SYNTHESIS OF FUNCTIONALISED, CROSSLINKED POLYMER NETWORKS

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University of Strathclyde

Declaration of authenticity

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Abstract

Crosslinked polymer networks that are largely insoluble and tolerant to various chemical environments are useful in solid-supported chemistries and as sorbents in chemical separation. The selective retention of organic pollutants is an area of environmental importance for the clean-up of contaminated water sources, and a family of ion-exchange hypercrosslinked polymer particles have recently been developed for use as mixed-mode solid phase extraction sorbents. Expanding this family of polymers will give rise to a greater range of materials with enhanced selectivity and capacity for specific analytes of environmental concern. This work is an investigation of methods to synthesise and functionalise crosslinked polymer networks with ion-exchange character.

The approaches explored for the synthesis of crosslinked polymers containing aromatic rings bridged by methylene ($–CH_2$) chains include phenol-formaldehyde inspired chemistry, precipitation polymerisation, and Friedel Crafts chemistry. Weak anion-exchange character was imparted by the introduction of tertiary amine groups through post-polymerisation chemical modification reactions. Furthermore, by utilising amino acid derived alkyl esters it was proposed that there could be potential to exploit the weak cation-exchange character of the deprotected carboxylic acid and harness and exploit zwitterionic character.

The use of precipitation polymerisation to synthesise poly(divinylbenzene-*co*-vinylbenzyl chloride) yielded spherical particles of low polydispersity in the 2-5 µm size range, with tuneable particle size and porosity (0-740 m^2/g). Subsequent hypercrosslinking using Friedel-Crafts chemistry produced microporous polymers with a high concentration of micropores and ultra-high specific surface areas (1,900 m^2/g). Amino acid derived esters, including sarcosine methyl and ethyl esters, and the L- and D- enantiomers of phenylalanine methyl ester, were utilised in a post-polymerisation amination study to impart weak anion-exchange character into the polymers at a level of around 1 mmol/g. Through evaluation of the macroreticular and hypercrosslinked variants as mixed-mode solid phase-extraction sorbents for the extraction of spiked organic analytes from ultra-pure water, it was found that these sorbents possess additional and useful functionality that is distinct from the weak anion-exchange polymeric sorbents that have been reported previously.

Abbreviations

ACN	Acetonitrile
2-EH	2-Ethyl-1-hexanol
AIBN	2,2'-Azo*bis*(isobutyronitrile)
AMPSA	2-Acrylamido-2-methylpropane sulfonic acid
AS	4-Acetoxystyrene
BET	Brunauer-Emmett-Teller
BuAc	Butyl acetate
CMPs	Conjugated microporous polymers
COFs	Covalent organic frameworks
DCE	1,2-Dichloroethane
DMF	*N,N'*-Dimethylformamide
DVB	Divinylbenzene
EtOH	Ethanol
FRP	Free radical polymerisation
FS	Formaldehyde solution
FT-IR	Fourier-Transform infrared
GCBs	Graphitised carbon blacks
HEMA	2-Hydroxyethyl methacrylate
HMTA	Hexamethylenetetramine
HPLC	High-performance liquid chromatography
IEC	Ion-exchange capacities
IUPAC	International Union of Pure and Applied Chemistry
MAA	Methacrylic acid
MEK	Methyl ethyl ketone
MeOH	Methanol
MMA	Methyl methacrylate
MOFs	Metal organic frameworks
NMP	*N*-Methyl pyrrolidone
NSAID	Non-steroidal anti-inflammatory drug
PCPs	Personal care products
PETRA	Pentaerythritol triacrylate

PF	Paraformaldehyde
PGCs	Porous immobilized graphitic carbons
PIMs	Polymers of intrinsic microporosity
SAX	Strong anion-exchange
SCX	Strong cation-exchange
SEM	Scanning electron microscopy
SPE	Solid-phase extraction
SSA	Specific surface area
THF	Tetrahydrofuran
VBC	4-Vinylbenzyl chloride
VP	4-Vinylphenol
WAX	Weak anion-exchange
WCX	Weak cation-exchange
θ	Theta

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Chapter 1 - Introduction

1.1. Polymerisation

Polymerisation is the process of synthesising a macromolecule by linking together monomer units, which are small repeat units. The mechanisms of polymerisation are generally classified into one of two categories: chain-growth polymerisation or step-growth polymerisation.

1.1.1. Step-growth polymerisation

In step-growth polymerisation, the polymer chains are built up stepwise by the combination of monomer molecules to form dimers, trimers and higher order species. This method requires at least two functional groups to be present on each monomer molecule; this functionality can be the same, as in the case of A_2 and B_2 difunctional monomers, or different like AB difunctional monomers. Simple, step-growth polymerisations involving the reaction of difunctional monomers produces linear polymers, however when a trifunctional (or higher order) monomer is introduced this leads to chain branching in the polymer.

Common examples of polymers that are synthesised by step-growth polymerisation include polyesters, polyurethanes, polyamides (nylons) and phenol-formaldehyde resins.

The monomers are consumed rapidly leading to high levels of monomer conversion; however, the molecular weight of the polymer increases slowly due to the formation of dimers, trimers and other oligomers, and only increases rapidly at high monomer conversion. There is no requirement for a polymerisation initiator in these reactions as the mechanism proceeds without initiation, propagation and termination stages. In some instances a catalyst may be required. The polymer end groups are usually always reactive; the reaction only ends when the source of monomer has been depleted.

1.1.2. Chain-growth polymerisation

Chain-growth polymerisation involves the successive addition of monomer molecules to the end of a propagating chain. This process involves the generation of reactive intermediates, sometimes referred to as active centres, *e.g.*, free radicals, cationic or anionic species.

1.1.3. Free radical polymerisation

Free radical polymerisation (FRP) is a widely used form of chain-growth polymerisation which often utilises vinyl monomers. With 1-substituted alkenes, the substituent, R, normally aids the stabilisation of the free radical on the substituted carbon. If this R group is an alkyl group, *e.g.*, a methyl group in the case of the monomer propylene, then the radicals will be poorly stabilised and it will be difficult to polymerise by free radical polymerisation under mild conditions. Common examples of monomers used frequently in FRP are styrene, methyl methacrylate and vinyl chloride.

The choice of initiator used is dependent on the conditions employed in the polymerisation. To be effective, the initiator should undergo homolytic fission into radical species of higher reactivity. Some common free radical initiators include those that are susceptible to thermal or photochemical dissociation. Typical oil-soluble initiators are organic peroxides, like benzoyl peroxide, or azo compounds such as 2,2'-azo*bis*(isobutyronitrile) (AIBN). The propagation stage involves the growth of the macromolecular chain by successive and identical addition of monomer to the growing chain. The propagation occurs at the reactive chain end and continues until a termination reaction halts the growth, or until all of the monomer has been consumed. The process of chain termination can occur throughout the reaction by combination, in which two reactive propagating species combine, or by disproportionation, which involves hydrogen atom abstraction from one reactive chain end to produce one unsaturated double bond and two dead polymer chains. In these termination reactions the free radical species are destroyed. The mechanism of termination will vary depending on the monomers and polymerisation conditions used. It has been reported that polystyrene and poly(methyl methacrylate) are terminated mainly by combination and disproportionation, respectively.[1] An additional chain-transfer reaction can occur throughout the polymerisation, which exchanges the active centre between molecules during a collision. In this mechanism the free radicals are not destroyed, but instead they are transferred from one chain to another, and in so doing they stop the growth of one chain and activate the growth of another, therefore shortening the average length of the polymer chains.[2]

In contrast to step-growth reactions, the molecular weight increases rapidly even at low monomer conversion. Long polymer chains can be produced using low levels of initiator. The rate of propagation is very fast so that high molecular weight polymers can be synthesised very rapidly. At any one time in the reaction there will be unreacted monomer and initiator, propagating chains and high molecular weight chains that have terminated.[3]

1.1.4. Crosslinked polymers

Polymerising mono-functional vinyl monomers produces linear polymers which are normally soluble in chemically compatible solvents. However, when there are two or more polymerisable vinyl groups within the monomer molecule then a variety of polymer architectures can be created. These multi-functional monomers, called crosslinkers, have the ability to crosslink multiple linear polymer chains. An example of an industrially important crosslinked polymer network is Merrifield resin, poly(styrene-*co*-divinylbenzene), which can be prepared by copolymerising styrene with the crosslinking monomer divinylbenzene (DVB), Figure 1.1.[3]

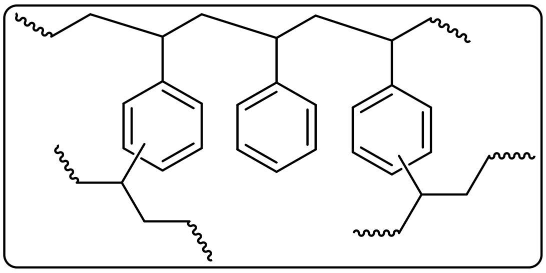


Figure 1.1: Example structure of poly(styrene-*co*-divinylbenzene)

Local concentration effects can contribute to determining the probability of inter- or intramolecular crosslinking. Intermolecular crosslinking will be favoured at higher concentrations of polymer in solution due to the higher number of polymer chains within close proximity to one another. In dilute solutions the polymer chains will be localised further away from one another within solution, and so intramolecular crosslinking will be favoured as there would be a shortage of neighbouring polymer chains to react with.

When a crosslinker such as DVB has been added to a polymerisation, it cannot be guaranteed that both of the vinyl groups on all of the molecules will react, and it is often found that there are unreacted pendent vinyl groups remaining in the polymer.[4] However, these vinyl groups can be used advantageously as sites for further chemical modification and functionalization.[5-7]

1.1.4.1. Microgel, gel-type and macroreticular polymers

The morphology and nature of the crosslinked polymers obtained can be governed by the crosslink ratio, and both the volume and type of solvent used. The crosslink ratio is defined as the ratio of crosslinker to total monomer in the feed (including crosslinker)[3], and is usually expressed as a percentage as shown in Equation 1.[8]

$$\%\,crosslinker = \frac{mol\ or\ mass\ of\ crosslinker\ (divinyl\ monomer)}{total\ mol\ or\ mass\ of\ monomers}10^2$$

Equation 1

When crosslinking under very dilute reaction conditions in the presence of a high volume of solvent, the primary polymer particles that form can remain in a non-aggregated state and can be recovered as powders. The primary particles are called microgels and the resulting powder is called a microgel powder. By comparison, if a higher monomer concentration in solvent had been used the microgels would typically fuse together to form gel-type polymers or macroporous polymers by macrogelation. Microgels have been considered as intermediates to branched and insoluble macroscopic polymer networks.[9]

Intramolecular crosslinking reactions are favoured because there is a higher local concentration of pendent vinyl groups available for reaction intramolecularly, in comparison to the number of vinyl groups available on other polymer chains. This probability is described by the Ziegler dilution law.[9]

The preparation of gel-type polymers will often require a low crosslinker ratio, or a higher crosslinker ratio but in the presence of a low volume of thermodynamically compatible solvent.[10]

A gel-type product prepared by polymerisation will be lightly solvated and prone to collapse upon drying to form an amorphous, glassy, gel-type polymer. These materials possess very low specific surface areas in the dry state due to the polymer chains being in close molecular contact, and also good swelling capacity in thermodynamically good or compatible solvents. The swelling capacity will have an elastic limit, beyond which it is not possible to swell the polymer any further even with very ‘good’ solvents. This is due to the crosslinks that link the polymer chains together and prevent the polymer chains from migrating apart. These gel-type particles will not swell in thermodynamically bad or non-compatible solvents.

In comparison to gel-type polymers, macroporous (or macroreticular) polymers will possess much higher specific surface areas in the dry state and also allow thermodynamically ‘bad’ or non-compatible solvents to access the pores within the structure.[10] They can be produced by using a higher crosslink ratio.

Macroporous polymers are otherwise known as macroreticular polymers.[4] Unfortunately, the macroporous terminology can be a little bit misleading as it does not reveal anything about the size of pores within the polymer, but in fact only signifies that there is a permanent and well-defined pore network present in the dry state. Pore sizing within permanently porous polymers shall be discussed later in Section 1.4.

1.2. Phenolic resins

Phenolic resins are products of step-growth polymerisation. Phenols and aldehydes were reacted together to produce resinous substances in the 1870s by Adolf von Baeyer, however the first successful commercialisation of these materials was when Leo Baekeland formed the General Bakelite Co. in the USA in 1910. By incorporating wood or mineral fibres into the resin and moulding under heat and pressure, Baekeland managed to mould and cure the resins into tough, temperature-resistant materials with low void content.[11] Although phenolic resins are based mainly upon phenol itself, higher analogues have also been used, such as cresols (methylphenols), xylenols (dimethylphenols) and resorcinols (diols).

The polymers produced during the production of phenol-formaldehyde resins can be classified into categories that reflect their physical properties:[12]

- A-stage resin: The polymer may be liquid or solid, but is soluble in simple organic solvents and fusible.
- B-stage resin: The resin is solid and insoluble, although may be swollen by solvents. The resin is infusible, but softened by heat.
- C-stage resin: The resin is solid, insoluble and infusible. It will not swell in solvents or be softened by heat.

1.2.1. The reaction between phenol and formaldehyde

Phenol has a functionality of three, which means that it can react with formaldehyde at the three *ortho*- and *para*- positions with respect to the phenol hydroxyl moiety, and formaldehyde has a functionality of two. Therefore the reaction between phenol and formaldehyde results in a three-dimensional structure.

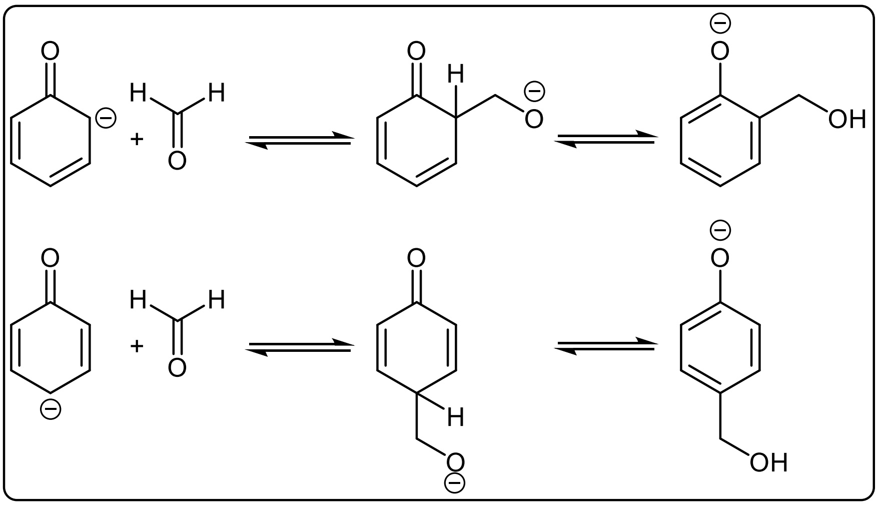
Baekeland investigated how the resulting resins were affected by varying the quantity of formaldehyde used in the phenol:formaldehyde ratio, in the presence of both basic and acidic catalysts. It was found that the reaction between phenol and formaldehyde using an excess of formaldehyde led to a heat-reactive product, that could only be controlled in the presence of basic catalysts and not acidic catalysts.[12] The two classes of phenol-formaldehyde polymers, namely resoles and Novolacs, are dependent on the molar ratio of the reactant phenol and formaldehyde and the pH conditions used.

Under basic conditions

Basic conditions and an excess of formaldehyde relative to phenol are required to prepare resole resins. The strong basic catalysts used in resole preparation include metal hydroxides, such as sodium hydroxide, calcium hydroxide or barium hydroxide. It is possible to prepare a crosslinked structure without the addition of a curing agent, therefore it is often written that resole resins are one-stage resins.[12]

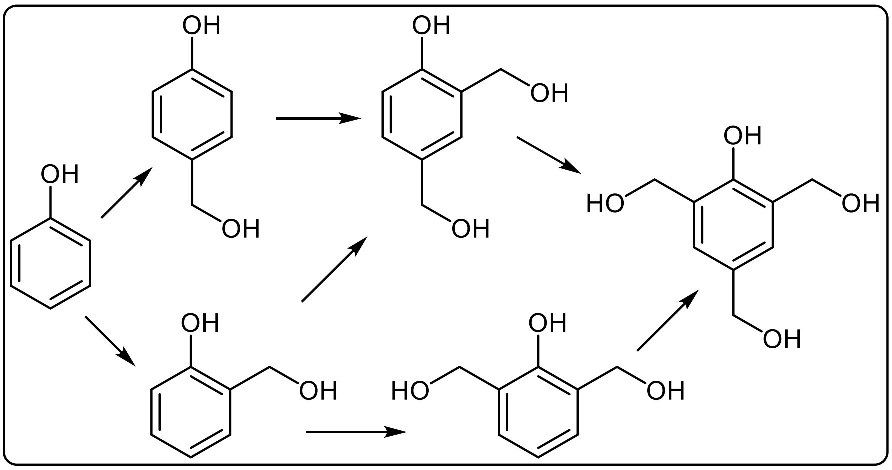
When formaldehyde is present in either acidic or basic aqueous solution, it exists as hydrated methylene glycols (methanediols). Due to reaction with alcohols such as the stabilising methanol in formaldehyde solution, or the hydroxyl moieties on the phenol products, a quantity of the formaldehyde may also exist as hemiformals.

Under basic conditions with a catalyst such as sodium hydroxide, the first stage of the reaction is the formation of the phenoxide anion by deprotonation of the phenol moiety. Delocalisation of the anionic charge around the ring is then possible. The reaction between the phenoxide anion and formaldehyde, as shown in Scheme 1.1, results in the formation of *ortho*- and *para*-methylolphenols.



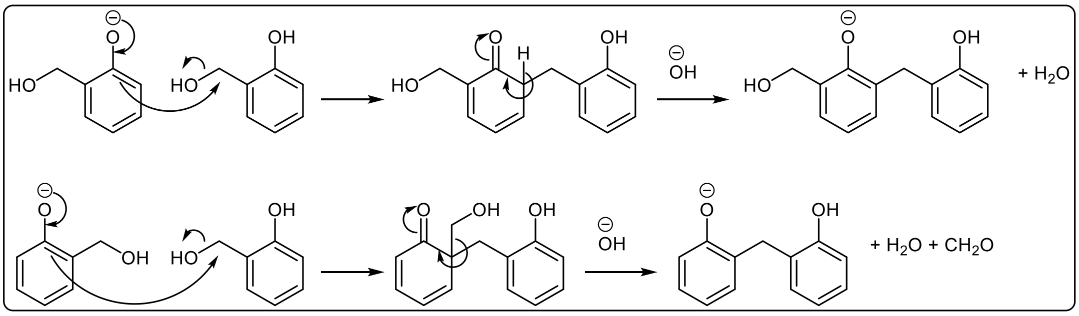
Scheme 1.1: The alkylation of phenol at the *ortho*- and *para*- positions to form methylolphenols

Methylolphenols are more reactive towards formaldehyde than the reactant phenol, so in the presence of excess formaldehyde they undergo further substitution to form di- and trimethylol derivatives as shown in Scheme 1.2.[13]



Scheme 1.2: The reaction from phenol to methylol phenol and the further reaction pathways to di- and trimethylol derivatives [12, 13]

These methylolphenols form di-, tri- and polynuclear phenols by a self-condensation mechanism, either involving two methylol groups or one methylol group reacting with a hydrogen atom at an unsubstituted *ortho*- or *para*- site on the methylolphenol ring. The S_N2 attack on the methylol carbon and displacement of the hydroxyl moiety leads to the subsequent elimination of either a proton or formaldehyde, depending on the applicable mechanism shown in Scheme 1.3.[12, 13]



Scheme 1.3: The self-condensation reaction between methylolphenols to form methylene bridging[12, 13]

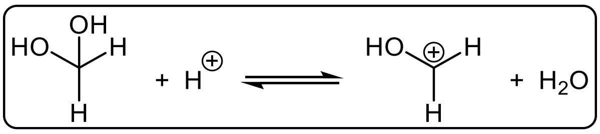
Competitive condensation reactions between two methylol groups may result in the formation of ether linkages between the phenolic nuclei, in addition to the methylene linkages. However, the formation of benzyl ethers is more probable under neutral or slightly acidic conditions, so under basic conditions the predominant reaction is methylene bridge formation.

The polynuclear phenols formed are the resole resin. This prepolymer is a low molecular weight, fusible and soluble A-stage resin. Upon heating and curing to a C-stage resin, the resole will transform to a crosslinked, infusible and insoluble solid 3-dimensional network called a resite. It is not necessary to add any additional crosslinker or curing agents to the resole as it is regarded as a one-stage process, where the initial quantity of formaldehyde present is sufficient to form these highly crosslinked resins.

Under acidic conditions

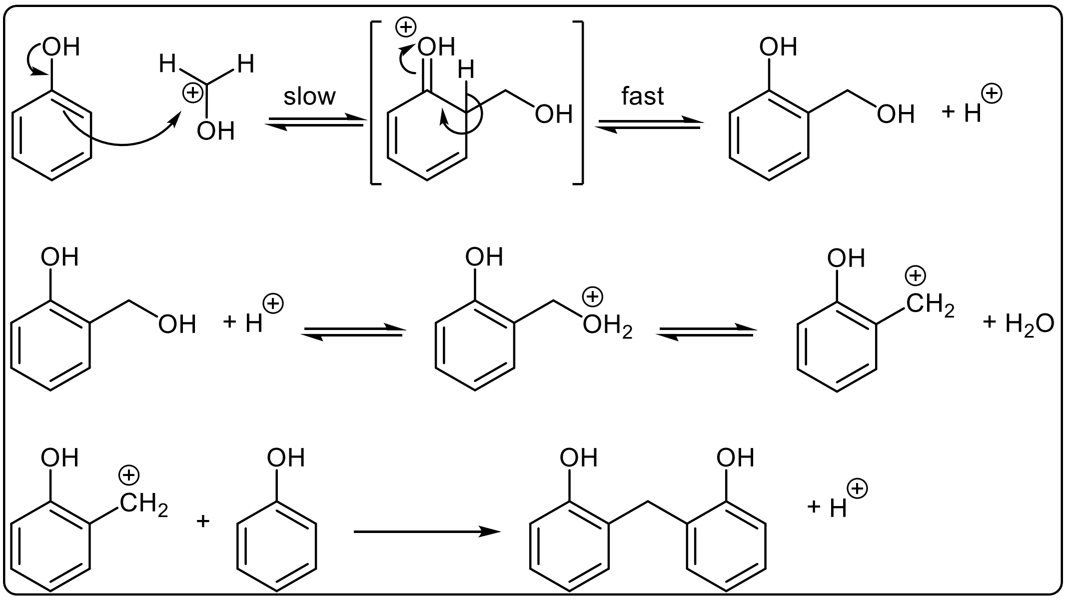
Acidic conditions and an excess of phenol relative to formaldehyde are utilised in the preparation of Novolac resins. The acidic catalysts commonly used include sulphuric acid, sulfonic acid, oxalic acid, phosphoric acid and hydrochloric acid.

At acidic pH the formaldehyde (as hydrated methylene glycol in solution) is protonated to the carbenium ion $^{+}CH_2OH$, as shown in Scheme 1.4.



Scheme 1.4: The formaldehyde (as methylene glycol) exists as a carbenium cation at acidic pH

The reaction scheme for the reaction of phenol and formaldehyde under acidic conditions is shown in Scheme 1.5. The rate determining step is the electrophilic substitution between phenol and the generated carbenium ion. The *ortho*- and *para*-methylolphenol groups are reactive and unstable under acidic conditions, so only exist transiently before eliminating water to form benzylic carbenium ions. The reaction between free phenol molecules and the benzylic carbenium ions produces the diphenylmethylene bridged products.[13]



Scheme 1.5: The reaction between phenol and formaldehyde, under acidic conditions, proceeds *via* a benzylic carbenium to generate dihydroxydiphenylmethane [12, 13]

In contrast to the preparation of resole resins, the first substitution of two phenol molecules to dihydroxydiphenylmethanes is deactivating towards further electrophilic substitution; therefore, there is often a high initial concentration of dinuclear phenols.[13] Polynuclear phenols are formed by further methylolation and methylene bridging between the rings, although the resins are often linear in nature. Only at higher molecular weights, when there are fewer end groups and less unreacted phenol present, is there a higher probability of polymer branching occurring.

Novolac resins are thermoplastic in nature, and require the addition of an auxiliary chemical curing agent to produce a crosslinked network. The most common additive for this is hexamethylenetetramine (HMTA), Figure 1.2.

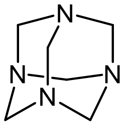


Figure 1.2: The auxiliary chemical crosslinking agent hexamethylenetetramine (HMTA) that is commonly added to cure Novolac resins

1.3. Heterogeneous polymerisation

Homogeneous methods of polymerisation include gas phase, bulk and solution polymerisation. These processes are termed homogeneous as the monomer, initiator and solvent (if required) are all mixed together in a single phase and the polymer does not precipitate during the course of reaction as the resulting polymer remains soluble within the medium. However, heterogeneous polymerisation involves two or more phases within the polymerisation. This can commonly utilise the immiscibility of two different liquids, or result from a change in polymer solubility during the course of the reaction. The particles formed often have an approximately spherical symmetry because this shape represents the lowest surface to volume ratio with the lowest interfacial tension.[14]

Some of the conventional and widespread heterogeneous techniques used to prepare polymer particulates include: suspension, dispersion, precipitation and emulsion polymerisations. Estimates of the particulate sizes that can be obtained from suspension, dispersion, precipitation and emulsion polymerisations have been given[14, 15] as: 20-2000 µm, 0.1-10 µm, 0.1-8 µm and 0.05-0.3 µm, respectively.

These techniques will be introduced briefly in the following sections, with particular emphasis placed on the discussion of precipitation polymerisation due to the importance of this technique for the preparation of polymer microspheres in the current investigation.

1.3.1. Emulsion polymerisation

Typically in classical emulsion polymerisation, the monomers will be insoluble in the continuous phase but will be stabilised by the presence of surfactant stabilisers. The monomer will partially exist within micelles coated with surfactant and emulsified in the continuous phase, and also be present as larger monomer droplets. A low level of the monomer will also be molecularly dissolved within the continuous phase depending on the solubility of the monomer.[15] In contrast to the other heterogeneous polymerisation techniques discussed, the free radical initiator is soluble in the continuous phase. Consequently, the initial locus of polymerisation is in the continuous phase. The dissolved monomer forms oligoradicals within the continuous phase that will subsequently result in surfactant-stabilised growing nuclei particles. These nuclei will continue growing until all of the monomer present has been consumed. Oil-in-water emulsions exist when the oil phase is emulsified within an aqueous phase, and conversely, water-in-oil emulsions will exist when the aqueous phase is emulsified within the oil.

1.3.2. Suspension polymerisation

The monomers (including crosslinker), initiator and porogen together constitute the dispersed organic phase that is suspended as droplets within the continuous phase containing the dispersion medium and stabilisers. Droplets form due to the low solubility of the monomers in the medium, which is commonly water. A stabiliser (suspension agent) is usually required to hinder the subsequent coalescence and break-up of droplets, and stirring is usually applied. In order to optimise the quality of the suspension particles it is important to consider the operational parameters and apparatus design. It has been reported that the shape and design of the reactor vessel and agitator/stirrer can influence the size distribution of particles, due to the need for uniform stirring throughout the mixture.[14, 15] The resulting polydispersity in particle size is often undesirable; however, the particles can be sieved to a specific size range after they have been isolated, if required.

Free radical polymerisation occurs within the droplets as the monomer soluble initiator dissociates into radical species. The porosity of the suspension particles can be tailored by careful selection of the porogen. In the presence of a thermodynamically good porogen, the phase separation occurs late, to yield porous particles. These particles are predominantly micro- and mesoporous with high specific surface area values and low pore volumes.

Alternatively, in the presence of a bad porogen, the phase separation occurs early, leading to particles with lower specific surface area values but higher pore volumes.[14] When intermediate pore sizes are required this can be achieved by employing a mixture of both solvent and non-solvent porogens.[16]

1.3.3. Dispersion polymerisation

In dispersion polymerisation the monomer, initiator, porogen and steric stabiliser are all dissolved in a suitable solvent to form a homogeneous solution. Agitation is required by means of a mechanical stirrer. The presence of the stabiliser adsorbed on the surfaces of the particles prevents the collisions between individual particles from resulting in aggregation. In the absence of a stabiliser the particles would most likely coagulate.[15] There is no requirement for crosslinkers to be present during dispersion polymerisation, so this is a technique which has the potential to produce non-crosslinked and non-porous particles, although crosslinkers can be incorporated at low levels if so desired.[14]

The initiators decompose under heating to initiate polymerisation and the formation of oligomers that are soluble within the media. As the oligomers grow the solution becomes cloudy due to precipitation and phase separation. These nuclei that have been formed are stabilised by the steric stabilisers, and grow by the addition of captured monomer and oligomers. If crosslinkers are required in the synthesis, ideally they must be added after the nucleation stage to ensure that the polydispersity of the particles remains low.

1.3.4. Precipitation polymerisation

The initial homogeneous reaction mixture consisting of monomer, initiator and solvent results in the formation of polymer that phase separates from the reaction medium as insoluble and crosslinked polymer microspheres in the low or sub-micron size range. In contrast to the suspension, emulsion and dispersion polymerisation techniques used to produce polymer particulates, there is no requirement for a surfactant/stabiliser.

Although they both begin as homogeneous solutions, there is a different growth mechanism in precipitation polymerisation compared to dispersion polymerisation due to the effect of the crosslinker and the absence of the stabiliser. The growth mechanism proposed by Stöver *et al.*[17] is shown in Figure 1.3. In the nucleation stage the soluble oligomers formed by free radical polymerisation are captured from solution by the reaction with pendent vinyl groups on the surfaces of the existing particles.[10] The desolvation can occur by either entropic or enthalpic precipitation. The particles that have undergone entropic precipitation have captured and crosslinked oligomers using the vinyl groups upon the particle surfaces. This growth mechanism has been tested by Stöver *et al.*[17] and it has been shown that the reactive vinyl groups at the particle surface are necessary for uniform particle growth to create monodisperse particles. If the surface vinyl groups were to be removed, or protected, then the particles would be unable to grow due to the lack of reactive sites for the capture of oligomers by radical reaction. The alternative hypothesis is enthalpic precipitation, which proposes that polymer deposits onto the surfaces of existing particles and this leads to phase separation because the polymer-polymer interactions are favoured over the polymer-solvent interactions.[9]

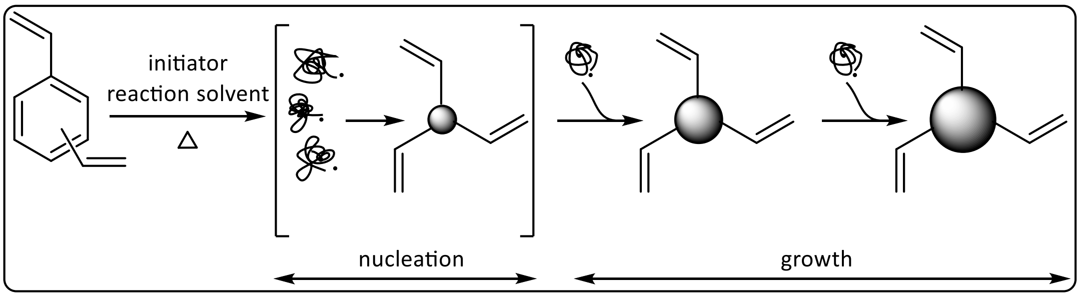


Figure 1.3: The proposed particle formation process for precipitation polymerisation showing the nucleation and growth stages[17]

A radical initiator commonly used in precipitation polymerisation is AIBN, added at a concentration of around 2 wt % relative to monomer or 2 mol % with respect to the total number of polymerisable double bonds. Dilute monomer loadings of 2-5 % (w/v) are required in order to allow the microspheres to grow individually without aggregating to form an interconnected network.[16, 18]

The presence of a crosslinker such as DVB is crucial to the success of precipitation polymerisations. If styrene was to be polymerised in neat acetonitrile without the presence of DVB this would fail to produce insoluble polymer particles. The resulting polymer formed would instead exist as soluble polymer dissolved in the solvent, due to acetonitrile being a thermodynamically good solvent for polystyrene.[19]

The high degree of crosslinking that exists within the particles produced by precipitation polymerisation prevents the coagulation of the particles as the particles are hard and resilient. Increasing the concentration of crosslinker present in the monomer feed results in the formation of particles with improved stability due to the higher degree of crosslinking.[19] At higher concentrations of DVB the solubility of the nuclei in acetonitrile is poorer, and therefore smaller sized final particles would be observed due to the high initial quantity of nuclei formed in the reaction.[19] However, crosslinking alone is not enough to form microspheres. The solvency of the continuous phase must be suitable for phase separation to occur. A theta (θ) solvent is a solvent that is at the borderline of being a thermodynamically good solvent and a precipitating medium for the polymer chains in the reaction.[20] The selection of a θ-solvent for a reaction will depend on the polymer and the temperature. At the θ-state there is an absence of long-range interactions, the polymer exhibits its unperturbed dimensions and the polymer solution represents an ideal solution.[21] Crosslinking of the polymer chains changes the polymer-solvent interactions and disturbs the θ-state, so microphase separation will occur during the final stages of polymerisation.[10, 20] The marginal, near-θ solvent commonly used for the precipitation polymerisation of DVB is acetonitrile.

Utilising a co-solvent improves the solvency of the reaction medium, which leads to a later onset of the phase separation between the polymer and solvent. This increase in the duration of time in which the polymer is in contact with the solvent allows the polymer network to entrap more molecules of solvent that lead to the formation of a pore system when the solvent has been removed. Studies have been performed that have investigated the use of toluene as a co-solvent at levels up to 40 % in acetonitrile.[16, 18]

Acetonitrile is a solvent that is frequently used in precipitation polymerisations due to its good miscibility with many organic monomers and solvents. However, the harmful properties of acetonitrile have provided motivation for recent research investigating the use of alternative solvents for precipitation polymerisation. Yan and Tan *et al*.[22, 23] have studied the successful synthesis of monodisperse crosslinked poly(DVB) and poly(styrene-*co*-DVB) in neat acetic acid, respectively. However, utilising acetic acid as a polymerisation solvent is far from ideal.

Agitation of the particles in the reaction vessel must be gentle to avoid particle coagulation. It is common practice to perform precipitation polymerisations on a motorised roller in order to gently rotate the sealed reaction vessel in a controlled and continuous manner which also prevents the particles from settling on the interior surfaces of the reaction vessel, Figure 1.4. The use of magnetic stir bars should be avoided where possible as this could damage the polymer microspheres by grinding. The resulting particles are often clean in appearance and free of ionic charge due to the absence of stabilisers.[16]

Figure 1.4: A typical low-profile roller and temperature controllable incubator system used in precipitation polymerisation reactions

1.4. Microporous solids

Microporous solids are a class of materials that contain permanent pores of less than 2 nm in diameter. The International Union of Pure and Applied Chemistry (IUPAC) reported and classified materials based upon their pore size. They define macropores as pores greater than 50 nm in diameter, mesopores as pores that have a diameter between 2-50 nm, and micropores as pores with a diameter that is no greater than 2 nm.[24] The materials listed here all contain porosity that has arisen due to the molecular structure, as opposed to pores that have been created by templating effects. Permanent porosity exists within a network if the structure remains porous when in the dry-state, *i.e.*, the pores do not collapse upon drying.[25]

Microporous materials are comprised of materials with both crystalline and amorphous structures. Well-ordered structures and uniform micropores are obtained from crystalline materials, whilst amorphous materials are typically disordered in their structure and have less regularity with respect to the pore structure.[25] Some examples of microporous solids include activated carbons, silicas, zeolites, metal organic frameworks (MOFs), covalent organic frameworks (COFs), conjugated microporous polymers (CMPs), polymers of intrinsic microporosity (PIMs) and hypercrosslinked polymers.[26] Within this, the title of microporous organic polymers has been designated to describe: triazine networks; covalent organic frameworks; polymers of intrinsic microporosity; conjugated microporous polymers; and hypercrosslinked polymers.[27, 28] Due to the importance of the topic in this thesis, a more in-depth introduction to hypercrosslinked polymers will be given in Section 1.5.

1.5. Hypercrosslinked polymers

Hypercrosslinked polymers are a heavily crosslinked class of microporous organic polymers which possess ultra-high specific surface areas and interesting sorption properties. Hypercrosslinked polymeric materials were introduced initially by Davankov's research group in the early 1970s and are sometimes referred to as Davankov resins.[29-34]

1.5.1. Synthesis of hypercrosslinked polymers by Friedel-Crafts chemistry

The original methodology involved the post-polymerisation crosslinking of linear polystyrene chains which were dissolved in a thermodynamically good solvent, anhydrous 1,2-dichloroethane (DCE), to form gels that were classified as macronet isoporous polymers.[33] The reaction was based upon Friedel-Crafts chemistry using a Lewis acid, $SnCl_4$, which enabled dissolved linear chains of polystyrene or swollen styrene-DVB copolymers to be crosslinked with the bifunctional compounds monochlorodimethylether (chloromethyl methyl ether) or 4,4'-*bis*(chloromethyl)-1,1'-biphenyl.

The structure of the crosslinking bridge between the polymer chains is dependent upon the crosslinking compound used.[32] The Lewis acid reacts with the crosslinking compound to generate an 'external' *bis*-electrophile that participates in Friedel-Crafts type hypercrosslinking. Figure 1.5 shows the structure of the bridge formed from compounds chloromethyl methyl ether, 4,4'-*bis*(chloromethyl)-1,1'-biphenyl and *p*-xylene dichloride. All of the bridges have limited conformational mobility, but the most rigid crosslink is methylene ($-CH_2-$) bridging. The first review paper published in 1990 cites some of the other crosslinking agents that have been employed, and the terminology used was changed to describe networks with a degree of crosslinking of > 40 % as being hypercrosslinked.[34]

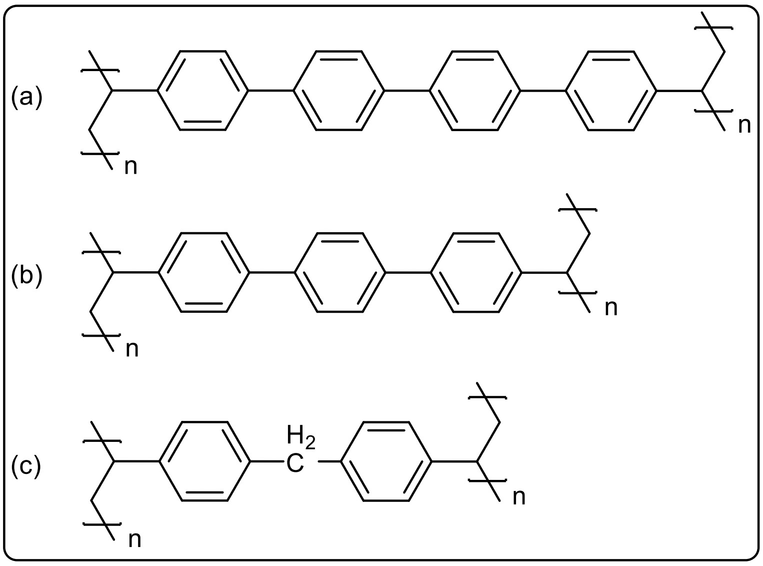


Figure 1.5: The structure of the bridges formed between the polymer chains using crosslinking compounds (a) 4,4'-*bis*(chloromethyl)-1,1'-biphenyl, (b) *p*-xylene dichloride and (c) chloromethyl methyl ether

A large quantity of thermodynamically good solvent was required for these hypercrosslinking reactions, and a concentration of 9 wt. % has been given for linear polystyrene dissolved in DCE.[32] The polymer solution becomes a gel upon crosslinking, taking the shape of the vessel but decreasing in volume as solvent is separated from the polymer by syneresis.

Introducing a large number of crosslinking bridges to the solvated polymer fixes the conformation of the polymer chains into an expanded state. When the gel is wetted in the presence of a good solvating medium, such as a θ-solvent, then there are no major internal stresses. However, upon drying the polymer and removing the solvent, the gel attempts to contract but is hindered by the rigid network that has been created.[29] Therefore, these polymer structures possess an unusual ability to swell even in poor solvents. In an effort to overcome the strained polymer-polymer interactions of the dry state for a favoured polymer-solvent interaction, hypercrosslinked polystyrene has been shown to swell in non-solvents such as hexane, methanol, ethanol and water until a new equilibrium state has been achieved through expansion of the network volume.[33]

A further benefit of these hypercrosslinked polymers is that the materials are microporous and often possess ultra-high specific surface areas in the region of 1,000 m^2/g and above.

The apparent specific surface area increases with increasing degree of crosslinking within the network.[30]

Tsyurupa and Davankov have studied extensively the porous nature of hypercrosslinked polystyrenes and presented this information in a mini-review.[29] The porosity of hypercrosslinked polymers differs substantially from the porosity of macroreticular polymers of poly(styrene-*co*-DVB) because the porosity does not result from phase separation, but from post-crosslinking of solvated polystyrene chains. The dry hypercrosslinked polymers increase in volume on contact with gases and liquids, therefore the porous network is somewhat flexible and changeable. The determination of the specific surface area by nitrogen sorption analysis is therefore a measure of the capacity available to nitrogen gas, and is the apparent specific surface area and not a true reflection of the surface area. Scanning electron microscopy and X-ray crystallography have been used to examine hypercrosslinked polystyrene, showing it to be a single-phase material.[29] The pores do not have walls or defined space but instead the porosity is a representation of the voids present between the loosely packed chains.

The ability to swell in thermodynamically good and non- solvents and the increasing specific surface area with increasing degree of crosslinking have been reported as the two fundamentally important properties of hypercrosslinked polymers that distinguish them from gel-type polymers.[30]

The hypercrosslinking methodology developed for linear polystyrene has been modified for use with alternative monomers and conditions, so this appears to be a generic methodology that can be manipulated and tailored. When the products possess different swelling properties and porosity to the classical polymers prepared, then these materials may belong to the hypercrosslinked family.[31]

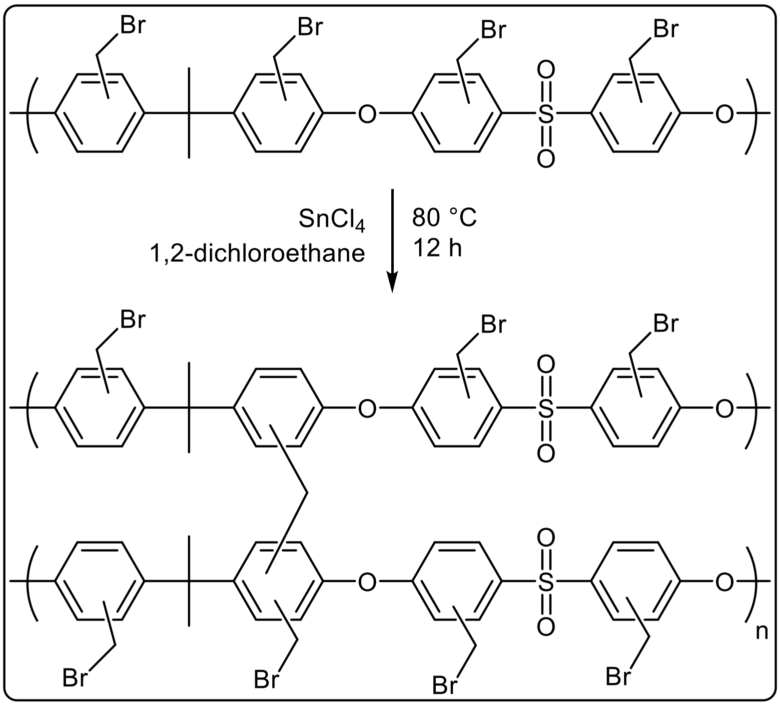
The chloromethylation of styrene-DVB copolymers to produce polymers with pendent chloromethyl moieties attached to the aromatic rings is one method of generating materials capable of crosslinking in the presence of a Lewis acid, without the need for an external crosslinking agent. The chloromethylated polystyrenes are macromolecular crosslinkers, therefore they effectively act as ‘internal’ electrophiles.[35, 36] This method of hypercrosslinking is a two-stage process, although chloromethylated precursors are readily available due to their use in ion-exchange and their synthesis is well described.

Veverka and Jeřábek have hypercrosslinked a gel-type chloromethylated polymer supplied by Spolchemie using $SnCl_4$ in DCE.[35] It was noted that the initial conversion of the chloromethyl groups to methylene bridges was rapid, and the conversion of the remaining reactive moieties proceeded more slowly. Of course, the crosslinking of chloromethylated polymers will result in some inaccessible chloromethyl moieties that are more difficult to crosslink. A later study by the authors investigated the hypercrosslinking of two macroreticular precursor polymers (of 10 mol % DVB and 17 mol % DVB) in addition to the gel-type polymer containing 5 mol % DVB.[36] These precursor polymers were supplied by Spolchemie and Research Institute of Resins and Varnishes.

A gel copolymer of poly(styrene-*co*-DVB) prepared by solution polymerisation and macroreticular poly(DVB) prepared by suspension polymerisation were crosslinked using distilled tetrachloromethane, in the presence of $AlCl_3$ or $FeCl_3$ and DCE.[7] The authors stated that tetrachloromethane is a less carcinogenic reagent than the crosslinking agent chloromethyl methyl ether that was commonly used in the early hypercrosslinking procedures.

Hypercrosslinked polyxylene has been prepared by the self-condensation of *p*-xylene dichloride. *p*-Xylene dichloride and $SnCl_4$ were dissolved in DCE and the solution was heated at 80 °C for 20 hours.[31] The values obtained for the specific surface area of hypercrosslinked polyxylene range from 900-1,000 m^2/g when the concentration of *p*-xylene dichloride in DCE was 250, 125 and 83 g/L.

Dissolving bromomethylated polysulfone in the thermodynamically good solvent DCE, in the presence of $SnCl_4$, has been shown to yield hypercrosslinked polysulfone, Scheme 1.6. The results obtained for the specific surface area were not particularly high, ranging from 10-72 m^2/g based upon the conditions utilised by the authors, but these values were greater than the experimental error of these measurements.[31] The polysulfone chains are more flexible than polystyrene chains due to the added oxygen link within the polymer backbone. The authors state that this conformational mobility may explain or justify the low values obtained, because this may give rise to denser chain packing within the structure and therefore less internal stress within the crosslinked polymer network. Additionally, the sulfone groups within the backbone deactivate the neighbouring aromatic rings and subsequently limit the number of rings available for crosslinking, thus resulting in a reduced number of bromomethyl groups able to participate in crosslinking.

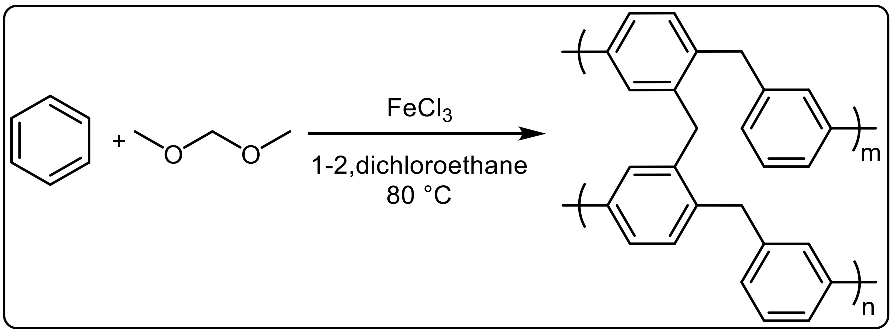


Scheme 1.6: Schematic representation of the crosslinking reaction of bromomethylated polysulfone in the presence of 1,2-dichloroethane and stannic chloride to form hypercrosslinked polysulfone[31]

Hypercrosslinked polyarylates have been prepared by the crosslinking of polyarylates using $SnCl_4$ in a dilute solution of DCE. Porosity was introduced to the hypercrosslinked polyarylates only when the concentration of polymer in solvent was very low, with specific surface areas of up to 380 m^2/g reported. The products were classified as belonging to the hypercrosslinking family due to their ability to swell in heptane, which is a precipitating solvent for polyarylate.[31]

Microporous hypercrosslinked polymers have been prepared by reacting small molecules such as benzene, biphenyl, 1,3,5-triphenylbenzene, methylbenzene, chlorobenzene and phenol using formaldehyde dimethyl acetal as the external bi-functional crosslinker in the presence of a Lewis acid.[37] Reacting phenol using this methodology leads to products with specific surface areas in the region of 400 m^2/g, whereas higher specific surface areas of

900-1,400 m^2/g can be obtained when materials are prepared by reacting benzene with varying levels of crosslinker, shown in Scheme 1.7.



Scheme 1.7: The schematic representation of the hypercrosslinking reaction between benzene and formaldehyde dimethyl acetal, using catalytic $FeCl_3$ in 1,2-dichloroethane[37]

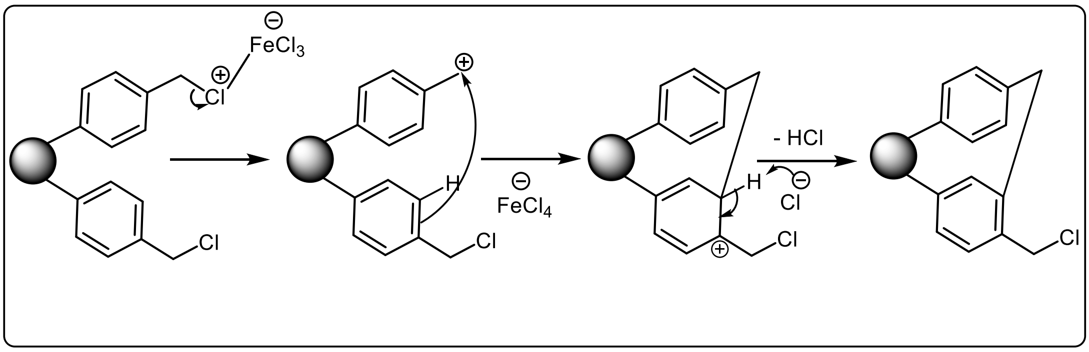
The Adams research group have utilised a similar protocol using the external crosslinker formaldehyde dimethyl acetal to hypercrosslink linear polystyrene synthesised by RAFT polymerisation, in the presence of $FeCl_3$ and 1,2-dichloroethane.[38]

The small molecule benzyl alcohol has been chloromethylated using chloromethyl methyl ether and then subjected to hypercrosslinking with $FeCl_3$ in nitrobenzene.[39] The measured specific surface area of the product was 525 m^2/g, and the average pore diameter fell within the microporous classification.

The phenyl groups of a templated phenolic resin were reacted with 1,4-*bis*(chloromethyl)benzene in a Friedel-Crafts alkylation reaction to produce a well-developed bimodal meso- and microporous framework of hypercrosslinked phenolic polymer. This polymer displayed improved CO_2 gas adsorption properties for CO_2 capture due to the bimodal micro-mesoporous framework, where micropores were incorporated on the mesoporous walls. [40]

In addition to the hypercrosslinking of linear polymers, there has been interest in the hypercrosslinking of spherical particulate polymers. Spherical precursor particles for hypercrosslinking are readily prepared by the heterogeneous polymerisation techniques of suspension,[30, 41] emulsion,[42] non-aqueous dispersion[43] and precipitation[43] polymerisations.

In the first stage, the copolymerisation of the functional monomer 4-vinylbenzyl chloride (VBC) and the crosslinking monomer divinylbenzene to give poly(divinylbenzene-*co*-vinylbenzyl chloride) (poly(DVB-*co*-VBC)) is carried out according to the relevant protocols for the technique. In the second stage, the lightly crosslinked poly(DVB-*co*-VBC) precursor particles are swollen in a thermodynamically good solvent, such as DCE, to an expanded state. Electrophilic aromatic substitution takes place when the Lewis acid, such as iron (III) chloride ($FeCl_3$), is added alongside the reactive, pendent chloromethyl moieties of the precursor polymer, as shown in Scheme 1.8. This Friedel-Crafts type reaction creates new C-C bonds and forms crosslinking methylene bridges between neighbouring aromatic rings.



Scheme 1.8: Poly(DVB-*co*-VBC) (left) undergoes electrophilic aromatic substitution to create new C-C bonds and produce a crosslinked product (right)

Ahn *et al.*[41] have investigated the choice of Lewis acids used to crosslink gel-type and macroreticular poly(DVB-*co*-VBC) precursor polymers prepared by suspension polymerisation. The Lewis acids $FeCl_3$, $AlCl_3$ and $SnCl_4$ were compared for hypercrosslinking. Although the research published by both the Davankov[31, 34] and Jeřábek[35, 36] groups have consistently used $SnCl_4$ within their reactions, Ahn and co-workers[41] discovered that $FeCl_3$ was the most active Lewis acid amongst the range that they examined. During the reaction between gel-type polymer precursors and $FeCl_3$, the measured chlorine content dropped rapidly within the initial 15 minutes of reaction and the specific surface area rose from zero (non-porous) up to 1,200 m^2/g. When the reaction was allowed to proceed for a total of 18 hours, it was found that the specific surface area increased further to 1,800 m^2/g after 2 hours of reaction but then rose only gradually for the remainder of the duration of the reaction.

Ahn *et al*. observed that the hypercrosslinking of non-porous gel-type precursor polymers yielded a unimodal population of micropores, whereas the products from the hypercrosslinking of macroreticular precursor polymers had a bimodal pore size distribution.[41] The proposed explanation was that the original pores of the macroreticular polymer are present alongside the micropores formed during the hypercrosslinking process.

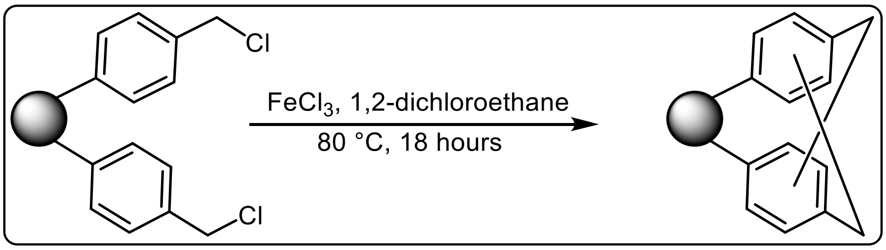
This bimodal porosity is potentially useful for facilitating mass transport within the macropores of the polymer. Xie has stated that the bimodal porosity could improve catalytic activity when hypercrosslinked polymers are used as catalytic supports, citing the work of Sidorov *et al*.[44, 45] Xie[44] has studied the preparation of hypercrosslinked polymers with a bimodal pore size distribution. The monomer concentration of precursor particles of poly(DVB-*co*-VBC) prepared by suspension polymerisation was varied prior to the hypercrosslinking reaction using $FeCl_3$ in DCE, and it was observed that the microporosity of the products was tuneable.

Li *et al*.[46] have also found that the pore structure of poly(DVB-*co*-VBC) hypercrosslinked polymers can be controlled by varying and adjusting the concentration of the crosslinking monomer DVB. They have observed that at DVB concentrations greater than 7 % it was possible to obtain a microporous structure without any meso- and macropores present.

The effect of the isomer composition of the functional monomer VBC was investigated for the synthesis of poly(DVB-*co*-VBC) particles by suspension polymerisation, and their subsequent hypercrosslinking.[47] The commercially available VBC monomer is available as either the *para*- isomer (4-vinylbenzyl chloride) or as a mixture of isomers (70 % *meta*- and 30 % *para*-isomers). The suspension polymerisation of DVB and VBC was performed twice in order to study the products prepared using the *para*-isomer (GpVBC-DVB) and the mixed isomers (GmixVBC-DVB). The precursor containing the *para*-isomer of VBC had lower chlorine content than the precursor prepared using the mixed VBC isomers. It has been proposed that hydrolysis of the benzyl chloride in the *para*- position to the polymer chain is more favourable than the *meta*- position, and oxygen has been detected inferred from elemental microanalysis results. This side reaction occurs due to the large quantity of water present in the suspension polymerisation. The products after hypercrosslinking possessed different surface areas, with specific surface areas of 908 m^2/g and 1,889 m^2/g for the *para*-isomer and mixed isomer products, respectively. The *para*- isomer has a higher content of hydroxyl moieties and performed better for the solid-phase extraction of polar compounds.

Macintyre *et al*. have synthesised hypercrosslinked polymers from poly(DVB-*co*-VBC) precursor particles prepared using surfactant-free emulsion polymerisation.[42] The hypercrosslinking procedure was performed using $FeCl_3$ in DCE, to yield nearly monodisperse particles of approximately 420 nm diameter. There was a substantial increase in the measured specific surface area from the non-porous precursor (10 m^2/g) to highly porous products with values of 1100-1300 m^2/g. A unimodal pore size distribution was obtained, with a high content of micropores. The solvent uptake experiments showed the characteristic properties of hypercrosslinked materials.

Micrometre-sized spherical particles have been prepared by the hypercrosslinking of particles prepared by non-aqueous dispersion or precipitation polymerisation.[43] Copolymers of poly(DVB-*co*-VBC) were reacted with $FeCl_3$ in DCE as shown in Scheme 1.9.



Scheme 1.9: A schematic representation of precursor poly(DVB-*co*-VBC) particles reacting with $FeCl_3$ to produce hypercrosslinked polymer microspheres

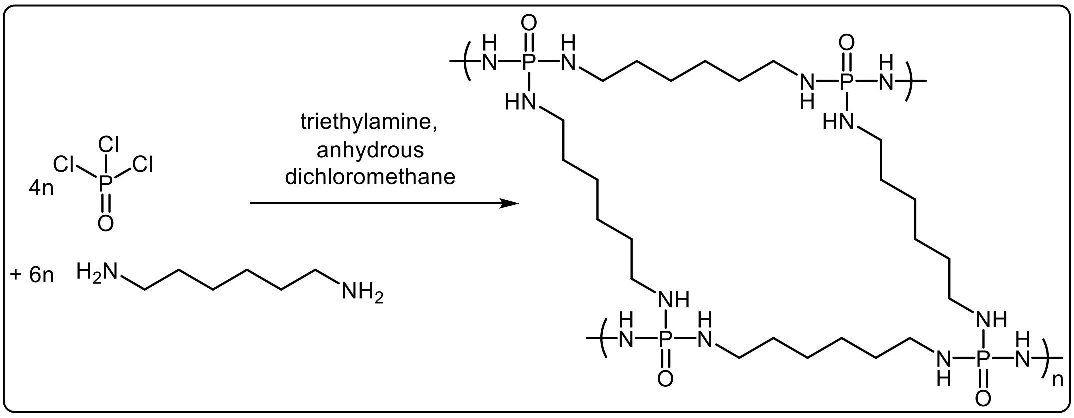
The precursor particles that were prepared by precipitation polymerisation yielded nearly monodisperse, spherical particles of approximately 4 µm in diameter. The precipitation polymerisation failed to generate particles by phase separation when the DVB content in the monomer feed was below 5 % (w/w) relative to VBC. As expected, the precursor microspheres had a very low specific surface area (5 m^2/g), but upon swelling in DCE and hypercrosslinking in the presence of $FeCl_3$ this specific surface area increased to 880-1320 m^2/g, depending on the DVB/VBC monomer ratio that was used. Precursor poly(DVB-*co*-VBC) particles with 25, 50 and 75 % (w/w) VBC content relative to DVB were used in hypercrosslinking reactions, and the results showed that the VBC content should be greater than 50 % in order to achieve specific surface areas greater than 1,000 m^2/g. Solvent uptake experiments for the solvents hexane, toluene and water were performed on the precursor particles from precipitation polymerisation and also on the products from hypercrosslinking. There was an increased capacity for water and toluene for the hypercrosslinked products compared to their precursors, confirming that the hypercrosslinked polymers have a greater capacity. The uptake of water is a particularly important result due to the hydrophobic nature of the polymers, indicating that this has arisen due to the rigid and strained crosslinked network in place.[43]

1.5.2. Synthesis of hypercrosslinked polymers by other methodologies

A brief outline of some alternative hypercrosslinking reactions and methodologies have been provided, to show that the area has been expanding and extending far beyond the original methodology that Davankov brought to attention many years ago.

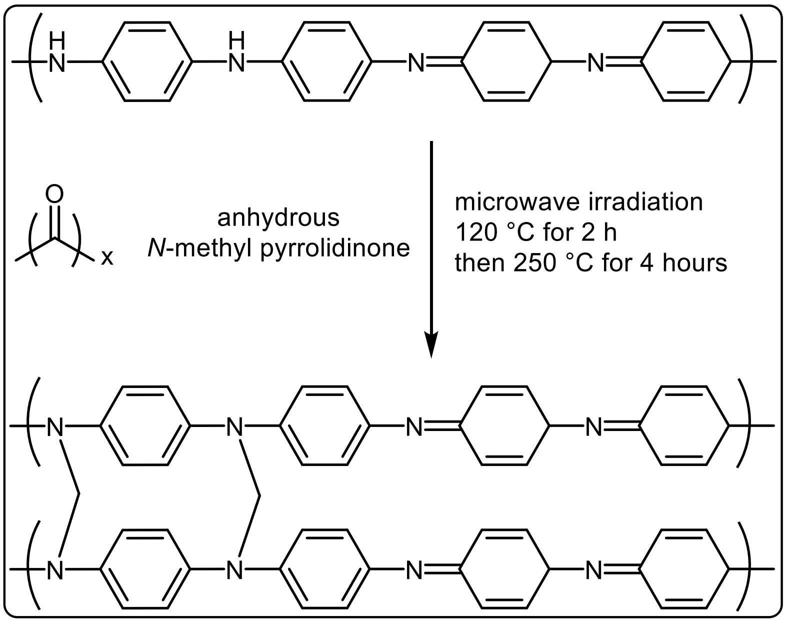
Two examples of hypercrosslinked polymers that have been reviewed by Tsyurupa and Davankov include: rigid polymers with specific surface area > 1,100 m^2/g prepared by reacting 4,4’-dilithio-biphenyl and dimethylcarbonate in tetrahydrofuran (THF) at -80 °C; and highly porous arylene-bridged polysilsesquioxane xerogel and aerogels.[31] These reactions do not follow Friedel-Crafts chemistry, but the properties of the products obtained have been deemed to belong to the hypercrosslinked class of materials.

Phosphoryl trichloride and hexane-1,6-diamine have been found to react together in the presence of triethylamine and anhydrous dichloromethane to form a novel hypercrosslinked polyphosphamide resin, as shown in Scheme 1.10.[48] This reaction was performed with stirring for 8 hours but in the absence of any external heating. The resulting pore structure was dominated by micropores, with a small level of mesopores, and a modest value of 15 m^2/g obtained for the specific surface area. The hypercrosslinked polyphosphamide material was evaluated for the removal of polar organic compounds in wastewater, and showed a good capacity for the adsorption of phenol.



Scheme 1.10: Schematic representation of the hypercrosslinking reaction that produces hypercrosslinked polyphosphonamide resin.[48]

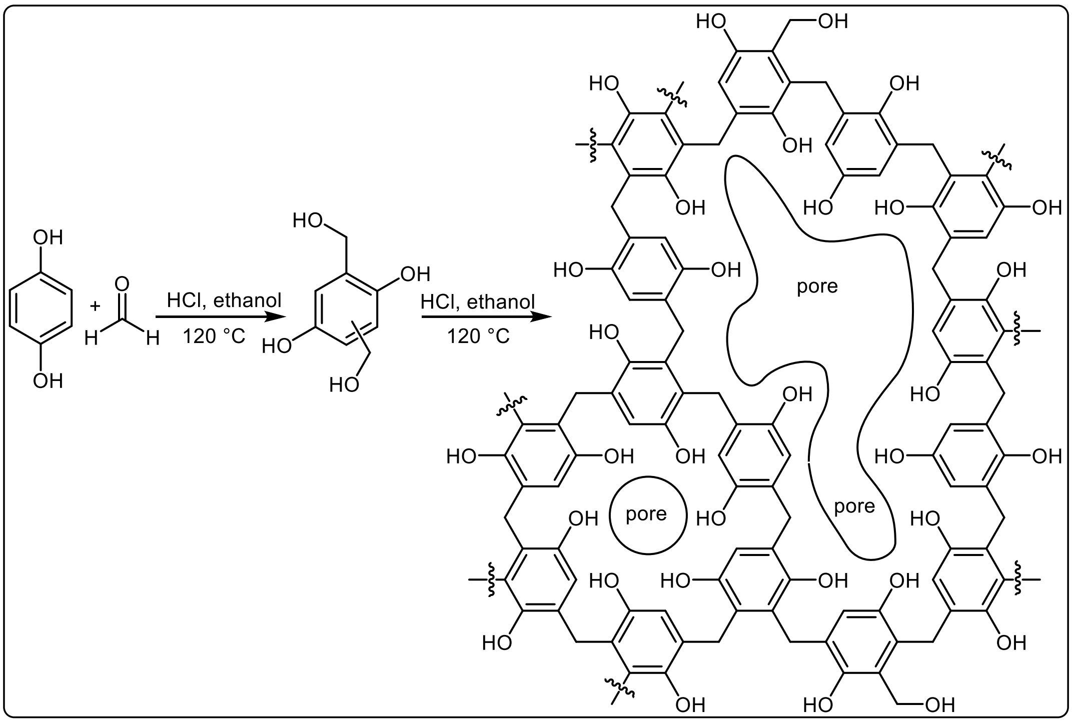
Germain *et al.*[49] have prepared hypercrosslinked polyanilines by the post-polymerisation crosslinking of a polyaniline base (emeraldine or leucoemeraldine) in the presence of diiodoalkanes or paraformaldehyde. In the paraformaldehyde reactions, shown in Scheme 1.11, they found that microwave-assisted synthesis led to a substantial improvement over the conventional thermal approach in terms of the specific surface areas that could be achieved (480 m^2/g compared to 0 m^2/g). It was proposed that the sealed microwave vials helped to retain the volatile formaldehyde gas and solvent, and the kinetics were improved by utilising higher reaction temperatures. The hydrogen storage capacities have been tested, and hypercrosslinked polyanilines gave rise to higher enthalpies of adsorption for hydrogen compared to hypercrosslinked polystyrenes and metal organic frameworks.



Scheme 1.11: The reaction between polyaniline and paraformaldehyde to form hypercrosslinked polyaniline[49]

Germain *et al*. have also prepared hypercrosslinked polypyrroles by hypercrosslinking with diiodomethane, iodoform or boron triiodide, in the presence of an inorganic base and solvent.[50] It was observed that the different sizes of these crosslinking units gives rise to different sized pores. The diiodomethane hypercrosslinked polypyrrole with methylene crosslinks provided the highest specific surface area and had the highest capacity for adsorbing hydrogen.

A novel hypercrosslinking methodology has been proposed that involves the one-pot condensation of hydroxybenzenes (hydroquinone, catechol or bisphenol A) and formaldehyde to form a porous resin, as shown in Scheme 1.12.[51, 52] The reaction was performed in a mixture of hydrochloric acid and ethanol at 120 °C for 24 hours in an autoclave, which is classified as solvothermal conditions. Specific surface areas up to 1,112 m^2/g have been reported for the hydroquinone formaldehyde resins prepared by this procedure. The solvent uptake capacity was measured and the hypercrosslinked polymers were shown to swell in ethanol, water, hexane and toluene. The motivation for developing this methodology was to introduce functional groups within the polymer, in this case hydroxyl moieties. These resins have been applied as oxidation-reduction interaction adsorbents for the recovery of precious metal ions, and have shown efficient adsorption for silver and gold ions, and excellent selectivity for gold ions.



Scheme 1.12: The condensation reaction between hydroquinone and formaldehyde under solvothermal conditions to form hypercrosslinked hydroquinone formaldehyde[51, 52]

1.6. Applications of crosslinked polymer particles

The particles resulting from heterogeneous polymerisation have found application in many fields. The applications that are suited to the polymer particles will depend on the particle size, size distribution, chemical functionality and porosity. Different combinations of these features and properties lead to a long list of potential uses and applications.

Spherical polymeric particles were used in early applications as ion-exchangers for water purification and treatment. For example, a polystyrene particle modified with sulfonic acid groups can exchange Na^+ ions with Ca^{2+} or Mg^{2+} to soften hard water, and because ion-exchange resins are able to form complexes with metals, they can also be utilised for removal of toxic heavy metals from water samples.

One of the largest applications of polymer beads is in chromatographic separation media. Polymer sorbents packed into chromatographic columns will preferably be monodisperse spherical particles that have a low coefficient of variation for uniform flow.[14, 53] The particle size will often be in the low micron size range and smaller particles (< 3 µm) will require higher pressures in the column. The ability to tune the particle pore size and porosity is a useful property for application in size-exclusion chromatography.[14]

Davankov *et al.* have nicely summarised that liquid chromatography is a collection of largely independent areas that includes: water treatment, hydrometallurgy, ion-exchange, ion-chromatography, sample preparation, and high-performance liquid chromatography (HPLC).[54] Davankov performed the first systematic HPLC studies using hypercrosslinked polymers as the stationary phase, and a recent publication from 2015 investigated hypercrosslinked polystyrene microspheres of 3.2 µm for reversed-phase HPLC under isocratic and gradient modes, and supercritical fluid chromatography.[55] The sorbents could be promising sorbents for the chromatography of small polar molecules and the analysis of mixtures of small molecule analytes alongside higher molecular weight species.

Polymer resins synthesised by heterogeneous polymerisation methods have been used extensively as solid supports in solid-phase synthesis, where organic molecules are attached to insoluble polymer supports. Advantages of using solid polymer supports in reactions include an easier reaction work-up and recovery because the species that are attached to the insoluble support are easily filtered and separated from anything that is non-supported or soluble. The 1980 book “Polymer-Supported Reactions in Organic Synthesis” has chapters specialising in polymer-supported reagents, polymer-supported catalysts, and polymer-supported synthesis.[56] Hodge has also published a review article to present what takes place in the bead during polymer supported organic reactions.[57] The polymer support may often be treated as the structural analogue of a low molecular weight species, by utilising identical chemistry in order to introduce functionality to the support.[4]

In 1963 Merrifield published a new method for peptide synthesis, called solid phase peptide synthesis. An amino acid was attached to a solid polymer by a covalent bond, and then further amino acids were sequentially attached to the growing chain in a stepwise manner.[58] The polymer that was found to work best at this time was a 200-400 mesh resin of chloromethylated poly(styrene-*co*-divinylbenzene) with a porous gel-type structure.

Phenolic polymeric resins have been synthesised for solid-phase peptide synthesis by suspension polymerisation[59] and precipitation polymerisation[60]. Styrene, DVB and *p*-

acetoxystyrene (AS) were polymerised and the acetoxy groups cleaved to generate the phenolic moieties. An acrylonitrile, DVB, and AS polymer was also synthesised and compared to the styrenic resin.[61] Hudson and Kenner have published the peptide synthesis on phenolic polymeric supports.[62]

Solid polymer supports have been utilised for combinatorial and parallel synthesis, with several reviews published.[63-65]

Polymer-supported catalysts are organic catalysts supported on insoluble polymer supports.[66] Recently, 4-(*N*-methylamino)pyridine was immobilised on hypercrosslinked polyHIPE materials to generate a polymer-supported version of the nucleophilic organocatalyst 4-dimethylaminopyridine.[67] Non-hypercrosslinked polyHIPEs and hypercrosslinked beads were also compared. The incorporation of chiral quaternary ammonium salts onto crosslinked polystyrenes has been published for the preparation of polymeric chiral organocatalysts with application in asymmetric reactions.[68]

Polymer microspheres of poly(DVB) prepared by precipitation polymerisation were modified and functionalised by the “grafting from” and “grafting to” approaches and reviewed by Barner.[69] Techniques such as atom transfer radical polymerisation, reversible-addition fragmentation chain-transfer polymerisation and anionic polymerisation were utilised in the “grafting-from” modification, and the modification of polymer microspheres by epoxide chemistry and click-chemistry were utilised in the “grafting-to” approach.

Chiral microspheres of optically active helical polyacetylenes were prepared by precipitation polymerisation. In this study the microspheres were applied to induce enantioselective crystallisation of D- and L-alanine racemates.[70]

Crosslinked polymer microspheres bearing magnetic properties have been synthesised by precipitating iron oxides into polymer sorbents, including gel-type, macroporous and hypercrosslinked polymers.[71] Superparamagnetic iron oxide nanoparticles were precipitated into the pores of sulfonated hypercrosslinked polymer microspheres to generate strongly magnetic polymer supports that can function as an immunomagnetic electrochemical biosensors for protein detection.[72]

The swelling properties of hypercrosslinked polymer microspheres lend them to the incorporation of metal complexes within the porous network of the polymer. A solution of

H_2PtCl_6 in methanol or THF was impregnated into the pores of hypercrosslinked polystyrene, and Pt(IV) was reduced to a Pt(II) complex that filled the cavities and formed Pt nanoparticles by H_2 reduction. The catalytic properties of hypercrosslinked polystyrene containing platinum nanoparticles were utilised to oxidise L-sorbose to 2-keto-L-gulonic acid, and it was found that the catalytic activity and selectivity did not deteriorate over 15 catalytic cycles.[45]

Hypercrosslinked polymers have utilised their high capacity for the adsorption of species including organic pollutants and gases. Hydrogen storage is an important area that is being studied and developed in order to harness hydrogen as a potential energy carrier and an alternative fuel source. McKeown has studied the use of hypercrosslinked polystyrene beads in a review on microporous organic polymers as potential hydrogen storage materials,[73] and Germain *et al.* have published an extensive review of porous polymers for hydrogen storage which includes background of the area and comparison to other materials.[74] Lee *et al.*, from the Cooper research group, have reported the adsorption of 3.04 wt. % hydrogen at 77 K and 15 bar H_2 pressure by a microporous hypercrosslinked polymer, with bead diameter of 50-200 µm and Brunauer-Emmett-Teller (BET) specific surface area of 1,470 m^2/g.[75] Gravimetric analysis was the method used to investigate the hydrogen sorption properties of this polymer, and the results showed 1.28 wt. % adsorption of H_2 at 1 bar, 2.75 wt. % adsorption at 10 bar, and 3.04 wt. % at 15 bar. The group also investigated an alternative route to synthesising hypercrosslinked polymer beads for hydrogen storage, based upon the step-growth polycondensation of dichloroxylene and other bischloromethyl monomers, and these polymers adsorbed up to 3.7 wt. % H_2 at 77 K and 15 bar H_2 pressure.[76] In the same year, Germain *et al.* synthesised hypercrosslinked polymers with a BET specific surface area of 1930 m^2/g that were able to adsorb 1.5 wt. % H_2 at 77 K and 1.2 bar pressure.[74, 77]

Monodisperse hypercrosslinked polystyrene particles of 500-600 nm average diameter have been synthesised with 2.13 wt. % hydrogen adsorption capacity at 163 K and 15 bar pressure, utilising a higher temperature than the 77 K utilised by other research groups.[78]

1.7. Solid-phase extraction

Solid-phase extraction (SPE) is an important analytical technique used to extract organic compounds or contaminants from a sample matrix prior to chromatographic analysis.[79]

Some common objectives of SPE include: the concentration and enrichment of samples for improved sensitivity and detection in subsequent analysis; the clean-up of samples by the removal of interfering compounds; the separation or fractionation of a sample into groups of compounds; exchange of the sample matrix for a more suitable matrix; storage of compounds within the cartridges.[80, 81] The compounds of interest in a sample are often referred to as analytes.

The principle of SPE is to isolate analytes from a sample matrix, by reversibly binding the analytes on a solid sorbent. The analytes are partitioned between the solid sorbent and the liquid sample matrix. The retention (adsorption) of analytes on the sorbent occurs when the analyte has a greater affinity for the sorbent than the sample matrix. Elution is therefore possible when the chosen elution solvent has a great affinity towards the analyte to break the analyte's interaction with the sorbent, and desorption from the sorbent occurs. The retention mechanisms, or the possible interactions between the sorbent and analyte, are shown in Table 1.1.

Table 1.1: Comparison of reversed phase, normal phase and ion-exchange interactions [82]

Reversed Phase	**Normal Phase**	**Ion-exchange**
Non-polar or hydrophobic interactions • van der Waals forces	*Polar or hydrophilic interactions* • hydrogen bonding • π-π interactions • dipole-dipole interactions • dipole-induced dipole interactions	*Electrostatic/ionic interactions* • Attraction of oppositely charged functional groups
Polar or aqueous sample matrix	Mid- to non-polar organic sample matrix	Aqueous or organic samples at appropriate pH
Mid- to non-polar analytes	Mid- to polar analytes displaying functional groups	Acidic and basic analytes with charged functionalities
Non-polar sorbent	Polar sorbent	Sorbent with ionisable functional groups

The SPE protocol

There are four key steps in the SPE protocol, as shown in Figure 1.6.

1. The first step is the conditioning of the extraction sorbent. The conditioning of the sorbent activates and solvates the material, by a wash with an organic solvent to increase the hydrophilicity, and then a subsequent equilibration wash with a pH modified aqueous solution to displace the organic solvent and prepare the sorbent to interact with the sample matrix.
2. The second step of the process is the loading of the analytes on to the solid sorbent. A slow flow of the sample matrix is passed through the sorbent to enable the analytes to be loaded and retained on the sorbent.
3. The third step is the washing of the sorbent with a solvent that aims to remove interferents from the SPE sorbent, but does not disturb the retained analytes of interest.
4. The fourth step of the SPE process is the elution of the analytes from the sorbent. An appropriate solvent is chosen that disrupts the interactions that bind the analytes to the sorbent, and allows the analytes to be eluted with the solvent. If the elution solvent is not suitable for injection into the HPLC for subsequent analysis, then an additional step of evaporation and reconstitution in an alternative solvent may be required.

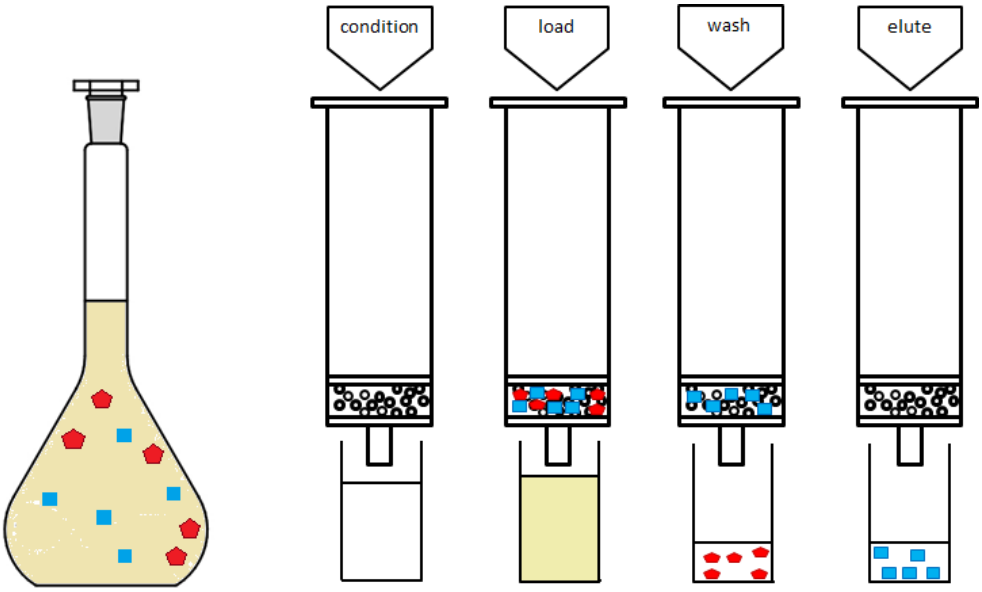


Figure 1.6: Illustration depicting the SPE protocol. On the left, a volumetric flask containing a dilute sample matrix of mixed analytes and interfering compounds. On the right, four SPE cartridges that show the conditioning, loading, washing and elution steps of the protocol.

1.7.1. Solid-phase extraction sorbents

The solid sorbents utilised in SPE are extraction materials that exist in either cartridge or disk form for off-line SPE, or are packed into a pre-column for on-line SPE systems. An SPE cartridge consists of a polypropylene or glass cartridge with a luer tip that connects to an SPE vacuum manifold. A loose, solid-phase sorbent is packed between porous metal or plastic frits in the cartridge. The SPE disk technology consists of particle-loaded membranes or particle embedded glass fibre disks.

The breakthrough volume for any particular analyte is the volume of sample at the point where the analyte begins to be eluted from the exit of the cartridge. This value is dependent on the sorbent utilised in the SPE cartridge, and is representative of the retention ability of the sorbent for each analyte.[80]

There is not a universal type of sorbent that is optimised for every application and set of compounds. Instead, there are many different SPE sorbents that have been developed and optimised for a particular range of conditions and compounds. The demand for new and improved sorbents is ongoing, as more and more problems and applications are being investigated. It is not feasible to discuss and review the full range of SPE materials available, so the intent of this section is only to introduce and provide an overview of some SPE sorbents that seem appropriate and relevant to the present study.

Silica sorbents

Chemically bonded silica is a very popular packing material for HPLC. Its commercial availability led to the extensive use and development of various bonded silica materials as SPE sorbents. Non-polar bonded silica sorbents are reversed phase sorbents that possess reversed phase interactions, or non-polar-non-polar attractive forces, between the bonded functional groups on the silica surface and the carbon chains of the analyte.[83] Some of the most commonly used silica sorbents have been modified with octyl (C_8) or octadecyl (C_{18}) chains, but other groups such as ethyl, butyl, phenyl, and cyclohexyl have been reported.[83, 84] Silica is a hydrophilic material; however the bonded hydrocarbon chains on the surface are hydrophobic.[85] Silica sorbents that have been modified with alkyl groups containing polar functionalities such as cyano, amine and hydroxyl moieties can also be used under normal phase conditions. These sorbents bonded with polar groups are more hydrophilic relative to the hydrocarbon bonded silica sorbents. The residual silanol groups on the surface of the silica sorbent may provide additional sites for interaction, and extract more polar analytes, however they also lead to limitations. Silica sorbents have only a narrow range of pH stability.[83]

Analytes may also bind to modified silica sorbents through the ion-exchange retention mechanism. Charged species bonded to silica sorbents are able to interact with oppositely charged functional groups on analytes by ionic interactions. The first commercially available mixed-mode sorbents were silica based materials with ion-exchange groups.[86]

Carbonaceous sorbents

Activated carbons were important in the development of solid-phase materials for the extraction of medium- to low-polarity organic compounds from aqueous matrices. However, in the 1960s the heterogeneous nature of the activated carbons did not satisfy the requirements for extraction sorbents, because the activated carbons suffered from irreversible adsorption and low recoveries for some analytes.[84, 87]

Graphitised carbon blacks (GCBs) were introduced later, obtained by the heating of carbon blacks.[83, 87] The surface of GCBs contains functional groups as a result of the oxygen chemisorption.[87] These functionalities are able to interact using an anion-exchange mechanism.

Porous immobilized graphitic carbons (PGCs) are sorbents with two-dimensional graphite layers immobilized on a silica surface.[83] The homogeneous and ordered structure can retain analytes by reversed-phase interactions and electronic interactions, and it has been shown to extract and retain non-polar, polar and water-soluble analytes from aqueous matrices.[84]

1.7.2. Polymeric sorbents

The development of polymeric sorbents has overcome some of the limitations of the silica sorbents, due to their greater stability at extreme pH and their compatibility with organic solvents.

Many SPE sorbents have a highly crosslinked, macroreticular structure based upon a hydrophobic poly(styrene-*co*-divinylbenzene) (PS-*co*-DVB) skeleton. The porosity of the polymer gives rise to an open structure that possesses the capacity to retain analytes. The active aromatic rings of PS-*co*-DVB can form π-π interactions with unsaturated moieties on analytes.

The first commercialised crosslinked polymeric sorbent based upon PS-*co*-DVB was Amberlite XAD-1, developed by Rohm and Haas. From this, the Amberlite series expanded to include other PS-*co*-DVB sorbents (XAD-2, XAD-4) and also poly(ethylene-*co*-dimethacrylate) sorbents (XAD-7, XAD-8).[84] The acrylate-containing polymers have a higher polarity, and were therefore suited for extraction of more polar compounds. A drawback of the early XAD sorbents was their requirement to be ground, sized and purified.[88]

Hydrophilic polymeric sorbents

The hydrophobic PS-*co*-DVB sorbents are limited in their ability to extract a wide range of organic compounds from a matrix, therefore there has been an extensive amount of research carried out to develop hydrophilic polymeric sorbents. The two main approaches taken involve the copolymerisation of a hydrophilic monomer possessing the desired functionality alongside a monomer such as DVB, or the post-polymerisation chemical modification of existing polymers with polar functional groups.

In the 1990s, there was a demand for new polymeric sorbents to extract polar compounds. It was found that polar analytes are retained more strongly by polymeric sorbents that possess polar functionalities.[89] The chemical modification of polymeric sorbents is a way of increasing the polarity of the sorbent surface and improving the contact between aqueous samples and the sorbent, resulting in improved recoveries of analytes compared to unmodified sorbents.[83]

In 1992, Sun *et al*. published the chemical modification of the PS-*co*-DVB sorbent Amberchrom GC-161 with hydroxymethyl and acetyl functional groups to increase the hydrophilicity of the sorbent.[90] It was found that the modified sorbent was superior to the unmodified polymeric sorbent and to the silica sorbents when studied comparatively for the recovery of polar organic compounds.

PS-*co*-DVB sorbents have also undergone modification with sulfuric acid to yield sulfonated sorbents that possess increased hydrophilicity, and have the ability to form ionic interactions with charged analytes. Later, Dumont investigated the effect of sulfonation on PS-*co*-DVB and found that the sulfonation occurred from the outside inwards, meaning that the surface became hydrophilic but the inside of the particles remained hydrophobic. It was reported that a capacity of 0.6 mequiv/g was the minimum level of sulfonation that was required to produce a hydrophilic surface.[89] The lightly sulfonated sorbents extract polar organic compounds more efficiently than the unmodified sorbents.[88]

Masqué *et al*. have also investigated the chemical modification of PS-*co*-DVB polymeric sorbents in order to increase the retention capacity for polar compounds such as phenols and pesticides. The commercially available Amberchrom GC-161 sorbent has been reacted with benzoyl chloride,[91] acetyl chloride,[5] and phthalic acid anhydride,[85] in a series of Friedel-Crafts reactions using aluminium (III) chloride and nitrobenzene. The modified sorbent was compared to several commercial sorbents (PLRP-S, Envi-Chrom P, LiChrolut EN and the unmodified Amberchrom GC-161) for the extraction of pesticides and phenolic compounds.[91] The resulting sorbents from these post-polymerisation modification reactions have polar benzoyl moietites,[91] acetyl moieties,[5] and *o*-carboxybenzoyl moietites[85] in place.[83]

Instead of undergoing post-polymerisation chemical modification, the popular and commercially available Oasis HLB sorbent (from Waters Corporation) has a poly(*N*-vinylpyrrolidinone-*co*-divinylbenzene) structure that is hydrophilic due to the polar lactam moieties in the vinylpyrrolidinone monomer. Two other widely used and commercially available hydrophilic polymeric sorbents with polar functionalities include Bond Elut Plexa (Agilent Technologies) and Strata-X (Phenomenex). Oasis HLB is a popular polymeric sorbent that has capacity and manages to retain a broad type of compounds, however, it lacks selectivity because it extracts groups of interferences from the matrix together.[92]

Hypercrosslinked polymeric sorbents

Hypercrosslinked polymers are neutral hydrophobic polymers. As SPE sorbents, their unusual sorption properties allow for the adsorption and desorption of analytes.[80] Although hydrophobic, the polymer swells in water in order to relieve the strained and rigid, porous network of the dry sorbent. The high content of micropores facilitates the incorporation of organic compounds from the aqueous sample matrix.[93] The high specific surface area (>1,000 m^2/g) of these sorbents results in a high capacity.

Tsyurupa *et al*. have reported that the retention ability of hypercrosslinked polymeric sorbents for phenols was greater than that of the other sorbents studied.[94] Hypercrosslinked sorbents achieved higher breakthrough volumes for phenolic analytes. Puig *et al*. also reported that the sorbents with high specific surface areas greater than 1,000 m^2/g have improved breakthrough volumes compared to the sorbents with less porosity and less active surface area available.[95]

The hypercrosslinked structures enhance reversed phase interactions and promotes analyte retention on the sorbent.[96] Aromatic sites on the hypercrosslinked sorbents allow π-π interactions with aromatic analytes *via* π-stacking, and a greater level of π-π interactions results in a higher adsorption capacity.[83, 85]

Mixed-mode polymeric sorbents

In 1993 Schmidt *et al*. published the simultaneous extraction of basic and neutral analytes in a two-step elution process using a sulfonated PS-*co*-DVB sorbent as a cation-exchange resin. An anion-exchange sorbent was also prepared by modifying the Amberchrom GC-161 sorbent with trimethylamine to separate neutral and acidic analytes.[88, 97] These are early examples of mixed-mode polymeric sorbents.

Mixed-mode polymeric sorbents are categorised according to whether they are strong or weak, and cationic or anionic. A strong anion-exchange (SAX) sorbent will have a high pKa (> 14), therefore the functional group on the sorbent will be charged at all pH values in aqueous solution. Weak anion-exchange (WAX) sorbents may have pKa of around 8-10, so the sample matrix must be applied at 2 pH units below this. Anion-exchange sorbents have an affinity for anionic, acidic analytes of interest. Examples of SAX and WAX sorbents are polymers that have been modified with quaternary amines or primary/secondary/tertiary amine moieties, respectively. A strong cation-exchange (SCX) sorbent will have a low pKa (<1), therefore the functional group on the SCX sorbent is charged over the whole pH range in aqueous solution. Weak cation-exchange (WCX) sorbents may have pKa of about 4.8, and the sample matrix must be applied 2 pH units above the pKa. Cation-exchange sorbents have an affinity for cationic, basic analytes of interest. Examples of SCX and WCX sorbents are polymers that have been modified with sulfonic acid or carboxylic acid moieties, respectively.

The research collaboration between the Cormack Polymer Group at the University of Strathclyde and the Group of Chromatography, Environmental Applications at Universitat Rovira i Virgili led to the first family of hypercrosslinked mixed-mode polymeric sorbents to be prepared "in-house", shown in Figure 1.7.

Several routes have been published for the synthesis of hypercrosslinked polymer particles that were prepared from precipitation polymerisation precursors and exhibit strong cationic-exchange character, HXLPP-SCX sorbents. Particles of hypercrosslinked poly(DVB-*co*-VBC) have been modified chemically with acetyl sulphate or lauroyl sulphate.[98] Alternatively, non hypercrosslinked polymers with SCX properties have been prepared too. The copolymerisation of monomers 2-acrylamido-2-methylpropane sulfonic acid (AMPSA), 2-hydroxyethyl methacrylate (HEMA) and pentaerythritol triacrylate (PETRA) have produced the SCX functionalised polymer poly(AMPSA-*co*-HEMA-*co*-PETRA), or post-polymerisation chemical modification of particles of poly(HEMA-*co*-DVB) with sulphuric acid generates a SCX polymeric sorbent.[92] Sorbent HXLPP-WCX was prepared by the copolymerisation of monomers DVB, VBC and methacrylic acid (MAA) to form particles of poly(MAA-*co*-VBC-*co*-DVB), followed by hypercrosslinking. The carboxylic acid moieties in the polymer give rise to the WCX character.[99]

Sorbent HXLPP-SAX is a SAX sorbent that was prepared by chemically modifying hypercrosslinked particles of poly(DVB-*co*-VBC) with dimethylbutylamine.[100] The WAX sorbents labelled as HXLPP-WAX have been prepared by the chemical modification of hypercrosslinked poly(DVB-*co*-VBC) particles with piperazine and ethylenediamine.[101]

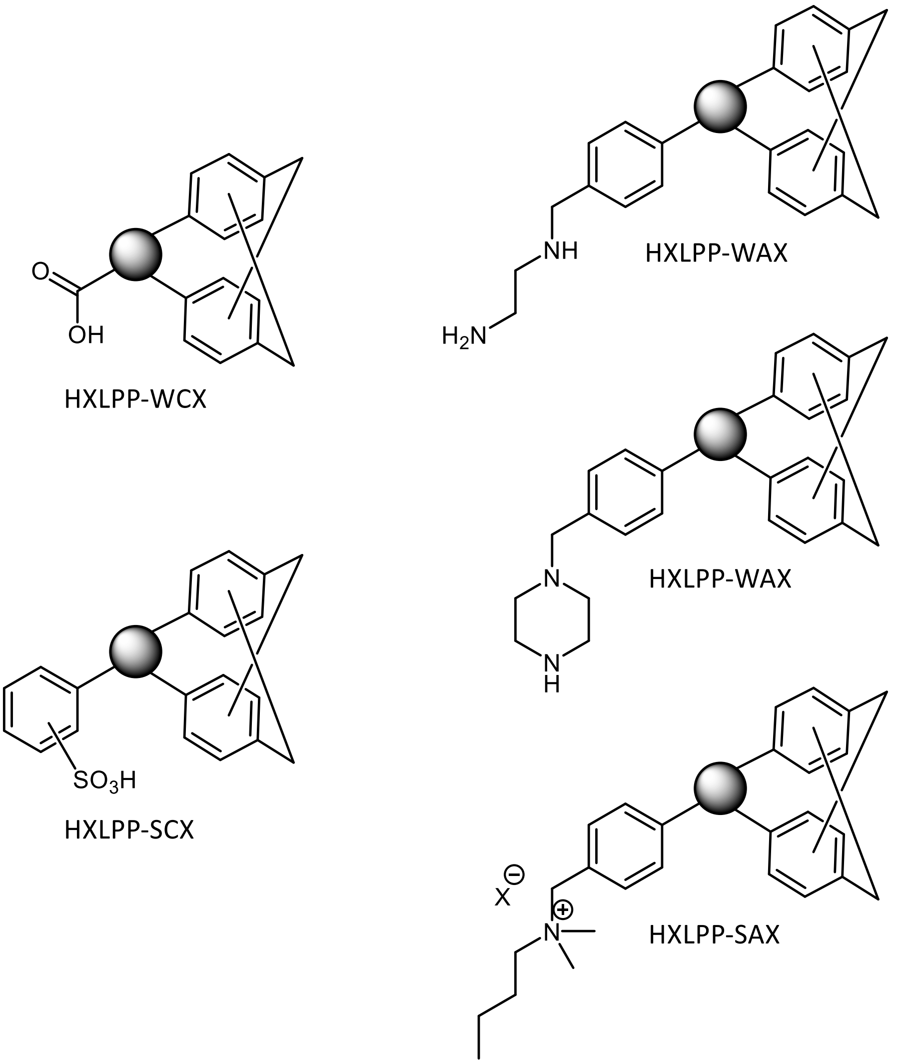


Figure 1.7: The family of hypercrosslinked polymer microspheres that have been modified for different ion-exchange interactions [98-101]

The washing step in the SPE protocol for mixed-mode sorbents removes the interfering compounds that are adsorbed to the sorbent by reversed-phase interactions, and as a result the analytes eluted in the elution step may be better quantified due to cleaner chromatograms.[93]

Strong ion-exchange sorbents are permanently charged; therefore, it is necessary to switch the chargeability of the analytes in order to elute the targeted analytes. In contrast, it is often the aim of the elution protocol to switch the chargeability of the weak ion-exchange sorbent instead of the analytes.[96] This ability to alter the charged state of both sorbent and analytes makes weak ion-exchange sorbents more pH tuneable.

Physico-chemical characteristics of sorbents

The physico-chemical parameters influence the efficiency of the polymeric sorbents. These parameters include the particle size, particle size distribution, specific surface area, degree of crosslinking, pore diameter and pore volume.[95]

Small particle diameters of 5-10 µm have been reported as the optimum particle size for efficient SPE.[80, 88, 93] Small particles promote better contact with the analytes during sample loading. An SPE column packed with larger particles may result in poorer retention of the analytes as the sample has longer pathways to flow between the particles. However, higher pressures are required to pass samples through a column packed with small particles; therefore particle sizes much below 5 µm are often undesirable.[88] The pressure increases exponentially with decreasing particle size.

In the van Deemter equation, shown in Equation 2, the A term describes Eddy diffusion. This relates to the flow and paths taken by the mobile phase through the sorbent and poorly packed SPE columns may result in band broadening. The effect of Eddy diffusion may be minimised by using particles with a low diameter, and a narrow particle size distribution. A small particle diameter decreases the distance that is required for an analyte to access and diffuse through its pores, therefore decreasing the diffusion time. Sorbents with a narrow distribution of particle size achieve a more homogeneous elution of analytes from the sorbent, resulting in an improved and narrower profile to minimise band broadening.[95] A short column of a few millimetres of sorbent is optimum; smaller columns may result in channelling and incomplete retention of analytes.[88]

$$HETP = A + \left(\frac{B}{u}\right) + (C \times u)$$

HETP = Height Equivalent to a Theoretical Plate (or H)

A = Eddy Diffusion

B/u = Longitudinal Diffusion

Cu = Resistance to Mass transfer

B = Random Molecular Diffusion

C = Mass transfer within particles caused by mobile phase

Equation 2

Many commercially available sorbents have large particle diameters (> 40 µm) but the HySphere range from Spark Holland have been designed with particle diameters ≤ 10 µm, especially for use in on-line SPE systems.[102]

1.8. Thesis objectives and content

A key objective in this work was to design and develop a novel and facile hypercrosslinking methodology that would result in crosslinked materials that are distinct from those prepared using the established Friedel-Crafts methodology, originally described by Davankov. The goal was to move away from the use of metal-based Lewis acids such as $FeCl_3$, and to develop materials containing a functional handle such as hydroxy moieties. Chapter 2 discusses the methods employed for the crosslinking of phenolic polymers using formaldehyde, and the subsequent characterisation of the materials.

The Cormack Group at the University of Strathclyde have prior experience in the synthesis of hypercrosslinked polymeric sorbents for use in SPE. It was a key objective of this thesis to expand the family of mixed-mode, spherical, polymeric SPE sorbents by synthesising materials with novel functionality and the capability to extract pollutants selectively from aqueous samples.

The post-polymerisation chemical modification of polymer microspheres using amino acid derived esters has been investigated and is described in Chapters 3 and 4. Chapter 3 is focused on macroreticular precursor polymer particles, whereas Chapter 4 is focused on the hypercrosslinked variants. Their application as mixed-mode polymeric SPE sorbents has been evaluated in Chapter 5, through an analytical study performed on a selection of these synthesised sorbents. The objective of this study was to assess the capability of the polymers to selectively extract acidic or basic compounds from aqueous mixtures of compounds in a novel manner, to further enhance the scope of mixed-mode SPE sorbents.

Chapter 2 - Synthesis of hypercrosslinked polymers *via* phenol-formaldehyde chemistry

2.1. Introduction

2.1.1. Hypercrosslinked polymers

Hypercrosslinked polymers, introduced in Chapter 1, are commonly (although not exclusively) synthesised using Friedel-Crafts chemistry to form new crosslinking C-C bonds. A recent review of Friedel-Crafts alkylation has been published by Rueping *et al*. to document its wide use in organic synthesis and describe some of the latest developments.[103] Hypercrosslinking by Friedel-Crafts chemistry is a tried-and-tested methodology pioneered by Davankov's research group, utilising transition metal catalysts such as the Lewis acid compounds $SnCl_4$, $AlCl_3$ and $FeCl_3$. Within the Cormack research group at the University of Strathclyde, $FeCl_3$ has been used in stoichiometric ratios with respect to the reactive benzyl chloride moieties present in the precursor polymers. This Friedel-Crafts reaction generates iron salts that colour the polymer orange if not removed fully, and hydrogen chloride gas.[37] Tsyurupa *et al*. have explained that this gas prevents air and moisture from coming into contact with the reactants.[104] These reactions are often performed under an inert atmosphere and the most commonly used solvent for hypercrosslinking is 1,2-dichloroethane (DCE), which unfortunately is halogenated, toxic and harmful.

There is ample scope for the evolution of a new, facile hypercrosslinking methodology that utilises more environmentally friendly and less hazardous reagents. The process would ideally involve an aqueous solvent, avoid the need for an inert atmosphere, produce products that are metal-free and be cost-effective. The established Friedel-Crafts hypercrosslinking of benzyl chloride containing polymers does not result in 100% conversion of the chloride moieties and they are therefore susceptible to subsequent chemical modification. The presence of functional species in the hypercrosslinked product is a desirable feature that should be incorporated into any new methodology.

2.1.2. Phenol-formaldehyde resins/phenolic resins

Crosslinked phenol-formaldehyde resins and phenolic resins have been in use for over 100 years, and the materials and topic have been the subject of continuous research and development. An introduction to phenol-formaldehyde chemistry has been discussed in Chapter 1.

Phenol-formaldehyde derived polymers were often transferred into moulds before curing to take on the shape of the mould, depending on the intended application. There is an interest in our research group at the University of Strathclyde in the synthesis of spherical, beaded polymer particles produced *via* heterogeneous polymerisation. Therefore, it is worth reporting the development of phenol-formaldehyde resins produced in spherical particulate form.

Crosslinked phenolic resins of phenol-formaldehyde have been prepared in spherical particulate form by suspension polymerisation.[105, 106] In these reactions, poly(vinyl alcohol) was added as stabiliser, hexamethylenetetramine as crosslinking agent and triethylamine as the basic catalyst.

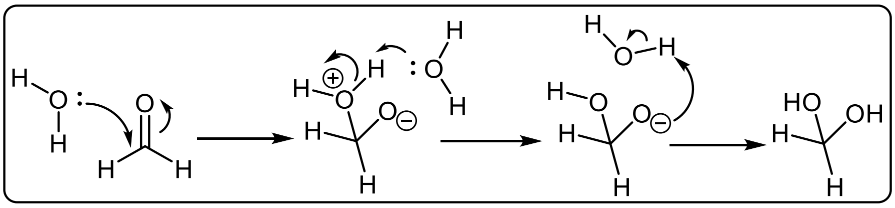
Spherical polymer particles of resorcinol-formaldehyde resin have been synthesised in an extended Stöber method by Liu *et al*.[107] Colloidal spheres with a narrow particle size distribution were prepared by the polymerisation of phenol and resorcinol in an alcohol and water mixture with aqueous ammonia as catalyst. The spherical particles were formed as emulsion droplets and polymerised to produce colloidal spheres with diameters ranging from 200 – 1,000 nm. Carbonisation of the resorcinol-formaldehyde resins resulted in spherical carbon spheres with a particle diameter slightly smaller than the original resorcinol-formaldehyde resin due to shrinkage. The carbon materials were reported as being microporous, with a BET specific surface area of 504 m^2/g. A similar, accelerated procedure was performed by Pol *et al*. under ultrasonic irradiation.[108]

Organic gels are a class of materials derived from phenol-formaldehyde chemistry, through the sol-gel polycondensation of phenol (or its substituted derivatives) and formaldehyde. Soft templating methods have been employed to impart a porous structure within the phenolic resin gels. The porosity is preserved by solvent-exchange or by complex drying procedures, and terminology has been assigned to describe the different dried gels.[109] The porous structure of the gels collapses when they are dried in air, and the low specific surface area product formed is called a xerogel. Using supercritical fluids such as supercritical CO_2 does not collapse the pores because of the small surface tension forces, and these products are called aerogels. Similarily, ambigels are formed by solvent-exchange using low surface tension solvents. Lyophilisation, or freeze-drying, can be used to produce porous cryogels by sublimation of the water in the pores. Organic gels are often carbonised to produce porous, carbonaceous materials.

2.1.3. Development of new methodology

A new crosslinking methodology to prepare hypercrosslinked polymers has been investigated on crosslinked polymeric particles prepared by heterogeneous polymerisation, and also on commercially available linear polymeric precursors. The polymeric precursors all contained phenolic functionality to impart useful hydroxyl functional groups to the crosslinked products, and to activate the aromatic ring towards electrophilic aromatic substitution. Following on from the traditional phenolic resins, formaldehyde was studied as the crosslinking species for hypercrosslinking.

Commercially available formaldehyde can be purchased in solid form, or in concentrated aqueous or alcohol solutions. Paraformaldehyde is a solid form of 95 % formaldehyde, which is particularly desirable when aqueous conditions should be avoided. Paraformaldehyde is a polymer with low to medium molar mass, composed of polyoxymethylene glycols with a degree of polymerisation between 8 and 100. The polymer decomposes in solution to regenerate formaldehyde. Formalin is the name commonly used for the concentrated aqueous solution of 37 % formaldehyde gas in water, with a small percentage of methanol added as stabiliser to prevent polymerisation. The aqueous solution of formaldehyde is completely hydrated to methylene glycol, as shown in Scheme 2.1, and contains essentially no CH_2=O.



Scheme 2.1: In aqueous solution formaldehyde exists in the hydrated form as methylene glycol (methanediol)

2.1.4. Aims

The objective of this chapter was to investigate the reaction between phenolic polymers and formaldehyde under basic conditions, with the aim of developing a new synthetic methodology for crosslinking polymers and achieving hypercrosslinked products.

2.2. Experimental

2.2.1. Materials

Divinylbenzene (DVB) (80 % technical grade) and 4-acetoxystyrene (AS) (97 %) were supplied by Sigma-Aldrich (UK) and passed through a short column of alumina (activated, neutral, Brockmann I, supplied by Sigma-Aldrich) to remove inhibitors before use. 2,2'-Azo*bis*(isobutyronitrile) (AIBN) (97 %) was supplied by BDH Lab Supplies (UK) and recrystallized from cold acetone.

Poly(4-vinylphenol) (Poly(VP)) (M_w 20,000 and M_w 25,000), poly(4-vinylphenol-*co*-methyl methacrylate) poly(VP-*co*-MMA), formaldehyde solution (FS) (≥ 36.5 %) and paraformaldehyde (PF) (95 %, prilled) were all supplied by Sigma-Aldrich and used as received.

Potassium hydroxide (KOH) (GPR Rectapur pellets) and sodium hydroxide (NaOH) (GPR Rectapur pellets) were supplied by VWR International and used as received to prepare aqueous solutions. Potassium bromide (KBr) (FT-IR grade) was supplied by Sigma-Aldrich and used as received. Hydrochloric acid (HCl) (36.5-38 %) was supplied by Sigma-Aldrich.

The solvents acetonitrile (ACN) (HPLC grade), *N*,*N*-dimethylformamide (DMF) (Chromasolv® Plus grade), 1,2-dichloroethane (DCE) (99.8 % anhydrous), tetrahydrofuran (THF) (Chromasolv® HPLC grade), 2-ethyl-1-hexanol (2-EH) (99.6 %), *N*-methyl pyrrolidinone (NMP) (99+ % spectrophotometric grade), *n*-heptane (99 % spectrophotometric grade), propan-2-ol (> 99.5 %), toluene, ethanol (EtOH), methanol (MeOH), and acetone were all supplied by Sigma-Aldrich and used as received. Diethyl ether was supplied by Fisher Scientific. Methyl ethyl ketone (MEK) (GPR™) was supplied by BDH Lab Supplies and used as received. Butyl acetate (BuAc) (99+ % extra pure) was supplied by Acros Organics and used as received. Water was double distilled in-house prior to use.

2.2.2. Equipment and instrumentation

Precipitation polymerisation experiments were carried out using a Stuart Scientific S160 incubator (Surrey, UK) and a Stovall low-profile roller system (NC, USA). Nalgene® plastic bottles were used as the reaction vessels in all experiments.

When required, samples were dried in Townson & Mercer Limited (England) vacuum ovens set to either 40 °C or 70 °C, at ~ 60 mbar pressure.

An Edwards Modulyo freeze-dryer was used to freeze-dry the samples specified. The temperature of the condenser was set at - 40 °C and the Pirani vacuum gauge read ~ 2 mbar. Sample KA46 was freeze-dried on a Christ Alpha 1-2 LDplus freeze dryer, with the temperature of the condenser set at - 59 °C and the vacuum at 0.27 mbar. All of the samples were pre-frozen prior to drying by swirling the flasks in liquid nitrogen.

C, H and N elemental microanalyses were determined using a Perkin Elmer 2400 Series II CHNS Analyser. The results were obtained as a percentage by weight, and are measured as a function of thermal conductivity. The chlorine content was determined by the Schöniger oxygen flask combustion technique, followed by ion chromatography using a Dionex DX-120 instrument. All analyses were performed by Denise Gilmour of the University of Strathclyde Microanalysis Service.

Optical microscopy was performed using an Olypmus Vanox microscope (Japan). Scanning electron microscopy (SEM) was performed using a Cambridge Instruments Stereoscan 90. All samples were sputter-coated in gold using a Polaron SC500A sputter coater prior to imaging. The mean particle diameter was obtained by manually measuring the diameter of a selection of the particles using iScan2000 imaging acquisition software, and obtaining the mean value. The SEM analysis was performed by Jim Morrow, University of Strathclyde.

Fourier-Transform infrared spectroscopy (FT-IR) spectroscopic analysis of the products was performed using several spectrometers. A Perkin-Elmer Spectrum One FT-IR spectrometer was used for analysing KBr pellets. Spectroscopic grade KBr was used to prepare the sample as a disc in a RIIC press at 8 tons, and the scanning range was 4,000-450 cm^{-1} in transmission mode. An Agilent 5500a FT-IR spectrometer was used without sample preparation, with a scanning range of 4,000-700 cm^{-1} in ATR mode, and a Shimadzu FT-IR spectrometer IRAffinity-1 was used without sample preparation, with a scanning range of 4,000 – 650 cm^{-1} in ATR mode.

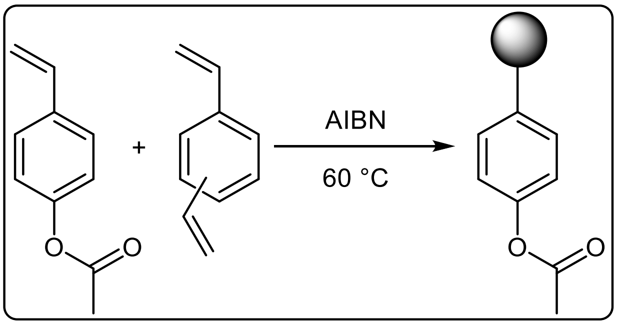
The porosity measurements were performed using a Micromeritics ASAP 2000. The samples were degassed under vacuum overnight at 100 °C prior to analysis. The analysis was carried out at – 196 °C *via* nitrogen sorption.

A Biotage® Initiator microwave fitted with a Robot Eight Sample Processor was used in the experiments that required microwave irradiation. The reaction vessels were Biotage® microwave vials (capacity 10-20 mL) and the parameters selected included 30 seconds pre-stirring and high absorbance level.

The solvent uptake measurements were performed using centrifuge tubes fitted with 0.2 µm modified nylon 500 µL centrifugal filter inserts (VWR, North America). The centrifuge used in these experiments was an Eppendorf Centrifuge 5804 (Hamburg, Germany) set to 3,000 rpm for 3 minutes.

2.2.3. Preparation of poly(4-acetoxystyrene-*co*-divinylbenzene) by precipitation polymerisation

The synthesis of polymer microspheres of poly(4-acetoxystyrene-*co*-divinylbenzene) (poly(AS-*co*-DVB)) is represented in Scheme 2.2.



Scheme 2.2: The reaction scheme for the precipitation polymerisation of monomers 4-acetoxystyrene and divinylbenzene to poly(AS-*co*-DVB)

A typical experimental procedure has been given for polymer sample KA10 (Procedure 2-i). The poly(AS-*co*-DVB) copolymers shown in Table 2.1 were synthesised according to an analogous procedure using different solvents and conditions.

Procedure 2-i

The comonomers DVB (0.547 mL, 0.5 g, 4 mmol) and AS (1.415 mL, 1.5 g, 9 mmol), and initiator AIBN (0.0547 g, 0.3 mmol, 2 mol % relative to the number of moles of polymerisable double bonds) were added to 2-ethyl-1-hexanol (100 mL) in a Nalgene® bottle (125 mL). The solution was sonicated for 10 minutes and then deoxygenated with N_2 at 0 °C for a further 10 minutes. The bottle was placed on a low-profile roller in a temperature-controllable incubator. The temperature was ramped from ambient to 60 °C over a period of approximately 2 hours and the polymerisation allowed to proceed at 60 °C for a further 46 hours and 35 minutes. An aliquot of the reaction mixture was taken to discern under an optical microscope whether bead formation had been successful. The resulting white particles were separated from the reaction medium by filtration on a 0.2 µm nylon membrane filter, washed successively with 2-ethyl-1-hexanol, methanol and diethyl ether, and then dried overnight in a vacuum oven at 40 °C/60 mbar (1.2591 g, 63%).

The mean particle diameter was < 1 µm.

Expected elemental microanalysis: 78.4 % C, 6.7 % H and 0.5 % N

Found elemental microanalysis: 78.5 % C, 6.3 % H and trace N

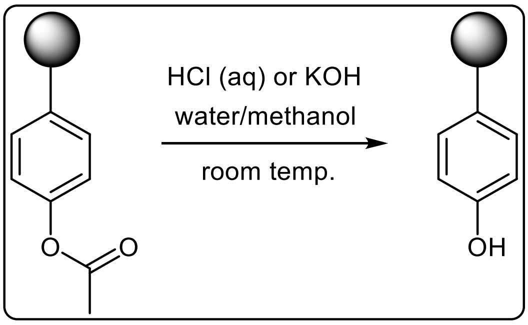
FT-IR: $\bar{\nu}/\text{cm}^{-1}$ (KBr): 3437 (O-H stretch, KBr), 3034, 2925, 1765 (C=O stretch, aryl ester), 1604, 1506, 1445, 1369, 1217, 1167 (C-O stretch), 1105, 1044, 1017, 990 (C-H bend, monosubstituted alkene), 940, 911 (C-H bend, monosubstituted alkene), 847 (1,4-disubstituted ring), 802 (1,3-disubstituted ring), 712, 594 and 553.

Table 2.1: The reagent quantities and conditions used for the preparation of poly(AS-*co*-DVB) by precipitation polymerisation

Polymer ref.	DVB (mL)	AS (mL)	AIBN (g)	ACN (mL)	MEK (mL)	Heptane (mL)	BuAc (mL)	2-EH (mL)	MeOH (mL)	Duration (h)	Phase separation?	Yield (g)	Yield (%)
KA4	2.735	7.075	0.27 (x2)	500	-		-	-	-	48+25	no	n/a	n/a
KA6	0.547	1.415	0.0533	-	50	50	-	-	-	48	no	n/a	n/a
KA9	0.547	1.415	0.0522	-	-	-	100	-	-	47	no	0.3839	19
KA10	0.547	1.415	0.0547	-	-	-	-	100	-	48	yes	1.2591	63
KA7	0.547	1.415	0.0547	-	-	-	-	-	100	48	yes	0.5331	27
KA8	0.547	1.415	0.0561	-	-	-	-	-	100	51	yes	0.6889	34
KA16	1.094	2.830	0.1070	-	-	-	-	-	100	49	yes	1.965	49
KA22	2.735	7.075	0.2709	-	-	-	-	-	250	48	yes	6.6	66

2.2.4. Preparation of poly(4-vinylphenol-*co*-divinylbenzene)

The hydrolysis of polymer microspheres of poly(AS-*co*-DVB) to poly(4-vinylphenol-*co*-divinylbenzene) (poly(VP-*co*-DVB)) is represented in Scheme 2.3.



Scheme 2.3: The reaction scheme for the hydrolysis of poly(AS-*co*-DVB) to poly(VP-*co*-DVB)

The procedure has been given for polymer sample KA12 (Procedure 2-ii), and for polymer sample KA17 (Procedure 2-iii). Table 2.2 summarises the conditions used.

Procedure 2-ii

This hydrolysis procedure was adapted from the procedure published by Kawabata *et al.*[110]

A mixture of concentrated HCl (2.5 mL), double distilled water (3.75 mL) and methanol (8.75 mL) were added to a round-bottomed flask with a magnetic stirrer bar. The poly(4-acetoxystyrene-*co*-divinylbenzene) copolymer KA10 (0.51 g) was added and stirred at RT for 71 hours. The resulting off-white solid was separated from the reaction medium by filtration on a 0.2 µm nylon membrane filter, washed with double distilled water until the pH of the effluent was neutral, and dried overnight in a vacuum oven at 40 °C/60 mbar (0.4123 g, 80 %).

The mean particle diameter was < 1 µm.

Expected elemental microanalysis: 84.4 % C, 6.7 % H and 0.5 % N

Found elemental microanalysis: 81.1 % C, 6.9 % H and trace N

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3414 (O-H stretch), 3021, 2923, 2852, 1761 (C=O stretch, aryl ester), 1739, 1612, 1597, 1513, 1445, 1370, 1232, 1173 (C-O stretch), 1106, 1016, 990 (C-H bend, monosubstituted alkene), 911 (C-H bend, monosubstituted alkene), 830 (1,4-disusbtituted ring), 802 (1,3-disusbtituted ring), 711 and 551.

Procedure 2-iii

This hydrolysis procedure was adapted from the procedure documented by Lewandowski *et al.*[111]

KA16 (1.85 g) was suspended in methanol (30 mL) and stirred in a 2-necked, round-bottomed flask connected to a mechanical stirrer. A solution of KOH (3.03 g) dissolved in double distilled water (15 mL) and methanol (15 mL) was added slowly to the flask and stirred at RT for 26 hours. The resulting off-white solid was separated from the reaction medium by filtration on a 0.2 µm nylon membrane filter, washed with double distilled water and methanol, and dried overnight in a vacuum oven at 40 °C/60 mbar (1.5654 g, 84 %).

Expected elemental microanalysis: 84.4 % C, 6.7 % H and 0.5 % N

Found elemental microanalysis: 78.1 % C, 6.9 % H and trace N

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3402 (O-H stretch), 3019, 2921, 2852, 1882, 1612, 1597, 1513, 1447, 1368, 1243, 1172 (C-O stretch), 1105, 1014, 992 (C-H bend, monosubstituted alkene), 905 (C-H bend, monosubstituted alkene), 830 (1,4-disubstituted ring), 799 (1,3-disubstituted ring), 712, 644 and 551.

Langmuir specific surface area: 7 m^2/g

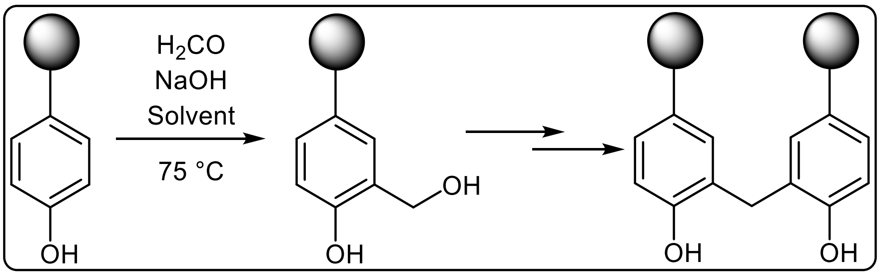
Mean pore diameter: 5.7 nm

Table 2.2: The reagent quantities and conditions used to prepare poly(VP-*co*-DVB).

Sample ref.	poly(AS-*co*-DVB) (g)	HCl (mL)	KOH (g)	Water (mL)	Methanol (mL)	Duration (h)	Yield (g)	Yield (%)
KA12	0.51, KA10	2.5	-	3.75	8.75	71	0.4123	80
KA17	1.85, KA16	-	3.03	15	45	26	1.5654	84
KA24	6.0, KA22	-	10.0	50	150	23	5.3765	90

2.2.5. Hypercrosslinking of poly(4-vinylphenol-*co*-divinylbenzene) using formaldehyde

The proposed hypercrosslinking reaction for polymer microspheres of poly(VP-*co*-DVB) using formaldehyde is represented in Scheme 2.4.



Scheme 2.4: The proposed reaction scheme for the hypercrosslinking of poly(VP-*co*-DVB) using formaldehyde under basic conditions

A typical experimental procedure has been given for polymer sample ref. KA18 (Procedure 2-iv). Table 2.3 and Table 2.4 show the quantities and conditions used in analogous experiments with paraformaldehyde and formaldehyde solution, respectively.

Procedure 2-iv

The poly(VP-*co*-DVB) precursor KA17 particles (0.4920 g) and ethanol (40 mL) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The reaction mixture was stirred at ~ 100 rpm for 3 hours. The oil bath was set to heat to 75 °C, then formaldehyde solution (0.9686 g, 11.8 mmol) and NaOH solution (303 µL, 12.2 M, 3.7 mmol) added to the flask once the bath had reached the desired temperature. The reaction mixture was left to stir and heat for 19 hours and 30 minutes. The resulting off-white solid was separated from the reaction medium by filtration on a 0.2 µm nylon membrane filter, washed with ethanol and diethyl ether, and dried overnight in a vacuum oven at 40 °C/60 mbar (0.5309 g).

Found elemental microanalysis: 73.8 % C, 6.6 % H and trace N

FT-IR: $\bar{\nu}/\mathrm{cm}^{-1}$ (KBr): 3402 (O-H stretch), 3020, 2922, 2852, 1612, 1513, 1484 (CH_2 deformations), 1445, 1368, 1337, 1239, 1173 (C-O stretch), 1105, 1012, 990 (C-H bend, monosubstituted alkene), 904 (C-H bend, monosubstituted alkene), 830 (1,4-disubstituted ring), 798 (1,3-disubstituted ring), 712 and 551.

Langmuir specific surface area: 5 m^2/g

Table 2.3: The reagent quantities and conditions used in an attempt to hypercrosslink the poly(VP-*co*-DVB) particles using paraformaldehyde (PF).

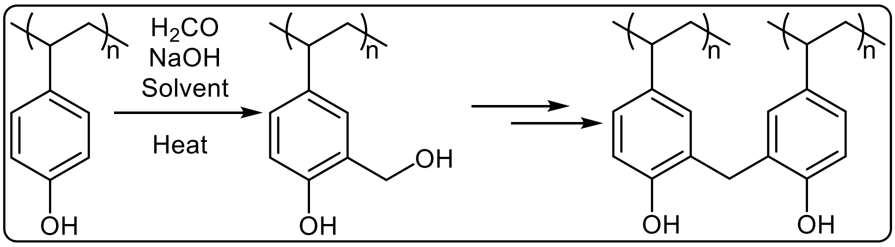
Sample Ref.	KA12 (g)	PF (g)	NaOH (μL, M)	Solvent	Volume (mL)	Duration (h)	Temp. (°C)	Yield (g)
KA15	0.16	0.12	100, 14.5	DCE	26	20	75	0.28

Table 2.4: The reagent quantities and conditions used in an attempt to hypercrosslink the poly(VP-*co*-DVB) particles using formaldehyde solution (FS).

Sample Ref.	KA17 (g)	FS (g)	NaOH (μL, M)	Solvent	Volume (mL)	Duration (h)	Temp. (°C)	Yield (g)
KA18	0.49	0.97	303, 12.2	ethanol	40	19.5	75	0.53
KA20	0.45	0.83	104, 16.2	ethanol	40	20.3	75	0.54

2.2.6. Hypercrosslinking of poly(4-vinylphenol) using formaldehyde

The proposed hypercrosslinking reaction for commercially available poly(4-vinylphenol) (poly(VP)) using formaldehyde is represented in Scheme 2.5.



Scheme 2.5: The proposed reaction scheme for the hypercrosslinking of poly(VP) using formaldehyde under basic conditions

Preparation using formaldehyde solution under basic conditions

For convenience, Table 2.5 lists all of the experiments using poly(VP), formaldehyde solution and sodium hydroxide solution. The name of the procedure followed for each experiment is identified, and the summary of the quantities and conditions used can be found in Table 2.6 (for experiments performed in ethanol), Table 2.7 (distilled water), Table 2.8 (tetrahydrofuran), Table 2.9 (1,2-dichloroethane) and Table 2.10 (*N*,*N*-dimethylformamide).

Table 2.5: Summary of the experiments using poly(4-vinylphenol), formaldehyde solution and sodium hydroxide solution

Polymer ref.	Quantities/ conditions	Procedure followed	Washing protocol	Oven temp. (°C)	Freeze-dried?
KA23	Table 2.6	Procedure 2-v	n/a	40	n/a
KA19	Table 2.6	Procedure 2-vi	ethanol	40	n/a
KA27	Table 2.6	Procedure 2-vi	ethanol, water	70	n/a
KA29	Table 2.6	Procedure 2-vi	n/a	40	n/a
KA34	Table 2.6	Procedure 2-vii	ethanol	40	n/a
KA36	Table 2.7	Procedure 2-vii	water	40	n/a
KA44	Table 2.7	Procedure 2-vii	water	40	n/a
KA46	Table 2.7	Procedure 2-vii	n/a	n/a	yes
KA45	Table 2.7	Procedure 2-vii	n/a	n/a	yes
KA35	Table 2.7	Procedure 2-vii	n/a	n/a	n/a
KA48	Table 2.7	Procedure 2-vii	water, ethanol	40	n/a
KA49	Table 2.7	Procedure 2-vii	n/a	n/a	yes
KA50	Table 2.7	Procedure 2-vii	n/a	n/a	yes
KA70	Table 2.7	Procedure 2-ix	water	70	n/a
KA71	Table 2.7	Procedure 2-ix	HCl (1 M), water	70	n/a
KA25	Table 2.8	Procedure 2-vi	tetrahydrofuran	70	n/a
KA26	Table 2.8	Procedure 2-vi	n/a	70	n/a
KA28	Table 2.9	Procedure 2-vi	ethanol	70	n/a
KA41 (1)	Table 2.10	Procedure 2-viii	ethanol	40	n/a
KA41 (3)	Table 2.10	Procedure 2-viii	HCl (1M), water, acetone	40	n/a
KA42 (1)	Table 2.10	Procedure 2-viii	ethanol	40	n/a
KA42 (2)	Table 2.10	Procedure 2-viii	n/a	n/a	n/a

Table 2.6: The conditions used for the poly(VP) experiments using formaldehyde solution (FS) under basic conditions in ethanol. [a] poly(4-vinylphenol) of M_w ~ 20,000, and [b] poly(4-vinylphenol) of M_w ~ 25,000.

Sample Ref.	Poly(VP) (g)	FS (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA23	0.54 [a]	0.5	0.24	50	~ 20-24	75	orange precipitate in yellow solution	0.43	62
KA19	0.50 [a]	1.39	0.22	50	21	75	orange solid	-	-
KA27	0.50 [a]	1.38	0.67	30	22	75	brown precipitate in orange/red solution	0.46	71
KA29	0.34 [a]	0.70	0.34	30	23	75	orange precipitate in colourless solution	0.48	109
KA34	0.43 [b]	2.97	0.14	30	24	75	orange precipitate in golden solution	0.53	96

Table 2.7: The conditions used for the poly(VP) experiments using formaldehyde solution (FS) under basic conditions in distilled water. [b] poly(4-vinylphenol) of M_w ~ 25,000.

Sample Ref.	Poly(VP) (g)	FS (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA36	0.47 [b]	3.21	0.16	30	23	75	yellow gel	0.62	102
KA44	0.45 [b]	3.13	0.15	30	24	75	yellow gel	0.61	105
KA46	0.46 [b]	3.18	0.15	30	24	75	yellow gel	1.01	168
KA45	0.53 [b]	3.63	0.18	30	47	75	yellow gel	0.82	120
KA35	0.39 [b]	2.66	1.29	32	23	75	yellow precipitate in brown solution	n/a	n/a
KA48	0.46 [b]	0.95	0.15	30	18	75	yellow precipitate (solvent evaporated)	0.65	110
KA49	0.46 [b]	0.98	0.15	30	43	75	no precipitate, orange/red solution	0.81	137
KA50	0.42 [b]	2.04	0.14	30	24	75	yellow gel	0.68	125
KA70	0.49 [b]	3.39	0.33	7	24	90	yellow gel in yellow solution	0.67	105
KA71	0.31 [b]	2.15	0.21	5	24	90	yellow gel in yellow solution	0.42	103

Table 2.8: The conditions used for the poly(VP) experiments using formaldehyde solution (FS) under basic conditions in tetrahydrofuran.

[a] poly(4-vinylphenol) of M_w ~ 20,000.

Sample Ref.	Poly(VP) (g)	Formaldehyde (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA25	0.47 [a]	1.31	0.21	30	24	65	orange precipitate in golden solution	0.56	92
KA26	0.51 [a]	1.39	0.68	30	24	65	brown precipitate in brown solution	0.99	150

Table 2.9: The conditions used for the poly(VP) experiments using formaldehyde solution (FS) under basic conditions in 1,2-dichloroethane.

[a] poly(4-vinylphenol) of M_w ~ 20,000.

Sample Ref.	Poly(VP) (g)	Formaldehyde (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA28	0.50 [a]	1.40	0.88	35	21	75	orange/red precipitate in colourless solution	1.29	200

Table 2.10: The conditions used for the poly(VP) experiments using formaldehyde solution (FS) under basic conditions in *N*,*N*-dimethylformamide.

[b] poly(4-vinylphenol) of M_w ~ 25,000.

Sample Ref.	Poly(VP) (g)	Formaldehyde (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA41	0.49 [b]	3.42	0.16	30	24	120	precipitate in red/brown solution	(1) 0.01 + (3) 0.46	73
KA42	0.43 [b]	2.95	0.14	30	24	140	precipitate in red/brown solution	(1) 0.01 + (2) 0.47	86

Procedure 2-v

The experimental procedure has been given for polymer sample ref. KA23 (Procedure 2-v).

Poly(VP) (0.5402 g, M_w ~20,000), solvent (50 mL) and formaldehyde solution (0.5 g, 6.1 mmol) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The reaction was stirred at ~ 100 rpm and heated using an oil bath. When the bath had reached the desired temperature, NaOH solution (661 µL, 9.06 M, 6.0 mmol) was added into the flask. The reaction was left to stir and heat overnight. An orange-coloured solid had precipitated from solution and was stuck to the flask. The solution was decanted off and the solid stuck to the round-bottomed flask was dried overnight at 40 °C/60 mbar and the solid residue collected (0.4311 g, 62 %).

Found elemental microanalysis: 64.6 % C and 6.2 % H

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3407, 3011, 2918, 2846, 1884, 1600, 1512, 1498, 1448, 1372, 1252 (C-O-C aryl ether), 1171, 1105, 1010, 885 (1,2,4,6-tetrasubstituted ring), 829 (1,4-disubstituted ring), 699, 644 and 551.

Langmuir specific surface area: < 1 m^2/g

Procedure 2-vi

Procedure 2-vi is the typical experimental procedure followed for polymer sample ref. KA19, KA25, KA26, KA27, KA28 and KA29. The procedure given is for KA27.

Poly(VP) (0.5030 g, M_w ~20,000) and solvent (30 mL) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The reaction was stirred at ~ 100 rpm and heated using an oil bath. When the bath had reached the desired temperature, formaldehyde solution (1.3766 g, 16.7 mmol) and NaOH solution (1149 µL, 14.6 M, 16.7 mmol) were added into the flask. The reaction was left to stir and heat for 22 hours. After cooling, the sticky brown solid was filtered on a 0.2 µm nylon membrane filter, washed with ethanol and distilled water, and dried overnight in a vacuum oven at 70 °C/60 mbar (0.4592 g, 71 %).

Found elemental microanalysis: 33.1 % C and 4.7 % H

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3424 (O-H stretch), 2923, 2846, 2505, 1776, 1602, 1444, 1258 (C-O-C aryl ether), 1171, 1104, 1077 (C-O-C ether), 1045, 880 (1,2,4,6-tetrasubstituted ring), 831 (1,4-disubstituted ring), 768, 701, 644 and 552.

Langmuir specific surface area: < 1 m^2/g

Procedure 2-vii

Procedure 2-vii is the typical experimental procedure followed for polymer sample ref. KA34, KA36, KA44, KA46, KA45 and KA35. The procedure given is for KA46.

Poly(VP) (0.4644 g, M_w ~25,000), solvent (30 mL) and NaOH solution (390 µL, 10 M, 3.9 mmol) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The reaction was stirred at ~ 150 rpm in order to dissolve the polymer and then the stirring rate was reduced to 100 rpm for the remainder of the reaction. The flask was heated using an oil bath, and when the bath had reached the desired temperature formaldehyde solution (3.18 g, 38.7 mmol) was added into the flask. The reaction was left to stir and heat for 24 hours and 20 minutes. The flask was left to cool and then all of the reaction mixture was transferred into a 250 mL round-bottomed flask with a small amount of water used to rinse the reaction flask. The flask was swirled in liquid nitrogen to freeze the contents, and then connected to a freeze dryer at 0.3 mbar pressure for ~ 72 hours. The product obtained was a bright yellow powder (1.0079 g, 168 %).

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3303, 2913, 2831, 2769, 2715, 1587, 1479 (CH_2 deformations), 1353, 1216, 1149, 1103, 1021 (C-O-C ether), 985, 935, 875 (1,2,4,6-tetrasubstituted ring), 773 and 748.

Langmuir specific surface area: 8.5 m^2/g

Procedure 2-viii

Procedure 2-vii is the typical experimental procedure followed for polymer sample ref. KA41 and KA42. The procedure given is for KA41.

Poly(VP) (0.4888 g, M_w ~25,000) and DMF (30 mL) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The reaction was stirred at ~ 100 rpm and NaOH solution (410 µL, 10 M, 4.1 mmol) was added into the flask. The reaction was heated using an oil bath, and when the bath had reached the desired temperature formaldehyde solution (3.42 g, 41.6 mmol) was added. The reaction was left to stir and heat for 24 hours. By the following day, the solution was clear and dark red/brown coloured with a small amount of precipitate settled on the flask wall. After cooling, the solid (labelled KA41 (1)) was filtered on a 0.2 µm nylon membrane filter, washed with ethanol and dried overnight in a vacuum oven at 40 °C/60 mbar (0.0051 g).

FT-IR KA41 (1): $\bar{\nu}/\mathrm{cm}^{-1}$ (KBr): 3437, 2923, 2852, 1627, 1598, 1513, 1503, 1469 (CH_2 deformations), 1383, 1351, 1221, 1169, 1141, 1097, 1040 (C-O-C ether), 941, 878 (1,2,4,6-tetrasubstituted ring), 829 (1,4-disubstituted ring) and 779.

The ethanol in the washing protocol caused a soluble solid in the filtrate to precipitate. This precipitate was filtered on a 0.2 µm nylon membrane filter, however the filtration was very slow so it was left overnight. After replacing the nylon membrane filter, the solid was washed with ethanol and acetone. A small portion of this solid was isolated and dried in a vacuum oven at 40 °C/60 mbar, and labelled KA41 (2).

FT-IR KA41 (2): $\bar{\nu}/\mathrm{cm}^{-1}$ (KBr): 3413, 3016, 2922, 2852, 1659, 1612, 1513, 1474 (CH_2 deformations), 1410, 1387, 1229, 1169, 1139, 1100, 1062 (C-O-C ether), 1040, 1021, 933, 881(1,2,4,6-tetrasubstituted ring), 830 (1,4-disubstituted ring), 746 and 663.

The solid remaining on the filter was washed with 1M hydrochloric acid, followed by distilled water and acetone. This was labelled as KA41 (3) and dried in a vacuum oven at 40 °C/60 mbar (0.4563 g, 73 %).

FT-IR KA41 (3): $\bar{\nu}/\mathrm{cm}^{-1}$ (KBr): 3413, 3016, 2921, 2703, 1700, 1651, 1612, 1513, 1475 (CH_2 deformations), 1446, 1383, 1366, 1220, 1172, 1139, 1104, 1056 (C-O-C ether), 1012, 937, 885 (1,2,4,6-tetrasubstituted ring), 830 (1,4-disubstituted ring), 744 and 619.

Procedure 2-ix

Procedure 2-ix is the typical experimental procedure followed for polymer sample ref. KA70 and KA71. The procedure given is for KA70.

Poly(VP) (0.4949 g, M_w ~25,000), distilled water (7 mL) and NaOH solution (820 µL, 10 M, 8.2 mmol) were added to a 20 mL carousel tube placed in a Radleys carousel with attached reflux head. After 17 hours of stirring at room temperature, formaldehyde solution (3.3886 g, 41.2 mmol) was added and the reaction heated. The reaction was stirred and heated at 90 °C for 24 hours. The precipitate was filtered on a 0.2 µm nylon membrane filter, washed with distilled water, and dried overnight in a vacuum oven at 70 °C/60 mbar (0.6725 g, 105 %).

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3329, 2914, 1643, 1607, 1479 (CH_2 deformations), 1452, 1352, 1211, 1148, 1065, 1013, 876 (1,2,4,6-tetrasubstituted ring), 826 (1,4-disubstituted ring) and 746.

Preparation using paraformaldehyde under basic conditions

For convenience, Table 2.11 lists all of the experiments using poly(VP), paraformaldehyde, and sodium hydroxide solution. The name of the procedure followed for each experiment has been listed, and the summary of the quantities and conditions used can be found in Table 2.12 (for experiments performed in ethanol), and Table 2.13 (for experiments performed in DMF).

Table 2.11: Summary of the experiments using poly(4-vinylphenol), paraformaldehyde and sodium hydroxide solution

Polymer ref.	Quantities/ conditions	Procedure followed	Washing protocol	Oven temp. (°C)	Freeze-dried?
KA14	Table 2.12	Procedure 2-x	ethanol, acetone	40	n/a
KA30	Table 2.12	Procedure 2-xi	ethanol	70	n/a
KA32	Table 2.12	Procedure 2-xi	ethanol	40	n/a
KA33	Table 2.12	Procedure 2-xi	n/a	40	n/a
KA31	Table 2.13	Procedure 2-xi	*N*,*N*-dimethylformamide	70	n/a
KA43	Table 2.13	Procedure 2-xi	ethanol	40	n/a

Table 2.12: The conditions used for the poly(VP) experiments using paraformaldehyde (PF) under basic conditions in ethanol. [a] poly(4-vinylphenol) of $M_w \sim 20,000$, and [b] poly(4-vinylphenol) of $M_w \sim 25,000$.

Sample Ref.	Poly(VP) (g)	PF (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA14	0.50 [a]	0.40	0.17	50	5	70	green precipitate in dark yellow solution	n/a	n/a
KA30	0.40 [b]	0.26	0.14	35	22	75	brown precipitate in brown solution	0.39	74
KA32	0.40 [b]	1.09	0.13	35	24	75	orange precipitate in orange solution	0.53	101
KA33	0.44 [b]	1.15	1.45	35	24	75	red/brown syrup in red/brown solution	0.73	130

Table 2.13: The conditions used for the poly(VP) experiments using paraformaldehyde (PF) under basic conditions in *N*,*N*-dimethylformamide. [a] poly(4-vinylphenol) of $M_w \sim 20,000$, and [b] poly(4-vinylphenol) of $M_w \sim 25,000$.

Sample Ref.	Poly(VP) (g)	PF (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)	Yield (%)
KA31	0.42 [a]	0.27	0.14	30	24	120	precipitate in dark red solution	0.18	34
KA43	0.42 [b]	1.11	0.14	30	48	120	orange precipitate in red/brown solution	0.52	95

Procedure 2-x

The experimental procedure has been given for polymer sample ref. KA14 (Procedure 2-x).

Poly(VP) (0.4998 g, M_w ~20,000) and ethanol (25 mL) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The mixture was stirred at ~ 100 rpm and heated using an oil bath. When the bath had reached the desired temperature, paraformaldehyde (0.4034 g, 12.8 mmol), ethanol (25 mL) and NaOH solution (1667 µL, 2.5 M in hot ethanol, 4.2 mmol) were added into the flask. The reaction was left to stir and heat for 5 hours. The solution was decanted off and the solid filtered on a 0.2 µm nylon membrane filter, washed with ethanol and acetone before drying overnight in a vacuum oven at 40 °C/60 mbar.

Found elemental microanalysis: 63.7 % C and 6.8 % H

FT-IR: $\bar{\nu}/\mathrm{cm}^{-1}$ (KBr): 3402, 3016, 2967, 2918, 2846, 1882, 1611, 1599, 1513, 1482 (CH_2 deformations), 1445, 1364, 1236, 1171, 1119, 1105, 1043 (C-O-C ether), 1012, 879 (1,2,4,6-tetrasubstituted ring), 827 (1,4-disubstituted ring), 771, 700 and 545.

Langmuir specific surface area: < 2 m^2/g

Procedure 2-xi

Procedure 2-xi is the typical experimental procedure followed for polymer sample ref. KA30, KA32, KA33, KA31 and KA43. The procedure given is for KA30.

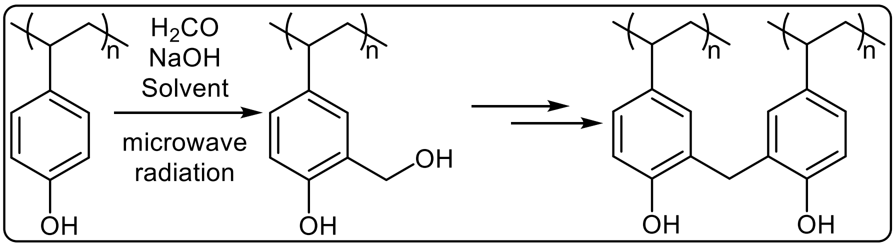
Poly(VP) (0.4019 g, M_w ~25,000) and solvent (35 mL) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. The mixture was stirred at ~ 100 rpm and NaOH solution (266 µL, 12.6 M, 3.3 mmol) was added into the flask. The reaction was heated using an oil bath set to 75 °C and when the bath had reached the desired temperature, paraformaldehyde (0.2618 g, 8.3 mmol) was added. The reaction was left to stir and heat for 22 hours. The solution was decanted off and the solids filtered on a 0.2 µm nylon membrane filter and washed with ethanol. After initial success, the product became difficult to filter through the membrane so the supernatant was decanted off and the product was transferred to a vacuum oven set at 70 °C/60 mbar to dry thoroughly (0.3856 g, 74 %).

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3424, 3016, 2973, 2919, 2846, 1882, 1611, 1597, 1513, 1445, 1377, 1237, 1171, 1104, 1044 (C-O-C ether), 1012, 880 (1,2,4,6-tetrasubstituted ring), 828 (1,4-disubstituted ring), 697, 644 and 547.

Langmuir specific surface area: < 1 m^2/g

2.2.7. Hypercrosslinking of poly(4-vinylphenol) using formaldehyde under microwave irradiation conditions

The proposed hypercrosslinking reaction for commercially available poly(4-vinylphenol) (poly(VP)) using formaldehyde is represented in Scheme 2.6.



Scheme 2.6: The proposed reaction scheme for the hypercrosslinking of poly(VP) using formaldehyde under basic conditions, by microwave irradiation

Preparation using paraformaldehyde under basic conditions

For convenience, Table 2.14 lists all of the microwave irradiation experiments using poly(VP), paraformaldehyde and sodium hydroxide solution. The summary of the quantities and conditions used are found in Table 2.15.

Table 2.14: Summary of the experiments using poly(4-vinylphenol), paraformaldehyde and sodium hydroxide solution

Polymer ref.	Quantities/ conditions	Procedure followed	Washing protocol	Oven temp. (°C)	Freeze-dried?
KA37	Table 2.15	Procedure 2-xii	ethanol, 1M HCl, ethanol	70	n/a
KA38	Table 2.15	Procedure 2-xii	ethanol, 1M HCl, ethanol	70	n/a
KA39	Table 2.15	Procedure 2-xii	ethanol	70	n/a
KA40	Table 2.15	Procedure 2-xii	ethanol	70	n/a

Table 2.15: The conditions used for the poly(VP) microwave irradiation experiments using paraformaldehyde (PF) under basic conditions in ethanol. [b] poly(4-vinylphenol) of M_w ~ 25,000.

Sample Ref.	Poly(VP) (g)	PF (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Pressure (bar)	Final appearance in flask	Yield (g)	Yield (%)
KA37	0.41 [b]	0.11	0.14	10	2	80	0-1	brown sticky solid	0.25	47
KA38	0.40 [b]	0.11	0.13	10	2	120	8-9	brown sticky solid	0.46	88
KA39	0.40 [b]	0.32	0.13	10	2	120	8-9	patchy brown solid	0.55	107
KA40	0.41 [b]	0.32	0.14	10	6	120	8-9	patchy brown solid	0.59	111

Table 2.16: The conditions used for the poly(VP) microwave irradiation experiments using formaldehyde solution under basic conditions in distilled water. [b] poly(4-vinylphenol) of M_w ~ 25,000.

Sample Ref.	Poly(VP) (g)	FS (g)	NaOH (g)	Volume (mL)	Duration (h)	Temp. (°C)	Pressure (bar)	Final appearance in flask	Yield (g)	Yield (%)
KA47	0.52 [b]	1.08	0.17	10	5	100	0-1	brown gel	0.88	131

Procedure 2-xii

Procedure 2-xii is the typical experimental procedure followed for polymer sample ref. KA37, KA38, KA39 and KA40. The procedure given is for KA37.

Poly(VP) (0.4071 g, M_w ~ 25,000) and ethanol (10 mL) were added into a 20 mL capacity microwave vial with a magnetic stirrer bar and stirred to dissolve the polymer. Paraformaldehyde (0.1090 g, 3.4 mmol) and sodium hydroxide solution (350 µL, 10 M, 3.5 mmol) were added to the vial and then the vial crimped immediately to seal. A white precipitate formed when the contents of the vial were stirred. After microwaving for 2 hours at 80 °C, the vial contained a brown liquid and dark brown precipitate. The supernatant was decanted off, the precipitate filtered on a 0.2 µm nylon membrane filter, and the brown/orange solid scraped out and washed with ethanol. The filtration was slow so 3 mL of HCl (1 M) solution was added causing the solvent to turn cloudy, but then flow successfully through the membrane filter. The remaining brown solid was washed with ethanol and dried in a vacuum oven at 70 °C/60 mbar (0.2482 g, 47 %).

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3419, 3060, 3010, 2918, 2846, 1883, 1601, 1498, 1449, 1369, 1266 (C-O-C aryl ether), 1171, 1101, 1048 (C-O-C ether), 1001, 878 (1,2,4,6-tetrasubstituted ring), 832 (1,4-disubstituted ring), 704, 644 and 551.

Preparation using formaldehyde solution under basic conditions

The summary of the quantities and conditions used are found in Table 2.16.

Procedure 2-xiii

The experimental procedure has been given for polymer sample ref. KA47 (Procedure 2-xiii).

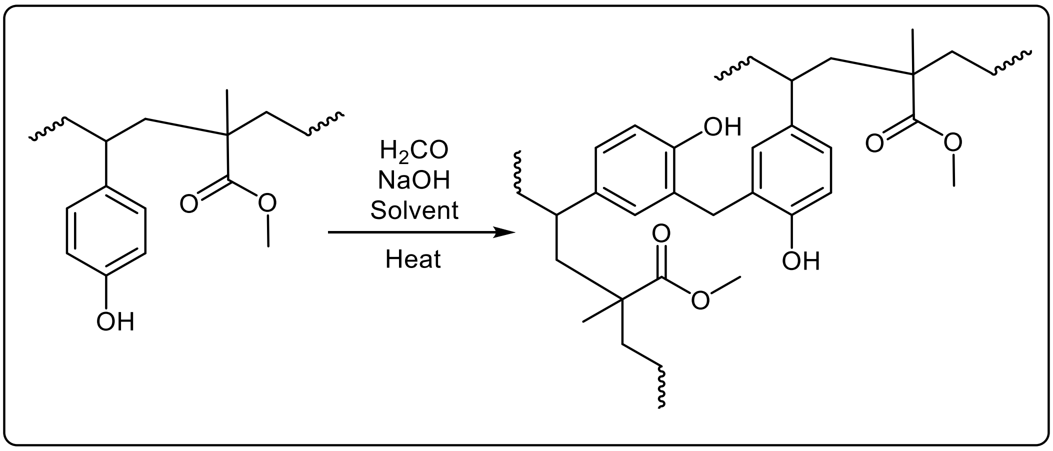
Poly(VP) (0.5166 g, M_w ~ 25,000), distilled water (10 mL) and sodium hydroxide solution (430 µL, 10 M, 4.3 mmol) were added into a microwave vial with a magnetic stirrer bar and stirred to dissolve the polymer. Formaldehyde solution (1.0816 g, 13.1 mmol) was added to the vial and the vial then crimped immediately to seal. The solution was clear and pale coloured, with no precipitate present. After microwaving for 5 hours at 100 °C, the vial contained a clear and very pale yellow liquid and brown, gel-like precipitate. The contents of the microwave vial were transferred into a 250 mL round-bottomed flask and swirled in liquid nitrogen to freeze the contents, and then connected to a freeze dryer at 2 mbar pressure for ~ 48 hours. The product obtained was a yellow/brown solid (0.8758 g, 131 %).

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3221, 2917, 1591, 1472 (CH_2 deformations), 1448, 1354, 1211, 1147, 1060 (C-O-C ether), 1008, 875 (1,2,4,6-tetrasubstituted ring), 825 (1,4-disubstituted ring) and 763.

Langmuir specific surface area: < 1 m^2/g

2.2.8. Hypercrosslinking of poly(4-vinylphenol-*co*-methyl methacrylate) using formaldehyde

The proposed hypercrosslinking reaction for commercially available poly(4-vinylphenol-*co*-methyl methacrylate) (poly(VP-*co*-MMA)) using formaldehyde is represented in Scheme 2.7.



Scheme 2.7: The proposed reaction scheme for the hypercrosslinking of poly(VP-*co*-MMA) using formaldehyde under basic conditions

For convenience, Table 2.17 lists of all the experiments using poly(VP-*co*-MMA), formaldehyde solution and sodium hydroxide solution. The name of the procedure followed for each experiment has been listed, and the summary of the quantities and conditions used can be found in Table 2.18 (for experiments performed using Procedure 2-xiv), Table 2.19 (for experiments performed using Procedure 2-xvi), and Table 2.20 (for experiments performed using Procedure 2-xv).

Table 2.17: Summary of the experiments using poly(4-vinylphenol-*co*-methyl methacrylate), formaldehyde solution and sodium hydroxide solution

Sample ref.	Quantities/ conditions	Procedure followed	Washing protocol	Oven temp. (° C)
KA51	Table 2.18	Procedure 2-xiv	water	40
KA52	Table 2.18	Procedure 2-xiv	ethanol	40
KA54	Table 2.18	Procedure 2-xiv	water	40
KA55	Table 2.18	Procedure 2-xiv	n/a	n/a
KA56	Table 2.18	Procedure 2-xiv	n/a	n/a
KA57	Table 2.18	Procedure 2-xiv	water, HCl (1M), water	70
KA58	Table 2.18	Procedure 2-xiv	water, HCl (1 M), water	70
KA59	Table 2.18	Procedure 2-xiv	ethanol	70
KA60	Table 2.18	Procedure 2-xiv	ethanol, water, ethanol	70
KA61	Table 2.19	Procedure 2-xvi	HCl (1 M), water	70
KA62	Table 2.20	Procedure 2-xv	HCl (1 M), water	70
KA63	Table 2.20	Procedure 2-xv	HCl (1 M), water	70
KA64	Table 2.20	Procedure 2-xv	HCl (1 M), water	70
KA65	Table 2.20	Procedure 2-xv	HCl (1 M), water	70
KA66	Table 2.20	Procedure 2-xv	water	70
KA67	Table 2.20	Procedure 2-xv	HCl (1 M), water	70
KA68	Table 2.20	Procedure 2-xv	water	70
KA69	Table 2.20	Procedure 2-xv	water	70
KA72	Table 2.20	Procedure 2-xv	water	70
KA73	Table 2.20	Procedure 2-xv	HCl (1 M), water	70
KA74	Table 2.20	Procedure 2-xv	water	70
KA75	Table 2.20	Procedure 2-xv	water	70

Table 2.18: The conditions used for the poly(VP-*co*-MMA) experiments following Procedure 2-xiv, using formaldehyde solution (FS)

Sample Ref.	Poly(VP-*co*-MMA) (g)	FS (g)	NaOH (10 M) (µL)	Solvent	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)
KA51	0.44	3.02	370	water	20	24	70	cream precipitate in yellow solution	0.27
KA52	0.44	3.04	370	ethanol	20	24	70	cloudy brown solution	0.02
KA53	0.44	3.05	370	methanol	20	24	70	no precipitate, yellow solution	0.00
KA54	0.44	3.05	370	water	10	24	70	cream precipitate in brown solution	0.36
KA55	0.48	1.81	220	ethanol/water	5	24	75	no precipitate, brown solution	0.00
KA56	0.48	3.24	400	ethanol/water	5	24	75	no precipitate, brown solution	0.00
KA57	0.50	3.39	410	water	5	24	75	beige precipitate in pale solution	0.51
KA58	0.51	1.88	230	water	5	24	75	beige precipitate in pale solution	0.50
KA59	0.51	3.50	430	ethanol	5	24	75	brown precipitate in brown solution	0.01
KA60	0.50	1.88	230	ethanol	5	24	75	yellow precipitate in brown solution	n/a

Table 2.19: The conditions used for the poly(VP-*co*-MMA) experiments following Procedure 2-xvi, using formaldehyde solution (FS)

Sample Ref.	Poly(VP-*co*-MMA) (g)	FS (g)	NaOH (10 M) (µL)	Solvent	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)
KA61	0.64	3.31	220	water	30	24	RT	beige precipitate in yellow solution	0.60

Table 2.20: The conditions used for the poly(VP-*co*-MMA) experiments following Procedure 2-xv, using formaldehyde solution (FS)

Sample Ref.	Poly(VP-*co*-MMA) (g)	FS (g)	NaOH (10 M) (µL)	Solvent	Volume (mL)	Duration (h)	Temp. (°C)	Final appearance in flask	Yield (g)
KA62	0.56	2.92	190	water	5	24	75	beige precipitate in yellow solution	0.61
KA63	0.60	3.12	620	water	5	24	75	beige precipitate in yellow solution	0.62
KA64	0.53	2.75	180	water	10	24	75	cream precipitate in pale solution	0.48
KA65	0.54	2.79	550	water	10	24	75	cream precipitate in pale solution	0.59
KA66	0.54	2.83	170	water	5	24	90	beige precipitate in colourless solution	0.57
KA67	0.54	2.82	340	water	5	24	90	beige precipitate in colourless solution	0.60
KA68	0.54	2.83	520	water	5	24	90	beige precipitate in colourless solution	0.62
KA69	0.55	2.84	690	water	5	24	90	beige precipitate in colourless solution	0.63
KA72	0.56	2.93	710	water	5	24	90	beige precipitate in colourless solution	0.64
KA73	0.51	2.68	320	water	5	24	75	beige precipitate in colourless solution	0.56
KA74	0.52	2.71	330	water	5	73	75	beige precipitate in colourless solution	0.55
KA75	0.52	2.71	330	water	5	240	75	beige precipitate in colourless solution	0.58

Procedure 2-xiv

Procedure 2-xiv is the typical experimental procedure followed for polymer sample ref. KA51, KA52, KA53, KA54, KA55, KA56, KA57, KA58, KA59 and KA60. The procedure given is for KA51.

Poly(VP-*co*-MMA) (0.4419 g), distilled water (20 mL), NaOH solution (370 µL, 10 M, 3.7 mmol) and formaldehyde solution (3.0211 g, 36.7 mmol) were added to a 20 mL carousel tube placed in a Radleys carousel with attached reflux head. The reaction was stirred and heated at 70 °C for 24 hours. After cooling, the solid was filtered on a 0.2 µm nylon membrane filter, washed with distilled water, and dried overnight in a vacuum oven at 40 °C/60 mbar (0.2652 g).

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3370, 2978, 2945, 1705 (C=O stretch, ester), 1614, 1586, 1513, 1446, 1382, 1202, 1174, 1149, 1101, 1015, 987, 883 (1,2,4,6-tetrasubstituted ring), 831 (1,4-disubstituted ring), 763 and 732.

Procedure 2-xv

Procedure 2-xv is the typical experimental procedure followed for polymer sample ref. KA62, KA63, KA64, KA65, KA66, KA67, KA68, KA69, KA72, KA73, KA74 and KA75. The procedure given is for KA62.

Poly(VP-*co*-MMA) (0.5608 g), distilled water (5 mL) and NaOH solution (190 µL, 10 M, 1.9 mmol) were added to a 20 mL carousel tube placed in a Radleys carousel with attached reflux head. After 17-21 hours of stirring at room temperature, formaldehyde solution (2.9156 g, 35.4 mmol) was added and the reaction heated. The reaction was stirred and heated at 75 °C for 24 hours. After cooling, HCl (1 M) was added to neutralise the solution (if pH was high). The solid was filtered on a 0.2 µm nylon membrane filter, washed with distilled water, and dried overnight in a vacuum oven at 70 °C/60 mbar (0.6126 g).

FT-IR: $\bar{\nu}$/cm^{-1} (KBr): 3337, 2990, 2936, 2914, 2901, 2857, 1703 (C=O stretch, ester), 1647, 1609, 1481 (CH_2 deformations), 1452, 1381, 1360, 1204, 1148, 1113, 1061, 1016, 986, 907, 878 (1,2,4,6-tetrasubstituted ring), 829 (1,4-disubstituted ring), 812, 750, 687 and 648.

Procedure 2-xvi

The experimental procedure has been given for polymer sample ref. KA61 (Procedure 2-xvi).

Poly(VP-*co*-MMA) (0.6381 g), distilled water (30 mL) and NaOH solution (220 µL, 10 M, 2.2 mmol) were added to a 100 mL 3-necked, round-bottomed flask connected to an overhead mechanical stirrer and condenser. After 20 hours of stirring at ~ 100 rpm at room temperature, formaldehyde solution (3.3131 g, 40.3 mmol) was added. The reaction was left to stir at room temperature for 24 hours. HCl (1 M) was added to neutralise the solution. The precipitate was filtered on a 0.2 µm nylon membrane filter, washed with distilled water, and dried overnight in a vacuum oven at 70 °C/60 mbar (0.6035 g).

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3381, 3252, 3198, 3003, 2945, 2909, 1703 (C=O stretch, ester), 1612, 1595, 1512, 1441, 1381, 1358, 1335, 1198, 1173, 1140, 1098, 1013, 988 (1,2,4,6-tetrasubstituted ring), 829 (1,4-disubstituted ring), 764, 725 and 712.

2.2.9. Solvent uptake experiments

The dry polymer sample was weighed into a centrifugal filter and 500 µL of ethanol was added into the filter top. The polymer was left to swell for 2 hours and 15 minutes. The excess solvent was removed by centrifugation at 3000 rpm for 3 minutes.

Table 2.21: The results of the solvent uptake experiments

Polymer ref.	Mass (g)	Ethanol (µL)	Swelling time (min)	Uptake of ethanol per 1 g dry polymer (g)
KA23	0.0305	500	135	0.40
KA19	0.0329	500	135	0.31
KA25	0.0314	500	135	0.19
KA26	0.0322	500	135	9.75
KA27	0.0304	500	135	10.71
KA28	0.0318	500	135	0.46
KA29	0.0311	500	135	8.90
KA34	0.0292	500	135	0.31
KA36	0.0318	500	135	0.18
KA35	0.0013	500	135	14.15
KA41 (1)	0.0071	500	135	2.92
KA42 (1)	0.0063	500	135	2.75

2.3. Results and discussion

In this chapter, novel methodologies and routes towards hypercrosslinked polymers based upon phenol-formaldehyde chemistry have been investigated. Crosslinked poly(VP-*co*-DVB) microspheres were synthesised by precipitation polymerisation, to be used in subsequent hypercrosslinking reactions that exploit phenol-formaldehyde chemistry.

2.3.1. The precipitation polymerisation of lightly crosslinked poly(4-acetoxystyrene-*co*-divinylbenzene) particles, and their subsequent hydrolysis to poly(4-vinylphenol-*co*-divinylbenzene) particles

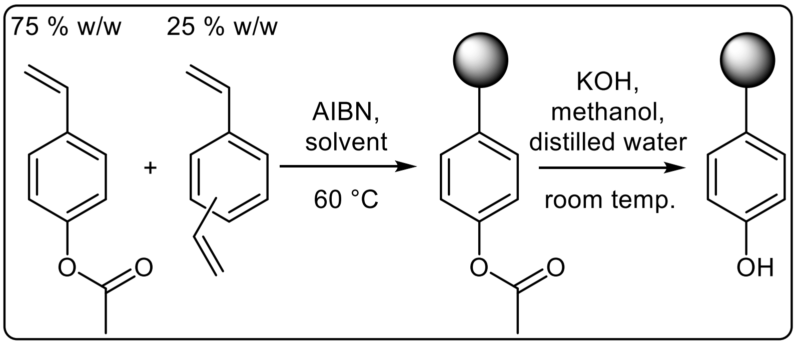
Heterogeneous polymerisation is used routinely for the synthesis of spherical polymer particles, and particles of varying size can be prepared depending on the methodology used.

The synthesis of poly(4-vinylphenol) (poly(VP)) usually occurs *via* the polymerisation of a protected 4-vinylphenol monomer, followed by the removal of the protecting group to reveal the phenolic moiety.[112] The synthesis and exploitation of poly(4-vinylphenol) has become more attractive since the monomer 4-acetoxystyrene (AS) became commercially available.[113] Other related, commercially available monomers include 4-*tert*-butoxycarbonyl oxystyrene and 4-*tert*-butoxystyrene.[111, 114]

The synthesis of insoluble, crosslinked poly(4-acetoxystyrene-*co*-divinylbenzene) and the hydrolysis to poly(4-vinylphenol-*co*-divinylbenzene) was described by Packham in 1964.[115] Electrophilic aromatic substitution reactions were performed on the polymer, and substitution was shown to occur at the *ortho*- position to the hydroxyl group. Spherical copolymers of poly(4-acetoxystyrene-*co*-divinylbenzene) and poly(4-acetoxystyrene-*co*-styrene) were synthesised by Arshady *et al.*, and the acetoxy groups were subsequently cleaved to generate phenolic groups within the polymer.[59] These phenolic groups were intended to provide an alternative linkage to peptide chains for solid-phase peptide synthesis, compared to crosslinked polystyrene supports containing chloromethyl groups. Following the earlier work by Arshady *et al.*,[59] Delueze *et al*. have synthesised spherical particles of poly(4-acetoxystyrene-*co*-styrene-*co*-divinylbenzene) by suspension polymerisation, and subsequently hydrolysed the acetoxy groups to phenolic groups using hydrazine hydrate in dioxane.[113] The typical particle size was 100-400 µm.

Heterogeneous polymerisation is used for the synthesis of spherical polymer particles, and different sized particles may be prepared depending on the methodology used. At the time of writing of this thesis, we were not aware of any publications documenting the synthesis of such polymers by precipitation polymerisation. Precipitation polymerisation was selected as the most appropriate polymerisation technique to prepare polymer microspheres of low particle diameter (in the low micron size range) and with a narrow particle size distribution.

Here, the synthesis of spherical particles of poly(4-vinylphenol-*co*-divinylbenzene) occurs *via* a two-stage reaction, shown in Scheme 2.8. In the first stage, lightly crosslinked particles of poly(AS-*co*-DVB) were synthesised by precipitation polymerisation of the functional monomer AS with the crosslinking monomer DVB.



Scheme 2.8: Outline of the two-stage synthetic procedure used to synthesise polymer particles of poly(VP-*co*-DVB)

Synthesis of poly(4-acetoxystyrene-co-divinylbenzene)

For precipitation polymerisation, dilute monomer loading of 2-5 % (w/w) in an appropriate θ-solvent are required in order to allow the growth of individual microspheres that do not aggregate. A monomer feed ratio of 75:25 % (w/w) of 4-acetoxystyrene and divinylbenzene was selected for all of the precipitation polymerisation reactions. This ratio of AS to crosslinker (DVB) was chosen to ensure that there was an adequate number of pendent acetoxystyrene groups present within the particles for further reaction, and also so that the particles were sufficiently lightly crosslinked with DVB to form swellable polymers.

Table 2.22 shows the quantities of the reactants and solvents used in each of the precipitation polymerisation reactions. The concentration of initiator (AIBN) was kept consistently at 2 mol % with respect to the total number of moles of polymerisable double bonds from the monomers. The choice of solvent was varied across the reactions to determine the most suitable solvent to use for this copolymer system. Acetonitrile was chosen in experiment KA4 as this was the most suitable solvent used for the precipitation polymerisation of poly(VBC-*co*-DVB). For experiment KA6, the solvent was replaced with a 50:50 mixture of *n*-heptane and methyl ethyl ketone (MEK). Butyl acetate was selected as the solvent in experiment KA9 as the theta temperature for poly(AS) in this solvent had been quoted in the literature at 26.8 °C.[116] As 2-ethyl-1-hexanol was utilised as the porogen in the suspension polymerisation reactions of poly(AS-*co*-DVB-*co*-styrene),[113] this was selected as a potential precipitation polymerisation solvent and used in experiment KA10 to deliver insoluble particles in an acceptable yield. Additionally, 2-ethyl-1-hexanol had been selected previously for the hydrocarbon/alcohol organic solvent mixture alongside isooctane for the suspension polymerisation of a poly(styrene-*co*-VP-*co*-DVB) copolymer.[117]

In experiments KA7 and KA8 it was observed that using methanol as a solvent for precipitation polymerisation successfully yielded insoluble polymer particles. Experiments KA16 and KA22 repeated this synthesis using an increased concentration of monomer, increased from 2% (w/v) up to 4% (w/v) relative to the solvent. The increased monomer concentration has notably increased the yield of insoluble polymer particles isolated from the reaction.

When the copolymer did not phase separate in the reaction solvent (samples KA4, KA6 and KA9), the soluble polymer fraction was recovered by precipitating into methanol. The soluble fraction was likely to be low molecular weight oligomers and lightly crosslinked or branched material, but it was useful to isolate for characterisation.

Table 2.22: The reagent quantities and conditions used to prepare poly(AS-*co*-DVB) particles by precipitation polymerisation.

Polymer ref.	DVB (mL)	AS (mL)	AIBN (g)	ACN (mL)	MEK (mL)	Heptane (mL)	BuAc (mL)	2-EH (mL)	MeOH (mL)	Duration (h)	Phase separation?	Yield (g)	Yield (%)
KA4	2.735	7.075	0.27 (x2)	500	-		-	-	-	48+25	no	n/a	n/a
KA6	0.547	1.415	0.05	-	50	50	-	-	-	48	no	n/a	n/a
KA9	0.547	1.415	0.05	-	-	-	100	-	-	47	no	0.38	19
KA10	0.547	1.415	0.05	-	-	-	-	100	-	48	yes	1.26	63
KA7	0.547	1.415	0.05	-	-	-	-	-	100	48	yes	0.53	27
KA8	0.547	1.415	0.05	-	-	-	-	-	100	51	yes	0.69	34
KA16	1.094	2.830	0.10	-	-	-	-	-	100	49	yes	1.97	49
KA22	2.735	7.075	0.27	-	-	-	-	-	250	48	yes	6.6	66

Characterisation of polymers by elemental microanalysis and FT-IR spectroscopy

The composition of the poly(AS-*co*-DVB) copolymers was confirmed by elemental microanalysis and FT-IR spectroscopy.

Elemental microanalysis was performed for the precipitation polymerisation products which successfully phase separated; the data is shown in Table 2.23. There was only a 1.4 % difference between the highest and lowest values of carbon for these samples, so it can be concluded that the chemical composition of these polymers were consistently similar.

Table 2.23: The elemental microanalysis results for the poly(4-acetoxystyrene-*co*-divinylbenzene) particles prepared by precipitation polymerisation

Sample Ref.	KA7	KA8	KA10	KA16	KA22
% C	79.0	79.1	78.5	79.9	79.1
% H	6.7	6.6	6.3	6.8	6.5
% N	Trace	Trace	Trace	Trace	Trace

FT-IR spectroscopy was utilised to confirm the incorporation of the monomers AS and DVB into the poly(AS-*co*-DVB). The FT-IR spectra were studied to look for characteristic signals that indicate the presence of the expected functional groups in the materials.

The FT-IR spectra of the insoluble precipitated polymers KA10, KA7, KA8, KA16, and KA22 listed in Table 2.22, all show bands in the spectra that suggest that that the chemical compositions of the polymers are very similar irrespective of the solvent used in the precipitation polymerisation protocol.

There is a very strong and intense signal at 1765 cm^{-1} that corresponds to the C=O stretch of the acetoxy ester group and is confirmation that the AS monomer has been incorporated successfully into the polymeric products. There is a very intense and broad signal from 1220 to 1170 cm^{-1} that is due to the C-O stretch in the ester. The unreacted, pendent vinyl groups from DVB gave rise to out of plane C-H bending vibrations associated with mono substituted unsaturated C=C bonds at 990 cm^{-1} and 911 cm^{-1}. The presence of these vibrations together with the acetoxy ester and unreacted vinyl groups serves as further confirmation that both AS and DVB have been copolymerised together successfully. The signals at 847 cm^{-1} and 802 cm^{-1} arise from the out of plane C-H bending on the substituted aromatic rings of the monomers, corresponding to 1,4- and 1,3-disubstitution, respectively.

The morphology of poly(AS-co-DVB)

After the completion of the precipitation polymerisation reactions, an aliquot from each reaction mixture was withdrawn and observed under an optical microscope to discern the morphology and appearance of the particles. In the reactions where there was no phase separation (KA4, KA6 and KA9) there were no particles to be observed under the microscope. The remaining reactions all showed successful particle formation, however the particles were aggregated and non-uniform in appearance.

The SEM micrograph of KA7, shown in Figure 2.1, reveals a broad particle size distribution of particles ranging from 0.7 µm to 3.7 µm. There was significant aggregation of the particles, with non-spherical shaped particles present.

Although spherical particles with a narrow particle size distribution were targeted, it was decided that these materials would be suitable for initial experiments to test the chemical properties of the polymers. Optimisation of the precipitation polymerisation procedure may be explored in the future, to improve the quality of the particles.

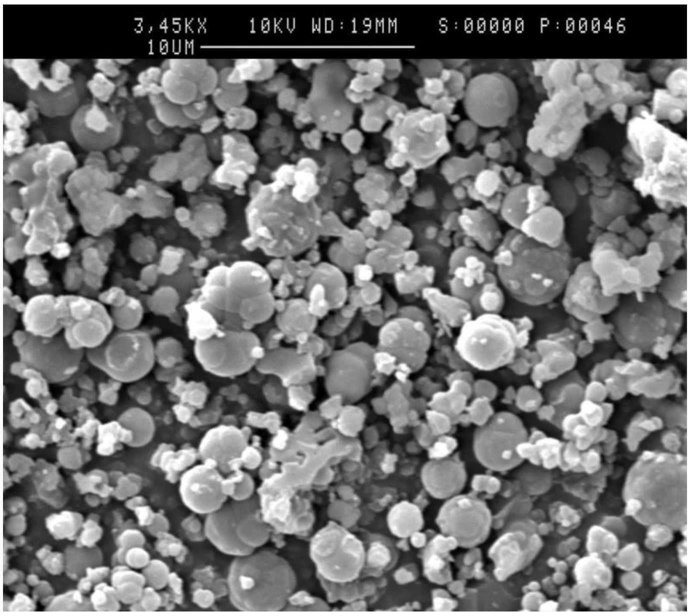


Figure 2.1: An SEM micrograph of poly(AS-*co*-DVB) particles, sample KA7

Synthesis of poly(4-vinylphenol-co-divinylbenzene)

As shown in Scheme 2.8, the acetoxy moieties of poly(AS-*co*-DVB) were deprotected by a hydrolysis reaction to yield poly(VP-*co*-DVB). The experimental protocols for these reactions were adapted from the scientific literature. For the synthesis of sample KA12, the acidic conditions used were described in a protocol by Kawabata *et al.*[110] Basic conditions using potassium hydroxide in an alcohol/aqueous mixture were described by Lewandowski *et al.*,[111] and these conditions formed the protocol for the synthesis of samples KA17 and KA24.

Characterisation of polymers by elemental microanalysis and FT-IR spectroscopy

The change in the chemical composition of the materials on converting the poly(AS-*co*-DVB) copolymers to poly(VP-*co*-DVB) was confirmed by elemental microanalysis and FT-IR spectroscopy. The elemental microanalysis results for the poly(VP-*co*-DVB) materials are displayed in Table 2.24.

Table 2.24: The elemental microanalysis results of poly(AS-*co*-DVB) and poly(VP-*co*-DVB)

poly(AS-*co*-DVB)	% C	% H	% N	poly(VP-*co*-DVB)	% C	% H	% N
KA10	78.5	6.3	Trace	KA12	81.1	6.9	Trace
KA16	79.9	6.8	Trace	KA17	78.1	6.9	Trace
KA22	79.1	6.5	Trace	KA24	68.7	6.1	Trace

There was a 12.4 % difference in the carbon values from the highest value to the lowest value across the three samples prepared. Compared to the close results obtained from the precipitation polymerisation of poly(AS-*co*-DVB), this larger discrepancy suggests that the extent of reaction and the composition of the product is dependent on the hydrolysis protocol and conditions used.

The decrease in the oxygen content of the copolymer upon hydrolysis should result in an increased weight percentage of carbon, and this would be observable in the elemental microanalysis. The hydrolysis of KA10 to KA12 under acidic conditions has resulted in a 2.6 % increase in the carbon content. However, the basic hydrolysis reactions from KA16 to KA17, and KA22 to KA24, have not shown an increase in the % C. One possible explanation for this is that the products contain residual impurities from the potassium hydroxide, methanol or water used in the reaction.

The FT-IR spectra obtained for poly(VP-*co*-DVB) samples KA12, KA17 and KA24 were very similar to that of a commercial linear poly(VP) purchased from Sigma-Aldrich, although the poly(VP-*co*-DVB) samples also have characteristic bands at 990 cm^{-1} and 911 cm^{-1} that correspond to C-H out of plane bending of unreacted DVB alkenes.

The FT-IR spectrum of poly(VP-*co*-DVB) (KA12) is compared to the spectrum of poly(AS-*co*-DVB) (KA10) in Figure 2.3. The chemical structures are presented in Figure 2.2. 2,925 cm^{-1} and 1,506 cm^{-1} bands are present in both the precursor and product, so these signals may be used in the qualitative comparison of the peaks in the spectra. The most distinguishable change in the spectra is the decrease in intensity of the very strong signal at 1,765 cm^{-1} which was attributed to the C=O stretch of the acetoxy ester, therefore this confirms that the ester moieties have been hydrolysed and are not present at the same concentration in KA12. The increase in intensity and broadening of the 3430 cm^{-1} O-H stretch in KA12 versus KA10 is a good indictation that phenolic hydroxy moieties have been incorporated.

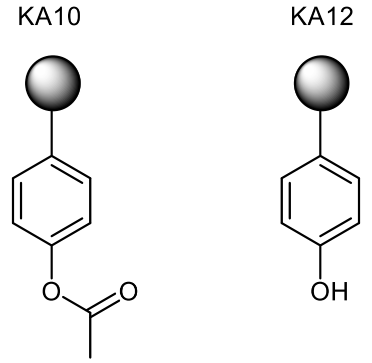


Figure 2.2: The chemical structures of lightly crosslinked polymer particles of poly(AS-*co*-DVB) (KA10) and poly(VP-*co*-DVB) (KA12)

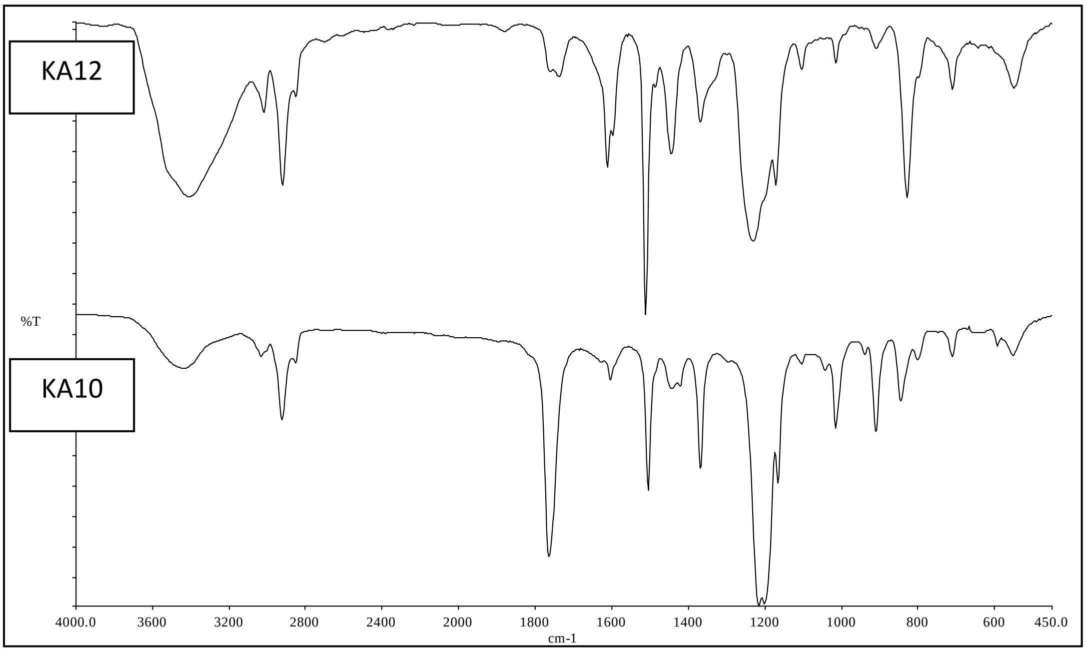


Figure 2.3: FT-IR spectra of poly(AS-*co*-DVB) KA10 particles (bottom) and the corresponding hydrolysed poly(VP-*co*-DVB) KA12 particles (top)

2.3.2. Hypercrosslinking of poly(4-vinylphenol-*co*-divinylbenzene) using formaldehyde

The crosslinking reaction that exists between formaldehyde and phenol has been studied for well over one hundred years. Phenol and formaldehyde are reacted together under either acidic or basic conditions to form crosslinked structures that consist of phenolic rings bridged together by methylene ($-CH_2-$) linkages. These methylene bridges between aromatic rings are also a feature of many traditional hypercrosslinked polystyrene-based polymers prepared by Friedel-Crafts chemistry, typically referred to as Davankov resins.

Previously published work from the University of Strathclyde has utilised poly(DVB-*co*-VBC) spherical particles in Friedel-Crafts type hypercrosslinking procedures to generate hypercrosslinked spherical particles in different size ranges: 50-500 µm particles by suspension polymerisation; 1-10 µm particles by precipitation polymerisation and non-aqueous dispersion polymerisation; and ~ 400 nm particles prepared by emulsion polymerisation.[41-43, 47]

In this chapter, the poly(VP-*co*-DVB) particles prepared in-house by precipitation polymerisation (0.5-4 µm) were subjected to reaction with formaldehyde under basic conditions. The aim of these reactions was to investigate whether it would be possible to form a new class of hypercrosslinked polymers through the crosslinking of the aromatic rings in solvent-expanded poly(4-vinylphenol-*co*-divinylbenzene) particles to generate a 3-dimensional, porous network and materials with increased specific surface areas.

The sodium hydroxide used in these experiments acted as a base to deprotonate the phenol groups in poly(VP-*co*-DVB) to form the phenoxide salt. This charged polymer acts as a polyelectrolyte. The anionic charges will repel each other by coloumbic repulsion, and it is expected that the polymer chains will move, where possible, in such a way where the polymer is in a more expanded state. This is favourable for the proposed hypercrosslinking mechanism because it is desired that the polymer will form crosslinking bridges whilst in an expanded state so as to maximise the porosity and specific surface area of the hypercrosslinked material.

A molar excess of formaldehyde was used relative to the phenol residues of the poly(VP-*co*-DVB). Each phenol ring in poly(VP-*co*-DVB) has a functionality of two, with two available sites for reaction at the positions *ortho*- to the hydroxyl moiety. Both forms of formaldehyde were tried in these experiments – aqueous formaldehyde solution (FS) and polymeric paraformaldehyde (PF). The reaction quantities and conditions explored are summarised and tabulated in Table 2.25.

Table 2.25: Summary of the reaction conditions used to hypercrosslink poly(4-vinylphenol-*co*-divinylbenzene) particles with PF or FS under basic conditions

Sample Ref.	**poly(VP-*co*-DVB) (g)**	**PF (g)**	**FS (g)**	**NaOH (µL, M)**	**Solvent**	**Volume (mL)**	**Duration (h)**	**Temp. (°C)**
KA15	0.16 KA12	0.12	-	100, 14.5	DCE	26	20	75
KA18	0.49 KA17	-	0.97	303, 12.2	ethanol	40	20	75
KA20	0.45 KA17	-	0.83	104, 16.2	ethanol	40	20	75

The solvents chosen for these particular reactions were 1,2-dichloroethane (DCE) and ethanol. DCE was the solvent used in the Friedel-Crafts hypercrosslinking reactions on the polymer microspheres of poly(DVB-*co*-VBC), selected for its ability to swell the microspheres to an expanded state prior to hypercrosslinking.[43] Due to the anhydrous grade of DCE used in the synthesis of KA15, solid paraformaldehyde was used to avoid introducing of water to the reaction. For the synthesis of KA18 and KA20, the polymer particles of poly(VP-*co*-DVB) were treated with aqueous formaldehyde solution in ethanol.

Characterisation of the products by elemental microanalysis and FT-IR spectroscopy

Elemental microanalysis and FT-IR spectroscopy were used to monitor the success of the hypercrosslinking reactions under the conditions tested.

The elemental microanalysis results are presented in Table 2.26. A successful hypercrosslinking reaction would install new C-C bonds between adjacent aromatic rings and lead to an increase in the mass of polymer, and an increase in the percentage carbon content relative to the oxygen content. The percentages of C, H and N in the products were measured and compared to their precursor poly(VP-*co*-DVB) particles. It was found that the carbon and hydrogen content of all of the polymers decreased upon reaction with formaldehyde, which suggests that there must be a higher percentage of other elements present in the samples, *e.g.*, an increased percentage of oxygen, or sodium ions from the phenoxide salt.

Table 2.26: The elemental microanalysis results for poly(VP-*co*-DVB) particles hypercrosslinked with formaldehyde, and the precursor results for comparison

poly(VP-*co*-DVB)	% C	% H	% N	crosslinked product	% C	% H	% N
KA12	81.1	6.9	Trace	KA15	69.5	6.3	Trace
KA17	78.1	6.9	Trace	KA18	73.8	6.6	Trace
				KA20	70.6	6.4	Trace

The inclusion of methylene bridging to the copolymers does not result in any great change in the FT-IR spectra, although small differences should be expected in the regions associated with C-H bonding. The region at 1,480-1,470 cm^{-1} is associated with CH_2 asymmetrical deformations, and an increase in the intensity of this band would be expected. Out of plane C-H bending on the substituted aromatic rings of the monomers can provide information on the level of substitution on the aromatic ring. The strong signal at

830 cm^{-1} arises from 1,4-disubstitution, corresponding to the *para*-substituted VP and DVB moieties connected to the polymer backbone. Changes within the region of the FT-IR spectrum from 900 to 700 cm^{-1} would confirm that there are other substitution patterns present, such as tri- and tetra-substituted aromatic rings from the new C-C bond formation and methylene bridging. A decrease in intensity of the 830 cm^{-1} signal corresponding to 1,2-disubstituted aromatic rings would be expected as methylene bridging ensues.

The FT-IR spectra for products KA15, KA18 and KA20 did not show any observable differences from their precursor poly(VP-*co*-DVB) particles. These results suggest that the formaldehyde did not as a crosslinker to link the aromatic rings by methylene bridging, or at least not at a level sufficient to give an observable change in the FT-IR spectra.

If the crosslinking bridges have not been put into place within the polymer particles, then the rigid structural supports that are necessary to create a permanent 3-dimensional network of pores will not be present. The dry-state porosity of the polymers was measured by nitrogen sorption analysis. The specific surface area of the products was 5 m^2/g, which is tells us that the products were essentially non-porous.

In conclusion, the methods of characterisation used here (elemental microanalysis, FT-IR spectroscopy and nitrogen sorption analysis) all suggest that the hypercrosslinking was unsuccessful. The phenoxide moieties may not be positioned in an easily accessible manner for the formaldehyde, so further experimental optimisation would be required. The solvent choice is an important parameter because the lightly crosslinked particles should be swollen to an expanded state before reaction takes place in order to allow access of formaldehyde to the phenol moieties and, in turn, to impart porosity.

2.3.3. Hypercrosslinking of poly(4-vinylphenol) using formaldehyde

The handling of, and chemical reactions on, insoluble, polymer particles has several practical differences compared to when working with the linear, non-crosslinked and soluble variants. It was thought that the insoluble poly(VP-*co*-DVB) particles studied in Section 2.3.2. had poor compatibility with the solvents tested since it was not possible to identify conditions suitable for a new and successful hypercrosslinking methodology.

Instead of pursuing further experimentation with the insoluble, crosslinked particles of poly(VP-*co*-DVB), it was decided that it would be worthwhile to investigate a linear polymeric alternative. Linear polymers have been used in established hypercrosslinking methodologies,[31] so the commercially available poly(4-vinylphenol) [poly(VP)] was selected as a starting point for this hypercrosslinking investigation.

2.3.3.1. Reaction of poly(4-vinylphenol) with formaldehyde solution

These reactions were performed using linear poly(VP), formaldehyde solution, sodium hydroxide solution and a solvent. Ethanol, distilled water, tetrahydrofuran (THF), 1,2-dichloroethane (DCE) and *N*,*N*-dimethylformamide (DMF) were all tested as the reaction solvent. The experimental conditions used in these reactions are tabulated and presented in Table 2.6, Table 2.7, Table 2.8, Table 2.9 and Table 2.10.

The molar equivalents of formaldehyde and sodium hydroxide were varied relative to the moles of oxygen in poly(VP). Other parameters that were investigated included changes to the solvent, reaction duration, and oil bath temperature. The concentration of the polymer in solution was not kept constant during these preliminary experiments as the initial focus was on finding conditions that led to crosslinked materials.

The reactions performed in distilled water began as clear, golden-coloured solutions, but as the reaction proceeded the solution turned white and cloudy with precipitate, and then a yellow/lime green colour developed, shown in Figure 2.4 for sample KA46. Most of the products had a gel- or slush-like appearance. There was no solid precipitate in the solution for experiment KA49, so the orange solution was freeze dried and a yellow solid was recovered.

Figure 2.4: Product KA46

The change in appearance was monitored across the duration of the reaction for samples KA70 and KA71. Figure 2.5 shows the reaction carousel tubes after the addition of formaldehyde solution (left), after 7 hours of reaction (centre), and then after 24 hours of reaction (right).

Figure 2.5: Products KA70 and KA71

For the ethanol-based reactions, poly(VP) was soluble in ethanol and formaldehyde solution, but a precipitate formed upon addition of sodium hydroxide solution. The precipitate usually turned orange in colour and the solution darkened as the reaction progressed, usually to a yellow or brown colour. The solution in KA29 was clear and colourless; the precipitate is shown in Figure 2.6.

Figure 2.6: Photograph of the product obtained (sample KA29) from the reaction of poly(VP), formaldehyde solution, sodium hydroxide solution and ethanol

The poly(VP) was easily dissolved in THF and formaldehyde solution, but a white precipitate formed when NaOH (aq.) was added to the reaction mixture. The white precipitate changed colour to yellow then orange, and gas was evolved. The product was sticky and viscous, settling to the bottom of the flask and darkening in colour to brown with increasing reaction duration.

Poly(VP) is not soluble in DCE at room temperature, so the polymer was left to stir at 75 °C for one hour to give the polymer an opportunity to swell if it was able to. Upon addition of the formaldehyde solution and NaOH solution, an orange precipitate formed.

As with the other solvents, the polymer dissolves in DMF and a white precipitate formed on addition of NaOH solution. When formaldehyde was added, the precipitate turned orange in colour. After leaving overnight, the solution had turned clear dark red and there was very little solid precipitate present, shown in Figure 2.7. The soluble polymer present in the filtrate precipitated out when washed with ethanol, and this solid was recovered.

Figure 2.7: Photographs of KA41 after the addition of NaOH (left), at the end of the reaction (centre), and of the polymer stuck to the flask after the reaction mixture was removed (right)

Phase separation usually occurred during the course of the reaction, to produce an insoluble precipitate. It was anticipated that this was an indication that a degree of crosslinking had been introduced between the aromatic rings of poly(VP). The reactions with a higher yield of insoluble precipitate were generally regarded as those more likely to have formed a higher degree of crosslinking within any given reaction period. Any soluble polymeric products were likely to have a lower degree of crosslinking between the chains, or be branched.

Characterisation of the products by elemental microanalysis and FT-IR spectroscopy

Elemental microanalysis and FT-IR spectroscopy were utilised to examine the change in composition of the poly(VP) polymer after reaction with formaldehyde solution under the various conditions evaluated.

The precursor poly(VP) materials with M_w of 20,000 and 25,000 had 75.7 % C, 6.6 % H and 76.9 % C and 6.6 % H, respectively. Some of the samples prepared in these reactions with formaldehyde solution were analysed by elemental microanalysis to obtain the relative quantities of carbon and hydrogen within the samples, to compare with the precursor poly(VP).

The results arising from these analyses are tabulated and presented in Table 2.27. The composition of the products shows lower percentages of carbon and hydrogen with respect to their poly(VP) precursor. The remaining composition of the polymer must be composed of other elements. In addition to oxygen, it is likely that there are sodium ions present from the sodium phenoxide salt, and other impurities and moisture from solvents. The sodium ions may be trapped within the crosslinked polymer structures after reaction; therefore these ions may be difficult to remove. An improved washing protocol and clean-up of these materials would be necessary in order to obtain elemental microanalysis results which shed more light upon the success of the reactions.

Table 2.27: The elemental microanalysis results for the experiments involving the hypercrosslinking of poly(VP) with formaldehyde solution under basic conditions.

Sample Ref.	KA23	KA19	KA25	KA26	KA27	KA28	KA29
% C	64.6	60.0	45.3	47.3	33.1	39.0	49.5
% H	6.2	5.7	5.1	5.6	4.7	4.7	5.6

The FT-IR spectra of these polymeric products were compared to the FT-IR spectrum obtained of the commercially available precursor polymer poly(VP) to observe any differences due to reaction with formaldehyde.

The installation of methylene bridging into the polymers will result in small and subtle differences to the FT-IR spectrum regions associated with C-H bonds and variations in the substitution patterns of aromatic rings. The region at 1,480-1,470 cm^{-1} is associated with CH_2 asymmetrical deformations, and an increase in the intensity of this band would be expected. The region from 900-700 cm^{-1} corresponds to the out-of-plane vibrations of the aryl C-H bonds. When the aromatic ring has undergone substitution, and an H atom has been replaced with a CH_2 group, the associated vibrations will change to reflect the positions of the remaining unsubstituted H atoms and therefore provide information on the positions of aromatic substitution. In the FT-IR spectrum of the poly(VP) precursor, there was a very strong signal at 830 cm^{-1} which corresponded to 1,4-disubstitution, fitting with the *para*- orientation of the OH group relative to the aliphatic polymer backbone. A decrease in intensity of the 830 cm^{-1} signal corresponding to 1,2-disubstituted aromatic rings would be expected as methylene bridging ensues. The presence of a peak at 830 cm^{-1}

in the FT-IR spectra of the products prepared in these reactions suggests that there was a substantial quantity of unsubstituted precursor poly(VP) residues remaining.

Out of the reactions performed in ethanol, the spectra for samples KA27 and KA34 showed the most convincing evidence for reaction and substitution on the aromatic rings of the polymer. When compared to the spectrum for poly(VP) in Figure 2.8, the spectrum for KA34 has a strong signal at 1,482 cm^{-1} for CH$_2$ deformations, a new broad signal at 1,061 cm^{-1}, a new signal at 880 cm^{-1} that si evidence of 1,3,4,5-tetrasubstitution on the aromatic ring, and a decrease in the intensity of the peak at 828 cm^{-1} that is evidence of 1,4-disubstitution on the aromatic ring.

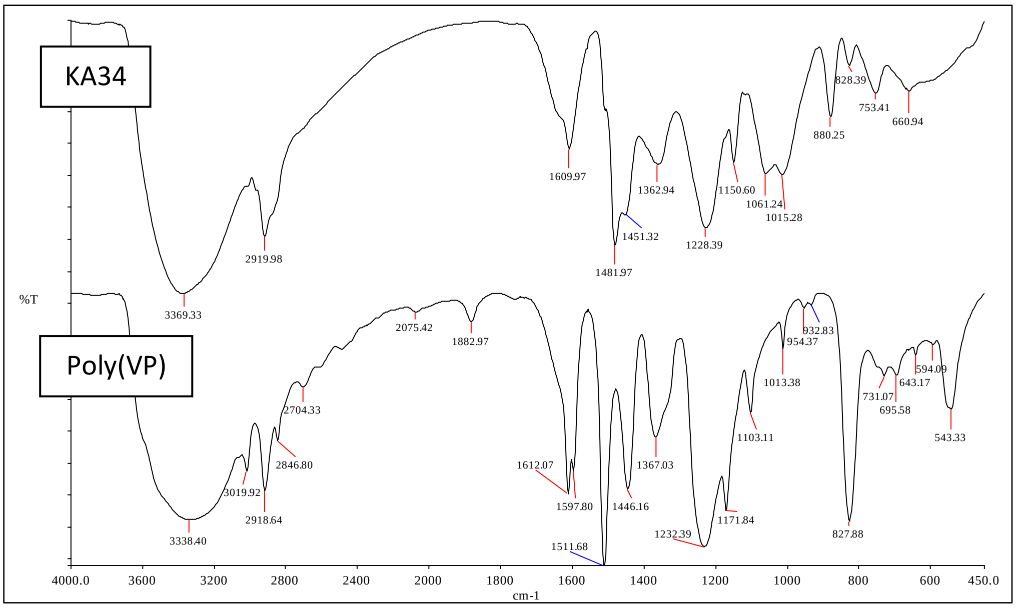


Figure 2.8: FT-IR spectra of KA34 (top) and the poly(VP) precursor (bottom)

The FT-IR spectra of the reaction products obtained from the reactions in THF, DCE and DMF all show low intensity peaks at 880 cm^{-1} that correspond to higher order substitution of the aromatic ring, which is consistent with hypercrosslinking. However, the 1,4-disubstitution signal at 830 cm^{-1} is more intense and dominant in the spectra. Some level of substitution has clearly occurred, but these solvents do not appear to be the most favourable for hypercrosslinking using the experimental conditions tested in these reactions.

Distilled water appears to be the most favourable solvent tested in these reactions between poly(VP) and formaldehyde solution. All of the FT-IR spectra show evidence of additional CH_2 moieties: strong and intense signals from 1,485 to 1,470 cm^{-1}; the appearance of a new broad band at 1,060 to 1,015 cm^{-1}; the appearance of a strong signal at 880 cm^{-1} that is evidence of 1,3,4,5-tetrasubstitution on the aromatic ring; and the disappearance or decrease in intensity of the 1,4-disubstituted signal at 830 cm^{-1}. The FT-IR spectrum for KA46 is shown in Figure 2.9.

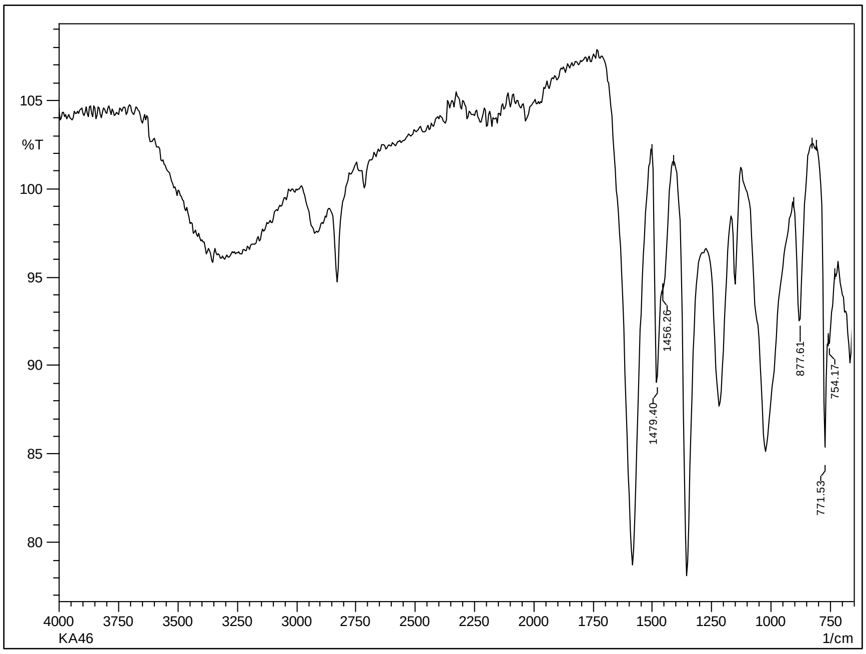


Figure 2.9: The FT-IR spectrum of KA46

Measured porosity of the polymers after reaction with formaldehyde

The dry-state porosity of the commercially available poly(VP) samples were measured by nitrogen sorption porosimetry. As expected for linear polymers of this type, the results in Table 2.28 confirm that these materials do not possess any permanent pore structure (very low specific surface area and pore volume measured).

Table 2.28: The nitrogen sorption porosimetry measurements for the poly(VP) precursors with M_w of 20,000 and 25,000

Sample Ref.	BET specific surface area (m^2/g)	c value	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)
poly(VP) M_w ~ 20,000	< 1	-13	< 1	0.003
poly(VP) M_w ~ 25,000	< 2	-27	< 2	0.005

Hypercrosslinked polymers typically possess ultra-high specific surface areas, often in excess of 1,000 m^2/g due to a high content of micropores. The dry-state porosity of the products isolated from the reactions with formaldehyde solution was measured to determine whether a permanently porous structure has been imparted during the crosslinking of poly(VP).

The reactions that produced a suitable quantity of insoluble material (greater than 300 mg) were analysed by nitrogen sorption analysis. The results show that all of the products obtained from the reactions in ethanol, THF, DMF and DCE do not have a porous structure because the specific surface areas obtained were all under 5 m^2/g. From the discussion of the FT-IR spectra, it was established that the degree of crosslinking may be quite low for these products. A low percentage of crosslinking in the material would result in a network lacking in the rigid supports required to sustain porosity after the removal of solvent. An alternative explanation is that the precursor polymer was not in a solvent-expanded state during the reactions with formaldehyde.

The FT-IR spectra of the products of the reactions performed in distilled water showed a greater level of crosslinking, therefore it was hoped that the nitrogen sorption analyses would show evidence for the formation of a permanent pore structure. Unfortunately, the specific surface areas that were generated from the analyses were all very low. Thus, methylene bridges are being installed into the products but not porosity. These results show that a high degree of crosslinking is not sufficient to form and maintain a permanent pore structure. The polymer needs to be in a suitably expanded and swollen state when the crosslinks are put in place, to create pores. If the polymer is not swollen sufficiently by the reaction solvent then the crosslinking bridges will form an insoluble polymer network that lacks porosity.

The best result achieved was for sample KA46, with a Langmuir specific surface area of 8 m^2/g and a pore volume of 0.02 m^2/g. This experiment was interesting because the reaction mixture was subjected to freeze-drying instead of the conventional vacuum filtration process through a membrane filter followed by drying in a vacuum oven. It was proposed that there may have been porous networks in place within the flask after the reaction, but that they had collapsed during the conventional work-up procedure. It was anticipated that freeze-drying the material would prevent the formation of a vapour-liquid interface which could induce mechanical stress on the pores of the polymer and lead to the collapse of the structure. Although this particular experiment yielded a product with a specific surface area of only 8 m^2/g, this is a route with potential to explore further.

Howdle *et el*. have synthesised macroporous resins that possess reversibly collapsible pore structures. They discovered that the porous morphology of their macroporous resins could collapse if the resins were left in contact with particular solvents before drying, to produce dry resins with a pore structure and specific surface area more akin to gel-type resins. However, this collapse was reversible because the porous morphology could be re-established and fully recovered by contacting the resin with an appropriate solvent and drying again.[118]

Solvent uptake experiments

An important feature of hypercrosslinked polymers is their unusual sorption ability towards thermodynamically good and bad solvents. Solvent uptake experiments were performed for some of the polymer products obtained after reaction with formaldehyde solution to measure the solvent uptake capacity available of the products. A selection of solvents with different polarities should be tested because hypercrosslinked polymers swell in order to relieve the strain of their rigid, highly crosslinked structures, and do not differentiate between good and bad solvents.

Unfortunately, the solvent uptake experiments discussed here are not a good representation of the swelling ability of the materials as only ethanol has been tested on a selection of the materials prepared. Control experiments were run using four centrifugal filters that contained only ethanol, in the absence of polymer. Different quantities of ethanol were retained on the filter top for all four tests; therefore these tests were not reproducible.

Some of the polymer samples were non-wettable; therefore the ethanol remained on top of the polymer sample instead of swelling them. The large increase in mass measured for samples KA26, KA27 and KA29 was not a good indication of their uptake ability. The large increase in mass measured for samples KA35, KA41, and KA42 was thought to be due to the errors associated with these particular experiments, as very low masses of polymer were used. The other polymer samples tested all retained less than 0.5 g of solvent per 1 g of dry polymer.

2.3.3.2. Reaction of poly(4-vinylphenol) with paraformaldehyde

The reaction between poly(VP) and formaldehyde has also been investigated using paraformaldehyde, the solid form of formaldehyde. The reactions were performed in either ethanol or DMF. The experimental conditions used for these reactions are tabulated and presented in Table 2.12 and Table 2.13. The reactions proceeded in a similar fashion to those reactions described in Section 2.3.3.1. using formaldehyde solution.

The solid material that precipitated out from each of the reactions was characterised using FT-IR spectroscopy. The FT-IR spectra for these products show evidence of C-H bonds that have arisen from methylene substitution on the aromatic ring and C-C bond formation. However, the peaks were not as intense as the peaks that correspond to the precursor poly(VP) spectrum. The intense signal at 826 cm^{-1} ascribed to 1,4-disubstituted aromatic rings signifies that there were aromatic rings present in the product which had not undergone substitution.

The level of crosslinking in these products was thought to be too low for the materials to be hypercrosslinked. Nitrogen sorption analysis was used to measure the dry-state porosity of the products obtained from reaction in ethanol (KA14, KA30, KA32 and KA33). All analyses confirmed that the products were non-porous, with specific surface areas less than 5 m^2/g.

2.3.4. Hypercrosslinking of poly(4-vinylphenol) with formaldehyde under microwave irradiation conditions

Based upon the results from the reactions between commercially available poly(VP) and formaldehyde, it was considered that there may be an experimental flaw in the design of the procedure which was limiting the success of these reactions. To try and improve the reaction conditions, and/or eliminate any doubts regarding the reaction protocol set in place, an alternative procedure utilising microwave irradiation was investigated.

The potential benefits of using microwave reactors in a synthetic procedure as an alternative to heating reaction vessels in an oil bath were numerous.[119] It was thought that the sealed reaction vials used inside of a microwave reactor would trap any formaldehyde gas evolved in the reaction that might otherwise have escaped through poorly-sealed joints and outlets that were common to the 3-necked round-bottomed flask apparatus utilised in the earlier reactions. By using microwaves as the source of energy, it was possible to utilise higher reaction temperatures above the standard boiling point of the solvent. This also led to safely utilising an increased pressure within the vial; an internal pressure of about 8-9 bar on some occasions was noted. Owing to these factors described above, it was possible to increase the rate of reaction and therefore decrease the duration of the reaction which would lead to an improved synthetic procedure. A final obvious advantage of utilising microwave irradiation was the ability to control the reaction conditions to a greater degree based upon the programmable functions entered at the beginning of the reaction, thus minimising the experimental errors and temperature fluctuations often present during standard laboratory practice.

Further to these points, it was also observed by S. Zeng *et al*. that they could produce hypercrosslinked hydroquinone-formaldehyde resins with higher specific surface areas when using microwave irradiation rather than conventional thermal heating.[51, 52]

The experimental conditions used are tabulated and presented in Table 2.15 for the reactions in ethanol, and Table 2.16 for the reactions in distilled water.

Paraformaldehyde was used in the experiments performed in ethanol. For reaction KA37, the molar equivalents of the reactants were all chosen to be equal, with a short reaction period of 2 hours and at a temperature of 80 °C which resulted in no increase in pressure inside the reaction vial. The temperature was increased to 120 °C in KA38 with an associated pressure of 8-9 bar, then the molar equivalents of formaldehyde was increased 3-fold in KA39, and finally, in experiment KA40, the reaction duration was increased to 6 hours.

Figure 2.10 shows a series of photographs for KA40. The appearance of the reaction was monitored before the reaction commenced, after the microwave reaction stage and then after removing the excess solvent from the vial. When the reactants were added into the vial they were predominantly insoluble with an off-white and expanded solid formed after stirring the reactants with a magnetic stirrer bar. After removing the vial from the microwave reactor, a brown precipitate had formed at the bottom of the vial around the magnetic stir bar. The clear brown filtrate was removed by decantation and the solid was isolated.

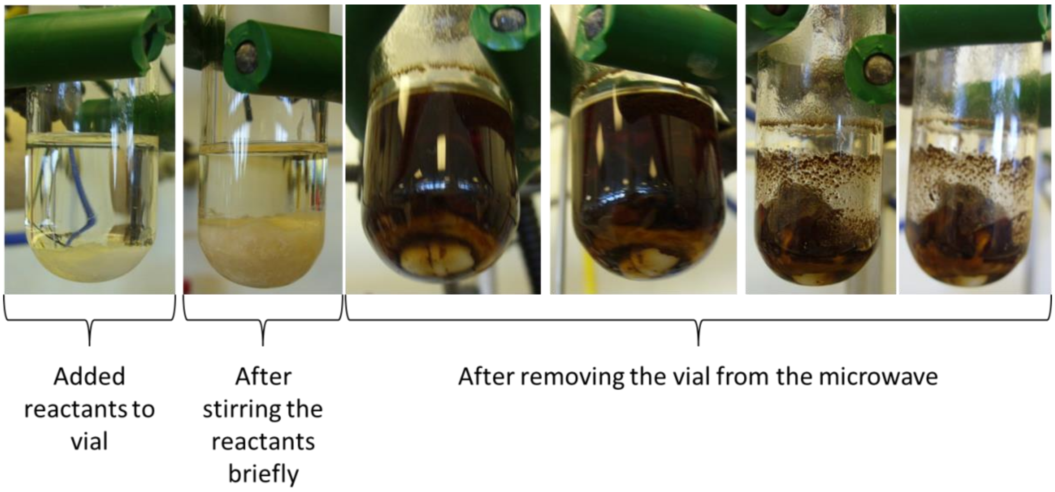


Figure 2.10: Photographs of the reaction vial for KA40 at different stages of the reaction, to monitor the changes in appearance that took place

The reaction performed with distilled water as the solvent used aqueous formaldehyde solution as the source of formaldehyde. A photograph of the microwave vial at the end of the reaction is shown in Figure 2.11. It was discussed in Section 2.3.3.1. that the reaction of poly(VP), formaldehyde solution and NaOH in distilled water resulted in products with a yellow, gel-like appearance and texture. Here, the gel-like material was brown in colour. The solid material of KA47 was isolated by freeze drying, instead of the more conventional filtration and oven drying.

Figure 2.11: Photograph of product KA47 at the end of the reaction

The microwaved samples were analysed by FT-IR spectroscopy to look for peaks associated with crosslinking. Products KA37, KA38, KA39 and KA40 from the reactions performed in ethanol gave rise to FT-IR spectra that resembled very closely the starting material, poly(VP). There were subtle differences and the appearance of some new low intensity peaks, but overall it would indicate that the level of crosslinking in these products was quite low. The formation of a brown, solid precipitate during the reaction would suggest that the material had undergone crosslinking; however that has not been shown unequivocally by this insensitive method of characterisation.

The FT-IR spectrum obtained for sample KA47 prepared in distilled water is shown in Figure 2.12. Compared to the spectra for the products arising from the other microwave reactions, this sample has very clear bands in the regions associated with the proposed crosslinking reaction. There is a peak at 1,472 cm^{-1} corresponding to CH_2 deformations, the appearance of a new broad band at 1,060 cm^{-1}, a strong and defined signal at 875 cm^{-1} that has been assigned as a 1,2,4,6-tetrasubstituted aromatic ring, and an almost complete disappearance of the peak at 825 cm^{-1} that arises from 1,4-disubstitued rings.

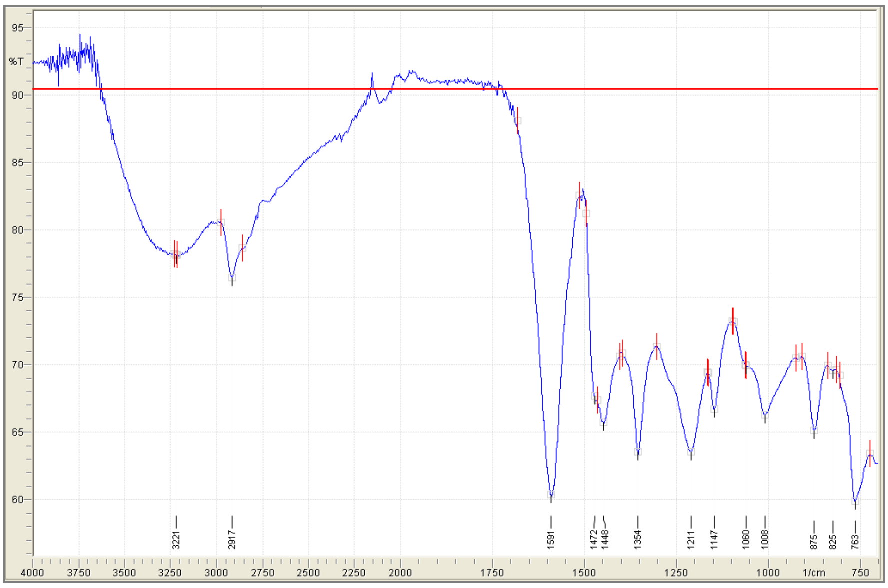


Figure 2.12: The FT-IR spectrum of KA47

Values of < 5 m^2/g were obtained for the specific surface area of the materials, as measured by nitrogen sorption analysis. These values correspond to non-porous materials with non-permanent porosity. The lack of a permanent pore structure suggests that the materials have been hypercrosslinked but not whilst the precursor is in an expanded state.

2.3.5. Hypercrosslinking of poly(4-vinylphenol-*co*-methyl methacrylate) using formaldehyde

The experiments between poly(VP) and formaldehyde have so far been unsuccessful in producing a 3-dimensional crosslinked structure with a permanent pore network in place.

The results from FT-IR spectroscopy indicate that a reaction has taken place to form products that are distinct from the starting materials, and that there has been substitution on the aromatic ring based upon the changes to the arrangement of the aryl C-H bonds. The formation of insoluble, coloured, solid products is also evidence that the poly(VP) precursor has been modified by chemical reaction. The products do not show evidence of any porosity, and it is possible that crosslinking has occurred between neighbouring aromatic rings on the same chain and not between neighbouring aromatic rings on different polymer chains.

The insoluble nature of these materials makes it difficult to differentiate between intramolecular and intermolecular crosslinking. Figure 2.13 shows structures representing two different poly(VP) chains crosslinking together, and intramolecular crosslinking between aromatic groups on the same poly(VP) chain to form a putative 10 atom ring structure, in an imperfect ladder-type structure.

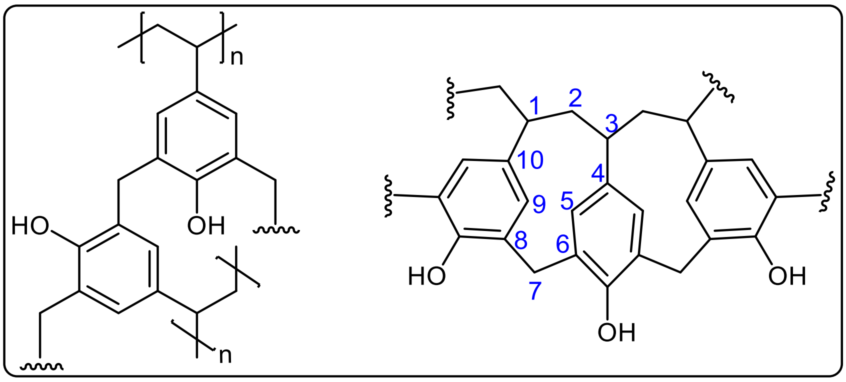


Figure 2.13: Representative structures of poly(VP) forming intermolecular crosslinking bridges with other poly(VP) chains (left), and poly(VP) intramolecularly crosslinking with neighbouring aromatic rings to form an imperfect ladder-type polymer (right)

One method to decrease the probability of the aromatic rings on one poly(VP) chain from reacting with an adjacent aromatic ring on the same chain is to add spacer monomers that are non-reactive under the reaction conditions, and increase the distance between the neighbouring aromatic moieties. The copolymer poly(4-vinylphenol-*co*-methyl methacrylate), shown in Figure 2.14, is commercially available so this was selected because it has pendent vinylphenol moieties that have been spaced by the additional presence of methyl methacrylate (MMA) moieties.

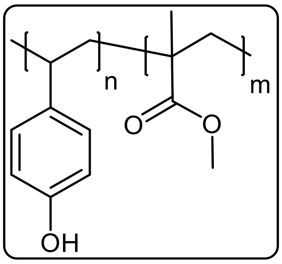


Figure 2.14: The chemical structure of poly(4-vinylphenol-*co*-methyl methacrylate) (poly(VP-*co*-MMA))

The concentration of the copolymer in solution was also varied. There are more polymer chains present in closer proximity at higher concentrations of poly(VP-*co*-MMA) in solvent, so it is more likely that crosslinking would occur between different polymer chains at higher polymer concentrations. At lower concentrations, when there are less copolymer chains in proximity to each other, it is more likely that crosslinking bridges would form between aromatic moieties on the same chain. This idea is illustrated in Figure 2.15.

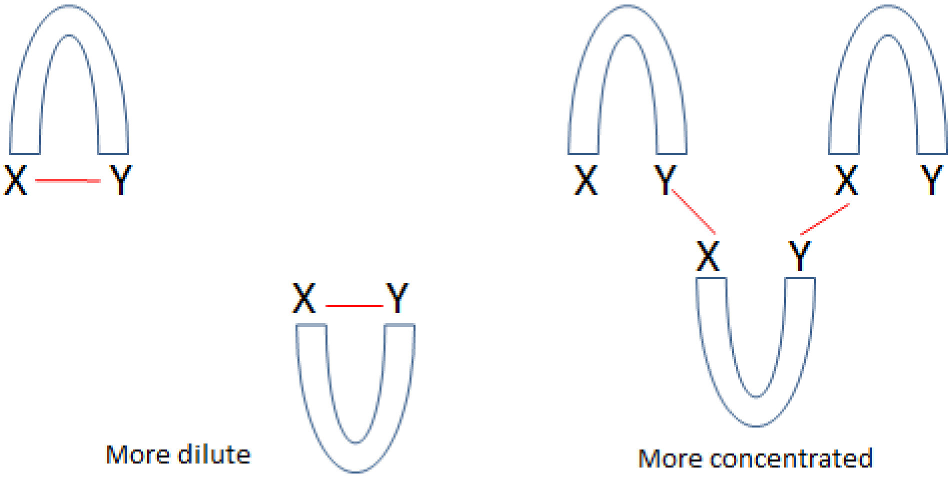
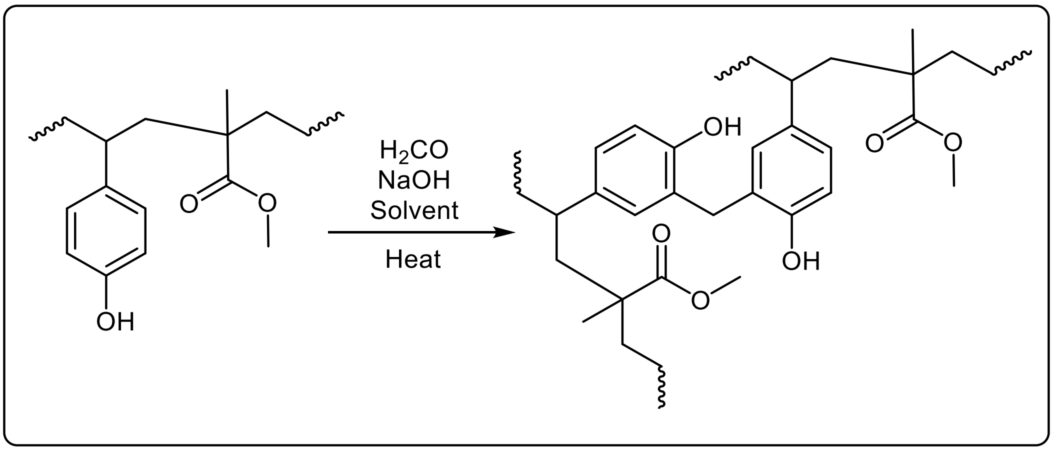


Figure 2.15: Representation of polymer chains at lower and higher concentrations in solution, and the intra- and intermolecular crosslinking, respectively.

Based upon the discussion of the earlier experiments using commercially available poly(VP), it was decided that the most suitable solvents for the reactions with poly(VP-*co*-MMA) would be distilled water and ethanol (Scheme 2.9). Methanol was tested as a reaction solvent in a parallel reaction alongside reactions in ethanol and distilled water, however the methanol reaction did not lead to the precipitation of any solid material so it was abandoned.



Scheme 2.9: The proposed reaction scheme for the hypercrosslinking of poly(VP-*co*-MMA) using formaldehyde under basic conditions

Formaldehyde solution was the source of formaldehyde in all of these experiments. Three different reaction protocols using poly(VP-*co*-MMA) were studied. In Procedure 2-xiv the reactants were all added at once to carousel reaction tubes and left to react, whereas Procedure 2-xv and Procedure 2-xvi had delayed addition of the formaldehyde solution. The reason for the delayed addition of formaldehyde was to determine whether or not it made any difference to the reaction products by allowing the polymer sufficient time to dissolve, expand, and deprotonate to form the reactive phenoxide anion before the formaldehyde was added.

Characterisation of the products by FT-IR spectroscopy

As with the experiments using poly(VP), FT-IR spectroscopy was the primary method of characterisation for these materials. The FT-IR spectrum of the precursor poly(VP-*co*-MMA) shows a C=O stretch at 1,720-1,700 cm^{-1} that corresponds to the carbonyl stretch of the ester moiety. This signal is present in all of the FT-IR spectra obtained for the products, confirming that the ester is present.

Parallel reactions were performed in a Radleys carousel reactor, to monitor the effect of changing parameters on simultaneous experiments.

The concentration of the reactants in solvent was varied, to deduce whether a more concentrated or more dilute solution is optimum for these crosslinking reactions. Following Procedure 2-xiv, a reaction using 20 mL of distilled water was compared to a reaction using only 10 mL of distilled water. The product obtained from the more concentrated solution of

10 mL, KA54, resulted in an improved FT-IR spectrum that showed evidence of a greater extent of crosslinking compared to KA51. Figure 2.16 shows a photograph of the carousel tubes of KA51 and KA54 after 24 hours, at the end of the reaction duration.

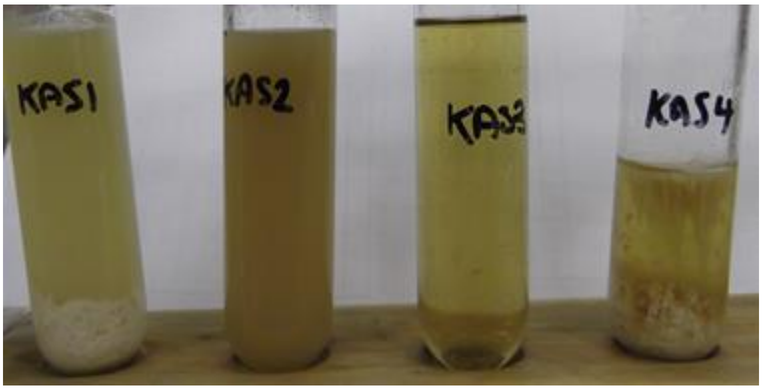


Figure 2.16: Photograph taken at the end of the reaction of KA51, KA52, KA53 and KA54 (left to right, solvents are water, ethanol, methanol and water). KA54 was designed to be a more concentrated experiment of KA51.

Similarly, following the alternative Procedure 2-xv, reactions KA62 and KA63 were performed in 5 mL of distilled water, and comparable reactions KA64 and KA65 were performed in 10 mL of distilled water (Figure 2.17). In both cases, the more concentrated solutions resulted in higher intensity signals at 889-878 cm^{-1} attributed to 1,2,4,6-tetrasubstituted aromatic rings. It appears, by the high intensity 1,4-disubstituted peak at 829 cm^{-1}, that there are more unsubstituted phenol moieties remaining when dilute solutions are used.

These results fit with the hypothesis that the crosslinking reaction is more favourable when there are more aromatic rings in closer proximity to each other.

Figure 2.17: Photograph of the carousel stirring hotplate, and reactions KA62-KA65

In addition to varying the concentration of the polymer in solution by utilising different volumes of solvent, the concentration of sodium hydroxide was also varied. KA63 had a higher concentration of NaOH solution compared to KA62 (620 µL versus 190 µL), and KA65 had a higher concentration of NaOH compared to KA64. The solutions containing a greater quantity of NaOH were noticeably darker in colour, although the colour difference became less apparent as the reaction progressed, as shown in Figure 2.18. The top photograph shows KA62, KA63, KA64 and KA65 prior to adding formaldehyde solution, the centre photograph shows the same reaction tubes after 4.5 hours of heating in the presence of formaldehyde, and the bottom photograph was taken at the end of the reaction after it had cooled and settled.

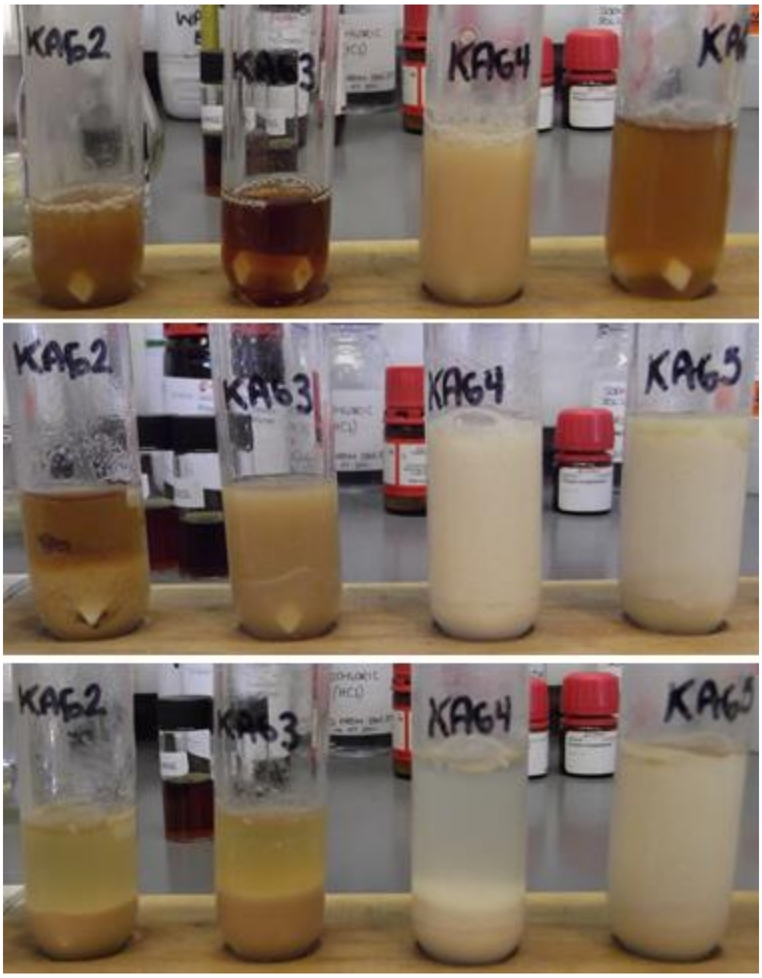


Figure 2.18: Photographs of KA62-KA65 prior to the addition of formaldehyde (top), after 4.5 hours of heating in the presence of formaldehyde (centre), and at the end of the reaction after settled out (bottom).

Four experiments were performed in parallel, with a different concentration of NaOH present in each reaction. The quantity of NaOH solution was increased by 170 µL from 170 µL, to 340 µL, to 520 µL and finally to 690 µL, for samples KA66, KA67, KA68 and KA69, respectively. The FT-IR spectra recorded for these products show that the lowest quantity of sodium hydroxide was not sufficient for adequate crosslinking as the spectrum contains peaks resembling the spectrum of poly(VP-*co*-MMA), however the higher quantities used all gave rise to peaks in the FT-IR spectra that are characteristic of the crosslinking reaction. It was not possible to establish if there was any difference between the experiments using 340 µL or 690 µL NaOH because the spectra were so similar.

It was mentioned previously that the experiment using methanol as a solvent, KA53, did not produce any precipitate by following Procedure 2-xiv. Only a very low yield of solid material was generated from the experiments performed in ethanol. As precipitate readily forms when distilled water was used as the solvent, it was investigated whether an aqueous/alcohol mixture may be used. Experiments KA55 and KA56 were performed in a 50:50 (v/v) mixture of ethanol and distilled water, but the reactions failed to yield any solid precipitate. Distilled water is the preferred solvent for these reactions.

A series of comparable experiments were performed lasting 24 hours (KA73), 73 hours (KA74) and 240 hours (KA75). It was not possible to observe any differences in these products by FT-IR spectroscopy, as the spectra all had similar signals.

Measured porosity of the crosslinked poly(VP-co-MMA) materials

The dry-state porosity of the polymers prepared in this section were measured by nitrogen sorption analysis, to ascertain whether the polymers possessed a permanent pore network. The commercially available precursor poly(VP-*co*-MMA) was non-porous, with a measured specific surface area of < 2 m^2/g. The nitrogen sorption analysis results from the products all showed very low specific surface areas (with the majority < 2 m^2/g) and low pore volumes. The highest specific surface area recorded was 7 m^2/g for sample KA64, with a pore volume of 0.01 cm^3/g. These values are well below the values desired, and it can be concluded that the crosslinking reactions have not managed to successfully impart a permanent pore network within the polymer.

2.4. Conclusions

Particles of poly(4-acetoxystyrene-*co*-divinylbenzene) (poly(AS-*co*-DVB)) were synthesised by precipitation polymerisation in a selection of different solvents, and it was found that 2-ethyl-1-hexanol and methanol were both able to precipitate polymer of poly(AS-*co*-DVB) within the 48 hour reaction duration. The standard monomer concentration of 2% (w/v) in methanol was increased to 4% (w/v) and produced a higher yield of insoluble polymer compared to the lower concentration experiments. It was not possible to synthesise uniform spherical particles of the same particle diameter using any of these reaction conditions, instead the particles were aggregated and possessed a distribution of particle sizes from 0.5 to 4 µm. An improved polymerisation protocol could be developed in the future.

The success of the hydrolysis reaction from poly(AS-*co*-DVB) to poly(4-vinylphenol-*co*-divinylbenzene) (poly(VP-*co*-DVB)) was monitored by FT-IR spectroscopy. The deprotected poly(VP-*co*-DVB) particles were subsequently reacted with formaldehyde with the aim of forming a highly crosslinked, three dimensional porous network with pendent phenolic moieties. Based upon the characterisation results of these materials, there was no evidence to suggest that this reaction had taken place and this set of experiments were set aside for future development.

A commercially available linear polymer, poly(4-vinylphenol) [poly(VP)], was reacted with formaldehyde under a variety of conditions because it was thought that the linear polymer had a greater chance of successfully crosslinking with formaldehyde compared to the in-house prepared particles.

Reaction of poly(VP) with formaldehyde solution in the presence of base was performed in ethanol, distilled water, tetrahydrofuran, 1,2-dichloroethane and *N*,*N*-dimethylformamide. The most promising solvent was distilled water, based upon the changes observed in the FT-IR spectrum of the product versus the poly(VP) precursor. This was a promising finding because water is a cheap and environmentally friendly solvent with low hazards. Although the products were highly crosslinked, with clear spectroscopic evidence of methylene bridging between aromatic rings, this reaction procedure was not sufficient to introduce a permanently porous network (as measured in the dry-state by nitrogen sorption analysis).

A series of reactions were performed that utilised microwave irradiation instead of thermally heating the reaction by conventional oil baths or by a heating mantle. The reaction of poly(VP) with paraformaldehyde in ethanol produced a solid precipitate, but when examined and characterised it was discovered that it lacked porosity and the key spectroscopic signals associated with the desired product. However, the reaction of poly(VP) with formaldehyde solution in distilled water generated a gel-like material that displayed the characteristic FT-IR vibrations of methylene bridging and higher substitution on the aromatic rings. Although there has not been extensive characterisation, the evidence suggests that distilled water leads to improved conditions for the crosslinking of the aromatic rings of poly(VP) using formaldehyde to generate three-dimensional insoluble polymer networks but they are unfortunately lacking in permanent dry-state porosity.

In order to limit intramolecular crosslinking between neighbouring aromatic rings on the same polymer chain that could limit the pore volume of the material, a copolymer variant of poly(VP) was investigated that contains the spacer monomer methyl methacrylate that would be unable to take part in any crosslinking reaction. Linear poly(4-vinylphenol-*co*-methyl methacrylate) was reacted with formaldehyde solution in solvent (distilled water, ethanol, or methanol). Methanol failed to precipitate any solid polymer so this was excluded from the study, and distilled water was preferred to ethanol or an ethanol/water mixture. A series of reactions was performed that varied the volume of distilled water added to the polymer, to alter the concentration of polymer in solution. It was observed that the more concentrated (lower volume) samples achieved higher crosslinking based upon spectroscopic characterisation evidence. The crosslinking of the vinylphenol moieties was successful, however these experiments failed to impart the desired porosity that was targeted and the products were non-porous in the dry-state.

2.5. Future work

It was shown to be possible to crosslink the aromatic rings of linear poly(4-vinylphenol), under basic conditions with formaldehyde solution and distilled water. However the crosslinking procedure failed to impart a permanently porous network that was accessible during dry-state measurements by nitrogen sorption analysis. The aim of this work was to synthesise hypercrosslinked polymers by a new and alternative methodology to the Davankov route, and the materials ought to be both highly crosslinked and porous with ultra-high specific surface areas (typically in the region of 1,000 m^2/g).

The methylene bridges that have formed between the aromatic rings meet the extensive crosslinking criteria, but further work will be required in order to develop materials with ultra-high specific surface areas. Examining the reaction conditions and solvent properties in the reaction is a suggestion for further development, because it is necessary to form the crosslinking bridges between aromatic rings that are distant enough to generate a pore network and void space. Perhaps the crosslinking bridges were formed when the polymer was in a collapsed state, or the level of crosslinking was insufficient to prevent the collapse of the network when the polymers were dried. Freeze-drying the polymers after reaction is a potential route to explore further, as this led to the highest specific surface areas obtained.

The precipitation polymerisation protocol to synthesise poly(4-acetoxystyrene-*co*-divinylbenzene) successfully incorporated both monomers to produce particles bearing the correct chemical functionality, however the physical format of the particles was not ideal because spherical and nearly monodisperse particles were targeted. In precipitation polymerisation, the solvent choice is an important parameter that affects the formation and growth of the polymer particles. It must be the correct solvent in order to solvate the polymer just enough, but not too much. The solvents tested were not the most appropriate solvents for the ratio of monomers used, and therefore this is a key area to study thoroughly. A systematic approach should be employed to test a variety of solvents and draw conclusions based upon the observed particle morphology of the resultant products. Once spherical particles of low size distribution have been synthesised, the particle size may be tailored by altering the duration of polymerisation or by adding co-solvents that alter the solvation properties of the polymer to delay or bring forward the onset of particle formation. The particle size targeted should be targeted based upon the intended application for the materials, but will fall within 0.5 – 10 µm.

The deprotected particles of poly(4-vinylphenol-*co*-divinylbenzene) were reacted with formaldehyde but the experiments failed to show evidence of extensive crosslinking between the aromatic rings from the phenolic moieties. It was anticipated that the spherical particles would be more difficult to functionalise than the linear polymer variants due to the lightly crosslinked network that is distributed throughout the entire diameter of the particle that restricts the movement of the formaldehyde within the particle. A suggestion for future experimentation would be to select the conditions that gave the best results for linear poly(4-vinylphenol) as a starting point, and see if the particles have been altered by reaction through characterisation and analysis.

Chapter 3 – Synthesis of polymer microspheres with functionality derived from amino acid moieties

3.1. Introduction

Functionality can be introduced to polymer microspheres either by the copolymerisation of monomers possessing the desired functional groups, or by the post-polymerisation chemical modification of existing polymers using functional small molecules. In this work, the latter method has been employed to introduce amine containing small molecules to chloromethyl containing polymers by nucleophilic aromatic substitution.

Crosslinked polymers that possess amine moieties within their structure have been applied as anion-exchange materials. Examples published in the literature have documented the reaction of chloromethylated poly(styrene-*co*-DVB), or poly(DVB-*co*-VBC), with diethylenetriamine,[120, 121] ethylenediamine,[101, 122, 123] piperazine,[101] and dimethylamine[124].

In a 1976 publication, Davankov reported that chloromethylated polymers were aminated by reaction with methyl esters of α-amino acids, and the ester group was subsequently hydrolysed for ion-exchange.[33] Sorbents based upon the natural amino acids were investigated for application in ligand-exchange chromatography. Amino acids have been reacted with chloromethylated crosslinked poly(styrene-*co*-DVB) resins in a process to prepare chiral-bonded ligand exchange stationary phases.[125-127]

All DNA-encoded α-amino acids, with the exception of glycine, contain a stereogenic carbon atom that has four different substituents including an amino group, a carboxylic acid group, a hydrogen atom and an R group. The isoelectric point is the pH at which an amino acid is electrically neutral. Simple amino acids that contain only one acid and amine group will form a zwitterionic species at the isoelectric point, when there is both one cationic and one anionic charge. Figure 3.1 shows the changes in ionisation state of polymer resins containing amino acid moieties when subjected to changes in pH.

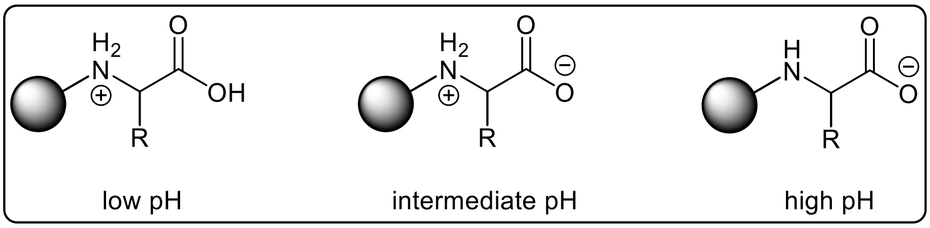


Figure 3.1: The ionisation state of amino acids may be modified according to pH

To utilise enantiomerically pure amino acids immobilised on polymer microspheres as sorbents and stationary phases opens up opportunities for chiral resolution. It should be possible to separate enantiomers in a racemic mixture of compounds based upon their differing affinity for the amino-acid-derived chiral stationary phase.

3.1.1. Aims

The objective of this chapter was to synthesise novel polymeric sorbents in a convenient and attractive microsphere format by the post-polymerisation chemical modification of polymer microspheres with amino acid derived esters. The preparation of polymer microspheres containing amino acid moieties provides a route into exploiting their zwitterionic character for both anionic- and cationic-exchange. The polymeric sorbents are designed for applications as mixed-mode solid-phase extraction sorbents for the selective extraction of organic compounds, an application of which is demonstrated in Chapter 5.

3.2. Experimental

3.2.1. Materials

Divinylbenzene (DVB) (80 % technical grade) and 4-vinylbenzyl chloride (VBC) (≥90 %) were supplied by Sigma-Aldrich (UK) and passed through a short column of alumina (activated, neutral, Brockmann I, supplied by Sigma-Aldrich) to remove inhibitors before use. 2,2’-Azo*bis*(isobutyronitrile) (AIBN) (97 %) was supplied by BDH Lab Supplies (UK) and recrystallized from cold acetone.

Sarcosine ethyl ester hydrochloride (99 %), sarcosine methyl ester hydrochloride (≥97.0 %), *L*-phenylalanine methyl ester hydrochloride (98 %) and *D*-phenylalanine methyl ester hydrochloride (98 %) were supplied by Sigma-Aldrich (UK) and used as received.

Potassium carbonate (K_2CO_3) (anhydrous), potassium hydroxide (KOH) (GPR Rectapur pellets), sodium hydrogen carbonate (Ph. Eur.), and sodium sulfate (anhydrous) were all supplied by VWR International and used as received. Sodium iodide (NaI) (99+ %), ethylenediamine (99 %), hydrochloric acid (36.5-38 %) and acetic acid (glacial, 99.8-100.5 %) were supplied by Sigma-Aldrich.

The solvents acetonitrile (HPLC grade), *N*,*N*-dimethylformamide (DMF) (Chromasolv® Plus grade), dichloromethane (puriss, ≥99 %), 2-methyl-2-butanol (99 %), toluene, ethanol, methanol and acetone were all supplied by Sigma-Aldrich and used as received. Water was double distilled in-house prior to use.

The reagents for the Kaiser test (potassium cyanide (KCN), pyridine, ninhydrin and phenol) were prepared and supplied by Morag Watson of the Jamieson Research Group at the University of Strathclyde.

3.2.2. Equipment and instrumentation

Precipitation polymerisation experiments were carried out using a Stuart Scientific S160 incubator (Surrey, UK) and a Stovall low-profile roller system (NC, USA). Nalgene® plastic bottles were used as the reaction vessels in all experiments.

The prepared samples were dried in a Townson & Mercer Limited (England) vacuum oven set to 70 °C, at ~ 60 mbar pressure.

C, H and N elemental microanalyses were determined using a Perkin Elmer 2400 Series II CHNS Analyser. The results were obtained as a percentage by weight, and are measured as a function of thermal conductivity. The chlorine content was determined by the Schöniger oxygen flask combustion technique, followed by ion chromatography using a Dionex DX-120 instrument. All analyses were performed by Denise Gilmour and Alex Clunie of the University of Strathclyde Microanalysis Service.

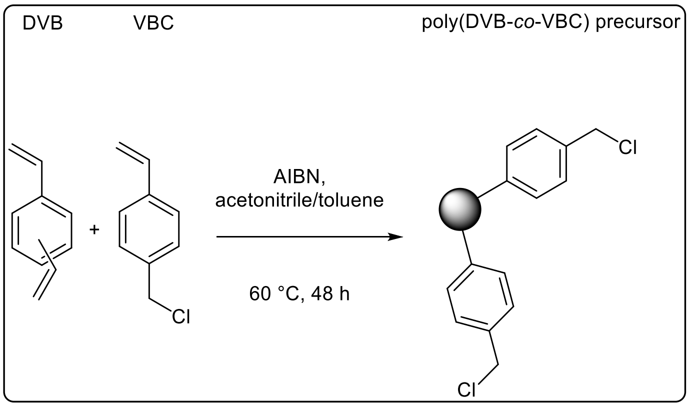
FT-IR spectroscopic analysis was performed on a Shimadzu FT-IR spectrophotometer IRAffinity-1, with a scanning range of 4,000 – 650 cm^{-1} in ATR mode.

The porosity measurements were performed using a Micromeritics ASAP 2000. The samples were degassed under vacuum overnight at 100 °C prior to analysis. The analysis was carried out at – 196 °C *via* nitrogen sorption.

SEM was performed using a Cambridge Instruments Stereoscan 90. All samples were sputter-coated in gold using a Polaron SC500A sputter coater prior to imaging. The mean particle diameter was obtained by manually measuring the diameter of a selection of the particles using iScan2000 imaging acquisition software, and obtaining the mean value.

3.2.3. Typical preparation of poly(DVB-*co*-VBC) microspheres by precipitation polymerisation

The polymer microspheres were synthesised using an adapted precipitation polymerisation protocol,[43] as outlined in Scheme 3.1.



Scheme 3.1: A schematic representation of the polymerisation of monomers DVB and VBC to produce polymer microspheres of poly(DVB-*co*-VBC)

A typical experimental procedure has been given for polymer sample ref. KA122 (Procedure 3-i). All of the poly(DVB-*co*-VBC) copolymers shown in Table 3.1 were synthesised according to an analogous procedure.

Procedure 3-i

The comonomers DVB (7.5052 g, 57.6 mmol) and VBC (2.5154 g, 16.5 mmol), and initiator AIBN (0.3964 g, 2.41 mmol, 2 mol % relative to the polymerisable double bonds) were added to a mixture of acetonitrile (375 mL) and toluene (125 mL) in a Nalgene® bottle (1 L) fitted with a screw cap. The solution was placed in an ultrasonic bath for 10 minutes and then deoxygenated by sparging with N_2 at 0 °C for a further 10 minutes. The bottle was then sealed under N_2 and placed on a low-profile roller in a temperature-controllable incubator. The temperature was ramped from ambient to 60 °C over a period of approximately 2 h and the polymerisation allowed to proceed at 60 °C for a further 46 h.

The resulting milky suspension of white particles were separated from the reaction medium by filtration on a 0.2 µm nylon membrane filter, washed successively with acetonitrile, methanol, toluene and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (5.0866 g, 51 %).

The mean particle diameter was 3.4 µm.

Expected elemental microanalysis: 86.2 % C, 7.5 % H, 0.7 % N and 5.6 % Cl

Found elemental microanalysis: 87.0 % C, 7.6 % H, 0.5 % N and 2.6 % Cl

Estimated ion-exchange capacity (Cl content from elemental microanalysis): 0.7 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3017, 2920, 2853, 1632, 1601, 1555, 1537, 1512, 1485, 1445, 1265 (Ar-CH_2-Cl wag), 1207, 1169, 1115, 1016, 988 (C-H bend, monosubstituted alkene), 970, 905 (C-H bend, monosubstituted alkene), 831 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 710 and 685 (C-Cl stretch).

Langmuir specific surface area: 740 m^2/g

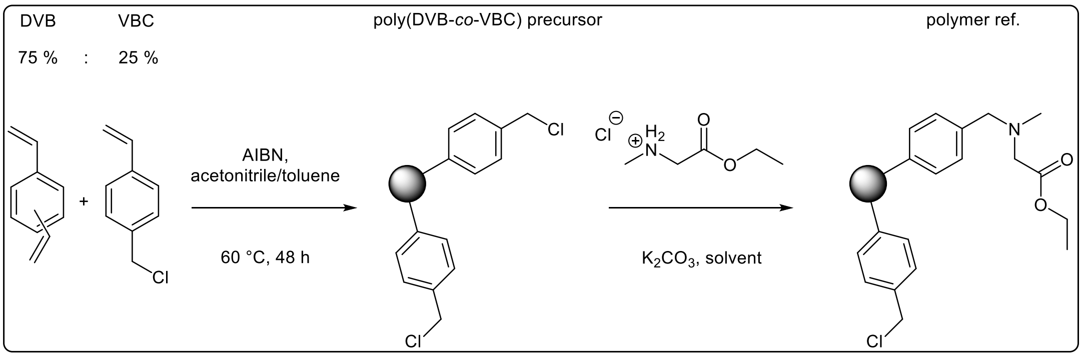
Mean pore diameter: 2.2 nm

Table 3.1: The reagent quantities for the preparation of poly(DVB-*co*-VBC) by precipitation polymerisation

Polymer ref.	DVB (g)	VBC (g)	AIBN (g)	Acetonitrile (mL)	Toluene (mL)	Yield (g)	Yield (%)
KA2	7.5	2.5	0.39	500	-	4.27	43
KA85	7.5	2.5	0.39	500	-	3.94	39
KA112	7.5	2.5	0.39	500	-	5.05	50
KA76	7.5	2.5	0.39	375	125	4.27	43
KA84	7.5	2.5	0.39	375	125	3.88	39
KA122	7.5	2.5	0.39	375	125	5.09	51

3.2.4. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using sarcosine ethyl ester hydrochloride

Polymer microspheres of poly(DVB-*co*-VBC) were modified using sarcosine ethyl ester hydrochloride, as depicted in Scheme 3.2.



Scheme 3.2: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with sarcosine ethyl ester hydrochloride

A typical experimental procedure is given for polymer sample ref. KA132 (Procedure 3-ii). Polymer ref. numbers KA78, KA79, KA80, KA81 and KA104 shown in Table 3.2 were synthesised according to an analogous procedure. A less rigorous washing protocol was used at the time of synthesising these other modified polymers. At a later date, all polymers were subsequently rewashed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

Procedure 3-ii

KA122 (1.5167 g, 0.93 mmol/g of chlorine, 1.41 mmol) and ethanol (40 mL) were added to a three-necked, round-bottomed flask (100 mL) fitted with an overhead mechanical stirrer and a reflux condenser. The contents of the flask were stirred for 1.5 h (for polymer ref. KA132 only). Sarcosine ethyl ester hydrochloride (1.6352 g, 10.64 mmol) and potassium carbonate (1.4918 g, 10.79 mmol) were dissolved in distilled water (40 mL) and added to the flask. The mixture was heated to 75 °C, with stirring at 100 rpm, for 18 h. The reaction mixture was cooled to room temperature and the white particles filtered on a 0.2 μm nylon membrane filter, washed successively with ethanol (reaction solvent), methanol, 1:1

mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (1.5470 g).

The mean particle diameter was 3.2 µm.

Expected microanalysis of KA132: 84.9 % C, 8.1 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA132: 86.0 % C, 8.4 % H, 1.1 % N and 1.4 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.8 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3050, 3017, 2920, 2845, 1738 (C=O stretch, ester), 1730, 1697, 1651, 1632, 1601, 1557, 1537, 1510, 1445, 1418, 1368, 1310, 1265 (Ar-CH$_2$-Cl wag), 1180 (C-O ester stretch), 1117, 1047, 988 (C-H bend, monosubstituted alkene), 903 (C-H bend, monosubstituted alkene), 831 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 710 and 687 (C-Cl stretch).

Langmuir specific surface area: 12 m^2/g

Mean pore diameter: n/a

A typical experimental procedure is given for polymer sample ref. KA106 (Procedure 3-iii). Polymer ref. numbers KA109, KA114, KA115, KA116, KA117, KA118, KA119, KA126 and KA127 shown in Table 3.2 were synthesised according to an analogous procedure.

Procedure 3-iii

KA84 (0.1776 g, 1.10 mmol/g of chlorine, 0.20 mmol), sarcosine ethyl ester hydrochloride (0.1553 g, 1.01 mmol), potassium carbonate (0.1358 g, 0.98 mmol), ethanol (10 mL) and a small quantity of sodium iodide (0.02 g) were added into a carousel reaction tube. The reaction tube was positioned into a carousel equipped with a water-cooled reflux head. The mixture was heated to 70 °C, with stirring using a stirring bar, for 90 h. The reaction mixture was cooled to room temperature and the white particles filtered on a 0.2 µm nylon membrane filter, washed successively with ethanol (reaction solvent) and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.2317 g).

A less rigorous washing protocol was used at the time of synthesising the other modified polymers. At a later date, all polymers were subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 2.9 µm.

Expected microanalysis of KA106: 84.9 % C, 8.1 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA106: 86.3 % C, 7.8 % H, 0.9 % N and 1.1 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.6 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3044, 2970, 2920, 2855, 1732 (C=O stretch, ester), 1601, 1584, 1510, 1485, 1445, 1371, 1263 (Ar-CH$_2$-Cl wag), 1180 (C-O stretch, ester), 1119, 1049, 1018, 989 (C-H bend, monosubstituted alkene), 966, 903 (C-H bend, monosubstituted alkene), 833 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 710 and 689 (C-Cl stretch).

Langmuir specific surface area: < 5 m^2/g

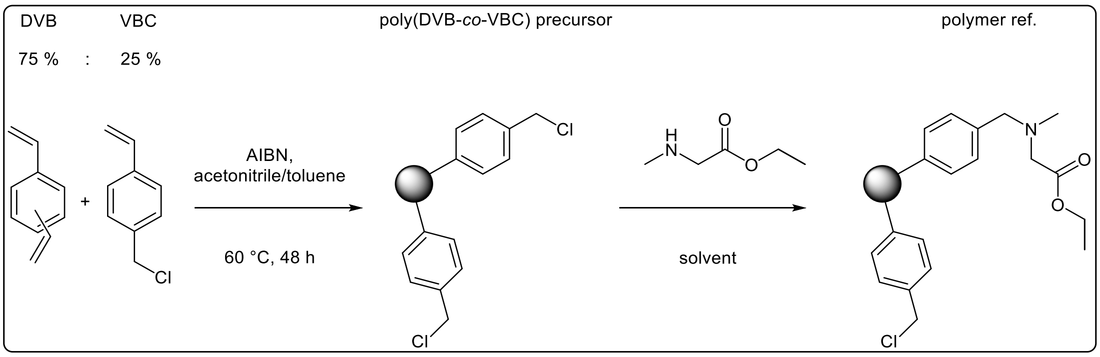
Mean pore diameter: n/a

Table 3.2: Experimental parameters for the post-polymerisation modification of poly(DVB-*co*-VBC) with sarcosine ethyl ester hydrochloride

Polymer ref.	Precursor (g)	Ester (g)	K_2CO_3 (g)	Solvent(s) (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) rewashed	% N (w%)	% Cl (w%)
KA78	0.2561 KA76	0.2876	0.2560	30 DMF	60	5	0.2303	0.1822	1.0	2.4
KA79	0.2514 KA76	0.2890	0.2553	30 ethanol & NaI	75	5.5	0.2463	0.0470	1.3	1.8
KA80	0.2505 KA76	0.2841	0.2586	30 DMF	60	22	0.2447	0.1418	1.0	1.8
KA81	0.2528 KA76	0.2844	0.2566	30 ethanol & NaI	75	21	0.2366	0.0207	1.6	1.3
KA104	1.0094 KA84	0.8545	0.7677	30 DMF	80	5	1.0236	0.9398	1.1	1.8
KA106	0.1776 KA84	0.1553	0.1358	10 ethanol & NaI	70	90	0.2317	0.1135	0.9	1.1
KA109	0.1808 KA84	0.1571	0.1399	10 toluene	70	90	0.3361	0.1591	0.8	3.9
KA114	0.0405 KA84	0.0390	0.0307	5 ethanol & NaI	70	0.167	0.0618	0.0159	0.6	3.5
KA115	0.0417 KA84	0.0375	0.0329	5 ethanol & NaI	70	0.5	0.0602	0.0166	0.5	3.2
KA116	0.0413 KA84	0.0346	0.0337	5 ethanol & NaI	70	1	0.0646	0.0175	0.8	2.9
KA117	0.038 KA84	0.0340	0.0286	5 ethanol & NaI	70	2	0.0656	0.0145	0.9	n/a
KA118	0.0406 KA84	0.0351	0.0308	5 ethanol & NaI	70	21	0.0651	0.0206	1.0	1.7
KA119	0.0436 KA84	0.0402	0.0386	5 ethanol & NaI	70	44	0.0562	0.0158	1.3	1.1
KA126	0.4447 KA112	0.3789	0.3381	10 ethanol	75	5	0.6969	0.4119	1.8	2.1
KA127	0.4490 KA122	0.3797	0.3410	10 ethanol	75	5	0.7020	0.4286	1.3	1.7
KA132	1.5167 KA122	1.6352	1.4918	40 ethanol & 40 water	75	18	n/a	1.5470	1.1	1.4

3.2.5. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using the freebase of sarcosine ethyl ester

Polymer microspheres of poly(DVB-*co*-VBC) were modified using the free base of sarcosine ethyl ester, as illustrated in Scheme 3.3.



Scheme 3.3: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with the freebase of sarcosine ethyl ester

The experimental procedure is given for polymer sample ref. KA99 (Procedure 3-iv). The freebase of sarcosine ethyl ester was prepared prior to the experiment.

Procedure 3-iv

Sarcosine ethyl ester hydrochloride (2.0458 g) was neutralised with an aqueous solution of potassium carbonate (4.0245 g, 29.1 mmol). The sarcosine ethyl ester was extracted three times with dichloromethane. The combined extracts were washed with water and subsequently dried over anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to yield the free base form of sarcosine ethyl ester (KA98) (0.8495 g).

KA85 (0.5209 g, 1.04 mmol/g of chlorine, 0.54 mmol), sarcosine ethyl ester KA98 (0.8191 g) and toluene (30 mL) were added to a three-necked, round-bottomed flask (100 mL) fitted with an overhead mechanical stirrer and a reflux condenser. The mixture was heated to 80 °C, with stirring at 100 rpm, for 24 h. The reaction mixture was cooled to room temperature and the cream/white particles filtered on a 0.2 µm nylon membrane filter, washed successively with toluene (reaction solvent), methanol and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.5342 g).

At a later date, KA99 was subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 3.7 µm.

Expected microanalysis of KA99: 84.9 % C, 8.1 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA99: 86.0 % C, 7.5 % H, 1.4 % N and 2.2 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 1.0 mmol/g

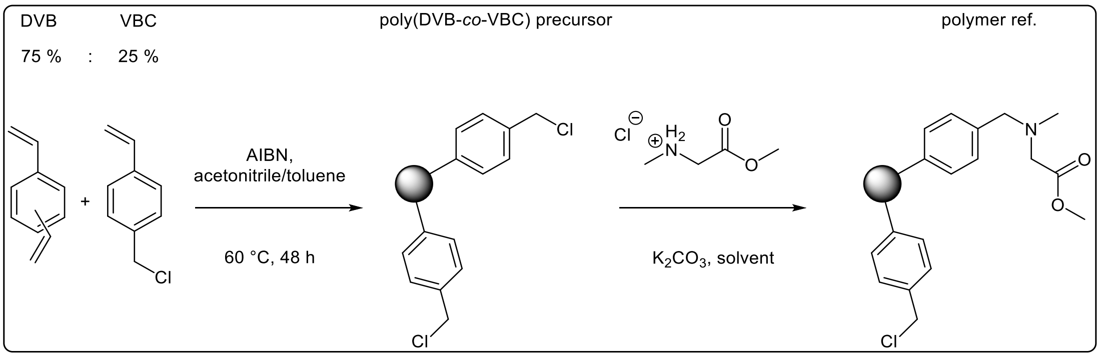
FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3179, 3017, 2918, 2851, 2789, 1734 (C=O stretch, ester), 1701, 1628, 1601, 1560, 1508, 1485, 1445, 1408, 1368, 1356, 1308, 1287, 1265 (Ar-CH$_2$-Cl wag), 1240, 1180 (C-O stretch, ester), 1117, 1096, 1047, 1034, 1018, 989 (C-H bend, monosubstituted alkene), 966, 943, 905 (C-H bend, monosubstituted alkene), 835 (1,4-disubstituted ring), 797 (1,3-disubstituted ring), 750, 710 and 689 (C-Cl stretch).

BET specific surface area: < 5 m^2/g

Mean pore diameter: 4.65 nm

3.2.6. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using sarcosine methyl ester hydrochloride

Polymer microspheres of poly(DVB-*co*-VBC) were modified using sarcosine methyl ester hydrochloride, as outlined in Scheme 3.4.



Scheme 3.4: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with sarcosine methyl ester hydrochloride

A typical experimental procedure is given for polymer sample ref. KA100 (Procedure 3-v). Polymer ref. numbers KA82, KA83 and KA87 shown in Table 3.3 were synthesised according to an analogous procedure.

Procedure 3-v

KA85 (2.0273 g, 1.04 mmol/g of chlorine, 2.11 mmol), sarcosine methyl ester hydrochloride (1.4990 g, 10.7 mmol), potassium carbonate (1.4594 g, 10.6 mmol) and DMF (30 mL) were added to a three-necked, round-bottomed flask (100 mL) fitted with an overhead mechanical stirrer and a reflux condenser. The mixture was heated to 60 °C, with stirring at 100 rpm, for 5 h. The reaction mixture was cooled to room temperature and the white particles filtered on a 0.2 µm nylon membrane filter, washed successively with DMF (reaction solvent), 2-methyl-2-butanol, glacial acetic acid, 2-methyl-2-butanol and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (3.8758 g).

A less rigorous washing protocol was used at the time of synthesising the modified polymers. At a later date, all polymers were subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M) distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 3.2 µm.

Expected microanalysis of KA100: 84.9 % C, 7.9 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA100: 90.2 % C, 7.9 % H, 1.0 % N and 2.0 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.7 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3518, 3005, 2916, 2847, 1755, 1736 (C=O stretch, ester), 1706, 1630, 1601, 1580, 1508, 1485, 1445, 1421, 1356, 1319, 1265 (Ar-CH$_2$-Cl wag), 1171 (C-O stretch, ester), 1115, 1016, 988 (C-H bend, monosubstituted alkene), 899 (C-H bend, monosubstituted alkene), 837 (1,4-disubstituted ring), 826, 795 (1,3-disubstituted ring), 758, 710 and 687 (C-Cl stretch).

BET specific surface area: < 5 m^2/g

Mean pore diameter: 0.47 nm

A typical experimental procedure is given for polymer sample ref. KA107 (Procedure 3-vi). Polymer ref. number KA110 shown in Table 3.3 was synthesised according to an analogous procedure.

Procedure 3-vi

KA84 (0.1761 g, 1.10 mmol/g of chlorine, 0.19 mmol), sarcosine methyl ester hydrochloride (0.1358 g, 0.97 mmol), potassium carbonate (0.1350 g, 0.98 mmol), ethanol (10 mL) and a small quantity of sodium iodide (0.02 g) were added into a carousel reaction tube. The reaction tube was positioned into a Radleys carousel equipped with a water-cooled reflux head. The mixture was heated to 70 °C, with stirring using a stirring bar, for 90 h. The reaction mixture was cooled to room temperature and the white particles filtered on a 0.2 µm nylon membrane filter, washed successively with ethanol (reaction solvent) and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.2493 g).

A less rigorous washing protocol was used at the time of synthesising the modified polymers. At a later date, all polymers were subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 2.6 µm.

Expected microanalysis of KA107: 84.9 % C, 7.9 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA107: 86.0 % C, 7.7 % H, 1.2 % N and 0.9 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.9 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3082, 3046, 3017, 2920, 2845, 1742 (C=O stretch, ester), 1630, 1603, 1585, 1510, 1485, 1445, 1366, 1283, 1265 (Ar-CH$_2$-Cl wag), 1175 (C-O stretch, ester), 1115, 1049, 1018, 989 (C-H bend, monosubstituted alkene), 903 (C-H bend, monosubstituted alkene), 837 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 710 and 689 (C-Cl stretch).

Table 3.3: Experimental parameters for the post-polymerisation modification of poly(DVB-*co*-VBC) with sarcosine methyl ester hydrochloride

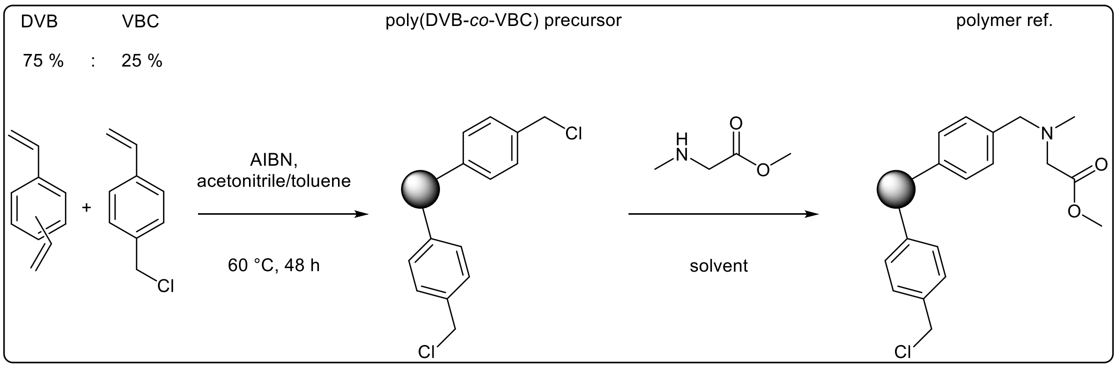
Polymer ref.	Precursor (g)	Ester (g)	K_2CO_3 (g)	Solvent(s) (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) re-washed	% N (w%)	% Cl (w%)
KA82	0.2510 KA76	0.2609	0.2557	30 DMF	60	22.5	0.2400	0.1423	1.3	1.9
KA83	0.2515 KA76	0.2613	0.2582	30 DMF	60	5	0.2494	0.1920	1.1	2.6
KA87	0.2561 KA76	0.2889	0.2736	30 toluene	60	25	0.2336	0.1276	0.8	3.1
KA100	2.0273 KA85	1.4990	1.4594	30 DMF	60	5	3.8758	1.1150	1.0	2.0
KA107	0.1761 KA84	0.1358	0.1350	10 ethanol & NaI	70	90	0.2493	0.1452	1.2	0.9
KA110	0.1785 KA84	0.1392	0.1420	10 toluene	70	90	0.3401	0.1515	0.8	4.8

Table 3.4: Experimental parameters for the post-polymerisation modification of poly(DVB-*co*-VBC) with sarcosine methyl ester (KA95)

Polymer ref.	Precursor (g)	Ester (g)	K_2CO_3 (g)	Solvent(s) (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) re-washed	% N (w%)	% Cl (w%)
KA96	0.4924 KA76	1.01	-	30 toluene	60	24	0.4771	0.4077	0.8	3.6
KA97	0.5003 KA76	1.0052	-	30 toluene	80	24	0.4980	0.4054	1.2	2.4

3.2.7. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using the free base of sarcosine methyl ester

Polymer microspheres of poly(DVB-*co*-VBC) were modified using the free base of sarcosine methyl ester, as outlined in Scheme 3.5.



Scheme 3.5: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with the freebase of sarcosine methyl ester

The experimental procedure has been given for polymer sample ref. KA96 (Procedure 3-vii). Polymer sample ref. KA97 shown in Table 3.4 was synthesised according to an analogous procedure. The free base of sarcosine methyl ester was prepared prior to the experiment.

Procedure 3-vii

Sarcosine methyl ester hydrochloride (10.0661 g) was neutralised with an aqueous solution of potassium carbonate (12.4776 g, 90.3 mmol). The sarcosine methyl ester was extracted twice with dichloromethane. The combined extracts were washed with water and subsequently dried over anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to yield the freebase form of sarcosine methyl ester (KA95) (2.235 g).

KA76 (0.4924 g, 1.46 mmol/g of chlorine, 0.72 mmol), sarcosine methyl ester KA95 (1.01 g) and toluene (30 mL) were added to a three-necked, round-bottomed flask (100 mL) fitted with an overhead mechanical stirrer and a reflux condenser. The mixture was heated to 60 °C, with stirring at 100 rpm, for 24 h. The reaction mixture was cooled to room temperature and the white particles filtered on a 0.2 µm nylon membrane filter, washed successively with toluene (reaction solvent), methanol and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.4771 g).

A less rigorous washing protocol was used at the time of synthesising the modified polymers. At a later date, all polymers were subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 6.8 µm.

Expected microanalysis of KA96: 84.9 % C, 7.9 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA96: 86.2 % C, 7.4 % H, 0.8 % N and 3.6 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.6 mmol/g

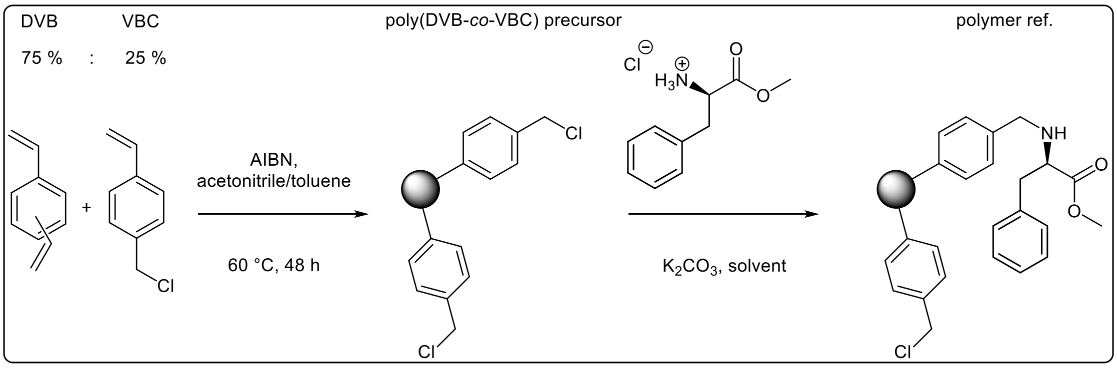
FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3646, 3084, 2980, 2918, 2857, 1738, 1732 (C=O stretch, ester), 1694, 1682, 1628, 1601, 1557, 1510, 1485, 1445, 1371, 1265 (Ar-CH_2-Cl wag), 1242, 1169 (C-O stretch, ester), 1117, 1016, 988 (C-H bend, monosubstituted alkene), 966, 903 (C-H bend, monosubstituted alkene), 893, 831 (1,4-disubstituted ring), 795 (1,3-disubstututed ring), 750, 710 and 687 (C-Cl stretch).

BET specific surface area: < 5 m^2/g

Mean pore diameter: 0.56 nm

3.2.8. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using *L*-phenylalanine methyl ester hydrochloride

Polymer microspheres of poly(DVB-*co*-VBC) were modified using *L*-phenylalanine methyl ester hydrochloride as outlined in Scheme 3.6.



Scheme 3.6: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with *L*-phenylalanine methyl ester hydrochloride

A typical experimental procedure has been given for polymer sample ref. KA105 (Procedure 3-viii).

Procedure 3-viii

KA84 (1.0006 g, 1.10 mmol/g of chlorine, 1.10 mmol), *L*-phenylalanine methyl ester hydrochloride (1.1840 g, 5.49 mmol), potassium carbonate (0.7621 g, 5.51 mmol) and DMF (30 mL) were added to a three-necked, round-bottomed flask (100 mL) fitted with an overhead mechanical stirrer and a reflux condenser. The mixture was heated to 80 °C, with stirring at 100 rpm, for 5 h. The reaction mixture was cooled to room temperature and the creamy/white particles filtered on a 0.2 µm nylon membrane filter, washed successively with DMF (reaction solvent), distilled water and methanol, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.9978 g).

At a later date, KA105 was subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 2.7 µm.

Expected microanalysis of KA105: 85.8 % C, 7.7 % H, 2.4 % N and 0 % Cl

Found microanalysis of KA105: 86.6 % C, 7.5 % H, 0.6 % N and 3.0 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.4 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3831, 3086, 3019, 2922, 2845, 2808, 2569, 2548, 2438, 2207, 2091, 1732 (C=O stretch, ester), 1682, 1603, 1585, 1510, 1487, 1444, 1408, 1312, 1265 (Ar-CH_2-Cl wag), 1200, 1171 (C-O stretch, ester), 1115, 1016, 988 (C-H bend, monosubstituted alkene), 903 (C-H bend, monosubstituted alkene), 829 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 710 and 687 (C-Cl stretch).

Langmuir specific surface area: 413 m^2/g

Mean pore diameter: 1.82 nm

A typical experimental procedure is given for polymer sample ref. KA108 (Procedure 3-ix). Polymer ref. numbers KA111, KA128 and KA129 shown in Table 3.3 were synthesised according to an analogous procedure.

Procedure 3-ix

KA84 (0.1817 g, 1.10 mmol/g of chlorine, 0.20 mmol), *L*-phenylalanine methyl ester hydrochloride (0.2225 g, 1.03 mmol), potassium carbonate (0.1409 g, 1.02 mmol), ethanol (10 mL) and a small quantity of sodium iodide (0.02 g) were added into a carousel reaction tube. The reaction tube was positioned into a Radleys carousel equipped with a water-cooled reflux head. The mixture was heated to 70 °C, with stirring using a stirring bar, for 90 h. The reaction mixture was cooled to room temperature and the cream/white particles filtered on a 0.2 µm nylon membrane filter, washed successively with ethanol (reaction solvent) and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.2653 g).

A less rigorous washing protocol was used at the time of synthesising the modified polymers. At a later date, all polymers were subsequently re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

The mean particle diameter was 2.7 µm.

Expected microanalysis of KA108: 85.8 % C, 7.7 % H, 2.4 % N and 0 % Cl

Found microanalysis of KA108: 85.4 % C, 7.4 % H, 0.8 % N and 3.6 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.6 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3015, 2920, 2853, 1734 (C=O stretch, ester), 1701, 1630, 1603, 1587, 1510, 1485, 1445, 1422, 1265 (Ar-CH$_2$-Cl wag), 1169 (C-O stretch, ester), 1117, 1034, 1016, 989 (C-H bend, monosubstituted alkene), 905 (C-H bend, monosubstituted alkene), 833 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 750, 708 and 689 (C-Cl stretch).

BET specific surface area: 6 m^2/g

Mean pore diameter: n/a

Table 3.5: Experimental parameters for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with L-phenylalanine methyl ester hydrochloride

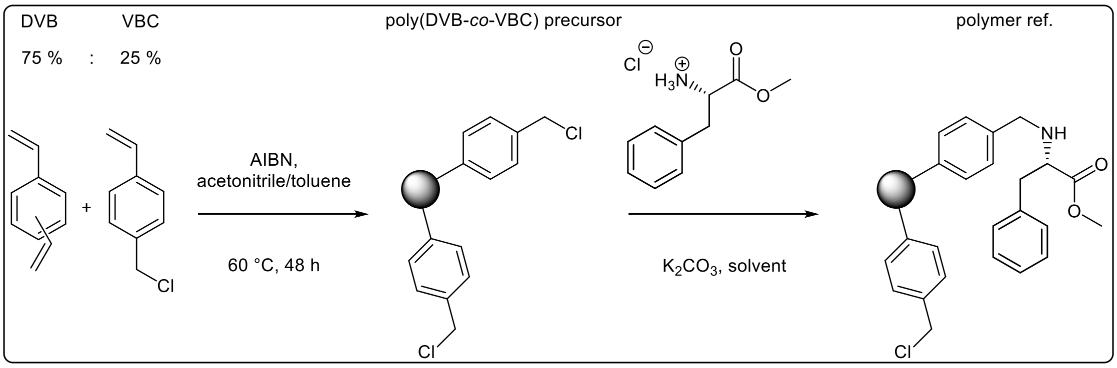
Polymer ref.	Precursor (g)	Ester (g)	K_2CO_3 (g)	Solvent(s) (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) re-washed	% N (w%)	% Cl (w%)
KA105	1.0006 KA84	1.1840	0.7621	30 DMF	80	5	0.9978	0.8129	0.6	3.0
KA108	0.1817 KA84	0.2225	0.1409	10 ethanol & NaI	70	90	0.2653	0.0734	0.8	3.6
KA111	0.1792 KA84	0.2232	0.1370	10 toluene	70	90	0.3998	0.1552	1.1	3.1
KA128	0.4445 KA112	0.5279	0.3392	10 ethanol	75	5	0.7331	0.3979	0.7	2.8
KA129	0.4431 KA122	0.5250	0.3373	10 ethanol	75	5	0.7158	0.4205	0.5	2.7

Table 3.6: Experimental parameters for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with D-phenylalanine methyl ester hydrochloride

Polymer ref.	Precursor (g)	Ester (g)	K_2CO_3 (g)	Solvent(s) (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) re-washed	% N (w%)	% Cl (w%)
KA130	0.4487 KA112	0.5366	0.3418	10 ethanol	75	5	0.7303	0.4120	0.6	2.7
KA131	0.4454 KA122	0.5292	0.3393	10 ethanol	75	5	0.7289	0.4354	0.6	2.5

3.2.9. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using *D*-phenylalanine methyl ester hydrochloride

Polymer microspheres of poly(DVB-*co*-VBC) were modified using *D*-phenylalanine methyl ester hydrochloride as outlined in Scheme 3.7.



Scheme 3.7: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with *D*-phenylalanine methyl ester hydrochloride

The experimental procedure is given for polymer sample ref. KA130 (Procedure 3-x). Polymer sample ref. KA131 shown in Table 3.6 was synthesised according to an analogous procedure.

Procedure 3-x

KA112 (0.4487 g, 1.10 mmol/g of chlorine, 0.49 mmol), *D*-phenylalanine methyl ester hydrochloride (0.5366 g, 2.49 mmol), potassium carbonate (0.3418 g, 2.47 mmol) and ethanol (10 mL) were added into a carousel reaction tube. The reaction tube was positioned into a Radleys carousel equipped with a water-cooled reflux head. The mixture was heated to 75 °C, with stirring using a stirring bar, for 5 h. The reaction mixture was cooled to room temperature and the cream/white particles filtered on a 0.2 µm nylon membrane filter, washed successively with ethanol (reaction solvent), methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.4120 g).

The mean particle diameter was 3.9 µm.

Expected microanalysis of KA130: 85.8 % C, 7.7 % H, 2.3 % N and 0 % Cl

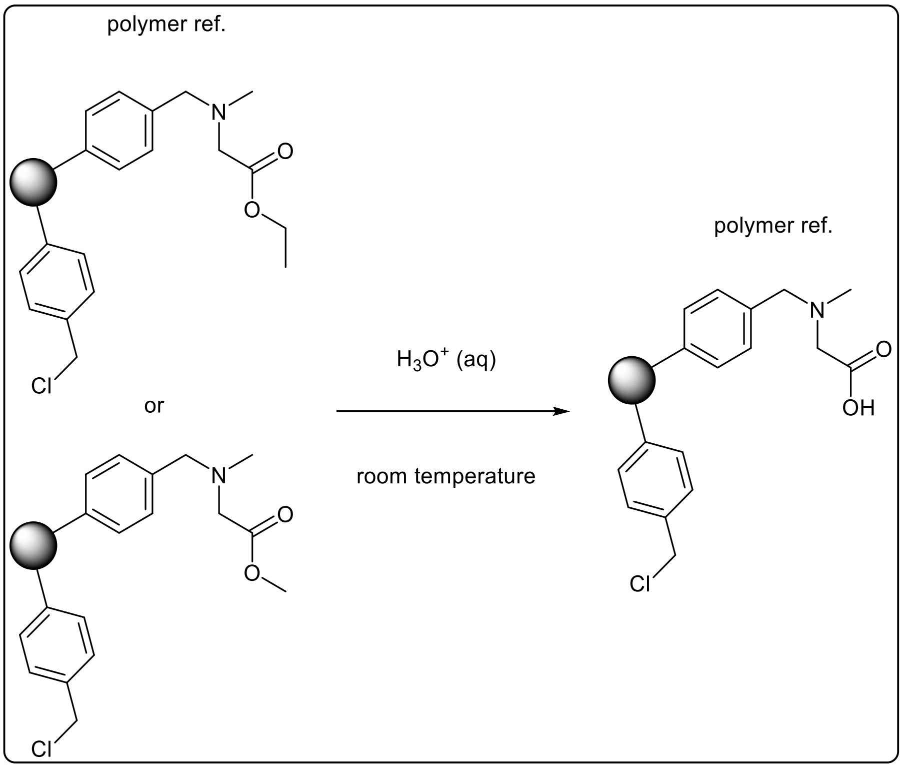
Found microanalysis of KA130: 85.7 % C, 7.5 % H, 0.6 % N and 2.7 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.4 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3607, 3127, 3046, 3011, 2918, 2851, 1732 (C=O stretch, ester), 1695, 1653, 1628, 1601, 1558, 1531, 1510, 1485, 1445, 1410, 1265 (Ar-CH_2-Cl wag), 1209, 1169 (C-O stretch, ester), 115, 1076, 1067, 1016, 989 (C-H bend, monosubstituted alkene), 966, 901 (C-H bend, monosubstituted alkene), 827 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 748, 708 and 685 (C-Cl stretch).

3.2.10. Ester hydrolysis of the polymers to the amphoteric amino acid form

The ester-functionalised polymer microspheres were further modified by acid-catalysed ester hydrolysis in order to form the carboxylic acid, as outlined in Scheme 3.8.



Scheme 3.8: Reaction scheme for the acid catalysed ester hydrolysis of the polymer-bound methyl or ethyl ester to the carboxylic acid

The experimental procedure is given for polymer sample ref. KA86 (Procedure 3-xi). Polymer ref. numbers KA88, KA89, KA90 and KA91 shown in Table 3.7 were synthesised according to an analogous procedure.

Procedure 3-xi

KA81 (0.1047 g) was added to a flask followed by distilled water (10 mL) and aqueous HCl (5 mL, 1 M). The contents of the flask were agitated gently and left for 30 minutes. The particles were filtered on a 0.2 µm nylon membrane filter, washed thoroughly with distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.0907 g).

The mean particle diameter was 7.4 µm.

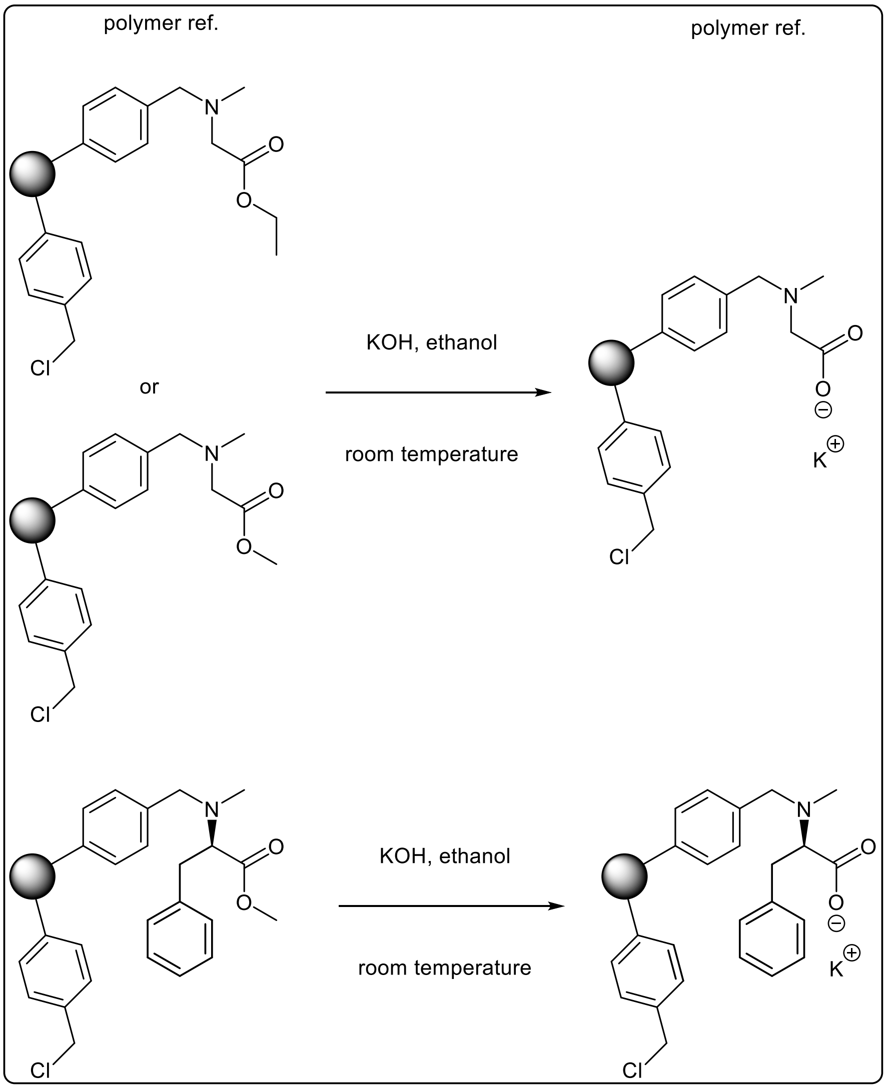
Expected microanalysis of KA86: 84.9 % C, 8.1 % H, 2.6 % N and 0 % Cl

Found microanalysis of KA86: 85.6 % C, 7.7 % H, 1.4 % N and 1.2 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 1.0 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3082, 3044, 2916, 2849, 1738 (C=O stretch, ester), 1730, 1694, 1680, 1632, 1603, 1555, 1537, 1510, 1485, 1445, 1368, 1306, 1263 (Ar-CH_2-Cl wag), 1238, 1179 (C-O stretch, ester), 1115, 1045, 1018, 988 (C-H bend, monosubstituted alkene), 903 (C-H bend, monosubstituted alkene), 889, 829 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 708 and 689 (C-Cl stretch).

The ester functionalised polymer microspheres were also modified by base-catalysed ester hydrolysis in order to form the carboxylate anion, as outlined in Scheme 3.9.



Scheme 3.9: Reaction scheme for the base-catalysed ester hydrolysis of the methyl or ethyl ester on the polymer microspheres to the carboxylate anion

The experimental procedure is given for polymer sample ref. KA133 (Procedure 3-xii). Polymer ref. numbers KA101, KA120, KA121 and KA125 shown in Table 3.7 were synthesised according to an analogous procedure.

Procedure 3-xii

A potassium hydroxide solution was prepared by dissolving KOH (1.0685 g) in ethanol (20 mL). KA132 (0.6423 g) and the KOH solution were added to a sealed glass Kimax tube and agitated gently on a Stovall low-profile roller within a temperature controllable incubator at room temperature for 31 hours. The particles were filtered on a 0.2 µm nylon membrane filter, washed thoroughly with ethanol, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.6325 g).

The mean particle diameter was 3.3 µm.

Found microanalysis of KA133: 79.9 % C, 8.2 % H, 1.1 % N and 1.2 % Cl.

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.8 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3044, 3017, 2918, 2853, 2776, 1751, 1701, 1655, 1597 (asymmetric –CO_2^- stretch), 1560, 1508, 1485, 1443, 1406 (symmetric –CO_2^- stretch), 1364, 1314, 1265 (Ar-CH_2-Cl wag), 1180 (C-O stretch), 1171, 1117, 1016, 989 (C-H bend, monosubstituted alkene), 903 (C-H bend, monosubstituted alkene), 829 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 710 and 687 (C-Cl stretch).

Langmuir specific surface area: 605 m^2/g

Mean pore diameter: 2.03 nm

All other polymers listed in Table 3.7 were re-washed with an additional washing protocol of methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate (0.5 M), distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar.

Table 3.7: Experimental parameters for the hydrolysis of the ester moieties

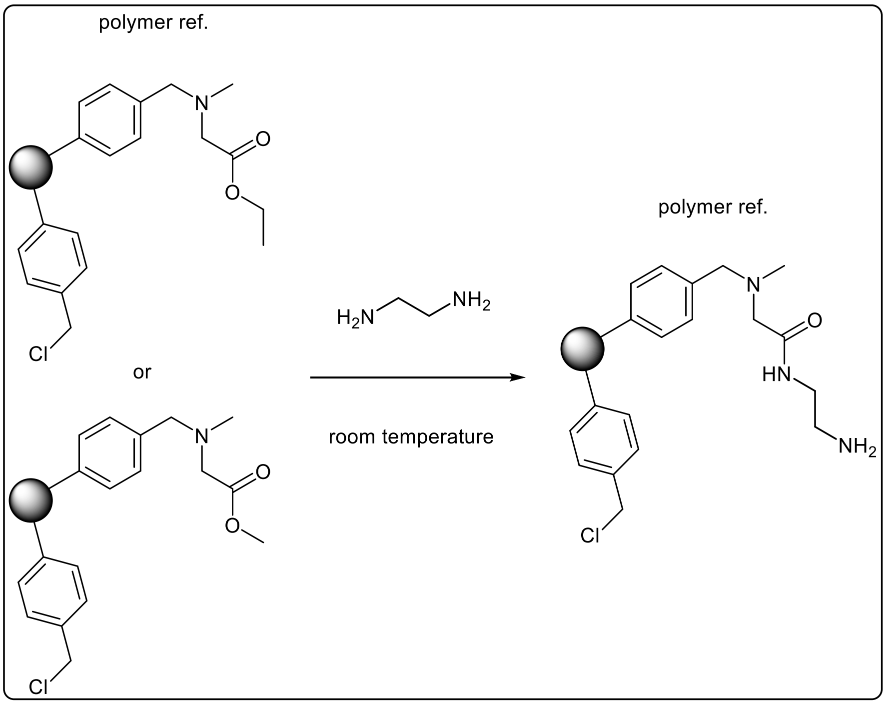
Polymer ref.	Precursor (g)	KOH (g)	HCl 1M (mL)	Solvent(s) (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) re-washed	% N (w%)	% Cl (w%)
KA86	0.1047 KA81	-	5	10 distilled water	RT	0.5	0.0907	0.0814	1.4	1.2
KA88	0.0590 KA80	-	30	-	30	22	0.0498	0.0159	1.2	1.7
KA89	0.0558 KA81	-	30	-	30	22	0.0407	0.0341	1.5	1.2
KA90	0.0573 KA82	-	30	-	30	22	0.0442	0.0369	1.0	1.8
KA91	0.0545 KA87	-	30	-	30	22	0.0480	0.0416	0.7	2.6
KA101	1.0070 KA100	0.5010	-	10 ethanol	RT	66	0.6480	0.4650	1.1	1.8
KA120	0.1048 KA105	0.5155	-	10 ethanol	RT	51	0.1001	0.0906	0.7	2.2
KA121	0.1129 KA108	0.5155	-	10 ethanol	RT	51	0.1008	0.0674	1.1	1.0
KA125	0.0499 KA79	0.2129	-	10 ethanol	RT	24	0.0390	0.0229	1.3	0.9
KA133	0.6423 KA132	1.0685	-	20 ethanol	RT	31	N/A	0.6325	1.1	1.2

Table 3.8: Experimental parameters for the preparation of ethylenediamine modified polymers

Polymer ref.	Precursor (g)	ethylenediamine (mL)	Temp. (°C)	Duration (h)	Yield (g) initial	Yield (g) re-washed	% N (w%)	% Cl (w%)
KA102	0.5453 KA100	40	RT	64	0.3820	0.2172	2.7	1.6
KA124	0.0828 KA79	10	RT	24	0.0537	0.0393	2.2	0.4

3.2.11. Reaction of the amino acid derived esters with ethylenediamine

Ethylenediamine was introduced to the polymer microspheres that had been functionalised with sarcosine methyl or ethyl esters, as shown in Scheme 3.10.



Scheme 3.10: Reaction scheme for the reaction of ethylenediamine with the methyl and ethyl ester moieties on the polymer microspheres

The experimental procedure is given for polymer sample ref. KA102 (Procedure 3-xiii). Polymer ref. number KA124 shown in Table 3.8 was synthesised according to an analogous procedure.

Procedure 3-xiii

KA100 (0.5453 g) and ethylenediamine (40 mL) were added to a flask and agitated gently at room temperature for 64 hours. The particles were filtered on a 0.2 µm nylon membrane filter, washed successively with DMF, 2-methyl-2-butanol, glacial acetic acid, 2-methyl-2-butanol, acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (0.3820 g).

The mean particle diameter was 2.9 µm.

Expected microanalysis of KA102: 83.3 % C, 8.2 % H, 6.4 % N and 0 % Cl

Found microanalysis of KA102: 84.7 % C, 7.7 % H, 2.7 % N and 1.6 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 1.9 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3154, 3086, 3019, 2920, 2911, 2853, 2018, 1948, 1734 (C=O stretch, ester), 1701, 1601, 1541, 1508, 1485, 1443, 1368, 1300, 1265 (Ar-CH_2-Cl wag), 1171 (C-O stretch), 1117, 1016, 989 (C-H bend, monosubstituted alkene), 899 (C-H bend, monsubstituted alkene), 835 (1,4-disubstituted ring), 795 (1,3-disubstituted ring), 741, 710 and 689 (C-Cl stretch).

Kaiser test with ninhydrin

The Kaiser test solutions (A, B and C) were prepared and provided by Morag Watson of the Jamieson Research Group at the University of Strathclyde, as shown in Figure 3.2.

A. 100 µL of KCN solution (16.5 mg of KCN in 25 mL of double distilled water) in 4.9 mL of pyridine
B. 100 mg of ninhydrin in 2 mL of ethanol
C. 4 g of phenol in 2 mL of ethanol

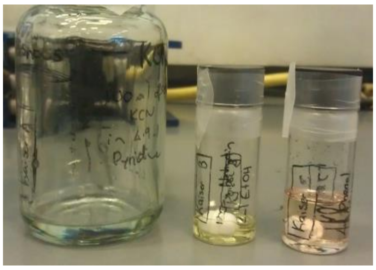
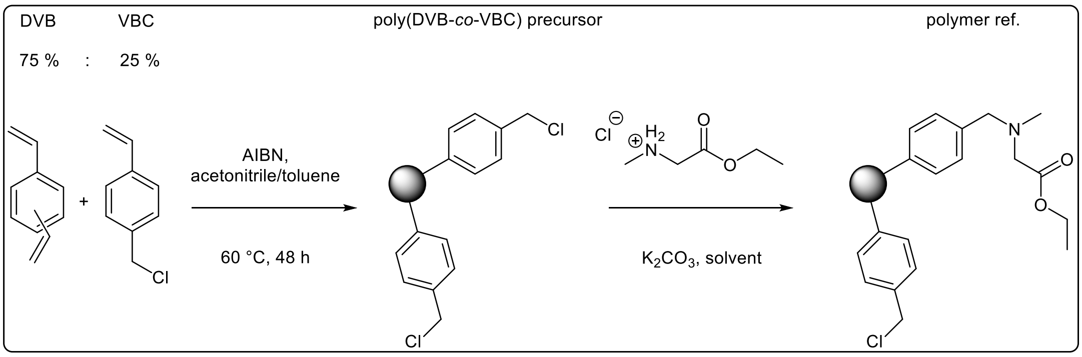


Figure 3.2: The Kaiser test solutions A, B and C (left to right)

The polymer particles (KA100 and KA102) were washed thoroughly using methanol prior to use. Three stoppered test tubes were prepared that contained a small quantity of sample KA100, a small quantity of sample KA102, and a blank control containing no polymer, respectively. Three drops of each Kaiser test solution (A, B and C) were added to all three test tubes. The test tubes were heated in parallel using a Radley’s carousel at 110 °C for 5 minutes.

3.3. Results and discussion

Polymer microspheres with particle diameters in the low micron size range are useful materials that have found application as sorbents in SPE. The post-polymerisation chemical modification of polymer microspheres imparts functionality that can be utilised to selectively extract pollutants from environmental samples. Poly(DVB-*co*-VBC) microspheres were synthesised and the pendent chloromethyl groups reacted with amino acid derived esters as shown in Scheme 3.11. Figure 3.3 shows the chemical structures of the four different esters used, sarcosine ethyl ester, sarcosine methyl ester, *L*-phenylalanine methyl ester and *D*-phenylalanine methyl ester, respectively.



Scheme 3.11: The two-stage reaction scheme for the synthesis of polymer microspheres containing reactive pendent chloromethyl residues, and the subsequent nucleophilic aliphatic substitution reaction with amino acid derived esters.

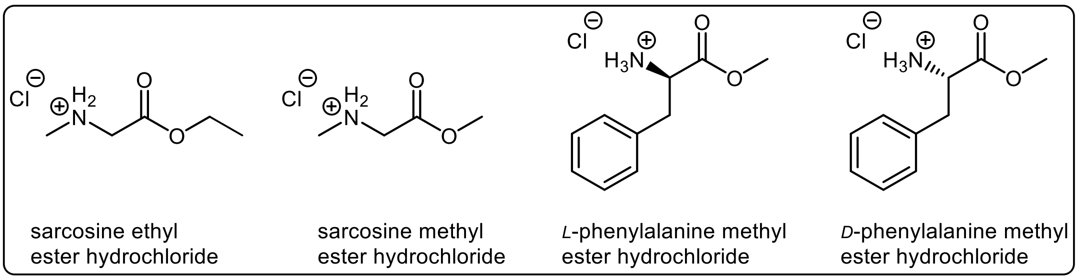
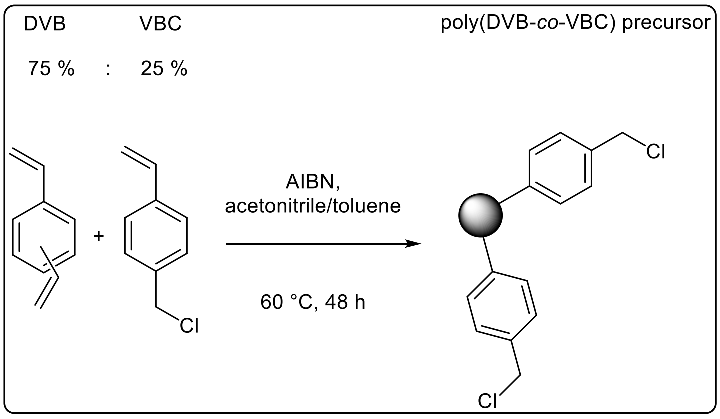


Figure 3.3: The amino acid derived esters used in the post-polymerisation chemical modification of poly(DVB-*co*-VBC) microspheres

Amino acid derived esters contain ionisable nitrogen moieties that can be exploited in anion-exchange, and the ester functionality can be hydrolysed to a carboxylic acid to be exploited in cation-exchange. Therefore a single sorbent has the potential to contain two ionisable moieties for exploitation as both an anionic- and cationic-exchange SPE sorbent.

3.3.1. Synthesis of poly(DVB-*co*-VBC) microspheres

Given that we were targeting polymer microspheres of low particle diameter and with a narrow particle size distribution, precipitation polymerisation was selected as the most appropriate polymerisation methodology for the synthesis. Precipitation polymerisation is a method used to prepare crosslinked and spherical polymer particles in the micron size range. Polymer microspheres of poly(DVB-*co*-VBC) were synthesised by polymerising a functional monomer (4-vinylbenzyl chloride, VBC) with a crosslinking monomer (divinylbenzene, DVB), as depicted in Scheme 3.12.



Scheme 3.12: Outline of the synthetic procedure used to synthesise polymer particles of poly(DVB-*co*-VBC) by precipitation polymerisation

The polymer microspheres were prepared in a single preparative step, using a 75:25 (w/w) feed ratio of DVB to VBC. The high loading of the crosslinking monomer DVB, with its two reactive vinyl groups, resulted in a 40-50 % yield of insoluble, highly crosslinked particles.

Polymer characterisation by elemental microanalysis and FT-IR spectroscopy

The composition of the poly(DVB-*co*-VBC) copolymers was confirmed by elemental microanalysis and FT-IR spectroscopy. The microanalysis results, shown in Table 3.9, were compared to the expected values generated on assumption that all monomer added would polymerise to generate a copolymer. Although this assumption was made, the insoluble poly(DVB-*co*-VBC) recovered after precipitation polymerisation accounted for 40-50% yield and the remainder of the mixture was soluble.

Table 3.9: Microanalysis values obtained for the poly(DVB-*co*-VBC) copolymers

Sample ref.	Elemental Microanalysis			
	% C	% H	% N	% Cl
Expected values	**86.2**	**7.5**	**0.7**	**5.6**
KA2	86.5	7.5	0.5	2.8
KA85	86.8	7.7	0.6	2.1
KA112	86.2	7.6	0.6	2.7
KA76	87.3	7.5	0.5	3.1
KA84	86.4	7.5	0.5	3.6
KA122	87.0	7.6	0.5	2.6

The chlorine content of the polymers reflects the level of VBC that has been incorporated from the monomer feed. The predicted value of 5.6 % would be obtained if the DVB and VBC monomers copolymerised according to the ratio of the monomer feed (75:25 (w/w) DVB:VBC). The actual results found, in Table 3.9, show that the level of chlorine is always lower than the expected value so the copolymer may consist of a slightly higher ratio of polymerised DVB to VBC. The copolymerisation of monomers DVB and VBC may be influenced by compositional drift.[2] However, the percentages for C, H, N and Cl do not tally up to 100 % so there may be an error associated with the chlorine values. The monomers DVB and VBC do not contain any nitrogen, so the only source of nitrogen within this copolymer is from the incorporated isobutyronitrile residues from the dissociated free radical initiator AIBN.

In all of the poly(DVB-*co*-VBC) FT-IR spectra, there were characteristic bands present that confirmed the presence of chloromethyl groups within the polymers, namely the Ar-CH_2-Cl wag at 1,265 cm^{-1} and the C-Cl stretching vibration at 677 cm^{-1}. In addition, the unreacted pendent vinyl groups from the DVB give rise to out of plane C-H bending vibrations associated with mono-substituted unsaturated C=C bonds at 988 cm^{-1} and 901 cm^{-1}. The presence of these vibrations associated with chlorine and unreacted vinyl groups serves as further confirmation that both VBC and DVB have been copolymerised together successfully. The intense signals at 827 cm^{-1} and 795 cm^{-1} arise from out of plane C-H bending on the substituted aromatic rings of the monomers.

Effect of the reaction solvent used in precipitation polymerisation

Although the composition of the copolymers should all be similar, it is expected that the choice of solvent utilised in the precipitation polymerisation reactions will have an effect on both the particle porosity and morphology. Neat acetonitrile is a commonly used solvent for the synthesis of DVB-containing polymers, and was utilised for the synthesis of samples KA2, KA85 and KA112. The other three samples (KA76, KA84 and KA122) were synthesised with toluene present as an additional co-solvent, in a 75:25 (v/v) ratio of acetonitrile:toluene, to increase the specific surface areas of the products.

In comparison to acetonitrile, toluene has a solubility parameter closer to poly(DVB) (the δ values for acetonitrile, toluene and poly(DVB) are 24.3, 18.2 and around 17-18 $\text{MPa}^{½}$, respectively).[4, 21] When copolymerising DVB by precipitation polymerisation, the solvation of the polymer phase can be improved by utilising an acetonitrile/toluene mixture. Studies have been performed that have investigated the use of toluene as a co-solvent at levels up to 40 % in acetonitrile.[16, 18] By improving the solvency of the reaction medium, there is a delay in the onset of phase separation between the polymer and solvent which allows the polymer network to entrap more molecules of solvent that lead to the formation of a pore system when the solvent is removed. Therefore, it was expected that the precipitation polymerisation reactions using the acetonitrile/toluene mixture would lead to products with larger particle diameters and well-developed, permanent pore structures.

The pore structure of poly(DVB-co-VBC)

Nitrogen sorption analysis was the technique utilised to obtain information about the pore structure of the poly(DVB-*co*-VBC) particles, namely the specific surface area, pore volume and average pore diameter. The calculation used to determine the specific surface area of any sample will depend upon the most appropriate model of gas adsorption. The Langmuir method is employed for monolayer coverage of the solid sample by nitrogen molecules, and this may be represented by a Type I adsorption isotherm. The Brunauer, Emmet and Teller (BET) model of adsorption is employed when incorporating multi-layer coverage of the solid sample, and this may be represented by a Type II or Type IV adsorption isotherm. The porosity data obtained for the six poly(DVB-*co*-VBC) particles has been tabulated in Table 3.10.

The isotherms plotted of quantity of nitrogen adsorbed versus relative pressure for the non-porous poly(DVB-*co*-VBC) particle samples (KA2, KA85, KA112 and KA76) do not fit closely with any of the six isotherm classifications published by IUPAC, as shown in Figure 3.4.[24] The values measured for the specific surface area confirm that these samples are non-porous, with low values obtained under 20 m^2/g.

As expected, the only poly(DVB-*co*-VBC) particles to show any significant porosity were those prepared in the presence of an acetonitrile/toluene co-solvent mixture (25 % v/v toluene). Samples KA84 and KA122 gave rise to Type I isotherms associated with Langmuir (monolayer) adsorption and had specific surface areas of 681 and 740 m^2/g, respectively. The pore volumes measured approached 0.3 cm^3/g.

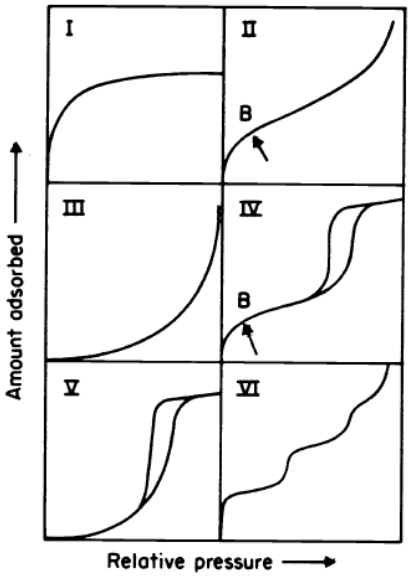


Figure 3.4: The six types of physisorption isotherm [24]

The values given in Table 3.10 for the average pore diameter range from about 1-3 nm. The IUPAC classification for physisorption states that pore widths between 2 and 50 nm are called mesopores and pore widths not exceeding about 2 nm are called micropores.[24]

Although the average pore diameter for samples KA2, KA85 and KA112 prepared in neat acetonitrile are within the microporous classification, caution should be taken because these samples are essentially non-porous. The polymer samples that were prepared in the co-solvent mixture have an average pore size that lies near the borderline between microporous and mesoporous, which is at 2 nm.

Table 3.10: The nitrogen sorption analysis data for poly(DVB-*co*-VBC) particles prepared in acetonitrile and in an acetonitrile/toluene mixture

Sample ref.	Reaction solvent	Isotherm classification[24]	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA2	acetonitrile only	Type II	20	-76	30	0.020	0.7
KA85		n/a	< 5	-16	< 5	n/a	n/a
KA112		n/a	< 5	-32	< 5	0.001	1.8
KA76	acetonitrile & toluene	n/a	14	-73	18	0.010	2.8
KA84		Type I	503	-365	681	0.271	2.2
KA122		Type I	543	-404	740	0.295	2.2

The morphology of poly(DVB-co-VBC)

Figure 3.5 shows the SEM micrographs of the poly(DVB-*co*-VBC) particles prepared by precipitation polymerisation in neat acetonitrile (images a, b and c) and in the acetonitrile/toluene mixture (images d, e and f). The tabulated values of the mean particle diameter for each sample are shown in Table 3.11.

The copolymer particles have smooth, spherical or nearly spherical morphology as expected for polymer microspheres. However, a bumpy, cauliflower-like imperfection to the surfaces of the particles can be observed in image b (for sample KA85). The narrow particle size distribution shown in the SEM micrographs is good for efficient packing should the particles be used in an application such as chromatographic stationary phases. The three samples prepared using the acetonitrile/toluene co-solvent mixture (images d, e and f) all show improved, almost monodisperse particles compared to their counterparts prepared in neat acetonitrile.

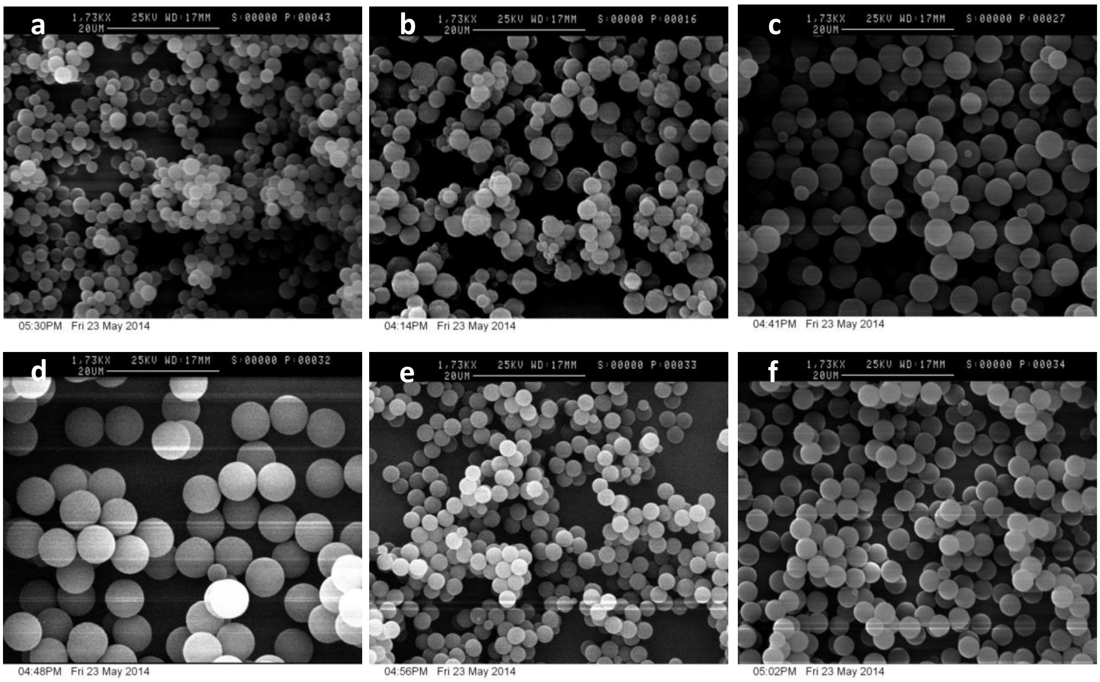


Figure 3.5: SEM micrographs of poly(DVB-*co*-VBC) prepared by precipitation polymerisation. KA2 (a), KA85 (b), KA112 (c) were prepared using neat acetonitrile, whereas KA76 (d), KA84 (e) and KA122 (f) were prepared with an acetonitrile/toluene mixture

Table 3.11: The mean particle diameter of the poly(DVB-*co*-VBC) particles (a-f) shown in Figure 3.5

Sample ref.	Image	Mean particle diameter (μm)
KA2	a	2.52
KA85	b	3.15
KA112	c	3.92
KA76	d	6.80
KA84	e	2.82
KA122	f	3.45

3.3.2. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) particles using sarcosine ethyl ester

The post-polymerisation chemical modification introduces amino acid functionality to the polymer microspheres that will enable them to be exploited as a novel class of functionalised weak anion-exchange sorbents.

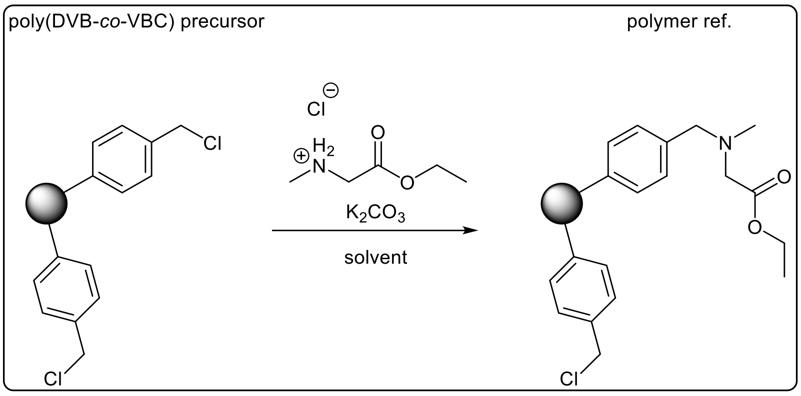
The precursor poly(DVB-*co*-VBC) microspheres contain chloromethyl groups distributed throughout the microspheres that serve as a functional handle for subsequent modification. The loading of chlorine present ranges from 1 to 1.5 mmol/g, although the actual number of chloromethyl groups available for reaction will be lower than the theoretical maximum because some sites may be inaccessible to the nucleophile.

The solvent choice was varied, in an effort to determine the conditions that were most favourable for the reaction. Water and ethanol are both polar protic solvents, DMF is a polar aprotic solvent, and toluene is a non-polar solvent. In a previous study, toluene was the solvent of choice for the substitution of the unreacted chloromethyl residues in hypercrosslinked polymer microspheres with 1,2-ethylenediamine and piperazine.[101] Although the polymer microspheres in this chapter have not been hypercrosslinked, it was considered reasonable to investigate toluene as a solvent for the amination of these poly(DVB-*co*-VBC) particles.

A Finkelstein type reaction was also used in order to exchange the pendent chloride ions of poly(DVB-*co*-VBC) microspheres with iodide ions, a more favourable leaving group for nucleophilic aliphatic substitutions. A small quantity of sodium iodide (NaI) was added to reactions in ethanol, to exchange with the chloride ion and shift the equilibrium forward by precipitating sodium chloride.

The secondary amine used in these reactions was sarcosine ethyl ester. As the hydrochloride salt of sarcosine ethyl ester was purchased commercially, it was necessary to include potassium carbonate within the reaction in order to deprotonate the amine for it to act as a nucleophile and to mop up the HCl which is formed as a reaction by-product. A 5-fold molar excess of the amine was used relative to the chloromethyl moieties.

The reaction shown in Scheme 3.13 between poly(DVB-*co*-VBC) and sarcosine ethyl ester hydrochloride, under basic conditions, resulted in a functionalised product containing a tertiary amine and an ester moiety.



Scheme 3.13: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with sarcosine ethyl ester hydrochloride

Evidence of successful amination by elemental microanalysis and FT-IR spectroscopy

The change in composition of the poly(DVB-*co*-VBC) particles upon functionalisation with sarcosine ethyl ester hydrochloride was confirmed by elemental microanalysis and FT-IR spectroscopy.

The expected elemental microanalysis values were calculated on the assumption that all of the chloromethyl groups would be consumed during the reaction. Table 3.12 shows the percentages of carbon, hydrogen, nitrogen and chlorine that were measured by elemental microanalysis. In all of the functionalised products, there is an observed increase in the % N content and a decrease in the % Cl content, relative to the precursor poly(DVB-*co*-VBC), that is evidence of the presence of sarcosine ethyl ester residues in the sample.

The elemental microanalytical data provides an indication of the maximum N content that may be available for ion-exchange; the estimated ion-exchange capacities (IEC) have been tabulated and are shown in Table 3.12.

.

Table 3.12: Microanalysis results obtained for the products after undergoing functionalisation with sarcosine ethyl ester hydrochloride

Sample ref.	Elemental Microanalysis						IEC
	% C	% H	% N	% Cl	% O	% unaccounted for	mmol/g
Expected values	**84.9**	**8.1**	**2.6**	**0.0**	**4.5**	**0.0**	**1.9**
KA78	86.1	7.5	1.0	2.4	-	3.0	0.7
KA79	86.5	7.7	1.3	1.8	-	2.7	0.9
KA80	86.4	7.6	1.0	1.8	-	3.2	0.7
KA81	85.7	7.6	1.6	1.3	-	3.8	1.1
KA104	86.5	7.7	1.1	1.8	-	2.9	0.8
KA106	86.3	7.8	0.9	1.1	-	3.9	0.6
KA109	84.8	7.4	0.8	3.9	-	3.1	0.6
KA114	85.7	7.4	0.6	3.5	-	2.8	0.4
KA115	85.7	7.5	0.5	3.2	-	3.1	0.4
KA116	85.1	7.4	0.8	2.9	-	3.8	0.6
KA117	85.8	7.5	0.9	n/a	-	n/a	0.6
KA118	85.7	7.6	1.0	1.7	-	4.0	0.7
KA119	85.3	7.7	1.3	1.1	-	4.6	0.9
KA126	85.4	7.7	1.8	2.1	-	3.0	1.3
KA127	86.1	8.2	1.3	1.7	-	2.7	0.9
KA132	86.0	8.4	1.1	1.4	-	3.1	0.8

The FT-IR spectrum of precursor KA122 is presented in Figure 3.6, and the FT-IR spectrum of KA132 after the post-polymerisation functionalisation with sarcosine ethyl ester is presented in Figure 3.7.

Upon functionalisation, the intensity of the chloromethyl-derived bands (observed in the FT-IR spectra of KA122) are diminished and a new band ascribed to carbonyl groups associated with the ester appears in the spectrum of KA132. The significant and isolated band present at 1,738 cm^{-1} correlates with the 1,742 cm^{-1} C=O stretching band present in the FT-IR spectrum of sarcosine ethyl ester hydrochloride, and the appearance of a broad band at 1,180 cm^{-1} confirms the presence of a C-O ester stretch. The change in the FT-IR spectra following reaction confirms that the chemical modification has resulted in a product that is distinct from the precursor poly(DVB-*co*-VBC) particles.

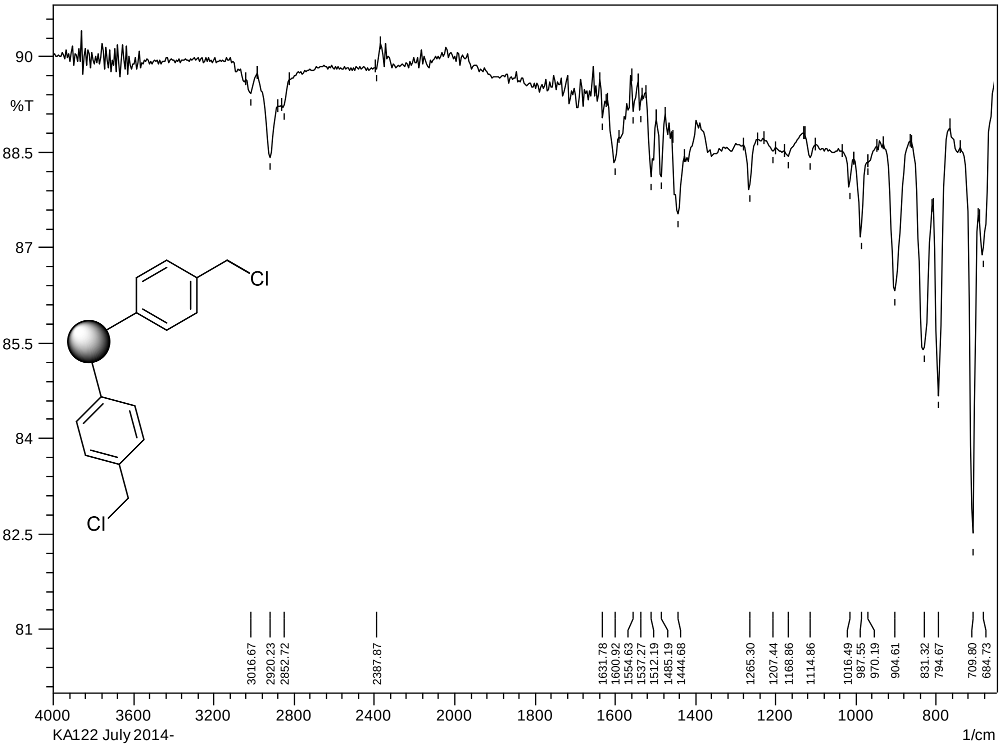


Figure 3.6: The FT-IR spectrum of KA122

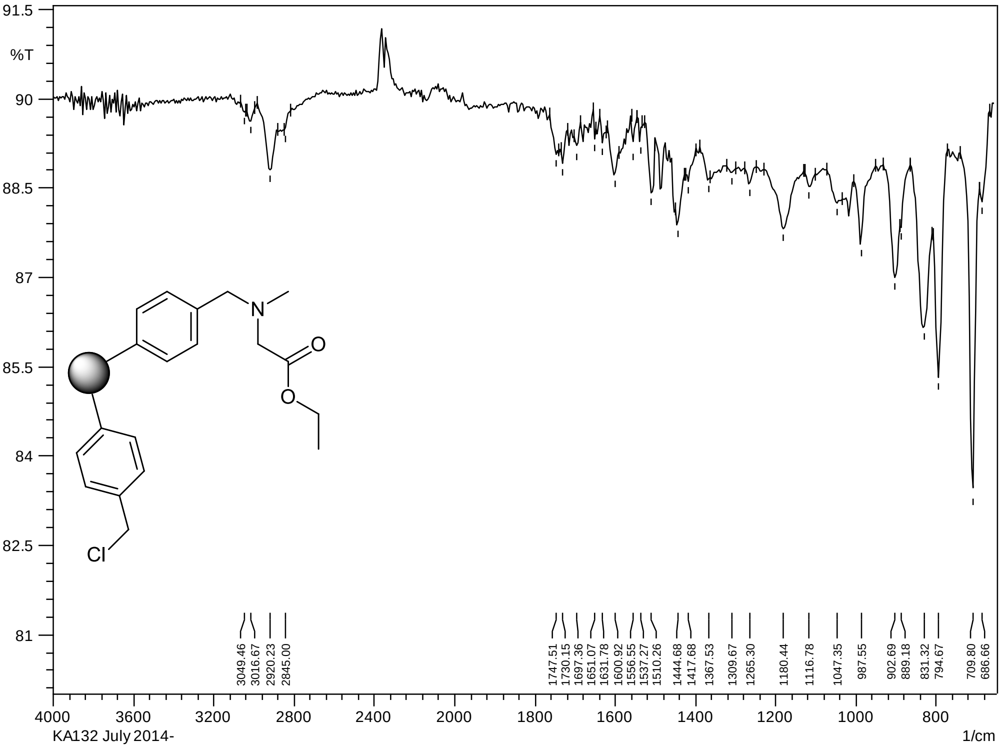


Figure 3.7: The FT-IR spectrum of KA132

Effect of altering the washing protocol for particle clean-up

On completion of the reaction, the insoluble, functionalised products were washed with a simple washing protocol involving the reaction solvent, an alcohol, and acetone. This was washing protocol A. After the elemental microanalysis was completed and the results interpreted, the polymer samples were subjected to further washing using washing protocol B. Protocol B includes successive washing with the following solvents: methanol, 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate, distilled water and acetone.

As a representative example, the microanalysis results for sample ref. KA106 using washing protocol A were measured to be 59.0 % C, 4.8 % H, 0.7 % N and trace (< 0.3 %) Cl. Upon rewashing the sample with washing protocol B, the measured microanalysis results were 86.3 % C, 7.8 % H, 0.9 % N and 1.1 % Cl. This suggests that the original washing protocol (A) was insufficient to remove the salts and impurities present within the sample, therefore, implementing the more thorough washing protocol B to clean up the samples was necessary. The basic sodium hydrogen carbonate neutralises the acidic HCl evolved, so it is recommended that a protocol such as B be used to clean up the polymer particles after chemical modification.[101]

Summary of the reaction conditions

The reaction conditions for the functionalisation of poly(DVB-*co*-VBC) with sarcosine ethyl ester are summarised in Table 3.13. This table should help to highlight what effect changing the solvent, temperature, or duration has on the resulting product.

Table 3.13: Summary of the reaction conditions used in the reaction of poly(DVB-*co*-VBC) with sarcosine ethyl ester hydrochloride

Polymer ref.	poly(DVB-*co*-VBC) precursor	Solvent(s)	Volume (mL)	Temp. (°C)	Duration (h)
KA78	KA76	DMF	30	60	5
KA79	KA76	ethanol & NaI	30	75	5.5
KA80	KA76	DMF	30	60	22
KA81	KA76	ethanol & NaI	30	75	21
KA104	KA84	DMF	30	80	5
KA106	KA84	ethanol & NaI	10	70	90
KA109	KA84	toluene	10	70	90
KA114	KA84	ethanol & NaI	5	70	0.167
KA115	KA84	ethanol & NaI	5	70	0.5
KA116	KA84	ethanol & NaI	5	70	1
KA117	KA84	ethanol & NaI	5	70	2
KA118	KA84	ethanol & NaI	5	70	21
KA119	KA84	ethanol & NaI	5	70	44
KA126	KA112	ethanol	10	75	5
KA127	KA122	ethanol	10	75	5
KA132	KA122	ethanol/water	80	75	18

Effect of changing the reaction solvent

When all other variables were kept constant (such as precursor, solvent volume, temperature and duration), it was possible to examine the effect of changing the reaction solvent on the characterisation results for the functionalised particles.

Upon changing the reaction solvent from ethanol (with sodium iodide) (sample KA106) to toluene (sample KA109), the % Cl observed in the microanalysis results shown in Table 3.12 increased from 1.1 to 3.9 %. The large percentage of chlorine remaining in sample KA109 suggests that the substitution of the chloride with sarcosine ethyl ester has not proceeded to high conversion. The corresponding change in the percentage of nitrogen was not as pronounced and within experimental error, with 0.9 % N found in sample KA106 compared to 0.8 % in sample KA109. Therefore, the conclusion from this comparison is that ethanol (with or without sodium iodide) is a preferred solvent to toluene for the reaction with sarcosine ethyl ester. Further experimentation using a larger sample set would be required to form a more robust conclusion.

Effect of changing the temperature

Upon increasing the reaction temperature from 60 °C (in KA78) to 80 °C (in KA104), the % Cl observed in the microanalysis results decreased from 2.4 to 1.8 %. The corresponding increase in % N was again only a change of 0.1 % which falls within experimental error. A clear relationship cannot be extracted from this set of results, but perhaps higher temperatures should be favoured to encourage a greater level of substitution.

Effect of changing the reaction duration

To monitor what effect the reaction duration has on the extent of functionalisation, a parallel synthesis was performed whereby the reactions were terminated at varying durations of time. The percentage of N and Cl present in the polymer was measured for the six samples and plotted against the corresponding reaction duration, shown in Figure 3.8. There is no Cl analysis for sample KA117, at 2 hours duration, because there was insufficient material available for analysis. The graph shows a trend of increasing N as the reaction duration is increased, and a trend of decreasing Cl. This relationship shows that the extent of functionalisation with sarcosine ethyl ester increases with time, with more chloride residues being substituted for sarcosine ethyl ester (measured as % N) at longer reaction durations. After 10 minutes there was 3.5 % Cl and 0.6 % N present, but after 44 hours the values were 1.3 % Cl and 1.1 % N.

The polymer microspheres of poly(DVB-*co*-VBC) are crosslinked particles where the reactive, pendent chloromethyl moieties are distributed throughout the entire space of the sphere. When the particles are in contact with the sarcosine ethyl ester for a short period of time, only the freely available chloromethyl groups (most likely at or near to the outer surface of the bead) will be able to react. However, when the reaction duration is increased there is an increased probability that the sarcosine ethyl ester will be able to penetrate through the pore network within the particles and reach the more inaccessible chloromethyl moieties.

The findings from this study have been observed and confirmed in other experiments of a similar nature. The comparison of experiments KA78 and KA80 show that there is 2.4 % Cl in the polymer after 5 hours, but a reaction of 22 hours produces a polymer with only 1.8 %

Cl. Similarly, by comparing a reaction of 5.5 hours (KA79) with a reaction of 21 hours (KA81) the chloride content decreases from 1.8 % to 1.3 %, respectively.

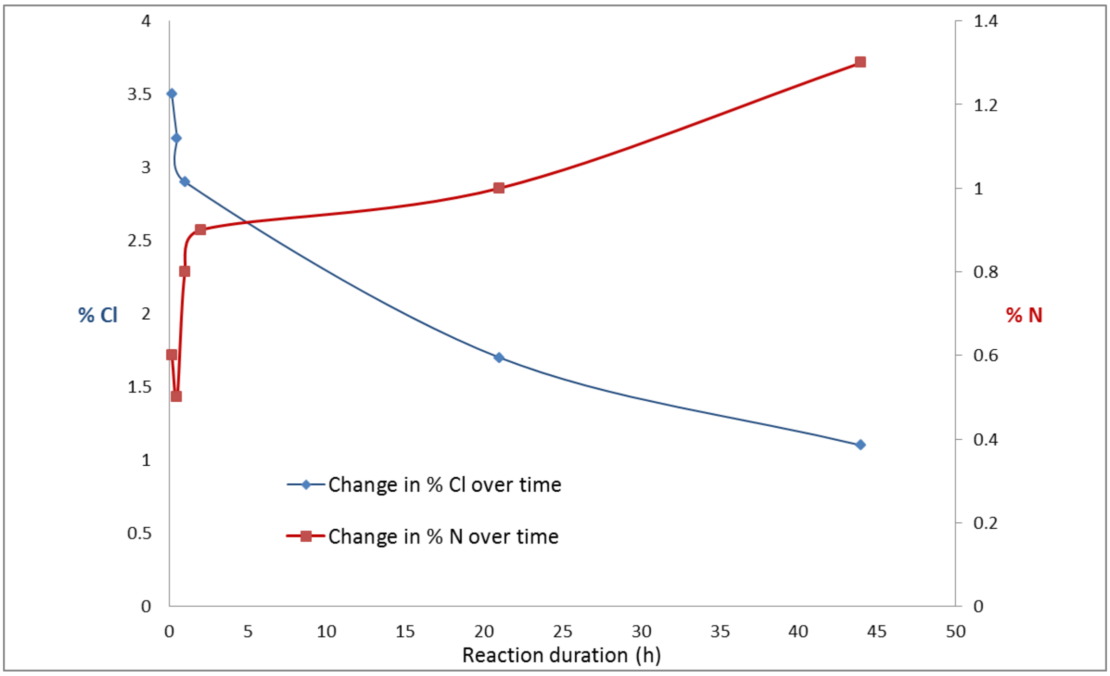


Figure 3.8: Effect of reaction duration on the extent of functionalisation

Measured porosity of the polymer particles after functionalisation

Nitrogen sorption analysis was utilised to measure the permanent porosity of these polymers in the dry state. A decrease in the specific surface area (measured as m^2/g) is to be expected upon functionalisation, because the mass of the polymer is increased but the surface area has remained unchanged. However, the measured specific surface area observed for the functionalised products (presented in Table 3.14) has decreased to the level of being non-porous.

The average pore size of the poly(DVB-*co*-VBC) precursor polymer was about 2.2 nm in diameter, so it is feasible to suggest that the functionalisation with sarcosine ethyl ester may have blocked some of these pores so that they became inaccessible, whilst in the dry state. The pore network was formed during the crosslinking and polymerisation of the monomers DVB and VBC, so the substitution of the chloride group with a bulkier sarcosine ethyl ester chain may have decreased the available porosity by pore blocking.

There is a critical crosslinking density that is required for the formation of a pore network, so this may point towards an issue with the pore stability that has resulted in collapse. A monomer ratio of 75:25 (w/w) DVB to VBC has been used in the preparation of the poly(DVB-*co*-VBC) precursor particles. Samples KA84 and KA122 were porous, as shown in Table 3.10, but upon functionalisation the porous structure may have reversibly or irreversibly collapsed.

Howdle *et al*. have previously described reversibly collapsible macroporous resins of polymerised styrene and divinylbenzene.[118] Instead of possessing permanent, measureable porosity in the dry state, these materials may instead behave like gel-type polymers that expand and open up to re-establish a porous network when solvated. To determine whether the collapse of the pore structure is reversible, it should be noted whether the closed pores (that are inaccessible to gas or guest molecules) can be reopened upon swelling in an appropriate solvent.

An experimental investigation to examine this in further detail should include a study of the methods used to dry and isolate the particles from the reaction mixture. In this thesis the functionalised particles were isolated by vacuum filtration and washed with solvents to remove any impurities. Freeze-drying may be a suitable alternative because it is anticipated that this would prevent the formation of a vapour-liquid interface that could induce mechanical stress on the pores of the material resulting in collapse.

However, the functionalised polymers that have been prepared in these experiments are not intended to be used in their dry state. Therefore these nitrogen sorption analysis measurements are useful as a guide, but not a representative measure of the porosity of these samples when they are solvated and utilised in real-life applications.

Further experimentation and development to deepen our understanding of these materials could include the use of inverse size-exclusion chromatography to measure the porosity of these samples in the wet state.

Table 3.14: The nitrogen sorption analysis data for the products after functionalisation with sarcosine ethyl ester

Sample ref.	Precursor poly(DVB-*co*-VBC)	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA104	KA84	3	-29	3	0.004	6.08
KA106		< 1	-15	<1	n/a	n/a
KA109		1	-23	1	n/a	n/a
KA127	KA122	4	+125	6	0.005	4.84
KA132 #1		10	-21	12	n/a	n/a
KA132 #2		2	-15	3	n/a	n/a
KA132 #3		8	+290	11	0.008	3.99

The morphology of the particles after functionalisation

The poly(DVB-*co*-VBC) particles were synthesised by precipitation polymerisation because spherical, nearly monodisperse particles in the low micron size range were targeted for applications as polymeric sorbents in solid-phase extraction and stationary phases in liquid chromatography. Therefore, a requirement of the post-polymerisation chemical modification is that the quality of the polymer microspheres is not affected during the process.

Figure 3.9 shows the SEM micrographs of the poly(DVB-*co*-VBC) particles prior to functionalisation, (a), and then after the subsequent functionalisation with sarcosine ethyl ester (b). There is no observable deterioration in the quality of the particles upon functionalisation. The product particles are uniform polymer microspheres with narrow particle size distribution and smooth, spherical morphology. The mean particle diameter has been measured to be approximately 3.2 µm, which is in good agreement with the mean particle diameter of 3.5 µm prior to modification. The size of these particles fit within the desired size range for application as solid-phase extraction sorbents or chromatographic packing materials, in the low micron range.

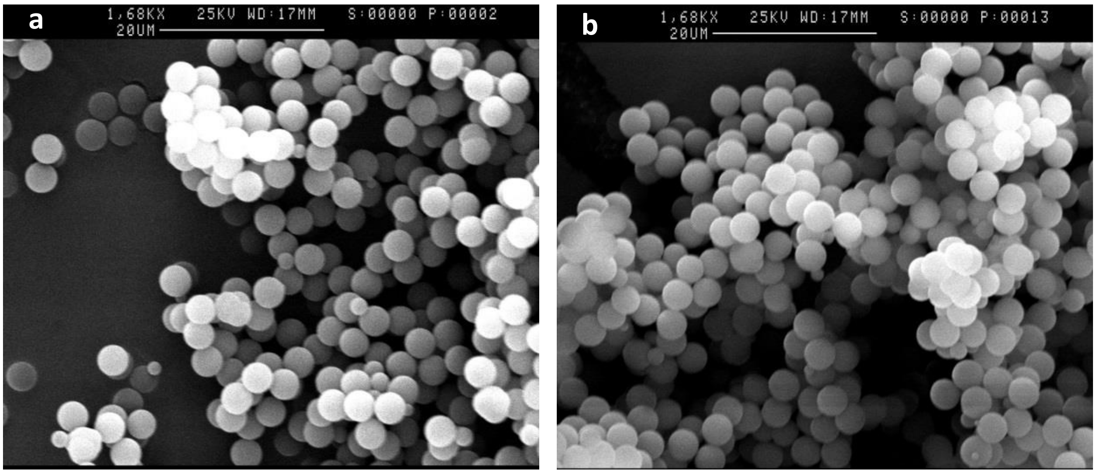
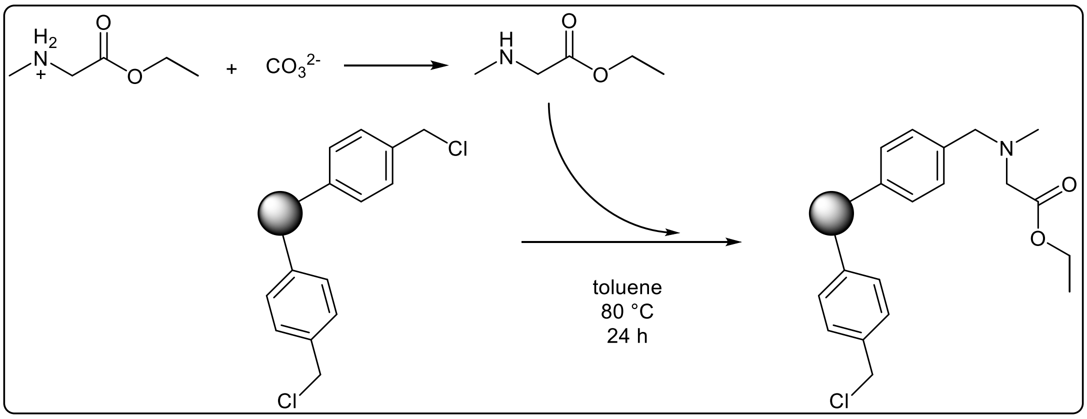


Figure 3.9: The SEM micrographs of poly(DVB-*co*-VBC) sample KA122 (a) and the product after functionalisation with sarcosine ethyl ester, KA132, (b)

Effect of reacting the free base of sarcosine ethyl ester instead of the hydrochloride salt

Sarcosine ethyl ester hydrochloride is unable to act as an effective nucleophile, so it is necessary to deprotonate the nitrogen with a base such as potassium carbonate. Previously in this chapter, this was performed *in situ* by including potassium carbonate in the reaction flask alongside the polymer microspheres of poly(DVB-*co*-VBC), sarcosine ethyl ester hydrochloride and the reaction solvent. The characterisation results showed this procedure to be successful for the partial substitution of the chloride ion with sarcosine ethyl ester, to produce functionalised polymer microspheres that have a mixture of unreacted chloromethyl residues and those successfully modified with the amino acid.

However, in order to determine if this one stage reaction was the most appropriate and convenient methodology for this reaction, a two-step reaction was investigated whereby the free base of sarcosine ethyl ester was prepared in advance and then subsequently combined with the polymer microspheres and reaction solvent. An outline of the reaction scheme for this procedure is shown in Scheme 3.14.



Scheme 3.14: Reaction scheme for the two-step post-polymerisation modification of poly(DVB-*co*-VBC) using sarcosine ethyl ester

The percentages of chlorine and nitrogen from elemental microanalysis are a good indication of the extent of modification that has occurred during the reaction. Upon reaction with sarcosine ethyl ester, there was an increase in the nitrogen content from 0.4 to 1.4 %, and a decrease in the chlorine content from 3.7 % to 2.2 %. These changes in the composition of the polymer confirm that the product has incorporated some sarcosine ethyl ester, by substituting the chloride on some of the pendent chloromethyl residues.

The intensity of the chloromethyl-derived signal at 1,265 cm^{-1} in the FT-IR spectrum of KA99, relative to the spectrum of precursor polymer KA85, has diminished which suggests that partial substitution has occurred during the modification step. Two new bands ascribed to the ester have appeared: the carbonyl C=O stretch at 1,734 cm^{-1}, and the broad C-O stretch at 1,180 cm^{-1}. This serves as further confirmation that sarcosine ethyl ester chains have been incorporated into the particles.

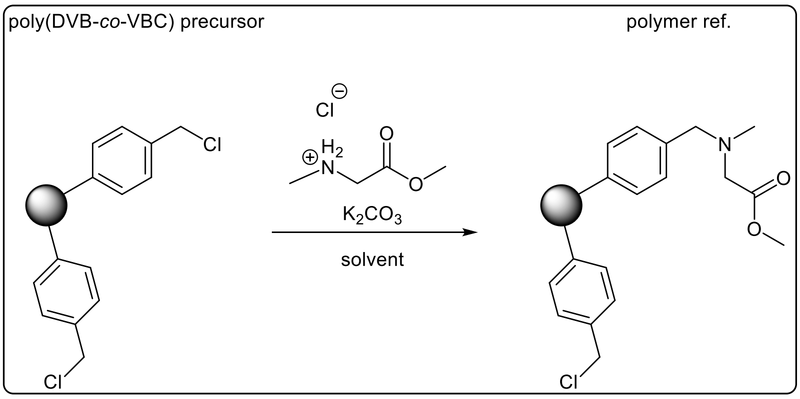
A very low specific surface area was obtained (< 5 m^2/g) following analysis of the pore structure by nitrogen sorption analysis. Unfortunately, the poly(DVB-*co*-VBC) precursor used (KA85) did not possess a permanent pore network in the dry state, so this subsequent analysis only shows that there has been no generation of measurable porosity following modification with sarcosine ethyl ester. A porous precursor ought to be used to obtain information on whether the two-step chemical modification results in preserved or diminished porosity.

The morphology of KA99, observed by SEM, indicated that there was no noticeable change in the size or shape of the particles resulting from the chemical modification.

The two-step reaction to prepare the free base before adding this to the polymer did not show any significant improvement to the quality of the resulting functionalised polymer particles, when compared with the one-step protocol. Therefore, the additional steps taken to prepare the free base are probably unnecessary in this chemical modification, and the easier and more time-efficient one-step synthesis using the hydrochloride salt with added potassium carbonate is recommended at the time of writing.

3.3.3. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using sarcosine methyl ester

Following on from the chemical modification reactions using sarcosine ethyl ester hydrochloride, this section reports the results of the experiments performed using sarcosine methyl ester hydrochloride. The compounds are chemically very similar, so it is expected that they will behave in the same way when reacted with poly(DVB-*co*-VBC). The scheme for the reaction between poly(DVB-*co*-VBC) and sarcosine methyl ester hydrochloride, under basic conditions, is presented in Scheme 3.15.



Scheme 3.15: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with sarcosine methyl ester hydrochloride

Evidence of successful amination by elemental microanalysis and FT-IR spectroscopy

Elemental microanalysis and FT-IR spectroscopy were utilised to examine the change in composition of the poly(DVB-*co*-VBC) particles after functionalisation with sarcosine methyl ester hydrochloride.

The percentages of carbon, hydrogen, nitrogen and chlorine measured in each of the functionalised polymer samples were tabulated and are shown in Table 3.15. Relative to the elemental microanalysis data for the precursor particles of poly(DVB-*co*-VBC), each sample shows an increased % N content and a decreased % Cl content. The precursor particles contained values of up to 0.4 % N prior to functionalisation, therefore the increase in nitrogen content is evidence that the amine has been incorporated into the polymer particles upon functionalisation. The decrease in the chlorine content confirms that substitution of the chloride has taken place. The expected values calculated and shown in Table 3.15 are based upon the assumption that all of the chloride ions will be replaced by sarcosine methyl ester. If there is 0.4 % N from the precursor when no substitution has occurred, and a maximum value of 2.6 % N obtainable after 100 % reaction, then these results show that an intermediate level of functionalisation has occurred.

The estimated values for the IEC are shown in Table 3.15, based upon the theoretical quantity of nitrogen that may be available for ion-exchange.

Table 3.15: The elemental microanalysis results and ion-exchange capacities of poly(DVB-*co*-VBC) particles that have been modified with sarcosine methyl ester hydrochloride

Sample ref.	Elemental Microanalysis					IEC
	% C	% H	% N	% Cl	% O	mmol/g
Expected values	**84.9**	**7.9**	**2.6**	**0.0**	**4.6**	**1.9**
KA82	85.7	7.5	1.3	1.9	-	0.9
KA83	85.9	7.4	1.1	2.6	-	0.8
KA87	86.7	7.4	0.8	3.1	-	0.6
KA100	90.2	7.9	1.0	2.0	-	0.7
KA107	86.0	7.7	1.2	0.9	-	0.9
KA110	84.3	7.3	0.8	4.8	-	0.6

The reaction conditions for the functionalisation of poly(DVB-*co*-VBC) with sarcosine methyl ester are summarised in Table 3.16, to help highlight what effect changing the solvent and duration has on the resulting product.

Table 3.16: Summary of the reaction conditions used in the reaction of poly(DVB-*co*-VBC) with sarcosine methyl ester hydrochloride

Polymer ref.	Precursor	Solvent(s)	Volume (mL)	Temp. (°C)	Duration (h)
KA82	KA76	DMF	30	60	22.5
KA83	KA76	DMF	30	60	5
KA87	KA76	toluene	30	60	25
KA100	KA85	DMF	30	60	5
KA107	KA84	ethanol & NaI	10	70	90
KA110	KA84	toluene	10	70	90

Poly(DVB-*co*-VBC) sample KA84 was reacted with sarcosine methyl ester hydrochloride under the same conditions but using either ethanol (with added NaI) or toluene as the reaction solvent. A comparison of the microanalysis % N and % Cl values shows that sample KA107 prepared in ethanol with NaI has a greater extent of functionalisation than sample KA110, which was prepared using toluene as solvent.

As expected, the extent of functionalisation with sarcosine methyl ester hydrochloride appears to increase with increasing reaction duration, which is a continuation of the trend already discussed in Section 3.3.2. KA83 and KA82 were products resulting from two similar experiments performed in parallel over different durations. After the longer duration of 22.5 hours (KA82) there was less chlorine present in the product than after the reaction of only 5 hours (KA83).

The FT-IR spectrum of sample KA107 presented in Figure 3.10 is a typical spectrum of polymer particles that have been modified with sarcosine methyl ester. Interpretation of the FT-IR spectrum delivers supplementary evidence to the microanalytical data, demonstrating the successful reaction between poly(DVB-*co*-VBC) precursor KA84 and sarcosine methyl ester hydrochloride to produce product KA107. The appearance of the characteristic ester carbonyl stretch at 1,742 cm^{-1} and the C-O band at 1,175 cm^{-1} confirm the presence of ester residues within the particles. The substitution of the chloride is indicated by the diminished intensity of the 1,265 cm^{-1} wag and the 688 cm^{-1} stretch associated with the chloromethyl residues.

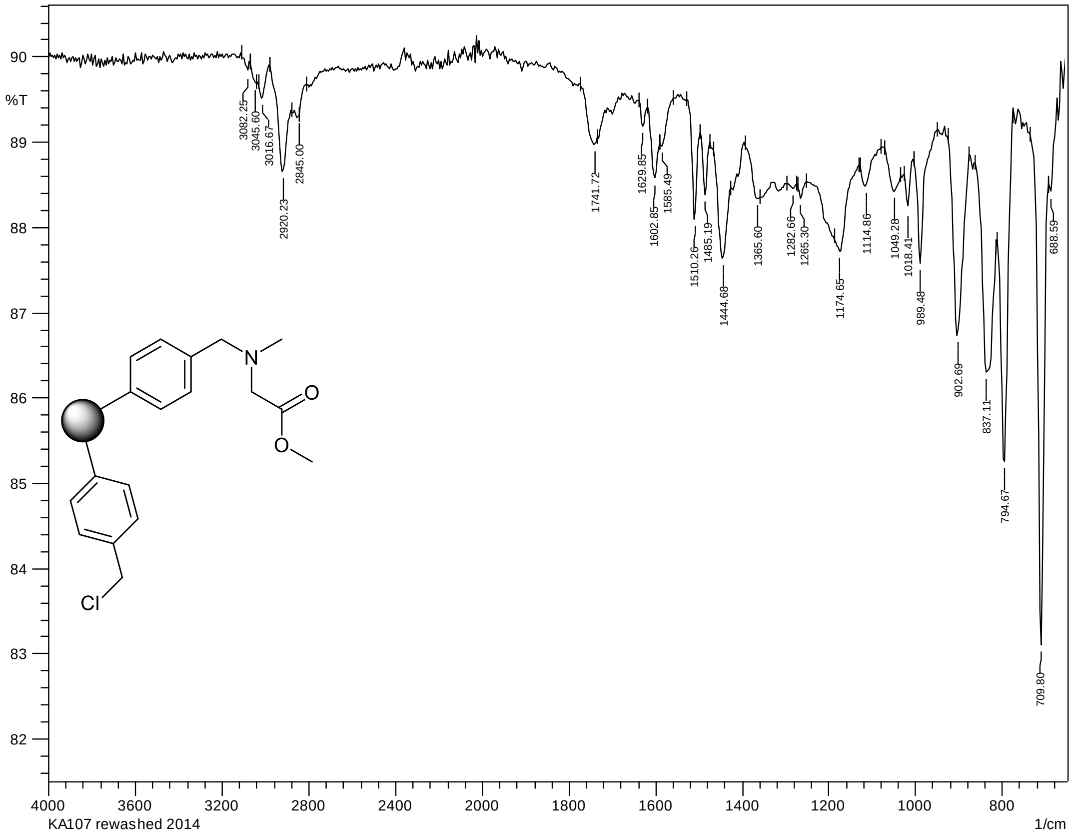


Figure 3.10: The FT-IR spectrum of KA107

Measured porosity of the particles after functionalisation

The permanent dry-state porosity of sample KA100 was measured by nitrogen sorption analysis. The results in Table 3.17 indicate that this sample does not possess any permanent pore structure, owing to the very low specific surface area and pore volume. This result was expected due to the absence of porosity in the poly(DVB-*co*-VBC) precursor, which was prepared by precipitation polymerisation in neat acetonitrile. Porosity measurements were not collected for the other samples in this section because the mass of sample was lower than the recommended quantity required for the instrument.

Table 3.17: The nitrogen sorption analysis data for sample KA100

Sample ref.	Precursor poly(DVB-*co*-VBC)	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA100	KA85	< 5	-25	< 5	0.002	0.47

The morphology of the particles after functionalisation

Figure 3.11 shows the SEM micrographs of the poly(DVB-*co*-VBC) particles prior to chemical modification (a), and also after the reaction with sarcosine methyl ester hydrochloride (b). As discussed in Section 3.3.2., the post-polymerisation chemical modification has not altered or deteriorated the quality of the polymer microspheres. The mean particle diameter was 2.8 µm prior to functionalisation and 2.6 µm after reaction; therefore there has been no significant change to the particle size. The particles are smooth, spherical and have a narrow particle size distribution.

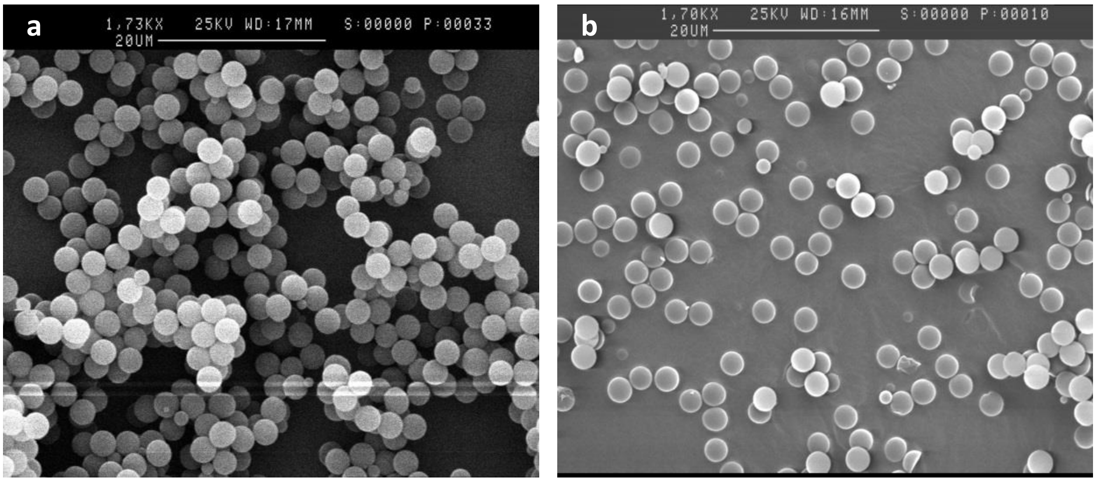
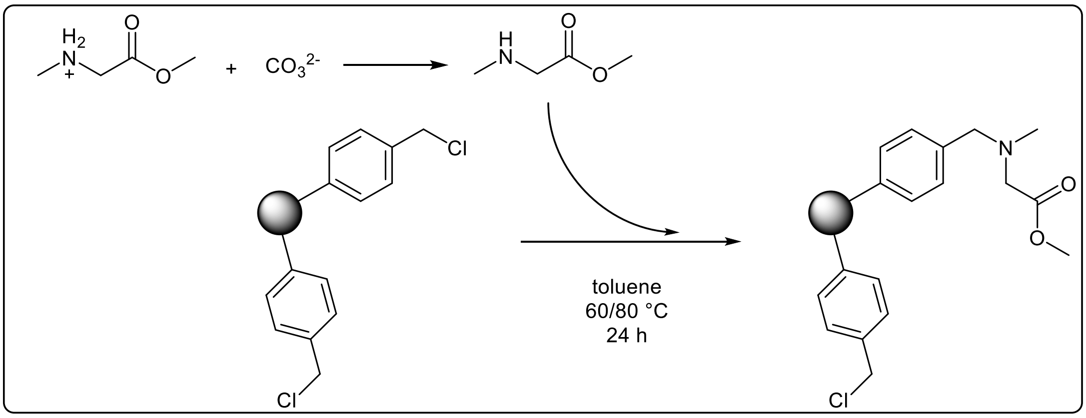


Figure 3.11: The SEM micrographs of poly(DVB-*co*-VBC) sample KA84 (a) and the product after functionalisation with sarcosine methyl ester, KA107 (b)

Effect of reacting the free base of sarcosine methyl ester instead of the hydrochloride salt

In a similar experiment to that already described in Section 3.3.2., involving the free base of sarcosine ethyl ester, this section aims to investigate what effect reacting the free base of sarcosine methyl ester has on the particles of poly(DVB-*co*-VBC). A two-step reaction was investigated whereby the free base of sarcosine methyl ester was prepared in advance and then combined subsequently with the polymer microspheres and reaction solvent. An outline of the reaction scheme for this procedure is shown in Scheme 3.16.



Scheme 3.16: Reaction scheme for the two-step post-polymerisation chemical modification of poly(DVB-*co*-VBC) using sarcosine methyl ester

As with all reactions discussed, the results of the elemental microanalysis and FT-IR spectroscopy are of great importance when monitoring the success of the functionalisation reaction. The percentages of C, H, N and Cl confirm that the products KA96 and KA97 are distinct from KA76, their poly(DVB-*co*-VBC) precursor. A trace quantity of nitrogen was present in the precursor which has increased to 0.8 % and 1.2 % in products KA96 and KA97, respectively. Likewise, the initial 5.2 % of chlorine has decreased to 3.6 % and 2.4 % after reaction with the free base of sarcosine methyl ester, suggesting that the chloride had been partially substituted by the amine. The two reactions were performed at different reaction temperatures, with the reaction performed at higher temperature showing a greater extent of functionalisation.

Nitrogen sorption analysis was used to assess the pore structure of the polymers; however, the functionalisation with sarcosine methyl ester has not generated any measurable porosity. The micrographs obtained by SEM show that the chemical modification of the particles has not had any negative effect on the quality of the polymer microspheres.

3.3.4. Post-polymerisation chemical modification of poly(DVB-*co*-VBC) using *L*- and *D*-phenylalanine methyl ester

There is an additional motivation to react polymer microspheres of poly(DVB-*co*-VBC) with the *L*- and *D*-enantiomers of phenylalanine methyl ester, shown in Figure 3.12, to generate polymeric sorbents with chiral separation potential.

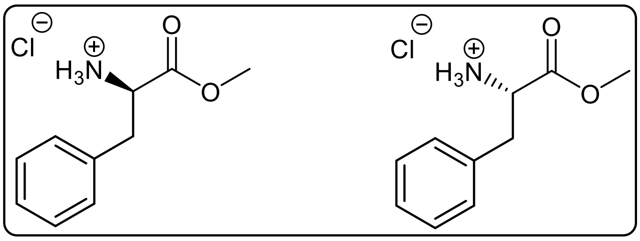
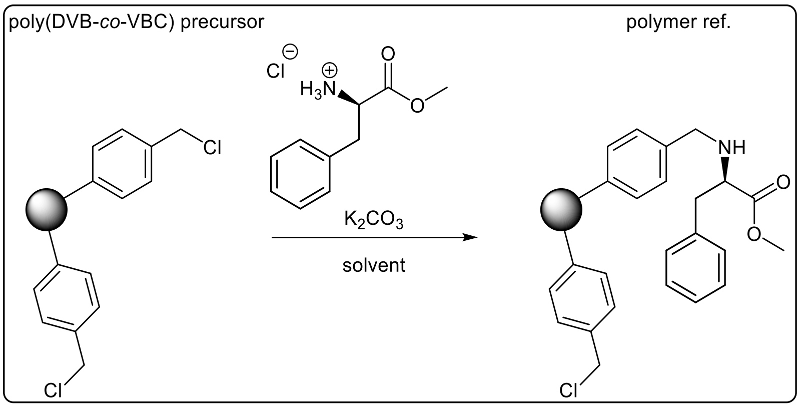


Figure 3.12: The chemical structures of *L*-phenylalanine methyl ester hydrochloride (left) and *D*-phenylalanine methyl ester hydrochloride (right)

Leading on from the secondary sarcosine methyl and ethyl ester salts discussed in Sections 3.3.2. and 3.3.3., phenylalanine methyl ester is a primary amine that will react by nucleophilic aliphatic substitution with poly(DVB-*co*-VBC) particles to produce modified polymer particles containing a secondary amine, an ester, a benzyl side-chain, and a stereogenic carbon centre. The use of enantiomerically pure polymer microspheres as sorbents and stationary phases opens up opportunities for chiral resolution. It should be possible to separate the enantiomers in a racemic mixture of compounds based upon their differing affinity for the amino-acid-derived chiral stationary phase.

The hydrochloride salt of the phenylalanine methyl esters was purchased, therefore potassium carbonate was included within the reaction in order to deprotonate the amine for nucleophilic attack. The reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with *L*-phenylalanine methyl ester hydrochloride has been shown in Scheme 3.17.



Scheme 3.17: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with *L*-phenylalanine methyl ester

Evidence of post-polymerisation chemical modification by elemental microanalysis and FT-IR spectroscopy

Elemental microanalysis and FT-IR spectroscopy were used to monitor the change in composition of the poly(DVB-*co*-VBC) particles upon functionalisation with *L*- or *D*-phenylalanine methyl ester hydrochloride.

The expected elemental microanalysis values were calculated on the assumption that all of the chloromethyl groups would be consumed during the reaction. Therefore, successful functionalisation with phenylalanine methyl ester would result in a decrease in the % Cl observed and an increase in % N. Table 3.18 shows the percentages of carbon, hydrogen, nitrogen and chlorine that were measured by elemental microanalysis.

KA105, KA108 and KA111 were all prepared from the sample precursor, KA84. Although there was a small increase in the % N and a small decrease in the % Cl, the change in composition is not substantial. Of the three solvents that were studied in this set of experiments (DMF, ethanol with NaI, and toluene), toluene has shown the greatest change in the compositional values, suggesting that product KA111 has undergone greater functionalisation.

The experiments to produce products KA128, KA129, KA130 and KA131 were run in parallel and the reaction conditions are summarised in Table 3.19. Precursor KA112 was reacted with *L*- and *D*-phenylalanine methyl ester hydrochloride to produce products KA128 and KA130, respectively. There was negligible change in the elemental microanalysis compositional values on going from precursor to product, inferring that substitution of the chloride with either enantiomer of phenylalanine methyl ester was unsuccessful. KA129 and KA131 are the respective products from reaction of precursor KA122 with the *L*- and *D*-phenylalanine methyl ester. Although the compositions of the products are not significantly different to the precursor, the change is greater than that observed when using precursor KA112. After comparing the elemental microanalysis values, it can be noted that there has been no difference between reactions using either the *D*- or *L*-enantiomer.

Table 3.18: The elemental microanalysis results and ion-exchange capacities of poly(DVB-*co*-VBC) particles that have been modified with the *L*- and *D*-phenylalanine methyl ester hydrochloride

Enantiomer	Sample ref.	Elemental Microanalysis					IEC
		% C	% H	% N	% Cl	% O	mmol/g
	Expected values	**85.8**	**7.7**	**2.4**	**0.0**	**4.1**	**1.7**
L-	KA105	86.6	7.5	0.6	3.0	-	0.4
L-	KA108	85.4	7.4	0.8	3.6	-	0.6
L-	KA111	85.2	7.4	1.1	3.1	-	0.8
L-	KA128	86.1	7.5	0.7	2.8	-	0.5
L-	KA129	85.2	7.4	0.5	2.7	-	0.4
D-	KA130	85.7	7.5	0.6	2.7	-	0.4
D-	KA131	86.7	7.6	0.6	2.5	-	0.4

Table 3.19: Summary of the reaction conditions used in the reaction of poly(DVB-*co*-VBC) and *L*- and *D*-phenylalanine methyl ester hydrochloride

Polymer ref.	Precursor	Enantiomer	Solvent(s)	Volume (mL)	Temp. (°C)	Duration (h)
KA105	KA84	*L*-	DMF	30	80	5
KA108	KA84	*L*-	ethanol & NaI	10	70	90
KA111	KA84	*L*-	toluene	10	70	90
KA128	KA112	*L*-	ethanol	10	75	5
KA129	KA122	*L*-	ethanol	10	75	5
KA130	KA112	*D*-	ethanol	10	75	5
KA131	KA122	*D*-	ethanol	10	75	5

The FT-IR spectra were studied to look for characteristic signals that indicate the presence of functional groups in the materials. The appearance of low intensity bands at 1,732 cm^{-1} and 1,180 cm^{-1} for C=O and C-O stretching, respectively, confirms that there are ester groups present within the polymer microspheres. However, the intensity is relatively low compared to the Ar-CH_2-Cl wag at 1,265 cm^{-1}. Based upon the elemental microanalysis and the FT-IR spectra, the results suggest that only a modest amount of chloride has been substituted by phenylalanine methyl ester.

Compared to the earlier reactions discussed with sarcosine ethyl ester and sarcosine methyl ester, the nucleophilic aliphatic substitution reaction with phenylalanine methyl ester hydrochloride has not been as successful. The reason for this has not been investigated due to time constraints, but perhaps the bulky benzyl substituent on the molecule has made it more difficult to access the pendent chloride moieties distributed throughout the microspheres.

Measured porosity of the particles after functionalisation

Nitrogen sorption analysis was performed for those products that were prepared from a poly(DVB-*co*-VBC) precursor polymerised in an acetonitrile/toluene solvent mixture. Samples KA128 and KA130 were not analysed because their precursor, KA112, was prepared in neat acetonitrile and lacked porosity. The results are tabulated and presented in Table 3.20.

Table 3.20: The nitrogen sorption analysis data for the products after reaction with phenylalanine methyl ester hydrochloride

Sample ref.	Precursor poly(DVB-*co*-VBC)	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA105	KA84	304	-243	413	0.138	1.82
KA108		6	-25	8	n/a	n/a
KA111		n/a	n/a	n/a	n/a	n/a
KA129	KA122	27	-54	36	0.015	2.23
KA131		7	-130	10	0.006	3.33

The poly(DVB-*co*-VBC) precursors KA84 and KA122 have measured Langmuir specific surface areas of 681 and 740 m^2/g. Therefore, it is surprising that only sample KA105 has maintained a high level of porosity after reaction with phenylalanine methyl ester, with a specific surface area of 413 m^2/g and a pore volume of 0.138 cm^3/g.

The morphology of the particles after functionalisation

The spherical and smooth nature of the poly(DVB-*co*-VBC) microspheres has been retained after these particles were subjected to chemical modification with *L*- and *D*-phenylalanine methyl ester hydrochloride under basic conditions. The SEM micrographs for precursor KA112 and product KA130 are shown in Figure 3.13. The mean particle diameter for both samples has been measured as 3.9 µm, and the particle size distribution is narrow.

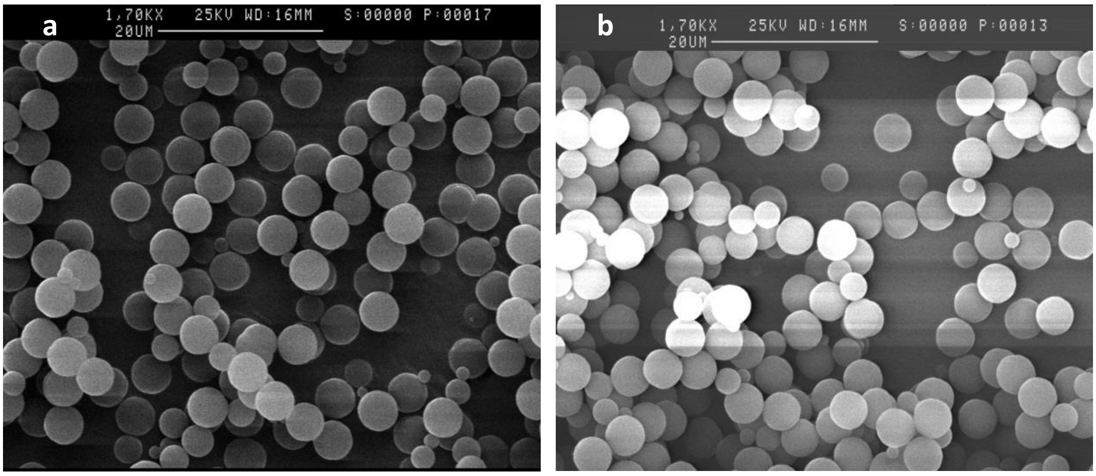


Figure 3.13: The SEM micrographs of poly(DVB-*co*-VBC) sample KA112 (a) and the product after functionalisation with *D*-phenylalanine methyl ester, KA130 (b)

The stereochemistry of the phenylalanine methyl ester microspheres

Enantiomerically pure *L*- and *D*-phenylalanine methyl ester hydrochloride were purchased to react in nucleophilic aliphatic substitution reactions with the reactive, pendent chloromethyl residues on poly(DVB-*co*-VBC) microspheres. If the stereochemistry of the phenylalanine methyl ester is preserved after the amine has reacted with the polymer, then this will open up opportunities for the material to be used in chiral separations.

Enantiomers possess optical activity, which is the ability to rotate plane-polarised light. Polarimetry is the technique commonly used to measure the extent of rotation for a sample in solution. When light is rotated to the right it is given a positive value, and rotation to the left is assigned a negative value. These polarimetric measurements provide valuable information on whether a sample is enantiomerically pure, or whether it exists as a mixture of enantiomers.

Due to the insoluble, solid nature of the polymer microspheres, it is not possible to characterise the stereochemistry of the sample directly. Therefore, in order to evaluate whether the stereochemistry has been retained, the functional pendent phenylalanine methyl ester moieties on the microspheres could be cleaved off to separate them from the insoluble polymer.

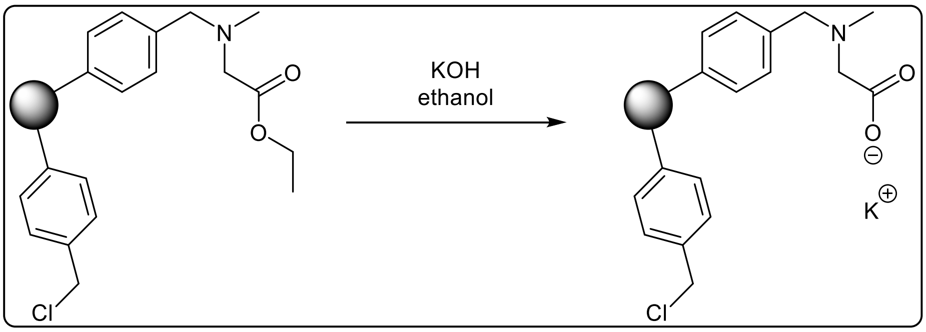
Alternatively, it should be possible to indirectly ascertain the stereochemistry of the functionalised polymer microspheres in solid-phase extraction experiments. A solid sorbent packed into a solid-phase extraction cartridge would show selectivity for chiral analytes if the chirality of the phenylalanine methyl ester has been preserved.

Regardless of whether the materials have retained their stereochemistry or if they have racemised due to the post-polymerisation chemical modification, particles of poly(DVB-*co*-VBC) functionalised with *L* or *D*-phenylalanine methyl ester are useful materials with a secondary amine that could be tested for its ion-exchange abilities, an aromatic side chain and an ester moiety.

3.3.5. Ester hydrolysis of the polymers to the amphoteric amino acid form

The ester moieties on the functionalised polymer microspheres may be converted to carboxylic acids by ester hydrolysis, to generate the amino acid species. The polymer microspheres will exist as crosslinked spherical particles that possess amino acid moieties distributed throughout the particles. Amino acids contain both amino and carboxylic acid groups, therefore they are amphoteric and will exist in zwiterionic form at their isoelectric point. One motivation for this work is that the preparation of polymer microspheres with pendent amino acids offers a route into polymeric sorbents that contain both anionic and cationic character for mixed-mode solid-phase extraction sorbents.

Ester hydrolysis may be performed under acidic or basic conditions. Acidic conditions using aqueous HCl would form the protonated carboxylic acid, although the tertiary amine may be protonated at low pH to form the quaternary hydrochloride salt. A subsequent basic wash would be required to neutralise the amine, although care would need to be taken to avoid deprotonating the carboxylic acid. Alternatively, hydrolysing under basic conditions would produce the amine free base and deprotonated carboxylate anion. The hydrophobic polymer microspheres do not interact well with water so it was decided that an alcoholic solution of potassium hydroxide dissolved in ethanol would be more suitable, as shown in Scheme 3.18.



Scheme 3.18: Reaction scheme for the hydrolysis to the amphoteric species possessing the ability to form a zwitterionic species

The conversion of the ester to the carboxylic acid or carboxylate form does not result in a substantial compositional change that can be tracked by elemental microanalysis. The crosslinked polymer is a large hydrocarbon structure with some functional chains that have been incorporated through chemical modification, so it is difficult to observe changes to the carbon and hydrogen content by this method.

FT-IR spectroscopy is an important characterisation tool for the detection of functional groups within materials. Sample KA133 was the product obtained after the basic hydrolysis of KA132, the poly(DVB-*co*-VBC) particles modified with sarcosine ethyl ester. The FT-IR spectra of KA132 and KA133 are presented in Figure 3.14 and Figure 3.15, respectively. The formation of a carboxylate anion results in asymmetric and symmetric C=O stretching that is distinct from the C=O stretch of an ester. The characteristic wavenumbers quoted for these signals are quoted as 1,610-1,550 cm^{-1} for the asymmetric stretch and 1,420-1,300 cm^{-1} for the symmetric stretch.[128] Upon comparing the precursor spectrum of KA132 to the product spectrum of KA133, qualitative observations have been made. The C=O stretch at 1738 cm^{-1} that was assigned to the ester is absent in the spectrum of KA133, although there is noise in this region of the spectrum, and the intensity of the band at 1,180 cm^{-1} for C-O stretching has decreased. The broadening of the peak from 1,600-1,560 cm^{-1} is due to asymmetric carboxylate ion stretching, and the increase in the intensity of the signal at 1,406 cm^{-1} is due to symmetric carboxylate ion stretching.

Characterisation of the products generated from the hydrolysis reactions performed under acidic conditions shows that these conditions were not as successful as the basic conditions using potassium hydroxide and ethanol.

The porosity of the hydrolysed products KA101 and KA133 were measured by nitrogen sorption analysis and the results have been tabulated and presented in Table 3.21. Sample KA101 does not possess a porous structure in its dry-state, but the polymer it was prepared from was also non-porous. The specific surface area of sample KA133 was high, 605 m^2/g, and had a pore volume of 0.2 cm^3/g. The average pore diameter is 2 nm, which suggests that there is high micropore content.

Table 3.21: The nitrogen sorption analysis data for samples KA101 and KA133

Sample ref.	Precursor	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA101	KA100	< 5	+486	< 5	0.004	0.54
KA133	KA132	449	-363	605	0.228	2.03

The morphology of the particles was examined by scanning electron microscopy, before and after the hydrolysis reaction. The SEM micrographs show that the reaction did not have any detrimental effects on the quality of the particles because they appear unchanged.

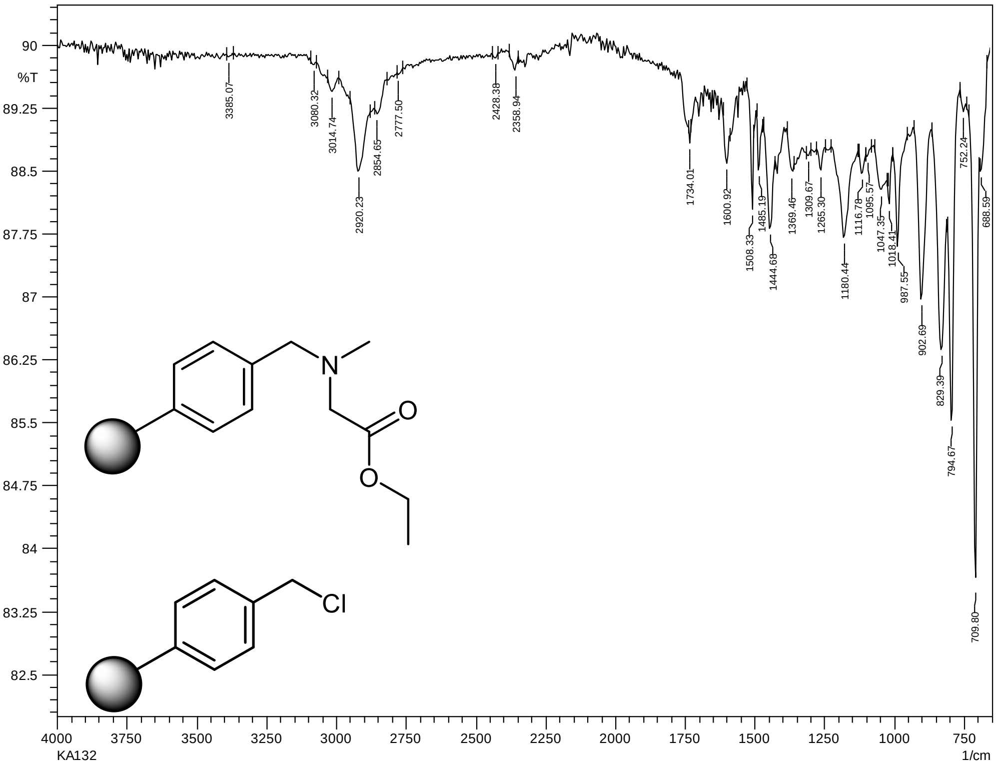


Figure 3.14: The FT-IR spectrum of KA132

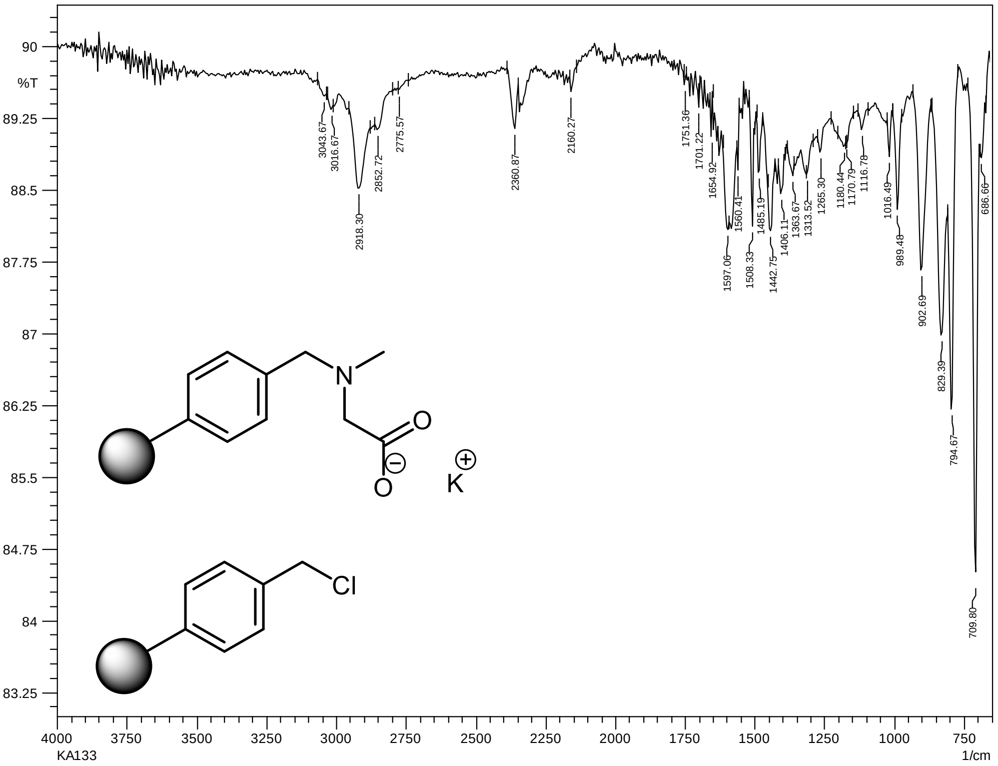
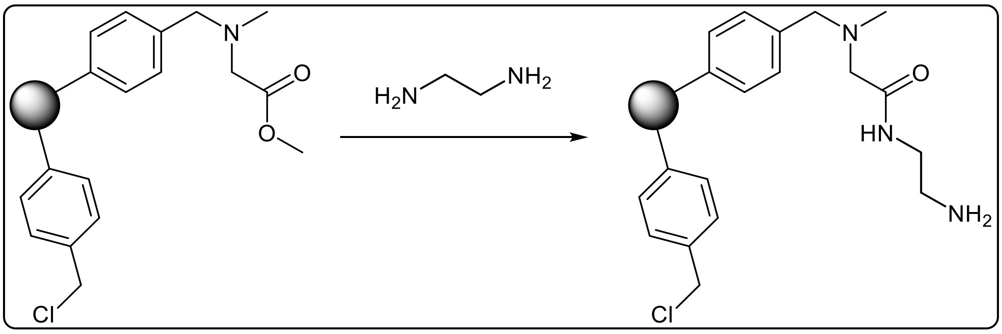


Figure 3.15: The FT-IR spectrum of KA133

3.3.6. Reaction of the amino acid derived esters with ethylenediamine

The ester moieties on the microspheres of poly(DVB-*co*-VBC) functionalised with amino acid derived esters are a potential functional handle for further chemical modification. In this work, ethylenediamine was reacted with the ester to convert the functional group and generate microspheres containing an amide and a pendent primary amine, shown in Scheme 3.19.



Scheme 3.19: Scheme for the reaction with ethylenediamine. The unreacted chloromethyl moieties from poly(DVB-*co*-VBC) have been shown pendent to the crosslinked microsphere.

The reaction between chloromethylated polymer microspheres of poly(DVB-*co*-VBC) and ethylenediamine has been reported previously.[101] However, here the chloromethylated poly(DVB-*co*-VBC) particles were first reacted with sarcosine methyl ester and then subjected to reaction with ethylenediamine. The product generated contains a tertiary amine, an amide and also a primary amine.

The expected changes in the composition of the functional chains in the microsphere on going from the ester to the ethylenediamine modified product includes an additional two N atoms per chain, one less O atom per chain, and an increase in the hydrocarbon content. The actual composition of the sample was measured by elemental microanalysis, and the results are shown in Table 3.22.

Two reactions were studied in this preliminary investigation. The reaction from sample KA100 to KA102 shows an increase in the nitrogen content from 1.0 % to 2.7 %. Similarly, the reaction from KA79 to KA124 resulted in an increase in the N content from 1.3 % to 2.2 %. In addition to the rise in the nitrogen composition, there was a decrease in the chlorine content from 1.8 % to 0.4 %. The most likely explanation for this is that some of the unreacted chloromethyl residues, shown in Scheme 3.19, have taken part in the substitution reaction with ethylenediamine.

Table 3.22: The elemental microanalysis results and ion-exchange capacities for the ethylenediamine modified samples

Sample ref.	Elemental Microanalysis					IEC
	% C	% H	% N	% Cl	% O	mmol/g
Expected values	**83.3**	**8.2**	**6.4**	**0.0**	**2.2**	**4.6**
KA102	84.7	7.7	2.7	1.6	-	1.9
KA124	85.7	7.8	2.2	0.4	-	1.6

After reaction with ethylenediamine, the product is expected to contain a tertiary amine, primary amine and an amide bond. The C=O ester and C-O stretches should be absent if the ester has been successfully consumed by reaction to form the amide. The primary amine –NH_2 should give rise to two bands between 3,500-3,300 cm^{-1} due to asymmetric and symmetric stretching of the N-H bond, and N-H bending between 1,650-1,560 cm^{-1}. For the amide, two bands are expected for N-H stretching at 3,460-3,400 cm^{-1} and 3,100-3,070 cm^{-1}. The C=O stretching bands for amide I and amide II are expected at 1,680-1,630 cm^{-1} and 1,570-1,515 cm^{-1}, respectively.

The FT-IR spectra obtained for products KA102 and KA124 do not show conclusive evidence that primary amines and amide bonds have been incorporated. The ester C=O stretch at 1,734 cm^{-1} and the C-O stretch at 1,171 cm^{-1} remain present, and there are no distinguishable peaks in the 3,500-3,300 cm^{-1} region associated with N-H stretching.

These results suggest that the procedure used in these reactions should be modified to find optimum conditions that lead to more conclusive evidence upon characterisation that the ethylenediamine has been successfully incorporated.

The Kaiser test is a qualitative experiment that indicates the presence or absence of free primary amino groups. The yellow colour of the compound ninhydrin turns to a deep blue colour upon reaction with primary amines.

An experiment was performed with samples KA100, KA102 and a blank control. Figure 3.16 shows the photographs of the colorimetric test. The blank control contained only the mixture of Kaiser test solutions to show the colour in the absence of polymer particles. The precursor polymer KA100 contains tertiary amine groups in the sarcosine methyl ester moieties. After being exposed to ninhydrin, the colour changed to a light blue colour. The product polymer KA102 that has been reacted with ethylenediamine was subjected to the same treatment, and the colour changed to a deep blue colour. The deep blue colour confirms that primary amino groups have been detected.

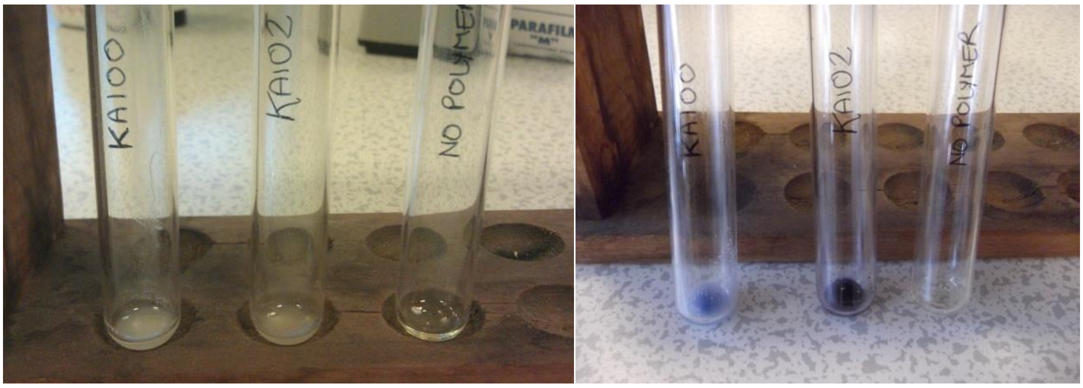


Figure 3.16: Photographs of the test tubes just after the addition of the Kaiser test reagents (left), and then again after a couple of minutes (right)

3.4. Chapter conclusions

Polymer microspheres of poly(divinylbenzene-*co*-vinylbenzyl chloride) were synthesised by precipitation polymerisation from a monomer loading of 75% divinylbenzene and 25% vinylbenzyl chloride. The toluene/acetonitrile co-solvent mixture yielded 39-51% of smooth and spherical particles with a narrow particle size distribution in the low micron size range. Specific surface areas of 681 and 740 m^2/g were achieved.

Post-polymerisation chemical modifications introduced amino acid functionality to the polymer microspheres that will enable them to be evaluated as a novel class of functionalised weak anion-exchange sorbents. The application of the polymers is reported in Chapter 5.

The reaction between poly(DVB-*co*-VBC) and either sarcosine ethyl ester hydrochloride or sarcosine methyl ester hydrochloride, under basic conditions, resulted in a functionalised product containing a tertiary amine and an ester moiety. The success of the reaction was assessed primarily by elemental microanalysis and FT-IR spectroscopy. Experimenting with the reaction conditions showed that ethanol was a preferred reaction solvent to toluene, and higher temperatures are thought to lead to increased substitution. The extent of functionalisation increases with increasing reaction duration. This has been ascribed to the fact that the pendent chloromethyl moieties are distributed throughout the space of the particle and it may take time to penetrate the crosslinked network and create opportunities for substitution to occur. It was not possible to detect porosity for the particles when measured in the dry-state so it appears that the porous network may have collapsed or been restricted after functionalisation, but it would be useful to measure the extent of porosity for swollen and expanded particles. There was no observable deterioration in the quality of the particle morphology after chemical modification; the particles were smooth and spherical with a narrow particle size distribution. The particle size was in the low micron size range that is desirable for application as solid-phase extraction sorbents.

The objective for synthesising polymer particles containing pendent phenylalanine methyl ester moieties was to produce polymeric sorbents that contain additional selectivity for chiral resolution and separation. The level of substitution was not significant, and only modest quantities of the chloride moieties have been substituted by the phenylalanine methyl ester. By systematically changing the reaction solvent in a series of experiments it was found that toluene was the solvent that led to the greatest level of modification. It has not been investigated why this ester has not been able to react with the poly(DVB-*co*-VBC) microspheres so readily as the sarcosine alkyl esters, however steric effects resulting from bulky benzyl substituents may have restricted access throughout the polymer network.

Hydrolysis of the ester moieties was performed in order to generate an amino acid like species that will offer zwitterionic properties for ion-exchange. The only useful method of characterisation was FT-IR spectroscopy due to its ability to detect the changes in vibration resulting from functional groups, and the spectra show characteristic changes that confirm that the ester was hydrolysed to a carboxylate ion. The hydrolysed polymers synthesised have been evaluated for application as solid-phase extraction sorbents, and this work is presented in Chapter 5.

A second post-polymerisation chemical modification reaction using ethylenediamine was performed on the poly(DVB-*co*-VBC) particles that had been reacted with sarcosine methyl ester, to incorporate amide and primary amine functionality in addition to the tertiary amine that was in place. The ester functionalised microspheres are susceptible to further functionalisation and therefore could be useful as sorbents with an added reactive functional handle for extension. A change in the chemical composition was observed by elemental microanalysis; however it was not obvious by FT-IR spectroscopy. The qualitative calorimetric Kaiser Test was employed for another assessment and confirmed that there were primary amine groups present in the sample.

3.5. Future work

This chapter has shown that the post-polymerisation chemical modification of poly(DVB-*co*-VBC) microspheres with amino acid derived alkyl esters was successful, but the process requires optimisation and improvement. A design of experiments (DOE) could be performed to investigate systematically the conditions and factors that influence the quality of the product. The effect of solvent choice would be a good starting point, as only a few organic solvents have been considered in this thesis and it is thought that the poly(DVB-*co*-VBC) particles should be in an expanded and swollen state prior to functionalisation for porosity to be preserved. For the purpose of characterisation, it would be useful to measure the porosity of the particles in the wet-state because in real-life application as solid-phase extraction sorbents the polymers would be wet with solvent and the available porosity of the network may differ to the dry-state porosity given by nitrogen sorption analysis.

The extent of reaction between poly(DVB-*co*-VBC) and phenylalanine methyl ester has not been as significant compared to the sarcosine alkyl esters tested in this chapter. The obvious difference between the esters is that the phenylalanine has a bulky side chain aromatic residue that may be more sterically difficult to navigate through the pore network of the particles. It would be worth investigating other amino acid derived esters in future experiments to see how wide the scope is for the synthesis of novel and functional polymeric sorbents. One could also synthesise polymers with bigger pores. Reactions with the *L*- and *D*- enantiomers of different amino acids would show great opportunity for developing polymeric sorbents that are capable separating chiral molecules from a racemic mixture. Looking at the other amino acids may also lead to polymeric sorbents with increased/different ion-exchange capacities as solid-phase extraction sorbents. For example, lysine has two basic amino groups due to the primary NH_2 on the side-chain in addition to the backbone, and glutamic acid and aspartic acid contain an additional acidic functionality on their side-chains that could increase their capacity for cationic-exchange.

The primary amine ethylendiamine was reacted with the ester of the functionalised poly(DVB-*co*-VBC) particles, to generate novel sorbents containing an amide bond and also primary and tertiary amino groups. By linking molecules and generating amide bonds, this shows potential for the sorbents to participate as solid supports in solid phase peptide synthesis or solid phase organic chemistry. The reaction conditions for the synthetic protocol should be re-examined in order to obtain more conclusive results through characterisation and analysis.

Chapter 4 – Synthesis of hypercrosslinked polymer microspheres with functionality derived from amino acid moieties

4.1. Introduction

The post-polymerisation chemical modification of gel-type and macroreticular polymer microspheres was described in Chapter 3, using amino acid derived esters such as sarcosine ethyl ester. Following on from this work, this chapter focuses on the chemical modification and functionalisation of hypercrosslinked polymer microspheres. In contrast to gel-type and macroreticular polymers, hypercrosslinked polymers possess ultra-high specific surface areas, typically greater than 1,000 m^2/g, owing to their high content of micropores. When hypercrosslinked polymers are applied as SPE sorbents for the extraction of analytes from complex media there is an increased capacity for adsorbing organic compounds due to the high micropore content and accessibility to small molecules.[93] Hypercrosslinked polymers uptake water in order to relieve the strained and rigid network of the dry porous sorbent, therefore they are good sorbents for the adsorption and desorption of analytes in aqueous media since they are water compatible.[80]

4.1.1. Aims

The objective of this chapter was to synthesise novel polymeric sorbents by the post-polymerisation chemical modification of hypercrosslinked polymer microspheres with an amino acid derived ester, sarcosine ethyl ester. The hypercrosslinked polymeric sorbents were designed to be exploited as mixed-mode SPE sorbents for the selective extraction of organic compounds from aqueous environments, as discussed in Chapter 5. The zwitterionic nature of amino acid moieties may potentially enable the sorbents to be utilised in both anionic- and cationic-exchange modes.

4.2. Experimental

4.2.1. Materials

Divinylbenzene (DVB) (80 % technical grade) and 4-vinylbenzyl chloride (VBC) (≥90 %) were supplied by Sigma-Aldrich (UK) and passed through a short column of alumina (activated, neutral, Brockmann I, supplied by Sigma-Aldrich) to remove inhibitors before use. 2,2'-

Azo*bis*(isobutyronitrile) (AIBN) (97 %) was supplied by BDH Lab Supplies (UK) and recrystallized from cold acetone.

Iron (III) chloride (97 %), nitric acid (65 % puriss.) and sarcosine ethyl ester hydrochloride (99 %) were supplied by Sigma-Aldrich (UK). Potassium carbonate (K_2CO_3) (anhydrous), potassium hydroxide (KOH) (GPR Rectapur pellets), and sodium hydrogen carbonate (Ph. Eur.) were all supplied by VWR International and used as received.

The solvents acetonitrile (ACN) (HPLC grade), 1,2-dichloroethane (DCE) (99.8 % anhydrous), diethyl ether, toluene, ethanol, methanol and acetone were all supplied by Sigma-Aldrich and used as received. Water was double distilled in-house prior to use.

4.2.2. Equipment and instrumentation

Precipitation polymerisation experiments were carried out using a Stuart Scientific S160 incubator (Surrey, UK) and a Stovall low-profile roller system (NC, USA). Nalgene® plastic bottles were used as the reaction vessels in all experiments.

Products were dried in a Townson & Mercer Limited (England) vacuum oven set to 70 °C, at ~ 60 mbar pressure.

C, H and N elemental microanalyses were determined using a Perkin Elmer 2400 Series II CHNS Analyser. The results were obtained as a percentage by weight, and were measured as a function of thermal conductivity. The chlorine content was determined by the Schöniger oxygen flask combustion technique, followed by ion chromatography using a Dionex DX-120 instrument. All analyses were performed by Denise Gilmour and Alex Clunie of the University of Strathclyde Microanalysis Service.

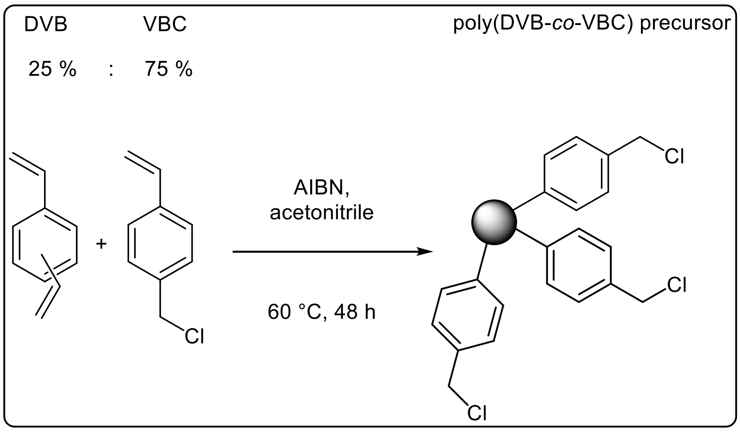
FT-IR spectroscopic analysis was performed on a Shimadzu FT-IR spectrophotometer IRAffinity-1, with a scanning range of 4,000 – 650 cm^{-1} in ATR mode.

The porosity measurements were performed using a Micromeritics ASAP 2000. The samples were degassed under vacuum overnight at 100 °C prior to analysis. The analysis was carried out at – 196 °C *via* nitrogen sorption.

SEM was performed using a Cambridge Instruments Stereoscan 90. All samples were sputter-coated in gold using a Polaron SC500A sputter coater prior to imaging. The mean particle diameter was obtained by manually measuring the diameter of a selection of the particles using iScan2000 imaging acquisition software, and obtaining the mean value.

4.2.3. Typical preparation of gel-type poly(DVB-*co*-VBC) microspheres by precipitation polymerisation

The polymer microspheres were synthesised using an adapted precipitation polymerisation protocol,[43] as outlined in Scheme 4.1.



Scheme 4.1: A schematic representation of the polymerisation of monomers DVB and VBC to yield polymer microspheres of poly(DVB-*co*-VBC)

A typical experimental procedure is given for polymer sample ref. KA93 (Procedure 4-i). All of the poly(DVB-*co*-VBC) copolymers shown in Table 4.1 were synthesised according to an analogous procedure.

Procedure 4-i

The comonomers DVB (8.206 mL, 7.5 g, 57.6 mmol) and VBC (20.776 mL, 22.5 g, 147.4 mmol), and initiator AIBN (0.8300 g, 5.1 mmol, 2 mol % relative to the polymerisable double bonds) were added to acetonitrile (1,500 mL) in a Nalgene® bottle (2 L) fitted with a screw cap. The solution was placed in an ultrasonic bath for 10 minutes and then deoxygenated by sparging with N_2 at 0 °C for a further 10 minutes. The bottle was then sealed under N_2 and placed on a low-profile roller in a temperature-controllable incubator. The temperature was ramped from ambient to 60 °C over a period of approximately 2 h and the polymerisation allowed to proceed at 60 °C for a further 46 h. The resulting milky suspension of white particles were separated from the reaction medium by filtration on a

0.2 µm nylon membrane filter, washed successively with acetonitrile, methanol, toluene and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (4.5151 g, 15 %).

The mean particle diameter was 3.3 µm.

Expected microanalysis: 76.0 % C, 6.5 % H, 0.5 % N and 17.0 % Cl

Found microanalysis: 77.1 % C, 6.3 % H, Trace N and 13.8 % Cl

Estimated potential ion-exchange capacity (Cl content from elemental microanalysis): 3.9 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3049, 2918, 2866, 2851, 1695, 1605, 1578, 1568, 1510, 1487, 1472, 1443, 1418, 1354, 1265 (Ar-CH$_2$-Cl wag), 1213, 1184, 1111, 1080, 1018, 988, 970, 912, 901, 835 (1,4-disubstituted ring), 822, 795, 744, 708 and 675 (C-Cl stretch).

BET specific surface area: < 1 m^2/g

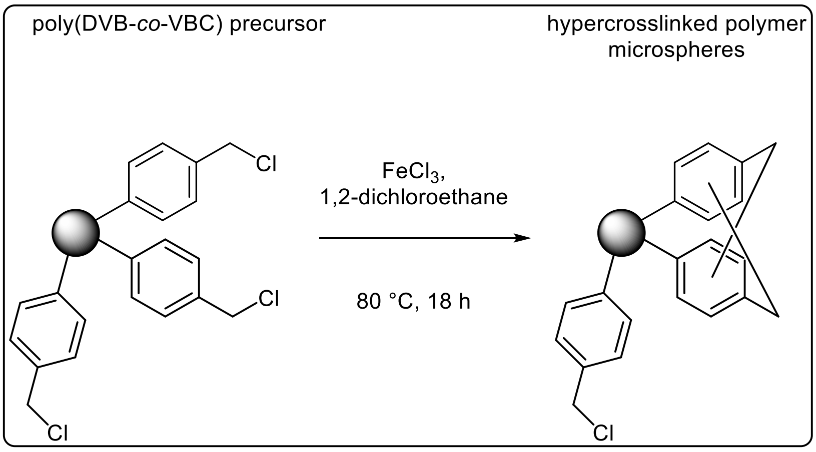
Mean pore diameter: n/a

Table 4.1: The reagent quantities and conditions used for the preparation of gel-type poly(DVB-*co*-VBC) polymer microspheres by precipitation polymerisation

Polymer ref.	DVB (g)	VBC (g)	AIBN (g)	Acetonitrile (mL)	Duration (h)	Yield (g)	Yield (%)
KA1	2.5	7.5	0.2796	500	47	2.2445	22
KA77	2.5	7.5	0.2783	500	48	2.0331	20
KA93	7.5	22.5	0.8300	1500	48	4.5151	15
KA113	7.5	22.5	0.8312	1500	48	7.7851	26
KA123	2.5	7.5	0.2769	500	48	2.6023	26

4.2.4. Typical preparation of hypercrosslinked polymer microspheres from poly(DVB-*co*-VBC)

The following hypercrosslinking procedure was derived from previous protocols used within the group.[43]



Scheme 4.2: A schematic representation of the hypercrosslinking of precursor poly(DVB-*co*-VBC) polymer microspheres to give hypercrosslinked polymer microspheres

A typical experimental procedure is given for polymer sample ref. KA103 (Procedure 4-ii). Polymer ref. KA3 shown in Table 4.2 was synthesised according to an analogous procedure.

Procedure 4-ii

Precursor particles KA93 (4.0109 g, 15.6 mmol of Cl) were added to DCE (80 mL) in a round-bottomed flask (250 mL) equipped with a reflux condenser and an overhead stirrer. The particles were left to swell under N_2 at room temperature for 1 hour. Iron (III) chloride (2.5439 g, 15.7 mmol) was dissolved in DCE (60 mL) and added to the mixture before heating to 80 °C for 18 hours. The resulting purple-coloured particles were isolated from the dark purple reaction medium by filtration, and washed successively with methanol, 2 M aqueous nitric acid, methanol and acetone. The now orange-coloured particles were washed further overnight with acetone in a Soxhlet extractor, followed by final filtration washes with methanol and diethyl ether on a 0.2 µm nylon membrane filter. The resulting particles were dried overnight in a vacuum oven at 70 °C/60 mbar (3.4367 g, 99 %).

The mean particle diameter was 3.9 µm.

Expected microanalysis: 92.9 % C, 7.1 % H, Trace N and 0.0 % Cl

Found microanalysis: 86.3 % C, 6.3 % H, Trace % N and 7.0 % Cl

Estimated potential ion-exchange capacity (Cl content from elemental microanalysis): 2.0 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3049, 2918, 2833, 1705, 1667, 1651, 1605, 1593, 1539, 1510, 1487, 1470, 1454, 1445, 1420, 1366, 1354, 1304, 1265 (Ar-CH_2-Cl wag), 1212, 1200, 1165, 1115, 1090, 1072, 1018, 966, 910, 889 (1,2,4,6-tetrasubstituted ring), 822, 793, 768, 712 and 683 (C-Cl stretch).

Langmuir specific surface area: 1,895 m^2/g

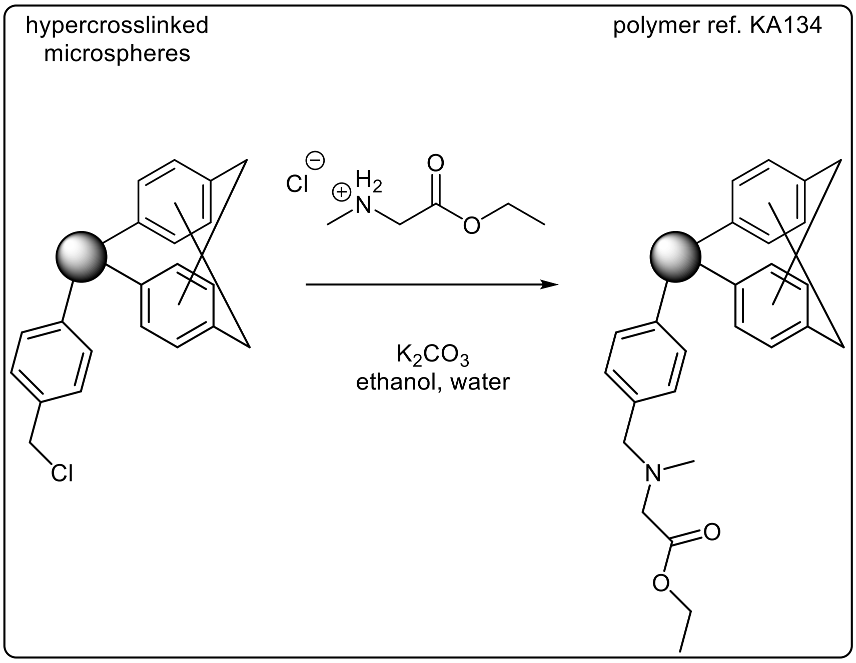
Mean pore diameter: 2.2 nm

Table 4.2: The reagent quantities and conditions used for the preparation of hypercrosslinked polymer microspheres

Polymer ref.	Precursor (g)	$FeCl_3$ (g)	1,2-dichloroethane (mL)	Duration (h)	Yield (g)	Yield (%)
KA3	1.2000 KA1	0.9300	60	18	0.7987	78
KA103	4.0109 KA93	2.5439	140	18	3.4367	99

4.2.5. Post-polymerisation modification of hypercrosslinked polymer microspheres using sarcosine ethyl ester hydrochloride

Hypercrosslinked polymer microspheres were modified using sarcosine ethyl ester hydrochloride as outlined in Scheme 4.3.



Scheme 4.3: Reaction scheme for the post-polymerisation modification of hypercrosslinked polymer microspheres with sarcosine ethyl ester hydrochloride

The experimental procedure is given for polymer sample ref. KA134 (Procedure 4-iii).

Procedure 4-iii

KA103 (1.5172 g, 1.97 mmol/g of chlorine, 2.99 mmol) and ethanol (40 mL) were added to a three-necked, round-bottomed flask (100 mL) fitted with an overhead mechanical stirrer and a reflux condenser. The contents of the flask were stirred for 1.5 h. Sarcosine ethyl ester hydrochloride (2.2959 g, 14.95 mmol) and potassium carbonate (2.0649 g, 14.94 mmol) were dissolved in distilled water (40 mL) and added to the flask. The mixture was heated to 75 °C, with stirring at 100 rpm, for 18 h. The reaction mixture was cooled to room temperature and the orange coloured particles filtered on a 0.2 µm nylon membrane filter, washed successively with ethanol (reaction solvent), methanol, a 1:1 mixture of methanol/water, aqueous sodium hydrogen carbonate, distilled water and acetone, before drying overnight in a vacuum oven at 70 °C/60 mbar (1.5020 g).

The mean particle diameter was 3.7 µm.

Expected microanalysis of KA134: 89.7 % C, 7.6 % H, 2.7 % N and 0.0 % Cl

Found microanalysis of KA134: 85.5 % C, 7.1 % H, 0.7 % N and 1.2 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.5 mmol/g

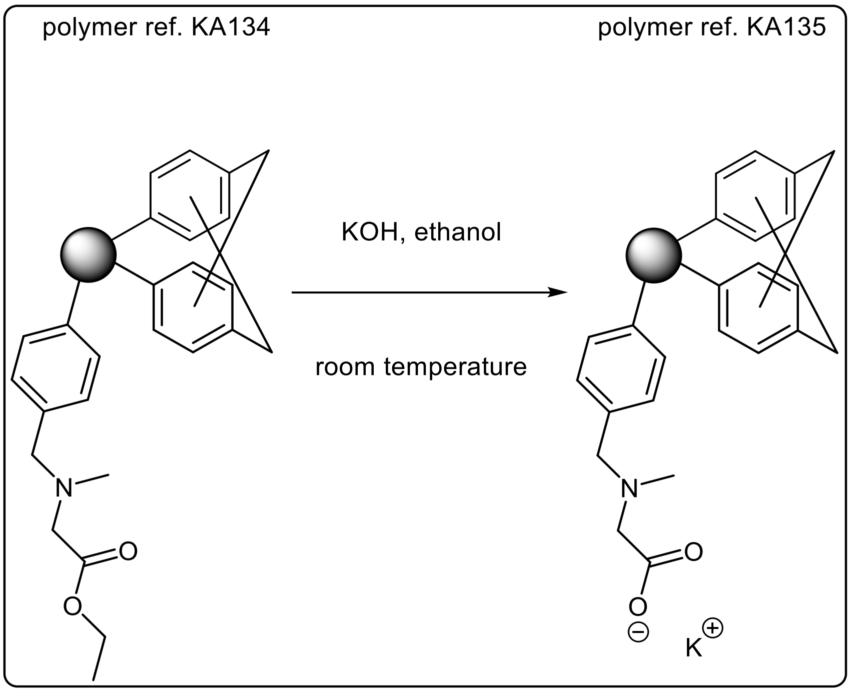
FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3015, 2918, 1859, 2779, 1792, 1734 (C=O stretch, ester), 1709, 1605, 1510, 1447, 1420, 1368, 1304, 1265 (Ar-CH$_2$-Cl wag), 1180 (C-O stretch, ester), 1113, 1051, 1018, 964, 889 (1,2,4,6-tetrasubstituted ring), 816, 795 and 710.

Langmuir specific surface area: 1,655 m^2/g

Mean pore diameter: 2.1 nm

4.2.6. Ester hydrolysis of the polymers to the amphoteric amino acid form

The ester functionalised polymer microspheres were modified further by base-catalysed ester hydrolysis in order to form the carboxylate anion, as outlined in Scheme 4.4.



Scheme 4.4: Reaction scheme for the base-catalysed ester hydrolysis of the ethyl ester on the polymer microspheres to the corresponding carboxylic acid

The experimental procedure is given for polymer sample ref. KA135 (Procedure 4-iv).

Procedure 4-iv

A potassium hydroxide solution was prepared by dissolving KOH (1.0161 g) in ethanol (20 mL). KA134 (0.6362 g) and the KOH solution were added to a sealed glass Kimax tube and agitated gently on a low-profile roller within a temperature controllable incubator at room temperature for 24 hours. The particles were filtered on a 0.2 μm nylon membrane filter, and washed thoroughly with ethanol before drying overnight in a vacuum oven at 70 °C/60 mbar (0.6305 g).

The mean particle diameter was 3.7 μm.

Found microanalysis of KA135: 79.4 % C, 7.1 % H, 0.6 % N and 1.1 % Cl

Estimated ion-exchange capacity (N content from elemental microanalysis): 0.4 mmol/g

FT-IR: $\bar{\nu}$/cm^{-1} (ATR): 3046, 3015, 2970, 2920, 2855, 1761, 1728, 1674, 1603 (asymmetric –CO_2^- stretch), 1510, 1447, 1418, 1371, 1362, 1315 (symmetric –CO_2^- stretch), 1267 (Ar-CH_2-Cl wag), 1182 (C-O stretch), 1169, 1113, 1018, 964, 891 (1,2,4,6-tetrasubstituted ring), 816, 795 and 710.

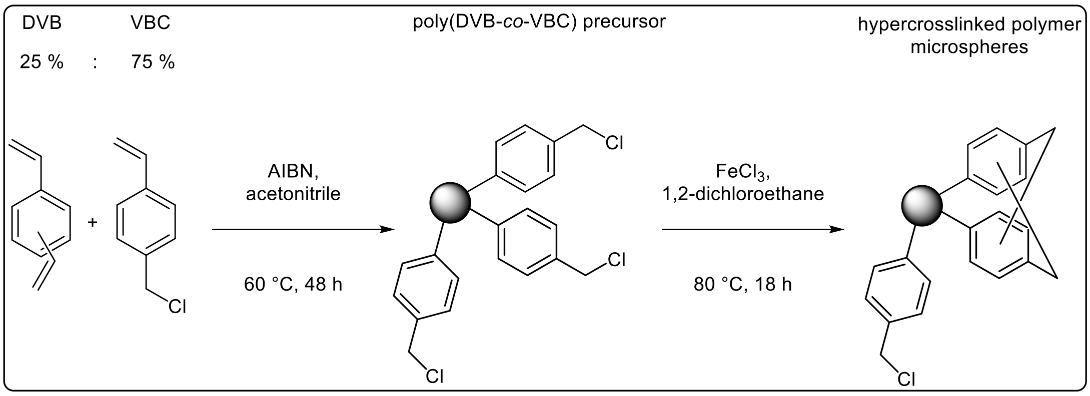
Langmuir specific surface area: 1,473 m^2/g

Mean pore diameter: 2.1 nm

4.3. Results and discussion

Polymer microspheres with particle diameters in the low micron size range are useful materials that have found application as sorbents in SPE. The post-polymerisation chemical modification of polymer microspheres imparts functionality that can be utilised to selectively extract pollutants from environmental samples. Poly(DVB-*co*-VBC) microspheres were synthesised and hypercrosslinked using Friedel-Crafts chemistry, and subsequently treated with an amino acid derived ester, sarcosine ethyl ester. Amino acid derived esters contain ionisable nitrogen moieties that can be exploited in anion-exchange, and the ester functionality can be hydrolysed to a carboxylic acid to be exploited in cation-exchange. Therefore a single sorbent has the potential to contain two ionisable moieties for exploitation as both an anionic- and cationic-exchange SPE sorbent.

A two-step synthetic protocol was required to prepare the microporous microspheres, and this is shown in Scheme 4.5. The route to the intermediate hypercrosslinked polymer microspheres was derived from an optimised protocol developed previously within our group.[43]

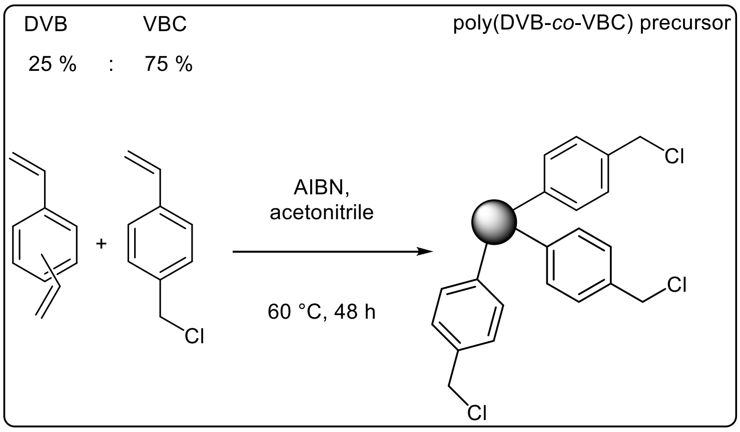


Scheme 4.5: Outline of the two-step synthetic protocol used to synthesise hypercrosslinked polymer microspheres from monomers DVB and VBC

4.3.1. Synthesis of gel-type poly(DVB-*co*-VBC) microspheres

As discussed previously in Chapter 3, precipitation polymerisation was the heterogeneous polymerisation technique chosen to synthesise polymer microspheres of poly(DVB-*co*-VBC). In this chapter, gel-type polymer particles that have a low particle diameter in the micron size range, and a narrow particle size distribution, were targeted. These gel-type particles will act as the precursor species in the subsequent hypercrosslinking reaction, discussed in Section 4.3.2.

It is necessary to include a crosslinking species in the monomer feed to generate polymer microspheres by precipitation polymerisation. The crosslinking monomer divinylbenzene (DVB) was copolymerised with the functional monomer 4-vinylbenzyl chloride (VBC) in a 25:75 (w/w) feed ratio, as depicted in Scheme 4.6. The low DVB content is sufficient to produce lightly crosslinked polymer microspheres, and the high content of pendent, reactive chloromethyl residues from VBC is necessary to participate in post-polymerisation hypercrosslinking *via* Friedel-Crafts chemistry for high specific surface area polymers to be generated.



Scheme 4.6: Outline of the synthetic procedure used to synthesise gel-type poly(DVB-*co*-VBC) microspheres by precipitation polymerisation

Polymer characterisation by elemental microanalysis and FT-IR spectroscopy

The composition of the poly(DVB-*co*-VBC) copolymers was confirmed by elemental microanalysis and FT-IR spectroscopy. The values obtained from elemental microanalysis, shown in Table 4.3, were compared to the expected values that were calculated on the assumption that all monomer added would polymerise to generate the poly(DVB-*co*-VBC) copolymer.

The chlorine content of the polymers reflects the level of VBC that has been incorporated from the monomer feed. The high level of VBC in the monomer feed (75 % w/w) equates to a predicted 17 % chlorine in the poly(DVB-*co*-VBC) copolymer, which would be achieved if both the DVB and VBC polymerised together according to the ratio of the feed and the monomer conversion was 100 %. The results shown in Table 4.3 show that the actual chlorine content of all the polymers prepared is lower than expected. This could be due to a higher proportion of DVB to VBC being incorporated into the copolymer than the 25:75 (w/w) monomer loading ratio, known as composition drift, backed up by a higher percentage of carbon in the products than their expected value of 76 %. The yield of polymer was not 100 %; therefore the assumption in the calculation that all monomer was fully converted to polymer is also untrue. It should be noted that there are errors associated with elemental microanalysis, and the results shown here do not tally up to exactly 100 %.

Table 4.3: The elemental microanalysis results and ion-exchange capacities of poly(DVB-*co*-VBC) microspheres prepared by precipitation polymerisation

Sample ref.	Elemental Microanalysis					IEC
	% C	% H	% N	% Cl	% O	mmol/g Cl
Expected values	**76.0**	**6.5**	**0.5**	**17.0**	**0.0**	**4.8**
KA1	77.4	6.6	0.3	15.2	-	4.3
KA77	77.8	6.5	0.4	13.1	-	3.7
KA93	77.1	6.3	Trace	13.8	-	3.9
KA113	77.5	6.5	0.3	11.4	-	3.2
KA123	77.6	6.5	0.3	13.9		3.9

The assignment of characteristic bands in the FT-IR spectra of the polymers is a useful method of establishing which functional groups are present within the sample. In the spectra obtained for gel-type poly(DVB-*co*-VBC) there were intense bands present that correspond to the presence of chloromethyl groups within the polymers, namely the Ar-CH_2-Cl wag at 1,265 cm^{-1} and the C-Cl stretching vibration at 677 cm^{-1}. The unreacted vinyl groups on DVB also gave rise to out of plane C-H bending vibrations associated with mono-substituted unsaturated C=C bonds at 988 cm^{-1} and 901 cm^{-1}. These characteristic bands confirm that both monomers have been incorporated in the copolymer of poly(DVB-*co*-VBC). VBC has a 1,4-disubstituted aromatic ring, and DVB is a mixture of the 1,3- and 1,4-disubstituted isomers. There is a strong signal at 835-822 cm^{-1} that corresponds to the substitution pattern of a 1,4-disubstituted ring.

Measured porosity of gel-type poly(DVB-co-VBC) microspheres

Nitrogen sorption analysis was used to investigate the pore structure of the gel-type poly(DVB-*co*-VBC) particles. The information obtained, shown in Table 4.4, includes measurement of the specific surface area, pore volume and pore diameter.

Due to the modest loading of crosslinking monomer DVB, at 25 % (w/w) relative to the functional monomer VBC, the resulting polymers are lightly crosslinked particles that do not possess a permanent pore structure in the dry state. Across all of these samples, the specific surface area was measured as < 5 m^2/g, and the pore volume was very low. In Table 4.4, n/a has been used to denote pore volume and pore size data that was not computed since there were too few points in the sorption isotherm to satisfy the calculations. Gel-type polymers prepared by precipitation polymerisation are typically non-porous, but swellable in thermodynamically good solvents.

Table 4.4: The nitrogen sorprtion analysis data for poly(DVB-*co*-VBC) microspheres prepared with a 25:75 (w/w) ratio of monomers DVB and VBC

Sample ref.	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA1	4	+81	7	0.009	15
KA77	< 1	-10	< 1	n/a	n/a
KA93	< 1	-12	< 1	n/a	n/a
KA113	2	+214	3	0.002	4.1
KA123	< 1	-13	< 1	n/a	n/a

The morphology of poly(DVB-co-VBC)

Scanning electron microscopy (SEM) was used to observe the morphology of the polymer particles and provide an estimate of the particle size and particle size distribution because the quality of the microspheres is an important parameter for packing into SPE cartridges and columns. Homogeneous, small particles are preferred for reasons of improved efficiency by better packing and minimised diffusion. The SEM micrographs captured for sample KA93 are shown in Figure 4.1. The copolymer particles have a spherical morphology, although there is a bumpy, cauliflower-like imperfection visible on the surfaces of some of the particles. This bumpy surface has been observed previously for precipitation polymerisation particles that have been prepared with low levels of crosslinker, and has been attributed to an extended homo-coagulation.[18] The mean particle diameter of KA93 is in the low micron size range that had been targeted, at 3.3 μm. Although the polymerisation has produced a mixture of differently sized particles, the particle size distribution is quite narrow and typical for this gel-type poly(DVB-*co*-VBC) composition.

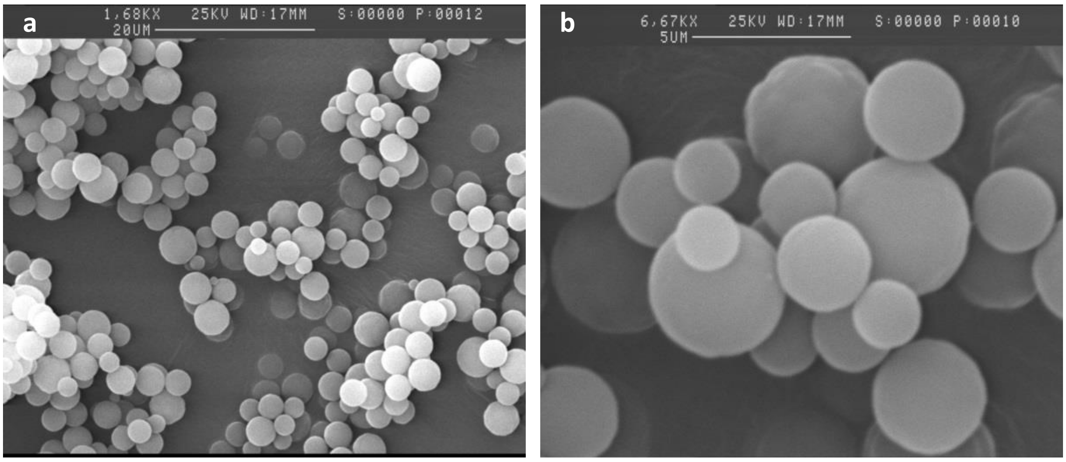
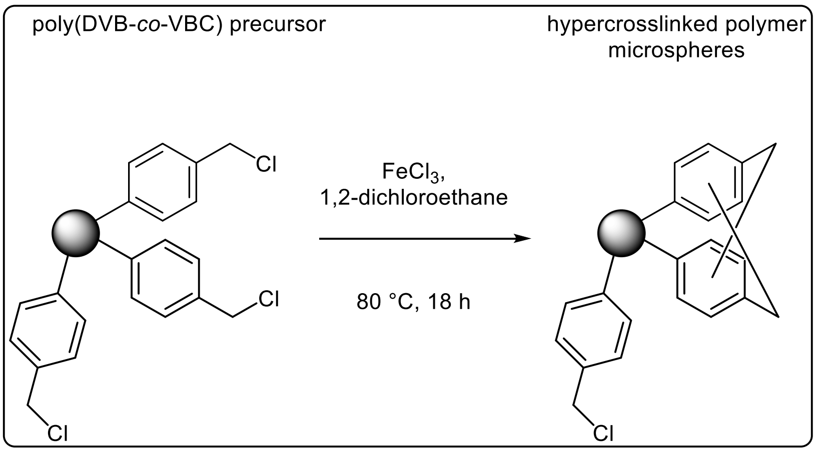


Figure 4.1: SEM micrographs of poly(DVB-*co*-VBC) sample KA93 at 1,680x (a) and 6,670x magnification (b)

4.3.2. Hypercrosslinking of poly(DVB-*co*-VBC) microspheres

In the second stage of the two-step procedure to hypercrosslinked polymer microspheres, the poly(DVB-*co*-VBC) precursor was swollen to an expanded state in an excess of DCE. After the poly(DVB-*co*-VBC) microspheres had been expanded, iron (III) chloride ($FeCl_3$) was added to catalyse the formation of crosslinking methylene bridges between neighbouring aromatic rings *via* Friedel-Crafts chemistry. Scheme 4.7 shows an outline of the reaction scheme for the hypercrosslinking of poly(DVB-*co*-VBC) microspheres.



Scheme 4.7: An outline of the reaction scheme for the hypercrosslinking of gel-type poly(DVB-*co*-VBC) microspheres in the presence of $FeCl_3$ and 1,2-dichloroethane

Confirmation of hypercrosslinking by elemental microanalysis and FT-IR spectroscopy

Upon hypercrosslinking, it was expected that the chlorine content of the polymer microspheres would decrease as the chloromethyl residues are consumed by reaction. The expected values given in Table 4.5 are based on the assumption that 100 % of the chlorine atoms are lost through the Friedel-Crafts reaction with $FeCl_3$. The resulting polymer would therefore be composed of only carbon, hydrogen, and trace levels of nitrogen derived from the AIBN used during synthesis of the poly(DVB-*co*-VBC) precursor polymer. In practice, however, it is not possible to engage all of the pendent chloromethyl residues in reaction due to difficulties in accessing all of the functional groups. During the crosslinking and polymerisation of DVB and VBC, the unreacted vinyl and chloromethyl groups are distributed throughout the entire body of the polymer microsphere. Therefore, there will undoubtedly be some functional groups that are inaccessible to reagents in post-polymerisation chemical modifcation reactions.

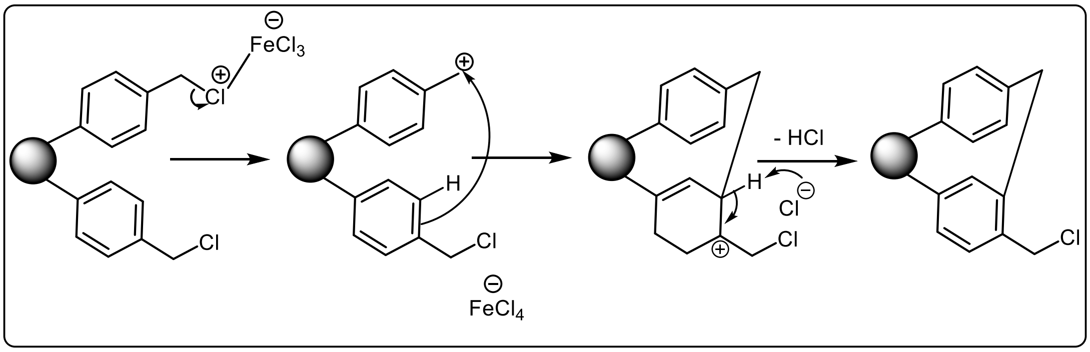
Table 4.5 shows the measured elemental composition of the hypercrosslinked polymers. The % Cl has decreased from 15.2 % in precursor KA1 to 4.4 % in hypercrosslinked KA3, and from 13.8 % (15.6 mmol/g) in precursor KA93 to 7.0 % Cl (2.0 mmol/g) in hypercrosslinked product KA103. These values reflect the level of residual VBC moieties present in the microspheres that have not been consumed by hypercrosslinking. In the literature, the hypercrosslinking of poly(DVB-*co*-VBC) microspheres synthesised under similar conditions led to a decrease in the % Cl from 14.5 % to 4.5 % Cl.[43]

Table 4.5: The elemental microanalysis and ion-exchange capacities of the hypercrosslinked poly(DVB-*co*-VBC) microspheres

Sample ref.	Elemental Microanalysis					IEC
	% C	% H	% N	% Cl	% O	mmol/g Cl
Expected values	**92.9**	**7.1**	**Trace**	**0.0**	**0.0**	-
KA3	85.7	6.3	0.3	4.4	-	1.2
KA103	86.3	6.3	0.3	7.0	-	2.0

Evidence of successful hypercrosslinking can also be inferred by examination of the FT-IR spectrum of KA103, and comparing this to the spectrum of the poly(DVB-*co*-VBC) precursor

KA93. The hypercrosslinking mechanism, shown in Scheme 4.8, shows the transformation from poly(DVB-*co*-VBC) to the hypercrosslinked product.



Scheme 4.8: A representation of the hypercrosslinking mechanism for poly(DVB-*co*-VBC). Poly(DVB-*co*-VBC) (left) undergoes electrophilic aromatic substitution to create new C-C bonds and produce a hypercrosslinked product (right)

The FT-IR spectrum of the precursor poly(DVB-*co*-VBC) particles has an intense signal at 835-822 cm^{-1} corresponding to a 1,4-disubstituted aromatic ring, where VBC is 1,4-disubstituted and DVB is a mixture of 1,3- and 1,4-disubstituted isomers.

Upon hypercrosslinking, the FT-IR spectrum shows a decrease in the intensity of the 822 cm^{-1} band, and the appearance of a new signal at 898 cm^{-1} which corresponds to higher substitution on the aromatic rings. Hypercrosslinking creates new C-C bonds between the aromatic rings, methylene bridging, therefore increasing the substitution of the aromatic rings from disubstituted to tri- or tetrasubstituted. The loss of chloride from the pendent chloromethyl residues of VBC is confirmed by the substantial decrease in the intensity of the 1,265 cm^{-1} and 683 cm^{-1} signals, relative to the poly(DVB-*co*-VBC) precursor.

Measured porosity of hypercrosslinked poly(DVB-co-VBC) microspheres

Nitrogen sorption analysis was the technique utilised to obtain information about the pore structure of the hypercrosslinked particles, namely the specific surface area, pore volume and average pore diameter.

The isotherm generated for sample KA103 is shown in Figure 4.2, and its shape is typical of a Type I isotherm, also known as a Langmuir isotherm. The most appropriate model of gas adsorption will be the Langmuir model, which assumes monolayer coverage, as opposed to multilayer coverage on a surface that is modelled by the BET model.

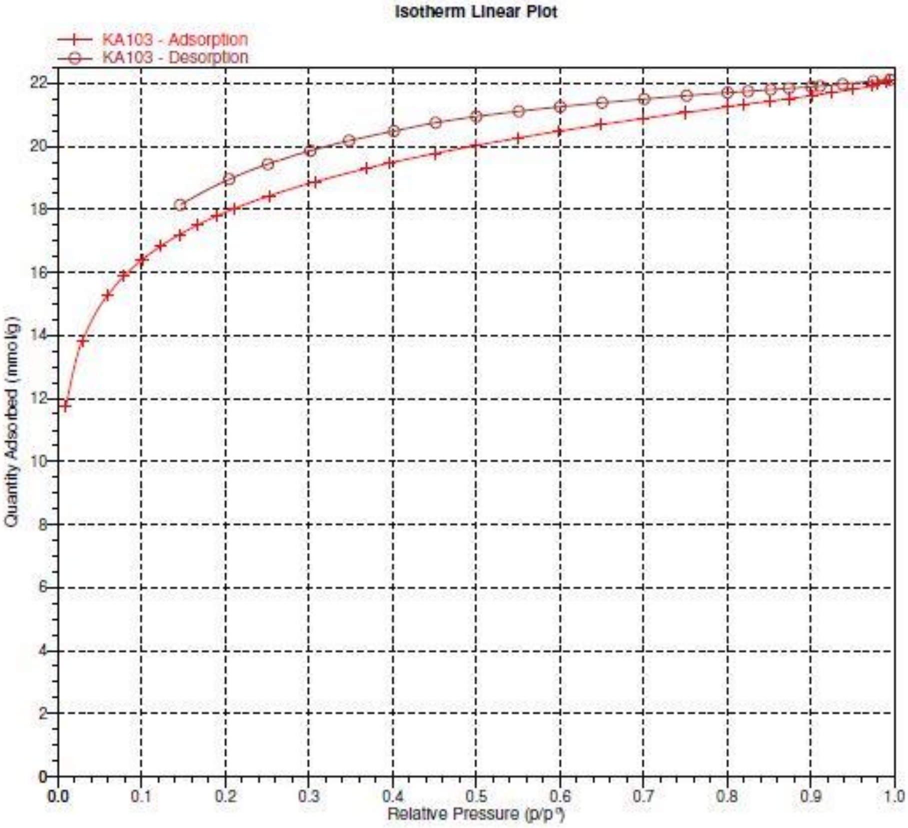


Figure 4.2: The isotherm of quantity of N_2 adsorbed against relative pressure for hypercrosslinked sample KA103

The specific surface area, pore volume and average pore size are tabulated and shown in Table 4.6. The results show that ultra-high specific surface areas have been achieved through hypercrosslinking, with sample KA103 achieving a calculated specific surface area value of 1,895 m^2/g and a pore volume of 0.767 cm^3/g. This pronounced increase in the specific surface area serves as clear confirmation that the pore structure of the precursor polymer has changed dramatically due to hypercrosslinking. A new material has been produced that possesses a rigid, 3-dimensional and permanently porous network.

The IUPAC classification for physisorption states that pore widths between 2 and 50 nm are called mesopores and pore widths not exceeding about 2 nm are called micropores.[24] As presented in Table 4.6, the average pore diameter of 2.2 nm for sample KA103 is right on the borderline between meso- and microporous.

Table 4.6: The nitrogen sorption analysis data for hypercrosslinked samples KA3 and KA103

Sample ref.	Precursor poly(DVB-*co*-VBC)	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA3	KA1	1,046	-54	1,557	0.598	2.4
KA103	KA93	1,391	-347	1,895	0.767	2.2

The morphology of hypercrosslinked particles

The quality of the hypercrosslinked particles shown in Figure 4.3 was dependent on the precursor poly(DVB-*co*-VBC). The SEM micrographs show that the post-polymerisation hypercrosslinking reaction has not resulted in any deterioration of the particle quality since the particles appear unchanged from their precursor. The measured mean particle diameter of 3.9 μm falls within the targeted size range (slightly larger than the precursor particles with a mean particle diameter of 3.3 μm) and the size distribution was narrow.

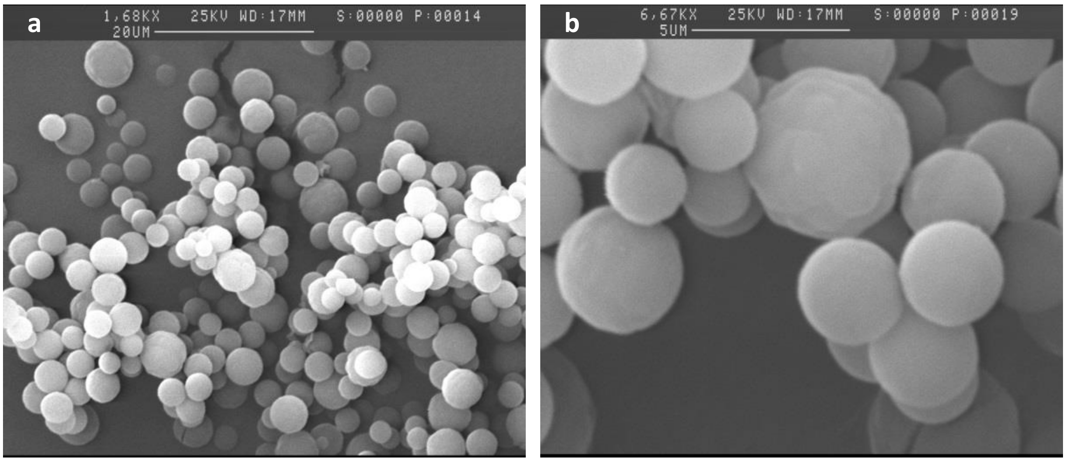
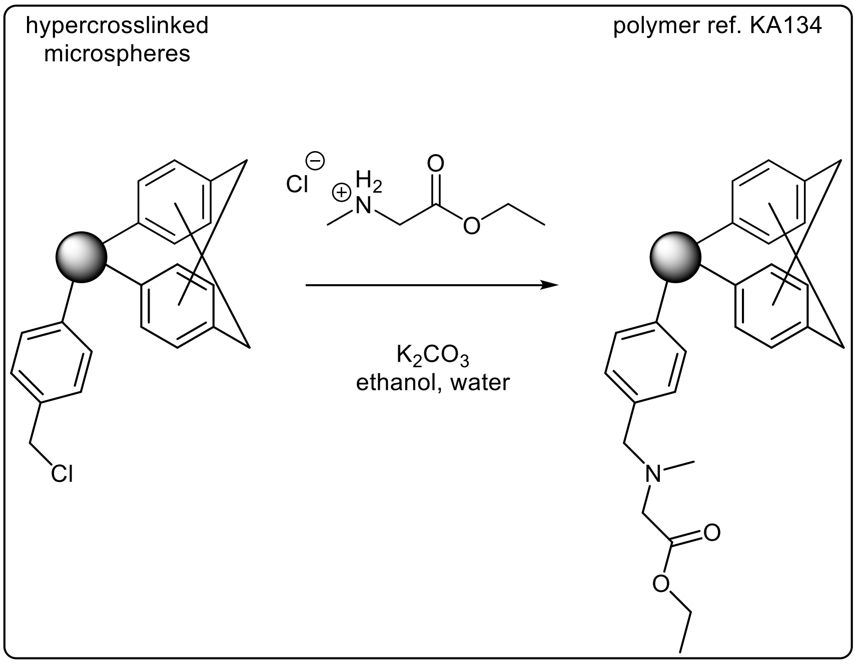


Figure 4.3: SEM micrographs of hypercrosslinked poly(DVB-*co*-VBC) KA103 at 1,680x (a) and 6,670x magnification (b)

4.3.3. Post-polymerisation chemical modification of hypercrosslinked poly(DVB-*co*-VBC) using sarcosine ethyl ester

The post-polymerisation chemical modification of poly(DVB-*co*-VBC) with sarcosine ethyl ester was discussed in Chapter 3. In this chapter, this chemistry has been applied to gel-type poly(DVB-*co*-VBC) copolymers that have undergone post-polymerisation hypercrosslinking. These hypercrosslinked polymers are a distinct family of materials that possess ultra-high specific surface areas and a high micropore content. The reaction scheme for the functionalisation of hypercrosslinked polymer KA134 with sarcosine ethyl ester hydrochloride is presented in Scheme 4.9.

Although the hypercrosslinking reaction consumed many of the chloromethyl groups in the process of creating the crosslinking C-C bridges, it is the unreacted chloromethyl groups that serve as a functional handle and allow the polymer to be further modified, int his case using nucleophilic aliphatic substitution reactions.



Scheme 4.9: Reaction scheme for the post-polymerisation chemical modification of poly(DVB-*co*-VBC) with sarcosine ethyl ester hydrochloride

Evidence of functionalisation by elemental microanalysis and FT-IR spectroscopy

The change in composition of the hypercrosslinked particles after functionalisation with sarcosine ethyl ester hydrochloride was confirmed by elemental microanalysis and FT-IR spectroscopy.

The expected microanalysis values were calculated based on some assumptions, so the values represent a guide composition but not a true prediction. It was not possible to base this calculation upon the expected elemental microanalysis results for the precursor hypercrosslinked sample KA103, because those values were generated using the assumption that there would be no chlorine remaining after hypercrosslinking. This was not the case, and in fact the residual Cl residues that were not consumed during the hypercrosslinking reaction provide the functional handle for this subsequent chemical modification with sarcosine ethyl ester hydrochloride. Therefore, the expected elemental microanalysis values for KA134 have been generated from the found results of sample KA103. The oxygen content was not measured by elemental microanalysis, so it has been assumed that there was 0 % oxygen in the hypercrosslinked sample KA103 prior to functionalisation with sarcosine ethyl ester hydrochloride. Finally, it was assumed that all of the chloromethyl moieties present in the hypercrosslinked sample KA103 would be consumed and the functionalised product KA134 would contain no chloride residues.

The expected and found elemental microanalysis compositions for sample KA134 are shown in Table 4.7. The hypercrosslinked precursor contained trace levels of nitrogen, and 7.0 % of chlorine. After the chemical modification with sarcosine ethyl ester hydrochloride, to generate product KA134, the nitrogen content increased to 0.7 % and the chlorine content decreased to 1.2 %. This change in elemental composition confirms that amine moieties have been incorporated within the particle by substitution of the chloride, and suggests that the functionalisation with sarcosine ethyl ester hydrochloride has been successful although not at full conversion.

The elemental microanalytical data provides an indication of the maximum nitrogen content that may be available for ion-exchange. As shown in Table 4.7, a nitrogen content of 0.7 % equates to 0.5 mmol of nitrogen to be exchanged per gram.

Table 4.7: The elemental microanalysis results and ion-exchange capacity for sample KA134

Sample ref.	Elemental Microanalysis					IEC
	% C	% H	% N	% Cl	% O	mmol/g N
Expected values	84.8	7.2	2.6	0.0	5.5	1.9
KA134	85.5	7.1	0.7	1.2	-	0.5

The FT-IR spectrum of sample KA134 is presented in Figure 4.4. The intensity of the chloromethyl-derived bands at 1,265 cm^{-1} and 683 cm^{-1} from hypercrosslinked sample KA103 have been diminished, and a new, strong band ascribed to carbonyl stretching of the ester has appeared in the spectrum of KA134 at 1,734 cm^{-1}. The strong, broad C-O stretch that has appeared at 1,180 cm^{-1} is further confirmation of the presence of an ester in the product

The changes observed in the elemental microanalysis and the FT-IR spectra show that sample KA134 is distinct from hypercrosslinked sample KA103, and it has been modified successfully with sarcosine ethyl ester hydrochloride.

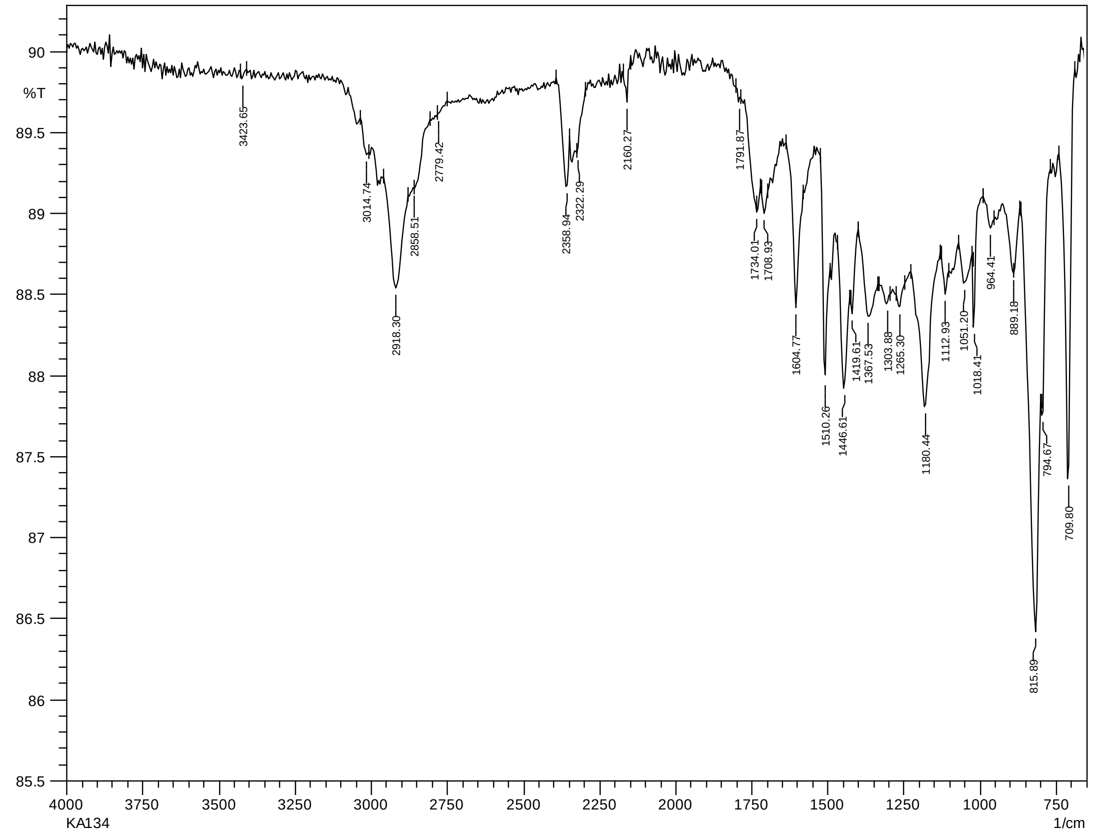


Figure 4.4: FT-IR spectrum of KA134

Measured porosity of the hypercrosslinked particles after functionalisation

The pore structure of the particles was investigated by nitrogen sorption analysis after functionalisation with sarcosine ethyl ester hydrochloride, and the results are shown in Table 4.8. The Langmuir specific surface area of sample KA134 was measured as 1,655 m^2/g, which showed that the ultra-high specific surface area observed for the hypercrosslinked precursor KA103 had been retained. The pore volume of 0.654 cm^3/g and average pore diameter of 2.1 nm are to be expected, and confirm that there has been no unfavourable changes to the measured dry-state porosity of the polymer microspheres.

Table 4.8: The nitrogen sorption analysis data for the hypercrosslinked polymer after functionalisation with sarcosine ethyl ester hydrochloride

Sample ref.	Precursor	BET specific surface area (m^2/g)	c	Langmuir specific surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
KA134	KA103	1,219	-261	1,655	0.654	2.1

The morphology of the particles after functionalisation

The SEM micrographs of KA134 shown in Figure 4.5 show that the post-polymerisation chemical modification with sarcosine ethyl ester has not affected the quality of the microspheres from their precursor KA103. This is important because the materials have been designed to be spherical, with a small particle size distribution in the low micron size range for application as polymeric sorbents in SPE. The mean particle diameter of KA134 is 3.7 µm.

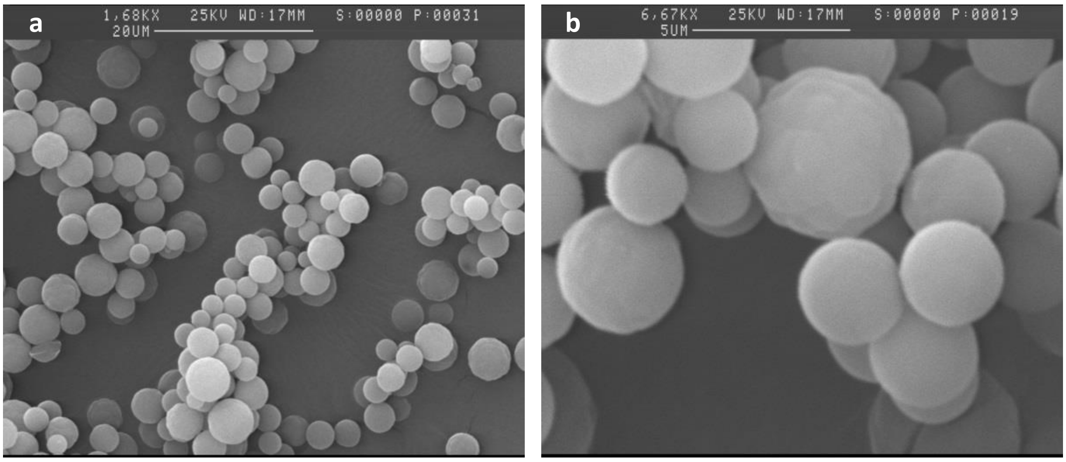
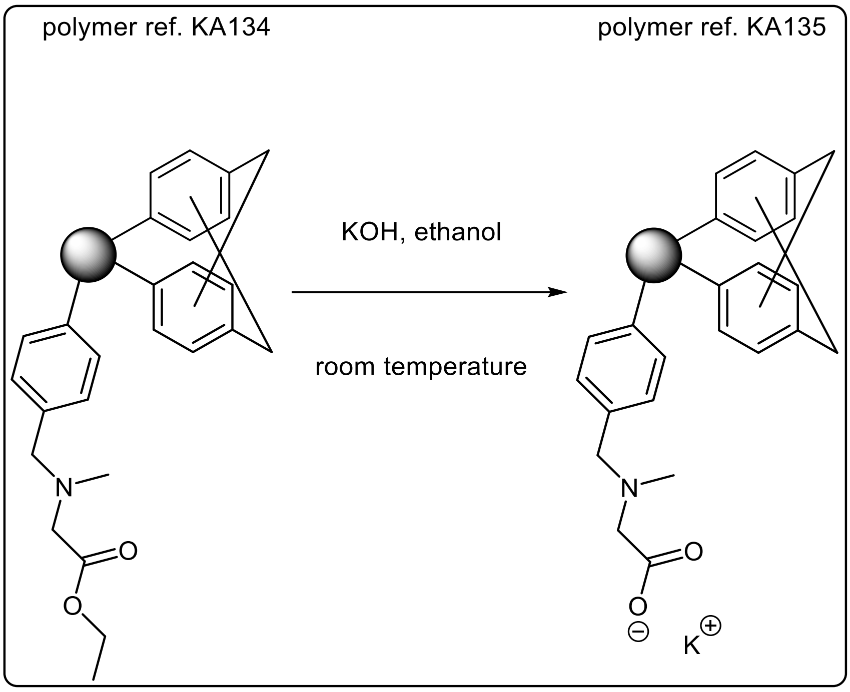


Figure 4.5: SEM micrographs of sample KA134 at 1,680x (a) and 6,670x magnification (b)

4.3.4. Ester hydrolysis of the polymers to the amphoteric amino acid form

The conversion of the amino acid derived esters to the amino acid form by ester hydrolysis was introduced in Chapter 3. Here, the hypercrosslinked polymer particles with pendent sarcosine ethyl ester moieties have been hydrolysed to produce the amphoteric form containing both amine and acid functionality. One motivation for this work is that the preparation of polymer microspheres with pendent amino acids offers a route into polymeric sorbents that contain both anionic and cationic character for mixed-mode solid-phase extraction sorbents. A base-catalsyed ester hydrolysis was performed using potassium hydroxide in ethanol, as shown in Scheme 4.10.



Scheme 4.10: Reaction scheme for the base-catalysed ester hydrolysis of the ethyl ester on the polymer microspheres to the carboxylate anion

The functional groups within the polymer microspheres may be detected by FT-IR spectroscopy; therefore, this is a useful characterisation tool to monitor the success of the reaction. The formation of a carboxylate anion results in asymmetric and symmetric C=O stretching in the FT-IR spectrum that is distinct from the C=O stretch of an ester. The characteristic wavenumbers quoted for these signals are 1,610-1,550 cm^{-1} for the asymmetric stretch and 1,420-1,300 cm^{-1} for the symmetric stretch.[128] The FT-IR spectra of KA134 and KA135 are presented in Figure 4.6 and Figure 4.7, respectively.

The C=O stretch at 1,734 cm^{-1} that was assigned to the ester is absent in the spectrum of KA135, and the intense band at 1,180 cm^{-1} for C-O stretching has decreased in intensity. These results suggest that the ester moiety has been hydrolysed. The broadening of the peak at 1,603 cm^{-1} is due to asymmetric carboxylate ion stretching, and the increase in the intensity of the signal at 1,315 cm^{-1} is due to symmetric carboxylate ion stretching.

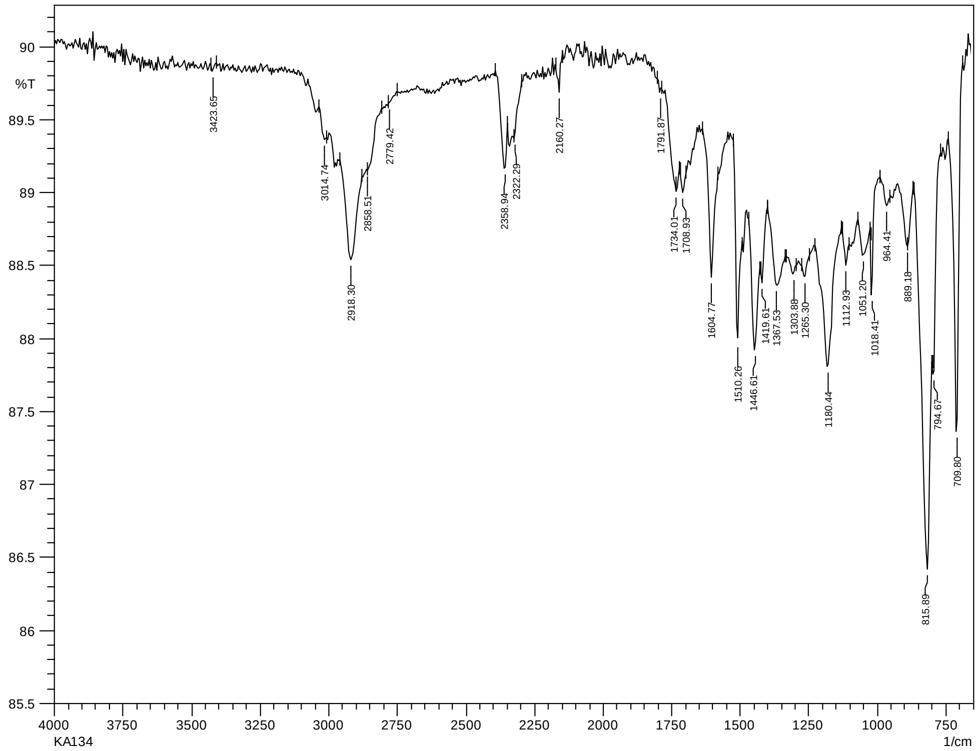


Figure 4.6: The FT-IR spectrum of KA134

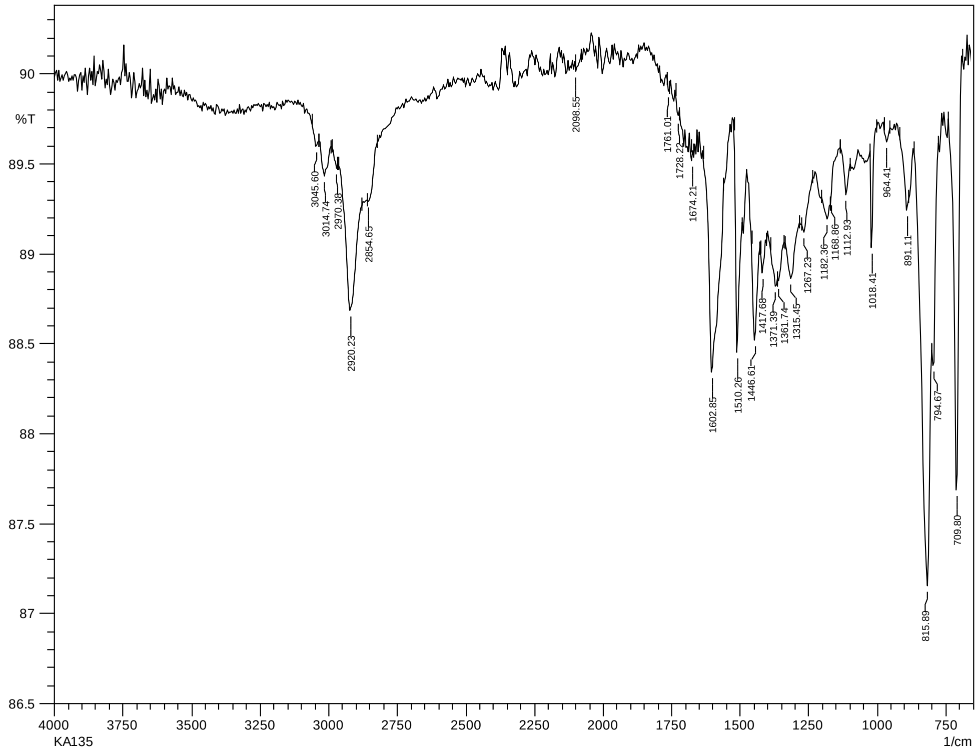


Figure 4.7: The FT-IR spectrum of KA135

The high specific surface area associated with hypercrosslinked polymers has been maintained. Nitrogen sorption analysis has measured the Langmuir specific surface area as 1,473 m^2/g, the pore volume as 0.573 cm^3/g and the average pore diameter as 2.1 nm. These results confirm that the hydrolysis reaction has not interfered with the rigid, highly porous network set in place through hypercrosslinking.

The morphology of the particles was examined by SEM, before and after the hydrolysis reaction. The SEM micrographs show that the reaction has not had any detrimental effect on the quality of the particles because they appear unchanged. The mean particle diameter is 3.7 µm.

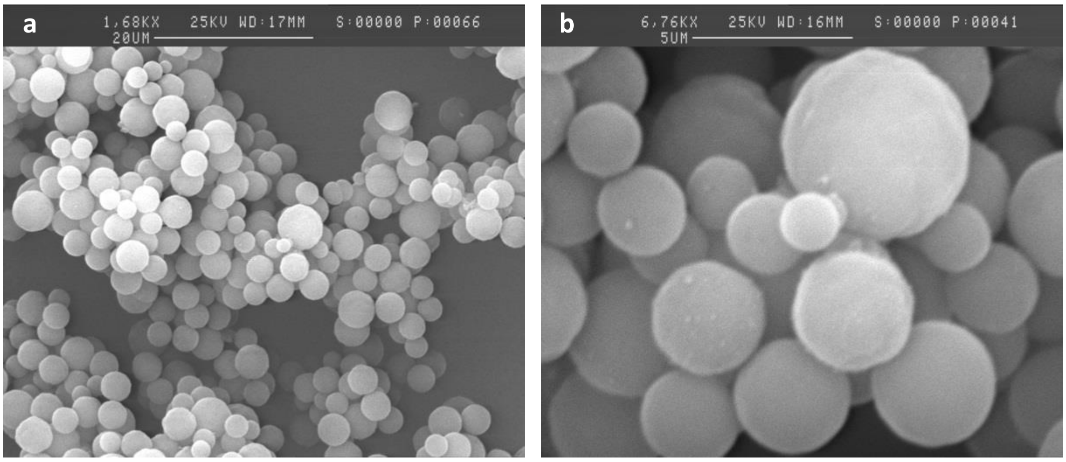


Figure 4.8: SEM micrographs of sample KA135 at 1,680x (a) and 6,670x magnification (b)

4.4. Chapter conclusions

Gel-type polymer microspheres of poly(divinylbenzene-*co*-vinylbenzyl chloride) were synthesised by precipitation polymerisation from a monomer loading of 25% divinylbenzene and 75% vinylbenzyl chloride. The gel-type particles do not possess any permanent porosity with essentially zero specific surface area in the dry state, and have a spherical morphology with some bumps that have been ascribed to homo-coagulation.

The gel-type particles were swollen and subjected to hypercrosslinking by Friedel-Crafts chemistry using $FeCl_3$. Elemental microanalysis was used to monitor the disappearance of chlorine during hypercrosslinking, and one typical example shows the available Cl decreasing from 15.6 mmol/g to 2.0 mmol/g. The appearance of signals in the FT-IR spectrum ascribed to both methylene bridging and higher substitution on the aromatic rings confirms that C-C bond formation has occurred, and the vibration associated with –CH_2Cl moieties from the vinylbenzyl chloride in the polymer decreased in intensity. A key feature of hypercrosslinked polymers is their ultra-high specific surface area, and values up to 1,895 m^2/g have been measured by nitrogen sorption porosimetry analysis to show that the polymers have a porosity characteristic of hypercrosslinked species.

The unreacted chloromethyl moieties that were not consumed during hypercrosslinking, up to 2 mmol/g of Cl, were reacted with sarcosine ethyl ester hydrochloride in the presence of a base to form hypercrosslinked polymers with pendent sarcosine ethyl ester functionality. The polymeric product was shown to contain 0.3 mmol/g of Cl and 0.5 mmol/g of N. Compared to the functional polymers produced in Chapter 3, this class of polymers have a much higher specific surface area and higher micropore content. The specific surface area of the particles after chemical modification with sarcosine ethyl ester was 1,655 m^2/g, and a pore volume of 0.65 cm^3/g was measured. This pore capacity is desirable for using these materials as SPE sorbents, and the functionality imparted from the tertiary amine moieties allows them to be exploited as selective weak anion-exchangers. The mean particle diameter of 3.7 µm is in the appropriate size range suited to SPE sorbents, and the narrow particle size distribution leads to better packing of the microspheres in a cartridge. The particles are not perfectly spherical and uniform because of the bumpy cauliflower like coagulation on the surfaces that was formed during the precipitation polymerisation of the monomers in the earlier stage of the process.

The hypercrosslinked polymers with pendent sarcosine ethyl ester moieties were hydrolysed using potassium hydroxide to convert the ester to a carboxylic acid functional group. The presence of both an amine and a carboxylic acid within the polymer particles enable it to be used like an amino acid, and the ionisation state of the polymer is dependent on pH. Under acidic conditions the nitrogen will be protonated to act as a weak anion-exchange resin, whilst under basic conditions the acid will be deprotonated to act as a weak cation-exchange resin. The presence of the carboxylate species was observed by FT-IR spectroscopy.

The particles produced in this chapter have been evaluated in Chapter 5 as SPE sorbents for the selective extraction of organic compounds from aqueous samples.

4.5. Future work

Although not critical, the precipitation polymerisation of divinylbenzene and 4-vinylbenzylchloride could be investigated to improve the particle morphology. It would be an improvement to produce perfectly spherical particles free of imperfections, and also lower the particle size distribution in favour of monodisperse particles. The subsequent hypercrosslinking and chemical modification steps do not alter the quality of the particles, so the improvements would need to be made during the formation of the gel-type polymer.

The post-polymerisation chemical modification of the hypercrosslinked particles using sarcosine ethyl ester hydrochloride followed a protocol described in Chapter 3. It would be useful to functionalise hypercrosslinked particles with different amino acid derived esters by a similar procedure to generate a family of functional resins that could be applied as SPE sorbents.

Chapter 5 - Application of polymer microspheres as solid-phase extraction sorbents for the selective extraction of pharmaceuticals from water

5.1. Introduction

Solid-phase extraction (SPE) is an important analytical technique that has been applied to complex environmental and biological samples for the pre-concentration, selective separation and purification of organic compounds of interest (analytes).

Pharmaceutical and personal care products are categorised as emerging organic contaminants and are the subject of many analytical investigations due to their widespread presence in daily human life, including drugs, cosmetics and food. A review written by Pedrouzo *et al*. has been published on extraction and chromatographic techniques for personal care products (PCPs) in the environment.[129] The examples of PCPs listed include organic UV filters, preservatives, antimicrobials, fragrances, insect repellents and siloxanes. SPE has been used to determine the occurrence of artificial sweeteners in the environment, including acesulfame, aspartame, cyclamate, saccharin, neohesperidin dihydrochalcone, and sucralose.[130] More recently, Arbeláez *et al*. have performed a study that is the first to also consider the determination of stevioside and glycyrrhizic acid in environmental water samples.[131]

Pharmaceutically active compounds that are present in the environment as pollutants are of serious concern for many countries, evident from the vast number of research papers published on the topic and studies of various water sources across the world.[132-137] The contaminants have been detected in environmental water samples that include surface water, groundwater, and sewage from water treatment plants. The consumption and excretion of both human and veterinary medicines provides one pathway for the release of pharmaceutical products into the environment and the contamination of water systems. Heberer has produced a scheme to represent some of the sources and pathways for the occurrence of pharmaceutical compounds in environmental waters, as shown in Figure 5.1.[136]

A study has been published that provides a method for the determination of different antibiotic substances in hospital sewage water, and studied the temporal variation in concentration of analytes over one day.[138]

In 2014, Gilart *et al*. published their work on the selective determination of a range of pharmaceuticals (atenolol, propranolol, metoprolol, ranitidine, trimethoprim, salicylic acid, clofibric acid, diclofenac and ibuprofen) and illicit drugs (morphine, cocaine, methadone and codeine) using polymeric SPE sorbents.[92]

Some of the most common classes of pharmaceutical medicines that have been detected and monitored include analgesics and anti-inflammatory drugs, antibiotics, antiepileptic drugs, beta-blockers, lipid regulators, contrast media, cytostatic drugs, and steroids and hormones.[132, 136] After the pharmaceutical products have been identified and quantified, there is further investigation to elucidate their transformation pathways, assess any potential biological effects, and then apply advanced treatment processes for their removal or mineralisation.[132]

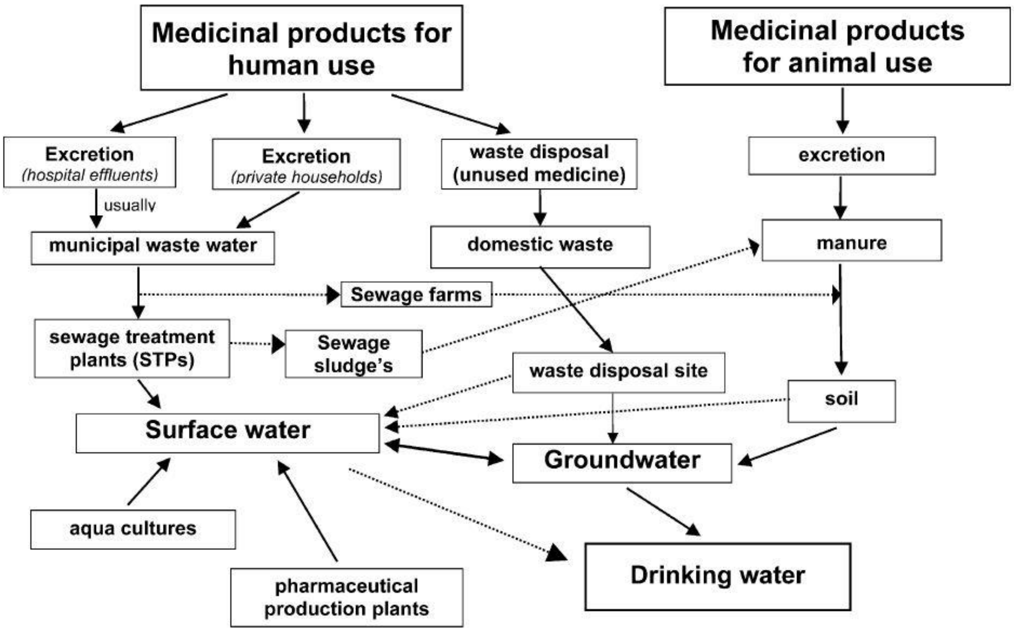


Figure 5.1: Scheme presenting some of the possible sources and pathways that lead to the occurrence of pharmaceutical compounds in environmental waters[136]

The nature of pharmaceutical drugs presents a challenge for analytical chemists, because a sample of pharmaceutical products may contain a mixture of acidic, basic and neutral compounds. The drugs may exist as small molecules or larger proteins, and may possess an assortment of complex chemistries.

SPE is a technique that has been used to extract pharmaceuticals present in water samples at low concentration levels. SPE makes identification and quantification easier by improving the sample preparation step. By removing the interfering compounds and contaminants from large volumes of aqueous samples, the drugs of interest are retained selectively and eluted at a higher concentration.

5.1.1. Mixed-mode polymeric sorbents for the extraction of organic compounds

Mixed-mode polymeric sorbents have a dual retention mechanism, which benefits from the ability to extract organic compounds using hydrophobic, reversed-phase interactions in addition to ionic interactions between ionisable groups.

The combination of both capacity and selectivity within the sorbent is what makes mixed-mode polymeric sorbents so interesting. The polymer backbone gives rise to a great capacity for reversed-phase interactions between the sorbent and the ‘R’ groups of the analytes, and the functional groups present on the polymer give rise to selective adsorption and desorption with functional and ionisable analytes. Figure 5.2 shows the different reversed-phase interactions and ionic attractions that occur in the four main categories of mixed-mode polymer sorbents: strong cation-exchange (SCX); weak cation-exchange (WCX); strong anion-exchange (SAX); and strong cation-exchange (SCX).

Our research group was the first to synthesise hypercrosslinked polymer micropsheres possessing mixed-mode properties for SCX,[92, 98] WCX,[99] SAX[100] and WAX[101]. Hypercrosslinked polymers were modified chemically with either piperazine or ethylenediamine moieties to generate mixed-mode polymeric sorbents (HXLPP-WAX) displaying WAX character, as shown in Figure 5.3.

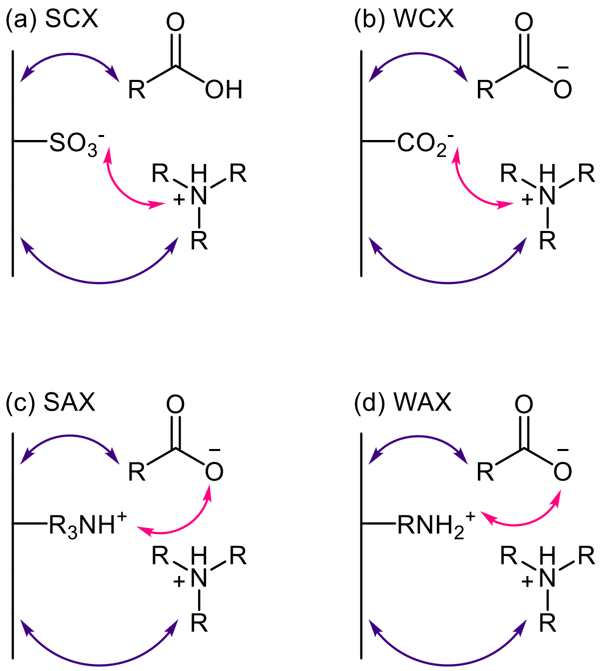


Figure 5.2: Representation of the reversed-phase interactions (purple) and ionic attractions (pink) between analytes and (a) SCX sorbent at pH 3, (b) WCX sorbent at pH 7, (c) SAX sorbent at pH 7 and (d) WAX sorbent at pH 7

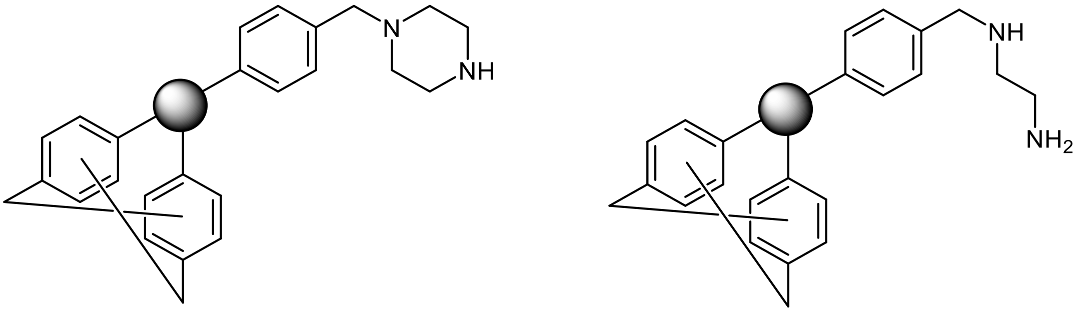


Figure 5.3: Structures of the piperazine and ethylenediamine modified HXLPP-WAX sorbents that have been prepared previously[101, 123]

5.1.2. Development of weak anion-exchange polymeric sorbents

In Chapters 3 and 4, the synthesis of novel WAX mixed-mode sorbents was described. Instead of utilising the existing protocol with amines such as piperazine and ethylenediamine, a new protocol was designed to utilise amines with greater functionality, namely amino acid derived esters such as sarcosine ethyl/methyl ester and phenylalanine methyl ester. Sorbents KA132 and KA134 were the products of chemical modification of poly(DVB-*co*-VBC) and hypercrosslinked poly(DVB-*co*-VBC), respectively, with sarcosine ethyl ester, shown in Figure 5.4. For simplicity, the residual benzyl chloride moieties have not been shown on either sorbent.

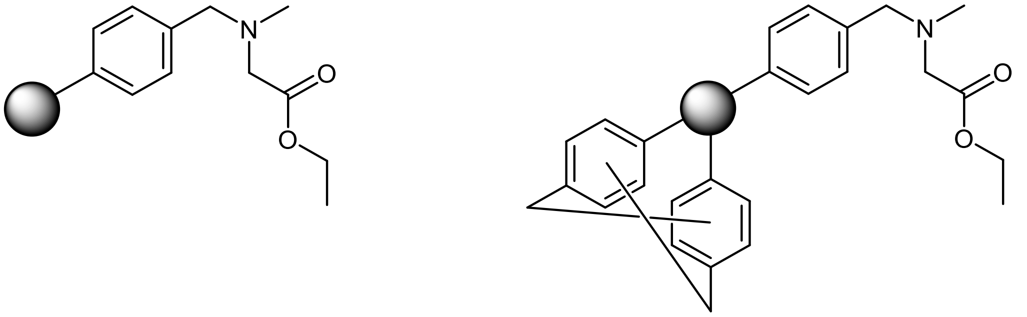


Figure 5.4: Structures of polymeric sorbents KA132 (left) and KA134 (right)

Although chemically similar, the physical properties of the two sorbents are in fact quite distinct. The hypercrosslinked sorbent KA134 has a much higher specific surface area (SSA) (1,655 m^2/g) compared to the macroreticular sorbent KA132 (12 m^2/g). A higher specific surface area corresponds to increased sorbent capacity, and allows for a greater level of interaction between the sorbent and the analytes, and this has been shown to increase the efficiency of the sorbents.[20, 139] The porosity of KA134 is greater than KA132, with a higher content of micropores within the sorbent. Both sorbents possess a mean particle diameter between 3 and 4 µm and a narrow particle size distribution. The ion-exchange capacity (IEC) has been calculated based upon the content of nitrogen measured in the sorbents by elemental microanalysis, to indicate the maximum available quantity of ionisable nitrogen present per gram. A summary of the measured physical properties of sorbents KA132 and KA134 is presented in Table 5.1.

Table 5.1: Physical properties of the WAX sorbents KA132 and KA134

Sample Ref.	IEC (mmol/g)	Langmuir SSA (m^2/g)	Pore volume (cm^3/g)	Pore diameter (nm)	Mean particle diameter (µm)
KA132	0.8 N	12	n/a	n/a	3.2
KA134	0.5 N	1,655	0.654	2.1	3.7

5.1.3. Development of zwitterion-exchange polymeric sorbents

The WAX sorbents KA132 and KA134 display weak anion-exchange character due to the tertiary (ionisable) amine, but the design was such that the ethyl ester moieties could be hydrolysed to the carboxylic acid form. This procedure has been discussed in Chapters 3 and 4. As carboxylic acids are commonly utilised in WCX resins, these novel polymers contain ionisable amines and ionisable carboxylic acid moieties within the same material and ought to be able to be applied as zwitterion-exchange materials. The structure of sorbents KA133 and KA135 are shown in Figure 5.5, but for simplicity the residual benzyl chloride moieties have not been shown on either sorbent.

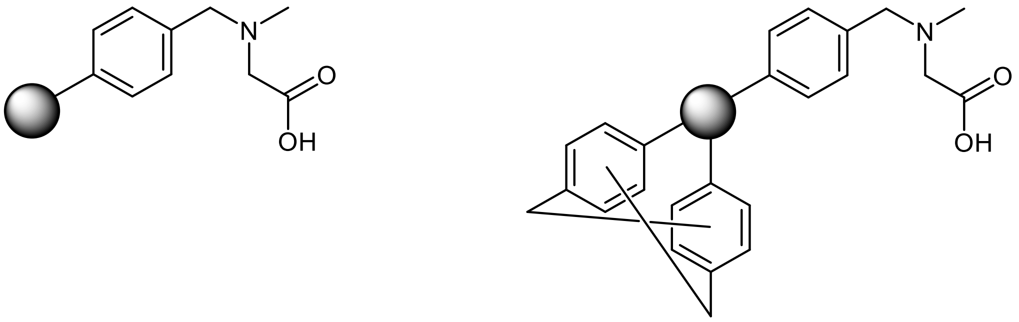


Figure 5.5: Structures of sorbents KA133 (left) and KA135 (right)

A summary of the measured physical properties of sorbents KA133 and KA135 is presented in Table 5.2.

Table 5.2: Properties of the zwitterion-exchange sorbents KA133 and KA135

Sample Ref.	IEC (mmol/g)	Langmuir SSA (m^2/g)	Pore volume (cm^3/g)	Pore diameter (nm)	Mean particle diameter (µm)
KA133	0.8 N	605	0.228	2.0	3.3
KA135	0.4 N	1,473	0.573	2.1	3.7

5.1.4. Aims

The aim of this chapter was to evaluate four different polymeric materials, namely KA132, KA133, KA134 and KA135, as SPE sorbents for the selective extraction of organic compounds by weak anion- and cation-exchange.

The SPE evaluation of the sorbents was performed by the author in the laboratories of the Departament de Química Analítica i Química Orgànica at Universitat Rovira i Virgili, Tarragona. Ultrapure MilliQ water was spiked with a mixture of analytes. Ultimately, in the future, real complex environmental water samples from rivers and waste water treatment plants would be used to evaluate the polymer sorbents as SPE extraction materials.

5.2. Experimental

5.2.1. Materials

Polymer sorbents KA132 and KA133 were prepared in-house, as described in Chapter 3. Polymer sorbents KA134 and KA135 were prepared in-house, as described in Chapter 4.

Ammonium hydroxide (ACS reagent), formic acid (≥ 95 %) and hydrochloric acid (37 %) were supplied by Sigma-Aldrich. Sodium hydroxide (97 %) was supplied by Panreac. Montplet & Esteban SA, Barcelona, Spain. MilliQ water was obtained from an Elga Purelab Ultra purification system, supplied by Veolia Water Solution and Technologies, Barcelona, Spain. Methanol (HiPerSolv Chromanorm for HPLC) was supplied by VWR International, France. Acetonitrile (LiChrosolv® Reagent Ph Eur) was supplied by Merck, Germany. Acetone was supplied by Quifransa, Constantí, Spain.

4-Hydroxy-1-naphthalenesulfonate (~70-75 %, sodium salt) and 2-naphthalenesulfonate (> 90 %, sodium salt) were supplied by Fluka Chemie, Switzerland. Trimethoprim (min. 98 % TLC), caffeine (anhydrous), salicylic acid (ACS reagent), clofibric acid (97 %), ibuprofen (meets USP specifications), diclofenac (sodium salt) and propranolol hydrochloride (99 %) were all supplied by Sigma-Aldrich, Germany. Antipyrine (99 %) was supplied by Acros Organics, USA.

Standard stock (mother) solutions of each analyte in methanol were prepared at a concentration of 1,000 ppm (1,000 mg/L).

5.2.2. Equipment and instrumentation

Preparation of SPE cartridges

SPE cartridges were packed manually by adding 60 mg of sorbent into 6 mL blank polypropylene cartridges (Spe-ed Accessories), sandwiched between two Isolute® frits (10 µm, 6 mL, Biotage) with an additional metal frit (2 µm, ½” OD, Supelco Analytical) at the bottom of the sorbent bed (to prevent the loss of the microspheres which were sub 10 µm).

Off-line SPE apparatus

The SPE cartridges were connected by a luer connection to an SPE vacuum manifold (Teknokroma), as shown in Figure 5.6. The vacuum manifold is a clear glass chamber to which vacuum (20 in Hg, 330 mbar) is applied to draw the sample through the SPE cartridge. An adjustable rack was placed inside the manifold to accommodate the sample collection vessels.

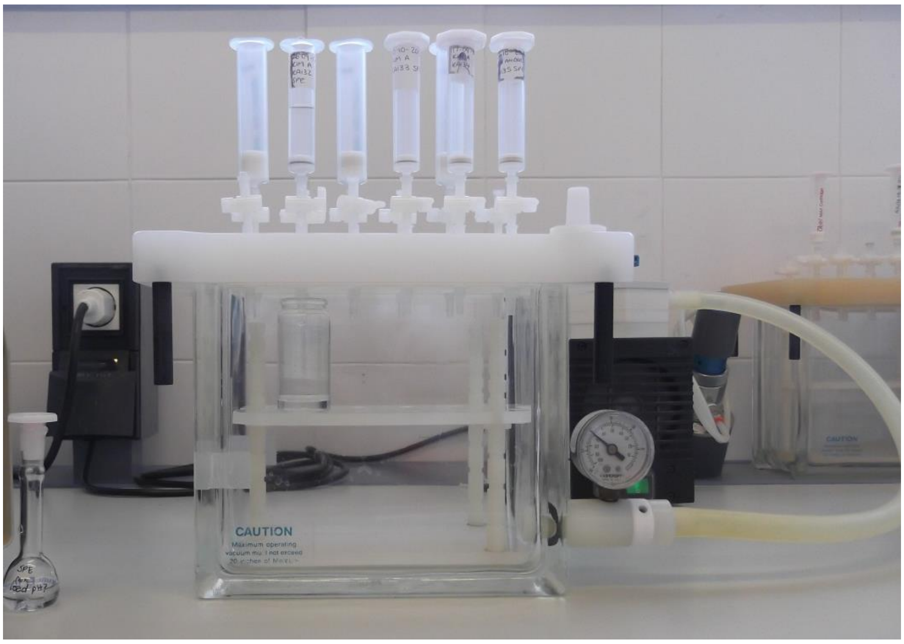


Figure 5.6: SPE vacuum manifold equipped with SPE cartridges packed with polymeric sorbents

HPLC instrumentation

The chromatographic experiments were performed with a Hewlett Packard Series 1100 Iso Pump with an Agilent Technologies 1200 Series Variable Wavelength Detector. An Agilent Technologies 1260 Infinity Degasser, Thermostatted Column Compartment, and Manual Injector were used. The analytical column was a Kromasil 100 C_{18}, 5 µm, 250 mm x 4.6 mm column obtained from Teknokroma.

5.2.3. SPE evaluation of new mixed-mode polymeric sorbents

Sample preparation

Mother solutions of each of the analytes were prepared at 1,000 ppm (1,000 mg/L) in methanol. Analyte mixtures at 100 ppm were prepared by withdrawing aliquots of each of the analytes from their mother solutions to a volumetric flask, and diluting to 100 ppm with methanol.

For the various SPE experiments, analyte mixtures containing 10 ppm of each analyte (shown in Table 5.3) were prepared by diluting an aliquot of the 100 ppm analyte mixture in methanol with MilliQ water modified to the pH of the SPE experiment. HCl and NaOH aqueous solutions were used to adjust the pH value towards acidic or basic pH, respectively.

Table 5.3: The basic and acidic analytes present in each analyte mixture

Analyte mixture A	Analyte mixture B	Analyte mixture C
trimethoprim	trimethoprim	trimethoprim
caffeine	caffeine	caffeine
antipyrine	-	antipyrine
-	-	propranolol
salicyclic acid	salicylic acid	-
clofibric acid	clofibric acid	clofibric acid
diclofenac	-	diclofenac
ibuprofen	-	ibuprofen
-	sodium 2-napthalenesulfonate	-
-	sodium 4-hydroxy-1-napthalenesulfonate	-

Solid-phase extraction for WAX sorbents at pH 7

Procedure 5-i

Sorbent KA134 was conditioned with 50 cm^3 methanol, followed by 50 cm^3 MilliQ water adjusted to pH 7 using dilute NaOH solution. The 10 cm^3 sample of analyte mixture A at 10 ppm concentration was loaded onto the sorbent using a slow flow rate, and the loading solution was collected for HPLC analysis after passing through the sorbent. The sorbent was washed with 2 x 5 cm^3 fractions of methanol. Each fraction was collected after passing through the sorbent and made up to 10 cm^3 with MilliQ water adjusted to pH 7. An elution solvent of 5% ammonium hydroxide (NH_4OH) in methanol was prepared, and 3 x 5 cm^3 fractions were passed through the sorbent. The three fractions collected were made up to 10 cm^3 with MiliQ water adjusted to pH 7.

Procedure 5-i has been repeated with some alterations to the protocol:

- The washing step with methanol was omitted. The sample was loaded onto sorbent KA134 and then eluted directly using the 5% NH_4OH solution in methanol.
- The concentration of analytes in the sample matrix was decreased to 1 ppm. The sorbent was washed with 5 x 1 cm^3 fractions of methanol, and the collected wash fractions were made up to 2 cm^3 using MilliQ water adjusted to pH 7.
- The procedure was repeated using sorbent KA132. The sorbent was washed with 2 x 2 cm^3 fractions of methanol, and the collected wash fractions were made up to 5 cm^3 using MilliQ water adjusted to pH 7. Elution was performed using 2 cm^3 of 5% NH_4OH solution in methanol, and the collected solution was made up to 5 cm^3 using MilliQ water adjusted to pH 7.

Solid-phase extraction for WAX sorbents at pH 5

Procedure 5-ii

Sorbent KA132 was conditioned with 50 cm^3 methanol, followed by 50 cm^3 MilliQ water adjusted to pH 5 using dilute HCl solution. The 10 cm^3 sample of analyte mixture A at 5 ppm concentration was loaded onto the sorbent using a slow flow rate, and the loading solution was collected after passing through the sorbent for HPLC analysis. The sorbent was washed with 2 x 2 cm^3 fractions of methanol. Each fraction was collected after passing through the sorbent and made up to 5 cm^3 with MilliQ water adjusted to pH 5. An elution solvent of 5% ammonium hydroxide (NH_4OH) in methanol was prepared, and 2 x 2 cm^3 fractions were passed through the sorbent. The two fractions collected were made up to 5 cm^3 with MilliQ water adjusted to pH 5.

Solid-phase extraction for WAX sorbents at pH 3

Procedure 5-iii

Sorbent KA134 was conditioned with 50 cm^3 methanol, followed by 50 cm^3 MilliQ water adjusted to pH 3 using dilute HCl solution. The 10 cm^3 sample of analyte mixture B at 10 ppm concentration was loaded onto the sorbent using a slow flow rate, and the loading solution was collected after passing through the sorbent for HPLC analysis. The sorbent was washed with 2 cm^3 of methanol. The solvent was collected after passing through the sorbent and made up to 5 cm^3 with MilliQ water adjusted to pH 3. An elution solvent of 5% ammonium hydroxide (NH_4OH) in methanol was prepared, and 2 x 5 cm^3 fractions were passed through the sorbent. The two fractions collected were made up to 10 cm^3 with MilliQ water adjusted to pH 3. However, in order to inject into the HPLC the solutions were evaporated to dryness and reconstituted in a 50:50 solution of methanol and MilliQ water adjusted to pH 3.

Procedure 5-iii has been repeated using sorbents KA135, KA133 and KA132.

Solid-phase extraction for WCX sorbents at pH 7

Procedure 5-iv

Sorbent KA135 was conditioned with 50 cm^3 methanol, followed by 50 cm^3 MilliQ water adjusted to pH 7 using dilute NaOH solution. The 10 cm^3 sample of analyte mixture A at 10 ppm concentration was loaded onto the sorbent using a slow flow rate, and the loading solution was collected after passing through the sorbent for HPLC analysis. The sorbent was washed with 2 cm^3 of methanol. The solvent was collected after passing through the sorbent and made up to 5 cm^3 with MilliQ water adjusted to pH 7. An elution solvent of 5% formic acid (HCOOH) in methanol was prepared, and 2 x 5 cm^3 fractions were passed through the sorbent. The two fractions collected were made up to 10 cm^3 with MilliQ water adjusted to pH 7.

Solid-phase extraction for WCX sorbents at pH 9

Procedure 5-v

Sorbent KA135 was conditioned with 50 cm^3 methanol, followed by 50 cm^3 MilliQ water adjusted to pH 9 using dilute NaOH solution. The 10 mL sample of analyte mixture C at 10 ppm concentration was loaded onto the sorbent using a slow flow rate, and the loading solution was collected after passing through the sorbent for HPLC analysis. The sorbent was washed with 2 cm^3 of methanol. The solvent was collected after passing through the sorbent and made up to 5 cm^3 with MilliQ water adjusted to pH 9. An elution solvent of 5% formic acid (HCOOH) in methanol was prepared, and 2 x 5 cm^3 fractions were passed through the sorbent. The two fractions collected were made up to 10 cm^3 with MilliQ water adjusted to pH 9.

5.2.4. HPLC conditions

The HPLC conditions were optimised to give good separation of all of the analytes in the sample mixture. The optimum conditions are presented in Table 5.4. The gradient profile for the mobile phase is shown in Table 5.5.

Table 5.4: HPLC conditions for the determination of the target analytes

Mobile phase	(A) MilliQ H_2O modified to pH 3, (C) acetonitrile (ACN)
Stationary phase	Kromasil 100 C_{18} column, 5 µm, 250 mm x 4.6 mm
Temperature	30 °C
Flow rate	1 cm^3/min
Injection volume	20 µL loop
Wavelength	210 nm (KA132, KA133, KA135) and 230 nm (KA134)
Initial conditions pressure	114 bar

Table 5.5: Mobile phase gradient profile for the determination of the target analytes

Time (min)	% C (ACN)	% A (H_2O pH 3)
0	10	90
4	10	90
19	60	40
35	100	0
37	100	0
40	10	90

5.2.5. Calculation of the % recovery of analytes after SPE

The solutions collected after the loading, washing and elution steps of the SPE experiments were injected into the HPLC for determination and quantification of the analytes. When possible, blank solutions of 50 % methanol and 50 % MilliQ water adjusted to the pH of the SPE experiment were also injected into the HPLC as a reference for comparison. Calibration graphs were plotted of the peak area against concentration for analyte mixture A at concentrations of 1 ppm, 5 ppm, 10 ppm and 20 ppm. The purpose of the calibration graphs was to determine the concentration of analyte recovered after each SPE step based upon the area of its peak. The % recovery of analyte could be calculated from Equation 3:

$$\% \, Recovery = \frac{Concentration \ (obtained \ after \ SPE)}{Concentration \ (sample \ prepared)} \times 100$$

Equation 3

Due to time constraints, calibration graphs were prepared only for analyte mixture A. If time had allowed, calibration graphs would have been prepared for analyte mixtures B and C as well. An alternative method of calculating the % recovery of analytes depended on a reference sample of analytes injected into the HPLC for comparison. This reference was prepared using the same mixture of analytes as those loaded onto the SPE sorbent, but in a 50:50 solution of methanol and MilliQ water at modified pH. The reference was injected directly into the HPLC without undergoing SPE. Therefore the area under the peaks of each analyte in this reference should represent the initial concentration of analytes that were loaded on to the SPE sorbent in the experiment, and be used to calculate the % recovery from Equation 4 and Equation 5.

$$Concentration \ (obtained) = \frac{Area \ (obtained) \times Concentration \ (reference)}{Area \ (reference)}$$

Equation 4

$$\% \, Recovery = \frac{Concentration \ (obtained)}{Concentration \ (reference)} \times 100$$

Equation 5

5.3. Results and discussion

The polymeric sorbents described in Chapters 3 and 4 were designed to perform as weak anion-exchange sorbents for solid-phase extraction. The ethyl ester functional group is a novel feature for this class of materials as it influences the nature of the tertiary amine and, through hydrolysis to the carboxylic acid, has provided a route into zwitterionic-exchange materials. Figure 5.7 shows the structures of the four polymeric sorbents that have been studied in this work. Sorbents KA132 and KA134 (shown as (a) and (b), respectively) have been designed to act as WAX sorbents, whereas sorbents KA133 (c) and KA135 (d) contain carboxylic acid moieties that could lend themselves to WCX in addition to the WAX function of the amine. Structures (b) and (d) possess a hypercrosslinked polymer backbone that has an increased specific surface area and higher micropore content.

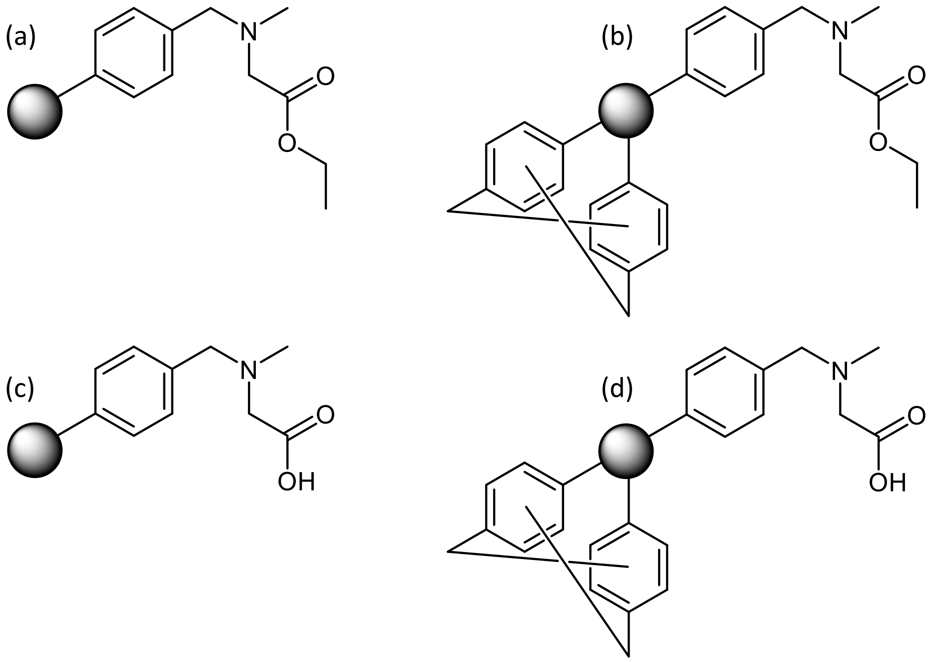
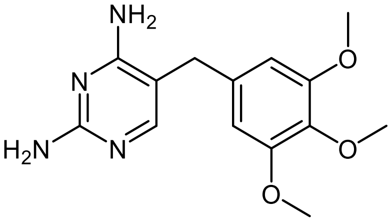
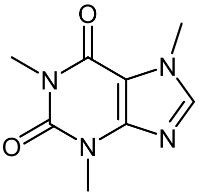
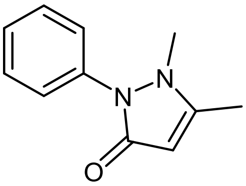
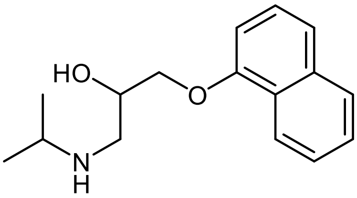
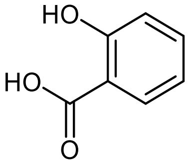
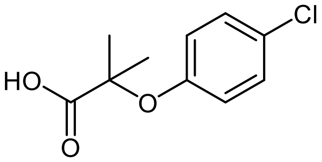
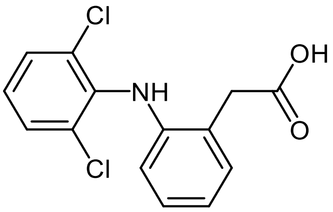
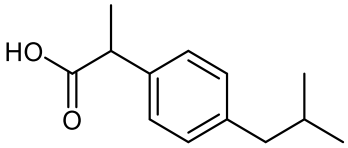
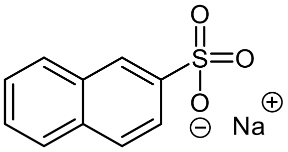
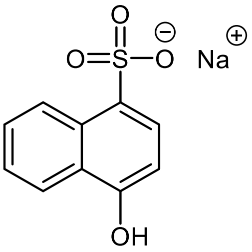


Figure 5.7: Structures of polymeric sorbents KA132 (a), KA134 (b), KA133 (c) and KA135 (d)

Mixtures of organic analytes in aqueous solution were percolated through the synthesised SPE sorbents. A table listing the name and structure of each analyte has been provided for reference in Table 5.6. Trimoethoprim, caffeine, antipyrine and propranolol are basic pharmaceutical compounds; salicylic acid, clofibric acid, diclofenac, and ibuprofen are acidic pharmaceutical compounds; 2-naphthalenesulfonate and 4-hydroxy-1-naphthalenesulfonate are strongly acidic organic compounds.

Table 5.6: The analytes used in the SPE evaluation of the polymeric sorbents

Analyte (and use)	pKa	Acidic/Basic	Structure
trimethoprim (antibacterial)	7.2	basic	
caffeine (stimulant)	13.3	basic	
antipyrine (analgesic/antipyretic)	13.5	basic	
propranolol (β-blocker)	9.5	basic	
salicylic acid (analgesic/antifungal)	3.0	acidic	
clofibric acid (lipid regulator)	3.2	acidic	
diclofenac (NSAID/antipyretic)	3.9	acidic	

ibuprofen (NSAID/antipyretic)	4.6	acidic	
sodium 2-naphthalenesulfonate	<1	strongly acidic	
sodium 4-hydroxy-1-naphthalenesulfonate	<1	strongly acidic	

5.3.1. Solid-phase extraction of WAX sorbents

All of the polymeric sorbents have a tertiary amine group, as shown in Figure 5.7, which can be protonated to form a quaternary ammonium group and thereby act as a weak anion-exchange sorbent. Analyte mixtures were prepared by spiking MilliQ water with a selection of acidic and basic organic compounds at known concentration. In order to trial these new sorbents for the selective extraction of acidic analytes from the mixture of organic compounds, the initial SPE protocol chosen was that used previously for amine-containing WAX sorbents, as shown in Table 5.7.[101]

Table 5.7: Recommended SPE protocols for mixed-mode polymeric sorbents[101]

SPE step	Strong cation-exchange (SCX)	Weak cation-exchange (WCX)	Strong anion-exchange (SAX)	Weak anion-exchange (WAX)
Conditioning	Pure methanol, followed by aqueous solution at pH of sample			
Loading	Aqueous sample at pH 3	Aqueous sample at pH 7	Aqueous sample at pH 7	Aqueous sample at pH 7
Washing (optional)	5 % formic acid aqueous solution	5 % ammonium hydroxide aqueous solution	5 % ammonium hydroxide aqueous solution	5 % formic acid aqueous solution
Washing	Pure methanol	Pure methanol	Pure methanol	Pure methanol
Elution	5 % ammonium hydroxide in methanol	5 % formic acid in methanol	5 % formic acid in methanol	5 % ammonium hydroxide in methanol

Targeted analytes to elute	weakly basic analytes	(strongly) basic analytes	weakly acidic analytes	(strongly) acidic analytes

Methanol was used to condition the SPE cartridge and wet the sorbent. A subsequent conditioning step with MilliQ water adjusted to pH 7 was performed to prepare the sorbent for the introduction of the aqueous sample matrix. The sample of mixed analytes in MilliQ water at pH 7 was loaded onto the sorbent. At pH 7, it was assumed that the basic analytes (trimethoprim, caffeine and antipyrine) would be retained reversibly on the sorbent by reversed-phase interactions, whilst the charged acidic analytes (salicylic acid, clofibric acid, diclofenac and ibuprofen) would be retained on the sorbent by both reversed-phase interactions and ionic interactions. Washing the sorbent with methanol breaks the reversed-phase interactions with all of the analytes. The basic analytes would be desorbed and washed from the sorbent, whilst the ionic interactions that bind the deprotonated acidic analytes to the protonated amine on the sorbent would remain in place. This step selectively separates the acidic and basic analytes. In order to desorb the acidic analytes from the sorbent, the ionic interactions must be broken. A basic solution of 5 % ammonium hydroxide (NH_4OH) in methanol was used to neutralise the charge on the sorbent, and disrupt the ionic interactions. The methanol present in the elution solvent prevents any new reversed-phase interactions from forming, and ensures that the sorbent is cleaned of analytes.

SPE experiments were performed under typical conditions at pH 7 using sorbents KA132 and KA134, and analyte mixture A. The analytes were loaded successfully onto the sorbents by reversed-phase interactions, showing that the sorbents are capable of extracting and adsorbing organic compounds from an aqueous sample matrix. However, it was found that washing with methanol removed all of the analytes due to the disruption of the reversed-phase interactions. The SPE protocol at pH 7 was unable to selectively retain the acidic analytes by ionic interactions. The sorbent and analytes must be oppositely charged for ionic interactions to occur, therefore this result confirms that sorbents KA132 and KA134 do not exist as charged WAX sorbents at pH 7.

Based upon this result, the next logical experiment was to adjust the SPE protocol to pH 5 in an attempt to protonate the amine on the sorbent. It has been reported that the pH of the sample matrix should be two pH units above the value of the pKa for anionic, acidic analytes to be charged.[82] Therefore, lowering the pH of the sample matrix may decrease the quantity of charged acidic analytes. The pKa values for the acidic analytes present in analyte mixture A are presented in Table 5.8.

Table 5.8: The pKa values of the acidic analytes in analyte mixture A

Analyte	pKa
salicylic acid	3.0
clofibric acid	3.2
diclofenac	3.9
ibuprofen	4.6

Sorbent KA132 was used in the SPE experiments performed at pH 5. The analysis of the loading, washing and elution fractions injected into the HPLC showed that all of the analytes were again eluted in the wash step with methanol. Therefore, these new conditions have failed to selectively retain analytes on the sorbent by ionic interactions.

In order to determine if it was a problem with the charge of the sorbent or the charge of the analytes which prevented the retention of analytes by ionic interactions, a new analyte possessing permanent charge was introduced to sorbent KA132. The strongly acidic analyte sodium 2-naphthalenesulfonate was added to MilliQ water at pH 5 and loaded onto the sorbent. The pKa of 2-naphthalenesulfonate is <1 and will therefore exist in its anionic form under the SPE conditions used. The load, wash and elution fractions were injected into the HPLC and the results showed that the analyte was present in all fractions. This confirms that the sorbent has difficulty retaining charged analytes, and therefore the amine is not sufficiently charged at pH 5.

Based upon this knowledge that the sorbent has not been behaving in an analogous manner to other familiar WAX sorbents,[101] the conditions for the SPE evaluation were reassessed. At an even lower pH of 3, the amine on the sorbent must surely be protonated and able to interact ionically with oppositely charged species.

Sorbent KA132 was once again loaded with the analyte 2-naphthalenesulfonate, at pH 3. The new protocol used was in fact that designed for SCX sorbents. The washing step remained the same, with a small volume of methanol. It would not be possible to protonate the anionically charged 2-naphthalenesulfonate, so the purpose of the elution solvent was to deprotonate the charged amine on the sorbent and disrupt the ionic interaction. The chromatograms were examined to detect the presence of the analyte in the SPE fractions. 2-Naphthalenesulfonate was retained successfully on the sorbent after washing with methanol, confirming that there were additional ionic interactions present binding it to the sorbent. Upon washing with 5% NH_4OH in methanol, the analyte was eluted fully and recovered.

The weak acids from analyte mixture A, shown in Table 5.8, would not exist in a deprotonated state at pH 3. Only strongly acidic analytes with pKa <1 would be able to interact ionically with the protonated amine on the sorbent. A new analyte mixture (B) was designed that includes two strongly acidic analytes, two weakly acidic analytes and two weakly basic analytes. Table 5.9 lists the chosen analytes and their pKa values, alongside their respective retention times observed using the optimised chromatographic conditions.

Table 5.9: The pKa and retention times for analyte mixture B, under the optimised HPLC conditions

Analyte	pKa	Retention time (min)
trimethoprim	7.2	11.03
caffeine	13.3	9.95
salicylic acid	3.0	16.17
clofibric acid	3.2	21.69
2-naphthalenesulfonate	<1	12.14
4-hydroxy-1-naphthalenesulfonate	<1	8.01

The elution fractions collected contain basic NH_4OH which interacts with the analyte 4-hydroxy-1-naphthalenesulfonate by increasing the polarity of the analyte (the base deprotonates the hydroxyl group on 4-hydroxy-1-naphthalenesulfonate). This causes the analyte to pass through the chromatographic column too quickly, and decreases the retention time on the chromatogram. In order to inject a suitable solution into the HPLC, the elution fraction was evaporated to dryness and then reconstituted with a 50:50 solution of methanol and MilliQ water at pH 3.

All four WAX sorbents (KA132, KA134, KA133 and KA135) were evaluated for the solid-phase extraction of analyte mixture B at pH 3.

KA132 and KA133 are macroreticular sorbents with specific surface areas of 12 m^2/g and 605 m^2/g, respectively. The hypercrosslinked sorbents possess much higher specific surface areas, at 1,655 m^2/g and 1,473 m^2/g for KA134 and KA135, respectively. Sorbents with higher specific surface areas are expected to have higher capacities, and greater π-π interactions between analytes and sorbent.[83]

The mean pore diameter of the sorbents is in the range of 2-4 nm, which is just on and beyond the boundary between micropores and mesopores, according to the IUPAC classification.[24]

Due to time constraints, it was not possible to prepare and analyse a reference sample of analytes when the SPE evaluation was performed using sorbent KA132. Therefore, the reference sample from the previous day's experimental study was used to calculate the percentage recoveries for this experiment (KA132). Table 5.10 shows the calculated percentage recoveries of each analyte from the load, wash and elution steps of the SPE experiments performed at pH 3, using the WAX sorbents KA132 and KA134.

Table 5.10: The calculated recoveries (%) of analytes during the load, wash and elution steps for the SPE experiments at pH 3 for sorbents KA132 and KA134. The dash – denotes a recovery which was ≤1 %.

	KA132			KA134		
	Load	Wash	Elution	Load	Wash	Elution
trimethoprim	83	19	-	-	97	-
caffeine	-	91	-	-	99	-
salicylic acid	-	94	2	-	92	-
clofibric acid	-	91	2	-	94	-
2-naphthalenesulfonate	-	-	100	-	-	93
4-hydroxy-1-naphthalenesulfonate	-	-	95	-	-	91

The HPLC chromatograms of the samples collected from the SPE experiment at pH 3 using sorbent KA132 are shown in Figure 5.8 (load), Figure 5.9 (wash) and Figure 5.11 (elution).

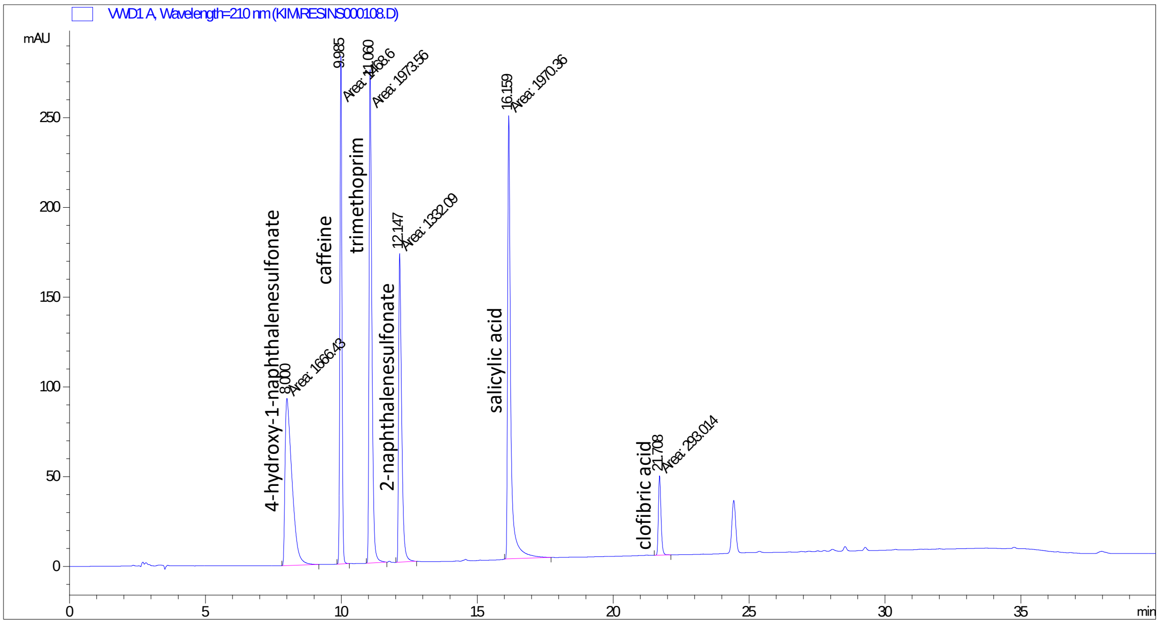


Figure 5.8: Chromatogram for the reference mixture of 6 analytes at pH 3

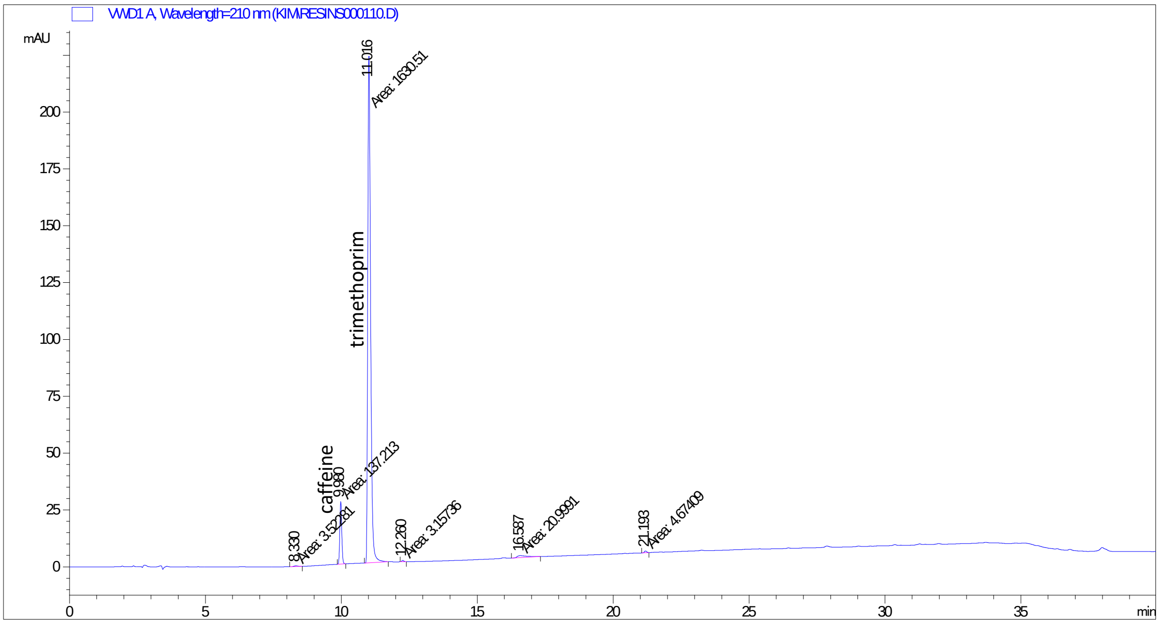


Figure 5.9: Chromatogram of the collected SPE load solution passed through sorbent KA132 at pH 3

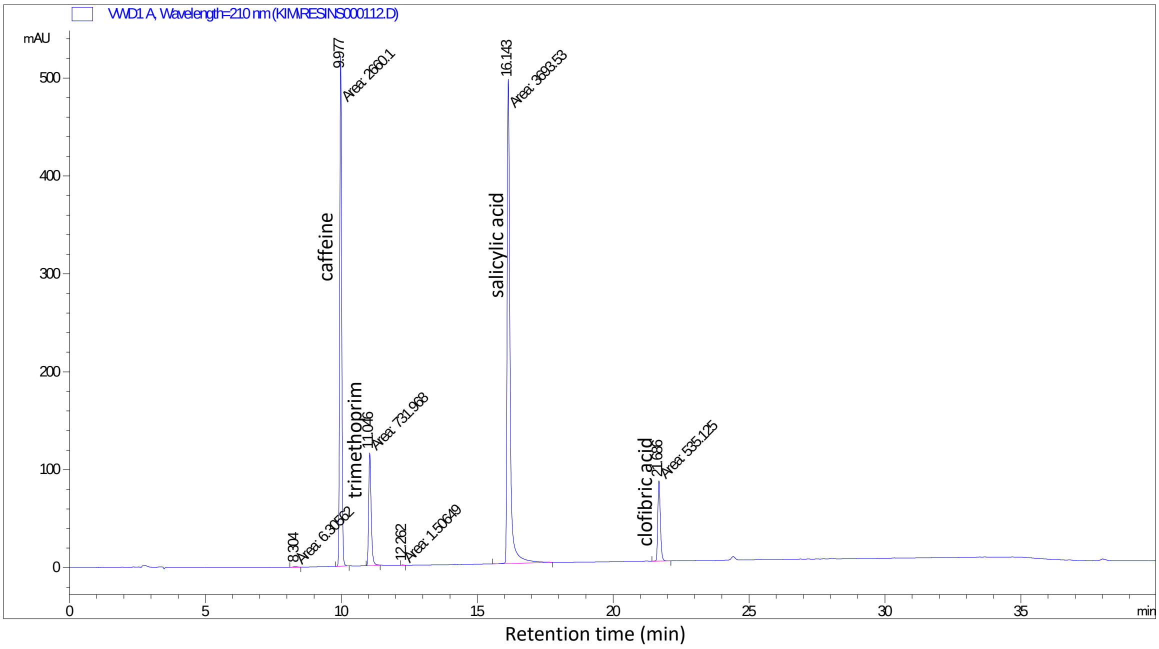


Figure 5.10: Chromatogram of the collected SPE wash solution passed through sorbent KA132 at pH 3

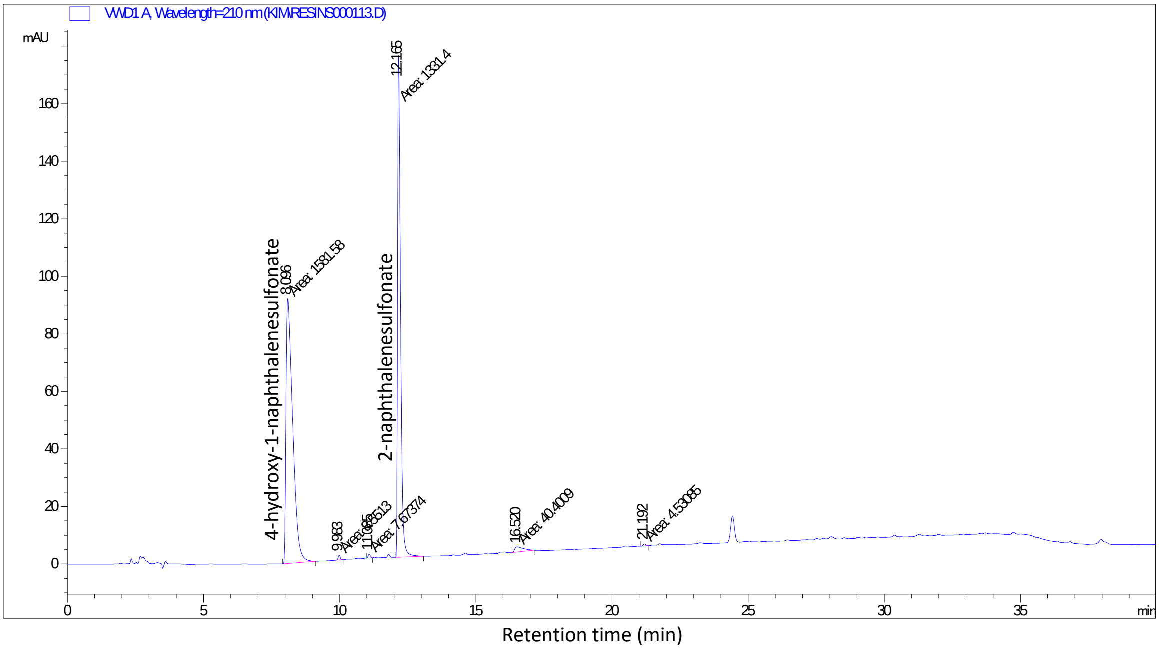


Figure 5.11: Chromatogram of the collected SPE elution solution passed through sorbent KA132 at pH 3

The most striking difference between these results tabulated in Table 5.10 is the breakthrough of trimethoprim during the loading of analytes onto sorbent KA132. The cause of this breakthrough is currently unknown, whether it be due to the flow rate during loading, or from an incompatibility between the structure of the sorbent and analyte. Repetition of the experiments would be required to gain additional data for firmer conclusions. The high percentage recoveries show that the weakly acidic analytes (salicylic acid and clofibric acid) and the weakly basic analytes (trimethoprim and caffeine) were removed from the sorbent upon washing with methanol. The strongly acidic 4-hydroxy-1-naphthalenesulfonate and 2-naphthalenesulfonate were retained selectively by the sorbent during loading and washing, and then eluted in the elution step using basic NH_4OH solution in methanol.

Table 5.11 shows the calculated percentage recoveries of each analyte from the load, wash and elution steps of the SPE experiments performed at pH 3, using the WAX sorbents KA133 and KA135.

Table 5.11: The calculated recoveries (%) of analytes during the load, wash and elution steps for the SPE experiments at pH 3 for sorbents KA133 and KA135. The dash – denotes a recovery which was ≤1 %.

	KA133			KA135		
	Load	Wash	Elution	Load	Wash	Elution
trimethoprim	35	52	14	-	81	-
caffeine	-	96	3	-	82	-
salicylic acid	-	84	7	-	79	-
clofibric acid	10	89	6	2	81	-
2-naphthalenesulfonate	-	-	98	-	2	95
4-hydroxy-1-naphthalenesulfonate	-	-	92	-	17	72

There are some slight differences between sorbents KA133 and KA135 in terms of the analyte recoveries presented in Table 5.11. There has been a breakthrough of analytes trimethoprim and clofibric acid during the loading of the analytes onto sorbent KA133. The subsequent wash with methanol recovered high percentages of the weak analytes, without desorbing the analytes 2-naphthalenesulfonate and 4-hydroxy-1-naphthalenesulfonate.

However, there a small proportion of the weakly acidic and basic analytes were eluted from sorbent KA133 after the NH_4OH wash. Perhaps using a greater volume of methanol in the wash step would help to remove these analytes fully, and prevent them from being eluted alongside the strongly acidic analytes. The recovery of the strongly acidic analytes is very good, with 98 % recovery of 2-naphthalenesulfonate and 92 % recovery of 4-hydroxy-1-naphthalenesulfonate.

Sorbent KA135 has retained successfully the analytes during the loading step, except for a minor trace of clofibric acid, showing that the analytes were able to be bound to the sorbent by reversed-phase interactions. The wash step recovered about 80 % of the four weak analytes, but there was some breakthrough of 4-hydroxy-1-naphthalenesulfonate that was desorbed when the reversed-phase interactions were disrupted. The elution using a basic solution recovered 95 % of 2-naphthalenesulfonate and 72 % of 4-hydroxy-1-naphthalenesulfonate.

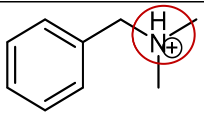
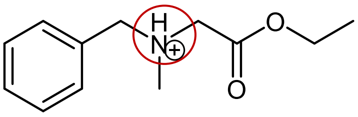
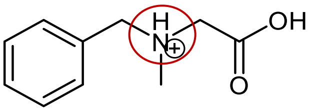
Investigation of pKa

The pH is an important parameter to consider when planning SPE experiments. Mixed-mode polymeric sorbents have a dual retention mechanism, which benefits from the ability to extract organic compounds using hydrophobic, reversed-phase interactions in addition to ionic interactions between ionisable groups. The pH plays an important role because both the sorbent and the analytes of interest need to exist in their appropriate charged state to participate in complimentary ionic interactions.

The SPE evaluation of sorbents KA132, KA134, KA133 and KA135 was performed at pH 7, pH 5 and pH 3. The initial SPE conditions at pH 7 were chosen due to the successful SPE evaluation of similar hypercrosslinked polymeric sorbents that had been modified with ethylenediamine and piperazine to form mixed-mode WAX sorbents, HXLPP-WAX.[101, 123] However, it became apparent that the sorbents prepared in this thesis behaved differently and the conditions were subsequently changed to a new SPE protocol at pH 3.

The ionisation behaviour of the amine (and sorbent) is dependent on its pKa value. In order to explain the results, theoretical, predicted pKa values have been calculated using ACD/Labs I-Lab 2.0 software. For convenience, the polymer backbone has been omitted and only the functional, small molecule sections of the polymers have been considered. The predicted pKa values are 5.9 for the ethyl ester sorbent, and 8.6 for the carboxylic acid sorbent, as shown in Table 5.12. The value of 5.9 was lower than expected for a tertiary amine, so it became clear that the β-carbonyl of the ester has perturbed the pKa value. The pKa of a comparable amine without the carbonyl functionality has been predicted at 8.6 (Table 5.12).

Table 5.12: Predicted values of pKa (generated using ACD/Labs I-Lab 2.0) for the small molecule equivalent of the sorbents, and comparison to a structure with an amine only.

Structure	Comparable to sorbent	pKa
	-	8.6
	KA132, KA134	5.9
	KA133, KA135	8.6

At pH values below the pKa of the amine, the nitrogen will be protonated and exist as a quaternary ammonium group. The inductive effect of the alkyl chain will donate electron density to stabilise the protonated amine. However, the polar carbonyl group of the ester has a δ+ charge on the carbon atom which is electron-withdrawing and destabilises the positively charged nitrogen. The consequence of this is a decrease in the base strength and a decrease in the pKa. The carboxylic acid has a more stabilising effect towards the protonated amine, compared to the ester.

This study has shown that low pH (pH 3) was required for the selective solid-phase extraction of strongly acidic analytes using these mixed-mode polymeric WAX sorbents. The retention mechanism offers a method of differentiating between strong and weak acids, and the SPE protocol allows for their separation.

5.3.2. Solid-phase extraction with zwitterionic sorbents

Sorbents KA133 and KA135 contain both tertiary amine and carboxylic acid moieties; therefore it should be possible to use these sorbents as zwitterion-exchange materials in solid-phase extraction. The amine can act as a weak anion-exchanger, and the carboxylic acid can act as a weak cation-exchanger.

Following on from the evaluation of the amine as a mixed-mode WAX sorbent in Section 5.3.1., the WCX properties of KA135 were investigated by testing the SPE protocols and modifying the conditions. The initial conditions selected were derived from a recommended protocol for the selective extraction of basic analytes using a WCX sorbent, at pH 7.[99] At pH 7, the carboxylic acid moieties on the acidic analytes will be deprotonated, the carboxylic acid on the sorbent will be deprotonated, and the basic analytes will be protonated.

The objective of the washing step in the SPE protocol is to remove interferences from the sample and desorb the acidic and neutral compounds that bind to the sorbent through reversed-phase interactions. The elution step was performed with an acidic solution of 5 % formic acid in methanol, to elute the basic compounds that were bound to the sorbent by ionic interactions. The protonation of the carboxylic acid groups on the sorbent disrupts the ionic interactions, and releases the basic analytes to collect in the elution solvent.

The percentage of analytes recovered from sorbent KA135 in the load, wash and elution steps of the protocol are presented in Table 5.13. A reference sample of the analyte mixture at pH 7 was not available; therefore the data from the chromatogram was compared to a reference sample of the analyte mixture at pH 3. The results show the relative amounts of each analyte recovered for comparison, although the values do not tally to 100 %.

Table 5.13: The calculated recoveries (%) of analytes during the load, wash and elution steps for the SPE experiment at pH 7 for sorbent KA135. A dash – denotes a recovery which was ≤1 %.

	KA135 pH 7		
	Load	Wash	Elution
trimethoprim	-	131	3
caffeine	-	75	57
antipyrine	-	136	-
salicylic acid	81	57	-
clofibric acid	-	136	-
diclofenac	-	123	11
ibuprofen	-	130	5

The binding of salicylic acid onto sorbent KA135 at pH 7 has been relatively unsuccessful as the majority of the analyte was collected in the loading solution after percolating through the sorbent. This was a surprising result, as the analyte had been shown to bind to sorbent KA135 in the WAX SPE experiments at pH 7 discussed in Section 5.3.1. The remainder of the analytes were bound successfully to the sorbent by reversed-phase interactions. The sorbent has failed to retain the basic analytes by ionic interactions, as evidenced by the high recovery of each analyte collected after washing the sorbent with methanol. Just under half of the concentration of caffeine was retained on the sorbent and eluted using formic acid.

The performance of KA135 as a WCX sorbent under these conditions was poor; therefore the conditions should be modified and optimised. THE SPE capabilities of KA135 loaded with analyte mixture A was evaluated at pH 9, but the experiment did not demonstrate selective separation of the analytes to fit with the requirements for a good mixed-mode WCX sorbent. The percentage recoveries were tabulated and are presented in Table 5.14.

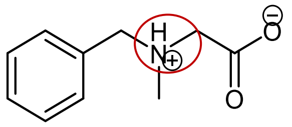
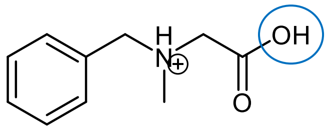
Table 5.14: The calculated recoveries (%) of analytes during the load, wash and elution steps for the SPE experiment at pH 9 for sorbent KA135. A dash – denotes a recovery which was ≤1 %.

	KA135 pH 9		
	Load	Wash	Elution
trimethoprim	-	100	2
caffeine	-	52	47
antipyrine	-	102	-
propranolol	2	26	72
clofibric acid	-	102	2
diclofenac	-	89	13
ibuprofen	-	100	3

Although it was reported by Bratkowska *et al*. for their sorbent HXLPP-WCX,[99] KA135 was not washed with a basic solution of 5 % NH_4OH in methanol in place of pure methanol during the washing procedure. The SPE protocol should be investigated further to find suitable conditions that give rise to selective extractions for this sorbent and exploits their cation-exchange ability.

Sorbent KA135 has tertiary amine and carboxylic acid functionalities, so depending on the pH of its environment it can exist with a net positive, negative or neutral charge. At pH 7, KA135 should exist as the zwitterionic species with a charged amine and charged carboxylate anion – with a net neutral charge. At pH 9 the sorbent should be negatively charged because the amine is neutral and only the carboxylate anion is charged. The theoretical, predicted pKa values for sorbents KA133 and KA135 are shown in Table 5.15.

Table 5.15: Predicted values of pKa (generated using ACD/Labs I-Lab 2.0) for the small molecule equivalent of sorbents KA133 and KA135

Structure	pKa
	8.6
	1.8

5.4. Conclusions

The in-house prepared mixed-mode polymeric sorbents KA132, KA134, KA133 and KA135 were packed into solid-phase extraction cartridges and evaluated as sorbents for the selective extraction of organic analytes in ultrapure water. The sorbents were treated as weak anion-exchange sorbents but failed to show evidence of any ionic interactions with the charged analytes at pH 7 and pH 5. It was discovered that the pKa value for the tertiary amine in the novel sorbents was lower than expected due to a perturbation of the pKa by the adjacent β-carbonyl. When a sample of analytes was loaded onto the SPE cartridge at pH 3, the N atoms in the sorbent were positively charged and successfully formed ionic interactions with the strongly acidic analytes present in the sample. The SPE procedure that was developed allowed selective separation of the analytes: the weakly acidic and basic analytes were removed during the washing step whereas the strongly acidic analytes were retained and eluted during elution. Thus interesting and useful SPE sorbents have been developed which are structurally and functionally distinct to commercially available sorbents.

Sorbents KA133 and KA135 contain both an ionisable amine and an ionisable carboxylic acid; therefore have the potential to perform as zwitterion-exchange materials by both weak anion-exchange and weak cation-exchange. In the short period of time allocated to this study it was not possible to develop an SPE protocol to selectively extract basic analytes from the mixture by weak cation-exchange.

5.5. Further work

There is great potential to continue this analytical study and invest more time to evaluate these polymeric materials as solid-phase extraction sorbents.

The successful results shown in this chapter for the selective separation of strongly acidic and weakly acidic analytes from a mixed analyte mixture should be challenged further by choosing conditions that closer resemble real-life conditions found in the environment.

Only 10 cm^3 of sample was loaded onto the SPE sorbent, so it would be necessary to increase the sample loading volume to test the SPE sorbent using greater quantities of aqueous solution, *e.g*. 100 cm^3, 250 cm^3, 500 cm^3 and then 1,000 cm^3. Organic pollutants are often found at low concentrations in the environment, so it would be useful to test the SPE sorbents to determine how well they are able to perform when the concentration of analytes in the sample are very low.

Another limitation in this study was that the mixture of analytes at known concentration was spiked in ultrapure MilliQ water from the laboratory. To progress further with the evaluation, the water source should be upgraded to more challenging tap water spiked with analytes. The key goal would be to progress to passing real, environmental samples of complex mixtures of analytes and interfering compounds through the SPE sorbents and measuring their performance.

Should the sorbents in this study continue to perform successfully in these additional experiments, then they should be tested against commercial sorbents to observe how they compare in terms of selectivity and capacity.

Chapter 6 - References

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