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ASSESSMENT OF BISPHENOL A IN THE MARINE ENVIRONMENT

By

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This thesis is the result of the author’s original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed:

Date:

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Abstract

Bisphenol A (BPA) is a high production volume chemical that is used widely in the production of polycarbonate plastics, epoxy resins, flexible polyvinyl chloride (PVC) plastics, and thermal paper. It is an endocrine disrupting chemical (EDC) and is considered as an emerging pollutant in the marine environment due mainly to plastic litter. This study developed analytical methodology for the measurement of BPA in marine waters and sediments so that ambient concentrations and the partition

coefficients that influence environmental fate and toxicity of BPA in the marine environment could be determined.

The experimental partition coefficients (octanol-water partition coefficient (K_{ow}), water solubility (S_w), and sorption capacity (K_d and K_{oc})) of BPA were affected by water conditions (salinity, pH and temperature). The sorptive process was not affected by only water conditions, but also solid properties (organic carbon (OC) and minerals (silica and calcium)) in the solid samples. The calculation equations to estimate each partition coefficient studied at any specific water conditions were also provided. These equations demonstrate the use of simple and inexpensive proxies (*e.g.*, salinity, pH and temperature) along with the modelling programme EPI Suite™ to assess environmental impacts associated with these partition coefficients. A climate change scenario was used as a case study to illustrate the effects of changing environmental conditions on the behaviour of BPA in the marine environment.

The concentrations of BPA were analysed using high performance liquid chromatography (HPLC). The extraction and pre-treatment methods to measure BPA in marine samples (seawater and sediments) were developed. For seawater, the extraction methods studied were conventional solid-phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME), and molecularly imprinted solidphase extraction (MISPE). The accelerated solvent extraction (ASE) was used to extract BPA from the marine sediment prior to clean-up of the sample with MISPE cartridges, and analysis with HPLC.

Publications

Borrirukwisitsak, S., Keenan, H. E. and Gauchotte-Lindsay, C. (2012). Effects of salinity, pH and temperature on the octanol-water partition coefficient of bisphenol A. *International Journal of Environmental Science and Development*, *3*(5), 460-464.

Borrirukwisitsak, S., Keenan, H. E., Gauchotte-Lindsay, C. and Cormack, P. A. G. (2013). Effects of climate change on environmental fate, bioavailability, bioaccumulation and toxicity of bisphenol A (BPA) in the marine environment. In: Proceedings of the 2nd Asian Conference on Civil, Material and Environmental Sciences, Osaka, Japan, 7th – 9th November 2013. Higher Education Forum, 329-334.

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List of Abbreviations

4-VP	4-Vinylpyridine
ACN	Acetonitrile
AFC	Atom/Fragment Contribution
AIBN	2, 2’-azo*bis*isobutyronitrile
APHA	American Public Health Association
ASE	Accelerated Solvent Extraction
ASTM	American Society for Testing and Materials
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BET	Brunauer, Emmett and Teller
BP	Boiling Point
BPA	Bisphenol A
Ca	Calcium
$CaCO_3$	Calcium Carbonate
CAS	Chemical Abstract Service
C_e	Equilibrium Concentration of Chemical in Solution

ChV	Chronic Value
C_s	Salt Concentration in Solution
DLLME	Dispersive Liquid-Liquid Microextraction
DVB	Divinylbenzene
EC_{50}	Median Effective Concentration
ECOSAR	Ecological Structure Activity Relationships
EDC	Endocrine Disrupting Chemical
EC	Electrical Conductivity
EEA	European Environmental Agency
EFSA	European Food Safety Authority
EPA	United States Environmental Protection Agency
EPI Suite	Estimation Programs Interface Suite
EWG	Environmental Working Group
f_{oc}	Fraction of Organic Carbon
FDA	United States Food and Drug Administration
FT-IR	Fourier-Transform Infra-Red Spectroscopy
G	Gibbs Energy
GC-MS	Gas Chromatography-Mass Spectrometry
GHG	Greenhouse Gas
H	Enthalpy
HCl	Hydrochloric Acid
HL	Biotransformation Half-Life
HLC	Henry’s Law Constant
HPLC	High Performance Liquid Chromatography

IPCC	Intergovernmental Panel on Climate Change
IUPAC	International Union of Pure and Applied Chemistry
KCl	Potassium Chloride
K_d	Sediment-Water Partition Coefficient
K_H	Henry's Law Constant
K_{OA}	Octanol-Air Partition Coefficient
K_{oc}	Organic Carbon Partition Coefficient
k_{oc}	Salting Coefficient of Organic Carbon Partition Coefficient
K_{om}	Organic Matter Partition Coefficient
K_{ow}	Octanol-Water Partition Coefficient
k_{ow}	Salting Coefficient of Octanol-Water Partition Coefficient
K_s	Setschenow Constant
LC_{50}	Median Lethal Concentration
LC-MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit of Detection
LOEC	Lowest Observed Effect Concentration
M	Molar Concentration
MCI	Molecular Connectivity Index
ME	Absolute Mean Error
MIP	Molecularly Imprinting Polymer
MISPE	Molecularly Imprinted Solid-Phase Extraction
MP	Melting Point
MPL	Maximum Permissive Level
MW	Molecular Weight

NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NIP	Non-Molecularly Imprinting Polymer
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NRDC	Natural Resources Defense Council
NSW	Natural Seawater
NTP	National Toxicology Program
OC	Organic Carbon
OECD	Organisation for Economic Co-operation and Development
OH	Hydroxyl Group
OM	Organic Matter
pK_a	Acid Dissociation Constant
PP	Precipitation Polymerisation
psu	Practical Salinity Unit
Q_e	Equilibrium Concentration of Chemical in Solid Phase
QSARs	Quantitative Structural-Activity Relationships
R	Ideal Gas Constant
R^2	Correlation Coefficient
RfD	Reference Dose
RP-HPLC	Reverse Phase-High Performance Liquid Chromatography
RSD	Relative Standard Deviation
S	Entropy
SD	Standard Deviation

SEM	Scanning Electron Microscope
SFM	Shake Flask Method
SI	International System of Units
SiO_2	Silica Oxide
SMILES	Simplified Molecular Input Line Entry System
SML	Specific Migration Limit
SPE	Solid-Phase Extraction
SST	Sea Surface Temperature
S_w	Water Solubility
T	Temperature
TDI	Tolerable Daily Intake
T:FM:X ratio	Template : Functional Monomer : Crosslinker Ratio
TOC	Total Organic Carbon
VP	Vapour Pressure
WWF	World Wide Fund for Nature
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence

Chapter 1 Introduction

1.1 Background

In recent years, the concern about the level of contamination of man-made chemicals in the environment has been increasing due to their potential adverse effects on both organisms and the environment itself. One interesting man-made organic chemical is bisphenol A (BPA) because of its high production volumes and toxicity. BPA is an endocrine disrupting chemical (EDC) that exhibits estrogen-like action and can interfere with the function of various hormone systems. BPA can be detected in the aquatic environment, including the marine environment. It is an emerging pollutant in the marine environment because it exists in wastewater from industry and leaching from landfill and is also released from plastic debris in the ocean. Its persistence in the marine environment is possibly longer than that in freshwater.

An environmental modelling programme (Estimation Programs Interface Suite™, EPI Suite™) has been used in environmental and risk assessment (EPA, 2012b). The model provides physicochemical properties, partitioning data and toxicity of chemicals. However, the programme does not take environmental conditions into consideration. Despite evidence that the environmental conditions influence the behaviour of pollutants in the environment, the default results retrieved from the model, although based on inherent chemical and physical properties, are thus only estimate values and refer to standard conditions (*e.g.*, 25 °C, pH 7, no salinity).

Results are not site specific and may lead to unreliable environmental and risk assessment of compounds.

The most pressing concern regarding environmental issues and human impact is climate change (IPCC, 2007), which affects ocean conditions (*e.g.*, temperature, sea level, salinity and ocean acidity). The effects of climate change on fate and transport of organic pollutants in the marine environment are often overlooked. Most research regarding the fate and transport of BPA focus on freshwater systems because BPA is a synthetic chemical that is originating from industry and landfill. It is normally released into freshwater system and/or groundwater. As BPA easily degrades under aerobic conditions, the amounts of BPA remaining in the aquatic system and contaminating into the marine system may decrease with time after release. Hence, there are few investigations relating the effects of marine environmental conditions on the fate and transport of BPA. This study was undertaken to fill these knowledge gaps.

1.2 Scope of Thesis

This research focuses on BPA in the marine environment. In this work, a costeffective analytical method for BPA has been developed using HPLC-UV. The natural seawater and sediment used in this study were collected from Prestwick, United Kingdom (UK). In the sorption study, different sediments were used to investigate the effects of solid properties on the sorptive process of BPA. These solid samples included sediments collected from Thailand (Rayong, Pattaya, Samet Island, and Kudee Island) and UK (Prestwick); solid samples from organisms (mussel shells and prawn shells); and a high organic carbon sample (biochar). The effects of seawater conditions (salinity, pH and temperature) on the important partition coefficients used in computer-based environmental assessment were evaluated. These partition coefficients were the octanol-water partition coefficient (K_{ow}), water solubility (S_w), and solid sorption coefficients (K_d and K_{oc}). The computer model used was EPI Suite™ version 4.11, and the experimental partition coefficients obtained from this study were compared with the default partition coefficients retrieved from the model. In climate change scenario, the concomitant change in the predicted environmental fate of BPA was investigated experimentally.

To monitor BPA in marine samples within the detection limit of the developed costeffective analytical method, effective extraction methods have been developed. The extraction methods were a conventional solid-phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME), and molecularly imprinted solid-phase extraction (MISPE). Optimal SPE conditions were developed experimentally, while the DLLME method was carried out following the method stated in the literature. MISPE experiments were performed using commercial MISPE cartridges and inhouse prepared MISPE cartridges. The optimal molecularly imprinted polymer (MIPs) used as the in-house MISPE sorbent was developed experimentally, as well as MISPE conditions, as part of this thesis.

1.3 Research Aim and Objectives

The aim of this study is to assess the partitioning behaviour, fate, transport and toxicity of BPA in the marine environment. This assessment provides insight into the potential for human and environmental exposure to BPA from the marine environment. To achieve this aim, the objectives of this study are:

(i) Identify possible sources of BPA leaching to the marine environment (ii)

Establish a suitable analysis method for BPA using HPLC-UV.

(iii) Determine the partition coefficients of BPA (*i.e.*, the octanol-water partition coefficient, water solubility and sorption coefficients) and compare these experimental values with the default values from the computer model.

(iv) Determine the effects of water conditions (*i.e.*, salinity, pH and temperature) on the studied partition coefficient, and additional solid properties on sorption coefficients.

(v) Evaluate the fate of BPA based on experimental values at different environmental conditions.

(vi) Investigate the environmental fate of BPA in marine environments under a climate-change scenario.

(vii) Establish an effective extraction method for BPA from marine environmental samples suitable for used with the developed analysis method.

By achieving the stated objectives, a better understanding of the current behaviour of BPA in the marine environment and the impacts of climate change may be evaluated. The findings may correlate with other organic pollutants known to contaminate sensitive marine environments. There is limited understanding of this issue, and this project provides a framework to investigate chemical fate in an ever changing environment.

Chapter 2 Literature Review

2.1 Introduction to Bisphenol A

2.1.1 Background of Bisphenol A

In recent years, the concern about the level of contamination of man-made chemicals in the environment has been increasing due to their potential adverse effects on both organisms and the environment. Of particular interest to this study is the man-made organic chemical bisphenol A (BPA) or 4,4'-isopropylidenediphenol (CAS number 80-05-7). BPA can be toxic and is also considered an endocrine disrupting chemical (EDC). Its structure is as shown in Figure 2.1 (Environment Canada, 2008).

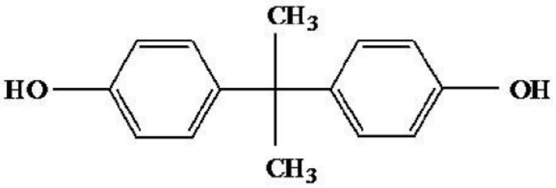


Figure 2.1 Chemical structure of BPA

The first reported synthesis of BPA from phenol and acetone (see Figure 2.2) was published by Thomas Zincke of the University of Marburg, Germany, in 1905. At that time, the key physical properties of BPA (*e.g.*, molecular composition, melting point, solubility in common solvents) were reported, but no specific use was proposed (The Bisphenol A Global Industry Group, 2002).

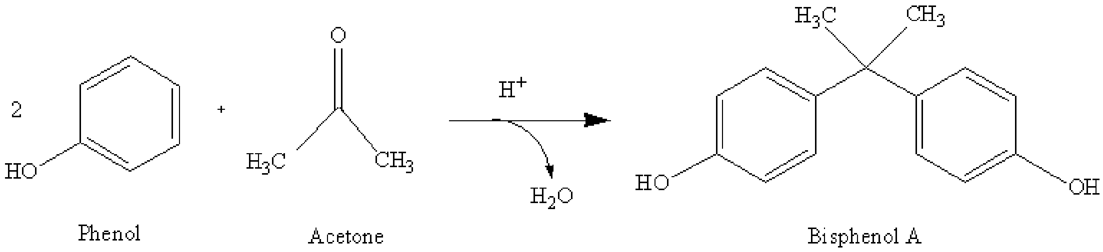


Figure 2.2 The synthesis reaction of BPA

In the 1930s, BPA was considered for clinical use as a synthetic oestrogen before the discovery of diethylstilboestrol, which was found to be a more effective synthetic oestrogen. In the 1950s, Bayer chemist, Hermann Schnell, rediscovered BPA to produce polycarbonate plastic (PC) by reacted BPA with phosgene, as shown in Figure 2.3. Subsequently, BPA has been widely used to produce PC plastics (Oehlmann *et al.*, 2009).

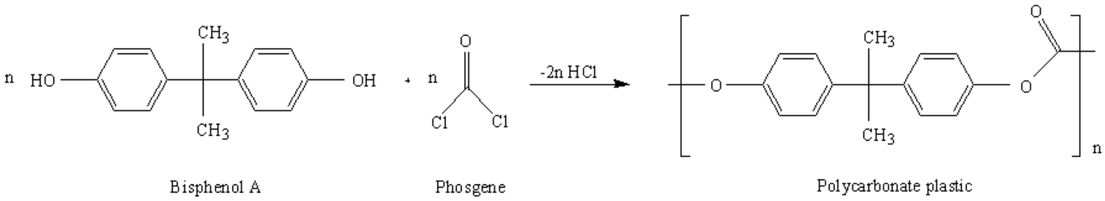


Figure 2.3 The synthesis reaction of polycarbonate plastic

2.1.2 Applications of Bisphenol A

BPA is one of the highest volume chemicals that is manufactured, with approximately three billion kilograms produced in 2003 (Burridge, 2003) and nearly four billion kilograms in 2006 (Environment Canada, 2008). BPA has been produced in many countries throughout the world, especially Germany, the Netherlands, the USA, Japan and Thailand (WWF, 2000). The global market growth was 5.7% annually from the

1990s to 2003, and increased to almost 10% annually from 2003 to 2006. Recently, global consumption of BPA was expected to be in the range of 0% – 5% per year. The highest growth of 5% per year was expected for Asia, particularly China, while a flat rate (approximately ± 1%) was expected in Europe. The apparent decrease in the rate of growth of global demand may be due to increasing concerns related to its toxicity (Burridge, 2008).

BPA has been commonly used as a monomer and plasticiser to produce polycarbonate plastics (PCs), epoxy resins, and other polymeric materials. About 90% of the BPA produced is used in PC and epoxy resin production (about 65% in PC production and about 25% in epoxy resin production) and in other manufacturing (the remaining 10%), as shown in Figure 2.4 (WWF, 2000). PCs are widely used in a variety of products due to their properties, including heat resistance, hardness, clearness, and shatter-resistant plastic. Epoxy resins are used for lining metal cans to prevent corrosion and food contamination; industrial protective coatings; electrical laminates; and adhesives. BPA-containing products include baby bottles; drinking and food containers; polyvinyl chloride (PVC) stretch films for food packaging; toys; water pipes; medical equipment; dental sealants; eyeglass lenses; CDs and DVDs; and consumer electronics. BPA is used as a colour developer in thermal paper, which is used in cash receipts. In addition, BPA is used in the manufacture of the flame retardant tetrabromobisphenol as well as in flexible PVC production and processing as a polymerisation inhibitor and antioxidant (WWF, 2000; The Bisphenol A Global Industry Group, 2002; EPA, 2010; Mendum *et al*., 2010).

In general, there are seven classes of plastics. Type 7 is a category that includes all other plastics not covered by types 1 - 6 and includes PC plastics and epoxy resins. Type 3 plastic is PVC, some of which may contain BPA. Therefore, some type 3 and 7 plastics may be made with BPA, while type 1, 2, 4, 5 and 6 plastics are unlikely to contain BPA (NTP, 2008).

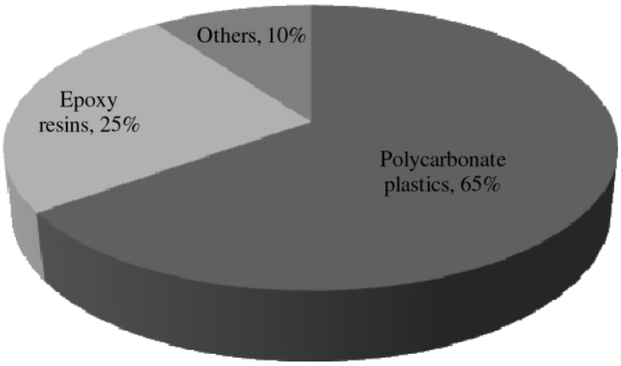


Figure 2.4 The proportion of the uses of the BPA produced (modified from WWF, 2000)

Health problems associated with BPA has created pressure on the plastic industry to produce BPA-free products. The BPA-free PC-replacement products can be made from acrylic, cyclic olefin copolymer (COC), cyclic olefin polymer (COP), glycolmodified polyethylene terephthalate (PETG), polyethersulfone (PES), polystyrene (PS), Tritan™ copolyester, or bisphenol S (BPS) (Bittner *et al*., 2014).

2.1.3 Physicochemical Properties of Bisphenol A

The physicochemical properties of a chemical are important in environmental and human exposure assessment because the behaviour of a substance in the environment is controlled by its physical and chemical properties. The important physicochemical properties of BPA that are relevant to its environmental fate, transport and toxicity are shown in Table 2.1.

Table 2.1 Physicochemical properties of BPA

Properties	Type	Conditions	Values	Reference
Molecular formula			$C_{15}H_{16}O_2$	European Commission, 2000[a]
Molecular weight (g mol^{-1})			228.29	European Commission, 2000[a]
Melting point (°C)	Experiment		150 – 157	Howard, 1989[b]; Dow Europe, 1993[b]
	Model		132	MPBPWIN, 2000[b]
Boiling point (°C)	Experiment		220 – 398	Howard, 1989[b]; Dow Europe, 1993[b]
	Model		364	MPBPWIN, 2000[b]
Density (kg m^{-3})	Experiment	25 °C	1,195	Sax and Lewis, 1996[b]
Water solubility (S_w, mg L^{-1})	Experiment	22 – 24 °C	253 – 257	Lee and Peart, 2000b[b]
		Room Temperature	301	Bayer, 1988[b]
		25 °C	120	Dorn *et al*., 1987[b]
	Model	25 °C	173	WSKOWWIN, 2000[b]
Other solubilities (mg L^{-1})	Experiment (alcohol)		Soluble	Lewis, 2000[b]
Vapour Pressure	Experiment	25 °C	5.3×10^{-6}	Bayer, 1988[b]

(Pa)	Model	25 °C	3.0 x 10^{-5} (2.27 x 10^{-7} mmHg)	MPBPWIN, 2000[b]

Properties	Type	Conditions	Values	Reference
Henry's law constant (Pa m^3 mol^{-1})	Experiment		1.0 x 10^{-6} (1.0 x 10^{-11} atm m^3 mol^{-1})	Hine and Mookerjee, 1975[b]
	Model	25 °C	9.3 x 10^{-7} (9.16 x 10^{-12} atm m^3 mol^{-1})	HENRYWIN, 2000 (Bond estimation)[b]
			S_w = 120 mg L^{-1}: 4.0 x 10^{-5} (3.95 x 10^{-10} atm m^3 mol^{-1})	HENRYWIN, 2000 (VP/S_w estimation)[b]
			S_w = 257 mg L^{-1}: 4.7 x 10^{-6} (4.7 x 10^{-11} atm m^3 mol^{-1})	HENRYWIN, 2000 (VP/S_w estimation)[b]
Log K_{ow} (dimensionless)	Experiment (Shake Flask)	pH 3	3.32	Howard, 1989[b]; Hansch *et al.*, 1995[b]
		Ambient pH	3.40	Staples *et al.*, 1998[c]
	Experiment (HPLC)		2.20	Eadsforth, 1983[c]
	Model		3.64	KOWWIN, 2000[b]
Log K_{oc} (dimensionless)	Experiment		2.53 – 2.85 (pH 4.5 – 5.9)	Loffredo and Senesi, 2006[b]

Properties	Type	Conditions	Values	Reference
	Calculation		2.85	ECB, 2003[b]
	Model		4.88	PCKOCWIN, 2000[b]
Log K_{OA} (dimensionless)	Model		12.7	KOAWIN, 2000[b]
pK_a (dimensionless)	Experiment		9.59 – 11.30	Staples *et al.*, 1998[c]
	Model		9.73 – 10.48	ACD, 2005[b]
			9.6 – 10.2	EPA, 1994[c]
Biodegradation			Readily (aerobic)	Howard, 1989[b]
Half-lives (days)			2.5 – 4	Staples *et al.*, 1998[c]
BCFs (dimensionless)			5 – 68	Staples *et al.*, 1998[c]
Toxicity			Slightly to moderately	Staples *et al.*, 1998[c]

Sources: [a] = European Commission, 2000;
[b] = Environment Canada, 2008;
[c] = Staples *et al.*, 1998

According to the physical and chemical properties of BPA in Table 2.1, BPA is moderately hydrophobic; has low volatility; and a moderate organic carbon sorption. The high pK_a range of 9.59 to 11.30 indicates that BPA is a very weak acid and it has greater solubility at alkaline pH values (high pH). When released into the environment, it will likely partition to all available environmental phases as a result of its measurable vapour pressure (VP), organic carbon-air partition coefficient (K_{OA}), water solubility (S_w), organic carbon-water partition coefficient (K_{oc}), and octanol-water partition coefficient (K_{ow}). BPA is readily biodegradable under aerobic conditions, but the biodegradation rate is less under anaerobic conditions (Environment Canada, 2008). Although BPA is expected to have low persistence (half-lives from 2.5 – 4 days) and low bioaccumulation potential (BCFs 5 – 68), it has been commonly detected in the environment due to near-continuous release (Staples *et al.*, 1998; Environment Canada, 2008).

2.1.4 Toxicity of Bisphenol A

With regard to toxicity, BPA can possibly harm both human health and the environment. BPA can irritate eyes, lungs and skin. It is considered an endocrine disrupting chemical (EDC) because it can mimic the action of oestrogen, a reproductive hormone, and interfere with the function of various hormone systems. Moreover, BPA can disrupt cell function and may cause breast and prostate cancer. BPA has the potential for health effects on brain development, prostate gland development, and behaviour of children with initial effects noted in the earliest stages of foetal development (Environment Canada, 2008; NTP, 2008; Meeker *et al.*, 2009; Talsness *et al.*, 2009; EPA, 2010; Ayyanan *et al.*, 2011; FDA, 2014). As a result, tolerable daily intake (TDI) of BPA was established at 50 µg of BPA kg^{-1} body weight day^{-1} (EPA, 1993; EFSA, 2006) and is currently under review for potential reduction in the near future (see Section 2.1.5).

In terms of environmental toxicity, BPA was found to cause reproductive and developmental disturbances in a variety of wildlife species (Crain *et al.*, 2007;

Oehlmann *et al*., 2009; Flint *et al*., 2012). For example, Aarab *et al*. (2006) studied the effects of BPA on gonadal development and reproduction in common mussels (*Mytilus edilus*) and found that BPA affected spawning in male and female mussels as well as phospho-protein expression in females. Moreover, BPA-exposed female mussels had severe damaged ovarian follicles and ovocytes. Canesi *et al*. (2007) also found that BPA altered gene expression and functional parameters (*e.g*., activities of enzymes involved in redox balance, lysosomal function) in the hepatopancreas of the bivalve mollusc *Mytilus galloprovincialis*. Biggers and Laufer (2004) found that BPA affected larval metamorphosis in American lobster (*Homarus americanus*), suggesting that BPA was affecting hormone expression in juveniles because of its role in stimulating larval metamorphosis.

Some studies at doses well below the TDI of 50 μg of BPA kg^{-1} body weight day^{-1} have shown detrimental physiological effects, known as low-dose effects (Nagel *et al*., 1997; vom Saal *et al*., 1998; Koch and Calafat, 2009; Wadia *et al*., 2013; Vandenberg, 2014). Low-dose effects are defined as “biological changes that occur at environmentally relevant exposure levels or at doses that are lower than those typically used in EPA’s standard toxicity testing paradigm” or “the effects of doses below the currently accepted No Observed Adverse Effect Level (NOAEL) for that substance” (NTP, 2008). The examples regarding low-dose effect are shown in Table 2.2.

Table 2.2 Studies of low-dose toxicity of BPA

Daily BPA Exposure (μg kg^{-1} body weight day^{-1})	Toxic Effect	Study Year
0.0001	Alterations in cell signalling pathways on the cell surface that control calcium influx in cells	2005

Daily BPA Exposure (μg kg^{-1} body weight day^{-1})	Toxic Effect	Study Year
0.025	Persistent changes to breast tissue, predisposes cells to hormones, and carcinogens	2005
0.025	Permanent changes to genital tract	2005
0.2	Decrease antioxidant enzymes	2003
0.25	Altered growth, cell size and lumen formation in mammary epithelium of mouse fetuses.	2007
2	Increased prostate weight 30%	1997
2	Increased aggression at 8 weeks of life	2003
2.4	Decreased time from vaginal opening to first estrus, possibly earlier puberty	1999
2.4	Lower bodyweight, increase of anogenital distance in both genders, signs of early puberty, and longer estrus.	2002
2.4	Decline in testicular testosterone	2004
2.5	Immune system impacts	2003
2.5	Breast cells predisposed to cancer	2006
10	Decreased maternal behaviors	2002
10	Prostate cells more sensitive to hormones and cancer	2005
10	Prostate cells more sensitive to hormones and cancer	2006
10	Insulin resistance develops in 2 days, chronic hyperinsulinemia at day 4	2006

20	Damage to eggs and chromosomes	2003
20	Brain effects - disrupted neocortical development by accelerating neuronal differentiation and migration	2006
20	Damage to eggs	2007
30	Reversed the normal sex differences in brain structure and behavior	2003
30	Hyperactivity	2004

Source: Reproduced from EWG, 2007

These results about low-dose effects of BPA are increasing public concern about toxicity. Debates continue regarding the “real safe” limit of BPA because the current safe limit is predicted based on high dose exposures (Aschberger *et al.*, 2010; Vandenberg, 2014).

2.1.5 Legislation and Regulations for Bisphenol A

Since the early 1980’s, BPA has been evaluated as a chemical of potential concern by the United States of America (USA), European Union (EU), and Japan as well as other countries (EPA, 2010). According to the risk assessment study, TDI or oral reference dose (oral RfD) of BPA was established at 50 µg kg^{-1} body weight day^{-1}. This value was estimated based on the NOAEL of 5 mg kg^{-1} body weight day^{-1} from reproductive and developmental effects in animal studies and applying an uncertainty factor of 100 (EFSA, 2006; EPA, 2010). It was noted that the effects of BPA are agedependent. The toxicity will be higher on infants, and even higher on newborns. As a result, for the worst case scenario, the dietary exposure to BPA for infants was set at 0.2 µg kg^{-1} body weight day^{-1} in 3-month-old breastfed babies, whereas at 13 µg kg^{-1} body weight day^{-1} in 6-12-month-old infants. The worse case dietary exposures to BPA for young children and adults were estimated at 5.3 and 1.5 µg kg^{-1} body weight day^{-1}, respectively (EFSA, 2006). Due to the adverse effects of BPA, many countries have established regulations on the use of BPA, as shown in Table 2.3.

Table 2.3 states that many countries ban the use of BPA in baby bottles and food contact materials for infants, resulting from the public concern about the effects of BPA on infants.

Table 2.3 Regulations on the use of BPA

Country	Regulation	Date of Decision or Application
USA	Ban BPA in infant formula packaging	Decided July 2013
France	Ban BPA in all food containers intended for direct contact with food	Voted 24/12/12 Applied 1/01/15
France	Ban BPA in food contact materials infants and young children under the age of three	Voted 24/12/12 Applied 01/01/13
Belgium	Ban BPA in food contact materials intended for children up to the age of three	Voted September 2012 Applied 01/01/13
Sweden	Ban BPA in food packaging intended for children under the age of three	Applied beginning 2013
Austria	Ban BPA in pacifiers or teethers made with BPA	1 February 2012

USA	Ban BPA from use in infant and toddler products such as baby bottles and sippy cups	Decided July 2012
China	Ban BPA in infant feeding bottles	Applied 1/06/11
Russia	Maximum Permissive Level (MPL) for BPA in water – 10 µg L^{-1}	
EU	No BPA to be used for the manufacture of polycarbonate infant feeding bottles	Decided 28/01/11 Applied 1/06/11
EU	Specific migration limit (SML) of BPA in food of 0.6 mg kg^{-1}	Decided 28/01/11 Applied 01/02/11
Canada	Ban BPA in baby bottles	Decided August 2008

Source: Reproduced from Polyintell, 2014

In the EU, BPA was authorised to be used as a monomer and additive to produce plastics and food contact materials by the Commission Directive 2002/72/EC on 6 August 2002. From May 2011, the use of BPA in EU in the Commission Directive 2002/72/EC was replaced by Regulation (EU) No 10/2011. In this regulation, BPA has been banned in PC infant feeding bottles and maintained a specific migration limit (SML) of BPA to foodstuffs at 0.6 mg kg^{-1} food (EFSA, 2014). In January 2014, the European Food Safety Authority (EFSA) released the *Draft Scientific Opinion on the Risks to Public Health Related to the Presence of BPA in Foodstuffs* (Question No EFSA-Q-2012-00423) for public consultation (EFSA, 2014). In the draft report, the new TDI was recommended to be lowered to 5 µg kg^{-1} body weight day^{-1}. Due to current uncertainties of the potential health hazards of BPA, this new TDI is recommended as a temporary TDI (t-TDI) until the results of research from the US National Toxicology Program (NTP) are released because the outcome of the NTP research should address many of the current uncertainties.

2.1.6 Environmental and Human Exposures to Bisphenol A

The extent of environmental exposure is indicated by the environmental fate and transport of chemicals. Evaluation of the available data makes it practicable to determine the behaviour of a substance in environmental media (soil, sediment, water, and air); potential bioavailability; and potential bioaccumulation in organisms. Environmental fate and transport are related to:

- The partitioning of chemicals between environmental media
- The transport properties of the media
- The degradation rates of chemicals

The tendency of BPA to partition to a particular environmental medium can be estimated from the physical and chemical properties of the chemical (as described in Section 2.1.3). The trend for transportation of BPA depends on the properties of the media into which the chemical is released partitions as well as its lifetime in each medium.

Contamination of BPA in the environment is restricted to human activities as it is a synthetic substance with no known natural source. It is released into environment during manufacturing, processing, use or disposal of the chemical or BPA-containing products. Nevertheless, BPA is discharged to air and water; discharges to water are likely to be of more concern since it is expected to persist longer. This is because BPA in the air is expected to break down fairly rapidly. Once BPA is released into the aquatic environment, it is expected to dissolve in water, sorb to sediments, and be absorbed by aquatic and benthic organisms. BPA can bioaccumulate in organisms because it is a lipophilic compound and subsequently magnify in the food chain.

Humans become exposed through the ingestion of BPA-laden biota. Human-derived solid wastes and sewage sludges that containing BPA contribute additional BPA contamination to the soil compartment (WWF, 2000; Environment Canada, 2008).

Unlike the extensive data available for fresh surface water, there is limited data related to BPA in sediments and even less for the marine environment (Klečka *et al*., 2009). BPA is an emerging pollutant of the marine environment and public concern about BPA in marine ecosystems is currently increasing because of increased plastics in the ocean either from litter or land-based sources (Sajiki and Yonekubo, 2003; Ryan *et al*., 2009). Plastics are one of the potential sources of BPA in the marine environment since the plastics can break down into smaller pieces (plastic debris) or be degraded (Teuten *et al*., 2009). Sajiki and Yonekubo (2003) observed the leaching of BPA from PC tubes into control water (BPA free), seawater and river water. The degradation of BPA in these samples was also investigated. The results found that BPA leaching into seawater was fastest, and the degradation rate of BPA in seawater was lowest. Kang and Kondo (2005) indicated that BPA could persist longer in seawater than in freshwater without any degradation for about 30 days and possibly accumulate more in marine organisms than that in freshwater organisms. BPA was observed to persist and accumulate in anoxic estuarine sediments with no degradation observed over 100 days (Voordeckers *et al*., 2002; Ying and Kookana, 2003). Consequently, the behaviour of BPA in the marine environment is an interesting issue that requires further research.

BPA is present in many environmental compartments. Numerous studies indicate that BPA has been detected in freshwater, seawater, landfill leachates, air, dust particles and biota, and finally, transfers to human (Heemken *et al*., 2001; Yamamoto *et al*., 2001; Belfroid *et al*., 2002; Basheer *et al*., 2004; Matsumoto *et al*., 2005; Kang *et al*., 2006; Sternbeck, 2007; Klečka *et al*., 2009; Thompson *et al*., 2009; Mita *et al*., 2011; Wei *et al*., 2011). For example, the concentrations of BPA in the Elbe River in

Germany were detected at 0.009 to 0.776 µg L^{-1} in water and at 66 - 343 µg kg^{-1} dry weight in sediment (Heemken *et al.*, 2001). BPA was measured in surface water in the Netherlands at concentrations up to 330 ng L^{-1}, and one sample was observed at a level of 21 µg L^{-1}. The concentrations of BPA in fish in the Netherlands were also detected at the levels ranging from 2 to 75 ng g^{-1} in the liver and 1 to 11 ng g^{-1} in the muscle (Belfroid *et al.*, 2002). BPA was detected in seawater in Singapore at levels up to 2.47 µg L^{-1} and in all seafood commercially in a Singapore supermarket (prawn, crab, blood cockle, white clam, squid, and fish) between 13.3 and 213.1 ng g^{-1} wet weight (Basheer *et al.*, 2004). Based on 89 investigations published between 1997 and 2007, the concentrations of BPA in fresh surface waters in North America (n = 1,068) and Europe (n = 848) were reported at the median concentrations of 0.081 and 0.01 µg L^{-1}, respectively. The 95th percentiles were 0.47 and 0.35 µg L^{-1}, respectively. The median concentrations in freshwater sediments in North American (n = 71) and Europe (n = 249) were 0.6 and 16 ng g^{-1} dry weight, respectively (Klečka *et al.*, 2009). Mita *et al.* (2011) found that most of fish caught in two different sites of the Tyrrhenian Sea (Italy), which were the Gulf of Naples and Litium coasts, were contaminated with BPA. They also found that BPA had polluted fish livers 2.5 times higher than muscle. Therefore, people consuming fish caught in these areas were likely exposed to BPA.

Significantly, literature indicates that BPA-derived products can release BPA into food and drinks even under normal conditions of use, especially cans with epoxy resin linings (Brotons *et al.*, 1995; Maia *et al.*, 2009; Cao *et al.*, 2011). BPA can be released from BPA-containing products into foodstuffs by diffusion of residual BPA in the products and/or hydrolysis of plastic catalysed by hydroxide (OH^-). The release of BPA depends on contact time, temperature, and pH of food (Aschberger *et al.*, 2010). Likewise, many reusable food and drink containers contain BPA (*e.g.*, Tupperware, baby bottles), and are also markets for use in the microwave, although repeated use and heating may increase BPA leaching levels (Biles *et al.*, 1997a; Biles *et al.*, 1997b; Brede *et al.*, 2003; Nérin *et al.*, 2003; Wong *et al.*, 2005). It was also found that BPA in PVC stretch film for food packaging can contaminate into foodstuffs (López-

Cervantes and Paseiro-Losada, 2003). For instance, BPA leaching from twelve PC baby bottles after use was observed by placing 100 °C of water in the bottles for 1 h. The results found that BPA was measured in the new bottles at the level of 0.23 µg L^{-1}, while the concentrations of 8.4 and 6.7 µg L^{-1} were measured after dishwashing 51 times and 169 times, respectively (Brede *et al.*, 2003). Also, residual BPA was detected in 19 out of the 28 PC baby milk bottles in Singapore at the range of 4.01 - 141 mg kg^{-1}, with an average of 28.1 mg kg^{-1} and a median of 17.2 mg kg^{-1} (Wong *et al.*, 2005). Consequently, opportunities for human BPA exposure abound.

Humans can be exposed to BPA from the environment (air, soil, aquatic environment) and food. Oral ingestion is the main human exposure of BPA, although dermal exposure by contacting BPA-contaminated water and purchase receipts is also a possible route (Kang *et al.*, 2006; Vandenberg *et al.*, 2007). Several researchers (Catafat *et al.*, 2005; Engel *et al.*, 2006; Fernandez *et al.*, 2007; Catafat *et al.*, 2008; Dekant and Völkel, 2008; Talsness *et al.*, 2009) have monitored the levels of BPA in human tissue, blood, urine and other fluids. For example, Catafat *et al.* (2005) measured BPA in urine samples from 394 adults in the United States; the results found that 95% of the samples contained BPA at or above levels of 0.1 µg L^{-1} of urine, with the mean, median and the 95th percentile concentrations of 1.33, 1.28 and 5.18 µg L^{-1} of urine, respectively. BPA was observed in 11 out of 20 samples of adipose tissue in women (55%) at the levels above 0.5 ng mL^{-1} (Fernandez *et al.*, 2007). These results reveal that most people are exposed to BPA.

In addition, the NTP in the U.S. Department of Health and Human Services summarised the highest estimated daily intake of BPA in people based on source of exposure, as shown in Table 2.4 (NTP, 2008).

Table 2.4 Estimated daily intakes of BPA in people based on sources of exposure

Population		Daily Intake of BPA (µg kg^{-1} body weight day^{-1})
Infant	0 – 6 months Formula-fed	1 - 11
	0 – 6 months Breast-fed	0.2 - 1
	6 – 12 months	1.65 – 13
Child	1.5 – 6 years	0.043 – 14.7
Adult	General Population	0.008 – 1.5
	Occupational	0.043 - 100

Source: NTP, 2008

Despite low BPA exposure in adults (below TDI), the effects of BPA on humans (especially newborns and infants) needs to be put into perspective. This is because humans can be exposed to other potential endocrine disrupting chemicals, either from synthetic or natural materials. This is possibly leading to the cumulative and/or synergetic impacts of mixtures of EDCs on human health (Ascheberger *et al*., 2010). Moreover, doubt about reliable safe limits (*e.g*., TDI) of BPA is rising due to the low-dose toxicity study (EFSA, 2014).

Many researchers have studied BPA in fresh surface water (Klečka *et al*., 2009). However, contamination of BPA in the marine environment is also likely to have large impacts on human health and the environment. This is because plastic debris in the ocean can act as a source of BPA, and BPA is likely to be more persistent and accumulate in marine ecosystems. This is also leading to widespread contamination of BPA. To date, there is little research regarding the impacts of water conditions on the environmental behaviour and toxicity of BPA in the ocean despite the increasing concern related to BPA in the marine environment.

2.2 Environmental Modelling Programme

Computer modelling programmes can be used to estimate the environmental fate and toxicity of organic chemicals once released into the environment. Therefore, models are important tools in environmental and risk assessments of chemicals, especially for new chemicals.

2.2.1 Quantitative Structure-Activity Relationships (QSARs)

Environmental modelling was first developed using quantitative structural-activity relationships (QSARs), which were originally used in medicinal chemistry and drug discovery. QSARs have been used in order to relate the physicochemical properties of a chemical to its biological activity, which commonly depend on its molecular structure (Jensen, 2007). Physical, structural and chemical properties have been taken into account to obtain equations used for determining biological activity. It has been noted that properties of most organic chemicals vary consistently, and can predict the changes in molecular structure (Mackay, 2001; Patrick, 2005).

QSARs are multiple regression analyses searching for statistically significant correlations between biological response and chemical properties, as shown in equation 2.1 (Draber and Fujita, 1992).

$$\mathrm{Log}A = a_0 + \sum a_i x_i + \sum b_i x_i^2 \qquad \text{Equation 2.1}$$

Where:

A = biological response variable x_i = molecular formulae of substituent parameters for each molecule a_i, b_i = coefficients that relate physicochemical parameters to the response The most important property of biologically active substances is lipophilicity as cell membranes are composed of lipids. The biological activity of chemicals generally increases with increasing hydrophobicity. The hydrophobicity of a chemical can be measured by the octanol-water partition coefficient (K_{ow}), see details in Chapter 3. The lipophilicity of an organic compound is also related to its bioaccumulation in organisms and bioconcentration in aquatic organisms. In aquatic systems, high K_{ow} values tend to have high bioconcentration factors (BCFs), see equation 2.2, as aquatic biota commonly uptake most pollutants by passive diffusion through membranes of organisms. BCF can be determined by equation 2.3.

$$\text{Log BCF} = a \log K_{ow} + b \qquad \text{Equation 2.2}$$

$$\text{BCF} = \frac{\text{Concentration of chemical in biota}}{\text{Concentration of chemical in water}} \qquad \text{Equation 2.3}$$

QSARs are useful to predict the biological activity of chemicals but they are limited to predict the fate of organic compounds in the complex system such as the environment. In general, the environmental fate of a chemical is predicted by two main factors (Mackay *et al.*, 1992):

(i) The inherent properties of the compound (*i.e.*, S_w, VP)

(ii) The properties of the environment (temperature, flow of water, solids properties)

To predict the environmental fate of chemical, fugacity modelling is a popular method.

2.2.2 Fugacity Modelling

The concept of fugacity was introduced by G. N. Lewis in 1901 (Mackay, 2001), but applied to environmental modelling in the 1970s by Mackay (Mackay, 1979). Fugacity uses thermodynamic equilibrium partitioning behaviour of chemicals to explain the chemical potential of the compound in each phase (Mackay, 1979).

The word “fugacity” comes from the Latin root *fugere*, meaning to flee or escape. Thus, fugacity describes the escaping tendency of a compound. If the escaping tendency between two phases is equal, the phases are at equilibrium. Fugacity is equal to partial pressure in ideal gases and has units of pressure (Pascal, Pa). It is linearly or nearly linearly proportional to concentration at low concentrations, which covers most cases of interest. To predict the movement of substance across environmental compartments, the models use partition coefficients and mass balance equations (Mackay, 1979). The assumptions of fugacity (f) are each compartment is homogeneous and at equilibrium (this is not realistic). But tend to be valid for very persistent substances that are a major concern. Therefore, the partitioning behaviour of a chemical can be described by thermodynamics (Mackay, 1979).

Fugacity, which is assumed to be linearly proportional to the concentration (C), is measured in terms of fugacity capacity (Z), see equation 2.4 and 2.5 (Walker *et al.*, 1996).

$$f = \frac{C}{Z}$$

Equation 2.4

At equilibrium, fugacity in each phase is similar. For example, in two-compartment system, $f_1 = f_2$, then;

$$\frac{C^1}{Z_1} = \frac{C^2}{Z_2} = k_{1,2}$$

Equation 2.5

Where:

f = fugacity

C = concentration of substance in the

compartment Z = fugacity capacity, mol m^{-3} Pa

$k_{1,2}$ = the partition coefficient between compartment one and two

The Z value depends on the properties of the substance and the compartment it is present in, and is also a function of ambient temperature and pressure. The relationship between fugacity capacities and partition coefficients is shown in Figure 2.5. The definitions of Z values are described in Table 2.5 (Samiullah, 1990).

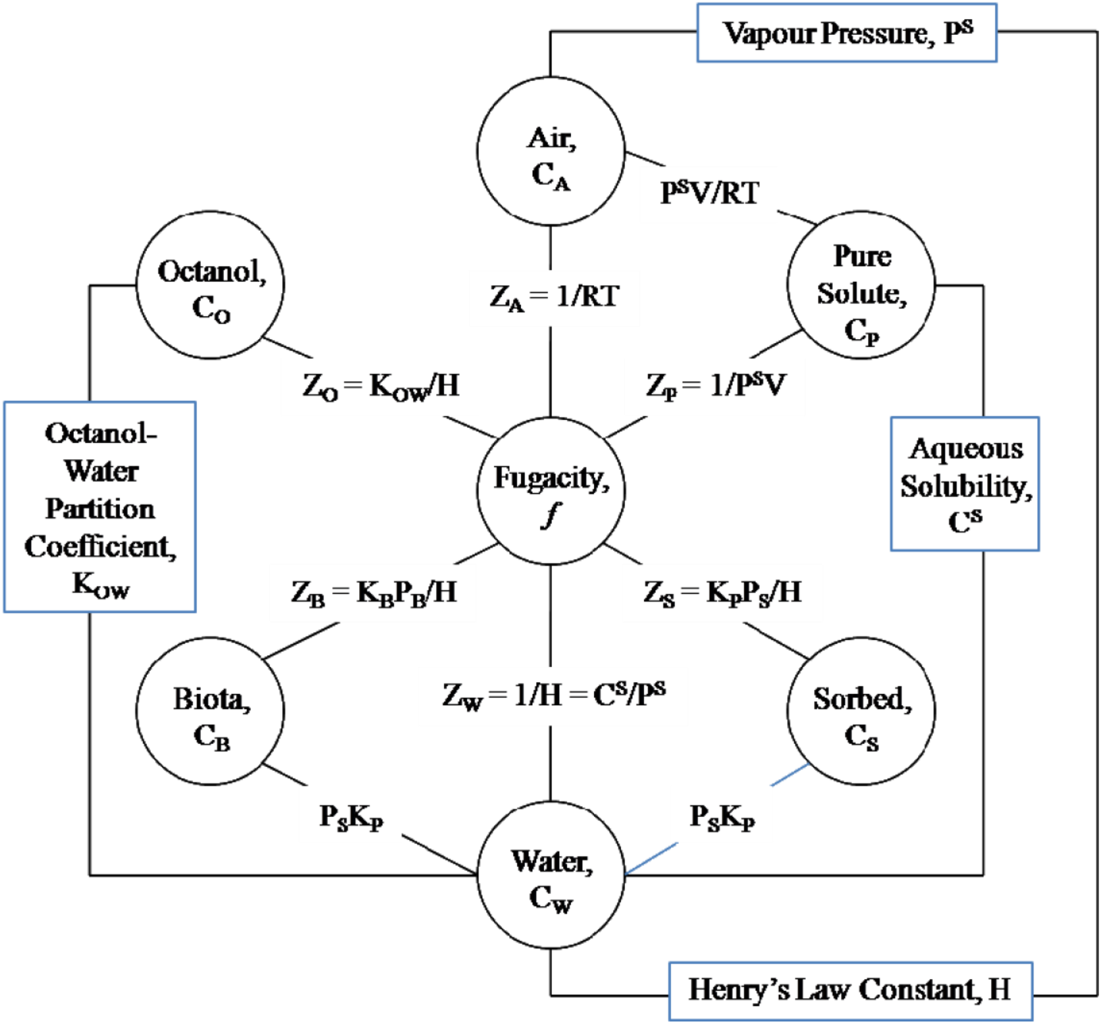


Figure 2.5 Relationship between fugacity (*f*), fugacity capacities (Z) and partition coefficients (K) (modified from Samiullah, 1990)

Table 2.5 The definitions of fugacity capacities (Z) for each compartment

Compartment	Definition of Z ($mol\ m^{-3}\ Pa$)	Definition of Terms
Air	$Z_A = 1/RT$	$R = 8.314\ Pa\ m^3\ mol^{-1}\ K^{-1}$ T = temperature (K) K_{AW} = air/water partition coefficient
Water	$Z_W = 1/H$ or C^S/P^S	H = Henry's law constant ($Pa\ m^3\ mol^{-1}$) C^S = aqueous solubility ($mol\ m^{-3}$) P^S = vapour pressure (Pa)
Soil Sorbent	$Z_S = K_{SW}P_S/H$	K_{SW} = soil-water partition coefficient P_S = phase density ($kg\ L^{-1}$)
Biota	$Z_B = P_SK_B/H$	K_B = bioconcentration factor
Pure Solute	$Z_P = 1/P^SV$	V = solute molar volume ($m^3\ mol^{-1}$)
Octanol	$Z_{OW} = K_{OW}/H$	K_{OW} = octanol-water partition coefficient

Source: Samiullah, 1990

For example, the interactions between five environmental compartments (air, soil, water, sediment, and aquatic biota) are represented in Figure 2.6.

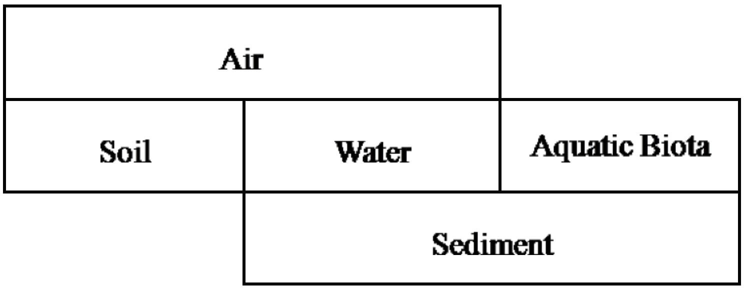


Figure 2.6 Interactions between five environmental compartments

There are four levels of fugacity, resulting from complexity of the environment of interest. The Level III fugacity model is the most popular. It uses the physicochemical properties of a chemical to predict the concentration of a chemical in each phase by applying mass balance equations in each phase instead of a whole system. To simplify the calculation, this model accounts for only four compartments: air, water, soil, and sediment. The Level III model assumes that the substance is continuously discharged at a constant rate and achieves a steady-state condition (input rate = output rate), but not equilibrium. However, direct discharges to sediment are assumed not to occur. Chemicals can be lost by degrading reactions and advection (Mackay *et al.*, 1992).

One of the widely used environmental modelling programmes, which is based on a Level III fugacity model, is EPI Suite™. This programme has been used in the prediction of environmental fate and toxicity of Persistence Organic Pollutants (POPs) and in the estimation of the partition coefficients of chemicals from inherent physical and chemical properties (EPA, 2012b).

2.2.3 EPI Suite™

The Estimation Program Interface (EPI) Suite™ version 4.11 used in this study has been developed by the United States Environmental Protection Agency (EPA) and Syracuse Research Corporation (SRC). EPI Suite™ can estimate several parameters, including the percentage of the substance expected in each environmental compartment (air, water, soil, and sediment), by using the physical and chemical properties of the compound along with the K_{oc}. The proportion of toxic substance in each medium can be used to forecast availability, accumulation and biomagnification.

EPI Suite™ is a model that provides users with screening level estimations of physicochemical properties and environmental fate. To obtain these data, the model uses a single input to run 17 individual programmes. Those programmes are presented in Table 2.6 (EPA, 2012b).

Table 2.6 Programmes in EPI Suite™

Programme	Description
AOPWIN™	Estimates the rate constant for the atmospheric, gas-phase reaction between hydroxyl radicals and organic chemicals
KOWWIN™	Estimates the log K_{ow} of organic chemicals, see Chapter 3
BIOWIN™	Estimates probability of aerobic and anaerobic biodegradation of organic chemicals in the presence of mix microorganisms
MPBPWIN™	Estimates melting point (MP), boiling point (BP) and vapour pressure (VP) of organic chemicals
WSKOWWIN™	Estimates the water solubility of organic chemicals using log K_{ow} of the chemicals, see Chapter 4
WATERNT™	Estimates water solubility of organic compounds at 25 °C, see Chapter 4
HENRYWIN™	Estimates Henry’s law constant (HLC) of substances at 25 °C
KOAWIN™	Estimates the octanol-air partition coefficient (K_{OA}) of organic chemicals using log K_{ow} and HLC of the chemicals
KOCWIN™	Estimates the K_{oc} of organic compounds, see Chapter 5
BCFBAF™	Estimates fish BCF, BAF and biotransformation rate of organic substances using log K_{ow} of the substance
HYDROWIN™	Estimates aqueous hydrolysis rate constants for these chemical classes: esters, carbamates, expoxides, halomethanes, selected alkylhalides, and phosphorus esters

Programme	Description
BioHCwin™	Estimates biodegradation half-life values for petroleum hydrocarbons
DERMWIN™	Estimates the dermal permeability coefficient (K_p), the dermally absorbed dose per event (DAevent) and Dermal Absorbed Dose (DAD) of organic substances *via* water contact
WVOLWIN™	Estimates volatilisation half-lives from river and lake
STPWIN™	Estimates the removal of compound in a conventional activated sludge wastewater treatment plant
LEV3EPI™	Level III fugacity model that predicts partitioning of substances between air, water, soil, and sediment
ECOSAR™	Estimates aquatic toxicity of chemicals using log K_{ow} of the chemical

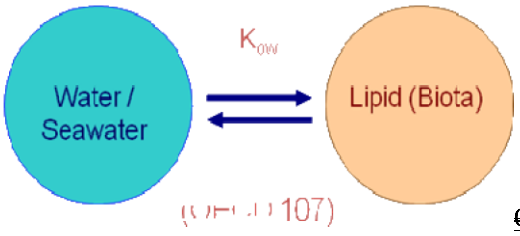
Source: EPA, 2012b

Several programmes in the EPI Suite™ estimate values using the values from other programmes. EPI Suite™ also contains a large experimental database of organic compounds. Unfortunately, EPI Suite™ does not take environmental conditions into consideration, and the obtained data are normally determined at standard conditions (*e.g.*, 25 °C and no salinity). Nevertheless, the model allows users to enter their experimental properties of chemicals as well as properties of a studied site, which leads to more site-specific assessment (EPA, 2012b). Thus, this study will incorporate laboratory-derived experiment values with EPI Suite™ in order to investigate the environmental fate and transport of BPA in the marine environment and also evaluate the impacts of changing water conditions.

Chapter 3 The Octanol-Water Partition Coefficient (K_{ow}) of Bisphenol A

3.1 Background

As described in Chapter 2, the partition coefficient is the ratio of the concentration of a substance equilibrated in two phases. The octanol-water partition coefficient (K_{ow}) is an important parameter to measure as it is directly correlated to the bioconcentration and bioaccumulation of BPA in the aquatic environment. The two main phases to consider in the aquatic environment are the aqueous phase and the lipid phase, which represents the biota, as shown in Figure 3.1. The lipid phase is a measure of the potential of BPA to bioaccumulate in the biota.

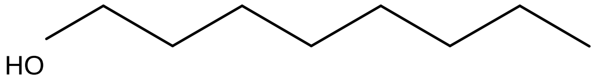


~~OECD~~ 107

(OECD, 1995b)

Figure 3.1 The partition coefficient between water and biota

The K_{ow} is a measurement of the hydrophobicity of a substance. It is the equilibrium ratio of concentration of a dissolved substance between the octanol phase and the water phase at a specified temperature (OECD, 1995b). The K_{ow} represents the membrane lipid-water barrier because octanol ($C_7H_{15}CH_2OH$) represents lipids in living organisms due to the similar carbon, hydrogen and oxygen ratio (OECD, 1995b). The chemical structure of octanol is shown in Figure 3.2.



octanol

Figure 3.2 Chemical structure of octanol

K_{ow} is used as an indicator of bioconcentration and bioaccumulation and a predictor of uptake by aquatic organisms (Gossett *et al.*, 1983; Sangster, 1989; Fisk *et al.*, 1998; Meylan *et al.*, 1999; Geyer *et al.*, 2000; Müller and Nendza, 2007). Due to the wide range of potential K_{ow} values, it is generally reported in logarithmic form. The K_{ow} is determined as:

$$K_{ow} = \frac{\text{concentration of substancein octanol}}{\text{concentration of substancein water}} \qquad \text{Equation 3.1}$$

which can also be written as:

$$K_{ow} = \frac{[\text{Octanol}]}{[\text{Water}]}$$

The K_{ow} has been extensively utilised in many areas such as research into drugs and pharmaceuticals; prediction of environmental distribution of organic compounds; and environmental modelling and toxicology of compounds. Such broad usage is possible because the K_{ow} is related to other physicochemical properties and partition coefficients such as water solubility (S_w), molecular weight (MW), organic carbon partition coefficients (K_{oc}), and bioconcentration factors (BCFs) for aquatic life (Briggs, 1981; Lyman *et al.*, 1990). The octanol-air partition coefficient (K_{OA}) can also be predicted from K_{ow} and Henry's Law Constant (HLC or K_H) (Meylan and Howard, 2005). The examples of general correlations (for organics) between these coefficients are shown in following equations (EPA, 2012b):

$$\text{Log } S_w \text{ (mol L}^{-1}\text{)} = 0.796 - 0.854 \log K_{ow} - 0.00728 \text{ MW} \qquad \text{Equation 3.2}$$

$$\text{Log } K_{oc} \text{ (L kg}^{-1}\text{)} = 0.8679 \log K_{ow} - 0.0004 \qquad \text{Equation 3.3}$$

$$\text{Log BCF (L kg}^{-1}\text{)} = 0.06598 \log K_{ow} - 0.333 \qquad \text{Equation 3.4}$$

According to these relationships, it is implied that chemicals with higher K_{ow} values tend to have greater solid adsorption capacities, higher bioconcentration and bioaccumulation factors, more persistence, and lower water solubilities and bioavailablities than more polar compounds (Briggs, 1981; Rand, 1995). These factors impact the overall fate of compounds in the aquatic environment. Therefore, a crucial aspect of this thesis was to measure accurately the K_{ow} and investigate the effects of salinity, pH and temperature of water on the K_{ow}.

3.1.1 Methods for Determining K_{ow}

According to the literature (van Haelst *et al.*, 1994; Finizio, *et al.*, 1997; Griffin *et al.*, 1999; Meylan and Howard, 2000; Finizio and Guardo, 2001; Paschke *et al.*, 2004), there are many methods used to determine K_{ow} either experimentally (both direct and indirect methods) such as the Shake Flask Method (SFM), Slow Stirring Method, Reverse Phase - High Performance Liquid Chromatography (RP-HPLC), or by calculation methods such as ClogP and Atom/Fragment Contribution (AFC).

Table 3.1 shows an assessment of the commonly used methods.

Table 3.1 Comparison of different determination methods of K_{ow}

Methods	Optimal log K_{ow} range	Strengths	Weaknesses
Shake Flask Method (SFM): OECD 107 (OECD, 1995b)	-2 to 4	- Direct method - High accuracy	- Time-consuming - Expensive - Unreliability to high lipophilicity substances (log K_{ow} > 5) due to the formation of octanol emulsions in water - Sensitive to the presence of impurities
Slow Stirring Method: OECD 123 (OECD, 2006)	up to 8.2	- Direct method - Suitable for very hydrophobic substances - High accuracy and good reproducibility	- Very time-consuming (2-8 days to achieve equilibrium) - Expensive
Reverse Phase-method High Performance Liquid Chromatography (RP-HPLC): OECD 117 (OECD, 1989)	0 to 6	- Fast - Convenient - Inexpensive - Good reproducibility - Less sensitive to the presence of impurities	- Indirect method - Require at least six appropriate reference compounds - Reliability to substances in the range of log K_{ow} 0 to 6 - Unreliability if the deviation from the SFM value is more than 0.5 log units

Methods	Optimal log K_{ow} range	Strengths	Weaknesses
Computational model - ClogP	Various	- Simplify and convenient - Inexpensive - Reliability for highly lipophilic classes - Correlates well with experimental values for neutral species	- Estimated values - Preferred if experimental data not available - Unreliable on complex molecules and compounds having many functional groups - Have a “missing fragments” problem for some compounds
Computational model – Atom/Fragment Contribution (AFC) used in EPI Suite™	Various	- Simple and convenient - Inexpensive - Reliable for highly lipophilic classes - Correlates well with experimental values	- Estimated values - Preferred if experimental data not available - Unreliable on complex molecules and compounds having many polar group
Calculation from water solubility	Various	- Simple and convenient - Inexpensive	- Least precise estimation method - Need special care to select the appropriate correlation equation - Only use for preliminary screening

In general, experimental K_{ow} values obtained from direct measurement methods are preferred over literature or modelling programme default values. If the experimental values are not available, SFM is a classical experimental method suitable to determine K_{ow} of hydrophobic substances in the range of log $K_{ow} < 4$; however, due to the occurrence of octanol emulsion this approach is not recommended for $K_{ow} > 5$ as these values can be overestimated (OECD, 1995b). RP-HPLC is also an experimental method and reliable for substances having log K_{ow} between 0 and 6; however, it is an indirect method and tends to be unreliable if the result is different from the SFM value by more than 0.5 log units (OECD, 1989). As a result, the slow stirring method has been developed for highly hydrophobic substances (log $K_{ow} > 4$) (OECD, 2006).

Calculation methods are useful and generally used to determine K_{ow} values when experimental values have not been determined or are difficult to measure. In addition, these methods can be used for screening of new substances. The estimation method ClogP has a missing fragments problem for some compounds. Consequently, the AFC method has been developed and this method is used by EPA in KOWWIN™, which is the log octanol-water partition coefficient programme in EPI Suite™ (EPA, 2012b). The K_{ow} values can also be calculated using property-property correlation equations, using for example, the calculation from water solubility but this method is the least accurate method and should be only used for preliminary screening.

Previous studies have indicated that environmental variables such as salinity, pH and temperature influence the K_{ow} values of hydrophobic substances (Major *et al.*, 1991; Escher and Schwarzenbach, 1996; Geyer *et al.*, 2000; Heinonen *et al.*, 2002; Eggleton and Thomas, 2004). For example, Bangkedphol *et al.* (2009) found that the log K_{ow} values of tributyltin (TBT) increased from 3.96 to 4.92 when increasing salinity from 0 to 32 psu (practical salinity units). Major *et al.* (1991) also found that K_{ow} values of methylmercury were higher at higher salinities, but decreased with increasing pH from 5 to 8. Previous work by Kang and Kondo (2005) found that BPA can persist longer in seawater than in freshwater without any degradation (~30 days). Therefore, due to the longer term of bioavailability, BPA can possibly accumulate to higher concentrations in marine organisms rather than in freshwater organisms.

The K_{ow} is one of the most important physicochemical characteristics used in environmental models to predict or estimate the partitioning between environmental phases, persistence, bioaccumulation in organisms and the toxicity of substances. Consequently, K_{ow} plays an important role in risk assessment and environmental assessment (Briggs *et al.*, 1982; Calamari and Vighi, 1990; Mackay, 2001). However, it is necessary to choose reliable K_{ow} values used in the mathematical models in order to get accurate determinations (Pontolillo and Eganhouse, 2001). Also, taking environmental conditions, such as salinity, pH and temperature, into account improves the accuracy of the model, moving away from a default model to something more site specific. This also allows the evaluation of changing environmental conditions on the impact of chemicals in marine environment (EPA, 2012b).

3.1.2 K_{ow} for BPA

According to the literature, the log K_{ow} values of BPA vary from 2.20 – 3.4 (Staples *et al.*, 1998; European Communities, 2003; Environment Canada, 2008). However, in order to evaluate and assess the impact of BPA in various sampling sites K_{ow} was measured using the SFM method and RP-HPLC method as well as the K_{ow} from the computational model, EPI Suite™, which is calculated using the Atom/Fragment Contribution (AFC). A sample of natural seawater (NSW) was collected from Prestwick (see Section 3.2.5) and analysed for salinity and pH. The K_{ow} of NSW was determined and compared with the K_{ow} value of distilled water. The effects of major variables on the K_{ow} values in aquatic environments were evaluated. Finally, the risk assessment and environmental assessment of BPA were conducted by inserting the experimental K_{ow} values into EPI Suite™ and therefore producing a more sitespecific evaluation.

3.2 Experimental

The three methods used in this study are:

1. The SFM which is a direct method using OECD guideline 107 (OECD, 1995b)

2. High Performance Liquid Chromatography (HPLC) or RP-HPLC which is an indirect method using OECD guideline 117 - (OECD, 1989)

3. The calculation method using EPI Suite™ version 4.11 (EPA, 2012b)

The comparison between these three methods was tabulated (see Section 3.3.4). The effects of salinity, pH and temperature of water on the K_{ow} values of BPA were investigated by using the direct shake flask method of (OECD, 1995b). The seawater sample was analysed for salinity and the K_{ow} value. This K_{ow} value was compared with the K_{ow} values of artificial seawater. Furthermore, the resulting data regarding environmental fate and toxicity of BPA in aquatic environment from inputting experimental K_{ow} value into EPI Suite™ programme were compared to the default data from the programme.

3.2.1 Determination of K_{ow} by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) Method

The Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method is based on substance partitioning between a non-polar stationary phase and a mobile solvent phase in RP-HPLC column. The determination of K_{ow} of BPA by RP-HPLC method was done by following OECD guideline 117 – partition coefficient (noctanol/water), HPLC method (OECD, 1989). The retention time and the capacity factor (k) of substances can be related to K_{ow} as a RP-HPLC system uses a polar mobile phase and non-polar column (long chain hydrocarbon column), such as a C_{18} column. Therefore, partitioning is similar to the shake flask method. Subsequently, this method determined the K_{ow} of a test substance by a calibration graph of the correlation between the measured capacity factor (k) of reference compounds and their K_{ow} values. The capacity factor (k) can be calculated from retention time from HPLC as shown in equation 3.5.

$$k = \frac{t_R - t_0}{t_0}$$ Equation 3.5

Where: k = capacity factor

t_R = the retention time of the test substance

t_0 = the dead-time, *i.e.*, the average time a solvent molecule needs to pass through the column

At least six reference compounds of known log K_{ow} values recommended by OECD guideline (OECD, 1989), which cover K_{ow} values of test substance, were used. Using the value as stated by Environment Canada (2008) of log K_{ow} 3.32, the reference substances chosen for this study are shown in Table 3.2.

Table 3.2 Reference compounds of the determination of K_{ow} by RP-HPLC method

Reference compounds	Log K_{ow}^{a}	Wavelength (nm)
Aniline	0.9	208
4-Methoxyphenol	1.3	220
Acetophenone	1.7	208
Cinnamyl alcohol	1.9	208
4-Chlorophenol	2.4	220
1-Naphthol	2.7	212
Naphthalene	3.6	216
Biphenyl	4.0	208
Phenanthrene	4.5	208

Triphenylamine	5.7	264

[a] Known log K_{ow} values from OECD Guideline 117 (OECD, 1989)

Table 3.2 also shows the maximum wavelengths, λ_{max}, of reference compounds, which were determined by spectrophotometer which was scanning from 190 nm to 400 nm (UNICAM UV-Vis spectrophotometer, Vision 4.30 software). The wavelength giving the highest response (λ_{max}) was used for the HPLC analysis.

RP-HPLC was carried out on a Dionex HPLC equipped with an LPG-3400A HPLC pump, an ACC-3000T autosampler with a 20 µL sample loop, and a VWD-3400 UV detector (Dionex, UK). The UV detector was set wavelengths (λ) for the reference compounds as shown in Table 3.2, and wavelength of 224 nm for BPA (wavelength as used by Rezaee *et al.*, 2009). BPA and all reference compounds were purchased from Sigma-Aldrich (UK). Separations were carried out on a Techsphere 5 ODS C18 column (25 cm x 4.6 mm) from HPLC Technology (UK). The column was held at a constant temperature of 25 ˚C within the housing unit. Deionised water (Barnstead Nanopure, Triple Red Limited, UK) and methanol (HPLC grade, Fisher Scientific, UK) (25:75 (v/v)) at a flow rate of 1 mL min^{-1} were used as the mobile phases (OECD, 1989). The compounds were injected three times with injection volume of 20 µL.

3.2.2 Determination of K_{ow} by Shake Flask Method

The determination of octanol-water partition coefficient (K_{ow}) of BPA by OECD guideline 107 – shake flask method (OECD, 1995b) was done at 25 ± 1 ˚C. Analytical grade 1-octanol was purchased from Rathburn (UK). Before the determination, 1-octanol (saturated with water) and distilled water (saturated with 1octanol) were prepared. The saturated solutions were prepared by visibly adding the saturating solvent until an excess was apparent (~ 50 mL L^{-1}). This was then shaken at desired temperature for 24 h, then left to separate prior to use. An initial concentration of 200 mg L^{-1} of BPA was prepared in 1-octanol, which was, in turn, mixed with water to obtain the study ratios. The volume ratios between 1-octanol and water studied were 1:20, 1:10 and 1:5. Each ratio was run in triplicate, the log K_{ow} value was obtained from the average results (n = 9) from 3 ratios and the results fell within a range of ± 0.3 units. K_{ow} value is defined as seen in equation 3.1 (OECD, 1995b).

The analysis of BPA was carried out by HPLC-UV detector (Dionex, UK) with Techsphere 5 ODS C18 column (HPLC Technology, UK) at wavelength of 224 nm as described in Section 3.2.1. The mobile phases were deionised water (Barnstead Nanopure, Triple Red Limited, UK) and HPLC grade acetonitrile (Fisher Scientific, UK) (55:45 (v/v)) at a flow rate of 1 mL min^{-1}. The run time of HPLC was 15 min and the retention time of BPA was 5.2 min. The water fractions were analysed and concentrations of BPA in the octanol phase were determined by subtraction from the total amount of BPA in the system (200 mg L^{-1}).

3.2.3 Determination of K_{ow} by Environmental Modelling Programme

To obtain the result, EPI Suite™ requires only a chemical structure that is input by SMILES (Simplified Molecular Input Line Entry System) notation. EPI Suite™ provides 2 default log K_{ow} values. One value is obtained from KOWWIN™ version 1.68, which is log K_{ow} 3.64. The other value is an experimental value in the "expkow" database of EPI Suite™ obtained from Hansch *et al.* (1995), which is log K_{ow} = 3.32.

The methodology of KOWWIN™ to estimate the log K_{ow} of organic substances uses the AFC method (EPA, 2012b). The AFC method determines log K_{ow} values of organic compounds by summing 2 sets of data from 2,351 compounds. The first set is atom/fragment contribution values, whereby a multiple linear regression of 1,120 compounds that have been measured for K_{ow} is used. The second set is correction factors occurring in a chemical structure which was obtained from 1,231 compounds. The K_{ow} estimated by the AFC method is shown in equation 3.6 (EPA, 2012b).

$$\log K_{ow} = \sum(f_i n_i) + \sum(c_j n_j) + 0.229 \qquad \text{Equation 3.6}$$

$$(n = 2351, R^2 = 0.982, SD = 0.216)$$

Where:

f_i = coefficient for each atom/fragment
n_i = the number of times the atom/fragment occurs in the structure

c_j = coefficient for each correction factor n_j = the number of times

the correction factor occurs in the molecule

3.2.4 Study of Effects of Environmental Parameters on K_{ow}

The major parameters that influence K_{ow} were studied in order to predict the environmental fate of BPA in the aquatic environment. This understanding leads to more efficient modelling and toxicity assessment. These studies were done by using OECD guideline 107, the shake flask method (OECD, 1995b), as mentioned in Section 3.2.2. The parameters studied were salinity, pH and temperature. The study of salinity on K_{ow} was done in two experiments. In the first experiment, the salinities studied were 0.5, 10, 35 and 50 psu to cover the range of brackish water to seawater. Usually, freshwater salinity is less than 0.5 psu; brackish or estuarine water is between 0.5 and 17 psu; and seawater salinity is between 32 and 37 psu with the average at 35 psu (APHA, 1999). Salinities of 50 psu and above are uncommon, but possible in extreme environments. To determine the correlation between salinity and K_{ow}, more salinities in the range from 0 to 100 psu were varied in the second experiment. The salinity was adjusted by adding KCl (Fisher Scientific, UK) because of its use as a reference chemical in the standard method for determination of salinity of water (APHA, 1999).

The salinity and pH of water were measured using a SevenMulti model (Mettler Toledo, UK) meter that measures both pH and conductivity and converts electrical conductivity (EC) to salinity. The effect of pH was studied by varying pH at 6, 7, 8 and 10 that covered the range of pK_a of BPA. The pH of water was adjusted with 0.1 M HCl (Fisher Scientific, UK) or 0.1 M NaOH (Fisher Scientific, UK) (Burton *et al.*, 2004). The temperatures studied were at 5, 25, 35 and 45 ˚C maintained in an incubator shaker (Stuart® orbital incubator SI500, Bibby Scientific Limited, UK). The range of temperature covered subtropical to tropical zones, including all conditions used in this research study.

3.2.5 Sample Collection

Natural seawater (NSW) was collected from Prestwick, United Kingdom (55˚49’40.82"N, 4˚62’9.30"W) in June 2012, as shown in Figure 3.3. NSW from Prestwick was chosen because of its convenience to sampling and the lack of local chemical industry activity. Prestwick is located along a major shipping channel for the West of Scotland, so it was not expected to be entirely free of pollution, but BPA was not expected to be present. The NSW sample was measured for pH and salinity (SevenMulti meter, Mettler Toledo, UK), and the K_{ow} value of BPA using NSW was examined by the shake flask method.

Figure 3.3 Map of Sampling site for natural seawater (Prestwick, UK) (Google Maps, 2014a)

3.2.6 Prediction of Environmental Fate and Toxicity from K_{ow}

Computation modelling programmes are useful in environmental studies because of the wide range of conditions that can be explored. The accuracy and reliability of results from the model depend on the accuracy of data input into the model. Moreover, the default modelling results tend to be at standard conditions, such as 25˚C, without taking site-specific environmental conditions into account. The use of experimental data can improve model accuracy and the ability to represent the real environmental conditions (Chakka *et al.*, 2010; EPA, 2012b).

In this study, the default data were obtained from EPI Suite™. Two default K_{ow} values are obtained from EPI Suite™. One obtained from KOWWIN™ version 1.68 which is used for toxicity prediction (ECOSAR™ version 1.00), another one is an experimental value in the database of EPI Suite™ used in the physicochemical properties and fate prediction. Prediction data obtained from experimental K_{ow} values were compared to default data.

3.3 Results and Discussion

The dimensionless octanol-water partition coefficient (K_{ow}) is a measurement of a lipophilicity of a substance and is defined as the ratio of the equilibrium concentrations of a dissolved substance in octanol and water at a specified temperature (OECD, 1995b) as seen in equation 3.1. It is used to predict the possible environmental fate and toxicity of organic compound. Although the estimated K_{ow} can be obtained from an environmental modelling programme, the experimental values are preferable. Additionally, it is necessary to use reliable values in order to get an accurate and reliable environmental risk assessment, which is also sitespecific. Consequently, this study determined the experimental K_{ow} values of BPA and evaluated the effect of environmental conditions in the marine environment on environmental and toxicity assessments by using computational model.

3.3.1 Determination of K_{ow} by RP-HPLC Method

RP-HPLC method is widely used to measure K_{ow} of organic compound due to its advantages, *e.g.*, fast, inexpensive and less sensitive to the presence of impurities. In the RP-HPLC system, the long chain hydrocarbons (*e.g.*, C_{18}) chemically bonded onto a silica column represents the lipid phase and mobile phase represents the aqueous phase, which corresponds to the octanol and water in the shake flask method. Therefore, the hydrocarbon-water partitioning coefficient, derived from the partitioning occurring in a RP-HPLC column between the hydrocarbon stationary phase and mobile solvent phase, represents the K_{ow}. This method determined K_{ow} of test compound from a correlation of known log K_{ow} of reference compounds with their retention times. According to the standard method, at least six appropriate reference compounds that cover the predicted K_{ow} value of test substance are required. Log K_{ow} of reference compounds used in this experiment were obtained from OECD Guideline 117 (OECD, 1989) and the log k (capacity factor) values of reference compounds and test substance, BPA, were calculated from HPLC retention times by using equation 3.5. The results are shown in Table 3.3.

Table 3.3 Log k (capacity factor) of substances from RP-HPLC method

Chemicals	Log K_{ow} derived from OECD[a]	Experimental log k ± %RSD
Aniline	0.9	0.370 ± 0.20
4-Methoxyphenol	1.3	0.258 ± 0.60
Acetophenone	1.7	0.409 ± 0.69
Cinnamyl alcohol	1.9	0.359 ± 0.84
4-Chlorophenol	2.4	0.368 ± 0.58
1-Naphthol	2.7	0.423 ± 0.45
Naphthalene	3.6	0.765 ± 0.71
Biphenyl	4.0	0.910 ± 0.13
Phenanthrene	4.5	1.094 ± 0.13
Triphenylamine	5.7	1.287 ± 0.00
BPA	unknown	0.368 ± 0.20

[a] Known log K_{ow} values derived from OECD Guideline 117 (OECD, 1989)

The log K_{ow} value of BPA was calculated by using linear regression equation obtained from the correlation graph of log k and log K_{ow} for appropriate reference substances from standard method as showed in Figure 3.4.

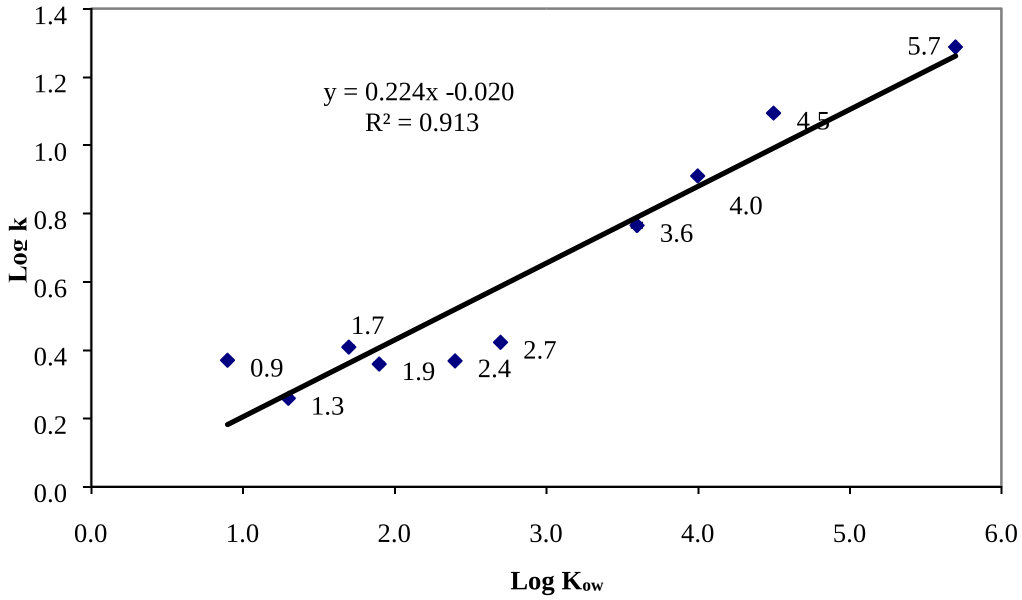


Figure 3.4 The correlation graph of log k and log K_{ow} for reference compounds

According to the relationship between log k and log K_{ow} (Figure 3.4), the linear regression equation was used to calculate log K_{ow} of BPA is:

Box 3.1 Calculation of log K_{ow} of BPA from a standard curve

$$\text{Log k} = 0.2247 \text{ Log } K_{ow} - 0.0209 \qquad \text{Equation 3.7}$$

From Table 3.3, log k value of BPA was 0.368 so the calculation equation is

$$\text{Log } K_{ow} = \frac{(0.368 + 0.0209)}{0.2247} \qquad \text{Equation 3.8}$$

Thus, $\text{Log } K_{ow} = 1.73$

The log K_{ow} value obtained from the experiment was 1.73. According to the literature review, the log K_{ow} of BPA using a RP-HPLC method, which was determined by Eadsforth, was reported at 2.20 (European Communities, 2003). The log K_{ow} in this study differs from reported value with a difference of 0.47 log units. This difference is possibly because of the reference compounds used in this study differed from Eadsforth's study. As Paschke *et al.* (2004) indicated that the choice of the reference substances used to get the regression equation plays an important role in this method. Unfortunately, there is no data regarding the reference compounds used in Eadsforth's study.

3.3.2 Determination of K_{ow} by Shake Flask Method

The shake flask method (SFM) is a conventional direct experimental method to measure K_{ow} for compounds having log K_{ow} in the range of -2 to 4. This method is considered to be more accurate than the RP-HPLC method (which is an indirect method). But the SFM method is more time-consuming, more expensive, and more sensitive to the presence of impurities compared to the RP-HPLC method. The K_{ow} determination was carried out by OECD Guideline 107 – shake flask method (OECD, 1995b). In this study, the volume ratios between octanol and water were 1:20, 1:10 and 1:5. An initial concentration of BPA in octanol is 200 mg L^{-1}. K_{ow} value was calculated as shown in equation 3.1.

Table 3.4 showed the log K_{ow} obtained from this experiment at 25 ± 1 °C was 3.42 ± 0.03. The result is supported by log K_{ow} value for BPA measured by Bayer at ambient pH values which was 3.4 (Staples *et al.*, 1998). The K_{ow} of natural seawater (NSW) from Prestwick was determined. In this study, the log K_{ow} of NSW at 25 ± 1°C was 3.52 ± 0.04. The salinity and pH of distilled water and NSW used in this study were also measured, as shown in Table 3.4.

Table 3.4 The K_{ow} value of BPA at 25 ± 1 °C from shake flask method

Samples	Log K_{ow} 1:20 (n=3)	Log K_{ow} 1:10 (n=3)	Log K_{ow} 1:5 (n=3)	Log K_{ow} Average $\pm$ SD (n = 9)	Salinity (psu) (n = 9)	pH (n = 9)
Distilled water	3.43	3.41	3.40	3.42 $\pm$ 0.03	0 $\pm$ 0^{a}	6.2 $\pm$ 0.03^{a}
NSW	3.51	3.56	3.48	3.52 $\pm$ 0.04	29 $\pm$ 0.3^{a}	7.8 $\pm$ 0.04^{a}
Reference[b]	N/A[c]	N/A[c]	N/A[c]	3.4	N/A[c]	N/A[c]

[a] Measured salinity and pH of water in this study

[b] Source: Staples *et al.,* 1998

[c] N/A = No reported data

The higher log K_{ow} in the NSW is possibly due to the salinity and pH. Geyer *et al.* (2000) suggested that the pH of water affects K_{ow} value of organic compounds. Copolovici and Niinemets (2007) found that increasing salinity by increasing KCl concentration increased K_{ow} of organic monoterpenes. In this study, the NSW had a salinity of 29 psu and a pH of 7.8 whereas distilled water had zero salinity and a pH of 6.2. Therefore, the effects of these factors on partitioning behavior were studied.

3.3.3 Determination of K_{ow} by Environmental Modelling Programme

KOWWIN™ is used to predict the log K_{ow} of organic substances in EPI Suite™ by using atom/fragment contribution (AFC) method (EPA, 2012b). The AFC method estimates K_{ow} values by summing atom/fragment contribution values with correction factors as described in Section 3.2.3. The estimated K_{ow} values predicted by this method are different from experimental K_{ow} values within the range of ± 0.8 log units for over 96% of 8,406 compounds (Meylan and Howard, 1995). The EPI Suite™ version 4.11 (EPA, 2012b) was used in this study. The KOWWIN™ version 1.68 is used to estimate log K_{ow} values of organic substances. The log K_{ow} value of BPA from this programme was 3.64.

In addition to calculated values, EPI Suite™ also provides the experimental values when they are available. These experimental values are in expkow database in the programme. For BPA, the experimental value from Hansch *et al.* (1995) was available in the database which was 3.32.

3.3.4 Comparison of the Different Determination Methods of K_{ow}

The K_{ow} value obtained from RP-HPLC was compared against the value measured by SFM to evaluate the reliability of the value from RP-HPLC method in accordance with guidelines in standard method. Furthermore, the experimental values from this study were compared to the default values from the computational programme, EPI Suite™, as shown in Table 3.5.

Table 3.5 Comparison of log K_{ow} values determined by different methods

	RP-HPLC	AFC	SFM
Experimental values	1.73	N/A[a]	3.42

KOWWIN™ (EPI Suite™)	N/A[a]	3.64	N/A[a]
expkow database (EPI Suite™)	N/A[a]	N/A[a]	3.32
Literature	2.20[1]	N/A[a]	3.32 – 3.4[1,2]

[1] European Communities, 2003

[2] Staples *et al.*, 1998

[a] N/A = No data

As mentioned in Section 3.1, the SFM method provides more reliable values than values obtained from RP-HPLC because SFM is a direct method while RP-HPLC is an indirect method. Finizio *et al.* (1997) suggested that if the difference of K_{ow} value between RP-HPLC method and SFM is more than 0.5 log units, the K_{ow} from RPHPLC is unreliable. However, RP-HPLC method has some advantages over SFM method such as its lower cost, faster analysis, and lower sensitivity to the presence of impurities (Paschke *et al.*, 2004). Thus, this study determined and compared log K_{ow} values using both methods (RP-HPLC and SFM), as shown in Table 3.5. The results found the deviations of log K_{ow} values from these two methods were more than 0.5 log units either from this study or from published literature (European Communities, 2003). Based on these results, the RP-HPLC method was deemed to be unsatisfactory in the determination of K_{ow} of BPA. Paschke *et al.* (2004) also suggested that K_{ow} in the range of < 4 obtained from SFM method is more accurate than value from RP-HPLC. According to the literature (Eadsfort and Moser, 1983; Rapaport and Elsenrelch, 1984; Li *et al.*, 1999), this deviation may be because of the difference between hydrocarbon-water partition coefficient in the RP-HPLC partitioning system and in the octanol-water partitioning system. Also, Li *et al.* (1999) indicated that this difference is greater for compounds that easily form hydrogen bonds. The RP-HPLC system is more complicated in hydrogen bonding interactions than those in the octanol-water system. These compounds include BPA as it has 2 hydroxyl (OH) functional groups that easily form hydrogen bonds.

Although the RP-HPLC method determines K_{ow} of a substance from the relationship between its retention time in a reverse-phase chromatographic system and its partition coefficient, the retention mechanism in a reverse-phase chromatography is still unclear. Vailaya and Horváth (1998) suggested that the two possible retention processes are the partition mechanism and the adsorption mechanism of the solute to the hydrocarbon stationary phase. Consequently, the K_{ow} values of BPA from RPHPLC can disagree with the values from SFM.

Table 3.5 also shows the deviations between the experimental K_{ow} values in this study, KOWWIN™ database and the literature values (Staples *et al.,* 1998; European Communities, 2003) and also between the experimental values and the calculated K_{ow} value from modelling programme. The experimental K_{ow} value from SFM in this study (log K_{ow} = 3.42) is in good agreement with the experimental values obtained from KOWWIN™ database (log K_{ow} = 3.32) and literature (log K_{ow} = 3.32 – 3.4) (Staples *et al.,* 1998). Subsequently, the comparison between the experimental K_{ow} values with the estimated value from AFC method in KOWWIN™ in EPI Suite™

study (log K_{ow} = 3.64) indicated that this programme slightly overestimates the K_{ow} value.

3.3.5 Effect of Salinity on K_{ow}

Previous studies indicated that the salinity of the aqueous phase affects water solubility and the partitioning of organic chemicals into other phases. This effect, referred to as the salting-out or salting-in effect, can change the K_{ow} value and is relevant to the environmental behaviour of organic compounds (Rydberg, 2004; Endo *et al.,* 2012).

Therefore, the salinity dependence on K_{ow} of BPA was determined by measuring K_{ow} at various salt concentrations and calculating the salting coefficients.

3.3.5.1 Determination of K_{ow} at Various Salinities

The effect of salinity on K_{ow} value was studied by using OECD Guideline 107 – shake flask method (OECD, 1995b). Salinity of artificial water was adjusted by adding KCl. In the first experiment, the K_{ow} values at various salinities of water were investigated in a range from 0.5 to 50 psu. These artificial salt waters were used to make up the octanol/water ratios as previously described in Section 3.2.2. These salinities represented the range from estuarine (0.5 psu) to seawater (32 psu). Usually, freshwater salinity is less than 0.5 psu, brackish or estuarine water is between 0.5 and 17 psu, and seawater salinity is between 32 and 37 psu with the average at 35 psu (APHA, 1999). Additionally, the natural seawater (NSW) from Prestwick was characterized by its properties, *i.e.*, pH and salinity, and also the K_{ow} was determined in order to evaluate the correlation between the K_{ow} values of artificial seawater and their salinity. The results are presented in Table 3.6 and Figure 3.5.

Table 3.6 Log K_{ow} of BPA at different salinities of artificial seawater and natural seawater

Samples	Salinity (psu)	Log K_{ow} ± SD (n = 9)	%RSD
Distilled water	0.5	3.44 ± 0.03	0.98
	10	3.48 ± 0.03	0.89
	35	3.53 ± 0.02	0.86
	50	3.55 ± 0.02	0.67
Natural seawater from Prestwick	29	3.52 ± 0.04	0.62

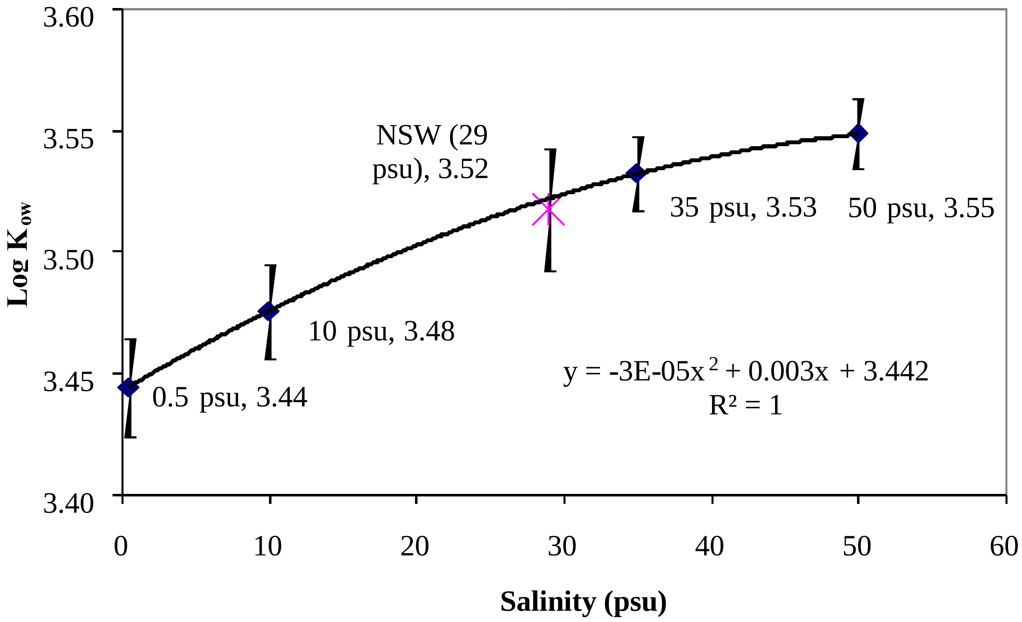


Figure 3.5 Log K_{ow} of BPA of artificial water at various salinities (0.5 – 50 psu). The NSW sample is natural seawater from Prestwick (see Figure 3.3). (Error bars indicate 95% Confidence Intervals, n = 9)

From the results, the correlation equation obtained from artificial water (salinity range 0.5 – 50 psu) is shown in following equation.

Box 3.2 Correlation equation between log K_{ow} and salinity (0.5 – 50 psu)

$$\text{Log } K_{ow} = [-0.00003 \text{ Salinity}^2 \text{ (psu)}] + [0.0036 \text{ Salinity}] + 3.4423,$$

$$R^2 = 1 \qquad \text{Equation 3.9}$$

Therefore, an increase in the salinity increases the log K_{ow}. This curve fitted a polynomial curve. The log K_{ow} value of the NSW from Prestwick coast (salinity 29 psu, pH 8) almost fell within the range of log K_{ow} values of the synthetic waters that were tested. From this correlation equation (equation 3.9), the log K_{ow} of the natural seawater from Prestwick was calculated as log K_{ow} = 3.52. This agrees with the measured value of 3.52.

Because of the very low degree of freedom from the first experiment, a second experiment was carried out by varying more salinity in the range from 0 to 100 psu which represented range from freshwater to extreme saltwater in order to test the relationship between salinity of water and log K_{ow}. The results are shown in Table 3.7 and Figure 3.6.

Table 3.7 Log K_{ow} of BPA at different salinities

Salinity of water (psu)	Log K_{ow} ± SD (n = 9)	%RSD
0	3.25 ± 0.02	0.75
0.2	3.28 ± 0.02	0.57
0.4	3.29 ± 0.03	0.82
0.5	3.29 ± 0.03	0.83
1	3.29 ± 0.03	0.98
5	3.29 ± 0.04	1.13
10	3.29 ± 0.02	0.74
14	3.33 ± 0.02	0.51
17	3.33 ± 0.03	0.83
25	3.32 ± 0.04	1.26
32	3.36 ± 0.03	1.01
35	3.39 ± 0.02	0.57
50	3.43 ± 0.04	1.13
100	3.64 ± 0.04	1.06

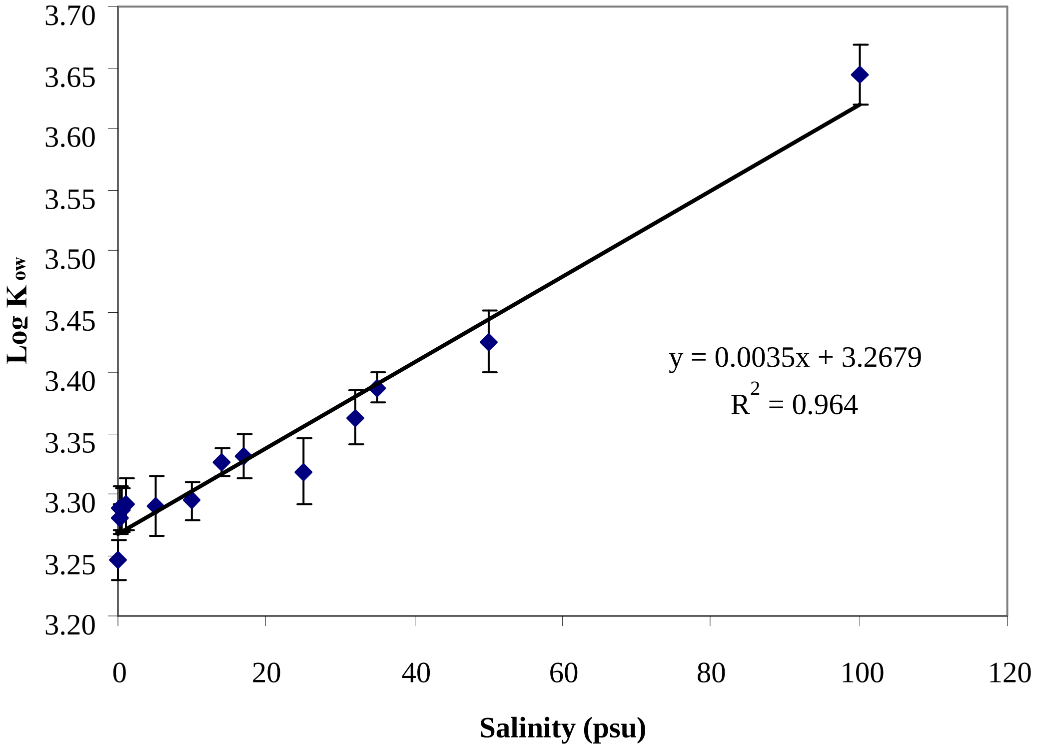


Figure 3.6 Log K_{ow} of BPA at various salinities (0 – 100 psu) of artificial water (Error bars indicate 95% Confidence Intervals, n = 9)

From the results, the regression obtained from artificial water (salinity range 0 – 100 psu) is a linear regression as shown in equation 3.10 (Box 3.3).

Box 3.3 Correlation equation between log K_{ow} and salinity (0 – 100 psu)

$$\text{Log } K_{ow} = 0.0035\,(\text{Salinity}) + 3.2679,$$

$$R^2 = 0.964 \qquad \text{Equation 3.10}$$

This equation represents freshwater, brackish, seawater and extreme salt bodies, although it does not cover the most extreme saline environments. The expanded graph from Figure 3.6 for brackish water to extreme salt waters (0.5 – 100 psu) is shown in Figure 3.7.

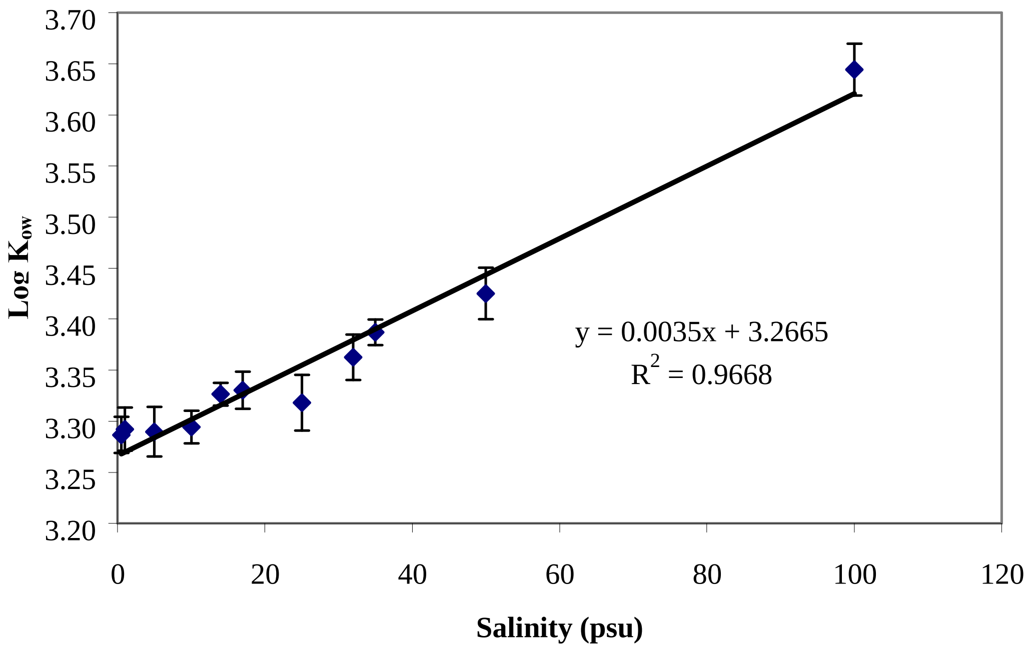


Figure 3.7 Expanded log K_{ow} of BPA for artificial brackish water to extreme saltwater (Error bars indicate 95% Confidence Intervals, n = 9)

The results can be divided into 3 groups in accordance with freshwater (salinity less than 0.5 psu), brackish or estuarine water, and saltwater (salinity more than 32 psu) (APHA, 1999). The comparison of K_{ow} values of each type of water is showed in Table 3.8 and Figure 3.8.

Table 3.8 Relationship between K_{ow} and type of water

Type of water	Salinity (psu)	Log K_{ow} ± SD	%RSD
Freshwater (n = 3)	0 – 0.4	3.27 ± 0.004	0.13
Brackish water (n = 7)	0.5 - 25	3.31 ± 0.008	0.25
Saltwater (n = 4)	32 - 100	3.45 ± 0.009	0.27

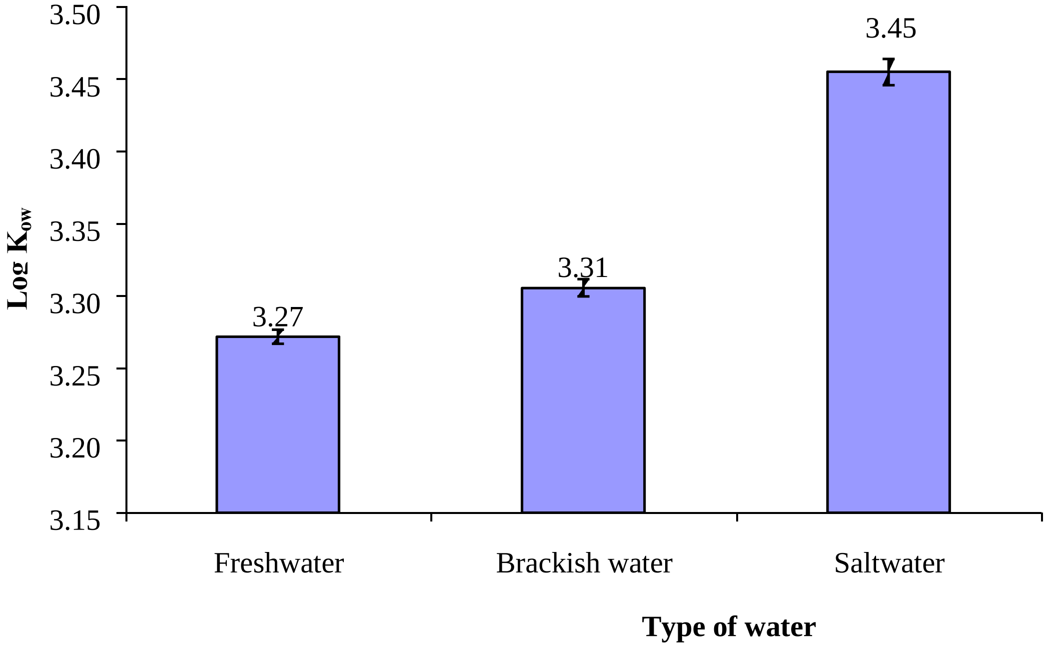


Figure 3.8 Relationship between K_{ow} and type of water

(Error bars indicate 95% Confidence Intervals, n = 3)

The results show that when BPA has been released into the aquatic environments, the organisms in saltwater tend to have a greater potential for bioconcentration and bioaccumulation than freshwater organisms because the order of K_{ow} values of BPA in each type of water is saltwater > brackish water > freshwater. This study supports previous work (Kang and Kondo, 2005) in that the bioaccumulation of BPA in marine organisms is possibly higher than that in freshwater organisms.

These results indicate that increasing salinity results in increased log K_{ow}. This finding suggests a salting-out effect as salinity reduces the polarity of the solute molecule and is able to shift the equilibrium partition of organic solute toward nonaqueous phase, *e.g.*, octanol phase, resulting in increase K_{ow} value (Endo *et al.,* 2012). The effects of salt on K_{ow} in this study agree with findings by Copolovici and Niinemets (2007) that K_{ow} of limonene and linalool were greater at a higher concentration of KCl. Consequently, greater salinity would indicate the potentially higher bioaccumulation of BPA in the lipid of aquatic organisms and it also means that it could also persist for longer in the marine environment as greater adsorption to soil/sediments could also occur. However, it would be less bioavailable to pelagic species. The converse is also true in that benthic organisms have the potential to bioaccumulate through a greater concentration in sediments.

3.3.5.2 Calculation of Salting Coefficients for K_{ow}

In general, the Setschenow equation is used to describe the salt effect on the solubilities of chemicals in aqueous salt solutions. However, Ni and Yalkowsky (2003) indicated that the K_{ow} is the best descriptor of the salting-out effect after determining the Setschenow constants (K_s) by NaCl of 101 compounds using molar volume, aqueous solubility (S_w) and K_{ow}, followed by comparing the relationships between K_s and those three parameters.

Because of the inverse relationship between S_w and K_{ow}, the Setschenow equation can be used to illustrate the effect of salt concentration on K_{ow} by determining the salting coefficient (k_{ow}) using this corresponding equation (Copolovici and Niinemets, 2007):

$$\mathrm{Log}\left(\frac{K_{ow}}{K_{ow,0}}\right) = k_{ow}C_s$$

Equation 3.11

Where:

K_{ow} = the octanol/water partition coefficient of aqueous salt solution

$K_{ow,0}$ = the octanol/water partition coefficient of pure water

k_{ow} = the salting coefficient (M^{-1} or L mol^{-1})

C_s = the salt concentration in solution (M or mol L^{-1})

In order to determine the salting coefficient (k_{ow}), the K_{ow} values from both experiments were used. Salt used in this study is potassium chloride (KCl), which has molecular weight at 74.55 g mol^{-1} (Sigma-Aldrich, 2013). The data used in the estimation of salting constant on K_{ow} of BPA were calculated and shown in Table 3.9. According to equation 3.11, the salting coefficient (k_{ow}) of this study was then obtained from slope of correlation graph plotting between $\mathrm{Log}\left(\frac{K_{ow}}{K_{ow,0}}\right)$ and salt concentration (C_s, mol L^{-1}), as shown in Figure 3.9.

Table 3.9 K_{ow} values and KCl concentration (M) using in estimation of k_{ow} on K_{ow} of BPA

Salinity (psu)	C_s (M)	$K_{ow}/K_{ow,0}$	Log ($K_{ow}/K_{ow,0}$)
0	0.000	1.000	0.000 [a,b]
0.5	0.006	1.066	0.028 [a]
10	0.124	1.146	0.059 [a]
35	0.435	1.306	0.116 [a]
50	0.622	1.356	0.132 [a]
0	0.000	1.000	0.000 [b]
0.2	0.002	1.081	0.034 [b]
0.4	0.005	1.105	0.043 [b]
0.5	0.006	1.099	0.041 [b]
1	0.012	1.114	0.047 [b]
5	0.062	1.109	0.045 [b]
10	0.124	1.118	0.049 [b]
14	0.174	1.204	0.080 [b]
17	0.211	1.216	0.085 [b]
25	0.311	1.185	0.074 [b]
32	0.398	1.310	0.117 [b]
35	0.435	1.384	0.141 [b]
50	0.622	1.515	0.180 [b]
100	1.243	2.509	0.399 [b]

[a] Data from 1st experiment

[b] Data from 2nd experiment

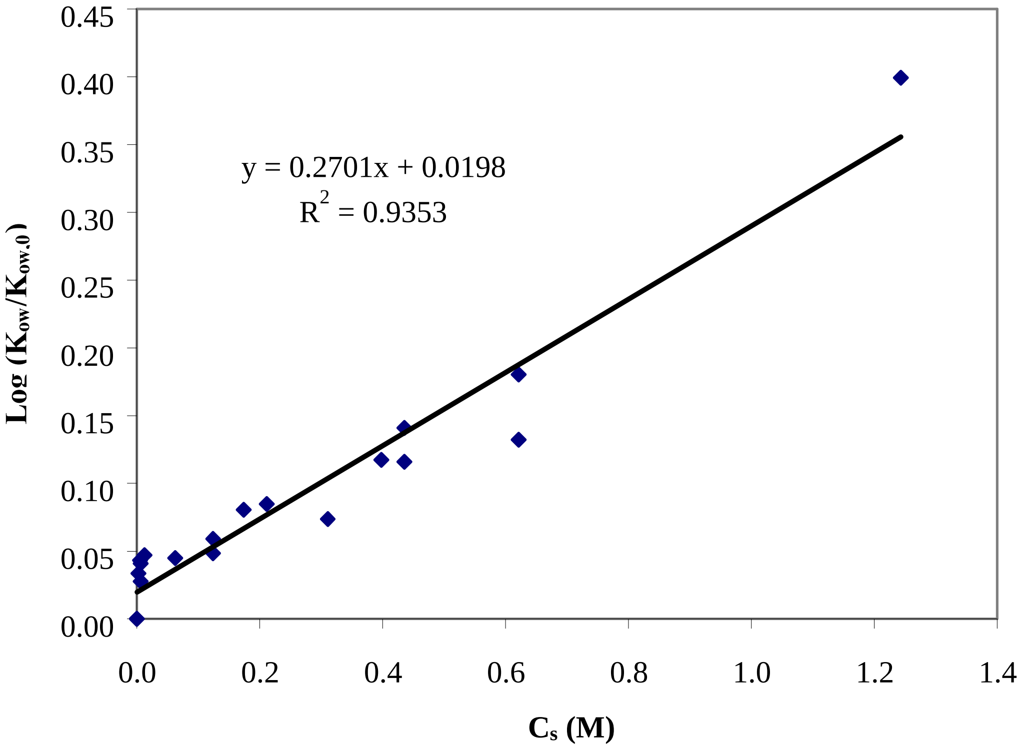


Figure 3.9 Salting effect on K_{ow} of BPA

The results show that the K_s of the KCl effect on the K_{ow} of BPA in this study is 0.2701 M^{-1}, refers to salting-out effect, and the correlation equation between K_{ow} and salt concentration is:

Box 3.4 Correlation equation between K_{ow} and salt concentration

$$\text{Log}\left(\frac{K_{ow}}{K_{ow,0}}\right) = 0.2701 * C_s + 0.0198, \quad R^2 = 0.9353 \qquad \text{Equation 3.12}$$

Equation 3.12 will be useful to estimate new K_{ow} values at specific molar salt concentration solution. According to two experiments in this study, the range of log K_{ow} of pure water obtained are 3.20 to 3.46 with the average of 3.33 (n = 18), SD = 0.09 and %RSD = 2.76%. This value is in good agreement of the log K_{ow} values from literature, which are between 3.32 and 3.4 (Staples *et al.,* 1998; European Communities, 2003). Consequently, the log $K_{ow,0}$ of 3.33 from this study is used in equation 3.12 to obtain the salting constant.

As a result, equation 3.12 is very helpful not only in the assessment of effects of BPA at a specific salt concentration, but also in the prediction of its effects in the environment under changing salinity scenarios at a specific site. Moreover, salinity is possibly used as a proxy for measuring K_{ow}, which is more time-consuming, difficult and expensive.

3.3.6 Effect of pH on K_{ow}

The pH is an important factor in controlling the partitioning of BPA. To understand the effect of pH on the K_{ow}, the K_{ow} values at different pH (6, 7, 8 and 10) by using shake flask method (OECD, 1995b) was studied. This pH range covers neutral and ionised forms of BPA as the pK_a of BPA is 9.6 (Staples *et al.*, 1998). This pH range was selected because the dissociation forms of organic substances, ionised form or neutral form, have effects on K_{ow} (Rand, 1995). The results are shown in Table 3.10 and Figure 3.10.

Table 3.10 Log K_{ow} of BPA at various pH

pH	Log K_{ow} ± SD (n = 9)	%RSD
6	3.39 ± 0.03	0.78
7	3.43 ± 0.03	0.87
8	3.47 ± 0.03	0.92
10	2.99 ± 0.04	1.26

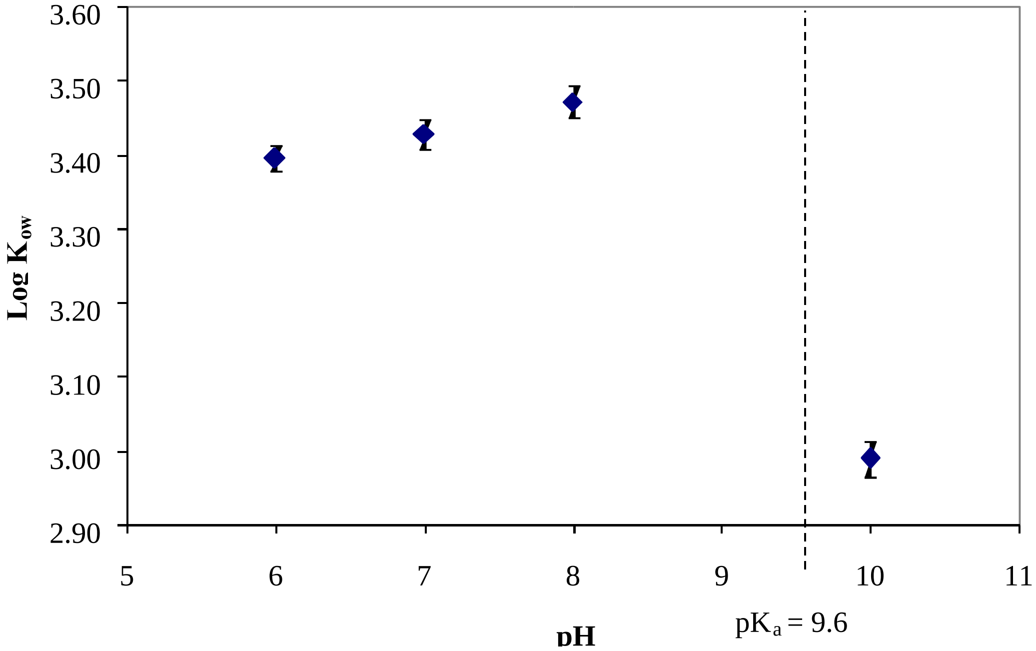


Figure 3.10 Log K_{ow} of BPA at various pH

(Error bars indicate 95% Confidence Intervals, n = 9)

The results show the log K_{ow} of BPA increased slightly at pH from 6 – 8 but decreased significantly at pH 10. A very likely reason is the dissociation of BPA in the system, as pH 10 is within its pK_a range (9.6 - 10.2) (Staples *et al.*, 1998). In fact, BPA is a weak organic acid and can be deprotonated to exist in the system as an anionic form and/or neutral form of BPA, as shown in Figure 3.11.

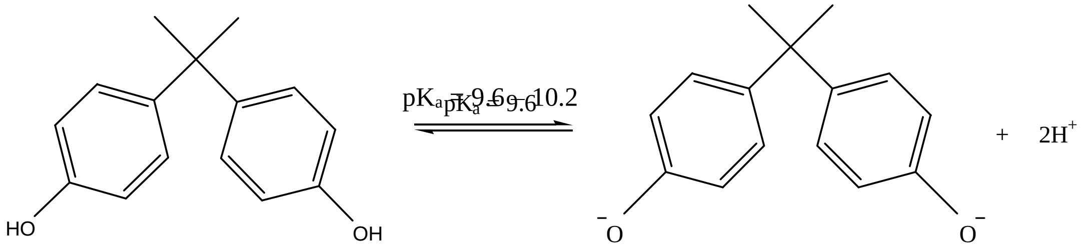


Figure 3.11 Dissociation system of BPA

If pH > pK_a, the ionised form of BPA is predominant and it has been suggested that the K_{ow} value is less if the chemical is in the ionised form because of its polarity. The ionised form is polar so the chemical tends to be more soluble in the aqueous phase (water) than in the octanol phase, resulting in low K_{ow} (Geyer *et al.*, 2000). The study of Kuramochi *et al.* (2008a) supports the results from this study. They measured the K_{ow} at 25 °C of 2,4,6-tribromophenol (TBP) at pH 6 to 12 using the shake flask method. The dissociation of the phenolic group of TBP began at a pH above 4 and will be 100% at pH > 9. As a result, log K_{ow} of TBP decreased from 3.73 to 1.24 with increasing pH of water from 6 to 9, then almost constant (log K_{ow} = 0.8 – 0.9) at pH above 9. TBP is completely in its ionic form at pH > 9, resulting in drastically decreased log K_{ow}.

3.3.7 Effect of Temperature on K_{ow}

Temperature is another important factor that influences the partition process of chemical in environment. According to Le Chatelier's principle, the equilibrium constant changes with changing temperature in order to minimise the effect of changing the temperature (Atkins, 2001). In this study, K_{ow} values of BPA at different temperatures were determined and thermodynamics of partitioning were applied in order to determine temperature dependence on K_{ow} of BPA.

3.3.7.1 Determination of K_{ow} at Various Temperatures

The temperature dependence of K_{ow} value was studied by conducting shake flask test following the standard method (OECD, 1995b) at temperatures between 5°C and 45°C using an incubator shaker, reflecting a wide range of potential temperatures in the marine environment. The results are shown in Table 3.11 and Figure 3.12.

Table 3.11 Log K_{ow} of BPA and ΔG° at various temperatures

Temperatures (°C)	Log K_{ow} $\pm$ SD	%RSD	ΔG° (kJ mol^{-1})
5	3.61 $\pm$ 0.05	1.44	-19.22
25	3.42 $\pm$ 0.03	0.98	-19.52
35	3.34 $\pm$ 0.03	0.87	-19.70
45	3.18 $\pm$ 0.05	1.59	-19.37

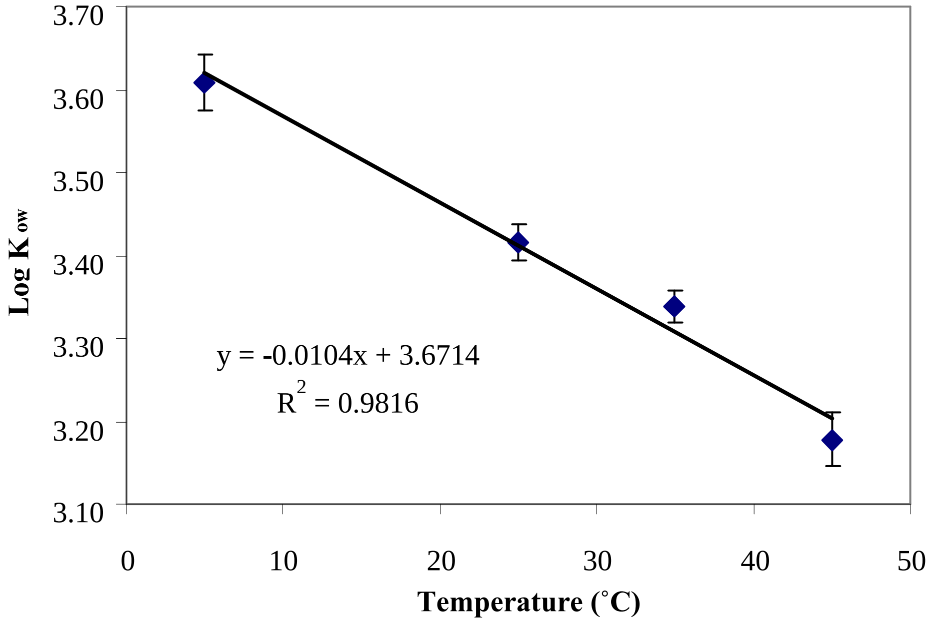


Figure 3.12 Log K_{ow} of BPA at various temperatures

(Error bars indicate 95% Confidence Intervals, n = 9)

The results indicate that increasing the temperature by one degree tends to decrease the log K_{ow} values of BPA with an average value of 0.01 unit. The K_{ow} is related to water solubility of the substance, which is a function of the Gibbs energy to transfer that substance from the aqueous phase to the non-aqueous phase. Thus, the partitioning of BPA between octanol and water is related to temperature as well. Gibbs energy relates to temperature and can be shown in the following equation (Sangster, 1989).

$$\Delta G^{\circ} = -RT\ln K_{ow} \quad \text{Equation 3.13}$$

Where:

ΔG° = the change in standard Gibbs energy (kJ mol^{-1})

R = the ideal gas constant = 8.314462 J mol^{-1} K^{-1}

T = temperature (Kelvin, K)

The standard Gibbs energy for transfer calculated by equation 3.13 using the experimental K_{ow} data in this study is also shown in Table 3.11. The results show that $\Delta G^{\circ} < 0$ at all temperatures (Table 3.11); therefore, the transfer of BPA in wateroctanol system is a spontaneous process (Atkins, 2001).

The effect of temperature on K_{ow} is specific for each chemical, depending on the nature of the interactions between that chemical and water. The K_{ow} can be higher or lower with increasing temperature (Mackay *et al.*, 2006). According to the results from this study, increased temperature leads to reduced K_{ow} values of BPA with concomitant increased water solubility.

3.3.7.2 Determination of the Temperature Dependence on K_{ow}

The van't Hoff equation is used to determine the effects of temperature on equilibrium constant of chemicals. It assumes that the change in standard enthalpy (ΔH°) and entropy (ΔS°) are constant over the temperature range of interest, which cannot be too wide (Atkins, 2001). The van't Hoff equation and a linear form of the van't Hoff equation for K_{ow} are shown in equation 3.14 and equation 3.15, respectively.

$$\frac{\ln K_{ow}}{1/T} = -\frac{\Delta H^{o}}{R} \qquad \text{Equation 3.14}$$

$$\ln K_{ow} = -\frac{\Delta H^{o}}{RT} + \frac{\Delta S^{o}}{R} \qquad \text{Equation 3.15}$$

Where:

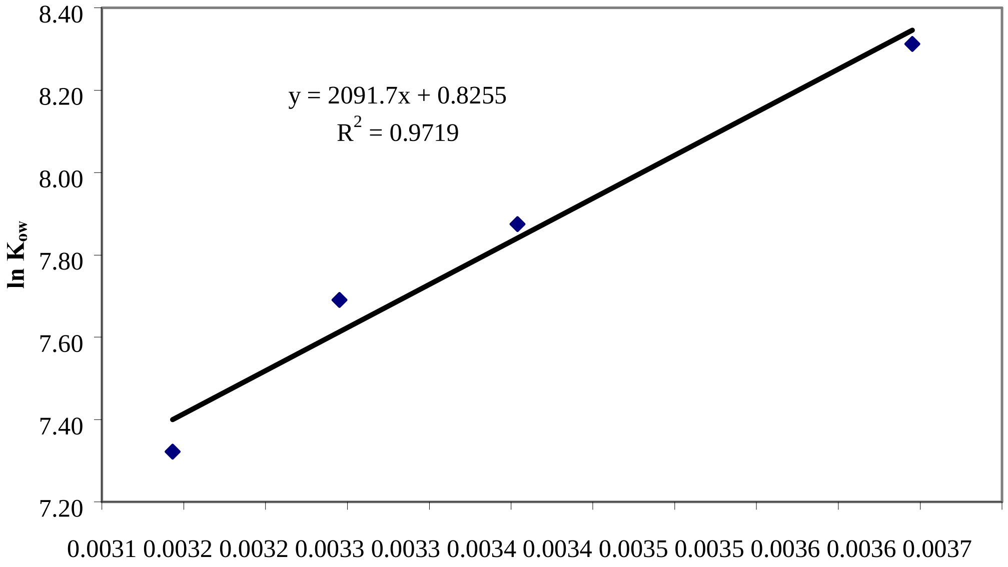
ΔH° = the change in standard enthalpy (kJ mol^{-1})

ΔS° = the change in standard entropy (J K^{-1})

Previous studies indicated that the van't Hoff plot can be used to determine the temperature dependence of the system and also assess the reaction of the system in accordance with the linear form of the van't Hoff equation (equation 3.15). For example, Congliang *et al.* (2007) used the van't Hoff to determine the thermodynamic relationship of K_{ow} of seven sulfonamides and its temperature dependence (from 25 ˚C to 60 ˚C). The results showed that increasing temperature tends to decreased K_{ow}. Bahadur *et al.* (1997) found that the K_{ow} of seven chlorobenzenes increased by 10% - 14% over the temperature range 5 ˚C to 45 ˚C with the linear van't Hoff plot. The results of this study are shown in Table 3.12 and Figure 3.13.

Table 3.12 Data using to determine temperature dependence on K_{ow}

Temperature (K)	K_{ow}	T_{-1} (K_{-1})	ln K_{ow}
278.15	4073.80	0.00360	8.31
298.15	2630.27	0.00335	7.87
308.15	2187.76	0.00325	7.69
318.15	1513.56	0.00314	7.32



T^{-1} (K^{-1})

Figure 3.13 The van’t Hoff plot of K_{ow}

The positive slope of the van’t Hoff plot of partitioning in octanol-water system (Figure 3.13) indicates that the enthalpy change is negative; the positive slope can imply that this reaction is an exothermic reaction and heat is released from the system (Atkins, 2001). The decrease in K_{ow} with increasing temperature is in accordance with Le Chatelier’s principle.

The van’t Hoff equation of this study is shown in equation 3.16. This equation can be used along with equation 3.15 to estimate the change in enthalpy (ΔH°) and entropy (ΔS°) in the reaction as following:

Box 3.5 Calculation of change in enthalpy (ΔH°) and entropy (ΔS°) in the reaction

$$\ln K_{ow} = 2091.7\left(\frac{1}{T}\right) + 0.8255, \quad R^2 = 0.9719$$ Equation 3.16

From equation 3.15, slope $= -\frac{\Delta H^o}{R}$ Equation 3.17

Thus, slope $= -\frac{\Delta H^o}{R} = 2091.7$

$$\Delta H^\circ = -17391.36 \text{ J mol}^{-1} = -17.39 \text{ kJ mol}^{-1}$$

And intercept $= \frac{\Delta S^o}{R}$ Equation 3.18

Thus, intercept $= \frac{\Delta S^o}{R} = 0.8255$

$$\Delta S^\circ = 6.86 \text{ J K}^{-1}$$

The results show that ΔH° is negative (< 0) whereas ΔS° is positive (> 0); thus, octanol-water partition reactions (water-to-octanol transfer) are expected to be spontaneous reactions at all temperatures (Atkins, 2001).

As a result, the van't Hoff equation used in the prediction of K_{ow} ($K_{ow,2}$) at any other temperature (T_2) can be expressed by:

$$\ln\frac{K_{ow,2}}{\quad} = -\frac{\Delta H^o}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$ Equation 3.19

$K_{ow,1}$

The examples of the estimated log K_{ow} values of BPA ($K_{ow,est}$) at 5, 35 and 45 ˚C calculated by equation 3.19 are shown in Table 3.13. The $K_{ow,exp}$ at the reference temperature (25 ˚C) is used as the $K_{ow,1}$. The results are compared to the K_{ow} values from this experiment ($K_{ow,exp}$). The results are shown in Table 3.13 and Figure 3.14.

Table 3.13 Comparison of experimental K_{ow} and estimated K_{ow} at a given temperature

Temperature (˚C)	Log $K_{ow,exp}$	Log $K_{ow,est}$	Log $K_{ow,exp}$ - Log $K_{ow,est}$
5	3.61	3.64	-0.03
35	3.34	3.32	0.02
45	3.18	3.23	-0.05

The results show that the estimated K_{ow} ($K_{ow,est}$) values at 5, 35 and 45 ˚C are in good agreement of K_{ow} values from this experiment at least in the studied range. Consequently, ΔH˚ obtained from equation 3.17 is good enough to predict K_{ow} value of BPA at specific temperature in the temperature range of 5 ˚C to 45 ˚C.

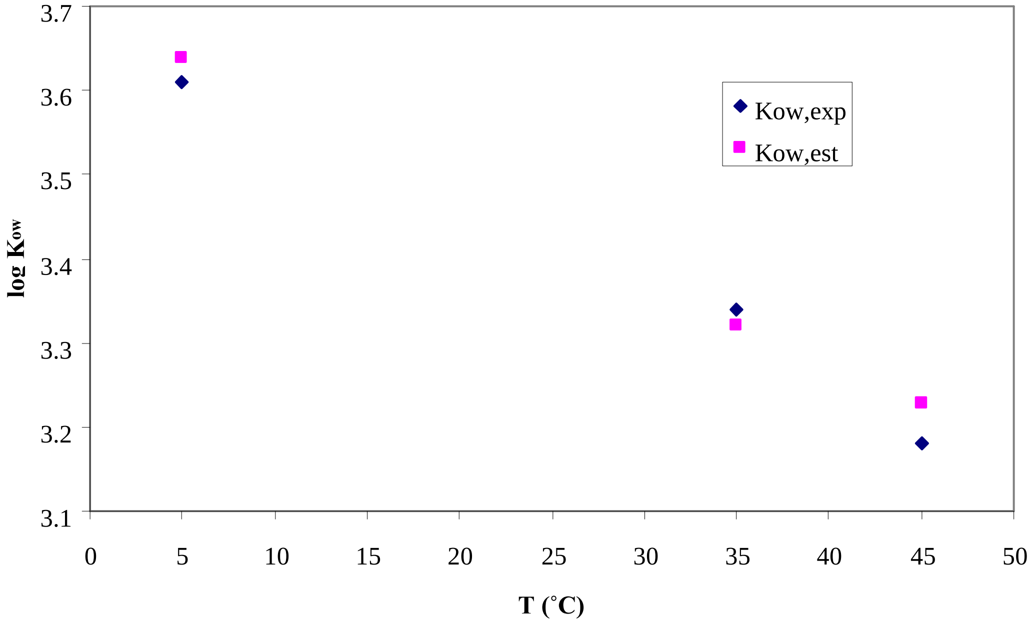


Figure 3.14 Comparison between K_{ow} from experiment and calculation at specific temperature

3.3.8 Determination of Water Condition-dependent K_{ow} of BPA

As mentioned in Section 3.3.5 to Section 3.3.7, salinity, temperature and pH have effects on K_{ow}. Those sections also provide the relationships of each parameter and K_{ow}. In this section, the correlations between these three parameters and K_{ow} were determined by using multiple linear regressions. The independent parameters were salinity (0 – 50 psu), pH and temperature (5 – 45 °C). The pH range used was pH 6 – 8 because of the limit of its linear correlations with K_{ow}. The data used are shown in Table 3.14.

Table 3.14 Dataset used to obtain the correlation equation and comparison between experimental log K_{ow} and calculated log K_{ow}

Salinity (psu)	Temperature (˚C)	pH	Experimental log K_{ow}	Calculated log K_{ow}	Differences[a]	% Difference
0.5	25	6.2	3.44	3.42	-0.02	-0.2
10	25	6.2	3.48	3.45	-0.03	-0.8
35	25	6.2	3.53	3.52	-0.01	-0.4
50	25	6.2	3.55	3.56	0.01	0.4
0	5	6.2	3.61	3.63	0.02	0.5
0	25	6.2	3.42	3.42	0.00	0.0
0	35	6.2	3.34	3.31	-0.03	-0.8
0	45	6.2	3.18	3.21	0.03	1.0
0	25	6	3.39	3.41	0.02	0.5
0	25	7	3.43	3.44	0.01	0.4
0	25	8	3.47	3.47	0.00	-0.1

[a] Calculation. S_w – Experimental S_w

The results of multiple linear regressions in Excel (n = 99) are shown in Appendix A.1. The results indicated that all of these three parameters (salinity, temperature and pH) are statically significantly associated with the K_{ow} values of BPA at 95% confidence levels (p-values < 0.05, $R^2 = 0.89$). The correlation between log K_{ow} and salinity (psu), pH and temperature (°C) is shown in equation 3.20.

Box 3.6 Correlation equation between log K_{ow} and water conditions

$$\text{Log } K_{ow} = 3.5078 + 0.002911\,(\text{Salinity}) - 0.01046\,(\text{Temperature}) + 0.02766\,(\text{pH}) \qquad \text{Equation 3.20}$$

Note: Salinity = 0 – 50 psu; Temperature = 5 – 45 °C; pH = 6 – 8

Table 3.14 also shows the differences and % differences between the experimental log K_{ow} values and log K_{ow} calculated from equation 3.20. The results indicate the differences between these values were in the range of ± 1%. Therefore, equation 3.20 can be used to predict log K_{ow} value of BPA at given salinity, temperature and pH at least in the range of studied conditions.

This finding is very useful in terms of environmental and risk assessment at any specific water conditions. It is possible to obtain the K_{ow} at any given conditions by measuring salinity, temperature and pH, which are less time-consuming, easier, and inexpensive.

3.3.9 Prediction of Environmental Fate and Toxicity from K_{ow}

The K_{ow} is widely used in many environmental hazard modelling programmes to obtain prediction data to input into an environmental and risk assessment. The calculation programmes in EPI Suite™ (*i.e.*, WSKOWWIN™, BCFBAF™, KOAWIN™, KOCWIN™, ECOSAR™ and DERMWIN™) use K_{ow} values, either KOWWIN's estimation value or experimental values in the database, to predict water solubility, bioconcentration-bioaccumulation, octanol-air partition coefficients, soil sorption capacity, aquatic toxicity, and dermal permeability coefficients, respectively (EPA, 2012b). These calculated values are estimated values and not sitespecific as the default values are not taking environmental conditions into account, possibly resulting in undermining environmental models and assessments (Renner, 2002). More importantly, these default values could be unable to predict the effects of changing environmental conditions such as changing temperature, salinity and pH of water from climate change. As a result, in order to improve the models to generate good and reliable results, it is important to input reliable data and take environmental conditions into consideration.

The experimental values of log K_{ow} obtained from this study were compared to estimated and experimental values from EPI Suite™. Also, the comparison of the prediction data of bioconcentration factor (BCF), soil/sediment sorption coefficient (K_{oc}), water solubility (S_w), acute toxicity in fish (LC_{50}), and chronic toxicity in fish (ChV) from the EPI Suite™ using those K_{ow} values was carried out. The results are shown in Table 3.15.

Table 3.15 shows the differences between experimental log K_{ow} values obtained from this study and the values given from the estimation programme, the default values are presented in bold font. Compared to the experimental values, the experimental value from the database in the programme is quite underestimated, but not by much, whereas the predicted log K_{ow} value from KOWWIN™ in EPI Suite™ which is estimated by using an AFC method is overestimated. Furthermore, there is no K_{ow} value for seawater in EPI Suite™. These lead to disparity predictions of fate and toxicity, especially in the marine environment, which represents 96.5% of all water on earth (USGS, 2014). Subsequently, the experimental K_{ow} was input into EPI suite™ to get a more accurate prediction of environmental fate for future studies.

Table 3.15 The K_{ow} and comparison of the predictions obtained from the EPI Suite™

Parameters	Log K_{ow}	BCF^{a} (L kg^{-1} wet-wt)	K_{oc}^{a} (L kg^{-1})	S_{w}^{a} (mg L^{-1})	Toxicity in fish^{a} (mg L^{-1})	
					LC$_{50}$^{b} (96 h)	ChV^{c} (30 d)
Distilled water	3.42^{d}	83.85	1414	146.9	3.173	0.773
Prestwick seawater	3.52^{d}	97.61	1606	116.5	2.773	0.664
50 psu	3.55^{d}	102.2	1669	109.9	2.663	0.634
pH 10	2.99^{d}	43.63	817.8	330.4	5.663	1.489
45 ˚C	3.18^{d}	58.23	1042	227.4	4.384	1.114
EPI Suite™: KOWWIN™	3.64^{e}	117.1	1871	92.04	**2.350**^{g}	**0.550**^{g}
EPI Suite™: “expkow” database	3.32^{f}	**72.03**^{g}	**1245**^{g}	**172.7**^{g}	3.631	0.900

[a] The prediction data using K_{ow} as the input values

[b] Lethal concentration 50 is the concentration of a chemical that will kill 50% of the test subjects, indicating acute toxicity

[c] Chronic value is the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration LOEC) (Mayo-Bean *et al.*, 2012)

[d] The experimental values from this study

[e] ™

™

The estimated value obtained from KOWWIN version 1.68 in EPI Suite , default value for toxicity prediction (ECOSAR™ version 1.00).

[f] The experimental value from Hansch *et al.* (1995) in database in EPI Suite™, default value in physicochemical properties and fate prediction.

[g] The default values from EPI Suite™ version 4.11 (EPA, 2012b)

Table 3.15 presents a comparison of these prediction data, using both experimental values from this study and default values from EPI Suite™. The differences between predicted data of BPA depended on the input values. The experimental prediction for Prestwick coast showed lower toxicity but higher adsorption capacity to sediment and higher bioaccumulation than the default model. As a result, organisms might have greater opportunity to accumulate BPA than the programme predicts.

The results indicate that salinity, pH and temperature all affect log K_{ow} with a subsequent influence on toxicity, bioaccumulation and sorption. Even small changes in these parameters can have an impact on behaviour. Therefore, the experimental values are necessary to predict accurately the environmental impact. Experimental values aid the understanding of real environmental behaviour of BPA in specific sites, which can lead to more appropriate prevention and remediation technology and more effective exposure assessment.

3.4 Summary

The K_{ow} is an important physicochemical property of substance. It is used to predict many properties, the environmental fate, transport, behaviour, and toxicity of substances. There are many standard methods to measure the K_{ow} value either using an indirect experimental method, direct experimental method or calculation method. This study found that the RP-HPLC method is an unsatisfactory method to determine the K_{ow} of BPA and the shake flask method (SFM) is a satisfactory determination method. Comparing to the calculated K_{ow} of BPA from the atom/fragment contribution (AFC) method in KOWWIN™, the results show that the predicted value is overestimated relative to the experimentally-derived value.

The study of the effects of major factors (salinity, pH and temperature) on the octanol-water partitioning in aquatic environments indicates that the K_{ow} values of BPA are greater with increasing salinity, but decreasing temperature of water (at least in the range of this study). For pH, the partition between octanol phase and water phase depends on dissociation form of BPA that is influenced by pK_a of BPA. At pH in the range of pK_a, the results show that the K_{ow} decreased significantly because the predominant form in this system is an ionised form that prefers a polar phase (water) to a non-polar phase (octanol). These findings are likely to help the understanding and prediction of behaviour of BPA in the real aquatic environment.

Furthermore, it is possible to estimate K_{ow} value of BPA at given salt concentration by using the salting-out constant (k_{ow}). Also, the enthalpy of reaction (ΔH°) obtained by plotting ln K_{ow} and T^{-1} along with any K_{ow} (such as K_{ow} at 25 °C) is useful to determine the K_{ow} value at any other given temperature.

More interestingly, the multiple linear regression equation obtained from the correlations between these three water conditions (salinity, temperature and pH) and K_{ow} may be used in the determination of K_{ow} value of BPA at any specific conditions by measuring only simple parameters (*i.e.*, salinity, temperature and pH).

EPI Suite™ used K_{ow} to estimate many of the physicochemical properties related to the environmental fate and the toxicity of a molecule. The K_{ow} was also used to assess toxicity in the aquatic environment. The computation programme, EPI Suite™, is useful as a screening-level tool for environmental and risk assessment when acceptable measured data are unavailable. It provides partition coefficients by calculation from the inherent chemical and physical properties of the compound, without taking environmental conditions into account. According to this study, the bioaccumulation, sorption capacity, persistence, bioavailability, and toxicity of BPA in aquatic environments vary with changes of environmental conditions such as salinity, pH and temperature. Subsequently, the interpretation of results implies that BPA in seawater potentially may inflict greater adverse effects than those from a freshwater environment. Moreover, these could help to evaluate the effects of changing environmental conditions on the environmental behaviour of BPA in the aquatic environment. For example, the changing from climate change in the marine environment, which is associated with lower salinity and pH of seawater, and higher sea and air temperatures, will result in less bioaccumulation, sorption capacity, persistence, and toxicity of BPA in the marine environment. In contrast, water solubility and bioavailability of BPA will be increasing. Also this study shows that due to the correlation of K_{ow} and salinity and temperature that simple tests such as salinity and temperature could act a proxy for the more difficult to measure K_{ow}. Therefore, site specific assessments and monitoring over a wider range of geographical areas becomes possible. This is of particular importance when global climate change is considered.

In conclusion, it is necessary to use good and reliable data and to take the environmental conditions into consideration, normally by determining the partition coefficients experimentally, in order to get more accurate data to assess environmental fate and ecological risk of the chemicals and also to develop more appropriate remediation methods for BPA contamination.

Chapter 4

The Water Solubility (S_w) of Bisphenol A

4.1 Background

The water solubility (S_w) is the saturated mass concentration of the substance called solute in water at a specified temperature and pressure. It is determined as an equilibrium partition coefficient between the pure chemical and water phases at a given temperature and pressure as shown in Figure 4.1. The unit of S_w is expressed in mass/weight of substance per volume of solution. Although the SI (International System of Units) unit is kg m^{-3}, the unit g L^{-1} is used regularly (OECD, 1995a).

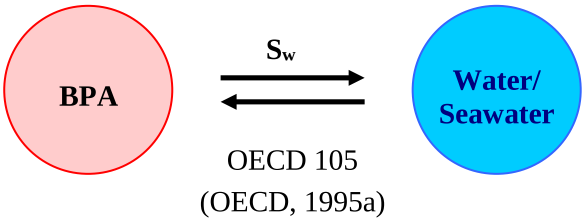


Figure 4.1 The partition coefficient between BPA and water

Water solubility (S_w) is important because of the following reasons (Rand, 1995; EPA, 1998; Ying *et al.*, 2002):

(i) S_w is used to determine the mobility of substances as highly water-soluble chemicals have more transport potential in water and tend to be less volatile and less sorptive to the solid phase.

(ii) S_w is used to determine bioavailabilities of substances as highly water soluble chemicals have more exposure routes for humans and other living organisms.

(iii) S_w is used to estimate other partition coefficients by using the correlation equations as mentioned in equation 3.2 – 3.4, such as K_{ow}, K_{oc} and BCF.

(iv) S_w is used to determine persistence of a substance as highly water-soluble substances tend to have less persistence since they tend to be less sorptive onto solid phases.

(v) S_w is used to calculate HLC along with VP.

Because of the reasons given above, aqueous solubility affects environmental fate and transport of organic substances in aquatic environments and is also used as an input parameter for estimation programmes (Shareef *et al.*, 2006). Unfortunately, reliable S_w values for a number of compounds are still problematic because of significant uncertainties in reported S_w values (Pontolillo and Eganhouse, 2001; Renner, 2002). BPA is one of these problematic compounds with experimental

reported S_w values of 0.120 – 0.300 g L^{-1} (Staples *et al.*, 1998). Consequently, the S_w of BPA was measured following the standard guidelines.

According to the standard guidelines (OECD, 1995a; EPA, 1998), there are two experimental methods to determine S_w, the column elution method and the shake flask method. However, neither of them is suitable for volatile substances. Because there is no single available method covering the whole range of S_w, the solubility range of a substance is a limitation of these methods, which are described below (OECD, 1995a; EPA, 1998):

(i) The column elution method is used for compounds with low solublities, below 10^{-2} g L^{-1} (or 10 mg L^{-1}).

(ii) The flask method is used for substances with solubilites above 10^{-2} g L^{-1}.

Therefore, the method used to determine the S_w of BPA in this study was the flask method due to reported S_w values, which are above 10^{-2} g L^{-1}.

The estimated S_w of organic compounds at 25 ˚C can be obtained by calculation methods such as by using correlations based upon other partition coefficients. The calculation methods used in EPI Suite™, the environmental modelling programme used in this study, are (i) correlation equations between K_{ow} and S_w (EPA, 2012b), and (ii) the atom fragment method (Meylan and Howard, 1994a).

It has been reported in the literature that environmental variables such as salinity, pH and temperature have effects on dissolution of substances in water (Gold and Rodriguez, 1989; Inaba *et al.*, 1995; Xie *et al.*, 1997; Poulson *et al.*, 1999; Noubigh *et al.*, 2007). For example, Inaba *et al.* (1995) reported that the aqueous solubilities of four organotin compounds were affected by varying salinity, pH and temperature. Increasing salinity led to decreases in S_w values of these compounds because of the salting-out effect. On the other hand, the S_w increased by an approximate factor of 2 with an increase of temperature from 10 ˚C to 25 ˚C. They also found that the S_w values increased when the pH of the water was less than pH 6 (which was less than the pK_a);

therefore, the compounds were in an ionised form. Whereas the S_w did not change much in the pH range 6 – 8. Shareef *et al.* (2006) found that the aqueous solubilities of estrone, 17β-Estradiol, 17α-ethynylestradiol and bisphenol A did not change much at pH between 4 and 7, but were greater at pH higher than pK_a (pH 10). The effects of temperature and salinity on the water solubility of six phenolic compounds (gallic acid, ferrulic acid, vanillic acid, protocatechuic acid, vanillin and syringic acid) were studied by Noubigh *et al.* (2007). The results showed that the solubilities in pure water and water containing chloride salts (KCl, NaCl and LiCl) increased concomitant with an increase of temperature from 20 °C to 45 °C.

This chapter describes S_w values of BPA obtained using the shake flask method and calculation programmes WSKOWWIN™ and WATERNT™ in EPI Suite™, which predicts S_w from correlations with K_{ow} as well as the fragment constant method, respectively (EPA, 2012b). Furthermore, the effects of changing water conditions of salinity, temperature and pH were determined. These experimental S_w values were compared with the estimated values calculated from the experimental K_{ow} values in Chapter 3. Lastly, the S_w values were inserted into EPI Suite™ to evaluate their effects on the prediction of environmental fate.

4.2 Experimental

4.2.1 Determination of S_w by Flask Method

The S_w of BPA was determined by using the flask method described in the OECD guideline 105 (OECD, 1995a) at 25 ± 0.5 ˚C. Pulverised BPA was dissolved in water in three glass vessels at a concentration of 1.5 g L^{-1}, a concentration five times greater than the reported maximum S_w of 0.300 g L^{-1} (Staples *et al.*, 1998) at 10 °C greater than the test temperature for 1 day. The temperature was then lowered to the desired test temperature and the mixtures were analysed for concentrations of BPA in water after 1, 2 and 3 days. In each case, S_w was calculated as the average of the results from the three experiments because the results did not differ by more than 15%.

The concentrations of BPA dissolved in water were analysed using the Dionex HPLC-UV detector under the same conditions described in Section 3.2.2.

4.2.2 Determination of S_w by Environmental Modelling Programme

EPI Suite™ version 4.11 provides three default S_w values, two values from two calculated methods and one from the experimental database in the programme. The calculated S_w values were obtained from the WSKOWWIN™ version 1.42 and WATERNT™ version 1.01. The WSKOWWIN™ calculates S_w using the correlation between S_w and K_{ow}, while WATERNT™ version 1.01 uses the fragment constant method. Moreover, WATERNT™ provides the experimental S_w values from the special database in the programme (EPA, 2012b).

4.2.2.1 Correlations with K_{ow} Value

In WSKOWWIN™, the equations used are shown as follows (Meylan and Howard, 1994a; Meylan and Howard, 1994b; Meylan *et al.*, 1996):

$$\text{Log } S_w\ (\text{mol L}^{-1}) = 0.796 - 0.854 \log K_{ow} - 0.00728\ \text{MW} + \text{Corrections} \qquad \text{Equation 4.1}$$

$$(R^2 = 0.934,\ SD = 0.585,\ ME\ (\text{mean error}) = 0.442\ \text{log units})$$

$$\text{Log } S_w\ (\text{mol L}^{-1}) = 0.693 - 0.96 \log K_{ow} - 0.0092\ (T_m - 25) - 0.00314\ \text{MW} + \text{Corrections} \qquad \text{Equation 4.2}$$

$(R^2 = 0.97$, $SD = 0.409$, $ME = 0.313$ log units) Where:

MW = molecular weight

T_m = melting point (MP) in °C (used only for solids)

Corrections = depends on the structural types of solute (15 structure types in database, including alcohols, acids, selected phenols, nitros, amines alkyl pyridines, amino acids, polyaromatic hydrocarbons (PAHs), multi-nitrogen types; Appendix B.1)

Equation 4.1 is used when a measured melting point (MP) is not available; however, if a measured MP is available, equation 4.2 is used. A dataset of 1,450 compounds with measured log K_{ow}, S_w and MP was used in order to determine these equations. The statistical accuracy from a validation dataset of 817 compounds is shown by the correlation coefficient (R^2) = 0.902, standard deviation (SD) = 0.615 and absolute mean error (ME) = 0.48 log units (Meylan *et al.*, 1996). For BPA, the default S_w values obtained from the WSKOWWIN™ version 1.42 were calculated by equation 4.1 (EPA, 2012b).

4.2.2.2 Fragment Constant Method

The second programme, WATERNT™, predicts S_w from a fragment constant method or Atom/Fragment Contribution (AFC) method using SMILES notation which is the same method of the KOWWIN™ programme described in Section 3.2.3 (EPA, 2012b). To estimating S_w, the general equation used in WATERNT™ version 1.01 is:

$$\log S_w (\text{mol L}^{-1}) = \sum(f_i n_i) + \sum(c_j n_j) + 0.24922 \qquad \text{Equation 4.3}$$

(n = 1128, R^2 = 0.940, SD = 0.537)

Where: f_i = coefficient for each atom/fragment n_i = the number of times the atom/fragment occurs in the structure c_j = coefficient for each correction factor n_j = the number of times the correction factor occurs in the molecule

4.2.2.3 Experimental S_w Value from the Database

WATERNT™ provided an experimental S_w value obtained from a special S_w database of 6,200 organic compounds. The programme was used to generate a structural representation by entry in SMILES notation. An experimental S_w value is shown if an exact atom-to-atom connection match of structure-representation between an inputted structure substance and a database structure is found.

4.2.3 Study of Effects of Environmental Parameters on S_w

Major variables known to influence S_w were studied in order to assess the effects of these parameters on the environmental fate and toxicity of BPA in the marine environment. These studies were conducted by using OECD guideline 105 (OECD, 1995a) as mentioned in Section 4.2.1. The variables studied were salinity, temperature and pH of water. The salinities studied were 0.5, 10, 35 and 50 psu to cover the range of typical estuarine to seawater conditions, omitting the most extreme saline conditions. As required in the standard method, KCl (Fisher Scientific, UK) was used to adjust the salinity of the water (APHA, 1999). The effect of pH was studied by setting the pH to 4, 6, 7, 8, 9 and 12 to cover the range of acidic to alkaline conditions. The pH was adjusted by adding either 0.1 M HCl (Fisher Scientific, UK) or 0.1 M NaOH (Fisher Scientific, UK) (Burton *et al.*, 2004). The temperatures studied were 5, 25, 35 and 45 ˚C, which covered the range of subtropical to tropical zones, and the temperature was maintained in an incubator shaker, Stuart® orbital incubator SI500 (Bibby Scientific Limited, UK).

4.2.4 Prediction of Environmental Fate and Toxicity from S_w

Generally, S_w is used in the determination of Henry's Law Constant (HLC or K_H) along with vapour pressure (VP) of the substance. HLC is the distribution equilibrium of concentrations of chemicals between gas and aqueous phases. It is used to indicate the volatilisation of chemicals from water into air (Sander, 1999).

The HLC can be used to understanding the partitioning behaviour of organic compounds between air and environmental matrices along with the K_{ow} by predicting the octanol-air partition coefficient (K_{OA}) (Meylan and Howard, 2005). If the S_w of compound is less than 1 mol L^{-1}, HLC can be expressed using this equation (EPA, 2012b)

$$\mathrm{HLC} = \frac{\mathrm{VP}}{S_w} \qquad \text{Equation 4.4}$$

Where:

HLC = Henry's Law Constant (atm m^3 mol^{-1})

VP = Vapour Pressure (atm)

S_w = Aqueous Solubility (mol L^{-1} or mol m^{-3})

For comparison purposes, EPI Suite™ version 4.11 also provides the HLC at 25 ˚C calculating by equation 4.4 using estimated S_w values from WSKOWWIN™ or userentered S_w values (EPA, 2012b). The default HLC value from this method is 3.948 x 10^{-10} atm m^3 mol^{-1}. The VP at 25 ˚C is estimated from MPBPWIN™ version 1.43 which is 2.27 x 10^{-7} mmHg. The estimated S_w value is 0.173 g L^{-1}.

This study compared the prediction environmental data obtained from inserting experimental S_w values along with default data from EPI Suite™. Additionally, the comparison between prediction data at specified conditions obtained from inputting experimental S_w values and from inputting experimental K_{ow} values (Chapter 3) was also done.

4.3 Results and Discussion

4.3.1 Determination of S_w by Flask Method

Data obtained from the shake flask method were found to be less than 3%, significantly below the criteria established in the standard method of less than 15%. The S_w of BPA at 25 ± 0.5 ˚C was calculated as 0.279 ± 0.009 g L^{-1} (n = 3). The pH of water used in this experiment was 6.

4.3.2 Determination of S_w by Environmental Modelling Programme

WSKOWWIN™ version 1.42 and WATERNT™ version 1.01 were used to estimate the S_w of substances at 25 ˚C in EPI Suite™ version 4.11. This modelling programme also provides experimental S_w value obtained from its database (EPA, 2012b).

The estimated S_w of BPA using its correlation with K_{ow} from WSKOWWIN™ (version 1.42) derived from equation 4.1 was 0.173 g L^{-1}. The log K_{ow} used in this calculation was 3.32, obtained from the experimental database in EPI Suite™. The MW was 228.29 and the correction for phenol was 0.580, the calculation is displayed below:

$$\text{Log } S_w (\text{mol L}^{-1}) = 0.796 - (0.854 \times 3.32) - (0.00728 \times 228.29) + 0.580$$

Equation 4.5

$$S_w = 0.000757 \text{ mol L}^{-1}$$

$$= 0.173 \text{ g L}^{-1}$$

The second estimated S_w of BPA at 25 ˚C was obtained from WATERNT™ version 1.01, which used the fragment constant method. This method divides a structure of substance into fragments to obtain atom/fragment contribution values and adds the coefficient values of each fragment or group to estimate the solubility as shown in equation 4.3. The estimated S_w of BPA from this method was 0.146 g L^{-1}.

Additionally, WATERNT™ also provides an experimental S_w value from the database if a match is found between an exact atom-to-atom connection of the test substance and the database substance. The experimental S_w of BPA obtained from WATERNT™ version 1.01 was 0.120 g L^{-1}, which was derived from Dorn *et al.* (1987).

As a result, the three S_w values of BPA retrieved from WSKOWWIN™ version 1.42 and WATERNT™ version 1.01 in EPI Suite™ version 4.11 were in the range of 0.120 – 0.173 g L^{-1}.

4.3.3 Comparison of the Different Determination Methods of S_w

The S_w value measured by the flask method in this study was compared against the values from EPI Suite™ and reported experimental values which were collected by the European Commission (2000), as shown in Table 4.1.

Table 4.1 The S_w value of BPA from this study, EPI Suite™ and literature

References	Temperature (°C)	pH	S_w (g L^{-1})
Flask method (This study) (n = 3)	25 ± 0.5	6	0.279 ± 0.009
WSKOWWIN™:			
- correlations with K_{ow}	25	N/A[b]	0.173
WATERNT™:			
- fragment constant method	25	N/A[b]	0.146
- exp database	25	N/A[b]	0.120

References	Temperature (°C)	pH	S_w (g L^{-1})
Dow Deutschland Inc Stade 5, 1988[a]	25	N/A[b]	0.301
	25	7	0.298
	25	8	0.318
	83	N/A[b]	≤ 0.34
Daltrade Ltd London[a]	25	N/A[b]	0.120
Shareef *et al.*, 2006 (n = 6)	25 ± 0.5	4	0.298 ± 0.002
	25 ± 0.5	7	0.300 ± 0.005
	25 ± 0.5	10	0.319 ± 0.006

[a] Source: European Commission, 2000

[b] N/A = No available data

Table 4.1 indicates that BPA is moderately soluble because the S_w values are in the range of 0.1 – 1 g L^{-1} (EPA, 2009). According to the Dataset report of BPA prepared by European Commission (2000), the reported S_w values at 25 °C varied between 0.120 g L^{-1} and 0.319 g L^{-1}. The S_w values from the programme were 0.120 g L^{-1} to 0.173 g L^{-1}. In this study, the experimental S_w was 0.279 ± 0.009 g L^{-1} ($n = 3$). The results show that the estimated S_w values from the programme and the experimental value from this study fall within the range of S_w values from literature. However, the estimated values were lower than experimental values. Using the criteria from OECD guideline 105 (OECD, 1995a), the S_w from this study was in good agreement with the S_w from Dow Deutschland Inc Stade 5 (European Commission, 2000) and Shareef *et al.* (2006) as the difference between these values was less than 15% (OECD, 1995a). Furthermore, the results in Table 4.1 show that either increasing temperature or pH increased the solubility of BPA because the S_w values increased when increasing pH (from 7 to 8) and increasing temperature (from 25 °C to 83 °C). Consequently, the effects of these water conditions on water solubility of BPA were studied.

4.3.4 Effect of Salinity on S_w

Generally, aqueous solubility of an organic compound is less in the presence of inorganic salts in solution, a phenomenon known as the salting-out effect (Xie *et al.*, 1997). But S_w may increase in salt solution (salting-in effect) as well (Huerta-Diaz and Rodriguez, 1992). The Setschenow equation (Setschenow, 1889) is frequently used to describe salting effect (Ni and Yalkowsky, 2003). The solubility of an organic compound in water is an important factor in environmental assessment, especially in the marine environmental assessment. In this study, the water solubilities (S_w) of BPA at various salinities were determined and also the Setschenow constant was predicted.

4.3.4.1 Determination of S_w at Various Salinities

The effect of salinity on S_w value of BPA was studied by using the flask method (OECD, 1995a) at salinities ranging from 0.5 psu to 50 psu to cover the range of salt water from estuarine to seawater. In addition, the estimated S_w values at various salinities were also retrieved from WSKOWWIN™ by using K_{ow} values from Chapter 3. The experimental S_w and estimated S_w values are shown in Table 4.2 and Figure 4.2.

Table 4.2 S_w of BPA at various salinity from experiment and estimation programme

Salinity (psu)	Experimental S_w ± SD (Exp., g L^{-1}) (n = 3)	Estimated S_w (Est., g L^{-1})	Differences (Exp. – Est., g L^{-1})
0.5	0.283 ± 0.002	0.136	- 0.146
10	0.272 ± 0.019	0.126	- 0.146
35	0.225 ± 0.004	0.114	- 0.111
50	0.209 ± 0.012	0.110	- 0.099

The results show that increasing salinity leads to a decrease of the S_w of BPA since the salt content in the aqueous phase has effects on the partitioning of organic chemicals, known as salting-out effect (Xie *et al.*, 1997). Consequently, greater salinity leads to increased volatilisation, persistence, bioaccumulation, and toxicity as BPA may accumulate higher in the lipid of marine organism and persist longer in marine environment (Gosset *et al.*, 1983); however, there may be reduced bioavailability of BPA from seawater because of lower S_w (Allam *et al.*, 2011). The estimated S_w values from K_{ow} by using equation 4.1 were less than the experimental S_w at all salt concentrations with an average of 0.125 g L^{-1}. The results also show that the differences between these methods tend to be lower at higher salt content.

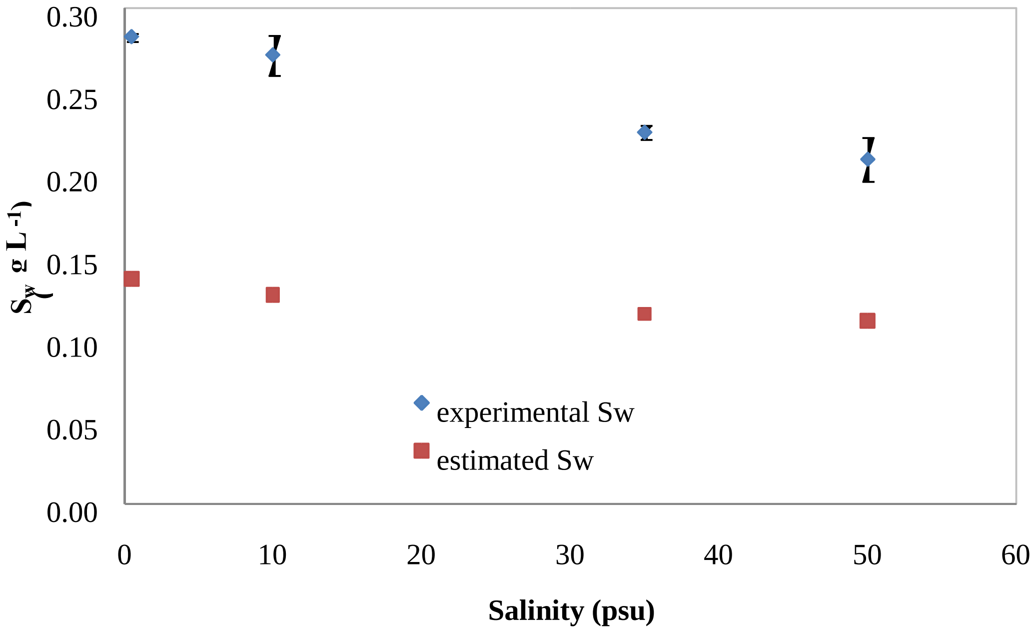


Figure 4.2 S_W of BPA of artificial water at various salinity (0.5 – 50 psu) (Error bars indicate 95% Confidence Intervals, n = 3)

The salting-out effect on solubility of BPA from this study is in good agreement with other organic compounds from the literature (Inaba *et al.*, 1995; Poulson *et al.*, 1999; Saab *et al.*, 2011; Endo *et al.*, 2012). For example, the study of Poulson *et al.* (1999) showed that toluene solubility decreases with increasing salt concentration. The salting-out effect on S_W of organic compounds is possibly because of reduction of the ability of free water to dissolve the compound because of the formation of hydration shells around the ionic species (Poulson *et al.*, 1999; Saab *et al.*, 2011). Endo *et al.* (2012) suggested that the inorganic salt ions in solution increase the cavity formation energy and the interaction energy between solute and solvent, resulting in increasing the activity coefficient. Solubility is inversely proportional to the activity coefficient, which means that water solubility of some organic compounds decreases with increasing salt concentration. Xie *et al.* (1997) indicated that the presence of salts in solution increases hydrophobicity of organic compounds resulting in decreasing water solubility while increasing evaporation, adsorption, bioaccumulation, and toxicity of these compounds in seawater compare to freshwater.

4.3.4.2 Calculation of Salting Coefficients for S_w

The Setschenow equation is an empirical equation used to describe the effects of salt on the aqueous solubilities of organic compounds (Setschenow, 1889). The Setschenow equation is defined as:

$$\mathrm{Log}\left(\frac{S_{w,0}}{S_w}\right) = K_s C_s \qquad \text{Equation 4.6}$$

Where:

$S_{w,0}$ = the solubilities of organic solute in pure water (mol L^{-1})

S_w = the solubilities of organic solute in aqueous salt solutions (mol L^{-1})

K_s = the salting coefficient or Setschenow constant (M^{-1} or L mol^{-1})

C_s = the molar concentration of salt (M or mol L^{-1})

In general, the partition coefficients used in the prediction model do not take salt effects into account, resulting in inaccurate assessment under salt conditions. The salting coefficient (Setschenow constant, K_s) is useful in terms of enhancing the accuracy and reliability of the prediction of environmental fate and transport of pollutants in marine environments (Endo *et al.*, 2012). Subsequently, many researchers (Xie *et al.*, 1997; Ni *et al.*, 2000; Ni *et al.*, 2003; Schröder *et al.*, 2011) offered prediction methods to estimate K_s.

For example, Xie *et al.* (1997) suggested that the factor $K_s = 1.36$ can be used to assess a reduction of solubility of a various organic compounds in seawater ($C_s = 0.5$ mol L^{-1}). The K_s in the study was determined by:

$$K_s = 0.0018\ V_{LeBas}$$ Equation 4.7

Where:

V_{LeBas} = the Le Bas molar volume (cm^3 mol^{-1})

Also, Ni *et al.* (2000) generated the prediction equation for NaCl effect on the solubility of organic compound from the correlations between K_s and K_{ow} of 62 organic compounds and was validated on a test set of 15 additional compounds. The equation is shown in equation 4.8.

$$K_s = 0.039 \log K_{ow} + 0.117, \quad R^2 = 0.9257$$ Equation 4.8

$$\log\left(\frac{S_w}{S_{w,0}}\right) = -(0.039 \log K_{ow} + 0.117)\ C_s$$ Equation 4.9

Equation 4.9, which combines equations 4.6 and 4.8, indicates that if K_{ow} of compound is more than zero (positive K_s), S_w will decrease with increasing salt concentration, known as salting-out effect. Inversely, if K_{ow} is much less than zero (negative K_s), S_w will increase with increasing salt, known as salting-in effect. K_s is useful to evaluate the effects of each salt since the salting effects depend on type of salt (Huerta-Diaz and Rodriguz, 1992; Noubigh *et al.*, 2007).

In this study, the salting coefficient (K_s) was obtained from the slope of correlation graph plotting between Log$\left(\frac{S_{w,0}}{S_w}\right)$ and salt concentration (C_s, mol L^{-1}) in accordance with equation 4.6. The results are shown in Figure 4.3.

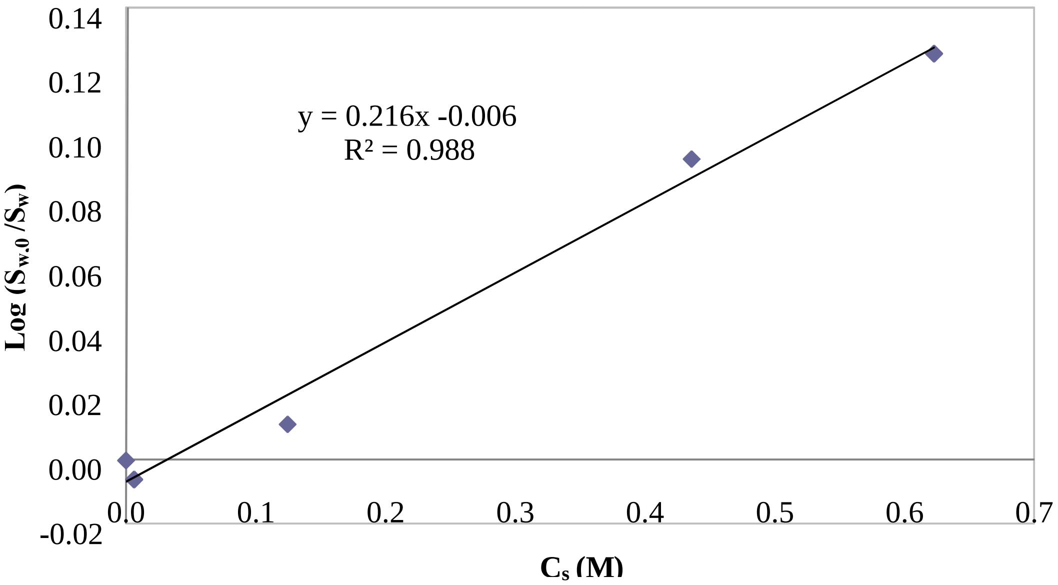


Figure 4.3 Salting effect on S_w of BPA

The results show that the K_s was 0.216 M^{-1}. Since the K_s is positive, the KCl effect on S_w of BPA is a salting-out effect; increasing KCl concentration leads to decreasing S_w. The salting-out effect of KCl on S_w of BPA in this study is consistent with the effect observed with many organic compounds. KCl had the salting-out effect on solubility of toluene with K_s of 0.188 M^{-1} (Poulson *et al.*, 1999). Saab *et al.* (2011) found that the solubilities of metalaxyl in aqueous KCl solutions at 25 °C decreased from 0.0296 mol kg^{-1} to 0.0161 mol kg^{-1} with increasing C_s from 0.01 M to

1.50 M. Noubigh *et al.* (2007) studied the effects of some chloride salts (KCl, NaCl and LiCl) on the solubility behaviour of some phenolic compounds, ferrulic acid and syringic acid, in water at 25 °C. The results showed the salting-out effects of these chloride salts on both ferrulic acid and syringic acid. Huerta-Diaz and Rodriguz (1992) observed the salting-out effect on the S_w of carbaryl in NaCl, Na_2SO_4, $CaCl_2$ solutions, and also in natural and artificial seawater. In addition, Pérez-Tejeda *et al.* (1987) compared the salting-out coefficients of the major salt in seawater (NaCl) and KCl of S_w of caffeine, theophylline and theobromine, they found that the salt effects of NaCl and KCl on these compounds were similar. In this study, the correlation equation between S_w and salt concentration is shown in Box 4.1.

Box 4.1 Correlation equation between S_w and salt concentration

$$\text{Log}\left(\frac{S_{w,0}}{S_w}\right) = 0.216{*}C_s - 0.006, \qquad R^2 = 0.988 \qquad \text{Equation 4.10}$$

Therefore, S_w of BPA at different salt concentrations (C_s) at 25 °C can be estimated by using equation 4.10. Use of this equation will help to improve the accuracy of prediction data from environmental model for marine environment. Also, the solubility is correlated to other partition coefficients and physicochemical properties (*i.e.*, see equation 3.2). These correlations can be corrected for salinity.

4.3.5 Effect of pH on S_w

The dissociation of an organic compound is pH-dependent, so the pH affects solubility of compounds (Rand, 1995). Because BPA is a weak acid with a pK_a in the region of

9.6 – 10.2 (Staples *et al.*, 1998), the pH range in this study (4, 6, 7, 8 and 12) covers ionic and non-ionic forms of BPA. The OECD Guideline 105 – flask method (OECD, 1995a) was used to obtain experimental S_w values. These values were then compared with estimated values calculated from K_{ow} values in Chapter 3 using the WSKOWWIN™ programme. The results are shown in Table 4.3 and Figure 4.4.

Table 4.3 S_w of BPA at various pH from experimental results and estimation programme

pH	Experimental S_w ± SD (Exp., g L^{-1}) (n = 3)	Estimated S_w (Est., g L^{-1})	Differences (Exp. – Est., g L^{-1})
4	0.286 ± 0.011	N/A[b]	N/A[a]
6	0.279 ± 0.009	0.151	- 0.128
7	0.314 ± 0.014	0.139	- 0.175
8	0.350 ± 0.010	0.129	- 0.222
10	N/A[a]	0.330	-
12	1.330 ± 0.046	N/A[b]	-

N/A[a] = No data. pH 10 was not studied because it is in the range of pK_a of BPA.

N/A[b] = No data. pH 6 – 10 was the range of study because it is suitable for living organisms (around neutral, pH 7).

The study of the effect on S_w of changing the pH of water shows that S_w was relatively insensitive to changes in the pH when the pH was below the pK_a (pH 4 to 8), but that significantly greater changes in S_w were observed at pH above the pK_a. BPA is a weak organic acid that is significantly ionised when the pH in water is greater than the pK_a. The ionised form is more soluble in water than the non-ionised form (Geyer *et al.*, 2000; Kuramochi *et al.*, 2008a). Shareef *et al.* (2006) found that the difference of S_w of BPA at between pH 4 to 10 is not large (S_w 0.298 g L^{-1} – 0.319 g L^{-1}). Kuramochi *et al.* (2008a) indicated that the ionic form of the phenolic group of 2,4,6-tribromophenol results in increasing its S_w significantly.

Figure 4.4 shows the comparison between the experimental S_w and estimated S_w obtained from the modelling programme. The results show the same pattern as previous sections that the estimated S_w values from K_{ow} by using equation 4.1 were underestimated with an average of 0.175 g L^{-1}. Figure 4.4 also shows that these differences tend to be greater in alkaline solutions.

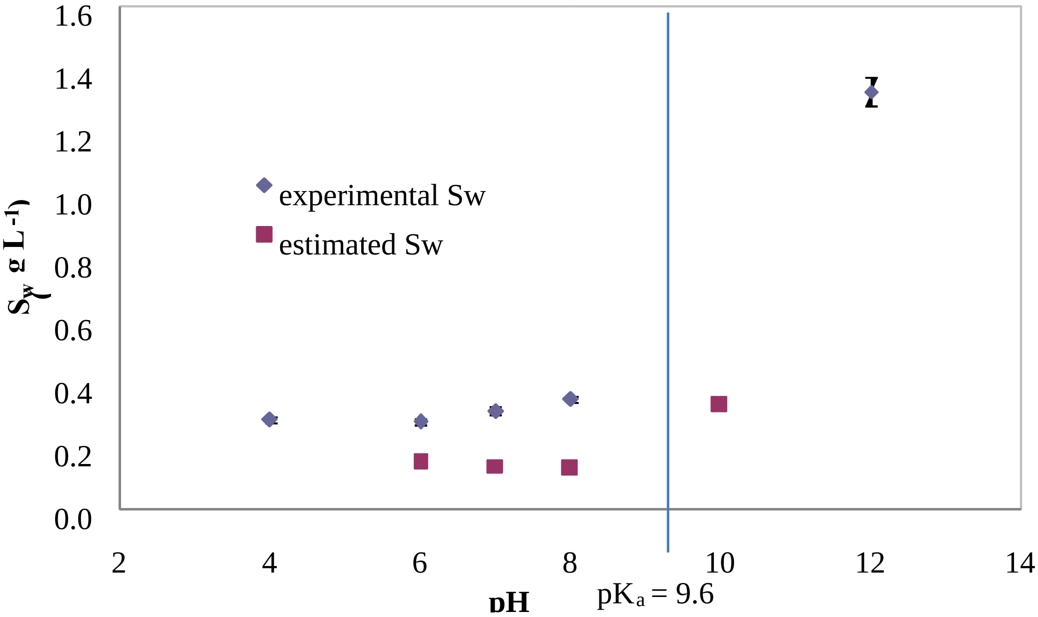


Figure 4.4 S_w of BPA at various pH

(Error bars indicate 95% Confidence Intervals, n = 3)

4.3.6 Effect of Temperature on S_w

It has been long known that solubility is a function of temperature in accordance with Le Chatelier's principle (Atkins, 2001). The effects can be positive or negative depending on the compound. Usually, most compounds show increased solubility with increasing temperature, but some compounds are less soluble. For example, potassium nitrate (KNO_3) is more soluble in water with increasing temperature, while calcium acetate ($Ca(OAc)_2$) is less soluble in water (Behera *et al.*, 2010). Unfortunately, the aqueous solubilities of compounds are commonly reported at standard temperature, 25 °C, as shown in Table 4.1. Therefore, the S_w values of BPA at different temperatures were measured in this study. Also, the thermodynamic relationship between S_w, temperature and free energy (ΔG°) was investigated.

4.3.6.1 Determination of S_w at Various Temperatures

In this study, the effects of temperature on S_w of BPA were investigated by using the flask method (OECD, 1995a) at temperature range 5 °C to 45 °C. The estimated S_w values from the programme, WSKOWWIN™, were also obtained. The results are shown in Table 4.4 and Figure 4.5.

Table 4.4 S_w of BPA and ΔG° at various temperatures from experiment and S_w from estimation programme

Temperatures (°C)	Exp. $S_w \pm$ SD (g L^{-1}) (n = 3)	Exp. ΔG° (kJ mol^{-1})	Est. S_w (g L^{-1})
5	0.189 ± 0.015	16.41	0.098
25	0.279 ± 0.009	16.63	0.142
35	0.405 ± 0.005	16.23	0.166
45	0.540 ± 0.018	16.00	0.227

Exp. = Experimental value; Est. = Estimated value

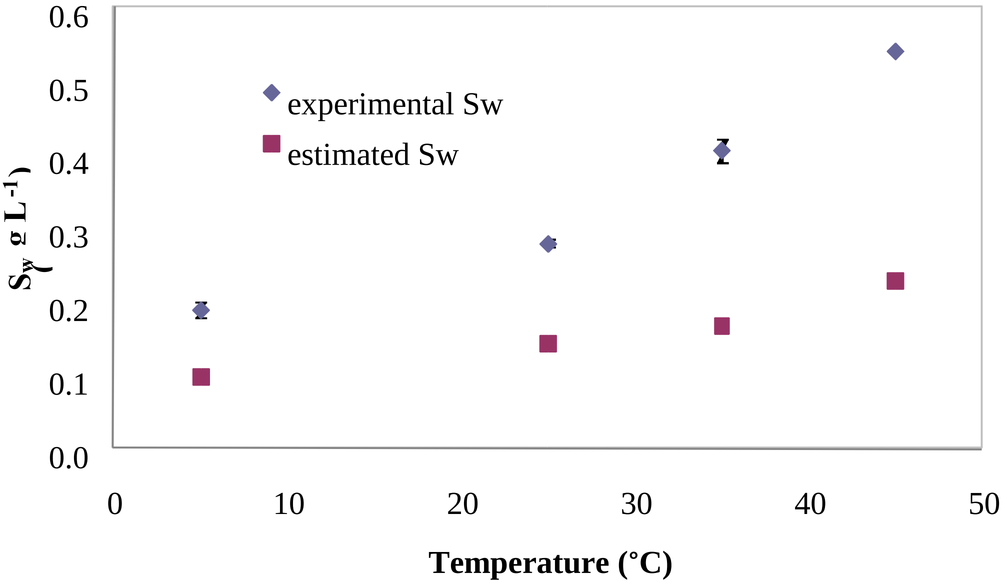


Figure 4.5 S_w of BPA at various temperatures

(Error bars indicate 95% Confidence Intervals, n = 3)

In this study, the experimental S_w of BPA increases from 0.189 g L^{-1} to 0.540 g L^{-1} over the studied temperature range (5 – 45 °C). This trend is also observed in the estimated S_w values at certain temperature retrieved from the modelling programme using the experimental K_{ow} in Section 3.3.7. By comparing the experimental and estimated S_w, the underestimation of S_w is observed. Figure 4.5 shows that the deviation between experimental and estimated S_w of BPA seems to be less at low temperature, while it tends to be larger at high temperature. Moreover, the effects of temperature on S_w tend to be greater at higher temperature. The experimental S_w increases at a rate of 0.005 g L^{-1} °C^{-1} from 5 °C to 25 °C and 0.013 g L^{-1} °C^{-1} from 25 °C to 45 °C. For estimated S_w, the S_w is changing from 5 °C to 35 °C at the increasing rate of 0.002 g L^{-1} °C^{-1}, then the rate doubles (0.004 g L^{-1} °C^{-1}) when the temperature changes from 35 °C to 45 °C. Therefore, increasing the temperature of water leads to increase solubility which is correlated with increased bioavailability and mobility of BPA in the aquatic environment. In contrast, the potentials of volatilisation, bioaccumulation, and accumulation in sediment will decrease

(Eggleton and Thomas, 2004; Allam *et al*., 2011).

The increasing S_w of BPA with temperature observed in this study is in agreement with this effect on other compounds (Inaba *et al.*, 1995; Kuramochi *et al.*, 2004; Noubigh *et al*., 2007; Kuramochi *et al.*, 2008b). For instance, Kuramochi *et al.* (2008b) also observed this trend on the S_w of tetrabromobisphenol A (TBBP-A) over the temperature range of 10 °C to 35 °C.

As mentioned in Section 3.3.7, water solubility is a function of Gibbs energy which is a function of temperature. The standard Gibbs energy of solution (ΔG°) can be calculated by equation 4.11

$$\Delta G^o = -RT \ln S_w \qquad \text{Equation 4.11}$$

Table 4.4 also shows the ΔG° at certain temperatures, which is estimated by using experimental S_w data in the present study. The results show that all ΔG° are positive in the studied range. Thus, the dissolution reactions of BPA are non-spontaneous processes at all temperatures in this study (Atkins, 2001).

4.3.6.2 Determination of the Temperature Dependence on S_w

The temperature-dependent equilibrium constant can be determined by using the van't Hoff equation. S_w is also a constant at equilibrium so the van't Hoff equation and its linear form for S_w are shown in equation 4.12 and equation 4.13, respectively.

$$\frac{\ln S_w}{1/T} = -\frac{\Delta H_o}{R} \qquad \text{Equation 4.12}$$

$$\ln S_w = -\frac{\Delta H_o}{RT} + \frac{\Delta S_o}{R} \qquad \text{Equation 4.13}$$

Where:

S_w = the aqueous solubility in mol kg^{-1}

The enthalpy change of solution (ΔH°) demonstrates the amount of heat released or absorbed associated with the dissolution of a substance in a solvent at constant pressure under standard conditions (1 atm and 298 K) (Atkins, 2001). Regarding the van't Hoff relationship (equation 4.12), the temperature dependence of S_w can be described by ΔH° (Atkins, 2001; Horn *et al.,* 2001). Many researchers (Horn *et al.,* 2001; Wick *et al.,* 2003; Kuramochi *et al.,* 2008b) investigated the temperature dependence of S_w by plotting ln S_w (mol kg^{-1}) as a function of T^{-1}, known as van't Hoff plot. The ΔH° can be determined by multiplying the slope of this plot with -R (the ideal gas constant, 8.314462 J mol^{-1} K^{-1}). The results are shown in Table 4.5 and Figure 4.6.

Table 4.5 Data used to determine temperature dependence on S_w

Temperature (K)	ln S_w (mol kg^{-1})	T^{-1} (K^{-1})
278	-7.097	0.0036
298	-6.708	0.0034
308	-6.334	0.0032
318	-6.047	0.0031

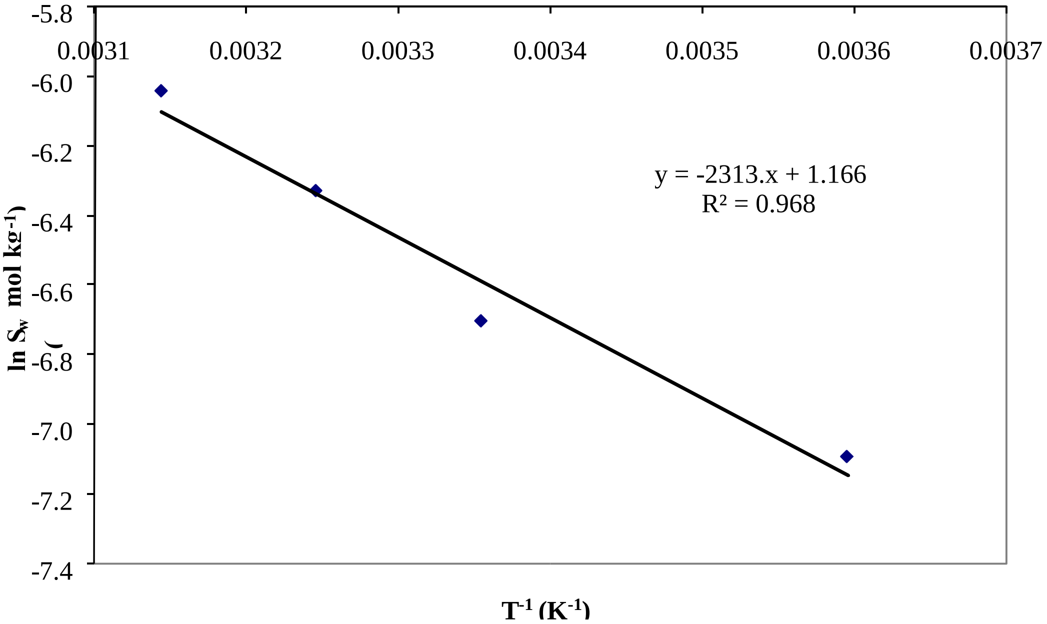


Figure 4.6 The van’t Hoff plot of S_w

The results show the negative slope of the van’t Hoff plot of water solubility of BPA (Figure 4.6) so the enthalpy change (ΔH˚) is positive. This can imply that the dissolution of BPA in water is an endothermic reaction and heat is absorbed to the system (Atkins, 2001). Regarding the Le Chatelier’s principle, BPA is more soluble in water when increasing temperature.

Equation 4.14 shows the van’t Hoff equation obtained from this study. The change in enthalpy (ΔH˚) and entropy (ΔS˚) in this reaction can be estimated by using equation 4.14 and equation 4.15 as following:

Box 4.2 Calculation of change in enthalpy (ΔH˚) and entropy (ΔS˚) in the reaction

$$\ln S_w = -2313.1\left(\frac{1}{T}\right) + 1.1662, \quad R^2 = 0.9688 \qquad \text{Equation 4.14}$$

From equation 4.12, slope $= -\frac{\Delta H^{\circ}}{R} = -2313.1$ Equation 4.15

$$\Delta H^{\circ} = 19232.18\ \text{J mol}^{-1} = 19.23\ \text{kJ mol}^{-1}$$

And intercept $= \frac{\Delta S^{\circ}}{R} = 1.1662$ Equation 4.16

$$\Delta S^{\circ} = 9.7\ \text{J K}^{-1}$$

The results above show that both ΔH° and ΔS° are positive (> 0), which may imply that the dissolution of BPA in water reactions is non-spontaneous at low temperatures where ΔH outweighs TΔS. This is because ΔG can be calculated from ΔH and ΔS as shown in equation 4.17 (Atkins, 2001).

$$\Delta G = \Delta H - T\Delta S \qquad \text{Equation 4.17}$$

Therefore, the van't Hoff equation used in the prediction of S_w ($S_{w,2}$) at any other temperature (T_2) can be expressed by:

$$\ln \frac{S_{w,2}}{S}\ \Delta H^{\circ}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

Equation 4.18

$$= -$$

$$R$$

$$_{w,1} \quad ?$$

Table 4.6 and Figure 4.7 show the experimental S_w values of BPA at 5, 35 and 45 ˚C in this study compared to the estimated S_w of BPA ($S_{w,est}$) at the same temperature which are calculated by equation 4.18. The $S_{w,1}$ is the $S_{w,exp}$ at 25 ˚C which is standard temperature (Atkins, 2001).

Table 4.6 Comparison of experimental S_w and estimated S_w at different given temperatures

Temperature (˚C)	$S_{w,exp}$ (g L^{-1})	$S_{w,est}$ (g L^{-1})	$S_{w,exp}$ - $S_{w,est}$ (g L^{-1})
5	0.189	0.160	0.029
35	0.405	0.359	0.047
45	0.540	0.454	0.086

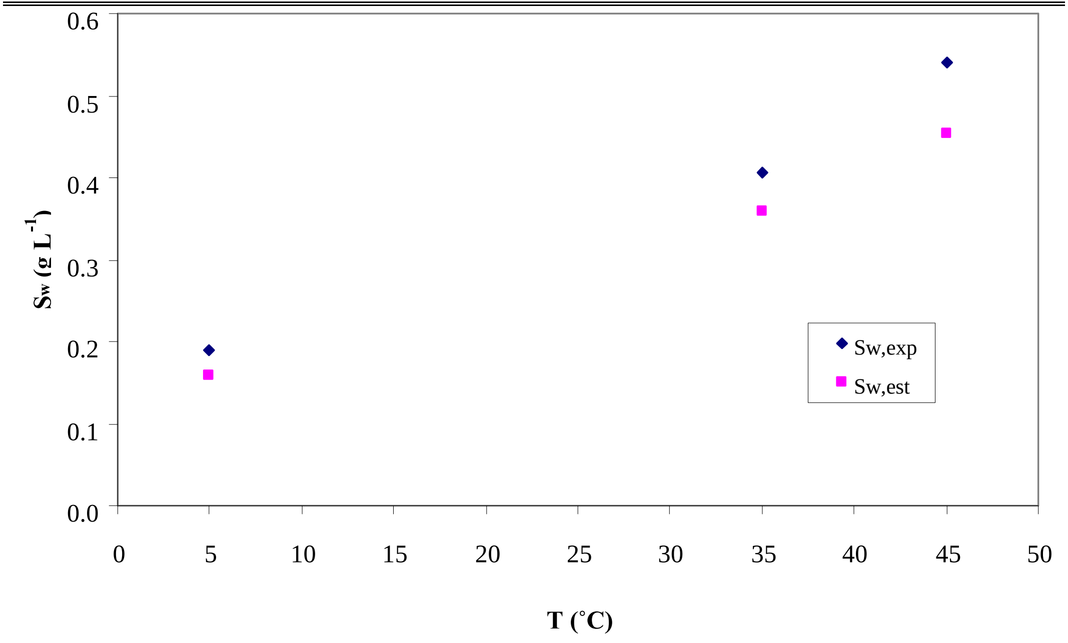


Figure 4.7 Comparison between S_w from experiment and calculation at specific temperature

The results show that the estimated S_w values tend to differ from the experimental S_w values at higher temperatures. However, the estimated S_w ($S_{w,est}$) values at 5, 35 and 45 ˚C using equation 4.18 are still in good agreement with the S_w values from this experiment (at least in the studied range), comparing with the range of reported S_w of BPA at 25 ˚C which has deviation of 0.18 g L^{-1} (0.120 g L^{-1} to 0.300 g L^{-1}) (Staples *et al.*, 1998). Consequently, the equation 4.18 is possible to use to predict S_w value of BPA at specific temperature in the temperature range of 5 ˚C to 45 ˚C.

4.3.7 Determination of Water Condition-dependent S_w of BPA

The equation to determine the water condition-dependent S_w of BPA can be obtained by using multiple linear regression with the experimental data in Section 4.3.4 to Section 4.3.6 (see Table 4.7). The independent parameters were salinity (0 – 50 psu), pH, and temperature (5 – 45 °C). The pH range used to retrieve the equation was pH 4 – 9 due to the limit of its linear relationship with S_w.

Table 4.7 Summary of comparison between experimental S_w and calculation S_w in this study at specified water conditions

Salinity (psu)	Temperature (˚C)	pH	Exp. S_w[a] (g L^{-1})	Cal. S_w[b] (g L^{-1})	Differences[c] (g L^{-1})	% Difference
0.5	25	6	0.283	0.314	0.031	11.0
10	25	6	0.272	0.291	0.020	7.3
35	25	6	0.225	0.233	0.008	3.7
50	25	6	0.209	0.198	-0.010	-4.9

0	5	6	0.189	0.132	-0.056	-29.9
0	25	6	0.279	0.315	0.036	12.9
0	35	6	0.405	0.406	0.001	0.1
0	45	6	0.540	0.497	-0.043	-7.9
0	25	4	0.286	0.298	0.012	4.3

Salinity (psu)	Temperature (˚C)	pH	Exp. S_w[a] (g L^{-1})	Cal. S_w[b] (g L^{-1})	Differences[c] (g L^{-1})	% Difference
0	25	6	0.279	0.315	0.036	12.9
0	25	7	0.314	0.323	0.009	3.0
0	25	8	0.350	0.332	-0.019	-5.3
0	25	9	0.323	0.340	0.018	5.4

[a] Exp. S_w = Experimental S_w

[b] Cal. S_w = Calculation S_w

[c] Cal. S_w – Exp. S_w

The results from running multiple linear regression in Excel ($n = 42$, $R^2 = 0.90$) indicated that S_w correlates with salinity (psu), pH and temperature (°C) with 95% confidence (see details in Appendix A.2). The multiple linear regression equation obtained is shown in equation 4.19.

Box 4.3 Correlation equation between S_w and water conditions

$$S_w\ (\text{g L}^{-1}) = 0.03626 - 0.00233\ (\text{Salinity}) + 0.009115\ (\text{Temperature}) + 0.008438\ (\text{pH}) \qquad \text{Equation 4.19}$$

Note: Salinity = 0 – 50 psu; Temperature = 5 – 45 °C; pH = 4 – 9

The S_w values calculated by equation 4.19 as well as the differences between the experimental S_w and the calculated S_w are shown in Table 4.7. The results show that the difference between experimental S_w and calculated S_w were less than 15% except only S_w at 5 °C, pH 6 and non-salt water. Therefore, this equation (equation 4.19) can possibly be used to estimate S_w of BPA at any specific site by measuring salinity, pH and temperature. However, this correlation equation may not suitable for water near the limits of the temperature range.

4.3.8 Prediction of Environmental Fate and Toxicity from S_w

The S_w is commonly used in environmental assessments such as the air-to-water partitioning assessment. EPI Suite™ version 4.11 (EPA, 2012b) provides the air-towater partitioning coefficient, also known as HLC, calculated from S_w and VP for comparison purposes, as described in Section 4.2.4. The default HLC for BPA from the programme and the examples of HLC values given by the model at specified condition using experimental S_w values from this study are shown in Table 4.8.

Table 4.8 Comparison of the S_w of BPA and the predictions derived from the EPI Suite™

Parameters	S_w (g L^{-1}) ± SD (n = 3)	HLC (atm m^3 mol^{-1})
Default values (25 °C)	0.173	3.948×10^{-10}

Salinity		
10 psu	0.272 ± 0.019	2.510 x 10^{-10}
35 psu	0.225 ± 0.004	3.032 x 10^{-10}
Temperature		
25 °C	0.279 ± 0.009	2.446 x 10^{-10}
35 °C	0.405 ± 0.005	1.682 x 10^{-10}
pH		
7	0.314 ± 0.014	2.172 x 10^{-10}
8	0.350 ± 0.010	1.947 x 10^{-10}

Comparing the HLC at 25 °C, the results show that the default value from the model is overestimated. Subsequently, the concentration of BPA in water will be higher than the default prediction. This prediction implies that there will be more bioavailable BPA to aquatic organisms but less volatility. The results also indicate that the changing of water conditions such as salinity, pH and temperature can have impacts on the environmental behaviour of BPA in the aquatic environment. Therefore, using experimental data when available and taking environmental conditions into account will help to understand the real environmental behaviour and produce a more effective assessment.

4.4 Summary

S_w is one of the physicochemical properties used in the prediction models. The S_w of a substance can be derived from an experiment and an estimation method. This study compared the experimental S_w values derived from this study and the literature with the estimated values from EPI Suite™ version 4.11. The results show that default S_w values at 25 ˚C were underestimated. As a result, the experimental values are more preferable in order to get more accurate assessment.

The results from this study indicate that water conditions such as salinity, pH and temperature have effects on the solubility of BPA in water. Increasing salinity leads to decreasing S_w due to the salting-out effect. In contrast, S_w increases with increasing temperature of water, at least in the studied range, which implies competing salinity and temperature have competing influences. Small changes of S_w were observed at pH less than the pK_a but the S_w drastically increased at pH above pK_a because BPA in the system is in the ionised form, which tends to be more soluble in water because of its polarity. These findings will be useful in the environmental assessment of BPA in the aquatic environment. For example, the S_w of BPA in local waters in Scotland is expected to be lower than S_w in waters in Thailand because an average annual water temperature of waters in Scotland (subtropical zone, average water temperature of 6 – 10 ˚C) is less than waters in Thailand (tropical zone, average water temperature of 27 – 31 ˚C). Thus, BPA may be more widespread and bioavailable in waters in Thailand but less persistent, less volatile, and less able to bioaccumulate.

In addition, the salting-out constant (K_s) from this study will help to estimate the S_w value of BPA at specified salt concentrations. Due to the study of effects of temperature on S_w, the S_w value at any given temperature can be estimated from the equation using the enthalpy of reaction (ΔH°) along with any S_w (such as S_w at 25 ˚C).

More importantly, it is possible to estimate S_w at any site specific just from known salinity, pH and temperature. The measurement of these water conditions is easier and cheaper compared with the measurement of S_w. However, the equation in this study is not suitable for temperatures outside of the range of study (5 – 45 °C) and may not be suitable for temperatures at the low end of the range (*i.e.*, 5 °C).

The S_w is used in the calculation of the air-to-water partitioning coefficient (HLC) in

EPI Suite™. The programme allows users to input their S_w values to get the specific HLC under specified conditions. The results show that the default HLC values are overestimated so the concentration of BPA in water will be higher and the volatility will be lower.

The computation model, EPI Suite™, uses the physicochemical properties of a substance to get data for the environmental assessment. However, the default data retrieved from the programme are not taking environmental conditions into consideration; thus, in order to improve the assessment for real environment, inputting the experimental data is preferable.

The results of this study imply that the direct physiological adverse effects of exposure to BPA may be less severe to marine species than those in freshwater. However, persistence and bioaccumulation would be greater in the marine environment.

In summary, to obtain more accurate data for reliable environmental and risk assessments of chemicals, experimental data are preferred to estimated data. Improved environmental analysis will lead to more appropriate prevention and remediation methods for BPA contamination.

Chapter 5

The Solid Sorption Coefficient of Bisphenol A

5.1 Background

The solid phase (soil and/or sediment) is an important phase in the marine system as the sorption of pollutants to solid phases tends to retard its mobility. The word "sorption" is the generic term of adsorption and absorption processes. Adsorption is the accumulation of a pollutant from solution onto the outer surface of solid particles, while the uptake of a pollutant into the structure of a solid particle is absorption. In a sediment-water system, the important partition coefficients are the sediment-water partition coefficient or sorption distribution coefficient (K_d) and the organic carbon partition coefficient or organic carbon normalised sorption coefficient (K_{oc}), as shown in Figure 5.1. These coefficients indicate the sorption capacity of pollutants on sediment in the aquatic environment and correlate with other physicochemical properties of chemicals such as water solubility and K_{ow}. Also, they are generally used in the prediction of behaviour and migration of organic pollutants in the environment (EPA, 1999).

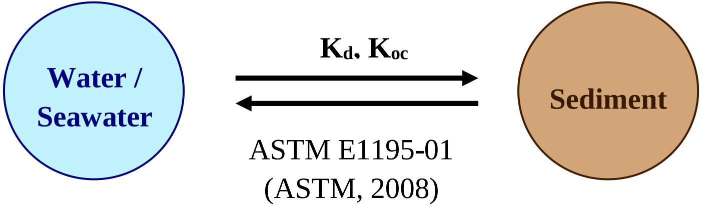


Figure 5.1 The partition coefficient of BPA in sediment-water system

K_d (solid-water partition coefficient or sorption distribution coefficient) is defined as the ratio of the concentrations of chemical in the solid phase (C_s) and water (C_w) at equilibrium at a specified temperature, as shown in equation 5.1 (Mackay, 2001).

$$K_d = \frac{\text{Concentration in the solid phase } (C^s)}{\text{Concentration in the solution phase } (C_w)} \qquad \text{Equation 5.1}$$

The common units of K_d are mg kg^{-1} (ppm), mmol kg^{-1} and mol g^{-1}. K_d depends upon the properties of pollutants, soil/sediment, aqueous phase, and temperature. In general, the sorption of non-polar organic compounds is related to the organic materials of the solid phase such as organic carbon (OC), organic matter (OM). The relationship is such that solid particles with high organic carbon tend to have high K_d, indicating high sorption to the solid phase and low mobility. Despite the assumption that the organic matter of a solid is the main factor influencing the sorption of non-polar organic chemicals, the organic carbon content (f_{oc}) is considered to be the main dependent factor for sorption instead of OM content. Therefore, to reduce the variability of K_d among solids, the K_d can be normalised based on organic carbon content (f_{oc}), known as organic carbon normalised sorption coefficient (K_{oc}), as shown in equation 5.2 (Site, 2001; ASTM, 2008)

$$K_{oc} = \frac{K^d}{f_{oc}} \quad \text{or} \quad K_{oc} = \frac{K^d}{\%OC} \times 100$$

Equation 5.2

Where:

f_{oc} = fraction of organic carbon, $\frac{\%OC}{100}$

%OC = the percentage of organic carbon in solid

The K_{oc} is often the preferred method of assessing solid sorption because it considers organic carbon, unlike K_d. This coefficient is also used in the estimation of K_d for any solids, along with the organic carbon content of that soil or sediment (ASTM, 2008).

Normally, sorption isotherms are used to determine K_d. These are curves related to the concentrations of compounds remaining in the solution and sorbed on solid particles at a fixed temperature. A plot between the equilibrium concentration in solution (C_e) and the amount of compound sorbed per unit mass of sorbent (Q_e), called adsorption or sorption isotherm, is used in the study of the behaviour of sorption. There are two methods to evaluate the sorption isotherm (Do, 1998):

(i) Identified from the shape and curvature of experimentally-derived sorption isotherms; and

(ii) Statistical modelling of sorption isotherm

For experimentally-derived sorption data, there are four particular isotherm shapes that have been observed and used to classify the sorption behaviour of solutes from the aqueous phase (Figure 5.2). The C-type curve is a linear isotherm that generally occurs at low concentrations. It suggests that the ratio of the concentrations of compound sorbed on solid to the concentration remaining in aqueous solution is constant across all concentrations. This ratio is known as the "distribution coefficient" or "partition coefficient" (K_d, L kg^{-1}). The L-type indicates that the solid has a high affinity for the compound. The curve of this isotherm type may or may not reach the maximum sorption capacity of the solid. The S-type has a sigmoid curve. This type indicates that the solid has a high affinity for the solute. The typical case of this isotherm type is non-polar organic compounds. In spite of their low affinity with clays, other organic molecules are sorbed more easily when the clay surface is already covered with those compounds. The H-type is a special type of the L-type isotherm when the initial slope is very high. It is also called a high affinity curve and shows the high affinity of solute to solid (Weber, 1970; Limousin *et al*., 2007).

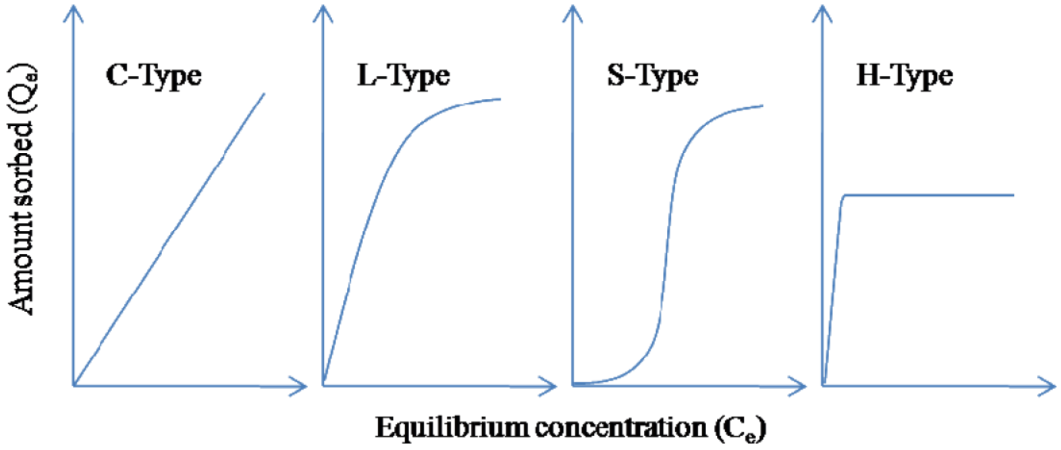


Figure 5.2 The four main types of sorption isotherm identify by shape and curvature (modified from Weber, 1970)

The second method uses statistical modelling of sorption reactions in the study of the sorption processes (Do, 1998; Site, 2001; Tolls, 2001; Limousin *et al*., 2007; *and others*), which is the approach used in this study. The common types of modelling of sorption isotherms are described in the sections that follow.

5.1.1 Freundlich Sorption Isotherms

This sorption isotherm represents increasing sorption with increasing concentration, but decreasing positive slope with increasing concentration in the aqueous phase. Generally, the sorption of many diluted organic and inorganic compounds on irregular surface sorbents and non-homogeneous media such as sediment and clay follow this equation (Do, 1998). The Freundlich equation can be described as:

$$Q_e = \frac{X}{m} = KC_e^{1/n}$$

Equation 5.3

Where:

Q_e = the amount of chemical sorbed per unit mass of

sorbent X = the amount of chemical sorbed m =

the amount of sorbent

K = constant, capacity of the sorbent for the sorbate

C_e = the aqueous concentration of the solute at equilibrium

1/n = constant, measures the strength of sorption, characteristic of system

The equation is linear when "1/n" nears 1. However, taking the log of both sides of equation 5.3 for non-linear isotherms will result in linear isotherms (see equation 5.4).

$$\log Q_e = \log K + \frac{1}{n}\log C_e$$ Equation 5.4

The study of sorption behaviour of BPA on sediment in this study used the Freundlich equation because aqueous concentrations were dilute and the solid media were non-homogenous sediments and other natural materials.

5.1.2 Langmuir Sorption Isotherms

This sorption isotherm originally derived from the sorption study of gases onto solids. The Langmuir isotherm assumes that all sorption sites are the same, has monolayer sorption, and all sites are independent of the sorbed quantity. The Langmuir equation can be described as:

$$Q_e = \frac{X}{m} = \frac{Q_{max} K C_e}{1 + K C_e} \qquad \text{Equation 5.5}$$

Where:

Q_{max} = the maximum amount of chemical sorbed onto a monolayer sorbent

The Langmuir equation suggests that the saturated surface of sorbents and the maximum sorption occur at high concentration (C_e), resulting in a constant X/m value at high concentrations, as shown in Figure 5.3.

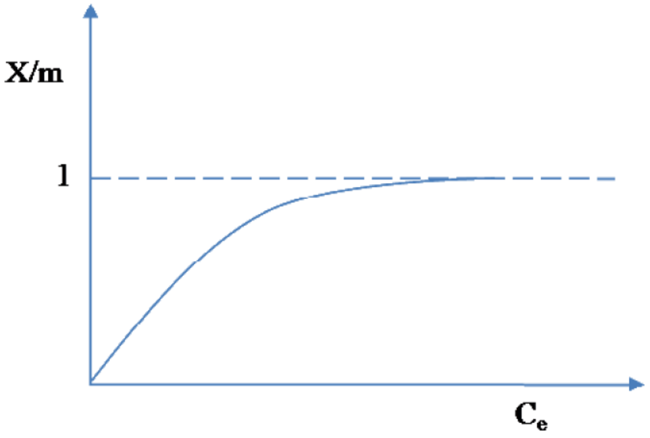


Figure 5.3 The relationship between X/m and C_e following the Langmuir equation (modified from Tan, 1998)

At very low concentrations, the value of $KC_e << 1$ (equation 5.5) so it can be ignored. Thus, the equation 5.6 is obtained. This is the Freundlich equation (see equation 5.3) where $1/n = 1$ and $K_1 = Q_{max}K$.

$$Q_e = \frac{X}{m} = K_1C_e \qquad \text{Equation 5.6}$$

The Langmuir isotherm at low concentration may be nearly linear. As a result, the linear equation of Langmuir isotherm can be obtained, as shown in equation 5.7.

$$\frac{C_e}{Q_e} = \frac{1}{KQ_{max}} + \frac{C_e}{Q_{max}} \qquad \text{Equation 5.7}$$

The distribution coefficient (K) can be obtained from the intercept of linear plotting between C_e/Q_e and C_e, which is $1/KQ_{max}$ (equation 5.7).

5.1.3 Brunauer, Emmett and Teller (BET) Sorption Isotherms

The BET equation involves multilayer sorption and was developed by Brunauer, Emmett and Teller for the adsorption of non-polar gases on multilayers. Then, the equation was revised, called BET-BDDT equation (equation 5.8).

$$\frac{P}{\quad} = \frac{1}{V_mC} + \frac{C-1}{V_mC}\left(\frac{P}{P_0}\right) \qquad \text{Equation 5.8}$$

$$\left[V\left(P_0 - P\right)\right] \qquad V_mC$$

Where:

P = equilibrium vapour pressure

P_0 = saturation vapour pressure

V = volume of gas sorbed

V_m = volume of gas sorbed when solid is covered with a monolayer

C = constant related to heat of sorption

The assumption of this equation is that gas is strongly sorbed on the surface on the first layer, and then the attractions gradually weaken on the second and subsequent layers. However, multilayer sorption does not occur in natural conditions. BET is currently used in surface area measurement. The determination can be carried out when the monolayer capacity and the area of a monolayer are known.

Even though there are many other isotherms, the classical sorption isotherms described above are commonly used. Some classical sorption isotherm models and analytical formulas can be derived as shown in Table 5.1 (Limousin *et al*., 2007).

Table 5.1 Some classical equilibrium sorption isotherms with analytical formulas

Curve Shape	Model	Formula
C-type	Linear	$Q_e = K_dC_e$

L-type or H-type	Freundlich	$Q_e = KC_e^{1/n}$
	Langmuir	$\frac{Q_e}{Q_{max}} = \frac{KC_e}{1+KC_e}$
S-type	Sigmoidal Langmuir	$\frac{Q_e}{Q_{max}} = \frac{KC_e}{1 + KC_e + \frac{s}{c_e}}$

Q = solid concentration; C = aqueous concentration

Source: Limousin *et al*., 2007

5.1.4 Sorption Behaviour of BPA in Environmental Media

Once BPA is released into the marine environment, the BPA in liquid phase can be sorbed onto sediment. Many researchers (Ying *et al*., 2002; Yamamoto *et al*., 2003; Clara *et al*., 2004; Shareef *et al*., 2006; Tian *et al*., 2009; Sen *et al*., 2010) have suggested that the behaviour of BPA in the solid-water system is affected by solid properties, aqueous conditions and physicochemical properties of chemicals. In general, the sorption of pollutants from water to sediment is based on the Freundlich equation (EPA, 1999; ASTM, 2008). Previous studies of BPA sorption on solid phases have used Freundlich sorption isotherms (Clara *et al*., 2004; Tian *et al*., 2009). Therefore, the K_d values in this study were determined by the application of the linear relationship of Freundlich equation to experimental data. Although, a single point K_d value obtained from equation 5.3 has been used and reported, this result has poor accuracy (Langston and Pope, 1995; Keenan *et al*., 2008). In order to improve the correlation coefficient and assess the sorption behaviour of BPA over a wider range of aqueous concentrations, the K_d values in the current study were obtained from the intercept of a log plot of the Freundlich equation (equation 5.4).

This chapter describes how the K_d and K_{oc} of BPA were obtained from the experiments; the effects of sediment properties (*e.g.*, organic carbon content) and water conditions (*e.g.*, salinity, pH and temperature); and predictions of the environmental fate of BPA from the EPI Suite™ modelling program.

5.2 Experimental

In order to understand the solid-water interactions of BPA, the partition coefficients (K_d and K_{oc}) were determined as well as the effects of influencing factors on these partition coefficients.

5.2.1 Sample Collections

Sediments used in this study were collected from various locations in the UK and Thailand. UK samples were collected from the coast of Prestwick in 2011 and 2012 (see Section 3.2.5). Samples were also collected from the Eastern part of Thailand, around Rayong province, near the largest petrochemical industrial park. The samples were collected from Samet Island beach, Kudee Island beach, Rayong beach and Pattaya beach in 2011. Sample locations from Thailand are shown in Figure 5.4. Figure 5.5 shows the air-dried sediment samples (particle size < 2 mm) used in this study.

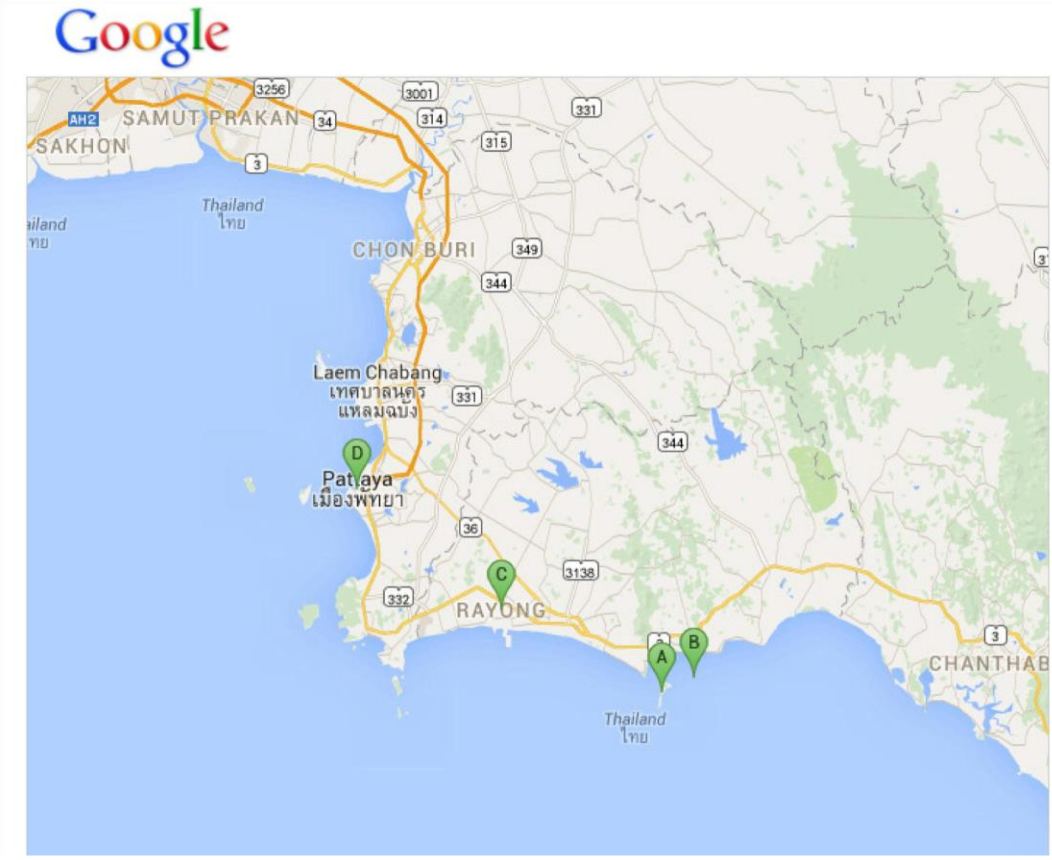


Figure 5.4 Map of sampling sites for sediments in Thailand: Samet Island (A), Kudee Island (B), Rayong beach (C), and Pattaya beach (D) (Google Maps, 2014b)

Figure 5.5 Sediment samples used in this study

Furthermore, biochar, mussel shells and prawn shells were used to examine the influence of other solid properties on the sorption of BPA in the solid-water system. Biochar represented a medium containing high organic carbon content, while mussel shells represented high calcium content medium. Mussel shells were found in sediment samples. Prawn shells were chosen as they had high amount of organic carbon like biochar but they were from living organism. Biochar was made from willow wood by the UK Biochar Research Centre at the University of Edinburgh in 2010. Mussel and prawn shells were bought in Glasgow, UK, and dried in the oven at 105 ± 5 ˚C before grinding.

5.2.2 Sample Characterisations

Firstly, air-dried samples were sieved through a 2 mm sieve to remove the coarser sediment fraction. These samples were then used in the experiments. The sample characterisations were carried out for pH, OM, TOC, XRF, and XRD.

5.2.2.1 Determination of Sediment pH

Sediment pH was determined by American Society for Testing and Materials (ASTM) Standard D4972-01 method (ASTM, 2007a). Sample pH was measured in a sediment slurry with a sediment to water ratio of 1:1 (w/v). Before the measurement, the slurry was stirred for 30 minutes and left for one hour. The measurement of pH was performed using a SevenMulti pH meter (Mettler Toledo, UK).

5.2.2.2 Determination of Organic Matter (OM)

Organic matter (OM) and moisture content of solid samples were determined by the ASTM Standard D2974-07a method (ASTM, 2007b). At first, the samples were weighed and dried in the oven at 105 ± 5 ˚C for at least 16 h, resulting in the removal of the moisture content of sample (equation 5.9). After oven-drying, the samples were weighed and then put into the muffle furnace at temperature 440 ± 22 ˚C over night or until there was no change in mass in the range of 0.01 g (equations 5.10 and 5.11).

$$\text{Moisture Content (\%)} = \frac{(A - B) \times 100}{A} \qquad \text{Equation 5.9}$$

$$\text{Ash Content (\%)} = \frac{C \times 100}{B} \qquad \text{Equation 5.10}$$

$$\text{Organic Matter Content (\%)} = 100 - D \qquad \text{Equation 5.11}$$

Where:

A = mass of as-received sample (g)

B = mass of oven-dried sample (g)

C = mass of ash (g)

D = ash content (%)

5.2.2.3 Determination of Total Organic Carbon (TOC)

Total organic carbon (TOC) analysis was carried out using a TOC analyser – TELEDYNE TEKMAR Model 183 TOC Boat Sampler coupled with Apollo 9000 Combustion TOC Analyser.

5.2.2.4 Determination of Trace Metals by X-Ray Fluorescence (XRF)

Trace metals in samples may affect sorption due to their influence on binding sites and the type of bonding involved. Therefore, X-Ray fluorescence (XRF) was used in the elemental analysis of samples. XRF analysis was performed using a BRUKER model S1 TURBO^{SD} – Hand-held XRF Analysers (Bruker, UK) with 10 minute scan run.

5.2.2.5 Determination of Crystal Lattice Analysis by X-Ray Diffraction (XRD)

The type of clay minerals in sediment samples was determined by X-Ray diffraction (XRD) analysis, which identifies the crystal lattice structure of a sample. XRD analysis was performed using a XRD analyser – PANalytical model X'Pert PRO at the National Metal and Materials Technology Center, Bangkok, Thailand.

5.2.3 Determination of Distribution Coefficients (K_d)

The solid-water partition coefficients (K_d) were determined using the ASTM Standard Test Method E1195-01 (ASTM, 2008). The K_d value was determined as the intercept of log plot of Freundlich equation (between log C_e and log Q_e), as indicated in equation 5.4. According to the standard method, the water used in this study was deionised water (Barnstead Nanopure, Triple Red Limited, UK). The solid sample used was Prestwick sediment. To establish the sorption behaviour, the first experiment was performed to obtain the equilibration time of the sorption interactions. To establish the equilibrium time, the appropriate water to solid ratio used was estimated to achieve chemical sorption efficiency between 20 – 80%, as described in the standard method (ASTM, 2008). Because sediments from Prestwick had OC in the range of 0.15 – 0.16%, the water to solid ratio was 2:1. Previous studies indicated that BPA sorption by marine sediments would reach equilibrium approximately 3 h as a guide (Tian *et al*., 2009); therefore, the equilibration time experiments were performed by varying the sorption time from 5 min to 24 h (5 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 8 h and 24 h). The equilibrium time determined was then set for subsequent experiments.

20 mL glass universal bottles were used as the reaction vessels. The BPA concentrations in water used to determine K_d were 2, 5, 10, 20 and 40 mg L^{-1}. Solid sample and water-containing BPA were put together at 2:1 water to solid ratio, and then mixed on a horizontal shaker until reaching equilibrium. The vessels were centrifuged at 3,800 rpm for 10 min. The supernatant was filtered using 0.45 µm filter (Sigma-Aldrich, UK), and then injected into the HPLC-UV to analyse the concentrations of BPA remaining in water. Conditions of HPLC were as described in Section 3.2.2. Vessels containing sediment and non-spiked BPA deionised water were also carried out at the same time using the same conditions, representing blank samples. This is to reduce the error in case the solid samples were already contaminated with BPA.

5.2.4 Determination of Organic Carbon Partition Coefficient (K_{oc})

The organic carbon partition coefficient (K_{oc}) or the organic carbon normalised sorption coefficient was calculated from the experimental K_d value using equation 5.2. Furthermore, K_{oc} values were provided from KOCWIN™ version 2.00 in EPI Suite™ version 4.11. The K_{oc} values were obtained from Molecular Connectivity Index (MCI) regression methodology and log K_{ow} regression methodology. These regression equations were obtained from 447 non-polar compounds training set. The methodology to estimate the MCI-based K_{oc} and the K_{ow}-based K_{oc} for BPA are shown as equation 5.12 and 5.13, respectively (EPA, 2012b).

$$\text{Log } K_{oc} = 0.5213\ \text{MCI} + 0.60 + \Sigma P_f N \qquad \text{Equation 5.12}$$

$$(n = 447, R^2 = 0.9, SD = 3.4)$$

$$\text{Log } K_{oc} = 0.55313 \log K_{ow} + 0.9251 + \Sigma P_f N \qquad \text{Equation 5.13}$$

$$(n = 447, R^2 = 0.855, SD = 0.396)$$

Where:

MCI = Molecular Connectivity Index

$\sum P_f N$ = the summation of the products of all applicable correction factor coefficients ($\sum P_f$, see Appendix B.2 and Appendix B.3 for MCI and log K_{ow} method, respectively) multiplied by the number of times (N) that factor is counted for the structure

5.2.5 Study of Effects of Environmental Parameters on Sorption of BPA

Improved understanding of the major influence parameters on BPA sorption behaviour and fate in the marine environment leads to more efficient modelling and remediation processes. The parameters studied were solid type (*e.g.*, organic carbon (OC)) and water conditions (*e.g.*, salinity, pH and temperature). To compare variations in solid type, Prestwick sediment, Rayong sediment, Pattaya sediment, Samet Island sediment, Kudee Island sediment, biochar, mussel shells and prawn shells were used. The water to solid ratios used were 2:1 for sediments from Prestwick, Rayong, Pattaya, Samet Island and Kudee Island, whereas 20:1 for mussel shells, and 100:1 for biochar and prawn shells. To ensure that the reactions reach equilibrium, the vessels were mixed for 24 h. The study on water conditions used Prestwick sediments and the water-solid ratio was 2:1. The salinities were 0.5, 10, 35 and 50 psu to cover the range of estuarine water to seawater. KCl (Fisher Scientific, UK) was used to adjust the salinity of water as it is stated in the standard method (APHA, 1999). The effect of pH was studied by setting the pH to 4, 7, 8, 9 and 12, ranging from acidic to alkaline conditions. The 0.1 M HCl (Fisher Scientific, UK) and 0.1 M NaOH (Fisher Scientific, UK) were used to adjusted pH of water (Burton *et al.*, 2004). To cover seawaters in subtropical zone to tropical zone, the temperatures studied were 5, 25, 35 and 45 ˚C, which was maintained in an incubator shaker (Stuart® orbital incubator SI500, Bibby Scientific Limited, UK).

5.2.6 Prediction of Environmental Fate from K_d

In general, K_{oc} is required to run the fugacity model in EPI Suite™. The fugacity model predicts the mass amount of an organic compound in each environmental compartment (air, soil, sediment, and water). There are three options for K_{oc} used in the fugacity model as follows (see Section 6.2.2.3) (EPA, 2012b).

(i) Estimation using log K_{ow} regression in KOCWIN™

(ii) Estimation using MCI regression in KOCWIN™

(iii) User-entered value

The MCI-based K_{oc} is selected as a default value for the fugacity model due to being slightly more accurate overall than the K_{ow}-based K_{oc} (EPA, 2012b). This study compared the default results from the fugacity model and the results obtained from inputting experimental K_{oc} values into the fugacity model in order to create a more accurate predicted environmental partitioning.

5.3 Results and Discussion

5.3.1 Characterisation of Solids

The properties of solid samples (sediments, biochar, mussel shells and prawn shells) used in this study are shown in Table 5.2. The most important factor is organic carbon content as it is used to determine K_{oc}.

Table 5.2 Properties of collected sediments and other solids used in this study

Sample	pH	OM (%)	TOC (%)	SiO_2 (ppm)	Ca (ppm)
Prestwick (2011)	8.2	0.68	0.15	284000	8333
Prestwick (2012)	8.2	0.73	0.16	306250	11500
Rayong	8.1	0.28	0.03	314000	13600
Pattaya	8.5	0.18	0.05	359250	5080
Samet Island	8.8	1.19	0.09	176200	366800
Kudee Island	8.8	1.99	1.34	24000	878667
Biochar	6.3	62.46	15.23	24000	5193
Mussel shells	9.3	3.44	2.03	N/A	N/A
Prawn shells	8.4	57.00	21.27	N/A	N/A

SiO_2 (silica oxide) and Ca (calcium) from XRF; N/A = No data

Table 5.2 shows that pH of marine samples were > 8, while biochar had pH = 6.3. For marine samples, mussel shell sample had highest pH (9.3). Prawn shells had highest TOC. The correlations between TOC and OM retrieved are shown in Figure 5.6.

Since most of the data were compressed at the low end and two data (biochar and prawn shells) were at the high end, the log-log graph (Figure 5.6) was used to represent these relationships with more even spacing of the data points. The results show a positive relationship between log OC and log OM with $R^2 = 0.946$ and $n = 9$. This correlation showed that samples in this study with high organic matter content tended to have high organic carbon content. The XRF results indicated that silica (SiO_2) and calcium (Ca) were the main elements in the samples, while aluminum (Al) and potassium (K) were not detected. The mussel shells and prawn shells were not analysed. The results indicated that most of samples had Si > Ca; however, samples from islands (Samet and Kudee) showed the opposite trend, which may be due to the mussel shells present in those samples.

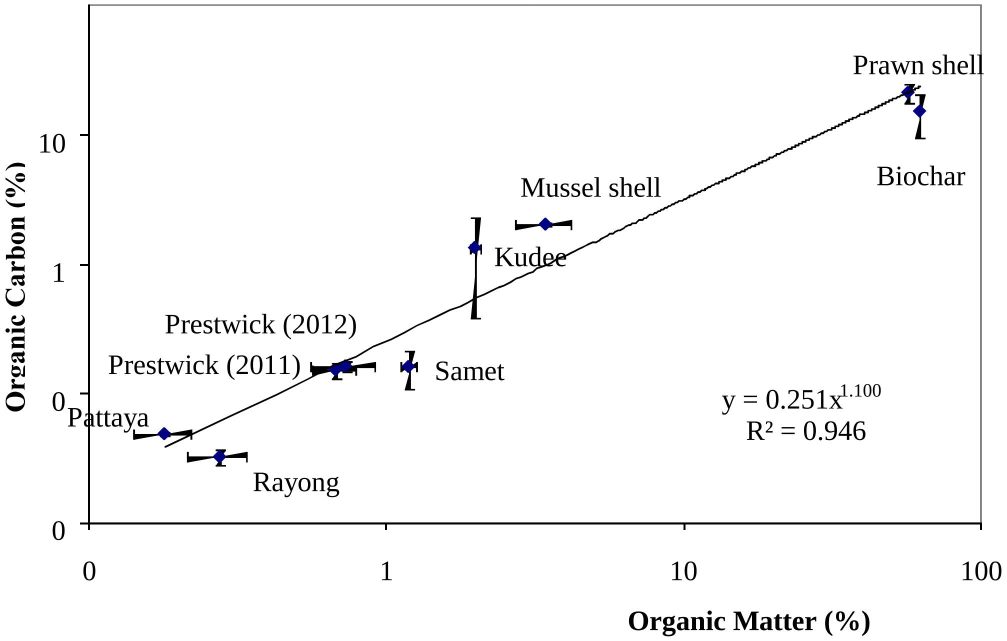


Figure 5.6 Log-log plot between organic carbon (%) and organic matter (%) (Error bars indicate 95% Confidence Intervals, n = 3)

XRD was also used to identify clay minerals in samples. All XRD spectra are shown in Appendix C.1. By taking XRF results into the consideration, a summary of the XRD results is presented in Table 5.3.

Table 5.3 Identification of minerals in samples from XRD analysis

Sample	Minerals
Prestwick (2011)	Quartz (SiO_2)
Prestwick (2012)	Quartz (SiO_2)
Rayong	Quartz (SiO_2)
Pattaya	Quartz (SiO_2)
Samet Island	Quartz (SiO_2), Aragonite ($CaCO_3$) and Calcite ($CaCO_3$)
Kudee Island	Aragonite ($CaCO_3$), Quartz (SiO_2), Calcite ($CaCO_3$) and Wollastonite ($CaSiO_3$)
Biochar	Amorphous
Mussel shells	Calcite ($CaCO_3$), Aragonite ($CaCO_3$)
Prawn shells	Calcite, magnesium (Mg 0.06 Ca 0.94)(CO_3), Lanthanum Oxide (La_2O_3)

The results (Table 5.3) show quartz was the most abundant mineral identified in Prestwick samples (both 2011 and 2012), Rayong, and Pattaya. While biochar could not be identified as the spectrum indicated an amorphous, or non-crystalline, structure. On the other hand, the spectrum of sediments from islands and marine organism shells represent $CaCO_3$ in samples.

5.3.2 Determination of Distribution Coefficients (K_d) and Organic Carbon Normalised Sorption Coefficient (K_{oc})

5.3.2.1 Selection of Water to Solid Ratio

Sorption experiments were mainly performed using Prestwick sediments. In this experiment, the water to solid (L:S) ratio is an important factor. Subsequently, K_d values were estimated from reported K_{oc} values and the fraction of organic carbon (f_{oc}) before starting the experiments. The K_{oc} values of BPA were reported as 708 (log K_{oc} = 2.85) (Staples *et al.*, 1998) and 1,524 (Howard, 1989). The experimental organic carbon (%OC) of Prestwick sediment sample was 0.15%. According to equation 5.2, estimation of K_d was completed by multiplying the K_{oc} by the %OC divided by 100, as shown in equation 5.14.

$$K_d = \frac{K_{oc} \times \%OC}{100} \qquad \text{Equation 5.14}$$

The estimated K_d values calculated by equation 5.14 were 1.06 and 2.29. Appropriate water to sediment ratios were selected based on relationships between percent sorbed and K_d (Figure 5.7) or between the water to sediment ratio and K_d (Figure 5.8).

The appropriate water to sediment ratio was chosen as 2:1 as it covered 20% to 80% sorption range for K_d values of 1 to 20, approximately. This ratio was used in the following experiments, which used Prestwick sediments. Using the same method, the water-solid ratios for Rayong, Pattaya, Samet Island and Kudee Island were 2:1. Whereas mussel shell sample tests were performed at 20:1, and the ratio of 100:1 was used for high OC samples (*e.g.*, biochar and prawn shells).

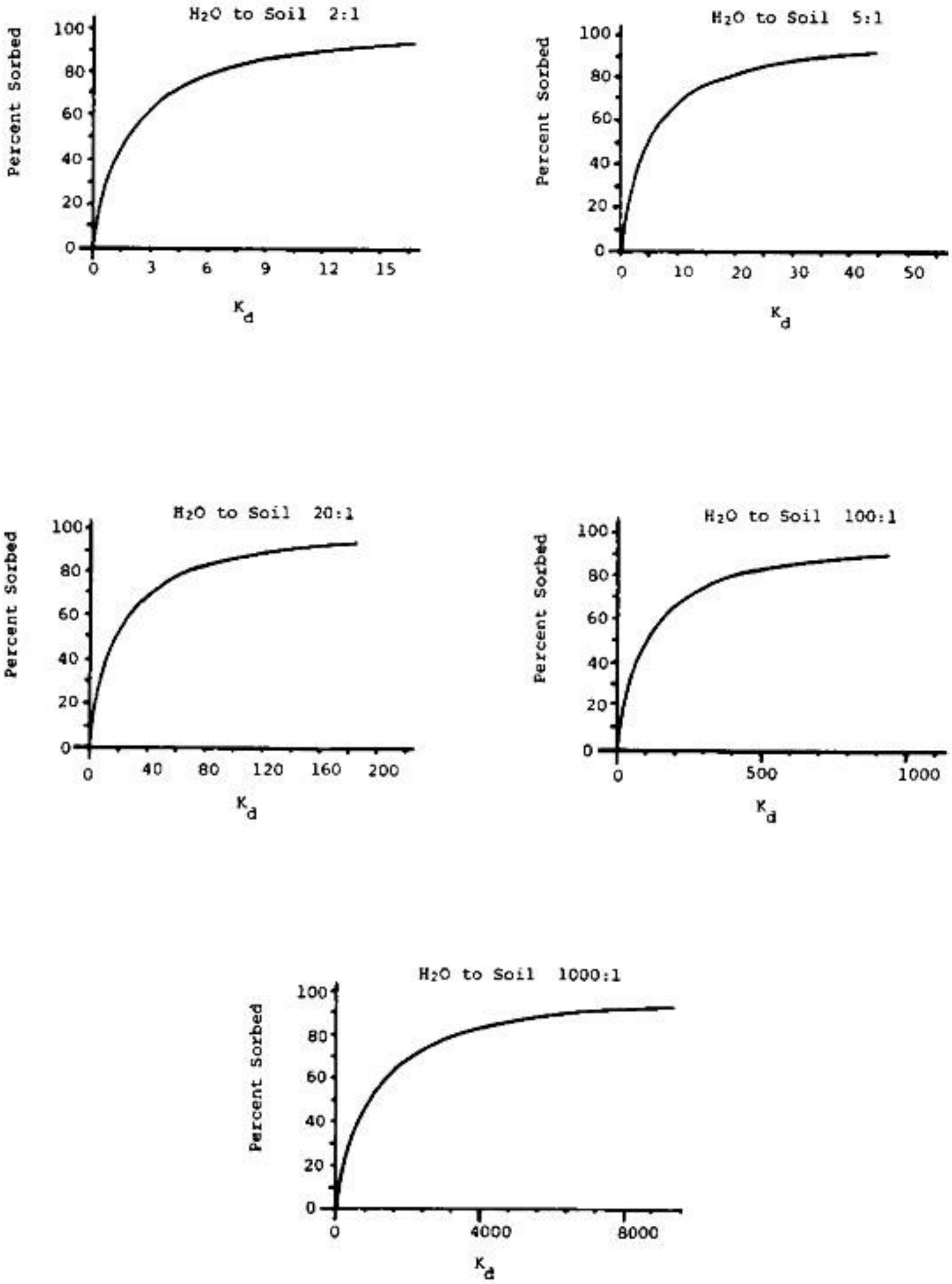


Figure 5.7 Relationship between percentage sorbed and K_d at various ratios of water to soil solids (reproduced from ASTM, 2008)

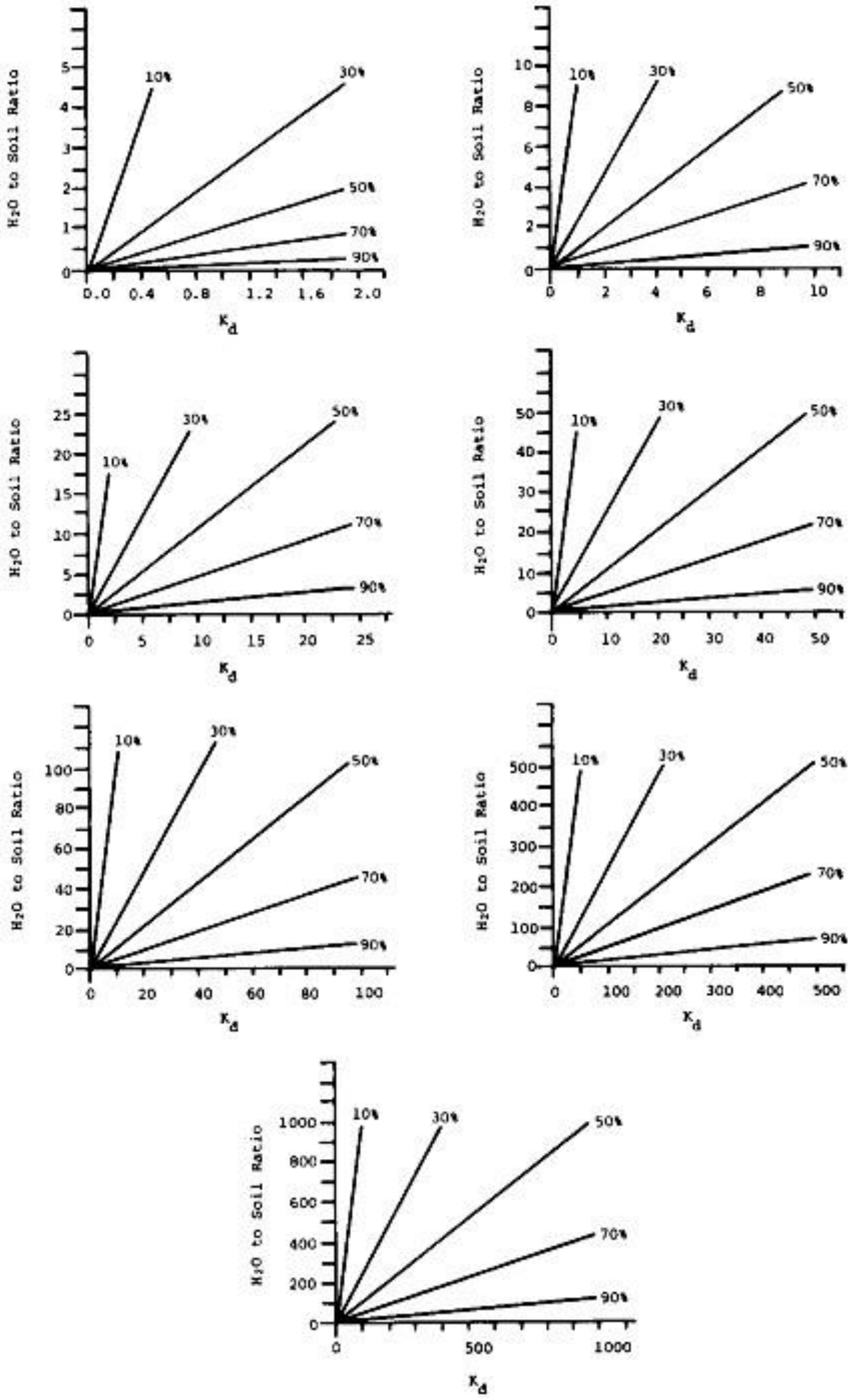


Figure 5.8 Relationship between water to soil ratios and K_d at various percentages of sorbed material (reproduced from ASTM, 2008)

5.3.2.2 Equilibrium Study

To determine K_d, the sediment/solution mixture should be retained for the equilibrium time. The water-sediment ratios used were stated in the previous section (Section 5.3.2.1). The concentration of BPA in water used in this experiment was 20 mg L^{-1}. The time scales were varied from 15 min to 24 h at 25 °C. The equilibrium study was performed on sediments from Prestwick (collected in 2011), Pattaya, Rayong, and Samet Island. The results are represented in Figure 5.9.

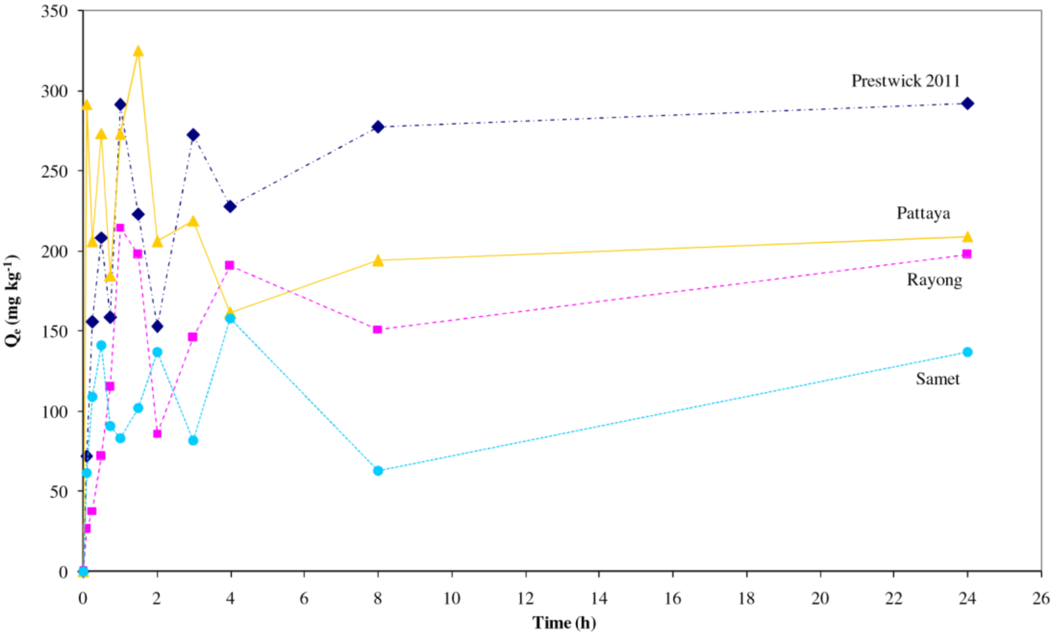


Figure 5.9 Equilibrium graph of BPA sorption onto different sediment samples

The results show that the sorptions of BPA onto these four sediments (Prestwick 2011, Pattaya, Rayong, and Samet Island) were almost steady after 2 h. These are in good agreement with the studies of Clara *et al*. (2004) and Tian *et al*. (2009) that indicated that sorption equilibrium of BPA onto sewage sludge and sediment occurred within 3 h. Therefore, the sorption experiments were performed by fixing the shaking time at 6 h for all sediments sample (including Kudee Island sample) to complete the steady state on the sorption. However, the sorption time was set at 24 h for other solid samples (biochar, mussel shell and prawn shell) to ensure that the partitioning reached equilibrium.

5.3.2.3 Determination of Sorption Coefficients (K_d and K_{oc})

The K_d values were determined using a linear form of Freundlich isotherm (equation 5.4). The experiments were conducted on marine sediments from different sources, shells from marine organisms, and biochar. Log K_d values at 25 °C were obtained from the intercepts of the log plot between Q_e (amount sorbed onto the solid) and C_e (amount remaining in aqueous solution) as shown in Figure 5.10 and Table 5.4.

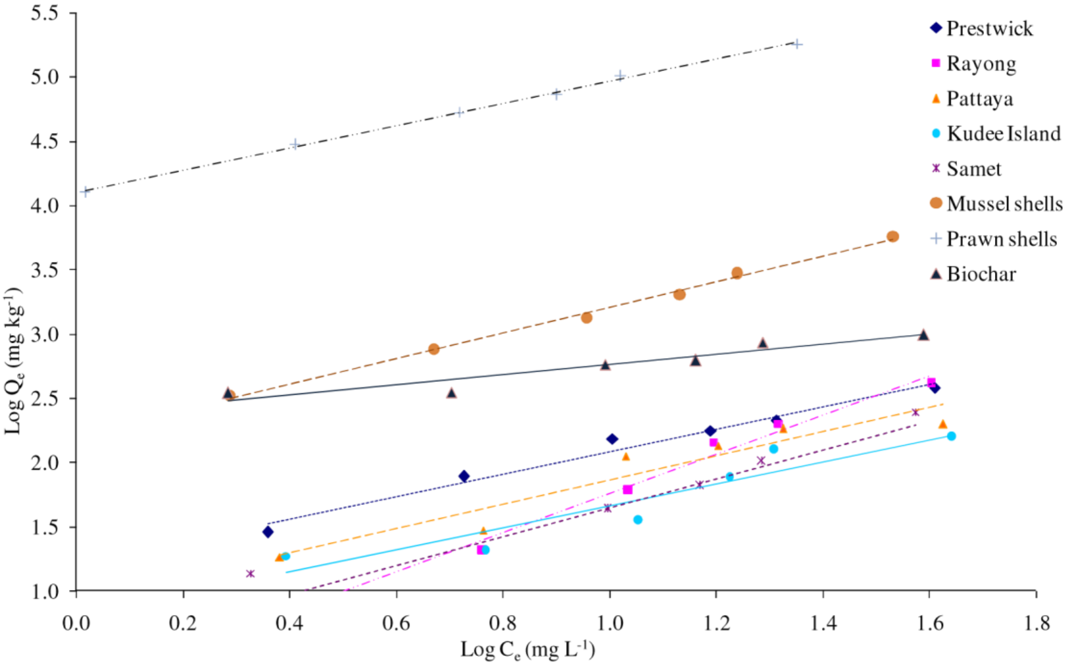


Figure 5.10 Freundlich sorption isotherm of BPA on various solid samples

Table 5.4 Freundlich sorption isotherms of BPA on various solid samples

Sample	Freundlich Sorption Isotherm	R^2
Prestwick	y = 0.876x + 1.212	0.977
Rayong	y = 1.520x + 0.245	0.988
Pattaya	y = 0.946x + 0.919	0.903
Samet Island	y = 1.128x + 0.523	0.856
Kudee Island	y = 0.853x + 0.815	0.880
Biochar	y = 0.390x + 2.371	0.903
Mussel shells	y = 0.993x + 2.214	0.995
Prawn shells	y = 0.862x + 4.101	0.998

The K_d value for each sample is shown in Table 5.5. Moreover, the K_{oc} values were calculated using equation 5.2 along with organic carbon (OC) content in each sample, whereas, organic matter (OM) content was used in equation 5.2 instead of OC in order to calculate K_{om}. The results are represented in Table 5.5. Furthermore, the K_{oc} values from literature and EPI Suite™ are also represented in Table 5.5. EPI Suite™ provides two K_{oc} values (MCI-based and K_{ow}-based). The calculations of these values are shown as follows.

MCI-based K_{oc} value was calculated by equation 5.12:

MCI for BPA = 7.998

Correction factor for aromatic hydroxyl (Appendix B.2) = -0.09661

Number of aromatic-OH in BPA structure = 2

$$\text{Thus, Log } K_{oc} = (0.5213 \times 7.998) + 0.60 + (-0.09661 \times 2) \quad \text{Equation 5.15}$$

$\text{Log } K_{oc} = 4.58$

K_{ow}-based K_{oc} value was calculated by equation 5.13

Log K_{ow} used = 3.32

Correction factor for aromatic hydroxyl (Appendix B.3) = 0.166847

Number of aromatic-OH in BPA structure = 2

$$\text{Thus, Log } K_{oc} = (0.55313 \times 3.32) + 0.9251 + (0.166847 \times 2) \quad \text{Equation 5.16}$$

$\text{Log } K_{oc} = 3.10$

Table 5.5 Experimental sorption coefficients (K_d and K_{oc}) of BPA on solid samples

Sample	Log K_d	K_d	Log K_{oc}	Log K_{om}
Prestwick (2011)	1.21	16.29	4.04	3.38
Rayong	0.24	1.76	3.74	2.80
Pattaya	0.92	8.30	4.22	3.66
Samet Island	0.52	3.34	3.56	2.45
Kudee Island	0.81	6.53	2.69	2.52
Biochar	2.37	235.34	3.19	2.58
Mussel shells	2.21	163.83	3.91	3.68
Prawn shells	4.10	12641.54	4.77	4.35
Average ± SD (n = 8)			3.76 ± 0.64	3.18 ± 0.69
MCI method (EPI Suite™ - Default)			4.58	N/A

K_{ow} method (EPI Suite™)	3.10	N/A
Literature (Howard, 1989)	3.18	N/A
Literature (Staples *et al*., 1998)	2.85	N/A

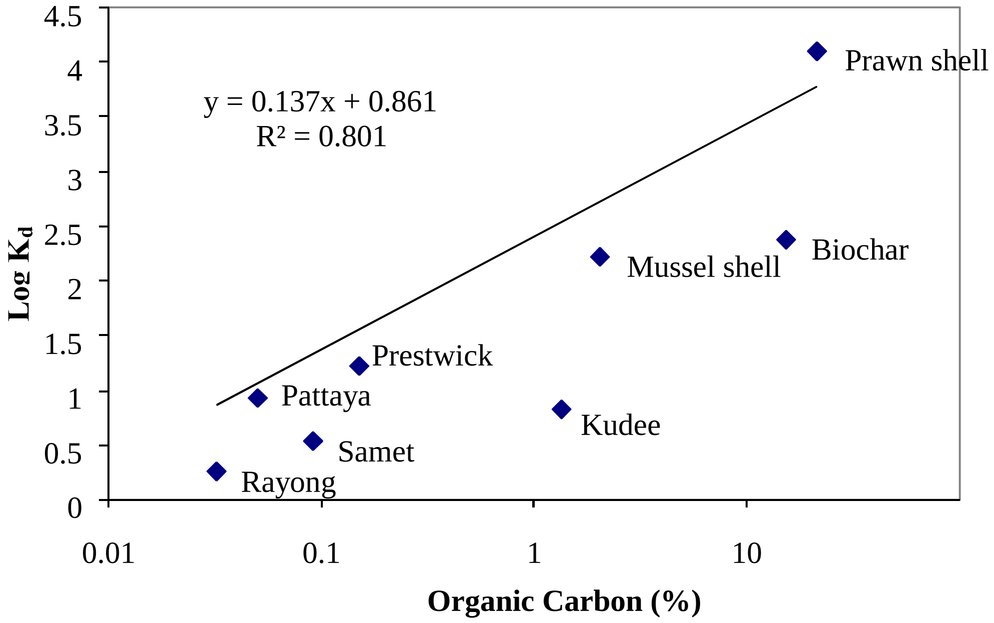
OC = organic carbon; OM = organic matter; N/A = No data

The experimental log K_{oc} and K_{om} ± SD (n = 8) were 3.76 ± 0.64 and 3.18 ± 0.69, respectively. The differences between each sample indicate that the sorption behaviour of BPA onto solids may not only be affected by OC and/or OM, but also by other factors such as clay minerals and sorption forces (Tan, 1998; Tian *et al*., 2009). The comparison between the average experimental K_{oc} value from this study and the values from other sources (model and literature) shows that most of the experimental log K_{oc} values were in the range of log K_{oc} from other source (log K_{oc} range of 2.85 – 4.58).

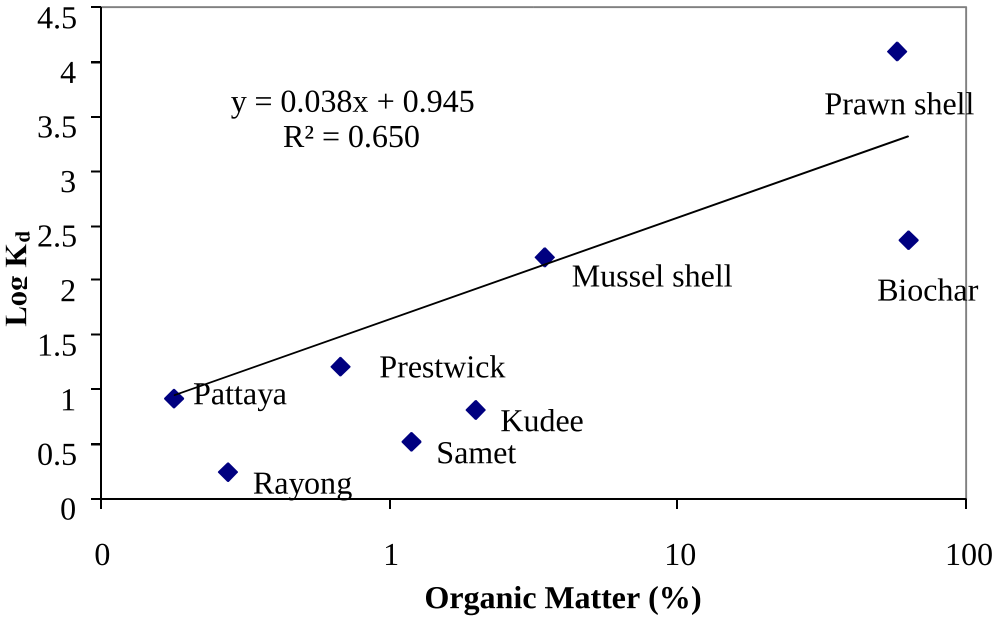
5.3.3 Effect of Solid Properties on Sorption of BPA

This sorption determination assumes that OC in solids is the main factor influencing the sorption of non-polar organic compounds (ASTM, 2008). Thus, the log K_d – OC relationship and log K_d – OM relationship are shown in Figure 5.11.

The results (Figure 5.11) indicate that the highest OC or OM content tend to have a larger sorption of BPA onto solid samples. Sorptive processes of BPA likely depend on OC than that on OM, with the R^2 approximately 0.8 for log K_d-OC and 0.65 for log K_d-OM (n = 8). However, K_d was not always directly proportional to OC or OM in all media; for example, Kudee Island sediment had higher OC and OM but lower K_d than that in Prestwick sediment, which may be due to the influence of clay mineral surfaces in both sediments (Tian *et al*., 2009). Therefore, the minerals in the samples were identified using XRD and also the metal elements were measured by XRF. The results from the XRF and XRD are shown in Table 5.2 and Table 5.3, respectively (see Section 5.3.1).



(a)



(b)

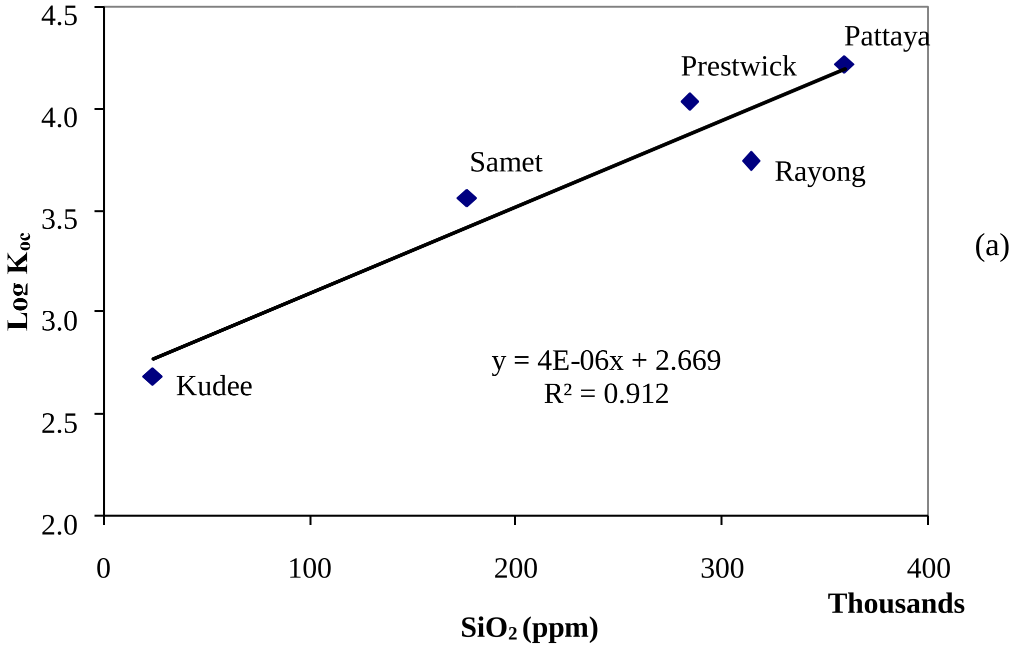
Figure 5.11 Correlations between log K_d and: (a) organic carbon (%OC), and (b) organic matter (%OM) in samples

To determine the relationship between sorption capacity and clay minerals, the K_{oc} was used instead of K_d in order to reduce the effect from OC. The solid samples in this analysis were restricted to the sediment samples. Clay minerals were not expected to be present in biochar due to its source material and amorphous morphology (and were not identified by XRD). Mussel shell and prawn shell samples were not analysed. The results indicate that samples containing quartz (sediments from Prestwick, Pattaya and Rayong) tend to have a higher sorption capacity than samples containing quartz and $CaCO_3$ (sediments from Samet Island and Kudee Island). It was observed that Pattaya sediment (highest amount of SiO_2 but lowest amount of Ca and lowest OC content) had the highest K_{oc}, whereas, Kudee Island sediment (lowest amount of SiO_2 but highest amount of Ca and highest OC content) had the lowest K_{oc} value (see Table 5.6).

Table 5.6 Comparison of K_{oc} values and sediment properties

Sediment Sample	Organic Carbon	SiO_2	Calcium	K_{oc}
Pattaya	Lowest	Highest	Lowest	Highest
Kudee Island	Highest	Lowest	Highest	Lowest

Subsequently, correlations between main metals in samples (silica (SiO_2) and calcium (Ca)) and log K_{oc} were determined in accordance with the results from XRF and XRD, as shown in Figure 5.12.



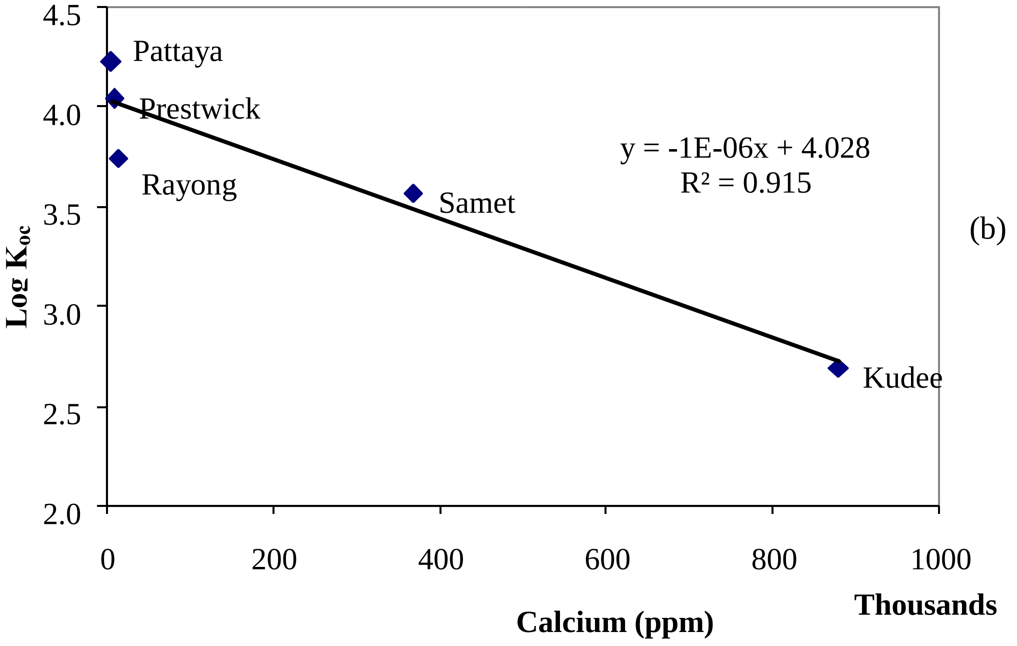


Figure 5.12 Correlations between log K_{oc} and: (a) silica (SiO_2), and (b) calcium (Ca) in samples

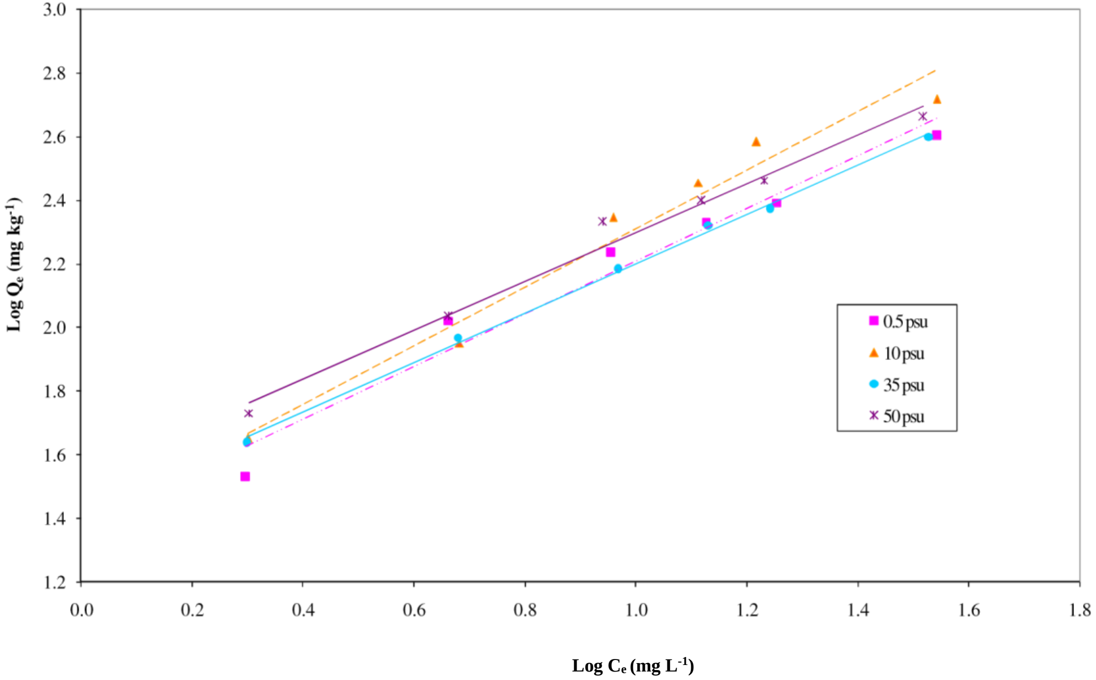
Correlations between log K_{oc} and minerals show that increasing SiO_2 was more likely to increase log K_{oc} ($R^2 = 0.91$, n = 5). On the contrary, the correlations between log K_{oc} and calcium were log K_{oc} decreasing with increasing calcium ($R^2 = 0.91$, n = 5). The results show that the sorption process of BPA was not only influenced by OC but also minerals present in sediment. Oxygen molecule of the silica (SiO_2) on the surfaces can bond with the hydroxyl groups in BPA by hydrogen bonding. In contrast, calcium has a negative effect on the sorption. This may be because calcium easily forms hydration complexes, resulting in a decrease in the available sorption surfaces (Site, 2001). It can be concluded that the sorption of BPA on solid surfaces depends upon the properties of the solid materials such as organic content, type of solids and mineral composition of the sample.

5.3.4 Effect of Salinity on Solid Sorption of BPA

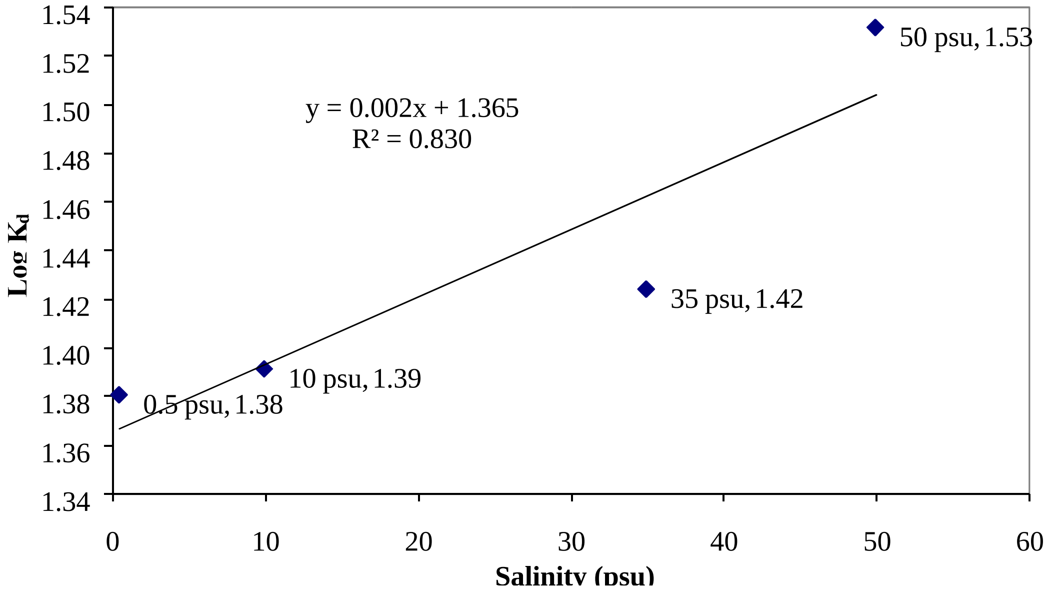
Previous studies found that salinity of water has an effect on the sorption of organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and tributyltin (TBT) (Tremblay *et al*., 2005; Bangkedphol *et al*., 2009). Thus, the effect of salinity on sorption coefficient of BPA was studied. Also, the Setschenow constant, which is the salting coefficient (as described in Section 4.3.4.2), was estimated.

5.3.4.1 Determination of Sorption Coefficients at Various Salinities

The effect of salinity on the sorption coefficient (K_d) was studied using the Prestwick sediment (collected in 2012) and deionised water with salinity adjusted within the range of 0.5 psu to 50 psu. The results are shown in Figure 5.13 and Table 5.7.



(a)



(b)

Figure 5.13 (a) Freundlich sorption isotherms of BPA on Prestwick sediment at various salinities of water, and (b) correlation between log K_d and salinity of water

Table 5.7 Freundlich sorption isotherms and log K_d of BPA on Prestwick sediment at various salinities of water

Salinity (psu)	Freundlich Sorption Isotherm	R^2	Log K_d
0.5	y = 0.826x + 1.380	0.962	1.38
10	y = 0.921x + 1.391	0.969	1.39
35	y = 0.776x + 1.424	0.997	1.42
50	y = 0.767x + 1.531	0.985	1.53

The results show that the sorption of BPA is likely to increase with increasing salinity of water (R^2 = 0.83, n = 4). The sorption from aqueous phase onto sediment is generally associated with S_w of chemicals. The findings in the sorption study agree with the results from Chapter 4 that showed higher salinity of water resulted in lower S_w. This effect is referred to as the salting-out effect (Xie *et al.*, 1997). Based on these results, marine sediments are expected to have higher sorption capacities than freshwater sediments and benthic organisms in the marine environment may be vulnerable to greater BPA exposure than benthic organism in freshwater environments, potentially resulting in more bioaccumulation.

5.3.4.2 Calculation of Salting Coefficients of Sorption Coefficient of BPA

As described in Chapters 3 and 4, the salting coefficient of K_{oc} (k_{oc}) was also determined. The K_{oc} values were calculated using K_d values in Section 5.3.4.1 along with %OC of Prestwick sediment (collected in 2012), which was 0.16%. Because of the similar inverse relationships of K_{ow} and K_{oc} with S_w, the equation used to determine the salting coefficient of K_{oc} was modified from the equation used for K_{ow} (equation 3.11), as shown in equation 5.17.

$$\mathrm{Log}^{??}\,\rule{2em}{0.4pt}K^{oc\ ?}_{?} = k_{oc}C_s \qquad \text{Equation 5.17}$$

$\Big({}_{oc,0} \Big)$

Where:

K_{oc} = the sorption coefficient in aqueous salt solution

$K_{oc,0}$ = the sorption coefficient in pure water

k_{oc} = the salting coefficient or Setschenow constant (M^{-1} or L mol^{-1})

C_s = the molar concentration of salt (M or mol L^{-1})

$\Big(K \quad \Big)$

According to equation 5.17, the salting coefficient (k_{oc}) was obtained from the slope of correlation graph plotted between $\text{Log}\left(\frac{K_{oc}}{K_{oc,0}}\right)$ and salt concentration (C_s, mol L^{-1}). The results are shown in Figure 5.14.

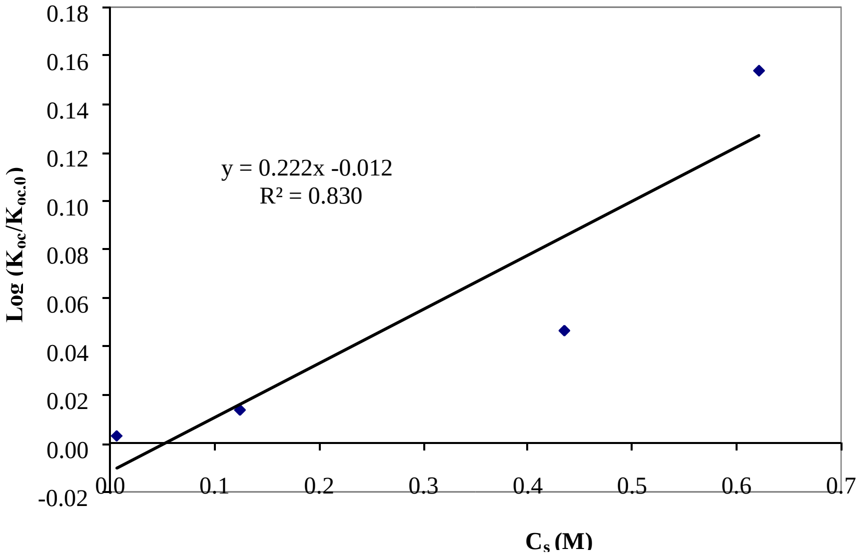


Figure 5.14 Salting effect on K_{oc} of BPA

The k_{oc} obtained was 0.222 M^{-1}. The positive k_{oc} indicates the salting-out effect of KCl on the K_{oc} of BPA on sediment. The correlation equation between K_{oc} and salt concentration is shown in Box 5.1.

Box 5.1 Correlation equation between K_{oc} and salt concentration

$$\text{Log}\left(\frac{K_{oc}}{K_{oc,0}}\right) = 0.222*C_s - 0.012, \quad R^2 = 0.83 \qquad \text{Equation 5.18}$$

Equation 5.18 can be used to predict K_{oc} at specified salt concentration (C_s) at 25 °C by measuring only the salt concentration in solution. Under the same conditions, the K_d for a specific medium can be predicted if the organic carbon content is known.

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5.3.5 Effect of pH on Solid Sorption of BPA

The pH of water can control the partitioning of BPA between the solid phase and the aqueous phase. Previous chapters show that the species of BPA depends upon the pH of the water. In a sediment-water system, the pH also affects the surface properties of sediment (EPA, 1999). The pH-dependent sorption coefficient was carried out on the Prestwick sediment (collected in 2011) with deionised water. The range of pH of water studied was 4 – 12 which covers acidic and alkaline conditions. The plot between concentration of BPA in sediment (Q_e) and in water (C_e) is shown in Figure 5.15.

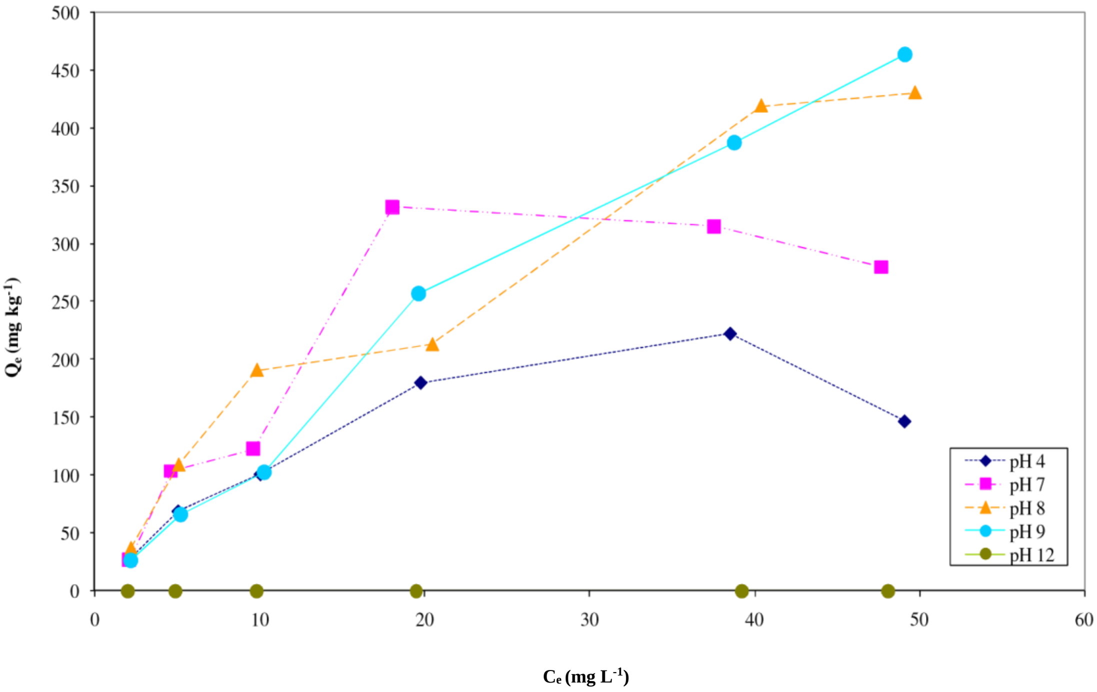
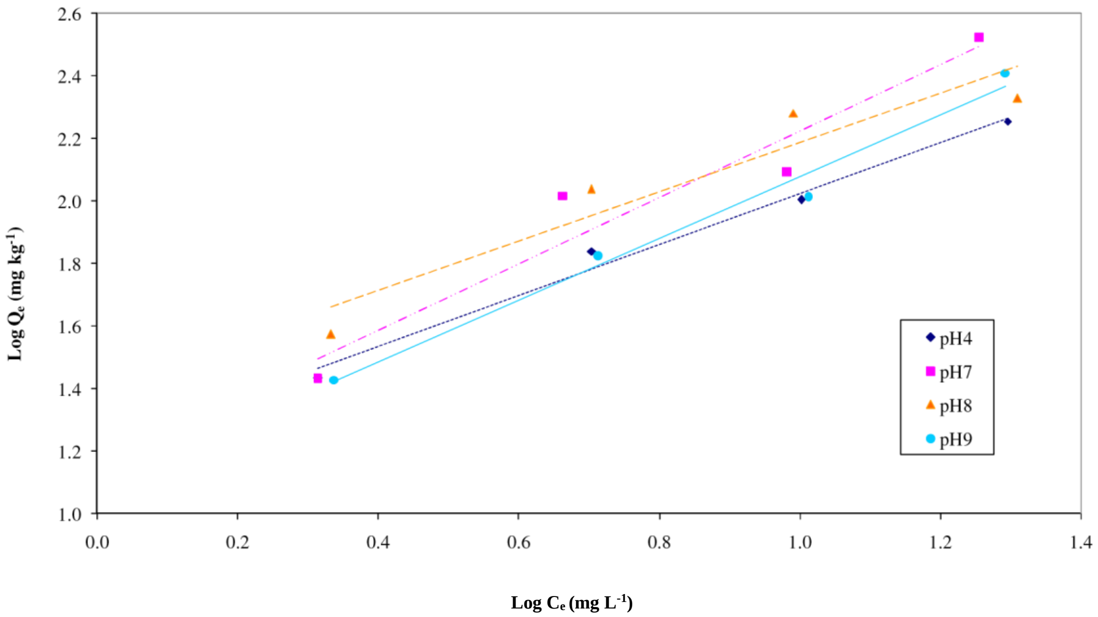
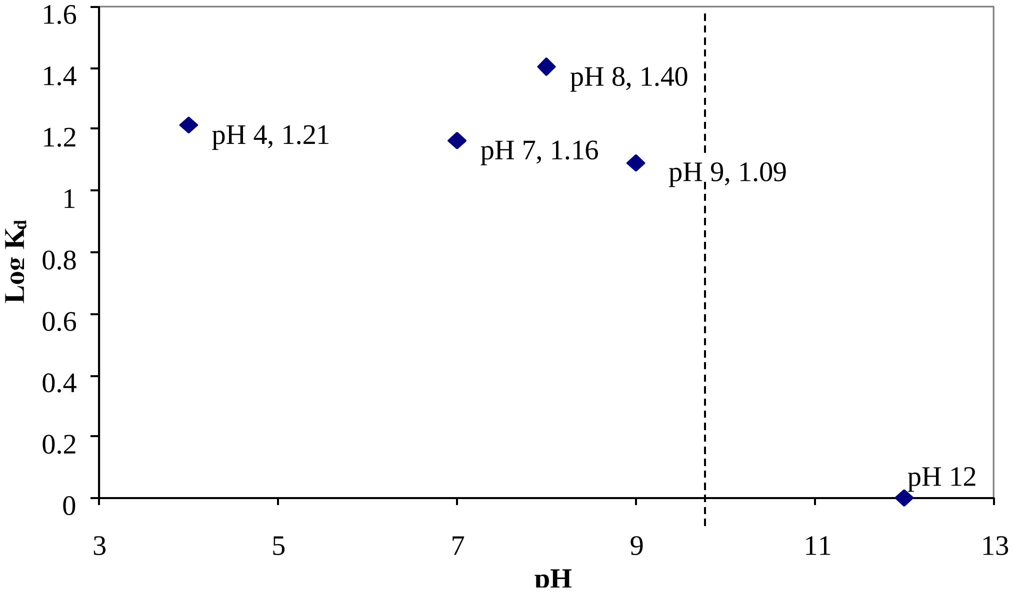


Figure 5.15 Relationship between concentration of BPA in sediment (Q_e) and in water (C_e) at various pH

Figure 5.16 indicates no sorption at pH 12. It also shows that a BPA concentration of 20 mg L^{-1} was the limit of the linear range for pH of water at 4, 7 and 8. The K_d values in the pH study were obtained from BPA concentration over the range of 2 to 20 mg L^{-1}. The K_d values these pH values are shown in Figure 5.16 and Table 5.8.



(a)



(b)

Figure 5.16 (a) Freundlich sorption isotherms of BPA on Prestwick sediment at various pH of water, and (b) relationship between log K_d and pH of water

(**Table 5.8** Freundlich sorption isotherms and log K_d of BPA on Prestwick sediment

at various pH of water

pH	Freundlich Sorption Isotherm	R^2	Log K_d
4	$y = 0.814x + 1.210$	0.987	1.21
7	$y = 1.196x + 1.069$	0.802	1.07
8	$y = 0.787x + 1.400$	0.900	1.40
9	$y = 0.987x + 1.090$	0.983	1.09
12	-	-	-

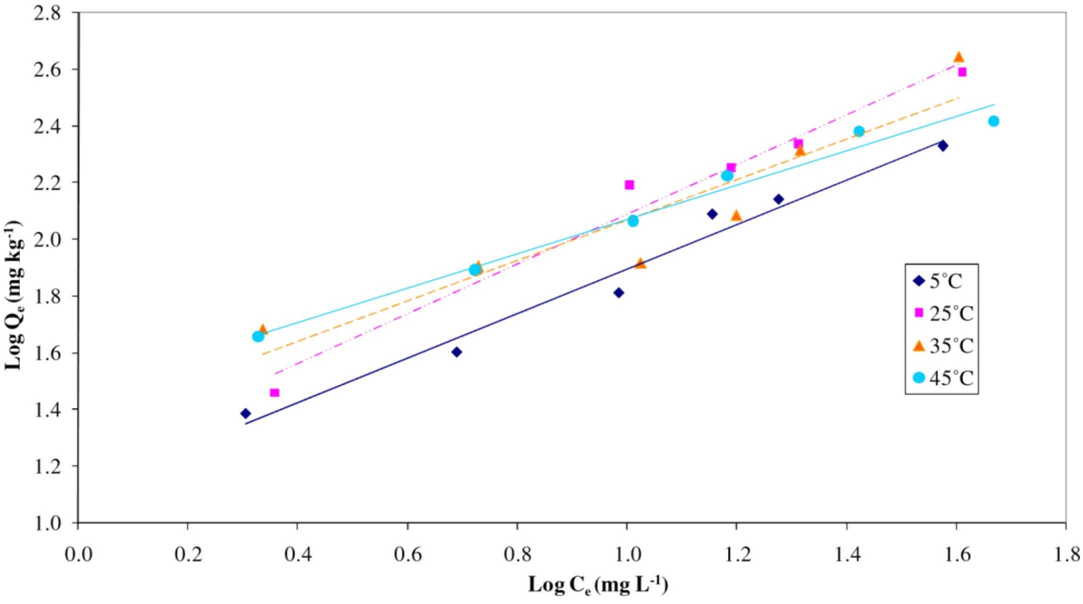
The results show that there was no sorption at pH 12. This finding is in good agreement with previous studies that stated the desorption process of BPA started at pH values higher than 9 and almost the whole of BPA desorbed into the aqueous phase by pH 12 (Clara *et al.*, 2004). The results indicate that desorption processes were more likely to occur at pH values in the range of pK_a values (pK_a range of 9.6 – 11.3, Staples *et al.*, 1998). This is because pK_a has an effect on BPA species in the system. BPA is a weak organic acid and can be deprotonated to exist in solution as the anionic form and/or neutral form. If $pH < pK_a$, the neutral species are predominant in the system, whereas the ionic species predominate when $pH > pK_a$. Neutral species are equal to ionic species when $pH = pK_a$. Moreover, the negatively charged surfaces of marine sediments vary with pH, as they become more negative in more alkaline water (Rand, 1995). At pH below pK_a, neutral BPA is the predominant species in water and the dissociation of BPA is negligible. Based on these factors, the neutral form of BPA is expected to have a much higher sorption coefficient than that of the ionised form of BPA. At pH above pK_a, the anionic form of BPA and the negatively charged surfaces of marine sediment increase; therefore, the amount of BPA sorbed onto marine sediments decreases (Tian *et al.*, 2009).

5.3.6 Effect of Temperature on the Solid Sorption of BPA

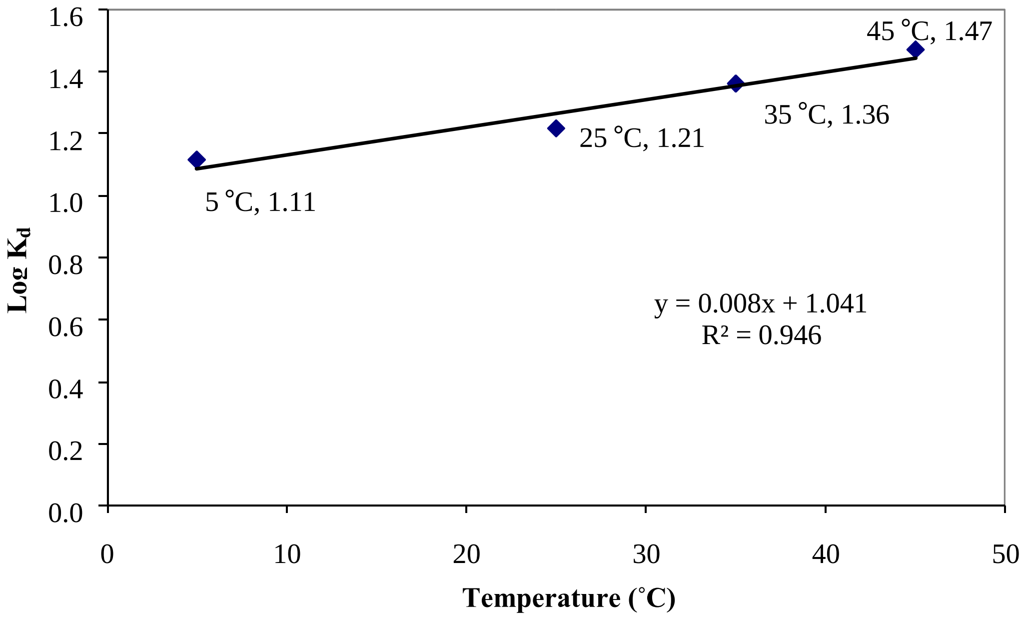
Temperature is an important factor in sorption processes. In terms of thermodynamics, sorption occurs when the free energy (ΔG) of the sorptive exchange is negative. There are two types of driving forces in sorption processes: enthalpy-related and entropy-related forces. An example of entropy-related forces is hydrophobic bonding. Hydrophobic sorption refers to a combination of London dispersion forces (instantaneous dipole-induced dipole or loosely van der Waals forces) and large entropy changes resulting from the removal of compound in solution. In contrast, enthalpy-related forces are greater for polar compounds because of the additional exothermic contribution of the electrostatic interactions to the sorption enthalpy (ten Hulscher and Cornelissen, 1996; Site, 2001). Thus, the effects of temperature on sorption coefficient of BPA were investigated as well as the thermodynamic relationship between the sorption coefficient, temperature and free energy (ΔG°).

5.3.6.1 Determination of Sorption Coefficient at Various Temperatures

The effect of temperature was studied on the Prestwick sediment (collected in 2011) with deionised water at the temperature range of 5 °C to 45 °C. The results are shown in Figure 5.17 and Table 5.9.



(a)



(b)

Figure 5.17 (a) Freundlich sorption isotherms of BPA on Prestwick sediment at various temperatures, and (b) relationship between log K_d and temperature **Table 5.9** Freundlich sorption isotherms and log K_d of BPA on Prestwick sediment at various temperatures

Temperature (°C)	Freundlich Sorption Isotherm	R^2	Log K_d
5	y = 0.785x + 1.111	0.975	1.11
25	y = 0.876x + 1.212	0.977	1.21
35	y = 0.712x + 1.357	0.874	1.36
45	y = 0.605x + 1.466	0.981	1.47

The results show that the sorption of BPA on Prestwick sediment tends to increase slightly at higher temperature. This can be described by the sorption of molecules on surfaces and interlayer of sediments as related to the Gibbs sorption isotherm. This sorption isotherm states the Gibbs surface concentration equation that indicates that the sorption process is correlated with surface tension, as shown in equation 5.19 (Ip and Toguri, 1994; Tan, 1998; Bajpai and Vishwakarma, 2003).

$$\Gamma = -\frac{a}{RT}\left(\frac{\partial \gamma}{\partial a}\right)_T \qquad \text{Equation 5.19}$$

Where:

Γ = the surface concentration of sorbed material (mol cm^{-2})

a = activity of solute (mol)

R = the ideal gas constant = 8.314462 J mol^{-1} K^{-1}

T = temperature (K)

γ = the surface tension (dynes cm^{-1})

Regarding equation 5.19, $\partial\gamma / a\partial$ is negative when the solute decreases tension, leading to the sorption of solute on the sorbent surfaces, but it is impossible to measure surface tension of solid surfaces. The surface tension of water tends to reduce with increasing temperatures (Rinker *et al.*, 1994), resulting in a higher Gibbs surface concentration (Γ), as shown in equation 5.19. Therefore, these factors lead to increasing sorption of BPA on the sediment

surfaces.

This study observed the directly proportional relationship between sorption coefficients and temperature. However, due to small changes of the sorption coefficients (slope = 0.008), it can be concluded that temperature has little influence on the binding of BPA to sediment.

Like other equilibrium partitioning, the sorption process is also a function of the Gibbs energy which is related to temperature. The standard Gibbs energy (ΔG°) of the sorptive exchange can be calculated by equation 5.20.

$$\Delta G^{o} = - RT\ln K_{oc} \qquad \text{Equation 5.20}$$

The K_{oc} values were calculated from K_d value at certain temperature using equation 5.2. The %OC of Prestwick sediment (2011) was 0.15%. The results are shown in Table 5.10.

Table 5.10 Log K_{oc} of BPA and standard Gibbs energy (ΔG°) at various temperatures

Temperature (°C)	Log K_{oc}	ΔG° (kJ mol^{-1})
5	3.94	-20.96
25	4.04	-23.04
35	4.18	-24.66
45	4.29	-26.13

The results show that ΔG° of the sorptive exchange were negative at all studied temperatures; thus, the sorption process of BPA occurs in the temperature range of this study and it is a spontaneous process (Atkins, 2001; Site, 2001).

5.3.6.2 Determination of Temperature Dependence on the Sorption Coefficient

The van't Hoff equation was used to determine the temperature-dependent equilibrium sorption partitioning constant. The van't Hoff equation and its linear form for K_{oc} are shown in equation 5.21 and equation 5.22, respectively.

$$\frac{\ln K_{oc}}{1/T} = -\frac{\Delta H_o}{R} \qquad \text{Equation 5.21}$$

$$\ln K_{oc} = -\frac{\Delta H_o}{RT} + \frac{\Delta S_o}{R} \qquad \text{Equation 5.22}$$

The linear form of the van't Hoff equation can be used to assess the reaction of the system and to determine the temperature dependence of the system. The sorption process involves bonding of the chemical with sorbent and the solubility of the chemical in solution. Thus, sorption effect and solubility effects contribute to the difference of sorption coefficients from temperature. In the sorption study, the enthalpy change (ΔH°) demonstrates the energy changes between the sorbent and the chemical and between the solution and the chemical (Site, 2001). The van't Hoff plot is shown in Figure 5.18.

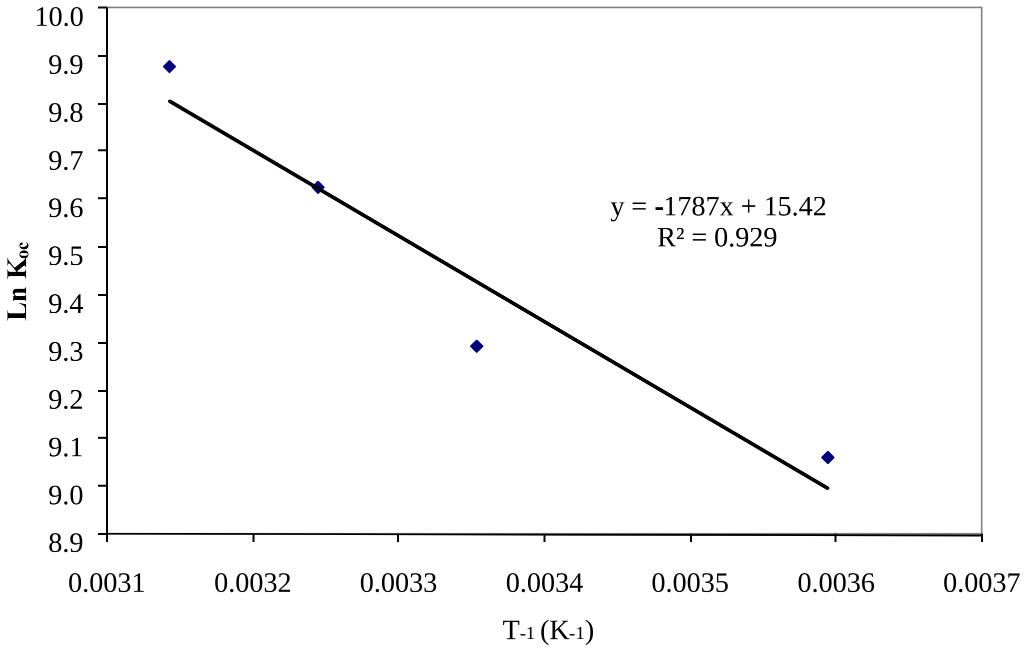


Figure 5.18 The van't Hoff plot of K_{oc}

The negative slope of the van't Hoff plot of sorption of BPA indicates the positive ΔH°, which may imply that the sorption process of BPA on Prestwick sediment is an endothermic reaction and the system absorbed heat (Atkins, 2001). Therefore, increasing temperature results in more sorption of BPA onto sediment in regards with Le Chatelier's principle.

Equation 5.23 shows the van't Hoff equation obtained from this study. Using this equation along with equation 5.22, it is possible to estimate the change in enthalpy (ΔH°) and entropy (ΔS°) in the reaction, as shown in Box 5.2.

Box 5.2 Calculation of change in enthalpy (ΔH°) and entropy (ΔS°) in the reaction

$$\ln K_{oc} = -1787\left(\frac{1}{T}\right) + 15.42\ , \quad R^2 = 0.929 \qquad \text{Equation 5.23}$$

From equation 5.22, slope $= -\dfrac{\Delta H^{o}}{R} = -1787$ Equation 5.24

$$\Delta H^{\circ} \quad = \quad 14857.94\ \text{J mol}^{-1} \quad = \quad 14.86\ \text{kJ mol}^{-1}$$

And intercept $= \dfrac{\Delta S^{o}}{R} = 15.42$ Equation 5.25

$$\Delta S^{\circ} \quad = \quad 128.23\ \text{J K}^{-1}$$

The results above show that both ΔH° and ΔS° were positive (> 0), whereas ΔG° was negative (Table 5.10), which may imply that sorption of BPA on sediment from water is spontaneous at high temperatures where $T\Delta S$ outweighs ΔH. ΔG can be calculated from ΔH and ΔS as shown in equation 4.17 (Atkins, 2001). Due to large entropy changes, these results may indicate that sorption is an entropy-driven process (Site, 2001).

The van't Hoff equation used in the prediction of K_{oc} ($K_{oc,2}$) at any other temperature (T_2) can be expressed by:

$$\ln \frac{K_{oc,2}}{K_{oc,1}} = -\frac{\Delta H^{o}}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

Equation 5.26

The comparison between the experimental log K_{oc} values of BPA ($K_{oc,exp}$) at 5, 35 and 45 ˚C and the estimated K_{oc} of BPA ($K_{oc,est}$) at the same temperature, calculated by equation 5.26, is shown in Figure 5.19. The $K_{oc,1}$ is the $K_{oc,exp}$ at 25 ˚C, which is the standard temperature for analysis (Atkins, 2001).

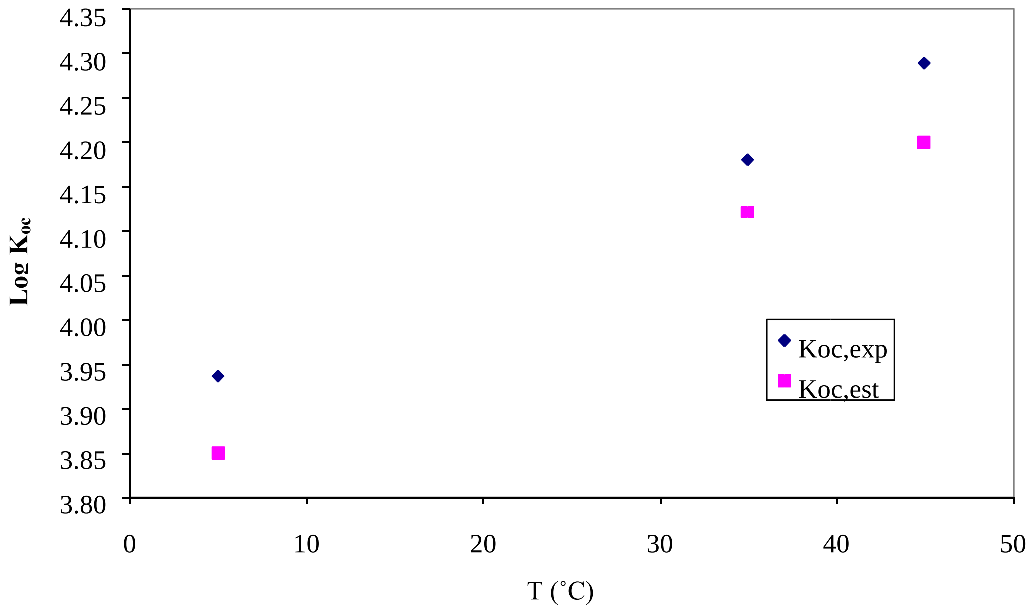


Figure 5.19 Comparison between K_{oc} from experiment and calculation at specific temperature

The results show that the estimated log K_{oc} values are likely to agree with the experimental log K_{oc} values in this study, at least in the range of study (variation between these values were 0.06 – 0.09 log unit). Consequently, from equation 5.26 along with ΔH˚ (from equation 5.24), it is possible to predict K_{oc} value of BPA at specific temperatures in the range of 5 ˚C to 45 ˚C.

5.3.7 Determination of the Water Condition-dependent Sorption Coefficient of BPA

Experimental data in this sorption study analysed by multiple linear regression provides the equation to estimate water condition-dependent sorption coefficient (K_{oc}) of BPA with independent parameters of salinity (0– 50 psu), pH (4 – 8), and temperature (5 – 45 °C). The pH range was limited to its linear relationship with the sorption coefficient.

The results were divided into two datasets. The training set of 11 experimental log K_{oc} values obtained from the effects of salinity, pH and temperature on the sorption coefficient of BPA was used to obtained the multiple linear regression to predict log K_{oc} of BPA at specific water conditions. These experiments (Section 5.3.4 – Section 5.3.6) were carried out on Prestwick sediments collected in 2011 and 2012 (n = 11). A separate validation set was the experimental log K_{oc} values of BPA stated in Section 5.3.2 (n = 8) that were not used to derive the regression equation. The variation between experimental log K_{oc} and calculated log K_{oc} was determined in order to verify the regression equation. The data used and the results are shown in Table 5.11.

The results from running multiple linear regression in Excel (n = 11) indicated that log K_{oc} is significantly related to salinity (psu), pH and temperature (°C) with 95% confidence (p-values < 0.05, $R^2 = 0.90$), see details in Appendix A.3. The multiple linear regression equation obtained is shown in equation 5.27 in Box 5.3.

Table 5.11 Summary of comparisons between experimental and calculated log K_{oc} in this study at specified water conditions

Sample	Salinity (psu)	T a (˚C)	pH	Exp. Log $K_{oc\ a}$	Cal. Log K_{oc} ^{a}	Variation^{b}	% Variation
Prestwick	0	25 25	7.3	4.17	4.14	-0.03 -	-0.7 -
(2012)	0.5	25 25	7.3	4.18	4.14	0.04	0.9
	10 35		7.3	4.19	4.18	-0.01	-0.4
			7.3	4.22	4.26	0.04	0.9
	50	25	7.3	4.33	4.31	-0.02	-0.5
Prestwick	0 0	5	7.4	3.94	3.98	0.04	1.1
(2011)	0 0 0	25 35 45 25	7.4	4.13	4.15	0.02	0.4
			7.4	4.18	4.23	0.05	1.1
			7.4	4.29	4.31	0.02	0.4
			4.0	4.03	4.02	-0.01	-0.2
	0	25	8.0	4.22	4.17	-0.05	-1.2
Rayong	0	25	8.1	3.74	4.17	0.43	11.5
Pattaya	0	25	8.5	4.22	4.19	-0.03	-0.8
Kudee	0	25	8.8	2.63	4.20	1.57	59.6
Samet	0	25	8.8	3.56	4.20	0.64	17.9
Prestwick (2011)	0	25	6.2	4.04	4.10	0.07	1.6
Mussel	0	25	9.3	3.91	4.21	0.30	7.8
Prawn	0	25	8.4	4.77	4.18	-0.59	-12.3
Biochar	0	25	6.3	3.19	4.11	0.92	28.7

[a] T = temperature; Exp. Log K_{oc} = Experimental Log K_{oc}; Cal. Log K_{oc} = Calculated Log K_{oc}

[b]

Cal. Log K_{oc} – Exp. Log K_{oc}

Box 5.3 Correlation equation between K_{oc} and water conditions

$$\text{Log } K_{oc} = 3.6738 + 0.003299\,(\text{Salinity}) + 0.008159\,(\text{Temperature}) + 0.0362\,(\text{pH}) \qquad \text{Equation 5.27}$$

Note: Salinity = 0 – 50 psu; Temperature = 5 – 45 °C; pH = 4 – 8

The calculation of log K_{oc} values using equation 5.27 compared with the experimental log K_{oc} is shown in Table 5.11. The results show that most of the variations were less than 20% except for Kudee Island sediment and biochar. Thus, equation 5.27 may be used to estimate log K_{oc} of BPA at any specific water condition by measuring salinity, pH and temperature, at least for screening purposes. Knowing K_{oc} values means it is also possible to predict the K_d value at any specific site if the organic carbon content of the marine sediment in that site is known.

5.3.8 Prediction of Environmental Fate from Sorption Coefficient

The fugacity model in EPI Suite™ used K_{oc} to calculate the amount of organic compound in each phase. The MCI-based K_{oc} is used as a default value in the program (EPA, 2012b). This study compares the prediction data from the fugacity model using the default value and experimental value at specified conditions. The results are presented in Table 5.12.

Table 5.12 Comparison of the predictions obtained from fugacity in the EPI Suite™

Parameters	Air (%)	Water (%)	Solid (%)	Persistence time (h)
Default values	7.95 x 10^{-5}	8.31	91.7	2.22 x 10^{3}
Salinity				
10 psu 35 psu	8.36 x 10^{-5}	9.8	90.21	2.01 x 10^{3}
	8.31 x 10^{-5}	9.7	90.29	2.03 x 10^{3}
Temperature				
25 °C 35 °C	8.56 x 10^{-5}	10.2	89.83	1.97 x 10^{3}
	8.37 x 10^{-5}	9.82	90.21	2.01 x 10^{3}
pH				
7 8	8.71 x 10^{-5}	10.5	89.46	1.93 x 10^{3}
	8.3 x 10^{-5}	9.69	90.27	2.03 x 10^{3}

The results indicate differences arising with changing conditions. The default prediction is slightly overestimated in the amount of BPA in the solid phase, resulting in fewer amounts in other phases. The persistence is associated with the concentration of the compound in the solid phase (EPA, 2012b); higher amounts in solids lead to longer environmental persistence. BPA is not very volatile; the amount in air is smaller than that in both water and solid phases. It also indicates that temperature and pH have more influences on partitioning in the environment than the salinity of water. These findings imply that the mobility of BPA will be higher than that predicted by the default value. Therefore, experimental values are necessary for site-specific investigations in order to develop a more accurate assessment and better reflect environmental conditions in the evaluation of the environment fate of BPA.

5.4 Summary

The sorption coefficients (K_d and K_{oc}) are important factors in environmental assessment. These coefficients can be retrieved from both experimental data or modelling. However, to understand the real environment and to be site specific, experimental values are preferred.

This study investigated the influences of sediment properties (*e.g.*, organic carbon, minerals) and water conditions (*e.g.*, salinity, pH and temperature) on sorption of BPA to solids. The sediment properties played important roles in the sorption interactions of BPA in the solid-water system. Higher organic carbon content in solids tended to have greater sorption capacity for BPA. The possible effects of silica (SiO_2) and calcium on the sorption of BPA from water to sediment were observed, which were directly and inversely proportional, respectively. The salting-out effect was observed as the sorption capacity increased with increasing salinity. The same trend was also observed in the temperature study because of the increasing surface concentration resulting from the reduction of surface tension. In the pH study, desorption was observed at pH > 9 which is near the pK_a of BPA, and there was no sorption at pH 12 when BPA was in its ionised form and did not interact with the charged surfaces of the sediment. At higher pH (> pKa), the ionised form of BPA is predominant and surface charges on sediment become more negative, leading to greater water solubility of BPA.

This study determined the salting-out constant (k_{oc}) of K_{oc}, which will help to estimate the K_{oc} value of BPA at specified salt concentrations. The study of effects of temperature on K_{oc} provided the equation to predict the K_{oc} value at any given temperature this used the enthalpy of reaction (ΔH°) along with any K_{oc} (such as K_{oc} at standard temperature, 25 ˚C).

More interestingly, the equation to estimate K_{oc} at any specific site using its correlation with salinity, pH and temperature was determined with multiple linear regression. As a result, the K_{oc} value can be obtained through measuring salinity, pH and temperature of that site. These are parameters that are easy and inexpensive to measure using probes. They do not require a lab or trained staff, but the information gained can be used to assess changes in K_{oc} with changing water temperature and salinity.

K_{oc} is used in the fugacity model in EPI Suite™, leading to predictions of the amount of BPA in each environmental phase. The default prediction is obtained from using MCI-based K_{oc} but the programme also allows users to input their experimental K_{oc} values. Slight overestimation of BPA concentration in the solid phase was observed, which means that the amount of BPA dissolved in water will be higher. As a result, the persistence is expected to be shorter but the amount of BPA that is bioavailable is expected to be greater and may increase bioaccumulation in some pelagic species.

The results of this study imply that the persistence and bioaccumulation of BPA may be higher for species in the marine environment compared to those in freshwater due to higher salinity (35 psu) and pH (approximately 8).

In conclusion, while models are useful screening tools to understand the behavior of BPA, experimental data is preferred over model estimates where possible to better reflect environmental conditions. An accurate understanding will help in the prevention and remediation for contamination of BPA in all aquatic environments.

Chapter 6

Environmental Modelling: Impacts of Climate Change on Bisphenol A

6.1 Background

An often-overlooked aspect of global climate change is how it can affect organic pollutants in the marine environment. BPA is considered an emerging pollutant in the ocean. Previous chapters indicate that changing water conditions (*i.e.*, water temperature, pH, and salinity) influence the partition coefficients of BPA, as shown in Table 6.1. Changing partitioning, in turn, affects the fate, transport, and toxicity of BPA in the environment. Evidence indicates that climate change will lead to changing water conditions; therefore, the fate and transport of BPA in the marine environment is also affected. The modelling programme EPI Suite™ uses partition coefficients of a chemical to predict its fate and toxicity in the environment. Because the default results of the programme are normally based on standard conditions (*e.g.*, 25 °C, fresh water) and may be derived from either estimated and/or experimental data, the programme allows users to change partition coefficients and chemical properties, improving accuracy and site-specific predictions. In this study, EPI Suite™ is used along with experimental values obtained from previous chapters in order to evaluate the potential effects of climate change on the behaviour of BPA in the ocean.

Table 6.1 Effects of increasing salinity, pH and temperature on partition coefficients of BPA from this study (Chapter 3 to Chapter 5)

Partition Coefficients	Salinity	pH	Temperature
Octanol-water partition coefficient (K_{ow})	K_{ow} increases with increasing salinity	K_{ow} increases slightly with increasing pH, then decreases significantly when pH > pK_a	K_{ow} decreases with increasing temperature
Water solubility (S_w)	S_w decreases with increasing salinity	S_w increases slightly with increasing pH, then increases significantly when pH > pK_a	S_w increases with increasing temperature
Organic carbon – water sorption coefficient (K_{oc})	K_{oc} increases with increasing salinity	K_{oc} is unaffected between pH 4 – 9; no adsorption at pH 12 (pH > pK_a)	K_{oc} increases with increasing temperature

6.1.1 Climate Change

The climate of the Earth is changing. The proposed main reasons for this are global greenhouse gas (GHG) emissions from human activities such as fossil fuel use and agriculture. The important GHGs are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (IPCC, 2007; IPCC, 2013). The observed changes in Northern Hemisphere spring snow cover, Arctic summer sea ice extent, average upper ocean (0 – 700 m) heat content, and global average sea level (Figure 6.1) show the increasing trend in global surface temperature and sea level, but lower in snow cover (IPCC, 2013).

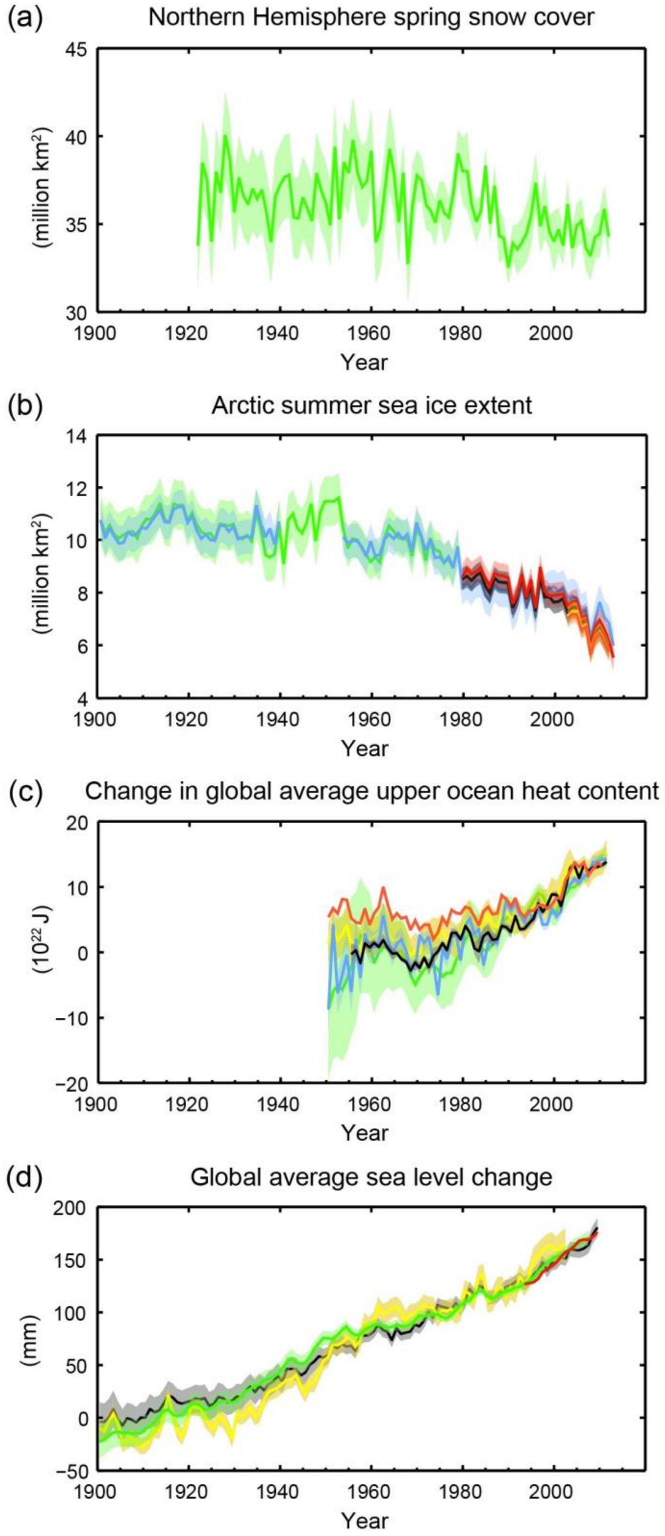


Figure 6.1 Observed changes in: (a) Northern Hemisphere spring snow cover, (b) Arctic summer sea ice extent, (c) global average upper ocean (0 – 700 m) heat content, and (d) global average sea level (reproduced from IPCC, 2013) Increasing

CO_2 also causes ocean acidification due to reaction between CO_2 and seawater as shown in equation 6.1 (NRDC, 2009; Gattuso, 2011).

$$CO_2 + H_2O + CO_3^{2-} \rightarrow 2HCO_3^- \qquad \text{Equation 6.1}$$

The term “ocean acidification” refers to the lowering of pH, but does not imply that the pH of surface seawater will become acidic (below 7), only that it will become less alkaline. Subsequently, climate change affects water conditions by increasing average ocean temperatures, global average sea levels, and ocean acidity with an associated decrease in ocean salinity (IPCC, 2007; IPCC, 2013).

Figure 6.2 shows the time series of the annual average sea surface temperature (SST) anomaly. It indicates that the average ocean temperatures are increasing and the deviations are also increasing. The European Environmental Agency (EEA) used the average temperature in the global ocean and in each of the Europeans seas between 1970 and 2012 to generate this chart (EEA, 2014).

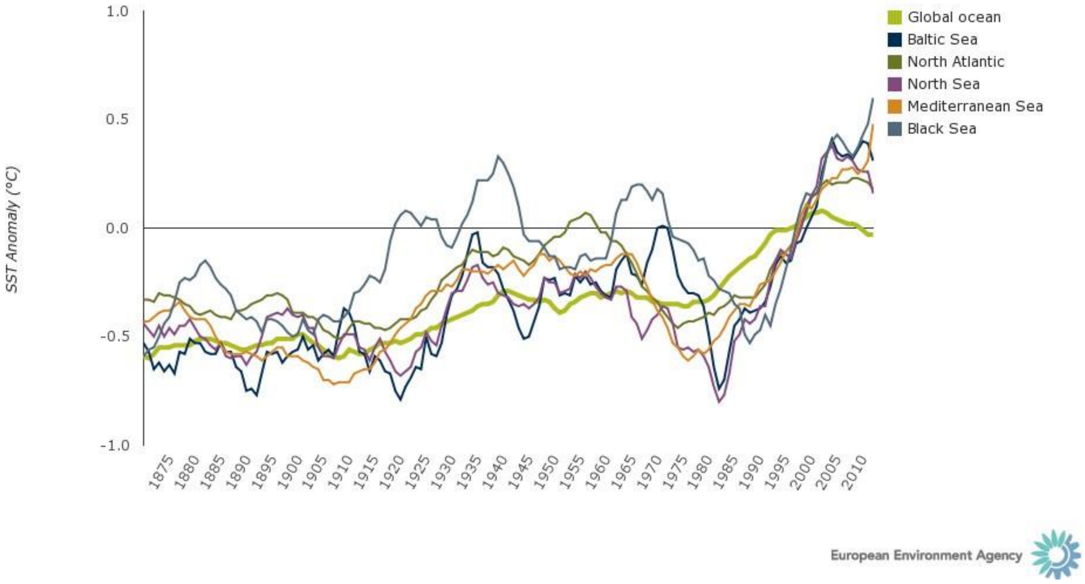


Figure 6.2 Annual average sea surface temperature anomaly (reproduced from EEA, 2014)

At present, the global ocean seawater conditions are (Anderson, 2008; Zeebe, 2011):

- Average salinity is approximately 35 psu.
- The range of surface ocean temperature varies from -2 °C in polar regions to above 30 °C in tropical regions.
- The pH of surface seawater is generally in the range of 7.5 to 8.5 with the average at approximately 8.2.

6.1.2 Environmental Modelling

Environmental modelling is an important tool in environmental monitoring and environmental and risk assessment as it provides estimations of the environmental fate, transport, and toxicity of organic chemicals in the environment. Chemical fate in the environment is complex and difficult to describe accurately due to the complexity of ecosystems and the difficulty in collecting enough data to represent environmental conditions. With modeling, it is possible to simulate the movement of chemicals between the environmental compartments and their reactions when discharged into the environment (Schnoor, 1996). Modelling is a cost-effective and powerful tool to predict the environmental behaviour and potential impacts of chemicals in the environment, especially for a substance that has not been studied widely. Modelling can be used to predict the potential environmental fate and transport of chemicals for various scenarios, leading to effective assessment and appropriate prevention and treatment methods to reduce the potential hazards to ecosystems (Mackay *et al.*, 2001). The model gives an output from the available input data either from its database or from user-defined inputs. As a result, the accuracy of the retrieved data depends on the accuracy of the input data. Consequently, actual monitoring, measurement and assessment are preferable if possible (Dunnivant and Anders, 2006).

In this study, the environmental modelling programmes EPI Suite™ (EPA, 2012b) was used to predict the environmental behaviour of BPA in the marine environment because of these following advantages that it offered:

- No licensed required
- Easy to use
- Contain large database
- Prediction based on partitioning ratios
- Easy to interpret

Based on the fugacity model, the migration of a substance between environmental phases such as soil, water and biota is predicted by using partition coefficients and mass balance equations (Mackay, 1979). The calculation in EPI Suite™ is based on a Level III fugacity model that typically has four compartments (air, water, soil, and sediment) and four fugacities that apply to each compartment. The assumptions of the Level III model are that compartments are homogeneous, conditions are at steady-state (*i.e.*, constant with time), and conditions are not at equilibrium. The programme calculates the values from the inherent chemical and physical properties of the chemical (EPA, 2012b).

EPI Suite™ contains many estimation programmes to predict the values. The programmes provide estimated values and also experimental values from the database if available. To obtain the result, EPI Suite™ requires only the chemical’s name, Chemical Abstract Service (CAS) number or SMILES (Simplified Molecular Input Line Entry System) notation, as show below.

Name: Bisphenol A

Chemical name:	Phenol, 4,4 -(1-methylethylidene)bis-
CAS number:	80-05-7
SMILES:	Oc(ccc(c1)C(c(ccc(O)c2)c2)(C)C)c1

The model allows the users to input site-specific data (*e.g.*, water depth, water flow, and wind velocity) and also physicochemical properties (*e.g.*, K_{ow}, S_w and K_{oc}) of the chemical under certain conditions, leading to site-specific data under the conditions of interest.

Interpreting the results from the model is also an important process. The general criteria and meaning of the important properties, values, and effects (from ECOSAR™) in this study are described in Table 6.2 and Table 6.3.

Table 6.2 Interpreting criteria and meaning for EPI Suite™ results

Parameters	Range	Meaning
Melting point (°C)	< 25	Liquid
	> 25	Solid
Boiling point (°C)	< 25	Gas
Vapour pressure (mm Hg)	$> 10^{-4}$	Exists mostly in the gas phase
	10^{-5} - 10^{-7}	Exists in the gas and particulate phase
	$< 10^{-8}$	Exists mostly in the solid phase

Log K_{ow} (octanol-water partition coefficient)	2 – 4	Tend to absorb well through biological skin
	> 4	Tend to not absorb well through biological membranes
	5 – 6	Tend to bioconcentrate in the lipid of the biological membranes

Parameters	Range	Meaning
S_w (water solubility, mg L^{-1})	> 10,000	Very soluble
	> 1,000 – 10,000	Soluble
	> 100 – 1,000	Moderate solubility
	> 0.1 – 100	Slightly soluble
	< 0.1	Negligible solubility
HLC (Henry's law constant, atm m^3 mol^{-1})	> 10^{-1}	Very volatile from water
	10^{-1} - 10^{-3}	Volatile from water
	10^{-3} - 10^{-5}	Moderately volatile from water
	10^{-5} - 10^{-7}	Slightly volatile from water
	< 10^{-7}	Nonvolatile
Log K_{oc} (organic carbon normalised sorption coefficient)	> 4.5	Very strong sorption to soil/sediment, negligible migration to ground water
	3.5 – 4.4	Strong sorption to soil/sediment, negligible to slow migration to ground water
	2.5 – 3.4	Moderate sorption to soil/sediment, slow migration to ground water

	1.5 – 2.4	Low sorption to soil/sediment, moderate migration to ground water
	< 1.5	Negligible sorption to soil/sediment, rapid migration to ground water
BCF (Log BCF) (bioconcentration factor)	> 5000 (≥ 3.7)	High bioconcentration potential
	1000 – 5000 (3)	Moderate bioconcentration potential
	< 1000 (< 3)	Low bioconcentration potential

Source: EPA, 2012a

Table 6.3 Interpreting criteria and meaning for ECOSAR™ results

Criteria	Toxicity Concern
Lowest acute toxicity value is < 1 mg L^{-1} (chronic value < 0.1 mg L^{-1})	High toxicity concern
Lowest predicted acute value is between 1 – 100 mg L^{-1} (chronic value between 0.1 – 10 mg L^{-1})	Moderate toxicity concern
Lowest predict acute value is > 100 mg L^{-1} (chronic value > 10 mg L^{-1}); or there are no effects predicted at saturation (when S_w of chemical is > toxicity value); or log K_{ow} is greater than the cutoff of 8.	Low toxicity concern

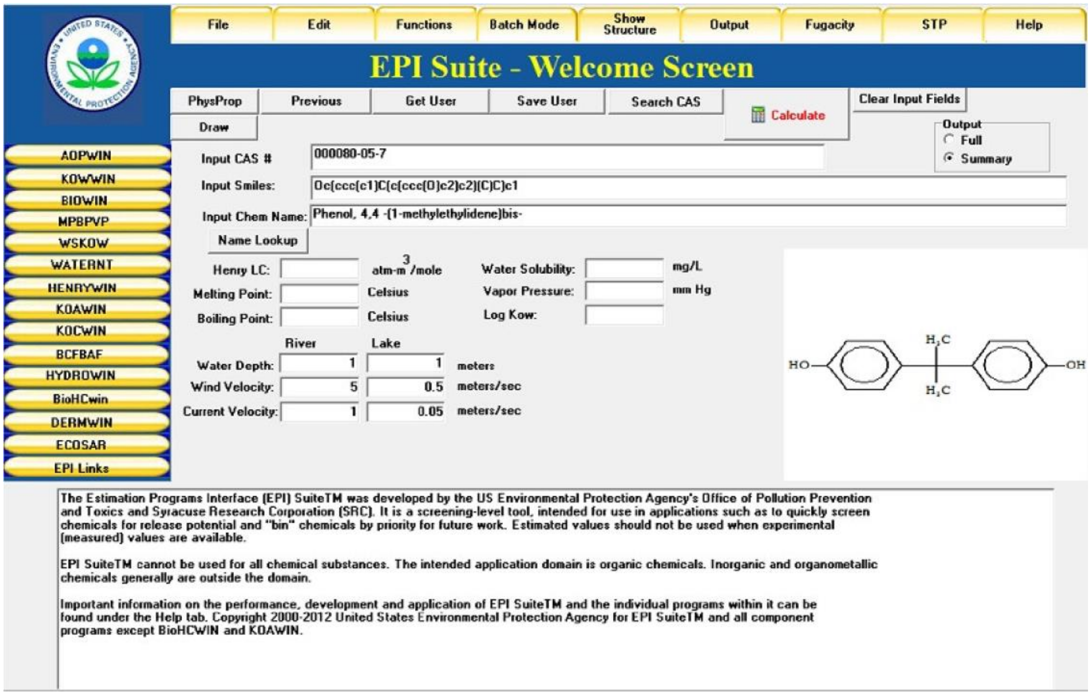
Source: EPA, 2012a

In this work, EPI Suite™ was used to investigate the possible impacts of climate change on the environmental fate and transport of BPA in the marine environment. In this study, experimental K_{ow}, S_w and K_{oc} values from previous chapters (Chapter 2 to Chapter 5) were entered into the model. The values obtained from the model at present ocean conditions were compared to the values at predicted conditions associated with climate change in order to evaluate the effects of global climate change on the environmental fate, transport, and ecotoxicity of BPA in the marine environment.

6.2 Methodology

6.2.1 Estimation of Environmental Fate by Computer Modelling

EPI Suite™ Version 4.11 (EPA, 2012b) is used to evaluate the effects of climate change on the environmental fate of BPA in this study. The calculations and theory used in the software are described in the reference material that is included in EPI Suite™. The user-friendly interface is shown in Figure 6.3



Output
○ Full
● Summary

Input CAS # 000080-05-7

Input Smiles: Oc(ccc(c1)C(c(ccc(O)c2)c2)(C)C)c1

Input Chem Name: Phenol, 4,4 -(1-methylethylidene)bis-

	River	Lake	
Water Depth:	1	1	meters
Wind Velocity:	5	0.5	meters/sec
Current Velocity:	1	0.05	meters/sec

The Estimation Programs Interface (EPI) SuiteTM was developed by the US Environmental Protection Agency's Office of Pollution Prevention and Toxics and Syracuse Research Corporation (SRC). It is a screening-level tool, intended for use in applications such as to quickly screen chemicals for release potential and "bin" chemicals by priority for future work. Estimated values should not be used when experimental (measured) values are available.

EPI SuiteTM cannot be used for all chemical substances. The intended application domain is organic chemicals. Inorganic and organometallic chemicals generally are outside the domain.

Important information on the performance, development and application of EPI SuiteTM and the individual programs within it can be found under the Help tab. Copyright 2000-2012 United States Environmental Protection Agency for EPI SuiteTM and all component programs except BioHCWIN and KOAWIN.

Figure 6.3 EPI Suite™ homepage (EPA, 2012b)

The partitioning behaviour, fate and toxicity of BPA in the marine environment were predicted using the model incorporating the experimental K_{ow}, S_w and K_{oc} values which were determined in the previous chapters.

6.2.2 User-Changed Parameters

The programme allows users to input many parameters, including physicochemical properties of BPA (*i.e.*, HLC, S_w, K_{ow}, etc.) and environmental conditions (*i.e.*, water depth, wind velocity, current velocity). In this study, three important parameters (log K_{ow}, S_w and K_{oc}) of BPA in the marine environment at specified conditions were input into the model. The environmental behaviours of BPA in the marine environment under present ocean conditions in Thailand (salinity 35 psu, pH 8 and temperature 30 °C) were compared with conditions under an extreme climate change scenario (salinity 25 psu, pH 7.5 and temperature 45 °C).

6.2.2.1 Log K_{ow} Values

A known log K_{ow} can be entered on the model homepage as shown in Figure 6.3. The log K_{ow} values of BPA under conditions of interest (see above) were estimated by using equation 3.20 (see Section 3.3.8). The results are shown in Table 6.4. These log K_{ow} values were entered into the model.

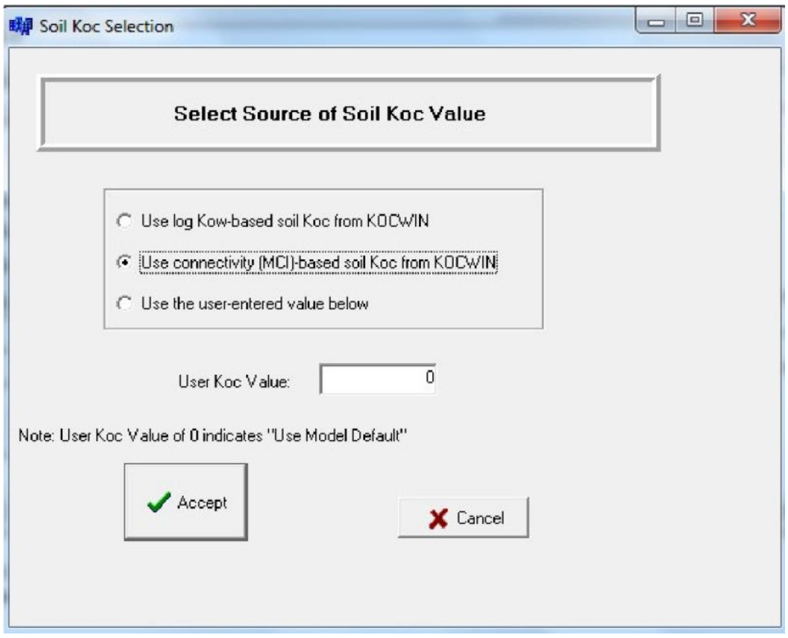
6.2.2.2 S_w Values

The programme also allows users to enter a known S_w on the homepage, as shown in Figure 6.3. The S_w of BPA under the same conditions as stated above were calculated using equation 4.19, see Section 4.3.7. The obtained S_w values, shown in Table 6.4, were then input into the model.

6.2.2.3 K_{oc} Values

The programme allows users to select source of soil K_{OC} values used in the prediction from three different options (Figure 6.4). The first option is log K_{ow}-based sediment K_{oc} which calculate K_{oc} based on K_{ow}. The second option is a Molecular Connectivity Index (MCI) which is a default source of K_{oc}. The last option is a userentered value. The user-specified K_{oc} values were used in this study.

The K_{oc} values calculated by equation 5.27 in Section 5.3.7 under the same scenarios described above were enter to the model, those values are shown in Table 6.4.



Soil Koc Selection

Select Source of Soil Koc Value

○ Use log Kow-based soil Koc from KOCWIN
● Use connectivity (MCI)-based soil Koc from KOCWIN
○ Use the user-entered value below

User Koc Value: 0

Note: User Koc Value of 0 indicates "Use Model Default"

Figure 6.4 Options of K_{oc} value for fugacity model in EPI Suite™ (EPA, 2012b)

6.2.3 Running the Fugacity Model

The fugacity model provides the mass amount distribution of the substance in each environmental compartment (air, water, soil, and sediment). The fugacity model uses K_{ow} and K_{oc} to run the model. It also requires a half-life ($t_{1/2}$) value in each compartment to run. A half-life is the time is required to remove half of the chemical from that compartment. Like K_{ow} and K_{oc}, the fugacity model allows users to enter known half-life values into the programme. However, if this value is not known, the half-life will be automatically calculated by the BIOWIN™, a different model in EPI Suite™. This model calculates both aerobic and anaerobic biodegradation and the water:soil:sediment biodegradation ratio assumed in this model is 1:2:9. The model does not allow user to change this ratio (EPA, 2012b). In this study, the environmental behaviour of BPA was evaluated by using the estimated partition coefficients value at certain conditions as stated in Section 6.2.2.

6.2.4 Bioconcentration (BCF) and Bioaccumulation (BAF)

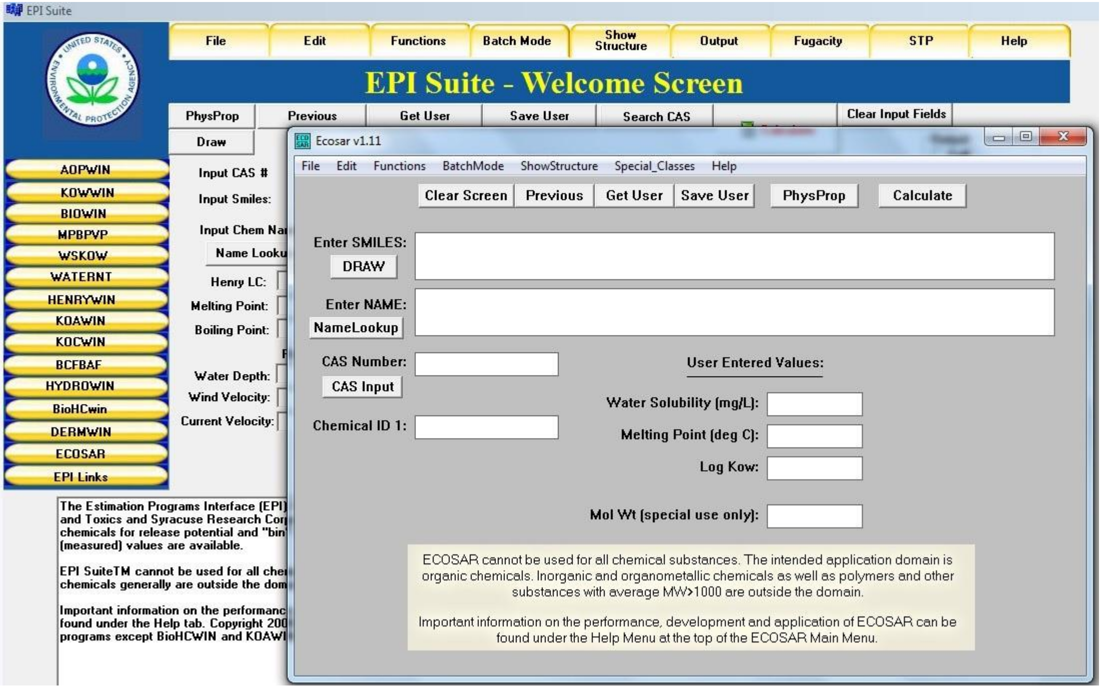
K_{ow} is used to estimate the bioconcentration factor (BCF) and bioaccumulation factor (BAF). High K_{ow} indicates high BCF because the amount of chemical in octanol, which represents the lipid phase in organisms, is increased. Using the estimated partition coefficients at certain conditions (Section 6.2.2) obtained from this study, BCF and BAF were obtained from BCFBAF™ programme in EPI Suite™, which predicted BCF and BAF from log K_{ow} of experimentally-obtained data for BPA (EPA, 2012b).

6.2.5 Henry's Law Constants (HLC)

As described in Chapter 4, HLC calculated from S_w and vapour pressure (VP) is provided from EPI Suite™. Thus, the HLC values predicted under two specified conditions (as stated in Section 6.2.2) were investigated.

6.2.6 Ecological Structure Activity Relationships (ECOSAR)

ECOSAR™ is a stand-alone programme in EPI Suite™. This programme uses computerised Quantitative Structure Activity Relationships (QSARs) to estimate acute (short-term) and chronic (long-term) toxicity to aquatic organisms. QSAR predicts the aquatic toxicity of chemicals based on their similarity to chemicals for which the measured aquatic toxicity data are available. In the ECOSAR™ programme, QSAR is calculated based on a linear relationship between the predicted K_{ow} and the measured toxicity values of the compound of interest. In order to minimise potential variables from laboratory measurement, the predicted K_{ow} values obtained from KOWWIN™, subprogramme in EPI Suite™, are generally used in default results (EPA, 2012b). The ECOSAR™ homepage is shown in Figure 6.5.



The Estimation Programs Interface (EPI)
and Toxics and Syracuse Research Cor
chemicals for release potential and "bin
(measured) values are available.

EPI SuiteTM cannot be used for all che
chemicals generally are outside the dom

Important information on the performanc
found under the Help tab. Copyright 20
programs except BioHCWIN and KOAWI

ECOSAR cannot be used for all chemical substances. The intended application domain is organic chemicals. Inorganic and organometallic chemicals as well as polymers and other substances with average MW>1000 are outside the domain.

Important information on the performance, development and application of ECOSAR can be found under the Help Menu at the top of the ECOSAR Main Menu.

Figure 6.5 ECOSAR™ homepage (EPA, 2012b)

Two ECOSAR™ scenarios were run for BPA using the estimated partition coefficients (log K_{ow} and S_w) obtained from this study as described in Section 6.2.2.

This study compared the predicted environmental fate and aquatic toxicity of BPA in the marine environment under two scenarios: present ocean conditions and hypothetical ocean conditions resulting from extreme climate change. The comparison was carried out by inserting the calculated partition coefficients (K_{ow}, S_w and K_{oc}) at specified conditions into the model to retrieve the environmental and toxicity data.

6.3 Results and Discussion

6.3.1 Estimation of Environmental Fate and Transport of BPA

To investigate the effects of climate change on environmental fate and toxicity of BPA in the marine environment, the EPI Suite™ model was used along with the calculated partition coefficient values under certain conditions. The predicted values were calculated by using multiple linear regression equations obtained from the experiments in this study (illustrate in Chapter 3 – Chapter 5). The comparison of the important physicochemical properties of BPA retrieved from running the model and the partition coefficients used are shown in Table 6.4.

Table 6.4 Physicochemical properties of BPA under two ocean conditions of Thailand

Parameters	Present Ocean Scenario	Extreme Climate Change Scenario
Water conditions	Salinity 35 psu / pH 8 / Temperature 30 °C	Potential changes to Salinity 25 psu / pH 7.5 / Temperature 45 °C
Log K_{ow}^{a}	3.52	3.32
S_w^{a} (g L^{-1})	0.30	0.45
K_{oc}^{a} (L kg^{-1})	21,069	24,827
Log K_{oc}^{a}	4.32	4.39
Melting point (°C)	131.76	131.76
Boiling point (°C)	363.54	363.54
Vapour pressure (mm Hg, 25 °C)	2.27×10^{-7}	2.27×10^{-7}
HLC (atm m^3 mol^{-1})	2.273×10^{-10}	1.515×10^{-10}
Log BCF	1.989	1.858

Log BAF	2.384	2.238

Parameters	Present Ocean Scenario	Extreme Climate Change Scenario
Biotransformation halflife (HL, days)	0.3029	0.2629
Mass amount (%)		
- Air	8.13×10^{-5}	8.00×10^{-5}
- Water	9.36	9.09
- Sediment	90.6	90.9

[a] Calculated values using equations obtained from this study

Data in Table 6.4 can be divided into three sections. The first part is the estimated partition coefficients (log K_{ow}, S_w and log K_{oc}) of BPA, which were calculated using equations obtained from the experiments described in previous chapters of this study. According to Table 6.2, the results of both scenarios demonstrate that BPA is likely to be absorbed through biological membranes (log K_{ow} 2 – 4), has moderate solubility (S_w 0.1 – 1 g L^{-1}) and strong sorption to solid phases (log K_{oc} 3.5 – 4.4). The model predicts that BPA will become more soluble in the aqueous phase but less soluble in the lipid phase in the extreme climate change conditions. These predictions can be interpreted to imply that global climate change will influence BPA to become more distributed in seawater and, therefore, more bioavailable to marine organisms. Although BPA may become more available, the model predicts lower potential for bioconcentration in organisms. For sorption capacity, a small difference was observed, indicating that BPA may become slightly more sorbed onto sediments in case of climate change, which means that benthic organisms may have greater potential exposure to BPA. These estimated values were input into the modelling programme to retrieve other important values for the environmental assessment, which are described below.

The second part of Table 6.4 shows the properties of BPA obtained from the model that were not changed with water conditions: melting point (MP), boiling point (BP) and vapour pressure (VP). These properties demonstrate the physical states (solid, liquid and gas) of a chemical and potential exposure routes for humans. The results indicate that BPA exists as a solid and human exposure to it is possible by ingestion, dermal contact, and inhalation (MP > 25 °C). However, the VP of BPA was less than 10^{-6}, so the concern for inhalation exposure is low relative to other exposure pathways (EPA, 2012a).

The last part of Table 6.4 shows the properties that were dependent on the water conditions: HLC, BCF, BAF, biotransformation half-life, and mass amount of BPA in each compartment (Table 6.4). HLC was retrieved from the proportional relationship between VP and S_w. The HLC values ($< 10^{-7}$ atm m^3 mol^{-1}) indicate that BPA is considered to be nonvolatile from the aqueous phase in both scenarios; BPA tends to be even less volatile from seawater in the climate change scenario because the HLC decreased from 2.273×10^{-10} to 1.515×10^{-10} atm m^3 mol^{-1}.

BCF and BAF are indicators of accumulation potential of a chemical in biota. BCF is the amount of chemical accumulated in an organism from the aqueous phase only. BAF is the amount of chemical accumulated in an organism from all sources, including air, water, soil, sediment, and food. The accumulation potential is important as the pollutant may be accumulated and potentially harm the organism. Once a pollutant is in the food chain, it may become more concentrated and toxic for organisms at higher trophic levels, which is known as biomagnification. Nevertheless, the toxicity of chemicals in organisms generally depends on uptake, biotransformation, and elimination processes (Newman and Unger, 2003). In EPI Suite™ programme, BCF and BAF are estimated based on the linear regression relationships with log K_{ow} because an organic chemical tends to accumulate in the lipid layers in organisms. The log BCF and BAF values of BPA in both conditions suggest that BPA has low bioconcentration potential in the marine biota (log BCF < 3). It also shows that BPA accumulation in aquatic organisms tends to be lower under climate change conditions. This is associated with increasing amount of BPA in aqueous phase.

EPI Suite™ also provides the half-life of biotransformation rate in fish. Biotransformation is defined as "the change of the parent substance to another molecule or a conjugated form of the parent substance" (EPA, 2012b). The biotransformation half-life (HL) states the rate to transform chemical to another form within living organism body; however, the metabolites may have lower (detoxification) or higher (bioactivation) toxicities than the parent substance.

Generally, most lipophilic chemicals are transformed into more water-soluble forms (hydrophilic) that are easier to eliminate from the body (Monosson, 2012). The model considers only the parent chemical but does not consider metabolic products. If the metabolites are known, they have to be re-entered into the model. The results suggest that BPA in climate change conditions (HL = 0.2629 days) will be changed to another form faster than in present ocean conditions (HL = 0.3029 days). Therefore, BPA will be excreted more readily from the marine organisms under climate change conditions.

The mass amounts of BPA in each compartment are estimated by the level III fugacity model in EPI Suite™. Fugacity model predicts the partitioning behaviour of chemicals mainly from K_{oc}. The fugacity models indicate that when BPA is released into the marine environment, it will be sorbed mainly onto the solid compartment. Some BPA will remain in the water phase while very small amounts of BPA will be volatile to the atmosphere. Small variations of the partitioning of BPA in each environmental compartment under these two scenarios were observed. The possible reason is because of the similarity of K_{oc} values (21,069 and 24,827 L kg^{-1}, see Table 6.4).

It can be concluded that climate change will have influences on environmental behaviour of BPA in the marine environment. There is a slight effect on sediment sorption capacity, resulting in small effect on partitioning behaviour in each environmental compartment. Although BPA may be less accumulated in biota, it may be more soluble in the aqueous phase and, therefore, it may be more bioavailable to aquatic organisms. Due to increased solubility, it may have more widespread distribution in the ocean, resulting in organisms currently unaffected by BPA to become exposed.

6.3.2 Estimation of Ecotoxicology of BPA

ECOSAR™ is used to estimate the ecotoxicity of BPA in this study. Because K_{ow} is a major property relating to chemical structure and toxicity for neutral organic substances, the ECOSAR™ programme uses the relationship between the log K_{ow} value and the median toxicity (LC_{50} and EC_{50}) values to estimate LC_{50} (median lethal concentration) and EC_{50} (median effective concentration) values. These median toxicity values illustrate acute ecotoxicological effects. The mean value between the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) is used to estimate chronic (ChV) effects of chemicals. The programme generally provides estimated acute and chronic toxicity for three freshwater species, which are fish, daphnia (representing aquatic invertebrates), and green algae (representing aquatic plants). The programme provides LC_{50} as a shortterm toxicity for fish and daphnia, and EC_{50} for green algae. ChV value is given for all three species. Also, chronic values for earthworms are provided for some substances (Mayo-Bean *et al.*, 2012). The estimated log K_{ow} and S_w values were input into the programme. Even though the toxicity values are calculated using only log K_{ow} value, the S_w is important for solid chemicals as it is used to compare with the predicted effect level. If the effect concentration is less than the S_w of the solid chemical, the toxicity concern of that chemical is based on the effect concentration. In contrast, the solid chemical may not have effects at saturation (low hazard concern) if the toxicity value is greater than the S_w by at least a factor of 10 (EPA, 2012a; EPA, 2012b). The comparison of acute and chronic effects between the present ocean conditions and climate change conditions of water in Thailand using estimated log K_{ow} at specified condition obtained from this study is shown in Table 6.5.

Table 6.5 ECOSAR™ predicted toxicity of BPA under two ocean conditions of Thailand

Organism	Duration	Toxicity	Predicted Toxicity Values (mg L^{-1})	
			Present Ocean Scenario	Extreme Climate Change Scenario
Fish	96-h	LC_{50}	1.520	2.000
Daphnid	48-h	LC_{50}	6.510	9.273
Green algae	96-h	EC_{50}	1.465	1.712
Fish		ChV	0.664	0.900
Daphnid		ChV	2.221	3.202
Green algae		ChV	0.245	0.278

Comparing the toxicity concentrations of BPA with its S_w, the results show that all toxicity values were less than the S_w. Also, the log K_{ow} of BPA did not exceed the QSAR log K_{ow} cutoff. Thus, the effect concentration of BPA from ECOSAR™ can be used to assess the hazard concern. According to the criteria described in Table 6.3, BPA has moderate toxicity for aquatic organisms in both scenarios since the acute toxicity values were in the range of 1 – 100 mg L^{-1} and ChV values were between 0.1 and 10 mg L^{-1}.

Comparison between the two scenarios found that the aquatic toxicity of BPA under climate change conditions was lower than that in present ocean condition. This was associated with decreasing log K_{ow} in climate change scenario. Despite the lower toxicity, more populations of aquatic organisms are expected to be exposed to BPA because it is predicted to increase in solubility. Moreover, a low-dose effect of BPA has been recently reported (Nagel *et al*., 1997; vom Saal *et al*., 1998; Koch and Calafat, 2009); thus, BPA may have greater possible adverse effects than the model predicts.

6.4 Summary

Global climate change influences ocean conditions (ocean temperature, sea level, and ocean acidity). Because the fate and toxicity of chemicals are associated with physicochemical properties, climate change may affect the environmental behaviour and toxicity of chemicals. In this study, EPI Suite™ was used in order to assess the impact of climate change on the fate, transport and toxicity of BPA in the marine environment. The important partition coefficients (K_{ow}, S_w and K_{oc}) at specified conditions were input into the programme. The coefficients at specified conditions were estimated using the correlation equations between each partition coefficient and water conditions obtained from this study, as described in Chapter 2 – Chapter 5.

According to this study, climate change will result in increasing the amount of BPA in the water compartment and thus its bioavailability. This is associated with decreasing bioaccumulation and volatilisation. Because BPA is more soluble in water, it may be excreted from biota faster. However, greater water solubility and bioavailability will lead to more widespread distribution and more biota will be exposed to BPA. Slightly increased sediment sorption capacity was predicted under the climate change scenario in this study, which results in greater potential exposure of BPA to benthic organisms and increases persistence in marine sediments. The toxicity of BPA is predicted to decrease under climate change conditions as it will be less accumulated in the lipid content of organisms. However, endocrine disruption can occur at concentrations less than those stated for toxicity. Currently, there are arguments about “safe” exposure limits regarding endocrine substances as some researchers observed the adverse effects of BPA at levels lower than what is considered the safe exposure limit at present.

In conclusion, changing environmental conditions affects environmental behaviour and toxicity of chemicals. The modelling programme is useful as a screening tool for environmental and risk assessments. However, to get a reliable and accurate assessments, taking environmental conditions into consideration is necessary. Continuous production and the continuing disposal of plastics will lead to pristine areas being contaminated. The threat of endocrine disruption to all species continues and greater bioavailability means greater physiological adverse effects can be expected with more species than before affected.

Chapter 7

Analysis of Bisphenol A in the Marine Environment

7.1 Background

BPA is an endocrine disrupting chemical that is used widely in many products (EPA, 2010). It can disturb reproduction and development in a variety of species including mussels and lobsters (Biggers and Laufer, 2004; Aarab *et al*., 2006; Canesi *et al*., 2007). BPA is considered an emerging pollutant in the marine environment because it can be released from plastic debris in the ocean and it can persist longer in seawater than freshwater (Kang and Kondo, 2005). Its physical and chemical properties indicate that BPA can dissolve in seawater and sorb to sediment (Staples *et al.*, 1998). Previous Chapters identify the influences of water conditions on the partition coefficients of BPA, and also the potential impact of BPA in the marine environment using environmental modelling. However, the model cannot replace an actual measurement of a chemical in the environment because the values obtained are only predicted values (EPA, 2012b). In aquatic samples, the BPA concentrations reported were in the wide ranges, from ng L^{-1} (ppt) to µg L^{-1} (ppb) for aqueous samples, and ng g^{-1} (ppb) to µg g^{-1} (ppm) for solid samples (EPA, 2000; Staples *et al*., 2000; Belfroid *et al.*, 2002; Basheer *et al*., 2004; Luo *et al*., 2014). Thus, this study aims to set in place a robust, reliable and effective analytical method to enable the determination BPA concentrations in marine environmental samples.

An environmental sample may have a complex matrix, resulting in a lot of interference in sample extracts. Consequently, the results from column chromatography can be poorly resolved due to the separation capacity of a column being exceeded or co-elution whereby the signal for the target compound is masked by interfering compounds. One or more clean-up steps are necessary for environmental analysis in order to remove non-target compounds, particularly for complex matrix samples (Lucci *et al.*, 2012).

The analytical instrument used in this study was a conventional HPLC coupled with a UV detector (Section 3.2.2). This instrument is simpler and cheaper than some other advanced chromatographic separations such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) techniques. HPLC has less sensitivity and less selectivity than these other methods as it identifies compounds by retention time (RT). MS techniques use the mass of a chemical for identification and are less prone to interferences. HPLC is unable to analyse samples that have interference peaks arising at the same retention time as the target analyte and is more prone to these interferences when analysing environmental samples. Therefore, effective sample preparation is needed to purify samples and concentrate target compounds to enable effective analysis of BPA at very low concentrations with HPLC (Lucci *et al.*, 2012).

Ideally, sample preparation methods should be fast, accurate, precise, inexpensive, easy to operate, and consume low volumes of solvent and sample (Lucci *et al.*, 2012). In this study, three sample treatment techniques were used in the analysis of BPA in marine environmental samples (*i.e.*, natural seawater and sediment from Prestwick, UK). These techniques were solid-phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME), and molecularly imprinted solid-phase extraction (MISPE). For aqueous samples, SPE, DLLME, and MISPE were used as the sample preparation method prior to HPLC-UV analysis. For sediment samples, accelerated solvent extraction (ASE) was used to extract BPA from samples, which were subsequently cleaned-up and concentrated by MISPE prior to HPLC-UV analysis.

7.1.1 Solid-Phase Extraction (SPE)

The solid-phase extraction (SPE) technique is the most popular sample preparation method for environmental samples. It is an alternative sample treatment technique to the classical method of liquid-liquid extraction (LLE) that has become more popular and developed due to its advantages over LLE. SPE uses smaller volumes of organic solvents and it is faster than LLE (Hennion, 1999). There are also many types of commercially available sorbent that suit many applications. The mechanism of action of SPE involves the partitioning between a liquid phase that initially contains analytes and a solid sorbent phase that will take up the analytes (Lucci *et al.*, 2012). The basic SPE procedure consists of four steps: conditioning, loading, washing, and elution (Figure 7.1).

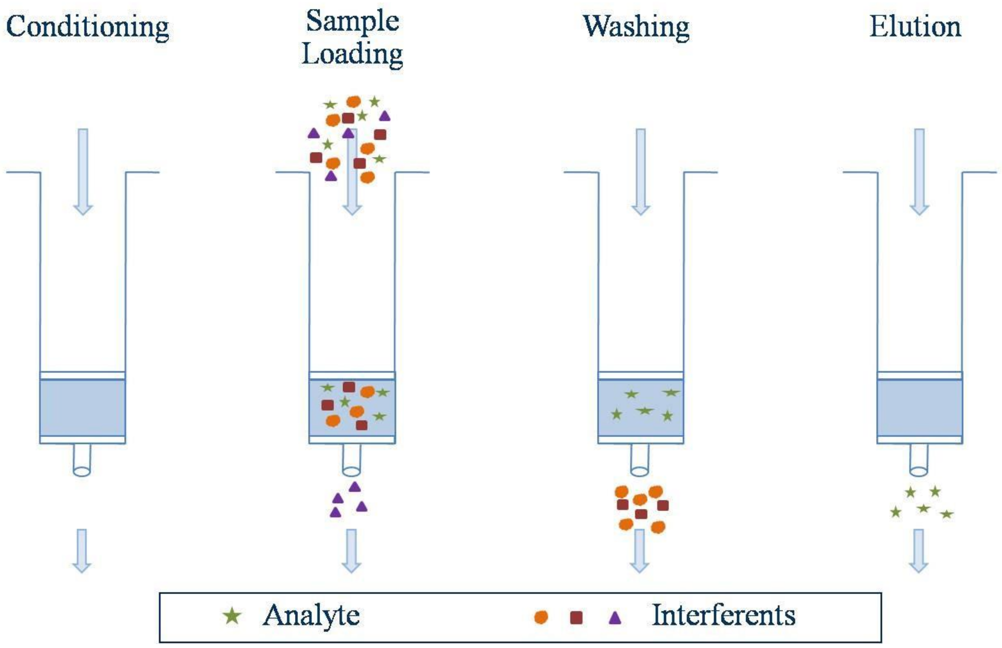


Figure 7.1 General SPE procedure (modified from Lucci *et al.*, 2012)

The purpose of the conditioning step is to wet the sorbent bed and, where appropriate, to activate the functional groups of the sorbent bed to make them ready to interact with sample. Loading of the sample onto the sorbent follows conditioning. During this step, the target analytes and some interferences are retained on the sorbent. Thirdly, the retained impurities are removed by appropriate washing solutions. The washing solutions should be strong enough to remove the impurities and weak enough to allow the sorbent to retain the target analytes. Lastly, the target analytes are eluted using appropriate solvents. The eluting solvents should be selective enough to not elute any strongly retained impurities from the sorbent (Lucci *et al.*, 2012).

Many researchers have used SPE for the extraction, concentration, and clean-up of BPA from food and environmental samples (Pedersen and Lindholst, 1999; VegaMorales *et al*., 2010) as well as many others target analytes. For example, Pedersen and Lindholst (1999) used SPE (a Sep-Pak C18 extraction tube) coupled with LCMS for the quantification of the xenoestrogens 4-*tert*-octylphenol (tOP) and BPA in water. Ahn *et al.* (2007) developed HLB-SPE (hydrophilic-lipophilic balanced copolymer (HLB) sorbent with a poly(divinylbenzene-co-*N*-vinylpyrrolidone) phase and Florisil-solid-phase extraction (SPE)) cartridges coupled with freezing-lipid filtration to extract and purify nine endocrine-disrupting phenols (eight alkylphenols and BPA) from fish followed by GC-MS for analysis. Zhao *et al*. (2009) determined BPA, nonylphenol and 4-octylphenol from river water and seawater samples by using microporous bamboo charcoals as the SPE sorbent along with HPLC-UV.

One of the key factors of an SPE method is selecting the most suitable SPE cartridge for any given target analyte and sample. As the examples shown above illustrate, several commercially available SPE cartridges have been used for the determination of BPA. For BPA analysis, Shao *et al*. (2005) evaluated the efficiency of three commercial SPE cartridges for use as a pre-concentration technique prior to quantification of BPA in beverage samples (drinking water and soda beverages) with liquid chromatography-electrospray ionisation tandem mass spectrometry (LC-ESIMS/MS). These cartridges were Sep-Pak octadecylsilica cartridges with 500 mg materials (Sep-Pak C18), Oasis® HLB solid-phase extraction cartridges with 200 mg *N*-vinylpyrrolidone-divinylbenzene copolymer (Oasis® HLB), and Supleclean ENVICarb solid-phase extraction cartridges (GCB). The results indicated that Oasis® HLB provided the highest recovery for BPA. Also, Basheer *et al.* (2004) used Oasis® HLB cartridges to clean-up extracts prior to injection into GC-MS for the measurement of alkylphenols and BPA in coastal waters and supermarket seafood (*e.g.*, prawn, crab, squid, fish) from Singapore.

In accordance with previous studies, the sample preparation for this study used 6 CC polypropylene Oasis® HLB cartridges packed with 200 mg sorbent. The optimum SPE conditions for the analysis of BPA in water samples were developed and modified mainly from the study of Shao *et al.* (2005).

7.1.2 Dispersive Liquid-Liquid Microextraction (DLLME)

The dispersive liquid-liquid microextraction (DLLME) method was introduced by Rezaee *et al.* (2006) as a powerful technique to extract organic compounds from water samples. The DLLME method uses syringes to rapidly inject the appropriate mixture of extraction solvent and disperser solvent into an aqueous sample to form a cloudy solution. It has become popular because it is simple; rapid; inexpensive; uses small amounts of sample and solvents; and has high recovery and sensitivity (Rezaee *et al.*, 2006; Herrera-Herrera *et al.*, 2010). There are two steps in the DLLME method (Rezaee *et al.*, 2010; Zgola-Grześkowiak and Grześkowiak, 2011):

(i) A mixture of disperser and extraction solvents is injected rapidly into an analyte-containing water sample. As a result of this step, a cloudy solution (dispersion) is formed and analytes are extracted from the water sample.

(ii) The cloudy solution is centrifuged, and then the solid phase (containing analytes) is collected by syringe for analysis.

The basic steps of DLLME are shown in Figure 7.2. The key factors to consider in the DLLME technique are appropriate extracting solvent and disperser solvent, and also the volumes of these two solvents (Rezaee *et al*., 2010).

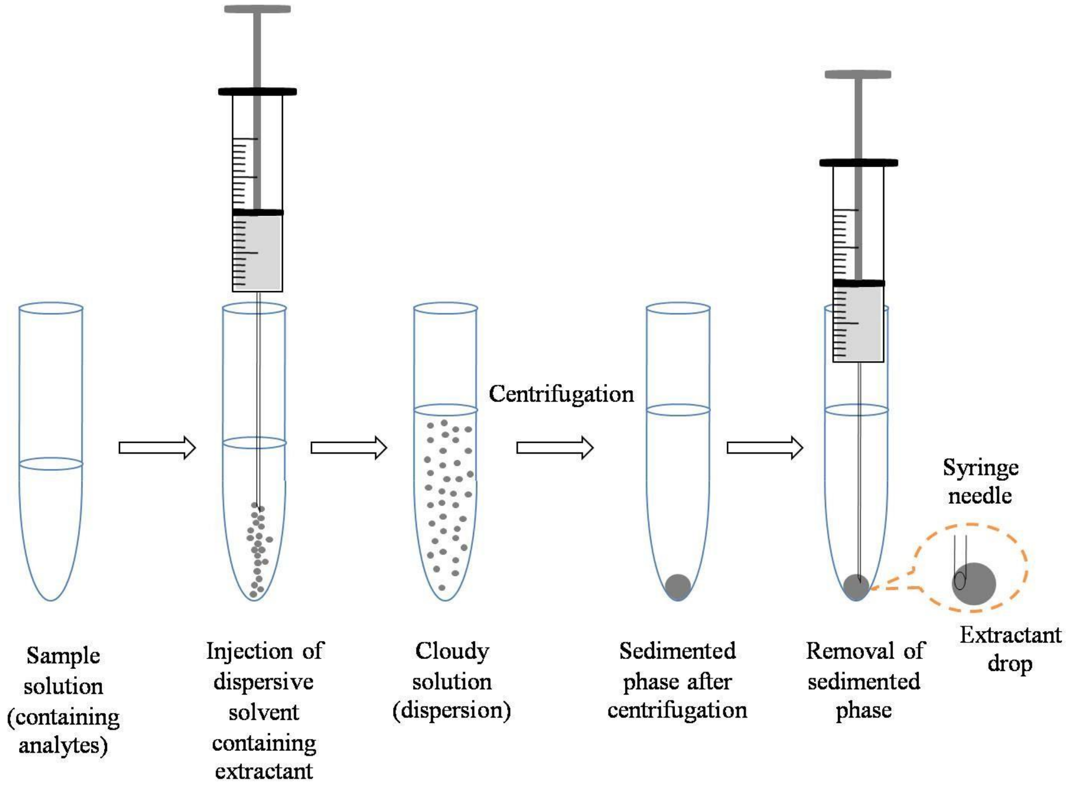


Figure 7.2 The basic DLLME procedure

(modified from Zgola-Grześkowiak and Grześkowiak, 2011)

This study determined BPA in aqueous samples using the DLLME method coupled with an HPLC-UV method that was developed and optimised by Rezaee *et al*. (2009). The optimum conditions involved a mixture of 2 mL of acetone as the disperser solvent containing 142 µL of chloroform as the extracting solvent.

7.1.3 Molecularly Imprinted Solid-Phase Extraction (MISPE)

The selectivity of the sorbent is an important factor contributing to a satisfactory sample treatment step by SPE. A selective clean-up step reduces the effects of impurities in complex samples, resulting in higher efficiencies. To realise this, SPE based on molecularly imprinted polymer (MIP) sorbents, known as MISPE, has been an interesting development. Recently, MISPE has been developed and applied as a pre-treatment step in the determination of target compounds in drug, food, and environmental and biological samples (He *et al.*, 2007; Pichon, 2007; Tamayo *et al.*, 2007; Pichon and Chapuis-Hugon, 2008; Turiel and Martín-Esteban, 2010; Lucci *et al.*, 2012).

7.1.3.1 Molecularly Imprinted Polymers (MIPs)

MIPs are synthetic organic macromolecules with pre-determined molecular recognition properties that render them capable of binding to target molecules. MIPs are obtained by template-directed polymerisation, which uses a molecular template (T) to organise functional monomer (FM) residues into well-defined positions within a three-dimensional porous macromolecule. A typical molecular imprinting process commences by combining the template and functional monomers into a templatemonomer assembly by covalent and/or non-covalent chemical bonds. The next step is polymerisation of the template-monomer assembly with an excess of a crosslinking agent (X) in a solvent, also called porogen. The role of the solvent is to solubilise the template-monomer assembly, crosslinking agent, and free radical initiator (I) and, if necessary, stabilise the assembly. The product of the crosslinking reaction is a porous, crosslinked macromolecule. The template is removed from the polymers network to reveal binding sites with shape, size, and chemical functionality complementary to the target compound. As a result, MIPs are molecularly selective for the target compound. The resulting MIPs are stable and durable to a wide range of pH values, solvents and temperature. Moreover, MIP production is cheap, simple and easy (Cormack, 2004).

In terms of templates, the most effective templates tend to be lower molar mass (< 500 Da) that are able to interact strongly with functional monomers. In cases where the template is not the target analyte, the template must have shape, size, and chemical functionality similar to the target analyte. The functional monomers, crosslinkers, free radical initiators, and solvents are chosen based upon the nature of the template (solubility, stability, chemical functionality, *etc*.), as well as the physicochemical properties desired from the imprinted product (Cormack, 2004; Pichon and Chapuis-Hugon, 2008).

MIPs are used widely in many applications, for example in analytical chemistry (*e.g.*, HPLC), biomimetic sensors, drug discovery, affinity separation, and catalysis (Kawaguchi *et al*., 2005; Jiang *et al*., 2006; He *et al*., 2007; Pichon and ChapuisHugon, 2008; Lin *et al*., 2012). The use of MIPs as selective sorbent materials in solid-phase extraction (SPE) has been commercialised (Biotage, 2014; Polyintell, 2014). In recent years, molecularly imprinted solid-phase extraction (MISPE) has been developed extensively and used as a sample-preparation method prior to the final determination of analytes (Martín-Esteban, 2013).

7.1.3.2 Synthesis of MIPs

There are three different synthetic approaches to MIPs that depend on the templatefunctional groups interactions. These are the Covalent Approach, the Non-Covalent Approach, and the Semi-Covalent Approach (Caro *et al*., 2002; Cormack, 2006).

Covalent Approach

The covalent approach, introduced by Wulff and Sarhan (1972), involves the formation of labile covalent bonds between template and functional monomers. Following the synthesis of the polymer, which involves copolymerisation of the polymerisable template with an excess of a crosslinking agent in solvent, the template is removed from the polymer by cleavage of the reversible covalent bonds that bind the template to the polymer. These covalent bonds are reformed upon rebinding of the template (analyte). The template-monomer interaction is covalent in nature, leading to a relatively homogenous population of binding sites and, in principle, no non-specific sites. However, the covalent approach has not been used as routine because of limited opportunities for the formation of reversible covalent bonds between the template and monomer complex under mild conditions.

Non-Covalent Approach

The non-covalent approach, introduced by Arshady and Mosbach (1981), involves the formation of non-covalent bonds, including electrostatic forces, hydrogen bonding and hydrophobic effects, between template and functional monomers before polymerisation. This method is used as a routine method for the preparation of MIPs as it is rather simple and there are many functional monomers available commercially. However, the non-covalent approach normally has the disadvantage that an excess of functional monomer is required. Excess functional monomer leads to excess of functional monomer residues polymerised at the final product and formation of non-selective binding sites.

Semi-Covalent Approach

Finally, the semi-covalent approach is an intermediate option that was developed by combining the advantages of the covalent approach with the advantages of the noncovalent approach (Whitcombe *et al.*, 1995). The template and functional monomers are brought together by labile covalent bonds; therefore, there is no redundancy of monomers in the product. After polymerisation, the labile covalent bonds are broken, thereby releasing the template. However, the template rebinding is based only on non-covalent bonds.

In the present study, the non-covalent approach was used to prepare MIPs for BPA due to the simplicity of the approach. The prepared in-house MIPs were synthesised by two simple free radical polymerisation techniques to give either imprinted polymer monoliths or imprinted polymer microspheres, the latter *via* a precipitation polymerisation (PP) method.

Conventional Free Radical Solution Polymerisation (Monolith Production)

Due to its simplicity, the polymer monolith method is the most commonly used MIP synthesis method. The copolymerisation of an excess of crosslinking agent with functional monomer(s) in a relatively low volume of porogen provides a highly crosslinked monolithic polymer. The monolith needs to be crushed, ground and sieved to yield MIP particles with a desired particle size range. The small particles (fines) can be removed by sedimentation. Although this method is simple, there are some drawbacks. It is time-consuming, wasteful (typically less than 50% yields of useable particles), and provides irregularly sized and shaped particles due to the grinding process. The particle size and shape are important parameters for some applications, including SPE and HPLC. A convenient particle size range for SPE is 25 – 38 µm. The particle shape is not a real limitation for off-line SPE, but it is more important in LC applications since irregular particles can cause broad asymmetric peaks. Thus, other polymerisation methods have been developed in order to deliver spherical, monodisperse MIP particles (Wang *et al.*, 2003; Pichon and ChapuisHugon, 2008).

Precipitation Polymerisation (PP)

MIP microspheres can be prepared by the precipitation polymerisation (PP) technique, which is related to the polymerisation of monomers in dilute solution (< 5% (w/v)). The spherical particles arise since the polymer chains formed during the polymerisation process become insoluble, precipitate and grow radially. This method is attractive due to its simplicity and its ability to deliver high quality imprinted spherical particles.

Moreover, it is possible to control the size and porosity of the MIPs by controlling the polymerisation conditions. However, this method uses up to 50x larger volume of porogen than what would be used in the monolith method. The diameters of the particles produced by the PP method are generally in the range 0.1 - 10 µm, which makes them well suited for many applications, including SPE. Divinylbenzene (DVB) has been introduced as a good crosslinker for PP, producing monodisperse MIPs with diameters of around 5 µm (Downey *et* al., 1999; Wang *et al*., 2003).

In recent years, many researchers have produced selective bisphenol A-molecularly imprinted polymers (BPA-MIPs) to use as packing materials in SPE and HPLC, and as recognition elements in sensors (Sanbe *et al*., 2003; Navarro-Villoslada *et al*., 2004; Kawaguchi *et al*., 2005; Jiang *et al*., 2006; Cela-Pérez *et al*., 2011). BPA is a good template for imprinting because it has a low molar mass (228.29 Da), two phenolic groups, and is soluble in a range of organic solvents. The MISPE technique has become widely developed to analyse BPA in biological and environmental samples due to its advantages over conventional SPE (Kawaguchi *et al*., 2005; Zhang *et al*., 2006; Jiang *et al*., 2007; Sasaki *et al*., 2010; Lin *et al*., 2012; da Silva *et al*., 2012; Shareena and Faizal, 2012). Table 7.1 shows some examples of MISPE for the analysis of BPA in biological and environmental samples.

Additionally, MISPE cartridges for BPA are available commercially from Polyintell (Polyintell, France), and are called AFFINIMIP® SPE Bisphenol A. However, to the best of our knowledge, the effectiveness of an AFFINIMIP® SPE Bisphenol A-based method to determine BPA in marine samples has not been reported so far (Polyintell, 2014).

Table 7.1 Relevant MISPE Applications for BPA

Template	Monomer	Crosslinker	Porogens	Polymerisation Method	Samples	Reference
BPA	4-VP	EGDMA	Toluene	Multi-step swelling and polymerisation	River water	Sanbe *et al.*, 2003
BPA-d_{16}	4-VP	EGDMA	Toluene	Suspension polymerisation	River water	Kawaguchi *et al.*, 2005
BPA	4-VP	TRIM	Acetonitrile	Precipitation polymerisation	Tap and lake water	Jiang *et al.*, 2006
BPA	4-VP	TRIM	Acetonitrile : Toluene	Precipitation polymerisation	Human serum, pig urine, tap water and shrimp	Zhang *et al.*, 2006

VP = 4-vinylpyridine; EGDMA = ethylene glycol dimethacrylate; TRIM = trimethylolpropane trimethacrylate

In the present study, BPA-MIPs were synthesised *via* non-covalent imprinting methods using both monolith and PP methods. In order to evaluate the selectivity of the BPA-

MIPs, non-imprinted (control) polymers (NIPs) were synthesised alongside the MIPs. The polymers were then packed into SPE cartridges. Suitable SPE conditions were evolved and optimised to determine satisfactory extraction efficiency for BPA in real environmental marine samples. The efficiencies of two inhouse produced polymers were compared with the AFFINIMIP® SPE Bisphenol A material (Polyintell, France).

3 mL polypropylene AFFINIMIP® SPE Bisphenol A cartridges were used in this study. Each cartridge contains 100 mg of MIP sorbent with a particle diameter range of 25 – 80 µm. The template used in the production of these MIPs was a BPA analogue in order to circumvent template bleeding issues (Polyintell, 2014).

In summary, three types of MISPE cartridges, two in-house MISPE cartridges and one commercial MISPE cartridge, were used to extract BPA from marine samples in this study.

7.1.4 Accelerated Solvent Extraction (ASE)

The pressurised liquid extraction (PLE) technique termed accelerated solvent extraction (ASE) has been used widely to extract analytes including BPA from solid matrices due to its simplicity, effectiveness and broad-range of applications (Belfroid *et al*., 2002; Carabias-Martínez *et al*., 2006; Shao *et al*., 2007; Ballesteros-Gómez *et al*., 2009). For instance, Shao *et al*. (2007) used ASE to extract alkylphenols and BPA from different meats (*i.e*., pork, fish, rabbit, duck and chicken), cleaned-up the extracts with amino-propyl solid-phase extraction cartridges, and finally quantified with LC-ESI-MS/MS. Therefore, this study used ASE to extract BPA from marine sediments prior to clean-up with MISPE and quantification with HPLC-UV.

To get satisfactory extraction efficiencies, ASE requires the use of appropriate extraction solvents, extraction temperatures, extraction times, and number of static cycles. Carabias-Martínez *et al*. (2006) developed an ASE extraction method for the determination of endocrine-disrupting compounds, including BPA, along with SPE and LC-ESI-MS. This optimised ASE method was used in the present study.

This Chapter aims to deliver a satisfactory sample preparation method to enable the measurement of BPA concentrations in marine environmental samples by HPLCUV. SPE, DLLME, and MISPE techniques were used in the determination of BPA in aqueous samples. The sample treatment efficiencies of these methods were then compared. For MISPE, in-house MISPE cartridges were produced and compared with commercial MISPE cartridges. Furthermore, BPA in solid samples was determined by using ASE and MISPE as the sample preparation method.

7.2 Experimental

This study was carried out by using three different sample pre-treatment techniques prior to HPLC-UV analysis (for the HPLC method used, see Section 3.2.2). For aqueous samples, experiments commenced using deionised water samples (Barnstead Nanopure, Triple Red Limited, UK) before moving to field-obtained seawater samples (NSW), which are more complex. ASE together with MISPE was tested as the sample pre-treatment method for real marine sediment. Marine samples (both seawater and sediment) were collected from Prestwick, UK (see location details in Section 3.2.5).

7.2.1 Conventional Solid-Phase Extraction (SPE)

Samples were spiked with BPA concentrations range from 1 – 500 µg L^{-1}. A 50 mL to 200 mL volume of sample was used; the volume of sample used depended upon the BPA concentration. Spiked samples were acidified to pH 3 with 10 mM ammonium formate buffer, pH 3 (Carabias-Martínez *et al*., 2006). The 10 mM ammonium formate buffer was prepared from ammonium formate and formic acid obtained from Sigma-Aldrich (UK). Dichloromethane (DCM), methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (UK). Before loading of the sample, an Oasis® HLB 6 CC cartridge (Waters, UK) was conditioned sequentially with 10 mL dichloromethane-methanol (DCM:MeOH, 80:20 (v/v)), 10 mL methanol, and 10 mL water with 10 mM ammonium formate buffer, pH 3. The acidified sample (pH 3) was then passed through the SPE cartridge (Shao *et al*., 2005). After that, the interferences were washed away with 3 mL 10% methanol in water, and dried the cartridge (Ballesteros *et al*., 2006). The analyte was eluted with 5 mL dichloromethane-methanol (80:20 (v/v)) (Shao *et al*., 2005). The eluate was dried under a gentle stream of nitrogen, and reconstituted with 1 mL mobile phase (water-acetonitrile (55:45 (v/v)). The resulting solution was filtered using a 0.45 µm filter (Sigma-Aldrich, UK) and injected onto the HPLC.

7.2.2 Dispersive Liquid-Liquid Microextraction (DLLME)

The DLLME method was carried out by using a method developed by Rezaee *et al*. (2009). HPLC grade organic solvents (acetone, chloroform and methanol) were obtained from Fisher Scientific (UK). A 10.0 mL volume of spiked sample (1 – 1000 µg L^{-1} of BPA) was placed in a 50 mL tube with a conical bottom. A 5.0 mL gastight syringe was used to rapidly inject 2.0 mL acetone, as disperser solvent, containing 142 µL of chloroform, into the sample solution, and the solution mixed thoroughly by vortex. The cloudy solution produced was centrifuged for 15 min at 3,800 rpm. Then, the sediment phase was transferred into another test tube and the solvent evaporated by a heating block (80 °C) and with a gentle nitrogen purge. The residue was dissolved in 30 µL methanol. The solution was then filtered with a 0.45 µm filter, and injected onto the HPLC.

7.2.3 Molecularly Imprinted Solid-Phase Extraction (MISPE)

Polymer synthesis and characterisation was performed at the Department of Pure and Applied Chemistry, University of Strathclyde, whereas the SPE cartridge packing steps and HPLC analyses were carried out at the Department of Civil and Environmental Engineering, University of Strathclyde.

7.2.3.1 Synthesis of Bisphenol A-Molecularly Imprinted Polymers (BPA-MIPs)

BPA-MIPs and NIPs were synthesised using precipitation polymerisation (PP) and conventional polymerisation (monolith). All chemicals and solvents used in the polymerisations were purchased from Sigma-Aldrich, UK. BPA was used as the template (T). Divinylbenzene-80 (DVB-80, containing 80% DVB isomers and 20% ethyl vinylbenzene) and 4-vinylpyridine (4-VP) were the crosslinker (X) and monomer (FM), respectively. DVB-80 and 4-VP were purified prior to use by passing through a short column of neutral alumina. AIBN (2,2’-azo*bis*isobutyronitrile) was the initiator and it was recrystallised from acetone at low temperature before use. BPA, 4-VP, DVB-80 and AIBN were dissolved in a mixture of acetonitrile and toluene (3:1 (v/v)) in the reaction vessels, which were either Nalgene® or Kimax® culture tubes (see details of polymerisation conditions in Table 7.2). The solution was degassed to remove dissolved oxygen gas (Cormack and Elorza, 2004). The deoxygenation processes were carried out in an ultrasonic bath for 10 min, and the monomer solutions purged with oxygen-free nitrogen while cooling on an ice bath for 10 min. The reaction vessels were then sealed with a screw cap and put in a Stuart Scientific S160 incubator (Surrey, UK) coupled with a Stovall low-profile roller system (USA) for PP or oil bath for monolithic polymers. The temperature was ramped from room temperature to 60 ˚C within 2 h, and then held at 60 ˚C for 24 or 48 h. The PP polymers were separated from the reaction medium by filtration on a 0.45 µm nylon membrane filter, washed with 100 mL acetonitrile and 100 mL methanol, sequentially, to remove the template and unreacted monomers. Finally, the polymers were dried in a vacuum oven (Townson & Mercer Limited, England) overnight at 70 ˚C and 60 mbar before yields were determined. The monolithic polymers were manually crushed, ground using Fritsch Pullveristette metal ball mill, and manual wet sieved in acetone to get the require size range (25 – 38 µm). Template and impurities were removed from the polymers using Soxhlet extraction (500 mL ethanol, overnight). To prevent problems arising from small particles in SPE, fine particles were removed from the acetone by sedimentation. Air-dried polymers were then dried in a vacuum oven overnight at 70 ˚C and 60 mbar prior to characterisation.

Table 7.2 Overview of polymerisation conditions

Polymer Code	Type of Polymer	T	FM (Mole Ratio)	X	Monomer Concentration (% (w/v))	Initiator Concentration (mol %)	Type of Reaction Vessel	Monomer Scale (g)	% Yield (%)
SB1	NIP	-	4	20	2	2	Nalgene®	4	65
SB2	MIP	1	4	20	2	2	Nalgene®	4	64
SB3	NIP	-	4	20	4	3	Nalgene®	4	84
SB4	MIP	1	4	20	4	3	Nalgene®	4	86
SB5p	MIP	0.5	2	20	4	3	Glass	1	87
SB6p	MIP	0.25	1	20	4	3	Glass	1	85
SB7p	MIP	0.1	0.4	20	4	3	Glass	1	88
SB8	NIP	-	2	20	4	3	Nalgene®	4	86
SB5	MIP	0.5	2	20	4	3	Nalgene®	4	88
SB9	NIP	-	2	20	4	3	Glass	1	78
SB10	MIP	0.5	2	20	4	3	Glass	1	72

T = template; FM = functional monomer; X = crosslinker; NIP = non-imprinted polymer; MIP = molecularly imprinted polymer

Table 7.2 Overview of polymerisation conditions (continued)

Polymer Code	Type of Polymer	T	FM Mole Ratio)	X	Monomer Concentration % (w/v))	Initiator Concentration mol %)	Type of Reaction Vessel	Monomer Scale (g)	% Yield %)
SB11	NIP	-	4	20	4	3	Glass	1	75
SB12	MIP	1	4	20	4	3	Glass	1	76
SB13	NIP	-	2	20	75	3	Glass	5	40
SB14	MIP	0.5	2	20	75	3	Glass	5	41

T = template; FM = functional monomer; X = crosslinker; NIP = non-imprinted polymer; MIP = molecularly imprinted polymer

7.2.3.2 Characterisation of Polymers

In this study, the polymers synthesised in-house were characterised in terms of their chemical and morphological properties, in the following manner.

The shape and size of polymers was determined using an optical compound microscope (Olympus, Germany) and a scanning electron microscope (SEM). The SEM used was a Cambridge Instruments Stereoscan 90. The SEM samples were coated with argon gas using Polaron SC500 Sputter Coater prior to imaging.

Nitrogen sorption porosimetry was performed using a Micromeritics ASAP 2000 instrument. This technique provides the specific surface area (m^2 g^{-1}), specific pore volume (mL g^{-1}) and average pore diameter and pore size distribution by measuring the amount of nitrogen gas uptake as a function of pressure and the application of the Brunauer-Emmett-Teller (BET) theory (Cormack and Elorza, 2004). Prior to analysis, samples of around 0.3 – 0.4 g were degassed overnight at 100 °C under vacuum.

Fourier-Transform Infra-Red (FT-IR) spectroscopy analysis was carried out on an Agilent 5500a FT-IR with ATR. The 4000 – 700 cm^{-1} scanning range in transmission mode was used.

The % mass of carbon (C), hydrogen (H) and nitrogen (N) in the polymer samples was measured by elemental microanalysis using a Perkin Elmer 2400 Series II analyser at the University of Strathclyde Microanalysis Service.

7.2.3.3 Optimum Conditions for MISPE

The optimum conditions for AFFINIMIP® SPE Bisphenol A (Polyintell, France) were recommended by the company, and are as follows. Firstly, the AFFINIMIP® SPE Bisphenol A (3 mL polypropylene cartridge) was conditioned sequentially with 5 mL methanol-2% acetic acid, 5 mL acetonitrile, and 5 mL water. Then, 10 mL of a BPA-containing aqueous sample was passed through the cartridge. After that, the cartridge was washed with 10 mL water and 6 mL water:acetonitrile (60:40 (v/v)), consecutively. After drying for 30 seconds, the analyte was eluted by 3 mL methanol. The eluate was dried under a gentle nitrogen stream, and reconstituted with 1 mL mobile phase (water:acetonitrile (55:45 (v/v)). Then, the solution was filtered using a 0.45 µm membrane filter prior to HPLC analysis.

In-house polymers (100 mg), both MIPs and NIPs, were mixed with methanol and packed into 6 mL polypropylene SPE cartridges between two frits (Sigma-Aldrich, UK). For the monolith-derived polymers, polyethylene (PE) frits (20 µm pore size) were used, while stainless steel frits (diameter ½ inch, pore size 2 µm) were used for the polymer microspheres. Then, the MISPE cartridges were washed with 200 mL methanol-2% formic acid and 200 mL methanol to remove any remaining template and impurities, and dried in air before use. The optimum conditions for the use of the in-house MISPE cartridges were obtained by modifying the second washing step recommended for the commercial MISPE cartridge. 3 mL of a mixture of water and acetonitrile (60:40 (v/v)) was used instead of 6 mL.

All chemicals used were HPLC grade. Formic acid, 0.45 µm syringe filters, PE frit (20 µm pore size) and stainless steel frits (diameter ½ inch, pore size 2 µm) were purchased from Sigma-Aldrich (UK), while other chemicals (methanol, acetonitrile and acetic acid) were purchased from Fisher Scientific (UK).

7.2.4 Accelerated Solvent Extraction (ASE)

The ASE conditions used in this study were modified from a procedure detailed in Carabias-Martínez *et al.* (2006). A 1 mg kg^{-1} BPA-spiked sample was prepared using a marine sediment sample obtained from Prestwick, UK. The bottle containing the spiked sample was capped, covered with aluminum foil, and stored in the dark for approximately 12 h before extraction. 2.5 g of sample was weighed in a small beaker and thoroughly mixed with diatomaceous earth (Thermo Fisher Scientific Inc., UK) and packed into a 10 mL extraction cell. Two glass fibre filters were placed at the bottom of the cell and one glass fibre filter at the top. Methanol (Fisher, UK) was used as the extraction solvent. Accelerated solvent extraction using an ASE 350 system (Dionex, Camberley, UK) was performed at a fixed pressure of 1500 psi, using two cycles of 10 min at 120 °C. The methanolic ASE extract was evaporated using a Büchi Syncore® Analyst (Oldham, UK) and nitrogen purge until dry. Then, the sample was reconstituted with 10 mL of deionised water (Barnstead Nanopure, Triple Red Limited, UK) prior to clean-up with MISPE followed by HPLC analysis.

7.3 Results and Discussion

7.3.1 Synthesis of Polymers

Due to the advantages of precipitation polymerisation (PP) over the conventional method of MIP production (monolith synthesis), such as the facts that PP is less time-consuming, easier and gives higher yields of useful particulate products, PP was used to deliver the most uniform MIP particles used in this study. Monolithic polymers were prepared using nominally similar synthesis conditions. BPA and 4vinylpyridine (4-VP) formed a template-monomer assembly, and AIBN was used as an initiator. Then, the BPA-4-VP assembly was copolymerised with an excess of crosslinking agent (DVB-80) in a mixed solvent system (acetonitrile:toluene, 3:1 (v/v)). When the polymerisation process was complete, BPA was extracted from the imprinted polymers by washing with solvents or by Soxhlet extraction (Figure 7.3).

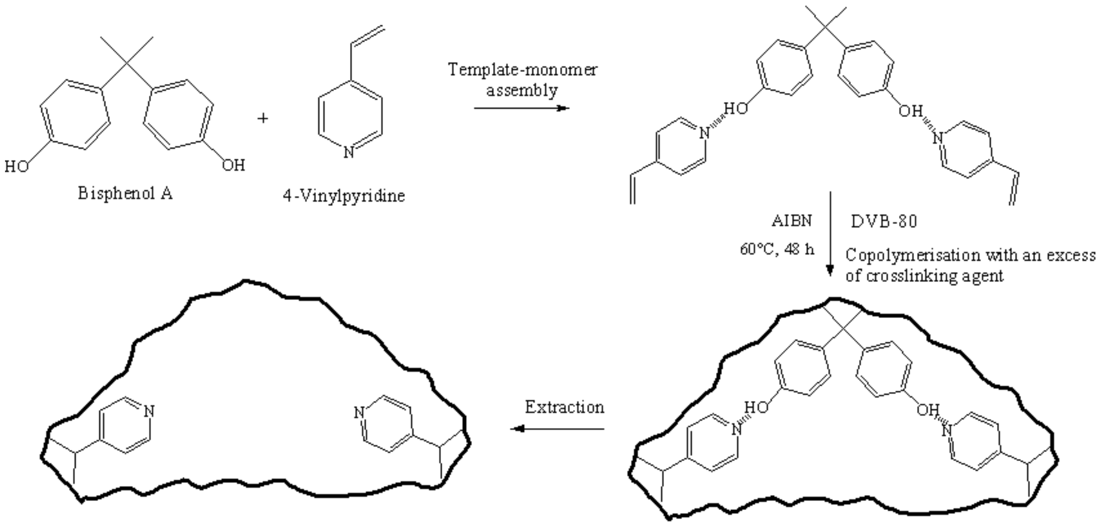


Figure 7.3 A schematic representation of the molecular imprinting of BPA

The mixture of acetonitrile and toluene (3:1 (v/v)) was used to prepare porous MIPs with reasonable specific surface areas (Li and Stöver, 1998).

To deliver suitable molecularly imprinted polymer microspheres for SPE, the T:FM:X ratio, monomer concentration, initiator concentration, and type of reaction vessel were varied systematically (Cormack and Elorza, 2004). The synthesis conditions used, morphology, and % yield for each of the polymers synthesised in this study are shown in Table 7.3.

Table 7.3 Synthetic overview of polymers synthesised

Polymer Code	Type of Polymer	T	FM	X	Monomer Concentration (% (w/v))	Initiator Concentration (mol %)	Type of Reaction Vessel	Monomer Scale (g)	Shape of Polymers (Microscope)	Particle Size (µm)	% Yield (%)
		(Mole Ratio)									
SB1	NIP	-	4	20	2	2	Nalgene®	4	Irregular	< 1	65
SB2	MIP	1	4	20	2	2	Nalgene®	4	Irregular	<1	64
SB3	NIP	-	4	20	4	3	Nalgene®	4	Beads	1 – 2.5	84
SB4	MIP	1	4	20	4	3	Nalgene®	4	Beads	1 – 2.5	86
SB5p	MIP	0.5	2	20	4	3	Glass	1	Beads	5	87
SB6p	MIP	0.25	1	20	4	3	Glass	1	Beads	2.5 – 10	85
SB7p	MIP	0.1	0.4	20	4	3	Glass	1	Beads	5 – 10	88
SB8	NIP	-	2	20	4	3	Nalgene®	4	Beads	0.8 – 2	86
SB5	MIP	0.5	2	20	4	3	Nalgene®	4	Beads	0.8 – 1	88
SB9	NIP	-	2	20	4	3	Glass	1	Beads	2.5 – 8	78
SB10	MIP	0.5	2	20	4	3	Glass	1	Beads	3 – 5	72

T = template; FM = functional monomer; X = crosslinker; NIP = non-imprinted polymer; MIP = molecularly imprinted polymer

Table 7.3 Synthetic overview of polymers synthesised (continued)

Polymer Code	Type of Polymer	T	FM	X	Monomer Concentration (% (w/v))	Initiator Concentration (mol %)	Type of Reaction Vessel	Monomer Scale (g)	Shape of Polymers (Microscope)	Particle Size (µm)	% Yield (%)
			(Mole Ratio)								
SB11	NIP	-	4	20	4	3	Glass	1	Beads	2.5 – 5	75
SB12	MIP	1	4	20	4	3	Glass	1	Beads	<1 – 2.5	76
SB13	NIP	-	2	20	75	3	Glass	5	Irregular*	25 - 38	40
SB14	MIP	0.5	2	20	75	3	Glass	5	Irregular*	25 - 38	41

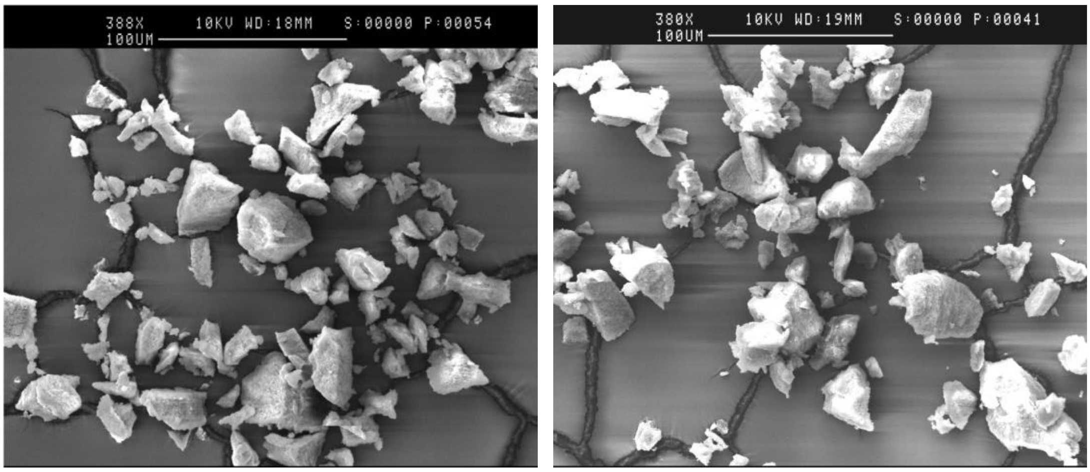
T = template; FM = functional monomer; X = crosslinker; NIP = non-imprinted polymer; MIP = molecularly imprinted polymer

The results show that increasing the monomer concentration from 2 to 4 % (w/v) whilst also increasing the AIBN concentration from 2 mol % to 3 mol % provided larger microspheres as well as a higher yields (> 70%) for both NIPs and MIPs (Wang *et al*., 2007). The initial polymers obtained were too small for SPE work so the T:FM:X ratios were changed and the polymerisations conducted on a smaller scale (SB5p, SB6p and SB7p). The mole ratio of 1:4:20 is a common ratio used in non-covalent imprinting. The results indicated that the decreasing amount of template relative to FM and X resulted in bigger particles. However, fewer recognition sites would also be anticipated (Cormack and Elorza, 2004). Thus, the conditions used in the preparation of SB5p were used in the following syntheses. The experiments also revealed that the type of reaction vessel used affected the outcomes of the polymerisation processes. Glass vessels (Kimax® culture tubes) provided better quality polymers compared to Nalgene® bottles. Thus, SB9 (NIP), SB10 (MIP) and SB5p were characterised fully. SB9 and SB10 were used in the MISPE experiments to evaluate their extraction efficiencies and the differences between nonimprinted polymers and imprinted polymers.

Optimised conditions were then used in conventional polymerisations to yield monolithic polymers which were ground subsequently into polymer particles, SB13 (NIP) and SB14 (MIP). The % yields of NIP and MIP in the required range size (25 – 38 µm) was 40% and 41%, respectively (Table 7.3). These polymers were also analysed further and utilised in the MISPE experiments.

7.3.2 Characterisation of Polymers

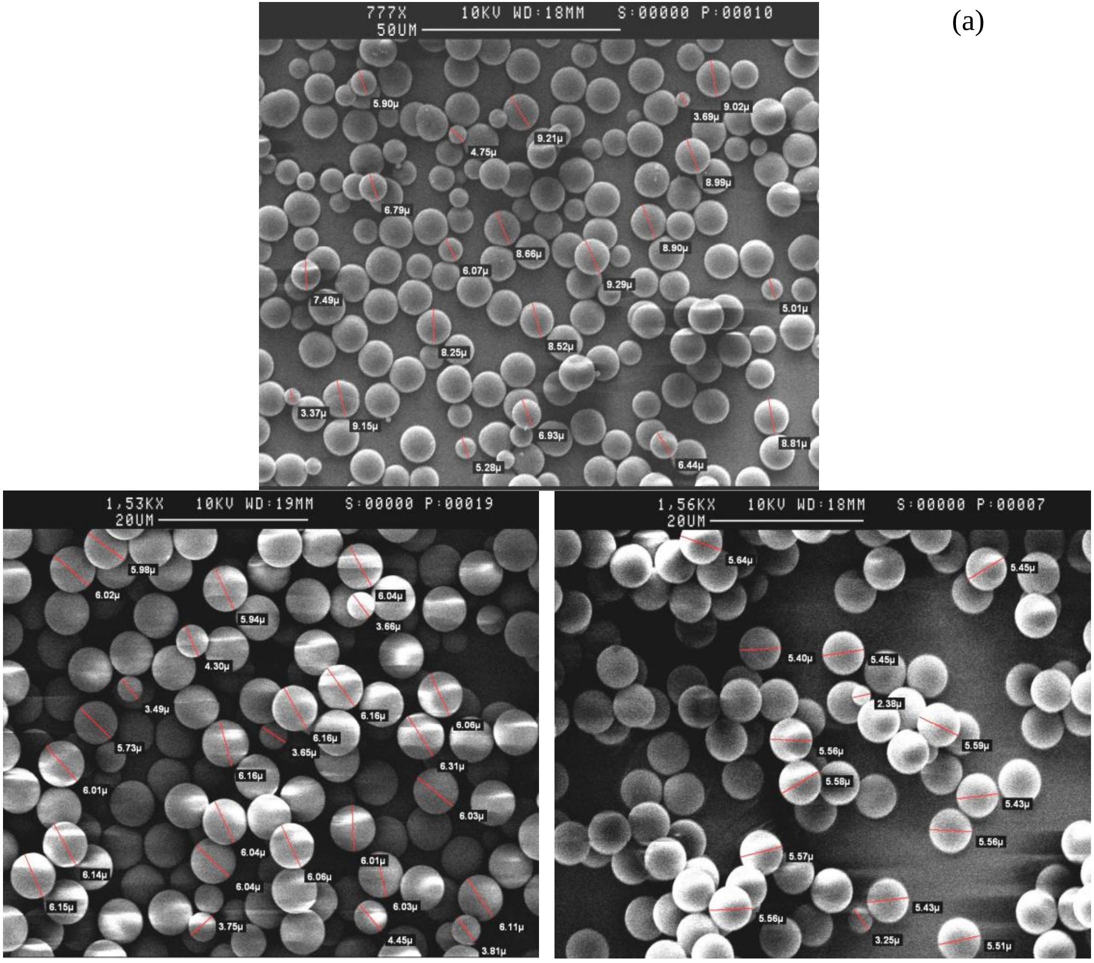
The polymers selected from both polymerisation techniques were characterised in terms of their size, shape, and porous morphology. The shapes of the particles were determined using SEM. For the monolith-derived polymers (SB13 and SB14), the particle size range was 25 – 38 µm and the particles were irregularly shaped (a consequence of the grinding process). The SEM micrographs of SB13 (NIP) and SB14 (MIP) are shown in Figure 7.4.



(a) (b)

Figure 7.4 SEM micrographs of: (a) SB13 (NIP), and (b) SB14 (MIP)

For the polymer microspheres synthesised *via* precipitation polymerisation, the shape, size, and size distribution were determined using SEM and ImageJ (a free image processing programme available at http://imagej.nih.gov/ij/index.html). The SEM micrographs of NIPs (SB9) and MIPs (SB10 and SB5p) are shown in Figure 7.5.



(a)

(b) (c)

Figure 7.5 SEM micrographs of: (a) SB9 (NIP), (b) SB10 (MIP), and (c) SB5p (MIP)

To determine the sizes of the polymer microspheres, SEM micrographs were processed with ImageJ. The average bead diameter and standard deviation (SD) were used in the calculation of the coefficient of variation (CV) and polydispersity index (PDI), terms which are used frequently to evaluate the size distribution of microspheres. The calculation method for CV and PDI as well as the dispersity criteria were described by Vega *et al*. (2013). The dispersity criteria are:

(i) Monodisperse when CV ≤ 3.04% and PDI < 1.003

(ii) Quasi-monodisperse when 3.04% ≤ CV < 16% and 1.003 < PDI < 1.077

(iii) Polydisperse when CV > 16% and PDI > 1.077

Table 7.4 Size and size distribution data of polymer microspheres

Polymer Code	Diameter (µm) ± SD (n = 100)	%CV[a]	PDI[b]	Dispersity
SB5p (MIPs)	5.3 ± 0.6	11.25	1.04	quasi-monodisperse
SB9 (NIPs)	7.8 ± 1.4	18.26	1.10	polydisperse
SB10 (MIPs)	5.6 ± 0.7	12.14	1.04	quasi-monodisperse

[a] CV = coefficient of variation = SD/average diameter; %CV = CV x 100 [b] PDI = polydispersity index ≈ $1+3(CV)^2$ if CV ≤ 0.3

The SEM images indicated that both the NIP and MIPs obtained from the PP method were in the form of spherical microspheres (Figure 7.5), whereas the monolithderived polymers were irregularly shaped due to the grinding processes (Figure 7.4). According to the data relating to the size and size distribution of polymer microspheres prepared by PP (Table 7.4), the results show that the non-imprinted polymers are bigger than the imprinted polymers, but that the NIPs were not uniform whereas the MIPs were quasi-monodisperse. The monolith-derived materials were larger in terms of particle size and size range.

Nitrogen sorption porosimetry was used to analyse the porous morphology (*e.g.*, specific surface areas and pore size) of these polymers. The data is presented in Table 7.5.

Table 7.5 The porous properties of the polymers synthesised in-house

Polymer Code	BET Specific Surface area (m^2 g^{-1})	Single Point Total Pore Volume (cm^3 g^{-1})	Average Pore Diameter (nm)
SB5p	631	0.31	1.94
SB9	598	0.31	2.07
SB10	591	0.29	1.97
SB13	518	1.14	8.77
SB14	607	1.19	7.86

The specific surface areas of typical silica-based SPE sorbents are between 50 and 500 m^2 g^{-1} (Pesek and Matyska, 2000). The results shown in Table 7.5 indicated that all polymers produced in-house had high specific surface areas, greater than 500 m^2 g^{-1} in all cases. SB13 had the lowest specific surface area. The monolith-derived particles had higher pore diameters and pore volumes than the polymer microspheres. According to the IUPAC (International Union of Pure and Applied Chemistry) classifications (Sing *et al.*, 1985), the MIP microspheres (SB5p and SB10) are microporous (pore diameter < 2 nm), while the other polymers are mesoporous (as pore diameters fell within the range of 2 – 50 nm).

FT-IR spectroscopy was used as a qualitative analysis method for these polymers. The FT-IR spectra are shown in Appendix C.2. The results obtained, as expected, show that all polymers synthesised in-house have similar chemical structures. Table 7.6 lists the diagnostic FT-IR for SB5p, as an example.

Table 7.6 Diagnostics bond in the FT-IR spectrum of SB5p

Wavenumber (cm^{-1})	Assignment	Monomer Residues Responsible
3019	C-H aromatic stretch	4-VP and DVB
2913	C-H aliphatic stretch	4-VP and DVB
2845	C-H aliphatic stretch	4-VP and DVB
1587	C-C or C-N aromatic stretch	4-VP and DVB
827	1,4 – disubstituted aromatic ring	DVB
795	1,3 - disubstituted aromatic ring	DVB
708	1,3 - disubstituted aromatic ring	DVB

The FT-IR results show that these polymers are dominated by DVB, and this is to be expected because DVB was used in significant excess relative to 4-VP in the monomer feeds. Elemental microanalysis was done to quantify the 4-VP in the samples (Table 7.7) because 4-VP has nitrogen (N) in the structure, unlike DVB.

This analysis can be used to evaluate the chemical composition of these polymers.

The results from the elemental microanalysis of the polymers synthesised in-house (Table 7.7) show that the amount of carbon and hydrogen between these all polymers were very similar, suggesting similar chemical composition for all these polymers. The %N found in all the polymers was slightly lower than would be expected based upon the monomer feeds and assuming statistical in corporation of the two comonomers. The results indicate that DVB was incorporated into the polymers at a faster rate than 4-VP.

Table 7.7 Elemental microanalysis data of polymers synthesised in-house

Polymer Code	Type of Polymer		Carbon, C (%)	Hydrogen, H (%)	Nitrogen, N (%)
SB5p	MIP	Expected (%)	89.6	7.9	1.9
		Found (%)	90.0	8.0	1.3
SB9	NIP	Expected (%)	90.0	8.0	2.0
		Found (%)	90.2	7.9	1.1
SB10	MIP	Expected (%)	89.6	7.9	1.9
		Found (%)	89.6	7.9	1.0
SB13	NIP	Expected (%)	90.0	8.0	2.0
		Found (%)	89.3	7.7	1.3
SB14	MIP	Expected (%)	89.6	7.9	1.9
		Found (%)	92.5	7.9	1.3

7.3.3 Optimum Conditions for MISPE

The optimum conditions for the in-house prepared MISPE cartridges were modified from the recommended conditions for the commercial MISPE for BPA (AFFINIMIP® SPE Bisphenol A cartridges, Polyintell, France). Polymers SB13 and SB14 were used in the optimisation of the MISPE conditions due to the greater amount of polymers obtained. To achieve optimised conditions for marine samples (seawater and sediment), the loading and washing solutions were varied (Table 7.8). Firstly, the extraction efficiency of AFFINIMIP® SPE Bisphenol A cartridges were determined using deionised water containing BPA in the range of 0.5 – 500 µg L^{-1} (n = 5). Due to the limitation of HPLC-UV, the % recoveries were obtained only for

BPA concentrations in the range 0.005 – 0.5 mg L^{-1} (n = 3); the values were between 65% and 72%, with an average (± SD) of 68% ± 4. The limit of detection (LOD; n

= 5) of this method was 1.4 µg L^{-1} (calculated using a method described by Miller and Miller (2005), as shown in Appendix D.4).

Table 7.8 Optimisation of MISPE conditions for BPA

MISPE Cartridge Type	Sample Loading Solution	BPA Concentration (mg L^{-1})	Washing Solution	Volume of Washing Solution (mL)	% Recovery (%)	
					NIP (SB13)	MIP (SB14)
AFFINIMIP®	Deionised water	0.0005 – 0.5	40%/60% ACN:Water	6	N/A	68
AFFINIMIP®	Cyclohexane	1	40%/60% ACN:Water	6	N/A	37
In-house	Cyclohexane	1	40%/60% ACN:Water	6	2	5
AFFINIMIP®	40%/60% ACN:Water	1	40%/60% ACN:Water	6	N/A	52
In-house	40%/60% ACN:Water	1	40%/60% ACN:Water	6	3	20
In-house	Deionised water	1	40%/60% ACN:Water	6	23	26
In-house	Deionised water	1	ACN	1	9	45
In-house	Deionised water	1	40%/60% ACN:Water	3	36	67

ACN = acetonitrile; N/A = no data

In order to achieve an effective extraction method for sediment samples, the loading solutions were varied. The loading solutions tested in this study were 100%

cyclohexane, 40% acetonitrile in water, and 100% water. The results showed that loading BPA with water provided the highest recovery for both commercial MISPE cartridge and in-house prepared MISPE cartridges derived from ground polymer monoliths (Table 7.8). However, the percent recoveries on the in-house MISPE cartridges (NIPs and MIPs) were still low. An analysis of each fraction (loading, first washing, and second washing step) was performed to identify the step or steps in which the analyte was lost.

The results indicate no loss during the loading step (when loading with water) or the first washing step (washing with 10 mL water), but there were significant losses during the second washing step (Table 7.9). Consequently, the washing solutions for the second washing step were optimised. The results show that using 3 mL of a mixture of acetonitrile and water (40%/60% (v/v)) provided the best result for both NIPs and MIPs (Table 7.9). Therefore, this washing solution was used in the experiments using the in-house prepared MISPE cartridges.

Table 7.9 Loss of BPA during each MISPE process using MISPE cartridges based on monolith-derived particles

Polymer	Variation of 2nd Washing Solutions	Loading with water (%)	1st Washing (10 mL water; %)	2nd Washing (%)
NIP (SB13)	Wash with 6 mL 40%/60% ACN:Water	ND	ND	49
MIP (SB14)	Wash with 6 mL 40%/60% ACN:Water	ND	ND	65
NIP (SB13)	Wash with 1 mL ACN	ND	ND	46
MIP (SB14)	Wash with 1 mL ACN	ND	ND	37
NIP (SB13)	Wash with 3 mL 40%/60% ACN:Water	ND	ND	42
MIP (SB14)	Wash with 3 mL 40%/60% ACN:Water	ND	ND	4

ACN = acetonitrile; ND = not detected

According to Table 7.8, loading with water and washing with 3 mL 40%/60% acetonitrile:water provided the highest extraction efficiencies, with recoveries of 36% for the NIP and 67% for the MIP. Consequently, it can be concluded that the optimum conditions for the in-house prepared MISPE cartridges used in this study were loading with water, washing with 10 mL water, washing again with 3 mL 40% acetonitrile in water, and eluting with 3 mL methanol. Moreover, from the results, it can be seen that MISPE is more effective than SPE with non-imprinted polymers in terms of recovery.

7.3.4 Analysis of Bisphenol A in Aqueous Samples

In this study, three sample treatment techniques (conventional Oasis® HLB SPE, DLLME, and MISPE) were used prior to HPLC-UV for the analysis of BPA. The SPE and MISPE conditions used are summarised in Table 7.10. Three types of MIPs were used in this study, comprising two distinct in-house prepared MIPs (monolithderived particles and polymer microspheres) and one commercial MISPE for BPA (AFFINIMIP® BPA). Additionally, two in-house prepared NIPs (synthesised along with the in-house prepared MIPs) were tested in order to evaluate the effect of molecular recognition.

Table 7.10 SPE conditions for Oasis® HLB SPE, AFFINIMIP® BPA and in-house MISPE

	Oasis® HLB SPE	AFFINIMIP® BPA	In-house MISPE
Conditioning	1. mL DCM:MeOH (80:20 (v/v))	1. mL MeOH – 2% Acetic acid	1. mL MeOH – 2% Acetic acid
	2. mL MeOH	2. mL ACN	2. mL ACN
	10 3. mL water at pH 3	5 3. mL ACN:Water (40:60 (v/v))	5 3. mL ACN:Water (40:60 (v/v))
Loading 10	– 200 mL acidified water sample 5	mL water	5 mL water
	10 pH 3)	10	10
Washing	(mL 10% MeOH in water 5	1. mL water 5	1. mL water
50		10 2. mL ACN:Water (40:60 (v/v))	10 2. mL ACN:Water (40:60 (v/v))
Elution 3	mL DCM:MeOH (80:20 (v/v))	mL MeOH	mL MeOH

DCM = dichloromethane; MeOH = methanol; ACN = acetonitrile 3 3

6 3

5

These methods (except for MISPE with polymer microspheres) were used for deionised water samples to determine their extraction efficiencies before moving to natural seawater (NSW) samples. The results are show in Table 7.11.

Where the LOD could be computed, the results (Table 7.11) show that AFFINIMIP® BPA provided the lowest limit of detection (LOD = 1.4 µg L^{-1}) with 68% recovery. The conventional SPE cartridge with Oasis® HLB gave an LOD of 8 µg L^{-1}, whereas the LOD from the DLLME method was the highest (at 18 µg L^{-1}). Experiments using SPE cartridges packed with the in-house prepared polymers were performed once only. Due to the limited amount of polymers available, it was not possible to obtain the LOD. Due to the efficiency of the HPLC-UV detector, it was not possible to measure BPA concentrations at less than 0.005 mg L^{-1}, resulting in no recovery at very low BPA concentrations (< 0.005 mg L^{-1}). The results show that Oasis® HLB provided the highest recovery (81%), while the in-house prepared non-imprinted polymers had the lowest recovery (36%). Based on these results, all techniques except the in-house prepared NIPs, were suitable for use in a sample pre-treatment for BPA in water samples. However, SPE cartridges (conventional and MISPE) were more effective than DLLME in terms of detection limit.

Natural seawater (NSW) collected from Prestwick, UK, was spiked with BPA at various concentration levels in order to evaluate the extraction method for BPA in seawater. For spiked NSW samples, the extraction efficiencies of Oasis® HLB SPE cartridges; DLLME; AFFINIMIP® BPA cartridges; in-house prepared monolithderived MISPE cartridges; and in-house prepared polymer microsphere MISPE cartridges were compared. The results are shown in Table 7.12.

Table 7.11 Comparison of extraction efficiencies of BPA from spiked deionised water samples

Parameters	DLLME	Oasis® HLB SPE	AFFINIMIP® BPA	Monolith-derived NIPs (SB13)	Monolith-derived MIPs (SB14)
Sample volume (mL)	10	50 – 200	10	10	10
BPA concentration (mg L^{-1})	0.001 - 1	0.001 – 0.5	0.0005 – 0.5	1	1
R^2	0.9998	0.9997	1	N/A	N/A
Number of concentration levels	6	5	5	1	1
Limit of detection (LOD, µg L^{-1})	18	8	1.4	N/A	N/A
% Recovery range (%)	69 - 78	77 - 84	65 - 72	36	67
- Average (%)	73	81	68	36	67
- SD	3	3	4	N/A	N/A
- %RSD (%)	5	4	5	N/A	N/A
- Number of data points (n)	5	4	3	1	1

DLLME = dispersive liquid-liquid microextraction; N/A = no data

Table 7.12 Comparison of extraction efficiencies of BPA from spiked natural seawater samples

Parameters	DLLME	Oasis® HLB SPE	AFFINIMIP® BPA	Monolith-derived NIPs (SB13)	Monolith-derived MIPs (SB14)	NIP Microspheres (SB 9)	MIP Microspheres (SB10)
Sample volume (mL)	10	50 – 200	10	10	10	10	10
BPA concentration (mg L^{-1})	0.1 - 1	0.005 – 0.5	0.0005 - 1	0.1 - 1	0.1 - 1	0.1 - 1	0.1 - 1
Number of concentration levels	3	2	5	3	3	3	3
Limit of detection (LOD, µg L^{-1})	N/A	N/A	30	N/A	N/A	N/A	N/A
% Recovery range (%)	24 - 61	72 - 94	50 - 59	38 - 49	67 - 87	42 - 49	52 - 53
- Average (%)	40	83	55	45	76	46	52
- SD	19	15	4	6	10	3	0.7
- %RSD (%)	47	18	8	13	14	7	1.4
- Number of data points (n)	3	2	3	3	3	3	3

DLLME = dispersive liquid-liquid microextraction; N/A = no data

The results in Table 7.12 indicate that the extraction efficiencies of the DLLME method returned the lowest average recoveries with large %RSD and were not reproducible. Therefore, the DLLME method was deemed unsuitable for seawater samples because interferences in the seawater sample seemed to affect the

effectiveness of the method (Rezaee *et al*., 2010).

The LOD was obtained only for AFFINIMIP® BPA (BPA concentration range = 0.0005 – 1 mg L^{-1}; n = 5) due to the limited availability of the other SPE cartridges. The LOD obtained for the AFFINIMIP® BPA for the extraction of BPA from NSW was 30 µg L^{-1} (Table 7.12). In this study, the efficiencies of the Oasis® HLB SPE cartridges and in-house prepared monolith-derived MISPE cartridges were similar (83% and 76%, respectively) and higher than the efficiencies of AFFINIMIP® BPA and polymer microsphere MISPE (55% and 52%, respectively). Despite higher effectiveness, the %RSD of the Oasis® HLB SPE cartridges and the monolithderived MISPE cartridges (18% and 14%, respectively) were greater than that of AFFINIMIP® BPA and the polymer microsphere MISPE (8% and 1.4%, respectively). The results show that polymer microsphere MISPE had a fair effectiveness; this may be because the MISPE conditions used may not be the optimum conditions for this cartridge. The optimum conditions used were those for the in-house prepared monolith-derived MISPE cartridges.

Despite the similar efficiencies of Oasis® HLB SPE and monolith-derived MISPE for seawater (Table 7.12), MISPE can be considered as the more effective method since MISPE requires lower amounts of sample and solvents; less toxic solvents; and is more environmentally friendly (Table 7.10). It can be concluded that the in-house prepared monolith-derived MISPE cartridge is suitable in the pre-treatment step for BPA in seawater with reasonable % recovery.

The comparison between NIPs and MIPs indicates that MIPs were more effective than NIPs (Table 7.12). It also helps in the demonstration of the selectivity of sorbent to analyte, as is shown in Figure 7.6 and Figure 7.7. The chromatograms of spiked BPA extracted with AFFINIMIP® BPA compared to NSW spiked with BPA are shown in Figure 7.8.

The results (Figure 7.6 – Figure 7.8) show that the BPA peaks were more intense and the chromatograms cleaner after passage through SPE cartridges. The decrease of the notable interference peaks (retention time around 2 min) indicates that the selectivity of the SPE cartridges is effective. The results also indicate that the MISPE cartridges were more selective to BPA than the NIP cartridges (less intense interference peaks). It can be concluded that both of the in-house prepared MISPE cartridges are effective in the pre-concentration of BPA and the removal of interferences.

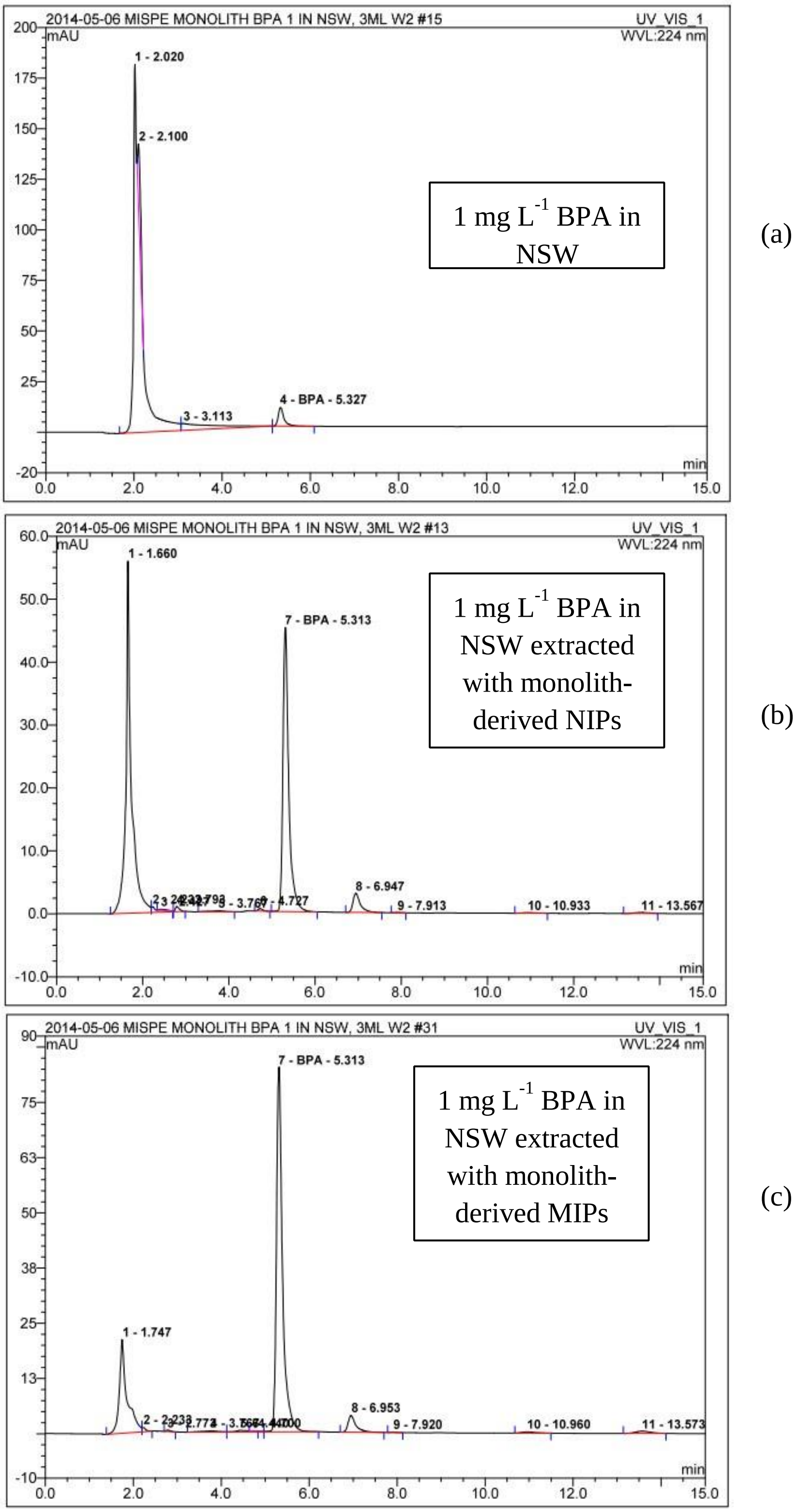
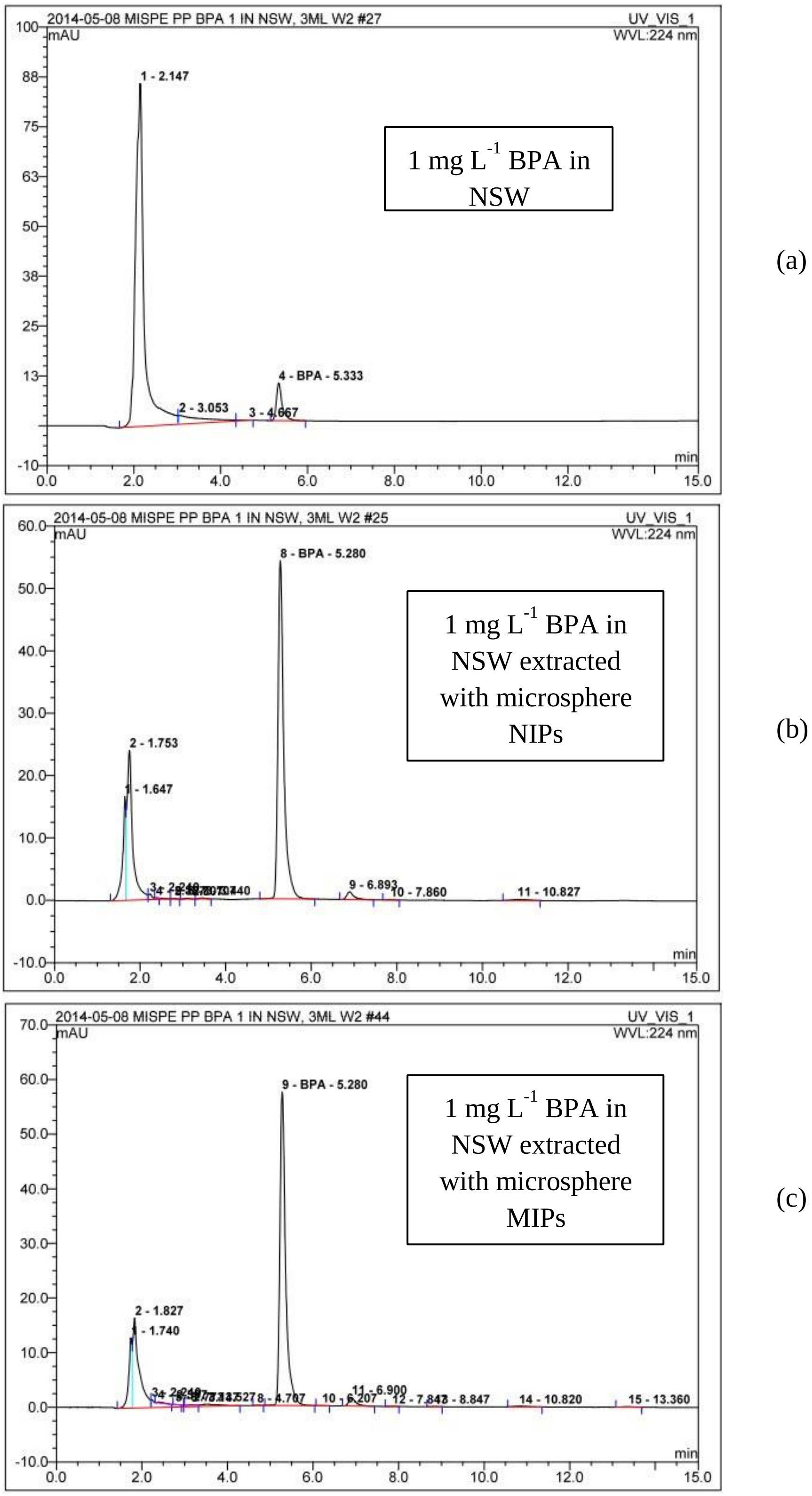


Figure 7.6 Chromatograms of: (a) spiked NSW, (b) spiked NSW extracted with monolith-derived NIP cartridge, and (c) spiked NSW extracted with monolith-

derived MISPE



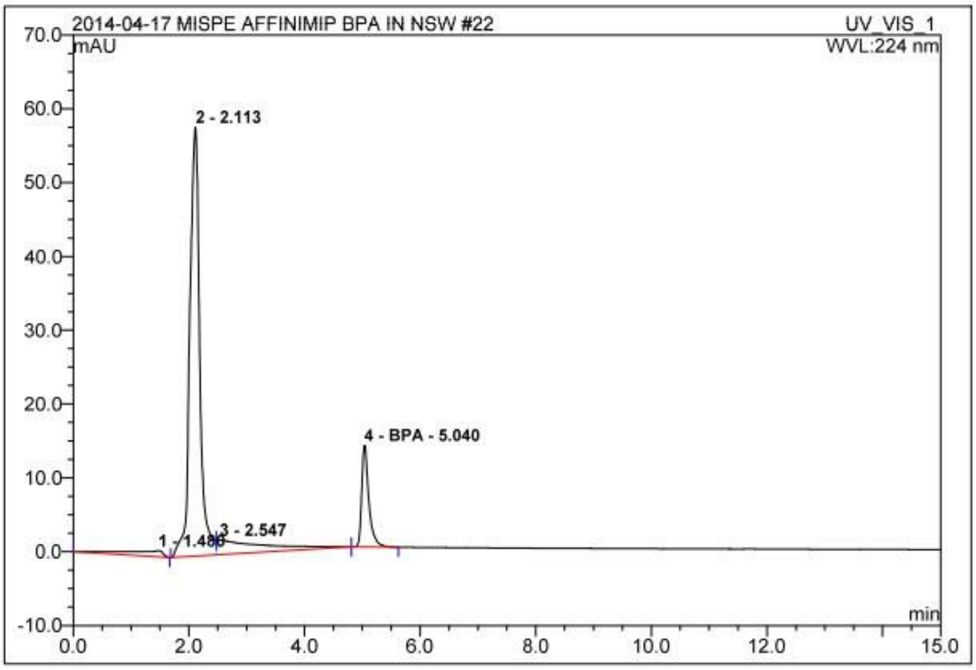
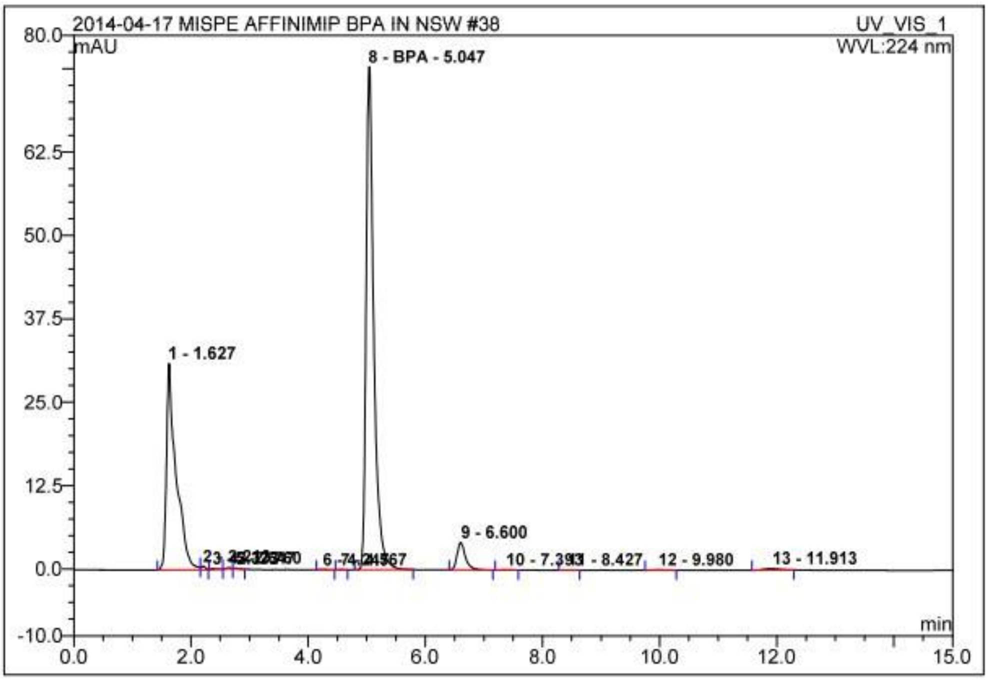


Figure 7.7 Chromatograms of: (a) spiked NSW, (b) spiked NSW extracted with microsphere NIP cartridge, and (c) spiked NSW extracted with microsphere MISPE

1 mg L^{-1} BPA in
NSW

(a)



1 mg L^{-1} BPA in NSW extracted with
AFFINIMIP® BPA

(b)

Figure 7.8 Chromatograms of: (a) spiked NSW, and (b) spiked NSW extracted with AFFINIMIP® BPA cartridge

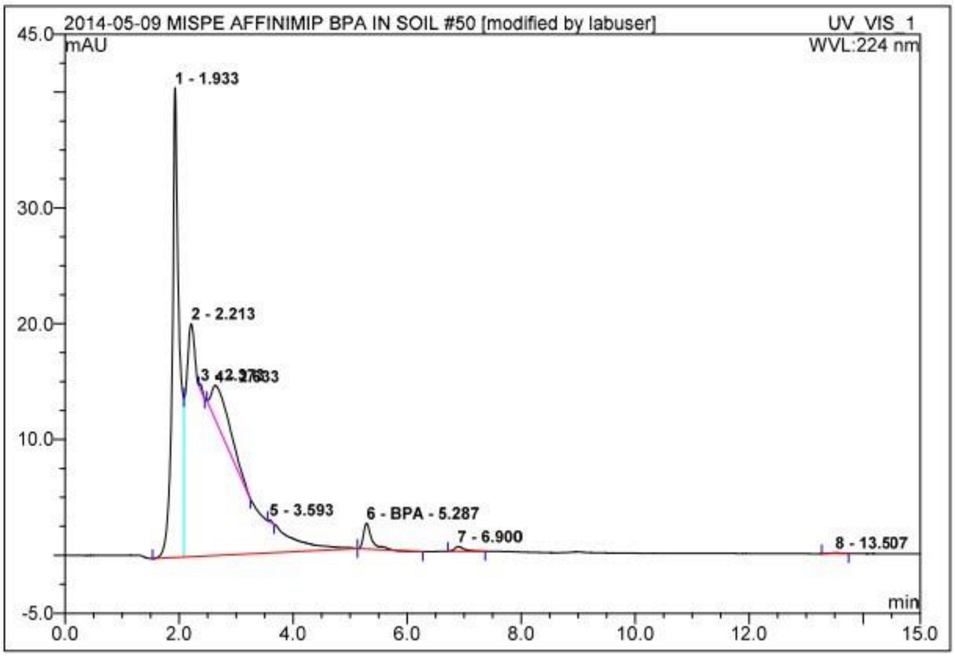
7.3.5 Analysis of Bisphenol A in Sediment Samples

Sediment samples collected from Prestwick, UK, were spiked with BPA at a concentration of 1 mg kg^{-1} and used for the analysis of BPA in marine sediment. ASE was used to extract BPA from the sediment prior to clean-up of the sample with the MISPE cartridges, and analysis with HPLC-UV. The extracted solvent from ASE was dried and reconstituted with 10 mL deionised water for MISPE. Regarding the results in previous section (Section 7.3.4), AFFINIMIP® BPA cartridges were used as they provided the lowest detection limit; required low volumes of sample and solvents; and were more environmentally friendly. The efficiency of the overall sample treatment process (ASE-AFFINIMIP® BPA) as well as the efficiency of each process, is shown in Table 7.13.

Table 7.13 % Recovery of sample treatment for BPA in sediment samples

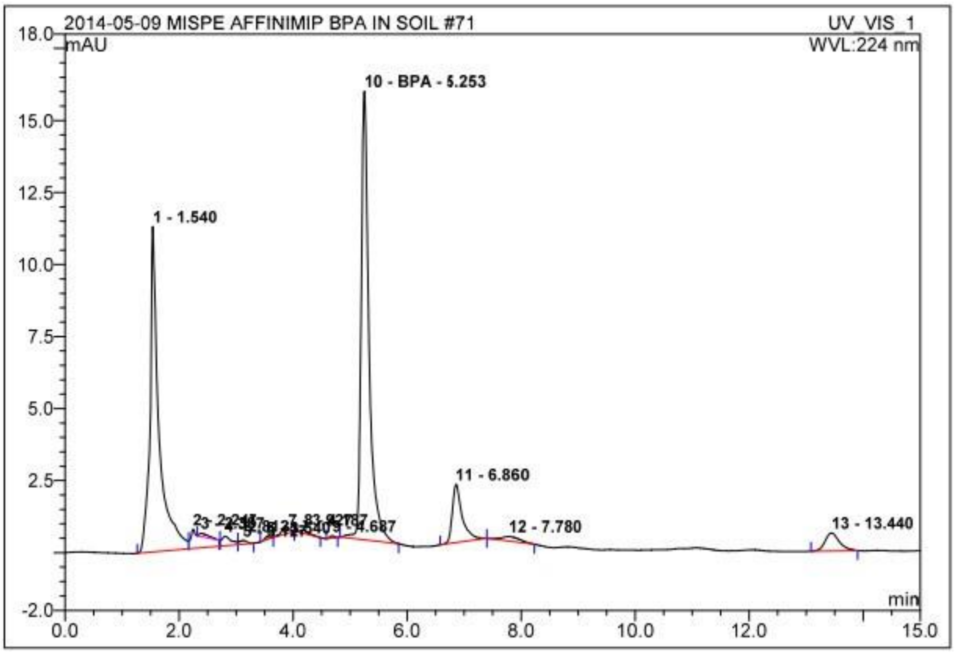
Process	% Recovery (%) (n = 2)
ASE	79
AFFINIMIP® BPA	63
ASE - AFFINIMIP® BPA	50

The analyte recovery obtained for this sample preparation method (ASEAFFINIMIP® BPA) was 50% overall, with 79% from ASE and 63% from AFFINIMIP® BPA. The efficiency of AFFINIMIP® BPA was similar to the extraction efficiency in MISPE (Table 7.11). This implies that there is no loss during the reconstitution step with 10 mL deionised water. Extraction with ASE provided only 79% recovery, thus resulting in reduced effectiveness. Figure 7.9 indicates that AFFINIMIP® BPA was an effective sample treatment method for a sediment sample as it provided lower intensity impurities peaks but higher intensity BPA peak.



Spiked sediment
+ ASE

(a)



Spiked sediment + ASE
+ AFFINIMIP® BPA

(b)

Figure 7.9 Chromatograms of: (a) spiked sediment extracted with ASE, and (b) spiked NSW extracted with ASE-AFFINIMIP® BPA cartridge

7.4 Summary

It is necessary to have an effective analysis method in order to enable the measurement of BPA in the marine environment. To achieve this goal, the sample preparation process is one of the most important steps because marine samples tend to be more complex than freshwater samples. As a result, three sample preparation methods were investigated in this study. These methods were conventional SPE, dispersive liquid-liquid microextraction (DLLME), and SPE using MIP-based sorbents (MISPE).

Two kinds of in-house prepared MIPs were synthesised and used in this study, monolith-derived particles and polymer microspheres. Also, non-imprinted polymers (NIPs) were synthesised along with the MIPs. The optimum conditions for MISPE were developed to extract BPA from real seawater samples. In-house prepared polymers were applied in MISPE and their efficiency was compared to commercially available MISPE BPA cartridges (AFFINIMIP® BPA). All of the MISPE cartridges were more selective and effective than the NIP-based cartridges.

The extraction efficiencies for spiked deionised water samples were in the range of 67% - 81%, which implies that all of the extraction methods studied (conventional SPE, DLLME, and MISPE) can be used to extract BPA from freshwater samples. The results also indicate that DLLME is not a suitable method for seawater samples due to poor reproducibility (40% recovery with 47% RSD). The average recoveries were as follows: Oasis® HLB SPE was 83% with 18% RSD; AFFINIMIP® BPA was 55% with 8% RSD; monolith-derived MISPE was 76% with 14% RSD; and microsphere MISPE was 52% with 1.4% RSD. It can be concluded that the Oasis® HLB SPE and MISPE cartridges can be used for seawater samples. However, use of
MISPE is more environmentally friendly and less toxic than use of Oasis® HLB SPE.

For marine sediment samples, the overall recovery obtained from the preparation method using ASE-AFFINIMIP® BPA was 50% with 79% recovery from ASE and 63% recovery from AFFINIMIP® BPA. Moreover, the chromatograms show lower intensity impurity peaks but a higher intensity BPA peak. Therefore, ASE-MISPE prior to HPLC-UV analysis can be used for extraction and pre-treatment steps to determine BPA concentrations in marine sediments.

Chapter 8

Conclusions and Recommendations for Future Work

8.1 Summary Conclusions

BPA is widely used in the manufacturing of polycarbonate plastics, epoxy resins, flexible PVC plastics, and thermal paper. It is a high production volume chemical. BPA can harm both human health and the environment. It is considered as an emerging pollutant in the marine environment because of its continuous release into the aquatic environment from industrial wastewater treatment plants, landfill leachate, BPA-containing products, and plastic debris in the ocean (Chapter 2). More importantly, it may persist longer and is more toxic in marine ecosystems.

The aim of this study was to assess the environmental fate and transport of BPA in the marine environment. This aim was achieved through a systematic evaluation of the environmental fate of BPA in the marine environment using a combination of laboratory experiments and computer modelling.

Assessment of the environmental behaviour and toxicity of BPA in the marine environment was successfully achieved as described in the relevant chapters. Three important partition coefficients (K_{ow}, S_w and K_{oc}) of BPA in various water conditions (salinity, pH and temperature) were determined following standard methods (Chapter 3 – Chapter 5). The results obtained were compared with the estimated values obtained from the EPI Suite™ programme in order to investigate the effects of water conditions on environmental fate and transport of BPA in the aquatic environment, and also the difference between freshwater and saltwater systems.

To measure the K_{ow} values of BPA (Chapter 3), the RP-HPLC method was unsatisfactory, given the large discrepancy between the methods used. Therefore, the shake flask method (SFM) was used to determine the experimental values. The measured K_{ow} obtained from the SFM in this study was compared with literature values and the estimated value from modelling programs. The measured value was similar to literature values derived experimentally but the estimated value from the programme was slightly overestimated. The K_{ow} values obtained indicate that BPA tends to absorb well through biological membranes. The study of the effects of water conditions on the K_{ow} found that the K_{ow} of BPA was greater with increasing salinity, whereas, increasing temperature of water resulted in lower K_{ow}. For pH, it was observed that K_{ow} significantly decreased at pH in the range of pK_a. K_{ow} has linear relationships with bioconcentration, sorption, and toxicity in aquatic organisms. Higher K_{ow} leads to higher bioconcentration and bioaccumulation in aquatic biota, and also more toxicity. This also leads to higher sorption on soil and sediment, but lower mobility.

Water solubility (S_w) of BPA was also measured (Chapter 4). According to the S_w, BPA is moderately soluble in water. The comparison between the experimental and estimated values shows that the S_w value determined from the model was an underestimate. The impacts of water conditions on the solubility of BPA in water are that the S_w decreased with increasing salinity but increased with increasing temperature. An increase of S_w at pH of water above pK_a was observed because BPA was in its more soluble ionised form. Higher S_w is expected to result in lower volatility but greater migration.

The measurement of sorption capacity (K_d and K_{oc}) was carried out by using sediments from UK and Thailand as well as biochar, mussel shells and prawn shells in order to investigate the influence of solid properties on the sorptive processes of BPA in the solid-water system (Chapter 5). Each sample was examined and its properties (*i.e.*, pH, OM, TOC and mineral content) measured. The measured K_{oc} values obtained in this study were compared with the literature values and estimated values from the modelling programme. The K_{oc} values (Table 5.5) indicate that BPA has moderate to strong sorption to soil or sediment; however, the K_{oc} value of prawn shells indicates very strong sorption to that medium. It was also observed that the sorption behaviour of BPA onto solids depends on OC and/or OM as well as other factors. This study observed that sorption capacity is higher in the solid sample that contains higher OC and/or OM. The possible effects of silica (SiO_2) and calcium on the sorption of BPA show that K_{oc} was more likely to increase with increased silica but decrease with increased calcium. The study of effects of water conditions on the sorption behaviour of BPA indicates a higher sorption capacity at greater salinity and temperatures. From the pH study, the desorption process was observed at a pH around pK_a and no sorption occurred at pH 12. The fugacity model in EPI Suite™ uses K_{oc} in the prediction of the amount of chemical in each environmental compartment. Greater K_{oc} indicates more persistence and bioaccumulation of the chemical but lower mobility.

In order to investigate the effects of salt on the partition coefficients (K_{ow}, S_w and K_{oc}), the salting coefficient, known as Setschenow constant, was determined (see Section 3.3.5, Section 4.3.4 and Section 5.3.4 for K_{ow}, S_w and K_{oc}, respectively). The temperature dependence on equilibrium constant was also determined using the van't Hoff equation (see Section 3.3.7, Section 4.3.6 and Section 5.3.6 for K_{ow}, S_w and K_{oc}, respectively). The calculation equations to estimate each partition coefficient at specific salinity and at specific temperature were also provided. Moreover, the regression equations between all studied parameters (*i.e.*, salinity, pH and temperature) and each partition coefficient were stated (see equation 3.20 in Section 3.3.8, equation 4.19 in Section 4.3.7 and equation 5.27 in Section 5.3.7 for K_{ow}, S_w and K_{oc}, respectively). The correlations between these parameters indicate that simple and inexpensive proxies of salinity (range 0 – 50 psu), pH (range 4 – 9), and temperature (range 5 – 45 °C) may be used to assess bioaccumulation potential and other impacts of BPA associated with K_{ow} and water solubility at locations that lie within these salinity, pH and temperature ranges. While exploration of further experimental conditions may be beneficial and competing influences outside of salinity, pH and temperatures should be explored, the scope of this finding is far reaching. If such a correlation exists for BPA then it is possible that other organic contaminants have similar relationships. The assessment of chemicals under different climatic conditions would be easier to predict. It also opens up assessment for chemicals where methods of analysis are prohibitively expensive or have not yet been developed for such low concentrations. Ultimately, there is the possibility of a single correlation suitable to a range of common contaminants.

The results obtained from Chapter 3 – Chapter 5 indicate that changing water conditions influences the behaviour and toxicity of BPA due to changing partition coefficients, which are used in the environmental and risk assessment. Thus, the values obtained in this study were used along with the modelling programme to evaluate the impacts of changing water conditions (Chapter 6). A climate change scenario was developed based on potential changes in water conditions in the ocean. The results obtained from current seawater conditions were compared with the results under the climate change scenario. The results predict that BPA will tend to remain in seawater and sediment when it is released into the ocean. Climate change is predicted to increase the amount of BPA in seawater and also the bioavailability, but decrease the bioaccumulation and volatilisation. It also slightly increases the sediment sorption capacity of BPA. Because BPA is more soluble in water, it may be excreted from marine organisms faster and have less bioaccumulation and toxicity. However, BPA is predicted to have a more widespread distribution of BPA in the marine environment in the climate change scenario, leading to a larger exposed population of marine organisms. Longer persistence in marine sediments and greater potential exposure for benthic organisms are predicted. At present, the doubt regarding real "safe" exposure limit of BPA is increasing due to recent results about low-dose toxicity of BPA, which may indicate that endocrine disruption of BPA can occur at concentrations lower than the existing safe exposure limit. Therefore, increasing potential exposure of BPA and widening the range of organisms exposed may produce greater adverse effects.

This research demonstrated the utility of the computer software EPI Suite™ as a screening tool for environmental fate and ecological risk of a chemical. In particular, it demonstrated the improvements that can be made by using experimentallyobtained data. This approach allows for improved representation of environmental conditions and leads to more reliable and accurate assessments. Improvements in environmental assessments should, in turn, lead to development and application of more appropriate remediation methods for BPA contamination.

This study used HPLC-UV to analyse the concentration of BPA. The optimum HPLC conditions were obtained; however, it is necessary to employ an effective sample treatment method to enable the measurement of BPA in marine samples (Chapter 7). The techniques used to extract BPA from seawater sample in this study were conventional SPE cartridge (Oasis® HLB SPE), DLLME, and MISPE. For MISPE cartridges, two types of in-house MIPs (monolith-derived and microsphere) were produced by optimised polymerisation conditions obtained from this study, and then packed into cartridges. These in-house MISPE cartridges were compared in extraction efficiencies for seawater samples with commercial MISPE (AFFINIMIP® BPA). For non-salinity water sample, the MISPE used were only monolith-derived MISPE and AFFINIMIP® BPA. The SPE and MISPE conditions were optimised and used to obtain the extraction efficiency. The results show that all methods can be used in the extraction of BPA from freshwater samples (recoveries range from 67% to 81%). However, for seawater, DLLME is not appropriate (40% recovery with 47% RSD), while SPE cartridges (83% recovery with 18% RSD), AFFINIMIP® BPA cartridges (55% recovery with 8% RSD), monolith-derived MIPSPE cartridges (76% recovery with 14% RSD), and microsphere MISPE cartridges (52% recovery with 1.4% RSD) provided acceptable recoveries. Pre-treatment with ASE and MISPE (AFFINIMIP® BPA) prior to HPLC-UV analysis was carried out in the analysis of BPA in the natural marine sediment. The MISPE were used as it is more likely to more selective than SPE. The recovery obtained from this method was 50%. Based on the investigation of extraction efficiency from each step (ASE and MISPE), the extraction efficiency of BPA from marine sediment may be improved by modifying ASE conditions. Extraction procedures for marine waters and sediments were evaluated so that a full assessment of BPA under different environmental conditions could be carried out.

Accordingly, this thesis contributes towards the understanding of environmental fate and toxicity of BPA in the marine environment, including prediction equations for partition coefficients at a wide range of environmentally relevant water conditions of interest. A further contribution of this thesis is the development and evaluation of extraction and analysis methods that are suitable for marine samples.

8.2 Recommendations for Future Work

Despite the achievement of the aim and objectives of this work, further research regarding BPA in the marine system is necessary. Recommendations for future work include:

- This research observed the effect of KCl salt on the partition coefficients of BPA and obtained the salting coefficients. It would be interesting to observe the effects of other salts, and also using natural seawater. These additional parameters would enhance the in-depth understanding of the effect of each salt on the partition coefficients.

- This study suggests that it is possible to estimate the partition coefficients of BPA by measuring only simple parameters and also provides the calculation equations. These equations may be improved through the collection of more experimental data under a wider range of conditions (*e.g.*, varying more salinity, pH and temperature). Also, the determination of experimental values by varying all of those three parameters is recommended in order to qualify the equations.

- In this study, it was observed that mineral content (silica and calcium) in solid samples may have effects on the sorption of BPA. Further sorption determination experiments using various solid type samples are recommended coupled with detailed analysis of mineralogy to evaluate the mechanisms affecting sorption in these materials.

- The sorption of BPA to prawn shells and mussel shells was observed. The experiments about bioconcentration and bioaccumulation are strongly recommended.

- This study found that BPA can be sorbed to solid samples. Further experiments on desorption processes in environmental media are recommended, including samples from organisms.

- Determining the optimum MISPE conditions for microsphere MIPs will lead to improvements in extraction methods for BPA in marine samples. Microsphere MIPs have wide-ranging applications and methods can be developed for many more compounds of environmental relevance.

- Developing improvements in the extraction efficiency of ASE would lead to improvements in the extraction procedures for the analysis of BPA in marine sediments and other media. Extraction efficiency may be improved with the use of in-house MISPE cartridges coupled with ASE to extract BPA from marine sediment. This approach may be applicable to other compounds of interest in marine samples.

- Degradation of BPA in various water conditions should be explored and quantified to inform environmental assessment practices.

Further experimental studies will provide valuable data for BPA over a wider range of environmental conditions, which will, in turn, improve model prediction capabilities for BPA fate in the environment. These techniques can be applied to other compounds of interest in the marine environment to make similar improvements in evaluating their fates, particularly in complex systems with multiple contaminants and multiple potential confounding factors.

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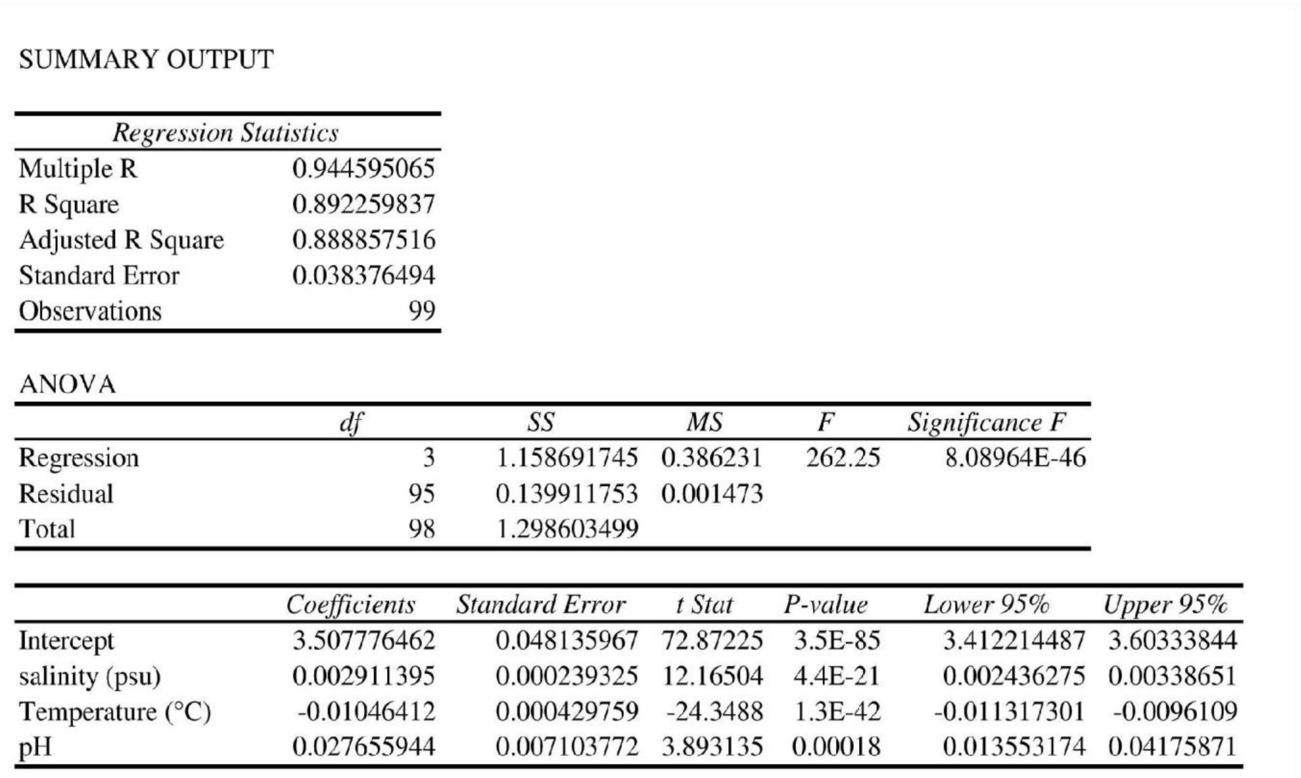
Appendix A

Statistics Results

A.1 Statistics Results for K_{ow}

Table A.1 Statistics results for K_{ow}



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.944595065
R Square	0.892259837
Adjusted R Square	0.888857516
Standard Error	0.038376494
Observations	99

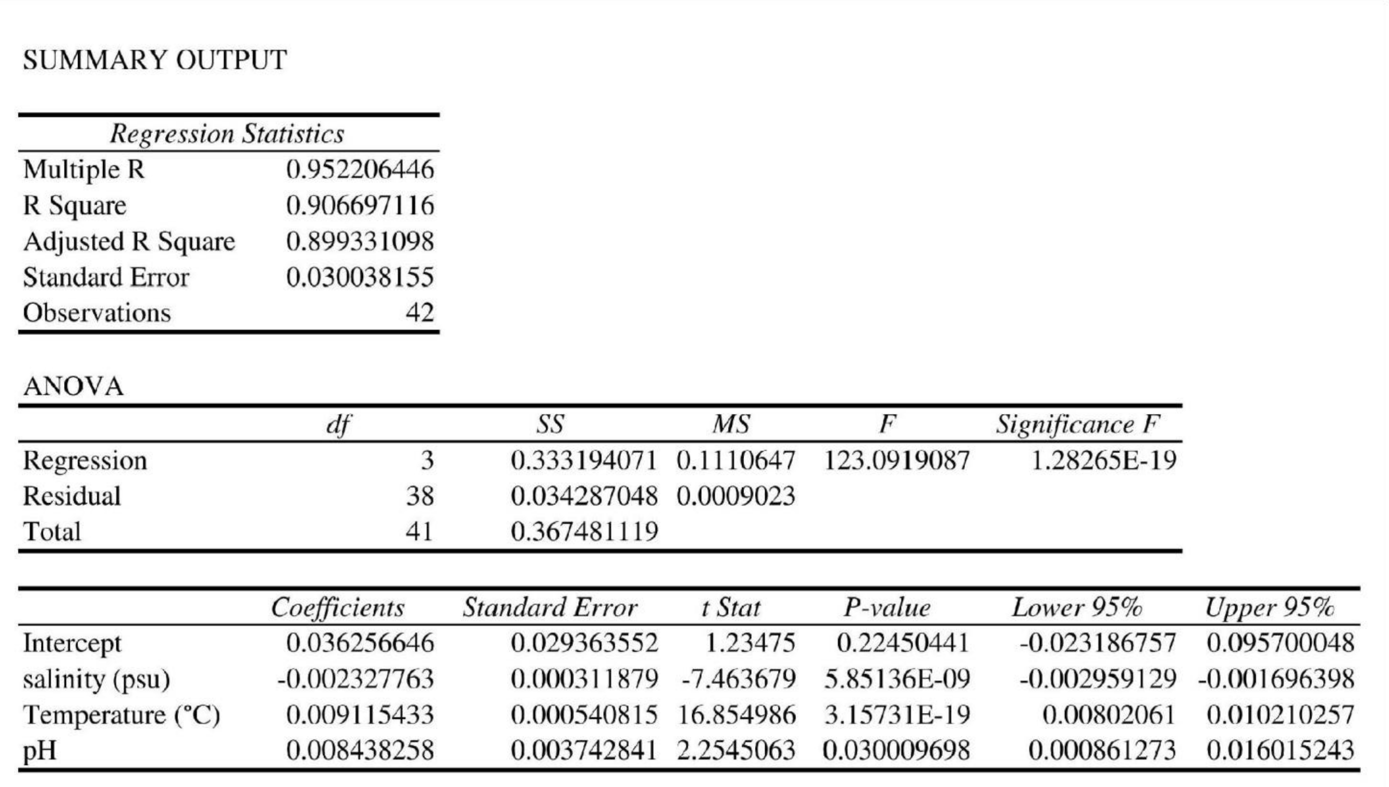
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	1.158691745	0.386231	262.25	8.08964E-46
Residual	95	0.139911753	0.001473		
Total	98	1.298603499			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	3.507776462	0.048135967	72.87225	3.5E-85	3.412214487	3.60333844
salinity (psu)	0.002911395	0.000239325	12.16504	4.4E-21	0.002436275	0.00338651
Temperature (°C)	-0.01046412	0.000429759	-24.3488	1.3E-42	-0.011317301	-0.0096109
pH	0.027655944	0.007103772	3.893135	0.00018	0.013553174	0.04175871

A.2 Statistics Results for S_w

Table A.2 Statistics results for S_w



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.952206446
R Square	0.906697116
Adjusted R Square	0.899331098
Standard Error	0.030038155
Observations	42

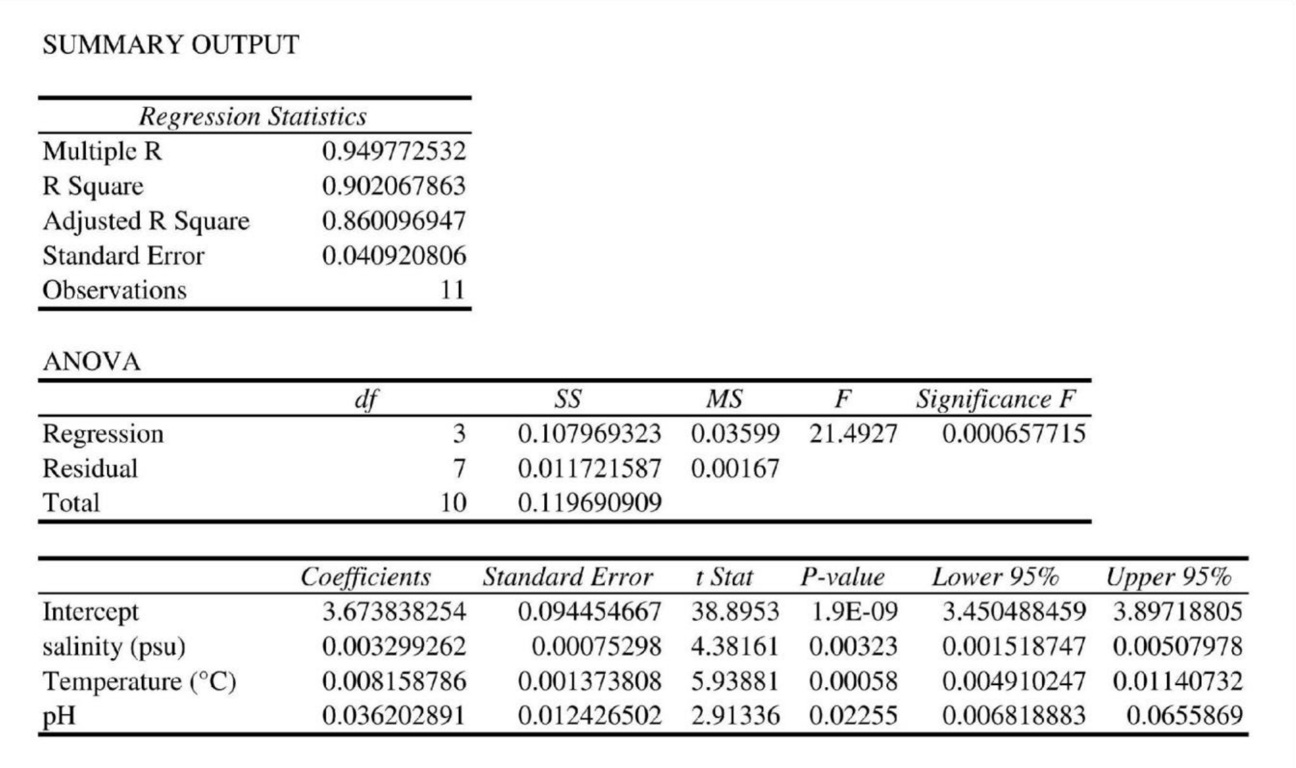
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	0.333194071	0.1110647	123.0919087	1.28265E-19
Residual	38	0.034287048	0.0009023		
Total	41	0.367481119			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.036256646	0.029363552	1.23475	0.22450441	-0.023186757	0.095700048
salinity (psu)	-0.002327763	0.000311879	-7.463679	5.85136E-09	-0.002959129	-0.001696398
Temperature (°C)	0.009115433	0.000540815	16.854986	3.15731E-19	0.00802061	0.010210257
pH	0.008438258	0.003742841	2.2545063	0.030009698	0.000861273	0.016015243

A.3 Statistics Results for K_{oc}

Table A.3 Statistics results for K_{oc}



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.949772532
R Square	0.902067863
Adjusted R Square	0.860096947
Standard Error	0.040920806
Observations	11

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	0.107969323	0.03599	21.4927	0.000657715
Residual	7	0.011721587	0.00167		
Total	10	0.119690909			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	3.673838254	0.094454667	38.8953	1.9E-09	3.450488459	3.89718805
salinity (psu)	0.003299262	0.00075298	4.38161	0.00323	0.001518747	0.00507978
Temperature (°C)	0.008158786	0.001373808	5.93881	0.00058	0.004910247	0.01140732
pH	0.036202891	0.012426502	2.91336	0.02255	0.006818883	0.0655869

Appendix B

Correction Factors

B.1 Correction Factors Applied to the WSKOWWIN™

Table B.1 Correction factors applied to the WSKOWWIN™

Correction Factor shown in WSKOWWIN™	With MP Coefficient	Without MP Coefficient	No. in Dataset
Alcohol, aliphatic	0.4240	0.5100	18
S(=O) Type	-0.8650	-1.0510	36
Acid, aliphatic	0.6500	0.3950	70
Acid, aromatic	0.8980	0.0000	46
Nitrile	-0.3620	-0.2650	23
Azo (-N=N-)	-0.3410	-0.4320	12
Phenol	0.9610	0.5800	91
Nitro (-NO_2)	-0.5050	-0.3900	75
Hydrocarbon	-0.4410	-0.5370	33
Amine, aliphatic	0.8380	1.0080	37
Polyfluoro alkane	-0.9450	-0.7420	8
Pyridine, alkyl	1.2430	1.3000	11
Amino acid	-	-2.0700	11
PAH Type	-	-1.1100	13
Multi-Nitrogen Type	-	-1.3100	44
Amino/sulfonic acid	-0.4000	-2.0000	
Linear Siloxane	-	-	
Cyclic Siloxane	-	-	

Note ... 1,450 compound dataset ... each correction factor counted a maximum of once per structure.

The following discusses correction factors in the table:

(1) Aliphatic Alcohol: applies to compounds containing one -OH group attached to an aliphatic carbon. Compounds with multiple -OH groups (*e.g.*, ethylene glycol) are excluded. Compounds containing acetamide, amino-azo-, or -S(=O) type functions (*e.g.*, sulfonamides or sulfoxides) are also excluded; the reason for this is not clear, but it's very evident from the dataset.

(2) Sulfonamide, Sulfone, Carbonyl [SO]: this is actually two different factors, but since they are closely related, only one factor is needed. It applies to any aromatic compound having a sulfonamide substituent attached to the ring plus any other substituent that is either a ketone, sulfone or sulfonamide. It also applies to any aliphatic unit that has the structure -S(=O)-C-C(=O)-C which is an aliphatic carbon with a ketone attachment and either a sulfone or sulfoxide attachment.

(3) Aliphatic Acid: applies to compounds with the aliphatic acid function. Amino acids and compounds with the C(=O)-N-C-COOH structure are excluded.

(4) Aromatic Acid: applies to compounds with the aromatic acid function. If the aromatic ring has any amino-type substituent (*e.g.*, NH_2, -NH-C(=O)), this correction factor is excluded.

(5) Nitrile: applies to both aliphatic and aromatic compounds with the nitrile function. It excludes any aliphatic unit with the N-C-C≡N structure.

(6) Azo [-N=N-]: applies to both aliphatic and aromatic compounds with the azo N=N- function; both nitrogens must have carbon attachments. No exclusions.

(7) Phenols: applies to phenols, a phenyl ring with an -OH substituent. Excludes any phenol ring with a nitro function or an alkyloxy function in an ortho-position to the OH. It also excludes phenol rings that have any amino-type substituent in either an ortho or non-ortho position (*e.g.*, NH_2, -NH-C(=O)).

(8) Nitro [-NO_2]: applies to both aliphatic and aromatic compounds with the nitro NO_2 function; if attached to another nitrogen (N-NO_2) it is excluded; if attached to an aromatic ring, it is excluded if the ring has an -OH or amino-type substituent (therefore, it does not apply to nitrophenols or nitroanilines).

(9) Plain Aliphatic Hydrocarbon [Hydrocarbon]: applies to any aliphatic hydrocarbon that contains just carbons and hydrogens. Includes alkanes, alkenes and alkynes.

(10) Liquid Amines [Amine, Aliphatic]: applies only to liquids that contain a primary, secondary or tertiary amine. The amine must have only aliphatic carbon attachments (as with all correction factors, a carbon attachment does not include the carbonyl function). For equation 4.1 (which includes solids and liquids), it also applies to the same amines; however, any structure that contains an acetamide, acid or imide is excluded.

(11) Polyfluorinated Alkane [Fluoro Alkane]: applies to any alkane with two or more fluorine attachments. No exclusions.

(12) Alkyl Pyridines [Alkyl Pyridine]: applies to pyridine and any pyridine ring with only alkyl-type substituents; it is excluded if any halogen, -OH, acid, nitro, or non-alkyl-type substituents are present. Alkyl-type means that the initial aliphatic carbon attachment to the ring must be either -CH_3, -CH_2- or -CH<; the next attachment aliphatic carbon (alkyl function) can be an alcohol, amine, etc.

Three additional correction factors were developed for equation 4.1 that applies to both liquids and solids and does not use a melting point parameter.

(13) Amino Acids [Amino acids]: applies simply to amino acids.

(14) PAHs [PAH]: applies to polyaromatic hydrocarbons. For the purposes of this study, a PAH must contain at least three rings of which two must be aromatic; this allows structures such as fluorene to be included. Also included are

structures such 9,10-anthracenedione and dibenzo-p-dioxin because they are three-ring structures with two aromatic rings.

(15) Multi-Nitrogen [Multi-Nitrogen]: applies to selected compounds that contain two or more nitrogens and have log K_{ow} values of less than 0.35. Applies to any compound with two or more aliphatic nitrogens and one of the nitrogens is connected to C(=O), S(=O), or C(=S). Applies to compounds with four or more aromatic nitrogens. Applies to compounds with two or more aromatic nitrogens and one or more aliphatic nitrogens connected to C(=O), S(=O), or C(=S). Nitrogens in nitriles, nitros, or azo functions are not counted. Barbiturate and metal compounds are excluded.

Siloxanes:

Siloxanes were added to the correction factors after the initial training regression using the initial 1,450 compounds. Experimental siloxane water solubilities were made available from Dow Corning (private communication).

The Siloxane correction factors are based on the number of Si-O-Si units in the structure as follows:

Linear Siloxanes:

with a melting point (MP):

Coefficient = -0.641 ((number of Si-O-Si units) - 1) - 0.842 without

a melting point (MP):

Coefficient = -0.396 ((number of Si-O-Si units) - 1) - 0.778

Cyclic Siloxanes:

with a melting point (MP):

Coefficient = 0.211 (((number of Si-O-Si units)-3))-0.946) - 0.857

(where > 3 units; else -0.857) without

a melting point (MP):

Coefficient = 0.457 (((number of Si-O-Si units)-3))-0.959) - 0.513

(where > 3 units; else -0.857)

Note: Each correction factor that occurs in a compound is counted only once, no matter how many times it occurs. For example, the nitro factor in 1,4-dinitrobenzene is counted just once. A compound either contains a correction factor or it doesn't. With the possible exception of a very few phenols (*e.g.*, catechol, resorcinol), the regression is more accurately developed when the value of each correction factor is either 0 or 1.

B.2 Correction Factors Applied to the MCI Regression Methodology in the KOCWIN™

Correction factors for Molecular Connectivity Index (MCI) regression methodology for 447 Compound Training Set

Table B.2 Correction factors applies to the MCI regression methodology

Correction Factor Descriptor	Coefficient (new model)	Occurrence (No. of compounds)	Occurrence (max per structure)	Previous Coefficient

Nitrogen to non-fused aromatic ring	-0.522510[a]	154	2	-0.7770
Ether, aromatic (-C-O-C-)	-0.679088[a,b]	64	2	-0.6431
Nitro (-NO$_2$)	-0.488879[a]	43	2	-0.6317
N-CO-C (aliphatic carbon)	-1.027725[a]	39	1	-0.8112
Urea (N-CO-N)	-1.003794[a]	57	1	-0.9222
Nitrogen to Carbon (aliphatic) (-N-C)	-0.212730[c]	133	5	-0.1242
Carbamate (N-CO-O) or (N-CO-S)	-0.960128[a]	26	2	-1.0249
Triazine ring	-0.225664	18	1	-0.7521
Nitrogen-to-Cycloalkane (aliphatic)	-0.861602	8	1	-0.8220
Uracil (-N-CO-N-CO-C=C-ring)	-1.731498	4	1	-1.8060
Organic Acid (-CO-OH)	-1.624874[f]	21	1	-1.7512
Ketone (-C-CO-C-)	-1.129027[a]	7	1	-1.2477
Aliphatic Alcohol (-C-OH)	-1.317914[a]	21	1	-1.5193
Nitrile/Cyanide (-C≡N)	-0.667701[a]	11	2	-0.7223

Correction Factor Descriptor	Coefficient (new model)	Occurrence (No. of compounds)	Occurrence (max per structure)	Previous Coefficient
Thiocarbonyl (C=S)	-0.570124[f]	5	1	-1.1002
OrganoPhosphorus [P=S]	-1.159919[a]	30	2	-1.2634

OrganoPhosphorus [P=O], aliphatic	-1.493958[f,d]	10	1	-1.6980
N-CO-O-Phenyl Carbamate	-1.647182	19	1	-2.0022
Ether, aliphatic (-C-O-C-)	-0.871597	20	2	-1.2643
Ester (-C-CO-O-C-) or (HCO-O-C)	-1.296957[a]	50	2	-1.3089
Sulfone (-C-SO$_2$-C-)	-1.313632[a]	10	2	-0.9945
Azo (-N=N-)	-0.647525	3	1	-1.0277
N-CO-O-N	-1.870532	8	1	-1.9200
Aromatic ring with 2 nitrogens	-0.596418	17	1	-0.9650
OrganoPhosphorus [P=O], aromatic	-2.425689[a]	10	1	-2.8781
Misc (C=O) Group (aliphatic attach)	-1.604672	20	1	-1.2000[e]
Pyridine ring	-0.308035	12	1	-0.7001
Sulfoxide (-C-SO-C-)	-1.184143[a]	5	1	-0.9000
Miscellaneous S(=O) group	-1.298009[a]	11	1	-0.9000
Multi-Nitrogen aromatic	-1.204448	10	1	----
Poly-Chlorinated Aromatic	0.343783	42	1	----
Dithiocarbonyl (-S-C(=S)-N)	0.197791[f]	2	1	----
Carbonyl Hydrazide (-C(=O)-N-NH$_2$)	-0.957393	2	1	----
Quinone (diketone) ring	1.298555	1	1	----
NH$_2$-Phenyl/Di-ortho halogens	0.823576	3	1	----
Aromatic Hydroxy (aromatic-OH)	-0.096610	27	1	----

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[a] Counted up to twice per structure, regardless of number of occurrences.

[b]

Either one or both carbons aromatic; if both carbons aromatic, cannot be cyclic.

[c] Any nitrogen attached to double bond is not counted; also, carbonyl and thiocarbonyl are not counted as carbons.

[d] This is the only fragment counted, even if other fragments occur.

[e] Not included in regression derivation; estimated from other carbonyl fragments.

[f] Counted only once per structure, regardless of number of occurrences

B.3 Correction Factors Applied to the Log K_{ow} Regression Methodology in the KOCWIN™

Correction factors for log K_{ow} regression methodology for 447 Compound Training Set

Table B.3 Correction factors applies to the Log K_{ow} regression methodology

Correction Factor Descriptor	Coefficient
Nitrogen to non-fused aromatic ring	-0.021606
Ether, aromatic (-C-O-C-)	0.055920
Nitro ($-NO_2$)	0.219081
N-CO-C (aliphatic carbon)	-0.003790
Urea (N-CO-N)	-0.010230
Nitrogen to Carbon (aliphatic) (-N-C)	-0.021787
Carbamate (N-CO-O) or (N-CO-S)	-0.082513
Triazine ring	-0.123923
Nitrogen-to-Cycloalkane (aliphatic)	-0.357564
Uracil (-N-CO-N-CO-C=C- ring)	0.030643
Organic Acid (-CO-OH)	-0.769374
Ketone (-C-CO-C-)	0.195596
Aliphatic Alcohol (-C-OH)	-0.411443
Nitrile/Cyanide (-C≡N)	0.392168
Thiocarbonyl (C=S)	0.300427
OrganoPhosphorus [P=S]	-0.091725
OrganoPhosphorus [P=O], aliphatic	0.103347
N-CO-O-Phenyl Carbamate	-0.074344
Ether, aliphatic (-C-O-C-)	-0.090599

Ester (-C-CO-O-C-) or (HCO-O-C)	-0.065594
Sulfone (-C-SO$_2$-C-)	0.023884

Correction Factor Descriptor	Coefficient
Azo (-N=N-)	0.431031
N-CO-O-N	-0.023725
Aromatic ring with 2 nitrogens	0.398424
OrganoPhosphorus [P=O], aromatic	-0.226272
Misc (C=O) Group (aliphatic attach)	-0.229281
Pyridine ring	0.176372
Sulfoxide (-C-SO-C-)	0.094975
Miscellaneous S(=O) group	0.161446
Multi-Nitrogen aromatic	0.072898
Poly-Chlorinated Aromatic	0.144394
Dithiocarbonyl (-S-C(=S)-N)	1.275262
Carbonyl Hydrazide (-C(=O)-N-NH$_2$)	0.340727
Quinone (diketone) ring	0.508606
NH$_2$-Phenyl/Di-ortho halogens	0.590880
Aromatic Hydroxy (aromatic-OH)	0.166847

The correction factors and occurrences are determined exactly the same as for the MCI regression.

Appendix C

XRD and FT-IR Spectra

C.1 XRD Spectra of Solid Samples

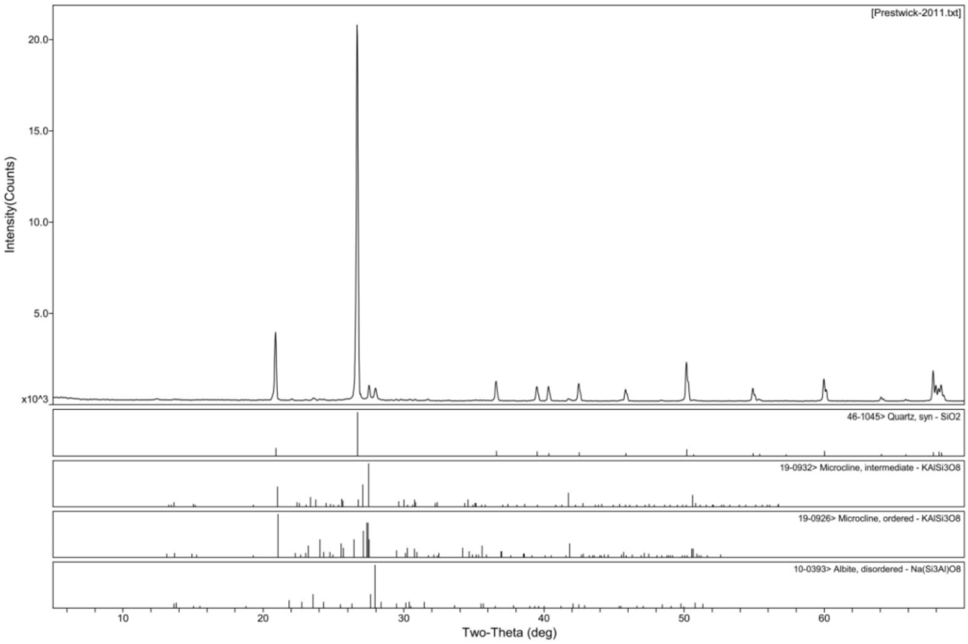

Figure C.1 XRD spectra of Prestwick 2011 sediment sample

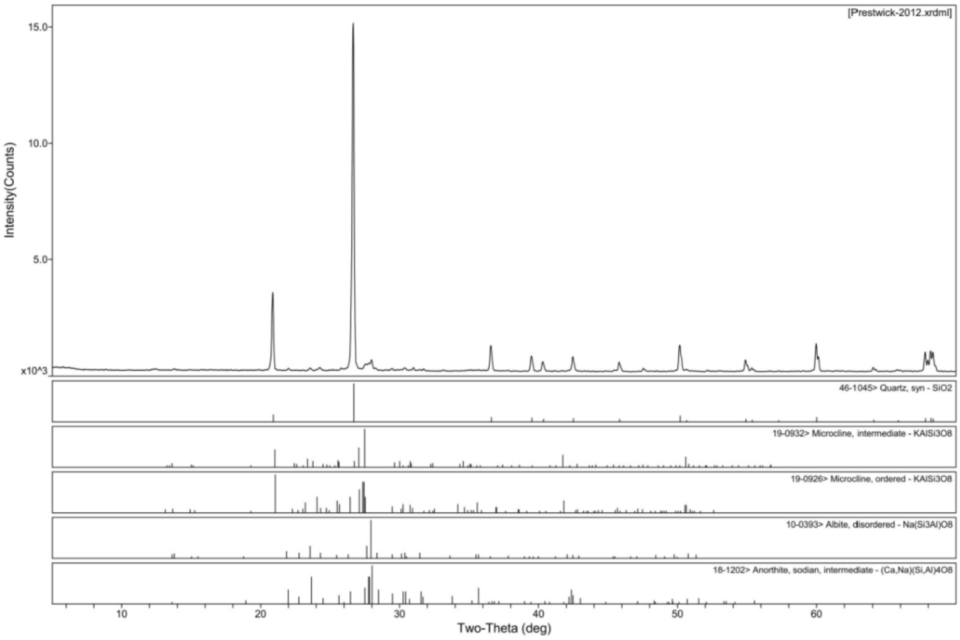

Figure C.2 XRD spectra of Prestwick 2012 sediment sample

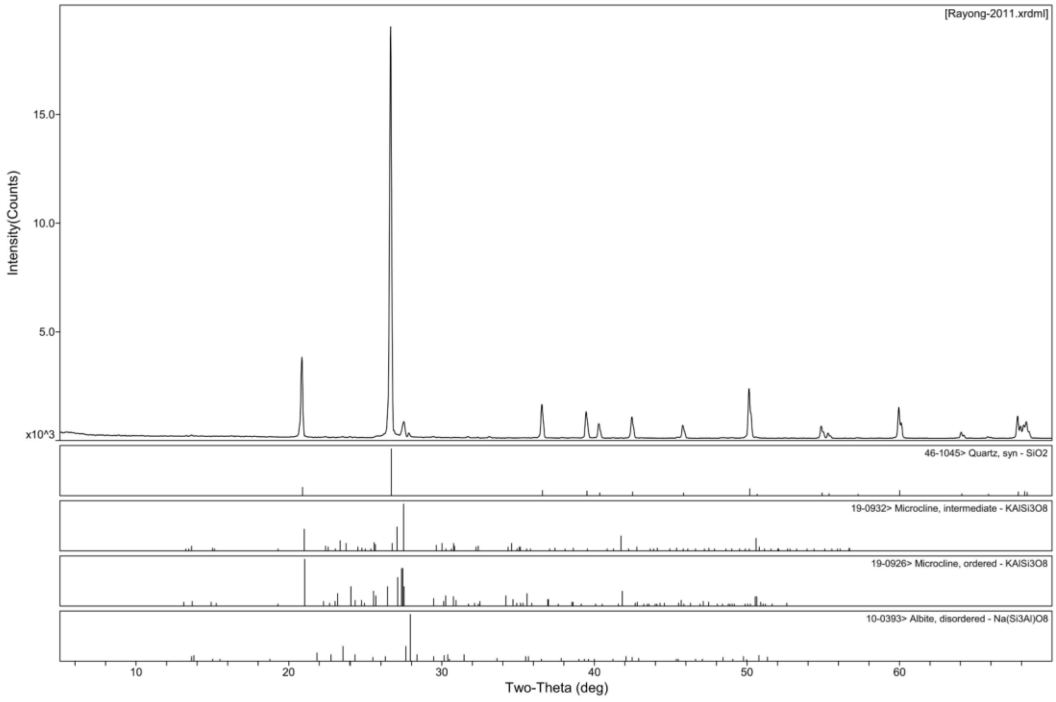

Figure C.3 XRD spectra of Rayong sediment sample

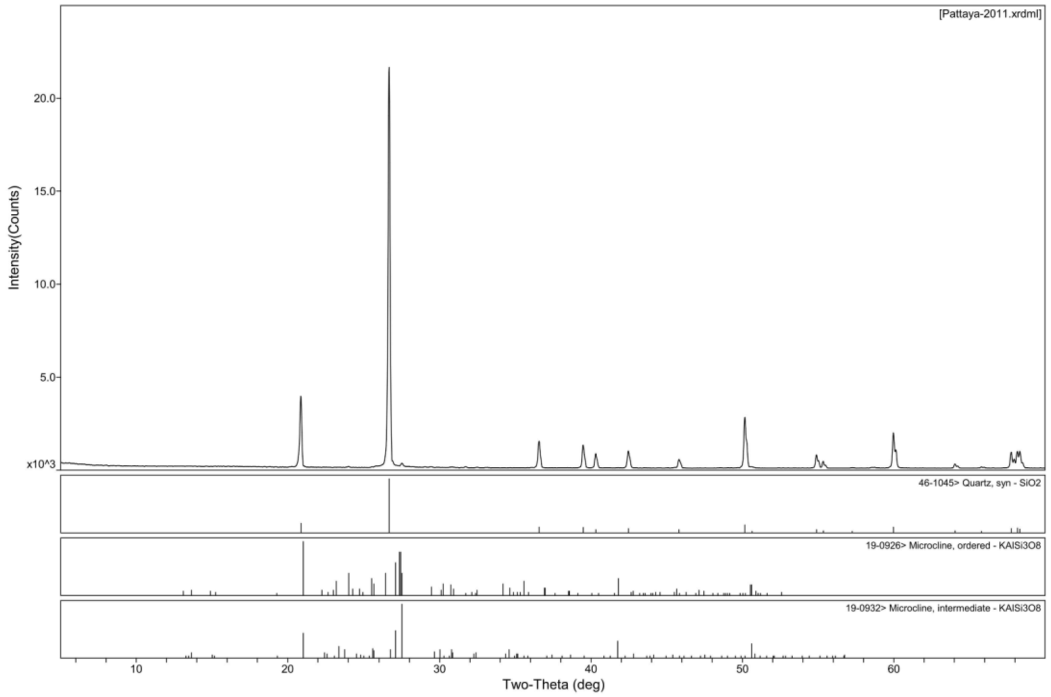

Figure C.4 XRD spectra of Pattaya sediment sample

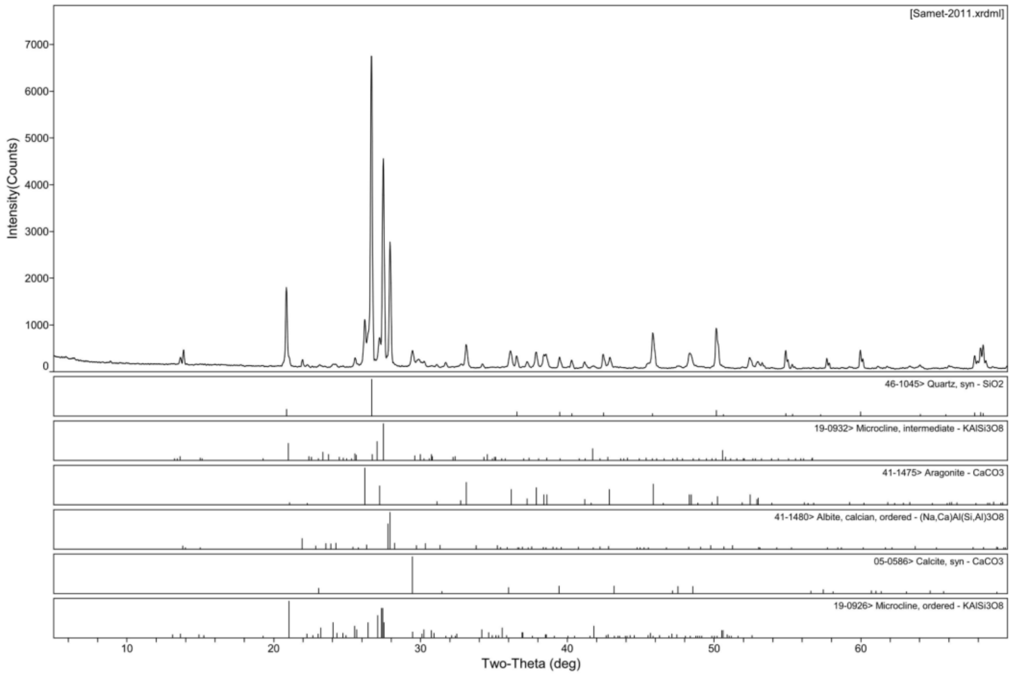

Figure C.5 XRD spectra of Samet Island sediment sample

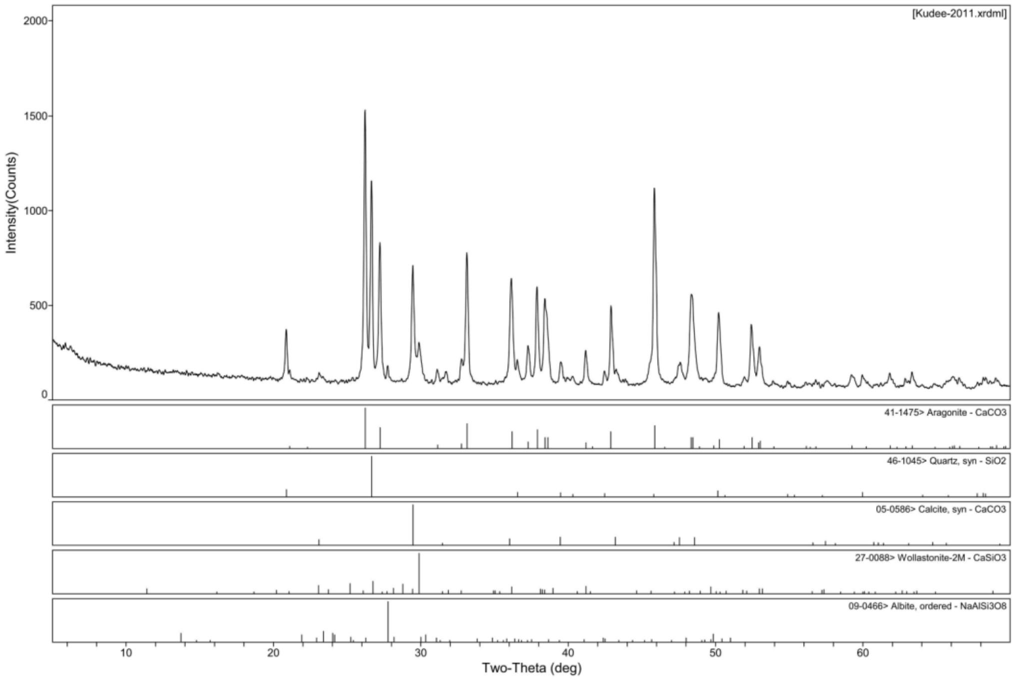

Figure C.6 XRD spectra of Kudee Island sediment sample

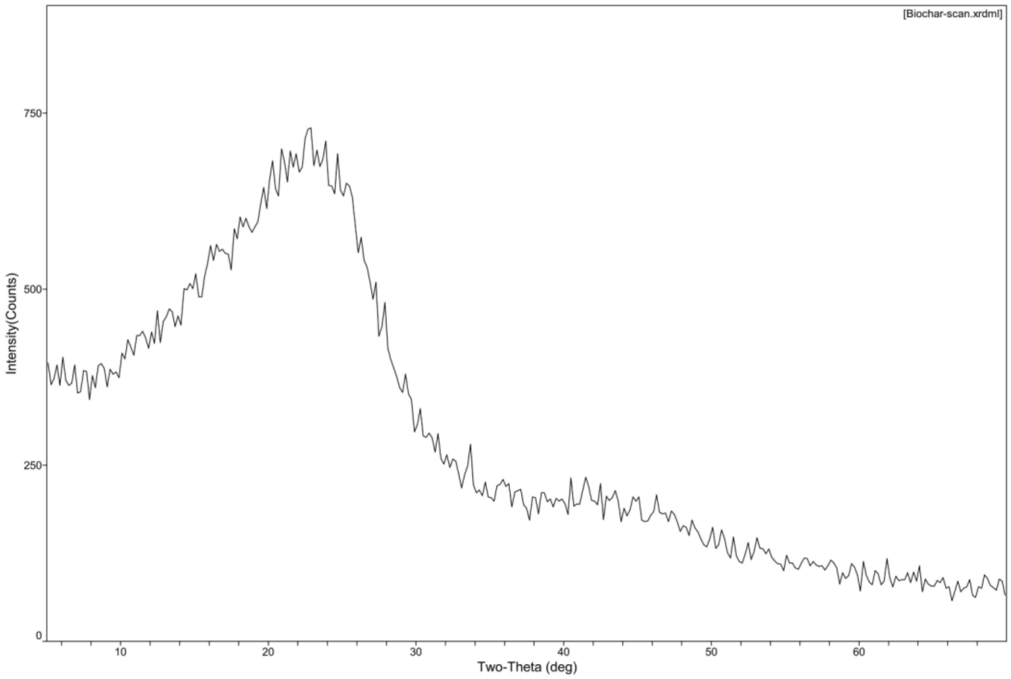

Figure C.7 XRD spectra of biochar sample

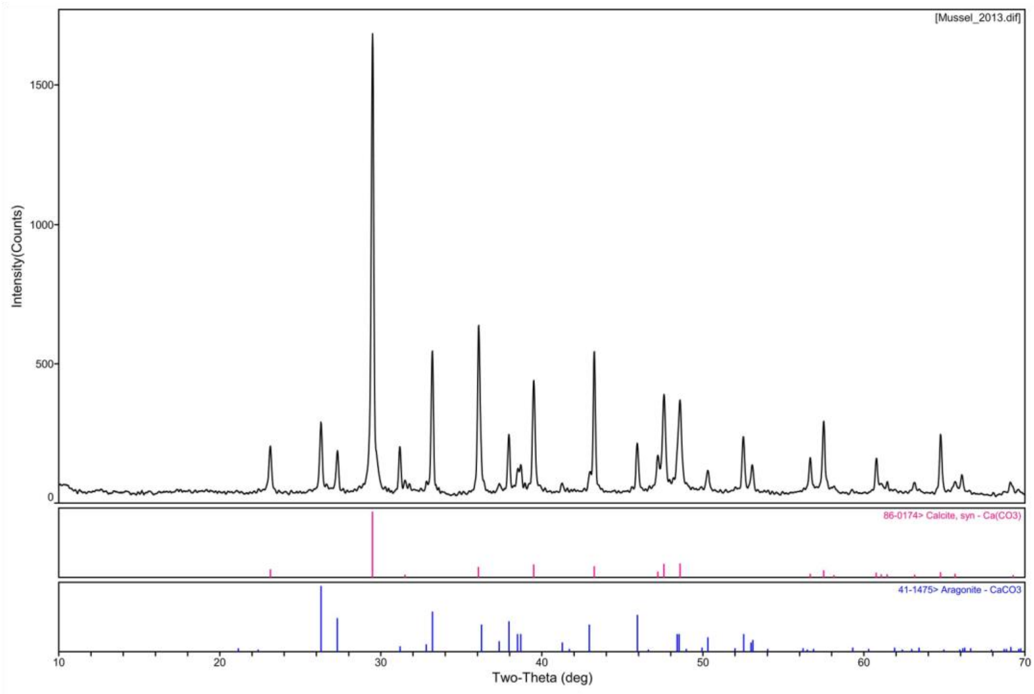

Figure C.8 XRD spectra of mussel shell sample

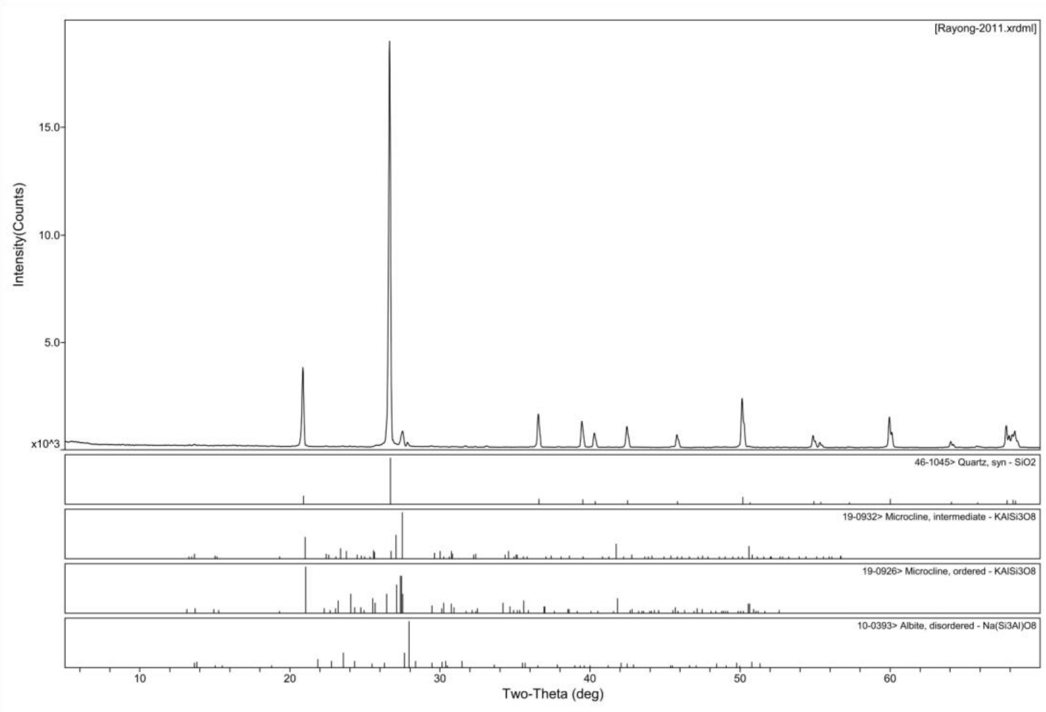

Figure C.9 XRD spectra of prawn shell sample

C.2 FT-IR spectra for polymers prepared in-house

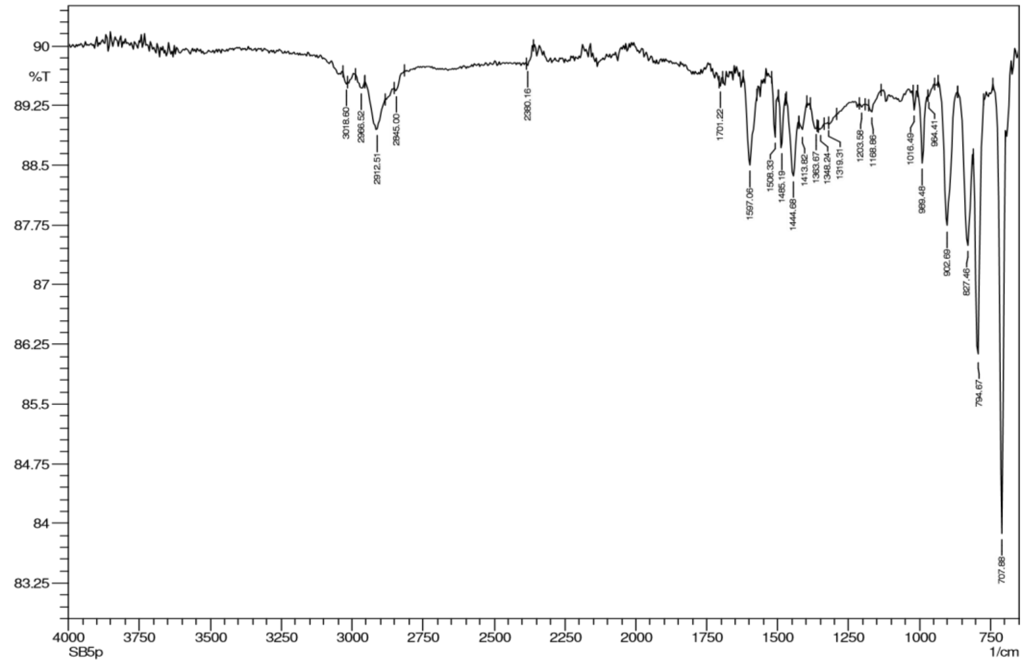

Figure C.10 FT-IR spectra of SB5p

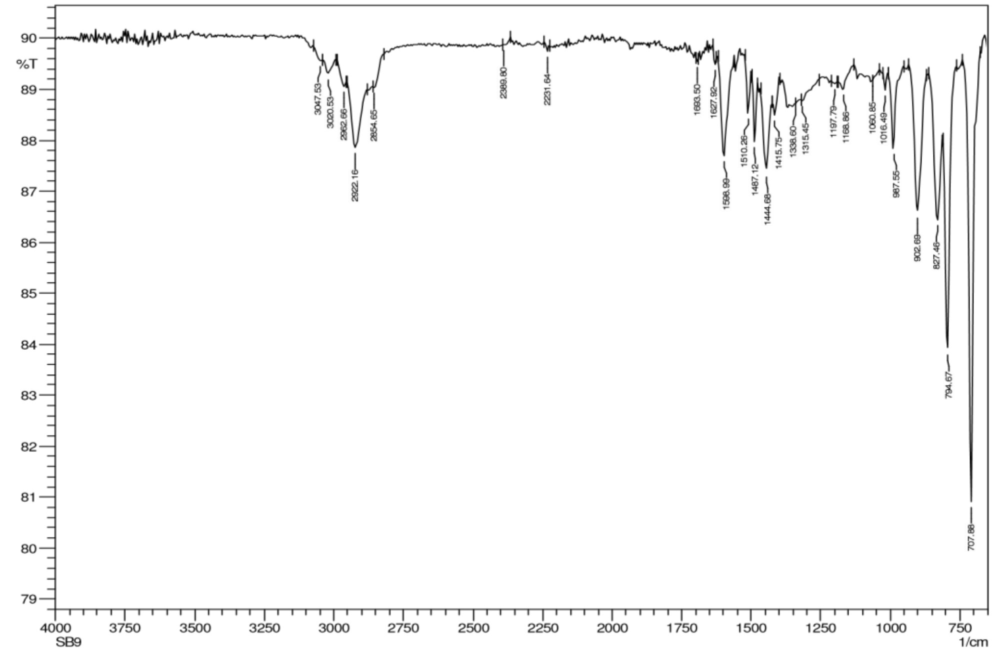

Figure C.11 FT-IR spectra of SB9

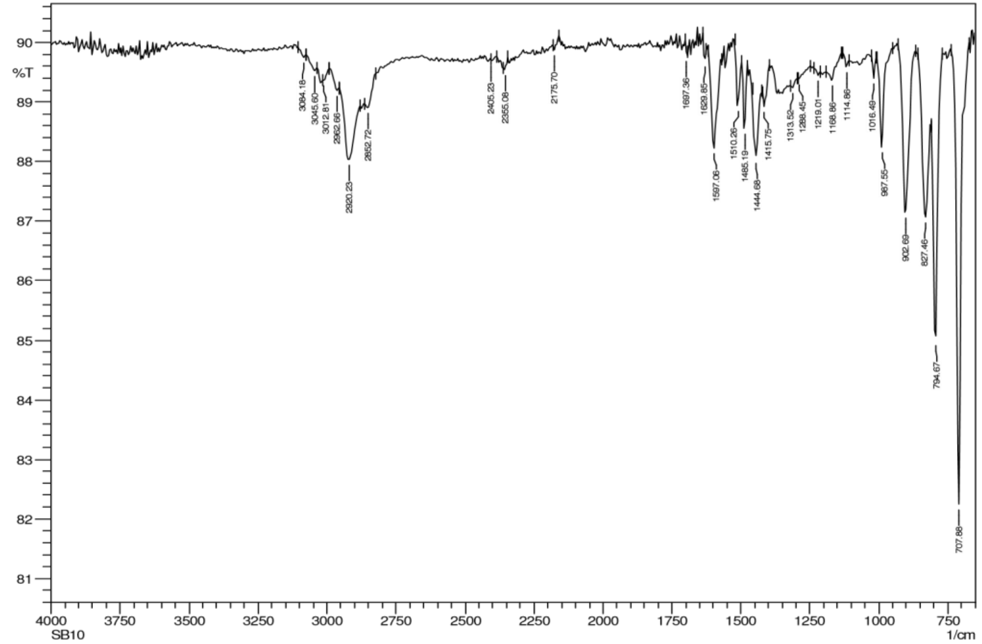

Figure C.12 FT-IR spectra of SB10

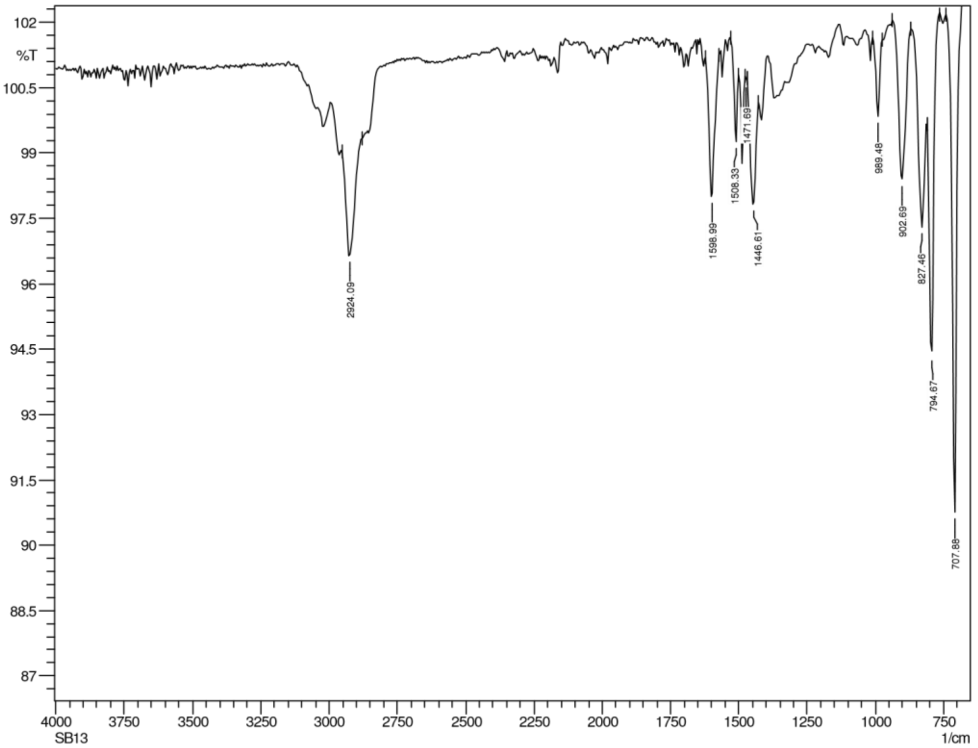

Figure C.13 FT-IR spectra of SB13

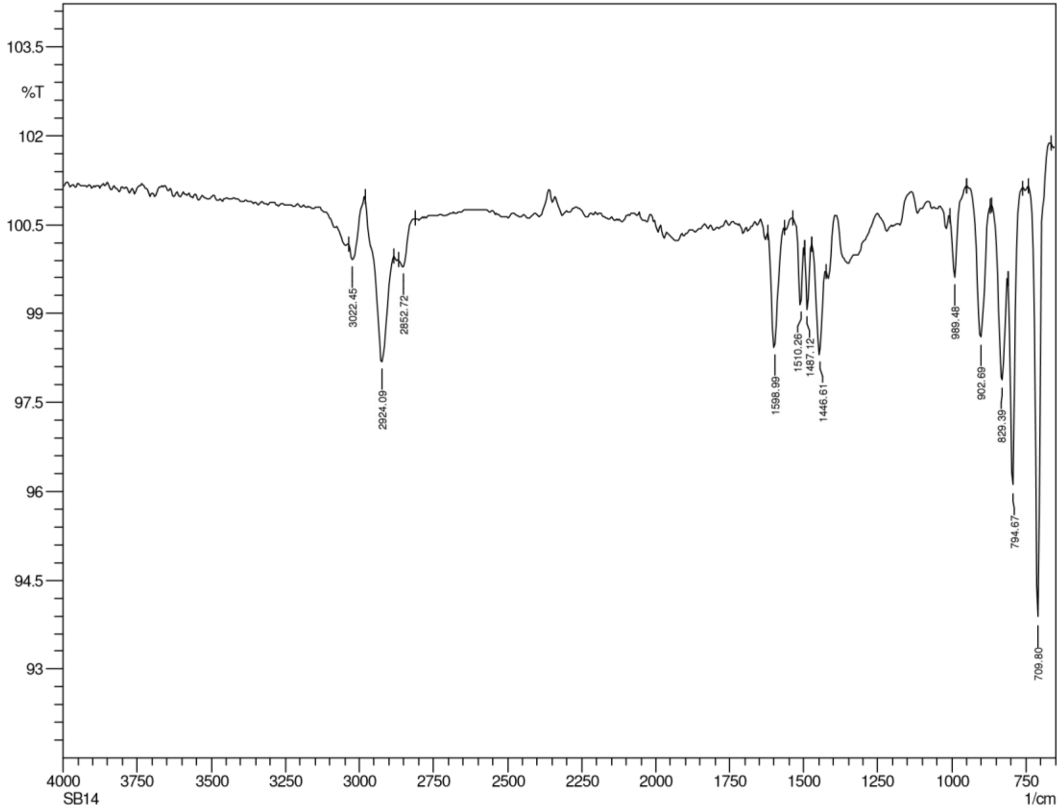

Figure C.14 FT-IR spectra of SB14

Appendix D

Limit of Detection (LOD)

D.1 Calculation of Limit of Detection (LOD) for BPA Determined by HPLC

Table D.1 Calculation data of LOD for BPA determined by HPLC

Concentration (mg L^{-1})	Average peak area, y_i	al from regression line, [$\hat{y}$] $y = 1.6088x + 0.5774$ $R^2 = 0.9993$	y-residuals $[y_i - \hat{y}]_2$
0.005	0.0130	0.5854	0.3277
0.01	0.0249	0.5935	0.3233
0.04	0.1031	0.6418	0.2901
0.08	0.1919	0.7061	0.2644
0.1	0.2491	0.7383	0.2393
0.2	0.4726	0.8992	0.1820
0.4	0.9020	1.2209	0.1017
0.6	1.3384	1.5427	0.0417
0.8	1.7540	1.8644	0.0122
1	2.1456	2.1862	0.0017
2	4.0038	3.7950	0.0436
4	7.7608	7.0126	0.5598
6	11.1222	10.2302	0.7957
8	14.5051	13.4478	1.1180
10	17.6686	16.6654	1.0065
20	33.6392	32.7534	0.7846
40	64.3159	64.9294	0.3764
50	80.6231	81.0174	0.1555

$$\text{LOD Signal} = a + 3S_{yx}$$

/

Where:

a = intercept of regression line

$$S_{y/x} = \sqrt{\frac{\sum (y_i - \hat{y})^2}{n-2}}, \quad n = 18$$

Therefore:

LOD Signal = (0.5774) + 3(0.6434)

= 2.5077

Using the equation from the regression line: $y = 1.6088x + 0.5774$

When: y = LOD Signal, and x = LOD Therefore:

LOD = 1.20 mg L^{-1}

D.2 Calculation of Limit of Detection (LOD) for BPA Determined by DLLME and HPLC

Table D.2 Calculation data of LOD for BPA determined by DLLME and HPLC

Concentration (mg L^{-1})	Average peak area, y_i	ıl from regression line, [ŷ] y = 8.6473x - 0.0045 R^2 = 0.9998	y-residuals [y_i - ŷ]^2
0.001	0.0057	0.0041	2.35 x 10^{-06}
0.005	0.0422	0.0387	1.22 x 10^{-05}
0.01	0.0807	0.0820	1.64 x 10^{-06}
0.1	0.8166	0.8602	1.91 x 10^{-03}
0.6	5.2731	5.1839	7.96 x 10^{-03}
1	8.5937	8.6428	2.41 x 10^{-03}

$$\text{LOD Signal} = a + 3S_{y/x}$$

Where:

a = intercept of regression line

$$S_{y/x} = \sqrt{\frac{\sum (y_i - \hat{y})^2}{n-2}}, \quad n = 6$$

Therefore:

$$\text{LOD Signal} = (-0.0045) + 3(0.0554) = 0.1618$$

Using the equation from the regression line: y = 8.6473x - 0.0045

When: y = LOD Signal, and x = LOD Therefore:

LOD = 18.19 µg L^{-1}

D.3 Calculation of Limit of Detection (LOD) for BPA Determined by SPE and HPLC

Table D.3 Calculation data of LOD for BPA determined by SPE and HPLC

Concentration ($mg\ L^{-1}$)	Average peak area, y_i	l from regression line, [ŷ] y = 192.57x - 0.4464 R^2 = 0.9997	y-residuals $[y_i - \hat{y}]^2$
0.001	0.1659	-0.2538	0.1762
0.005	0.8300	0.5165	0.0983
0.01	1.7596	1.4793	0.0786
0.1	17.5557	18.8106	1.5747
0.5	96.0804	95.8386	0.0585

$$\text{LOD Signal} = a + 3S_{y/x}$$

Where:

a = intercept of regression line

$$S_{y/x} = \sqrt{\frac{\sum (y_i - \hat{y})^2}{n-2}}, \quad n = 5$$

Therefore:

LOD Signal = (-0.4464) + 3(0.8137) = 1.9946

Using the equation from the regression line: y = 192.57x - 0.4464

When: y = LOD Signal, and x = LOD Therefore:

LOD = 8.04 µg L^{-1}

D.4 Calculation of Limit of Detection (LOD) for BPA Determined by AFFINIMIP® SPE Bisphenol A cartridges and HPLC

Table D.4 Calculation data of LOD for BPA determined by AFFINIMIP® SPE Bisphenol A cartridges and HPLC

Concentration ($mg\ L^{-1}$)	Average peak area, y_i	al from regression line, [ŷ] $y = 13.167x + 0.0095$ $R^2 = 0.9997$	y-residuals $[y_i - \hat{y}]^2$
0.0005	0.0123	0.0161	0.0000
0.001	0.0168	0.0227	0.0000
0.005	0.0775	0.0753	0.0000
0.01	0.1489	0.1412	0.0001
0.5	6.5929	6.5930	0.0000

$$\text{LOD Signal} = a + 3S_{y/x}$$

Where:

a = intercept of regression line

$$S_{y/x} = \sqrt{\frac{\sum (y_i - \hat{y})^2}{n-2}}, \quad n = 5$$

Therefore:

LOD Signal = (0.0095) + 3(0.0061) = 0.0279

Using the equation from the regression line: $y = 13.167x + 0.0095$

When: y = LOD Signal, and x = LOD Therefore:

LOD = 1.40 µg L^{-1}