Studies of a Lyophilised Nasal Delivery System

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By

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Dedication

Dedicated to my god brother, T. B. Thapa and to my wife, Bhimu for their love, inspiration and encouragement.

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Abstract

The *in vitro* and *in vivo* drug release characteristics and physico-mechanical properties of a putative lyophilised nasal dosage form, prepared using hydroxypropyl methylcellulose (HPMC), as a hydrophilic gel forming excipient have been investigated. The *in vitro* release of model compounds (nicotine and UK-92, 480) was studied in a diffusion cell, which, with a minimum dissolution volume on the donor side, was intended to mimic the low hydration environment of the nasal mucosa. *In vitro* drug release rate decreased with the concentration of HPMC used, whether the donor side of the cell was presented with a solution, powder or lyophilised plug of the drug – excipient mixture. However at any particular HPMC concentration, drug (nicotine) release rate was faster from solution than from lyophilised plug. The difference in release rate between solution and lyophilisate was greater at high polymer concentrations and indicated that the high viscosity solution that resulted from hydration of the lyophilised plug in the diffusion cell was responsible for the difference. However, release (nicotine) rate was found to be relatively independent of HPMC molecular weight. The bioavailability of nicotine from lyophilised plug after absorption from the sheep nasal cavity was found to be higher than from other intranasally administered dosage forms such as powder and solution.

The mechanical properties of hardness, fracturability, springiness and resilience of the (HPMC) plugs and adhesivity of hydrated plugs, which are important parameters for handling, insertion and residence time on the nasal mucosal surface, were studied using a Texture Analyser. These parameters could be manipulated by changing the concentration of individual formulation components. For lyophilisates in the weight range 5-40 mg, the quantity of water required for complete hydration, which occurred in about 15 sec, lay in the range 50-120 µl. A manufacturing process for the production of lyophilised nasal inserts is also described.

Abbreviations

AC	Acyl carnitines
AG	Ammonium glycyrrhizinate
AUC	Area under the plasma level curve
BS	Bile salts
BG	Blood glucose
CD	Cyclodextrin
CHI	Chitosan
CM	Camostat mesilate
C_{max}	Maximum concentration
CPAA	Cross-linked polyacrylic acid
DSM	Degradable starch microspheres
EI	Electron impact
FD	Freeze drying
GA	Glycyrrhetenic acid
GC	Gas chromatograph
GC-MS	Gas chromatography – mass spectroscopy
HCO	Hydrogenated caster oil
HPC	Hydroxypropyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
LDE	Lyophilised dry emulsion
LPC	L-α-lysophosphatidyl choline

LPG	Lysophosphatidyl glycerol
L-9	Polyoxyethylene-9-lauryl ether
MCC	Microcrystalline cellulose
NHT	Nicotine hydrogen tartrate
PAA	Polyacrylic acid
SEM	Scanning electron micrograph
SCF	Supercritical fluid processing
SDC	Sodium deoxycholate
SGC	Sodium glycocholate
STDHF	Sodium taurodihydrofusidate
ST	Sodium taurocholate.
T_{max}	Maximum plasma concentration

Declaration

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McInnes, F.; **Thapa, P.;** Stevens, H.N.E.; Baillie, A.J.; Watson, D.G.; Nolan, A. and Gibson, I. Nasal Absorption of Nicotine in Sheep from a Lyophilised Insert Formulation. Abstract submitted for Annual meeting of American Association of Pharmaceutical Scientists **(AAPS 2000),** to be held in Indiana Polish, USA.

Chapter 1 General Introduction

1.1 Background

The nasal route of drug administration has been considered in recent years as an means of systemic drug delivery and attempts to use it as an alternative to intravenous administration of drugs such as diazepam (Bechgaard, Gizurarson & Hjortkjjaar, 1997; Gizurarson *et al.*, 1999) are typical. The nasal mucosa is an attractive site for the non-parenteral delivery of therapeutic proteins (Illum & Fisher, 1997) and for the uptake of antigens for the induction of mucosal immunisation (Almeida & Alpar, 1996). The intranasal administration of drugs exploits the permeability of nasal mucosa which is higher than some other mucosal surfaces (Fisher *et al.*, 1992; Hosoya *et al.*, 1993; McMartin *et al.*, 1987). It is classified as a leaky epithelium that allows restricted passive permeation of relatively high molecular weight molecules through intercellular pathways. Among the other advantages offered by this route is the useful accessible mucosal surface of approximately 150 cm^2, accessibility and ease of administration that would encourage patient compliance. The nasal mucosa is highly vascularised and the venous flow that drains it avoids the portal system, allowing the blood to reach various tissues and organs before the liver, thus avoiding a first-pass metabolism

(Verhoef & Merkus, 1994). Nasal drug delivery is attractive for drugs which are active at low dose and have no or minimal oral bioavailability (Illum, 1991). This has opened up a venue for further development of improved nasal dosage forms.

1.2 The nasal cavity as a site for drug absorption

The nasal route was, until recently, largely limited to elicitation of local effects on the mucosa. However the available nasal mucosal surface area, the high vascularisation and the avoidance of first-pass effects, indicate that absorption across the nasal membrane is a reasonable proposition (Table 1.1).

Table 1.1 The nasal cavity as a site for drug absorption (Harris, 1993).

• Large surface area (150 cm^2). Epithelial cells are covered with microvilli
• Porous endothelial basement membrane
• Highly vascularised epithelial layer
• High total blood flow per cm^3 tissue
• Venous return avoids first-pass liver metabolism.

1.2.1 Function of the nose

The normal nose is characterised by slit-like passages, which provide the efficient heat and moisture exchange. Breathing through the mouth can sustain life but, without the air conditioning effect, heating and humidification of the nose, the air flow is both unpleasant and potentially harmful. Another important function of the nose is olfaction. The nose also has a 'piggy bank' function in that the body saves

about 100 ml of water per day, due to condensation of exhaled water in the anterior part of the nose which has a temperatures 3-4°C lower than that of the lungs (Mygind & Dahl, 1998).

The nose is an important site of particle deposition although the efficacy of the nasal filter depends on the diameter of the inhaled particles. Almost all particles larger than 10 μm (pollen grain) are retained in the nose during breathing at rest, while most particles smaller than 2 μm (mould spores) pass through the nose (Mygind, 1978; Mygind & Dahl, 1998). The nose also acts as a protective filter against water-soluble gases such as sulphur dioxide and formaldehyde. Inhaled particles, trapped in the nasal filter, are cleared from the nose within 30 minutes by mucociliary transport.

The properties of air conditioning, air cleaning and olfaction make up the major functions of nasal cavity. Consideration of the nasal delivery of drugs for a systemic effect should acknowledge that these essentially defensive functions remain intact and are not or minimally compromised by the delivery system.

1.2.2 Anatomy of the nose

The nose is divided by the median septum into the two nasal cavities; each of which opens onto the face through the nostril and extends posteriorly to the nasopharanyx with a length of approximately 10 cm. The combined surface area of both cavities is about 150 cm^2 and the volume about 15ml. Each cavity is convoluted into the folds

of the conchae (Fig 1.1 & 1.2) which engender increased resistance to airflow and produce intimate contact between inspired air and the mucosa (Mygind, 1981). There are three distinct functional zones in the nasal passage, which are arranged anterio-posteriorly in the following sequence: (i) the vestibular area, which serves as a baffle system and whose surface is covered by a common pseudostratified epithelium and long hairs, probably to provide filtering of airborne particles; (ii) the respiratory area, which has a pseudostratified columnar epithelial surface and is normally covered by a layer of mucus that is constantly moving toward the posterior aperture of the nasal cavity by the ciliary system; and (iii) the olfactory region, which is approximately 10 cm^2 in area. The respiratory region accounts for most of the surface area (Chien, Su & Chang, 1989; Mygind, 1978).

1.2.2.1 Epithelium morphology

The nostrils are covered by skin, the anterior one-third of the nasal cavity by transitional squamous epithelium which is not attractive as a drug absorption site, the upper part of the cavity by an olfactory epithelium and the remainder by typical airway epithelium, which is ciliated, pseudostratified and columnar. The ciliated and olfactory epithelia have large numbers of microvilli in close association with the cilia. The pseudostratified epithelial membrane rests upon a layer of connective tissue fibrils, the basement membrane, beneath which is the submucosa (lamina propria) with its dense network of fenestrated capillaries that supply the tissue with a large volume of blood. Nasal epithelium (Fig. 1.3) consists of four major cell types (Gizurarson, 1993; Mygind, 1981). *Ciliated cells*, which propel the mucus gel

toward the pharynx by coordinated, wave-like movements of the cilia. *Goblet cells*, which produce the complex secretion which forms an important part of the protective layer. The number of goblet cells seems to increase with age. *Basal cells*, which lie on the basement membrane, are progenitors of the other cell types, and do not reach the airway lumen. Non-ciliated *columnar cells*, the function of which is thought to be related to their high fluid-transport capacity. In addition, non-epithelial cells such as inflammatory cells are occasionally found and intra-epithelial nerve endings (axons) are also present.

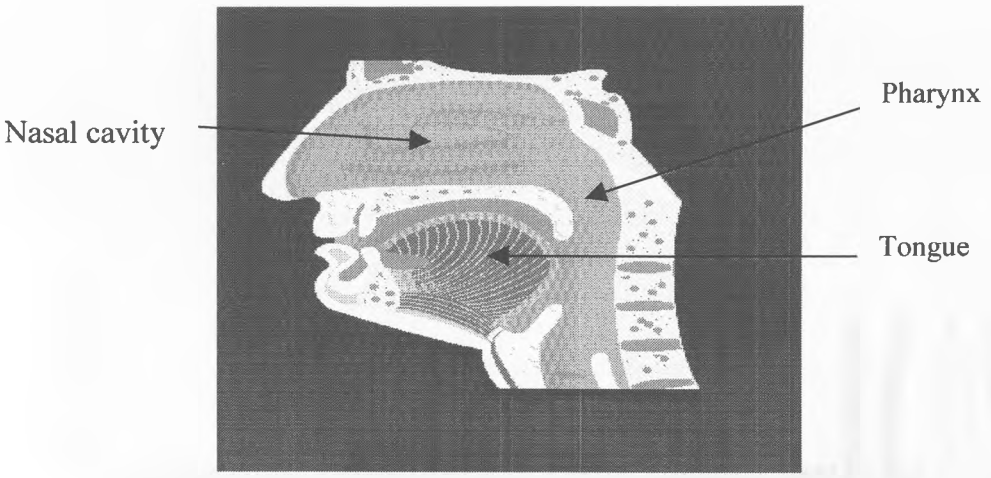


Figure 1.1 Mouth and esophagus showing nasal cavity (reproduced from Body Works CD ROM).

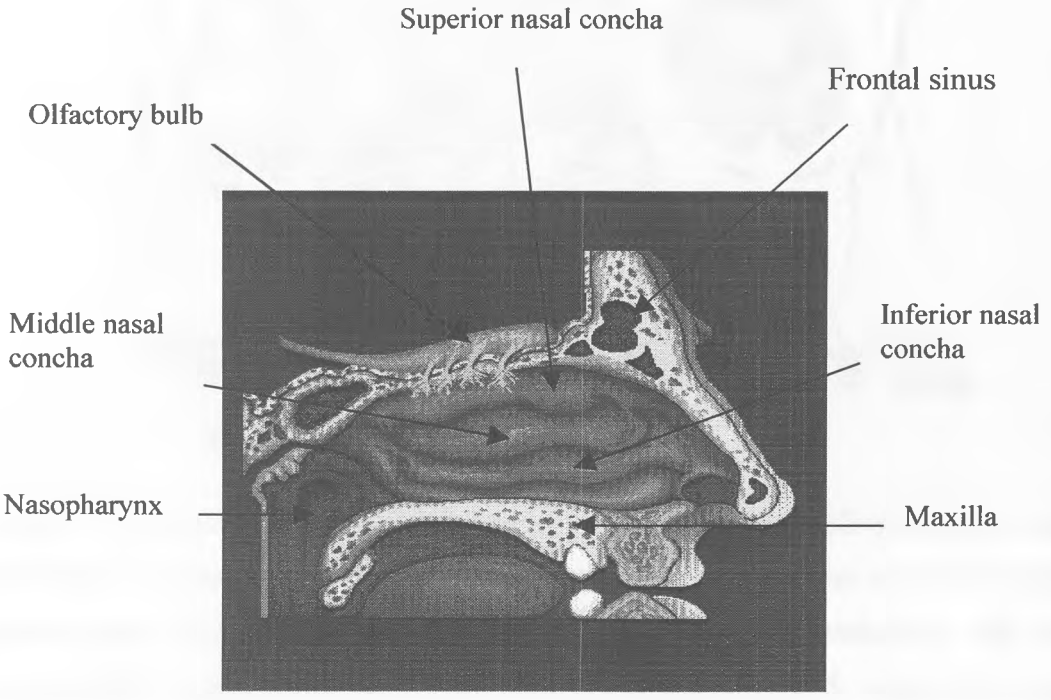


Figure 1.2 Anatomy of the nasal cavity (reproduced from Body Works CD ROM).

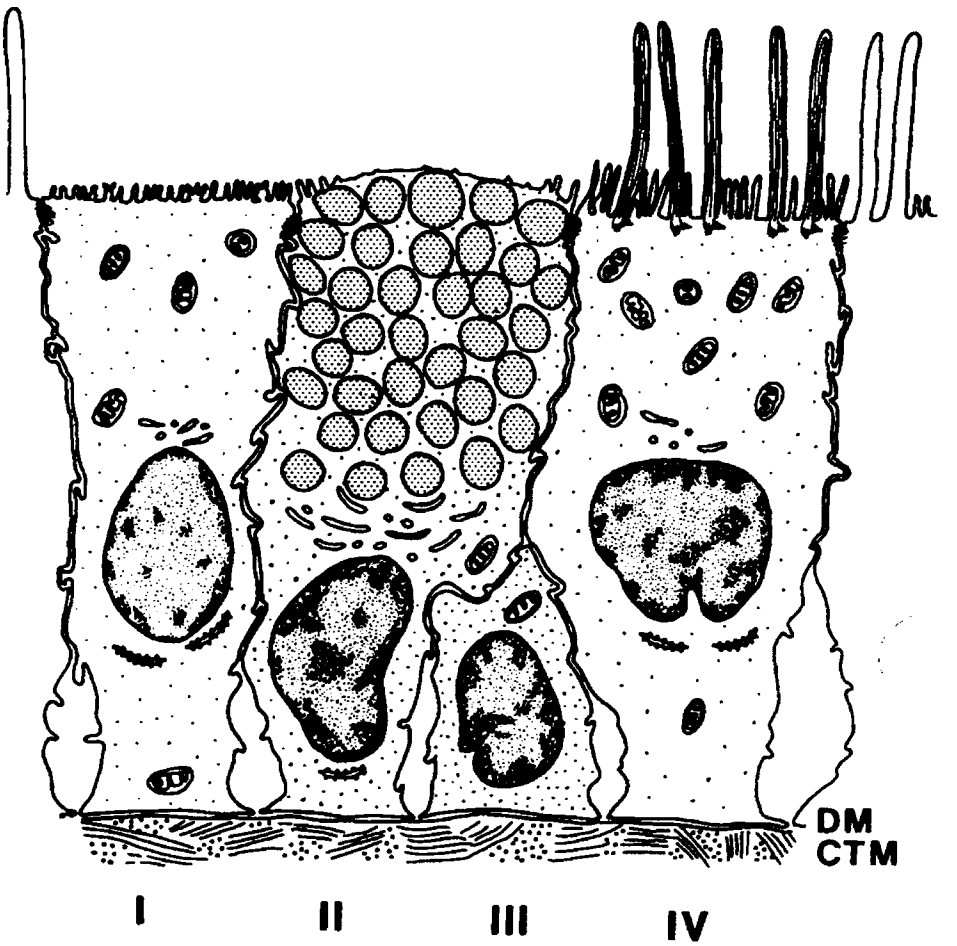


Figure 1.3 Transmission electron microscopic view of various cell types in the nasal epithelium. **I**, nonciliated columnar cell with microvilli, **II**, goblet cell with mucous granules and Golgi apparatus, **III**, basal cell, **IV**, ciliated columnar cell with mitochondria in the apical part, **DM**, double membrane, **CTM**, connective tissue membrane (reproduced from Chien *et al.*, 1989).

1.2.2.2 Mucus and mucociliary clearance

Mucus

The nasal mucosa has a high secretory capacity and in humans, for example, about 1-2 litres of secretion are produced daily (Chien *et al.*, 1989). There is a diurnal variation, with reduced nocturnal secretion rate (Mygind & Thomsen, 1976) when the clearance of secretion is also markedly reduced (Bateman, Pavia & Clarke, 1978). Under normal conditions, the nasal mucosa is covered with mucus which is arranged in two layers; a viscous, dense external layer and a fluid, serous internal layer. The thickness and composition of the double layer is important for mucociliary transport.

Airway mucus is composed of water (95%), mucus glycoproteins (2%), other proteins including albumin, immunoglobulins, lysozyme and lactoferin (1%), inorganic salts (1%) and lipids (<1%) (Kaliner *et al.*, 1984; Mygind & Dahl, 1998).

Nasal secretion in the adult has a pH in the range of 5.5 to 6.5 (Chien, 1995). Maintenance of pH 6.5 or less is believed to prevent growth of pathogenic microorganisms in the nasal passage. The composition of nasal secretion is shown in Fig. 1.4.

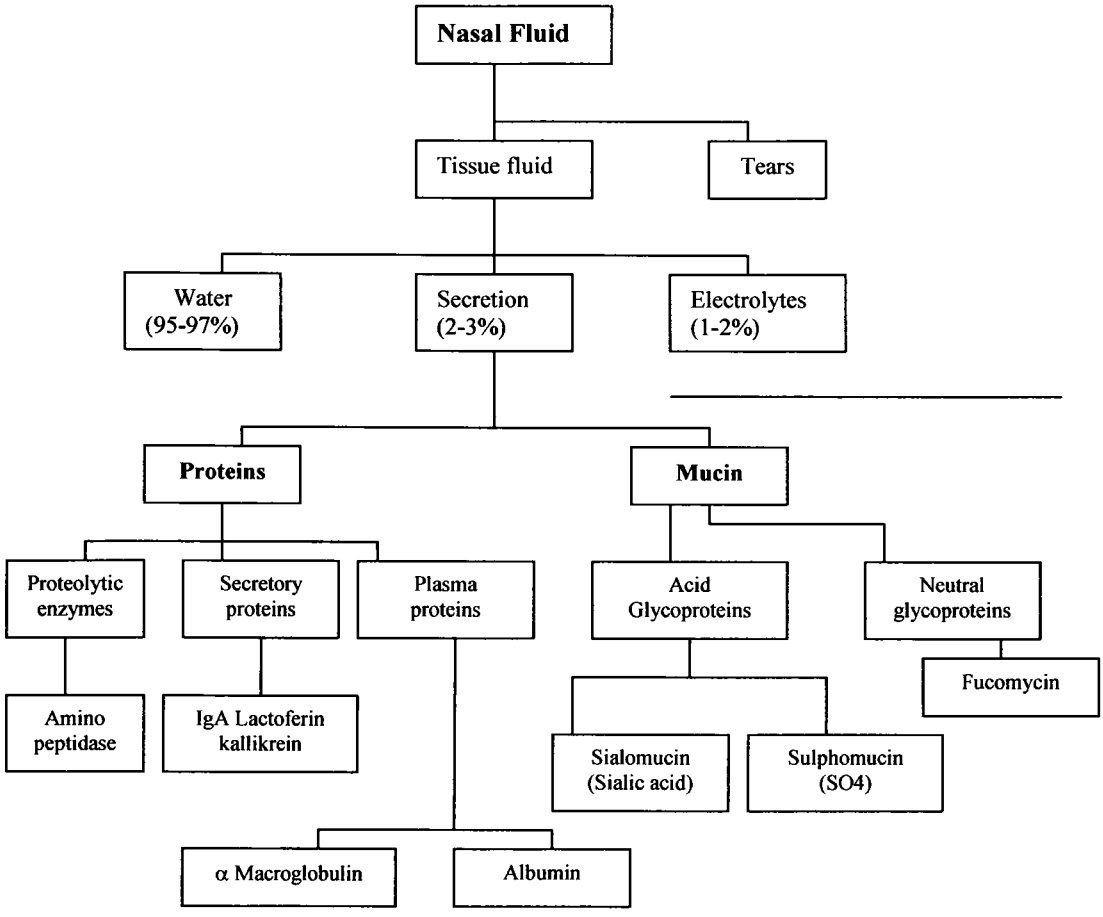


Figure 1.4 The composition of nasal secretion (Harris, 1993).

Mucociliary transport

Mucociliary transport is one of the most important physiological defence mechanism of the respiratory tract, which protects the body against inhaled noxious materials (Chien, 1995). It is probably the most significant physiological factor which might influence drug absorption (Schipper, Verhoef & Merkus, 1991). The speed of mucus

flow is an important factor in the residence half-life of the formulation within the nasal cavity and hence the time a drug remains at its absorption site.

Cilia beat about 1000 times per minute in the backward direction and thereby convey the mucus, with its trapped inhaled particles, to the throat where it is swallowed. Transport rates of 3-25 mm/min (average 6 mm/min) have been reported in normal subjects (Proctor, 1982). Thus, the nasal mucus layer is replaced every 10-15 min and under normal conditions, inhaled substances or a drug delivery system are cleared from the nose within 15-20 min (Anderson & Procter, 1983). Although ciliary beating is the major component of mucociliary clearance, there is also the additional influence of mucus secretion, swallowing, sniffing and gravity. To achieve good drug absorption with only a minimal mucociliary effect, the absorption process must be rapid, preferably within the 15 min of administration (Gizurarson, Rasmussen & Larsen, 1991).

1.3 Factors that influence nasal absorption

Nasal drug absorption is influenced by number of factors that are described below:

1.3.1 Biological factors

1.3.1.1 Nasal blood flow

The nasal blood vessels play an important role in the thermal regulation and humidification of the inhaled air (Chien & Su, 1987) and the vascular nature of the

mucosa makes it a good membrane for drug absorption (Cauna, 1982) although blood flow and hence drug absorption will depend upon vasodilation and vasoconstriction.

Nasal mucosa blood vessels are surrounded by adrenergic nerves which when stimulated decrease blood flow and blood content in the human nose (Bende, 1983; Richarson & Seeboham, 1968). Nasal blood flow is affected by several external and physiological factors such as ambient temperature, humidity, presence of vasoactive drugs, trauma, and inflammation (Cauna, 1982) as well as some psychological factors such as emotion, fear, anxiety and frustration (Paulsson, Bende & Ohlin, 1985). Nasal blood flow is also sensitive to a variety of compounds that act both locally or systemically. Such effects, due to their effects on blood flow, are important in determining nasal drug absorption.

1.3.1.2 Enzymatic activity in the nose

Several enzymes, which are present in nasal secretions, may effect the metabolism of drugs. Cytochrome P-450 dependent mono-oxygenase has been reported to metabolise drugs such as cocaine, decongestant, nicotine (Dahl & Hadley, 1983) and progesterone (Brittebo, 1992). The few reports available on human nasal metabolic capabilities strongly suggest that significant metabolic activity is present in the human nasal mucosa (Behl *et al.*, 1998).

1.3.1.3 Physical condition of the nasal mucosa

Drug absorption can be affected by the condition of nasal mucosa. There are times when the mucosa is crusting, bleeding, or dry as a result of rhinorrhea, sinitis, or nasal infection. Excessive nasal secretion can wash away a formulation before the drug is absorbed through the mucosa or before acting locally (Proctor, 1985). Similarly a blocked or runny nose will affect the clearance of the formulation and drug absorption.

1.3.1.4 Volume of administration

In spite of the high permeability of nasal mucosa, many drugs may not be sufficiently absorbed. Lack of adequate aqueous solubility is often a problem. The optimal formulation volume for nasal administration is 25-100 μl per nostril and larger volumes than this will drain out of the nose. The most practical volume is 100 μl (Behl *et al.*, 1998). Identification of the volume of administration on drug absorption may be important during the development process.

1.3.1.5 Site of deposition of formulation in the nose

For greater nasal residence time and to allow more contact time between drug and mucosa, deposition of formulation in the anterior portion of the nose is desirable. Deposition in the posterior part of the nasal cavity allows faster ciliary clearance of the formulation. Ideally, slow absorbing drugs should be deposited in the anterior

part of the nasal cavity and fast absorbing drugs in the posterior part of the nasal cavity.

1.3.2 Physico-chemical properties

1.3.2.1 Drug molecular weight and size

It has been well established that the absorption process is generally conditioned by the size of the drug molecule. If the colloidal particles can be assumed to be approximately spherical, the following equation, suggested by Sutherland and Einstein, can be used to relate the diffusion coefficient of the particle to its radius (Martin, Swarbrick & Cammarata, 1983):

$$D = \frac{kT}{6\pi\eta r}$$

where D is the diffusion coefficient, k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solvent and r is the radius of the spherical particle.

Investigation of the effect of molecular size in a wide range of drugs with molecular weight varying from 160-34000 Da (McMartin *et al.*, 1987) indicated that nasal absorption decreased exponentially as a function of increasing molecular weight. This trend was observed for both rats and humans. The rate limiting molecular weight was found to be 1000 Da compared to 300 Da for the oral route. In other studies, the nasal absorption of a wide range of water-soluble substances with

different molecular weights, such as p-aminohippuric acid, sodium cromolyn, inulin and dextran was studied in rats (Fisher *et al.*, 1987; Maitani, Machida & Nagai, 1989). The results indicated a good linear correlation between the log of percent absorbed and the log of the molecular weight (an inverse relationship between absorption and molecular weight), suggesting the participation of aqueous channels in the nasal absorption of water-soluble molecules.

In another study using polyethylene glycols, 600, 1000, and 2000 in rats (Donovan, Flynn & Amidon, 1990) it was found that both oral and nasal absorption showed an inverse relationship between percent absorbed and molecular weight. Similarly in the study of nasal absorption of radio-labelled dextran compounds of molecular weight varying form 1260 to 45500 Da, an inverse relationship between percent absorbed and molecular weight was observed (Fisher *et al.*, 1992). A 36-fold increase in molecular weight resulted in a decline of nasal absorption from 52.7% to 0.6%, an 88-fold difference.

1.3.2.2 Drug solubility and dissolution rate

In general, drug solubility is perhaps the most studied and most important factor in determining absorption of drugs through biological membranes. The effect of drug solubility on absorption has been extensively explored for GIT and skin membranes. Similar information on nasal absorption needs to be generated (Behl *et al.*, 1998).

The dissolution rate of particulate nasal products, administered as either powders or suspensions is important since particles deposited in the nostril must dissolve prior to absorption. There will be no absorption if the drug remains in the particulate form in the nostrils, or if the particles are cleared from the nasal cavity prior to dissolution.

1.3.2.3 pKa and the partition coefficient of drug

Drugs are absorbed through biological membranes by various processes. During concentration gradient-dependant (passive) diffusion process, a drug may pass through the cell membrane, transcellular transport or it can pass between cells through tight 'junctions'. Some drugs can cross the cell membrane via some carrier-mediated means. Although many factors can affect these transport pathways, the most important factors are pH, pKa and the partition coefficient of a drug.

The pH of the nasal mucosal surface was reported to be 7.39 (Hirai *et al.*, 1981) and its importance to drug absorption was stressed. The pH of the formulation as well as the mucosal surface pH influence drug absorption and it was found that the nasal absorption of a weak electrolyte such as salicylic acid or aminopyrine in rats was highly dependant on the degree of ionisation. For aminopyrine, absorption rate increased with pH and the authors suggested that aminopyrine was absorbed through the nasal mucosa by a passive transport of the un-ionized form. For salicylic acid, the absorption rate decreased with pH.

The effects of dose, pH, and osmolarity on the nasal absorption of secretin have been studied in rats (Ohwaki *et al.*, 1987; Ohwaki *et al.*, 1985; Ohwaki *et al.*, 1989). It was found that absorption was much higher at a pH below 4.79 and was maximal at a pH of about 3 and minimal at pH > 7. The authors concluded that the pH effects seen with secretin were mainly due to pH mediated mucosal changes especially at pH 3.

The relationship between lipophilicity and the absorption rate constant for mucosal absorption of progesterone has been decribed (Corbo, Liu & Chien, 1990). For the three rabbit membranes (nasal, rectal and vaginal) studied, progesterone absorption increased linearly with partition coefficient. It was also found that the nasal mucosa was more permeable than the rectal and vaginal membranes.

In a study of the nasal transport of sulphisomidine as a function of pH in rats it was found that the unionised species of the drug was absorbed more than the ionised species supporting the pH-partition theory of drug absorption (Sakane *et al.*, 1993).

Similarly, in a study of effect of pH on the nasal absorption of benzioc acid (pKa = 4.19) from solution, as the pH of the solution was increased from 2 to 7.19, the amount absorbed decreased from 44% to 13%, presumably due to ionisation of the drug (Huang *et al.*, 1985a). The same group reported that the absorption of L-tyrosine was pH independent in the range of 4-7.4 (Huang *et al.*, 1985b). Based on their various studies these authors have suggested that drug partition coefficient can be discounted as the major factor determining the extent of nasal absorption.

However drug absorption through biological membranes is complex and no single factor can explain the dynamics of drug absorption.

1.3.2.4 Chemical states: prodrugs

Prodrugs have been used to overcome unpleasant tastes, poor solubility, insufficient stability (both chemical and enzymatic), incomplete absorption and metabolism to an inactive species. The nasal mucosa is rich in various enzymes such as aldehyde dehydrogenase, glutathione transferases, epoxi-hydrolases and cytochrome P-450-dependant monooxygenases. The presence of these enzymes make it possible to design prodrugs for better absorption and high systemic availability (Krishnamoorthy & Mitra, 1998). The chemical form in which a drug is presented to the (nasal) mucosa is important for its absorption. In the absence of the desired absorption properties, several options can be considered. One is to alter the drug by adding a bio-cleavable lipohililic moiety and such ester prodrugs can be made by simple esterification of hydroxyl or free carboxyl groups to yield a more favorable partition coefficient and better drug transport. These ester linkages may be cleaved either in the nasal mucosa or in the blood to provide the original compound. Such approaches have been used for nasal absorption of acyclovir (Shao, Hoffman & Mitra, 1994a; Shao & Mitra, 1994; Shao *et al.*, 1994b), and 5-iodo-2-deoxyuridine (Ghosh & Mitra, 1991; Ghosh & Mitra, 1992; Narukar & Mitra, 1988).

The prodrug approach has been similarly utilized to improve peptide absorption since they penetrate biomembranes poorly, suffer rapid enzymatic degradation, and

have short biological half lives. By derivatization the peptide molecule can be made more lipophilic and can be protected against enzymic degradation. The ideal peptide prodrug would exhibit enhanced membrane permeation characteristics and increased stability against enzymatic degradation. After crossing the membrane barrier, the prodrug should undergo spontaneous or enzymatic transformation to the pharmacologically active peptide. Recently, a cyclic acycloxylalkoxycarbamate prodrug of a model hexapeptide (H-Trp-Ala-Gy-Gly-Asp-Ala-OH) was synthesized to enhance membrane permeation and stabilize it against metabolism. It was suggested that an acyloxyalkoxy promoiety reduced the liability of the peptide to peptidase metabolism and substantially increased permeation through biological membranes (Pauletti *et al.*, 1996).

The prodrug approach may not however be a first-line strategy since it presents additional challenges which need to be overcome in the development process. The toxicity of the prodrug needs to be fully evaluated.

1.3.2.5 Physical state: particle size and morphology

Particle size and morphology are important characteristics of particulate nasal drug products. These parameters influence drug dissolution rate and should be controlled to obtain appropriate dissolution in the nostrils. Particle size and morphology also influence the feel of grittiness and possibly irritation of the nasal mucosa. Too fine particles, below 5 μm may be inhaled into the lungs and should be avoided for nasal

products (Behl *et al.*, 1998). Generally, particles in the 5-10 μm range are deposited in the nostrils.

1.3.3 Formulation Factors

Developing the appropriate formulation for a particular drug is important. A good drug candidate can be rendered useless, ineffective or mediocre by an inappropriate formulation. On the other hand, by selecting an optimal formulation, a mediocre drug can be converted into an effective, successful product. A considerable amount of work has been reported on nasal drug products with respect to general formulation variables and dosage forms including specialized drug delivery systems such as microspheres.

1.3.3.1 Dosage forms and delivery systems

Drugs are usually administered to the nasal cavity in the form of solution, suspensions, powders, microspheres, gels or inserts for local or systemic effects (Su, 1993). Drug absorption across the nasal mucosa depends on the physical form of the formulation (Hardy, Lee & Wilson, 1985; Harris, Hedner & Vilhardt, 1987; Pontiroli *et al.*, 1989; Schipper *et al.*, 1993). A powder was found to be more effective than a liquid formulation in delivering insulin in rabbits (Schipper *et al.*, 1993). Similar finding was reported when powder and solution dosage forms of cromoglycate were compared in humans with allergic rhinitis (Resta, Barbaro & Carnimeo, 1992). It was suggested that the powder form was better than the solution because it is less

readily washed out with the nasal secretions. However the nasal absorption of calcitonin from a spray or powder was same (Pontiroli *et al.*, 1989). In another study drops were found to deposit albumin in human nostril more efficiently than a spray (Hardy *et al.*, 1985).

Viscosity is another important formulation parameter and in general, a viscous formulation is less efficient in systemic nasal drug delivery. In the nasal delivery of desmopressin (Harris *et al.*, 1989) it was reported that addition of a viscous agent to a nasal formulations tended to produce a more sustained effect, delayed the onset of absorption and did not enhance total bioavailability. Viscous formulations such as gels might be more effective and appropriate for locally acting drugs and may also be attractive for drugs that have an unpleasant taste in the mouth via nasal drip.

Specific types of nasal dosage forms are important in determining the nasal absorption profiles of drugs. Choice of a particular dosage form generally depends on the drug being developed, its indication, the patient population, and marketing considerations. The following section describes the various different nasal dosage forms which have been developed.

Nasal bougies (buginaria)

Bougies were medicated pencils intended for insertion into the nostril. They were prepared in the same way as suppositories but differed in shape, resembling a pointed rod and were usually made from a gelato-glycerin base. Nasal bougies are rarely

used today probably due to their uncomfortable size for the patient and they were messy after melting. They were typically 1 - 3 inch long and weighed 10-18 grain (0.66 - 1 gm) (Gunn & Carter, 1965; Martindale, 1941; Martindale & Westcott, 1920). They required careful pouring of the molten mass due to the tapered mould geometry. However nasal bougies can be prepared by sucking the melted mass into previously lubricated glass tubing (Davis, 1961), a technology that could be improvised to prepare rapid hydrating nasal inserts of suitable size incorporating drug into hydrophilic polymer.

Nasal drops

Nasal drops are perhaps the simplest and most convenient means of administration of drugs to the nose. The disadvantage of this dosage form is that an exact amount of the formulation cannot be delivered easily and the formulation may be contaminated by the dropper. A major drawback of all water based dosage forms is microbiological stability, because the required preservatives impair mucociliary function (Batts *et al.*, 1989). Besides microbiological stability, the chemical stability of the dissolved drug and the short residence time of the formulation in the nasal cavity are other major disadvantages of such liquid formulations (Hardy *et al.*, 1985; Illum, 1987).

Sprays

Nasal spray devices include the squeezed bottle, the metered-dose spray pump and newer devices known as airless or preservative free spray pumps. Most of the nasal preparations on the market containing solutions, emulsions or suspensions are delivered by metered-dose spray pumps. Compared to squeezed bottles and continuous valve sprays, they allow administration of a defined dose with high dosing accuracy and a typical pattern (Kublik & Vidgren, 1998). Dose volumes between 25 and 200 µl are available as standard. Spray characteristics vary according to the precompression mechanism, the type of the pump and valve and the physical properties of the product. Viscosity, thixotropic behaviour, elasticity and surface tension of the liquid determine the spray pattern, the particle size of the drops, the dose and the dosing accuracy. Metered dose spray pumps include the container, the pump with valve and the actuator. The disadvantages of sprays are similar to nasal drops mainly due to the requirement of preservatives.

Suspension sprays

Suspension dosage forms can also be administered by using the metered-dose nasal actuator systems as described above. The actuator may have to be designed to take account of the size and morphology of the drug particle.

Powders

Dry powders are less frequently used in nasal drug delivery. Major advantages are the good stability of the formulation and the lack of preservatives. Compared to solutions, a powder can give prolonged contact with the nasal mucosa although it has been shown that small volumes of liquid and powder particles have almost the same clearance rate (Illum *et al.*, 1987). The addition of bioadhesive excipients decreases clearance. Dimethyl-β-cyclodextrin as penetration enhancer in a powder formulation of insulin gave higher bioavailability than a solution formulation (Schipper *et al.*, 1993). Similarly, the nasal administration of a lyophilised insulin - dimethyl-beta-cyclodextrin powder formulation in human volunteers resulted in a rapid nasal insulin absorption with a peak plasma level at 10 min. The mean absolute bioavailability of insulin absorption with this formulation was 5.1% (Merkus, Schipper & Verhoef, 1996).

In another study it was reported that powder preparations with mixed base materials such as blend of microcrytalline cellulose (MCC) and hydroxy propyl cellulose (HPC) gave better nasal absorption of a low molecular weight compound, 5-carboxy fluoroscein (CF) than a liquid formulation (Dohi *et al.*, 1997). MCC, a water-absorbable and water-insoluble excipient with a property that enhances the bioavailability of active ingredients by increasing its local concentrations on the nasal mucosa after insufflation. On the other hand, HPC is a water absorbable and gel-forming excipient that captures and slowly releases active ingredients onto the nasal mucosa. A suitable combination of two such base materials with different

properties might allow low molecular weight compound as well as peptides to be effectively absorbed.

Liquid preparations are easily cleared to the nasopharynx and oropharynx from where they enter the posterior part of the tongue and nasal powders may improve patient compliance, especially for children, if the smell and taste of the drug is unacceptable. A powder allows delivery of the highest mass of active ingredient without the necessity for a vehicle. However, the advantage of powder formulations over liquids is highly dependent on the solubility of the drug, its absorption rate, particle size and its irritation potential. Furthermore, the powder properties of size and shape, density and flow characteristics have an influence on distribution in the nose. Specialized delivery devices can be used to deliver metered doses of powders into the nose. To ensure high dose accuracy for intranasal powders, the administration by a mono-dose device, similar to a small unit-dose syringe, is possible. A new development of this device enables the administration of freeze dried powders lyophilised in the device (Kublik & Vidgren, 1998).

Powders can irritate the nasal mucosa and give a gritty feel. Such dosage forms are also difficult and expensive to manufacture due to particle/morphology considerations.

Nasal Gels

Nasal gels, which are either thickened/gelled solution or suspensions of drugs have been investigated as a means of prolonging the contact time of the formulation with the nasal mucosa. Experimentally, the formulations were administered by means of a syringe (Morimoto, Morisaka & Kamada, 1985). Until the recent development of metered dose gel devices, there was no precise method of delivery. The deposition of gel in the nasal cavity depends on the mode of administration, because due to its viscosity the formulation has poor spreading abilities. In the absence of special application techniques the gel covers a small area, where it is applied in the nasal cavity. Gels can offer the following advantages over other dosage forms (Behl *et al.*, 1998) by reduction of post nasal drip and taste problems and loss of the formulation from the nasal cavity; reduction of anterior leakage from the nasal cavity; sticking to the mucosa to optimize drug absorption.

For some drugs, the irritant potential of the drug or other excipients can also be reduced because the gel may permit incorporation of soothing agents or emollients which may not be possible with solution, suspension, or powder dosage forms. Gels can be developed for systemic and local drug delivery. For instance, a cyanocobalamin (vitamin B12) gel has been developed by Nastech Pharmaceutical Company for administration of the vitamin B12 deficiency anemia. This product is provided in a metered dose nasal gel delivery system.

Emulsion and ointment

These types of dosage form for nasal administration are usually used for locally acting drugs. Major disadvantages of nasal emulsions and ointments include poor acceptance by patients and consumers, problems of delivery of precise doses and a more complicated development process.

Specialized systems (microspheres and liposomes)

Recently, microsphere technology has been used in nasal drug delivery formulations. The primary rationale for such work is to allow a more intimate and prolonged contact between the drug and the mucosal membrane. It is well known that solutions, suspensions and powder are rapidly cleared from the nasal cavity and drugs which are not absorbed from such dosage forms, stand a better chance for absorption when formulated in 'gelling' microspheres made from biocompatible materials such as starch, gelatin, albumin and dextran.

The nasal delivery of gentamicin in rats and sheep was improved from a 1% absolute bioavailability from a solution formulation to 50% absolute bioavailability by incorporating the drug into microspheres (Illum *et al.*, 1988) along with the absorption enhancer, lycolecithin. Absorption of insulin through rat nasal mucosa was substantially improved by insulin starch microspheres (Bjork & Edman, 1988; Bjork & Edman, 1990). In another study, it was found that starch microspheres were more effective than dextran microspheres in optimizing insulin delivery in rats (Edman, Bjork & Ryden, 1992). It has been reported that hyaluronic acid insulin

microspheres produced a large and significant increase in nasal absorption in sheep (Illum *et al.*, 1994b). The bioavailability relative to a subcutaneous dose was found to be 11%.

Desmopressin in starch microspheres with and without a mild absorption enhancer, 1-α-lysophosphatidylcholine (LPC) when tested in a sheep model of nasal absorption (Critchley *et al.*, 1994) showed significantly improved absorption from microspheres which was further improved by microspheres which contained LPC. An erythrocyte based bioadhesive system for the nasal delivery of propanolol evaluated in the rat (Vyas *et al.*, 1993) showed good bioadhesion and drug blood levels which were maintained for periods of up to 10 hours.

In a study of the effect of nasally administered biodegradable starch microspheres (DSM) on the mucociliary system and the geometry of the nasal cavities in 15 healthy human volunteers no change in these parameters was found after repeated DSM administration (Holmberg *et al.*, 1992). This means that DSM had no adverse effects on human nasal mucociliary clearance and did not cause mucosal congestion.

The safety of starch microsheres has been further evaluated (Edman *et al.*, 1992) by 1-week dosing in healthy human volunteers who showed good tolerance. These authors provided a mechanistic insight into how the mucoadhesive microspheres could optimize nasal drug absorption. In a Caco-1 cell culture model, a temporary widening of the tight junctions in the presence of the microspheres was observed. This widening coincided with increased insulin absorption. It was hypothesized that

the microspheres dehydrate the nasal mucosa by moisture uptake which results in a reversible 'shrinkage' of the cells and separation of the intercellular junctions.

1.3.3.2 Formulation pH

A nasal formulation must be adjusted to an appropriate pH to avoid irritation of the nasal mucosa, to obtain efficient drug absorption and to prevent growth of pathogenic bacteria in the nasal passage.

Formulation pH should be between 4.5 and 6.5 to avoid nasal irritation. The nasal surface pH is 7.39 (Hirai *et al.*, 1981) and the pH of nasal secretion 5.5-6.5 in adults and 5.0-6.7 in infants and children (Behl *et al.*, 1998). The physicochemical properties of the drug influences formulation pH since drugs are absorbed well in the unionized form. A formulation pH < 4.5 as dictated by drug physicochemistry raises benefit to risk considerations. Acidic formulations inhibit the growth of bacteria,

1.3.3.3 Formulation osmolarity

The effect of osmolarity, and other factors, on nasal absorption of secretin in rats was studied (Ohwaki *et al.*, 1987; Ohwaki *et al.*, 1985; Ohwaki *et al.*, 1989) using sodium chloride and sorbitol formulations. Although optimal absorption was obtained with a hypertonic 0.462 M of sodium chloride solution, histological evaluation of the mucosa revealed shrinkage and structural changes compared to controls. Interestingly, when the nasal mucosa was treated with 0.924M sorbitol, the same

osmolarity as 0.462M sodium chloride solution, there was neither enhanced absorption nor structural changes in the epithelial cells. This was thought to be due to the ionic species of NaCl which acted more strongly on the epithelial cells than the sorbitol. Another study found that hypertonic and isotonic solutions caused minimal irritation while hypotonic solution caused extensive leakage of the enzyme markers lactate dehydrogenase and 5-nucleotidase from nasal membrane (Pujara *et al.*, 1995).

These studies show that although hypotonic or hypertonic solutions cause better drug absorption than isotonic solutions, the integrity of epithelial cells and ciliary beat frequency is best preserved with the later.

1.3.3.4 Type and concentration of buffer

Four different buffers (acetate, adipate, citrate, and phosphate) at a concentration 0.07 M, pH 4.75 were evaluated to determine their effects on the integrity of rat nasal mucosa (Pujara *et al.*, 1995). Acetate buffer was found to have most irritant potential perhaps because of the high lipid solubility of unionized acetic acid at pH 4.75. The damaging effect of pH 4.75 acetate buffer on nasal mucosa, evaluated at three concentrations (0.07, 0.14 and 0.21 M) was found to be concentration dependant and the highest concentration was the most damaging effect. The type and concentration of buffer should be chosen based on the solubility and stability of the drug and on safety concerns for the integrity of the nasal mucosa.

1.3.3.5 Drug concentration, dose and volume of administration

It has been shown that nasal absorption of 1-tyrosyl-L-tyrosine increased with concentration in an *ex vivo* rat nasal perfusion experiment (Huang *et al.*, 1985b). On the other hand, the effect of concentration of aminopyrine and salicylic acid on nasal absorption in rats was different (Hirai *et al.*, 1981). Aminopyrine absorption rate was constant against concentration whereas nasal absorption of salicylic acid decreased with concentration. The authors explained that salicylic acid at high concentration might alter permeability of the nasal mucosa as it is well known to induce gastric mucosal damage at high concentration. Another explanation given was the saturation of binding sites at the nasal mucosa at high concentration of salicylic acid. However further studies would be required to elucidate the transport process of the drug through the nasal mucosa.

The effect of three nasal spray concentrations of cetrizine on clinical efficacy (Clement *et al.*, 1994) showed that patients experienced no or only mild symptoms of rhinitis on 16.7%, 30.8%, 42.9%, and 26.7% of days for drug concentrations of 0.0% (placebo), 0.06%, 0.125%, and 0.25% respectively. It is difficult to judge if this relationship was true of the systemic absorption of cetrizine as well as a local effect on the mucosa.

Such drug concentration effects on nasal absorption are revealing, for if the primary mechanism of absorption was passive, there should be a positive relationship between absorption and drug concentration. However such a relationship is not always observed. Although this does not necessarily mean that nasal absorption does

not occur via a passive mechanism. There are other confounding factors which can influence the nasal transport and modify the absorption profile (Behl *et al.*, 1998).

Several studies have sought an effect of drug 'dose' on nasal absorption, for secretin (Ohwaki *et al.*, 1987; Ohwaki *et al.*, 1985; Ohwaki *et al.*, 1989), desmopressin (Harris *et al.*, 1988a; Harris *et al.*, 1988b), loperamide hydrochloride (Smith *et al.*, 1992) and insulin (Dondeti *et al.*, 1994). In general, higher nasal absorption or greater therapeutic effects were observed with increasing dose, although it is important to note how the dose was varied. If the dose is increased by increasing formulation volume, there may be a limit to the increase in nasal absorption. The nostrils can retain only a limited volume (0.05-0.15 ml), beyond which the formulation will drain out of the nasal cavity (Behl *et al.*, 1998).

1.4 Mechanism of drug enhancement across the nasal mucosa

Nasal mucosa is relatively impermeable to large drug molecules. To improve the absorption across nasal mucosa, a number of approaches have been taken such as co-administration of drugs with either penetration enhancers or bioadhesive polymers or combination of both. The mechanisms of drug absorption enhancement through nasal mucosa are not completely known. However, it is apparent that any formulation excipient will come into contact with the mucosal surface and exert some effect on it which may provide drug molecules an easier passage through various transport pathways.

1.4.1 Penetration enhancers

There are many hypothesis proposed by various investigators on enhancement mechanisms for a variety of penetration enhancers (Lee, Yamamoto & Kompella, 1991; Lee, 1990; Su, 1992; Su, 1993; Uchida *et al.*, 1991). These enhancers may improve drug absorption across biological membranes by one or more of the following mechanisms;

(i) increasing membrane fluidity and reducing the viscosity of the mucous layer;

(ii) inhibiting proteolytic enzymes at the absorption site;

(iii) transient loosening of tight junctions between epithelial cells;

(iv) increasing paracellular or transcellular transport by affecting membrane lipids and proteins;

(v) dissociating protein aggregates;

(vi) initiating membrane pore formation;

(vii) lowering membrane potential during the process of drug transport;

(viii) increasing nasal blood flow, thereby raising the concentration gradient across the nasal mucosa and;

(ix) enhancing the thermodynamic activity of peptides and proteins.

It is usually difficult to precisely predict the best enhancer for a given drug formulation. Generally, higher concentrations of enhancers are likely to cause nasal irritation and damage to the nasal mucosa. On the other hand, lower enhancer concentrations would generally provide lower or no improved absorption so that

many enhancers may be 'dangerous' to use beyond a certain concentration and be 'beneficial' below that concentration. The diagrammatic relationship between bioavailability and damage caused to nasal mucosa by various penetration enhancers is shown in Fig. 1.5.

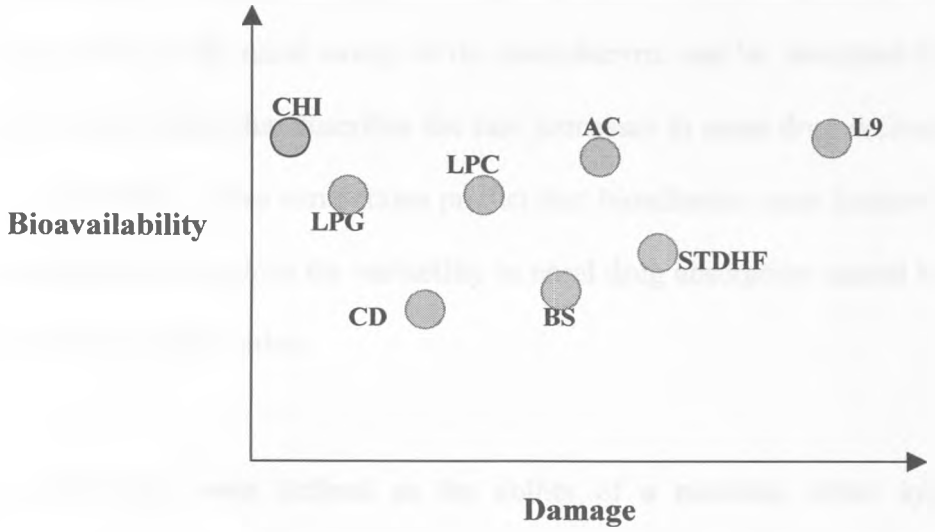


Figure 1.5 Diagrammatic representation of relationship between bioavailability and damage caused by different penetration enhancers used in nasal delivery systems (reproduced from Davis, 1999). AC = Acyl carnitines, BS = Bile salts, CD = Cyclodextrin, CHI = Chitosan, LPC = Lysophosphatidyl choline, LPG = Lysophosphatidyl glycerol, L-9 = Polyoxyethylene-9-lauryl ether, STDHF = Sodium taurodihydrofusidate.

1.4.2 Bioadhesive polymers

An alternative approach to improved nasal absorption of large molecules is the use of bioadhesive polymers which lack the toxic effects of penetration enhancers such as bile salts, chelators or surfactants. It has been hypothesized that one might improve the nasal absorption by increasing the retention time of drug in the nasal cavity via bioadhesion (Nagai & Machida, 1990). The effect of bioadhesive carrier systems on the reduction of mucociliary clearance rate constants for the transport from the posterior part of the nasal cavity to the nasopharynx can be simulated by using a mathematical model that describes the rate processes in nasal drug delivery (Gonda & Gipps, 1990). These simulations predict that bioadhesion may improve systemic bioavailability and reduce the variability in nasal drug absorption caused by variable patterns of drug deposition.

Bioadhesion has been defined as the ability of a material, either synthetic or biological, to adhere to a biological tissue for an extended period of time. Such materials, when applied to mucosal epithelia, may adhere primarily to the mucus layer. This phenomenon has been termed as mucoadhesion. In drug delivery systems, the polymeric/drug carrier is usually a non-biological macromolecular or hydrocolloid material that adheres primarily to the mucus layer, or alternatively may attach to the underlying epithelium.

A number of water-soluble polymers and water-insoluble polymers which are swellable networks joined by cross-linking agents exhibit bioadhesive properties.

Bioadhesion is dependent on the nature of the adherent polymer and has been reported to require two criteria (Bodde, DeVries & Junginger, 1990):

(i) the polymer should possess optimal polarity to make sure it is sufficiently 'wetted' by the mucus and
(ii) optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place.

When selecting a bioadhesive polymer for formulation, several characteristics should be considered (Dondeti, Zia & Needham, 1996) such as,

(i) molecular weight and chain length
(ii) charge and ionisation
(iii) hydrophilic functional groups and hydration
(iv) chain segment mobility.
(v) expanded nature of the polymer network
(vi) concentration of bioadhesive polymer.

The adhesion of water soluble polymer powders to the nasal mucosa was investigated both *in vitro* and *in vivo* (Nakamura *et al.*, 1996). The rank order of adhesion of the polymers to agar plates in two *in vitro* methods appeared to be quite similar to that of their mucoadhesion *in vivo,* which was measured by observing a dye mixed with the polymer after its application to the nasal cavity of rabbit, using a thin fibroscope. Xanthan gum showed the longest residence time in the nasal cavity,

followed by tamarind gum, hydroxypropylcellulose and polyvinyl alcohol (Nakamura *et al.*, 1996). In a study of nasal mucociliary clearance rates *in vivo* (Zhou & Donovan, 1996) of several bioadhesive polymers, clearance of 3% methyl cellulose was slowest and fastest for 0.2% polyacrylic acid. However, the total clearance of a polymer gel formulation was not dependent on its initial clearance and very viscous or fluid formulations demonstrated rapid initial bulk clearance, but if they were bioadhesive, their total clearance from the nasal cavity was limited (Zhou & Donovan, 1996).

The clearance characteristics of bioadhesive nasal delivery systems such as starch microspheres, chitosan microspheres and chitosan solution in humans evaluated using gamma scintigraphy (Soane *et al.*, 1999) showed that bioadhesive systems had much longer half-lives. The clearance of chitosan solution was 41 min compared to the control (21 min), whilst the clearance half-life of starch microspheres was 68 min and for chitosan microspheres, 84 min (Soane *et al.*, 1999). Apparently chitosan delivery systems can reduce the rate of clearance from nasal cavity, increasing the residence time of the delivery system and providing the potential for increasing the bioavailability of drugs incorporated into these systems.

It has been reported that the mucoadhesive properties of chitosan are most likely mediated by ionic interaction between the positively charged amino groups in chitosan and the negatively charged sialic acid residues in mucus. The optimum concentration of chitosan for maximal absorption of insulin in rats and sheep was

found to be 0.2% and 0.5% respectively. The increase in absorption of insulin was attributed to the mucoadhesive properties of chitosan (Illum, Farraj & Davis, 1994a).

A number of bio/ or mucoadhesive delivery systems have been investigated for nasal drug administration, for example, freeze-dried formulations of insulin and neutralized polyacrylic acid increased the nasal absorption of insulin in dogs (Nagai *et al.*, 1984).

It is evident from the literature that the use of bioadhesive polymers alone, either in the form of sprays, powders, microspheres or gels can improve nasal drug absorption to some extent. A significant additional improvement in bioavailability has been seen by incorporating penetration enhancers in the bioadhesive delivery systems. Bioadhesive polymers used in nasal formulations intended for systemic drug delivery are summarised in Table.1.2.

Table 1.2 Bioadhesive polymers used in nasal drug delivery studies (reproduced from Dondeti 1996).

Drug	Polymer+enhancer	Dosage form	Species	Bioavailability/ pharmacological effect
Dopamine	4% HPC	Solution	Dog	20% vs IV
	HPC + 1%HCO	Solution	Dog	25.7% vs IV
	HPC + 1%SDC	Solution	Dog	37.5% vs IV
	HPC + 5%Azone	Solution	Dog	63.8% vs IV
Gentamicin	DSM	Powder	Sheep	9.7% vs IV
	DSM + LPC	Powder	Sheep	51.3% vs IV
Propanolol HCL	1.5% Carbopol 934P PG:GF = 40:60	Gel	Dog	51.8% vs IV
Nifedipine	0.5% Carbopol 941 + PEG 400 (1:1)	Gel	Rat	Comparable to IV effect
Vasopressin	1% Na Hyaluronate	Solution	Rat	9.9% vs IV effect
	+ 50 mM CM	Solution	Rat	83.6% vs IV effect
Insulin	Carbopol 934	Powder	Dog	25% of IV effect
	HPC	Powder	Dog	20% of IV effect
	Lactose	Powder	Dog	15% of IV effect
Insulin	0.1% Carbopol	Solution	Rat	Not determined
	1% Carbopol	Gel	Rat	Not determined
Insulin	DSM	Powder	Rat	33% vs IV
Insulin	DSM	Powder	Sheep	4.5% vs IV; 10.7% vs SC
	DSM + LPC	Powder	Sheep	13.1% vs IV; 31.5% vs SC
Insulin	DSM	Powder	Rat	26% BG reduction
	Sephadex	Powder	Rat	24% BG reduction
	DEAE-Sephadex	Powder	Rat	8% BG reduction
Insulin	1.5%MCC	Spray	Rabbit	2.0 vs IV
	MCC + ST	Spray	Rabbit	8.4% vs IV
	MCC + AG	Spray	Rabbit	7.8% vs IV
	MCC + GA	Spray	Rabbit	2.2% vs IV
Insulin	70% Plastoid L50	Spray	Rabbit	2.3% vs IV
	Plastoid L50 + ST	Spray	Rabbit	5.9% vs IV
Insulin	PAA (SCF)	Powder	Rabbit	8.1% vs IV
	PAA (FD)	Powder	Rabbit	20.4% BG reduction
	CPAA (SCF)	Powder	Rabbit	3.6% vs IV
	CPAA (FD)	Powder	Rabbit	1.8% vs IV
	AG (SCF)	Powder	Rabbit	9.8% vs IV
	AG (FD)	Powder	Rabbit	2.9% vs IV
Insulin	0.5% Chitosan	Solution	Sheep	7 times higher AUC
Human GH	DSM	Powder	Sheep	2.7% vs SC
	DSM + LPC	Powder	Sheep	14.4 vs IV
Interferon-B	MCC + SGC	Powder	Rabbit	3% vs IV
Calcitonin	0.1% Carbopol	Solution	Rat	Not determined

AG = Ammonium glycyrrhizinate; BG = Blood glucose; CM= camostat mesilate; CPAA = cross-linked polyacrylic acid; DSM =degradable starch microspheres; FD = Freeze drying; GA = glycyrrhetenic acid; HPC=hydroxypropyl cellulose, HCO=hydrogenated caster oil; LPC = L-α-lysophosphatidyl choline; MCC = microcrystalline cellulose; PAA = polyacrylic acid; SCF = supercritical fluid processing; SDC = sodium deoxycholate; SGC = sodium glycocholate; ST = sodium taurocholate.

1.5 Summary

The nasal route of administration holds promise for the systemic delivery of low dose drug candidates such as peptides and proteins. A major factor in the low bioavailability of nasally administered drugs is their rapid removal from the nasal cavity by mucociliary clearance. Several methods used to enhance nasal drug absorption include chemical modification, salt or ester formation, formulation design and incorporation of surfactant into the nasal formulation. However, incorporation of penetration enhancers and the use of bioadhesive polymers in nasal formulations has been the dominant approach for the improvement of nasal drug absorption. It is evident from the literature that bioadhesive polymers alone, or in combination with penetration enhancers either in the form of sprays, powders, microspheres or gels can improve drug absorption to some extent. The effect of mucoadhesive polymers on the mucociliary clearance mechanism and its possible consequences in humans during chronic therapy have still to be studied. Consistent and reproducible therapeutic levels from these delivery systems has yet to be achieved.

Liquid nasal formulations in the form of sprays or drops are rapidly cleared from the nasal cavity due to their low viscosity and drug stability in such systems may be questionable. Nasal gel formulations may increase the residence time but patient acceptability is likely to be low. Nasal powders provide good retention in the nasal cavity, better stability of some drugs, better absorption of some drugs e.g. peptides and polar drugs. Moreover higher dose can be administered. However nasal powders require special delivery devices and accuracy of dosing at the site of

application is not always possible with such formulations. Nasal delivery systems and their various attributes are summarised in Table 1.3.

The development of nasal dosage forms for systemic delivery of challenging large molecules and other molecules is attractive. Such systems should possess the attributes of mitigating drug stability problems, dosing accuracy, patient compliance and residence time in the nose, with no interference of the self-cleaning capacity of the nose. One means of achieving such attributes can be by a solid unit dosage form in a primary package ready for insertion into the nose. There is no extensive literature on such nasal dosage forms but the typical characteristics one would expect of this type of nasal dosage form are rapid rehydration, satisfactory dispersion throughout the nasal cavity and increased residence time in the nose with no impairment of the normal physiology of the nose and improved patient compliance. Therefore it seems reasonable to attempt to design and develop a solid unit dosage form for insertion into the nose.

A rapidly hydrating solid dosage form for nasal administration can be achieved by incorporating the drug into hydrophilic gel forming polymer solution and then lyophilising the formulation in a suitable primary package. Lyophilisation is a low temperature process and causes minimum damage and loss of activity of delicate heat labile materials. In addition lyophilised products are porous in nature and can undergo rapid and complete rehydration with a remarkable speed when in contact with solvent or a moist surface. Such formulations also provide accurate dosing.

Table 1.3 Nasal delivery summary (Humphries & Morgan, 1999).

	Use	Technology	Patient compliance	Distribution in nasal cavity	Dosing control	Formulation	Delivery system
Liquid nasal drops	Not widely used	"Old"	Poor	Likely to be poor	Likely to be acceptable	Insoluble drugs?	Simple technology
Gel delivery	Not widely used	"Old"	Moderate	Moderate	Difficult to achieve	Insoluble drugs?	May be difficult to achieve
Liquid pump spray	Widely used	"New"	Likely to be high	Good	Acceptable	Preservative required?	Established
Dry powder Delivery	Widely used for oral inhalation	"New"	Potentially high	Potentially good	Acceptable	Powder formulation and handling?	Yet to be developed
Pressurised aerosol spray	Widely used for oral inhalation	"New"	Potentially high	Moderate	Acceptable	Formulation with non-CFC propellants	Well established

1.6 Aim and objectives of the project

Aim

To study formulations based on incorporating hydrophilic gel-forming excipients using lyophilisation technology and preparing in a suitable format for easy insertion into the nose.

Objectives

- To develop a lyophilised dosage form that becomes adhesive on the mucosal surface and releases drug in a controlled manner.
- To acquire *in vitro* drug release data for the dosage form (donor-acceptor, diffusion model).
- To study mechanical robustness of dosage form.
- To study the hydration characteristics of candidate lyophilisates.
- To acquire *in vivo* drug release data for the dosage form.
- To devise an outline manufacturing process.

Chapter 2 *In Vitro* Drug Release Studies

2.1 Introduction

The use of polymers to control drug release has become important in the formulation of pharmaceuticals. Hydroxypropyl methylcellulose (HPMC), a non-ionic cellulose ether, is typical of such pharmaceutically useful polymers and has been widely used in slow release hydrophilic matrix systems (Melia, 1991). Its popularity can be attributed to the polymer's non-toxic nature, the small influence of processing variables on drug release from an HPMC matrix, ease of compression, and its ability to accommodate high drug loading.

Drug release from such systems, involves the penetration of water causing polymer relaxation to a viscous rubber (gel layer). This gel controls drug release by viscous resistance to drug diffusion and/or gel matrix erosion. Classically, the diffusion of a water soluble drug through the gel layer can be shown to be linearly dependent on the square root of time (Korsmeyer *et al.*, 1983; Peppas, 1985; Shah *et al.*, 1993). To

accommodate a dual release mechanism, i.e. drug diffusion through the gel and erosion of the gel layer, the Peppas transport equation

$$\mathbf{M_t/M_\infty = kt^n} \qquad \textbf{(1)}$$

(where M_t/M_∞ is the fraction of the drug dissolved at time **t**, with **k** a constant and **n** the diffusional exponent for drug release) (Peppas, 1985; Ritger & Peppas, 1987a; Ritger & Peppas, 1987b) has been employed to describe initial 60% of drug release. Regardless of dosage form type and shape, the kinetics of drug release the magnitude of n can be used as an indication of diffusion controlled drug release (value of n between 0.45 and 0.5) or a gel erosion release mechanism ($0.45<n<0.89$) (Chebli *et al.*, 1999; Eyjolfsson, 1999; Ford *et al.*, 1987; Ranga Rao, Padmalatha Devi & Buri, 1988; Skoug *et al.*, 1991).

This chapter deals with the *in vitro* release of UK-92,480 mesylate and nicotine hydrogen tartrate from solutions and from lyophilisates prepared mainly from different concentrations of Methocel K (various molecular weight) and in some cases gelatin. We have used the Peppas equation as the basis for prediction of drug release from lyophilised dosage forms. This chapter also describes some aspects of the stability of nicotine in lyophilised plugs.

2.2 Materials & Method

2.2.1 Materials

2.2.1.1 Chemicals

The active compound UK-92, 480 (as the mesylate salt, molecular weight 570.7) was supplied by Pfizer Ltd., Sandwich, Kent, England. Nicotine (-) hydrogen tartrate (NHT) (lot 17H1206), authentic samples of nornicotine (lot 76H4052), cotinine (lot 97H4050), and phosphate buffer saline (PBS), pH 7.4, povidone (molecular weight 10,000) were purchased from Sigma Chemical Company. D (-) mannitol (GPR), potassium dihydrogen phosphate (HPLC grade) and potassium phosphate, dibasic trihydrate (HPLC grade) was purchased from BDH. Mucin (extracted from Hog stomach) was purchased from Fluka. The acetonitrile and water used in the preparation of buffer was of HPLC grade.

Hydroxypropylmethylcelluloses (HPMC), Methocels K4MP (lot KC31012N11), K15MP (lot KE03012N12), K100MP (lot KC04012N11) and K100LVP (lot JL08012N21) were obtained as a gift from the Dow Chemical Company, Michigan, USA. The numbers that follow K identify the viscosity (mPa.s) of that product at 2% concentration in water at 20°C, 'M' represents 1000, 'P' represents premium Methocel products and 'LV' represents special low viscosity grade. The different type K Methocels differ in the degree of substitution of methoxyl and hydroxypropyl groups in the molecule (The Dow Chemical Company, 1996). Gelatin (160 bloom strength) was purchased from BDH, Poole, England.

2.2.1.2 Apparatus

Diffusion cell

The diffusion chamber (Plexiglass) which consists of donor and receiver compartments (Fig. 2.1) with sampling ports and which was based on the design of Cornaz Gudet (1996) was fabricated by the workshop staff of the Department of Pharmaceutical Sciences, University of Strathclyde in Glasgow. Photographs of the diffusion cell, different parts of the cell, and a view of the donor compartment from the top with/ or without a hydrated sample placed on the membrane are shown in Figs. 2.2 (a & b), and 2.3 (a & b) respectively.

The *in vitro* release profile of UK-92, 480 and nicotine from various formulations was studied in the diffusion chamber, which with a minimal dissolution volume on the donor side, was intended to mimic the hydration of a lyophilised plug in the nasal mucosa. In effect the donor compartment contained minimal medium volume with existing air saturated with water which was achieved during the course of experiment after replacing the lid and the receptor compartment PBS, pH 7.4 at 37°C. The test sample (lyophilisate, powder or solution) was placed on the filter paper membrane (595 S & S filter paper, 90 mm diameter; Schliecher & Schuell, Germany, reference number 311609) which was maintained just in contact with the constantly agitated liquid phase of the receptor compartment (Cornaz Gudet *et al.*, 1996)

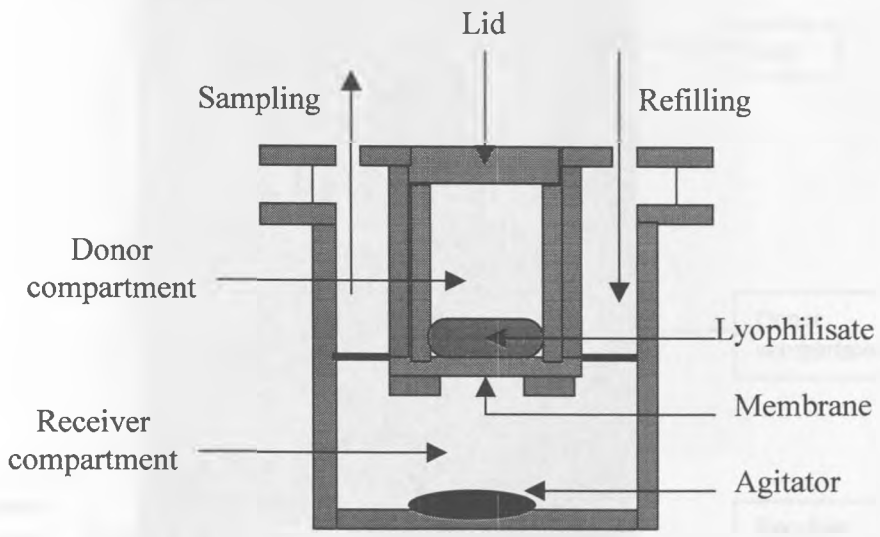


Figure 2.1 Schematic diagram of the diffusion chamber.

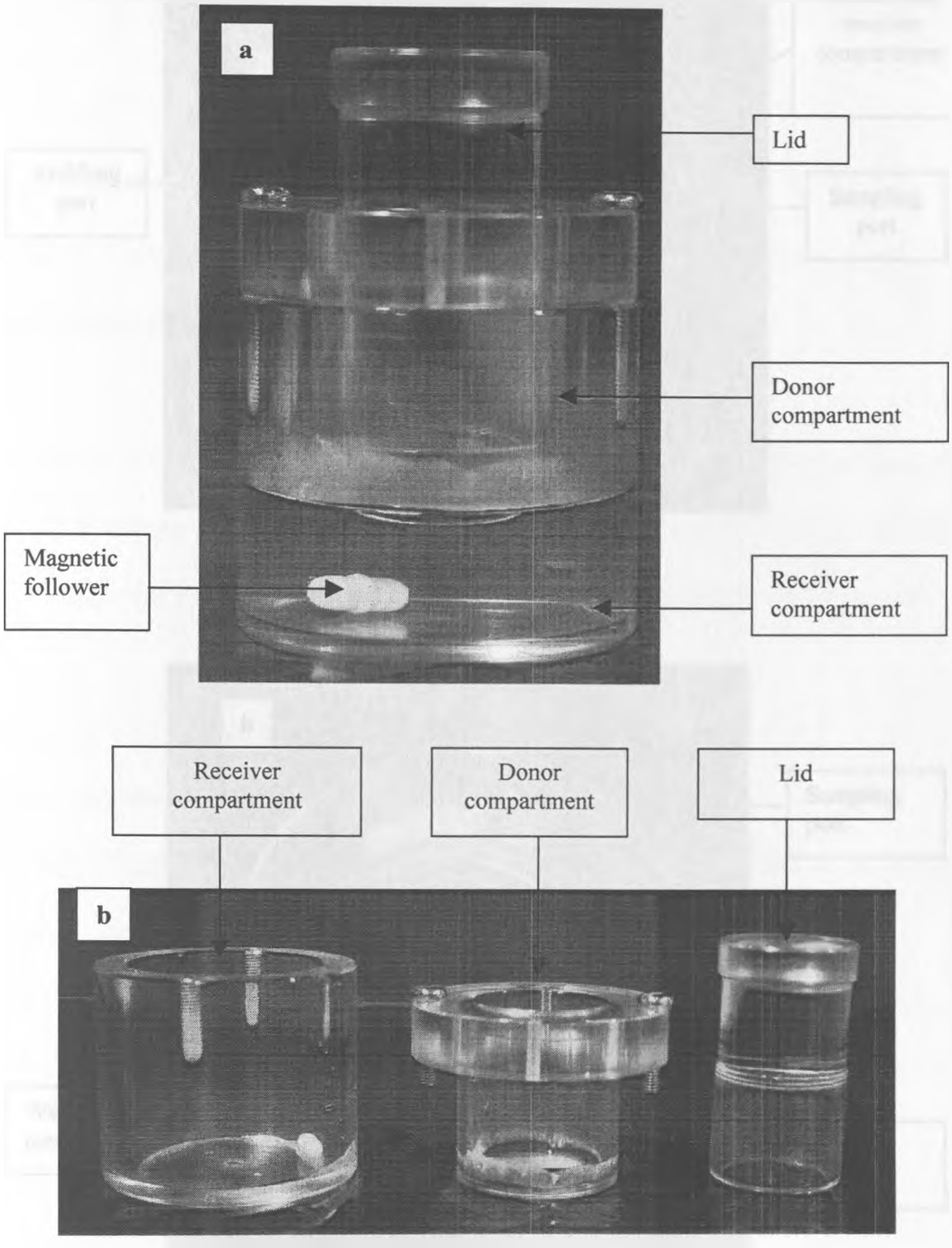


Figure 2.2 (a) Diffusion cell in complete assembly (b) different parts of diffusion cell.

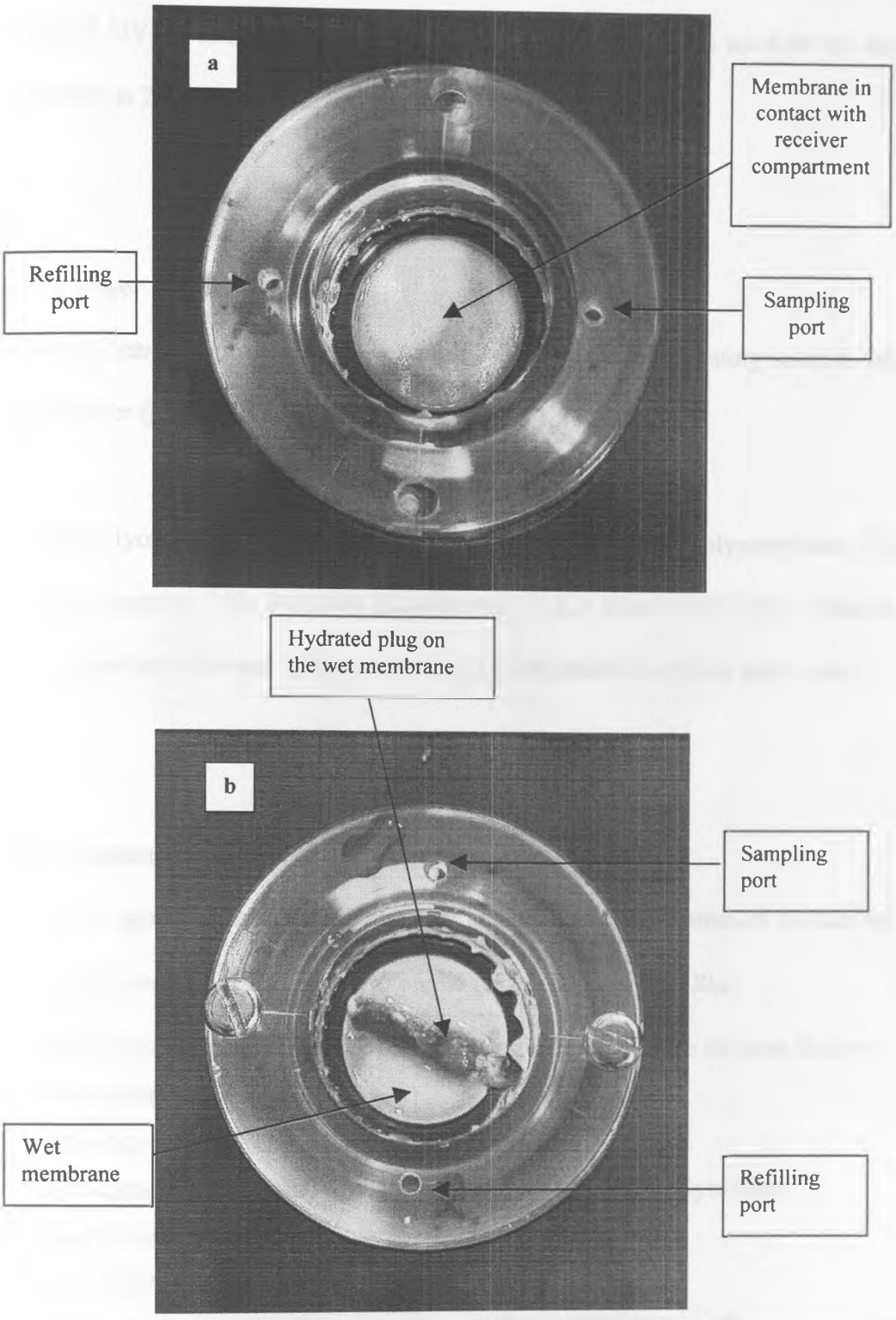


Figure 2.3 Donor compartment (as viewed from the top) (a) without plug and (b) with hydrated plug.

UV Spectrophotometer

UNICAM UV4 – 100 UV/Visible Spectrophotometer V4.15 was used for the assay of nicotine at 260 nm and UK-92, 480 at 292 nm.

Freeze Dryer

Different formulation solutions were lyophilised either in a laboratory size Modulyo Freeze Dryer (Edwards) or in VirTis Advantage EL Freeze Dryer.

To prepare lyophilisate plugs, snap seal micro centrifuge tubes (polypropylene, 0.2ml or 0.5ml capacity, Life Sciences International, U.K.) filled with 0.3ml solution in 0.2ml microfuge tube and 0.66 ml solution in 0.5ml microfuge tubes were used.

HPLC Systems

The HPLC apparatus used for the assay of nicotine and investigation of its stability is shown. Injection (by auto sampler) volume for all samples was 20µl.

The HPLC system used consists of the following as shown in the diagram below:

1~ Solvent reservoir.

2~ Degassing unit, four channels (Jour Research).

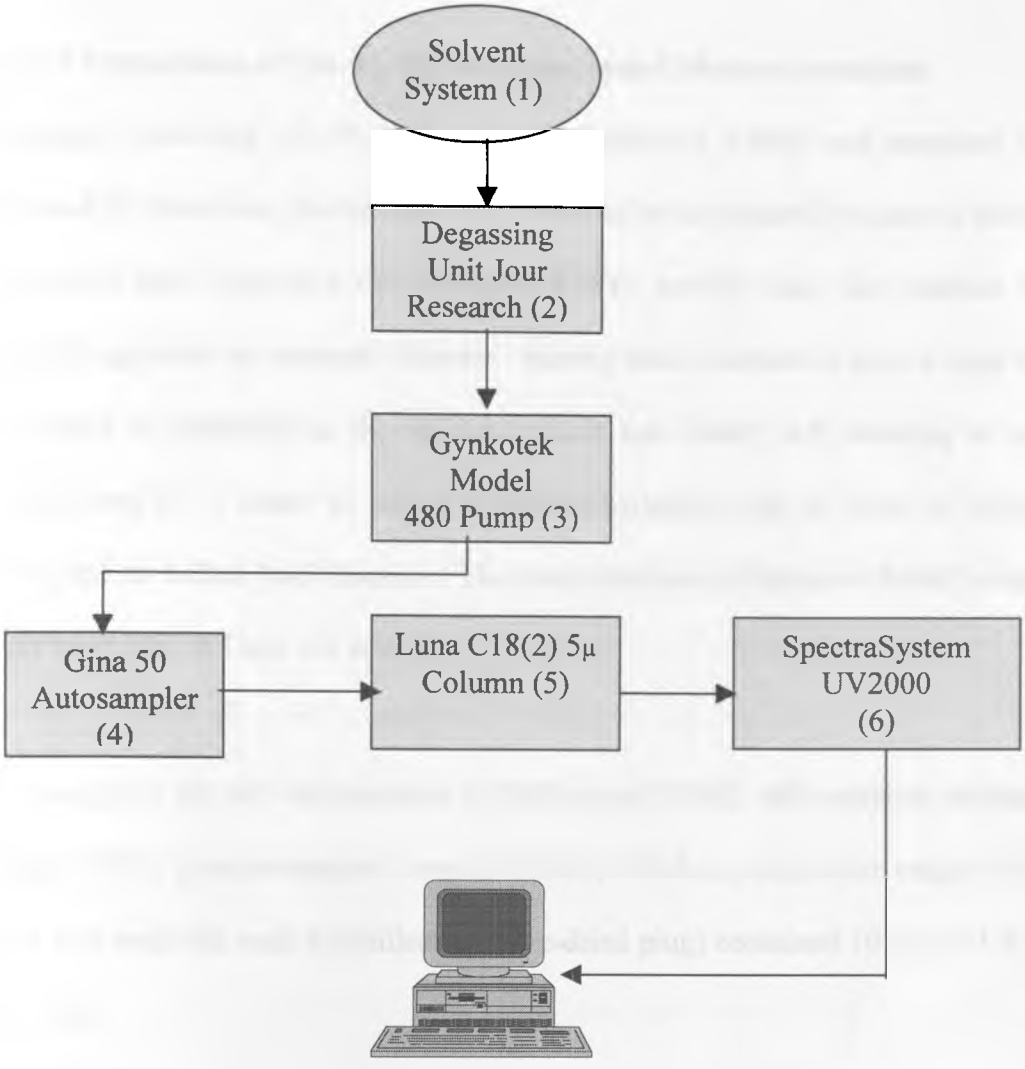
3~ High reproducibility pump Model 480 Serial No. 9632039 (Gynkotek).

4~ Gina 50 Autosampler, Serial No 9626002 (Gynkotek).

5~ Luna C18(2) 5µ column.

6~ SpectraSystem UV2000 detector, Serial No. 016/15624-5 (Thermoseparation products).

7~ Chromeleon integration package running on a Pentium PC.


Solvent System (1)
Degassing Unit Jour Research (2)
Gynkotek Model 480 Pump (3)
Gina 50 Autosampler (4)
Luna C18(2) 5µ Column (5)
SpectraSystem UV2000 (6)

2.2.2 Methods

2.2.2.1 Preparation of UK-92, 480 mesylate loaded Methocel solutions

Solutions containing UK-92, 480 mesylate, Methocel K4MP and mannitol were prepared by dissolving the mesylate and mannitol in the required volume of distilled water and then sprinkling the Methocel K4MP powder into this solution with constant agitation by magnetic follower. Stirring was continued to ensure there were no lumps of Methocel in the solution which was finally left standing at room temperature for 4 hours to ensure complete hydration and to allow to dissolve entrapped air before lyophilisation. The concentrations of Methocel K4MP solution used were 0.25, 0.5 and 1% w/w.

The weight of UK-92, 480 mesylate (1.2025 mg of UK-92, 480 mesylate, molecular weight 570.7, is equivalent to 1 mg of UK-92, 480 base, molecular weight 474.6) used was such that each lyophilisate (freeze-dried plug) contained 10 mg of UK-92, 480 base.

2.2.2.2 Preparation of UK-92, 480 mesylate loaded gelatin solutions

The required amount of gelatin was dissolved in half the required volume of water with gentle heat. Once the gelatin was completely dissolved and cooled, UK-92, 480 mesylate and mannitol were added. Methocel K4MP powder was added to these solutions as required with constant agitation by magnetic follower as described

above. The concentrations of gelatin solution used were 0.1 and 0.25% w/w with/or without 0.1% w/w Methocel K4MP.

2.2.2.3 Preparation of nicotine hydrogen tartrate loaded Methocel solutions

The different formulation solutions containing NHT, Methocel K and mannitol were prepared by dissolving NHT and mannitol in 1/3 of the required volume of distilled water and then sprinkling Methocel powder into this solution with constant agitation by either magnetic follower or high shear stirrer. Concentrated solutions of Methocel required high shear stirring to avoid lumping. When the Methocel particles were thoroughly wetted and evenly dispersed, the remaining volume of water was added to make 100 grams of solution and stirring continued until no lumps were seen. These solutions were then left standing at 4°C overnight to ensure complete hydration and to allow to dissolve entrapped air.

The concentrations of Methocel K4MP solutions used were 0.25, 0.5, 1, 2, and 3% w/w. K15MP, K100MP and K100LVP were used at 1 and 2% w/w. In all cases the formulation solutions also contained 1% w/w mannitol. A 2% w/w solution of K100MP, containing NHT but no mannitol was also prepared to study the effect of mannitol on nicotine release.

The weight of NHT (molecular weight 462.4) used was calculated on the basis that the final lyophilisate (freeze dried plug) would contain 4 mg of nicotine base

(molecular weight 162.2) per plug (2.85 mg of NHT is equivalent to 1 mg of nicotine base).

2.2.2.4 Preparation of lyophilisates containing UK-92, 480 mesylate

A volume of 0.3 ml, which gave 10 mg of UK-92, 480 base per plug, was filled into micro-centrifuge polypropylene tubes (internal diameter of the mouth roughly 4 mm, 1.7 cm long). The filled tubes were pre-frozen by dipping into liquid nitrogen. The tubes were lyophilised for 24 hours on a programmable VirTis Advantage EL Freeze Dryer with a shelf programmed such that the shelf temperature started at 10 and rose 21°C over a period of 24 hours and chamber pressure from 100 mTorr down to 40 mTorr with condenser temperature of -90°C. A slow pre-freeze was achieved by freezing the filled tubes on the chamber shelf by an appropriate programme on the freeze dryer as shown in Table 2.1.

Table 2.1: Freeze drying programme.

Thermal Treatment		
Step	Temp (°C)	Time (Min.)
1	-30	60
2	-40	60
3	-50	60
4	-60	120

Primary Drying			
Step	Temp (°C)	Time (Min.)	Vacuum (mTorr)
1	10	600	100
2	15	600	40
3	20	240	40
Post heat	25	10	10

Freeze	-30
Extra freeze	30 min
Condenser	-40
Vacuum	200 mTorr

Large plugs were similarly prepared using fill volumes of 0.66 ml, 10 mg of UK-92, 480 base per plug, into tubes with dimensions of, internal diameter of the mouth 6.0 mm by 2.5 cm long. These were lyophilised as described above. The length of the lyophilised plug was 2.5 cm approximately.

The compositions on a dry weight basis of the various types of UK-92, 480 lyophilisates are presented in Table 2.2.

Table 2.2 Composition of the selected formulation solutions and the corresponding lyophilisates composition on a dry weight base.

Solution (% w/w)			Lyophilisates		
			Calculated composition (mg)	*Mean weight (mg)	Calculated moisture content (%)
1.	UK-92, 480 mesylate	1.82	12.02	20.97±0.16	3.45
	K4MP	**0.25**	1.65		
	Mannitol	1	6.6		
	Distilled water to	100			
	(Fill vol. = 0.66 ml)				
2.	UK-92, 480 mesylate	4.0	12.025	15.97±0.48	1.24
	K4MP	**0.25**	0.75		
	Mannitol	1	3.0		
	Distilled water to	100			
	(Fill vol. = 0.3 ml)				
3.	UK-92, 480 mesylate	4.0	12.025	18.65±0.35	3.47
	K4MP	**1**	3.0		
	Mannitol	1	3.0		
	Distilled water to	100			
	(Fill vol. = 0.3 ml)				
4.	UK-92, 480 mesylate	4.0	12.025	Not done	
	K4MP	**0.5**	1.5		
	Mannitol	1	3.0		
	Distilled water to	100			
	(Fill vol. = 0.3 ml)				
5.	UK-92, 480mesylate	4.0	12.025	15.90±0.40	3.8
	Gelatin	**0.1**	0.3		
	Mannitol	1	3.0		
	Distilled water to	100			
	(Fill vol. = 0.3 ml)				
6.	UK-92, 480 mesylate	4.0	12.025	16.08±0.46	1.93
	Gelatin	**0.25**	0.75		
	Mannitol	1	3.0		
	Distilled water to	100			
	(Fill vol. = 0.3 ml)				
7.	UK-92, 480 mesylate	4.0	12.025	16.72±0.70	7.00
	Gelatin	**0.1**	0.3		
	K4MP	**0.1**	0.3		
	Mannitol	1	3.0		
	Distilled water to	100 g			
	(Fill vol. = 0.3 ml)				

*(n=5)±S.D.

2.2.2.5 Preparation of lyophilisates containing nicotine hydrogen tartrate

A volume of 0.66 ml, which gave 4 mg of nicotine base per plug, was filled into micro centrifuge polypropylene tubes (bullet shaped, internal diameter at mouth 6.0 mm, and 2.5 cm long). The filled tubes were pre-frozen by dipping into liquid nitrogen. 'Slow' pre-freezing involved storage of filled micro-centrifuge tubes at -15°C for 24 hours followed by freezing in liquid nitrogen. The tubes were lyophilised for 24 hour using Modulyo Freeze Dryer (Edwards) at a condenser temperature of -55°C and 0.08mbar pressure. At the end of the process the chamber was vented with air. The length of lyophilised plug was 2.5 cm approximately.

The composition of lyophilisates on a dry weight basis is presented in Table 2.3.

Table 2.3 Composition of the selected formulation solutions and the corresponding lyophilisates composition on a dry weight base.

Solution (% w/w)		Lyophilisates		
		Calculated composition (mg)	*Mean weight (mg)	Calculated moisture content (%)
1. NHT	1.73	11.4	24.94±0.54	1.38
K4MP	**1**	6.6		
Mannitol	1	6.6		
Distilled water to	100			
2. NHT	1.73	11.4	30.84±1.44	1.15
K4MP	**2**	13.2		
Mannitol	1	6.6		
Distilled water to	100			
3. NHT	1.73	11.4	40.92±0.35	8.25
K4MP	**3**	19.8		
Mannitol	1	6.6		
Distilled water to	100			
4. NHT	1.73	11.4	34.62±1.12	10.96
K15MP	**2**	13.2		
Mannitol	1	6.6		
Distilled water to	100			
5. NHT	1.73	11.4	32.34±1.6	3.65
K100MP	**2**	13.2		
Mannitol	1	6.6		
Distilled water to	100			
6. NHT	1.73	11.4	32.07±0.69	2.78
K100LVP	**2**	13.2		
Mannitol	1	6.6		
Distilled water to	100			
7. NHT	1.73	11.4	26.1±1.08	6.09
K100MP	**1**	6.6		
Mannitol	1	6.6		
Distilled water to	100 g			

*(n=10) ±S.D.

2.2.2.6 Preparation of K4MP and NHT powder mix

Three different batches of K4MP powder formulation were prepared in such a way that the per-unit-dose for the first batch contained the same quantity of K4MP, mannitol and NHT as lyophilisates prepared from 1%w/w K4MP solution. Similarly, the second and third batches contained the same quantity of K4MP, mannitol and NHT as lyophilisates prepared from 2 and 3%w/w K4MP solutions respectively.

The required amount of K4MP, mannitol and NHT were accurately weighed (total weight of each batch = 10 g), hand shaken for few minutes and mixed for one hour using Turbula W. A. B mixer (Basel, Switzerland).

To study the release of nicotine, 25, 31.5 and 38 mg of the first, second and third powder mixes were used respectively. These weights contained 11.4 mg of NHT (equivalent to 4 mg of nicotine base) and were equivalent to the weight of lyophilisates prepared from 1, 2, and 3% K4MP solutions respectively.

2.2.2.7 Standard curve for UV assay of UK-92, 480

To prepare the stock standard solution, 6.0125 mg of UK-92, 480 mesylate (equivalent to 5 mg of base, UK-92, 480) was accurately weighed and dissolved in 25 ml freshly prepared distilled water to produce a solution of 200 μg/ml base. The stock solution was diluted to obtain concentrations of base in the range 10 μg/ml to 50 μg/ml. These dilute solutions were analysed by UV spectrophotometry at 292 nm

to obtain a linear relationship between absorbance and base concentration (Figure 2.4).

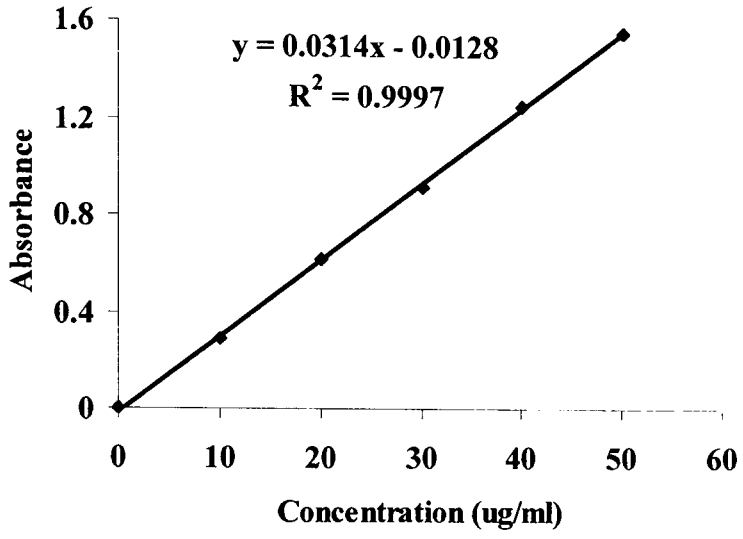


Figure 2.4 Calibration curve for the UV analysis of UK-92,480 at 292 nm in freshly prepared distilled water.

2.2.2.8 Total content of UK-92, 480 in lyophilisates

To determine the UK-92, 480 content of individual plugs, they were dissolved in 250 ml of freshly prepared distilled water and analysed by UV spectrophotometry at 292nm against a water blank. The UK-92, 480 concentration was then calculated from the standard curve.

2.2.2.9 Standard curve for UV nicotine assay

To prepare the stock standard nicotine solution, 28.5 mg of NHT powder (equivalent to 10 mg of nicotine base) was accurately weighed and dissolved in 100 ml, pH 7.4 PBS to produce a solution of 100 μg/ml as nicotine base. This stock solution was diluted to obtain nicotine base concentrations in the range 5 μg/ml to 50 μg/ml. These dilute solutions were analysed by UV spectrophotometry at 260 nm. Absorbance was then plotted against nicotine concentration.

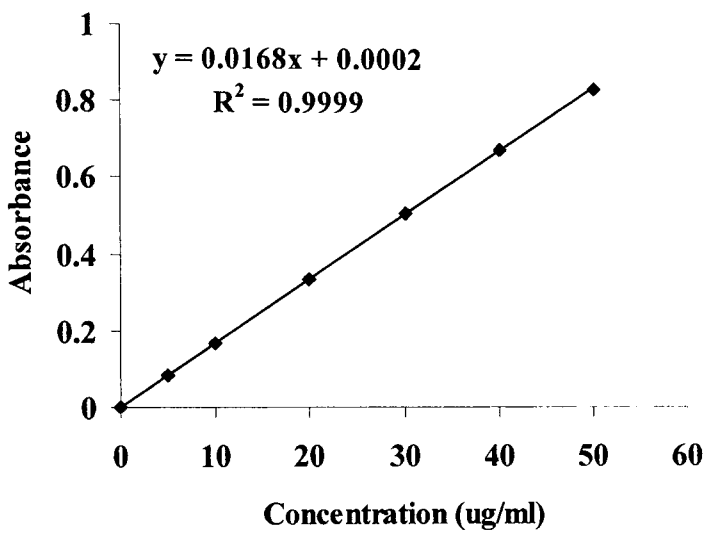


Figure 2.5 A typical curve for the spectrophotometric analysis of nicotine in PBS at 260 nm.

2.2.2.10 Total content of nicotine in powder mix, lyophilisate, and formulation solution

To determine their nicotine content, individual plugs were dissolved in 100 ml pH 7.4 PBS and analysed by UV spectrophotometry at 260 nm against a PBS blank. Nicotine concentration was then calculated from the standard curve. The nicotine content of formulation solutions and powder mixes was similarly determined.

2.2.2.11 *In vitro* release of UK-92, 480

The *in vitro* release profile of UK-92, 480 was studied in the diffusion chamber described in section 2.2.1.2. Samples of 0.5 ml were withdrawn at regular time intervals from the acceptor compartment, replaced by fresh medium and after appropriate dilution to the assay concentration range, spectrophotometrically analysed at 292nm. Unless otherwise stated, all the release profile values were calculated as base (UK-92, 480).

The release profile of UK-92, 480 from the following preparations was measured:

UK-92,480 mesylate powder as supplied.

Lyophilisates prepared from solutions containing 1% w/w mannitol and 0.25, 0.5 or 1.0% w/w K4MP or 0.1 or 0.25% w/w gelatin.

Lyophilisates prepared from a solution containing gelatin and K4MP (both 0.1% w/w) and 1% mannitol.

2.2.2.12 *In vitro* release of nicotine

Similarly, the *in vitro* release profile of nicotine was studied in the diffusion chamber described in section 2.2.1.2. Samples of 0.5 ml were withdrawn at regular time intervals from the acceptor compartment and spectrophotometrically analysed at 260 nm after appropriate dilution to the assay concentration range. Each sample volume taken from the acceptor compartment was replaced with fresh medium.

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The release profile of nicotine from the following preparations was measured:

Lyophilisates prepared from different concentrations (0.25, 0.5, 1, 2, 3% w/w) of K4MP solution, each containing 1% w/w mannitol.

Solutions of 0.25, 0.5, 1, 2 & 3% w/w of K4MP each containing 1% w/w mannitol.

Lyophilisates prepared from 1 & 2% w/w solutions of different molecular weight grade K Methocels (K4MP, K15MP, K100MP, and K100LVP) each containing 1% w/w mannitol.

Solutions of 2% w/w of K4MP, K15MP, K100MP containing 1% w/w mannitol.

Lyophilisates prepared from 2% w/w solution of K100MP without mannitol.

Lyophilisates prepared from 2% w/w solution of K100MP without mannitol (slow pre-freezing).

Nicotine hydrogen tartrate (NHT) powder.

Solutions of 5 and 10% w/w mannitol solution.

Lyophilisates prepared from NHT in 1, 5 or 10% w/w mannitol solution.

Powder mixes of NHT in K4MP and mannitol.

2.2.2.13 Chromatographic conditions for nicotine and its degradation products

A Luna reversed phase C_{18} (2) column (150 mm x 4.6 mm inner diameter, 5μm packing, Phenomenex U.K. Ltd.) was used. The solvent system was 0.01M phosphate buffer (pH 7.4)/acetonitrile 80/20 (v/v), isocratic for 10 minutes at 1.0ml/min. For nicotine stability studies the solvent system composition was 0.01M phosphate buffer (pH 7.4)/acetonitrile 90/10 (v/v), isocratic for 30 minutes at 1.0 ml/min. The detection wavelength was 260 nm and all experiments were performed at ambient column temperature.

2.2.2.14 Calibration curve for nicotine HPLC assay

To prepare the nicotine standard stock solution, 285 mg of NHT powder (equivalent to 100 mg of nicotine base) was accurately weighed and dissolved in 100ml of 0.01M phosphate buffer (pH 7.4), which was prepared by dissolving 1.36 gm of potassium dihydrogen orthophosphate and 2.28 gm of potassium phosphate, dibasic trihydrate in 1000 ml of HPLC grade water. Five standard solutions were prepared in the concentration range 200 µg/ml to 600 µg/ml nicotine base from stock solution by serial dilution. The calibration curve was constructed by plotting peak areas versus nicotine base concentration (Fig.2.6).

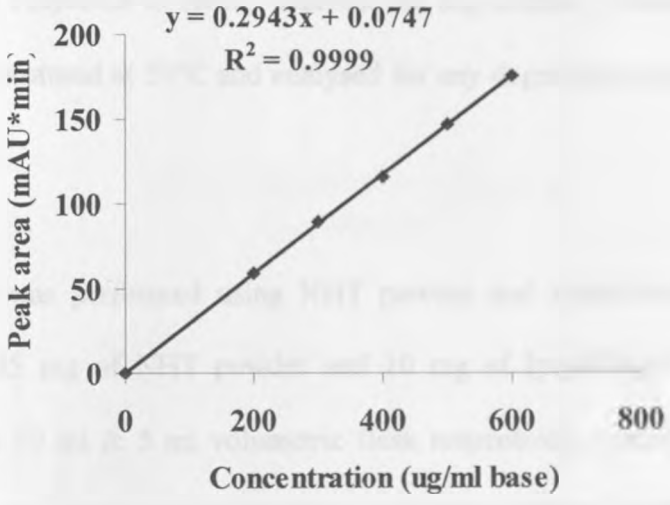

Figure 2.6 Calibration curve for the HPLC analysis of nicotine in acetonitrile and phosphate buffer (20:80, pH 7.4) solvent system

2.2.2.15 Samples for nicotine assay from different sections of lyophilisate

Three lyophilisates prepared from 2% w/w solution of K100LVP were chosen and sectioned into three parts i.e. top, middle and bottom, keeping the weight of the

sample constant as far as possible. Each section was dissolved in 5 ml water (class A volumetric flask) and the concentration of nicotine base (retention time 5.6 min) determined by HPLC (20%acetonitrile: 80% phosphate buffer, pH 7.4 mobile phase, flow rate 1.0ml/min).

2.2.2.16 Samples for stability testing

The NHT solutions used as the boiled and 50°C storage samples had concentrations of 200 μg/ml and 250 μg/ml of nicotine base equivalent respectively. NHT solution was boiled (100°C) for half an hour, the volume adjusted to 50 ml to compensate for evaporation then subjected to HPLC analysis for degradation products. 10 ml of NHT solution was stored at 50°C and analysed for any degradation products at 0, 8, 12, and 32 days.

A similar study was performed using NHT powder and lyophilisates containing NHT. Here, 2.85 mg of NHT powder and 10 mg of lyophilisate was weighed accurately into a 10 ml & 5 ml volumetric flask respectively, and stored at 50°C. After 32 days the samples were analysed for degradation product after dissolution in water.

2.3 Result and Discussion

2.3.1 In vitro release of UK-92, 480

UK-92, 480 is a weak base with two pKa values of 6.3 and 8.7. It has a good aqueous stability, and withstands conventional autoclaving at 121°C (The Pfizer Limited, 1999). The mesylate salt has a good aqueous solubility (> 70 mg/ml in water at ambient temperature) although above pH 4.7 the solubility decreases to approximately 1 mg/ml. The pH dependence of UK-92, 480 mesylate solubility is shown in Fig. 2.7. Based on this relationship freshly prepared distilled water was chosen as the dissolution medium for the *in vitro* release study as its pH was found to be around 4.5.

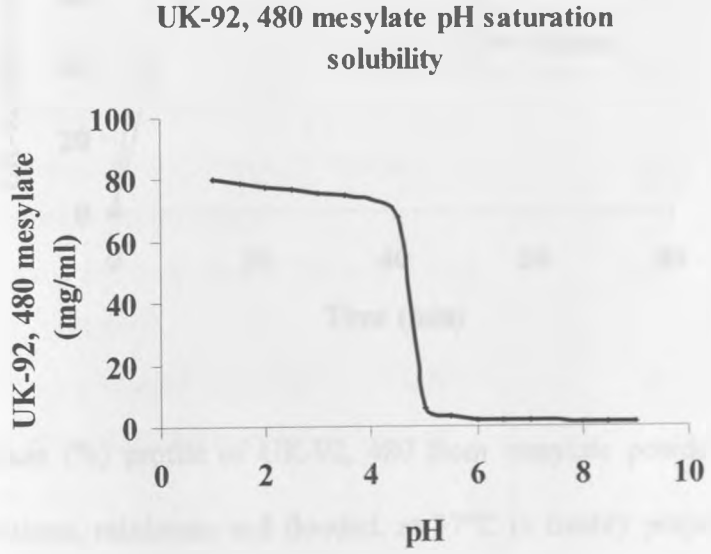

Figure 2.7 UK-92, 480 mesylate pH solubility (graph redrawn from the original data supplied by Pfizer).

2.3.1.1 Release from UK-92, 480 mesylate powder

The UK-92, 480 release profile from mesylate powder was studied under two donor side environments. In the first, the volume of medium was maintained at a minimum and in the second, the donor side was slightly flooded with medium. As is evident from Figure 2.8, the flooded condition left-shifted the release curve, that is, the drug went into solution more quickly than under the minimum fluid condition at the donor side. The times required to effect 50% drug release ($t_{50\%}$) were <10 and <5 minutes for minimum and flooded donor side conditions respectively (Table 2.4).

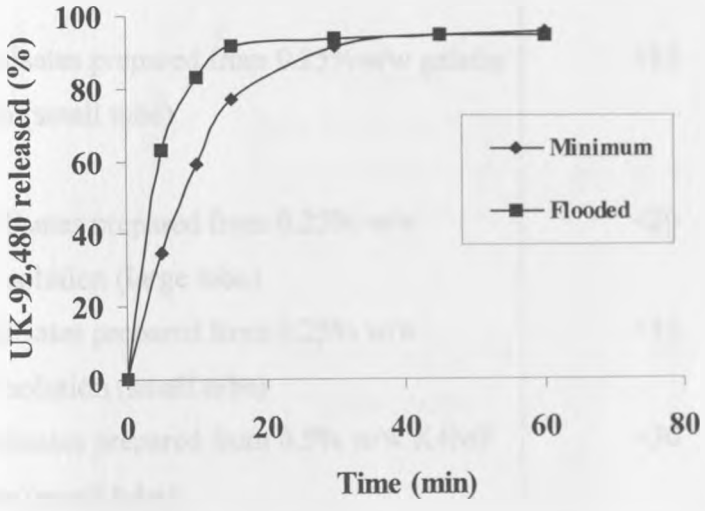

Figure 2.8 Release (%) profile of UK-92, 480 from mesylate powder under two donor side conditions, minimum and flooded, at 37°C in freshly prepared distilled water.

Table 2.4. Time required to release 50% ($t_{50\%}$) of UK-92, 480 in freshly prepared distilled water from various lyophilised formulations prepared from different K4MP/gelatin solutions. All the formulation solutions (unless otherwise stated), except drug powder, also contained 1% w/w mannitol.

Formulations	$t_{50\%}$ (minutes)
1. UK-92,480 mesylate powder	<10
2. UK-92,480 mesylate powder (slightly flooded with medium at donor side)	<5
3. Lyophilisates prepared from 0.1% w/w gelatin solution (small tube)	<10
4. Lyophilisates prepared from 0.1% w/w gelatin and 0.1% w/w Methocel K4MP solution (small tube)	<15
5. Lyophilisates prepared from 0.25%w/w gelatin solution (small tube)	<15
6. Lyophilisates prepared from 0.25% w/w K4MP solution (large tube)	<20
7. Lyophilisates prepared from 0.25% w/w K4MP solution (small tube)	<15
8. Lyophilisates prepared from 0.5% w/w K4MP solution (small tube)	<30
9. Lyophilisates prepared from 1.0% w/w K4MP solution (small tube, slightly flooded with medium)	<30

2.3.1.2 Release of UK-92, 480 from lyophilisates prepared from gelatin solution with and without admixture of K4MP

At the gelatin contents used, the release of UK-92, 480 from the lyophilised plugs was not significantly different from release from the mesylate powder. When K4MP was incorporated in the plugs, release was slowed (Fig. 2.9). The $t_{50\%}$ for the different formulations containing gelatin are shown in Table 2.4.

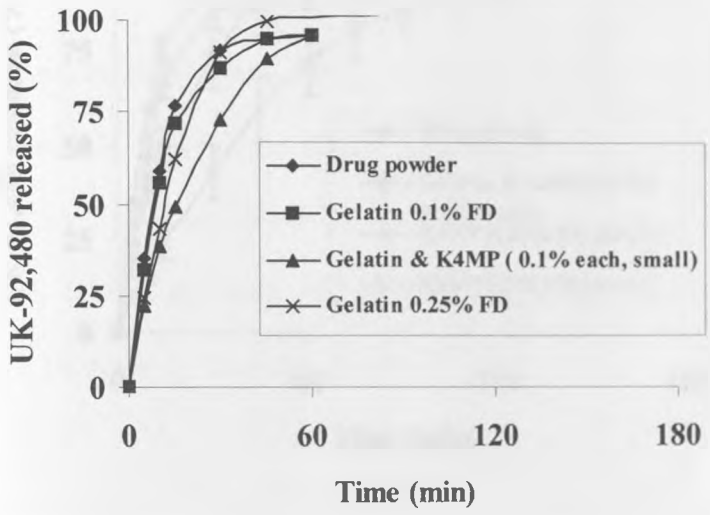

Figure 2.9 Release (%) profile of UK-92, 480 from gelatin lyophilisates prepared from 0.1 & 0.25% solutions with and without K4MP. 'Small' in the graph represents plugs prepared in small microfuge tube (internal diameter at mouth 4.0 mm and length 1.7 cm with formulation fill volume 0.3 ml). Each data point is the mean of three experiments. For clarity error bars not shown.

2.3.1.3 Release of UK-92, 480 from lyophilisates prepared from Methocel K4MP solution with and without gelatin

The release profiles of UK-92, 480 from lyophilisates prepared from different concentration of Methocel K4MP solution are shown in Fig. 2.10 & 2.11. As described above release rate decreased with K4MP content. The $t_{50\%}$ for the different formulations containing K4MP are shown in Table 2.4.

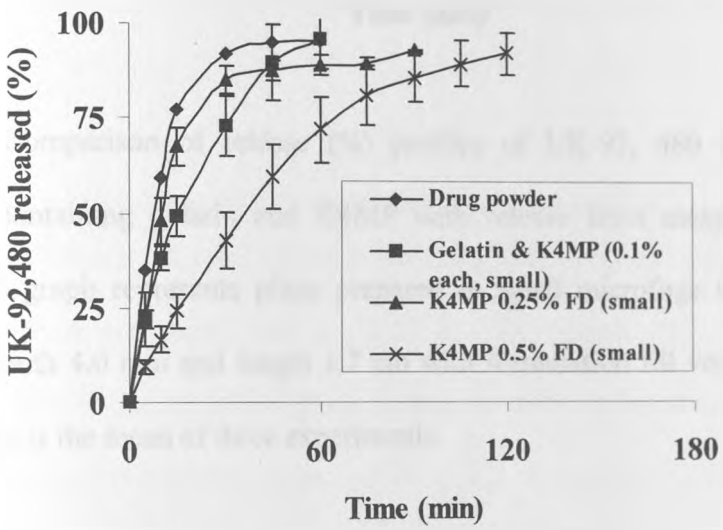

Figure 2.10 Release (%) profile of UK-92, 480 from lyophilisates prepared from different concentration of K4MP solutions (0.1, 0.25, 0.5%) with and without gelatin. 'Small' in the graph represents plugs prepared in small microfuge tube (internal diameter at mouth 4.0 mm and length 1.7 cm with formulation fill volume 0.3 ml). Each data point is the mean of three experiments.

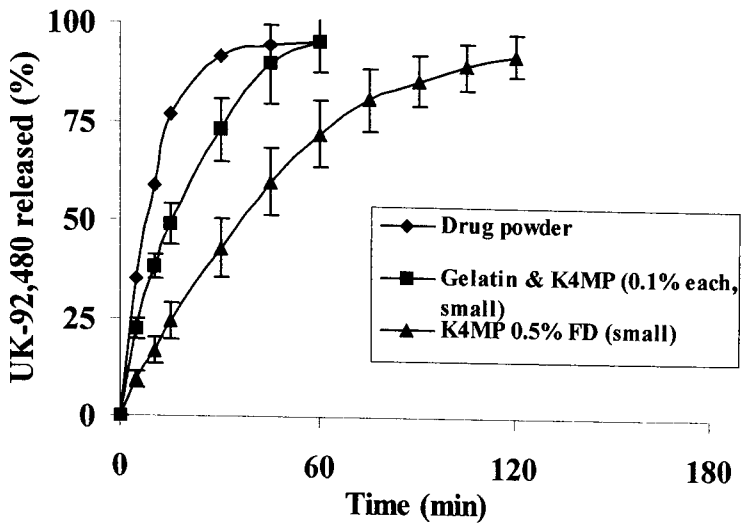

Figure 2.11 Comparison of release (%) profiles of UK-92, 480 from various lyophilisates containing gelatin and K4MP with release from mesylate powder. 'Small' in the graph represents plugs prepared in small microfuge tube (internal diameter at mouth 4.0 mm and length 1.7 cm with formulation fill volume 0.3 ml). Each data point is the mean of three experiments.

In the range studied, the release profile of UK-92, 480 was dependent on the concentration of gelatin and K4MP. It was also observed that drug release depended upon the availability of medium volume at the donor side.

2.3.2 *In vitro* release of nicotine

2.3.2.1 Nicotine content of formulation solutions, lyophilisates, and powder mixtures

The nicotine content was found to be within ±5% of theoretical content of nicotine base. In most cases the nicotine content was less than the calculated content which could be due to variation in fill volume, weight of the powder taken, efficiency of powder mixing, moisture content of powdered materials and lyophilisates.

2.3.2.2 Nicotine release from K4MP solutions and lyophilisates

The *in vitro* nicotine release profile from Methocel K4MP solutions and lyophilisates prepared from them are shown in Figs. 2.12 & 2.13 respectively. Release rates whether from the solutions or lyophilisates decreased as the concentration of polymer increased. The time required to release 50% ($t_{50\%}$) of nicotine from concentrated solution (3% w/w) and lyophilisates prepared from it was 45 and 75 minutes respectively. Nicotine release rates were always faster from K4MP solutions than from K4MP lyophilisates (Fig. 2.14).

The observed variability in the release rate data decreased as the polymer concentration was increased. Some of the reasons of this variability could be attributed to the experimental factors which became less critical at higher polymer concentrations when more structured systems (gels) were formed. However the role

of gel erosion in the release process may be also have contributed to the variability at low gel strength.

The release data were plotted as % nicotine released against t^n (Figs 2.16 & 2.17) and the slopes of these plots are shown for the different concentrations of K4MP solutions (Table 2.5) and lyophilisates (Table 2.6). It can be seen (Tables 2.5 & 2.6) that the best fit to the plot of % nicotine released against t^n was obtained for n = 0.7. The value of the exponent indicates that in this system, NHT release was best described by an anomalous diffusion mechanism and was at least in part dependent on erosion or dissolution of the polymer gel. Similar results have been described for the release of an insoluble drug from tablets prepared from different ratios of Methocels K4MP and K100LVP (Eyjolfsson, 1999) and for the release of alprazolam from sustained release HPMC tablets (Skoug *et al.*, 1993).

The effect of K4MP concentration (solution or lyophilisate) on the slope of the t^n plots is shown in Fig. 2.18. The best fit to these data showed remarkably good agreement between the exponent of x for solution $y = 7.3975x^{-0.70}$ and lyophilisate $y = 4.7973x^{-0.67}$. This can be interpreted as meaning that the dependence of release mechanism on viscosity was the same for K4MP solutions and lyophilisates.

The observed difference in NHT release rate between a K4MP solution (Fig. 2.12) and its corresponding lyophilisate (Fig. 2.13 & 2.14) can be explained on the basis of the outcome of the hydration of the freeze dried plug in the diffusion cell. Under the experimental conditions, hydration of the lyophilisate was very rapid (instantaneous)

and the formation of a penetration front (Skoug *et al.*, 1993) which could influence subsequent nicotine release can be discounted. The open, porous structure of a typical K4MP lyophilisate (Fig. 2.15) would explain the rapid hydration of the plugs observed during the experiments. Importantly the concentration of K4MP solution formed on hydration of a lyophilisate appeared to be greater than the concentration of the solution from which it was dried. In effect, the result of hydrating a freeze dried K4MP plug (e.g. prepared from a 3% K4MP solution) in the diffusion cell was a K4MP solution with a concentration >3% from which nicotine release was slower than from a 3% solution. Lyophilisation allowed rapid hydration and the formation of a very concentrated K4MP solution, containing the NHT, from which release of nicotine was slow.

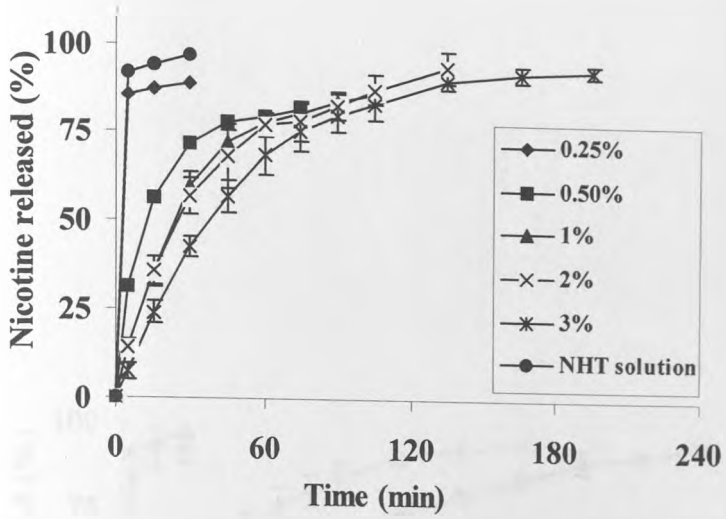

Figure 2.12 Release (%) profile of nicotine into pH 7.4 PBS at 37°C from different indicated concentrations of K4MP solution. A solution of nicotine hydrogen tartrate (NHT solution) with the same nicotine content (6.06 mg/ ml) as the K4MP solutions was used as a control. Each point is the mean ±S.D. of 4 experiments.

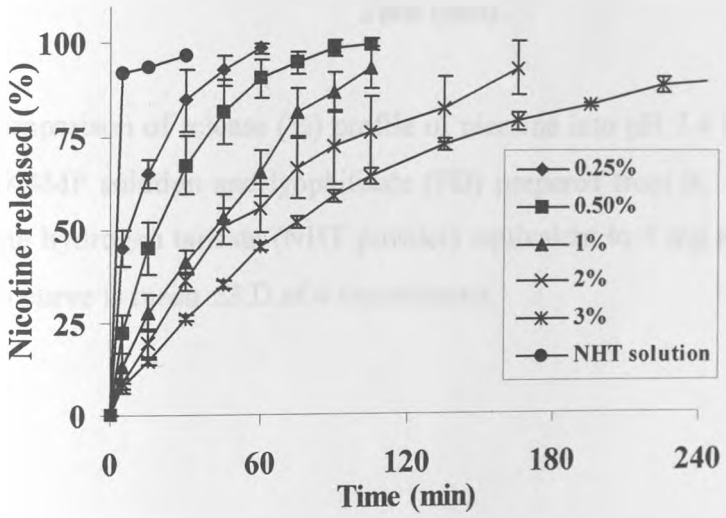

Figure 2.13 Release (%) profile of nicotine into pH 7.4 PBS at 37°C from lyophilisates prepared from different indicated concentrations of K4MP solution. A solution of nicotine hydrogen tartrate (NHT solution) with the same nicotine content (6.06 mg/ ml) as the K4MP lyophilisates was used as a control. Each point is mean ±S.D.of 4 experiments.

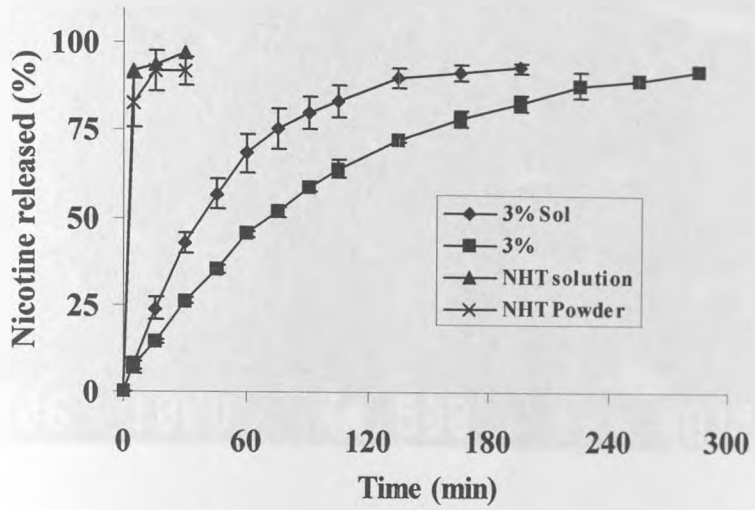

Figure 2.14 Comparison of release (%) profile of nicotine into pH 7.4 PBS at 37°C from 3% w/w K4MP solution and lyophilisate (FD) prepared from it. As controls, 11.4 mg nicotine hydrogen tartrate (NHT powder) equivalent to 4 mg nicotine base was used. Each curve is mean ±S.D.of 4 experiments.

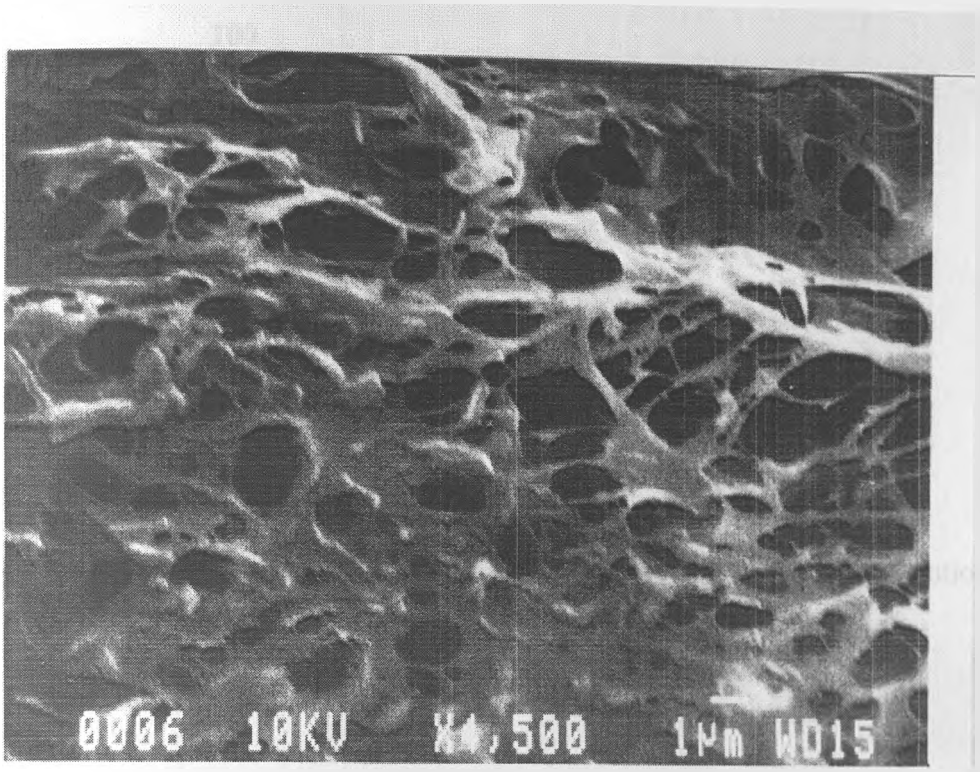

Figure 2.15 SEM of a lyophilisate (20mg) prepared from 2% K4MP solution containing 11.4 mg NHT.

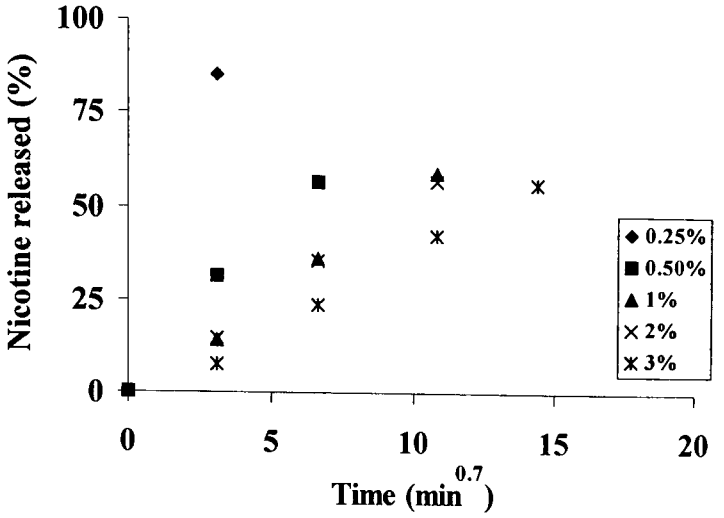

Figure 2.16 Plot of the release data, up to 60% release, from various K4MP solutions (Fig. 2.12) as % nicotine released against $t^{0.7}$.

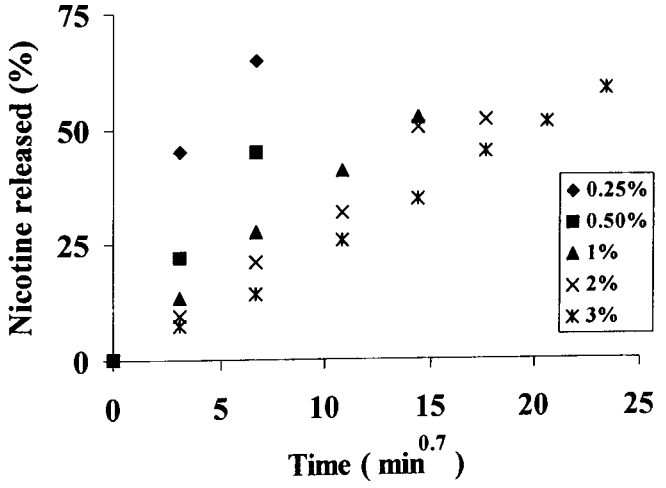

Figure 2.17 Plot of the release data, up to 60% release, from various K4MP lyophilisates (Fig. 2.13) as % nicotine released against $t^{0.7}$.

Table 2.5 Release rate (slope) of nicotine, up to 60% release, from K4MP solutions. Three different values for the diffusional exponent (n) were used in the equation (1) (% released vs. t^n) to determine the best fit to the experimental data. For 0.25% K4MP, data shown was for up to 85% release.

[K4MP][a] (%w/w)	[b]Slope for n = 0.7	R^2 for n = 0.7	[c]Slope for n = 0.5	R^2 for n = 0.5	R^2 for n = 0.3
0.25	27.73	1	38.30	1	1
0.5	8.78	0.9867	14.46	0.9992	0.9669
1	5.44	0.9964	9.98	0.9523	0.8399
2	5.28	0.9979	9.69	0.9620	0.8563
3	3.881	0.9886	7.65	0.9304	0.7951

[a] All formulations contained 1% w/w mannitol.

R^2 Square of the correlation coefficient.

[b] %released/time$^{0.7}$

[c] %released/time$^{0.5}$

Table 2.6: Release rate (slope) of nicotine, up to 60% release, from K4MP lyophilisates prepared from the K4MP solutions shown. Three different values for the diffusional exponent (n) were used in the equation (1) (% released vs. t^n) to determine the best fit to the experimental data.

[K4MP][a] (%w/w)	[b]Slope for n = 0.7	R^2 for n = 0.7	[c]Slope for n = 0.5	R^2 for n = 0.5	R^2 for n = 0.3
0.25	14.61	1	20.18	1	1
0.5	6.83	0.9989	11.19	0.9894	0.9344
1	3.79	0.9940	7.55	0.9885	0.8989
2	3.16	0.9815	6.62	0.9536	0.8389
3	2.58	0.9979	5.68	0.9523	0.8155

[a] All formulations contained 1% w/w mannitol.

R^2 Square of the correlation coefficient.

[b] %released/time$^{0.7}$

[c] %released/time$^{0.5}$

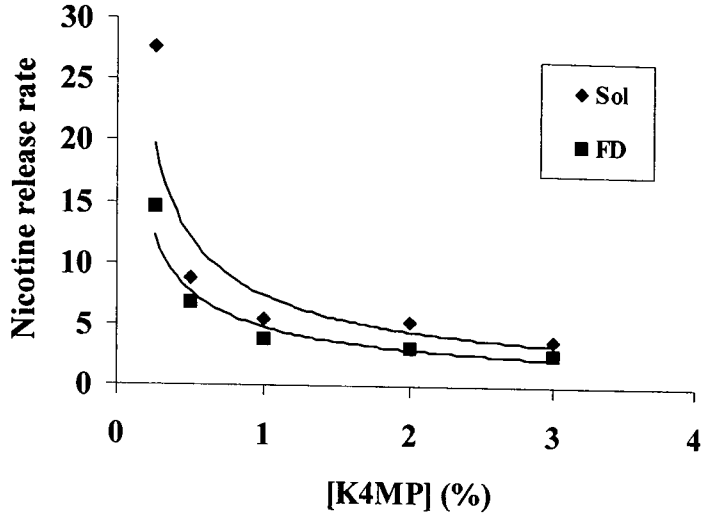

Figure 2.18 The effect of K4MP concentration (%) either in the form of a solution (Sol) or a lyophilisate (FD) on the slope of the % nicotine released against $t^{0.7}$ data from Figs. 7 & 8 respectively. The best power fits to the data points gave $y = 7.3975x^{-0.70}$ ($R^2 = 0.8525$) for solutions and $y = 4.7973x^{-0.67}$ ($R^2 = 0.9403$) for lyophilisates.

2.3.2.3 Nicotine release from different viscosity grade (molecular weight) Methocel solutions and lyophilisates

Release profiles of nicotine from different viscosity grade (molecular weight) Methocels, K4MP, K15MP, K100MP and K100LVP (solutions and lyophilisates prepared from them) are shown in Figs. 2.19 & 2.21. As is evident from the plots, nicotine release rates were not different among the Methocels K of different molecular weights irrespective of the type of formulation i.e. solution or lyophilisate.

However for any particular Methocel, release was faster from solution than from lyophilisate (Fig. 2.20). The $t_{50\%}$ was 30 minutes from Methocel solution and 45-75 minutes from lyophilisates. As described above for K4MP, nicotine release was significantly decreased with increased polymer concentration (Fig. 2.22 and Table 2.7 & 2.8), a trend observed with Methocels K15MP, K100LVP, K4MP.

Release rates (slopes) and corresponding R^2 values obtained from the plots of % nicotine released versus time (t^n) are shown in Table 2.7 & 2.8 for lyophilisates prepared from 1 and 2 % w/w solutions and in Table 2.9 for 2 % w/w solutions of different molecular weight Methocel K. The marked effect on release rate, of varying K4MP concentration (Table 2.5 & 2.6), was not observed by varying Methocel molecular weight (Table 2.8 & 2.9). However, a trend to slower release was observed between K4MP and K15MP (Fig. 2.23), although there was no significant difference in release rate between K15MP and K100MP. This result is supported by those of a previous study (Sung *et al.*, 1996) of HPMC matrix tablets using adinazolam mesylate as a model drug which suggested that drug release rate was fastest for a low viscosity grade (K100LV) formulation and that a K4M formulation exhibited a faster drug release rate than either K15M or K100M formulations. Here, K15M and K100M formulations had identical drug release profiles which suggested the existence of a 'limiting HPMC viscosity', around 15000 cps, for the systems studied at which drug release rate no longer decreased with viscosity. In other words, nicotine release rate decreased with increasing Methocel molecular weight for various low molecular weight HPMC and became independent of molecular weight for high molecular weight HPMC. In this present study, a

'limiting viscosity' effect on release rate was also observed at high K4MP concentration (Fig.2.18).

Similarly, in another study of HPMC matrix tablets using promethazine hydrochloride (Ford, Rubinstein & Hogan, 1985a); propanolol hydrochloride and aminophylline (Ford, Rubinstein & Hogan, 1985b); propanolol hydrochloride and tetracycline hydrochloride (Mitchell *et al.*, 1993b); buflomedil pyridoxalphosphate (Bettini *et al.*, 1994); isoniazid, anhydrous caffeine, theophylline, salicylic acid and indomethacin (Kurahashi, Kami & Sunada, 1996), it was found that in spite of a large difference in release rates among low viscosity grade HPMC, little difference was observed among higher molecular weight matrices. It may be argued that release rate is independent of molecular weight at least for a homologous series of polymers, which chemically have the same monomeric repeating units (building blocks). The influence of type of the substitution on the performance of methyl cellulose and HPMC in gels and matrices have been studied using propanolol as a model drug (Mitchell *et al.*, 1993a). This study suggested that propanolol dissolution rates varied according to the drug/cellulose ether ratio within the matrix but the performance differences of the three grades of HPMC (K4M, E4M, F4M) could not be distinguished.

A plausible explanation for the nicotine release data is that although various HPMC solutions and HPMC gels formed after hydration of various lyophilisates have different macro-viscosities, they might have the same micro-viscosity as far as nicotine is concerned. Nicotine, as the diffusing species, has a much smaller

molecular weight than the Methocels and could be seen as percolating through the non-viscous environment of the solution in the void spaces between the polymer chains. In other words, the effects of the polymer molecules on the macroscopic flow properties of the system (i.e. on macroscopic movement, evaluated as viscosity) do not necessarily correlate with effects on diffusion (i.e. movement at the microscopic scale). This has led some workers to suggest that micro-viscosity (i.e. a measure of viscosity at the microscopic scale) should be used instead of macro-viscosity as a predictor of drug diffusion rate in systems such as hydrophilic cellulosic and non-cellulosic polymers (Al-Khamis, Davis & Hadgraft, 1986; Smidt, Offringa & Crommelin, 1991). However, the power relationship observed between macro- and micro-viscosity suggested that the former may be of some value for the prediction of diffusion rates (Alvarez-Lorenzo *et al.*, 1999).

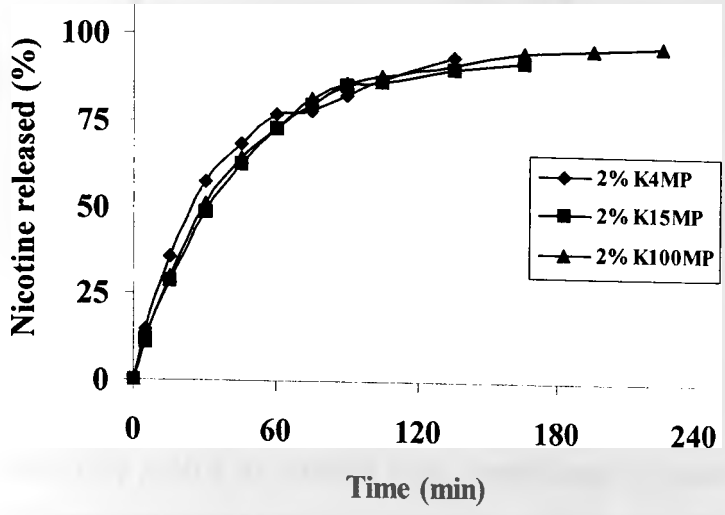

Figure 2.19 Release (%) profile of nicotine into PBS, pH 7.4 at 37°C from 2% solutions of different molecular weight Methocels; K4MP, K15MP, K100MP. Each curve is mean of 3 experiments.

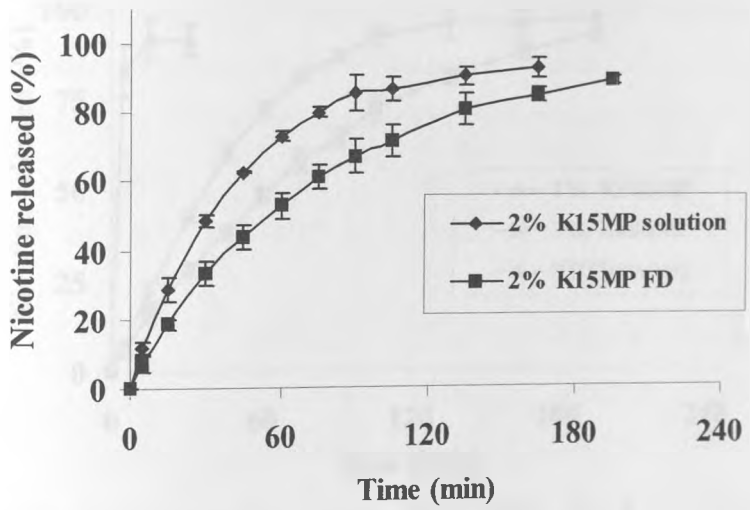

Figure 2.20 Comparison of release (%) profile of nicotine into PBS, pH 7.4 at 37°C from 2% w/w K15MP solution and lyophilisate (FD) prepared from it. Each curve is mean ±s.d. of 3 experiments.

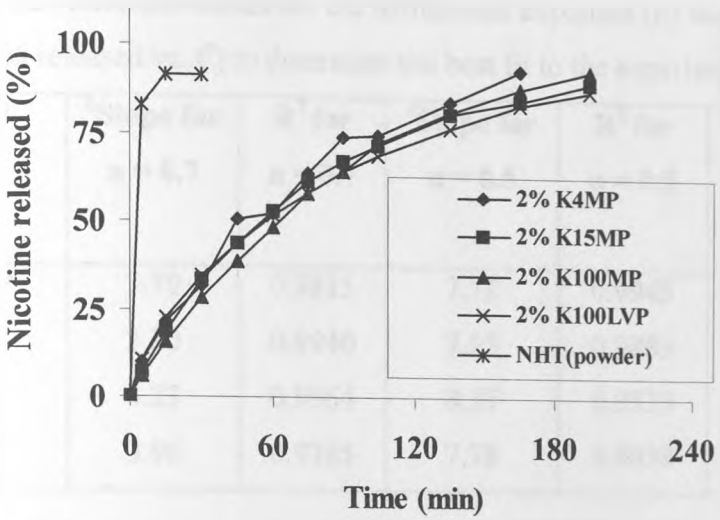

Figure 2.21 Release (%) profile of nicotine from lyophilisates prepared from 2% w/w solution of different molecular weight Methocel K solutions into PBS, pH 7.4 at 37°C. Each curve is mean of 3 experiments.

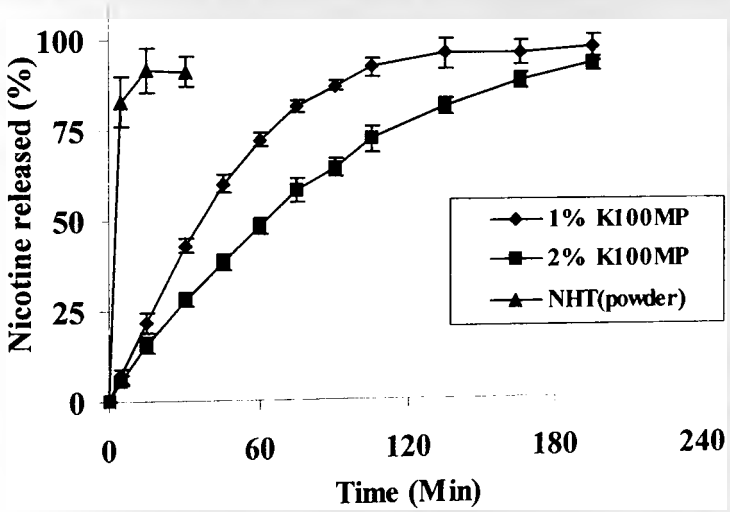

Figure 2.22 Release (%) profile of nicotine into PBS, pH 7.4 at 37°C from lyophilisate prepared from 1% and 2% K100MP solutions. Each curve is mean ±s.d. of three experiments.

Table 2.7: Release rate (slope) of nicotine, up to 60% release, from lyophilisates prepared from 1% solution of different viscosity grade (molecular weight) Methocels. Three different values for the diffusional exponent (n) were used in the equation (1) (% released vs. t^n) to determine the best fit to the experimental data.

Methocel grade[a]	[b]Slope for n = 0.7	R^2 for n = 0.7	[c]Slope for n = 0.5	R^2 for n = 0.5	R^2 for n = 0.3
K100LVP	3.70	0.9815	7.72	0.9945	0.9142
K4MP	3.80	0.9940	7.55	0.9885	0.8989
K15MP	4.23	0.9964	8.37	0.9523	0.8297
K100MP	3.96	0.9765	7.78	0.9038	0.7587

[a] All formulations contained 1% w/w mannitol.
R^2 Square of the correlation coefficient.
[b] %released/time$^{0.7}$
[c] %released/time$^{0.5}$

Table 2.8: Release rate (slope) of nicotine, up to 60% release, from lyophilisates prepared from 2% solution of different viscosity grade (molecular weight) Methocels. Three different values for the diffusional exponent (n) were used in the equation (1) (% released vs. t^n) to determine the best fit to the experimental data.

Methocel grade[a]	[b]Slope for n = 0.7	R^2 for n = 0.7	[c]Slope for n = 0.5	R^2 for n = 0.5	R^2 for n = 0.3
K100LVP	2.98	0.9922	6.53	0.9876	0.8869
K4MP	3.15	0.9803	6.60	0.9514	0.8361
K15MP	3.00	0.9972	6.55	0.9574	0.8264
K100MP	2.73	0.9920	5.92	0.9344	0.7907

[a] All formulations contained 1% w/w mannitol.
R^2 Square of the correlation coefficient.
[b] %released/time$^{0.7}$
[c] %released/time$^{0.5}$

Table 2.9: Release rate (slope) of nicotine, up to 60% release, from 2 % solution of different viscosity grade (molecular weight) Methocel. Three different values for the diffusional exponent (n) were used in the equation (1) (% released vs. t^n) to determine the best fit to the experimental data.

Methocel grade[a]	[b]Slope for n = 0.7	R^2 for n = 0.7	[c]Slope for n = 0.5	R^2 for n = 0.5	R^2 for n = 0.3
K100LVP	ND	ND	ND	ND	ND
K4MP	5.27	0.9979	9.68	0.9620	0.8563
K15MP	4.41	0.9967	8.09	0.9519	0.8393
K100MP	4.60	0.9931	8.42	0.9415	0.8231

[a] All formulations contained 1% w/w mannitol.
R^2 Square of the correlation coefficient.
ND – not done
[b] %released/time$^{0.7}$
[c] %released/time$^{0.5}$

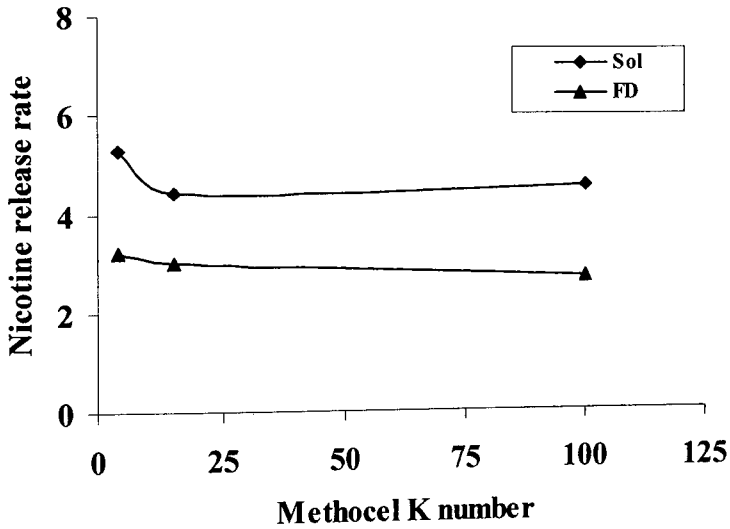

Figure 2.23 The effect of Methocel K numbers (molecular weight) on the release rate of nicotine from 2% solutions (Sol) and lyophilisates (FD) prepared from these solutions.

2.3.2.4 Nicotine release from Methocel lyophilisates prepared by quench cooling and slow pre-freezing

In these experiments, comparison was made of lyophilisates prepared from 2% w/w K100MP solution with and without mannitol using two different freezing techniques. One group of samples was pre-frozen at -15^{0}C for 24 hours followed by quench cooling with liquid nitrogen and the other group of samples was quench cooled from solution with liquid nitrogen. Lyophilisates from the first group were glassy in appearance. The second group of lyophilisates (quench cooled) had the amorphous white appearance of most of the lyophilisates described in this work.

The nicotine release profiles for the two types of lyophilisate are shown in Fig. 2.24. For lyophilisates without mannitol, nicotine release was faster from the glassy than from the amorphous lyophilisates. The $t_{50\%}$ for glassy lyophilisates was 45 minutes and for amorphous lyophilisates, 60 minutes.

From amorphous lyophilisates with mannitol, nicotine release was slower ($t_{50\%}$ =75 min) than from amorphous lyophilisates without mannitol ($t_{50\%}$ = 60 min). One reason may be that lyophilisates with mannitol had a higher solid content (31.5mg) than those without mannitol (25 mg) and might take longer to hydrate and go into solution. However it is apparent that the glassy/ amorphous form of the lyophilisate also influenced release rate.

The release rates of nicotine (slope) obtained from the Peppas equation (% released vs t^n) are shown in Table 2.10. The release rate was slower from lyophilisates

prepared with mannitol than from those without mannitol. Similarly, the release rate from 'glassy' lyophilisates was faster than from amorphous lyophilisate.

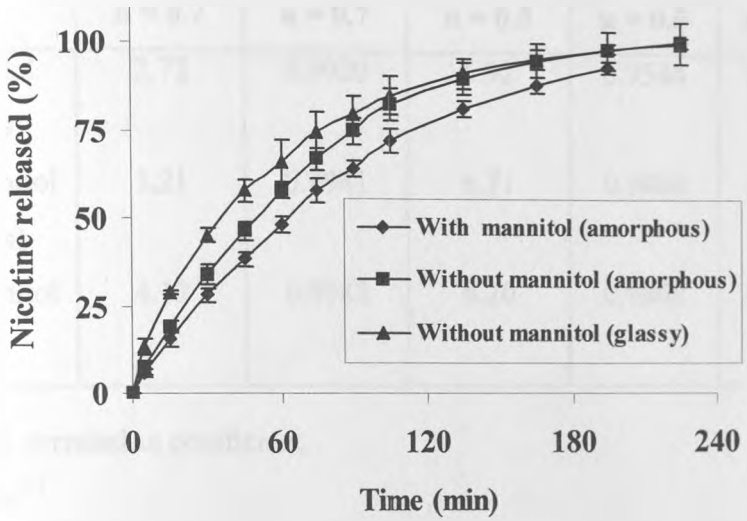

Figure 2.24 Comparison of release (%) profile of nicotine into PBS, pH 7.4 at 37°C among lyophilisates prepared from 2% w/w solution of K100MP with & without mannitol in the formulation and amorphous vs glassy lyophilisates. Each curve is mean ±s.d of four experiments.

Table 2.10: Release rate (slope) of nicotine, up to 60% release, from lyophilisates prepared from 2% solution of K100MP with & without mannitol (quenched cooling), and glassy lyophilisates (pre-freezing). Three different values for the diffusional exponent (n) were used in the equation (1) (% released vs. t^n) to determine the best fit to the experimental data.

Lyophilisate	[a]Slope for n = 0.7	R^2 for n = 0.7	[b]Slope for n = 0.5	R^2 for n = 0.5	R^2 for n = 0.3
With mannitol (amorphous)	2.72	0.9920	5.92	0.9344	0.7907
Without mannitol (amorphous)	3.21	0.9941	6.71	0.9406	0.8037
Without mannitol (glassy)	4.12	0.9982	8.20	0.9802	0.8792

R^2 Square of the correlation coefficient.

[a] %released/time$^{0.7}$

[c] %released/time$^{0.5}$

2.3.2.5 Nicotine release from powder mixes

Release profiles of nicotine from K4MP powder blends are shown in Fig. 2.25. The powder blends were such that they had the same composition as the corresponding lyophilisate. Nicotine release rates from different powder blends did not differ with polymer content ($t_{50\%}$ <15 minutes for K4MP content in the range 26-52% w/w) although release rates were lower than from NHT powder, $t_{50\%}$ = 3 minutes. In the powder blends, crystalline NHT was present as part of a simple powder mix which lacked the porous but homogenous structure of lyophilisates (Fig 2.15) and from the results it is apparent that NHT could dissolve rapidly and largely independently of the hydration of the K4MP particles. There is thus an explanation for the similar and

relatively rapid, nicotine release rates from powder blends of different K4MP contents.

A comparison of the release profiles of nicotine from powder blend, lyophilisate and NHT powder are shown in Figs. 2.26 & 2.27. Release was slower from lyophilisates ($t_{50\%}$ =45 and 60 min for lyophilisates prepared from 2 and 3% w/w solutions respectively) than from powder blend ($t_{50\%}$ <15 min).

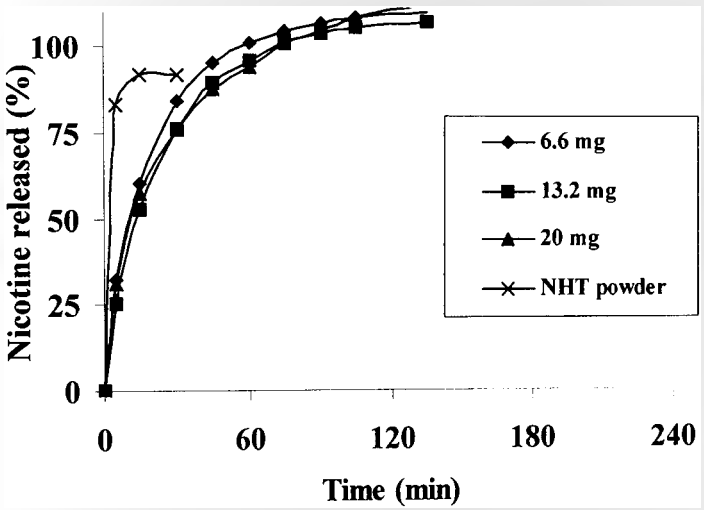

Figure 2.25 Release (%) profile of nicotine from K4MP powder blends, each containing 4 mg nicotine base (as hydrogen tartrate). The amount of K4MP in the powder mixes indicated, 6.6, 13.2 and 20 mg, is the same as in lyophilisates prepared from 1, 2 and 3% K4MP solutions respectively. Each curve is mean of three experiments.

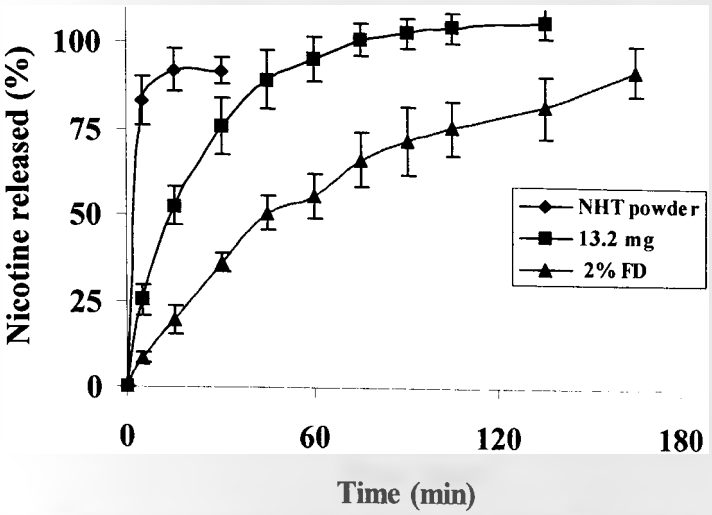

Figure 2.26 Comparison of release (%) profiles of nicotine from a powder blend (K4MP), a lyophilisate prepared from 2% w/w K4MP solution and as a control from 11.4 mg NHT powder equivalent to 4 mg nicotine base. The amount of K4MP in the powder mix indicated, 13.2 mg, is the same as in lyophilisates prepared from 2% K4MP solution. Each data point is mean ±s.d. of three experiments.

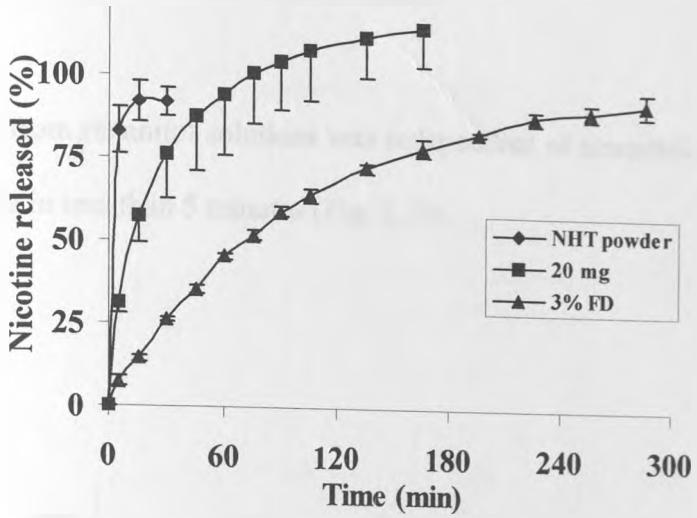

Figure 2.27 Comparison of release (%) profiles of nicotine from a powder blend (K4MP), a lyophilisate prepared from 3% w/w K4MP solution and as a control from 11.4 mg NHT powder equivalent to 4mg nicotine base. The amount of K4MP in the powder mix indicated, 20 mg, is the same as in lyophilisates prepared from 3% K4MP solution. Each data point is mean ±s.d. of three experiments.

2.3.2.6 Nicotine release from mannitol solutions and lyophilisates

Nicotine release profiles from lyophilisates prepared from different concentrations (1, 5 and 10% w/w) of mannitol solution are shown in Fig. 2.28. Nicotine release decreased with mannitol concentration. However, release was very fast in comparison to lyophilisates incorporating Methocel K. The $t_{50\%}$ of nicotine from lyophilisates containing the highest amount of mannitol was less than 20 minutes. Although there was an effect of mannitol concentration on nicotine release, it was small compared to the effect of polymer concentration. It is possible that the mannitol effect could be attributed to the volume of medium available for plug hydration. If at a high mannitol content in the plug, the quantity of hydration

medium was insufficient for complete mannitol dissolution it is apparent that this would also influence NHT dissolution and release.

Nicotine release from mannitol solutions was independent of mannitol concentration and was complete in less than 5 minutes (Fig. 2.29).

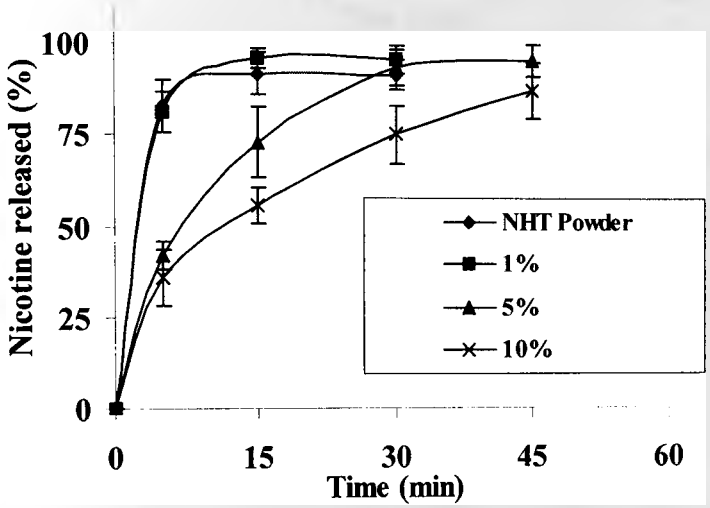

Figure 2.28 Release (%) profile of nicotine into pH 7.4 PBS at 37°C from lyophilisates prepared from different indicated concentrations of mannitol solution. As a control, 11.4 mg nicotine hydrogen tartrate (NHT powder) equivalent to 4 mg nicotine base was used. Each curve is a mean ±s.d. of 3 experiments.

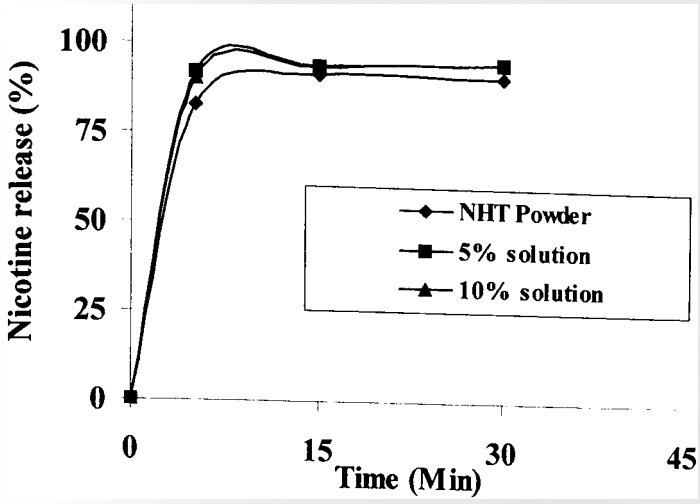

Figure 2.29 Release (%) profile of nicotine into pH 7.4 PBS at 37°C from different indicated concentrations of mannitol solution. As a control, 11.4 mg nicotine hydrogen tartrate (NHT powder) equivalent to 4 mg nicotine base was used

2.3.2.7 Nicotine content determination from different section of lyophilised plug

The nicotine content of different sections of a lyophilisate prepared from 2% w/w solution of Methocel K100LVP is summarised in Table-2.11. It was found that the nicotine content in sections, top, middle, bottom, of the plug were the same. These data suggest that there was no NHT solute migration during freezing of the formulation solution. Such migration is theoretically possible so that during freezing, solute concentration may vary along the length of the plug and be particularly high where phase transformation (liquid to solid) last occurs. Solute migration would tend to be more prominent during slow freezing when solute

molecules have more time to accumulate in still liquid regions of the freezing plug than during quench cooling in liquid nitrogen.

Table-2.11 Assay of nicotine (by HPLC) of different portion of sectioned lyophilised plug prepared from 2% w/w K100LVP solution to observe the possibility of solute migration during freezing process.

Sample ID	Wt. of plug taken (mg)	Nicotine content (mg)	Nicotine content/mg of plug
Top	10.86	1.3264	0.1221
Top	10.37	1.2879	0.1242
Top	10.55	1.3122	0.1244
Middle	10.69	1.3154	0.1230
Middle	11.23	1.4201	0.1264
Middle	10.93	1.3806	0.1263
Bottom	11.18	1.3687	0.1224
Bottom	11.4	1.4586	0.1279
Bottom	11.4	1.435	0.1258

2.3.2.8 Stability studies of nicotine

The chemical structures of known degradation products of nicotine (Carlisle, Chicoine & Wygant, 1992; Kyeremanten *et al.*, 1987) and related substances are represented in Fig. 2.30. Authentic samples of nornicotine (Fig. 2.30b) and cotinine (Fig. 2.30c) were analysed and the time of elution of these materials under various chromatogrpahic conditions determined. A typical chromatogram of nicotine with

the known degradation products is illustrated in Fig. 2.31, 2.32 & 2.33. The concentration of nornicotine and cotinine in this chromatogram of nicotine was 500 μg/ml and 300 μg/ml respectively. Chromatograms of individual components were obtained and then chromatograms of nicotine, nornicotine, and cotinine mixes were obtained.

Initial studies with mobile phase composition (20% acetonitrile/ 80% 0.01M phosphate buffer pH 7.4 by volume) did not give well separated peaks, so the polarity of the mobile phase was increased (10% acetonitrile/ 90% 0.01M phosphate buffer pH 7.4) to give better peak separation. Retention times of the various analytes was dependent on mobile phase composition.

Nicotine degradation products were not detected by HPLC from boiled nicotine solution and from solution stored at 50^0C for 8, 12, and 32 days. Similarly NHT powder and lyophilisate, stored at 50^0C for 32 days did not show degradation products. This result is in agreement with a previous study (Cornaz Gudet & Buri, 1998) which evaluated nicotine in solution at different pH and storage condition, as well as in dry dosage forms (freeze dried microspheres). The predicted degradation products of nicotine were not observed, confirming the good stability of nicotine in liquid and solid dosage forms.

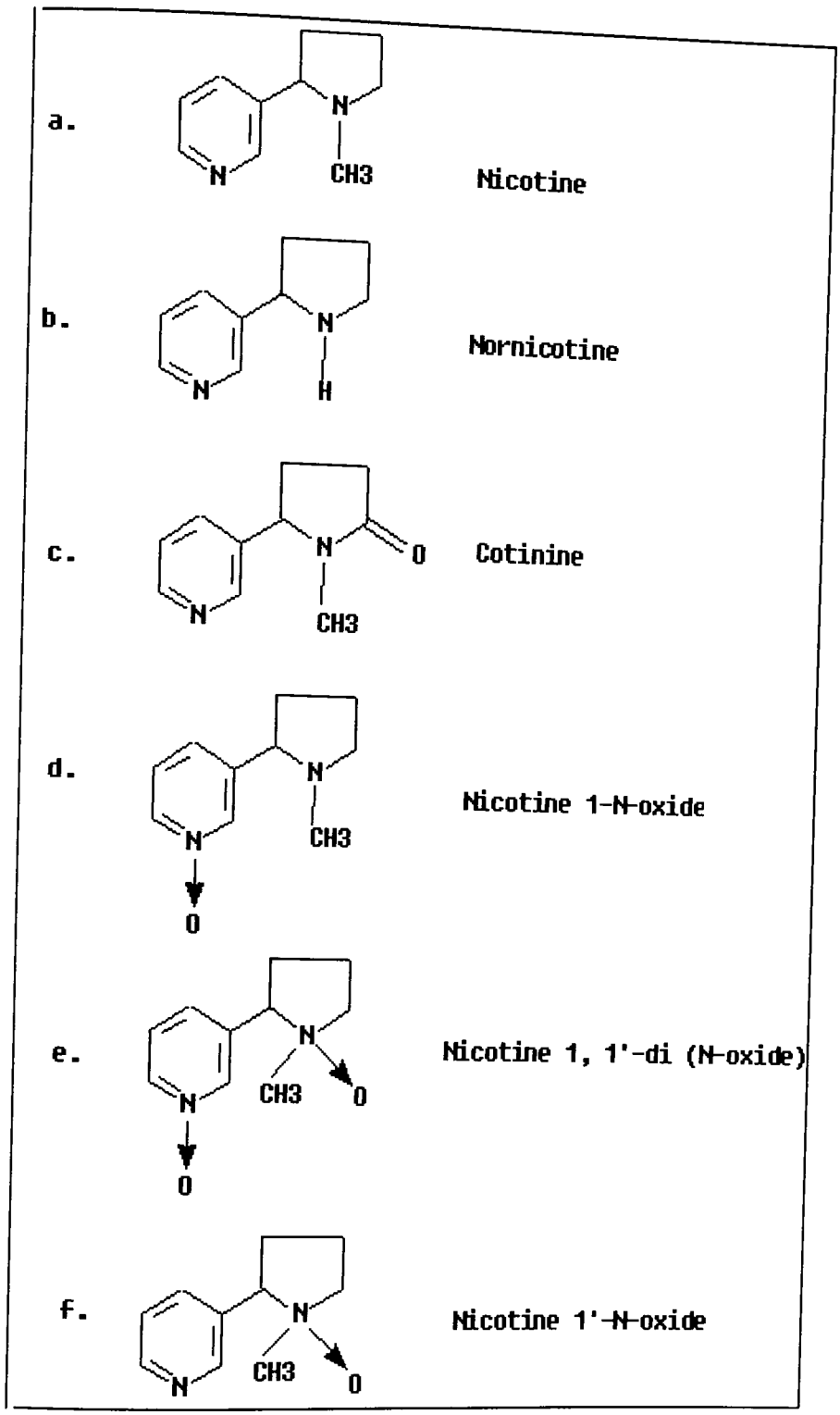

Figure 2.30 Chemical structure of nicotine and its known degradation products.

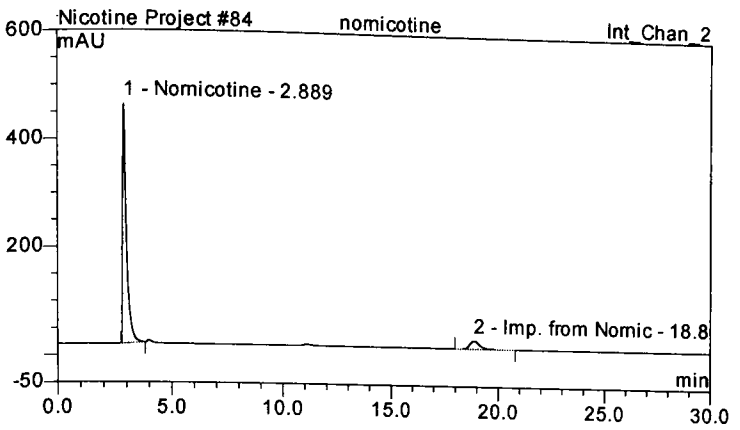

Figure 2.31 HPLC chromatogram of nornicotine. Mobile phase: 10% acetonitrile/ 90% 0.01M phosphate buffer pH 7.4 by volume.

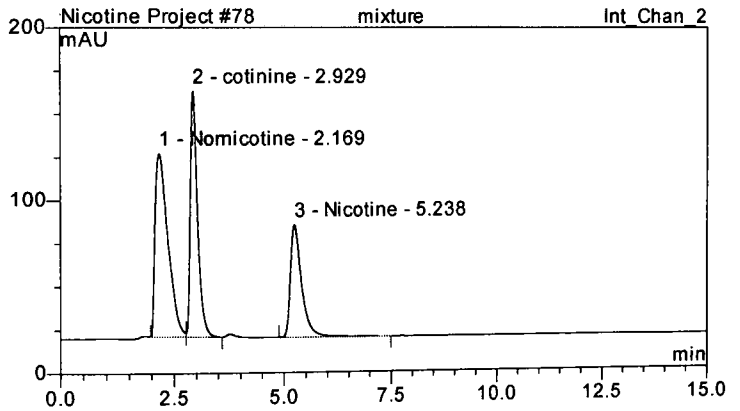

Figure 2.32 HPLC chromatogram of nicotine, nornicotine and cotinine. Mobile phase: 20% acetonitrile/ 80% 0.01M phosphate buffer pH 7.4 by volume.

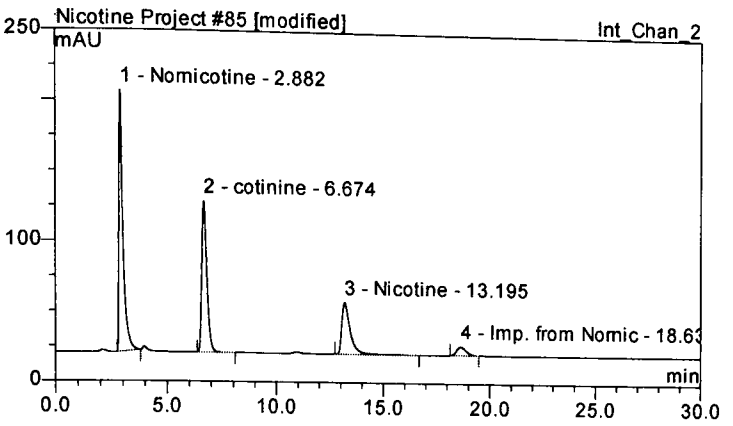

Figure 2.33 HPLC chromatogram of nicotine, nornicotine and cotinine. Mobile phase: 10% acetonitrile/ 90% 0.01M phosphate buffer pH 7.4 by volume.

On hydration, the mechanism of drug release from these lyophilised plugs appears to be similar to the release characteristics described for various other types of solid dosage forms incorporating HPMC matrices. The predictability of the *in vitro* release behaviour indicates that polymer concentration could be used to manipulate drug release and perhaps design drug specific release profiles.

No degradation products of nicotine were observed, confirming the good stability of nicotine in freeze-dried plug.

Chapter 3 Physico-mechanical Properties of Lyophilised Dosage Form

3.1 Introduction

Texture profile analysis (TPA) is extensively employed in the food industry for the characterisation of semi-solid products (Breene, 1975; Friedman, Whitney & Szczniak, 1963). TPA has also been applied in the mechanical characterisation of pharmaceutical semisolids and in the investigation of the interactions between the components of bioadhesive polymeric formulations (Jones, Woolfson & Brown, 1997a; Jones *et al.*, 1997b; Jones, Woolfson & Djokic, 1996a; Jones *et al.*, 1996b). Some of the parameters, relevant to a solid dosage form which may be measured by TPA are as follows;

- hardness (the force required to attain a given deformation),
- fracturability (the first significant peak where the force falls off during the first penetration),

- springiness (how well the product physically springs back after it has been deformed)
- resilience (how well a product `fights to regain its original position'). It can be thought of as instant springiness, since resilience is measured on the withdrawal of the first penetration, before the waiting period is started.).

A typical TPA plot ('two bite' system) is shown as a schematic in Fig. 3.1 (Bourne, 1982) and the basis of TPA calculations used by the texture expert software to measure the parameters described above is shown in Fig. 3.2. Thus using the definitions in Fig. 3.2, hardness is the peak force of the first penetration into the product. The peak force need not occur at the point of deepest penetration, although for most products, typically it does. Not all products fracture; but when they do, the fracturability point occurs where the plot has its first significant peak during the probe's first penetration into the product.

Springiness is measured at the down stroke of the second penetration and the waiting time between the two penetrations can be important. In some cases, an excessively long waiting time will allow a product to spring back more than it might under the in use conditions of interest. Springiness can be measured in several ways, but most typically, it is the time to the peak force on the second penetration (Length 2, Fig. 3.2) divided by the time to the peak force on the original penetration (Length 1).

Resilience is calculated by the area during the withdrawal after the first penetration, divided by the area under the peak of the first penetration (Area 5/Area 4 on the Fig.3.2).

Adhesivity (adhesiveness) has been defined as the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe (Jones *et al.*, 1996b). The adhesive properties of the formulations can be examined in various ways and in TPA the work required to remove a polymeric probe from the test formulation is commonly used. Adhesivity can also be quantified as the (detachment) force required to overcome the adhesive bond between a formulation and a substrate, say for example, glass surface, mucin disc or mucosae. A typical plot in adhesive mode is shown in Fig. 3.3. Here, for example, the test force of 2g was applied for 30 seconds and the detachment force (f) was measured. In this mode, the probe travels down to a predefined distant with a predefined speed in to sample and remains at that position for a predefined duration and maintains the predefined test force through out. And at the end of the predefined duration, the probe withdraws from the sample. The machine measures this force (detachment) as adhesive force.

Polymers which interact adhesively with biological substrates offer certain advantages for drug delivery, including, prolonged residence time and improved location on, e.g. the gastrointestinal tract, buccal cavity, nasal and vaginal tracts, the eye and the cervix (Bouckaert *et al.*, 1992; Ch'ng *et al.*, 1985; Jones *et al.*, 1996b; Nagai *et al.*, 1984; Woolfson *et al.*, 1995b). Cellulose polymers, such as,

hydroxyethylcellulose, hydroxypropylcellulose, sodium carboxymethylcellulose, are polymers that have been reported to possess bioadhesive properties. Pharmaceutically, water soluble cellulose polymers have found widespread applications for instance in the formulation of solid dosage forms, in aqueous disperse systems as viscosity enhancing agents and in products for topical application (Peppas, 1987).

In the development of any dosage form, several desirable attributes that contribute to the ultimate patient acceptability and clinical efficacy of the product may be defined. Some of these attributes may include optimal mechanical properties, good bioadhesion (to ensure retention at the site of application), acceptable viscosity, drug release and absorption.

This chapter describes the physico-mechanical properties of a lyophilised hydroxypropyl methylcellulose (HPMC) dosage form.

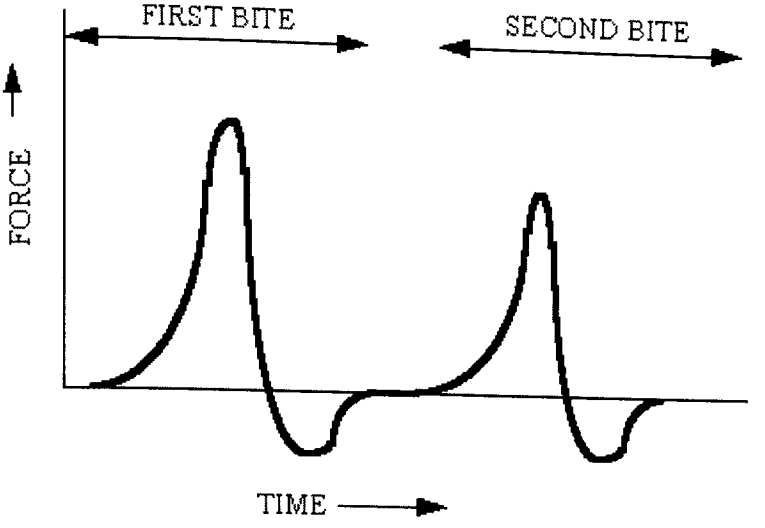

Figure 3.1 A typical schematic texture plot in texture profile analysis (TPA) mode ('two bite' system).

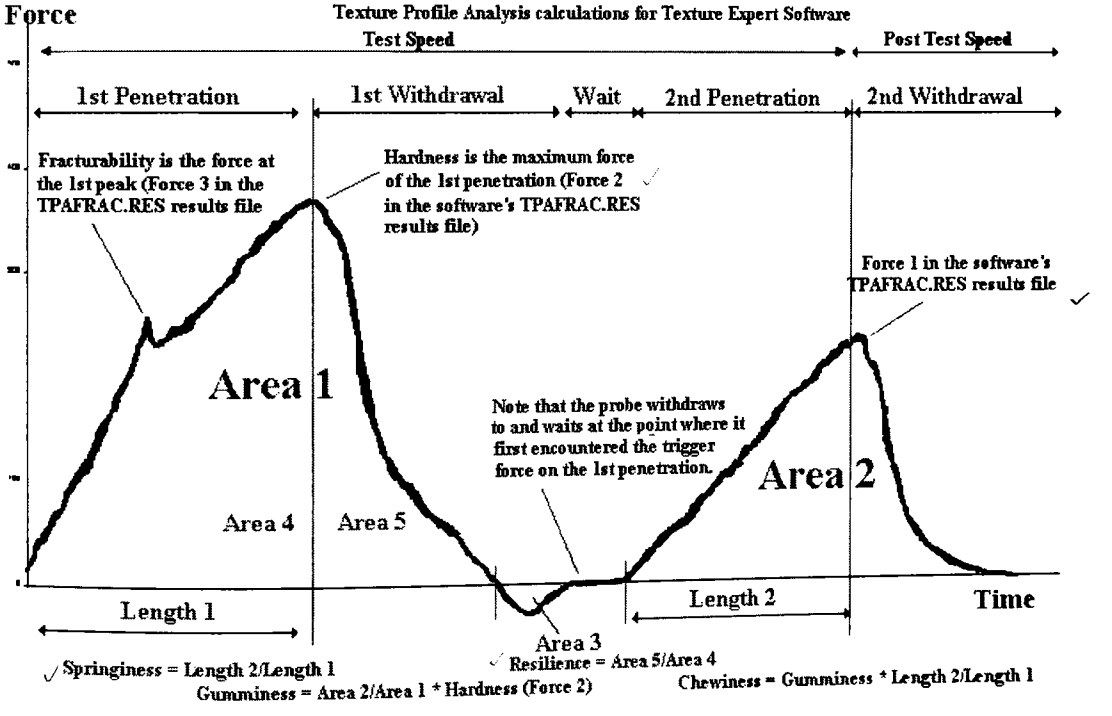

Figure 3.2 Texture profile analysis (TPA) calculations for texture expert software.

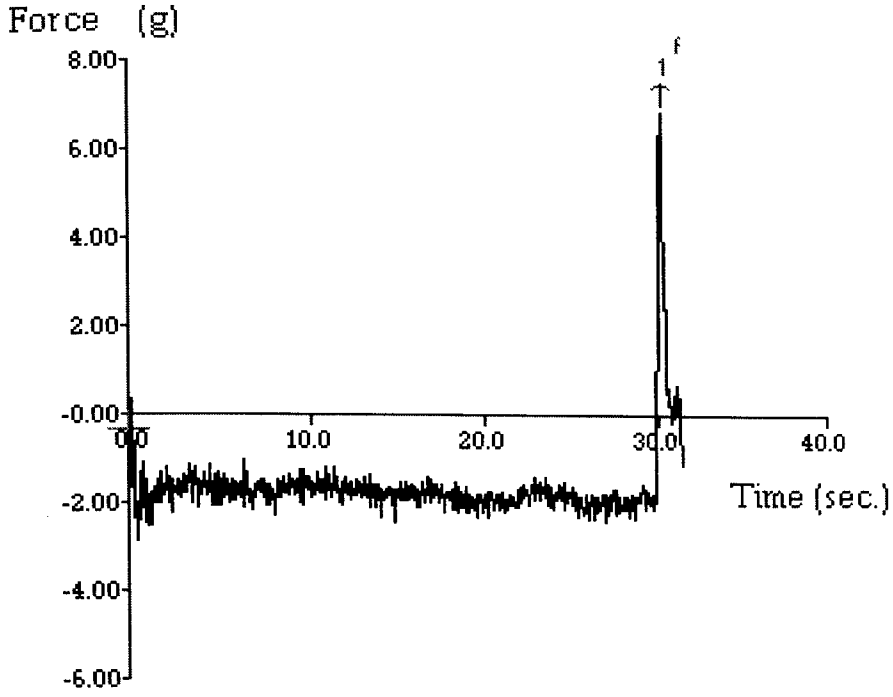

Figure 3.3 A typical plot in adhesive mode. The test force of 2g was applied for 30 seconds and in this case the adhesive force was 6.79g.

3.2 Materials and Methods

3.2.1 Materials

Chemicals used are described in section 2.2.1.1.

3.2.2 Methods

3.2.2.1 Apparatus

The measurement of the mechanical properties of the dry plugs and the adhesivity and gel strength of hydrated plugs were performed on TA.XT2 Texture Analyser with interchangeable load cell capacity of 5 Kg and 25 Kg (Stable Micro Systems, Goldalming, surrey, England) and data were processed using a Texture Expert (version 1.19) software package (Stable Micro systems).

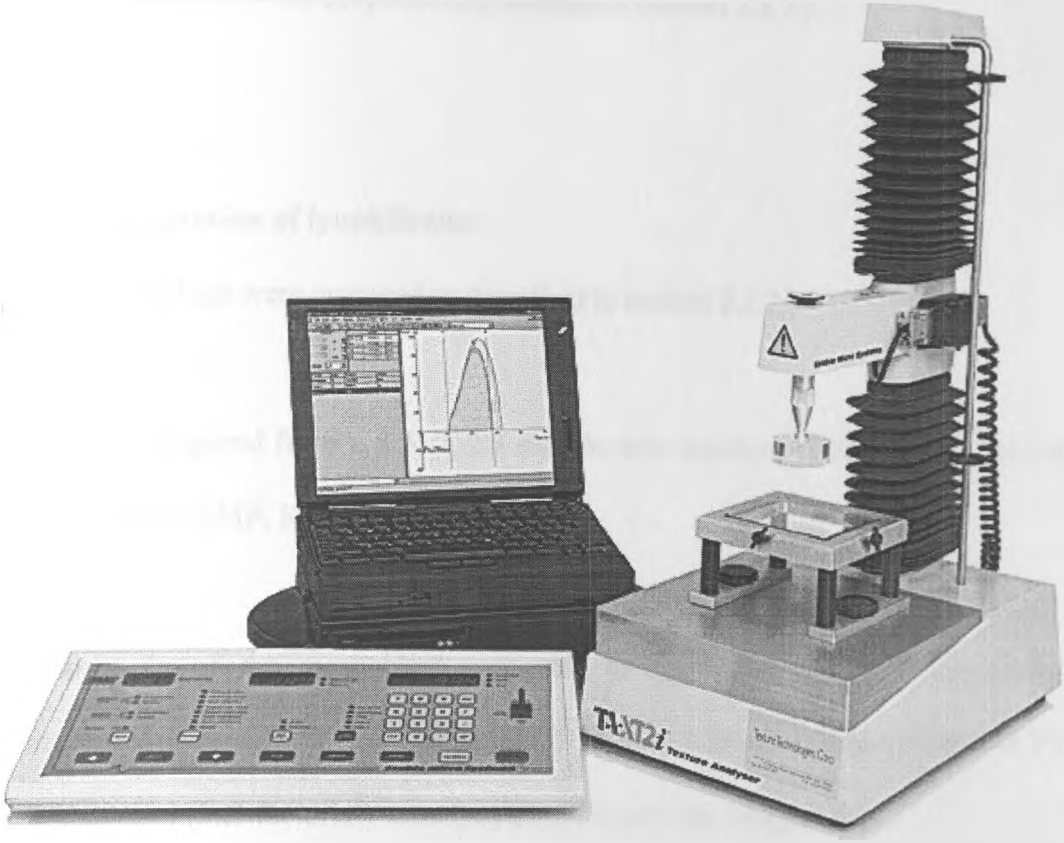

Figure 3.4 TA.XT2 Texture Analyser (reproduced from Stable Micro Systems, internet homepage).

3.2.2.2 Preparation of Methocel solutions

Methocel solutions were prepared as described in section 2.2.2.3.

3.2.2.3 Preparation of lyophilisates

Lyophilised plugs were prepared as described in section 2.2.2.5.

Plugs were prepared from 1, 1.5, 2, 2.5 and 3% w/w solutions of K4MP and 2% w/w solutions of K15MP, K100MP and K100LVP.

In the study of the mechanical properties of lyophilised plugs, 9 formulation solutions containing 1, 2 & 3% w/w K4MP; 1, 2 & 3 % w/w of povidone; and 3% w/w mannitol (3x3 factorial) were lyophilised to provide sample plugs.

Throughout, Methocel solutions and lyophilisates used in the studies did not contain NHT.

3.2.2.4 Selected operating parameters for the study of the mechanical properties of lyophilised plugs using the Texture Profile Analysis programme

TA-XT2 settings:

Option:	TPA mode
Pre-test speed:	1.0 mm/s
Test speed:	1.0 mm/s
Post-test speed:	1.0 mm/s
Distance:	10 mm
Time:	5 seconds
Trigger type:	Auto 20g
Data acquisition rate:	200pps
Accessory:	3mm cylindrical stainless steel probe.

Test conditions

The end of the lyophilised plug was cut by a Histoline razor to get a flat uniform surface at the top. The plug was replaced in the microfuge tube which was positioned so that the probe hit the sample plug at the centre of its circular cross section. The probe did not touch the wall of the microfuge tube during the test.

At each compression of the 'two bite' TPA test, the probe penetrated the sample to a depth of 10 mm at a rate of 1.0mm/sec. There was a delay period of 5 second

between the end of first and beginning of the second compression. All analyses were performed on 10 replicate samples.

3.2.2.5 Selected operating parameters for the measurement of gel strength (compression force)

TA-XT2 settings

Mode:	Measure force in compression
Option:	Return to start
Pre-test speed:	1.0 mm/s
Test speed:	0.5 mm/s
Post-test speed:	1.0 mm/s
Distance:	10mm
Trigger type:	Auto 0.1g
Data acquisition rate:	200pps
Accessory:	25G-needle probe using 5 Kg load cell.

Test conditions

The gel strength (compression force and adhesivity) of solutions of grade K Methocels and of hydrated lyophilisates prepared from them was determined using a needle probe.

The volume of water (150 µl) for hydration of the lyophilisates was sufficient for the full hydration of the heaviest lyophilisate, 20 mg plug prepared from 3% w/w Methocel solution.

The water was added to the lyophilisate in the microfuge tube, the resulting thick gel was stirred by the help of small spatula in order to make sure it lay uniformly in the lower part of the tube which was whirl-mixed for few minutes to remove entrapped air and to help settle the gel. The compression force and adhesivity (if any) was measured using TA-XT2 parameters described above.

The tube containing the gel was positioned such that probe hit the centre of the gel surface during test. The probe was programmed to descend 10mm after attaining the trigger force of 0.1g in compression mode and then to return to the start position. The probe did not touch any part of the tube during measurements. Each measurement was repeated at least 10 times.

3.2.2.6 Selected operating parameters for the study of the effect of test force and contact time on adhesive force, and for measurement of the adhesivity of plugs on a wet mucosal surface

TA-XT2 settings:

Mode:	Adhesive test
Pre-test speed:	1.0 mm/s
	0.5 mm/s (to measure adhesivity by surface hydration).
Test speed:	1.0 mm/s
	0.5 mm/s (to measure adhesivity by surface hydration).
Post-test speed:	1.0 mm/s
Test force:	5, 10, 25, and 50g (to study effect of test force),
	10g (to study effect of contact time and to measure adhesive force on mucosal surface), and
	2g (to measure adhesivity by surface hydration).
Time:	60 sec (to study effect of test force and to measure adhesive force on mucosal surface),
	30, 60, 90, 120 seconds (to study effect of contact time),
	30 seconds (to measure adhesivity by surface hydration).
Distance:	0.1 mm (to measure adhesivity by surface hydration),
	1 mm (for different contact time and test force), and
	2 mm (to measure adhesive force on mucosa).

Trigger type:	Auto 1g
Data acquisition rate:	200pps
Accessory:	Stainless steel cylindrical probe (6mm diameter) 25 Kg load cell except for the measurement of adhesive force on mucosa where a circular polystyrene disk (40 mm diameter) probe was used for the attachment of the plug.

3.2.2.6.1 Test conditions for the measurement of the effect of test force

Lyophilised plugs prepared from 1.5 and 2.5% w/w solutions of K4MP were stuck to the probe using double-sided tape. The required volume of water (30 µl, enough to hydrate at least half of the lyophilised plug) was pipetted onto a glass slide and positioned such that when lowered, the probe with the attached plug hit the centre of the water droplet. Care was taken to avoid lifting of the glass slide at the end of the hold time by weighting both ends of the slide. Adhesive force was measured at different applied test force. Each test run was repeated at least 10 times.

3.2.2.6.2 Test conditions for the measurement of the effect of contact time

The sample plug (lyophilised from 1.5% w/w K4MP solution) was set up as described in section 2.2.6.1 except that the applied test force of 10g was maintained for different test times. The 10g test force was chosen on the basis of the

compressibility of the lyophilised plugs which at this force were compressed by about one third. Each test was repeated 10 times.

3.2.2.6.3 Test set up to measure adhesive force on a wet mucosal surface

Bovine tracheal mucosa was separated from the underlying tracheal tissue and stuck to a glass slide with super glue at 25°C. Hydration of the mucosal sample was maintained by adding three drops of isotonic saline each time a test was run with a new plug.

Lyophilised plugs were prepared from each of three formulation solutions, containing K4MP 1, 2.0 or 3.0% w/w, Povidone 1% w/w and mannitol 3.0%. Plugs were attached to the probe with double-sided tape. The probe was programmed to descend 2 mm onto the mucosa after the trigger and to remain at that position for one minute before withdrawing. Each test was repeated five times.

3.2.2.6.4 Test conditions for the measurement of adhesive force between a hydrated plug and a wet glass surface

Lyophilised plugs prepared from 1.5, 2, 2.5 or 3%w/w K4MP solutions were stuck to the probe using double-sided tape. The probe was programmed to travel to a distance of 0.1mm after coming in contact with the glass slide surface which was wetted with 5 µl water for each sample plug. Methylene blue was dissolved in the water to monitor how far the water was absorbed into the plug. The 5 µl volume was

chosen to just hydrate the surface of the sample plug. Only plugs with a uniform surface were used in the test. The lowest possible test force was used to ensure that the partially hydrated plug did not collapse.

3.2.2.7 Hydration characteristics (water uptake) of lyophilisates

To study how rapidly a lyophilisate hydrated (collapsed in medium), it was dropped into 5 ml 1.5% w/v mucin solution on a watch glass at room temperature and the collapse time recorded as that time at which there was no remaining visual evidence of the plug.

In another series of experiments, a range of Carbopol 934P solutions (1.5-10% w/w) were used instead of mucin solution. As for mucin, the time required to undergo complete hydration was recorded.

To measure how much water was required for complete hydration, lyophilised plugs were placed on a glass slide and water was added with Hamilton syringes until there were no visual evidence of the plug.

3.2.2.8 Morphological study of lyophilisates by scanning electron microscopy (SEM)

The morphology of lyophilised K4MP plugs and as control, of blends of K4MP and NHT powder were examined by SEM. Lyophilised plugs were stuck onto the aluminium stub with carbon tape, and coated with gold using a Polaron E5000

sputter coating apparatus. Powder blends were thinly spread over the aluminium stub and carbon disc or tape used to stick the sample to the stub. These samples were then scanned by using a JOEL JSM840A scanning electron microscope at 10-15 kV under varying magnifications.

3.3 Result and discussion

3.3.1 Mechanical properties of lyophilised plugs

The mechanical properties of hardness, fracturability, springiness and resilience, of the formulations under examination are summarised in Table 3.1. The effect of the K4MP and PVP content of lyophilised plugs on hardness and springiness are shown in Figs. 3.5 and 3.6 respectively. The hardness and fracturability of lyophilised plug increased with the K4MP or PVP content in the formulations whereas springiness and resilience decreased. Similar trends have been observed in a study of pharmaceutical semisolids (Jones *et al.*, 1996a).

A knowledge of the physical properties of pharmaceutical products, whether semisolid or solid, is of value in predicting the performance of the product under various of conditions. For a semi-solid in particular, behaviours during product filling (Pena, Lee & Stearns, 1994), spreadibility and bioadhesion on mucosal or non-mucosal surfaces (Gandhi & J.R., 1994), perceived 'feel' of the product on the skin, and the reproducibility of such product characteristics on removal from the final packaging are important. However, similarly for a solid unit dosage form such as the

lyophilisates under study here, the properties of hardness, fracturability (brittleness), springiness, and resilience are useful determinants of ease of handling and patient compliance during product use, in this case by insertion into the nose.

Table 3.1 The effect of K4MP and PVP (mol.wt. 10,000) content on hardness (**H**, g), fracturability (**F**, g), springiness (**S**, no unit), and resilience (**R**, no unit) of lyophilised plugs each containing 19.5 mg of mannitol, as determined by texture profile analysis. Each value represents the mean ±s.d of 10 replicates.

K4MP (mg)	PVP (mg)	H (g)	F (g)	S	R
6.5	6.5	636.06±104.74	471.73±273.68	0.403±0.135	0.053±0.013
6.5	13	1178.15±212.20	1165.90±177.06	0.214±0.07	0.035±0.007
6.5	19.5	1758.78±322.16	1016.00±570.75	0.294±0.105	0.040±0.014
13	6.5	896.67±236.54	797.67±315.54	0.385±0.168	0.058±0.022
13	13	1294.42±187.70	1266.98±207.32	0.257±0.115	0.046±0.008
13	19.5	1929.81±247.46	1551.76±478.80	0.199±0.074	0.038±0.016
19.5	6.5	1293.51±181.20	956.25±561.8	0.292±0.110	0.058±0.012
19.5	13	2304.69±316.22	2232.97±409.71	0.189±0.050	0.044±0.008
19.5	19.5	3566.68±450.40	3303.70±450.40	0.245±0.068	0.047±0.004

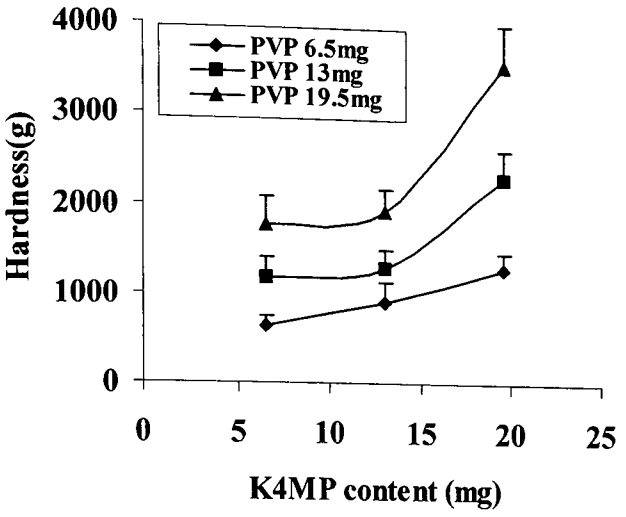

Figure 3.5 K4MP/PVP plugs containing 19.5 mg mannitol. The effect of K4MP and PVP contents on mean (n=10) plug hardness.

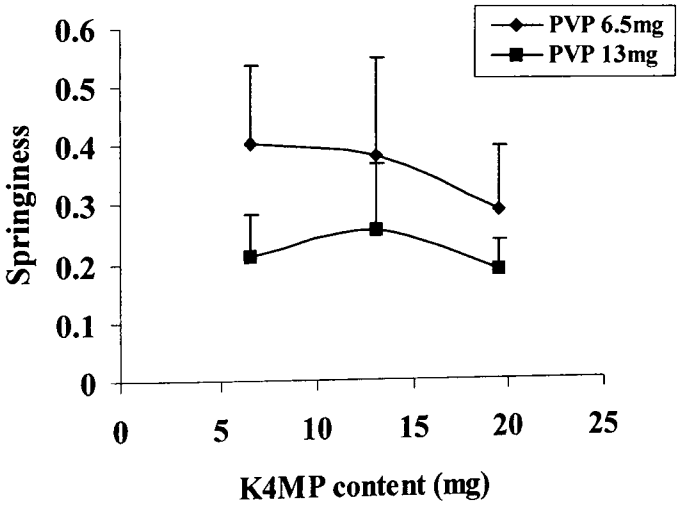

Figure 3.6 K4MP/PVP plugs containing 19.5 mg mannitol. The effect of K4MP and PVP contents on mean (n=10) plug springiness.

3.3.2 Gel strength of Methocel K solutions and hydrated lyophilisates

The mean (n=10) gel strength (compression force), measured by a 25G needle probe, of different concentrations of K4MP solution and of hydrated lyophilisates prepared from these solutions are summarised in Table 3.2 and 3.4 respectively. Similarly the gel strength of different molecular weight Methocel K (K100LV, K4MP, K15MP, K100MP) solutions (2% w/w) and the corresponding hydrated lyophilisates are shown in Table 3.3 and 3.5 respectively. No difference among Methocel K solutions in compression force and detachment force (adhesive force) was observed. The obvious reason could be that the probe contact area and therefore resistance to probe penetration was not sufficient for a difference to be detected in the range of solutions studied (1, 2 and 3% w/w K4MP and 2% w/w of the different molecular weight K Methocels). However marked differences in gel strength were observed among fully hydrated lyophilisates prepared from these solutions (Table 3.3 and 3.5, and Figs. 3.7 and 3.8). On the basis of measured gel strengths and adhesive forces, it would appear that hydrated lyophilisates contained higher concentrations of polymer solution than the solutions from which they were prepared since they exerted higher resistance to probe penetration and withdrawal. These results for the hydrated lyophilisates are in agreement with a previous study in which the increased gel strength (Ferrari *et al.*, 1994) of aqueous hydroxypropyl methyl cellulose gels was shown to be a function of polymer concentration. In another study (Jones *et al.*, 1997a; Jones *et al.*, 1997b) increased gel strength as a function of the concentration of cellulose polymers was also described.

Table 3.2 Summary of mean (n=10) gel strength (compression force) and adhesivity of different concentrations of Methocel K4MP solution.

K4MP conc. (% w/w)	Mean gel strength (g) ±sd	Coefficient of variation (%)	Mean adhesive force (g) ±sd	Coefficient of variation (%)
1	0.216±0.030	13.829	Not measured	-
2	0.208±0.023	10.975	0.979±0.048	4.901
3	0.197±0.038	19.258	1.012±0.043	4.266

Table 3.3 Summary of mean (n=10) gel strength (compression force) and adhesivity of 2% w/w solution of different molecular weight grade K Methocels.

Methocel	Mean gel strength (g) ±sd	Coefficient of variation (%)	Mean adhesive force (g) ±sd	Coefficient of variation (%)
K100LVP	0.206±0.031	14.898	1.048±0.041	3.919
K4MP	0.208±0.023	10.975	0.979±0.048	4.901
K15MP	0.246±0.049	20.053	1.012±0.062	6.137
K100MP	0.279±0.028	10.098	0.958±0.057	5.975

Table 3.4 Summary of mean (n=10) gel strength (compression force) and adhesivity of lyophilisate prepared from different concentration of Methocel K4MP solution, volume of water used for hydration = 150 µl.

Weight of lyophilisate (mg)	Mean gel strength (g) ±sd	Coefficient of variation (%)	Mean adhesive force (g) ±sd	Coefficient of variation (%)
06.6	0.365±0.060	16.397	0.880±0.070	7.894
13.0	1.445±0.464	32.112	1.677±0.545	32.491
19.5	3.477±0.769	22.102	3.644±0.703	19.280

Table 3.5 Summary of mean (n=10) gel strength (compression force) and adhesivity of 13 mg lyophilisates prepared from 2% w/w solution of different molecular weight. grade K Methocel, volume of water used for hydration = 150 µl.

Methocel	Mean gel strength (g) ±sd	Coefficient of variation (%)	Mean adhesive force (g) ±sd	Coefficient of variation (%)
K100LVP	0.304±0.026	8.592	0.931±0.044	4.769
K4MP	1.445±0.464	32.112	1.677±0.545	32.491
K15MP	2.491±0.459	18.439	2.935±0.362	13.303
K100MP	3.629±0.482	13.290	3.451±0.494	14.325

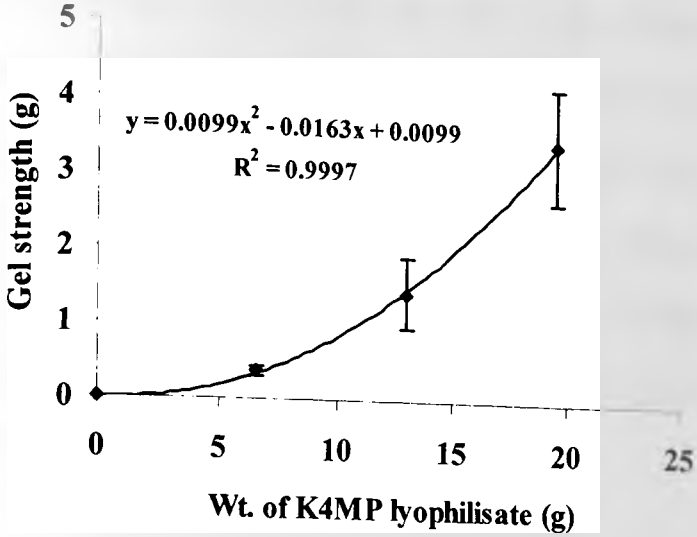

Figure 3.7 Effect of K4MP content on the mean (n=10) gel strength of hydrated lyophilisates prepared from 1, 2 and 3% K4MP solutions.

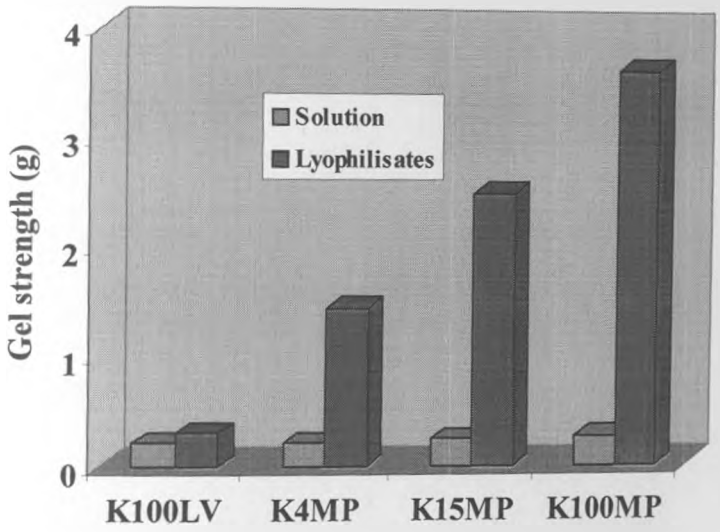

Figure 3.8 Effect of molecular weight of Methocel K on the mean (n=10) gel strength of hydrated lyophilisates prepared from 2% solutions.

3.3.3 Effect of test force on adhesive force of lyophilised plug

For the four contact forces, 5, 10, 25 and 50g used to test adhesivity between hydrated plugs and the glass slide surface, it was found that adhesion (detachment force) increased with the test (applied) force (Fig. 3.9). This trend is in accordance with the previous work done on pig gastric mucosa (Tobyn, Johnson & Gibson, 1993) and on tanned goat leather (Blanco-Fuente *et al.*, 1996). The high adhesivity at higher applied force must be due to the fact that the two surfaces have brought into more intimate contact.

The variability of measured adhesive force was high. Among the reasons for this could be due to non-uniform wetting of sample surface and size of the sample. In spite of the variability, the difference in adhesivity of different plugs e.g. 1.5 and 2.5% K4MP (Fig. 3.9) could be detected at all applied forces used.

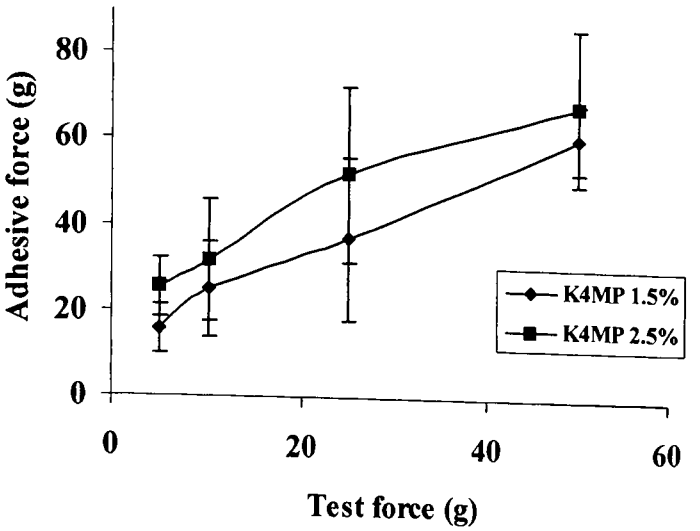

Figure 3.9 Effect of test force on mean adhesive force (n=10) of hydrated K4MP lyophilisates (contact time 1 minute, vol. of water 30 µl) as measured by TA.XT2 Texture Analyser, V 5.19, load cell capacity 25 kg.

3.3.4. Effect of contact time on the adhesivity of hydrated lyophilised plug

The contact times used were 30, 60, 90 and 120 sec with a glass slide as a substrate, 30 µl water for hydration and 10 g test force. The effect of these contact times on adhesive force is shown in Fig. 3.10 where it can be seen that adhesive force of the hydrated lyophilisates increased with time which is in accordance with a previous study of adhesivity of Carbopol 940 tablets on tanned leather (Blanco-Fuente *et al.*, 1996). They observed that adhesivity increased with the contact time and adhesive work was more or less constant after 20 minutes. The obvious reason for the

increased adhesion with time was the greater opportunity for gel mediated adhesive bonds to develop between the probe and the glass surface. The volume of water used for hydration was however critical and a large hydration volume gave a dilute gel which was insensitive to contact time. In other words, there was an optimum level of hydration for the contact time.

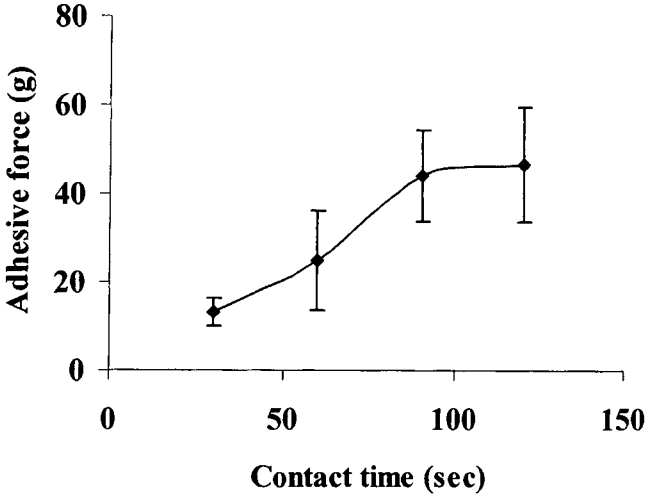

Figure 3.10 Effect of contact time on mean adhesive force (n=10) of lyophilised plug of K4MP (prepared from 1.5% w/w solution, wt of plug 5.25mg) at 10 g test force and hydrated with 30 µl water as measured by TA.XT2 texture analyser (V 5.19R).

3.3.5 Surface hydration and adhesive force of lyophilised plug

The mean (n=20) adhesive force between the glass slide surface and the probe mediated by the hydration of lyophilised plugs using a 5 μl hydration volume is shown in Fig. 3.11. The lowest available test force, 2g, was used because higher test forces tended to over-compress the plug. Care was taken to ensure uniform surface hydration of the whole diameter of the plug. The mean adhesive strength of different K4MP lyophilised plugs was not significantly different. There was a trend of decreasing adhesivity with polymer concentration, so that apparently as the K4MP concentration increased, the extent of plug hydration with a fixed volume (5 μl) decreased leading to decreased adhesivity. The volume of water used for hydration was critical and although the very porous nature of lyophilised system permits rapid entry of water, for heavier plugs, some of the plug may be unhydrated. The most important information obtained from this experiment is that as far as surface hydration is concerned the adhesive force of lyophilised K4MP plugs was not significantly different irrespective of K4MP content.

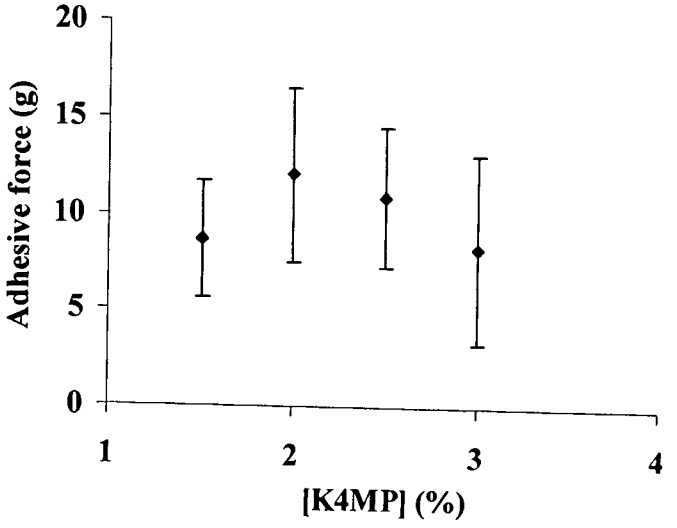

Figure 3.11: Plot showing mean (n=20) adhesive force measurement of partially hydrated (surface hydration) lyophilised plugs prepared from different concentration of K4MP solution.

3.3.6 Adhesive force with a wet mucosal surface

The mean adhesive force of three formulations hydrated on a wet mucosal surface, is shown in Fig. 3.12. It was observed that mean adhesive force was not significantly different among these formulations. However as described above for adhesivity on a glass surface, the extent of hydration of the plug is critical. Because hydration plays an important role on bioadhesion. The optimum level of water or hydration liquid leads to gel formation and facilitates the mobility of the polymeric chains, thus these can diffuse and form bioadhesive bond much more easily (Smart, Kellaway & Worthington, 1984). However an excessive degree of hydration may modify the

mechanical resistance of the hydrogel (Blanco-Fuente *et al.*, 1996). At a particular polymer content (plug weight), if the mucosal surface was very wet, "flooded", the formulation may not have its intended adhesive effect. This argument is supported by the results of a previous study in which decreased bioadhesive strength was reported to be due to over-hydration of the interface between the two adherant phases (Woolfson *et al.*, 1995a). This again emphasises the need for an optimum level of hydration for an adhesive effect. The hydration condition of the mucosal surface was kept as constant as far as possible. Alternatively, if the lyophilised plug on contact with mucosa, was under-wetted, the differences among formulations would be minimised.

Among several parameters, adhesive force also depends on the area of the contact surface. A high contact area will give a high adhesive strength and vice versa. Adhesive force also depends on molecular weight and contact time (Smart *et al.*, 1984).

A product designed for topical application to a biological substrate, e.g. mucous membranes or skin, should preferably possess adhesive properties, as these will enhance the residence time at the site of application and hence improve clinical efficacy (Woolfson *et al.*, 1992). Although, the polymer examined in this study has been described as bioadhesive (Smart *et al.*, 1984), it is less bioadhesive than Carbopol, hydroxyethylcellulose and sodium carboxymethylcellulose (Smart *et al.*, 1984).

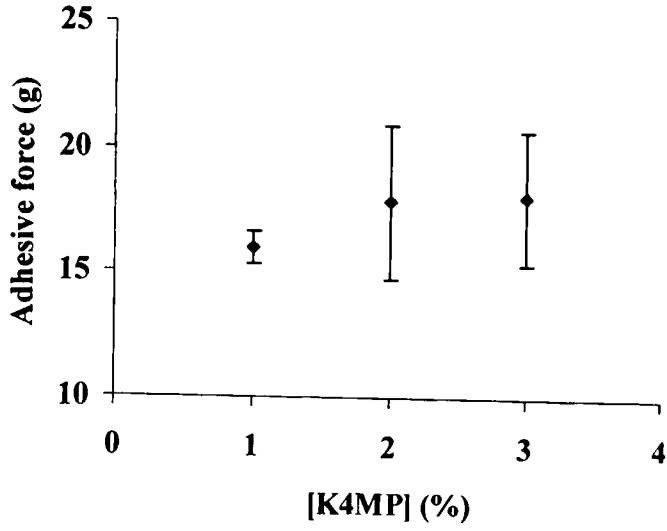

Figure 3.12 Plot showing the comparison of mean (n=5) adhesive force (g) of lyophilised plug of three formulations prepared from 1.5%, 2% and 3% K4MP on wet mucosal surface with contact time of 1 minute. The diameter of the unhydrated lyophilised plug was roughly 5mm.

3.3.7 Water uptake studies (hydration characteristics of lyophilisate)

The volume of water required to hydrate a plug (plug collapse) was dependent on the lyophilisate weight (5-40 mg) and lay in the range 50 - 165 µl (Fig 3.13). The hydration (collapse) times for these lyophilisates in 5 ml of 1.5% mucin solution was less than 15 seconds. However the hydration time of lyophilisates in different concentrations of Carbopol 934P was slower (Table 6), an effect that was more pronounced at higher Carbopol concentrations. This means that Carbopol solution

do not have sufficient free water available for instant lyophilisate hydration. Only on prolonged contact with Carbopol solution the lyophilisates did hydrate.

The characteristic of how fast a lyophilised plug hydrates and collapses when it comes into contact with water or nasal secretion, and how much water is required for such collapse (just to make it completely hydrated) are important product attributes. Unless the plug collapses on contact with nasal secretion in a reasonably (short) time period, it would not serve its intended purpose and may be unacceptable to the patient.

Since the water uptake was so rapid it was not possible to measure hydration kinetics. However it would be interesting to measure such phenomena.

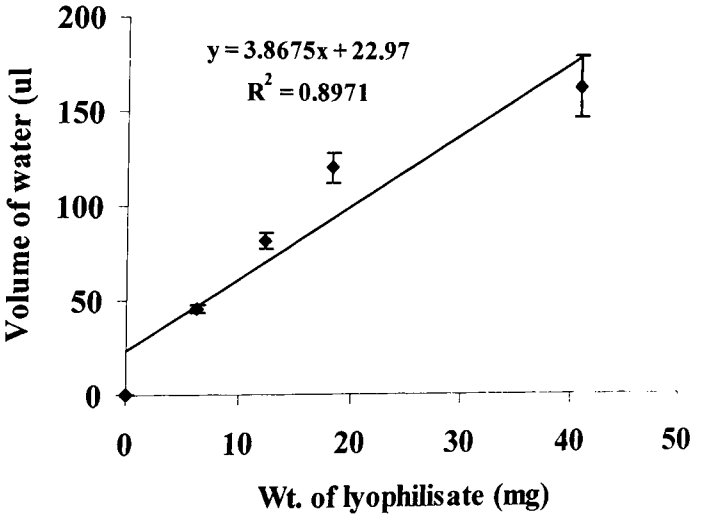

Figure 3.13 Relationship between lyophilisate weight and the mean (n = 5) volume of water required (μl) for complete hydration.

Table 3.6 Summary of hydration time of lyophilisate prepared from 3% w/w K100MP solution measured in different concentration of Carbopol 934P solutions.

Carbopol concentration (% w/w)	Time taken for complete hydration (minutes)
1.5	16
3	66
4	68
6	88
10	88

3.3.8 Morphological studies of K4MP lyophilisates and powder blends

SEM of lyophilisate prepared from 1 and 2% K4MP solution containing NHT and mannitol are shown in Fig. 3.14 (a and b). Similarly SEM of lyophilisates prepared from 1, 2 and 3% K4MP solution without NHT and mannitol are shown in Fig. 3.15 (a, b and c) respectively. As is evident from the microgrpahs it can be observed that lyophilised plugs have very porous net like (matrix) structure which had been previously occupied by ice. Moreover there was a trend of decreasing porosity with nominal solid content (Fig. 3.14a-b; 3.15a-c).

Fig. 3.16 and 3.17 are the SEM of powder blends of K4MP, NHT and mannitol at high and low magnifications which show how small particles are adhered onto the large K4MP particle and they do not have a net like structure as observed in lyophilisates. Since the powder blends are simple physical mixtures of the individual components in which NHT crystals can be seen, such systems do not form matrix.

Ice crystal size determines the size of the pores or channels created during ice sublimation which determines the surface area of the porous solid produced by the sublimation process. In other words, pore size vary with different rates of freezing. Rapid freezing produces smaller ice crystals and when freeze-dried yield large surface area (due to higher degree of porosity) leading to faster hydration at the time of reconstitution (Dawson & Hockley, 1991). The slow freezing yields large ice crystal hence creates large pores and it promotes the migration of water soluble constituents to the surface of the plug. This impeds removel of water during ice sublimation and destabilise the preparation after the lyophilisation due to the non-uniform pH or salt concentration throughout the lyophilisate. This may lead to product instability. Migration of the various constituents of a preparation is prevented by rapid freezing.

In the course of lyophilization it was observed that there was a change in the physical appearance of Methocel lyophilisates which depended on the method of prefreezing. The final appearance of the lyophilisate was glassy (rather than 'normal' white powdery) when the sample was prefrozen in the deep freeze, rather than subjected directly to liquid nitrogen freezing. The glassiness of the lyophilisate seemed to depend upon the duration of prefreezing. Three sets of samples were prefrozen in the deep freeze for 12, 24, 48 hours and lyophilised for 24 hours. The extent of glassiness was intense for the lyophilisate, which was subject to longer time (48 hours). Moreover these 'glassy' lyophilisates had laminated structure as shown in Fig. 3.18 (a and b).

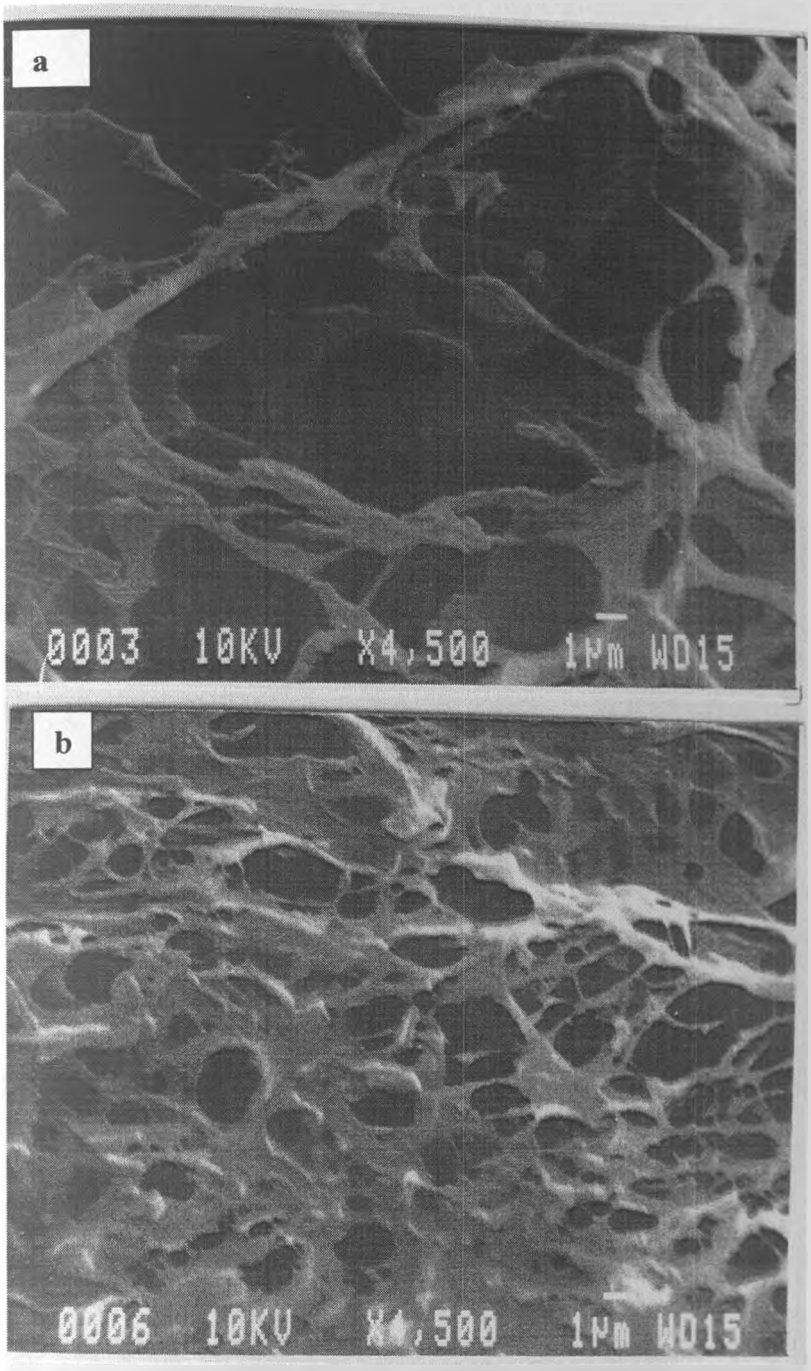

Figure 3.14 SEM of lyophilised plugs prepared from (a) 1% and (b) 2% K4MP solution containing NHT and mannitol.

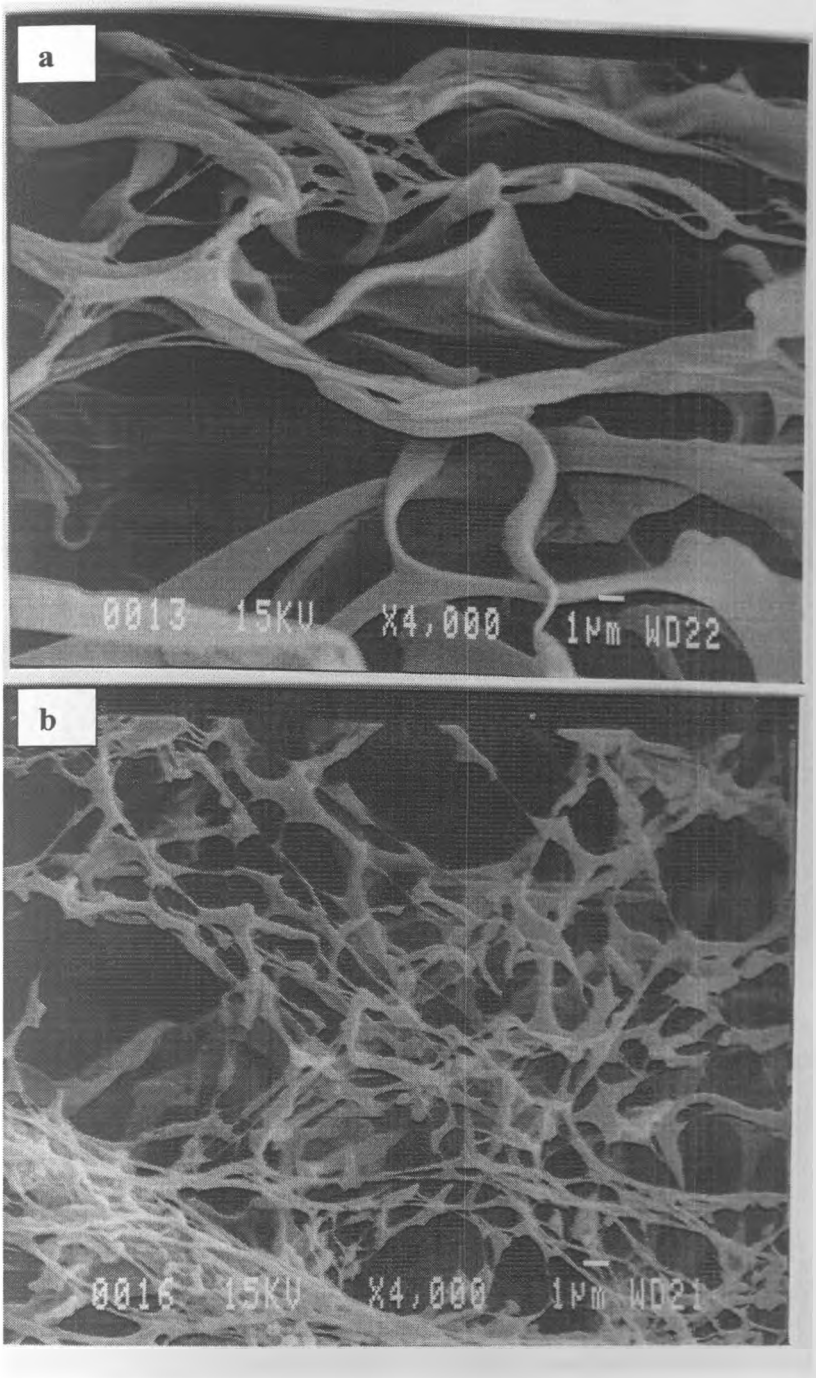

Figure 3.15 SEM of lyophilised plugs prepared from (a) 1% and (b) 2% K4MP solution without NHT and mannitol.

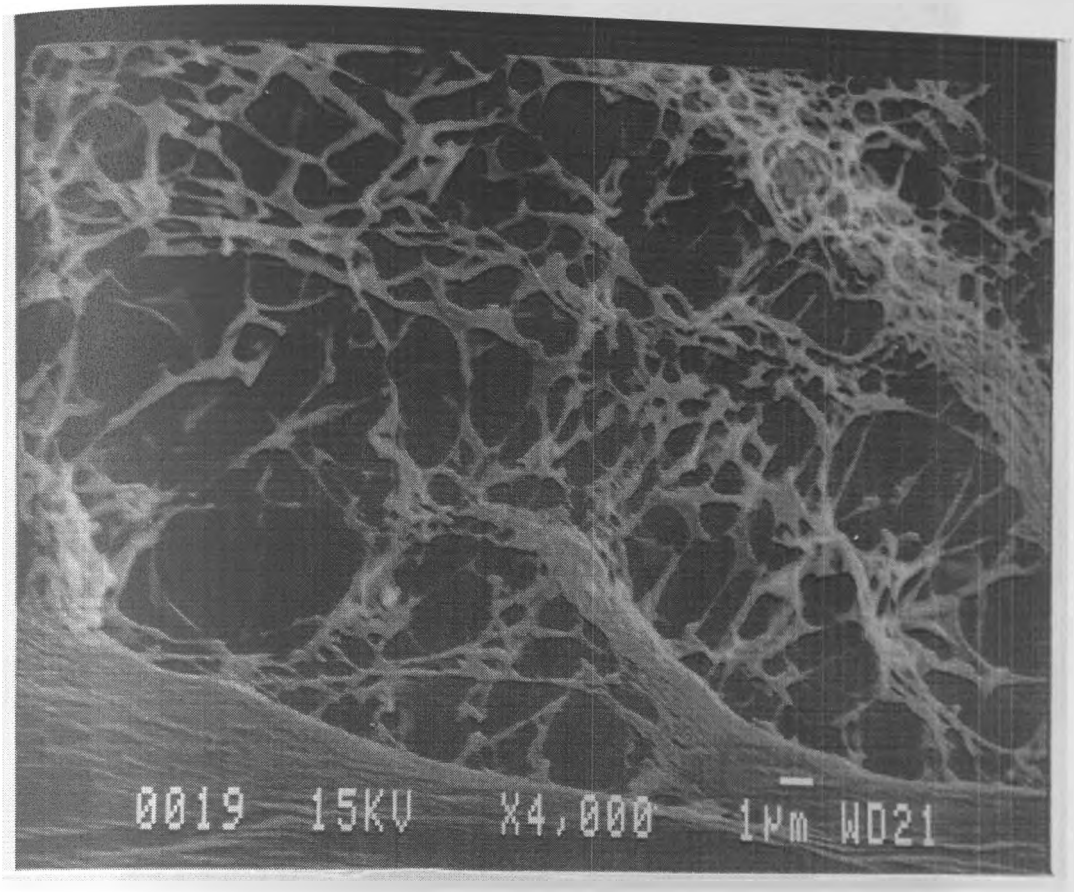

Figure 3.15C SEM of lyophilised plug prepared from 3% K4MP solution without NHT and mannitol.

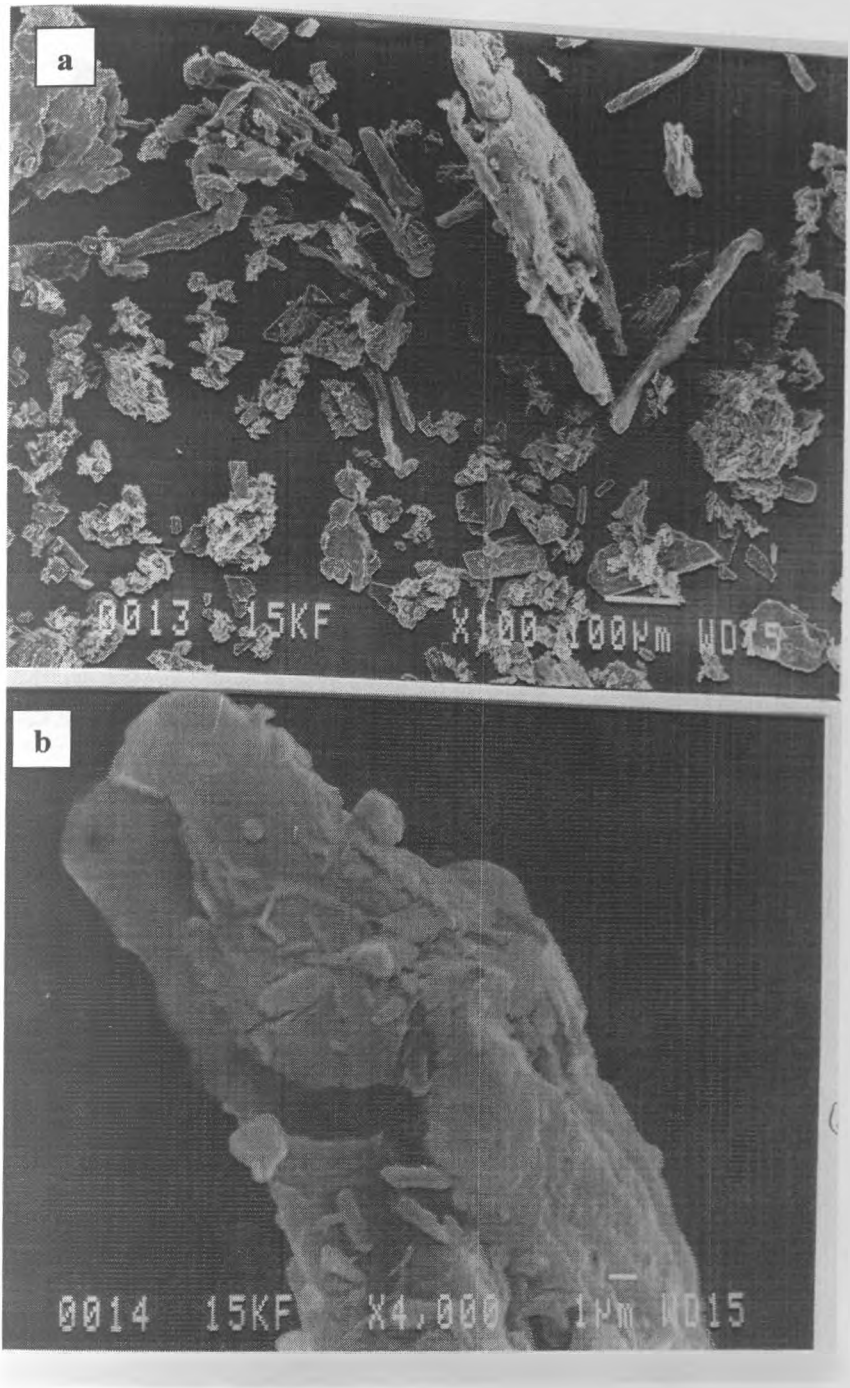

Figure 3.16 SEM of powder blend of K4MP (6.5 mg) NHT (11.4 mg) and mannitol (6.5 mg) at (a) x100 magnification and (b) x4000 magnification.

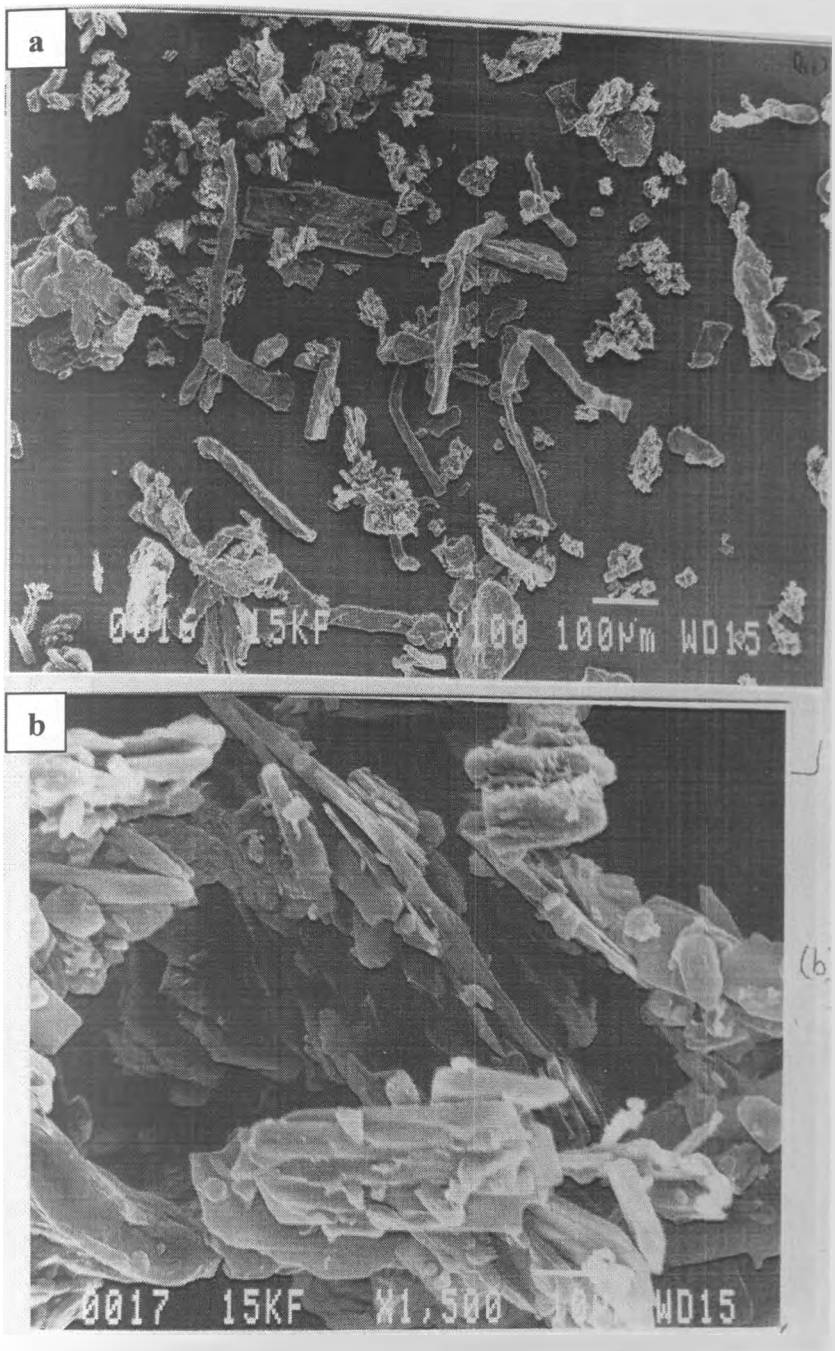

Figure 3.17 SEM of powder blend of K4MP (13.5 mg) NHT (11.4 mg) and mannitol (6.5 mg) at (a) x100 magnification and (b) x1500 magnification.

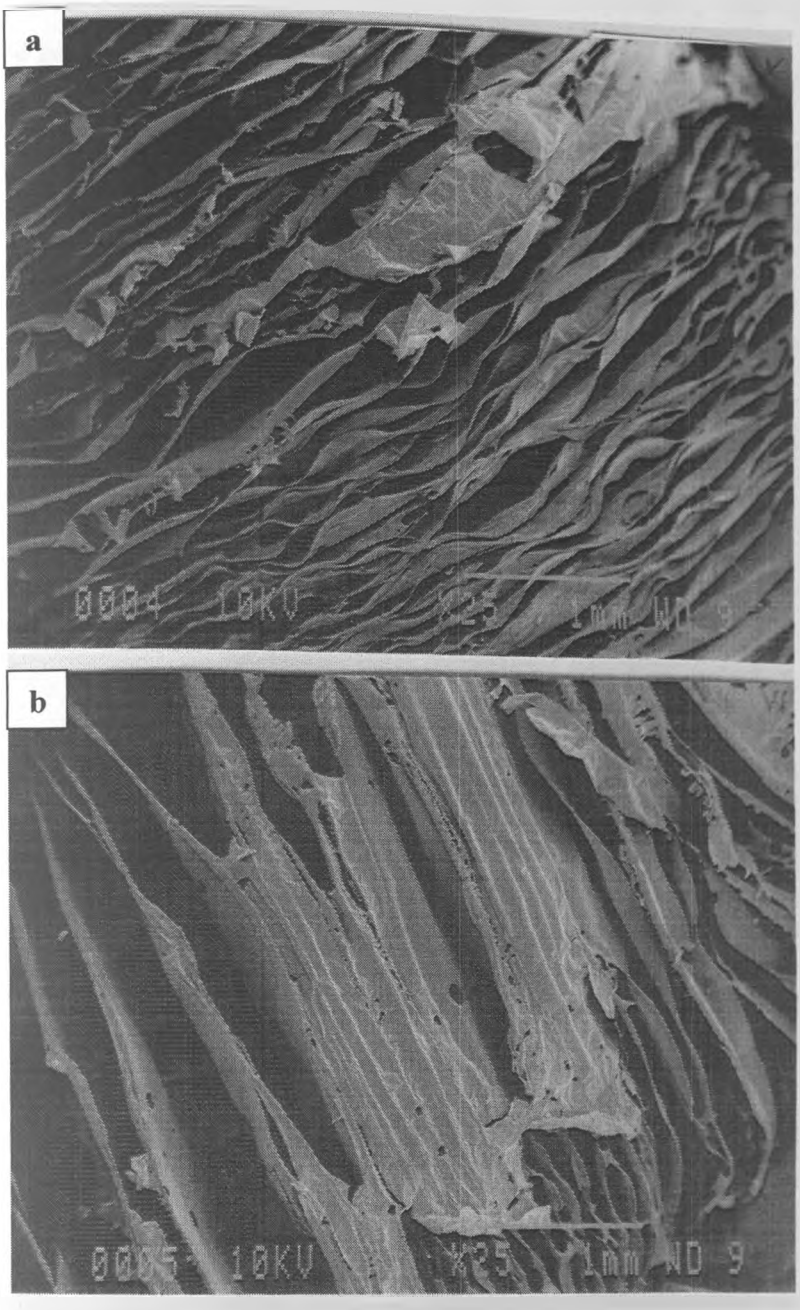

Figure 3.18 SEM of lyophilised 'glassy' plugs prepared from (a) 1.5% and (b) 2% K4MP solution at x25 magnification.

The *in vitro* and *ex-vivo* adhesive force data suggest that the formulation (dosage form) has got some adhesive property on glass and mucosa. The mechanical properties of lyophilised plug can be manipulated over a significant range, which was dependent upon individual component present in the formulation. Measurement of gel strength of hydrated plugs could also be manipulated in this novel dosage form. Hydrated plug viscosity might be used to manipulate drug release rate.

Moreover, hydration time and volume of water needed to make lyophilisate collapse are reasonable for nasal application where there is a low hydration environment.

Chapter 4 *In Vivo* Studies

4.0 Introduction

Animal models are used in drug delivery research as a testing ground for both experimental and clinical hypotheses since the whole animal is required to probe the important aspect of absorption, distribution, metabolism and excretion (ADME) and hence the performance of the delivery system. There is a continuing need for the systematic use of animal models in such studies due to many experimental and methodological limitations in the use of the human species (Gizurarson, 1990). For certain classes of drugs such as peptide and protein hormones the intranasal route offers significant advantages. The choice of a nasal absorption animal model should consider whether the aim of the proposed study is merely the screening of drugs and excipients such as potential absorption enhancers (i.e. obtaining comparative results in the same aminal models) or whether extrapolation of the data to man (e.g. in terms of extent of absorption) is required (Illum, 1996). The selection of the correct animal model, which includes species choice, is of great importance.

As described in Chapter 1, the outcome of nasal drug delivery is influenced by factors such as the anatomical and physiological condition of nose, the dosage form

and the techniques and devices used for administration. Species specific anatomical differences are summarised in Table 4.1.

Table 4.1 Comparative nasal cavity characteristics of man and various animal species (Table reproduced from Illum 1996)

Species	Weight (kg)	Surface area (cm^2)	Surface area /kg body weight	Volume (cm^3)	Length (cm)	Turbinate complexity	Olfactory region (cm^2)
Man	70	181.0	2.5	19.0	8.0	Simple scroll	20.0
Monkey	7	61.6	7.7	8.0	5.3	Simple scroll	8.0
Dog	10	220.7	22.0	20.0	10.0	Very complex membraneous scroll	169.5
Sheep	40	327	8.2	114.0	18.0	Double scroll	-
Rabbit	3	61.0	20.3	6.0	5.2	Membraneous complex scroll	9.3
Guinea Pig	0.6	27.0	45.0	0.9	3.4	Complex scroll	-
Rat	0.25	10.4	41.6	0.4	2.3	Complex scroll	20.0

In the case of simple liquid formulations, the volume of drug administered into the nasal cavity of individual animal model should not exceed those summarised in Table 4.2. These data are only theoretical, and the underlying calculations are based on the assumption that the maximal volume that can be administered into each nostril (humans) without loss due to drips and /or swallowing is 150 µl and also assumes

that the volume of solution administered is distributed uniformly in all of the species (Gizurarson, 1990).

Table 4.2 Volume of drug administered to obtain the same volume per surface area as in man (Table reproduced from Gizurarson 1990).

	Volume administered per nostril (μl)
Man	150
Dog	207
Guinea pig	25
Mouse	3
Monkey	58
Rabbit	58
Rat	13
Sheep	307

The animal species that have been used in intranasal drug delivery studies including intranasal vaccination are shown in Table 4.3. More detail of the sheep and dog model which were used in the present study is provided below.

The sheep model

The sheep has been used to study nasal absorption of protein and peptides such as insulin (Farraj *et al.*, 1990; Lee *et al.*, 1991; Longenecker *et al.*, 1987), human growth hormone (Baldwin *et al.*, 1990; Illum *et al.*, 1990) and desmopressin

(Critchley *et al.*, 1994) and the nasal absorption of gentamicin (Illum *et al.*, 1988) and 17-beta-oestradiol (Kublik *et al.*, 1996).

The size of the sheep with the large space required for housing and the need for grazing facilities, has however limited its use in nasal absorption studies. However, sheep are cheaper than dogs and are docile animals well suited to nasal absorption studies with easy blood sampling from the jugular vein and they can be used repeatedly with a washout period between experiments. The strains most commonly used in such studies are crossbred Suffolk & Texel and Dorset sheep with body weights in the range 40-60 kg. It has been reported that results obtained in the sheep model closely mirror those obtained in man (Illum, Farraj & Davis, 1994) and the sheep offers a reliable model for nasal absorption of all types of drug formulation.

The surface area and volume of the ovine nasal cavity are 327 cm^2 and 114 cm^3 respectively. The surface area per kg body weight is 8.2 for a 40 kg sheep compared to 2.5 for man and 22.0 for the dog (Table 4.1). The two halves of the slightly pyramidal shaped nasal cavity are incompletely separated by the nasal septum. The morphology of ovine and human nasal mucosae are similar.

Depending on the nature of the pharmaceutical formulation under test, dosing can be performed through a cannula inserted into the nostril through which the dose is blown into the nasal cavity. To avoid sneezing during administration, the sheep can be sedated, for instance with a low dose of ketamine hydrochloride (Illum, 1996) which lasts for less than 3 minutes and has little effect on nasal clearance. Blood

samples are obtained from the jugular vein via an in-dwelling cannula which can be left in place for prolonged periods provided it is kept patent by flushing with heparinised saline.

Various other animal models can be used for the study of nasal absorption. Small animals, such as guinea pigs, hamsters, mice and rats are easy to handle and inexpensive but unfortunately, are not suited for studies of powder formulations. However, they are useful for initial absorption studies and the effects on absorption of formulation strategies such as absorption enhancing agents and enzyme inhibitors. With blood volumes sufficiently large to permit frequent sampling, the dog, monkey, sheep and rabbit are more useful in pharmacokinetic and formulation studies.

The dog model

The beagle dog is the most commonly used strain of dogs for nasal absorption studies because beagle dogs are very docile in nature as compared to other strains, easy to handle and have large and open nostril which are well suited for administration of nasal formulations. They can be trained to receive drugs intranasally without being anaesthetised or sedated, depending upon the characteristics of the drug and the purpose of the study. If the nasal formulation is expected to irritate, then sedation or anaesthesia should be recommended. The dog is well suited for clinical studies. They frequently are maintained in environments similar or identical to man. Dog has been used to study nasal absorption of a wide variety of drugs such as propranolol (Hussain, Hirai & Bawarshi, 1980; Vyas *et al.*,

1993), dopamine (Ikeda *et al.*, 1992), insulin (Nagai *et al.*, 1984), human fibroblast interferon (Igawa *et al.*, 1990) and calcitonin (Manzoni, Monti & Valente, 1989).

The length and volume of dog nasal cavity is similar to man but has a complex system of turbinates resulting in a relatively large surface area compared to the body weight (22 cm^2/kg) (Table 4.1). The mucous membrane has a watery surface caused by goblet cells and the lateral serous glands and is highly vascularised (Gizurarson, 1990). The nasal mucosa has two functionally separate passageways, a high flow and high pressure system draining the anterior part of the nasal cavity via the dorsal nasal vein and a low flow and low pressure system draining the posterior part of the nasal cavity via the sphenopalatine vein. The blood from both veins drains ultimately into the jugular veins of the dog (Illum, 1996).

Nasal formulations such as solution can be administered with a nasal nebuliser spray or with a pipette inserted about 1 cm inside the nostril. Similarly powders can be administered using a catheter inserted 1-2 cm inside the nostril (Illum, 1996). Blood samples can be collected from the foreleg vein or the hind leg vein.

This chapter describes the study of the intranasal absorption of nicotine from a novel lyophilised plug, a solution spray and a powder formulation in comparison to I.V. administration in sheep. This chapter also describes the study of nasal absorption of UK-92, 480 from two formulations administered as lyophilised plug and a nasal spray.

Table 4.3 Animals most commonly involved in intranasal delivery studies (Table reproduced from Gizurarson 1990).

Animals/Strain:
Dogs
Beagles
Guinea pigs
Dunkin-Hartley
Hamsters
Sprague-Dawley Syrian
Mice
C57BL
Balb/c
Outbred Swiss CDI (Charles river)
SPF (ICR)
White (Connaught Med. Lab.)
Monkeys
Rhesus monkey
Rabbits
New Zealand White
Japanese white
Rats
Wistar
Sprangue-Dawley
Fischer-344
Lewis
SHR
Sheep
Suffolk & Texel
Dorset

Section 4.1 Nasal absorption of nicotine in sheep

4.1.1 Materials and Methods

4.1.1.1 Materials

Nicotine-methyl-d_3 manufactured by Isotech Inc. and potassium dihydrogen phosphate were purchased from Aldrich Chemical Company. Naso-pharyngeal tubes (internal diameter 7.0 mm) from Portex Limited, Kent, England, different pore size (0.45 and 0.2 micron) membrane filters and 0.45 μm 13 mm GD/X nylon syringe disk filters from Whatman, Lithium heparin beaded tubes (monovette) from Sarstedt Ltd. and SCX column (Isolute SCX 100 mg/1ml column reservoir, part no. 530-0010A) from International Sorbent Technology Ltd., Mid Glamorgan, UK were similarly purchased.

4.1.1.2 Preparation of nicotine solution for intravenous administration

57 mg of NHT powder (equivalent to 20 mg of nicotine base) was dissolved in 50 ml of freshly prepared distilled water and the final volume adjusted to 100 ml to give a concentration of 0.2mg nicotine base/ml. 7 ml of this solution was filled into glass vials by sterile filtration through 0.45 and 0.2 μm membrane filters. The aseptically filled vials were sealed and capped and stored in the fridge until use.

4.1.1.3 Drug administration and sampling procedure

As a pilot to a larger study in eight sheep, the four nicotine formulations were studied in two sheep identified as Sheep One and Sheep Two. On the first occasion, Sheep One received a lyophilised nasal insert, containing 4 mg of nicotine base, into one nostril. The lyophilised insert used in this study was prepared from 2 % K4MP solution. Sheep Two received, intravenously, a single dose of 5 ml solution containing 1mg nicotine (1mg nicotine base/5ml). After a seven day wash out period, the second experiment was carried out in which Sheep One received 11.4 mg of NHT powder (equivalent to 4 mg nicotine base) intra-nasally and Sheep Two received 200μL of aqueous nicotine solution in the form of a spray (4 mg nicotine base/200 μL) intra-nasally.

The details of the administration systems used to deliver the formulations into the sheep nostril are shown in Fig. 4.1, 4.2, and 4.3. Each intra-nasal formulation was administered via a naso-pharyngeal tube (internal diameter 7.0 mm) which was introduced into one of the nostril up to the mark shown in the Fig. 4.1 (approximately 7 cm into the nasal cavity) to ensure that the formulation was delivered to the respiratory region of the nasal cavity (Illum 1996). The cylindrical lyophilised insert (Fig. 4.4) was placed inside the naso-pharyngeal tube and gently pushed out by a flexible nylon tube of defined length to deliver the insert into the nasal cavity. To deliver the powder, a 200μL pipette tip was cut 2 cm from the narrow end, then a tiny plug of cotton-wool placed into the narrow end to ensure the powder remained in the tip and NHT powder (11.4 mg) packed into this tip followed by an equivalent

weight of mannitol. This tip was connected to a silicon tube attached to 12 ml plastic syringe and the silicon tube with the tip inserted into the naso-pharyngeal tube up to a pre-defined length such that the powder packed tip was located just at the end of the distal end of the naso-pharyngeal tube (Fig. 4.2). The powder was blown out by rapidly depressing the plunger of the syringe. For the nicotine solution, a Gilson pipette was used to draw 200μL into a naso-gastric tube which at the proximal end was attached by the Leur connector to a 12mL plastic syringe. The naso-gastric tube was placed inside the naso-pharyngeal tube up to a pre-defined length and the solution sprayed into the nostril by rapidly depressing the syringe plunger (Fig. 4.3). After delivery of the formulations, the naso-pharyngeal tube was removed.

Following drug administration, blood samples were taken during the first 12 hours via an in-dwelling a jugular catheter and thereafter samples were taken by jugular venepuncture. Following intravenous dosing blood samples (6 ml) were collected into lithium heparin beaded tubes (Monovette: Sarstedt Ltd) using a 1 inch 20 gauge needle at 0, 0.083, 0.166, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 36, 48 hours and following intra-nasal dosing at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 36, 48 hours. Blood samples were centrifuged at 1850 x g for 20 min at $4^{O}C$ and decanted into 5mL plastic storage tubes and were stored at $-20^{O}C$ until analysis.

The catheter was flushed through with heparinised saline each time a blood sample was drawn.

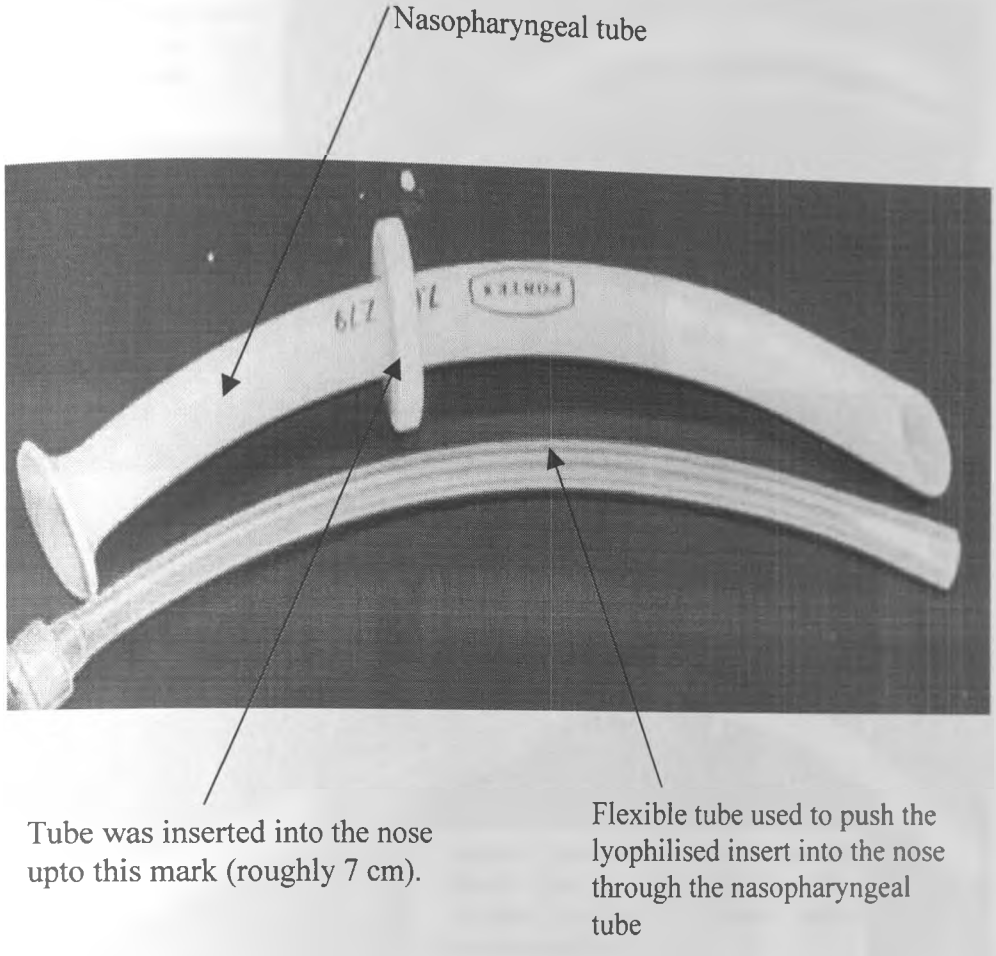

Figure 4.1 Nasopharyngeal tube used for inserting lyophilised insert into the sheep nose.

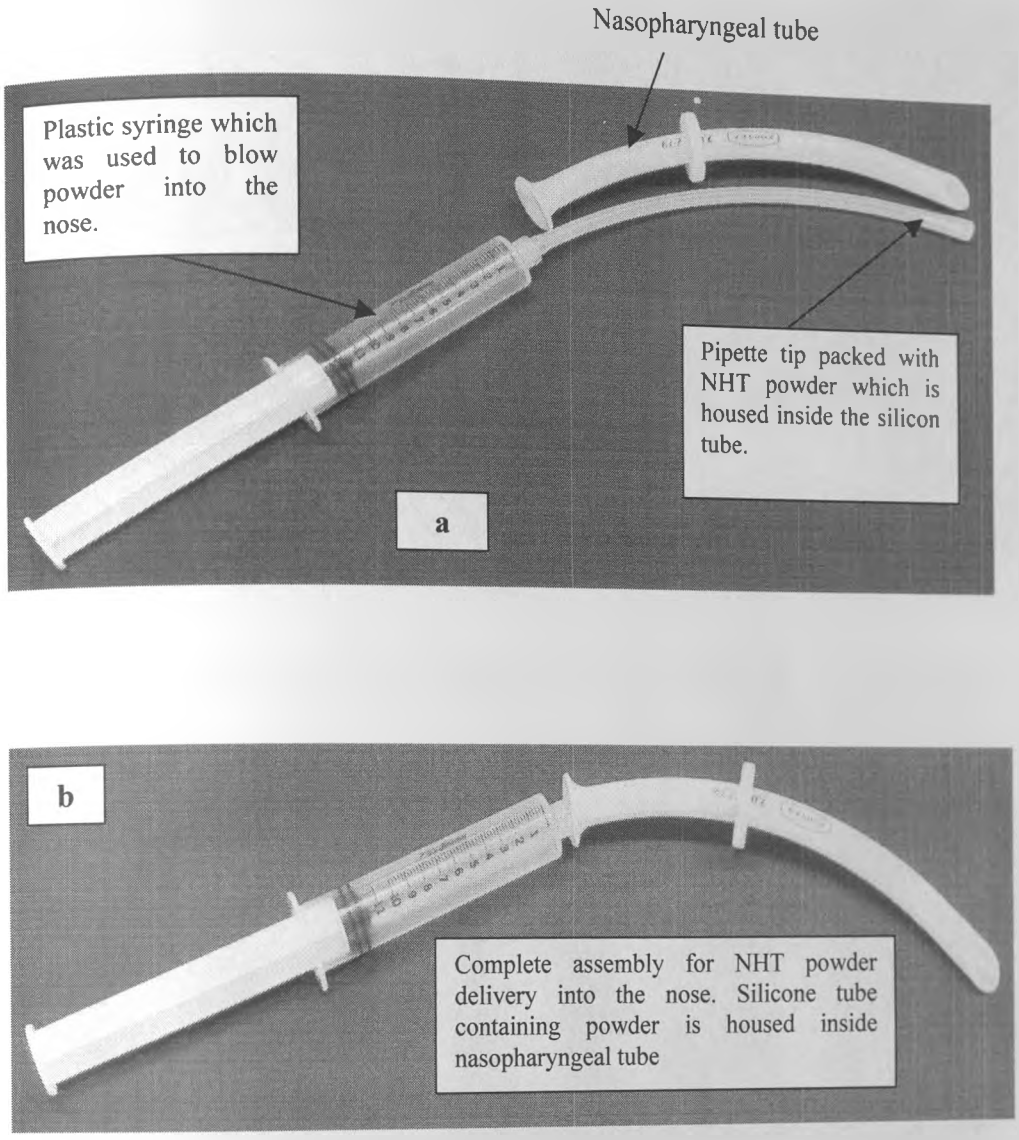

Figure 4.2 Arrangement for the administration of NHT powder into the sheep nose.

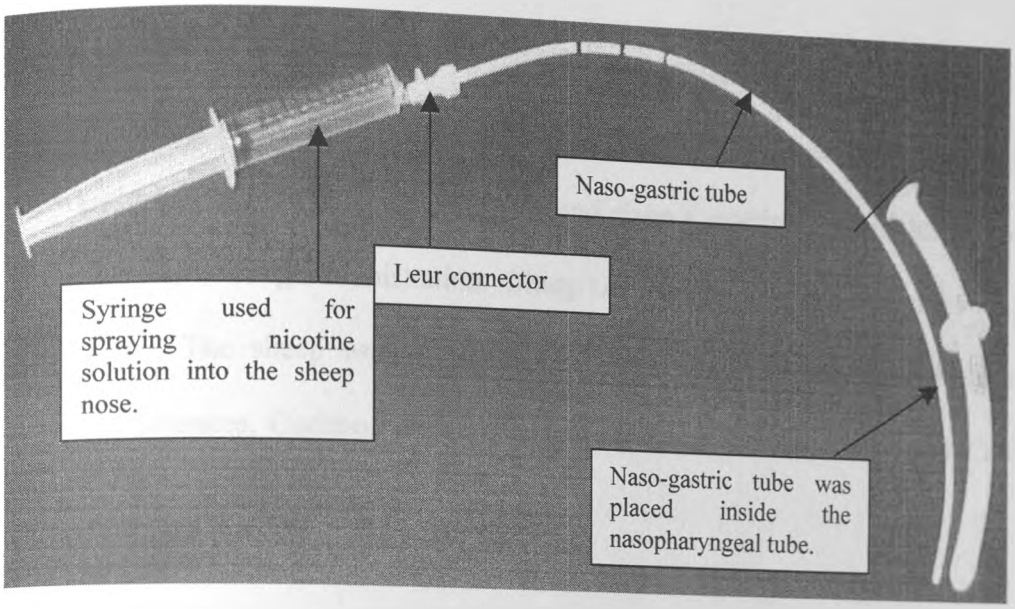

Figure 4.3 Arrangement for the delivery of nicotine solution into the sheep nose.

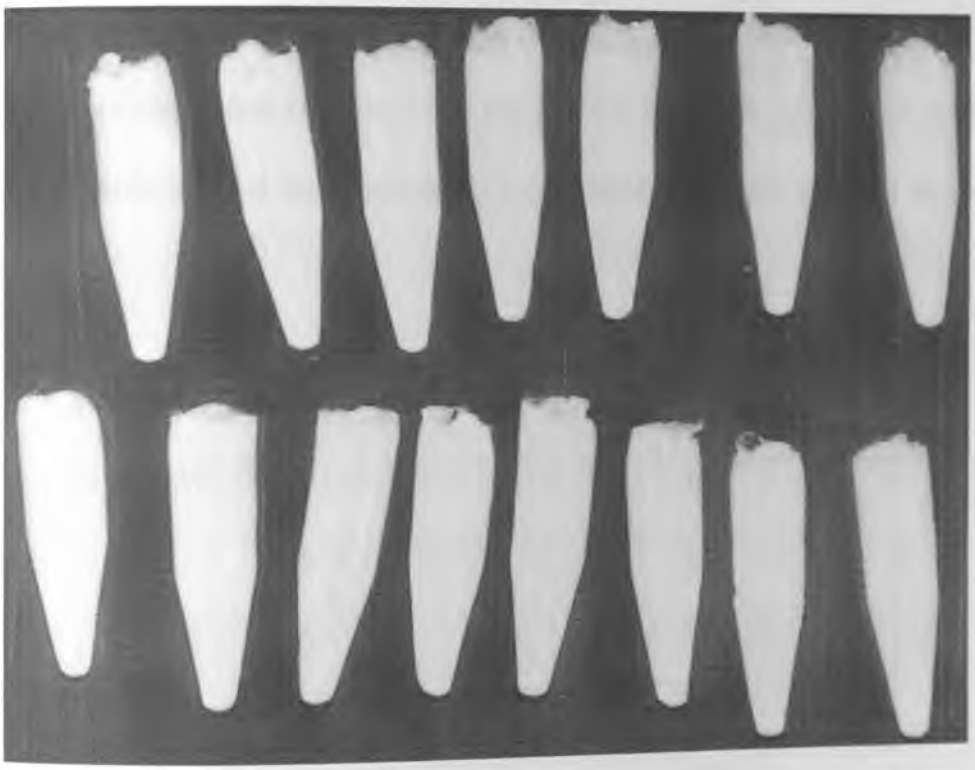

Figure 4.4 Lyophilised plugs containing nicotine.

4.1.1.4 Animals and husbandry

Grey Face sheep were purchased from The Moredun Research Institute. The sheep were weighed (in a Premier livestock scale) and given a veterinary health inspection prior to the day of drug administration. Sheep One and Two weighed 51 and 66 Kg respectively. The sheep were accommodated at Glasgow University School of Veterinary Sciences, Cochno Farm and Research Centre, Hardgate, Clydebank and had free access to hay and water.

4.1.1.5 Analysis of nicotine in plasma by GC-MS

Solid phase extraction of nicotine from sheep plasma

To prepare calibration samples, 1 ml each of ovine plasma was spiked with standard nicotine solution and nicotine-methyl-d_3 (as internal standard) solutions as shown below:

Nicotine (ng/ml)	Nicotine-methyl-d_3 (ng/ml)
0	100
10	100
20	100
40	100
80	100

The spiked plasma was extracted by mixing 1 ml spiked plasma with 1 ml 0.2M phosphate buffer pH 2.5 and filtering through 0.45 μm Whatman 13 mm GD/X nylon syringe disk filters. An SCX column (Isolute^R SCX 100 mg/1ml column reservoir) was washed with 1 ml methanol, 1ml water and then 1ml 0.2M phosphate buffer pH 2.5 before loading with the filtered plasma sample. The column was washed with 1 ml water, then 2 ml methanol and then eluted with 1 ml of 5% v/v ammonia solution in methanol. The methanol/ammonia eluate was blown under a stream of nitrogen to almost dryness and the remaining solution transferred into sample vials for GC-MS.

The nicotine from plasma samples obtained after administration of the different dosage forms was extracted as above but using 0.5 ml of plasma along with the addition of 100 ng/ml nicotine-methyl-d_3 as internal standard in each sample.

GC-MS conditions for nicotine analysis

A Trace 2000 Gas Chromatograph (GC) was fitted with ZB50 column (15m X 0.5 mm I.D. X 0.25 μm film thickness) and programmed as follows: 80°C (0.5 min. isothermal), increased at the rate of 10°C/min to 170°C and then further increased at 30°C/min to 215°C with the carrier gas (helium) at constant flow rate of 1.2ml/min. Automass was in the electron-impact (EI) mode at 70 eV. Ions at *m/z* 84 for nicotine and *m/z* 87 for d_3-nicotine were monitored. The retention time was 5.51 min.

The concentration of nicotine in plasma sample was calculated by using following calibration equation (Fig. 4.5).

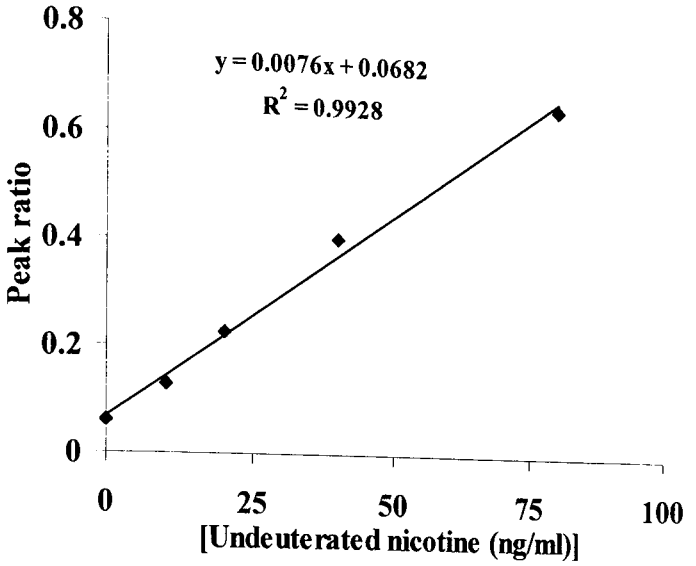

Figure 4.5 Calibration curve for the estimation of nicotine in sheep plasma by GC-MS.

4.1.2 Result and Discussion

The plasma concentrations of nicotine following intranasal administration of various dosage forms and intravenous injection in sheep are illustrated in Fig. 4.6 and 4.7 respectively. The observed maximum concentration (C_{max}) and the time of maximum plasma concentration (T_{max}), together with the area under the plasma concentration curve (AUC) (calculated by trapezoidal rule) and bioavialability (relative to the lyophilised plug) are shown in Table 4.4.

After the intravenous bolus dose of 1 mg, a fast decline of plasma nicotine level was observed (Fig. 4.7). The observed plasma levels (normalised) after intravenous administration were low, C_{max}= 22 ng/ml, T_{max} = 5 min. One explanation would be that the nicotine had distributed rapidly and extensively to body tissues owing to its lipid solubility. A previous study on intravenous nicotine (single i.v. dose of 2μg/kg/min for 10 minutes) in healthy men supports this argument (Rosengerg *et al.*, 1980). It was found that nicotine had a volume of distribution 1.7 times body weight, suggesting that it distributed extensively in extra-vascular spaces. It was found that nicotine also underwent moderately rapid metabolism (nonrenal clearance of approximately 800 ml/min) and variable renal elimination (2% to 23% of administered dose) depending on urinary pH.

Using a volume of distribution of 1.7 times bodyweight and a normalised dose of 4 mg in a 66 kg sheep, the calculated C_{max} is equal to 35.65 ng/ml. This calculated value, which does not take any metabolic and renal excretion events into consideration, is in good agreement with the experimental value (22 ng/ml) obtained. However this explanation is based on a single experiment and inter-subject variability has not been taken into consideration. Therefore the full eight subject 4 way cross-over study would clarify this explanation.

For the intranasally administered nicotine formulations, the time to reach maximum plasma concentration was 60 minutes for the lyophilised plug and 15 minutes for the powder and the solution. The maximum plasma concentrations observed were 129.9,

73.3 and 13.3 ng/ml for lyophilised plug, powder and solution respectively (Table 4.4).

The AUC of lyophilised plug, powder, solution and intravenous injection were 157.3, 30.08, 5.0 and 5.44 ng.h./ml respectively (Table 4.4). A plausible explanation of the higher nicotine bioavailability (AUC) of lyophilised plug is that it forms a viscous gel when in contact with the moist nasal mucosa and the gel has a long residence time that allows more time for nicotine absorption from it to occur. This could be expected on the basis of the *in vitro* nicotine release from lyophilised plugs which was depended upon polymer concentration (chapter 2). The relatively slow rise in plasma concentration during first 45 minutes after nasal administration of the lyophilised plug (Fig. 4.6) could be an indication of effect of viscosity of gel formed after hydration of lyophilised plug in the nasal cavity. Another reason could be due to the osmotic effect of the lyophilised plug during hydration. The hydrated plug in contact with the mucosa could draw moisture from mucosal surface because of the concentration of the non-diffusible polymer in the resultant gel. The transient dehydration of the mucosa (shrinkage) could open tight junctions thereby enhancing mucosal permeability. As a small lipophilic molecule nicotine may also be absorbed via transcellular pathways through the mucosal surface.

The higher bioavailability of the powder (AUC, 30 ng.h/ml) compared to the solution (5 ng.h/ml) suggested that all the powder did not go into solution at once because of the low hydration condition of the nasal cavity. Although *in vitro* nicotine release from powder and solutions was similar (Chapter 2) bioavailability from solution was

less than that of the powder because solution was more likely to be swallowed or run out of the nose.

Due to the rich vasculature of the nose, intranasal drug administration generally provides rapid absorption and T_{max} values in the range of 10-30 min have previously been reported (Chien, 1985). T_{max} of 7-15 minutes for nasal nicotine solution have been reported in man (Johansson *et al.*, 1991; Russell *et al.*, 1983) and in the present study, T_{max} for powder and solution formulations was at 15 minutes. The lyophilised plug T_{max}, 60 minute is another indication of effect of viscosity which at least *in vitro* delayed/sustained nicotine release and which may increase the total absorption of nicotine.

Table 4.4 Summary of plasma data following administration of single dose of nicotine in different intranasal dosage forms and intravenous bolus to sheep.

Dosage forms	C_{max} (ng/ml)	T_{max} (minute)	AUC (ng.h/ml)	Relative AUC values[a] (%)
Lyophilised plug	129.9	60	157.3	100
Powder	73.3	15	30.08	19.1
Solution	13.3	15	5.0	3.2
Intravenous*	22.0	5	5.44	3.48

[a]AUC of lyophilised plug taken as 100%.

*normalised i.v.data.

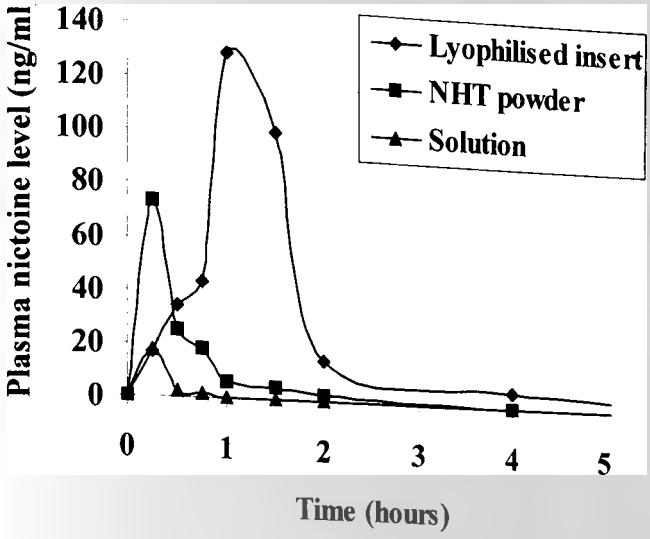

Figure 4.6 Nicotine plasma concentration versus time curves after intranasal administration of nicotine (4 mg nicotine base) in various dosage forms in the sheep. Data represent single experiment.

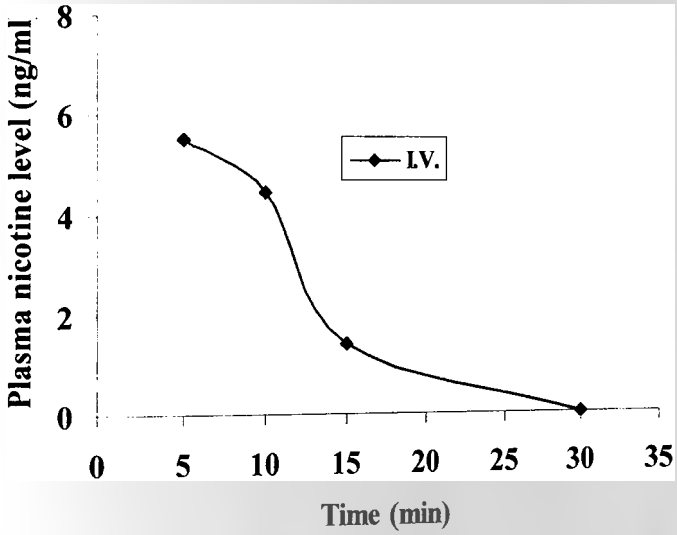

Figure 4. 7 Nicotine plasma concentration versus time curve after a single intravenous bolus of 1 mg nicotine base in 5 ml. Data represent a single experiment.

In terms of AUC (bioavailability) the lyophilised plug looks better than the powder, and the solution. Comparing Sheep One and Sheep Two there seems to be large inter-subject variability although these comments are based on single administration in a pilot study. The full 8 subject 4 way cross over study would clarify whether lyophilised insert has better performance or not in comparison to other dosage forms.

Chapter 5 Lyophilisation of unit dose pharmaceutical dosage forms

5.1 Introduction

Freeze drying, or lyophilisation, is a widely used technique for the stabilisation and preservation of heat labile substances such as biological products and pharmaceuticals. Its use in the preservation of microorganism, food stuffs, biological products and pharmaceuticals is well documented (Carpenter *et al.*, 1997; Couriel, 1980; Goldblith, Rey & Rothmayr, 1975; Greaves, 1962; King, 1971; Pikal, 1990a; Rey & May, 1999; Williams & Polli, 1984). Lyophilisation is the term given to the process whereby ice is sublimed from frozen solutions, generally under reduced pressure, leaving a dry porous mass of approximately the same size and shape as the original frozen mass. A freeze drying cycle essentially consists of three stages; freezing, primary drying, and secondary drying. In the first stage, the material is cooled until it is completely frozen. This stage has the effect, at least in part, separating the water from the solutes. The second stage, which is usually accomplished under vacuum and by supplying heat to the product, involves the sublimation of the ice in the product. The last stage involves the removal of sorbed water and is normally carried out at elevated product temperature to achieve efficient

water removal. Since freeze drying takes place at a lower temperature than spray-drying it is normally considered to be less destructive especially to protein products (Pikal, 1990b). In addition, control of sterility and foreign particulate matter is relatively straightforward in lyophilisation, and accurate, clean dosing into final product containers is simple. Because of the porous nature of freeze-dried products, rehydration is fast and complete. Hence, albeit inherently expensive from the viewpoints of plant cost and energy consumption, lyophilisation is often the processing method of choice for protein products.

Freezing

Freezing is required to achieve the solid state before drying by sublimation. During this period, the solvent (usually water) crystallizes or hardens into a complex mixture of crystals, eutectics and/ or amorphous 'glassy' materials, giving the product a (typical) structure which will be retained in the final dry cake. The freezing stage is of critical importance, since the internal structure of the product, its specific surface area all result directly from it (Rey, 1991). Freezing should be carried out under strictly controlled conditions to achieve optimum cake structures.

Primary Drying

Primary drying involves direct sublimation from the frozen mass and is carried out at low temperature under moderate vacuum. Primary drying must be done from the solid state which implies that the material is maintained below the 'minimum

temperature of incipient melting'. This, in turn, requires that an appropriate balance be established between heat input and mass transfer (Deluca & Lachman, 1965; Nail, 1980) which can be delicate operation in a continuously evolving drying system. Indeed, the sublimation interface (drying front) moves down the frozen mass and is progressively covered by an increasingly thick layer of dry porous material which acts as a filtering plug, hindering vapour flow and behaves, under vacuum, as a thermal insulator. To achieve a good compromise between quality and economy, the operating parameters, heat flux, chamber pressure, position, shape and temperature of the condenser, have to be carefully adjusted.

The end of the sublimation of primary drying is an important control point in the freeze drying process. Traditionally, the end of sublimation drying was determined by product temperature, or rapid rise in product temperature after the temperature sensor loses thermal contact with ice, being taken as evidence of the complete removal of ice from that container. However, product temperature is measured by temperature sensors placed in a relatively small number of vials, and the product in these probed vials may behave differently from the batch as a whole. An alternative method of end point detection has been developed which depends on a property of the whole batch by using an electronic moisture sensor (Pikal & Roy, 1989) and they considered that the partial pressure of water vapour in the drying chamber would better represent the batch. Electronic moisture sensors have the sensitivity and precision to provide an unambiguous criterion for the end of primary drying.

Secondary drying

When all 'crystalline solvent' has been removed, the temperature of the product can be raised to allow the extraction of the remaining 'unfreezable' fluids. This operation, usually referred to as secondary drying, is generally done at relatively high temperatures and is substantially enhanced by high vacuum. Again all the necessary parameters need to be monitored and controlled accurately if mild heat denaturation or even scorching the material is to be avoided.

The primary and secondary drying processes, respectively sublimation and isothermic desorption, can be controlled automatically by using temperature and physical measurements carried out during the process, directly or remotely. The design and operation of freeze dryers must take these factors into account and modern equipment provides means of adjusting such parameters in process and carrying out standardised operations.

Packaging and Storage

The freeze-drying process is stopped when the residual solvent content is consistent with long-term preservation of the product at room temperature. This is not an easy decision since, for some products, over-drying can be as deleterious as under-drying and for this reason selected excipients (cryoprotectants) such as mannitol, trehalose, PVP etc. may be included in the formulation to help 'buffer' the residual water content of the finished product.

When the operation is ended, the freeze-dried material is extracted from the vacuum chamber and stored. Bulk product is not handled in the same way as sterile vials, the latter generally being stoppered under vacuum or dry inert gas inside the chamber.

The product needs to be protected adequately from the outside environment because it has been processed under vacuum and presents a large 'virgin', internal surface, produced under low pressure by direct sublimation and further cleaned by a long desorption, which is highly sensitive to contamination. Any trace contaminant can result in an almost irreversible 'print' of the contamination on this active surface and this is why precautions are taken to avoid contact of the freeze-dried product with oxygen or water vapour which together with light might induce progressive denaturation.

Lyophilised pharmaceutical formulations and dosage forms

The development of a suitable formulation and freeze-dry cycle for a pharmaceutical dosage form requires knowledge of some basic properties such as the eutectic temperature, if one exists; the effect of temperature on drug solubility, the degree of super cooling of the material during freezing, the heat transfer properties of the frozen product, and equipment design and capability. The desired characteristics, that can be achieved, by the proper formulation of the solution to be freeze-dried and by employing an optimum freeze-drying cycle, include an intact cake with sufficient strength, uniform colour and rapid reconstitution.

The last decade has seen the emergence of some interesting ideas such as 'soft ice' and 'supporting' matrix technology in the lyophilisation of pharmaceuticals (Rey, 1991). Some products are not stable if they are allowed to remain in solution for too long at temperature above zero and they may also not be readily soluble and difficult to maintain in stable suspensions for the time needed for freezing. In such situations, the 'soft ice technology' may be useful. A homogeneous solution of the main constituents is progressively frozen in a scraped surface heat exchanger and extruded as a soft ice sherbet into a refrigerated vessel where it is mixed with these powdered ingredients which were not compatible with the original solution. To this flow can be added a second sherbet coming from another line or other streams of low-temperature-stable emulsions, oils and aromatics. The whole mass is mixed as a plastic, cold paste and finally extruded into appropriate molds, of the conventional drug tablet type, which are then brought to low temperatures for final hardening. The frozen product is then lyophilised and when reconstituted, it gives an instantaneous metastable mix which is sufficiently durable for immediate therapeutic use.

Similarly, the 'supporting matrix' concept is particularly useful in the freeze drying of highly dilute solutions of very active biologicals such as human hormones and vaccine antigens. Such solutions are difficult to dry due to a lack of structure and during sublimation the frozen mass disintegrates under vacuum and is, partially lost in the vapour stream. Although such 'blow out' can be overcome by the use of 'bulking' agents, these excipients may have side effects or may interfere with the biological activity of the active compound. An alternative is to adsorb the solution of

active onto a neutral porous material which can be handled, as a granular solid and may easily undergo, freezing, drying and storage. Porous materials such as sintered glass, ceramics, aluminium and zirconium oxides, expanded polymers or porous natural supports (chitin, chitosan, cellulose) can be used for this purpose. The support can be shaped as threads, beads, plates, granules, manufactured by machining moulding or extrusion as a dry, inert matrix carrying the active substance. At the time of reconstitution, the material is washed and extracted with excess suitable solvent, and the active substance released from the support.

Recently, freeze drying technology has been applied in the manufacture of unit, fast dissolving dosage forms (Gole *et al.*, 1997; Seager, 1998), such as rapidly disintegrating tablets (Corveleyn & Remon, 1997), and lyophilised dry emulsion (LDE) tablets (Corveleyn & Remon, 1998b) for the oral delivery of drugs. Freeze dried tablets may be of benefit to patients who find it difficult to swallow conventional tablets or hard gelatin capsules and therefore tend to be non-compliant. This applies to paediatric and geriatric patients, but also to the bedridden and to some active, working, patients who are busy or travelling, and have no ready access to water. Such problems can be mitigated by fast dissolving tablets and the high bioavailability of drugs from freeze-dried tablets is in general significantly greater than from conventional tablets (Corveleyn & Remon, 1998a; Seager, 1998). Lyophilisation has also been used for the manufacture of a vaginal suppository in a unit dose applicator (Brummer *et al.*, 1997).

An attempt has also been made to combine extrusion and freeze drying technologies to produce particles containing active ingredient (Nguyen & Jacquont-Leyder, 1998). Protein inhalation powders, prepared by spray freeze drying, have superior aerosol performance as they are light and porous and have better aerodynamic properties than powders prepared by spray drying only (Maa *et al.*, 1999).

Recently, there has been an increasing interest in the possibility of administering pharmaceutically active peptides via the nasal route e.g. in the form of a nasal ointment or gel, or for greater accuracy of dosing, in the form of a liquid nasal spray. The concept of a solid nasal insert for peptide drug delivery may give improved results, e.g. in term of patient compliance (e.g. facilitated self-application), achievable accuracy of dosing/ bioavailability when compared to a liquid nasal spray (Azria, 1991).

Many active agents are unsuitable for oral delivery as they are broken down by the digestive activity of the gut. If such agents can be absorbed across the buccal and/or nasal mucosae, these tissue sites represent alternative routes of administration. Conventional freeze drying, however, generally results in or solid with very low bulk density and poor flow characteristics which may make it difficult to administer it reproducibly, for instance, to the nasal or buccal mucosae. Thus, it would be desirable to produce a lyophilised formulation which would expedite delivery to such mucosal surfaces.

As described above, in conventional freeze drying of a pharmaceutical, a glass vial is partially filled with a solution of the substance to be lyophilised and placed in the freeze dryer. The partially stoppered vial remains open throughout the drying process to allow water vapour to escape from the frozen solution and on completion of lyophilisation cycle a 'plug' of dry material is left in the vial. In certain pharmaceutical applications, for example freeze dried pharmaceutical tablets for oral administration it may be desirable to produce the freeze-dried product in a packaged form, ready for administration. WO-A-9412142 (Kearney, Thompson & Yarwood, 1994) discloses a freeze-drying manufacture method in which the solution precursor of the dosage form is filled into and dried in the depressions of a blister film (Zydis technology RP Scherer). Following freeze drying, a plastic sheet is placed over the depressions and the blisters sealed. However, a freeze dried plug is usually fragile and it tends to disintegrate depending on the solid content of the plug. Thus the freeze-dried residue in the type of open blister pack described above is easily lost by static, air currents or by handling operations prior to film sealing.

In order to mitigate at least one of these problems, an attempt has been made to develop a lyophilisation process for a pharmaceutical unit dosage form which comprises a container closed with an impermeable membrane pierced with one or more holes through which the material in the container can be lyophilised. The hole or holes in the membrane have to be sufficiently large to allow water vapour to escape but small to ensure that the material is kept within the container.

5.2 Materials and Methods

5.2.1 Materials

D (-) mannitol (GPR) was purchased from BDH. Methocels of different viscosity grades were obtained as a gift from Dow Chemical Company, USA. Different pore size membrane filters used were purchased from Whatman. The dialysis membrane was from Medicell Ltd and other polymers were purchased from the source described in Table 5.3.

5.2.2 Method

5.2.2.1 Apparatus

A laboratory Modulyo Freeze Dryer (Edwards) was used to freeze dry the sample. Throughout unless stated otherwise, all solutions were quench cooled in liquid nitrogen before drying.

5.2.2.2 Preparation of Methocel solutions

Methocel solutions were prepared as described in section 2.2.2.3.

5.2.2.3 Preparation of other polymer solutions

The polymers in Table 5.3 were dissolved in at least half of the required amount of water under constant agitation by magnetic follower and the solution adjusted to 100 ml.

5.2.2.4 Lyophilisation of mannitol and polymer solutions through different pore size membranes

1 ml each of samples I to IV (Table 1) were filled in 10 ml glass vials and placed in the freeze dryer and lyophilised for 24 hours at a temperature of -55°C and a chamber pressure of 0.08mbar. Samples II to IV were sealed with an impermeable membrane (polythene sheet) with the help of super glue. Holes of 300 and 550 μm were made in the impermeable membranes of samples III and IV respectively.

In another series of experiments, 1 ml 10% mannitol solution was filled into the vials and sealed with various pore size filter membranes filters (0.0029, 0.2, 0.45, 0.8, 1.2 and 5 μm). These samples were lyophilised as described above and the % weight loss calculated.

To study the relationship between the rate of water loss and number of holes present in the impermeable membrane, 2 ml 1.5% Methocel F50 Prem LV and 1.5% Carbopol 934P was placed in each of seven vials I to VII (Table 5.2). Vial I was left open. Each remaining vial was covered with a polythene membrane approximately 80 μm thick. The polythene membrane was held in place by a rim of rubber plug, which formed a sealing engagement with the vial mouth. Each plug had a hole bored through leaving a portion of the sheet exposed.

Holes were made in the exposed polyethylene membrane the vials by piercing a 460 μm needle. 5 holes were made in the membrane of vial II, 10 in vial III, 15 in vial

IV, and 20 and 25 holes in vials V and VI respectively. These samples were lyophilised as described above. The % weight loss (water) was calculated.

5.2.2.5 Lyophilisation of different polymer solutions

1ml volumes of each of the 1.5% w/v solution of the polymers listed in Table 5.3 were filled into 10 ml vials. The vials were lyophilised as described above.

Similarly, different concentrations of Carbopol 934P (Table 5.4) were lyophilised for 24 hours.

5.2.2.6 Drying kinetics of different solutions

0.4 ml each of 1.5 and 10% w/w mannitol and 1.5% w/w Methocel K4MP and K100LVP solutions were filled into thirty, 0.5 ml capacity, snap seal polypropylene microfuge tubes (Life Sciences International UK Ltd.) of internal diameter 6 mm. These 30 tubes were grouped into three sets of 10 tubes. The mouths of one set were left open, the second set was snap sealed and the third set snap sealed but with one 600 μm hole bored through the lid. After pre-freezing in liquid nitrogen the tube contents were lyophilised for 2, 4, 6, 12 and 24 hours. This experiment was carried out in triplicate.

Similarly, different fill volumes (0.1, 0.2 and 0.4ml) of 1.5% w/w K4MP solution were filled into the microfuge tubes, grouped as described above and the contents lyophilised for 2, 4, 6 and 12 hours.

5.3 Results and discussion

The results of these lyophilisation studies are shown in Figures 5.1-5.5 and Tables 5.1-5.5. Figure 5.1 depicts the effect of pore size on weight loss (%) relative to an open vial. Comparison of relative weight losses among a sealed vial, a single pierced hole of 300 and 550 μm and an open vial during lyophilisation is shown in Figure 5.2. It can be seen that there was no significant difference in weight loss through different pore size membranes ranging from 0.2-5 μm during 24 hours lyophilisation. The weight loss was 75% and 90% through the 300 and 550 μm holes respectively when compared to an open vial.

The relationship between the rate of weight (water) loss during freeze drying and the number of holes (surface area) present in the impermeable membrane is shown in Table 5.2. The number of holes did not have an appreciable effect on drying rate.

The result obtained after lyophilisation of different polymers for 24 hours indicate that weight loss (water) from this polymer solution was 98% (Table 5.3). Weight loss was 90 to 98% from different concentrations of (1-5 % w/v) Carbopol 934P (Table 5.4) after lyophilisation for 24 hours.

The drying rate (% water vapour loss per hour) through a single hole (diameter of 600 μm) and open container (6.5 mm aperture) was essentially the same (Table 5.5). The fill volume had an appreciable effect on drying rate (Fig. 5.4).

The water loss (sublimation) from product during lyophilisation involves heat and mass transfer. Heat transfer takes place by direct conduction from the shelf to the vial via points of direct contact between the vial and shelf, conduction through the gap (gap filled with heat conducting materials) between the vial bottom and the shelf, and radiant heat transfer (Pikal, 1985). The amount of heat transfer is not only controlled by the temperature difference between vial and shelf but also by the pressure in the chamber. As the heat conductivity increases with the chamber pressure, a higher pressure shortens the drying time, that is, a significant rate limiting resistance to conductive heat transfer is formed by the gas phase under the vial where there is poor contact between the shelf and the vial.

The sublimation rate is also limited by mass transfer. Pikal suggested that mass transfer (water vapour flow) is impeded by various mass transfer barriers: the partially dried layer, the stoppered opening and chamber to condenser pathways (Pikal, 1985; Pikal, Roy & Shah, 1984). The dried product resistance is an important controlling factor of drying at a fixed temperature. The rate of freezing effects the size of the ice crystals in the frozen mass, it determines the size of the resulting pores in the material and generally, the resistance in the dried layer is lower when the size of the pores is bigger (Pikal *et al.*, 1983).

In this study it was observed that % weight loss (sublimation) was dependent on hole size (area) upto a certain level (Fig. 5.1 and Table 5.1). However, weight loss was similar to that from an open vial when 'pierced' hole diameter reached 550 μm (Fig. 5.2 and Table 5.5). Under the conditions used here, a hole diameter of 500-600 μm appeared to be 'cut-off' size for the efficient passage of water vapour. This argument was further supported by the fact that no weight loss difference was observed when freeze drying was carried out through different numbers of 460 μm diameter holes (Table 5.2).

In a previous study, sublimation rates (weight loss of ice) from vials through capillaries of different size (2.71, 2.17 or 1.15 mm diameter) were found to be equal to that from open vials (Ybema *et al.*, 1995). However, during the initial phase of primary drying, while heating the shelves from –40 to 40°C, sublimation rate of the vial with a smallest restriction was slowest. This phenomenon might be explained by the fact that the pressure in the partially closed vials tends to be slightly higher than in an open vial. The narrower the restriction, the higher the vapour pressure during sublimation and consequently, higher the sublimation temperature at which sublimation was occurring. As a result, the temperature difference ($T_{shelf} - T_{subl}$) is slightly reduced. As this temperature difference is the driving force for heat transfer, the increased sublimation temperature results in slower heat transfer. At the beginning of the primary drying the temperature difference is small because of the low shelf temperature and a change in sublimation temperature has a relatively large influence. However, when the shelf approaches its maximum temperature, this effect is negligible compared to the temperature difference (Ybema *et al.*, 1995). This

effect would be similarly negligible in our study, since drying was performed at a fixed shelf temperature (room). The sublimation rate (water vapour loss rate) from the frozen solution would depend on the dried layer of concentrated solution and the 'pierced' hole size at the container mouth. Usually the dried cake is regarded as an assembly of minute capillaries formed by sublimation of ice crystals. And if the fill height and solid content is high, the resistance to escape of water vapour would be increased and melt back or collapse during the lyophilisation (Fig.5.5) might occur even if the container mouth is left open as resistance to vapour flow from the capillaries in the dried layers would be higher than from pierced hole. The sublimation rate increases as the product temperature increases and therefore primary drying should be carried out at the highest temperature possible although there is an upper temperature limit. This temperature is the eutectic temperature of the frozen solute system if it crystallises or the collapse temperature for a solute system that remains amorphous (Pikal, 1990b). If the product temperature rises above the maximum allowable temperature, the solute-water system gains sufficient fluidity to undergo viscous flow once the ice in that region has sublimed. Thus, the dried region adjacent to the ice will 'flow' and lose structure and the result is a mass of ill-defined geometry that is unacceptable even if the product activity is unaltered.

Under the conditions used here (vial size and fill volume) the 300 and 600 μm diameter holes were found to be sufficiently large to permit effective escape of water vapour during lyophilisation yet were sufficiently small to permit aggressive handling of the containers with no spillage and to offer protection from the manufacturing environment prior to freezing.

Figure 5.6 & 5.7 show illustrations of the types of unit dose packs that might be realised using the technology of lyophilisation through 'holes' in otherwise 'sealed' containers. It is apparent that at the end of the drying cycle an optional secondary packaging operation will afford a truly sealed pack. The technology has application for the manufacture of a range of dosage forms.

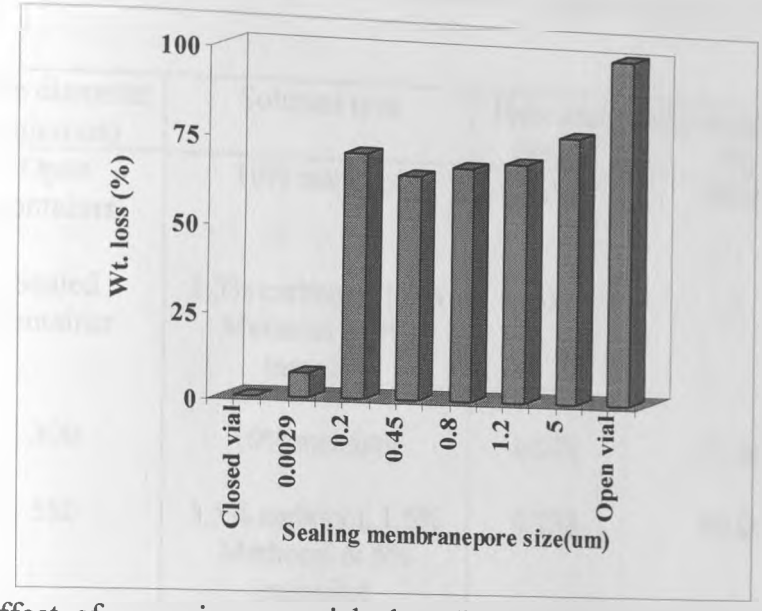

Figure 5.1: Effect of pore size on weight loss (%) relative to open vial during lyophilisation of 1ml 10 % mannitol solution for 24 hours.

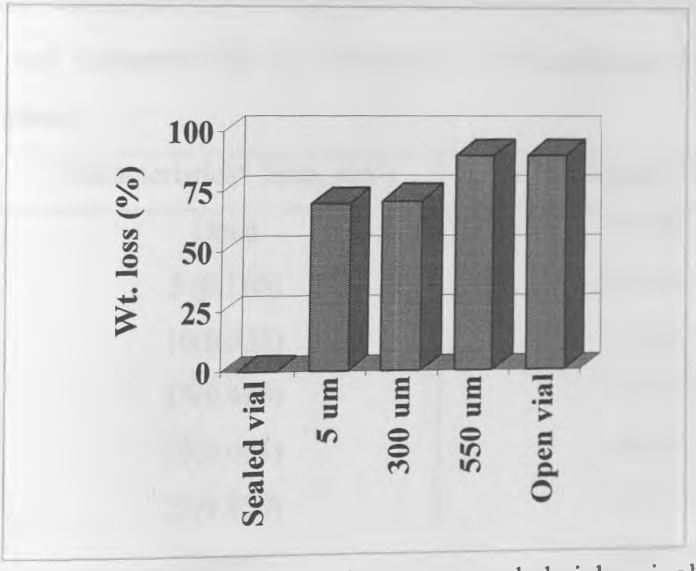

Figure 5.2: Comparison of weight loss (%) among a sealed vial, a single pierced hole of 300 or 550 μm and a open vial (13 mm) during lyophilisation of 1 ml 10% mannitol solution for 24 hours.

Table 5.1.The relationship between hole diameter and rate of water loss during freeze drying process for 24 hours at -55°C condenser temperature and 0.08 mbar pressure.

Sample	Hole diameter (microns)	Solution type	Hole area (mm^2)	24 h weight loss (%)
I	Open container	10% mannitol	132.67	90.00
II	Sealed container	1.5% carbopol, 1.5% Methocel & 5% mannitol	0	0
III	300	10% mannitol	0.071	71.00
IV	550	1.5% carbopol, 1.5% Methocel & 5% mannitol	0.238	90.00

Table 5.2. The relationship between the rate of water loss and the number of holes in the impermeable membrane seal during freeze drying of 1.5% w/v each of Methocel F50 Prem LV and Carbopol 934P for 24 hours at -55°C condenser temperature and 0.078 mbar pressure.

Vial	Characteristics* (area, mm^2)	24 h weight loss (%)
I	Open	92.00
II	5 (0.166)	92.50
III	10(0.332)	93.00
IV	15(0.498)	93.00
V	20(0.664)	92.00
VI	25(0.830)	92.00
VII	Sealed	0.00

*Number of 0.46mm diameter holes formed in membrane indicated.

Table 5.3. List of 15mg/ml polymer solutions used for feasibility study of freeze drying for 24 hours at -50°C condenser temperature and 0.08 mbar pressure. 1 ml of solution was dried from 10 ml open vials.

Polymer	Source	Conc.	24 h weight loss (%)
Carbopol 940	BF Goodrich	15 mg/ml	98.5
Carbopol941	BF Goodrich	,,	98.3
Carbopol 934P	BF Goodrich	,,	98.00
Carbopol 974P	BF Goodrich	,,	98.5
Carbopol 954	BF Goodrich	,,	98.00
Povidone (plasdone K-29/32)	ISP	,,	97.6
PEG 4000	BDH	,,	98.5
PEG 1500	BDH	,,	97.7
Na-CMC (low viscosity)	BDH	,,	98.5
HPMC (6 cps)	Shinetsu chem.	,,	98.6
MC (2500 cps, Celacol Gum)	Tokyo	,,	98.00
HPMC	Courtauds Ltd.	,,	98.00
	Courtauds Ltd.		

Table 5.4 The effect of Carbopol concentration on rate of freeze drying. 1 ml of solution was dried from 10 ml open vials.

Concentration of Carbopol 934P (% w/v)	24 h weight loss (%)
1	97.96
2	97.23
3	97.12
4	90.24
5	96

Table 5.5 Compilation of % vapour rate loss and r^2 values during freeze-drying (fill volume = 0.4 ml).

Fill solution (% w/w)	Open		1 hole (600μm)	
	Slope (% weight loss per hour)	r^2	Slope (% weight loss per hour)	r^2
Mannitol 1.5	8.1295	0.9631	8.124	0.9915
Mannitol 10	7.6185	0.9952	7.5295	0.9867
K100LVP 1.5	8.2193	0.9298	8.3528	0.9292
K4MP 1.5.	7.9721	0.8689	8.2128	0.9692

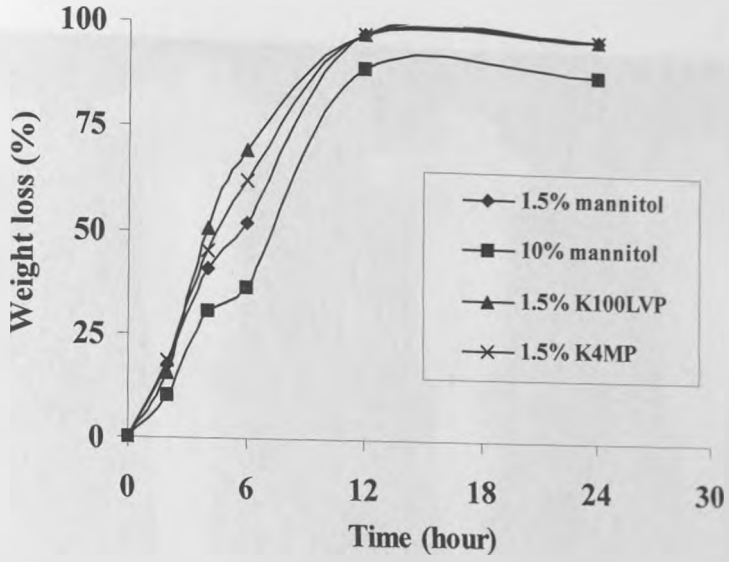

Figure 5.3 Lyophilisation of 1.5% solution of K4MP, K100LVP and 1.5 & 10% mannitol solution (fill volume = 0.4 ml and single hole of 600 μm).

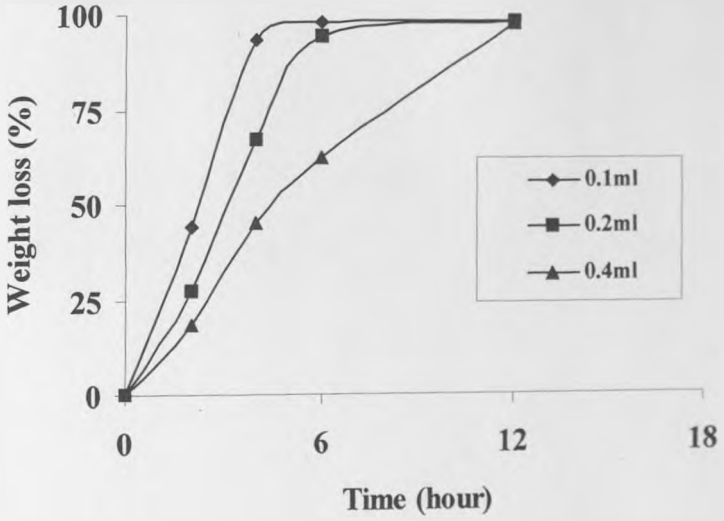

Figure 5.4 Effect of fill volume on drying rate during lyophilisation of 1.5% K4MP solution through a single hole of 600 μm.

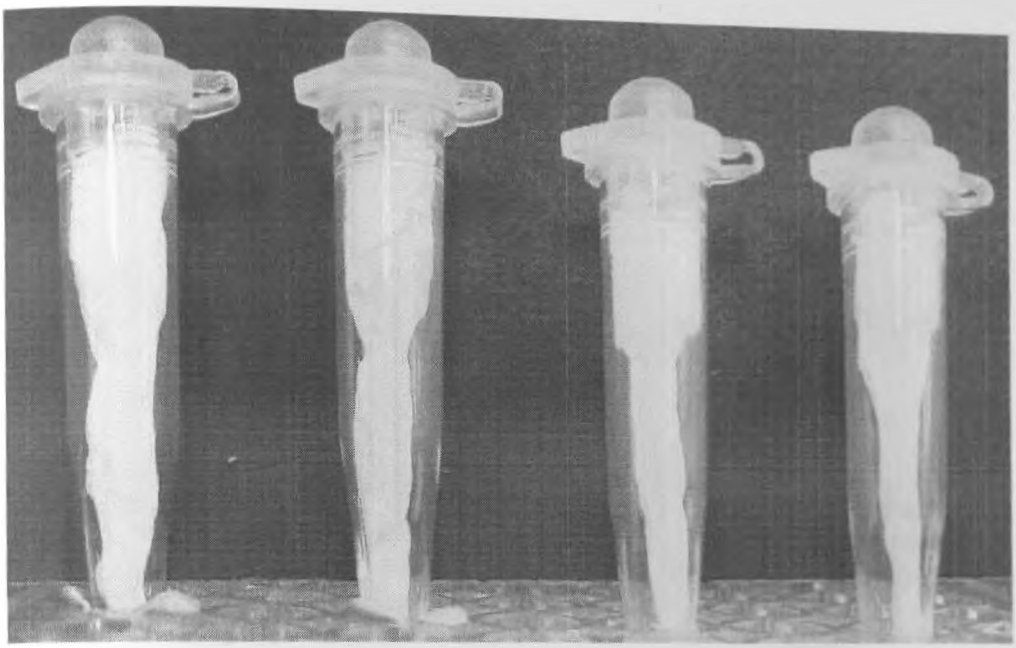

Figure 5.5 A typical example of 'meltback' observed at high solids content during the lyophilisation of 0.7 ml solution containing NHT, K4MP and mannitol (11.4, 14 and 7 mg) respectively in microfuge tubes. The mouth of the microfuge tube was left open during the cycle.

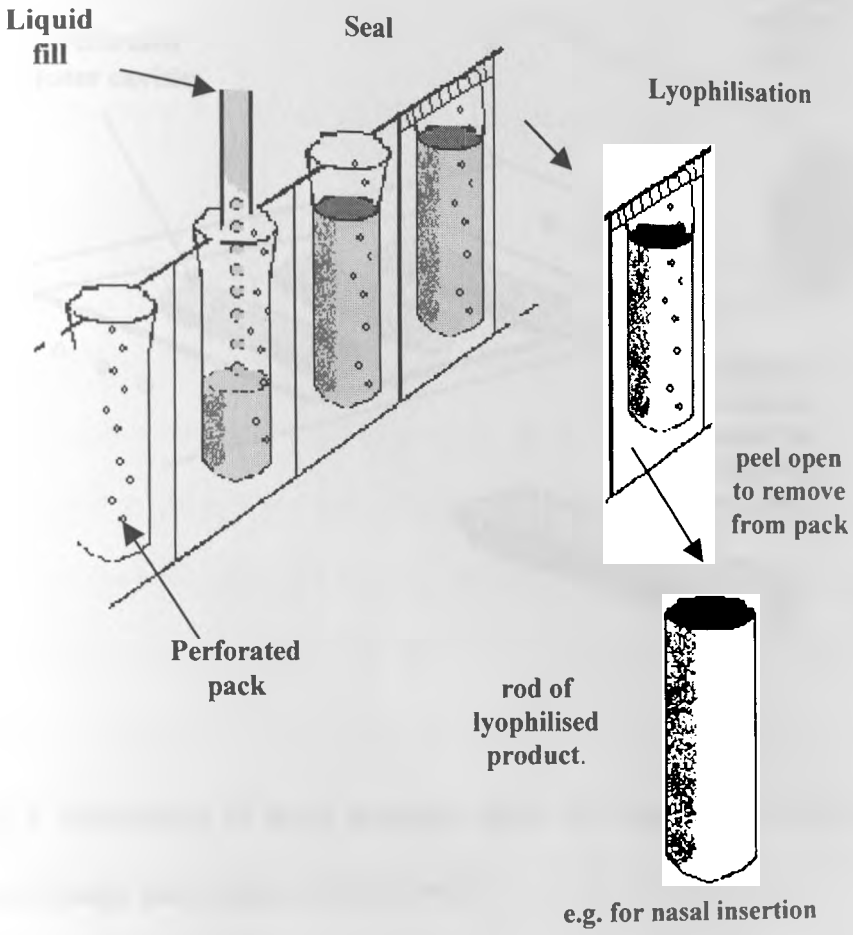

Figure 5.6: Illustrations of some proposed packs for manufacture of unit dose lyophilised dosage units (based on strip pack).

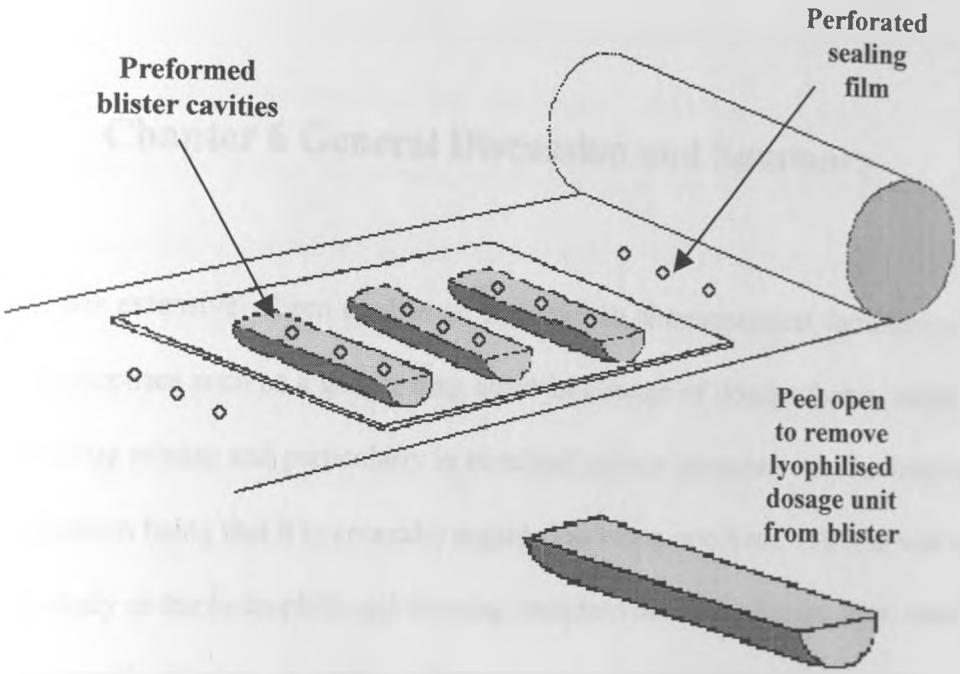

Figure 5.7 Illustrations of some proposed packs for manufacture of unit dose lyophilised dosage units (based on blister pack).

Lyophilisation from sealed, perforated, unit-dose packages is shown to be feasible. The technique offers a novel convenient means of lyophilising non-sterile products in their primary pack and increases the potential for the development of lyophilised formulations for non-parenteral applications.

Chapter 6 General Discussion and Summary

HPMC has extensively been used as an excipient in pharmaceutical formulation for various purposes such as a gel-forming agent in a range of dosage forms, mainly to control drug release and particularly in sustained release preparations. An important consideration being that it is generally regarded as being non-toxic. HPMC was used in this study as the hydrophilic gel-forming excipient basis of a dosage form intended for the nasal delivery of drugs. However the resultant lyophilised plugs could equally well be exploited for the delivery of drugs to various mucosal surfaces such as buccal and vaginal mucosae either for the fast or slow release of drugs for systemic absorption or for a local effect. This fast hydrating delivery system could also be utilised in wound healing medications.

There are three principal reasons for administering drugs via the nose. The first is convenience; the second is for a topical effect on the cutaneous lining of the nose, as in the use of antihistamines, steroids or other desensitising applications in conditions such as allergic rhinitis; the third is for a systemic effect made possible by the superficial blood vessels of the nasal mucosa which facilitate rapid absorption into the blood stream. The rapid passage of drugs across the nasal epithelium into the blood stream is a major advantage of the nasal route of delivery, making the nose an

alternative to the parenteral route especially in some acute treatments and maintenance therapies currently delivered via injection. The nasal route can be considered for those drugs which are ineffective orally, used chronically, used in small doses or requiring rapid entry into the systemic circulation (Hussain, 1998). A drug candidate for nasal administration should possess the appropriate aqueous solubility to provide the desired dose in the 25-150 µl volume of formulation that can be realistically be administered per nostril. Other attributes of nasal drug candidates would include appropriate absorption properties; no nasal irritation; a suitable clinical rationale for the nasal route e.g. rapid onset of action; low dose. generally below 25 mg per dose; no offensive odours and a suitable stability profile.

Some of the limitations of the nasal route include local irritation; rapid removal of the therapeutic agent from the site of deposition due to mucociliary clearance; low permeability of the nasal membrane to large molecules; degradation of macromolecules by nasal cavity (proteolytic) enzymes and pathological conditions such as cold or allergies which may significantly alter nasal bio-availability. However some of these limitations can be overcome by strategies such as the inclusion in the formulation of penetration enhancers, bioadhesive agents that increase nasal cavity residence time and enzyme inhibitors.

Attempts to increase the residence time of a formulation in the nasal cavity or on the nasal mucosal surface usually involve the use of polymeric materials which increase solution viscosity and/or have bio- or mucoadhesive properties. Bioadhesion involves interaction of the compound with the mucosal surface and will depend on

the nature and duration of contact between the bioadhesive (polymer) and the mucous surface (Huang *et al.*, 2000). Generally, it is believed that mucoadhesion can be used to increase the residence time of a drug formulation at various sites in the body and commonly used bioadhesives include water-soluble polymers and water-insoluble polymers with swellable cross-linked networks. Carbopol, carboxymethyl cellulose (CMC), microcrystalline cellulose (MCC), hydroxypropyl cellulose (HPC), HPMC and chitosan are examples of bioadhesive polymers.

As described in the previous chapters, bioadhesive drug delivery systems have been developed in the form of aqueous solutions, gels, powders and microspheres. Drug stability and loss of dosage volume after administration are problems that can be encountered with aqueous based formulations. The 'stability issue' may be overcome by using an anhydrous powder formulation or a solid dosage form, although 'accuracy of dose' with powders tends to be difficult.

In the present study an attempt has been made to combine the advantages of lyophilisation with the use of a polymeric excipient to prepare an anhydrous dosage form, in which drug stability was good, yet with the potential to become bioadhesive on hydration. Such a dosage form must have a certain minimum in-use mechanical strength in this case, for insertion into the nose and yet become rapidly hydrated in the nasal environment thereby sticking to, and releasing incorporated drug, at the site of absorption. This can be readily achieved by the use of lyophilisation since lyophilised systems are solvent loving due to their porous structure and in contact

with a dissolution medium, the loss of physical shape caused by the instantaneous hydration is important in nasal delivery for patient compliance and drug release.

The *in vitro* drug release of two 'model' drug molecules, nicotine and UK-92, 480, after hydration of lyophilised plugs was studied. Both of these molecules are small (MW 162.2, nicotine and 474.6, UK-92.480), potent and chemically stable although they have different clinical indications. As described in Chapter 2, *in vitro* release of UK-92, 480 depended upon the availability of dissolution medium at the donor side of the diffusion cell and release rates were faster when the donor side was flooded with medium. This was apparently due to the faster dissolution of the drug in abundant solvent. However the availability of medium in the normal nasal cavity can be assumed to be much lower and so most of the *in vitro* release studies were carried out with a minimum medium volume on the donor side. *In vitro* release of either model compound depended on the concentration of the HPMC incorporated in the various test formulations. High polymer contents gave, on hydration, viscous solutions or gels which had the effect of retarding drug release (Chapter 2). In the case of nicotine, release was faster from HPMC solution than from a lyophilisate which had been prepared from that solution. The reason deduced for this finding was that the lyophilisate, after hydration, formed an HPMC solution or gel which was more concentrated than the parent solutions from which it was lyophilised. At the higher end of the MW range of the various HPMC studied, an interesting, but unexpected finding was that nicotine release rate was independent of molecular weight (Chapter 2) although at the lower end of the MW range of the various HPMC used, nicotine release rate tended to decrease with increasing molecular weight. The

interpretation put on this finding was that of a 'limiting HPMC viscosity' beyond which drug release rate no longer decreased with viscosity. The diffusing nicotine molecule has a much smaller molecular weight (162.2) than the HPMC and can be considered as percolating through the solution in the void spaces between the macromolecular chains of the polymer. Thus the effect of the polymer on the macroscopic flow properties of a hydrated system, evaluated as viscosity, would not necessarily be the same at the micro-scale at which the diffusion (movement at the molecular scale) of the nicotine occurs. This is a similar conclusion to that of Alvarez-Lorenzo et al (1999) who found that although the macro- and micro-viscosities of HPMC gels were correlated, micro-viscosities determined by a dynamic light scattering technique were consistently lower than macro-viscosities as determined by capillary viscometry.

The release of nicotine from various powder blends with HPMC was little affected by HPMC concentration or molecular weight, although it was however faster from the blend than from a lyophilised plug containing the same quantity of polymer. In the powder blend, the nicotine was shown by SEM to be present as crystals of the hydrogen tartrate (NHT). Individual NHT crystals could be clearly seen as part of a simple powder mix with HPMC powder particles and the whole did not have the porous, homogenous, non-crystalline structure of the lyophilisates. It is apparent that on hydration of this powder mix, the NHT could dissolve rapidly and largely independently of the hydration of the HPMC particles leading to relatively rapid and similar nicotine release rates from various different powder blends (Chapter 2).

In the sheep, the absorption of nasally administered nicotine from different formulations including a lyophilised plug (Chapter 4) suggested that at least some of the formulation effects seen *in vitro* also had an impact on nasal absorption and that the inclusion of the nicotine in the lyophilised HPMC plug had a significant effect. The area under the nicotine plasma level curve (AUC) for a lyophilised plug, NHT powder, solution and intravenous injection were 157.3, 30.08, 5.0 and 5.44 ng.h/ml respectively. A plausible explanation of the relatively high bio-availability (AUC) of nicotine from the plug was that the viscous gel formed when the plug contacted the moist nasal mucosa gave an extended residence time that allowed more time for nicotine absorption to occur. This effect could be expected on the basis of the nicotine release from the lyophilised plugs *in vitro* which was dependent upon polymer concentration (Chapter 2). The observed T_{max} of 60 min for the lyophilised plug was another indication of the effect of the viscosity and the adhesive nature of the hydrated dosage form. Sticking to the absorption site would increase the time window for absorption but the viscous nature of the hydrated HPMC would hinder diffusion to the mucosal surface. There is also the possibility that the concentrated HPMC solution which resulted from the hydration of the plug *in situ* had an osmotic effect on the mucosal barrier that further enhanced absorption. The higher bio-availability from powder than from solution suggested that complete dissolution of the powder in the low hydration condition of the nasal cavity was not rate limiting. Although *in vitro* nicotine release from powder and solutions was similar (Chapter 2), the lower bio-availability from solution was probably because more of the solution was likely to be lost by swallowing or simply draining from the nose. At least in terms of bio-availability, the lyophilised plug would appear to be a better

nasal delivery system than a simple solution (drop or spray) for potent low MW drugs such as nicotine and perhaps other, larger molecules. The presumed osmotic effect of the concentrated HPMC solution on the nasal mucosa would be an important consideration in the absorption of large molecules.

In addition to its drug release characteristics, the type of solid unit dosage form, such as the lyophilisates under study here, should be robust enough to withstand transport and handling. Measurement of the properties of hardness, fracturability (brittleness), springiness and resilience were intended to be a determinant of ease of handling and product use, in this case by insertion into the nose and these mechanical properties must also take account of patient compliance. The increase in hardness and fracturability of the lyophilised plugs with their solids content was accompanied by a decrease in springiness and resilience (Chapter 3) and it is apparent that the hardness of a lyophilised plug for nasal application should not be too great as it might cause discomfort to the patient on application yet should be hard enough to retain its original structure during handling and maintain the porous structure essential for rapid hydration.

Another important measure of plug performance is gel strength after hydration. In this study marked differences in gel strength were observed among fully hydrated lyophilised plugs prepared from different concentrations of HPMC solution. Gel strength of hydrated plugs also increased with HPMC molecular weight (Chapter 3) and on this basis it was expected that drug release would decrease with HPMC molecular weight. At least in the case of nicotine, *in vitro* release rates from

hydrated plugs were independent of HPMC molecular weight at the higher end of the molecular range (Chapter 2).

The adhesivity of the hydrated plug is another important determinant of long residence time in the nasal cavity since this property would ensure whether the hydrated plug stuck to the mucosal surface or not. The lyophilised plugs under study here exhibited adhesiveness onto glass and pig tracheal mucosa. The adhesivity increased with applied force and contact time (Chapter 3) and in order to achieve interpenetration or diffusion of polymeric chains, a minimum force appears to be needed. Once such interpenetration is achieved, continued diffusion depends on contact time and increased contact time is known to give rise to deeper chain penetration into substrate (mucosa or skin) (Blanco-Fuente *et al.*, 1996).

The total adhesive force also depends on a number of other factors such as contact surface area and molecular weight. The hydration volume is another important parameter since hydration by water or other liquids is the key step in gel formation and facilitating the mobility of the polymer chains to form bioadhesive unions more readily (Smart, Kellaway & Worthington, 1984). However, excessive hydration and dilution of the gel may modify the mechanical strength of the gel leading to loss of adhesion.

The nasal cavity has a low hydration environment and gel dilution should not occur, however a solid dosage form intended for insertion into nose either for systemic or local effect should hydrate (collapse) in a minimum of water (hydration fluid) at a

speed which will impact patient compliance and drug release. In this study, the hydration volume (water) required was observed to depend on the lyophilisate weight (5-40 mg) and lay in the range 50-165 µl. Hydration occurred in less than 15 seconds in 1.5% mucin solution. However hydration times of lyophilisates placed on different concentrations of Carbopol gel were much slower indicating the requirement for sufficient free water for plug hydration and collapse (Chapter 3).

The characteristics of how fast the lyophilised plug hydrates and collapses when in contact with water or nasal secretion, and how much water is required for such collapse (just to make it completely hydrated) are important product attributes. The observed hydration time and volume of water needed to make lyophilised plug collapse were in a reasonable range for nasal application.

The speed of hydration of a lyophilised system is to a certain extent related to its porosity which in turn depends on the method of freezing before lyophilisation. Rapid freezing produces smaller ice crystals and when freeze dried yield large surface area leading to faster hydration when it comes in contact with moist surface or water. On the other hand slow freezing yields large ice crystals hence creates large pores effectively reducing surface area (degree of porosity) (Chapter 3).

As described above and in Chapter 5, a rapidly hydrating solid unit dosage form for nasal administration can be achieved by incorporating the drug into a hydrophilic gel forming polymer solution and then lyophilising the formulation in a primary package. WO-A-9412142 discloses a freeze drying manufacture method in which

the solution precursor of the dosage form is filled into and dried in the depressions of a blister film. Following freeze drying a plastic sheet is placed over the depression and the blisters sealed. However such freeze dried plugs are usually fragile and depending on the solid (binding agent) content, tend to disintegrate. Thus the freeze dried residue in the type of open blister pack described above is easily lost by static, air currents or handling operations prior to film sealing. In order to mitigate at least one of these problems, an attempt has been made to develop a primary package for the nasal insert unit dosage form which comprises a container closed with an impermeable membrane pierced with one or more holes through which the material in the container can be lyophilised. The holes in the membrane have to be sufficiently large to allow water vapour to escape but small enough to ensure that the dried material is kept within the container. In this study it was observed that weight loss (%, sublimation) was dependent on hole size (area) up to a certain level and under the lyophilisation conditions used, weight loss was similar to that from an open vial when 'pierced' hole diameter reached 550 μm (Chapter 5). Under the conditions used here, a hole diameter of 500-600 μm appeared to be 'cut-off' size for the efficient passage of water vapour. This argument was further supported by the fact that no weight loss difference was observed when freeze drying was carried out through a different number of 460 μm diameter holes.

In addition to other factors as described above and in Chapter 5, the water vapour loss from frozen solution also depends on the dried layer of concentrated solution. Usually the dried cake is regarded as an assembly of minute capillaries formed by sublimation of ice crystals and if the fill height and solid content is high, the

increased resistance to escape of water vapour can cause meltback or collapse during the lyophilisation, as resistance to vapour flow from the capillaries in the dried layers would be higher than from open container.

Under the conditions used here (vial size and fill volume) the 300 and 600 μm diameter holes were found to be sufficiently large to permit effective escape of water vapour during lyophilisation. Illustrations of the types of unit dose packs that might be developed using the lyophilisation through 'holes' technology in otherwise 'sealed' containers are described in Chapter 5. Such techniques may offer a convenient means of lyophilising non-sterile products in their primary pack and increases the potential for the development of lyophilised formulations for non-parenteral applications.

The lyophilised plugs under study here could perhaps serve as an alternative delivery system for nicotine replacement therapy (NRT). Smoking remains the single most important preventable cause of death in modern society. The major problem with nicotine is that it is highly addictive since of itself, it is neither carcinogenic nor mutagenic, nor does it act as a tumour initiator. Similarly, none of the major metabolites of nicotine are known to be carcinogenic unlike, tobacco and especially tobacco smoke which contain several potent carcinogens.

Nicotine addiction often manifests withdrawal syndromes, such as craving, frustration, anxiety and decreased heart rate and various methods have been devised to help smokers to quit smoking (Jarvis, 1997). Among these NRT has become a

popular, and continuous attempts are being made to develop improved delivery systems for nicotine such as transdermal patch (Lin, Ho & Chien, 1993), a chewing gum (Russell, Feyerabend & Cole, 1976) and nasal spray (Ferno, Helgertz & Ohlsson, 1986) for replacement therapy. Nasal delivery of nicotine may help patients through withdrawal symptoms.

It has been suggested that the rapid absorption of the nicotine when given nasally may be an important factor for smokers for whom other forms of nicotine replacement show too slow absorption (Sutherland *et al.*, 1992). Attempts are also being made to improve nasal nicotine replacement formulations to provide a 'buzz effect' by initial rapid release and absorption of nicotine followed by a more controlled release and absorption of nicotine to provide a sustained high level of absorbed nicotine (Illum, 1994).

The lyophilised plug may be useful in those smokers for whom chewing gum delivery is unsuitable due to dental problems and in those with allergy to transdermal patches. Since nasal absorption of nicotine is rapid, the lyophilised plug may also be able to provide a 'buzz effect' depending upon the level of polymer concentration (viscosity enhancing agent) in the formulation. This system may also be beneficial where nicotine is required for therapeutic reasons such as its use as a cognitive enhancer in ulcerative colitis, weight reduction, Parkinsons disease, Alzeimers disease, narcolepsy, depressions and sleep apnoea.

In summary it was found that a lyophilised plug offered a novel means of drug presentation to (nasal) mucosa and has the several advantages that the mechanical properties of the plug and its performance on hydration, adhesivity and drug release, can be manipulated over a significant range.

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