CHAPTER II

LITERATURE REVIEW

NASAL DRUG DELIVERY SYSTEMS

The nasal route appears to be an ideal alternative to the parenterals for administering drugs intended for systemic effect, in view of the rich vascularity of the nasal membranes and the ease of intranasal administration. Besides avoidance of hepatic first-pass elimination , the rate and extent of absorption and the plasma concentration versus time profile are relatively comparable to those obtained by IV medication, Nasal membranes are characterized by existence of a highly rich vasculature and a highly permeable structure for absorption. However, there are some factors which could potentially influence the efficacy of nasal absorption of drugs, such as method and technique of administration, the site of deposition, and rate of clearance (Chien et al., 1994). Nasal mucosa is a thin vascular tissue with a surface area of about 150 cm^2.

Nasal Passage

The nasal passage, which is 12-14 cm in depth, runs from the nasal valve to the nasopharynx. The three distinct functional zones in the nasal cavity are named the vestibular, respiratory, and olfactory areas. The nasal passage is composed of a horizontally skin lined vestibule with the passage being directed upward and backward, and is separated by cartilagenous bony nasal septum. The septum ends at the nasopharynx and the airways merge into one.

Nasal Epithelium

The nasal membane can be classified into olfactory and nonolfactory epithelia. The former is pseudostratified columnar in type and consists of specialized olfactory cells, supporting cells, and both the serous and mucous glands. There are two types of mucus covering the surface of the mucous membrane; one adheres to the tips of cilia

and the other fills the space among the cilia. Adequate moisture is necessary to maintain the normal functions of the nasal mucosa. Dehydration of the mucous blanket increases the viscosity of secretions and reduces the ciliary activity.

Nasal Secretion

A total of approximately 1500-2000 ml of mucus is produced daily , which contains 90-95% water, 1-2% salt, and 2-3% mucin. In addition to mucous glycoproteins, nasal secretions contain a variety of other proteins, enzymes and antibodies. The presence of excessive mucus has direct implications on the development of nasal bioadhesive drug delivery systems.

The normal pH of the nasal secretions in the adults ranges approximately from 5.52 to 6.5, whereas in infants and young children it ranges from 5.5 to 6.8. It is obvious that the distribution of the drug in the nasal carvity is an important factor for nasal medication. Because the method of delivery will affect drug distribution in the nose, it will subsequently influence the site of deposition and efficacy of drug. It is important that the integrity of the nasal clearance mechanisms should be kept intact so that it can remove dust, allergens, and bacteria. However, the mechanism can be influenced by drug and excipients in the formulation.

Several advantages can be achieved from delivery drugs intranasally: (a) avoidance of hepatic "first-pass" elimination, gut wall metabolism, and/or destruction in gastrointestial tracts; (b) the rate and extent of absorption and the plasma concentration vs. time profile are relatively comparable to that obtained by intravenous medication; and (c) the existence of a rich vasculature and a highly permeable structure in the nasal membranes for absorption. These advantages have made the nasal mucosa a feasible and desirable site for systemic drug delivery.

However, there are some factors that should also be considered for optimizing the intranasal adminstration of drugs: (a) methods and techniques of administration; (b) the site of disposition; (c) the rate of clearance; and (d) the existence of any

pathological conditions which may affect the nasal functions. These factors could potentially influence the efficiency of nasal absorption of drugs.

DRUG DISTRIBUTION IN THE NASAL CAVITY

The drug distribution in the nasal cavity is one of the important factors which affects the efficiency of nasal absorption. The mode of drug administration could affect the distribution of a drug in the nasal cavity, which in turn will determine the absorption efficiency of a drug. Using a cast of the human nose, it was demonstrated that a significant difference in drug distribution was observed by comparing different types of nasal delivery systems, like nose drops, plasic bottle nebulizer, atomized pump, and metered-dose pressurized aerosol. The results indicated that the atomized pump is the best nasal delivery system because it gives a constant dose and a very good mucosal distribution. The results also suggest that the use of a large volume of a weak solution is preferable to a small volume of a concentrated solution. This may be of particular importance when vasoconstrictor is used locally (Chien, 1985)

A simulated nasal cavity made of acryl resin was developed for studying the distribution of beclomethasone diporpionate (BD) areosol particles in the nasal cavity. No significant difference was found among gas, liquid, and powder preparations. The highest concentration of BD was usually found at the anterior portion of the middle turbinate.

Nasal mucociliary clearance is one of the most important limiting factor for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. However, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities thus enhancing drug absorption (Vidgren, 1991 and Ugwoke, 2000).

DRUG DEPOSITION IN THE NASAL CAVITY

Nasal deposition of particles is related to the individual's nasal resistance to air flow. The high linear velocity and the bend in the air stream in the anterior nares results in impaction of a large proportion of particles that are small enough to enter the nasal airway. With nasal breathing, nearly all the particles with an aerodynamic size of 10-20 μm are so deposited. A significant fraction of the very small particles is also deposited in the nose, though many particles which are smaller than 2 μm pass with the inspired air into the lungs. The capacity of removal for the upper respiratory tract is 100% for particles with a size larger than 10 μm, and approximately 80% for particles of 5μm. It drops progessively with further reduction in size and approaches zero for paticles at 1-2 μm. In soluble particles, which are deposited in the main nasal passage, are likely to be carried back by the ciliary movement and dispatched to the stomach (Proctor, 1985). If the drug is introduced as a vapor or a soluble particle, it may readily pass into the lining secretions and then be absorbed from there into the blood.

The deposition of aerosols in the respiratory tract is a function of particle size and respiration patterns. The density, shape, and hygroscopicity of the particles and the pathological conditions in the nasal passage will influence the deposition of particles, whereas the particle size distribution will determine the size of deposition and affect the subsequent biological response in experimental animals and man. It was reported that 91.5 and 83.2% of the mass of the aerosol produced, respectively, by the jet and ultrasonic nebulizer were deposited in the upper respiratory tract of 15 normal adults.

A uniform distribution of particles throughout the nasal mucosa could be achieved by delivering the particles from a new nasal spray using a pressurized gas propellant. A metered-dose delivery system developed for the nasal delivery of flunisolide, a synthetic fluorinated corticosteroid, was assessed to provide a consistent dose delivery and spray pattern which affects the deposition of droplets in the nasal cavity (Yu et al., 1983).

If the aerodynamic size distribution of an aerosol is known, respiratory tract deposition can be estimated from theoretical and experimental data relating deposition fraction to particle aerodynamic diameter. However, the estimation of deposition in the nasal cavity is complicated by the growth or shrinkage of particles resulting from water condensation or evaporation by humidity change (Hiller et al., 1980). When exposed to an environment with increasing humidity, a hygroscopic particle pesponds and absorbs moisture to the extent of a few molecular layers at low relative humidities and becomes dissolved as humidity increases. It then becomes a saturated droplet, and at the same time its size undergoes an abrupt increase and it grows larger as humidity increases further or reduces as humidity decreases. The change in the particle size of aerosol depends on relative humidity : The greater the humidity, the larger the aerosol particle size (Davis and Bubb, 1978).

The particle or droplet size of an aerosol is important for both efficacy and toxicity. For example, the metered-dose flunisolide solution discussed above requires that the majority of particles have a diameter of greater than 10 μm to achieve a localized delivery in the nasal cavity and to avoid any potential undesired effects resulting from any deposition of flunisolide aerosol in the lung(Yu et al, 1984). Numerous methods for sizing aerosol particles have been developed in the past. The most common ones are microscopy, light scattering, laser holography, and the cascade impactor method.

Of the three mechanisms usually considered for particle depositon in the respiratory tract, i.e., inertia, sedimentation, and diffusion, the inertail deposition is the dominant mechanism in the nasal deposition. Any particles with an aerodynamic diameter of 50 μm or greater do not enter the nasal passage. Several techniques, such as polydisperse and monodisperse aerosols, have been employed to determine the regional and / or total deposition in the nasal passage. The results demonstrated a generally monotonic increase in percent total deposition when plotted against the parameter D_A^2O. This curve demonstrates the primary role of inertia, either in laminar or turbulent flow studied. The site of maximum deposition was found at 2-3 cm behind the tip of the nostrils.

The deposition site within the nasal cavity depends upon the type of delivery system used and the technique of administration applied. The deposition and clearance of solution with a relatively large volume of administration, as nasal spray and nose drops, were compared and the results indicated that following administration by the nose drops, a greater coverage of the nasal walls is achieved, which is independent of the volume administered over the range of 0.1-0.75 ml (Aoki and Crawley, 1976). The continuous air stream from the posterior outlet of the nose had only a little effect on the amount of the particles deposited at each region. The particles once deposited at the anterior region of the nasal cavity might be again conveyed posteriorly by the inhaled air, ciliary movement and/or diffusion in the mucous layer.

Experiments were undertaken to clearify the deposition of drug in the nasal cavity using the gas and powder sprays. A nose model molded from a cadaver with some modifications to allow for postmortem shrinkage was developed. Three types of nasal cavities were designed from the original model, using dental compound and celluloid plate : (a) a straight septum with normal turbinates, (b) a concave septum with hypertrophic turbinates, and (c) a convex septum with atrophic turbinates. The inner surface of the cavity except the anterior portion (corresponding to the vestibulum) was covered with moistened filter paper. Beclomethasone dipropionate (BD) particles were emitted into each nostril by fluorinated Freon propellant. Results indicated that the shape of the nasal cavity produces a greater effect on the deposition of BD from the gas spray than from the powder spray. This difference could probably be due to the spray angle to the size and speed of the aerosol particles. The particles from the spray container hit the localized area in the spray axis and thus were deposited there most densely. A wider spray angle, therefore, more adequately advances the sprayed particles beyond any obstacles, such as a deviated septum or hypertrophic turbinates. The powder spray is, therefore, preferable with regard to the deposition and distribution of drug particles in the nasal cavity, and improvement of the delivery system and drug forms is necessary to achieve a better clinical effect and easier manipulation by the patients.
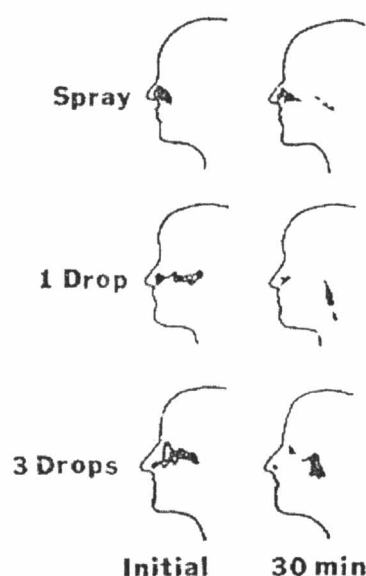
Figure 1 Diagram showing the sites of deposition and patterns of clearance following intranasal administration by nasal spray and nose drops. Each pair of images is of the same subject, but the three pairs are of different subjects.

The patterns of nasal deposition and the rates of clearance were studied in normal subjects using nasal spray and nose drops of ^{99m}Tc-labeled human serum albumin (Haray et al., 1995). The nasal spray deposited HAS anteriorly in the nasal cavity, with little of the dose reaching the turbinates. In contrast, the nose drops dispersed the dose throughout the length of the nasal cavity, from the atrium to the nasopharynx, and the dosing with three drops resulted in a greater coverage of the nasal walls compared with that of a single drop. The solution deposited anteriorly in the nasal cavity was slow to clear, especially with the spray administration. The nose drops cleared more rapidly than the dose administered as a spray.

NASAL MUCOADHESIVE DRUG DELIVERY SYSTEM

The goal of the development of bioadhesive is to duplicate, mimic, or improve biological adhesives. They should be both durable where required and degradable where necessary, and not toxic at all.

Mucoadhesive drug delivery systems utilize the property of bioadhesion of certain water-soluble polymers which become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended periods of time (Kamath and Park, 1994).

Mucus layer

The tissue layer responsible for formation of the adhesive interface is mucus. Mucus is a translucent and viscid secretion which forms a thin, continuous gel blanket adherent to the mucosal epithelial surface. The mean thickness of this layer varies from about 50 to 450 μm in humans (Kamath and Park, 1994).

The composition of mucus varies widely depending an animal species, anatomical location, and the normal or pathological state of the organism. It is secreted by the goblet cells lining the epithelia or by special exocrine glands with mucus cells acini. The lubrication propertiesof mucus secretions are a result of their viscous and gel-forming properties, and general stickness. Mucus has the following general composition (Rathbone and Hadgraft, 1991).

Water	95%
Glycoproteins and lipids	0.5-5%
Mineral salts	1%
Free proteins	0.5-1%

Mucus glycoproteins are high molecular proteins possessing attached oligosaccharide units. These units contain an average of about 8-10 monosaccharide residues of five different types. They are (a) L-fucose, (b) D-galactose, (c) N-acetyl - D-glucosamine, (d) N-acetyl-D-galactosamine, and (e) sialic acid. In humans the only important sialic acid is N-acetylneuramic acid, although in animals a number of other sialic acids occur, including N-glycollyneuramic acid and various O-substituted derivatives. Amino acids are principally serine, threonine, and proline. The mucus layer which covers the epithelial surface has various roles.

Mucoadhesion

For bioadhesion to occur, a succession of phenomena is required. The first stage involves an intimate contact between a bioadhesive and a membrane, either from a good wetting of the bioadhesive surface or from the swelling of the bioadhesive. In the second stage, after contact is established, penetration of the bioadhesive into the crevice of the tissue surface or interpenetration of the chains of he bioadhesive with those of the mucus takes place. Low chemical bonds can then settle (Ahuja et al., 1997).

One of the most important factors for bioadhesion is tissue surface roughness. Castellanos et al. showed that adhesive joints may fail at relatively low applied stresses if cracks, air bubbles, voids, inclusions, or other surface defects are present. Viscosity and wetting power are the most important factors for satisfactory bioadhesion. Wachem et al. studied in vitro interaction of human endothelial cells with polymeric substances processing different metabolites in a culure medium containing serum.

On a molecular level, mucoadhesion can be explained on the basis of molecular interactions. The interaction between two molecules is composed of attraction and repulsion. Attractive interactions arise from van der Waals forces, electrostatic attraction, hydrogen bonding, and hydrophobic interaction. Repulsive interactions occur because of electrostatic and steric repulsion. For mucoadhesion to occur, the attractive interaction should be larger than nonspecific repulsion (Kamath and Park, 1994).

Theories of Bioadhesion

Several theories have been proposed to explain the fundamental mechanisms of adhesion. In a particular system, one or more theories can equally well explain or contribute to the formation of bioadhesive bonds.

Electronic Theory

According to the electronic theory. Electron transfer occurs upon contact of an adhesive polymer with a mucus glycoprotein network because of differences in their electronic structures. This results in the formation of an electrical double layer at the interface. Adhesion occurs due to attractive forces across the double layer.

Adsorption Theory

According to the adsorption theory, after an initial contact between two surfaces, the material adheres because of surface forces acting between the atoms in the two surfaces. Two types of chemical bonds resulting from these forces can be distinguished:

1. Primary chemical bounds of covalent nature, which are undesirable in bioadhesion because their high strength may result in permanent bonds.
2. Secondary chemical bonds having many different forces of attraction, including electrostatic forces, van der Waals forces, and hydrogen and hydrophobic bonds.

Wetting Theory

Wetting theory is predominantly applicable to liquid bioadhesive systems. It analyzes adhesive and contact behavior in terms of the ability of a liquid or paste to spread over a biological system.

Diffusion Theory

According to diffusion theory, the polymer chains and the mucus mix to a sufficient depth to create a semipermanent adhesive bond. The exact depth to which the polymer chains pentrate the mucus depends on the diffusion coefficient and the time of contact. This diffusion coefficient, in turn, depends on the value of molecular

weight between cross-links and decreases significantly as the cross-linking density increases.

Factors Important to Mucoadhesion

The bioadhesive power of a polymer or of a series of polymers is affected by the nature of the polymer and also by the nature of the surrounding media.

Polymer-Related Factors

Molecular Weight

Numerous studies have indicated that there is a certain molecular weight at which bioadhesion is at a maximum. The interpenetration of polymer molecules is favorable for low molecular weight polymers, whereas entanglements are favored for high molecular weight polymers. The optimum molecular weight for the maximum bioadhesion depends on the type of polymer. Their nature dictates the degree of swelling in water, which in turn determines interpenetration of polymer molecules within the mucus. The bioadhesive force increases with the molecular weight of the bioadhesive polymer up to 100,000, and that beyond this level there is not much effect. To allow chain interpenetration, the polymer molecular must have an adequate length. Size and configuration of the polymers molecule are also important factors. For example, with polyethylene oxide adhesive strength increases even up to molecular weights of 4,000,000; these polymers are well know to contain molecules of hightly linear configuration, which contribute to interpenetration with dextran. Molecules with molecular weights as high as 19,500,000 do not exhibit better bioadhesion than molecules with a molecular weight of 200,000 (Smart et al., 1984).

Concentration of Active Polymer

Bremecker relates that there is an optimum concentration of polymer corresponding to the best bioadhesion. In highly concentrated systems, the adhesive strength drops significantly. In fact, in concentrated solutions, the coiled molecules

become solvent poor and the chains available for interpenetration are not numerous. This result seems to be of interest only for more or less liquid bioadhesive forms. For solid dosage forms such as tablets, showed that the higher the polymer concentration, the stronger the bioadhesion.

Flexibility of Polymer Chains

Flexibility is important for interpenetration and entanglement. As water-soluble polymers become cross-linked, the mobility of the individual polymer chain decreases. As the cross-linking density increases, the effective length of the chain which can penetrate into the mucus layer decreases even further and mucoadhesive strength is reduced.

Spatial Conformation

Besides molecular weight or chain length, spatial conformation of a molecule is also important. Despite a high molecular weight of 19,500,000 for dextrans, they have adhesive strength similar to that of polyeth-ylene glycol, with a molecular weight of 200,000. The helical conformation of dextran may shield many adhesively active groups, primarily responsible for adhesion, unlike PEG polymers, which have a linear conformation.

Environment-Related Factors

pH

pH was found to have a significant effect on mucoadhesion as observed in studies of polyacrylic polymers cross-linked with COOH groups. pH influences the charge on the surface of both mucus and the polymers. Mucus will have a different charge density depending on pH because of differences in dissociation of functional groups on the carbohydrate moiety and amino acids of the polypeptide backbone.

Robinson and his group observed that the pH of the medium was critical for the degree of hydration of highly cross-linked polyacrylic acid polymers, increasing between pH 4 and pH 5 continuing to increase sligntly at pH 6 and pH 7, and decreasing at more alkaline pH levels. This behavior was attributed to differences in charge density at the different pH levels.

Polycarbophil shows maximum adhesive strength at pH 3; the adhesive strength decreases gradually as the pH increases up to 5. Polycarbophil does not show any mucoashesive property above pH 5. This study (Smart et al., 1984), the first systematic investigation of the mechanism of mucoadhesion, clearly shows that the protonated carboxyl groups rather than ionized carboxyl groups react with mucin molecules, presumably by numerous simultaneous hydrogen bonding reactions. At pH above 5, polycarbophil swells to a larger extent than at pH 3 or below. At high pH, however, the chains are fully extended because of the electrostatic repulsion of carboxylate anions. The polymer chains are also repelled by the negatively charged mucin molecules. It has been also observed that, due to hydrogen bonding between hydroxypropyl cellulose and carbopol 934, interpolymer complexes form at pH values below 4.5.

Applied Strength

To place a solid bioadhesive system, it is necessary to apply a defined strength. Whatever the polymer, poly[acrylic acid/divinyl benzene poly (HEMA)] or carbopol 934, the adhesion strength increases with the applied strength or with the duration of its application , up to an optimum. The pressure initially applied to the mucoadhesive tissue contact site can affect the depth of interpenetration. If high pressure is applied for a sufficiently long period of time, polymers become mucoadhesive even though they do not have attractive interactions with mucin.

Initial Contact Time

The intial contact time between mucoadhesives and the mucus layer determines the extent of awelling and the interpenetration of polymer chains. Along

with the initial pressure, the initial contact time can dramatically effect the performance of a system. The mucoadhesive strength increases as the initial contact time increases. However, longer initial contact time should be based on tissue viability. In case of mucoadhesives that need to be polymerized at the application sites, the initial contact time is critical. It is easity controlled when mucoadhesives are applied to exposed areas such as eye, nose, or mouth. For the application of mucoadhesives to the GI tract, however, the initial contact time cannot be controlled, which is one of the difficulties in applying mucoadhesives to the GI tract (Chien et al., 1994).

Selection of the Model Substrate Surface

The handling and treatment of biological substrates during the testing of mucoadhesives is an important factor , since physical and biological changes may occur in the mucus gels or tissues under the experimental conditions. The viability of the biological substrate should be confirmed by examining properties such as permeability , electrophysiology , or histology. Such studies may be necessary before and after performing the in vitro tests using tissues.

Swelling

The swelling characteristic is related to the polymer itself , and also to its environment. Interpenetration of chains is easier as polymer chains are disentangled and free of interactions. Swelling depends both on polymer concentration and on water presence. When swelling is too great, a decrease in bioadhesion occurs; such a phenomenon must not occur too early, in order to lead to a sufficient action of the bioadhesive system. Its appearance allows easy detachment of the bioadhesive system after the discharge of the active ingredient.

Bioadhesive Polymer

To overcome the relatively short gastrointestinal time and improve localization for oral-controlled or sustained-release drug delivery systems,

bioadhesive polymers which adhere to the mucin/epithelial surface are effective and lead to significant improvement in oral drug delivery. Improvements are also expected for other mucus-covered sites of drug administration. Bioadhesive polymers find application in the eye , nose , and vaginal cavity as well as the gastrointestinal tract, including the buccal cavity and rectum (Park and Robinson, 1984).

Polymers that adhere to the mucin-epithelial surface can be conveniently divided into three broad categories: (a) polymers that become sticky when placed in water and owe their bioadhesion to stickness; (b) polymers that adhere through nonspecific, noncovalent interactions which are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant); (c) polymers that bind to specfic receptor sites on the cell surface. All three polymer types can be used for drug delivery. Polymers which can adhere to either hard or soft tissue have been used for many years in surgery and dentistry. Among these "superglues," polymers and monomeric alpha-cyanoacrylate esters have been most frequently investigated and used. Other synthetic polymers such as polyurethanes, epoxy resins, polystyrene, acrylates, and cements from natural products were also extensively investigated, as were glues.

An ideal polymer for a mucoadhesive drug system should have the following characteristics.

1. The polymer and its degradation products should be nontoxic and nonabsorbable from the gastrointestinal tract.
2. It should be a nonirritant to the mucous membrane.
3. It should preferably form a stong noncovalent bond with the mucin-epithelial cell surfaces.
4. It should adhere quickly to moist tissue and should possess some site specificity.
5. It should allow easy incorporation of the drug and offer no hindrance to its release.
6. The polymer must not decompose on storage or during the shelf life of the dosage form.

7. The cost of the polymer should not be high , so that the prepared dosage form remains competitive.

Robinson and his group (Park and Robinson, 1984), using the fluorescence techinque, concluded that:

1. Cationic and anionic polymers bind more effectively than neutral polymers.
2. Polyanions are better than polycations in terms of binding/potential toxicity; and further, that water-insoluble polymers give greater flexibility in dosage form design compared to rapidly or slowly dissolving water-soluble polymers.
3. Anionic polymers with sulfate groups bind more effectively than those with carboxylic groups.
4. Degree of binding is proportional to the charge density on the polymer.
5. Highly binding polymers include carboxymethyl cellulose, gelatin, hyaluronic acid, carbopol and polycarbophyl.

Role of pH on bioadhesion is of great importance; with maximum adhesion being observed from pH 5 to 6.

Bioadhesive Powder Systems

Nagai and coworkers investigated the use of bioadhesive power dosage forms for the administration of peptides such as insulin to the nasal cavity (Nagai et al., 1984; Nagai and Machida, 1985). The bioadhesive agents studied, in combination with freeze-dried insulin, were crystalline cellulose, hydroxypropyl cellulose (HPC), and Carbopol 934. All formulations tested gave significant decreases in plasma glucose level when administered nasally to the dog and rabbit models. Freeze-dried insulin powders alone had relatively good bioavailabilities, which were not improved by the addition of the soluble and nonbioadhesive material lactose. A very effective formulation was crystalline cellulose blended with freeze-dried insulin, which resulted in a fast decline in the glucose level to 49% of the control value. Addition of HPC or

Carbopol 934 to the freeze-dried insulin resulted in prolonged decreases of the plasma glucose levels but the extent of the decrease (the trough) was not affected. However, when Carbopol was freeze dried with the insulin and added to the crystalline cellulose powder, the effect of the formulation was considerably improved, leading to a hypoglycemia of the order of one third of the effect obtained after intravenous injection of the same dose of insulin. With the addition of increasing concentrations of Carbopol, the trough in the plasma glucose was increasingly delayed. The effect of the formulation was attributed to the formation of a gel in contact with the mucus, which prolonged the residence of the formulation in the nasal cavity. It was reported that the insulin-Carbopol-crystalline cellulose powder formulation was readily acceptable in term of tolerability when administered to human volunteers; however the plasma glucose levels obtained in the volunteers were quite variable (Nagai and Machida, 1985).

The potential of various powder formulations to enhance the nasal absorption of octreotide was studied in vivo in the anesthetized rat model (Oechslein et al., 1996). The powder formulations were also characterised in vitro in terms of calcium binding, water uptake, and drug ralease. The powder formulations were prepared by dry blending of octreotide with microcrystalline cellulose, semicrystalline cellulose, hydroxyethyl starch, cross-linked dextran, microcrystalline chitosan, pectin, and alginic acid. The bioavailabilities obtained for all of the powder formulations were low, with values ranging from 0.59% for the control to 5.56% for the cross-linked dextran powder formulation. The ranking of the formulations, in terms of absorption-enhancing effect, coincided with the ranking in terms of calcium binding properties, cross-linked dextran powder having the highest degree of calcium binding. No correlation was found between adsorption-enhancing effect and water uptake. The ralease of drug from all powder formulations was complete within 10 minutes, which may explain the low bioavailabilities obtained for the various formulations. These results were suprising in light of the results obtained by Nagai and coworkers for insulin and also because it had been suggested by several authors that a reason for the improvement in absorption obtained for bioadhesive powder systems was their ability to take up water, from a gel, and increase the residence time in the nasal cavity. An additional factor that has been proposed is that the uptake of water by the powder can

result in shrinkage of the cells and opening of tight junctions (Edman et al., 1992; Illum et al., 1994b).

It was shown by Provasi et al. (1992) that the administration of salmon calcitonin in powder formulations containing lactose and colloidal silica (a swellable polymer) can improve the nasal absorption of the drug in rats as compared with a simple nasal solution of salmon calcitonin. It was also shown that a colyophilized formulation was more effective than a dry blend formualtion. No bioavailability values were given in the paper, and hence the results are difficult to compare with the work of Oechslein et al. (1996). Sakr (1996) investigated the enhancing effect of dimethyl-β-cyclodextrin on the nasal absorption of glucagon when administered as a solution formulation or a freeze-dried powder formulation. The freeze-dried formualtion was prepared from the solution formulation. No bioadhesive agent was added. No improvement was found when using the freeze-dried formualtion as compared with the solution formulation, which is not surprising, because the formualtion was readily soluble. Similarty, Vermehren et al. (1996) used freeze-dried powders consisting of methylcellulose and α-cyclodextrin, with or without the phospholipid didecanoylphosphatidylcholine (DDPC), to improve the nasal absorption of human growth hormone. There was no direct comparison with similar solution formulations; however, the paper mentions previously obtained bioavailabilities of about 20% in rabbits. This value is very similar to the result obtained for the powder formulations. Likewise.Marttin et al. (1997) found no difference in bioavailability for dihydroergotamine administered nasally to rabbits as a spray formulation or as a powder with methylated β-cyclodextrin. This is probably not surprising, considering that no bioadhesive material was added to the powder formulation.

Also of interest is the study by De Ascentiis et al. (1996) in which the effect of particle size of nasal powders of β-cyclodextrin containing progesterone on delivery behavior (including bioavailability) was studied). Progesterone-β-cyclodextrin powders were prepared by granulation with polyvinylpyrrolidone or mannitol and sieved into particle fractions of 0-45, 45-63, 63-88, 88-125, 125-180, 180-250, and 250-355 μm, and the various fractions administered nasally with a nasal powder

insufflator (Miat S.p.A). It was found that the rate of delivery from the insufflator decreased with decreasing particle size, whereas the time needed for full emission of the dose through the nose adapter increased with decreasing particle size in the range 50-150 μm. Furthermore , it was found that the particle size had an effect on the compactness of the cloud produced, which again would influence the impaction or sedimentation pattern of the particles after administration to the nasal cavity.

Bioadhesive Microsphere Systems

The use of bioadhesive microspheres for the nasal delivery of drugs that were poorly absorbed was first suggested in 1987 by our group (Illum, 1987; Illum et al., 1987). The rationate behind this suggestion was that the application of bioadhesive microspheres (in powder form) with good bioadhesive properties would permit such microspheres to swell in contact with the nasal mucosa to form a gel and control (decrease) the rate of clearance from the nasal cavity, thereby giving poorly absorbed drugs a longer time to be available at the absorptive surface for absorption. Microspheres made from DEAE-dextran (DEAE-Sephadex), starch microspheres (Spherex), and albumin microspheres, about 25-50 μm in diameter, were radiolabeled, and it was shown in a gamma scintigraphy study in human volunteers that after nasal administration they were cleared significantly more slowly than solution and nonbioadhesive powder formulations. At 3 hours after administration, 50% of the initial amount of albumin and starch microspheres and 60% of the DEAE-dextran microspheres were still present in the nasal cavity, whereas the half-life of clearance of the control formulations was about 15 minutes (Illum et al., 1987). Similar results were later obtained by Ridley et al. (1995) for starch microspheres in human volunteers, with a half-life of clearance of about 20 minutes for a simple solution and nearly 2 hours for starch microspheres. No effect on the clearance pattern could be attributed to the posture (i.e.,seated or supine) during administration.

The promoting effect of bioadhesive microspheres on the absorption of a poorly absorbable drug, when given nasally, was first demonstrated by Illum et al. (1988) using gentamicin. In this study gentamicin, as a solution formulation, with and without the enhancer lysophosphatidylcholine (LPC) and freeze dried with starch

microspheres with and without the addition of LPC was admnistered nasally to sheep. It was shown that the drug alone was absorbed to a negligible degree and that the addition of LPC only marginally improved the absorption from a solution. In contrast, the use of the freeze-dried bioadhesive microsphere formulation increased the bioavailability to 10% and the further addition of the LPC improved the bioavailability to 57%. This study showed that the bioadhesive microsphere concept worked in that the absorption was considerably improved. Surprisingly, the enhancer LPC improved the effect five-to sixfold, although when given as a solution the LPC had only a marginal effect on the absorption of gentamicin.

Further work was carried out on the effect of starch microspheres on the nasal absorption of peptides and proteins, both by our group and by Edman and others. Farraj et al. (1990) showed that the nasal absorption of insulin in sheep was improved from a bioavailability less than 1% to 11% and 32% when administered as a freeze-dried powder with starch microspheres without and with the addition of LPC, respectively. Similar effects were found for desmopressin (Critchley et al., 1994) and growth hormone (Illum et al., 1990). Bjørk and Edman (1988) obtained similar results for insulin in the rat model, with the bioavailability being as high as 30%. The reason for the better effect in the rat model is probably the impairment in mucociliary function observed in this anesthetized animal model. The same group later investigated the effect of degree of cross-linking (and thereby the degree of swelling of the starch microspheres) on the absorption-promoting ability (Bjørk and Edman, 1990). These authors found that although the release of insulin from the microspheres was affected by the degree of cross-linking, with the slowest release from highly cross-linked microspheres, there was no singnificant effect on the absorption-promoting ability. It was later suggested that the mechanism of action of the starch microspheres was a combination of the bioadhesive effect keeping the formulation for a longer time in the nasal cavity and the dry microspheres absorbing water in the nasal cavity and thereby opening up tight junctions (Bjørk and Edman, 1990; Edman et al., 1992; Bjørk et al., 1995). It was also shown by the same authors that the starch microspheres had little toxic effect on the nasal mucosa after up to 8 weeks of daily application in rabbits (Bjørk et al., 1991) and no adverse effect on the human mucociliary clearance mechanism (Bjørk et al., 1992; Holmberg et al., 1994).

A range of other bioadhesive microspheres has been used for the nasal administration of peptides and other drugs. Sephadex and DEAE-Sephadex (cross-linked dextran) microspheres, which are water insoluble and water absorbable, were shown to improve, to a lesser degree than starch microspheres, the nasal absorption of insulin (Edman et al., 1992; Ryden and Edman, 1992a). The same microspheres have also been suggested for the nasal delivery of nicotine (Cornaz et al., 1996) but no in vivo data have so far been presented. Pereswetoff-Morath and Edman (1995b) found an apparent difference in absorption-promoting efffect between Sephadex G-25 and G-50 microspheres administered with insulin in rats; G-25 did not allow the absorption of insulin into the spheres whereas G-50 did. It was found that G-25 microspheres, where insulin was on the surface, had a greater absorption-enhancing effect, although no differences were seen in vitro between drug release rates. No apparent toxic effect was found from the Sephadex microspheres on cilia beat frequency (Pereswetoff-Morath et al., 1996). Nor were they found to be immunogenic (Pereswetoff-Morath and Edman, 1996). Microspheres produced from polyacrylic acid and incorporating disodium cromoglycate were shown in a human clinical trial to decrease the clearance rate in the nasal cavity, with 50% remaining after 30 minutes compared with 27% for a control (Vidgren et al., 1991).

The use of hyaluronic acid ester microspheres for the nasal delivery of insulin in sheep was investigated by our group (Illum et al., 1994b). The increase in nasal absorption of insulin so achieved was found to be independent of the dose of microspheres in the range 0.5-2.0 mg/kg. The mean bioavailability of the system was found to be 10%, similar to what was previously achieved for starch microspheres in the sheep model. The mucoadhesive properties of these microspheres were not measured, but hyaluronate esters are know to have excellent adhesive properties. Because the microspheres expressed a very low degree of swelling in water, the full mechanism of action for the absorption improvement (including bioadhesion) was not known.

Thus, a wealth of literature shows the excellent absorption-enhancing properties of bioadhesive microsphere systems in combination with both low-molecular-weight drugs and peptides and proteins. However, very few studies have

compared the effect of bioadhesive powder and microspheres made from the same material. Thus, it is difficult to conclude which would generally give the better effect. It was shown by Bjørk and Edman (1990) that microspheres made from starch and insoluble starch powder gave a similar decrease in glucose levels after nasal administration with insulin to rats. Soluble starch, however, showed no absorption enhancement. In comparison, our group has shown in sheep that starch microspheres gave a better absorption-enhancing effect than insoluble starch powder (unpublished data).

GENERAL FACTORS TO CONSIDER IN DEVELOPING A CELL CULTURE MODEL SYSTEM FOR DRUG TRANSPORT AND METABOLISM STUDIES

In order to mimic successfully a biological barrier with an in vitro cell culture system, the selection of the cell line becomes particularly important. The transport and metabolism properties of cultured cells can vary depending on (a) whether the cells are primary cultures, passaged lines, or transformed lines; (b) the number of times the cells have been passaged; (c) the phenotypic stability of the cell line; (d) the heterogeneity of the cell line; and (c) the inherent ability of the cell line to undergo differentiation. Once the cell line has been selected, the properties may vary depending on (a) the cell seeding density; (b) whether the cells have reached confluency; (c) the stage of cellular differentiation; and (d) the presence or absence of essential nutrients growth factors, or associated cells that produce trophic factors. During transport experiments the properties may change depending on (a) the composition of the transport medium (e.g., concentration of the solute, temperature, pH, presence or absence of a metabolic source of energy or ions, presence or absence of proteins that might bind the solute, presence or absence of competing solutes) and (b) whether the solute is added to the apical or basolateral side of the monolayer. All of these factors need to be carefully optimized and regulated so as to best mimic the biological barrier in vitro.

The development of a cell culture system that will mimic a specific biological barrier requires not only an appropriate cell line but also a microporous membrane,

which by itself or after treatment with an appropriate matrix material (e.g., collagen will support cell attachment and cell growth. Ideally, the microporous membrane should also be (a) sufficiently translucent so that the development of the cell monolayer can be verified by microscopic techniques; (b) readily permeable to hydrophobic solutes; and (c) readily permeable to both low and high molecular weight solutes.

Many microporous membranes for cell culture (e.g., polycarbonate, nitrocellulose) are now commercially available in different surface areas and different pore sizes. Careful selection of the microporous membrane, including the physical-chemical properties of the membrane, its pore size and surface area, and the nature and thickness of the supporting matrix (e.g., collagen), is critical so as to avoid generating artifactual data in transport experiments. As illustrated in Fig. 1, the ideal diffusion characteristics of a cell culture model system occur when the major diffusion barrier is provided by the cell monolayer and not the microporous membrane or the supporting matrix. In conducting transport studies with cell cultures on microporous membranes, it is essential that control experiments be conducted using the microporous membrane alone and the microporous membrane coated with the supporting matrix. The results of these experiments will assure that the solute is freely permeable through the microporous membrane and the supporting matrix and that the diffusion barrier is provided by the cell monolayer.

Another critical factor, particularly in the study of the transport of lipophilic molecules, is the selection of the diffusion apparatus. Whether the diffusion apparatus is stagnant or stirred can influence the thickness of the aqueous boundary layer on the surface of the cell monolayer (Figure 2) and, thus, the permeability of lipophilic solutes. The types of diffusion apparatus currently employed for studying transport across cell monolayers include (a) the unstirred cell-insert system (Figure 2A, B), (b) the side-by-side diffusion system stirred mechanically (Figure 2C), and (c) the side-by-side diffusion system stirred by gas lift (Figure 2D). The gas lift system was recently developed in our laboratory specifically for conducting transport studies on cell cultures grown in "mini" cell inserts. The stirring provided by the O_2 / CO_2 gas

lift system produces minimal damage to the cell monolayer and also minimizes the thickness of the aqueous boundary layer (Hidalgo et al., 1989).
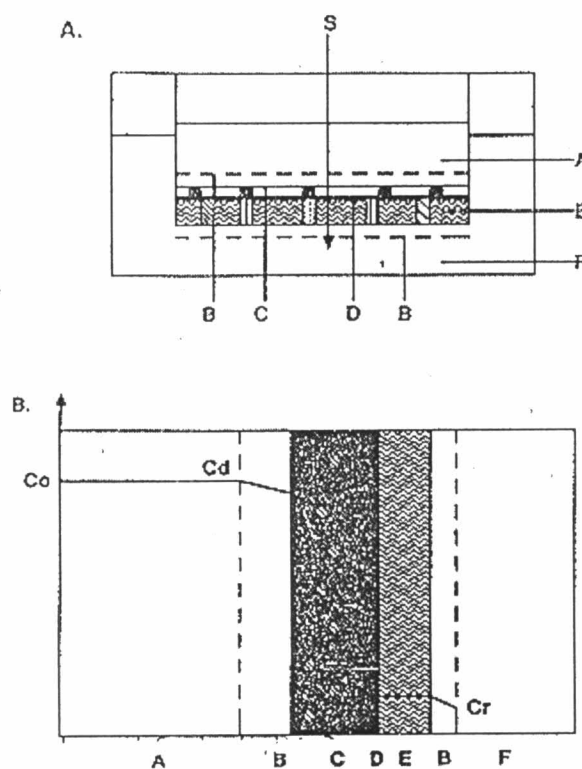

Figure 2 Potential barriers to solute transport in a cell culture systems grown onto a microporous membrane. (A) Monolayer cell culture system grown onto a microporous membrane; (B) concentratial profile for solute (S) with the largest concentration drop product by the cell monolayer. A, apical side; B, aqueous boundary layer; C, cell monolayer; D, supporting matrix; E, microporous membrane F, basolateral side; Co, original concentration of solute; Cd, donor side concentration; Cr, receiver side concentration.

Thus, in developing a cell culture model system mimic an in vitro biological barrier, care needs to be taken selecting the cell line, the microporous membrane, the supporting matrix, the culting conditions, the conditions for conducting transport studies, and the diffusion apparatus. Once these variables have been optimized, the system can be utilized by the pharmaceutical scientists for drug transport and metabolism studies.

Since it is possible readity to manipulate the experimental conditons in a cell culture system, these in vitro model have tremendous potential to help in the elucidation of the various pathways by which a drug could penetrate a biological barrier. Experiments can be designed to determine whether the permeability of a small solute is via passive diffusion, active or facilitated diffusion, and / or paracellular diffusion. For macromolecules, experiments can be design to determine whether the molecule penetrates the barrier of a paracellular or transcellular mechanism (e.g., fluid phase absorptive, or receptor-mediated transcytosis. These systems are also potentially useful in elucidating the mechanism by which adjuvants enhance intestinal absorption and why some drugs partition preferentially into the lymphatic system. Most importantly, these systems may provide scientists with new, basic information about transport mechanisms biological barriers that will permit them to develop novel strategies for targeting drugs to specific tissue compartments or enhancing drug permeability through now impermeable biological barriers.

Nasal Mucosal Cells

The existence of a large surface area for absorption, extensive underlying vascularization, and the absence of first-pass metabolism are a few of the characteristics that make the nasal cavity a desirable site for durg delivery. Davelopment of the systemic delivery systems, particularly for peptides and proteins, through the intranasal route has been more or less continuously pursued since the beginning of this century.

Two basic cell types line the nasal cavity, a stratified squamous and a pseudostratified columnar epithelia. The former cell type is an extension of the facial skin and extends from the anterior portion of the nose to the skull entrance of the nasal cavity. This epithelium contains hair and sebaceous glands and presents the first defense against particulate material. Absorption of substances in this area would be subject to restrictions similar to those observed for dermal drug delivery. The second cell type of the nasal cavity, the pseudostratified columnar epithelium, begins at the shull entrance and extends down throughout the tracheobronchial tree. At the skull entrance the nasal cavity is divided by the septum centrally and convolutes laterally

into turbinate projections, substantially increasing the surface area of inside the skull. The absorption area for most drug formulations, and therefore the subject of our discussion here, generally extends from the skull entrance to the nasopharyngeal region of the nasal cavity. Absorption of substances in this area of the nasal cavity is subject to interactions with at least a mucin layer, degradative enzyme systems, and passage across a single columnar epithelial layer before reaching the systemic circulation. (Audus et al., 1990)
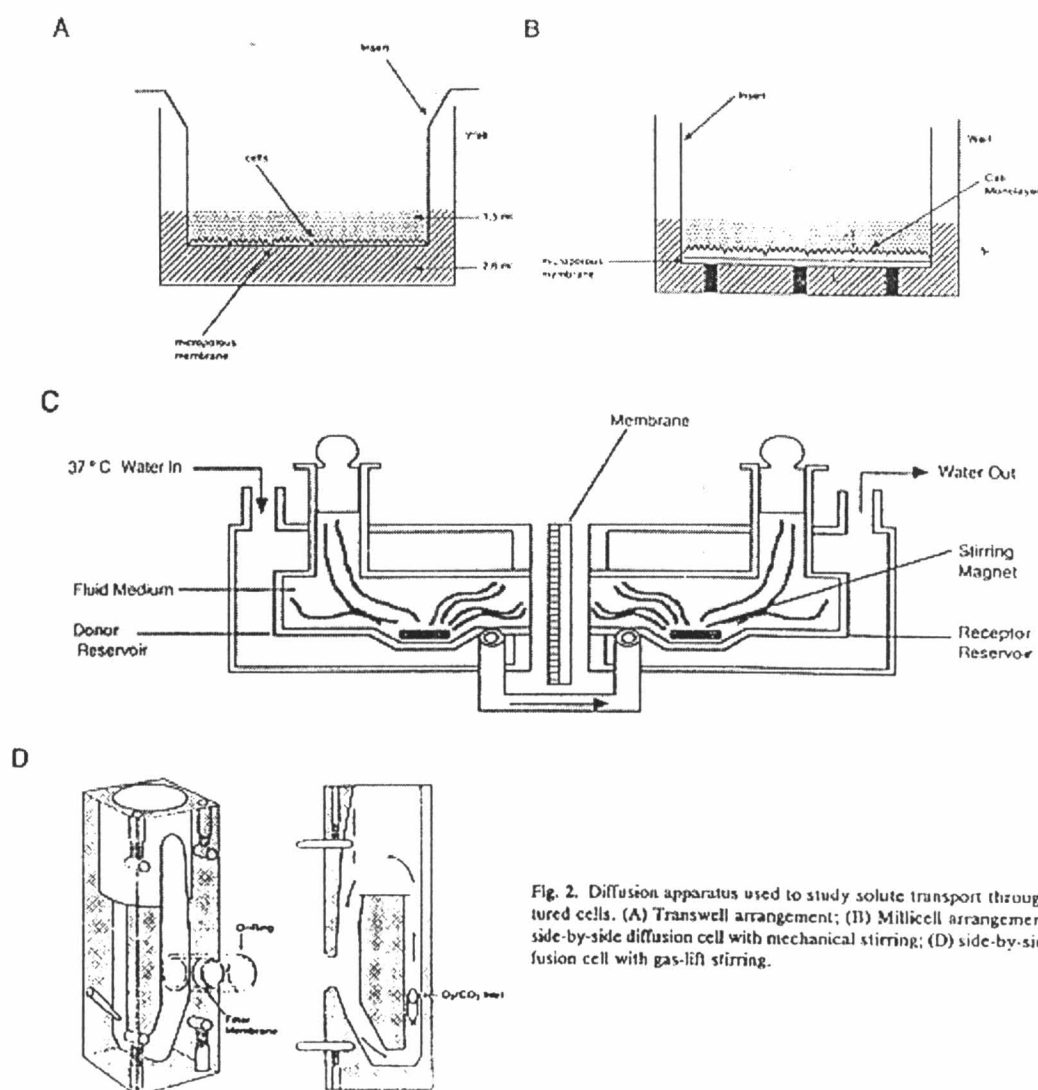

Fig. 2. Diffusion apparatus used to study solute transport through cultured cells. (A) Transwell arrangement; (B) Millicell arrangement; (C) side-by-side diffusion cell with mechanical stirring; (D) side-by-side diffusion cell with gas-lift stirring.

Figure 3 Diffusion apparatus used to study solute transport through cultured cells. (A). Transwell arrangement; (B) Millicell arrangement; (C) side-by-side diffusion cell with mechanical strring; (D) side-by-side diffusion cell with gas-lift stirring.

Development of a nasal cell culture model requires establishment of a complex mixture of cell types. In a representaive region of normal nasal mucosa, the turbinate region, the pseudostratified columnar epithelium is the predominate cell type, existing in either ciliated (15-20%) or nonciliated (60-70%) form. Both cell types express numerous microvilli. The role of the cilia is protective and moves the overlying mucous layer containing entrapped particulates to the nasopharyngeal area for expectoration or swallowing. The columnar epithelial cells also express substantial numbers of mitochondria, suggesting a high metabolic activity. A third cell type, the goblet cells, comprises about 5-15% of mucosal cells, depending on age, and are responsible for secretion of carbohydrate-rich constituents of the mucous layer. Columnar epithelial and goblet cells within this single layer of cells form tight intercellular junctions near the apical surface which forces transepithelial diffusion or transport of materials from the nasal cavity to the systemic circulation. Minor numbers of other cell types are found in this region which do not extend to the nasal cavity surface and include the progenitor basal cells which differentiate to the above cell types, occasional macrophages, leukocytes, and neurosecretory cells. Occasional submucosal glands can extend to the nasal cavity surface and are responsible for serous and mucous secretions. (Audus et al., 1990).

Several laboratories, including our own, are exploring the potential application of nasal cell cultures in drug delivery reserch. At least two problems quickly confront researchers in this area. The first problem is the absence of a substantial literature base on the fundamental biochemistry, cell biology, and physiology of nasal epithelium in vitro, which is important for validating an in vitro model. The second problem is the complexity of the nasal mucosa (e.g., the multiple cell types present), somewhat analogous to the gastrointestinal mucosa. For the purposes of this review we identify three general types of nasal tissue culture systems currently in use. These systems include explants, primary cultures, and cell lines.

The first type of nasal tissue culture system has been developed from either excised rat nasal septal epithelium or excised human and hamster tracheal epithelium. In both instances, the tissue explants attached and grew in culture to form a morphologically similar to the parent tissue. Rat septal explants grown in this system

have been proposed as a model to study the role of nasal mucosa in the activation of nitrosoamines as nasal-specific carcinogens. The hamster tracheal explants have been used in structure-activity to screen retinoids for ability to prevent cancer and, more recently, to study the role of cigarette smoking and vitamin A in modulating epithelial cell growth and function. As in other explant cell culture systems, the possibility exists that normally minor numbers of nonepithelial cell types may overgrow in culture and introduce complicating factors into interpretation of experimental results.

The second type of nasal tissue culture systems has been developed by the enzymatic dissociation and isolation of human and animal nasal epithelial cells. Several examples of this system have been described as origination from tissues extracted form either the turbinate regions of the nasal cavity or the tracheobronchial regions of the respiratory system (Warner and Thomas, 1995). These culture systems are generally comprised of monolayers of nasal epithelial cells exhibiting biochemical characteristics similar to those of the in vivo parent tissue. Attempts at duplicating obvious morphological properties (e.g.,columnar shape, cilia, microvilli, and extensive intercellular interdigitation) in vivo, however, have been most successful in those in vivo systems employing tracheal epithelium. This has been disappointing from the standpoint of intranasal drug delivery applications, however, since development of an appropriate tissue culture system from epithelia of the septal or turbinate regions of the nasal cavity seems more applicable. These tissue culture systems are currently being utilized to identify basic functional and biochemical characteristics that distinguish both normal and diseased tissues. For example, recent studies have examined ion permeability, electrical resistance, lectin histochemistry, hematopoietic growth factor production, and proteoglycan synthesis in the various tissue culture systems derived from either normal or cystic fibrosis tissue. The emerging information on the permeability and biochemistry of these primary culture systems should provide an excellent basis for exploring the applications of these models for drug delivery problems.

The third general nasal cell culture type is represented by at least three cell lines. These cell lines are RPMI 2650 (i.e., derived from cancerous human septum) and BT (i.e., derived from normal bovine turbinate), both available from American

Type Culture Collection (Rockville, MD), and NAS 2BL [i.e., derived from rat nasal epithelial squamous carcinoma]. Cell lines in tissue culture work can offer convenience and reproducibility in many applications. However, little information on the use of nasal epithelial cell lines in drug delivery studies exists. Preliminary studies in our laboratory have been performed with the BT cell line (M.R.Tavakoli-Saberi and K.L. Audus, unpublished results). These cells will grow to form monolayers on various collagen matrices both on plastic and in Transwell culture systems. In contrast to the typical pseudostratified columnar epithelium, the cells are flattened but express a few cilia and microvilli. Manipulation of culture conditions and growth substrates may improve morphological characteristics, but this remains to be demonstrated. The cells do retain expression of three aminopeptidases with kinetic parameters (M.R.Tavakoli-Saberi and K.L.Audus, unpublished results) similar to those described for rabbit nasal aminopeptidases. As indicated above, little published information is available on the potential research applications of these cell lines aside from that mentioned previously and preliminary carciogenesis studies.

The availability of several tissue culture systems now provides the opportunity to conduct much needed basic research on the nasal mucosa. The availability of these models also offers immediate and convenient in vitro systems to explore drug effects on nasal epithelial cell growth and function.

The Analysis of Dissolution Data of Controlled Release System.

The Release Mechanism of Controlled Release System.

In order to analyze the mechanism of the drug from the matrices, the dissolution data may be analyzed using the semiempirical equation of Peppas (1985) given below

$$\frac{M_t}{M_\infty} = kt^n \qquad [1]$$

Where M_t/M_∞ is the fraction of drug released up to and time t
t is the release time
k is a constant incorporating structural and geometric characteristics
of the controlled device

and   n is the diffusional release exponent indicative of the mechanism of release

The determination of the exponent n is valid for the first 70% of the total released drug ($M_t/M_\infty \leq 0.7$), which also applied only to the early times of release.

Clearly, a desirable mechanism for many applications is that which leaded to n equal to 1, which characterized zero-order release behavior. Table 1 summarized the general dependence of n on the diffusional mechanism (Peppas, 1985).

Table 1 Interpretation of diffusional release mechanisms form drug release data from thin polymeric film

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5 < n < 1.0	Anomalous (non-fickian) Transport	t^{n-1}
1.0	Case- II transport	Zero-order release (time-independent)
n > 1.0	Super-Case-II transport	t^{n-1}

The empirical equation 1 could be modified for the application to non-planar geometries. The relationship between the diffusional exponent (n) and the corresponding release mechanism is clearly dependent upon the geometry employed as shown in Table 7 (Ritger and Peppas, 1987A).

In non-swellable metrices, the values of n are 0.45 and 1.00 for Fickian and Case-II transport, respectively. Case II transport is a special case readily identified and characterized by the constant velocity of the moving solvent front and the resulting linear weight gain with time. However, its characteristics are not as well understood, nor are they as fundamental in origin as those of Fickian diffusion (Tyle, 1990). When the value of n is in the range of 0.45 to 1.00, the release mechanism was said to be non-Fickian diffusion (Ritger and Peppas, 1987A). A value of n=1, however, mean that the drug release is independent of time, regardless of the geometry. Thus, zero-order release can exist for any geometry.

In swellable controlled release systems, Case-I (Fickian diffusion) and Case-II solute release behaviors are unique in that each can be described in the term of a single parameter. Case-I transport is described by a diffusion coefficient, while Case-II transport is described by a characteristic relaxation constant. Non-Fickian behavior, by comparison, required two or more parameters to describe the coupling of diffusion and relaxation phenomena.

Table 2 Diffusional exponent and mechanism of diffusional release from various non-swellable controlled release systems.

Diffusional Exponent, n			Drug Release Mechanism
Thin Film	Cylindrical Sample	Spherical Sample	
0.5	0.45	0.43	Fickian diffusion
$0.5 < n < 1.00$	$0.45 < n < 1.0$	$0.43 < n < 1.00$	Anomalous(non-Fickian) Transport
1.0	1.0	1.0	Zero-Order Release

In swellable matrices, when the system did not swell more than 25% of its original volume, the values of n are 0.45 and 0.89 for Fickian and Case-II transport, respectively. When the value of n is >0.45 and <0.89, the release is said to be non-Fickian (Ritger and Peppas, 1987B). While the value of n is greater than that of the Case-II transport, the release is said to be Super Case-II transport. Table 3 summarized the range of values of diffusional exponent (n) and the related transport mechanism for each geometry (Ritger and Peppas, 1987B). A value of n is as same as 1 mean that drug release is not a function of time, regardless of geometry. Thus, zero-order release kinetic can exist for any geometry; only for slabs did this release coincide with Case-II transport.

Hogan (1989) examined the dissolution curves of the drugs (promethazine hydrochloride, aminophylline and propranolol hydrochloride) with different HPMC quantity and concluded that as the polymer fraction increase, the dissolution of the drug from device are decrease. The kinetics of drug release can be investigated by using equation 7. The promethazine hydrochloride and diazepam matrices appear slightly higher values of n at low level HPMC content. The values of n are nearly similar (0.65-0.71) for highly soluble drugs promethazine hydrochloride, aminophylline and propranolol hydrochloride and additional theophylline (0.64). The values of n for these drugs are closely to the values predicted for diffusional release. The n values for the two poorly soluble drugs are 0.82 and 0.9 for diazepam and indomethacin, respectively. Thus the values of n obtained for indomethacin and diazepam merely emphasized that release kinetic for these drugs is not Fickian-controlled and indicates large contribution by tablet erosion to drug release. The anomalous behavior for tetracycline matrices with a value of n=0.45 emphasized the complexity of release. Peppas (1985) did not interpret n values of n < 0.5 but stated that such occurrences are an indication of statistical analysis problems or are due to diffusion through a polymeric network where diffusion can occur partially through a swollen matrix and partly through water-filled pores. It is possible that tetracycline hydrochloride undergoes a complexation reaction with HPMC in the gel state in the hydrating matrix in which retarding its release

Table 3 Diffusional exponent and mechanism of drug from various swellable controlled release systems.

Diffusional Exponent (n)			Drug Release Mechanism
Thin Film	Cylindrical Sample	Spherical Sample	
0.5	0.45	0.43	Fickian diffusion
$0.5 < n < 1.00$	$0.45 < n < 0.89$	$0.43 < n < 0.85$	Anomalous (non-Fickian) transport
1.0	0.89	0.85	Case-II transport

The release pattern of controlled release system

The pattern of delivery achieves by a controlled release system can vary over a wide range, but most of all release profiles are categorized into three major types:
1. Zero order release pattern
2. Square root time release pattern
3. First order release pattern

Zero-order model

An ideal controlled release device is one which can deliver the drug at a constant rate until the device is exhausted of active agent. Mathematically, the release rate from this device is given as

$$\frac{dM_t}{dt} = k \qquad [2]$$

where k is a constant, t is time and the mass of active agent released was M_t.

This pattern of release is so called zero–order release model.

Square root of time model (Higuchi model)

The second common release pattern, frequently referred to square-root-of-time or $t^{1/2}$ release, provides compound release that is linear with the reciprocal of the square root of time. The release rate then given as

$$\frac{dM_t}{dt} = \frac{k}{\sqrt{t}} \qquad [3]$$

In contrast to first-order release, the release rate here remained finite as the device approached exhaustion.

The release pattern of this type can be described by Higuchi equation (Higuchi, 1963)

$$Q = \left[\frac{D\varepsilon}{\tau} (2A - \varepsilon C_s) C_s t \right]^{1/2}$$ [4]

Where Q = weight in grams of drug released per unit surface area
D = diffusion coefficient of drug in the release medium
ε = porosity of the matrix
τ = tortuosity of matrix
Cs = solubility of drug in the release medium
A = concentration of drug in the tablet, expressed as g/ml

The assumptions made deriving equation 4 are as follows.

1. A pseudo-steady state is maintained during release.
2. A >> C s, i.e., excess solute is present.
3. The system is in perfectly sink condition in which Cs approximately to zero at all time.
4. Drug particles are much smaller than those in the matrix.
5. The diffusion coefficient remains constant.
6. No interaction between the drug and the matrix occurs for purposes of data treatment, equation 4 is usually reduced to

$$Q = k_H t^{1/2}$$ [5]

Where k_H is Higuchi constant. Therefore, the plot of amount of drug released from matrix versus the square root of time should be increased linearly if drug release from the matrix is diffusion controlled. Although the above equation is based on release from a single face, it may be used to describe diffusion-controlled release from all surface matrixes.

In order to further verify that the release followed Higuchi model, Higuchi equation is converted into logarithmic form as

$$\log Q = \log k_H + \frac{1}{2} \log t$$ [6]

The plot of log Q versus log t must not only yield a straight line, but must have a slope of 0.5.

First order model

The first order pattern is the third common type of the release model. The release rate in this case is proportional to the mass of active agent contained within the device. The rate is then given as

$$\frac{dM_t}{dt} = k(M_0 - M_t) \qquad [7]$$

where M_0 is the mass of agent in the device at t=0. On rearrangement, this gave

$$\frac{dM_t}{dt} = kM_0\exp^{-kt} \qquad [8]$$

In first order model, therefore, the rate declined exponentially with time, approaching a release rate of zero as the device approached exhaustion.

On the assumption that the exposed surface area of matrix decreased exponential with time, Wagner (1969) suggested that drug release from most controlled-release matrices could be described by apparent first order kinetics, thus:

$$A_t = A_0 e^{-k_1 t} \qquad [9]$$

Where k_1 = first order release constant
A_0 = initial amount of drug
A_t = amount of drug remaining in the matrix at time t

Simplifying and taking the logarithm of equation 15 yielded

$$\log A_t = \log A_0 - \frac{k_1 t}{2.303} \qquad [10]$$

First order pattern can be predicted by plotting the logarithm of the percent of drug remaining against time. If first order model, linear relationship were obtained.

Sa, Bandyopadhyay and Gupta (1990) reported that the initial curvature of the plot may be obtained because of the presence of surface drug and they suggested to be ignored.

Since both the square root of time release and first order release plots are linear, as indicated by correlation coefficient, it is necessary to distinguish between the models.

The treatment was based upon use the differential forms of the first order and square root time equations (Schwartz, Simonelli and Higuchi, 1968).

For Higuchi model, the rate will be inversely proportional to the total amount of drug release in accordance with equation (Sa, Bandyopadhyay and Gupta, 1990)

$$\frac{dQ'}{dt} = \frac{k_H^2 S^2}{2Q'} \qquad [11]$$

where Q' = Q*S (S is the surface area of matrix). The rate predicted by first order model is given by:

$$\frac{dQ'}{dt} = kA_0 - kQ' \qquad [12]$$

where A = A_0- Q'. This indicated that rate will be proportional to Q'. The rates of release are determined by measuring the slopes at different points on the percent of drug release versus times curves.

The plot of rates of release versus 1/Q' is linear, indicating that the release was fitted with Higuchi model. If the plot of rate of release versus Q' is linear, indicating that first order model was operative.