THE ROLE OF SURFACTANT IN, AND A COMPARISON OF, THE PERMEABILITY OF PORCINE AND HUMAN EPITHELIA TO VARIOUS CHEMICAL COMPOUNDS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my original work and that I have not previously submitted it, in its entirety or in part, at any other university for a degree.

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SUMMARY

In this thesis, research results are reported on the role of natural and synthetic surfactants on the *in vitro* permeability characteristics of various chemical compounds across porcine (buccal, bronchial, arterial, venous and rectal) and human (vaginal) tissues. The permeability flux values of the different compounds (arecoline, 17β-estradiol, hydrocortisone, dexamethasone, vasopressin, oxytocin, zidovudine and isoniazid) were determined using a continuous flow-through diffusion system. Mean steady state flux values were compared statistically by means of a t-test at a significance level of 5% as well as an F-test using whole curve comparisons. The results indicated that the synthetic pulmonary surfactant Biopolsurf is an effective enhancer for the permeation of chemical compounds through most of the tissues tested and that molecular weight, electrostatic charge, partitioning of the molecules in surfactant and surfactant concentration play an important role in trans membrane diffusion.

In addition the epithelial permeability of the different types of tissues for various chemical compounds (arecoline, 17β-estradiol, hydrocortisone, dexamethasone, vasopressin and oxytocin) across the above tissues were compared. The results obtained showed that the permeability flux values of the compounds across porcine bronchial and human vaginal tissues were consistently similar and that porcine buccal tissue had the lowest permeability of all tissues tested. This was in agreement with previous *in vitro* studies. It was concluded that a wide variation in the permeability characteristics of different epithelia exists and that the pulmonary epithelium, due to its high permeability, is probably the most effective epithelium for drug delivery purposes, especially for drugs that undergo extensive gastrointestinal or hepatic first-pass metabolism.

OPSOMMING

In hierdie tesis, word die navorsingsresultate van die effek van natuurlike en sintetiese surfaktante op die *in vitro* deurlaatbaarheidseienskappe van verskeie chemiese stowwe deur vark (bukkaal, bronchiaal, arteriëel, veneus en rektaal) en menslike (vaginaal) weefsels bespreek. Die diffusie vloedwaardes van die chemiese stowwe (arekolien, 17β-estradiol, hidrokortisoon, deksametasoon, vasopressien, oksitosien, zidovudien en isoniasied) oor weefsels is bepaal met ‘n deurlopende-vloei perfusie apparaat. Gemiddelde vloedwaardes by vaste vlak toestande is statisties vergelyk deur middel van ‘n t-toets asook vergelykings van volle kurwes met ‘n F-toets. Die resultate het aangetoon dat sintetiese pulmonêre surfaktant Biopolsurf die deurlaatbaarheid van chemiese stowwe deur die meeste van die mukosa verhoog het en dat molekulêre gewig, elektrostatiese lading, partisie van molekules in surfaktant en surfaktantkonsentrasie ‘n belangrike rol speel in membraandiffusie.

Die deurlaatbaarheidsvloede tussen verskillende tipes weefsels is ook vergelyk ten opsigte van verskeie chemiese stowwe (arekolien, 17β-estradiol, hidrokortisoon, deksametasoon, vasopressien en oksitosien). Die resultate van die studie het aangetoon dat die diffusie vloedwaardes van chemiese stowwe deur vark bronchiale en mens vaginale weefsels baie ooreenstem met mekaar. Die vark bukkale weefsel het die laagste deurlaatbaarheidsvloede van alle weefsels getoon. Dit het ooreengestem met vorige *in vitro* studies. Deur die studie is dus vasgestel dat pulmonêre weefsel, as gevolg van die hoë deurlaatbaarheid, waarskynlik die effektiefste mukosa is vir aflewering van terapeutiese middels. Dit geld veral vir middels wat ekstensiewe gastrointestinale en hepatiese afbraak ondergaan.

DEDICATION

I dedicate this thesis to my parents, for their love, support and guidance, without which this wouldn't have been possible; especially my mother, who has been a source of encouragement and inspiration throughout my life.

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CONTENTS

1. INTRODUCTION

An increasing amount of research is focused on optimising drug delivery as a method of improving pharmacotherapy. As oral administration is probably a non-optimal delivery system for many therapeutic agents, growing attention is given to the potential of absorptive mucosae, including nasal, ocular, rectal, vaginal, buccal, sublingual and pulmonary tissue, as a non-invasive route of administration for systemic drug delivery. Furthermore, as proteins and peptides are emerging as a major class of therapeutic agents, the unusual characteristics of these compounds present considerable challenges to pharmaceutical scientists in selecting a suitable route of administration and formulation of these drugs. In the past the most common route of protein and peptide drug delivery has been parenteral. However, this route is associated with pain on administration, resulting in poor patient compliance and the formulation needs to be sterile. When administered by the oral route, many drugs are subjected to acid hydrolysis and extensive gut and/or hepatic first-pass metabolism, thus, exhibiting poor oral bioavailability. On the other hand, administration via absorptive mucosae often requires delivery vehicles and/or permeability enhancers, which assist in transfer across the delivery site and into the systemic circulation. As with the route of administration, targeting a specific site of action often requires the aid of a delivery vehicle that relies on the specific properties of the drug to be delivered, as well as the unique properties of the tissue being targeted.[1]

The human respiratory tract has the potential to provide means for non-invasive drug delivery of molecules that cannot be efficiently, reproducibly, or rapidly delivered without injecting them into the body. The large surface area, good vascularisation, immense capacity for solute exchange and ultra-thinness of the alveolar epithelium are unique features that can facilitate systemic delivery of drugs. Advances in engineering procedures aimed at controlling the dispersibility of drugs, the particle size of vectors, and the consistency of the delivery have made pulmonary delivery a feasible approach for many therapeutic agents. As clinical experience and data accumulate in support of the efficacy and safety of inhaled drugs,

pharmaceutical developers are recognising that pulmonary delivery offers unique advantages. This delivery route has substantial impact on four areas of pharmaceutical development: proteins and peptides, fast-acting drugs, localised lung infections and other pulmonary disease treatments as well as vaccines.[2]

Inhaled versions of proteins and peptides, which are currently administered via injection to avoid breaking down in the gastrointestinal tract, have shown promise. Pulmonary insulin, in particular, is in late-stage human clinical testing, its most advanced version being Exubera®, which is under development by Pfizer, Aventis and Nektar. Clinical data for Exubera® indicate that the drug can provide better glycaemic control than combinations of oral diabetic therapies.[2] Fast onset is a key consideration for many drugs, particularly for treating pain, nausea, anxiety and hypertension.

Infections remain a leading cause of death worldwide, pneumonia and other lung infections account for a large proportion of these deaths.[2] Such diseases respond well when treated locally with pulmonary-administered antibiotics. A drug delivered via the pulmonary route acts directly at the infected site, offering the potential for faster, and more efficient therapy with reduced risk of drug resistance and fewer side effects.[2] Similar treatment benefits may be found with pulmonary drugs destined for local treatment of lung diseases such as severe asthma, emphysema or cancer.[3] In addition, inhaled vaccines are of great interest to pharmaceutical developers because many currently approved vaccines address respiratory diseases.[2] Almost all of these vaccines are currently injected; one intranasal vaccine against flu is nearing commercialisation. Pulmonary vaccines may offer major benefits, including greater efficacy through the stimulation of mucosal and humoral immunity, which could help stop infections at their port of entry.

The permeability barrier of the lungs presents a major problem for most drugs when formulated without an absorption enhancer/promoter.[4] In an attempt to circumvent this problem, many novel absorption promoters have been tested for enhancing the systemic availability of drugs from the lungs. Various protease inhibitors, surfactants, lipids, polymers and agents from other

classes have been tested for their efficacy in improving the systemic availability of protein and macromolecular drugs after pulmonary administration.[4]

The mechanisms of action by which the surface-active agents increase the alveolar-capillary transfer of solutes are not well characterised. They may involve an increase in transcellular transport via an interaction and/or fluidisation of the cell membrane, which subsequently makes it more permeable, a modulation of tight junctions and increase in paracellular permeability, or some combination of both.[4]

Surfactant treatments are currently being assessed in a variety of different scenarios as permeation enhancers, not just in the traditional role of Surfactant Replacement Therapy (SRT) for neonates.[5] These different scenarios may include targeted drug delivery to specific areas of the lung, or uniform chemical coverage, that in turn, may require different initial doses or, indeed, different methods of delivery. Some of the areas where surfactants are now being utilised to improve drug delivery are in animal and clinical studies, e.g. in antimicrobial therapy in pneumonia.[5-7] Several other lung diseases for which surfactant could prove useful, include the delivery of bronchodilators for asthma[8], anti-inflammatory agents for bronchopulmonary dysplasia[9], vasodilators for persistent pulmonary hypertension[10], growth factors for lung hypoplasia[11], drugs enhancing epithelial integrity, anti-neoplastic drugs for cancer, factors for accelerating lung maturation for respiratory distress syndrome (RDS) and lung lavage with surfactant replacement as well as drugs for alveolar proteinosis.[12]

The typical spreading behaviour of surfactant, combined with the ability to disperse drugs that are insoluble in aqueous solutions, has also instigated research in new application fields for ‘surfactant-like’ solutions. Surfactant has been used as an emulsifier with lipophilic cancer drugs, clearing the way for potential parenteral use.[13] The ability of surfactant to decrease the interfacial tension between water and an organic solution, thus increasing emulsification, helps to stabilise droplets of non-soluble drugs and prevent aggregation or

coalescence.[14] Although these studies used non-pulmonary based surfactants they show promise for future research and alternative applications for surfactant as a carrier agent.

2. LITERATURE REVIEW

2.1 Administration of drugs

The quest for successful drug delivery requires consideration of numerous aspects of which the most important are efficacy and safety. First and foremost, drug concentration should be sufficiently high at the site of action in order to have a therapeutic effect, but at the same time it should not be too high, since this may result in detrimental side effects. For safe and efficient therapy, drug concentration should preferably be essentially constant within this “therapeutic window”. This confers requirements not only on the drug itself but also on its formulation. Drug delivery systems should preferably be designed such that a preferential accumulation of drug is reached at the site of action, whereas the drug concentration elsewhere in the body should be as low as possible. The reason for this need of “targeting” is that a high concentration of the drug in tissues or cells other than those being targeted may cause problems related to side effects. A typical example of the latter is cancer therapy, where accumulation of chemotherapeutic agents in areas other than the tumour frequently causes severe side effects. Furthermore, the drug should be stable against degradation during storage and administration, and the formulation designed such that drug degradation is minimised. Once the drug has performed its action, however, it should either be excreted or metabolised to harmless compounds.

Another important aspect of drug delivery systems is the route of administration. Naturally, the preferred situation is that the drug can be administered by the patient himself/herself, without unpleasant sensations. However, depending on a number of different aspects, other routes may also be chosen. The complete therapeutic strategy therefore involves choice not only of the drug but also of the drug delivery system and the route of administration.

Different routes of administration place different requirements on the drug delivery system. In oral drug delivery, the drug and its carrier have to pass through the stomach with its low pH, which tends to affect drug stability and

drug solubility, as well as the properties of the drug carrier system. In addition, the absorption in the intestine places some rather demanding requirements on the drug and its formulation, especially for hydrophobic drugs and/or large molecules (e.g., protein and peptide drugs). During transdermal administration the penetration of the stratum corneum poses a severe limitation in the therapy efficacy, which puts special demands on the drug delivery system. During intravenous drug delivery, the uptake of the drug and its carrier in the reticuloendothelial system frequently reduces bioavailability and results in dose-limiting side effects. The major disadvantage of injections, intravenous or otherwise, however, is that they are highly inconvenient and cause discomfort, especially when repeated doses must be given to treat chronic conditions and they increase the risk of infection.[15]

2.2 Pulmonary mucosa

The absorptive surfaces used for delivery of therapeutic agents each have a physiology and cell structure that governs the amount of drug that can be absorbed into the systemic circulation. In order to understand the mechanism by which absorption occurs, the structural features of the pulmonary mucosa must be examined. The respiratory tract is a highly specialised organ with a surface area of more than 75 m^2. It can be divided into two areas: the upper conducting airway and the lower respiratory regions.[17] The conducting region consists of the nose, nasal passages, mouth, pharynx, oesophagus, larynx, and trachea. The respiratory region consists of bronchi and alveoli (Fig. 1). The alveoli constitute about 95% of the absorptive surface area and are primarily involved in gas exchange. Pulmonary drug absorption into blood circulation occurs here. The tracheobronchial tree, which is part of the upper conducting region, is composed of the trachea and is further subdivided into the central airway (bronchi) and the peripheral airway (bronchioles). The bronchioles further branch into alveolar ducts and sacs. A group of ducts and sacs can be collectively referred to as a lobule. In an adult human lung, there are approximately 130,000 lobules, each with a diameter of 3.5 mm, and almost 300 million alveoli.[18]

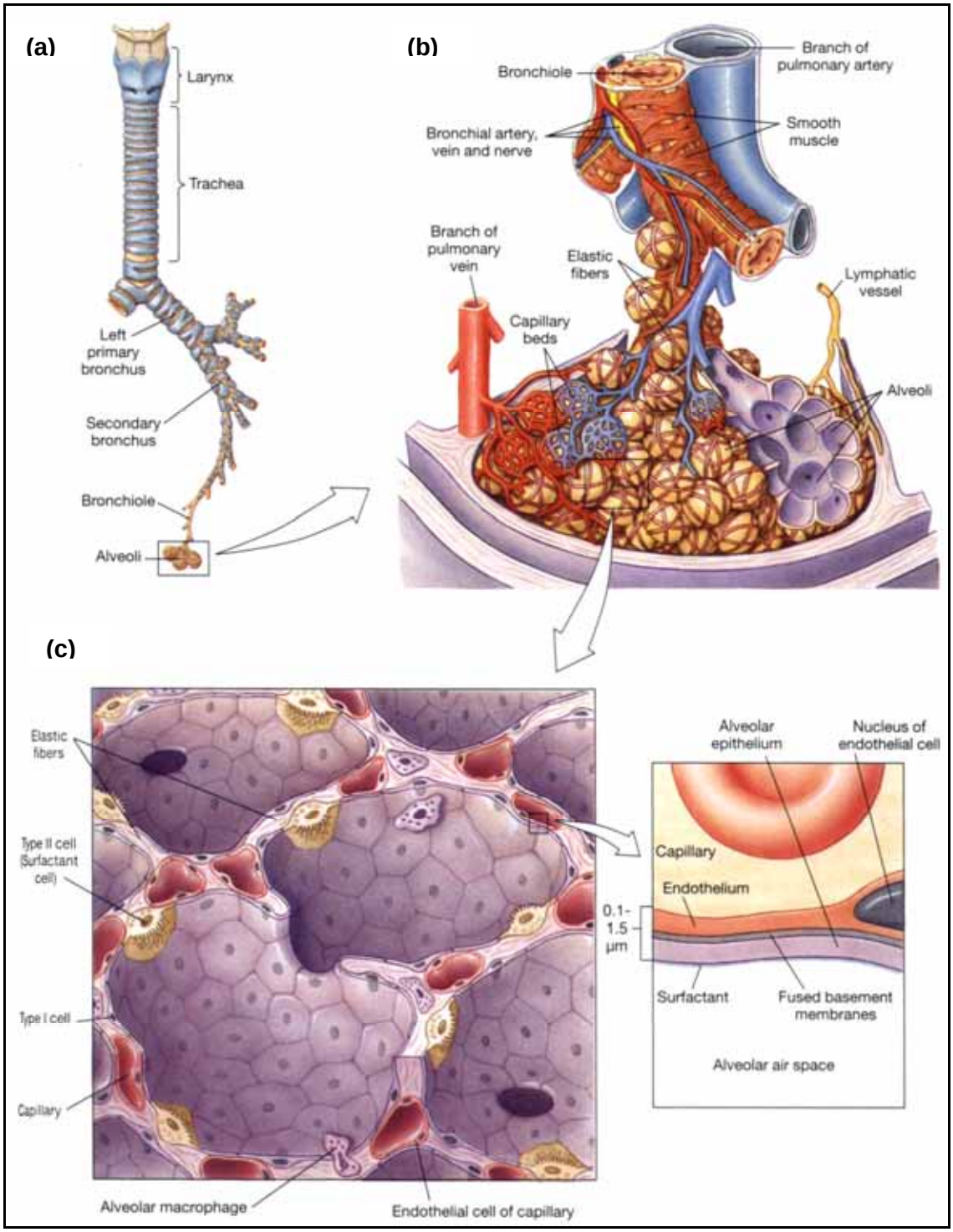

Figure 1. (a) Branching of airways. The trachea branches into two bronchi, one to each lung. Each bronchus branches 22 more times, terminating in a cluster of alveoli. (b) Structure of lung lobule. Each cluster of alveoli is surrounded by elastic fibres and a network of capillaries. (c) Alveolar structure. The alveoli are composed of type I cells for gas exchange and type II cells that synthesise surfactant. Alveolar macrophages ingest foreign material that reaches the alveoli.[16]

The upper airway is covered by a thick, pseudostratified, ciliated, columnar epithelium similar to that of the nasal and gastrointestinal epithelia, while the terminal bronchioles are lined with a simple, ciliated, cuboidal epithelium (Fig. 2). The alveolar epithelium consists of three types of cells. Approximately 97% of alveolar cells are type I (flat squamous) cells, which are about 5 µm thick. Attached to the basement membrane and interspersed with the squamous cells are the type II (septal) cells. These are approximately 10-15 µm in thickness and secrete a surfactant, which is responsible for the decrease in surface tension at the alveolar capillary interface. A few type III (brush) cells and macrophages are also found in the lung.[19] The macrophages are capable of removing foreign particles and are themselves continually being removed by the ciliary escalator from the lungs and into the lymph nodes.[20]

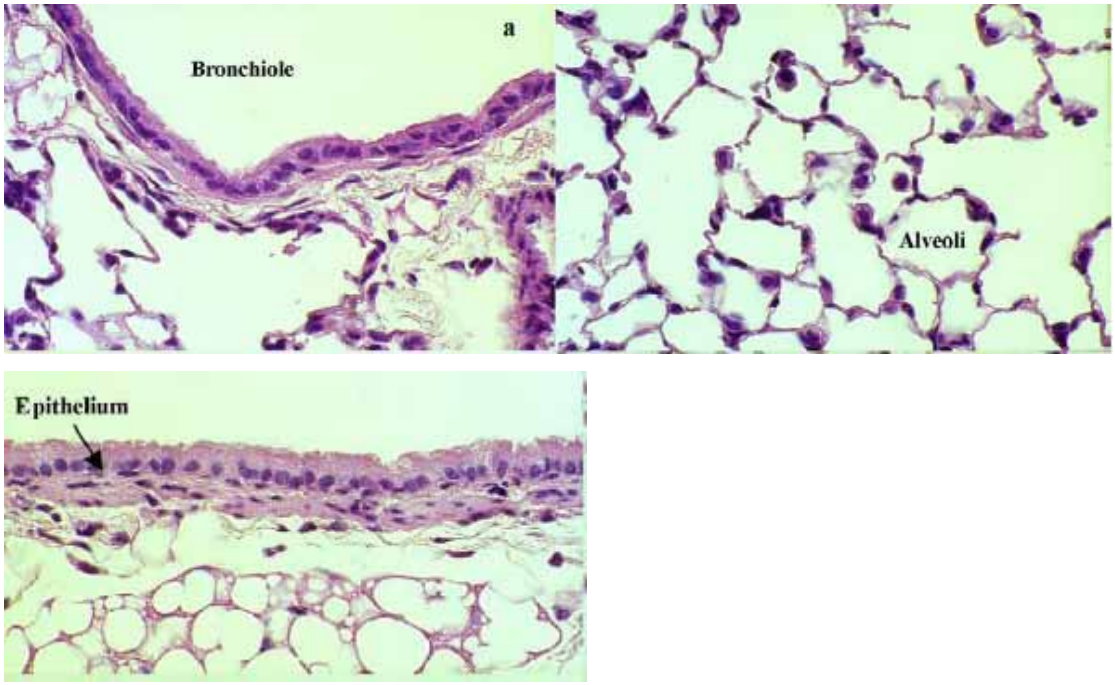

Figure 2. These micrographs illustrate the bronchiole, alveoli and epithelium.[2]

The blood capillary and alveolar gases are separated by the alveolar-capillary membrane. This membrane is composed of a continuous epithelium 0.1-0.5 µm thick, a collagen fibre network, and the interstitium, which itself is made up of ground substance, basement membrane, and capillary endothelium. The interstitium further forms the region where alveoli and

capillaries are attached. Drug absorption would presumably occur where this interstitium is at its thinnest (about 80 nm).[18]

The alveolar epithelium and capillary endothelium have a very high permeability to water, gases, and lipophilic substances. However, the presence of tight junctions in the type I cells effectively creates a barrier for absorption of large, hydrophilic substances and ionic species, as has been observed for sucrose and horseradish peroxidase.[21,22] The tight junctions limit penetration of molecules larger than 1.2 nm in diameter. On the other hand, both the endothelial junctions, which have gaps of 4-6 nm, and the microvascular endothelium, which has much larger intercellular gaps, are more permeable to proteins.[18]

A mucus blanket about 5 μm thick, which covers the entire internal surface of the respiratory tract, and the mucociliary clearance are additional barriers to drug penetration. Furthermore, the lung natural surfactant, which maintains the integrity of the lung, provides another barrier to drug absorption. However, the surfactant, which is composed of phospholipids, mucopolysaccharides, and proteins, may be affected by a drug, which in turn affects the structural integrity and permeability characteristics of the lung.

The alveoli are lined with a rich blood capillary and lymphatic system. The alveolar capillaries, which provide a surface area of 60-80 m^2 for absorption and a volume of 100-200 ml, maintain good sink conditions for drugs that penetrate the alveolar-capillary membrane. For successful delivery, a drug must first traverse the mucus blanket and lung surfactant coating before reaching the alveolar epithelium.[18]

2.3 Transmembrane diffusion processes

Free diffusion or passive transport of substances through liquids, solids, and membranes is a process of substantial importance in the pharmaceutical sciences. The transmembrane diffusion process is passive in nature, requiring a concentration differential as the driving force and each molecule requires kinetic energy to achieve a net movement down this gradient. The diffusion of

a molecule through a membrane, biological or synthetic, *in vivo* or *in vitro*, would normally occur as follows:[23]

1. The molecule must diffuse through the vehicle in which it is contained to the membrane interface and partition from the vehicle into the upper lamina of the membrane.
2. The molecule diffuses within the membrane, equilibrating laterally and emerges under steady state conditions, from the distal surface of the medium. Adsorptive interaction may be extensive in this layer, forming a reservoir of the molecule.
3. The molecule then partitions either into the adjacent membrane strata or into the receptor fluid under the influence of the concentration gradient, and adsorption may occur once again. Diffusion through any one of the layers or any of the partitioning events may control the overall rate of permeation.

There are two general mechanisms by which soluble macromolecules can be absorbed from the mucosa into the body. They can either pass through the cells (absorptive transcytosis) or between the cells (paracellular or intercellular transport). Absorptive transcytosis may occur independently of a plasma membrane receptor (fluid phase or adsorptive transcytosis) or it may involve receptor mediated binding followed by vesicular transport (receptor mediated transcytosis). Paracellular transport is usually thought to occur through the junctional complex between two or three cells. Another classification of diffusion processes is into polar (aqueous channels in mucosa) and non-polar (partitioning into the lipid bilayers of the mucosa) pathways. It would seem that the passage of most molecules across mucosa appears to be a simple first-order diffusion process (Fig 3).[24]

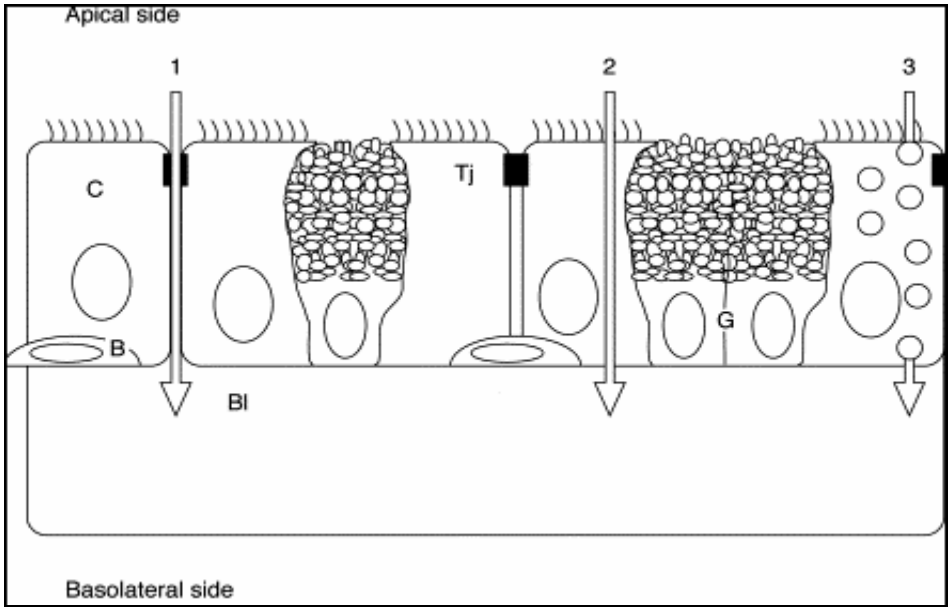

Figure 3. Schematic representation of transport routes across nasal respiratory mucosa: (1) paracellular across tight junctions, (2) transcellular and (3) transcytotic. Mucus-secreting goblet cells (G), ciliated columnar cells (C) and tight junctions (Tj) are represented. Basal cells (B) are located on the basal lamina (Bl).

The physicochemical properties of a drug such as molecular weight, lipophilicity, ionisation, surface charge and chemical nature influence drug absorption. The governing factor for which pathway a molecule will follow when it diffuses through a membrane is the partition coefficient. Hydrophilic molecules are expected to predominantly permeate via the intracellular route while lipophilic molecules are expected to permeate predominantly via the intercellular route.[25] A mixed permeation model has been proposed where drugs diffuse through tissue predominantly via the continuous intercellular lipid domains. Both the lipid and polar regions of the lipid bilayers could thus provide the micro-routes through which the permeant diffuses, depending on its partition coefficient.[25] Surfactant molecules in the donor vehicle may enhance partitioning by reducing the surface tension between the vehicle and the membrane surface, but may also disrupt the barrier layers of the membrane.[26]

2.4 Drug delivery

The lungs represent a relatively unexploited route of delivery for large therapeutic molecules that otherwise must be delivered by injection. Considerable effort has been spent on finding ways to deliver macromolecules via the more obvious and convenient gastrointestinal, nasal, buccal (cheek), rectal, vaginal and transdermal (skin) routes. However, these body surfaces are either fundamentally impermeable, have a restricted surface area, limited residence time, or other factors may make efficiency of penetration very low to all but the most potent small peptides.[15] Administration via these routes often requires delivery vehicles and/or permeability enhancers, which assist in transfer across the delivery site and into the systemic circulation.[1]

There are numerous advantages in delivering therapeutic drugs to the lungs including:

- Non-invasive method of delivery
- A large surface area for absorption (~75 m^2)
- Thin (0.1 to 0.5 μm) alveolar epithelium, permitting rapid absorption
- Absence of first-pass metabolism
- High bioavailability
- Rapid onset of action

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Dry or liquid particles can be prepared and inhaled with the aid of dry-powder dispersers, liquid-aerosol generators or nebulisers. These devices produce particles that range in size from 1 μm to > 10 μm. Although inhaled particles more than 10 μm in diameter are trapped in the nasal passages, throat, larynx and bronchial walls, those less than 5 μm in diameter may penetrate deeply into the lung. There have been many attempts to use inhalation to deliver insulin for the treatment of diabetes. These early approaches failed because the aerosol generators (jet nebulisers and metered dose inhalers) had low and variable delivery to the lung. Inhalation therapy for the treatment of diseases of the respiratory tract, especially asthma, underwent enormous growth in the last three decades. The discovery of potent drugs that could be administered in one or two puffs from a low cost hand-held device, such as a metered dose inhaler or dry powder inhaler, led to the acceptance of aerosols as the

preferred mode of therapy of asthma. One of the main advantages of targeting the lung was the minimisation of systemic side effects of these drugs. However, systemic absorption of the inhaled drugs was observed in the early development of these asthma products.[27]

Early work on peptides such as insulin and some proteins certainly indicated that such molecules were absorbed from the lung. Hastewella *et al.* reported a summary of extensive studies in which the absolute bioavailabilities of several proteins and peptides were measured in rat models of pulmonary, nasal, and colonic absorption.[28] The compounds were human and salmon calcitonins, the human parathyroid hormone and its 34-peptide fragment, hirudin, and a hybrid α-interferon. All routes of administration exhibited general reduction of bioavailability with increasing molecular weight. However, the pulmonary route (via intratracheal administration) resulted in much higher values than the other two routes. Thus, pulmonary delivery was known to be extremely promising among the non-invasive routes of delivery. Industrial and academic research demonstrated the need for targeting of the drug into the “deep lung” to increase the efficiency of delivery and in particular to avoid the highly variable deposition in the oropharyngeal cavity. The conventional therapeutic aerosol generators used for delivery of asthma drugs to the airways were not designed with these requirements in mind. New types of delivery systems had to be developed that could generate very fine particles with aerodynamic properties suitable for drug delivery to the distal part of the respiratory tract with large highly absorptive surfaces.[27]

In comparison to subcutaneous injection, the bioavailabilities of small peptides and insulin (< 6 kDa) placed into the lungs can approach or exceed 100%.[29-31] Furthermore, larger proteins (molecular mass 18-22 kDa), such as granulocyte colony stimulating factor (GCSF)[32,33], α-interferon[34] and human growth hormone[35,36] have exhibited pulmonary bioavailabilites approaching or exceeding 50% compared to subcutaneous injection. High lung bioavailability may stem from immediate access to a tremendous surface area provided by pulmonary delivery (by liquid instillation or aerosol), and/or the slow clearance

from the deep lung as well as this organ's significant extracellular protease inhibitory activity. Regardless of the growing appreciation of the remarkable systemic bioavailability of peptides and proteins administered via the lung, the mechanisms of absorption are poorly understood. It is still unclear how molecules such as insulin (5.7 kDa, diameter 2.2 nm) get from the airspaces into the blood.[37]

Macromolecule absorption is inversely related to molecular weight. Smaller molecules would diffuse through tissue at a faster rate than bigger molecules. However, most conventional therapeutic drugs (small organic molecules) selected for transmucosal drug delivery tend to lie within a very narrow range of molecular weights (100-500 Da). Within such a narrow range the influence of molecular weight on drug flux appears to be relatively minor if compared to the influence of, for example, changes in partition coefficients. The transmucosal flux is much more apparent when using larger molecules like peptides and proteins. It is well known that most organic molecules, which have a high melting point, have lower aqueous solubility at normal temperatures and pressures. This leads to the assumption that the more lipophilic the molecule, the faster it would permeate through any tissue. Since many drugs are weak electrolytes, the pH may change their degree of ionisation and affect the absorption of the drug. Weak acids and weak bases will dissociate to various degrees depending on the pH of the formulation used and the pH of the membrane through which it must diffuse. In the unionised state, the drugs will be more lipid soluble and will diffuse through membranes at a higher rate than in the ionised state.[25]

Inhaled and instilled macromolecules above approximately 40 kDa (i.e. >5-6 nm diameter which includes almost all plasma proteins) are slowly absorbed over many hours from the airspaces and appear in high concentrations (relative to plasma) in pulmonary lymph following absorption.[37] Albumin (7 nm diameter) is absorbed slowly (1-2 %/h) and peaks in the blood at 12-24 h.[38] Although lymph concentrations may be high, the majority of molecules such as α_1-antitrypsin (52 kDa) and albumin (68 kDa) are absorbed

from the airspaces directly into the blood.[39] Macromolecules injected into the subcutaneous space also partition between blood and lymph in a molecular weight dependent manner with higher molecular weight proteins partitioning more readily into the lymph.[40] Peptides and proteins < 40 kDa (i.e. < 5-6 nm in diameter) can rapidly appear in blood following instillation or inhalation into the airways.[34] Most cytokines (18-22 kDa, ~ 3-4 nm in diameter), insulin (5.7 kDa, 2.2 nm in diameter) and many smaller peptides are absorbed rapidly and peak in the blood in 5-90 min in humans and animals.[29-36,41,42] Therefore, based on the peptide and protein absorption data, there should be one or more populations of equivalent pores in both the airway epithelium and vascular endothelium that are large enough to allow relatively rapid (5-90 min) absorption of macromolecules with diameters ≤ 5-6 nm, and relatively slow absorption (hours to days) of macromolecules ≥ 5-6 nm across the airspace epithelium and capillary endothelium.[37]

The alveolar epithelium is the major site of absorption of peptides and proteins in the lungs because of the large surface area and the low permeability of the upper airways to proteins. The alveolar epithelial cells and not the underlying endothelial cells are the major barrier to transport.[21] The lung epithelium is composed of polarised epithelial cells with tight junctions between the cells. Though the alveolar epithelium is highly permeable to water, gases, and lipophilic substances, the permeability of large hydrophilic substances, such as proteins is limited.[43]

The apical surfaces of these cells contain a high level of actin, which strengthen them and inhibit endocytic activity, making the uptake of macromolecules more difficult. Rapid clearance and degradation of drug in the lungs will limit the amount available for transport. While mucociliary clearance does not play a major role in the alveoli, the epithelial surface is covered with a complex surfactant (a mixture of lipids and proteins), which can limit absorption. Protease and peptidase degradation and/or alveolar phagocytosis may also hamper the delivery of protein/peptide drugs systemically.[44,45]

It is important to merge theoretical estimations of equivalent pore dimensions and populations derived from whole tissue absorption studies with the real ultrastructure of the barrier. Molecular diameters of some relevant molecules are shown in Table 1.[37] A variety of studies with many epithelia have been done where the absorption of different test molecules was measured from one side of the tissue to the other. Based on the absorption rates, concentrations, surface areas, molecular dimensions and diffusion coefficients of the test molecules, different populations of equivalent pores and pore sizes are mathematically derived to account for the absorption data.

Table 1

Molecular weights and diameters of molecules

Molecule	Molecular weight (Da)	Diameter (nm)	Reference
Water	18	0.3	46, 47
NaCl	58	0.46	48
Urea	60	0.44-0.48	46, 47, 49
Glucose	180	0.74	48
Mannitol	182	0.86	48
Sucrose	340	1.04	46-48
DTPA*	495	1.2	50
Cyanocobalamin	1 400	1.4	38
Inulin	5 000	2.8	46, 49
Insulin	5 700	2.2-2.6	38
Cytochrome C	12 800	3.0	48
Myoglobin	17 800	3.3-3.8	51
HRP*	40 000	4.5-5.0	52, 53, 48
Hemoglobin	64 500	6.0	54
Albumin	69 000	7.0-7.2	39, 55
IgG	150 000	11.0	38
Catalase	240 000	10.4	48
Ferritin	440 000	10.0-11.0	56, 53

*DTPA – Diethylenetriamine penta-acetic acid, HRP – Horseradish peroxidase

In general, most studies suggest that there are at least two populations of pores, small and large (with sometimes a third ultra-large pore) (Table 2).[37] The small pores may correspond to the thin slits that exist between cells, the tight junctions, or to transcellular pathways (i.e. pathways through the plasma membrane, into the cell and out of the opposing plasma membrane). The large pores may also occur in the junctions between cells and/or they may represent vesicular transport (transcytosis) a process whereby small membrane vesicles called caveolae carry engulfed or bound material across the cell. Transport studies have shown that the small pores in the alveoli and trachea have estimated diameters of approximately 1-5 nm. These small pores may be the ones responsible for the absorption of peptides and proteins $\leq$ 5-6 nm such as insulin (2.2 nm diameter). In addition, the pore studies have indicated that there are also populations of larger pores in alveolar and tracheal epithelium, which may be responsible for the absorption of macromolecules ≥ 5-6 nm in diameter. [37]

Table 2

Estimated pore size diameters (nm)

Barrier	Species	Small pore	Large pore(s)*	Reference
Alveolar	Dog	1.2-2	ND**	22
Alveolar	Dog	ND**	>16	57
Alveolar	Rat	0.4	50	46
Alveolar	Frog	5	26	46
Alveolar	Dog	4	10 (16%), 98	46
Alveolar	Frog	1	10 (4%)	47
Alveolar	Dog	2	80 (30%), 800	55
Endothelium	Dog	8-12	-	22
Tracheal	Dog	3.3	13.8	58
Tracheal	Dog	0.4	50	59
Tracheal	Guinea pig	ND**	20	60
Tracheal	Rat, Guinea pig	ND**	14-19	61

*Percentage indicates fraction of total transport mediated by pore.

**Not determined

2.5 Permeation enhancers

Permeation enhancers promote the transport of drugs through a mucosal membrane. Several classes of enhancers have been evaluated for potential use in enhancing transmucosal delivery. Among the agents studied extensively are bile salts, dihydrofusidates, cyclodextrans, surfactants, and fatty acids and their derivatives. It is clear from the literature that the choice of a permeation enhancer must be based on the following characteristics: effectiveness, safety, chemical inertness, lack of biological activity, and rapid reversibility of (or recovery from) toxic and/or pharmacological effects, if any.

Permeation enhancers exert their action by (1) interacting with phospholipids and/or proteins to increase membrane fluidity, (2) interacting with Ca^{++} ions to open tight junctions, (3) altering the rheological properties of the mucus layer, and (4) inhibiting enzyme activity.[62,63] Although classification of the enhancers may be based on their mechanism of action, most enhancers affect the cell membranes as well as the tight junctions.[64] (Fig. 4)

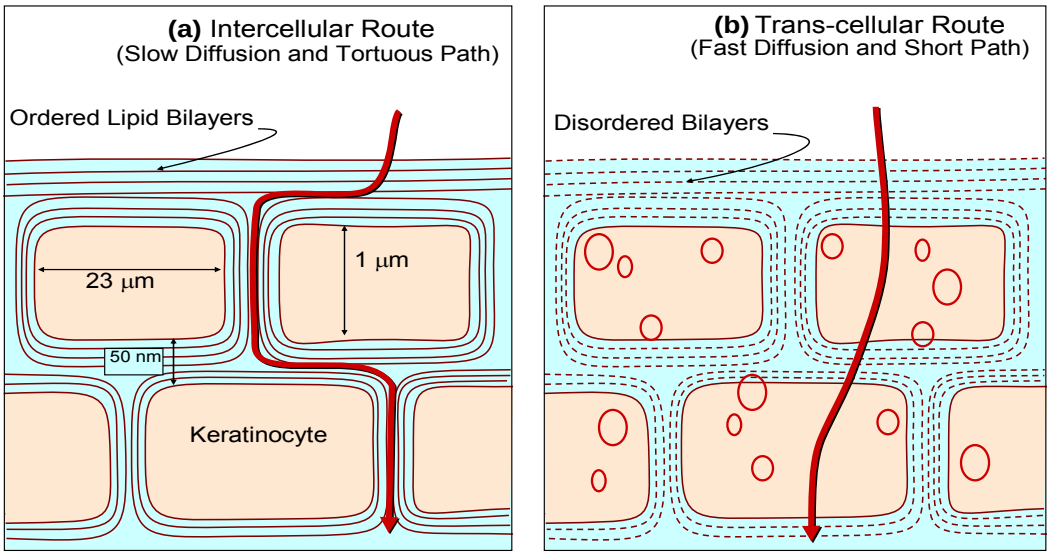


Figure 4. (a) Permeation of drugs through the epithelial layer via the intercellular route. (b) Permeation of drugs through the epithelial layer via the transcellular route following disordering of the lipid layers by surfactant.

Drugs, such as proteins and peptides, which are poorly absorbed through alternate routes, demonstrate an improved bioavailability when administrated

though the lungs due to the large absorptive surface area, high rate and volume of blood supply, and highly permeable blood barrier in the alveoli.[34,65] However, peptide delivery through the pulmonary route is still considerably less efficient than through the injectable route due to the protective nature of the pulmonary epithelium. The permeability barrier of the pulmonary airspaces is controlled largely by the epithelial cells, which are joined by tight junctions.[66] Altering the permeability of these tissues with amphipathic enhancers like surfactants can yield promising bioavailabilities.

The advantages of using surfactant for sustained and targeted drug delivery can improve the therapeutic index of a drug by one or more of the following: [67]

- Increasing the proportion of drug that reaches its site of action (be it intracellular or extracellular)
- Enhance the solubility (dispersion) of drug
- Improving the transport of the drug to its site of action
- Allowing co-localised deposition of protein with other proteins or excipients (e.g. protein and protease inhibitor)
- Improving the stability of the drug *in vivo*
- Reducing clearance, thus prolonging the residence time of the drug at its site of action
- Decreasing the non-specific delivery of the drug to non-target tissues
- Decreasing irritation caused by the drug
- Decreasing toxicity due to high initial doses of the drug
- Altering the immunogenicity of the protein

The pulmonary absorption of salmon calcitonin and insulin is more efficient with absorption enhancers and enzyme inhibitors.[68-71] The use of enzyme inhibitors is not limited to systemically delivered proteins. The efficacy of locally acting proteins/peptides may also be improved by decreased degradation. Formulation of absorption enhancers and enzyme inhibitors with drugs in carriers, such as liposomes and microspheres, would provide not only effective co-administration but also co-localisation within the lungs.

Microparticles themselves can aid the absorption process. Edwards *et al.*[72] enhanced insulin absorption using large, porous polylactic-co-glycolic acid (PLGA) particles and prolonged action for up to 96 h. Both microspheres[72,73] and liposomes[74,75] have been used to enhance the systemic effect of insulin after inhalation. The effect of microspheres is attributed to the sustained release of the insulin from the polymeric carriers. The effects of liposomes on systemic absorption depend on the concentration, charge, and acyl chain length of the phospholipid components.

The attachment of bioadhesive and targeting ligands to the carriers may also be used as a strategy to alter the systemic absorption of proteins/peptides. Ligands that specifically trigger transcytosis, for example, by harnessing calveolae-mediated transport, of the conjugated/encapsulated proteins may be preferable from a safety perspective to generalised permeation enhancement that allows the non-specific passage of other particles across the airways.[76]

Pulmonary drug delivery through nasal sprays or tracheal instillation, offers an alternative mode of delivery to intravenous and oral systems. However, the disadvantage is that it is very wasteful and a relatively large amount of drug is necessary to ensure that patients receive the correct dose. For this reason mixing of a pharmaceutically active agent with a pulmonary surfactant may provide an attractive method of improving drug delivery through the natural barriers of the lung. In particular, the spreading behaviour of surfactant, combined with the ability to disperse drugs that are insoluble in aqueous solutions. These characteristics are ideal to instil therapeutic agents into the lung, because it enables high local therapeutic levels while systemic side effects are minimised. The reason for using enhancers is to find a therapeutic level that does not damage or irritate the tissue but still increase drug absorption.[77]

Pulmonary surfactant is produced by the alveolar type II cells in the lung and it is composed of two major fractions i.e. lipids and surfactant-specific proteins.[78] Lipids comprise up to ~90% of pulmonary surfactant with

phospholipids forming the bulk of the lipids. Other lipids found in surfactant are cholesterol, triacylglycerol, and free fatty acids. Phosphatidylcholine (PC) is identified as the most abundant component of surfactant and is consistently present in a quantity of 70-80% of the total amount of lipids. Approximately 50-70% of PC is saturated, especially in the dipalmitoylated form (DPPC). The anionic phosphatidylglycerol (PG) accounts for ~8%. Other lipids present are phosphatidylethanolamine (PE, ±5%), phosphatidylinositol (PI, ±3%), and small quantities of phosphatidylserine (PS), lysophosphatidylcholine and sphingomyelin (less than 2%).[79]

Although pulmonary surfactant is mainly composed of lipids, it also contains 10% protein. To date the four surfactant proteins that have been described can be divided into two groups: the hydrophilic proteins SP-A and SP-D, and the hydrophobic proteins SP-B and SP-C.[80]

The normal physiological functions of the pulmonary surfactant system include the following:

- Mechanical stabilisation of lung alveoli; during deflation of the lung a static high surface tension would tend to promote alveolar collapse. The dynamic surface tension behaviour of surfactant prevents this.[81]
- Transport of mucus and inhaled particles; it acts as an antiglue factor, preventing the development of large adhesive forces between mucus and the bronchial wall.[82]
- Protection against lung oedema; another important function of surfactant is stabilisation of the fluid balance in the lung, especially across the alveolar capillary membrane.[83,84]
- Local defence against infection; it has been demonstrated that surfactant, in particular SP-A and probably SP-D, enhances the antibacterial and antiviral defence properties of alveolar macrophages.[85,86]

The spreading properties and the inherent therapeutic potential of surfactant can be used to facilitate delivery of antimicrobial agents to the lung

parenchyma. Direct administration of antibiotics to the airway offers many potential advantages in the treatment and prevention of pneumonia.[5-7] Delivery directly to the airways should increase the local effectiveness (higher levels of antibiotics locally) and reduce the risk for systemic toxicity caused by antibiotics, e.g. aminoglycosides (lower levels of antibiotics systemically).[87] Locally administered antibiotics for prevention and/or treatment of lower respiratory tract infections have been extensively studied. However, despite the high antibiotic dose delivered to the lung, the question of efficacy remains controversial; explanations for this include failure of the antibiotic to reach the infected lung area. When delivered as an aerosol, only a small amount of nebulised antibiotics (around 10%) is actually deposited in the lung.[88] Furthermore, with increased airway obstruction, atelectasis and lung damage, the amount of aerosol deposited will be even lower.

Lung distribution of intratracheally-instilled antibiotic solutions has been poorly studied. It is known, however, that distribution of intratracheally-instilled saline is largely limited to the central regions of the lung.[5] Due to the small diameter of peripheral airways, fluid with a high surface tension (such as saline or water) requires high pressures for passage through these airways.[89] Studies have shown that pulmonary surfactant is superior to saline in distributing a radioactive colloid within healthy lungs, as indicated by the more homogenous and peripheral lung distribution. The effectiveness of surfactant as carrier was even more evident at lower volumes of fluid.[5] Furthermore, surfactant re-expands atelectatic regions, which are most likely to be the infected areas. It is therefore anticipated that intratracheally-instilled antibiotics will be more effective when using pulmonary surfactant as a carrier to optimise the distribution within the lung.[77]

Although the idea to use surfactant as a carrier agent was proposed several years ago, data from *in vivo* experimental studies are scarce. Van't Veen *et al*. was the first to study the effect of a surfactant-tobramycin mixture on mice suffering from *Klebsiella pneumoniae* repiratory infection.[90] It was demonstrated that an intratracheal instilled surfactant-tobramycin mixture is more effective in protecting mice from death due to a respiratory *Klebsiella*

pneumoniae infection than an intratracheal instilled tobramycin alone or of surfactant alone. These results were the first to indicate that exogenous surfactant is an effective drug-carrier agent. It is suggested that one of the advantages of locally administered drugs is the minimisation of systemic side effects of the drugs. Van't Veen *et al.* also studied the clearance rate of intratracheally-instilled labelled [^{99m}Tc] tobramycin with either surfactant or saline as a carrier agent.[91] Lung clearance of intratracheally-instilled [^{99m}Tc] tobramycin was increased when surfactant was used as a carrier agent, which was correlated with the increase in spatial lung distribution due to surfactant. Thus, when administering antibiotics with surfactant as a carrier, there will be higher systemic levels of antibiotics, but still lower than with systemic application.

In near term newborn rabbits suffering from group B streptococcal (GBS) pneumonia, Herting *et al.* studied the effect of adding specific antibacterial polyclonal antibody (IgG fraction) to surfactant.[92] The addition of a specific immunoglobulin against GBS reduced bacterial proliferation more effectively than either specific antibody alone or surfactant treatment alone. This usage of specific opsonising antibodies could afford the clinician with new tools in the fight against the increasing resistance of several pathogens. Because all opsonised bacteria are phagocytised and consequently killed intratracheally, the net result will be substantially lower levels of 'free' bacterial toxins and cell wall components; reducing severe adverse systemic reactions such as endotoxin shock or pulmonary hypertension. Thus, severely ill patients requiring ventilatory support and whom have atelectatic lung areas infected by multi-resistant pathogens could benefit from specific immunoglobulin therapy in combination with exogenous surfactant.[77]

Because of its unique properties particularly in the lung environment and its involvement in several lung diseases, it has been proposed to use surfactant as a carrier for several other therapeutic agents. Compared with saline, the delivery of experimental recombinant adenoviral vectors into the lung of rats showed a more uniform distribution pattern when surfactant was used as a carrier. Surfactant improved lobular distribution of adenovirus delivered

beta-galactosidase expression, the difference was even more striking at lower volumes. The activity of the gene expression of beta-galactosidase was also significantly greater when surfactant was used instead of saline.[93] Given prophylactically, surfactant can alleviate an asthma attack and addition of surfactant to bronchodilators may improve their effectiveness in a severe asthma attack.[94] In a rabbit model, corticosteroids were delivered intratracheally with surfactant as a carrier and it was shown that the amount of corticosteroid delivered was greater than with nebulisers, while minimising systemic side effects.[95]

Premature children suffering from respiratory distress syndrome (RDS) can now be treated successfully with exogenous surfactant. A small percentage of these children, however, require prolonged mechanical ventilation with high oxygen concentrations. The fetal lung is deficient of surfactant and antioxidase enzymes thus increasing its susceptibility for oxygen toxicity, chronic lung injury and bronchopulmonary dysplasia.[96] Walther *et al*. showed that intratracheal instillation of surfactant liposomes can boost lung antioxidant capacity in premature rabbits exposed to hyperoxia.[97] When CuZn-superoxide dismutase (an antioxidant enzyme) was added to the surfactant liposomes a further elevation in antioxidant activity was observed, reducing the amount of harmful free oxygen radicals.

The unique spreading properties of surfactant, combined with the ability to dissolve drugs that are insoluble in aqueous solutions, has also instigated research in new application fields for ‘surfactant-like’ solutions. The ability of surfactant to decrease the interfacial tension between water and an organic solution, thus increasing emulsification, will help stabilise droplets of non-soluble drugs and prevent aggregation or coalescence.[14] These characteristics are ideal for the delivery of therapeutic agents into the lung, because it enables high local therapeutic levels while minimising systemic side effects.

2.6 Other mucosa for drug delivery

2.6.1 Buccal mucosa

There is currently a resurgence of interest in the oral mucosa as a route for drug delivery. The buccal cavity is an attractive route for non-invasive, controlled transmucosal delivery of both local and systemic therapeutic agents. Due to the rich vasculature of the oral mucosa and the absence of gastrointestinal and first-pass hepatic degradation it is very useful for administering of drugs.[98] The permeation of drugs through the oral mucosa is governed by its permeability barrier, which prevents the entry of potentially harmful substances and thus affects the passage of drugs. Although barrier function is very complex, the physical barrier appears to be located in the region of the upper third of the epithelium, consisting primarily of a lipid-containing layer.[99] The buccal mucosa may be very well suited for the development of sustained-delivery systems and may be particularly suited to deliver peptide drugs, especially of low molecular weight, high potency and/or long biological half-life.[24] Permeation enhancers and enzyme inhibitors have been added to transbuccal tablet formulations of insulin[100,101] and calcitonin to evaluate their effects on buccal absorption in rabbits.[102,103]

2.6.2 Rectal mucosa

Although the rectal route has been used for many years for drug delivery, its use for peptides and proteins is relatively new. The rectal region has a rich blood supply, with lower venous drainage directly connected to the systemic circulation. If a peptide or protein is able to permeate the mucosa, it can avoid the first-pass effect. The presence of lymphatic vessels in the region also provides an opportunity for targeted delivery of drugs used in oncology. The paracellular route is the suggested pathway for rectal permeation of hydrophilic peptides and proteins.[104]

2.6.3 Vascular tissue

The complex architecture of the blood vessel wall would naturally lead one to believe that there are substantial barriers to the entry and transport of therapeutic agents, and that drugs with one set of physicochemical properties

may be handled entirely differently by the artery than drugs with radically different attributes. An appreciation of arterial permeability to different drugs would be of great value in understanding and developing of arterial drug delivery systems. Blood vessel walls form a selective barrier to the transport of materials between blood and tissue, and the endothelium contributes significantly to this barrier function. Vascular endothelium plays strategic roles in many drug delivery paradigms, both as an important therapeutic target itself and as a barrier for reaching tissues beyond the vascular wall. Local vascular drug delivery provides elevated concentrations of drug in the target tissue while minimising systemic side effects. Various means are being developed to improve vascular drug delivery for poorly water-soluble therapeutic agents.[105] Surfactant-like enhancers may incorporate non-soluble drugs and effectively deliver them into pathological areas with compromised vasculature (tumours, infarcts) via enhanced permeability. Optimal control of drug delivery through this mucosa would require the vehicle to be the rate-limiting domain.

2.6.4 Vaginal mucosa

In recent years, the vaginal route has been rediscovered as a potential route for systemic delivery of peptides and other therapeutically important macromolecules. However, successful delivery of drugs through the vagina remains a challenge, primarily due to the poor absorption across the vaginal epithelium. The rate and extent of drug absorption after intravaginal administration may vary depending on formulation factors, vaginal physiology, age of the patient and menstrual cycle. The presence of a dense network of blood vessels has made the vagina an excellent route of drug delivery for both systemic and local effect. The main advantages of vaginal drug delivery over conventional drug delivery are the ability to by-pass first pass metabolism, ease of administration and high permeability for low molecular weight drugs.[106] With the development of effective permeation enhancers that tend to favourably modify the transport barrier inside the epithelium, controlled drug delivery can be achieved. Permeation enhancers create favourable conditions for the shift of the rate control from the epithelium to the dosage form.[106]

2.7 Comparative studies of chemical markers through different epithelia

While all the advantages of mucosal delivery seem appealing and a great deal of research has been devoted to alternate drug delivery, the actual clinical use of the epithelial membranes as delivery sites thus far has been limited, mainly because of low drug bioavailability. The unfavourable properties of the epithelia to drug absorption, i. e., high degree of hydrophobicity, charge selectivity and tight junctions, as well as those of the peptide and protein molecules, i. e., large molecular size and high charge density, make delivery of these compounds difficult. For most drugs, it is generally accepted that the transport through the cellular epithelial barriers occurs via a non-specific diffusion process governed primarily by a concentration gradient. Under these conditions, the physicochemical properties of the diffusing solute and the physiological function of the cell layer involved are the important factors dictating the transport rate. For hydrophilic drugs which do not partition well into the cell membrane, as is the case for most peptides and proteins, leakage through the paracellular and/or transcellular routes should be the major transport pathways. The transport barrier of the paracellular pathway has two properties that influence the absorption rates of drugs, particularly those with charges: first, the general permeability or magnitude of the barrier, which is controlled mainly by the tight junctions and second, the permselectivity of the barrier, which is a quantitive measure of the ability of the epithelia to discriminate or show preference for the transport of molecules of different charges.[107] These two membrane properties have been studied by several investigators, but only a few have compared their relative magnitude and their subsequent effect on drug absorption, particularly in the epithelia relevant to drug delivery.[108,109] Thus, it remains unclear which of these membranes may represent the best alternate route for drug delivery purposes. Furthermore, the differences in experimental design and conditions used in previous studies, i. e., type of drugs, species of animals, and analytical techniques used, make it impossible to compare the transport data.

2.8 Permeability studies for *in vitro* drug testing

A number of experimental methods have been developed to study the transmembrane permeability of drugs in an attempt to isolate physical and chemical factors that govern drug absorption. There are several benefits of utilising an *in vitro* flow cell system for the initial testing of drug diffusion in the laboratory. The environment, specific permeation parameters and variables may be controlled in an attempt to reveal specific factors affecting the kinetic processes, prior to undertaking studies in human volunteers. Experimental design that allows the greatest number of variables and different variable permutations to be investigated by the same diffusion apparatus is highly desirable.

The different flow cells (Fig. 5) that can be used, include the conventional static Franz cells, where a precise volume of sample must be removed with a simultaneous media replacement and the receptor fluid is agitated with a magnetic bar stirrer. A variation on these designs is a side-by-side cell which allows both donor and receptor compartments to be stirred at the same time. In contrast to the static cells, flow-through diffusion cells attempt to mimic *in vivo* conditions with a flow-through receptor phase equating to the blood supply, and with an unstirred donor phase equivalent to a drug formulation.[25, 110,111]

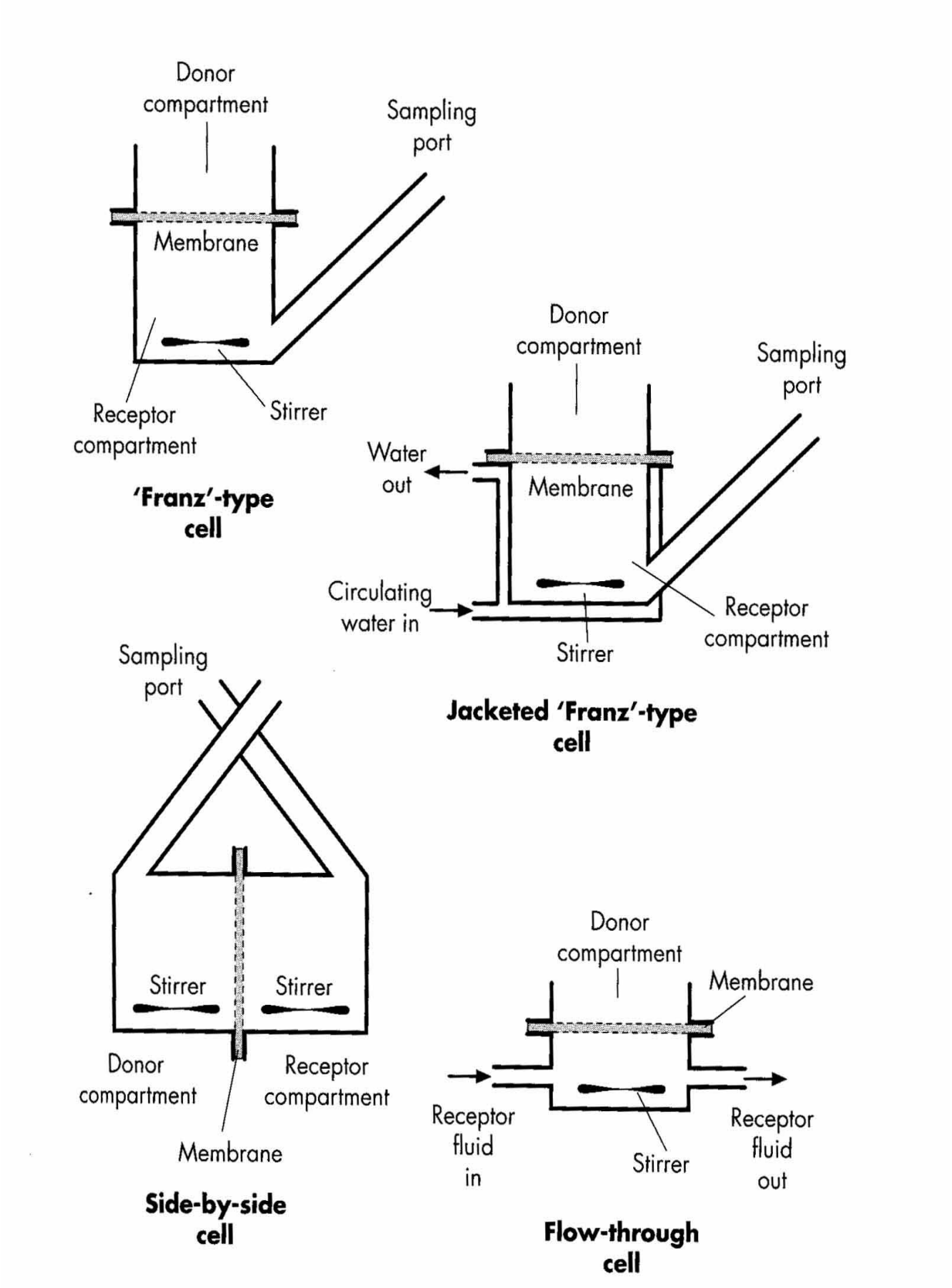


Figure 5. Examples of commonly used diffusion cell designs.[25]

In contrast to the static Franz cells, the flow-through diffusion cells offers automation with the addition of a pump that offers an accurate, constant flow rate of buffer. The addition of a fraction collector is the only other requirement.[110] The drug is added to the donor compartment of the flow cell and collected in the fraction collector from the acceptor compartment of the flow cell for the required time period at a constant flow speed. The amount of drug present in the effluent can be detected by various means e.g. measuring labelled drug (radio-labelled, fluorescently-labelled) by means of scintillation counting, UV spectroscopy, fluorimetry or high-performance liquid chromatography (HPLC).

The use of small tissue samples (4 mm∅) and the maintenance of a continuous high gradient of permeant across the biological barrier membrane are major benefits of this system.

2.9 Aim

To investigate the role of surfactant in, and compare the permeability of, porcine and human epithelia to various chemical compounds.

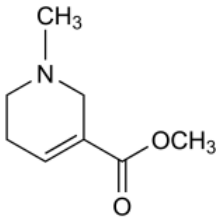
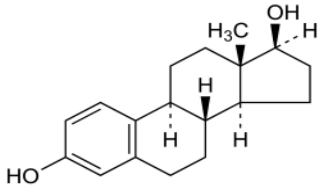
3. MATERIALS

3.1 Chemicals

1,2 Dipalmitoyl-L-α-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-L-α-phosphatidylglycerol (PG), cetyl alcohol, tyloxapol (4-(1, 1, 3, 3-tetramethylbutyl) phenol polymer with formaldehyde and oxirane), Zidovudine (AZT) and Isoniazid (INH) were purchased from the Sigma Chemical Company, St. Louis MO. Phospholipid purity was verified by thin-layer chromatography (TLC) [112]. Sterile water for injection was used in the preparation of surfactant. Chloroform used was HPLC grade. All other chemicals used were of the highest analytical grade.

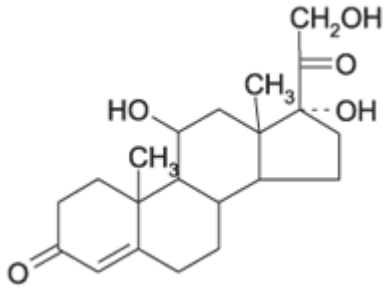
3.2 Radioactive compounds

^{3}H-17β-estradiol was supplied by Sigma Chemical Company (St. Louis, Mo). ^{3}H-reduced arecoline, ^{3}H-Dexamethasone and ^{3}H-Vasopressin were obtained from Amersham Laboratories (Amersham Place, Little Chalfont, UK). ^{3}H-Hydrocortisone and ^{3}H-Oxytocin were obtained from Perkin Elmer Life Sciences (Boston).

CHEMICAL STRUCTURES OF PERMEANTS	
Arecoline (Mw = 141 Da) This alkaloid has been associated with oral cancer as well as submucous fibrosis.  	**17β-estradiol** (Mw = 272 Da) This drug is used for hormone replacement therapy during the female climacteric. 

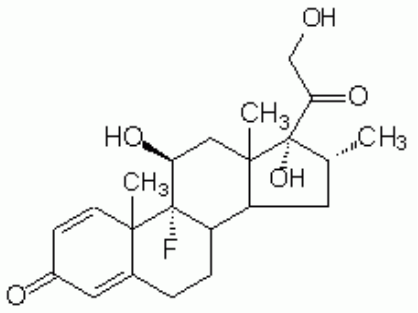
Hydrocortisone (Mw = 362 Da)

This is a naturally occurring corticosteroid used principally for its anti-inflammatory and immunosuppressive actions, or for hormone replacement therapy.



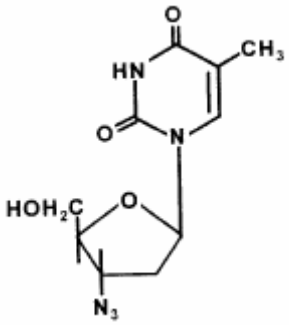
Dexamethasone (Mw = 392 Da)

This is one of the potent synthetic analogues of cortisol used for the prevention and treatment of cerebral oedema and it is the drug of choice for suppression of ACTH production.



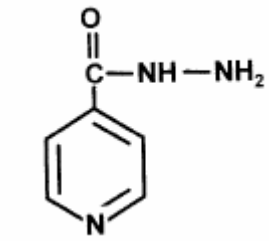
Zidovudine (AZT) (Mw = 267 Da)

This is an analogue of thymidine. In retroviruses – such as the HIV virus – it is an active inhibitor of reverse transcriptase.



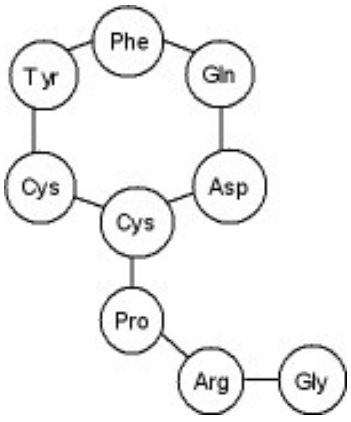
Isoniazid (INH) (Mw = 137 Da)

This is an antituberculosis drug.



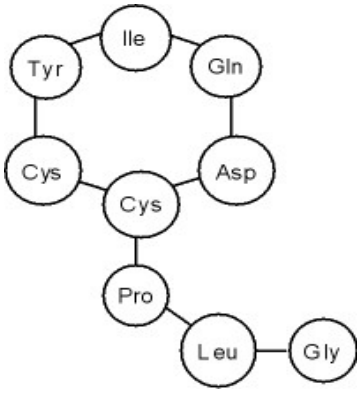
Vasopressin (ADH) (Mw = 1084 Da)

This is the antidiuretic peptide hormone important for its actions on the kidney.



Oxytocin (Mw = 1007 Da)

The principal uterus-contracting and lactation-stimulating hormone of the posterior pituitary gland.



3.3 Natural surfactants

Curosurf® and Survanta® were obtained from Safeline Pharmaceuticals (Florida R.S.A.) and Abbott Laboratories (Aeroton, Johannesburg), respectively.

4. METHODS

4.1 Synthetic lung surfactants

Biopolsurf preparations were prepared by mixing DPPC, cetyl alcohol and PG in a 10:1.1:1 ratio (w/w) in chloroform. The organic solvent was then removed by rotary evaporation and the mixture was dried under a continuous stream of nitrogen at room temperature. The dried phospholipid film was then hydrated with 0.1 M NaCl. A Branson B-15P ultrasonicater fitted with a microtip was then used to sonicate the mixture on ice under a stream of nitrogen (power of 20 watts for 7 x 13 s; 60 s intervals). Hereafter, tyloxapol was added to the preparation to a final concentration of 1 mg/ml and the tube was sealed under nitrogen before use. The Biopolsurf suspension contained 13.5 mg/ml DPPC and 1.35 mg/ml PG. An “in-house” Exosurf® was also prepared in a similar fashion as described above and consisted of three components: DPPC / cetyl alcohol / tyloxapol [13.5:1.5:1 (w/w)]. Biopolsurf used in experiments contained a phospholipid concentration of 14.85 mg/ml (full), 7.425 mg/ml (half) and 3.713 mg/ml (quarter) respectively. Dilutions were done with 0.1 M NaCl.

4.2 Tissue preparation

Porcine tissue specimens of bronchial, buccal and rectal mucosa as well as bronchial artery and vein tissue were obtained from the Tygerberg Animal House. Human vaginal specimens were obtained from excess tissue removed from 4 postmenopausal patients, mean age 67 ± 7 S.D. (range 60 – 80) years, following vaginal hysterectomies at the Louis Leipoldt Hospital, Bellville, South Africa.

All the above tissue specimens were immediately placed in a transport medium consisting of phosphate-buffered saline (PBS, pH 7.4) or Eagle's Minimum Essential Medium (MEM) without L-glutamine and $NaHCO_3$ and transferred to the laboratory within 1 h. Excess connective and adipose tissue were trimmed away and tissue specimens were snap frozen in liquid nitrogen and stored at -85°C. No specimens were included in the study where there was any clinical evidence of disease that might influence the permeability characteristics of the mucosa. The Ethics Committee of the Stellenbosch University and Tygerberg Hospital approved the study.

4.3 Permeability experiments

The diffusion kinetics of chemical compounds through porcine: bronchial, buccal, rectal, arterial and venous tissue as well as human vaginal mucosa were determined with a flow-through diffusion system in the presence or absence of different surfactants (Fig 6).

Figure 6. Flow-through diffusion apparatus used in the present study.

Before each permeability experiment, tissue specimens were equilibrated for 10 min at room temperature in phosphate-buffered saline (PBS, pH 7.4). Thereafter the specimens were carefully cut, so as not to damage the

epithelial surfaces, into 4 mm^2 sections and mounted in flow-through diffusion cells (exposed areas 0.039 cm^2) as previously described.[113-124] Permeation studies were performed on 7 tissue replicates for each experiment. Before beginning each experiment, tissue disks were equilibrated for 10 min with PBS (pH 7.4) at 20°C in both the donor and receiver compartments of the diffusion cells. After equilibration, the PBS was removed from the donor compartment and replaced with 0.5 ml of chemical marker/drug mixed with either PBS or surfactant. Aliquots (100 μl) were removed within 1 min from each of the seven donor compartments for determination of donor cell concentration at time zero. PBS at 20°C was pumped through the receiving chambers at a rate of 1.5 ml/h and collected, by means of a fraction collector, at 2-h intervals for 24 h. The permeability study was performed under sink conditions, i.e. at the completion of each run the concentration of permeant in the acceptor chamber never reached 10% of that in the donor compartment. Scintillation cocktail (10 ml) (PCS, Complete Phase Combining System for liquid scintillation counting, Amersham Biosciences UK Limited, Little Chalfont, England) was added to samples containing radioactive compounds and counted in a liquid scintillation counter (Beckman LS 5000TD) until a 2-s value of 1% was reached. Quenching for each sample was automatically corrected for in the counter.

4.4 HPLC detection of AZT and INH

Permeant-containing effluent samples, collected from the acceptor compartments of the perfusion apparatus over the 2 – 14 h sampling intervals, were analysed using an Hewlett Packard 1100 series high-performance binary liquid chromatograph (Agilent Tecnologies, Waldbron, Germany) with an Agilent Eclipse (XDB-C18) Zorbax analytical column (5 µm particle size), 150 mm x 4.6 mm (ID). The latter column was preceded by a 30 x 2.1 mm (ID) C_{18} guard column (40 µm particle size). The temperature was maintained at 40˚C, the injection volume was 20 µl and flow rates of 1.0 ml/min were used. The mobile phase consisted of a mixture of two solvents, A (50 mM KH_2PO_4, pH 5.42) and B (acetronitrile-isopropanol; 4:1 v/v). All reagents used for the mobile phase were HPLC grade (Burdick & Jackson,

Honeywell International Inc, Muskegon, MI, USA) and were filtered through a 0.45 µm filter. Deionised water was used for preparing all aqueous standard and buffer solutions. For the determination of AZT an isocratic mixture of 60% A: 40% B was used. AZT was detected at 266 nm (retention time 4.5 min). A standard calibration curve (R^2 = 0.999) was constructed over the expected concentration range (1-20 µg/ml AZT in PBS, pH 7.4) and used for quantitation of AZT. Spiked standards over the expected concentration range (1-20 µg/ml) were randomly included in each batch. The area under the curve of the respectively obtained peaks was used to calculate the AZT content of effluent samples.

For the detection of INH, 400 µl of 10% TCA (trichloroacetic acid) and 400 µl of the INH-containing effluent sample were mixed and centrifuged for 1 min at 10 500 x g. 40 µl of 1% methanolic cinnamaldehyde were added to 200 µl of supernatant for derivatisation. Peaks for INH detection were recorded at 340 nm. For the first min of chromatography the mobile phase contained 40% of solvent B, which was then increased linearly to reach 70% after 10 min. For re-equilibration of the column 4 min was allowed between consecutive runs.[125]

A standard calibration curve was constructed over the expected concentration range (1-15 µg/ml INH in PBS, pH 7.4). The area under the curve of the respectively obtained peaks was used to calculate the INH content of effluent samples. Spiked standards over the expected concentration range (1-15 µg/ml) were included in each batch. Calibration was linear over the entire concentration range (R^2 = 0.9993). Recording and integration peaks were performed by means of an Agilent Chem Station.

4.5 Calculation of flux values

Flux (J) values of radioactive compounds across membranes were calculated by means of the relationship: $J = Q / A \times t$ (dpm $\times$ cm^{-2} $\times$ min^{-1}), where Q = quantity of substance crossing membrane (dpm), A = membrane area exposed (cm^2), and t = time of exposure (in min).

Flux (J) values of other chemical compounds across membranes were calculated by means of relationship: $J = Q / A \times t$ (µg x cm^{-2} $\times$ min^{-1}), where

Q = quantity of substance crossing membrane (µg), A = membrane area exposed (cm^2), and t = time of exposure (in min).

4.6 Steady state kinetics

When no statistically significant differences ($p<0.05$) (ANOVA and Duncan's multiple range test) between flux values were obtained over at least two consecutive time intervals, a steady state (equilibrium kinetics) was assumed to have been reached for a particular specimen and chemical compound.

4.7 Statistical analyses

Non-linear regression analyses (third order polynomial) were performed using a GraphPad Prism, Version 3, 2004 Computer program.[126] A F-test was used to compare entire curves. An unpaired t test with Welch's correction was used to investigate possible differences between flux means of tissues at 2-h intervals. A significance level of $P<0.05$ was used for all tests and comparisons.

4.8 Histological assessment

Trimmed bronchial artery and venous tissue were placed in 4% neutral buffered formaldehyde solution. Tissue was then dehydrated in increasing concentrations of alcohol. Thereafter, the specimens were embedded in paraffin wax before sectioning on a microtome. The specimens were then stained according to the Massons Trichrome staining method.

5. RESULTS

The mean steady state flux values of arecoline across: Porcine bronchial tissue (n = 33), buccal (n = 12), rectal (n = 11) and human vaginal mucosa (n = 27) as well as porcine bronchial artery tissue (n = 24) are shown in Fig. 7. Arecoline steady state flux values were obtained after 18 h. Compared to arecoline flux values across porcine bronchial tissue, arecoline flux values across porcine buccal mucosa were 40% lower ($p = 9.49E^{-6}$), flux values across porcine rectal mucosa were 35% higher (p = 0.00012), flux values across porcine bronchial arterial tissue were 93% higher (p = 0.00015). Arecoline flux values across human vaginal mucosa were 5% higher (p = 0.0115) compared to the flux values across porcine bronchial tissue.

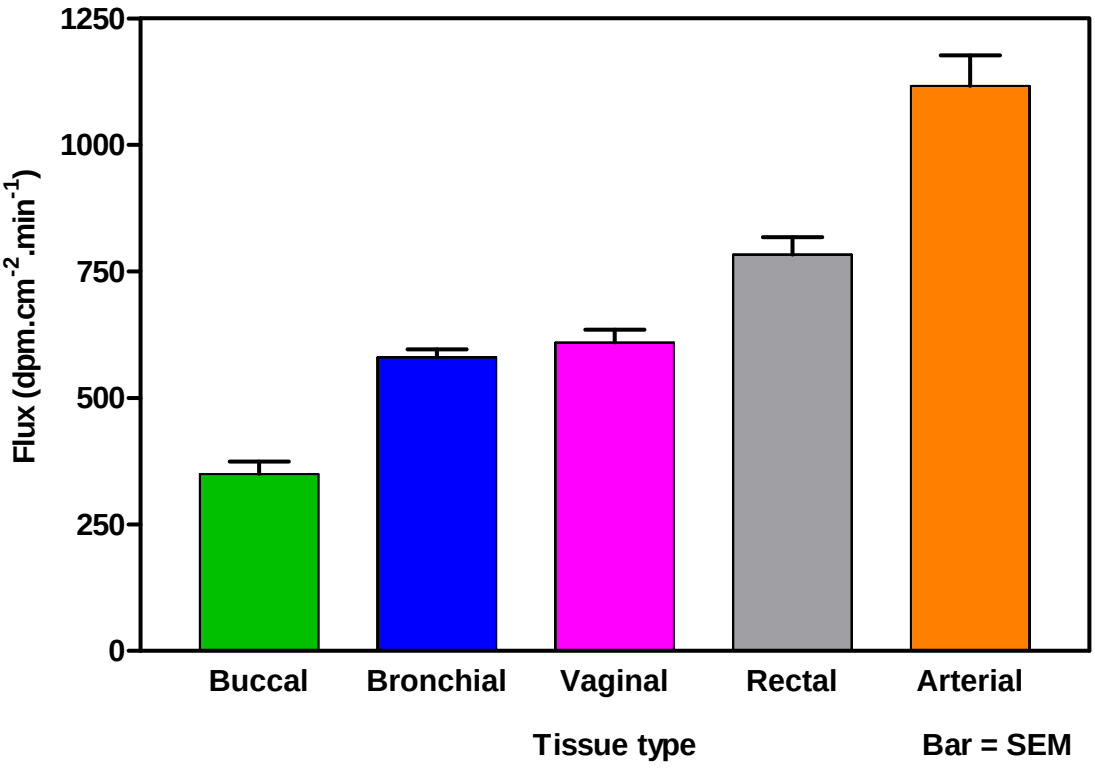


Figure 7. Mean steady state flux values of arecoline across: Porcine bronchial, buccal, rectal and human vaginal mucosa as well as porcine bronchial arterial tissue.

The overall mean flux values of arecoline across porcine bronchial tissue with and without Biopolsurf and Exosurf as penetration enhancers are shown in Fig. 8. No statistically significant differences between the arecoline control (n = 33) and arecoline with Exosurf (n = 17) could be demonstrated (p = 0.9554). The flux values of arecoline with Biopolsurf (n = 31) were enhanced by 12% when compared to the arecoline control (p = 3.75311 E^{-11}).

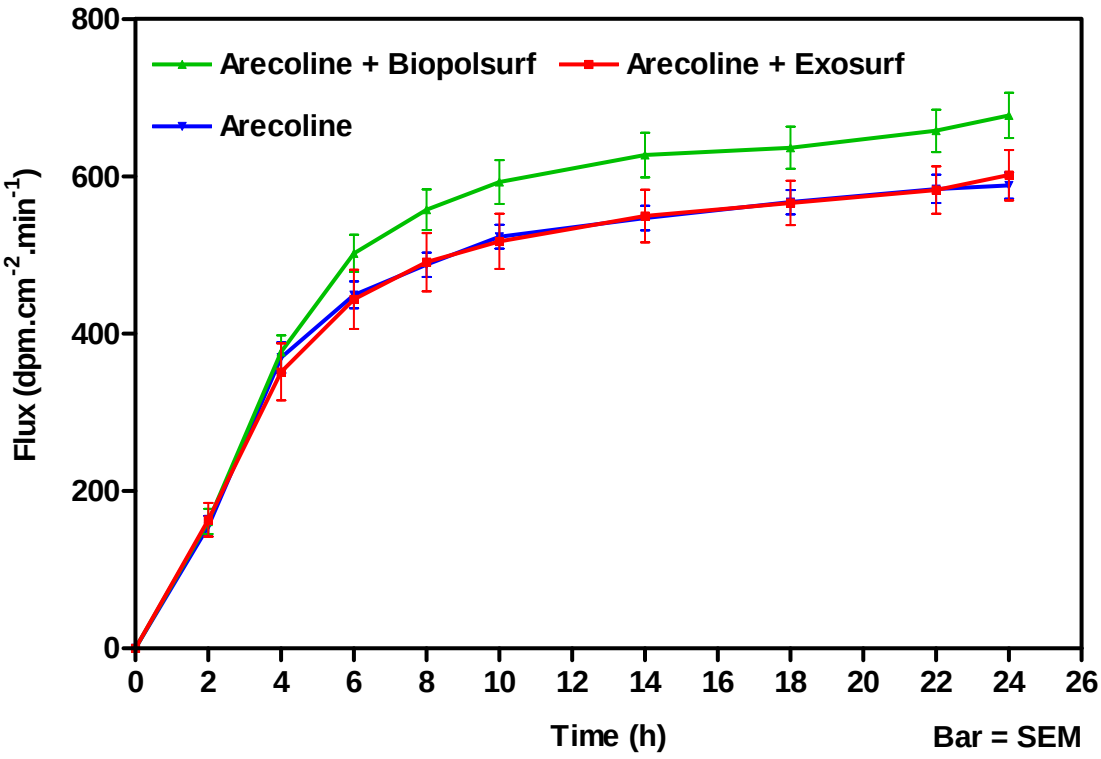


Figure 8. Overall mean flux values of arecoline across porcine bronchial tissue with and without Biopolsurf and Exosurf as penetration enhancers.

The overall mean flux values of arecoline across porcine bronchial tissue with and without Biopolsurf, Curosurf® and Survanta® as penetration enhancers are shown in Fig. 9. Both Biopolsurf (n = 31) and Curosurf® (n = 17) significantly enhanced the flux values of arecoline across porcine bronchial tissue by 14% ($p = 3.75\ E^{-11}$) and 19% ($p = 1.7\ E^{-10}$), respectively, when compared to arecoline control (n = 33).There were no statistical differences between Biopolsurf and Curosurf® when compared to each other (p = 0.4745). While Survanta® (n = 16) gave no significant enhancement (p = 0.1183).

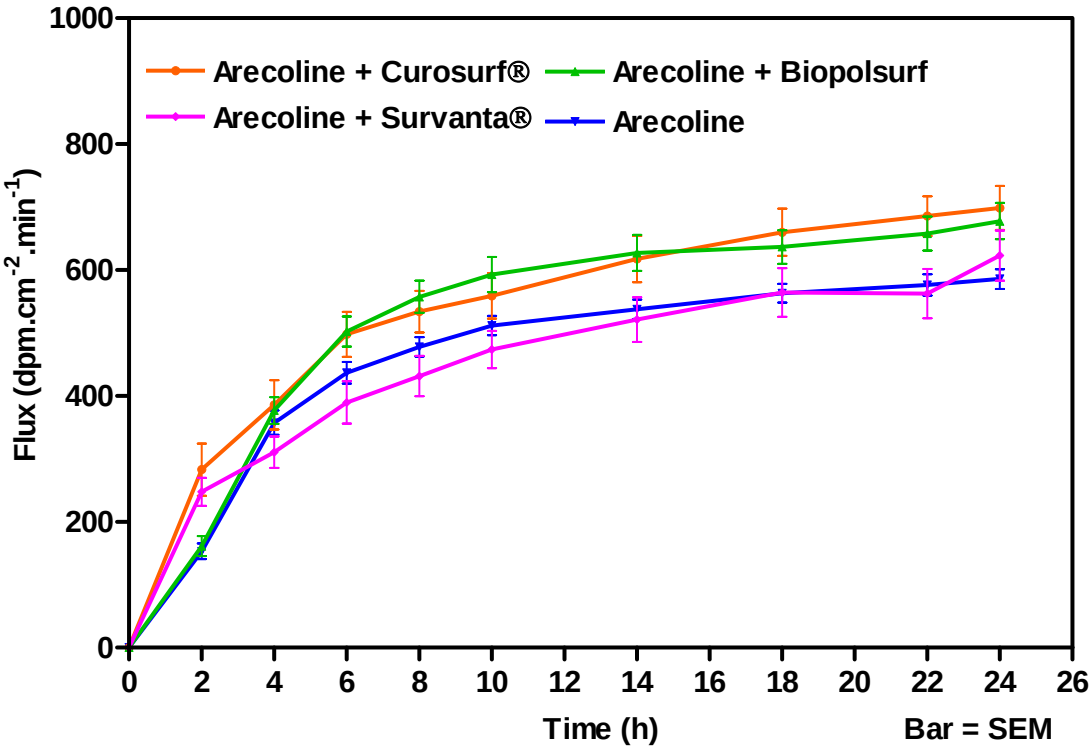


Figure 9. The overall mean flux values of arecoline across porcine bronchial tissue with and without Biopolsurf, Curosurf® and Survanta® as penetration enhancers. Phospholipid concentration in Curosurf® and Survanta® preparations was adjusted to be similar to that of Biopolsurf.

The overall mean flux values of arecoline across human vaginal mucosa with and without Biopolsurf and Exosurf as penetration enhancers are shown in Fig. 10. Compared to the arecoline control both Biopolsurf (n = 18) and Exosurf (n = 17) significantly enhanced the flux value of arecoline (n = 27) across human vaginal mucosa by 22% ($p = 1.0388\ E^{-5}$) and 24% ($p = 1.807\ E^{-14}$), respectively.

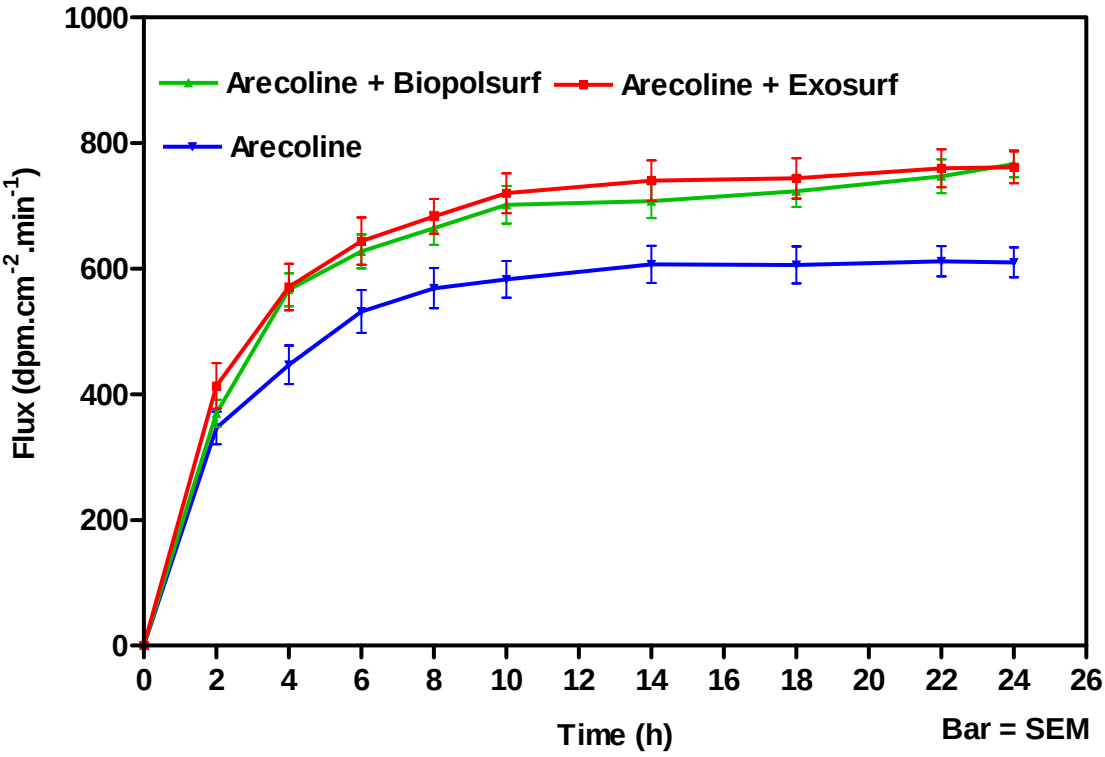


Figure 10. Overall mean flux values of arecoline across human vaginal mucosa with and without Biopolsurf and Exosurf as penetration enhancers.

Fig. 11 shows the overall mean flux values of arecoline across porcine buccal mucosa with and without Biopolsurf and Exosurf. The presence of Biopolsurf (n = 11) significantly increased the flux values of arecoline by 20% ($p = 5.58\ E^{-10}$) compared to arecoline control (n = 12). Arecoline with Exosurf (n = 17) did not cause any significant enhancement.

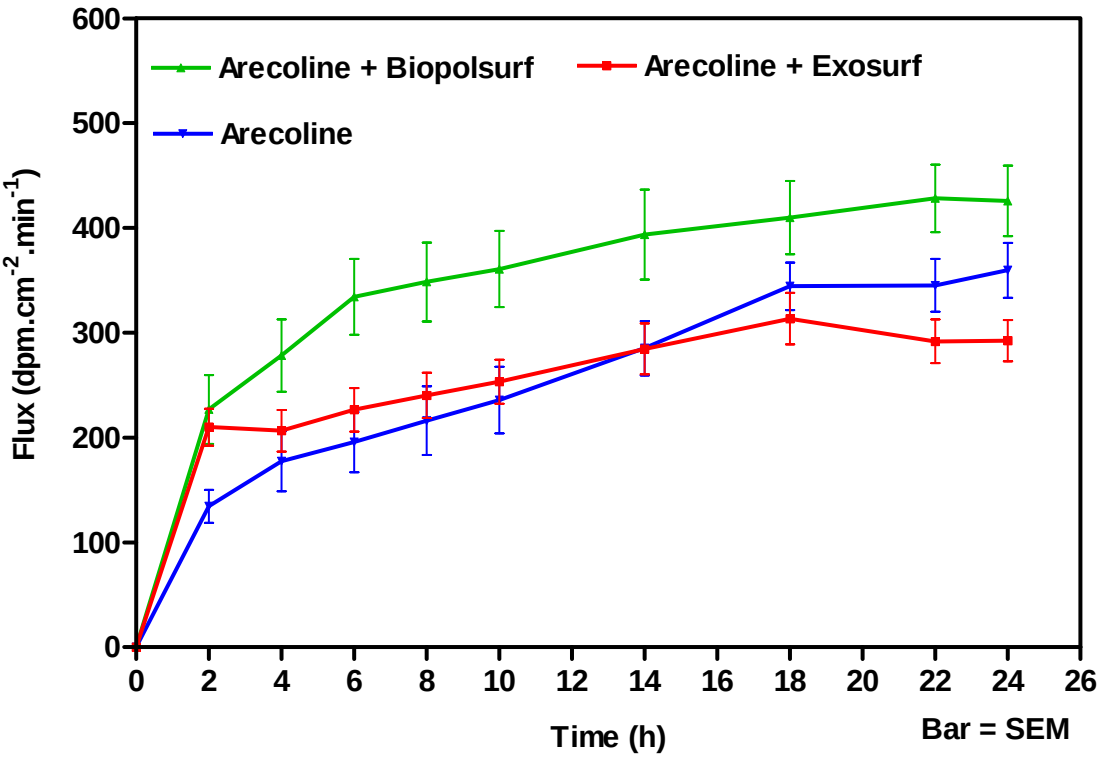


Figure 11. Overall mean flux values of arecoline across porcine buccal mucosa with and without Biopolsurf and Exosurf as penetration enhancers.

Fig. 12 shows the overall mean flux values of arecoline across porcine rectal mucosa with and without Biopolsurf as penetration enhancer. The flux value of arecoline with the Biopolsurf (n = 14) was significantly enhanced by 14% (p = 0.0081) when compared to the arecoline control (n = 11).

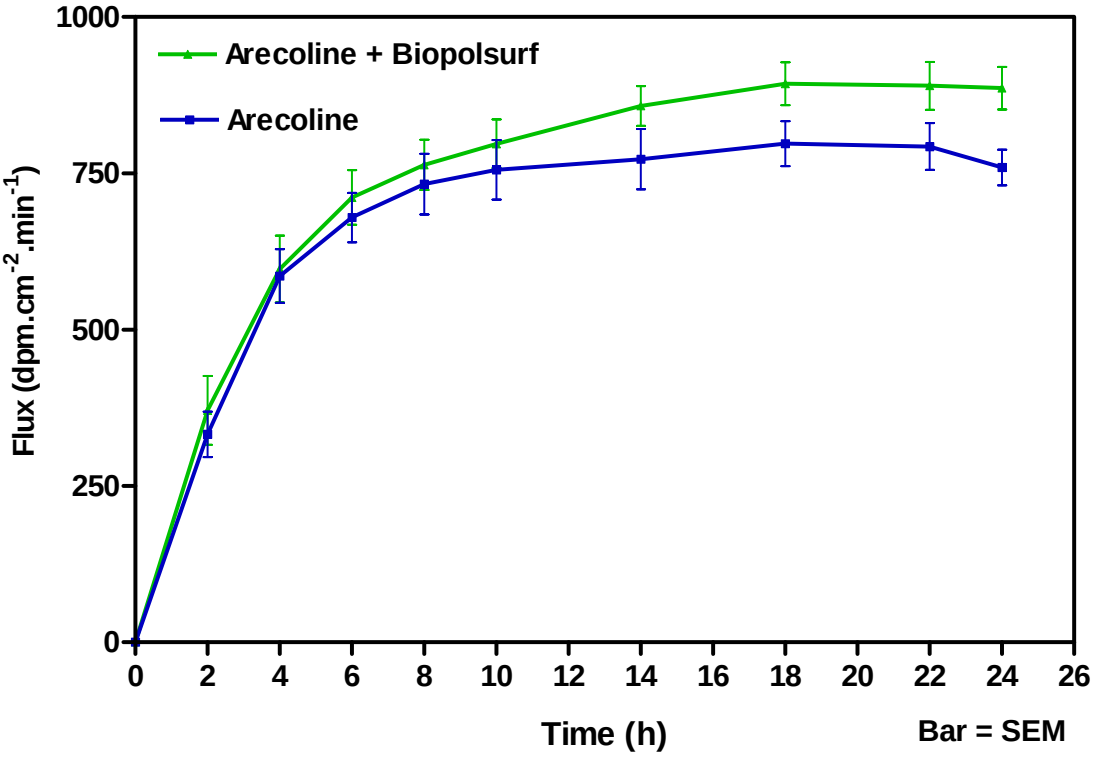


Figure 12. Overall mean flux values of arecoline across porcine rectal mucosa with and without Biopolsurf as penetration enhancer.

The mean estimated steady state flux values of 17β-estradiol across: Porcine bronchial (n = 15) and human vaginal mucosa (n = 24) as well as porcine bronchial arterial (n = 10) and venous tissue (n = 11) are shown in Fig. 13. For 17β-estradiol steady state conditions were not reached over the 24 h time course over which the experiment was conducted, however for comparative purposes, steady state fluxes were estimated by averaging flux rates at 18, 22 and 24 h. Compared to 17β-estradiol flux values across porcine bronchial tissue, 17β-estradiol flux values across porcine bronchial venous tissue were 191% higher (p = 0.00037), 17β-estradiol flux values across porcine bronchial arterial tissue were 94% higher (p = 0.00052) and 17β-estradiol flux values across human vaginal mucosa were 24% higher (p = 0.14).

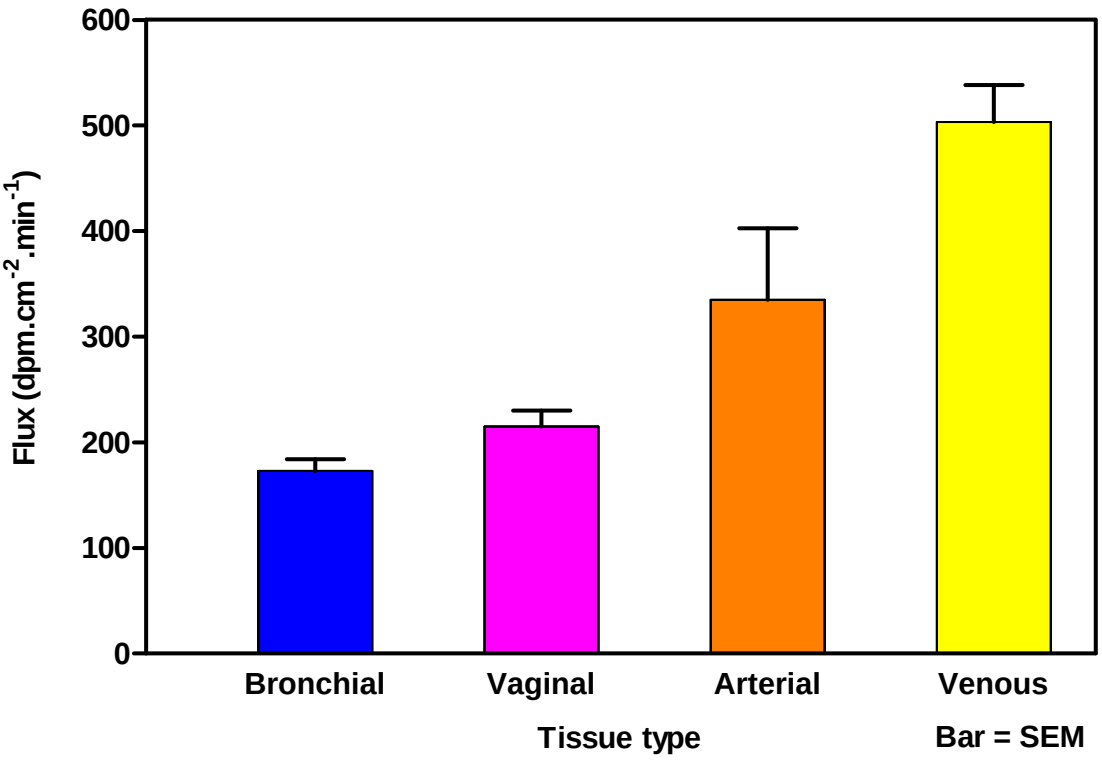


Figure 13. Mean estimated steady state flux values of 17β-estradiol across: Porcine bronchial and human vaginal mucosa as well as porcine bronchial arterial and venous tissue.

Overall mean flux values of 17β-estradiol through porcine bronchial tissue with and without Biopolsurf and Exosurf as penetration enhancers are shown in Fig. 14. The presence of Biopolsurf (n = 24) and Exosurf (n = 13) significantly increased the 17β-estradiol flux values with 43% ($p = 4.51\ E^{-26}$) and 11% ($p = 8.9\ E^{-10}$) respectively, when compared to 17β-estradiol control values (n = 15). Although 17β-estradiol flux values with the Biopolsurf were significantly higher (28%) when compared to the flux values with Exosurf ($p = 1.3\ E^{-9}$), steady state was not reached in 24 h.

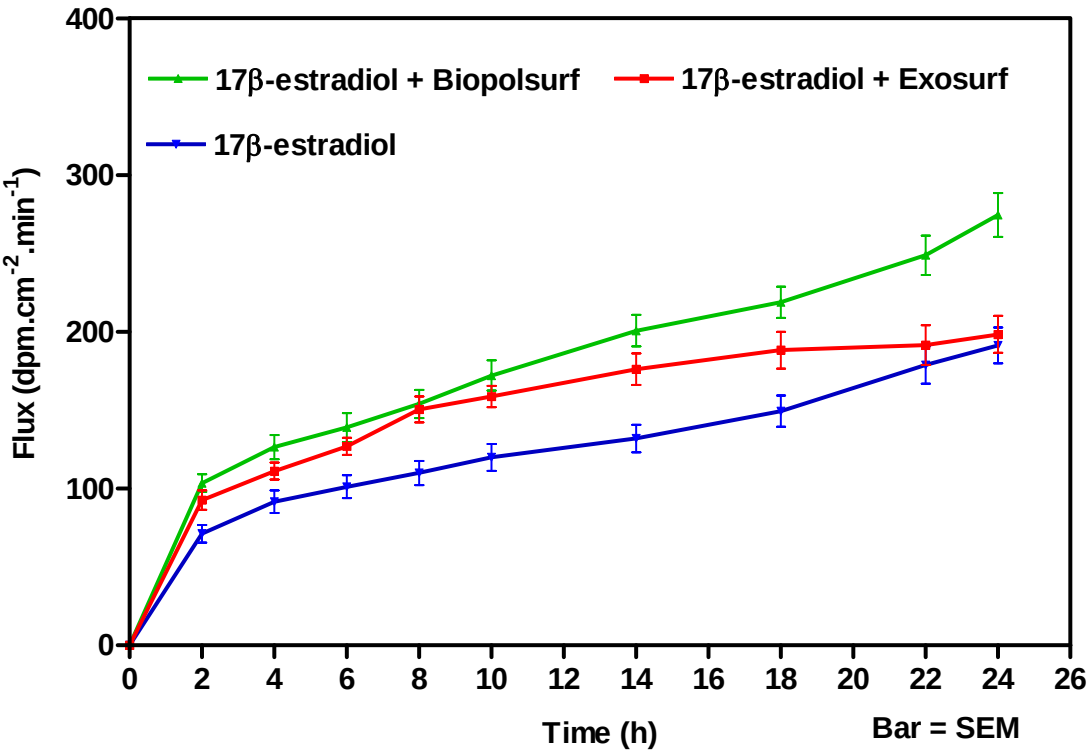


Figure 14. Overall mean flux values of 17β-estradiol across porcine bronchial tissue with and without Biopolsurf and Exosurf as penetration enhancers.

The overall mean flux values of 17β-estradiol across porcine bronchial arterial tissue with and without Biopolsurf are compared in Fig. 15. In the presence of Biopolsurf (n = 14) the flux value of 17β-estradiol across porcine bronchial arterial tissue was significantly enhanced by 22% ($p = 8.15\ E^{-6}$) compared to the 17β-estradiol control (n = 10).

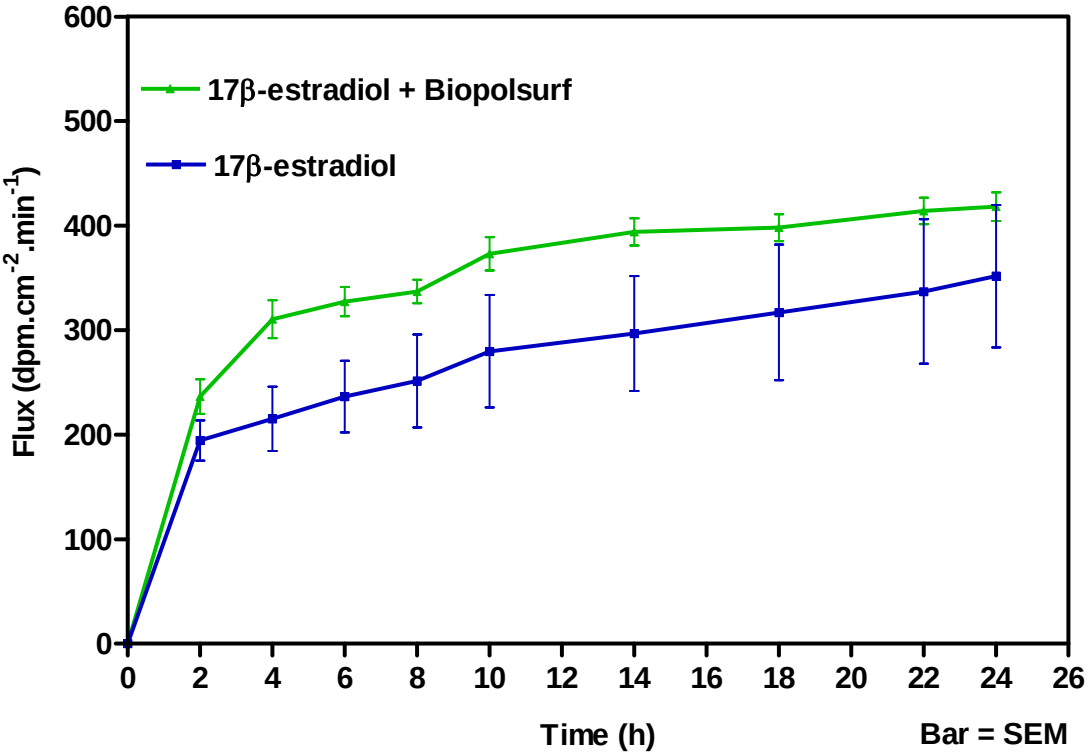


Figure 15. The overall mean flux values of 17β-estradiol across porcine bronchial arterial tissue with and without Biopolsurf as penetration enhancer.

The overall mean flux values of 17β-estradiol across porcine bronchial venous tissue with and without Biopolsurf are compared in Fig. 16. In the presence of Biopolsurf (n = 15) the flux value of 17β-estradiol across porcine venous tissue (bronchial) was significantly enhanced (p = 0.00545), although it was only for the first 12 h compared to 17β-estradiol control (n = 11).

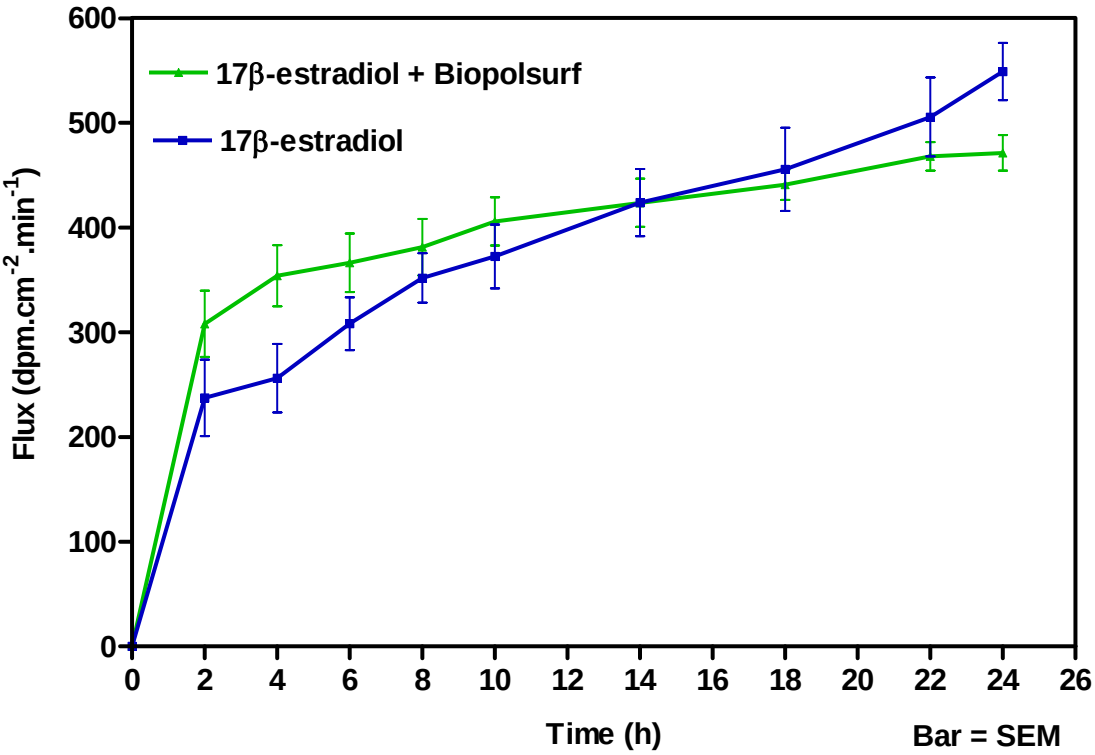


Figure 16. The overall mean flux values of 17β-estradiol across porcine bronchial venous tissue with and without Biopolsurf as penetration enhancer.

The mean steady state flux values of hydrocortisone across: Porcine bronchial (n = 26) and human vaginal mucosa (n = 14) are shown in Fig. 17. The flux values of hydrocortisone across human vaginal mucosa were 33% higher (p = 0.127) compared to porcine bronchial tissue.

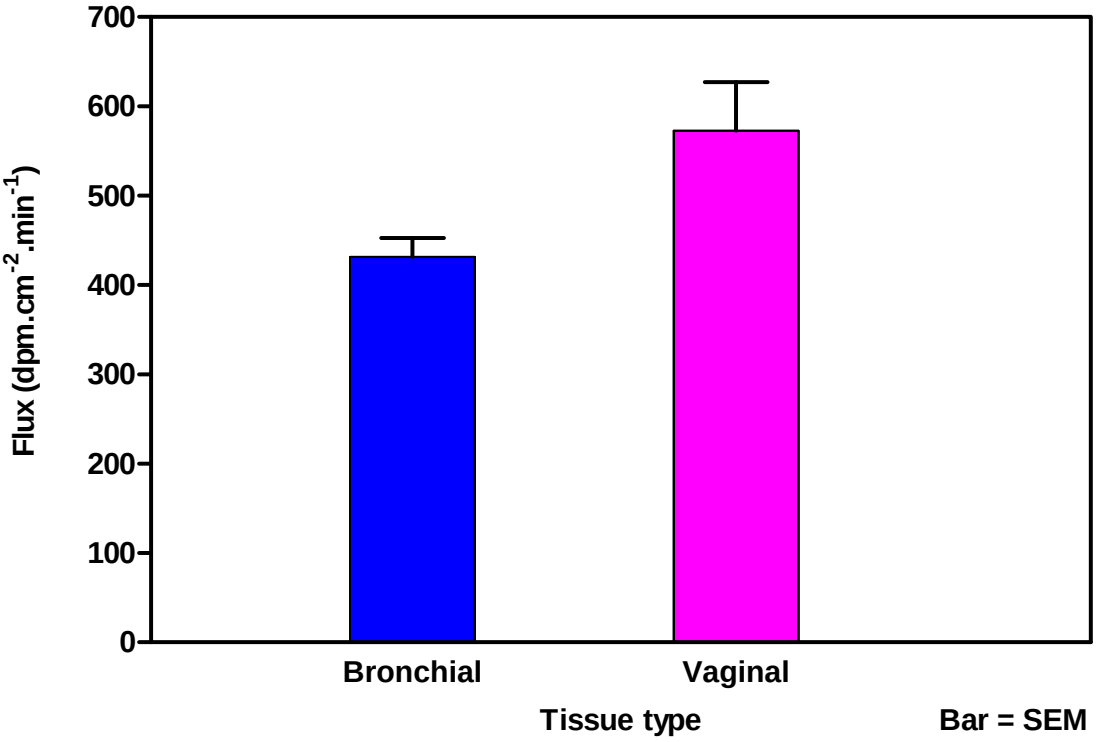


Figure 17. Mean steady state flux values of hydrocortisone across porcine bronchial and human vaginal mucosa.

As can be observed in Fig. 18, the presence of Biopolsurf (n = 24) significantly increased the flux value of hydrocortisone across porcine bronchial tissue by 17% ($p = 4.37E^{-5}$) compared to the flux value of the hydrocortisone control (n = 26).

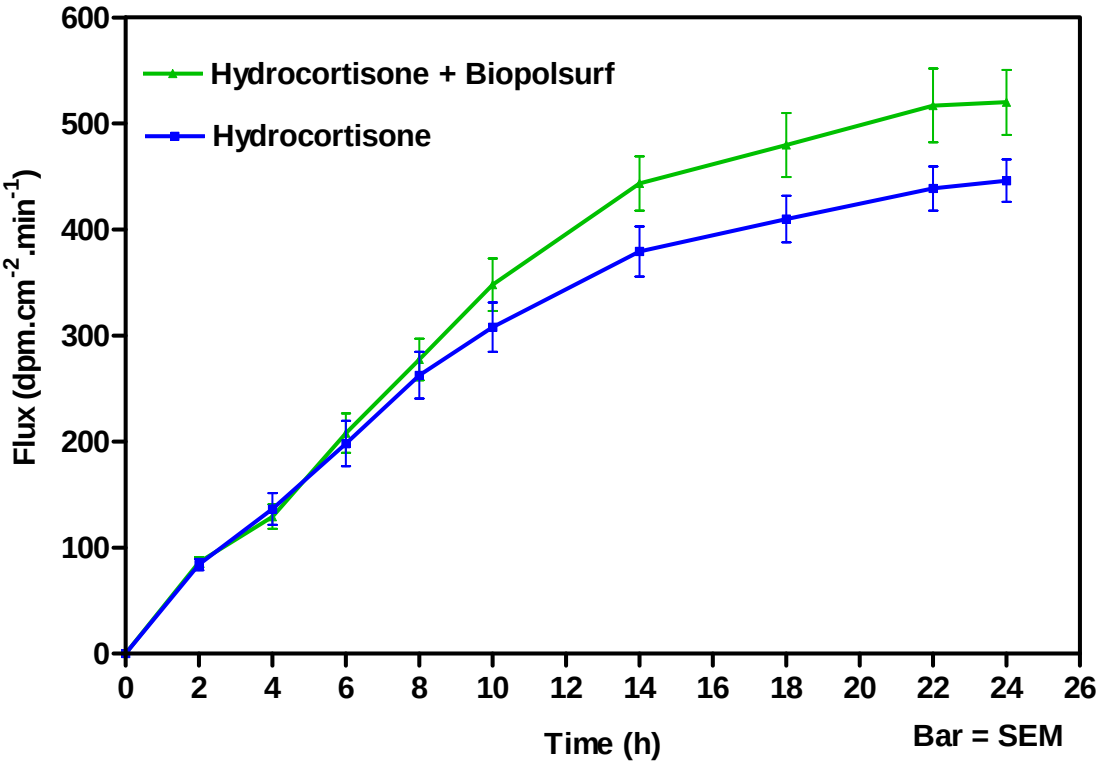


Figure 18. Overall mean flux values of hydrocortisone across porcine bronchial tissue with and without biopolsurf as penetration enhancer.

The mean estimated steady state flux values of dexamethasone across: Porcine bronchial (n = 22) and buccal mucosa (n = 13) as well as human vaginal mucosa (n = 14) are shown in Fig. 19. Steady state was not achieved for dexamethasone and estimated flux values were used (means of values obtained at 18, 22 and 24 h.) Dexamethasone flux values across human vaginal mucosa and porcine buccal mucosa were 8% (p = 0.303) and 62% (p = 0.00047) lower compared to flux values across porcine bronchial tissue, respectively.

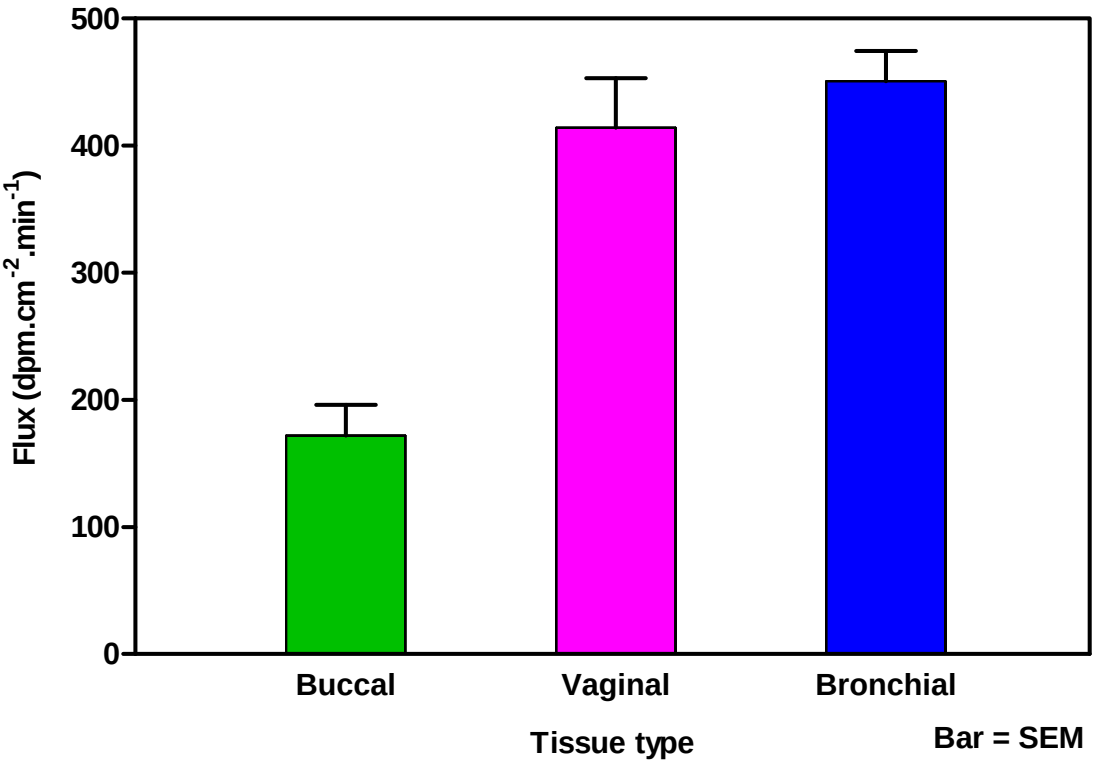


Figure 19. Mean estimated steady state flux values of dexamethasone across: Porcine bronchial, buccal and human vaginal mucosa.

Fig. 20 shows the overall mean flux values of dexamethasone across porcine bronchial tissue with and without various concentrations of Biopolsurf (Biopolsurf concentration study). Compared to the dexamethasone control (n = 22) the half concentration Biopolsurf (n = 18) significantly enhanced the flux value of dexamethasone across porcine bronchial tissue by 6% (p = 0.00014) and the quarter concentration Biopolsurf (n = 20) significantly enhanced the flux value of dexamethasone by 17% (p = $2.02E^{-11}$). The full concentration Biopolsurf (n = 21) retarded the flux value of dexamethasone across the bronchial tissue.

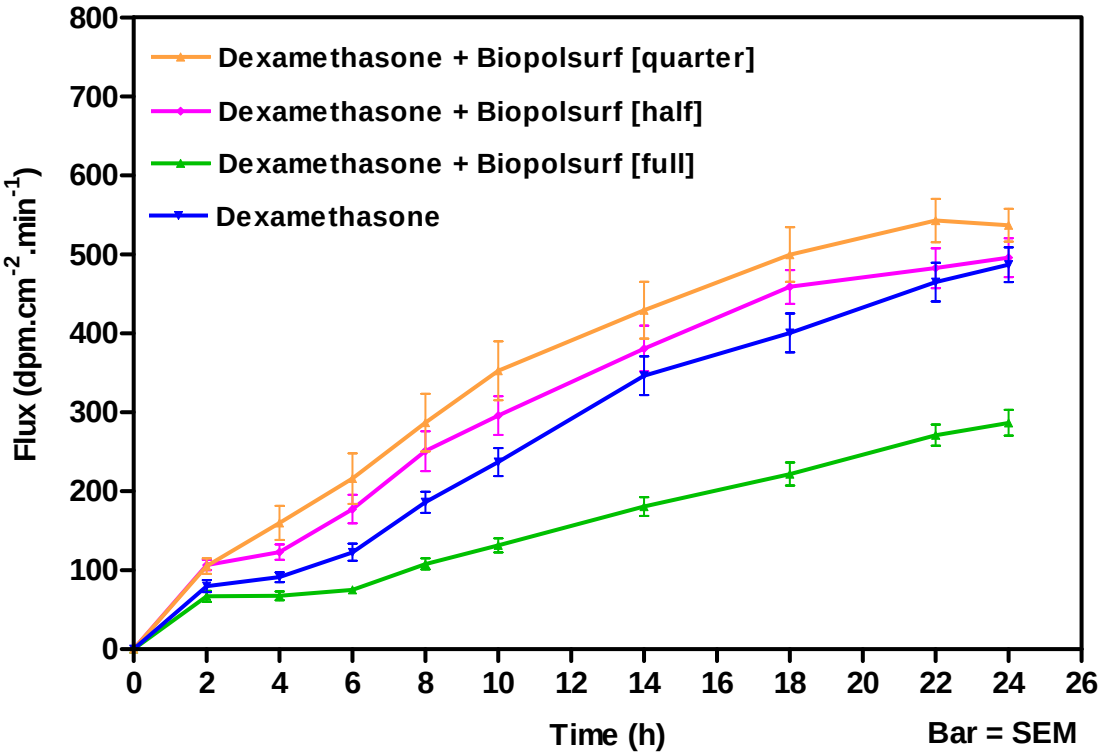


Figure 20. Overall mean flux values of dexamethasone across porcine bronchial tissue with and without full, half and quarter concentrations of Biopolsurf as penetration enhancers.

The mean estimated steady state flux values of vasopressin across: Porcine bronchial (n = 26) and human vaginal mucosa (n = 16) are shown in Fig. 21. Steady state conditions were not achieved for vasopressin and estimated flux values were used (means of values obtained at 18, 22 and 24 h). The flux values of vasopressin across human vaginal mucosa were 85% higher (p = 0.00032) compared to porcine bronchial tissue.

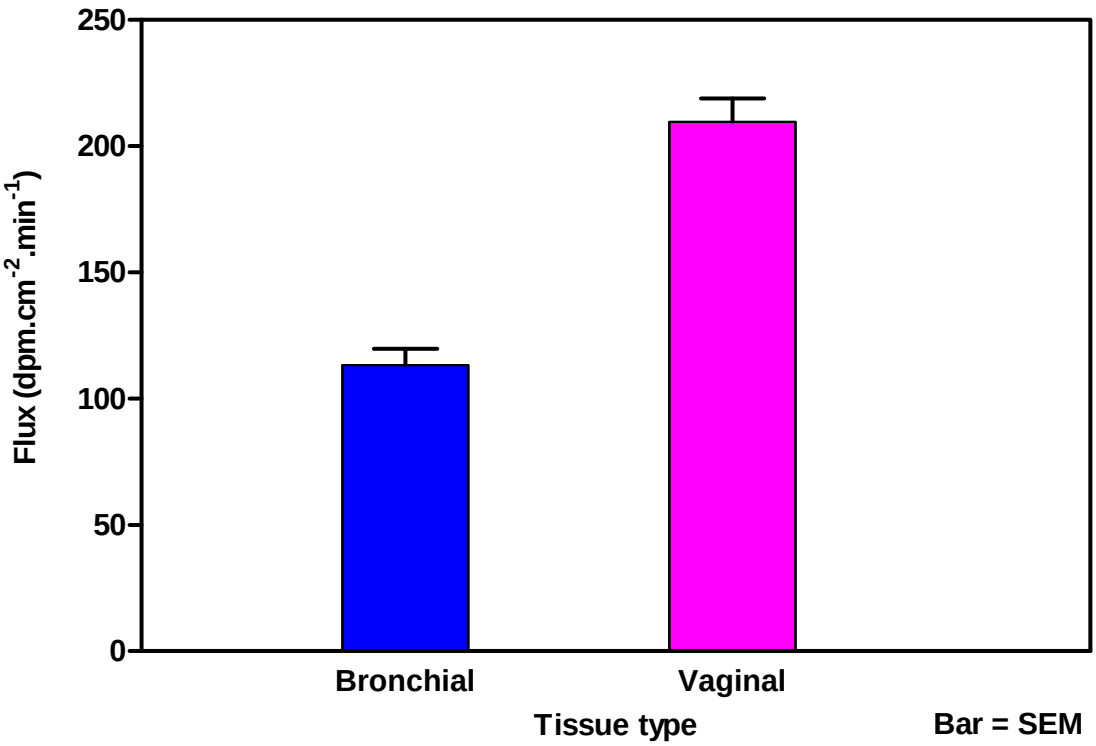


Figure 21. Mean estimated steady state flux values of vasopressin across: Porcine bronchial and human vaginal tissue.

Fig. 22 shows the overall mean steady state flux values of vasopressin across porcine bronchial tissue with and without Biopolsurf at different concentrations. Full concentration Biopolsurf (n = 18) and half concentration Biopolsurf (n = 15) were used as penetration enhancers in a concentration study. Compared to the vasopressin control (n = 26), full concentration Biopolsurf enhanced the flux value of vasopressin across porcine bronchial tissue by 32% ($p = 2.4\ E^{-25}$). The half concentration Biopolsurf caused a 42% increase in the flux value of vasopressin ($p = 2.44\ E^{-35}$).

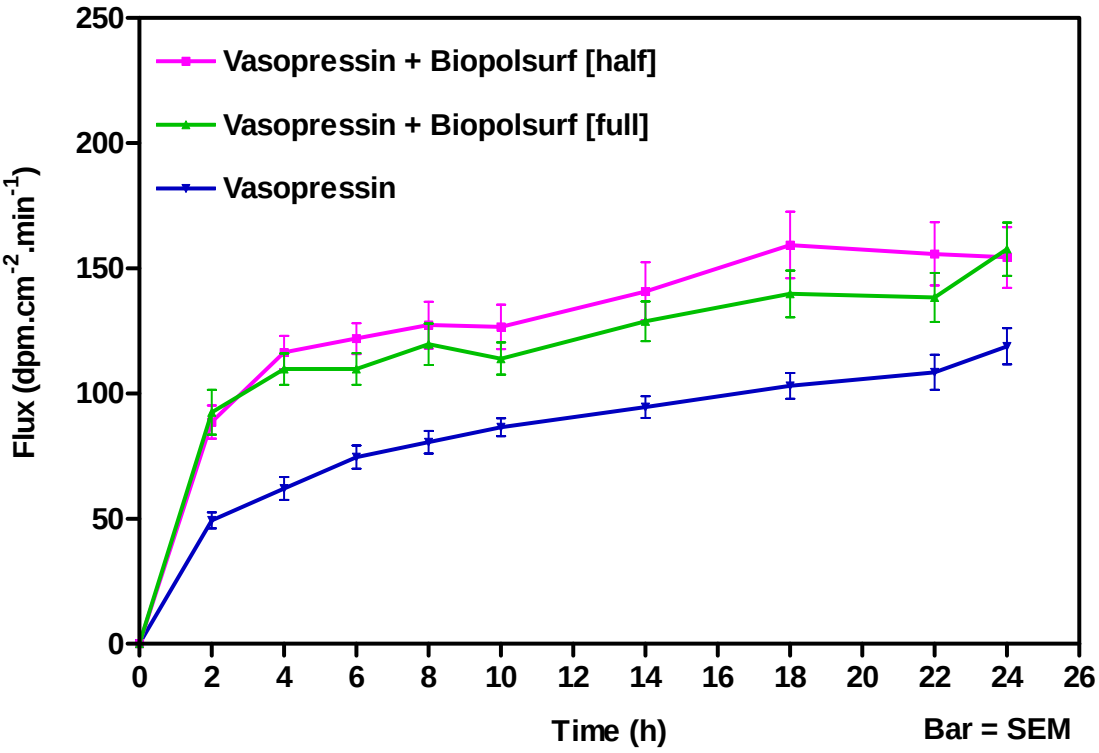


Figure 22. Overall mean steady state flux values of vasopressin across porcine bronchial tissue with and without Biopolsurf full and half concentrations as penetration enhancers.

The mean steady state flux values of oxytocin across porcine bronchial (n = 29) and human vaginal mucosa (n = 43) are shown in Fig. 23. Steady state conditions were achieved for oxytocin across bronchial tissue at 18 h and across human vaginal mucosa after 16 h. There were no significant differences between the flux values of oxytocin across human vaginal mucosa and porcine bronchial tissue (p = 0.999).

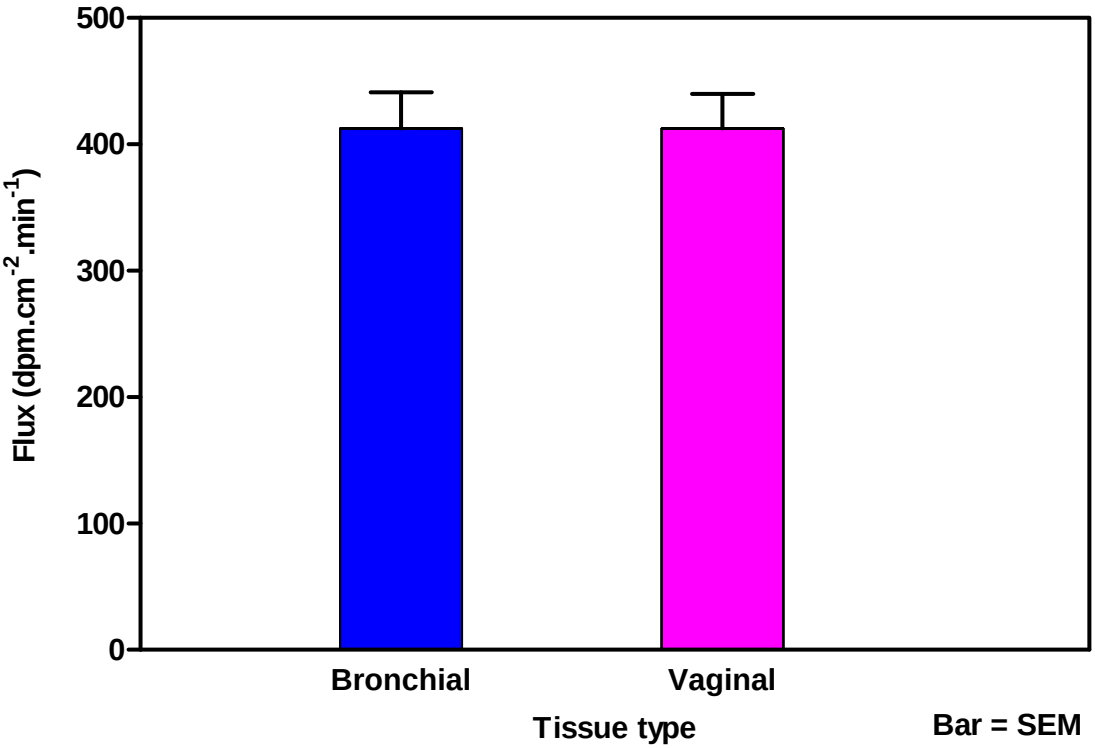


Figure 23. Mean steady state flux values of oxytocin across porcine bronchial and human vaginal mucosa.

The overall mean flux values of oxytocin across porcine bronchial tissue with and without Biopolsurf full and half strength concentrations are shown in Fig. 24. There was no enhancement in flux values across porcine bronchial tissue with either of the penetration enhancers used.

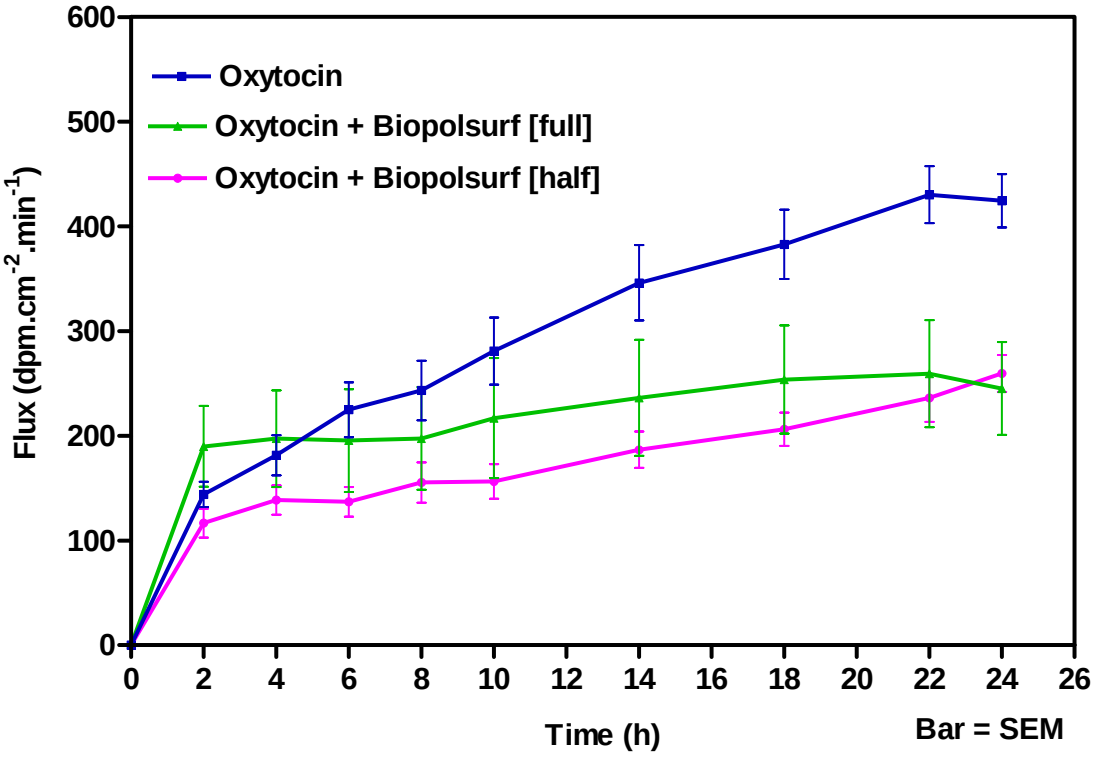


Figure 24. Overall mean flux values of oxytocin across porcine bronchial tissue with and without Biopolsurf full and half strength concentrations as penetration enhancers.

The overall mean flux values of AZT (5 mg/ml solution) across porcine bronchial tissue with and without Biopolsurf are shown in Fig. 25. The presence of Biopolsurf (n = 12) with AZT increased the flux value non-significantly by 12% (p = 0.16408) compared to the flux rate of the AZT control (n = 16).

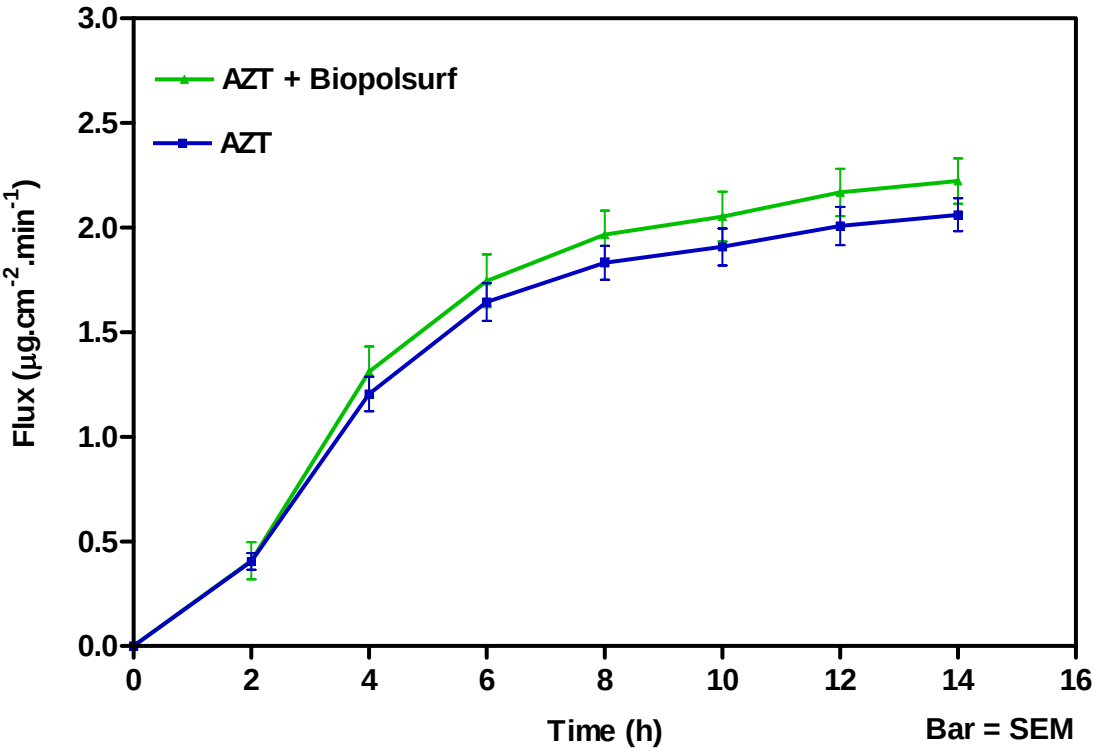


Figure 25. Overall mean flux values of AZT (Zidovudine) across porcine bronchial tissue with and without Biopolsurf as penetration enhancer.

The overall mean flux values of INH (1 mg/ml solution) across porcine bronchial tissue with and without Biopolsurf full and half concentration as penetration enhancers are shown in Fig. 26. The flux value of the INH with the Biopolsurf half concentration (n = 8) was non-significantly enhanced by 5% (p = 0.996293) when compared to the INH control (n = 9). However the flux value of the INH with the Biopolsurf full concentration (n = 4) was significantly enhanced by 19% (p = 0.000811) when compared to the INH control.

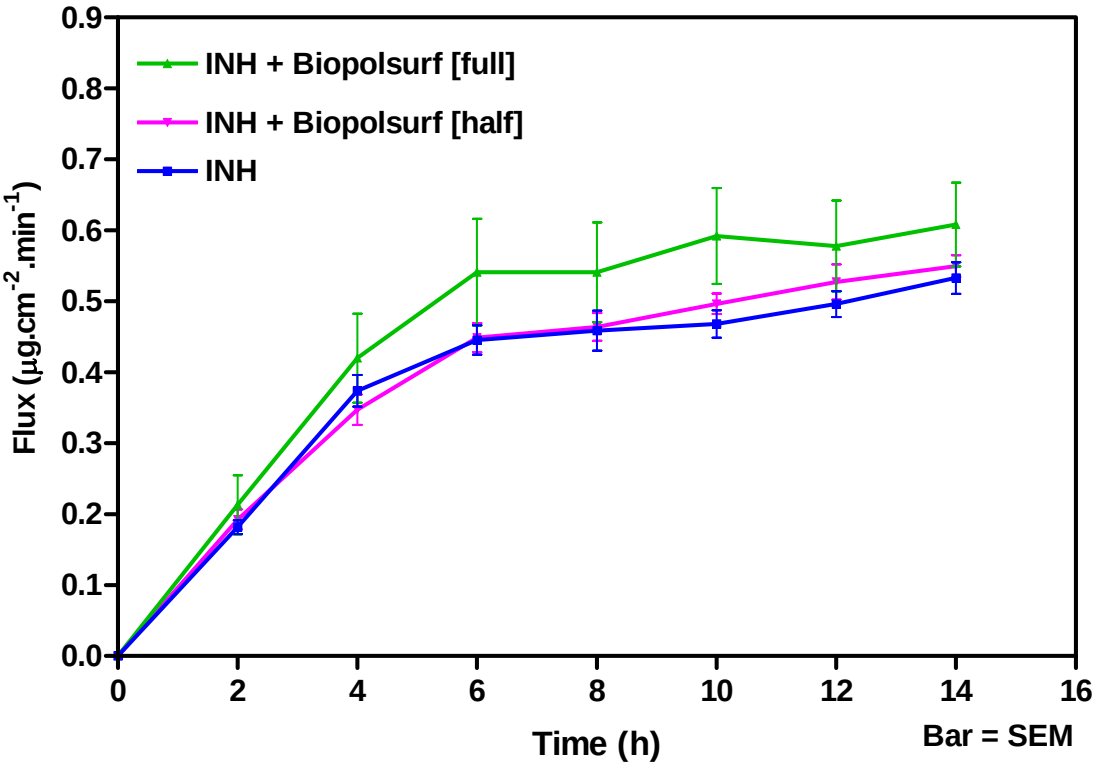


Figure 26. Overall mean flux values of isoniazid (INH) across porcine bronchial tissue with and without Biopolsurf full and half concentration as penetration enhancers.

Fig. 27 shows the overall mean flux steady state values for various permeants across porcine bronchial tissue. Mean steady state flux values for water (8-24 h); arecoline (18-24 h); hydrocortisone (18-24 h) and oxytocin (16-24 h) across porcine bronchial mucosa were 3193 ± 136, 580 ± 16, 432 ± 21 and 413 ± 29 dpm. cm^{-2}. min^{-1}, respectively. Mean estimated steady state flux values for 17β-estradiol (18-24 h); flunitrazepam (18-24 h); dexamethasone (18-24 h) and vasopressin (18-24 h) across porcine bronchial mucosa were 173 ± 11, 735 ± 29, 451 ± 24 and 113 ± 6 dpm. cm^{-2}. min^{-1}, respectively. In general, as would be expected, the magnitude of the flux rates of the various permeants across porcine bronchial tissue were related to molecular size, the larger compounds, having lower flux rates.

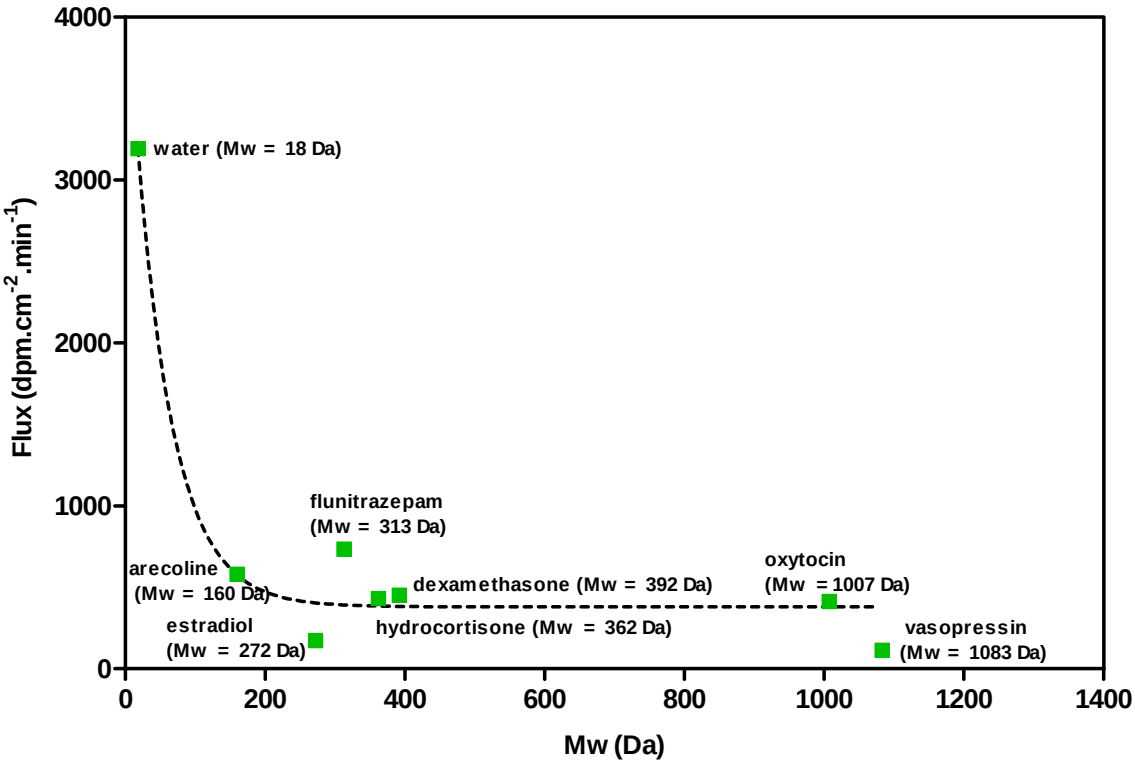
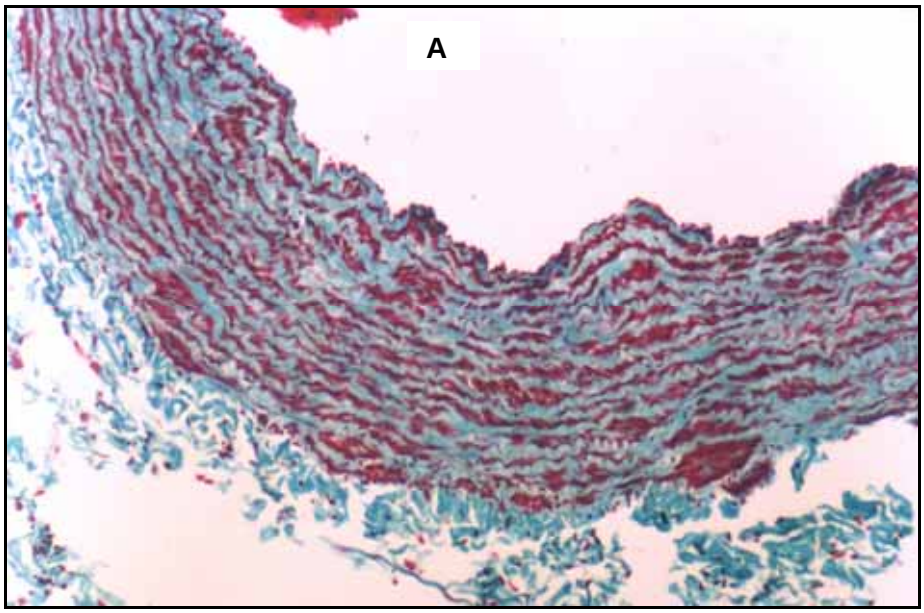
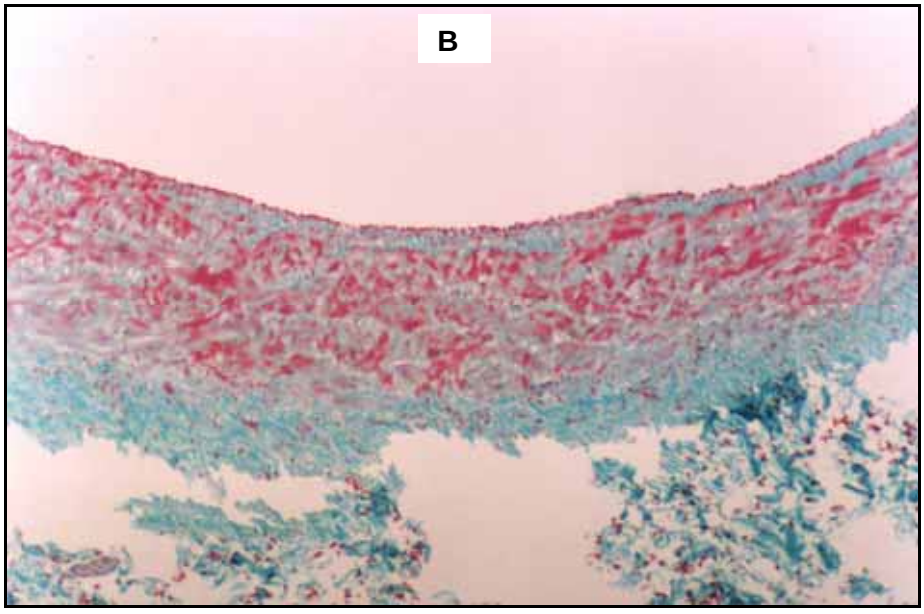


Figure 27. Overall true and estimated mean flux steady state values for various permeants across porcine bronchial tissue versus molecular weight.

Fig. 28 shows a comparative light-microscopic study of porcine bronchial artery and vein tissue. Histological studies showed that the arterial wall contains a thicker layer of smooth muscle elastic fibres than the vein.



← Tunica media composed of a thick layer of smooth muscle elastic fibres



← Tunica media containing less smooth muscle elastic fibres

Figure 28. Micrograph illustrates bronchial artery (A) and bronchial vein (B) (Masson's trichrome x 10).

6. DISCUSSION

The future of pharmacotherapy for many disorders may lie in drug delivery routes other than oral administration. In particular, growing interest has been given to the lung as well as other absorptive mucosae as non-invasive administration routes for systemic delivery of therapeutic agents. For delivery of proteins and peptides, the lung provides direct entry into the systemic circulation, thus avoiding the hepatic first-pass effect and degradation in the gastrointestinal tract. It also provides ease of administration and the ability to terminate delivery when required. Although few drugs are delivered by inhalation for non-pulmonary therapy, this appears set to change as there has been an increasing amount of interest in the transmembrane diffusion kinetics of a variety of chemical compounds across different tissues. This can be attributed to a better understanding of factors that influence or govern transmembrane diffusion processes of chemical compounds in the context of: rates of transmembrane absorption, permeability enhancer characteristics and actions as well as medicinal-agent delivery formulations. As only limited information is available on the *in vitro* diffusion kinetics of chemical agents across bronchial tissue, we decided to study it. We also tested different surfactants as permeation enhancers of various chemical markers through porcine: bronchial, buccal, rectal, arterial and venous tissue and human vaginal mucosa.

It is well known that the ability of substances to diffuse through epithelial barriers depends not only on the properties of the barriers involved, but also on the chemical nature, size and conformation, lipid/water partition coefficient, and degree of ionisation of the permeant molecules tested.[113,116,119] Cellular membranes are barriers to macromolecules and to most polar compounds, but they are relatively permeable to water and small lipophilic molecules. This presents one of the major problems limiting the effective use of absorptive mucosa as alternate delivery routes for drugs. Rojanasakul *et al.* compared the permeability of the membrane barrier and charge selectivity in various rabbit epithelia and demonstrated its significance on transepithelial movement

of charged solutes.[107] Their results indicated a wide variation in the magnitude of barrier permeability among different epithelia, with a rank order of most permeable to least permeable of: intestinal ~ nasal ≥ bronchial ≥ tracheal > vaginal ≥ rectal > corneal > buccal > skin. Results also indicated that all epithelia were selective to positively charged solutes when compared to their oppositely charged counterparts, with the magnitude of selectivity being comparable in most epithelia.[107] These authors therefore concluded that the intestinal and pulmonary epithelia, due to their high membrane permeability and large absorptive surface area, are probably two of the most effective mucosal surfaces for drug delivery purposes.

The molecular diffusion of drugs across bronchial tissue can be viewed as consisting of a continuous epithelium, collagen fibre network and interstitium. Depending on the physicochemical properties of a permeant, the diffusional resistance offered by the individual layers can vary greatly. Bronchial epithelium, which is lipoidal in nature and consists of pseudo stratified, ciliated columnar epithelium, is the main barrier offering high resistance to the diffusion of hydrophilic and charged molecules.[127]

Arecoline, an alkaloid that is associated with oral cancer as well as submucous fibrosis, is a small lipophilic unionised molecule of 160 Dalton. As it permeates relatively fast through most epithelial membranes, it was chosen as a model compound for the comparative study in different tissues (Fig. 7).[120] From this result it was found that the rank order (most permeable to least permeable) to arecoline was arterial >rectal > vaginal ≥ bronchial >buccal. This is in agreement with the results obtained in previous studies that showed that permeation of arecoline through porcine buccal mucosa was consistently lower than that for human buccal or vaginal mucosa.[124,120] The very similar permeability characteristics of the vaginal and bronchial tissues and the low permeability of the buccal tissue found, also correlates with the permeability rank order found in the study of Rojanasakul *et al*.[107] Although porcine rectal tissue in the present study was more permeable than rabbit rectal tissue,[107] this may probably be related to the substantial differences in the digestive tracts of these two species. The porcine bronchial arteries were found to have

the highest permeability to arecoline of all the tissues tested, probably because squamous endothelium of the arterial wall is very permeable to small, unionised, lipophilic molecules.[105]

In a study by Kukowska-Latallo *et al.* [128], it was reported that the non-ionic surfactant component in Exosurf, tyloxapol, enhanced the efficiency of gene expression in pulmonary cell cultures of human bronchial and small airway epithelium cell lines. They concluded that tyloxapol did not markedly alter cell permeability as measured by dual staining with propidium dodicide and fluorescein diacetate. When we compared Biopolsurf and Exosurf as enhancers of arecoline flux across bronchial tissue, Biopolsurf was able to increase the permeability and an increase in flux rate of 12% was found (Fig. 8). Both surfactants used in our experiments contain the amphipathic phospholipid DPPC, as well as cetyl alcohol and tyloxapol. When used in respiratory distress syndrome (RDS) in neonates, the blend of these three components in the Exosurf formulation achieves the biophysical properties necessary for pulmonary surfactant to prevent alveolar collapse during expiration. Although experiments using surfactant in whole-tissue models are complex and can be difficult to interpret, we argue that the enhancement in flux rate of arecoline through lung tissue with Biopolsurf as surfactant might be attributed to the presence of the anionic surface-active phosphatidylglycerol in the formulation. On the other hand, the finding that flux values of arecoline across vaginal mucosa were enhanced with Exosurf (Fig. 10), argues against the formulation difference between the two surfactants. It could rather relate to the type of tissue and surfactant used in the experiment. This view was substantiated when Biopolsurf significantly enhanced the permeability of arecoline through porcine buccal mucosa (20%), while there was no enhancement with Exosurf (Fig. 11). This trend was also found when we compared the enhancement of arecoline across porcine bronchial tissue with Biopolsurf (synthetic) and the two natural surfactants Curosurf® and Survanta® (Fig. 9). Arecoline flux values were significantly enhanced by 14% across porcine rectal mucosa in the presence of Biopolsurf as a penetration enhancer (Fig. 12). Mainly, these results show that although arecoline diffuses with relative ease across the various tissues, permeation is significantly

enhanced when specific surfactants are added. The natural (Curosurf® and Survanta®) and synthetic (Biopolsurf and Exosurf) surfactants may typically act by destabilizing the intercellular barrier via lipid and protein extraction, which in turn increases membrane fluidity and reduces restriction to molecular movement through the cellular layers. Another possible explanation could be that the surfactants “loosen” the tight junctions between cells and increase paracellular permeability.

Estrogen undergoes substantial intestinal breakdown and first-pass hepatic metabolism if orally ingested and as a result alternative routes of administration are very attractive. The higher molecular mass (Mw = 272 Da) and lipophilicity of 17β-estradiol cause it to have lower permeability characteristics compared to arecoline. Steady state conditions were not reached for the tissues tested during the 24 h interval over which the experiment was conducted. These observations are in agreement with those of studies performed on the passage of 17β-estradiol across human vaginal and buccal mucosa.[115] The reason for this could be due to the wider distribution of 17β-estradiol into the lipophilic domains in the tissues. The rank order (most permeable to least permeable) for the membrane permeability of 17β-estradiol (Fig. 13) was vein > artery > vaginal ≥ bronchial. The permeability values of 17β-estradiol across porcine bronchial vein in these results are almost identical to the values found in a previous study performed on human saphenous vein.[122] Furthermore, porcine bronchial vein was found to be more permeable than the porcine bronchial artery which may be because the artery wall has a tunica media composed of a thicker layer of smooth muscle elastic fibres than the vein (Fig. 28). The very similar permeability characteristics of the human vaginal and porcine bronchial tissues found (Fig. 13) also correlates with the permeability rank order study of Rojanasakul *et al*.[107] as well as with the results obtained in previous studies on 17β-estradiol permeation through human vaginal mucosa.[123]

The flux values of 17β-estradiol were significantly enhanced across the bronchial epithelium in the presence of Biopolsurf (~ 43%) and Exosurf (~ 11%). The permeation of 17β-estradiol was also significantly enhanced by

22% in the presence of Biopolsurf through the porcine bronchial artery (Fig. 15). Furthermore, enhancement across porcine bronchial venous tissue (Fig. 16) was only observed during the first 12 h, there-after a plateau was reached. This enhancement could possibly be attributed to the surfactant's ability to cause solubilisation and functional alterations in the barriers of the epithelium. A further possible explanation may be that the passage of 17β-estradiol through the epithelium could have been facilitated by the surfactant which, by its very nature, alters oil/water-partitioning coefficients of lipophilic molecules, "dispersing" more of the drug in the aqueous phase.

Hydrocortisone, an unionised, lipophilic corticosteroid, has a higher flux rate across porcine bronchial and human vaginal tissues (Fig. 17) than 17β-estradiol even though it has a higher molecular weight (362 Da). Although the flux values of hydrocortisone across human vaginal mucosa are higher than those across the porcine bronchial tissue, there was no statistical difference between these values. This is in agreement with the permeability rank order study of Rojanasakul *et al*.[107] The overall flux values of hydrocortisone across porcine bronchial tissue were significantly enhanced by 17% with Biopolsurf. However, this was only observed after 8 h of the experiment (Fig. 18). This enhancement might also be attributed to the surfactant's ability to cause solubilisation and functional alterations in the epithelial barriers of the bronchial epithelium.

Dexamethasone, one of the therapeutically potent synthetic analogues of cortisol, is also an unionised, lipophilic molecule. It has a molecular weight of 392 Da. Its flux values across porcine buccal, bronchial and human vaginal mucosa are shown in Fig. 19. Once again the flux values across the buccal epithelium were the lowest, which is in agreement with the results obtained in previous studies that showed that porcine buccal mucosa had a consistently lower permeability than human vaginal mucosa for a number of permeants.[120] The very similar permeability characteristics of the vaginal and bronchial tissues and the low permeability of the buccal tissue found also correlates with the permeability rank order study of Rojanasakul *et al*.[107] In this case the vaginal permeability was lower than the bronchial permeability, although this

difference was not statistically significant. No plausible explanation for this observation is evident. Dexamethasone flux values across porcine bronchial tissue were compared with and without various concentrations of Biopolsurf (Fig. 20). The full concentration of Biopolsurf significantly retarded the flux values of dexamethasone, although half and quarter concentrations significantly enhanced flux rates by 6% and 17% respectively. This indicates that there may be a possible interference of diffusion processes at high concentrations of surfactant.

Vasopressin is an endogenous nonapeptide hormone (Mw = 1083) used in the treatment of vasopressin sensitive diabetes insipidus and, due to its pressor activity, it is used in the control of esophageal and gastrointestinal bleeding.[114] Because vasopressin is susceptible to enzymatic degradation in the gastrointestinal tract, it is usually administered intranasally or by injection. Since the transmucosal route may be useful for the administration of small peptides, this drug was considered to be an appropriate model compound to be evaluated. The flux values across porcine bronchial and human vaginal tissue are shown in Fig. 21. Compared to the other molecules used in this study the flux value of vasopressin across membranes is relatively low. This can be attributed to its larger molecular weight, hydrophilicity and its positive charge. The permeability of vasopressin across human vaginal mucosa is in agreement with that found in previous studies.[121] Moreover, it was found to be less than that of bronchial tissue which again correlates with the permeability rank order study of Rojanasakul *et al*.[107]

Vasopressin flux values across porcine bronchial tissue were compared with and without two concentrations of Biopolsurf (Fig. 22). The full concentration of Biopolsurf significantly enhanced the flux values of vasopressin by 32%, although the half concentration significantly enhanced flux rates by 42%. This is indicative of the possible interference of diffusion processes by high concentrations of surfactant. By disrupting intercellular lipids, which act as a rate-limiting barrier for hydrophilic molecules, it reduces restriction to molecular movement through the cellular layers.

Oxytocin, the principal uterus-contracting and lactation-stimulating hormone, has a molecular weight of 1007 Da. It is a nonapeptide that differs in structure from vasopressin by substitution of the amino acids Phe and Arg with the hydrophobic amino acids Ile and Leu. This brings about an unionised lipophilic molecule contrary to vasopressin that is positively charged at pH 7.4. The flux values of oxytocin across porcine bronchial and human vaginal tissue are shown in Fig. 23. Although it has a much larger molecular weight than most other permeants investigated, the flux value across membranes is relatively high. This confirms the principle that drugs in the unionised state will be more lipid soluble and will diffuse through membranes at a higher rate.[27] Furthermore, the permeability characteristics of the human vaginal tissue were also found to be equal to that of bronchial tissue. However, when we compared the enhancement of oxytocin across porcine bronchial tissue with full and half concentrations of Biopolsurf (Fig. 24), we instead found retardation. The reason for this could be that the oxytocin flux rates through bronchial tissue, which were four times higher than that of vasopressin, were already at a maximum. Addition of surfactant may merely have increased competition with the oxytocin molecules for the transmembrane permeation routes. Another possible explanation for this could be that the surfactant may have aided solubilisation of the lipophilic oxytocin molecule, thereby negatively affecting its partitioning from solution into the mucosa. In this respect the net effect of the complex interactions between the constituents of the mucosa, oxytocin, and the components in the surfactant that might enhance drug permeability or retain it in the formulation are difficult to predict.

Zidovudine (3'-azido-3'-deoxythymidine, AZT) is an active inhibitor of reverse transcriptase in retroviruses such as the human immunosuppressant virus (HIV). During antiretroviral therapy it is crucial to maintain the systemic drug concentration within the therapeutic level throughout the treatment course. Oral AZT has a short elimination half-life and low bioavailability, and frequent high doses are required to maintain the therapeutic level. As a result, dose-dependant toxic side effects are frequently observed. Although the molecule has a highly hydrophilic character, it permeated well across

bronchial tissue (Fig. 25). In this regard enhancement with Biopolsurf was non-significant (12%).

Pulmonary tuberculosis (TB) continues to be a major infectious burden worldwide. The primary drug used in the treatment is isoniazid (INH), the hydrazide of isonicotinic acid, which is a small molecule (Mw = 137) that is highly water-soluble. One of the main difficulties associated with current therapy however, include dose related drug toxicity, prolonged duration of treatment and the fact that drugs may not reach atelectatic areas of the lung. Thus the administration of INH via the respiratory route is an exciting possibility. Recently the idea to use the spreading properties of DPPC as a drug delivery agent of INH was studied by Chimote and Banerjee.[129] As this approach could serve the dual purpose of antitubercular drug delivery to the site of infection as well as alveolar stabilisation by surfactant action, we decided to investigate the permeation and enhancement of INH with Biopolsurf through porcine bronchial tissue. A significant enhancement of INH flux value of 19% was found with Biopolsurf (Fig.26). Although this is only a first step to study the effects of a exogenous surfactant in the permeation of a antitubercular drug through bronchial tissue, the finding holds positive ramifications towards the development of surfactant loaded with INH and other antitubercular drugs in the treatment of one of the most dreaded diseases.

The flux values of various permeants across porcine bronchial tissue versus molecular weight (Da) were also plotted (Fig. 28). One of the major advantages of this study is that all experiments were performed on the same apparatus under standardised conditions. Relative comparisons of the data for the above tissues were thus not subjected to interlaboratory variations. It is clear that the magnitude of the flux rates of the various permeants across bronchial tissue were found to be related to molecular size, the larger compounds with Mw > 300 Da having lower flux rates. This is in agreement with the results obtained in previous studies on human vaginal, small intestinal and colonic mucosa.[123]

In conclusion, the results indicated that a wide variation in the permeability characteristics of different epithelia exists and that the pulmonary epithelium, due to its high membrane permeability, is probably one of the most effective mucosal surfaces for drug delivery. Furthermore, the results of this study showed that Biopolsurf, in most cases, significantly enhanced the permeability of drugs across different epithelia, especially of the peptide drug vasopressin that normally permeates slowly across membranes. This study also demonstrated the usefulness of the *in vitro* flow-through diffusion model to study the absorption characteristics of different epithelia and the effects of different absorption enhancers.

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