EUROPEAN JOURNAL OF INFLAMMATION Vol. 6, no. 2, 65-71 (2008)

PHYSICOCHEMICAL CHARACTERISTICS OF MOLECULES AND THEIR DIFFUSION ACROSS HUMAN VAGINAL MUCOSA

A.D. VAN EYK, P. VAN DER BIJL and L.M. MOLL

Division of Pharmacology, Department of Medicine, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa

Received December 31, 2007 – Accepted March 3, 2008

The diffusion rate of permeant molecules through mucosal tissue depends on the physicochemical characteristics of the molecules themselves as well as the properties of the tissue. In this study the diffusion kinetics of various molecules was examined through intact as well as de-epithelialised human vaginal mucosa. The molecules studied included tritium-labelled water, 17β-estradiol, reduced-arecoline, vasopressin, oxytocin, bradykinin, tacrolimus, cyclosporin A, dihydro-alprenolol, cimetidine and benzylpenicillin. Freshly harvested human vaginal tissue was frozen in liquid nitrogen and stored at –85°C. A flow-through diffusion apparatus was used for the *in vitro* permeability studies (24 h, 20°C, 1.5 ml/h). The mean estimated – or mean steady-state flux values for all the molecules studied across intact human vaginal mucosa, were generally found to be lower than those of the corresponding de-epithelialised tissue. Using an F-test and comparing whole curves, statistically significant differences in the diffusion rates of tacrolimus, reduced-arecoline, vasopressin, bradykinin, benzylpenicillin, water and cimetidine were found when comparing intact and de-epithelialised vaginal mucosa. Generally, smaller permeant molecules diffused at a higher rate than larger molecules. The epithelial layer retarded the diffusion rate of molecules carrying charges at physiological pH. Damage to the epithelial layer did not necessarily increase the diffusion rate of all molecules tested and small lipophilic molecules did not necessarily diffuse at higher rates than hydrophilic molecules.

One of the most common and convenient routes whereby drugs may be delivered topically as well as systemically to the body, is via mucosal surfaces(1). Transmucosal delivery of drugs, involves delivery of therapeutic agents across mucosal linings of the vagina, oral and nasal cavities, rectum and eyes (2). Each drug will traverse these mucosal linings in a unique way depending on its physicochemical characteristics (e.g. molecular weight, size, lipophilicity, partition coefficient and polarity). Two major pathways exist by which drug molecules diffuse passively across mucosal membranes i.e. the paracellular (passage between adjacent cells) and the transcellular (passage through cells) routes (1-12).

The paracellular (intercellular) route of diffusion seems to predominate for most lipophilic and hydrophilic drugs when they cross mucosal membranes. There are two types of pathways that exist within the intercellular space: 1) the hydrophobic pathway through the lipid bilayers and 2) a parallel hydrophilic pathway along the narrow aqueous regions that are associated with the polar

Key words: diffusion, human vaginal mucosa, permeability, physicochemical characteristics

Mailing address: Dr A.D. van Eyk,
Division of Pharmacology,
Department of Medicine, Faculty of Health Sciences,
Stellenbosch University,
Private Bag X1, Tygerberg 7505, South Africa
Tel: ++27 21 938 9326 Fax: ++27 21 932 6958
e-mail: armorel.vaneyk@wits.ac.za

1721-727 (2008)

head groups of the lipids. Generally speaking, as the lipophilicity of a permeant molecule increases, so the propensity for diffusion of the molecule across the epithelial barrier also seems to increase (5, 8, 13).

The diffusion of drug molecules across the vaginal mucosa is also dependant on a concentration gradient across the mucosal layer according to Fick's first law:

$$J = P \times C_{Aq}$$

where J = drug flux rate (mass/area/time), P = permeability coefficient through the lipophilic membrane and C_{Aq} = drug concentration at the aqueous exterior (14-15). Most drug molecules are either weak acids or weak bases and according to the pH-partition theory, it is assumed that the uncharged (lipophilic) form will penetrate the vaginal mucosa to a greater extent than the charged (hydrophilic) form (9-10). It is also generally accepted that large molecular weight lipophilic and hydrophilic drugs permeate vaginal mucosa at a slower rate than smaller molecules. Pathology, with associated mucosal damage of the epithelium of the mucosa, is also assumed to result in increased diffusion rates of drug molecules (2).

In this study the *in vitro* diffusion kinetics of various permeants, having different physicochemical properties, through human vaginal mucosa were compared. Conditions, when there is epithelial damage due to pathology, were mimicked by heat de-epithelialisation of the mucosa (16). Diffusion kinetics under these circumstances were compared with those of intact mucosa. It was envisaged that the results from this study would help to understand the effect of physicochemical characteristics of different chemical molecules on their diffusion rates across human vaginal mucosa in the *in vivo* state. This could be helpful in the design process of chemical entities to improve their absorption rate and thus bioavailability when they are going to be used therapeutically.

MATERIALS AND METHODS

Human vaginal mucosa

Human vaginal specimens were obtained from excess tissue removed from 58 postmenopausal patients, ages 51-80 years (mean age 59 ± 11 yr SD), following vaginal hysterectomies at the Louis Leipoldt Hospital, Bellville, South Africa.

All the specimens were placed in a transport fluid, prepared as previously described and transferred to our laboratory within 1 h (17). Excess connective and adipose tissue were carefully removed from all specimens, and hereafter these were snap-frozen in liquid nitrogen and stored at -85°C. No specimens were obtained where there was clinical evidence of any disease or other damage that might have influenced the permeability characteristics of the mucosa. The study was approved by the Ethics Committee of Stellenbosch University and the Tygerberg Academic Hospital.

Permeability Experiments

The diffusion kinetic behaviour of 9 tritium-labelled permeant molecules, water, oxytocin, vasopressin, bradykinin, 17β-estradiol, benzylpenicillin, reduced-arecoline (r-arecoline), cimetidine and dihydro-alprenolol) as well as 2 previously tested permeant molecules (cyclosporin A, tacrolimus) (18) across intact as well as de-epithelialised human vaginal mucosa were compared. Frozen specimens were thawed in PBS (pH 7.4) for 10 min prior to each experiment. Intact thawed mucosal specimens were carefully cut, so as not to damage the epithelial surfaces, into 4 mm diameter sections. De-epithelialised mucosal specimens were prepared by dipping intact mucosa for a period of 30 seconds in 80°C distilled water and the epithelial layer gently removed with tweezers (18-19). Either intact or de-epithelialised mucosal sections were then mounted in flow-through diffusion cells (exposed areas 0.039 cm^2) as previously described, and six permeation experiments were performed for each permeant molecule tested (20). Tissue disks were equilibrated for 10 min in PBS (pH 7.4) at 20°C in both the donor and acceptor compartments of the diffusion cells prior to each permeability experiment. The PBS was removed from the donor compartment and 0.5 ml of PBS added, containing either 1.4 μCi ^{3}H-17β-estradiol, 1 μCi ^{3}H-reduced arecoline (r-arecoline), 1 μCi ^{3}H-dihydro-alprenolol, 1 μCi ^{3}H-cimetidine, 1 μCi water, 0.1 μCi ^{3}H-bradykinin, 0.15 μCi ^{3}H-oxytocin, 0.093 μCi ^{3}H-vasopressin or 1 μCi ^{3}H-benzylpenicillin. PerkinElmer Life Sciences Inc. (Boston, MA) supplied the ^{3}H-oxytocin, and all the other radioisotopes were obtained from Amersham Laboratories (Little Chalfont, Amersham, UK). For the determination of donor cell concentration at time zero, 100 μl aliquots were removed within minutes from each of the seven donor compartments. Fractions were collected at 20°C, by means of a fraction collector, at 2-h intervals for 24 h and a flow-rate of 1.5 ml/h. All permeability studies were performed under sink conditions, i.e. at the completion of each run the concentration of tritiated permeant in the acceptor chamber never reached 10% of that in the donor compartment. Radioactivity was determined using a liquid scintillation counter (Beckman LS 5000TD) after addition of 10 ml scintillation cocktail (PCS scintillation cocktail;

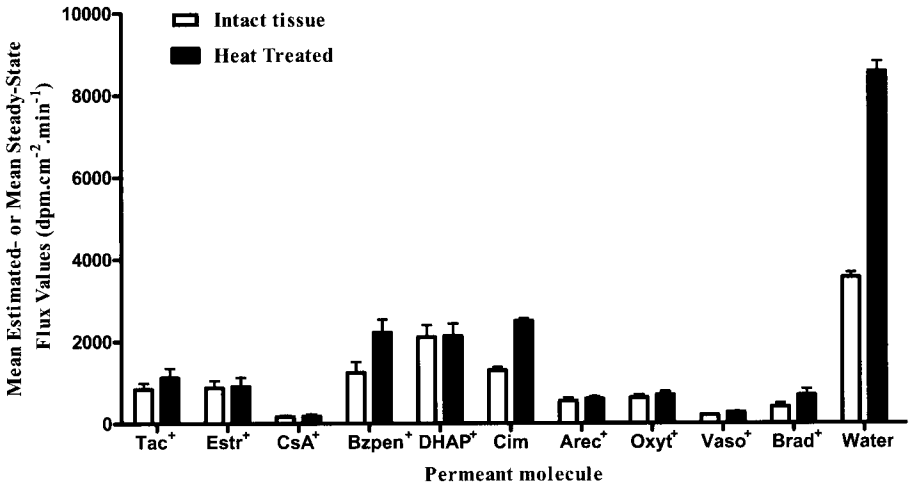

Fig. 1. *The diffusion of tacrolimus (Tac) (18), cyclosporin A (CsA) (18), 17β-estradiol (Estr), benzylpenicillin (Bzpen), dihydro-alprenolol (DHAP), cimetidine (Cim), vasopressin (Vaso), arecoline (Arec), bradykinin (Brad), oxytocin (Oxyt) and water across intact and heat de-epithelialised human vaginal mucosa. (+ Mean estimated steady-state flux values).*

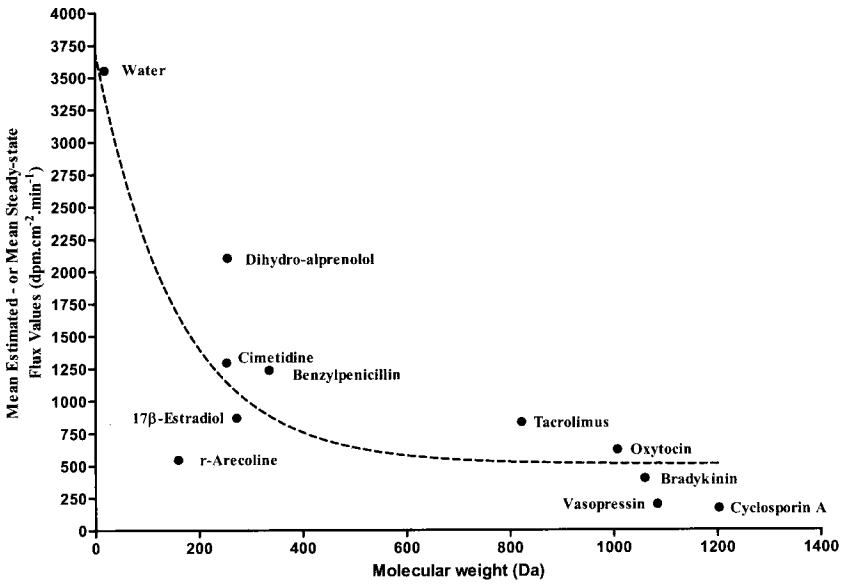

Fig. 2. *The relationship between molecular weight of permeant molecules and mean estimated - or mean steady-state flux values across intact human vaginal mucosa.*

Amersham Bio-Sciences, SE-75184, Uppsala, Sweden) to each sample collected. The counting of the samples was continued until a 2-s value of 1% was reached. Quenching for each sample was automatically corrected in the counter.

Calculation of Flux Values

Flux values (J) across the human vaginal mucosa were calculated using the following relationship:

$$J = Q / A \times t \quad (dpm.cm^{-2}.min^{-1})$$

where Q = quantity of permeant crossing mucosa (in dpm), A = mucosal area exposed (in cm^2) and t = time of exposure (in min).

Steady-State Kinetics

Steady-state (equilibrium kinetics) was assumed to have been reached for a particular mucosal specimen and tritiated permeant, when no statistically significant differences ($P<0.05$, t-test) between flux values were obtained over at

Table 1. *Mean estimated – or mean steady-state flux values obtained for the various permeant molecules across intact and heat de-epithelialised human vaginal mucosa.*

Permeant Molecule	Mean Steady-State flux values $dpm.cm^{-2}.min^{-1}$ Intact	Mean Steady-State flux values $dpm.cm^{-2}.min^{-1}$ Heat Treated	Increase in flux values %*	$Log_{10}P$ (lipophilicity)	Statistical significance using an F-test *(P value)**
Water (18 Da)	3554 ± 119	8567 ± 252	141	0	<0.0001
Dihydro-alprenolol (255 Da)[+]	2106 ± 293	2129 ± 305	1.1	0.5	0.9955
Cimetidine (253 Da)	1296 ± 72	2495 ± 58	92.5	0.4	<0.0001
Benzylpenicillin (335 Da)[+]	1239 ± 260	2215 ± 320	78.8	1.8	<0.0001
17β-Estradiol (272 Da)[+]	871 ± 172	905 ±219	3.9	4.0	0.9713
Tacrolimus (822 Da)(18)[+]	836 ± 143	1117 ± 233	33.6	5.4	0.0010
Oxytocin (1007 Da)[+]	623 ± 70	692 ± 82	11.1	< 1	0.4937
Arecoline (160 Da)[+]	547 ± 77	592 ± 60	8.2	0.4	<0.0001
Bradykinin (1060 Da)[+]	400 ± 88	682 ± 153	70.5	< 1	<0.0001
Vasopressin (1084 Da)[+]	200 ± 20	265 ± 22	32.5	< 1	<0.0001
Cyclosporin A (1203 Da)(18)[+]	170 ± 31	186 ± 43	9.4	3.0	0.6221

* *Between flux rates across intact and de-epithelialised mucosa*

[+] *Mean estimated steady-state flux values*

least 2 consecutive time intervals.

Statistical Analysis

Non-linear regression analyses (third order polynomials) were performed using a GraphPad Prism, Version 5, 2007 computer programme. An F-test was used to compare entire curves (21). A significance level of 5% was used. When steady-state was not reached for a particular permeant molecule the mean estimated steady-state flux value was calculated (= mean flux values at 16, 20, 24 h).

RESULTS

The mean estimated, or mean steady-state flux values of water, tacrolimus (18), cyclosporin A (18), 17β-estradiol, r-arecoline, cimetidine, dihydro-alprenolol, benzylpenicillin, vasopressin, bradykinin and oxytocin versus time across intact and de-epithelialised human vaginal mucosa are shown in Fig. 1. The relationship between flux rate and molecular weight for different molecules is shown in Fig. 2, and on average the mean estimated – or mean steady-state flux values obtained for all the permeant molecules tested across the intact mucosa were lower than those obtained across the de-epithelialised mucosa (Table I). Using an F-test and comparing whole curves, statistically significant differences in flux values ($P < 0.05$) across intact and de-epithelialised human vaginal mucosa were found for water, tacrolimus, vasopressin, r-arecoline, bradykinin, benzylpenicillin and cimetidine. De-epithelialisation had a minimal effect ($P > 0.05$) on

the flux values of dihydro-alprenolol, cyclosporin A, 17β-estradiol and oxytocin.

DISCUSSION

One of the major problems that limits the effective use of mucosal surfaces as alternative delivery routes for drugs is the epithelial barrier layer. The latter lipid-rich layer limits the movement of drug molecules due to its high degree of hydrophobicity, charge selectivity and the presence of tight junctions (22). It is generally accepted that passage of drugs across the epithelial barriers of mucosal surfaces occurs as a non-specific diffusion process, driven primarily by a concentration gradient. The physicochemical characteristics of the drug molecules as well as the properties of the membranes involved, will thus be the most important factors determining the diffusion rate (22). A better understanding of how the physicochemical properties of different molecules affect their diffusion across the mucosal epithelial barrier layer may be helpful in designing more effective drug products that will have higher absorption rates as well as greater bioavailability (11). Conditions affecting the epithelial integrity of the vaginal mucosa were simulated by heat de-epithelialisation (18-20). Vaginal mucosa, of which the epithelial layer has become damaged due to various conditions (e.g. lichen planus, pemphigus, viral infections and allergic reactions), may be expected to become more permeable to chemical compounds (2). During a previous study, histological examination of heat de-epithelialised mucosa indicated effective removal of the epithelial layers by this process, with only small islands of epithelial cells remaining (18). All permeant molecules diffused at a higher rate across de-epithelialised mucosa than intact mucosa, however, only for water, tacrolimus, vasopressin, r-arecoline, bradykinin, benzylpenicillin and cimetidine were these differences in flux rates statistically significantly increased (Fig. 1 and Table I). It is clear that generally the molecules diffused across intact vaginal mucosa at flux rates commensurate with their molecular weights (Fig. 2). The rank order of flux rates was: water (18 Da)> dihydro-alprenolol (255 Da) > cimetidine (253 Da) > benzylpenicillin (335 Da) > 17β-estradiol (272 Da) > tacrolimus (822 Da) > oxytocin (1007 Da) > r-arecoline (160 Da) > bradykinin (1060 Da) > vasopressin (1084 Da) > cyclosporin A (1203 Da), with r-arecoline being the exception (Table I). Although r-arecoline is a small molecule with a molecular weight of 160 Da, it diffused at a rate much slower than larger molecules. The latter alkaloid has a pKa of ~ 7.8 and is a relatively hydrophilic ($\log_{10} P = 0.4$) tertiary amine containing a large proportion of the ionised species at physiological pH (pH 7.4) (23). At this pH value, approximately 71% of the r-arecoline will be positively charged and hence its paracellular movement retarded, due to the ionic interaction with negatively charged molecules lining the pathway between the cells (22-23). The diffusion rate of water was very rapid across the human vaginal mucosa due to its small size, and removal of the epithelial layer increased the diffusion rate significantly. Water was chosen as one of the permeants because it is used as a standard marker in many *in vitro* permeability studies. It is, however, not the preferred permeant to be used for comparative studies on paracellular diffusion because it permeates the mucosal barrier not only by osmosis but also by passive paracellular diffusion (24).

The three nonapeptides, oxytocin, vasopressin and bradykinin, are all hydrophilic molecules which will primarily traverse the epithelial barrier via the paracellular pathway. Their transmucosal passage is largely restricted by their size and charge will play a lesser role, since the intercellular tight junctions of the epithelium only allow access to molecules of a finite size (Table I). The nonapeptides oxytocin and vasopressin differ only in respect of two amino acids residues, the former containing isoleucine and leucine and the latter phenylalanine and arginine. This difference will render oxytocin more lipophilic and smaller (1007 Da vs 1084 Da) than vasopressin and could explain the higher flux rate of the former compared to that of the latter. At physiological pH the arginine residue in vasopressin would almost certainly carry a positive charge. Lower lipophilicity, larger size and higher charge, would all be contributing factors to the slower diffusion rate of vasopressin compared to that of oxytocin. Bradykinin contains two arginine residues, which most probably confer a net positive charge at pH 7.4 on this peptide. The epithelial layer seems to retard

the passage of the positively charged bradykinin to a large degree, possibly due to ionic interactions that occur between the negative charges in the intercellular pathways of the membrane and those of the peptide (Fig. 1 and Table I). The importance of the epithelial barrier to the entry of bradykinin into the mucosa is evident in that, when this layer is removed, the flux rate of bradykinin increases by up to ~71%.

Cyclosporin A (11 amino acids) is a fairly large, neutral cyclic peptide molecule that is highly lipophilic ($\log_{10}$ P ~ 3). Of the 11 amino acids, 7 are methylated (25-26). However, due to its large size which partially offsets its favourable (lipophilic) epithelial barrier penetrating properties, its diffusion rate is slow and even in the absence of the epithelial barrier layer, the permeation of this molecule does not increase significantly. Tacrolimus (a cyclic macrolide, $\log_{10}$ P = 5) and 17β-estradiol ($\log_{10}$P = 4, pKa = 10.4) are also highly lipophilic molecules that are unlikely to be charged at physiological pH values. Both these molecules diffuse through the mucosa at similar flux rates, despite an approximately 3-fold size difference of tacrolimus compared to 17β-estradiol (Table I). Cimetidine (pKa = 6.8) is a small, fairly hydrophilic molecule ($\log_{10}$P = 0.4) which may contain a significant proportion of ionised species at physiological pH. This molecule diffuses across the mucosal barrier at a high rate due to its small size. In the absence of the epithelial layer, the flux rate increases by ~92%, possibly indicating the existence of ionic interactions between the positively charged species and negatively charged molecules in the diffusion pathways of the epithelial layer. Dihydro-alprenolol is also a small, relatively hydrophilic molecule ($\log_{10}$P = 0.5) with a pKa ~ 9.5. At physiological pH, a significant amount of the molecules may be charged, however, due to its small size, it also diffuses rapidly across the mucosal barrier. Very little, if any ionic interaction occurs in the epithelial layer, since de-epithelialised mucosa does not yield a significantly higher flux rate. Benzylpenicillin is a small molecule which is relatively lipophilic ($\log_{10}$ P = 1.8) and is highly acidic (pKa = 2.8). At pH 7.4 a large quantity of negatively charged molecules will probably be present and these will be repelled by the negatively charged molecules lining the paracellular pathways. Upon de-epithelialisation, the flux rate increased by ~79%. After removal of the epithelial layer, the benzylpenicillin molecules now penetrate the mucosa more freely since ionic interactions are greatly reduced.

It can be concluded from the above results that although the physicochemical properties of permeants and those of the membranes themselves, undoubtedly play an important role to predict the passage of chemical compounds through human vaginal mucosa, these cannot be solely relied upon. As can be seen from the results, while size does predict to a large extent diffusion rate, this is not always the case (e.g. r-arecoline). Other physicochemical factors also have to be considered. Generally, lipophilicity and charge are also very important predictors of the diffusion rate, as can be seen in the results obtained from this study (Fig. 2). The necessity to perform *in vitro* permeability experiments before embarking on *in vivo* studies can therefore not be overemphasised. These results will deepen our understanding of how the physicochemical characteristics of different drug molecules affect their diffusion across mucosal epithelium. Furthermore, the results could be helpful in the future design of more effective drugs in the pharmaceutical development process. This will hopefully lead to designing better dosage forms, yielding higher absorption rates and better bioavailability.

ACKNOWLEDGEMENTS

The authors would like to thank the Stellenbosch University and the South African Medical Research Council for their financial support. Special thanks are extended to Dr C le Grange for supplying human vaginal mucosa.

DISCLAIMER: Any opinion, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the South African Medical Research Council does not accept any liability in regard thereto.

REFERENCES

1. Prego C, García M, Torres D, Alonso MJ. Transmucosal macromolecular drug delivery. J Contr Rel 2005; 101:151-62.
2. Wani MS. Current status in buccal delivery systems. http://www.pharmainfo.net/reviews/current-status-in-buccal-delivery-systems. 2007; accessed 15 November 2007.
3. Lee K-J, Johnson N, Castelo J, Sinko PJ, Grass G, Holme K, Lee Y-H. Effect of experimental pH on the

in vitro permeability in intact rabbit intestines and Caco-2 monolayer. Eur J Pharm Sci 2005; 25:193-200.

4. Boisset M, Botham P, Haegele KD, Lenfant B, Pachot JI. Absorption of angiotensen II antagonists in Ussing chambers, Caco-2, perfused jejunum loop and *in vivo*: Importance of drug ionisation in the *in vitro* prediction of *in vivo* absorption. Eur J Pharm Sci 2000; 10:215-24.
5. Karlsson J, Yngell A-L, Gråsjö J, Artursson P. Paracellular drug transport across intestinal epithelia: influence of charge and induced water flux. Eur J Pharm Sci 1999; 9:47-56.
6. Wertz PW, Squier CA. Cellular and molecular basis of barrier function in oral epithelium. Crit Rev Therap Drug Carrier Syst 1991; 8(3):237-69.
7. Mahalingam R, Ravivarapu H, Redkar S, Li X, Jasti BR. Transbuccal delivery of 5-aza-2′- deoxycytidine: effects of drug concentration, buffer solution, and bile salts on permeation. AAPS Pharm Sci Tech 2007; 8(3):Article 55. DOI: 10.1208/pt0803055.
8. Salama NN, Eddington ND, Fasano A. Tight junction modulation and its relationship to drug delivery. Ad Drug Del Rev 2006; 58:18-28.
9. Justin-Temu M, Damian F, Kinget R, Van den Mooter G. Intravaginal gels as drug delivery systems. J Women's Health 2004; 13(7):834-44.
10. Mashru R, Sutariya V, Sankalia M, Sankalia J. Transbuccal delivery of lamotrigine across porcine buccal mucosa: *in vitro* determination of routes of buccal transport. J Pharm Pharm Sci 2005; 8(1):54-62.
11. Aurora J, Pathak V. The fate of drugs and drug development: an overview. Drug Deliv Tech 2004; 4(3). Online version: http://www.drugdeliverytech.com.
12. Nielsen HM, Rassing MR. Nicotine permeability across the buccal TR146 cell culture model and porcine buccal mucosa *in vitro*: effect of pH and concentration. Eur J Pharm Sci 2002; 16(3):151-57.
13. Senel S, Kremer M, Nagy K, Squier C. Delivery of bioactive peptides and proteins across oral (buccal) mucosa. Curr Pharm Biotechnol 2001; 2(2):175-86.
14. Loftsson T, Konráðsdóttir F, Másson M. Influence of aqueous diffusion layer on passive drug diffusion from aqueous cyclodextrin solutions through biological membranes. Pharmazie 2006; 61(2):83-9.
15. Loftsson T, Brewster ME, Másson M. Role of cyclodextrins in improving oral drug delivery. Am J Drug Deliv 2004; 2(4):261-75.
16. Nicolazzo JA, Reed BL, Finnin BC. The effect of various *in vitro* conditions on the permeability characteristics of the buccal mucosa. J Pharm Sci 2003; 92(12):2399-410.
17. Van der Bijl P, Van Eyk AD, Thompson IOC. Permeation of 17β-estradiol through human vaginal and buccal mucosa. Oral Surg Oral Med Oral Path Oral Radiol Endo 1998; 85:393-8.
18. Moll LM, Van Eyk AD, Van der Bijl P. *In vitro* permeability of intact and de-epithelialised human vaginal mucosa to tacrolimus and cyclosporin A. Eur J Inflam 2007; 5(1):7-14.
19. Basson E, Van der Bijl P, Van Eyk AD. Transvaginal diffusion of synthetic peptides. Eur J Inflam 2007; 5(1):15-20.
20. Van Eyk AD, Van der Bijl P. Comparative permeability of various chemical markers through human vaginal and buccal mucosa as well as porcine buccal and mouth floor mucosa. Arch Oral Biol 2004; 49(5):387-92.
21. Motulsky H. Intuitive Biostatistics. In: Graphpad Software, Oxford University Press, New York, 1995.
22. Rojanasakul Y, Wang L-Y, Bhat M, Glover DD, Malanga CJ, Ma JKH. The transport barrier of epithelia: a comparative study on membrane permeability and charge selectivity in the rabbit. Pharm Res 1992; 9(8):1029-34.
23. Krogsgaard-Larsen P, Frydenvang K. Acetylcholine and histamine receptors and receptor-ligands: medical chemistry and therapeutic aspects. In Textbook of drug design. P. Krogsgaard-Larsen, T. Liljefors and U. Madsen, ed. CRC Press, United States, 2002; p282.
24. Nielsen HM, Rassing MR. TR146 cells grown on filters as a model of human buccal epithelium: IV. Permeability of water, mannitol, testosterone and β-adrenoceptor antagonists. Comparison to human, monkey and porcine buccal mucosa. Int J Pharm 2000; 194:155-67.
25. Mahato RI, Narang AS, Thoma L, Miller DD. Emerging trends in oral delivery of peptide and protein drugs. Crit Rev Therap Drug Carrier Syst 2003; 20153-214.
26. Akhlaghi F, Trull AK. Distribution of cyclosporine in organ transplant recipients. Clin Pharmacokinet 2002; 41(9):615-37.