International Journal of Pharmaceutics 413 (2011) 87–92

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Concentration and surface of absorption: Concepts and applications to gastrointestinal patches delivery

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ARTICLE INFO

Article history:
Received 3 February 2011
Received in revised form 11 April 2011
Accepted 14 April 2011
Available online 21 April 2011

Keywords:
Peptide absorption
Drug delivery
Intestinal absorption
Multiparticulate dosage forms
Gastrointestinal patches

ABSTRACT

Gastrointestinal patches represent a novel multiparticulate drug delivery system able to increase the intestinal absorption of drugs with poor bioavailability. The number of patches to administer is a critical issue since it is related to the surface and drug concentration at the absorption site.

The objective of this article is to evaluate the effect of the number of administered patches on the final absorption of leuprolide, a peptide chosen as model drug, assuming complete adhesion of all the devices to the intestinal membrane.

The same dose of leuprolide was encapsulated into 2, 4 and 6 patches; the resulting intestinal absorption profiles were measured with the Ussing chamber *ex vivo* experimental setup and compared between them.

The results showed that varying the number of patches, the final absorption does not present statistically significant changes, indicating that changes in concentration are balanced by change in absorption surface. These experimental findings can also be explained considering the equation that links the drug flow to surface and concentration at the absorption site, showing that the drug flow is related only to the geometry of each individual patch.

1. Introduction

Oral delivery is a convenient and patient-friendly route for drug administration compared to injections but unfortunately it presents different drawbacks that limit the oral absorption for some classes of drugs, such as insoluble molecules and hydrophilic macromolecules like proteins, peptides and nucleic acids. Active substances appertaining to this latter group are often extremely instable in the gastrointestinal environment, with a consequent low absorbed fraction.

Faced to the challenge of conceiving efficient dosage forms to deliver such molecules, various strategies have been proposed in order to improve their oral absorption, e.g. the co-administration of permeation enhancers or enzymatic inhibitors. These strategies are intended to increase and/or to control the flux of absorption F (amount absorbed per unit of time), which in case of passive transport is basically controlled by three main parameters, namely the surface of absorption S, the concentration at the absorption interface C and the apparent permeability of the intestinal tissue to the drug P_{app} (Eq. (1)).

$$F = \frac{dQ}{dt} = S \times C \times P_{app} \quad (1)$$

Available strategies are generally addressed to improve one of these parameters, normally the one that has been identified to limit the absorption for a specific drug. The use of permeation enhancers to increase the apparent permeability of the epithelium has been proposed, especially for enhancing the absorption of hydrophilic macromolecules (Aungst, 2000; Uchiyama et al., 1999). Alternatively, the co-administration of enzyme inhibitors (Yamamoto et al., 1994) can be used to decrease the presystemic metabolism of the drug, which would result in an increase of C. Drug encapsulation in micro or nanoparticles (Dogru et al., 2000; Garcia-Fuentes et al., 2002), in liposomes (Takeuchi et al., 2003) or complexed in hydrogels (Lowman et al., 1999) have been used for temporary masking the drug to an unfavourable environment resulting at the same time in an increase of C and, when a mucoadhesive polymer is used in the formulation, an increase in the surface area S available for the absorption. In fact, in order to increase the residence time in the intestine and the local concentration in proximity of the intestinal wall it has been proposed to associate these molecules to particulate systems coated with mucoadhesive

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doi:10.1016/j.ijpharm.2011.04.032

polymers (Carino and Mathiowitz, 1999; Ponchel and Irache, 1998; Ponchel et al., 1997; Takeuchi et al., 2003).

Particulate dosage forms appear very convenient for protecting the drug from degradation; however, it is rather difficult to locally maximize the drug concentration *C*, ideally at the surface of the intestinal epithelium, since several issues limit the applicability of these particles systems. Specifically: (i) drug release cannot be directed towards the mucosal surface, but on the contrary it is multidirectional, leading thus to the loss of a certain fraction of the drug into the luminal fluids; (ii) since the particle surface is exposed to intestinal fluids, drugs encapsulated in these particles may not get sufficient protection against proteolytic degradation, and (iii) in case of co-administration of an enzyme inhibitor or a permeation enhancer, it will be also diluted into the intestinal lumen, with a loss in activity and the risk of locally generate adverse effects.

In order to overcome these limitations the oral administration of intestinal patches has been proposed (Eaimtrakarn et al., 2003; Tao and Desai, 2005), since they can protect the drug from degradation, optimize the concentration gradient and localize the effect of enzyme inhibitors or permeation enhancers, allowing consequently to reduce the amount administered of these substances. These devices are in general composed of three different layers: (i) a pH-sensitive layer able to protect the molecule until the patch reaches the intestinal environment; (ii) a mucoadhesive layer, which gives mucoadhesive properties to the patch and which is loaded with the drug and (iii) a backing layer, made from a non permeable polymer, which prevents any back-diffusion of the drug. It has been already shown in literature that these patches could represent a convenient and efficient way for increasing the intestinal absorption of hydrophilic macromolecules like insulin (Grabovac et al., 2008; Whitehead et al., 2004), erythropoietin (Venkatesan et al., 2006), granulocyte colony-stimulating factor (G-CSF) (Eiamtrakarn et al., 2002) and interferon-alpha (Ito et al., 2005). This effect could be attributed to the patches ability to locally increase the drug concentration at the absorptive surface. Despite the number of publication showing the improvement in absorption for these delivery devices, any of them investigate the relationship between the number of devices administered and the resulting absorption. In fact in the case of patches, as also for the other multiparticulate systems, the surface involved in the absorption process is related to the number of administered dosage units. Moreover, considering a constant drug dose administered, dividing this dose in a different number of dosage units would imply a different local concentration of the drug after this is released from the delivery devices. The aim of the present work is to study the implications of the number of dosage units administered on the resulting drug absorption. In order to achieve that, mucoadhesive patches were used as a delivery tool to easily modulate the surface and concentration involved in the absorption process, when an identical dose is divided in a different number of devices. The Ussing chamber has been chosen as experimental setting in order to measure the intestinal absorption (Lennernas, 1998).

2. Materials and methods

2.1. Materials

Leuprolide acetate was purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Trifluoroacetic acid and glutamine were purchased by Sigma–Aldrich (Saint-Quentin Fallavier, France). Acetonitrile for analysis was purchased from Carlo Erba (Val de Reuil, France). All products were used as received.

2.2. Ex vivo absorption experiments

The patches used in the study consist of a two layer film composed by a backing layer realized with an impermeable polymer and a mucoadhesive drug loaded layer. The pH-sensible layer, normally also included in the gastrointestinal patches formulations, was not included in this specific case since the devices were placed directly in contact with the intestinal membrane when studied in the Ussing chamber.

Leuprolide acetate was chosen as a model peptidic drug, and its transport across rat intestine was studied in Ussing chambers (Fig. 1).

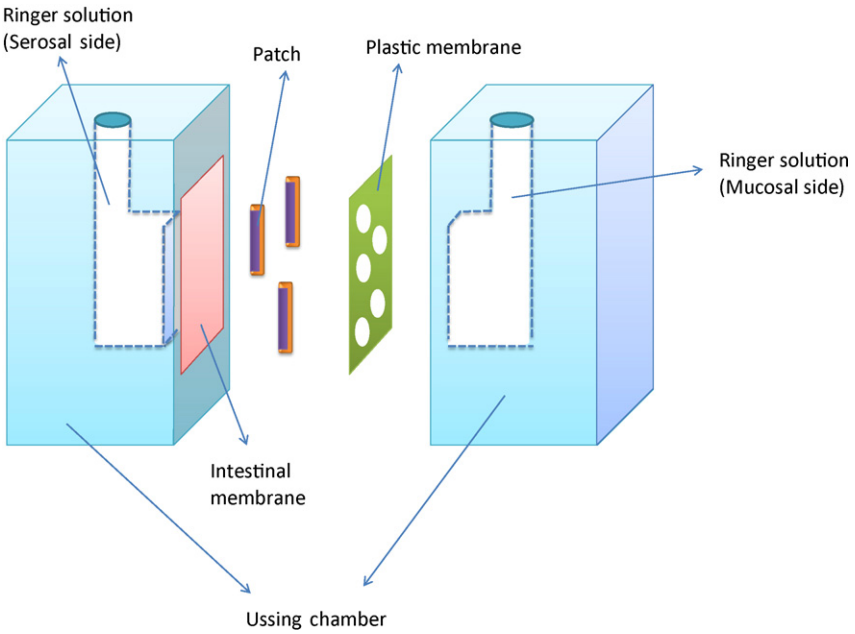

Fig. 1. Schematic representation of Ussing chamber. The rat intestine (in red) is placed between the two half-chamber. After the equilibration of the solutions (approx. 30 min.) the chamber is opened, patches are placed on the intestine and a plastic membrane (in green) is placed on the patches in order to assure the adhesion of the devices. This plastic membrane contain opening of 2 mm in order to assure a freely diffusion in the chamber. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.2.1. Preparation of the intestinal tissue

All animal experiments were performed in an accredited laboratory in accordance with the official regulations provided by the French Ministry of Agriculture and with local ethical guidelines. Experiments were approved by the institutional animal care and use committee.

Jejunum from fresh small intestine of sacrificed male Wistar rats (200–250 g) (Charles River, France) was surgically withdrawn, rinsed with chilled physiological saline solution (NaCl 0.9%) and cut into segments of 2–3 cm length. After visual examination of the tissue, section containing Peyer's Patches were discarded from the studies.

2.2.2. Permeation experiments

Jejunum portions were mounted in Ussing chambers (the intestinal surface in the chambers was of 1 cm^2). Chambers were closed, filled with Ringer solution at pH 6 containing glutamine 1 mmol/l. The system was maintained at 37 °C and continuously oxygenated with O_2/CO_2 95%/5%. After 30 min of equilibration, the leuprolide solution (as control) or the leuprolide loaded patches were introduced in the donor chamber. When patches have to be placed on the intestinal membrane, the solutions in the donor and acceptor chambers were totally withdrawn and patches were placed on the intestinal mucosa. A plastic membrane with openings of 2 mm in diameters was placed up to patches in order to ensure their adhesion to the intestinal lumen and at the same time allow the medium diffusion on the intestinal surface (Fig. 1). Finally, chambers were re-filled with the already equilibrated solutions. Assays were carried out for 4 h, and any sample taken was replaced by the same amount of fresh Ringer solution. The experiments were repeated on different days and with different rats. The membrane integrity has been checked along the experiments by measuring the electrical parameters to determine the tissue viability and the tight-junctions integrity has been checked at the end of the experiment.

2.2.3. Stability of leuprolide acetate in Ussing chambers

A stability study has been performed by incubating a leuprolide solution into the Ussing chamber in the same experimental conditions with an equal concentration at the two sides of the chamber; this situation corresponds to a status of equilibrium. A solution of 150 μg of leuprolide acetate in MilliQ® water were placed in both donor and acceptor sides of the chambers replacing the intestine with a dialysis membrane (Spectra/Por 3 membrane MWCO: 3500).

2.2.4. Leuprolide acetate formulations

The effect of different formulations on intestinal permeation was tested, aiming to modify the drug concentration and surface of absorption. A leuprolide solution in Ringer as control buffer was used at the concentration of 30 μg/mL, corresponding to the introduction of 150 μg of leuprolide in the 5 mL volume of the donor chamber. Further, 6 intestinal patches (2–3 mm in diameter) loaded with 25 μg of leuprolide each (with a total dose of 150 μg) were prepared and placed at the surface of the intestinal tissue in Ussing chambers. In order to vary the local concentration, experiments were also conducted using 2 and 4 patches keeping the total dose constant to 150 μg.

2.3. HPLC quantification of leuprolide

The HPLC system consisted of an automatic samples injector (Waters 717 plus autosempler), a pump (Waters 1525 binary HPLC pump), a separation column (Waters Symmetry C18 column 4.6 mm × 150 mm) maintained at 25 °C, an UV detector (Waters 2487 dual λ absorbance detector) reading at 220 nm and a software package for the data acquisition (Breeze Software). A gradient separation was used, consisting of two mobile phases: phase A, 10% of acetonitrile +90% of water with 0.1% trifluoroacetic acid; phase B, 90% of acetonitrile +10% water with 0.1% trifluoroacetic acid. The injection volume was 50 μl and the flow was 1 mL/min. The limit of detection was found to be 0.2 μg, the limit of quantification was 1 μg, precision was 0.67% Relative Standard Deviation and the accuracy was 96–104%. Leuprolide quantification was calculated on a calibration curve with amounts ranging from 1 to 120 μg in Ringer solution (R^2 = 0.9999). A statistical analysis of the linear regression was obtained with Statgraphics 5 Plus (Statpoint Technologies Inc., USA).

2.4. Statistics

All values are expressed as their mean ± S.D. Statistical differences were assumed to be significant when $p < 0.05$ with a Friedman test of repeated measurements using GraphPad Prism 5 software (Demo version of GraphPad Software Inc., La Jolla, USA). The AUCs of the absorption profiles were computed using the R package PK (R Foundation for Statistical Computing)(R Development, 2010). Michaelis–Menten degradation curve for leuprolide was simulated using Microsoft Excel 2003 (Microsoft Corporation, Redmond, USA).

3. Results and discussion

Gastrointestinal mucoadhesive patches are multiparticulate delivery devices with interesting potentials improving the absorption of highly instable molecules, like peptides and proteins. These patches present the characteristic to change the absorption surface and concentration depending on the drug dose but also on the number of devices administered. With the purpose to study the effect of change in local concentration and absorption surface, the absorption of a fixed dose of leuprolide (150 μg) was studied, comparing the administration as solution and splitting this amount into a different number of patches (2, 4 and 6). In this way, since the number of devices changes, but the total dose remains constant, there is a different amount of drug contained in each patches and consequently a different local concentration once that each patch releases the drug. At the same time a change in the number of devices will affect the total surface involved in the absorption. This situation corresponds to a simultaneous change of the parameters S and C in Eq. (1). The concentration of the drug at the absorption interface and the surface involved in the absorption affect directly the drug absorption flow according the Eq. (1) where F, S and C represent the apparent absorptive flux, the surface and the concentration respectively and P_{app} represent the apparent permeability for the considered molecule.

Fig. 2 is a visual representation of the different components involved in the absorption when patch-like devices are used for the intestinal delivery.

Intestinal permeation of leuprolide from the patches has been measured using rat intestinal tissues placed in Ussing chambers. Compared to cell cultured membranes, this technique offers the advantage of being more representative of the living tissues, especially when pharmacokinetic experiments in animals are foreseen.

Because peptides are likely to be adsorbed on glass or plastic surfaces and also to degrade, some controls were conducted previously to permeation experiments. Leuprolide stability in our experimental condition is reported in Fig. 3. As can be seen, a short lag-time of approx. 10 min is needed in order to obtain a homogeneous concentration in the Ussing chamber, but after that the concentration remains stable, showing the absence of any adsorption on the chambers materials.

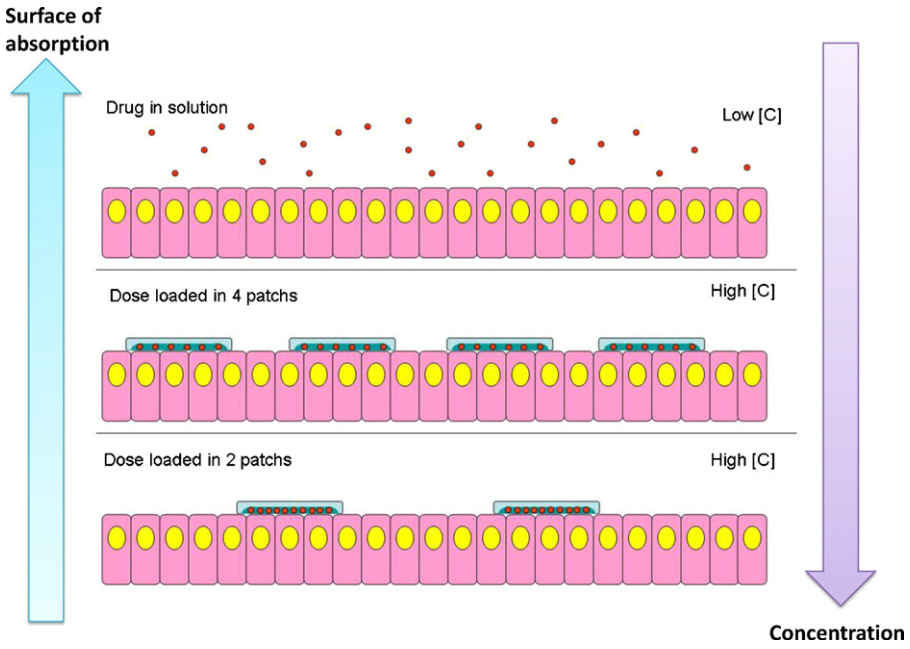

Fig. 2. Schematic representation of the same dose administered as a solution and loaded into patches (2 or 4) in contact with the intestinal mucosa.

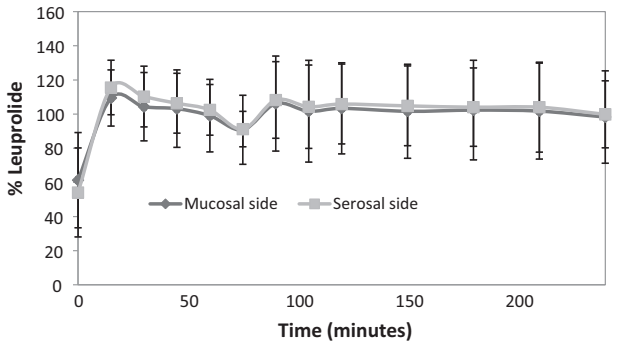

Fig. 3. Stability of leuprolide in the Ussing chambers with a dialysis membrane replacing the intestinal tissue. Each one of the two side of the chamber contain 150 μg (data are the mean of $n = 4 \pm SD$).

Leuprolide permeation through the intestinal mucosa has been determined for one dose of leuprolide (150 μg) either divided between 2, 4 or 6 patches or as a solution in the donor chamber. Leuprolide permeation when delivered from patches was considerably enhanced compared to the control solution, increasing the fraction of dose absorbed from 0.5% for the solution to 2.7% for the patches (Fig. 4). This enhancement is probably related to the increase in local concentration at the intestinal membrane when the drug is encapsulated into the patches. In fact, also if with the patches there is a decrease of the surface involved in the absorption (from 100 mm^2 to approx. 3.14 mm^2), the increase in local concentration is capable to balance this "lost" in flow and still produce an increase in the resulting absorption (Fig. 2).

Permeation kinetics of leuprolide encapsulated into a different number of patches are presented in Fig. 5, by plotting directly the permeated amounts in mass against time. The apparent perme-

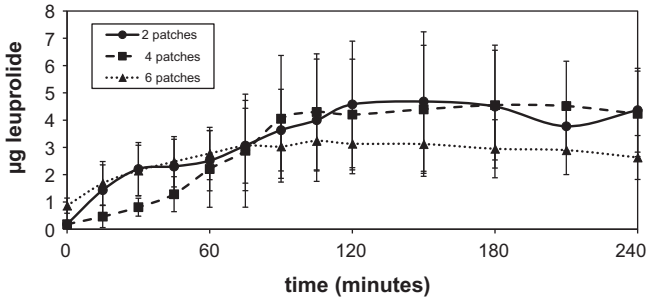

Fig. 5. Permeated amount of leuprolide from 2, 4 or 6 patches. The total dose was maintained equal to 150 μg (data are the mean of $n = 4 \pm SD$). The AUCs of the absorption profiles are also reported as mean and confidence interval: AUC_{0-t} (2 patches) = 896.82 (793.91; 988.14); AUC_{0-t} (4 patches) = 1055.34 (863.02; 1242.61); AUC_{0-t} (6 patches) = 896.81 (725.85; 1047.08).

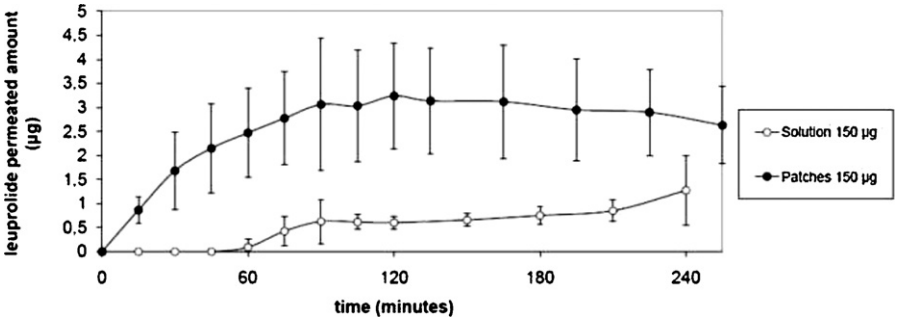

Fig. 4. Permeated amount of leuprolide from a leuprolide solution or encapsulated into 6 patches. The total dose administered was maintained equal to 150 μg (data are the mean of $n = 4 \pm SD$).

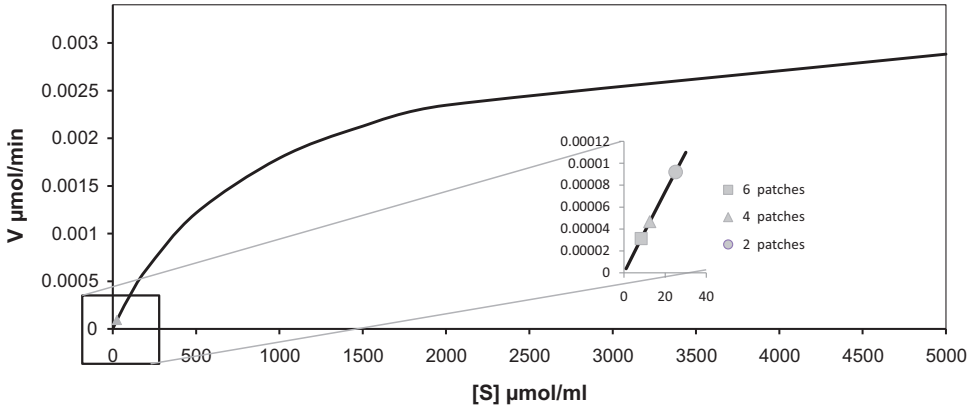

Fig. 6. Simulation of the Micaelis–Menten curve for leuprolide in intestinal homogenate according to the parameters presented in leterature (Zheng et al., 1999). On the curve are also reported the concentration estimated at the interface between patches and intestinal membrane (in black).

ability of 150 μg leuprolide in solution was 2.80×10^{-6} cm/s. In comparison, Guo et al. (2004) reported permeabilities in the range of 1×10^{-5} to 4×10^{-5} cm/s in rat or rabbit, using the reverted gut sac technique. Those discrepancies could result from differences in proteolysis in the different experimental conditions, leading to decreases in the actual leuprolide concentrations depending on the dose. In some experimental situations when proteolysis was reduced, these authors reported also that leuprolide permeability was almost independent from the dose, suggesting passive intestinal absorption. In the case of patches, adhesion at the intestinal membrane is not expected to modify the intrinsic permeability of the membrane. Attempts were made to calculate the apparent permeability of the membrane in this situation, but only an estimation of the permeability may be obtained since when delivering the drug inside the patches it is not possible to know the exact drug concentration at the absorption surface.

Whatever the exact leuprolide concentration created at the interface between the patches and the intestinal membrane, it seems reasonable to consider that this concentration was much higher than the one produced in the donor chamber by the solution. Indeed, compared to patches a considerable dilution of leuprolide occurred when directly added in the 5 mL of chamber medium, which lowered considerably the concentration gradient and thus the permeation.

Further, under experimental conditions and although it would require experimental confirmation, it was reasonable to consider that the intestinal permeability to leuprolide was not really affected by the delivery device itself (solution or patch).

As showed in Fig. 2, intestinal patches constitute very attractive devices, since they allow modifying the drug flow changing surface and concentration. For further investigating this aspect, the dose of leuprolide (150 μg) was divided between 6, 4 or 2 patches. As shown in Fig. 5, the amounts of leuprolide permeating through the mucosa were not significantly different ($p = 0.29$), whatever the amount of patches used for dividing the 150 μg dose. This absence of effect results from a balance between the surface of absorption and the leuprolide concentration generated by the patches in front of the absorptive membrane. This can be confirmed theoretically as follows.

Indeed, according to the Eq. (1), for a drug which presents passive absorption the flow (F) generated by the patches depends on the apparent permeability of the molecule (P_{app}), the surface involved in the absorption (S) and the concentration at the absorption interface (C), where F, S and C corresponds to the sum of the individual contributions of each patch.

If all the patches have the same size, like in our experimental conditions, the global area involved in the absorption corresponds to the sum of the individual areas of each patch (S_{ip}):

$$S = n \times S_{ip} \tag{2}$$

If we consider that following adhesion, the drug-containing layer of each patches hydrates and form a solution of volume V_{ip}. Thus, the concentration C corresponds to the dose (D) divided by the total hydratation volume of all the patches ($n \times V_{ip}$):

$$C = \frac{D}{n \times V_{ip}} \tag{3}$$

Substituting Eqs. (2) and (3) in Eq. (1) we obtain that

$$F = \frac{P_{app} \times D \times S_{ip}}{V_{ip}} \tag{4}$$

Eq. (4) shows how the flux produced by the patches during absorption is related to the drug permeability (P), the dose administered (D) and the physical geometry of individual patch, represented by its surface and its hydratation volume. It can be seen that a given dose divided into a variable number of patches leads to the same flux of absorption because the reduction in surface is balanced by an increase in local concentration provided that the patch geometry (height/surface) remains constant. This finding is of practical interest when considering the need for minimizing variability under *in vivo* conditions. It suggests that the dose can be conveniently divided into a large number of patches to form a multiparticulate delivery system. Indeed, such system are known to minimize the absorption variability *in vivo*. Among the sources of variability, patches adhesion in a reliable way to the mucosal surface cannot be guaranteed, which has to be considered. By multiplying the number of patches, adhesion *in vivo* will become statistically more likely, driving to a more reliable fraction of the dose absorbed. On the other side, dividing the same dose in a higher number of patches lead to a reduction of the concentration gradiant produced by each device, since each patch would be loaded with a smaller fraction of the dose (Fig. 2).

Finally, it should be stressed that despite the fact that patches generated locally high drug concentrations compared to conventional solutions, it could be estimated that no saturation of enzymatic pathways occurred. In fact, using the Michaelis–Menten constants for the intestinal degradation of leuprolide reported in literature (Zheng et al., 1999) it was possible to verify that under our experimental conditions, a saturation of enzymatic degradation process of leuprolide was unlikely (Fig. 6). However, in some situations and for some drugs, a saturation of the metabolism cannot be excluded. In this case a change in the number of patches it is expected to affect the absorption profile, since an increase in the

local concentration may lead to a local saturation of the metabolic pathway for the considered drug.

4. Conclusions

Mucoadhesive gastrointestinal patches present interesting and useful properties that allow controlling the surface and the concentration involved in the absorption process when the adhesion to the intestinal membrane is assured. In this article was investigated the effect of the number of devices used for the delivery of an unchanged total dose of leuprolide. Experimental results showed that if the same dose was divided into a different number of patches, the final absorption was not significantly different. In order to investigate the reasons of this finding, it was demonstrated that indeed the global flow generated by the patches is related to the surface of each individual device and to the hydratation volume of this surface. However, it was point out that this consideration are on the theoretical perspectives, and that in some cases the number of patches may influence the absorption, particularly considering the statistical adhesion of the devices *in vivo* and the possible saturation of the metabolic pathway for the drug considered. On the other side, although if limited to specific circumstances, these consideration lead to a better understanding of the process involved in the intestinal absorption of highly degraded drugs when multiparticulate dosage forms are used as delivery system.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

Authors would like to thank Valérie Domergue for her support in animal studies and Dr. Raquel Diaz-Lopez for help reviewing the manuscript. Moreover authors would like to thank the Ministère National de la Recherche et de la Technologie of France for financial support.

References

Aungst, B.J., 2000. Intestinal Permeation Enhancers. J. Pharm. Sci. 89, 429–442.
Carino, G., Mathiowitz, E., 1999. Oral insulin delivery. Adv. Drug Deliv. Rev. 35, 249–257, Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10837700.
Dogru, S.T., Calis, S., Oner, F., 2000. Oral multiple w/o/w emulsion formulation of a peptide salmon calcitonin: in vitro-in vivo evaluation. J. Clin. Pharm. Ther. 25, 435–443.
Eaimtrakarn, S., Rama Prasad, Y.V., Puthli, S.P., Yoshikawa, Y., Shibata, N., Takada, K., 2003. Possibility of a patch system as a new oral delivery system. Int. J. Pharm. 250, 111–117.
Eiamtrakarn, S., Itoh, Y., Kishimoto, J., Yoshikawa, Y., Shibata, N., Murakami, M., et al., 2002. Gastrointestinal mucoadhesive patch system (GI-MAPS) for oral administration of G-CSF, a model protein. Biomaterials 23, 145–152.
Garcia-Fuentes, M., Torres, D., Alonso, M.J., 2002. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloids Surf. B: Biointerfaces 27, 159–168, doi:10.1016/S0927-7765(02)00053-X.
Grabovac, V., Föger, F., Bernkop-Schnürch, A., 2008. Design and in vivo evaluation of a patch delivery system for insulin based on thiolated polymers. Int. J. Pharm. 348, 169–174, doi:10.1016/j.ijpharm.2007.06.052.
Guo, J., Ping, Q., Jiang, G., Dong, J., Qi, S., Feng, L., et al., 2004. Transport of leuprolide across rat intestine, rabbit intestine and Caco-2 cell monolayer. Int. J. Pharm. 278, 415–422, doi:10.1016/j.ijpharm.2004.03.031.
Ito, Y., Tosh, B., Togashi, Y., Amagase, K., Kishida, T., Kishida, T., et al., 2005. Absorption of interferon alpha from patches in rats. J. Drug Target. 13, 383–390, doi:10.1080/10611860500331506.
Lennernas, H., 1998. Human intestinal permeability. J. Pharm. Sci. 87, 403–410.
Lowman, A.M., Morishita, M., Kajita, M., Nagai, T., Peppas, N.A., 1999. Oral delivery of insulin using pH-responsive complexation gels. J. Pharm. Sci. 88, 933–937, doi:10.1021/js980337n.
Ponchel, G., Irache, J., 1998. Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract. Adv. Drug Deliv. Rev. 34, 191–219, Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10837678.
Ponchel, G., Montisci, M.-J., Dembri, A., Durrer, C., Duchene, D., 1997. Mucoadhesion of colloidal particulate systems in the gastro-intestinal tract. Eur. J. Pharm. Biopharm. 44, 25–31.
R Development, C.T., 2010. R: a language and environment for statistical computing. In: R. D. C. Team (Ed.), Vienna Austria R Foundation for Statistical Computing. R Foundation for Statistical Computing, Retrieved from http://www.r-project.org.
Takeuchi, H., Matsui, Y., Yamamoto, H., Kawashima, Y., 2003. Mucoadhesive properties of carbopol or chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats. J. Control. Release 86, 235–242.
Tao, S.L., Desai, T.A., 2005. Gastrointestinal patch systems for oral drug delivery. Drug Discov. Today 10, 909–915.
Uchiyama, T., Sugiyama, T., Quan, Y.-S., Kotani, A., Okada, N., Fujita, T., et al., 1999. Enhanced permeability of insulin across the rat intestinal membrane by various absorption enhancers: their intestinal mucosal toxicity and absorption-enhancing mechanism of n-lauryl-β-D-maltopyranoside. J. Pharm. Pharmacol. 51, 1241–1250, doi:10.1211/0022357991776976.
Venkatesan, N., Uchino, K., Amagase, K., Ito, Y., Shibata, N., Takada, K., 2006. Gastrointestinal patch system for the delivery of erythropoietin. J. Control. Release 111, 19–26, doi:10.1016/j.jconrel.2005.11.009.
Whitehead, K., Shen, Z., Mitragotri, S., 2004. Oral delivery of macromolecules using intestinal patches: applications for insulin delivery. J. Control. Release 98, 37–45, doi:10.1016/j.jconrel.2004.04.013.
Yamamoto, A., Taniguchi, T., Rikyuu, K., Tsuji, T., Fujita, T., Murakami, M., et al., 1994. Effects of various protease inhibitors on the intestinal absorption and degradation of insulin in rats. Pharm. Res. 11, 1496–1500, doi:10.1023/A:1018968611962, Springer, Netherlands.
Zheng, Y., Zheng, Y.J., FuLu, M. -y., Qiu, Y., Reiland, T.L., 1999. Enzymatic degradation of leuprolide in rat intestinal mucosal homogenates. Pharm. Dev. Technol. 4, 539–544, Informa UK Ltd UK.