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The study of drug permeation through natural membranes

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Abstract

In this study, natural membranes such as the outer membrane of *Prunus persica* (peach) and *Lycopersicon esculentum* (tomato), the inner layer of the egg of *Gallus domesticus* (hen) and the middle membrane of the *Allium cepa* (onion) were used as controlling barriers for permeation of some model drugs with different MW and lipophilicities. Drug permeation studies were done by using modified Franz diffusion cell. The permeation of drugs through these natural membranes was compared to permeation of them through human skin and synthetic cellophane membrane. Results showed that the rate and amount of diclofenac permeated through onion membrane was not significantly different from that with tomato ($p > 0.17$), egg ($p > 0.29$) and human skin ($p > 0.93$). Permeation of diclofenac through tomato skin and cellophane was not significantly different ($p > 0.35$). Permeation of diclofenac through all studied membranes except for human skin that follows the Fickian kinetic followed non-Fickian mechanism and their permeabilities were not significantly different from each other ($p > 0.05$). Permeation of metronidazole through onion membrane and tomato skin were not significantly different from human skin ($p > 0.053$ and 0.38, respectively). All membranes were significantly different from each other ($p < 0.0001$) for permeation of erythromycin as a relatively large molecular weight and lipohilic molecule through human skin and other studied membranes. Permeation of diclofenac through human skin and metronidazole through egg and tomato skin followed Fick's first law. Diffusion of diclofenac through onion, tomato, egg, cellophane, and peach; metronidazole through onion, peach, cellophane, and human skin, and erythromycin through all studied membranes followed non-Fickian mechanism for diffusion. Statistical analysis showed the most similarity between onion and human skin for diclofenac, tomato and human skin for metronidazole, onion and cellophane for erythromycin.

Keywords: Drug; Permeation; Natural; Membrane; Diffusion

1. Introduction

Control of drug release has very considerable importance in novel drug delivery system (NDDS) design. Coupled with this, methods for evaluation of drug release are in the center of attention in NDDS. The keystone of any NDDS is based on the pharmacokinetic and permeability properties of the drug. Drug release can be controlled or evaluated by monitoring of its permeation through natural (Godin and Touitou, 2004; Barry, 2002; Jiang et al., 1998) or synthetic membranes (Shimamura et al., 2004; Iordanskii et al., 2000; Dinarvand and Ansari, 2003; Ano et al., 2004). Because, permeation of drug through the skin is very important in transdermal drug delivery design, most permeation models were designed to simulate skin permeation (Dureja et al., 2001; Puglia et al., 2001), although permeation of drug through GI tract was also recognized (Bermejo et al., 2004; Sugano et al., 2003; Bij and Eyk, 2003). The ideal way to determine percutaneous absorption of a compound in human is to do the actual study in humans (Shah and Maibach, 1993). However, many compounds are potentially too toxic to test in vivo in humans. Beside of this, evaluation of these systems in vivo using human beings is difficult from the viewpoint of cost, time consumption, and ethical restrictions. Therefore the studies must be conducted in vitro using excised skin (human cadaver, animals) (Pongjanyakul, 2000; Wester and Maibach, 1975; Roberts and Mueller, 1990; Wester and Noonan, 1980). The animal skins differ significantly from human skin (HS) due to differences in thickness, nature of stratum cornea, density of hair follicles and sweat glands (Barry, 1983). In this respect, in vitro studies are generally conducted using a diffusion cell system with either static or a flow-through cell (Franz, 1975). The difficulty in obtaining excised skin and the variation in their permeability due to race, age, sex, anatomical site and concern for restricted use of animals has led the workers to use simulated skin or

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artificial membrane (Li et al., 2003). It is not clear that natural membranes such as peach and tomato skin, inter-lamellar layer of the onion, and inner layer of the egg could be applied in spite of HS, synthetic, simulated or artificial skin in in vitro drug permeation studies. The objective behind the work was to use these natural membranes in permeation studies of some model drugs such as diclofenac sodium, metronidazol, and erythromycin having different lipophilicities and molecular weight in comparison to HS and synthetic membranes to estimate possibility of their substitution in permeation studies.

2. Experimental

2.1. Materials

Erythromycin (Er), Emad Darman Co. Iran; diclofenac Na (DS), Alborz Daru Co., Iran; metronidazole (Me), Tehran Chimi, Iran; orthonitrobenzaldehyde, acetic acid, ethanol, hydrochloric acid, Merck (Darmstadt, Germany). All of reagents were used as received. In all steps of the study, double distilled water was used.

2.2. Apparatus

UV–vis spectrophotometer, 2100, Shimadzu, Japan; Magnetic stirrer, Heidolph, Germany; Ultrasonic bath, Strasonic, Italy; Analytical balance 0.0001 g, Sartorious, Germany; Modified Franz diffusion cell, home made. Circulation pump, Water bath, Memmert, Germany.

2.3. Methods

2.3.1. Drug analysis

2.3.1.1. Analysis of erythromycin in the receptor compartment of Franz diffusion cell. This has done with the following steps.

2.3.1.2. Preparation of orthonitro benzaldehyde reagent. 0.4 g of orthonitrobenzaldehyde was transferred to a 100 mL volumetric flask. This reagent was dissolved by glacial acetic acid and diluted to volume with glacial acetic acid.

2.3.1.3. Alcoholic phosphate buffer pH 8. Fifty milliliters of 0.05 M KH_2PO_4 was mixed with 63 mL NaOH 0.05 M in a 500 mL volumetric flask and diluted to volume with diluted alcohol (alc:water; 4:3).

2.3.1.4. Standard stock preparation. One hundred milligrams of erythromycin in a 100 mL volumetric flask was dissolved by glacial acetic acid and diluted with it to volume. Ten milliliters of the above solution was transferred to a 50 mL flask and diluted to volume by glacial acetic acid.

2.3.1.5. Standard preparation and procedure. 0.25, 0.5, 1.0, 1.25, 1.5 and 2.0 mL of this solution were transferred to 10 mL flask and to each 3 mL of HCl and 2 mL of reagent was added. Solution was shaking well before the following step.

The flasks were stand for 15–17 min in ambient temperature. All were diluted to volume with glacial acetic acid and absorbance were recorded at 486 nm (note: all of the steps must not exceed 28 min).

2.3.1.6. Determination of metronidazole in the receptor compartment of Franz diffusion cell. The stock solutions for calibration standards were prepared by dissolving 10 mg of metronidazole in 100 mL water. Standard solutions of the drug containing concentration ranges of 5–20 mg/L were prepared in water. UV absorbances of the solutions were directly recorded at 319 nm and calibration curve was constructed by plotting the absorbance versus concentration.

2.3.1.7. Analysis of diclofenac in the receptor compartment of Franz diffusion cell. The stock solutions for calibration standards were prepared by dissolving 10 mg of diclofenac in 100 mL water. Standard solutions of the drug containing concentration ranges of 2–25 mg/L were prepared in water. UV absorbances of the solutions were directly recorded at 270 nm and calibration curve was constructed by plotting the absorbance versus concentration.

2.3.2. Preparation of model membranes

Outer skin of the *Prunus persica* (peach) and *Lycopersicon esculentum* L. (tomato) were pealed from the ripped fruits, the outer shell membrane of the egg of *Gallus domesticus* that just located inside the shell exactly under the hard calcified layer was prepared by immersing the egg in HCl 0.01N solution for 6 h to dissolve the calcified layer without any further process and then cutting the membrane cautiously to expel the content of the egg and washing it with normal saline solution, and the middle membrane of the *Allium cepa* L. (onion) were pealed or separated with caution to prepare at least 10 cm^2 uniform membrane without any crack or orifice. All the membranes were inspected by a microscope to assure about their integrity and uniformity. Their thicknesses were measured by a caliper, and tried to use membranes that their thickness are very similar to cellophane. Samples of adult HS were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 °C for 2 min, after which stratum cornea and epidermis (SCE) were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at 25% relative humidity. The dried samples were wrapped in aluminum foil and stored at 4 °C until use.

2.3.3. Skin permeation studies

The in vitro permeation was studied across the skin of peach, tomato, egg, onion and peach and the results were compared to which that obtained from breast HS removed from healthy women during plastic surgery as a comparator and cellophane as a synthetic membrane. The permeation study was conducted with Franz diffusion cells (Franz, 1975) in the static mode (Ashke Shisheh, SIRO, Teh) with a diffusion area of 4.7 cm^2. The capacity of receptor compartments was 37 mL, and temperature was maintained at 37 °C by means of circulating the content of a water bath by a pump through the surrounding layer of the cell.

Drug concentrations in donor compartment were 20 mg/mL in ethanolic buffer, 7.5 mg/mL in water, and 10 mg/mL in water for erythromycin, metronidazole, and diclofenac Na, respectively. Receptor solution was Sorensen's phosphate buffer (pH 7.4) for DS and Me and ethanolic buffer for Er, which was continuously stirred at 600 rpm with a Teflon-coated bar magnet placed inside the cell. Skin samples were mounted between donor and receptor compartments of the cells and clamped with the dermal side in contact with the receptor medium. Then, at time zero, 2 mL of the drug solution was placed in the donor compartment and the cell was covered with Parafilm® to avoid solvent evaporation. Samples of 2 mL were taken from receptor compartments and immediately replaced with 2 mL of the receptor solution, at the same temperature. Initial experiments confirmed the maintenance of sink conditions by this procedure. The amounts of drug permeated from receptor solutions at predetermined times were analyzed by the method previously mentioned. Three parallel experiments were conducted with each drug and each membrane.

2.3.4. Data analysis

The solute release data were analyzed using Peppas equation:

$$\frac{M}{M_\infty} = kt^n$$

in which M is the amount of drug permeated to time t, and M_∞ is the amount drug permeated to infinite time. M/M_∞ is the fractional solute release, t is the release time, k is a kinetic constant, and n is an exponent which characterizes the mechanism of release of the solutes (Korsmeyer et al., 1983). Based on the diffusional exponent, n, the drug transport is classified as Fickian diffusion for $n \leq 0.5$, anomalous (non-Fickian) diffusion for $0.5 < n < 1.0$, case II transport or zero order (time independent release) for $n = 1.0$, and super case II transport for $n > 1.0$, but in this study for simplicity we categorized as Fickian for $n \leq 0.5$ and non-Fickian for $n \leq 0.5$.

According to this equation, logarithm of fractional solute release each time was plotted against logarithm of time to calculate n and k. On the basis of the above mentioned guidance and calculated n, release profiles were classified which considers the skins to be a one plane barrier membrane that used to calculate the permeability coefficients.

2.3.5. Statistical analysis

Results are expressed as the means of at least three experiments ± S.D. Statistical data analysis was performed using one-way ANOVA for erythromycin and a non-parametric Kruskal–Wallis test for metronidazole and diclofenac. All tests have $p < 0.05$ as a minimal level of significance.

3. Results and discussion

In this study peach and tomato skin, inter-lamellar layer of the onion, and inner layer of the egg were selected on the basis of their availability, price, and ethics. Diclofenac sodium is water soluble with medium MW, metronidazole is intermediate lipophilic with low MW, and erythromycin is lipophilic with high MW. Permeation profiles of these drugs through different membranes were shown in Figs. 1–3.

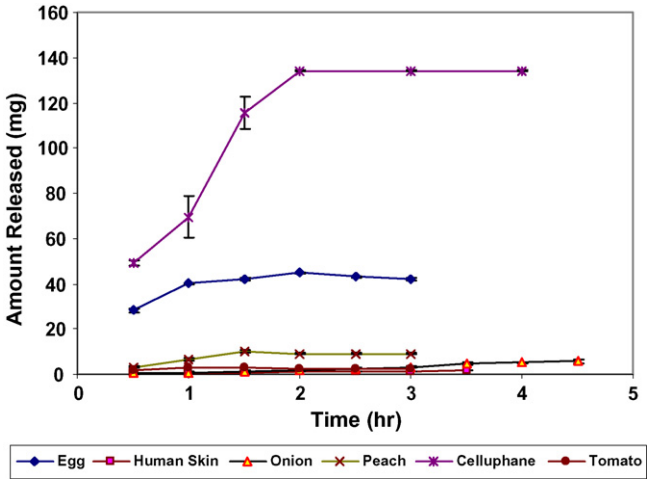

Fig. 1. Permeation profiles of metronidazole through different membranes.

As can be seen in Figs. 1 and 2, permeation from cellophane for metronidazole and diclofenac with low MW were very higher than all of other membranes, however permeation of erythromycin as a relatively high MW drug from this membrane is low. From the results were showed in these figures we can obtain that for permeation study of low MW drugs, cellophane cannot be membrane of choice in in vitro studies instead of HS, but rather some of these natural membranes are better models for such drugs. The results of calculation of n and k as the Peppas equation parameters were indicated in Table 1, and the diffusion manners for each drug from membranes were shown in Table 2.

As can be seen in Table 2, permeation of model drugs from membranes of onion, peach and cellophane follows the same diffusion manner. Metronidazole permeates through onion, peach, cellophane and human skin with similar diffusion mechanism, however its diffusion through egg and tomato skin are the same.

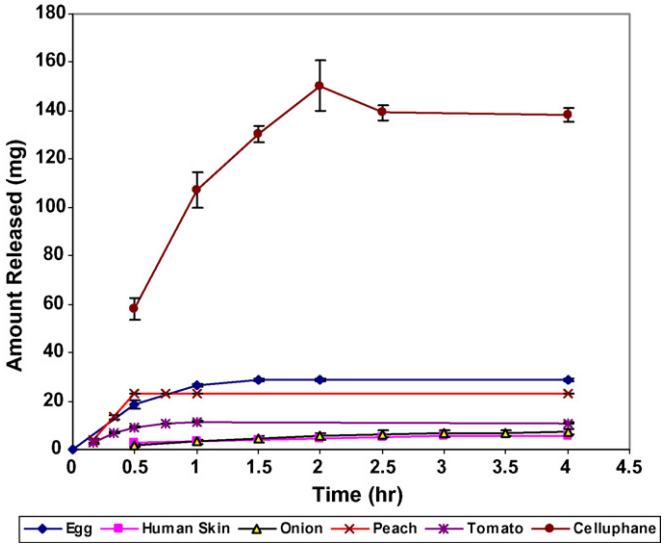

Fig. 2. Permeation profiles of diclofenac sodium through different membranes.

Table 1
Calculated parameters of Peppas equation

Drug membrane	Metronidazole			Diclofenac Na			Erythromycin		
	Exponent (n)	Kinetic constant (k)	r	Exponent (n)	Kinetic constant (k)	r	Exponent (n)	Kinetic constant (k)	r
Onion	1.46	0.11	0.9918	0.85	0.34	0.9767	1.10	0.15	0.9799
Egg	0.33	0.82	0.9608	0.98	0.57	0.9802	0.93	0.25	0.9942
Peach	1.06	0.65	0.9998	1.51	2.92	0.9989	0.62	0.62	0.9908
Tomato	0.36	0.89	0.9802	0.94	1.42	0.9672			
Cellophane	0.75	0.59	0.9782	0.69	0.65	0.9876	0.75	0.28	0.9181
HS	0.61	0.45	0.9774	0.43	0.59	0.9932	0.85	0.32	0.9842

$M/M_\infty = kt^n$ for each drug and each membrane studied.

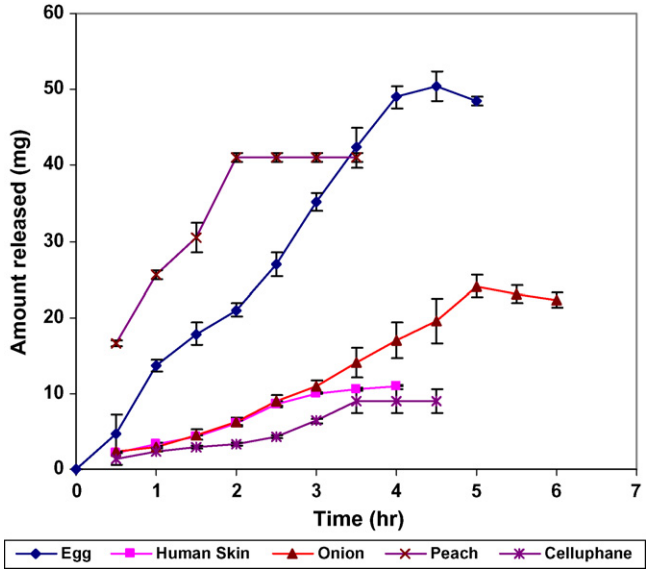

Fig. 3. Permeation profiles of erythromycin through different membranes.

Table 2
Estimated diffusion models for permeation of drugs according to Peppas equation

Drug	Membrane					
	Onion	Egg	Peach	Tomato	Cellophane	HS
Metronidazole	NF	F	NF	F	NF	NF
Diclofenac Na	NF	NF	NF	NF	NF	F
Erythromycin	NF	NF	NF		NF	NF

F, Fickian; NF, non-Fickian.

For diclofenac, diffusion mechanisms are the same in membranes except for the human skin but for erythromycin, diffusion takes place in a similar way through all skins studied. Also we can see that onion, peach, cellophane and human skin are similar in view of metronidazole and erythromycin permeation. But as mentioned earlier, diffusion mechanism for diclofenac transport in human skin is different from all other membranes studied even for cellophane. This similarities and dissimilarities may be related to solubility of the drug molecules, so in this case metronidazole and erythromycin are lipophilic drugs whereas diclofenac sodium is a hydrophilic drug. However by considering to the permeability of diclofenac in Table 1, it can be seen that human skin, cellophane and in some extent onion have the same effects on drug permeation.

Table 3 shows the permeability of each drug from the membranes and Table 4 indicate the significant level of difference ($p = 0.05$) from the post hoc test of ANOVA for each membrane and drug.

As indicated in Table 4, onion, tomato, egg and human skin are similar in view of amount and rate of diclofenac permeated through them. Also between tomato and cellophane was not significantly different ($p > 0.35$) for permeation of diclofenac. Permeation of metronidazole through onion membrane and tomato skin were not significantly different from human skin ($p > 0.53$ and 0.38, respectively). All membranes were significantly different from each other ($p < 0.0001$) for permeation of erythromycin as a relatively large (MW = 733.94 g/mol) and lipohilic molecule through human skin and membranes. Permeation of diclofenac through peach skin and metronidazole through onion, peach, cellophane and human skin and erythromycin through onion, peach, egg, cellophane and human skin followed zero order kinetic and Fick's diffusion law. Diffusion of diclofenac through onion, tomato, egg, cellophane, human skin and metronidazole through egg and tomato membrane followed Higuchi law. Statistical analysis of permeability coefficients of drugs through each membrane by ANOVA test showed the most similarity between onion and human skin for diclofenac, tomato and human skin for metronidazole, onion and cellophane for erythromycin.

Table 3
Calculated permeability (cm/h) of drugs from each membrane

Permeability (cm/h) membrane	Metronidazole ($n = 3$), MW = 171.2	Diclofenac Na ($n = 3$), MW = 318.13	Erythromycin ($n = 3$), MW = 733.94
Onion	11.7e−4 ± 2.6e−4	8.9e−4 ± 1.8e−4	15.8e−4 ± 4.3e−4
Egg	88.2e−4 ± 3.16e−4	98.1e−4 ± 12.1e−4	36.8e−4 ± 1.5e−4
Peach	57.3e−4 ± 5.71e−4	354.7e−4 ± 7.3e−4	50.1e−4 ± 2.7e−4
Tomato	69.1e−4 ± 1.5e−4	54.4e−4 ± 9.5e−4	
Cellophane	511.9e−4 ± 11.3e−4	351.1e−4 ± 89.7e−4	7.2e−4 ± 1.23e−4
HS	2.7e−4 ± 1.1e−5	6.8e−4 ± 1.5e−4	89.5e−4 ± 3.43e−4

Table 4
p-Values calculated from ANOVA test for each drug and membrane comparison

	Onion	Egg	Peach	Tomato	Cellophane	HS
Onion		Er: 0.000; M: 0.000; D: 0.079	Er: 0.000; M: 0.261; D: 0.001	Er: –; M: 0.377; D: 0.171	Er: 0.021; M: 0.001; D: 0.285	Er: 0.000; M: 0.53; D: 0.925
Egg	Er: 0.000; M: 0.000; D: 0.079		Er: 0.002; M: 0.046; D: 0.001	Er: –; M: 0.001; D: 0.129	ER: 0.000; M: 0.001; D: 0.435	Er: 0.000; M: 0.003; D: 0.076
Peach	Er: 0.000; M: 0.026; D: 0.001	Er: 0.002; M: 0.048; D: 0.001		Er: –; M: 0.036; D: 0.000	Er: 0.000; M: 0.000; D: 0.9999!	Er: 0.000; M: 0.043; D: 0.001
Tomato	Er: –; M: 0.377; D: 0.171	Er: –; M: 0.001; D: 0.129	Er: –; M: 0.036; D: 0.000		Er: –; M: 0.002; D: 0.347	Er: –; M: 0.308; D: 0.157
Cellophane	Er: 0.021; M: 0.001; D: 0.285	Er: 0.000; M: 0.001; D: 0.435	Er: 0.000; M: 0.000; D: 0.999!	Er: –; M: 0.002; D: 0.347		Er: 0.000; M: 0.002; D: 0.282
HS	Er: 0.000; M: 0.053; D: 0.925	Er: 0.000; M: 0.003; D: 0.076	Er: 0.000; M: 0.043; D: 0.001	Er: –; M: 0.308; D: 0.157	Er: 0.000; M: 0.002; D: 0.284	

Er, erythromycin; M, metronidazole; D, diclofenac Na.

4. Conclusion

From the results obtained, can be concluded that natural membranes used, have pores and channels with hydrophilic properties which permeates small to middles size hydrophilic drugs to diffuse in a manner similar to human skin, and because of its availability can be used in in vitro diffusion studies. Besides of this, these membranes can be used in controlled drug delivery in a natural manner.

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