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Simulation of skin permeability in chitosan membranes

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Abstract

To provide an alternative means of evaluating transdermal drug delivery systems, membranes of chitosan were developed. The membranes were prepared by cast-drying method. The effects of chitosan concentration, sodium tripolyphosphate (NaTPP) concentration and crosslinking (CL) time on flux and lag time were studied using central composite design. It was observed that chitosan membrane at a particular composition simulated the permeation of diclofenac sodium through rat skin The mathematical model developed in the present study can be used to simulate the permeation of drugs through different species of animal skins. © 2001 Published by Elsevier Science B.V.

Keywords: Simulated skin; Chitosan membrane; Percutaneous permeation; Central composite design

1. Introduction

Transdermal drug delivery systems have been recognized as an interesting way to provide controlled delivery of drugs to the systemic circulation. Evaluation of these systems in vivo using human beings is difficult from the viewpoint of cost, time consumption and ethical restrictions. An alternative to in vivo studies is in vitro experiments with excised skin (human cadaver, animal). The animal skin differ significantly from human skin due to difference in thickness, nature of stratum corneum, density of hair follicles and sweat glands (Barry, 1983). Hatanaka et al., (1994) have reported various causes for differences in the in vitro skin permeation and in vivo percutaneous absorption of drugs. In addition, the content of skin microconstituents in the normal skin differs from that of the diseased skin (Nardo et al., 1998). 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 artificial skin. Simulated membranes offer several advantages over biological membranes in terms of ease of preparation, controlled composition and reproducibility (Feldstein et al., 1998). In the present study, the aim was to study critical variables affecting the preparation of chitosan membranes and permeability of these simulated skins using statistical experimental design. The objective behind the work was to formulate an artificial skin that is capable of simulating permeation of drugs through rat skin.

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2. Materials and methods

2.1. Materials

Chitosan-degree of deacetylation-95% (Central Institute of Fisheries Technology, Cochin), diclofenac sodium (Crystals Pharmaceuticals, Ambala), sodium tripolyphosphate (NaTPP) (Loba Chemie Pvt. Ltd., Bombay).

2.2. Methods

2.2.1. Preparation and conditioning of rat skin

The dorsal skin of albino Wistar rats were shaved with mechanical hair clipper and subsequently, depilatory cream was applied to remove small hair. The animal was sacrificed after 24 h and the skin was excised. After removing the adhering fat, visceral debris and washing with physiological saline, the whole skin was clamped on Franz diffusion-cell with stratum corneum facing the donor compartment. The skin was stabilized for 4 h (by stirring pH 7.4 buffer in receptor compartment) before in vitro permeability study.

2.2.2. Preparation and crosslinking of chitosan membranes

Simulated skin membranes were prepared by modifying the method reported by Remunan-Lopez and Bodmeier, 1997. Solution of chitosan (2–5% w/v) was prepared in 10 mM acetic acid (2% v/v) solution. The solution was homogenized (Teflon pestle, 2000 rpm) and filtered through cheesecloth to remove debris. The solution (50 ml) was degassed and membranes were casted on polycarbonate petridish (cross-sectional area 50.286 cm^2) to achieve thickness close to full thickness of hairless rat skin. The dried membranes (60°C for 40 h) were stored in polyethylene bags till use.

Dried chitosan membranes were crosslinked with NaTPP (10 ml of 3–12% w/v for 3–40 min). These membranes were washed three times with water to remove excess of NaTPP. Freshly crosslinked membranes that retained flexibility and integrity for more than 24 h were used for in vitro permeation study.

2.2.3. Experimental design

Chitosan membranes were formulated according to the central composite design to study the effect of independent variables on the permeation parameters of diclofenac sodium (model drug). To evaluate three factors (n) at two levels (k), the central composite design consisted of eight batches (IF–8F), k^n, on factorial points; six batches (IS–6S), $2n$, on axial points and four replicates (IC–4C) at the center point.

2.2.4. In vitro permeability study

Vertical Franz diffusion-cell apparatus was used to study the permeability of diclofenac sodium employing infinite dose technique (Shah et al., 1994). The apparatus assembly consisted of clamped preconditioned rat skin or crosslinked chitosan membrane onto glass diffusion-cell (3.63 cm^2) between donor and receptor compartments. In the receptor compartment (600 rpm, 37°C),phosphate buffer pH 7.4 (25 ml) contained formaldehyde (0. 1% w/v of 37–41% v/v solution) to prevent microbial growth (Michniak et al., 1994).The donor compartment contained saturated solution of diclofenac sodium in phosphate buffer pH 7.4. The aliquots (1 ml) withdrawn at various intervals were immediately analyzed for drug concentration spectrophotometrically (276 nm), directly or after appropriate dilution with phosphate buffer. The aliquot withdrawn was replenished with equal amount of phosphate buffer pH 7.4.

3. Results

Preliminary studies were carried out to prepare chitosan membranes that could maintain its integrity for at least 24 h during diffusion studies. It was observed that drying temperature, concentration of chitosan, concentration of NaTPP and crosslinking (CL) time affects the properties of membrane.

Although Lim et al., (1999) reported that temperature above 80°C changes the physical properties of chitosan, in the present study temperature above 60°C resulted in non-uniform membrane. Thickness close to rat skin was obtained upon

crosslinking with NaTPP, when the membrane was prepared using 3.5% (w/v) chitosan solution. However, the thickness of membrane varied depending upon the concentration of crosslinking agent.

To study the effect of concentration of chitosan, NaTPP and CL time on the permeation, crosslinked chitosan membranes were formulated according to the central composite design (Table 1). The permeation parameters, i.e. flux and lag time were calculated from the linear portion of the graph (cumulative amount of diclofenac sodium permeated versus time) and the intercept of the extrapolated linearity on the time axis, respectively. To determine the magnitude of contribution of different factors towards lag time and flux, multiple linear regression analysis was performed. The effect of coefficients on flux and lag time is shown in Figs. 1 and 2. The correlation of factors with lag time was found to improve after omitting the second order terms associated with significant coefficients, i.e. b4 and b7, whereas the correlation of factors with flux was found to improve after omitting the interaction term associated with insignificant coefficients, i.e. b7 and b8. Further, on comparison of flux and lag time of rat dorsal-skin, batch 3S and IF of chitosan membranes, respectively, exhibited minimum difference. The model, developed from multiple linear regression, to estimate flux (Y) can be represented mathematically as

$$Y = 4.817 - 0.466X_1 - 0.342X_2 - 0.087X_3 - 0.318X_1^2 + 0.318X_2^2 + 0.387X_3^2 + 0.012X_1X_2 - 0.072X_2X_3 - 0.108X_1X_3$$

Where, X_1 = concentration of chitosan; X_2 = concentration of NaTPP; X_3 = CL time.

Analysis of variance (ANOVA) was applied (Table 2) to study the fitting and significance of the mathematical model to estimate flux. The point to point correlation between cumulative permeation of diclofenac sodium through rat skin and batch 3S is shown in Fig. 3.

Table 1
Formulation of chitosan membrane and permeation parameters[a]

Batch number	X_1 (% w/v)	X_2 (% w/v)	X_3 (min)	Flux $\times 10^{-5} \pm$ S.D (mg cm^{-2} s^{-1})	Thickness $\times 10^{-5} \pm$ S.D. (cm)	Lag time $\times 60 \pm$ S.D. (s)
IF	−1 (3)	−1 (5)	−1 (10)	6.948 ± 0.016	0.650 ± 0.010	5.881 ± 0.031
2F	−1 (3)	−1 (5)	1 (30)	7.285 ± 0.023	0.693 ± 0.006	4.664 ± 0.014
3F	−1 (3)	1 (10)	−1 (10)	6.596 ± 0.004	0.723 ± 0.006	8.114 ± 0.011
4F	−1 (3)	1 (10)	1 (30)	6.206 ± 0.015	0.743 ± 0.006	6.658 ± 0.013
5F	1 (4)	−1 (5)	−1 (10)	6.290 ± 0.008	0.823 ± 0.006	3.650 ± 0.003
6F	1 (4)	−1 (5)	1 (30)	5.755 ± 0.008	0.837 ± 0.006	10.213 ± 0.025
7F	1 (4)	1 (10)	−1 (10)	5.548 ± 0.019	0.857 ± 0.006	6.621 ± 0.009
8F	1 (4)	1 (10)	1 (30)	5.165 ± 0.004	0.857 ± 0.006	6.756 ± 0.004
IS	−1.68 (4.34)	0 (7.5)	0 (20)	4.553 ± 0.012	0.923 ± 0.006	10.286 ± 0.016
2S	1.68 (2.66)	0 (7.5)	0 (20)	5.793 ± 0.015	0.703 ± 0.006	5.707 ± 0.007
3S	0 (3.5)	−1.68 (11.7)	0(20)	4.607 ± 0.005	0.867 ± 0.006	10.166 ± 0.011
4S	0 (3.5)	1.68 (3.3)	0 (20)	5.739 ± 0.016	0.683 ± 0.006	5.440 ± 0.009
5S	0 (3.5)	0 (7.5)	−1.68 (36.8)	5.303 ± 0.001	0.840 ± 0.006	7.186 ± 0.003
6S	0 (3.5)	0 (7.5)	1.68 (3.2)	5.433 ± 0.001	0.797 ± 0.006	9.887 ± 0.004
IC	0 (3.5)	0 (7.5)	0 (20)	4.844 ± 0.005	0.833 ± 0.006	7.299 ± 0.009
2C	0 (3.5)	0 (7.5)	0 (20)	4.867 ± 0.012	0.833 ± 0.006	7.075 ± 0.012
3C	0 (3.5)	0 (7.5)	0 (20)	4.875 ± 0.001	0.837 ± 0.006	8.289 ± 0.027
4C	0 (3.5)	0 (7.5)	0 (20)	4.875 ± 0.010	0.833 ± 0.006	5.746 ± 0.004
Rat skin	–	–	–	4.660 ± 0.012	0.710 ± 0.006	5.991 ± 0.007

[a] F, factorial points; S, star points; C, center points. X_1, concentration of chitosan; X_2, concentration of NaTPP; X_3, CL time. The values in brackets besides the transformed values of factor X_1, X_2 and X_3, represent real values.

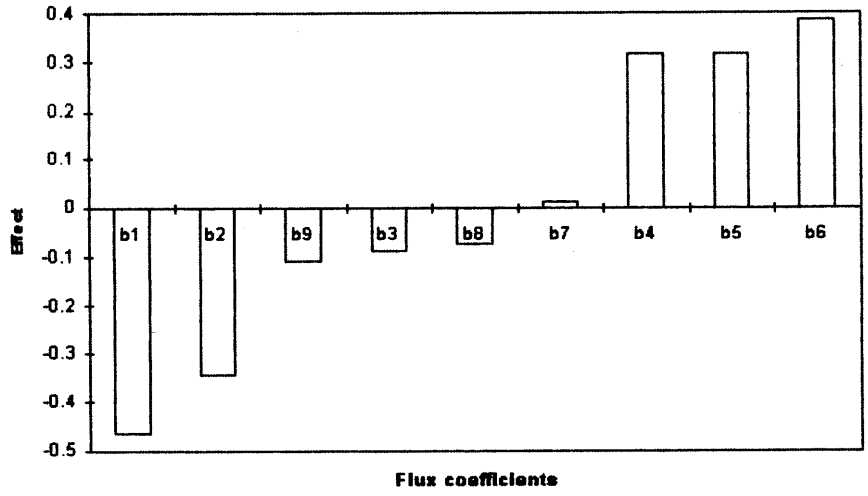

Fig. 1. Effect plot of coefficients of flux (bl, b2, b3 = coefficients of main effects; b4, b5, b6 = coefficients of square terms; b7, b8, b9 = coefficients of interaction terms).

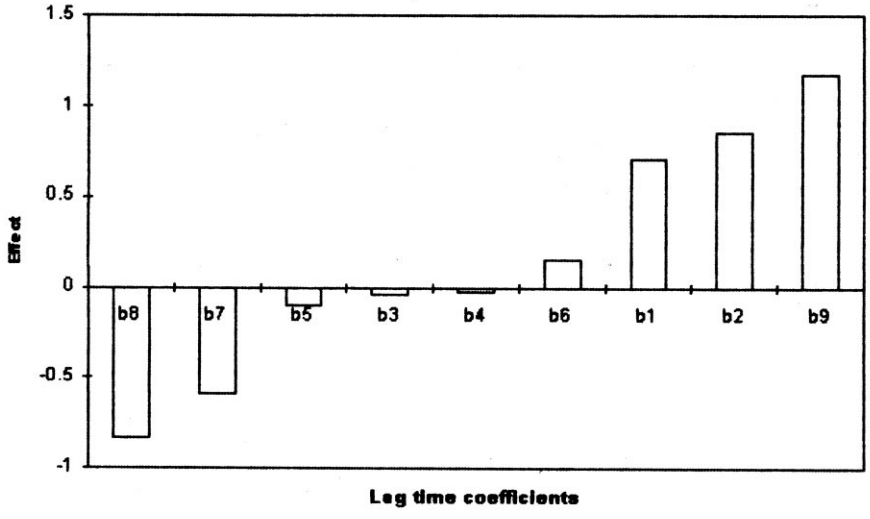

Fig. 2. Effect plot of coefficients of lag time (bl, b2, b3 = coefficients of main effects; b4, b5, b6 = coefficients of square terms; b7, b8, b9 = coefficients of interaction terms).

Table 2
ANOVA of the regression (Flux)

	Degree of freedom	Sum of squares	Mean square	*F*	*F*-significance
Total	17	11.122			
Regression	6	7.826	1.304	4.354	0.017
Residual	11	3.296	0.300		

4. Discussion

Central composite design was used to study the effect of critical variables on the permeation characteristics of cross-linked chitosan membranes. The real values of the factors were transformed to facilitate orthogonality of results and easy calculations.

From Table 1, it was observed that membrane thickness increases at high level of chitosan concentration. During preliminary studies, when concentration of NaTPP was more than 5% (w/v) and CL time was more than 10 min, the swelling of chitosan membranes was insignificant ($< 10\%$) in phosphate buffer pH 7.4.

Results show that lag time decreases with increase in the concentration of chitosan at low value of CL time and low level of NaTPP except at high concentration of chitosan and CL time. Also lag time decreases at high level of CL time except at high concentration of chitosan. The interaction of chitosan concentration–CL time has maximum effect on lag time, whereas square of chitosan concentration has minimum effect. The CL time, interaction of NaTPP–CL time and interaction of chitosan–NaTPP concentration has inverse relation with lag time. Interaction of chitosan concentration–CL time, concentration of NaTPP, concentration of chitosan and square of CL time has direct relationship with lag time.

Flux of diclofenac sodium increased at low level of chitosan concentration with decrease in CL time. However, at low concentration of chitosan and NaTPP, flux was found to increase with CL time. The effect of chitosan concentration is maximum on flux, whereas the interaction of chitosan–NaTPP is insignificant. The chitosan concentration, NaTPP concentration and interaction of chitosan concentration–CL time are inversely related to flux, whereas square of CL time, interaction of NaTPP–CL time is directly related to flux. The multiple regression data show that square terms should be retained in the mathematical model to explain the curvature of flux-response. The ratio $F = 4.354$ shows regression to be significant. The estimated model, therefore, may be used as response surface for flux.

The permeation of diclofenac sodium from chitosan membranes was compared with that across dorsal skin of Wistar rats. It was observed that batch 3S simulated the permeation of diclofenac sodium through rat skin. A good correlation ($R^2 = 0.989$) was found for permeation of diclofenac sodium between chitosan membrane of batch 3S and rat skin. It appears that chitosan membrane simulates flux of diclofenac sodium across

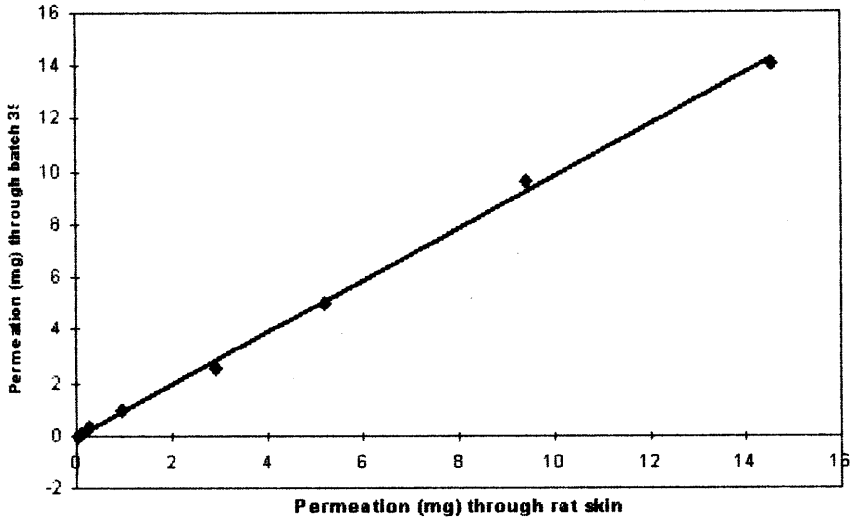

Fig. 3. In vitro permeation profile of diclofenac sodium through rat skin versus batch 3S.

rat skin, although the lag time is more than that of rat skin. The increased lag time can be attributed to the difference in thickness of chitosan membrane (batch 3S) and rat skin. However, chitosan concentration and CL time needs to be optimized to obtain desired lag time. Alternatively, composite membrane can be designed because chitosan membrane of batch 3S simulates flux, whereas batch 1F simulates lag time. To match permeation through rat skin, the top layer of composite membrane may consist of batch 1F and bottom layer of composite membrane may consist of batch 3S.

5. Conclusions

The unquestionable feasibility of transdermal drug therapy has been recognized during the past decade and this therapeutic system has become established as an effective alternative to other dosage forms. However, the growing concern for the restricted use of animals for research work may delay the development of these dosage forms. Under these circumstances, the characterized polymeric membranes can be used as skin imitating barrier in the evaluation during developmental stages of transdermal drug delivery systems and routine quality control tests of these drug delivery systems. Although chitosan membrane (batch 3S) was found to simulate the permeation of diclofenac sodium through rat skin in the present study, trials with drugs with different lipophilicity are advocated.

It is proposed that similarly derived mathematical models can be used for the estimation of drug permeation through other species of animal skins. Retrospectively, this model can be used to design chitosan membranes to simulate the permeation of drugs through different species of animal skins.

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References

Barry, B.W., 1983. Methods for studying percutaneous absorption. In: Dermatological Formulations. Percutaneous Absorption. Marcel Dekker Inc, New York, pp. 138–150.

Feldstein, M.M., Raigorodskii, I.M., lordanskii, A.L., Hadgraft, J., 1998. Modeling of percutaneous drug transport in vitro using skin-imitating carbosil membrane. J. Control. Rel. 52, 25–40.

Hatanaka, T., Katayama, K., Koizumi, T., Sugibayashi, K., Morimoto, Y., 1994. In vitro-in vivo correlation of percutaneous absorption: Isosorbide dinitrate and morphine hydrochloride. Biol. Pharm. Bull. 17, 826–830.

Lim, L.Y., Khor, E., Ling, C.E., 1999. Effects of dry heat and saturated steam on the physical properties of chitosan. J. Biomed. Mater. Res. 48, 111–116.

Michniak, B.B., Player, M.R., Fuhrman, L.C., Christensen, C.A., Chapman, J.M., Sowell, J.W., 1994. In vitro evaluation of a series of azone analogs as dermal penetration enhancers III. Acyclic amides. Int. J. Pharm. 110, 231–239.

Nardo, A.D., Wertz, P., Giannetti, A., Seidenari, S., 1998. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. Acta Derm.-Venereol. 78, 27–30.

Remunan-Lopez, C.R., Bodmeier, R., 1997. Mechanical water uptake and permeability properties of crosslinked chitosan glutamate and alginate films. J. Control. Rel. 44, 215–225.

Shah, C., Kaka, L., Tenjarla, S., Lau, S.W.J., Chow, D., 1994. Analysis of percutaneous permeation data II. Evaluation of the lag time method. Int. J. Pharm. 109, 283–290.