Permeation Kinetics of Diclofenac Sodium from Pseudolatex Transdermal Formulations through Lipidized and Delipidized Mouse Skin

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A transdermal drug delivery system of diclofenac sodium was developed for prolonged and controlled release of drug. The designed system essentially based on polymeric dispersion system. To achieve the desired release rate, different combinations of hydrophilic and hydrophobic polymers were used for the preparation of pseudolatex systems. The permeation kinetics of diclofenac sodium from transdermal system through hairless delipidized and lipidized mouse skin was done. The permeation profile and the related kinetic parameters of diclofenac sodium alone and in presence of an enhancer isopropyl myristate at different concentrations were studied. The observed permeation flux, permeation coefficient found to increase in presence of enhancer isopropyl myristate. The effect was found to be the maximum with isopropyl myristate at a concentration of 10 percent. The more pronounced enhancing effect of isopropyl myristate regarding permeability flux, permeation coefficient, diffusion coefficient was attributed with solubility parameter being nearer to the skin lipid solubility parameter and probably due to its passage across the skin barrier through the lipid pathway.

Scores of literature are available for permeation profile of drugs through various types of animal skin. However, information about drug permeation through human skin is scarce and does not follow the same pattern as animal skin[1]. However, various animal skins have been used as models for prediction of drug permeation through human skin[2,3]. Diclofenac sodium (DS) is a potent nonsteroidal antiinflammatory drug. It is used for the treatment of rheumatoid arthritis and other rheumatic disorders[4]. It possesses a narrow therapeutic index due to short biological half-life[5]. Diclofenac sodium is a weak base therefore it exists in the cationic form at the pH of human skin i.e. 4.2 to 5.6[6] and hence permeation through the skin is expected to be subtherapeutic under this conditions. Therefore permeation enhancers are required to be combined for higher permeation rate.

There are report describing the use of Eudragit RL and RS pseudolatex types of transdermal or otherwise for controlled released of drug[7]. Pseudolatex may be easily prepared by the solvent change technique, which consists of dissolving the polymer in a water miscible organic solvent or in a mixed water-miscible organic solvent system followed by dispersion in deionized water with the help of agitation. The organic solvent is then removed to leave stable latex. Once the product is dried pseudolatex is obtained. This property of the pseudolatex films gives it a character of not either too extremely hydrophobic or extremely hydrophilic. In the present study pseudolatex patches containing DS have been developed alone with different concentration of isopropyl myristate (IPM) i.e. 5, 7.5, 10 percentage as permeation enhancers. Further, the effect of different concentrations of permeation enhancer on the permeation profile and release kinetic parameters of DS alone and in presence of IPM have been reported.

Diclofenac sodium was obtained from M/s. Cipla Ltd., Mumbai, Eudragit NE-30D, Eudragit RS 100, RL 100 have been procured from M/s. Rohm Pharma, Germany and dibutyl phthalate was supplied by M/s. C.D.H. Pvt. Ltd., All other reagents used were of analytical grade.

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The partition coefficient was determined between n-octanol and phosphate buffer solution of pH 7.2 at ambient temperature 37±1° following the method of Leo *et al.*[8] Equal volumes (10 ml) of phosphate buffer solution pH 7.2 and n-octanol are added to 10 mg of accurately weighed drug in a glass stoppered tube. 10 mg of drug was accurately weighed and added to this solvent mixture. The mixture was shaken for 24 h at room temperature with the help of test tube shaker (wrist action shaker). The drug concentrations in aqueous and n-octanol phase were spectrophotometrically estimated at 277 nm[9]. The partition coefficients were then calculated using the equation[10], DC=(Ca–Cb)Vw/CbVo, where DC is the distribution coefficient, Ca and Cb represent the drug concentration in the aqueous layer at the beginning and at equilibrium, respectively, Vw and Vo represent the volume of aqueous layer and n-octanol layer, respectively.

The partitioning of the drug between saline phosphate buffer pH 7.2 and skin epidermis was determined by the method reported by Valia *et. Al.*[11] and Tajo *et al.*[12]. A piece of whole excised albino mouse skin was weighed accurately (90~100) mg and put into glass stopper test tube containing a 10 ml solution of the drug in saline phosphate buffer. The pieces of skin were equilibrated with buffer for 24 h at 37±1°. The solution was filtered and spectrophotometrically estimated at 277nm. The partition coefficient of DS was calculated as described by the equation[13],

Partition coefficient=Cs–CeqX1000/Ceq.We, where Cs, Ceq and We are the initial and equilibrium concentrations of DS in the buffer and weight of epidermis, respectively. Dry weight of the epidermis was considered for calculating the partition coefficient.

Backing membrane[14] was prepared using Eudragit NE 30D suspension after dried at room temperature of 25±5°. The film thus obtained was crushed into pieces. Two hundred and fifty milligrams of polymer was dissolved in 5 ml of acetone and was poured within an aluminum foil of area 16 cm^2 and dried overnight at room temperature. The dryness of the films was monitored by visually observing the transparency of these films.

The films were prepared using 7 ml of solutions containing 50 mg of drug and different combinations of the polymer (Eudragit RL 100 and RS100) by dissolving in a mixture of acetone and buffer solution pH 7.2, viz.1:0, 1:1, 1:2, 1:3, 2:3 caste over backing membrane. Dibutyl phthalate was used as a plasticising agent. The casted films were dried at controlled condition. The dried films were stored in a desiccator. Films containing different concentrations of IPM as permeation enhancer were prepared in a similar manner. To achieve an effective plasma concentration of DS (2 mg/h) the required absorption rate through the skin was calculated to be 0.178 mg/h. The absorption rate was calculated using

TABLE 1: PERMEATION OF DS THROUGH MOUSE EPIDERMIS

Skin type	IPM %	J (mg/cm min)	t_Lh	D×10^{-9} cm^2/sec	P×10^{-4}cm/sec	K	E
Epidermis	Control	0.353	1.75	9.09±0.65	14.2±0.42	2.9	1
	10%	0.741	1.25	12.7±0.72	29.8±0.58	4.35	2.1
	7.5%	0.617	1.35	11.8±0.95	24.9±0.65	3.91	1.7
	5%	0.529	1.60	9.94±0.58	21.3±0.73	3.98	1.5
Delipidized Epidermis	Control	0.388	1.70	7.19±0.45	15.6±0.91	3.53	1
	10%	1.047	1.00	12.2±0.69	42.2±0.53	5.61	2.7
	7.5%	0.834	1.20	10.2±0.85	33.6±0.61	5.36	2.1
	5%	0.659	1.50	8.20±0.81	26.6±0.43	5.30	1.7

J stands for permeation flux, t_lh represents lag time, D is diffusion coefficient, P stands permeability coefficient, K is the calculated partition coefficient and E is the enhancement factor of diclofenac sodium form various formulation containing different concentration of permeation enhancer IPM (isopropyl myristate) from lipilized and delipilized mouse epidermis. Standard deviation is in parentheses, n=6, P and D were calculated from individual steady state flux in fig. 1 and 2. K calculated from the relation K=Ph/D, where, h is the thickness of the barrier. Control films were prepared with 2:3 proportion of Eudragit RL-100 and RS-100.

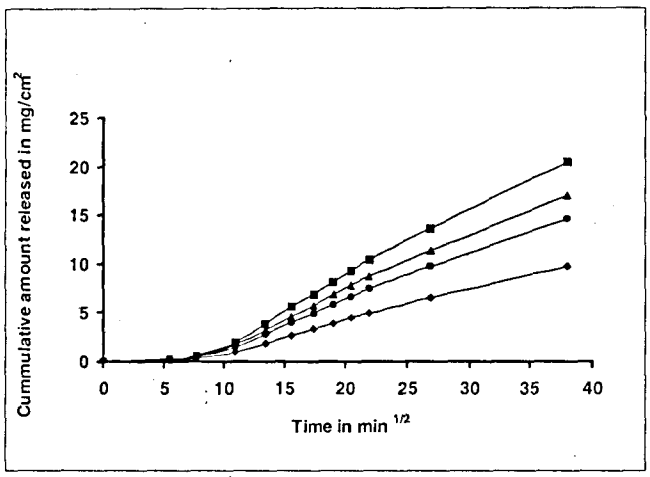

Fig. 1: Permeation profile of diclofenac sodium from various formulations through lipidized mouse epidermis

Permeation profile of DS from various formulation containing different concentrations of permeation enhancer IPM.viz. Control (-◆-); IPM 10 % (-◘-); IPM 7.5 % (-▲-) and IPM 5 % (-●-)

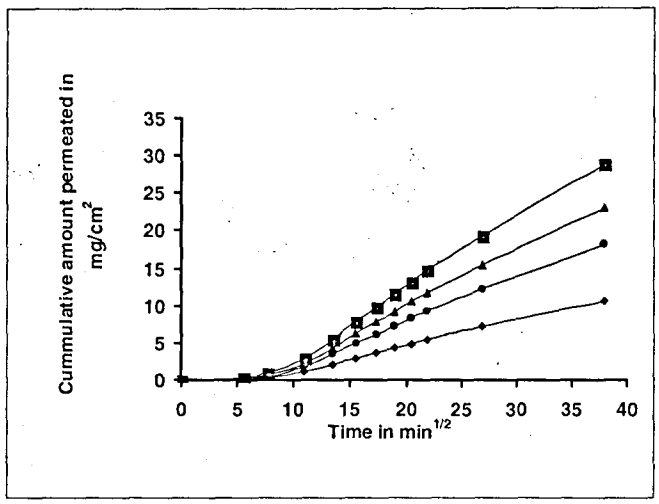

Fig. 2: Permeation profile of diclofenac sodium from various formulations through delipidized mouse epidermis

Permeation profile of DS from various formulation containing different concentrations of permeation enhancer IPM.viz. Control (-◆-); IPM 10 % (-◘-); IPM 7.5 % (-▲-) and IPM 5 % (-●--).

the pharmacokinetic parameters in the equation suggested by Guy *et al.*[15].

The epidermal thickness was measured microscopically from 5 μm thickness microtomed sections after staining with haematoxylineosin. The thickness was found to be 1.853×10^{-2} cm±0.12 for lipidized skin and 1.625×10^{-2}±0.21 cm for delipidized skin from three measurements. A saturated DS solution in phosphate saline buffer of pH 7.2 was prepared by equilibrating the excess DS with the vehicle for 24 h. The temperature of the solution was maintained at 37±1° using a circulating water bath. The sample was filtered and appropriately diluted for estimation of saturation solubility of DS. The DS concentration was found to be 4.135 mg/ml.

Pretreated abdominal skin of albino mice was used in the Keshary-Chein diffusion cell. Hair from the abdominal region was carefully removed by means of fine forceps, and the full thickness skin was taken out, and then trimmed to remove the adherent fatty material. The skin sample was then examined microscopically to ensure its integrity[16].

The film sample was fixed on the skin sample previously fixed in between the donor and receptor compartment of Keshary-Chein cell. Both the effective skin area and the area of the film sample placed on the skin were 3.14 cm^2. But in total, a slightly large size of skin was taken to help its fixation on the slot of the cell assembly. Each patch contains 15.9 mg of drug in it. The receptor compartment was filled with phosphate saline buffer, pH 7.2. The temperature of the elution medium was thermostatically controlled at 37±1° by a surrounding water jacket and the medium was stirred magnet at 500 rpm, using a magnetic stirrer[17]. Aliquots withdrawn at predetermined intervals over 24 h. were spectrophotometrically estimated at 277 nm. Blanks were run for each set as described above without using films and calculated accordingly. Films containing different concentrations of permeation enhancer were studied in the same manner and calculated accordingly.

The permeability coefficient (P) was calculated using the relation derived from Fick's first law [18], P=J.h/C, where J is the steady state permeation flux and C is the initial concentration. Diffusion coefficient was calculated using the relation derived from Fick's second law[19], D=h2/6L, where h is the thickness of the skin and L is the lag time.

The epidermal flux of lipidized and delipidized albino mice skin was determined from Fick's law. The stable state flux was determined from the slope of the linear portion of the cumulative amount release, square root of time plot. The lag time was determined by extrapolating the linear portion of Q *Vs* $t^{1/2}$ curve to the abscissa. The enhancement factor (E)[20] was calculated as the ratio of the permeation rate of DS from the formulation with enhancer to that from the controlled system without enhancer. Table 1 summarizes the

permeation flux, lag time, diffusion coefficient, permeability coefficient, enhancement factor, calculated partition coefficient. The permeation flux was highest with 10 % IPM as enhancer both with whole and delipidized epidermis. The calculated partition coefficient was also highest with 10 % IPM as enhancer. It appears from the calculated partition coefficient that the epidermal permeability of DS had been altered. The passage of solution through the skin follows two main routes i.e. polar route, associated with protein component of the epidermis and non polar route, which is related with the lipid component of skin[21]. Varying partition coefficient of DS in presence of different concentrations of enhancer used implies that the solubility of the drug in the epidermis had altered. Therefore, it may be assumed that the enhancer used acts through the non polar route to increase the passage of DS transport across the barrier. The major rate limiting factor for the permeation of many drugs through epidermis is lipid and the removal of it is known to reduce the barrier property of the epidermis[22]. Permeation of DS through delipidized epidermis proves that the removal of lipids increases the passage of drugs through skin. Partition coefficient was calculated using the relation K=Ph/D, where, h is the thickness of the barrier, P is the permeability coefficient and D is the diffusion coefficient. The calculated partition coefficient was in agreement with the above. There is no significant difference in lag time both in lipidized and delipidized epidermis. The presence of enhancer increases the enhancement factor up to 2.0 in case of lipidized skin where as it was 2.70 in case of delipidized epidermis, probably due to loss of barrier properties. When the diffusion coefficient with enhancer impregnated epidermis and delipidized epidermis was compared it was seen that the diffusion coefficient value was highest for 10 percentage IPM with delipidized epidermis suggesting the reduction of resistance to the diffusion of DS. This result reaffirm that DS permeation through skin took the lipid pathways and 10 % IPM was proved to be the best amongst the three different enhancer concentrations used. The solubility parameter of the lipid of biological membrane is 8.7±1.03 as reported[23] is very near to the solubility parameter of 10 % IPM i.e. 8.02 and this helps in greater permeation through both the normal and delipidized epidermis[24].

From the above result the formulations prepare with 10 % IPM as enhancer was found to be most satisfactory. It follows the first order kinetics over a period of 24 h. The permeation kinetic profile indicates that with increase in the enhancer concentration the diffusion coefficient, permeability coefficient and enhancement factor increases. Thus the controlled release rate of DS can be achieved by altering the enhancer concentration in the formulation.

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