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Effect of some enhancers on the permeation of haloperidol through rat skin in vitro

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Abstract

The objective of this work is to enhance the permeation of haloperidol through the rat skin in vitro by using various enhancers at a concentration of 1 mg/ml in the saturated drug solution and analysing the dose-dependent diffusion profile for the enhancers which significantly increased permeation at this concentration compared with the control. Enhancers belonging to various chemical classes like the vitamins (ascorbic acid), surfactants (cetrimide, polysorbate 20), sulfoxides (dimethyl sulfoxide), glycols (polyethylene glycol 400, propylene glycol) and amides (urea) were used. Amber glass Franz-type diffusion cells were used for the permeation studies and haloperidol was made soluble in aqueous solution with the aid of lactic acid. Ascorbic acid and cetrimide increased flux and permeability coefficient significantly. From the dose-dependent permeation studies, it was concluded that ascorbic acid enhanced the permeation by increasing the solubility of the drug in the vehicle thus providing a high concentration gradient across the skin, whereas cetrimide enhanced the permeation by increasing the thermodynamic activity which may be due to solubilization of skin lipids by micelles. Polysorbate 20 decreased the enhancer index by decreasing the thermodynamic activity. None of the enhancers changed the lag time except for urea which decreased the lag time probably by its binding with keratin. Dimethyl sulfoxide, polyethylene glycol 400 and propylene glycol did not have a significant effect on haloperidol permeation compared with control.

Keywords: Transdermal; Haloperidol; Penetration enhancers; Rat skin

1. Introduction

Haloperidol (HP), a butyrophenone derivative, is widely prescribed to treat mania, aggressiveness, agitated states, hallucinations associated with acute and chronic psychosis including schizophrenia and psychotic reactions in adults with organic brain damage (Daniels and Jorgensen, 1982). HP can be given up to a maximum oral dose of 200 mg daily in acute psychosis (Reynolds, 1996). Once the acute psychosis is resolved and the patient is free from overt psy-

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chotic symptoms, maintenance therapy is used to sustain the minimum therapeutic concentration, thereby avoiding relapse of the disease (Stimmel, 1992). In maintenance therapy, long-acting formulations are more meaningful since frequent dosing may lead to non-compliance. Haloperidol decanoate (HPD), the only long-acting formulation of its ester in oily injection, is available as a depot formulation in the market. HPD is stored in fat tissues and released slowly to plasma and hydrolysed to HP by esterases (Groves and Mandel, 1975).

A depot formulation has several advantages such as: (1) less frequent administration — an intramuscular injection of 50–100 mg of HPD is sufficient for 2–3 weeks; (2) improved patient compliance; (3) relapse rate is very low among the patients even after the discontinuation of treatment compared with oral therapy (Nyberg et al., 1997); (4) fewer extra pyramidal side effects (Pabis et al., 1996). However this formulation has some disadvantages, namely (1) oily injection can cause pain at the site of injection due to slow dispersion; (2) since esterases vary in individuals and with age, the rate of liberation of free drug from its prodrug varies from individual to individual (Cheng et al., 1998); (3) since HP and HPD have different pharmacokinetic parameters, a complex dosing schedule is required while converting from oral therapy to depot therapy (Toney et al., 1989); (4) depot formulations showed marked plasma concentration variations which are clinically undesirable (Tuninger and Levander, 1996). Because of these disadvantages with depot formulation, the development of alternative long-acting formulations could be beneficial.

Transdermal drug delivery offers many advantages such as sustained drug delivery, improved patient compliance, reduced side-effects, elimination of first-pass effect, interruption or termination of treatment when necessary, and so on (Kydonieus, 1987). So HP can be a good candidate for the development of transdermal dosage form because of its clinical need and suitable molecular weight (375.9) and maintenance dose (3–10 mg/day) (Reynolds, 1996). However, many drugs require some mechanism to enhance penetration through the excellent skin barrier. Chemical agents have been shown to enhance the penetration of drugs through the skin by either increasing the solubility of the drug in stratum corneum (SC) or disrupting the lipid matrix of SC or interacting with the intracellular protein (Williams and Barry, 1991). Since it was reported that HP concentration was at the sub-therapeutic level in the absence of enhancers in in vitro permeation studies through cadaver human skin, it would require penetration enhancement (Almirall et al., 1996). In the present study, we analysed the influence of enhancers belonging to various chemical classes like the vitamins (ascorbic acid), cationic surfactants (cetrimide), non-ionic surfactants (polysorbate 20), sulfoxides (dimethyl sulfoxide), glycols (polyethylene glycol 400 and propylene glycol) and amides (urea) on the permeation of HP through the rat skin in vitro. Those enhancers which increased permeation of HP significantly at a concentration of 1 mg/ml of donor solution, were tried at various concentrations to characterize dose-dependent permeation.

2. Materials and methods

2.1. Chemicals and reagents

Haloperidol, droperidol, DL-lactic acid, L-ascorbic acid, polyethylene glycol 400 (PEG 400), propylene glycol (PG), polysorbate 20, urea, antibiotic antimycotic solution (100 X) and sodium acetate trihydrate were purchased from Sigma, dimethyl sulfoxide (DMSO) from BDH (Poole, UK) and cetrimide from Chempure (Singapore). All other chemical reagents were of at least reagent grade and all materials were used as supplied. Sprague–Dawley (SD) male rats were obtained from the university Laboratory Animal Centre.

2.2. Analytical method

Drug concentrations were determined by reversed phase HPLC with a fixed wavelength UV detector at 254 nm. The HPLC system was equipped with a Waters model 510 pump; model 440 absorbance detector (Waters Chromatogra-

phy, MA) and Shimadzu C-R3A chromatopac printer (Shimadzu, Japan). Separation of compounds was carried out on a C_{18} ODS hypersil column (250×4 mm) with 5-μm particle size. (Hewlett Packard, Germany). Flow rate was 1 ml/min and injection volume was 10 μl.

Mobile phase consisted of acetate buffer (pH 5) and acetonitrile in the ratio of 40:60. Acetate buffer was prepared by dissolving 50 mM of sodium acetate trihydrate in 1 liter of Milli-Q water and pH was adjusted to 5 with glacial acetic acid. Drug concentrations in the receptor phase samples were interpolated from a calibration curve established by using droperidol as internal standard. Retention times of internal standard and drug were approximately 4.5 and 7.5 min, respectively. A series of standard samples was prepared by dissolving haloperidol and droperidol in 0.03% v/v lactic acid. Peak area ratios of the drug and internal standard were linearly related to the concentrations for samples containing 2–120 μg/ml. Calibration curves had a correlation coefficient of 0.999 ($n = 3$) and coefficients of variation at all concentration level ranged from 1.29 to 6.09%.

2.3. Solubility studies

An excess of haloperidol was added to 0.03% v/v lactic acid containing antibacterial antimycotic solution (1 in 100 dilution) with and without enhancers and amber coloured bottles were kept in a water bath on an immersible magnetic stirring bed at 32 ± 1°C. The samples were stirred with a magnetic bar for 36 h to ensure saturation and filtered through 0.45-μm Teflon syringe filter units using a gas tight syringe (Hamilton, Switzerland). To minimize oxidation of ascorbic acid, the lactic acid solution was degassed before adding the enhancer, the bottles were filled to the level of the neck and lids were tightly closed. Saturated drug concentrations were determined by HPLC in triplicate after appropriate dilution.

2.4. Preparation of the rat skin

Abdominal skin of SD rat, male, weighing around 250 g, was used for the permeation studies. The rat was sacrificed with ether and its abdominal fur was removed using clippers. Skin samples in full thickness were cut, removed and washed with water. Fat and connective tissues were carefully removed with a scalpel. Skin was observed for any damage through a magnifying lens. Thickness of the skin was measured by micrometer and was in the range of 0.3 ± 0.1 mm.

2.5. Permeation studies

Amber glass Franz-type diffusion cells were used for permeation studies. The receptor compartment was filled with 5.9 ml of 0.03% v/v lactic acid solution containing antibacterial antimycotic solution (1 in 100 dilution). To both donor and receptor compartment solutions, antibacterial antimycotic solution was added to maintain the integrity of the skin throughout the experiment and minimize microbial contamination in samples during analysis. Freshly excised full thickness rat skin was mounted between donor and receptor compartments and securely clamped together. SC was arranged to face towards the donor compartment. Available skin area for permeation was approximately 1 cm^2. High vacuum silicone grease was applied onto donor and receptor compartments and excessive skin at the sides was trimmed off to minimize lateral diffusion. Skin was allowed to equilibrate with receptor phase for half an hour to enhance the permeation thereby the activity of the enhancers could be measured and compared. A 1-ml saturated solution with and without enhancers was added to the donor compartment, which was later covered with Para-film and aluminium foil to minimize the evaporation of the solution and degradation of the drug from light. The sampling port was also occluded with aluminium foil for the same purpose. Saturated drug solutions were used to maximize thermodynamic activity. Cells were placed on an immersible magnetic stirring bed in a water bath with temperature adjusted to 37 ± 1°C. The receptor compartment was stirred with a magnetic bar at high and constant speed. Aliquots of 300 μl were withdrawn periodically and replaced with the same volume of receptor fluid for 48 h. Donor and receptor compartments were replaced with fresh

solutions at least three times during the experiment to maintain sink conditions. Each experiment was done in triplicate. The drug concentrations were measured by reversed phase HPLC.

2.6. Calculation of permeation parameters

The cumulative amount of drug (Q) permeating through the skin in time (t) from the donor solution at constant concentration (Co) to the receptor phase at the sink condition was described by Okamato et al. (1986) according to the following equation:

$$Q = AhKCo\left[\frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{(-Dn^2\pi^2t/h^2)}\right] \quad (1)$$

A is the area of application, h is the thickness of the skin, K is the partition coefficient of the drug between the skin and donor solution and D is the diffusion coefficient. Since it is generally accepted that most molecules permeate through SC mainly by a tortuous intercellular route, the thickness of the skin (h) is not equal to the diffusional pathlength (Williams and Barry, 1991). But it is difficult to determine the diffusional pathlength correctly, so Kh and D/h^2 are replaced with K' and D', respectively. The equation becomes:

$$Q = AK'Co\left[D't - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{(-D'n^2\pi^2t)}\right] \quad (2)$$

where K' is the partition parameter and D' is the diffusion parameter. A non-linear regression program (Graph Pad Prism™, San Diego, CA) with $n = 5$ was used to fit the equation to the experimental data and K' and D' values were calculated. The following equations were used to calculate the permeability coefficient (P), lag time (Lt) and flux (J) from K' and D' values:

$$P = K' \times D' \quad (3)$$

$$Lt = 1/6D' \quad (4)$$

$$J = P \times Co \quad (5)$$

where Co is the saturated solubility of the drug. The following equations were used to evaluate the effect of the enhancers on permeability coefficient, lag time and flux:

$$EI = \frac{[P] \text{ with enhancer}}{[P] \text{ without enhancer}} \quad (6)$$

$$Kr = \frac{[K'] \text{ with enhancer}}{[K'] \text{ without enhancer}} \quad (7)$$

$$Dr = \frac{[Lt] \text{ without enhancer}}{[Lt] \text{ with enhancer}} \quad (8)$$

$$Cr = \frac{[Co] \text{ with enhancer}}{[Co] \text{ without enhancer}} \quad (9)$$

$$Ps = Kr \times Dr \quad (10)$$

$$Js = Kr \times Dr \times Cr \quad (11)$$

where EI is the enhancer index, Kr is the coefficient of relative activity, Dr is the coefficient of relative diffusion, Cr is the relative solubility of HP, Ps is the relative index of permeability coefficient and Js is the relative index of flux.

3. Results

Tables 1 and 2 show permeation parameters of enhancers at a concentration of 1 mg/ml of drug solution through rat skin. At this concentration, ascorbic acid and cetrimide significantly increased the flux ($P < 0.001$, $P = 0.003$) and the permeability coefficient ($P = 0.002$, $P = 0.002$) of haloperidol. These two enhancers at concentrations of 0.5 and 0.75 mg/ml were used to analyse the dose-dependent diffusion profile and to find out the factor mainly responsible for the increased permeation (Tables 3–6). DMSO, PEG 400, PG and urea did not show any influence on the flux and permeability coefficient. Polysorbate 20 decreased the flux ($P = 0.015$) and permeability coefficient ($P = 0.017$) of HP significantly.

Except for ascorbic acid, none of the other enhancers had any effect on the solubility of HP in 0.03% lactic acid solution. Ascorbic acid was found to increase the solubility of HP. Table 3 shows that as ascorbic acid concentration increased, the solubility of HP and flux also increased gradually, though the enhancement index remained almost constant. Table 5 indicates that

Table 1
Partition parameter (K') and diffusion parameter (D') obtained from the Eq. (2), solubility of haloperidol (Co) and estimated lag time (Lt), permeability coefficient (P), flux (J) and enhancer index (EI) in the absence and presence of the enhancers at the concentration of 1 mg/ml in the saturated drug solution[a]

Enhancer	K'	$D' \times 10^2$	Co (mg/ml)	Lt (h)	$P \times 10^2$ (cm/h)	$J \times 10^2$ (mg/h per cm^2)	EI
Control	1.44 ± 0.58	1.48 ± 0.32	1.02 ± 0.01	11.71 ± 2.85	2.00 ± 0.39	2.04 ± 0.40	–
Ascorbic acid	1.88 ± 0.34	1.97 ± 0.39	2.77 ± 0.17***	8.70 ± 1.66	3.62 ± 0.13**	10.00 ± 0.40***	1.81
Cetrimide	3.13 ± 0.34	1.27 ± 0.04	0.96 ± 0.02	13.13 ± 0.44	3.96 ± 0.32**	3.80 ± 0.25**	1.98
DMSO	0.65 ± 0.34	3.07 ± 1.40	1.07 ± 0.04	6.66 ± 4.04	1.70 ± 0.56	1.83 ± 0.67	0.85
PEG 400	0.55 ± 0.41	2.95 ± 1.59	0.97 ± 0.03	6.61 ± 3.56	1.29 ± 0.34	1.24 ± 0.29	0.64
Polysorbate 20	0.45 ± 0.26	2.91 ± 1.15	0.97 ± 0.03	6.60 ± 3.37	1.12 ± 0.06*	1.09 ± 0.06*	0.56
PG	0.84 ± 0.29	1.67 ± 0.58	1.08 ± 0.01	10.66 ± 3.73	1.31 ± 0.01	1.41 ± 0.00	0.66
Urea	0.69 ± 0.16	2.62 ± 0.60	0.99 ± 0.01	6.58 ± 1.38*	1.75 ± 0.31	1.67 ± 0.26	0.88

[a] Mean $\pm$ S.D. ($n = 3$).
* $P < 0.05$.
** $P < 0.005$.
*** $P < 0.001$.

gradual increase in the concentration of cetrimide did not change the solubility of haloperidol but increased the flux, permeability coefficient and enhancer index, with cetrimide at 0.75 mg/ml showing the greatest effect. Figs. 1–3 represent mean cumulative amount of the drug released per cm^2 of rat skin for 48 h for various enhancers and for different concentrations of ascorbic acid and cetrimide, respectively. No enhancer except urea altered lag time. Urea decreased the lag time significantly from that of the control ($P = 0.049$).

4. Discussion

Since HP is lipophilic in nature, it was dissolved in water with the aid of lactic acid (US Pharmacopoeia, 1990; Reynolds, 1996). The same concentration of lactic acid solution (0.03%) was added to the receptor compartment to create a sink condition. HP forms a soluble salt, haloperidol lactate, with lactic acid. Apart from ascorbic acid, no other enhancer increased the solubility of HP; probably solubility was limited by the availability of lactic acid for salt formation. The enhancer increases the permeation of the drug through the membrane either by increasing the thermodynamic activity or decreasing the tortuous intercellular pathway in the skin or creating a high concentration gradient across the skin (Mitra and Wirtanen, 1989; Almirall et al., 1996). Vitamin C at a concentration of 1 mg/ml increased the flux significantly from that of the control by creating a high concentration gradient across the skin due to the increased solubility (Js, Table 2). This finding was confirmed by the fact that the solubility of HP increased with increase in the concentration of vitamin thus increasing the concentration gradient across the skin resulting in a gradual increase in flux (Co, J, Table 3 and Js, Table 4). Ascorbic acid did not enhance the flux either by increasing the partition coefficient of the drug between the

Table 2
Effect of the enhancers at a concentration of 1 mg/ml in the saturated drug solution on the permeation profile of haloperidol expressed in relation to the coefficient of relative activity (Kr), coefficient of relative diffusion (Dr), relative solubility (Cr), relative index of permeability coefficient (Ps) and relative index of flux (Js)

Enhancer	Kr	Dr	Cr	Ps	Js
Ascorbic acid	1.31	1.35	2.72	1.76	4.78
Cetrimide	2.17	0.89	0.94	1.94	1.82
DMSO	0.45	1.76	1.05	0.79	0.83
PEG 400	0.38	1.77	0.95	0.67	0.64
Polysorbate 20	0.31	1.77	0.95	0.56	0.53
PG	0.58	1.10	1.05	0.64	0.67
Urea	0.48	1.78	0.93	0.85	0.79

Table 3
Partition parameter (K') and diffusion parameter (D') obtained from the Eq. (2), solubility of haloperidol (Co) and estimated lag time (Lt), permeability coefficient (P), flux (J) and enhancer index (EI) in the absence and presence of ascorbic acid at various concentrations in the saturated drug solution[a]

Ascorbic acid (mg/ml)	K'	$D' \times 10^2$	Co (mg/ml)	Lt (h)	$P \times 10^2$ (cm/h)	$J \times 10^2$ (mg/h per cm^2)	EI
–	1.44 ± 0.58	1.48 ± 0.32	1.02 ± 0.01	11.71 ± 2.85	2.00 ± 0.39	2.04 ± 0.40	–
0.50	1.44 ± 0.07	1.30 ± 0.10	1.80 ± 0.09	12.84 ± 0.94	1.88 ± 0.18	3.38 ± 0.49	0.94
0.75	1.35 ± 0.03	1.62 ± 0.03	2.32 ± 0.05	10.32 ± 0.16	2.18 ± 0.05	5.04 ± 0.05	1.09
1.00	1.88 ± 0.34	1.97 ± 0.39	2.77 ± 0.17	8.70 ± 1.66	3.62 ± 0.13	10.00 ± 0.40	1.81

[a] Mean ± S.D. ($n = 3$).

Table 4
Effect of ascorbic acid at various concentrations in the saturated drug solution on the permeation profile of haloperidol expressed in relation to the coefficient of relative activity (Kr), coefficient of relative diffusion (Dr), relative solubility (Cr), relative index of permeability coefficient (Ps) and relative index of flux (Js)

Ascorbic acid (mg/ml)	Kr	Dr	Cr	Ps	Js
0.50	1.00	0.91	1.76	0.91	1.61
0.75	0.94	1.13	2.27	1.06	2.41
1.00	1.31	1.35	2.72	1.76	4.78

Table 5
Partition parameter (K') and diffusion parameter (D') obtained from the Eq. (2), solubility of haloperidol (Co) and estimated lag time (Lt), permeability coefficient (P), flux (J) and enhancer index (EI) in the absence and presence of cetrimide at various concentrations in the saturated drug solution[a]

Cetrimide (mg/ml)	K'	$D' \times 10^2$	Co (mg/ml)	Lt (h)	$P \times 10^2$ (cm/h)	$J \times 10^2$ (mg/h per cm^2)	EI
–	1.44 ± 0.58	1.48 ± 0.32	1.02 ± 0.01	11.71 ± 2.85	2.00 ± 0.39	2.04 ± 0.40	–
0.50	1.60 ± 0.85	1.88 ± 1.22	1.06 ± 0.03	11.34 ± 5.84	2.40 ± 0.35	2.53 ± 0.34	1.20
0.75	2.43 ± 0.25	1.75 ± 0.05	1.03 ± 0.00	9.50 ± 0.27	4.25 ± 0.36	4.38 ± 0.37	2.13
1.00	3.13 ± 0.34	1.27 ± 0.04	0.96 ± 0.02	13.13 ± 0.44	3.96 ± 0.32	3.80 ± 0.25	1.98

[a] Mean ± S.D. ($n = 3$).

donor solution and the skin or by decreasing the diffusional pathlength (Ps, Table 4). Fig. 4 shows that the plots of coefficient of relative activity (Kr) and coefficient of relative diffusion (Dr) versus concentration of ascorbic acid (Ca) remained almost parallel to the x-axis whereas the plot of relative solubility (Cr) gradually increased with increase in vitamin concentration, with the equation and r-value as follows:

$$Cr = 1.73Ca + 0.97 \quad r = 0.9979$$

Like lactic acid and tartaric acid, ascorbic acid probably increased the solubility of HP in aqueous solution by forming haloperidol ascorbate salt. Fig. 5 shows that the solubility of the HP increased linearly as the vitamin concentration was increased ($r = 0.9982$). Table 3 shows that every 0.25 mg increase of vitamin per ml increased the solubility of haloperidol by an average 0.44 mg. If 1 mol of ascorbic acid forms salt with 1 mol of haloperidol, 0.25 mg of ascorbic acid has to dissolve 0.53 mg of the drug, which is approximate to the obtained value. The obtained

value was about 0.09 mg short of the theoretical value and this could be explained as when saturated drug solutions were prepared, excess of drug was added to the solutions containing definite concentrations of the vitamin. As the drug dissolved, the volume of the solution would expand thereby decreasing the enhancer concentration.

Cetrimide, a cationic surfactant, at a concentration of 1 mg/ml enhanced the permeation of the drug significantly from that of the control by increasing the thermodynamic activity (*Kr*, Table 2). This was further confirmed by a gradual increase in the thermodynamic activity as the cetrimide concentration was increased (*Kr*, Table 6). Since the relative diffusion coefficient (*Dr*) and the relative solubility (*Cr*) were almost at unity for all concentrations, cetrimide did not enhance the permeation either by decreasing the diffusional pathlength or by increasing the concentration gradient. Plots of the coefficient of relative diffusion (*Dr*) and relative solubility (*Cr*) versus concentration of cetrimide (*Cc*) in Fig. 6 are parallel to the *x*-axis whereas the plot of coefficient of relative activity (*Kr*) linearly increased with the concentration of the enhancer. The correlation could be described by the equation:

Table 6
Effect of cetrimide at various concentrations in the saturated drug solution on the permeation profile of haloperidol expressed in relation to the coefficient of relative activity (*Kr*), coefficient of relative diffusion (*Dr*), relative solubility (*Cr*), relative index of permeability coefficient (*Ps*) and relative index of flux (*Js*)

Cetrimide (mg/ml)	*Kr*	*Dr*	*Cr*	*Ps*	*Js*
0.50	1.11	1.03	1.04	1.15	1.19
0.75	1.69	1.23	1.01	2.08	2.10
1.00	2.17	0.89	0.94	1.94	1.82

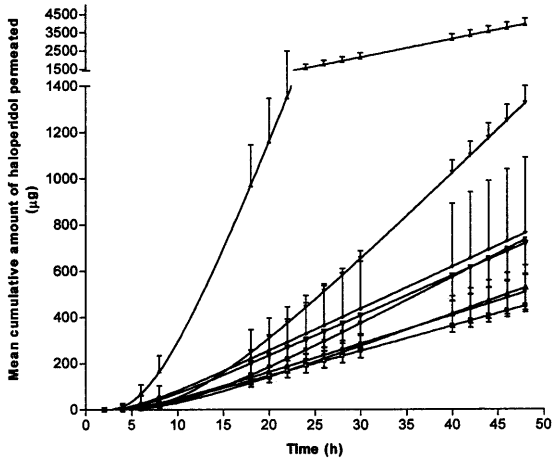

Fig. 1. Mean cumulative amount of haloperidol permeated through 1 cm^2 of rat skin for 48 h in the absence and presence of the enhancers at the concentration of 1 mg/ml in saturated drug solution. Key (■) control, (▲) ascorbic acid, (▼) cetrimide, (□) DMSO, (◆) PEG 400, (□) polysorbate 20 (△) PG and (▽) urea. Each point represents mean ± S.D. ($n = 3$).

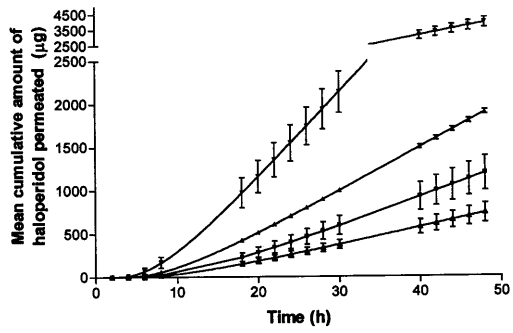

Fig. 2. Mean cumulative amount of haloperidol permeated through 1 cm^2 of rat skin for 48 h in the absence and presence of ascorbic acid at various concentrations in the saturated drug solution. Key (△) control, (□) ascorbic acid 0.5 mg/ml, (▲) ascorbic acid 0.75 mg/ml and (▼) ascorbic acid 1 mg/ml. Each point represents mean ± S.D. ($n = 3$).

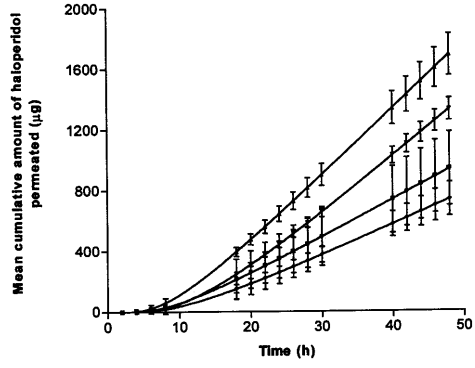

Fig. 3. Mean cumulative amount of haloperidol permeated through 1 cm^2 of rat skin for 48 h in the absence and presence of cetrimide at various concentrations in the saturated drug solution. Key (◆) control, (□) cetrimide 0.5 mg/ml, (▲) cetrimide 0.75 mg/ml and (■) cetrimide 1 mg/ml. Each point represents mean ± S.D. ($n = 3$).

$Kr = 1.16Cc + 0.84 \quad r = 0.9109$

Though the thermodynamic activity gradually increased with an increase in cetrimide concentration, the flux and permeability coefficient were higher for cetrimide at 0.75 mg/ml than at 1 mg/ml (Table 5). This may be due to the fact that

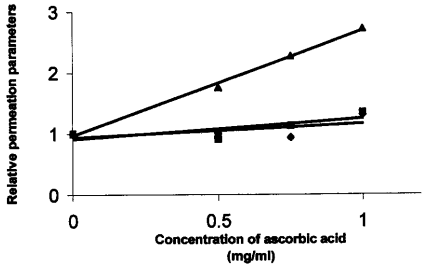

Fig. 4. The relationship between the concentration of ascorbic acid and the relative permeation parameters. Key (◆) coefficient of relative activity (*Kr*), (■) coefficient of relative diffusion (*Dr*) and (▲) relative solubility (*Cr*).

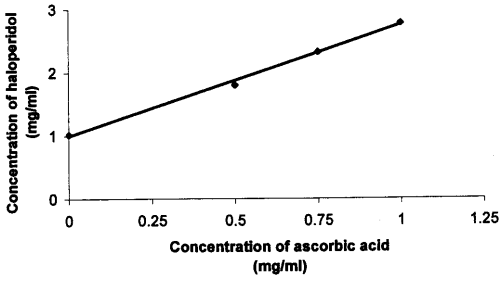

Fig. 5. Linear relationship between the concentration of ascorbic acid and solubility of haloperidol in 0.03% lactic acid solution.

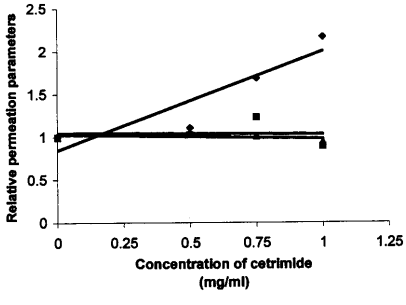

Fig. 6. The relationship between the concentration of cetrimide and the relative permeation parameters. Key (◆) coefficient of relative activity (*Kr*), (■) coefficient of relative diffusion (*Dr*) and (▲) relative solubility (*Cr*).

though cetrimide did not alter lag time significantly from that of the control, cetrimide at 0.75 mg/ml had less mean lag time than that of cetrimide at 1 mg/ml. Provided the enhancer does not influence the lag time, such a difference in the lag time may be due to experimental and animal variations and these variations can be minimized either by increasing the sample size or increasing the enhancer concentration difference. All three concentrations chosen were above the critical micelle concentration (CMC) of cetrimide ($> 0.01\%$) (Wade and Weller, 1994), therefore drug solutions would presumably contain an identical number of monomers but different quantities of micelles increasing with the surfactant concentration. Enhancer dose-dependent thermodynamic activity of haloperidol above CMC can be attributed to micelle-dependent solubilization of skin lipids (Loden, 1990). Though it is generally accepted that micelles do not penetrate the skin on the account of bulkiness, they may solubilize specific components within the intercellular lipid matrix (Ruddy, 1995). So an increase in the thermodynamic activity was proportional to the number of micelles present. Since the enhancer index of cetrimide at 0.5 mg/ml was only 1.2, monomers probably did not show any enhancement activity.

Polysorbate 20, a non-ionic surfactant, significantly decreased the flux and permeability coefficient by decreasing the thermodynamic activity (*Kr*, Table 2). PG, PEG 400 and DMSO are generally effective at high concentrations and they are mostly used as co-solvents for permeation enhancement. For example DMSO requires high concentrations ($> 60\%$ in water) for enhancement action (Barry, 1987). In the present work, the concentration of these enhancers may be too low to show any influence on the permeation. Though urea decreased lag time significantly, the flux and permeability coefficient remained almost the same as that of the control. This is because the decrease in diffusional pathlength was undermined by a decrease in partition coefficient of the drug between donor solution and the skin (*Dr*, *Kr*, Table 2). Decrease in the lag time may be due to the interaction of urea with the protein components of the skin thereby affecting drug–keratin binding (Williams, 1995).

In conclusion, ascorbic acid enhanced the permeation of haloperidol from the saturated solution by a high concentration gradient across the rat skin, cetrimide enhanced permeation by increasing the thermodynamic activity, polysorbate decreased permeation by decreasing partition coefficient, urea decreased lag time probably by protein binding in the skin and other enhancers did not change the permeation significantly from that of the control. Many of the enhancers show side effects like skin irritation, allergy, damage and cutaneous toxicity. Since cationic surfactants cause more skin damage than anionic and nonionic surfactants (Ruddy, 1995), use of cetrimide as an enhancer may be limited. As vitamin C has been proven to stimulate the synthesis of collagen in cultured human skin fibroblasts in the wound healing process (Yamamoto et al., 1992), to protect the porcine skin from sunburn when applied topically (Darr et al., 1991) and with the minimal skin irritation (Ash et al., 1998), it would be more compatible with the human skin. Therefore ascorbic acid should be further explored as a potential enhancer for transdermal studies.

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