Effect of Cetrimide and Ascorbic Acid on *in Vitro* Human Skin Permeation of Haloperidol

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Received April 23, 2001; accepted July 29, 2001

Permeation of haloperidol through the human skin *in vitro* was studied with two enhancers, cetrimide and ascorbic acid, at various concentrations. 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. Donor solutions were prepared by adding excess of haloperidol to 0.03% (v/v) lactic acid solution with or without enhancers at concentrations 0.1, 0.3 and 0.6% (w/v) and stirred for 36 h at 32 °C before filtering. Ascorbic acid gradually increased the solubility of the haloperidol from that of the control where as cetrimide did not show any effect. Cetrimide concentration dependent increase in the permeability coefficient of haloperidol was observed. Mechanism of enhancement by cetrimide was probed with the diffusion profile kinetics and Fourier transform infrared (FT-IR) spectroscopy. Cetrimide was found to increase the thermodynamic activity of the drug in the skin. IR spectra of the stratum corneum treated with cetrimide showed time-dependent decrease in the intensity of the spectrum and dose-dependent decrease of lipid band but no change in the protein conformation. Cetrimide appears to interact with both the dermal keratin and lipids and this interaction was found to be irreversible. Ascorbic acid although increased the flux of haloperidol to the same extent at all concentrations from that of the control, decreased the permeability coefficient and enhancer index in a concentration dependent manner and this is due to the increased solubility of the drug in the vehicle. Both the enhancers did not change the lag time from that of the control.

Key words haloperidol; transdermal; cetrimide; ascorbic acid; Fourier transform infrared spectroscopy

Haloperidol (HP) is given in the treatment of acute psychosis as well as in maintenance therapy to prevent the relapse of the psychosis.[1] Long-acting formulations are more meaningful in the maintenance therapy since long-term daily oral dose of 3 to 10 mg of HP[2] may lead to noncompliance. HP decanoate oily injection, the only long acting formulation available at present, has some disadvantages namely; complex dosing schedule while converting from oral therapy to depot therapy,[3] pain at the site of injection, individual to individual variation in the liberation of active drug from the parent drug[4] and marked variations of HP plasma concentration.[5] Because of these disadvantages, the development of alternative long-acting formulations could be beneficial.

Drug delivery *via* skin 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.[6] However, many drugs require some mechanism to enhance their penetration through the excellent skin barrier to achieve therapeutic plasma concentration. One common approach is using chemical enhancers, which act by increasing the solubility of the drug in stratum corneum (SC) or disrupting the lipid matrix of SC or interacting with the intracellular protein.[7] HP, which was reported to penetrate only sub-therapeutically through the human skin *in vitro*, would require penetration enhancers.[8] In this study, cetrimide and ascorbic acid, effect of which were studied in our earlier work on the permeation of HP through the rat skin *in vitro*,[9] were used at various concentrations 0.1, 0.3 and 0.6% (w/v) on human skin and the mechanism of enhancement by cetrimide was probed with diffusion profile kinetics and Fourier transform infrared (FT-IR) spectroscopy.

Experimental

Materials HP, droperidol, DL-lactic acid, L-ascorbic acid, antibiotic antimycotic solution (100×) and sodium dihydrogen phosphate monohydrate were purchased from Sigma Chemical Company and cetrimide from Chempure Pte Ltd., Singapore. All other chemical reagents were of at least reagent grade and all materials were used as supplied.

Analytical Method Drug concentrations were determined by reversed phase HPLC (C_{18} column, Hewlett Packard Pte Ltd., Germany) at 254 nm. Mobile phase consisted of 0.05 M phosphate buffer (pH 3) and acetonitrile in the ratio of 50 : 50. Droperidol was used as an internal standard. Flow rate was 1.3 ml/min and injection volume was 100 μl. Retention times of the internal standard and drug were approximately 4 and 6.5 min. Mean peak area ratios of the drug and internal standard in 0.03% (v/v) lactic acid were linearly related to the drug concentrations for the samples containing 1 to 7 μg/ml (r=0.9999). Intraday and interday coefficient of variations for all concentrations varied from 1.29 to 11.64%.

Solubility Studies Excess of HP was added to 0.03% (v/v) lactic acid containing antibacterial antimycotic solution (1 in 100 dilution) with and without enhancers and stirred for 36 h at 32±1 °C over an immersible magnetic stirring bed kept in a water bath. These samples were filtered through 0.45 μm teflon filter units using a gas-tight syringe (Hamilton Pte Ltd., Switzerland). To minimize the oxidation of ascorbic acid, degassed milli Q water was used, amber colored bottles were filled to the neck level, surface air was replaced with nitrogen and lids were tightly closed. Saturated drug concentrations were determined by HPLC in triplicate after appropriate dilution. These saturated solutions were used for permeation studies.

Preparation of Human Epidermis Cadaver chinese male abdominal skin was obtained from the Singapore General Hospital, Singapore. Epidermal membranes were prepared by heat separation technique.[10] Whole skin was immersed in water at 60 °C for 2 min, followed by careful removal of the epidermis. Samples were stored at −80 °C until used. Prior to permeation experiments, membranes with SC side up were floated over 0.9% (w/v) sodium chloride solution containing antibacterial antimycotic solution (1 in 100 dilution) for 3 d.

Preparation of SC Human epidermal membranes with SC side up were incubated in petri-dishes over filter papers imbibed with 0.1% (w/v) trypsin in 0.5% (w/v) sodium bicarbonate solution at 37±1 °C for 3 h.[10] SC was removed, thoroughly washed and dried in vacuum dessicator. After 24 h, SC was dipped in acetone solution for 20 s to remove sebaceous lipids and dried again.

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Table 1. Partition Parameter (K'), Diffusion Parameter (D'), Solubility (Co), Lag Time (Lt), Flux (J), and Permeability Coefficient (P) of HP and Enhancer Index (EI) in the Absence and Presence of the Enhancers at Various Concentrations

Enhancer	Enhancer concentration % (w/v)	$K'*10^2$	$D'*10^2$	Co ($mg \cdot ml^{-1}$)	Lt (h)	$J*10^4$ ($mg \cdot h^{-1} \cdot cm^{-2}$)	$P*10^4$ ($cm \cdot h^{-1}$)	EI
Control	—	2.36±0.92	1.43±0.06	0.98±0.03	11.71±0.54	3.28±1.20	3.36±1.30	—
Cetrimide	0.1	3.43±0.21	1.40±0.05	1.03±0.03	11.88±0.46	4.96±0.07	4.81±0.14	1.43
	0.3	4.35±0.20	1.36±0.10	1.07±0.06	12.30±0.92	6.37±0.64*	5.93±0.68*	1.77
	0.6	5.17±0.23	1.63±0.15	0.99±0.03	10.28±0.94	8.37±0.68*	8.42±0.62*	2.51
Ascorbic acid	0.1	1.98±0.70	1.31±0.21	2.75±0.08*	12.92±1.88	6.85±1.41	2.60±0.15	0.77
	0.3	1.15±0.36	1.47±0.16	5.10±0.14*	11.40±1.26	8.50±1.91*	1.70±0.06	0.51
	0.6	0.45±0.07	1.57±0.20	9.48±0.22*	10.72±1.49	6.64±0.81	0.71±0.02	0.21

a) Mean±S.D. (n=3). *b*) $p<0.05$. One-way Anova Tukey posthoc test.

Permeation Studies Amber glass Franz-type diffusion cells were used for permeation studies. Fully hydrated human epidermis was mounted between donor and receptor compartments and excessive skin at the sides was trimmed off to minimize lateral diffusion. SC was arranged to face towards donor compartment and available skin area for permeation was approximately 1 cm^2. Prior to mounting, high vacuum silicone grease was applied onto the donor and receptor compartments. One milliliter of saturated drug solution in 0.03% (v/v) lactic acid solution with or without enhancers was added to the donor compartment and 0.03% (v/v) lactic acid solution containing antibacterial antimycotic solution was taken in receptor compartment to create a pseudo-sink condition. Receptor solution was thoroughly degassed to prevent the formation of bubbles beneath the membrane. Antibacterial and antimycotic solution was added to both donor and receptor solutions to maintain the integrity of the skin throughout the experiment and to minimize the microbial contamination in samples during the analysis. Donor compartment was covered with parafilm to minimize the evaporation of the solution. Sampling port was occluded with the aluminum foil for the same purpose. Cells were placed over a heater/stirrer block (PermeGear, U.S.A.), which was covered to minimize the degradation of the drug from light and the content of the receiver compartment was stirred at 37±1 °C. Aliquots of 300 μl were withdrawn periodically and replaced with the same volume of receptor fluid for 48 h.

FT-IR Spectroscopy SC was cut into small circular discs with approximate diameter of 1.5 cm and floated over 0.9% (w/v) sodium chloride solution containing antibacterial and antimycotic solution for 3 d. Then these discs were thoroughly blotted over filter paper and FT-IR (JASCO, FT/IR-430) spectra were recorded in the frequency range 4000 to 400 cm^{-1}, with 2 cm^{-1} resolution. Each spectrum was an average of 60 scans. This spectrum was considered to be taken at 0 h. SC discs were kept in 1.7 ml of various concentrations of cetrimide solutions (1 cm^2 of the SC treated with 1 ml of cetrimide solution) for 2 d at 21±1 °C. IR spectrum was taken for each sample at 12, 24 and 48 h. After 2 d, SC discs were thoroughly washed, dried in vacuum dessicator for 24 h and FT-IR spectra were taken to observe the reversibility of the enhancer action. Each sample served as its own control.

Calculation of Permeation Parameters The cumulative amount of drug 'Q' permeated through the skin with area 'A' 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.*[11] according to the following equation:

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

Where K' is the activity parameter and D' is the diffusion parameter. A nonlinear regression program (Graph Pad Prism™, San Diego, CA, U.S.A.) 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.

$$P=K'*D' \quad (2)$$

$$Lt=1/6D' \quad (3)$$

$$J=P*Co \quad (4)$$

Where Co is the saturated solubility of the drug. Following equations were used to evaluate the effect of the enhancers on permeability coefficient, lag time and flux.

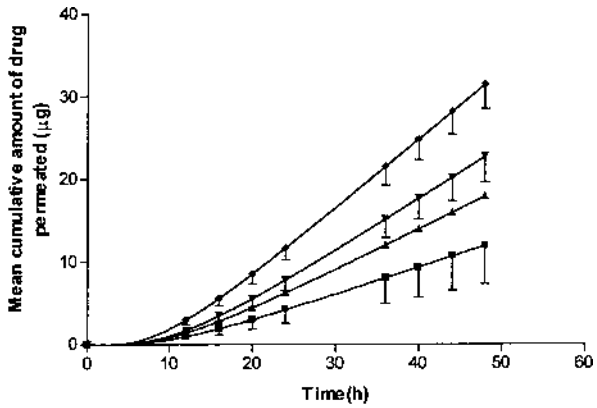

Fig. 1. Time Course of Mean Cumulative Amounts of HP Permeated through 1 cm^2 of Human Epidermal Membrane in the Absence and Presence of Cetrimide at Various Concentrations

Key (■) control, (▲) cetrimide 0.1%, (▼) cetrimide 0.3% and (◆) cetrimide 0.6%. Each point represents mean±S.D. (n=3).

$$EI=\frac{[P] \text{ with enhancer}}{[P] \text{ without enhancer}} \quad (5)$$

$$Kr=\frac{[K'] \text{ with enhancer}}{[K'] \text{ without enhancer}} \quad (6)$$

$$Dr=\frac{[Lt] \text{ without enhancer}}{[Lt] \text{ with enhancer}} \quad (7)$$

Where EI is the enhancer index, Kr is the coefficient of relative activity, Dr is the coefficient of relative diffusion.

Results and Discussion

The flux and the permeability coefficient of HP through the skin increased significantly with cetrimide 0.3% (w/v) and 0.6% (w/v) concentration ($p<0.05$, one-way Anova Tukey posthoc test) and not significantly with cetrimide 0.1% (w/v) from that of the control (J, P, Table 1, Fig 1). Cetrimide concentration-dependent enhancement of drug flux and permeability coefficient was observed. As the cetrimide concentration increased, enhancer index also increased gradually from that of the control (EI, Table 1). The drug flux increased significantly with ascorbic acid 0.3% (w/v) and not significantly with ascorbic acid 0.1% (w/v) and 0.6% (w/v) from that of the control (J, Table 1, Fig. 2). The flux remained relatively unchanged at all ascorbic acid concentrations. The permeability coefficient and enhancer index decreased as the concentration of the vitamin increased (P, EI, Table 1). Cetrimide did not increase the solubility of drug

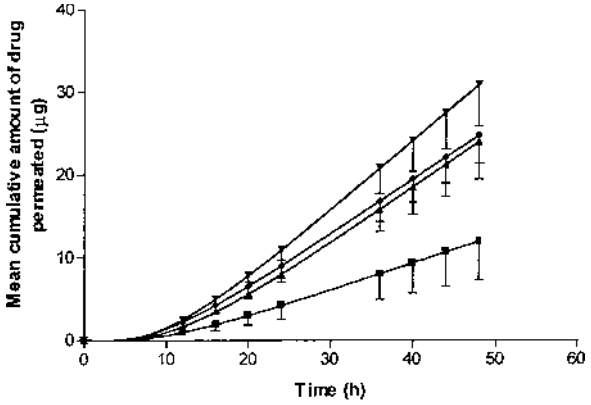

Fig. 2. Time Course of Mean Cumulative Amounts of HP Permeated through 1 cm^2 of Human Epidermal Membrane in the Absence and Presence of Ascorbic Acid at Various Concentrations

Key (■) control, (▲) ascorbic acid 0.1%, (▼) ascorbic acid 0.3% and (◆) ascorbic acid 0.6%. Each point represents mean±S.D. (n=3).

Table 2. Effect of Enhancers at Various Concentrations on the Permeation Profile of HP Expressed in Relation to the Relative Activity Parameter (*Kr*) and Relative Diffusion Parameter (*Dr*)

Enhancer	Enhancer concentration % (w/v)	*Kr*	*Dr*
Cetrimide	0.1	1.45	0.98
	0.3	1.84	0.95
	0.6	2.19	1.14
Ascorbic acid	0.1	0.84	0.91
	0.3	0.49	1.03
	0.6	0.19	1.09

whereas ascorbic acid was found to increase the solubility in a dose-dependent manner (*Co*, Table 1). Both these enhancers at all concentrations did not alter the lag time significantly from that of the control.

As the relative diffusion parameter (*Dr*) was almost at unity for all cetrimide concentrations and relative activity parameter (*Kr*) gradually increased with the cetrimide concentration (Table 2), cetrimide did not increase the permeation of HP by decreasing the diffusional path length but by increasing the thermodynamic activity of the drug in human skin. Similar pattern of cetrimide concentration-dependent drug permeation profile was also obtained with the rat skin.[9] Plot of relative diffusion parameter (*Dr*) *versus* concentration of cetrimide (*Cc*) in Fig. 3 is parallel to *X*-axis whereas the plot of relative activity parameter (*Kr*) linearly increased with the concentration of the enhancer. The correlation could be described by the equation with *r*-value as

$$Kr = 1.862Cc + 1.1545, \quad r = 0.9620$$

Enhancer-induced changes in SC, which is a complex matrix of protein and lipids, can be studied from the IR spectrum. Many bands in the spectrum can be either related to protein or lipids or both. The keratin backbone was reported to give amide A, amide I and amide II peaks at *ca.* 3300, *ca.* 1650 and *ca.* 1550 cm^{-1} respectively. Asymmetric and symmetric CH_2 vibration absorption bands at 2920, 2850 cm^{-1}, carboxyl group vibrations at 1740, 1715 cm^{-1}, OH stretching vibrations at *ca.* 3400 cm^{-1} are primarily due to the SC

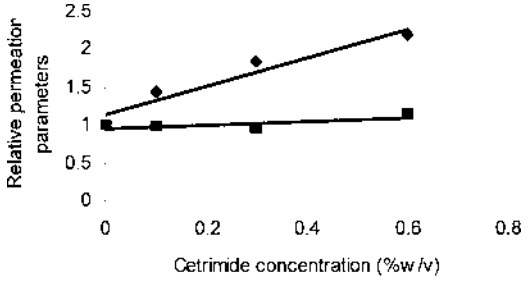

Fig. 3. The Relationship between the Concentration of Cetrimide and the Relative Permeation Parameters

Key (◆) relative activity parameter (*Kr*), (■) relative diffusion parameter (*Dr*).

lipids.[12—14] In the present study, cetrimide was found to enhance the flux and permeability of HP through SC. The SC was treated with various concentrations of cetrimide and IR spectra were obtained at different time intervals to study the mechanism of these actions. Superimposition and subtraction of spectra were used to analyze the cetrimide interaction with the keratin and lipids. Superimposition of spectra obtained from the SC treated with cetrimide 0.6% (w/v) showed gradual decrease in the absorbance from 0 to 48 h (a to d in Fig. 4). The same trend was also observed with the SC treated with cetrimide 0.1 (w/v) and 0.3% (w/v) (not shown). Subtraction of the 48 h spectrum from 0 h spectrum showed positive bands throughout the spectrum with two negative peaks at 2917, 2850 cm^{-1} (f in Fig. 4). Decrease in the absorbance of amide A and amide II bands could possibly be due to the cetrimide interaction with the keratin. Since amide I is very complex and intensive,[15] change in the intensity of this band was not taken into consideration. However amide I band, which appears at different regions between 1600 to 1700 cm^{-1} according to the protein conformation,[16] did not shift to the other regions from 1652 cm^{-1} at all cetrimide concentrations and this infers that cetrimide upto 0.6% (w/v) concentration, like 5% (w/v) sodium lauryl sulphate,[17] failed to produce any conformational change.

All the three concentrations chosen were above the critical micelle concentration (CMC) of cetrimide [≈0.01% (w/v)],[18] therefore cetrimide solutions would contain identical number of monomers but different quantities of micelles increasing with the surfactant concentration. Monomers can penetrate SC and interact with the keratin where as micelles can not penetrate the skin due to their bulkiness but can solubilize specific components of skin lipids[19] and can also supply monomers when they break up. So gradual decrease in protein peaks with the time may be due to the continuous supply of monomers by micelles. Surfactant dose-dependent permeation of HP above CMC can be mainly attributed to micelle-dependent solubilization and removal of the skin lipids.[20] In SC spectrum, extraction of lipids should decrease the intensity of bands at 2920, 2850 cm^{-1}.[21] Ironically, in our study, increase in peak height was observed upon treatment with cetrimide as shown by the negative bands in the difference spectrum (f, Fig. 4). This increase in peak intensity is due to the keratin-bound cetrimide, which has long hydrocarbon chain and therefore exhibits asymmetric and symmetric CH_2 absorption bands at the same wave numbers as that of the SC lipids; 2917, 2850 cm^{-1} (g, Fig. 4). Because of this overlapping of cetrimide bands with the SC lipid bands, the

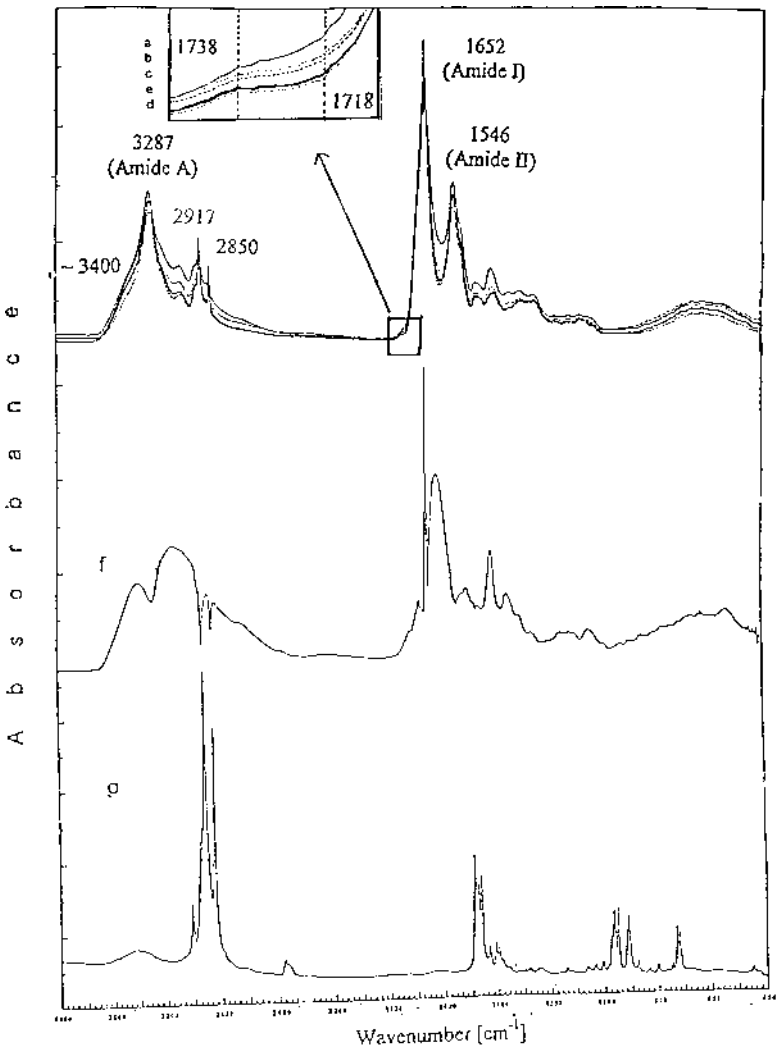

Fig. 4. FT-IR Spectra of SC Treated with Cetrimide 0.6%

Spectra represented with various types of lines (a) 0 h ——, (b) 12 h ·····, (c) 24 h ------, (d) 48 h –··–, (e) 24 h after washing and drying ——, (f) difference spectrum a—d (g) cetrimide in KBr pellets.

decrease in peak intensity due to the extraction of the lipids was probably undermined by the protein-bound cetrimide. Alternatively, information about the SC lipids can be deduced from the bands at *ca.* 3400, 1738, 1718 cm^{-1}. Decrease in the intensity at these bands due to the extraction of the lipids was confirmed from the spectra obtained before and after treating the SC with chloroform and methanol in 2 : 1 ratio for 24 h and extracted lipids after evaporation of organic solvent on sodium chloride transmission windows (a, b, c, Fig. 5). The time-dependent decrease in the absorbance at these bands with all cetrimide concentrations could infer that micelles gradually extracted the lipids (a, b, c, d, Fig. 4). To assess the dose-dependent action of cetrimide on lipids, SC discs were treated with various concentrations of cetrimide solutions continuously for 48 h. Intensity at *ca.* 3400 cm^{-1} band gradually decreased with the increase of cetrimide concentration (Fig. 6). This is probably due to the extraction of the lipids by micelles in a concentration-dependent manner. HP is a weak base with pK_a 8.3 and therefore exists as cation in lactic acid solution (pH *ca.* 3). The removal of lipids by cetrimide from the SC could gradually enhance the penetration of this water-soluble cation through the skin. Reversibility of the spectrum was not seen after the SC, at the end of 48 h of cetrimide treatment, was washed and dried for 24 h (e, Fig. 4) and this indicates that interaction of the cetrimide with the SC is irreversible. It is in agreement with the earlier study that the interaction of the cationic surfactants with the SC is irreversible particularly in *in vitro* studies where the repair mechanism is absent.[22)]

Ascorbic acid probably did not have any activity on the skin. Gradual decrease in the permeability coefficient of HP with the increase of vitamin concentration was due to the gradual increase of the drug solubility in the vehicle as per the equation $P=J/Co$ (P, Co, Table 1). The flux increase was only marginal and not significant at 0.1 and 0.6% (w/v) ascorbic acid concentrations. Increase in the amount of the drug applied on to the skin alone may not increase the permeation in the absence of any enhancement mechanism. In the case of the rat skin also,[9)] ascorbic acid seemed to have no activity on the skin as its enhancement index remained at unity at all concentrations.

In conclusion, cetrimide enhanced HP permeation by interacting with the SC proteins and lipids and its dose-dependent enhancement was primarily due to the extraction of SC lipids. Ascorbic acid did not increase the permeation of the drug but increased the solubility of the drug in the vehicle.

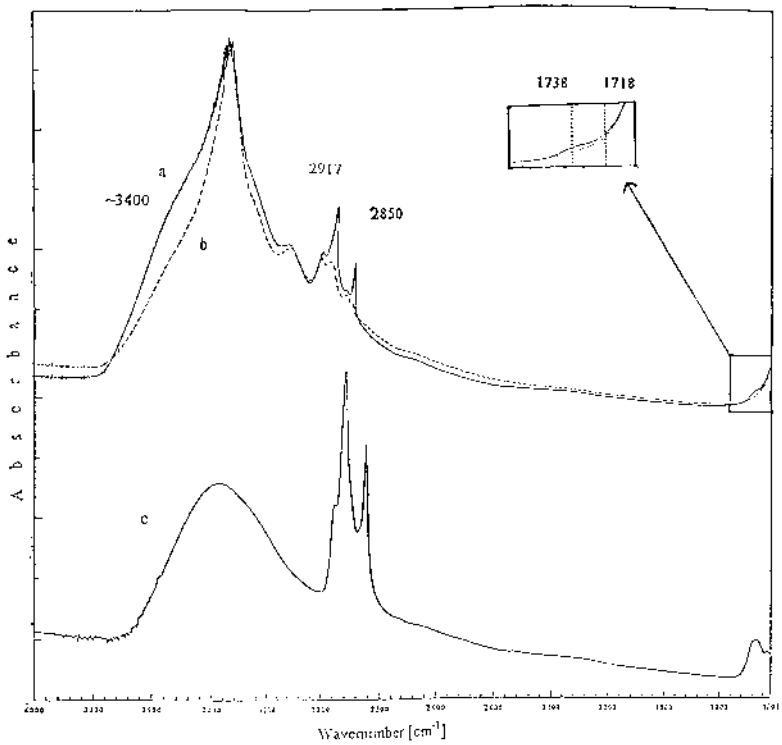

Fig. 5. Spectra of SC before and after Treated with Chloroform and Methanol in 2 : 1 for 24 h and of Extracted Lipids

Spectra represented with various types of lines (a) SC ——, (b) SC with out lipids -----, (c) lipids.

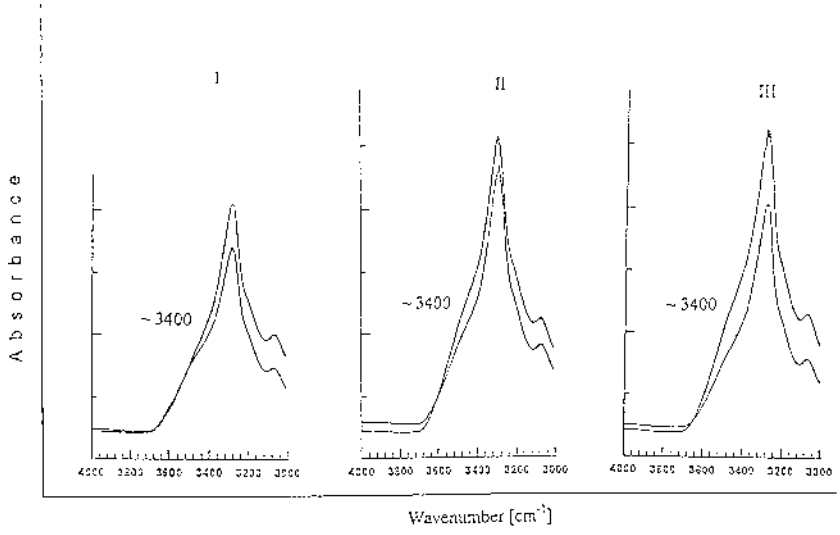

Fig. 6. Effect of Cetrimide Concentration on the O–H Stretching Band of Lipids at *ca.* 3400 cm^{-1}

Top: 0 h, bottom: 48 h, (I) cetrimide 0.1%, (II) cetrimide 0.3%, (III) cetrimide 0.6%.

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