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PROTECTIVE AND RETENTIVE EFFECTS OF LIPOSOMES ON WATER-DEGRADABLE HYDROCORTISONE ACETATE IN DERMATOLOGICAL APPLICATIONS

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Abstract – Various dermatological samples containing liposomes as a drug carrier were prepared, and the effects of variations in the dermatological formulations, such as liposomal encapsulation, base materials, and the purity of lipid products, on drug stability and characteristics for effective topical drug delivery were investigated. Hydrocortisone-21-acetate, a hydrophobic and water-degradable, anti-inflammatory agent, was used as the model drug. It was found that the liposomally encapsulated drug was more stable than the free-form drug in an ointment formulation. Also, the hydrogel base was found to be effective in maintaining drug stability in spite of its high water content. Another evidence that liposomes were surrounding drug particles in the base was obtained from an *in vitro* test of drug permeation from liposome-hydrogel through the pig ear skin. The permeability of hydrocortisone acetate through the skin membrane was found to be 2.7-fold lower in the case of liposome-hydrogel than in the case of free-drug hydrogel. The results also suggested that liposomes play a role in localizing drug molecules in the skin membrane.

Key words: Liposomes, Topical Drug Delivery, Stability, Skin Permeability, Hydrogel

INTRODUCTION

As liposomes show highly appreciable effects as drug carriers in a number of pharmaceutical and medical applications, attempts have been made to use liposomes in topical drug delivery [Braun-Falco et al., 1992]. When liposome-encapsulated drugs are applied onto the surface of the skin, liposomes are known to enhance drug permeation through the stratum corneum, localize the drug molecules in the epidermis and dermis, and reduce unnecessary drug diffusion into the blood vessels [Wholrab and Lasch, 1987; Mezei and Gulasekharam, 1982].

However, since dermatological products for the healing of skin diseases usually need to be applied for prolonged time periods, the drugs as well as liposomes are expected to remain stable on such long-term storage. Many dermatological drugs show instability in the presence of water. Water molecules react with drug molecules, causing the drug to undergo degradation, and as a consequence, the drug activity is gradually reduced. Thus, encapsulating such water-degradable drugs into liposomes, thus into their interior water chamber, may decrease the drug efficacy. On the other hand, the risk of vesicle fusion or membrane breakage during storage is not negligible. Overall, the liposomal encapsulation of drugs may result in undesirable effects and reduced efficacy because of the instability of both drugs and liposomes. Therefore, in order to verify the potency of the liposomal delivery of dermatological drugs, the stability of drugs and liposomes on long-term storage should be confirmed.

In this work, the stability of a dermatological drug in the liposomal system on long-term storage was investigated. We have so far carried out studies on the stability of liposomes in the suspension state [Kim and Kim, 1991; Lee and Kim, 1995], but this time we stabilized liposomes in semisolid media to give them minimal mobility. Hydrocortisone-21-acetate (HCA), an anti-inflammatory agent degradable in water [Dick and Scott, 1992], was entrapped in egg phosphatidylcholine (PC) liposomes, and three types of dermatological bases, such as hydrogel, cream, and poly(ethylene glycol) ointment, were prepared with the liposomal suspension. Then, the effects of liposome entrapment, base materials, base viscosity, and lipid purity on the drug stability were investigated. In addition, the *in vitro* drug permeability through the skin was studied with the hydrogel formulation with liposome-encapsulated drug (liposome-hydrogel) and the hydrogel formulation with non-encapsulated, free-form drug (free-drug hydrogel). Hence, the overall adequacy of liposomal ointment formulation for topical drug delivery was investigated.

EXPERIMENTAL

1. Materials

The phospholipids used in all experiments were egg phosphatidylcholine (PC) of 60 % purity. One exceptional case was the experiment to observe the effects of liposomal lipids on the drug stability, in which soybean phosphatidylcholine of 90 % purity (Epikuron 200) was used in comparison with egg PC.

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Egg PC and hydrocortisone-21-acetate were purchased from Sigma Chemical Co. (St. Louis, MO) and soybean PC was obtained from Lucas-Meyer (Hamburg, Germany). Stearyl alcohol, poly(ethylene glycols) of molecular weights 400, 1,500, 3,350, and 8,000 and two surfactants, namely poly(oxyethylene sorbitan monooleate) and sorbitan sesquioleate, were purchased from Aldrich Chemical Co. (Milwaukee, WI). White petrolatum and the sodium salt of carboxymethylcellulose were purchased from Yakuri Pure Chemicals Co., Osaka, Japan and Junsei Chemical Co., Tokyo, Japan, respectively. All the above chemicals were used without further purification. Other chemicals and solvents were of reagent grade.

2. Preparation of Liposome Suspension

All liposomal suspensions used in this study were prepared by the precipitation method [Kim et al., 1997a]. Hydrocortisone-21-acetate (HCA) (600 mg) and phosphatidylcholine (PC) (1,200 mg for preparation of liposome-hydrogel and 1,800 mg for other ointments) were dissolved together in excessive ethanol, and purified water was added to the ethanol solution. The ratio of water to ethanol was approximately 1 : 6 by volume. The mixture was warmed to 33 °C in a water bath, while being stirred with a magnetic stirrer, so that all the drug particles and lipid clusters were completely dissolved. Ethanol from the mixture was then evaporated under mild vacuum provided by an aspirator. The temperature of the water bath was kept constant at 33 °C and vacuum evaporation and stirring were continued until a yellowish liposome suspension was formed. Aspiration was removed only when the volume of the liposome suspension left was smaller than the original input volume of water, in order to ensure that only an infinitesimal amount of ethanol was present in the suspension. Thus formed liposome suspension was stored in the refrigerator until used in the preparation of liposomal semisolid formulations.

3. Preparation of Semisolid Formulations

We prepared six semisolid formulations, which were grouped into three types by the base material, namely, hydrogel, cream, and PEG hydrophilic ointment, according to the procedures described below. Table 1 summarizes the ingredients and water contents. Liposome-hydrogel was prepared by swelling carboxymethylcellulose (4.5 wt%) with the previously prepared egg PC liposome suspension and adding propylene glycol (7 wt%) as an excipient. The mixture was continuously stirred until a homogeneous semisolid was obtained. In the case of free-drug hydrogel, HCA was treated, in the absence of PC, with a 1 : 6 water-ethanol mixture and vacuum evaporation, as in the liposome preparation procedure; and the HCA suspension in water was mixed with carboxymethylcellulose and propylene glycol, so that the contents of the gel were the same as those in the liposome-hydrogel formulation. Liposome-cream was prepared by melting stearyl alcohol (15 wt%), mineral oil (40 wt%) and surfactants (5 wt%) together and quickly emulsifying with egg PC liposomal suspension with simultaneous cooling to room temperature. Liposome-PEG hydrophilic ointment was prepared by mixing melted poly(ethylene glycol) of different molecular weights (70 wt%) and white petrolatum (10 wt%) with egg PC liposomal suspension at 40 °C. Liposome-cream and liposome-PEG ointment samples containing soybean PC liposomes were prepared in addition, according to the same procedure. For the samples prepared for the stability test, the contents of HCA were all set to 1 wt%, and the water contents were 84 wt% in hydrogel, 34 wt% in cream, and 14 wt% in PEG ointment. For the hydrogel samples prepared for the skin permeation test, the contents of HCA and phosphatidylcholine were set to 2 wt%, respectively, and the water contents were reduced in compensation.

4. HPLC Analysis of Drug

A JASCO HPLC pumping set with JASCO UV detector was used in the quantitative analysis of HCA. The wavelength for the absorbance was 214 nm, and the pumping flow rate was 1.5 ml/min. The mobile phase was a mixture of deionized water, methanol, and acetonitrile in the ratio of 5 : 2 : 3. The separation column used in this experiment was a C18 (octadecyl silica) column with 10-μm particles.

5. Stability Test

The stability of HCA contained in various six different formulations on a one-month storage was studied. Variations in the semisolid formulations were made to give three different comparisons that allowed observations of the effects of liposomal encapsulation, different kinds of semisolid bases, and lipid purity on the drug stability.

Previously described semisolid formulation samples were packaged in sealed aluminum tubes such that each semisolid sample was distributed in six tubes. Those tubes were stored in desiccators, where supersaturated brine was contained to provide an appropriate relative humidity, maintained at 75 %, at room temperature and 30 °C. However, the hydrogel samples were stored additionally at 40 °C in order to accelerate destabilization. Since other semisolid samples underwent phase sep-

Table 1. Six different types of ointment formulations and lists of their ingredients and water content

Type	Liposomal hydrogel	Free-drug hydrogel	Liposomal cream	Liposomal cream	Liposomal PEG oint.	Liposomal PEG oint.
Lipid	egg PC (60 %)	none	egg PC (60 %)	soy PC (90 %)	egg PC (60 %)	soy PC (90 %)
Model drug	HCA		HCA		HCA	
Ingredients	Carboxymethyl cellulose		Stearyl alcohol		PEG 8000	
			Mineral oil		PEG 3350	
	Propylene glycol		Sorbitan sesquioleate		PEG 1500	
	Water		Poly(oxyethylene sorbitan monooleate)		PEG 400	
					White petrolatum	
			Water		Water	
Water contents	84 wt%		34 wt%		14 wt%	

aration or liquefaction at 40 °C, they were not tested for accelerated destabilization. The semisolid samples were stored for four weeks under the given temperature and humidity conditions, and the drug content in each tube was analyzed at an interval of one week. From each sample tube, 0.50 g of ointment formulation was taken and analyzed by HPLC. Thus, each point on all of the graphs for the stability test represents the mean average of six data values, and the error bar represents the statistically calculated standard error range.

6. Viscosity Test

The base viscosity was measured with Haake Rotovisco® RV 20. Approximately 6 ml of a base sample was placed in between two cylinders, the smaller of which rotated to exert torque onto the sample, and shear viscosity was measured. The viscosity data were obtained at room temperature, 30 °C, and 40 °C for each base sample.

7. Pig Skin Permeation Test

Diffusion cells, made of acrylic plastics, were designed to hold pig ear skin membranes horizontally between two chambers. Fig. 1 depicts the design of the cells. The procedure for the preparation of pig ear skin membrane followed the method of Dick and Scott [Dick and Scott, 1992]. Pig ears were obtained from a local abattoir. The ears were washed under cold running water, and the whole skin membrane, including the dermis and the subdermal tissues, was detached from the underlying cartilage. The whole skin membrane was soaked in 60 °C water for 70s, and the stratum corneum and epidermis were carefully separated from the remaining tissues by blunt dissection. The average final thickness of the skin membranes was 1.2±0.1 mm. The microscopic photographs of the prepared pig ear skin membrane are given in Fig. 2. Prepared pig skin membranes, which consisted of the stratum corneum and epidermis, were preliminarily soaked in purified water and were mounted, with the stratum corneum facing upward, on the lower chambers of the cells, which had been filled with purified water. This water, bathing the epidermal side of the skin membrane, was stirred continuously by a magnetic stirrer. The total volume of the water filled in the lower chamber was 140 ml.

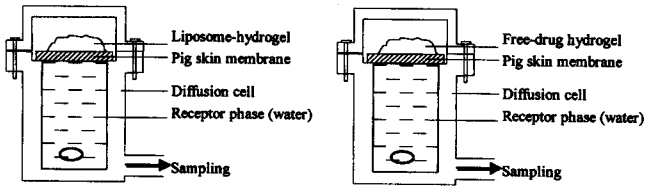

Fig. 1. Schematic of the diffusion cells designed for this experiment.

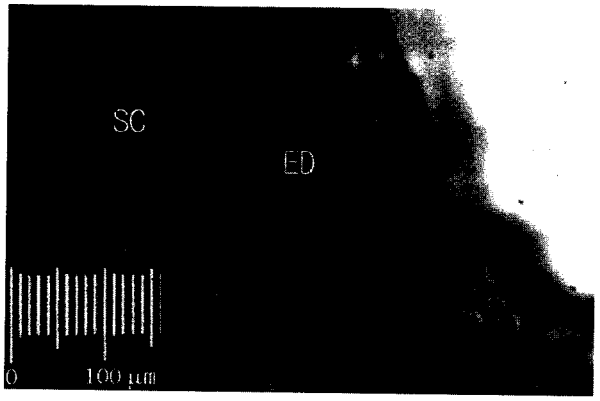

Fig. 2. Microscopic photographs of the pig ear skin membrane. Compact stratum corneum and soft epidermal tissue show clear distinction.
SC: stratum corneum, ED: epidermis

Liposome-hydrogel and free-drug hydrogel were taken for the test such that 2.0 g of each ointment sample was evenly applied on the stratum corneum side of the skin mounted on a cell, covering an effective diffusional area of 7.07 cm^2. The upper chamber of the cell was placed above the skin membrane with hydrogel on it, and the membrane was held in place between two O-rings attached to the upper and lower chambers. The two chambers were then screw-fixed to each other. The diffusion cells were kept in a water bath which was maintained at 37±1 °C.

Two ml of the aqueous medium in the receptor side of each cell was taken at regular time intervals and was analyzed for HCA concentration by HPLC. Also, 2 ml of fresh water was supplemented into the receptor chamber after every sampling.

RESULTS AND DISCUSSION

1. Preparation of Liposome Suspension

The principle of the precipitation method is to make PC undergo an environmental (solvent) change gradually from an ethanol-rich environment to a water-rich environment, thereby inducing self-assembly of the lipid molecules. This method can be compared with the ethanol injection method, in which lipid molecules are exposed to ethanol and then water. In this work, hydrocortisone acetate was hydrophobic, and therefore as the solvent environment became rich in water, the drug molecules must have aggregated to reduce the surface area exposed to water. It is suggested that the hydrophobic tails of PC molecules have aggregated together with the drug aggregates and lipid bilayers assembled upon the aggregates, thereby minimizing the exposure of HCA to water.

Multilamellar liposomes were produced by the method and had a wide size distribution. However, since the liposomes were not to be used parenterally, the size was not critical in this work. Fig. 3 shows the negative staining TEM photographs of the liposomes prepared by this method. The magnification was 115,000 times. Multilamellar membranes of the liposomes are observable in the pictures.

2. Stability Test

The degradation kinetics and its products of hydrocortisone in aqueous media have been studied intensively, and the drug degrades into analogues of decreasing activity [Hansen and Bundgaard, 1979, 1980a, b]. For instance, according to Hansen and Bundgaard, hydrocortisone loses its activity by 60% within 30 hours in 0.1 M borate buffer solution of pH 9.21 at 50 °C [Hansen and Bundgaard, 1980b]. However, when the degradation pattern of hydrocortisone-21-acetate (HCA) was observed, the intermediate degrading compounds first showed an increase and then a decrease in activity (data not shown). Since the UV

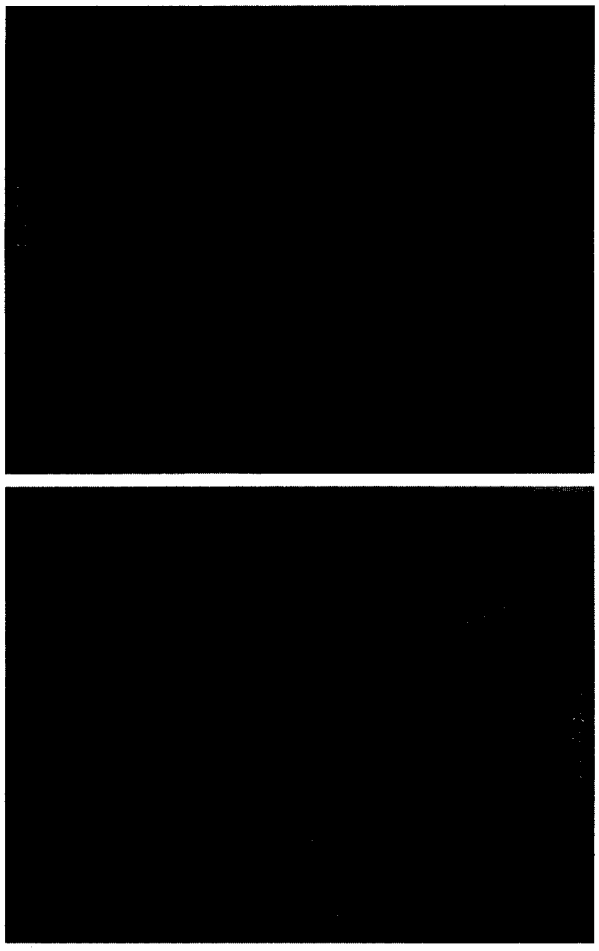

Fig. 3. Negative staining electromicrographs of multilamellar vesicles prepared by the precipitation method.

detector in the HPLC set detects the polarity of functional groups in the molecules under test, it is possible that HCA molecules have reacted with water to become analogues with more polar functional groups. Therefore, the apparent increase of drug concentration in the following results seems to be due to the above reason, not because of changes in drug amount.

Three comparisons were made to analyze the effects of liposome encapsulation, base materials, and lipid purity on the stability of HCA. First, the stabilities of liposomal HCA and free-drug HCA contained in hydrogel bases were compared, and liposomal HCA was found to be more stable than free HCA on a long-term storage. Fig. 4 illustrates the results. It is obvious that liposomes had influence on the drug stability in a protective manner. The HCA precipitate particles staying within the hydrophobic domain of the liposomal membranes may have a lower probability of getting in contact with water molecules dispersed in the base. Therefore, degradation reaction may occur to a lesser degree in the liposomal case than in the free-drug case.

Secondly, when the three bases were all prepared with HCA encapsulated in egg PC liposomes, the stability of HCA was maintained higher in the hydrogel base than the other two bases, cream and PEG ointment. The tendency is indicated in Fig. 5. Since hydrogel contained the largest percentage of water by

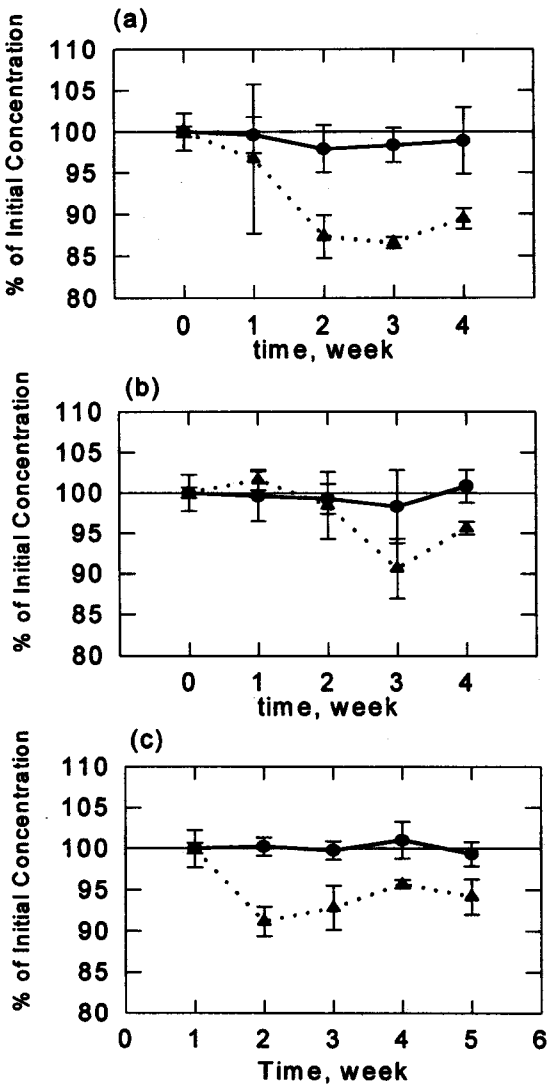

Fig. 4. Effects of liposomal encapsulation of HCA on the drug stability.
Samples were stored at (a) room temperature, (b) 30 °C and (c) 40 °C; ●: HCA in liposome-hydrogel, ▲: HCA in free-drug hydrogel

weight, it was expected to result in the lowest drug stability. However, the carboxymethylcellulose polymer chains might have caused reductions in the diffusivities of the drug particles and of the vesicles. Hydrogel polymer chains generally form an extensive network, trapping the liposomes in the network and holding water molecules by hydrogen bonding. Therefore, liposomes might have remained in an intact form and segregated HCA particles from water molecules. Fig. 6 illustrates the putative morphology of the three semisolid formulations containing liposomally entrapped HCA. In the hydrogel formulation, liposomes are surrounded by an aqueous environment but are immobilized by the polymer chain network. The enhancement of liposome stability by immobilizing the vesicles in a gel matrix has been proven to be effective [Yamanaka et al., 1997]. Therefore, the environment provided by the hydrogel base was favorable for the physical stability of liposomes, extending its influence onto the drug stability. In the case of liposome-cream,

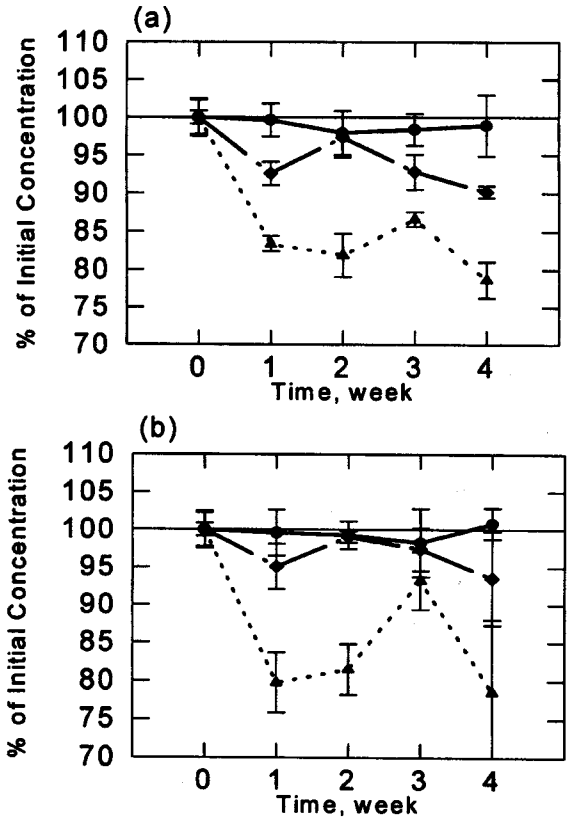

Fig. 5. Effects of base materials on the stability of liposomally encapsulated HCA.

Samples were stored at (a) room temperature and (b) 30 °C; ●: HCA in liposome-hydrogel, ◆: HCA in liposome-PEG ointment, ▲: HCA in liposome-cream

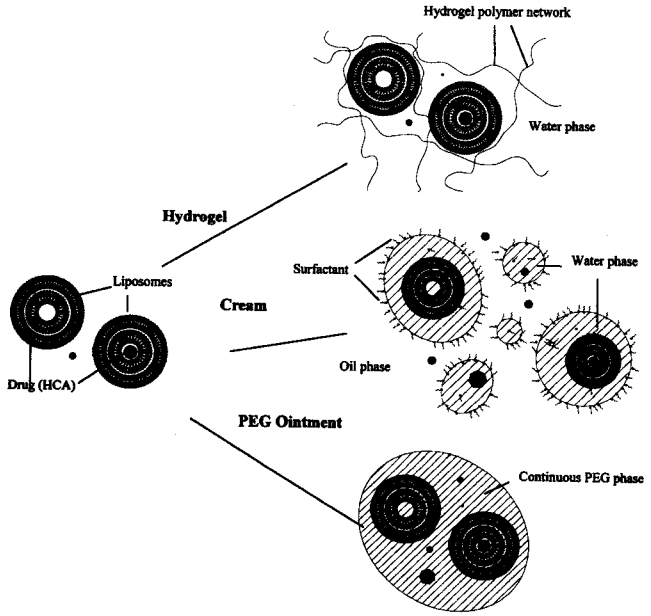

Fig. 6. Putative morphology of the semisolid formulations containing liposomally encapsulated hydrocortisone acetate.

as shown in Fig. 6, the surfactant molecules added as emulsifiers might have penetrated into the liposomal membranes and

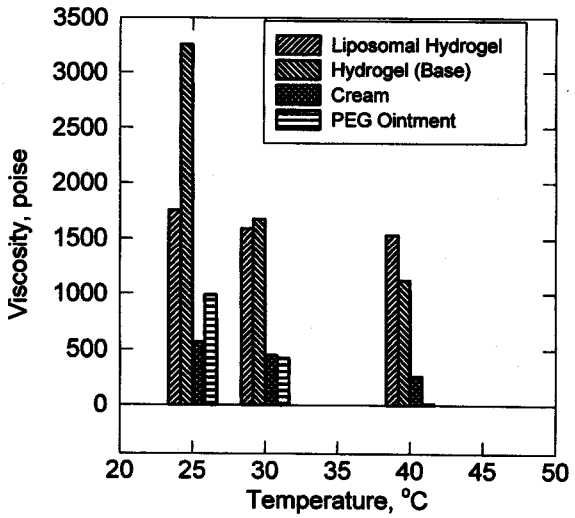

Fig. 7. Changes of viscosities of the three semisolid bases and of the liposome-hydrogel upon elevation of temperature.

caused defects or breakage in the membranes. Then, the evenly dispersed water phase domains could have attacked the HCA particles in exposure. In the case of liposome-PEG ointment, drug degradability was low when compared with the liposome-cream, because its water content was lower than the latter. However, the drug degradability was higher compared to that of the liposome-hydrogel. It is possible that the lipid molecules dispersed in the PEG base were not in proper assembly because of the low water content.

The base viscosities were compared as in Fig. 7. The viscosity data of the bases indirectly indicate the degree of molecular diffusion within the bases. Greater activation energy will be expected in diffusional movement in a highly viscous medium than in a less viscous medium. Hydrogel, which shows the highest viscosity among the three bases, has the highest drug stability. But, cream and PEG ointment, having low viscosities, may allow more molecular movement to occur within the bases and provide more chances of molecular collision for reaction. The molecular movement effect may be also extended to the preservation of the liposomal assembly. Lipid molecules tend to go to thermodynamic equilibrium by molecular diffusion [Gruner, 1987], and therefore liposomal bilayers are susceptible to breakage. But, the high viscosities of the semi-solid bases, especially that of Hydrogel, may prevent lipid molecules from breaking away from the bilayer arrangement, thereby maintaining the protective role for HCA particles.

Lastly, the cream and PEG ointment samples were prepared with egg PC (60 % purity) liposomes and soybean PC (90 % purity) liposomes in separate batches. The stability of HCA was better in the samples with liposomes made of high purity lipid than in those of low purity lipid, as shown in Fig. 8. The impurities associated in the 60 % PC could be mainly proteins and non-amphiphilic lipids. Those macromolecules may penetrate into the liposomal membranes and deform the overall vesicle structure. Thus, when surfactant molecules attack the vesicles, as in the liposome-cream, the vesicles are relatively easily destroyed. Meanwhile, 90 % PC could maintain the liposome structure nearly intact and could endure the attack of surfac-

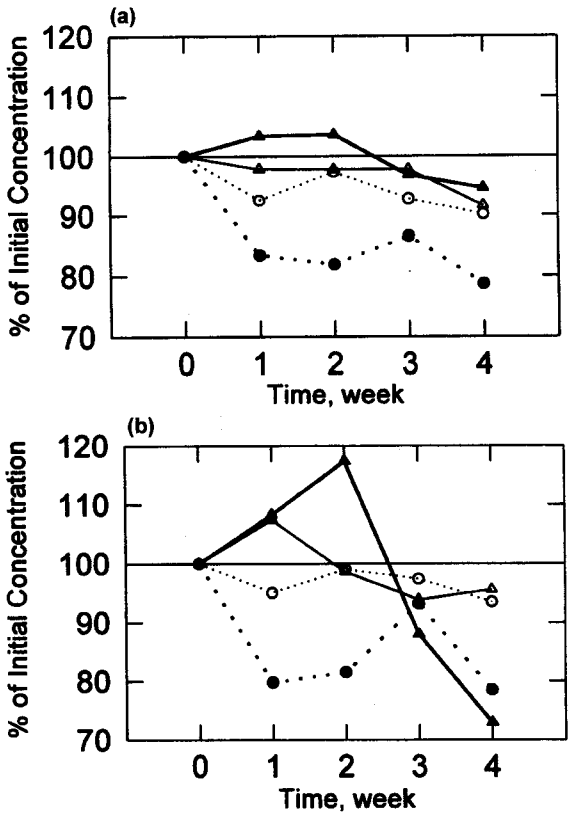

Fig. 8. Effects of the purity of a lipid product on the stability of HCA.

Samples were stored at (a) room temperature and (b) 30 °C;
▲ : 90 % PC in liposome-cream
△ : 90 % PC in liposome-PEG ointment
○ : 60 % PC in liposome-PEG ointment
● : 60 % PC in liposome-cream

tant molecules to a certain extent. The PEG ointment base had no apparent influence on the lipid type because liposomes could not stay intact in the PEG environment where water was insufficient.

3. Skin Permeation Test

Liposomes are vesicles with bilayer membranes of lipids assembled in water as a result of microphase separation, and the membranes are known to act as a permeation barrier to small molecules dissolved in the medium. So far it has been understood that lipids enhance penetration of drug molecules through the stratum corneum, the impermeable top layer of the skin, and therefore liposomes should enhance penetration as well [Wholrab and Lasch, 1987]. However, it is ironic that macromolecules like liposomes would ever penetrate into the skin in an intact form. As shown in Fig. 2, the compact structure of the stratum corneum can be clearly distinguished from the epidermal tissue with a looser, softer structure. Therefore, it would be fairly difficult for macromolecules such as liposomes to penetrate into the stratum corneum and downward. Hence, the mass fluxes of drug molecules from the liposome-hydrogel and free-drug hydrogel through an excised piece of the pig ear skin were compared in this study.

Fig. 9 shows the concentration of HCA collected in the receptor phase of the diffusion cell after 8 hours in the skin permeation test. The plot represents three repetitions of the same experiment under the same conditions. The results show that the final amount of drug escaping from the free-drug hydrogel was approximately three times higher than that from the liposome-hydrogel, when all the experiment parameters and conditions were the same for both cases.

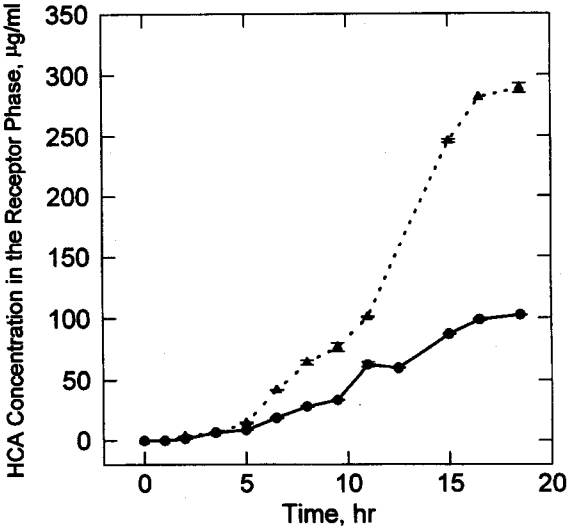

Fig. 9. Change in HCA concentration in the receptor phase as a result of drug permeation through the stratum corneum and epidermis of the pig ear skin.

●: HCA released from liposome-hydrogel
▲: HCA released from free-drug hydrogel

The mass fluxes from the two hydrogels through the pig skin membrane were calculated from the early steady regions of the results shown above, using Eq. (1);

$$\dot{M} = \frac{dM}{dt} = n''_{HCA} A \tag{1}$$

where, $\dot{M}$ stands for the mass transport rate, n''_{HCA} for the mass flux of HCA, and A for the diffusion area. Since the experiments were prolonged for 18 hours, the skin membranes could have undergone deformation, composition changes, and property changes, thereby affecting the drug permeation. Thus, we selected the early steady regions in the results (up to 9.5 h) to calculate the mass fluxes. The value of $\dot{M}$ for liposome-hydrogel, measured from the graph, was 0.188 μg/s, and that for free-drug hydrogel was 0.494 μg/s. The fluxes were 0.0266 μg/s·cm^2 and 0.0699 μg/s·cm^2, respectively. Since the drug diffusion was a complex phenomenon involving the drug transport through the liposome bilayer membrane as well as through the skin membrane, calculation of the diffusion coefficient from the mass flux value was meaningless. Instead, permeability of HCA through the skin membrane was calculated for both formulations, using Eq. (2);

$$n''_{HCA} = -D_{HCA} k_{HCA} \frac{dc}{dx} = -P_{HCA} \frac{dc}{dx} \Rightarrow P_{HCA} = \frac{n''_{HCA} \cdot X}{\Delta C} \tag{2}$$

where, D_{HCA}, P_{HCA}, and k_{HCA} represents the diffusivity and permeability of HCA through the membrane, and the partition coefficient of HCA between water and membrane, respectively. C and X represent the HCA concentration and the membrane

Table 2. Comparison of experiment parameters and mass flux values

Sample	Initial HCA concentration (mg/cm^3)	Effective diffusion area (cm^2)	Membrane thickness (mm, Ave.)	M ($\mu g/s$)	n''_{HCA} ($\mu g/s \cdot cm^2$)	P_{HCA} (cm^2/s)
Liposome-hydrogel	23.86	7.07	1.2	0.188	0.0266	1.34×10^{-7}
Free-drug hydrogel	23.30	7.07	1.2	0.494	0.0699	3.60×10^{-7}

thickness, respectively. The permeability values were found to be 1.34×10^{-7} cm^2/s for the liposome-hydrogel and 3.60×10^{-7} cm^2/s for the free-drug hydrogel. The permeability of HCA was approximately 2.7 fold higher in the free-drug hydrogel. Table 2 lists part of the experimental parameters and calculation results.

The results imply that liposomes can somehow localize a drug within the stratum corneum and the epidermis, preventing the drug molecules from further diffusing into the lower layers of the skin. The stratum corneum consists, in a large part, of various lipids, and therefore lipid exchange may occur between the skin layer and the liposomes in contact with it [Blume et al., 1993]. Then, it is unlikely that liposomes maintain their bilayers intact in such a hydrophobic, lipid-rich milieu. Thus, the possibility of liposomes holding the drug molecules without releasing into the skin is quite low. Rather, drug release from the liposomes could have been retarded because of the time taken for all of their multilamellar membranes to be destroyed and expose the internal contents. The results also imply that liposomes have played the main role in retarding the diffusion rate of the drug molecules in the pig skin membrane. Hydrocortisone and its derivatives are hydrophobic, and therefore they may tend to remain in the skin, where it is lipid-rich, rather than dissolve into the water in the receptor chamber. However, a large amount of HCA dissolved in the receptor phase water--even the final concentration surpassed the normal solubility of HCA in water. The receptor phase, which was pure water in the beginning, gradually became turbid, as lipids from the pig skin membrane dissolved into the receptor phase. Hence, the receptor phase became more lipophilic and provided a favorable environment for HCA to dissolve in it. Such dispersion of lipids in the receptor phase was observed to occur in both diffusion cells because both phases turned turbid. But, there was a great difference in the final HCA concentrations. Since the experimental parameters and conditions, except the presence of liposomes, were controlled to be the same for both diffusion cell systems, the results signify the effects of liposomal encapsulation. Other researchers also investigated such retarding effect of liposomes in *in vivo* topical drug delivery, and it was found that liposomes significantly reduced the rate of drug (hydrocortisone acetate) penetration into the skin [Kim et al., 1997b].

On the other hand, the pig ear skin prepared as the membrane for drug permeation was removed of the dermis and the subcutaneous fatty tissue. Thus, any quantity of drug that had penetrated through the membrane and collected in the receptor phase of the diffusion cell could be considered to have high potential to diffuse further into the blood vessels that are richly embedded in the dermis and the subcutaneous tissue. Hence, the diffusion-retarding effect of liposomes is beneficial in preventing systemic side effects and drug waste [Wholrab and Lasch, 1987; Mezei and Gulasekharam, 1982].

CONCLUSION

The HPLC analysis results for the HCA concentrations in those three samples showed that HCA was stable as being entrapped in liposomes. The protective hydrophobic lipid bilayer prevented the contact between HCA and the aqueous phase. The HPLC results for the HCA concentrations in the control samples containing free HCA without liposomal encapsulation showed that the contact between the drug and water molecules was crucial in drug degradation. Also, the hydrogel formulation containing liposomes was found to be highly effective for the stability of a drug in long-term storage and also for the delivery of a drug to the skin. Since the stability of the drug reflects the stability of liposomes contained in dermatological bases, the liposomal stability was shown to be the best in the hydrogel base among the three base samples.

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