Enhanced permeability of gentamicin sulfate through shed snake-skin and liposomal membranes by different enhancers

*E. Moghimipour, S. A. Sajadi Tabassi, **M. Ramazani, *** Raimar Löbenberg

**Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences and Pharmaceutical Sciences Research Center of Bu Ali Institute, Mashhad, Iran*

***Department of Pharmacognosy, School of Pharmacy, Mashhad Medical Sciences University, Mashhad, Iran*

****Faculty of Pharmacy and Pharmaceutical Science, Dentistry/Pharmacy Centre, University of Alberta, Edmonton, Alberta, Canada*

Abstract

The current work provides information on the surfactant induced membrane permeability. The membrane model was a Franz diffusion cell with shed snake-skin as membrane. Also, liposomes made by cholesterol and phosphatidylcholine, prepared by freeze-thawing method were tested as synthetic lipid membrane models. Three hydrophilic molecules, gentamicin sulfate, 5(6)-carboxyfluoresceine and phenol red were used as drug models. Absorption enhancers were sodium lauryl sulfate (SLS), benzalkonium chloride (BC), that are anionic and cationic surfactants, respectively, and *Quillaja saponaria* (QTS) and *Acanthophyllum squarrusom* (ATS) total saponins, two naturally originated enhancers. Surface tension changes and hemolytic activities were also determined to find out any probable relationship between surfactant properties and its enhancing activity.

The obtained results showed that maximum enhancing effect occured below the surfactant critical micelle concentration (cmc), while they reach their maximum hemolytic activity with concentrations more than their cmc. The results also indicated that there was no enhancing effect induced by benzalkonium chloride when applied on the skin model, probably due to the similarity of electric charges on the skin surface and surfactant ions. Among surfactants tested, SLS showed maximum enhancing effect in the diffusion cell model, while benzalkonium chloride had maximum activity in liposome membrane model. Both QTS and ATS showed a moderate activity in both diffusion cell and liposome models. The precise enhancement mechanism of surfactants tested in this study is not clearly known.

Keywords: Absorption enhancer, *Acanthophyllum squarrosum*, saponin, liposome.

Introduction

Gentamicin sulfate is produced by species of bacteria of the genus Micromonospora and was discovered in 1963. It is not absorbed after oral administration and is usually administered by intravenous or intramuscular routes(21). Therapeutic index of gentamicin is moderately narrow, and blood concentrations above 10 to 12 μg/ml are considered potentially toxic(22).

There have been many efforts to enhance systemic absorption of gentamicin sulfate. The utility of a semisynthetic Quillaja saponin, DS-1, from *Quillaja saponaria*, was investigated as a permeation enhancer for nasal, ocular and rectal delivery of gentamicin and a significant transport was observed(19). Singh *et al.* used iontophoretic force to increase transdermal absorption of gentamicin(21). Also, it has been reported that labrasol, a novel emulsifier, has a good gastrointestinal absorption enhancing effect on poorly absorbable drugs such as gentamicin sulfate(7).

Although *in vivo* studies represent the most crucial test for any formulation, *in vitro* release studies have many advantages. They allow the study of each factor that affect the process. Test conditions may easily be established and maintained and the resulting data are derived both quickly and less costly (13). An *in vitro* model, which is often used to evaluate drug permeability is diffusion cell containing animal skin as a model membrane. The skin is a good, but partial barrier to xenobiotics. When materials come

into contact with the skin, they have to pass through both the stratum corneum and the viable tissue before reaching the systemic circulation(6). Transdermal permeation or percutaneous absorption can be defined as the passage of a substance, such as a drug, from the outside of the skin through its various layers into the blood stream(18). Many drugs diffuse through human skin too slowly to produce satisfactory systemic effects(9). The uptake into the blood and the subsequent distribution of drug molecules are usually rapid relative to the permeation process, unless the material itself constricts the blood vessels and thereby decreases its own clearance(6). The success of percutaneous therapy depends upon absorption characteristics of the drug, and its access to the skin with sufficient speed and suitable quantities from the vehicle or dosage system employed (13). In recent decades, transdermal drug delivery has been an active field of pharmaceutical research with rapid development in both the extent and the depth of investigation (25). In the development of a transdermal delivery system, a series of inter-related elements must be taken into consideration. These factors can be classified into five basic areas: bioactivity of drug, skin characteristics, formulation, adhesion and dosage form design.

Absorption enhancers increase drug absorption through the skin and mucosal barrier, but the mechanism by which these agents act is unclear. Their success rate varies from one drug to another(3). The use of surface active agents for improving permeability has been widely investigated, although general use in human therapeutic has not established yet(24). For example, using an improved Franz diffusion cell, it has been shown that sodium lauryl sulfate can increase the permeation of diazepam across rat skin (20).

Several advantages have been ascribed to transdermal delivery systems such as, increasing patients compliance, controlling plasma levels and avoiding GI distress. Meanwhile, skin irritation or sensitization and economical considerations are probable disadvantages of such systems(18).

Another *in vitro* model to evaluate enhanced permeability is measurement of the release of entrapped drug from liposomes. Liposomes are defined as artificial vesicles composed of concentric lipid bilayers, separated by water compartments. The typical characteristics of bilayer-forming lipids is their amphiphilic nature, a polar head group covalently attached to one or two hydrophobic hydrocarbon tails(2). The most important use of liposomes is their ability to retain solutes for long periods of time(2). Different types of liposomes have been used for this purpose. Another main use of liposomes is their ability to act as a membrane. Xia WJ *et al.* described a method of liposome preparation using reverse phase evaporation method. They used 5(6)-carboxyfluoresceine to evaluate release rate from liposomes(24).

The ability of surfactants to rupture erythrocyte has been used for decades as a detection and quantification method. The most frequently measured parameter is the change in absorbance of the supernatant of an erythrocyte suspension after being hemolysed by a surfactant solution(5). In saponins, for example, hemolytic activity varies considerably with the structure of glycoside. The phenomenon involves a reduction in interfacial tension between the aqueous and lipid phases of the erythrocyte membrane, causing emulsification of the lipids and their subsequent release from the membrane(5).

Drug models used were gentamicin sulfate (GS), 5(6)-carboxyfluoresceine (CF) and phenol red (PR), which are water-soluble compounds.

The goal of the present study was to evaluate drug permeation through an *in vitro* animal skin model, and to compare the

results with data from a synthetic membrane model, liposome. For permeation enhancement studies, we used sodium lauryl sulfate (SLS), benzalkonium chloride (BC), which are anionic and cationic surfactants, respectively, and *Quillaja saponaria* total saponin (QTS) and *Acanthophyllum squarrusom* total saponin (ATS) that are naturally originated enhancers. In addition to permeability studies by two membrane models, we determined surface characteristics and hemolytic activity of the enhancers to find out any possible correlation between them and their absorption enhancing effect.

Materials and Methods

GS, PR, CF, QTS, SLS, BC and Triton X_{100} were all analytical grade and purchased from Sigma – Aldrich Company.

Perparation of ATS: ATS was extracted using the method described by Lacaille-Dubois *et al.*(11). Briefly, *Acanthophyllum squarrusom* roots were collected from around Tabas, south Khorassan, identified, dried and powdered. Then powdered roots were defatted in a soxhelet with petrolium ether. The air-dried powder was extracted with methanol yielding after evaporation a syrupy brown residue. Then it was extracted with water saturated *n*-butanol. The residue was dissolved in least amount of methanol and precipitated by addition of 5 volumes diethyl ether. After filteration, the residue was dissolved again in methanol and precipitated by adding diethyl ether (x2). Finally, total saponin was freeze-dried (Freeze Dryer 3, LABCONCO) and stored at room temperature.

cmc determination: Using wilhelmy-plate apparatus (Model $K_{12,}$ Kruss Processor Tensiometer) surface tension of different aqueous concentrations of surfactants and critical micelle concentration (cmc) were determined. Approximately, 20 ml of surfactant solution was placed in a clean beaker in the instrument chamber. After thermal equilibrium, a vertical plate of platinum-iridium, attached to a balance was immersed in the solution and the force due to wetting was determined.

Preparation of liposomes (reverse-phase evaporation or RPE method): Cholesterol (50mg) and phosphatidyl choline (150 mg) were dissolved in 5 ml chloroform and diethyl ether (1:1), and mixed with drug (CF or GS) isotonic solution (0.1% w/v, pH 7.4) of an organic to aqueous ratio of 1:1. Gentle sonication (Bath ultrasonic, Metler Electronics) led to formation of a w/o type emulsion. The organic solvent was evaporated under reduced pressure (Rotaevaporator, IKA-WERK, Janke & Kunkle) at room temperature. Unentrapped drug was removed from the liposomal suspension by washing with buffer and centrifuging at 17000g (Model LB-55, Beckman Instrument Inc., Rotor type 50.2 T_1) for 30 minutes three times(24).

Preparation of liposomes (freeze-thaw or FT method): Lipids (phosphatidyl choline-cholesterol) were used as equimolar ratio and in solvent (chloroform-methanol 1:1) were coated onto a round-bottom flask using a rotary evaporator. The flask was incubated in a 43 ^{o}C vacuum for 2 hours to remove the last trace of remaining solvent. To this was added 2 ml 0.1% drug solution in 45 ^{o}C water bath and then vortexed (Model K-550-GE, Vortex-Genie, Scientific Industries Inc.) until lipids were rehydrated. The flask was then frozen in dry ice/acetone, thaw for 20 min., incubated at 45^{o}C for 5 min., and vortexed for 5 min. The process was repeated three times. Liposomes were collected by ultracentrifugation (20000g) for 30 min., and washed twice with phosphate buffer solution (PBS)(12,16).

***Liposome size determination*:** Liposome size was determined using Zetasizer (*Zetasizer, Model LB-55, Malvern Instrument, UK*). Size was measured at 25 ^{o}C and the samples were diluted with deionized water.

***Encapsulation efficiency of liposomes*:** To evaluate drug entrapment in two types of liposomes, simply 20 µl of each CF containing liposome suspension was mixed with 2.98 ml Triton

X-100 (0.2% w/v) in PBS. When liposomes were completely dissolved and total CF released, the intensity of fluorescence was measured using the fluorimeter. Finally, encapsulation rate was calculated using equation 3,(12).

Preparation of red blood cell suspension: Approximately 10 ml of blood from a healthy volunteer was taken and added to a heparinized tube and put in to ice. The plasma and buffy coat were removed by centrifuging at 2200g for 10 min and the erythrocytes were washed three times, at least five times their volume of McIlvaine's buffer, pH 7.0. The erythrocytes were adjusted to approximately 12% haematocrit by resuspending them in buffer to 3.33-times their original weight. The erythrocyte suspension was stored on ice at 4^{o}C and was used within 48h of collection(4).

Hemolysis experiments: 200 µl of RBC suspension was incubated for the required time with an equal volume of the test sample of enhancer solution, prepared in McIlvaine's buffer, at 37^{o}C. The incubation time was 30min in all experiments.

After incubation, the mixtures were spun in a microcentrifuge at 3200g for 15s, and 200 µl of the resulting supernatant was added to 3 ml Drabkin's reagent to assay for the amount of haemoglobin released. Positive controls consisted of 200 µl samples, taken from uncentrifuged mixtures of erythrocyte suspensions (200 µl) and buffer (200 µl), which were added to 3 ml Drabkin's reagent to obtain a value for 100% haemolysis. A negative control, included to assess the levels of spontaneous haemolysis, comprised 200 µl buffer mixed with 200 µl erythrocyte suspension. After centrifugation for 15s, a 200 µl sample of supernatant was added to 3 ml Drabkin's reagent. The absorbance (540 nm) of the samples was determined spectrophotometrically and the values expressed as the percentage of maximum haemolysis (4).

Skin preparation: 3x3 cm^2 skin specimens from Vipera labetina (Razi Institute, Karaj, Iran) were placed between two aluminum foils and refrigerated. Prior to test, samples were thawn and placed in PBS for 40 minutes to ensure complete hydration(8).

Diffusion cell experiment: Franz diffusion cell (figure 1) was designed to study drug transport through the skin. The cell included two main parts, donor compartment and receptor compartment. Skin specimens were placed between two glass chambers with the stratum corneum side contacting the donor phase(8), and fastened using polymer washers and stainless steel clips. Upper part (donor compartment) contained 5 ml of liquid sample. Test drugs (phenol red and gentamicin) in combination with enhancer dissolved in PBS (total volume of 5 ml) were placed in donor compartment. The lower part composed of receptor compartment, circulating water jacket, and sampling ports. In the receptor compartment, 20 ml PBS (pH 7.4) was placed. To assure sink condition, the solution was mixed during the experiment using a magnet. The cells were connected to a circulating water bath to adjust the temperature at 37°C. At 30 minute intervals, 1 ml sample was removed from receiver phase and replaced by same volume of PBS. To ensure intact skin, after finishing each test, the solution was removed from donor chamber and replaced by ethanolic solution of gentian violete. The skin specimen was intact if there was no color in receiver phase after one hour. For each test, three cells were used simultaneously.

Phenol red (PR) assay: Stock solution of 100 µg/ml phenol red in PBS was prepared. Then 10, 25, 50 and 75 µg/ml concentrations were made using the stock solution and their absorbance at 435 and 500 nm were measured. Finally, phenol red absorbance in samples was determined at 435 nm.

5(6)-Carboxyfluoresceine (CF) assay: The intensity of fluorescence emitted by CF in samples was determined using fluorimeter (Fluoro Max, SPEX Industries Inc., USA) at 588 nm wave length and reverted to percent released using equation 2.

Gentamicin sulfate (GM) assay: Gentamicin sulfate assay was performed using an agar diffusion method discussed by Philips *et al.*(15), that is one of the most suitable method for GS assay(23). Briefly, large flat-bottomed glass assay dishes were filled to a depth of approximately 5mm with antibiotic medium seeded with *Serratia marcescense*.Wells of 7mm diameter were filled with standard and test solutions, and the plates were held for two hours at room temperature and then incubated at 37°C for 18 hours. Then the diameter of zone of growth inhibition were measured and compared to standard values.(10,15)

Calculations: For calculations regarding encapsulation efficiency, the following equation was used,

$$\% \text{ encapsulation} = 100\ (C_x - C_c)/C_o - C_c \quad (1)$$

where, C_X is the total concentration of CF after all liposomes were dissolved,

C_o is the concentration of drug in buffer solution before exposure to liposomes and C_c is the total concentration drug in buffer solution after exposure to liposomes.

Concentrations of GS and CF released by liposomes were calculated using equation 2,

$$\%\text{drug released} = 100\ (C_t - C_c)/C_T - C_c \quad (2)$$

where, C_T is the total concentration of drug after all liposomes were dissolved by Triton X_{100} (final concentration of 0.2%),

C_c and C_t are concentrations of drug in buffer alone (PBS 10 mM phosphate, pH 7.4) and in combination with enhancer, respectively, at a given time.

For diffusion cell studies, using equation 3, real concentration of gentamicin and phenol red in samples were calculated.

$$C_{tn} = C_{on} + \frac{\sum v}{V} C_{on-1} \quad (3)$$

where, C_{tn} is real concentration of sample n,

C_{on} is apparent concentration of sample n,

C_{on-1} is apparent concentration of sample n-1,

v is the volume of sample,

And V is the total volume of receiver phase.

Statistics: Each data point presents the mean of triplicates and an average of duplicate experiments. Results are shown as M±SEM and compared using student t-test. ($p<0.05$ assumed significant).

Results

Surface tension stud*ies:* According to the data obtained in this study (table 1), the average surface tension of pure water at room temperature (21°C) was 72.62 ± 0.67 mN/m. Critical micelle concentrations (cmc) for BC and SLS were found to be 25 and 30 µg/ml, respectively.; and 50-100 µg/ml, for ATS and QTS (figure 2).

Liposome size and encapsulation studies: Particle size analysis showed a mean diameter of 812.0 nm and 278.6 nm for liposomnes prepared by FT and RPE methods respectively, and encapsulation efficiencies for CF were 26.01±2.16 and 11.43±5.23% for RPE and FT liposomes, respectively. According to the results, FT liposomes had a much greater entrapment capacity than that of RPE liposomnes. Therefore, it was chosen for liposome permeation studies.

Hemolysis studies: Table 2 shows hemolytic activity of BC, SLS, ATS and QTS. In general, maximum hemolytic effect occurs at concentrations above cmc. There was a sharp increase in hemolysis with increasing concentration. Figure 3 indicates that among surfactants tested, BC has the maximum efficacy for disruption of red blood cell membrane.

Skin permeation studies: Figures 4 and 5 indicate the effect of BC, SLS, ATS and QTS on gentamicin sulfate and phenol red permeation through shed snake-skin. The effect of surfactants on membrane permeation is considerable, especially after one hour of application. The obtained results, as shown in tables 4 and 5, and figures 4 and 5 indicate that benzalkonium chloride could not significantly increase the passage of drugs through skin model. In contrast, SLS had a considerable effect on drug permeation. In comparison to negative control and BC, ATS and QTS also increased drug transport significantly ($p<0.05$).

enhanced permeaeility of gentamicin sulfate in membrane models

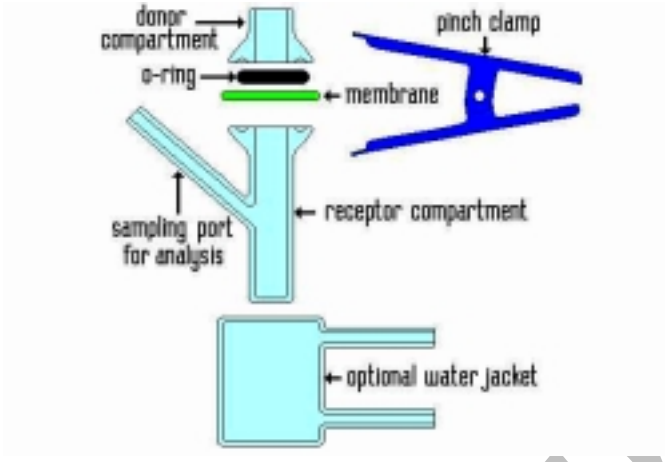

Figure 1: Franz diffusion cell designed for skin permeation study

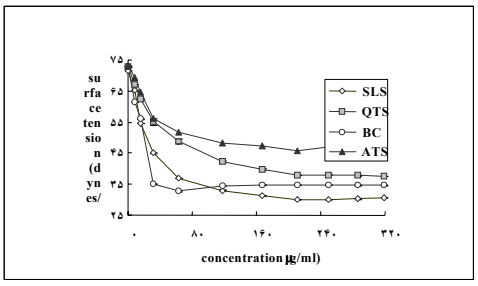

Figure 2: Surface tension changes with increasing enhancer concentration

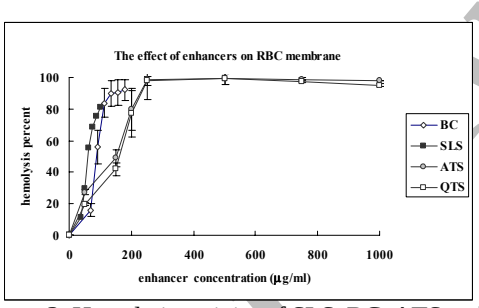

Figure 3: Hemolytic activity of SLS, BC, ATS and QTS

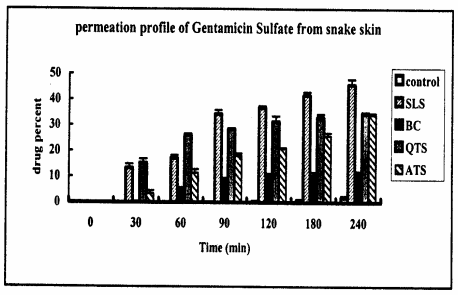

Figure 4: Permeation profile of GS in the presence of SLS, BC, ATS and QTS

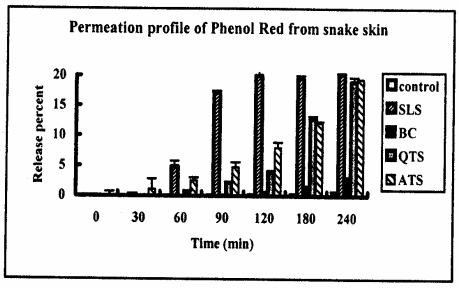

Figure 5: Permeation profile of PR in the presence of SLS, BC and QTS

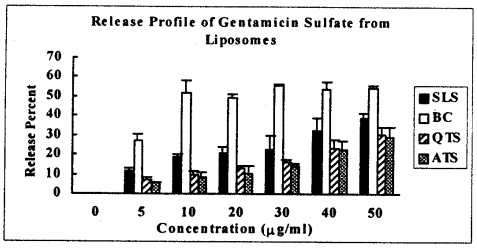

Figure 6: Release profile of GS from liposomes in the presence of SLS, BC, ATS and QTS

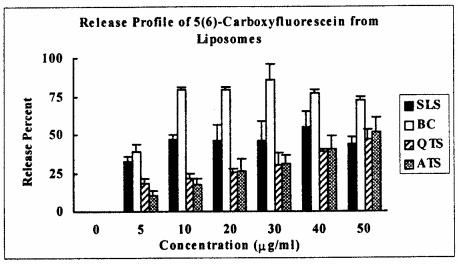

Figure 7: Release profile of CF from liposomes in the presence of SLS, BC and QTS

Table 1: Results from surface tension studies of SLS, BC,ATS and QTS

Concentration (µg/ml)	Surface tension (mN/m)			
	SLS	QTS	ATS	BC
0	71.563±0.10	72.948±0.010	71.500±0.377	72.810±0.393
8	65.157±0.074	67.113±0.010	66.333±0.311	61.567±0.119
16	54.710±0.000	62.500±0.000	62.000±0.361	56.157±0.074
32	44.948±0.147	55.157±0.947	53.011±0.361	34.920±0.034
62.5	36.825±0.024	48.649±0.810	51.677±0.445	32.743±0.097
117.65	32.878±0.101	42.300±0.339	50.333±0.473	34.270±0.000
166.67	31.213±0.024	39.740±0.250	48.334±0.298	34.690±0.000
210.53	30.058±0.015	37.880±0.000	47.333±0.153	34.649±0.081
250	29.910±0.000	37.948±0.184	46.671±0.261	34.600±0.000
285.71	30.158±0.015	37.658±0.119	45.333±0.323	34.598±0.097
319.18	30.498±0.015	37.650±0.000	44.670±0.211	34.563±0.147

Table 2: Hemolytic effect of BC, QTS and SLS

Concentration (µg/ml)	Hemolysis percent (Mean ±SD)			
	BC	QTS	SLS	ATS
0	0.000	0.000	0.000	0.000
37.5	15.942±4.238	20.195±0.982	11.570±1.780	19.144±4.111
50	55.861±10.587	24.039±4.092	29.420±2.470	27.300±1.001
62.5	83.953±8.913	27.820±0.982	55.191±1.493	30.113±2.227
75	89.747±8.254	31.539±3.110	68.519±3.118	35.023±3.333
87.5	90.54±8.161	35.195±3.601	75.754±0.401	39.197±0.923
100	92.259±6.728	38.789±0.818	81.111±2.588	47.745±3.333
150	94.718±4.721	48.800±2.890	87.465±1.618	48.85±3.0202
200	97.121±3.941	80.121±2.904	91.963±8.276	80.000±0.861
250	98.859±5.248	98.779±2.601	99.020±3.198	98.848±0.780
500	98.045±5.801	98.800±0.311	98.365±4.238	99.347±2.100

Table 3: Percentage of GS and CF released from liposomes in the presence of enhancers

Conc. (µg/mL)	Percent Released							
	ATS		QTS		SLS		BC	
	GS	CF	GS	CF	GS	CF	GS	CF
0	0	0	0	0	0	0	0	0
5	5.516±0.164	10.516±2.986	7.147±1.364	17.869±3.411	11.513±1.668	32.895±3.033	27.316±3.065	39.023±4.262
10	8.119±2.761	17.234±4.017	9.479±1.900	21.064±3.167	18.797±1.615	46.993±3.032	51.810±6.721	79.707±1.108
20	10.417±3.552	26.492±7.371	12.762±1.193	25.524±2.65	20.771±3.079	46.157±10.262	49.148±1.773	79.148±1.773
30	14.479±1.003	31.003±5.267	16.472±0.877	29.949±8.327	22.930±6.892	45.860±12.530	55.471±1.235	85.340±10.340
40	22.711±4.649	40.323±8.661	23.116±4.580	38.526±1.594	32.470±6.636	54.843±10.533	54.052±3.619	77.217±2.262
50	28.938±5.161	51.929±9.463	30.617±3.649	47.102±6.082	38.615±2.553	44.024±4.186	54.148±1.583	72.198±2.469

Table 4: Percentage of PR passed through the skin to the receptor compartment

Time (min)	PR percent permeated				
	Control	SLS	BC	QTS	ATS
0	0	0	0	0	0
30	0	0.192±0.264	0	0	1.091±0.741
60	0	4.873±0.888	0	0.750±0.077	2.511±1.733
90	0.063±0.019	17.229±0.250	0.125±0.097	2.203±0.173	4.795±0.516
120	0.189±0.086	20.019±0.841	0.628±0.153	3.997±0.217	8.005±0.792
180	0.255±0.128	19.563±0.460	1.515±0.198	12.888±0.343	12.121±0.916
240	0.635±0.122	20.283±0.164	3.046±0.096	19.018±0.740	19.415±0.250

Table 5: Percentage of GS passed through the skin to the receptor compartment

Time (min)	GS percent permeated				
	Control	SLS	BC	QTS	ATS
0	0	0	0	0	0
30	0	6.633±1.567	0.076±0.066	7.590±1.596	3.423±1.066
60	0.010±0.171	8.548±1.126	2.584±0.616	13.036±0.606	7.119±1.334
90	0.104±0.257	17.130±1.620	4.403±0.680	14.165±0.392	11.250±0.987
120	0.417±0.034	18.388±0.655	4.985±1.261	15.681±2.176	13.011±0.414
180	0.894±0.030	20.842±1.192	5.151±1.420	16.539±1.155	16.125±1.169
240	1.756±0.738	20.842±1.192	5.456±1.148	17.254±0.542	20.003±0.667

Permeability of liposomes: The permeation profiles of CF and GS in the presence of SLS, BC, ATS and QTS through liposomes are shown in table 3 and figures 6 and 7. As indicated in figure 7 for CF, there was an increase in drug release with increasing surfactant concentration. Also, it is evident that the effect of BC is more considerable than the other enhancers. These results demonstrate enhancement in release of GS from liposomes in the presence of surfactants, although the effect is not as significant as for CF (figure 6).

Discussion

This article describes the effect of four surface active agents on the permeability of two membrane models to GS. Among various methods available for testing, *in vitro* permeation studies, diffusion cells possess many advantages. Among them, they permit the study of factors affecting the process and the compatibility of components, both with each other and with the drug(13).

Many drugs permeate human skin too slowly to produce therapeutic plasma concentrations. Various substances have been identified that increase percutaneous absorption of given drugs. Some probably act as cosolvent for the drug, allowing an increase in its activity within the vehicle. Others appear to produce some structural change in the skin tissues, thereby altering the skin resistance to drug permeation(9). In addition to the absorption characteristics, the success of percutaneous therapy depends on its access to the skin with sufficient speed and quantities from the system employed(13). Application of these enhancers has been limited, however, probably due to the skin irritation they cause. Therefore the development of any successful drug delivery agent will require a thorough analysis of local and systemic toxicity(8).

The main determining step in skin permeation rate is the drug diffusion through stratum corneum, that is the main barrier for skin permeation. One of the most important properties of stratum corneum, is its high hydration potency, which modulate chemical permeation through the skin(16). Although human skin is the most preferred for all permeation studies, its use is not always possible because of limitation in availability(1). In the present study shed snake-skin was used, which is hairless and consists of pure keratinic cells to evaluate transport of GS. Advantages of shed snake-skin include its biochemical similarity to human stratum corneum, the availability of multiple samples from the whole shed skin, easy handling and relatively small sample variation(8). It has been reported that shed snake-skin response to lipophilicity and molecular weight is very similar to human stratum corneum. In addition, evaporation rates of water from shed snake-skin and human skin are similar(16).Various substances have been identified that increase percutaneous absorption of certain drugs. Among them, surfactants are believed to differ skin's resistance to drug absorption. We used SLS, BC, ATS and QTS that are anionic, cationic and two naturally origin surfactants, respectively. In addition to GS, we used PR as a water soluble drug model. PR is a drug model usually used in drug delivery studies. GS and PR are highly soluble in water, therefore, they may pass through intercellular spaces between keratinic cells (13).

The obtained results suggest that there is an increase in drug permeability due to presence of ATS, QTS and especially, SLS. However in spite of high surface activity and hemolytic effect, BC had no effect on drug permeation. The lack of efficacy can be described in charge interaction term. It is proved that the potential for using surfactants as absorption enhancing agents for percutaneous delivery of water-soluble drugs would depend on several factors, including electric charge of the molecule. According to a theory, surfactants increase percutaneous absorption of drugs by producing some structural changes in the skin tissues and thereby altering the skin's resistance to drug permeation(9). Possible repulsive forces between charges of shed snake skin surface and BC ion may inhibit the interaction between enhancer molecule and the membrane, which is necessary for absorption promoting action of formulation, and could be the probable cause of the lack of efficacy of BC (17).

Another membrane model used in our study was liposome made by cholesterol and phosphatidylcholine. Liposome membrane is different from the skin model in nature. Liposomes are composed of lipid molecules that are arranged so that their hydrophilic portions are in contact with aqueous media. In addition to GS, we used CF as another water-soluble drug model to evaluate drug release from liposomes. CF is a fluorescent material that can be easily assayed in trace amounts using a fluorimeter.

Based on the results, liposome permeability was increased upon the addition of enhancers. The effect was more considerable in GS model, where up to 80% of drug was released at higher concentrations of enhancers. The maximum increase is in permeability was found to occur below the critical micelle concentrations, suggesting that micelle formation is not the main mechanism of action. The precise mechanism of surfactant action is not known. However, the results of the study by Pillion *et al.* are consistent with the present study in that saponins can cause a change in membrane structure to make it more permeable(17). There could be a similar mechanism for other surfactants. Reduction of the rate of transport of the drug combined with enhancer after critical micelle concentration is attributed to

the ability of surfactants to form micelles and is normally seen only if interaction between micelle and the drug occurs. Solubilization of the drug by surfactant micelles decrease the thermodynamic activity of the drug, and hence, decreases the driving force of the drug absorption. Therefore, the overal effect of a enhancer on the rate of drug permeation across a membrane will be a combination of the influence of these two opposing effects(20).

One further point is that no correlation was found between maximum hemolytic activity, which occurs at concentrations higher than cmc, and maximum permeation enhancement, which generally is seen with concentrations below the cmc of surfactants in this study.

Conclusion

Based on the results of the present study, it is suggested that direct effect of surfactants on shed snake-skin and liposomal membranes is responsible for their enhancing effects. Also it can be concluded that the extent of enhancement action of surfactants depends not only on the surfactant nature, but also on other properties of surfactant such as electric charge and polarity of both enhancer and the membrane model. This study also showed that enhancer concentration has a significant effect on skin permeability.

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