ELSEVIER

International Journal of Pharmaceutics 212 (2001) 73–80

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Release of salicylic acid, diclofenac acid and diclofenac acid salts from isotropic and anisotropic nonionic surfactant systems across rat skin

Nancy H. Gabboun [a], Naji M. Najib [a], Hussein G. Ibrahim [b,*], Shereen Assaf [a]

[a] *Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan*
[b] *Arab Company for Drug Industries and Medical Appliances (ACDIMA), P.O. Box 925161, Amman, Jordan*

Received 22 March 2000; received in revised form 29 August 2000; accepted 21 September 2000

Abstract

Release of salicylic acid, diclofenac acid, diclofenac diethylamine and diclofenac sodium, from lyotropic structured systems, namely; neat and middle liquid crystalline phases, across mid-dorsal hairless rat skin into aqueous buffer were studied. Release results were compared with those from the isotropic systems. The donor systems composed of the surfactant polyoxyethylene (20) isohexadecyl ether, HCl buffer of pH 1 or distilled water and the specific drug. High performance liquid chromatography (HPLC) methods were used to monitor the transfer of the drugs across the skin barrier. Results indicated that the rate-determining step in the transport process was the release of the drug from the specified donor system. Further, apparent zero order release was demonstrated with all systems. Except for diclofenac sodium, drug fluxes decreased as the donor medium changed from isotropic to anisotropic. The decrease in fluxes was probably due to the added constrains on the movement of drug molecules. By changing the anisotropic donor medium from neat to middle phase, drug flux decreased in case of salicylic acid and diclofenac sodium. In the mean time, flux increased in case of the diethylamine salt and appeared nearly similar in case of diclofenac acid. Rates of drug transfer across the skin from the anisotropic donors seemed to be largely controlled by the entropy contribution to the transport process. The type and extent of drug-liquid crystal interactions probably influenced the latter.

Keywords: Liquid crystals; Nonionic sufactant; Rat skin; Rate of drug release; Franz diffusion cell; Drug-liquid crystalline system interactions

1. Introduction

Certain surfactants in water, at given temperature and concentration, afford good examples of lyotropic liquid crystals (LLC). As the concentra-

* Corresponding author.

0378-5173/01/$ - see front matter
PII: S0378-5173(00)00585-8

tion of the sufactant is increased, different LLC polymorphic forms could be obtained. More than one form of liquid crystals could exist. Neat (lamellar), middle (hexagonal) and cubic phases are the three well known forms of LLC (Brown, 1972; Florence and Attwood, 1988). Formation, properties and characteristics of these mesophases are adequately described in the literature (Brown, 1972; Martin, 1993; Ibrahim, 1989; Ibrahim et al., 1993).

Lyotropic mesophases could be considered as ordered micelles with molecular arrangement characterized by alternate hydrophobic and aqueous hydrophilic regions. Solutes dissolved in LLC usually reside in these two regions and also could be incorporated in the surfactant bilayer of the mesophase (Muller-Goymann and Frank, 1986). Lipophilic and amphiphilic drug molecules reside in the lipid bilayer of the structure (Larrson, 1994). Molecules dissolved in such systems suffer from structure-imposed constrains, which would affect their extent of mobility and direction of movement. Drug molecules present in the aqueous region of the mesophase in an undissociated form, are the main molecular species that would be able to diffuse out and cross lipophilic barriers. However, solute-solvent interactions play an important role in controlling rate of drug transport from these systems. Therefore, solutes in the LLC systems would be released at rates depending on the mesophase structure and type of dissolved solutes. These properties made it possible to use liquid crystals as drug carriers, solublizing vehicles and to prepare controlled-release formulations (Ericsson et al., 1991; Norling et al., 1992; Wyatt and Dorsehel, 1992; Larrson, 1994).

As a consequence of solute-solvent interactions, dissolved solutes, under certain conditions, could induce changes in the molecular packing of the LLC vehicle. This effect could influence the rate of release from these donor systems. Ibrahim and co-workers investigated the effects of solute characteristic and concentration on neat and middle phases (Ibrahim et al., 1993). Also, other workers investigated the nature of drug interaction with components of mesomorphic vehicles (Muller-Goymann and Frank, 1986). In both investigations, in-vitro drug release was correlated with apparent effects of interactions of drug molecules with the vehicles.

In a therapeutic transdermal system (TTS), the vehicle reservoir, the membrane, or the adhesive layer could be made rate determining to control transport of the drug across the skin. It would be of interest to assess the potential use of LLC as rate determining-vehicle reservoir in transdermal patches. In line with this notion, the current study aimed to characterize the release of representative drugs, namely; salicylic acid, diclofenac acid, and its salts, from two forms of LLC across rat skin. The mesomorphic vehicles composed of the nonionic surfactant polyoxyethylene (20) isohexadecyl ether in water or hydrochloric acid buffer and the drug. In addition, effects of interactions of solutes with the isotropic and anisotropic donor media on release across rat skin were delineated.

2. Materials and methods

Polyoxyethylene (20) isohexadecyl ether, was obtained from ICI Americas, USA, the surfactant is marketed under the name Arlasolve 200. Salicylic acid (SA) LR, potassium dihydrogen orthophosphate LR, disodium hydrogen orthophosphate LR and concentrated hydrochloric acid (HCl) were obtained from Laboratory Rassyan, India. Diclofenac sodium (DS), diclofenac diethylamine (DDEA) and flufenamic acid were donated by Dar Al Dawa Development and Investment Co. LTD, Jordan. Phenacetin was obtained from Cambrian Chemicals, UK. Ammonium acetate AR and acetic acid glacial were obtained from C.B.H. LAB. Chemicals, UK. Sodium acetate trihydrate was obtained from JCLE, UK. Potassium chloride was obtained from Panreac, Spain. Normal saline B.P. was obtained from Bieffe Medital, Italy. Acetonitril HPLC grade was obtained from LABSCAN, Ireland. Octanol was obtained from Merck, Germany. All material was used as supplied.

Hydrochloric acid buffer of pH 1 and phos-

phate buffer of pH 7.4 were prepared according to USP XXI (1985), and BP (1993), respectively. The water used was deionized and distilled in an all glass still, and it will be referred to as distilled water. A 0.1 μm cellulose nitrate millipore filter was obtained from Sartorius, Germany.

A crown glass Franz diffusion cell, USA, a Haake water-heater circulator, Germany and a Labinco L 34 magnetic stirrer, Holland, were used as parts of permeation set-up. A Desaga STA-peristaltic pump, England, was used in the permeation studies through filter membrane. Toshiba digital clinical thermometer, Japan, was used for measuring the temperature of the receiving medium in the diffusion cell. A Shimadza UV-1201 spectrophotometer, Japan, was used.

The high performance liquid chromatography (HPLC) system used consisted of Spectra-Physics Isochrom LC pump, USA, Beckman injection head of 50 μl loop volume, USA, Jasco 875-UV detector (range 0.08), Japan, Spectra-Physics Chromget integrator (attenuation 8), USA, and SGE ODS column (5 μm size, 250 cm length), Australia. Other HPLC accessories were also used in this research, and they include Beckman 114 M pump, Beckman 340 injection head of 20 μl loop volume and Beckamn L65 detector (range 0.05), USA, or Varian 2050 detector (range 0.025), USA. These two detectors were connected to a Spectra-Physics 4270 integrator (attenuation 8, 2, respectively), USA.

Lietz, Orholux II POL-BK microscope, Germany, was used to examine L.C. systems for their birefringence and texture between crossed polarizers. Alietz Orthomat camera, Germany, fitted to the microscope was used for taking photomicrographs of LC systems.

A Shimadzu infrared spectrophotometer-435, UV–VIS spectrophotometer-240 Graphicord, Graphic printer PR-1, Japan, Gllen lamp melting point apparatus, Germany, and Hana Instrument 8520-pH meter, Italy, were used. Assistant Reamix 2789 vortex, Eppendorph centrifuge 54145, Holland, Julabo, SW-20c, water-bath and Moser 1400 electric hair clipper, Germany, were used. GFL 2008 distillator, and Seral Seradest 5600 deionizer, Germany, were used for distillation and deionization of water, respectively.

2.1. Preparation of diclofenac acid

Diclofenac acid was prepared from diclofenac sodium by acid precipitation method. 20 g of diclofenac sodium were dissolved in 2 l water and stirred well while protecting from light. A 1 N HCl solution was added dropwise with stirring until complete precipitation was obtained. The precipitate was filtered and washed with distilled water and then air dried to a constant weight. Infrared spectroscopy and melting temperature measurements were used for the identification of DA, and its salts DDEA, and DS. The results were checked against reported data (Moffat et al., 1986).

2.2. Preparation of the permeation barriers

In part of the study, millipore filter membranes were impregnated with octanol and were used as lipophilic barrier. In the main bulk of the study skin from Sprague–Dawley adult male rats of about 350 gm each were used in the permeation studies. The animals were scarified by cervical dislocation after anesthetizing them with ether. Hair was removed using electric hair clipper. One circular piece of skin was taken from the dorsal area of each rat using surgical blades. Subcutaneous tissue was removed using forceps, and full thickness skin was used. The skin was washed with distilled water and normal saline, and kept between two filter papers impregnated with normal saline, in a plastic bag at 4–8°, to be used within 7 days. Before mounting the skin in to the diffusion cell, it was washed with distilled water and phosphate buffer of pH 7.4.

2.3. Preparation and examination of donor systems

A known weight of the sufactant was melted at 60°C in a water bath. An accurate weight of the drug was added to the surfactant melt to achieve the required concentration. Known weight of either HCl buffer of pH 1 or distilled water was added in one portion with gentle stirring. All systems were allowed to equilibrate for 48 h before use.

Donor systems were examined macroscopically for their fluidity and general appearance, and microscopically between crossed polarizers, for their birefringence and texture. Photomicrographs of different L.C. systems were taken at room temperature and the indicated magnifications.

2.4. Permeation set-up

In all permeation studies, a Franz diffusion cell, consisting of two compartments; an upper one for the donor system and a lower one for the receiving medium, was used. The lower compartment has a volume of 11.2 ml. The cell was connected to a water-heater circulator operating at 37°C. Phosphate buffer of pH 7.4 was used as the receiving medium. The solution was continuously stirred using a 7 mm magnet, and a magnetic stirrer operating at a constant rotation. The impregnated filter membrane or rat skin was mounted between the two compartments of the diffusion cell. The assembled set-up, with the receiving medium and the permeation barrier were left to equilibrate to the experimental temperature. About 2.5 gm of the donor system previously equilibrated to the experimental temperature was carefully placed in the upper compartment of the diffusion cell. Preheating of the highly viscous unflowable donor systems (those containing 44% w/w of the surfactant) was necessary to make them easily pourable into the donor compartment.

2.5. HPLC analysis

SGE ODS column was used through out the permeation studies. The HPLC conditions were as follows: the pump was operated at a flow rate of 1.5 ml/min, and the UV detector was operated at $\lambda = 295$ nm to monitor SA, and at $\lambda = 280$ nm for DA, DDEA and DS. The operating ranges and attenuations of the instruments were adjusted so that the detection limit would be 2 μg/ml for SA, and 0.02 μg/ml for the other drugs.

The mobile phase used for SA assay was prepared by adding 15 ml acetic acid and 0.25 g ammonium acetate to 300 ml acetonitrile. The volume was completed to 1000 ml using distilled water. The final ratio of acetonitrile to water was (30:70), and pH was 3.0.

The mobile phase used for DA, DDEA and DS assay was prepared by adding 10 ml acetic acid and 10 ml sodium acetate to 600 ml acetonitrile. The volume was completed to 1000 ml using distilled water. The final ratio of acetonitrile to water was (60:40), and pH was 4.8.

2.6. Typical release experiment

Release experiments were run at least in duplicate for 24 h. At suitable time intervals, samples of 100 μl each were carefully removed from the receiving medium, using a microliter syringe. Samples were withdrawn from the sampling port of the diffusion cell and collected into eppendorphe tubes. Samples were replaced with equal volume of fresh receiving medium.

20 μl of phenacetin solution having a concentration of 400 μg/ml was added as internal standard for the sample of SA. Similarly 20 μl of flufenamic acid solution having a concentration of 3 μg/ml, was added for the samples of other drugs. Samples were mixed using a vortex mixer, then centrifuged for 1 min using Eppendorphe centrifuge. The concentrations of the permeating drugs were determined using HPLC methods.

3. Results and discussion

The type and stability of the donor systems containing the probe drugs were verified using polarizing microscope. Comparison of donor systems with controls and published data (Ibrahim, 1989) confirmed the phases of the donor systems under study and indicated that presence of the different probes at the given concentrations did not disrupt the liquid crystal structure. Further, preliminary release studies using octanol-impregnated filter membrane and rat skin from different locations in the dorsal area, demonstrated the reproducibility of the donor systems, the reliability of the procedure and that the skin of choice is from the mid-dorsal area. Table 1 describes the macroscopic and microscopic appearance and characteristics of the systems used in the study.

The fluidity of the donor systems ranged from pourable liquid, to viscous liquid and unflowable gel. It is significant to know that, the different donor systems were prepared using the same surfactant. The use of the same surfactant provided environment of similar chemical nature around the dissolved molecules; hence, qualitatively maintaining the same enthalpy interactions in both the isotropic and anisotropic media.

Fig. 1 shows release profiles of SA from isotropic, neat and middle phases across mid-dorsal rat skin. From Fig. 1, it is apparent that, after a lag time period, a zero-order steady state flux was established in each of the three systems. Flux values were calculated from the slope of the straight-line portion of the release profiles. Further, lag times were obtained from the intercept of the extrapolated straight line with the time axis. Assuming that the thickness of the diffusion layer in the three permeation systems is nearly similar, the differences in the observed lag times of Fig. 1 could be interpreted as a consequence of different diffusivities of SA in the different phases. Release data of the other probe drugs were processed in a similar fashion. Fig. 2 depicts a plot of the steady state fluxes of SA as a function of the surfactant concentration. The close similarity of the flux values from the two isotropic donors, which had an 8% difference in surfactant concentration, suggests similar overall effects on release arising from the enthalpy and entropy interactions of SA with the two isotropic solvent media. In ideal solutions, the enthalpy of diffusive mixing is zero. In real solutions, on the other hand, there is an enthalpy input in the overall free energy of diffusive mixing; however, its magnitude is relatively small and the diffusion process can be regarded as an entropy driven process (Flynn et al., 1974). Release data from the two isotropic donors suggest that the difference in their chemical composition had a minimum effect on rate of release across the skin. Hence, it is safe to conclude that the enthalpy input arising from the 8% difference in surfactant concentration was insignificant. Release data are subsequently interpreted in light of this characteristic.

Correlation between the published phase diagram of the used surfactant (Ibrahim, 1989) and Fig. 2, indicates that increasing the surfactant concentration from 30 to 38% induced a phase change from isotropic to a neat anisotropic, with a concomitant decrease in the flux of about 51.8%. Further increase in surfactant concentration from 38 to 44% induced another phase change from neat to middle LLC and an accompanying decrease in the flux of SA of about 59.6%. The fact that fluxes of SA across the skin were (a) very similar from systems within the isotropic range; (b) differed as one examined release from isotropic, neat and middle phases in relation to one another, suggests that the controlling step in the transport of SA is the donor medium. Plots of the steady state fluxes of DA and its salts DDEA and DS as a function of the surfactant concentration of the donor systems are shown in Fig. 3. Examination of these plots sug-

Table 1
Macroscopic and microscopic characteristics of donor systems containing different surfactant concentrations and different drugs* under polarized light and at room temperature[a]

Surfactant conc.(%w/w)	SA		DA		DDEA		DS	
	Fluidity	Phase	Fluidity	Phase	Fluidity	Phase	Fluidity	Phase
22	Liquid	Isotropic	Liquid	Isotropic				
30	Liquid	Isotropic	Liquid	Isotropic	Liquid	Isotropic	Liquid	Isotropic
38	Viscous L.	Neat	Viscous L.	Neat	Viscous L.	Neat	Viscous L.	Neat
44	Unflowable gel	Middle	unflowable gel	Middle	unflowable gel	Middle	unflowable gel	Middle

[a] L, Liquid; *, drug concentration was maintained at about 0.50% (w/w) in all the systems.

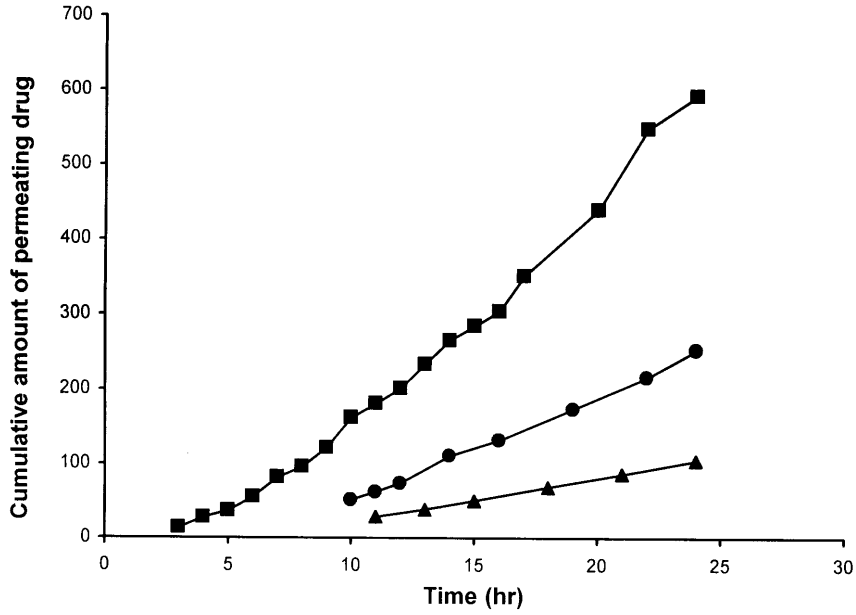

Fig. 1. Cumulative amounts (ug) of SA permeated through mid dorsal hairless rat skin, form isotropic and anisotropic donor systems, containing 0.50% w/w SA, at 37°C. Key: (■) Isotropic donor containing 30% w/w of the surfactant. (●) Anisotropic neat donor system containing 30% w/w of the surfactant (▲) Anisotropic middle donor system containing 44% w/w of the surfactant.

gests the same conclusion arrived at for SA, i.e. the controlling step in the transport of DA and its salts, at the given concentration and temperature, were the donor media and not the skin.

Figs. 2 and 3 show slower fluxes for SA, DA, and DDEA from anisotropic media than from isotropic media. These results correlate with reports in the literature documenting higher activation energy of diffusion and lower diffusivities for drug agents diffusing from structured environment in comparison to isotropic media (Muller-Goymann and Frank, 1986; Ibrahim, 1989; Tiemessen et al., 1990). It is probable that constraints imposed on the movement of molecules dissolved in structured environment decrease their random motion and lower the probability of reaching the skin for diffusion. These constraints could arise from the imposed directional diffusion and the need of dissolved molecules to partition from the hydrophobic to the hydrophilic or aqueous domains of the structured lyotropic donors to diffuse through the skin.

The anomalies in release of DA, DS, and DDEA as shown in Fig. 3 probably were due to differences in their structural characteristics and ability of the different media to accommodate them. An attempt to explain these anomalies is as follows. (a) Fluxes of DA from the neat and the middle donors were nearly similar. Based on preliminary release studies from the isotropic media, it is reasonable to assume that, the 6% difference in surfactant concentration between the neat and the middle phase donors could also have minimal enthalpy contribution to transport, i.e. the transport process is mainly entropy controlled. Consequently, one could conclude that the net entropy effects on transport of DA from the lamellar and the hexagonal phases were nearly equal resulting in equal rates of transport from the two donors. (b) Flux of DDEA from the hexagonal middle phase was slightly faster than from the lamellar neat phase. This finding suggests that the polarity, configuration, and size of DDEA molecules afforded higher compatibilty between the dissolved drug and the lamellar structure than with the hexagonal structure. In other words, entropy changes in the donor system favored release of DDEA across the skin from the hexagonal phase

more than from the lamellar phase. (c) Fluxes of DS from the isotropic and neat donors were very similar and were more than double that from the middle hexagonal donor. DS is an organo-metalic salt, which is expected to be ionizable in the aqueous environment. From release data, it is possible that a significant proportion of DS molecules was squeezed-out from the hydrophobic region as well as the lamellae of the neat phase, and probably resided in the aqueous region. Thus, similar fluxes from the isotropic and the neat donors. It is also possible that DS molecules were accommodated to a high degree by the hexagonal array of the long cylindrical miscelles of the middle LLC; thus, limiting their availability for diffusion from the aqueous environment of the mesophase across the skin.

The results of this investigation indicate that, transport of DA and its salts across the rat skin were more than thirty times slower than SA. These results could be attributed to structural differences between the diclofenac compounds and SA in so far molecular weight, shape and size, as well as polarity, polarizability and configuration of the molecules are concerned. It is also concluded that solute–solvent interactions in the anisotropic environment, in contrast to such interactions in the isotropic medium, caused the release of DA and its salts to proceed at different rates. Therefore, as an application to these results, the notion of using LLC as a rate-determining vehicle-reservoir in a transdermal patch could be put to test. In the process, a specified drug could be delivered across the skin at a zero-order rate using a lyotropic vehicle along with a noncontrolling membrane. Attainment of a specified rate could be achieved through the proper choice of the mesophase and the drug concentration.

Acknowledgements

The authors would like to thank The Arab Company for Drug Industries and Medical Appliances (ACDIMA) for supporting the publication.

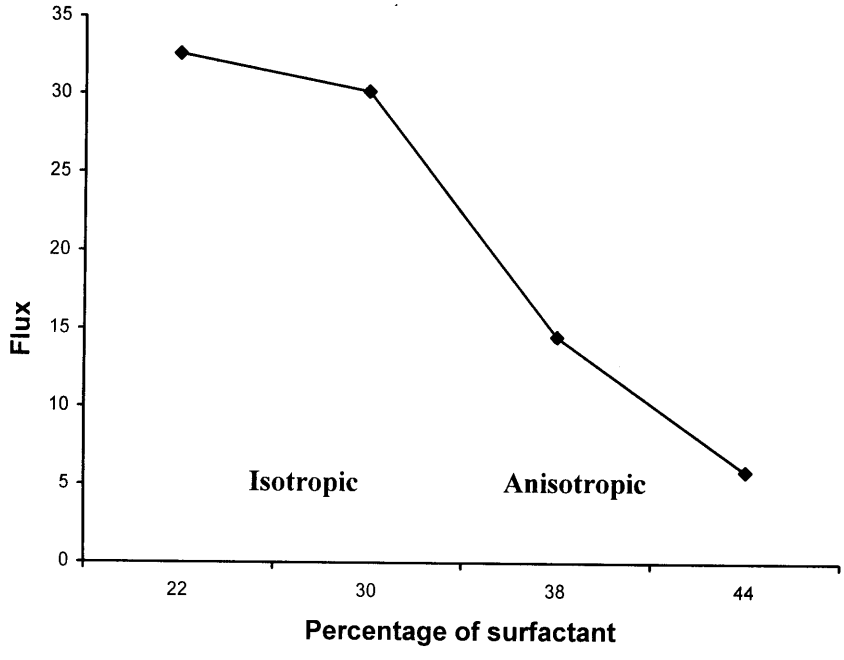

Fig. 2. Steady-state fluxes (ug/hr) of SA through mid-dorsal hairless rat skin, from isotropic and anisotropic donor systems at 37°C, as a function of the surfactant concentration.

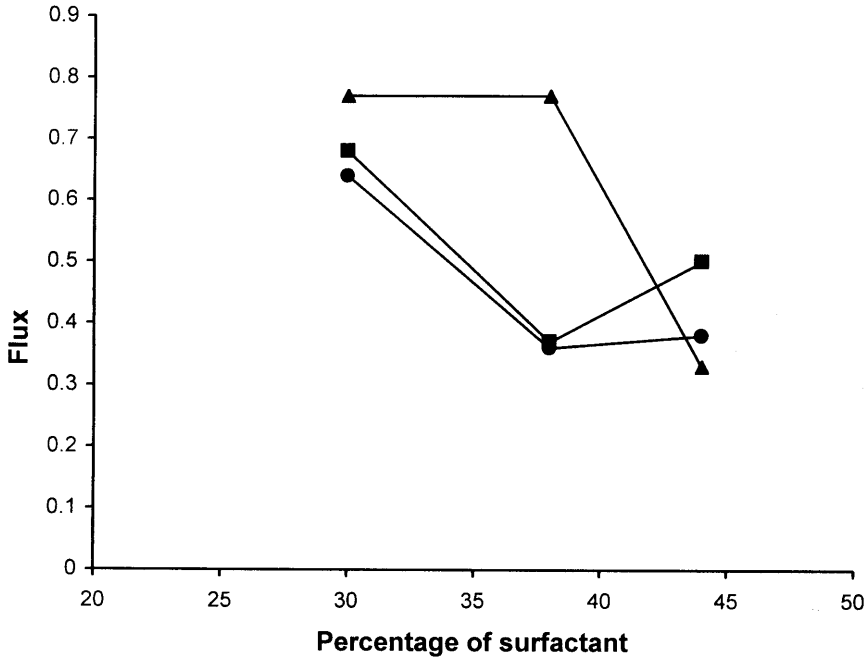

Fig. 3. Steady-state fluxes (ug/hr) of DA, DDEA, DS through mid dorsal hairless rat skin, from isotropic and anisotropic donor systems at 37°C, as a function of the surfactant concentration. Key: (●) DA, (■) DDEA, (▲) DS.

References

Brown, G.H., 1972. Liquid crystals and their roles in inanimate and animate systems. Am. Scientist 60, 64–73.

Ericsson, B., Eriksson, P.O., Lofroth, J.E., Engstrom, S., 1991. Polymeric drugs and drug delivery systems. Am. Chem. Soc. 22, 251–265.

Florence, A.T., Attwood, D., 1988. Physicochemical Principles of Pharmacy, second ed., Macmillan, New York, Chapter 6, pp. 209, 246–247.

Flynn, G.L., Yalkowsky, H.L., Roseman, T.J., 1974. Mass transport phenomena and models: theoretical concepts. J. Pharm. Sci. 63, 479–510.

Ibrahim, H.G., 1989. Release studies from lyotropic liquid crystal. J. Pharm. Sci. 78, 683–687.

Ibrahim, H.G., Sallam, E., Takieddin, M., Habboub, M., 1993. Effects of solutes characteristics and concentration on a lyotropic liquid crystal: solute-induced phase change. Pharm. Res. 10, 737–742.

Larrson, K., 1994. Lipids-Molecular Organization, Physical Functions and Technical Applications. The Oily Press, Dondee, p. 171.

Martin A., 1993. Physical Pharmacy Lea and Fiber Philadelphia, 4th ed., Chapter 2, pp. 24, 36–37.

Moffat, A.C., Jackson, C.V., Moss, M.S., Widdop, B., 1986. Clarke's, isolation and identification of drugs. In: Pharmaceuticals, Body Fluids and Post-Mortem Material, second ed. The Pharmaceutical Press, London, pp. 533–534 Part 2.

Muller-Goymann, C.C., Frank, S.G., 1986. Interaction of lidocaine and lidocaine HCl with the liquid crystal structure of topical preparation. Int. J. Pharm. 29, 147–159.

Norling, T., Lading, P., Engstrom, S., Larsson, K., Krog, N., Nissen, S.S., 1992. Formulation of a drug delivery system based on a mixture of monoglycerides and triglycerides for use in the treatment of periodontal disease. J. Clin. Periodontal 19, 687–692.

Tiemessen, H.L.M., Maarel, J.R.C.V., Bodde, H.E., Jousman, H., Junginger, H.E., 1990. Probing the microstructure of liquid crystalline surfactant systems, physicochemical study using reometry, DTA and ^{17}O NMR. J. Control. Rel. 13, 33–81.

Wyatt, D.M., Dorsehel, D., 1992. A cubic-phase delivery system composed of glyceryl mono-oleate and water for sustained release of water-soluble drugs. Pharm. Technol., 116–130.