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Interaction of phloretin and 6-ketocholestanol with DPPC-liposomes as phospholipid model membranes

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Abstract

Phloretin and 6-ketocholestanol are penetration enhancers for percutaneous delivery of certain topically applied drugs. In the present study some physicochemical experiments have been performed to elucidate the mechanism of action of phloretin and 6-ketocholestanol. The penetration enhancing effect of phloretin and 6-ketocholestanol is believed to be due to their increase of the fluidity of the intercellular lipid bilayers of the stratum corneum. Phospholipid vesicles were chosen as a simple model to represent these bilayers. The effect of phloretin and 6-ketocholestanol on phase transition temperature and enthalpy was studied using differential scanning calorimetry. Beside of that the size of liposomes was monitored when the amount of penetration enhancer in the liposome preparation was changed. Addition of increasing amounts of phloretin and 6-ketocholestanol to the bilayer resulted in lowering of phase transition temperatures and increasing the enthalpy. Additionally the size of the liposomes was increased when penetration enhancer was added. The results suggest that phloretin as well as 6-ketocholestanol would interact with stratum corneum lipids in a similar manner, both reduce the diffusional resistance of the stratum corneum to drugs with balanced hydrophilic–lipophilic characteristics.

Keywords: Phloretin; 6-ketocholestanol; DPPC; Liposomes; DSC

1. Introduction

In the last years, the role of the stratum corneum in the barrier function of the skin has been thoroughly investigated (Moore et al., 1988; Wertz and Downing, 1989; Barry, 2001; Hadgraft, 2001). Two major routes of drug penetration through the human stratum

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corneum have been proposed: the transcellular and the intercellular pathways (Michaels et al., 1975; Elias, 1983; Barry, 1987). For most drugs, the major barrier effect of the stratum corneum has been attributed to the intercellular lipids, mainly to their nature and to their ordered multilayers (Madison et al., 1987; Williams and Elias, 1987; Wertz and Downing, 1989). Consequently, in order to extend the variety of drugs that might be administered via the skin and also to increase the local activity of topically applied drugs, considerable attention has been focused on the mechanism of action of skin penetration enhancers (Stoughton, 1982; Aungst et al., 1986; Barry, 1987; Beastall et al., 1988; Walters, 1989).

Most of the penetration enhancers have been proved to interact in some way with the stratum corneum lipid structure, generally by increasing the fluidity of the intercellular lipid bilayers (Barry, 1988). In recent studies two new penetration enhancers have been proposed. It could be shown that 6-ketocholestanol enhanced the flux of bacitracin, sodium fluorescein and 5-ALA (Cladera et al., 2003; Auner et al., 2003a, 2003b, 2004a) and it could be demonstrated that phloretin enhanced the permeation of lidocaine and progesterone (Auner and Valenta, 2004b; Valenta et al., 2001).

The aim of the present study was, to show interactions of phloretin and 6-ketocholestanol with multilamellar vesicles (MLV or liposomes) of dipalmitoylphosphatidylcholine (DPPC), concerning the phase transition temperature as well as the enthalpy. Since thermodynamic parameters for the gel-to-liquid crystalline phase transition of liposomes are best obtained by DSC, the effect of phloretin and 6-ketocholestanol on the thermodynamic properties of MLV was investigated using this technique. Besides that additional size measurements of liposomes was performed. The stability of the vesicles was checked employing a microreaction calorimeter.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Lipoid (Switzerland).

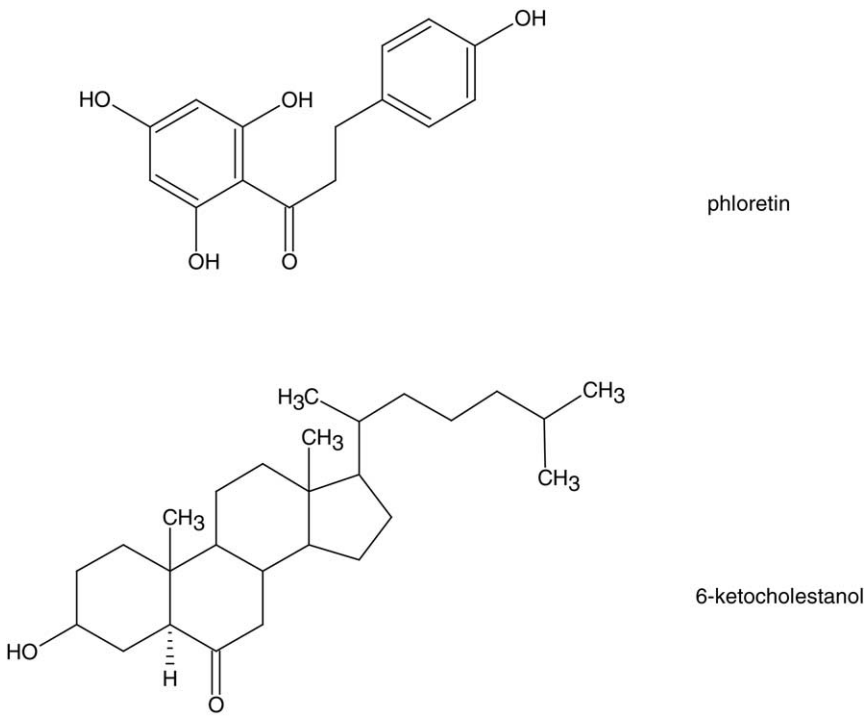

Fig. 1. Chemical structure of phloretin and 6-ketocholestanol.

The product was Lipoid PC 16:0/16:0. According to its specification the content of phosphatidylcholine related to the dry weight was at least 99%. Phloretin and 6-ketocholestanol were obtained from Sigma (St. Louis, USA). All other chemicals used in the study were of analytical reagent grade and were used as received without any further purification.

The chemical structures of the two penetration enhancers phloretin and 6-ketocholestanol are presented in Fig. 1.

2.2. Liposome preparation

Neutral liposomes (MLV) were prepared from DPPC using a procedure derived from the solvent evaporation method, originally described by Bangham et al. (1974). In brief, MLVs were made by dissolving DPPC in chloroform/ethanol (3–4, freshly prepared) in a round-bottomed flask. The flask was attached to a rotary evaporator and held at 40 °C with vacuum applied for 15 min for solvent evaporation and thin film formation. After all solvent has evaporated the phospholipid film was hydrated with 60 °C deionised water (temperature above the transition temperature of DPPC) to give a lipid concentration of 62.5 mg/ml by shaking for 10 min and annealing for 2 h. The suspension was then left overnight at 6 °C in order to achieve maximal liposome hydration. Penetration enhancers (from 5 to 30 mol%) were incorporated in the liposome structure by first dissolving phospholipids and enhancers together in chloroform/ethanol. The preparation of liposomes was then carried out as described above. In order to ensure sample uniformity the liposomes were carefully homogenised using an automatic pipette.

2.3. Size measurement of MLVs

Liposome size was measured using laser diffraction with a Mastersizer (Malvern Instruments).

2.4. Differential scanning calorimetry (DSC)

DSC was performed using an HSDSC (Setaram, DSC III). 0.8 ml of the liposomal suspension containing an average of 50 mg DPPC were sealed in a pan. The samples were analysed by heating at a scanning rate of 1 °C/min over the temperature range 20–65 °C, using deionised water as reference.

2.5. Stability experiments

Stability experiments were performed on a Microreaction Calorimeter (AnalytiCal^{TM}). One millilitre of liposomal preparation was analysed in the calorimeter against a reference of deionised water over a period of 5 h at a constant temperature of 25 °C.

To ensure that liposomes remain stable over the whole observation period stability experiments with a microreaction calorimeter were performed. Results showed that liposomes were stable over the whole time period and did not degrade at any rate.

2.6. Data analysis

Results are expressed as the means of at least three parallel experiments ± S.D. Thermal transitions were analysed using Origin software. After baseline subtraction, raw power data were converted to molar heat capacity data. Baselines were fitted to the pre-transition and main transition regions using a linear baseline function so that transition temperatures and enthalpies of reaction could be calculated for each enhancer concentration. Linear onset was calculated by extrapolating the up-shift of the peak to the baseline.

Statistical data analysis was performed using the Student's *t*-test with $p < 0.05$ as the minimal level of significance. Calculations were done using the software Xlstat Version 5.0.

3. Results and discussion

Recent developments in transdermal drug delivery have brought about a renewed interest in the mechanisms of percutaneous absorption. In order to extend the range of drugs which can be administered via the skin and also to increase the efficiency of locally acting drugs it is necessary to include penetration enhancers in the formulations. The mechanism of action of penetration enhancers is not clearly understood. However, if these can be identified it should be possible to rationalise and optimise their structural design.

DPPC liposomes were prepared with increasing enhancer content and their size was monitored. The

Table 1
The effect of incorporation of phloretin into DPPC liposomes on the volume median diameter (VMD) and the span are presented

Mol%	VMD (μm)	Span
0	10.46 ± 0.35	2.35 ± 0.12
5	9.54 ± 0.09	2.58 ± 0.07
10	10.72 ± 0.35	2.32 ± 0.16
20	25.79 ± 0.91	2.65 ± 0.07
30	23.3 ± 3.06	4.94 ± 0.22

Span = (90% undersize − 10% undersize)/VMD. Indicate values are means (±S.D.) of at least three experiments.

Table 2
The effect of incorporation of 6-ketocholestanol into DPPC liposomes on the volume median diameter (VMD) and the span are presented

Mol%	VMD (μm)	Span
0	10.46 ± 0.35	2.35 ± 0.12
5	11.37 ± 0.30	1.83 ± 0.03
10	10.64 ± 0.14	2.59 ± 0.59
20	20.89 ± 0.08	2.72 ± 0.68
30	21.15 ± 0.75	4.80 ± 1.00

Span = (90% undersize − 10% undersize)/VMD. Indicate values are means (±S.D.) of at least three experiments.

results of the size measurements are presented in Table 1 for the incorporation of phloretin and for 6-ketocholestanol in Table 2. It can be clearly seen that the size of the liposomes increases with increasing enhancer concentration but just till the amount of 20 mol%. Further increase of enhancer concentration does not increase the MLVs size (Fig. 2), which suggests that liposomes are saturated with enhancer.

As observed in Tables 3 and 4 for pure DPPC liposomes the excess specific heat reaches a maximum at about 38 °C for the pre-transition and at about 44 °C for the main transition. This is in agreement with literature values (Mabrey-Gaud, 1981; Constantinides et al., 1986; Rolland et al., 1991; Kyrikou et al., 2004). These two endothermic transitions have been generally attributed firstly to rotations of the phospholipid head group or transformation in the lamellar structure and changes in hydrocarbon chain packing (pre-transition temperature) and secondly to chain-melting transition (phase transition temperature) (Janiak et al., 1979; Mabrey-Gaud, 1981; Grunder, 1987; Kennedy et al., 2002; Milhaud, 2004). At the phase transition temperature, liposomes undergo distinct structural changes. Below the phase transition temperature, lipids in the bilayers are in a highly ordered gel state, the hydrocarbon chains being in an all-*trans* configuration. At the pre-transition temperature, lipids change from tilted one-dimensional arrangements to two-dimensional arrangements with periodic undulations. Above the phase transition temperature, the lipids become more fluid as a consequence of a *trans-gauche* rotational isomerisation along the chains resulting in a lateral expansion and decrease in thickness of the bilayer, and revert to one-dimensional arrangements (Mabrey-Gaud, 1981; Fendler, 1982). This so-called gel-to-liquid crystalline phase transition of multilamellar liposomes is specific of the phospholipid structure and occurs for DPPC liposomes at about 44 °C.

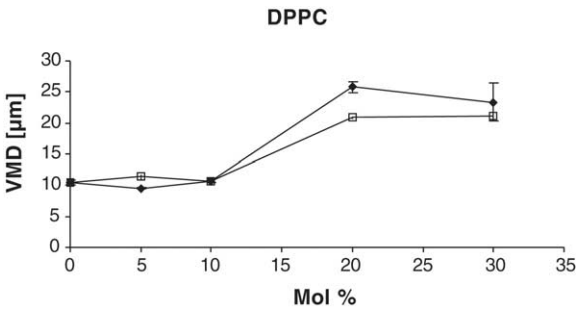

Fig. 2. Volume median diameter (VMD) of phloretin (♦) and 6-ketocholetsanol (□) containing DPPC liposomes. Indicated values are means (±S.D.) of at least three experiments.

The pre-transition is quite sensitive to the presence of molecules in the polar region of the phospholipid bilayer (Balasubramanian et al., 1997). Therefore, the disappearance of the pre-transition with addition of phloretin or 6-ketocholestanol, respectively, indicated that the enhancer interacted with the polar region of the phospholipid bilayer and interfered with the tilting of phospholipid acyl chains (Albertini et al., 1990; Pedrosa de Lima et al., 1990).

The incorporation of phloretin as well as 6-ketocholestanol into these lipid bilayers decreased the phase transition temperature. This is similar to the effects of a number of other molecules employed as dermal penetration enhancers, such as azone, oleic acid, dimethyl sulphoxide and dodecyl-2-(*N*,*N*-dimethylamino)propionate (Beastall et al., 1988; Rolland et al., 1991; El Maghraby et al., 2004; Wolka et al., 2004). It can be related to the ability of Ph and KC in disrupting the dipole potential of bilayers (Rokitskaya et al., 2002; Franklin and Cafiso, 1993).

Table 3
Transition temperature and enthalpy values of DPPC liposomes containing phloretin

Mol%	T_{max} (°C)	Linear onset (°C)	Enthalpy (J/M)
0	38.48 ± 0.03 (pre-transition)	36.94 ± 0.07 (pre-transition)	3424.47 ± 81.30 (pre-transition)
0	44.03 ± 0.02 (main transition)	43.13 ± 0.05 (main transition)	23104.45 ± 177.53 (main transition)
5	42.50 ± 0.10	41.00 ± 0.00	42456.97 ± 5762.17
10	40.17 ± 0.06	37.59 ± 0.08	27561.39 ± 711.71
20	37.1 ± 0.01	35.97 ± 0.06	31242.15 ± 1860.24
30	38.07 ± 0.08	36.78 ± 0.07	33810 ± 4428.92

Indicated values are means (±S.D.) of at least three experiments.

Table 4
Transition temperature and enthalpy values of DPPC liposomes containing 6-ketocholestanol

Mol%	T_{max} (°C)	Linear onset (°C)	Enthalpy (J/M)
0	38.48 ± 0.03 (pre-transition)	36.94 ± 0.07 (pre-transition)	3424.47 ± 81.30 (pre-transition)
0	44.03 ± 0.02 (main transition)	43.13 ± 0.05 (main transition)	23104.45 ± 177.53 (main transition)
5	41.20 ± 0.24	38.37 ± 0.33	29821.61 ± 7522.77
10	42.69 ± 0.48	40.94 ± 0.05	25655.74 ± 3193.47
20	41.50 ± 0.07	36.08 ± 0.17	23471.21 ± 9029.17
30	41.50 ± 0.44	34.37 ± 0.49	17131.43 ± 3103.41

Indicated values are means (±S.D.) of at least three experiments.

Increasing the enhancer: DPPC molar ratios leads to a dramatic effect on the thermotropic behaviour of the phospholipid. Thus, the pre-transition vanishes at low phloretin and 6-ketocholestanol concentrations of 5 mol%. The increase in enhancer: lipid molar ratios broadens the gel-to-liquid crystalline transition peak and shifts it to lower temperatures. The influence of phloretin concentration on the phase transition of DPPC as well as on the enthalpy is shown in Table 3 and the influence of 6-ketocholestanol is given in Table 4. The changes of the enthalpy are depicted in Fig. 3.

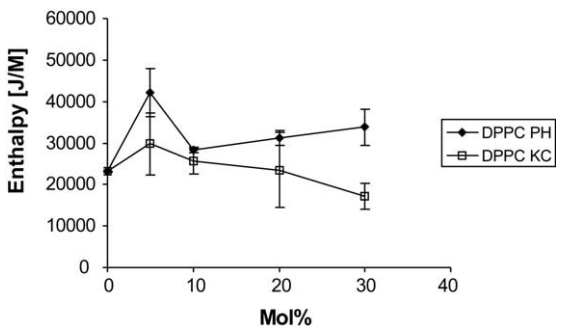

Fig. 3. Effect of phloretin (♦) and 6-ketocholetsanol (□) on the transition enthalpies (T_{max}) of DPPC liposomes. Indicated values are means (±S.D.) of at least three experiments.

Since the transition temperatures observed in skin lipids are near 35 and 70 °C (Rehfeld and Elias, 1982), it is likely that partitioning of topically applied phloretin or 6-ketocholestanol could increase fluidity, which can be thought to affect the lipid molecules also on molecular level in the stratum corneum.

Phase transitions in lipids are cooperative phenomena; that is, the behaviour of a particular molecule in one phase is dependent upon the state of the other molecules around it. The sharpness of the transition depends on the number of molecules forced to cooperate in the transition (Jain and Wagner, 1980). The broadening of the phase transition on addition of phloretin as well as of 6-ketocholestanol to the MLVs suggests that phloretin and 6-ketocholestanol, both intercalates into the bilayer and affects the number of other lipid molecules that a single lipid molecule may influence. This is also in correlation with recent results (Cseh et al., 2000; Castile et al., 2001; Valenta et al., 2004). The more phloretin or 6-ketocholestanol present within the MLVs, the greater the reduction in the size of the cooperative unit.

Main and pre-transition enthalpies in control liposomes were 23.1 ± 0.08 and 3.4 ± 0.2 kJ/mol, respectively. These results are consistent with previous reports (Mabrey and Sturtevant, 1976; Correa-Freire et al., 1979; Stümpel et al., 1981; Lewis et al., 1987;

Hata et al., 2000; Wolka et al., 2004). Main transition enthalpy values are depicted in Fig. 3. Pre-transition enthalpy values vanished by addition of enhancer. Enthalpies of reaction decreased especially for increasing 6-ketocholestanol concentrations. This indicates that the addition of the enhancer destabilised both the gel phase and in the liquid crystal phase, which are associated with the pre-transition and main transition, respectively.

4. Conclusion

Results indicate that phloretin as well as 6-ketocholestanol interacts not only with the surface of the membrane (as indicated by changes in the pre-transition with enhancer treatment) but also with the core of the bilayer (as indicated by changes in the main transition). Enhancer treatment decreases the cooperative unit of the main transition. Furthermore, phloretin as well as 6-ketocholestanol, destabilise both gel and liquid crystal phases within the phospholipid bilayer.

This behaviour is likely to increase the drug flux, by increasing membrane fluidity of the skin.

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References

Albertini, G., Donati, C., Phadke, R.S., Ponzi Bossi, M.G., Rustichelli, F., 1990. Thermodynamic and structural effects of propranolol on DPPC liposomes. Chem. Phys. Lipids 55, 331–337.

Auner, B.G., Valenta, C., Hadgraft, J., 2003a. Influence of phloretin and 6-ketocholestanol on the skin permeation of sodium-fluorescein. J. Contr. Release 89, 321–328.

Auner, B.G., Valenta, C., Hadgraft, J., 2003b. Influence of lipophilic counter-ions in combination with phloretin and 6-ketocholestanol on the skin permeation of 5-aminolevulinic acid. Int. J. Pharm. 255, 109–116.

Auner, B.G., Petzenhauser, E., Valenta, C., 2004a. Influence of 6-ketocholestanol on skin permeation of 5-aminolevulinic acid and evaluation of chemical stability. J. Pharm. Sci..

Auner, B.G., Valenta, C., 2004b. Influence of phloretin on the skin permeation of lidocaine from semisolid preparations. Eur. J. Pharm. Biopharm. 57, 307–312.

Aungst, B.J., Rogers, N.J., Shefter, E., 1986. Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–234.

Balasubramanian, S.V., Straubinger, R.M., Moris, M.E., 1997. Salicylic acid induces changes in the physical properties of model and native kidney membranes. J. Pharm. Sci. 86, 199–204.

Bangham, A.D., Hill, M.W., Miller, N.G.A., 1974. Preparation and use of liposomes as models of biological membranes. In: Korn, E.D. (Ed.), Methods in Membrane Biology, vol. 1. Plenum Press, New York, pp. 1–68.

Barry, B.W., 1987. Mode of action of penetration enhancers in human skin. J. Contr. Release 6, 85–97.

Barry, B.W., 1988. Action of skin penetration enhancers—the lipid protein partitioning theory. Int. J. Cosmetic Sci. 10, 281–293.

Barry, B.W., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.

Beastall, J.C., Hadgraft, J., Washington, C., 1988. Mechanism of action of azone as a percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects. Int. J. Pharm. 43, 207–213.

Castile, J.D., Taylor, K.M.G., Buckton, G., 2001. The influence of incubation temperature and surfactant concentration on the interaction between dimyristoylphosphatidylcholine liposomes and poloxamer surfactants. Int. J. Pharm. 221, 197–209.

Cladera, J., O'Shea, P., Hadgraft, J., Valenta, C., 2003. Influence of molecular dipoles on human skin permeability: use of 6-ketocholestanol to enhance the transdermal delivery of bacitracin. J. Pharm. Sci. 92, 1018–1027.

Constantinides, P.P., Inouchi, N., Tritton, T.R., Sartorelli, A.C., Sturtevant, J.M., 1986. A scanning calorimetric study of the interaction of anthracyclines with neutral and acidic phospholipids alone and in binary mixtures. J. Biol. Chem. 261, 10196–10203.

Correa-Freire, M.C., Freire, E., Barenhloz, Y., Biltonen, P.L., 1979. Thermotropic behaviour of monoglucocerebroside-dipalmitoylphosphatidylcholine multilamellar liposomes. Biochemistry 18, 442–445.

Cseh, R., Hetzer, M., Wolf, K., Kraus, J., Bringmann, G., Benz, R., 2000. Interaction of phloretin with membranes: on the mode of action of phloretin at the water–lipid interface. Eur. Biophys. J. 29, 172–183.

Elias, P.M., 1983. Epidermal lipids, barrier function, and desquamation. J. Invest. Dermatol. 80, 44s–49s.

El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2004. Interactions of surfactants (edge activators) and skin penetration enhancers with liposomes. Int. J. Pharm. 276, 143–161.

Fendler, J.H., 1982. Vesicles. In: Fendler, J.H. (Ed.), Membrane Mimetic Chemistry. Characterization and Applications of Micelles, Microemulsions, Monolayers, Bilayers, Vesicles, Host–Guest Systems, and Polyions. Wiley, New York, pp. 113–183.

Franklin, J.C., Cafiso, D.S., 1993. Internal electrostatic potentials in bilayers: measuring and controlling dipole potentials in lipid vesicles. Biophys. J. 65, 289–299.

Grunder, S.M., 1987. Materials properties of liposomal bilayers. In: Osto, M.J. (Ed.), Liposomes. From Biophysics to Therapeutics. Dekker, New York, pp. 1–38.

Hadgraft, J., 2001. Skin, the final frontier. Int. J. Pharm. 224, 1–18.
Hata, T., Matsuki, H., Kaneshina, S., 2000. Effect of local anaesthetics on the bilayer membrane of dipalmitoylphosphatidylcholine: interdigitation of lipid bilayer and vesicle–micelle transition. Biophys. Chem. 87, 25–36.
Jain, M.W., Wagner, R.C., 1980. Introduction to Biological Membranes. Wiley, New York.
Janiak, M.J., Small, D.M., Shipley, G.G., 1979. Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin. J. Biol. Chem. 254, 6068–6078.
Kennedy, A., Hmel, P.J., Seelbaugh, J., Quiles, J.G., Hicks, R., Reid, T.J., 2002. Characterization of the main phase transition in 1,2-dipalmitoyl-phosphatidylcholine LUVs by 1H NMR. J. Liposome Res. 12, 221–237.
Kyrikou, I., Daliani, I., Mavromoustakos, T., Maswadeh, H., Demetzos, C., Hatziantoniou, S., Giatrellis, S., Nounesis, G., 2004. The modulation of thermal properties of vinblastine by cholesterol in membrane bilayers. Biochim. Biophys. Acta 1661, 1–8.
Lewis, R.N.A.H., Mak, N., McElhaney, R.N., 1987. A differential scanning calorimetric study of the thermotropic phase behaviour of model membranes composed of phosphatidylcholines containing linear saturated fatty acyl chains. Biochemistry 26, 6118–6126.
Mabrey, S., Sturtevant, J.M., 1976. Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. Proc. Natl. Acad. Sci. U.S.A. 73, 3862–3866.
Mabrey-Gaud, S., 1981. Differential scanning calorimetry of liposomes. In: Knight, C.G. (Ed.), Liposomes: From Physical Structure to Therapeutic Applications. Elsevier/North-Holland, Amsterdam, pp. 105–138.
Madison, K.C., Swartzendruber, D.C., Wertz, P.W., Downing, D.T., 1987. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. J. Invest. Dermatol. 88, 714–718.
Michaels, A.S., Chandrasekaran, S.K., Shaw, J.E., 1975. Drug permeation through human skin: theory and in vitro experimental measurement. AIChE J. 21, 985–996.
Milhaud, J., 2004. New insights into water–phospholipid model membrane interactions. Biochim. Biophys. Acta 1663, 19–51.
Moore, L., Chien, Y.W., Vatlia, K.H., 1988. Transdermal drug delivery, a review of pharmaceutics, pharmacokinetics and pharmacodynamics. Crit. Rev. Ther. Drug Carrier Syst. 4, 285–349.
Pedrosa de Lima, M.C., Chiche, B.H., Debs, R.J., Düzgünes, N., 1990. Interaction of antimycobacterial and anti-pneumocystis drugs with phospholipid membranes. Chem. Phys. Lipids 53, 361–371.
Rehfeld, S.J., Elias, P.M., 1982. Mammalian stratum corneum contains physiologic lipid thermal transitions. J. Invest. Dermatol. 79, 1–3.
Rokitskaya, T.I., Kotova, E.A., Antonenko, Y.N., 2002. Membrane dipole potential modulates proton conductance through gramicidin channel: movement of negative ionic defects inside the channel. Biophys. J. 82, 865–873.
Rolland, A., Brzokewiez, A., Shroot, B., Jamoulle, J.C., 1991. Effect of penetration enhancers on the phase transition of mulitlamellar liposomes of dipalmitoylphosphatidylcholine. A study by differential scanning calorimetry. Int. J. Pharm. 76, 217–224.
Stoughton, R.B., 1982. Enhanced percutaneous penetration with 1-dodecylazacycloheptane-2-one. Arch. Dermatol. 118, 474–477.
Stümpel, J., Nickschi, A., Eibl, H., 1981. Calorimetric studies on saturated mixed-chain lecithin–water systems. Nonequvalence of acyl chains in the thermotropic phase transition. Biochemistry 20, 662–665.
Valenta, C., Nowak, M., Hadgraft, J., 2001. Influence of phloretin and 6-ketocholestanol on the permeation of progesterone through porcine skin. Int. J. Pharm. 217, 79–86.
Valenta, C., Steininger, A., Auner, B.G., 2004. Phloretin and 6-ketocholestanol: membrane interactions studied by a phospholipid/polydiacetylene colorimetric assay and differential scanning calorimetry. Eur. J. Pharm. Biopharm. 57, 329–336.
Walters, K.A., 1989. Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery. Dekker, New York, pp. 197–246.
Wertz, P.W., Downing, D.T., 1989. Stratum corneum: biological and biochemical considerations. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery. Dekker, New York, pp. 1–22.
Williams, M.L., Elias, P.M., 1987. The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. Crit. Rev. Ther. Drug Carrier Syst. 3, 95–122.
Wolka, A.M., Rytting, J.H., Reed, B.L., Finnin, B.C., 2004. The interaction of the penetration enhancer DDAIP with a phospholipid model membrane. Int. J. Pharm. 271, 5–10.