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# The interaction of the penetration enhancer DDAIP with a phospholipid model membrane

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**Abstract**

Differential scanning calorimetry (DSC) was used to investigate the mechanism of action of a proprietary skin penetration enhancer, dodecyl-2-(*N*,*N*-dimethylamino)propionate (DDAIP) in dipalmitoylphosphatidylcholine (DPPC) liposomes. Furthermore, the effect of enhancer concentration on lipid thermotropic transitions was investigated. With increasing concentrations of DDAIP (from 5 to 50 mol%), the main transition peak shifted to lower temperatures and became more broad. The pretransition peak also shifted to lower temperatures with increasing concentrations of DDAIP and disappeared completely above an enhancer concentration of 20 mol%. Main transition and pretransition enthalpies of reaction decreased with increasing DDAIP concentration, indicating that enhancer treatment destabilized both rippled gel and liquid crystal phases within the bilayer. At and above a DDAIP concentration of 33.3 mol%, an additional transition was evident, indicating the presence of two phases of enhancer–lipid complex. Results suggest that DDAIP enhances drug transport by interacting with the polar region of the phospholipid bilayer and also by increasing the motional freedom of lipid hydrocarbon chains.


*Keywords:* Skin penetration enhancer; DDAIP; Mechanism; Dipalmitoylphosphatidylcholine liposomes; Differential scanning calorimetry

## 1. Introduction

It is well known that the stratum corneum acts as a formidable barrier to systemic delivery of therapeutic agents (Barry, 1983). For this reason, chemical penetration enhancers are of significant interest in transdermal drug delivery, since they can reversibly reduce this barrier function. Dodecyl-2-(*N*,*N*-dimethylamino)propionate (DDAIP) (Fig. 1) is particularly attractive as a chemical penetration enhancer. Not only does it enhance the delivery of a variety of drugs (Wong et al., 1989; Büyüktimkin et al., 1991, 1993), but also it is biodegradable (Büyüktimkin et al., 1993). Studies have indicated that one mechanism of enhancement could involve the interaction of DDAIP with the lipid portion of the stratum corneum (Turunen et al., 1994).

In the present study, the effect of DDAIP on the thermotropic phase behavior of dipalmitoylphosphatidylcholine (DPPC) liposomes was investigated using differential scanning calorimetry (DSC) in order to yield further information about the mechanism of enhancer–membrane interactions. Furthermore,

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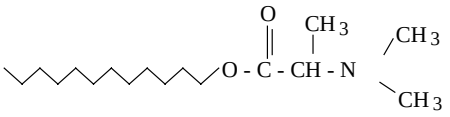


Fig. 1. Chemical structure of DDAIP.

the effect of enhancer concentration on lipid phase behavior was investigated.

## 2. Materials and methods

### 2.1. Materials

DPPC (99%) was obtained from Sigma (St. Louis, MO, USA) and was stored at −15 °C. DDAIP was provided by NexMed (Robbinsville, NJ, USA) and was stored at 4 °C.

### 2.2. Preparation of liposomal dispersions

Multilamellar vesicles (MLVs) were prepared according to the following method. A fixed amount (10 mg) of DDPC was dissolved in 2 ml of chloroform–methanol (2:1, v/v), and the solvent was evaporated under a stream of nitrogen gas to form a thin film on the bottom of a 20-ml glass scintillation vial. The lipid film was dispersed in 3 ml of distilled water to give a final lipid concentration of 3.33 mg/ml. The dispersions were heated to 50 °C (above the lipid transition temperature) and vortexed in turn until the lipid film dissolved. Liposomes were allowed to swell at room temperature for 2 h, and then were stored at 4 °C overnight. For enhancer studies, the appropriate amount of a 4 mg/ml DDAIP stock solution (prepared in chloroform–methanol (2:1, v/v)) was added to the DPPC solid before the addition of the solvent. DDAIP was studied at concentrations of 5, 10, 16.7, 20, 25, 33.3, and 50 mol%. All samples were analyzed by DSC the day after preparation.

### 2.3. DSC studies

Thermal scans were performed using a Nano II DSC (Calorimetry Sciences Corp., Spanish Fork, UT, USA) ($n = 3$ per liposome preparation). Capillary cells with volumes of 0.3268 ml held the reference and sample. After sample addition, the cells were pressurized to 3 atm to remove air bubbles, and the sample and cell temperatures were allowed to equilibrate before each scan was initiated. The sample and reference cells were heated from 10 to 70 °C at a rate of 1 °C/min, and then cooled at a rate of 2 °C/min.

### 2.4. Data analysis

Thermal transitions were analyzed using CpCalc software, Version 2.1 (Applied Thermodynamics Corp., Longwood, FL, USA). After baseline subtraction, raw power data were converted to molar heat capacity data. Baselines were fitted to the pretransition and main transition regions using a linear baseline function so that transition temperatures and enthalpies of reaction could be calculated for each enhancer concentration.

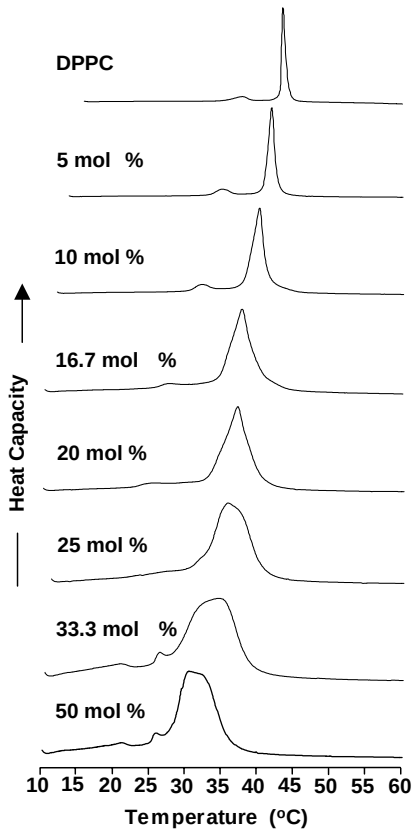


Fig. 2. Representative DSC scans of DPPC liposomes containing 0–50 mol% DDAIP.

## 3. Results

Representative DSC thermograms of DPPC liposomes containing various concentrations of DDAIP are shown in Fig. 2. Furthermore, effects of DDAIP on main and transition temperatures and enthalpies of reaction are presented in Figs. 3 and 4, respectively. Unless obvious, the error bars are within the size of the symbols.

The DPPC bilayer exists in the gel phase at temperatures below 32 °C and in the liquid crystalline phase at temperatures above 44 °C. The gel-to-liquid crystalline transition was observed at 41.7 ± 0.1 °C for liposomes composed of DPPC alone, and a pretransition peak around 35.9±0.3 °C was also evident. These results are consistent with previous reports (Mabrey and Sturtevant, 1976; Correa-Freire et al., 1979; Stümpel et al., 1981; Wilson and Dahlquist, 1985; Lewis et al., 1987; Balasubramanian et al., 1997; Hata et al., 2000).

With increasing concentrations of DDAIP (from 5 to 50 mol%), the main transition peak shifted to lower temperatures and became more broad. The pretransition peak also shifted to lower temperatures with increasing concentrations of DDAIP, and this transition disappeared completely above enhancer concentrations of 20 mol%. An additional transition was evident at DDAIP concentrations of 33.3 and 50 mol%. The presence of two separate peaks suggests different modes of interaction of DDAIP with DPPC resulting in the formation of two phases of enhancer–phospholipid complex.

Main and pretransition enthalpies in control liposomes were 33.5±1.8 kJ/mol and 4.9±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). Main transition enthalpy values decreased with the addition of up to 10 mol% DDAIP. However, the main transition enthalpy of reaction

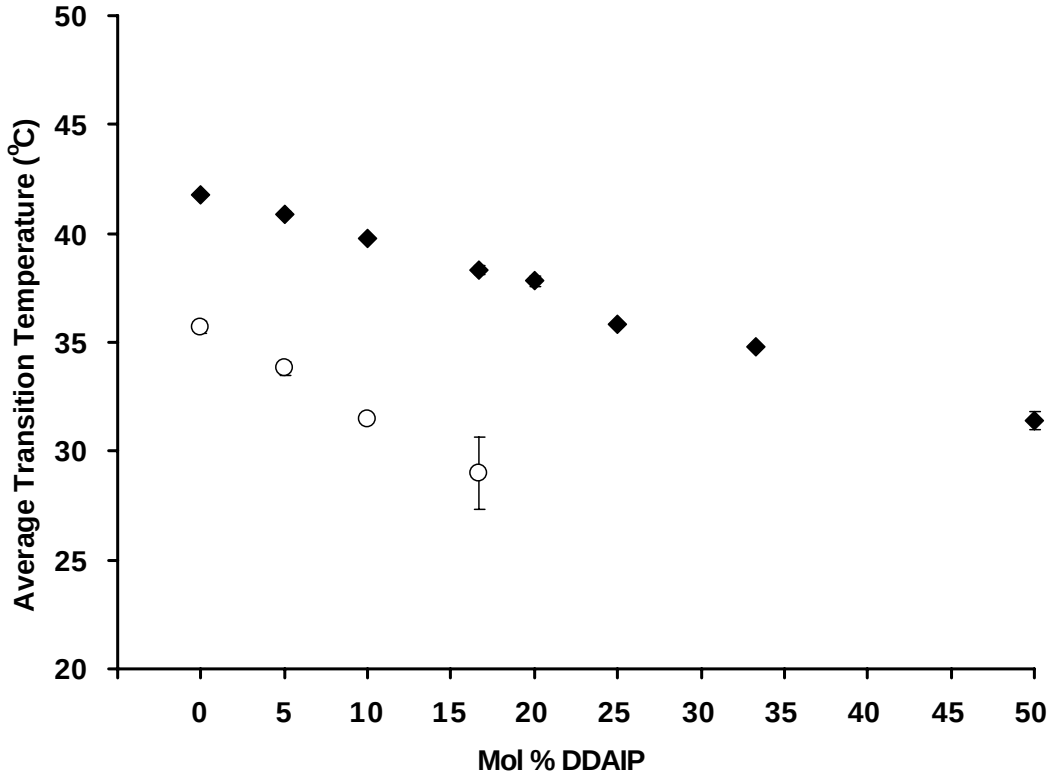


Fig. 3. Effect of DDAIP concentration on main (◆) and pretransition (○) temperatures of transition in DPPC liposomes.

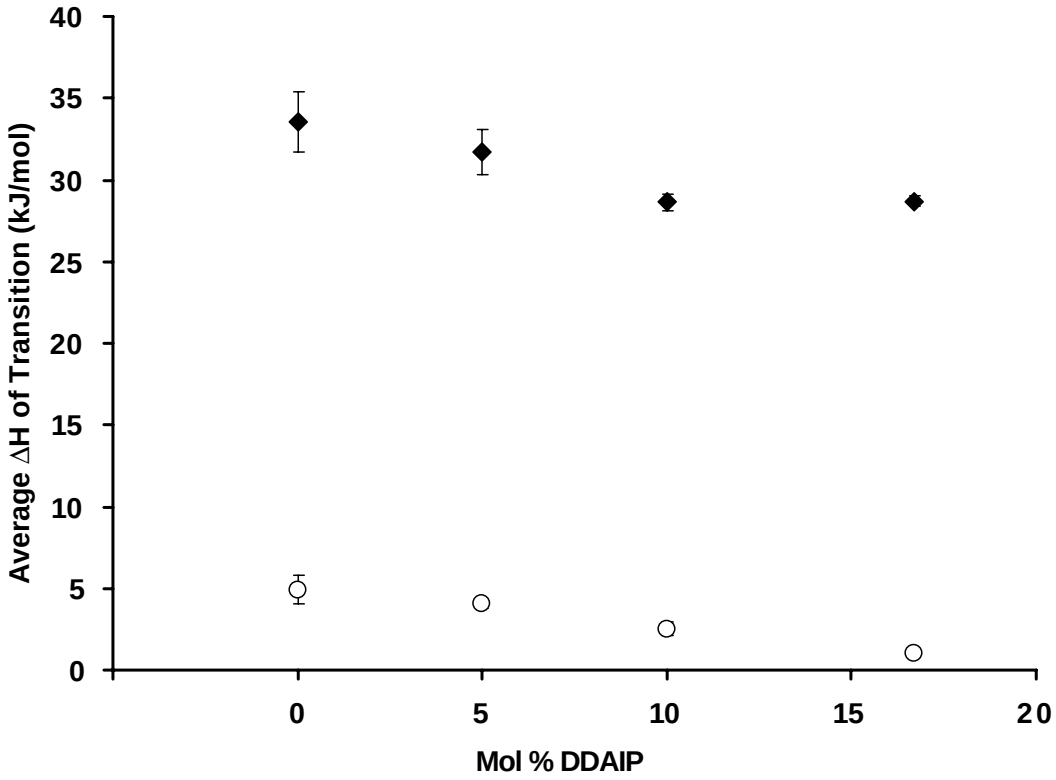


Fig. 4. Effect of DDAIP concentration on main (◆) and pretransition (○) enthalpies of transition.

was not lowered further by the addition of 16.7 mol% DDAIP. Pretransition enthalpy values decreased with increasing enhancer concentrations. Individual main and pretransition enthalpies could not be measured at and above enhancer concentrations of 20 mol% since the peaks could not be adequately separated and due to the presence of an extra peak at high concentrations of DDAIP.

## 4. Discussion

An understanding of the mechanism of action of any skin penetration enhancer is critical in assessing its commercial potential and in optimizing transdermal formulations. A number of drugs and several lipophilic penetration enhancers have been shown to partition into membranes and thereby alter physical properties of the bilayer (Demel and de Kruyff, 1976; Ahmed et al., 1980; Antunes-Madeira and Madeira, 1985; Fujisawa et al., 1987; Pedroso de Lima et al., 1990; Sarkar et al., 1993; Balasubramanian and Straubinger, 1994; Balasubramanian et al., 1997; Savva et al., 1999; Mavromoustakos et al., 2001). Results of the current study indicate that DDAIP acts by a similar mechanism. The apparent partitioning of DDAIP into the lipid bilayer is not surprising based on the lipophilicity of this enhancer (log $P$ = 5.40; calculated using Log Kow online database, http://esc.syrres.com/interkow/kowdemo.htm).

Three separate phase transitions are known to occur with increasing temperature in DPPC lipid bilayers (Mabrey and Sturtevant, 1976; Correa-Freire et al., 1979; Stümpel et al., 1981; Lewis et al., 1987; Hata et al., 2000). The $L_c \rightarrow L\beta'$ subtransition from the lamellar crystal phase to the lamellar gel phase was not observed since this transition is evident only in vesicles incubated at low temperature for several days (Chen et al., 1980). The pretransition which occurred around 35.9 °C in these liposomes reflected the

$L\beta'$ (lamellar gel) $\rightarrow P\beta'$ (rippled gel) transition in the gel phase (Janiak et al., 1976; Balasubramanian and Straubinger, 1994; Balasubramanian et al., 1997; Ueda et al., 1994). The orientational order of phospholipid acyl chains was reflected in the main $P\beta'$ (rippled gel) $\rightarrow L\alpha$ (liquid crystal) transition that occurred at 41.7 °C in these liposomes.

The pretransition which occurred around 35.9 °C in these liposomes reflected the transition from the lamellar gel phase to the rippled gel phase (Janiak et al., 1976; Balasubramanian and Straubinger, 1994; Balasubramanian et al., 1997; Ueda et al., 1994). Rather than being a core property of the phospholipid bilayer, such as the main transition, the pretransition depends on the surface structure of the membrane (Ueda et al., 1994; Hata et al., 2000) and is related to the reorientation of the DPPC head group and water (Ueda et al., 1994; Okamura et al., 1990).

The pretransition 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 pretransition with increasing concentrations of DDAIP 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; Pedroso de Lima et al., 1990).

The main transition ($P\beta'$ to $L\alpha$) at 41.7 °C reflected an increase in the motional freedom of the lipid hydrocarbon chains (Ahmed et al., 1980; Ueda et al., 1994). During this highly cooperative acyl chain "melting", *trans-gauche* isomerization took place in the acyl chain conformation of lipid molecules, effectively increasing the fluidity of the phospholipid bilayer (Campbell et al., 2001; Mavromoustakos et al., 2001).

The main transition from a highly ordered all-*trans* conformation to a less ordered conformation in which some hydrocarbon chains exist in the *gauche* conformation depends not only on temperature, but also on perturbations due to the presence of enhancer molecules intercalating between the phospholipids. Therefore, the decrease in the main transition temperature was interpreted as a "fluidizing" effect of DDAIP on the phospholipid bilayer (Albertini et al., 1990; Pedroso de Lima et al., 1990). Furthermore, the increase in disorder of lipid hydrocarbon chains with enhancer treatment occurred in a concentration-dependent manner. Broadening of the main transition peak, on the other hand, indicated that DDAIP interacted with the hydrophobic acyl chains and interfered with the cooperativity of the acyl chain melting (Pedroso de Lima et al., 1990; Balasubramanian and Straubinger, 1994; Balasubramanian et al., 1997).

The appearance of a peak on the low-temperature side of the main transition at and above enhancer concentrations of 33.3 mol% indicated the formation of two partially separated phases, one rich in phospholipid and the other in enhancer (Ortiz et al., 1988). This result suggests two different modes of interaction of DDAIP and DPPC (Pedroso de Lima et al., 1990).

Main and pretransition enthalpies of reaction decreased with increasing enhancer concentration, indicating that the addition of DDAIP destabilized both the $P\beta'$ and $L\alpha$ phases which are associated with the pretransition and main transition, respectively.

Results indicate that DDAIP interacts not only with the surface of the membrane (as indicated by changes in the pretransition 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, DDAIP destabilizes both rippled gel and liquid crystal phases within the phospholipid bilayer. Phase separation occurs at high enhancer concentrations, suggesting the formation of two distinct phases of enhancer–phospholipid complex.

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