# DSC:A valuable tool to study drug:membrane interactions using Differential Scanning Calorimetry

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## 1. Introduction

DSC is a thermodynamic technique suitable for studying the effects of drugs on the phase transitions of membrane bilayers The technique, as its name signifies is based on the differential heat flow between a sample that undergoes a phase transition in the temperature range under study and an inert reference material that lacks such a phase transition in the same temperature range. When a phase transition occurs, the sample undergoes a thermally-induced event. The following parameters in an endothermic or exothermic event are recorded during the phase transition: (a) *Tm* (maximum of the peak) (b) *Tonset* (the starting temperature of the phase transition); (c) $T_{m1/2}$ (the half-height width of the phase transition); (d) the area under the peak which represents the enthalpy change during the transition *(ΔH*).

Due to the high complexity of the biological membranes and their instability, the study of their phase transitions is not an easy task. Therefore, lipids and especially phospholipid bilayers which share many of the conformational and dynamic properties of the natural membranes are used as a model membranes.

In this plenary lecture, examples of the effects of bioactive molecules with lipid bilayers will be given and the complementarity information of various other techniques will be mentioned.

## 2. Materials and Methods

*Materials:* Lα-dipalmoty-phosphatidylcholine (Lα-DPPC, 99+%) was purchased from Avanti Polar Lipid Inc. and $CHCl_3$ from Sigma Aldrich. Valsartan was kindly donated by Novartis.

*Methods*: Appropriate amounts of DPPC and valsartan were diluted in chloroform and were mixed, dried under a stream of $N_2$ and then stored under vacuum overnight. After dispersing in water (50% w/w), ca. 5 mg were sealed in stainless steel capsules obtained from Perking-Elmer. The drug concentrations used were $x$=0.01, $x$=0.02, $x$=0.05, $x$=0.10 and $x$=0.20.

## 3. Results and Discussion

Fig. 1 shows the calorimetric scan of La dipalmitoyl phosphatidylcholine multilamellar vesicles in the absence and presence of valsartan. These bilayers have been studied

extensively since they show two endothermic transitions, a broad low enthalpy pretransition (*Tm*=35.3 $^{o}$C) and a main phase transition (Tm=41.2 $^{o}$C). Below the pretransition the phospholipid molecules are arranged in a one-dimensional lamellar gel phase ($L_{\beta}'$), while above the main transition they exist in the liquid crystalline phase ($L_{\alpha}'$). At temperatures between the ripple phase ($P_{\beta}'$) [1] and main phase transition (La) based on solid-state NMR evidence, the bilayers are composed of coexisting gel and liquid crystalline components.

Below pretransition temperature, the chains are in all-trans conformation [2] and are tilted with respect to membrane normal about 32$^{o}$ [3]. Above the phase transition a trans:gauche isomerization is evident. At only 1 mol% of valsartan, the pretransition is almost suppressed, the enthalpy is lowered by a factor of three and the transition width spans over 7-8 $^{o}$C. This illustrates the interface activity of the drug molecule. Increased of drug concentration leads to the decrease of the phase transition monotically. From the 5-20% valsartan concentration there is a splitting of the main phase transition into two components (Table 1). The total enthalpy increased quite significantly from 7.5 to 9.8 kcal/mol for 1-20 mol%. This enthalpy change as it is confirmed by x-ray diffraction data and Raman spectroscopy is due to the induce of a partial interdigitation of the alkyl chains.

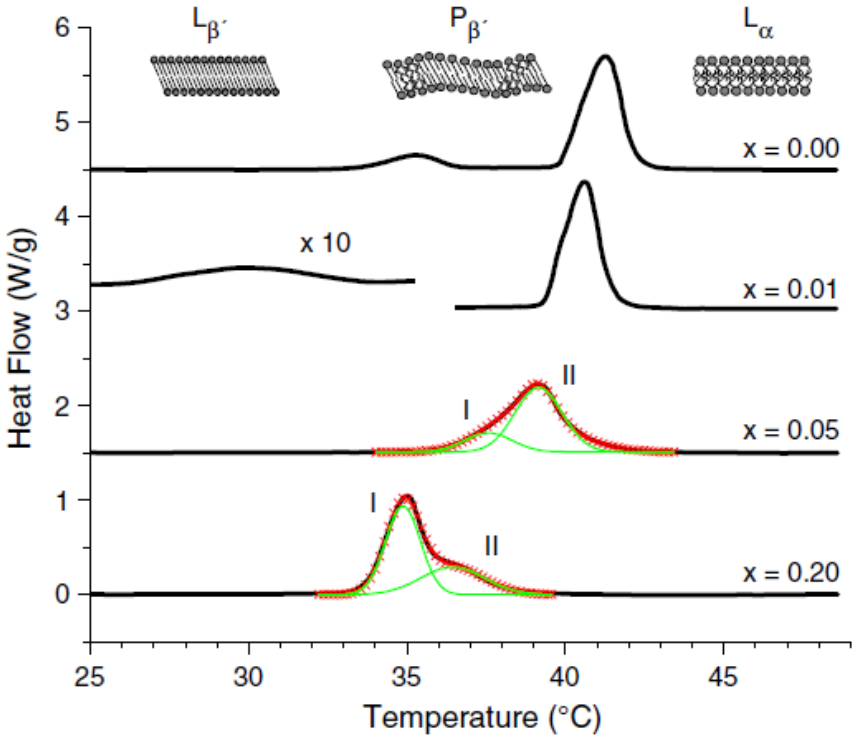


**Figure 1**. DSC scan for DPPC bilayers alone and ascending concentrations of valsartan.
The mechanism of action of valsartan on membrane bilayers in gel and liquid crystalline phase base on a combination o biophysical results is shown in Figure 2.

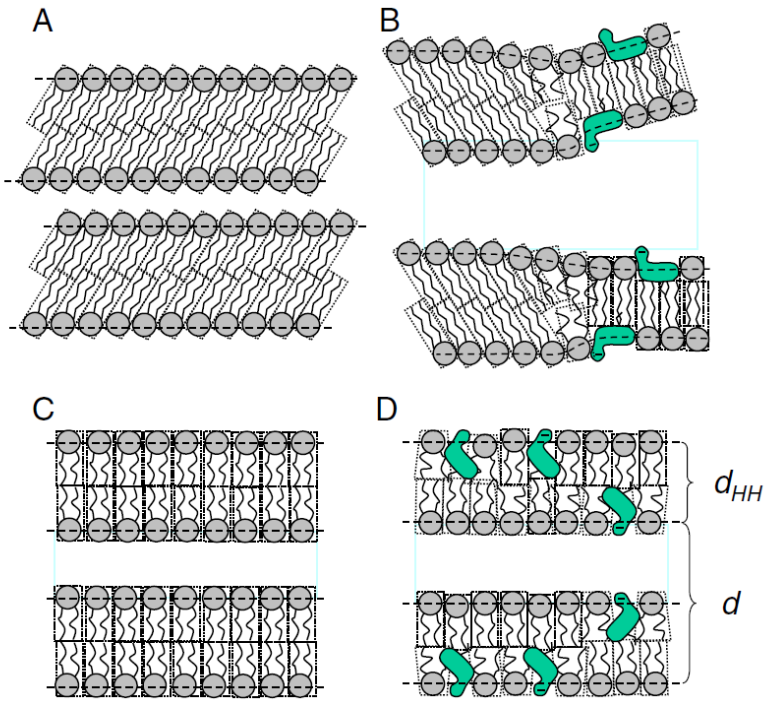


**Figure** 2. Proposed perturbations induced by valsartan when it is incorporated in lipid bilayers. A and C Figures show DPPC bilayers at gel and liquid crystalline phases. B shows the effect of valsartan (20% mol) in the gel phase and D in the liquid crystalline phase. In the gel phase valsartan induces the formation of valsartan rich-domains with chain interdigition ($L_{\beta I}$), This partial interdigitation is also evident in the liquid crystalline phase as depicted by the decrease of the thickness of lipid bilayers [4].

**Conclusions** Valsartan is hypothesized that exerts its action probably through the lipid bilayers. DSC in combination with x-ray diffraction and Raman spectroscopy defined its topographical position and its effects on lipid bilayers. It is evident from this study that valsartan is possible to perturb lipid bilayers inducing partial interdigitation. This is not a common feature for the rest seven AT1 commercially available antagonists. This indicates that partial interdigitation is based on subtle structural features.

**References**

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