Kinetic study of the thermodynamic behavior of lipid bilayers in the presence of aminoadamantane drugs

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- **Introduction**

Differential scanning calorimetry (DSC) has been proven to be a very useful and fast technique towards the revealing of interactions between drug molecules and lipid biomembranes [1]. The scope of the present study is to assess the interactions of two congener amphiphilic drug molecules, namely amantadine and its synthetic analog spiro[pyrrolidine-2,2′-adamantane] compound (AK13), with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) model membranes. Apart from the calorimetric profile of the systems, we attempted to evaluate their thermodynamic properties upon time and monitor their kinetic mode.

- **Material and Methods**

The amantadine drug (Amt) (Merck Schuchardt OHG – Hohenbrunn) and its synthetic analog AK13 (synthesized in the lab of Prof. A. Kolocouris) were mixed with DMPC phospholipid (Avanti Polar Lipids, Inc.) at four drug molar concentrations (5, 8, 20 and 50%) and dissolved in methanol that was evaporated at room temperature under a gentle flow of argon. Subsequently, the samples were placed under vacuum for 24 hours in order to form a thin lipid film. The prepared samples were fully hydrated with phosphate buffer saline (PBS). Samples were hydrated immediately after preparation (indicated as "new"), as well as two weeks later (indicated as "old") and were subjected to DSC scans. Moreover, the DSC measurements of all the samples were repeated after 15 days. An 822^e Mettler-Toledo instrument (Schwerzenbach, Switzerland) was used, while all thermal ($T_{onset,m}$, T_m) and thermodynamic (ΔH_m) parameters were calculated with the Mettler-Toledo STAR^e software.

- **Results and Discussion**

Starting from the pure DMPC bilayers (**Figure 1**), some differences between the old and the new samples were observed. This observation implies that the preparation protocol can influence significantly the thermal properties of a sample.

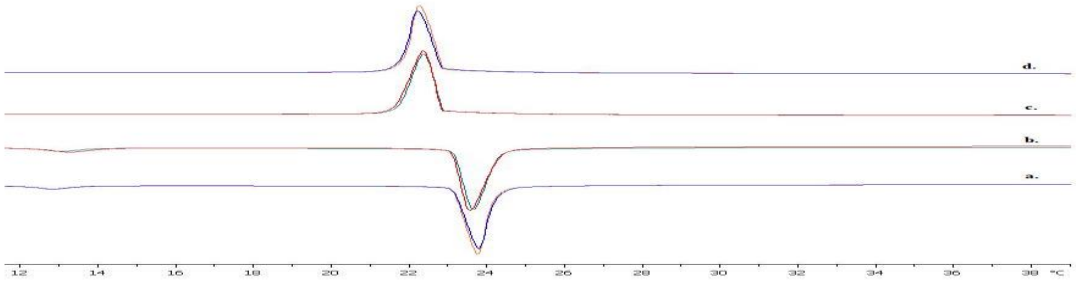

Figure 1: Thermodynamic stability after 15 days during heating of (a) old DMPC bilayers; (b) new DMPC bilayers; during cooling of (c) new DMPC bilayers; and d) old DMPC bilayers. The DSC measurements were repeated twice.

Referred to the DMPC bilayers that were incorporated with the two lipophilic drug molecules, it can be assumed that both molecules affected significantly the calorimetric parameters of the main transition of DMPC bilayers (**Table 1**), while they also caused abolishment of the pretransition, reflecting alterations of the membrane organization [2,3]. The curves of **Figures 2** and **3** suggested a strict dependence of the thermodynamic behavior on the drug concentration.

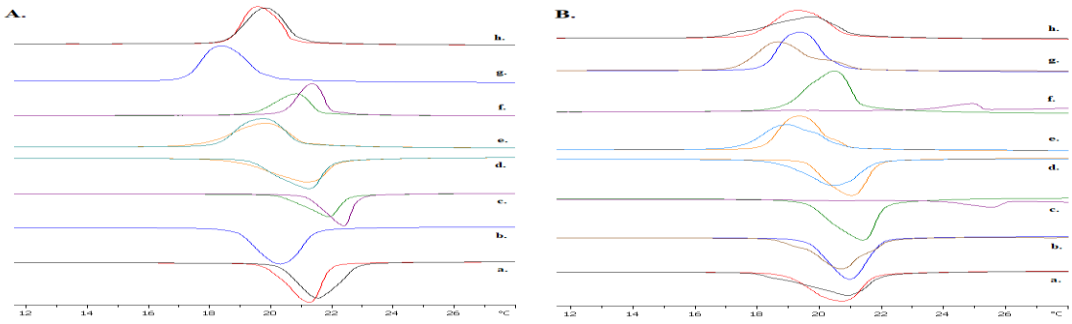

Figure 2: Thermodynamic stability after 15 days of heating and cooling A) a+h) DMPC:Amt 5% old, b+g) DMPC:Amt 5% new, c+f) DMPC:AK13 5% old, d+e) DMPC:AK13 5% new and B) a+h) DMPC:Amt 8% old, b+g) DMPC:Amt 8% new, c+f) DMPC:AK13 8% old, d+e) DMPC:AK13 8% new. The DSC measurements were repeated twice.

Moreover, many differences appeared between the old and new samples (**Figure 2**), highlighting that the long interval between sample preparation and hydration affected the thermal behavior. Finally, the changes observed upon the time reflected some kinetic instability of the thermodynamic behavior of all the studied samples, especially for AK13. More specifically, the drug topography, orientation and the formation of various domains depicted in the scans with complex peaks and using high concentrations are very sensitive to the drug preparation and sample equilibration conditions.

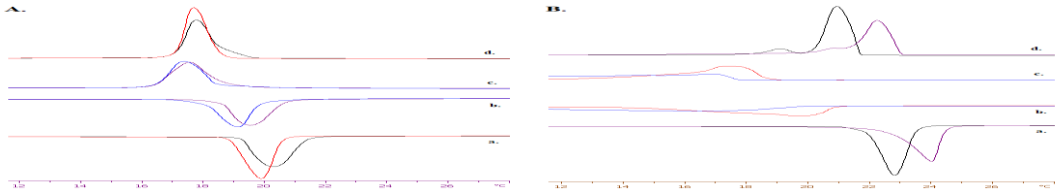

Figure 3: Thermodynamic stability after 15 days of A) a+d) DMPC:Amt 20%, b+c) DMPC:AK13 20% and B) a+d) DMPC:Amt 50%, b+c) DMPC:AK13 50%. The DSC measurements were repeated twice.

Table 1: Calorimetric profiles of DSC runs and reruns of bilayers (heating cycle).

Sample	Day	Drug Molar%	$T_{onset,m}$ (°C)	T_m (°C)	$\Delta T_{1/2,m}$ (°C)	ΔH_m (J g^{-1})
DMPC:Amt	1	5 old	20.21	21.40	1.54	41.23
DMPC:Amt	15	5 old	19.88	21.13	1.21	43.22
DMPC:AK13	1	8 old	19.51	21.36	1.57	50.34
DMPC:AK13	15	8 old	23.72	25.57	1.33	24.02
DMPC:AK13	1	8 new	19.48	20.90	1.48	38.35
DMPC:AK13	15	8 new	18.92	20.29	1.82	39.60
DMPC:Amt	1	20	19.20	20.12	1.38	46.97
DMPC:Amt	15	20	18.82	19.69	1.01	48.45
DMPC:AK13	1	20	18.53	19.40	1.34	41.59
DMPC:AK13	15	20	17.98	19.02	1.10	41.50
DMPC:Amt	1	50	21.89	22.60	0.92	52.74
DMPC:Amt	15	50	22.70	23.88	0.96	43.80
DMPC:AK13	1	50	16.27	19.69	3.93	41.98
DMPC:AK13	15	50	10.63	16.67	6.53	26.30

- **Conclusions**

This study showed that DSC thermodynamic technique is very sensitive to the sample equilibration conditions. Distinct scans are observed with DMPC bilayers containing amantadine or AK13 at different concentrations using different preparation protocols. The consequences of these observations are significant. Kinetic instability modifies the thermodynamic parameters and thermal scans. Thermal scans reflect the topographical position and orientation of drugs incorporated in membrane bilayers. As thermal scans are modified according to protocol preparation is inferred that topographical position and orientation of the drug embedded in lipid bilayers are also modified.

- **References**

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