ARTIFICIAL MEMBRANES BY THE SELF-ASSEMBLY OF N-DODECYL AND N-METHYL PYRIDINIUM SALTS WITH LONG ALKYL SUBSTITUENTS*

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Four series containing N-dodecyl- and N-methylpyridinium perchlorates having different long alkyl substituents give self-organized assemblies.
The effect of change in the chemical structure of the compounds resulting from the variation of the number and length of the alkyl groups was studied using differential scanning calorimetry (DSC) and dynamic light scattering (DLS).
DSC and DLS investigations are mostly in good agreement and show that the transition temperatures, size distributions and diameters depend on the nature and the length of the substitutents of the pyridinium ring.
The relationship phase transition temperatures *vs* number of carbon atoms of alkyl side chains for these N-dodecyl and N-methyl amphiphiles is also reported.

INTRODUCTION

In the previous papers we described the preparation of new 2,3,4,6-tetra substituted pyrylium and derived pyridinium salts.[1] Synthetic membranes formed by the self-assembly of some pyridinium salts with N-triethyl-triamino or N-ethyleneamino- and with two long alkyl substituents (undecyl, palmitoyl, stearoyl) in the 2,6-(α) positions were also reported.[1]

Pyridinium salts with different substituents are important heterocyclic compounds which possess antimicrobial, antifungal, magnetic and optical properties. Recent developments showed that cationic amphiphiles have many interesting properties in the domains of physics, chemistry and biology as ionic liquid catalysts[2] - which are a new generation of green solvents – or as liquid crystals.[3] These compounds are useful in many different applications such as an excellent inhibitor for acid secretion or for controlled drug delivery. [4] They were used for preparation of nanocomposites[5] and ultrathin films.[6]

Recently a number of discotic ionic molecules containig pyridinium salts have been reported by Veber and Kumar. The decrease of molecule ordering degree with temperature variation implies simple or complex first order phase transitions which were observed by polarizing optical microscopy (POM) and differential scanning calorimetry (DSC).[7]

The most important application of pyridinium salts with long alkyl substituents is as carriers for DNA delivery into cells. So, recent papers have been focused on the characterization and the aggregation properties of amphiphilic transfection agents derived from cationic pyridinium salts namely SAINTs (Synthetic Amphiphiles Interdisciplinary). Besides the fundamental interest of analysing and understanding the phase behavior of novel synthetic amphiphiles, the determination of the aggregation properties of gene delivering systems associated with high transfection efficiencies are described.[8]

A simple attractive alternative to phospholipid vesicles in some applications may be offered by surfactant vesicles, formed by mixing single-tailed cationic surfactants as N-alkyl pyridinium halides (decyl, dodecyl or cetylpyridinium chloride). These compounds form "catanionic" vesicles which have several advantages over conventional phospholipid vesicles. For example, they form vesicles spontaneously without the need for additional sonication or extrusion, have an extremely long shelf life and the raw materials are inexpensive compared to synthetic or purified phospholipids.[9]

Pyridinium salts with long alkyl substituents and several substituents at nitrogen atom give self-organized systems. We have shown previously that the cationic amphiphile's chemical structures have a crucial influence on the membrane stability (phase separation) and aggregate morphologies. These systems contain bilayer vesicles, globules or twisted globules and vesicles or complex structures and have different hydrodynamic diameters as shown by optical microscopy (OM) and DLS experiments. [1,10,12]

The present paper is focused on the characterization of the synthetic membranes derived from two classes of pyridinium salts. The effect of a lower (methyl) or a higher (n-dodecyl) alkyl substituent at nitrogen atom from corresponding amphiphiles on the aggregation properties has been studied by DSC and DLS. Characterization of the pure pyridinium salts by DSC is also presented.

This paper presents the preliminary results. Systematic investigations and details (as the texture of mesophases) in order to situate these compounds in the liquid crystal's class will be presented elsewhere.[11]

RESULTS AND DISCUSSION

Materials and Methods

The different amphiphiles of series **A**, **B**, **C** and **D** are obtained with good yields (75-88%). They were prepared from the corresponding pyrylium and n-dodecyl- (freshly distilled amine) or methylamine (20% in ethanol) according to the classical procedure. The chemical structure and the purity of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, UV spectroscopy and elemental analysis. [1,10,12]

Tabel 1 presents the various compounds relevant for this paper.

Two classification criteria have been adopted: (i) by the nature of the substituent on nitrogen R^1 (n-dodecyl for series **A** and **B** or methyl for series **C** and **D**) and (ii) by the substituent (H or CH_3) in the β- position of the pyridinium ring (series **A** / **B** or **C** / **D**).

All new compounds were fully characterized by ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz), IR and UV spectra and by elemental analysis.

Artificial membranes derived from these pyridinium salts with different long alkyl substituents were obtained by sonication.

Sonication was performed in a cleaning bath (Langford Sonomatic, T175) having the frequency of 33 kHz, using a 50 mL Erlenmeyer flask with a bottom area of 20 cm² containing water-pyridinium salts suspension.The total power was in the range of 0.5 watts/cm².

Characterization of pyridinium salts with long alkyl substituents by DSC

The phase transition temperature (T_m, temperature at a peak maximum of DSC thermogram) for the pure pyridinium salts or derived membranes in the aqueous dispersion state was measured with a differential scanning calorimeter DuPont 2000. The samples were sealed in DSC pans and run at 5°C/min under nitrogen or argon atmosphere.

The phase transitions appear at 43-94°C (pure compounds) or 10-33°C (aqueous dispersions) by DSC. After the first scan, successive heating scans on the same sample always gave superimposable thermograms. Transition enthalpies were determined using the software provided with the calorimeter.

Table 1

The alkylpyridinium amphiphiles
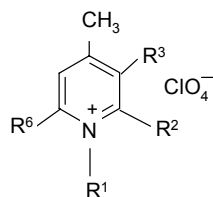
Cpd	R^1	R2	R^3	R^6
Series A				
1	$C_{12}H_{25}$	$C_{11}H_{23}$	H	CH_3
2	$C_{12}H_{25}$	$C_{11}H_{23}$	H	$C_{11}H_{23}$
3	$C_{12}H_{25}$	$C_{15}H_{31}$	H	$C_{15}H_{31}$
4	$C_{12}H_{25}$	$C_{17}H_{35}$	H	$C_{17}H_{35}$
Series B				
1	$C_{12}H_{25}$	$C_{11}H_{23}$	CH_3	$C_{11}H_{23}$
2	$C_{12}H_{25}$	$C_{15}H_{31}$	CH_3	$C_{15}H_{31}$
3	$C_{12}H_{25}$	$C_{17}H_{35}$	CH_3	$C_{17}H_{35}$
Series C				
1	CH_3	$C_{11}H_{23}$	H	CH_3
2	CH_3	$C_{11}H_{23}$	H	$C_{11}H_{23}$
3	CH_3	$C_{15}H_{31}$	H	$C_{15}H_{31}$
4	CH_3	$C_{17}H_{35}$	H	$C_{17}H_{35}$
Series D				
1	CH_3	$C_{11}H_{23}$	CH_3	$C_{11}H_{23}$
2	CH_3	$C_{15}H_{31}$	CH_3	$C_{15}H_{31}$
3	CH_3	$C_{17}H_{35}$	CH_3	$C_{17}H_{35}$

The transition temperatures and enthalpies obtained by DSC for all pure compounds described here, present clear trends when the chain length is increased. High values were obtained for amphiphiles having a new methyl group in β-position of the heterocyclic ring (series **B** / **A** and **D** / **C**). The results were similar to that observed for other compounds with long alkyl side chains.[8c, 13]

N-dodecylpyridinium perchlorates (series **A** and **B**) have a single melting transition with the associated enthalpy change varying linearly with alkyl chain length (**Figure 1**). In this case, very probably, the peak was composed of two nonseparable peaks. Melting is associated with changes in the conformation of the alkyl chains.
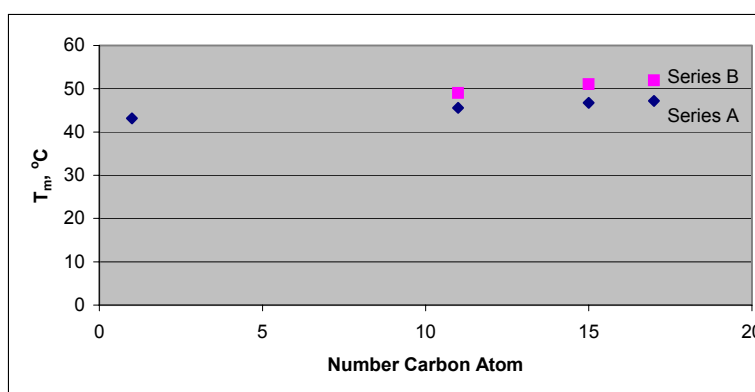

Fig. 1 – The relationship phase transition temperatures *vs* number
of carbon atoms of pure amphiphiles from series **A** and **B**.

For N-methylpyridinium perchlorates (series **C** and **D**) two different transitions corresponding for two different mesophases, appear in the DSC thermograms, exo- at heating and endothermic

process at cooling the corresponding compounds. The experimental results from the first heating and cooling cycle are presented in **Table 2**. Details for these mesophases were reported elsewhere.[11]

Table 2

The transition temperatures and the melting enthalpy of pure amphiphiles

Cpd.	Number carbon atom R^6	First heating scan		First cooling scan	
		T_m °C	$\Delta_m H$ kJ/mol	T_m °C	$\Delta_m H$ kJ/mol
C_1	1	58.72 72.41	27.01 9.74	55.85 71.18	11.77 0.43
C_2	11	66.91 78.23	28.88 10.44	56.71 67.43	12.55 0.20
C_3	15	78.99 91.67	29.82 12.54	57.08 68.33	14.23 0.31
C_4	17	84.14 93.32	37.68 12.92	60.12 72.87	14.52 0.74
D_2	11	68.45 79.25	34.95 10.61	58.44 70.16	19.17 0.97
D_2	15	79.12 95.74	35.12 12.87	59.47 69.92	19.79 1.01
D_3	17	89.03 96.53	38.42 14.01	60.75 75.11	20.49 1.21

Characterization of artificial membranes derived from pyridinium salts with long alkyl substituents by DSC

All amphiphiles presented form bilayer membranes in aqueous media. Differential scanning calorimetry experiments were performed in order to determine the phase transition temperature of respective surfactant bilayers.

Previously[10] we showed that there is a substantial difference between the stability of the bilayers derived from some N-dodecyl *vs* N-methyl amphiphiles, a long chain substituent increasing significantly the phase separation times. Optical microscopy confirmed the bilayer membrane formation from these newly synthesized amphiphiles.[10]

In order to examine the properties of these artificial membranes a portion of the solution after sonication was cooled to -25 °C and was analysed in comparison with the rest of the solution at the same concentration.

The experimental results are presented in **Table 3**. For A_1 and A_2 the temperature dependence of the heat flow ($\Delta H/\Delta T$) is presented in **Figure 2**. The insert shows a OM picture of the corresponding membranes.
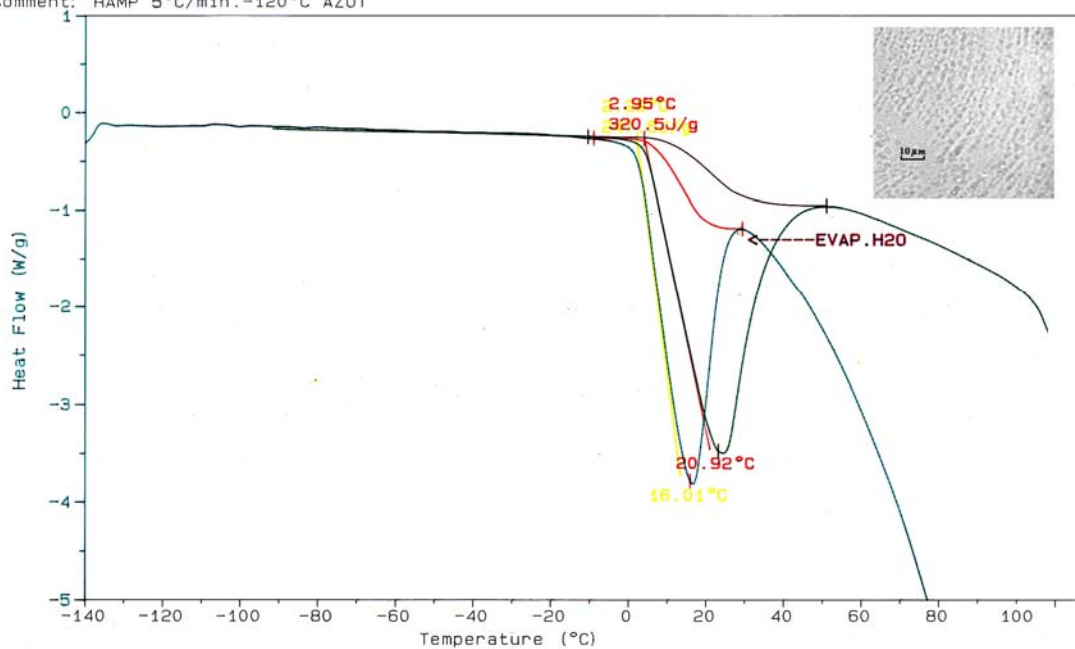
Table 3

The transition temperatures of synthetic membranes derived from pyridinium salts

Bilayer	Sonicated Sample	Frozen Sample
	T_m, °C	T_m, °C
A_1	16.01	20.92
A_2	24.37	25.44
A_3	27.56	32.44
A_4	31.44	41.25
B_1	26.33	27.97
B_2	28.44	29.55
B_3	39.83	32.03
C_1	13.31	20.24
C_2	13.58	22.42
C_3	15.18	25.61
C_4	16.43	29.96
D_1	14.25	20.98
D_2	16.96	21.25
D_3	17.43	30.56

(a)
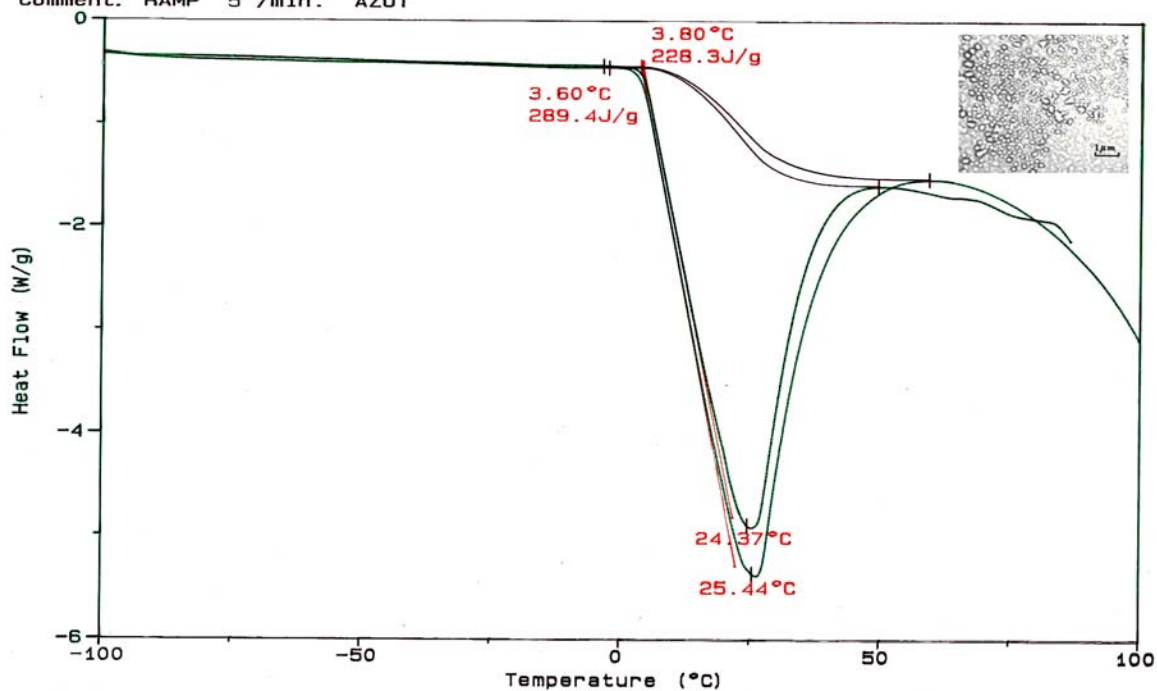
(b)

Fig. 2 – The temperature dependence of the heat flow for membranes derived from **A₁** (a) and **A₂** (b).

DSC reveals a remarkable increase in the thermal stability of the membranes formed from N-dodecylpyridinium salts **A** and **B**.

Thus, aggregates prepared from amphiphiles corresponding to series **A** and **B** underwent a phase

transition at 16-39°C. The DSC curve does not show any other transition.

In comparison with some previous experimental data for pure amphiphiles from series **C** and **D**, the corresponding synthetic membranes have only one phase transition, and, as expected, small values for T_m are obtained. In the light of our previous study, the results for N-dodecyl *vs* N-methyl were consistent with the formation and morphology of stable bilayers.

By cooling after sonication the increase of time till phase separation and thus the stability of the membranes were observed. In these conditions, a high T_m was obtained. A similar effect was observed for all series **A** – **D** and was also noticed for long chain alkyl ammonium salts which were extensively studied by Kunitake and coworkers.[14]

Characterization of artificial membranes derived from pyridinium salts with long alkyl substituents by DLS

To understand the effect of the chemical structure of amphiphiles on the corresponding membrane formation, we monitored the aggregation process of **A** – **D** in aqueous solutions in time.

So, the hydrodynamic diameter of the aggregates derived from pyridinium amphphiles was measured and the morphological self-assembly process was observed *in situ* by DLS. Experimental results, size (hydrodynamic diameter d) / distribution of corresponding aggregates and distinctive stabilization time as a function of chemical structure of amphiphiles was presented in **Table 4**. Both cases, sonicated and frozen sample were described.

Table 4

Size, distribution and distinctive time of self-assembling process observed by DLS

Bilayer	Sonicated Sample		Frozen Sample	
	Time, min.	Diam., nm / %	Time, min.	Diam., nm / %
A_1	16.30	2001.0/100	15.8	330.0/100
A_2	24.11	432.1/8.1 2331.3/91.9	22.24	738/.1100
A_3	26.03	520.2/10.0 2560.0/90.0	24.10	780.0/100
A_4	26.52	590.0/11.2 2690.4/88.8	24.30	830.3/100
B_1	18.47	3362.0/37.2 5403.3/62.8	14.12	299.9/18.1 1798.0/81.9
B_2	21.15	3500.4/40.1 5708.2/59.9	18.40	480.7/20.0 1806.5/80.0
B_3	20.40	3555.2/45.0 5896.6/55.0	20.03	500.8/29.1 1903.7/70.9
C_1	14.40	2250.1/100	11.12	1121.7/100
C_2	13.25	264.5/3.6 2548.9/96.4	10.17	970.3/100
C_3	10.15	301.4/2.0 9641.0/98.0	9.16	112.4/0.2 2104.3/99.8
C_4	9.40	355.6/0.8 9851.9/99.2	8.53	123.6/1.0 2253.4/99.0
D_1	12.56	196.3/3.5 1723.8/11.2 6701.3/85.3	10.01	270.4/11.8 2305.8/88.2
D_2	10.01	230.6/1.5 1909.7/14.2 6919.2/84.3	8.59	305.9/10.5 2455.1/89.5
D_3	9.23	299.8/0.8 2112.5/16.9 7209.8/82.3	8.42	395.1/9.7 2600.9/90.3

The aggregation observed by dynamic light scattering is interpreted here (**Table 4**) as being due to the noncovalent rearrangement of preexisting large species to give rise to even larger entities (d up to 2 μm). This process is expected to be complete in 1–2 h, as the average aggregate size levels off in the dynamic light scattering results.

The primary difference is that, for N-dodecyl pyridinium amphiphiles (**A** and **B**) vs N-methyl amphiphiles (**C** and **D**), a higher time (by DLS

determinations) was needed to form stable aggregates with a certain shape. For **C** and **D**, larger sizes of derived aggregates with higher distributions than for **A** and **B** were obtained.

The formation of larger aggregates in all series **A** – **D**, must be caused by increased interparticle aggregation. Previous work[1b,c,10a] has shown that these more substituted amphiphiles having different long alkyl side chains show tendency to aggregate and present lower phase stability with the increase in alkyl chain length. For the last compounds of the presented series, complex structure were obtained.

The freezing process generated stabilization of all systems and smaller values for size, time and distribution were obtained. Similar processes were described in some previous reports.[10a,b] The small aggregates resulting from the freezing process were stable for three months (for series **A** and **B**) and two-four weeks (for series **C** and **D**) since there was no precipitation and their transparency did not change during storage at room temperature.[15]

Aggregates with diameters of 2000 – 2200 nm and with unique morphology were generally obtained for the sonicated systems derived from pyridinium salts A_1 or C_1 which contain only two long alkyl substituents in vicinal or 2, 6 – positions respectively. All the evidence (OM) shown above suggests a different assembling of these pyridinium salts. Some similar processes were described in the corresponding frozen systems.

N-methyl amphiphiles **C** or **D** form less stable aggregates. Probably, the lower methyl group at the nitrogen atom may prevent molecules to assemble in stable bilayers. On the other hand, before ultrasonication, these pyridinium salts form aggregates with much larger diameters; the size distribution was bimodal or trimodal, as shown in **Table 4**. However, after the freezing process, the distribution became unimodal (for C_2) or bimodal (for D_2 and D_3) and smaller mean diameters were obtained. The phase stability increased as visually indicated by turbidity changes. Some cationic surfactants were reported to also form vesicles by themselves on hydration, but some others needed a second component such as cholesterol to form vesicles.[16]

Incorporation of a new methyl group in the β-position of the heterocyclic ring increases the hydrodynamic diameter of the aggregates as shown in **Table 4**. In time, the chains self-assembled into aggregates with different size distributions and complex architecture were obtained.[15]

EXPERIMENTAL PART

The DSC experiments were performed with a DuPont 2000 equipment. The dynamic light scattering (DLS) experiments were carried out using a NICOMP 370 equipment.

General procedure for the preparation of membranes

In all cases, given amounts (0.1 –10 mM) of pyridinium salts were first suspended and then sonicated in distilled water.

Sonication times in the ultrasonic cleaning bath were 20 min for all compounds and the concentration was about 1 mM or 30 min if the concentration was about 10 mM. The obtained solutions which were either opalescent, emulsions or (micro)dispersions were stored in closed vessels until analysed.

Differential scanning calorimetry (DSC)

The phase transition temperatures (T_m, temperature at a peak maximum of DSC thermogram) for the pure pyridinium salts or derived membranes in aqueous dispersion state were measured with a differential scanning calorimeter DuPont 2000. The samples were sealed in DSC pans and run at 5°C/min under nitrogen or argon atmosphere. After the first scan, successive heating scans on the same sample always gave superimposable thermograms. Transition enthalpies were determined using the software provided with the calorimeter.

Dynamic light scattering (DLS)

The hydrodynamic aggregates diameters were determined using a submicron particle sizing system based on DLS technique using a NICOMP 370 equipment. The scattering data were processed with two types of distributions: a Gaussian or a multimodal distribution referred to as Nicomp Analysis Distribution (NAD).

The experiments were done at room temperature with dilution of the previous sample at optimal DLS concentration (in the range of $1.5\text{-}2\text{X}10^{-5}$ M).

Every sample derived from pyridinium amphiphiles **A** – **D**, has a characteristic time when the system is stable. At this time, the corresponding aggregates have different morphologies, size and distribution which were evaluated with the DLS tehnique.

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