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Review Paper

THERMOTROPIC MESOPHASES OF IONIC AMPHIPHILES
II. Ionic amphiphiles in aqueous media

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Abstract

In part I of this article the thermotropic mesophases of anhydrous ionic amphiphiles were discussed. In this part the thermotropic mesophases of ionic amphiphiles in aqueous media, as determined by thermal analysis, microscopic studies, X-ray diffraction and other techniques are reviewed.

Abbreviations used:
α-lecithins: 1,2 or 2,3 diacylphosphatidylcholines;
α-cephalins: 1,2 or 2,3 diacylphosphatidylethanolamines.;

DLPC:	dilauroylphosphatidylcholine;
DLPE:	dilauroylphosphatidylethanolamine;
DMPC:	dimyristoylphosphatidylcholine;
DMPE:	dimyristoylphosphatidylethanolamine;
DPPC:	dipalmitoylphosphatidylcholine;
DPPE:	dipalmitoylphosphatidylethanolamine;
DSPC:	distearoylphosphatidylcholine;
DSPE:	distearoylphosphatidylethanolamine;
DOPC:	dioleoylphosphatidylcholine;
DAPE:	diarachinoylphosphatidylethanolamine;
DBPC:	dibehenoylphosphatidylcholine;
OSPC:	oleoylstearoylphosphatidylcholine;
SOPC:	stearoyloleoylphosphatidylcholine.

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The fatty acids saturated or unsaturated that are found in the above molecules are: Lauric acid ($C_{12}H_{24}O_2$); myristic acid ($C_{14}H_{28}O_2$); palmitic acid ($C_{16}H_{32}O_2$); stearic acid ($C_{18}H_{36}O_2$); arachic acid ($C_{20}H_{40}O_2$); behenic acid ($C_{22}H_{44}O_2$); oleic acid ($C_{18}H_{34}O_2$).

Keywords: determination of thermotropic mesophases, ionic amphiphiles in aqueous media

Introduction

An attempt was made in Part I [1] to explain the origin of the ability of ionic amphiphiles to form thermotropic mesophases – a critical parameter for the exhibition of liquid crystallinity. Thermotropic mesophases are, however, formed not only in anhydrous amphiphiles and in various hydrated phases of these but also in amphiphiles in excess water. The ionic amphiphiles, particularly the phospholipids in excess water, tend to disperse without disruption of their organization and orientation, forming closed vesicle-shaped structures [2].

The amphiphilic orienting force is important from both biological and chemical viewpoints. In biology, the amphiphilic force is probably the single most important force for structural organization in living matter [3]; consequently, it enables phospholipid bilayers to play a structural part constituting a matrix for protein membranes [4]. Moreover, phospholipid bilayers are functional constituents that interact with other membrane components to induce biological activity [5]. In chemistry, amphiphile orientation is of interest because it provided the first biomembrane-like bilayer structures from a totally synthetic organic compound, electron micrographs showing that it forms vesicles indistinguishable from those of dipalmitoyllecithin [6].

One of the main characteristics of membrane bilayer structures is the thermotropic transitions that occur – particularly those between the 'solid' and fluid states, (referred to as the gel to the liquid crystal transition) as determined by various methods including DSC and X-ray diffraction. The transition temperature provides a reference point characterizing fluidity, since the amphiphile bilayers undergo a change from a rigid relatively immobile condition to a fluid structure when heated above this temperature [7].

The ionic amphiphiles in aqueous media reviewed here are:

A. (Anionic, cationic and zwitterionic types)-H_2O phase diagrams, from the anhydrous state to excess water.

B. Ionic amphiphiles in excess water that can be classified as follows:

1. Lecithins and cephalins (the most abundant biologically active phospholipids for constructing biological membranes) as well as their mixtures, in particular, those with cholesterol.

2. Synthetics: a) With two alkyl chains, and with a head group such as ammonium, phosphate, sulphonate, and carboxylate: these constitute the best known synthetic bilayer membranes. Also, the double-chain ammonium amphiphiles with linkage in the hydrophobic tails. b) With a single long chain and counter-ion that can be derived: (i) from fatty acids or fatty alcohols at a suitable *pH*; (ii) from a flexible tail, a rigid segment and a hydrophilic head group; (iii) from all included in b with the addition of a spacer group and an additional interacting group. c) Amphiphiles with three or more alkyl chains and ammonium head group. The thermal behaviour of three hydrocarbon chains is compared when one, two or three of them are replaced by fluorocarbon chains.

Phase diagrams of ionic amphiphile–water systems

Because the lyotropic mesophases in ionic amphiphile–water systems (which occur as various hydrated phases) also exhibit thermotropic mesophases, each particular phase obtained is a function of water content and of temperature and is revealed by the phase diagram.

Anionics

The phase diagrams of sodium laurate, myristate, palmitate, stearate and oleate, with respect to water content and temperature have been constructed by various methods such as visual and microscopic observations supplemented by dilatometric measurements. All these amphiphiles (soaps) yielded phase diagrams of similar type with differences in the solubility curves defining the field of isotropic liquid. A characteristic for all these diagrams was that, at very high soap concentrations, they pass through a large number of mesomorphic phases before melting to an isotropic liquid, the number being the same as for the corresponding anhydrous compounds and the water merely lowering the temperature at which a phase initially appeared. Further addition of water produced different phases that probably could not exist in the absence of water [8–10].

The mesomorphic states of sodium and potassium myristate, palmitate, stearate, oleate and sodium lauryl sulphate in water have also been investigated by X-ray diffraction and thermomicroscopy. From these phase diagrams, giving the concentration of the above amphiphiles in water in relation to temperature, intermediate phases appeared up to the isotropic state. An essential feature of all phases was the (disordered) liquid-like configuration of the paraffin chains [11].

A DTA study of the transitions and characteristics of the phase diagrams of the sodium laurate-water system established that: (a) the upper limit of the mesomorphic state was represented by a curve, T_1 [12]; (b) the neat phase of the

anhydrous soap in the presence of water represented one unique mesomorphic state and the lower limit of this phase, curve T_c, lay between the anhydrous state at 241°C (a temperature in good agreement with that given in Table 1 Part I [1]) and the extended state about 36% into the concentration in water at 70°C [13]; (c) bound, intermediate and free water existed [14].

The thermal behaviour of the sodium myristate–water system, as determined by DTA, was very similar to that of the sodium laurate–water system; thus the main regions of the phase diagrams were confirmed [15].

Cationics

The thermal behaviour of dioctadecyldimethylammonium chloride (DODAC) samples ranging from a sample completely dehydrated under high vacuum to one with about 85 wt% water content were examined by DSC over −20 to 150°C. This enabled the phase diagrams for all the samples to be constructed from the transition temperatures and enthalpies of successive changes, mainly of the coagel to gel (T_{gel}) and gel to liquid crystal (T_c) transitions. These changes appeared because of the increasing structural disorder of the polar head groups and the hydrocarbon chains, respectively. Three types of water were also shown to exist – namely, bound, intermediate and free. Below 11 wt% water content only bound water strongly attached to the DODAC molecules (which form the coagel phase) existed, the water molecules being strongly bound to the polar head group of the DODAC molecule. In the 11–18 wt% region intermediate water existed: this relatively weakly bound water formed the gel phase, and its transition temperature was lowered to a limiting value. The weakly bound water could be converted to free (ordinary) water on annealing. For the unannealed samples, the peak at 0°C was not observed until the water content reached 18%. This water does not crystallize even on cooling to −20°C. From 18 wt% to 85 wt%, the three kinds of water can coexist. In this region the enthalpy changes at the gel to liquid crystal transition are nearly constant, while the transition temperatures have limiting values represented by a straight line parallel to the water content axis wt% at the phase diagram [16].

The thermal behaviour of the dioctadecyldimethylammonium bromide (DODAB)-water system containing from 0 to about 99 wt% water was also examined by DSC over the temperature range from 20°C to the temperature of the liquid crystal transition T_c. Comparison of this phase diagram with that for DODAC showed differences in detail, the most notable being the thermodynamic stability of the gel phases: the gel phase of the chloride counter-ion was stable only in the temperature region between the T_{gel} and T_c transitions, whereas the gel phase of the bromide counter-ion was metastable over all temperatures below the T_c transition [17].

Spectroscopic data have shown that, above the T_c transitions, all these double-chain amphiphiles in the presence of water formed bilayer lamellar liquid crystals – in contrast with the micellar solution formed by homologous amphiphiles with a long single chain [18].

The difference in thermodynamic data for the DODAC– and DODAB–water phase diagrams can be attributed to the hydration enthalpies of the different halide-ions in the polar head groups. The same difference is observable for the single chain amphiphiles of octadecyltrimethylammonium chloride and bromide (OTAC, OTAB) in systems with water. The hydration enthalpy, due to a change of the interaction of the positive and negative ions of the polar head group of amphiphiles with water molecules, associated with the main transition, according to DSC increased in the order OTAC<OTAB. Therefore, the different counter-ions are a major factor in determining the thermodynamic stability of all these amphiphiles [19].

Zwitterionics

The phase diagram of the 1,2 dipalmitoyl-*L*-phosphatidylcholine (DPPC)–water system (Fig. 1) was constructed from results obtained by a variety of physical methods, including X-ray diffraction, thermal analysis, and spectroscopic techniques.

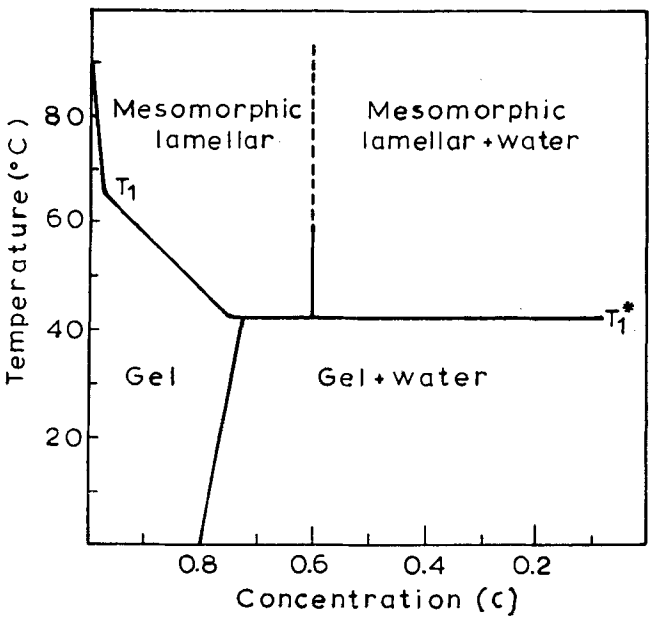

Fig. 1 Schematic phase diagram of 1,2-dipalmitoyl-*L*-phosphatidylcholine (lecithin) in water (from [20])

In the concentration range 1.0≥0.8 of DPPC the main endothermic transition temperature T_1 decreased steadily to a limiting value T^*. In the gel region no endothermic peak was present at about 0°C probably because of formation of hydrated structure associated with its polar group. Quantitative studies showed that a proportion of the water was bound to the DPPC in a fixed ratio of 1:4 by weight. Above the T_1 line the DPPC–water system existed in a mesomorphic lamellar phase in which the hydrocarbon chains were in a liquid-like state. This composition of the system remained to ~40 wt% water. On addition of more than 40 wt% water, the system dissociated into two phases, the mesomorphic lamellar phase and water [20]. The phase diagrams of different chain length lecithin-water system, were essentially similar to the system DPPC–water [20].

More details about the phase behaviour on heating and about structural features were obtained by DSC and X-ray diffraction of synthetic lecithins, and particularly of DMPC, as a function of temperature (–10 to 60°C) and degree of hydration (7.5 to 60 wt%). In these regions, four distinct phase transitions were observed. The temperature-composition phase diagram of hydrated DMPC was constructed and the phase-transition structures were described [21].

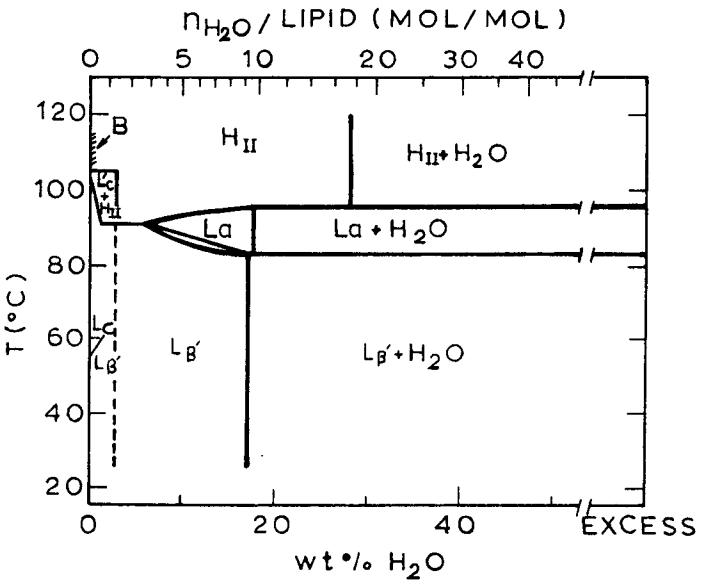

Fig. 2 Schematic phase diagram of diarachinoylphosphatidylethanolamine (cephalin) in water (from [23])

The structures of a variety of lecithin–water phases, observed by X-ray diffraction below the 'melting' temperature of the hydrocarbon chains, have been

described. These phases belong to the extended region of the phase diagrams, often called the 'coagel' of the lecithin–water region [22].

The phase diagram of the (DAPE)–water system obtained from DSC, and X-ray diffraction data is shown in Fig. 2 [23].

L_{β}' corresponds to a gel region, L_{α} to the fluid bilayer and H_{II} to the hexagonal region (which is a class of liquid crystal phases). The L_c transition is the same as that of the untilted crystalline form, and the L_c' corresponds to a tilted crystalline form. The sample with 2.2 wt% water, on standing overnight at room temperature, showed in addition to the transition at 92°C a peak at 95°C on the first heating scan, indicating metastability of the gel phase of DAPE at a very low water content.

To determine the effect of varying the chain length of this homologous series on the structure of their different phases, DLPE (C_{12}), DMPE (C_{14}), DPPE (C_{16}), DSPE (C_{18}) and DAPE (C_{20}) in water were examined by the same methods. It was noted that the gel phase, especially within a small temperature range, of the short-chain-length compounds di-C_{12} to di-C_{14} was metastable in excess water, reverting spontaneously within hours to the dehydrated crystalline form. Reversion of the gel phase of di-C_{16} to a state with a more crystalline chain packing has also been observed but only after incubation for several days at temperatures below 6°C and without complete dehydration. Thus, spontaneous metastable reversion to the unhydrated crystalline forms becomes slower with increasing chain length and it is no longer observed for DAPE (C_{20}) [23].

The tendency of the cephalins to remain or become dehydrated is a direct consequence of the strong hydrogen-bonding and electrostatic interactions that exist between the lipid head groups, both in the plane of the bilayer and between adjacent bilayers. The result is the limited hydration of the gel phase of cephalins in comparison with lecithins. In lecithin gel phases there are ~15 molecules of water per lecithin molecule, whereas for cephalins there are only ~9 molecules [21, 23].

Ionic amphiphiles in excess water

Phospholipids

When water is added to a dry phospholipid, swelling takes place leading to the formation of liquid crystal phases of different kinds. Further addition of water tends to lead to dispersion of this lipid without disruption of the ordered aggregated structures – a process probably caused by the tendency of bilayers to form vesicles, – i.e. closed structures – in the presence of water [24]. Phospholipids with this molecular arrangement, which became known as liposomes, were first described by Bangham in the early 1960's [2, 25], and a recent col-

loquium entitled 'Liposomes: From membrane model to therapeutic applications', organized to honour Bangham's contribution to the understanding of cell membranes revealed the importance of his experiments. Thus, it was demonstrated that the formation and properties (e.g. molecular organization, fluidity and permeability) determined the various applications (such as drug carriers, fusogenic agents, immunological adjuvants, etc.). Moreover, their use in understanding the significance of membrane lipid diversity and a wide variety of phenomena were covered by this colloquium, which revealed the widespread interest of liposomes [26].

Liposomes can essentially be divided into three types based on the size of vesicular structures: a) multilamellar (multicompartment) vesicles (MLV), which consist of a series of concentric lipid bilayers, each separated by an aqueous compartment and range in size up to fractions of a millimeter; b) small unilamellar vesicles (SUV), which are produced by different methods and usually from the multilamellar vesicles on exposure to ultrasonic irradiation: vesicles of this type consist of a single lipid bilayer surrounding an aqueous compartment ranging in diameter over ~200–500 Å; c) large unilamellar vesicles (LUV), which are prepared by various methods including reverse-phase evaporation, which consist of a single lipid bilayer with an aqueous compartment and which range in diameter from 600 Å to several microns. All three types of liposomes develop thermotropic behaviour [27, 28]. Moreover, their thermotropic behaviour reveals the unstable types of 'lipidic particles' that have a very high curvature with a diameter usually <130 Å [29].

The mechanism of formation of phospholipid vesicles of different size is not well understood but it is known that the free energy increases from MLV to SUV and that transformations are induced by changing the temperature [30, 31]. DSC is thus an excellent technique for studying the thermodynamics of transitions in these phospholipid structures.

As there is an extensive literature on relevant researches and, in particular, on the characteristic of their phase transitions, it would be difficult to consider all here; consequently, reference is made only of some special relevant reviews [32–34]. However, some conclusions from and results of some characteristic experiments are given to show the contribution thermal analysis has made in this important field.

Most of the early experiments referred here were performed by DSC [35, 36], instruments for which are being continuously improved in sensitivity and accuracy. DSC, and to lesser extend DTA, studies of the thermally induced transition of phospholipids in excess water revealed that the transition from a relatively ordered crystalline-like state (gel), existing at lower temperatures, to a relatively disordered fluid-like state (liquid crystal phase) at higher temperature was the main transition. The 'solid' and 'fluid' states, which are sometimes

referred to as the gel and the liquid crystal phases have been observed by a large variety of methods such as thermal analysis and calorimetry, X-ray diffraction, NMR, EPR and Raman spectroscopy [7].

The gel to liquid crystal transition is considered a two-state first order endothermic process and arises from a cooperative melting of hydrocarbon chains in the phospholipid molecule. An application of DSC that provides important information of the phase transition cooperativity, is based on a comparison of ΔH_{vH} and ΔH_{cal}. ΔH_{vH} is independent of ΔH_{cal} and can be determined from the value of $\Delta T_{1/2}$ (temperature width at half height) and T_m (the transition temperature) on the excess specific heat curve. From the ratio $\Delta H_{vH}/\Delta H_{cal}$ the cooperative unit (CU) (in molecules) can be determined. The degree of intermolecular cooperation between bilayer phospholipid molecules during the phase transition is a measure of cooperative unit [37–39].

As the size of the cooperative unit is particularly affected by the impurities, a purified synthetic sample of DPPC in multilamellar suspensions required a high sensitivity DSC to study the nature of the gel to liquid crystal transition which closely approximated to an isothermal first order transition. For this transition $\Delta H_{cal} = 8.7$ kcal·mole^{-1} and the calculated $\Delta H_{vH} = 12\,000$ kcal·mole^{-1} so that the average cooperative unit was 1 400 lipid molecules. However, the excess specific heat curve was slightly asymmetric, suggesting a slightly impure sample [37].

Pure phospholipids

Suspensions of multilamellar phospholipids

Two characteristic endothermic transitions were originally observed for multilamellar vesicles of pure lecithins. The major chain-melting transition occurred over a narrow temperature range and was considered the main transition. About 3–12°C below this occurred an additional broader transition, or pretransition, corresponding to lower-enthalpy lamellar-gel bilayer transformation. The interval between the two, decreased with the increasing chain length.

Multilamellar suspensions of saturated cephalins yielded a single transition, which was clearly asymmetric and was frequently attributed to the gel to liquid crystal transition. This transition occurred at 20–25°C higher than that for the corresponding lecithin.

Table 1 lists the values of thermodynamic parameters for the transitions of some lecithins and cephalins in aqueous suspensions, as determined by different researchers [40, 38–39, 41– as indicated by a, b, c and d respectively]. All the data for each parameter are in good agreement among themselves, although the concentrations of aqueous suspensions were not the same. It can also be deduced that:

(a) The main transition temperatures and enthalpies of saturated lecithins with acyl groups containing 12, 14, 16, 18 and 22 carbon atoms increase progressively. On a plot of enthalpy change against transition temperature, the points for lecithins with increasing number of carbon atoms lie approximately on a straight line. Extrapolation of this line would indicate that saturated lecithins with hydrocarbon chains shorter than 12 carbon atoms cannot, under ambient conditions, form a stable bilayer.

(b) The main transition temperatures and enthalpies of the unsaturated lecithins are significantly lower than those the corresponding saturated lecithins. In biological membranes the acyl chain of phospholipid bilayers have usually more than 12 carbon atoms so that stability against dissociation into monomers can be guaranteed [42].

Table 1 Transitions thermodynamic data for various phospholipidis in multilamellar aqueous suspensions

Phospholipid	T_{tr} / °C				CU / molec.	or	$\Delta T_{1/2}$ / °C	ΔH / kcal·mole^{-1}			
	a	b	c	d	b	c	d	a	b	c	d
DLPC			–1.8			980				1.7	
(C12)											
DMPC		13.5	14.2		200	280			1.1	1.0	
(C14)	23	23.7	23.9	24	200	330	0.13	6.6	6.3	5.4	5.4
DPPC		34	35.3		70	290			2.3	1.8	
(C16)	41	41.7	41.4	41.4	70	260	0.14	8.7	9.7	8.7	8.5
DSPC		49.1	51.5		230	160			1.4	1.8	
(C18)	58	58.2	54.9		80	130		10.7	10.8	10.6	
DOPC	–22							7.6			
(C18)											
DBPC	75							14.9			
(C22)											
DLPE				30.5			0.3				3.5
(C12)											
DMPE											
(C14)			49.5	49.1		140	0.5			5.8	5.7
DPPE				63.1			0.2				8.8
(C16)											

The experimental thermodynamic data in Table 1 for the α-lecithins have been compared with theoretical data derived from a statistical mechanical partition function with adjustable parameters. By use of optimum values for these parameters, agreement between calculated and experimental data is quite reasonable for both pure α-lecithins and their binary mixture [43].

The thermal behaviour of multilamellar vesicular various lecithins not only with different lengths of acyl chains but also in different positions (1,2 or 1,3 of the acyl chains in the glycerol backbone) has been evaluated by calorimetry and X-ray studies. The results show that the difference in thermodynamic data is due to the variation in the acyl chain length, to their positioning in the glycerol backbone and to differences in the thermal history of the sample [44, 45].

a) Lamellar bilayer lecithins

i. Multilamellar (vesicles) suspensions of DPPC samples, after being maintained at 0°C for prolonged periods of time, reveal a third endothermic transition or 'subtransition', which has been detected by both DSC and X-ray diffraction. This transition is less cooperative than the other two, occurs 18°C below that of the pretransition and is accompanied by an enthalpy change about half that of the main transition. When the subtransition is not clearly observed, and the pre- and main transitions are identical with those observed on the initial heating run, the sample must be cooled to well below 0°C for the subtransition to occur. The rate of reversal of this transition on cooling is extremely slow. Prolonged storage of samples at a low temperature converts them into more ordered stable bilayer crystal forms, probably accompanied by a change in hydration at the polar head. Moreover, this is clear evidence that the subtransition represents a transition from a bilayer crystal form with an ordered hydrocarbon chain into a more regular form than that of the usual gel [46–48].

The acyl chain length affects the metastability of aqueous liposomes of saturated lecithins. A plot of the main and subtransition enthalpies (as measured by DSC) against the acyl chain length, n, reveals that the sum of the main and subtransition enthalpies is almost constant between $n = 14$ and $n = 20$. For $n>14$ the subtransition enthalpy decreases with increasing n and extrapolates to zero above $n = 20$, implying that the main and subtransition phases become one [49].

ii. Small unilamellar vesicles may be prepared from MLV. Thus, SUV of a homogeneous size of DPPC were prepared by sonication of MLV of synthetic DPPC. DSC curves of the MLV exhibited two distinct transitions at 35.4 and 41.2°C with enthalpy changes of 1.6 and 8.2 kcal·mole^{-1}, respectively – data in good agreement with values reported in the Table. DSC curves for the SUV, however, displayed two distinct broadened endotherms at 36.9 and 41.2°C with enthalpy changes of 3.9 and 2.9 kcal·mole^{-1}, respectively; moreover, the ther-

mal characteristics of the two types of liposomes were quite different. The thermal characteristics of MLV were stable with respect to time and reproducible over several DSC scans, whereas those of SUV were dependent on the history of the sample. As the number of scans increased, the enthalpy associated with the higher temperature transition increased while that associated with the lower temperature transition decreased. The thermotropic behaviour of the latter sample was similar to the MLV, especially if the sample was stored in the frozen state for some hours [50]. In addition, incubation of these sonicated phosphatidylcholine vesicles at temperatures close to the main endotherm for several hours usually led to curves similar to that of MUV [51]. Similar DSC observations in DSC curves were made for DMPC vesicles [52].

An instability dependent on temperâture and time is thus characteristic of SUV of lecithins. Stored in frozen state, incubation at temperature below the transition and repeated scans through the transition all cause a change in the DSC curves revealed by the shapes of endotherms and their temperatures and enthalpies; these DSC curves were similar to those of stable MLV. This is probably due to the small radius of curvature of SUV with the 'strained' configuration and the trend to form larger structures involving either fusion or molecular diffusion. DSC is an accurate method for obtaining evidence on the extent and the kinetics of mixing of lamellar vesicles and enables controversial results in the literature to be explained [50–52].

iii. Large unilamellar vesicles with low curvature and a higher internal aqueous volume are particularly interesting, as they would be more suitable as model membrane and would be useful for permeability measurements as well as encapsulation of drugs and macromolecules.

The DSC curves of LUV composed of DPPC and prepared by reverse-phase evaporation were similar to that of MLV but the gel to liquid crystal transition was at a slightly lower temperature and covered a broader temperature range. The temperature width at half height of the LUV was slightly greater than that of MLV, suggesting that the cooperative unit of the transition consisted of a smaller number of molecules. The pretransition phase was not observed. Repeated scanning of LUV caused the DSC curve to become identical with that of MLV [53].

b) Cephalin aqueous suspensions and dispersions

Cephalins in the presence of water exhibit a complex thermotropic behaviour that depends on factors such as the source from which the lipid was obtained, thermal history, procedure of preparation and presence of ions.

DSC, IR, and X-ray diffraction of a series of aqueous dispersions of synthetic saturated cephalins revealed that samples previously heated above the gel

to liquid crystal transition points gave values of thermodynamic parameters approximately the same as those listed in Table 1 for the main transitions. However, when these samples had been prepared from fresh dispersions by vortexing or sonicating without allowing the temperature to reach the gel to liquid crystal transition level, this transition took place at a higher temperature and had a considerably greater energy change. The unusual thermotropic behaviour of these saturated cephalins was demonstrated to be due to the nature of water-head group interaction and to acyl chain melting [54, 55].

A high-sensitivity DSC study, at scan rates of 0.02–1.0 deg·min^{-1}, of aqueous suspensions of a series of cephalins not heated above room temperature and heated demonstrated that, at all scan rates, the unheated samples yielded a single asymmetric transition peak, whereas multilamellar dispersions of heated samples exhibited transition with a fine structure (which could be fitted by the sum of three two-state component transitions) at low scan rates, but only a single asymmetric transition at 1.0 deg·min^{-1}. At all scan rates the transitions of heated samples occurred at lower temperature than those of unheated samples [56].

DLPE incubated in excess water for several hours at temperatures below the gel to liquid crystal transition was shown by DSC and X-ray diffraction to be metastable and to exhibit polymorphism, two or, for some samples, three phase transitions being observed [57]. Two or three transitions were also detected on DSC curves for samples of DMPE bilayers. The two additional endotherms are displayed by samples incubated near 0°C for long periods of time and the gel phase appears to be metastable over its entire temperature range [58].

The metastability of DLPE dispersions in water, depended on the DLPE source, the temperature of initial hydration and the time of incubation at low temperatures. Their DSC curves thus usually show one or two new phases at different temperature on the first heating scans, whereas all the second heating scans are identical, showing one phase transition [59].

The thermal behaviour of cephalins in excess water can also be influenced by different ions. The lipidic particles, which probably formed are closely related to the hexagonal II (H_{II}) phase transition, as recognized by X-ray diffraction [29]. DSC indicated that H^+ and Ca^{2+} destabilized cephalins MLVs with the appearance of another phase transition at low temperature [60].

Lipid mixtures

Nature can vary the molecular architecture of phospholipids by altering their hydrocarbon region depending on whether a rigid or flexible membrane is needed. Therefore, membrane at different times contain different mixture lipids. Consequently, it has been suggested that transitions in biomembrane are not

just a physical phenomenon but that they have real biological relevance as well [61, 62].

Research on the interactions that occur in multilamellar suspensions of phospholipid mixtures and phospholipid–cholesterol mixtures should improve our understanding of the structure and organization of phospholipid systems, which serve as models for membranes.

a) Phsopholipid mixtures

A high-sensitivity DSC study of the thermotropic behaviour of binary mixtures of synthetic phospholipids, such as lecithins, DLPC (C_{12}), DMPC (C_{14}), DPPC (C_{16}) and DSPC (C_{18}), in multilamellar aqueous suspensions revealed that, in contrast to the individual phospholipids, which exhibited sharp highly cooperative phase transitions, the mixtures containing different hydrocarbon chains melted over a broad temperature range. Moreover binary systems with acyl groups containing 14 and 16 or 14 and 18 carbon atoms exhibited essentially complete miscibility in both gel and liquid crystal states. However, the system with acyl groups containing 12 and 18 carbon atoms (i.e. difference of six carbon atoms) showed only limited miscibility and led to lateral phase separation in the gel state [39].

Binary mixtures of cephalins and lecithins, such as DMPE (C_{14})–DSPC (C_{18}) or DPPE (C_{16})–DMPC (C_{14}), exhibited complete miscibility in gel and liquid crystal phases, forming mixed bilayers and having transition temperatures intermediate between those of the pure components [39, 63].

b) Phospholipid–cholesterol mixtures

Aqueous suspensions at very high as well as very low concentrations of synthetic *a*-lecithins DMPC, DOPC, DPPC mixed with cholesterol showed clearly defined interactions on DSC examination. The gel to liquid crystal transition of lecithin bilayers gradually broadened and diminished as the amounts of cholesterol increased until it disappeared at 33–50 mole% cholesterol [64, 65]. Study of aqueous multilamellar suspensions of DPPC and DMPC with cholesterol, employing a highly sensitive and stable scanning microcalorimeter, revealed that the broad endothermic transition became undetectable above 50 mole% cholesterol [66].

The transition enthalpies, as indicated by high-sensitivity DSC, of aqueous dispersions of cephalin–cholesterol mixtures were nearly the same as those of aqueous dispersions of lecithin–cholesterol mixtures. But the transition temperatures of aqueous dispersions of cephalin–cholesterol mixtures were higher than those for the corresponding lecithins. Moreover, when cholesterol was

added to equimolar mixtures of cephalins and lecithins, preferential affinity for either of the phospholipids was not shown calorimetrically [67].

Aqueous dispersions of mixtures of cholesterol with *a*-lecithins (DSPC, DOPC, SOPC or OSPC) have been examined by DSC at both low and high concentrations of cholesterol. At low fixed cholesterol concentrations the endotherms obtained, had a different shape for each lecithin and the endotherms could be resolved into broad and narrow components. At high cholesterol concentrations, all the mixtures gave one broad endotherm, but the concentration of cholesterol at which the endotherms could not be resolved from the base line was different for each lecithin. Quantitative differences in the interactions between cholesterol and phospholipids were determined by the phospholipid structure, including the nature and location of acyl chains [68].

During the last few decades fatty acids with no counterparts in terrestrial sources have been discovered in phospholipids of sponges. These are characterized by very long chain lengths (C_{24}–C_{30}) and unexpected locations of double bonds. The effect of cholesterol or marine sterols on liposomal bilayers of natural or synthetic cephalins and lecithins with C_{26} acyl groups having the double bonds in different place were studied by DSC. It was concluded that these cephalins and lecithins hardly incorporated cholesterol into their liposomal bilayers as their endothermic transitions were not affected to an appreciable extent: however, their short chain (C_{18}–C_{24}) synthetic analogues with the same double bond pattern readily incorporated cholesterol. It would appear, therefore, that chain length is the predominant factor in determining the interaction of these phospholipids with sterols, although the location of the double bond may also play a contributing role [69, 70].

Synthetic amphiphiles

a) Double-chain amphiphiles

Interest in the orientation of synthetic amphiphiles began with the first observation of the formation of biomembrane-like bilayer structures from didodecyldimethylammonium bromide in water [6]. Moreover, some of the simple double-chain ammonium amphiphiles in water display various morphologies, such as vesicles, rods, tubes and disks, whereas bilayer vesicles of chiral amphiphiles undergo metamorphoses to large helical aggregates [71]. In addition to ammonium hydrophilic head groups anionic groups such as phosphate, sulphonate, carboxylate and zwitterionic group can also be used as the head group [72]. Phase transitions, mainly that between gel and liquid crystal, (T_c), as revealed by DSC, are, just as for lecithin liposomes, fundamental characteristics of the bilayer membrane of synthetic amphiphile vesicles [7].

From DSC measurements on samples of a variety of dialkyl (C_{12} to C_{18}) amphiphiles in water (1–2 wt%) prepared by different methods (sonication, freezing and dispersion) the following important conclusions can be drawn.

1. The DSC curve obtained for a particular samples, depended on the sample preparation procedure: thus, frozen samples of the ammonium and sulphonate bilayers yielded endotherms at temperature higher than those for the corresponding sonicated samples by 3–30°C. The separation increased with increasing alkyl chain length. This discrepancy arose from the different dispersion states of the amphiphiles, and DSC curves of dispersions of frozen samples were transformed to those for the sonicated samples after 3–5 heating scans.

2. The transition temperatures, T_c, (0–80°C) for a series of these amphiphiles increased progressively with increasing alkyl chain lengths. However, the transition enthalpy (2–12 kcal·mole^{-1}) did not obey any simple relation and varied with the alkyl chain lengths in a peculiar way.

3. Amphiphiles with anionic head groups (phosphate and sulphonate) and with fixed alkyl chain length, gave a higher T_c than the other types (cationic and zwitterionic). The T_c values for zwitterionic (phosphocholine) were located in between those for anionic and cationic types. This suggests that the phosphocholine (zwitterionic) head group does not favour bilayers stabilization [73].

Double-chain ammonium amphiphiles which possessed ether linkage in the alkyl chains of the hydrophobic tails, sonicated in deionized water, gave flexible aggregates under the microscope. DSC curves of these aggregates, in comparison with other aggregates and with those of corresponding amphiphiles which either no ether linkage or with an oleyl chain in the alkyl tail, gave very sharp peak for the main transition at 33°C and ΔH = 67 kJ·mole^{-1} (~16 kcal·mole^{-1}), whereas the aggregates without ether linkage gave main transition at 45°C and ΔH = 41 kJ·mole^{-1} (~9.8 kcal·mole^{-1}). When aggregates of amphiphiles with ether linkage were kept on the microscope stage at temperatures above the main transition temperature for about 30 min, flexible aggregates, such as large vesicles, tubes, and fibres, were observed. These were subject to rapid Brownian motion and the shear force of water flow aligned the fibres in one direction. Flexible double-helical rods were also seen, the double-helix growing with simultaneous twisting. When these aggregates were kept on the microscope stage, at room temperature (below the main transition temperature) crystalline helical forms were observed. Thus, the ether linkage can make bilayers more fluid without losing their component cooperativity, as is indicated by the low temperature of the DSC main transition and its sharpness [74]. Similar dynamic structural transformations were also observed in lecithin liposomes [75]. It may be deduced therefore, that this polymorphism can be achieved by eliminating

the net charge in the head group, as in the lecithin, or by incorporating an ether linkage, as in ammonium amphiphiles.

b) Single-chain amphiphiles

Vesicles, heterogeneous in size, have been prepared from dilute aqueous dispersions of C_8–C_{18} single-chain amphiphiles derived from fatty acids or fatty alcohols. They formed only under specific conditions, such as during acid titration of alkaline micellar solutions of fatty acids, and could be transformed by heating into lamellar fluid vesicles. These transformations occurred over a *pH* (designated as standard value) that varied with the degree of unsaturation and chain length of the hydrocarbons. Determinations of their thermotropic transitions were made using a thermistor-equipped hollow microscope slide which permitted phase-contrast microscopy over a temperature range of 10–60°C. It was assumed that the visible transitions corresponded to the major transition from the gel to the liquid crystal phase. This melting point transition, occurred 7–10°C below the melting point of the bulk (anhydrous) material [76]. The importance of liposome formation from monoalkyl amphiphiles reinforces the suggestion that such compounds must have been present before the accumulation of proteins, polynucleotides and other complex molecules that contribute to the formation of semipermeable membranes in prebiotic conditions [77].

Single-chain amphiphiles with rigid aromatic segments also give rise to vesicle structures. The essential structural elements of these amphiphiles are, generally, the flexible tails – i.e. the linear methylene chains of length C_7 or more – the rigid segments, usually consisting of two benzene rings (biphenyl, diphenylazomethine, azobenzene) and the hydrophilic head group – e.g. trimethylammonium, phosphate or sulphonate. The amphiphile with a C_{12} hydrocarbon chain, diphenylazomethine as the rigid segment and trimethylammonium as the hydrophilic head group, diluted in aqueous dispersion (1.5 wt%) gave a DSC endotherm that can be attributed to the transition at 40°C, suggesting the liquid crystal nature of its aqueous aggregates. The enthalpy change at the 40°C peak was 0.51 kcal·mole^{-1}, which is only about 6% of the enthalpy change of (although approximately at the same temperature as) the transition reported for the DPPC in Table 1. It is possible, therefore, that the change in molecular motion at the phase transition becomes less drastic when the dialkyl chain is replaced by the partially rigid, single chain. The 40°C peak moved to lower temperatures and the peak height decreases simultaneously as the hydrocarbons tail became shorter [78].

The structural elements of single-chain amphiphiles, which are used to improve the assemblage and intermolecular alignment of aggregates to be transformed into vesicles, are a flexible tail, a rigid segment, a hydrophilic head

group, a spacer group and an additional interacting group. Aqueous aggregates of such amphiphiles a methylene chain as the spacer group and an ester group as the additional interacting group (connector) at the tail end yielded DSC endotherms that could be attributed to the gel to liquid crystal transitions of the assemblage. The enthalpy changes were 0.4–3.8 kcal·mole^{-1} and the transition temperature were 18–47°C [79].

c) Triple- and four-chain ammonium amphiphiles

Electron microscopy of a series of ammonium amphiphiles possessing three long-chain alkyl tails, (C_{12} or C_{16}) and a series of ammonium amphiphiles in which one, two or three of the long chains were fluorocarbons indicated the formation of bilayer aggregates and vesicles in aqueous dispersion. The presence of connectors, such as esters, and spacers, such as methylene groups, between the hydrophilic and hydrophobic units seemed to stabilize in particular the molecular arrangements which are then maintained in sonicated and frozen samples and are effective in the formation of well-developed bilayers. DSC thermodynamic data also indicated that (a) the transitions temperature and enthalpy are always greater for a frozen sample than for a corresponding sonicated one; (b) the transition shifts to lower temperatures as the spacer methylene lengthened from C_2 to C_{11}; (c) the transition appeared at a lower temperature when one of the hydrocarbon chains was replaced with a fluorocarbon chain, whereas introduction of two or three fluorocarbon chains shifted it to higher temperature. The enthalpy change of this transition decreases as the number of the fluorocarbon chains increased [80, 81].

A series of amphiphiles in which four long alkyl chains were connected to the single trimethylammonium head group via amino acid residues gave aqueous dispersions on sonication and the formation of bilayer membranes was confirmed by electron microscopy, DSC and fluorescence depolarization. DSC transition temperatures and enthalpies were higher for frozen samples than for those prepared by sonication [82].

Remarks on the DSC behaviour of synthetic amphiphiles:

(a) The variation in thermodynamic data for single-chain amphiphiles could not be correlated with molecular structure to the extent possible for double-chain amphiphiles. This is attributed to interdependent influences of the various structural elements on the DSC behaviour. (b) The entropy changes for single-chain amphiphiles with spacers and aromatic rigid segments were much smaller than those for bilayer forming double-chain amphiphiles, while those for triple-chain amphiphiles were much larger [83]. (c) The enthalpy and entropy values

for the four-chained ammonium amphiphile bilayers were greater than those for the corresponding bilayers of double-chain amphiphile [82].

Conclusions

The evidence given in this review demonstrates that thermal analysis and complementary techniques can be very useful for characterizing thermotropic mesophase structures of ionic amphiphiles, both in the anhydrous state and when in aqueous medium. In particular, when in excess water these natural or synthetic amphiphiles aggregate to form liposomes-vesicles, DSC can be used to reveal their thermotropic behaviour. The phase transition characteristics of ionic amphiphiles so obtained are also important in determining their physical behaviour. Those molecular assemblies that possess different sites for intermolecular binding can be combined with molecules, ions, and electrons to create a supramolecular architecture of great importance for their applications, (Refs [7, 26, 62, 84 and 85]).

* * *

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Zusammenfassung — In Teil I dieses Artikels wurden die thermotropischen Mesophasen von wasserfreien ionischen Amphiphylen behandelt. In vorliegendem Teil wird ein Überblick über die thermotropischen Mesophasen ionischer Amphiphylen in wäßrigem Medium, bestimmt mittels Thermoanalyse, mikroskopischen Untersuchungen, Röntgendiffraktion und anderen Techniken, gegeben.