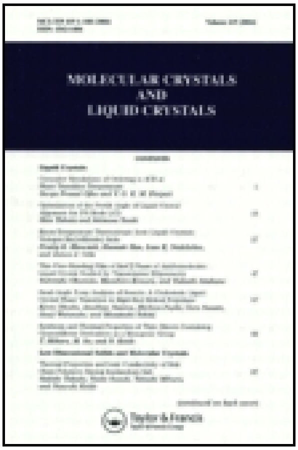

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Size and Surface Effects Related to Phase Transitions in Human Plasma Low Density Lipoprotein

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Size and surface effects on liquid crystal transitions are studied by absorbing samples in porous silica (Porasil) and observing the phase transitions by differential scanning calorimetry. The transitions are depressed and broadened and the transition enthalpy decreases. The smectic–cholesteric–isotropic transitions of cholesteryl oleate absorbed in 150 Å porous silica are measured. These transitions are compared with previously reported liquid crystal transitions in human plasma low density lipoproteins (LDL). We show that the broad transition and its low enthalpy observed in LDL can be explained, at least partially, in terms of size effect phenomena.

INTRODUCTION

The general statistical treatment of a phase transition is in the "thermodynamic limit" of an infinite system. This approximation is valid for macroscopic systems. However, as the size of the system is reduced to atomic dimensions, the arguments based on the thermodynamic limit must break down. In the range of sizes between these two extremes, a thermodynamic approximation can be made by considering the surface, which is now a significant part of the sample. This is the approach adopted by Gibbs and Kelvin and accounts for the observed melting point depression of a small sample relative to the bulk material.[1,2]

There is continuing interest in the size dependence of the melting of small particles.[3] This is naturally related to the nucleation theory of phase transitions. The nuclei of the new phase are small systems, and their thermodynamic description is a size effect problem.[2]

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Biological processes often occur at surfaces or interfaces (e.g. biological membranes) and size effects become important in the thermodynamic description of such systems. Plasma lipoproteins provide another example of biological assemblies exhibiting size and surface effects. For example, low density lipoprotein (LDL)[4] is a complex particle 200 Å in diameter which circulates in the bloodstream. Figure 1 illustrates our current view of the structure of human LDL. The surface region consists primarily of phospholipid and apoprotein. The central region contains a large proportion of cholesterol esters, primarily cholesteryl oleate and linoleate. Triglyceride and cholesterol are also present in small amounts in the central region.

LDL is a major carrier of serum cholesterol in man. Elevated serum LDL is positively correlated with an increased incidence and severity of atherosclerosis.[5] Elucidation of the precise structure and function of LDL will help to clarify the causes of this disease.

A phase transition has recently been observed in LDL and identified with a reorganization from a smectic-like to a liquid-like arrangement of the cholesteryl ester core component[6,7] (see Figure 1). This phase transition is supportive evidence that the cholesteryl esters are agglomerated and not dispersed in the particle. In LDL the smectic transition takes place at about body temperature, suggesting that it may be significant in the metabolism of the particle. It is speculated that the smectic state of the core region may

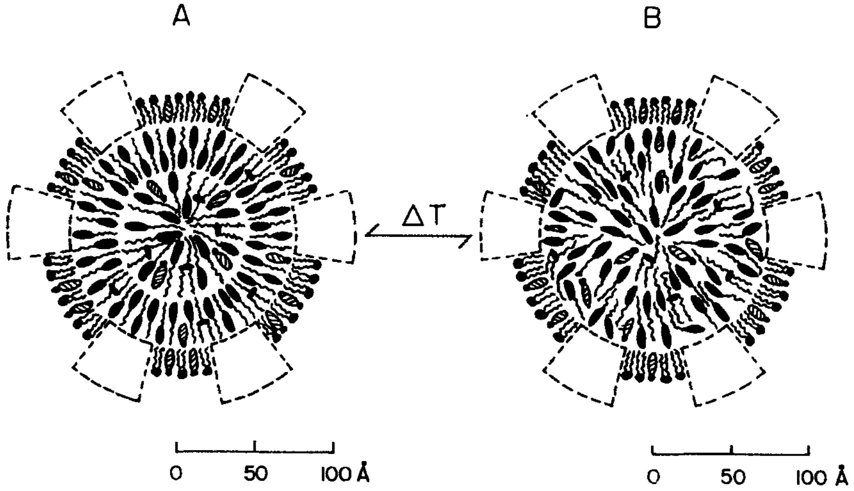

FIGURE 1 Schematic representation of the distribution of lipids in LDL phospholipid; cholesterol, cholesterol ester; triglyceride. A, 10°C; B, 45°C. The phase transition associated with the cholesteryl ester-rich central region is illustrated. The outer surface is mainly phospholipid and apoprotein (taken from Ref. 7).

influence the surface structure of LDL and alter its interaction with cell membrane receptors.[7]

We have developed a simple means of investigating size and surface effects on phase transitions.[8] The sample is absorbed in porous silica of uniform pore diameter and the phase transition is then observed by differential scanning calorimetry (DSC). The application of this technique to liquid crystal phase transitions and the relation to thermal transitions in LDL are presented here.

EXPERIMENTAL

Materials

We are investigating small depressions in transition temperature attributed to size and surface phenomena. Since the transition temperatures are sensitive to impurities, it is necessary to use high purity samples.

The liquid crystals *p*-azoxyanisole (PAA), and 4,4'-Bis(Heptyloxy) azoxybenzene (HAB) (Eastman Kodak) are purified by recrystallization from *n*-pentanol and then ethanol. PAA is further purified by zone refining. Cholesteryl myristate (Eastman Kodak) is purified by recrystallization from *n*-pentanol and then acetone. Cholesteryl oleate (Nu-Chek) is more than 99% pure and used without further purification.

The following (non-liquid crystal) materials are also utilized: naphthalene (Fisher thermometric standard), octadecane (Eastman Kodak), and water, purified by distillation. A DSC estimate of purity from the melting transition indicated that the cholesteryl oleate and octadecane are better than 98% pure. The remaining materials are better than 99.9% pure.

Low density lipoprotein

Low density lipoprotein is isolated from human plasma by ultracentrifugation as previously described.[6,7]

Porous silica and absorption

A technique of producing porous silica of controlled pore size has been developed.[9] This utilizes the phase segregation properties of borosilicate glasses, where one of the phases is removed by an acid treatment. The material has been developed for chromatography and is available under the commercial name Porasil.[10] It is provided in six pore sizes: A, <100 Å; B, 100–200 Å; C, 200–400 Å; D, 400–800 Å; E, 800–1500 Å and F, >1500 Å. A surface deactivation treatment with ammonium chloride is also available. The deactivated version is generally used with liquid crystal samples.

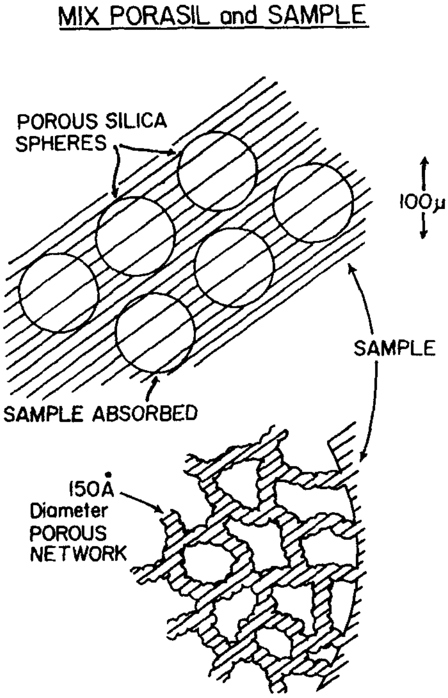

FIGURE 2 Sample and porous silica (Porasil) mixture illustrating absorption of sample into porous microcapillaries.

Figure 2 illustrates the structure of Porasil and the behavior of a molten sample in contact with it. Porasil is supplied in the form of 100 μ spheres. Each sphere has a porous structure which is a system of interconnecting microcapillaries of known diameter. The diameter shown in Figure 2 is an average value for Porasil B, 100–200 Å.

A liquid brought into contact with Porasil will be absorbed by capillary action if the contact angle favors this. We experience no difficulty in absorbing molten samples into Porasil, judging from the effects on the transition.

It is essential that the silica should not introduce impurities into the samples, since any change in sample purity will also be reflected by a change in the transition temperature. The Porasil is baked at 150°C in the nitrogen atmosphere of the DSC for several hours to remove volatile surface contamination. The sample is then added without removing the Porasil from the calorimeter. A less stringent method using a vacuum oven to bake out the Porasil does not affect the result.

Differential scanning calorimetry

Differential scanning calorimetry is performed using the Perkin–Elmer DSC 2.

THEORETICAL BACKGROUND AND RESULTS

DSC of Porasil-absorbed samples

The sample is absorbed in Porasil as described above. A fraction of the sample remains in the interstices between Porasil particles. The interstitial dimensions are of order of the particle size (100 μ). At sizes of greater than 1 μ the effect of size on the transition behavior is negligible.[11] Therefore the interstitial material behaves as the normal bulk material.

A typical DSC thermogram for naphthalene in Porasil is shown in Figure 3. Two peaks are observed. The higher temperature peak is identical to that for the melting transition of the normal bulk material and we associate this peak with the melting of the interstitial material. The broad peak at lower temperature is attributed to the melting of the material absorbed in the 150 Å microcapillaries of the Porasil.

The temperature difference ΔT between the two transitions is the temperature depression induced by size and surface effects. Temperature gradient errors are minimized by measuring ΔT at constant power as shown in Figure 3. The lower peak is broadened by the pore size distribution, and

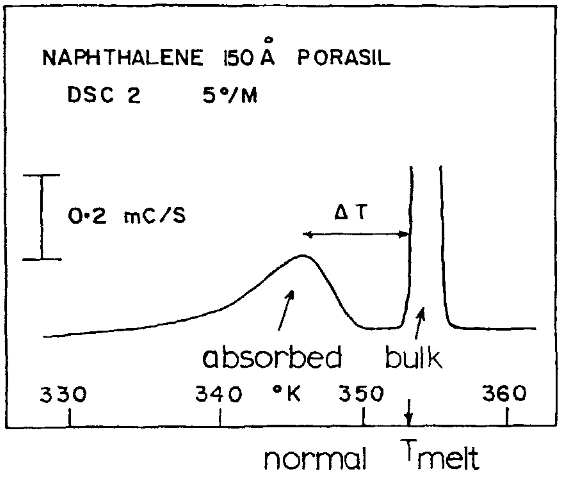

FIGURE 3 DSC thermogram of naphthalene mixed with Porasil B, 150 Å pore size.

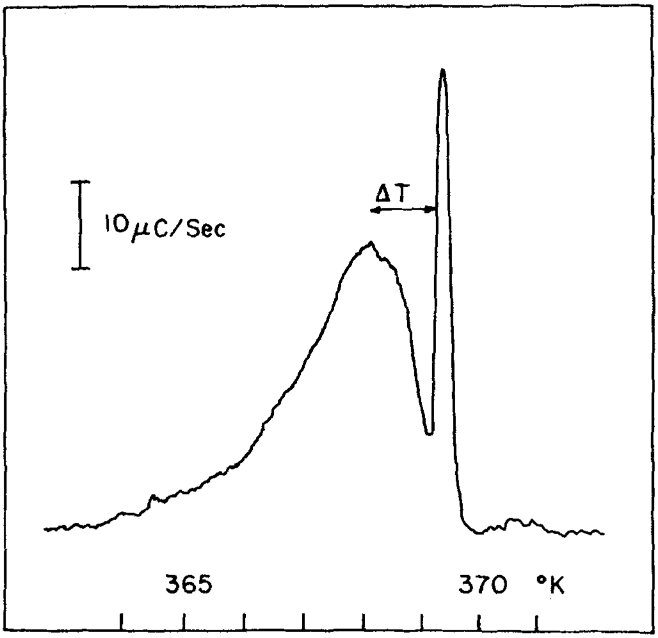

FIGURE 4 DSC thermogram of smectic to nematic transition of 4,4′-bis(heptyloxy)azoxybenzene mixed with Porasil B, 150 Å pore size. Heating rate, 1.25°C/min.

we correlate the average pore size with a transition temperature corresponding to the peak.

The behavior of a liquid crystal sample HAB is shown in Figure 4. This thermogram shows the smectic to nematic transition for an absorbed sample. Again, the sharply defined higher temperature peak represents the interstitial material, and the broad lower peak the absorbed material.

The area under the high temperature peak is used to calculate the weight of bulk material, and the weight of the absorbed material can be derived from the total sample weight. The transition enthalpy of the absorbed material is given by the area under the lower peak. The transition enthalpy is always smaller for the absorbed material. The results for several materials are listed in Table I.

Solid Melting

The melting depression of a small crystal can be accounted for by a surface energy term leading to the well-known Gibbs–Kelvin expression.[1,2] Various refinements of this approach have been prompted by experiments on the melting of microscopic metallic particles.[3]

TABLE I

Sample	Transition[a]	Pore diam. (Å)	ΔT (°C)	$\Delta H/\Delta H$ Bulk	σ	σ Nucleation (erg/cm^2)
Naphthalene	C → I	150	7.2	0.4	30	30[b]
Water	C → I	150	3.0	0.7	25	31[b]
Octadecane	C → I	150	3.0	0.7	13	9.6[c]
PAA	N → I	300	0.3	0.4	0.03	0.05[d]
HAB	S → N	150	1.2	0.5	0.09	
CM	S → Ch	300	0.6	0.7	0.04	
CO	S → Ch	150	1.5	0.6	0.05	

[a] C = crystal, I = isotropic, N = nematic, S = smectic, Ch = cholesteric.

[b] D. G. Thomas and L. A. K. Stavely, *J. Chem. Soc.*, 4569 (1952).

[c] D. Turnbull and R. L. Cormia, *J. Chem. Phys.*, 34, 820 (1961).

[d] Ref. 17.

The behavior of samples absorbed in microcapillaries is a more complicated problem.[12,13] Some approximation is required in describing the geometry of the absorbed sample, but this should be a constant for all samples. The sample-substrate interaction is a major difficulty. We make a simplifying assumption that the sample-substrate interface is a strained region and melting will begin here.[13] These factors give the following version of the Gibbs–Kelvin equation:

$$\Delta T = \sigma b T \cos \alpha / r \Delta H,$$

where ΔT is the depression of transition temperature; b is a geometric factor, we assume a cylindrical geometry with length much greater than diameter, giving $b = 1$; T = normal transition temperature; σ = interfacial energy between molten and solid sample; α = solid–liquid–substrate contact angle, representing the substrate interaction, which we simplify by assuming $\cos \alpha = -1$; r = pore radius; ΔH = transition enthalpy/cm^3.

Negative pressures are possible in a capillary system[12] and this could have a significant effect on the transition temperature. The absorbed material is in contact with interstitial material which is at atmospheric pressure. This will maintain atmospheric pressure throughout the system provided there is sufficient sample material remaining in the interstitial region. The results are insensitive to increased sample loading of the Porasil which indicates that pressure effects are not significant.

Liquid crystal transitions

It is generally accepted that fluctuations close to the transition have a correlation length of order 100 Å.[14,15] This should be an approximation to the thickness of the interfacial region. Therefore a description in terms of surface energy is not physically realistic for the absorbed liquid crystal samples.

It is possible to account for the depression of the transition in terms of the elastic continuum theory of liquid crystals.[14,15] The elastic distortion free energy $G \doteq 0.5K\,(\mathrm{div}^2\, n + \mathrm{grad}^2\, n)$ where K is an elastic constant. Assuming the direction n changes completely across the diameter d of the pore, gives the approximation $G = K/d^2$. Applying the usual thermodynamic approximation $G = \Delta T \Delta H/T$ yields $\Delta T = TK/\Delta H d^2$. Substituting the available values for PAA[14,15] gives ΔT of order 1°C, which is comparable with the experimental value 0.3°C. Thus the observations are readily accounted for by elastic strain energy.

Transition width

The transition width of a sample divided into small particles increases as the size of the particle decreases. The simplest description of this effect is attributed to Van't Hoff.[16] For a two state transition, the width of the transition at half height $\Delta T = 4kT^2/\Delta H d^3$ where k is the Boltzmann constant, ΔH is the transition enthalpy/cm^3 and d^3 represents a volume. Putting $d = 100$ Å gives for typical transition enthalpy: crystal melting $\Delta T = 0.1$°C, liquid crystal transition $\Delta T = 10$°C. The Van't Hoff approximation for ΔT is an underestimate when additional intermediate transition states are significant. We conclude that Van't Hoff broadening is an important factor in the liquid crystal transitions (see, for example, Figure 4). There will also be a broadening of the transition on account of the pore size inhomogeneity. This will be most effective where the transition is more sensitive to pore size, as in the solid-melt transitions.

Comparison with Nucleation

Classical nucleation theory[2] describes the stability of a nucleus in terms of an interfacial energy σ and a volume free energy $G = \Delta T \Delta H/T$. This is the same approach we adopt for the melting of a small particle. Therefore the σ derived from nucleation experiments should be comparable with the σ derived from the Porasil experiments.

Table I shows the results for several solids and liquid crystals. The σ values are in reasonable agreement. This suggests that the theoretical basis for our interpretation of the Porasil experiments is correct.

The σ values for the liquid crystal transition have little physical significance as discussed earlier. However, σ does have the correct order of magnitude as shown by comparison with a figure derived from interface stability experiments.[17,18] These values are also of the same order as theoretical predictions.[19] Small interfacial energies are also consistent with the difficulty of observing supercooling in these transitions.

Low Density Lipoproteins

Figure 5 shows the DSC thermogram for a 5 weight % solution of LDL in 0.19 m NaCl. Thermograms (a) and (b) show a reversible transition centered at 30°C and of half width 10°C. There is a marked absence of supercooling of this transition. Attempts to induce crystallization by lowering the temperature to −60°C or maintaining the temperature at 0°C for 24 hrs. does not affect the transition (see thermogram (c)).

The transition enthalpy derived from these thermograms is 0.69 cal/gm of LDL cholesteryl ester, which is comparable with the 1.0 cal/gm observed for the extracted esters.[6,7]

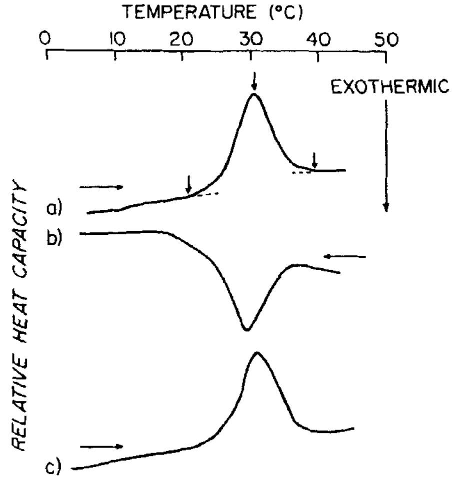

FIGURE 5 DSC thermogram of human low density lipoprotein showing reversible transition centered at ~30°C. (a) initial heating curve 0 → 45°C, (b) cooling curve 45 → 0°C, (c) heating curve 0 → 45°C after cooling to −60°C (taken from Ref. 7).

The behavior of one of the major esters in LDL, cholesteryl oleate, is shown in Figure 6. The upper thermogram (a) is from pure cholesteryl oleate, while (b) shows the transition when cholesteryl oleate is absorbed in 150 Å diameter Porasil. The small sharply defined peak in (b) is associated with the interstitial material smectic transition. By using a smaller ratio of Porasil to sample, both smectic and cholesteric peaks of the interstitial material can be resolved.

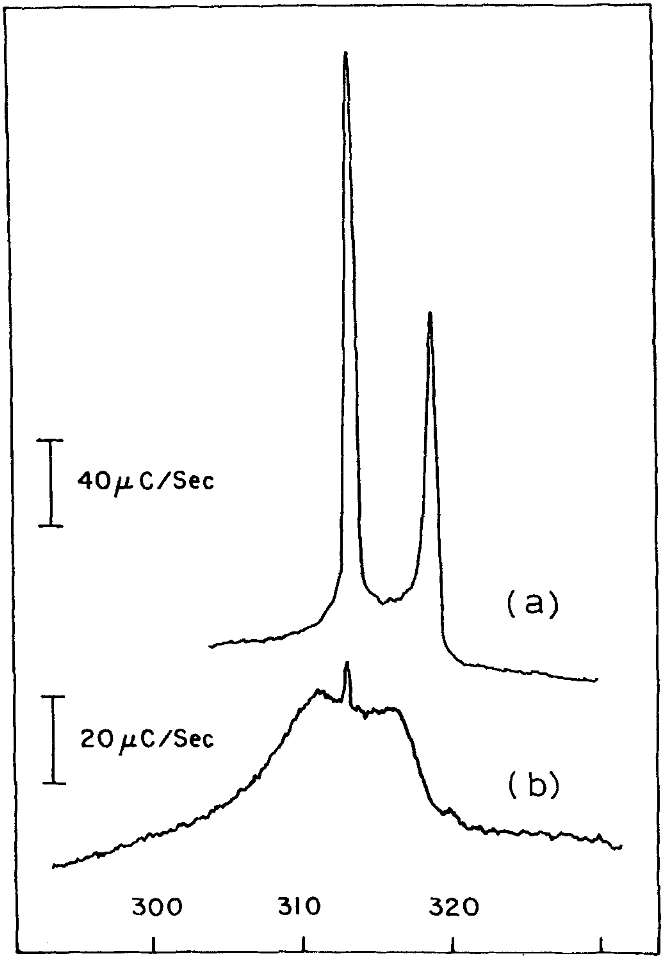

FIGURE 6 DSC thermogram of cholesteryl oleate. Upper, pure bulk sample. Lower, similar sample absorbed in Porasil B, 150 Å pore size. Heating rate 5°C/min.

In thermogram (b), judging from the peak areas, virtually all of the sample is absorbed in the Porasil. This transition of absorbed cholesteryl oleate closely resembles that shown by native LDL (see Figure 5). Moreover, the transition enthalpy falls to about 65% of the bulk material, again in accord with the LDL transition. Therefore it is possible to explain the broad LDL transition and its lower enthalpy in terms of a size effect.[6,7] The mixture of esters and the small amounts of cholesterol and triglyceride in the core of LDL will also broaden and depress the transition.[7]

A significant characteristic of LDL is the absence of crystallization of the cholesteryl esters in the particle. Although crystallization is drastically reduced in the Porasil-absorbed cholesteryl oleate, it is not eliminated.

DISCUSSION

Using Porasil we have shown that liquid crystal transitions are observed by calorimetry with samples of order 100 Å dimensions. This reinforces the view that liquid-crystal arrangements can exist in small particles such as LDL.[6,7] Furthermore, the reduced transition enthalpy in LDL can be attributed, at least partially, to size or surface effects.

There are a number of processes which can be identified with the size dependence of the transition enthalpy. If the sample dimensions approach the interaction range of the intermolecular forces, then ΔH would be expected to decrease. Alternatively, the molecular layer in contact with the silica surface may not take part in the transition, with a consequent reduction in the observed ΔH.

Currently, we are experimenting with surface coating of the Porasil. With suitable surface conditions it may be possible to suppress crystallization of the absorbed sample. Boundary surfaces which promote a uniform orientation of the liquid crystal may raise the transition temperature.

Acknowledgements

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