The Effect of Temperature on the Surface Nature of an Adsorbed Layer of Poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) Block Copolymers

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Purpose. To investigate the influence of the temperature at which adsorption takes place and the temperature at which the adsorbed surface is studied on the polarity of Poloxamer adsorbed to a hydrophobic surface. The implication is that changes in surface nature of adsorbed Poloxamer may subsequently be related to functionality, such as changes in opsonisation of Poloxamer coated latex in animals.

Methods. The surface energies of Poloxamer surfactant have been calculated following adsorption to silanised glass plates. The adsorption to the plates was undertaken at a range of concentrations and at different controlled temperatures. The contact angles were measured using three different liquids on each surface, at a range of controlled temperatures. The surface energies were calculated using the harmonic mean and the acid-base models, via Wilhelmy plate contact angle measurements. These data were compared with previously published adsorption and hydrophobic interaction chromatography studies.

Results. The apolar surface energy term remained consistent, but the polar contribution (which was totally of the electron donor type) changed depending upon the temperature of adsorption (and to a lesser extent the temperature at which the surface energy was measured). The polar nature was most elevated at the critical micelle concentration/temperature. The data are consistent with estimates of surface hydrophobicity made using hydrophobic interaction chromatography.

Conclusions. It is argued that the changes in surface energy, which result from the different adsorption conditions, can be expected to influence the functionality of the adsorbed coat, especially for application such as drug targeting.

KEY WORDS: poloxamer; surfactant; adsorption; targeting; surface energy.

INTRODUCTION

Poloxamers (Pluronics and Synperonics) are nonionic surfactants which exist as aba block copolymers of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene). Many combinations of hydrophobe (poly(oxypropylene), POP) and hydrophile (poly(oxyethylene), POE) molecular weights exist such that a wide variety of structure and function are obtainable from the series. The uses of poloxamers include inclusion in cream and aqueous suspension formulations, as well as a large literature concerning the role of adsorbed poloxamers in controlling organ distribution of colloidal particles which have been injected into animals (see for example (1)). Much remains to be understood about the structure and function of adsorbed poloxamers. It is known that poloxamers adsorb to hydrophobic surfaces by means of the POP region, which binds to the surface in a "loop and chain" structure. This form of adsorption results in practical irreversibility of the adsorption process (as all bonds with the surface are unlikely to break simultaneously). The hydrophilic chains then project some considerable distance from the surface of the solid. The physico-chemical properties of poloxamer solutions have been the subject of many publications in recent years, most of which have been reviewed elsewhere (2).

Latex coated with different poloxamers, which all have sufficiently large coating thickness to achieve steric stabilisation of the particles and which all apparently have similarly hydrophilic surfaces, are known to accumulate in different organs when injected into animals (e.g. 3). There is considerable uncertainty about why different poloxamers will alter organ distribution of injected colloids on which they have been adsorbed. It is apparent that the organ distribution may relate to the POP molecular weight (1), which in turn may relate to the number of proteins which adsorb to the surfactant coated surface (which then function as triggers for removal to different sites by various phagocytic cells of the body) (e.g. 4).

The phase behaviour of poloxamers has been investigated in dilute aqueous solution (5–8) and in more concentrated systems (e.g. 9–10). Dilute aqueous solutions of the poloxamers show major changes in association as a function of temperature. Critical micelle temperatures (T_m) have been identified (11), and obviously the T_m is concentration dependent, with a higher T_m being seen with decreasing concentration (8). It has been shown that the T_m, and thermodynamic parameters relating to this transition, correlate with the POP content of the surfactants, rather than with either total molecular weight or the POE content (6). Such theories have been reviewed in detail elsewhere (12).

Previously (13) we have shown that the amount of material adsorbing on a hydrophobic surface is related to the difference between the temperature at which adsorption takes place and the T_m. At any selected equilibrium surfactant concentration the amount adsorbed increases at the T_m. With further increases in temperature (above T_m) the amount adsorbed falls (see Figure 1, for experimental details see Carthew et al 13). The major jump in adsorption behaviour at the T_m shows that the liquid state conformation of the surfactant affects adsorption. The increase in adsorption at the T_m is in keeping with the proposal that the hydrophobe dehydrates and contracts at this point, thus more surfactant is able to adsorb to unit area of the solid surface. In our previous work (13) we were able to show that the amount adsorbed to a surface was not changed substantially if the temperature was altered subsequent to adsorption being completed.

The fact that the organ distribution of poloxamer coated colloidal particles correlates with POP content (1, 14), as does the T_m, may well mean that the proximity to the phase transition has an influence on the structure of the coated surface, which in turn could be the cause of the significant influence on functionality. Recently (15), we have examined the surface of polystyrene latex coated with poloxamer using hydrophobic interaction chromatography. It was reported that the hydropho-

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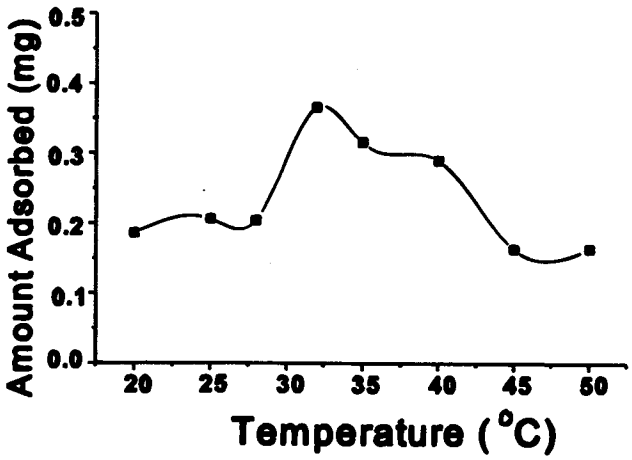

Fig. 1. The amount of P407 adsorbed to a sample of atovaquone as a function of the temperature of adsorption (for further details see Carthew *et al.* 13). The maximum corresponds to the T_m for this surfactant at this equilibrium concentration (32°C at an equilibrium concentration of 5 mg/L). A secondary maxima is seen at ca 40°C, which is also observed by the surface energy and HIC techniques (Figures 2 and 3).

bicity of the coated surface changes in a manner related to the amount of poloxamer adsorbed (i.e. is greatly influenced by the temperature of adsorption in relation to the T_m). The coating layer thickness, however, was essentially unchanged by the temperature of adsorption (i.e. not related to the amount adsorbed), but was influenced by the temperature at which the sizing was undertaken, (this is due to dehydration of the POE with increasing temperature). Hydrophobic interaction chromatography only gives a ranking of hydrophobicity of different surfaces, thus the purpose of this study was to characterise and quantify the differences fully.

METHOD

Two surfactants were used as received from ICI (from exactly the same batch of material used in Carthew et al 15), these were P407 and P338. The notional structural detail of these surfactants is for P407 a POP chain of 67 and two POE chains of 98 monomer units; for P338 the POP chain is 54 and the two POE chains are 128 monomer units, however, these surfactants are notoriously polydisperse.

Preparing Model Hydrophobic Surfaces

Hydrophobic surfaces were prepared by coating glass slides (microscope cover slips 22 mm by 22 mm) with chlorotrimethylsilane (CTMS). The plates were coated using CTMS diluted 5 ml to 500 ml in cyclohexane. The plates were equilibrated for 20 minutes, and rinsed with water. Samples of plates were tested for uniformity of coating by measuring the advancing contact angle with double distilled water (using a Wilhelmy plate method, Cahn DCA). If a contact angle of less than 85° was measured on any of five samples taken from a batch (ca 20 plates) the batch was rejected. If the angle was over 85° the plates were then used for surfactant adsorption studies.

Coating with Surfactants

The silanised plates were held in a perspex stand in a trough, which had been rinsed previously with poloxamer solution. Temperature equilibrated poloxamer solution (of desired concentration) was then added to the trough to cover the plates. The trough was then stored in a water bath at the desired temperature for 24 hours. After this period the liquid was removed and the plates were dried. We have already shown that the addition and removal of the solution with which the surface is in equilibrium does not result in substantial changes in the amount of surfactant adsorbed (13). The only difference in this experiment over those reported previously (15) is that following adsorption of poloxamer to polystyrene latex spheres the material was not dried prior to hydrophobic interaction chromatography investigation.

For P338, surfactant coating was undertaken at concentrations of 50, 250, 500 and 5000 mg/L of surfactant, at temperatures of 25, 30, 37 and 45°C. For P407, the surfactant was adsorbed from a solution of 250 mg/L at 15, 24, 28, 32, 37, 45 and 50°C.

Surface Energy Determination for Coated Surfaces

The surface energies of the plates were assessed by measuring contact angles with three different liquids; water, formamide and diiodomethane. Contact angles were measured using a Wilhelmy plate approach (Cahn DCA). Five measurements were taken and means and standard deviations calculated for the cosine of the contact angle (16).

Surface energies were calculated using both the harmonic mean equation (17) using the data for water and diiodomethane, and the electron donor-electron receptor approach (18) using the contact angles for all three liquids.

RESULTS AND DISCUSSION

Surfactant P338

The contact angle data are not presented due to space limitations. The hydrophobic interaction chromatography shown in Figure 2, reveals that the surface of the adsorbed surfactant became more hydrophilic when adsorption occurred at the T_m. The surface energy data (Table I) do not show this difference (for simplicity of comparison only the harmonic mean surface energy data have been shown in Table I). However, an exact comparison between the HIC data (Figure 2) and the surface energies in Table I reveals no difference in hydrophobicity following adsorption of P338 at 30, 37 or 45°C. Only at one point, 32°C, did the surface become more hydrophilic. Thus it can be concluded that both sets of data are consistent and that the surface only becomes more hydrophilic at the T_m and not when the adsorption is undertaken a few degrees above or below this temperature. The slightly lower polar contribution for samples where adsorption was undertaken at 25°C is also in keeping with the HIC data.

Although the surface energy data in Table I show only minor variations, a common theme is the trend for the polar component of surface energy to decrease with increases in measurement temperature (largely irrespective of the temperature at which adsorption occurred). This fall is in keeping with the dehydration of the POE with increasing temperature, and

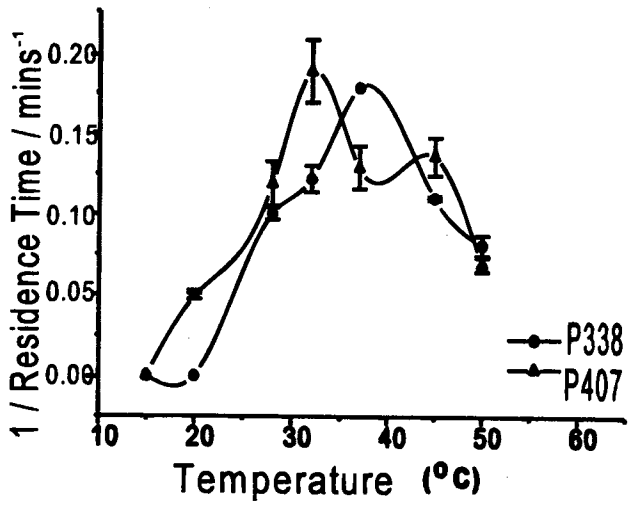

Fig. 2. Hydrophobic interaction chromatography data showing the reciprocal of retention time on a propyl-agarose column as a function of the temperature at which adsorption was undertaken (for experimental details see Carthew *et al.* 15). The maxima on these plots represent regions where the coated surfaces are most hydrophilic. The maxima coincide with the T_m for each surfactant (i.e. ca 27°C for P407 and ca 37°C for P338 at concentrations of 250 mg/L, N.B. the T_m values are lower than seen in Figure 1 due to the higher surfactant concentration used in the solution from which adsorption occurred).

shows that the reduction in size which is seen in heating the adsorbed material (which we reported previously (15)) is mirrored by a small reduction in surface polarity.

Surfactant P407

The harmonic mean surface energy data are shown in Table II. It can be seen that the polar component of surface energy rises at 28°C. The polar contribution to surface energy can be split into electron donor and electron receptor contributions, using the acid-base model proposed by Fowkes (18). The use of the acid-base approach results in identical values for the apolar (dispersive) contribution, and with a polar contribution which is monopolar (zero electron acceptor and a finite electron donor (γ^-) contribution). The electron donor contribution is plotted in Figure 3 as a function of temperature of adsorption, and mirrors the polar component obtained using the harmonic mean approach (Table II). It can be seen that a peak is observed at 28°C which is in keeping with the HIC data (Figure 2). There is also a secondary maxima at 45°C, which also is in keeping with the HIC data. It is probable that this secondary peak is reflecting a polydispersity in the P407. The adsorption behaviour of this batch of surfactant (Figure 1) to a model drug also shows a similar secondary maxima at the same adsorption temperature. Assuming that the adsorption to the model drug (atovaquone) is mirrored in the adsorption behaviour to the silanised plates used in this study, then it can be seen that the amount of surfactant adsorbed correlates with the polarity of the surface produced. The amount adsorbed is highest at the T_m, and for P407 there is also a secondary maxima. These observations may provide some explanation for the changes in organ distribution observed (3) following injection of polystyrene latex coated with different batches of P407 (each with slightly different molecular weight distributions). The changes

Table I. Surface Energies of P338 Adsorbed to Silanised Glass Plates Calculated Using the Harmonic Mean Approach, as a Function of Temperature Used During Adsorption and During Contact Angle Measure

Ia) Adsorbed at 25°C

Measured at (°C)	20		28		37		45	
Concentration of Surfactant (mg/L)	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p
50	33.6	10.6	34.3	10.8	33.3	9.5	32.2	8.5
250	33.5	10.3	32.9	10.7	32.0	9.5	32.5	9.1
500	33.3	10.2	33.7	9.8	33.3	8.5	34.2	10.0
2500	30.7	11.3	34.4	9.4	34.2	9.0	33.9	8.7

Ib) Adsorbed at 30°C

Measured at (°C)	20		28		37		45	
Concentration of Surfactant (mg/L)	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p
50	30.1	13.0	29.9	11.4	29.6	10.8	31.5	9.9
250	30.7	12.9	30.3	12.6	30.9	11.0	31.4	10.6
500	31.2	14.4	31.1	12.5	31.9	12.1	34.2	10.0
2500	32.4	19.3	32.1	13.2	32.2	12.7	35.5	10.8

Ic) Adsorbed at 37°C

Measured at (°C)	20		28		37		45	
Concentration of Surfactant (mg/L)	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p
50	31.4	12.2	31.8	11.7	32.7	11.1	34.2	10.7
250	31.1	12.7	31.7	12.2	32.1	11.8	32.5	10.5
500	32.0	12.8	32.7	11.8	32.7	11.8	34.1	10.0
2500	35.2	13.5	33.3	12.9	33.1	12.6	32.3	12.0

Id) Adsorbed at 45°C

Measured at (°C)	20		28		37		45	
Concentration of Surfactant (mg/L)	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p	γ^d	γ^p
50	31.6	12.9	31.3	12.9	32.4	12.0	34.6	11.2
250	31.7	13.5	32.2	13.0	33.2	12.3	34.7	11.7
500	32.2	14.2	32.8	13.4	34.3	13.5	34.2	10.0
2500	33.0	14.8	33.9	15.2	33.8	14.2	33.7	13.1

Table II. The Surface Energies of Surfaces Coated with P407 (measured at 25°C), According to the Harmonic Mean Equation, as a Function of Temperature of Adsorption ($mJ.m^{-2}$)

Temperature	γ^d	γ^p
15	30.5	9.4
24	31.5	8.7
28	29.6	12.0
32	30.4	9.3
37	30.9	8.7
45	31.6	9.0
50	31.2	8.5

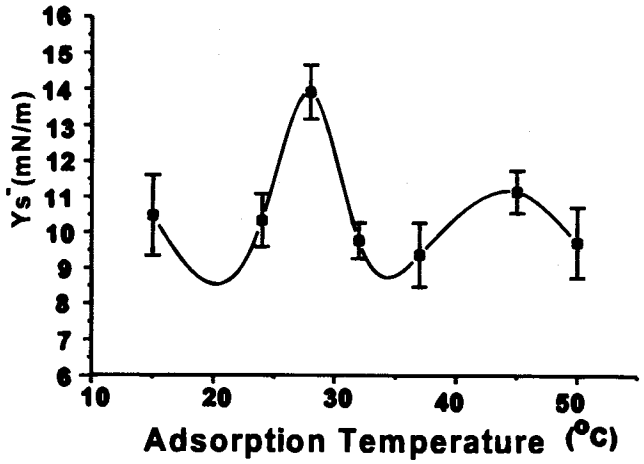

Fig. 3. Electron donor contribution of surface energy for the surface of a CTMS plate with P407 adsorbed at different temperatures (the maximum corresponds with that seen in Figure 2, as adsorption was also from a solution of 250 mg/L, the secondary maximum also fits with the data in Figure 2).

T_m, which in turn will influence the nature of the adsorbed layer. The interaction of opsonins with the adsorbed layer can be expected to change as a function of surface energy of that layer, and as such the biological fate can also be expected to alter.

CONCLUSIONS

It can be concluded that the surface energy data for a layer of poloxamer adsorbed to silanised plates are reflecting the same surface changes as were seen by HIC, following adsorption of the same surfactants to polystyrene latex. These changes are a consequence of the amount of material adsorbed which in turn is a consequence of a change in aggregation of the surfactant in solution i.e. it has been shown to change at the T_m. The functionality of adsorbed layers of poloxamer surfactant is to act as a barrier to adsorption of other species (for example of opsonising proteins in colloidal drug targeting). It is known that different surfactants result in varying amounts of adsorption of different opsonins. The data presented here show that the surface nature of the coated layer varies depending upon the surfactant used and the proximity to T_m at the point of adsorption. There is a need to consider both the temperature and the concentration of surfactant used during adsorption, to allow the influence on processes such as drug targeting to be understood more fully.

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