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Adsorption of ethoxylated surfactants on nanoparticles. I. Characterization by hydrophobic interaction chromatography

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Summary

Hydrophobic interaction chromatography (HIC) was employed to assess the surface hydrophobicity of model drug carriers (polystyrene particles) after surface modification by adsorption of ethoxylated surfactants (coating). The hydrophobicity of Poloxamer and Poloxamine 908 coated particles decreased with increasing polyethylene oxide chain length and increasing thickness of the coating layer. A reversed effect was observed for ethoxylated nonylphenols. Particles coated with Antarox CO 660 containing 10 moles ethylene oxide were least hydrophobic despite forming the thinnest coating layer. The Antarox results elucidate the contribution of the polymer solvency and affinity to the surface to the properties of the adsorption film. In conclusion, altering the hydrophobic and hydrophilic part of the surfactant molecule can be used to adjust solvency and affinity to form optimised coating layers with low hydrophobicity.

Introduction

Polymeric nanoparticles are possible carriers for controlled drug delivery and targeting by the intravenous route (Davis, 1981; Müller, 1990). The surface of the particles can be modified to reduce the uptake by the macrophages of the reticuloendothelial system (RES), mainly the liver and spleen macrophages. To escape RES recognition the particles need to be non-charged (Wilkins and Myers, 1966) and of low surface hydrophobicity to avoid opsonization (Van Oss et al., 1975, 1978, 1984). The adsorption of nonionic blockcopolymers on particles (coating), e.g. Poloxamer and Poloxamine polymers, covers the particle charge (Müller et al., 1986). The adsorption layers are hydrophilic minimizing opsonization (Wallis and Müller, 1990). The hydrophobicity of Poloxamer and Poloxamine adsorption layers was found to decrease with increasing length of the polyethylene oxide chain and thickness of the coating layer (Müller and Blunk, 1989; Müller, 1990). These surface hydrophobicity data correlated with the observed in vivo organ distributions. Poloxamer 188 with medium thickness (76 Å) and hydrophobicity reduced the liver/spleen uptake (Illum et al., 1986), 60 nm polystyrene particles coated with a thick

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layer of Poloxamine 908 (134 Å) avoided liver/spleen uptake. The particles circulated in the blood (Davis et al., 1986; Illum et al., 1987a). Considering the correlation between coating layer thickness and reduction of phagocytosis in vitro (Illum et al., 1987b) and in vivo (Davis et al., 1986; Illum et al., 1986, 1987a) a minimum coating layer thickness of 100 Å was postulated to avoid RES uptake (Davis and Illum, 1988).

However, the results could not be transferred from non-biodegradable polystyrene particles to biodegradable drug carriers (e.g. poly(lactic acid) nanoparticles (Wallis, 1990). The surface of biodegradable particles is distinctly less hydrophobic than the one of polystyrene particles (Müller, 1990) leading to little or no adsorption of Poloxamer and Poloxamine blockcopolymers. The affinity of the polymers to the particle surface is too low, the hydrophobic interaction between the particle surface and the hydrophobic anchor part (polypropylene oxide chain) of the polymers too weak. Enhanced binding could be achieved by increasing the surface hydrophobicity of the biodegradable particles (Müller and Wallis, 1989; Wallis 1990). Alternatively, the hydrophobicity of the anchor part in the coating polymer can be altered. A series of ethoxylated nonylphenols (Antarox CO) was used to investigate the effect of a more hydrophobic anchor part on the properties of the coating layer on polystyrene model carriers. Antarox CO surfactants with increasing length of the polyethylene oxide chain were used to assess whether a similar correlation between structure and layer thickness and the resulting surface hydrophobicity of the coated particles existed as observed for Poloxamer/Poloxamine.

Materials and Methods

Materials

Polystyrene particles with a size of 60 nm and 140 nm were purchased from Polysciences Inc. (Warrington, U.K.). Styrene and potassium persulphate for polystyrene particle production were purchased from Sigma (Deisenhofen, F.R.G.). The styrene was purified prior to use by distillation at 5 mmHg and 36 °C to remove the stabilizer (free radical inhibitor). The potassium persulphate was recrystallized twice from double distilled water prior to use.

The ethoxylated blockcopolymers Poloxamer and Poloxamine were supplied by ICI Chemicals (Manchester, U.K.). Ethoxylated nonylphenols (Antarox CO) were a gift from Gattefossé (Manchester, U.K. and Frechen, F.R.G.).

The chemicals for the buffer solutions ($NaH_2PO_4 \cdot 2H_2O$, $Na_2HPO_4 \cdot 2H_2O$, NaCl, Triton X-100) were of analytical grade and purchased from Sigma. The alkyl-agaroses (propylamine-agarose, pentylamine-agarose) were obtained from Sigma.

Methods

Commercial polystyrene latex particles were analysed by Static Secondary Ion Mass Spectrometry (Davies and Brown, 1987; Davies et al., 1987) for surfactant residues. No surfactant residues could be detected on 60 nm and 140 nm particles but contamination was found on larger sized commercially available particles (Davies, 1988). The surfactant-free 60 nm and 140 nm particles were used in this study whereas larger surfactant-free particles were produced by ourselves.

Polystyrene particles were produced surfactant-free as described previously (Müller, 1990). Briefly, the polymerisation of styrene (0.2 ml in 50 ml distilled water) was performed at 80 °C for 6 h. The addition of 0.09 g potassium persulphate led to a particle size in the range of 200 nm. Free initiator was removed by dialysis.

The coating of the particles was performed by mixing of equal volumes of particle suspension (2.5% w/w) and surfactant solution (2.5% w/w) and incubation overnight at room temperature.

Photon correlation spectroscopy (PCS) (Cummins and Pike, 1974, 1977; Müller and Müller, 1984) was employed to measure the particle size, width of distribution and the coating layer thickness on the nanoparticles. The width of the distribution is given by the PCS polydispersity index ranging from zero (monodisperse particles) to 0.5 (polydisperse distribution). Values above 0.5 indicate a very broad distribution which cannot be correlated any more to a logarithmic normal distribution. The layer thickness was obtained by

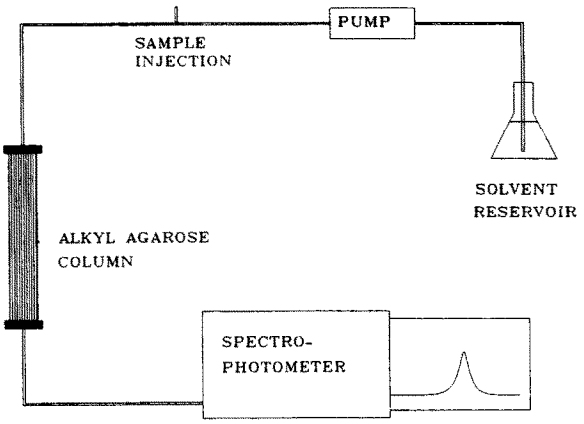

Fig. 1. Set up for hydrophobic interaction chromatography (HIC).

subtraction of the radii of coated and uncoated particles (Müller, 1990). The standard deviation of the mean radii was 0.5%.

For the HIC, a column set-up was used as described previously (Müller, 1990; Fig. 1). The bed volume was 7.85 ml, column diameter 1.0 cm, and column height 10 cm. The elution of the particles was performed with a phosphate buffer (pH 6.8, 0.02 M) containing 0.3 M NaCl. Hydrophilic particles pass the column without interaction, particles with increased hydrophobicity show a retarded elution or are retained by the column. In the next step washing of the column was performed with the buffer containing 0.1% Triton X-100. The particles were detected by absorption measurements at 350 nm using a Kontron Spectrophotometer (Kontron, München, F.R.G.). The injected particle volume was 200 μl containing 0.04–0.5% (w/w) coated particles to yield particle elution peaks with a maximum absorption up to 0.5.

The chromatograms were analysed with regard to the elution volume, the area under the curve (AUC) and calculating the AUC ratio of elution peak to wash peak (EP/WP). The AUC was also used to assess if any of the particles loaded on the column were irreversibly bound.

TABLE 1

Coating layer thickness of Poloxamer polymers with different molecular weights and Poloxamine 908 on 60 nm polystyrene particles (Müller, 1990)

Polymer		Number of units		Molecular weight	Coating layer thickness (Å)
		PO	EO		
Poloxamer	184	30	13	2900	24
	234	39	22	4200	29
	235	39	27	4600	35
	333	54	20	4950	58
	188	30	76	8350	76
	407	67	98	11500	119
	288	47	122	13500	130
	338	54	128	14000	154
Poloxamine	908	23	123	27000	134

Results and Discussion

Hydrophobicity of Poloxamer / Poloxamine coating layers

The coating layer thickness of Poloxamer polymers was found to increase with the molecular weight (Kayes and Rawlins, 1979; Müller, 1990) (Table 1). The relative surface hydrophobicities of the Poloxamer coating layers were determined by elution from a propyl-agarose column. Polymers forming thick adsorption layers led to particles which passed the column without interaction (e.g. coated 140 nm polystyrene particles). Particles with thinner layers comprised an increasing elution volume with decreasing layer thickness. Particles with very thin adsorption layers were retained on the column (Table 2).

The polymers adsorb in a Langmuirian type manner with a plateau above the CMC (Kayes and Rawlins, 1979) whereby the polyoxypropylene (POP) part adsorbs flatly onto the surface by hydrophobic interaction. The polyoxyethylene (POE) chains protrude into the dispersion medium forming a surface less hydrophobic than that of the latex particles. Low molecular weight polymers with short POE chains expose most of the flatly adsorbed hydrophobic POP parts resulting in a relatively hydrophobic surface. Higher molecular weight polymer with longer POE chains are able to cover the POP parts more efficiently by a thick POE coat. This leads to a decrease in hydro-

TABLE 2

Elution behaviour of polystyrene particles (140 nm) coated with Poloxamer polymers with increasing molecular weight

Polymer		Molecular weight	Elution volume (ml)
Poloxamer	184	2900	retained
	234	4200	retained
	235	4600	7.97
	402	5000	7.20
	217	6600	7.12
	188	8350	6.53
	288	13500	no interaction
	407	11500	no interaction
	338	14000	no interaction
Poloxamine	908	27000	no interaction

Most hydrophobic particles are retained on the column, less hydrophobic can be eluted, least hydrophobic particles pass without interaction (Müller, 1990).

phobicity of the coated particles with increasing layer thickness.

Differences in the amount of adsorbed polymers on surfaces are attributed to differences in the hydrophobicity of the surfaces (Kayes and Rawlins, 1979; Law and Kayes, 1983). The coating layer thickness of a given polymer is therefore not constant on polystyrene particles, for example Poloxamer 407 comprises a thickness of 119 Å on 60 nm particles but only 75 Å on less hydrophobic 140 nm particles (Müller, 1990). However, the relative hydrophobicities of the polymer coats are persistent. As shown by HIC, coats from high molecular weight polymers are less hydrophobic than ones with lower molecular weight polymers (Müller, 1990).

In vivo organ distributions obtained with Poloxamer and Poloxamine coated particles showed a decreased clearance by the RES with increasing coating layer thickness. Poloxamer 338 (Illum et al., 1986) and Poloxamine 908 (Illum et al., 1987a) proved to be more efficient than Poloxamer 188 (Illum et al., 1986). A minimum thickness of 100 Å was even postulated which provides least hydrophobicity and leads to particles avoiding the RES (Davis and Illum, 1988).

Hydrophobicity of Antarox CO coating layers

Polystyrene particles of two different sizes (60 nm, 190 nm) were coated with a series of Antarox CO surfactants. On both particles, the coating layers increased with increasing molecular weight as described for the Poloxamer and Poloxamine 908 polymers (Table 3).

The surface hydrophobicities of the Antarox coating layers on 190 nm latex were determined by HIC using a propyl-agarose column. To allow a comparison with Poloxamer and Poloxamine polymers, the least hydrophobic coating of these polymers was used as reference (Poloxamine 908). The chromatograms obtained with Poloxamine 908 coated 60 nm and 190 nm particles showed a distinct difference in the hydrophobicity of the coating layers. The Poloxamine 908 forms a thick layer of 134 Å on the more hydrophobic 60 nm latex. As described above this results in a very hydrophilic coat. The coated 60 nm particles passed the propyl column without interaction as indicated by the large elution peak (Fig. 2). The surface of the 190 nm polystyrene particles is less hydrophobic (Müller 1990) leading to less strong hydrophobic interactions with the adsorbed Poloxamine 908. The adsorption layer is thinner (102 Å) and consequently more hydrophobic. Only about 50% of the coated particles could be eluted with buffer. The rest was too hydrophobic and bound by the column but could be washed off with Triton X-100 (Fig. 2). Analysing the area

TABLE 3

Coating layer thickness of a series of Antarox surfactants with increasing molecular weight on 60 nm and 140 nm latex particles

Antarox CO	Number of EO units	Molecular weight	Coating layer thickness on	
			60 nm (Å)	190 nm (Å)
660	10	660	10	not detectable
730	15	880	10.6	11.6
850	20	1100	14.2	17.7
970	50	2420	61.4	35.9
990	100	4620	72.9	51.5

The larger latex comprises a less hydrophobic surface resulting in thinner layers compared to the more hydrophobic 60 nm particles.

under the curve revealed that washing with Triton could remove all of the particles from the column, none were irreversibly bound.

Antarox CO 990 possesses a POE chain with 100 units which is comparable to Poloxamer 407 (98 units) and Poloxamine 908 (123 units). However, the coating layer is thinner (51 Å). The Antarox coat proved to be distinctly more hydrophobic than the Poloxamine 908 coated reference particles. Little could be eluted with the buffer, almost 100% of the particles were retained and could only be washed off with Triton X-100 (Fig. 3).

The thinner Antarox coating layers were thought to be even more hydrophobic. No elution peaks were expected considering the dependency between layer thickness and hydrophobicity of the coat established for Poloxamer and Poloxamine.

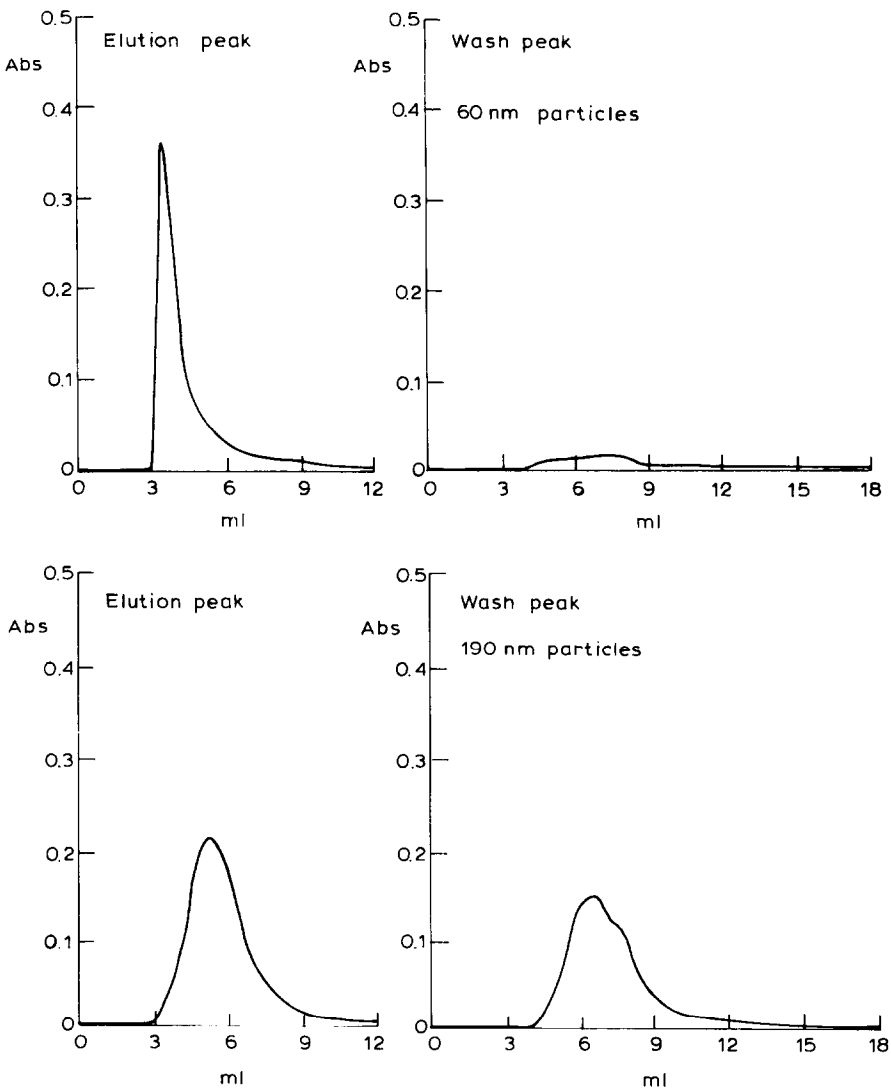

Fig. 2. Chromatograms obtained with Poloxamine 908 coated 60 nm (upper) and 190 nm (lower) polystyrene latex particles. The coating layer is thicker on small (134 Å) than on the larger particles (102 Å) resulting in a decreased hydrophobicity (60 nm: EP/WP = 8.45; 190 nm: EP/WP = 1.17).

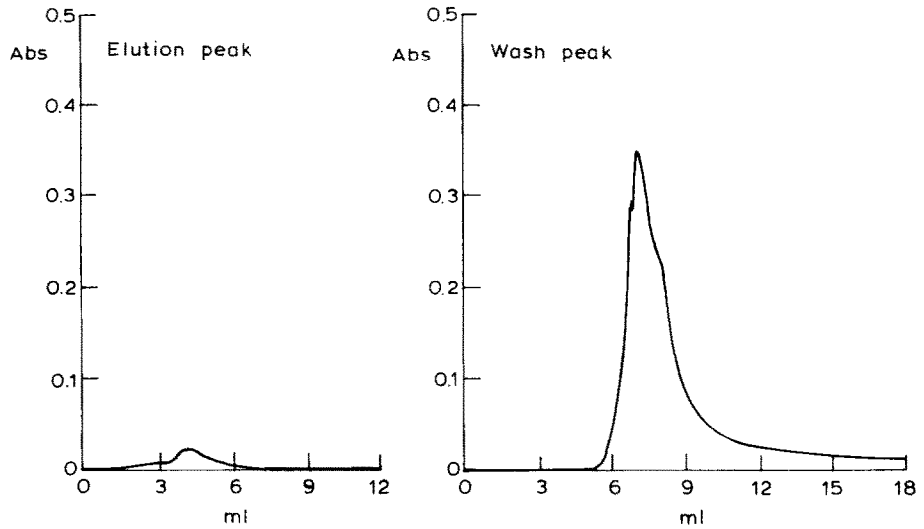

Fig. 3. Chromatograms obtained with Antarox CO 990 coated 190 nm polystyrene latex particles (coating layer 52 Å). The particle coat is more hydrophobic than in Fig. 4 as indicated by the ratio EP/WP = 0.06.

However, for particles coated with a thinner layer (36 Å) of Antarox CO 970 a slightly larger elution peak was obtained (Fig. 4). This trend continued with Antarox CO 850 coated particles possessing a layer of only 17 Å (EP/WP = 0.22). Antarox CO 730 coated particles with a layer thickness of 12 Å showed an EP/WP ratio of 0.76 (Fig. 5). Least hydrophobicity was observed for particles with the thinnest coating layer, Antarox CO 660 coated particles possessed a large elution peak (Fig. 6). Therefore they were even less hydrophobic than the reference particles with a thick Poloxamine

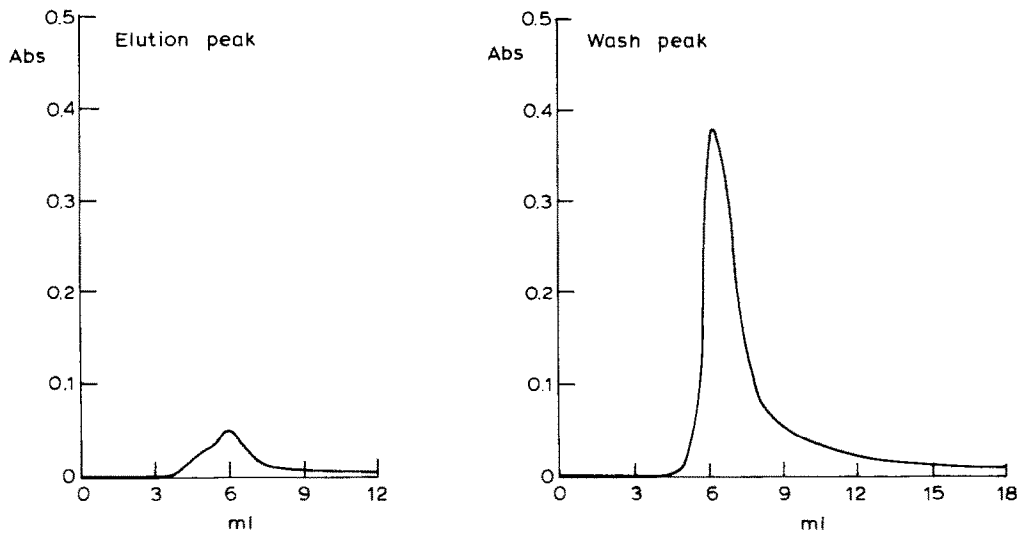

Fig. 4. Chromatograms obtained with Antarox CO 970 coated 190 nm polystyrene latex particles. The particle coat is thinner (36 Å) but slightly less hydrophobic than in Fig. 3 as indicated by the ratio EP/WP = 0.12.

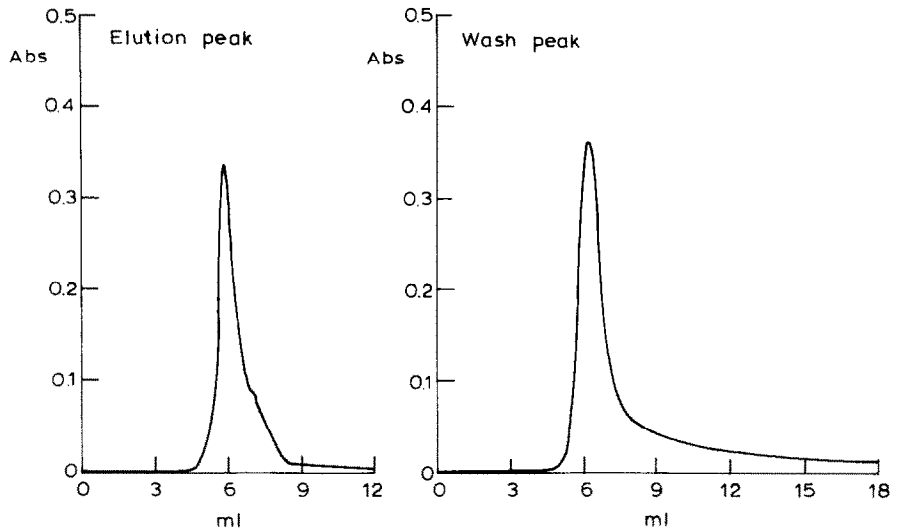

Fig. 5. Chromatograms obtained with Antarox CO 730 coated 190 nm polystyrene latex particles. The particle coat is distinctly thinner (12 Å) but much less hydrophobic than in Fig. 4 as indicated by the ratio EP/WP = 0.76.

908 coat of 102 Å. In contrast to the observations with Poloxamer and Poloxamine polymers, the hydrophobicity of the Antarox coat decreased with decreasing thickness of the adsorbed layer.

The driving forces of adsorption are according to Kronberg (1983):

(1) the gain in interaction energy with the surface when water is replaced by hydrocarbon on the surface,

(2) the difference in surfactant-water interaction in the surface and bulk phases (lower number of hydrocarbon-water contacts in the surface phase) whereby the surfactant-water interactions are regarded as the main driving force. Ethoxylated

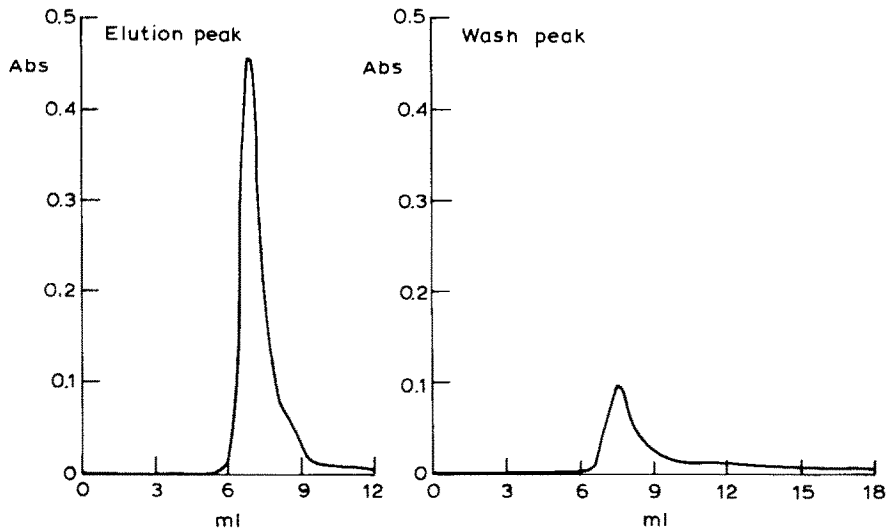

Fig. 6. Chromatograms obtained with Antarox CO 660 coated 190 nm polystyrene latex particles. The particles comprise the thinnest coat but are least hydrophobic (EP/WP = 3.16).

nonylphenols lie flat on the surface at low coverage but rise up when the surface is completely covered (Kronberg, 1983). Adsorption studies of nonylphenols on polymeric particles different in surface hydrophobicity revealed little difference in the amount adsorbed per surface unit (Kronberg et al., 1984). It was concluded that the driving forces for adsorption are the solution properties of the nonylphenols and not the specific interaction with the surface. An increased adsorption of non-ionic surfactants was found at reduced solvency (Tadros and Vincent, 1980). The affinity for the particle surface decreases with increasing POE chain length (Kronberg et al., 1981) due to the improved solvency in water. The solvency of the nonylphenols decreases with decreasing POE chain length leading to a higher affinity of the short-chained Antarox CO surfactants (e.g. 730 and 660). The high affinity leads to a close packing on the surface, the molecules rise up (Fig. 7). The close packing is further enhanced by the adsorption-free-energy gained by the orientation of the surfactants on the surface replacing water-hydrophobic moiety contacts by surfactant-surfactant contacts. About 80% of the adsorption-free-energy is due to the orientation of the surfactants on the surface, only 20% are influenced by the nature of the adsorbent surface (hydrophobicity, polarity) (Kronberg et al., 1984).

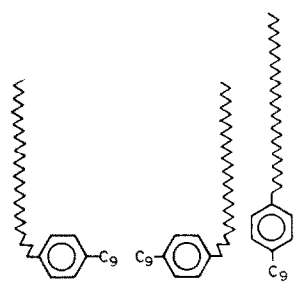

Fig. 7. Surface coverage of nonylphenols with high solvency and low adsorption pressure (upper) and vice versa (lower).

Conclusions

The achieved reduction in surface hydrophobicity is directly related with the increasing length of the POE chain for Poloxamer and Poloxamine polymers. Modification of the adsorbing hydrophobic POP part by using a nonylphenol changes the adsorption behaviour. Differences in solvency and affinity of the ethoxylated nonylphenols are important factors determining the packing and conformation of the surfactants on the surface. In contrast to Poloxamer least hydrophobic adsorption layers were obtained with decreasing length of the POE chain. This demonstrates the possibilities for future synthesis of improved coating materials by optimization of the hydrophobic anchor part and the POE chain length.

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