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## Adsorption of ethoxylated surfactants on nanoparticles. II. Determination of adsorption enthalpy by microcalorimetry

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### Summary

The adsorption of ethoxylated surfactants on differently sized polystyrene nanoparticles was followed by microcalorimetric titration. Titration allowed three phases of the adsorption process to be distinguished: (a) a first, exothermic phase due to hydrophobic interaction of surfactant and particle surface; (b) a second endothermic phase due to interfacial micelle formation; and (c) a third exothermic phase due to molecular rearrangements in the adsorption layer. The driving force for the adsorption of poloxamer and poloxamine block copolymers is the interfacial micelle formation, signifying that it is a mainly entropy-driven process. A third phase of molecular rearrangement could only be detected for high molecular weight poloxamers. Nonyl- and dinonylphenols (AntaroX CO and DM series) with short polyethoxy chains adsorb by hydrophobic interaction via a single-phase process, whilst long-chain derivatives exhibit all three phases. The thicker adsorption layers on small latex particles could be attributed to promotion of interfacial micelle formation.

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### Introduction

The surface hydrophobicity of intravenously injected nanoparticles is one of the main factors determining the organ distribution of the carriers and their fate in vivo (Müller, 1991). The surface hydrophobicity determines the quantity and composition of adsorbed blood components after i.v. injection. The resulting adsorption pattern mediates the recognition by the immune system (e.g.,

via complement C3b), the interaction with cells possessing the appropriate surface receptors (e.g., apolipoprotein E on hepatocytes) or can mask the surface leading to nanoparticles circulating in the blood. To adsorb a protein pattern with a masking effect the nanoparticle surface needs to be hydrophilic. Hydrophilic surfaces can be obtained by adsorption of ethoxylated block copolymers or surfactants (coatings). Coating of polystyrene nanoparticles with poloxamine 908 led to their circulation in the blood (Davis et al., 1986). Investigation of the protein adsorption pattern by two-dimensional polyacrylamide gel electrophoresis (PAGE) showed totally different adsorption patterns on uncoated and poloxamine coated

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nanoparticles (Blunk et al., 1991). Recognition by the immune system could also be minimized for hydrophilic liposomes. Using ethoxylated phospholipids led to hydrophilic vesicles, the so-called 'stealth' liposomes (Woodle et al., 1991).

An adjustment of the surface hydrophobicity of nanoparticles is essential for the controlled development of site-specific i.v. drug carriers. Sufficiently hydrophilic surfaces could be created by coating with polymers to protect non-biodegradable nanoparticles against immunological recognition. In contrast, the coating of biodegradable polylactide co-glycolide particles is very poor (Müller and Wallis, 1992). It is therefore necessary to optimize the coating process by modification of the molecular structure of the coating materials. Hydrophobic interaction chromatography (HIC) was presented as a suitable characterization method to quantify surface hydrophobicity (Carstensen et al., 1991). HIC only allows the hydrophilicity of the adsorption (coating) layer to be assessed and provides no information about the mechanism of the adsorption process.

Microcalorimetry enables the contribution of hydrophobic interactions and entropy effects to be determined. This should allow the molecular parts of the coating materials determining the adsorption process to be optimized specifically, e.g., hydrophobic anchor parts. The hydrophobicity of the adsorption layers of homologous series of block copolymers (poloxamer, poloxamine) and ethoxylated nonylphenols (Antarox) was assessed previously (Carstensen et al., 1991). In this paper, the mechanisms of their adsorption are studied by microcalorimetry.

## Materials and Methods

### Materials

Polystyrene particles with a size of 60 nm were purchased from Polysciences Inc. (Warrington, U.K.). Particles with a diameter of 200 nm were polymerized free of surfactants as described previously (Carstensen et al., 1991). Styrene and the polymerisation initiator potassium persulphate were purchased from Sigma (Deisenhofen, Germany).

Ethoxylated block copolymers poloxamer 188, 238, 338, 407 and poloxamine 908 were supplied by ICI Chemicals (Manchester, U.K.). Poloxamer 184, 235, 335 and poloxamine 1508 were provided by Erbslöh (Düsseldorf, Germany). Ethoxylated nonylphenols (Antarox CO and DM) were a gift from Gattefossé (Manchester, U.K. and Frechen, Germany). Details of the surfactants are listed in Tables 1 and 2.

### Methods

To determine the adsorption (coating) layer thickness of surfactants equal volumes of particle suspension (2.5% (w/w) for 60 nm particles, 5.0% (w/w) for 200 nm particles) and surfactant solution (2.5% (w/w)) were mixed and incubated overnight at room temperature. The adsorption layer thickness was measured by photon correlation spectroscopy (PCS) as described previously (Carstensen et al., 1991).

Microcalorimetric measurements were performed with a Thermal Activity Monitor (TAM 2277) system from Thermometric AB (Stockholm, Sweden) (Suurkuusk and Wadsö, 1982; Görman Nordmark et al., 1984). A titration cell (double ampoule 2277-318) with a titration volume of 1.0 ml was used. Titrations were carried out at 25 °C over 6 h. Calibration of the system was performed with an internal calibration resistor and additionally by titration of HCl with NaOH. The baseline noise was less than  $\pm 0.3 \mu\text{W}$ . The calorimetric cell was initially filled with 250  $\mu\text{l}$  of polystyrene latex suspension (2.5% (w/w) of 60 nm and 5.0% (w/w) of 200 nm particles) and titrated with the surfactant solutions (2.5% (w/w) of poloxamer and poloxamine solutions; 0.005 M Antarox solutions). The block copolymer solutions were prepared on a per cent basis rather than molarity due to the large differences in molecular weight. Equimolar solutions of the gel forming high molecular weight block copolymers would be too viscous. In cases when a positive or negative enthalpy could still be detected for the last titration steps the titration was continued with a part of the first sample until no further heat flow was detected. To determine the heat flow caused by the dilution of the surfactant a similar experiment was performed using distilled water as a titrand

TABLE 1

Composition and molecular weight of poloxamer block copolymers  $EO_n-PO_m-EO_n$  and poloxamine block copolymers  $(EO_n-PO_m)_2-N-CH_2-CH_2-N-(PO_m-EO_n)_2$  and coating layer thickness on 60 nm (after Carstensen et al., 1991 and Müller, 1991) and on 200 nm polystyrene latex particles

Polymer	Number of units		Molecular weight	Coating layer thickness on	
	EO ( <i>n</i> )	PO ( <i>m</i> )		60 nm (Å)	200 nm (Å)
Poloxamer					
184	13	30	2 900	24	26
188	76	30	8 350	76	48
235	27	39	4 600	35	12
238	97	39	11 800	81	46
335	38	54	6 500	53	16
338	128	54	14 000	154	105
407	98	67	11 500	119	76
Poloxamine					
908	123	23	27 000	134	87
1 508	136	26	30 000	115	103

instead of a particle suspension. The exothermic peak obtained during the deaggregation of the micelles provided information about differences between the polymers in the enthalpy effect during micelle formation.

Titration was performed stepwise by adding 5  $\mu$ l at a titration velocity of 1  $\mu$ l/s followed by a pause of 120 s. This mode of titration allowed measurements with a small volume of nanoparticle suspension despite the relatively low heat flow (range of a few microwatts). This avoided the need to use larger volumes of the cost-intensive nanoparticle suspensions. The stirring velocity was

60 rpm which proved to cause least baseline noise.

In the titration curves the thermal heat flow ( $\mu$ W) is plotted vs the time (h). This results in positive peaks for exothermic reactions corresponding to a negative enthalpy. The heat flow curves presented were corrected by the thermal heat flow resulting from the dilution of the surfactant. The enthalpy was calculated via the area under the curve in mJ per m<sup>2</sup> surface area according to Norde and Lyklema (1978). In the case of endothermic and exothermic effects during one titration the positive and negative peak areas

TABLE 2

Composition and molecular weight of Antarox CO (*p*-nonylphenol- $EO_n$ ) and Antarox DM (*p,m*-dinonylphenol- $EO_n$ ) surfactants and coating layer thickness on 60 nm (after Carstensen et al., 1991 and Müller, 1991) and on 200 nm polystyrene latex particles

Polymer	Number of units	Molecular weight	Coating layer thickness on	
	EO ( <i>n</i> )		60 nm (Å)	200 nm (Å)
Antarox CO				
730	15	880	11	5
970	50	2 420	61	26
990	100	4 620	73	41
Antarox DM				
710	15	1 006	25	7
880	50	2 546	61	35
970	150	6 946	89	60

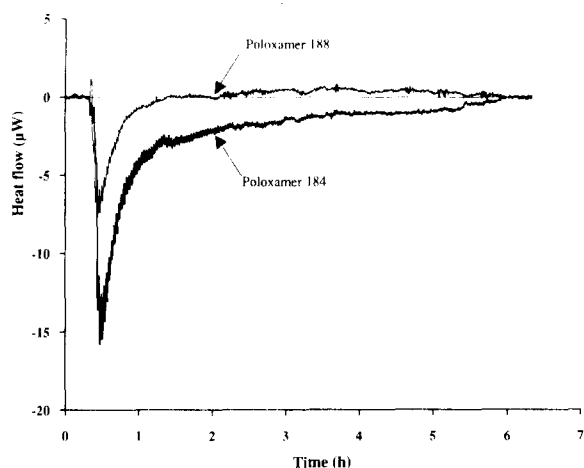


Fig. 1. Heat flow curves of adsorption of poloxamers 184 and 188 on 200 nm polystyrene latex particles. Constant length of POP chain: 30 units PO; increasing length of POE chain: 13 and 75 units EO, respectively.

were calculated separately. They were summed up to characterize the overall heat effect.

## Results and Discussion

The adsorption behaviour of poloxamer polymers is affected by the length of the POP and POE chains in the molecule. To study these effects poloxamers with similar length of the POP chain and increasing length of POE chains (and vice versa) were selected for the microcalorimetric measurements. The adsorption of poloxamer 184 and 188 was found to be a purely endothermic process (Fig. 1, 200 nm latex). The peaks correspond to enthalpies  $+129.9$  and  $+8.5$   $\text{mJ}/\text{m}^2$ , respectively. As described by Lindheimer et al. (1990), the initial anchoring of nonionic surfactants onto silica gel is an exothermic process. After this first phase of adsorption an interaction between the nonpolar parts of the nonionic surfactants is described. This leads to the formation of interfacial aggregates which is an endothermic process. According to Kronberg et al. (1984), one driving force for the adsorption of ethoxylated nonylphenols on latices is the reduction of contacts between water and the hydrocarbon moiety of the surfactant which is the same

driving force as for micellization. The gain in entropy of the water caused by the destructuring of the water molecules around the hydrophobic parts of the surfactant leads to the formation of micelles (Keh et al., 1985). Micelle formation on different surfaces by nonionic and anionic surfactants was also reported by Denoyel and Rouquerol (1991). In calorimetric studies they found an exothermic reaction followed by an endothermic process and attributed the latter to interfacial micelle formation. Apart from hydrophobic interaction the formation of interfacial micelles can therefore be considered as a second driving force for the adsorption of the nonionic surfactants onto latex particles. The superposition of an exothermic effect due to adsorbent-adsorbate interaction and an endothermic effect due to inter-

TABLE 3

*Adsorption enthalpies of poloxamer, poloxamine and Antarox on 60 nm and 200 nm polystyrene latex particles ( $\text{mJ}/\text{m}^2$  latex surface)*

Polymer	Adsorption enthalpy (mJ/m <sup>2</sup> )		Dilution enthalpy (mJ)
	60 nm latex	200 nm latex	
Poloxamer			
184	172.1	129.9	− 15.2
188	85.8	8.5	− 9.2
235	192.6	144.9	− 12.5
238	50.5	33.6	− 8.2
335	229.7	27.6	− 79.7
338	127.6	65.5	− 29.9
407	47.4	− 20.7	− 15.6
Poloxamine			
908	157.0	79.7	− 6.3
1508	289.1	99.1	− 13.1
Antarox CO			
730	− 22.4	− 23.9	− 1.3
970	− 12.7	− 17.8	− 4.4
990	15.5	2.3	− 10.0
Antarox DM			
710	− 51.0	− 47.9	− 0.6
880	− 15.3	− 26.1	− 3.7
970	− 3.2	− 108.8	− 9.7

The heats of dilution obtained from the reference titrations are also given as a measure for the enthalpy of micelle formation ( $\text{mJ}/\text{dilution of } 250 \mu\text{l water with } 750 \mu\text{l surfactant solution}$ ).

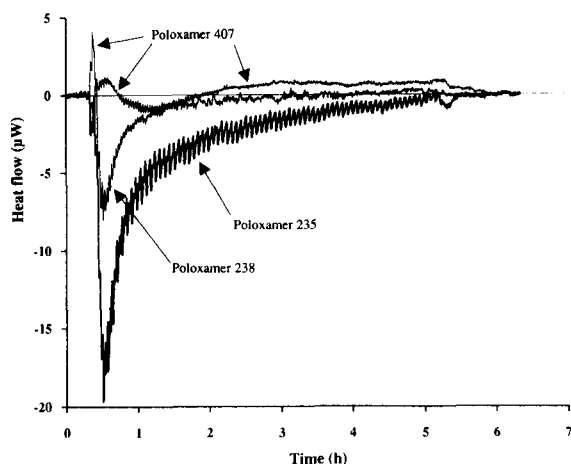


Fig. 2. Heat flow curves of adsorption of poloxamers 235, 238 and 407 on 200 nm polystyrene latex particles. Length of POP chains: 30 units PO (poloxamers 235 and 238), 67 units PO (poloxamer 407).

facial micelle formation determines the shape of the titration curve. In the case of poloxamer 184 and 188 the latter is the main driving force for the adsorption. The adsorption enthalpy of the low molecular weight poloxamer 184 is distinctly larger than for the higher molecular weight poloxamer 188. This is partly attributed to the larger enthalpy for the micelle formation concluded from the reference titration (Table 3). A different extent of interfacial micelle formation might additionally contribute to the difference between poloxamer 184 and 188 (Wesemeyer et al., in preparation).

Poloxamers 235 and 238 are of higher molecular weight but possess similar ratios of POE and POP chain lengths compared to 184 and 188 ( $n(\text{EO})/m(\text{PO})$  approx. 0.5 and 2.5, respectively). The POP part is increased to 39 instead of 30 units PO. The shapes of the heat flow curves were identical, but the enthalpies are more positive (Fig. 2, Table 3). Increasing the molecular weight at similar EO/PO ratio led to a more positive enthalpy for molecules with POP chains up to 39 units. Increasing the length of the POP chain to 67 units at a constant length of the POE chain (98 units) yielded a totally different heat flow curve. A triphasic curve was obtained for the adsorption of poloxamer 407 (Fig. 2). The first

phase is an exothermic reaction followed by an endothermic and again an exothermic process. The triphasic curve was reproducible and such an effect was also observed for other block copolymers (poloxamer 188, 335 and 338 on 200 nm latex particles). The first, exothermic phase can be interpreted in terms of hydrophobic interaction between the particle surface and predominantly the most hydrophobic parts of the non-ionic surfactants (POP chains). This effect was minor and not detectable with nonionic surfactants containing short POP chains. However, a slight peak appeared in the heat flow curves of poloxamers with a longer POE chain, i.e., poloxamer 238. The second, endothermic phase is attributed to interfacial micelle formation. The third, exothermic phase is explained by a rearrangement of adsorbed molecules in the adsorbed layer. Multimolecular adsorption can be excluded as an explanation for this third phase on the basis of small angle neutron scattering (SANS) studies (Cosgrove et al., 1983) describing the loop and tail structure of the layer. In addition, adsorption isotherms published in the literature indicate a Langmuirian isotherm (Kayes and Rawlins, 1979; Lindheimer et al., 1990). Langmuirian type isotherms were also obtained for all surfactants used in this study (Wesemeyer et al., in preparation). However, time-dependent rearrangement appears to be possible. Kronberg (1983) reported changes in the conformation of the adsorbed polymers depending on the polymer concentration in the water: at low surface coverage the molecules lie flat on the surface, at higher coverage the molecules protrude into the external phase. For high molecular weight block copolymers an increase in thickness of the adsorption layer was measured within 24 h (Müller, 1991). This was attributed to the replacement of low molecular weight polymers with a short POP central region by higher molecular weight polymers with a larger POP region and higher affinity for the surface. The commercial block copolymers show a heterogeneity in their molecular weight distribution. Very high molecular weight polymers are present at a low concentration. They will gradually displace the low molecular weight polymers on the surface. Taking this into consid-

eration, we suggest a process of rearrangement which is dependent on the surfactant concentration in the surrounding water phase. This rearrangement process involves the protrusion of POE chains from the surface, the increased formation of hydrogen bonding between hydrated extended and protruding POE chains of the polymers and possibly displacement effects. Possibly a close-packed monolayer is formed by the reorganization of adsorbed surfactant molecules.

In contrast, the high molecular weight polymers poloxamine 908 and 1508 showed a biphasic behaviour. An initial, very sharp exothermic peak is present similar to the high molecular weight poloxamers. It is followed by a relatively large endothermic effect more similar to the low molecular weight poloxamers. A third phase was missing. The gain in entropy of water correlated to the formation of interfacial micelles was therefore considered as the driving force for the adsorption of the poloxamine polymers.

The enthalpies calculated for the adsorption of poloxamer and poloxamine polymers on 60 nm latex particles were up to 10-times more positive than on 200 nm (Table 3). The adsorption layers are distinctly thicker on the small latex particles (Table 1). This increased thickness was explained by the increased hydrophobicity of the smaller latex particles. The surface of the 60 nm particles is more hydrophobic due to the polymerisation conditions employed. This leads to the enhanced adsorption of block copolymers (Müller, 1991). From this one might expect a more distinct first exothermic phase but the endothermic peak is enlarged. This can be explained by the promotion of interfacial micelle formation by denser packing of the adsorbed block copolymers.

In contrast to low molecular weight poloxamers, the adsorption of low molecular weight nonylphenols Antarox CO led to a single exothermic peak. Hydrophobic interaction between adsorbent and particle surface dominates, resulting in a monophasic adsorption process (Antarox CO 730, Fig. 3). Interfacial micelle formation could not be detected. Increasing the length of the POE chain from 15 to 50 units in Antarox CO 970 led to the appearance of the above-described second, endothermic phase indicating interfacial

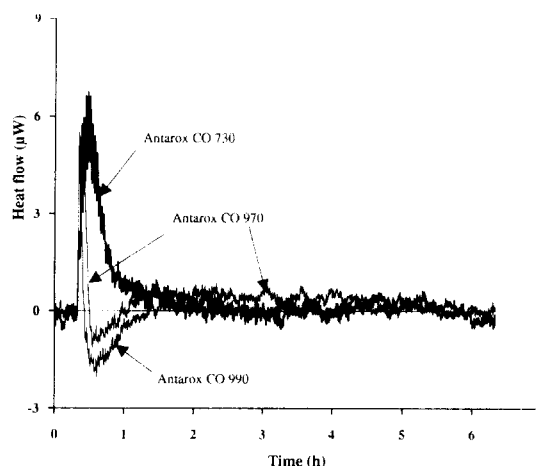


Fig. 3. Heat flow curves of adsorption of the nonylphenols Antarox CO 730, 970 and 990 on 200 nm polystyrene latex particles. Length of POE chains: 15, 50, 100 units EO, respectively.

micelle formation. The enthalpy of the endothermic peak increases with increasing length of the POE chain from +2.6 (Antarox CO 970) to +7.7 mJ/m<sup>2</sup> (Antarox CO 990 with 100 EO units) for adsorption on 200 nm latex (Fig. 3). However, the enthalpies are a factor 10–100 smaller compared to those for poloxamer and poloxamine polymers (Table 3). This proves the minor contribution of interfacial micelle formation. The main driving force for the adsorption of Antarox CO surfactants is the hydrophobic interaction represented by the first exothermic peak. A third phase was also observed, indicating the above phenomenon of rearrangement in the adsorption layer. The enthalpies of this third peak are similar to those of corresponding poloxamer polymers with similar lengths of the POE chains. This supports the assumption that this peak is due to interactions and rearrangements of the POE chains in the molecules.

The dinonylphenols with short and medium POE chain lengths (Antarox DM 710, DM 880) exhibited similar heat flow curves compared to the corresponding nonylphenols (Fig. 4). Comparing the enthalpies of the first peak between corresponding CO and DM surfactants (similar POE chain length) yields distinctly higher exothermic values for the DM series. For Antarox CO 730

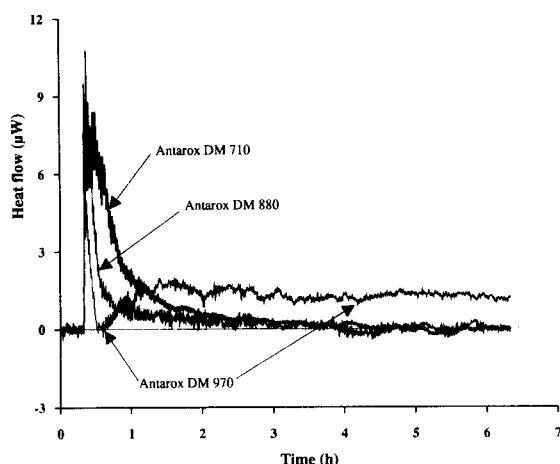


Fig. 4. Heat flow curves of adsorption of the dinonylphenols Antarox DM 710, 880 and 970 on 200 nm polystyrene latex particles. Length of POE chains: 15, 50, 150 units EO, respectively.

and DM 710 (both 15 EO units) enthalpies of  $-23.9$  and  $-47.9$   $\text{mJ/m}^2$ , for Antarox CO 970 and DM 880 (50 EO units) enthalpies of  $-17.8$  and  $-26.1$   $\text{mJ/m}^2$  were obtained for the adsorption on 200 nm latex. This indicates that the introduction of a second nonyl group promotes the hydrophobic interaction. Antarox DM 970 with a long POE chain of 150 units shows again a triphasic behaviour (Fig. 4). The first phase consists of a very sharp peak, while the second phase is represented by a minimum in the curve (an endothermic peak was only obtained after adsorption on 60 nm latex particles). The third phase dominates the heat flow curve indicating a distinct rearrangement in the adsorption layer. The Antarox heat flow curves show that the length of the POE chain is the factor determining the shape of the curve.

Kronberg et al. (1984) reported a less dense packing of surfactants in the adsorbed layer for an increasing surface polarity. As presented in Table 2, the adsorption layers of Antarox CO and DM were also found to be thicker on the more hydrophobic 60 nm latex particles. This corresponds to an increased endothermic enthalpy due to interfacial micelle formation as discussed above. This effect is only distinguishable for Antarox with longer POE chains. The enthalpy of

the second peak of Antarox CO 970 is  $+2.6$   $\text{mJ/m}^2$  on 200 nm latex particles,  $+4.6$   $\text{mJ/m}^2$  on 60 nm latex particles.

## Conclusions

The reduction in surface hydrophobicity of nanoparticles after adsorption of poloxamer and poloxamine polymers was found to be correlated with the adsorption (coating) layer thickness (Carstensen et al., 1991). To enhance the adsorption it was suggested that hydrophobic parts of the adsorbent should be enlarged. The microcalorimetric data could prove that poloxamer and poloxamine adsorption is entropy driven, and that hydrophobic interaction plays only a minor role. Increasing the length of the hydrophobic part in the molecule (polypropylene oxide) had little effect. Increasing the surface hydrophobicity of the nanoparticle itself, however, promoted interfacial micelle formation and created thicker adsorption layers. This results in two options for further development of nanoparticles surface-modified by ethoxylated block copolymers: (a) increasing the coating layer thickness of a block copolymer by creating a more hydrophobic nanoparticle surface and (b) modification of the polyethoxy chains to promote interfacial micelle formation whereby the POE chains need to be of a minimum length to cover the hydrophobic parts of the molecule (POP).

For the ethoxylated nonylphenols the heat flow curves confirmed the dominant role attributed to the hydrophobic anchor part (Carstensen et al., 1991). Hydrophobic interaction is the driving force for adsorption. Future developments can therefore be based on the modification of the hydrophobic regions in the molecule.

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