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The effect of surface coverage and conformation of poly(ethylene oxide) (PEO) chains of poloxamer 407 on the biological fate of model colloidal drug carriers

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Abstract

Poloxamer 407 was adsorbed onto the surface of model colloidal drug carriers, polystyrene nanoparticles of 40, 70 and 137 nm in diameter, and the effect of the degree of surface coverage and the conformation of the poly(ethylene oxide) (PEO) chains on biological fate was studied. The relationship between the physicochemical and the biological properties of the nanoparticle systems was also investigated. The adsorbed layer of poloxamer 407 was characterised in terms of percentage surface coverage, thickness of the adsorbed layer and average surface area per PEO chain. Computer modelling of the adsorbed layer was performed (applying the self-consistent field technique), to obtain the structural information of the PEO chains in the layer. The in vitro interaction of the nanoparticles with different degrees of poloxamer 407 surface coverage with serum components and the in vivo biodistribution in the rat model were assessed. The results demonstrated that an increase in the surface coverage with poloxamer 407 resulted in an increased volume fraction of the PEO in the adsorbed layer, further extension of the PEO chains from the surface and closer packing of the chains at the surface. With regard to the interaction with the serum components, an increased surface coverage resulted in a reduction of the amount of serum proteins adsorbed, and, importantly, affected the type of proteins adsorbed. High molecular weight proteins were not adsorbed onto the nanoparticles with a surface coverage above approx. 25%. Following the intravenous administration to rats, even the nanoparticles with the lowest degree of surface coverage (approx. 5%) showed improved circulation profiles relative to the uncoated nanoparticles. The effect was more pronounced for the 40 nm nanoparticles. A further increase in the surface coverage to approx. 25% resulted in a significant increase in circulation time, as compared to uncoated and 5% coated systems, for all sizes of nanoparticles. Importantly, it was found that a long in vivo blood circulation time could be achieved for nanoparticles with a relatively low degree of surface coverage with PEO chains.

Keywords: Poloxamer 407; Biodistribution; Poly(ethylene oxide); Surface density; Nanoparticle

1. Introduction

The surface engineering of colloidal carriers (poly-

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meric particles, emulsions and liposomes) in the nanometre size range has been demonstrated to provide opportunities for the site-specific delivery of drugs following injection into the general circulation or the lymphatic system. Targets have included the liver (both Kupffer cells and hepatocytes), tumours, endothelial cells, sites of inflammation and lymph nodes [1–5]. Following our original report in 1984 [6], various authors have shown that colloidal carriers, in the form of nanoparticles, surface modified with poly(ethylene oxide) (PEO) have prolonged circulating times in the systemic circulation and a reduced uptake by the organs of the reticuloendothelial system [7–11]. This same concept has been applied with success to liposomal carriers and the development of the so-called 'stealth liposome' [12–14].

The major challenge in this field remains to be the establishment of a correlation between the physicochemical properties of the colloidal carriers (to include surface characteristics), and their resulting site(s) of deposition within the body after injection. Studies have been previously conducted to assess the characteristics of the PEO chains at the carrier surface, the nature of which has been suggested to be responsible for the interaction of the system with the biological environment [2,15,16]. However, these studies have been performed generally with colloidal particles that had a surface coverage of PEO at a level that corresponded to a plateau of adsorption or a maximum PEO incorporation, since it was assumed that such a level of coverage would be necessary in order to obtain nanoparticles with surface characteristics that would provide prolonged systemic circulation.

A critical stage that influences the biodistribution of a colloidal carrier is the process of particle coating by blood components, a process known as opsonisation [3]. The nature, rate and extent of adsorption of plasma protein onto nanoparticles is known to depend on the surface characteristics of the particle and that sterically stabilised hydrophilic particles are less prone to protein adsorption than uncoated hydrophobic particles [2,3]. Jeon et al. [17] conducted a theoretical evaluation of the extent to which surface adsorbed or grafted polymer molecules would resist protein adsorption. They predicted an optimal surface density of PEO groups at which resistance to protein adsorption was greatest. More recently Schroen et al. [18] have evaluated experimentally the influence of preadsorbed block copolymers on protein adsorption to surfaces. They found that small amounts of adsorbed poloxamer (Pluronic F-108) (10% surface saturation) greatly reduced the initial adsorption rate of an enzyme. In the case of a saturated layer of block copolymer no protein adsorption took place.

In a previous study, we have prepared polystyrene particles from copolymers of styrene and methoxypoly(ethylene glycol) acrylate (MW 2000). The particles had increasing amounts of PEO2000 on the surface. It was demonstrated that the level of PEO2000 on the nanoparticle surface affected the biodistribution pattern of the nanoparticles after intravenous injection in rats [19,20]. Particles with the higher surface density showed increased circulatory times. However, the use of surface chemical analysis by X-ray photoelectron spectroscopy (XPS) combined with static secondary ion mass spectrometry (SSIMS) for the characterisation of these nanoparticles allowed only measurement of the relative level of surface bound PEO, but not the determination of the actual amount of PEO chains per unit surface area. Hence, the information obtained on the relationship between the conformation, the surface density and the layer thickness or chain extension of the PEO on the surface of these particles, and the in vivo behaviour was limited.

Recently, Araujo et al. [21] investigated the influence of coating methyl methacrylate nanoparticles of 130 nm in size with different levels of the block copolymer poloxamine 908 or the nonionic surfactant polysorbate 80 by suspending the nanoparticles in concentrations of the coating agents in the range 0.001–5%. The excess coating polymers were not removed neither was any attempt made to physically characterise the resultant surfaces for example in terms of the surface density of the hydrophilic moiety, surface hydrophobicity, ζ potential or surface layer thickness. The nanoparticle systems were injected intravenously to groups of rats and the organ distribution of the nanoparticles was determined. It was shown that below a coating concentration of 0.1% the nanoparticles behaved like uncoated particles, whereas at a concentration of 0.1% and above for poloxamine 908, and at 0.5% and above for polysorbate 80, the liver deposition of the particles was

greatly decreased. The area that one polymer molecule would cover at a concentration of 0.1% was calculated to be 8.7 nm^2 for poloxamine 908. However, these calculations did not take into account information from other studies which indicated that it was most likely that not all of the surfactant in the solution would be adsorbed to the surface of the nanoparticles. Apparently adsorption isotherms were not measured in the study.

The present work set out to investigate in detail the effect of the surface characteristics of nanoparticles on the in vitro adsorption of plasma protein and the in vivo biodistribution after intravenous injection in rats. Polystyrene nanoparticles were chosen as a model system since it is to be expected that a coating block copolymer of the poloxamer series containing hydrophilic (PEO) and hydrophobic blocks (PPO), will be strongly adsorbed to the hydrophobic polystyrene surface, with the hydrophilic blocks capable of forming a hydrophilic barrier to protein adsorption [3,10]. We have described previously how data obtained from model disperse particles such as polystyrene microspheres and nanoparticles can be used to design biodegradable drug carrying particles (such as those formed from polymers such as polylactide and polylactide coglycolide) that can have extended circulation times in the blood [22]. The polystyrene nanoparticles were coated with poloxamer 407, and the adsorption isotherms measured for nanoparticles of diameters 40 nm, 70 nm and 137 nm. The nanoparticles were characterised in terms of the PEO surface density and hydrodynamic layer thickness. Furthermore, a self-consistent field modelling approach was used to model the adsorption of the copolymer and to gain information on the adsorbed layer thickness and volume fraction profiles of the PEO chains.

2. Materials and methods

2.1. Materials

Polystyrene nanoparticles of particle size $40 \pm 19.8\%$ nm (8.4% w/v aqueous suspension), $71 \pm 10.9\%$ nm (8.4% w/v suspension) and $137 \pm 2.3\%$ nm (8.0 w/v suspension) were purchased from Interfacial Dynamics (Portland, USA). The size was determined by the supplier using transmission electron microscopy. The hydrodynamic diameter of the particles was determined in our laboratory using photon correlation spectroscopy (*z*-average). As stated by the supplier, the specific surface area was 142.86 m^2/g, 81.63 m^2/g and 41.71 m^2/g for 40, 70 and 137 nm sized nanoparticles, respectively. We had shown previously that such particles had apparently similar surface properties as determined by particle electrophoresis and conductometric titration. Poloxamer 407 (Pluronic F-127) was a gift from BASF (Parsippany, USA) and was used as supplied. The poloxamer is a linear tri-block copolymer of poly(ethylene oxide) and poly(propylene oxide) ($PEO_nPPO_mPEO_n$), where n is 98 and m is 67 units. The average molecular weight of the copolymer was 12 600, with each PEO chain of approx. 4400. Iodine-125 for radiolabelling of the nanoparticles was obtained from NEN-Dupont (UK) in the form of $Na^{125}I$.

SDS–PAGE reagents used for the protein adsorption studies were dithiothreitol (DTT), sodium dodecyl sulphate (SDS), ammonium persulphate, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), bromophenol blue, Coomassie brilliant blue R250, glycine, molecular weight markers, acrylamide/bisacrylamide (ratio 37.5:1) 40% solution and acrylamide/bisacrylamide (ratio 29:1) 30% solution and were all purchased from Sigma. Dialysis tubing with molecular weight cutoff of 100 000 was obtained from Spectrum, Pierce-Warriner (Chester, UK). Whatman 0.02 μm Anopore microcentrifuge filters were purchased from Fisher Scientific UK (Loughborough, UK). All other reagents were at least analytical grade. McIlvaine's buffer pH 7.4 was used which contained 1.8 mM disodium phosphate, 90 μM citric acid and 65 μM potassium chloride. A reducing buffer was prepared by mixing together 1 ml of 1 M Tris–HCl pH 6.8, 1.6 ml glycerol, 4.2 ml of 22.86% w/v of SDS, 0.8 ml of 100 mM DTT and 0.4 ml of 0.05% w/v of bromophenol blue.

2.2. Adsorption isotherms

The method used to obtain the adsorption isotherms has previously been described [15]. A suspension of polystyrene nanoparticles (containing 4.2 mg of solid material) was incubated with the copolymer

solution overnight at room temperature (20 ± 2°C). After the incubation, the suspension was centrifuged at 46 000 rpm (Beckman L8-60M ultracentrifuge, USA), using Polyallomer ultracentrifugation tubes (Beckman Instruments, USA) to sediment the nanoparticles. Preliminary experiments, measuring light scattering of the supernatant, showed that 2 h of centrifugation was sufficient to sediment 137 and 70 nm nanoparticles, while for 40 nm nanoparticles 5 h of centrifugation was required. The residual concentration of the copolymer in the supernatant was determined using a differential refractive index detector (Gilson, France) thermostatted at 20 ± 0.5°C (flow through water bath, Grant, UK) and connected to a digital voltmeter (Farnell Instruments, UK).

A control experiment was conducted in parallel, to eliminate effects of adsorption of the copolymer onto the ultracentrifugation tubes and glass vials. The samples were treated in the same way as in the adsorption experiment with the exception that no polystyrene nanoparticles were added. To ensure that the centrifugation conditions did not lead to any separation of the copolymer in the solution, refractive index increments (dn/dc) for the control experiment and a calibration curve were compared. The same absolute values indicated that no copolymer sedimentation occurred during the centrifugation process. The adsorption isotherm for each of the particle sizes was constructed from the results of at least three separate experiments, where in each experiment three readings were taken for each point and the mean value and standard deviation calculated. The surface coverage of poloxamer 407 on the polystyrene nanoparticles at various points along the isotherm was calculated, assuming a 100% surface coverage at the plateau of the isotherm.

2.3. Hydrodynamic layer thickness

The thickness of the adsorbed layer was measured at each sampling point of the adsorption isotherm using photon correlation spectroscopy (PCS) (Malvern 4700, Malvern Instruments, UK). Data analysis was performed using the CONTIN programme. For each point, a sample was taken and diluted with filtered double distilled water (0.2 μm membrane filter). Ten measurements were performed on each sample. The mean value and standard deviation were calculated for the particle size and polydispersity. The hydrodynamic layer thickness (δ) was calculated as follows:

$$\delta = \frac{d_a - d_o}{2} \quad (1)$$

where d_a is the nanoparticle diameter after poloxamer adsorption and d_o is the initial nanoparticle diameter.

2.4. Polystyrene (PS) nanoparticles with different surface coverage

PS nanoparticles (40, 70 and 137 nm in diameter) were incubated with poloxamer 407 to obtain particles with different percentages of surface coverage (0, 3, 27, 63, 93 and 100%). For this purpose, 150 μl of the relevant nanoparticle suspensions was incubated overnight in 2.85 ml of deionised water containing increasing volumes (0, 35.6, 60.0, 177.0, 521.0, and 1350.0 μl) of a 1% (w/v) solution of poloxamer 407. The excess poloxamer 407 was removed by dialysis of the particles for 48 h against deionised water.

2.5. Adsorption of serum components on nanoparticles

Rat serum was obtained from healthy laboratory animals (Wistar) and stored at −20°C. To evaluate the adsorption of serum components, PS nanoparticles with different percentages of surface coverage of poloxamer 407 (300 μl of a 0.42% (w/v) suspension) were incubated for 2 h at room temperature with an equal volume of rat serum. Free protein was removed from particle bound protein by centrifugation at 10 000 × g for 60 min and washing of the particle pellet with 600 μl McIlvaine's buffer pH 7.4. Supernatants were set aside for determination of protein concentration. The final particle pellet was either resuspended in 10 mM phosphate buffered saline (PBS) or in a reducing buffer. The former samples were further used for the determination of the quantity of protein adsorbed onto the particles by a Lowry assay and the latter samples were used for further qualitative analysis of the adsorbed proteins by SDS–PAGE. Previous studies had shown that the Lowry method was not affected by the presence of the poloxamer [23].

Control suspensions containing PS nanoparticles only in McIlvaine's buffer were subjected to the same centrifugation steps to determine any loss of the nanoparticles. For this purpose, the light scattering of the supernatants was measured using a spectrofluorometer at $\lambda = 480$ nm for both emission and excitation wavelengths. As further controls rat serum mixed with buffer or buffer alone was treated in the same way to ensure that precipitation of serum proteins during the procedures did not occur.

2.6. Determination of protein concentration and particle concentration

The protein concentration in the different supernatants and final particle suspensions was determined by a Lowry assay. Just prior to the reading of the optical density of the samples containing the final pellets, the particles were precipitated by centrifugation at $10\,000 \times g$ as described previously by Basinska and Smolowski [24].

2.7. PAGE analysis of proteins adsorbed onto the nanoparticles

SDS–PAGE was used to analyse the quality and quantity of the proteins adsorbed on the surface of the nanoparticles during the protein adsorption studies outlined in Section 2.5. Before submitting the proteins to SDS–PAGE all proteins were removed from the particles by incubating the final particle pellets, resuspended in reducing buffer, in a boiling water bath for 10 min. Subsequently, proteins were separated from the particles employing Whatman 0.02 μm Anopore microcentrifuge filters. Centrifugation was carried out at 10 000 rpm for 20 min. Particles on the top of the filter were rinsed once with 50 μl of the reducing buffer followed by centrifugation. The two filtrates were pooled together and used for SDS–PAGE analysis. SDS–PAGE gels were prepared in a $80 \times 70 \times 1.5$ mm casting chamber. The slab gels consisted of a polyacrylamide resolving gel with 12.5% total acrylamide (T) and 2.6% bisacrylamide cross-linker (C) and a stacking gel with 5.2% T and 3.3% C. The separation of 20 μl samples was performed at a constant voltage (200 V) in a Mini-Protean II chamber employing a Bio-Rad Power PAC 300 power supply. Protein bands were stained with Coomassie brilliant blue R250. The stained gels were scanned with SharpJX-330P scanner (Sharp). Data were analysed using Phoretics 1D Advanced software distributed by Phoretics International (Newcastle upon Tyne, UK).

2.8. Computer modelling

The self-consistent field approach was applied in order to model the adsorption of poloxamer 407 on the surface of polystyrene particles [25–27]. For the purpose of modelling, poloxamer 407 ($PEO_{100}PPO_{65}PEO_{100}$) was equated to a structure B100[A65]B100 to describe the polymer in terms of hydrophobic anchor (A) blocks and hydrophilic buoy (B) blocks. A flat surface was used to simulate the adsorbing hydrophobic polystyrene surface. The interaction parameters used to simulate the adsorption were as follows. The interaction of the A blocks (PPO) with the surface (S) was considered strongly attractive with the χ_{as} interaction parameter set as -10, the interaction of B blocks (PEO) with the surface was considered repulsive, hence χ_{bs} was set as 0, whilst the attraction of the solvent (W) to the surface (S) was also considered weak with χ_{ws} set as 0. The buoy blocks were simulated in a good solvent with χ_{wb} being 0, whilst the anchor blocks were considered to repel the solvent with χ_{wa} being set at 0.5. The parameter used to describe the interaction between the A and B blocks was set to $\chi_{ab} = 0.5$. The amount adsorbed on the surface was varied from 0 to 6, which could be related to the experimentally adsorbed amount on the surface and to the bulk concentration of the simulated polymer [28]. The temperature was set to 298 K. A hexagonal lattice with 80 layers was used to simulate the adsorption. The results obtained included the determination of the layer thickness and the volume fraction profiles of the anchor and buoy layers with increasing mounts of adsorbed polymer.

The following equation was used to convert modelling into experimental values [28]

$$\Gamma = \frac{\theta \cdot m}{N_L \cdot l_L^2} \quad (2)$$

where Γ is experimental adsorbed amount, θ is theoretical adsorbed amount, m is molecular weight of the copolymer, N_L is number of monomers present in

one polymer chain (total number of monomeric units in the copolymer is 265) and l_L is length of monomeric unit in metres. The value of 0.3×10^{-9} m was taken to be the length of one monomer unit [29].

2.9. In vivo studies

For in vivo studies, polystyrene nanoparticles were radiolabelled with $Na^{125}I$ using a procedure based on that of Huh et al. [30]. Radiolabelled polystyrene nanoparticles were incubated overnight with different concentrations of poloxamer 407 solution, as described for the non-labelled nanoparticles. Free polymer was removed by dialysis against chromatographically purified water (tubing of molecular weight cutoff 100 000 was used, Spectropor, UK).

Groups of three male SBW rats were injected intravenously via the tail vein with 0.5 mg of the various radiolabelled nanoparticles suspended in phosphate buffer pH 7.4. Blood samples (20 ml) were taken from the contralateral tail vein at various time intervals (5, 15, 30 min, 1, 2 and 3 h). The rats were sacrificed after 3 h by an intravenous injection of pentobarbitone (0.4 ml, 60 mg/ml) and the liver, spleen, kidney and thyroid were removed and excess blood removed by a blotting process. The carcass associated activity was counted using a large sample volume γ counter (EG and G Ortec, UK). The blood associated activity and that in the organs were counted using a γ counter (LKB 182 Compugamma CS, LKB Wallac, Finland). A total blood volume per rat of 7.5% of body weight was assumed [31]. The results are expressed as a percentage of the dose injected and are the mean of values obtained for the three rats ± S.D. Uncoated radiolabelled polystyrene nanoparticles were used as control systems. (The values obtained for the various organs may represent a slight overestimate since not all blood could be removed from the organ samples using a standardised blotting process; for example the liver of the rat is known to contain about 25% of the circulating blood pool.)

3. Results and discussion

3.1. Adsorption isotherms and adsorbed layer thickness

The isotherms for the adsorption of poloxamer 407 onto 40, 70 and 137 nm sized polystyrene nanoparticles are shown in Fig. 1. The isotherms obtained followed a Langmuirian-type profile as characterised by a steep initial slope at low copolymer equilibrium concentration and an adsorption plateau reached above a certain equilibrium concentration. The values for the amount of poloxamer 407 adsorbed at the plateau and the adsorbed layer thickness are listed in Table 1. The plateau values obtained are in broad agreement with values obtained for similar adsorption systems reported in the literature [15,32]. In the present study, the amounts of poloxamer 407 ad-

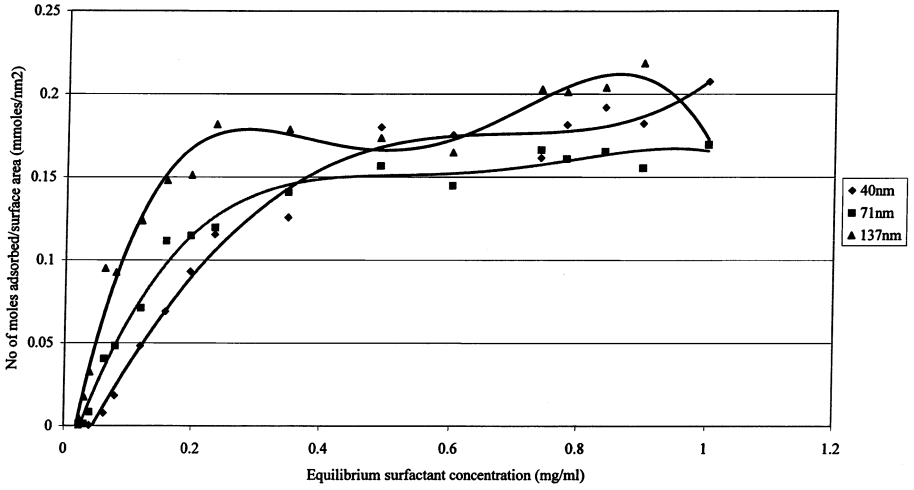

Fig. 1. Adsorption isotherms for poloxamer 407 on polystyrene nanospheres of different sizes. Particle diameters: ♦, 40 nm; ■, 71 nm; ▲, 137 nm.

Table 1
Adsorption of poloxamer 407 onto 40 nm, 70 nm and 137 nm sized nanoparticles: average adsorbed amount, surface area and hydrodynamic layer thickness

Particle size (nm)[a]	Average amount adsorbed (mg/m^2)	Averaged amount adsorbed (μmol/m^2)	Average area per molecule (nm^2)	Hydrodynamic layer thickness (nm)[b]
40	2.3 ± 0.3	0.18 ± 0.03	12.0 ± 1.9	8.5 ± 0.1
70	2.0 ± 0.5	0.16 ± 0.04	10.8 ± 0.6	7.7 ± 0.3
137	2.4 ± 0.2	0.19 ± 0.02	9.6 ± 0.9	7.7 ± 0.5

[a]As determined by the supplier.
[b]Determined from particle size analysis by photon correlation spectroscopy.

sorbed per surface unit at the plateau level of the isotherm were 0.183, 0.160 and 0.193 μmol/m^2 for the 40 nm, 70 nm and 137 nm particles, respectively, and were not significantly different. This is in agreement with work by Faers and Luckham [32] who found that for polystyrene particles in the size range between 70 and 500 nm, the amount of the copolymer Synperonic F-108 (of similar structure to poloxamer 407) adsorbed on the surface was independent of particle size. On the contrary, Caldwell and co-workers [10,33] have shown in earlier studies for the same F-108 copolymer, that the amount, expressed as chains/nm^2 or molecules/nm^2, adsorbed on the surface of polystyrene nanoparticles of sizes from 69 nm to 482 nm increased with increasing particle size from 5.2 ± 0.5 mol/nm^2 to 11.2 ± 1.0 mol/nm^2, respectively. On the other hand, in the paper by Caldwell and co-workers, the adsorption isotherms for 69 nm and 109 nm nanoparticles showed no significant difference in the amount of F-108 adsorbed per m^2 surface area [10].

On theoretical grounds, it is to be expected that an increase in particle size would provide an increase in adsorbed layer thickness up to the point where the curvature of the surface was essentially the same as a plane surface. This behaviour is consistent with the suggestion that decreasing the curvature of the particle causes additional lateral crowding in the adsorbed layer which is expected to increase the thickness. Wijmans et al. [34] have used a self-consistent field theory to study the adsorption of di-block copolymers onto small colloidal particles. They concluded that the adsorbed amount should increase with increasing curvature and that the hydrodynamic layer thickness should also increase with increasing curvature. The experimental data in the literature largely supports this conclusion but a close examination reveals certain discrepancies. The work of Li et al. [35] has shown that the thickness of adsorbed poloxamers to polystyrene microspheres increases with particle size over the size range 60–270 nm for the Pluronic materials F-108, F-88, F-68 but not for P-105. These results were obtained by field flow fractionation. Interestingly, the same group had reported earlier [10] that the adsorbed layer thickness was method dependent. Field flow fractionation demonstrated an increase in adsorbed layer thickness with particle size for Pluronic F-108 on polystyrene (69–261 nm) whereas photon correlation spectroscopy showed the opposite trend for particles coated with F-108 and the Tetronic block copolymers T-908 and T-1508. Interestingly, for particles of 69, 85 and 109 nm, the adsorbed layer thickness was effectively constant when measured by PCS. Baker and others [36,37] measured the layer thickness for various poloxamer block copolymers on polystyrene particles using photon correlation spectroscopy and concluded that the specific adsorption of their copolymers on polystyrene was independent of particle size and that adsorbed layer thickness decreased with increase in particle radius. Bevan and Prieve [38] have reviewed the literature on the adsorption of Pluronic F-108 on polystyrene and have quoted a value of 12 nm for the adsorbed layer thicknesses on 140 nm particles and 15 mm for 400 nm particles.

In contrast, Greenwood et al. [39] have reported that the adsorption layer thickness of poloxamer 407 on polystyrene particles as determined by rheological measurements was strongly size dependent increasing from about 11 nm on 40 nm particles to as large as 37 nm on 217 nm particles. They pointed out that their results were expected on purely geometrical grounds, but cautioned the reader that the reason for the strong dependence on size for the moment

remained a mystery. They cited the work of Faes and Luckham [32], who had shown that for polystyrene particles the amount of poloxamer adsorbed was within experimental error, independent on particle size over the range 70–500 nm. Thus, the exact relationship between adsorbed layer thickness and particle size (curvature) is not yet totally defined and experimental data demonstrate that the method used to determine the thickness, the nature of the adsorbed block copolymer and the nature of the particle can all have an influence. As far as we could ascertain the particles used in the present experiments all had similar surface properties in terms of surface groups and surface hydrophobicity, but it is possible that subtle differences in surface properties could have led to the lack of a defined particle size effect. It is known that the nature of the surface of a particle can strongly affect adsorption. The polystyrene particles used in the present work had sulphate groups on the surface. Recently, Gorg et al. [40] have described studies on polystyrene particles having different radii and numbers of sulphate groups on the surface and how this can affect effective charge and counterion association. Furthermore, Ahmed et al. [41] have reported that the surface adsorption of poloxamers (Pluronic P-105 and F-68) on carboxylated polystyrene microspheres decreased by approx. 100-fold as compared to plain microspheres of an almost identical size (1.94 and 2.00 μm, respectively). (There was no evidence to suggest that our particles carried carboxyl moieties.)

At selected points on the adsorption isotherms the adsorbed layer thicknesses were determined, as shown in Fig. 2. As would be expected the layer thickness increased as the surface coverage increased, reaching a maximum value at the plateau of adsorption. The results clearly reveal a dependence of the layer thickness on the copolymer concentration in the solution and the surface coverage, the latter being a function of the concentration.

Table 2 lists the data at selected points on the adsorption isotherm for the adsorption of poloxamer 407 onto 40 nm sized nanoparticles. The values illustrate the changes in the conformation of the molecule at the nanoparticle surface when the amount of polymer adsorbed increases. When low amounts of poloxamer 407 are adsorbed, with a corresponding low surface coverage, the calculated larger area per one

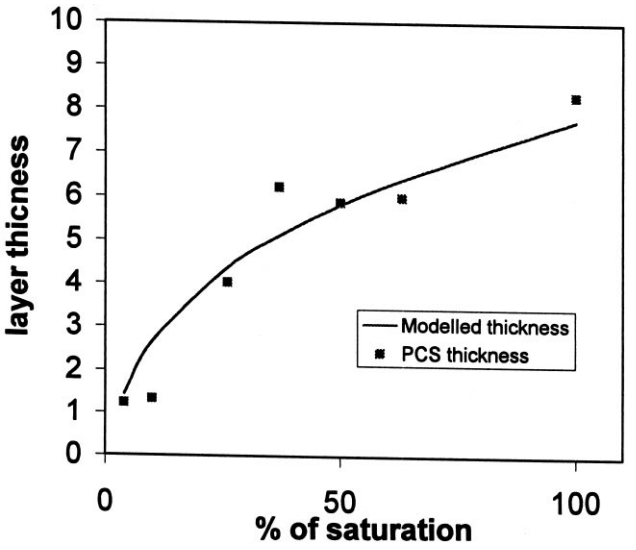

Fig. 2. Adsorbed layer thickness of poloxamer 407 on polystyrene nanospheres with increasing % saturation of the surface: comparison of experimental (■) and modelled data (———).

PEO chain and the low value for the adsorbed layer thickness indicate that the poloxamer 407 molecules are less densely packed on the surface, giving the PEO more space to spread laterally. At higher amounts of adsorbed poloxamer the surface is more crowded and the area that one polymer molecule or PEO chain occupies decreases and the adsorbed layer thickness increases. This indicates a closer packing of the PEO chains and suggests that the PEO chains are in a more extended conformation in a direction perpendicular to the surface. Finally, at the adsorption plateau, the hydrodynamic layer thickness is more than twice the radius of gyration (Rg) for the corresponding PEO chain in an aqueous solution ($2Rg = 5.7$ nm for MW 4400 PEO) [42]. This implies that the most probable conformation of the PEO chains in the adsorbed layer at the plateau would be a laterally compressed elongated coil. This suggested conformation of the poloxamer 407 adsorption agrees with those presented previously in the literature [15,32]. Faes and Luckham [32] reported that the adsorbed layer thickness increased with molecular weight of the PEO moiety as one would expect and that the results scaled with $n\mathrm{EO}^{1/2}$. This would suggest that the ethylene oxide chain was arranged as an elongated coil, which extended from the surface. (If the polymer was a linear chain, then the dependence would be nEO). Interest-

ingly, decreasing the coverage of the selected poloxamers (Pluronic 103) coverage from full to one twelfth coverage resulted only in a slight reduction of the adsorbed layer thickness. The authors believed that at such twelfth coverage, the individual poloxamer molecules would be isolated on the surface. They concluded that the PEO chains were not adsorbed onto the surface but still protruded in the form of extended coils. At the plateau, the chains would be laterally compressed leading to a slightly more extended conformation.

Cosgrove et al. [43] have reported results from small angle neutron scattering experiments for two PEO–PPO–PEO block copolymers, (32–56–32) and (140–56–140), adsorbed to deuterated polystyrene particles. Cutoff values for adsorbed layer thickness were 9 nm and 18 nm, respectively, and extended tails at high coverage were proposed.

As shown in Table 2, from the information on the layer thickness and the area occupied by one PEO chain it is possible to define the average space occupied by one PEO chain in the adsorbed layer. However, such a representation of the PEO is only approximate. More detailed information of the structural composition of the chains within that space would be advantageous. Experimentally this information can be gained from small angle neutron scattering (SANS), which gives a volume fraction profile for the adsorbed polymer [44,45]. This profile is related to the concentration of the polymer expressed as a function of the distance from the surface but it is important to note that SANS is insensitive to the tail fraction of the adsorbed layer. In the present work we have combined the experimental data (amount adsorbed and layer thickness) with data obtained from the modelling of the adsorption of poloxamer 407 on a surface in order to gain an insight into the conformation of the PEO chains within the adsorbed layer.

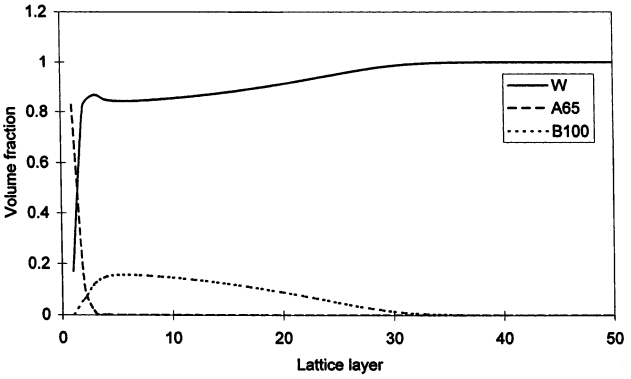

Fig. 3. Computer modelled volume fraction profiles of a copolymer on a flat surface. W, water; A, anchor block (PPO); B, buoy block (PEO).

3.2. Computer modelling

Fig. 3 is a typical result obtained from the modelling of the adsorption of the copolymer B100A65B100 on a flat surface. It shows the volume fraction profiles of the anchoring 'A' blocks, the buoy 'B' blocks and the solvent 'W' molecules with the distance from the surface (expressed as the number of lattice layers). The plot demonstrates that the anchoring A blocks are in the vicinity of the solid surface and do not extend further than the fifth lattice layer from the surface while the buoy B blocks protrude into the solvent up to the 32th layer. Most of the solvent W molecules are in a region of the

Table 2
Different points of the adsorption isotherm of poloxamer 407 on 40 nm sized polystyrene nanoparticles: surface coverage, area occupied per one molecule or PEO chain and hydrodynamic layer thickness

Amount adsorbed ($nmol/m^2$)	Amount adsorbed (mg/m^2)	Surface coverage (%)	Average area per molecule (nm^2)	Average area per PEO chain (nm^2)	Hydrodynamic layer thickness (nm)
7.72	0.097	4	215	107.5	1.23
18.3	0.230	10	90.7	45.4	1.33
48.3	0.608	26	34.4	17.2	4.03
69.0	0.869	37	24.1	12.0	6.22
93.1	1.173	50	17.8	8.9	5.88
115	1.449	63	14.4	7.2	6.00
182	2.293	100	9.12	4.5	8.32

buoy blocks. A conversion of the modelling into physical values, by the use of Eq. 2, shows the following. If the theoretical adsorption value of 4.0 (the point where the plateau was reached on the curve in Fig. 4) is selected, a modelled adsorbed amount of 3.52 mg/m^2 is obtained. This is of the same order of magnitude as the experimental values of 2.30, 2.01 and 2.43 mg/m^2, respectively, determined for the adsorption of poloxamer 407 onto 40, 70 and 137 nm sized nanoparticles (Table 1). Moreover, the use of a conversion factor of 0.3 nm per lattice layer [28] transforms a modelled hydrodynamic thickness of 25.82 layers into a value of 7.75 nm for the modelled PEO layer thickness, while the experimental values obtained were 8.5 ± 0.1, 7.7 ± 0.7, 8.5 ± 0.1 nm for 40, 70 and 137 nm sized nanoparticles, respectively (Table 1).

The data on modelled hydrodynamic layer thickness at varying theoretical adsorbed amounts (Θ) are listed in Table 3. The points were chosen to correspond to the points from the experimental adsorption isotherm (Table 2). A comparison of these two sets of data demonstrates that there is a good agreement between experimental results, which show an increased hydrodynamic layer thickness as the adsorbed amount increases (Table 2), and the modelled adsorption, where the layer thickness also increases with increased adsorbed amount (Table 3). These comparisons confirm that the use of the modelling parameters (i.e. the parameter χ_{as} to describe the strong interaction between the PPO blocks and the polystyrene surface and the χ_{wb} parameter that allows favourable interaction between PEO and the solvent molecule) produced a description of the adsorption of the B100A65B100 copolymer that agrees with a representation of PEO–PPO–PEO adsorption onto a hydrophobic surface, obtained from the experimental results above and the literature [46,47]. This modelling procedure and the parameters used can therefore be used for the exemplification of adsorption mechanisms for poloxamer 407.

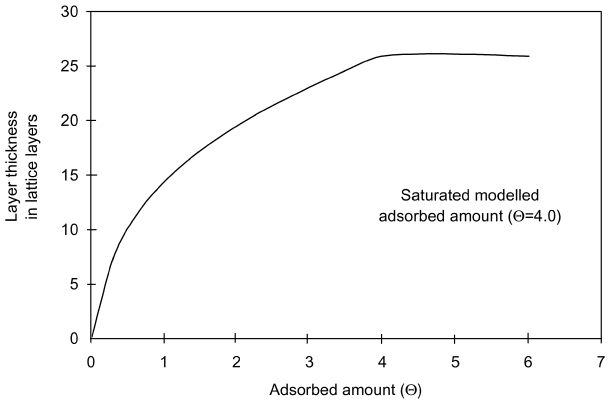

Fig. 4. Computer modelled adsorbed layer thickness (expressed in number of lattice layers) of a copolymer on a flat surface.

It should be noted that in the above discussion values for the theoretical adsorbed amount (Θ) were taken from Fig. 4. The figure represents the changes in the layer thickness of an adsorbed polymer (expressed as number of lattice layers) as the theoretical adsorbed amount (Θ) increases. The plot shows that the thickness increases as the theoretical adsorbed amount increases, reaching a plateau value at a certain theoretical adsorbed amount. The point where the curve reaches the plateau (4.0) was taken as the modelled saturated adsorbed amount (which corresponds to a plateau value for experimentally determined adsorption isotherm). The assumption was made that the adsorbed layer thickness does

Table 3
Theoretical (modelled) adsorption of poloxamer 407; conversion of modelled to physical values

% of saturated modelled adsorbed amount (Θ)	% of saturated modelled adsorbed amount – converted (mg/m^2)	Modelled hydrodynamic thickness (lattice layer)	Modelled hydrodynamic thickness – converted (nm)
4	0.14	4.78	1.43
10	0.35	8.83	2.65
26	0.92	14.56	4.37
37	1.30	17.07	5.12
50	1.76	19.43	5.83
63	2.20	21.32	6.40
100	3.52	25.82	7.75

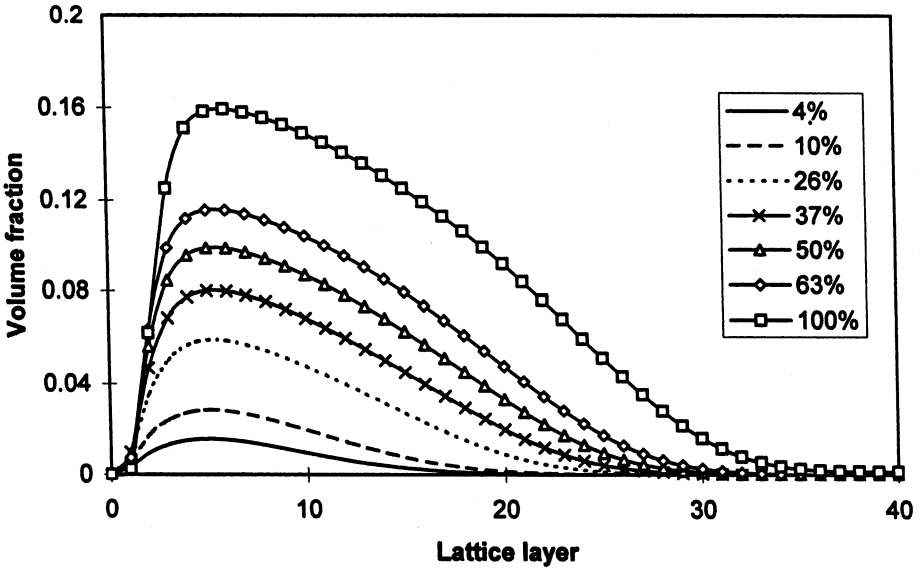

Fig. 5. Computer modelled volume fraction profiles of a copolymer on a flat surface with different surface coverage from 4 to 100%.

not increase significantly once the adsorbed amount reaches the plateau, as the experimental results shown in Fig. 2 demonstrate.

Fig. 5 shows the volume fraction profiles for the buoy block only (i.e. PEO chains) as the modelled adsorbed amount increases up to the modelled saturated adsorption. An important feature to note is the shape of the profiles, which gives information on the possible structure of the PEO segments in the adsorbed layer. The shape of the curves demonstrates that the volume fraction (concentration) of the PEO chains is higher closer to the surface, and decreases gradually over a distance from the surface. This means that within the space that one PEO chain occupies on the surface, as estimated from the experimentally determined surface area per one PEO chain and the PEO layer thickness (Table 1), the PEO chain does not occupy a randomly fluctuating three-dimensional (random coil) conformation. There is a higher probability that the PEO segments are located closer to the surface with 'tails' extending into the solution. Also it should be noted that the shape of the volume fraction profiles in Fig. 5 is gradually changing from a relatively shallow curve into a steeper curve with higher values for maximum volume fraction, as the theoretical adsorbed amount increases. Hence, there is a higher density of the PEO segments in the adsorbed layer as the adsorption proceeds and this higher density of PEO extends further into the solution with the higher adsorbed amount.

3.3. Adsorption of serum proteins

Fig. 6 shows the amount of the serum protein adsorbed onto the PS nanoparticles with varying surface coverages of poloxamer 407. The results demonstrate that the protein adsorption decreases with increasing surface coverage of poloxamer 407 from 0 to 68%. For uncoated particles about 136 μg protein were adsorbed per mg of particles. This was reduced to 40 μg for nanoparticles with 68% surface

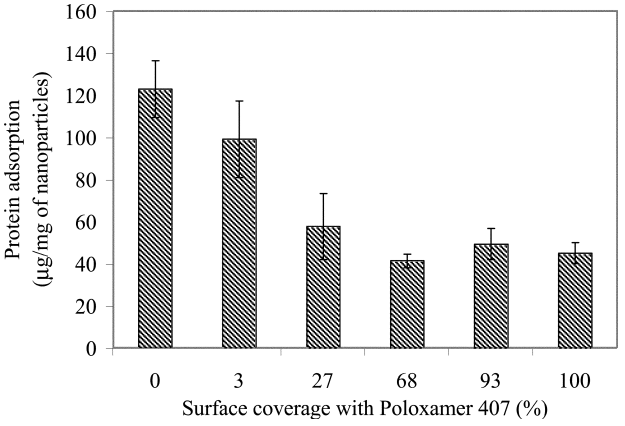

Fig. 6. Amount of protein adsorbed on polystyrene nanospheres with different surface coverage of poloxamer 407.

coverage. Interestingly, above 68% surface coverage the amount of adsorbed protein did not change significantly, but was still relatively high, 40% of the amount that was adsorbing onto uncoated nanoparticles. Comparable observations have been reported for PEGylated liposomes by Blume and co-workers [48], who showed that increasing the concentration of PEGylated distearylphosphatidylethanolamine (PEG-DSPE) in the lipid bilayer from 0 to 15 mol% resulted in a decrease of the amount of protein adsorbed onto the vesicles.

Quantitative data on the adsorption of protein on 137 nm nanoparticles are given in Table 4, which show difference in the molecular weight and relative proportion of the adsorbed proteins as a function of the surface coverage. The control system, uncoated polystyrene nanoparticles, adsorbed a variety of proteins with molecular weights from approx. 180 000 to under 24 000. Interestingly as much as 32% of the protein adsorbed onto uncoated nanoparticles had a molecular weight of approx. 120 000, while the relative proportion of smaller molecular weight proteins was low. Relatively high molecular weight proteins, with the molecular weight between approx. 130 000 and 85 000, were also found on the surface of nanoparticles with 3% and 27% surface coverage, but the relative proportion was lower than for the uncoated nanoparticles. An important change in the adsorption pattern of proteins from blood appears to have occurred between 25% and 68% surface coverage, so that at and above a value of 68% coverage only proteins with a molecular weight of about 68 000 and under 36 000 could be detected. Further increase in the surface coverage did not result in any significant changes to the pattern of protein adsorption. An identification of the type of proteins adsorbed was not attempted. Previously, we have used SDS–PAGE in combination with densitometry to evaluate the adsorption of plasma and serum proteins to polystyrene microspheres coated with block copolymers of the poloxamer and poloxamine series [23]. Immunoblotting studies revealed the adsorption of immunoglobulin G, complement C3, transferrin and fibronectin. A diffusion chamber method was subsequently used [49]. This was implanted intraperitoneally in the rabbit. Using a desorption technique follow by SDS–PAGE six proteins of molecular weights 94 000, 86 000, 66 000, 53 000, 40 000 and 20 000 were identified, the last two proteins dominated the protein profile. In general the coating of polystyrene with the block copolymers reduced the amount of protein bound to the microspheres in vivo but did not change the type of protein bound. The plasma proteins adsorbed to polystyrene particles modified with poloxamer 407 have also been identified by Blunk et al. [50,51] using two-dimensional polyacrylamide gel electrophoresis. The major proteins (75% of total) were apoprotein J, apoprotein C III and an unidentified protein. Fibrinogen, albumin, and a range of other apoproteins were also present at lower amounts. As found in the present work, the quantity of protein adsorbed was dramatically reduced as compared to the uncoated particles. Thus, our results like those of others show that the amount of adsorbed poloxamine on the surface of a particle greatly reduces the quantity of proteinaceous material adsorbed but does not dramatically affect the qualitative aspects.

It has been proposed that the presence of PEO on the surface of particles can lead to the selective uptake of certain blood components. For example, recently Vert and Domurado [52] have proposed that the effect of PEO on a surface could be one due to albumin compatibility rather than protein repulsion. This proposal was made on the basis of known interactions between albumin and polyethylene glycol but would seem an unlikely mechanism. The work of Green et al. [53] and, more lately, by Pavey and Olliff [54] using surface plasmon resonance methods have shown that poloxamer coated hydrophobic surfaces can minimise or totally prevent the uptake of proteins (to include BSA).

The adsorption of Pluronics F-127, F-108 and F-68 to hydrophobic glass surfaces has been described by McPherson et al. [55], who also investigated the mechanics of how PEO grafted surfaces prevented protein adsorption. In a similar manner to the present work, the surface protein concentration decreased as the surface density of the (grafted) PEO increased. McPherson et al. suggested that at low surface coverage the PEO chains themselves could be adsorbed to the surface but as the surface coverage increased, PPO–PEO repulsions started to dominate and the PEO desorbed from the surface. Their view was that the plateau-like region of the isotherm corresponded to the surface coverage where

Table 4
Quantitave and qualitative evaluation of protein adsorption on polystyrene particles with different surface coverage of poloxamer 407

Rat serum		Uncoated PS		PS-407 (3%)		PS-407 (27%)		PS-407 (68%)		PS-407 (93%)		PS-407 (100%)	
MW	Band %	MW	Band %	MW	Band %	MW	Band %	MW	Band %	MW	Band %	MW	Band %
± 220 000	1.3												
178 000	8.0	180 000	3.0										
152 000	9.0												
132 500	3.2			131 500	6.7								
		120 000	32.0										
119 000	3.4												
107 000	3.6	95 000	9.9	97 500	6.8	98 000	6.7						
		83 500	6.9	82 500	4.2	85 000	6.3						
78 000	12.2												
		68 500	14.3	71 000	5.3							68 500	40.7
				66 000	5.9					66 500	25.9		
60 500	34.6			62 000	9.2	61 500	14.8	62 600	36.3				
		58 000	9.1										
		53 000	8.5	54 000	6.6								
		49 500	5.9	48 000	7.0	49 000	7.6						
46 000	6.7												
		39 000	3.0										
36 500	3.5	36 500	2.9										
35 000	1.7	35 000	2.2	34 500	7.8	35 000	13.7	34 500	18.0	35 000	14.0	35 500	18.7
		32 000	2.7	32 500	4.0	32 500	14.0	32 000	12.8				
31 000	3.0	30 000	3.5	30 000	6.6	30 000	10.6			31 200	9.4	31 000	13.1
		29 000	3.1					29 000	16.4				
28 400	1.9					28 500	20.0			28 500	18.9	28 500	11.2
27 900	1.3			28 000	15.4								
26 000	2.0							25 000	31.1	25 000	31.9	25 000	16.4
24 700	2.9					24 500	14.0						
24 400	3.1	24 000	6.3	24 000	20.8	24 200	18.4						

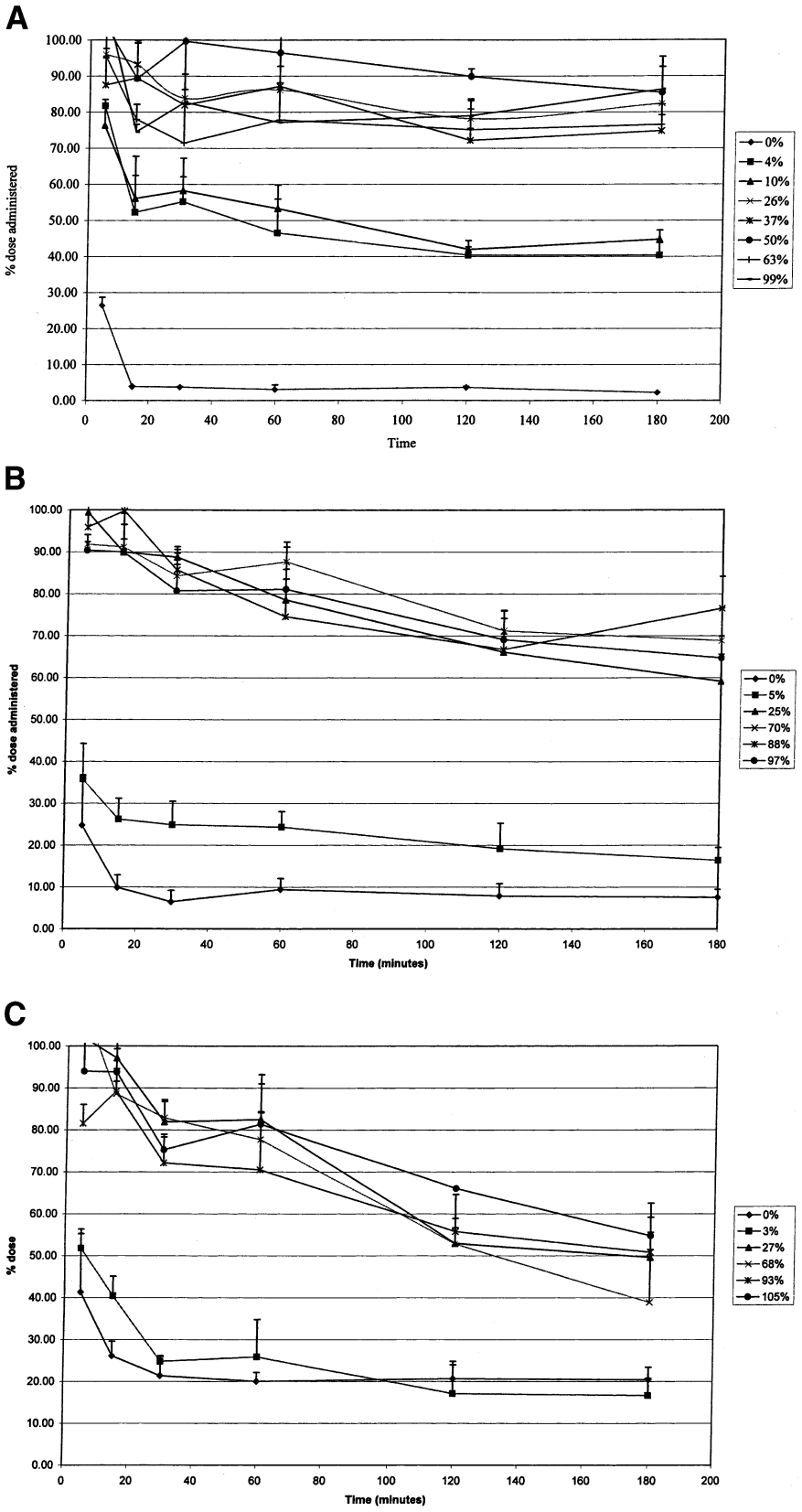

Fig. 7. The plasma concentration in rats of polystyrene nanospheres of different sizes coated with poloxamer 407 at different percentage of surface coverage. (A) Particle diameter 40 nm; (B) particle diameter 71 nm; (C) particle diameter 137 nm.

the interplay between repulsive PPO–PEO interactions and PEO surface attractions were such that one was in a region where a 'pancake to brush' transition is predicted. Interestingly, McPherson et al. have suggested that the mechanism for the prevention of protein adsorption by attached PEO on hydrophobic surfaces results from the ability of the PEO to block the adsorption sites for protein. They showed that the PEO layer on the surface did not have to exist as the commonly referred brush and that the surface coverings and polymer chain lengths that were effective were far from those necessary to reach the true brush regime.

In the present work it is possible to consider the decrease in protein adsorption and change in molecular weight of proteins adsorbed at higher poloxamer surface coverage, by using the information on the conformation of the PEO chains at different surface coverage as described above. We believe that our results can be explained in terms of surface accessibility and the ability of the PEO chains to block protein absorption sites. Assuming a random coil conformation of the PEO chains on the surface and taking into account the radius of gyration (Rg) for an MW 4400 PEO chain to be 2.8, the estimated adsorbed layer thickness at an adsorption plateau would be 5.7 nm. An estimation of the projected area that such a PEO chain would occupy on the surface is about 25.5 nm^2.

As shown in Table 2, at a surface coverage of 26%, the PEO chains have formed an adsorbed layer that is approx. 4 nm thick, whereby each chain occupied approx. 17 nm^2 of the surface. It can further be seen that below 26% surface coverage, the experimentally determined area that the PEO chain occupied on the surface was much higher than the estimated value (107 nm^2 and 45 nm^2, for 4% and 10% surface coverage, respectively). This means that the PEO chains have more space to spread than their random conformation suggests. Also, the values for hydrodynamic layer thicknesses (Table 2), which are less than 2Rg for the corresponding PEO chain, confirm that this is the case. In addition, the results from the modelling show that the volume fraction profiles for situations where there is less than 26% surface coverage are in the form of shallower curves with lower maxima for volume fraction, in comparison to the corresponding profile for saturated surface coverage (Fig. 5). The protein adsorption studies indicate that for particles with less than 27% surface coverage, the surface was accessible for the adsorption of higher amounts of protein and that the adsorbed protein molecules were of relatively higher molecular weight than were those on particles with a greater surface coverage (Table 4).

At a surface coverage of approx. 26% and higher, the estimated surface area for an MW 4400 PEO chain is lower than what was experimentally determined (17.2, 12.0, 8.9, 7.2, 4.5 nm^2 for 26, 37, 50, 63 and 100% surface coverage, respectively) (Table 2). At the same time, measured hydrodynamic layer thicknesses at these surface coverages became larger than the estimated value (Table 2). This indicates that when the surface is covered by more than 26% of poloxamer, the PEO chains are packed in a conformation that is less laterally spread than for a truly random coil, and is more extended in a direction perpendicular to the surface. The density of the PEO segments in the surface layer, at surface coverage of 26% and higher, was much higher than estimated, i.e. 25.5 nm^2 per chain as compared to 17.2, 12.0, 8.9, 7.2, 4.5 nm^2, respectively, for 37%, 50%, 63% and 100% surface coverage. Protein adsorption was reduced and proteins with relatively lower molecular weights were preferentially adsorbing (Table 4) onto the surface of these nanoparticles.

Schroen et al. [18] measured the adsorption of a model protein (lipase, 60 000 molecular weight) to a hydrophobic surface covered with block copolymer layers with various degrees of saturation. At 70–100% saturation with poloxamer 338 ($PEO_{129}PPO_{56}PEO_{129}$) no measurable adsorption of lipase took place. However, if the adsorbed amount of polymer was decreased below this level the adsorption of lipase began to increase but remained low until a coverage with 10% poloxamer 338 was reached. They reasoned that the protein molecule could begin to penetrate into the poloxamer 338 layer when the coverage fell below 70%, but the number of 'holes' in the poloxamer 338 layer would remain very low until 10% saturation or less was reached. It is difficult to directly compare the present results with their work due to the different surface used for adsorption and the different poloxamer.

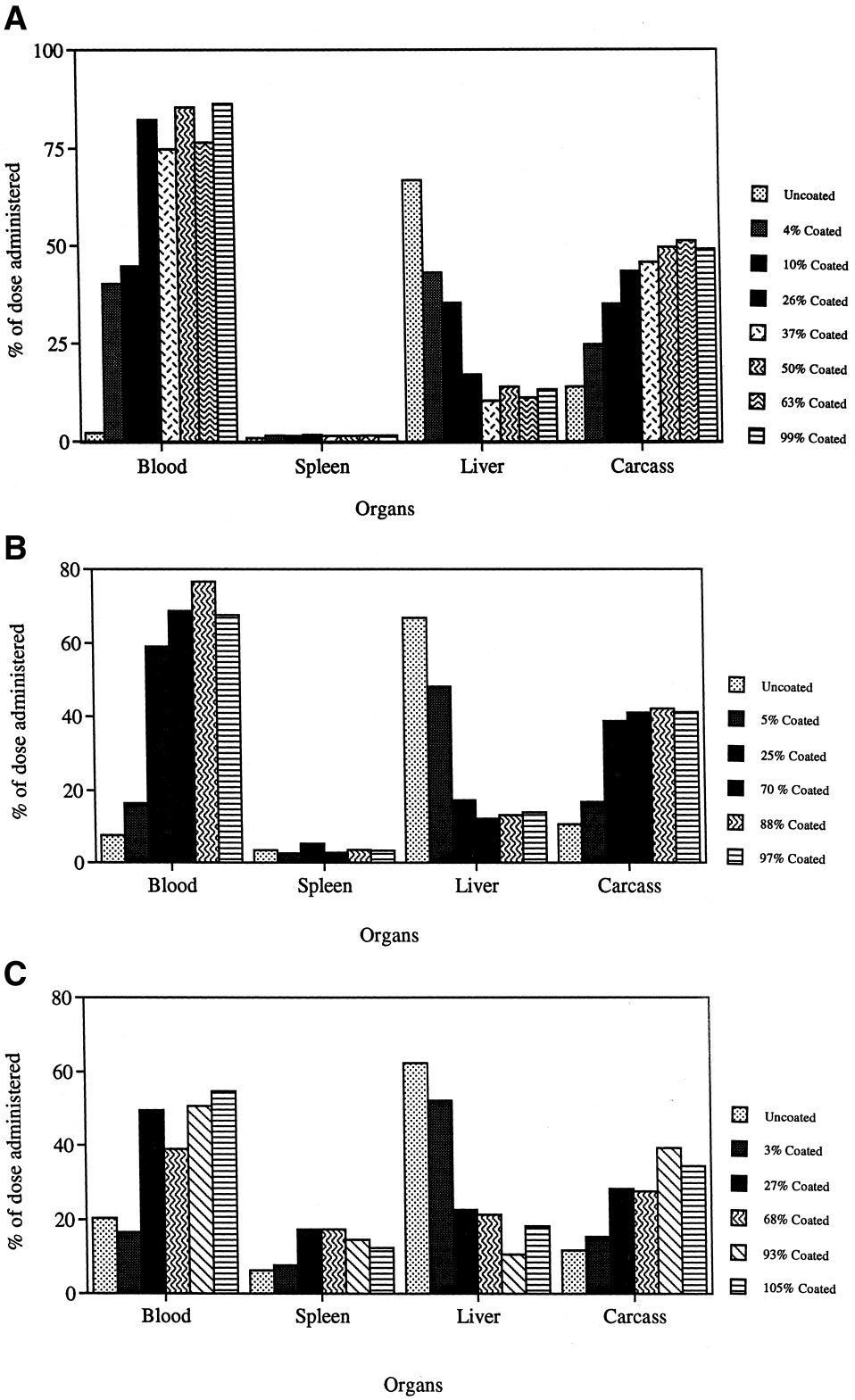

Fig. 8. The organ distribution in rats of polystyrene nanospheres coated with poloxamer 407 at different percentage of surface coverage. (A) Particle diameter 40 nm; (B) particle diameter 71 nm; (C) particle diameter 137 nm.

3.4. Biodistribution of nanoparticles

At selected points of the poloxamer 407 adsorption isotherms (surface coverage) the in vivo biodistribution of radiolabelled nanoparticles (40, 70 and 137 nm) was followed after intravenous injection into rats. The blood clearance profiles are shown in Fig. 7. These data reveal that as expected uncoated polystyrene nanoparticles of all investigated sizes were rapidly cleared from the systemic circulation, less than approx. 10% of the injected dose was still present in the systemic circulation 1 h after injection. Data for the organ distribution of the nanoparticle systems 3 h post injection (Fig. 8) demonstrate that most (about 65%) of the uncoated nanoparticles were deposited in the liver. Such uptake is known to involve phagocytic macrophages [3].

With a minimal (5%) surface coverage of poloxamer 407 the circulation profiles of the nanoparticles were improved, the effect being more pronounced for the smaller 40 nm sized nanoparticles. Further increase in the surface coverage of poloxamer to approx. 25% resulted in a significant increase in the circulation time for all nanoparticle systems. The improved circulation time was dependent on the particle size. After 3 h, $82.3 \pm 3.0\%$, $59.1 \pm 6.5\%$, and $49.6 \pm 1.8\%$ of the injected dose of 40, 71 and 137 nm nanoparticles with 25–27% surface coverage, respectively, remained in the circulation (Fig. 8). It should be noted that the increased surface coverage of poloxamer on the 137 nm polystyrene nanoparticles also resulted in an increased spleen uptake. Such splenic uptake has been shown previously to be due a mechanical filtration of particles by the spleen. Sterically stabilised nanoparticles and liposomes of about 100 nm or larger, that are not captured by the macrophages of the liver, show an increased deposition in the spleen [3,11,56].

The three sizes of nanoparticles, at maximum poloxamer 407 surface coverage, showed differences in their biodistribution, with the smallest (40 nm) nanoparticles having the highest blood concentration at 3 h after injection followed by the 70 nm and the 137 nm nanoparticles. Furthermore, at 3 h after injection the liver uptake was highest for the largest particles and lowest for the smallest particles. These results suggest that the size of the nanoparticles could give rise to conformational differences of the PEO on the surface as described on theoretical grounds by Wijmans et al. [34] and thereby to differences in subsequent protein adsorption following injection which would modify the biodistribution. However, as discussed above, the amount of poloxamer adsorbed to particles of different sizes and the adsorbed layer thicknesses were particle size independent.

An increase in surface coverage of poloxamer 407 above a value of 25% did not further increase the circulation times of the nanoparticles significantly. A reduction in liver uptake was obtained with values reaching a plateau at 25% coverage and above. This is an important observation and demonstrates that a nanoparticle surface does not need to be saturated with PEO chains, as would be obtained at the adsorption plateau, in order for long nanoparticle circulation times and a decreased liver deposition to be achieved.

Araujo et al. [18] also found that the biodistribution of nanoparticles (methyl methacrylate) coated with a hydrophilic polymer (poloxamine 908) was highly dependent on the quantity of polymer added to a nanoparticle suspension (and assumed to be at least partly adsorbed to the surface). It was shown that below a polymer concentration of 0.1% the nanoparticles behaved like uncoated particles whereas at a concentration of poloxamine 908 of 0.1% and above the liver deposition of the particles was highly decreased. Similarly, the blood concentration increased sharply at a polymer concentration of 0.1% and above. No adsorption isotherms were produced in the study nor were calculations done to estimate the surface coverage of poloxamine 908 at each concentration.

It is tempting to compare the results from the present work with those obtained for liposomes carrying PEG functions. Here, for example, it has been reported that long circulation times can be obtained when the PEG-PE content of a phospholipid mixture is equal or greater than 5% [57–59]. An upper limit of 10 mol% PEG has even been proposed recently because of the spontaneous formation of PEG-PE micelles at higher concentrations [60]. However, such comparisons need to be made with caution since the properties of a PEO moiety adsorbed via a poloxamer will be different to a PEO incorporated into a phospholipid bilayer. Indeed, McPherson et al. [55]

have concluded for the case of lipid-PEO layers [61] and liposomes, where the PEO is not attracted to particle surface, the PEG moieties act more as a kinetic barrier to proteins in solution rather than acting to block the actual protein adsorption process.

The present studies show that in order for nanoparticles of sizes 40 nm, 70 nm and 137 nm to be fully stabilised with a steric barrier so as to provide low protein adsorption and subsequent long blood circulation times after injection and with low degree of deposition in the various organs especially the liver, the surface needs to have a layer of PEO chains with a sufficient thickness and surface density. Such a surface can be obtained with poloxamer 407 at a surface coverage of about 26%.

The relationship between physicochemical and biological properties can be well evaluated by means of adsorption isotherm determinations, conformational modelling of the PEO chains on the surface and protein adsorption studies. At about 26% surface coverage there is a significant change in the hydrodynamic PEO layer thickness accompanied by a sharp increase in the surface layer density and a change in the modelled volume fraction profile (from a relatively shallow curve into a steeper curve with higher values for maximum volume fraction). The density of the PEO segments in the adsorbed layer increases with increased poloxamer adsorption and the higher density region extends further into the solution. While the present results have been obtained using model particles in the form of polystyrene it should be possible to use this information to create biodegradable nanoparticles based on suitable polymers with similar characteristics.

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