Real-time evidence of surface modification at polystyrene lattices by poloxamine 908 in the presence of serum: in vivo conversion of macrophage-prone nanoparticles to stealth entities by poloxamine 908

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Received 24 April 2003; revised 11 June 2003; accepted 11 June 2003

First published online 26 June 2003

Edited by Veli-Pekka Lehto

Abstract Intravenously injected polystyrene nanoparticles, which are prone to rapid sequestration by professional phagocytes, are converted to stealth entities by prior bolus intravenous injection of poloxamine 908. This behaviour is not due to alteration in macrophage phagocytic activity. Laser Doppler anemometry and surface plasmon resonance were used to unravel the mechanisms fundamental to generation of such stealth entities in vivo by poloxamine 908. Electrophoretic mobility of poloxamine pre-coated monodisperse polystyrene nanoparticles in serum, which behave as stealth entities in vivo, was similar to that of uncoated nanoparticles incubated in poloxamine pre-treated serum. This observation supported the notion that poloxamine in serum can modify the surface of nanoparticles with similar topography to that of stealth poloxamine pre-coated particles, i.e. with polyoxyethylene chains projected from the surface. Surface plasmon resonance optical phenomenon was used for real-time monitoring of protein–poloxamine interactions and adsorption at the polystyrene interface. It was found that poloxamine can not only adsorb to a serum-modified surface but in addition poloxamine in serum can form macromolecular complexes with high affinity for adsorption to a polystyrene lattice. A role for serum albumin in surface modification of nanoparticles by poloxamine 908 is also identified. Hence, our biophysical observations correlate precisely with the in vivo longevity of uncoated polystyrene nanoparticles in poloxamine pre-treated rats. This rational and sensitive biophysical approach has unravelled the probable mechanism fundamental to generation of stealth entities in vivo and therefore has application in the design and nano-engineering of stealth colloidal carriers for optimal biological performance.

Key words: Colloidal carrier; Drug delivery system; Poloxamine; Polymer–protein interaction; Serum; Serum albumin; Stealth nanoparticle; Surface plasmon resonance

1. Introduction

The biological performance of colloidal particles in vasculature can be altered by surface modification with non-ionic block copolymers such as poloxamer 407 and poloxamine 908 [1,2]. For instance, following intravenous injection into rats or mice, polystyrene nanoparticles (sub-100 nm) that are pre-coated with a sufficient surface density of either poloxamer 407 or poloxamine 908 escape rapid detection and clearance by the body's scavenger cells [3,4]. As a result, these surface-modified nanoparticles exhibit prolonged circulation times when compared to untreated particles. In contrast to this classical approach, a recent study from this laboratory demonstrated that the circulatory half life of intravenously injected uncoated polystyrene nanoparticles, which are prone to rapid phagocytosis, can equally be increased by prior intravenous injection of either poloxamine 908 or poloxamer 407 (140 mg/kg) to rats [5,6]. To date, the in vivo observations support the notion that nanoparticles in the blood are spontaneously converted to long circulating entities by prior injection of such copolymers. The precise mechanism(s) of this conversion remains unknown but is presumably linked to generation of a steric barrier by poloxamine 908 molecules to combat phagocytic recognition. Interestingly, serum proteins are known to rapidly coat the surface of nanoparticles; a process that leads to surface opsonisation. This raises the question as to how such small quantities of copolymers in competition with serum proteins can re-arrange the interfacial surface dynamics to generate stealth entities under in vivo shear forces.

The aim of this paper is therefore to understand the biophysical basis of nanoparticle surface modification by poloxamine 908 in the presence of serum proteins. We have used two complementary approaches to determine the nature of such interactions. Firstly, the mobility of monodispersed polystyrene nanoparticles was measured in a defined electric field [7,8]. Thus, we compared the electrophoretic mobility of poloxamine-coated polystyrene nanoparticles both in the absence and in the presence of serum to that of uncoated polystyrene nanoparticles incubated in poloxamine pre-treated serum. Therefore, a similar mobility profile between the two treatments would suggest that the poloxamine in serum can coat the surface of nanoparticles with similar topography to that of stealth poloxamine pre-coated particles (e.g. with polyoxyethylene chains projected from the particle surface). With the aid of surface plasmon resonance (SPR) [9,10] we monitored the real-time protein–poloxamine interactions and the assembly of possible macromolecular complexes at the polystyrene interface, which may impart the stealth-like behaviour observed in vivo. Our observations reflect precisely the in vivo longevity of uncoated model polystyrene nanoparticles in po-

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0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00707-5

loxamine pre-treated rats. These approaches may be adopted for surface engineering of biodegradable nanoparticles such as poly(lactide-co-glycolide) (PLGA) nanospheres for optimal therapeutic performance.

2. Materials and methods

2.1. Nanosphere treatments and electrophoretic mobility measurements

Monodispersed polystyrene nanospheres (2.5% w/v), 56 ± 4.9 nm in diameter, manufactured by Polysciences (Warrington, PA, USA), were purchased from Park Scientific (Northampton, UK) and this batch was used in all experiments. Poloxamine 908 was a gift from BASF Corp. (Mt. Olive, NJ, USA) and was used as received. Polystyrene nanospheres (0.8 mg) were incubated with different concentrations of poloxamine 908 or physiological concentration of bovine serum albumin (BSA), 40 mg/ml, or bovine IgG (10 mg/ml), in 10 mM McIlvaine buffer, pH 7.2, for 1 h at room temperature in a total volume of 1.0 ml. In some experiments, poloxamine (final concentration in the range of 0.5–5.0 mg/ml) was incubated with foetal calf serum (final concentration 50% v/v) for 15 min at room temperature. To this mixture, uncoated polystyrene nanospheres (0.8 mg) were added. The total volume was adjusted to 1.0 ml with 10 mM McIlvaine buffer and the pH was maintained between 7.0 and7.2 and the suspension was incubated for a period of 15 min. The electrophoretic mobility of all nanospheres was measured in 10 mM McIlvaine buffer, pH 7.2, using a Zetasizer 3000 system (Malvern Instruments, UK) [7]. Furthermore, the electrophoretic mobility of poloxamine-coated nanospheres was also evaluated in the presence of 50% v/v foetal calf serum. All electrophoretic mobility measurements were determined at a dielectric constant of 78.3, current of 1.4 mA, fluid refractive index of 1.35, cell field of 29.3 V/cm, conductivity of 0.7 mS/cm and viscosity of 0.89 cP [7]. In a further series of experiments, polystyrene nanospheres (0.8 mg) were incubated either in 50% v/v fresh or heat-denatured (56°C/30 min) foetal calf serum for 30 min at room temperature to allow for serum protein adsorption. Following such treatment, poloxamine 908 (5.0 mg) was added and the mixture was incubated for a further period of 15 min at room temperature. The total volume was kept to 1.0 ml and the pH was maintained in the range of 7.0–7.2. Following all incubations, the electrophoretic mobility of nanospheres was measured as described above.

2.2. Slide preparation, surface assessment and SPR studies

SPR experiments were performed using a prototype Kretchmann configuration instrument (Johnson and Johnson Orthoclinical Diagnostics, Chalfont St. Giles, UK), as described in detail elsewhere [10]. Briefly, SPR slides with silver dielectric layer (obtained from the same source) were modified prior to use with a layer of polystyrene. Prior to modification, the surfaces of the silver slides were analysed by atomic force microscopy (AFM) using a Digital Instruments Nanoscope (Digital Instruments, Cambridge, UK) in contact mode with silicon nitride tips. Slides were optically matched to the instrument prism using low-viscosity microscope immersion oil (Resolve, Stephens Scientific, Riverdale, NJ, USA). The analysis confirmed that the surfaces were smooth for a 5 μm scan. Polystyrene (280 000 Mw) was dissolved in high-performance liquid chromatography (HPLC) grade toluene (0.05% w/v) and spun-coated (2000 rpm) from a glass pipette (3 × 250 μl droplets) centrally, at a height of 5 mm above the spinning slide. The presence of the polystyrene coating was confirmed by contact angle measurements [11]. The sessile drop contact angle with 2 μl HPLC water droplet was 25.6 ± 1.2° and 89.5 ± 1.1° ($n = 6$) for silver SPR slides and polystyrene-coated SPR slides, respectively. Uniformity and thickness of the applied layers was investigated with scanning electron microscopy (SEM), on both flat surfaces and Z-axis-sectioned slides, as well as AFM. For SEM, polystyrene-coated slides were treated with palladium (2 nm) and visualised at 5 keV using a Joel 6310 scanning electron microscope (Joel, Welwyn Garden City, UK). The polystyrene coating was continuous and the thickness was estimated 95 ± 10 nm on Z-axis-sectioned slides. A minor degree of polystyrene surface roughness was detected by AFM for a 5 μm scan. Three flow channels were described on the surface of the SPR slide, with a thermally controlled head unit at 25 ± 0.1°C. Liquid flow across the surface of the slides was achieved using a Shimadzu LC-9A dual piston HPLC pump. The pump was connected in series with a Rheodyne 9125 all-PEEK™ MBB six way injection valve and 20 μl PEEK™ loop, through which all samples were introduced to the flow stream. Flow rate for all experiments was fixed at 10 μl/min. The slide surfaces were treated first with buffer and subsequently with either foetal calf serum (50% v/v) or heat-denatured (56°C/30 min) calf serum (50% v/v) or poloxamine (5 mg/ml) or poloxamine-treated calf serum (5 mg/ml poloxamine in 50% v/v serum). In some experiments the surface of the SPR slide was treated first with serum followed by immediate passage of poloxamine. Changes in the SPR angle are expressed as millidegree angle change (mda) and were recorded at 2 s intervals using instrument specific WinSPR V1.1 software with initial data analysis carried out using similarly specific Wbplot software [10].

2.3. Viscosity measurements

The viscosity of foetal calf serum (50% v/v) and poloxamine (5 mg/ml)-treated serum was measured at 25 ± 0.1°C by Thermo Haake RheoStress® 1 rheometer (Gebrüder HAAKE GmbH, Germany). A C60 cone-plate sensor system with 1° angle and a gap of 0.054 mm was used. Data analysis was carried out with the Thermo Haake software RheoWin Pro.

2.4. Biodistribution studies

Polystyrene nanoparticles were surface-labelled with Na[^{125}I] by a radiation-induced bonding of iodine as described in detail previously [12]. The labelled particles were cleaned by dialysis prior to surface modification with copolymers and serum [12]. Male Wistar rats (body weight, 150 ± 10 g, mean ± standard deviation) in groups of three were injected intravenously via one of the lateral tail veins with either uncoated or surface-modified [^{125}I]-radiolabelled polystyrene nanoparticles of 60 nm in diameter (0.8 mg polystyrene/150 g body weight). In some experiments particles were pre-incubated in various media (e.g. serum, BSA, IgG, serum and proteins pre-treated with poloxamine 908) prior to intravenous injection. In all experiments the total volume of injection was between 0.4 and 0.6 ml/animal. The circulatory activity of the nanoparticles was monitored by removal of the blood (20 μl) from the second tail vein at selected time intervals, and animals were killed 3 h post-nanosphere administration for the analysis of the radioactivity present in key macrophage-rich organs (liver and spleen). The whole liver and spleen were counted for radioactivity. To determine the amount of nanospheres in the blood, a total blood volume per rat of 7.5 ml/100 g of the body weight was assumed [3,5,6]. A correction factor for the blood content in the liver was determined as described earlier [13]. The results are presented as a percentage of the administered dose ± standard error of mean for each group.

Table 1
Electrophoretic mobility and body distribution of poloxamine-coated polystyrene nanoparticles

Poloxamine (mg/ml)	Mobility (10^{-8} m^2/V s)		Distribution at 3 h post-injection (% of injected dose)	
	In buffer	In serum	Liver+spleen	Blood
0	−3.94 ± 0.19	−2.66 ± 0.03	63.6 ± 2.0	1.0 ± 0.1
0.5	−0.99 ± 0.29	−1.09 ± 0.02	20.6 ± 3.7	48.2 ± 1.9
1.0	−0.82 ± 0.24	−0.92 ± 0.06	9.3 ± 1.6	63.4 ± 3.9
2.0	−0.76 ± 0.13	−0.88 ± 0.02	8.6 ± 1.4	61.2 ± 2.2
5.0	−0.73 ± 0.05	−0.90 ± 0.03	9.9 ± 1.2	58.6 ± 3.6

3. Results and discussion

As shown in Table 1 the electrophoretic mobility of nanospheres is changed dramatically following poloxamine treatment. The mobility values are similar, irrespective of the initial poloxamine concentration. Since the equilibrium poloxamine concentration in all incubations is at the plateau region of the adsorption isotherm, the polyoxyethylene chains of the poloxamine are expected to be closely packed and should exhibit a compressed 'brush-like' configuration that extends from the particle surface [7]. This steric protection by poloxamine 908 molecules explains the sharp drop in the electrophoretic mobility values [7,8]. In the presence of 50% v/v foetal calf serum the electrophoretic mobility values of poloxamine-coated nanoparticles are slightly changed, which could arise from some interaction between the adsorbed poloxamine molecules and serum proteins. This was also confirmed by SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis; data not shown) but the extent of protein adsorption was considerably lower than that of uncoated particles. This further supports the notion that the surface-adsorbed poloxamine molecules provide some resistance to protein adsorption and particle opsonisation [7,14]. Indeed, biodistribution studies also show that the sequestration of nanoparticles by scavengers of the reticuloendothelial system is dramatically suppressed by prior poloxamine coating, and again this is irrespective of the initial poloxamine concentration. As a result a large fraction of nanoparticles remains within the vasculature at the time of killing. The time of killing was chosen as 3 h for direct comparison with previous studies [5,7]; however, such surface-modified particles are known to circulate for longer periods of time [5].

In order to mimic the previous in vivo conditions [5], serum was pre-treated with relevant concentrations of poloxamine 908 (similar to those encountered in the systemic circulation) prior to nanoparticle addition. If poloxamine in serum can coat the surface of nanoparticles in a similar manner to incubations with no proteins, then close electrophoretic mobility values are expected. The results in Table 2 demonstrate that the incubation of uncoated nanoparticles with poloxamine-treated serum can indeed modify their body distribution and produce similar biodistribution as native poloxamine-coated nanoparticles. The effect is best seen with 2.0 mg poloxamine/ml of serum, although at lower concentrations poloxamine can still suppress particle retention in the liver and the spleen. Remarkably, the electrophoretic mobility of nanoparticles in poloxamine pre-treated serum (e.g. 2.0 and 5.0 mg poloxamine/ml of serum) also resembles that of poloxamine pre-coated nanoparticles in the serum. These observations further confirm that even in the presence of serum proteins poloxamine can equally modify the surface of nanoparticles. Previously, we [14] demonstrated that some unidentified serum proteins act synergistically with the effect of poloxamine coating to further suppress nanoparticle recognition by the liver macrophages in vitro. Therefore, it is plausible that poloxamine can form complexes with such proteins in serum with high affinity for the polystyrene surface. Such complexes may even displace some of the adsorbed proteins from the polystyrene surface. Conversely, serum proteins, particularly albu-

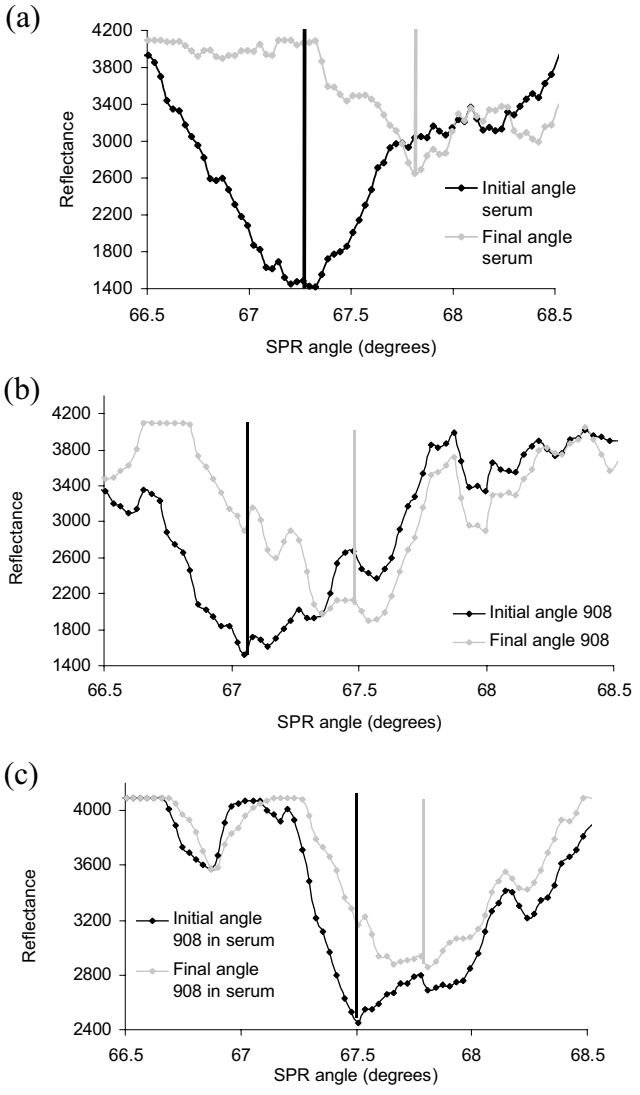

Fig. 1. The SPR curves from measurements of the reflected intensity as a function of angle. The curves are obtained following (a) injection of foetal calf serum (50% v/v), (b) poloxamine 908 (5 mg/ml) and (c) poloxamine-treated foetal calf serum.

Table 2
Electrophoretic mobility and body distribution of polystyrene nanoparticles following incubation in poloxamine-treated foetal calf serum

Poloxamine in serum (mg/ml)	Mobility (10^{-8} m^2/V s)	ζ potential (mV)	Distribution at 3 h post-injection (% of injected dose)	
			Liver+spleen	Blood
0	−2.66 ± 0.03	−33.6 ± 0.4	66.8 ± 3.6	1.3 ± 0.8
0.5	−1.26 ± 0.06	−15.9 ± 0.8	33.3 ± 1.5	33.6 ± 1.8
1.0	−1.11 ± 0.04	−14.0 ± 0.5	27.7 ± 1.2	38.1 ± 2.3
2.0	−0.88 ± 0.03	−11.1 ± 0.4	10.2 ± 1.3	61.4 ± 3.7
5.0	−0.81 ± 0.05	−10.2 ± 0.7	9.1 ± 3.1	63.2 ± 5.2

min and IgG, are known to readily coat the surface of polystyrene nanospheres [7,15–18]. Hence, the mechanism of poloxamine coating may simply arise from hydrophobic interaction between the polyoxypropylene segments of the poloxamine and surface-adsorbed proteins. Since albumin is the most abundant protein in serum, its role in surface modification of nanoparticles by poloxamine was investigated. The results in Table 3 demonstrate a sharp drop in the electrophoretic mobility of BSA-coated nanoparticles following the addition of poloxamine 908. Interestingly, this also results in generation of nanoparticles with long circulating behaviour. Furthermore, the mobility of nanoparticles is further reduced when incubated in poloxamine-treated BSA. Again, the resultant nanoparticles exhibit stealth behaviour when injected intravenously in a manner similar to that of poloxamine-treated serum incubations. When BSA was replaced by bovine IgG (10 mg/ml), poloxamine 908 failed to significantly alter the electrophoretic mobility of nanoparticles; subsequently these pre-treated nanoparticles were sequestered rapidly by the liver and the spleen following intravenous injection (data not shown). Therefore, a role for BSA–poloxamine complexes in surface modification of nanoparticles seems probable.

In order to understand how poloxamine 908 in the presence of serum proteins can modify a polystyrene surface SPR experiments were performed. SPR is an evanescent wave technique, which allows the real-time monitoring of interactions with surfaces [9]. The wave profile is dependent on the thickness and refractive index of the layer above the polystyrene film [19]. Changes in this layer require a new angle of incident light to initiate resonance [19]. Therefore, change in the SPR angle with time is measured using a photodiode detector, along with the depth and shape of the 'dip angle'. SPR results are presented as time-resolved traces as well as in the form of the reflectivity versus angle curves [10,19]. Serum (50% v/v) was found to adsorb strongly to a polystyrene SPR-coated slide surface with a value of 635 ± 35 mda and an initial slope of 0.48 mda/s. A second injection of serum had no further effect. The SPR reflectance curve, as the initial and final scans during the adsorption process, confirmed the presence of the adsorbed layer of serum proteins on the polystyrene surface (Fig. 1a). The significant shift in the baseline SPR angle from 67.25° to 67.9° with a concomitant flattening of the profile suggests the adsorption of a relatively thick layer of proteinaceous material [19]. This experiment mimicked the condition where polystyrene nanoparticles are in continuous contact with serum. In the case of poloxamine 908 (Fig. 1b) the adsorption profile generated 0.5° shift in baseline angle with a small decrease in reflectivity, hence suggesting a presence of a thin poloxamine layer on the sensor surface. Again, the addition of poloxamine to serum (Fig. 1c) resulted in a significant shift in angle (0.3°) with a concomitant flattening of the curve. In both cases of poloxamine and poloxamine-treated serum, the pre-existing small secondary minimas suggest some underlying inhomogeneous roughness on the polystyrene-coated slide [19]. Interestingly, the adsorption profiles for poloxamine and poloxamine-treated serum appeared similar and were 78 ± 3 and 97 ± 5 mda, respectively. The similarity of the values suggests that in the case of poloxamine-treated serum, poloxamine and/or poloxamine–protein complexes are adsorbed on to the polystyrene surface. However, the rate of adsorption is ~ 3 times higher with poloxamine-treated serum when compared to the passage of serum and poloxamine under identical flow conditions (1.19 vs. 0.42 mda/s). The rate of adsorption is known to be dependent on mass transport and intrinsic kinetics [20,21]. However, the final concentrations of both serum and poloxamine were constant in all systems, and furthermore there was no significant change in serum viscosity following poloxamine addition. On the basis of these observations the mass transport can not explain the higher rate of adsorption seen following the passage of poloxamine-treated serum. We therefore conclude that poloxamine in serum can form macromolecular complexes and these presumably have higher affinity for adsorption to a polystyrene surface.

Previously, we also reported an intriguing observation in the body distribution of intravenously injected polystyrene nanospheres that were pre-coated first with either rat serum or heat-denatured rat serum then followed by addition of poloxamine [5]. The results in Table 3 also confirm these observations with foetal calf serum. Again, poloxamine is able to exert some degree of protection to nanospheres that were pre-coated with serum, but not with heated serum, against hepatic sequestration. In addition, we also show that poloxamine can coat the surface of serum pre-treated nanoparticles since there is a dramatic drop in the electrophoretic mobility of nanospheres when compared to serum-coated nanoparticles. This stealth property may arise simply by adsorption of poloxamine to the surface-adsorbed proteins or as a result of protein displacement from the surface by poloxamine, or both. Conversely, the electrophoretic mobility measurements confirm that poloxamine is unable to adsorb efficiently on to surface of particles that were pre-treated with heated serum.

Table 3
Electrophoretic mobility and body distribution of BSA- and serum-pre-treated polystyrene nanoparticles following poloxamine addition

	Incubation protocol	Mobility (10^{-8} m^2/V s)	ζ Potential (mV)	Distribution at 3 h post-injection (% of injected dose)	
				Liver+spleen	Blood
A	BSA (40 mg/ml)	-2.52 ± 0.11	-31.8 ± 1.4	63.3 ± 1.2	0.8 ± 0.2
B	908 (5 mg/ml)	-0.76 ± 0.07	-9.5 ± 1.5	8.9 ± 2.2	62.3 ± 4.8
C	As (A) then poloxamine added (5 mg/ml)	-1.05 ± 0.11	-13.2 ± 1.4	20.3 ± 4.9	33.8 ± 5.8
D	Poloxamine (5 mg/ml)-treated BSA (40 mg/ml)	-0.46 ± 0.10	-5.8 ± 1.3	9.7 ± 2.5	58.1 ± 2.3
E	Heat-treated BSA (40 mg/ml)	-1.97 ± 0.37	-24.9 ± 4.6	60.1 ± 5.2	1.2 ± 0.4
F	As (E) then poloxamine added (5 mg/ml)	-0.96 ± 0.07	-12.2 ± 0.8	26.4 ± 3.9	33.2 ± 2.3
G	Poloxamine (5 mg/ml)-treated heated BSA (40 mg/ml)	-0.62 ± 0.23	-7.9 ± 3.1	9.7 ± 3.1	67.2 ± 5.2
H	Serum (50% v/v)	-2.38 ± 0.11	-30.2 ± 1.3	65.2 ± 3.6	2.3 ± 1.7
I	As (H) then poloxamine added (5 mg/ml)	-1.44 ± 0.18	-18.2 ± 2.2	28.7 ± 4.2	35.6 ± 3.4
J	Heat-denatured serum (50% v/v)	-2.43 ± 0.04	-30.7 ± 0.5	59.7 ± 4.5	2.7 ± 0.9
K	As (J) then poloxamine added (5 mg/ml)	-2.05 ± 0.07	-25.9 ± 1.0	60.6 ± 2.9	1.8 ± 0.6

In order to confirm and determine the mode of poloxamine adsorption on to a protein-coated surface, SPR studies were conducted. To mimic the in vivo conditions, the polystyrene-coated SPR slide was first enriched with serum proteins followed by immediate passage of poloxamine and then serum again. The SPR trace in Fig. 2 clearly indicates that poloxamine 908 does not desorb serum proteins from the polystyrene surface. Interestingly, the poloxamine adsorbs to the protein-coated surface as evident from a further increase of 20 ± 2 mda and an initial slope of 0.06 mda/s. Furthermore, subsequent passage of serum induces a transient intermediate change (an intermittent protein adsorption/desorption process) but the final SPR angle value remains unchanged. These observations may be indicative of the underlying ex vivo coating mechanisms and their inherent stability under in vivo shear flow and are in agreement with electrophoretic mobility and biodistribution studies. From these observations we also speculate the possibility of some surface re-arrangements with poloxamine-pre-coated particles on contact with serum or plasma. The validity of the SPR approach is further confirmed with the passage of heat-denatured serum followed by poloxamine washing. The SPR trace in Fig. 3 confirms modification of the polystyrene surface with heat-denatured serum with a final change of 35 ± 4 mda. In contrast to serum, heat-denatured serum seems to adsorb poorly on to the SPR slide. However, subsequent passage of poloxamine shows very little change in SPR angle shift and therefore is in agreement with both the electrophoretic mobility and biodistribution studies. These results indicate that the nature and conformation of

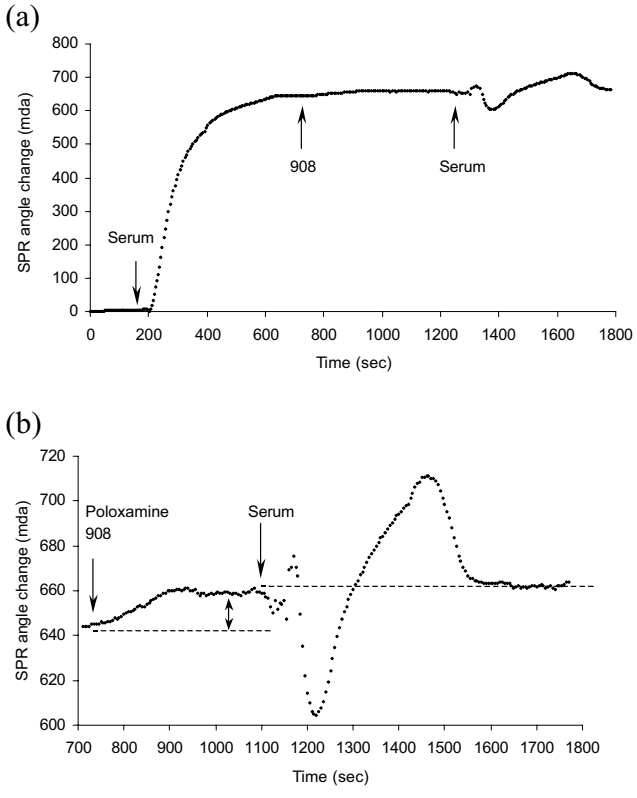

Fig. 2. Sensorgram of SPR angle change with time for the cumulative injection of foetal calf serum (50% v/v) followed by poloxamine 908 (5 mg/ml) and then serum again (arrows indicate injection points). The lower trace is an enlarged portion showing SPR angle changes following the injection of poloxamine 908.

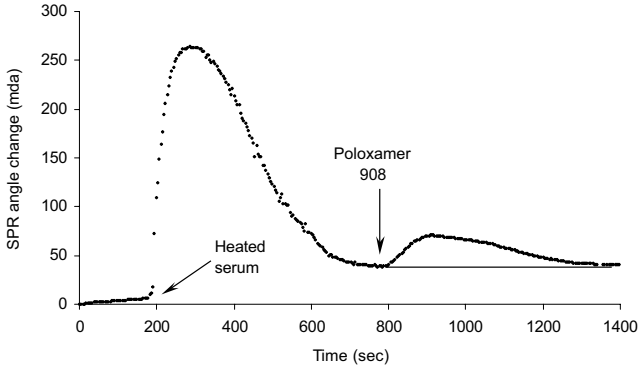

Fig. 3. Sensorgram of SPR angle change with time for the cumulative injection of heat-denatured (56°C/30 min) foetal calf serum (50% v/v) followed by poloxamine 908 (5 mg/ml).

pre-adsorbed proteins on the surface of nanospheres can control subsequent interactions with poloxamine and/or poloxamine–protein complexes. Due to the observed differences between fresh and heat-denatured sera, one may consider a regulatory role for surface-deposited complement and complement regulatory proteins in subsequent poloxamine binding.

On the basis of these observations we also evaluated the role of serum albumin after heating at 56°C/30 min on electrophoretic mobility and body distribution of polystyrene nanospheres in the presence of poloxamine 908. The results (Table 3) are strikingly similar to those of native albumin and further support the involvement of other serum proteins in preventing the adsorption of poloxamine on to the surface of nanoparticles pre-treated with heat-denatured serum.

In conclusion, electrophoretic mobility measurements and SPR studies have effectively demonstrated plausible mechanisms for surface modification of model polystyrene lattices by poloxamine 908 in the presence of serum proteins. The SPR approach can perhaps be translated to re-evaluate the interaction between block copolymers and biodegradable polymers used for nanosphere manufacturing (e.g. PLGA) as well as model bilayers. Such investigations may lead to novel approaches for surface engineering and development of stealth biodegradable carriers as well as to understand how block copolymers can span through biological membranes [2,3,22].

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