Polystyrene-Poly (Ethylene Glycol) (PS-PEG2000) Particles as Model Systems for Site Specific Drug Delivery. 2. The Effect of PEG Surface Density on the *in Vitro* Cell Interaction and *in Vivo* Biodistribution

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The effect of differing densities of poly (ethylene glycol-2000) (PEG2000) at the particle surface of polystyrene-poly (ethylene glycol-2000) (PS-PEG2000) particles was assessed in terms of hydrophobic interaction chromatography (HIC) and the *in vitro* and *in vivo* behaviour of the particles. The particles, with different surface densities of PEG, were prepared by varying the copolymerizing reaction of styrene with a PEG macromonomer. There is a clear relationship between the surface density of PEG as determined by X-ray photoelectron spectroscopy and surface hydrophobicity as assessed by hydrophobic interaction chromatography (HIC). Similarly, the interaction of the particles with non-parenchymal liver cells in *in vitro* studies was shown to decrease as the surface density of PEG increases. The *in vivo* study investigating the biodistribution of the PS-PEG particles after intravenous injection into rats reveals that a relationship exists between the surface density of PEG and the extent to which the particles remain in the circulation, avoiding recognition by the reticuloendothelial system. Particles with the higher surface densities show increased circulatory times which compared well with data for particles prepared with the surface adsorbed PEO-PPO block copolymer, Poloxamine 908.

KEY WORDS: polystyrene; polyethylene glycol; particles; rats; non-parenchymal liver cells; blood clearance.

INTRODUCTION

Microspheres produced from polymers can serve as parenteral carrier systems for site-specific delivery of drugs due to the versatility of the types of microspheres which may be produced in terms of size, surface charge, surface hydrophobicity/hydrophilicity and surface composition (1). However, like other colloidal carriers intended for drug delivery, such polymeric microspheres, after intravenous (iv) administration, are normally retained by the reticuloendothelial system (RES), comprising mainly the Kupffer cells of the liver and the macrophages of the spleen. Thus, the liver constitutes a major obstacle to the efficient targeting of colloidal carriers to other organs of the RES, the spleen and bone marrow, and to other target sites outside the RES, such as the circulation and the endothelial lining, tumours and sites of inflammation (2).

The presence of poly (ethylene oxide), PEO, on the surface of polymers used for medical devices confers platelet-, thrombo- and protein resistance (3,4,5). Illum and coworkers were the first to use polyethylene oxide (PEO) containing poloxamer and poloxamine block co-polymers for modification of the surface characteristics of colloidal drug carriers (6,7). They demonstrated for model polystyrene microspheres that the presence of surface orientated PEO decreased the extent of protein adsorption (opsonisation) and phagocytosis of the microsphere by non-parenchymal liver cells *in vitro*. Further, after intravenous administration specific polymer coatings were able to prevent sequestration of the particles by the liver and spleen and permit interaction with the endothelial cells of the bone marrow in rabbits (8). The copolymer adsorbs from solution onto the hydrophobic surface of the polystyrene particle by way of the hydrophobic poly(propylene oxide) (PPO) domains, with the hydrophilic PEO chains extending into the surrounding aqueous phase, thereby creating a steric barrier preventing (or decreasing) the opsonisation process and the interaction with phagocytic cells.

The PEO moiety is now being utilized to modify the surface of other drug carrier systems including liposomes, in order to minimise protein uptake and recognition by the macrophages of the liver and spleen. Harper *et al.* (9) found that the uptake by rat Kupffer cells of polystyrene microspheres stabilised with covalently grafted PEO was lower or equivalent to that of control polystyrene microspheres stabilised by the adsorption of Poloxamer 238 or methoxy-PEO. For liposomes, Klibanov *et al.* (10) have shown in the rat model, that incorporating a PEO-phosphatidylethanolamine (PEO-PE) of molecular weight 2,000 into liposomes increased clearance half times from less than 30 minutes for control liposomes to 5 hours for PEO-PE modified liposomes. Blume and Cevc (11) have shown increased circulation times of liposomes with covalently attached PE-PEG5000 compared to control liposomes. Senior *et al.* (12) covalently attached monomethoxypoly(ethylene glycol) (MPEG5000) via dipalmitoylphosphatidylethanolamine to preformed liposomes and found that the liposomes were cleared from the blood circulation up to 30% more slowly than liposomes without MPEG5000. Various workers have investigated the effects of varying PEO chain length on the particle clearance *in vivo* (13,14,15). Liposomes containing PEO-distearoylphosphatidylethanolamine conjugates (PEG-PE) of molecular weight 120 were shown to have the lowest blood levels post dose compared with levels for PEG-PE conjugates of molecular weight 1900. Extending the PEG chain length to 5,000 D had no additional suppression effect on RES uptake (13). Maruyama *et al.* (15) found that liposomes coated with PEG of molecular weight 1000–2000 D had the longest circulation times, whereas Mori *et al.* (14) found that a PEG chain of 5000 D led to the longest circulation time.

The precise surface characteristics of colloidal carriers required for successful RES avoidance and specific targeting have been poorly defined, particularly in terms of the surface conformation and density of PEG chains required. We have prepared model spherical polystyrene particles with covalently bound PEG chains by copolymerising styrene with

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a PEG macromonomer. The composition of the copolymerisation reaction mixture was varied in order to prepare particles with varying amounts of macromonomer on the particle surface (16). Surface chemical analysis measurements using X-ray photoelectron spectroscopy (XPS) indicated an increased surface density of PEG with an increase in the ratio of the bulk content of the macromonomer, which was accompanied by a decrease in particle size (16). The aim of the present work was therefore to investigate the structure-activity relationship between the surface composition of the polystyrene particles with surface immobilised poly(ethylene) chains (MW 2000 D), denoted by PS-PEG2000, and their biological behaviour in terms of *in vitro* interaction with non-parenchymal liver cells and in vivo biodistribution in rats.

In the *in vitro* and *in vivo* studies described, uncoated polystyrene microspheres and those coated with Poloxamine 908 were used as controls. Poloxamine 908 was chosen, as it has been established that it has the ability to produce a dramatic increase in circulating levels of coated particles, compared to uncoated particles in the rabbit (17) and in the rat animal models (18).

EXPERIMENTAL

Materials

Polystyrene microspheres of particle size 60 nm ± 9.1% (8.4% w/v), 100 nm ± 5.3% (8.4% w/v) and 156 nm ± 4.2% (9.9% w/v) were obtained from Interfacial Dynamics Corporation, Portland, Oregan, U.S.A. Poloxamine 908 (BASF Corporation, Parsippany, New Jersey, U.S.A.) was used as purchased. Iodine for radiolabelling was obtained from Amersham International, UK, in the form of Nal-125. Water used in the preparation of samples was double distilled. All other materials were obtained from Sigma, unless otherwise stated.

Preparation of PS-PEG2000 particles

The surfactant-free emulsion copolymerisation of styrene (Aldrich Chemicals, Dorset, U.K. with the macromonomer methoxypoly(ethylene glycol) acrylate (ICI Paints, Slough, U.K.) of molecular weight 2000 D (PEG2000) was based on a method used by Ottewill and Satgurunathan (19) using potassium persulfate (Analar grade, BDH Chemicals, Ltd., Poole, U.K.) as the initiator, as detailed by Brindley *et al.* (20). By varying the weight of the macromonomer added to the copolymerisation reaction, a range of (PS-PEG2000) polystyrene particles with different amounts of surface PEG and hence different surface compositions were produced (20), as shown in Table I. The particles were filtered and dialysed exhaustively for 14 days as described by Brindley *et al* (20).

Particle characterisation

The methods used for the characterisation of the particles have previously been described (20) and a brief outline is given here. Particle sizes were analysed in double distilled water at 25°C (n = 10) using photon correlation spectroscopy (PCS) (Malvern Instruments, Malvern, U.K.). The effective particle surface charge (zeta potential) of the series of PS-PEG2000 particles were determined in constant ionic strength (0.001M) McIlvaine's type buffer (n = 4) using a Zetasizer 4 (Malvern Instruments, Malvern, U.K.).

Table I. Input molar ratios styrene:macromonomer, % ether carbon (% C – O) level on the particle surface, particle size and zeta potential.

Particle	Input molar ratio styrene: macromonomer	% C – O	Size (nm)	Zeta potential
PS	—	4	60.7	−43.8
PS-908	—	20	74.0	−1.9
PS-PEG2000a	1:0.004	8	155.3	−18.2
PS-PEG2000b	1:0.025	15	101.5	−5.5
PS-PEG2000c	1:1.053	41	66.9	−2.0
PS-PEG2000d	1:1.061	51	60.7	−1.2

The surface hydrophobicity/hydrophilicity of the particles was assessed by hydrophobic interaction chromatography (HIC). A butyl agarose column (Sigma Chemical Company, Poole, U.K.) of bed volume 2.5 ml, was equilibrated with 5–10 bed volumes of 0.2 M phosphate buffer (pH 6.8) containing 0.2 M sodium chloride (elution buffer). Identical weights of particles (equivalent to 0.15 ml of a 0.3% w/v latex) were applied to the column in each instance to minimise variations in elution behaviour due to column loading. Particles were eluted with the buffer, the eluting particles being detected by UV absorption at 380 nm using a flow through cell in a Cecil CE272 UV spectrometer (Cecil Instruments, Cambridge, U.K.) connected to a Metrohm Labograph E478 chart recorder (Metrohm, Switzerland). The column was then washed with sufficient buffer containing 0.1% Triton X-100 (wash buffer) (octylphenoxyethoxyethanol, Sigma Chemical Company, Poole, U.K.) to recover any particles that did not elute with the buffer alone. The areas under the curves (AUC) for the elution and wash peaks were determined by a cut and weight method and the HIC ratio was calculated as follows:

$$\text{HIC ratio} = \frac{\text{AUC elution peak}}{\text{AUC wash peak}} \times 100$$

Thus, an increase in the HIC ratio denotes a greater hydrophilicity of the particle.

Radiolabelling of PS and PS-PEG2000 particles.

For *in vitro* and *in vivo* studies PS and PS-PEG2000 particles were surface labelled with Nal-125 using a procedure based on that of Huh *et al.* (21). 2.5 μl of Nal-125 solution (3.7 GBq/ml at the activity reference date, Amersham International, U.K.) was mixed with 1 ml of the particle dispersions (2% w/v) and exposed to a Cesium-137 source (activity 5.54×10^4 ± 0.67% Rads per hour on 19/1/90) for 48 hours. After irradiation, excess free iodine was removed by dialysis (Spectropor® dialysis tubing, molecular weight cut off 100 kD) against double distilled water, for three days with daily changes of the dialysis medium. The labelling efficiency was found to decrease with increasing

amount of surface PEG being 10% for the PS-PEG2000a and 2% for the PS-PEG2000d particles.

Preparation of Poloxamine 908 coated polystyrene particles (PS-908)

Radiolabelled polystyrene particles were coated by mixing a dispersion of the particles (2% w/v) with an equal volume of an aqueous solution of Poloxamine 908 (2% w/v) and incubating at room temperature overnight. The coated particles were not separated from free polymer.

Interaction of non-parenchymal liver cells with particles.

Non-parenchymal rat liver cells were isolated as described by Moghimi *et al.* (22). Briefly, male Wistar rats (150–250 g) were anaesthetised by intraperitoneal injection of 0.2–0.3 ml of 60 mg/ml pentobarbitone (Sagatal, May and Baker, Dagenham, UK.). The livers were perfused with 100 ml calcium and magnesium free Hanks' balanced salt solution (HBSS) supplemented with 0.033% collagenase type IV and 0.033% calcium chloride at a flow rate of 3.2 ml/min until the liver became gelatinous. The livers were removed and the cell suspension passed through a nylon gauze. The majority of hepatocytes were removed by low speed centrifugation (200 rpm, 3 min.). The supernatants were retained and centrifuged at 2,500 rpm for 5 minutes. The pellet was resuspended in 10 ml HBSS. Two aliquots were overlayed onto two 7 ml volumes of Nycodenz density gradient (Nycomed, Norway). The tubes were mixed by inversion and 1 ml of HBSS overlayed onto each gradient. Separation was achieved by centrifugation at 2,420 rpm for 15 minutes. The cells (primarily Kupffer (70–80%) and endothelial cells (10–15%)) (22) were collected, washed twice with HBSS and were resuspended to a concentration of 2×10^6 cells/ml for use in *in vitro* particle-cell interaction experiments. Cell viability (greater than 80%) were determined using trypan blue exclusion.

Non-parenchymal liver cells (1 ml) were incubated in triplicate with the various radiolabelled particles (45 μg), given in Table I, for 1 h at 37°C in the absence and presence of fresh rat serum (5% w/v) in a total volume of 2.0 ml of HBSS. After incubation the particle suspensions were placed on ice to stop the reaction and were centrifuged at 2,500 rpm for 5 minutes. The supernatant was discarded and the cell pellet resuspended and washed twice by centrifugation. The cell associated activity was counted using a gamma counter (LKB 182 Compugamma CS, LKB Wallac, Finland.). The ratio of mass of particles to the number of cells were kept constant in all experiments. The cell associated radioactivity (uptake) is expressed as the percentage activity of the initial dose incubated, and refers to both particles internalised and surface bound.

In a further experiment the effect of the size of the PS-PEG2000 particles on uptake by non-parenchymal liver cells was studied. 2×10^6 cells were incubated in triplicate with radiolabelled polystyrene particles 60 nm, 100 nm and 156 nm in diameter (45 μg) for 1 h at 37°C in a total volume of 2.0 ml HBSS. In this experiment the fraction of the particles internalised as opposed to adsorbed was measured by trypsinisation of cells at 37°C (23).

Biodistribution in rats

For each system, three Wistar rats (150 ± 10 g) were injected via the tail vein with 100 μl of a 0.3% w/v suspension of either PS-PEG2000a, b, d or polystyrene particles (60 nm) uncoated (PS) or coated with Poloxamine 908 (PS-908). 20 μl samples of blood were taken from the tail vein at various time intervals (1, 3, 5 and 24 hours). The rats were sacrificed after 24 hours using 0.3 ml pentobarbitone solution (60 mg/ml) and the liver, spleen, lungs, kidney, one femur (left hind leg) and the thyroid were removed. The organs and blood associated activity were counted using a gamma counter (LKB 182 Compugamma CS, LKB Wallac, Finland). A total blood volume per rat of 7.5% of body weight was assumed (24). The results are expressed as a percentage of the injected dose and are a mean of the three rats ± standard deviation (S.D.).

RESULTS

Surface characterisation studies

The size of the PS-PEG2000 particles was found to decrease with increasing bulk PEG macromonomer employed in the polymerisation reaction from 155.3 nm for PS-PEG2000a to 60.7 nm for PS-PEG2000d particles (Table I) (20). In all cases, the polydispersity indices of the particles were in the range 0.05–0.07, indicating a near monodisperse particle nature (16). The reduction in particle size with increasing amounts of macromonomer arises due to the increased PEG surface density stabilising the formation of smaller particles. The zeta potential of the PS-PEG2000 particles showed a dramatic reduction in values to near zero (Table I). This is due to the fact that the increasing levels of surface PEG chains shift the shear plane out into the medium which will manifest itself as a decrease in zeta potential (16).

The surface chemical analysis of the range of PS-PEG2000a–d particles was previously performed by XPS (20). In this paper it was shown that the high resolution C1s spectrum from the XPS analysis allows the determination of the level of carbon atoms in a hydrocarbon environment ie $\underline{C}-C/\underline{C}-H$ and ether carbon environment, $\underline{C}-O$ for these particles. The $\underline{C}-C/\underline{C}-H$ environment will arise entirely from the polystyrene structure with only a small contribution of $\underline{C}-O$ arising from the carbon adjacent to sulphate end groups. In contrast, the PEG monomer gives rise to only the $\underline{C}-O$ ether environment $[-O-(CH_2-CH_2-O)_n-]-$. Therefore, the level of $\underline{C}-O$ in C1s spectra for the range of PS-PEG2000 particles is employed to determine the surface PEG presence. For clarification these data from Brindley et al. (20) are given in Table I together with other particle characteristics. It may be seen that there is a steady increase in the level of $\underline{C}-O$ with increasing bulk macromonomer content reaching over 50% for PS-PEG2000d. This increase in PEG surface levels correlates well with the noted changes in size, charge and hydrophilicity with bulk macromonomer content.

The surface hydrophobicity/hydrophilicity of the PS-PEG2000 particles with different surface PEG density was compared to the hydrophobicity of a commercially available homopolymer polystyrene particle (PS). The HIC ratios are presented in Table II. It is evident that, with the introduction of low concentrations of the PEG2000 macromonomer into

Table II. HIC ratios for PS, PS-908 and PS-PEG2000 particles

Particle	HIC ratio
PS	0.00
PS-908	1.272
PS-PEG2000a	UNSTABLE
PS-PEG2000b	0.12
PS-PEG2000d	0.24

the particle surface layers and the resultant low density of surface PEG, the hydrophilicity of the surface of the PS-PEG2000b is increased only slightly. However, as the proportion of macromonomer in the polymerisation reaction is increased and thereby the surface density of PEG, the HIC ratio is raised correspondingly. Thus, with the increasing macromonomer levels in the polymerisation reaction, a more extensive coverage of the particle surfaces with PEG2000 is implied which, in turn, increases the hydrophilic nature of the resultant colloidal particles. The PS-PEG2000d particles with the highest density of PEG surprisingly show a HIC ratio lower than that of PS-908. A likely explanation for this phenomenon is the difference in surface conformation of the PEG chains when adsorbed to the surface of the particles as opposed to terminal grafting.

In vitro cell interaction studies.

A study was performed to investigate whether the difference in size seen for PS-PEG2000 particles with high and low surface PEG density would influence the uptake by non-parenchymal liver cells. The incubation of the cells with uncoated PS particles with diameters of 60, 100 and 150 nm showed no significant increase in uptake with increasing particle size (Table III). The study was performed without the presence of serum in order to see the maximal size effect, if any. Trypsinisation of the cells after separation showed that about 55% of the particles were internalised by the cells after 1 hour incubation and that particle size did not influence this process.

The *in vitro* cell interaction studies of the PS-PEG2000 particles demonstrated a clear relationship between the surface density of PEG (% C–O) and the ability of these particles to avoid recognition by isolated non-parenchymal liver cells (Table IV). As the surface density of PEG of these particles increased, a dramatic reduction in their uptake by the cells was seen in the absence of serum (Figure 1). The uptake of PS-PEG2000 particles with 15% (b), 41% (c) and 51% (d) C–O at the surface was significantly decreased compared to uncoated polystyrene particles. The PS-PEG2000c and PS-PEG2000d particles showed similar cell uptake to that seen for the PS-908. Increasing the % C–O from 41% to 51% had no significant effect on the macrophage uptake. In the presence of serum, the uptake of all systems (except PS-PEG2000d) was significantly reduced compared to values obtained in the absence of serum. The extent of the reduction for PS-PEG2000 particles was less significant as the surface density of PEG on the particles increased.

Table III. The effect of the different size of PS particles on *in-vitro* non-parenchymal liver cell uptake in the absence of serum.

Particle	Incubation 1h/37°C	Incubation 1h/37°C + trypsinisation	% internalised
60 nm	20.2 ± 4.7%	11.7 ± 1.5%	57.9
100 nm	26.8 ± 2.1%	14.8 ± 3.1%	55.2
156 nm	23.7 ± 2.2%	13.0 ± 1.1%	54.9

Table IV. The *in-vitro* uptake of PS-PEG2000 particles and PS particles uncoated and coated with Poloxamine 908 by non-parenchymal liver cells.

Particle	Incubation 1h/37°C No serum	Incubation 1h/37°C 5% v/v serum
PS	27.9 ± 5.23%	1.26 ± 0.03%
PS-908	1.13 ± 0.08%	0.39 ± 0.03%
PS-PEG2000a	21.7 ± 3.7%	1.3 ± 0.25%
PS-PEG2000b	3.4 ± 0.73%	0.51 ± 0.15%
PS-PEG2000c	0.85 ± 0.14%	0.64 ± 0.07%
PS-PEG2000d	0.84 ± 0.19%	0.96 ± 0.05%

In vivo studies

The results presented in Figure 2 show significant decreases in uptake by the liver of the PS-PEG2000 particles as the surface density of PEG increases. The liver uptake of the PS-PEG2000a particles with the least amount of PEG at the surface is 32.2 ± 3.8% and as the amount of PEG at the surface increases the liver uptake decreases, the uptake for the PS-PEG2000b particles and the PS-PEG2000d being 13.1 ± 1.4% and 7.9 ± 0.27%, respectively. For the uptake in the spleen the levels for all PS-PEG2000 particles were generally higher than for the control PS particles.

The decrease in uptake by the liver is accompanied by increases in circulating blood levels with the percentage activity in blood at 24 hours post injection being 1.1 ± 0.21%,

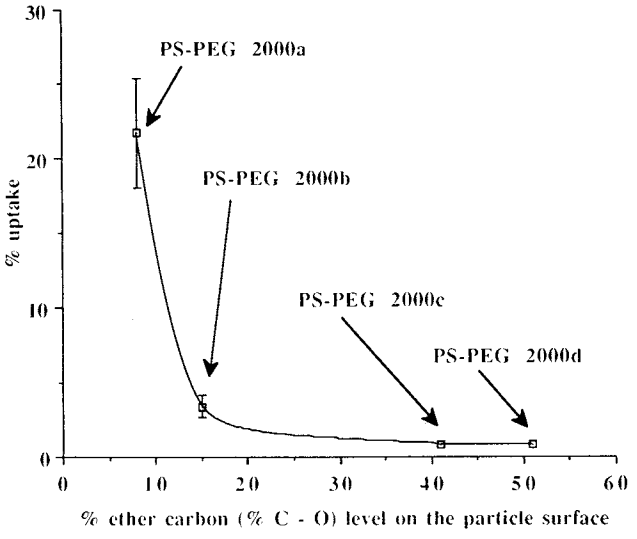

Figure 1. The relationship between surface density of PEG (% C–O) on the PS-PEG2000 particle and the *in vitro* uptake of these particles by isolated non-parenchymal liver cells of the rat in the absence of serum.

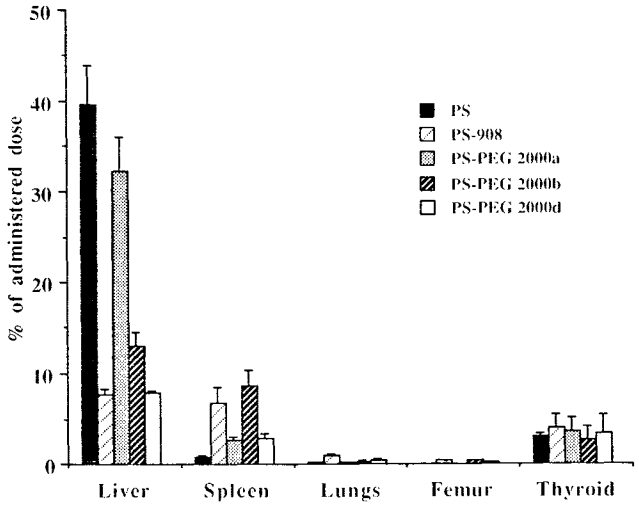

Figure 2. Biodistribution profiles for PS, PS-908 and PS-PEG2000 particles in the rat 24 hours post intravenous administration.

4.6 ± 3.3% and 15.8 ± 1.5% for PS-PEG2000a, b and d, respectively (Fig. 3). The organ distribution and circulation clearance time of the PS-PEG2000d particles is similar to that seen for PS-908. The uptake by the liver is significantly reduced for PS-908 particles compared to the corresponding value for PS particles. This reduction in uptake of particles by the RES cells of the liver results in a corresponding increase in circulatory level of PS-908 particles in the blood (% activity in blood at 24 hours post injection, PS: 0.5 ± 0.078%, PS-908: 13.2 ± 2.6%) (Figure 3). A similar pattern of biodistribution has been observed in rabbits with the circulation times similar to those obtained previously for PS-908 observed for the PS-PEG2000d particles whereas the PS-PEG2000a particles were taken up avidly by the liver (data not shown).

DISCUSSION

It has previously been shown that the interaction of sterically stabilised polystyrene particles with macrophages *in vitro* is highly dependent on the thickness of the adsorbed layer of a range of PEO/PPO block copolymers (17,25). In general terms, the greater the adsorbed layer thickness or the longer the PEO chain of the polymer the lower the relative phagocytic index. In the present studies we have investigated the effect of increasing the surface density of PEG, of constant chain length, on the uptake by non-parenchymal liver cells. It is demonstrated that the surface density of the PEO is an important factor in determining the extent of the particle-cell interaction. Previously, Harper *et al.* (9) produced similar PEG2000 grafted polystyrene particles (1 μm in diameter) and showed that the uptake by isolated Kupffer cells was lower or equivalent to that of control polystyrene particles stabilised by the adsorption of Poloxamer 238 which has a PEO chain length of 104 oxyethylene units. It was also shown that this effect could be enhanced synergistically by the additional adsorption of Poloxamer 238 on the surface of the PEG grafted particles. It is possible that this increased effect was due to the increased density of PEG chains on the surface. However, PS-PEG2000 particles with only one density of PEG were produced and no indication of the surface density of PEG from XPS studies was given.

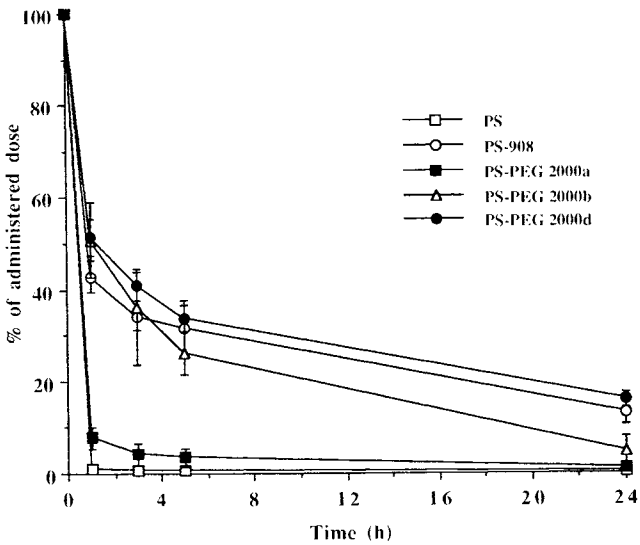

Figure 3. Blood elimination curves for PS, PS-908 and PS-PEG2000 particles in the rat.

The results from the present studies on isolated non-parenchymal liver cells were supported by the *in vivo* biodistribution studies in rats. Here, the sequestration by the liver was directly related to the surface density of the PEG with the highest uptake seen for the PS-PEG2000a particles with the lowest surface PEG density, the uptake being only a fraction lower than that seen for uncoated polystyrene particles. The PS-PEG2000d particles with the highest surface PEG density were taken up to a degree similar to the PS-908 particles. Twenty four hours after the injection, the blood levels of the PS-PEG2000 particles with the highest densities of PEG on the surface were still elevated in line with the PS-908 particles. The spleen levels were generally shown to be higher for particles with surface exposed PEG. This phenomenon has previously been described by Moghimi *et al.* (26) and is due to a mechanical filtration process with the capture increasing with increasing particle size.

It has previously been suggested by various authors that the reason for the decreased cell-particle interaction and the low *in vivo* liver uptake of particles with PEG present on the surface is due to the creation of a steric barrier that prevents or decreases the adsorption of plasma proteins on the surface of the particles; the so-called opsonisation process (7,27,28). The adsorption of proteins was found to be highly dependent on the PEG chain length (28,29) with less protein adsorbed to particles coated with polymers with longer PEG chains. However, lately it has been suggested that it is not only the reduction in the adsorption of plasma components but also the selective uptake of certain plasma components, that act as dysopsonins, that prevent the recognition of particles by macrophages (27). Thus, the mechanism controlling particle uptake by macrophages, and hence the biodistribution, is suggested to be controlled by a balance of competing opsonic and dysopsonic processes and factors. This theory is supported by the data in Table IV which show that in the presence of serum the uptake by non-parenchymal liver cells of the uncoated polystyrene particles is reduced from 27.9% to 1.26%. Further, it can also be seen that the low uptake of

the particles with PEG on their surface is further reduced by the addition of serum. This suggests that a dysopsonic compound present in the serum is taken up on the sterically stabilised particles and acts synergistically to prevent particle-cell interaction. In the similar work by Muir *et al.* (18) on PS-908 particles, it was proposed that two serum components may be responsible for exerting the dysopsonic effect; namely a component of molecular weight below 30,000 D and a second component greater than 100,000 D.

From adsorption isotherms it can be calculated that for 60 nm polystyrene particles the density of Poloxamine 908 molecules on the surface at plateau levels is 9×10^{-8} mole/m^2 (27). However, such calculations can not easily be performed for surface grafted particles. Using XPS it is possible to get a comparative indication of the level of surface exposed PEG by measuring the percentage of C–O bonds within the C1s spectra for the various systems. It can be seen from Table I, IV and Figure 2 that although the % C–O on the surface of the PS-PEG2000c and d particles is higher than on the PS-908, the resultant effect on particle-cell interaction and avoidance of liver uptake is not further improved. This indicates that only a certain density of PEG on the particle surface is necessary to provide the desired result, provided that the PEG chain length is sufficiently long to create a steric stabilisation effect. Previously, it has been shown for "grafted" liposome systems that a chain length of PEG of about 2000 D seems to be sufficient to provide prolonged circulation times *in vivo* (13,15). These results are consistent with data from Blume and Cevc (30) who recently showed that the half-time of circulation of distearoylphosphatidylethanolamine – PEG110 / distearoylphosphatidylcholine (DSPE-PEG/DSPE) liposomes was dependent on the surface density of the PEG groups rather than the molar ratio between DSPE-PEG and DSPE and that the half-time of circulation plateaued at a certain PEG density for such gel-phase bilayer liposomes.

For particle systems with adsorbed block copolymers it has been shown that the PEG chains exist on the surface in a coil-like chain conformation where the relationship between surface layer thickness and PEG chain length is governed by the equation $\delta_h = a^{0.84}$, where a is the number of PEO units in the PEG chain (28). Grafted PEG chains would be expected to exert a more rod-like conformation governed by the equation $\delta_h = a^{1.00}$. Hence, in terms of a steric stabilisation layer of polymer it can be expected that a grafted PEG2000 chain (equivalent to about 50 oxyethylene units) will appear similar in thickness to an adsorbed poloxamer of PEO chain length about 106 oxyethylene units. We note that Poloxamer 238, with an oxyethylene chain length of 104 units, has indeed been shown after adsorption on polystyrene particles (60 nm) to be very effective in terms of steric stabilisation and protein resistance effect (9,17,28).

The grafted PEG-polystyrene particles studied in this paper were designed to study the importance of PEG chain density as opposed to PEG chain length. Such information will be needed in the design of biodegradable PEG stabilised carrier systems with optimal characteristics for drug targeting to specific sites.

In conclusion, a direct relationship has been shown to exist between the particle surface density of PEG and the particle-cell interaction for a polymer colloidal particle system both in *in vitro* Kupffer cell studies and studies *in vivo*. The uptake by non-parenchymal liver cells decreased with increasing PEG density until an optimal density was reached. A similar picture was seen for *in vivo* biodistribution studies where only the least dense PEG surface coatings led to considerable uptake of particles by the liver and a particle circulation time comparable to uncoated polystyrene particles. The results obtained for these model grafted particles will guide the design of sterically stabilised drug carriers produced from biodegradable polymers.

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