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Long circulating microparticulate drug carriers

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Abstract

To exert its activity a drug must reach its pharmacological site(s) of action(s) within the body. One of the current approaches to achieve site specific delivery utilises the use of a carrier. This review focuses on the physicochemical and biological properties of polymeric particulate carriers in the nanometre size range surface modified by poly(ethylene oxide) (PEO). Such systems are able to bypass the normal physiological defence processes occurring after the intravenous injection of particulates and, depending on the particle size and PEO layer properties, remain for a prolonged period of time in the systemic circulation, or have a degree of selectivity for sites of deposition within the body.

Keywords: Particulate (colloid) drug delivery system; Poloxamer; Poloxamine; Poly(ethylene oxide); PEG copolymer; Drug targeting; Biodegradable material; Protein adsorption; Biodistribution

Contents

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1. Introduction

1.1. PEO-coated particles

Since the time of Erlich it has been an ultimate aim of drug therapy to deliver a drug exclusively to its pharmacological site(s) of action(s) in a controlled manner appropriate to the disease. One current approach to achieve site-specific delivery involves the use of a carrier which should alter the normal physiological distribution of the drug and direct it to its pharmacological site(s) of action. One such carrier, composed of polymeric particles in the nanometre size range, the surface of which is covered by a layer of poly(ethylene oxide), can bypass the normal physiological defence processes occurring after intravenous injections of particulates (Fig. 1). Furthermore, depending on the particle size and properties of the PEO layer, particles remain for a prolonged period of time in the systemic circulation or show a certain extent of selectivity in the site of deposition within the body.

The design of these systems is based on collective knowledge gleaned from different research fields. During the nineteen sixties the concept of incorporating a drug into a polymeric or macromolecular particulate carrier (microencapsulation) was introduced by pharmaceutical scientist as a means to modify the physicochemical and biological properties of the entrapped drug [1]. In the field of colloid science, in 1971 Napper and Netchey [2] published their classic study on the principle of steric stabilization of colloid particles by an adsorbed macromolecular or polymer layer on the surface. Later, in 1978, van Oss [3] showed that many pathogenic bacteria possess a surface that consists of a highly hydrophilic hydrated layer of protein, polysaccharide and glycoprotein which reduces their interaction with blood components and inhibit phagocytosis of the bacteria by the cells of reticuloendothelial system. At about the same time Abuchowski and Davis [4] reported that covalent attachment of poly(ethylene oxide) to proteins gives conjugates that are nonimmunogenic and nonantigenic and have greatly increased serum lifetimes.

Few years later, in pivotal studies Illum and Davis [5–7] used a range of amphipathic poly(ethylene oxide) containing block copolymers to modify the surface of model particulate drug carrier (polystyrene latex) by forming a steric barrier on the particle surface which resulted in the carrier having a greatly prolonged blood circulation lifetime due to reduced uptake by the cells of reticuloendothelial system. Following this lead various research groups have recently reported on the preparation of biodegradable carriers with PEO-modified surface which have size and the surface properties comparable to the model polystyrene coated latex [8–11]. Some of these systems, including liposomes, have been shown to have prolonged systemic circulation and reduced deposition in the RES.

1.2. The reticuloendothelial system

During the last twenty years extensive studies on the potential of various particulate carriers to serve as targetable systems have identified that the major obstacle to active targeting is the ability of the cells of reticuloendoethelial system (RES) to rapidly remove intravenously applied particulates from the systemic circulation [12–

16]. The design of long circulating particulate systems is therefore reliant upon a proper understanding of mechanism(s) by which particulates are cleared by the macrophages of the RES, a process which is still poorly understood.

1.3. Opsonization and dysopsonization

The clearance process is mediated by an array of blood components that interacts with particulates introduced into the circulation, the so called opsonization process. It renders particles recognisable to the RES. It is now recognized that phagocytosis by elements of the RES in the liver (Kupffer cells) is regulated by the presence and balance between two groups of blood components: opsonins, that promote the phagocytosis, and dysopsonins, that suppress the process [17–20]. The former have been described as proteinaceous components of the blood that adsorb onto the surface of particulates and/or cells, thereby making foreign material more 'palatable' to phagocytes [18,19]. Immunoglobulins and components of the complement system (particularly C3 and C5) are known as classical opsonin molecules, while fibronectin, C-reactive protein and tuftsin have also been shown to enhance recognition of various particulates by different macrophages. Recently, the presence of 'organ-specific' opsonins has been proposed by Moghimi and Patel [18] where, for instance, the liver specific opsonin can enhance the uptake of particulates by Kupffer cells, whereas spleen specific opsonins could mediate the uptake of particulates by spleen macrophages. Immunoglobulin IgA and secretory IgA are the best known dysopsonins. The mode of their action is not known, although a high hydrophilicity of the molecule has been suggested as a possible explanation [20]. Recently, two serum components, one with molecular weight below 30 kDa and the second heat stable, greater than 100 kDa were proposed to be responsible for exerting the dysopsonic effect when polystyrene latex sterically stabilized with Poloxamine 908 (poly(ethylene oxide)-based copolymer) was injected into the blood stream and was partly cleared by the Kupffer cells [21,22]. The detailed description of the opsonization process and its effects on the uptake of particulates can be found elsewhere [19,23] and in this volume.

1.4. Particle characteristics and opsonization

Very little is known about the effects that the surface of injected particles has on the opsonization process. However, due to the protein(aceous) nature of blood components that interact with a particle, the factors that determine adsorbability of proteins to solid surfaces, such as surface chemistry, charge and hydrophilicity [24–28] undoubtedly play an important role. The interaction of the particles with blood proteins will, depending on their surface properties, be qualitatively and quantitatively different. This may account for the different patterns of blood clearance and specific sites of deposition in the body. The hydrophilicity/hydrophobicity of the particle surface affects their attractive forces, thereby influencing the opsonization process and interaction forces that govern adhesion of the particle to the cell. In general, a higher protein adsorbability of hydrophobic relative to hydrophilic surfaces [24,29,30] has been related to enhanced uptake of more hydrophobic particles by phagocytes in vitro [31,32] and rapid removal of hydrophobic particles in vivo [7]. The charge on the particle surface influences the electrostatic interactions with components in a surrounding milieu. It should be noted that the range of electrostatic interactions decreases with increasing ionic strength and in blood (having an ionic strength of approximately 0.15 M), the range is less than 1 nm. Some of the basic aspects of the attractive and repulsive interactions between particles and blood components or a cell surface can be rationalized by the classical DLVO theory, well known in colloid science in the form of a potential energy diagram [33,34]. However in a biological environment the situation is far more complex and, although, for instance, the surface charge has been recognised for many years as an important determinant of particule clearance from the circulation, there is conflicting evidence regarding its effect [35–37]. Nevertheless, it is a general view that negative surface charge increases the clearance of particulates

from the circulation, relative to neutral or positively charged ones.

2. Surface modification

2.1. Surface hydrophobicity

The concept of surface modification of particulate carriers to control the opsonization process and the specific interaction of particulate carriers with phagocytic cells as well as the non-specific interaction with blood components and phagocytic cells, raises a question about the optimal coating material and optimal surface properties of the carrier. There are many water compatible hydrophilic macromolecules and polymers which, in principle, may form the hydrophilic, hydrated steric barrier on the particle surface. However, the effect of a gelatin coating on polyactide/polyglycolide microspheres on their in vitro phagocytosis illustrates that any hydrophilic layer does not necessarily have the anti-phagocytic effect [38]. Gelatin encourages the uptake of fibronectin, a blood component that provides particle recognition. Another highly hydrophilic polymer, dextran, failed to prevent the accumulation of coated poly(butyl cyanoacrylate) nanoparticles in the RES (mainly liver) after intravenous administration to rabbits [39]. The relative rigidity of this polymer, arising from the hindered rotation of individual glycopyranosil units around glucosidic linkages, and therefore an inability to create high density conformational clouds on the surface, could be a reason for such behaviour [40].

2.2. PEO-coated surfaces

Since 1977, [4] PEO has been the most successful synthetic material used to modify the interactions of solid surfaces with biological media. This has been demonstrated for particulate carriers with either grafted PEO [41] or adsorbed amphipathic PEO-copolymers, liposomes with incorporated PEO-derivatives [42–44] and biomedical implants with grafted PEO chains [45–47]. A range of amphipathic PEO copolymers with poly(propylene oxide) (PPO) has been used to modify the surface of model polystyrene carrier rather than PEO homopolymer. This rationale is based on the experience in colloid science where amphipathic copolymers have been shown to provide effective steric stabilization [48,49]. When adsorbed onto a solid surface, the hydrophobic moiety anchors the copolymer and acts to prevent its desorption. Various amphipathic PEO and PPO copolymers of different composition and molecular weights are commercially available as Poloxamers and Poloxamines (BASF, Wyandote, USA), where Poloxamers have ABA block structure with central PPO block and two terminal PEO blocks (PEO-PPO-PEO), while Poloxamines are tetrafunctional block copolymers with four PEO-PPO blocks joined together by a central ethylene diamine bridge ($(PEO\text{-}PPO)_2$-x-$(PPO\text{-}PEO)_2$).

Other commercially available poly(ethylene oxide)-containing surfactants have also been tested. For instance a range of poly(ethylene oxide) sorbitan mono oleates (Polysorbates 20, 40 and 80) have been used for surface modification of poly(methyl methacrylate) nanoparticles. In vitro measurements showed that these surfactants failed to provide a hydrophilic steric barrier on the particle surface and therefore the presence of flocculated particles in the circulation is most likely the reason for an observed high accumulation in the lungs after intravenous application [50].

In the field of liposome research, along with PEG-surface modification, a prolonged systemic circulation in mice has also been achieved by imitating the surface composition of the red blood cells via incorporation of monosialoganglioside GM1 into the lipid phase [51–53]. The GM1 effect was suggested to be related to the reduction in opsonization brought about through increasing surface hydrophilicity (a sterically hindered negative charge on sialic acid), suppression of complement dependent phagocytosis and a barrier effect of the carbohydrate chains. A similar approach has recently been applied to poly(isobutyl cyanoacrylate) nanoparticles where orosomucoid, a sialic acid rich glycoprotein, was used [54]. However, in vitro results showed that the adsorbed orosomucoid layer can be desorbed/replaced with serum proteins and it is

unlikely that this system would demonstrate acceptable in vivo behaviour.

Interestingly, it has recently been demonstrated that a relatively low molecular weight sugar-based surfactants (less than 2000 Da) are able to supress the in vitro phagocytosis of sub-micrometre polyester particles [55]. These surfactant materials are produced by attaching an oleophilic group (alkyl, cycloalkyl or aryl group) to a sugar molecule with 3 to 7 -CHOH- units in the chain.

3. Biological effects of PEO surfaces

3.1. Polystyrene particles

The PEO coating on the polystyrene latex surfaces produced by adsorption of Poloxamers and Poloxamines has been shown to significantly reduce interactions with blood components and thereby increase circulation lifetimes of the particles after intravenous injection [5–7, 21,22,57,58]. For instance, coating of the latex with Poloxamine 908 (contains four 5000 Da PEO chains and four 1000 Da PPO chains, $(5000–1000)_2 \times (1000–5000)_2$ Da) resulted in a prolonged circulation time in the vascular compartment while Poloxamer 407 (4400–3800–4400 Da) and Poloxamine 904 ($(670–1000)_2 \times (1000–670)_2$ Da) coatings resulted in particles with a reduced level of liver/spleen accumulation and in a redirection of a significant portion of the administered particles to the endothelial cells of the bone marrow of rabbits. Such discrimination in biological properties has been suggested to be due to the different surface properties produced by these copolymers and, consequently, differences in the interaction with plasma proteins. This determines the degree and balance of opsonins and dysopsonins that are adsorbed at the nanoparticle surface [21].

3.2. Poly(methyl methacrylate) particles

When used to modify the surface of another model carrier, poly(methyl methacrylate) nanoparticles, Poloxamers 407 and 338 (5600–3100–5600 Da) also reduced the liver uptake to a considerable extent. However, in these studies the spleen uptake was always higher than for polystyrene latex coated with the same copolymers. As a result the overall reticuloendothelial uptake was not reduced significantly [50,59].

3.3. Block copolymer structure

Within the Poloxamer and Poloxamine series, only polymers with a certain optimal PEO and PPO molecular weight and composition have an ability to alter biological properties of nanoparticles. The low molecular weight polymers with shorter PEO chains, such as Poloxamers 108 (2000–950–2000 Da), 184 (570–1740–570 Da) and 235 (1190–2260–1190 Da) do not provide such an effective steric barrier towards in vitro phagocytosis [60]. Poloxamers with higher PEO molecular weight, 188 (M_w 3300–1740–3300 Da) and 338 (M_w 5630–3130–5630 Da) both form an effective steric barrier but, while the higher molecular weight Poloxamer 338 maintained its ability to suppress in vitro phagocytosis in the presence of serum and prolong systemic circulation of the particles after intravenous injection [6], for Poloxamer 188 this effect was reduced [61]. Although the composition of the copolymers is the same (80% PEO and 20% PPO), it seems that the higher molecular weight of PPO part of Poloxamer 338 is important to provide sufficient anchoring of the copolymer to the particle surface towards desorption/displacement in the biological milieu.

3.4. Particle size

It should be emphasised that, together with the surface characteristics, the size of the PEO modified particulate systems also determines biological fate. For instance, Poloxamer 407 was found effective in diverting nanoparticles smaller than 150 nm to the bone marrow of the rabbit whereas particles of 250 nm were mostly sequestered by the liver and spleen and only a small fraction reached the bone marrow [57]. An enhancement of the spleen uptake and decreased blood level was observed for the Poloxamine 908 coated latex greater than 200 nm following intravenous administration to rats [62]. A similar size-depen-

dent long circulating effect was also observed for GM1 and PEG-modified liposomes. Liposomes of diameters greater than approximately 300 nm had a significantly increased accumulation in the spleen [63]. It has been suggested that the mechanism of removal of the particles above approximately 200 nm by the spleen is a result of mechanical filtration (a function of splenic construction and intrasplenic microcirculation), followed by eventual phagocytosis in red pulp macrophages [62]. On the other hand, it has been demonstrated that long circulating effect has a lower size limit. For instance, irrespective of their composition, liposomes with diameters below approximately 70 nm demonstrated an increased accumulation in the liver [64]. This might be due to a possible penetration of such small particles through fenestrae in the endothelial lining of the liver and resulting association with parenchimal cells and/or a higher adsorption of blood proteins onto PEG layer formed on particles with such high curvature [64]. The effects of the particle size (curvature) on a formation and properties of PEO coating layer are described in section 5.4. Hence, the effect of the surface modification with PEO on prolonged circulation and the site of deposition of a particulate carrier appears to be limited to a relatively narrow size range of 70–200 nm.

4. Biodegradable long circulating particulate carriers

The studies on Poloxamers and Poloxamines modified polystyrene latices have demonstrated that by optimizing the particle size and by creation of a suitable PEO coating layer on the particle surface, an increase in circulation lifetime (and a certain extent of selectivity in delivery) may be achieved. However, for targeted systems to have a potential use in human therapy, polystyrene latex needs to be replaced by carriers made from biodegradable and biocompatible materials. Hence, our efforts and those of other research groups have been directed to a production of biodegradable and biocompatible particulate carriers. An appreciation of the importance of the design properties that successful targeted systems should meet (e.g., size and surface characteristics) places specific demands on the optimized production method. The first problem to solve has been the design of a preparation method for the production of particles with the size in the 70–200 nm range (referred to as nanoparticles or nanospheres). Until recently polymerization was the main method for the production of colloid particles in the nanometre size range. Hence polystyrene and polyacrylate particles have been used extensively as prototype carrier. Additionally, polystyrene latices are useful models since they are physicochemically well characterised systems and are commercially available in a broad range of particle sizes.

In the last few years modifications of classic emulsification solvent evaporation and phase separation methods [1,65–67] have resulted in nanosized biodegradable carriers becoming available (Fig. 1) [68–77].

4.1. Emulsification procedures

A reduction in particle size to the nanometre range using an oil-in-water emulsification solvent evaporation method has been achieved by high speed stirring [72,73,75], sonication [75,76,78], or/and microfluidization [74,79]. For example, 250 nm sized polylactide nanoparticles were produced using high speed stirring followed by sonication [75,76] in the presence of polyvinyl alcohol or sodium dodecyl sulphate as the surfactant. However, attempts to modify subsequently the surface of these nanoparticles by adsorption of Poloxamers and Poloxamines did not lead to a formation of detectable coating layer. Secondary ion mass spectrometry analysis clearly demonstrated that, despite extensive cleaning, residual quantities of the surfactant used in the preparation were still present on the particle surface, which, most likely, inhibited Poloxamer and Poloxamine adsorption [80].

Particle size reduction to a sub-micrometre range can also be achieved by introducing a water miscible organic solvent (e.g. acetone) into the organic phase [73,81]. This effect has been attributed to the interfacial turbulence between

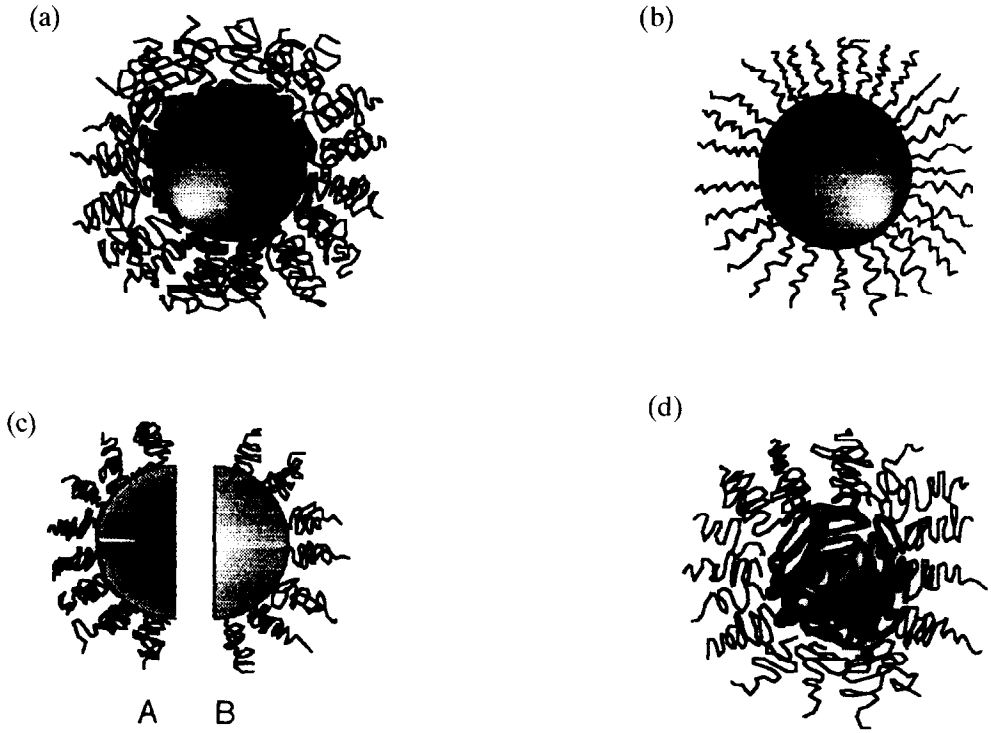

Fig. 1. Schematic diagram illustrating long-circulating nanoparticulate systems. (a) Polystyrene nanoparticles coated with Poloxamers and Poloxamines [5–7,21,22,57,62]. (b) Polystyrene nanoparticles with grafted PEO [41,95]. (c) Polylactide/glycolide nanoparticles with polylacide/glycolide-PEO copolymers, coated (A) or prepared from common solvent (B) [9,10,86,88]. (d) 'Self-forming' polylactide/glycolide-PEO block copolymers systems [11,98].

the organic phase and the water phase arising from the rapid diffusion of acetone across the interface, which spontaneously produces a larger interfacial area and results in finer droplets/particles.

4.2. Phase separation procedures

Modifications of phase separation methods involve a simple mixing of a solution of a biodegradable matrix forming material in a water miscible organic solvent with an aqueous solution of the coating material under moderate stirring. The resulting nanoparticles are within a 100–300 nm size range [9,10,68,71,77,82–85]. The nanospheres are formed due to the desolvation of the polymer from its solution caused by mixing with the non-solvent. The presence of the coating material in the aqueous phase results in simultaneous surface modification of the particles during production. This approach also enables the production of a suspension of surfactant free nanoparticles which are stabilized in the suspension by an electrostatic repulsion arising from ionized groups on the particle surface [10,71,87]. These particles have been proven to be useful in assessing the surface properties of surfactant free biodegradable colloids and the effect that subsequent coating with amphipathic PEO copolymers has on such properties [10,87].

4.3. Common solvent approach

Another approach to prepare biodegradable PEO-modified nanoparticles is to co-dissolve a coating polymer together with a matrix forming material in a common organic solvent [8,9,88]. For instance, 200 nm nanospheres were prepared by dissolving both the matrix forming polylactide/polyglycolide (PLGA) and coating polylac-

tide/polyglycolide-polyethylene glycol (PLGA-PEG) copolymer in a common solvent. This was followed by emulsification of the solution into a water phase. During evaporation of the organic solvent, the PLGA-PEG slowly reorientates with its PEG parts towards the outer water phase. It may be expected that such procedure would result in an entrapment of the PLGA block of PLGA-PEG copolymer into the forming nanoparticle matrix, resulting in a higher stability of the coating layer towards desorption/displacement.

4.4. PEO-grafted biomaterials

The polylactide/polyglycolide copolymers have predominantly been used in nanoparticle production [8–10,75,76]. However, at the present time the most promising materials in this area appear to be copolymers formed by grafting of PEO to a biodegradable moiety, such as poly-(lactide)-PEG [8,89–91], poly(caprolactone)-poly(ethylene oxide) [88], or albumin-PEG [92] copolymers. So far, some of these materials have been introduced for nanoparticle preparation and modulation of the surface properties [8–11,88,89,91].

4.5. Coated systems

For the term 'coated systems' we refer to systems consisting of biodegradable polymeric nanoparticles where the surface is modified by adsorption of PEO containing copolymers. Polylactide/polyglycolide (PLGA) nanoparticles, surface modified with a range of polylactide/polyethylene glycol copolymers (PLA-PEG) or Poloxamers, are examples of such systems [10,93,94]. The nanoparticles were prepared by the modified phase separation method and coated with either PLA-PEG 2:5 copolymer (5000 Da PEG chain) or 3:4 copolymer (2000 Da PEG chain; the PLA chain of both copolymers approximately 2000 Da). A comparison of the physicochemical properties of surfactant free uncoated and the PLA-PEGs coated nanoparticles clearly demonstrated that the PLA-PEGs form adsorbed layers on the surface of the nanoparticles. The surface layer thickness and related decrease in surface negative charge, increased hydrophilicity and efficiency of the steric stabilization of the nanoparticles towards sodium sulphate induced flocculation and adsorption of model protein, were all found to be dependent on the molecular weight of the PEG moiety (Table 1). Such in vitro characterization indicated that the PLA-PEG 2:5 coated PLGA nanoparticles would be a good candidate system for the achievement of prolonged blood circulation since it had similar surface properties to the model Poloxamine 908 coated polystyrene latex. Indeed, in vivo results (Fig. 2) demonstrate a prolonged systemic circulation and reduced liver/spleen accumulation of this system in comparison

Table 1
The physicochemical properties of uncoated and polystyrene and polylactide/glycolide (PLGA) nanoparticles coated with polylactic acid-poly(ethylene glycol) copolymer 2:5 (PLA-PEG 2:5) or Poloxamine 908.

	PLGA			Polystyrene		
	Uncoated	PLA:PEG 2:5	Poloxamine 908	Uncoated	PLA:PEG 2:5	Poloxamine 908
Particle size (nm)	140.9 ± 2.7	161.7 ± 3.7	160.4 ± 3.8	164.3 ± 3.2	178.7 ± 3.5	182.7 ± 2.6
Coating layer (nm)		10.5 ± 1.4[a]	9.8 ± 1.5		7.2 ± 1.5	9.2 ± 1.3
Zeta potential (mV)	−40.3 ± 2.3	−18.5 ± 1.4	−14.9 ± 1.8	−44.7 ± 1.7	−21.3 ± 1.6	−15.8 ± 2.2
CFC (mol l^{-1})	0.05	0.45	0.55	0.05	0.50	0.55

[a] Standard error of difference.

The particle size was measured by photon correlation spectroscopy (Malvern Instruments, UK), the zeta potential by laser doppler anemometry (Malvern Zetasizer IV, Malvern Instruments, UK) in 1×10^{-3} M HEPES buffer, the critical coagulation/flocculation concentration (CFC) was determined by measuring the nanoparticle suspension turbidity as a function of the electrolyte (sodium sulphate) concentration. The PLGA nanoparticles were prepared by the precipitation solvent evaporation method from poly(D,L-lactide-co-glycolide) 75:25, M_w 63 kDa, polystyrene latex 156 ± 4.2 nm was obtained from Interfacial Dynamic Corporation, Portland, OR, USA.

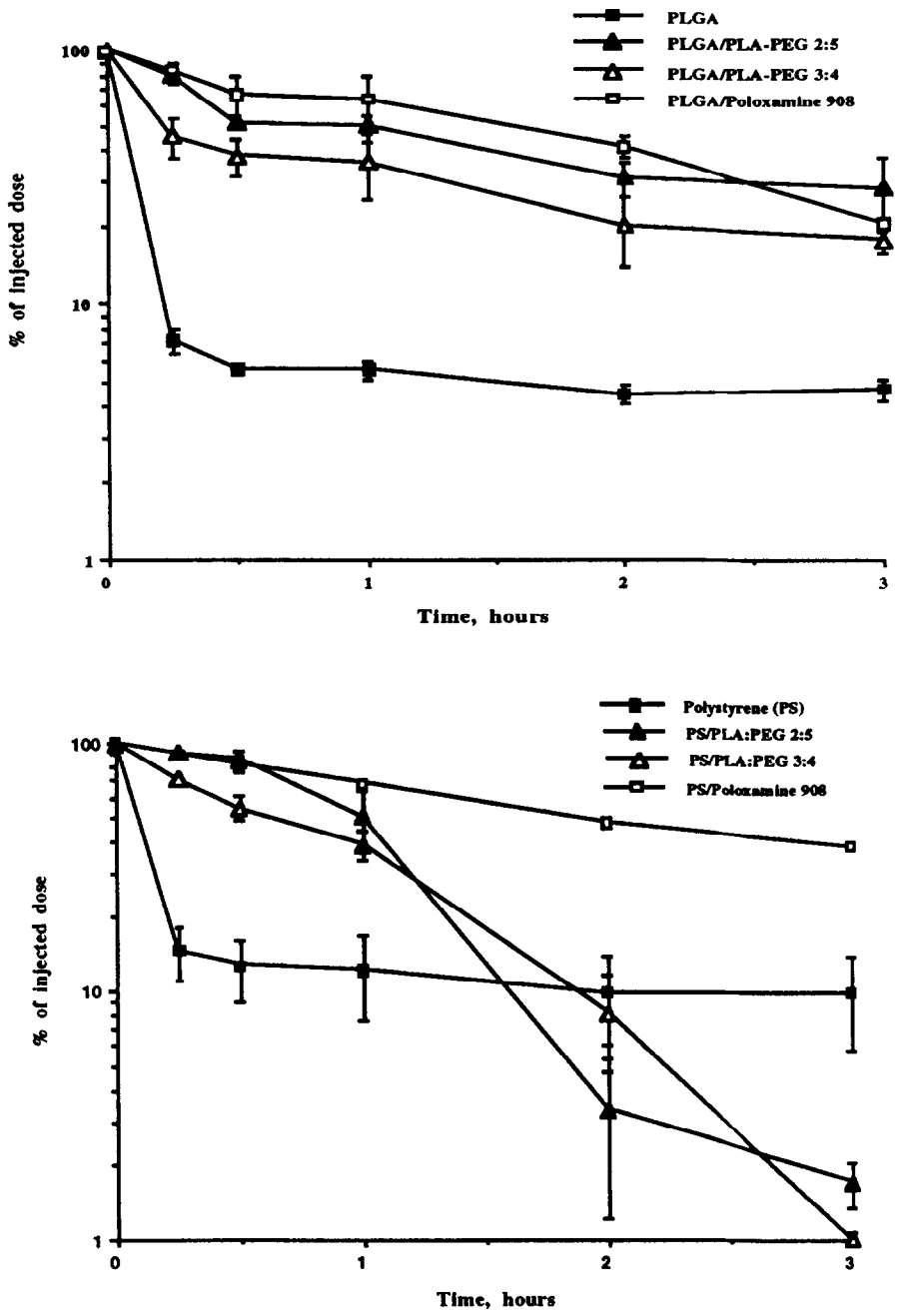

Fig. 2. Blood clearance profiles for uncoated and PLA:PEG 2:5, PLA:PEG 3:4 and Poloxamine 908 coated PLGA (top) and polystyrene (bottom) nanoparticles in rats post intravenous injection. The PLGA nanoparticles were radiolabeled by entrapment of the Indium-111-oxine complex, while the polystyrene latex was surface labelled with $Na^{125}I$. For each system three rats were injected via the lateral tail vein and the blood samples taken at various time intervals. The results are expressed as a percentage of the dose and a mean value of the three rats ± standard deviation.

with uncoated PLGA colloid; the effects being comparable to those of the model Poloxamine 908 coated polystyrene latex system.

4.5.1. In vivo results

Contrary to the in vitro results, the in vivo results reveal a distinct difference in the effect of

PLA-PEGs coatings on PLGA and polystyrene nanoparticles. The coating layer of PLA-PEGs on polystyrene latex produces systems with an initial high circulation level, but after 3 h the organ deposition data show values similar to uncoated polystyrene nanoparticles (Fig. 2). Such a behaviour has been attributed to a gradual desorption/displacement of PLA-PEG coating from the polystyrene surface in vivo, which implies a difference in stability of the PLA-PEGs coating on polystyrene and PLGA nanoparticles. This may in turn be attributed to the higher affinity of the PLA anchor part of the PLA-PEG copolymers for the PLGA surface with the similar structure than for the polystyrene.

Langer and co-workers [9,86,88] have prepared PLGA nanoparticles in the presence of the range of PLGA-PEG copolymers with 5000, 12000 or 20000 Da PEGs. Increased blood circulation lifetimes in mice were seen as the PEG chain molecular weight increased, which the authors attributed to an effect of increased thickness of the protective PEG layer. However, in contrast to Langer's group, Spenlehauer et al. [8] have produced PLA particles using PLA-PEG copolymers with a relatively short PEG chain of 2100 Da for surface modification. After intravenous administration to rats, 140 nm sized nanoparticles prepared from a range of PLA and PLA-PEG 2100 mixtures showed a trend of an increasing blood circulation half-time from 2 to 43 min as the PLA-PEG 2100 to PLA ratio increased from 0 to 73 percent. These results show a similar trend to our own work on the range of the polystyrene nanoparticles with an increasing level of surface grafted PEG 2000 Da [95]. This would suggest that a sufficient surface coverage with relatively short PEG chains (2100 Da) can inhibit opsonization and produce nanoparticles with prolonged circulation times.

4.6. 'Self-forming' systems

As discussed above, the block copolymer Poloxamer 188 failed to produce an effect similar to other highly hydrophilic Poloxamers when adsorbed to particles [7,96]. It has been suggested that the same copolymer can be desorbed/displaced from poly(isobutyl cyanoacrylate) nanospheres in vitro by serum components [11]. These facts, taken together with our own experience with the PLA:PEGs coated polystyrene latex, clearly demonstrate how essential is the stability of the coating layer in modification of the particle interaction with the biological environment. The recent introduction of novel biodegradable copolymers with grafted PEG chains [8,9,11,89] for the production of PEG modified particulate carriers appears to be an elegant way to eliminate the possibility of desorption/displacement of the adsorbed PEG coating layer. In a selective solvent these copolymers form structures comparable to micelles with a core comprising of the relatively more hydrophobic blocks and a corona of the relatively more hydrophilic PEG blocks. Our present work on a series of PLA-PEG copolymers demonstrates that, depending on the composition and molecular weight of the copolymer, more dynamic (micelle like) or solid (particle like) structure can be formed [98]. The degree of association and the micelle size are dependent on the molecular weight of the copolymer and molecular weight of hydrophilic portion and its relative content [97,98]. This provides a possibility of producing the particles with different sizes and surface properties. For example, 116 nm sized nanoparticles were prepared from PLA-PEG copolymer consisting of a 30000 Da polylactide chain linked to 2000 Da PEG chain using a simple separation of the copolymer from its solution [11]. So far, in vitro complement activation tests performed on this system show a very low deposition of complement proteins, which would indicate effective PEO protection of the particles towards recognition.

5. Properties of PEO layer

It is evident from results with both model non-biodegradable and biodegradable systems that the surface modification of particulate carriers by the creation of a coating layer composed of PEO chains can result in the avoidance of the physiological processes that would normally occur after parenteral administration of the particles. The mechanism(s) leading to this effect

has not been fully evaluated. Hence, systems exhibiting altered biological properties have been subjected to the extensive in vivo and in vitro investigations. As injected particles interact with the body components via their surface, the surface properties of the successful model system (e.g., surface chemistry, hydrophilicity, surface charge, coating layer thickness and arrangements of the PEO chains on the surface) have been extensively studied. This assists in our better understanding of the interactions of PEO modified surfaces with the biological environment and, consequently, in designing new long circulating and targetable systems.

5.1. Adsorption of block copolymers

The adsorption of Poloxamers or Poloxamines on a model polystyrene system suspended in water is driven by a selective solvation of the copolymer, since water is good solvent for the PEO and a non-solvent for the PPO block. The process is apparently controlled by the composition of the copolymer blocks and the properties of the surface. The arrangements of the PEO-PPO-PEO molecules on the surface, responsible for the interaction of the system with the biological environment, would be best characterised by a segment density profile. For the adsorption of block copolymers the self-consistent field model predicts that the adsorbing blocks are located predominantly in vicinity of the surface, with the segment density profile nearly the same as for a homopolymer with the same structure and molecular weight [99]. Buoy blocks protrude far into the solution with a profile that is about the same as that of a corresponding homopolymer. Experimentally, small angle neutron scattering provides the most comprehensive description of adsorbed layer conformation, by allowing the segment density distribution to be determined. However, the method is still relatively inaccessible and there are very few data reported. The segment density profiles for two Poloxamers (with PEO chains of 15000 and 6000 Da) on D-polystyrene latex have been determined, and show that for both copolymers the segment distribution declines exponentially from the surface without any peaks, resembling that of adsorbed PEO homopolymer [100]. This has been attributed to both PPO and PEO blocks adsorbing to the surface, although the authors left open a possibility of alternative surface conformations in which there is a more organized structure.

5.2. Polymer conformation

To obtain an idea of the adsorbed polymer conformation other approaches and techniques have been used in a complementary manner. These tend to combine the data from an adsorption isotherm (determined usually from difference in a polymer concentration in bulk solution before and after the adsorption) with adsorbed layer thickness (determined by photon correlation spectroscopy, viscosimetry, centrifugation or electrophoresis) and fraction of segments in contact with the surface (determined by infrared spectroscopy, magnetic resonance or microcalorimetry) (these methods are reviewed in Refs. [101–103]). It should be noted that in applying different experimental techniques the values measured for the coating layer thickness will differ, as each of them probe different property of the layer [104].

The layer thickness of PEO-PPO-PEO copolymers adsorbed onto polystyrene latex increases proportionally as the molecular weight of the PEO increases [60,105–110]. The theoretical model for block copolymer adsorption from selective solvent [99,111–114] has been applied to the layer thicknesses data. To describe the dependence of the hydrodynamic layer thickness on the molecular weight of the PEO chain an expression $\partial = M^a$ can be used. Values for the scaling factor a of 0.55 [115], 0.84 [108], 0.5 [110] or 0.55 [106] have been calculated from experimental results, in comparison to the theoretical value of 0.7 for adsorption of asymmetric selectively solvated copolymers with the adsorbing block relatively small in comparison to the buoy block [112]. This suggests that the adsorption of hydrophilic PEO-PPO copolymers with higher molecular weight PEO chains is dominated by osmotic repulsion between the PEO blocks. This leads to a larger separation between the anchoring sites and hence lower surface coverage, which in turn causes less stretching of

the buoy chains from the surface. A nonlinear dependence of the layer thickness on the number of monomer units in the buoy chain is then found. (An extremely strong surface affinity of anchor PPO block may overcome the osmotic repulsions between buoy chains, resulting in greater surface coverage and further stretching of the buoy chains form the surface. In this case, the scaling exponent would increase towards 1).

In order to assess the conformation of the PEO chain in the adsorbed layer, the coating layer thickness has been compared with the dimensions of the isolated PEO chains free in solution. Such a comparisons has shown that the hydrodynamic layer thickness is, at a sufficient surface coverage, greater than twice the hydrodynamic radius of polymer coil in its dilute solution (referred to as radius of gyration, R_g). For instance, when adsorbed onto 67 nm sized polystyrene latex, PEO-PPO-PEO copolymers with 880, 1630 and 5680 Da for each PEO chain form an adsorbed layer of 10.5, 12.5 and 13.5 nm, respectively. The calculated values of twice the radius of gyration are 1.8, 2.2 and 4.4 nm, respectively [110]. This indicates that the PEO chains of the molecules on the surface are not arranged in the form of a true random coil, as in solution (the layer thickness $2R_g$ would be expected), but are stretched out towards the surrounding solution as elongated coils [108,110]. Similar coil elongation has been observed for PEO homopolymer on polystyrene latex [104]. It should be noted that at lower surface coverage (below the plateau of adsorption) the chains are less compressed and adopt a structure that lies more on the surface (similar to a mushroom shape).

The PPO block of the PEO-PPO-PEO copolymers adsorbed onto hydrophobic surfaces is believed to lie flat as a compact coil or disc on the surface [105,106,110] and contribute negligibly to the adsorption layer thickness. Recent studies on adsorption of a PS-PVP block copolymer from a selective solvent [116] show that there is an effect of the anchoring block on the adsorbed layer thickness, where the maximum layer thickness occurs at a certain ratio of anchoring to buoy blocks. Such a maximum is predicted by the mean field theory [114], but the results of Webber and Anderson [116] show a sharper variation of the hydrodynamic layer thickness with the size of the adsorbing block which may be attributed to a few factors. A clustering of the adsorbing blocks at the surface due to selective solvent conditions is one of these factors. The importance of an optimal size of the PPO block to prevent desorption/displacement of the coating layer in contact with biological systems was discussed earlier.

5.3. Nature of solid surface

As already mentioned, the nature of the solid surface influences the adsorption process [117–120]. This should be appreciated when designing long circulating biodegradable particulate carriers using the same procedure adopted for the model polystyrene latex systems (i.e., via adsorbtion of amphipathic PEO copolymers onto the surface). The PEO-PPO-PEO copolymers exhibit high affinity for a hydrophobic polystyrene latex [105,110,117,121] but the differences in chemistry, polarity and hydrophilicity between the polystyrene and biodegradable surfaces can lead to a different adsorption behaviour and, consequently, different physicochemical and biological properties of the carrier. For instance, adsorption of Poloxamine surfactants onto biodegradable colloidal particles of poly(beta-malic acid-co-benzyl malate) is influenced by the existence of electrostatic interactions between the tertiary nitrogens in Poloxamine molecules and the carboxyl groups present on the surface of the particles. Such behaviour was not observed for polystyrene latex [77]. Similarly, the amount of PEO-PPO-PEO copolymer adsorbed onto hydrophilic colloidal silica particles is much lower than onto a hydrophobically modified surface. The model calculations suggest a different distribution of the PEO and PPO block segments in the adsorbed layer. The PEO blocks, interacting with the water and hydrophilic surface, are preferentially located in the proximity of the surface and in the outer part of the layer, while PPO blocks, interacting poorly with both water and the hydrophilic surface, are situated primarily in the middle part of the adsorbed layer [122].

5.4. Particle size

Another effect that the substrate has on the adsorbed PEO-PPO-PEO layer is that of particles size (curvature) on the layer thickness. Within the particle size range required for targetable systems (70–200 nm) a thicker layer is formed on larger particle; the effect being less pronounced as the particle radius increases and particle surface becomes less curved. The effect has been attributed to the steric effects of the chains on the surface [106,109,123]. Recently, Wijmans et al. [124] used the self-consistent mean field theory to discuss the effect of particle size (i.e., curvature) on the adsorption and layer thickness of a diblock copolymer. The model shows that curved geometry of smaller particles allows more lateral freedom for buoy blocks which makes the adsorption process entropically favourable and results in an increased adsorbed amount on smaller particles while, interestingly, the layer thickness decreases on smaller particles, which implies that the volume fraction profile of buoy moiety falls off more quickly than on the larger particles. However, the authors emphasised that this curvature effect depends strongly on the way in which the adsorbed layer thickness is defined.

The particle size also affects the mobility of the PEO chains [109]. The same PEO-PPO-PEO copolymer adsorbed onto particles in the size range from 69 to 272 nm showed higher mobility on smaller particles, which would indicate more lateral movements of the chains on smaller particles. Together with the fact that the particle size affects the adsorbed amount and layer thickness, these differences in the properties of the adsorbed layer on the particles of different size would imply that a presentation of the layer to the body after administration may differ, resulting in different biological properties. This may be related to the differences in biodistribution seen for 250 and 150 nm sized polystyrene latex coated with the same Poloxamer 407 polymer (as described above in section 3.4.).

The so-called 'self-forming systems' have not yet been so extensively characterised, but analogy with micellar systems suggests that there would be differences in arrangement of the PEO chains in the corona depending on the 'micelle' size and PEO molecular weight [97,125].

The PEO layer formed on long circulating particles produces systems which are effectively sterically stabilized [10,60,121] have decreased surface potential [10,60,126] and increased surface hydrophilicity relative to uncoated controls [77,95,127]. The extent of all these effects is proportional to the size (molecular weight) of the PEO chain (Table 1). These physicochemical characterizations have been used to quantify the PEO effects on the in vitro and in vivo properties of the coated systems, and also to assess the potential of candidate systems to achieve desired effects. For instance, our recent results have established a correlation between hydrophilicity/hydrophobicity of the surface of nanoparticles covered with a range of Poloxamers and Poloxamines and interstitial drainage and regional lymph node distribution in rats after subcutaneous administration [128]. From the established relationship on optimum chain length for maximum regional lymph node uptake could be predicted.

6. Adsorption of plasma proteins onto PEO coated carriers

The long circulating PEO-coated carriers exhibit reduced adsorption of blood proteins [58,129–136]. This effect of PEO on protein adsorption has been attributed to several factors involving, among others, the unique solution properties and molecular conformation of PEO in aqueous solution. It has been suggested that PEO segments nicely fill out voids in the water structure and minimally perturb the structure of water itself, thereby minimizing the tendency for hydrophobic interactions. PEO produces a surface that is in a liquid-like state with the polymer chains exhibiting considerable flexibility and mobility [137–139]. The high mobility of PEO chains has been proposed to repel approaching proteins from the surface because the protein does not have sufficient contact time with the mobile chains to adsorb [138]. Furthermore, the reduced mobility of the PEO chains on a highly crowded surface (produced by grafting of bran-

ched PEO derivatives) has been suggested as the reason for higher protein adsorption on this system relative to the surface modified with linear PEO chains [140]. The effect was clearly evident for the PEO up to 1700 Da. It should be noted that recent studies [109] have shown a reduced mobility of PEO chains when Poloxamer 108 (1850–930–1850 Da) was adsorbed onto larger 272 nm than onto smaller 69 nm sized polystyrene latex. One may speculate that this could generate differences in adsorption characteristics of these surfaces. The second factor contributing the protein resistance arises from unfavourable compression of conformationally-random PEO chains on the approach of a protein [138]. Another possible contributing factor is suggested to be a minimum interfacial free energy of water-PEO interfaces. Proteins at or near to PEO on a surface will not feel any greater attraction from the surface than they do from the bulk solution [141].

6.1. Optimal protein resistance

An optimal protein resistant effect of PEO on solid surfaces is considered to be dependent on both the polymer chain length (i.e. the molecular weight) and the surface density of the chains. By considering the relative contribution of each of the components to the overall repulsion it has been suggested that a lower surface density of high molecular weight PEO is more effective in reducing protein adsorption than a higher surface density of the low molecular weight polymer [142,143]. However, Jeon et al. [144,145] suggested that the surface density of PEO chains have a greater effect than their length. To explain the molecular weight dependence of PEO on protein adsorption it has been suggested that lower molecular weight PEO chains may not provide sufficient lowering of the interfacial free energy [141]. Also, small PEO chains have been shown to be less mobile than the larger ones [138]. Antonsen et al. [139] hypothesized that the protein resistance of the PEO modified surface is molecular weight dependent due to the unique way in which the PEO molecule binds water. The water molecules, that are hydrogen bonded to the ether oxygens of PEO, are believed to form a protective hydration shell around the molecule (so called structured water). At low molecular weights only tightly-bound water is associated with the PEG chain. As the molecular weight of a PEO molecule rises, the cloud temperature also increases, suggesting an increased amount of bound water. It has also been suggested that as the molecular weight increases, the chain begins to fold in on itself, forming segment-segment interactions as it traps additional, more loosely bound water between the segments. Thus, the folding of the PEG chain into a hydrated coil results in the formation of a repulsive hydrated layer. This folding can occur only above a certain molecular weight of the PEG chain, with molecular weights of 1500 and 3500 Da being suggested as the critical values [139,142,146]. An increase in PEO molecular weight above these values did not result in further reduction in protein adsorption [140,142,146]. This can be compared with the biological properties of PEO modified nanoparticulate carriers, where layers of relatively short 2000 Da PEO are shown to be able to produce long circulating nanoparticles [8,10,95].

6.2. Computer modelling

Recently, computer molecular simulations have been performed to study interactions of PEO surfaces with proteins [40,147]. The work by Lim and Herron [147] demonstrates that the mobility and density of PEO chains on the surface determines the extent of protein-polymer surface interaction. On a rigid crystalline PEO surface the protein remains virtually stationary while it was swept along and drifted from the surface of mobile PEO chains. The protein moved away much faster from a more densely covered surface (18 Å separation distance between PEO chains) than from less densely covered surface (24 Å separation). Unfortunately, due to limits in computation time, water molecules in the surrounding environment were not included into these simulations. It is critical that water is taken into account when comparing experiments with such computer simulations.

6.3. Critical plasma components

As mentioned earlier, according to the current hypothesis it is the interaction of particulate carriers with an array of blood components and the balance in opsonic-dysopsonic effects, and not just protein rejection from the surface, that determine the processes of recognition and site specific deposition. Hence, research has been directed towards identifying the specific serum components that interact with the particular carrier surface [58,134,135,148]. It has recently been shown that the pattern of protein adsorption onto a range of PEO-PPO-PEO modified polystyrene latices reveals quantitative and qualitative differences in the protein adsorption depending on the PEO coating [135]. Poloxamer 238 (4580–2620–4580 Da) coated particles exhibited much higher protein adsorption than Poloxamine 908 coated particles. Two dimensional polyacrylamide gel electrophoresis detected 550 spots for the former (the fibrinogen spot being dominant) and only 200 spots for the Poloxamine 908 system. In their recent work, Moghimi et al. [21] suggested that the longevity of Poloxamine 908 coated particle in circulation is because Poloxamine 908 suppresses opsonization but allows dysopsonins in the serum to act. The nature of these dysopsonic factors and the mechanism by which they interact with PEO layer to provide the observed effects remain to be established.

7. Concluding remarks

A detailed consideration of the factors that determine the adsorption of amphipathic PEO copolymers onto a particle surfaces and factors that influence interactions of a PEO covered surface with proteins can be made. It is then not difficult to imagine that PEO coatings on different systems could result in particulate carriers with different surface properties that would, consequently, differ in their interaction with plasma proteins and the degree and the balance of adsorbed opsonins and dysopsonins which determine the site of deposition of the carrier. Long circulating systems have been produced by forming a PEO layer on the surface of polymeric particles with a particle size between 60 and 200 nm. The PEO layer may be produced by grafting, adsorption of amphipathic copolymers or through the use of grafted PEG copolymers. The PEG molecular weight should be about 2000 Da or higher. A certain selectivity in the site of deposition can be achieved by different coatings and particle size. To increase selectivity or deposition, methods for directing the carrier within the body (possibly by attachment of a targetable moeity (antibody) at the PEO chain end) have already been suggested [88]. The major challenge in this field is still to establish a correlation between the physicochemical properties of the systems and their interaction with blood components and resulting site of deposition. This correlation would then open a possibility of designing targetable systems with desirable biological properties.

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