REVIEW ARTICLE

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Protected Particulate Drug Carriers for Prolonged Systemic Circulation - a Review

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The field of protected particulates with longer circulation period *in vivo* is reviewed. Long circulating pharmaceutical carriers are the fastest growing field in the pharmaceutical research, today. Then uptake of particles by reticuloendothelial system poses problem in delivering the drug or active molecule to the appropriate site for a required period of time. This review discusses the possibility of using poly (ethylene glycol) and poly (ethylene oxide) as a coat to prolong the circulation half-life of the microparticulate system *in vivo*.

In the field of pharmaceutical formulation research, there has long been the desire to achieve selective delivery of drugs to specific sites in the body in order to maximize drug action and minimize side effects. When a pharmaceutical agent is encapsulated within or attached to a polymer or lipid, drug safety and efficacy can be improved. This has provided the impetus for active study of the design of suitable carriers, intelligent delivery systems and approaches for delivery through different portals in the body[1].

One current approach to achieve site-specific delivery involves the use of carriers, which should alter the normal physiological distribution of the drug and direct it to its pharmacological site of action. Once such carrier, composed of polymeric particles in the nanometre size range, the surface of which is covered by a layer of poly (alkane oxide) can by pass the normal physiological defence processes occurring after intravenous injections of particulates. Depending on the particle size and properties of the poly (alkane oxide) 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[2].

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COLLOIDAL PARTICLES AS DRUG CARRIERS

The use of polymeric and colloidal carriers for the site-specific delivery of drugs has attracted much attention[3]. The concept of incorporating a drug into a polymeric or macromolecular particulate carrier was introduced by the pharmaceutical scientist as a means to modify the physicochemical and biological properties of entrapped drug[4]. Colloidal polymer particles include nanoparticles, nanocapsules, microspheres and microcapsules. Nanoparticles and microspheres are monolithic devices, with a rate-controlling polymer matrix, throughout which, the drug is dissolved or dispersed. In contrast to them, nanocapsules and microcapsules are reservoir devices consisting of a shell-like dosage form with the drug contained within a rate controlling membrane[5]. Nanoparticles are in the size range from 10 to 1000 nm. Microspheres are in the range of 1 to 200 μm. These carriers can be used not only for the delivery of drugs but also for vaccines and diagnostic imaging[6].

Most of these carriers are administered by parenteral route which brings the carrier in direct contact with the blood components. Polymeric microparticles being hydrophobic in nature are immediately coated by plasma components as soon as they enter the bloodstream (a process known as opsonization), which makes them

viable to be taken up by the reticuloendothelial system (RES). So the major obstacle to active targeting is; the ability of the cells of RES to rapidly remove intravenously applied particulates from the circulation[7]. The design of long circulating particulate systems, therefore depends upon the proper understanding of mechanism by which particulates are cleared by the macrophages of the RES, a process which is still poorly understood.

Opsonization and Dysopsonization:

The clearance process is mediated by an array of blood components that interact with particulates introduced into the circulation, the so called opsonization process[8]. 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[8]. Opsonins are proteinaceous components that adsorb onto the surface of particulates and/or cells, thereby making foreign material more recognizable to phagocytes[9]. The major and minor opsonins and their role in phagocytosis is discused briefly. The best known dysopsonins are immunoglobulin A and secretory immunoglobulin A. Their mode of action may be owed to their tremondous hydrophilicity[10].

Major Opsonins:

Major specialized opsonins of normal blood include immunoglobulins, fibronectin and C-reactive protein. Immunoglobulins are the most specialized opsonins that can recognize and selectively bind specific fragments of macromolecular species[11]. Fibronectins are large proteins abundant in plasma and extracellular matrix. Fibronectin consists of several domains connected by relatively flexible joints and is believed to be capable of unfolding[12]. Fibronectins are believed to participate in hepatic uptake of particulates[13]. Complement (C-reactive) comprises a set of proteins that work to eliminate microorganisms and other antigens from blood[14]. The third complement protein, C3, has a central role in complement function. One of the most important functions of complement is to mark antigens with fragments of C3, thus making them recognizable by phagocytes bearing C3 receptors.

Minor Opsonins:

Several proteins present in blood in small concentrations are also recognition proteins bearing binding sites, receptor recognizable domains and sometimes, sites for binding another recognition molecule. Most minor opsonins are lectins[15]. Recently, the presence of organ specific opsonins has been proposed by Moghimi and Patel[9], where, the liver specific opsonins can enhance the uptake of particulates by Kupffer cells, whereas spleen specific opsonins could mediate the uptake of particulates by spleen macrophage.

Particle characteristics and opsonization:

It is clear that, the surface chemistry, charge and hydrophilicity of the particulates play an important role in the clearance/opsonization process[16,17]. The hydrophilicity/hydrophobicity of the particle surface affects their attractive forces, thereby influencing the opsonization processes and interaction forces that govern adhesion of the particle to the cells. In general, a higher protein adsorbability of hydrophobic surfaces relative to hydrophilic surface has been related to enhanced uptake of hydrophobic particles by phagocytosis *in vitro*[18] and rapid removal of hydrophobic particles *in vivo*[19].

The charge on the particle surface influences the electrostatic interactions with components in the surrounding milieu. It is a general view that negative surface charge increases the clearance of particulates from the circulation as compared to neutral and positively charged particles[2]. This may be attributed to the increased electrostatic interaction between blood components and the particulates. Another important factor responsible for the uptake of particles is the particle size. Particles of size greater than 200 nm are said to be recognized by the RES, which clear them from circulation. Hence, it is necessary to have particles of a size range below 200 nm, which correspond to sizes of natural carriers such as viruses or lipoproteins.

In order to overcome the above discussed shortcomings, it was thought appropriate to coat the particles with polymers which render them undected by the RES and thereby increasing the residence time of the microparticulate carrier in the systemic circulation[19].

BASIS OF PARTICULATE PROTECTION

In general, to impart *in vivo* longevity to drug carriers, certain synthetic and natural polymers are being studied. These polymers have been shown to protect individual molecules and solid particulates from interaction with different solutes[20]. Stealth particulate systems can be achieved by:

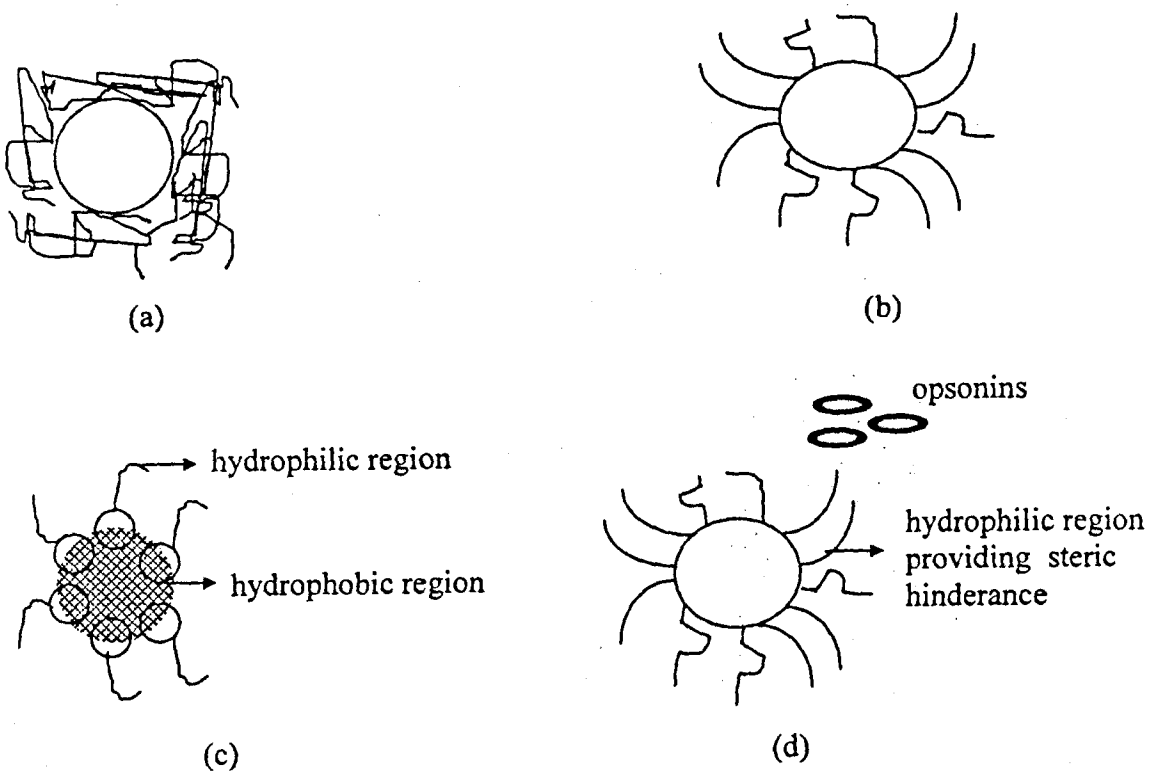

Fig. 1 : Schematic illustration of 'Stealth' particulate system formation and mechanism

a) Coated particulate surface; microparticles coated with poloxamer or polymer
b) Grafted particulate surface; microparticles grafted with poloxamer or polymer
c) Self-forming particulate system in which the inner core represents the hydrophobic region and outer (polar) surface represents the hydrophilic surface
d) Mechanism of providing 'stealth' nature; the hydrophilic region of the grafted particulate surface provides steric hinderance thereby preventing it from opsonins

a. coating of the polymer onto the particle surface[19,21-24]
b. grafting of the polymer onto the particle surface[25,26]
c. self-forming systems[27]

Coating of the particle is achieved either by absorption or incorporation of polymers containing substituents onto the surface via hydrophobic interaction. In any case, as mentioned above, 'stealth' nature is brought about due to the exposure of hydrophilic fragments into the solution, where in they prevent the particulates from interaction with plasma proteins in the blood[28] Fig. 1.

COATED PARTICULATE CARRIER FOR LONG CIRCULATION

Poly (ethylene oxide) coated particulates:

The principle of steric stabilization of colloidal particles by an adsorbed macromolecular or polymeric layer on to the surface was studied by Napper and Netchey in 1971[29]. Later in the year 1978, Van Oss showed that many pathogenic bacteria possess a surface that consists of a highly hydrophilic hydrated layer of protein, polysaccharide and glycoprotein which inhibit phagocytosis of the bacteria by the cells of RES[30]. At about the same time, it was reported that covalent attachment of poly (ethylene oxide) (PEO) to proteins gives conjugates that are non-immunogenic and non-antigenic and have greatly increased serum lifetimes[31] Table 1.

Illum and Davis, in their pivotal studies, used a range of amphiphathic PEO 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 prolonged

TABLE 1 : VARIOUS MATERIALS USED IN THE PROCESS OF COATING PARTICULATES AND THE MECHANISM BY WHICH COATING OFFERS PROTECTION TO THE PARTICULATES FROM BEING CLARED FROM THE BODY FLUIDS

Materials	Mechanism	References
PEO and its copolymers; PPO	A amphipathic copolymer. The hydrophobic moiety anchors onto the solid surface preventing desorption projecting hydrophilic surface. The polymeric chain exhibits considerable flexibility and mobility which has been proposed to repel approaching proteins from the surface	25,31,35,36,38
PEG	Formation of a conformation 'cloud' of flexible polymeric chains which protects the hydrophobic core of the particle from opsonizing proteins with hydrophilic surface and steric hinderance	51,52
Orosomucoid (a sialic acid rich glycoprotein)	Increasing surface hydrophilicity and a sterically hindered negative charge imparted by sialic acid	53

blood circulation due to reduced uptake by the cells of RES[19,21,22]. Following this, various research groups have recently reported on the preparation of biodegradable carriers with PEO-modified surface. Some of these systems, have been shown to have prolonged systemic circulation and reduced deposition in the RES[27,32,33]. The long circulating PEO-coated carriers exhibit reduced adsorption of blood proteins[34]. This steric effect of PEO on protein adsorption has been attributed to several factors involving among other, 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. It also provides considerable flexibility and mobility to the particles[35]. 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[36].

The second factor contributing the protein resistance due to unfavorable compression of conformationally random PEO chains on the approach of protein[36]. Another possible contributing factor is suggested to be a minimum interfacial free energy of water-PEO interfaces.

So proteins at or near the PEO on a surface will not feel any greater attraction from the surface than they do from the bulk solution[37]. Antonsen and Hoffman[38], 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 sheath around the molecule.

Polyethylene glycol coated particulates:

It has been shown that polyethylene glycol (PEG) coated particulate carriers have a prolonged circulation half-life in the circulations and a reduced rate of uptake by the liver when compared to uncoated particulates[39-43]. This opsonin scavenging effect is due to the presence of hydrophilic PEG chain located on the surface of the carrier. These chains act by concealing the carrier and thus, by preventing its recognition by the mononuclear phagocytic system (MPS)[44]. It has also been suggested that as the molecular weight of PEG 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[45].

PEG-coated nanospheres using amphiphilic PEG-polyester diblock copolymer showed that the protective effect against MPS is a function of PEG molecular weight and PEG density on the nanosphere surface[44,46]. Coating of poly (D, L-lactide) nanoparticle with PEG (PLA-PEG)

resulted in increased plasma half-life of the colloidal carrier. In this way, the PLA-PEG nanoparticle half-life was increased to about 6 h as compared to few minutes for PLA/albumin-or PLA/poloxamer 188-coated nanoparticles[47].

The fact that particles can be engineered to avoid capture by the liver opens up opportunities for targeting within as well as outside the vascular compartment[48]. It has been shown that particles with longer circulation time, can reach sites of inflammation as well as escape to the interstitium of tumors[49], as a result of the leakiness of the endothelial cells and the associated basement membrane of the local vasculature.

Surface modified human serum albumin (HSA) nanospheres with a size of around 100 nm were prepared from poly (amidoamine)-poly(ethylene glycol) copolymer grafted human serum albumin (HSA-PAA-PEG) and poly (thiotheramido acid)-poly (ethylene glycol) copolymer grafted human serum albumin (HSA-PTACC-PEG). The surface modified nanospheres showed a reduced plasma protein adsorption on the particle surface compared with unmodified particles[50].

SURFACE PROPERTIES OF COATED PARTICLES

Surface hydrophobicity:

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, may form the hydrophilic, hydrated steric barrier on the surface of the particle. It is possible to inhibit the uptake of polystyrene nanoparticles completely in the small intestine by adsorbing poloxamer surfactants on to the nanoparticle surface, which converts the hydrophobic nanoparticle surface to hydrophilic one[54].

However, the effect of gelatin coating on polylactide microspheres[55] and dextran coating on poly(butyl cyanoacrylate) nanoparticles[56] failed to prevent the capture by RES after intravenous administration to rabbits. This illustrates that any hydrophilic layer does not necessarily have the antiphagocytic effect.

PEO coated surface:

PEO has been the most successful material used to modify the surface properties of the particulate carriers. A range of amphipathic PEO copolymers with poly(propylene oxide) (PPO) has been used to modify the surface of polystyrene carrier rather than PEO homopolymer. This rationale is based on the experience in colloidal science where amphipathic copolymers have been shown to provide effective steric stabilization[57]. When absorbed on to a solid surface, the hydrophobic moiety anchors the copolymer and acts to prevent its desorption. Poloxamers and poloxamines are the commercially available PEO and PPO copolymers of different composition and molecular weights.

The low molecular weight polymers with shorter PEO chains, such as poloxamer 108, 184 and 235 do not provide an effective steric barrier towards *in vitro* phagocytosis[58]. Poloxamers with higher PEO molecular weight, 188 and 338 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 the systemic circulation of the particles after intravenous administration, for poloxamer 188 this effect was reduced[59]. 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 in the biological milieu.

IN VIVO FATE OF PEO-COATED PARTICLES

Effect of PEO surface on Polystyrene particles:

Polystyrene coated with poloxamers and poloxamins have been shown to increase the circulation life times of the particles after intravenous injection[23,34]. For instance, coating of the latex with poloxamine 908 resulted in prolonged circulation time in the vascular compartment while poloxamer 407 coatings resulted in a reduction in the levels of accumulation of particles in liver and spleen 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[24].

Effect of PEO surface on Poly (methyl methacrylate) particles:

Surface modifications of poly (methyl methacrylate) nanoparticle with poloxamers 407 and 338 resulted in reduced liver uptake to a considerable extent. However,

the spleen uptake was higher than for polystyrene latex coated with the same copolymer[60].

Particle size:

It is clear that, together with surface characteristics, the size of the PEO modified particulate systems also determines its biological fate. Poloxamer 407 was found to be effective in diverting nanoparticles smaller than 150 nm to the bone marrow of the rabbit, whereas particles of 250 nm were mostly captured by the liver and spleen and only a small fraction reached bone marrow[23]. An enhancement of the spleen uptake and decreased blood level was observed for the poloxamine 908-coated latex greater than 200 nm following intravenous injection to rats[61]. Hence, the effect of 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[2].

CONCLUSIONS

It has been proved that particulate carriers can be engineered (coated) to avoid capture by the liver and can be targeted within as well as outside the vascular compartment. The selectivity in the site of deposition can be achieved by different coatings and particle size and the attachment of cell-specific ligands can lead to increased selectivity. Recently, computer-aided molecular simulations have been performed to study interactions of coated surfaces with proteins. But, due to the limitations in computation time, water molecules in the surrounding environment were not included into these simulations. It is critical that water is taken into account is such computer simulations[62]. According to the clearance hypothesis, it is the interaction of particulate carriers with an array of blood components and the balance in opsonic-dysopsonic effects. The nature of these dysopsonic factors and the mechanism by which they interact with coated layer remain to be established. Future research shall be directed to establish a correlation between the physicochemical properties of the systems and their interaction with blood components and resulting site of deposition. This correlation can then be exploited in designing a system with desirable biological properties to target into specific sites.

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