Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles

Donald E. Owens III, Nicholas A. Peppas

Abstract

The process of opsonization is one of the most important biological barriers to controlled drug delivery. Injectable polymeric nanoparticle carriers have the ability to revolutionize disease treatment via spatially and temporally controlled drug delivery. However, opsonin proteins present in the blood serum quickly bind to conventional non-stealth nanoparticles, allowing macrophages of the mononuclear phagocytic system (MPS) to easily recognize and remove these drug delivery devices before they can perform their designed therapeutic function. To address these limitations, several methods have been developed to mask or camouflage nanoparticles from the MPS. Of these methods, the most preferred is the adsorption or grafting of poly(ethylene glycol) (PEG) to the surface of nanoparticles. Addition of PEG and PEG-containing copolymers to the surface of nanoparticles results in an increase in the blood circulation half-life of the particles by several orders of magnitude. This method creates a hydrophilic protective layer around the nanoparticles that is able to repel the absorption of opsonin proteins via steric repulsion forces, thereby blocking and delaying the first step in the opsonization process.

Keywords: Opsonization, Poloxamer, Poloxamine, Poly(ethylene glycol), PEGylation, Stealth nanoparticles
by the mononuclear phagocytic system (MPS), also known as the reticuloendothelial system (RES), is a major obstacle to the realization of these goals.

The macrophages of the MPS have the ability to remove unprotected nanoparticles from the bloodstream within seconds of intravenous administration, rendering them ineffective as site-specific drug delivery devices (Gref et al., 1994). These macrophages, which are typically Kupffer cells, or macrophages of the liver, cannot directly identify the nanoparticles themselves, but rather recognize specific opsonin proteins bound to the surface of the particles (Frank and Fries, 1991). Broadly speaking, opsonins are any blood serum component that aids in the process of phagocytic recognition, but complement proteins such as C3, C4, and C5 and immunoglobulins are typically the most common. Several methods of camouflaging or masking nanoparticles have been developed, which allow them to temporarily bypass recognition by the MPS and increase their blood circulation half-life (Illum and Davis, 1984; Gref et al., 1994; Kaul and Amiji, 2002). Many of these systems make use of surface treatments that interfere with the binding of opsonin proteins to the particle surface as a means of imparting stealth, or MPS-avoidance characteristics to nanoparticles. This review focuses on those systems that utilize polyethylene glycol (PEG) and PEG-containing surface treatments because these systems seem to hold the most promise and show the lowest occurrence of harmful effects in vivo.

2. Opsonization and phagocytosis

Opsonization is the process by which a foreign organism or particle becomes covered with opsonin proteins, thereby making it more visible to phagocytic cells. After opsonization, phagocytosis can occur, which is the engulfment and eventual destruction or removal of foreign materials from the bloodstream. Together these two processes form the main clearance mechanism for the removal of undesirable components larger than the renal threshold limit from the blood. In the case of polymeric nanoparticles, which cannot normally be destroyed by the phagocytes, sequestration in the MPS organs typically occurs. If the polymeric nanoparticle is non-biodegradable, then accumulation of particles in these organs, most commonly the liver and spleen, can occur leading to toxicity and other negative side effects (Illum et al., 1986; Perachia et al., 1999a; Plard and Bazile, 1999).

Opsonization typically takes place in the blood circulation and can take anywhere from a matter of seconds to many days to complete. The exact mechanism through which this process is activated is very complicated and not yet fully understood, but the important components involved are, for the most part, well known. Immunoglobulins and components of the complement system such as C3, C4, and C5 are known to be common opsonins as well as other blood serum proteins such as laminin, fibronectin, C-reactive protein, type I collagen and many others (Frank and Fries, 1991; Johnson, 2004). The importance of these proteins in the clearance process has been indirectly demonstrated in many in vivo animal studies of inherited and induced C3 deficient animal models. For instance, research has shown that these animal models are often times more susceptible to certain diseases which are easily controlled by phagocytosis in non-C3 deficient animal models (Singer et al., 1994). The opsonins, which are present throughout the blood, are thought to come into contact with injected polymeric nanoparticles typically by random Brownian motion. However, once sufficiently close to the surface of a particle, any of several attractive forces including van der Walls, electrostatic, ionic, hydrophobic, hydrophilic, and others can be involved in the binding of opsonins to the surface of the nanoparticle.

After opsonization has occurred, the next step in the clearance process is the attachment of the phagocyte to the nanoparticle via surface bound opsonins. Without the presence of surface bound or adsorbed opsonin proteins, the phagocytes will typically not be able to bind or recognize the foreign particles. One method of attachment occurs when the bound opsonin proteins undergo conformational changes from an inactive protein present in the blood serum to an activated protein structure that can be recognized by phagocytes. Phagocytic cell surfaces contain specialized receptors that interact with the modified conformation of these various opsonins thus alerting them to the presence of a foreign material.

A second method of phagocyte attachment is the non-specific adherence of phagocytes to surface adsorbed blood serum proteins which can result in the stimulation of phagocytosis as well (Frank and Fries, 1991). This process is typically due to the association of opsonin proteins with a more hydrophobic particle surface. The third significant method of phagocyte attachment is complement activation. The complement system can be activated by one of several mechanisms including the classical, alternative, and lectin pathway. The exact details of these mechanisms are beyond the scope of this review, but several excellent sources are available on this subject (Frank and Fries, 1991; Singer et al., 1994; Morgan, 1995; Johnson, 2004). Regardless of the pathway of complement activation, the final result is the binding and phagocytosis of the foreign particle by the mononuclear phagocytes.

The third and final step in the clearance process is the ingestion of foreign materials by phagocytes. This step in the process typically involves the endocytosis of the particle or foreign material by a phagocyte. Following endocytosis of the particle, the phagocytes will begin to secrete enzymes and other oxidative-reactive chemical factors, such as superoxide, oxyhalide molecules, nitric oxide, and hydrogen peroxide, to break down the phagocytosed material (Mitchell, 2004). Unfortunately, most non-biodegradable polymeric nanoparticles cannot be degraded significantly by this process and, depending on their relative size and molecular weight, will either be removed by the renal system or sequestered and stored in one of the MPS organs. As a first approximation, removal by the renal system occurs only for molecules with a molecular weight of around 5000 or less, but can be as high as 100,000 for more dense polymers such as dendrimers. Therefore, non-biodegradable particles and degradation molecules with a molecular weight higher than the renal threshold, typically become sequestered in the MPS organs. The final biodistribution of this sequestration depends on several factors and is discussed in more detail in the biodistribution and pharmacokinetics section of this paper.
Since the initial opsonization of particles is so critical to the process of phagocytic recognition and clearance from the bloodstream, most research in the area of stealth drug delivery has focused on trying to stop or block this step of the process. There are no absolute rules or methods available to completely and effectively block the opsonization of particles, but research over the last 30 years has found some trends and methods that can be effective at slowing this process, thus increasing the blood circulation half-life and effectiveness of stealth devices. As a general rule, the opsonization of hydrophobic particles, as compared to hydrophilic particles, has been shown to occur more quickly due to the enhanced adsorbability of blood serum proteins on these surfaces (Carstensen et al., 1992; Muller et al., 1992; Norman et al., 1992).

A correlation between surface charge and opsonization has also been demonstrated in vitro, with research showing that neutrally charged particles have a much lower opsonization rate than charged particles (Roser et al., 1998). Therefore, one widely used method to slow opsonization is the use of surface adsorbed or grafted shielding groups which can block the electrostatic and hydrophobic interactions that help opsonins bind to particle surfaces. These groups tend to be long hydrophilic polymer chains and non-ionic surfactants. Some examples of polymer systems that have been tried in the literature as shielding groups include polysaccharides, polyacrylamide, poly(vinyl alcohol), poly(N-vinyl-2-pyrrolidone), PEG, and PEG-containing copolymers. Of all the polymers tested to date, the most effective and most commonly used are the PEG and PEG-containing copolymers. These polymers are typically very flexible and highly hydrophilic, which can help shield even hydrophobic or charged particles from blood proteins. They are also typically charge neutral, which lessens the effect of electrostatic interactions.

3. PEGylation

As previously mentioned, the preferred method of imparting stealth, or sterically stabilized properties to nanoparticles is through the PEGylation of these particles. PEGylation simply refers to the decoration of a particle surface by the covalently grafting, entrapping, or adsorbing of PEG chains. Also, in the case of biodegradable nanoparticles, PEG chains can be incorporated as copolymers throughout the particle so that some surface PEG chains are always available even after the degradation of surface layers. The purpose of these PEG chains is to create a barrier layer to block the adhesion of opsonins present in the blood serum, so that the particles can remain camouflaged or invisible to phagocytic cells. Experimental research using freeze-fracture transmission electron microscopy (TEM) has even been able to demonstrate visually the protein rejecting capabilities of PEGylated surfaces (Peracchia et al., 1999b).

Many different types of PEG-containing polymers have been tested for their ability to impart stealth characteristic to polymeric nanoparticles. The basic repeating units of poly(ethylene glycol) and poly(propylene glycol) are shown below. Because of the chemical structure of the repeating units, these polymers are also known as poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO).

![Polymers](image)

Tables 1 and 2 contain a representative listing of PEG-containing polymers for adsorbed and covalently attached surface coatings, (adapted from Storm et al., (1995)). From Table 1, it is evident that the vast majority of research in PEG surface coatings has involved surface adsorbed poloxamers and poloxamines. These polymers are amphiphilic block copolymers consisting of blocks of ethylene oxide (EO) and propylene oxide (PO) monomer units, which are typically formed by anionic polymerization.

The important difference between these structures is the additional methylene group of the PO unit, which makes it more hydrophobic, while the EO unit is more hydrophilic. Therefore, the hydrophobic sections of the polymer which contain PO units can be used to adsorb and anchor the surfactant molecule to the nanoparticle surface, while the hydrophilic EO containing polymers or PEG sections can extend into solution and shield the surface of the particle. This method has the advantage of being fairly simple to achieve and can impart increased MPS-avoidance characteristics to the particles. Conversely, it has the draw back that surface adsorbed PEG polymers can also desorb, leaving holes in surface coverage where opsonins can bind (Neal et al., 1998). The situation is even worse when PEG polymers are surface adsorbed on biodegradable polymer nanoparticles.
Table 1

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Surface coating</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(butyl 2-cyanoacrylate) (PBCA)</td>
<td>Poloxamer-338</td>
<td>Douglas et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Poloxamine-908</td>
<td>Douglas et al. (1986)</td>
</tr>
<tr>
<td>Poly(e-caprolactone) (PCL)</td>
<td>PEG (6000, 20,000)</td>
<td>Leroux et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-407</td>
<td>Jackson et al. (2000)</td>
</tr>
<tr>
<td>Poly(lactic acid) (PLA)</td>
<td>Poloxamer-184</td>
<td>Troster et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-188</td>
<td>Les et al. (1984); Troster et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-338</td>
<td>Troster et al. (1990); Troster and Kreuter (1992)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-407</td>
<td>Troster et al. (1990); Jackson et al. (2000)</td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid) (PLGA)</td>
<td>Poloxamer-235</td>
<td>Norman et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-237</td>
<td>Illum et al. (1987a,b); O’Mullane et al. (1990); Norman et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-238</td>
<td>Illum et al. (1987a,b); Harper et al. (1991); Norman et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-338</td>
<td>Illum et al. (1983, 1984); Illum et al. (1986, 1987b); O’Mullane et al. (1990); Watrous-Pelletier et al. (1992); Muller and Wallis (1993); Tan et al. (1993)</td>
</tr>
<tr>
<td>Poly(lactic acid)-poly(ethylene-co-vinyl acetate) (PLA:eva) 50:50</td>
<td>Poloxamer-184</td>
<td>Troster et al. (1990); Troster and Kreuter (1992)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-338</td>
<td>Illum and Davis (1983, 1984); Illum et al. (1986, 1987b); O’Mullane et al. (1990); Watrous-Pelletier et al. (1992); Muller and Wallis (1993); Tan et al. (1993)</td>
</tr>
<tr>
<td>Polystyrene (PS)</td>
<td>Poloxamer-338</td>
<td>Illum et al. (1986, 1987b); Illum et al. (1988, 1987b); Illum et al. (1993); Mughn and Gray (1997); Neal et al. (1998); Stolnik et al. (2001); Mughn (2003)</td>
</tr>
<tr>
<td></td>
<td>Poloxamer-908</td>
<td>Mughn et al. (1993)</td>
</tr>
</tbody>
</table>

Notes: Studies of the opsonization of polymeric nanoparticles with surface adsorbed PEG and PEG containing polymer layers.
In this case, not only can desorption occur, but biodegradation of the particle can also increase the loss of surface bound PEG moieties. Because of these issues, several different methods have been developed in the literature, see Table 2, to covalently attach PEG chains to the surface of nanoparticles. Some research has directly shown that particles with covalently bound PEG chains achieve longer blood circulation half-lives than similar particles with only surface adsorbed PEG (Harper et al., 1991; Bazile et al., 1995). Nevertheless, there are some disadvantages to this method as well. It is sometimes hard to ensure that covalently binding of the PEG chains occurs at the surface and not in the bulk of the material, if surface coverage is the goal. Also, as a result of this, it can be much more difficult to control and optimize the surface coverage density and conformation. On the other hand, the covalent bonding of PEG chains throughout the particle maybe preferred for biodegradable particles, due to the availability of surface exposed PEG chains during the entire degradation and erosion process.

To create these types of nanoparticle systems, most researchers use a copolymer of PEG with another biodegradable polymer, such as poly(lactic acid), poly(lactic acid-co-glycolic acid), or poly(alkylcyanoacrylates). In this case, a surface PEG layer is typically created by addition of PEG containing copolymers to the reaction mixture prior to polymerization. Since these reactions typically employ an emulsion, precipitation or dispersion polymerization in aqueous media, the PEG portion of the copolymer is able to orient itself within the non-reacting water phase, while the biodegradable portion of the copolymer is covalently bonded or physically entangled inside the polymerizing nanoparticle matrix. Alternatively, PEG moieties might also be
of motion will be greatly restricted and they will most often
be located closer to the surface of the particle. Very low surface
coverage can also lead to gaps in the PEG protective layer where
protein resistant (i.e. opsonization resistant) properties to materials
which supports the hypothesis that PEGylation can add pro-
tein resistant (i.e. opsonization resistant) properties to materials
(Jeon et al., 1991).

This theory makes the argument that the hydrophilic and flex-
able nature of the surface PEG chains allows them to take on a
more extended conformation when free in solution. Therefore,
when opsonins and other proteins are attracted to the surface of
the particle, by van der Waals and other forces, they encounter the
extended surface PEG chains and begin to compress them. This
compression then forces the PEG chains into a more condensed
and higher energy conformation. This change in conformation
creates an opposing repulsive force that, when great enough,
can completely balance and/or overpower the attractive force
between the opsonin and the particle surface. It is important
to note that for effective blocking or repulsion of opsonins to
occur, the surface coating layer needs to exceed a minimum
layer thickness. The exact thickness of the layer required can
vary depending on the situation and is sometimes hard to con-
trol. Therefore, layer thickness is usually correlated to other
factors such as PEG molecular weight, surface chain density,
and conformation.

Most research indicates that a surface PEG chain molecular
weight of 2000 or greater is required to achieve increased MPS
avoidance characteristics. This minimum MW is most likely
due to the loss in flexibility of shorter PEG chains. Also, it has
been shown that as molecular weight is increased above 2000,
the blood circulation half-life of the PEGylated particles is also
increased, which may be due in part to the increased chain flex-
ibility of higher MW PEG polymers (Gref et al., 1994; Leroux
et al., 1994; Leroux et al., 1995; Peracchia et al., 1997; Peracchia,
2003). In addition to chain molecular weight, surface chain density and confor-
mation are also critical factors to achieving improved stealth
characteristics, although these two aspects are much more inter-
related. For instance, at low surface coverage, the PEG chains
have a larger range of motion and will typically take on what is
termed a "mushroom" configuration, where on average they will
be located closer to the surface of the particle. Very low surface
coverage can also lead to gaps in the PEG protective layer where
opsonin proteins can freely bind to the nanoparticle surface. On
the other hand, at high surface coverage the PEG chains range
of motion will be greatly restricted and they will most often
exhibit a semi-linear or "brush" configuration. Although a high
surface coverage ensures that the entire surface of nanoparticle
is covered, this method also decreases the mobility of the PEG
chains and thus decreases the steric hindrance properties of the
PEG layer (Storm et al., 1995). A 3D schematic diagram of the
PEG "brush" and "mushroom" configurations is illustrated in
Fig. 1.

Therefore, the optimal surface coverage is located some-
where in between the "mushroom" and "brush" configurations,
where most chains are in a slightly constricted configuration,
but are present at a high enough density to ensure that no gaps
or spaces on the particle surface are left uncovered. As a gen-
eral guideline, researchers have pointed to a minimum effective
hydrodynamic layer thickness of roughly 5% of the particle’s
diameter, or one that is greater than twice the hydrodynamic
radius of the polymer coil in its dilution solution conformation
(Stofnik et al., 1995; Storm et al., 1995). It should also be noted
that this analysis of surface coverage was developed primarily
for solid surfaces, which is not always the case in drug delivery
systems. For instance, when the surface PEG chains of swollen
hydrogel materials are compressed, there is a finite probability
that these chains will penetrate back into the hydrogel matrix
itself, instead of being compressed into a higher energy confor-
mation, thereby making the surface coating layer less effective
(Huang et al., 2001). Currently, this effect has not been fully
studied in stealth nanoparticles and should therefore be taken
into consideration when designing stealth hydrogel systems.

4. Biodistribution and pharmacokinetics

Typically once a polymeric nanoparticle is opsonized and
removed from the bloodstream, it is sequestered in one of the
MPS organs. In the case of "naked" nanoparticles, or nanopar-
ticles that have not been PEGylated and lack stealth properties,
sequestration in the MPS organs is very rapid, typically a matter of minutes, and usually concentrates in the liver and spleen (Illum et al., 1987a; Gref et al., 1995; Panagi et al., 2001). However, for PEGylated stealth nanoparticles the speed of clearance and final biodistribution is dependent on many factors.

Research has shown that particle size plays a key role in the final biodistribution and blood clearance of stealth particles. As discussed earlier, molecules that have a molecular weight less than 5000, or even higher for dense polymers such as dextrans, can be removed from the body via the renal system. For large molecules and particles that cannot be removed by the renal system, research has shown that particles with hydrodynamic radii of over 200 nm typically exhibit a more rapid rate of clearance than particles with radii under 200 nm, regardless of whether they are PEGylated or not (Moghimi et al., 1993b). In other words, a 250 nm PEGylated nanoparticle would be cleared from the bloodstream much more rapidly than a 70 nm PEGylated particle. Likewise, a 250 nm “naked” nanoparticle would be removed more quickly than a 70 nm “naked” nanoparticle, but both “naked” nanoparticles and the 250 nm PEGylated particle would be removed orders of magnitude more quickly than the 70 nm PEGylated nanoparticle. Besides blood clearance rate, the final biodistribution is also affected by particle size. In the case of PEGylated nanoparticles, a hydrodynamic radius of less than 150 nm was shown to produce an increased uptake of particles in the bone marrow of rabbits, where as particles of 250 nm in diameter where mostly sequestered in the spleen and liver, with 150 nm was shown to produce an increased uptake of particles in the bone marrow (Porter et al., 1992a; Hunter and Moghimi, 2002).

The summarized work above demonstrates that the study of stealth nanoparticles and their opsonization by the mononuclear phagocytic system remains a very active and developing area of research. Although the proteins and blood serum components involved in this process are fairly well known, the mechanism by which they activate specific cellular responses and interact with stealth nanoparticles is still not fully understood. Also, the lack of a comprehensive study of these responses across multiple cell lines and animal models and the inherent variability in these systems has hindered our understanding of these mechanisms and produced conflicting results. Furthermore, there are still several
major factors that have not been adequately addressed in this area of research. They include the extent of molecular-weight polydispersity and particle size distribution in polymeric systems and intermediate polymer degradation products on stealth properties and biocompatibility. Until comprehensive and systematic studies can be conducted to account for all of these critical factors, there will be some difficulty in achieving truly exceptional stealth properties in polymeric nanoparticle systems. However, despite these issues, great strides have been made over the past several decades at improving the overall MPS-avoidance characteristics and stealth properties of PEGylated polymeric carriers. While this and other research has led to exciting discoveries in the field of stealth nanoparticles, a significant amount of work remains before these systems can be considered safe for use in humans. Hopefully, with more characterization and understanding of the factors that affect stealth materials, long circulating stealth nanoparticle drug delivery in humans will soon become a reality.

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References


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