Poloxamers for surface modification of hydrophobic drug carriers and their effects on drug delivery

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Poloxamers for surface modification of hydrophobic drug carriers and their effects on drug delivery

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Abstract

Tri-block copolymer poloxamers are successfully employed for reducing adsorption of proteinous molecules onto hydrophobic surfaces, which will protect them from quick engulfing by macrophages. For sustained systemic circulation of hydrophobic drug carriers, particle surfaces need suitable modification for avoiding phagocytosis and this can be successfully done by poloxamers. They can affect the drug release profile, which makes them a very promising agent for targeted delivery. This review discusses the structure, characteristics and advantages of poloxamers. Poloxamer adsorption onto hydrophobic surfaces and adlayer thickness, relative phagocytic uptake and drug release profiles of coated drug loaded particles have been described in detail.

Keywords: poloxamer; micelle; drug release; surface modification ; nanoparticles; microparticles.

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1. Introduction

Since 1980s, scientists have been trying to develop potent drug carriers. The primary cause behind tremendous development of medical industry is recognizing essentiality for developing current therapies for drugs already marketed and yet to be marketed. Drugs face tremendous hindrance inside the human body that needs to get over so that they can arrive at the target site(s). If they fail to reach, they cannot execute their biological function(s). The plasma proteins of our body adsorb exogenous particles which is very significant part of defense mechanism of the human body[1, 2, 3]. Our body clears foreign materials with the help of MPS[4, 5, 6, 7, 8, 9]. Non-ionic surfactants are now in widespread applications in the pharmaceutical sciences for the coating of surfaces to facilitate drug targeting. Sustainable circulation can be obtained by proper modification of hydrophobic NPs and MPs which will help them fending off phagocytosis[10]. Poloxamers are block copolymers[11, 12, 13, 14]. They have hydrophilic ethyleneoxide (EO) and hydrophobic propyleneoxide (PO) units. Polymer chain PEO and PPO blocks form A-B-A type structure. This structure helps them showing surfactant characters. They also have the capability of interacting with hydrophobic particles, biological membranes, etc.[15, 16, 17].

Several reviews have been published in the last decade on the application of poloxamers for drug delivery[18, 19]. Kabanov et al. in 2002 [20] also reviewed drug and gene delivery, and described poloxamer micelles and micellar drug formulations, drug release from micelles and pharmacokinetic and biodistribution of poloxamers. Brain and oral bioavailability of poloxamer was also discussed. In 2008, Batrakova et al. published a

review on drugs and genes delivery using poloxamers[21]. Micellar formulations and unimer-associated biological response modifying effects of poloxamers were described in that study showing exceptional potentiality of application in pharmaceutical industries. Multiple effects of poloxamers in multidrug resistance (MDR) cells were also highlighted. To the best of our knowledge, no reviews have been published focusing on surface modification of hydrophobic drug carriers, adsorption isotherm, adlayer thickness and drug delivery profile of poloxamer coated hydrophobic drug carriers.

2. Structure and characteristics of poloxamers

Poloxamer (also known as "Pluronic") is a triblock amphiphilic copolymer of EO and PO. The simplest structure is: $(EO)_x$–$(PO)_y$–$(EO)_x$ (Figure 1). Unit number in poloxamers (e.g. x and y) can be varied.

Poloxamer synthesis is simple and can be done by adding monomers sequentially. Preferred catalyst is alkaline type e.g. KOH, NaOH, etc.[22]. Initiation step is the polymerization of the PO block. After that PEO chains grow on the two sides of PPO chain. Table 1 shows characteristics of some poloxamers of BASF Corporation, which have been discussed in this review. Poloxamers having various EO (x) and PO units (y) are characterized by their own HLB (hydrophilic-lipophilic balance)[23].

The most important benefits of these compounds in surface modification of drug carrier particles are:

(1) They have amphiphilic structures and show surfactant behavior. So, it is possible to improve hydrophobic surface's solubility in aqueous solution. The miscibility of the substances having various hydrophobicities can also be improved. The control of various

amphiphilic property is possible with controlled use of blocks length (structural control) and temperature[24, 25].

(2) These compounds have low toxicity and lack of immunogenic activity[26, 27, 28].

(3) They can affect the drug release profile, which makes them very potent constituents existing in the field of controlled drug delivery.

(4) In water, above critical micelle concentration (CMC) and temperature (CMT), poloxamer micelle formation is observed[29, 30, 31, 32, 33]. Poloxamer micelles are potential carriers of different types of drugs.

Poloxamers have much more advantageous applications that are not being mentioned here, since they are not relevant to the area of our review.

Micellization

Individual molecules (also known as 'unimers') of amphiphilic block copolymers generally have the tendency of being assembled to different micellear conformation in water when the concentration is higher than CMC. This process is known as 'micellization'[34, 35, 36]. Simplest definition of CMC is concentration at which micelle formation takes place. Poloxamer's CMC is highly dependent on the length of its two block. Increase in PPO chain length elevates the overall hydrophobicity. It leads to the segregation of PPO chains inside the micelle core[37, 38, 39]. Consequently, the CMC decreases with the increase in PPO chain length[40]. Conversely, increasing PEO block length will reduce core hydrophobicity destabilizing the micelle. Therefore, enhancement of PEO block length will increase the CMC[40, 41].

Common methods used for determining CMC of poloxamers are: viscosimetry [42], surface tension measurements[43], chromatographic analysis[44], small angle neutron scattering (SANS)[45], small angle X-ray scattering (SAXS)[46], light scattering[47], differential scanning calorimetry[48], acoustic measurements[49], etc. Typically, poloxamers that are used to deliver different drugs possess CMC in the range 1 μM-1 mM[20].

The usual diameter for poloxamer micelles ranges between 10 nm and 200 nm[50]. Micelles can have different shapes: sphere, rod, lamellar, etc. The micelle shapes mainly depend on the length of the PEO and PPO chains. Concentration of poloxamer and temperature also play significant role in the formation of different shapes. Every poloxamer micelle consists of PEO shell and PPO core. After reaching CMC, the number of polymers existing in micelle is known as 'aggregation number'[51]. The sphere-shaped micelles generally show aggregation numbers ranging from few to more than hundred. PPO core has the ability to act like 'pool'. It offers hydrophobic drugs to incorporate inside the micelle(s). This process is termed as 'solubilization'[52].

3. Advantages of tri-block poloxamers over its PEO block

For modification of hydrophobic surfaces, poloxamers show advantages over hydrophilic PEO. PPO being hydrophobic, a polymer composed of PO blocks alone is not used for the modification of hydrophobic polymeric surfaces. Poloxamers bind to the hydrophobic surface(s) in a more stable way by adapting their structure due to having both PEO and PPO chains. On the other hand, PEO alone binds with loose association and possesses high dynamicity. Surface-adhering patterns of PEO polymer and copolymer having PEO were investigated by Tan et al.[53]. The protein adsorption to

polystyrene (PS) colloids treated with PEO or poloxamer 338 (P338) was compared. If PEO chain is long, it resists proteins more than that having shorter chain. Thus, it was expected that after treating with PEO, improved or leastwise same effect can be observed like that of P338 in case of stable attachment for both of them. P338 coated PS has very low protein uptake[54]. On the other hand, protein uptake pattern by PEO treated and untreated compounds cannot be distinguished. It was concluded that it is easy for protein(s) to be adsorbed on the particles by dislocating homopolymer PEO. In case of P338 coating, dislocation by protein(s) is not so easy resulting in intact seam. For hydrophobic compounds, attraction is weak for homopolymer PEO and stronger for copolymer poloxamers.

4. Benefits of poloxamers in drug formulation

Different poloxamer excipients have been extensively used in pharmaceutical industries[55]. They are used as emulsifier[56], solubilizer for hydrophobic drugs[57] and suspension stabilizer. They also find application in parenteral dosage forms[58]. Intravenous formulation of poloxamer 188 (P188) is being marketed by the name RheothRx injection. It finds application in the clinical studies. An example of application is the thrombolysis in myocardial infarction. Poloxamers can serve different purposes: plasticizer, wetting agent, lubricant, etc. They have been using to formulate gel since they exhibit thermoreversible gelation in solid dosage forms, however this topic is not the object of the present review.

In a thorough review Moghimi et al.[59] studied poloxamer application in nanoparticle engineering and medicine, such as long-circulating particles, macrophage

stimulation, inhibition of multidrug resistance and adjuvant activities, as well as antithrombotic, haemorheological activities, cell membrane sealing, phagocyte activation and neutrophil degranulation.

As mentioned before, poloxamer helps drug loaded NPs/MPs to become stealth. In previous decades, some interesting studies have been published showing the higher existence period of poloxamer in the bloodstream with lower phagocytic uptake[60, 61, 62, 63]. Poloxamer coated NPs/MPs are not recognized by macrophages and hence not engulfed by them. Macrophageal uptake study for P188 coated and uncoated PLGA NPs was made by RAW 264.7 cell lines[64]. Prussian blue staining detected existence of NPs inside cells. Uncoated NPs subjected to cell showed blue staining which was the indication of uptake by macrophages. On the other hand, same test didn't show any stain indicating non-biorecognitive properties of P188 coated NPs. These results confirmed the potential to prolong NP circulation when administered in vivo. Further, uptake study using normal liver cells showed a dose dependent uptake of NPs functionalized by P188. Thus controlled adsorption may enlarge the binding of the stabilizer onto NPs.

Poloxamer coating is also useful for drug delivery to regional lymph nodes[65, 66]. Therapeutic and diagnostic agents can be delivered to the regional lymph nodes using biodegradable poloxamer coated nanospheres following interstitial administration[67]. It was established that poloxamer coated PLGA NPs are suitable for diagnostic and therapeutic applications in clinical and experimental medicine[67]. NPs modified with poloxamers showed improved lymphatic uptake (11 %) compared to the control NPs (3 %) in rat. Successful delivery to lymphatic system was also observed by Sanjula et al., who investigated lymphatic uptake of carvedilol-loaded NPs using male Wister rat[68].

Modified PS MPs with P407 were found to be efficient for bone marrow application in rabbit[69]. Approximately 50% deposition of the injected MPs in the bone marrow of rabbits was measured. The uptake by marrow endothelial cells suggests that a specific interaction mechanism enables the penetration through the steric repulsive barrier of the cell surface[69]. In section 6.3, delivery to brain, heart, liver, spleen, lungs and kidneys by poloxamer coated particles have also been discussed. Overall, it can be concluded that poloxamer coating can find widespread application for drug delivery in various parts of the body.

5. Adsorption pattern and adlayer thickness of poloxamers

Poloxamers readily dissolve in water. They are also soluble in many organic solvents. Based on the preparation method of hydrophobic NPs and MPs, they can be added in a different way and stage of preparation for the modification of hydrophobic surfaces.

By increasing the hydrophobicity of substrate surface, the binding between poloxamer and substrate is enhanced[54]. It is well known and can be easily understood that adsorption pattern is strongly dependent on the nature of the surface of a particle. Hydrophobic PPO has much stronger attraction for hydrophobic surface(s). Conversely, hydrophilic PEO has stronger attraction for water and extends towards aqueous phase. In general, the adsorption of poloxamer onto a hydrophobic polymeric nano- or microparticle surface is shown in Figure 2. When concentration is small, poloxamer molecules are adsorbed singly onto hydrophobic polymers. The result is monolayer formation on the substrate in which PEO blocks build “mushroom-type” conformation

(Figure 2c)[70]. Nevertheless, efficient steric stabilization is observed for a denser adsorption layer of poloxamer (Figure 2a). It means when the surface concentration is comparatively high, extension of PEO blocks is stronger, which will eventuate "brush-type" conformation (Figure 2b) in accordance with Gennes et al.[71]. It is interesting to note that adlayer thickness is strongly affected by hydrophobicity and used poloxamer's HLB. P188 showed 20 nm adlayer onto PLGA NPs. At concentration close to or higher than CMC, thicker layer growth is observed because of hemimicelle adsorption[72]. The adlayer thickness depends on the method[53]. For coating PS with F108, field flow fractionation method gave higher adlayer thickness than photon correlation spectrophotometer (PCS) method.

Poloxamer adsorption on hydrophobic drug carriers increases the carrier's diameter and broadens the size distribution. The effect of surface characters on sterically stable PS MPs was investigated by mouse peritoneal macrophages[73]. Various poloxamers and poloxamine were applied to coat PS MPs, and the adlayer thickness varied between 3.5-15.8 nm depending on the conditions. For lower concentration of P338 (0.01%), adsorption layer was almost 12 nm thick, while it was almost 16 nm at high concentration (2%)[73].

A sharp plateau was found for adsorption isotherm of F68 onto PLGA NPs when the concentration was under the CMC[72]. This result was expected and agreed with the study performed by Kayes et al.[74] whose plateau adsorption value was close to $10\times10^{-4}g/m^2$. For PS particles, Tadros et al.[75] and Baker et al.[76] found about (8.5 and 9.5)$\times10^{-4}g/m^2$ plateau-adsorption value respectively, which coincide with the value obtained by Santander-Ortega[72], although just below the CMC the isotherm suffers from

a little change. AFM studies revealed that homogeneous layers are not always formed by poloxamers. As mentioned before, under certain conditions nonionic surfactants show the tendency to construct micelles. That is why sometimes steep-isotherm can be obtained, nevertheless there is available theoretical explanation for them in the literature[77,78]. Isotherm curve alteration can be attributed to hemimicelle adsorption on PLGA particles[72]. The calculated adlayer thickness was around 20 nm measured using PCS. For a PS latex having 56 nm diameter, Baker et al.[76] obtained lower thickness which was only 6 nm using the identical polymer and optical measurement technique. The adsorption of poloxamer on hydrophobic surface is based on the hydrophobic attraction among PPO chain and the target surface. Lower hydrophobicity of PLGA related to PS might result in less compact adlayer onto PLGA surface by poloxamer.

The adsorption isotherms of PS NPs with P407 were measured for NPs of diameters 40 nm, 70 nm and 137 nm (Figure 3)[79].They follow a Langmuirian-type profile initially showing steep slope, when equilibrium surfactant concentration is low, i.e. below 0.4 mg/ml. Above certain equilibrium concentration, an adsorption plateau is observed. The plateau values greatly agree with the results of another study[80] using the same type of adsorption system(s). P407 plateau adsorption value on NPs can be seen from Table 2. 0.19, 0.16 and 0.18 μmol/m^2 of P407 was adsorbed per surface unit for the 137 nm, 70 nm and 40 nm particles, respectively (Figure 3). These results are in accordance with that obtained by Faers et al.[81]. Stolnik et al.[79] also measured adlayer depth for every sampling point of isotherm. The hydrodynamic adlayer thickness for PS NPs of diameters 137 nm, 70 nm and 40 nm coated with poloxamer is also shown in Table 2[79] (obtained using PCS).

Table 3[79] lists the values of chosen points on isotherm showing how P407 adsorbs on 40 nm PS NPs. It illustrates conformational changing of molecules onto NPs for the increase in quantity of P407 adsorbed. In case of adsorption of low amounts of P407 having less surface coverage, area counted for single PEO chain was larger. Adlayer thickness was also low indicating the absence of dense packing for P407 onto PS NPs. This less dense packing allows more space for lateral spreading of PEO. The surface is more crowded, when the amount of poloxamer adsorbed is higher, and the area occupied by single P407 decreases, although the adlayer becomes thicker. It is the indication of close packing of PEO chains. It also suggests perpendicular extension of PEO chains from NP surfaces.

In our most recent experiments (not published yet), we have investigated poloxamer adsorption onto PLGA NPs co-encapsulating model drug human serum albumin and magnetite. Double emulsion solvent evaporation method was used for NP preparation. Different concentrations of poloxamer F68 (0.25, 0.5 and 1% wt/vol) were used to coat the NPs which were redispersed in phosphate buffered saline (PBS) solution before the addition of poloxamer. The attachment of poloxamer onto PLGA surface was checked by size measurement carried out by Zetasizer Nano ZS (Malvern Instruments, UK). The volume mean size of our control sample was 199.8 nm and for coating with 0.5% F68, we got 56 nm enhancement in size which is comparable to the result obtained by Greenwood[82]. Figure 4 shows the size distribution of control and modified PLGA NPs obtained in our experiments. It was found that for 0.25 and 0.5 % poloxamer concentrations, the size distribution of poloxamer covered PLGA NPs shifted towards higher particle size region with significant simultaneous increase in volume mean particle

size. Santander-Ortega et al.[72] found a sharp increase in adsorption isotherm for F68 coating onto PLGA particles for low F68 concentration (up to 100 mg/L) and above that concentration, the increase was quite steady and reached a plateau. In our study, we got sharp increase in size up to the concentration of 0.5%. If number of poloxamer attached is higher, the surface will be highly crowded. Consequently, adlayer thickness increases[80]. Increase in poloxamer concentration from 0.5 to 1% resulted in both smaller and bigger particles than the control (Figure 4) indicating the formation of micelles, since the diameter of poloxamer micelle generally ranges from 10-200 nm as mentioned above.

From these studies it can be concluded that the method employed to find adlayer depth, the surfactant and the MP/NP characteristics significantly influence the adsorption pattern. Relationships of adlayer thickness with MP/NP size and with poloxamer units are discussed in section 7.

6. Poloxamer coated hydrophobic carriers for drug delivery

6.1. Surface modification by poloxamer to improve drug delivery

Hydrophobic NPs loaded with drugs when administered intravenously face quick clearance from body system. They are cleaned by MPS and end up in spleen/liver. Thus, hydrophobicity of drug carriers limits their use in pharmaceutical fields. Short lifetime of hydrophobic drug carriers can be increased by modifying their surface, which will yield "stealth" particles and would not be detected by MPS[10, 83]. Poloxamers are well applicable for this purpose.

The PPO blocks of poloxamers is capable of attaching the hydrophobic polymeric surfaces and the PEO blocks, which are extended outside the surface of hydrophobic polymeric surfaces, transforming the surface of drug loaded hydrophobic NPs hydrophilic to make NPs stealth. As a result, the MPS fails to recognize the NPs due to their hydrophilic nature.

The fact can be lightened more explicitly with the Figures 5 and 6. PLGA NPs which are one of the most widely used hydrophobic drug carriers has been selected in the figures as an example. PLGA NPs form negatively charged surfaces in solution since they have carboxyl group in their structure (Figure 5a). These negatively charged drug loaded hydrophobic PLGA NPs adsorb plasma proteins after intravenous administration. For example, human serum albumin (HSA) will be adsorbed on PLGA NPs according to Figure 5b. Plasma protein adsorption enlarges the size of hydrophobic drug carrier particles. As a result, the particles will be visible to macrophages and will be removed quickly from the bloodstream. Poloxamer is adsorbed on the surface of a PLGA NP according to the mechanism shown in Figure 6a and protein adsorption is prevented by the mechanism shown in Figure 6b. As mentioned before poloxamer coating will make the particle "stealth" and macrophages will not be able to detect and engulf the particle.

Due to the quick detection of drug loaded particles by macrophages, they are engulfed by macrophages and removed quickly from the blood stream. Hence, they cannot reach the target site(s) or don't get sufficient time to release the drug, which will lead no effect of medication. Poloxamers, when adsorbed onto hydrophobic drug carriers, can depress quick clearance of those carriers from the blood stream. This is an essential point especially in case of targeted and sustained drug delivery. Adsorption of poloxamers onto the hydrophobic particles yields hydrophilic surface possessing reduced surface charge. The formation of such a surface not only limits the adsorption of opsonins resulting in extended circulation in the plasma, but also increases possible distribution to different organ sites. It can be mentioned here that opsonins are any blood serum component that aid in the process of phagocytic recognition. Opsonization is the process by which foreign particle(s) or organism(s) becomes covered with opsonin proteins, thereby making it more visible to phagocytic cells[84]. Phagocytosis can occur after

opsonization. Phagocytosis is the engulfing and eventual destruction or removal of foreign materials from the bloodstream. For non-biodegradable polymeric NPs, accumulation of particles in organs (most commonly the liver and spleen) can lead to toxicity and other negative side effects[83, 85, 86].

6.2. Poloxamer to depress quick clearance from the blood stream

Illum and Davis[87] coated the surface of hydrophobic PS with poloxamer. If the PS was coated with P188, significantly higher amount of particles can get to the lung. P338 coated particles show high-level in lungs, carcass and spleen. The effect of the used P188 and P338 with different M_w and characteristics was investigated. Hydrophilic NPs/MPs having high M_w P338 coating showed appropriate colloidal stability with very low adhesive-properties[88]. Hence, high M_w poloxamer (P338) has better effectivity when compared to those with lower M_w.

Illum et al.[86] studied phagocytic uptake of PS coated with poloxamers. In general it was found that the higher the adlayer thickness, the lower the relative phagocytic uptake as shown in Figure 7[74].

These results are in agreement with the predictions of the various theories that explain the phenomenon of steric stabilization which can also be applied to the interaction of particles with phagocytic cells. It can be observed if the regression line (Figure 7) is extrapolated to zero, an adlayer of 23 nm thick will be needed to get over van der Waals attractive forces between macrophages and 5.25 μm MPs. Prediction of adlayer thickness, needed to achieve equal stabilizing effect for little NPs (e.g. 60nm), is not very easy. Van der Waals attractive force has direct relation to particles radii (a).

According to the equation (1) it is expected that a 10 nm thick adsorbed layer will be enough for providing both steric stabilization of 60 nm PS particles in terms of their aggregative propensity and a lack of interaction with macrophages[86]:

$$VA \approx \frac{aA_{eff}}{12h} \text{----------(1)}$$

where, VA = van der Waals attractive forces.

a = particles radii.

A_{eff} = composite Hamaker constant.

h = Planck's constant.

Phagocytosis of PS latex particles coated with 22 different poloxamers was studied by Rudt et al.[89]. A tendency was found for reduction of phagocytic uptake for poloxamers coated particles if M_w is more than 4000. This is obviously a generalization, although few exceptions were observed, notably poloxamer 108 and 333. They had the same M_w but different relative phagocytic uptakes (94.5% and 3.3%, respectively). When considering poloxamers with the same PPO chain length, variations in hydrophilic PEO chain length did not seem to show significant effect on phagocytic uptake. As an example, relative phagocytic uptakes of poloxamers 331,333,334,335, and 338 were 22.9, 3.3, 2.5, 7.2 and 6.0 %, respectively. However, great effect is observed for PPO chain length variation. If more than 39 units are present in PPO chains, better protection against phagocytosis is observed, which might be because of more secure adsorption onto particle surfaces. The secure adsorption is capable of preventing displacement of the adsorbed poloxamer very well by other species, thus limiting the phagocytosis of drug carriers. Another reason which imparts a little part in this mechanism is steric

stabilisation effects. However, too short PEO chains cannot provide sufficient hydrophilicity to the surface of the drug carrier to prevent opsonisation, and there exists possibility of steric stabilization as well. Finally, it was concluded that a PPO block of about 40 units will be the minimum length for achieving great decrease or preventing phagocytosis (Figure 8). This type of minimum length can be required for strong anchoring of poloxamers onto drug carrier surface which will provide enough thickness and hydrophilicity.

Thus, a conclusion can be drawn: poloxamers adsorption onto hydrophobic polymeric drug carrier surfaces produces hydrophilic surfaces reducing surface-charge. These sufficiently hydrophilic particles can prevent opsonisation, which in turns increase the life time of carrier particles in blood circulation as well as giving the possibility of distribution to different organ sites.

6.3 Drug delivery and release profile from poloxamer coated drug carriers

Poloxamer coating is capable to generate higher residence time in the plasma than uncoated NPs[90, 91, 92]. Fluorescently labelled PLGA particles coated with PEG and Pluronic F127 had higher plasma concentration than the uncoated ones while used in oral formulations[90]. PLGA carriers without poloxamer coating were detected in the brain, heart, liver, spleen, lungs and kidneys over a period of 7 days. Interestingly, not even a single particle was detected in the plasma in the lack of surface functionalization. The biodistribution profile for 1% F127 and PEG coated particles administered orally is shown in Figure 9. Though NPs were accumulated in spleen and brain, the NPs in these tissues at high doses were established to be safe[91].

Loperamide, an opioid drug cannot cross the blood brain barrier (BBB) although effective as antinociceptive if injected directly to the brain. P188-coated PLGA-PEG-PLGA (PEP) triblock copolymer NPs containing the drug showed 14.4 to 21.2% penetration[93], which was much higher than either with PLGA NPs (4.3%) or with PEP NPs (8.2%). The in vitro BBB permeation percentage of PEP obtained was 13.7 folds higher than just the loperamide solution. It shows that PEP is capable of encapsulating and transporting drugs across BBB which is normally impossible. Moreover, P188 coated PEP resulted in improved cellular uptake in BBB model compared to PEP coated with polysorbate80 (Ps80). Biopsy studies also confirmed increased penetration for P188 coated PEP over non-coated one. In brain tissue(s), NPs deposition for PEP coating with P188 showed significantly higher concentration than both PEP and PLGA NPs. Maximal possible antinociception effect (MPE) for the P188-coated PEP was 21 to 35 % after 2 and half hours of intravenous loperamide administration whereas that of just the PEP was 11.6%.

Various proteins quickly attach onto foreign NPs after being exposed to serum or plasma[94,95]. The type and the relative amount of adsorbed proteins can decide the fate of particles. In 1989, this concept was originally defined as "differential protein adsorption"[95]. Based on this concept, the hypothesis was postulated that an adsorption of the ApoE or ApoB (apolipoprotein E or B) on Ps80-coated NPs could be responsible for the interaction with the BBB and the subsequent endocytosis[95, 96, 97]. ApoE attaches the particles to the ApoE receptor of the endothelial cells of the BBB[98]. As a result, the particles are taken up by endocytosis into the cells. In the cells, the drug is released from the particle and diffuses into the surrounding brain tissue. Schematically the brain uptake

of surfactant coated NPs can be illustrated as shown in Figure 10, which is based on endocytosis.

Hydrophobic polybutyl cyanoacrylate (PBCA) is an effective drug carrier. PBCA and PLGA NPs having P188 coating loaded with doxorubicin (Dox) appears to be interesting and hopeful for treating brain tumors. These NPs yield 40% of long-term survivors. P188 was an effective coating agent for both PLGA and PBCA NPs. However, coating with Ps80 showed effectiveness only for PBCA and not for PLGA NPs[99]. Ps80- and P188-coated PBCA loaded with Dox NPs for anti-tumor effect resulted in 35% and 20 % of long-term survivors.

Recently, seven different formulations including Dox was evaluated according to their anti-tumor efficacy[100]. All formulations increased the survival time in brain tumor-containing specimens by comparing with control one that is shown in Figure 11. Poloxamer (P188) containing formulations represented the highest effectiveness. Long-term remission (more than 100 days without tumor) in 40% of the specimens could be achieved. Dox-PLGA having P188/Ps80 resulted in more than 20% of long-term survivors[101, 102, 103]. Nevertheless, the efficacy of uncoated NPs (without poloxamer and polysorbate) was almost the same like Dox–solution. For uncoated NPs, just a single specimen survived after day 30. Free Dox concentration in the brain remained below the detection limit of 0.1 μg/ml. Dox concentration delivered to the brain by Ps80 coated PBCA NPs was found to be comparatively very high (6 μg/g tissue), showing significantly improved Dox-delivery to the brain because of used coating material[104]. Stabilizers yielded NPs of similar size[103], although dextran-stabilized PBCA NPs had higher polydispersity and less negative surface charge enabling higher loading of Dox.

On the other hand, drug-loaded P188-stabilized NPs showed lower drug loading (45% for P188 and 65% for dextran) than that of dextran-stabilized particles.

Cytotoxicity of Dox loaded PBCA NPs, coated with Ps80, P188 or poloxamine 908 pharmaceutical surfactants was investigated in rat glioma cell lines[105]. Drug accumulation determined by confocal scanning microscopy revealed higher accumulation of nanoparticle-bound Dox after coating the particle surface with Ps80, which corroborated the results of the previous studies. Nanoparticle-associated Dox showed a higher cytotoxicity than the free drug in both tests, when the Ps80 was used to coat the NPs. On the other hand, P188 and poloxamine 908 showed negligible influences. This phenomenon can be attributed to more efficient transport of the Ps80-coated NPs into the cells.

Poloxamer is able to totally inhibit protein adsorption onto the PLGA 37.5/25 layer[106]. When P188 was present in the formulation, it exhibited a continuous release of lysozyme over 3 weeks without any burst effect (Figure 12). After 3 weeks, a plateau was reached due to additional destabilising mechanisms concomitant with polymer degradation. To promote the release of lysozyme in the latter stage of release, a PEP triblock copolymer was used, which resulted in continuous lysozyme release over 45 days in a biologically active form with limited initial burst (9%). Considering the wettability of the polymer, after 45 days the poloxamer and the PEG segments are leached thereby limiting their protective function, and according to the adaptation of hydration kinetics, the microspheres might provide optimal protein release.

As a summary, the investigations undoubtedly indicate the protein adsorption hindrance and thus, possibility of achieving prolonged residence time in the bloodstream

using poloxamer surfactants even via oral administration. Detection of the comparatively high amount of NPs in kidney, lungs and liver might be because of nonhomogeneous attachment of coating agents onto NPs. Poloxamers have the ability to (i) be incorporated into membranes and to change microviscosity (ii) decrease ATP (Adenosine triphosphate) levels (iii) resist drug efflux transporters, which might result in enhanced penetration and drug transport through the BBB. These features help the efficient use of microencapsulated active agents functionalized with poloxamers in controlled drug delivery systems. It is noted that results suggest that for NP formulations in vitro observations cannot easily correlate or represent the in vivo behavior of the NPs.

7. Correlation of particle size, poloxamer units, adlayer thickness and drug release

7.1. Effect of particle size on adlayer thickness

A small particle adsorbs less polymeric molecules per unit area in comparison to a large one resulting in lower thickness than larger particles as shown in Figure 13[55]. This can be also explained by the following equation showing how the surface area filled by unit poloxamer molecule depends on the particle diameter [54]:

$$\frac{Surface\ area}{Molecule} = \frac{\pi\ d_A^2 MW}{N_A\ m_B}$$

where d_A = diameter of core NP or MP

MW = molecular weight of adsorbed poloxamer

m_B = mass of adsorbed poloxamer

N_A = Avogadro's number.

From the equation it can be seen that the surface area covered by poloxamer is directly proportional to the square of the diameter of core NP or MP (d_A).

The thickness of the adsorbed poloxamers to PS MPs was studied using field flow fractionation technique, and it was found that the thickness increases with particle size[54].

The thickness increased from 60 nm to 270 nm applying F108, F88, F68 coating but not for P105[77]. As a contrary, Baker and Berg[76] and Killmann et al.[107] measured adlayer thickness of poloxamer on PS particles using PCS. They found that the adsorption does not depend on the particle size, and the adlayer thickness decreases with increasing NPs/MPs radii. For adsorption of F108 on PS, Bevan et al.[108] has quoted 12 nm and 15 nm thick adlayer onto 140 nm and 400 nm NPs, respectively. Greenwood et al.[82] found strong size dependence of adlayer thickness for P407 onto PS NPs. The adlayer showed a tendency to increase from 11 nm to 37 nm onto NPs of size 40 nm and 217 nm, respectively. Li et al.[54] observed strong dependence of surface concentration and adsorbed layer thickness on substrate size. On little PS-latex (69 nm), poloxamer adsorption layer was almost double thick of radius of gyration (Rg) of its PEO blocks. On the other hand, for bigger size (272 nm PS), PEO blocks showed large extension and adlayer was about 4Rg thick. On small particles, the surface-density of adsorbed polymeric molecules is little. The consequence is highly mobile adsorbed polymer chains. Conversely, on large particles, the adlayer is mobile to a lesser extent due to more crowded surface. An adsorbed polymeric chain moves more freely if surface packing is less (little NPs/MPs) in comparison to highly dense packing (big NPs/MPs).

7.2. Effect of units of the poloxamers on adlayer thickness

Adlayer thickness strongly depends on EO units and not influenced by PO number[73]. Figure 14 depicts the direct dependence of adlayer depth on EO and PO content. This is the indication that the PO adsorption occurs on substrate surface whereas looping or tailing is observed due to the extension of EO to aqueous phase. This can be

more clearly understood from the Table 4 obtained by Illum et al.[73] who studied adsorption of poloxamer on polystyrene particles. P338 having 128 EO units gives 15.8 nm adlayer thickness whereas P188 having 75 EO units gives less thick adlayer (7.6 nm). This result is in agreement with the one found by Kayes et al.[74]. Faers[81] observed increase in adlayer thickness for enhancement of M_w of PEO chain.

7.3. Effects of the adlayer thickness on release profile

The release pattern depends on many factors e.g. matrix structure, type of drug loaded, size of drug molecules, distribution of drug molecules inside the matrix, etc. It is known that drug loading and drug particle size distribution determine the geometry and the topology of pores and channels which determine drug diffusion from the hydrophobic matrix[109]. It is not an easy task to make a correlation between the coating thickness and the release profile for drug loaded NPs and MPs. Only the coated layer covering the surface i.e. PPO block affects the release profile. Nano- and microparticle surfaces are porous and may also contain cracks which originate from fabrication process. Drugs escape from the polymeric matrices through the cracks and pores. Poloxamer, used for coating, may cover many of the pores (not all of them). After surface covering, the coated portion will prevent diffusion of drug and initial burst release.

The prevention of the drug diffusion has direct effect on the drug release. If the diffusion is low, the drug release time will be extended. On the other hand, PEO blocks extends to the outer aqueous phase and make the particles stealth to macrophages. It can be assumed that they do not have direct effect on the release profile. However, if the extended chain length of PEO is not sufficiently thick, they won't be able to prevent protein adsorption on the particle surface as shown in Figure 6b. It is well known that the drug release rates decrease with increasing coating thickness due to enhanced length of diffusion pathways for tablet coating, which cannot be applied directly for poloxamer coating, nevertheless, it can provide an approximation for estimating the poloxamer effect. In addition, the surface coating will decrease the initial burst release[110].

Diffusion-controlled drug release from matrix-type drug-delivery systems can be expressed by the popular equation given by Higuchi[111],

$$M_t = A\sqrt{\frac{D_i \varepsilon\ C_s (2C_d - \varepsilon\ C_s)t}{\tau}}$$

where M_t is the drug amount released at time t, A is the surface area of the matrix, D_i is the diffusivity of the drug, ε is the porosity of the matrix, τ is tortuosity of the matrix, C_s is the solubility, C_d is the concentration of the drug.

Poloxamer attachment increases the size of NPs and MPs. Consequently, surface area A will decrease, and smaller value of M_t, i.e. lower drug release will be obtained.

8. Conclusion

Poloxamers are triblock copolymers having amphiphilic character and have the ability to interact with hydrophobic drug carrier surfaces and biological membranes. Drug carriers are confronted with difficulties during the route to the target organ that need to overcome before they reach its target site(s) within the body. The most important barrier is the adsorption of plasma proteins which make them more visible to phagocytic cells, then, they are immediately engulfed by macrophages and removed quickly from the blood stream. Poloxamer coating makes hydrophobic carriers "stealth", hence they can reach the target site(s), where they can perform their biological roles. Poloxamers are adsorbed onto hydrophobic carriers by their PPO block, whereas PEO block is extended toward the aqueous dispersant and make the surface of the carrier hydrophilic. The adsorption pattern and the adlayer thickness are strongly affected by the nature of the surface of carrier particles. The concentration of poloxamer is also vital since the adlayer thickness and thus the final size of drug carriers generally show the tendency to grow with the increase in poloxamer concentration. The particle size also influences the adlayer thickness, since the smaller particles take up fewer poloxamer molecules per unit

area than the larger ones, and in turn show lower thickness than the adlayer found on bigger particles. Poloxamer coated stealth carriers possess increased life time in the blood stream and show decreased phagocytic uptake. Most widely investigated biodegradable polymers for drug delivery, PLGAs show excellent life time in the blood stream after being coated by poloxamers. In vitro and in vivo studies certified that improved drug delivery and better release profile can be obtained from poloxamer coated drug carriers. It is expected that in the future more in vivo studies will be conducted, and more clinical trials will be performed to utilize promising coating agent poloxamer in the field of drug delivery.

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Abbreviations

Apo = apolipoprotein

BBB = blood brain barrier

CMC = critical micelle concentration

Dox = doxorubicin

EPR = enhanced permeability and retention

HLB = hydrophilic-lipophilic balance

M_w = molecular weight

MPS = mononuclear phagocytic system

MPs = microparticles

NPs = nanoparticles

PBCA = poly(butyl cyanoacrylate)

PEG = poly(ethylene glycol)

PLGA = poly(lactide-co-glycolide)

PLGA-PEG-PLGA = PEP

P188 = poloxamer 188

P338 = poloxamer 338

P407 = poloxamer 407

F88 = Poloxamer 238

PS = polystyrene

Ps80 = polysorbate 80

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Legends of Figures

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Figure 2: Schematic representation of poloxamer adsorption onto hydrophobic drug carrier surfaces: (a) PPO binds with carrier surface and PEO block extends to outer phase; (b) "brush-type" conformation and (c) "mushroom-type" conformation[54].

Figure 3: Adsorption isotherms for P407 on PS NPs. Particle diameters: 40 nm (♦), 71 nm (■) and 137nm (▲) (Taken from Ref. 79, with permission from American Chemical Society, Copyright 2001).

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Legends of Tables

Table1: Parameters of poloxamers which are discussed in this review.

Table 2: Adsorption pattern of P407 onto 40, 70 and 137 nm PS NPs[79].

Table 3: Points of adsorption isotherm for P407 adsorption onto 40 nm PS NPs showing hydrodynamic layer thickness and others[79].

Table 4: Thickness of adlayer for different poloxamers having different EO and PO units[73].

Table_1: ~~Some properties~~Parameters of poloxamers ~~(only those~~ which are discussed in this review~~)~~.

Copolymer	Molecular Weight	Critical micelle concentration ~~(CMC)~~[a] (M)	Average no. of EO units (x)[b]	Average no. of PO units (y)[b]	hydrophilic -lipophilic balance
L35 (Poloxamer 105)	1900	5.3×10^{-3}	21.59	16.38	19
F68 (Poloxamer 188)	8400	4.8×10^{-4}	152.73	28.97	29
F88 (Poloxamer 238)	11400	2.5×10^{-4}	207.27	39.31	28
L101 (Poloxamer 331)	3800	2.1×10^{-6}	8.64	85.97	1
P103 (Poloxamer 333)	4950	6.1×10^{-6}	33.75	59.74	9
P104 (Poloxamer 334)	5900	3.4×10^{-6}	53.64	61.03	13
P105 (Poloxamer 335)	6500	6.2×10^{-6}	73.86	56.03	15
F108 (Poloxamer 338)	14600	2.2×10^{-5}	265.45	50.34	27
F127 (Poloxamer 407)	12600	2.8×10^{-6}	200.45	65.17	22

[a]CMC values obtained with the use of pyrene probe[24].

[b]The average numbers of EO and PO units were calculated using the average molecular weights.

Table 2: Adsorption pattern of P407 onto 40, 70 and 137 nm PS NPs[797].

Particle size (nm)	Average amount adsorbed ($\mu mol/m^2$)	Average amount adsorbed (mg/m^2)	Hydrodynamic layer thickness (nm)
137	0.19±0.02	2.4±0.2	7.7±0.5
70	0.16±0.04	2.0±0.5	7.7±0.3
40	0.18±0.03	2.3±0.3	8.5±0.1

Table 3: Points of adsorption isotherm for P407 adsorption onto 40nm PS NPs showing hydrodynamic layer thickness and others[797].

Amount adsorbed (mg/m^2)	Amount adsorbed ($nmol/m^2$)	Hydrodynamic layer thickness (nm)	Average area per molecule (nm^2)	Average area per PEO chain (nm^2)	Surface coverage (%)
0.097	7.72	1.23	215	107.5	4
0.23	18.3	1.33	90.7	45.4	10
0.608	48.3	4.03	34.4	17.2	26
0.869	69	6.22	24.1	12	37
1.173	93.1	5.88	17.8	8.9	50
1.1449	115	6	14.4	7.2	63
2.293	182	8.32	9.12	4.5	100

~~Table 4: Average size of PLGA NPs coated with poloxamer F68.~~

~~Percentage of poloxamer~~	~~Size (nm)~~
~~0% (control)~~	~~199.8~~
~~0.25% F68~~	~~207.4~~
~~0.5% F68~~	~~256.3~~
~~1% F68~~	~~201.3~~

Table 4: Thickness of adlayer for different poloxamers having different EO and PO units[73].

Type of poloxamer	**Molecular block average values (mol)**			**Thickness of adlayer (nm)**
	EO	PO	EO	
P188	75	30	75	7.6
F88	97	39	97	13.2
P338	128	54	128	15.8
P407	98	67	98	15.4

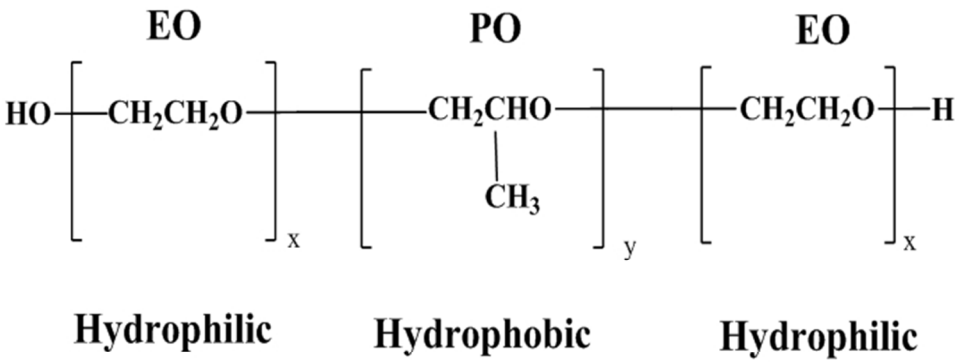

Figure 1: Structure of poloxamer.
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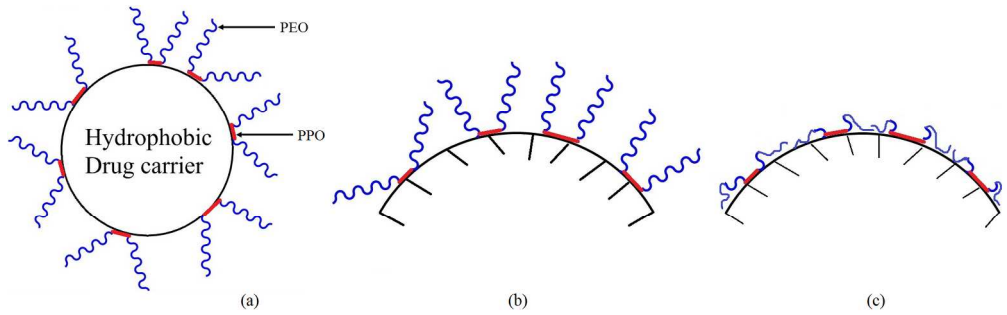

Figure 2: Schematic representation of poloxamer adsorption onto hydrophobic drug carrier surfaces: (a) PPO binds with carrier surface and PEO block extends to outer phase; (b) "brush-type" conformation and (c) "mushroom-type" conformation54.
569x179mm (96 x 96 DPI)

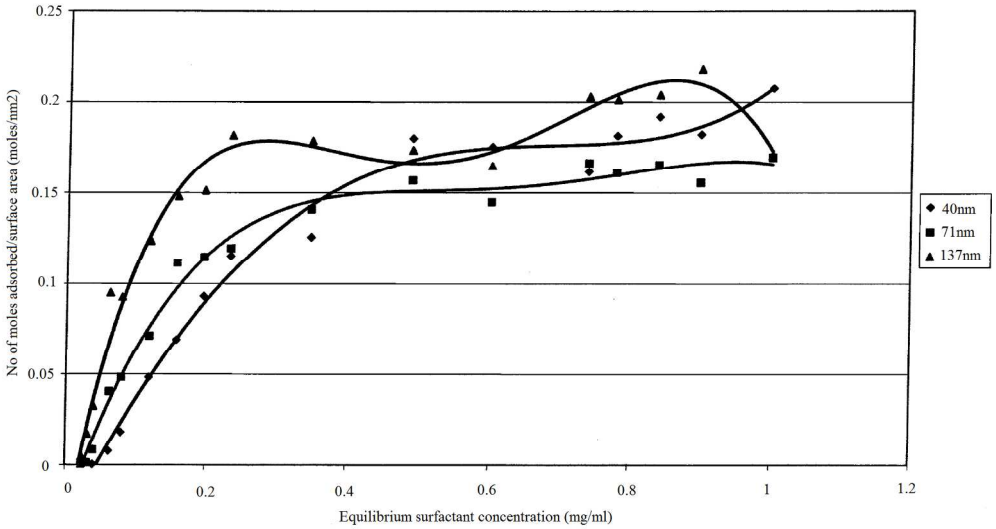

Figure 3: Adsorption isotherms for P407 on PS NPs. Particle diameters: 40 nm (♦), 71 nm (■) and 137nm (▲) (Taken from Ref. 79, with permission from American Chemical Society, Copyright 2001).
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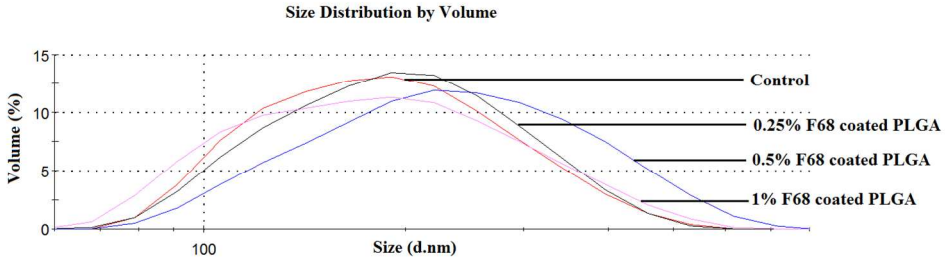

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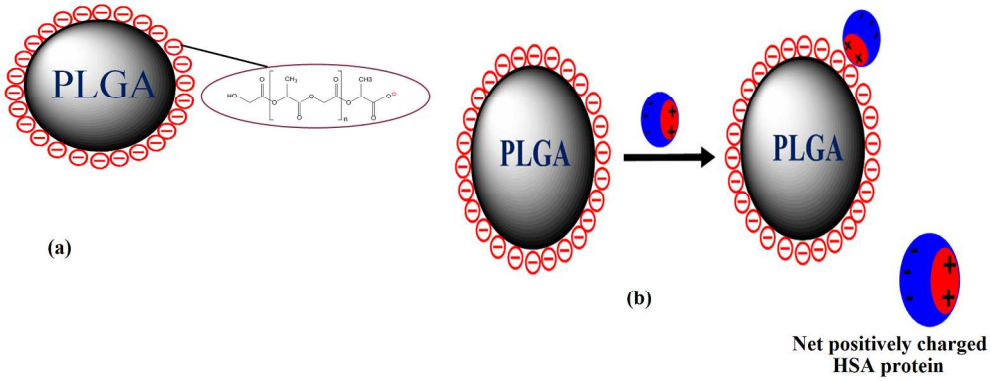

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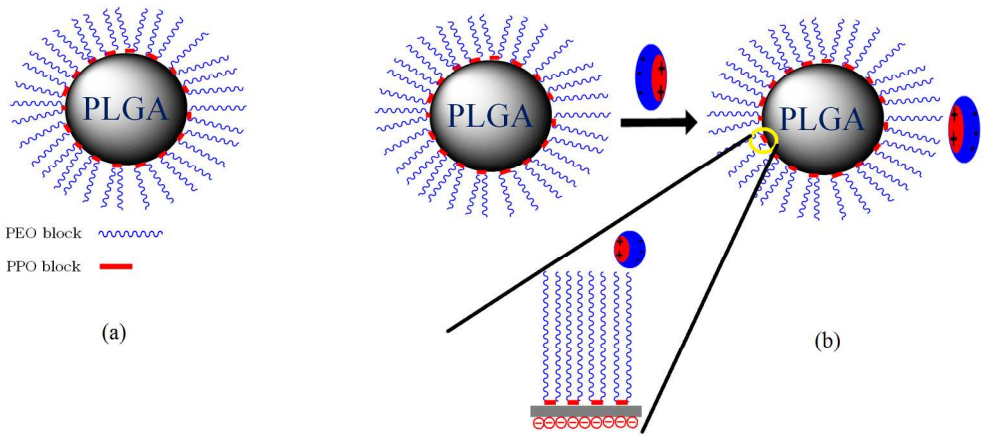
614x240mm (96 x 96 DPI)



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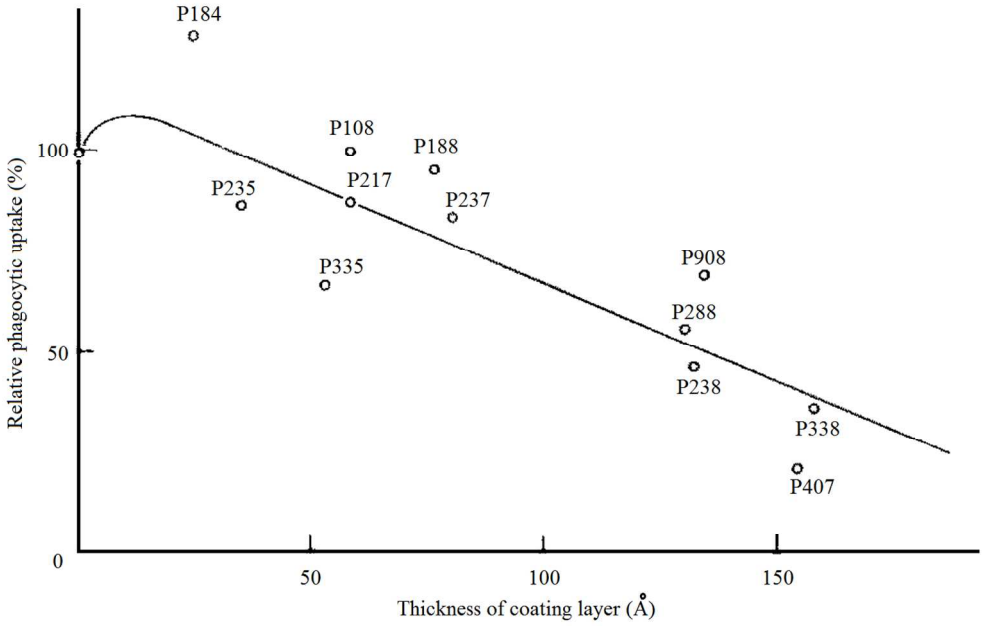

Figure 7: Relationship among thickness of the coating layer of poloxamers and poloxamine on PS particles and their relative phagocytic uptake by mouse peritoneal macrophages (Taken from Ref. 73, with permission from Elsevier, Copyright 1987).
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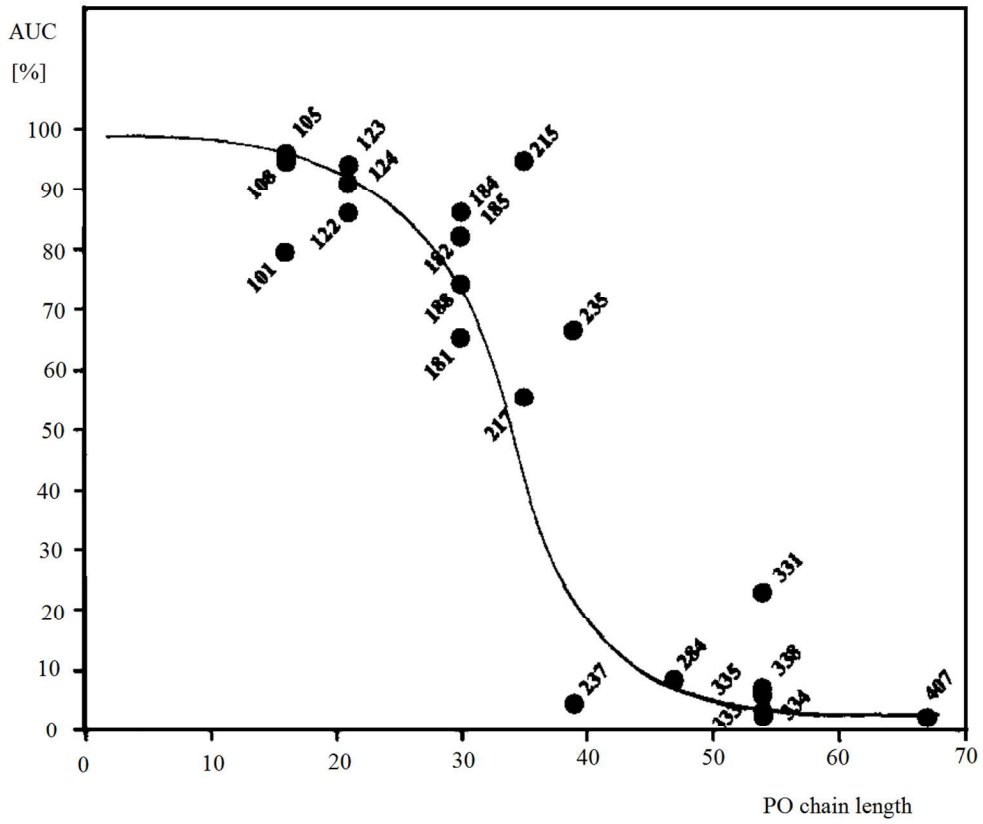

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357x306mm (96 x 96 DPI)

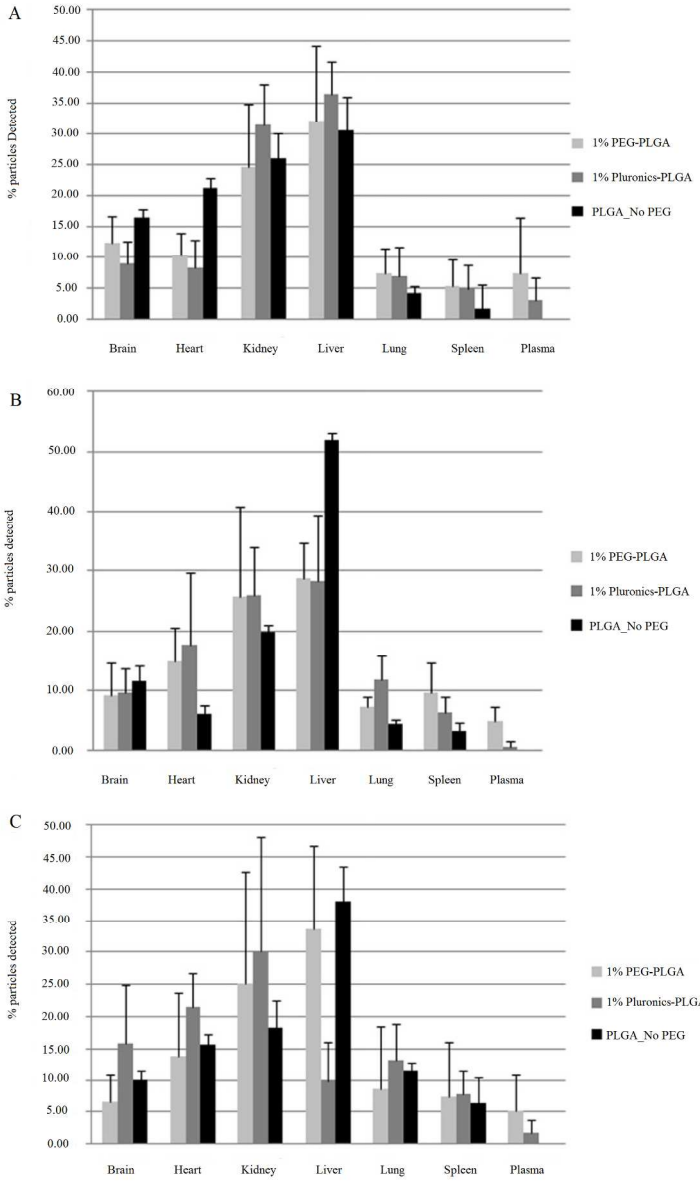

Figure 9: Biodistribution of Rhodamine labelled PLGA NPs. Days: (A) One; (B) Three; (C) Seven (after oral administration). (Taken from Ref. 90, with permission from Elsevier, Copyright 2012).
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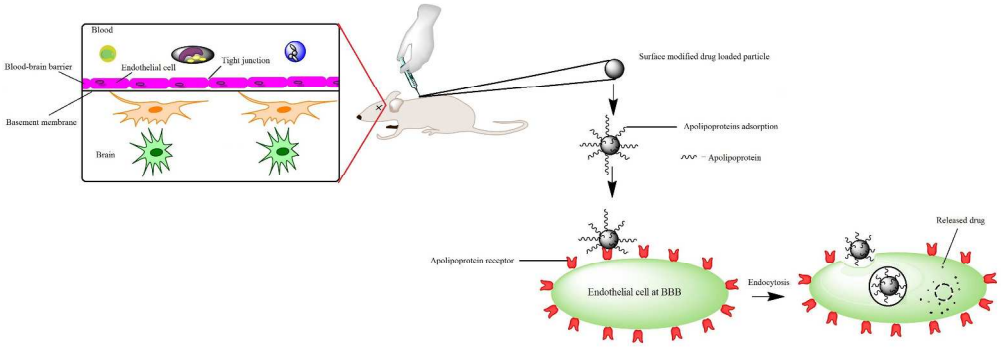

Figure 10: Schematic representation of the probable mechanism of surfactant coated NP's brain uptake. Drug releases from NPs upon endocytosis through the BBB endothelial cells.
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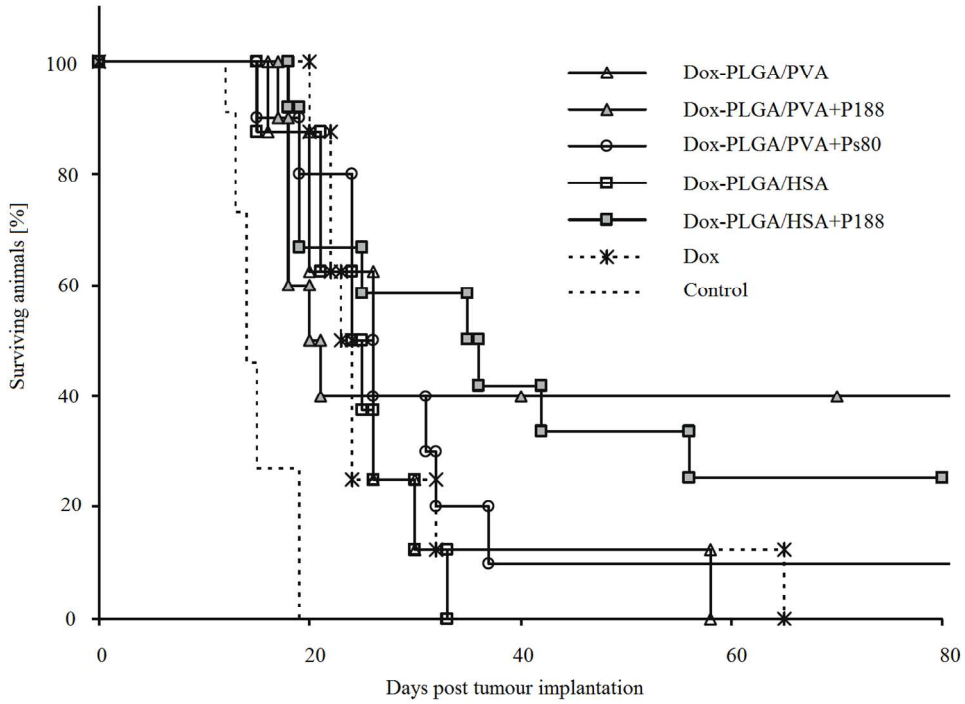

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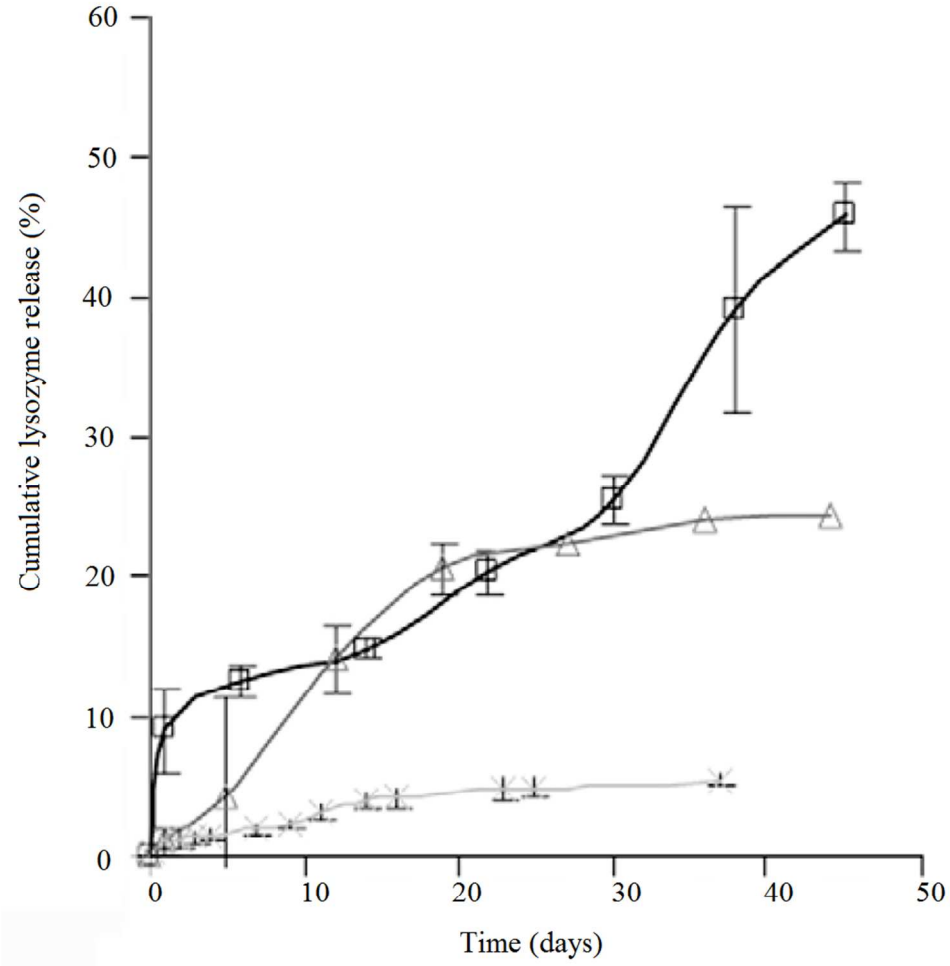
397x294mm (96 x 96 DPI)



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278x285mm (96 x 96 DPI)

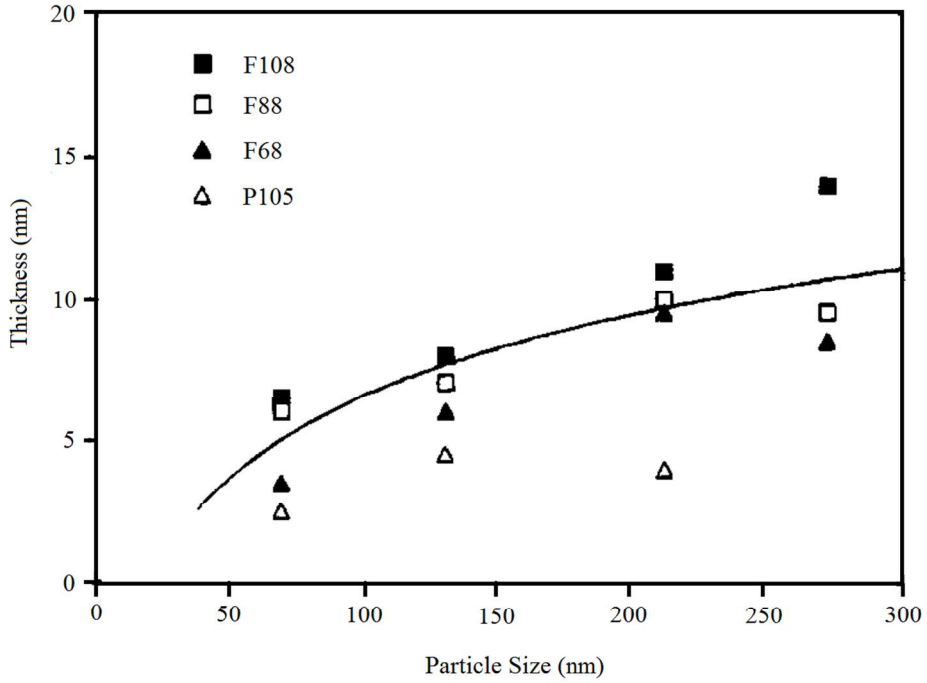

Figure 13: Curvature-dependent adlayer thickness for poloxamers (Taken from Ref. 54, with permission from American Chemical Society, Copyright 1994).
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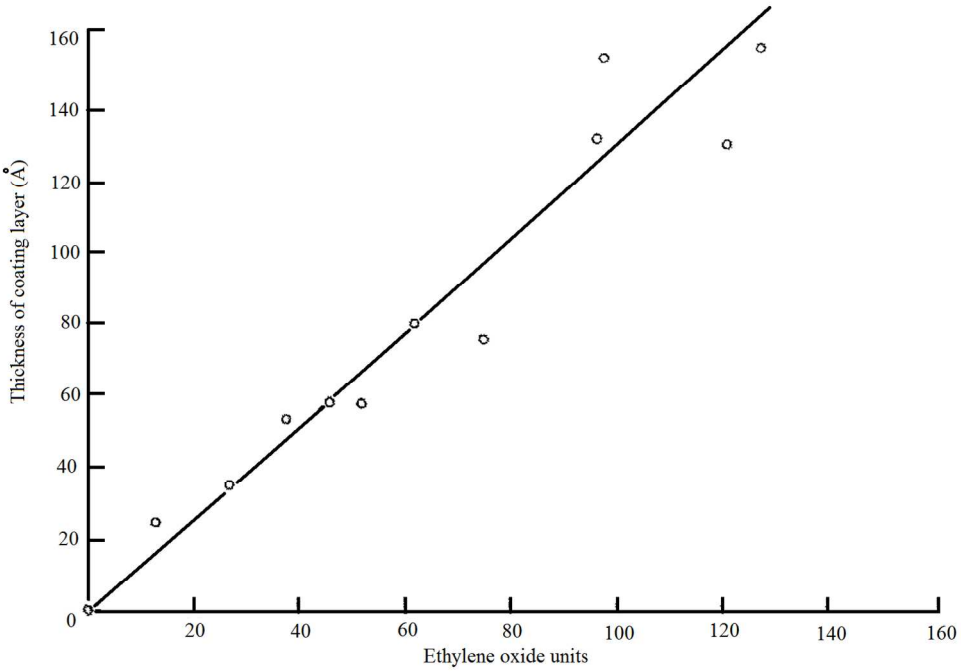

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411x285mm (96 x 96 DPI)