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Poloxamers and poloxamines in nanoparticle engineering and experimental medicine

S. Moein Moghimi and A. Christy Hunter

Poloxamers and poloxamine nonionic surfactants have diverse applications in various biomedical fields ranging from drug delivery and medical imaging to management of vascular diseases and disorders. Although this is a progressive, rapidly advancing field in biotechnology, the future will depend on the recognition and rectification of a range of toxicity issues, which have to be addressed but have frequently been ignored until now.

Poloxamers and poloxamines (Fig. 1) are also known as Pluronic® and Tetronic® macromolecules, respectively. They are a family of more than 50 different amphiphilic nonionic block polymers of hydrophobic propylene oxide (PO) and hydrophilic ethylene oxide (EO), covering a range of liquids, pastes and solids. Poloxamers consist of a central polyoxypropylene (POP) molecule, which is flanked on both sides by two hydrophilic chains of polyoxyethylene (POE). A slightly different structure is exhibited by the poloxamines, which are tetrafunctional block copolymers with four POE–POP blocks joined together by a central ethylene diamine bridge. These surfactants were first introduced in the 1950s (by BASF, NJ, USA) and since then have found a wide range of

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diverse applications in the pharmaceutical and biomedical fields (Table 1). This article addresses the recent applications of these polymers in nanoparticulate engineering and experimental medicine, as well as the problems inherent in the use of commercially available products.

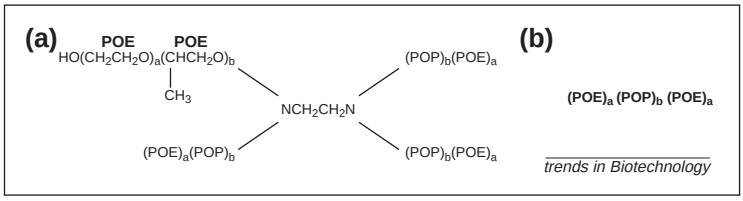

Figure 1
The chemical structure of (**a**) poloxamines and (**b**) poloxamers.

Nanoparticulate engineering: site-specific targeting and medical imaging

Long circulating particles

Poloxamers and poloxamines adsorb strongly onto the surface of hydrophobic nanospheres [e.g. polystyrene, poly(lactide-co-glycolide), poly(phosphazene), poly(methyl methacrylate) and poly(butyl 2-cyanoacrylate) nanospheres] via their hydrophobic POP centre block[1]. This mode of adsorption leaves the hydrophilic POE side-arms in a mobile state because they extend outwards from the particle surface. These side-arms provide stability to the particle suspension by a repulsion effect through a steric mechanism of stabilization, involving both enthalpic and entropic contribution[2,3]. The strength of polymer adsorption, and the resultant polymer conformation, is dependent on the proportion and the size of both the POP and POE segments, as well as on forces that include the initial nanoparticle surface charge, hydrogen bonding between the polyoxyethylene ether groups and the constituent groups on the particle surface, hydrophobic forces among the polymer chains and polymer–solvent interactions. For example, with the help of field-flow fractionation, electron-spin resonance and conventional labelling techniques, Li *et al.*[4] have accomplished the detailed analytical characterization of the adsorption complexes that are formed between poloxamers and polystyrene beads of different sizes. These studies in particular, indicate the importance of the effect of bead surface curvature on polymer chain mobility and conformation. For a given triblock polymer, it was found that both surface concentrations and adlayer thicknesses are strongly related to the particle size, such that smaller particles (sizes <100 nm) take up fewer polymer molecules per unit area than larger particles[4]. The reduced crowding around each POE chain results in thinner adlayers and higher chain mobilities. For a particle of a given size, it is the size of the hydrophobic centre block (POP) of the surfactant, rather than the size of its flanking tails, that determines the surface concentration. Thus, triblocks of similar POP size show comparable surface concentration, whereas the longer POE chains are associated with thicker adlayers, as well as greater chain dynamics[4].

Such engineered nanoparticles exhibit reduced adsorption of proteins and blood opsonins compared with uncoated particles and, as a result, resist ingestion by phagocytic scavenger cells[2,3]. This is due to the flexibility of the highly hydrated and mobile POE chains, which generate effective conformational clouds on the

Table 1. Selected examples of poloxamers and poloxamines[a]

Polymer	Average molecular weight[b]	EO units	PO units	HLB value[b]	Suggested applications
Poloxamers					
188	8400	2×52	30	29	Antithrombotic, haemorheological activities, cell membrane sealing, phagocyte activation (stimulation of phagocytosis and superoxide anion production) and neutrophil degranulation
401	2000	2×5	67	3	Nanoparticle engineering (lymphotropic particles), inhibition of multidrug resistance and adjuvant activities
402	2500	2×11	67	7	See poloxamer-401
407	12600	2×98	67	22	Long circulating particles, slow release gels, macrophage stimulation, stimulating the production of EGF
Poloxamines					
904	6700	4×15	4×17	15	Nanoparticle engineering (lymphotropic particles) and macrophage stimulation
908	25000	4×119	4×17	31	Long circulating particles and macrophage stimulation

[a]EGF, epidermal growth factor; EO, ethylene oxide; HLB, hydrophile–lipophile balance; PO, propylene oxide.
[b]Data supplied by BASF (NJ, USA).

Box 1. Splenic targeting of nanoparticles

The interendothelial cell slits (IESs) in sinusoidal spleens provide resistance to flow through the reticular meshwork[65,66]. From *in vivo* studies we know that ~20% of the total IES present anatomically allow passage of red blood cells during any 5 min period. Retention of blood cells and blood-borne particles at IES depend on their bulk properties, such as size, sphericity and deformability. These cell slits are the sites where rigid inclusions of diseased erythrocytes (e.g. Heinz bodies and malarial plasmodia) are believed to be 'pitted'[65,66].

Polystyrene particles are rigid and nondeformable. The rat spleen can filter 40–50% of the dose of poloxamine-908-coated polystyrene spheres of 220 nm within a few hours after intravenous administration at IES. Interestingly, all filtered poloxamine-coated spheres are eventually phagocytosed by the splenic red pulp macrophages, presumably because of intrasplenic loss of the coating polymer and thus the steric barrier[67]. Nevertheless, intravenous administration of poloxamine-based splenotropic particles will have numerous applications in clinical and experimental medicine. For example, in medical imaging, such strategies might allow assessment of splenic function before spleen-specific drug carriers are administered therapeutically. Splenotropic particles might also prove to be useful in the detection of abnormal spleen positions, occupying lesions and focal defects, splenic enlargement, and determination of accessory spleens following splenectomy.

In experimental physiology, splenotropic particles might be useful for assessing the circulatory dynamics and the microcirculation of the healthy spleen, and a variety of splenomegalies. For example, in healthy rats >95% of the blood travels the open route of splenic circulation (blood flows through the reticular meshwork of the red pulp or marginal zone to reach the venous system), whereas in phenylhydrazine-induced anaemic rats ~70% of the blood takes the open route and the rest travels the closed route (blood flows through a direct connection between arterial capillaries and venous vessels). The reduction in the proportion of the blood travelling via the open circulation in the spleen of anaemic rats is the result of congestion in the open route by erythrocytes. Such a decrease in both the open pulp circulation and total splenic blood flow can reduce the filtering capacity of the spleen. Indeed, this has been demonstrated for 220 nm poloxamine-coated beads in splenic congestion associated with phenylhydrazine-induced haemolytic anaemia[68].

particle surface. The most effective polymers in suppressing the recognition of hydrophobic nanoparticles (15–200 nm in diameter) by macrophages are those that contain at least 40 central PO units and adjacent POE segments consisting of at least 70 EO units (e.g. poloxamine-908, poloxamine-1508 and poloxamer-407; Ref. 1). Following intravenous administration into mice, rats and rabbits, such engineered nanoparticles were reported to remain in the systemic circulation for prolonged periods (the reported half life varies from 2 to 12 hours depending on the particle size and its surface hydrophobicity as well as the copolymer type; Refs 1,2). These prototype nanovehicles might have several clinical applications, which include medical imaging (e.g. blood pool visualization, detection of vascular malformations, selected pathologies and measurement of gastrointestinal bleeding) and drug delivery (for controlled release of therapeutic materials within vasculature, as well as in the delivery of therapeutic agents to pathologies with 'leaky' vasculature).

Splenotropic particles

By simple engineering, intravenously injected long circulatory poloxamer- or poloxamine-coated particles can be effectively redirected to sinusoidal spleens (e.g. rat and human). This simply requires a rigid nanosphere in the size range, equivalent to or greater than the reported width of splenic interendothelial cell slits in the sinus walls – usually in the order of 200–500 nm (Box 1; Ref. 5).

Conversion of 'phagocyte-prone' particles to long-circulatory or splenotropic particles *in vivo*

Interestingly, intravenously injected, uncoated polystyrene beads (which are cleared rapidly by hepatic Kupffer cells) behaved like long circulatory or splenotropic particles, depending on their size, if they are injected shortly (up to 3 h) after an appropriate dose of poloxamine-908 or poloxamer-407 (Ref. 6). The altered biodistribution profile of the beads is apparently independent of the 'hepatic-blockade' concept, which would have been caused by the administered copolymer. Polystyrene beads were suggested to acquire a coating of copolymer and/or copolymer–protein complexes *in vivo*[6]. These events explain altered tissue distribution of the beads[6,7]. In contrast to these copolymers, previous administration of poloxamer-188 fails to prevent hepatic sequestration of the beads[6]. This is not surprising, given that beads coated with poloxamer-188 are also cleared rapidly from the blood by hepatic macrophages (see also the section on long circulatory particles).

Lymphotropic nanoparticles

One of the most intriguing applications of poloxamer and poloxamine engineered nanoparticles is in the medical imaging of lymphatics, as well as in drug delivery to regional lymph nodes following subcutaneous administration. A correlation was found to exist between the length of the stabilizing POE chains of the block copolymer surfactants, and polystyrene bead (60 nm) drainage from interstitium and passageway across dermal lymphatic capillaries in the rat footpads[8]. The longer the POE chains are, the greater the reduction in particle aggregation, as well as particle interaction with interstitial elements, and thus drainage and delivery into the lymphatic system are faster. For example, 70% of the administered dose of copolymer-coated beads, composed of more than 70 EO units per POE chain (e.g. poloxamine-908 and poloxamer-407), were drained from the injection site into initial lymphatics within two hours, escaped clearance by macrophages of the regional nodes, and reached the systemic circulation where they remained in the blood for prolonged periods[8]. Such prototype vehicles might have applications in visualizing the lymphatic chain and assessing lymph flow by an imaging modality (e.g. scintigraphy and magnetic resonance imaging), as well as for controlled delivery and release of bioactive molecules into the systemic circulation. However, for efficient and

rapid delivery to lymph node macrophages, interstitially administered nanoparticles must be coated with copolymers that consist of POE chains of 5–15 EO units in length (Fig. 2). The steric barrier imposed by short POE chains not only suppresses particle aggregation at the interstitial sites but also allows surface opsonization processes to proceed either in the interstitium or lymph, thus aiding macrophage recognition[8].

Slow-release gel systems

One particular copolymer, poloxamer-407 exhibits reversible thermal gelation in aqueous solution at concentrations >20% w/v. Therefore, a solution of poloxamer-407 is liquid at low temperatures but rapidly gels at ~25°C. Such systems have been administered subcutaneously for the slow release of peptides and therapeutic proteins, which include interleukin-2, urease and human growth hormone[9–11]. Following administration, the gels slowly dissolve and release the entrapped protein molecules over a period of 1–2 days. A substantial fraction of this poloxamer eventually undergoes renal excretion.

Poloxamer gels also satisfy the major characteristics of an optimal dressing material for early management of skin burns[12]. Indeed, poloxamer-407 gels with entrapped bacteriostatic or bacteriocidal agents have been used as an artificial skin in the treatment of third-degree burns[13]. Because of its surfactant nature, the poloxamer also cleanses the wound of tissue detritus. It has been suggested that poloxamer-407 can significantly increase the rate of wound healing possibly by stimulation of endogenous production of epidermal growth factor[13].

Immunological activities

Adjuvant activity and vaccine formulation

Several poloxamer and poloxamine copolymers in micellar or aggregate forms have proved to be powerful adjuvants for increasing antibody formation to a variety of antigens following subcutaneous administration[14–16]. Similar results have also been obtained with oil-in-water emulsions stabilized by such copolymers[17,18]. The adjuvant activity of copolymers has been suggested to be influenced by both their size and POE content; maximal activity is reported for copolymers with low POE content (5–15%) and a molecular size of 10–12 kDa (Ref. 16). The POE content is also believed to regulate the type of immune response. Copolymers with 10% POE were successful in significantly augmenting Type 2 helper T-lymphocyte responses, whereas copolymers with lower POE contents increased both Type 1 and Type 2 responses[16]. The mechanisms behind these observations still remain to be determined. Nevertheless, it seems that copolymers with low hydrophile–lipophile balance (HLB) values (an index of the relative strengths of their hydrophilic and hydrophobic portions) can channel protein and peptide antigens into the major histocompatibility complex (MHC) class I pathway of macrophages and antigen-presenting cells (APCs) by destabilization of the plasma membrane or the membrane of internalized vacuoles (pinosome or phagolysosome). The more hydrophilic copolymers, presumably have a reduced ability to disrupt lipid bilayers, and tend to increase the efficacy of antigen delivery to the MHC class II pathway and augument primarily Type 2 responses. The increased delivery of antigen to APCs (also see previous section on lymphatic delivery) and the subsequent activation of these cells by copolymers might also explain their adjuvant activity. For example, poloxamer-231 was reported to enhance the expression of

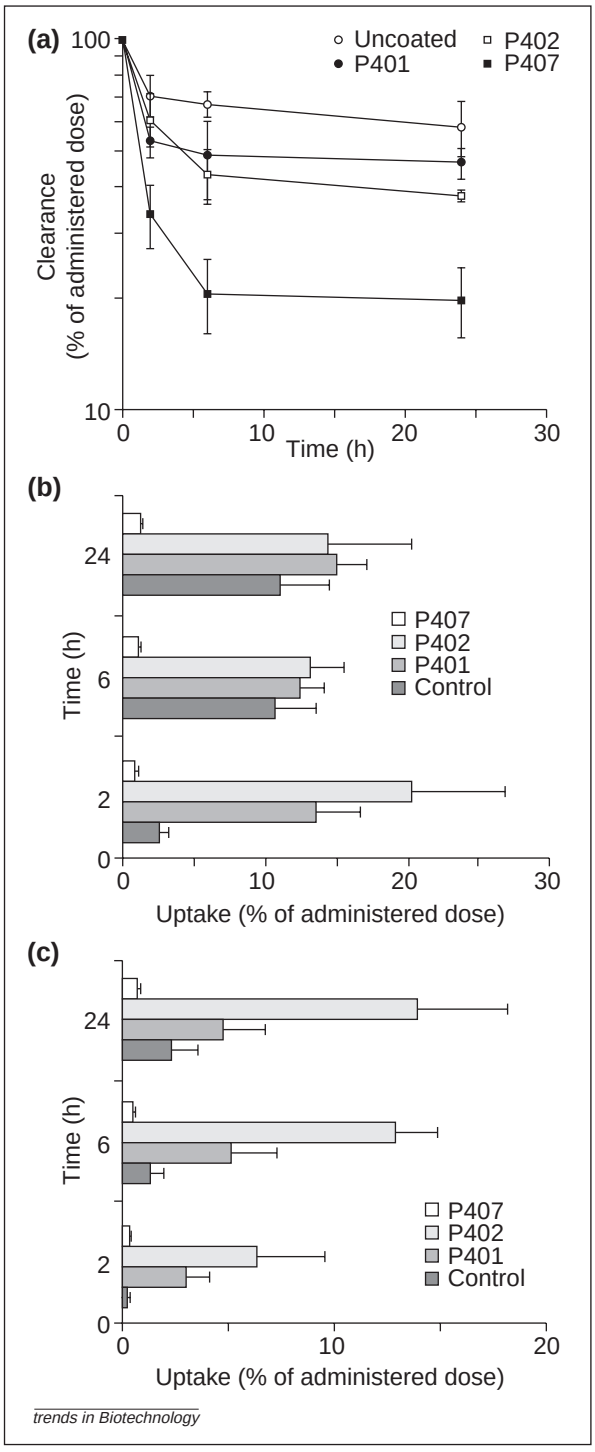

Figure 2

The kinetics of (**a**) drainage and (**b,c**) regional lymph node uptake of uncoated and poloxamer-conditioned polystyrene beads (Poloxamer-401, -402 and -407) following subcutaneous administration into rat footpads. Popliteal nodes are referred to in (b) and iliac nodes are referred to in (c). Poloxamer-401- and -402-coated particles spread well from the injection site and provide good uptake in the regional nodes.

macrophage MHC class II (Ia) molecules in mice following intraperitoneal administration in the absence of mature lymphocytes[19]. Poloxamer-231-induced macrophages were also primed for the secretion of superoxide anions, which can be stimulated by interferon-γ and lipopolysaccharide to lyse tumour cells[19]. A newly synthesized poloxamer member (CRL-1072) was also shown to stimulate the production of interleukin-8, tumour necrosis factor-α and granulocyte-macrophage colony-stimulating factor by macrophages in a dose-dependent manner[15]. Other members, such as poloxamer-188, poloxamer-407 and poloxamine-908, can stimulate phagocytic activity of human neutrophils and rodent tissue macrophages[20–22]. Such stimulated macrophages even have the ability to phagocytose long-circulatory or 'phagocyte-resistant' particles[21,22]. It therefore appears that the adjuvant activity of copolymers is initiated and directed primarily through macrophages and the cytokines that these cells subsequently produce.

Complement activation

Numerous studies have demonstrated that poloxamer and poloxamine copolymers, regardless of their HLB values, can activate the human complement system causing conversion of complement factor 3 (C3) through the alternative pathway[18,23]. Of particular interest is poloxamer-188, which has been used for stabilizing Fluosol-DA, a perfluorocarbon artificial blood substitute emulsion. In a human trial, the copolymer content of Fluosol-DA was responsible for activating the complement system and for damaging the pulmonary endothelium[23]. Interestingly, these deleterious effects were not observed in a Japanese clinical trial[23]. It is possible that copolymers, depending on their structure in plasma, activate human complement by the classic pathway as a result of the binding of naturally occurring anticholesterol antibodies to the hydroxyl-rich surface of copolymer micelles or copolymer-coated particles, because the epitope that they recognize contains a hydroxyl group[24]. Although these antibodies are abundant in most human plasma their level varies considerably among individuals.

To date, no correlation has been found between copolymer structures and the degree of complement activation. The mechanisms for these observations still remain to be elucidated but might also be related to the degree of the purity and homogeneity of the copolymers, as well as polymer degradation products. So far, evidence in support of complete polymer purity and homogeneity has not been demonstrated. Thus, the presence of antioxidants and traces of low molecular weight impurities, such as acetaldehyde and formic acid in commercial preparations of copolymers, might be responsible for these observations.

Inhibition of multidrug resistance

A major problem in chemotherapy of many human malignancies is the development of drug resistance[25,26]. Multidrug resistance is the ability of tumour cells to develop resistance to the cytotoxic effects of several chemically unrelated anticancer drugs. This phenomenon has been associated with the overexpression of membrane transport proteins belonging to the superfamily of the ATP-binding cassette[25,26]. Examples include the P-glycoprotein and a newly identified 190–210 kDa multidrug-resistance-associated protein (MRP), which has different substrate characteristics and different inhibitor specificity from P-glycoprotein[25–28]. These drug efflux transport proteins are also expressed in normal tissues, such as the epithelial cells of the intestine (P-glycoprotein) and brain microvessel endothelial cells (P-glycoprotein and MRP), where they have an important protective and detoxification function[27,28].

A classic approach for overcoming multidrug resistance in experimental cancer chemotherapy involves the use of a P-glycoprotein inhibitor coadministered with the anticancer agent. Interestingly, recent studies have demonstrated that poloxamer block copolymers can act as potent inhibitors of both P-glycoprotein and MRP efflux systems in several cancer cells, as well as human intestinal epithelial cells and bovine brain microvessel endothelial cell monolayers[29–33]. However, the extent of inhibition is dependent on HLB properties and the size of poloxamer molecules. The most effective copolymers were those with long POP segments and short POE arms at concentrations below their critical micelle concentration (i.e. block copolymer single chains or unimers); this implies a mechanism based on interaction of the hydrophobic POP segment of copolymers with the cell membrane[33]. It is probable, therefore, that incorporation of selected poloxamers in pharmaceutical preparations or in combination with particulate drug delivery vehicles might lead to increased drug bioavailability, as well as drug accumulation in selected organs (e.g. the brain); and might overcome the problem of drug resistance, which limits the effectiveness of many therapeutic agents. Indeed, a recent study has successfully demonstrated gastrointestinal absorption of the aminoglycoside amikacin following oral administration to mice in the presence of poloxamer CRL-1605 (Ref. 34). For systemic activity, amikacin cannot be delivered orally probably because of the efflux of drug by the P-glycoprotein pump in the brush border of the intestine.

Applications in vascular medicine

Commercial grade poloxamer-188 has been reported to exhibit haemorrheological, antithrombotic and neutrophil-inhibitory properties, presumably via hydrophobic interaction with the surface of blood cells, vascular endothelial cells, and/or alteration of plasma protein properties (particularly fibrinogen, soluble fibrin and albumin)[35–42]. Indeed, poloxamer-188 at a clinically relevant concentration, decreases erythrocyte aggregation, and reduces blood viscosity in a concentration and shear-dependent manner[35]. Therefore, poloxamer-188 appears to lubricate cell surfaces and thus reduce the friction caused by cell–cell adhesion. Experimental and clinical studies[37,38,41] have confirmed that intravenous administration of poloxamer-188 is of significant benefit in the management of stroke and myocardial infarction, in which poloxamer-188 accelerates thrombolysis (presumably via interaction with the adhesive fibrin clots), reduces reocclusion and ameliorates reperfusion injury[41]. It is also conceivable that the therapeutic effects of poloxamer-188 in ischaemic injury might be, in part, because of inhibition of platelet aggregation in the microcirculation. Poloxamer-188 also inhibits leucocyte infiltration into the myocardium

in models of acute myocardial infarction and acute lung injury[37]. Recently, RheothRx (CytRx Corporation, GA, USA), a clinical form of poloxamer-188, was withdrawn from a multinational Phase III Core trial because of renal toxicity, which was suggested to be caused by the presence of unspecified impurities in the RheothRx preparation[39].

Some studies have highlighted the therapeutic potential of poloxamer-188 in the management of vaso-occlusive crisis associated with sickle cell disease[40]. For example, in a pilot study, RheothRx was reported to significantly reduce the total analgesic use and pain intensity in patients with sickle cell disease, thus resulting in a shorter duration of painful episodes and of total days of hospitalization[40]. Again a possible lubricating effect of poloxamer on cell surfaces (blood cells and vascular endothelial cells) was suggested as an explanation for these observations. By contrast, a more recent Phase III trial with a fractionated sample of poloxamer-188 (Flocor™) was not successful in demonstrating any clinical benefit[43]. A possible explanation for the failure of this formulation might lie in the fact that an essential component(s) was removed from the copolymer during the fractionation process.

From the preceding discussion, it appears that the long-term application of such copolymers in vascular medicine could also affect the host defence system, in particular the induction of macrophage activation and production of proinflammatory cytokines (see 'Immunological activities' section), and therefore their use should be viewed with caution. This also extends to the application of those copolymers (e.g. Pluronic® L-81) that have been reported to modify the hepatic secretion of very low-density lipoprotein–cholesterol particles and thus reduce the incidence of atherosclerosis (since macrophages play a key role in development of atherosclerosis).

Cell membrane sealing

Several copolymers, such as poloxamer-188 and poloxamine-1107, are capable of sealing electroporated and radiopermeabilized cell membranes in a dose-dependent manner; preventing rapid exhaustion of high-energy cellular compounds and thus resultant cellular necrosis[44,45]. These observations further support the notion that these polymers are ineffective membrane-solubilizing agents and therefore adhere to the cell surface or damaged spots in the membrane. These copolymers might be of potential therapeutic use for victims of electrical trauma and high doses of ionizing radiation.

Organs removed for transplantation might suffer from the tissue damage caused by ischaemia-reperfusion. Because of their effect on blood flow, as well as cell membrane sealing, poloxamers and poloxamines might find an application in transplantation medicine. To reduce such ischaemic tissue damage, the perfusion media, which can also be whole blood or plasma, could be supplemented with surface-active copolymers, thus reducing the damage to the tissue.

A poloxamer for your thoughts

The occurrence of pharmacological and immunological responses *in vivo* raises several questions that must be addressed regarding the commercially available poloxamers and poloxamines, if they are to be used safely and be amenable to both scientific and clinical rigor. These include:

- What impurities and/or additives are present in commercial polymers, are they immunogenic or do they exhibit pharmacological activity?
- How stable are the polymers to degradative processes once the package is opened?
- What is the role of the different molecular weight polymers in each sample in reported biological activity?
- Are the polymers structurally modified *in vivo* and what is the effect of such changes on the humoral and/or cellular immune system (e.g. complement activation and cytokine release), as well as the haemostasis system?

Low molecular weight contaminants are present in these polymers from their initial synthesis[46–48]; post-polymer processing will involve the addition of an antioxidant as these polymers undergo oxidative decomposition[49,50] processes *ex-vivo*. However, the concentrations and types of additive supplied in these polymers is proprietary information that has not been made readily available. The inherent nature of an antioxidant gives it high reactivity and low stability, the decomposition products of which are highly complex and might also play a role in immune-system modulation. An example of an antioxidant used to stabilize poloxamers is butylated hydroxytoluene (ditertiobutyl-cresol)[48], which is known to cause hypersensitivity reactions when applied topically[51]. It is possible that antioxidants of similar structure might initiate several immunological events once administered parenterally.

Poloxamers and poloxamines are known to undergo both photo- and thermally induced oxidative degradation[49,50]. Photooxidation might result from poor storage of these materials if they are exposed to light sources. The first step in photo-oxidation is abstraction of hydrogen on the polymeric backbone by a free radical formed by photonic excitation of chromophoric species. A macroradical is generated, which reacts with oxygen and leads to the formation of a peroxy radical. This forms a hydroperoxide following abstraction of a labile hydrogen atom. Hydroperoxides can undergo both thermal and photochemical decomposition to give alkoxy and hydroxyl radicals, which can initiate further oxidation reactions with the polymer. Thermal oxidation can occur in either the absence or presence of oxygen and is dominated by the initial formation of hydroperoxides, which form primarily at secondary carbons on the polymer backbone. The presence of oxygen will accelerate the reaction[52]. The volatile products of thermal degradation include acetaldehyde, acetone and methanol. High temperature and pressure conditions used in copolymer synthesis[46] might induce some degree of thermal degradation; this offers a possible explanation for polymeric contaminants in commercial samples. The official monograph for poloaxamers in USP (Ref. 53) states under 'packaging and storage' to 'preserve in tight containers'. It is possible that further polymer degradation might be enhanced not only by moisture but also by atmospheric oxygen, prolonged exposure to light, and elevated temperatures.

Five poloxamers are listed in the current USP (Ref. 53), and include poloxamer-188 and -407, which

are being widely researched for their use in the clinical environment. The average molecular weight copolymer distribution, which conforms to the USP requirement for poloxamer-188, is 7680–9510 Da (average MW difference = 1830 Da) and for poloxamer-407 is 9840–14 600 Da (average MW difference = 4760 Da). It is clear from currently available publications that the composition of these commercially available polymers is relatively unknown and ignored. To date, it has not been convincingly demonstrated whether the lower molecular weight copolymer fractions have the same *in vivo* activity as the higher molecular weight fractions. The current USP methodology does not take into account the possibility of variable types of polymer (e.g. homopolymers of POP and POE) being present within commercial samples. An accurate method for the assessment of the presence of varying polymer populations within a sample uses aqueous gel permeation chromatography (GPC); this is hindered by the fact that the use of the technique is relatively new and not many directly applicable standards are available for comparison with these polymers. Examination of three commercial polymer samples (supplied by BASF, NJ, USA) using GPC revealed the presence of a range of different molecular weight polymers in each of the samples, which, in some cases, did not conform to the suppliers' listed average molecular weights[54] and indicated the presence of contaminant homopolymers (A.C. Hunter and S.M. Moghimi, unpublished results). The question thus arises as to the reproducibility of the published data in this field, using these compounds if there is batch-to-batch variation. A study investigating such parameters has not yet been fully undertaken. Evidence in support of variable biological activity from different suppliers has been observed with poloxamer-407 (Ref. 55). In this case, only one of three samples of the poloxamer was found to be capable of directing model polystyrene colloids to sinus endothelial cells of bone marrow in rabbits. A range of varying bimodal molecular weight distribution profiles were observed following GPC analysis of the same poloxamer type from each different supplier. Nuclear magnetic resonance (NMR) spectroscopy also indicated variable EO content. Another example is Flocor™ (CytRx Corporation, GA, USA), a defined fraction of poloxamer-188 (MW 8964 Da, polydispersity 1.0280) obtained by GPC (Ref. 56). This copolymer was shown to reduce nephrotoxicity by 68% in a clinical trial of unspecified patient number, when compared with RheothRx (http://www.cytrx.com/FLOCORBroch.html). In this case, clearly the isolation of a specific molecular weight fraction only reduced the total amount of nephrotoxicity observed, it did not remove it totally. This might be achieved by further purification steps or it might be found that such copolymers are inherently toxic to specific individuals.

Attempts have been made to remove the contaminants from these polymers. One method uses the passage of the polymer through a column containing silica and amberlite resin[47]. Curiously, these investigators did not use NMR spectroscopy or any other technique to identify the presence of the polymer post-processing, but did find a reduction in immune reactivity in the recovered solution. Another method that has been employed to purify a poloxamer-188 is supercritical fluid fractionation[48] (SFF). However, the stability of the poloxamer following SFF (CO_2 at 160 atmospheres and 40°C) was questionable, with evidence of macromolecular cleavage. They suggested that a mixture of intact surfactant, POE monomers and POP monomers was produced. Preparative GPC was not used to isolate the monomers and this hypothesis was confirmed with supporting spectroscopic evidence.

The available toxicology LD50 data[54] for poloxamers and poloxamines is based on oral and dermal dosing, and is less than 5 g kg^{-1} body weight. The kinetics and fate of radiolabelled poloxamers following intravenous and oral administration has been determined in rats and dogs[57–59]. These studies have indicated that the primary route of polymer excretion is renal and that the minor route is biliary. Rodgers *et al.*[59] have demonstrated that following intraduodenal infusion of a radiolabelled analogue of Pluronic® L-81 (poloxalene 2930), ~20% of an infused dose was recovered in bile, which indicates that hydrophobic poloxalenes can be adsorbed and perhaps metabolized by the liver. Radiolabelled poloxamer-188 has been found in all organs, particularly in the liver, lung and muscles, 24 h post intravenous administration into the dog[58]. Long-term effects of accumulated polymers in organs are unknown. Furthermore, no studies have so far determined to what extent these copolymers are excreted intact or in a modified form compared with the starting material.

Copolymers of EO and PO at varying ratios have been shown to undergo bacterial degradation[60]. In general, the rate of biodegradation was irrespective of copolymer ratio but was found to be dependent on molecular weight; the greater the molecular weight, the lower the rate of biodegradation as determined using GPC. This might have implications in medicinal formulations, that are designed for use against bacterial infection, where pathogenic organisms might be able to use poloxamers and poloxamines as a carbon source. A loss of synergistic activity of the poloxamer in combination with antitubercular drugs in both cell lines and murine models has been reported[61] with changes in either the POE or POP chains, which reduce or abolish the synergistic activity of the surfactant. As previously mentioned, after oral administration selected copolymers can inhibit P-glycoprotein; if commensal gut organisms are capable of degrading these macromolecules would the metabolite(s) also have the ability to initiate and/or potentiate inhibition of P-glycoprotein? Furthermore, it is also known that following oral administration small amounts of certain poloxamers (e.g. Pluronic® L-81) can inhibit intracellular transport of chylomicrons by the enterocytes[62].

Future prospects

Clearly, the purification and characterization of poloxamers and poloxamines for use within the medical and pharmaceutical areas has only undergone a cursory examination. It is imperative that those involved in this field must become aware of the potential hazards lurking within polymer toxicology. This will only be achieved if multidisciplinary groups are formed to tackle the challenges inherent in this work; groups must include immunologists, toxicologists and polymer chemists to add an informed knowledge base into this arena. It would also be naïve to think that the problem

of polymer toxicity is restricted to poloxamers and poloxamines. Other structurally related materials include poly(ethylene glycols), Cremophor-EL (ethoxylated emulsifiers), poly(lactide-co-glycolides) poly(lactide-co-glycolide)-poly(ethylene glycol) and POE-polybutadiene-POE. An example of a fatal clinical toxicity was encountered with paclitaxel (Taxol®), which was linked to its vehicle Cremophore-EL (e.g. complement activation and histamine release) (Refs 24,63,64).

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Animal-cell damage in sparged bioreactors

Yusuf Chisti

The gas sparging of culture broth causes damage to suspended animal cells. However, despite this, sparged bioreactors remain the preferred means of cell culture because sparging is a robust method of supplying oxygen, especially on a large scale. This article examines the underlying mechanisms involved in bubble-associated cell damage and the methods available for controlling such damage.

Animal cells cultured in bioreactors are widely used to produce various therapeutic proteins, vaccines and diagnostic monoclonal antibodies[1,2]. Cells need oxygen to grow and thrive, and many schemes have been developed to ensure that the cells receive sufficient levels of oxygen[2–4]. Although many options are available for supplying oxygen, sparging of the culture broth with a gas mixture remains the most practicable method of supplying oxygen[4,5], especially in large-scale culture[6–8]. (Sparging, or submerged aeration, is the process of bubbling air or another gas through a relatively deep pool of the culture broth, usually inside a bioreactor. A sparger is the device through which the gas enters the bioreactor.)

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Of the many kinds of cell-culture system available[2], stirred tanks and air-lift bioreactors are the most commonly used in commercial processes, and they both rely on sparged aeration. This situation is not likely to change in the foreseeable future because direct sparging is effective and simple. However, sparging damages animal cells. This article examines the underlying mechanisms of cell damage in sparged bioreactors and the methods that might be used to protect the cells.

Bubble-associated damage

Cell lines differ tremendously in their sensitivity to aeration[9]. In bubble-free media, mouse-cell lines are more sensitive to turbulence than human and insect cells[4]. However, in sparged bioreactors, mouse hybridomas are generally more robust than insect cells (e.g. the Sf9 cell line from *Spodoptera frugiperda*[10]). In all

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