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The effect of adsorbed coats of Poloxamers 237 and 338 on the in vitro aggregation and in vivo distribution of polystyrene latex (PSL) particles

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Summary

Poloxamer-coated PSL has been used to examine the effect of adsorbed hydrophilic layers on the biological dispersion of intravenously injected hydrophobic particles. The localisation of uncoated particles in the lungs and liver is caused by protein adsorption and aggregation, which has been verified by in vitro observations. Increasing the thickness – and presumably the packing – of the adsorbed polymer layer results in the redistribution of particles from the lungs to other tissues.

Data are becoming available to show that the biological dispersion of intravenously injected colloids is dependent on interfacial physicochemical events (Tomlinson, 1987). Hydrophobic colloids are avidly cleared by cells of the mononuclear phagocytic system (MPS), notably the liver and spleen, after adsorption of plasma proteins (Bradfield, 1984). Adsorbed hydrated polymer layers can reduce protein adsorption (Norde, 1984; O'Mullane et al., 1988) and, as we show here, particle aggregation. The hydration effect is enthalpic in origin and the resulting stabilisation of the colloidal particles against aggregation is determined by osmotic and mixing components which are entropically derived (Ottewill, 1977). The effectiveness of the hydration shell in stabilising the particles is governed by the physicochemistry of the stabilising polymer, the thickness of the adsorbed coat and the density of the surface coverage (Napper, 1983; Tomlinson, 1987). There is also evidence from studies on ideal systems that the free polymer in solution influences the stabilisation of particles in solution (Rukenstein and Rao, 1986).

Poloxamers are A-B-A block copolymers where A is poly(oxyethylene) and B is poly(oxypropylene). These were first used by Illum, Davis and their co-workers (1982, 1983, 1984) in order to measure the effect of the adsorbed layers on the avoidance of MPS clearance. We have selected Poloxamer 237 and 338 for studies of the effect of the polymer on the aggregation and biological dispersion of particles as they differ in the relative

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molecular weights of the two blocks (Douglas et al., 1985). The Poloxamers were adsorbed onto PSL particles under conditions previously defined by Kayes and Rawlings (1979); the plateau-adsorbed layer thickness from the adsorption isotherm was found to be 12.8 nm for Poloxamer 237 and 18.5 nm for Poloxamer 338.

We have previously shown that the adsorption of a single plasma protein, namely fibrinogen, onto Poloxamer-coated PSL particles is reduced with respect to uncoated particles (O'Mullane et al., 1988). The effect of the adsorption of fibrinogen on the aggregation of the PSL has been monitored using the turbidimetric method. Samples were mixed at a speed of 500 rpm and maintained at a temperature of 37°C. The turbidity of the solution was recorded at a wavelength of 605 nm, and the output was measured using a pen recorder at a chart speed of 1 mm/min. It can be seen from Fig. 1 that coated PSL particles remain stabilised under conditions that cause uncoated PSL to aggregate readily. At increasing concentrations of fibrinogen, Poloxamer 237-coated particles exhibited a dispersed aggregation in solution which was not apparent for the Poloxamer 338-coated particles under similar conditions (Fig. 2). In order to demonstrate that the stabilising effect of Poloxamer 338 was due to an adsorbed coat and not merely the presence of Poloxamer in solution, fibrinogen was added to a suspension of uncoated latex particles together with an amount of Poloxamer equivalent to the amount added in the other experiments. In this case, the particles aggregated in a manner similar to that of uncoated particles (Fig. 3).

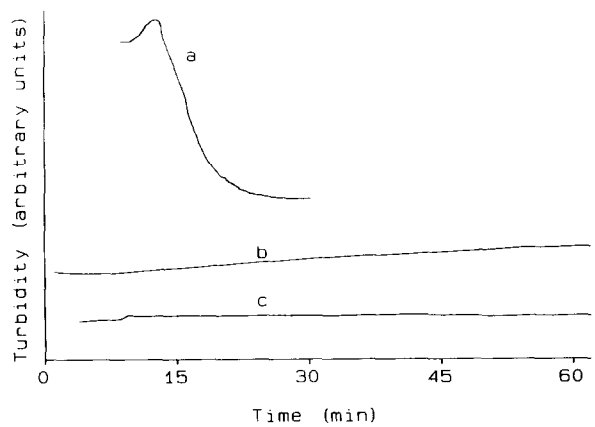

Fig. 1. The effect of the addition of 0.5 mg fibrinogen to (a) uncoated, (b) Poloxamer 237-coated and (c) Poloxamer 338-coated latex. The traces for (b) and (c) have been off-set for clarity purposes.

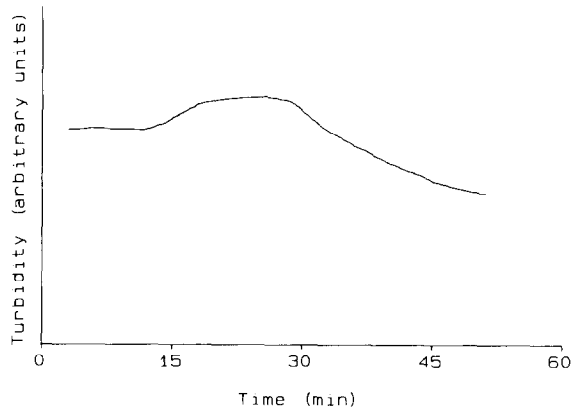

Fig. 2. The effect of the addition of 1 mg of fibrinogen to Poloxamer 237-coated PSL. The Poloxamer 338-coated latex resulted in a flat base-line (as for Fig. 1c).

A tissue extraction assay was developed in order to quantify the distribution of fluorescently labelled PSL. Blood and other tissue samples were homogenised for 2 min in 21 ml of dichloromethane : methanol (1 : 2 v/v). The homogenates were centrifuged at $750 \times g$ for 10 min at 4°C, and the fluorescence in the supernatants measured at an excitation wavelength of 450 nm and an emission wavelength of 540 nm. The extraction procedure was validated for blood and tissue samples from control animals which were injected with doses of particles ranging from 0.0025 to 2.5% solids (w/v), thus establishing a calibration curve for each tissue.

PSL particles (diameter 800 nm), were injected into the tail vein of groups of mice ($n = 4$). The animals were killed after 10 min by rapid decapitation and the levels of fluorescence in the blood, liver, spleen and lungs were assayed.

Fig. 4 gives the results on the in vivo distribution of uncoated, Poloxamer 237-coated and Poloxamer 338-coated particles 10 min after their intravenous administration into mice. For the uncoated particles, a third of the injected dose is found in the lungs, and the remainder in the liver.

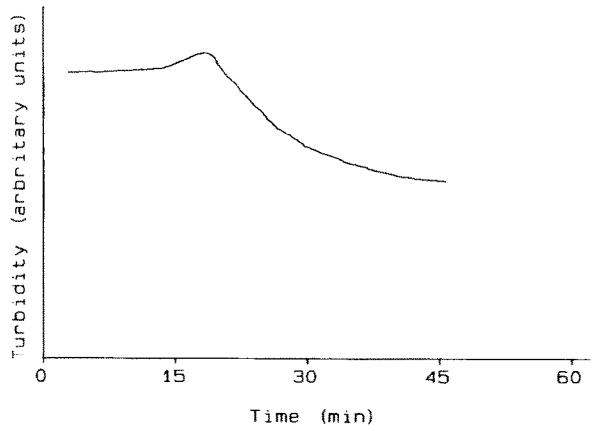

Fig. 3. The effect of the addition of 0.5 mg fibrinogen to a mixture of PSL and Poloxamer 338 without allowing time for polymer adsorption to the PSL.

This can be understood with reference to the in vitro data, where fibrinogen caused extensive aggregation of the particles. If this were to occur in vivo, the particles would become physically trapped in the first capillary bed which they encounter, i.e., the lung (which has a minimum capillary lumenal diameter of around 4 μm (Tomlinson, 1983)).

For the Poloxamer 237-coated particles the dose is found virtually all (approx. 80%) in the liver, with small amounts found in the spleen, lung and blood. This is consistent with the in vitro data which show that these particles do not extensively aggregate. The in vivo data show that these particles are insufficiently protected by the hydrophilic coat to avoid opsonisation and clearance by the liver.

In the case of the Poloxamer 338-coated particles, approx. 80% of the injected dose remains in the bloodstream after 10 min. This shows that as well as avoiding aggregation these particles are able to avoid MPS clearance at least up to this time point.

In conclusion, we have shown, using a simple turbidimetric procedure, that we can examine factors affecting the aggregation of particles in vitro and that these data can be readily compared with the results giving particle distribution profiles in vivo. Specifically, there appears to be a correlation between fibrinogen adsorption in vitro and the behaviour of the particles in vivo. It is clear from our analysis that effective steric stabilisation reduces protein adsorption, prevents protein-induced particle aggregation and reduces particle-cell interactions. We suggest, therefore, that a routine test of biological stability and fate in vivo could be achieved by measuring the aggregation behaviour of test colloids with fibrinogen in vitro.

A prime consideration in the design of colloidal drug delivery carriers must be the physical stability of the dispersion; thus if the dispersion aggregates upon injection, its potential to be site-directed will be limited. In such situations it is also difficult to interpret in vivo distribution profiles and to assess the true targeting potential of the carriers.

The presence of an adsorbed steric coat is only one way of modifying the surface properties of a colloidal suspension. We recognise that the stabilisation is imperfect as the adsorbed coat may be desorbed by molecules having a higher affinity for the surface. A knowledge of the factors governing MPS uptake will, however, enable the development of a system designed to achieve such an effect.

These results argue that colloidal particles intended for drug delivery can have an extended residence time in the blood compartment due to their ability to shield the hydrophobic core from undesirable protein interactions.

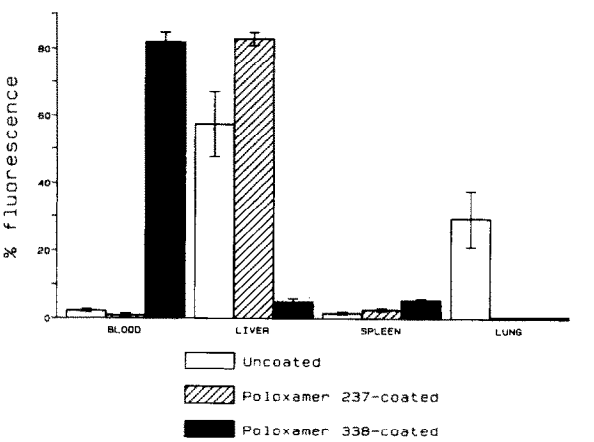

Fig. 4. The tissue distribution of fluorescently labelled PSL, 10 min after intravenous injection in mice (see text for details).

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