International Journal of Pharmaceutics, 34 (1986) 145–152
Elsevier

IJP 01151

Biodistribution of poly(butyl 2-cyanoacrylate) nanoparticles in rabbits

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(Received 25 June 1986)
(Accepted 22 July 1986)

Key words: Nanoparticle; γ-Scintigraphy; Biodistribution; Poloxamer 338; Poloxamine 908; Technetium-99m–dextran; Poly(butyl 2-cyanoacrylate)

Summary

Poly(butyl 2-cyanoacrylate) nanoparticles were radiolabelled with a technetium-99m–dextran complex and the biodistribution pattern of the intravenously injected particles determined in rabbits using γ-scintigraphy. The nanoparticles were found to localise partly in the liver/spleen region (about 60%) and partly to stay in the circulation (about 30%) before degrading and releasing the radiolabel. Coating the nanoparticles with the block copolymer poloxamer 338 or poloxamine 908 did not significantly influence the biodistribution pattern.

Introduction

A major limiting factor to the systemic use of particulate drug delivery systems is the rapid clearance of the carrier from the circulation by the reticuloendothelial system (RES) (Poste, 1985). Several methods have been investigated in attempting to overcome this problem such as suppression of the RES (Proffitt et al., 1983; Illum and Davis, 1984a) and change of surface characteristics by coating the particles with block copolymers (Illum and Davis, 1984a). Of these, the latter approach has been shown to be highly effective in altering the biodistribution pattern of radiolabelled colloidal particles (Illum and Davis, 1984b; Leu et al., 1984). Although coating polystyrene particles with the block copolymer poloxamer 338 greatly reduces (50%) the normally predominant liver and spleen uptake, the particles not taken up by the liver are rapidly cleared by the RES cells of the bone marrow (Illum and Davis, 1984b). However, this may be prevented by coating the particles with poloxamine 908 which totally suppresses RES capture throughout the vascular compartment leading to prolonged circulation times (Davis et al., 1986; Illum et al., 1986). Although the above-mentioned studies were performed using non-degradable polystyrene particles, the technique has been shown to be applicable to biodegradable systems in the form of fat emulsions (Illum et al., 1986).

Nanoparticles composed of biodegradable poly(alkyl 2-cyanoacrylate) have been proposed as

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drug targeting systems since they meet many of the criteria of an ideal drug carrier (Couvreur et al., 1984) but in common with most other intravenously injected colloids, these particles are normally deposited mainly in the liver and spleen (Grislain et al., 1983). This can lead to accumulation in these organs of drugs entrapped in the nanoparticles (Kante et al., 1980). RES uptake has been implicated in the enhanced toxicity observed when certain cytotoxics were injected in nanoparticle form (Kreuter and Hartmann, 1983). The technique of coating the particle surface with non-ionic surfactants could well alleviate this problem. This paper describes the use of γ-scintigraphy to determine the biodistribution pattern of intravenously injected poly(butyl 2-cyanoacrylate) nanoparticles radiolabelled with a technetium-99m–dextran complex. The effects of coating these particles with poloxamer 338 and poloxamine 908 have also been investigated.

Materials and Methods

Nanoparticle formation

Nanoparticles were formed by an aqueous dispersion polymerisation technique which has been described in detail elsewhere (Douglas et al., 1985). Briefly, this involved adding 0.5 ml of butyl 2-cyanoacrylate (Sichel Werke, F.R.G.) to 24.5 ml of 2% w/v dextran 10 (Sigma, U.K.) in 0.01 N hydrochloric acid. The mixture was stirred rapidly at room temperature for 2 h and the resulting nanoparticles isolated by centrifugation and lyophilisation.

Adsorption of poloxamer 338 and poloxamine 908 onto nanoparticles

Nanoparticle suspensions (1 mg/ml) were prepared in various concentrations ($1-50 \times 10^{-3}$ % w/v) of poloxamer 338 (Pechiney Ugine Kuhlmann, U.K.) or poloxamine 908 (Pechiney Ugine Kuhlmann, U.K.) in distilled water and gently agitated at 20°C for 2 h. Each sample was then centrifuged (20,000 *g*) and the surfactant concentration in the supernatant determined according to the method of Baleux (1972). From these results adsorption isotherms were constructed.

Preparation of radiolabelled nanoparticles

Ten ml of a 10% w/v dextran 10 solution in deoxygenated distilled water was added to 0.05 ml of a solution of stannous chloride (Sigma, U.K.) in concentrated hydrochloric acid (6 mg/ml). To 2.2 ml of this solution was added 2 ml of the eluate from a technetium generator (Amersham International, U.K.) containing 400 MBq of activity. After 10 min this was diluted to 11 ml with deoxygenated distilled water and 9.8 ml filtered through a sterile 0.22 μm membrane filter. Butyl 2-cyanoacrylate (0.2 ml) was added to this mixture as described above to give the nanoparticle suspension. The radiolabelled nanoparticles were isolated from free radiolabel by passing them through a Sepharose CL4B (Pharmacia, U.K.) gel filtration column using sterile phosphate-buffered saline (pH 7) as the eluant. Details of this procedure and its validation have been given elsewhere (Douglas and Davis, 1986).

The nanoparticle fraction from the gel filtration column (2.5 ml) was diluted to 3.3 ml with 0.9% w/v sodium chloride or 0.9% w/v sodium chloride containing 4.13% w/v poloxamer 338 or poloxamine 908. This gave a final activity of approximately 4 MBq/ml, a nanoparticle concentration of 10 mg/ml and a surfactant concentration (if added) of 1% w/v. The suspensions were left to equilibrate for 15 min prior to injection. The adding of surfactant to the nanoparticle suspension did not affect the labelling efficiency with 88% of the activity in nanoparticle form and 12% free in solution.

Preparation of free technetium-99m–dextran 10

Dextran 10 (0.5 g) was dissolved in deoxygenated water (5 ml) and this was added to 0.025 ml of a solution of stannous chloride (30 mg) in concentrated sulphuric acid (5 ml). 0.4 ml of this was filtered (0.2 μm sterile membrane filter) into a sterile vial and 0.2 ml of eluate from a ^{99m}Tc generator was added to this (total activity 20 MBq). After 10 min 3.4 ml of sterile phosphate-buffered saline (pH 7) was added to give a 1% w/v solution of ^{99m}Tc–dextran 10 with an activity of 5 MBq/ml. This solution was filtered through a sterile 0.2 μm membrane filter prior to injection.

Animal experiments

New Zealand White (NZW) rabbits about 2.5 kg in weight were randomly divided into 4 groups of 3. Each experimental group was injected with uncoated, poloxamer 338-coated or poloxamine 908-coated nanoparticles, with each rabbit receiving 1 ml of suspension via the right marginal ear vein. The control group of 3 animals were each injected with 1 ml of the ^{99m}Tc–dextran 10 solution. Injections were flushed through with 1 ml of sterile 0.9% w/v sodium chloride solution. The animals were positioned on a γ-camera (Maxi Camera II, General Electrics, U.S.A.) and data acquisition commenced immediately. Dynamic images (45 × 20 s) were recorded during the first 15 min and static images (60 s) taken at 1, 2, 4 and 8 h post-injection. The data were stored and processed by a dedicated computer system (Micas 2020 Medical Computer System, Nodecrest, U.K.)

Data processing

Regions of interest (ROIs) were created around the liver/spleen, lung/heart, lower kidney and bladder, and the activity within each region calculated. The data for each ROI were normalised by subtracting the background count for an equivalent sized ROI taken from an image recorded without an animal on the camera. For the dynamic data, a decay correction was applied to account for the radioactive decay of ^{99m}Tc during the dynamic phase. Results for each ROI during the dynamic phase are expressed as a percentage of the total activity in the whole animal. For the static images the total body activity at 1 h post-injection was used to calculate the total theoretical activity at 2, 4 and 8 h, after allowing for radioactive decay. The activity in each ROI was then expressed as a percentage of this value.

Due to the image of the upper kidney (situated on the right of the image) usually being superimposed on the liver image, the activity in the lower, well resolved kidney was subtracted from the liver activity to give values due to liver uptake alone. In calculating the total kidney and bladder activities the value from the lower kidney was doubled and added to that of the bladder. All activities are quoted as the mean of the 3 animals within each group together with the standard deviations.

Results and Discussion

Adsorption of poloxamer 338 and poloxamine 908 onto nanoparticles

Poloxamer 338 and poloxamine 908 were rapidly adsorbed by nanoparticles with equilibrium being established in a few minutes. The adsorption isotherms given in Fig. 1 show a maximum uptake of approximately 85 mg/g of nanoparticles for poloxamer 338 and 60 mg/g for poloxamine 908. At a nanoparticle concentration of 10 mg/ml the minimum concentrations of surfactants needed to ensure full surface coverage would be approximately 0.29% w/v for poloxamer 338 and 0.36% w/v for poloxamine 908.

On the basis of these findings the nanoparticles were coated with surfactant by incubating the suspensions for 15 min in 1% w/v solutions of poloxamer 338 or poloxamine 908.

Biodistribution of uncoated and surfactant-coated nanoparticles in rabbits

Following injection into NZW rabbits via the marginal ear vein approximately 50% of the uncoated nanoparticles (measured activity) were found to localise in the liver/spleen region within 2 min (Fig. 2). Coating with poloxamer 338 or poloxamine 908 caused a slight delay in liver/

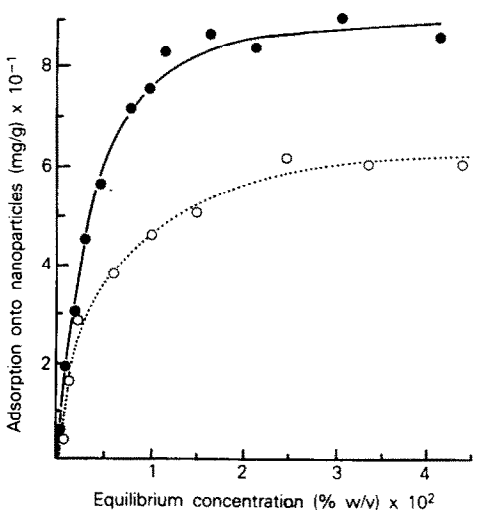

Fig. 1. Adsorption isotherms of poloxamer 338 (●) and poloxamine 908 (○) onto nanoparticles at 20°C.

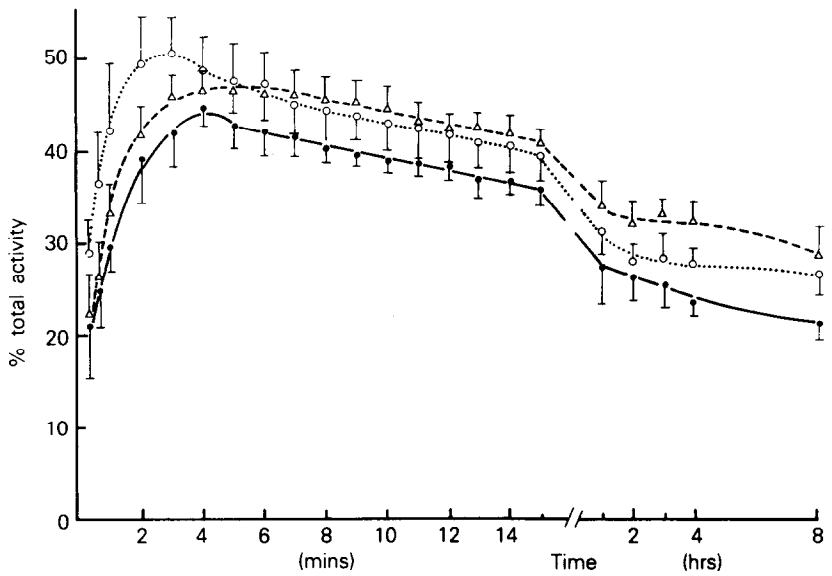

Fig. 2. Activity–time profiles for the liver/spleen region of interest following injection of uncoated (○), poloxamer 338-coated (●) and poloxamine 908-coated (△) nanoparticles labelled with ^{99m}Tc–dextran 10 ($n = 3$; bar = S.D.).

spleen clearance with maximum uptake occurring after 4 min (Fig. 2). There was no significant difference, however, in the final liver/spleen uptake between the coated and uncoated systems. The loss of activity from this region after 3 to 4 min is due to nanoparticle degradation resulting in the release of the water-soluble ^{99m}Tc–dextran radiolabel. In contrast, non-biodegradable polystyrene particles give a constant liver/spleen activity profile over the same time period (Illum and Davis, 1984b).

As the liver/spleen activity decreased, the activity found in the kidneys and bladder steadily increased as the radiolabel was filtered by the kidneys and accumulated in the bladder (Fig. 3). The kidney activity maintained a constant level of approximately 14% during the dynamic phase as the label was filtered and discharged to the bladder, in which activity steadily increased with time. After 4 h, between 50 and 60% of the total injected activity was found in the kidneys and bladder. This value fell sharply during the next 4 h when the animals urinated, giving activities of approximately 25% at 8 h post-injection. No significant difference was found between the activity–time profiles for the coated and uncoated nanoparticles given in Fig. 3. The initial rapid filtration by the kidneys of about 15% of the total injected activity was due to the presence of free radiolabel which constituted 12% of the injected dose.

There was no significant accumulation of radioactivity in the lung/heart ROI indicating that the nanoparticles were not mechanically filtered by the capillary bed of the lungs. This is consistent with the small diameter of the injected nanoparticles (126 nm, determined by photon correlation spectroscopy) since lung entrapment normally requires a minimum particle diameter of 7–12 μm (Illum et al., 1982).

The distribution pattern can be observed clearly in the scintiscans given in Fig. 4 for a rabbit injected with uncoated nanoparticles. Following injection into the marginal ear vein the suspension is delivered to the heart and then the lungs before returning to the heart and entering the general circulation. Hence a relatively high level of activity is observed initially in the lung/heart region (Fig. 4a) before the nanoparticles are cleared by the RES. A similar pattern has been observed for polystyrene particles (Illum and Davis, 1984b). However, even at this early stage the outline of the

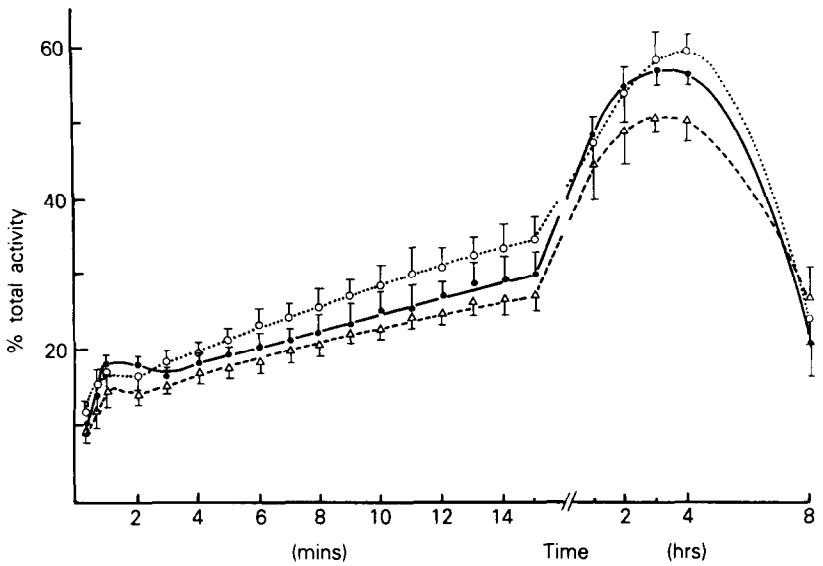

Fig. 3. Activity–time profiles for the kidney and bladder regions of interest following injection of uncoated (○), poloxamer 338-coated (●) and poloxamine 908-coated (△) nanoparticles labelled with ^{99m}Tc–dextran-10 ($n = 3$; bar = S.D.).

liver is clearly distinguishable. At 4 min post-injection (Fig. 4b) the outline of the two kidneys can be seen (normally the kidney seen on the right in the scintiscan was obscured by the liver image) and 2 min later (Fig. 4c) the bladder image is resolved. This distribution pattern was maintained throughout the dynamic phase (first 15 min) with the intensity of the bladder image gradually increasing. In all these scintiscans the outline of the animal was well defined indicating a relatively high level of circulating activity (blood activity was not measured). After 1 h (Fig. 4d) the bladder was seen to contain most of the activity although the liver and kidneys were still distinguishable.

The activity–time profiles for the control animals injected with free ^{99m}Tc-dextran 10 are given in Fig. 5. This compound was rapidly cleared by the kidneys and accumulated in the bladder. The kidney and bladder activities in these animals at a given time were much higher compared with the experimental groups, since all of the radiolabel is essentially free in the circulation and capable of being filtered by the kidneys. The low levels of activity found in the liver/spleen region are evidence for the lack of ^{99m}Tc–tin colloid formation during the radiolabelling procedure. Any liver uptake of colloidal contaminants would have been observed in the first few minutes following injection. Due to the rapid clearance of the labelled dextran from the circulation, data are only given for the dynamic phase. At 8 h post-injection there were only residual traces of activity left in the animals.

Taking into account the level of free radiolabel in the nanoparticle injection (approximately 12%) the maximum liver/spleen uptake for the uncoated particles was 57%. Since at the same time the maximum activity in the kidneys and the bladder is about 18%, the remaining activity can be assigned to circulating particles. This figure for liver uptake is very much lower than that reported by Illum and Davis (1984b) for uncoated (hydrophobic) polystyrene particles and by Leu et al. (1984) for polymethylmethacrylate particles. It is also lower than values found by Grislain et al. (1983) for dextran stabilised poly(isobutyl 2-cyanoacrylate) nanoparticles that would be expected to be more hydrophilic in their surface properties. In the last two studies the experimental model was the rat and direct comparison of results between various studies is not possible due to the large number of variables which include the na-

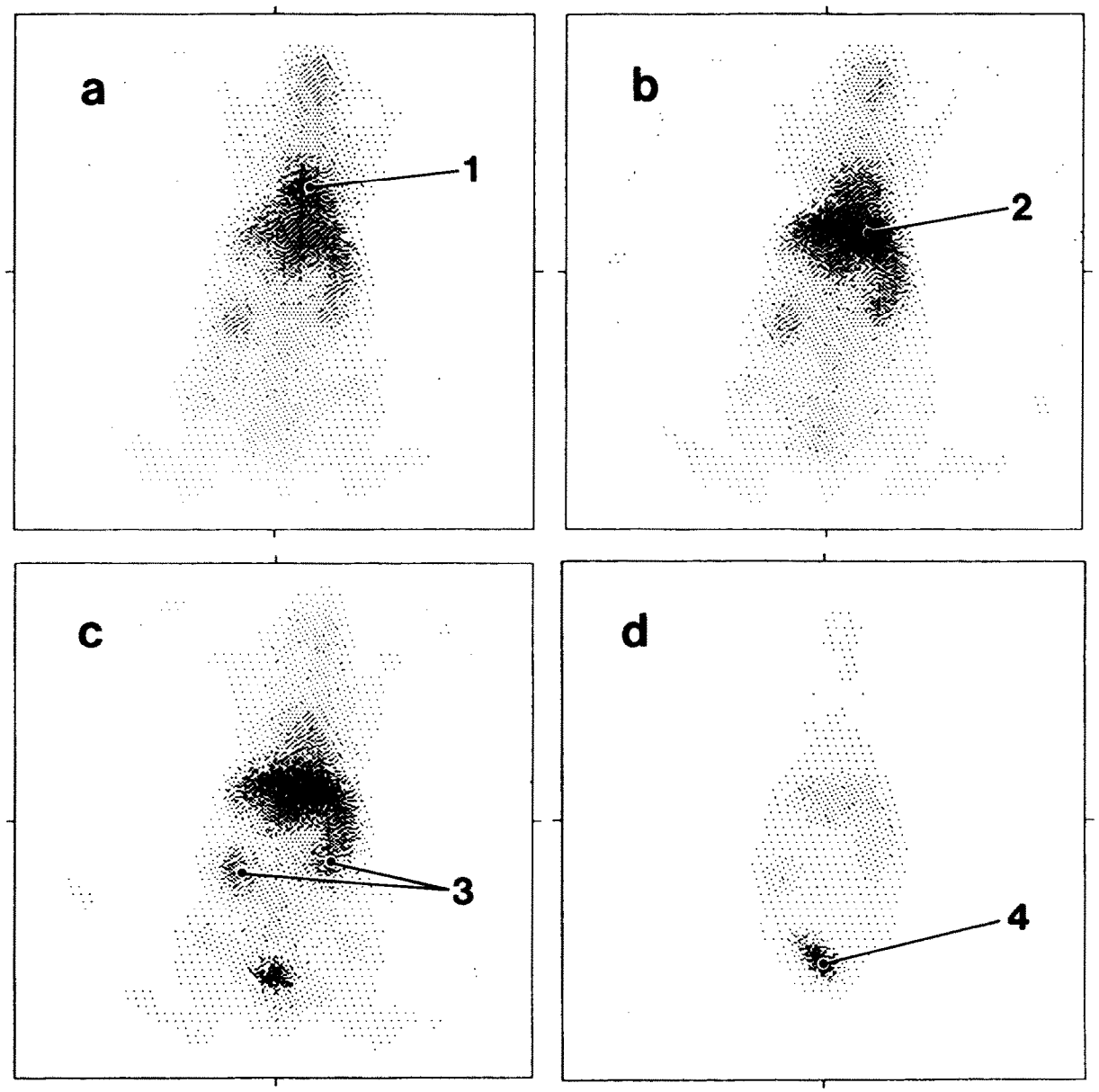

Fig. 4. Scintiscans of a rabbit injected with uncoated nanoparticles labelled with ^{99m}Tc–dextran 10 at various times after injection. Normally the kidney seen on the right was obscured by the liver/spleen image (ROI designation: 1 = lung/heart, 2 = liver/spleen, 3 = kidneys and 4 = bladder). Time after injection: (a) 1 min; (b) 4 min; (c) 6 min; (d) 1 h.

ture of the particles (size, surface charge, composition, hydrophobicity), radiolabelling procedures, animal species, radiation detection techniques and dosage levels. The results obtained here imply that poly(butyl 2-cyanoacrylate) nanoparticles stabilised by dextran 10 are partly removed by the liver and/or spleen and are rapidly degraded in these organ sites and possibly also in circulation.

The coating of nanoparticles with surfactants did not significantly alter the distribution. This is

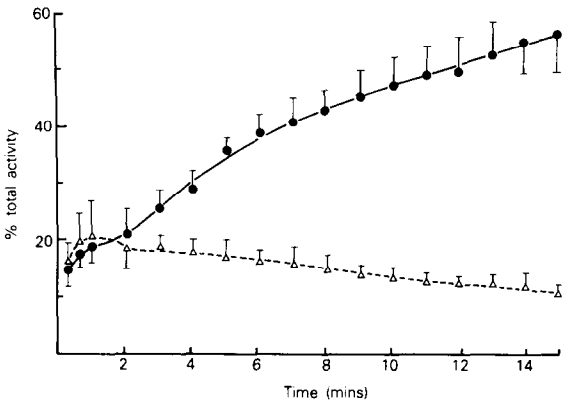

Fig. 5. Activity–time profiles for the control animals injected with free ^{99m}Tc–dextran 10. △, liver/spleen region; ●, kidneys and bladder regions. ($n = 3$; bar = S.D.)

in contrast to other reports for polystyrene by Illum and Davis (1984b) as well as for polymethylmethacrylate nanoparticles by Leu et al. (1984). It has been proposed (Davis et al., 1986) that the interfacial coating of surfactants decreases particle capture by macrophages of the RES due to the formation of a hydrophilic surface and an enhanced steric repulsion preventing close approach and particle/cell interactions. Since the nanoparticle system under investigation here already possesses a strong steric barrier provided by the surface layer of covalently linked dextran (Douglas et al., 1985), the addition of further stabiliser to the system may fail to significantly increase the existing repulsive steric potential energy. Furthermore, although the adsorption isotherm indicates uptake of the block copolymers on the surface of the nanoparticles, and for poloxamer 338 the plateau level is similar to that reported for uptake on polystyrene (Kayes and Rawlins, 1979), the adsorption energies per chain may be different for the two surfaces (Klein and Pincus, 1982). In addition, their surface conformation could be different, e.g. loops vs tails. It has been shown by Davies (1986) that on a hydrophilic surface (aluminium foil) poloxamine 908 will adsorb with the hydrophilic polyoxyethelene chains on the surface whereas the hydrophobic polyoxypropylene chain extends into the external environment. A similar possibility may exist for dextran-stabilised nanoparticles. Interestingly, de Gennes (1981) and Klein and Pincus (1982) have discussed the adsorption of neutral flexible polymers on solid surfaces. Their theoretical analyses show that a non-ionic flexible polymer is well adsorbed from a good solvent for the polymer and that the high polymer molecular weight ensures that even for a weak adsorption energy per monomer the adsorption energy per chain may be large. This coupled with slow polymer diffusion leads to effectively irreversible adsorption even when the solid/polymer system is washed with pure solvent. However, in some cases, particularly with grafted surfaces, the polymer will not be taken up and in extreme cases even repelled and a so-called depletion layer created. While adsorbed polymer may not be desorbed by washing with pure solvent (de Gennes, 1981) it may be desorbed by the competitive uptake of plasma proteins. For example, it is known that poloxamer 188 is well adsorbed to polystyrene surfaces and remains so in aqueous buffer but apparently is easily displaced by serum components (Illum et al., 1986). Other factors such as the rapid degradation of nanoparticles in vivo as shown here, may result in loss of the coating surfactant molecules as the particle surface is eroded.

The radiolabelled nanoparticles described in this work could be exploited as a diagnostic tool. Alternatively, their distribution to the liver and general circulation could be utilised in the treatment of disseminated infections. Encouraging results on the treatment of Chagas disease using similar carriers have been obtained by Avila (1983).

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