RADIOLABELLING OF POLY(BUTYL 2-CYANOACRYLATE) NANOPARTICLES WITH A TECHNETIUM-99m-DEXTRAN COMPLEX

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SUMMARY

Poly(butyl 2-cyanoacrylate) nanoparticles have been radiolabelled with a ^{99m}Tc-dextran complex to allow their pattern of biodistribution to be followed by using the technique of gamma scintigraphy. The method involves the preforming of a ^{99m}Tc-dextran complex and using this as a polymeric stabiliser during the formation of nanoparticles. Copolymerisation of this complex with the cyanoacrylate monomer results in the radiolabel being covalently linked to the nanoparticle matrix with a labelling efficiency of approximately 18%. The radiolabelled nanoparticles slowly degrade and release the activity into buffer solution. The release rate was relatively unaffected by the presence of plasma proteins indicating that the system should be suitable for use in vivo.

Keywords: Nanoparticle, copolymerisation, technetium-99m-dextran, poly(butyl 2-cyanoacrylate)

INTRODUCTION

Synthetic microspheres have attracted much attention in recent years as

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potential drug carriers for use in site specific drug delivery[1]. These particles are capable of sorbing drug molecules and thereby modifying the tissue distribution of a wide range of compounds. This in turn can lead to a marked increase in the therapeutic index of the drug[2]. Of the systems currently under investigation, nanoparticles composed of poly (alkyl 2-cyanoacrylate) have shown promise and meet many of the criteria required of a drug carrier system[3].

The factors affecting the interaction of injected colloids with serum components and their subsequent biodistribution are well understood and include particle size, surface charge and surface hydrophobicity[4]. In order to determine the affect of these parameters on the distribution pattern of poly(butyl 2-cyanoacrylate) nanoparticles in the body it is necessary to label the system with a suitable radiolabel. Nanoparticles have been labelled previously[5,6] with carbon-14. However, the initial preparation of the ^{14}C-labelled monomer is difficult since this compound is prone to spontaneous polymerisation and yields nanoparticle systems with a wide particle size distribution. The subsequent in vivo studies are highly time consuming and the possible interference from the degradation products of labelled particles is hard to detect and eliminate since the biodistribution of these components cannot be determined readily.

Alternatively, if nanoparticles could be radiolabelled with a gamma-emitting radionuclide the distribution could be followed by gamma scintigraphy. This approach has several advantages over beta counting. In particular, it requires small numbers of animals yet provides much data on the kinetics of uptake. For this reason gamma scintigraphy has been used to determine the fate of colloidal systems such as liposomes[7], microspheres[8] and emulsions[9] in a range of animals (rats, mice, rabbits, dogs).

This paper describes a simple procedure for the radiolabelling of poly(butyl 2-cyanoacrylate) nanoparticles using a technetium-99m-dextran complex.

MATERIALS

Dextran 10 (molecular weight 9000), bovine serum albumin and stannous chloride were purchased from Sigma (UK). Butyl 2-cyanoacrylate was a gift from Sichel-Werke (FRG) and was stored desiccated at 4°C.

Sepharose CL 4B was purchased from Pharmacia (UK) and sterilised by autoclaving according to the manufacturer's instructions. All other chemicals were of reagent grade and used as purchased. $^{99m}TcO_4^-$ was eluted in normal saline from a generator (Amersham International plc (UK)).

Silica gel impregnated glass fibre sheets (1 TLC-SG) for thin layer chromatography were obtained from Gelman Instruments (USA). The chromatograms were scanned by a radiochromatogram scanner constructed by the Department of Medical Physics, Queen's Medical Centre, Nottingham. Gamma counting was performed using an Intertechnique (France) CG4000 automatic gamma well counter.

The particle size of the nanoparticles was determined by photon correlation spectroscopy[10] using a commercially available system (Malvern (UK)).

METHODS

Incorporation of a technetium-99m-dextran complex into nanoparticles

The ^{99m}Tc-dextran complex was preformed by a modification of the method described by Henze et al[11]. This involved adding, with vigorous mixing, a 10% w/v dextran 10 solution in deoxygenated distilled water (10ml) to a solution of stannous chloride in concentrated hydrochloric acid (6mg/ml) (0.05ml). This solution (2.2ml) was filtered (0.2μm membrane filter) and mixed with the eluate (1ml) from a ^{99m}Tc generator containing 2MBq of activity. After 10 minutes this was diluted to 11ml with deoxygenated distilled water and 9.8ml was filtered (0.2μm membrane filter) into a McCartney vial fitted with a glass covered magnetic stirrer bar. To this solution was added butyl 2-cyanoacrylate (0.2ml) and the mixture rapidly stirred for 1.75 hours. After this time

0.5M phosphate buffer (pH7) (1ml) was added and stirring continued for a further 15 minutes. The suspension (2.5ml)was then passed through a 1 x 20cm Sepharose CL 4B column which had been pre-conditioned with 'cold' nanoparticle suspension (2ml) flushed through with phosphate buffered saline (PBS) (pH7) (30ml). The labelled nanoparticles were eluted with PBS (pH7) and the activity in each 1ml fraction was determined by gamma counting and expressed as a percentage of the total activity added to the column. The total nanoparticle elution volume was found to be 2.5ml which had a solids content of 33mg.

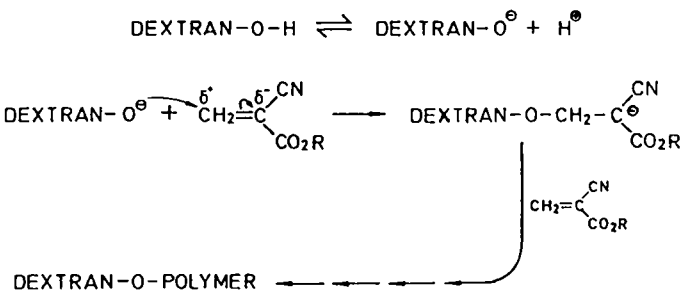
Thin layer chromatography was performed on the ^{99m}Tc-dextran solution prior to addition of the monomer and on the nanoparticle solutions before and after gel filtration. Silica gel impregnated glass fibre sheets were used with 0.9% w/v sodium chloride as the solvent and the radioscans of the chromatograms recorded.

Release of activity from ^{99m}Tc-dextran labelled nanoparticles

The labelling procedure was repeated as above and column fractions containing a total of 50mg of labelled nanoparticles were combined (obtained from two separate column elutions). The suspension was then flocculated by freezing rapidly with liquid nitrogen and then thawing. Flocculation of the suspension allowed rapid centrifugation of the particles using a low speed centrifuge. The flocculated suspension was then dispersed in 100ml of PBS (pH 7.4) or PBS (pH7.4) containing 1% w/v bovine serum albumin. These suspensions were stirred at 37°C for 24 hours and 5ml samples were withdrawn periodically. The samples were centrifuged rapidly and the activity free in solution determined by gamma counting and expressed as a percentage of the total activity added to the dissolution medium.

Particle size analysis

The nanoparticle suspension (0.2ml)obtained from the gel filtration column was diluted to 20ml with filtered (0.2μm membrane filter) distilled water. This sample was then analyzed by photon correlation spectroscopy[10] to give an average value (n=15) for the diameter of the nanoparticles.



SCHEME 1

Proposed mechanism of the covalent linkage of dextran to the cyanoacrylate polymer.

RESULTS AND DISCUSSION

Nanoparticles are formed by an aqueous dispersion polymerisation of an alkyl 2-cyanoacrylate (eg. butyl) in the presence of a polymeric stabilising agent such as a dextran or nonionic surfactant[12]. Weak nucleophiles, such as water or alcohol, are capable of initiating polymerisation due to the polarisation of the double bond of the monomer caused by the highly electronegative alkoxycarbonyl and nitrile groups. In this way polyhydroxyl stabilisers such as dextrans become covalently attached to the nanoparticle matrix when added to the polymerisation medium as shown in scheme 1[12]. Therefore, the ability of ^{99m}Tc to form a complex with dextran, that is stable both *in vitro* and *in vivo*[11,13], can be utilised for the radiolabelling of nanoparticles.

The ^{99m}Tc-dextran complex is formed after reduction of pertechnetate with stannous ion according to the following mechanism[11]

$$TcO_4^- + 2[C_6H_{10}O_5] + Sn^{2+} + H_2O \quad \text{----}> \quad [TcO(C_6H_8O_5)_2]^- + 4H^+ + [SnO_2(OH)_2]^{2-}$$

This complex was relatively simple to prepare and could be used directly in place of ordinary dextran for the formation of nanoparticles. The resulting particle size was unaffected by the labelling procedure, giving an average

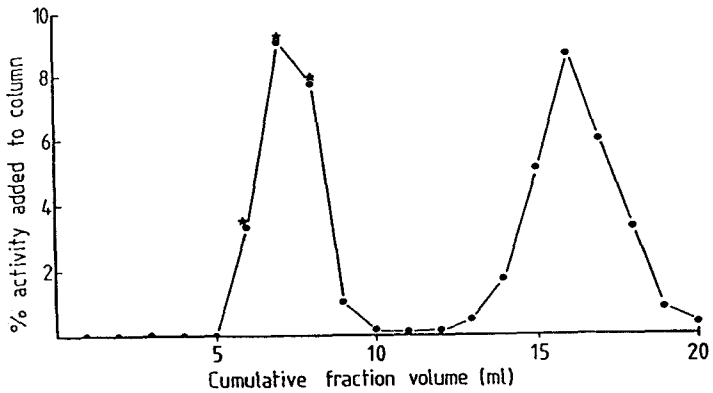


FIGURE 1

Radioactivity found in each 1ml eluted from a Sepharose CL4B gel filtration column expressed as a percentage of the total activity added to the column. *denotes the major nanoparticle fractions.

diameter of 126nm. Labelled nanoparticles were readily separated from free radiolabel by gel filtration chromatography. On passing through a Sepharose CL 4B column, the small dextran molecules were retained to a much greater extent than the relatively large nanoparticles which were eluted in the column void volume. The use of dextran 10, as opposed to higher molecular weight dextrans, enhanced the separation and allowed the use of relatively short columns. This was necessary in order to keep dilution of the nanoparticle fraction to a minimum. The elution-activity profile for this system is given in figure 1. The combined nanoparticle fractions were found to contain approximately 21% of the activity added to the column. Centrifugation (20000 rpm) of these particles indicated that 88% of this activity was firmly bound to the nanoparticles, giving a labelling efficiency of 18.5%.

Thin layer chromatography of the ^{99m}Tc-dextran complex showed that most of the activity moved at the solvent front (figure 2a). This indicated that ^{99m}Tc labelled tin colloid, which would remain at the origin, was not formed to any significant extent, in agreement with Henze et al[11]. It was important to demonstrate the absence of tin colloid from the labelled dextran solution since this substance could interfere with the subsequent work in vivo. Both ^{99m}Tc-tin

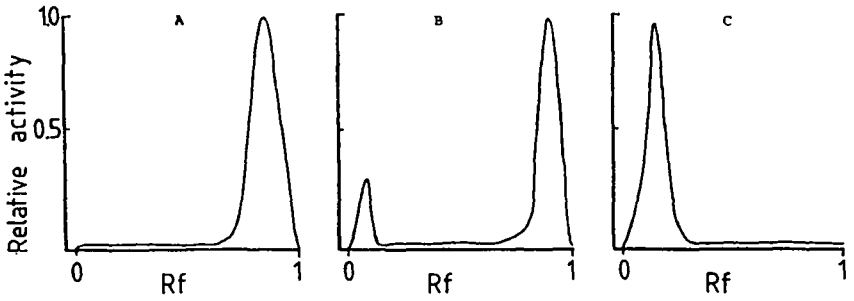


FIGURE 2

Radiochromatograms of (A) ^{99m}Tc-dextran 10, (B) the nanoparticle suspension isolated after polymerisation and (C) the nanoparticle suspension isolated from the gel filtration column.

colloid and the nanoparticles would be expected to show predominant hepatic clearance which would be difficult to resolve.

Similar chromatographic analysis of the nanoparticle suspension prior to gel filtration showed approximately 18% of the activity to remain at the origin due to incorporation into nanoparticles (figure 2b). This value is in good agreeement with the results obtained after centrifugation of the nanoparticles eluted from the column. Chromatographic analysis of nanoparticles isolated from the column showed that most of the activity remained at the origin and no distinct band of activity, due to the free complex, could be detected at the solvent front (figure 2c).

When these radiolabelled nanoparticles were dispersed in PBS or PBS containing bovine serum albumin a low but steady release was obtained over a period of 24 hours. This release was relatively unaffected by the presence of protein as shown in figure 3. Since the technetium-99m-dextran complex is known to be highly stable the release of activity is believed to be due to degradation of nanoparticles giving rise to free ^{99m}Tc-dextran in solution. The stability of the radiolabel in the presence of bovine serum albumin is an important factor considering the intended use of the system in vivo. It has been found that other

non-covalently linked radiopharmaceuticals, such as ^{131}I-labelled rose bengal and indium-111-oxine despite displaying good stability in buffer, are rapidly lost from nanoparticles in the presence of serum[14].

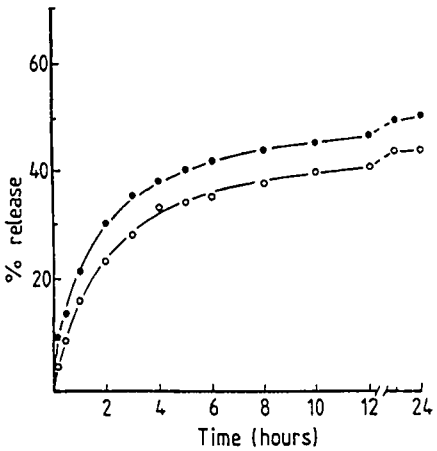


FIGURE 3
Release of radioactivity from ^{99m}Tc-dextran labelled nanoparticles in phosphate buffered saline (pH7.4)(O), and in phosphate buffered saline (pH7.4) containing 1% w/v bovine serum albumin (O) at 37°C.

CONCLUSIONS

Despite the low incorporation efficiency, the technique described is regarded as a suitable method for radiolabelling dextran stabilised nanoparticles. Although the slow release of activity observed *in vitro* could cause interference when determining the fate of the colloid *in vivo*, rapid filtration by normal kidneys of the free label would be expected to minimise such effects.

For *in vivo* experiments it is necessary to increase the level of added activity (in the form of $^{99m}TcO_4^-$) to approximately 400 MBq. This provides a final suspension of sufficient specific activity to allow the nanoparticle distribution to be followed in rabbits over a period of approximately 12 hours after the injection of nanoparticle suspension (0.8ml).

Studies are now in progress to determine the fate of radiolabelled poly (butyl 2-cyanoacrylate) nanoparticles in rabbits following intravenous injection. It is intended to alter the normal predominant hepatic clearance of these nanoparticles by controlling factors such as particle charge, size and hydrophobicity.

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