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Labeling efficiency and biodistribution of Technetium-99m labeled nanoparticles: interference by colloidal tin oxide particles

Tanima Banerjee[b], A.K. Singh[b], R.K. Sharma[b], A.N. Maitra[a,*]

[a] *Department of Chemistry, University of Delhi, Delhi 110007, India*
[b] *Division of Biocybernetics and Radiopharmaceuticals, Institute of Nuclear medicine and Allied Sciences, Brig SK Mazumdar Road, Delhi 110054, India*

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Abstract

The interference of colloidal tin oxides on the biodistribution of ^{99m}Technetium radiolabeled chitosan nanoparticles has been overcome by using sodium borohydride instead of commonly used stannous salts as reducing agent for the reduction of ^{99m}Tc (VII) to lower valency states. Biodistribution of radiolabeled chitosan nanoparticles prepared by using stannous chloride method revealed localization of the radioactivity mainly in the liver and spleen while that of radiolabeled chitosan nanoparticles prepared by using sodium borohydride method manifested the presence of radioactivity in blood up to an extent of 10% even after 2 h. Interestingly, the reduction of radioactivity in the latter case with the progress of time was not manifested through an increase in activity in the liver. Rather, a time dependent increased accumulation of radioactive materials was observed in the stomach. From the results it has been concluded that the biodistribution is strongly influenced by the presence of colloidal particles of tin oxides and ^{99m}Tc labeled chitosan nanoparticles are RES evading and long circulating in blood when Tc (VII) is reduced by sodium borohydride and not by stannous chloride during radiolabeling process.

Keywords: ^{99m}Tc; Chitosan nanoparticles; Gamma scintigraphy; Hydrogels

1. Introduction

Nanometer particles have easy accessibility in the body and can be transported to different body sites through systemic circulation of blood (Moghimi et al., 2001). Therapeutic efficacy of drugs can be enhanced by targeting the drug through polymeric nanoparticles as carrier. But like other colloidal carriers used for drug targeting, they are sometimes taken up by the reticuloendothelial system (RES) such as kupffer cells of liver and macrophages of spleen (Stolnik et al., 1995). Numerous recent investigations have been aimed at re-

* Corresponding author. Tel.: +91 11 27666596; fax: +91 11 27666596.
E-mail address: maitraan@yahoo.co.in (A.N. Maitra).

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ducing the RES uptake and increasing the concentration of the particulate carriers at the desired sites in the body. The most promising among the various strategies followed till now is by reducing the particle size and making the particle surface hydrophilic by conjugating with an amphiphilic polymeric compound made of polyethylene oxide such as poloxamers, poloxamines etc (Calvo et al., 2001; Storm et al., 1995; Peracchia et al., 1999). One of the most suitable methods of studying the biodistribution is to label these nanoparticles with ^{99m}Tc and to measure their radioactivity in various tissues or to perform gamma imaging of the whole body after their administration. The starting material for technetium nucleus is pertechnetate ions, which are usually reduced by acidic stannous chloride or other stannous salts (Nowotnik, 1990), although other reducing agents are also often used (Kowalsky and Perry, 1987). There have been few reports on the biodistribution studies of ^{99m}Tc labeled nanoparticles (Douglas et al., 1986) and in all these studies stannous chloride has been used as a reducing agent for reduction of Tc (VII) ions. In these studies, the major liver uptake as well as rapid blood clearance of radiolabeled nanoparticles were reported. Recent studies in our laboratory have demonstrated that nanoparticles of size less than 100 nm diameter and made of hydrogel polymers can evade the RES and have a long circulation time in the blood (Gaur et al., 2000). Nanoparticles of hydrogel materials such as chitosan can, therefore, be suitably used for examining their distribution in various tissues in the body and to explore their potential for tissue targeting. Chitosan, used in the present study as nanoparticles materials, is a pH sensitive polymer which protonates at lower pH. Chitosan is known to form complex through its dextran moiety [β(1,4)-D-glucosamine] with reduced ^{99m}Tc ions and at pH ≥ 7, the amine groups of the polymer is also expected to bind to ^{99m}Tc ions.

We have used sodium borohydride as a reducing agent to reduce ^{99m}Tc (VII) to lower valency states. Boric acid, the oxidized product formed in this redox reaction, remains in the ionic form in solution at neutral pH and can be easily removed through dialysis. In this paper, we have reported the use of both sodium borohydride and stannous chloride as reducing agents for reduction of ^{99m}Tc (VII) ions and subsequent complexation with chitosan nanoparticles. Radiolabeled nanoparticles prepared by both these methods have been used for biodistribution and blood clearance studies and the results have been compared.

2. Materials and methods

2.1. Materials

Chitosan (M.W. 400 kD), glutaraldehyde, and surfactant-sodium bis(ethylhexyl) sulfosuccinate (AOT), were purchased from Sigma (St. Louis, MO, USA). All other reagents used were purchased from local market and were of analytical grade. ^{99m}Tc-pertechnetate freshly eluted from ^{99}Mo by solvent extraction method was procured from Regional Centre for Radiopharmaceuticals (Northern region), Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy, India. Strain A mice were procured from animal house of Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi.

2.2. Preparation of ultrafine chitosan nanoparticles

Chitosan nanoparticles were prepared by the method reported earlier (Banerjee et al., 2002). A brief outline of the method is as follows. The surfactant, AOT, was dissolved in *n*-hexane. To 40 ml of 0.04 M AOT solution, 400 μl of 0.1% chitosan solution in acetic acid, 76 μl aqueous solution/Tris–HCl buffer (0.01%; pH 8.0), 40 μl ammonium chloride + ammonium hydroxide buffer (pH 10), 4 μl of 0.01% glutaraldehyde solution were added, with continuous stirring at room temperature. The solution was homogenous and optically transparent. The solvent was, then, evaporated off in a rotary evaporator, and the dry mass was resuspended in 20 ml of Tris–HCl buffer (pH 8.0) by sonication. Four milliliters of 30% $CaCl_2$ solution was added dropwise to precipitate the surfactant as calcium salt of diethyl sulphosuccinate ($Ca(DEHSS)_2$). The precipitate was pelleted by centrifugation at 6000 rpm for 15 min at 4 °C.The pellet was dissolved in *n*-hexane and the hydrocarbon solution was washed each time with water and the aqueous layer was drained out. The supernatant solution along with washing liquid was dialyzed for about 2–3 h using a spectrapore membrane dialysis bag (12 kD cut off) and lyophilized to dry powder for subsequent use.

2.3. Radiolabeling of placebo chitosan nanoparticles

Four hundred micrograms of lyophilized chitosan nanoparticles was dispersed in 0.5–1.0 ml of 0.01% Tris–HCl buffer of pH 7.5. To this nanoparticles solution, 200 μl ^{99m}Tc (2.2 mCi) in saline (obtained by elution from molybdenum) was added. To this mixture, either (a) 25 μl of stannous chloride (2.0 mg/ml) solution in 0.1 N HCl or (b) 100 μl of aqueous solution of sodium borohydride (50 mg/ml) and containing 50 μl of 1% w/v NaOH solution, as the case may be, were added. Nitrogen gas was passed to degas all the solutions prior to the mixing. The resulting solution was incubated for 20 min at room temperature, after adjusting the pH at 7.2 in case of stannous chloride system with the help of 0.1% w/v NaOH. The contents were transferred into an evacuated sterile sealed vial for subsequent use.

2.4. Labeling efficiency and stability study.

The labeling efficiency of ^{99m}Tc to chitosan nanoparticles by both the methods (stannous chloride and sodium borohydride method.) was assessed by ascending instant thin layer chromatography (ITLC) using silica gel coated fiber sheets (Gelman Sciences Inc., Ann Arbor, MI, USA). The ITLC was performed using acetone as the mobile phase. The contaminants were identified as reduced/hydrolyzed (R/H) technetium, colloids of tin hydroxides and free ^{99m}-pertechnetate. The free pertechnetate which moved with the solvent front (rf = 0.9) was estimated as 15–20% of the total radioactivity added. Labeling efficiency was calculated using the following equation

$$\text{labeling efficiency \%} = \frac{\text{total counts} - \text{counts of free pertechnetate}}{\text{total counts}} \times 100.$$

2.5. Blood clearance of radiolabeled chitosan nanoparticles

Four hundred micrograms of chitosan nanoparticles were dispersed in 1.0 ml Tris buffer and were radiolabeled by both the methods separately. New Zealand rabbits of weight 2.0–2.5 kg were taken, and radiolabeled nanoparticle solution (injected dose: 2.2–2.5 mCi in 1.0 ml) was injected intravenously through the dorsal ear vein of the rabbits. Blood was withdrawn from the other ear (100–200 μl) at fixed interval of time and the counts were recorded in the gamma spectrometer. Further, specific counts per gram of blood were calculated for each time interval.

2.6. Biodistribution of radiolabeled chitosan nanoparticles

Biodistribution of ^{99m}Tc-chitosan nanoparticles was studied in 2–3-month-old strain A mice weighing 25–30 g. One hundred microliters of sterile radiolabeled nanoparticle suspension (concentration: 1 mg/ml) was administered through the tail vein of each mice, (injected dose: 1 μCi/μl). The animals were sacrificed by cervical dislocation at different time intervals and different organs were removed, washed with normal saline, and dried in paper folds. The radioactivity in each organ was counted using well-type gamma spectrometer, and expressed as percent injected dose per gram of the organs.

2.6.1. Statistical analysis

Data have been represented as the mean of six individual observations with standard error of mean. Significance has been calculated using Student's *t*-test.

2.7. Gamma scintigraphy

The body distribution profile in rabbit was recorded in a gamma camera, till 4 h post injection with ^{99m}Tc labeled chitosan nanoparticles.

For gamma-imaging studies, New Zealand rabbit of 2.0–2.5 kg body weight was used. After intravenous administration of radiolabeled chitosan nanoparticles (injected dose: 400 μg in 2.5–3.0 ml Tris buffer, activity: 2.2–2.5 mCi) radiolabeled nanoparticles, the animal was anesthetized by intramuscular injection of 1 ml ketamine hydrochloride and 1 ml calmpose, few minutes before imaging. The animal was fixed on a board in posterior anterior position and imaging was performed at different time intervals using a planar gamma camera.

3. Results and discussion

3.1. Labeling efficiency and stability of ^{99m}Tc labeled nanoparticles

Fig. 1(a) and (b), show the labeling efficiency and stability of ^{99m}Tc labeled nanoparticles prepared by using stannous chloride method and borohydride method, respectively. The figures depict that there is not much difference in the labeling efficiency of chitosan nanoparticles by method I and method II. Both the reducing agents were efficient to reduce the pertechnetate ion and enable it to form complex with the ligand (here nanoparticles) to an extent of 90%. But with progress of time there was a decline in the stability of the radiolabeled complex prepared by borohydride reduction method. In 60 min, the labeling efficiency is more than

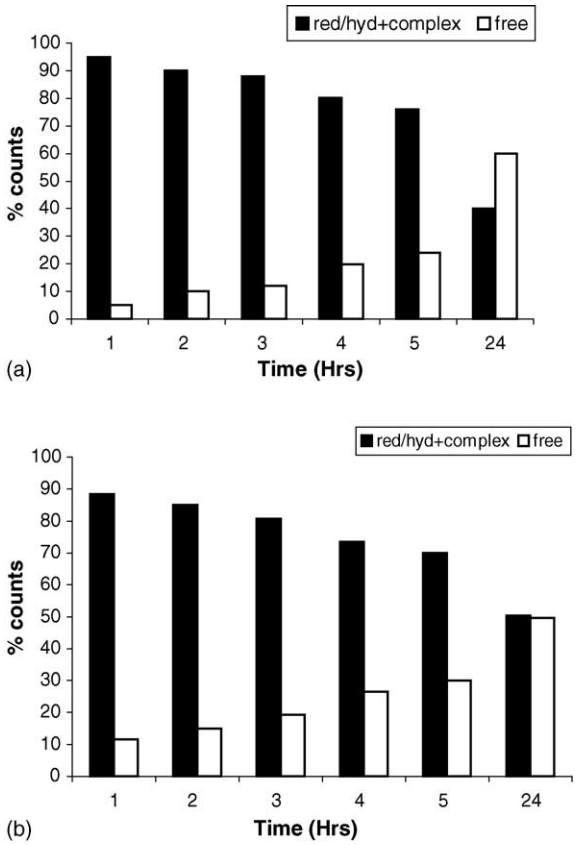

Fig. 1. Stability of the radiolabeled chitosan nanoparticle complex prepared by stannous chloride method (a) and sodium borohydride method (b).

90% in stannous chloride reduction method whereas it is around 80–85% by using borohydride reduction method. We presume that the high labeling efficiency is due to the synergistic effect of ^{99m}Tc-chitosan nanoparticles complex and partial adherence of the radiolabeled chitosan nanoparticles on the surface of the colloidal tin oxide particles, which could not be resolved and quantified in the ITLC paper. This has been confirmed by performing the biodistribution experiments in mice in absence of any chitosan nanoparticles by reducing ^{99m}Tc ions with stannous chloride as well as sodium borohydride methods. Huge amount of colloid formation took place in the stannous chloride method, which got trapped in the liver, spleen and lungs (data not shown). The stability of the complex is also higher in presence of colloidal tin oxide particles compared to that of nanoparticles prepared through sodium borohydride reduction method. In 4 h, the complex is stable up to 85% in presence of tin oxide colloids while it is only 70% when we use sodium borohydride as a reducing agent as seen in Fig. 1(a) and (b). Fig. 2 shows the blood clearance kinetics of radiolabeled chitosan nanoparticles in rabbit. Stannous chloride reduction method indicates that the labeled nanoparticles are practically removed from the blood circulation within 30 min of introduction into the circulatory system. On using sodium borohydride as reducing agent, a longer circulation time of the nanoparticles is seen, such that after 30 min 17% activity is observable in blood and about 10% activity is observed after 120 min. The biodistribution profiles of these hydrogel nanoparticles in mice show same trends of results using the two different reducing agents. Fig. 3(a) shows that using stannous chloride, as reducing agent, the radiolabeled nanoparticles are accumulated in the RES organs such as liver, spleen and lungs within half-hour of injection. On changing the reducing agent by sodium borohydride, these nanoparticles demonstrate lower accumulation in the RES organs and longer residence time in the blood circulation as depicted in Fig. 3(b). These observations are further validated by studying the gamma scintigraphic images of rabbit as shown in Fig. 4(a) and (b). On using the protocol of ^{99m}Tc reduction using stannous chloride, concentration of radioactivity is highlighted in the liver and spleen (Fig. 4(a)), whereas on using the sodium borohydride method, the accumulation in the liver and spleen is less, while the blood pool is clearly visible even up to 2 h post injection (Fig. 4 (b)). As is evi-

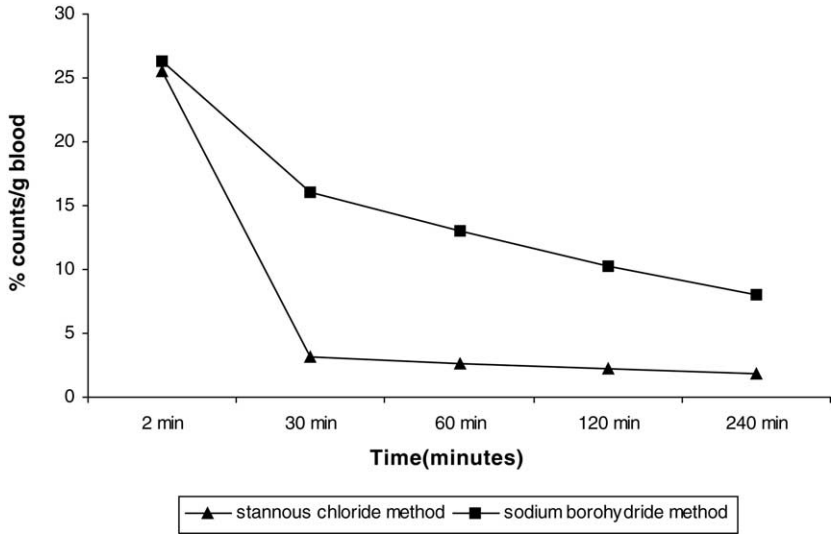

Fig. 2. Blood clearance study of radiolabeled chitosan nanoparticle complex prepared by stannous chloride method (a) and sodium borohydride method (b).

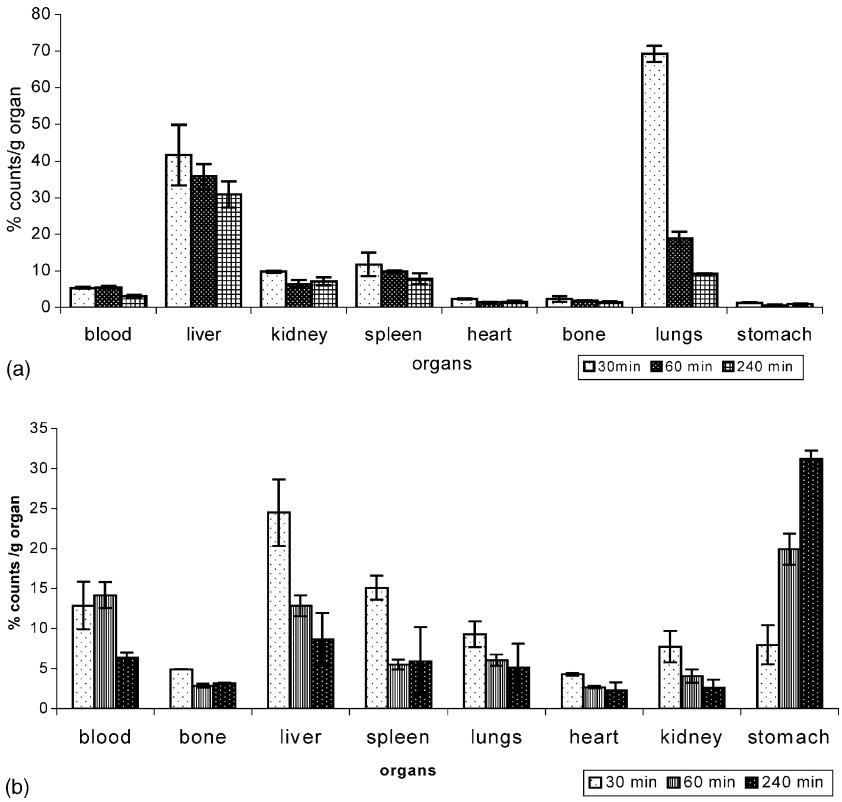

Fig. 3. Biodistribution of radiolabeled chitosan nanoparticle complex prepared by stannous chloride method (a) and sodium borohydride method (b).

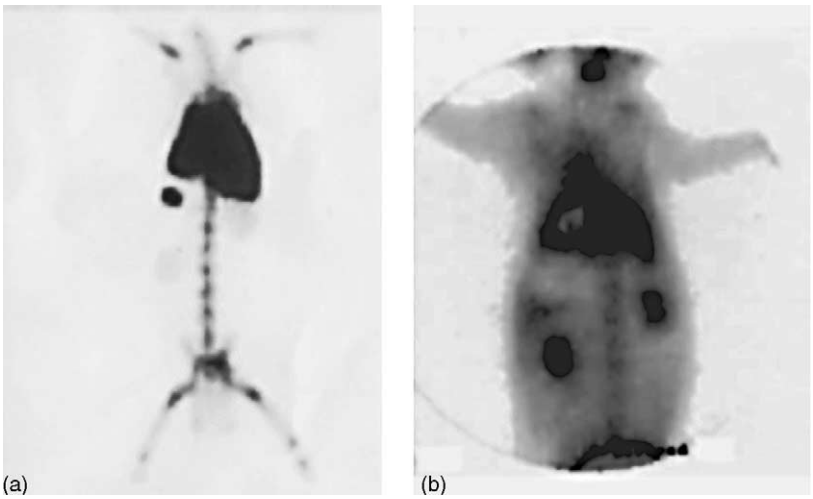

Fig. 4. Gamma images at 2 h post injection of radiolabeled chitosan nanoparticles complex by stannous chloride method (a) and sodium borohydride method (b).

dent from the above observations, the results obtained using stannous chloride as the reducing agent are contradictory to our expectations of nanometer sized hydrogel nanoparticles, as was previously reported from our laboratory (Gaur et al., 2000). Our assumption is that during the reduction of pertechnetate ions with acidic stannous chloride, the stannous chloride gets oxidized in the process to tin oxides and hydroxides suspended in aqueous solution in the form of highly polydispersed and micron sized colloidal particles. These colloidal tin oxides cannot be separated from chitosan nanoparticles before injection. The nanoparticles are probably adhered on the surface of these tin oxide colloids due to adhesive nature of chitosan polymer (Bernkop-Schnurch and Krajicek, 1998). As a result, the distribution of colloidal tin oxides contributed primarily to the results of biodistribution of chitosan nanoparticles. While reducing the pertechnetate ions with sodium borohydride, boric acid and its sodium salt are formed as the oxidized products, which remain in the solution and do not add to the radiolabeled impurities. As a result, these nanoparticles interact independently with the various body tissues while on circulation and are not hindered by any other colloidal particulate matter in the system. This is clearly evident from the completely different biodistribution profile of chitosan nanoparticles following sodium borohydride reduction method (Fig. 3(b)). Chitosan, being a hydrogel polymer, is expected to have least interaction with the amphiphilic proteins (opsonins) in the blood serum. However, the RES uptake of the nanoparticles should also reduce. This is manifested through the relatively longer residence time of these nanoparticles in blood (Fig. 2) and reduced activity in the liver and spleen as seen in the body distribution study (Fig. 3(b)) as well as gamma image (Fig. 4(b)). However, due to poor stability of ^{99m}Tc-chitosan complex, the dissociation of the complex becomes significant after 4 h and the free technetium ions are collected in the kidney and bladder.

4. Conclusion

While studying the pharmacokinetics and biodistribution of ^{99m}Tc labeled chitosan nanoparticles, it was found that stannous chloride and other stannous salts, when used as reducing agents, lead to the formation of colloidal tin (IV) oxide particles, and the nanoparticles are adsorbed on the surface of these colloidal particles. As a result, the biodistribution of radiolabeled chitosan nanoparticles is governed by the biodistribution of tin oxide colloids. When we used sodium borohydride as a reducing agent, no such interference of colloidal particles was observed and the hydrogel chitosan nanoparticles exhibit long circulation and less RES uptake as expected.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.ijpharm.2004.09.022.

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