Albumin Microspheres as Carriers for the Antiarthritic Drug Celecoxib

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ABSTRACT

The present study investigates the preparation of celecoxib-loaded albumin microspheres and the biodistribution of technetium-99m (^{99m}Tc)-labeled celecoxib as well as its microspheres after intravenous administration. Microspheres were prepared using a natural polymer BSA using emulsification chemical cross-linking method. The prepared microspheres were characterized for entrapment efficiency, particle size, and in vitro drug release. Surface morphology was studied by scanning electron microscopy. Biodistribution studies were performed by radiolabeling celecoxib (CS) and its microspheres (CMS) using ^{99m}Tc and injecting arthritic rats intravenously. The geometric mean diameter of the microspheres was found to be 5.46 μm. In vitro release studies indicated that the microspheres sustained the release of the drug for ∽6 days. Radioactivity measured in different organs after intravenous administration of celecoxib solution showed a significant amount of radioactivity in the liver and spleen. In case of celecoxib-loaded microspheres, a significant amount of radioactivity accumulated in the lungs. No significant difference ($P > .1$) in the radioactivity was observed between the inflamed joint and the noninflamed joint following intravenous injection of ^{99m}Tc-CS. However, in case of the microspheres (CMS), the radioactivity present in the inflamed joint was 2.5-fold higher than in the noninflamed joint. The blood kinetic studies revealed that celecoxib-loaded albumin microspheres exhibited prolonged circulation than the celecoxib solution.

KEYWORDS: microspheres, albumin, celecoxib, technetium-99m, biodistribution

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INTRODUCTION

Albumin is a major plasma protein constituent, accounting for ∽55% of the total protein in human plasma.[1] Since they were first described by Kramer,[2] albumin microspheres have been extensively investigated in controlled release systems as vehicles for the delivery of therapeutic agents to local sites. The exploitable features of albumin include its reported biodegradation into natural products,[3] its lack of toxicity,[4,5] and its nonantigenicity. Albumin microspheres are metabolized in the body, and the size of particles, degree of stabilization, and site of metabolism are the main factors influencing the extent of metabolism.[3] Drug release from the microspheres can be widely modulated by the extent and nature of cross-linking, size, the position of the drug, and its incorporation level in the microspheres.[6] Colloidal forms of albumin have been considered as potential carriers of drugs for their site-specific localization or their local application to anatomically discrete sites.[7] Albumin has been used as a carrier for targeting drugs to tumors, and since the synovium of the rheumatoid arthritic patients shares various features observed in tumors, albumin-based delivery systems can be used to target drugs to the inflamed joint. Intravenous administration of the drugs coupled with albumin has been reported to improve the targeting efficiency of the drug to arthritic regions.[8] The circulation half-lives of the drugs have been reported to dramatically increase when the drug is conjugated with albumin.[9] Increasing the circulation half-life of the formulation by reducing its uptake by the reticuloendothelial system has been shown to improve the targeting efficiency of the formulation to the arthritic paws of rats.[10] There are several reports on the use of long circulating liposomes to target the drugs to the arthritic joints.[10-12] However, there are only a few reports on the use of microspheres for targeting the drugs to the arthritic joints.[13] Hence in this study, the use of intravenously administered biodegradable microspheres as a targeted drug delivery system for delivering celecoxib to the arthritic joints of rats is reported. Celecoxib is a selective cyclo-oxygenase-II (COX-II) inhibitor and is associated with a lower incidence of gastrointestinal side effects than the traditional nonsteroidal anti-inflammatory drugs (NSAIDs). But since COX-II is constitutively present in some organs, such as kidney

and brain, and can be induced in other tissues, even the specific COX-II inhibitors are not devoid of side effects. Thus, celecoxib is associated with increased cardiovascular and renal side effects.[14-17] Achieving higher concentrations of the drug at the arthritic joint and minimizing its distribution to the other tissues would minimize the side effects associated with the drug. Targeting drugs to the inflamed joints, in the treatment of rheumatoid arthritis, would reduce the amount of drug required to control the disease, with possible additional reduction or even elimination of adverse side effects.[18] Colloidal particles introduced in the circulation can concentrate in the inflammatory lesions.[19] Thus, in this investigation, celecoxib-loaded bovine serum albumin (BSA) microspheres were prepared and the biodistribution of the microspheres was compared with that of celecoxib after intravenous administration in arthritic rats. Albumin, being biodegradable, biocompatible, and nontoxic, was chosen as a matrix material for the preparation of the microspheres.

MATERIALS AND METHODS

The drug celecoxib was a gift from Sun Pharmaceutical Advanced Research Centre (Vadodara, India). BSA and pepsin were purchased from S.D. Fine Chemicals Ltd (Mumbai, India). The cross-linking agent glutaraldehyde solution (25%) was purchased from E-Merck India Ltd, Mumbai, India. Diethylene triamine penta acetic acid (DTPA) and stannous chloride ($SnCl_2.2H_2O$) were purchased from Sigma Chemical Co (St Louis, MO). Sodium pertechnetate separated from Molybdenum-99 by solvent extraction method was procured from Regional Center for Radiopharmaceutical Division (Northern region), Board of Radiation and Isotope Technology (Delhi, India). Complete Freund's adjuvant was purchased from Bangalore Genei Ltd, Bangalore, India. All other chemicals and solvents were of analytical grade and used without further purification.

Preparation of Microspheres

Albumin microspheres were prepared using emulsion polymerization technique.[20] A weighed amount of BSA (30% wt/wt) was dissolved in distilled water. Tween-80 was added at a concentration of 2% wt/wt. Celecoxib was finely triturated in a mortar and passed through sieve no. 400. Ten milligrams of finely powdered celecoxib was added to the above solution and sonicated to obtain a uniform dispersion. One milliliter of this dispersion was injected into a mixture of 20 mL of heavy liquid paraffin and 0.5 mL of span-85, while stirring at 2500 rpm. Stirring was continued for 10 minutes to obtain a water/oil (w/o) emulsion. One milliliter of glutaraldehyde was added into the emulsion to cross-link the albumin present in the internal phase of the emulsion. Microspheres formed were then separated by centrifugation and washed with 30 mL of petroleum ether to remove the liquid paraffin. The microspheres were then suspended in 10 mL of 5% wt/vol sodium bisulphite solution and stirred on a magnetic stirrer for 10 minutes to remove the residual glutaraldehyde. Finally, the microspheres were washed with 100 mL of water until they were free from residual glutaraldehyde; then they were dried at room temperature and stored in a dessicator until further use.

Characterization of Microspheres

Entrapment Efficiency

A weighed amount of microspheres were suspended in 0.1 N hydrochloric acid containing 2% wt/vol pepsin and allowed to stand for 24 hours. The dispersion was then shaken with methylene chloride to extract celecoxib. The organic extract was evaporated to dryness and the residue dissolved in methanol. The absorbance of the resulting solution was measured at 250 nm on a Shimadzu-UV 1601 spectrophotometer (Shimadzu, Kyoto, Japan) to determine the amount of celecoxib present in the microspheres.

Particle Size

The particle size distribution of the microspheres was determined by laser light scattering on a Malvern particle size analyzer (Malvern Master Sizer 2000, SM, Malvern, United Kingdom). The microspheres were added to the sample dispersion unit containing the stirrer and stirred to reduce the aggregation between the microspheres; laser obscuration range was maintained between 15% and 20%. The average volume-mean particle size was measured after performing the experiment in triplicate.

In Vitro Drug Release

Drug release from the microspheres was determined using phosphate buffer (pH 7.4) containing 2% wt/wt Tween-80 as the release medium. A weighed amount of microspheres, equivalent to 2.5 mg of celecoxib, were suspended in 50 mL of the dissolution medium in 100-mL glass vials and stirred on a magnetic stirrer at 50 rpm in a thermostated bath at 37°C. Two-milliliter samples were withdrawn at appropriate time intervals and centrifuged at 5000 rpm. Supernatants were diluted suitably, and absorbance of the resulting solution was measured at 250 nm using the dissolution medium as blank. The residue was redispersed in 2 mL of the fresh dissolution medium and replaced back into the vial.

Surface Morphology of Microspheres

Scanning electron microscopy (SEM) of the albumin microspheres was performed to examine the surface morphology.

The microspheres were mounted on metal stubs and then coated with a 150-Å layer of gold. Photomicrographs were taken using a Jeol scanning electron microscope (Jeol, JSM-5610LV SEM, Tokyo, Japan).

Radiolabeling of Celecoxib and Its Microspheres

Celecoxib (CS) and albumin microspheres (CMS) were labeled with technetium-99m (^{99m}Tc) by direct labeling method as described earlier.[21] Briefly, 1 mL of ^{99m}Tc (2 mci/mL) was mixed with 60 μL of stannous chloride solution in 10% vol/vol acetic acid (1 mg/mL), and the pH was adjusted to 6.5 using 0.5 mol/L sodium bicarbonate solution. To this mixture, 1 mL of the celecoxib solution (2 mg/mL) or the microsphere suspension (30–40 mg/mL) was added and incubated for 10 minutes at room temperature. The quality control was performed as per the method described earlier.[22] The effects of stannous chloride concentration, incubation time, and pH on the labeling efficiency were studied by varying the factor in question and keeping the other factors constant.

Labeling Efficiency

The radiochemical purity of ^{99m}Tc celecoxib and the microspheres was estimated by ascending instant thin layer chromatography (ITLC) using silica gel (SG)-coated fiber sheets (Gelman Sciences Inc, Ann Arbor, MI). The ITLC was performed using 100% acetone or 0.9% saline as the mobile phase. Around 2 to 3 μL of the radiolabeled complex was applied at a point 1 cm from the end of an ITLC-SG strip. The strip was developed in acetone or 0.9% saline, and the solvent front was allowed to reach 8 cm from the origin. The strip was cut into 2 halves, and the radioactivity in each segment was measured in a well-type gamma ray counter (Gamma ray spectrometer, type GRS23C, Electronics Corporation of India Ltd, Mumbai, India). The free pertechnetate that moved with the solvent (Rf = 0.9) was determined. The reduced/hydrolyzed (R/H) technetium along with the labeled complex remained at the point of application. The amount of R/H ^{99m}Tc (radiocolloids) was determined using a mixture of pyridine, acetic acid, and water in the ratio of 3:5:1.5 vol/vol as mobile phase. The radiocolloids remained at the point of application, while free pertechnetate and the labeled complex moved away with the solvent front. By subtracting the activity moved with the solvent front, using either acetone or saline, from that using pyridine/acetic acid/water as a mixture, the net amount of radiolabeled celecoxib as well as microspheres was calculated.

Stability Study of ^{99m}Tc-celecoxib/Microsphere Complexes

The stability of the radiolabeled complex was determined in vitro using 0.9% sodium chloride and serum by instant thin layer chromatography (ITLC). A volume of 0.1 mL of the radiolabeled complex was mixed with 1.9 mL of normal saline and 0.9 mL of human serum and incubated at 37°C. ITLC was performed at different time intervals to determine the stability of the complex.

Transchelation With DTPA

In order to check the stability and strength of the bonding of ^{99m}Tc with celecoxib/microspheres, 0.5 mL of 1.0 mM solutions of DTPA in saline were taken in separate 5-mL vials. To these vials, 0.1 mL of the radiolabeled celecoxib/microspheres was added. After brief mixing, each vial was incubated for 1 hour at 37°C. As a control, 0.5 mL of saline was mixed with 0.1 mL of the labeled preparation and incubated for 1 hour at 37°C. The effect of DTPA on the labeling efficiency was determined using ITLC-SG using normal saline as the mobile phase, which allowed the separation of free pertechnetate and DTPA-complex (Rf = 0.8–1) from ^{99m}Tc-celecoxib/microsphere complex, which remained at the point of application (Rf = 0).

In Vivo Studies

Adjuvant-induced Arthritis

Male Sprague-Dawley rats weighing between 300 and 350 g were used for in vivo studies. Mono-articular arthritis was induced in left knee joints of the rats by injecting 0.1 mL of the Complete Freund's adjuvant[23] through the suprapatella ligament using a 27-gauge needle. The development of arthritis was monitored regularly by measuring changes in the knee joint diameter using Varnier calipers; the mean of 3 readings was taken with the joint flexed at 90°. Four days after the induction of the arthritis, the diameter of the arthritic joint was 19.4 ± 0.2 mm, while that of the control joint was 12.2 ± 0.3 mm.

Biodistribution Studies

All animal experiments conducted were approved by the Institutional Animal Ethics Committee. Biodistribution of the ^{99m}Tc-celecoxib/microspheres complexes was studied in arthritic Sprague-Dawley rats weighing 300 to 350 g. Four days after the induction of the arthritis, 250 μL (250 μCi) of the radiolabeled preparation was injected intravenously via the tail vein into each rat. Groups of 3 rats per group per time point were used in the study. The rats were humanely killed at 1 hour, 4 hours, and 24 hours after intravenous injections, and blood was obtained by cardiac puncture. Subsequently, tissues (heart, lung, liver, kidney, spleen, intestine, stomach, and lungs) were taken out. The knee joints, both the left and the right, were exposed and were cut into fragments. All the tissues were washed with normal saline, blotted dry, and weighed; then their radioactivity was measured in a well-type gamma scintillation counter. To correct for physical decay and to calculate radiopharmaceutical uptake in each organ as

Table 1. Effect of Albumin Concentration, Span-85 Concentration, and Volume of Glutaraldehyde on Particle Size and Entrapment Efficiency*

Batch Code	BSA Concentration (% wt/wt)	Span-85 Concentration (% wt/wt)	Volume of Glutaraldehyde (mL)	Particle size (μm)	Entrapment Efficiency (%)
A	20	2.0	0.5	4.42 ± 0.28	70.52 ± 1.40
B	20	2.0	1.0	4.11 ± 0.13	68.31 ± 1.77
C	20	5.0	0.5	3.22 ± 0.19	55.24 ± 1.68
D	20	5.0	1.0	3.05 ± 0.12	57.19 ± 0.88
E	30	2.0	0.5	6.29 ± 0.52	88.58 ± 0.95
F	30	2.0	1.0	5.46 ± 0.31	90.63 ± 1.90
G	30	5.0	0.5	3.71 ± 0.08	82.64 ± 3.66
H	30	5.0	1.0	4.13 ± 0.10	80.51 ± 2.72

*BSA indicates bovine serum albumin.

a fraction of the injected dose, aliquots of the injectate, containing 1% of the injected dose, were counted simultaneously at each time point.

Blood Kinetics

The clearance of the ^{99m}Tc-celecoxib/microspheres into the systemic circulation was studied in arthritic rabbits weighing 3 to 3.5 kg. Arthritis was induced in the left knee joints of the rabbits by injecting 0.5 mL of the Complete Freund's adjuvant. Assessment of arthritis was done by measuring the knee joint diameter by means of Varnier calipers. The diameter of the arthritic joint after 4 days of induction was 33.5 ± 0.4 mm, while that of the control joint was 25.4 ± 0.3 mm. Five hundred microliters (500 μCi) of the labeled preparation was injected intravenously through the left marginal ear vein of each rabbit of known weight. Blood was withdrawn at different time intervals from the marginal ear vein. The radioactivity was measured in a well-type gamma ray counter (Gamma ray spectrometer, type GRS23C, Electronics Corp of India Ltd, Mumbai, India). The blood was weighed, and the radioactivity present in the whole blood was calculated by keeping 7% of the body weight as the total weight of the blood.[24]

Statistical Analysis

The results are expressed as mean ± SD and were analyzed using a Kruskal-Wallis multiple comparison test followed by post hoc Dunn's test at the significance level of $P < .05$ and $P < .005$.

RESULTS AND DISCUSSION

Characteristics of Microspheres

Albumin microspheres with high entrapment efficiency were prepared using the emulsification chemical cross-linking technique. The various factors affecting the characteristics of the microspheres were studied. The entrapment efficiency was found to be mainly dependent on the concentration of albumin and concentration of span-85. As shown in Table 1, an increase in the concentration of albumin from 20% wt/wt to 30% wt/wt led to a significant increase ($P < .05$) in the entrapment efficiency. The entrapment efficiency increases with an increase in the albumin concentration because with an increase in the albumin concentration, more viscous solutions are formed that can more efficiently prevent the dissolution of celecoxib in the external phase of the emulsion. At a lower concentration of albumin, a major amount of the drug remained as free drug. There was a significant decrease in the entrapment efficiency with an increase in the concentration of span-85 from 2% wt/wt to 5% wt/wt. The decrease in the entrapment efficiency with an increase in the emulsifier concentration is because of dissolution of celecoxib in the external phase of the emulsion at higher concentration of span-85. This decrease in the entrapment efficiency was more pronounced at a lower concentration of albumin.

The geometric mean diameter of the microspheres was found to be 5.46 μm. The particle size was found to be mainly dependent on the albumin concentration, span-85 concentration, and stirring speed. An increase in the albumin concentration from 20% wt/wt to 30% wt/wt led to a significant increase in the particle size. An increase in the span-85 concentration led to a significant decrease ($P < .05$) in the particle size. The decrease in the particle size of the microspheres with an increase in the concentration of span-85 is due to a decrease in the interfacial tension between the aqueous droplets and organic suspension medium, as a result of an increase in the emulsifier concentration. The increase in the particle size with an increase in the albumin concentration is due to the formation of bigger droplets of the internal phase because of the increase in the viscosity.

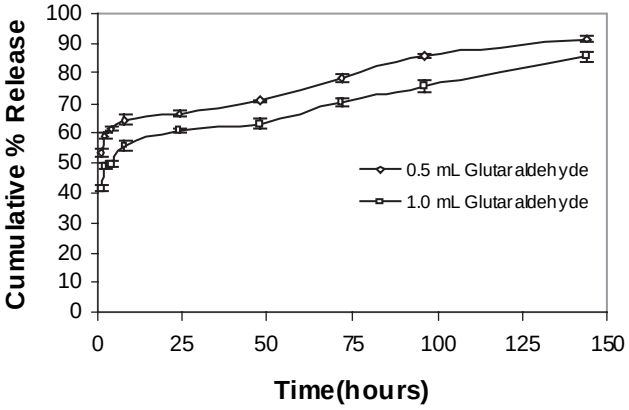

Figure 1. In vitro release profile of celecoxib-loaded albumin microspheres. The error bars in the chart represent the SD (n = 3) values.

There was a decrease in the particle size with an increase in the stirring speed from 1500 rpm to 2500 rpm. The decrease in the particle size with an increase in the emulsifier concentration was more pronounced at a lower concentration of albumin. The volume of glutaraldehyde had no significant influence on the entrapment efficiency, and the particle size of the microspheres as shown in Table 1.

In order to evaluate the effect of concentration of cross-linking agent and duration of cross-linking on the release characteristics of the drug from the microspheres, glutaraldehyde was used in 2 different concentrations (0.5 mL and 1.0 mL). It was found that there was a decrease in the drug release with an increase in the concentration of cross-linking agent as shown in Figure 1. The decrease in the drug release with an increase in the concentration of the cross-linking agent is because of the formation of denser polymer cross-links leading to an increase in the diffusional path length that the drug molecules have to traverse. This is in accordance with the earlier reports.[25] The kinetics of release of celecoxib from BSA microspheres showed a biphasic drug release pattern with an initial burst release followed by a slower release. This burst effect can be attributed to the presence of drug crystals on the surface of the microspheres as evident from the SEM (Figure 2). The burst release is followed by a slow and controlled release phase resulting from controlled diffusion of the entrapped drug. To examine the drug release kinetics, the release data were fitted to models representing zero order, first order, and Higuchi's square root of time kinetics. The coefficient of determination (R^2) values (calculated from the plots of Q vs t for zero order, log(Qo-Q) vs t for first order and Q vs $t^{1/2}$ for Higuchi model, where Q is the amount of drug released at time t, (Q_0-Q) is the amount of drug remaining after time t) was much closer to 1 for Higuchi kinetics indicating that after the initial burst release, the drug release from the microspheres followed a diffusion controlled mechanism.

The toxicity of the residual glutaraldehyde employed for cross-linking proteins and polysaccharides has been of concern, but the residual glutaraldehyde can be completely removed by bisulphite wash.[26] The nucleophilic addition of bisulphite across the pi bond of the carbonyl group of glutaraldehyde produces a water-soluble sodium salt of an organic sulphite, which can be easily removed by water washing. Thus, sodium bisulphite was used to remove the residual glutaraldehyde, and the residual glutaraldehyde determined by reaction with hydroxylamine hydrochloride[27] was found to be less than 5 ppm.

The formulation prepared using 30% albumin, 0.5 mL span-85, and 1.0 mL glutaraldehyde, with 3 hours stirring at 2500 rpm, was chosen for radiolabeling and in vivo studies because of its high entrapment efficiency and sustained drug release.

Radiolabeling of Microspheres

Albumin microparticles of various sizes have been used as carriers for radioactive diagnostics and therapeutic mole-

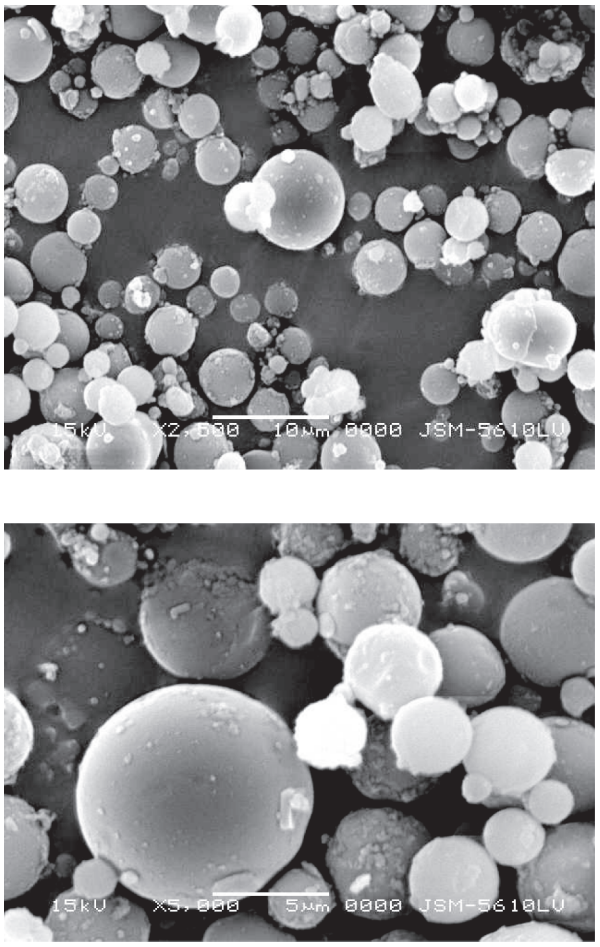

Figure 2. SEM of celecoxib-loaded albumin microspheres. The photomicrographs were taken at 2 different magnifications (original magnification ×2500 and ×5000).

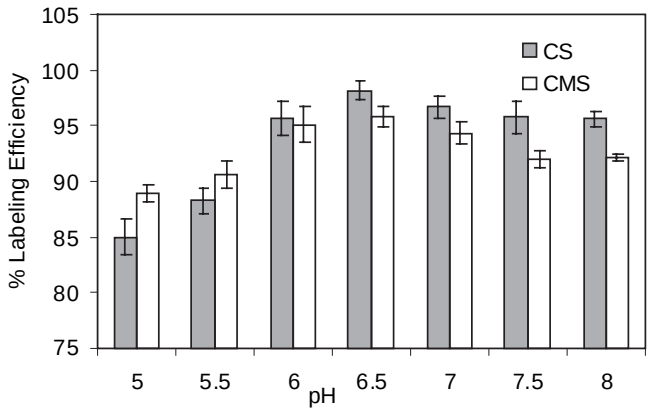

Figure 3. Effect of pH on the labeling efficiency of celecoxib (CS) and celecoxib-loaded albumin microspheres (CMS). Error bars in the graph represent the SD (n = 3) values.

cules.[7] Tc-99m microspheres of human serum albumin have been widely used for clinical nuclear medicine, particularly for lung scanning since 1969.[28,29] Celecoxib and its microspheres were labeled with high efficiency by the direct labeling technique using reduced ^{99m}Tc. Data on radiochemical purity and stability of the labeled complex were obtained by ascending chromatography using saline or 100% acetone as the solvent. The radiochemical impurities are free pertechnetate and reduced/hydrolyzed ^{99m}Tc (radiocolloids) in the ^{99m}Tc-labeled complexes. Figure 3 depicts the effect of pH on the labeling efficiency. As the pH increases from 5 to 6.5, the labeling efficiency also increases from 85% to 98.17% for CS and from 88.91% to 95.85% for CMS. With further increase in the pH, there was a decrease in the labeling efficiency.

The influence of the $SnCl_2.2H_2O$ concentration on the labeling efficiency CS and CMS is shown in Table 2. The labeling efficiency of CS increased from 86.8% to 98.65% when the stannous chloride concentration was increased from 30 μg to 60 μg. Further increase in the concentration of stannous chloride led to a decrease in the labeling efficiency. In the case of CMS, increase in the concentration of stannous chloride from 30 μg to 60 μg led to increase in the labeling efficiency from 85.91% to 95.85%. Further increase in the stannous chloride concentration led to decrease in the labeling efficiency. Also in both the drug and the microspheres, when lower concentration of stannous chloride was used, the remaining activity was as free TcO_4^-, while at higher concentrations of stannous chloride, the amount of radiocolloids increased.

The incubation time required for high labeling efficiency was found to be 10 minutes for CS and CMS.

In Vitro Stability

Stability of the labeled complex with time was studied in saline and in serum (human) at 37°C as shown in Table 3. There was no significant difference ($P > .1$) between the percentage radiolabeled CS or CMS with time indicating a high stability of ^{99m}Tc-CS and ^{99m}Tc-CMS. It is evident from the results that there is insignificant detachment of the radioisotope from the complex. Even after 24 hours of incubation, there is no significant reduction in the radiolabeled compound, indicating its high stability and suitability for in vivo use. Further, the stability was confirmed by challenging the labeled complex with DTPA solution. As shown in Table 4, there was no significant difference ($P > .1$) in the percentage radiolabeled CS or CMS when challenged with DTPA, indicating a high stability of the radiolabeled complexes.

Blood Clearance

The radioactivity present in the blood at various time intervals, after intravenous injection of CS as well as CMS, is shown in Figure 4. The blood clearance of the ^{99m}Tc-celecoxib and ^{99m}Tc-microsphere conducted in rabbits showed that the half-life of the drug when entrapped in microspheres was greater than the drug in its free state. As shown in Figure 4, the drug is very rapidly cleared from the blood, only 5% radioactivity being detected 5 minutes after intravenous injection. On the other hand, in the case of microspheres, 15% of the injected dose is detected at 5 minutes post injection. Twenty-four hours post injection, only 1% of the injected radioactivity is present in the blood in the case of

Table 2. Effect of Stannous Chloride Concentration on the Labeling Efficiency of Celecoxib and Its Microspheres*

	CS			CMS		
$SnCl_2.2H_2O$ μg	% Bound	% Colloids	% Free	% Bound	% Colloids	% Free
30	86.8 ± 1.95	1.44 ± 0.07	11.76 ± 1.01	85.91 ± 0.76	5.05 ± 1.74	9.04 ± 1.66
60	98.65 ± 1.01	0.88 ± 0.17	0.47 ± 0.13	95.85 ± 0.46	3.46 ± 1.11	0.69 ± 0.38
100	95.51 ± 1.09	2.70 ± 0.37	1.79 ± 0.14	91.87 ± 1.29	5.64 ± 1.16	2.49 ± 1.42
150	88.65 ± 1.75	7.84 ± 1.21	3.51 ± 0.26	87.75 ± 1.11	11.52 ± 1.08	0.73 ± 0.79

*CS indicates celecoxib; and CMS, celecoxib microspheres. Data are expressed as mean ± SD (n = 3).

Table 3. Stability of the ^{99m}Tc-Celecoxib and ^{99m}Tc-Microspheres in Physiological Saline and Serum In Vitro at 37°C*

	In Saline		In Serum	
Time in Hours	**CS**	**CMS**	**CS**	**CMS**
0	98.65 ± 1.01	95.85 ± 0.99	98.48 ± 1.43	95.72 ± 0.99
0.25	98.40 ± 0.58	95.61 ± 1.25	98.30 ± 1.17	95.18 ± 1.99
0.5	98.32 ± 1.46	95.35 ± 2.17	97.56 ± 1.18	94.63 ± 1.08
1	97.81 ± 1.57	95.28 ± 1.38	97.14 ± 1.33	94.17 ± 1.82
2	97.46 ± 0.76	94.91 ± 1.90	96.82 ± 0.82	94.85 ± 1.25
4	97.20 ± 1.14	94.56 ± 1.10	96.14 ± 1.42	93.71 ± 1.18
24	96.65 ± 0.93	93.82 ± 1.06	94.50 ± 0.80	92.66 ± 2.37

*CS indicates celecoxib; and CMS, celecoxib microspheres. The samples were subjected to instant thin layer chromatography using silica gel-coated fiber sheets with 100% acetone as the mobile phase. Data are expressed as percentage of the total radioactivity in sample.

the drug solution, whereas in the case of microspheres, ∽8% of the injected radioactivity is present, indicating an 8-fold increase. Thus, albumin microspheres exhibit a longer residence time in blood as compared with celecoxib. This is owing to the high protein binding affinity of albumin. Albumin is not highly immunogenic,[30] which probably accounts for the ability of the particles to remain in the circulation long enough to enter into the inflamed tissues of the joint. The endothelium at the site of inflammation is more permeable and leaky, leading to preferential accumulation of the long-circulating microspheres in these regions. Thus, albumin microspheres have a prolonged presence in the circulation and consequently ample time to escape from the circulation to the inflamed region through the leaky endothelium.

Biodistribution Study of ^{99m}Tc-CS and ^{99m}Tc-CMS

The biodistribution of ^{99m}Tc-CS after 1 hour, 4 hours, and 24 hours is shown in Table 5. The percentage radioactivity is expressed per whole organ or tissue. The percentage injected dose/per whole organ of rats at different times after intravenous injection of the ^{99m}Tc-CMS is depicted in Table 6. In the case of ^{99m}Tc-CS, the radioactivities present in the whole organ/tissue at 1 hour postinjection were found as follows: blood (0.861%), liver (33.44%), spleen (5.59%), lungs (0.98%), and kidney (7.1%). Thus, celecoxib is extensively distributed in organs such as liver, kidney, and spleen, whereas in the case of ^{99m}Tc-CMS, the major amount of the injected activity accumulated in lungs, 6.2% being present per whole organ 1 hour postinjection. The radioactivities present in the whole organ/tissue at 1 hour postinjection of ^{99m}Tc-CMS were found as follows: blood (3.21%), liver (1.58%), spleen (0.138%), and kidney (1.34%). The radioactivity present in these organs postintravenous injection of ^{99m}Tc-CS was significantly higher ($P < .05$) than that after the injection of ^{99m}Tc-CMS, indicating that the microspheres are not taken up significantly by the liver, spleen, or kidney. A major amount of the injected radioactivity was present in blood and lungs. As reported by earlier workers, a particle of diameter 3 to 12 μm becomes entrapped within the capillary networks of the lungs, liver, and spleen.[7] The particle size of the microspheres was around 5 μm. Thus, a major amount accumulated in the lungs. In the case of ^{99m}Tc-CS, a negligible activity was detected in the inflamed joint, indicating that the celecoxib is not distributed to the inflamed joint. There

Table 4. Transchelation of the Radiolabeled Complexes with DTPA*

	% Radiolabeled	
	Control	**DTPA**
Celecoxib-^{99m}Tc complex	97.56 ± 1.21	95.35 ± 1.36
Microspheres-^{99m}Tc complex	95.67 ± 0.75	91.46 ± 0.81

*DTPA indicates diethylene triamine penta acetic acid. Data are expressed as mean ± SD (n = 3).

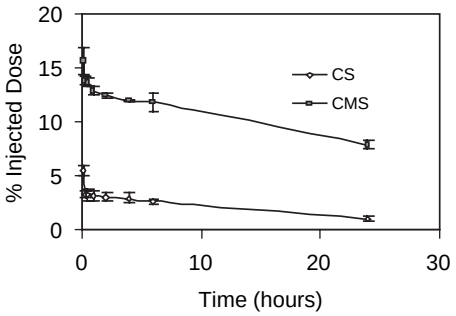

Figure 4. Blood clearance studies of celecoxib solution and celecoxib-loaded albumin microspheres. The rabbits were administered 0.5 mL (500 μci) of CS and CMS intravenously through left marginal vein. Blood samples were collected at different time intervals, and the radioactivity present was determined. Error bars in the graph represent the SD values.

Table 5. Biodistribution of ^{99m}Tc-Celecoxib in Sprague-Dawley Rats After Intravenous Administration*

Organ/ Tissue	% Injected Dose/Whole Organ or Tissue (±) SD		
	1 Hour	4 Hours	24 Hours
Blood	0.861 ± 0.136	0.543 ± 0.060	0.085 ± 0.028
Heart	0.030 ± 0.019	0.133 ± 0.014	0.106 ± 0.088
Liver	33.442 ± 4.002	27.181 ± 3.638	17.11 ± 3.300
Spleen	5.59 ± 0.584	5.58 ± 0.947	5.295 ± 1.494
Kidney	7.104 ± 1.521	3.99 ± 0.900	3.266 ± 0.806
Lung	0.98 ± 0.159	1.33 ± 0.242	1.28 ± 0.139
Intestine	0.361 ± 0.182	0.321 ± 0.089	0.336 ± 0.103
Stomach	0.430 ± 0.024	0.409 ± 0.172	0.331 ± 0.084
Noninflamed joint	0.044 ± 0.015	0.033 ± 0.020	0.063 ± 0.018
Inflamed joint	0.071 ± 0.013	0.048 ± 0.030	0.077 ± 0.022

*Each value is expressed as mean ± SD (n = 3).

Table 6. Biodistribution of ^{99m}Tc-Microspheres in Sprague-Dawley Rats After Intravenous Injection*

Organ/ Tissue	% Injected Dose/Whole Organ or Tissue (±) SD		
	1 Hour	4 Hours	24 Hours
Blood	3.210 ± 0.905	3.531 ± 0.459	2.433 ± 0.416
Heart	0.054 ± 0.013	0.059 ± 0.028	0.022 ± 0.013
Liver	1.58 ± 0.305	0.980 ± 0.138	0.997 ± 0.177
Spleen	0.138 ± 0.045	0.094 ± 0.028	0.121 ± 0.035
Kidney	1.342 ± 0.580	1.358 ± 0.056	0.580 ± 0.157
Lung	6.207 ± 0.657	3.390 ± 0.051	2.952 ± 0.113
Intestine	2.44 ± 0.412	0.119 ± 0.021	0.151 ± 0.018
Stomach	0.646 ± 0.049	0.174 ± 0.066	0.129 ± 0.013
Noninflamed joint	0.073 ± 0.013	0.0691 ± 0.022	0.042 ± 0.018
Inflamed joint	0.255 ± 0.058	0.204 ± 0.161	0.106 ± 0.018

*Each value is expressed as mean ± SD (n = 3).

was no significant difference ($P > .1$) in the radioactivity in the inflamed or noninflamed joint. While in the case of ^{99m}Tc-CMS, the radioactivity present in the inflamed joint was 2.5-fold higher than the noninflamed joint. Because of the prolonged circulation, around 0.25% of the injected dose was able to reach the inflamed tissue in the case of ^{99m}Tc-CMS, which was significantly higher ($P < .05$) than the ^{99m}Tc-CS. This result indicates that the increased capillary permeability in the inflamed joint is responsible for the accumulation of the activity. The higher particle size of the microspheres does not allow the redistribution of the microspheres to the other organs. Thus, there is no significant difference ($P < .05$) between the radioactivity present in the inflamed joint 4 hours and 24 hours postinjection.

A negligible amount of free label (^{99m}Tc) is observed over the course of the experiment, which is evidenced by less activity in stomach. Celecoxib is also reported to have a beneficial effect on lung carcinoma.[31–35] The high concentrations of the celecoxib-loaded microspheres detected in the lungs for a prolonged period of time after intravenous injection indicate its potential use as targeted drug delivery system for lung carcinoma.

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