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PREPARATION, ANALYSIS AND APPLICATION OF ^{99m}Tc-SULPHUR MICROCOLLOID FOR BONE MARROW AND LYMPH SCINTIGRAPHY

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Kits were developed for labeling sulphur microcolloids with ^{99m}Tc. The microcolloids were prepared to get the desired particle size. The stannous chloride was treated with sulphide ions released from thioacetamide in the presence of carboxymethyl cellulose and the pH of the reaction was adjusted to 3.0. The contents of single reaction vial were reacted with ^{99m}Tc, the radiochemical yield was higher than 95%. Sulphur-microcolloid kits were stable and the stability was followed for 6 hours. The freeze-dried kits were followed more than three months after production and were found stable. Bone marrow uptake in rabbits was determined to be about 36%. The preparation of ^{99m}Tc-sulphur microcolloid is performed in single step process and axcellent node scintigraphy was obtained using experimental animal.

Lymph node scanning was introduced in nuclear medicine as a diagnostic procedure in 1968.[1] Gold-198 has been used for visualization of lymph node for more than 15 years,[1] now ^{99m}Tc-colloids are used.[2] The drawbacks of injected dose, are low bone-marrow concentration and a urinary excretion which alter the clarity of the marrow imaging.[3] The above disadvantages make it necessary to improve the colloidal properties of ^{99m}Tc-S-colloids. STERN et al.[4] developed a procedure for preparing ^{99m}Tc-S-colloids for liver by reducing $^{99m}TcO_4^-$ with an acid in the presence of sodium thiosulphate and carboxymethyl cellulose as a stabilizer. Other authors[5] used thioacetamide instead of thiosulphate for preparing a kit for liver and spleen. In this paper we used the same procedure as in Reference 5 with some modification to get the desired particle size for the preparation of sulphur microcolloid kits to be labeled with technetium in single step process with high labelling efficiency.

Experimental

Preparation of ^{99m}Tc-S-microcolloids: Low viscosity carboxymethyl cellulose sodium salt was obtained from Sigma Chemical Company, USA. Hydrated stannous chloride and thioacetamide were obtained from Riedel-De-Haen, Seelze-Hannover,

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FRG. $^{99m}TcO_4^-$ was eluted from ^{99}Mo–^{99m}Tc generator (Radiochemical Centre-Amersham).

All reagent solutions were made up in distilled water or physiological saline and were purged with nitrogen. For preparation of ^{99m}Tc-S-microcolloid from Nuclear Research Centre, Baghdad, Iraq (NRC), briefly the formation of sulphide precolloid was prepared by adding thioacetamide (5 mg/ml) stabilized with carboxymethyl cellulose (6 mg/ml). The solution was heated in boiling water, then coold down to room temperature. Tin chloride solution (10 mg/ml) was added to the reaction mixture to form tin(II)-sulphur-colloid and the excess hyrogen sulphide was removed by purging with nitrogen gas. The reagent was sterilized by membrane filtration (0.1 μm) and the pH was adjusted to 3.0 using 0.3M phosphate buffer. 3 ml portions were placed in vials for lyophilization. The contents of single reaction vial performed (0.2 mg) tin(II) sulphide and 3.75 mg carboxymethyl cellulose were reacted with ^{99m}Tc in 3 ml saline.

Chromatographic method (paper chromatography): Paper chromatography was used for determining the labelling efficiency using Whatman No. 1 paper strips of 1.5 cm width. The active samples were placed at 5 cm distance from the lower ends of the strips and dried in air. Ascending chromatography was carried out in sealed glass cylinders using 85% methanol as an eluent. The R_f value of $^{99m}TcO_4^-$ and radiocolloids are 0.7 and 0, respectively.

Determination of particle size: Gel-chromatography column was used to determine the relative particle size.[6] A column with an inner diameter of 15 mm and 330 mm length was filled with swollen Sepharose cl-4B to a hight of about 300 mm. The sample to be analyzed was applied on the top of the column in a volume of 0.1 – 0.2 ml and developed with 10 ml saline. The column was then sealed and scanned with a silt-collimated (1 mm) NaI crystal. The radioactivity was distributed throughout the GCS-profile zones according to their particle size. Thus, ^{99m}Tc-reduced hydrolyzed with the particle size < 1000 nm were occupying a zone of 15–30 mm in the top of the column, ^{99m}Tc-pertechnetate with the particle size of 100 – 500 nm occuping a zone of 50 – 65 mm below, ^{99m}Tc-S-microcolloid with a particle size of 5 – 10 nm and 10–100 nm occupying a zone of 65–100 mm and 100–200 mm, respectively, below the top of the column.

Calibration of the gel filtration column: Columns (15 mm × 330 mm) filled with Sepharose Cl-4B to a hight of 300 mm. Sample of 0.1 ml was applied on the top of the column and eluted with 10 ml saline. The columns were calibrated using Blue-Dextran 2000 to determine the volume of maximum operating range. ^{99m}Tc-pertechnetate were used to determine the volume of minimum operating range. Gold-198 colloid with a particle size of 5 nm were used to determine the particle size which have an activity peak 95 mm from the top of the column. ^{99m}Tc-antimony sulfide obtained from Mallincrodit (U.S.A.) was also used for calibration the column. Thus a particle size

range 5 – 15 appaer at 100 mm from the top of the column. A particle size range (1000 nm) and < 130 nm appeared at 15 and 250 mm from the top of the column. Whereas the profile of ^{99m}Tc-sulphur colloids which obtained from Banna (Switzerland) shows a particle size range < 130 nm appear at 250 mm from the top of the column.

The migration of ^{99m}Tc-pertechnetate and various radiocolloids through the column were studies by recording a set of scanning profiles of the above reagents and compared with scanning profiles of our ^{99m}Tc-S-microcolloids.

Preparation, labelling and using of commercial kits: ^{99m}Tc-antimony sulfide colloid (Techne Scan) which is obtained from Mallinckrodt (U.S.A.) were prepared by adding 12 mCi ^{99m}Tc-O_4^- in 2 ml. The reaction mixture were heated in boiling water bath for 15 minutes, the vial was then cooled down to room temperature in tap water. Citrate buffer solution from ampoule was introduced to the vial. Clear aqueous solution were obtained. The radiocolloids are ready for use.

Liver-spleen scintigraphy can be obtained 30 minutes after i.v. injection of the agent, bone-marrow scintigraphy can be obtained 30 – 60 minutes after i.v. injection of the agent, while lymph nodes scintigraphy can be obtained 1–6 hours after subcutaneous injection of 0.2 – 4 mCi. Whereas ^{99m}Tc-sulfur colloid which is obtained from Banna (Switzerland) can be prepared, by adding $^{99m}TcO_4^-$ (5 – 6 mCi) in a volume of 0.5 – 1 ml into the vial. The reaction mixture was then heated in a water bath at 100 °C for 10 minutes and then cooled down to room temperature. The radiocolloid are ready for use. Scintigraphy can be obtained 1 – 2 hours after interdigitial webs injection of 0.2 – 0.3 ml (1 mCi) of the agent.

The yields of labeling of the two agents were high and the free pertechnetate fraction was less than 2% as determined by paper chromatography in 85% methanol.

Tissue distribution: Tissue distribution of ^{99m}Tc-sulphur microcolloid was studied in New Zealand albino rabbits weighing 1.5–2.0 kg by i.v. injection of about 500 μCi into the ear vein. The rabbits were sacrified at different time intervals. The organs of interest (liver, spleen, lungs kidneys, bone marrow of the right femur) and samples of blood and urine were measured using well-type scintillation counter (Berthold MAG 132). The results obtained were normalized to the total injected dose. Blood volume and total bone marrow weight were approximately estimated as 7% and 1.6%[7] of whole bodyweight, respectively. The blood clearance studies for the radiocolloid in rabbits were performed by taking multiple blood samples at different time intervals (3 – 240 minutes) after intravenous administration of 0.5 ml (500 μCi) from the radiocolloid, via ear vein. The blood samples were counted as mentioned before. The results obtained were normalized to the total adminstered radioactivity and corrected for physical decay of ^{99m}Tc. Blood radioactivity expressed in percent of injected dose per ml, was plotted on semilogarithnic paper against time in minutes. The curves were resolved into two exponential components and the biological half-time was calculated.

In-vivo biokinetic studies: Lymph node scintigraphy was performed in New Zeland albino rabbits, weighing (1.5 – 2 kg). The animals were anesthetized with pentabarbitone and fixed in supine position. The radiocolloid 0.5 ml (230 mCi) was injected subcutaneously bilaterally just below the xiphoid process. Lymphoscintiphotos were recorded as a function of time using scintillation large field gamma-camera (General Electronic) equiped with general purpose low energy parallel holes collimator.

Results and discussion

Preparation of ^{99m}Tc-S-microcolloids

Paper chromatography was used to determine ^{99m}Tc-species in the preparation as a function of pH and tin concentration concerning the radiochemical purity of ^{99m}Tc-S-microcolloids. Figure 1 shows the effect of using different concentration of tin

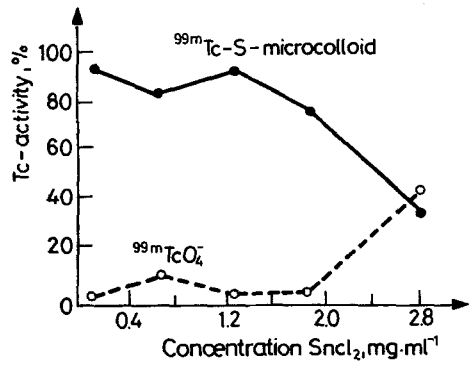

Fig. 1. Percent of bound and unbound ^{99m}Tc-fractions in ^{99m}Tc-S-microcolloid preparation using different concentrations of $SnCl_2$

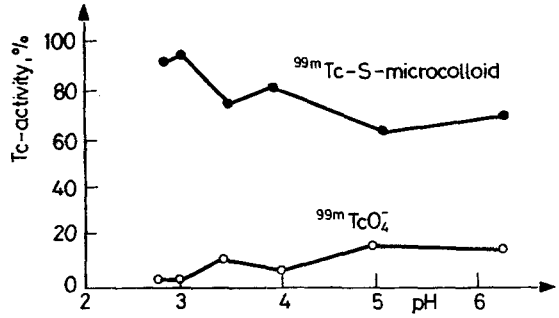

Fig. 2. Effect of pH-values on the labeling yield of ^{99m}Tc-S-microcolloid

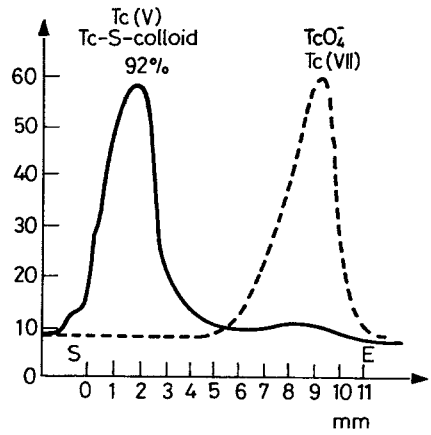

Fig. 3. Radiochromatogpam of the oxidation state of Tc-S-microcolloids and TcO_4^- using 3 mm paper strips developed with 0.3 NH_4Cl

Table 1
The formation rate and stability of ^{99m}Tc–S-microcolloid as obtained from PLC-technique

Time, min	^{99m}Tc-activity under profile zone of	
	$^{99m}TcO_4^-$	^{99m}Tc–S-microcolloid
10	8.4	91.6
30	5.3	94.7
60	4.0	96.0
120	3.30	96.7
360	2.0	98.0

chloride and constant amount of thioacetamide on the labeling yield. The data from the profile indicated that high labeling yield could be obtained by using 1.22 mg/ml of tin chloride and 187 mg/ml of thioacetamide in the final preparation. Figure 2 shows the effect of pH values of the labeling yield of radiocolloids. Thus, high labeling yield (95%) could be obtained at pH 3.0. The formation rate and stability of the radiocolloids were studied for several hours and the results are presented in Table 1. The data indicated that at least 30 minute incubation with ^{99m}Tc was needed to complete formation of ^{99m}Tc-S-microcolloids, that might be due to the formation of Tc(V)[8] as shown in Fig. 3. The data of Table 1 indicated that the radiocolloid is stable for at least 6 hours, which is the usual period of keeping radiopharmaceuticals for clinical use.

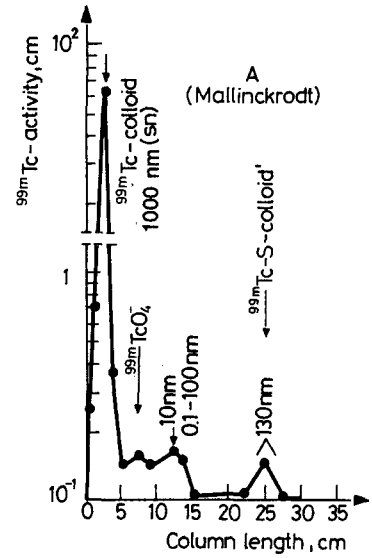

Fig. 4. Profiles of ^{99m}Tc-S-radiocolloids eluted with 10 ml saline from Sepharose Cl–4B column: Antimony sulfide colloids profiles

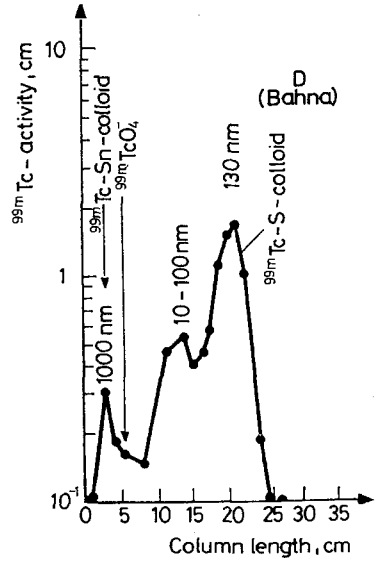

Fig. 5. Profiles of ^{99m}Tc-S-radiocolloids eluted with 10 ml aline from Sepharose Cl–4B column: Sulphur colloids profiles

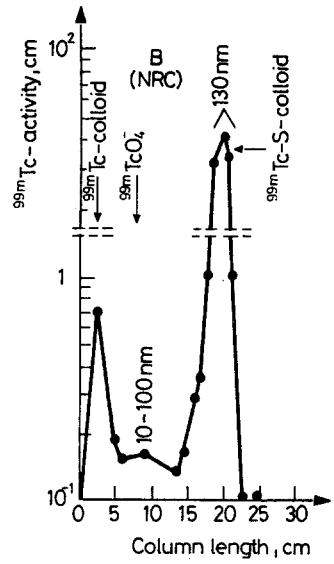

Fig. 6. Profiles of ^{99m}Tc–S -radiocolloids eluted with 10 ml saline from Sepharose Cl–4B column: In-house Tc-S-microcolloids profiles

The choice of the agents for lymph or bone marrow scintigraphy depends on the size of the particles. This parameter has been shown to effect the biological behavior of the tracer, and ultimate quality behavior of the diagnostic study.[9] Using Sepharose-4B column eluted with 10 ml saline, ^{198}Au-colloid with particle size of 5 nm was distributed in the column in a Zone of 95 mm from the top of the column. Antimony sulfide colloids (obtained from Malinckrodt) having particle size of about 5–15 nm are distributed in the column in a zone of 100 mm, while sulphur colloid (obtained from Banna) with a particle size 400–1000 nm is found in the front of the column and 65% of the activity is distributed in the column in a zone of 210 mm from the top of the column as shown in Figs. 4 and 5. While home-made preparation ^{99m}Tc-S-microcolloid was distributed in the column in a zone of 170 mm from the top, the column and the particle size obtained could be in the range of 10–100 nm (Fig. 6) as compared with the profiles of the above radiocolloids.

In vivo biokinetic studies

The organ distribution of ^{99m}Tc-S-microcolloid prepared at different pH values was evaluated in rabbits with 30 minute post injection as shown in Table 2. Soft tissue uptake (liver, kidneys, G.I. muscle, blood, urine) was increased as pH value increased. Bone marrow uptake was greatly decreased with the increase or decrease of pH value

Table 2
Effect of pH on the organ distribution of ^{99m}Tc–sulphur microcolloid on rabbits 30 minutes after injection

pH	Injected dose, %							
	Blood	Urine	Liver	Spleen	Kidneys	Lungs	Bone marrow	Marrow/ liver
2.8	3.9	0.2	69	1.92	0.2	0.18	26.0	0.38
3.0	2.0	1.0	43	2.0	1.0	1.20	36.0	0.84
3.5	4.4	2.4	51	2.3	2.14	0.52	18.7	0.37
4.0	6.6	6.5	4.8	0.2	9.0	0.60	2.3	0.48
5.0	19.0	16.0	18.0	0.5	5.0	0.70	6.0	0.33
6.0	31.0	5.0	40	2.0	7.4	0.60	8.8	0.22

Mean of three rabbits per each point.

Table 3
Studies on uptake of ^{99m}Tc–sulphur microcolloid in RES. A comparison with other commercially available microcolloid kits, 30 minutes after i.v. injection

Organ	Injected dose, %		
	^{99m}Tc–Sb_2S_3-colloid from Mallinckrodt	^{99m}Tc–S-microcolloid from Banna	^{99m}Tc–S-microcolloid from NRC
Blood	13.6 ± 0.72	2.0 ± 0.05	2.84 ± 0.84
Urine	0.65 ± 0.20	1.65 ± 1.25	1.92 ± 0.55
Liver	28 ± 2.8	66.4 ± 2.0	48 ± 3.8
Spleen	0.28 ± 0.2	0.95 ± 0.05	2.1 ± 1.42
Kidneys	1.10 ± 0.25	3.6 ± 0.13	1.44 ± 0.42
Lungs	0.31 ± 0.11	0.2 ± 0.07	0.76 ± 0.47
Bone marrow	48.8 ± 2.6	22 ± 0.92	36 ± 2.07
Blood (ml)	0.20 ± 0.02	0,025 ± 0.004	0.03 ± 0.008
Liver (g)	0.70 ± 0.25	1.65 ± 0.04	1.24 ± 0.51
Bone marrow (g)	2.55 ± 0.2	0.65 ± 0.04	1.16 ± 0.23
Ratios:			
Bone marrow (g) / Blood (ml)	12.80	26.0	38.70
Bone marrow (g) / Liver (g)	3.64	0.39	0.94

the 3.0. These results suggest that cleavage and agglomeration of ^{99m}Tc-S-microcolloids take place partly leading to increase of $^{99m}TcO_4^-$ activity which cause high accumulation in soft tissue[5] and low activity uptake in bone marrow. The organ distribution of ^{99m}Tc-S-microcolloid was compared with other two commercial sources of

Table 4
Biodistribution of ^{99m}Tc-S-microcolloids lyophilized and non-lyophilized in rabbits, 30 minutes after i.v. injection

Organ	Lyophilized kit	Non-lyophilized kit
Blood	4.36	2.50
Urine	2.43	0.90
Liver	51.0	58.0
Spleen	2.3	1.1
Kidneys	2.14	1.30
Lungs	0.52	0.20
Bone marrow	26.0	36.0

Mean results for three rabbits used for each evaluation.

^{99m}Tc-S-microcolloids kits (Mallinckrodt and Banna Products) in rabbits (Table 3). These data indicates that the clearance rate of NRC and Bana products from the blood seems to be about the same but the blood retained higher activity residue using Mallinckrodt products compared with others. Our results are in good agreement with that obtained earlier.[7] The increased uptake of the bone marrow follows the order: Mallinckrodt > NRC > Banna products accompanied by increased uptake in the liver with the order Banna > NRC > Mallinckrodt product. NAGAI et al.[7] showed that about 20.6% and 10% of the administered activities were deposited in the bone marrow of rabbit after i.v. injection of ^{99m}Tc-antimony sulfide and ^{99m}Tc-sulphur colloid, respectively. However, we found that about 36% of the administered activity were deposited in the bone marrow of rabbits after i.v. injection of ^{99m}Tc-S-microcolloids (Table 3). The advantages of ^{99m}Tc-S-microcolloids prepared by modified procedure are (1) high bone marrow uptake (36%), (2) low liver uptake (48%) (Table 3) and (3) the radiochemical yield is higher than 90% in the range of 10–100 nm (Fig. 2). The life-time of sulphur-microcolloid kit is long enough for clinical evaluation, and can be used for imaging bone marrow and lymph node.

The lyophilized kits cause decreased in bone marrow uptake from 36% to 26% and increase in liver uptake from 51% to 58%. These results could be attributed to the formation of sulphur colloid particles with sizes bigger than 100 μm (Table 4).

Experimental animal

A rabbit was injected subcutanously with the radiotracer in the left side of abdomen. Lymph node scintigrams were taken, 30 and 60 minutes after injection of ^{99m}Tc–S-microcolloid. Sequential gamma-imaging was performed over the injection site and regions of interest at 1 minute interval per image for 30 minutes. The results obtained at 30 and 60 minutes later showed that a considerable amount of radiotracer was found in the auxilliary lymph node.

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