^{90}Y AND ^{105}Rh LABELLED PREPARATIONS: POTENTIAL THERAPEUTIC AGENTS

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Abstract. ^{90}Y and ^{105}Rh formulations were studied with an aim to prepare therapeutic radiopharmaceuticals. ^{90}Y obtained from a ^{90}Sr-^{90}Y generator as chloride was complexed with known ligands such as DTPA, EDTMP and DOTA as well as a few other phosphonate ligands. Particulates such as ^{90}Y labelled ferric hydroxide macroaggregates (FHMA) and ^{105}Rh-sulphur colloid were prepared and studied for their stability in buffers and human serum. The studies on the complexation of ^{90}Y and the preparation of radiolabelled particulates are described. ^{90}Y complexed nearly quantitatively with DTPA, DOTA and EDTMP under optimised conditions of reaction pH, temperature and ligand concentrations. Both ^{90}Y-FHMA and ^{105}Rh-S colloid could be prepared in high yields under optimised conditions. The labelled particulates were measuring 20–100 μm and 1–20 μm, respectively and were found to be very stable in buffers as well as human serum at 37 °C. The particulates have the potential for use as radiosynovectomy agents and for therapy of cancers such as hepatomas.

1. INTRODUCTION

The potential of ^{90}Y ($T_{1/2}$ 64.4 h, β^- decay, E_{max} 2.25 MeV) and ^{105}Rh ($T_{1/2}$ 35 h, β^- decay, E_{max} 0.56 MeV,70% & 0.25 MeV,30%, γs 319 keV,19% and 306 keV,5%) as therapeutic radionuclides has been realised since long [1–3]. Apart from their amenable physical characters for therapy, the feasibility of producing carrier free grade ^{90}Y (from a ^{90}Sr-^{90}Y generator) and ^{105}Rh {^{104}Ru (n, γ)^{105}Ru → ^{105}Rh} adds to the potential for their use in therapy. ^{90}Y labelled antibodies have been of interest since long [4] and have been studied in many laboratories [5–7]. Complexation properties of the ^{90}Y obtained from a locally developed ^{90}Sr-^{90}Y generator was studied with the ligands such as DTPA, DOTA, EDTMP to adjudge the product for further use in radiolabelling proteins and polypeptides, and are described here.

^{90}Y labelled particulates were also prepared with an aim to use them for radiosynovectomy. Treatment of arthritis with radionuclides in the form of colloids and particulates such as ^{198}Au-colloids have been reported since long. Leaching of activity from the synovial joints has been the major problem causing poor efficacy of such agents. Currently, agents such as ^{166}Ho-chitosan, ^{166}Ho-FHMA, ^{90}Y-FHMA, ^{165}Dy-FHMA and ^{166}Ho-HA are under clinical trials in different countries [8]. Availability of a wide range of beta emitting radionuclides and the feasibility of making radionuclide incorporated bio-degradable particulates have facilitated a great deal of studies in this area [8]. Attempts to prepare labelled Ferrichydroxide macroaggregates (FHMA) and Sulphur colloid with ^{90}Y and ^{105}Rh were made and the preparations were studied for their stability. The studies on these particulates are also described here.

2. MATERIALS AND METHODS

DTPA (diethylene triamine pentaacetic acid) was purchased from Sigma Chemical Co. EDTMP (ethylene diamine tetramethyl phosphonate), DOTA (1,4,7,10-tetraaza cyclododecane N,N′,N″,N‴tetraacetic acid) and a cyclic phosphonate, tetraaza cyclo tetradecane N,N′,N″,N‴ tetramethylene phosphonate (CTMP) were synthesised in the laboratory by following reported procedures. All other reagents were either from Sarabhai M.Chemicals or S.D. Fine Chemicals, India. The solvents used in these studies were from Merck (India).

^{90}Y was supplied as chloride, by the Fuel Reprocessing Division, BARC from a ^{90}Sr-^{90}Y generator developed by them [9]. The absence of ^{90}Sr was tested by beta spectrometry in the initial

stages. The purity of ^{90}Y and absence of ^{90}Sr were also ascertained by following the decay of ^{90}Y activity. ^{105}Rh-chloride, processed from irradiated Ru target [10] was supplied by our colleagues.

Both ^{90}Y and ^{105}Rh were counted in NaI(Tl) scintillation detector; Bremstrahllung radiations from ^{90}Y were measured while the window was adjusted for the γs from ^{105}Rh.

2.1. Preparation of ^{90}Y complexes

^{90}Y complexes of the ligands DTPA, DOTA, EDTMP and CTMP were optimised for pH, time, reagent concentrations etc. Initially excess reagents were reacted for a period of 4 h or more, to study the reaction at different pH, ranging from highly acidic (<2) to pH≥ 9.5. 1 M acetate buffer was used to maintain a pHupto 5.5–6, 1 M phosphate buffer for pH7–7.5 and 1 M Bicarboante buffer for pH9.5. 2.5 μM–25 mM solutions of the ligands were prepared in double distilled water. Reagent concentrations and time of reaction were then optimized under the optimum pH. Typically, 25 μL of ^{90}Y-chloride (500–900 kBq) was mixed with 50 μL of the appropriate buffer, to which was added 50 μL of the ligand solution. In the case of DOTA, the effect of heating on reaction yield and time required was also studied. After incubation of the reaction mixture, the complexation yields were determined by paper chromatography/TLC using pyridine:ethanol:water (1:2:4) elution, in all the cases.

2.2. Preparation of ^{90}Y-FHMA

^{90}Y-FHMA was prepared by precipitating ferrichydroxide as fine particulates in the presence of ^{90}Y under alkaline conditions [11]. ^{90}Y-chloride, (both at tracer levels as well as with addition of ~0.3 nanomoles inactive YCl_3) was added to 0.5 mL of 0.02 M ferrous sulphate solution and mixed well. 3 mL of 0.2 N NaOH was added dropwise to this mixture with stirring. The solution was mixed well and then centrifuged to remove the supernatant, washed with N saline thrice and resuspended in N saline. The labelling yield was calculated by determining the percentage of ^{90}Y activity associated with the particulates. The effect of addition of polyvinyl pyrollidone (net 0.6%) during precipitation on the yield of labelling and on the particle size distribution was studied.

2.3. Preparation of ^{105}Rh-Sulphur Colloid

^{105}Rh labelled sulphur colloid was prepared as reported for rhenium labelled sulphur colloid [12]. 18–37 MBq of ^{105}Rh chloride was added to 2 mL of 0.1 M sodium thiosulphate containing 1% Haemmacel. This solution was acidified with 1 mL of 1 N HCl. The colloidal sulphur formed was then mixed and placed in a water bath at 80°C for 4–5 minutes followed by rapid cooling in an ice bath for 5 minutes. The colloid was centrifuged to remove the acidic supernatant and reconstituted in saline. This procedure was repeated twice to remove traces of acid. The yield of ^{105}Rh-sulphur colloid was determined as in the case of ^{90}Y-FHMA. The effect of the presence of 0.1% Haemmacel in the reaction mixture on the reaction yield and particle size distribution was also studied.

2.4. Stability Studies

The stability of the particulate preparations, namely, ^{90}Y-FHMA and ^{105}Rh sulphur colloid was studied in saline and phosphate buffered saline (0.04M, pH7.5) at ambient temperature (25°C) and in human serum at 37°C. At each time point, the particulates were centrifuged, separated from the liquid phase and counted to estimate the extent of leaching of activity from the particles.

2.5. Estimation of the particle Sizes

In order to estimate the particle size distribution, both Y-FHMA and Rh-Sulphur Colloid were made under optimal conditions, using inactive yttrium chloride and rhodium chloride, keeping the amounts of Y and Rh identical to those in active preparations. The particles were dispersed in N

saline and analysed by laser diffraction for size distribution at the Powder Metallurgy Division, BARC.

3. RESULTS AND DISCUSSION

3.1. ^{90}Y complexes

^{90}Y was free from any detectable ^{90}Sr activity as estimated by following the decay of separated ^{90}Y. DTPA, EDTMP and DOTA showed good complexation with ^{90}Y. Paper chromatography using pyridine:ethanol:water (1:2:4) solvent was found to be the most suitable with R_f of the complex ~1 and the uncomplexed ^{90}Y retained at R_f value of 0. The cyclic phosphonate, CTMP, did not complex ^{90}Y under various reaction conditions, perhaps indicating the importance of the cavity size available for complexation, which is observed with most metal ions including Y and several lanthanides.

The effect of pHon the complexation yields is depicted in Figure-1.

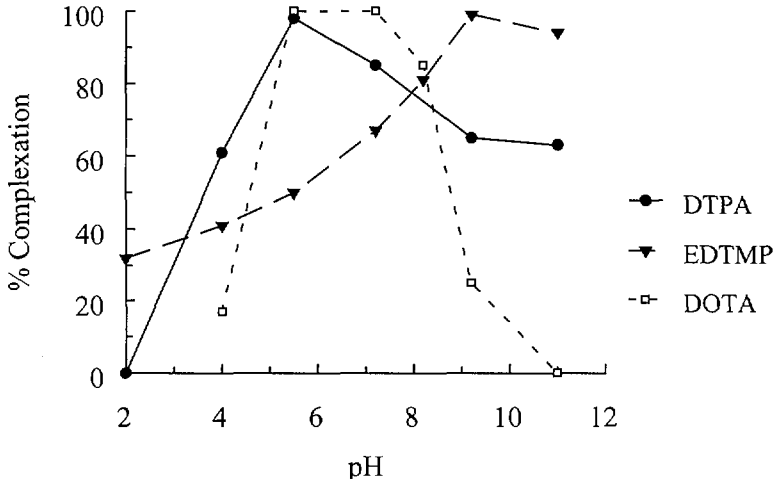

FIG. 1. Effect of pHon the complexation yields

It was observed that pHwas an important factor and DTPA complexed ^{90}Y at a pHof ~ 5–5.5 in acetate buffer to the extent of >99%. In the case of EDTMP, it was imperative to maintain the reaction pHat ~ 6–7 to obtain reasonable complexation yields and high yields of complexation was possible only under stronger alkaline conditions, pH≥ 9. Acetate buffer was found to be suitable for the range 5–6 pHand often ^{90}Y complexes are reported via formation of an acetate prior to the addition of the ligand. However, in the case of EDTMP where the complexation required pH>9, acetate addition did not alter the yield and was not necessary. As reported by several workers earlier, DOTA formed stable complexes with Y, at a pH~ 5.5–6. Figure-2 gives the effect of ligand concentration on complexation yield for the three ligands, DTPA, EDTMP and DOTA.

It was observed that a minimum of 1 μg (~2.5 nanomoles) of DTPA was required to completely complex trace amounts of ^{90}Y (~0.5 picomoles). However, when the complexation was carried out with ~270 picomoles of carrier Y (which corresponds to 0.185 GBq of ^{90}Y activity) the amount of ligand required was much higher (80 μg) corresponding to a ligand: metal ratio of ~750:1. In the case of EDTMP, a much larger amount, 50 μg was required for complete complexation of even trace amounts of Y, but the addition of carrier Y did not alter the complexation yield. At least 5 μg of DOTA was necessary to quantitatively complex trace levels of "no carrier added" (n.c.a.) ^{90}Y. Addition of carrier Y (0.3 nanomoles) however resulted in better complexation at lower amounts of DOTA and quantitative complexation could be obtained at ~2.5 μg of DOTA. Figure 3 shows the complexation yields of the three ligands with respect to time.

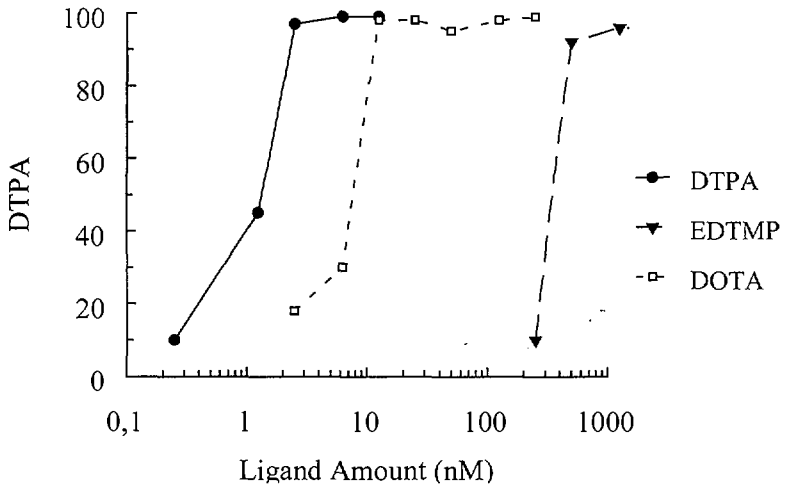

FIG. 2. Effect of the ligand amount on the complexation yields.

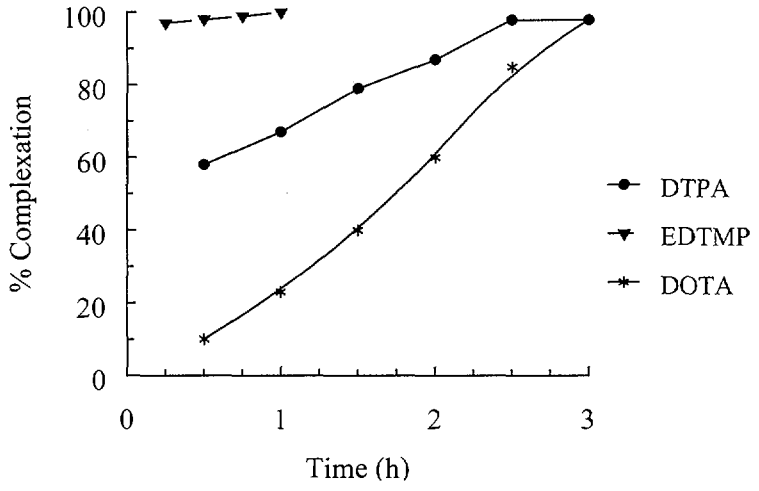

FIG. 3. Effect of Reaction Time on the Complexation Yield.

Mild heating (~37°C) of the reaction mixture helped in reducing the reaction time from a minimum of 2.5 h to 1 h for DTPA, and from 3 h to 2 h for DOTA. But there was no effect on the minimum ligand amount required for near quantitative complexation. In the case of EDTMP, there was no complexation when the reaction mixture was heated. On placing the ^{90}Y-EDTMP complex in a water bath at 37°C, disintegration of the complex was observed. In brief, the ligands DTPA, EDTMP and DOTA complexed ^{90}Y quantitatively under optimal conditions of pHand reagent concentrations and required a minimum of 2.5 h, 15 min and 3 h, respectively for completion of reaction at ambient temperature. Of these, the EDTMP complexes were not stable, pre-empting their use for in-vivo applications.

3.2. ^{90}Y -FHMA and ^{105}Rh-sulphur colloid particulates

^{90}Y-FHMA could be prepared in very high yields of 98 ± 3% (n = 5) with ease, both at tracer level and when carrier Y was added. Although the precipitation of ferric hydroxide could be observed with lower amounts of alkali, the variations in the acidity of the ^{90}Y-chloride solution warranted different amounts of alkali to be used for complete precipitation. Hence a moderate excess of alkali was used in the optimised procedure. The particles were ranging between 20–100 μm size and addition of polyvinyl pyrrolidone did not affect the yield or the particle size distribution.

^{105}Rh-Sulphur Colloid formed in considerable yields and 85 ± 4% activity was associated with the colloid. Addition of 0.1% Haemaccel was essential to keep the colloidal particles from clustering and sticking to the walls of the container. However, Haemaccel did not have any effect on the labelling yield. The Rh-suphur colloid particles were smaller in size and ranged between 1–20 μm.

Both the preparations were stable and no significant activity (<0.5%) was lost on repeated washings with water, saline or buffer. Table I shows the percentage of ^{90}Y or ^{105}Rh activity retained with the particulates along with the storage duration. Both ^{90}Y-FHMA and ^{105}Rh-S-Colloid particles are seen to be very stable even in human serum at 37°C for several days.

TABLE I. STABILITY OF ^{90}Y-FHMA AND ^{105}RH-S-COLLOID PARTICLES

Preparation	% Activity retained		Storage duration
	In PBS, 25°C	In human serum 37°C	
^{90}Y-FHMA	>99.5	~98	10 days
^{105}Rh-S colloid	>99	~97	5 days

4. CONCLUSION

^{90}Y complexes could be made with ease with the tested ligands such as DOTA, DTPA and EDTMP, which proves the usability of the locally made ^{90}Sr-^{90}Y generator. The particulate preparations, ^{90}Y-FHMA and ^{105}Rh-S-Colloid, could both be obtained in good yields and were found to be very stable. These particles hence may have potential for use in radiosynovectomy and the ^{105}Rh-S-Colloid in treatment of cancers such as hepatomas.

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