Research article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 06 October 2015, Revised 27 January 2016, Accepted 05 February 2016 Published online 9 March 2016 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3384

An easy and effective method for synthesis and radiolabelling of risedronate as a model for bone imaging

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This study aimed to provide an easy method for synthesis of 1-hydroxy-2-(3-pyridyl) ethylidene bisphosphonic acid monosodium (sod. risedronate) with a high yield of 71%. The synthesized risedronate was labeled with technetium-99 m using two different reducing agents ($SnCl_2.2H_2O$ and $NaBH_4$) where $NaBH_4$ gave stable complex and higher radiochemical yield more than $SnCl_2.2H_2O$. The results showed that, the radiochemical purity of $^{99m}Tc(NaBH_4)$-risedronate was 99.2 ± 0.6% and its stability was up to 6 h. Biodistribution study showed high uptake and long retention of $^{99m}Tc(NaBH_4)$-risedronate in bone starting from 15 min (29 ± 2.5% ID/organ) up to 4 h (35.1 ± 3.2 ID/organ) post injection. This research could introduce an easy and effective method for synthesis and labeling of risedrionate and affording a good tracer for bone imaging.

Keywords: Bisphosphonate; ^{99m}Tc; Synthesis; Risedronate; Imaging; Bone

Introduction

Bisphosphonates (BPs) are an important class of compounds that are used in the treatment of a variety of bone diseases, which are associated with high bone resorption, such as metastatic bone disease, Paget's disease, and osteoporosis.

The efficient and early diagnosis of bone cancer in its early stages allows the management of bone cancer patients with conventional therapeutic strategies (chemotherapy, hormone therapy, external beam radiation, and analgesics) and controlling bone pain[1] or with the systemic radiopharmaceutical therapy, which shows safe and effective management of the advanced disease.[2,3]

Technetium-99 m represents the radionuclide of choice in most performed diagnostic nuclear medicine applications[4,5] because of its good physical properties: ($t_{1/2}$ = 6.01 h, 140 keV γ-ray energy, and very low abundance β-emission).^{99m}Tc-bisphosphonate derivatives are good bone targeting and used for bone imaging.[6]

Technetium-99 m labeled methylenediphosphonate, ethylenediamine tetramethylene phosphonate, dicarboxy propanediphosphonate, hydroxyethylidinediphosphonate, hydroxyl methylene-diphosphonate, 1-thioethylidene-1,1-disodium phosphonate (TEDP), 1-hydroxypropylidene-1, 1-disodium phosphonate, and ethane-1-amino-1, 1-diphosphonic acid represent radiopharmaceuticals of choice for bone scintigraphy especially in bone metastases, Paget's disease, and osteoporosis.[7–16]

The main disadvantage of these ^{99m}Tc-labeled diphosphonates (DPs) is an interval of 2–6 h is needed between injection and bone imaging.[14,17] Shortening this interval would decrease the burden on patients in terms of the total length of the examination and the dose of radiation absorbed. To enable imaging at an earlier time after injection, a radiopharmaceutical with higher affinity for bone is required correspondingly.[18]

Risedronate and zoledronate are two of the most potent antiresorptive BPs in several animal models[19] because of the nitrogen atom within the heterocyclic ring. Zoledronic acid, one kind of the typical third-generation DPs, is currently the most potent bisphosphonate. In preclinical models of bone resorption, for example, zoledronate is at least 100 times more potent than clodronate and pamidronate, and it is at least 1,000 times more potent than etidronate.[20] A series of zoledronate derivatives MIPrDP, MIBDP and MIPeDP have been prepared and successfully labeled with ^{99m}Tc in a high labeling yield (with colloid formation) and good *in vitro* stability, showing high selective uptake in the skeletal system and rapid clearance from soft tissues.[21] One of the previous study is ^{99m}Tc-TEDP,[14] TEDP is a simple arm of bisphosphonate like hydroxyl ethylene diphosphonate, and the only difference between TEDP and hydroxyl ethylene diphosphonate is the replacement of (OH) group by (SH) group; but in our study, we use one of the third generation of bisphosphonate that contains heterocyclic ring containing nitrogen in the ring (risedronate), which is more potent than other generations of bisphosphonate; also, we use two reducing agent ($SnCl_2.2H_2O$ and $NaBH_4$) to overcome the problems that appear by using $SnCl_2.2H_2O$ as a reducing agent, and we conclude that $NaBH_4$ is more effective and easier than $SnCl_2.2H_2O$. Stannous chloride is used for reduction of sodium

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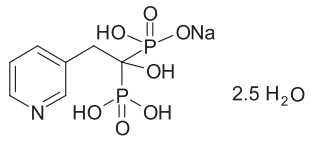

Figure 1. Sod. risedronate.

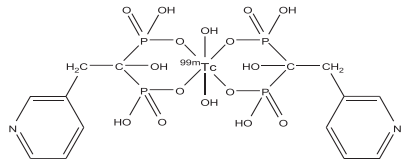

Figure 3. ^{99m}Tc-BPs, a proposed structure.

pertechnetate ($Na^{99m}TcO_4^-$) to obtain lower valency state of ^{99m}Tc, but during labeling by this method, radiocolloids are generated as main impurities.[22,23]

This article reports one-pot method for synthesis of sodium risedronate (Figure 1) with a high yield and also reports a rapid and effective method for radiolabeling of risedronate by ^{99m}Tc using sodium borohydride as a reducing agent. Sodium borohydride was used for reduction of ^{99m}Tc(VII) ions to the lower oxidation state, and subsequent complexation was done with risedronate molecules without forming radiocolloid. This article uses the two reducing agents ($SnCl_2$ and $NaBH_4$) for radiolabeling of risedronate with ^{99m}Tc and compare between them in terms of radiochemical yield and stability of the formed complex.

Experimental

All of the chemical reagents were of analytical reagent grade; Carbon-13 nuclear magnetic resonance (^{13}C NMR, Jeol JNM-Ex- 67.8 MHz FT NMR system using tetramethylsilane as an internal standard; Jeol Ltd, Hertfordshire, UK), and proton nuclear magnetic resonance (^{1}H NMR, Jeol EX-270 MHz FT using tetramethylsilane as an internal standard; Jeol Ltd, NRC, Cairo, Egypt) were used for the characterization of the synthesized sodium risedronate. Technetium-99 m was eluted as $^{99m}TcO_4^-$ from ^{99m}Mo/^{99m}Tc generator (radionuclidic purity: 99.99 %; radiochemical purity: 99.99 %; Activity: 1 Ci; Elutec, Brussels, Belgium). Albino mice, each of 20–25 g, were used for the biological distribution study. A NaI(Tl) γ-ray scintillation counter (Scaler Ratemeter SR7, Nuclear Enterprises, Edinburgh, UK) was used for the measurement of γ-ray radioactivity.

Synthesis of 1-hydroxy-2-(3-pyridyl)ethylidenebisphosphonic acid (risedronic acid)

Aqueous methanesulfonic acid (85%, 432.5 mL) was added to 3-pyridineacetonitrile (100.0 g, 0.847 mol) and heated to 98–100 °C for 8 h (Figure 2), then cooled the reaction temperature to 65 °C, and phosphorus trichloride (396.0 g, 2.880 mol) was added over 25 min. After 5 h of stirring at 65–70 °C, the reaction temperature was cooled to 30 °C, and pre-cooled water (1000 mL) was added very slowly in 30 min. The reaction temperature was heated to 98 °C. After 15 h of stirring, the temperature was cooled to 50 °C, and methanol (1500 mL) was added. After 2 h of stirring at 5–10 °C, the product was collected by filtration and dried to give risedronic acid (201.5 g, 79%) as a white monohydrate solid.

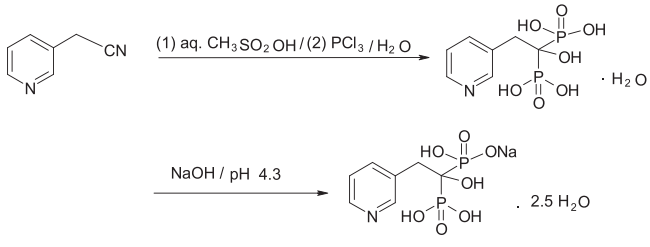

Figure 2. Synthesis of risedronic acid monosodium salt hemipentahydrate.

Synthesis of risedronic acid monosodium salt

Monosodium salt was obtained using the aforementioned procedure with 3-pyridineacetonitrile (100.0 g, 0.847 mol), but the pH was adjusted to 4.3 with 30% sodium hydroxide solution before diluting with methanol (1500 mL). The resulting product was filtered and dried to yield risedronic acid monosodium salt (210 g, 71%) as a white crystalline hemipentahydrate solid (Figure 2).

Preparation of ^{99m}Tc-risedrinate complex

Preparation of ^{99m}Tc(Sn)-risedronate complex

Exactly 5 mg of risedronate, dissolved in 1 mL N_2-purged distilled water, was separately transferred to evacuated penicillin vials. Exactly 1 μg of Sn(II) was added to each vial along with different amounts of 0.1 N NaOH or 0.1 N HCl to adjust pH in a range of 3–7. Then, 100 μL of freshly eluted $^{99m}TcO_4^-$ (~200 MBq) was added to each vial. The reaction mixtures were left at room temperature for 30 min. The same procedure was repeated with varying Sn(II) amounts (0.5–5 μg), varying risedronate amounts (1–20 mg), and different reaction times (5–360 min).

Preparation of ^{99m}Tc($NaBH_4$)-risedronate complex

Exactly 0.5 mg of risedronate, dissolved in 1 mL distilled water, was separately transferred to penicillin vials. Exactly 5 mg of $NaBH_4$ was added to each vial along with different amounts of 0.1 N NaOH or 0.1 N HCl to adjust pH in a range of 3–13. Then, 100 μL of freshly eluted $^{99m}TcO_4^-$ (~200 MBq) was added to each vial. The reaction mixtures were left at room temperature for 15 min. The same procedure was repeated with varying $NaBH_4$ amounts (2–20 μg), varying risedronate amounts (0.1–5 mg), and different reaction times (5–360 min).

Bisphosphonate compounds are strong chelating agents, the reaction of bisphosphonate compounds with technetium gave only three species (free pertechnetate $^{99m}TCO_4^-$, colloid, and ^{99m}Tc-complex) where the structure of the complex is formed from two molecules of bisphosphonate and one technetium atom like all previously reported bisphosphonates[21,24–28] as shown in Figure 3.

Radiochemical yield of ^{99m}Tc-risedronate complex

Paper chromatography analysis

The percent labeling yield of the labeled ^{99m}Tc-risedronate complex was determined using ascending paper chromatographic technique. Strips of Whatman No.1 paper chromatography (Whatman International Ltd, Maidstone, Kent, UK) of 13 cm long and 1 cm wide were marked at a distance of 2 cm from the lower end and lined into sections 1 cm each up to 10 cm.

In case of ^{99m}Tc(Sn)-risedronate complex, a spot from ^{99m}Tc(Sn)-risedronate complex solution was applied using hypodermic syringe, and then, the strip was developed in an ascending manner in a closed jar filled with N_2 gas to prevent oxidation of the labeled phosphonate spot. The developing solvents were acetone for developing one paper and saline for developing a second paper strip.

In case of ^{99m}Tc($NaBH_4$)-risedronate complex, a spot from ^{99m}Tc ($NaBH_4$)-risedronate complex solution was applied using hypodermic syringe, and then, the strip was developed in an ascending manner in a jar using only acetone as a developing solvent. After complete development, the strips were dried and cut into fragments 1 cm each. Then, the sections were counted in a NaI(Tl) γ-ray scintillation counter.

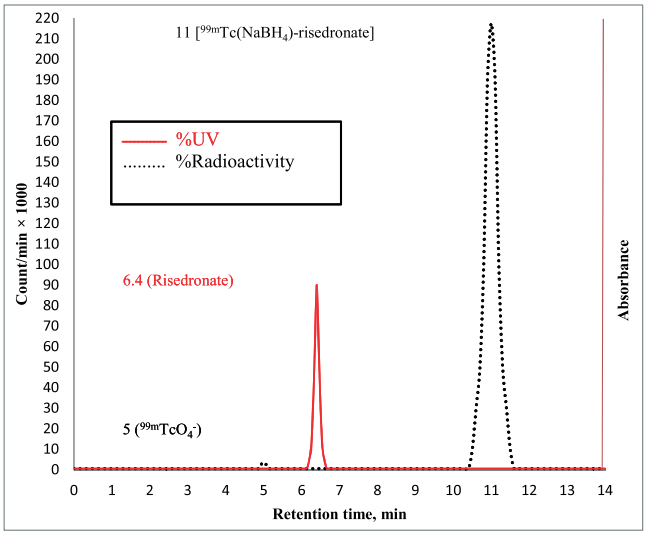

Figure 4. Radiochromatogram of ^{99m}Tc($NaBH_4$)-risedronate complex, UV radioactivity.

High performance liquid chromatography analysis

The radiochemical yield was further confirmed by HPLC. The sample volume was 20 µL injected into the column (C18 (4.6 × 150 mm, 5 mm, Make: Thermosil), and UV detection of 262 nm at ambient temperature). The column was eluted with mobile-phase mixture of acetonitrile/methanol/H2O mixture in ratio 30:30:40, and the flow rate was adjusted to 1/2 mL/min, then fractions of 1/2 mL were collected separately up to 60 fractions and counted in a well-type NaI(Tl) detector connected to a single-channel analyzer.

The HPLC radiochromatogram of ^{99m}Tc($NaBH_4$)-risedronate. Two peaks appeared at retention times 5 and 11 min for free $^{99m}TcO_4^-$ and ^{99m}Tc($NaBH_4$)-risedronate, respectively. The radiochromatogram showed 99.2 ± 0.6% radiochemical yield of ^{99m}Tc($NaBH_4$)-risedronate complex coinciding with the results of the analysis using ascending paper chromatography as shown in Figure 4.

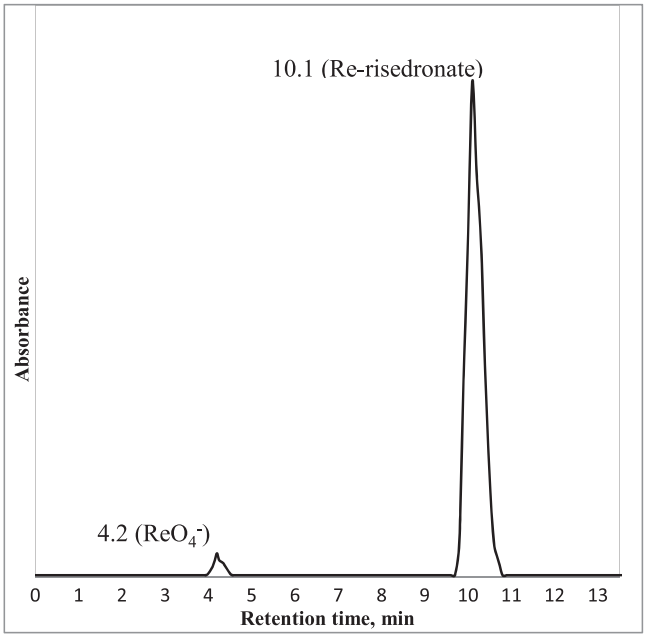

Figure 5. UV absorbance of Re-risedronate complex.

Re($NaBH_4$)-risedronate complex was prepared by the same method that of ^{99m}Tc($NaBH_4$)-risedronate and was examined using HPLC (Figure 5) with the same separation condition of ^{99m}Tc($NaBH_4$)-risedronate. The HPLC chromatogram of Re-risedronate showed one peak at retention time 10.1 min for Re-risedronate and another beak at 4.2 for ReO_4^-. Technetium complex was washed out with longer retention time than the corresponding rhenium complex, because technetium complex was more hydrophobic than the corresponding rhenium complex, because of the different atomic radii of technetium and rhenium.[29] Similar unmatched retention times have also been observed previously.[30]

Biodistribution study

Experiments were performed using the procedure that was approved by the Animal Ethics Committee and was in accordance with the guidelines set out by the Egyptian Atomic Energy Authority. Male Albino Swiss mice weighing 20–25 g were used.

In-vivo biodistribution studies were carried out in five groups of Albino mice where each animal was injected in the tail vein with 0.2 mL solution containing 5–10 kBq of ^{99m}Tc-risedronate. The mice were put in metabolic cages for the required time. The mice were sacrificed by cervical dislocation in groups at various time intervals after injection, and the organs or tissues of interest were removed, weighted and counted. The weights of blood, bone, and muscles were assumed to be 7, 10, and 40% of the total body weight, respectively.[31]

To correct for physical decay and to calculate uptake of the radiolabel compound in each tissue sample as a fraction of the injected dose, aliquots of the injected dose were counted simultaneously. The results were expressed as percentage injected dose per gram of tissue or organ (% ID/g or organ).

Results and discussion

Characterization of the synthesized 1-hydroxy-2-(2pyridyl) ethylidenebis-phosphonic acid monosodium (sod. risedronate)

The chemical structure of sod. risedronate (molecular formula, $C_7H_{10}NO_7P_2Na$. 2.5 H_2O) was confirmed by the following: IR (KBr, cm^{-1}) 3440, 3007, 2933, 1642, 1254, 1074; MS (*m/z*): 306 $[M+H]^+$; ^{1}H NMR (D_2O) δ 2.45 (OH, alcohol), 4.85 (t, 2H, J = 12.1 Hz), 7.45 (m, 1H), 8.20 (dd, 1H, J = 8.2, 5.5 Hz), 8.50 (d, 1H, J = 7.2 Hz), 8.70 (dd, 1H, J = 9.1, 8.2 Hz); ^{13}C-NMR (300 MHz, D_2O) δ 9($\underline{C}H_2$ aliphatic), 39($\underline{C}$, aliphatic), 122($\underline{C}$H, aromatic in position 5), 133($\underline{C}$H aromatic in position 4), 139($\underline{C}$, aromatic in position 3), 146($\underline{C}$H aromatic in position 6), 148($\underline{C}$H aromatic in position 2).; ^{31}P NMR (D_2O) δ 17.1.

Radiolabeling of ^{99m}Tc-risedronate complex

Using ascending paper chromatographic technique, the radiochemical purity of the formed ^{99m}Tc(Sn)-risedronate complex was checked by using acetone as a developing solvent where free $^{99m}TcO_4^-$ was moved with the solvent front (R_f = 1) while other species (reduced hydrolyzed-^{99m}Tc colloid and ^{99m}Tc(Sn)-risedronate complex) remained at the point of spotting. The percentage of the reduced hydrolyzed-^{99m}Tc was calculated using saline as a developing solvent where reduced hydrolyzed-^{99m}Tc colloid remained at the origin (R_f = 0), while free $^{99m}TcO_4^-$ and ^{99m}Tc(Sn)-risedronate complex migrate to the top of the paper. The percent of ^{99m}Tc(Sn)-risedronate complex were determined as follows:

$$\text{Labeling yield} = 100 - (\%\ \text{free}\ ^{99m}TcO_4^- + \%\ \text{reduced hydrolyzed}\ ^{99m}\text{Tc colloid}).$$

In case of ^{99m}Tc(*NaBH*$_4$)-risedronate complex, only one developing solvent is used (acetone). Free $^{99m}TcO_4^-$ migrate to

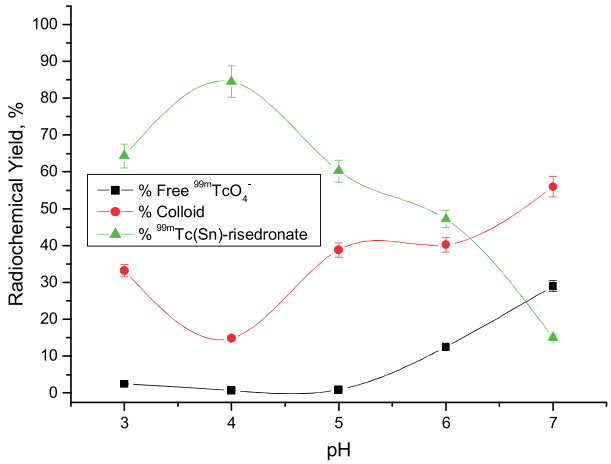

Figure 6. Radiochemical yield of ^{99m}Tc(Sn)-risedronate as a function of pH. Reaction conditions: 1 μg of Sn(II), 5 mg sod.risedronate, 100 μL(~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature, 30 min.

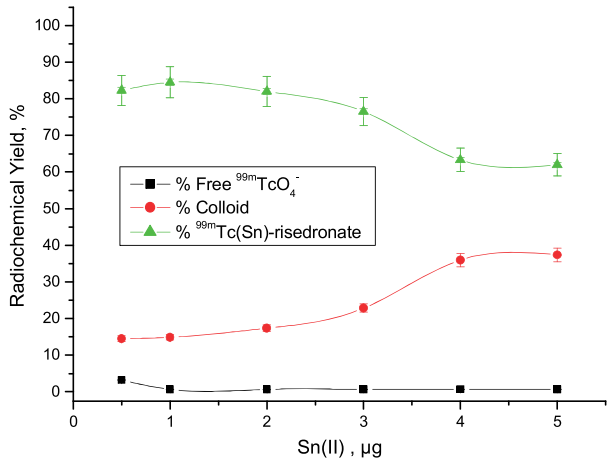

Figure 8. Radiochemical yield of ^{99m}Tc(Sn)-risedronate as a function of Sn(II) amount. Reaction conditions: 5 mg sod.risedronate, pH 4, 100 μL(~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature, 30 min.

the top of the paper, while ^{99m}Tc($NaBH_4$) risedronate complex still down (there is no colloid formed).

Effect of pH of the reaction medium

Figures 6 and 7 clearly show the effect of pH on radiochemical yield of both ^{99m}Tc(Sn)-risedronate and ^{99m}Tc($NaBH_4$)-risedronate complexes.

In case of ^{99m}Tc(Sn)-risedronate complex, at pH below or above the optimum pH of the reaction medium, the radiochemical yield was low because of the formation of RH-^{99m}Tc, which was the main radiochemical impurity; the optimum pH was 4, which give maximum radiochemical yield of 84.5 ± 3.5%.

In case of ^{99m}Tc($NaBH_4$)-risedronate complex (Figure 7) (there is no colloid formation), the radiochemical yield increased with increasing of the pH; where at pH 3, the radiochemical yield was low (8.9 ± 0.2%), while at pH 9, the maximum radiochemical yield (99.2 ± 0.6%) was obtained; where at this pH value, the risedronate combined all the reduced technetium. When the pH value was increased, above pH 9, the percent labeling yield was slightly decreased.

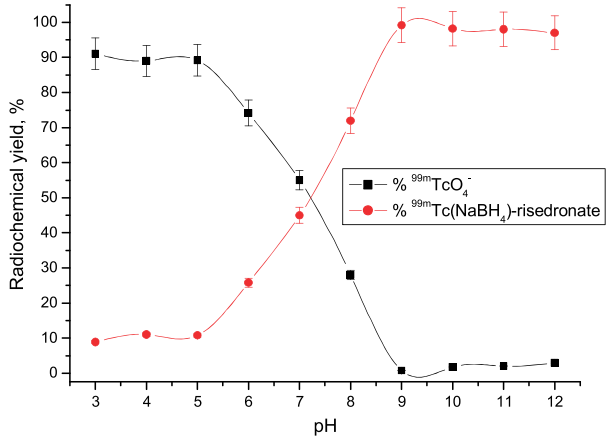

Figure 7. Radiochemical yield of ^{99m}Tc($NaBH_4$)-risedronate as a function of pH. Reaction conditions: 5 mg $NaBH_4$, 0.5 mg sod.risedronate, 100 μL(~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature, 15 min.

Effect of reducing agent content

Stannous chloride is the most commonly used reducing agent in acidic medium in preparations of most ^{99m}Tc-labeled compounds.[32,33] Although stannous chloride is the most commonly used reducing agent in preparations of most ^{99m}Tc-labeled compounds, but radiolabelling by $SnCl_2$ method generates radiocolloids, the biodistribution of desired molecule is affected by these radiocolloids. We have to optimize the amount of stannous salts and pH of the reaction for getting maximum labelling efficiency. Some time, we have to pass the mixture of compounds through a column to obtain radiocolloids free-labeled compound.[34] These processes are time consuming, so sodium borohydride is used as a reducing agent to avoid the aforementioned problems. Boric acid is generated as a by-product when sodium borohydride is used in radiolabelling purpose, which act as stabilizing agent.[35] Sodium borohydride ($NaBH_4$) was introduced into nuclear medicine by Suberamanian and McAfee, 1969.[36] The influence of Sn(II) content on the percent labeling yield of the ^{99m}Tc (Sn)-risedronate complex was investigated in a range of 0.5–5 μg as shown in Figure 8. Using too little stannous chloride may lead to incomplete reduction of free $^{99m}TcO_4^-$, and hence, unreliable yield of the ^{99m}Tc(Sn)-risedronate complex was obtained because of the low Sn(II) content, which was insufficient for the complete reduction of the free $^{99m}TcO_4^-$, giving rise to high percentage of the free $^{99m}TcO_4^-$. By increasing the Sn(II) content from 0.5 to 1 μg, the labeling yield increased from 82.3 to 84.5 ± 3.5%. By increasing Sn(II) greater than 1 μg, the labeling yield was decreased again because of formation of Tc-Sn-colloid. So, the optimum amount of Sn(II) content for the formation of maximum ^{99m}Tc(Sn)-risedronate complex (84.5 ± 3.5) was 1 μg.

Effect of $NaBH_4$ on the percent labeling yield of ^{99m}Tc ($NaBH_4$)-risedronate complex was shown in Figure 9; 1 mg $NaBH_4$ is insufficient to complete reduction of $^{99m}TcO_4^-$ to form ^{99m}Tc-complex, so the percentage of $^{99m}TcO_4^-$ was high and equal to 54.7 ± 0.4%. By increasing the amount of $NaBH_4$, the labeling yield was increased, where the maximum labeling yield was achieved at 5 mg $NaBH_4$. Increasing the $NaBH_4$ greater than 5 mg, the labeling yield was slightly decreased.

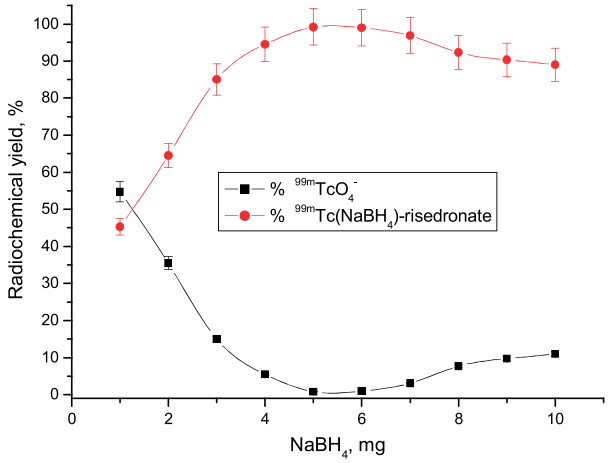

Figure 9. Radiochemical yield of ^{99m}Tc(NaBH$_4$)-risedronate as a function of NaBH$_4$ amount. Reaction conditions: 0.5 mg sod.risedronate, pH 9, 100 μL(~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature, 15 min.

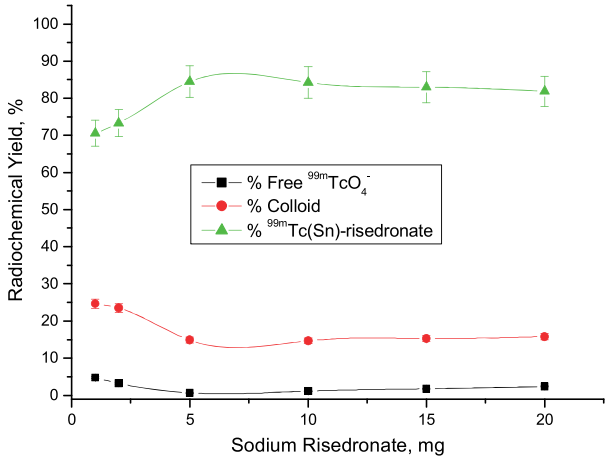

Figure 10. Radiochemical yield of ^{99m}Tc(Sn)-risedronate as a function of sod. Risedronate amount. Reaction conditions: 1 μg of Sn(II), pH4, 100 μL(~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature, 30 min.

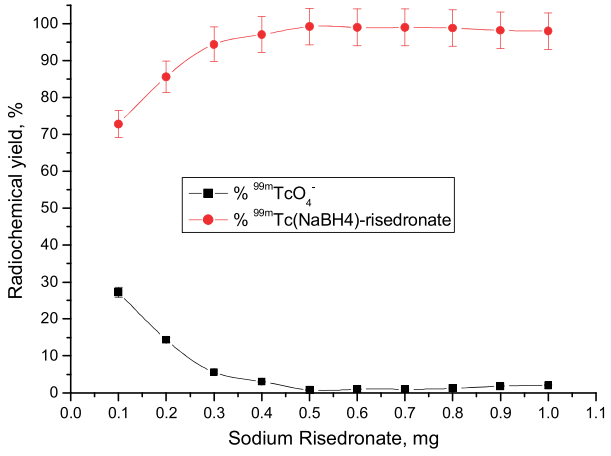

Figure 11. Radiochemical yield of ^{99m}Tc(NaBH$_4$)-risedronate as a function of sod. risedronatesod.risedronate. Reaction conditions: 5 mg of NaBH$_4$, pH 9, 100 μL (~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature, 15 min.

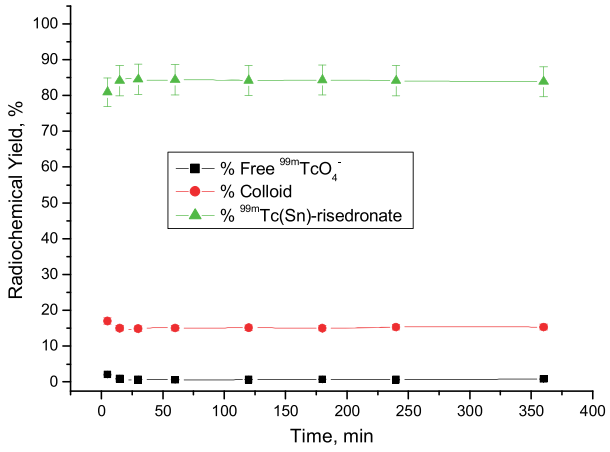

Figure 12. Radiochemical yield of ^{99m}Tc(Sn)-risedronate as a function of time. Reaction conditions: 1 μg of Sn(II), 5 mg sod.risedronate, pH 9, 100 μL(~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature.

Effect of sodium risedronate content

The effect of sodium risedronate content was studied in a range of 0.1–20 mg as shown in Figures 10 and 11. Low percent radiochemical yield (70.55 ± 2.5%) was obtained at low risedronate amount (1 mg) where the labeling yield was increased with increasing the risedronate amount until it reaching the highest yield of 84.5 ± 3.5% at 5 mg of risedronate, when Sn(II) is used as a reducing agent (Figure 10). Increasing the risedronate amount more than 10 mg, the labeling yield was slightly decreased.

But in case of ^{99m}Tc(NaBH$_4$)-risedronate complex (Figure 11), the maximum radiochemical yield (99.2 ± 0.6%) was obtained at 0.5 mg risedronate, At low risedronate amount (0.1 mg), the labeling yield was small and equal to 72.8 ± 0.5% because of the amount of risedronate was insufficient to form complex with all of the formed reduced technetium, so the percent of $^{99m}TcO_4^-$ was high and equal to 27.2 ± 0.3%. The radiochemical yield was increased with increasing the risedronate amount where a maximum labeling yield of 99.2 ± 0.6% was obtained at 0.5 mg risedronate. By increasing the risedronate amount above

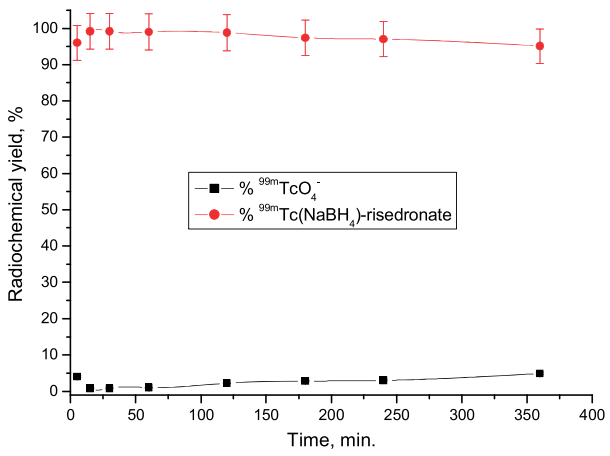

Figure 13. Radiochemical yield of ^{99m}Tc(NaBH$_4$)-risedronate as a function of time. Reaction conditions: 5 mg of NaBH$_4$, 0.5 mg sod.risedronate, pH 9, 100 μL (~200 MBq) of $^{99m}TcO_4^-$ solution, room temperature.

Table 1. Biological distribution of ^{99m}Tc-risedronate complex in mice as a function of time

Organs and body Fluids	% Injected dose/organ and body fluid at different time post injection (min)				
	15	30	60	120	240
Bone	29.2 ± 2.5	30.25 ± 3	33.85 ± 3.2	34.21 ± 3.1	35.1 ± 3.2
Muscles	14.25 ± 1.2	8.05 ± 0.9	3.56 ± 0.4	2.24 ± 0.21	2.11 ± 0.2
Kidneys	12.65 ± 1	7.23 ± 0.8	3.67 ± 0.3	2.2 ± 0.22	1.23 ± 0.1
Blood	9.45 ± 0.95	1.75 ± 0.09	1.26 ± 0.1	0.85 ± 0.07	0.62 ± 0.05
Intestine	3.22 ± 0.3	2.55 ± 0.12	1.73 ± 0.15	1.13 ± 0.1	1.1 ± 0.1
Liver	2.02 ± 0.25	1.83 ± 0.15	1.51 ± 0.12	1.17 ± 0.1	1.09 ± 0.09
Stomach	0.89 ± 0.1	0.8 ± 0.07	0.52 ± 0.03	0.45 ± 0.02	0.43 ± 0.02
Lungs	0.94 ± 0.1	0.92 ± 0.1	0.82 ± 0.07	0.82 ± 0.05	0.8 ± 0.05
Spleen	1.54 ± 0.14	1.1 ± 0.09	0.86 ± 0.08	0.73 ± 0.05	0.74 ± 0.05
Heart	0.41 ± 0.05	0.39 ± 0.03	0.23 ± 0.02	0.096 ± 0.01	0.06 ± 0.01
Urine	25.36 ± 2.7	45.18 ± 4	52.01 ± 4.1	56.15 ± 3.9	56.67 ± 4.5

0.5 mg, the labeling yield remained nearly stable at maximum labeling yield.

Effect of reaction time and in vitro stability

The influence of the reaction time on the percent radiochemical yield of ^{99m}Tc-risedronate complex (Figures 12 and 13) was investigated as a function of time from 5 min up to 6 h. It was found that the rate of complexation was relatively fast in case of $NaBH_4$, and high percent radiochemical yield was achieved (99.2 ± 0.6%) within about 15 min and then remained around maximum value up to 6 h. Figure 13

In case of Sn(II) method, the reaction starts slowly where the labeling yield was low (80.9 ± 2.9%) at 5 min and labeling yield was increased with time till reach its maximum labeling yield at 30 min. The formed ^{99m}Tc-risedronate complex has high *in vitro* stability up to 6 h in both methods.

Biological distribution study

Table 1 shows the biodistribution of ^{99m}Tc-risedronate complex in different body organs and fluids in mice at different time intervals after intravenous administration of ^{99m}Tc-risedronate complex. Radioactivity was eliminated from the body through urinary pathway where the urine was 56.67 ± 4.5% at 4 h post injection.

The bone uptake was start high and equal to ~29.2 ± 2.5% at 15 min, and then, the activity remained stable at this level (29.2 ± 2.5 to 35.1 ± 3.2%) up to 4 h post injection. So, this radiopharmaceutical could be used to scan body bones in time interval between injection and bone imaging ranging from 15 min to 4 h, which will assist in reducing the radiation absorbed by the patient and medical staff. Also, the long retention of the ^{99m}Tc-risedronate complex in bone indicated its high biological stability. The radioactivity in blood and muscle decreased rapidly with time because of both bone uptake and clearance through the kidneys, which probably occurs via the mechanism of glomerular filtration,[37] and this is the main reason for the presence of some activity in the kidneys.

Conclusion

A method for synthesis of risedronic acid monosodium salt hemipentahydrate is reported with a high yield, and the synthesized pharmaceutical compound was labeled with technetium-99 m using $NaBH_4$ as a reducing agent to give high radiochemical yield of 99.2 ± 0.6%, which was higher than the method used Sn(II) as a reducing agent that gives a radiochemical yield of 84.5 ± 3.5% because of forming radiocolloid impurities, so $NaBH_4$ is more effective and easier than $SnCl_2.2H_2O$. ^{99m}Tc($NaBH_4$)-risedronate showed high and long uptake of the radioactivity in bone starting from 15 min (29.2 ± 2.5 ID/g) to 4 h (35.1 ± 3.2 ID/g) showing the high affinity of ^{99m}Tc($NaBH_4$)-risedronate complex to the bone, so ^{99m}Tc($NaBH_4$)-risedronate could be used as a good radiopharmaceutical for skeletal imaging.

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