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Radiochemical and Biological Evaluation of ^{99m}Tc-Labeling of Phthalic Acid Using ^{99m}Tc-Tricabonyl and ^{99m}Tc-Sn (II) As a Model for Potential Hazards Imaging

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Abstract

In this study, ^{99m}Tc- phthalic acid (PA) firstly, was prepared with a high radiochemical yield up to 98% that confirmed with different chromatographic techniques by using 2 mg phthalic acid, 50 µg $SnCl_2 \cdot 2H_2O$ as a reducing agent, in solution of pH 7 at room temperature for 30 min. Secondly, ^{99m}Tc-tricarbonyl phthalic acid was prepared under 30 min heating at 100°C. Bio distribution studies were carried out in Albino Swiss mice in which ^{99m}Tc-PA and ^{99m}Tc-tricarbonyl PA were concentrated in kidneys and intestine. This work aims to evaluation of labeled PA as a model for Potential Hazards imaging *in vitro* and *in vivo* toxicity in Albino Swiss mice.

Keywords: Phthalic acid; Labeling; Bio-distribution; Technetium-99m; Potential hazards imaging

Introduction

Phthalates are used in a wide range of common products, and are released into the environment. There is no covalent bond between the phthalates and plastics; rather, they are entangled within the plastic as a result of the manufacturing process used to make PVC articles [1]. They can be removed by exposure to heat or with organic solvents. However, people are exposed to phthalates, and most Americans tested by the Centers for Disease Control and Prevention have metabolites of multiple phthalates in their urine [2]. Phthalate exposure may be through direct use or by indirect means through leaching and general environmental contamination. Diet is believed to be the main source of di(2-ethylhexyl) phthalate (DEHP) and other phthalates in the general population. Fatty foods such as milk, butter, and meats are a major source. In studies of rodents exposed to certain phthalates, high doses have been shown to change hormone levels and cause birth defects [3]. Among healthy workers exposed to di (2-ethylhexyl) phthalate, the excretion of metabolites with the urine (o-phthalic acid: 0.21–0.31 µg/ml; n = 9) was increased as compared with non-exposed workers (o-phthalic acid: 0.19 µg/ml; n = 8) [4,5]. Patients with renal failure are exposed to di (2-ethylhexyl) phthalate during dialysis since it leaches out of the plastic tubes to a slight extent. In the body, di (2-ethylhexyl) phthalate is hydrolyzed mainly to o-phthalic acid, which is detected in the serum and dialysate and is excreted unchanged in the urine [6,7]. Technetium-99m (^{99m}Tc) is the radionuclide of choice for diagnostic imaging due to its ideal nuclear properties (Eγ = 140 keV, $T_{1/2}$ = 6 h, no β-emission) and availability from a ^{99}Mo/ ^{99m}Tc generator [8,9]. Although the previous imaging methods using stannous chloride dihydrate as reducing agent or others was considered simple and rapid labeling method with the ^{99m}Tc isotope that have been developed for clinical applications but its disadvantage in less stability in saline or serum. So ^{99m}Tc-tricarbonyl precursor was developed to overcome this problem which considered more stable and can label many compounds by on bond only where other methods need four bond to label any compound [10,11]. Phithalic acid was labeled by ^{99m}Tc-tricarbonyl precursor to give accurate ratio of the concentrated of complex in different organs and that give us complete information about this compound than other method which is more difficult and not give this results. Factors affecting the labeling yield of ^{99m}Tc-phthalic acid complex (Figure 1) and biological distribution in Swiss Albino mice (25-30 gm) were studied in detail. The radiochemical yield of the complex was determined by paper chromatography, paper electrophoresis and High Performance Liquid Chromatography (HPLC).

Materials and Methods

Materials and instrumentation

Phthalic acid was purchased from Sigma Chemical Company, USA., Other chemicals were purchased from Merck and they were reactive grade reagent. Purged deoxygenated bidistilled water were used during all experiment. Electrophoresis apparatus model EC-3000 p-series programmable (E.C. Apparatus Corporation) power and chamber supply units using cellulose acetate strips (Albany, Orion Research, USA) was used. A well-type NaI scintillation γ-Counter model Scalar Ratemeter SR7 (Nuclear Enterprises Ltd., USA) was also used for radioactive measurement.

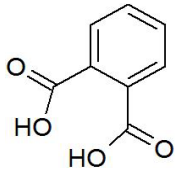

Figure 1: Chemical structure of phthalic acid.

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Preparation of stock solution of $SnCl_2.2H_2O$

One hundred and nintymilligramsof Tin (II) chloride dihydrate (stannous chloride dihydrate) were completely dissolved in 0.5 ml Conc. HCl (25%) by heating on a hot plate then the volume was completed to 10 ml by water. Each ml containing 19 mg of stannous chloride dehydrate was equivalent to 10 mg of tin (II). By dilution, different concentrations of $SnCl_2.2H_2O$ (38 to 760 µg ml^{-1}) were prepared. Each concentration was flushed with nitrogen gas for 15 min and kept at -20°C till use. These concentrations were equivalent to 20-400 µg $ml^{-1}$of Tin (II). Selection the optimum concentration of Sn (II) was done by examination the effect of each concentration on the labeling efficacy. Five hundred micro liter of each concentration (from 10 to 200 µg) was used to prepare the labeled mixture.

Labeling of ^{99m}Tc-phthalic acid

At the optimum amount of Sn (II) (50 µg), optimum pH (7) and optimum duration 30 min, different concentrations of phthalic acid (4 - 40 mg ml^{-1} water) were examined to select the optimum concentration to be labeled. Two hundred and fifty microliters of each concentration (containing from 1-10 mg) was transferred to a penicillin vial and evacuated. The previously prepared $SnCl_2.2H_2O$ (500 µl, 50ug Sn II) solution was added and the pH of the mixture was adjusted to 7. The volume of the mixture was finally adjusted to one ml by water. One ml of freshly eluted $^{99m}TcO_4^-$(200-400 MBq) was added to the above mixture. The reaction mixture was vigorously shaken and allowed to react at room temperature for sufficient time (30 min) to complete the reaction. The proposed structure (Figure 2) of the ^{99m}Tc-phthalic acid complex via. reaction of phthalic acid with ^{99m}Tc-pertechnetate in the optimum conditions made the oxidation state of ^{99m}Tc changed from +7 into +5 to form a complex with two molecules of phthalic acid. ^{99m}Tc-phthalic acid complex coordinated as a Tc (V) oxocore, leading to the formation of a complex in which a TcO_3^+ core existed. This complex formed by two molecules with one another where two (COOH) from every molecule shared to form a complex of ^{99m}Tc-phthalic acid.

Synthesis of ^{99m}Tc-tricarbonyl precursor

^{99m}Tc-tricarbonyl precursor was prepared by the addition of 1 ml of ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$, 740 – 3700 MBq) to a penicillin vial with 7.15 mg sodium carbonate, 4.5 mg sodium boranocarbonate, 2.85 mg sodium tetraborate and 8.5 mg sodium tartrate. After heating for 30 min in a boiling water bath and cooling, the basic solution (pH = 11) was brought to room temperature. The labeling yield and stability of the $[^{99m}Tc\ (CO)_3\ (H_2O)_3]^+$ ion were determined using (RP-HPLC).

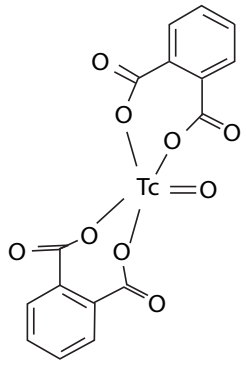

Figure 2: Proposed structure of ^{99m}Tc- PA.

The ^{99m}Tc-tricarbonyl precursor was successfully prepared with a high radiochemical yield (> 95%) (Figures 3-6) [12-14].

Radio labeling with ^{99m}Tc-tricarbonyl precursor

The tricabonyl complex ($^{99m}Tc\ (CO)_3$ PA) was performed by adding 1 ml of the prepared tricabonyl ion to 2 mg PA (10 mg 5/ml in DMF), at room temperature. Then the reaction vial was heated to100°C for 30 min. After cooling down to room temperature (25 ± 1°C), labeling yields were checked by radio-HPLC (Figure 7). According to the previously published results [12-15],$^{99m}TcO_4^-$ is reduced from the oxidation state +7 into +1 by forming ^{99m}Tc-tricarbonyl precursor. Therefore, $^{99m}Tc(CO)_3$ PA was formed by two bonded (OH^-) groups in the IDA part of the molecule. This complex is confirmed by HPLC results.

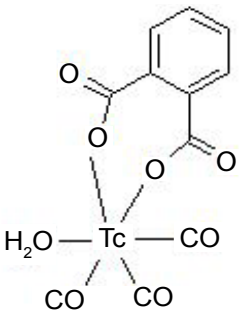

Figure 3: Proposed structure of ^{99m}Tc-tricarbonyl PA.

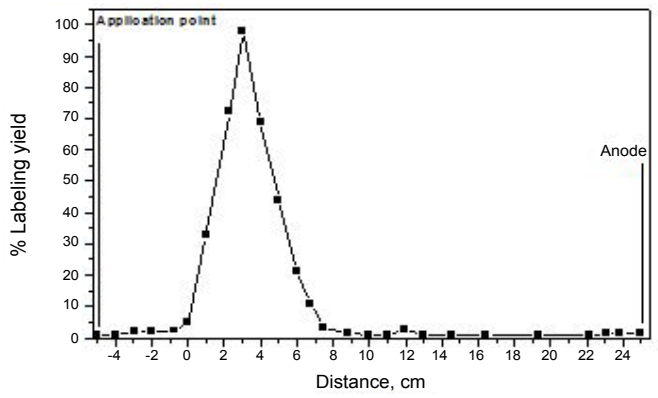

Figure 4: Electrophoresis radio chromatogram of ^{99m} Tc- phthalic acid.

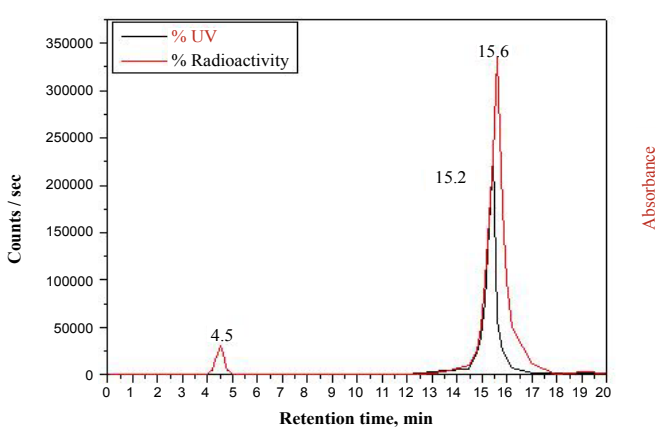

Figure 5: HPLC of ^{99m}Tc- phthalic acid complex.

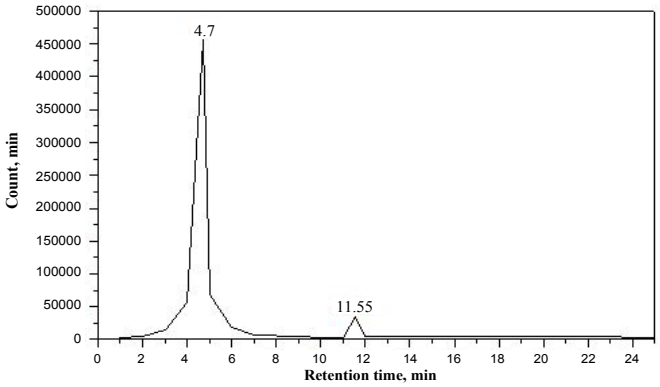

Figure 6: HPLC radiochromatogram of $[Tc(CO)_3(H_2O)_3]^+$ precursor, pH = 11, flow rate 0.6 ml/min, R_t = 4.5 min for $[Tc(CO)_3(H_2O)_3]^+$, R_t = 11.44 min for free $^{99m}TcO_4^-$ using 0.22 µm millipore filtration to eliminate unknown radiochemical impurities.

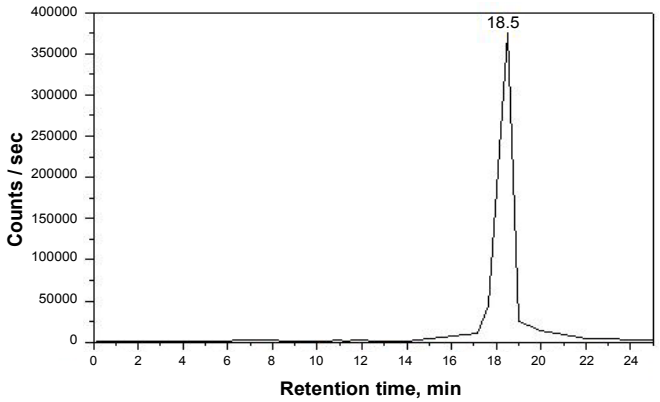

Figure 7: ^{99m}Tc-tricarbonyl PA (>99%) at pH = 7.

Labeling verification

The radiochemical yield and stability of ^{99m}Tc-phthalic acid were determined by paper chromatography method (PC), electrophoresis condition and High Performance Liquid Chromatography (HPLC). But ^{99m}Tc-$(CO)_3$ PA was confirmed by HPLC only.

Paper chromatography of ^{99m}Tc-phthalic acid

Radiochemical yield of ^{99m}Tc-phthalic acid was checked by paper chromatography method in which, the reaction product was spotted on ascending paper chromatography strips (10 × 1.5 cm). Free $^{99m}TcO_4^-$ used in the preparation was determined using acetone as the mobile phase. Reduced hydrolyzed technetium was determined by using ethanol: water: ammonium hydroxide mixture (2:5:1) as the mobile phase. After complete development, the strips were dried then cut into 0.5cm pieces and counted in a well-type γ-scintillation counter.

Electrophoresis conditions of ^{99m}Tc-phthalic acid

Electrophoresis was done with EC-3000 p-series programmable (E.C. Apparatus Corporation) power and chamber supply units using cellulose acetate strips. The strips were moistened with 0.05 M phosphate buffer pH 7.2 ± 0.2 and then were introduced in the chamber. Samples (5µl) were applied at a distance of 10 cm from the cathode with standing time for one and half hours and the applied voltage (300 v) were continued. Developed strips were dried and cut into 1 cm segments and counted by a well-typeNal scintillation counter (Figure 4).

HPLC analysis for ^{99m}Tc-phthalic acid

A High-Performance Liquid Chromatography (HPLC) was established for the simultaneous determination of phthalic acid. The mobile phase was methanol-water-ammonium acetate-acetic acid buffer (pH 4.70) (5/40/55/, v/v/v) at a flow rate of 0.2 ml min^{-1}, and UV absorption wavelength at 254 nm. Five microliters of the reaction mixture was injected into the column (RP-C_{18}-250 mm × 3 mm, 5µm, LiChrosorb) built in HPLC Shimadzu model which consisted of pumps LC-9A, Rheohydron injector. Fractions of 20 ml were collected separately using a fraction collector up to 20 ml and measured in a well-type-γ-scintillation counter (Figure 5) [16].

HPLC for ^{99m}Tc-$(CO)_3$ PA

A 10 µl aliquot of the ^{99m}Tc-$(CO)_3$ PA reaction mixture was injected into RP18 (Lichrosorb, 250 mm x 3 mm, 5 µm) column. The mobile phase consisted of methanol (solvent B) and 0.05 M TEAP (triethyl ammonium phosphate) (solvent A). Gradient system was made up following an isocratic elution (100% A) for the first 0~5 min; a linear gradient of 75% A/25% B to 100% A/0% B was obtained for 5~8 min; a linear gradient of 66% A/34%, B to 75%, A/25%, B was obtained for 8~11 min; a linear gradient of 0% A/100%, B to 66%, A/34%, B was obtained for 11~22 min; and an isocratic elution (100% B) was obtained for 22~25 min. The flow rate was 0.6 ml/min (Figure 7) [12-14].

In-vitro stability test of ^{99m}Tc-PA and ^{99m}Tc-tricarbonyl PA.

In-vitro stability of ^{99m}Tc- phthalic acid was studied in order to determine the suitable time for injection to avoid the formation of the undesired products that result from the radiolysis of complex. These undesired radioactive products might be accumulated in non-target organs. The results of stability showed that the ^{99m}Tc-phthalic acid was stable for 24 hours at 37°C as determined by (PC) sheets. Also, ^{99m}Tc-tricarbonyl PA remained stable during 24 h that can be determined by HPLC [12-14].

Stability in Serum

The stability in rat serum of purified ^{99m}Tc-tricarbonyl PA and ^{99m}Tc-phthalic acid was determined by mixing 0.1 ml of ^{99m}Tc-tricarbonyl PA or ^{99m}Tc-phthalic acid solution with 0.9 ml rat serum and kept at 37°C. At time intervals, the stability was assayed using HPLC and PC techniques [12-14].

Animal Studies

The study was approved by the animal ethics committee, Labeled Compound Department, and was in accordance with the guidelines set out by the Egyptian Atomic Energy Authority. The animals, normal Swiss Albino mice (30-35 gm), were intravenously injected with 100 µl (3.3 mg kg^{-1} body weight, 100–150 MBq) of sterile ^{99m}Tc-phthalic acid and kept alive in metabolic cage for different intervals of time under normal conditions. For quantitative determination of organ distribution, five mice were used for each experiment and the mice were sacrificed at different time post-injection. Samples of fresh blood, bone and muscle were collected in pre-weighed vials and measured. The different organs were removed, counted and compared to a standard solution of the labeled PA. The average percent values of the administrated percent injected dose per gram (% ID/organ ± SD) were calculated. Blood, bone and muscles were assumed to be 7, 10 and 40%, respectively, of the total body weight [17].

Statistical Analysis

Data were evaluated with one way analysis of variance test (name of used program). All the results were observed as mean ± SEM of five replicates. The level of significance was set at $p<0.05$.

Results and Discussion

Verification of labeling procedure

In case of ascending paper chromatographic method, acetone was used as the developing solvent, free $^{99m}TcO_4^-$ moved with the solvent front (R_f=1), while ^{99m}Tc- phthalic acid and reduced hydrolyzed technetium (colloid) remained at the point of spotting. After developing with the mixture of ethanol: water: ammonium hydroxide, reduced hydrolyzed technetium remains at the origin (R_f = 0) while other species migrate with the solvent front (R_f =1). The radiochemical purity was determined by subtracting the sum of the percent of reduced hydrolyzed technetium and free pertechnetate from 100%. The radiochemical yield was calculated from the mean value of five experiments.

The paper electrophoresis pattern revealed that ^{99m}Tc-phthalic acid complex moved towards the anode with 2.8 cm distance (Figure 4). Whereas, $^{99m}TcO_4^-$ moved towards the anode with 12 cm distance suggesting that it had a high negative charge. Radioactivity yield (%) = Peak activity of the ^{99m}Tc- phthalic acid × 100/Total activity.

HPLC chromatogram is presented in (Figure 5). Three sharp peaks was obtained, one at 4.5 min retention time, which corresponded to $^{99m}TcO_4^-$, second one at 15.2 min, which corresponded to the UV signal of phthalic acid. While the last peak was observed at 15.6 min which revealed to ^{99m}Tc-phthalic acid.

Factors effecting on the labeling yield

Effect of $SnCl_2.2H_2O$ concentration: The effect of $SnCl_2.2H_2O$ amount in the reaction mixture on the radiochemical yield of phthalic acid is illustrated in (Figure 8). At 10 µg of Sn (II), the labeling yield of ^{99m}Tc-phthalic acid was low (66%) due to the fact that $SnCl_2.2H_2O$ concentration was insufficient to reduce all pertechnetate so 20% of $^{99m}TcO_4^-$ remained in the solution. The labeling yield significantly increased by increasing the amount of $SnCl_2.2H_2O$ from 10 to 50 µg (optimum amount), at which a maximum labeling yield of 98% was obtained. By increasing the amount of $SnCl_2.2H_2O$, above 50 µg, the labeling yield decreased again because the excess $SnCl_2.2H_2O$ was converted to colloid (55% at 200 µg $SnCl_2.2H_2O$). This finding could be interpreted by the consumption most of the ligand molecules in the formation of complexes. Subsequently the pertechnetate was reduced to insoluble technetium (IV) $TcO_2. \times H_2O$ in the absence of ligand [18-20]. In other explanation, this decrease attributed to the fact that the excess amount of stannous chloride led to the formation of stannous hydroxide colloid $Sn(OH)^-_3$ in basic medium [21-23] as the very high Sn (II) concentration increased the reduction reaction rate to colloid formation and it became more competitive with respect to the complexation reaction thus decreasing the labeling yields.

Effect of the amount of phthalic acid: At constant conditions of Sn II amount (50 µg), pH (7) and duration (30 min), different amounts of phthalic acid were used (Figure 9). Using 1 mg of phthalic acid gave 80% radiochemical yieldin^{99m}Tc-phthalic acid complex. Whereas, 98% labeling yield was obtained by using 2 mg phthalic acid instead of 1 mg. Surprisingly, this yield did not change by increasing the amount of phthalicacideven at 10 mg. Similar findings were observed in case of tricabonyl and levosalbutamollebeling [24].

Effect of pH of the reaction mixture: Labeling of phthalic acid affected dramatically by changes in pH (4-12) (Figure 10). The optimum pH was found to be in basic medium (pH=7) that gave the maximum radio-chemical yield of 98%. This observation agreed with [25] who found that the base medium was the optimum level to label muscarinic receptors in rats. The radiochemical yield of ^{99m}Tc-phthalic acid complex in acidic medium (pH 4) was low (47%) with the appearance of free pertechnetate as predominant species 20%. The percentage of

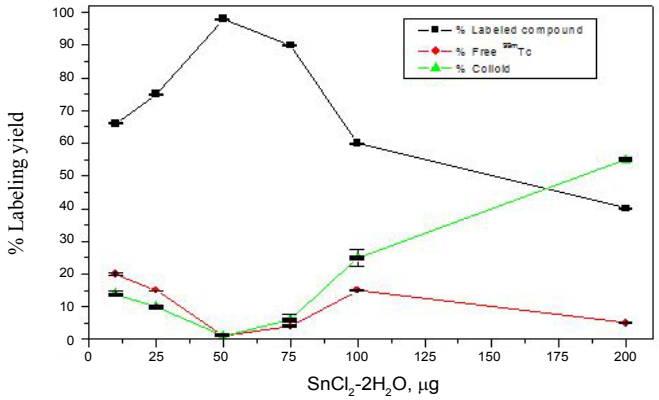

Figure 8: Effect of $SnCl_2.2H_2O$ amount on the radiochemical yield % of ^{99m}Tc-phthalic acid.

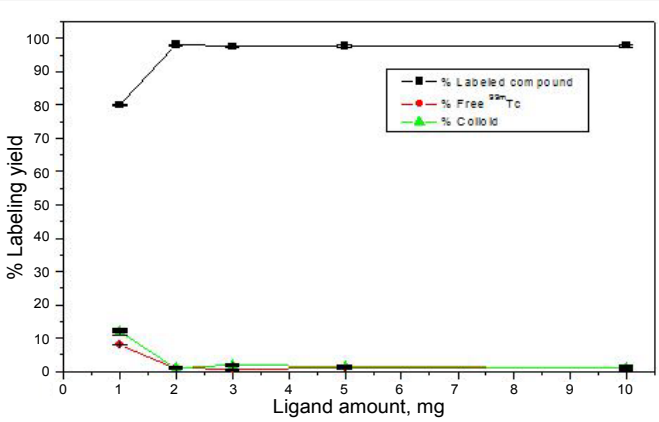

Figure 9: Percent radiochemical yield of ^{99m}Tc- phthalic acid as a function of substrate amount.

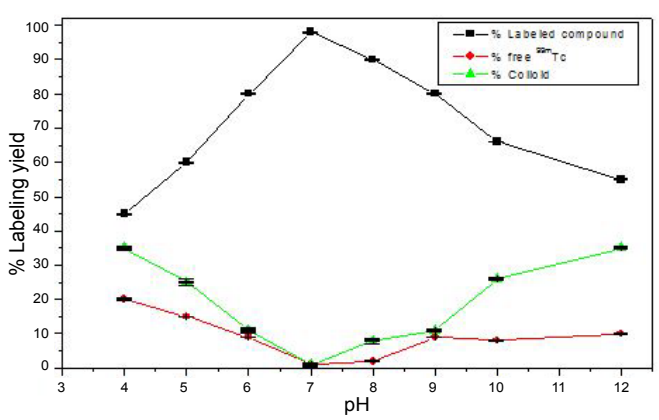

Figure 10: Effect of pH on the radiochemical yield % of ^{99m}Tc- phthalic acid.

^{99m}Tc-phthalic acid increased gradually by the increase of pH up to 7 which gave the maximum radiochemical yield. Increasing the pH of the reaction medium above pH 7 (8 to 12) decreased the radiochemical yield of ^{99m}Tc- phthalic acid from 88% to 55% respectively [26-28].

Effect of reaction time: Different duration time (1-120 min) was examined for its efficiency in radiochemical labeling (Figure 11). The radiochemical yield of ^{99m}Tc- phthalic acid complexe at 1 min post labeling was low (77%). Half an hour was the optimum duration gave the best yield (98%) which kept stable even after 120 min. Similar work done on ^{99m}Tc-cefprozil which increased with time until reaching its maximum value of 97.5% at 30 min [29].

In-vitro Stability Test of ^{99m}Tc-PA and ^{99m}Tc-tricarbonyl PA

As illustrated in (Figure 12). The ^{99m}Tc-phthalic acid complex considered stable during 24 h resulted in a small release of radioactivity as determined by (PC) sheets which decreased from 98 ± 0.11% to 95 ± 0.43% (Figures 13 and 14) shows that ^{99m}Tc-tricarbonyl PA remained stable during 24 h and decayed from > 99% to 96% as detected by RP-HPLC [12-14].

Stability in Serum

The stability of ^{99m}Tc- phthalic acid in normal serum at 37°C is as illustrated in (Figure 13) with a small release of radioactivity as determined by (PC) sheets which decreased from 98 ± 0.11% to 89 ± 0.22%. The stability in serum of ^{99m}Tc-tricarbonyl showed that small release of radioactivity (n = 5 experiments) which decreased from >99% to 94% (Figure 15) [30-33]

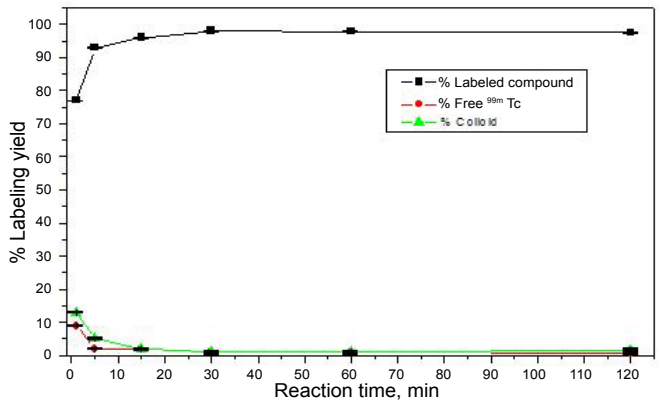

Figure 11: ^{99m}Tc- phthalic acid yields vs. reaction time.

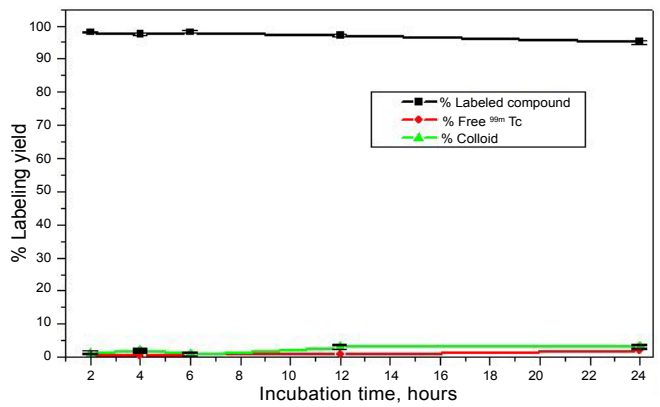

Figure 12: *In-vitro* stability of ^{99m}Tc- phthalic acid at optimum conditions.

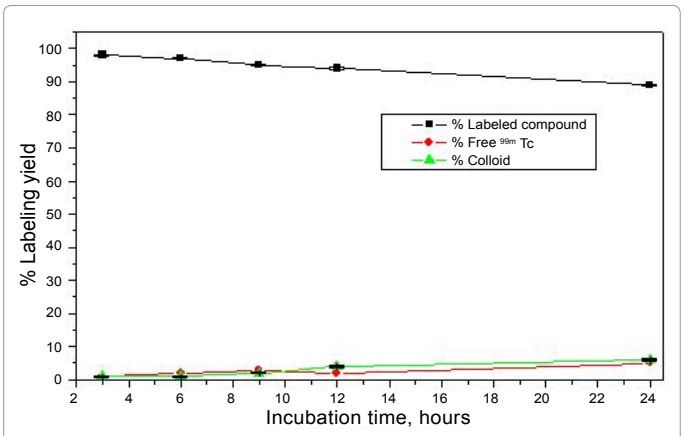

Figure 13: Stability in serum of ^{99m}Tc- phthalic acid at optimum conditions.

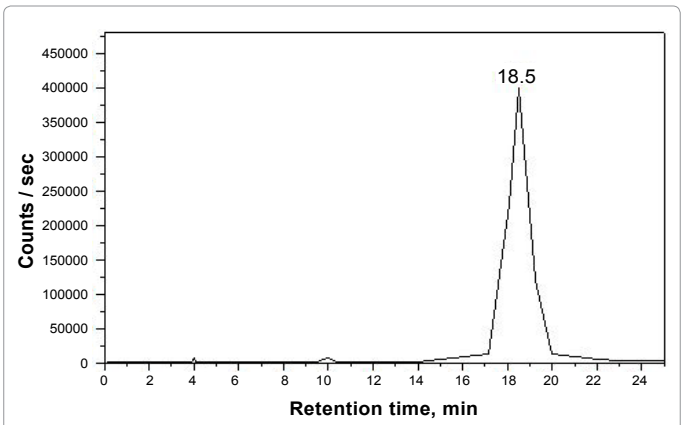

Figure 14: *In-vitro* stability of ^{99m}Tc-tricarbonyl PA (96%) at optimum condition.

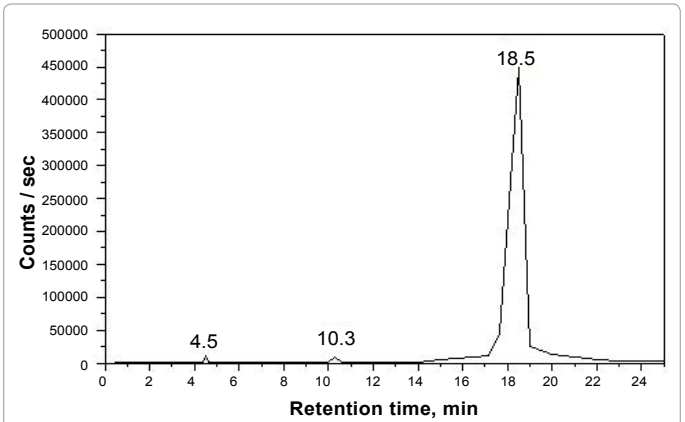

Figure 15: *In-vitro* stability of ^{99m}Tc-tricarbonyl PA in serum at 37°C.

Bio-Distribution

(Table 1) shows the *in vivo* behavior of the ^{99m}Tc- phthalic acid in mice at 30, 60, 120, and 240 min post intravenous injection. The bio distribution of ^{99m}Tc- phthalic acid in important body organs and fluids was evaluated. All radioactivity levels are expressed as average percent-injected dose per organ (%ID/ total organ in the most relevant organs

Organs and Body fluids	% Injected dose/organs and body fluids at different time post injection			
	30 min	1 h	2 h	4 h
Liver	3.60 ± 0.22	4.90 ± 0.10	6.00 ± 0.19	2.10 ± 0.66
Urine	4.30 ± 0.12	5.27 ± 0.22	13.00 ± 1.10	37.90 ± 0.50
Kidneys	5.90 ± 0.80	9.75 ± 0.15	15.80 ± 0.40	6.12 ± 0.13
Blood	8.27 ± 1.00	2.90 ± 0.80	1.10 ± 0.40	0.90 ± 0.01
Heart	1.10 ± 0.007	0.95 ± 0.001	0.70 ± 0.005	0.50 ± 0.003
Lung	0.90 ± 0.001	0.81 ± 0.003	0.60 ± 0.10	0.40 ± 0.0001
Stomach	1.60 ± 0.11	1.10 ± 0.02	0.90 ± 0.20	0.77 ± 0.001
Intestine	15.19 ± 0.09	22.50 ± 1.00	33.00 ± 1.11	14.55 ± 0.09
Spleen	1.10 ± 0.20	0.90 ± 0.001	0.80 ± 0.002	0.50 ± 0.002
Muscle	1.20 ± 0.002	1.11 ± 0.07	0.95 ± 0.06	0.60 ± 0.001
Bone	1.12 ± 0.10	1.00 ± 0.04	0.90 ± 0.07	0.60 ± 0.002
SD (mean of five experiments) ± Mean				

Table 1: Bio-distribution of ^{99m}Tc-phthalic acid in mice at different post-injection times.

Organs and Body fluids	% Injected dose /organs & body fluid at different time post injection			
	30 min	1 h	2 h	4 h
Liver	3.00 ± 0.21	7.80 ± 0.11	9.00 ± 0.17	4.90 ± 0.33
Urine	3.20 ± 0.19	6.29 ± 0.24	16.00 ± 0.90	39.95 ± 0.44
Kidneys	7.10 ± 0.22	12.68 ± 0.13	19.60 ± 0.20	8.13 ± 0.11
Blood	9.5 ± 0.77	3.11 ± 0.60	1.30 ± 0.22	0.80 ± 0.31
Heart	1.12 ± 0.01	0.95 ± 0.002	0.80 ± 0.003	0.60 ± 0.001
Lung	0.96 ± 0.002	0.81 ± 0.001	0.60 ± 0.001	0.50 ± 0.001
Stomach	1.10 ± 0.01	1.20 ± 0.001	0.96 ± 0.002	0.81 ± 0.002
Intestine	13.15 ± 0.01	19.90 ± 1.11	35.00 ± 0.15	16.00 ± 0.001
Spleen	1.00 ± 0.10	0.98 ± 0.002	0.91 ± 0.001	0.80 ± 0.001
Muscle	0.90 ± 0.001	1.00 ± 0.01	0.90 ± 0.002	0.80 ± 0.002
Bone	1.00 ± 0.01	1.10 ± 0.001	0.98 ± 0.002	0.80 ± 0.001
SD (mean of five experiments) ± Mean				

Table 2: Bio-distribution of ^{99m}Tc-tricarbony phthalic acid in mice at different post-injection times.

for the complex ± S. D) [34-37]. All organ like blood, heart, lung, stomach, spleen, muscle and bone were declined with time from 30 min up to 4 h. The uptake within the kidneys were increased from 5.90% at 30 min post injection to 15.80% at 2 h post injection which declined to 6.12% at 4 h post injection with increasing of urine uptake from 4.30 at 30 min to 37.90% at 4 h post injection. The uptake in liver increased from 3.60% at 30 min post injection to 6.00 at 2 h post injection that declined to 2.10% at 4 h post injection. Also, the uptake of intestine were increased from 15.19% at 30 min post injection to 33.00% at 2 h post injection which decline to 14.55% at 4 h post injection [38-40]. Also (Table 2) of ^{99m}Tc-tricarbonyl phthalic acid shows the same biodistribution without differences than ^{99m}Tc- phthalic acid. So, this complex was excreated through kidneys and intestine that is important to follow this compound in human body as a toxic substance [41-45].

Discussion

Phthalic acid esters (phthalates) are used as plasticizers in numerous consumer products, commodities, and building materials. Consequently, phthalates are found in human residential and occupational environments in high concentrations, both in air and in dust. Phthalates are also ubiquitous food and environmental contaminants [46]. An increasing number of studies sampling human urine reveal the ubiquitous phthalate exposure of consumers in industrialized countries. At the same time, recent toxicological studies have demonstrated the potential of the most important phthalates to disturb the human hormonal system and human sexual development and reproduction. Additionally, phthalates are suspected to trigger asthma and dermal diseases in children [47]. The metabolism of phthalates first produces phthalate monoesters, which can be metabolized further to oxidative products [48]. Many metabolites are glucuronidated and excreted in the urine and feces [49]. On this study, the labeled phthalic acid was studied in mice at 30,60, 120, and 240 min post intravenous injection. The biodistribution of labeled phthalic acid shows that it was excreated through kidneys and intestine.

Conclusion

Phthalic acid complex was labeled easily by tow methods firsly using $SnCl_2.2H_2O$ as a reducing agent and 30 min reaction time with a labeling yield of 98 ± 0.11% secondly by tricarbonyl core to give > 99% radiochemical yield. Its stability in saline and serum was studied in both cases. Depending on the data obtained from the biodistribution in case of ^{99m}Tc- phthalic acid and ^{99m}Tc-tricarbonyl PA. It was stated that this complex was concentrated in kidneys and intestine, on the other hand labeling of phthalic acid considered a new strategy to analyse phthalate as an end hydrolysis product of phthalate in the biological systems.

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