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Research Article

Synthesis, Characterization and Radiolabeling of Iminodiacetic Acid Derivative with Technetium-99m

MA Motaleb[1], M El-Tawoosy[1], W Hamouda[1*], M Abdallah[2], A Hassan[2]

[1]Labeled Compounds Department, Hot Labs Center, Atomic Energy Authority, P.O. Box 13579, Cairo, Egypt;[2]Department of Chemistry Department, Zagazig University, Egypt

ABSTRACT

N-(2,4,6-trimethylphenylcarbamoylmethyl)iminodiacetic acid (TMIDA) was successfully synthesized, characterized and radiolabeled with technetium-99m by direct technique using sodium dithionite as reducing agent. The labeling parameters including TMIDA concentration, sodium dithionite concentration, pH of the reaction mixture, reaction temperature and reaction time were optimized. The results showed that high radiochemical yield of 96.64 ± 0.11 % obtained at pH 7 without any notable decomposition at room temperature over a period of 8 h. The biodistribution studies of 99mTc-TMIDA complex in mice showed high liver uptake of 18.88% injected activity/g tissue organ at 10 min post-injection with fast biliary excretion.

Keywords: TMIDA concentration; pH; Temperature; Concentration

INTRODUCTION

Technetium-99m complexes are routinely used in the diagnosis of many cancers and non-cancer diseases involving various organs such as heart, bone, kidneys, liver, lungs and thyroid, etc., because the 99mTc radionuclide has favourable nuclear properties such as 140 KeV, γemitters, t1/2=6 h, low cost and readily availability. These good imaging characteristics and economic reasons make 99mTc an ideal radionuclide to radiolabel drugs [1–4]. Tc-99m is available as pertechnetate ion from the Mo-99/Tc-99m generator, but the pertechnetate ion cannot attach to any organic moiety. So reduction of pertechnetate ion to a lower oxidation state is essential for Tc-99m complex formation in high yield. Several reducing agents were used for this purpose such as, stannous ion, ferric chloride with ascorbic acid, sodium dithionite, electrolysis with zirconium electrodes...etc [5,6]. Stannous chloride is the most commonly used reducing agent in most preparations of 99mTc-labeled compounds, but during labeling by this method radiocolloids are generated. These radiocolloids affect the biodistribution of desired molecule. On the other hand, sodium dithionite instead of stannous salts was effectively used to obtain radiocolloids free radiolabeled complexes [7].

Derivatives of iminodiacetic acid (IDA) have been widely used as ligands for 99mTc in hepatobiliary radiopharmaceuticals [8,9]. In other words, the majority of 99mTc hephatobiliary agents are iminodiacetic acid derivatives including 99mTc-Disofenin, 99mTc-EHIDA [10], 99mTc-Mebrofenin [11–16], 99mTc-Lidofenin [17,18], 99mTc-JODIDA [12] and 99mTc-IOIDA [19]. The IDA group was chosen as the chelating function because it has a number of desirable features: it is relatively small, being roughly isosteric with diethylamine, and so involved minimal departure in molecular size; it forms stable complexes with most metals; and it can be synthetically incorporated into organic molecules with relative ease. All the 99mTc radiopharmaceuticals for hepatobiliary imaging show similar pharmacokinetic properties in animals and human. They are effectively extracted from the blood by the liver and excreted into the bile. Furthermore, they assess disease of hepatocytic function and the functional status of the cystic duct and gallbladder [19].

The present study report the synthesis, characterization, radiolabeling of TMIDA (Figure 1) and biological evaluation of 99mTc-TMIDA in mice as hepatobiliary imaging agent.

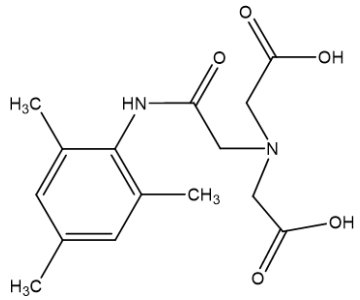

Figure 1: Structure of N-(2,4,6-trimethylphenylcarbamoylmethyl) iminodiacetic acid, (TMIDA).

Correspondence to: W Hamouda, Labeled Compounds Department, Hot Labs Center, Atomic Energy Authority, P.O. Box 13579, Cairo, Egypt; E-mail: Waleedhamoda@yahoo.co.uk

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MATERIAL AND METHODS

Chemicals

All chemicals used in this study were of analytical grade and used directly without further purification. Deionized water was used in all experiments for the preparation of all solutions. Technetium-99m was eluted as $^{99m}TcO4^-$ from 99mMo/99mTc generator (radionuclidic purity: 99.99%; radiochemical purity: 99.99%; Activity: 1Ci; Elutec, Brussels, Belgium).

Instrumentations

All melting points were determined with a Kofler Block apparatus. The 1H-NMR spectra were recorded on a Varian Gemini 200 NMR Spectrometer at 300 MHz with TMS as a standard. IR spectra were recorded on a Perking Elmer 1430 ratio recording infrared spectrophotometer with CDS data station using KBr Wafer technique, and Mass spectra were measured on a GC-MSQP 1000EX Shimadzu at micro analytical laboratory, Cairo University, Cairo, Egypt.

Synthesis

N-(2,4,6-trimethylphenylcarbamoylmethyl)iminodiacetic acid (TMIDA(was prepared in two steps as shown in Figure 2. The first one involves the synthesis of ω-chloro-2,4,6-trimethylacetanilide following the procedure of Lofgren [20], a solution of 2,4,6-trimethylaniline (5.616 ml, 40 mmol) in glacial HOAc (30 ml) was placed in an ice bath to maintain the temperature at below 5°C and 1.1 equivalents of chloroacetyl chloride (3.5 ml, 44 mmol) was added dropwise. Upon completing the addition, it was allowed to stand at room temperature for 1 h. Sodium acetate (19.7 g, 240 mmol) in water (100 ml) was added at one time and was allowed to stand for additional 1 h. The solid precipitate was filtered, washed with water and dried. The second step involves the synthesis of TMIDA following the procedure of Chiotellis and Valvarigou [21], a solution of ω-chloro-2,4,6-trimethylacetanilide (10 mmol) and iminodiacetic acid (10 mmol) in 50% aqueous ethanol was refluxed for 5 h at 85°C and adjusted to pH 11-12 with 10% NaOH every hour. The mixture was cooled to room temperature and the ethanol was removed using rotary evaporated. The mixture was extracted three times with diethyl ether. The aqueous layer was then adjusted to pH 2-3 with HCl and the precipitate was formed on cooling. The pecipitate was filtered off, dried and recrystallized with ethanol to give TMIDA (Yield: 23.5 % and Mp: 218-221 °C).

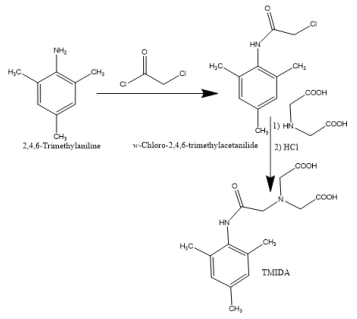

Figure 2: A Reaction scheme for the synthesis of TMIDA.

Labeling procedure study

TMIDA was labeled with technetium-99m by the direct technique using sodium dithionite ($Na_2S_2O_4$) as a reducing agent. To 100 µ of freshly eluted 99mTcO4- (400 MBq), the required concentration of solid sodium dithionite was added directly with continuous stirring followed by immediate addition of the required TMIDA concentration, which dissolved in 0.1 M NaOH. The pH of the preparation was adjusted followed by incubation at room temperature at specific reaction time [22]. TMIDA concentration, $Na_2S_2O_4$ concentration pH, reaction time and temperature were studied as factors affecting labeling efficiency. Each factor studying experiment was repeated three times. The forming 99mTc-IDA with a negative charge in which two ligand molecules are coordinated to one Tc(III)-core [15].

Radiochemical yield assay

The percent radiochemical yield of 99mTc–TMIDA was determined by ascending paper chromatographic technique using two strips of Whatman No.1 paper chromatography, one strip developed with acetone and the other developed with saline. A spot from 99mTc–TMIDA complex solution was applied using hypodermic syringe on the spotting point and then the strip was developed in an ascending manner in a closed jar filled with N2 gas to prevent oxidation of the labeled complex spot. After complete development, the strips were dried, cut into 1 cm pieces and separately counted using the NaI(Tl) γ-ray scintillation counter [22]. Each experiment was repeated three times.

Biodistribution study

The study was approved by the animal ethics committee and was in accordance with the guidelines set out by the Egyptian Atomic Energy Authority. A volume of 0.2 ml of 99mTc–TMIDA complex containing 100 kBq was intravenously injected in the tail vein of mice. The animals were anesthetized by chloroform at the predesigned time interval and their body organs and fluids were separated, weighted and their radioactivities were assayed using a NaI(Tl) γ-ray scintillation counter [23]. Biological distribution of 99mTc–TMIDA complex in mice organs and fluids was studied as a function of time 10, 30, 60 and 120 min post injection (p.i.). For blood, bone, and muscles, the activity distribution was determined for weighed fractions of the above-mentioned biological samples and was calculated assuming that blood represents 7%; bone, 10%; and muscles, 40% of the mouse body weight [24].The percentages of the injected dose/g organ or fluids were calculated. Experiment studying was repeated three times.

RESULTS AND DISCUSSION

Synthesis of N-(2,4,6-trimethylphenylcarbamoylmethyl) iminodiacetic acid (TMIDA)

Synthesis of TMIDA was accomplished according to the reaction sequence in two steps. The first one involves the reaction between 2,4,6-trimethylaniline derivative and chloroacetyl chloride to give ω-chloro-2,4,6-trimethylacetanilide. The second step involves condensation reaction between ω-chloro-2,4,6-trimethylacetanilide and iminodiacetic acid at alkaline pH in ethanol for 5 h to give TMIDA.

Characterization TMIDA

The synthesized compound, TMIDA, was confirmed by IR, mass and 1H-NMR spectra. The IR spectrum of TMIDA showed the most characteristic bands for carboxylic C=O group at 1748 cm-1, the sharp band for NH group at 3206 cm-1, and the stretching

vibration band for CH (Aromatic) group is observed at 3055 cm^{-1}. The 1H-NMR spectrum of TMIDA showed two signals appeared at γ=2.093 and 2.303 ppm for methyl groups. The aromatic protons appeared as singlet signal at γ=6.869 ppm. The protons of CH_2 attached to the amidic carbonyl group have a signal at 3.483 ppm. Two broad signals appeared for NH and OH groups at γ 9.363 and 12.550, respectively.

The mass spectrum of TMIDA showed a corresponding peak to its molecular ion at m/z 308. The suggested fragmentation pathway can be explained as follows: the radical cation of compound TMIDA loses H_2O molecule to give molecular ion peak at m/z 290 (M^+-H_2O) which loses CO molecule to give a radical cation at m/z 262. Also, other fragmentation pathways.

Radiolabeling of TMIDA

Factors affecting the percent radiochemical yield of 99mTc–TMIDA complex

Effect of TMIDA concentration

The effect of the radiochemical yield on the TMIDA concentration. The reaction was performed at different TMIDA concentrations, 20-150 μg/ml. Low percent radiochemical yield of 55.67 ± 0.31 % was obtained at low TMIDA concentration (20 μg/ml), because this concentration was insufficient to chelate all the reduced 99mTc, so the excess ^{99m}Tc was converted to colloid. The radiochemical yield was increased with increasing the TMIDA concentration until reaching the highest yield of 96.64 ± 0.11% at 60 μg/ml TMIDA. Increasing the TMIDA concentration more than 60 μg/ml, the radiochemical yield remained nearly stable at maximum yield.

Effect of reducing agent concentration

99mTc was used for direct labeling of TMIDA under reductive conditions using sodium dithionite ($Na_2S_2O_4$). The optimum concentration of sodium dithionite which gave a maximum radiochemical yield of 96.64 ± 0.11 % was 8 mg/ml. At lower concentrations of $Na_2S_2O_4$, high quantities of free pertechnetate were obtained (radiochemical yield of 61.34 ± 0.31 % at 2 mg/ml). This may be due to the used concentration of Na2S2O4 was insufficient for complete reduction of pertechnetate to form 99mTc-TMIDA. No further increase in radiochemical yield was obtained by increasing the concentration of $Na_2S_2O_4$ above 8 mg/ml.

Effect of pH of the reaction mixture

Data presented clearly show that the radiochemical yield of ^{99m}Tc–TMIDA is dependent on the pH of the reaction mixture. The maximum radiochemical yield of 99mTc–TMIDA (96.64 ± 0.11 %) was obtained at pH 7. At pH below the optimum pH, the radiochemical yield was significantly decreased by forming reduced hydrolyzed technetium-99m which was the main radiochemical impurity, where at pH 5, the radiochemical yield was found as 48.31 ± 0.12 %. The radiochemical yield was also decreased by increasing the pH above the optimum value, where at pH 10, the radiochemical yield was found as 33.90 ± 0.12% due to the hydrolysis of the 99mTc–TMIDA complex.

Effect of reaction time and in-vitro stability of 99mTc–TMIDA complex

The rate of formation of 99mTc-TMIDA complex is started relatively slowly. The maximum radiochemical yield of 99mTc-TMIDA complex (96.64 ± 0.11%) is achieved at 30 min reaction time. The radiochemical yield is reached the saturation value and was not affected by increasing the reaction time above 30 min up to 8 h.

Biodistribution of ^{99m}Tc–TMIDA complex

Biodistribution of ^{99m}Tc-TMIDA complex in mice is shown in Table 1. The body clearance of ^{99}mTc-TMIDA complex is mainly through the hepatobiliary pathway and the activity is not accumulated in specific organ. The major fraction of the injected dose of 99mTc-TMIDA was transported to the hepatocytes and cleared into the gallbladder and intestine. The liver uptake of ^{99m}Tc-TMIDA was 18.88 % at 10 min post injection, with the subsequent rapid clearance, 6.34 % ID/g at 10 min p.i. The whole-body clearance of radioactivity was fast as the radioactivity level for ^{99m}Tc- TMIDA complex in the blood was 15.14 % ID/g at 10 min p.i. followed by a decrease to 0.93 % at 120 min p.i. The low stomach uptake confirms *in-vivo* stability of ^{99m}Tc-TMIDA complex.

Table 2: *In-vivo* biodistribution study of ^{99mTc}-TMIDA complex in mice at different time intervals.

Body Organ (fluid)	% ID/g organ (fluid) at time intervals post injection			
	10 min	30 min	60 min	120 min
Blood	15.14 ± 0.1	4.17 ± 0.05	1.41 ± 0.01	0.93 ± 0.01
Kidneys	3.22 ± 0.01	3.38 ± 0.18	4.26 ± 0.02	5.32 ± 0.03
Liver	18.88 ± 0.23	14.15 ± 0.02	9.95 ± 0.75	7.35±0.31
Spleen	2.6 ± 0.08	1.42 ± 0.06	0.71 ± 0.03	0.45 ± 0.02
Intestine	35.13 ± 0.02	48.25 ± 0.02	55.94 ± 0.06	61.05 ± .02
Gallblader	4.66 ± 0.01	6.97 ± 0.01	4.20 ± 0.07	3.33 ± 0.02
Stomach	1.56 ± 0.09	1.96 ± 0.01	2.83 ± 0.07	2.07 ± 0.08
Lungs	0.41 ± 0.01	0.93 ± 0.01	1.66 ± 0.01	1.13 ± 0.02
Heart	7.91 ± 0.01	3.49 ± 0.04	3.09 ± 0.06	1.92 ± 0.06
Bone	3.50 ± 0.08	9.53 ± 0.11	8.50 ± 0.09	6.26 ± 0.10

CONCLUSION

N-(2,4,6-trimethylphenylcarbamoylmethyl)iminodiacetic acid (TMIDA) was synthesized using an easy and cheap method with an overall yield equal to 23.5 % and characterized using different analytical techniques like IR, 1H-NMR and mass spectroscopy. The synthesized iminodiacetic acid derivative (TMIDA) was labeled with 99mTc following the direct method, in which pertechnetate was added to an aqueous solution containing sodium dithionite as reducing agent at room temperature for 30 min at pH 7 and these were the optimum conditions with the percent radiochemical yield 96.64 % of 99mTc-TMIDA without any notable decomposition at room temperature over a period of 8 hr. Considering the biodistribution data results, 99mTc-TMIDA complex prepared in such way remains stable without any decomposition inside the body as a hepatobiliary imaging agent for an evaluation of the functional status of the hepatocytes and the patency of the biliary duct.

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