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Acid-base equilibria of 2,4-diiodo-6-methylphenylcarbamoylmethyl iminodiacetic acid and its labeling with technetium-99m

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Abstract: The acid-base equilibria of a novel hepatobiliary imaging agent, 2,4-diiodo-6-methylphenylcarbamoylmethyl iminodiacetic acid (DIIODIDA) were studied. The potentiometrically determined acidity constants of the second carboxylic group, amino and amide groups were $pK_2 = 2.52 \pm 0.02$; $pK_3 = 5.86 \pm 0.06$ and $pK_4 = 10.9 \pm 0.1$. The determinations were performed at 25 ºC and an ionic strength of 0.1 mol/dm^3 (NaCl). The acidity constants ($pK_1 = 1.3 \pm 0.4$) corresponding to the first carboxylic group was determined indirectly, on the basis of equilibrium constants obtained in a heterogeneous system, at 25 ºC and an ionic strength 1 mol/dm^3 (HCl, NaCl). DIIODIDA was labeled with technetium-99m, and the influence of pH on the yield of labeling was investigated. It was found that labeling within the pH range from 5.5 to 6.5 provided a radiopharmaceutical of high radiochemical purity (>98 %).

Keywords: iminodiacetic acid analogues, acidity constants, hepatobiliary agent, technetium-99m.

INTRODUCTION

The analogues of iminodiacetic acid (IDA) labeled with technetium-99m are used in nuclear hepatology for non-invasive and quantitative evaluation of numerous hepatobiliary diseases related to bile formation and excretion.[1] Functional imaging with suitable IDA analogues provides an objective basis for the choice of therapy and critical evaluation of the efficiency of the chosen therapy. The biological properties of complexes of ^{99m}Tc-IDA analogues depend on the blood bilirubin level, because the transport of these radiopharmaceuticals across hepatocytes is a carrier-mediated organic anion pathway, similar to the hepatic handling of biliru-

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bin. A high blood level of bilirubin leads to a decrease of both plasma and hepatic clearance of ^{99m}Tc-IDA analogues, thus reducing the hepatic accumulation and biliary excretion of these radiopharmaceuticals. Hence, hyperbilirubinemia represents the limiting factor in the application of ^{99m}Tc-IDA analogues as hepatobiliary imaging agents. The structural and physicochemical relationships between the lipophilic and polar groups in radiolabeled complexes play a key role in determining the affinity of the radiopharmaceuticals for binding to hepatic transport proteins, the efficiency of their uptake by hepatocytes and the excretion rate. Increasing lipophilicity leads to a higher degree of resistance against the competitive effects of bilirubin. Monohalogenic IDA analogues have diagnostic advantages under hyperbilirubinemia conditions compared to non-halogenic ones.[2–4]

Biological studies on rats showed that the newly synthesized diiodo-IDA analogue, 2,4-diiodo-6-methylphenylcarbamoylmethyl iminodiacetic acid (DIIODIDA) has all the characterstics of a good hepatobiliary imaging agent and a greater tolerance to bilirubin than the presently used monoiodo-IDA analogues.[5] The modification procedure for the synthesis of DIIODIDA was performed in order to improve the reaction yield.[6] Its clinical application could be possible after kit preparation, *i.e.,* the determination of the best conditions for the preparation of the ^{99m}Tc-DIIODIDA complex. From a chemical point of view, DIIODIDA represents a protolyte, with 4 potentially ionizable groups. Data on the acid-base equlibria of DIIODIDA, *i.e.*, the knowledge on the distribution of the equilibrium species, especially within the physiological pH range, is of great importance because it enables the biological behaviour of the radiopharmaceutical to be understood and the conditions for its labeling with ^{99m}Tc to be defined. This prompted us to determine the acidity constants of DIIODIDA and, hence, define the conditions for the labeling this compound with technetium-99m, in order to obtain a radiopharmaceutical of high radiochemical purity, which is a prerequisite for its clinical application.

EXPERIMENTAL

Apparatus

The pH values were determined on a pH meter PHM-82 with a combined GK 2401B electrode (Radiometer, Copenhagen, Denmark). The electrode was calibrated using standard buffer solutions, pH 4.01, 7.00 and 9.18 (Radiometer). The titrations were performed with a TTT-80 titrator and an ABU-80 autoburette (Radiometer) (with an accuracy of 0.001 cm^3), under a dynamic nitrogen atmosphere. The temperature was kept constant within ±0.1 ºC by using a thermostat HAAKE F3 (Karlsruhe, Germany).

The spectrophotometric measurements were performed on a GBC Cintra spectrophotometer (GBC Scientific Equipment Pty Ltd., Dandenong, Australia).

The radioactivities were measured in a Gamma 333 scintillation counter (ICN Tracerlab Belgium).

Chemicals and reagents

The details on synthesis of DIIODIDA were reported previously.[6] The purity of the DIIODIDA was checked by thin layer chromatography, m.p. (224–225 ºC) and elemental analysis (deviations

related to the calculated values was ±0.10 %). The solution of Na $^{99m}TcO_4$ was obtained from a $^{99}Mo/^{99m}Tc$-generator by eluating with 0.95 % NaCl (Vinča Institute of Nuclear Sciences, Serbia and Montenegro). All other chemicals (HCl, NaOH, NaCl and $SnCl_2 \cdot H_2O$) used in this study were analytical grade purity (Merck, Darmstadt, Germany). All solutions were prepared with double-distilled water.

Standardization of the HCl and NaOH solutions was performed potentiometrically. The NaOH solution was standardized with potassium hydogenphthalate. The standardized NaOH solution was used for the standardizacion of the HCl solution.

Determination of the constants K_2, K_3 *and* K_4

The acidity constants K_2, K_3 and K_4 were determined at a constant ionic strength of 0.1 mol/dm^3 (NaCl) at 25.0±0.1 ºC. A solution of DIIODIDA (5.0×10^{-4} mol/dm^3) was prepared in 0.1 mol/dm^3 NaCl, and 25 cm^3 aliquots were titrated with the standard HCl solution (0.1073 mol/dm^3) for the determination of the K_2 constant, or with the standard NaOH solution (0.05688 mol/dm^3) for the determination of the constants K_3 and K_4.

The value of $\bar{n}$ was calculated from the experimental data obtained by potentiometric titration of DIIODIDA according to the equation:

$$\bar{n} = \frac{3c_{\text{DIIODIDA}} + c_{\text{HCl}} - c_{NaOH} - [\text{H}_3\text{O}^+] + [\text{OH}^-]}{c_{\text{DIIODIDA}}} \quad (1)$$

where c_{DIIODIDA}, c_{HCl} and c_{NaOH} correspond to the stoichiometric concentrations of DIIODIDA, HCl and NaOH, respectively; $[H_3O^+]$ represents the equilibrium concentration of hydronium ion obtained from the pH measurements; $[OH^-]$ is the concentration of hydroxide ions. The measured pH values within the range from 2 to 12 were converted into pc_{H} values according to the relation:[7] p$c_{\text{H}} = -\log [H_3O^+] = \text{pH} - \text{A}$, where A represents a correction factor (0.05) obtained by titration of the standard HCl solution with the standard NaOH solution at 25 ± 0.1 ºC and ionic strength of 0.1 mol/dm^3 (NaCl). The ionic product of water, pK_{w} = 13.84 was determined by applying a previously procedure reported.[7]

Determination of equilibrium constants in a heterogeneous system

Saturated solutions within the pc_{H} range from 0 to 1 were obtained by treating an excess of solid DIIODIDA with a standard HCl solution (1.062 mol/dm^3), and a constant ionic strength of 1 mol/dm^3 was maintained by adding NaCl. The pc_{H} values were calculated according to the HCl concentrations. The samples were thermostated at 25.0±0.1 ºC with intensive stirring until equilibrium was established (4 h). The two phases were separated by filtration. Aliquots of the filtrate were diluted and the final concentration of HCl was adjusted to 0.1 mol/dm^3. The concentration of DIIODIDA in those solutions was determined spectrophotometrically at the absorption maximum of λ = 227 nm against 0.1 mol/dm^3 HCl as the blank. The Beer law had previously been verified for the concentration range of DIIDIDA $3\times10^{-6} - 3\times10^{-5}$ mol/dm^3.

Labeling with technetium-99m

Preparation of ^{99m}*Tc-DIIODIDA*. To a solution of 25 mg of DIIODIDA dissolved in 0.25 cm^3 1 mol/dm^3 NaOH, were added 3 cm^3 of water and 0.02 cm^3 of $SnCl_2 \cdot H_2O$ in 0.5 mol/dm^3 HCl (11 mg/cm^3). The pH was adjusted to 5.5–7.5 by the addition of an appropriate volume of a HCl or NaOH solution. To this solution, $Na^{99m}TcO_4$ (about 18 MBq) was added, making a final volume of 4 cm^3. The radiochemical purity was determined after 15 min.[5]

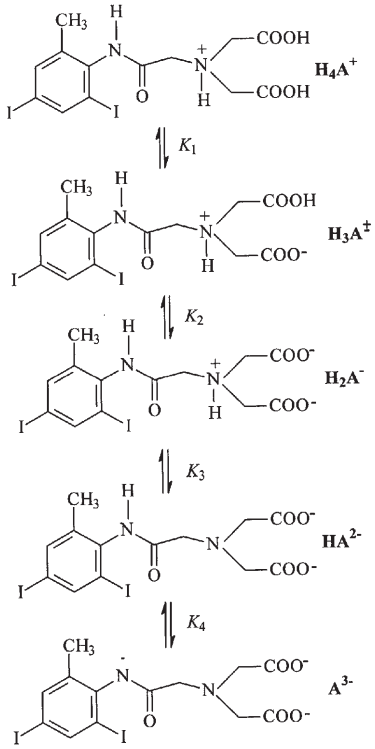
Determination of the radiochemical purity ^{99m}*Tc-DIIODIDA*. The radiochemical purity and the labeling yield were determined by instant thin-layer chromatography (ITLC).[8] ^{99m}Tc-DIIODIDA was separated from $^{99m}TcO_4^-$ using an ITLC SA strip with 2 mol/dm^3 NaCl as the mobile phase (R_{f} values of ^{99m}Tc-DIIODIDA and $^{99m}TcO_4^-$ were zero and 1.0, respectively). Hydro-

lysed ^{99m}Tc and ^{99m}Tc-DIIODIDA were separated using an ITLC SG strip with 80 % CH_3OH as the mobile phase (R_f values of (^{99m}Tc-DIIODIDA + $^{99m}TcO_4^-$) and hydrolysed ^{99m}Tc were 0.85 and zero, respectively).

RESULTS AND DISCUSSION

Determination of acidity constants

Considering that there are three acidic centers (two carboxylic groups and one amide group) and one basic center (amino group) in the DIIODIDA molecule, DIIODIDA undergoes complex acid-base equilibria in aqueous media (Scheme 1).



Scheme 1.

The neutral form of DIIODIDA (H_3A) rearranges spontaneously to the zwitterion ($H_3A^{\pm}$), due to the protolysis of the first carboxylic group and proton acceptance of the amino group. The protolysis of the second carboxylic, amino and amide groups afford H_2A^-, HA^{2-} and A^{3-}, respectively. In more acidic media, the H_4A^+ form exists, as well. The corresponding equilibrium constants are defined as follows:

$$K_1 = \frac{[H_3A^{\pm}][H_3O^+]}{[H_4A^+]} \tag{2}$$

$$K_2 = \frac{[H_2A^-][H_3O^+]}{[H_3A^\pm]} \tag{3}$$

$$K_3 = \frac{[HA^{2-}][H_3O^+]}{[H_2A^-]} \tag{4}$$

$$K_4 = \frac{[A^{3-}][H_3O^+]}{[HA^{2-}]} \tag{5}$$

The acidity constants K_2, K_3 and K_4 were determined potentiometrically by application of the formation function method ($\bar{n}$), *i.e.,* the average number of bound protons:[9]

$$\bar{n} = \frac{\sum_{1}^{n} n[H_nA]}{\sum_{0}^{n} [H_nA]} \tag{6}$$

(The charges in Eq. (6) are omitted for sake of simplicity).

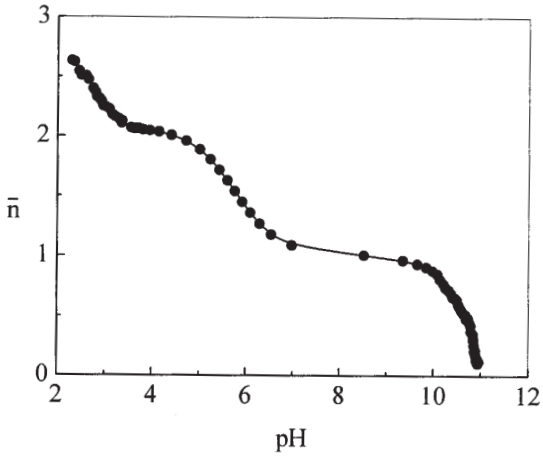


Fig. 1. Dependence of the formation function ($\bar{n}$) on pH.

The dependence of the experimentally determined function of formation ($\bar{n}$) on the pH value of the solution, (Eq. (1)), is shown in Fig. 1. The shape of the curve shows that at pH > 2, DIIODIDA participates in three separate acid-base processes ($\bar{n}$ within the ranges 3–2, 2–1 and 1–0). These three processes correspond to the K_2, K_3 and K_4 constants, while the process corresponding to the K_1 constant requires more acidic media, *i.e.,* pH values under 2.[10,11] Taking into account the fact that the acid-base processes corresponding to the K_2, K_3 and K_4 constants are separate ones, it was possible to determine their values independently of each other within the three

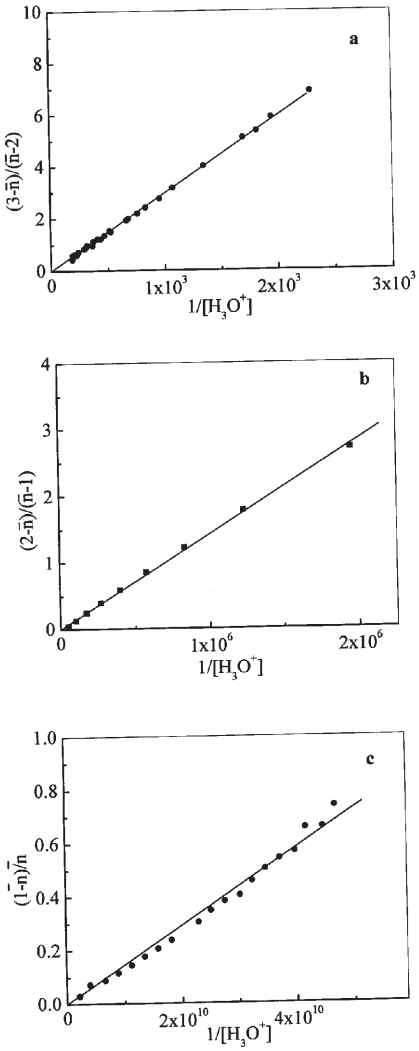


Fig. 2. Determination of DIIODIDA acidity constants K_2, K_3 and K_4 by applying equations: a) (7) K_2, b) (8) K_3, c) (9) K_4.

different pH ranges, each characterized by the dominance of only one acid-base pair species. The linear relationships that served to calculate the above constants are listed in Table I. These linear relationships were obtained on the basis of the theoretically defined function of formation ($\bar{n}$) for each individual pH range and the corresponding equation for the acidity constant (Eqs. (3)–(5)). By using Eqs. (7), (8) and (9) and the experimentally obtained $\bar{n}$ values within different pH ranges, the K_2, K_3 and K_4 constants were calculated from the slopes of linear relationships (Fig. 2). The obtained values of the constants are listed in Table II.

Bearing in mind that the acid-base process in which the first carboxylic group

of DIIODIDA participates occurs at pH values below 2, where DIIODIDA is only slightly soluble in water, it was impossible to directly determine the constant K_1 using pH-metrical titrations. Also, the classical spectrophotometric method could not be applied since no significant changes in the absorption spectra of DIIODIDA within the pH range from 0 to 4 were observed. Hence, the constant K_1 was determined indirectly, on the basis of equilibrium constants obtained in a heterogeneous system. In the solid state, DIIODIDA crystallized in its zwitterionic form, in a similar way to HIDA (2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid).[11,12] Since the zwitter ion of DIIODIDA is the least soluble[6,12] in aqueous solutions at pH<1, the following equilibria are possible between the solid phase, $(H_3A^{\pm})_s$, and the ions present in solution:

$$(H_3A^{\pm})_s \rightleftharpoons H_3A^{\pm} \qquad K_{s0} = [H_3A^{\pm}] \tag{10}$$

$$(H_3A^{\pm})_s + H_3O^{+} \rightleftharpoons H_4A^{+} + H_2O \qquad K_{s1} = \frac{[H_4A^{+}]}{[H_3O^{+}]} \tag{11}$$

TABLE I. Survey of the equations for the potentiometric determination the acidity constants K_2, K_3 and K_4 of DIIODIDA, obtained by linearizing the $\bar{n}$–pH curve

Constant	pH range	Dominant species	Formation function (theoretically defined)	Linear relationship	Equation
K_2	2.3 – 3.5	$H_3A^{\pm}$; H_2A^{-}	$\bar{n} = \frac{3[H_3A^{\pm}] + 2[H_2A^{-}]}{[H_3A^{\pm}] + [H_2A^{-}]}$	$\frac{3-\bar{n}}{\bar{n}-2} = K_2\frac{1}{[H_3O^{+}]}$	(7)
K_3	4.7 – 6.8	H_2A^{-}; HA^{2-}	$\bar{n} = \frac{2[H_2A^{-}] + [HA^{2-}]}{[H_2A^{-}] + [HA^{2-}]}$	$\frac{2-\bar{n}}{\bar{n}-1} = K_3\frac{1}{[H_3O^{+}]}$	(8)
K_4	9.2 – 11.0	HA^{2-}; A^{3-}	$\bar{n} = \frac{[HA^{2-}]}{[HA^{2-}] + [A^{3-}]}$	$\frac{1-\bar{n}}{\bar{n}} = K_4\frac{1}{[H_3O^{+}]}$	(9)

TABLE II. The equilibrium constants determined in homogeneous and heterogeneous systems of DIIODIDA

Constants	Value*
pK_1	1.3 ± 0.4
pK_2	2.52 ± 0.02
pK_3	5.86 ± 0.06
pK_4	10.9 ± 0.1
pK_{s0}	5.0 ± 0.4
pK_{s1}	3.69 ± 0.04

*Mean ± standard deviation (n = 4)

Hence, the following relationship between the acidity constant K_1 and the equilibrium constants in a heterogeneous system exists:

$$K_1 = \frac{K_{s0}}{K_{s1}} \tag{12}$$

The constants K_{s0} and K_{s1} were determined spectrophotometrically using the method of solubilility.[7] The solubility (S) of DIIODIDA at pH<1, where $H_3A^{\pm}$ and H_4A^+ forms are dominant, can be presented by the following expression:

$$S = H_3A^{\pm} + H_4A^+ \tag{13}$$

From Eqs. (10), (11) and (13), a linear relationship was obtained:

$$S = K_{s0} + K_{s1}\,[H_3O^+] \tag{14}$$

On the basis of the experimentally determined solubility of DIIODIDA in solutions of HCl (pc_H 0–1) and Eq. (14), by applying linear regression analysis, the constants K_{s0} (intercept) and K_{s1} (slope) were calculated (Fig. 3). Based on the obtained values for the constants in a heterogeneous system, the constant K_1 was calculated by applying Eq. (12).

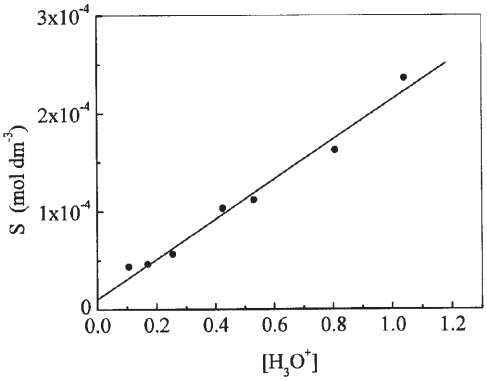


Fig. 3. Solubility of DIIODIDA (S) as a function of the hydronium ion (H_3O^+) concentration.

The values of all the determined equilibrium constants of DIIODIDA are given in Table II. The constants K_2, K_3 and K_4 were determined at a constant ionic strength of 0.1 mol/dm^3 (NaCl), while K_1 was determined at 1 mol/dm^3 (HCl and NaCl). The large value of the standard deviation of pK_1 could be explained by the insufficiently reliable determination of the pK in a strongly acidic medium. The obtained values for the acidity constants of DIIODIDA are in accordance to the values for monohalogenic IDA analogues, 4-bromophenylcarbamoylmethyl iminodiacetic acid (pK_1 1.6, pK_2 2.2, pK_3 5.9)[13] and 4-iodo-2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid (pK_1 1.7, pK_2 2.44, pK_3 6.29, pK_4 10.91).[11]

On the basis of the determined acidity constants of DIIODIDA, the distribution of the equilibrium species was calculated according to the following equations:

$$\chi_{H_4A^+} = \frac{[H^+]^2 K_1^{-1} K_2^{-1}}{1 + [H^+]^2 K_1^{-1} K_2^{-1} + [H^+] K_2^{-1} + [H^+]^{-1} K_3 + [H^+]^{-2} K_3 K_4} \tag{15}$$

$$\chi_{H_3A^{\pm}} = \frac{[H^+]K_2^{-1}}{1+[H^+]^2K_1^{-1}K_2^{-1}+[H^+]K_2^{-1}+[H^+]^{-1}K_3+[H^+]^{-2}K_3K_4} \quad (16)$$

$$\chi_{H_2A^-} = \frac{1}{1+[H^+]^2K_1^{-1}K_2^{-1}+[H^+]K_2^{-1}+[H^+]^{-1}K_3+[H^+]^{-2}K_3K_4} \quad (17)$$

$$\chi_{HA^{2-}} = \frac{[H^+]^{-1}K_3}{1+[H^+]^2K_1^{-1}K_2^{-1}+[H^+]K_2^{-1}+[H^+]^{-1}K_3+[H^+]^{-2}K_3K_4} \quad (18)$$

$$\chi_{A^{3-}} = \frac{[H^+]^{-2}K_3K_4}{1+[H^+]^2K_1^{-1}K_2^{-1}+[H^+]K_2^{-1}+[H^+]^{-1}K_3+[H^+]^{-2}K_3K_4} \quad (19)$$

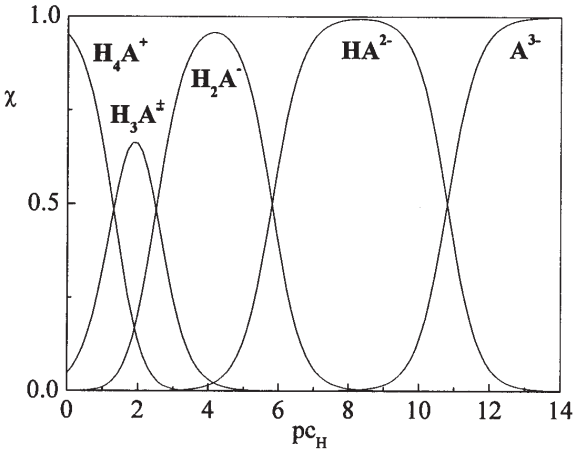


Fig. 4. The distribution of the ionic forms of DIIODIDA as a function of pc_H.

The corresponding distribution diagram of DIIODIDA is presented in Fig. 4.

Labeling with technetium-99m

TABLE III. The influence of pH on the labeling yield of ^{99m}Tc-DIIODIDA

pH	^{99m}Tc-DIIODIDA/%	Hydrolysed ^{99m}Tc/%	$^{99m}TcO_4^-$ /%
5.5	98.6	0.8	0.6
5.9	99.0	0.6	0.4
6.5	97.9	1.4	0.7
7.5	90.7	7.6	1.7

The labeling of IDA analogues with Tc-99m is a complex formation reaction. Tc-IDA analogues applied as hepatobiliary imaging agents represent Tc(III) chelates with a 1:2 mole ratio, where each ligand is coordinated to the Tc(III)- center through one imino nitrogen and two carboxylate oxygens.[14–16] These compounds

could be prepared by reaction of the ligand with reduced Tc-pertechnetate or by ligand substitution in a precursor, *e.g.* $[Tc(tu)_6]^{3+}$ (hexakis(thiourea) technetium(III) complex.[17,18] During this substitution, decomposition of the precursor $[Tc(tu)_6]^{3+}$ and/or of the main product (Tc-complex) results in an increased formation of TcO_2 as a radiochemical impurity (about 40 %).[16] According to the European Pharmacopoeia 4,[19] not less than 95.0 per cent of the radioactivity must correspond to technetium-99m complexed with IDA analogue. Bearing in mind all the above mentioned facts, DIIODIDA was labeled with ^{99m}Tc-pertechnetate using Sn(II) ions as the reductant within the pH range in which the dianion of DIIODIDA (HA^{2-}) is the dominant ionic species, as was determined from the distribution diagram (Fig. 4). The influence of pH on the labeling yield of ^{99m}Tc-DIIODIDA is presented in Table III. The results obtained by ITLC demonstrated that the ^{99m}Tc-DIIODIDA prepared within the pH range from 5.5 to 6.5 was of high radiochemical purity (> 98 %), while a further increase of the pH led to a decrease in the labeling yield. This may be explained by the fact that the reaction of this complex formation is followed by the competitive hydrolysis reaction of technetium and Sn(II) ions, resulting in the formation of various hydrolytic complexes as radiochemical impurities, particularly at higher pH values.[20]

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ИЗВОД

КИСЕЛИНСКО-БАЗНЕ РАВНОТЕЖЕ 2,4-ДИЈОД-6-МЕТИЛФЕНИЛКАРБАМОИЛМЕТИЛ ИМИНОДИСИРЋЕТНЕ КИСЕЛИНЕ И ОБЕЛЕЖАВАЊЕ ТЕХНЕЦИЈУМОМ-99m

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Испитане су киселинско-базне равнотеже новог хепатобилијарног агенса, 2,4-дијод-6-метилфенилкарбамоилметил иминодисирћетне киселине. Киселинске константе које одговарају другој карбоксилној групи, амино и амидној групи одређене су потенциометријски: pK_2 = 2,52 ± 0,02; pK_3 = 5,86± 0,06; pK_4 = 10,9 ± 0,1. Одређивања су изведена на 25 ºC и при константној јонској сили 0,1 mol/dm^3 (NaCl). Киселинска константа прве карбоксилне групе (pK_1 = 1,3±0,4) одређена је индиректно на основу равнотежних константи одређених у хетерогеном систему, на 25 ºC и јонској сили 1 mol/dm^3 (HCl, NaCl). DIIODIDA је обележена технецијумом-99m и испитан је утицај pH на принос обележавања. Утврђено је да се обележавањем у pH опсегу 5,5–6,5 добија радиофармацеутик високе радиохемијске чистоће (> 98 %).

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REFERENCES

1. G. Galli, C. L. Maini, *Technetium in Chemistry and Nuclear Medicine 2*, Cortina International, Verona, Raven Press, New York, 1986, p. 309

2. C. Aprile, U. Prati, R. Saponaro, M. Carena, T. Cebrelli, C. Tibaldeschi, *Technetium in Chemistry and Nuclear Medicine 2*, Cortina International, Verona, Raven Press, New York, 1986, p. 265
3. M. Jovanović, J. Brborić, S. Vladimirov, Lj. Suturkova, *J. Radional. Nucl. Chem.* **245** (2000) 555
4. R. Schwarzrock, J. Kotzerke, H. Hundeshagen, K. Böcker, B. Ringe, *Eur. J. Nucl. Med.* **12** (1986) 346
5. M. Jovanović, J. Brborić, S. Vladimirov, B. Zmbova, Lj. Vuksanović, D. Živanov-Stakić, V. Obradović, *J. Radioanal. Nucl. Chem.* **240** (1999) 321
6. J. S. Brborić, S. Vladimirov, M. S. Jovanović, N. Dogović, *Monatsh. Chem.* **135** (2004) 1009
7. L. B. Pfendt, D. M. Sladić, T. J. Janjić, G. Popović, *Analyst* **115** (1990) 383
8. V. Jovanović, T. Maksin, M. Jovanović, J. Bzenić, N. Terzić, *J. Radioanal. Nucl. Chem.* **98** (1986) 141
9. H. Rossotti, *The Study of Ionic Equilibria*, Longman Group Limited, London, 1978, p. 36
10. L. Haggman, C. Lindblad, H. Oskarsson, A. S. Ullstrom, I. Persson, *J. Am. Chem. Soc.* **125** (2003) 3631
11. M. S. Jovanović, G. Popović, V. Kapetanović, M. Orlić, S. Vladimirov, *J. Pharm. Biomed. Anal.* **35** (2004) 1257
12. B. Ribar, Cs. Meszaros, S. Vladimirov, D. Živanov-Stakić, M. Jovanović, B. Zmbova, P. Engel, *Struct. Chem.* **6** (1995) 119
13. G. Shtacher, *J. Inorg. Nucl. Chem.* **28** (1966) 845
14. C. E. Costello, J. W. Brodack, A. G. Jones, A. Davison, D. J. Johnson, S. Kasina, A. R. Fritzberg, *J. Nucl. Med.* **24** (1983) 353
15. M. D. Loberg, A. T. Fields, *Int. J. Appl. Radiat. Isotopes* **29** (1978) 167
16. P. S. Lundberg, L. Jacobsson, G. Stenhagen, J. Martensson, M. Fjalling, *Nucl. Med. Biol.* **22** (1995) 521
17. R. Gonzalez, C. Kremer, R. Chiozzone, J. Torres, M. Rivero, A. Leon, E. Kremer, *Radiochim. Acta* **81** (1998) 207
18. J. Torres, R. Gonzalez, C. Kremer, A. Leon, E. Kremer, *Radiochim. Acta* **77** (1997) 235
19. *European Pharmacopoeia*, 4th ed., Radiopharmaceutical Preparations, Council of Europe, Strasbourg, 2002, p. 2342
20. R. S. Tobias, *Acta Chem. Scand.* **12** (1958) 198.