Short communication

Determination of the ionization constants of 4-iodo-2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid

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Abstract

In order to develop a radiopharmaceutical for hepatobiliary scintigraphy with better hepatobiliary properties new ligand for complexation of ^{99m}Tc, 4-iodo-2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid (METHYLIODIDA), was synthesized. Acid–base equilibria of METHYLIODIDA were studied potentiometrically, because these data are important for determination of complex formation conditions. It was established that METHYLIODIDA undergoes a complex acid–base equilibrium due to its zwitterionic nature and four proton-binding sites. The stoichiometric ionization constants were determined at 25 °C and constant ionic strength 0.1 M (NaClO$_4$): pK_1 = 1.7 ± 0.1; pK_2 = 2.44 ± 0.07; pK_3 = 6.29 ± 0.02 and pK_4 = 10.91 ± 0.06, respectively.
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1. Introduction

The derivatives of iminodiacetic acid (IDA) labeled with technetium-99m are used in nuclear medicine for hepatobiliary scintigraphy. Biological properties of ^{99m}Tc-IDA derivatives are determined by chemical structure, lipophilicity, protein binding [1]. Biokinetics of these radiopharmaceuticals depends on the blood level of bilirubin, thus the hyperbilirubinemia decreases hepatic clearance and biliary excretion and increases urinary excretion and represents the restricting factor in application of IDA derivatives as hepatobiliary imaging agents [2–4]. In order to develop a new radiopharmaceutical with better hepatobiliary properties and larger tolerance on bilirubin 4-iodo-2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid (METHYLIODIDA), a ligand for complexation of the ^{99m}Tc was synthesized. The conditions of METHYLIODIDA-Tc-99m complex formation and an understanding of its structure depend on physicochemical properties of ligand, particularly on its ionization degree expressed by the pK values. Having in mind the above arguments as well as the fact that METHYLIODIDA is newly synthesized compound it was of interest to determine its ionization constants.

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2. Experimental

2.1. Apparatus and reagents

The following apparatus were used: a PHM-82 pH meter with a glass–calomel electrode assembly; titrator TTT-80 with an ABU-80 autoburette (Radiometer); PERKIN-ELMER 467 IR spectrophotometer; BRUKNER-NMR.

The most of reagents were from Merck, except chloroacetylchloride and iminodiacetic acid from Fluka and 2,6-dimethylaniline from Aldrich, and were of analytical reagent grade quality.

2.2. Synthesis of 4-iodo-2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid (METHYLIODIDA)

2.2.1. Phase I: synthesis of 4-iodo-2,6-dimethylaniline

The iodination reaction involves electrophilic substitution of 2,6-dimethylaniline (0.4 mol, 48.4 g) with iodine (0.4 mol, 101.2 g) in alkaline media (0.6 mol, 56 g NaHCO₃) at 12–15 °C for 3 h. 4-Iodo-2,6-dimethylaniline was separated by filtration in vacuum, dried and crystallized from ethanol (mp 48–49 °C).

2.2.2. Phase II: synthesis of 4-iodo-2,6-dimethylchloroacetanilide

To the solution of 4-iodo-2,6-dimethylaniline (0.16 mol, 40 g) in acetone (100 ml) was added chloroacetylchloride (20 ml), and mixture was cooled at 4 °C with constant stirring on a magnetic stirrer for 3 h. To this solutions, 8% HCl (200 ml) was added and stirred for 30 min. Solid mass of 4-iodo-2,6-dimethyl-chloroacetanilide was separated by filtration in vacuum, dried and crystallized from 80% ethanol (mp 174–176 °C).

2.2.3. Phase III: synthesis of 4-iodo-2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid

The condensation involves a reaction between equimolar amounts of obtained 4-iodo-2,6-dimethyl-chloroacetanilide with iminodiacetic acid in presence of Na₂CO₃ in ethanol solution (75%). After refluxion at 86 °C during 10 h, METHYLIODIDA

was precipitated at pH 2.25 with 5 M HCl, separated by filtration in vacuum, dried and crystallized from absolute ethanol (mp 207–209 °C).

The chemical purity of synthesized compound was investigated by TLC method on the silica-gel GF$_{254}$ plates (Merck) with chloroform as mobile phase. After development of chromatogram, the plates were exposed to ultraviolet light at 254 nm and noticed only one spot corresponding to METHYLIODIDA with Rf = 0.74.

2.3. Potentiometric investigations

2.3.1. Solutions

METHYLIODIDA of 8×10^{-4} M, 0.1000 M NaOH and 0.8820 M HClO₄ were used. Standardization of NaOH and HClO₄ was performed potentiometrically. The ionic strength was kept constant by addition of standardized NaClO₄ (5.8769 M).

2.3.2. Determination of ionization constants

Aliquots of 25 ml solution of METHYLIODIDA (8×10^{-4} M) were titrated with NaOH for determination of K_3 and K_4. For determination of K_1 and K_2 aliquots containing 8×10^{-4} M METHYLIODIDA and 2.8134×10^{-3} M HClO₄ were used. The titrations were carried out at 25 °C, at constant values of ionic strength 0.1 M NaClO₄. The conversion of measured pH value into pc$_H$ for $I = 0.1$ M was done by using the following relation [5]: pc$_H = -\log[\mathrm{H_3O^+}] =$ pH + 0.05.

3. Results and discussion

The iodination methods of aromatic compounds described in literature have same disadvantages: long time and high temperature of the reaction [6], the application of concentrated acid [7,8], secondary reaction of formation of mixture o- and p- isomers [9,10], low yield of reaction [11]. The proposed iodination method of 2,6-dimethylaniline (phase I of synthesis of METHYLIODIDA) compared to other is rather simple, faster and gives high yield (81%).

METHYLIODIDA was identified by IR and ^{1}H NMR spectroscopy and elemental analysis. IR (KBr pellet) (cm^{-1}): 3300 (νN–H), 3021 (ν arom. C–H), 1675 (νNH–CO), 1541 (νCOO–), 862 (tetrasub.

benzene). ^{1}H NMR (in DMSO-d_6, TMS as internal standard) (in ppm): 2.10 (s, 2CH$_3$), 3.16 (br.s, –NH), 3.49 (br.s, –N–CH$_2$–COO–), 3.52 (br.s, –N–CH$_2$COO–), 7.46 (s, arom. 2C–H), 9.51 (s, –COOH). Elemental analysis (C$_{14}$H$_{17}$N$_2$O$_5$I): calculated (%) C (39.99), H (4.09), N (6.67); found (%) C (39.79), H (4.02), N (6.69).

In aqueous media of METHYLIODIDA undergoes a complex acid–base equilibrium:
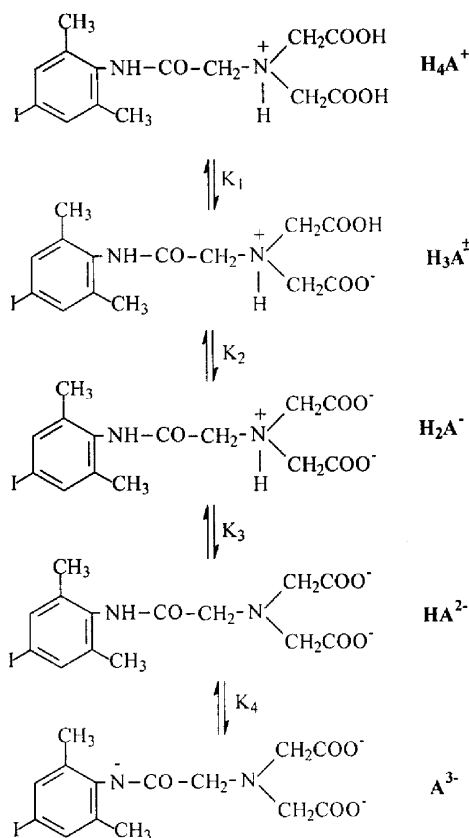
The neutral nonionic form of METHYLIODIDA is rearranged spontaneously to the zwitterion (H$_3$A$^{\pm}$) due to the protolysis of the first carboxylic group and proton acceptance of the amino group. The protolysis of the second carboxylic, amino and amide groups results in H$_2$A$^-$, HA^{2-} and A^{3-}. In more acidic media H$_4$A$^+$ form exists. The corresponding equilibrium constants are as follows:

$$K_1 = \frac{[\text{H}_3\text{A}^{\pm}][\text{H}^+]}{[\text{H}_4\text{A}^+]} \tag{1}$$

$$K_2 = \frac{[\text{H}_2\text{A}^-][\text{H}^+]}{[\text{H}_3\text{A}^{\pm}]} \tag{2}$$

$$K_3 = \frac{[\text{HA}^{2-}][\text{H}^+]}{[\text{H}_2\text{A}^-]} \tag{3}$$

$$K_4 = \frac{[\text{A}^{3-}][\text{H}^+]}{[\text{HA}^{2-}]} \tag{4}$$

Potentiometric determination of the ionization constants was performed by application of the formation function method [12], which is based on the determination of $\bar{n}$, i.e. the average number of bound protons:

$$\bar{n} = \frac{\sum_0^n n[\text{H}_n\text{A}]}{\sum_0^n [\text{H}_n\text{A}]} \tag{5}$$

The calculation of the $\bar{n}$ from experimental data of potentiometric titration was done according to the equation:

$$\bar{n} = \frac{3c_{\text{MI}} + c_{\text{HClO}_4} - c_{\text{NaOH}} - [\text{H}^+] + [\text{OH}^-]}{c_{\text{MI}}} \tag{6}$$

where c_{MI}, c_{HClO_4} and c_{NaOH} correspond to the stoichiometric concentration of METHYLIODIDA, HClO$_4$ and NaOH, respectively, and [H$^+$] and [OH$^-$] are the equilibrium concentration of hydrogen and hydroxyl ions obtained from pH measurements. The dependence of formation function, $\bar{n}$, versus pH is shown in Fig. 1. The shape of the curve indicates that the acid–base processes of METHYLIODIDA are partly separated. Namely, the acid–base processes which correspond to K_1 and K_2 are overlapping, and the other two processes corresponding to K_3 and K_4
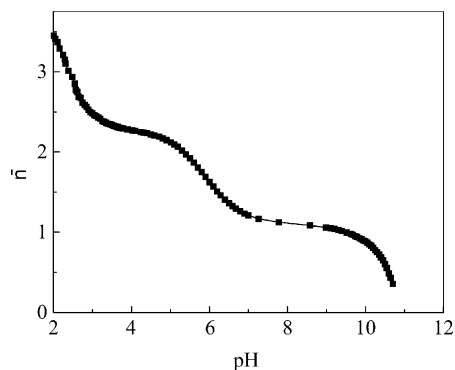

Fig. 1. Formation function dependence on pH.

are completely separated. From this reason determination of ionization constants was performed in three different pH ranges.

The constants K_1 and K_2 were determined in pH range 2–4, where the following acid base pairs H_4A^+–$H_3A^{\pm}$ and $H_3A^{\pm}$–H_2A^- are dominant. On the basis of the formation function in this pH range and Eqs. (1) and (2) the following linear dependence is obtained:

$$\underbrace{\frac{\bar{n}-3}{\bar{n}-2}[\mathrm{H}^+]}_{y} = -K_2 + \frac{1}{K_1}\underbrace{[\mathrm{H}^+]^2\frac{4-\bar{n}}{\bar{n}-2}}_{x} \quad (7)$$

By applying the linear regression analysis, constants K_1 (slope) and K_2 (intercept) were calculated.

In pH range 5–7, H_2A^- and HA^{2-} forms are dominant. By combination of Eqs. (3) and (5) the linear dependence is obtained:

$$\underbrace{\frac{2-\bar{n}}{\bar{n}-1}}_{y} = K_3\underbrace{\frac{1}{[\mathrm{H}^+]}}_{x} \quad (8)$$

Determination of K_4 was performed in pH range 10–12, where the acid–base pairs $HA^{2-} - A^{3-}$ are dominant. From Eqs. (4) and (5) the following dependence is obtained:

$$\underbrace{\frac{1-\bar{n}}{\bar{n}}}_{y} = K_4\underbrace{\frac{1}{[\mathrm{H}^+]}}_{x} \quad (9)$$

Constants of K_3 and K_4 were calculated from the slopes of linear dependence given by Eqs. (8) and (9) by applying the regression analysis.

The stoichiometric ionization constants are presented in Table 1. On the basis of the determined ionization constants, the relative concentrations of the equilibrium species depended on $[\mathrm{H}^+]$ were calculated according to the following equations:

Table 1
Stoichiometric ionization constants of METHYLIODIDA ($\mathrm{p}\bar{K} \pm s^a$); $t = 25\,^\circ\mathrm{C}$; $I = 0.1\,\mathrm{M}$ NaClO$_4$

$\mathrm{p}K_1$	1.7 ± 0.1
$\mathrm{p}K_2$	2.44 ± 0.07
$\mathrm{p}K_3$	6.29 ± 0.02
$\mathrm{p}K_4$	10.91 ± 0.06

[a] $n = 5$.
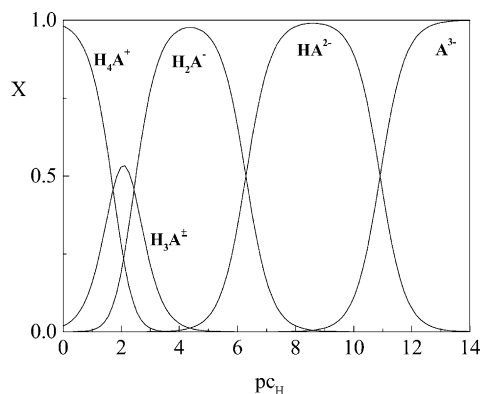
Fig. 2. The distribution diagram of METHYLIODIDA.

$$\chi_{\mathrm{H_4A^+}} = \frac{[\mathrm{H}^+]^2}{[\mathrm{H}^+]^2 + K_1[\mathrm{H}^+] + K_1K_2} \quad (10)$$

$$\chi_{\mathrm{H_3A^{\pm}}} = \frac{K_1[\mathrm{H}^+]}{[\mathrm{H}^+]^2 + K_1[\mathrm{H}^+] + K_1K_2} \quad (11)$$

$$\chi_{\mathrm{H_2A^-}} = \frac{K_2[\mathrm{H}^+]}{[\mathrm{H}^+]^2 + K_2[\mathrm{H}^+] + K_2K_3} \quad (12)$$

$$\chi_{\mathrm{HA^{2-}}} = \frac{K_3[\mathrm{H}^+]}{[\mathrm{H}^+]^2 + K_3[\mathrm{H}^+] + K_3K_4} \quad (13)$$

$$\chi_{\mathrm{A^{3-}}} = \frac{K_3K_4}{[\mathrm{H}^+]^2 + K_3[\mathrm{H}^+] + K_3K_4} \quad (14)$$

The corresponding distribution diagram of METHYLIODIDA is presented in Fig. 2.

METHYLIODIDA is precipitated at pH 2.25 (as cited in Section 2). Regarded to the distribution diagram, maximum concentration of the zwitterion is at that pH approximately, so it can be expected that METHYLIODIDA crystallized as a zwitterionic form. This consideration is supported by X-ray diffraction determination of crystal structures, of 2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid and its monomethylester, structurally similar compounds obtained in the same manner which crystallized as zwitterionic form [13].

According to the literature, iminodiacetic acid has $\mathrm{p}K_1$ and $\mathrm{p}K_2$ values, which correspond to the first and the second carboxylic group, in the range from 1.82 to 2.58 and $\mathrm{p}K_3$ (amino group) from 9.30 to 9.60 [14]. Substitution of hydrogen atom of amino group of IDA with short alkyl chain has no influence on $\mathrm{p}K_1$

Table 2
Ionization constants of some *N*-substituted iminodiacetic acids

Compound	R	pK_1	pK_2	pK_3
1	CH_3-	1.82	2.22	9.59
2	$n\text{-}C_6H_{13}-$	1.55	2.37	10.41
3	$n\text{-}C_{18}H_{37}-$		5.64–7.61	9.4–10.65
4	$4\text{-}BrC_6H_4NHCOCH_2-$	1.60	2.20	5.90
5	NH_2COCH_2-		2.30	6.60
6	$2,6\text{-}(CH)_3C_6H_3NHCOCH_2-$		2.70	6.20

and pK_2, but substitution with long alkyl chain has a great influence on these values [15] (Table 2) due to aggregation. The consequences of this are: the first ionization constant is very acidic and its value could not be determined and the second ionization constant is substantially less acidic $pK_2 = 5.5$–7.5. The acidic properties of the ammonium group are fairly independent of the state of aggregation ($pK_3 = 9.4$–10.6) [15]. Substitution with other groups substantially influences the protolysis of ammonium group, but does not influence the pK value of carboxylic groups [16–18]. This effect is shown in Table 2 (compounds **4**, **5** and **6**). The values of pK_1 and pK_2 obtained for METHYLIODIDA are in a good accordance to corresponding values in Table 2, except compound **3**. Slight differences between the pK_3 values of compounds **4**, **5**, **6** and METHYLIODIDA are caused by different ($-I$)-effect of substituents bounded to the amino group.

The value obtained for $pK_4 = 10.91$ (amide group) of METHYLIODIDA is in a good correlation with pK values 10.3–11.6, which correspond to deprotonation of amide group bounded to benzene ring as in a case of 1,4-benzodiazepine [19].

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