

COMPLEXATION BEHAVIOUR OF TIN(II) ION WITH DERIVATIVE OF p-AMINOHIPPURIC ACID

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Summary

Complex formation between tin(II) ion and p-(biscarboxymethyl)-aminomethyl-carbamino-hippuric acid (PAHIDA) in 0.5 M NaClO₄ ionic medium at 298 K, was studied by means of u.v. spectrophotometry and glass electrode potentiometric titrations. In the concentration range $0.09 \leq [\text{Sn}] \leq 0.150$, $0.5 \leq [\text{PAHIDA}] \leq 3.0$ (mmol dm⁻³) and $1.30 \leq \text{pH} \leq 6.5$, mathematical analysis of potentiometric data, with the aid of general least-squares programme Superquad, indicates the formation of the following complexes and their respective stability constants ($\log (\beta \pm \sigma)$): [Sn(H₂PAHIDA)] (12.98 ± 0.15), [Sn(HPAHIDA)] (9.38 ± 0.05), [Sn(PAHIDA)] (5.74 ± 0.01) and [Sn(OH)PAHIDA] (1.55 ± 0.09) as well as several pure hydrolytic complexes of tin(II).

The effect of tin(II)-PAHIDA complexation on labelling yield of PAHIDA with ^{99m}TcO₄⁻ was investigated. The optimum yield (90%) was achieved at PAHIDA to tin(II) concentration ratio 30:1 and pH = 5.5.

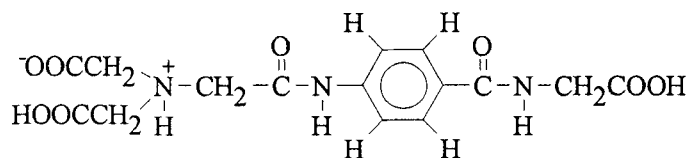
Key words: PAHIDA, Tin(II), Complexation, Tc-99m- PAHIDA labelling

Introduction

PAHIDA, p-[(biscarboxymethyl)-aminomethyl-carboxyamino] hippuric acid (Scheme 1) is a chelating agent whose complexes with ^{99m}Tc core, are widely used as a diagnostic tool

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in nuclear medicine, to provide assessment of renal function, glomerular filtration rate and renal plasma flow¹. The molecule of PAHIDA contains iminodiacetate and hippurate functional groups which could be attached to radionuclide as a pharmaceutical. In the labelling reaction of PAHIDA with ^{99m}Tc various complexes may be formed



Scheme I

depending on the composition of reaction mixture. PAHIDA is labelled with ^{99m}TcO₄⁻ using stannous chloride as a reducing agent. Numerous reactions between these components may occur in solution, *e.g.* complexation of tin(II) with PAHIDA, hydrolysis of tin(II) and Tc, oxidation of tin(II) to tin(IV), *etc.* In order to investigate the labelling behaviour of PAHIDA with pertechnetate these side reactions must be taken into account. The most important of them is complexation between tin(II) and PAHIDA. There is no available literature data on tin(II)-PAHIDA complexes. Therefore, in the present work the complex formation between tin(II) and PAHIDA was studied as a series of glass electrode emf and pH-metric titrations and by u.v. spectrophotometry. Since the final goal is to increase efficiency in the process of Tc-labelling of Sn(II)-PAHIDA complexes, the effect of varying PAHIDA concentrations and pH values on labelling yield was studied by instant thin layer chromatography (TLC) using acetonitrile-water solution as a developing agent.

Experimental

Reagents and Analysis

PAHIDA was synthesized and analyzed as described previously². A stock solution of PAHIDA (5.00 10⁻³ mol dm⁻³) was prepared by dissolving a weighed amount of the acid in doubly distilled water. Stock solution of tin(II) perchlorate was prepared from SnCl₂ · 2 H₂O p.a. (Merck, Darmstadt, F.R.G.) by precipitation with (NH₄)₂CO₃ and by subsequently dissolving the precipitate in 1:1 HClO₄ in the presence of tin metal pellets. The concentration of tin (0.1080 mol dm⁻³) was determined gravimetrically by precipitation with ammonia. The concentration of free HClO₄ in the stock solution of tin(II)-perchlorate was determined by potentiometric titration with standard NaOH, using a Gran's plot. The presence of tin(IV) was checked by pyrocatechol-violet. It was found that its content did not exceed 2%. The stock

solutions of HClO_4 , NaOH , and NaHCO_3 were made and analyzed as previously described³. Buffers for pH-meter calibration were prepared from potassium-hydrogenphthalate p.a., HCl , "suprapure", KH_2PO_4 , and Na_2HPO_4 p.a. (Merck) according to procedure after Perrin and Dempsey⁴. The buffers prepared had the pH values: 7.00, 4.008, 3.00 and 2.00 at 298 K. For instant TLC measurements silicagel impregnated glass fiber sheets (Merck) were utilized.

Instrumentation

U.V. spectra were taken on UVICON 810/820 (Kontron Instruments, Austria) spectrophotometer, in 195 - 350 nm wavelength range, in 10 mm quartz cells (matching pair). The reference cell was filled with corresponding tin(II)-free solutions. For potentiometric titrations pH-meter PHM 85 (Radiometer, Denmark) equipped with GK 2401C combined electrode was used. Precision of potential measurements was ± 0.1 mV and those of pH, ± 0.001 pH units. Titration agent (NaOH or NaHCO_3) was delivered using an automatic burette ABU 80 (Radiometer).

Procedure

Spectral measurements were made on tin(II)-PAHIDA solutions in the pH range 1.0 - 6.0. The pH of the solutions was adjusted with standard HClO_4 or NaOH solutions. Ionic medium was kept constant (0.5 mol dm^{-3}) by the addition of NaClO_4 . Some measurements were made in potassium-chloride medium (0.1 mol dm^{-3}). Concentration ratio of PAHIDA to tin was varied between 2:1 to 10:1, with the total concentration of tin $1 \times 10^{-4} \text{ mol dm}^{-3}$. Spectra of pure tin(II) and PAHIDA solutions, in perchlorate medium, at the same pH values as for complexation studies were also recorded.

Potentiometric titrations were made on test solutions (TS) of general composition: $\text{TS} = M \text{ Sn}^{2+}$, $H \text{ H}^+$, $C \text{ PAHIDA}^3$, $0.5 \text{ mol dm}^{-3} \text{ ClO}_4^-$, where M, H and C denote the total molar concentrations of corresponding species. The concentration of the sodium ion was omitted for simplicity. The electrochemical cell, filled with test solution, was immersed in a paraffin-oil thermostat; constant temperature was maintained to ± 0.1 K. Titrations were performed at 298 K. The nitrogen gas, purified by passing through the series of washing bottles, was bubbled through the cell, during the measurements. The concentration of hydrogen ion was decreased by the addition of standard NaOH (or, in some titrations, NaHCO_3). The concentration of free hydrogen ion, h , at each point of titration curve, was calculated from the measured emf of the cell, E , using the Nernst equation:

$$E = E^0 + Q \log h + E_j \quad (1)$$

where E^0 denotes the constant which includes the standard potential of the glass electrode, Q is the slope of the glass electrode response and E_j is liquid-junction potential, whose

contribution to E was found to be negligible. Potential readings were taken every minute, until stable potentials (within $\pm 0.2 - 0.3$ mV) were obtained (usually after 15 ~ 20 min.). The determination of E^0 and Q and calibration of the cell, in terms of hydrogen ion concentration, was performed as already described³.

To investigate labelling efficiency, the Sn^{2+} -PAHIDA kit was prepared by dissolving various amounts of PAHIDA in 3 mL of solutions containing $1.0 \mu\text{mol SnCl}_2$ in $0.1 \text{ mol dm}^{-3} \text{ HClO}_4$. The pH of solutions was then adjusted with NaOH, to 5.50 ± 0.01 . The solutions were purged with nitrogen for 10 min, filtered through $0.22 \mu\text{m}$ Millipore filter membrane and dispensed into sterilized vials. Each vial was lyophilized and closed under nitrogen atmosphere. Thus, the quantity range of the constituents covered was: $1 \mu\text{mol SnCl}_2$ and 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0 and $45.0 \mu\text{mol PAHIDA}$. The series of solutions with constant concentrations of both, tin and PAHIDA and with varying pH values was also prepared. Different pH values of solutions containing $1 \mu\text{mol SnCl}_2$ and $35 \mu\text{mol PAHIDA}$ were 4.0, 4.5, 5.0, 5.5 and 6.0 (± 0.01). In each vial 1 mL of $1.0 \text{ mCi } ^{99\text{m}}\text{TcO}_4^-$ was injected, followed by filling up to 5.0 mL with normal saline. The labelling efficiency was studied by instant TLC with acetonitrile:water (7:3 vol. %) as a developing agent. Chromatography was performed 15 min. after mixing.

Results

Spectrophotometric measurements

U.V. spectra of solutions containing $1 \times 10^{-4} \text{ mol dm}^{-3} \text{ Sn}^{2+}$ and varying PAHIDA concentration ($2 \times 10^{-4} - 1 \times 10^{-3} \text{ mol dm}^{-3}$) in the pH range 1.0 - 5.0, were recorded using a corresponding tin(II)-free solutions as a reagent blank. Spectra were taken 1 hr. after the preparation of the solutions. Part of the spectra obtained is shown in Fig. 1. Along with the

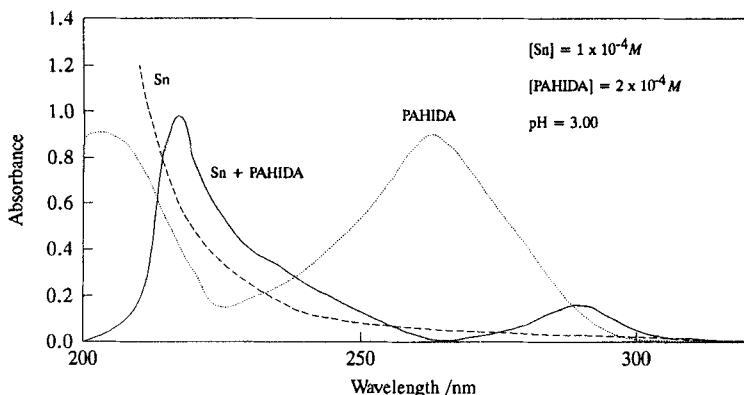


Figure 1. U.V. spectra of tin(II), PAHIDA and their mixture, $[\text{PAHIDA}] : [\text{Sn}] = 2:1$, in $0.1 \text{ mol dm}^{-3} \text{ KCl}$ medium.

spectrum of Sn-PAHIDA mixture, the spectra of hydrolysed tin(II) and pure PAHIDA are shown. As a reagent blank the solutions of the same composition and pH with exception of tin(II) or PAHIDA, respectively, were used. It may be seen that the spectrum of the mixture exhibits a new peak at ~ 295 nm. The intensity and position of this peak varies upon changing the concentration ratio of PAHIDA to tin as well as the pH of solutions. With increasing the concentration of PAHIDA ($\text{pH} = 4.2$, $[\text{Sn}] = 1 \times 10^{-4} \text{ mol dm}^{-3}$) the absorbance increases up to concentration ratio 3:1 and then remains constant, with slight tendency to decrease at concentration ratio higher than 6:1. The dependence of the absorbance at 295 nm on pH is shown in Fig. 2. The shape of the curve obtained indicates the formation of the several complexes in solution.

Preliminary treatment of spectrophotometric data indicates the formation of mononuclear complexes with PAHIDA to tin ratio 1:1. Small intensity of the peak of the complex did not provide a sound basis for the calculation of the stability constant(s), therefore, no reliable values of stability constants could be obtained from spectrophotometric data.

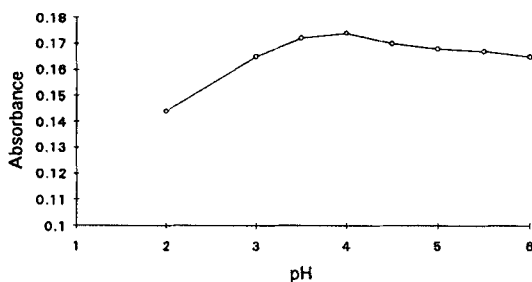


Figure 2. Dependence of absorbance at 295 nm on pH in Sn - PAHIDA solutions. $[\text{Sn}] = 1 \times 10^{-4} \text{ M}$, $[\text{PAHIDA}] = 3 \times 10^{-4} \text{ M}$.

Potentiometric measurements

The emf glass electrode measurements in $0.5 \text{ mol dm}^{-3} \text{ NaClO}_4$ medium, at 298 K were performed on solutions in which the concentration of the tin(II) ranged between ~ 0.07 and $0.15 \text{ mmol dm}^{-3}$, that of PAHIDA between ~ 0.5 and 3.0 mmol dm^{-3} ; thus the concentration ratio of PAHIDA to tin was between 5:1 to 20:1. The experimental data are summarized in Table 1.

Establishing of equilibrium in the system was relatively slow, especially at lower ligand/metal concentration ratios. At higher concentration ratios ($> 10:1$) the equilibrium was much more rapidly attained. Approaching to equilibrium was judged by stable potential readings of the glass electrode. Usually, stable potentials (within $\pm 0.3 \text{ mV}$) were attained 10 - 15 min after addition of the base. However, at pH values higher than 4.5 it was necessary to wait 15 - 25 min. to obtain stable potentials. Beyond pH ~ 6.0 slight turbidity of the solutions was observed, but potential remained stable, within specified range of values and period of time. Drifting potential readings were obtained at $\text{pH} > 7.5$ regardless of the particular concentration ratio of PAHIDA to tin. Visible precipitation begun to form at pH

Table 1. Summary of potentiometric measurements in Sn^{2+} - PAHIDA system in 0.5 mol dm^{-3} NaClO_4 medium, at 298 K. Total concentrations of the species are given in mmol dm^{-3} .

Run	[Sn]	[PAHIDA]	[HClO ₄]	[PAHIDA]/ [Sn]	pH range	Total No. of points
1.	0.100	0.500	7.860	5.0	1.540 - 9.160	41
2.	0.180	1.100	13.570	6.1	1.300 - 8.910	50
3.	0.150	1.500	0.900	10.0	2.503 - 8.841	51
4.	0.045	0.500	7.200	11.1	1.600 - 9.010	52
5.	0.090	1.110	13.600	12.2	1.300 - 9.000	44
6.	0.091	1.110	12.500	12.2	1.340 - 9.070	43
7.	0.084	1.500	1.200	17.9	2.400 - 8.910	32
8.	0.076	1.500	0.800	19.7	2.580 - 8.912	42
9*	0.152	3.000	0.810	20.0	2.579 - 6.866	54

* Measurement in water-ethanol mixture, $d = 0.96596 \text{ g/mL}$

~ 8.0. Titrations were continued up to pH ~ 9.0, but only the points in the pH range ~ 2.5 - 6.5 were included in calculations.

Part of the experimental data is shown in Fig. 3 as the dependence of pH on titration parameter. Titration parameter was calculated as the quotient (mmoles of base added - mmoles of strong acid present in solution)/ total mmoles of tin. Thus, negative values of

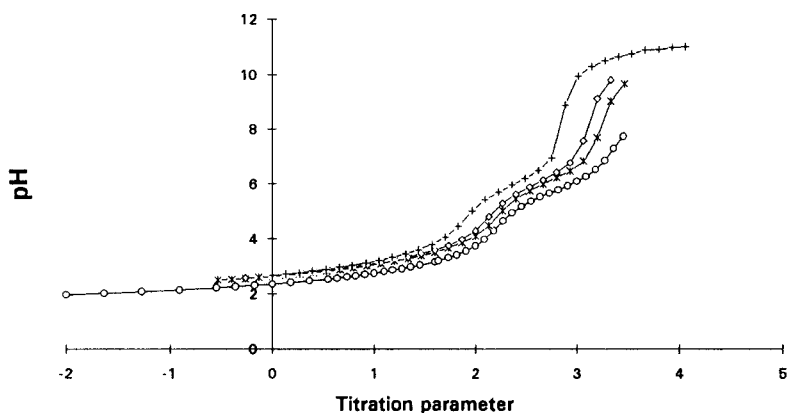
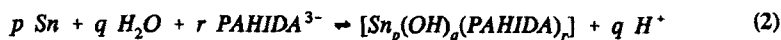


Figure 3. Titration curves of Sn-PAHIDA solutions in 0.5 M NaClO_4 , at 298 K. (x) PAHIDA, $1 \times 10^{-3} \text{ M}$; [PAHIDA]:[Sn] = (□) 20:1, (∗) 10:1, (○) 5:1.

titration parameter denote excess of strong acid in solution. Titration curves coincide in the pH range 2 - 5 and begin to diverge at pH > 5.0.

The equilibria in Sn²⁺ - PAHIDA system may be represented in a general form:



(Charges are omitted for clarity).

The stability constants of various (p,q,r) species formed in reaction (2) may be defined as:

$$\beta_{pqr} = C_{pqr} \cdot b^{-p} \cdot h^q \cdot c^{-r} \quad (3)$$

where C_{pqr} denotes the equilibrium concentration of the complex, b, h and c are the free equilibrium concentrations of tin, hydrogen and PAHIDA, respectively. In accordance with usual practice negative values of q denote hydroxo group while positive values denote protonated complexes.

In order to study speciation in three-component system Sn - H⁺ - PAHIDA, the binary equilibria *i.e.* the hydrolysis of the tin(II) and protonation of PAHIDA³⁻ ion must be studied under the identical experimental conditions as for complexation studies. These binary equilibria were studied in our previous papers^{3,5}, therefore the results obtained there are presented in Table 2.

Mathematical analysis of the experimental data was performed with the aid of a general least-squares program Superquad⁶. In Superquad calculations the identity and stability constants of the complexes which give the best fit to the experimental data are determined by minimizing the error-squares sum, U, of the potential residuals:

$$U = \sum w_i (E_{obs} - E_{calc})^2 \quad (4)$$

where w_i represents statistical weight assigned to each point of the titration curve. E_{obs} and E_{calc} refer to measured potential and calculated one assuming the specific model and trial values of constants. The best model was chosen using these criteria: (a) lowest value of U (eq. 4), (b) the standard deviation of calculated constants, σ, less than 0.2 log units, (c) standard deviations, s, in potential residuals less than 3.0 (with standard error in potential readings 0.1 mV and in volume readings 0.0005 mL), (d) goodness of fit statistics, χ² (Pearson's test) at 95% confidence level with 6 degree of freedom, less than 12.6 and (e) reasonably random scatter of potential residuals (E_{obs} - E_{calc}) against pH, without systematic trends. The following species were selected to find the best model: monomeric, (1,0,1), (1,0,2), (1,0,3), (1,1,1), (1,2,1), (1,-1,1), (1,-1,2), (1,-1,3), (1,-2,1), (1,-2,2), polymeric, (2,0,1), (2,0,2), (2,0,3), (2,1,1), (2,-1,1), (2,-1,2), (2,2,1), (2,-2,1), (2,-2,2), (3,-4,1). More than 20 models were tested. Firstly, each titration curve was treated separately. All complexes

found in such a way were used as a starting model for Superquad calculations. The following complexes were included after several cycles of calculations: (1,0,1), (1,1,1), (1,2,1), (1,-1,1), (1,-2,1), (2,-2,2). Then, the data belonging to all titration curves, referred to one particular PAHIDA to Sn concentration ratio, were treated together. During the calculations analytical concentrations of the reagents along with the stability constants of hydrolytic complexes of tin and protonation constants of PAHIDA were held constant, while E^0 values were allowed to float. In the final cycle of calculations, the total proton concentration was optimized, keeping the stability constants of the complexes and E^0 values, from the previous cycle as non-refinable parameters. In most titrations the calculated values deviated from the experimental one for 0.3 - 0.5%. However, in some titrations (with higher PAHIDA to tin concentration ratios) deviations were higher than 1%. The mathematical treatment of these titrations was repeated, but with the stability constants of pure hydrolytic complexes of tin as refinable parameters. As a result the polynuclear complexes, (2,-2,0) and (3,-4,0) were rejected and only the complex [Sn(OH)] was accepted with satisfactorily good values of statistics. The complexes found and their respective stability constants are given in Table 2.

Table 2. Stability constants of the complexes formed in Sn - PAHIDA solutions in 0.5 M NaClO₄ medium, at 298 K.

Species	$\log(\beta \pm \sigma)$	$\log(\beta \pm \sigma)^*$	χ^2	s
[Sn(OH)]	-4.0 ± 0.10		12.9	5.1
[Sn ₂ (OH) ₂] ^a	-4.59 ± 0.08			
[Sn ₃ (OH) ₄] ^a	-6.30 ± 0.10			
HPAHIDA ^b	5.99 ± 0.01		9.2	1.3
H ₂ PAHIDA ^b	9.16 ± 0.02			
H ₃ PAHIDA ^b	11.26 ± 0.03			
[Sn(H ₂ PAHIDA)]	12.98 ± 0.15	12.76 ± 0.03	11.8	3.9
[Sn(HPAHIDA)]	9.38 ± 0.05	8.71 ± 0.04		
[Sn(PAHIDA)]	5.74 ± 0.01			
[Sn(OH)PAHIDA]	1.55 ± 0.09			
*) Ref. 3, ^b) Ref. 5, * Measurement in ethanol-water solvent (50 wt. %)				

The obtained values of statistics, σ , s and χ^2 indicate a good fit of the data. It should be noted that at PAHIDA to tin concentration ratio 5:1 the complex $[\text{Sn}_2(\text{OH})_2(\text{PAHIDA})_2]$ was found, but with relatively large value of s (> 5.0). Therefore, this complex was rejected from the final model.

Effect of PAHIDA concentration and pH on Tc-PAHIDA labelling yield

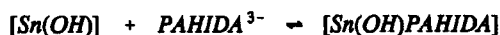
In order to label PAHIDA with $^{99\text{m}}\text{TcO}_4^-$, in the presence of stannous ion, the pertechnetate must be first reduced to lower oxidation state, preferably to bivalent Tc, and then complexed with PAHIDA⁷. The labelling yield was measured on SG bands ($R_f = 0.90$) with increasing PAHIDA concentration, at constant tin concentration and pH. At low PAHIDA to tin concentration ratios (up to 15:1) labeling yield was less than 10%. Increasing the PAHIDA concentration to ~ 20:1 the yield increases to 60%. Maximum yield (90 - 91%) was obtained at PAHIDA to tin concentration ratio 30 - 35:1 at pH between 5.0 and 6.0. Further increase in PAHIDA concentration did not improve the yield. The labeling yield also depends on pH. At constant PAHIDA and tin concentrations ($[\text{PAHIDA}] = 35 \mu\text{mol}$, $[\text{Sn}] = 1 \mu\text{mol}$) the yield increases with increasing the pH. At pH ~ 4.0 it is 45%, pH ~ 5.0, 60% and between pH 5.5 and 6.0 it is about 91%.

Discussion

The distribution diagram of various species formed in tin(II)-PAHIDA solutions is shown in Fig. 4. It may be seen from the Fig. 4. that at pH values higher than 4.0 the dominating species are pure $[\text{Sn}(\text{PAHIDA})]$ complex and hydrolytic complex $[\text{Sn}(\text{OH})\text{PAHIDA}]$. With increasing the pH the concentration of the hydrolytic complex increases, followed by polymerization and eventually, at pH > 7.5 begins the precipitation. The hydrolytic complex may be formed either by reaction:



or:



Both reactions are equally probable although the former one seems to proceed in higher extent than the latter one, bearing in mind the pH range of formation (Fig. 4) and the stability constant of $[\text{Sn}(\text{PAHIDA})]$ complex which is moderate.

High concentrations of PAHIDA are needed to stabilize bivalent state of the tin and to prevent initial hydrolysis or hydrolytic precipitation of the tin. At the concentration ratios of PAHIDA to tin and pH values utilized, most of the tin is bound to various complexes and

is therefore only partly available for reduction. The complexes of tin(II) with PAHIDA possess different composition and stability. Their distribution *i.e.* concentration in the reaction mixture depends on pH. Addition of Tc is probably, accompanied by partial replacement of the tin from PAHIDA complex(es). The experimental conditions should therefore, favour the formation of moderately stable complexes, from which Tc could easily replace the tin. Since this reaction proceeds in slightly acidic medium, the stability constant of the Tc-PAHIDA complex should be higher than the one of $[\text{Sn}(\text{PAHIDA})]$, ($> 10^5$). Released tin reduces the

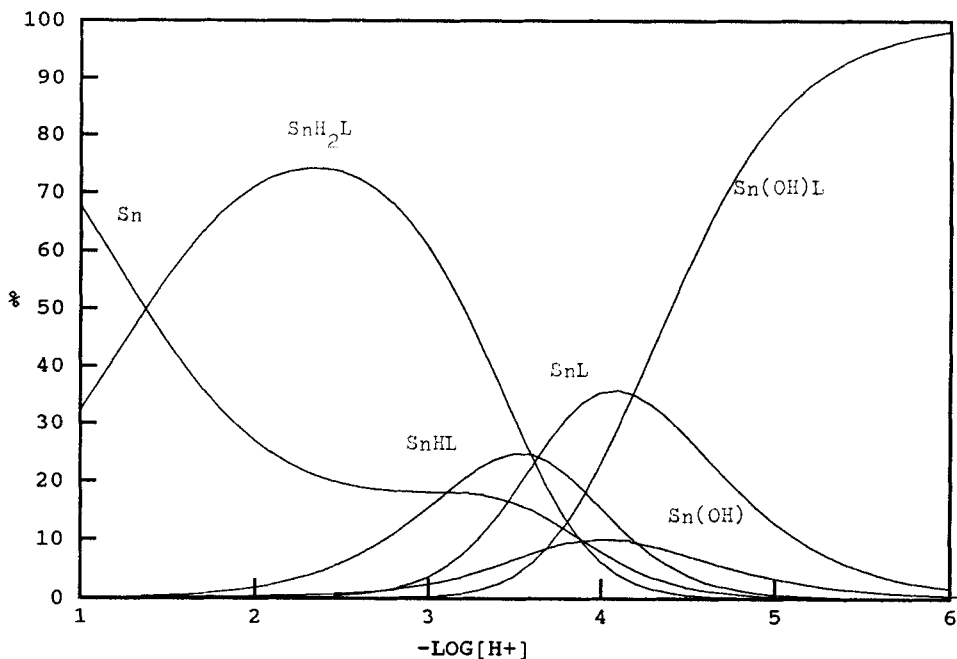


Figure 4. Distribution diagram of various complexes formed in Sn - PAHIDA solution. $[\text{Sn}] = 1 \times 10^{-4} \text{ M}$, $[\text{PAHIDA}] = 1 \times 10^{-3} \text{ M}$.

remaining pertechnetate, which is then complexed by PAHIDA. The dependence of the labelling yield on PAHIDA concentration and pH indicates that probably more than one Tc-PAHIDA complex is formed in solution. It is not surprising bearing in mind that at least three species TcO^{2+} , $\text{TcO}(\text{OH})^+$ and Tc^{2+} are involved in complexation⁸⁻¹⁰. Oxo and hydroxo species may be the result of incomplete reduction due to low stannous ion concentration or unfavourable pH. As seen from distribution diagram tin is much more easily replaced from mixed hydroxo complex than from pure PAHIDA complex. Therefore, the labelling yield increases with increasing pH, since at $\text{pH} > 5.0$ almost all available tin is bound to mixed

complex. Another mechanism or Tc - PAHIDA complexation could be the binuclear mixed complex formation where the iminodiacetate group is attached to both Tc^{2+}/TcO^{2+} and $Sn^{2+}/SnOH^+$ cores. In the mixed complex $[Sn(OH)PAHIDA]$ ligand is bound to the tin by acetate group only as indicated by similar values of the stability constants of this and acetate complex of the tin^{II}, thus, leaving the imino and second acetate group available for ligation.

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