

**POLAROGRAPHIC STUDY OF THE SYSTEMS Pb(II)-VALINE AND Pb(II)-VALINATE ION UNDER PHYSIOLOGICAL CONDITIONS**

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The polarographic method has been used to the study of the systems Pb(II)-valine and Pb(II)-valinate ion in aqueous medium, at  $I = 0.15 \text{ mol l}^{-1}$  ( $\text{NaClO}_4$ ) and  $37 \text{ }^\circ\text{C}$ . The stabilized complexes were found:  $[\text{Pb}(\text{HV})]^{2+}$ ,  $\beta_{10} = 9.0$ ;  $[\text{Pb}(\text{HV})_2]^{2+}$ ,  $\beta_{20} = 12$ ;  $[\text{Pb}(\text{V})]^+$ ,  $\beta_{01} = 1.8 \times 10^4$ ; and  $[\text{Pb}(\text{V})_2]$ ,  $\beta_{02} = 1.7 \times 10^6$ .

**Keywords:** Stability constants; Speciation; Amino acids; Valine; Lead complexes; Pb(II); Polarography; Electroreductions; Electrochemistry.

The study of coordination of metal ion and amino acids has become increasingly important in recently, from different viewpoints.

One of the most interesting procedures currently followed to investigate the structures and functions of complex macromolecules consists in the analysis of simple molecules that show properties similar to those characteristic of macromolecules. The model compounds are generally easier to study and the information obtained has frequently been successfully extrapolated to macromolecules. The first studies date from the 70s. For example, Freeman<sup>1</sup> has shown the utility of the information obtained in the study of some simple complexes for understanding the structure of active sites of carboxypeptidase.

Likewise, diverse observations have led to the study of the formation of low-molecular-weight metal ion complexes since this is the way in which metal ions are mobilized during prolonged periods of nutrition. The reliability of the stability constant values for simple and mixed metal complexes with simple amino acids is also of importance since they are the main form of both uptake of metals food and their excretion. The same de-

cade also saw the first reports on the reinvestigation of the system Zn(II)–cysteinate–histidinate<sup>2</sup> that has led to a reduction of the initial estimation of the daily dosage of zinc from 38 to 32 mg in some nutritive mixtures studied.

On the other hand, the studies of the coordinated systems metal ion–amino acid have, generally, taken into account only anions as ligands, disregarding the complexing nature of the neutral species. It was therefore thought worthwhile to undertake a systematic analysis of these systems considering the complexation of the metal ions by both species.

Lastly, must be pointed out that a majority of studies of coordinated systems metal ion–amino acid have been carried out by adjusting the ionic strength with weakly complexing electrolytes (*e.g.*, NO<sub>3</sub><sup>-</sup>). We have shown and verified experimentally<sup>3</sup> that the constants determined under these conditions are not true stability constants but, rather, correspond to groups of formation constants of simple and mixed species existing in the medium. The importance of this particular result became evident realizing that the use of this type of electrolyte is general. This provided a solid basis for questioning a greater part of the data previously reported in the literature on coordination species.

In this work we report on the analysis under physiological conditions of the coordination systems Pb(II)–valine and Pb(II)–valinate ion adjusting the ionic strength with NaClO<sub>4</sub>, which have been little studied<sup>4–6</sup> (Table I). The scatter of the results is quite remarkable and requires experimental revision.

## THEORETICAL

We describe below the approach that we have developed for the exhaustive analysis of the coordinated systems metal ion–amino acid.

TABLE I  
Stability constants reported in literature for the system Pb(II)–valine–valinate ion

Method	<i>T</i> , °C	Medium	$\beta_{01} \times 10^{-4}$	$\beta_{02} \times 10^{-6}$	Ref.
Voltammetry	30	1.0 M KClO <sub>4</sub>	1.0	0.78	4
Paper electrophoresis	35	0.1 M NaClO <sub>4</sub>	13	251	5
Polarography	25	1.0 M NaClO <sub>4</sub>	3.7	28	6

The  $F_{00\dots 0}$  function of Schaap and McMasters<sup>7</sup> for one metal ion with different ligands (X, Y, ..., Z) is given by:

$$F_{00\dots 0}(X, Y, \dots, Z) = \text{anti log} \left( \frac{(E_{1/2}^r)_L - (E_{1/2}^r)_M}{\frac{2.303RT}{nF}} + \log \frac{(i_d)_M}{(i_d)_L} \right) = \\ = \sum \beta_{j\dots z} [X]^i [Y]^j \dots [Z]^z,$$

where:  $(E_{1/2}^r)_M$  is the reversible half-wave potential for the solution in absence of ligands,  $(E_{1/2}^r)_L$  is the reversible half-wave potential for the solution in presence of ligands,  $(i_d)_M$  is the diffusion limit instantaneous current for the solution in absence of ligands,  $(i_d)_L$  is the diffusion limit instantaneous current for the solution in presence of ligand,  $n$  is the electron number in the electrochemical reduction,  $T$  is the absolute temperature,  $F$  is the Faraday constant and  $R$  is the gas constant.

If the molecule of valine (HV) and the valinate ion (V) are considered as possible ligands of Pb(II) and if we admit, as occurred in the studies of valine<sup>6</sup> and alanine<sup>8</sup>, that only two ligands can be coordinated, the  $F_{00}$  function will be developed as follows:

$$F_{00} = \{1 + \beta_{10}[\text{HV}] + \beta_{20}[\text{HV}]^2\} + \{\beta_{01} + \beta_{11}[\text{HV}]\}[\text{V}] + \{\beta_{02}\}[\text{V}]^2 \quad (1)$$

It is evident that at high pH values the complexes with the anion will predominate since their concentration will be significant and their stability constants must be much greater than those of the neutral form.

At sufficiently low pH values it is to be expected that the high concentration of the neutral species in comparison with that of the anionic species compensates greatly for its much lower stability constant and its complexes with the metal cations under study can be determined.

Within the limits mentioned, determination of the constants of mixed coordinated species is possible.

In any case, the fact should be taken into account that if the required pH is very low, the concentration of the protonated form of amino acid may be high, appreciably modifying the ionic strength. This effect can be minimized by decreasing the total concentration of amino acid.

Likewise, in every case it is necessary to work with sufficiently high concentration of complexing forms of amino acid for the complexation to appreciably predominate over the formation of hydroxo complexes.

The studies were carried out at constant pH but modifying the total concentration of amino acid, such that [HV] and [V] vary with the total concentration of valine ( $C_V$ ).

With regard to the expression  $K_{a2} = [V][H^+]/[HV]$ , Eq. (1) transforms to

$$F_{00} = 1 + \{\beta_{10} + (K_{a2}/[H^+])\beta_{01}\}[HV] + \{\beta_{20} + (K_{a2}/[H^+])\beta_{11} + (K_{a2}/[H^+])^2\beta_{02}\}[HV]^2 \quad (2)$$

The relationship at constant pH reduces to

$$F_{00} = 1 + B[HV] + C[HV]^2 \quad (3)$$

where, if  $m = [H^+]/K_{a2}$ , the  $B$  and  $C$  coefficients are defined as follows

$$B = \beta_{10} + \beta_{01}m^{-1} \quad (4)$$

$$C = \beta_{20} + \beta_{11}m^{-1} + \beta_{02}m^{-2} \quad (5)$$

and can be calculated by a linear regression procedure.

The knowledge of an appropriate number of values of the  $B$  and  $C$  coefficients would allow to determine the stability constants of the coordinated species present in the medium. Analogously, the development of  $F_{00}$  can be expressed as a function of [V]. At variable pH, the concentrations of different forms of the amino acid will change varying the pH. At a sufficiently high pH, where the concentration of  $[H_2V^+]$  is negligible, the sum of the concentrations of neutral and negative species is equal to the total concentration of the amino acid, *i.e.*

$$C_V = [HV] + [V] \quad (6)$$

In order to calculate the constants for the anionic form, the value of  $[HV]$  obtained from Eq. (6) is introduced into the Eq. (1), giving

$$F_{00} = \{1 + \beta_{10}C_V + \beta_{20}C_V^2\} + \{(\beta_{01} - \beta_{10}) + (\beta_{11} - 2\beta_{20})C_V\}[V] + \{(\beta_{02} - \beta_{11} + \beta_{20})\}[V]^2 \quad (7)$$

The terms in curves are constant, and therefore

$$F_{00} = A + B[V] + C[V]^2 \quad (8)$$

where

$$A = 1 + \beta_{10}C_V + \beta_{20}C_V^2 \quad (9)$$

$$B = (\beta_{01} - \beta_{10}) + (\beta_{11} - 2\beta_{20})C_V \quad (10)$$

$$C = \beta_{02} - \beta_{11} + \beta_{20} \quad (11)$$

The knowledge of an appropriate number of values of the  $B$  and  $C$  coefficients would allow to determine the stability constants of species present in the medium. Analogously, the development of  $F_{00}$  can be expressed as a function of the concentration of neutral form of the amino acid.

## EXPERIMENTAL

### Materials and Equipment

All the  $i$  vs  $E$  curves were registered by TAST polarography, using an Inelecsa PDC1212 electrochemistry system, in the range of potential from  $-100$  to  $-700$  mV, in 4 mV steps. An Ag|AgCl|NaCl saturated Metrohm EA427 electrode and a Pt (EA285) electrode were employed as reference and auxiliary electrode, respectively. The temperature was  $37 \pm 0.1$  °C and the drop time was set at 3 s. The ionic strength was maintained constant at  $I = 0.15$  mol  $l^{-1}$  by addition of NaClO<sub>4</sub>. The pH measurements were carried out with a pHM84 digital pH-meter and a Radiometer GK2401C combined electrode. The Lead perchlorate (Merck p.a.) solution was prepared by weighting and it was standardized complexometrically. Sodium perchlorate was also Merck p.a. and L-valine was from Sigma p.a. The values of the acid ionization constants<sup>9</sup> were  $pK_{a1} = 2.319$  and  $pK_{a2} = 9.218$ .

## Procedures

Of the three studies performed, two were carried out at constant pH varying the total concentration of valine within the limits indicated in Table II, and the remaining one at variable pH, as can be seen in Table II. The value of  $C_V$  is the starting value and does not reflect any volume correction.

In all cases, the plots in the absence of ligand were made at low pH  $\cong$  3.00 and  $[\text{Pb}^{2+}] \cong 1.0 \times 10^{-4} \text{ mol l}^{-1}$ . All adjustments were carried out utilizing Sigma Plot 4.0 for Windows.

## RESULTS

In all the solutions studied, the polarograms show good symmetry and a regular shape. The plot of  $\log [(i_d - i)/i]$  shows that the electrochemical reduction takes place by a two-electron process. The values of reversible half-wave potentials were obtained directly from those plots. The reproducibility of  $i$ - $E$  curves was excellent, and the precision of the half-wave potentials is better than  $\pm 0.5 \text{ mV}$ . The stability of the pH, during the polarographic measurements, was checked by measuring it before and after the polarographic wave recording.

The plot of the  $F_{00}$  function vs the ligand concentration allows to verify the quadratic dependence suggested. Adjusting  $F_{00}$  by least-squares treatment provides the values of  $B$  and  $C$  given in Table III. Figure 1 corresponds to study 1.

The studies performed were planned such that studies 1 and 2 correspond to a low value of pH, in which the complexes with neutral valine should prevail. The study carried out at higher pH values (study 3), allow the determination of the complexes with the anionic form of valine.

TABLE II  
Conditions for sets of experiments

Study	Constant pH	$C_V \times 10^2, \text{ mol l}^{-1}$	$[\text{HV}] \times 10^2, \text{ mol l}^{-1}$	$[\text{V}] \times 10^5, \text{ mol l}^{-1}$
1	4.13	2.00 – 18.0	1.943/17.68	1.153/15.83
2	4.23	3.00 – 22.0	2.948/21.56	3.174/20.69
Study	Variable pH	$C_V, \text{ mol l}^{-1}$	$[\text{HV}] \times 10^2, \text{ mol l}^{-1}$	$[\text{V}] \times 10^4, \text{ mol l}^{-1}$
3	7.09/7.91	0.10	0.988/10.86	5.853/36.69

### Determination of the $\beta_{10}$ and $\beta_{20}$ Constants

It can be shown that for the studies 1 and 2, performed at lower pH (Eqs (4) and (5)), the summands other than  $\beta_{10}$  and  $\beta_{20}$  are negligible in comparison with corresponding coefficients  $B$  and  $C$ , respectively, which leads to the following values of the formation constants of the complexes  $[\text{Pb}(\text{HV})]^{2+}$  and  $[\text{Pb}(\text{HV})_2]^{2+}$ :

$$\beta_{10} = 9.0 \pm 0.8$$

$$\beta_{20} = 12 \pm 1.4$$

TABLE III  
Coefficients of function  $F_{00}$

Study	Constant pH	$m^{-1} \times 10^6$	$B$	$C$
1	4.13	8.166	$8.246 \pm 0.261$	$10.40 \pm 1.89$
2	4.23	10.28	$9.770 \pm 0.232$	$13.26 \pm 1.35$
Study	Variable pH	$m^{-1} \times 10^6$	$B \times 10^{-4}$	$C \times 10^{-6}$
3	7.09–7.91	7.447–49.20	1.765–0.08	1.735–0.27

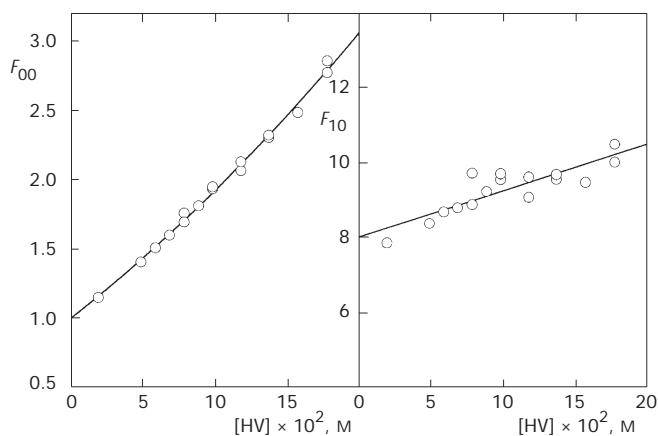


FIG. 1  
Plot of the functions  $F_{00}$  and  $F_{10}$  vs  $[\text{HV}]$  at pH 4.13

### Determination of the $\beta_{01}$ and $\beta_{02}$ Constants

For the study 3, performed at variable pH, it can be shown that Eqs (10) and (11) are approximately  $B \cong \beta_{01}$  and  $C \cong \beta_{02}$

From these coefficients, the values of the stability constants of the  $[\text{Pb}(\text{V})]^+$  and  $[\text{Pb}(\text{V})_2]$  complexes are:

$$\beta_{01} = (1.8 \pm 0.08) \times 10^4$$

$$\beta_{02} = (1.7 \pm 0.3) \times 10^6$$

### DISCUSSION

This is the first experimental confirmation of the stabilization, under physiological conditions, of the complexes  $[\text{Pb}(\text{HV})]^2+$ ,  $[\text{Pb}(\text{HV})_2]^2+$ ,  $[\text{Pb}(\text{V})]^+$  and  $[\text{Pb}(\text{V})_2]$ . Our results are in the range of the stability constants proposed in the two studies performed at ionic strength  $1 \text{ mol l}^{-1}$  and it questions the validity of the abnormally high data obtained by Singh<sup>5</sup>, performed under near-physiological conditions. Figure 2 shows the species distribution curves in per cent as a function of pH for the solution:  $C_{\text{pb}} = 1.0 \times 10^{-4} \text{ mol l}^{-1}$ ;  $C_{\text{V}} = 0.10 \text{ mol l}^{-1}$ .

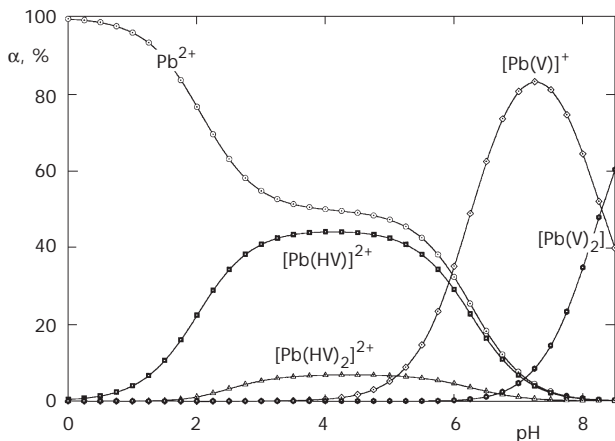


FIG. 2

Distribution curves of the lead-valine complexes as a function of pH



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