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Potentiometric investigation of the effect of the pH on the ionic transfer of some amino acids at the interface between two immiscible electrolyte solutions

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Abstract

The effect of the pH on the ionic transfer of glycine and β-alanine at the interface between two immiscible electrolyte solutions (ITIES) was investigated by a simple potentiometric method. Upon addition of small amounts of solution containing the investigated amino acids, a variation of the potential drop across the interface was recorded, which was found to be pH-dependent. This behavior was explained in terms of a preferential orientation of the amino acid molecules at the ITIES, induced by the different lipoficility of the functional groups. The results enabled the measurement of this voltage variation to be used as the basis for a simple and rapid method for determining the isoelectric point of the investigated compounds. The agreement between the pH_i values thus estimated and those reported in the literature suggests the possibility of using the method for the interpretation of processes occurring at the level of biological membranes.

Keywords: Potentiometric investigation; pH; Ionic transfer

1. Introduction

Over the past several years, there have been numerous studies devoted to the electrochemical phenomena of liquid/liquid interfaces. Attention has been given to the use of the results thus obtained, both for analytical applications in sensor design, and to uses in the interpretation of processes occurring at the level of biological membranes (see Refs. [1,2] and references therein). It is widely accepted that, in order to better understand the electrochemical behavior of an interface between two immiscible electrolyte solutions (ITIES), it is suitable to describe the above interface by means of an equivalent electrochemical cell [3–5]. Thus, considering the case of two immiscible electrolyte solutions (denoted I and II) that are brought into contact and contain the same redox couple (O/R), the following model holds for the interface thus created:

$$\mathrm{Pt/I,O,R//II,O,R/Pt} \quad (1)$$

The two platinum electrodes (denoted "Pt") immersed into the both solutions allow us to measure the voltage of the above electrochemical cell:

$$U = (\varphi_{\mathrm{PtI}} - \varphi_{\mathrm{I}}) + (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}}) + (\varphi_{\mathrm{II}} - \varphi_{\mathrm{PtII}}) \quad (2)$$

were φ represents the inner potential of each phase. At the equilibrium, the voltage drops to zero and the following equation can be written:

$$U = 0 = g_{\mathrm{PtI}} + g_{\mathrm{ITIES}} + g_{\mathrm{PtII}} \quad (3)$$

in which the terms g_{PtI}, g_{ITIES} and g_{PtII} stand for the differences of inner potentials $(\varphi_{\mathrm{PtI}} - \varphi_{\mathrm{I}})$, $(\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})$ and $(\varphi_{\mathrm{II}} - \varphi_{\mathrm{PtII}})$, respectively [6,7].

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The addition at the ITIES of a small amount of solution II containing the electrolyte CA, will give rise to a variation of the cell voltage, ΔU_1. Taking into account that the new electrochemical cell is:

$$\mathrm{Pt/I,O,R//CA,II//II,O,R/Pt} \quad (4)$$

its voltage will be:

$$U + \Delta U_1 = (\varphi_{\mathrm{PtI}} - \varphi_{\mathrm{I}}) + (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^* + (\varphi_{\mathrm{II}} - \varphi_{\mathrm{PtII}}) \quad (5)$$

and the voltage jump ΔU_1 can be obtained by subtracting Eq. (2) from Eq. (5):

$$\Delta U_1 = (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^* - g_{\mathrm{ITIES}} \quad (6)$$

It is worth noting that the term $(\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^*$ in Eq. (6) accounts for the effect of the electrolyte CA on the voltage across the ITIES.

For the case in which the amount of solution II added at the ITIES contains, besides the electrolyte CA, a buffer (improperly denoted "pH"), the following model holds for the electrochemical cell:

$$\mathrm{Pt/I,O,R//CA,pH,II//II,O,R/Pt} \quad (7)$$

and the voltage jump (ΔU_2) can be written as:

$$\Delta U_2 = (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{**} - g_{\mathrm{ITIES}} \quad (8)$$

Therefore, it seems reasonable to assume that the difference between the voltage jumps produced by the addition of a CA and a CA + buffer solution:

$$\Delta U_1 - \Delta U_2 = (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^* - (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{**} \quad (9)$$

will account for the effect of the concentration of protons on the transfer energy of the ions resulted from the dissociation of CA. Thus, if the dissociation equilibrium of CA is not affected by the variation of the pH, the term $(\Delta U_1 - \Delta U_2)$ in Eq. (9) drops to zero, or in other words, the same value of the voltage jump is to be expected, regardless the presence of the buffer. We should note however that, for a more accurate approach, the effect of the buffer itself on the potential drop across the ITIES has to be taken into account. Thus, due to the fact that the buffer is also an electrolyte, its addition at the ITIES (even without any amount of electrolyte CA) results in the formation of the following electrochemical cell:

$$\mathrm{Pt/I,O,R//pH,II//II,O,R/Pt} \quad (10)$$

the corresponding voltage jump being:

$$\Delta U_3 = (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{***} - g_{\mathrm{ITIES}} \quad (11)$$

Keeping in mind that the terms $(\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{**}$ and $(\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{***}$ account for the effects of the presence of either CA + buffer or of the buffer alone on the voltage across the ITIES, the equation:

$$\Delta U_3 - \Delta U_2 = (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{***} - (\varphi_{\mathrm{I}} - \varphi_{\mathrm{II}})^{**} \quad (12)$$

allows us to estimate the effect of the presence of CA, even when its dissociation equilibrium is pH-dependent. Among electrolytes the dissociation of which is affected by the variation of the pH, amino acids are of particular interest. This is because it was previously suggested [8] that, understanding the behavior of amino acids at ITIES could help in establishing useful in vitro models for some processes occurring at biological membrane level. It is expected that, at pH values close to the isoelectric point ($\mathrm{pH_i}$), the presence of the amino acid at the ITIES does not affect to a large extent the cell voltage, because the zwitterions forms prevail. Thus, under these experimental conditions, the following relation holds:

$$\Delta U_3 - \Delta U_2 \approx 0 \quad (13)$$

provided that the amino acid molecules are randomly oriented along the ITIES.

Based upon the above considerations, we propose a simple potentiometric method for studying the effect of the pH on the process of ionic transfer of amino acids at the interface between two immiscible electrolyte solutions. In the present paper we report the results of an investigation of the behavior of glycine and β-alanine. These compounds are good models for further studies of the electrochemical behavior of other amino acids at the ITIES.

2. Experimental

The experiments were carried out in a two-compartment glass cell with vertical symmetry, containing the two immiscible phases I and II, in the upper and lower compartments, respectively (Fig. 1). The geometry of the cell allows us to adjust the position of the ITIES (within the range of the length of the narrow neck between the two compartments) by means of the cock on the side arm. The surface area of the ITIES was ca. 0.3 $\mathrm{cm^2}$. A platinum electrode (3 $\mathrm{cm^2}$) was immersed into each compartment, in order to measure the voltage across the cell, by means of a high-impedance voltmeter (Instek GDM-8145) and/or a x–t recorder (Linseis LY 14100 II). It should be noted that the cell voltage was recorded by measuring the potential of the electrode in the lower compartment versus that of the electrode in the upper compartment. The modified micropipette attached to the upper compartment of the cell allows the addition of small, reproducible drops (ca. 0.03 $\mathrm{cm^3}$) of phase II with a content of investigated electrolyte (CA) of 0.2 M.

In the present work, water-saturated 1-butanol ($\mathrm{B_W}$) and butanol-saturated water ($\mathrm{W_B}$) were used as the immiscible phases I and II, respectively. The redox couple $\mathrm{K_3Fe(CN)_6/K_4Fe(CN)_6}$ was present in both compartments (at a concen-

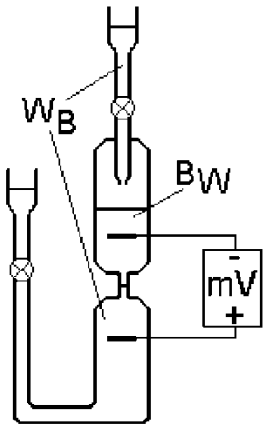

Fig. 1. Two-compartment cell employed for measuring the potential drop across ITIES. (W_B and B_W stand for water saturated with butanol and butanol saturated with water, respectively.)

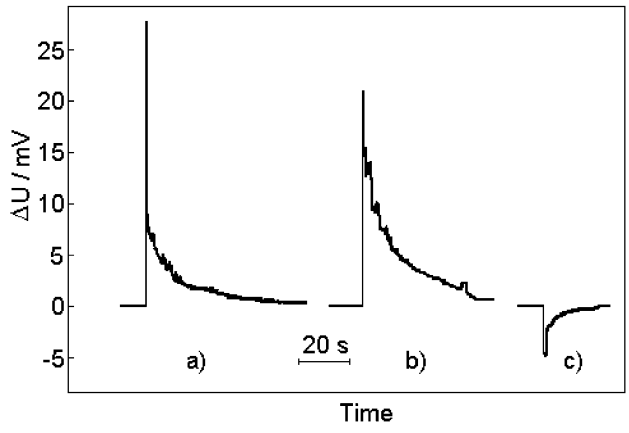

Fig. 2. Typical variation of ΔU as a function of time: (a) ΔU_3 for a pH of 5.8; (b) ΔU_2 for Gly at pH 5.8; (c) ΔU_2 for Gly at pH 7.5.

tration of 5 mM) in order to ensure the stability of the potential of each platinum electrode. In a typical experiment, a drop of W_B from the micropipette was allowed to flow through the upper B_W phase and, when the drop reached the ITIES, a voltage jump (ΔU) was measured between the two platinum electrodes. The investigated CA electrolytes were glycine (Gly) and β-alanine (Ala), and in some cases, NaCl was also used for comparison. The effect of the pH was studied (within the range 5.3–7.5) by using a phosphate buffer solution saturated with butanol, as a solvent for electrolyte CA in the micropipette. The pH of the buffer was adjusted by mixing appropriate volumes of 1/15 M KH_2PO_4 and 1/15 Na_2HPO_4 solutions. Glycine and β-alanine were purchased from Aldrich and were used without further purification. All the other substances were analytical reagent grade, and all solutions were prepared using Milli-Q water (Millipore). All the measurements were performed under aerated conditions, at room temperature.

3. Results and discussion

In order to assess the validity of the above theoretical considerations, the voltage jumps ΔU, observed when the drop reaches the ITIES, were recorded for three different compositions of the solution from the micropipette, as follows:

- ΔU_1 for the drops that contain the electrolyte CA (NaCl, Gly, Ala) dissolved in W_B;
- ΔU_2 for the case in which CA is dissolved in the buffered W_B, at several pH values;
- ΔU_3, corresponding to buffered W_B drops (at the same pH values as for ΔU_2), in the absence of the electrolyte CA.

Typical ΔU_3 and ΔU_2 responses are shown in Fig. 2. Elucidating the shape of the curves describing the variation of ΔU as a function of time is a difficult task and was beyond the scope of the present work.

Table 1 summarizes the results of all the above potentiometric measurements, together with the corresponding values of the relative standard deviation. It should be noted that, each ΔU value in Table 1 represents the average of 10 consecutive measurements. It was found that, the use of NaCl as electrolyte resulted in a value $\Delta U_1 = 53$ mV, in agreement with the results previously reported for the study of the potentiometric determination of the relative standard Gibbs energies of transfer [9]. As data in Table 1 shows, the values ΔU_2 obtained for the NaCl over the whole investigated pH range are rather equal to ΔU_1. This is not surprising because NaCl dissociation process is not affected by the variation of the pH. The same measurements carried out by using amino acids as the electrolyte CA, resulted in ΔU_1 values of -34 and -12 mV for glycine and alanine, respectively. It was also observed that, in the presence of the amino acids into the W_B drops, ΔU_2 gradually decreases by increasing the pH.

Fig. 3 illustrates the variation of ΔU_2 as a function of the pH for NaCl, Gly and Ala (curves a, b and c, respectively) together with the same variation of ΔU_3 (curve d in Fig. 3) obtained in the absence of the electrolyte. Curve a in Fig. 3

Table 1
Results of the potentiometric measurements performed for several pH values of the W_B drops, in the presence (ΔU_2) and in the absence (ΔU_3) of the investigated electrolytes

PH	ΔU_2/mV (RSD[a]/%)			ΔU_3/mV (RSD[a]/%)	$(\Delta U_3 - \Delta U_2)$/mV		
	NaCl	Gly	Ala		NaCl	Gly	Ala
5.3	52 (0.92)	28 (2.32)	27 (2.67)	34 (1.06)	−18	6	7
5.8	52 (1.10)	21 (3.33)	20 (3.40)	28 (0.96)	−24	7	8
6.3	53 (0.85)	9 (7.55)	8 (7.62)	20 (1.30)	−33	11	12
6.5	55 (0.80)	4 (7.50)	1 (16.83)	16 (3.75)	−39	12	15
6.8	52 (0.98)	−2 (12.50)	−6 (8.83)	10 (5.23)	−42	12	16
7.0	53 (1.23)	−4 (9.00)	−7 (8.85)	9 (5.44)	−44	13	16
7.5	52 (1.50)	−5 (8.60)	−9 (7.88)	8 (4.63)	−44	13	17

[a] Relative standard deviation of ΔU.

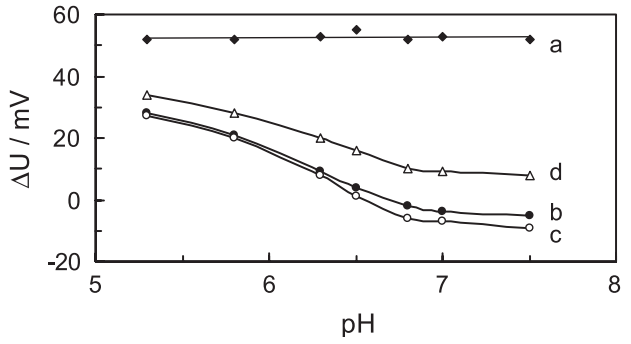

Fig. 3. The effect of the pH on the potentiometric response for the ionic transfer at the ITIES: (a) ΔU_2 for NaCl; (b) ΔU_2 for Gly; (c) ΔU_2 for Ala; (d) ΔU_3 for the buffer only.

clearly shows that the transfer of NaCl through the ITIES is not a pH-dependent process. This observation is in line with the fact that, in this case, the ionic transfer is controlled only by the difference in standard Gibbs energies of transfer of the involved ions [6,10]. Unlike NaCl, ΔU_2 linearly decreases with increasing pH for both investigated amino acids, tending to a pseudo-plateau at pH values higher than ca. 6.7 (curves b and c in Fig. 3).

According to Eq. (13), it would be expected that the curves describing the pH-dependence of ΔU_2 for Gly and Ala cross curve d (at pH values close to those of the pH_i of each amino acid), all on the condition that the molecules of the amino acids remain randomly oriented along the ITIES. Nevertheless, as Fig. 3 shows, our experimental data seem to indicate that this prerequisite is not fulfilled. A possible interpretation for this behavior is provided by the fact that, at the ITIES, the molecules of short aliphatic amino acids are most probably directed with the carboxylic moiety towards the aqueous phase. This orientation is favored by the hydrophilic character of the above functional group [11,12]. Based upon this observation, and taking into account that in aqueous solutions the ionization of amino acids depends on the pH [13], it seems reasonably to assume a distribution of Gly and Ala at the ITIES like that in Fig. 4. It is likely that, within the whole investigated pH range, the molecules are orientated with the carboxylic groups towards W_B, although the prevailing ionic form of the amino acid depends on the pH. Schemes from Fig. 4 could help in

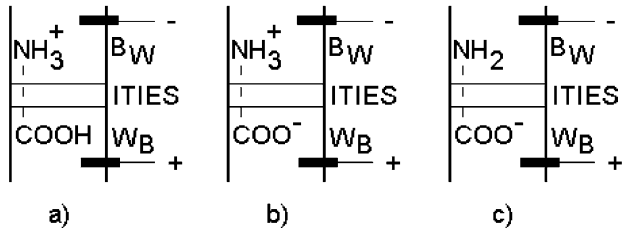

Fig. 4. Schematic representation of the hypothetical distribution at the ITIES of short aliphatic amino acids: (a) $pH < pH_i$; (b) $pH \approx pH_i$; (c) $pH > pH_i$.

understanding, at least in a qualitative manner, the results obtained under our experimental conditions (we should remind that all ΔU values were obtained by measuring the potential of the platinum electrode in the lower compartment of the cell versus that of the electrode in the upper one). Thus, at pH values close to pH_i, the distribution of the amino acid molecules at the ITIES (Fig. 4b) will have the effect of a reversed polarization of the interface, resulting in the decrease of ΔU_2 (in the presence of the amino acid) compared with the voltage jump ΔU_3 recorded for a W_B drop that contains the buffer only. In other words, an increase of the difference $(\Delta U_3 - \Delta U_2)$ is to be expected.

As Fig. 4a and c suggest, the presence of the amino acid at the ITIES will result in an increase of $(\Delta U_3 - \Delta U_2)$ even if the zwitterions form is not prevailing. Nevertheless, this increase should be less important than that observed at pH values close to pH_i, due to the fact that the reversed polarization is only partial.

Fig. 5 shows the variation of the difference $(\Delta U_3 - \Delta U_2)$ as a function of the pH, for the investigated amino acids. It can be observed that, for Gly and Ala the term $(\Delta U_3 - \Delta U_2)$ gradually increases with increasing pH (curves a and b in Fig. 5, respectively). It is interesting to note that, for both amino acids, this increase is more important within a certain pH range. If the above theoretical considerations hold, this pH range should correspond to the vicinity of pH_i, for each investigated amino acid. It was found that the shape of the curve obtained for NaCl is rather similar to that illustrating the pH-dependence of ΔU_3 (curve d in Fig. 3). This was not surprising because the dissociation process of NaCl is not affected by the pH.

Based upon the variation of $(\Delta U_3 - \Delta U_2)$ as a function of pH, an attempt to estimate the value of pH_i is shown in Fig. 5, both for Gly and for Ala. Thus, a graphical method (similar to that used for estimating the half-wave potential in polarography) applied on curves a and b in Fig. 5 yielded pH_i values of 6.14 ± 0.11 for Gly and 6.20 ± 0.09 for Ala, that reasonably agree with data from the literature, i.e. 6.06 and 6.11, respectively [14]. Nevertheless, it appears that, due to the errors of experiment, the pH_i values estimated under the above experimental conditions do not allow us to

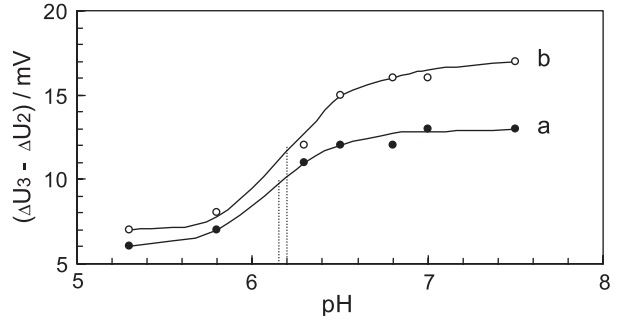

Fig. 5. The variation of $(\Delta U_3 - \Delta U_2)$ as a function of pH for Gly (a) and Ala (b).

discriminate between Gly and Ala. This was not surprising because this is a very difficult task also when using pH_i values estimated from titration curves [15].

4. Conclusions

The effect of the pH on the ionic transfer of gycine and β-alanine at the interface between two immiscible electrolyte solutions was investigated by a simple potentiometric method. Thus, the equilibrium of the ITIES was perturbed, by adding small amounts of solution containing the investigated amino acids, at several pH values. As a result, a variation of the potential drop across the interface was recorded, which was found to be pH-dependent, both for Gly and Ala. A possible explanation for this behavior is provided by assuming a preferential orientation of the amino acid molecules at the ITIES, as a result of the different lipoficility of the functional groups. Although this interpretation should only be considered as tentative, the good agreement between the pH_i values estimated by this potentiometric method and those reported in the literature is noteworthy. We are currently pursuing this approach in our laboratory and detailed results concerning the behavior of other amino acids will be published elsewhere. It is likely that these results will enhance our ability to understand the relationships between the structure of the amino acids and their behavior at the level of biological membranes.

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