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Determination of L-histidine by modified carbon paste electrode using tetra-3,4-pyridinoporphirazinatocopper(II)

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Abstract

This paper describes a potentiometric method for determination of L-histidine (L-his) in aqueous media, using a carbon paste electrode modified with tetra-3,4-pyridinoporphirazinatocopper(II) (Cu (3,4tppa)). The electrode exhibits linear response to the logarithm of the concentration of L-histidine from 2.4×10^{-5} to 1.0×10^{-2} M, with a response slope of -49.5 ± 1 mV and response time of about 1.5 min. The detection limit according to IUPAC recommendation was 2.0×10^{-5} M. The proposed electrode shows a good selectivity for L-his over a wide variety of anions. This chemically modified carbon paste electrode was successfully used for the determination of L-his in a synthetic serum and RANDOX control serum solutions.

Keywords: Carbon paste; Potentiometry; Cu (3,4tppa); L-histidine

1. Introduction

The carbon paste electrode (CPE) was introduced by Adams in 1958 [1] and generally consist of Teflon well into which is inserted a platinum, copper, steel, or graphite contact [2]. The well is filled with a paste made by mixing powdered graphite with a suitable mulling liquid. By adding modifier materials in paste we can improve the electrode selectivity and sensitivity. The amount of modifier in the paste usually varies between 10 and 30% (w/w), depending on the character of modifying agent and its capability of forming enough active sites in modified paste [3]. Chemically modified carbon paste electrode (CMCPE) preparation is very simple and fast, carbon pastes undoubtedly represent one of the most convenient materials for the preparation of modified electrodes.

Mostly CMCPEs used in the field of voltammetric determination and only very few of these types of the electrodes have been used in potentiometry [4,5].

One of the most important recognition elements that can be utilized in the development of potentiometric sensors involves specific metal–ligand interactions. Such interactions have been used in the development of anion-selective electrodes based on different ionophores [6]. The current methods for simultaneous determination of amino acids are based on gas chromatography [7], LC techniques [8–12], capillary electrophoresis [13], fluorimetry [14] and a potentiometric method which is based on a PVC-based Mn(III) porphyrin membrane coated on graphite electrode [6]. To our knowledge, only two carbon paste electrodes were reported for determination of L-histidine by the voltammetry [15,16]. Both techniques have a shorter linear range with our proposed potentiometry technique which has also the advantages of being inexpensive and simpler method.

2. Experimental

2.1. Materials

Graphite powder (Merck) and paraffin oil (Fluka) were used for the preparation of the carbon paste electrode. Cu (3,4tppa) (Fig. 1) was synthesized and purified according to the literature [17]. All amino acid reagents were of analytical reagent grade from Fluka. All solutions were prepared with doubly distilled de-ionized water.

A stock solution of L-histidine, 2.01×10^{-2} M, was prepared by dissolving an appropriate amount of L-histidine in

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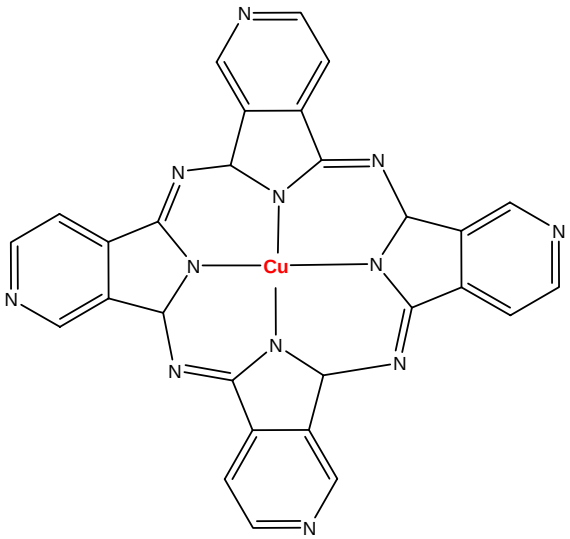


Fig. 1. Chemical structure of tetra-3,4-pyridinoporphirazinatocopper(II).

water. Solutions of interfering species were prepared by dissolving the appropriate amount of each compound in water. All of the working solutions were buffered at pH 9.5 using 0.05 M borax buffer solutions.

2.2. Preparation of CMCPE

CMCPE was prepared by mixing 0.0600 g of graphite powder and 0.0197 g of Cu (3,4tppa) complex in a 5 mL beaker and then was grinded in a mortar. Subsequently 0.0340 g of liquid paraffin was added to the mixture and then mixed well till a uniform paste was obtained.

Electrode bodies were made from a disposable 1 mL polyethylene syringe the tip of which had been cut off with a razor blade. These bodies were filled with approximately 0.2 mL of CMCP. Smooth surface were obtained by applying manual pressure to the piston while holding the electrode surface against a flat solid support, and was polished with the very smooth paper. After polishing, the working surface of the electrode was checked for pinholes by a usual lens.

Packing carbon paste electrodes to obtain a uniform and a reproducible surface is very important, i.e. it should be avoided to apply too much pressure when packing the well. This may result in separation of the carbon and oil, with a resulting high-resistance contact between paste and metal [2]. The electrical connection was made with a copper wire.

A fresh electrode surface can be obtained by cutting approximately 1 mm of paste and polishing the fresh surface over a smooth paper.

2.3. Apparatus

A Corning model of 125-pH/mV meter was used for potentiometric measurements. The reference electrode was a double junction saturated calomel electrode. A Metrohm potentiometer (pH meter model of 780) was used for pH controlling and a Heidoloph type of MR 2000 stirrer was used for stirring the solutions.

2.4. Procedure

The electrode was immersed directly in test solution. The pH of this solution was adjusted to 9.5 by borax buffer (0.05 M and also as supporting electrolyte). The solution stirred (100 rpm) until the response of the potentiometer became stable (20 min). Then certain amounts of stock solution (2.01×10^{-2} M L-his) were added to the test solution. After each addition, the potentiometer was remained to give a stable potential, then this potential was reported as a measured potential.

3. Results and discussion

3.1. Optimization of the amount of modifier in the electrode

For this purpose, six electrodes were prepared. The amounts of carbon powder and paraffin oil were constant in each electrode. The proportions of modifier in these six electrodes were 5.1, 9.2, 10.3, 12.3, 15.3, 17.3 and 19.3% of weight percentage of sensing element. The resulting Nernstian slopes and correlation coefficients are given in Table 1. According to these results, optimum amount of the modifier was 17.3%. In this optimum proportion the slope of the electrode was nearly Nernstian and its linear range also was large.

3.2. Effect of the ionic strength on the response of the electrode

The effect of ionic strength (0.05–0.1 M borax buffer) on the calibration curve was investigated. The electrode response changed slightly within the 0.05–0.1 M borax buffer solution. However, 0.05 M was chosen as an optimum value, since in this strength the linear range was wider than the other concentrations.

Table 1
Optimization of the amount of modifier

Percent of modifier	Slope[a]	Linear range	*R*-value
5.1	−43.3	4.4×10^{-4} to 1.0×10^{-2}	0.992
9.2	−44.7	2.4×10^{-5} to 1.0×10^{-2}	0.994
10.3	−48.4	6.4×10^{-5} to 1.0×10^{-2}	0.991
12.3	−45.8	2.4×10^{-5} to 1.0×10^{-2}	0.995
15.3	−43.0	7.0×10^{-5} to 1.0×10^{-2}	0.991
17.3	−49.5	2.4×10^{-5} to 1.0×10^{-2}	0.995
19.3	−44.3	6.4×10^{-5} to 1.0×10^{-2}	0.995

[a] The slopes are the average of five experiments.

3.3. Optimization of pH

The behavior of the electrode in relation to the variation of pH (3–10.5) at concentration of 1×10^{-3} M of L-his was studied (Fig. 2). For measuring the effect of pH, adjustments were made with the concentrated HCl and KOH solutions.

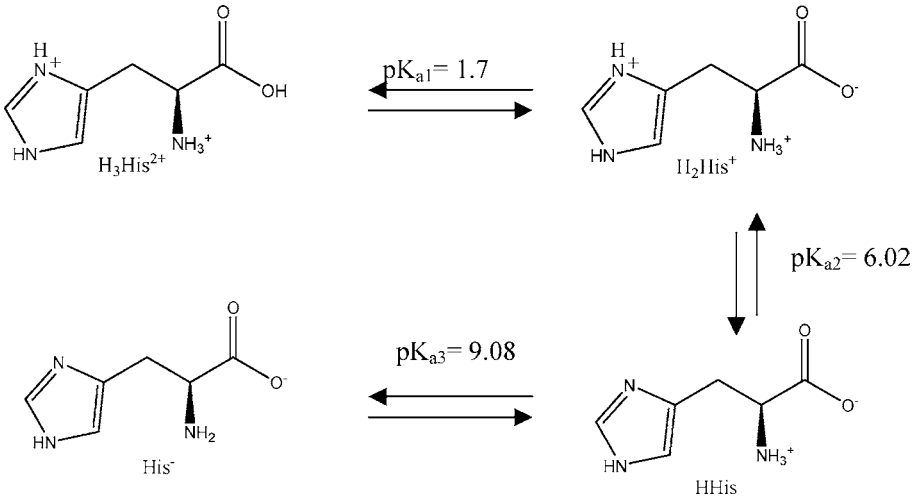
As shown in Fig. 2, a short range (from 8.2 to 9.5) of pH can be selected as optimum pH. The titration curves of L-histidine in buffer solutions with different pH values are illustrated in Fig. 3. According to Fig. 3 pH 9.5 offered a better slope and a wider linear range than in the other pH values. Decreasing pH, the slope and linear range decreased (Fig. 3) because the nitrogens in L-histidine are protonated, therefore the interaction between the L-histidine and the complex decreased. Actually at pH 5.0 no response of analyte was noticed since the pK_{2a} is 6.02 [18] and at pH lower than pK_{2a} the prevailing species is H_2His^+(see below schematic) which cannot readily coordinate with Cu (3,4tppa) ligand.

3.4. Response characteristics of modified and unmodified carbon paste electrode

The unmodified electrode showed no significant response under the optimum conditions (Fig. 4). The response time of the modified electrode was measured by using IUPAC recommendation [19]. The response time in variation of concentration from 5×10^{-4} to 5×10^{-3} M L-his was measured and was about 1.5 min. The limit of detection (2×10^{-5} M) was evaluated from the intersection of two extrapolated segments of the calibration graph according to IUPAC recommendations [20].

3.5. Electrode renewal and its reproducibility

The electrode surface should be renewed when the L-his solution is changed from higher to lower concentration in order to remove the residual L-his which still be adsorbed on



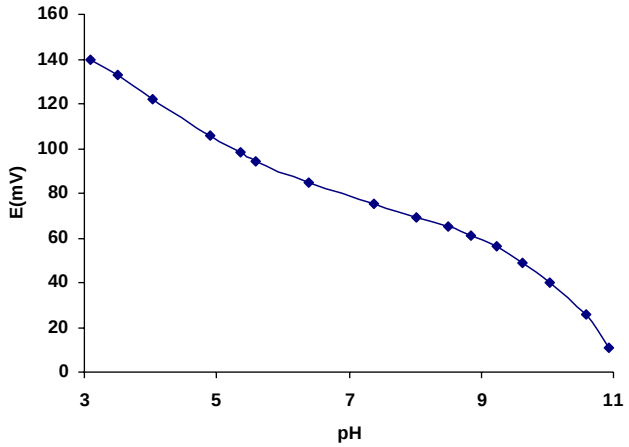


Fig. 2. The pH effect on potential response of the L-his electrode (conditions: 17.3% ionophore, L-his concentration was 1.0×10^{-3} M).

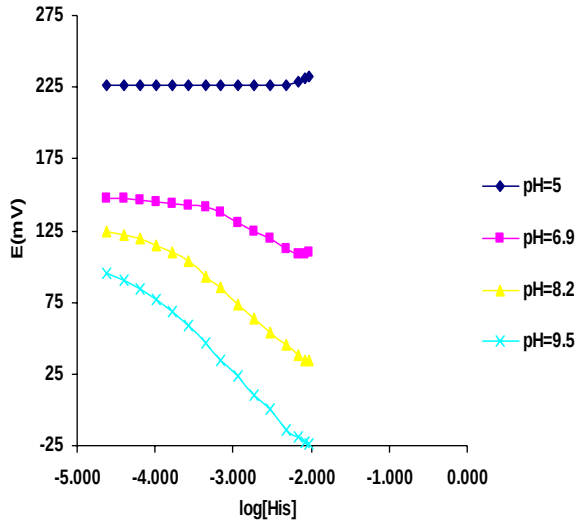


Fig. 3. Titration curves of the proposed electrode at various pH buffers (conditions: 17.3% ionophore, L-his concentration was 1.0×10^{-3} M).

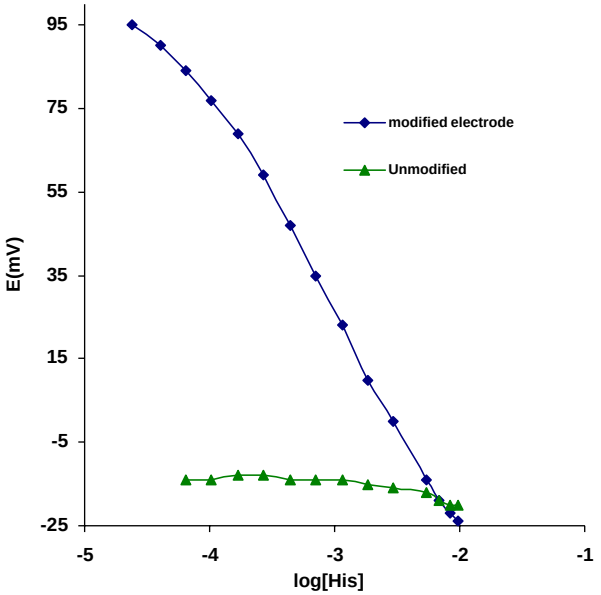


Fig. 4. Response of modified and unmodified carbon paste electrode under the optimum conditions (17.3% ionophore, pH 9.5).

the surface of carbon paste electrode, this process improve reproducibility. The appropriate renewal process is already described in procedure section. For this purpose, we applied the proposed electrode for measuring potential of 6.5×10^{-4} and 5.33×10^{-3} M L-his solutions. The results are given in Table 2. As these results show, the standard deviations are reasonable for these measurements.

3.6. Selectivity and interference

The selectivity coefficients of modified carbon paste electrode were evaluated by the fixed primary ion method. In this method the potential E was first measured in a known volume of the solution containing L-his at an activity a_1 and known volumes of a stock solution containing interference were added successively and the potential E' was measured after each addition. Thus a series of values of E' were obtained, each value corresponding to a set of values for the activities of L-his and interference in the same solution. The

Table 2
Measurement of two L-his solutions (6.5×10^{-4} and 5.33×10^{-3} M) by four electrodes

Determination (M)	1	2	3	4	$\overline{X}$	$X\delta_{n-1}$
6.5×10^{-4}	56	51	55	47	51.5	1.95
5.33×10^{-3}	16	17	17	15	16	0.447

Table 3
Selectivity coefficients for some common anions and amino acids fixed primary ion method at optimum conditions (pH 9.5 and 17.3% modifier)

Interference, j	$K_{i,j}^{pot}$	Interference, j	$K_{i,j}^{pot}$
Atenolol (drug)	1.6×10^{-3}	L-Glutamine	8.8×10^{-3}
Phenobarbital (drug)[a]	–	L-Isoleucine	4.5×10^{-3}
Acetaminophen (drug)	1.4×10^{-3}	L-Tryptophan	4.3×10^{-3}
Vitamin B4 (adenin)	5×10^{-5}	L-Phenylalanine	6.6×10^{-3}
Vitamin B3	4×10^{-4}	L-Methionine	2.3×10^{-2}
Vitamin B12	5.4×10^{-4}	L-Aspartic acid	5.7×10^{-4}
Vitamin B2[a]	–	L-Proline	3.8×10^{-3}
Caffein	5×10^{-4}	D-Leucine	1.0×10^{-2}
Nicotinamide	4.7×10^{-5}	L-Glycine	5.8×10^{-2}
Cl^-	6.8×10^{-5}	L-Alanine	3.2×10^{-2}
Citrate	7.1×10^{-4}	L-Threonine	1.1×10^{-2}
NO_3^-	6.7×10^{-5}	DL-Serine	6.5×10^{-3}
$C_2O_4^{2-}$	1×10^{-4}	D-Lysine	1.2×10^{-2}
SO_4^{2-}	9.2×10^{-4}	L-Valine	6.6×10^{-3}
$H_2PO_4^-$	8×10^{-4}	L-Asparagine	1.0×10^{-2}
Acetate	2×10^{-4}	D-Glutamic acid	2.0×10^{-5}

[a] Do not show any interfering effect at their most soluble concentration.

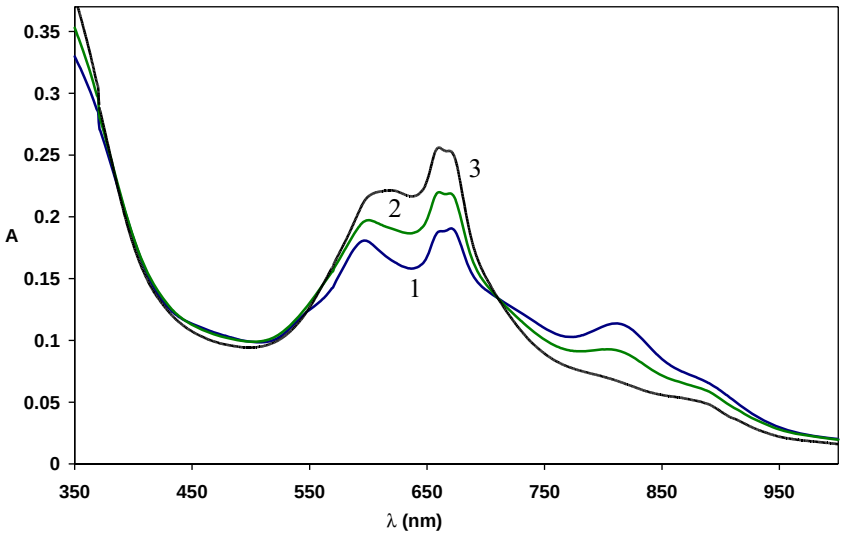


Fig. 5. Spectra of L-his interaction with Cu (3,4tppa) complex in DMSO and borax buffer (pH 9.5) solution (13:1) in three different concentrations of L-his (concentration of 3 (6×10^{-4} M) > 2 (3×10^{-4} M) > 1 (no L-his)).

Table 4
Composition of synthetic solution

Species	Concentration
L-Aspartic acid	4.13×10^{-4}
L-Leucine	3.74×10^{-4}
L-Proline	7.90×10^{-4}
L-Histidine	5.85×10^{-4}
$NaHCO_3$	2.79×10^{-3}
Na_2SO_4	2.34×10^{-3}
Na_2HPO_4	1.30×10^{-3}
KCl	4.28×10^{-3}

Table 5
L-His measurements in synthetic solution (conditions as Table 4)

L-His concentration in synthetic solution (M)	No. of measurements	Found	% Error
5.85×10^{-4}	5	5.70×10^{-4}	−2.56
1.55×10^{-3}	6	1.56×10^{-3}	0.65

selectivity coefficients were evaluated using the following equation:

$$\left(\exp\frac{E' - E}{RT/ZF}\right) a_{\text{his}} - a'_{\text{his}} = K a_j'^{Z/Y}$$

According to this equation if the left hand side of equation is plotted against $a_j'^{Z/Y}$, the slope of the graph should give the value of the selectivity ratio K.

$K_{\text{his},j}$ for several common anions, amino acids, some drugs and some important vitamins which have amino groups in their chemical structure were measured and the results are shown in Table 3.

The UV–vis spectrum of Cu (3,4tppa) in the absence and presence of L-histidine in DMSO and borax buffer solution (13:1) is shown in Fig. 5. There are three isobestic point in this spectrum and as shown in this figure by increasing the concentration of L-his, the absorption at 672 nm ($\lambda_{\max}$) increased, meaning that the absorptivity coefficient of the formed L-his complex is larger than the absorption coefficient of Cu (3,4tppa).

3.7. Practical application

Two synthetic solutions (Table 4) with different amounts of L-his were prepared. The results were obtained from five measurements for 5.85×10^{-4} M of L-his and from six measurements when 1.55×10^{-3} M of L-his were used in these solutions. These results are given in Table 5. To check the capability of applying the developed electrode in a biological matrix it was used to determine L-his in a normal RANDOX serum sample. In this case the serum was diluted approximately seven times and then a certain amount of L-his spiked into it. L-His concentration in this solution was 2×10^{-4} M which is close to L-his concentration in human serum (1.2×10^{-4} M) [6,21]. The average of the obtained measurements was 2.12×10^{-4} M ($n = 8$).

Acknowledgements

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References

[1] R.N. Adams, Anal. Chem. 30 (1958) 1576.
[2] P.T. Kissinger, W.R. Heineman, Laboratory Techniques in Electroanalytical Chemistry, Marcel Dekker Inc., New York, 1984, pp. 295–296.
[3] I. Švancara, K. Vytøas, J. Barek, J. Zima, Crit. Rev. Anal. Chem. 31 (4) (2001) 311.
[4] A. Abbaspour, S.M.M. Moosavi, Talanta 56 (2002) 91.
[5] J. Pi, Q. Yin, J. Zhong, Talanta 381 (1991) 1185.
[6] M.K. Amini, S. Shahrokhian, S. Tangestaninejad, Anal. Chem. 71 (1999) 2502.
[7] M.L. Lee, F.J. Yang, K.D. Bartle, Open Tubular Column Gas Chromatogrphy: Theory and Practice, Wiley, New York, 1984.
[8] J.R. Benson, Am. Lab. 4 (10) (1972) 60.
[9] E. Kasziba, L. Flancbaum, J.C. Fitzpatrick, J. Schneiderman, H. Fisher, J. Chromatogr. Nov. 18 (432) (1988) 315.
[10] V. Frattini, C. Lionetti, J. Chromatogr. A 5809 (1–2) (1998) 241.
[11] N. Tateda, K. Matsuhisa, K. Hasebe, N. Kitajima, T. Miura, J. Chromatogr. B Biomed. Sci. Appl. 6/718 (2) (1998) 235.
[12] M.A. Sanz, G. Castillo, A. Hernandez, J. Chromatogr. A 5/719 (1) (1996) 195.
[13] D.A. Skoog, F.J. Holler, T.A. Nienman, Principles of Instrumental Analysis, fifth ed., Sunders College Publishing, 1998.
[14] D.A. Gerber, Anal. Biochem. 34 (1970) 500.
[15] K. Sugawara, S. Tanaka, M. Taga, Bioelectrochem. Bioenerg. 31 (2) (1993) 229.
[16] G.S. Wang, T.Z. Peng, B.E. Shen, P.L. Zhu, L.M. Qu, Fenxi-Huaxue 23 (6) (1995) 703.
[17] M. Yokote, F. Shibamya, S. Takarin, Kogyo Kagaku Zasshi 67 (1964) 166.
[18] D.C. Harris, Quantitative Chemical Analysis, fourth ed., W.H. Freeman and Company, New York, 1995, p. 260.
[19] R.P. Buck, E. Lindner, Pure Appl. Chem. 66 (1994) 2527.
[20] Recommendations for Nomenclature of Ion Selective Electrodes, IUPAC Analytical Chemistry Division, Commission on Analytical Nomenclature, Pure Appl. Chem. 48 (1976) 127.
[21] C.P. Pau, G.A. Rechnitz, Anal. Chim. Acta 160 (1984) 141.