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Chemically Modified Carbon Paste Electrode For Potentiometric Analysis Of Cyproheptadine Hydrochloride In Serum And Urine

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ABSTRACT

A new carbon-paste electrode for cyproheptadine hydrochloride (CyCl) was prepared and fully characterized in terms of composition, response time, thermal stability and usable pH ranges. The electrode active recognition is by liquid ion-exchange mechanism via the use of the cyproheptadinium-tetraphenylborate (CY-TPB) as ion-exchanger dissolved in tricresyl phosphate (TCP) as a more suitable solvent mediator for the paste and sodium tetraphenylborate (Na-TPB) as additive. The modified electrode showed a Nernstian slope of 58.5±2 mV/decade over the concentration range of 5.2×10^{-6}-1.0×10^{-2} M with very low detection limit of 4.2×10^{-7}. The electrode exhibits good selectivity for CyCl with respect to a large number of inorganic cations, sugars, amino acids. The developed electrode was applied to the potentiometric determination of cyproheptadinium ion in its aqueous solution, pharmaceutical preparations and biological fluids (serum and urine). The proposed potentiometric methods offer the advantages of simplicity, accuracy, automation feasibility and applicability to turbid and colored sample solutions.

INTRODUCTION

Chemically modified carbon paste electrodes (CMCPEs) have been successfully applied as potentiometric sensors for determination of various species[1-3]. One of the most important active recognition elements that can be utilized in the development of potentiometric sensors involves specific ion-exchange mechanism. Such mechanism has been used in the development of very few cation-selective CMCPEs based on different ion-exchangers[4-9].

In comparison with ion-selective electrodes

based on polymeric membranes, CMCPEs possess advantages of ease of preparation, ease of regeneration, and very stable response in addition to the very low Ohmic resistance[10,11], probably due to the formation of very thin film of pasting liquid coated onto small particles of carbon powder[12]. Therefore, CMCPEs have found direct application in a variety of analytical situations, such as amperometry[13,14] and voltammetry[15], in addition to potentiometry[1-9].

Cyproheptadine hydrochloride 4-(5H-dibenzo [a,d]-cyclohepten-5-ylidene)-methyl-piperidine hydrochloride [969-33-5] is a serotonin and a histamine antagonist with antichloinergic, sedative effects and calcium-channel blocking activity. Cyproheptadine hydrochloride is used to treat some hormonal disorders and is used as an adjunct therapy in children who are taking human growth hormone[16].

The reported methods for the determination of cyproheptadine hydrochloride are mainly chromatographic, these methods are highly sensitive, but they are very expensive, involve the use of complex procedures with several sample manipulations and require long analysis times. Besides, none of them is easy to automate. They include high-performance liquid chromatography HPLC[17-22], reversed phase high-performance liquid chromatography RP-HPLC[23-27], thin layer chromatography TLC[28-31] and gas chromatography[32-36], Other alternatives include colorimetric methods[37,38] and UV-spectrophotometry[39,40], visible spectrophotometry[41-43]. The spectrophotometric methods of drug analysis usually suffer from poor selectivity. Only a few reports[44,45] have been devoted to the construction of ion selective electrodes for cyproheptadine. However, these electrodes have not been very fruitful as the developed electrodes have either one, two, or in some cases, all of the following problems, (1) high detection limit, (2) a narrow working concentration range, (3) long response time, (4) serious interferences from various cations, sugars, and amino acids.

In this paper, chemically modified carbon paste electrode was applied to improve the concentration range, limit of detection and working pH ranges, an optimized mixture of graphite, insoluble ion-pair of cyproheptadine-tetraphenylborate, with plasticizing solvent mediator tricresyl phosphate (TCP) and NaTPB as a lipophilic additive was prepared and used in construction of this electrode (Cy-CMCPE) as a potentiometric sensor for cyproheptadinium ion. The results presented in this paper show that the adopted sensor developed for cyproheptadinium ion have a wide concentration range, low limit of detection, good Nernstain slope, high selectivity over a wide variety of other cations, sugars and a mino acids and wider pH range than previous papers[44,45]

EXPERIMENTAL

Reagents and solutions

Cyproheptadine hydrochloride was obtained from Birzeit pharmaceutical company (Birzeit, Palestine) stock solution was prepared to contain 0.01 M CyCl and was standardized against standard NaOH solution[46]. The pharmaceutical preparations containing CyCl (Cyprodine, tablets and syrup) were obtained from the local drug stores. Graphite powder, bis(2-ethylhexyl) adipate (DOA), tricresyl phosphate (TCP) and dioctyl phthalate (DOP) and sodium tetraphenylborate (Na-TPB) and chloride or nitrates salts of all cations, investigated as interferences were used as received from Aldrich. All solutions were prepared with doubly distilled water.

Preparation of ion-exchanger

The ion-exchanger cyproheptadinium-tetraphenylborate (Cy-TPB) was prepared by adding 50 mL of 0.01 M CyCl hot solution to appropriate volume of 0.01 M of Na-TPB. The formed precipitates were filtered off, washed thoroughly with distilled water, dried at room temperature and ground to fine powders.

Preparation of the electrode

Modified carbon paste electrode was prepared by thoroughly mixing weighed amounts of ion pair (Cy-TPB), high purity graphite and tricrsylphosphate until obtaining a uniformly wetted paste. The mixture was packed in the end of a polypropylene syringe (3 mm i.e., 1 ml). Electrical contact to the carbon paste was made by a copper wire. The carbon paste was smoothed onto paper until it had a shiny a appearance and was used directly for potentiometric

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measurements without preconditioning requirements.

Apparatus

The potential measurements were carried out at 25±0.1 with a digital millivoltmeter (SR-MUL-3800). pH measured were made on a digital pH meter (HANNA pH 211). Ag-AgCl with KCl (saturated) were used as a reference electrode. The electrochemical system is represented as follows:

Cy-CMCPE/test solution/Ag-AgCl, KCl (saturated).

Selectivity

The selectivity coefficients $-\log K^{pot}_{CyJj} z+$ of the electrode towards different cationic species, sugars and amino acids were determined by the matched potential method (MPM)[47,48].

Potentiometric determination of CyCl

The standard addition method[49] was applied: small increments (10-100 μL) of standard CyCl solution (0.01) were added to 50 mL aliquot samples of various concentrations from the drug sample solution equivalent to 0.175-3.5 mg CyCl. The change in potential at (25 ± 0.1°C) was recorded for each increment, and this data was used to calculate the concentration of CyCl in the sample solution.

Determination of CyCl in cyprodine, tablets

The content of 35 tablets (4 mg CyCl/tablet) of cyprodine were powdered, and an accurately weighed portion equivalent to 100 mg was dissolved in 100 ml distilled water according the method of British Pharmacopoeia[50]. Different volumes of this solution (1.0-5.0 ml) were taken and subjected to the potentiometric determination using the present electrode.

Determination of CyCl in biological fluids

Different quantities of CyCl and 1.5 mL serum or 4 mL urine were transferred to a 50 mL measuring flask and completed to the mark with doubly distilled water to give solutions of concentrations ranging from 2.0×10^{-6} to 1.6×10^{-4} M CyCl. These solution are subjected to the standard additions method.

Potentiometric titration

An aliquot of CyCl solution containing 3.5-17.5 mg CyCl was transferred into 100 mL beaker, diluted to approximately 50 mL with distilled water and then titrated against a standard solution of Na-TPB. The end point were determined from the S-shaped curve by first and second derivative plots.

RESULT AND DISCUSSION

Composition, response behavior and characteristics of the electrode

It is well known that the selectivity, linear dynamic range and sensitivity obtained for a given CMCPE depends significantly on the paste composition[51], the nature of the solvent mediator[1,51] and any additive used[52]

Therefore, in the first step in this study, sixteen compositions of the paste containing various a mounts of graphite, plasticizers (DOP, DOA and TCP), ion-pair and additive (Na-TPB) were prepared.

The influence of the plasticizer type and concentration on the characteristics of the Cy-sensor was investigated by using three plasticizers with different polarities including DOP, DOA and TCP. It is noted that although significant cyproheptadine response was observed with most plasticizers, pastes containing the polar plasticizer TCP yielded the best detection limits and total EMF response. It appears that the relatively high polarity of this plasticizer (dielectric constant, ε(TCP)= 6.9 vs, ε(DOP)=5.1 and ε(DOA)=3.9) enables a stronger cooperative ion pairing Cy-TPB in the paste phase, thereby enhancing the extraction of cyproheptadine ions into the paste film. As is quite obvious from EMf pCy plots (Figure 1), the use of TCP results in a Nernstian linear plot over a wide concentration range, whereas in the case of other solvent mediators, the slopes of the potentiometric response are much different from the expected Nernstian value of 59.5 mV/concentration decade. As is quite obvious from emfpCy plots (Figure 1), the use of TCP results in a Nernstian linear plot over a wide concentration range, whereas in the case of other solvent mediators, the slopes of the potentiometric response are much different from the expected Nernstian value of 59.5 mV/concentration decade.

Besides the critical role of the nature and the

amount of plasticizer in preparing Cy-CMCPE, the influence of the amount of the ion-exchanger on the potential response of the electrode were investigated and the results are summarized in TABLE 1. As can be seen, the electrode without modifier ion exchanger (electrode No.1) showed a Nernstian slope of 44.3 mV/decade over the concentration range of 2.2×10^{-5}-1.0×10^{-2} M with detection limit of 9.3×10^{-6}. On other hand, the electrode made of 1.0% (w/w) ion-exchanger exhibits the best performance.

It should be noted that the presence of lipophilic anions in cation-selective electrodes not only diminishes the ohmic resistance and enhances the response behavior and selectivity but also, in case where the extraction capability is poor, increase the sensitivity of the electrode[53-55]. Moreover, the lipophilic additives may catalyze the exchange kinetics at sample-electrode interface. As TABLE 1 shows in the absence of Na-TPB, the electrodes exhibit near Nernstian slope (electrode No. 2), limited LOD and concentration ranges, while adding more a mounts of Na-TPB (electrode No. 4, 5) asuper Nernstian can be obtained. The electrode showed better potentiometric response by adding 1.0% Na-TPB (electrode No. 3).

Among the different compositions studied, the paste incorporating 55% graphite, 1.0% Cy-TPB, 43% TCP and 1.0% NaTPB (electrode No. 3) exhibit the best response characteristics (TABLE 1) . Therefore, this composition was used to study various operation parameters of the electrode. The electrochemical performance characteristics of this electrode were systematically evaluated according to the IUPAC recommendations[56].

The dynamic response time[56] of the electrode was tested by measuring the time required to achieve a steady state potential (within ± 1 mV) after successive immersion of the electrode in a series of CyCl solutions, each having a 10-fold increase in concentration from 1.0×10^{-6} to 1.0×10^{-2} M. The electrode yielded steady potentials within 10-15 s. It is observed that the response times for higher concentrations are shorter than those for the diluted solutions, this can be explained to be due to the enhancement of the ion exchange process of the electrode at higher concentrations. The potential reading stay constant, to within ± 1 mV, for at least 15 min.

The repeatability of the potential reading of the electrode was examined by subsequent measurements in 1×10^{-4} M of CyCl solution immediately after mea-

TABLE 1: Characteristics of the Cy-CMCPEs evaluated from the calibration curves

Composition (%)					Slope (mV/decade)	Linear range (M)	Detection limit (M)
No.	Graphite	Ion-pair	Plasticizer	NaTPB			
1	55.0	--	45.0 (TCP)	--	44.3	$2.2x10^{-5}$-$1.0x10^{-2}$	$9.3x10^{-6}$
2	55.0	1.0	44.0 (TCP)	--	54.2	$6.0x10^{-6}$-$1.0x10^{-2}$	$2.8x10^{-6}$
3	55.0	1.0	43.0 (TCP)	1.0	58.6	$3.8x10^{-6}$-$1.0x10^{-2}$	$4.6x10^{-7}$
4	54.0	1.0	42.0 (TCP)	3.0	64.8	$5.5x10^{-6}$-$1.0x10^{-2}$	$1.5x10^{-6}$
5	54.0	1.0	42.0 (TCP)	7.0	78.8	$5.5x10^{-6}$-$1.0x10^{-2}$	$2.0x10^{-6}$
6	54.0	5.0	41.0 (TCP)	--	60.1	$7.5x10^{-6}$-$1.0x10^{-2}$	$4.2x10^{-6}$
7	53.0	5.0	41.0 (TCP)	1.0	63.2	$5.3x10^{-6}$-$1.0x10^{-2}$	$2.7x10^{-6}$
8	51.0	8.0	38.0 (TCP)	--	69.2	$7.8x10^{-6}$-$1.0x10^{-2}$	$6.3x10^{-6}$
9	51.0	8.0	38.0 (TCP)	1.0	77.0	$6.3x10^{-6}$-$1.0x10^{-2}$	$4.1x10^{-6}$
10	50.0	10.0	40.0 (TCP)	--	65.0	$8.8x10^{-6}$-$1.0x10^{-2}$	$6.5x10^{-6}$
11	48.0	15.0	36.0 (TCP)	--	53.0	$9.5x10^{-6}$-$1.0x10^{-2}$	$7.3x10^{-6}$
12	55.0	1.0	44.0(DOP)	--	56.2	$7.3x10^{-6}$-$1.0x10^{-2}$	$5.7x10^{-6}$
13	55.0	1.0	43.0(DOP)	1.0	56.3	$5.0x10^{-6}$-$1.0x10^{-2}$	$2.3x10^{-6}$
14	54.0	5.0	41.0 (DOP)	--	53.1	$7.5x10^{-6}$-$1.0x10^{-2}$	$5.3x10^{-6}$
15	55.0	1.0	44.0(DOA	--	51.7	$1.2x10^{-5}$-$1.0x10^{-2}$	$8.3x10^{-6}$
16	55.0	1.0	43.0(DOA)	1.0	52.6	$8.3x10^{-6}$-$1.0x10^{-2}$	$6.8x10^{-6}$
17	54.0	5.0	41.0(DOA)	--	53.3	$9.5x10^{-6}$-$1.0x10^{-2}$	$7.3x10^{-6}$

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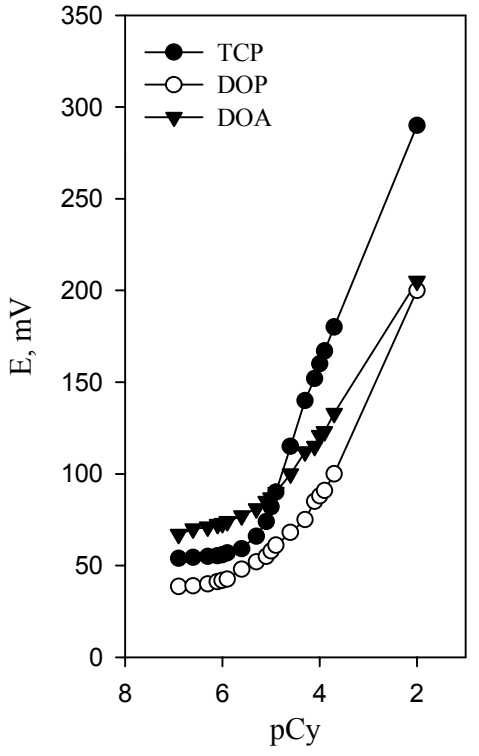

Figure 1: Effect of different plasticizers on the potential response of Cy-CMCPE

suring the first set of solutions at 1.0×10^{-3} M of CyCl. The standard deviation of measuring emf for 4 replicate measurements was found to be 1.854 in 1.0×10^{-4} M solution and 0.854 in 1.0×10^{-3} M solution. The slope of the calibration graph obtained by this electrode was found to decrease slightly after several times of use, which may be attributed to surface contaminations. In this case, a new section from the master paste was found to function very properly.

Effect of temperature

Calibration graphs (electrode potential, E_{elec} vs pCy) were constructed at different test solution temperatures: 20, 25, 35, 45ºC. The isothermal coefficient (dE_{elect}/dt) of the electrode was calculated[57] and found to be 0.0003 V/ºC and (dE_{cell}/dt) equals 0.0009 V/ºC. These values indicate fairly high thermal stability of the electrode within the temperature range investigated and shows no deviation from the theoretical Nernstian behavior.

Effect of pH

The effect of pH on the response of the Cy-CMCPE was examined with a series of sample solutions (1×10^{-4} and 1×10^{-3}M CyCl). The pH was adjusted by adding small volumes of (0.1-1 M) HCl acid or sodium hydroxide to the test solutions and the variation of potential was followed. As can be seen from figure 2, the variation in potential due to pH change is considered acceptable in the pH range 2.7-7.8. Nevertheless, at pH values lower than 2.5, the observed drift may be due to the protonation of Cy-TPB, therefore, causing its diminished tendency for ion-exchange. On the other hand, at pH values higher than 8.0 the potential decrease gradually, which can be attributed to the formation of the free cyproheptadine base in the test solution.

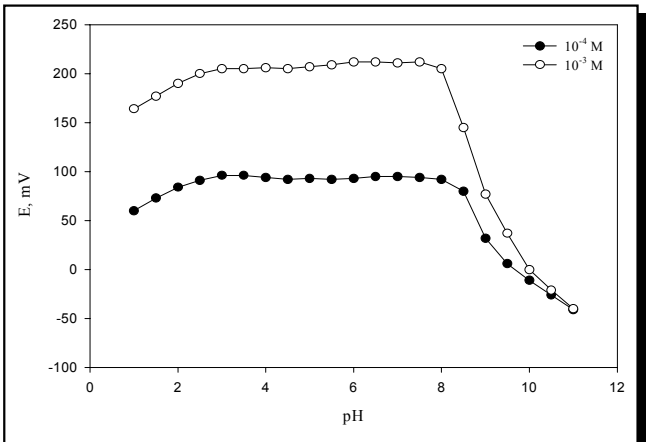

Figure 2: Effect of the test solution on the potential response of the Cy-CMCPE

Selectivity of the electrode

Selectivity is an important characteristic, which defines the nature of the device and the range to which it may successfully employed. It is measured in terms of potentiometric selectivity coefficient ($K_{M,J}$), The selectivity of Cy-CMCPE towards many inorganic cations, sugars and amino acids was measured by using the matched potential method[47,48]. Among the different mixed solution methods, the matched potential method is unique in that it depends neither on the Nicolsky-Eisenman equation nor on any of its modifications. This method was recommended in 1995 by IUPAC as a method that gives analytically relevant practical selectivity coefficient values. To determine the selectivity coefficient of different interfering ions for Cy-CMCPE, the potential of a reference solution of CyCl was measured and specified amounts of CyCl (a_{Cy}) in the range of 2×10^{-4} to 5×10^{-5}M were added to the refer-

ence solution, the potential was measured and the corresponding potential change (ΔE) is recorded. In a separate experiment, the interfering ions (J) (1.0×10^{-1} M) were successively added to an identical reference solution of CyCl until the change in potential matched the ΔE value. The values of selectivity coefficients, are then calculated using the following equation:

$$-\log K^{pot}_{Cy,J^{z+}} = \alpha_{c_y} / \alpha_J$$

where α_J is the activity of the added interferent

The selectivity coefficient values $-\log K^{pot}_{Cy,J^{z+}}$ of the electrodes listed in TABLE 2 reflect a very high selectivity of these electrodes for cyproheptadinium cation. The mechanism of selectivity is mainly based on the stereospecificity and electrostatic environment and it is dependent on how much fitting is present between the locations of the lipophilicity sites in the two competing species in the bathing solution side and those present in the receptor of the ion exchanger[57]. Inorganic cations do not interfere because of differences in ionic size, mobility and permeability. The electrodes are also selective to Cy^+ over number of sugars, amino acids,. The performance of the electrodes was examined in presence of different anions including chloride, nitrate, sulphate. No effect was observed from these anions except sulphate. However, this is not present in the formulations. For this reason, they do not prejudice the quality of the determinations.

TABLE 2: Selectivity coefficients $-\log K^{pot}_{Cy,J^{z+}}$ of various interfering ions

Interferent	$-\log K^{pot}_{Cy,J^{z+}}$
Na	3.12
K	2.48
NH4	1.62
Cu	1.43
Co	2.71
Mg	3.13
Ca	2.37
Ba	3.25
Glucose	4.12
Maltose	3.95
L-Histidine	3.82
L-Cystine	3.62
Lactose	3.05
Fructose	3.19

Analytical application

The Cy-CMCPE was used as indicator electrode in the potentiometric titration of CyCl with NaTPB, and the resulting titration curve is shown in figure 3. As seen, the amount of Cy ion in pure solution can be a accurately determined with this electrode. The standard additions method was applied for determination CyCl content in its pharmaceutical preparations (tablets and syrup), as can be seen in TABLE 3 the recovery of CyCl is almost quantitative.

Clinical pharmacological studies indicates that, after a single 4 mg oral dose of 14C-labelled cyproheptadine HCl in normal subjects, at least 40% of the administered radioactivity was excreted in the urine[58]. Accordingly we presented this method for determination of CyCl in serum and urine samples spiked with known amounts of CyCl. The standard

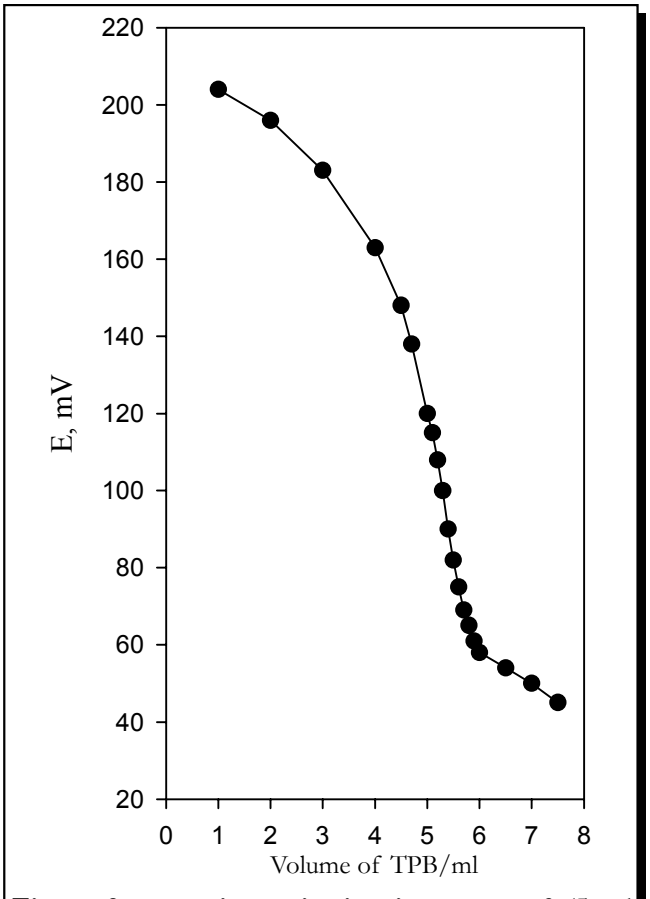

Figure 3: potentiometric titration curve of (5 ml of 1×10^{-2} M) CyCl with (1×10^{-2} M) Na-TPB using the proposed electrode as an indicator

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TABLE 3: Recovery of CyCl from cyprodine (syrup and tablets) and spiked serum and urine samples using proposed electrode Cy-CMCPE

Sample	CyCl[a] (mg)	Recovery[b] %	RSD %	$F^{3,3}$ value (9.28)	t-values
Syrup (Cyprodine)	0.399	97.85	0.019	1.28	2.78
	1.995	101.12	0.023	1.19	2.06
	3.990	100.53	0.017	3.26	2.66
Tablets (Cyprodine)	0.399	98.59	0.032	4.55	2.32
	1.197	103.32	0.038	5.83	1.75
	3.990	102.4	0.017	2.79	3.12
Serum	0.175	102.61	0.028	1.43	3.05
	1.050	96.27	0.016	2.96	4.96
	1.750	100.23	0.025	4.74	2.41
Urine	0.350	95.89	0.019	5.66	1.98
	1.050	101.82	0.028	3.63	2.88
	2.800	100.23	0.017	3.46	2.13

[a] Milligrams of CyCl spiked to serum or urine samples
[b] Average of five determinations

additions method was applied for the determination of CyCl in these real samples to overcome the matrix effect as given in TABLE 3.

The results of applying the above methods are compared with the values obtained from the official method. F-test was used for comparing the precisions of the two methods and t-test for comparing the accuracy[59]. The calculated F- and T- test TABLE 3 were less than the critical (tabulated) ones. Thus, there is no significant difference between the precisions or the accuracies of the two methods at 95% confidence levels.

CONCLUSIONS

The proposed chemically modified carbon paste electrode based on cyproheptadine tetraphenylborate as electroactive ion exchanger might be a useful analytical tool and interesting alternative for the determination of Cy^+ in pharmaceutical preparations and biological samples. The electrode shows high sensitivity, reasonable selectivity, fast static response, long term stability and applicability over a wide concentration range with minimal sample pretreatment.

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