Chemically Modified Carbon Paste Sensor for Potentiometric Determination of Doxycycline Hydrochloride in Batch and FIA Conditions

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The utility of carbon paste electrode for the determination of doxycycline HCl modified with doxycycline-tetraphenylborate ion-pair (in both batch and flow injection analysis (FIA) modes) is demonstrated. The electrode revealed a Nernstian response over a wide concentration range (1.99×10^{-5} - 3.19×10^{-3} mol L^{-1}). The detection limit of this sensor is 1.33×10^{-5} mol L^{-1}. The best performance was obtained with carbon paste composition of 3% doxycycline-tetraphenylborate, 48.5% graphite and 48.5% dibutyl phthalate (DBP). The sensor exhibits a very fast response time (9 s) and good selectivity in presence of inorganic cations, sugars and aminoacids. The proposed sensor shows a great improvement in comparison with other previously reported sensors. The sensor was successfully applied to monitoring of doxycycline in pure solution and pharmaceutical formulation (vibramycin and farcodoxin capsoules) with recovery ranges from 97.30 - 105.37% and coefficient of variation from 0.54 to 2.40%.

1. INTRODUCTION

Doxycycline, 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11 dioxonaphthacene -2-carboxamide, is a broad-spectrum antibiotic possessing the advantage of being readily absorbed into blood stream after oral administrations and used for treatment of malaria. Many methods for its determination were reported including spectrophotometry [1,2], chromatoghraphy [3-5], and electrometry [6,7]. Most of these methods, in spite of their good sensitivities, are very expensive, time consuming and some of them are not easy to automate. Potentiometric detection based on ion-selective electrode as a simple method offers several

advantages, such as speed, ease of preparation, simple instrumentation, relatively fast response, wide dynamic ranges, selectivity and low cost. Recently, a group of ion-selective electrodes have been used for doxycycline measurements including plastic membrane ion selective electrode [8] and solid contact electrode [9]. However, these electrodes lack good stability, sufficient selectivity and suffer from long response time.

Since innovation of carbon paste electrode (CPEs) by Adams, they are widely used in many fields such as voltammetry, amperometry and potentiometry [10]. In comparison with ion-selective electrodes based on polymeric membranes, chemically modified carbon paste electrodes (CMCPEs) possess advantages of ease of preparation, ease of regeneration, stable response, very low Ohmic resistance [11], in addition to, lower detection limits with respect to those with an internal reference solution.

This paper describes the construction, potentiometric characterization, and analytical application of doxycycline chemically modified carbon paste electrode (Dc-CMCPE) based on the use of doxycycline-tetraphenylborate ion-associate (Dc-TPB) as electroactive materials and dibutylphthalate (DBP) as plasticizer. The results presented show that the sensor constructed for doxycycline cation has a wide concentration range, low limit of detection, good Nernstian slope, and high selectivity over a wide variety of other cations and compounds compared with the previously reported electrodes [8,9].

2. EXPERIMENTAL

2.1. Reagents and materials

All chemicals were of analytical grade. Double distilled water was used throughout all experiments. Pure grade doxycycline hydrochloride was obtained from Kunshan chemical and pharmaceutical Co., Ltd. and the pharmaceutical preparation Farcodoxin and Vibramycin capsules were provided by Pharco Company for Pharmaceutical Industries, Alexandria, Egypt and Pfizer Company for Pharmaceutical Industries, Cairo, Egypt, respectively.

Sodium tetraphenylborate (NaTPB), dibutyl phthalate (DBP), dioctyl phthalate (DOP), tricresyl phosphate (TCP) and graphite powder were from Aldrich. Ethylhexyl adipate (EHA) was obtained from Fluka.

Stock solutions of doxycycline, 5×10^{-2} mol L^{-1}, were prepared by dissolving accurately weighed amount of pure solid in double distilled water, stored in dark bottles and kept in the refrigerator for no more than 3 days, solutions of low concentrations were prepared daily by appropriate dilution.

2.2. Apparatus

The potentiometric measurements in batch mode were carried out with a Jenway 3010 digital pH/mV meter, England. A Techne circulator thermostat Model C-100 (Cambridge, England) was used

to control the temperature of the test solution. A Water Treatment Warehouse Inc. (WTW) packed saturated calomel electrode (SCE) was used as an external reference electrode. All measurements were carried out at 25 ºC with a cell of the following type: Dc-CMCPE/ test solution/SCE. The flow injection system was set up as previously reported [12].

2.3. Preparation of the ion-exchanger

Doxycycline-tetraphenylborate (Dc-TPB) was prepared by mixing equal volumes of equimolar solutions of NaTPB and doxycycline HCl and kept for 12 h in dark place for complete precipitation. The pale yellow precipitate was filtered, washed till chloride free, dried and used as the active ingredient for preparing the chemically modified carbon paste electrode of doxycycline hydrochloride. The composition of the solid ion-exchanger was confirmed by elemental analysis and found to be 1:1 Dc-TPB.5H$_2$O. The C, H and N percentages are 65.1, 4.6 and 4.4%, the corresponding calculated ones are 64.7, 5.1 and 3.3%, respectively.

2.4. Preparation of the electrode

A teflon holder (12 cm length) with a hole at one end (7 mm diameter and 3.5 mm deep) for the carbon paste filling served as the electrode body [13]. The carbon paste was smoothed onto paper until it had a shiny appearance and was used for potentiometric measurements after soaking in 10^{-3} mol L^{-1} of Dc.Cl for 15 minutes. After several times of use (5 days), a fresh electrode surface can be obtained by squeezing out a small amount of the paste, scrapping off the excess and polishing the electrode on a smooth paper to obtain a shiny appearance again.

2.5. Calibration graph

The performance of the electrode was investigated by measuring its potential in prepared doxycycline solutions of a concentration range of 10^{-6} to 10^{-2} mol L^{-1} by serial dilution. Each solution was stirred and the potential was recorded using carbon paste electrode and saturated calomel electrode when it became stable. The potential readings of the stirred solutions were measured at 25±1 ºC and plotted versus the negative logarithmic of the drug concentration, pDc (-log[Dc.Cl]). The constructed calibration graphs were used for subsequent measurements of unknown Dc.Cl test solutions.

2.6. Selectivity of the electrode

Potentiometric selectivity factor was evaluated using the matched potential method [14]. According to this method, the activity of the analyte was increased from $a_A = 1.0 \times 10^{-5}$ mol L^{-1} (reference solution) to $a'_A = 1.4 \times 10^{-5}$ mol L^{-1}, and the change in potential (ΔE) corresponding to this increase in activity is measured. Then, 0.1 mol L^{-1} solution of an interfering ion is added to a new 1.0×10^{-5} mol L^{-1} analyte reference solution until the same potential change (ΔE) is recorded [15], the

concentration of the added amount is thus a_B. The selectivity coefficient $K_{A,B}^{MPM}$ for each interferent was calculated using the following equation:

$$K_{A,B}^{MPM} = \frac{a'_A - a_A}{a_B}$$

In FI, solutions that are 1.0×10^{-3} mol L^{-1} of interfering ions were prepared and the corresponding peak heights were measured. The peak heights were converted to millivolts and then compared to that obtained from the standard drug of the same concentration under the same conditions. The selectivity coefficients were calculated using the following equation:

$$\log K_{Drug,J^{Z+}}^{pot} = \frac{E_2 - E_1}{S} + \log [Drug] - \log [J^{z+}]^{1/z}$$

2.7. Potentiometric determination

The standard addition method was applied [16]. In this method, the proposed electrode was immersed into a sample of pure solution or pharmaceutical formulations (50 mL) with unknown concentration ($2.0 \times 10^{-4} - 1.0 \times 10^{-3}$ mol L^{-1}) and the equilibrium potential of E_u was recorded, then 0.2 mL of 1.0×10^{-2} mol L^{-1} of standard doxycycline solution was added into the test solution and the equilibrium potential (E_s) was measured. From the potential change ($\Delta E = E_s - E_u$), one can determine the concentration of the test sample using the following equation:

$$C_X = C_S \left(\frac{V_S}{V_X + V_S} \right) \left(10^{\,n(\Delta E/s)} - \frac{V_S}{V_X + V_S} \right)^{-1}$$

Where C_X is the concentration to be determined, V_X is the volume of the original sample solution, V_S and C_S are the volume and concentration of standard solution added to the sample to be analyzed, respectively, ΔE is the change in potential after addition of certain volume of standard solution, and S is the slope of the calibration graph.

2.8. Analysis of doxycycline in pharmaceutical formulations

The contents of ten capsules were accurately weighed and powdered in a mortar; the required amount from the capsules powder was dissolved in about 30 mL double distilled water and filtered in a 50 mL measuring flask. The residue was washed three times with double distilled water, and the volume was completed to the mark using double distilled water. The contents of the measuring flask were transferred into a 100 mL beaker, and subjected to potentiometric determination of Dc.Cl.

In FI, a series of solutions of different concentrations was prepared from the capsules and the peak heights were measured, and then compared with those obtained from injecting a standard solution of the same concentration prepared from pure Dc.Cl.

3. RESULTS AND DISSCUTION

3.1. Composition, response behavior and characteristics of the electrode

Doxycycline-tetraphenylborate (Dc-TPB) as an ion-sensor was found to be highly sensitive to Dc^+ with respect to several other cations. Therefore, the performance of the CMCPE containing this ion-exchanger in aqueous solutions was studied in details. It is well known that the selectivity, linear dynamic range and sensitivity obtained for a given CMCPE depend significantly on the paste composition [17], the nature of the solvent mediator [11,18] and additives used [19].

The amount of ion-exchanger in the paste affects the response of the electrode, so four paste compositions were prepared by varying the percentage of Dc-TPB. The results in Table 1 show that the electrodes containing 1, 2, 3 and 5% have slopes of 62.10, 63.97, 62.30 and 65.52 mV/decade, respectively. Although, The four electrodes have the same linear range and almost the same detection limit and slope except the paste containing 5% of Dc-TPB has narrow linear range and higher detection limit, the electrode with 3% of Dc-TPB has a long life time up to 5 days in comparison to other electrodes.
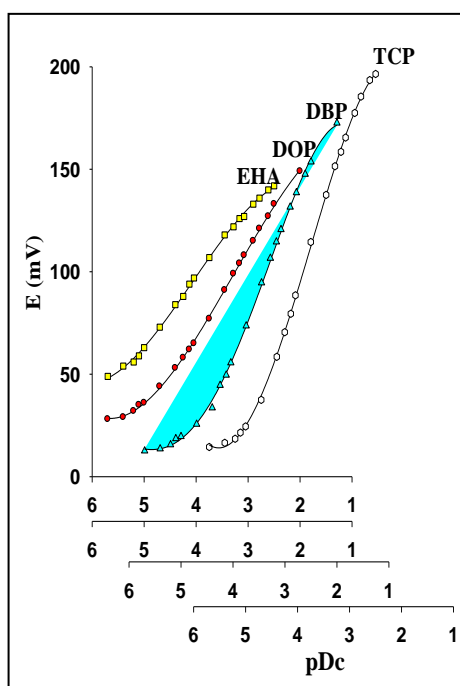
3.2. Effect of plasticizer

Figure 1. Effect of different plasticizers on the response of Dc-TPB CMCPE.

Two parameters are of importance when manufacturing a carbon paste: 1) its mechanical stability and 2) its active surface area. Mechanical stability can be interpreted as the ability of the carbon paste to avoid erosion in solution.

Table 1. Composition, slope, linear ranges, and detection limits of calibration curves for doxycycline chemically modified carbon paste electrode at 25±1 ºC and 15 minutes of soaking in 1.0×10^{-3} mol L^{-1} Dc.Cl solution.

Paste No.	Composition % w/w				Slope mV/ decade	Linear range (mol L^{-1})	LOD (mol L^{-1})
	sensor	Graphite	Plasticizer	Additive %			
1	1	49.5	49.5 (DBP)	---	62.10	1.99×10^{-5}-3.19×10^{-3}	1.00×10^{-5}
2	2	49.0	49.0 (DBP)	---	63.97	1.99×10^{-5}-3.19×10^{-3}	1.00×10^{-5}
3	*3	48.5	48.5 (DBP)	---	62.30	1.99×10^{-5}-3.19×10^{-3}	1.33×10^{-5}
4	5	47.5	47.5 (DBP)	---	65.52	3.98×10^{-5}-3.19×10^{-3}	2.37×10^{-5}
5	3	48.5	48.5 (TCP)	---	74.50	1.00×10^{-5}-3.19×10^{-3}	7.94×10^{-6}
6	3	48.5	48.5 (EHA)	---	33.26	6.30×10^{-6}-2.45×10^{-3}	3.98×10^{-5}
7	3	48.5	48.5 (DOP)	---	41.18	1.99×10^{-5}-1.00×10^{-2}	1.00×10^{-5}
8	3	48.25	48.25 (DBP)	0.5 NaTPB	73.06	3.98×10^{-5}-3.18×10^{-3}	1.25×10^{-5}
9	3	48.00	48.00 (DBP)	1.0 NaTPB	69.31	1.99×10^{-5}-3.18×10^{-3}	1.49×10^{-5}
10	3	48.25	48.25 (DBP)	0.5 KTPB	66.45	1.99×10^{-5}-3.18×10^{-3}	1.00×10^{-5}
11	3	48.00	48.00 (DBP)	1.0 KTPB	64.66	1.99×10^{-5}-3.18×10^{-3}	1.12×10^{-5}

The use of plasticizers will give some permeable properties to the paste and will improve its mechanical stability by promoting binding between grains [20]. In addition, the solvent mediator, in particular, has a dual function; liquifying agent, enabling homogenous solubilization and modifying the distribution constant of the active ingredient used. The proportion of solvent mediator must be optimized in order to minimize the electrical asymmetry of the paste, to keep the sensor as clean as possible, and to stop leaching to the aqueous phase [21]. In exploration for a suitable plasticizer for constructing this electrode, four plasticizers were used, with different values of dielectric constants, lipophilicity and molecular weight, respectively listed in parentheses, namely, DOP ($\varepsilon r = 5.1$, $P_{TLC} =$

7.0, M.wt. = 391), DBP (εr = 6.4, P_{TLC} = 4.5, M.wt. = 278), TCP (εr = 6.9, M.wt. = 368) and EHA (εr = 4.13, M.wt = 370.57) [22] to figure out the plasticizer with the best response. The sensor with DBP as a solvent mediator produced the best response, as shown in Figure (1), likely due to better dielectric characteristics of DBP comparing to other solvents, and the ability of DBP to extract doxycycline ions from the aqueous solution to the organic paste phase [23]. It is well known that the sensitivity and selectivity of the ion-selective sensors not only depend on the nature of ion-exchanger used but also significantly on the paste composition, the nature of plasticizers and any additives used, so NaTPB and KTPB were tested as additives on the paste ingredients as shown in Table 1. Addition of NaTPB and KTPB resulted in super Nernstian response with narrow linear range and high detection limit.

Among the different compositions studied, a paste containing ion-exchanger complex 3.0 wt% Dc-TPB, 48.5 wt% graphite, 48.5 wt% DBP exhibited the best response characteristics and the lowest detection limit. Therefore, this composition was used to study various operation parameters of the electrodes. The electrochemical performance characteristics of this electrode were systematically evaluated according to the IUPAC recommendations [24].

### 3.3. Life time of the electrodes

The performance of the electrodes was studied as a function of soaking time. Calibration plots (pDc versus E (mV)) were obtained after the electrode was soaked continuously in 1×10^{-3} mol L^{-1} Dc.Cl solution for 1/4, 1/2, 1, 2, and 3 hours and 1, 5, and 7 days. The results indicate that the slope of the calibration graph was 63.59 mV/decade after 1/4 hour of soaking, and then remained constant near 59.60 mV/decade for up to one day of soaking, then decreased reaching 52.60, and 46.66 mV/decade after 5, and 7 days of soaking, respectively.

These results indicate that the life span of Dc-TPB electrode is 5 days. The main property of using modified carbon paste electrodes is that the electrode surface can be renewed, so by cutting the exhausted surface and use a new surface of electrode, the slopes of electrode increase again to reach about 53.30 mV/decade.

3.4. Response time

The dynamic response time [24] 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 Dc$^+$ solutions, each having a 10-fold increase in concentration from to 1.0×10^{-5} to 1.0×10^{-3} mol L^{-1}. The electrode yielded steady potentials within 7-10 s. The potential reading stays constant, to within ±1 mV, for at least 2 minute.

To evaluate the memory effect of the electrode, a similar procedure with opposite direction was adopted. The measurements were performed in the sequence of high-to-low sample concentration and the results are shown in Figure 2. It shows that the potentiometric response of the sensor has no memory effect.
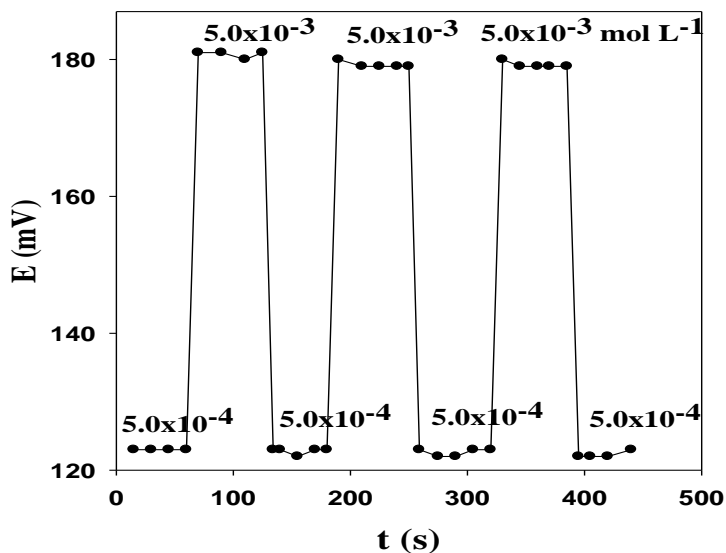
Figure 2. Dynamic response characteristics of Dc-TPB CMCPE for several high to low cycles

3.5. Effect of pH

The effect of pH on the potential values of the doxycycline electrode was tested by measuring the EMF of the cell in the tested solution in which the pH was varied by adding HCl and/or NaOH solution (each 0.1–1.0 mol L^{-1}).
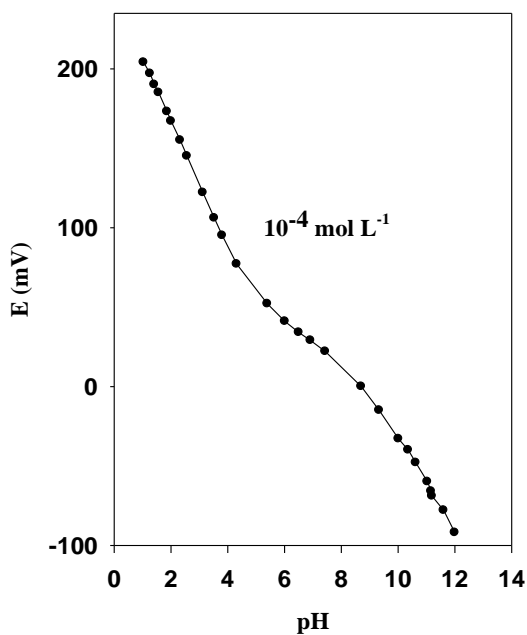
Figure 3. Effect of pH on the potential response of Dc-TPB CMCPE.

The electrode potential decreases markedly with increasing of pH till pH=12 (Figure 3). A similar effect of pH on the potential was also observed with the conventional PVC plastic membrane electrode [8] and solid contact electrode [9]. The three dissociation constants (pKai) of doxycycline are 3.40, 7.7, and 9.3, respectively. The remarkable effect of pH is due to the fact that doxycycline is an amphiprotic compound. It was also observed that the color of Dc changed with increasing of pH or time, this fact can be explained due to the cause of lactone isomerism of doxycycline molecule [9, 25]. In addition, dehydration of doxycycline takes place easily in a strongly acidic solution [25], so acetate buffer solutions with different pH values covering the acidic range (1.0-3.0 pH), at which the protonated form of doxycycline is present, were prepared and used as test solution. The response of the electrode in presence of acetate buffer solution (HCl+CH$_3$COONa) largely deviated from Nernstian value with narrow linear range and high detection limit in comparison with aqueous medium.

3.6. Effect of temperature

To investigate the thermal stability of the electrode, calibration graphs [E$_{cell}$ versus pDc] were constructed at different test solution temperature covering the range 20-50 ºC. The electrode exhibits good Nernstian behavior in this temperature range. The standard cell potentials (E$^o_{cell}$), were determined at different temperatures from the respective calibration plots as the intercepts of these plots at pDc = 0, and were used to determine the thermal coefficient (dE^o/dt) of the cell with the aid of the following equation [26]:

E$^o_{cell}$= E$^o_{cell\ (25ºC)}$ + (dE^o/dt)$_{cell}$ (t-25)

Plot of E$^o_{cell}$ versus (t−25) produced a straight line of slope equals to -2.24×10^{-3} V/◦C. This value is the thermal temperature coefficient of the cell. The values of the standard potentials of Dc-CMCPE were calculated at the different temperature from the relation:

E$^o_{electrode}$ = E$^o_{cell}$ + E$^o_{reference}$

Plot of E$^o_{electrode}$ versus (t-25) gave a straight line; its slope was taken as the thermal temperature coefficient of the electrode which amounts to -2.89×10^{-3} V/◦C. The small values of (dE◦/dt)$_{cell}$ and (dE^o/dt)$_{electrode}$ reveal the high thermal stability of the electrode within the investigated temperature range.

3.7. Flow injection (FI) response

The dispersion coefficient of Dc-TPB electrode was found to be 2.09, i.e. limited dispersion that aids optimum sensitivity and fast response of the electrodes [27]. Samples of different volumes (19.0 – 500.0 μL) were injected. In general, the higher the sample volume, the higher the peak heights and residence time of the sample at the electrode surface, thus, requires a longer time to reach a steady state and greater consumption of sample [28]. A sample loop of size 150 μL was used throughout this work, giving maximum peak height, less consumption of reagents, and a short time to reach the base line.
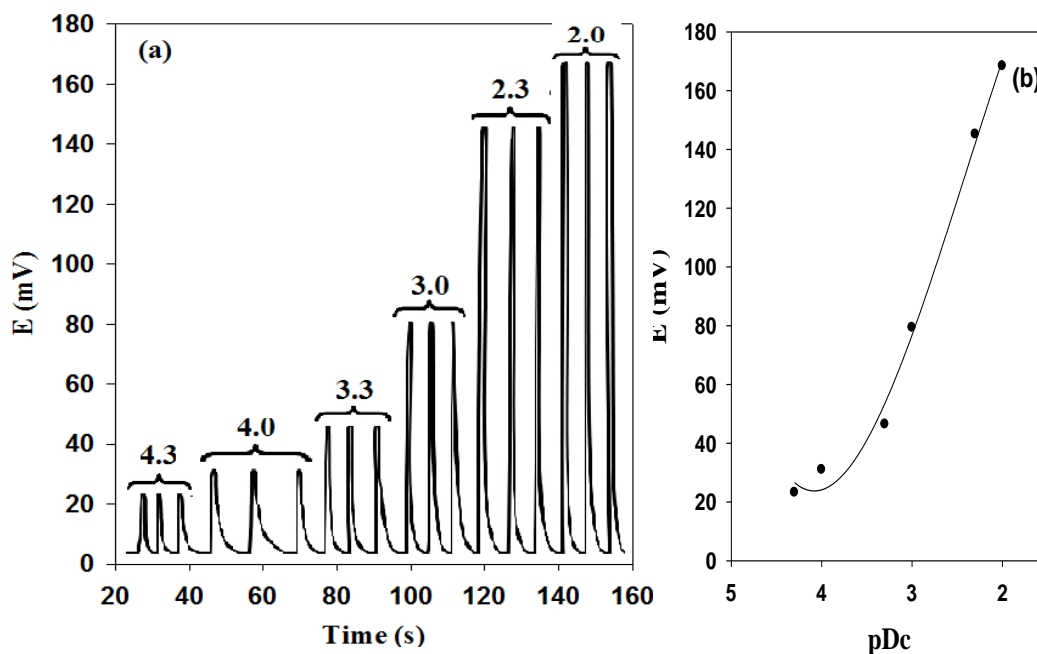
Figure 4. The recordings (a) and their corresponding calibration graph (b) obtained for Dc-TPB CMCPE at optimum FIA condition.

The dependence of the peak height and time to recover the base line on flow rate was studied. The response of the electrode to a solution 1.0×10^{-3} mol L^{-1} Dc.Cl was studied at different flow rates (7.50, 9.70, 12.50, 17.85, 23.25, 25.00, 27.00, and 30.00 mL/min). With constant injection volume (150 μL), the residence time of the sample was inversely proportional to the flow rate [29]. It was found that, as the flow rate increased, the peaks became higher and narrower until a flow rate of 25.00 mL/min. The peaks obtained above these flow rates were nearly the same. This flow rate was used throughout this work providing the maximum peak height, a shorter time to reach the base line and less consumption of the carrier solution.

In potentiometric detection, the electrode potential depends on the activity of the main ion sensed, but in flow conditions, the main unfavorable feature of this detection is the slow response of the electrode to concentration changes which is pronounced when low concentrations are measured [30]. An increase in the slope of the calibration plots in FI was observed compared with batch measurement where potential is measured under conditions very close to the equilibrium. The slopes of the calibration graphs obtained were 73.57 mV/decade compared to 62.30 mV/decade in batch conditions. The usable concentration range of the electrode in FI measurements was from 1.0×10^{-4} to 1.0×10^{-2} mol L^{-1}. The detection limit was 5.0×10^{-5} mol L^{-1}, which is higher than that obtained in batch mode. The lower sensitivities of the electrode in FI may be attributed to many factors such as mass transport rate, the sample dispersion and the effect of contact time between the sample and the electrode [31]. In general, this behavior is similar to that observed previously [32]. Figure (4) represents typical recordings and their corresponding calibration graphs obtained for Dc-TPB electrode.

3.8. Selectivity

The most important parameter of any potentiometric ion sensor is its response to the primary ion in the presence of other ions in solution, which is expressed in terms of the potentiometric selectivity ($K_{Dc,J}$).

Two specialized IUPAC committees were held concerning the determination of potentiometric selectivity coefficients [33]. In the first IUPAC committee, held in 1975 [34], the separate solution method (SSM) was recommended. Another method named the "matched potential method (MPM)" was recommended in the second IUPAC committee [14] especially when the primary ion and/or the interfering ion dissatisfy with the Nernst response or when the involved ions have unequal charges [35]. The selectivity coefficient values were calculated by applying the matched potential method and the resulting values, presented in Table 2, show that the electrode displays significantly high selectivity for doxycycline over many common inorganic catians as well as amino acids. In pharmaceutical analysis, it is important to test the selectivity towards the excipients such as lactose, glucose, maltose and fructose.

In FI conditions, the values of selectivity coefficient were calculated based on potential value measured at the top of the peak for the same concentrations of the drug and the interferent according to the separate solution method [34], since the matched potential and other mixed solution methods, in this case are time consuming due to the need of many solutions and perform many steps. The selectivity coefficient values $-\log K_{Dc,J^{z+}}^{MPM}$ of the electrodes listed in Table 2 reflect a very high selectivity of this electrode for doxycycline cation. The influence of some inorganic cations, sugars and amino acids on the behavior of the electrode was investigated graphically by plotting the potential responses for all different species against their concentration.

As it is seen from calibration curves (Figure 5) except for doxycycline, the slopes of the obtained curves are much lower than the expected Nernstian slope and the plots suffer from limited linear range. However, the doxycycline cation results in a Nernstain response over a concentration range of 1.99×10^{-5}-3.19×10^{-3} mol L^{-1}. This is most probably due to both the selective behavior of the ion-exchanger Dc-TPB towards doxycycline in comparison to other cations tested and the rapid exchange kinetics of the cation between the aqueous phase and paste**.**

Table 2. Selectivity coefficient values $-\log K_{Dc,J^{z+}}^{MPM}$ for Dc-TPB CMCPE

Interferent	$-\log K_{Dc,J^{z+}}^{MPM}$		Interferent	$-\log K_{Dc,J^{z+}}^{MPM}$	
	Batch	FIA		Batch	FIA
Na$^+$	2.56	0.65	Glucose	2.78	----
K$^+$	2.18	0.85	Lactose	2.40	----
NH$_4$$^+$	3.30	0.71	Maltose	4.17	----
Mg^{2+}	2.78	2.42	Fructose	3.36	----
Ca^{2+}	2.00	2.15	Glycine	2.88	----
Mn^{2+}	2.23	2.18	D-alanine	3.34	----
Co^{2+}	2.41	2.15	DL-asparagine	2.62	----
Ni^{2+}	2.27	2.03	L- threonine	3.19	----
Zn^{2+}	3.34	2.21	DL-methionin	2.88	----
Ba^{2+}	2.11	2.36			
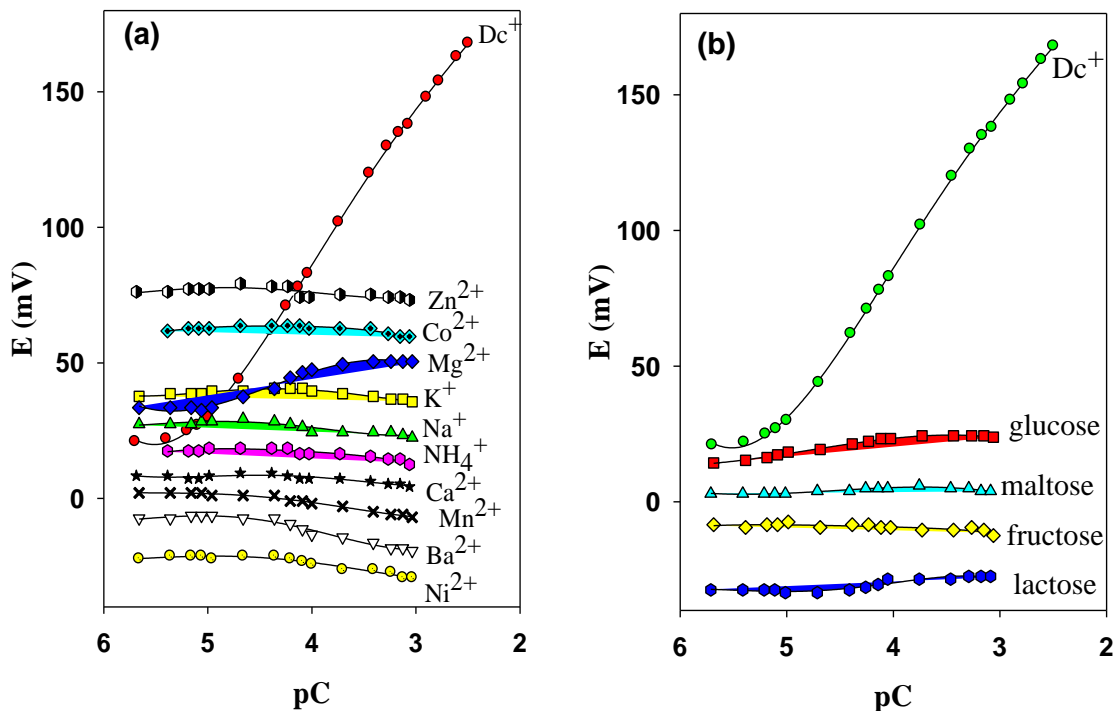
Figure 5. Calibration graphs of some inorganic cations (a) and sugars (b) by using Dc-TPB CMCPE.

3.9. Analytical applications

Table 3. Determination of doxycycline in pure solution and in pharmaceutical preparations using Dc-TPB CMCPE by applying standard addition method in batch condition and peak height comparison method in FIA.

Sample	Taken (mg)	Found (mg)	Recovery (%)	*RSD (%)
Batch method				
Pure solutions	4.80	4.70	97.92	0.62
	24.00	25.29	105.37	1.42
Farcodoxin (100 mg/capsule)	7.21	7.14	99.02	1.73
	9.62	9.56	99.38	0.64
Vibramycin (100 mg/capsule)	12.02	11.85	98.58	1.25
	24.00	24.50	102.08	2.07
FI method				
Farcodoxin (100 mg/capsule)	1.00×10^{-4}	0.990×10^{-4}	99.00	1.53
	2.00×10^{-4}	2.028×10^{-4}	101.40	*2.40
	4.00×10^{-4}	4.132×10^{-4}	103.30	0.82
	8.00×10^{-4}	7.979×10^{-4}	99.73	1.05
	1.00×10^{-3}	0.990×10^{-3}	99.00	*0.54
	3.00×10^{-3}	3.093×10^{-3}	103.10	**1.76
Vibramycin (100 mg/capsule)	2.00×10^{-4}	1.946×10^{-4}	97.30	**0.97
	4.00×10^{-4}	4.144×10^{-4}	103.60	1.10
	8.00×10^{-4}	8.144×10^{-4}	101.80	1.03
	1.00×10^{-3}	0.988×10^{-3}	98.80	**2.00
	3.00×10^{-3}	3.084×10^{-3}	102.80	*0.72

RSD: Relative standard deviation (Three determinations)
(*) (Four determinations)
(**) (Five determinations)

The electrode was successfully applied for potentiometric determination of doxycycline in pure solution and in pharmaceutical preparations by applying standard addition method in batch conditions and applying peak height comparison method in FI condition. The results shown in Table 3 indicate that recovery and R.S.D. values range between 97.30 and 105.37% of doxycycline, and 0.54 to 2.40%, respectively. It is noted that the results are accurate and reproducible. Thus, the sensor can be employed for quantification of doxycycline in commercial pharmaceutical samples

3.10. Statistical treatment of the results

The calculated F values [36] were less than the tabulated F values where $\upsilon_1 = 4$ and $\upsilon_2 = 2$ for batch condition at 95% confidence level. The t-test [36] was also done at 99.9% confidence level and the results are shown in Table 4. The applied method does not exhibit significant difference in comparison with the reference method [1] which reflects the accuracy and precision of this method.

4. CONCLUSION

The proposed chemically modified carbon paste electrode based on doxycycline tetraphenylborate as electroactive ion-exchanger might be a useful alternative analytical tool for the determination of Dc^+ in different samples. Table 5 compares the slope, response time, detection limit, and life time of the proposed doxycycline electrodes with those doxycycline-selective electrodes

Table 4. Statistical treatment of data obtained for determination of doxycycline using Dc-TPB CMCPE in pure solution and pharmaceutical preparation in comparison with the reference method [1].

Sample	Reference method Recovery %	Batch conditions			FIA		
		Recovery %	F value	t value	Recovery %	F value	t value
Pure solutions	98.10	99.60	4.18	0.95	----	----	----
Farcodoxin	102.30	98.80	0.54	2.85	103.00	1.50	0.66
Vibramycine	100.00	99.10	8.63	0.33	98.80	4.84	0.55

reported in the literature. We can observe that, our electrode shows a better slope and faster response time than those reported in the literature [8,9]. The electrode was used successfully for the determination of Dc^+ content in pure solution and in pharmaceutical formulations. The electrode shows a high sensitivity, reasonable selectivity, fast static response and long term stability with minimal sample pretreatment either it is used in batch mode or FIA.

Table 5. General performance characteristics of the previously developed doxycycline electrodes

Ion-recognition+ plasticizer + supporting material	Slope	Detection limit	Linear range	Response time	Reference
7%Dc-TPB/46.5% PVC/46.5% DOP	57.0	Not cited	7.9×10^{-5}-1.9×10^{-3}	spontaneous	[8]
GC/PPy/0.7%Dc-TPB/ 35.4% PVC/63.9%DBP	54.4	4.0×10^{-6}	1.0×10^{-5}-1.0×10^{-2}	≤ 15	[9]
3%Dc-TPB/48.5% graphite/48.5%DBP	62.30	1.33×10^{-5}	1.99×10^{-5}-3.19×10^{-3}	<8-10	This work

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