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ORIGINAL PAPER

Electrochemical behaviour of pyridoxine hydrochloride (vitamin B_6) at carbon paste electrode modified with crown ethers

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Abstract The electrochemical behaviour of pyridoxine hydrochloride (pyridoxine HCl) at the plain carbon paste electrode and the electrode modified with oxa crown ether has been studied using voltammetric and impedance measurements. The macrocycles used as modifiers were 18-crown-6, dibenzo-18-crown-6 (DB18C6), dicyclohexano-18-crown-6 and dibenzo-24-crown-8, out of which DB18C6 gave better response for pyridoxine HCl. Tris buffer (pH 10.3) was chosen as an appropriate medium among the several supporting electrolytes of varying pH studied. The characterization of the DB18C6-modified electrode (CME-DB18C6) using kinetic parameters such as number of electrons (n) and electron transfer coefficient (α) is studied by cyclic voltammetry. Electrochemical impedance spectroscopic measurements obtained confirm the current enhancement over the modified electrode. Analytical applications of this electrode have been studied for the determination of pyridoxine HCl. A sensitive linear working range of 0.6 to 100 μg cm^{-3} with a detection limit of 0.4 μg cm^{-3} by differential pulse voltammetry was observed for pyridoxine HCl on CME-DB18C6. However, on decreasing the scan rate to 5 mV s^{-1}, the detection limit lowered to 0.2 μg cm^{-3}. Interference from some vitamins like thiamine hydrochloride, riboflavin, nicotinamide, *para*-aminobenzoic acid, cyanocobalamin, folic acid and D-biotin and amino acid L-tryptophan was studied, and simultaneously, riboflavin, thiamine hydrochloride and pyridoxine HCl were determined over the modified electrode, CME-DB18C6. The modified electrode is successfully used for the determination of pyridoxine HCl in multivitamin pharmaceutical preparations.

Keywords Pyridoxine hydrochloride · Vitamins · Chemically modified electrodes · Crown ethers · Voltammetry

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Introduction

Vitamins are biologically active organic compounds with a diverse chemical nature. They enter in the human organisms with food in small amounts and play a major role as biocatalysts in metabolism. Both a lack and an excess of certain vitamins in an organism may cause significant disturbances of various functions of the organism, resulting in serious diseases [1–3]. The vitamins of the B_6 group are compounds that contain the pyridine ring in their molecules and are water-soluble vitamins. There are six forms of vitamin B_6: pyridoxal, pyridoxine, and pyridoxamine and their phosphate derivatives pyridoxal 5′-phosphate, pyridoxine 5′-phosphate and pyridoxamine 5′-phosphate. Pyridoxine was the first vitamin of the B_6 group to be isolated. It is essential in the diet for the maintenance of the body cells [4]. Several methods have been described in literature for the determination of pyridoxine, which include flow injections [5–8], high-performance thin-layer chromatography [9] and liquid chromatography with electrochemical detection [10]. Spectrophotometric determination in presence of other vitamins has also been described by multicalibration techniques [11, 12]. Algar et al. [13] have determined pyridoxine hydrochloride (pyridoxine HCl) in pharmaceutical preparations after sorption on Sephadex SP C-25 by native fluorescence method. A review on various

methods for determination of vitamin B_6 in pharmaceutical formulations has been presented [14].

A few papers concerning the determination of pyridoxine by voltammetric techniques have been reported. Söderhjelm and Lindquist [15] were the first to study the voltammetric determination of vitamin B_6 using a carbon paste electrode (CPE). The oxidation of pyridoxine and related compounds in ammonia buffer was evaluated by cyclic voltammetry. The determination of vitamin B_6 and its separation in pharmaceutical preparations and food by chromatography [16] and capillary electrophoresis [17] with amperometric electrochemical detection, using a carbon disc electrode as an electrochemical detector, have also been reported. The literature also reports that pyridoxine was studied in pharmaceutical preparations by cyclic voltammetry using carbon paste electrodes (CPEs) modified with copper (II) hexacyanoferrate (III) [18] and with *N*,*N*′-ethylenebis (salicylideneiminato) oxovanadium (IV) complex (VIV O [Salen]) [19]. The detection limit in the latter paper is reported to be as low as 3.7×10^{-5} mol dm^{-3} [19]. Furthermore, there are reports where the glassy carbon electrodes have been modified using various modifiers such as a novel carbon nanotube [20] and polymethylene blue [21]. Recently, the electrocatalytic oxidation of pyridoxine, an aluminium electrode modified with metallic palladium particles/iron (III) hexacyanoferrate (II) film [22], has been reported.

In electrochemistry, electrode modification has gained importance because of the enhancement in the sensitivity of specific analytes. In recent years, chemically modified CPEs have gained increasing attention because of their potential applications in various analyses, low background current and easy preparation technique and its regeneration [23]. Literature reports many modifiers such as clays, zeolites, metal oxides, metal phthalocyanines, metal porphyrins, resins, crown ethers, enzymes, polymers and surfactants [24–31]. Electrodes modified with crown ethers also have gained much importance in the past several years [32], and its major applications was in the area of metal analysis. Presently, an interest has been developed for the analysis of organic compounds, which are of great importance (biologically and pharmaceutically) to us. We have recently developed and reported chemically modified electrodes for the determination of riboflavin [33] *p*-aminobenzoic acid (PABA) [34], vitamin C [35], and various metal ions viz. lead [36] and copper [37] based on the macrocyclic compounds.

In the present piece of work, an effort has been made to develop an electrochemical sensor in the form of CPE modified with dibenzo-18-crown-6 (DB18C6) for determination of pyridoxine HCl. We have recently reported the complexation of pyridoxine HCl in dimethylsulphoxide with several macrocyclic compounds by conductometric and polarographic methods [38]. It is observed that DB18C6 forms a better complex as compared to that of other crown ether series. The influence of several parameters such as pH, scan rate, potential window and interferences of several compounds on the voltammetric response of pyridoxine HCl has been studied.

Experimental

Chemicals

All the chemicals were of analytical grade and used without any further purification. 18-Crown-6 (18C6) and DB18C6 from Aldrich and dicyclohexano-18-crown-6 (DCH18C6) and dibenzo-24-crown-8 (DB24C8) from Fluka were bought and used as such. The amino acid like L-tryptophan and vitamins like PABA, D-biotin (vitamin H), thiamine hydrochloride (thiamine HCl/vitamin B_1) and nicotinamide (vitamin B_3) were obtained from Lancaster, cyanocobalamin (vitamin B_{12}), folic acid (vitamin M) and riboflavin (vitamin B_2) from S.D.Fine chemicals and pyridoxine HCl (vitamin B_6) from Sigma. Tris–[hydroxymethyl] aminomethane (Tris buffer), graphite powder (<50 μM) and liquid paraffin (heavy) were obtained from S.D.Fine chemicals and were used as such.

Double-distilled, deionised water was used for preparation of all the solutions. All the voltammetric measurements were carried out using 0.05 mol dm^{-3} Tris buffer of pH 10.3 at 25±0.2 °C as the supporting electrolyte.

Instrumentation

All the voltammetric measurements were carried out on EG & G Princeton Applied Research 264A Potentiostat with model 303 A electrode assembly and X–Y chart recorder RE0089. The three-electrode system used for the study consists of a plain CPE or a modified electrode with 5% of the modifier in graphite paraffin matrix and used in conjunction with a saturated calomel reference electrode and a platinum counter electrode.

The electrochemical impedance studies (EIS) were undertaken using an Ecochemie, Electrochemical Work Station, model Autolab 30, Frequency Response Analyser. The instrument is driven by the FRA 4.9005 software. The conventional three electrodes used were the same as that of the voltammetric measurements.

Preparation of electrodes

Chemically modified CPEs were prepared by dispersing a weighed amount of crown ether crystals in absolute ethanol and adding the required amount of graphite powder into it. The slurry thus formed was stirred till all ethanol had evaporated and a complete dry powder was obtained.

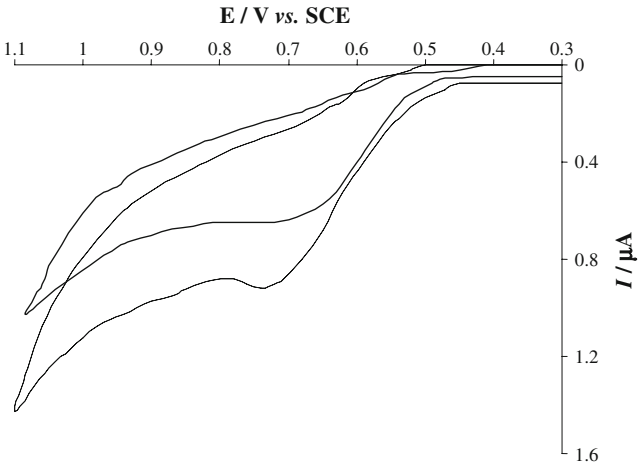


Fig. 1 Cyclic voltammograms obtained on PCPE (*solid line*) and CME-DB18C6 (*dashed line*) for 30 μg cm^{-3} pyridoxine HCl in 0.05 M Tris buffer pH 10.3 at 50 mV s^{-1}

Modified carbon pastes were prepared by thorough mixing of modified graphite powder and paraffin oil in a mortar. The final composition was 55:5:40% (w/w) of graphite powder, modifier and paraffin oil. Plain (unmodified) CPE (PCPE) was prepared in similar way, without the addition of the modifying agent with a composition of 60:40 of graphite powder and oil. The pastes were then packed in a syringe with a bore size of 1.5 mm. Electrical contact was established by means of a thin copper wire by-passing the syringe.

Procedure

Initially, differential pulse voltammograms (DPVs) of 30 μg cm^{-3} pyridoxine HCl in different electrolytes were recorded with a PCPE and CMEs at a scan rate of 10 mV s^{-1} from +0.2 to +0.9 V vs SCE. DPV was then used for the quantification of pyridoxine HCl on the DB18C6-modified electrode (CME-DB18C6) with a scan rate of 10 mV s^{-1} and pulse amplitude of 50 mV. Electrochemical behaviour of pyridoxine HCl and characterization of the modified electrode was also done by cyclic voltammetry on PCPE and CMEs in the buffer solution containing 30 μg cm^{-3} pyridoxine HCl at different scan rates. Before every successive scan, pressing out a small amount of the paste renewed the surface of the electrodes by scraping off the excess and polishing the tip on a zero-grade polishing paper until the surface had a shiny appearance. Deaeration was not required, except for the mixing of the solutions, as it did not have any effect on the signal obtained. The EIS measurements were recorded at three different potentials using an alternating current amplitude of 5 mV root mean square within the frequency range from 10^{-1} to 10^{5} Hz.

The interference study in the presence of other vitamins was studied by observing the effect on peak potential and peak current of pyridoxine HCl, by adding the increasing concentration of vitamins like thiamine HCl, riboflavin, nicotinamide, PABA, cyanocobalamin, folic acid, D-biotin and an amino acid L-tryptophan, to a fixed concentration of pyridoxine HCl. Results so obtained from interference studies were than utilized for simultaneous determination of pyridoxine HCl with riboflavin and thiamine HCl by keeping the concentration of pyridoxine HCl constant and varying the concentration of other two vitamins at different ratios, and DPVs were recorded. Synthetic samples having compositions of the vitamins; namely, riboflavin/thiamine HCl/pyridoxine HCl in the ratios 10:50:30, 10:50:20, 5:20:10 and 5:10:5 μg cm^{-3} were analysed.

Determination of pyridoxine HCl in the pharmaceutical preparations

The pharmaceutical preparations analysed were Neurobion Forte (Merck, India), B-Complex Prophylactic tablets (Omega Biotech, India), Polybion injection (Merck), Benadon (Nicholas Piramal, India) and B-long (Elder Pharmaceuticals, India).

In addition to pyridoxine HCl, Neurobion Forte contained thiamine mononitrate, vitamins B_2, B_3, B_{12} and calcium pantothenate. B-Complex Prophylactic tablets contained thiamine mononitrate and vitamins B_2, B_3 and B_{12}. Whereas, Polybion injection contained thiamine HCl, riboflavin sodium phosphate, vitamins B_3 and B_{12}, D-Panthenol and benzyl alcohol along with it. B-long and Benadon tablets contained only pyridoxine HCl as their major concomitant besides their binders.

Each tablet was crushed with a mortar and pestle and dissolved in the supporting electrolyte and sonicated, which was then filtered through a Whatman's filter paper no. 615 to remove traces of any undissolved matrix. The filtrate was transferred to a 100-cm^3 flask and diluted up to the mark with Tris buffer. The standard addition method was employed for the quantification of pyridoxine HCl wherein 10 cm^3 of the filtrate was taken into a cell as a supporting electrolyte and additions of 0.05 cm^3 of standard pyridox-

Table 1 Effect of scan rate on the behaviour of CME-DB18C6 for 30 μg cm^{-3} pyridoxine HCl in 0.05 mol dm^{-3} Tris buffer at pH 10.3 by cyclic voltammetry

Scan rate (mV s^{-1})	$\nu^{1/2}$	E_p (V)	I_p (μA)
2	1.41	+0.62	0.20
5	2.23	+0.66	0.29
10	3.16	+0.66	0.40
20	4.47	+0.67	0.55
50	7.07	+0.73	0.85
100	10.00	+0.76	1.10
200	14.14	+0.79	1.50
500	22.36	+0.97	1.68

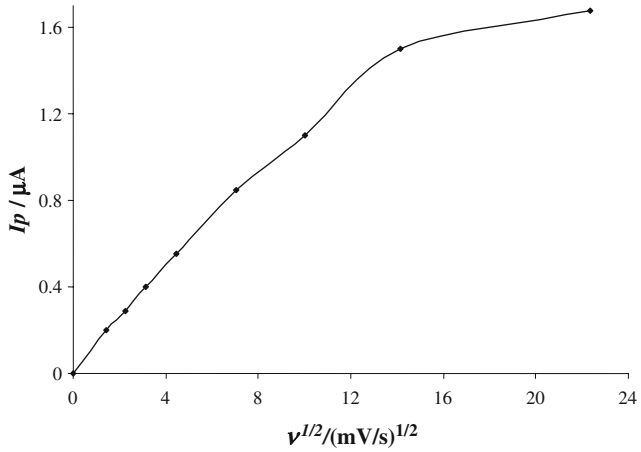


Fig. 2 Plot of I_p vs $\nu^{1/2}$ for 30 µg cm^{-3} pyridoxine HCl at CME-DB18C6 in 0.05 M Tris buffer

ine HCl (1,000 µg cm^{-3}) were done. A DPV with a pulse amplitude of 50 mV and a scan rate of 10 mV s^{-1} was used to record the signal. The samples were also analysed by the pharmacopoeial method [39] to compare the results obtained by the abovementioned method.

Results and discussion

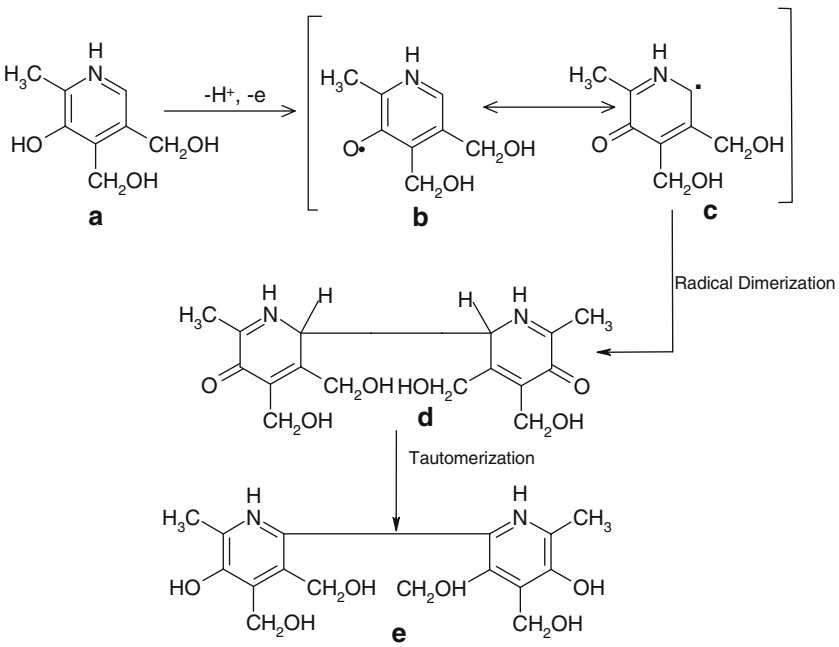
Effect of supporting electrolytes

The voltammetric response of pyridoxine HCl is affected by the type and concentration of the supporting electrolyte used, which was investigated by choosing different media of varying pH viz., 0.01 mol dm^{-3} HCl, 0.01 mol dm^{-3} HNO_3, 0.01 mol dm^{-3} CH3COOH, Britton and Robinson type (BR) buffer (pH 1.5), CH_3COONH_4 (pH 7) and Tris buffer (pH 10.3) on PCPE. Among the entire pH range studied, the BR and Tris buffers showed the maximum response. In the BR buffer, pyridoxine HCl gave a peak at around +1.15 V on PCPE. However, on CME-DB18C6, the signal of pyridoxine HCl got suppressed because CME itself gave a peak at around +1.3 V; because of which, it became difficult to study the pyridoxine HCl peak over CME.

In the Tris buffer, the peak potential of pyridoxine HCl shifted to much less positive potential (+0.58 V) as compared to that in the BR buffer. This became an advantage, to study pyridoxine HCl over CME in the Tris buffer, which was not possible in the BR buffer (pH 1.5) medium. Thus, by selecting the Tris buffer (~0.05 mol dm^{-3}), pH 10.3, as an appropriate medium, pyridoxine HCl was determined on PCPE and various CMEs based on 18C6, DB18C6, DCH18C6 and DB24C8. This vitamin is expected to be in an anionic form in strongly basic solution because of the deprotonation of the OH group bonded to the pyridinic ring [19].

Electrochemical behaviour of pyridoxine HCl

Initially, cyclic voltammograms were scanned at PCPE and CMEs based on 18C6, DB18C6, DCH18C6 and DB24C8 in the potential range of +0.2 to +0.9 V at a scan rate of 50 mV s^{-1}. On comparison of peak currents obtained on both the electrodes at a similar concentration, it is observed that electrode modified with DB18C6 gave the best response for pyridoxine HCl as compared to that of other



Scheme 1 The mechanism of pyridoxine oxidation followed by chemical transformation

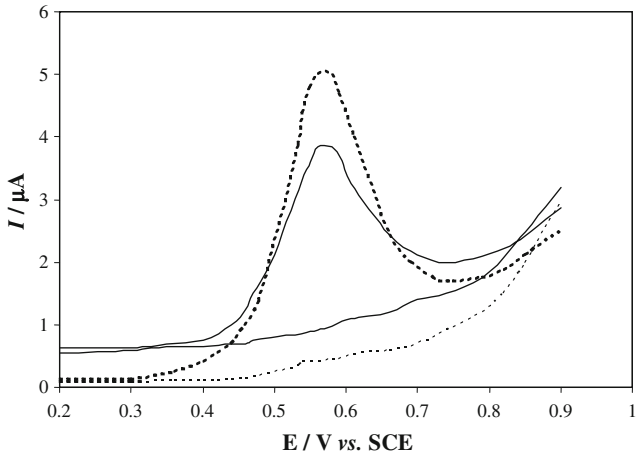


Fig. 3 Differential pulse voltammograms obtained by PCPE (*solid line*) and CME–DB18C6 (*dashed line*) for 30 µg cm^{-3} pyridoxine HCl in 0.05 M Tris buffer pH 10.3 at scan rate of 10 mV s^{-1} for the 50-mV pulse amplitude

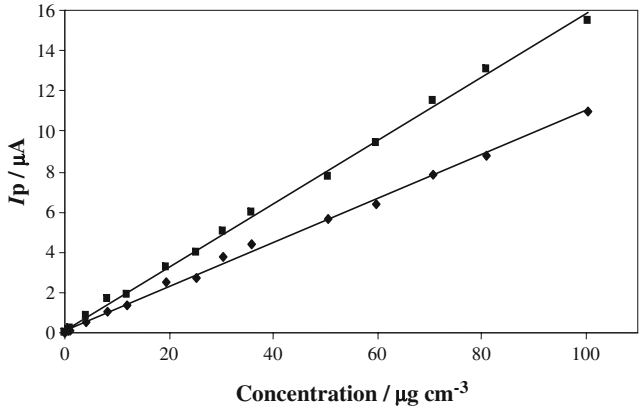


Fig. 4 Plot of peak currents (I_p) vs concentration of pyridoxine HCl by DPV for pyridoxine HCl at PCPE (*triangles*) and CME–DB18C6 (*squares*) in 0.05 M Tris buffer at a scan rate of 10 mV s^{-1} with50-mV pulse amplitude

CMEs. The cyclic voltammograms of pyridoxine HCl in 0.05 mol dm^{-3} Tris buffer on the PCPE and CME-DB18C6 scanned are shown in Fig. 1. It is noticed from the figure that it undergoes an irreversible oxidation process with an increase in peak current without any shift in peak potential.

To characterize the modified electrode, some kinetic parameters such as number of electrons (n) and electron transfer coefficient (α) for pyridoxine HCl on CME-DB18C6 were evaluated.

For an irreversible reaction, the electron transfer coefficient has been obtained using cyclic voltammetry by Tafel's plot [40] at a scan rate of 50 mV s^{-1}. The plot of log I vs E provides the value of slope:

$$\text{Slope} = (1-\alpha)F/2.3\,RT$$

The slope of the Tafel plot obtained from the cyclic voltammogram for the modified electrode as given in Fig. 1 was found to be 0.041 mV, which resulted in the value of α to be 0.76. This value of α has been utilized to calculate the value of n by cyclic voltammetry using the following equation [40],

$$\left|E_p - E_p/2\right| = 47.7/\alpha n$$

where, E_p is the anodic peak potential and $E_p/2$ is the potential where the current is half the peak value. The $E_p-E_p/2$ value was observed to be 70 mV at 50 mV s^{-1} scan rate, which is close to the theoretical value of 95 mV for a totally irreversible reaction. The value of n was calculated to be 0.90, which is close to one.

Table 2 Comparison of peak currents of various CMEs over PCPE by DPV for pyridoxine HCl at 30 µg cm^{-3}

Electrodes	E_p (V)	I_p (µA)
PCPE	+0.58	3.25
18C6	+0.58	4.10
DB18C6	+0.58	5.00
DCH18C6	+0.58	3.35
DB24C8	+0.58	4.00

As E_p is a function of scan rate for a totally irreversible wave, the effect of scan rate on CME-DB18C6 has been studied. It was found that the anodic peak current increases with the increase in scan rate and the peak potential also shifts towards a more positive side with increasing scan rate as given in Table 1. The plot of I_p vs $\nu^{1/2}$ shown in Fig. 2 indicates an initial linearity, which curves off at higher scan rates, suggesting that the reaction is initially diffusion controlled, but at faster scan rates, the electron transfer becomes rate determining.

Based on the above results, the mechanism of pyridoxine oxidation followed by chemical transformation is given in Scheme 1. Pyridoxine (A) undergoes one electron oxidation at the electrode surface resulting in a radical ion (B) formation, which produces the most stable of the resonating structure (C) that would lead to radical ion dimerization (D). Further, tautomerization of D will produce a stable product E.

The anodic peak enhancement was used for the determination of pyridoxine HCl by DPV within the potential range scanned (+0.2 to +0.9 V vs SCE). The effect of pulse amplitude, at 50 and 100 mV, was also studied. As expected, an increase in peak current was observed at 100 mV; however, the better signal to background current characteristic could be obtained with pulse amplitude of 50 mV. The optimum conditions for further studies were chosen as, pulse amplitude, 50 mV, and, scan rate, 10 mV s^{-1}. Figure 3 shows the DPV of 30 µg cm^{-3} pyridoxine HCl in Tris buffer on PCPE and CME-DB18C6 for the 50-mV pulse amplitude at a scan rate of 10 mV s^{-1}. On comparison of the peak currents of pyridoxine HCl under identical conditions on both these electrodes by DPV showed that the response of

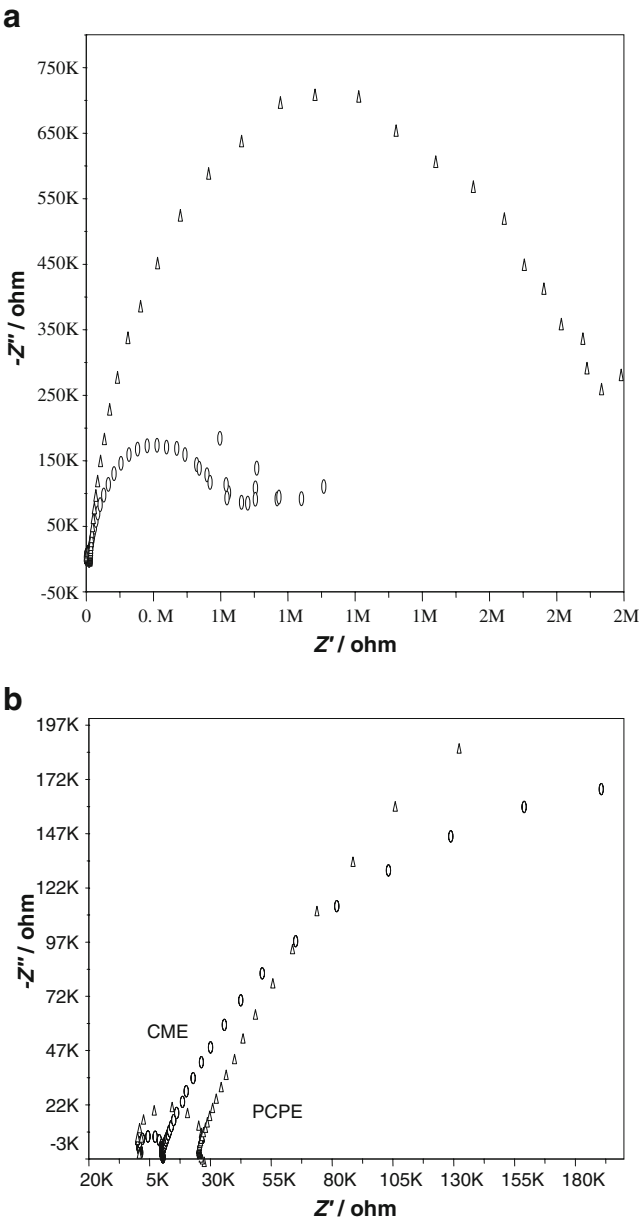


Fig. 5 **a** Impedance spectra for pyridoxine HCl (30 μg cm^{-3}) on PCPE (*triangles*) and CME-DB18C6 (*oblongs*) at +0.58 V in the frequency range of 10^{-1} to 10^{5} Hz. **b** Impedance spectra for pyridoxine HCl (30 μg cm^{-3}) on PCPE (*triangles*) and CME-DB18C6 (*oblongs*) at +0.58 V for the first semi-circle

the modified electrode is about 1.5 times higher than that of the unmodified one. Furthermore, the background current of CME in the Tris buffer is low as compared to PCPE. This enhancement in peak current and low background current lowered the detection limit over CME, and it takes place via non-covalent interactions. The site of the interaction of the vitamin with the crown ether may be the pyridine ring with the N atom of the ring approaching the crown cavity with the vitamin molecule being directly perpendicular to the plane of the crown ether. The interactions of amino acids with oxa

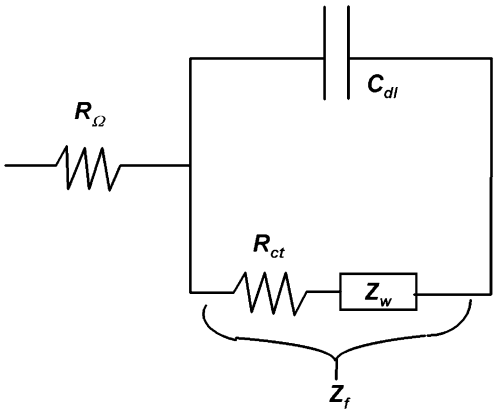


Fig. 6 Equivalent circuit for a charge-transfer process

crown ethers have been well studied [41]. In these cases, in NH_3^+ of an amino acid, each H atom interacts with one O atom of the crown ether via hydrogen bonding, and the N atom bonds with three O atoms via electrostatic attraction. A full participation of all macrocyclic donor atoms with the complexed cation is expected to give the highest possible stability to the resulting complex. This does not seem to be the case with pyridoxine because only nitrogen in the pyridine ring is available for complexation. Such behaviour is reflected in the weak stability of the complexes [38]. These interactions of pyridoxine HCl with crown ether were confirmed by 1:1 complexation reported between six-membered *N*-heteroaromatic cations and crown ether by Kiviniemi et al. [42]. Table 2 shows the effect of modification on the peak current by DPV. As DB18C6 offers an advantage of being water insoluble, no leaching took place in the solution; hence, CPE modified with 5% DB18C6 was used for further studies. As evident from Table 2, this increase in anodic peak current was then used for probing the linear working range, which is observed to be from 0.6 to 100 μg cm^{-3} (coefficient of variation=0.9932) with a detection limit of 0.4 μg cm^{-3} by DPV at a scan rate of 10 mV s^{-1} and pulse amplitude of 50 mV. However, on decreasing the scan rate to 5 mV s^{-1}, the detection limit lowered to 0.2 μg cm^{-3} (relative standard deviation=6.3%,

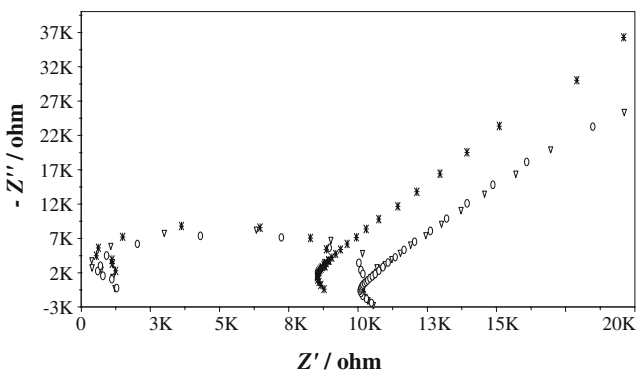


Fig. 7 Impedance spectra for pyridoxine HCl (30 μg cm^{-3}) on CME–DB18C6 at+0.4 (*asterisk*),+0.58 (*oblongs*) and +0.75 V (*triangles*) for the first semi-circle

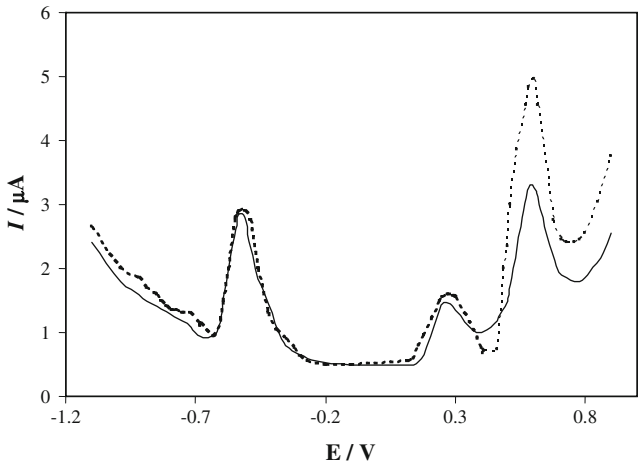


Fig. 8 Simultaneous determination of riboflavin (10 µg cm^{-3}), thiamine HCl (50 µg cm^{-3}) and pyridoxine HCl (30 µg cm^{-3}) on CME-DB18C6 in 0.05 M Tris buffer

for n=7). Although a lower scan rate showed better sensitivity, it was not considered here, as it also increased the analysis time. The plot of current (I_p) vs concentration (C) was linear on both the electrodes (Fig. 4).

Impedance studies

An EIS study was undertaken to evaluate the influence of surface kinetics of PCPE and CME-DB18C6 in 30 µg cm^{-3} pyridoxine HCl in 0.05 mol dm^{-3} Tris buffer as represented in Fig. 5. The EIS data obtained were further undertaken to examine the exact fitting equivalent circuit. As an evidence of the plot of Z(real) vs Z(imaginary), a mixed kinetic and diffusion control type of circuit was obtained on both the electrodes as shown in Fig. 6. A similar type of Nyquist plots were obtained on PCPE and CME-DB18C6 and given in Fig. 5a and b. In this figure, the semi-circle part (Fig. 5b) corresponds to the charge transfer-limited process in which the circuit seen in Fig. 6 is made of the electrolyte solution resistance (R_Ω) in series with the parallel circuit of Faradaic impedance (Z_f) and double-layer capacitance (C_{dl}). Z_f is composed of charge-transfer resistance (R_{ct}) and Warburg impedance (Z_w). The R_{ct} diameter obtained over CME-DB18C6 is less and depressed than on PCPE as observed from Fig. 5b. The experiments were performed at three different working electrode potentials covering the range ±200 mV of the peak potential observed in DPV. The R_{ct} diameter changes with the applied potential (Fig. 7) because the rate of the anodic reaction increases with an increase in the applied potential. Thus, EIS results reveal that the enhancement obtained through voltammetric results over CME-DB18C6 is kinetically facile.

Table 3 Accuracy and precision of the method for simultaneous determination of riboflavin, thiamine HCl and pyridoxine HCl in Tris buffer at pH 10.3 by DPV

Synthetic sample	Observed content		
Riboflavin/ thiamine HCl/ pyridoxine HCl (µg cm^{-3})	Riboflavin (µg cm^{-3})	Thiamine HCl (µg cm^{-3})	Pyridoxine HCl (µg cm^{-3})
10:50:30	10±0.9	50±1.8	30±1.2
10:50:20	10±0.9	50±1.8	20±1.0
5:30:10	5±0.7	30±1.6	10±0.8
5:20:5	5±0.6	20±1.2	5±0.8

Where n=5

Table 4 Determination of pyridoxine HCl in pharmaceutical samples by the proposed voltammetric method

Sample	Quoted content (mg/sample)	Observed content by the proposed voltammetric method (mg/sample)	Recovery (%)
Benadon	40	39.75±1.01	99.39
B-long	100	99.76±0.96	99.76
Neurobion Forte	3	2.70±0.03	91.00
B-Complex Prophylactic tablet	0.5	0.50±0.01	100.00
Polybion	4	4.01±0.02	100.25

Where n=5

Interference studies

To understand the sensitivity of this modified electrode towards pyridoxine HCl, the interference of several vitamins such as thiamine HCl, riboflavin, nicotinamide, cyanocobalamin, folic acid, D-biotin and PABA and amino acid L-tryptophan in 0.05 mol dm^{-3} Tris buffer. Among the above tested, only L-tryptophan (+0.6 V) and folic acid (+0.7 V) caused interference on the electrode response, as they undergo oxidation in the same potential range as that of pyridoxine HCl (+0.58 V). More than 20-fold excess in concentration of thiamine HCl that undergoes oxidation at +0.28 V affects the pyridoxine HCl signal, which may be due to the interaction of thiamine HCl with the crown ether sites. Riboflavin oxidizes at −0.53 V, which is far apart from the peak potential of pyridoxine HCl and hence does not interfere. Other vitamins such as nicotinamide, cyanocobalamin, PABA and D-biotin do not undergo oxidation in the said medium, and hence none of these water-soluble vitamins were found to interfere even when present in 100 times excess concentration of pyridoxine HCl within the potential range studied.

Simultaneous determination

From the interference studies, it was observed that pyridoxine HCl could be studied simultaneously with riboflavin and thiamine HCl. The ratio taken for the study was riboflavin (10 μg cm^{-3})/thiamine HCl (50 μg cm^{-3})/ pyridoxine HCl (30 μg cm^{-3}). During a simultaneous study, it was observed that although when a mixture of vitamins was present and peaks of the said vitamins were detected, on PCPE and CME, CME-DB18C6 selectively responded only to pyridoxine HCl; that is, the increase in peak current was much better. Figure 8 shows simultaneous determination of pyridoxine HCl in presence of riboflavin and thiamine HCl. The peak current for pyridoxine HCl for this synthetic mixture was the same as that obtained for pure pyridoxine HCl solution, which confirms that these ions do not interfere when present together in sample. Table 3 compares the actual and observed contents obtained for riboflavin, thiamine HCl and pyridoxine HCl in the mixtures of different proportions.

Analysis of pharmaceutical preparations

The proposed method was applied for the determination of pyridoxine HCl in five pharmaceutical formulations by the standard addition method. Voltammograms were recorded as in pure pyridoxine, the content was calculated, and the results are shown in Table 4. The results obtained over CME-DB18C6 are in good agreement with the declared pyridoxine HCl content. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are good. Out of the five samples analysed, three samples (Neurobion Forte, B-Complex Prophylactic tablet and Polybion injection) contained concomitants that did not interfere in the determination of pyridoxine HCl, using the proposed methods. Furthermore, the pharmacopoeial method was applied for B-long and Benadon tablets, which contained only pyridoxine HCl. The results obtained by this method were in good agreement with the ones obtained by the proposed voltammetric method.

The above experimental results show that the detection of pyridoxine HCl in pharmaceutical samples by DPV with CME-DB18C6 is more accurate than PCPE because of its selectivity, sensitivity and low detection limit. The detection limit of the proposed method is much lower as compared to the reported one [19]. Hence, the proposed method is considered useful for the routine determination of pyridoxine HCl at μg cm^{-3} levels.

Conclusions

DPVs show that the CPE modified with DB18C6 was sensitive towards pyridoxine HCl. The proposed electrode shows good operating characteristics such as sensitivity, selectivity, detection limit and wide linear working range. The background current lowered over CME-DB18C6, and the detection limit is lower as to that of the one reported in the literature. The electrochemical impedance spectra also support these results. The electrode is successfully applied for the simultaneous determination of pyridoxine HCl with riboflavin and thiamine HCl and also in pharmaceutical preparations. Along with these advantages, easy preparation and easy regeneration of the electrode surface make the system useful in constructing simple devices for its determination. It is also expected that the chemically modified electrode developed for pyridoxine HCl could be used for the routine determination at μg cm^{-3} levels using DPV in aqueous medium.

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