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ORIGINAL ARTICLE

Electrochemical behavior of nicotinamide using carbon paste electrode modified with macrocyclic compounds

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Abstract The electrochemical behavior of nicotinamide was studied at a carbon paste electrode and the electrodes modified with macrocyclic compounds using voltammetric and impedance measurements. The electrodes so formed were able to bind nicotinamide ions chemically and gave better voltammetric responses than the unmodified ones. The macrocycles used as modifiers for the electrode preparation were 18-crown-6, dicyclohexano-18-crown-6, dibenzo-18-crown-6, 7,16-dibenzyl-1,4,10,13-tetraoxa-7, 16-diazacyclooctadecane, 1,4,7,10,13,16-hexathiacyclooc tadecane (Hexathia), 1,4,7,10-tetratosyl-1,4,7,10-tetraaz acyclododecane, 1,4,8,11-tetraazacyclooctadecane, c-Methylcalix[4]resorcenarene and calix[8]arene. Among these macrocyclic modified electrodes, hexathia showed more affinity towards nicotinamide and a 2.3-fold increase in voltammetric signal was obtained. Impedance measurement was used to confirm this enhancement observed on modified electrode. This increase in anodic peak current was then used for finding linear working range, which was 0.1–500 μg mL^{-1} with a detection limit of 0.03 μg mL^{-1} by DPV. Interference from other vitamins like thiamine HCl (Vit. B_1), riboflavin (Vit. B_2), pyridoxine HCl (Vit. B_6) cynocobamine (Vit. B_{12}), *para*-aminobenzoic acid (PABA) and ascorbic acid (Vit. C) was also studied. The modified electrode could be used for the simultaneous determination of riboflavin, nicotinamide and pyridoxine HCl. It has also been utilized for the analysis of nicotinamide in pharmaceutical preparations.

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Introduction

Nicotinamide (3-pyridine carboxylic acid amide) commonly known as Niacin or vitamin B_3 is a water-soluble vitamin, required for cell respiration. It helps in release of energy and metabolism of carbohydrates, fats and proteins, proper circulation and healthy skin, functioning of the nervous system and normal secretion of bile and stomach fluid and so it is pharmacologically important compound [1]. A deficiency of nicotinamide causes pellagra [2]. Water-soluble vitamins might, however be lost through chemical reactions or by extraction and leaching during storage and process of food. In that sense, it is extremely important to have available preparations to replace the possible lack of the vitamins in daily diet, which is why multi-vitamins pharmaceuticals are becoming widely employed. On the other hand, excessive nicotinamide may have influence on human, too. Hence, the estimation of nicotinamide by analytical methods has great importance. Even though determination of nicotinamide has been extensively studied by different methods like luminescence [3], surface-enhanced Raman detection [4], reversed-phase liquid chromatography [5], micellar liquid chromatography [6], and high-performance liquid chromatography [7]; However, electrochemical methods are more sensitive and simple to analyze in complex matrix, due to its more negative reduction potential as compared to that of other vitamins [8]. Also, biologically important molecules like nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP) on decomposition give small

amount of nicotinamide, hence knowledge of the electrochemical properties of nicotinamide and its reduced forms are helpful in characterization of the nucleotides. Various polarographic methods have been reported to analyze nicotinamide in pharmaceutical preparations [8, 9]. Recently determination of nicotinamide along with nicotinic acid on polycrystalline gold electrode is reported [10]. An Ion selective piezoelectric sensor for nicotinamide assay in serum and urine has been reported [11]. Also, a biosensor for nicotinamide determination has been explored [12].

Electrode modification has a great importance in electrochemistry as they enhance the sensitivity of specific analyte. In recent years chemically modified carbon paste electrodes (CMEs) have received increasing attention due to their potential applications in various analyses, low background current and also due to the relative ease of electrode preparation and regeneration [13]. They consists of a mixture of carbon paste and a modifying reagents such as clays, zeolites, surfactants, metal oxides, metal phthalocyanines, metal porphyrins, resins, ligands, crown ethers, enzymes, polymers, etc. [14–18]. The use of macrocyclic compounds like crown ethers as a modifier has gained considerable attention in recent years [19, 20]. Initially, the most common application of CMEs happened to be in the area of metal analyses, but in the present decade, interest has been shifted to the analysis of organic compounds, which are of industrial and biological importance. We have recently developed and reported chemically modified electrodes for the determination of vitamins viz. riboflavin [21], *para*-aminobenzoic acid [22] and ascorbic acid [23] and metal ions viz. lead [24] and copper [25] based on macrocyclic compounds.

Nicotinamide is converted to the coenzymes, nicotinamide adenine dinucleotide and its phosphorylated form, which plays an important role in many biological redox processes. NAD is formed in the cells from nicotinamide when it joins with ribose and adenosine diphoshpate. Addition of phosphate group to the two position of the adenyl nucleotide through an ester linkage forms NADP. Since NAD and NADP easily undergo redox reaction, many electrochemical sensors have been reported [26–28]. However nicotinamide undergoes reduction at more negative side, which makes it difficult to study on a carbon paste electrode, and hence no electrochemical sensor based on carbon paste electrode has yet been developed. Based on its biological and pharmaceutical importants, it was thought to develop an electrochemical sensor for trace analysis. It is reported in literature that pyridine ring forms a stable complex with six member crown ether [29, 30] and stability of complex found to increase by substitution of oxygen by sulfur in the coronand ring [30]. Since the pyridine ring is an important group in nicotinamide that undergoes reduction on the electrode surface, the modification of the electrode surface, which attracts the pyridine ring, is supposed to increase the sensitivity of it. Therefore, in this paper we describe the effect of macrocyclic modified electrodes viz. crown ethers (18-crown-6, dicyclohexano-18-crown-6, dibenzo-18-crown-6, 7,16-diben zyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane, Hexathia, 1,4,7,10-tetratosyl-1,4,7,10-tetraazacyclododecane, 1, 4,8,11-tetraazacyclooctadecane) and calixarene (c-Methylcalix[4]resorcenarene, calix[8]arene) on the electrochem ical behavior of nicotinamide, and an increase in voltammetric signal is then used for its quantitative determination.

Experimental

Reagents and solutions

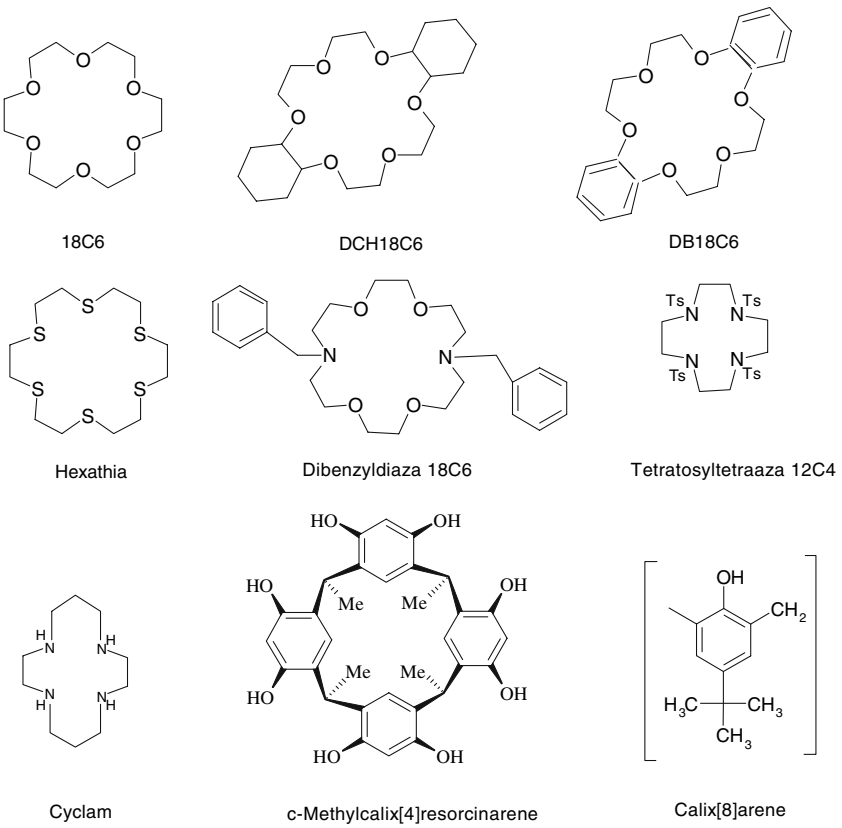
The macrocycles viz. 18-crown-6 (18C6), dibenzo-18-crown-6 (DB18C6) and hexathia were purchased from Aldrich; dicyclohexano-18-crown-6 (DCH18C6), 7,16-dib enzyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (dibenzyldiaza 18C6), 1,4,8,11-tetraazacyclooctadecane (cyclam), c-Methylcalix[4]resorcenarene and calix[8]arene from Fluka and were used as supplied, 1,4,7,10-tetratosyl-1,4,7,10-tetraazacyclododecane (tetratosyltetraaza 12C4) was synthesized by reported method [31] (Scheme 1). Vitamins viz. nicotinamide, PABA and thiamine HCl from Lancaster; riboflavin, cyanocobalamin and ascorbic acid from S. D. Fine chemicals and pyridoxine HCl from Sigma and were used as such.

Double distilled, deionised water was used for preparation of all solutions. All the voltammetric studies were carried out in 0.001 mol L^{-1} CH_3COOH at 25 ± 0.2 °C.

Preparation of electrodes

Chemically modified carbon paste electrodes were prepared by dispersing a weighed amount of macrocycle crystals in absolute ethanol, and adding the required amount of graphite powder into it. The slurry formed was stirred till all ethanol had evaporated and a complete dry powder was obtained. This dry powder was mixed with paraffin oil in a mortar to get the modified carbon paste. The final composition was 55:5:40% (w/w) of graphite powder, modifier and paraffin oil. Plain (unmodified) carbon paste electrode (PCPE) was prepared in similar fashion, without modifying agent with a composition of 60:40 of graphite powder and oil. The pastes were then packed ~2 cm in syringe with a bore size of 1.5 mm. Electrical contact was made by means of a thin copper wire by-passing the syringe. Smooth and fresh electrode

Scheme 1 Macrocyclic compounds studied in the present investigation



surfaces were obtained by squeezing out a small amount of paste from the syringe, scraping off the excess and polishing it against zero grade polishing paper until the surface had a shiny appearance.

Apparatus and voltammetric measurements

The voltammetric system used for the studies was an Eco Chemie, Electrochemical Work Station, model Autolab 30; the electrode assemblies being a 663 VA stand with GPES computer software for recording and analyses of the voltammogram. The three electrode system used for study consisted of carbon paste electrode or modified electrode as working, Ag/AgCl/3 mol L^{-1} KCl as reference and platinum as counter electrode. All potentials are quoted with respect to Ag/AgCl/3 mol L^{-1} KCl.

Initially, cyclic voltammograms of nicotinamide solutions in different electrolytes were recorded with a PCPE at a scan rate of 100 mV s^{-1} from −1.0 to −1.55 V. By choosing 0.001 mol L^{-1} CH_3COOH as a supporting electrolyte, effects of different carbon paste electrodes modified with 18C6, DCH18C6, DB18C6, dibenzyldiaza 18C6, hexathia, tetratosyltetraaza 12C4, cycalm, c-Methylcalix[4]resorcenarene and calix[8]arene were studied over a fixed concentration of nicotinamide under identical conditions. Differential pulse voltammetric (DPV) technique was then used for the quantification of nicotinamide by hexathia modified electrode, with pulse amplitude of 50 mV and a scan rate of 10 mV s^{-1}. Before each scan the solution was purged with nitrogen for 2 min. The impedance spectra were recorded by using three electrode systems in the frequency range from 10^{-1} to 10^{5} Hz and the AC amplitude was 5 mV.

Interference study in presence of other vitamins was carried out by observing the effect on peak potential and current of nicotinamide, by adding the increasing concentration of vitamins like thiamine HCl, riboflavin, pyridoxine HCl, cyanocobalamin, ascorbic acid and PABA to a fixed concentration of nicotinamide. Results of interference study were then utilized for simultaneous determination of nicotinamide with riboflavin and pyridoxine HCl. By keeping the concentration of nicotinamide constant and varying the concentration of other two vitamins at different ratios, the cyclic and differential pulse voltammograms were recorded. Synthetic samples having compositions of the three vitamins viz., riboflavin, nicotinamide and pyridoxine HCl in the ratios 10:50:500; 10:50:400; 5:25:200 and 2.5:10:100 μg mL^{-1} were prepared and their DPV were recorded.

Preparation and analysis of samples

The multivitamin tablets/capsule analyzed was Neurobion Forte (Merck, India), ZiComplex capsule (Mexin Medicaments Ltd., India), Omega B complex (Biotech Ltd., India), polybion capsule (Merck, India) and A to Z antioxidants, multivitamin, multimineral tablets (Alkem Laboratories Ltd., India). In addition to nicotinamide, Neurobion Forte contained thiamine mononitrate, vitamins B_2, B_6, B_{12} and calcium pantothenate; ZiComplex capsule contained thiamine mononitrate, vitamins B_2, B_6, B_{12}, C, folic acid, zinc sulphate monohydrate and calcium pantothenate; Omega B complex contained vitamins B_1, B_2, B_6 and $B_{12;}$ polybion capsule contained vitamin thiamine mononitrate, vitamins B_2, B_6, B_{12}, C, biotin and calcium pantothenate; whereas A to Z tablet contained vitamin A, E, C, B_2, B_6, B_{12}, folic acid, thiamine mononitrate, calcium pantothenate and some multimineral along with it.

Each tablet/contents of capsule was crushed with a mortar and pestle and dissolved in 0.001 mol L^{-1} CH_3COOH supporting electrolyte, sonicated for 20 min, which was then filtered through Qualigens (615) filter paper to remove trace of any undissolved matter. The filtrate was transferred to a 100 mL flask, and diluted up to the mark with supporting electrolyte. About 10 mL of the diluted solution was directly taken into a cell and a standard addition method was used for quantitative determination of nicotinamide by successive additions of 0.1 mL of standard nicotinamide solution (300 μg mL^{-1}). All these samples were also analyzed by the pharmacopoeial method [32] to compare the results obtained by above method.

Results and discussion

Effect of supporting electrolyte

From the study of electrochemical behavior of nicotinamide on carbon paste electrode in 0.01 mol L^{-1} HCl, 0.01 mol L^{-1} HNO_3, 0.01 mol L^{-1} H_2SO_4, 0.01 mol L^{-1} $HClO_4$, 0.01 mol L^{-1} CH_3COOH, BR buffer (pH 1.5), 0.05 mol L^{-1} TEAP, 0.05 mol L^{-1} ammonium acetate, 0.05 mol L^{-1} Tris buffer and 0.05 mol L^{-1} TBAOH, it was found that electrochemical behavior of nicotinamide was very sensitive to pH. A single well defined irreversible reduction peak was observed in acidic medium, which shifted towards more negative side with increase in pH. In strong acidic media (pH ∼ 2.0) like 0.01 mol L^{-1} HCl, 0.01 mol L^{-1} HNO_3, 0.01 mol L^{-1} H_2SO_4 and 0.01 mol L^{-1} $HClO_4$ peak observed at –1.28 V by cyclic voltammetry, which is at the extreme end of negative potential range of carbon paste electrode and hence peak observed to be at high background current of supporting electrolyte. With further increase in pH, nicotinamide peak shifted towards more negative side, which starts merging with supporting electrolyte current, hence basic medium was not found useful at carbon paste electrode. Among the entire pH range taken, 0.01 mol L^{-1} CH_3COOH showed maximum current response for the same concentration of nicotinamide, as supporting electrolyte current was very less. To find out the suitable medium for analysis of nicotinamide at trace level, different concentration of CH_3COOH like 0.1, 0.01 and 0.001 mol L^{-1} was tried, among these 0.001 mol L^{-1} CH_3COOH shows very less background current at nicotinamide reduction peak potential and hence taken for further study.

Voltammetric determination of nicotinamide and effect of modifier

The cyclic voltammograms of nicotinamide in 0.001 mol L^{-1} CH_3COOH solution on PCPE and hexathia modified carbon paste electrode, in the potential range of –1.0 to –1.55 V at scan rate of 50 mV s^{-1} are given in Fig. 1. It is observed from the cyclic voltammograms that it undergoes an irreversible reduction process. At lower pH nicotinamide is reduced to a neutral radical which very rapidly dimerizes to an apparent 6,6′ dimmer species [33] resulting in the irreversible reduction. By comparing the peak current of nicotinamide on PCPE and hexathia modified electrode at same concentration, a well sharp peak with increase in current was observed for modified electrode. A straight line obtained in Fig. 2 for I_p vs. $v^{1/2}$ plot on modified electrode indicates that the process is totally diffusion controlled. Table 1 represents the effect of scan rate on cyclic voltammogram of nicotinamide. With increase in scan rate, nicotinamide peak shifted towards more negative side by ∼30–39 mV for each 10-fold increase in scan rate. The theoretical full width at half maximum for nicotinamide is in the range of 111–127 mV and the $E_p - E_p/2$ value is in the range of 52–60 mV. The electron transfer coefficient obtained from the Tafel plot was 0.723. All these parameters show that nicotinamide undergoes one electron reduction.

Figure 3 shows DPV of nicotinamide in 0.001 M CH_3COOH on PCPE and hexathia modified electrode. It is observed that electrode modified with hexathia shows better response for nicotinamide as compared to that of PCPE, which results in a 2.3-fold increase in peak current. This enhancement of voltammetric signal of nicotinamide by CME is due to the non-covalent interaction between nicotinamide and hexathia crown ether. The site of the interaction of nicotinamide with the crown ether may be pyridine ring with the N atom of the ring approaching the

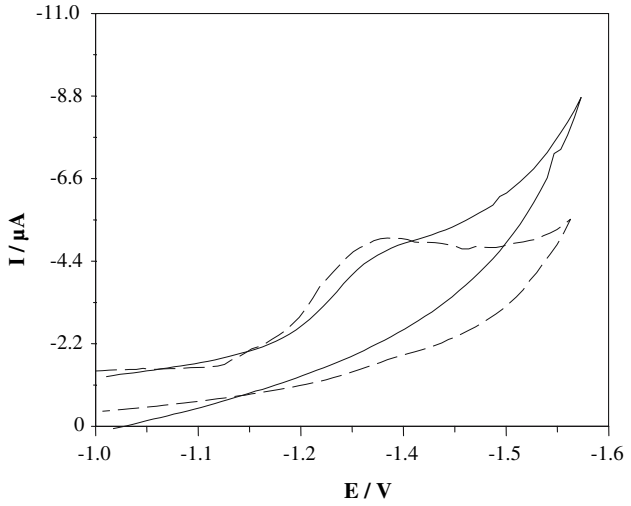


Fig. 1 Cyclic voltammograms obtained on PCPE (—) and hexathia modified electrode (- - -) for 100 µg mL^{-1} nicotinamide in 0.001 mol L^{-1} CH_3COOH at scan rate of 50 mVs^{-1}

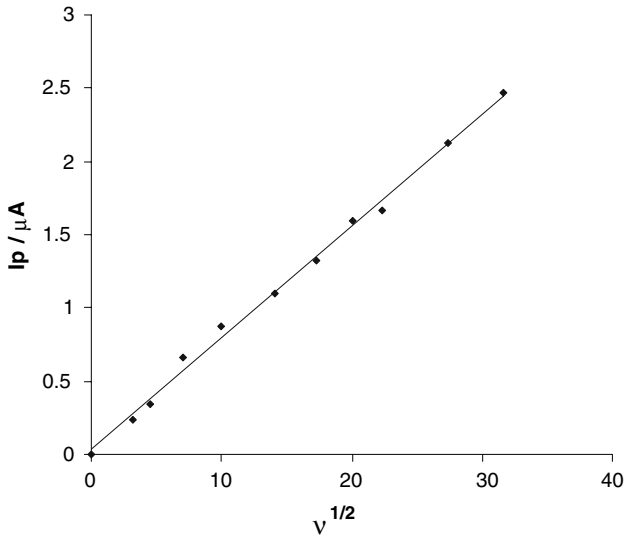


Fig. 2 Plot of I_p vs. $v^{1/2}$ for 50 µg mL^{-1} nicotinamide at hexathia modified electrode in 0.001 mol L^{-1} CH_3COOH

crown cavity with the nicotinamide molecule being directly perpendicular to the plane of the crown ether. The interactions of amino acid with oxa crown ethers have been well studied [34]. In these cases, each H atom from NH_3^+ of an amino acid 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 nicotinamide since only nitrogen in pyridine ring is available for complexation. Such behavior reflected in the weak stability of the complex as reported for the complexation of pyridoxine HCl with macrocyclic compounds, where

Table 1 Effect of scan rate on the behavior of hexathia modified electrode for 50 µg mL^{-1} nicotinamide in 0.001 mol L^{-1} CH_3COOH by CV

Scan rate (mV s^{-1})	$v^{1/2}$	E_p (V)	I_p (µA)
10	3.16	–1.294	0.23
20	4.47	–1.310	0.34
50	7.07	–1.325	0.66
100	10.00	–1.333	0.88
200	14.14	–1.341	1.10
300	17.31	–1.357	1.32
400	20.00	–1.365	1.60
500	22.36	–1.373	1.66
750	27.38	–1.373	2.13
1,000	31.62	–1.393	2.47

stability was found to increase by substitution of oxygen by sulfur in the coronand ring [30]. The interaction of nicotinamide with crown ether takes place through N atom present in the pyridine ring was confirmed by 1:1 complexation reported between six-membered N-Heteroaromatic cations and crown ether by Kiviniemi and coworkers [29]. Thus, the modification of electrode surface with macrocyclic compounds containing six-membered crown ether having more affinity towards pyridine ring causes an enhancement in voltammetric signal. Even bigger macrocycles like calixarenes, having the property to encapsulate the molecules are also tried, but on comparison with different CMEs made from crown ethers and calixarenes, maximum enhancement in peak current was obtained in case of hexathia. Although, DB18C6 and dibenzyldiaza 18C6, tetratosyltetraaza 12C4, cyclam, c-Methylcalix[4]resorcenarene and calix[8]arene also showed enhancement in peak current, the increase in current observed in the case of hexathia was found to be maximum. This may be due to interaction of pyridine ring to thia atoms. Table 2 shows the effect of modification on the peak current of nicotinamide by DPV. Other modified electrodes like 18C6, DCH18C6 show same current as that of PCPE, whereas cyclam gave less response towards nicotinamide even after having four nitrogen atoms, due to high supporting electrolyte current.

Effect of the presence of the modifier was also observed by making different compositions (2, 5 and 10%) of hexathia CME. With increasing the percentage of modifier the voltammetric signal of nicotinamide also increases. Though the 10% hexathia modified electrode showed maximum current, the current showed by 5% was considered suitable enough for trace analysis. As hexathia was water insoluble, no leaching out of it into solution took place.

It is evident from Table 2 that the sensitivity of the hexathia modified electrode is about 2.3 times higher than

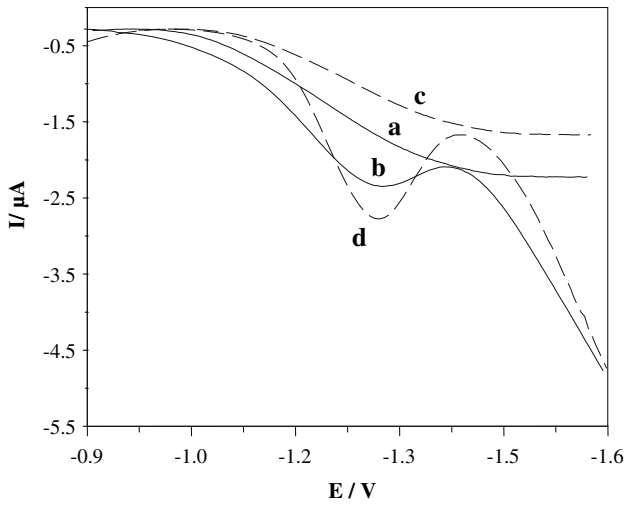


Fig. 3 DPV obtained by PCPE (—) and hexathia modified electrode (- - -) for 21 µg mL^{-1} nicotinamide in 0.001 mol L^{-1} CH_3COOH at scan rate of 10 mV s^{-1} for 50 mV pulse amplitude (a and c for background electrolyte, b and d for nicotinamide)

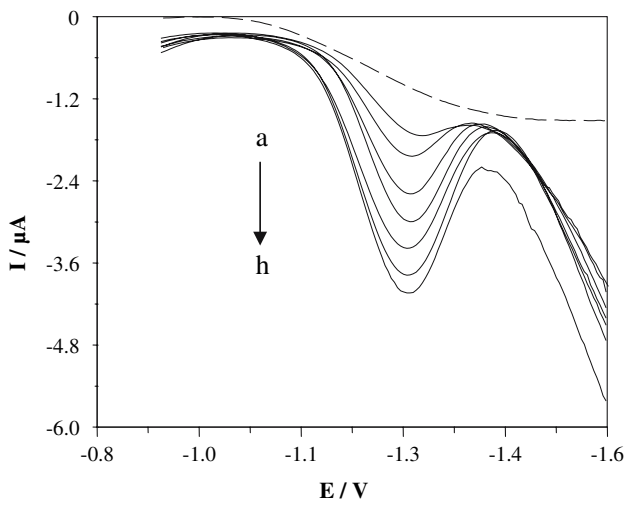


Fig. 4 DPV of nicotinamide on hexathia modified electrode at a scan rate of 10 mV s^{-1} and pulse amplitude 50 mV for linear concentration range (a) 0.0 (- - -), (b) 7.7, (c) 13.0, (d) 18.2, (e) 21.0, (f) 27.5, (g) 35.0 and (h) 40.0 µg mL^{-1} (—)

that of unmodified one, which lowers the detection limit. This increase in cathodic peak current was then used for probing the linear working range which is observed to be from 0.1 to 500 µg mL^{-1} (coefficient of correlation = 0.9996) with a detection limit of 0.03 µg mL^{-1} (RSD = 2.8%) at a scan rate of 10 mV s^{-1} and a pulse amplitude 50 mV by DPV. The detection limit was found to be lower than reported one (0.04 µg mL^{-1}) [10]. The voltammograms for different concentration of nicotinamide in 0.001 mol L^{-1} CH_3COOH on hexathia modified electrode are given in Fig. 4 and the calibration plots on PCPE and hexathia modified electrode are presented in Fig. 5 It was notice from Fig. 5 that on both the electrodes, I_p vs. c plots were linear.

Impedance measurement

Figure 6 represents the impedance spectra of 100 µg mL^{-1} nicotinamide on CPE and hexathia modified electrode in 0.01 mol L^{-1} CH_3COOH. As seen from the figure, the trend of impedance spectra for both electrodes is similar in nature, but charge transfer resistance (diameter of the semicircle, R_{ct}) value observed to be very less on hexathia modified electrode compared to PCPE. The nature of the plot is observed to be the result of mixed kinetic diffusion control type of circuit given in the Fig. 6 (inset). Hence charge transfer reaction is more favorable on modified electrode then PCPE. The peak potential of nicotinamide was observed to be at –1.276 V by DPV, hence the impedance spectra were recorded at three different potentials (–1.1, –1.3 and –1.5 V). It is observed from Fig. 7 that the R_{ct} value decreases with increase in the potential as indicated by the decrease in the diameter of the semicircle.

Table 2 Comparison of peak currents of various CMEs over PCPE by DPV for nicotinamide at 40 µg mL^{-1}

Electrodes	E_p (V)	I_p (µA)
PCPE	–1.271	1.28
Tetratosyltetraaza 12C4	–1.276	2.34
Hexathia	–1.276	2.84
DB18C6	–1.271	2.11
Dibenzyldiaza 18C6	–1.271	1.91
c-Methylcalix[4]resorcinarene	–1.286	1.45
Calix[8]arene	–1.291	1.79

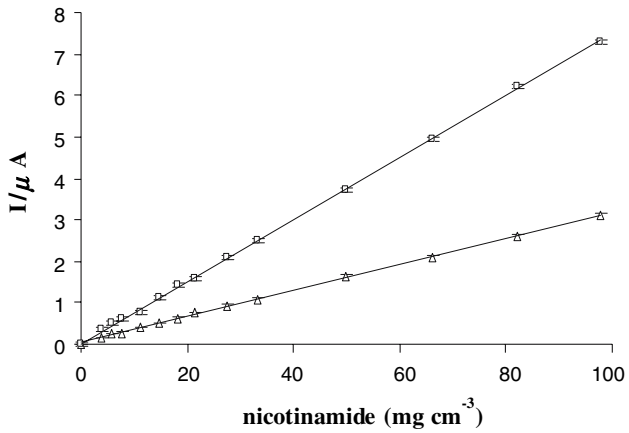


Fig. 5 Plots of peak current (I_p) vs. concentration of nicotinamide by DPV at PCPE (▲) and hexathia modified electrode (■) in 0.001 mol L^{-1} CH_3COOH at scan rate of 10 mV s^{-1} and 50 mV pulse amplitude

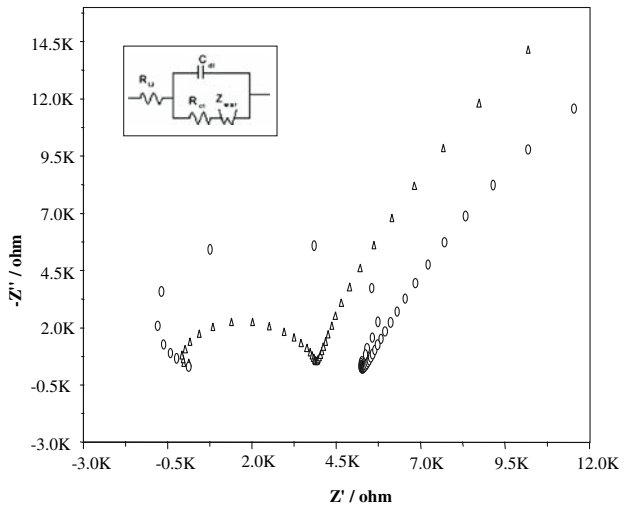


Fig. 6 Impedance spectra for nicotinamide (100 μg mL^{-1}) on PCPE (0) and hexathia modified electrode (△) at –1.3 V in the frequency range of 10^{-1} to 10^{-5} Hz. The top inset shows the equivalent circuit model of the impedance data

R_{ct} is expected to change with applied potential as the rate of the following cathodic reaction increases with the increase of applied potential. Hence, the above diagnosis of the observed impedance spectra reveals that reduction of nicotinamide is kinetically facile on the hexathia modified electrode.

Interference study

The interference study due to other vitamins viz. thiamine HCl, riboflavin, pyridoxine HCl, cyanocobalamin, ascorbic acid and PABA, which are the major components of multivitamin pharmaceutical preparations, was undertaken under the same conditions. None of them were found to affect the peak potential and peak current of nicotinamide even though when present in 100-fold excess concentration, indicating that there was no interaction between chemically modified electrode with these molecules. Riboflavin having the property to be adsorbed on the electrode surfaces [35, 36] was found to interfere in the reduction process of nicotinamide on Hg electrode, but on hexathia modified electrode, nicotinamide shows a well defined peak even in the presence of 100 times more concentration of riboflavin. Application of this was made for the simultaneous determination of nicotinamide with other vitamins. Out of the six vitamins taken, thiamine HCl is electroinactive in acidic medium, and ascorbic acid and PABA (0.914 V) undergo oxidation, hence simultaneous determination of nicotinamide was possible only with riboflavin (–0.239 V) and pyridoxine HCl (–1.499 V). Figure 8 shows the DPV of the synthetic samples containing riboflavin, nicotinamide and pyridoxine HCl. From Fig. 8 it was observed that hexathia modified electrode is sensitive towards nicotinamide. Due to the enhancement of nicotinamide peak and low supporting electrolyte current on CME, causes an well separation of nicotinamide and pyridoxine HCl peaks, which are merging with each other on PCPE. Even riboflavin peak current was observed to be less on modified electrode compared to PCPE. Synthetic samples containing different compositions of riboflavin, nicotinamide, and pyridoxine HCl, were studied and the peak current and the peak potential of nicotinamide was observed to be same as those obtained for pure nicotinamide solution with the same concentration. Table 3

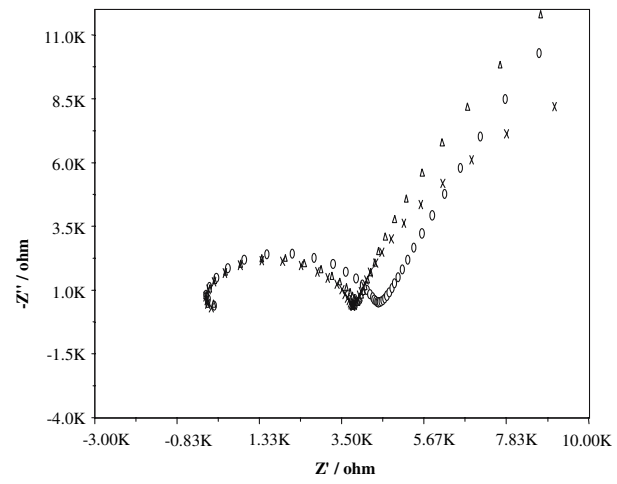


Fig. 7 Impedance spectra for nicotinamide (100 μg mL^{-1}) on hexathia modified electrode at –1.1 (0), –1.3 (△) and –1.5 V (×) in the frequency range of 10^{-1} to 10^{-5} Hz

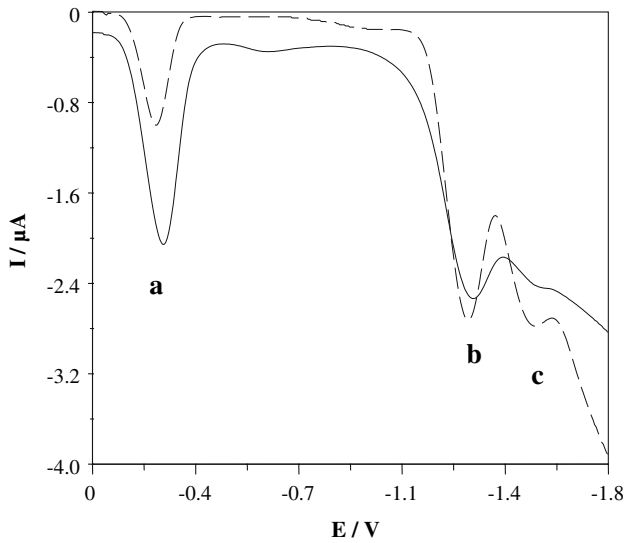


Fig. 8 Simultaneous determination of [a] riboflavin (5 μg mL^{-1}), [b] nicotinamide (25 μg mL^{-1}) and [c] pyridoxine HCl (100 μg mL^{-1}) on PCPE (—) and hexathia modified electrode (- - -) in 0.001 mol L^{-1} CH_3COOH by DPV

Table 3 Accuracy and precision of the method for simultaneous determination of riboflavin, nicotinamide and pyridoxine HCl in 0.001 mol L^{-1} CH_3COOH by DPV

Synthetic sample	Observed content		
Riboflavin:Nicotinamide: Pyridoxine HCl (μg mL^{-1})	Riboflavin (μg mL^{-1})	Nicotinamide (μg mL^{-1})	Pyridoxine HCl (μg mL^{-1})
10:50:500	10.02 ± 0.5 (n = 5)	50.00 ± 0.9 (n = 5)	500.10 ± 1.8 (n = 5)
10:50:400	10.08 ± 0.5 (n = 5)	50.02 ± 0.8 (n = 5)	400.08 ± 1.2 (n = 5)
5:25:200	5.10 ± 0.4 (n = 5)	25.04 ± 0.6 (n = 5)	200.12 ± 1.2 (n = 5)
2.5:10:100	2.5 ± 0.3 (n = 5)	10.00 ± 0.6 (n = 5)	100.06 ± 1.0 (n = 5)

compares the actual and observed contents obtained for riboflavin, nicotinamide, and pyridoxine HCl in the mixtures of different proportions. It shows that these ions do not interfere even when present together in the sample. Maintaining the same experimental conditions, pharmaceutical preparations having a complex matrix were analyzed and gave satisfactory results.

Determination of nicotinamide in pharmaceutical samples

The chemically modified electrode was used for the determination of nicotinamide in pharmaceutical samples by standard addition method. The results obtained using the proposed method are given in Table 4. The contents of nicotinamide in these samples determined by the present method using DPV at hexathia modified electrode are in good agreement with the labeled specifications. The results show that the detection of nicotinamide in pharmaceutical samples by DPV technique with CME is more accurate than PCPE, due to its selectivity, sensitivity, and lower detection limit. The results obtained for pharmaceutical samples by this method were also compared with the results obtained by pharmacopoeial method, and found to be in good agreement.

Conclusion

DPV showed that the hexathia modified electrode was sensitive towards nicotinamide. The proposed electrode has been shown to have good operating characteristics (sensitivity, selectivity, detection limit and wide linear working range). The interference of riboflavin being a highly adsorptive molecule at mercury electrode surface was not observed over hexathia modified electrode in determination of nicotinamide. Hence, the method could be successfully applied for the analysis of nicotinamide in presence of riboflavin in pharmaceutical preparations. Also, a simultaneous determination of nicotinamide with riboflavin and pyridoxine HCl was possible. The above advantages together with the very easy preparation and easy regeneration of the electrode surface by simple polishing make the system useful in constructing simple devices for determination of nicotinamide.

Table 4 Assay of nicotinamide in pharmaceutical preparations

Samples	Quoted content mg/(cap/tablet)	Observed content	
		DPV technique mg/(cap/tablet)	Standard method mg/(cap/tablet)
ZiComplex capsules	50.0	50.03 ± 0.8 (n = 5)	49.88 ± 0.8 (n = 5)
Neurobion Forte	45.0	45.02 ± 0.4 (n = 5)	45.04 ± 0.5 (n = 5)
A to Z tablets	50.0	49.92 ± 0.5 (n = 5)	50.08 ± 0.8 (n = 5)
Omega B complex	15.0	15.04 ± 0.3 (n = 5)	15.12 ± 0.6 (n = 5)
Polybion tablets	100.0	99.76 ± 0.5 (n = 5)	99.54 ± 0.7 (n = 5)

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