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Bioelectrochemistry and Bioenergetics 38 (1995) 229–245

BIOELECTROCHEMISTRY AND BIOENERGETICS

Review

The electrochemistry of neurotransmitters at conducting organic polymer electrodes: electrocatalysis and analytical applications

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Received 1 October 1994; revised 10 December 1994

Abstract

The electrooxidation of catechols, catecholamines and NADH at conventional electrode materials is generally characterized by high degrees of irreversibility as well as strong adsorption and, hence, fouling by reactants and/or products of the reactions. On the contrary, the rates of the electron transfer are highly catalysed by the use of conducting polymer films, such as poly(3-methylthiophene), polyphenylene, polyanaline and polypyrrole, as described here. Furthermore, the usual fouling problems are eliminated. Even interference from electroinactive large proteins, such as haemoglobin, and other surfactants are substantially reduced. Also, electron spectroscopy for chemical analysis, energy-dispersive analysis of X-rays, theoretical diffusion coefficient calculations, metal ion coordination, solution diffusion analyses of cyclic voltammograms etc. show that the electron transfer occurs at the polymer–solution interface and not at the inert electrode substrate surface after diffusion through the polymer matrix or through pores. The analytical application of these polymer electrodes as amperometric detectors for flow injection analysis and high performance liquid chromatography are given. In addition, selective potentiometric electrodes for catecholamines based on conducting polymer films of crown ethers, such as binaphthyl-20-crown-6, dibenzo-18-crown-6, etc., have been developed and characterized. These potentiometric detectors significantly decrease the usual interferences of ascorbic acid, uric acid and acetaminophen found in amperometric detection.

Keywords: Neurotransmitters; Catecholamines; Conducting polymer films; Cyclic voltammograms; ESCA; Crown ethers

1. Introduction

With respect to dynamic electroanalytical techniques the concept of the modified electrode is certainly one of the exciting developments of the last two decades and worldwide interest can be readily measured by the large number of publications in this field. The underlying motivations for electrode surface modifications stem from the desire for improved electrocatalysis and freedom from surface fouling effects. Alternatively, electrodes can be modified to prevent undesirable reactions from competing kinetically with the desired electrode process [1–4].

The most widely adapted scheme for electrocatalysis is to use soluble or surface-immobilized electron transfer mediators, so that oxidation or reduction of the desired substrate occurs at a potential nearer to its expected thermodynamic potential, e.g. the activation potential that exists at unmodified (bare) electrode is overcome. However, this scheme can only be useful if the formal potentials of mediator catalyst and substrate are similar. In other words, electron exchange via redox mediators is limited by the redox potential of the mediator, which generally provides only a very narrow useful potential window.

There is a multitude of polymeric coatings for a wide variety of electrode applications in electrochemistry [2,5]. Electrically conducting polymers, which constitute one group of a larger family of polymers (redox polymers, ion exchange polymers, conducting polymers and inert neutral polymers), appear to have a distinct advantage over redox mediators for catalysis. This advantage is inherent in the fact that conduction, and therefore interfacial electron transfer, can take place over a broad potential window.

Poly(3-methylthiophene) (P3MT) is an electronically conducting polymer which is easily deposited onto electrodes by electrooxidation of its monomer. Its applications have been discussed and emphasized in terms of electrochromic effects and energy storage and conversion [6]. In the field of catalysis, its use was customarily considered

SSDI 0302-4598(95)01846-8

in conjunction with metallic aggregates or clusters embodied into the polymer matrix to achieve high electrocatalytic activity for hydrogen generation [6].

The use of conducting polymers for electrochemical determination of some biologically important compounds has been demonstrated in a very limited number of works. For instance, Saraceno et al. [7] used a polypyrrole-coated glassy carbon electrode for resolving voltammetric waves of ascorbic acid and dopamine. They concluded that the current observed was mass transport limited and not limited by permeation into or through the polymer film. P3MT-coated electrodes were used by Wang and Li [8] to eliminate the passivation problems otherwise occurring at glassy carbon electrodes, in voltammetric measurements of phenolic compounds. They also observed significantly larger oxidation peaks for acetaminophen. In a different study Wang et al. [9] showed that conducting polymers (polyaniline, polypyrrole and polyphenol) could be used for controlling the size exclusion selectivity. The smaller the molecules, the more facile was their transport through the polymer film. Thus, bare electrode (platinum) responses were always larger than those obtained at the coated electrodes. However, these studies concerning the use of conducting polymers for non-mediated electron transfer were limited to a small number of compounds. Also, no observations were made regarding the effects of film thickness and electrolyte properties on the electrocatalytic ability of the conducting polymer electrodes.

In this paper we examine the electrochemical behaviour of a large number of biologically important compounds at a P3MT and other conducting polymer modified electrodes. Electrocatalytic effects of P3MT film are clearly demonstrated and selective voltammetric determination of even ternary mixtures is shown to be possible. Additionally, the P3MT-coated electrodes are also used for sensitive determinations in a flow injection analysis (FIA)–amperometric detection regime. Also, electron spectroscopy for chemical analysis (ESCA), energy-dispersive analysis of X-rays (EDAX), theoretical diffusion coefficient calculations, metal ion coordination and solution diffusion analyses of cyclic voltammograms are used to determine the sites of the electron transfer reactions.

With respect to potentiometric techniques, many synthetic macrocyclic host compounds have been developed as a promising class of potential-sensitive elements for ion-selective electrodes because of their chemical recognition function as well as their high chemical stability. The diversity in structure design possibilities of these macrocyclic compounds expands the scope of their application. A series of crown ether derivatives for metal cation selective electrodes has been reported [10–21]. However, few compounds have been designed as anion carriers and also for neutral organic molecules employing the concept of host–guest chemistry [19–21]. The development of direct potentiometric sensors for neutral molecules has been a major problem owing to the lack of surface interaction with a charged species. A fundamental problem remains as how to form a complex between host and neutral guest and subsequently to generate a charge separation at the interface between the electrode surface and the supporting electrolyte. One successful report of such neutral compound responses was discussed by Kimura et al. [22]. They suggested that 18-azacrown-6 (which is triprotonated) forms stable 1:1 complexes with catechol and its biological derivatives with loss of H^+ in neutral pH solutions. Umezawa et al. [23] developed a potentiometric adenosine triphosphate polyanion sensor using a macrocyclic polyamine as a sensory element.

Macrocyclic crown ethers function as cation carriers. The ion selectivities were mainly governed by the geometric dimension of the crown cavity. Some simple crown compounds were first found to complex with ammonium and alkylammonium salts [24,25]. On the basis of these fundamental findings, Cram and Cram [26] synthesized binaphthyl-20-crown-6 which was found to complex with both potassium and alkylammonium ions. The first organic amine molecular selective electrode using benzo-18-crown-6 as a host in a poly(vinyl chloride) (PVC) membrane for the potentiometric determination of amphetamine was investigated by Hassan and Elnemma [27]. Liu and Zhao [28] used binaphthyl-20-crown-6 to construct a potentiometric drug electrode for Micinlex. A serotonin molecular selective electrode based on this crown ether in PVC also has been investigated [29].

In the present study, a new potentiometric electrode was constructed by means of electrochemical polymerization of the binaphthyl-20-crown-6 on a platinum electrode for the selective static potentiometric determination of catecholamines. In addition, in this paper, new modified electrodes were made by means of electrochemical polymerization or adsorption of several different crown ether compounds onto a platinum electrode for the determination of catechol and catecholamines by not only static potentiometry but also by potentiometric FIA. Also, because of the importance of the determination of catecholamines in natural biological media which contain relatively high concentrations of ascorbic acid, uric acid and/or acetaminophen, a comparison of the effects of these interferents on the static potentiometric and potentiometric FIA response of these crown ether electrodes has been made. These interferents are a classical problem encountered in using simple dynamic electroanalytical detectors without prior separation or the use of modified electrodes [30,31]. The effect of the nature of the crown ethers on catecholamine response is also discussed.

2. Experimental details

2.1. Reagents

NADH, dopamine, epinephrine (Sigma), ascorbic acid, acetaminophen (Aldrich), *p*-aminophenol, catechol, hydro-

quinone and potassium ferricyanide (Fisher) were used as received. 3-methyl-thiophene and tetrabutylammonium tetraflouroborate were obtained from Aldrich and were not further purified. L-dopa, L-dopamine, L-epinephrine and D,L-norepinephrine, 3-(3,4-dihydroxyphenyl)-2methyl-L-alanine(methyl-L-dopa), 3,4-dihydroxybenzylamine hydrobromide (DHBA), 3,4-dihydroxyphenyl acetic acid (DHPAA), norephedrine, 3-(3,4-dihydroxyphenyl) propionic acid (DPPA), tyramine, *p*-cresol, *p*-nitrophenol, serotonin and tryptamine were purchased from Fluka Chemical Company and used as received. Resorcinol, L-phenylalanine, and *o*-phenyldiamine were purchased from Aldrich Chemical Company. Catechol was purchased from the Fisher Scientific Company. The acetonitrile was dried by double distillation over calcium hydride and stored in sealed containers. The phosphate buffer stock solution (pH 9.4, 1 M) for potentiometric studies was prepared with potassium dihydrogen phosphate and the pH was adjusted by the addition of KOH. The standard solution of L-dopa, L-dopamine, L-epinephrine and D,L-norepinephrine were prepared with 0.01 M HCl and stored in brown glass bottles. Redistilled acetonitrile (UV grade, Aldrich) was used as solvent for electrochemical polymerization. The Sörensen buffer, which was used as electrolyte in cyclic voltammetric, square wave and differential pulse voltammetric studies and also as mobile phase in amperometric flow injection unless otherwise stated, was prepared as follows: 60 ml Na_2HPO_4 (9.47 g l^{-1}) + 40 ml NaH_2PO_4 (9.208 g l^{-1}) + 0.460 g NaCl. The measured pH of this buffer was 6.9.

The lipophilic macrocyclic crown ethers, binaphthyl-20-crown-6, and benzo-15-crown-5, were synthesized in our laboratories and verified with IR spectroscopy and ^1H nuclear magnetic resonance (NMR). All other crown ethers were purchased (Sigma). All other chemicals used in the present study were of reagent grade. Deionized water of 17.8 MΩ resistance was used throughout.

2.2. Procedure and apparatus

Electrochemical polymerization of 3-methylthiophene was carried out in a one-compartment cell containing deaerated acetonitrile, 0.1 M tetrabutylammonium tetrafluoroborate and 0.05 M 3-methylthiophene. Either a potentiostatic or a galvanostatic mode was adapted for film growth. Cyclic voltammetry was done with films grown at a constant potential of +1.80 V for 45 s. In all other cases, films were grown under galvanostatic mode with a current density of 10 mA cm^{-2} for 25 s. After the polymerization, all films were kept at −0.20 V for 10 min to undope BF_4^- (original electrolyte anion).

A platinum (MF2013, Bioanalytical Systems, Inc. (BAS), West Lafayette, IN, USA) or a glassy carbon (MF2012, BAS) electrode, coated or otherwise, was used for the voltammetric (cyclic, differential pulse or square wave) measurements. These measurements were conducted with a BAS-100 electrochemical analyser and the voltammograms were recorded with a DMP-40 digital plotter from Houston Instrument. Analyte concentrations stated for voltammetric analysis were obtained by directly adding a carefully weighed analyte(s) into the electrolyte solution(s).

A thin layer detector cell with dual Pt–Pt electrodes (MF1012, BAS) was used for flow injection–amperometric detection of analytes. The mobile phase was driven by an Altex model 100 double reciprocating pump with a flow rate of 1.0 ml min^{-1}. A 20 μl sample injection loop was used throughout the experiment. The electrode potential was controlled by a BAS model CV-1B cyclic voltammetry unit and the signals were recorded with a Fisher series 5000 recorder. Analyte solutions of desired concentrations were prepared through serial dilution of 1000×10^{-6} M stock solutions. A mobile phase buffer was used as a solvent for the preparation of stock solutions and for other dilution procedures. All potential values were expressed vs. Ag/AgCl (3 M NaCl) (MF2020, BAS).

The poly(crown ether) electrodes were prepared as follows. A stationary platinum disk electrode with a 1.6 mm diameter (MF-2012, BAS) was used as the basic transducer element matrix. This electrode was polished with 600 grid emery paper to a mirror surface and then ultrasonicated. Finally, the electrode was washed with high performance liquid chromatography (HPLC) grade acetone and dried in air for about 15 min before the electropolymerization.

The polymerization of binaphthyl-20-crown-6 on the platinum electrode was carried out in a three-electrode single-compartment cell containing 20 mM of the crown and 200 mM tetrabutylammonium tetrafluoroborate as the supporting electrolyte in freshly distilled acetonitrile. The above-mentioned stationary platinum electrode was used as the working electrode, a platinum wire coil as the auxiliary electrode and an Ag/AgCl electrode (MF-2063, BAS) as the reference electrode. The electrochemical polymerization was performed with a PAR 175 potentiostat–galvanostat (EG & G) (Princeton Applied Research, USA), with an applied potential of +3.2 V vs Ag/AgCl for 5 min. Visible dark films on the electrode were observed. Surface coverage of polymer film was not successful at lower potentials than +3.2 V. At potentials above +3.2 V gas evolution was observed and the polymer film did not adhere. The polymerized electrode was then rinsed with acetone, air dried, and immersed in a three-electrode single-compartment cell containing monomer-free acetonitrile solution of TBATFB for about 25 min at an applied potential of +0.5 V. This polymerized electrode was then rinsed with water and dried in air for 20 min before use. Potentiometric measurements, both static and FIA, were made with an Orion model 601A Ionanalyzer using Ag/AgCl as the reference electrode filled with 3 M KCl. The electrode potential was measured in 25 ml of 0.1 M phosphate buffer (pH 9.4) solution while stirring at 22°C

and recorded using a chart recorder. The polymerized electrodes were conditioned in stirred water until a steady potential value was obtained before use. After use, the electrode was stored in air. For the FIA experiments, the above flow cell and FIA system was employed. A flow rate of 1.0 ml min^{-1} was used throughout.

The EDAX system was a Cambridge 100 X-ray instrument with a Cambridge model 2P goniometer and a cobalt anticathode and the ESCA unit was a Perkin Elmer model 5300 spectrometer.

3. Results and discussion

3.1. Neurotransmitter electroxidation kinetics at poly(3-methylthiophene) electrodes [32]

Fig. 1 shows the cyclic voltammetric behaviours of ascorbic acid, catechol, dopamine and *p*-aminophenol at P3MT electrodes. Except for ascorbic acid, these and other test substances (ferri/ferrocyanide, hydroquinone, acetamidophenol and NADH) all displayed reversible behaviour in 0.1M H_2SO_4 electrolyte. From the biochemical sensor application point of view, oxidation peak potential positions of these substances are more important than their reversibility characteristics. In this respect, all compounds demonstrate dramatically improved electrode kinetics at P3MT-coated platinum electrodes. The enhanced electron transfer abilities of the P3MT-coated electrodes are presented in Table 1 by tabulating the anodic peak potentials of test substances at three different electrodes (P3MT, platinum and glassy carbon).

Glassy carbon electrodes are often preferred for the analysis of biological systems owing to their relative resistance to surface fouling effects [24]. However, as can be seen from Table 1, the anodic peak potentials of all the compounds were always more positive than those obtained at platinum electrodes in the electrolyte chosen for this study (0.1M H_2SO_4). Table 1 clearly demonstrates the electrocatalytic ability of the P3MT electrodes.

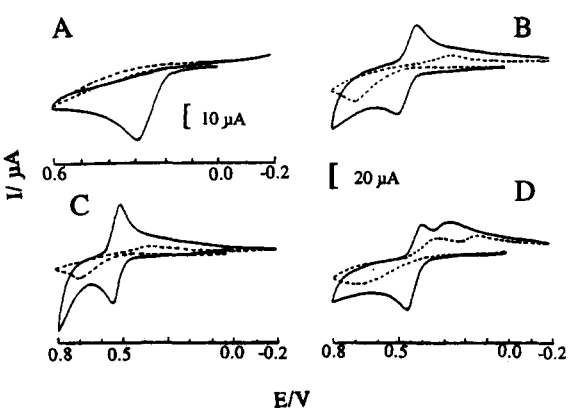


Fig. 1. Cyclic voltammograms for 5×10^{-3} M (a) ascorbic acid, (b) dopamine, (c) catechol and (d) *p*-aminophenol at P3MT-coated (———) and bare platinum (- - - - -) electrodes. Scan rate, 50 mV s^{-1}; electrolyte, 0.1 M H_2SO_4.

Table 1
Oxidation peak potentials of some biological compounds at three different elecrodes [a]

Compound	E_P [a] (V)		
	P3MT	Pt	Glassy carbon
Acetaminophen	0.492	0.600	0.904
p-Aminophenol	0.471	0.620	0.900
Ascorbic acid	0.296	— [b]	0.981
Catechol	0.520	0.689	0.849
Dopamine	0.510	0.690	0.831
Ephinephrine	0.440	0.735	0.940
Hydroquinone	0.492	0.567	0.729
Fe^{3+}/Fe^{2+}	0.317	0.437	0.568

[a] Values are obtained from the positive scan of first cycle electrolyte: 0.1 M H_2SO_4; analyte concentration, 5 mM; scan rate, 50 mV s^{-1}.
[b] No peak was observed up to 1.250 V.

One other characteristic of the voltammograms in Fig. 1 is that they all display large current envelopes. This kind of marked increase in the shapes of voltammograms which is also observed for analyte-free electrolytes appears to be an inherent property of conducting polymers. It seems that the large surface area provided by the three-dimensional porous structure of the coating greatly increases both faradaic and non-faradaic residual currents. This phenomenon, which is observed by other workers with polypyrrole [7] and with P3MT [8], greatly impairs the peak resolution for a given binary mixture, although the data in Table 1 otherwise indicate large differences in anodic peak potentials. For instance, the difference in anodic peak potentials for ascorbic acid and dopamine is 0.214 V. However, because of the general increase in the entire envelope of the voltammograms, the peak resolution is obscured to such an extent that their simultaneous observation by cyclic voltammetry is almost impossible.

Differential pulse voltammetry at a glassy carbon electrode of equimolar ternary mixture of ascorbic acid, *p*-aminophenol and catechol produces, as expected, a single overlapped peak (Fig. 2(a)). However, separation is easily achieved at P3MT-coated glassy carbon (Fig. 2(b)). This example, illustrating the separation of ascorbic acid, *p*-aminophenol and catechol has not yet been demonstrated at any other surface to the best of our knowledge and, on its own, deserves special attention.

Another example of electrocatalysis at a poly-3-methylthiophene electrode is the oxidation of reduced nicotinamide adenine dinucleotide (NADH) [33]. Problems associated with the electrooxidation of NADH, namely the considerable overpotentials, 1.1 V at carbon [34] and 1.3 V at platinum [35] electrodes (and the adsorbed oxidation product(s)) [36] result in the interference from more easily oxidizable species for the amperometric NADH detection in serum samples and electrode fouling at NADH concentrations above 0.1 mM respectively. The electrocatalytic

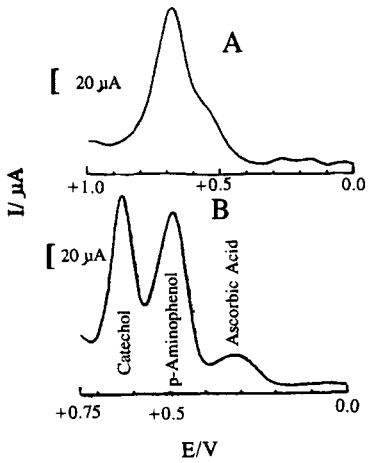


Fig. 2. Differential pulse voltammogram of a ternary mixture (5×10^{-3} M of each) of ascorbic acid, *p*-aminophenol and catechol at (a) glassy carbon and (b) P3MT electrodes. Scan rate, 4 mV s^{-1}: amplitude, 25 mV; electrolyte, Sörenson buffer (pH 6.9).

oxidation of NADH at modified electrode surfaces has been extensively investigated [37–42]. Transfer of electrons has been shown to be effectively catalysed by the introduction of mediators. However, these electrodes generally suffered from the lack of long-term stability.

Fig. 3 shows typical cyclic voltammetric behaviour of 0.01 M H_2SO_4 in the absence (curve A) and presence (curve B) of an electrode in 1 mM NADH. As may be seen from the results obtained on a P3MT electrode, the anodic peak potential values E_{Pa} for the NADH oxidation are $\approx$ 0.450–0.760 V, which is 0.250–0.850 V less positive at the modified electrode than at the non-treated platinum and carbon electrodes. However, the increase in the effective surface area is manifested by the increase in the background charging current in the case of P3MT. Summaries of the cyclic voltammetric results of NADH at a P3MT electrode in different electrolytes are given in Table 2.

The position of E_{Pa} was a function of both the nature of electrolyte and the pH employed. This might be attributed to different kinetic effects for different electrolytes used. The "doping" level by the anions and the conductivity of the polymer film electrode are a function of the charge : size ratio as reported by Garnier and co-workers [43]. This will also affect the electrochemical response of these films towards electroactive species on varying the supporting

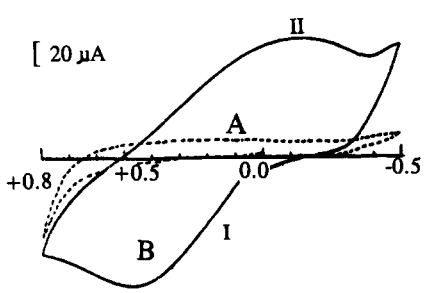


Fig. 3. Cyclic voltammetric response of P3MT grown on Pt in the absence (curve A) and in the presence (curve B) of 1 mM NADH (in 0.01 M H_2SO_4). Scan rate, 50 mV s^{-1}. I, anodic; II, cathodic.

Table 2
Effect of the electrolyte type on the peak potentials E_{Pa} and anodic peak currents I_{Pa} observed on the P3MT electrode (scan rate, 50 mV s^{-1})

Electrolyte [a]	E_{Pa} (V)	ΔE_{Pa} (V) [b]	I_{Pa} (μA)
Na_2SO_4	0.455	0.545–0.845	12.8
H_2SO_4	0.450	0.550–0.850	5.90
NaCl	0.472	0.528–0.828	9.07
$NaNO_3$	0.758	0.242–0.542	72.0
Buffer I [c]	0.448	0.552–0.852	157

[a] All electrolytes were 0.1 M in concentration in nano-pure water.
[b] Peak differences from those measured in glassy carbon and Pt electrodes.
[c] Buffer I: 5 mM Na_2HPO_4, 5 mM NaH_2PO_4 and 0.1 M NaCl.

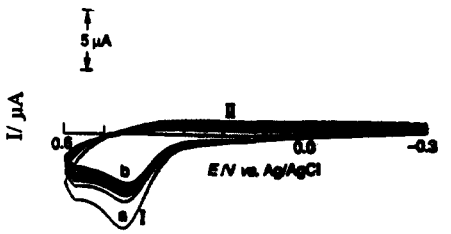


Fig. 4. Effect of repeated cycles on the stability of P3MT grown on Pt on the response towards 1 mM NADH (in 0.01 M Na_2SO_4) solution: curve a, cycle 1; curve b, cycle 100; I, anodic; II, cathodic.

electrolyte. However, the reversibility vs. irreversibility shown in Figs. 3 and 4 cannot be explained at this stage of the present work. The highest catalytic effect was observed with the use of buffer I. The stability of the polymer film was examined by repeatedly cycling the electrode within a narrow potential window as shown in Fig. 4. The usual adverse adsorption effect onto the electrode which resulted in the fouling of its surface and the significant attenuation of the voltammetric current signal was not observed. The anodic peak current values I_{Pa} correlate linearly with the square root $v^{1/2}$ of the scan rate for all the electrolytes used, indicating that the charge transfer process is controlled mainly by solution mass transport as illustrated in Fig. 5. Moreover, the peak potential E_{Pa} exhibited a positive linear shift with the logarithm of the scan rate indicating the irreversible nature of the electrode reaction. It could also be observed from the cyclic voltammogram

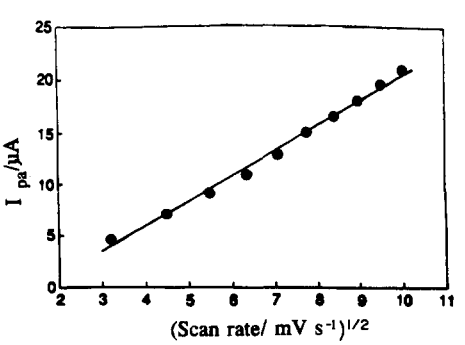


Fig. 5. Effect of increasing scan rate on anodic peak current I_{Pa}.

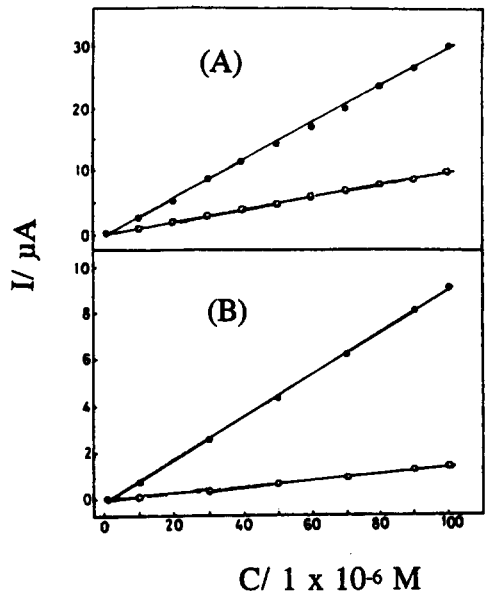


Fig. 6. Flow injection–amperometric detection calibration curves for (a) catechol and (b) ascorbic acid at P3MT (●) and Pt (○) electrodes. Sample size, 20 μl; mobile phase, Sörenson buffer (pH 6.9); flow rate, 1.0 ml min^{-1}; working electrode potential, 0.500 V.

shown in Fig. 1 that the electron transfer occurs through the polymer film at potentials well negative to its doped (oxidized) state, where the film becomes highly conducting [44–46].

Amperometric measurements under flow injections or liquid chromatographic conditions are particularly beneficial since the fouling problems are not as severe as in batch experiments, because of the small amount of product that is generated. This is especially true at low analyte concentrations.

Flow injection analyses of catechol, ascorbic acid, dopamine, epinephrine, NADH, *p*-aminophenol and acetaminophen were performed using Sörenson buffer as mobile phase; 20 μl of each test substance having concentration levels of 1×10^{-6}–100×10^{-6} M with 10×10^{-6} M increments were injected and current signals were measured from the recorded peak heights. The working electrode potential for each substance was set at a value that was slightly above the anodic peak potential of each compound shown for the P3MT electrode in Table 1. Fig. 6 shows the calibration curves for catechol and ascorbic acid obtained on P3MT and platinum electrodes. Current responses at the P3MT electrode were 4–10 times higher (depending on analyte type) than those obtained on platinum, and excellent linearity was observed for all the substances for the concentration range studied. For the estimation of detection limits 1×10^{-6} M, 10×10^{-6} M and 100×10^{-6} M solutions of the test substances were injected and the concentration which produced a current signal with a magnitude at least 2.5 times as high as the signal that is caused by the injection of buffer itself is accepted as a practical limit for detectability. Detection limits estimated from the procedure described above were 10×10^{-6} M for catechol, dopamine, epinephrine, NADH and *p*-aminophenol, 1×10^{-6} M for acetaminophen and 100×10^{-6} M for ascorbic acid.

Also, the signal stability was tested for 100 repetitive injections of 5 mM NADH. Coefficient of variation was found to be 8.1%. This implies that P3MT electrode is not subject to surface fouling by the oxidation product(s) of NADH, which is notorious for its surface fouling effects. Fig. 7 shows the chromatogram obtained for a sample of a mixture of norepinephrine, L-DOPA, epinephrine and dopamine with a P3MT electrode. The conditions for the separation and detection were as follows: column, PARTISIL 5 ODS-3; mobile phase, 0.15 M monochloroacetic acid, 50 mg SOS, pH 3.0; flow rate, 1.5 ml min^{-1}; electrochemical detector; P3MT electrode at an applied potential of +0.55 V. Equimolar amounts of the four analytes were used, namely 10^{-3} M. Comparison of this chromatogram with that shown in Fig. 8, where a glassy carbon electrode was used, reveals the following facts: (i) the current signals obtained for P3MT as the working electrode are higher than those obtained in the case of the glassy carbon electrode; (ii) the baseline of the first chromatogram exhibits a relatively higher stability when compared with that obtained in Fig. 8. The use of poly-*N*-methylpyrrole as the electrochemical detector dramatically affected the current signal and the baseline stability as depicted in Fig. 9.

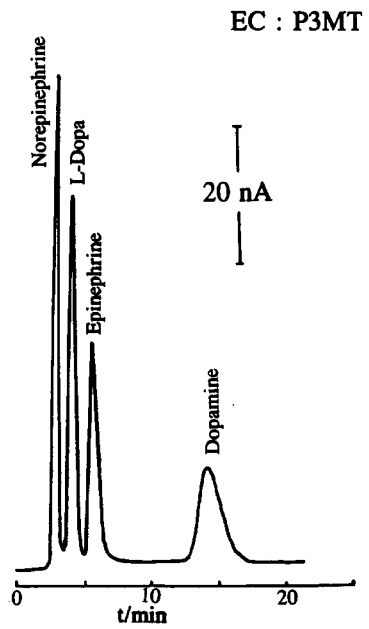


Fig. 7. Chromatogram of catecholamine mixture. Sample volume, 20 μl; PARTISIL 5 ODS-3 column; mobile phase, 0.15 M monochloroacetic acid, pH 3.00, 26°C; 100 nA full scale; flow rate, 1.5 ml min^{-1}; chart speed, 10 mm min^{-1}; detector BAS CV-1B; 0.55 V with P3MT working electrode.

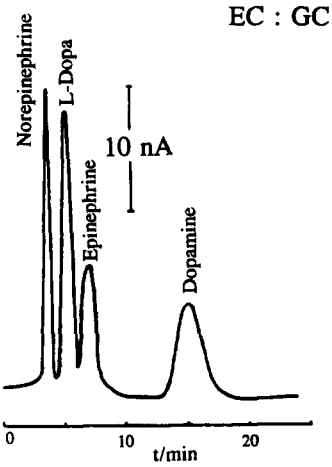


Fig. 8. Chromatogram of catecholamine mixture. Conditions as in Fig. 2 except glassy carbon used as working electrode; full scale is 50 nA.

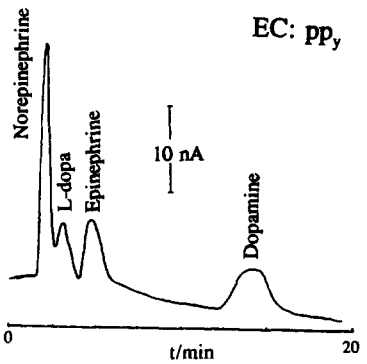


Fig. 9. Chromatogram of catecholamine mixture. Conditions as in Fig. 2 except poly-*N*-methylpyrrole used as working electrode; full scale is 50 nA.

3.2. Mechanism studies

As real samples of neurotransmitters will also contain large concentrations of non-electroactive organic surfac-

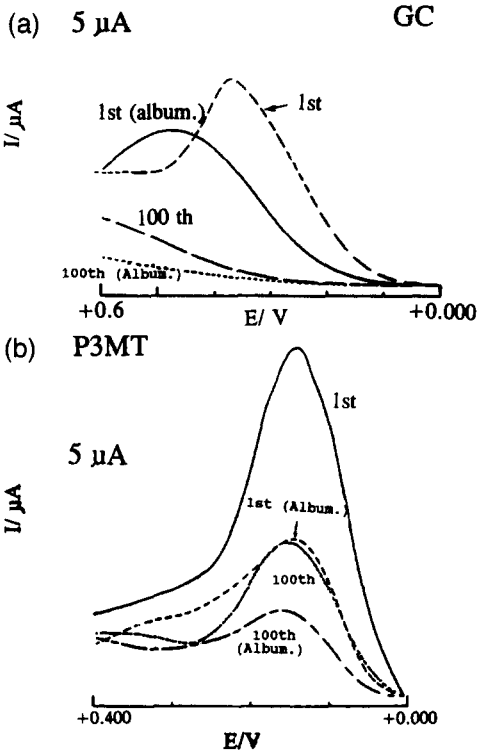


Fig. 10. Square wave voltammograms of 5 mM *p*-aminophenol in phosphate buffer (pH 6.9) in the presence and absence of 1000 mM albumin for (a) glassy carbon and (b) P3MT.

tants, the electrode response was examined in the absence and presence of some surface-active agents. Fig. 10(a) shows the square wave voltammetry of 5 mM catechol in the presence of 1000 mM albumin at P3MT, glassy carbon and Pt electrodes. The signal was attenuated in the case of Pt and glassy carbon electrodes and relatively unaffected in the case of P3MT. The effect of repetitive cycles on the electrochemical response of glassy carbon and P3MT was examined in the presence and absence of 1000 mM albumin for the analysis of *p*-aminophenol and the results are

Table 3
Electrolyte and substrate effect on the electrochemical response of P3MT

Electrolyte	pH	Pt [a]	P3MT/Pt [a]	Glassy carbon [a]	P3MT/glassy carbon [a]
H_2SO_4	1.60	0.524, 25	0.445, 45	0.633, 67	0.439, 170
Na_2SO_4	3.70	0.367, 30	0.340, 43	0.444, 67	0.335, 120
HCl	1.56	0.590, 24	0.427, 50	0.663, 66	0.435, 160
NaCl	3.23	0.439, 11	0.381, 22	0.512, 42	0.388, 130
HNO_3	1.25	0.568, 23	0.422, 36	0.667, 62	0.430, 160
$NaNO_3$	3.28	0.422, 16	0.317, 29	0.444, 69	0.321, 150
H_3PO_4	3.85	0.385, 19	0.291, 37	0.475, 46	0.298, 130

Solution, 5 mM ascorbic acid–100 mM electrolyte; scan rate, 100 mV s^{-1}.
[a] The values are voltages (volts) and currents (microamperes).

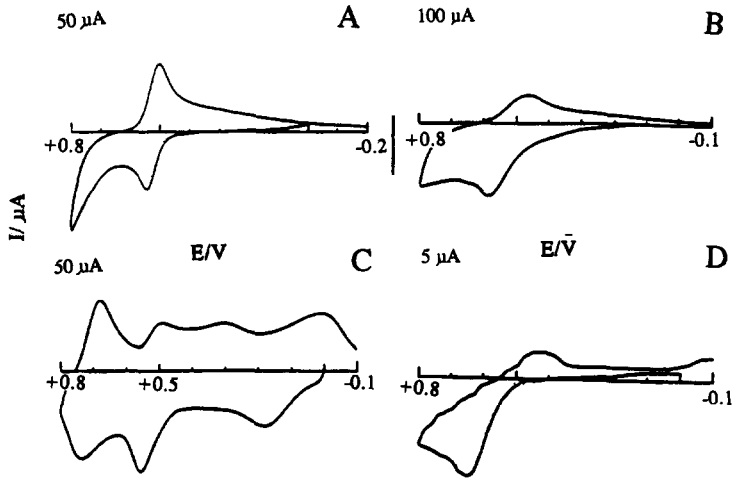


Fig. 11. Cyclic voltammetry of 5 mM catechol in 0.1 M H_2SO_4 at (a) P3MT, (b) poly-*N*-methylpyrrole, (c) polyaniline, PAn, and (d) polyfuran, PF electrodes. Scan rate, 50 mV s^{-1}. All films were prepared under similar conditions.

depicted in Fig. 10(b). The following observations could be noticed: (i) the position of the oxidation peak potential was shifted to a more positive value on the addition of albumin in the case of glassy carbon while relatively unaffected in the case of P3MT; (ii) the effect of repetition in the absence of albumin attenuated the current signal after 100 cycles, while on the contrary the P3MT electrode displayed only "partial" attenuation for the same number of repeated cycles. More interestingly the peak potential position remained relatively unchanged.

The analytical potential described above for the determination of neurotransmitters etc. raises many questions concerning the nature and mechanism of the electrocatalysis obtained at P3MT electrodes.

As mentioned briefly above, Wang et al. [9] have previously showed that, for many polymer-coated electrodes, the electroactive species diffuses through pores in the film and the actual electron transfer occurs at the surface of metal or carbon substrate. Thus, the redox characteristics, such as formal potentials, overpotentials, peak current separation etc., are the same as those obtained at the bare substrate itself. They used pore size to obtain electrochemical selectivity. However, careful inspection of Table 3 reveals an interesting fact; the electrocatalytic activity of the polymer film is independent of the nature of the solid substrate used for the film deposition [47]. As shown in the fourth and sixth columns of Table 3, the E_{ox} values for the different supporting electrolytes studied are the same for films grown at glassy carbon or Pt surfaces. On the contrary, the oxidation potential values E_{ox} are different for Pt and glassy carbon (third and fifth columns). This indicates that the oxidation process is taking place at the "surface" of the polymer film rather than at the substrate. This conclusion is supported by scan rate studies which exhibit a square root dependence (see Fig. 5 for example) and ΔE_p values which indicate that the cyclic voltammograms at P3MT electrodes have a large solution diffusion control component [32,33].

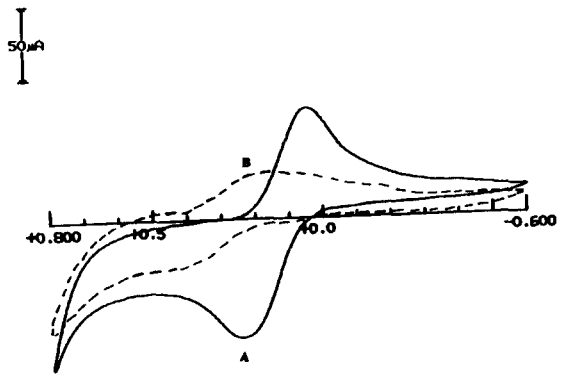


Fig. 12. Cyclic voltammograms of a 0.5 mM catechol + 0.1 M Na_2HPO_4 (pH 7.5) solution at a P3MT electrode: curve A, untreated; curve B, cycled in a 0.1 M $NaMnO_4$ buffer solution prior to use.

The fact that different conducting polymer electrodes exhibit large differences in the degree of electrocatalysis for the oxidation of the neurotransmitters, with polythiophene being the most effective (Fig. 11) [47,48], suggests that the heteroatom in the polymer may be the active site in the electron transfer. This supposition is supported by the comparison of the cyclic voltammograms in Fig. 12. Curve A is the cyclic voltammogram obtained for a 5 mM catechol–0.1 M phosphate buffer (pH 7.5) solution at a normal P3MT electrode and curve B is that obtained for the same electrode that was pretreated by cycling in a 0.5 M $NaMnO_4$ buffer solution prior to the cyclic voltammetry experiment. This pretreatment markedly decreases both the anodic and the cathodic peak currents and also gives a large shift of the peak potential values. The ESCA spectrum (Fig. 13) of the pretreated electrode film exhibits distinct molybdenum peaks. The cyclic voltammograms remain unchanged and the Mo ESCA peaks remain the same on repetitive cycling indicating that the Mo ion is strongly bound to the polymer surface. Assuming that the

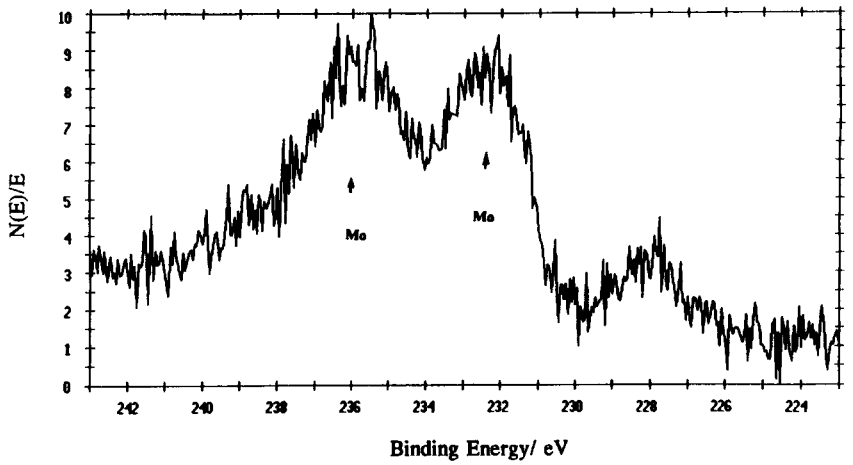


Fig. 13. ESCA spectrum cycled in a 0.1 M $NaMnO_4$, 0.1 M Na_2HPO_4 (pH 7.5) P3MT electrode. ESCA spectrum plotted $N(E)/E$ is the counting rate as a function of the kinetic energy of the electron versus the binding energy.

Mo ion is coordinated to the ring sulphur further suggests that the ring sulphur atom is directly involved in the electrocatalysis mechanism and that the electron transfer occurs at the polymer–solution interface. In addition, one can calculate "apparent" diffusion coefficients for the neurotransmitters in the polymer film matrix from double-potential step chronocoulometric experiments [49] using the method described by Chambers and coworkers [50,51]. Table 4 shows that values of the order of 10^{-14}–10^{-15} $cm^2\ s^{-1}$ are obtained [52]. Calculation of the diffusion coefficient of the redox process for the same neurotransmitters from cyclic voltammetry data gives values in the 10^{-5} $cm^2\ s^{-1}$ range suggesting that the diffusion rate limiting process takes place in the solution phase and not in the polymer film matrix. This also supports the contention that the electron transfer is occurring at the polymer–solution interface.

3.3. Neurotransmitter-selective potentiometric electrodes

We had previously found that an electropolymerized film of binaphthyl-20-crown-6 (BN-20-C-6) exhibited a selective potentiometric response to catechol and the neurotransmitters [53]. In this paper we describe the optimum experimental conditions for the response, selectivity, and analytical applications of BN-20-C-6 and other electropolymerized crown ethers for static potentiometric [53,54] and FIA determination [54,55] of these compounds.

Table 4
Apparent diffusion coefficients for different analytes at P3MT electrode (30 s growth time, 0.6 μm estimated thickness; analyte, 1 mM in 1 M KCl)

Compound	D_{ox} ($cm^2\ s^{-1}$)	D_{red} ($cm^2\ s^{-1}$)
Ferricyanide	2.7×10^{-14}	3.1×10^{-14}
Catechol	1.8×10^{-14}	2.2×10^{-14}
Dopamine	8.7×10^{-15}	9.4×10^{-15}
Norepinephrine	1.5×10^{-15}	2.1×10^{-15}

The mechanism of the interaction and nature of the response [53,55] is also presented. Fig. 14 shows the structures and designations of the various compounds studied in this section.

Fig. 15 shows the pH dependence of the observed potential for the BN-20-C-6 electrode in 0.1 M potassium phosphate buffer in the presence of 2×10^{-6} M dopamine or epinephrine (pH 6–12). As depicted in Fig. 15, this poly(crown) ether electrode response is highly pH dependent. The pH response of the electrode is linear with a nernstian slope of 56–59 mV $decade^{-1}$ in the pH range 6–8.5 (cf. Fig. 15). Constant response was obtained between pH 8.5 and 10. This is the pH region where only one hydroxy group of the catechol moiety is protonated. In the present case, potassium ions at the high concentrations which exist in the buffer solution would be expected to occupy the cyclic ring of crown ether as the electrode was preconditioned in the potassium solution. At pH values between 8.5 and 10, the monoprotonated catechol moiety probably interacts with the crown ether as discussed later. At lower pH values (below 8.5) amino groups will be protonated and would be expected to interact with crown ethers, resulting in a normal nernstian pH response behaviour. However, this is not the cause of the potential–pH response in this range as catechol and DHPAA have identical behaviours. For pH values over 10.5, hydroxy groups of catecholamines will be totally deprotonated [56] resulting in a dramatic shift of the electrode response to the negative direction as the pH increases. In the present study phosphate buffer solutions of pH 9.4 were chosen and used throughout the evaluation of the electrode response as it was only in this pH range and particular buffer that gave a "super-nernstian" concentration response for catechol as discussed below.

Typical calibration curves of the polycrown ether electrode for catecholamines are shown in Fig. 16. The general characteristics of the calibration curves of dopamine, epinephrine, and norepinephrine are similar. The calibra-

tion curve of dopa has a somewhat better linearity than that of the other catecholamines. The polycrown ether electrode has a linear response range of about 1×10^{-7}–5×10^{-4} M and a "super-nernstian" response slope of 110–130 mV decade^{-1} with a detection limit of about 3×10^{-8} M for the catecholamines. The usable analytical

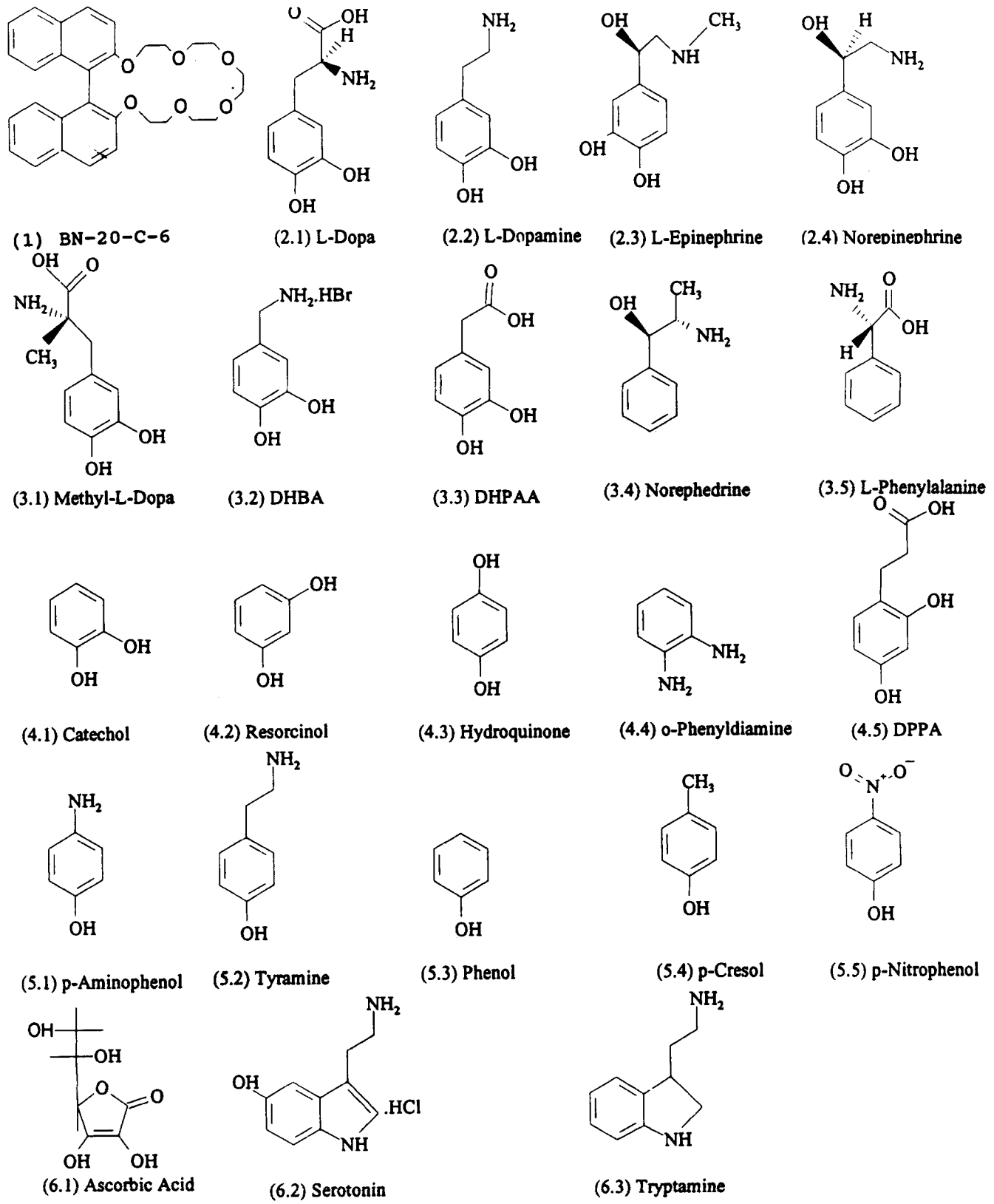


Fig. 14. Structures of compounds studied.

dynamic range of the various calibration curves was about 3×10^{-8}–2×10^{-5} M.

It is known that BN-20-C-6 can complex with potassium ions owing to the size matching of potassium ion (1.33 Å) and the crown cavity (1.35 Å) [23]. In the present case, high concentrations of potassium, up to 0.1 M, were used to make up the phosphate buffer solution. However, all interferences that might be caused by the interaction of potassium (control cation) and other inorganic anions are negligible compared with the dopamine response as shown in Fig. 5. The anions tested, Cl^-, F^-, Br^-, NO_3^-, NO_2^-, HCO_3^-, Ac^-, $B_4O_7^{2-}$, ClO_4^-, SCN^-, SO_4^{2-}, $S_2O_3^{2-}$ and I^-, exhibited relatively poor response as indicated by the shaded area of Fig. 17.

All alkyl derivatives of catecholamines and similar compounds listed in Fig. 14 were tested for their response behaviour and the results are shown in Figs. 18(a) and 18(b). Fig. 18(a) indicates that the electrode responded to methyl-L-dopa, 3,4-dihydroxybenzylamine (which have amino groups), and 3,4-dihydroxyphenyl acetic acid (without an amino group). The compounds to which the electrode responded have a common factor in their chemical structure; that is the presence of an *o*-dihydroxyphenyl moiety. Moreover, the presence or absence of the amino group in the compound did not significantly affect the electrode response. Therefore, it could be concluded that the sensor electrode has a selective response to the 3,4-dihydroxyphenyl derivatives of organic compounds. The above assumption was confirmed by inspecting the data of Fig. 18(b) as follows: (i) the response towards catechol

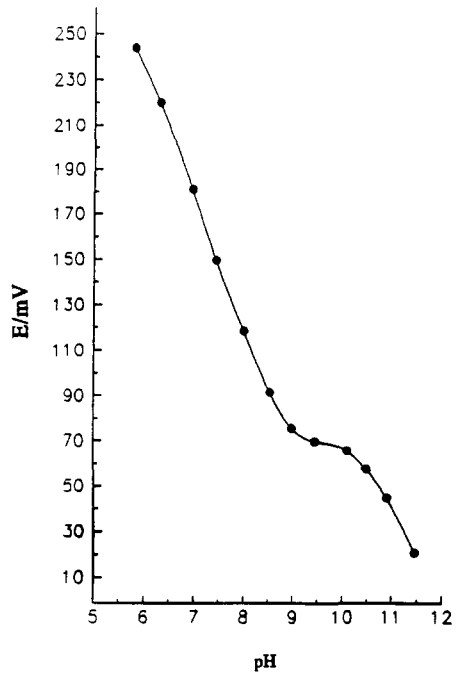


Fig. 15. The pH dependence of potentiometric response of poly-(binaphthyl-20-crown-6) electrodes. 0.1 M K phosphate buffer; 10^{-6} M dopamine and/or epinephrine.

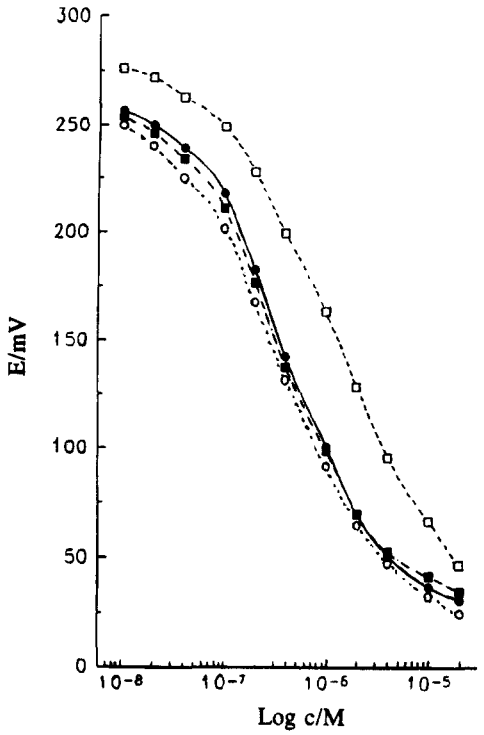


Fig. 16. Calibration curves of poly(crown ether) electrode for catecholamines: ●, dopamine; ■, norepinephrine; ○, epinephrine; □, L-dopa.

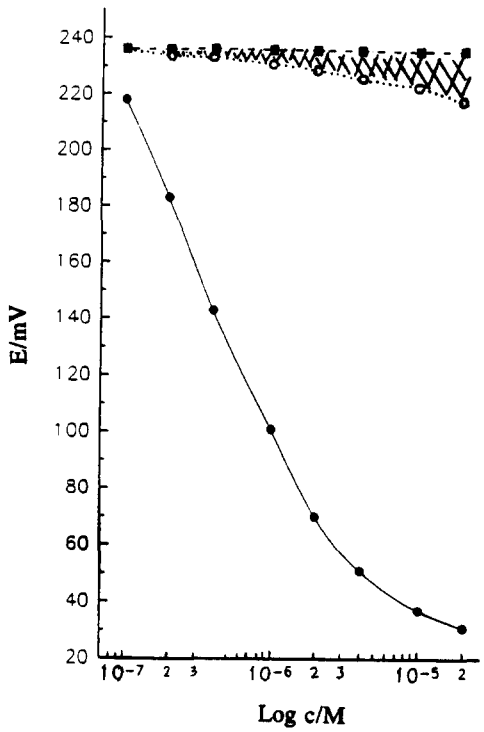


Fig. 17. The selectivities of potentiometric response to inorganic anions: ●, dopamine; ■, chloride; ○, iodide; curves for the anions F^-, Br^-, NO_3^-, NO_2^-, HCO_3^-, Ac^-, $B_4O_7^{2-}$, ClO_4^-, SCN^-, SO_4^{2-}, $S_2O_3^{2-}$ fall between those of chloride and iodide.

was relatively superior and, therefore, will be taken as a reference measure of the electrode responsive behaviour; (ii) a relatively poorer response was observed for the determination of *o*-phenyldiamine which bears two amino groups; (iii) the interaction of the other dihydroxy derivatives is also important as can be observed for the moderate response towards hydroquinone and the poor response towards resorcinol and DPPA. Fig. 18(c) shows further

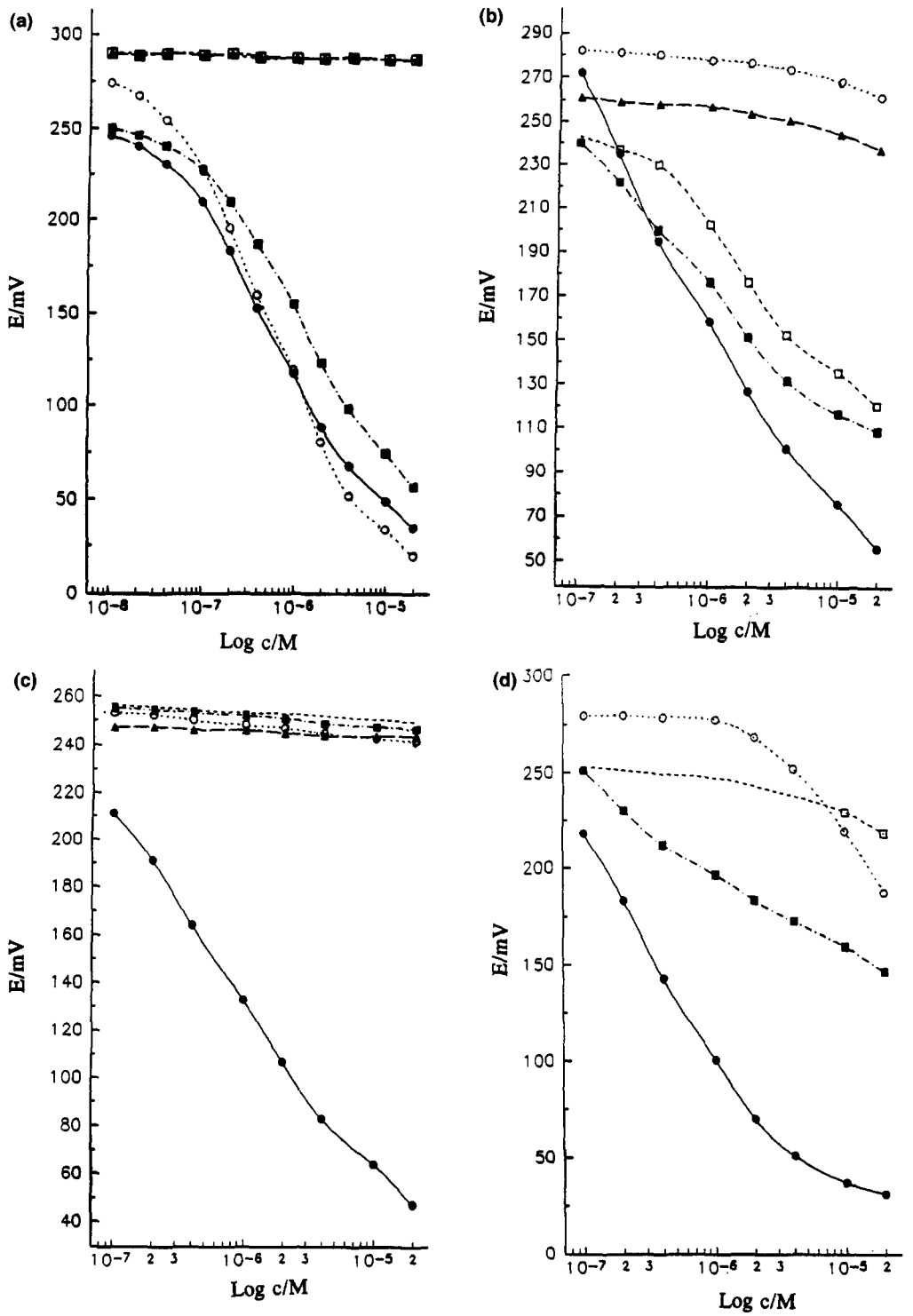


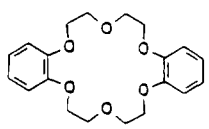
Fig. 18. Response to other derivatives of catecholamines. (a) Response to derivatives with and without catechol groups: ●, methyl-L-dopa; ○, dihydroxybenylamine; ■, dihydroxyphenylacetic acid; □, norephedrine; ▲, phenylalanine. (b) Effects of hydroxy position and substitution of amino group on potentiometric response: ●, catechol; ○, resorcinol; ■, hydroquinone; □, *o*-phenyldiamine; ▲, 3-(2,4-dihydroxphenyl) propanic acid (DPPA). (c) Response to phenol and its *p*-substituted derivatives: ●, 4-aminophenol; ○, tyramine; ■, phenol; □, *p*-cresol; ▲, *p*-nitrophenol. (d) Response to other dopamine-like derivatives (phosphate buffer, 0.1 M, pH 9.4): ●, dopamine; ○, ascorbic acid; ■, serotonin; □, Tryptamine.

evidence for this explanation; the electrode does not respond to phenol, *p*-cresol or *p*-nitrophenol. It is, however, important to note that *p*-aminophenol is an exception to the general trend. Moreover, another important aspect about the selective nature of the mechanism of interaction of the analyte and the crown ether can be observed from the data of Fig. 18(c) as tyramine and the *p*-aminophenol possess the same functional groups, amino and hydroxy groups. However, the steric effect exerted by the relatively long chain in tyramine could possibly explain the poor response compared with that of *p*-aminophenol, or the basicity differences of the aromatic and aliphatic amine groups in these compounds might cause the response difference.

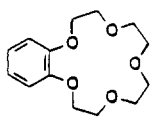
The comparison of these electrode responses to dopamine and its structurally similar compounds (ascorbic acid, serotonin and tryptamine) is shown in Fig. 18(d). The electrode exhibited a negligible response to tryptamine but had a moderate response to serotonin with a "sub-nernstian" slope of about 45 mV decade^{-1}. The electrode had virtually no response to ascorbic acid, except at high concentrations. This result shows that ascorbic acid below 3×10^{-5} M does not interfere for the potentiometric determination of catecholamines. This is important for the analysis of catecholamines in natural biological media which usually contain relatively high concentrations of ascorbic acid and represents a classical problem encountered in the amperometric determination of dopamine etc. in biological fluids.

Fig. 19 shows the structures of the other crown ethers used in this study. The effects of changing the crown ether polymerized or adsorbed onto a platinum electrode for the analyses of catechol and catecholamines by static potentiometric and potentiometric FIA were studied. A series of experiments were designed to determine which crown

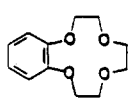
Dibenzo-18-Crown-6
(DB-18-C-6)



Benzo-15-Crown-5
(B-15-C-5)



Benzo-12-Crown-4
(B-12-C-4)



Tetraethylene Glycol Dimethyl Ether
(Open-C)

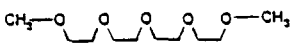


Fig. 19. Crown ether structures.

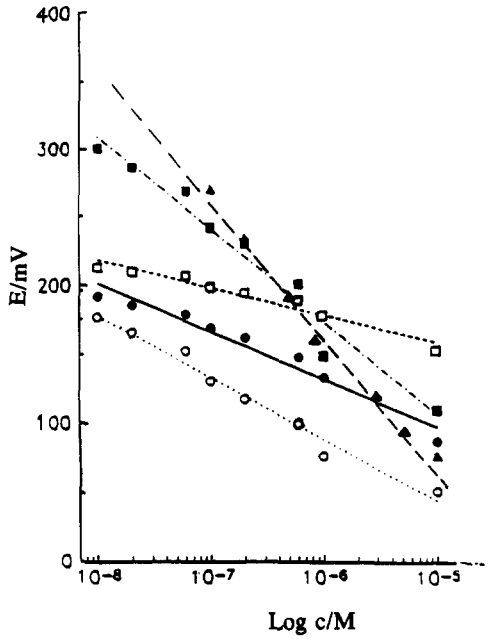


Fig. 20. Effect of changing the crown structure on the electrode response to catechol by static potentiometric detection: ●, DB-18-C-6; ○, B-15-C-5; ■, B-12-C-4; □, open crown; ▲, BN-20-C-6.

ether gave the best response to catechol and catecholamines.

Fig. 20 shows the effect of changing the crown ether structure on the response to catechol under static potentiometric conditions. The benzo-12-crown-4 potentiometric electrode exhibited "near-nernstian" response with a slope

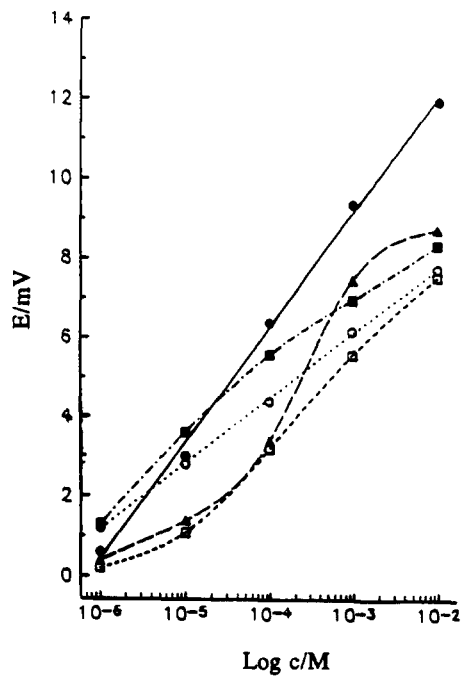


Fig. 21. Effect of changing the crown structure on the electrode response to catechol by potentiometric FIA: ●, DB-18-B-6; ○, BN-20-C-6; ■, B-15-C-5; □, B-12-C-4; ▲, open crown.

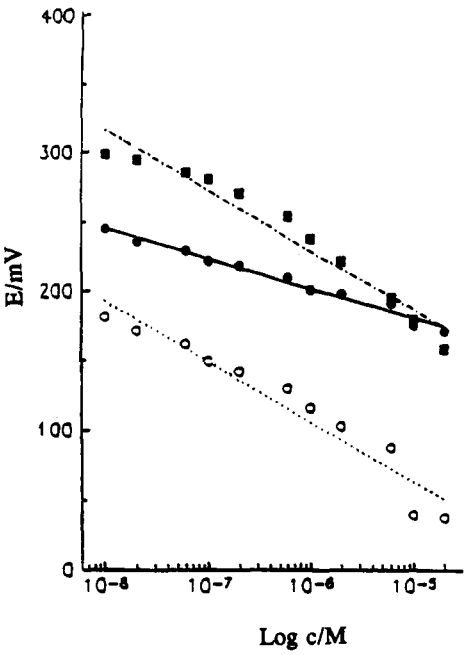


Fig. 22. DB-18-C-6 modified electrode under static potentiometric detection of various derivatives of catecholamines: ●, L-dopa; ○, catechol; ■, dopamine.

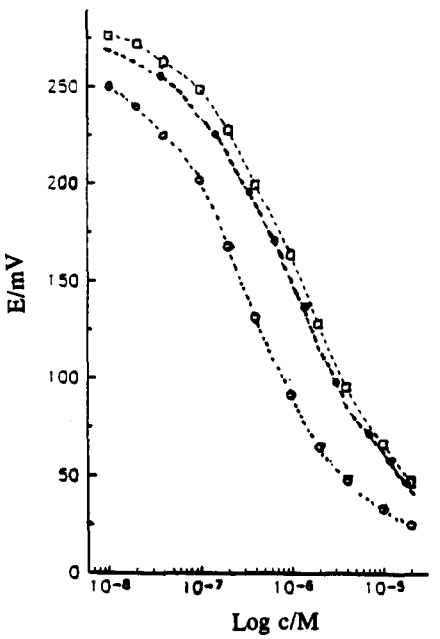


Fig. 23. BN-20-C-6 modified electrode under static potentiometric detection of various derivatives of catecholamines: □, L-dopa; ●, catechol; ○, dopamine.

of 67 mV decade^{-1} and the binaphthyl-20-crown-6 gave a "super-nernstian" slope of 110–120 mV decade^{-1}. All the other crown compounds exhibited "less than nernstian" slopes. The open crown with five oxygen atoms gave the smallest slope of all. The static potentiometric measurements achieved detection limits low as 0.5×10^{-8} M. There was no correlation of the crown structure (or number of oxygen atoms) on the response slopes. However, it is important to note that all the crowns gave a static potentiometric response.

Fig. 21 shows the potentiometric FIA response of the various crown electrodes for catechol. A general trend is exhibited for the various crowns polymerized onto the platinum electrode as potentiometric FIA detectors. For a decrease in the number of crown ring oxygen atoms, there is a decrease in the FIA response to catechol. The two crown-6_s and the crown-5 electrodes achieved detection limits low as 0.5×10^{-6} M; the benzo-12-crown-4 and the tetraethylene glycol dimethyl ether (open crown) achieved a detection limit of about 0.5×10^{-5} M and 0.5×10^{-4} M respectively.

It has been found that the different crowns exhibit some selectivity for the static potentiometric determination of specific catecholamines. For example Fig. 22 illustrates the effect of the dibenzo-18-crown-6 modified electrode response to various derivatives of the catechols. The binaphthyl-20-crown-6 modified electrode exhibits, however, less difference in response for the three catechols as shown in Fig. 23. In contrast, the potentiometric FIA mode (Fig. 24) illustrates that the binaphthyl-20-crown-6 was more selective towards the catechol than other catecholamines. The response characteristics of the aromatic crown ether electrodes under static potentiometric and potentiometric FIA determination of specific catecholamines were unchanged over 1–7 days of continuous use. The static potentiometric response of the open crown ether electrode was found to decrease after 1 h to about

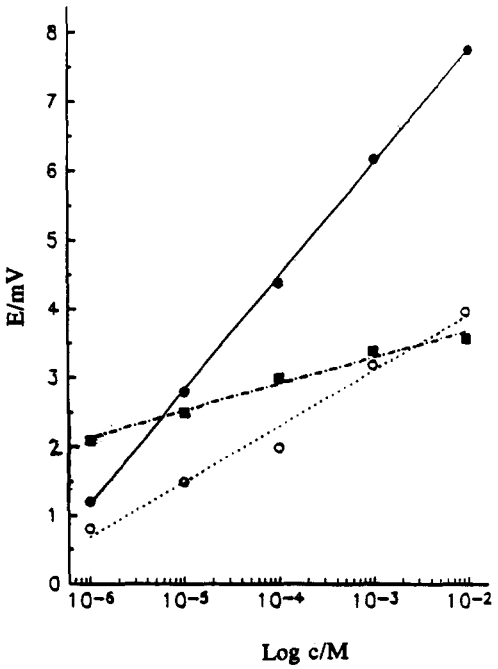


Fig. 24. BN-20-C-6 modified electrode under potentiometric FIA detection of various derivatives of catecholamines: ●, catechol; ○, L-dopa; ■, dopamine.

20% of the original signal. After 5 h, the potentiometric FIA of the open crown ether electrode did not respond to the catecholamines.

Common interferents such as ascorbic acid, uric acid and acetaminophen in the determination of catechols are of great concern in biomedical analyses. These interferents are oxidized at more negative potentials than the catechols and simple amperometric and voltammetric methods cannot be used without prior separation or use of an anionic membrane for FIA [55,57,58]. It has been found here that one can determine catechols in the presence of ascorbic acid, uric acid, or acetaminophen by FIA employing a potentiometric dibenzo-18-crown-6 electrode detector. These interferents, in as much as two order of magnitude excess compared with the catechols, did not significantly interfere, as shown in Fig. 25. For example, Fig. 26 displays the typical potentiometric FIA signal responses obtained for catechol in the absence and in the presence of ascorbic acid in equal concentrations. This demonstrates that, at equal concentrations of catechol and ascorbic acid, the FIA signal response remains identical to that in the absence of ascorbic acid. It also shows that the potentiometric response time of these electrodes is very fast compared with the residence time of the sample zone in contact with the electrode surface for this flow rate.

With respect to the mechanism, (i) the pH dependence shown in Fig. 15 of the BN-20-C-6 electrode does not result from the proton exchange of the complexes formed between the protonated amino group of catecholamine and poly(crown ether) at low pH values and (ii) a catechol-like structure with a 1,2-dihydroxy substituent is critical for the response (except for the two 1,4-difunctional species mentioned above). Before we consider a systematic response

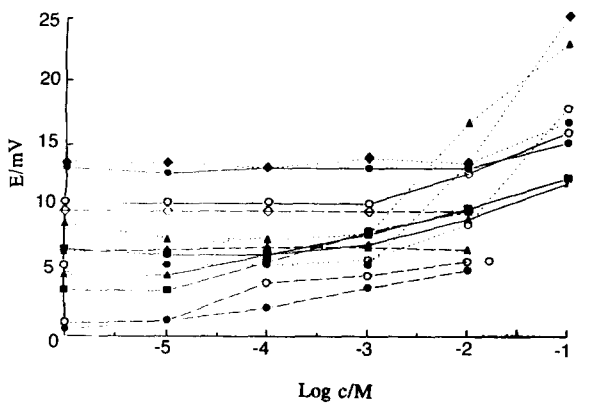


Fig. 25. Effect of interferents on the potentiometric determination of catechol by FIA using a DB-18-C-6 detector: — ● —, 10^{-2} M catechol + ascorbic acid; — ○ —, 10^{-3} M catechol + ascorbic acid; — ■ —, 10^{-4} M catechol + ascorbic acid; — ▲ —, 10^{-5} M catechol + ascorbic acid; — ● —, 10^{-6} M catechol + acetominophen; — ○ —, 10^{-5} M catechol + acetominophen; — ■ —, 10^{-4} M catechol + acetominophen; — ▲ —, 10^{-3} M catechol + acetominophen; — ◇ —, 10^{-2} M catechol + acetominophen; ··· ● ···, 10^{-4} M catechol + uric acid; ··· ○ ···, 10^{-4} M catechol + uric acid; ··· ▲ ···, 10^{-3} M catechol + uric acid; ··· ◆ ···, 10^{-2} M catechol + uric acid.

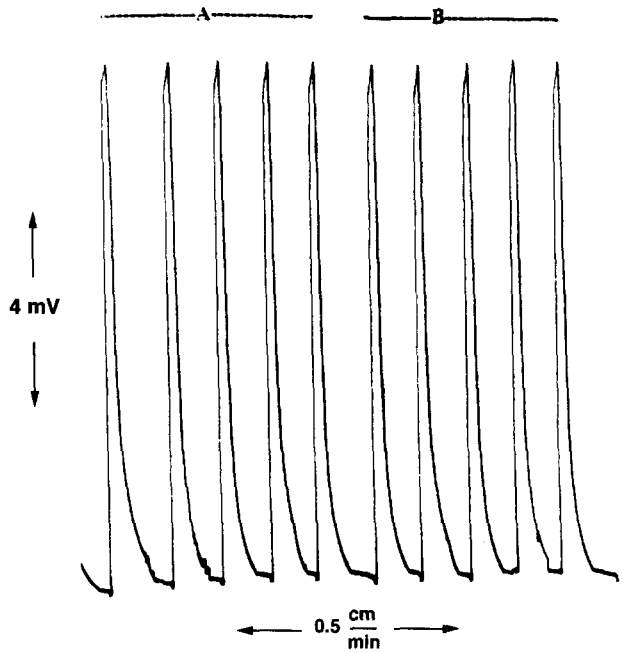


Fig. 26. Examples of FIA signals obtained on the modified DB-18-C-6 electrode. Sample size, 20 μl; mobile phase, potassium phosphate buffer (pH 9.4); curves A, 10^{-2} M catechol; curves B, 10^{-2} M catechol + 10^{-2} M ascorbic acid.

model of the poly(crown ether) electrode, the negative response of the electrode with increased concentration of catecholamines has to be considered. Umezawa et al. [23] suggested a possible response model of macrocyclic poly(amine) liquid membrane electrode for catechol. This poly(amine) liquid membrane electrode had a negative potential response for catechol in the acetate buffer of pH 6.1. It was thought that two protons on the aza crown cycle of polyamine were ejected owing to hydrogen bonding between the host and guest at the membrane surface. This model is suspect for two reasons. First, the electrode response to catechol was in acid buffer where the amine group is protonated. Second, the hydrogen bonds are possibly not strong enough to eject the protons bonded with ring nitrogen atoms. There must be other factors that caused the negative response for catechol. In the present case, the working pH of the poly(crown ether) electrode is 9.4. The catechol "functionality" of the guest compounds, catecholamines, have an approximate acid dissociation constant similar to that of catechol, $pK_{a1} \approx 9.3$ [56]. Thus, the catechol group loses one hydroxy-proton, producing the negative charge in phosphate buffer of pH 9.4. The poly(crown ether) group on the electrode surface would be expected to take up a potassium ion and the catechol monoanion would probably "sandwich" between two crown moieties as proposed recently for HS^- response to the binaphthyl-20-crown-6 electrode [59]. This would explain the "super-nernstian" slope. This assumption was not confirmed by the data obtained from EDAX of the electrode surface. A freshly prepared poly(binaphthyl-20-

crown-6) electrode exposed to a potassium–phosphate buffer solution alone and one exposed to a buffer–catecholamine solution were compared. Fig. 27 shows the results of the EDAX analyses. In this case, the potassium peak is high for the electrode preconditioned in the potassium buffer (0.1 M) only, as indicated in Fig. 27(a). If the electrode is treated with the buffer solution containing 1×10^{-5} M dopamine or epinephrine (Fig. 27(b)) the potassium peak intensities decrease significantly. ESCA spectra also confirm the loss of K^+ on exposure to catechols. This indicates that the monoanions of the catecholamines were interacting with the poly(crown ether) film and subsequently ejecting the K^+ ion. Furthermore, it was found that variation of the cation of the buffer (Li^+, Na^+, K^+, and Cs^+) had no effect on the electrode response. The "super-nernstian" slope observed does suggest that each binaphthyl-20-crown-6 unit may be arranged in a parallel layer (stacked) structure and the catechol moiety is "sandwiched" between two adjacent crown rings.

Further evidence that the catechol monanion does not enter the rings is given by a study that shows that even small ring crowns and the open crown (all of which do not coordinate with K^+) gave only slight decreases in potentiometric response which suggests that ring size is not a significant factor in the interaction occurring here. NMR studies with water-soluble crowns are underway to try to determine the interaction mechanism.

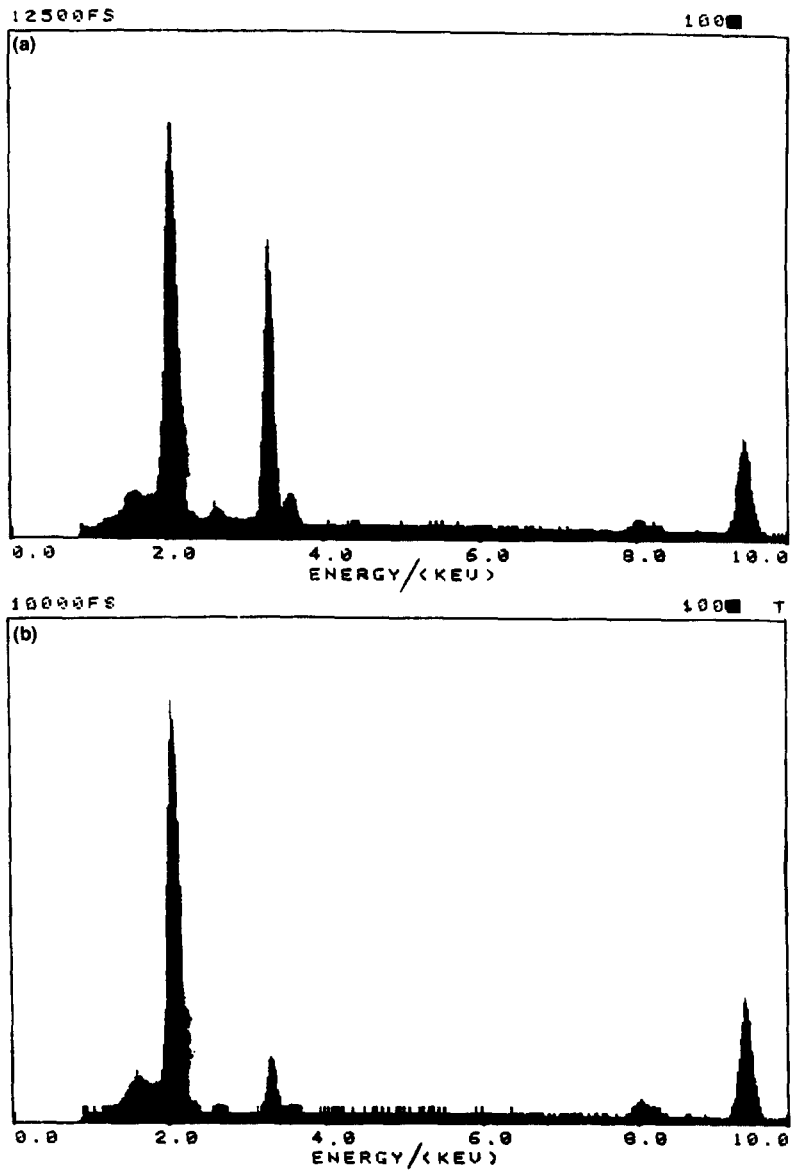


Fig. 27. EDAX analysis before and after electrode response to catecholamines: (a) 0.1 M phosphate buffer (pH 9.4); (b) buffer + 10^{-6} M dopamine or 10^{-6} M epinephrine.

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