The Application of Various Immobilized Crown Ether Platinum-Modified Electrodes as Potentiometric and Amperometric Detectors for Flow Injection Analyses of Catechol and Catecholamines

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Abstract
Different crown ethers were electrochemically polymerized or adsorbed onto a platinum electrode for the analysis of catechols by either static potentiometric, potentiometric FIA or amperometric FIA. The static potentiometric responses of all the crown ether modified electrodes were linear over about three decades of catechol concentration ($10^{-5}-10^{-8}$ M). The potentiometric FIA response of all the crown ether modified electrodes exhibited linear responses over four decades of catechol concentration ($10^{-2}-10^{-6}$ M). The amperometric FIA responses were linear over a $10^{-2}-10^{-5}$ M range. Comparing the different techniques; static potentiometric, potentiometric FIA, and amperometric FIA, all had markedly different response slopes for the different crown ethers. Overall, the crown ether modified electrodes, except for an open-crown ether, displayed excellent response stability for successive injections in both FIA modes. The lowest detection limits were about 0.5×10^{-6} M for the potentiometric FIA with minimal interference from ascorbic acid, uric acid and acetaminophen. For amperometric FIA the detection limits were about 0.5×10^{-5} M, but for ascorbic acid, uric acid, and acetaminophen the interference was significant. Experimental parameters which influence the mechanism of the potentiometric response are discussed.

Keywords: Crown ether platinum-modified electrodes, Potentiometric detector, Amperometric detector, Catechols, Catecholamines, Flow injection analysis

1. Introduction

The development of modified electrodes is certainly one of the most extensive areas of analytical chemistry research over the last two decades and the large number of publications in this field illustrates the world wide interest. The chemical function recognition and high chemical stability of immobilized synthetic macrocyclic host compounds in liquid or polymer matrices makes them ideal for ion selective electrodes (ISE). A series of various crown ether derivatives have been synthesized and ISE's based on these have been published [1–10]. A recent study showed that a binaphthyl-20-crown-6 ether could be used to construct a static potentiometric selective electrode for catechol and catecholamines [11].

Over the years a number of studies have used modified electrodes as FIA detectors. In the late 80's, the electrochemically grown conducting poly(pyrrole) films grown on an inert electrode substrates were first used to detect electroinactive anions in flow injection analysis (FIA) [12–14].

Previous studies in our laboratory have been concerned with the immobilization by electropolymerization of the crown ether binaphthyl-20-crown-6 onto a platinum electrode for the determination of neurotransmitters by static potentiometric detection [15]. Extending the previous study [15], in this article 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 and amperometric 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 acetaminophen, a comparison of these interferents on the potentiometric FIA and amperometric 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 [16, 17]. The effect of the nature of the crown ethers on catechol response is also discussed.

2. Experimental

2.1. Reagents

Reagent grade chemicals (Fisher Scientific Co; Aldrich Chemical Co.) and distilled water, further purified by a Sybron/Barnstead Pure II system, were used to prepare analyte stock solutions. The 0.1 M potassium phosphate buffer (pH 9.4) solution, used as the mobile phase throughout, was filtered and deaerated prior to use in FIA. The crown ether binaphthyl-20-crown-6 was synthesized and all others were purchased. Scheme 1 shows the various crowns studied and their structure.

2.2. Preparation of The Modified Electrodes

The polymerization of the crown ethers onto a platinum disk electrode, with a 2-mm diameter, purchased from Bioanalytical System (BAS: West Lafayette, IN) was carried out by a three electrode system. A platinum wire coil was the auxillary electrode and an Ag/AgCl (MF-2063, BAS) was the reference electrode. A PAR 175 Potentiostat/Galvanostat performed the electrochemical polymerization using an applied potential of +3.2 V (vs. Ag/AgCl) for five minutes in the monomer solution [15]. The monomer solution consisted of 0.025 M crown ether and 0.20 M tetrabutylammoniumtetrafluoroborate (TBATFB)

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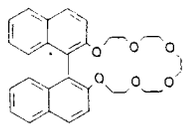
as the supporting electrolyte in acetonitrile. The polymerized electrode was then rinsed with HPLC grade acetonitrile, air-dried, and immersed in a monomer free TBATFB solution. A potential of +0.5 V (vs. Ag/AgCl) was then applied for approximately 25 min. The tetraethylene glycol dimethyl ether (open-crown) electrode was prepared in exactly the same manner as above. However, as this molecule is not electroactive, the open crown is simply chemisorbed onto the platinum. The fact that this electrode has a very short life time of response under FIA conditions compared to the electropolymerized aromatic crowns confirms this finding.

2.3. Flow Cell and Measurements

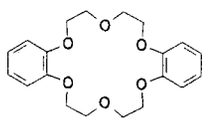
For amperometric measurements a BAS model (MF-1021) flow cell (working electrode, auxillary electrode and reference electrode) was used. The flow injection system consisted of an Altex injection 100 A double reciprocating pump followed with an Altex injection valve (CAT. NO: 905-42). Teflon tubing (55 cm in length with a 0.3-mm i.d.) was used between the injection valve and the detector cell. A 20 μl sample loop was used throughout the experiments. A BAS cyclic voltammetry unit (CV-IB) was employed to vary and control the potential applied to the working electrode. The FIA peaks were recorded on a Fisher Recordall Series 5000.

For both the static potentiometric and potentiometric FIA measurements, the polymer-modified electrode Teflon block was incorporated into a standard BAS thin layer flow cell with an Ag/AgCl electrode which acted as the second electrode. The potentiometric measurements were carried out with an Orion model 601A ionanalyzer and the recorder was a Fisher Recordall 5000 Series. A flow rate of 1.0 mL/min was used throughout for the FIA studies.

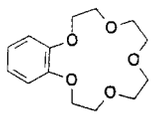
Binaphthyl-20-Crown-6
(BN-20-C-6)



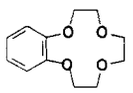
Dibenzo-18-Crown-6
(DB-18-C-6)



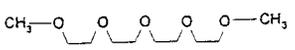
Benzo-15-Crown-5
(B-15-C-5)



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

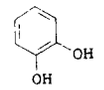


Tetraethylene Glycol Dimethyl Ether
(Open-C)

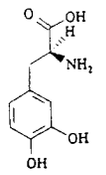


Scheme 1. Crowns.

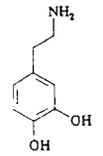
Catechol



L-DOPA



L-Dopamine



Scheme 2. Catecholamines.

3. Results and Discussion

3.1. Comparison of Different Detection Techniques Used for the Analyses of Catechols and Catecholamines

Scheme 1 shows the structure of the different crown ethers and Scheme 2 shows the structure of the different catecholamines used in this study. The effect of changing the crown ether polymerized or adsorbed onto a platinum electrode for the analyses of catechol and catecholamines by static potentiometric, potentiometric FIA and amperometric FIA were studied. A series of experiments were designed to determine which crown ether gave the best response to catechol and catecholamines.

Figure 1 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

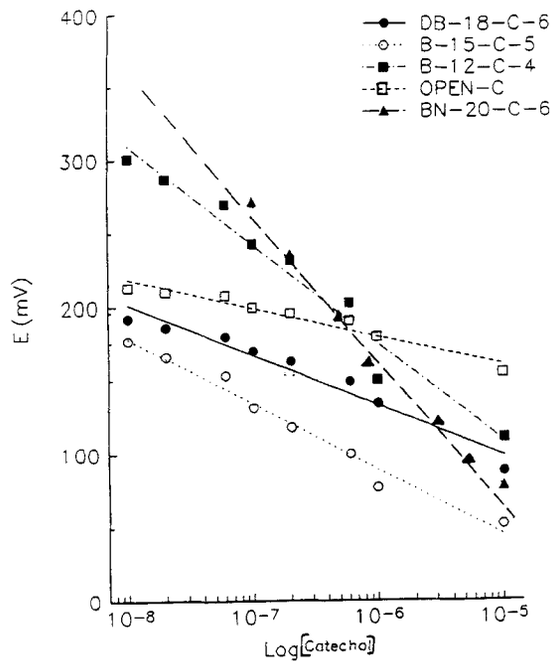


Fig. 1. Effect of changing the crown structure on the electrode response to catechol by static potentiometric detection.

exhibited 'near Nernstian' response with a slope of 67 mV/decade and the binaphthyl-20-crown-6 gave a 'super-Nernstian' slope of 110–120 mV/decade. All of the other crown compounds exhibited 'less than Nernstian' slopes. The open-crown with five oxygens 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 oxygens) on the response slopes. However, it is important to note that all the crowns gave a static potentiometric response.

Figure 2 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 oxygens, 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.

The amperometric-FIA studies of the various crown ether responses exhibited much less structure dependence as shown in Figure 3, which also shows that the response for bare platinum was greater than or equal to that of all the aromatic crowns. The binaphthyl-20-crown-6, dibenzo-18-crown-6, benzo-15-crown-5 and the benzo-12-crown-4 showed decreasing responses in that order. This decreasing response trend might reflect a decrease in conductivity of the polymer film in this series. One might consider that steric strain could reduce the size of the extended pi-systems in the series and, hence, the conductivity in these organic polymers. The tetraethylene glycol dimethyl ether and the bare platinum electrode exhibit similar responses because this crown film is only a monolayer (or a few monolayers) at best. Thus, the electron transfer can take place readily through the thin film or at holes in the adsorbed layer.

It has been found that the different crowns show some selectivity for the static potentiometric determination of specific catecholamines. For example Figure 4 illustrates the effect of the

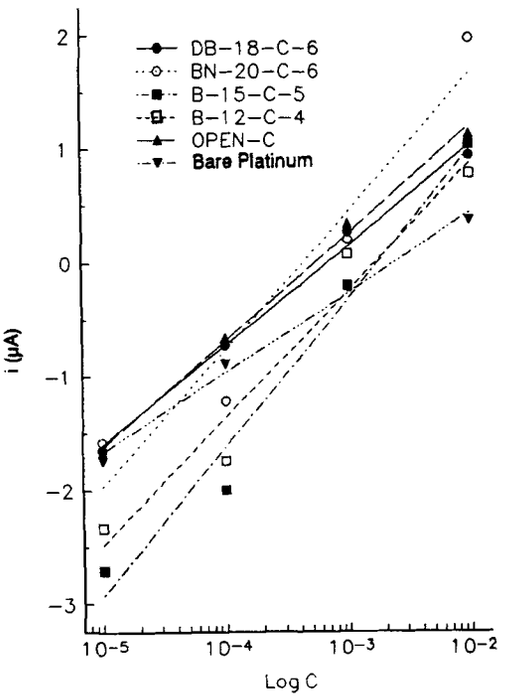


Fig. 3. Amperometric FIA studies of various crown ethers and bare platinum response to catechol.

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 Figure 5. In contrast, in the potentiometric FIA mode, Figure 6 illustrates that the binaphthyl-20-crown-6 was more selective towards the catechol that other catecholamines. The response characteristics of the aromatic crown ether electrodes under static potentiometric, potentiometric FIA and amperometric FIA determination of specific catecholamines were unchanged over 1 to 7 days of

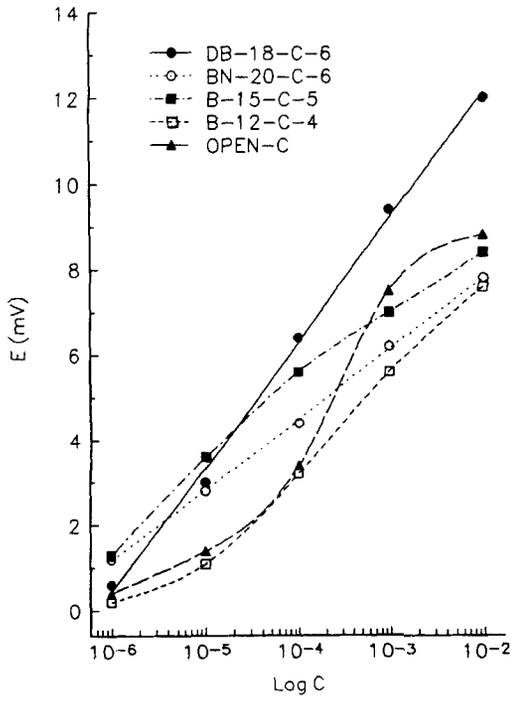


Fig. 2. Effect of changing the crown structure on the electrode response to catechol by potentiometric FIA.

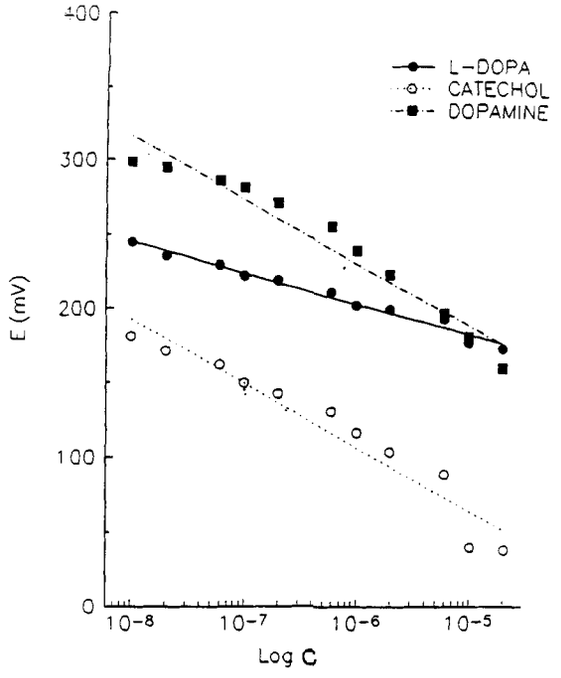


Fig. 4. DB-18-C-6-modified electrode under static potentiometric detection of various derivatives of catecholamines.

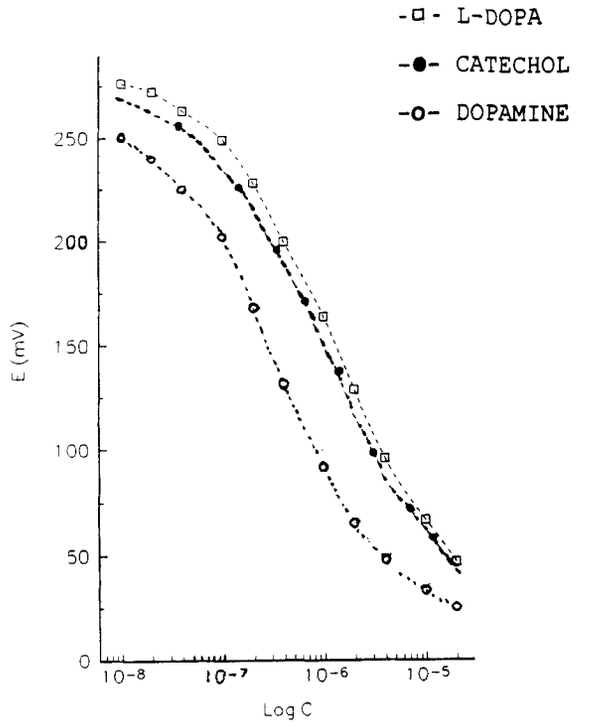


Fig. 5. BN-20-C-6-modified electrode under static potentiometric detection of various derivatives of catecholamines.

continuous use. The static potentiometric response of the open-crown ether electrode was found to decrease after 1 h to about 20% of the original signal. After 5 hours, the potentiometric FIA and amperometric FIA response of the open-crown ether electrode did not respond to the catecholamines.

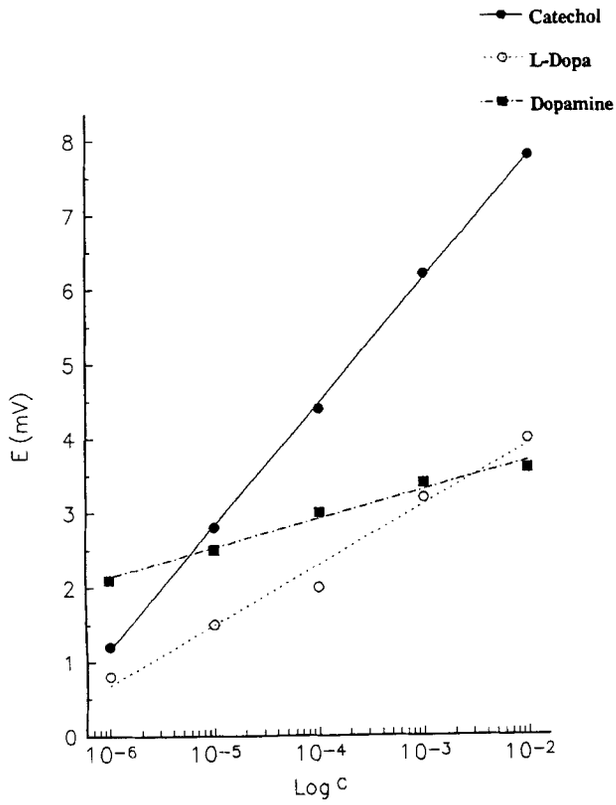


Fig. 6. BN-20-C-6-modified electrode under potentiometric FIA detection of various derivatives of catecholamines.

3.2. Interferent Study

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 flow injection analysis [16–18]. In a preliminary communication we showed that one can determine catechols in the presence of ascorbic acid and uric acid by flow injection analysis employing a potentiometric dibenzo-18-crown-6 electrode detector [19]. Ascorbic acid or uric acid in as much as two order of magnitude excess compared to the catechols did not significantly interfere. Table 1 shows the peak heights obtained in this study under potentiometric FIA using a DB-18-C-6-modified electrode for catechol in the presence of various acetaminophen concentrations. Acetaminophen shows the same trend as the other interferents previously studied [19]. For example, Figure 7 displays the typical potentiometric FIA signal responses obtained for catechol in the absence and in the presence of acetaminophen in equal concentrations. This demonstrates that, at equal concentrations of catechol and acetaminophen, the FIA signal response remains identical to that in the absence of acetaminophen. It also shows that the potentiometric response time of these electrodes is very fast compared to the residence time of the sample zone in contact with the electrode surface for this flow rate.

Amperometric FIA shows serious interference for ascorbic acid, uric acid and acetaminophen as expected.

4. Conclusions

Overall, our studies indicate that by using crown ether modified electrodes the potentiometric FIA response has

Table 1. Potentiometric flow injection analyses of catechol in the presence of acetaminophen.

Catechol [M]	*Acetaminophen [M]*	*Δ Potential [mV]*
10^{-2}	0	9.8
10^{-2}	10^{-2}	9.8
10^{-2}	10^{-3}	9.8
10^{-2}	10^{-4}	9.8
10^{-2}	10^{-5}	9.8
10^{-3}	0	6.6
10^{-3}	10^{-2}	6.8
10^{-3}	10^{-3}	7.0
10^{-3}	10^{-4}	7.0
10^{-3}	10^{-5}	6.8
10^{-4}	0	3.6
10^{-4}	10^{-2}	9.8
10^{-4}	10^{-3}	8.2
10^{-4}	10^{-4}	5.8
10^{-4}	10^{-5}	3.6
10^{-5}	0	1.00
10^{-5}	10^{-2}	5.9
10^{-5}	10^{-3}	4.8
10^{-5}	10^{-4}	4.2
10^{-5}	10^{-5}	1.2
10^{-6}	0	0.5
10^{-6}	10^{-2}	5.2
10^{-6}	10^{-3}	3.8
10^{-6}	10^{-4}	2.2
10^{-6}	10^{-5}	1.2

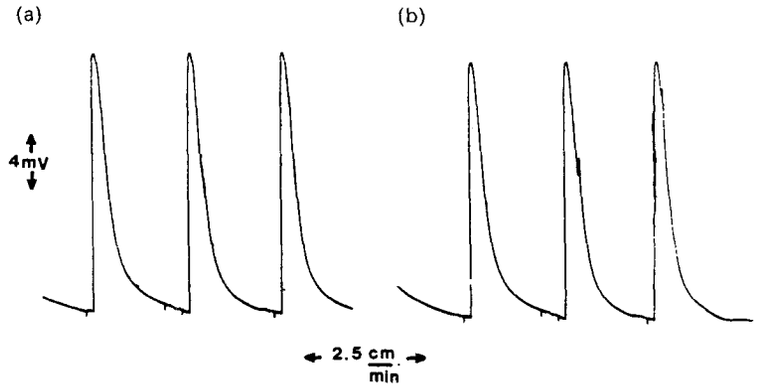


Fig. 7. FIA signals obtained on the modified DB-18-C-6 electrode. A) 10^{-3} M catechol and B) 10^{-3} M catechol + 10^{-3} M acetaminophen.

significant advantage over the amperometric FIA response as it shows minimal interference for ascorbic acid, uric acid and acetaminophen. Other advantages for the potentiometric FIA are the simple low cost instrumentation, rapid analysis time and low detection limits (0.5×10^{-6} M). Furthermore, the amperometric response using the different crowns is not really that different from the bare platinum response. Also, these conducting polymer films in the amperometric FIA mode showed no electrocatalytic activity as was found in our previous studies with poly(3-methylthiophene) electrodes. These exhibited significantly improved performance as an amperometric detector for FIA and HPLC analyses of catechols compared to bare platinum [20]. The amperometric crown polymer electrodes behave more like all other conducting polymer electrodes such as poly(N-methylpyrrole), poly(aniline) and poly(furan) [20]. The lack of selectivity for the crown polymers in amperometric FIA indicates that absorption is not a rate limiting step in the electrooxidation reaction.

In previous studies of the static potentiometric response of the binaphthyl-20-crown-6 electrode [15, 21], a potassium buffer was also employed. As this crown has a high affinity for K^+ one might speculate that the catechol monoanion at pH 9.4 would ion pair with K^+ in the crown. EDAX experiments did show that K^+ was indeed incorporated in the electrode when exposed to the buffer alone. However, when the catechol was present in the buffer medium, the EDAX results showed that the K^+ was expelled from the electrode matrix. This would seem to suggest, then, that the catechol monoanion entered the rings at least partially. However, this study shows that even small rings 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.

5. Acknowledgements

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