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# DETERMINATION OF CATECHOL DERIVATIVES ON PRETREATMENT AND COPOLYMER COATED GLASSY CARBON ELECTRODE

ANHUA LIU and ERKANG WANG*

Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China

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**Summary**—A glassy carbon electrode was pretreated electrochemically and was coated with a copolymer of maleic acid anhydride attached with Eastman-AQ55D (MA/AQ). The voltammetric behavior of a series of biologically important compounds, such as dopamine, L-DOPA, DOPAC, ascorbic acid and uric acid were examined at both pretreated and coated electrodes. Electrochemical pretreatment increased peak current of dopamine and L-DOPA while decreased that of ascorbic acid, uric acid and DOPAC. The copolymer coating caused a decrease of peak currents, but effectively hindered the anionic species (ascorbic acid, uric acid and DOPAC) access to the electrode surface. In flow injection and liquid chromatographic analysis. The dopamine and L-DOPA yielded the better selectivity response at MA/AQ electrode than at bare and AQ electrodes.

Some important catechol derivatives such as dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and L-3,4-dihydroxyphenylalanine (L-DOPA) can be oxidized through their two hydroxy groups, their determination by electrochemical techniques using carbon electrodes has attracted much attention. Unfortunately, they show a signal at the same potential, therefore, they could not be distinguished from each other.

The successful route to overcome problems with selectivity is to locate the electrode for *in vivo* measurement in the appropriate region of the brain where only one neurotransmitter is present, or where one neurotransmitter is present in great excess. However, with this method, it is impossible to exclude the contributions that other species may make to the obtained signal. For example, in every biological liquid in the brain a high concentration of ascorbic acid is present that gives a clear oxidation signal near the other ones.

Many attempts have been made to increase the selectivity and specificity of carbon electrodes toward catechol amines. Separation of ascorbic acid from catechols such as dopamine and DOPAC was achieved by means of treated carbon fiber electrodes.[1,2] Plotsky[3] employed a carbon fiber electrode and different pulse voltammetry techniques to increase selectivity. He demonstrated the importance of electrochemical pretreatment of the electrode. In this way, only the signal of dopamine was obtained from a mixture of ascorbic acid and dopamine. Later, Gonon *et al.*[4] demonstrated the application of a differential normal pulse voltammetry (DNPV), for measurements with carbon fiber electrodes. With this technique, the potential changes applicable in common differential pulse voltammetry are supplemented by a condition potential prior to the potential at which the measurement is carried out. Ascorbic acid yielded a peak at −0.105 V; dopamine and DOPAC gave peaks at 0.085 and 0.055 V, respectively, which are difficult to discriminate.

Further investigations led to the use of chemical modified electrode to change the surface properties of the electrode. This technique has been used since the middle of the 1970s, with a Nafion-coated carbon fiber electrode. Polymer-modified electrodes, in particular, hold great promise for increasing the selectivity, sensitivity, and reproducibility of voltammetric measurements enhanced electron-transfer kinetics, permselective transport. The attractive features of Nafion, cellulose acetate, polyaniline, polypyrrole or poly(vinylpyridine) have been particularly useful for voltammetric modified electrodes, including *in vivo* monitoring of primary catecholamine neurotransmitters.[5–11]

*To whom correspondence should be addressed.

Adams[12] deposited Nafion on a miniature graphite electrode which can be used to detect neurotransmitter-related species in rat brain. Wang *et al.*[13] investigated the selectivity and stability of Nafion polymer and catechol amines, and other neutral molecules response characteristics in flow injection analysis with Nafion-coated electrode. Wang[14] described the ion-exchange properties and permselective characteristics of Eastman-AQ55D for cationic and nonionic biologically important compounds, then a new polypyrrole/AQ55D composite coating film was introduced, PP/AQ offers unique coupling of cation-exchange and permselective properties.[15] In recent research, Peter[16] used a polymer of styrene and maleic acid anhydride to modify the electrode. The signal of ascorbic acid was greatly suppressed while that of DOPAC disappeared even after polymer coating.

In our investigation, we have discussed how to increase dopamine and L-DOPA selective response characteristics in a series of biologically importance-compounds. We used electrochemical pretreatment method, in which was applied the triangular pretreatment and followed by the application of constant potential. This pretreatment method increased peak current of dopamine and L-DOPA, while decreased significantly that of DOPAC, ascorbic acid and uric acid. Better selective responses were obtained in FIA and LC systems. In addition, we prepared a novel copolymer of maleic acid anhydride attached with Eastman-AQ55D coating glassy carbon (MA/AQ) electrode. Although this coating caused a decrease in peak current, it is of interest that substances of anionic character, such as ascorbic acid, uric acid and DOPAC, was hindered in their access to the electrode surface.

Eastman-AQ55D is a new poly(ester sulfonic acid) cation exchange available in a commercial

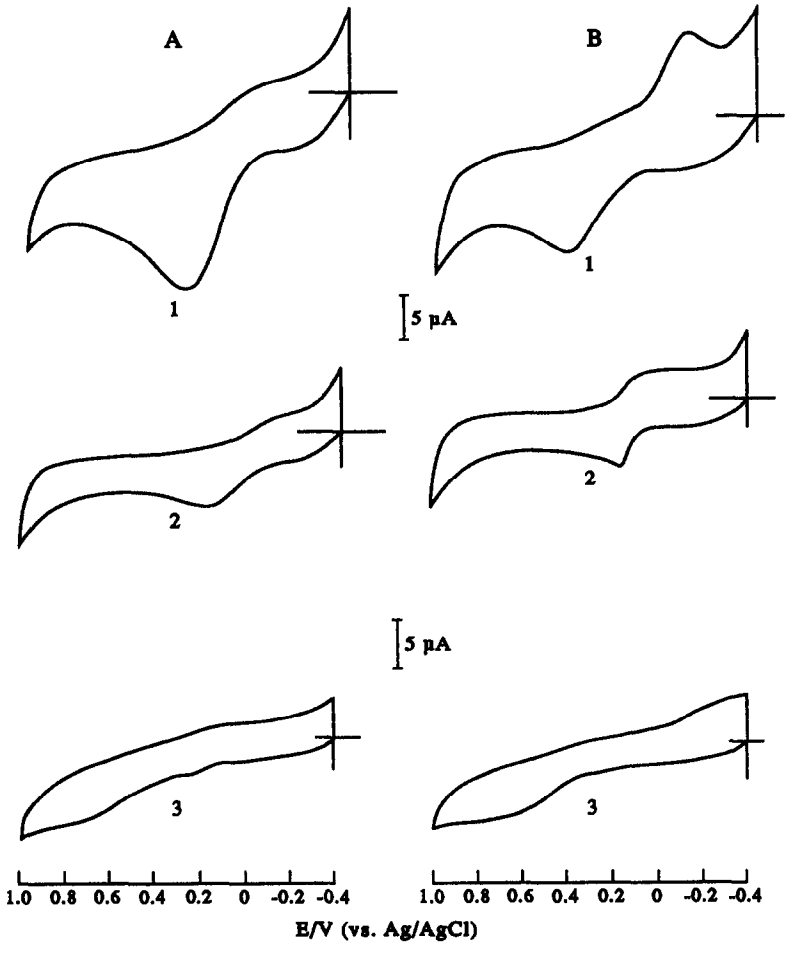


Fig. 1. Cyclic voltammograms of 1.0 m*M* ascorbic acid and DOPAC on bare glassy carbon (1), pretreated glassy carbon (2) and coated (3) electrode. Scan potential range: $-0.4 - +1.0$ V (*vs.* Ag/AgCl), scan rate 100 mV/sec, base electrolyte: 0.05 mol/l phosphate buffer (pH 7.4). (A) ascorbic acid; (B) DOPAC.

dissolved form. The Eastman-AQ coated electrode exhibited attractive permselectivity toward the cationic neurotransmitters.[15] Peter *et al.*[16] used a polymer of styrene and maleic acid anhydride to modify the glassy carbon electrode due to its anhydride unit and anionic structure, which results from slow hydrolysis of the anhydride in aqueous solution. An example of such a modification of maleic anhydride in a copolymer is shown by Rätzsch *et al.*[17] The reaction mechanism has been described with a penultimate model[18] or a complex model.[19] After the glass carbon electrode, which was coated maleic anhydride/Eastman-AQ copolymer was placed in water, the copolymer swelled, which allowed cationic molecules to pass through the layer and excluded anionic compounds from the electrode surface.

We first prepared this copolymer by "double coating step" deposition of maleic acid anhydride onto Eastman-AQ polymer. The selectivity was increased for dopamine and L-DOPA at MA/AQ electrode compared with at Eastman-AQ electrode. Unsatisfactorily, peak current and the sensitivity were decreased. Investigations have been made on the behaviors of dopamine, L-DOPA, DOPAC, ascorbic acid, and uric acid in CV and LCEC experiments.

## EXPERIMENTAL

### *Apparatus*

Electrochemical experiments were performed with a laboratory-built potentiostat[20] with a three-electrode cell containing a silver–silver chloride (saturated with potassium chloride) electrode and a platinum wire as a reference and a counter electrode, respectively.

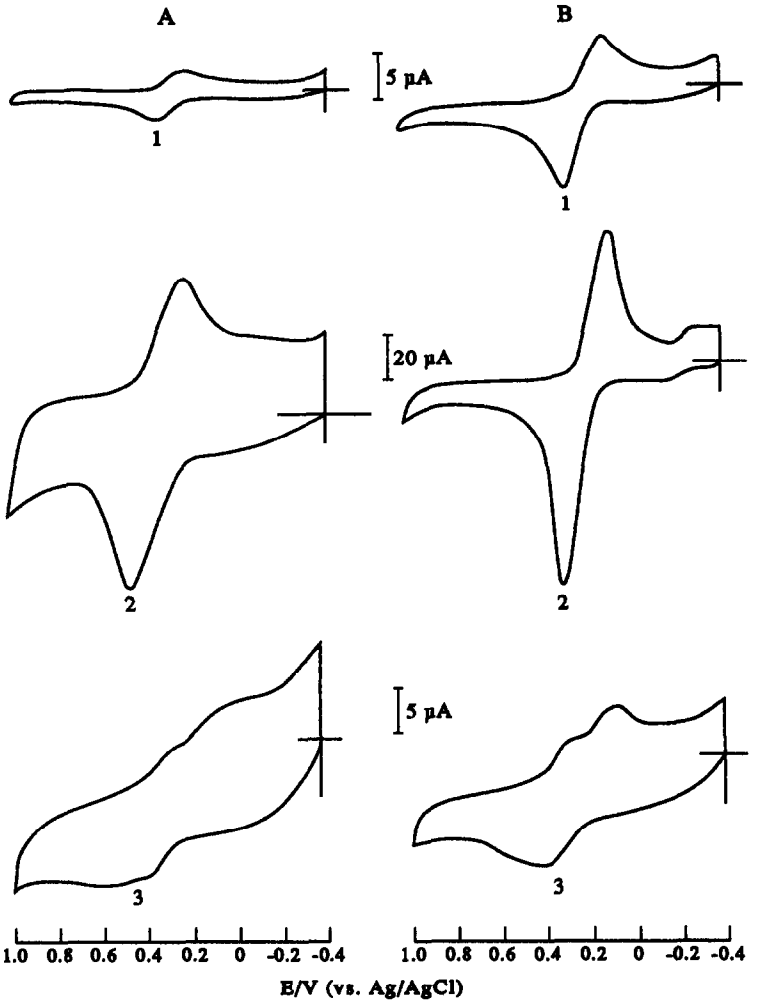


Fig. 2. Cyclic voltammograms of 0.5 m*M* L-DOPA and 1.0 m*M* dopamine on bare glassy carbon (1), pretreated glassy carbon (2) and coated (3) electrode. Scan potential range: $-0.4 - +1.0$ V (*vs.* Ag/AgCl), scan rate: 100 mV/sec, base electrolyte: 0.05 mol/l phosphate buffer (pH 7.4). (A) L-DOPA; (B) dopamine.

The flow injection analysis and liquid chromatographic (LC) experiment were performed using a Model 510 pump, a U6K injection valve (Waters Assoc., U.S.A.) and a Model TL-5A thin-layer glassy carbon (BAS Inc., U.S.A.) was used in CV and flow system experiments.

Liquid chromatographic (LC) experiments were carried out using a Model 510 pump, a U6k injection valve, a 10 $\mu$m particle size Nucleosil $C_{18}$ column (200 × 4.0 mm I.D.) as an analytical column.

FIA and LC system was controlled by a laboratory-made potentiostat[21] used for amperometric detection. The mobile phase was 0.05 mol/l phosphate buffer (pH 7.4) and was delivered at a flow rate of 1.0 ml/min. All potentials were measured and recorded with respect to a saturated calomel electrode (SCE), unless stated otherwise.

*Reagents*

Distilled water was used to prepare all solutions. A solution of the Eastman-AQ55D polymer (28% dispersion) was obtained from Eastman Kodak Co. MA solution ($1.0 \times 10^{-3}$ mol/l) was prepared by dissolving maleic acid anhydride (Beijing Chemical Co.) for modifying electrode. Dopamine hydrochloride, 3,4-dihydroxyphenylacetic acid (DOPAC) and L-3,4-dihydroxyphenylalanine (L-DOPA) was obtained from Sigma. Ascorbic acid and uric acid (Beijing Chemical Co.) were used. Solution of pH 7.4 phosphate buffer at 0.05 mol/l ionic strength was used in batch and flow experiments.

*Electrochemical pretreatment electrode*

Prior to its pretreatment and coating, a glassy carbon electrode with an area of 0.071 $cm^2$ was hand-polished with alumina slurries of 1 and 0.05 $\mu$m. Residual polishing material was removed from the surface by the sonication in a water bath for 5 min after each polishing process.

The pretreatment consisted of two parts: a triangular and a constant potential. First the electrode was pretreated with triangular voltage using the cyclic voltammetry mode of the polarograph in a range between 0.0 and 2.5 V (50 times), then between 0.0 and 1.3 V (100 times). The scan rate was 30 V/sec. Then constant potentials of 1.5 V, −0.8 V, 1.3 V and −0.4 V was applied, each for a duration of 5 sec. The electrochemical pretreatment was always done in the same solution in which the measurement was carried out.

*Maleic acid anhydride electrode attached with Eastman-AQ55D*

The polishing glassy carbon surface was modified by a double coating step: the disk and its surrounding were first coated with measured volume (5 $\mu$l) of the dilute (1:20 (v/v) Eastman-AQ:acetone) polymer solution. After the film was dried in air, the surface was coated with 10 $\mu$l of maleic acid anhydride solution ($10^{-3}$ mol/l). Finally, the CME was allowed to dry under an infrared lamp, a white deposit appeared on the glassy carbon surface ready for use. This white deposit demonstrated that maleic anhydride adhered to the Eastman-AQ film surface.

## RESULTS AND DISCUSSION

*Cyclic voltammetry behavior of signal substrates*

*Ascorbic acid.* The cyclic voltammograms on bare electrode show only a clearly anodic peak at *ca.* +0.3 V (Fig. 1(a)), electrochemical pretreatment did not increase the peak current but decreased it significantly. The anodic peak shifted 100 mV, negatively.

At coated electrode, the potential of the anodic peak shifted positively while the peak

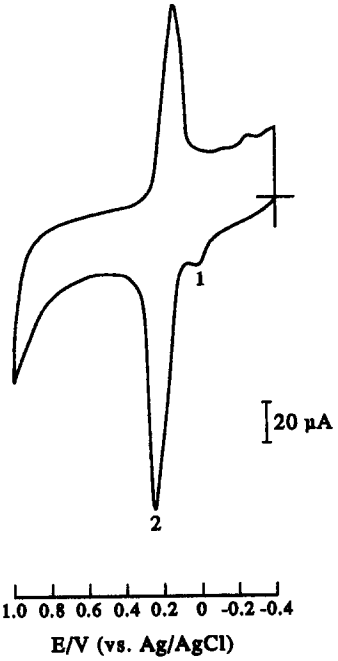


Fig. 3. Cyclic voltammograms of 1.0 m*M* dopamine with 2.0 m*M* ascorbic acid on pretreated electrode. 1—ascorbic acid, 2—dopamine. Other conditions as for Fig. 1.

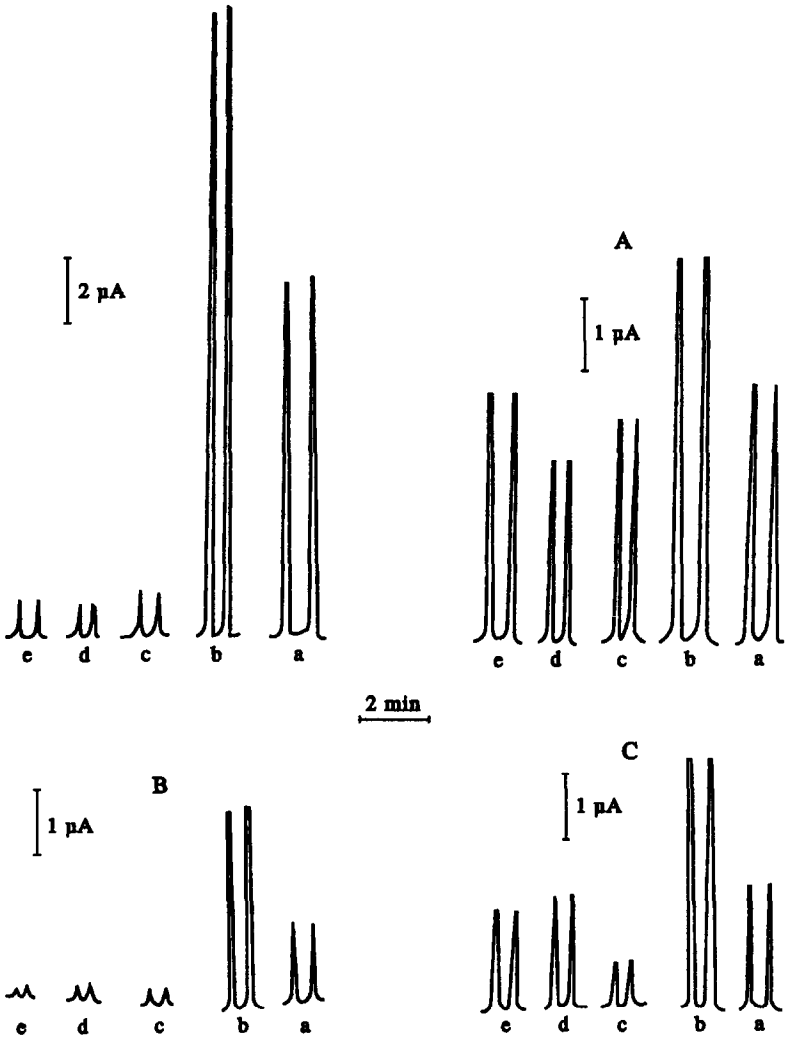


Fig. 4. Flow injection peaks for $1.0 \times 10^{-4}M$ dopamine (a), L-DOPA (b), ascorbic acid (c), DOPAC (d) and uric acid (e) at the bare glassy carbon electrode (A) and pretreated glassy carbon electrode (B) and AQ (C) and MA/AQ (D) coated electrode. Applied potential, +0.8 V (*vs.* SCE), carrier electrolyte, 0.05 mol/l phosphate buffer (pH 7.4).

current dramatically decreased. The peak shape almost became smooth. The reason for this behavior may be due to the anionic character of ascorbic acid, which may be excluded going through the copolymeric coating.

*DOPAC.* A fresh polished glassy carbon electrode yielded good voltammogram for DOPAC. A fully developed anodic peak and cathodic peak were seen (Fig. 1(b)) at *ca.* +0.4 V and −0.1 V. After pretreatment, the peak current decreased clearly. The anodic and cathodic peak shifted negatively and positively about 200 mV, respectively. Increased concentrations shifted the peak potentials apart, no increase of the peak currents was observed at concentration higher than 5 m*M*.

On a coated electrode a very small peak of DOPAC was detected. The reason for this behavior may be that the film coating did not allow DOPAC access to the electrode surface.

*L-DOPA.* On the bare electrode, better anodic and cathodic peaks were obtained at +0.35 V and +0.25 V. After electrochemical pretreatment, the peak current increased and the anodic peak potential shifted to +0.5 V (Fig. 2(a)), with increasing concentrations of L-DOPA, the peak currents did not increase appreciably with L-DOPA concentrations higher than 3 m*M*. This could be caused by high saturation of the electrode surface with L-DOPA.

On a coated electrode, two cathodic waves were formed and the potential of anodic peak

shifted positively while the peak current decreased. The peak shape became broader.

*Dopamine.* On the polished bare electrode, dopamine gave an anodic and a cathodic peak within the potential window (Fig. 2(b)). After electrochemical pretreatment, the anodic and cathodic peak currents are clearly increased *ca.* 10-fold. With increasing concentrations of dopamine, the anodic and cathodic peaks shifted positively and negatively, respectively.

On a coated electrode, two small cathodic peaks appeared and even lower than those on a polished electrode, but the peak potential did not shift.

From the curves of dopamine and L-DOPA obtained on a polished and electrochemically pretreated electrode, by the resulting from the difference of both curves it demonstrated the attributability of the electrochemical pretreatment. It is also shown from Fig. 2 that the increasing activity electrode surface by electrochemical pretreatment was favourable for dopamine and L-DOPA oxidation.

On the film coated electrode, the peak current clearly decreased. This may be due to the fact that the film hindered the dopamine directly penetrating the carbon electrode.

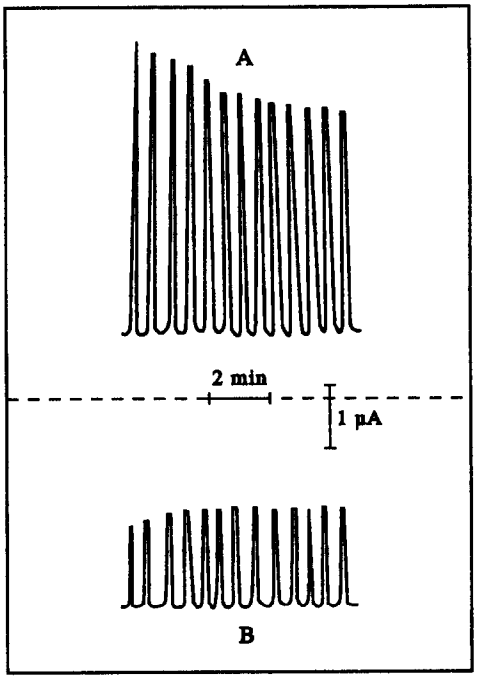


Fig. 5. Detection peaks for repetitive injections of $1.0 \times 10^{-4}M$ dopamine solution at bare glassy carbon electrode (A) and MA/AQ coated electrode (B). Flow rate, 1.0 ml/min, applied potential, +0.8 V (*vs.* SCE), electrolyte and carrier: 0.05 mol/l phosphate buffer (pH 7.4) with 20 $\mu$l injection.

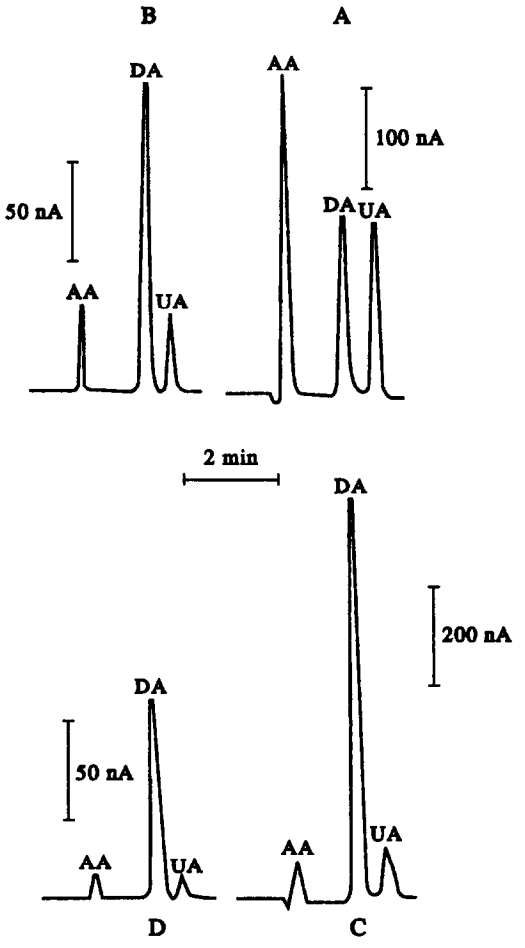


Fig. 6. Chromatograms of 4 ppm ascorbic acid (AA), 4 ppm dopamine (DA) and 5 ppm uric acid (UA) at the bare glassy carbon (A) and AQ electrode (B) and pretreated glassy carbon electrode (C) and MA/AQ electrode (D). Potential: +0.8 V (*vs.* SCE), mobile phase: 0.05 mol/l phosphate buffer (pH 7.4), flow rate: 1.0 ml/min.

*Simultaneous detection.* Figure 3 shows cyclic voltammograms of dopamine and ascorbic acid on the electrochemical pretreated electrode. The distinction peak of dopamine and ascorbic acid was obtained on pretreated electrode. After addition of ascorbic acid, the dopamine peak became higher and narrower, but the peak potential shift was not found. As has been shown in Fig. 3, the dopamine peak current can be increased by ascorbic acid because oxidized dopamine is regenerated by ascorbate near the electrode surface, and is oxidized again.[22] For this reason, we obtained the increasing dopamine response on pretreated electrode.

### *Flow injection analysis*

Analyte currents for dopamine, L-DOPA, DOPAC, ascorbic acid and uric acid were

compared with the four flow amperometric electrodes tested (Fig. 4). Flow injection measurements were mobile phase of 0.05 mol/l phosphate buffer (pH 7.4), applied potential of +0.8 V (*vs.* SCE) and for 20 $\mu$l $1.0 \times 10^{-4}$ mol/l samples were injected. Figure 4 shows the response characteristics of the five biologically compounds. Among the four amperometric electrodes tested, the electrochemical pretreatment electrode (B) yielded the highest and best selective response toward dopamine and L-DOPA. The responses increased significantly compared with bare GC (A). At the maleic anhydride/Eastman-AQ electrode (D), we have obtained better selectivity toward dopamine and L-DOPA than at Eastman-AQ electrode (C). We have also observed analogous result in cyclic voltammetric experiments. Unfortunately, after coating the electrode with copolymer, the dopamine and L-DOPA peak current decreased compared with AQ electrode.

The stability of MA/AQ electrode was illustrated in Fig. 5 which compared the stability of the response for 13 times of injections of sample containing $1.0 \times 10^{-4}$ mol/l dopamine, rapid loss of the electrode activity was observed at the bare electrode (A). In contrast, relatively stable analytical response on the MA/AQ electrode was observed (B). This behavior showed the binding of the using maleic acid anhydride to the Eastman-AQ surface was strong, the rapid and stable response is important for the MA/AQ coated electrode as in an analysis application.

*Analytical application*

We used a pretreatment electrode and MA/AQ electrode for chromatographic analysis, amperometric detection could not be easily applied to mixtures of species with similar redox properties. A mixture of ascorbic acid, dopamine and uric acid was tested by electrochemical detection with reverse-phase chromatographic separation. Figure 6 compared the chromatograms of this mixture obtained at the bare (A), AQ electrode (B), pretreatment (C) and MA/AQ coated (D) electrodes. In contrast, the response of DOPAC, ascorbic acid and uric acid at the pretreatment and MA/AQ coated electrode is considerably decreased. However, the response of dopamine increased at the pretreated electrode and decreased at the MA/AQ coated electrode.

## CONCLUSION

The cyclic voltammetric and the flow system experiments described above indicated that the better selectivity could be achieved by electrochemical pretreatment electrode and MA/AQ electrode for dopamine and L-DOPA detection. The ascorbic acid, DOPAC and uric acid signals were found to be suppressed to a high degree at pretreated and film coated electrodes. It is of interest that substrates of anionic character were hindered in their access to the pretreatment and film coated electrode. For this reason, the biologically important substances exhibiting neutral or cationic character could be detected without interference caused by DOPAC, ascorbic acid and uric acid.

Unfortunately, the copolymer coated electrode observed limiting sensitivity. The copolymer coating shifted peak potentials and made the peak shape broad. Further investigation should be directed to creating increased sensitivity of the coated electrode and extensive application of this copolymer.

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## REFERENCES

1. F. Gonon, M. Buda, R. Cespuglio, M. Jouvet and J. F. Pujol, *Nature*, 1980, **286**, 102.
2. F. Gonon, M. Buda, R. Cespuglio, M. Jouvet and J. F. Poujol, *Brain Res.*, 1981, **223**, 69.
3. P. M. Plotsky, W. J. de Greet and J. D. Neill, *Brain Res.*, 1982, **250**, 251.
4. F. G. Gonon, F. Navarre and M. J. Buda, *Anal. Chem.*, 1984, **56**, 575.
5. J. Wang and L. D. Hutchins, *Anal. Chem.*, 1985, **57**, 1536.
6. M. N. Szentirmay and C. R. Martin, *Anal. Chem.*, 1984, **56**, 1898.
7. L. A. Coury, E. M. Birch and W. R. Heineman, *Anal. Chem.*, 1988, **60**, 553.
8. N. Oyama, T. Ohsaka and T. Shimizu, *Anal. Chem.*, 1985, **57**, 1526.
9. J. A. Cox and K. P. Kulkarni, *Analyst*, 1986, **111**, 1219.
10. J. Ye and R. P. Baldwin, *Anal. Chem.*, 1988, **60**, 1979.
11. J. Wang and P. Tuzhi, *Anal. Chem.*, 1986, **58**, 3257.
12. G. A. Gerhardt, A. F. Oke, G. Nagy, B. Moghaddam and R. N. Adams, *Brain Res.*, 1983, **290**, 390.
13. H. Ji and E. Wang, *J. Chromatogr.*, 1987, **410**, 111.
14. J. Wang and T. Golden, *Anal. Chem.*, 1989, **61**, 1397.
15. J. Wang, Z. Sun and Z. Lu, *J. Electroanal. Chem.*, 1991, **310**, 269.
16. Peter Heiduschka and Jurgen Dittrich, *Electroanalysis*, 1992, **4**, 223.

17. M. Rätzsch and B. Wohlfarth, *Acta Polymer*, 1986, **37**, 708.
18. A. S. Brown and K. Fujimori, *Makromol. Chem.*, 1987, **188**, 2177.
19. P. C. Dep, *J. Polymer Sci.*, 1985, **23**, 233.
20. H. Ji, G. Zhu and E. Wang, *Anal. Chem. (Chinese)*, 1987, **15**, 897.
21. H. Ji and E. Wang, *J. Chromatogr. (Chinese)*, 1988, **6**, 1394.
22. M. A. Dayton, A. G. Ewing and R. M. Wightman, *Anal. Chem.*, 1980, **52**, 2392.