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Electrochemical Investigation of Dopamine and Pyrocatechol with Unmodified and Banana Tissue Modified Carbon Paste Electrodes

Elektrokemijsko istraživanje dopamina i pirokatehola s nemodificiranom i bananom modificiranom elektrodom od grafitne paste

B. S. Grabarić, I. Kruhak, J. Velikonja and M. Tkalčec*

Faculty of Chemical Engineering and Technology and *Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

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Summary

The electrochemical investigation of a neurotransmitter and drug, dopamine, and an air pollutant, pyrocatechol, was performed with unmodified and banana tissue modified carbon paste electrodes by cyclic voltammetry and differential pulse voltammetry in batch experiment and amperometrically in a continuous flow injection mode. It has been demonstrated that the electrochemical activation of the unmodified carbon paste electrode plays an important role in pyrocatechol determination, while for dopamine determination the catalytic effect of the (poly)phenol oxidase present in a banana tissue is predominant.

Sažetak

Provedeno je elektrokemijsko istraživanje dopamina, važnog neuroprijenosnika i pirokatehola, onečišćivača u zraku, s nemodificiranom elektrodom od grafitne paste i bananom modificiranom elektrodom od grafitne paste. Određivanja su obavljena diskontinuirano primjenom cikličke i pulsne voltametrije te kontinuirano uz injektiranje u protok primjenom amperometrijske tehnike. Ustanovljeno je da elektrokemijska prethodna obradba elektrode od grafitne paste ima znatan utjecaj na povećanje osjetljivosti određivanja pirokatehola. Pri određivanju dopamina odlučujući je katalitički učinak enzima polifenol-oksidaze u tkivu ploda banane.

Introduction

Natural selectivity of enzyme/substrate or antibody/antigen complexes represents models which barely ten years ago have been exploited in analytical chemistry. Development of biosensors is becoming the hotest field of the investigation due to their exceptional selectivity and very acceptable sensitivity and due to their application in (bio)chemistry, and quality control in life sciences, ecology and biotechnological processes monitoring. Many aspects of biosensors are reviewed in the literature *(1-7)*.

Electrochemical biosensors *(8-10)* are very simple to prepare and therefore it is obvious that besides biologists, biochemists, biochemical engineers and technologists, electrochemists show considerable interest in their development and application. First electrochemical biosensors were based on ion-selective electrodes *(8)*. Modified electrodes with unique recognition and binding properties based on immobilized enzymes, organelles, living tissue and microorganisms have been investigated and some attempts to produce immuno sensors based on lysis action or heterogeneous immunocomplex action have also been studied.

By investigating enzyme modified electrodes it has been found in many systems that enzymes in living tissue are giving better results concerning stability, durability and even sensitivity of the electrodes, than the electrode with immobilized enzyme alone. A possible reason of such behavior is the problem associated with enzyme immobilization and the fact that enzymes in living tissues are surrounded by their natural environment. Biosensors using whole cells have also shown high degree of stability *(11)*.

Banana tissue is a convenient source of a Cu-containing enzyme, (poly)phenol oxidase *(12-14)*, especially selective to an air pollutant, pyrocatechol, and a neurotransmitter and drug, dopamine.

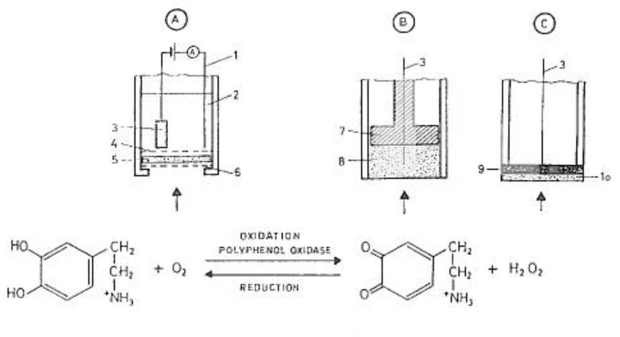

Fig. 1. Three principles for dopamine detection based on banana modified electrodes

A) Cell with thin slice of banana *(9)*
B) Banana modified carbon paste electrode *(14)*
C) Electrode with whole banana cells entrapped in poly(pyrrole) film *(11)*

Slika 1. Tri načela za određivanje dopamina na bananom modificiranim elektrodama

A) Ćelija s kriškom tkiva ploda banane *(9)*
B) Bananom modificirana elektroda od grafitne paste *(14)*
C) Elektroda s polipirolnim filmom na imobiliziranim stanicama banane *(11)*

1 Ag/AgCl electrode
Ag/AgCl-elektroda
2 KCl solution
Otopina KCl
3 Pt wire (electrode)
Pt-žica (elektroda)
4 Poly(propylene) membrane
Polipropilenska membrana
5 Banana slice (0.25 – 1.00 mm)
Kriška tkiva ploda banane (0,25 – 1,00 mm)
6 Dialysis membrane
Dijalizna membrana
7 Teflon piston
Teflonski stap
8 Unmodified or banana modified carbon paste
Nemodificirana ili bananom modificirana grafitna pasta
9 Au electrode
Au-elektroda
10 Poly(pyrrole) with entrapped Banana cells
Polipirolni film na imobiliziranim stanicama banane

Two electrochemical sensors have been proposed with banana tissue modified electrodes (BTMCPE) and one electrode modified with whole cells isolated from banana tissue. All three electrodes and principles (A, B and C) of dopamine detection are illustrated in Fig. 1.

The first dopamine detection principle (A) is based on the modification of the Clark type oxygen electrode with the thin slice of banana. The catalytic action of (poly)phenol oxidase from banana tissue takes up oxygen present in the solution. The decrease of current is proportional to oxygen depletion and therefore proportional to the dopamine concentration in the solution *(9)*. This detection is slow because two membranes were used, therefore, another more direct approach has been proposed using banana modified carbon paste *(14)*, principle B. The catalytic action of (poly)phenol oxidase gives, at the surface of the electrode, dopaquinone which can be directly reduced at the electrode. The third and most recent approach (principle C) is based on gold electrode on which a poly(pyrrole) film with entrapped whole banana cells is electrochemically deposited *(11)*.

The electrode is set at the potential where the oxidation of hydrogen peroxide, generated as the byproduct of dopamine oxidation by (poly)phenol oxidase from the entrapped whole banana cells, takes place.

In this paper we investigated in more detail the use of banana modified carbon paste electrode (BTMCPE) as an electrochemical biosensor sensitive to dopamine and pyrocatechol because Wang and Lin *(14)* investigated only the analytical application for selective determination of dopamine in batch mode at μM concentration level and qualitatively reported relative response of many relevant compounds to that of dopamine using the flow injection analysis (FIA) at 0.1 mM concentration level.

The neurotransmitter dopamine is used as an infusion drug for patients under shock of various origins, while pyrocatechol is an air pollutant, and therefore such an electrochemical biosensor can be used in pharmacy, life sciences and pollution monitoring.

Materials and Methods

All chemicals were of reagent grade. Dopamine hydrochloride was obtained as a drug in an ampoule from Farmakos Pharmaceutical Comp., Prizren, Macedonia. One ampoule of 5 mL contained 50 mg of dopamine hydrochloride (c = 52.7 mM). High purity water obtained from Millipore Milli Q system was used for preparation of all solutions.

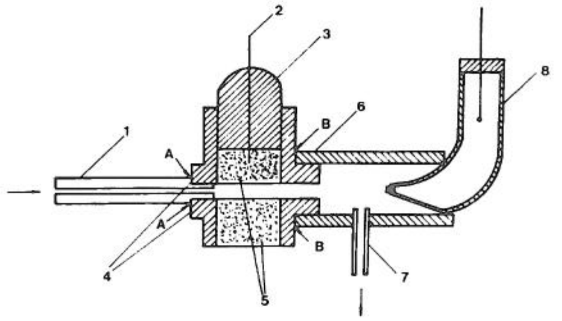

Fig. 2 Flow-trough microelectrochemical cell (V = 1.5 μL; A = 8.8 mm^2) with carbon paste electrode

Slika 2. Protočna mikroelektrokemijska ćelija (V = 1,5 μL; A = 8,8 mm^2) s grafitnom pastom

1 Stainless steel inlet capillary (l = 20 mm; $2r$(outer) = 1.5 mm; $2r$(inner) = 0.2 mm)
Ulazna cjevčica od nehrđajućeg čelika (l = 20 mm; $2r$(vanjski) = 1,5 mm; $2r$(unutrašnji) = 0,2 mm)
2 Pt wire
Pt-žica
3 Poly(ethylene) piston
Polietilenski stap
4 Poly(ethylene) cell body
Tijelo polietilenske ćelije
5 Carbon paste
Grafitna pasta
6 Silicon rubber tube
Cjevčica od silikonske gume
7 Stainless steel outlet capillary as auxiliary electrode
Izlazna cjevčica od nehrđajućeg čelika kao pomoćna elektroda
8 Ag/AgCl reference electrode
Ag/AgCl-referentna elektroda

A and B
Joints for easy disconnection and renewal of the carbon paste
Spojevi za rastavljanje ćelije i obnovu istisnute mase grafitne paste

Carbon paste working electrode was prepared as described in the literature *(14)*. The graphite powder (Fisher) was thoroughly mixed with mineral oil (Aldrich) in mass ratio 1.1 : 0.9 and with this carbon paste a glass tube ($2r$ = 4 mm) with teflon piston was filled to produce an unmodified working electrode.

The electrochemical activation of carbon paste electrode was made by keeping the electrode for two minutes at positive potential (+1.5 V) and then for another two minutes at negative potential (–0.2 V) with mixing.

The modified electrode was made by adding a mass fraction of banana tissue of 5 % to the same carbon paste, mixing and filling an identical glass tube with teflon piston. The electrical contact was made by Pt wire. Three-electrode electrochemical cell was used, the other two were Ag/AgCl reference electrode and stainless steel outlet capillary as auxiliary electrode. All measurements were made at t = (25.0 ± 0.1) °C in phosphate buffer solution of pH = 6.5.

For the flow injection experiments a ternary gradient pump Model 9010 (Varian) was used with a 2 cm stainless steel tube, $2r$(outer) = 1.5 mm; $2r$(inner) = 0.2 mm, between the injector and the flow through cell. Two identical flow-through electrochemical cells, V(cell) = 1.5 μL; A(electrode) = 8.8 mm^2 with unmodified and modified carbon paste were constructed for easy exchange. The construction of the flow-through cell is shown in Figure 2.

The cell can be disconnected at joints A and B, the fresh carbon paste can be pushed out vertically with a teflon piston, while the pushed paste can be removed with another piston pushing horizontally. The electrodes and cells with modified and unmodified carbon pastes were stored in refrigerator after usage.

All electrochemical measurements were made by polarographic analyzer EG & G PAR 264 A connected with XY recorder PL 3 (Lloyd).

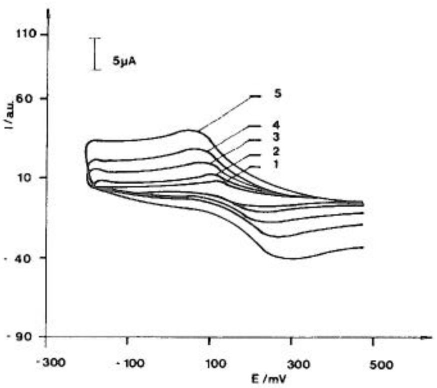

Fig. 3. Cyclic voltammograms at BTMCPE of 0.1 mM dopamine solution for different potential scan rates

Slika 3. Ciklički voltamogrami na bananom modificiranoj elektrodi od grafitne paste u 0,1 mM otopini dopamina za različite brzine promjene potencijala

Unmodified electrode:	BTMCPE:
Nemodificirana elektroda:	BTMCPE:
1 $\Delta E/\Delta t$ = 100 mV/s	$\Delta E/\Delta t$
	2 10 mV/s
	3 20 mV/s
	4 100 mV/s
	5 200 mV/s

Results and Discussion

Basic advantage of the investigated electrochemical biosensor for dopamine is its easy preparation, fast response, selectivity and micromolar sensitivity as it was shown in *(14)*. However in the paper of Wang and Lin only analytical aspects of this biosensor have been reported.

In this paper we investigated the behavior of the dopamine and pyrocatechol by cyclic voltammetry. Cyclic voltammograms of dopamine system (c = 0.1 mM) on BTMCPE at different scan rates are shown in Figure 3.

Similar cyclic voltammograms are obtained for pyrocatechol system (Figure 4A) and they show enhanced current peak relatively to dopamine (Figure 3) as it was reported in *(14)*. However, by recording cyclic voltammograms on an unmodified electrochemically activated carbon paste electrode (Figure 4B), better defined voltammograms are obtained with highest current peak. This suggests that the electrochemical pretreatment of an unmodified CPE compensates the catalytic action of (poly)phenol oxidase in banana tissue on pyrocatechol, which has been neglected so far and not mentioned in the literature.

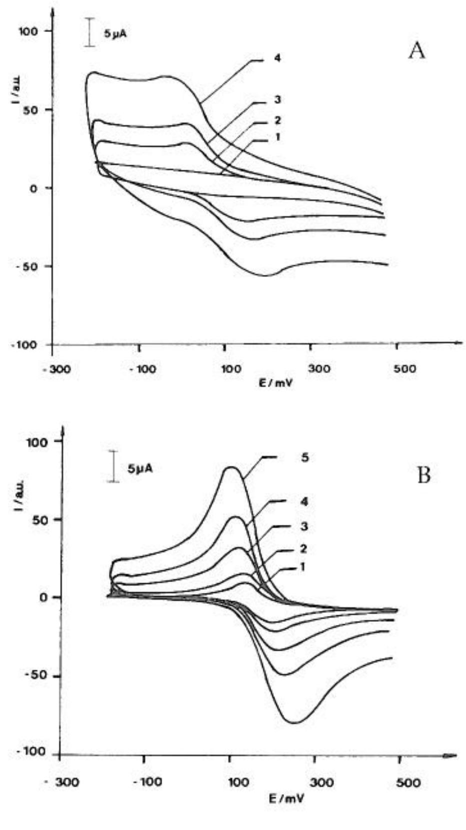

Fig. 4. Cyclic voltammograms at BTMCPE (A) and at electrochemically activated CPE (B) of 0.1 mM pyrocatechol solution for different potential scan rates

Slika 4. Ciklički voltamogrami na BTMCPE (A) i na elektrokemijski aktiviranoj elektrodi od grafitne paste (B) u 0,1 mM otopini pirokatehola za različite brzine promjene potencijala

$\Delta E/\Delta t$	
1 10 mV/s	4 100 mV/s
2 20 mV/s	5 200 mV/s
3 50 mV/s	

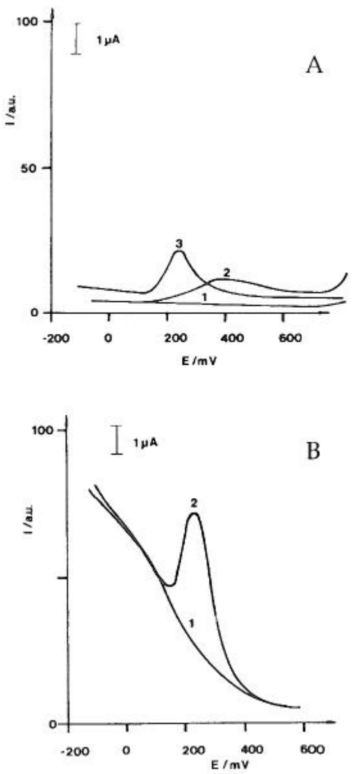

Fig. 5. Differential pulse voltammograms at CPE (A) and at BTMCPE (B) of 10 µM dopamine solution at $\Delta E/\Delta t$ = 10 mV/s

Slika 5. Diferencijalni pulsni voltamogrami na CPE (A) i na BTMCPE (B) u 10 µM otopini dopamina pri $\Delta E/\Delta t$ = 10 mV/s

A: 1 – CPE (buffer); 2 – CPE (dopamine);
3 – electrochemically activated CPE (dopamine)

A: 1 – elektroda od grafitne paste (pufer); 2 – CPE (dopamin);
3 – elektrokemijski aktivirana elektroda od grafitne paste (dopamin)

B: 1 – BTMCPE (buffer); 2 – BTMCPE (dopamine)

B: 1 – BTMCPE (pufer); 2 – BTMCPE (dopamin)

Differential pulse voltammograms (DPV) of a buffer solution, and 10 µM dopamine buffer solution on the electrochemically untreated and treated unmodified CPE are shown in Figure 5A, while that of a buffer solution and buffer solution with 10 mM dopamine recorded on BTMCPE are shown in Figure 5B. Again, the electrochemical treatment of the unmodified CPE shows an increase of the DPV current response, but for dopamine the catalytic effect is approximately 2.5 times greater at the investigated concentration level.

For 10 µM pyrocatechol system the increase of DPV current response on the electrochemically activated unmodified CPE is over ten times greater than that obtained on the unactivated CPE showing a very flat background current (Figure 6A). DPV obtained on BTMCPE shows a current peak enhancement relative to that of dopamine of 1.6 (2.0 was reported by Wang and Lin *(14)*), but on a relatively steep background current (Figure 6B).

These results suggest that BTMCPE is a very good biosensor for dopamine determination, but it seems that pyrocatechol might be better determined on the electrochemically activated graphite electrodes. The investigation of the electrochemical activation of graphite electrodes for pyrocatechol determination is now in progress.

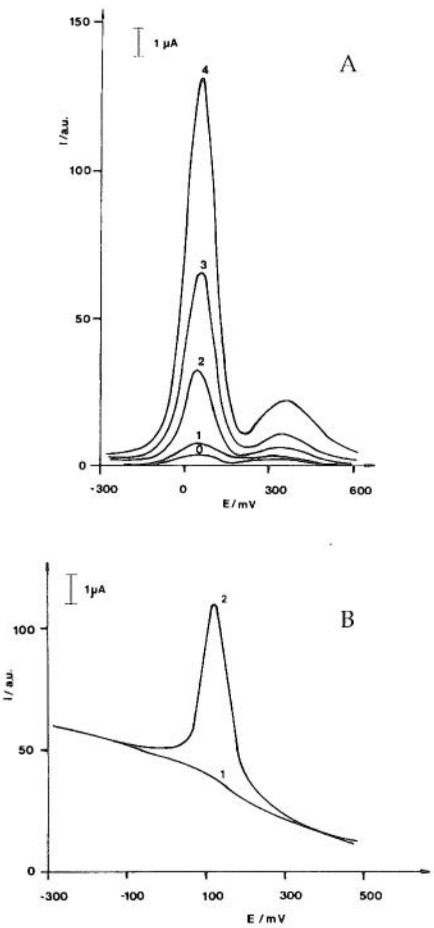

Fig. 6. Differential pulse voltammograms at CPE (A) and at BTMCPE (B) of pyrocatechol solution at $\Delta E/\Delta t$ = 10 mV/s

Slika 6. Diferencijalni pulsni voltamogrami na CPE (A) i na BTMCPE (B) u otopini pirokatehola pri $\Delta E/\Delta t$ = 10 mV/s

A: 0 – CPE (buffer); 1 – CPE (10 µM pyrocatechol);
2 – electrochemically activated CPE (5 µM pyrocatechol);
3 – electrochemically activated CPE (10 µM pyrocatechol);
4 – electrochemically activated CPE (20 µM pyrocatechol);

A: 0 – elektroda od grafitne paste (pufer);
1 – elektroda od grafitne paste (10 µM pirokatehol);
2 – elektrokemijski aktivirana elektroda od grafitne paste (5 µM pirokatehol);
3 – elektrokemijski aktivirana elektroda od grafitne paste (10 µM pirokatehol);
4 – elektrokemijski aktivirana elektroda od grafitne paste (20 µM pirokatehol);

B: 1 – BTMCPE (buffer); 2 – BTMCPE (10 µM pyrocatechol)

B: 1 – BTMCPE (pufer); 2 – BTMCPE (10 µM pirokatehol)

Using a flow injection analysis (FIA) in a microelectrochemical cell (Fig. 2) constructed in our laboratory good reproducibility is obtained at micromolar concentration range for dopamine on a BTMCPE and for pyrocatechol on the electrochemically activated CPE (Fig. 7).

The construction of the flow-trough cell allows an easy maintenance and renewal of the carbon paste. Its miniature size and compactness enabled the use of the same banana tissue modified carbon paste for over six months (stored in a refrigerator after usage). During this period the activity of the BMTCPE dropped by less than 10 %.

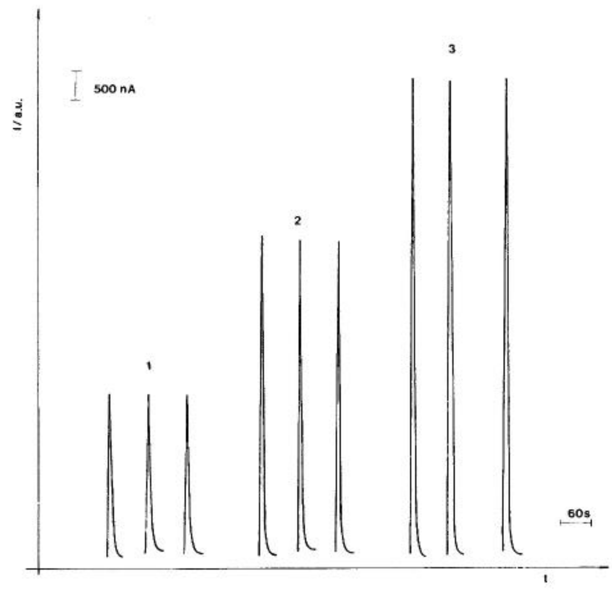

Fig. 7. Amperometric responses for pyrocatechol FIA determination on electrochemically activated CPE *c*(pyrocatechol): 1 – 5 μM; 2 – 10 μM; 3 – 15 μM

Slika 7. Amperometrijski odzivi pri određivanju pirokatehola na elektrokemijski aktiviranoj elektrodi od grafitne paste s pomoću kontinuirane analize uz ubrizgavanje u protok (flow injection analysis, FIA) *c*(pirokatehol): 1 – 5 μM; 2 – 10 μM; 3 – 15 μM

Conclusion

The microchemical cell construction for FIA is proposed, which enables easy handling of the unmodified and modified carbon paste electrode. Using amperometry, cyclic voltammetry and differential pulse voltammetry it has been shown that the banana tissue modified carbon paste electrode can be successfully used for dopamine detection while the unmodified electrochemically activated CPE is a better choice for the determination of pyrocatechol due to flatter background current response and with practically the same sensitivity as that obtained by catalytic activity of the enzyme poly(phenol) oxidase present in the banana tissue. The constructed microelectrochemical cell enabled the reproducible use of the banana tissue modified carbon paste for over six months decreasing the activity by less than 10 per cent.

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