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Electrochemistry and Detection of Some Organic and Biological Molecules at Conducting Polymer Electrodes. II. Effect of Nature of Polymer Electrode and Substrate on Electrochemical Behavior and Detection of Some Neurotransmitters

Ahmed Galal^a; Nada F. Atta^b; Judith F. Rubinson^c; Hans Zimmer^b; Harry B. Mark Jr.^b

^a Department of Chemistry College of Science, University of Cairo, Giza, Egypt ^b Department of Chemistry Edison Sensor Technology Center, University of Cincinnati, Cincinnati, OH, USA ^c Department of Chemistry, University of Dayton, Dayton, OH, USA

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**Electrochemistry and Detection of Some Organic
and Biological Molecules at Conducting Polymer Electrodes
II. Effect of Nature of Polymer Electrode and Substrate
on Electrochemical Behavior and Detection of Some Neurotransmitters[†]**

Key Words: Electrochemistry, Biological Compounds, HPLC, Detection, Conducting Polymers, Electrocatalysis, Sensors, Neurotransmitters.

Ahmed Galal,^{*} Nada F. Atta, Judith F. Rubinson,[§] Hans Zimmer & Harry B. Mark, Jr.^{**}

Department of Chemistry, Edison Sensor Technology Center, University of Cincinnati, Cincinnati, OH 45221-0172 USA.

^{*}Department of Chemistry, College of Science, University of Cairo, Giza, Egypt.

[§]Department of Chemistry, University of Dayton, Dayton, OH 45469, USA.

^{**}Author to whom all correspondence should be addressed.

[†] This work is dedicated to an eminent scholar and a friend W. Simon.

ABSTRACT

The electrochemical behavior and detection of some molecules of biological significance were studied. The separation and detection of some neurotransmitters, namely, norepinephrine, L-DOPA, epinephrine, and dopamine were accomplished using reverse phase high performance liquid chromatography(HPLC) and amperometric detection, respectively. The voltammetric behavior of these molecules was compared using different polymeric electrodes. Electrocatalytic "efficiency" decreases in the order of poly(3-methylthiophene)(P3MT), poly(N-methylpyrrole)(PNMP), poly(aniline)(PAn) and poly(furan)(PF). P3MT showed improved performance as an amperometric detector for HPLC analysis systems over other types of polymer and conventional electrodes examined. Detection limits as low as 10^{-8} - 10^{-9} M were achieved using the P3MT, compared to 10^{-6} - 10^{-8} M using glassy carbon or platinum electrodes.

The nature of the substrate used for the polymer deposition had no effect on the electrochemical characteristics of the compounds studied. The results describes the intrinsic catalytic property of the polymer electrode surface towards the redox behavior of the compounds studied. The polymer electrode showed promising antifouling resistant against common fouling agents.

INTRODUCTION

In the last decade, the need for high sensitivity and selectivity for the analysis of biomolecules has been increasing.¹ Electrochemical methods of detection following HPLC separation represent a cornerstone in the determination of catecholamines.² The electrode materials most commonly used in amperometric systems are glassy carbon,^{3a} platinum and gold electrodes.^{3b} Mercury electrodes, namely, dropping, thin-film mercury and amalgam electrodes have a relatively wide potential range because of the high hydrogen overvoltage at their surfaces.⁴ However, several limitations were reported in the application of mercury electrodes in flowing solutions such as their poor mechanical stability and the dissolution of mercury at low potentials which resulted in their inadequacy in the detection of organic and biological compounds. On the other hand, the performance of glassy carbon, pyrolytic graphite, platinum and gold electrodes depends mainly on the method and quality of surface polishing and pretreatment. Moreover, glassy carbon electrodes were found to be sensitive to high current densities and to aging.⁵ Carbon paste electrodes are cheaper, easier to prepare and replace than many of the aforementioned electrodes. However, the use of carbon paste electrodes is also limited when organic solvents are implemented and the pastes are not resistant toward mechanical damage exerted by relatively high flow rate of solutions.

Changes caused to the electrode surface due to the adsorption of the analytes, oxidative or reductive byproducts results in commonly known as the electrode surface fouling. This phenomenon represents a substantial challenge to the electrochemical detection of organic

compounds. While mercury electrodes are easily regenerated by forming a new surface and solid electrodes are efficiently cleaned by mechanical polishing, this regeneration process is time consuming and the mechanical polishing of the surface is tedious and requires disassembling of the detector compartment. Recently, the concept of chemically modifying the electrode surface has gained extensive attention among analytical chemists and others. The properties of the modifying "layer" coated onto the substrate are deliberately designed to alter the sensitivity and the selectivity of the measurement.⁶ The improvement in the electrode response is based on the catalytic effect imparted by the modifying layer to the analyte/electrode interaction and by the suppression of interfering and undesirable concomitant reactions. The natures of the modifying layers and the techniques adapted for their application at the electrode surface varies widely. Modification of the working electrode with redox mediators represents a dominant category, namely on carbon-based electrodes.⁷ The simplest technique of electrode modification is by dispersing of the modifier within a paste or by physically adsorbing it to the electrode surface.^{8a} Alternatively, the modifier could be covalently bonded to the electrode surface.^{8b,c} Although chemically modified electrodes showed impressive results, several disadvantages prevented these electrodes from practical applications. Among these disadvantages are the poor stability, relative low capacity in the case of thin films covalently bound to the surface and the relative slow response for polymeric films containing modifiers. The application of these modified electrodes in flowing systems demands even more restrictions on the mechanical properties and life time of the electrode. In previous work,⁹ we examined the electrochemical behavior of a large number of biologically important compounds at a P3MT electrode. The electrocatalytic property of the P3MT was demonstrated as well as the effect of the nature and the pH of the base electrolyte. The intrinsic catalytic property of the polymer electrode, in the absence of a "redox mediator", had not been shown for different conducting polymers. Moreover, the electrocatalytic property of the polymer films, which is independent of the nature of the substrate, was not yet demonstrated. Previous work by Wang and coworkers¹⁰ proved

that the selectivity of detection could be based on controlling the pore size of the polymer film and the electron transfer actually occurred on the substrate. In this work we examine and compare the electrochemical behavior of some neurotransmitters at different conducting polymer electrodes. The cyclic voltammetric behavior for the same compound at the same polymer film electrode using different substrate will be compared. Moreover, the advantages of using the polymer electrodes for the HPLC/EC separation and detection of some neurotransmitters will be presented. The detection limits, sensitivity, and sensor life time using the P3MT are also given. The polymer electrodes showed promising antifouling characters over the conventionally used glassy carbon and platinum electrodes. Examples will be provided for the detection of biological compounds in the presence of albumin, gelatin and Triton 100-X.

EXPERIMENTAL

Reagents:

Dopamine, epinephrine, L-Dopa (Sigma), catechol, disodium and monosodium hydrogen phosphates, sulfuric acid, monochloroacetic acid and sodium octyl sulfate (Fisher) were used as received. 3-Methylthiophene, N-methylpyrrole, furan, aniline, tetrabutylammonium tetrafluoroborate and acetonitrile (Aldrich) were distilled before usage. Water was distilled and then purified through a NaNOpure II-apparatus (17-19 Mohm, 0.22 μm pore size) (Barnstead Company, Newton, MA). All solutions were filtered in a solvent clarification apparatus (0.22 μm pore size) (Millipore). The pH was determined for buffers prepared at room temperature (± 0.5 °C) using an Orion digital pH-meter Model 601A1 and an Orion pH-indicator electrode Model 90-20 double-junction reference electrode (Orion, Inc., MA).

Procedure and Apparatus:

Electrochemical polymerization was carried out in an one compartment cell containing deaerated acetonitrile, 0.1 M tetrabutylammonium tetrafluoroborate and 0.05 M monomer (in case of methylthiophene, pyrrole or furan) and 0.1 M sulfuric acid and 0.05 M monomer (in case of aniline). The polymer films were deposited onto platinum or glassy carbon substrates using the

potentiostatic technique. The potential was controlled at a constant value depending on the type of monomer using a PAR 173 Potentiostat/Galvanostat equipped with a plug-in PAR Model 176 current-to-voltage converter and a PAR Model 379 Digital Coulometer (Princeton Applied Research, Princeton, NJ). Cyclic voltammetric experiments were performed using a BAS-100 Electrochemical Analyzer (BAS, Inc., West Lafayette, IN). All potentials were measured in reference to an 3M Ag/AgCl electrode. Data were reported using an HI PLOT Digital Plotter (Houston Instrument, Houston, TX). Liquid chromatography apparatus consisted of: an ALTEX Model 100A (ALTEX Scientific Inc., Berkeley, CA) dual reciprocating pump, Model 7120 injection valve fitted with a 20 μ L sample loop (Rheodyne, Cotati, CA), Model CV-1B cyclic voltammetry unit controlling a thin layer flow through cell Model AMP/Pt-Pt (AMP, Inc., Cincinnati, OH) working in the amperometric mode. The analytical column was a stainless steel Whatman(PARTISIL 5 ODS-3) 25 cm x 4.6 mm id prepacked with 3 μ m ODS (BAXTER, McGaw Park, IL), protected by an ODS guard column (BAXTER, McGaw Park, IL). The mobile phase consisted of a 0.15 M monochloroacetic acid, 1 mM/L sodium octylsulfate solution. The final pH was adjusted to 3.0 with 1.0 M acetic acid/1.0 M sodium acetate solution. The column was equilibrated with the mobile phase at least 2 hours before usage. The data were collected using a Fisher Y-t recorder Model FISHER RECORDALL Series 5000 (Fisher, Cincinnati, OH). A Fluka 8000A Digital Multimeter was used for monitoring the potential difference between the working and the reference electrodes in the flow cell. All test solutions were freshly prepared prior to injection using the mobile phase used for elution. The effluent was potential monitored at +0.500 V (vs. Ag/AgCl) unless otherwise stated. Full-scale sensitivities ranged between 1-100 nA according to the concentrations injected through the column. The pump flow rate was 1.5 mL/minute unless otherwise stated.

Results and Discussion

Redox behavior at different polymer electrodes:

Figure 1 shows the cyclic voltammetric behaviors of 5 mM catechol in 0.1 M sulfuric acid at P3MT, PNMP, PAn and PF electrodes. All cyclic voltammograms displayed reversible

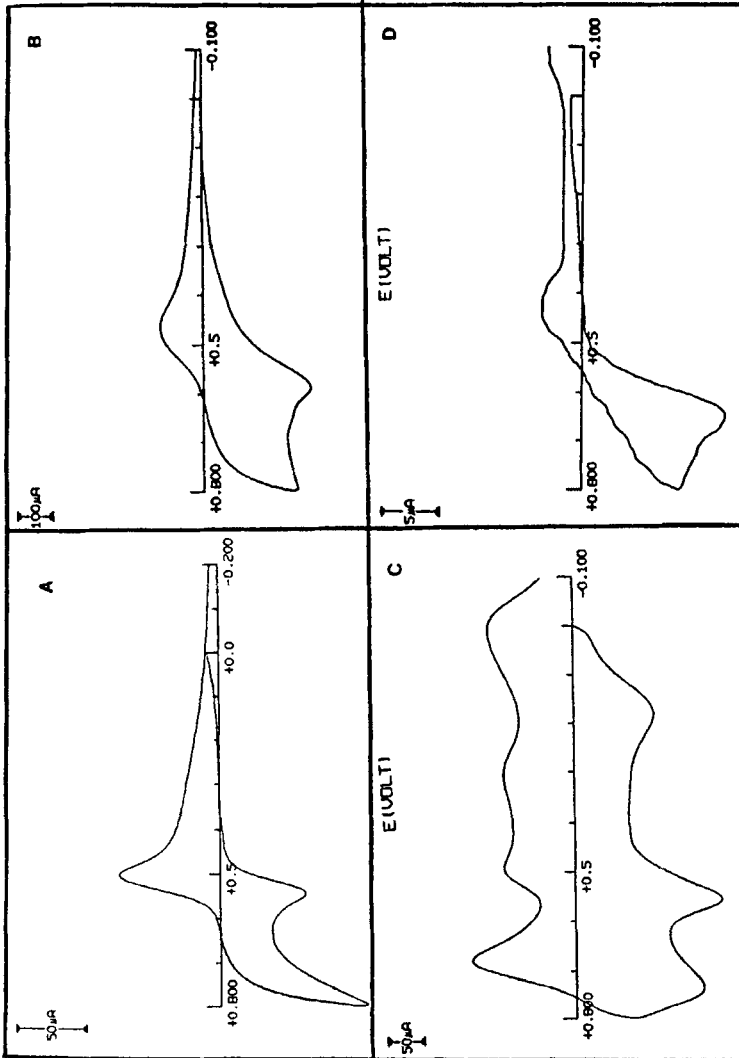


FIG. 1 Cyclic voltammetry of 5 mM catechol in 0.1 M H_2SO_4 , at P3MT (A), PNMP (B), PAN (C), and PF (D) electrodes. Scan rate 50 mV/s. All films were prepared under similar conditions.

TABLE 1.

Electrolyte and Substrate Effect on the Electrochemical Response of P3MT.
Solution (5 mM ascorbic acid/100 mM Electrolyte), Scan rate 100 mV/s.

Electrolyte	pH	Pt	P3MT/Pt	GC	P3MT/GC
H ₂ SO ₄	1.60	524(mV) 25 (μA)	445(mV) 45 (μA)	663(mV) 67 (μA)	439(mV) 170 (μA)
Na ₂ SO ₄	3.70	367 30	340 43	444 67	335 120
HCl	1.56	590 24	427 50	663 66	435 160
NaCl	3.23	439 11	381 22	512 42	388 130
HNO ₃	1.25	568 23	422 36	667 62	430 160
NaNO ₃	3.28	422 16	317 29	444 69	321 150
H ₃ PO ₄	1.63	469 29	393 60	564 72	410 160
Na ₃ PO ₄	3.85	385 19	291 37	475 46	298 130

redox peaks with distinctive peak separation values and different general potential-current features. From the analytical point of view, the sensitivity of the measurement could be regulated by the catalysis of the analyte/electrode charge transfer reaction. The comparison of the oxidation peak potential E_{ox} at these electrodes for 5 mM ascorbic acid in 0.1 M electrolyte (table 1) and of the oxidation for 5 mM catechol in 0.1 M electrolyte (table 2) showed two important facts: (i) All polymer electrodes have relatively lower E_{ox} values as compared to those obtained at Pt or GC electrodes, (ii) for the series of polymer electrodes studied, the E_{ox} values increase in the order of P3MT < PAN < PNMP < PF. The cyclic voltammograms (figure 1) and the peak separations, ΔE_p , of the anodic (E_{ox}) and cathodic (E_{red}) peak potentials (table 2), indicate the extent of the reversibility of the redox process. The peak separation ΔE_p displayed

TABLE 2

Electrochemical Data for Catechol at Different Conducting Polymer Electrodes.
Catechol and Electrolyte concentrations were 5 and 100 mM, respectively.
Scan Rate 100 mV/s. ΔE_p is the Difference between Anodic and Cathodic Peak Potentials.

Electrolyte	P3MT		PNMP		PF		PAn		Pt	
	Epa (mV)	ΔE_p	Epa (mV)	ΔE_p	Epa (mV)	ΔE_p	Epa (mV)	ΔE_p	Epa (mV)	ΔE_p
H ₂ SO ₄	560	69	587	122	643	183	556	67	702	364
Na ₂ SO ₄	544	238	574	238	600	325	568	107	628	455
HCl	559	77	599	137	675	372	564	64	634	251
NaCl	590	250	618	285	689	464	598	218	610	376
HNO ₃	559	79	578	121	673	323	55	67	658	278
NaNO ₃	556	225	591	244	683	461	601	251	641	408
H ₃ PO ₄	585	165	628	243	621	296	599	109	777	424
Na ₃ l O ₄	513	231	604	211	605	384	581	291	616	558

relatively smaller values for PAn when compared to PMeT electrodes and noticeable larger difference with respect to the PNMP and PF electrodes. However, the cyclic voltammogram in figure 1 for PAn electrode shows several peaks and represent complication in identifying the redox peaks for the analyte from those expected from the polymer film charge-discharge process. The PMeT electrode, therefore, represents a relatively superior behavior over the other polymer films under consideration. The large current envelopes depicted in figure 1 have been retained within all the polymer electrodes which is an inherent characteristics for this category of electrodes.¹¹ Moreover the reversibility of the redox process was found to be superior for the P3MT electrode as depicted by the amount of charge involved in the process, ca $Q_{ox} = 28.04$ mC/cm² and $Q_{red} = 26.89$ mC/cm².

Effect of substrate on the electrochemical behavior:

Careful inspection of table 2 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.

Previous work¹⁰ indicated that the selectivity of the detection was due to the permselective nature of the film and its porosity was mainly controlled by the synthesis conditions.

Inspection of columns three and five of table 2 indicate that the E_{ox} values for different supporting electrolytes studied are the same for films grown at GC or Pt surfaces. On the other hand, the oxidation potential values, E_{ox} , are different for Pt and GC. This indicates that the oxidation process is taking place at the "surface" of the polymer film rather than at the substrate.

Determination of catecholamines:

Figure 2 shows the chromatogram obtained for a sample of a mixture of norepinephrine, L-DOPA, epinephrine and dopamine using 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, electrochemical detector; and the PMeT electrode at an applied potential of 0.55 V (vs. Ag/AgCl). Equimolar amounts of the four analytes were used, namely 10^{-8} M. Comparison of this chromatogram to that shown in figure 3 where a glassy carbon electrode was used reveals the following facts: (i) the current signals obtained in the case of using P3MT as the working electrode are higher than those obtained in the case of using glassy carbon electrode, (ii) the base line of the first chromatogram shows a relatively higher stability when compared to that obtained in figure 3. The use of PNMP as the electrochemical detector proved to dramatically affect the current signal and the base line stability as depicted in figure 4.

Detection limits and sensitivities:

Calibration curves for the catecholamines analyzed using the P3MT and GC electrodes as the electrochemical detector are shown in figures 5a & 5b, respectively. The calibration curves describe the sensitive nature of the electrodes used towards the different catecholamines studied. The relative sensitivities and the detection limits (signal to noise ratio = 3) for the P3MT and GC electrodes are given in table 3. Again the P3MT electrode

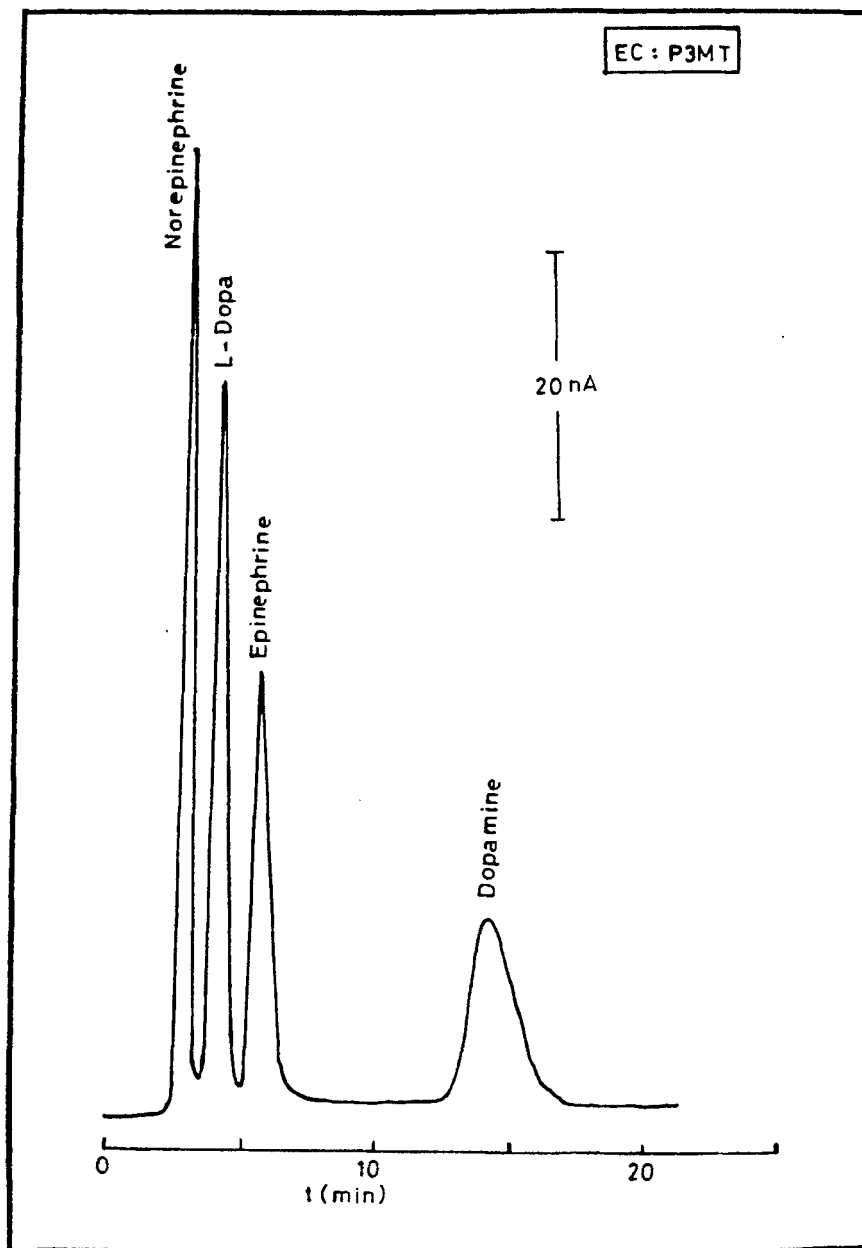


FIG.2 Chromatogram of catecholamines mixture. Sample volume 20 μ L, PARTISIL 5 ODS-3 column, mobile phase 0.15 M monochloroacetic acid, pH=3.00, 26 $^{\circ}$ C, 100 nA full scale, flow rate 1.5 mL/min, chart speed 10 mm/min, detector BAS CV-1B, 0.55 V with P3MT working electrode.

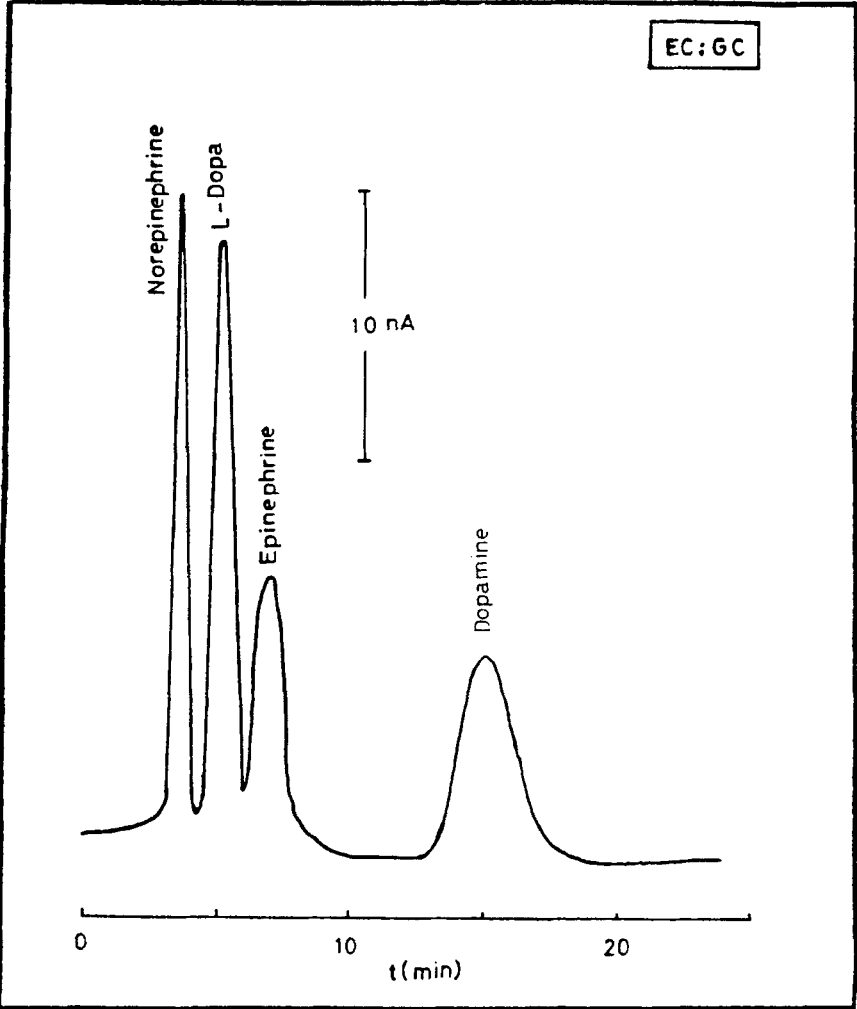


FIG.3 Chromatogram of catecholamines mixture. Conditions as in Figure 2 except GC used as working electrode, full scale is 50 nA.

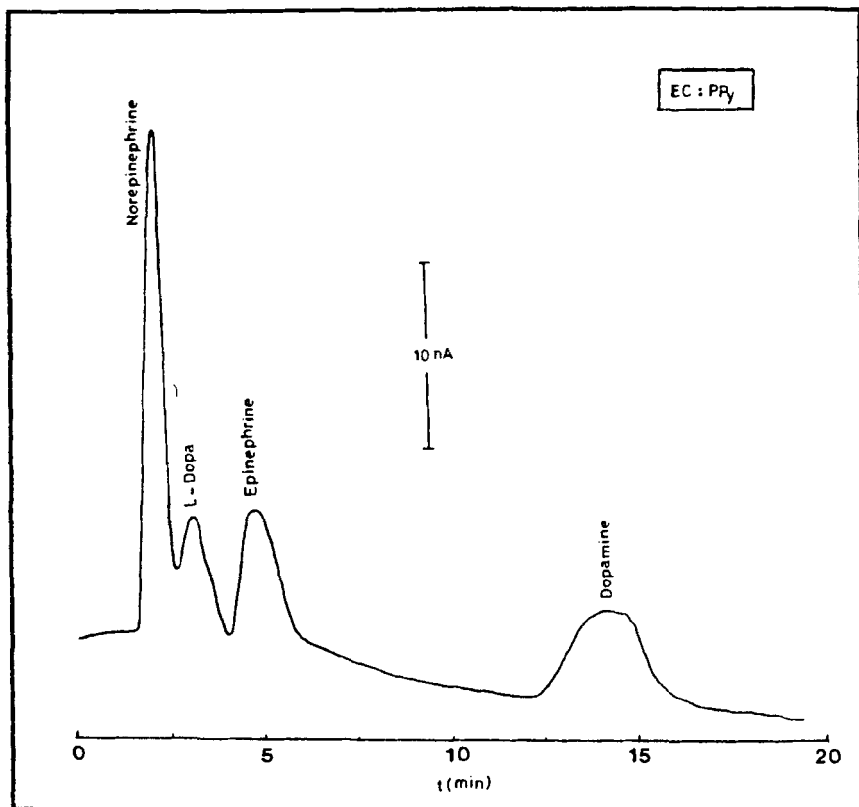


FIG.4 Chromatogram of catecholamines mixture. Conditions as in Figure 2 except PNMP used as working electrode, full scale is 50 nA.

showed relatively lower detection limits as compared to the GC electrode for the analysis of catecholamines. The effect of changing the flow rate on the current signals of the peaks obtained in the chromatograms are given in figures 6a & 6b, respectively. The data shows the relative fast response of the P3MT electrode when compared to that of GC. The mechanism of response proved to be similar when comparing the data obtained in figures 6a and 6b.

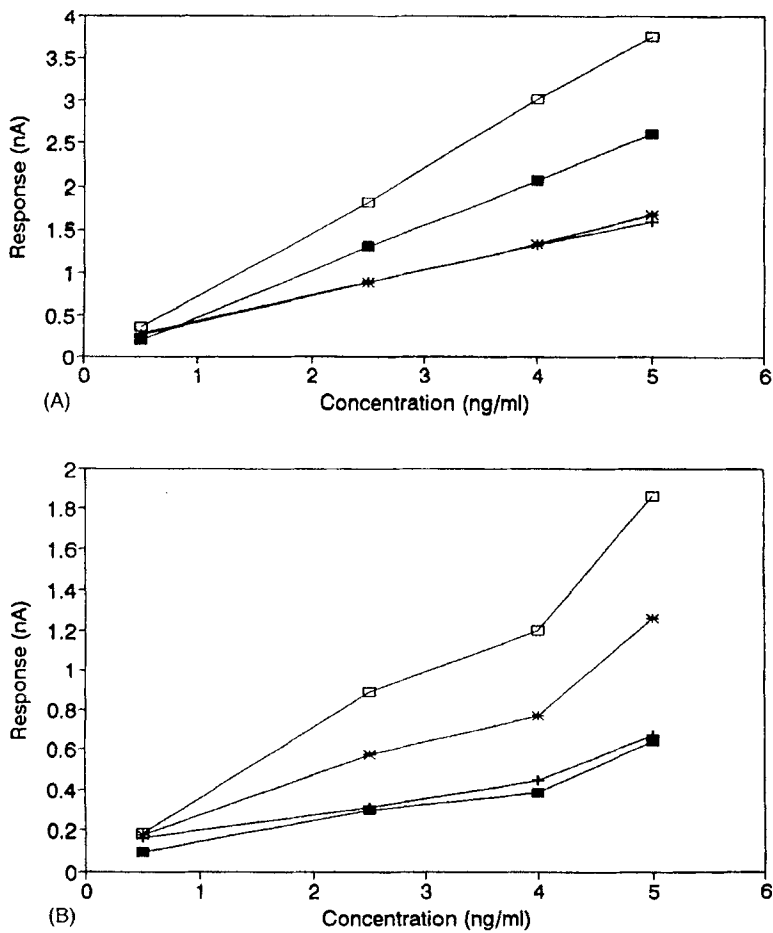


FIG.5

(a) Calibration curve for catecholamines mixture obtained under HPLC/EC conditions as indicated in Figure 2 at P3MT electrode, (■) epinephrine, (+) norepinephrine, (*) L-DOPA, (□) dopamine.

(b) Calibration curve for catecholamines mixture obtained under HPLC/EC conditions as indicated in Figure 2 at GC electrode, (■) epinephrine, (+) norepinephrine, (*) L-DOPA, (□) dopamine:

TABLE 3

Relative Sensitivities and Limits of Detections of P3MT and GC Electrodes in the HPLC/EC Detection of Some Neurotransmitters. Analysis Conditions: 20 μ L injection loop, 1.5 mL/min flow rate, 0.15 M (pH=3.0) monochloroacetic acid mobile phase, $E_{app}=0.55$ mM (vs. Ag/AgCl).

Analyte	P3MT Electrode		GC Electrode	
	Sensitivities nA/mg/mL	LOD ng/mL	Sensitivities nA/mg/mL	LOD ng/mL
Norepinephrine	0.298	0.153	0.106	2.54
L-Dopa	0.757	0.137	0.349	1.46
Epinephrine	0.533	0.105	0.112	1.59
Dopamine	0.309	0.218	0.223	1.87

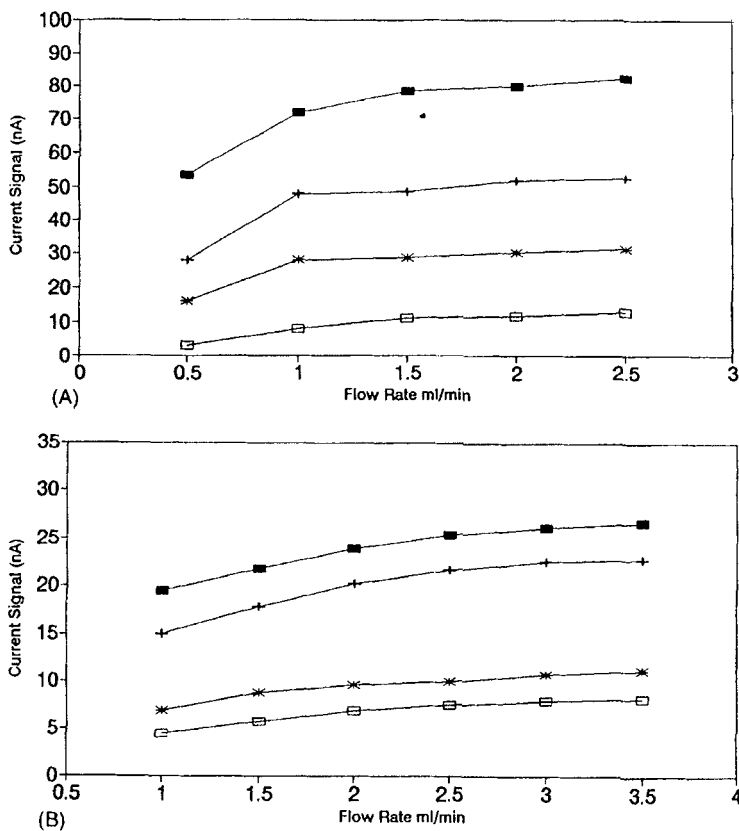


FIG. 6 Effect of flow rate on the current signal obtained at P3MT(a) and GC(b) electrodes, respectively. Chromatographic conditions as in Figure 2.

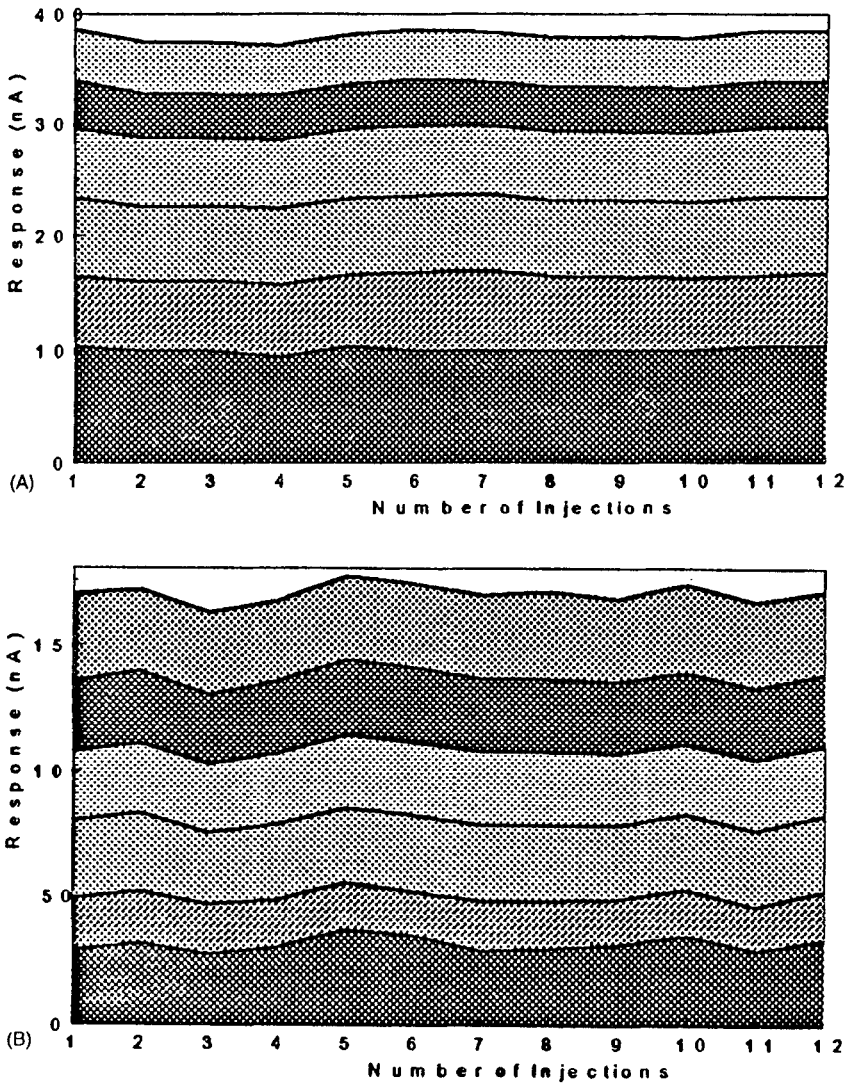


FIG. 7 Effect of extended use of P3MT(a) and GC(b) electrodes in the HPLC/EC analysis of catecholamines for a period 60 days. Data shown for 10 days intervals. Chromatographic conditions as in Figure 2.

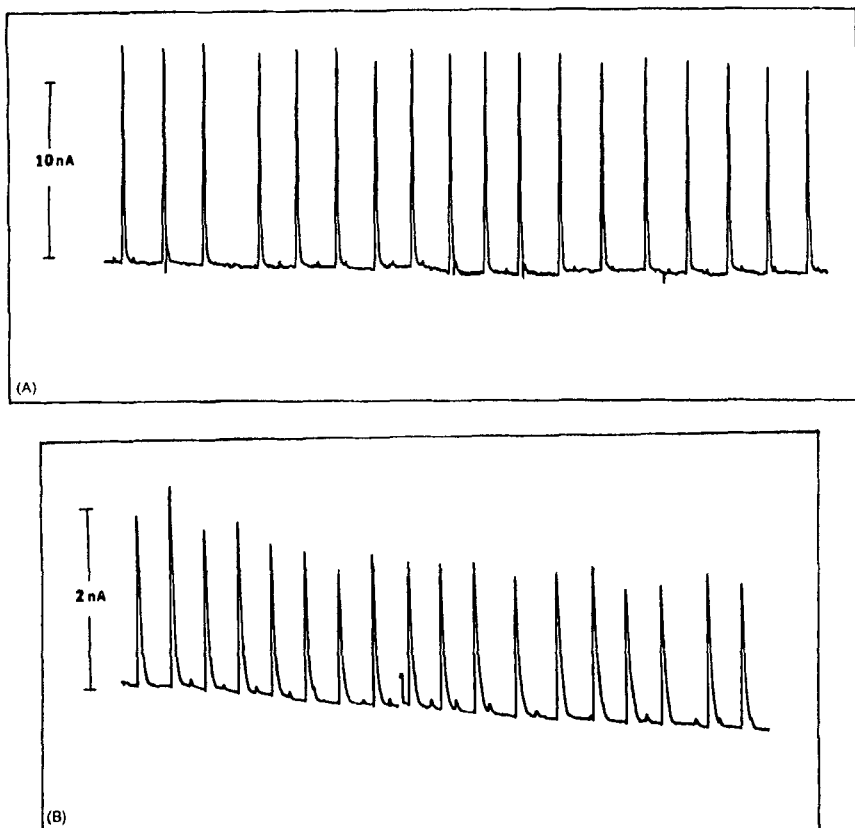


FIG. 8 Successive flow injection analysis of a sample of 5 mM norepinephrine at P3MT(a) and GC(b) electrodes. Flow rate 2 mL/min, E_{app} 0.50 V (vs. Ag/AgCl), Mobile phase 0.15M monochloroacetic acid, 8mM SOS, pH=3.00.

Stability of the electrode response and life time:

The P3MT and GC electrodes were examined for the analysis of catecholamines over an extended period of time of over 60 days. The electrodes were also tested for a successive injection in the flow analysis mode for over 20 injections. Figures 7a & 7b show the results obtained for extended use of the P3MT and GC electrodes in the HPLC/EC analysis of catecholamines. The following observations can be noticed from these results: (i) the current

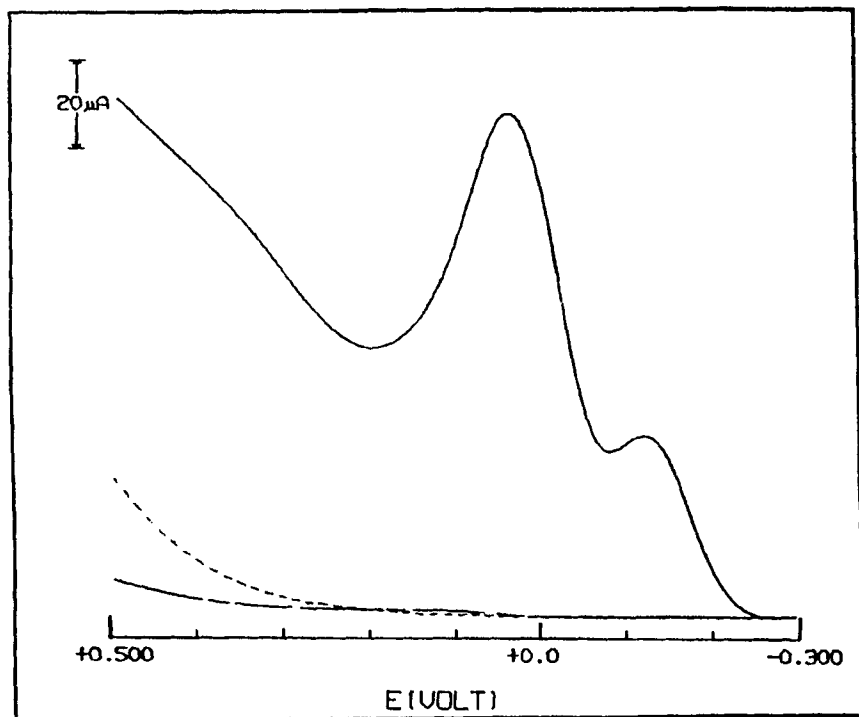


FIG.9 Square wave voltammogram of 5 mM catechol in the presence of 1000 ppm albumin at P3MT(-), GC (---), and Pt(---) electrodes in phosphate buffer (pH=6.9).

signal obtained at the P3MT electrode is relatively more stable than that obtained at the GC, (ii) the current signal showed erratic behavior for successive injections, (iii) the current signal shows 35% attenuation (calculated as the ratio between 60th day/1st day current signal) for the P3MT electrode compared to 69% attenuation for the GC. The flow injection current signals obtained for over twenty successive injections are shown in figure 8. The results shows a relatively high stability for the P3MT electrode when compared to the GC electrode. The P3MT electrode current signal showed only a relative decrease in magnitude of 0.75% up to an extended time of use of over 60 days.

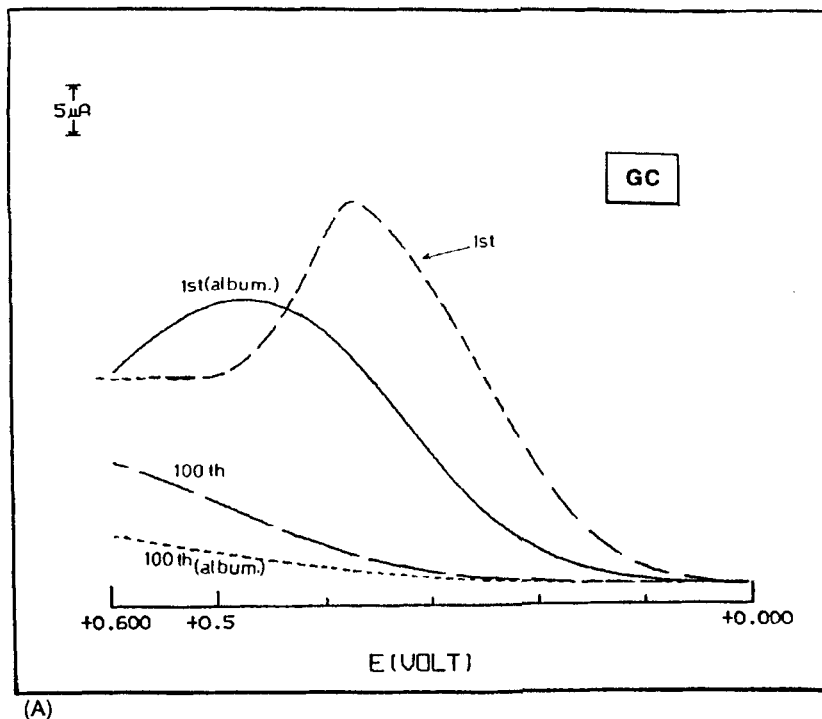
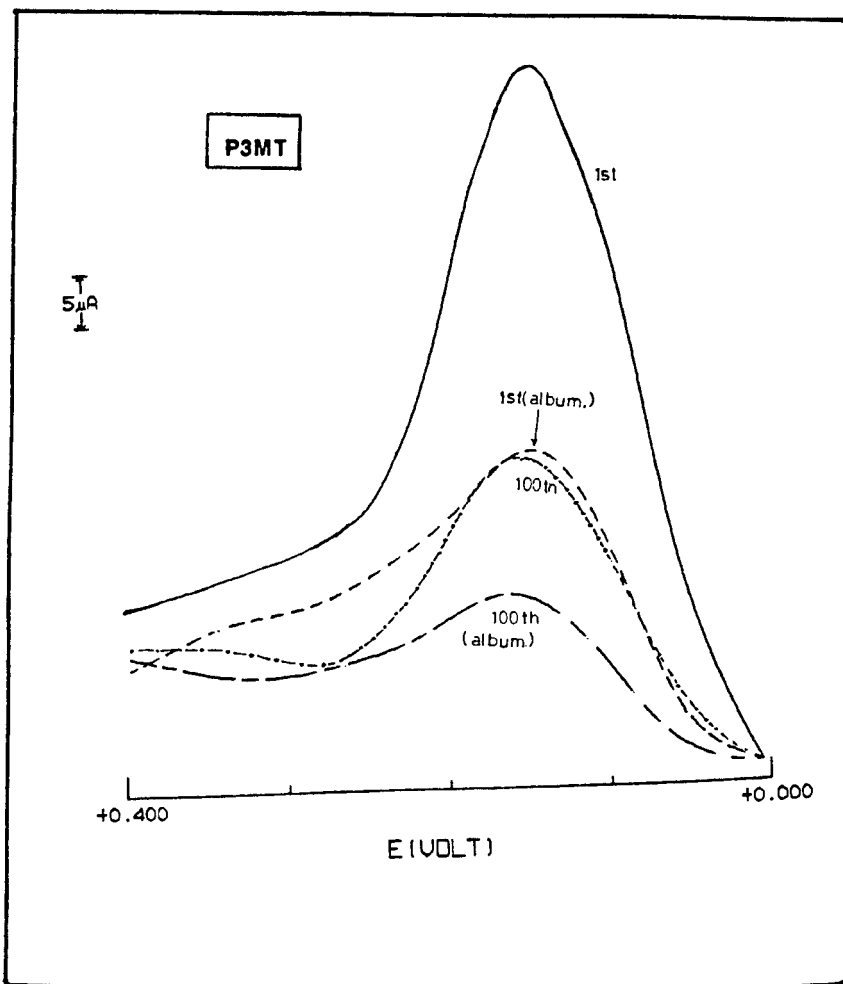


FIG.10 Square wave voltammograms of 5 mM p-aminophenol in phosphate buffer (pH=6.9) in the presence and absence of 1000 albumin for GC(a) and P3MT(b) electrodes.

Effect of surface active agents on the detector response:

The stability of the electrode response was examined in the absence and presence of some surface active agents. Figure 9 shows the square wave voltammetry of 5 mM catechol in the presence of 1000 albumin at P3MT, GC and Pt electrodes. The signal was attenuated in the case of Pt and GC electrodes and relatively unaffected in the case of using the P3MT. The effect of repetitive cycles on the electrochemical response of GC and P3MT was examined in the presence and absence of 1000 albumin for the analysis of p-aminophenol. The results are depicted in figures 10a & 10b. The following observations could be noticed:



(B)

FIG. 10 Continued

(i) the position of the oxidation peak potential was shifted to a more positive value upon the addition of albumin in the case of GC 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, on the other hand the P3MT electrode showed only "partial" attenuation for the same number of repeated cycles. More interestingly the peak potential position remained relatively unchanged.

Conclusions:

In this work we compared the electrochemical behavior of some catecholamines at different conducting polymer electrodes. The electrodes were prepared under similar conditions, the results showed that the four polymers studied exhibited improved electrocatalytic activity when compared to GC or Pt electrodes. However, the P3MT electrode showed better reversibility and stability over the other polymers studied. The use of P3MT in HPLC/EC proved to be superior over the use of GC. This polymer electrode showed to be stable and more sensitive than GC. The detection limits obtained using the polymer electrode were as low as 0.1-0.2 ng/mL. This polymer electrode showed promising results for the resistance against surface active agents.

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