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Newly synthesized poly(glycidyl methacrylate-*co*-3-thienylmethylmethacrylate)-based electrode designs for phenol biosensors

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ABSTRACT

A newly synthesized poly(glycidyl methacrylate-*co*-3-thienylmethylmethacrylate) [poly(GMA-*co*-MTM)] was designed to fabricate various HRP electrodes for detection of phenol derivatives. The results showed that the poly(GMA-*co*-MTM)/polypyrrole composite film microarchitecture provided a good electroactivity as a result of pyrrole and thiophene interaction, and provided chemical bonds for enzyme immobilization via the epoxy groups of poly(GMA-*co*-MTM). The glassy carbon-based working electrode displayed significantly higher performance for the same composite film configuration comparing to the gold-based working electrode. Poly(GMA-*co*-MTM)/polypyrrole/HRP coated glassy carbon electrode exhibited a fast response less than 3 s, a high sensitivity (200 nA μM^{-1} for hydroquinone), a good operational stability (%RSD values ranged between 2 and 5.1 for all phenolics), a long-term stability (retained about 80% of initial activity at the end of 40th day) and a low detection limit ranging between 0.13 and 1.87 μM for the tested.

1. Introduction

During the last years, an increasing demand for new methods of analysis and control in various fields of environmental monitoring and production sector can be noticed. To answer this request, the development of the sensitive, selective but fast in answer sensors, giving reproducible and easy to interpret information, represents for many researchers a major challenge [1]. Owing to their high selectivity and simple use, amperometric biosensors represent powerful tools for environmental monitoring [2,3].

In the construction of the amperometric biosensors, an important role is played by the method of electrode fabrication. Several methods of the electrode fabrication are used today. These methods are being developed by synthesizing new conducting polymers, which are essential parts of working electrodes [4–8]. Conducting polymers with conjugated double bonds are a new class of materials and have been gaining more and more attention because of their potential use in many applications [9–17]. Among the conducting polymers, polythiophene has a special place due to their electrical properties, rich synthetic flexibility, environmental stability in doped and undoped states, non-linear optical properties, and highly reversible redox switching [18].

Synthesis of a thiophene-functionalized methacrylate monomer [3-methylthienylmethacrylate (MTM)] via the esterification of 3-thiophene methanol with methacryloyl chloride can be prepared. Thus, the MTM monomer obtained has two polymerizable groups: the vinyl group is useful for radical polymerization while the thiophene ring, with substitution at the 3-position, can be employed in both oxidative polymerization and electropolymerization. It is also possible to prepare block and random copolymers of MTM with other acrylic or vinyl monomers at different compositions. Subsequently, constant-potential electrolyses can be employed for the synthesis of the graft copolymers of the side chain thiophene.

Various architectures of epoxy group possessing polymers have been developed in the literature. Copolymers of glycidyl methacrylate (GMA), an epoxy group containing methacrylate monomer, have received great interest. Epoxide is a three-membered cyclic ether and very reactive due to the large strain energy (about 25 kcal mol^{-1}) associated with the three-membered ring. Therefore, it can be employed into a large number of chemical reactions by ring opening. Various applications of chemically modified pendant copolymers, such as immobilization of enzymes, DNA, catalysts, and biomolecules, were reported [19,20].

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Copolymerization is the most effective and successful way among the existing polymerization techniques for incorporation of systematic changes in polymer properties. It does not require rigorous experimental conditions, and can be employed for the polymerization of a large variety of monomers leading to the formation of new materials. Reactive functional polymers can be prepared by incorporation of acrylates and methacrylates monomers containing side chain reactive functional groups into polymers.

In the present study, we reported the construction of an amperometric phenol biosensor based on the immobilization of HRP onto a novel copolymer electrode. For this purpose, random copolymer of electroactive 3-methylthienyl methacrylate (MTM) and side chain epoxy group containing glycidyl methacrylate (GMA) monomers was prepared via free-radical polymerization. The determination of phenolic compounds has great importance due to their toxicity and persistency in the environment. One of the phenol oxidases, HRP was immobilized using different immobilization methods including chemical bonding or entrapment or chemical bonding/entrapment to poly(GMA-*co*-MTM)-based electrodes for phenol detection. Chemical bonding was attributed via pendant epoxy groups of the poly(GMA-*co*-MTM) with amine groups of HRP while enzyme entrapment was carried out in cross-link networks of the poly(GMA-*co*-MTM)/PPy film formed during the electropolymerization step. The response dependences and amperometric characteristics including sensitivity, linear range, detection limit, standard deviation and stability of the electrodes were investigated. Various phenol derivatives were tested by using the optimized working electrode.

2. Experimental

2.1. Reagents

Horseradish peroxidase (E.C.1.11.1.7) with an activity of 10.000 U vial^{-1} (according to pyrogallol method performed by the supplier), aqueous solution of hydrogen peroxide (30%), lithium chloride, di-potassium hydrogen phosphate, citric acid, tri-sodium citrate, acetic acid (96%), sodium acetate tri-hydrate and potassium di-hydrogen phosphate were purchased from Merck. Phenol, *p*-benzoquinone, hydroquinone, 2,6-dimethoxyphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2-aminophenol, 4-methoxyphenol, pyrocatechol, guaiacol, *m*-cresol, *o*-cresol, *p*-cresol, catechol, 4-acetamidophenol, pyrogallol, 2,4-dimethylphenol, pyrrole (99%), CHES buffer and sodium dodecyl sulfate (SDS) were obtained from Sigma. α,α′-azobisisobutyronitrile (AIBN), tetrahydrofuran (THF) and dimethylformamide (DMF) were purchased from Riedel. The phenol reagents were used as purchased without any further pre-treatment. Stock solutions of various phenols were daily prepared in 0.1 M phosphate buffer solution (pH 7.0).

2.2. Apparatus

Electrochemical experiments were performed by using a CHI Model 840B electrochemical analyzer. A gold working electrode (2 mm diameter), a glassy carbon working electrode (2 mm diameter), a platinum wire counter electrode, a Ag/AgCl (3 M NaCl) reference electrode, and a conventional three-electrode electrochemical cell were obtained from CH Instruments.

2.3. Synthesis of poly(GMA-co-MTM)

Side chain thiophene containing monomer, 3-thienylmethylmethacrylate (MTM) was synthesized according to the procedure described in Refs. [21,22]. We have previously reported the copolymers of MTM with glycidyl methacrylate (GMA) [23]. Poly(GMA-*co*-MTM) was synthesized via radical polymerization of appropriate GMA/MTM feed mixture in the presence of AIBN as an initiator. Predetermined quantities of MTM, GMA and AIBN (1% of total weight of monomers) in DMF with a volume of 1.5 mL were placed in a Pyrex tube. The mixture was deoxygenated by flushing with oxygen-free argon for at least 15 min. The tube was tightly sealed and immersed in a thermostated oil bath at 60 ± 1 °C. The conversion was determined by gravimetric measurements. After the reaction, copolymer was precipitated in methanol, filtered off, and purified by reprecipitation from dichloromethane solution into methanol and finally dried in vacuo for 24 h. Molecular weight and molecular weight distribution of the poly(GMA-*co*-MTM) was measured by using Agilent Instrument (Model 1100) consisting of a pump, refractive index and UV detectors, and four Water Styragel Columns (HR 5E, HR 4E, HR 3, and HR 2) and using THF as eluent at a flow rate of 0.3 mL min^{-1} at 30 °C and toluene as an internal standard. Molecular weights were calculated with the aid of polystyrene standards.

2.4. Fabrication of the HRP-based working electrodes and electrochemical measurements

All electrochemical measurements were carried out in a 0.1 M phosphate buffer solution (pH 7.0) in the presence of 0.7 mg mL^{-1} lithium chloride with an applied working potential of −50 mV and a continuous stirring at 600 rpm in three-electrode cell. Various phenol derivatives were added to this reaction medium to produce current–time curves of amperometric measurements.

The fabrication of the electrodes was preceded by a cleaning phase of the electrode surface using gamma alumina powder then, rinsing with distilled water. Six various electrode configurations, hereafter referred to using the codes of A, B, C, D, E and F were designed. A gold electrode with a diameter of 2 mm was used for the preparation of A, B, C, D and E electrodes. Only the electrode F was prepared by using a glassy carbon electrode with the same diameter. Six milligrams of poly(GMA-*co*-MTM) was dissolved in 10 mL of THF. The polymer solution with a volume of 20 μL was directly spread onto the surface of the electrodes, except for the electrode E. The electrodes, A, B, C, D and F were then allowed to dry for solvent evaporation at room temperature. Electrode A was then coated with PPy in a polymerization medium contained 10 mL of 50 mM pH 6.5 citrate buffer including 0.01 M pyrrole, and 0.6 mg mL^{-1} SDS, which was used as supporting electrolyte, at a potential scan between −1.2 and +1.2 V for 4 min at a scan rate of 100 mV s^{-1}. The electrode A was dipped into a solution of 0.6 mg mL^{-1} HRP, dissolved in 0.1 M, pH 7.0 phosphate buffer, and stored at +4 °C overnight as a final step. The electrode B was dipped into the solution of HRP, and stored at +4 °C overnight. Afterwards, the electrode B was coated with PPy in the polymerization medium mentioned above. The electrode C was coated with PPy in the citrate buffer including 0.01 M pyrrole, 0.6 mg mL^{-1} of HRP and 0.6 mg mL^{-1} SDS at the same electropolymerization conditions. The electrode D was dipped into a solution of HRP and stored at +4 °C overnight. For the fabrication of electrode E, a bare gold electrode was coated with PPy at condition given above in the citrate buffer including 0.01 M pyrrole, 0.6 mg mL^{-1} of SDS and 0.6 mg mL^{-1} of HRP. Electrode F was fabricated by using completely same procedure given for the electrode C.

3. Results and discussion

The composition results of poly(GMA-*co*-MTM) are presented in Table 1. ^{1}H NMR spectrum of poly(GMA-*co*-MTM) is shown in Fig. 1.

Table 1
Composition data for free-radical copolymerization of GMA with MTM.

Copolymer	M_{GMA}[a]	m_{GMA}[b]	Time (min)	Conversion (%)	$M_W \times 10^{-3}$	$M_n \times 10^{-3}$	M_W/M_n
Poly(GMA-*co*-MTM)	0.28	0.30	150	81	67.3	18.4	3.66

[a] M_{GMA} and m_{GMA} are the mole fraction of GMA in the feed and copolymer, respectively.
[b] The composition of copolymer was calculated by comparing the integral peak areas of the methylene protons (–$COOCH_2$–) (d), exhibiting resonance signal at around at 5.0 pmm, to that of the methylene proton of epoxy ring (b) showing signal at about 3.1 mg L^{-1} (Fig. 1).

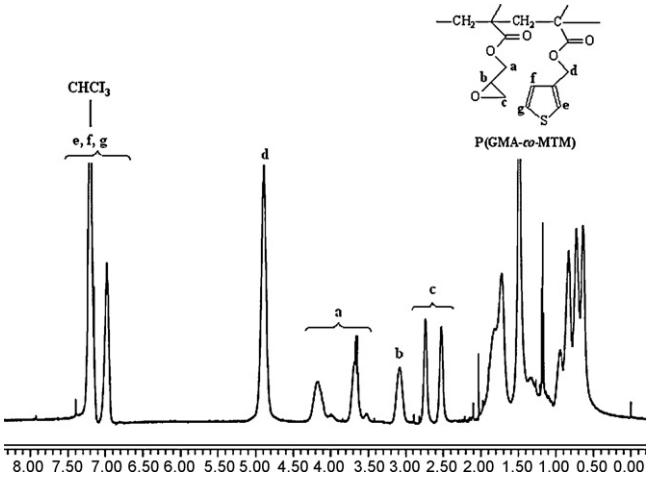

Fig. 1. ^1H NMR spectrum (solvent, $CDCl_3$; at rt) of poly(GMA_{30}-*co*-MTM_{70}).

The thiophene groups on the copolymer do not show any electroactivity (Fig. 2). In the case of pyrrole present in the system, the usual pyrrole polymerization peaks were drastically shifted. The shift of the redox peaks to higher potential values is known to be an indication for the reaction between pyrrole and the thiophene moiety of the copolymer [21,24,25].

3.1. Comparing the response of the designed working electrodes (A, B, C, D, E and F) to phenolic substance

Fig. 3 shows the calibration curves of the electrodes A, B, C and E, which were constructed from the current–time recordings of hydroquinone additions. The highest sensitivity and the lowest detection limit were obtained from the electrode C among the electrodes of A, B and E. This can be a result of the used enzyme immobilization process of the electrode C performed via chemical bonding/entrapment. While chemical bonding was performed via pendant epoxy groups of the poly(GMA-*co*-MTM) with amine groups of HRP, enzyme entrapment was carried out in cross-link networks of the poly(GMA-*co*-MTM)/PPy composite film formed during the electropolymerization process. No reproducible signal was obtained from the electrode D, which was only fabricated with poly(GMA-*co*-MTM), due to the limited electroactivity of poly(GMA-*co*-MTM) film. The sensitivity value of the electrode E obtained from the hydroquinone calibration is smaller than the value of the electrode C since the enzyme was immobilized only by means of entrapment in the cross-networks of PPy film. The electrodes A and B gave the lowest amperometric responses. The electrode A was coated with poly(GMA-*co*-MTM) and PPy resulting a composite polymeric film. Then, the composite film was dipped to the enzyme solution. It is showed that the electropolymerized poly(GMA-*co*-MTM)/PPy composite film was not allowed the enzyme to bound onto the polymeric film surface. The active epoxy groups of the poly(GMA-*co*-MTM) might become useless for enzyme immobilization after the electropolymerization with PPy. The electrode B consists of chemically bonded HRP to the poly(GMA-*co*-MTM) film via pendant epoxy groups. In that case, electropolymerization of pyrrole on the enzyme immobilized poly(GMA-*co*-MTM) film possibly prevented the immobilized enzyme contact with bulk solution where the enzymatic reaction occurred.

The calibration curve of the electrode F after the addition of hydroquinone with the same procedure was obtained, and compared with the calibration of the electrode C (Fig. 4). It can be clearly seen in Fig. 4 that the amperometric response of the electrode F was higher than the response of the electrode C.

The main characteristics of the electrodes A, B, C, D, E and F including sensitivity, linear range, detection limit and regression coefficient are listed in Table 2. It appears that the relative high sensitivity was obtained with the electrode F (200 nA μM^{-1}), which is nearly 7 times higher than the electrode C (30 nA μM^{-1}) and 100 times higher than the electrode B (2 nA μM^{-1}). The higher

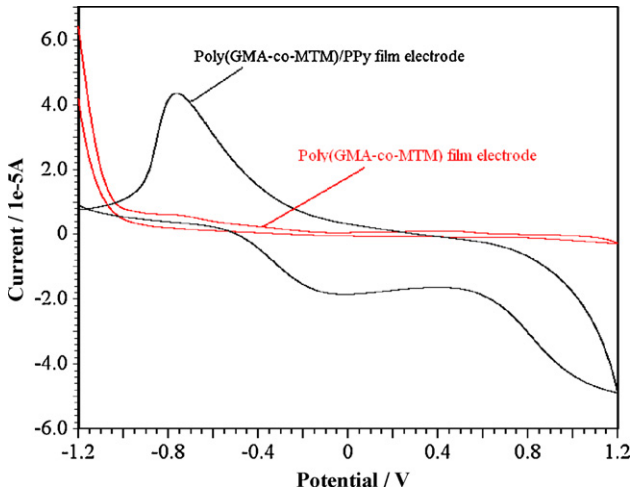

Fig. 2. Cyclic voltammograms obtained with poly(GMA-*co*-MTM)/PPy and poly(GMA-*co*-MTM) film coated electrodes in 10 mL of 0.1 M phosphate buffer solution (pH 7.0) contained 0.7 mg mL^{-1} of lithium chloride, at a scan rate of 100 mV s^{-1}.

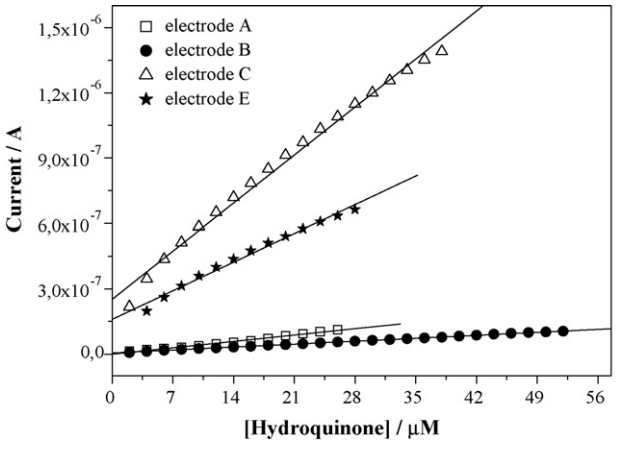

Fig. 3. Calibration curves of electrode A, B, C and E to increasing hydroquinone concentrations (initial phenolic concentration is 2 μM). Applied potential: −50 mV vs. Ag/AgCl, 3 M NaCl.

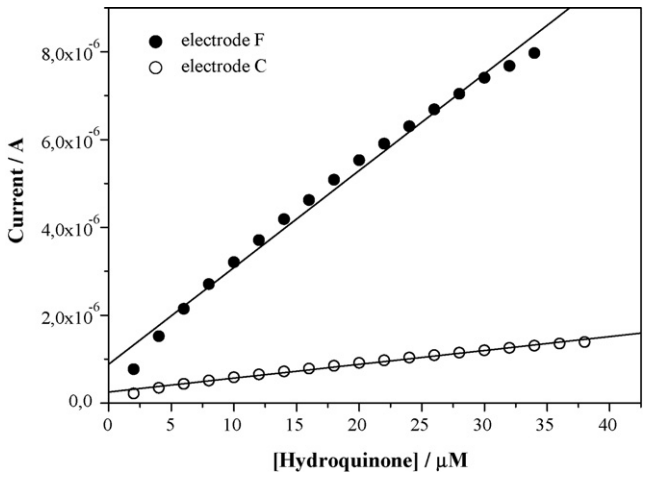

Fig. 4. Calibration curves of electrodes C and F to increasing hydroquinone concentrations (initial phenolic concentration is 2 μM). Applied potential: −50 mV vs. Ag/AgCl, 3 M NaCl.

sensitivity of the electrodes C and F can be attributed to the favorable microenvironment of the immobilization matrix and enzyme immobilization procedure, which was performed by both chemical bonding and entrapment. However, the type of the electrode material played an important role on the value of the sensitivity when the electrodes C and F were compared with each other. Glassy carbon electrodes (GCEs) have been widely used compared with metal electrodes due to its biocompatibility with tissue, having low residual current over a wide potential range and minimal propensity to show a deteriorated response as a result of electrode fouling [26–29]. Recently reported papers have stated that HRP is more compatible with carbon electrode materials [30–36]. Rabinovich and Lev have claimed that the response of a phenol biosensor is usually limited by the electrochemical back reduction of the quinone leading to the diphenolic compound. Carbon electrode material affects significantly the sensitivity of the biosensor, because the limiting electrochemical back reduction of the enzymatic products takes place on the grain of the carbon materials [37].

Detection limit (LOD) was calculated according to the $3s_b/m$ criteria in Ref. [38], where m is the slope of the linear range of the respective calibration plot (sensitivity parameter), and s_b is estimated as the standard deviation of the signals from different solutions of phenolics at the concentration level corresponding to the lowest concentration of the calibration plot. The lowest detection limit was found to be 0.13 μM (S/N = 3) for the electrode F comparing to the other working electrodes. Since the best response was obtained from the electrode F, this electrode was employed for the detection of eighteen phenolics for further experiments.

Table 2
Analytical parameters of electrode A, B, C, D, E and F calculated from the calibrations of hydroquinone additions.

Electrode	Linear range (μM)	LOD (μM)	Sensitivity (nA μM^{-1})	r^2
A	2–26	1.2	4	0.988
B	2–52	1.5	2	0.999
C	2–38	1.03	30	0.990
D		No reproducible response		
E	4–28	2.55	20	0.987
F	2–34	0.13	200	0.989

3.2. Response of the electrode F to various phenolics

3.2.1. Optimizing the working conditions of the electrode F

The applied potential is an important parameter for the response of a biosensor. Effect of working potential ranging between −80 and 20 mV was investigated for the electrode F. The concentration of hydroquinone was kept constant at 20 μM in the reaction medium at each working potential value. The amperometric response increased between 20 and −50 mV vs. Ag/AgCl and then remained practically constant until −70 mV. In the potential range between −50 and −70 mV, quinone species produced in the enzymatic reaction are reduced [39,40]. At the higher negative potentials than −70 mV, the amperometric response decreased sharply probably due to the irreversible HRP inactivation as described by Csoregi et al. [41]. In addition to this, fouling of electrode surface can occurred as a consequence of the polymerization of the enzymatic products at more negative potential values [42]. Thus, potential was set to be −50 mV since the highest amperometric response was obtained. This result is identical with the other papers related to HRP-based phenol biosensors [30,43–45].

The concentration of hydrogen peroxide is one of the other important factors affecting the response of HRP-based phenol biosensors, since HRP reaction is hydrogen peroxide dependent. The reaction between HRP and phenolic compounds bases on the so-called double displacement or "ping-pong" mechanism in which two substrates, a peroxide and the given phenolic compounds are involved [46,47]. Effect of hydrogen peroxide concentration ranging 2–40 μM was investigated for a fixed concentration of hydroquinone (10 μM) by using the electrode F. Excess amount of hydrogen peroxide has an inhibitor effect on HRP activity [48], while low concentrations restrict the enzymatic phenol reaction. Amperometric response increased up to the hydrogen peroxide concentration of 20 μM then sharply decreased possibly due to HRP inactivation (*not shown*). Thus, hydrogen peroxide concentration was fixed at 20 μM.

3.2.2. Producing the calibration curves

The typical amperometric responses and the calibration curves of the electrode F are illustrated in Fig. 5A and B, respectively after the addition of successive aliquots of phenolic compounds (phenol, catechol, *p*-benzoquinone, *m*-cresol, *o*-cresol, *p*-cresol, guaiacol, 2,4-dimethylphenol, 2,6-dimethoxyphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, hydroquinone, 4-acetamidophenol, pyrogallol, 4-methoxyphenol, pyrocatechol, 2-aminophenol) under continuous stirring at 600 rpm. The electrode F reached to the steady-state current of 95% in less than 3 s by reaching.

Table 3 summarizes the characteristics of the calibration plots obtained from the current–time recordings of phenol derivatives. The lowest detection limit was found to be 0.13 μM for hydroquinone, and the highest detection limit was found to be 1.87 μM (S/N = 3) for pyrocatechol among the tested phenol derivatives. LOD values calculated in this study are smaller than recently reported phenol biosensors where detection limit ranged between 0.16 and 6 μM (S/N = 3) for various phenol derivatives [34,49–55].

The sensitivity of HRP-based biosensor depends on the stability of the phenoxy radicals produced in the enzyme reaction, electrode material, HRP immobilization method and the magnitude of the applied potential [44]. In the detection of different phenolic compounds, the trend of the sensitivity was consistent with the ability of the substituents for forming electron-donor conjugation. Sensitivity is also depends on the ability of electron-donor conjugation. Kane et al. [56] reported that the phenol compounds with electron-donor substituents in an *ortho*-position gave no response. Similar with this report the electrode F did not give any response to *o*-cresol and 2-aminophenol among the tested phenolics contain-

Table 3
Analytical parameters of the biosensor fabricated with electrode F for 18 phenolic compounds.

Phenolic compound	r^2	Sensitivity (nA μM^{-1})	Linear range (μM)	LOD (μM)	%RSD	%Recovery
Hydroquinone	0.989	200	2–34	0.13	2.3	95
Catechol	0.915	30	2–12	0.87	4.5	81
p-Benzoquinone	0.922	30	2–10	0.85	5	87
2-Chlorophenol	0.972	10	4–10	1.62	4.1	91
3-Chlorophenol	0.978	20	2–12	1.31	5	70
4-Chlorophenol	0.998	60	1–34	0.55	2	93
2-Aminophenol			No response			
Phenol	0.987	90	2–12	0.3	2.1	91
Guaiacol	0.998	10	2–20	1.2	3.8	109
2,6-Dimethoxyphenol			No response			
4-Acetamidophenol	0.993	100	2–30	0.21	2.3	102
4-Methoxyphenol	0.997	100	2–70	0.25	3.2	105
2,4-Dimethylphenol			No response			
Pyrogallol			No response			
Pyrocatechol	0.981	3	2–22	1.87	2.8	94
m-Cresol	0.996	10	2–88	1.43	3.8	100
o-Cresol			No response			
p-Cresol	0.995	20	2–70	1.28	5.1	99

ing electron-donor substituents in an *ortho*-position. The sensitivity was calculated from the slope of the calibration curves. The different sensitivities varied between 3 and 200 nA μM^{-1} (Table 3) for the tested phenolics can be related to the formation of *o*-quinones during the enzymatic reaction [57]. The maximum sensitivity was found to be 200 nA μM^{-1} for hydroquinone. In addition to this, 4-methoxyphenol and 4-acetamidophenol showed higher sensitivity than the other phenolics. This can be dialed with the presence of –OCH_3 group of 4-methoxyphenol which enhances oxidation of the phenolic by HRP. Due to the strong ability of electron-donor conjugation of hydroquinone and 4-acetamidophenol, the corresponding conjugation structure could be easily formed. No response was obtained for 2,4-dimethylphenol, as expected, for the one having the *ortho*-position occupied by a methyl group. Not only *o*-cresol and 2,4-dimethylphenol but also 2-aminophenol, pyrogallol and 2,6-dimethoxyphenol gave no response. The obtained sensitivity values are higher than those of the recent studies [34,40,49–54,58–66]. The widest linear range of 2–88 μM was observed for *m*-cresol with the regression coefficient of 0.996. Zhou et al., have claimed that a loose in linearity at higher concentration of phenolic compounds is attributed to slow surface fouling by the reaction products [67]. In this study, the surface fouling by the enzymatic reaction products was minimized owing to the biocomposite structure of the poly(GMA-*co*-MTM)/PPy/HRP film.

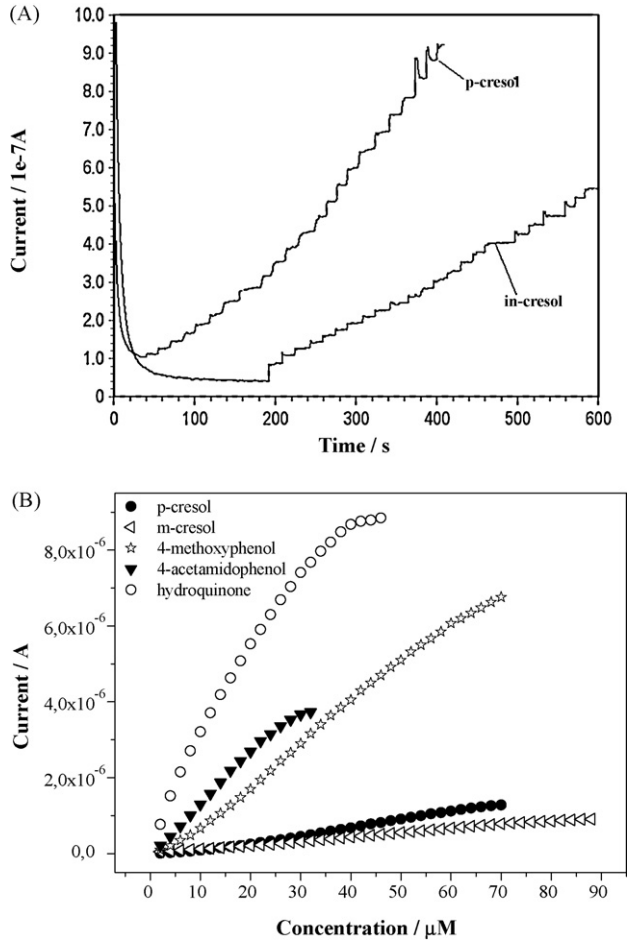

Fig. 5. Current–time recordings of electrode F to increasing *p*-cresol and *m*-cresol concentrations (initial phenolic concentration is 1 μM) (A) and calibration curves of electrode F to increasing phenolic concentrations (B). Applied potential: −50 mV vs. Ag/AgCl, 3 M NaCl.

The operational stability of the electrode F was monitored for a series of 20 successive additions of 2 μM phenolic compounds. Well-defined reduction responses were obtained for the tested phenolics with relative standard deviations (RSD) ranging between 2% and 5.1% as seen in Table 3. Relative standard deviation (%RSD) was calculated according to the (*standard deviation of the currents obtained by repetitive additions*/*mean values of the currents formed by these additions*) × 100 criteria. The high operational stability of the electrode F might be a result of the microarchitecture of the newly synthesized poly(GMA-*co*-MTM) creating an available surface attachment for the enzyme. Recovery values calculated according to the formula given as (*measured concentration*/*actual concentration*) × 100 for the most of the tested phenolics gave satisfactory results.

The electrode F retained about 80% of its initial activity at the end of the 40th day. Long-term stability of the electrode F was relatively higher than those of the previous phenol biosensors reported [68–78]. The entrapment of the enzyme via electropolymerization with PPy onto the poly(GMA-*co*-MTM) coated electrode, provided more stable microenvironment for the reactions. Furthermore, chemically bonding of HRP to poly(GMA-*co*-MTM) improved the stability of the electrode due to preventing enzyme deterioration and loss.

4. Conclusion

In this study, a newly synthesized poly(GMA-*co*-MTM) was used to fabricate various HRP electrodes for detection of phenol derivatives. The electrode F under the optimized experimental conditions

gave the best response among the prepared working electrodes. It showed a sensitivity of 200 nA μM^{-1}, a long-term stability of 80% of initial activity at the end of 40th day, and a detection limit of 0.13 μM for hydroquinone. The entrapment of the enzyme into cross-link networks of the poly(GMA-*co*-MTM)/PPy composite film provided stable conditions for enzymatic reaction and transferring electrons due to the mild microenvironment through the composite film. The response time of the electrode F was less than 3 s. In the detection of different phenolic compounds, the trend of the sensitivity was consistent with the ability of the substituents for forming electron-donor conjugation. The highest amperometric responses obtained from the electrodes C and F can also be attributed to the poly(GMA-*co*-MTM)/PPy composite film microstructure providing a good electroactivity as a result of pyrrole and thiophene interaction. Although, the same composite film and fabrication method were used for the preparation of the working electrodes of C and F, the sensing performance of the electrode F based on glassy carbon was significantly higher than that of electrode C based on gold. Because HRP is more compatible with carbon electrode materials as well as gassy carbon electrodes have low residual current over a wide potential range and minimal propensity to show a deteriorated response as a result of electrode fouling. Conclusively, newly synthesized poly(GMA-*co*-MTM) is a promising polymeric material for the fabrication of enzyme electrodes concerning its ability of forming chemical bonds with the amine groups of enzymes. Moreover, the thiophene moiety of poly(GMA-*co*-MTM) can be employed for the polymerization of a large variety of monomers leading to the formation of conductive copolymers.

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