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A novel amperometric biosensor based on single walled carbon nanotubes with acetylcholine esterase for the detection of carbaryl pesticide in water

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ABSTRACT

Amperometric biosensor is fabricated for the detection of carbaryl based on single walled carbon nanotubes (SWCNTs) and acetylcholine esterase (AchE). The dispersion of SWCNTs in positively charged polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA), possibly takes place due to weak supramolecular interaction between them, which then binds electrostatically to the negatively charged AchE at pH 7.4 using layer-by-layer (LbL) self-assembly technique. The optical intensity of UV/vis spectra increased with the number of layers, indicating the build up of a multilayer coating on the electrode. The activity of acetylcholine esterase on modified electrode of 3 mm in diameter was found to be 0.2 U. The biosensor showed good sensitivity and stability towards the monitoring of carbaryl pesticides in water with the detection limit of 10^{-12} g L^{-1} and recovery of 99.8 ± 2.7% to 10^{-10} g L^{-1}. This protocol can be used for the immobilization of other enzymes to fabricate a range of biosensors.

1. Introduction

The presence of pesticide residues and metabolites in food, water and soil currently represents one of the major issues for the environmental chemistry. Pesticides are, in fact, among the most important environmental pollutants because of their increasing use in agriculture [1]. Among the pesticides, organophosphate and carbamate species have been used extensively because of their high insecticidal activity and relatively low persistence [2]. Their toxicity arises mainly due to their inhibitory effect on acetylcholine esterase, a key enzyme for the nerve transmission. The toxicity of organophosphate and carbamate pesticides can vary considerably depending on the chemical structure of the pesticide [3].

Pesticides of the carbamate family have been progressively replacing more persistent species (mainly organophosphates) owing to their low persistence in the environment, biological activity and large spectrum of utilization. They are used as insecticides, fungicides, nematocides, miticides and molluscocides. Carbaryl (1-naphthyl-1-methylcarbamate) is one of the major active ingredients of many commercial formulations of pesticides [4]. Carbaryl, like other carbamates, is not only a powerful inhibitor of acetylcholine esterase activity in the organism but also presents a potential teratogenic capability [5], thus requiring continuous monitoring in food and potable waters. For this reason, a number of analytical procedures have been employed to monitor carbamate pesticides and to determine their concentrations. These techniques include spectrophotometry [6], infrared spectroscopy [7], flow-injection chemiluminescence [8], fluorimetry [9] and chromatography. Among chromatographic techniques, the liquid chromatography with ultraviolet diode array [10,11], fluorescence spectrometry [12] and mass spectrometry [13] detection probes have been used widely due to the moderately high polarity and thermolability of carbaryl. Many of the above-mentioned determination methods are though accurate and selective, but require relatively expensive instrumentation, relatively more time and make use of higher toxic organic reagents. As a consequence the development of alternative methods the requirements for "in situ" determinations or monitoring in real time have motivated to undertake a study that minimize the consumption of reagents and decrease the time of analysis without deteriorating the quality of measurements. In the last few decades, numerous biosensing methods for detection of pesticides have been developed using enzyme-based and affinity-based sensors as well as several types of transducers [13].

The accuracy, efficiency and detection limit of biosensor can be enhanced with the use of nanomaterials. In this regard carbon nanotubes (CNTs) have attracted considerable attention due to their high electrical conductivity, strong adsorptive ability, good mechanical strength and excellent biocompatibility [14]. These features have made CNTs highly suitable in the biosensor field

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[15]. Yet, untreated CNTs are extremely hydrophobic and prefer to assemble into bundles, making them difficult to process, and limit their usage. This has led to significant research efforts to devise some effective approaches to solubilize CNTs. Surface modification and non covalent bondings with dispersants are two main methods employed for this purpose. Surface modification of the CNTs includes oxidative treatment [16] and sidewall functionalization [17]. A major disadvantage of this approach is the intrinsic properties of CNTs were damaged during modification, therefore the usage of dispersant having non-covalent bonding with CNTs via either π–π interactions or electrostatic interaction between charged chemical groups may prove to be a better approach. Many dispersants such as Teflon [18], Nafion [19], chitosan [20], polyethylenimine [21] and poly(acrylic acid) have become fairly popular used for dispersing CNTs. The biocompatibility and versatility of these polymers make it possible to utilize them for solubilizing CNTs without destroying the intrinsic properties. But the treated CNTs still cannot display their function effectively. It would, therefore, be worthwhile to find an effective dispersant for CNT with all the above discussed characteristics. Poly(diallyldimethylammonium chloride) (PDDA), is a water soluble cationic polyelectrolyte, has been extensively used for the immobilization of biomolecules [22].

The present work explores to disperse SWCNTs in PDDA for developing new electrochemical transducers. There are many kinds of methods for immobilization of biomolecules such as sol–gel [23], Langmuir–Blodgett [24], avidin–biotin interaction [25], self-assembled monolayer [26], layer-by-layer (LbL) self-assembly technique [27] and so on. LbL self-assembly technique has been considered as one of the most promising immobilization methods because of its simple procedure and precise control over the film composition. Acetylcholine esterase (AchE) is one of the most extensive used enzymes and has been successfully employed for preparing biosensors for pesticide detection. Herein, AchE was chosen as a model enzyme to construct an amperometric biosensor by LbL self-assembled (PDDA–SWCNTs)/AchE multilayer films on glassy carbon electrode. The preparation of PDDA–SWCNTs aqueous dispersions, the assembly of multilayer films and the performance of amperometric biosensors were investigated. The $[(\text{PDDA–SWCNTs})/\text{AchE}]_5$ coated glassy carbon electrode developed in this work exhibited fairly high sensitivity as amperometric biosensor and has been employed for the first time for carbaryl detection in water sample(s).

2. Experimental

2.1. Materials

Acetylcholine esterase enzyme (Electric eel 686 U/mg), acetylthiocholine chloride, 5,5-dithio bis (2-nitrobenzoic acid) (DTNB) (Ellmans reagent), PDDA (MW: 100–200 kDa, 20 wt.%) and pesticide carbaryl were purchased from Sigma. SWCNTs (1–2 nm diameter, 0.5–1 μm length and >95% purity) were obtained from Chengdu Organic Chemistry Co., Ltd. (China), water was collected directly from the river at Harbin without any pre treatment. All other reagents were of analytical reagent grade and were used without further purification.

2.2. Equipments

Electrochemical measurements were carried out on an electrochemical analyzer Potentiostat–Galvanostat, Parstat 2273 Princeton Applied research with power suite software. Three-electrode system with a modified glassy carbon as working electrode (3 mm diameter), a platinum wire counter electrode (1 mm diameter) and a saturated calomel electrode (KCl) was employed. All experiments were performed in an electrochemical cell filled with 20 ml of 0.1 M PBS with 4 mM acetylthiocholine chloride at room temperature. In steady-state amperometric experiment, the potential was set at 400 mV under gentle magnetic stirring. All UV–vis spectral measurements were carried out on a Varian Cary 4000 and 5000 spectrophotometers using WinUV software. All optical spectra were recorded after immersing the modified electrode in a cuvette cell for about 5 min.

2.3. Methods

2.3.1. Dispersion of SWCNTs

3 mg of pristine SWCNTs was weighed and dispersed in 5 ml of solvent containing different concentrations of PDDA (0.1–1%) by sonication for different periods. The as-prepared dispersions were allowed to equilibrate for 24 h. Precipitates were removed by filtration using Whatman filter paper.

2.3.2. Fabrication of biosensor

PDDA is a highly positive charged species, which can adsorb on the surface of the glassy carbon electrode [28]. AchE can be used as a negatively charged material to get adsorbed on the polycationic PDDA at pH 7.4, because the isoelectric point (p*I*) of AchE lies at pH 5.6. To assemble PDDA–SWCNTs/AchE of multilayer films on glassy carbon electrodes, the cleaned electrodes were dipped in as-prepared PDDA–SWCNTs solution for 30 min, rinsed for about 15 min with PBS to remove the weakly adsorbed PDDA. Then, the PDDA–SWCNTs modified electrodes were dipped in AchE (10 U/ml) solution for 30 min, immersed in PBS for 15 min. A layer of PDDA–SWCNTs/AchE was thus formed. This procedure was repeated *n* times (*n* represents the number of layers). The multilayer films were deposited without drying in between after each deposition.

2.3.3. Estimation of thiocholine

Acetylthiocholine chloride is used as substrate and treated with the enzyme AChE. The thiocholine (Tch) produced by their interaction was then detected following Ellman's method [29]. DTNB reacts with thiocholine to give TNB, 2-nitro-5-thiobenzoic acid, a yellow-coloured product having the maximum absorption at 412 nm.

2.3.4. Determination of enzyme activity

The enzyme activity was determined by monitoring Tch estimated by the formed TNB following the absorbance at 412 nm after 5 min of reaction between Tch and DTNB. The formula used by the relation is $A = 0.133c$, where A corresponds to absorbance at 412 nm which is related to the enzyme activity $U = 0.075A$ [30]. After substituting the value of A, U comes out to be 0.2.

3. Results and discussion

3.1. Optimization of biosensing system

3.1.1. Dispersion of SWCNTs

3 mg of pristine SWCNTs is dissolved in the 5 ml of 1% PDDA solution. A black homogeneous ink like suspension is obtained. The solution remains black even after sonication for 60 min. The as prepared solution is allowed to stand for 24 h. A small amount of precipitate is seen at the bottom of test tube, which is removed by filtration using Whatman filter paper. The precipitate might contain the impurities of SWCNTs in PDDA.

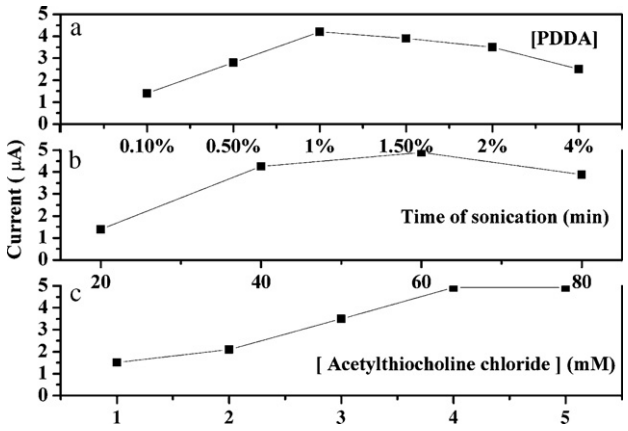

Fig. 1. (a) Effect of the concentration of PDDA on amperometric response of modified electrode. (b) Effect of sonication time on the amperometric response of modified electrode. (c) Effect of the concentration of acetylthiocholine chloride substrate on the amperometric response of modified electrode.

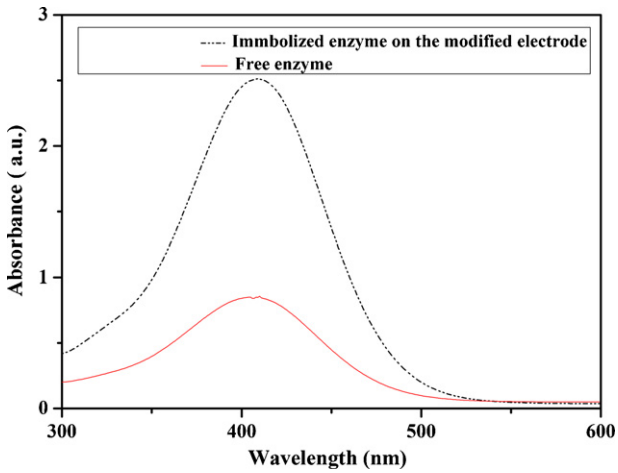

Fig. 2. Activity of the immobilized enzyme on the modified glassy carbon electrode.

3.1.2. PDDA concentration for dispersion of SWCNTs

The concentration of PDDA has a profound effect on the dispersion of SWCNTs; therefore, different concentrations of PDDA (0.1%, 0.5%, 1%, 1.5%, 2%, and 4%) were used to disperse SWCNTs. For these samples amperometric responses were recorded as a function of the concentration of PDDA, and are shown in Fig. 1a. For the modified electrode, the maximum current was obtained at a concentration corresponding to 1% of PDDA. A further increase of the concentration of PDDA resulted in a negative change of current response. Therefore, 1% PDDA was used throughout this study.

3.1.3. Sonication time

For the above optimized sample the value of current observed as a function of sonication time is given in Fig. 1b. It is seen that the value of current increases by about 3 fold as the sonication time increases from 20 min to 60 min and, thereafter, it decreases with a further increase in sonication time. A decrease in the magnitude of current with increasing sonication time might be caused by the degradation of PDDA under longer sonication treatment. Thus, the optimal sonication time of 60 min was used in this work.

3.1.4. Substrate concentration for the electrochemical measurements

Amperometric responses were recorded as a function of the concentration of acetylthiocholine chloride substrate (Fig. 1c). Current is observed to follow a complex variation with an increase in the concentration of acetylthiocholine chloride substrate. Initially, a mild increase in current is noted upto 2 mM substrate and, thereafter, a steep linear increase are depicted up to 4 mM of substrate. A further increase in concentration of substrate, however, did not bring any change in the current. For this reason, 4 mM substrate was chosen for the inhibition measurements. It is the lowest concentration of substrate, which produces maximum amount of thiocholine, and, thereby, results in the maximum amperometric response.

3.2. Activity of immobilized enzyme

The modified electrode was immersed in cuvette for 5 min and the activity of the immobilized enzyme was monitored by following the optical absorption due to thiocholine formed. Absorption spectra of the free and immobilized enzyme on the modified electrode are shown in Fig. 2.

An increase in the value of absorbance in the presence of substrate without causing any change in the absorption maximum due to enzyme, clearly suggests that the enzyme is immobilized successfully on the electrode without its denaturation.

3.3. Adsorption of SWCNTs and enzyme on the electrode

In general, the fabrication procedure is based on the adsorption of alternating layers of PDDA–SWCNTs/AchE on glassy carbon electrode, which is caused by the electrostatic attraction between the opposite charges on the top of the previously adsorbed layer and by enzyme molecules from solution. The dispersion of SWCNTs in positively charged PDDA possibly takes place due to weak supramolecular interaction between them, which may then bind electrostatically to the negatively charged AchE at pH 7.4 to this surface and is then deposited consecutively onto the surface of the electrode. By monitoring the optical absorption due to thiocholine, a gradual increase is observed in the intensity of UV/vis spectra of PDDA–SWCNTs/AchE multilayer films assembled as a function of deposition cycles (Fig. 3a), indicating the successful multi layer coating on the electrode. Growth characteristics were also followed in the absence of SWCNT along with enzyme (Fig. 3b).

Although there is an increase in the absorption in both the cases, but in the presence of SWCNTs an increase in absorption is relatively more pronounced. These data show that the absorbance increases drastically in the presence of SWCNTs at 412 nm compared to that in its absence. The increase of absorbance in the presence of SWCNTs is understood due to enhanced production of thiocholine under these conditions. It evidently indicates that the enzyme activity is significantly enhanced at the electrode in the presence of SWCNTs. The enhanced enzyme activity may be assigned to the high aspect ratio of SWCNTs which would have resulted by an increase in the working area of the electrode. But this mechanism is still needed to be explored.

3.3.1. Film assembling and characterization on quartz glass plate

The PDDA dispersed SWCNTs films were coated on the surface of quartz glass plate by using LBL approach. The UV–vis spectra of the deposited films was recorded as a function of the number of layers coated. An absorption band at 267 nm indicates the presence of SWCNT [31], indeed shows a linear variation with the number of layers coated, indicating the successful multilayer coating on the quartz glass plate. Data obtained is shown under supporting information in Fig. S1.

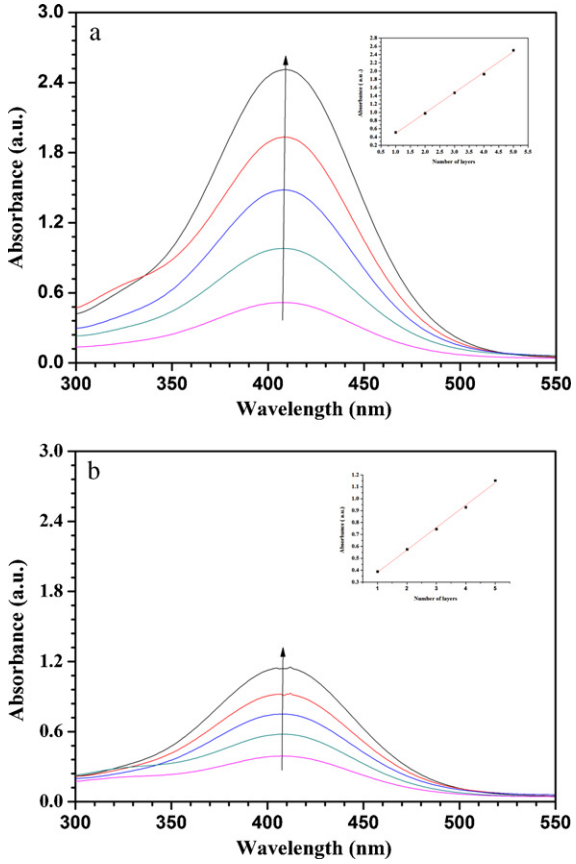

Fig. 3. UV/vis absorption spectra of modified GCE having (a) PDDA–SWCNTs/AchE multilayer. The inset shows the relationship of absorbance at 412 nm vs. number of coated bilayers. (b) PDDA/AchE multilayer. The inset shows the relationship of absorbance at 412 nm vs. number of coated bilayers.

3.4. Detection of carbaryl in aqueous medium

The above optimized electrode was employed to examine the electrochemical behaviour of thiocholine and for the detection of carbaryl.

3.4.1. Electrocatalytic activity of SWCNTs—oxidation of thiocholine

The electrochemical oxidation of thiocholine on the surface of GCE and $(PDDA/SWCNTs)_5$/GCE were investigated by cyclic voltammetry in the potential range of −0.2 to 1.0 V at a sweep rate of 100 mV s^{-1} in phosphate buffer of pH (7.4).

Typical cyclic voltammograms are shown in Fig. 4. In 5 mM thiocholine solution, anodic oxidation peaks were observed both on bare GCE and on $(PDDA/SWCNTs)_5$/GCE. It may further be noted that the anodic peak current of thiocholine on the $(PDDA/SWCNTs)_5$/GCE (I_{pa} = 55 μA) is enhanced about 1.5 times compared of that at bare GCE (I_{pa} = 35 μA) while the overvoltage of thiocholine at the $(PDDA/SWCNTs)_5$/GCE E_{pa} = 0.37 V is about a half less than that of bare GCE (E_{pa} = 0.69 V). This relatively low working potential needed for the anodic process is advantageous because it prevents the oxidation of possible interfering species existing in the samples to occur. The observed low oxidation overvoltage and high anodic oxidation peak of thiocholine at the $(PDDA/SWCNTs)_5$/GCE are attributed to the electrocatalytic activity of SWCNTs because of their edge plane-like sites at the tube ends.

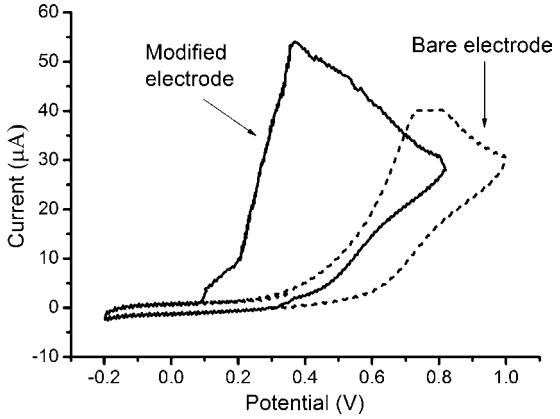

Fig. 4. Electrochemical behaviour of thiocholine (TCh) on modified GCE.

3.4.2. Determination of enzyme activity of the modified electrode

Enzyme activity of the modified electrode was determined, as described above in Section 2, to be 0.2 U.

3.4.3. Inhibition activity of carbaryl

The analyzed water samples were collected directly from river at Harbin. The pesticide (carbaryl) was determined in spiked real water samples without any pre-treatment.

Inhibition rate was determined after the 5 min immersion in different concentrations of pesticide solutions of enzymatic electrode by coating it five times with the PDDA–SWCNT/AChE bilayers. The same electrode was dipped sequentially in increasing concentration of pesticide. The decrease in peak current increased with increasing concentration of carbaryl. The observed trend in the peak current can be assigned to the binding interaction of carbaryl with the active target groups in enzyme, thus reducing the enzymatic activity to its substrate. From this variation a correlation between inhibition rate(s) and the current was worked out (Fig. 5). A formula for the calculation of inhibition rate of AChE is obtained as follows:

$$\%\,\text{Inhibition} = \left[\frac{I_0 - I_1}{I_0}\right] \times 100$$

I_0 was found to be 19.7 μA for 0.2 U activity of immobilized AChE, where I_0 and I_1 denote the current in the absence and presence of carbaryl, respectively.

Fig. 5 shows a good linearity between inhibition rates and −log[carbaryl] in a concentration range of 10^{-6} to 10^{-11} g L^{-1}.

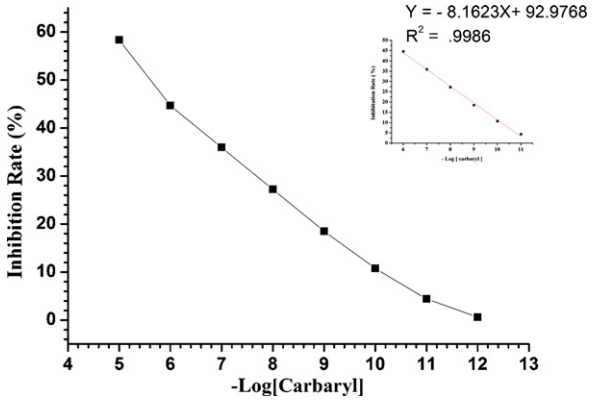

Fig. 5. Relationship between enzyme inhibition rate and −log[carbaryl]. Inset: linearity of enzyme inhibition rate and −log[carbaryl] between the concentration range of 10^{-6} to 10^{-11} g L^{-1}.

The recovery rate of carbaryl was obtained by the determination of standard sample of 10^{-10} g L^{-1}. An average recovery of 99.8 ± 2.7% was also obtained from seven times detection of 10^{-10} g L^{-1} carbaryl samples in water, which indicated that the enzymatic electrode has a good stability and specificity for the detection of pesticide(s) in water. The electrode was rinsed with water for 5 min after each measurement to remove the bonded carbaryl.

3.4.4. Detection limit

The detection limit (LD) of carbaryl was calculated by using the equation given below [32]:

$$\mathrm{LD} = \frac{3S_B}{b}$$

where S_B is the standard deviation of the blank solution and b is the slope of the analytical curve as shown in the inset of Fig. 5. The above graph shows a good inhibition rate with the detection limit of 10^{-12} g L^{-1} carbaryl in water.

4. Conclusions

The [(PDDA–SWCNTs)/AchE]$_5$/GCE shows excellent electrocatalytic activity towards the carbaryl pesticides in water, which is assigned to the higher conductivity and surface area of the SWCNTs coupled with advantages of the utilization of LbL assembly technique. It was found that the modified GCE exhibits an excellent detection limit in a wide linear range with good precision and operational stability. The fabrication of amperometric biosensor by LbL, based on adsorption, drastically increases the sensitivity for the pesticides estimation in relatively much lesser time. This biosensor might have great prospect for automatic monitoring of carbaryl pesticide(s) in river water. The fabrication method described in this work can also be used to immobilize other enzymes to construct a range of biosensors.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.09.028.

References

[1] FAO, Agriculture towards 2010, C 93/94 document of 27th SESSION of the FAO conference, Rome, 1993.
[2] Standard Methods for Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, 1998, 6/85–6/90.
[3] I. Liska, J. Slobodnik, J. Chromatogr. A 733 (1996) 235–258.
[4] G.Z. Tsogas, D.L. Giokas, P.G. Nikolakopoulos, A.G. Vlessidis, N.P. Evmiridi's, Anal. Chim. Acta 573 (2006) 354–359.
[5] M. Del Olmo, J. Laserna, D. Romero, J. Rohand, J.L. Vilchez, Talanta 44 (1997) 443–449.
[6] A. Tuncelli, H. Bag, A.R. Turker, J. Anal. Chem. 371 (2001) 1134–1138.
[7] Y. Daghbouche, S. Garrigues, M. Guardi, Anal. Chim. Acta 314 (1995) 203–212.
[8] J.A. Murillo Pulgar, A.A. Molina, P. Fernandez López, Talanta 68 (2006) 586–593.
[9] L.F. Capit'an-Valley, M.K.A. Deheidel, R. Avivad, J. Anal. Chem. 362 (1998) 307–312.
[10] M.C. Pietrogrande, G. Blo, C. Bighi, J. Chromatogr. 349 (1985) 63–68.
[11] B.D. McGarvey, J. Chromatogr. 642 (1993) 89–105.
[12] A. De Kok, M. Hiemstra, J. Assoc. Anal. Chem. 75 (1992) 1063–1072.
[13] W.H. Newsome, B.P.Y. Lau, D. Ducharme, D. Lewis, J. Assoc. Anal. Chem. 78 (1995) 1312–1321.
[14] S. Iijima, Nature 354 (1991) 56–58.
[15] L.Q. Rong, C.Y.Q.Y. Qian, X.H. Xia, Talanta 72 (2007) 819–824.
[16] G. Liu, Y. Lin, Anal. Chem. 78 (2006) 835–843.
[17] H. Kong, P. Luo, C. Gaoa, D. Yan, Polymer 46 (2005) 2472–2485.
[18] J. Manso, M.L. Mena, P.Y. Sedeno, J. Pingarron, J. Electroanal. Chem. 603 (2007) 1–7.
[19] S. Shahrokhian, H.R. Zare-Mehrjardi, Electrochim. Acta 52 (2007) 6310–6317.
[20] J. Tkac, J.W. Whittaker, T. Ruzgas, Biosens. Bioelectron. 22 (2007) 1820–1824.
[21] M.D. Rubianes, G.A. Rivas, Electrochem. Commun. 9 (2007) 480–484.
[22] X. Chen, X. Yan, K.A. Khor, B.K. Tay, Biosens. Bioelectron. 22 (2007) 3256–3260.
[23] T. Li, Z. Yao, L. Ding, Sens. Actuators B 101 (2004) 155–160.
[24] Y. Hou, N. Jaffrezic-Renault, A. Zhang, J. Wan, A. Errachid, J.-M. Chovelon, Sens. Actuators B 86 (2002) 143–149.
[25] N. Anicet, A. Anne, J. Moiroux, J.M. Saveant, J. Am. Chem. Soc. 120 (1998) 7115–7116.
[26] A. Ottova, V. Tvarozek, J. Racek, J. Sabo, W. Ziegler, T. Hianik, H.T. Tien, Supramol. Sci. 4 (1997) 101–112.
[27] B.Y. Wu, S.H. Hou, F. Yin, Z.X. Zhao, Y.Y. Wang, X.S. Wang, Q. Chen, Biosens. Bioelectron. 22 (2007) 2854–2860.
[28] D. Li, H. Wang, J. Zhu, X. Wang, L. Lu, X. Yang, J. Mater. Sci. 22 (2003) 253–255.
[29] G.L. Ellman, Arch. Biochem. Biophys. 82 (1959) 70.
[30] G. Liu, Y. Lin, Electrochem. Commun. 8 (2006) 251.
[31] M.N. Zhang, Y.M. Yan, K.P. Gong, L.Q. Mao, Z.X. Guo, Y. Chen, Langmuir 20 (2004) 8781–8785.
[32] I. Krull, M. Swartz, LC–GC 16 (1998) 922–924.