Electrodeposition of taurine on gold surface and electro-oxidation of malondialdehyde

M. Zamani-Kalajahi[1], M. Hasanzadeh*[2], N. Shadjou[3,4], M. Khoubnasabjafari[5], K. Ansarin[5], V. Jouyban-Gharamaleki[6] and A. Jouyban[2]

In this study, polytaurine film was formed on a gold surface using repetitive cyclic voltammetry (CV) from taurine and phosphate buffer solution (PBS) and used as an electrochemical nanobiosensor for determination of malondialdehyde (MDA). The electrochemical behaviour of polytaurine modified gold (PT/Au) electrode was studied using CV technique. The Electroanalytical behaviour of MDA was investigated at the PT/Au electrode in (PBS), using differential pulse voltammetry. Finally, the applicability of the method to direct assays of human serum and exhaled breath condensate (EBC) is described, and the results showed that this polymeric sensor is suitable for detecting total serum MDA levels of healthy people because the normal level of total MDA in serum is in the range of 0·78–3·10 mM. The results show that by using the proposed sensor, MDA can be determined with detection limits of 34 and 0·995 nM in human serum and EBC samples, respectively.

Keywords: Electrodeposition, Polytaurine, Nanosensor, Nanopolymer, Malondialdehyde

This paper is part of a special issue on electrodeposition

Introduction

Lipid peroxidation is a prominent manifestation of free radical activity in biological systems, and it is involved in the development of different diseases such as cancer or cardiovascular and liver diseases.[1] One particular breakdown product and an important biomarker of lipid peroxidation is malondialdehyde (MDA), which is an endogenous byproduct of prostaglandin and leukotriene biosynthesis.[2] Malondialdehyde constitutes both a biomarker of lipid oxidation and a potential concurrent cause of initiation of some serious diseases such as breast or lung, cervical, gastric and skin cancers.[1–3] Moreover, levels of MDA in living organisms have been found to be significantly modified in many pathological situations (e.g. gastric, lung or breast cancers and atherosclerotic or cardiovascular diseases).[4]

The significance of determining MDA in human plasma or serum can be considered from many aspects. Malondialdehyde is globally recognised as a scientifically accepted biomarker of oxidative stress. Another significant reason for the determination of MDA is its toxicity toward the cardiovascular system. MDA action on lipoproteins has been related to atherogenesis, and, probably, its reactivity toward collagen is responsible for the stiffening of the cardiovascular tissue. Further studies are needed to determine if the presence of this molecule at certain levels may predict the insurgence of vascular pathologies.[5]

Different analytical techniques have been employed for the determination of MDA in biological samples. These methods include fluorescence[6] high performance liquid chromatography with diode array detection,[7] gas chromatography with mass spectroscopy,[8] surface enhanced Raman spectra,[9] electron capture detection,[10] capillary electrophoresis[11] and electrochemical biosensing method.[12]

These analytical techniques can be divided into three categories; the most widely used detection methods are based on colorimetric sensing of the reaction products of MDA with thiobarbituric acid (TBA).[13] The drawback of this method is that TBA reacts not only with MDA but also with many other chemical species (e.g. amino acids, nucleic acids, proteins and carbohydrates),[14] and this causes interference in the MDA-TBA assay. Furthermore, the treatment of biological samples is usually carried out at high temperatures (~100°C) and in acidic media; this may result in further oxidation of the matrix and, therefore, variability in the estimation of MDA levels. To overcome the shortcomings of the TBA assay, other MDA derivatising methods have been used, which could be carried out in milder conditions. In this category, methods of detection are based on non-TBA derivatisation. This includes derivatisation with 1,3-diethyl-2-thiobarbituric acid,[15] diaminonaphthalene,[16] 2,4-dinitrophenylhydrazine[17] and pentafluorophenylhydrazine.[18] However, the techniques were developed and

[1]Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[2]Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[3]Department of Chemistry, Faculty of Chemistry, Urmia University, Urmia, Iran
[4]Department of Nanochemistry, Nano Technology Center, Urmia University, Urmia, Iran
[5]Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[6]Department of Mechatronic Engineering, International Campus, University of Tabriz, Tabriz, Iran

*Corresponding author, email mhmmd_hasanzadeh@yahoo.com

Published by Maney on behalf of the Institute
Received 19 March 2014; accepted 11 August 2014
DOI 10.1179/1743294414Y.0000000349

validated for the measurement of MDA in the field of pathology. The application of these methods has been limited because they are too expensive, are time consuming or need extraction procedures.[19] Many chromatographic methods provide high sensitivity and specificity, although they require the use of extensive extraction procedures, specialised staff, column cleaning or column regeneration as they lose efficiency and resolution with time and require expensive equipment and sample preparation, especially when biological samples are used.[20]

Owing to the significance of MDA as a marker of lipid peroxidation and because of its elevations in various diseases, the assessment of MDA with new and reliable assays, the development of a fast, sensitive and selective method to detect MDA with simple instruments is necessary. In the present work, an electrochemical method that enables the accurate, precise and selective detection of MDA in human serum and exhaled breath condensate (EBC) samples is developed. This method is a suitable assay for MDA detection and offers the advantages in that it is a rapid, valid, sensitive, low operation cost and easy to use method. It is also suitable for routine analysis of MDA in biological samples without further sample pretreatment that enables direct use of small amounts of sample without additional substances, such as TBA, and without any need of an extraction stage. These characteristics of the proposed method are more important, when routine analysis of MDA in clinical chemistry laboratories is considered.

The literature survey reveals that there is one report on the electroanalytical determination of MDA.[12] In the present work, we used polytaurine as a new polymer film for electro-oxidation and determination of MDA. The purpose of this work is to develop a sensitive, simple and rapid method for the determination of MDA in human serum and EBC samples. The aim of this study is to establish suitable experimental conditions, to investigate the voltammetric behaviour and oxidation mechanism of MDA by cyclic and differential pulse voltammetric (DPV) methods. To the best of our knowledge, this is the first report of the determination of MDA based on its direct electrochemical oxidation on polymer films. In addition, this work is the first statement on the determination of MDA in EBC samples using electrochemical methods.

Experimental

Chemicals and solutions

All chemicals used in this work were of analytical reagent grade. 1,1,3,3-Tetramethoxypropane was purchased from Merck (Hohenbrunn, Germany). Taurine was obtained from Sigma-Aldrich. Additional dilute solutions were prepared daily by accurate dilution just before use. MDA (produced from *in situ* hydrolysis of 1,1,3,3-tetramethoxypropane) solutions were stable, and their concentrations did not change with time. Acteonitrile, dipotassium phosphate and monopotassium phosphate were obtained from Scharlau. Deionised water was purchased from Ghazi Pharmaceutical Company (Tabriz, Iran). Stock standard solution of MDA (1000 μM) was prepared by dissolving an appropriate mass of 1,1,3,3-tetramethoxypropane in phosphate buffer solution (PBS) buffer (pH 6·8), and the daily standard working solutions of different concentrations were prepared by diluting the stock solution with buffer and were freshly used.

Preparation of serum and EBC samples

Human serum samples were obtained from the Iranian Blood Transfusion Research Center (Tabriz, Iran), and aliquots were transferred into polypropylene microtubes and frozen at −4°C until analysis. Serum samples that were frozen at −4°C were thawed at room temperature on the day of analysing and were vortexed to ensure homogeneity. Subsequently, 500 μL human serum acteonitrile was added with a 1 : 2 ratio and vortexed for 20 s. After this, it was centrifuged for 10 min at 6000 rev min^{-1}. Thus, the plasma proteins were precipitated; 1·0 mL of clear supernatant solution was transferred to a 10·0 mL volumetric flask; and an MDA standard solution of desired concentration was added up to the mark line.

EBC samples were obtained from healthy volunteers using a lab made set-up based on a cooling trap system patented in the national patent office. The set-up cools the exhaled breath to −25°C and condenses the EBC with acceptable efficiency. EBC sample (1·0 mL) collected from a healthy volunteer was transferred to a 10·0 mL volumetric flask, a standard solution of desired concentration was added up to the mark line, and electrochemical analysis was performed.

Instrumentation

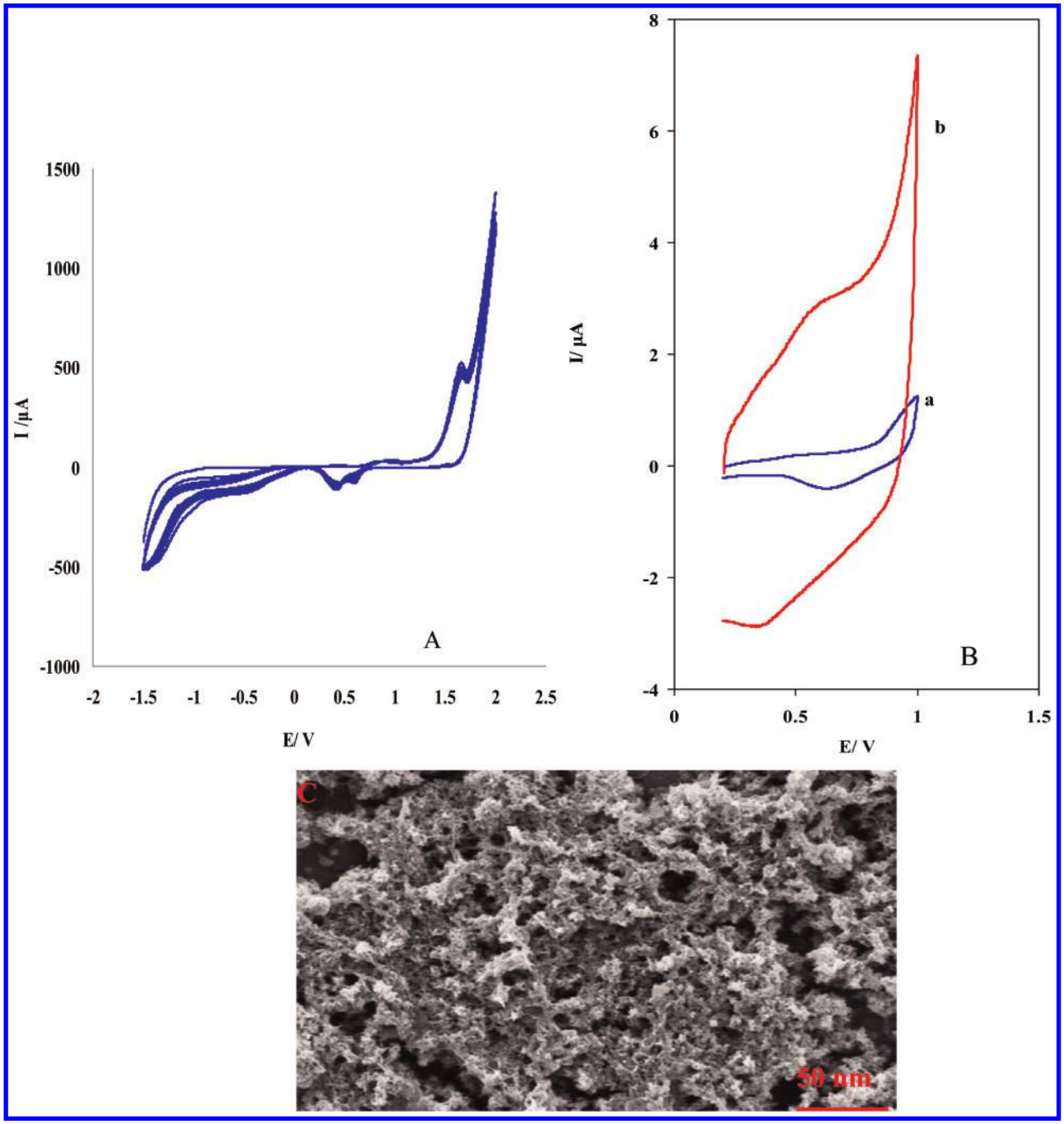
Electrochemical experiments were performed with a computer controlled AUTOLAB system with PGSTAT 302N (Eco Chemie, Utrecht, The Netherlands), driven with NOVA 1·7 software. A conventional three-electrode cell was used with an Ag/AgCl (Methrom, The Netherlands) as a reference electrode, and a Pt wire was used as a counterelectrode. The surface morphology of the modified electrodes was evaluated with a Vega-Tescan electron microscope (SEM, Hitachi Ltd, Tokyo, Japan).

Au electrode (2 mm in diameter) was polished to a mirror-like finish with 0·3 and 0·05 μm alumina slurry (Beuhler, USA) followed by rinsing thoroughly with double distilled water. Then, it was sonicated in acetone and double distilled water, and allowed to dry at room temperature. The results show that considerable amounts of PT with an average size of 50 nm were formed on the surface of Au electrode.

Results and discussion

Electrochemical behaviour

Cyclic voltammetry (CV) was used for the polymerisation of PT film on Au surface. Figure 1*a* presents 15 consecutive CVs of the Au electrode in the presence of 0·1M taurine containing 0·1M PBS in the range from −1·5 to 2·0 V with a scan rate of 100 mV s^{-1}. The CVs are similar to those reported in the literature.[21] The voltammograms of MDA at a bare Au and polytaurine modified gold (PT/Au) electrodes in PBS (pH 6·8) were shown in Fig. 1*b*. As seen in Fig. 1*b* (curve a), any oxidation signal was not exhibited in the bare Au electrode. This indicated the electroinactivity of MDA on the Au surface. Typical CVs of PT/Au electrode in 0·1M PBS are shown in Fig. 1*b* (curve b), where a potential sweep rate of 100 mV s^{-1} has been employed. One oxidation peak for MDA at the PT/Au surface can be observed. This observation is attributed to the high conductivity and inherent ability of PT. These results



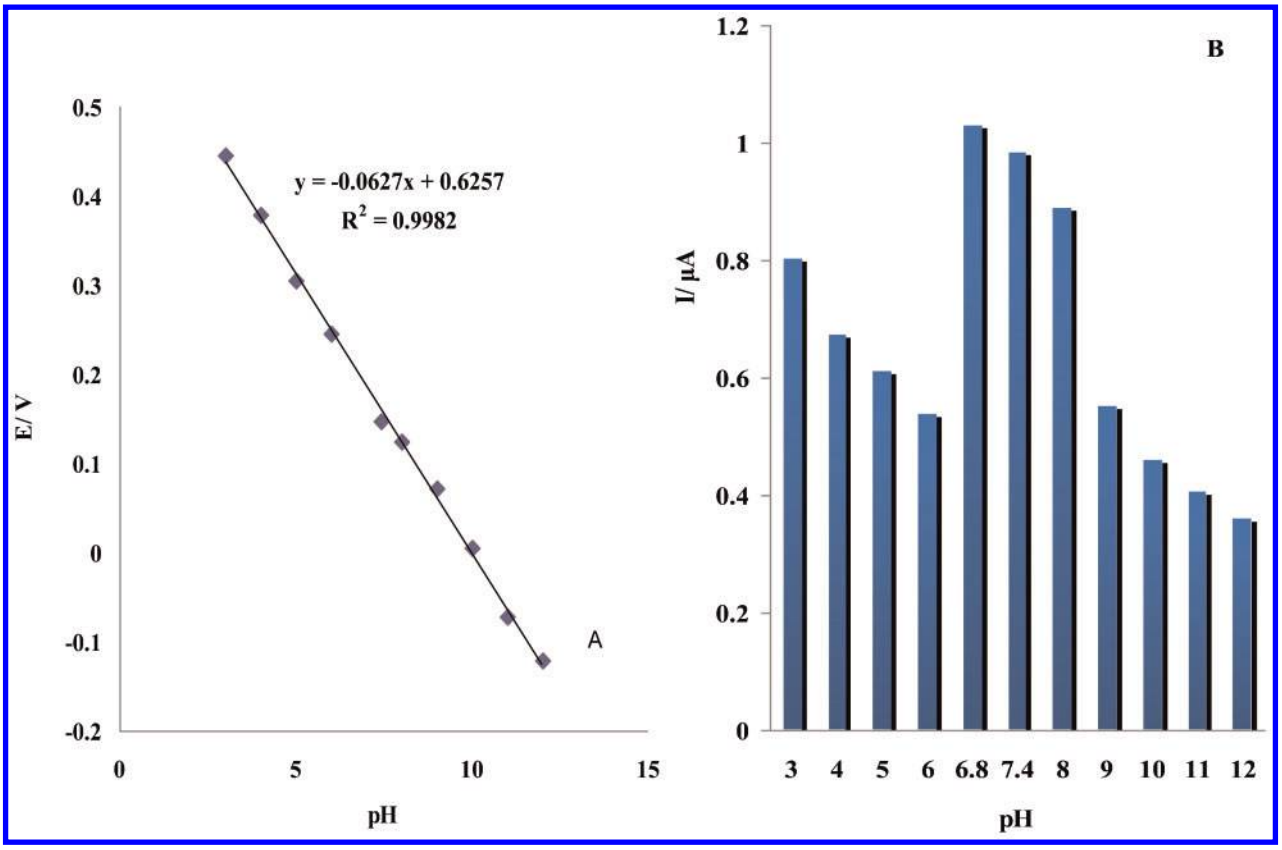
1 ***a*** **cyclic voltammetries obtained for 0·1M taurine containing 0·1M PBS using Au electrode; potential continuously between −1·5 and 2 V at 100 mV s^{-1}; *b* CV obtained with Au electrode (curve a) and PT/Au electrode (curve b) in 0·1M PBS; And *c* SEM images of surface of Au electrode after modification by PT film**

indicate that the PT film could accelerate the rate of electron transfer of MDA and has good electroactivity for a redox reaction of MDA. To investigate the surface morphology of the PT film formed on the Au surface, it was examined by scanning electron microscopy. Figure 1*c* shows the SEM images obtained for PT/Au electrodes. Figure 1*c* shows that considerable amounts of PT with an average size of 50 nm were formed on the surface of Au electrode. The SEM for PT/Au shows spongy morphology of PT, which can be helpful in the entrapment of MDA.

The effect of pH on the CV response of 1·0 mM MDA at the PT/Au electrode was studied by applying different buffer solutions ranging from pH 3·0 to 12·0 (Fig. 2). As can be observed, the electrochemical behaviour of MDA depends on the pH value of the aqueous solution. The anodic peak current of MDA is slightly increased when there is an increase in the pH solution until it reaches 6·80. Therefore, the optimum solution of pH selected was 4·00. On the other hand, the anodic peak of MDA shifted toward negative potentials with an increase in pH. These results indicated that the protons have taken part in their electrode reaction processes.

$$E_{p(MDA)}(Ag/AgCl) = -0{\cdot}0627pH + 0{\cdot}6257 \quad (1)$$

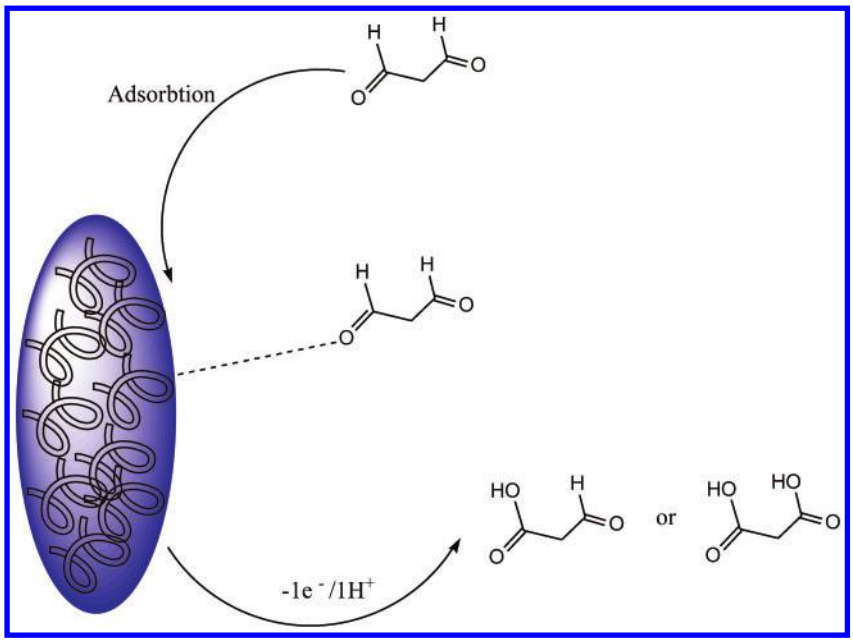
The slope of the plot of $E_{p(Ag/AgCl)}$(pH) was approximately −0·0627 V (R^2=0·9982). It was, therefore, deduced that both peaks correspond to irreversible processes involving one electron and one proton.



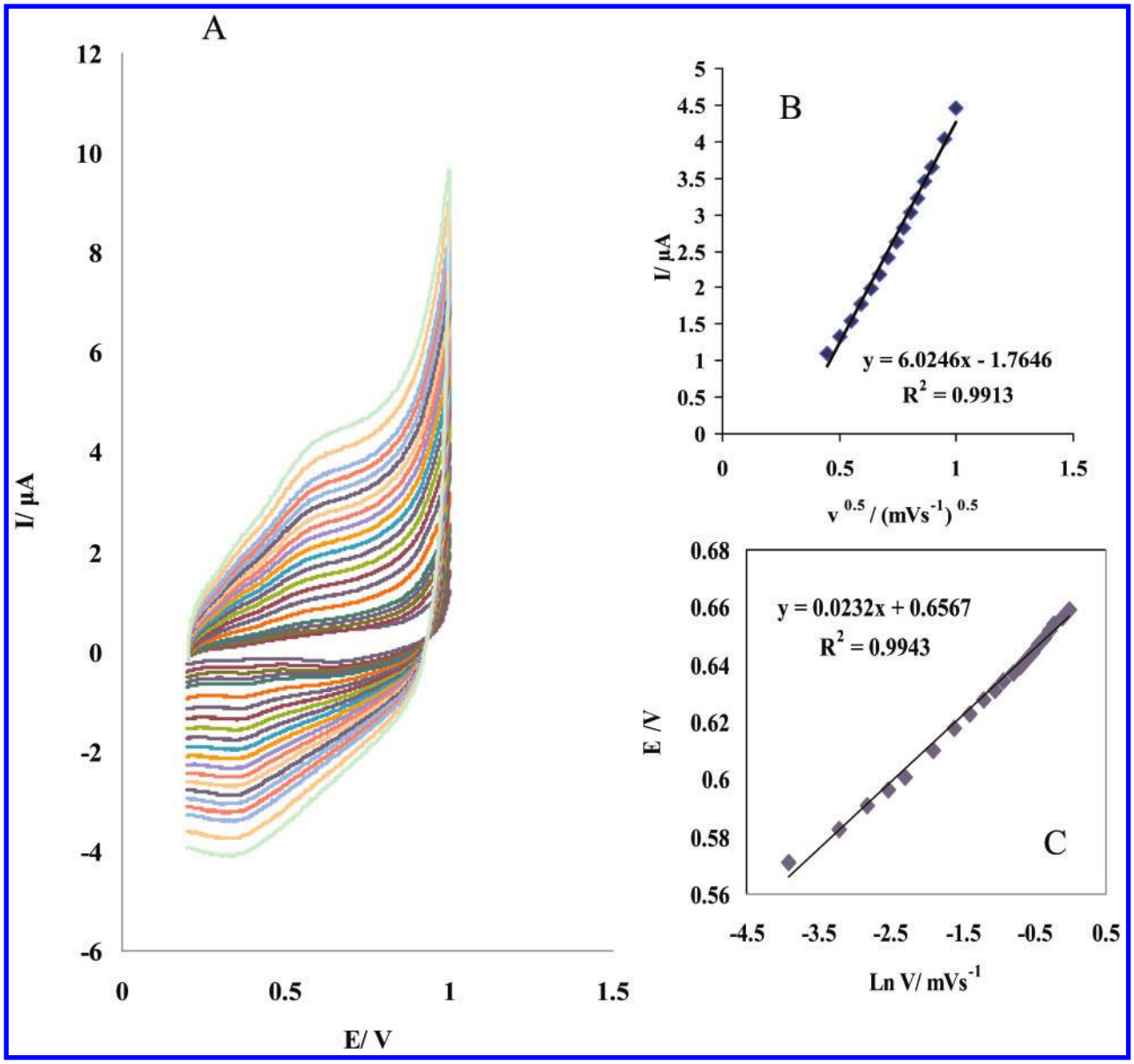
2 ***a*** **effect of pH on peak potential and** ***b*** **effect of pH on peak current**

This conclusion is in accordance with the known electrochemical reactions of MDA as shown in another investigation.[12] It has been known that MDA can be oxidised via one-electron and one-proton processes as shown in Fig. 3.

One of the most important applications of CV is for a qualitative diagnosis of chemical reactions in redox processes.[22,23] The occurrence of such chemical reactions, which directly affect the available surface concentration of the electroactive species, is common to redox processes of many biomarkers. Cyclic voltammetry studies of MDA oxidation on PT/Au electrode demonstrate the importance of this method as a tool for investigating the mechanism of MDA oxidation. From these results, electrocatalytic behaviour is observed for MDA oxidation at the surface of the PT/Au electrode via an EC ì catalytic mechanism. This mechanism is shown in Fig. 4. In this scheme, MDA is oxidised in the catalytic chemical reaction C_i by the oxidised form of PT that is produced via an electrochemical reaction *E*.



3 Proposed oxidation mechanism for MDA



4 ***a*** **cyclic voltametries of PT/Au electrode in 0·1M PBS in presence of 0·1 mM MDA in various potential sweep rates of 10, 20, 30, 40, 50, 70, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 and 700 mV s^{-1};** ***b*** **dependence of anodic peak currents during forward sweep versus square roots of potential scan rate;** ***c*** **dependence of anodic peak currents during forward sweep versus sweep rate; and** ***d*** **obtained from data of** ***a***

Therefore, when the PT is oxidised at the potential of 400 mV, the MDA can be oxidised as well at this potential.

Figure 4*a* illustrates typical CVs of the PT/Au electrode in 0·1M PBS + 0·1 mM MDA recorded at different potential sweep rates. As shown in Fig. 4*b*, the anodic peak currents increased with the square root of the potential sweep rate as a linear relationship. This observation revealed that the process of MDA oxidation via diffusion is controlled by a mass transfer phenomenon. The electron transfer coefficient of the reaction could be calculated using[24]

$$E_p = \left(\frac{RT}{2\alpha F}\right)\ln v + \text{constant} \qquad (2)$$

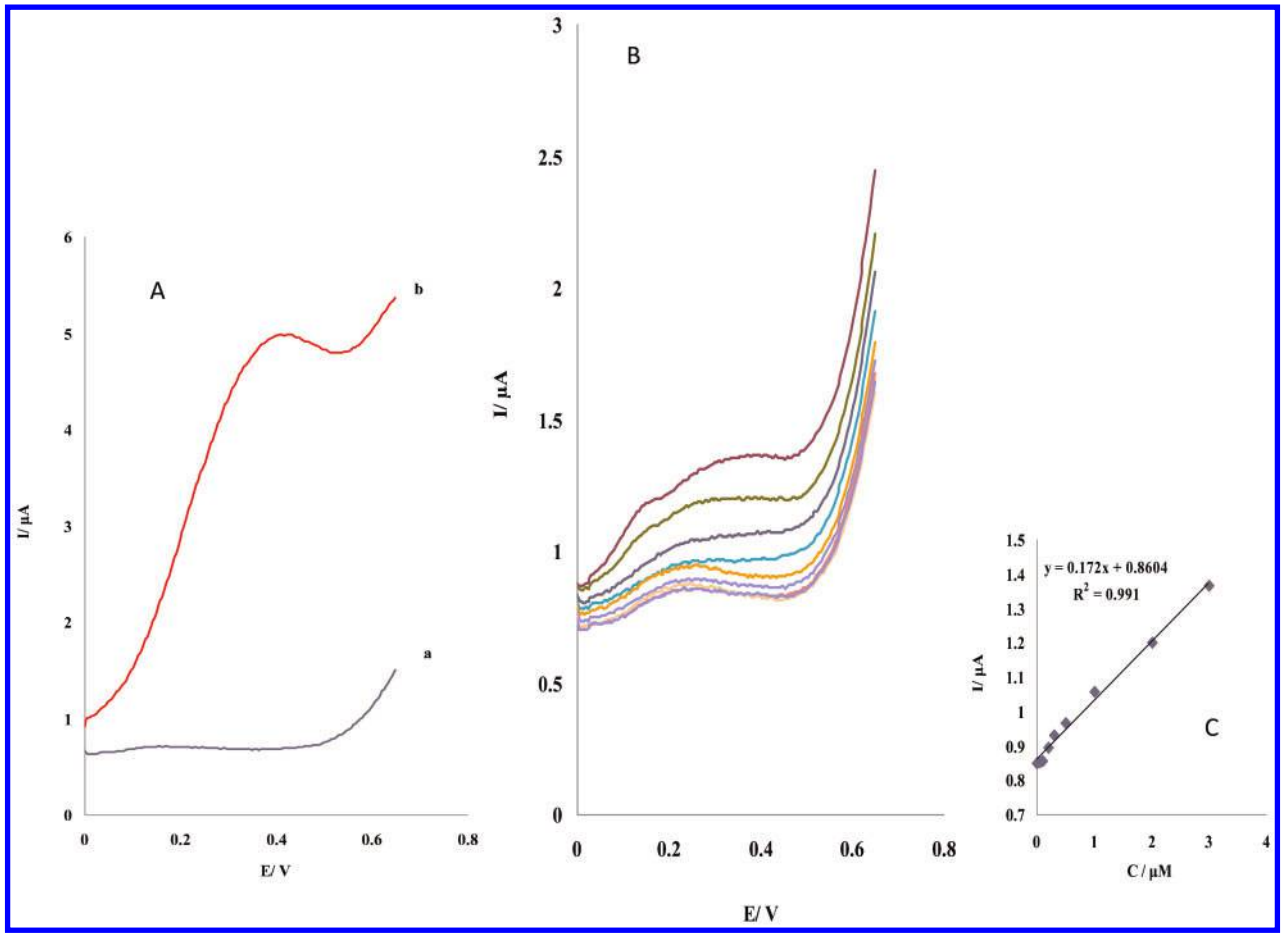
According to dependence of anodic peak potential on the Neperian logarithm of the potential sweep rate (as illustrated in Fig. 4*c*), the electron transfer coefficient obtained was 0·44.

On the basis of the slopes of the linear dependency of the anodic peak currents on the square root of the potential sweep rates (Fig. 4*b*), and the Randles–Sevcik equation[25]

$$I = \left(2{\cdot}99 \times 10^5\right)\alpha^{1/2} n^{3/2} A C^* D^{1/2} v^{1/2} \qquad (3)$$

where I_p is the peak current, A is the electrode surface area, D is the diffusion coefficient and C^* is the bulk concentration of MDA; the diffusion coefficient D for MDA was calculated to be $1{\cdot}24 \times 10^{-6}$ cm^2 s^{-1}.

Differential pulse voltammetrics of PT/Au electrode in the absence (curve a) and presence (curve b) of MDA in the 0·1 mM PBS with the potential sweep rate of 10 mV s^{-1} were obtained (*see* Fig. 5*a*). A comparison of the recorded DPVs in the absence and presence of MDA revealed that the anodic peak current on the PT/Au electrode is significantly enhanced, whereas the cathodic peak current is significantly decreased. These results indicate that PT could accelerate the rate of electron



5 ***a*** **differential pulse voltammetrics of PT/Au electrode in absence (a) and presence (b) of MDA,** ***b*** **DPVs recorded for determination of MDA in different concentrations (0·005, 0·05, 0·1, 0·2, 0·3, 0·5, 1, 2 and 3 μM) and** ***c*** **related calibration graph for MDA**

transfer of MDA and have good electroactivity activity for oxidation of MDA.

Analytical applications

In order to develop a voltammetric method for the determination of MDA, we selected the DPV mode, because the peaks are sharper and better defined at a lower concentration of MDA than those obtained by CV, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of MDA. The PBS was selected as the supporting electrolyte for the quantification of MDA, as it gave a maximum peak current. The peak at ~0·4 V was considered, as the analysis DPVs obtained with increasing amounts of MDA showed that the peak current increased linearly with an increasing concentration, as shown in Fig. 5*b*. Using the optimum conditions described earlier, a linear calibration plot was obtained for 0·02–3·00 μM MDA with a slope of 0·038 μA μM^{-1}. The linear regression equation was I_p (μA)=0·172C_{MDA} (μM)+0·86, with a correlation coefficient of 0·991 and a sensitivity of 0·172 μA μM^{-1}. The detection limit (LOD) and quantification limit (LOQ) were estimated to be 0·034 μM and 0·116 mM respectively. In addition, the characteristics of the proposed sensor for MDA determination in human serum and EBC are summarised in Table 1. The results demonstrate that the new sensor features a lower LOD as compared with the other ones.[12] Moreover, the sensor has a wide linear range for MDA determination, which can be used to readily detect MDA within the range of its physiological levels.

Precision and accuracy were assessed by performing replicate analyses of MDA samples. The precision of the method is calculated as the relative standard deviation (RSD). The procedure was repeated on the same day and the same solutions at concentrations in the range of the standard series. The intra-assay RSDs of the proposed method were determined on the basis of peak current for five replications. The accuracy of the proposed method was determined by spiked serum samples with different concentrations of MDA (Table 2).

Table 1 Validation data of proposed method for quantification of MDA in human serum and EBC

EBC/nM	Serum/μM	Parameters
1–100	0·005–3	Linear range
0·033	0·172	Slope
0·861	0·060	Intercept
0·990	0·991	Correlation coefficient
8	9	Number of data points
0·995	0·034	LOD
3·319	0·116	LOQ

Table 2 Assay precision and accuracy

Nominal concentration ($n=5$)/μM	Intra-assay precision ($n=5$)/RSD%	Inter-assay precision ($n=5$)/RSD%	Accuracy*/RE%
0·05	15·51	17·92	0·016
0·3	13·10	14·94	−0·199
2	16·70	15·83	0·045

*RE: relative error.

The high sensitivity of the proposed method and polytaurine enables the determination of MDA in spiked human serum samples. During the first electroanalytical determination of this biomarker, serum samples were not diluted with PBS that related to a specification of the sensor with the SH group for MDA with a similar functional group. The recovery of the analytes was measured by a spiked biomarker in undiluted serum samples (Table 3). The DPVs were recorded after the serum was spiked with various amounts of the MDA within the working concentration range. Recoveries were found to lie in the range of 98·35–119·96%. Good recoveries of MDA were obtained from this method; they show that application of the proposed biosensor to the analysis of MDA in biological fluids could be easily assessed.

Obtained results indicated that this biosensor is an appropriate platform for the determination of MDA. The prepared electrode shows voltammetric responses with a low LOD and a wide linear range for MDA in optimal conditions, which makes it suitable for the determination of this biomarker. It is observed that, by polymerisation of taurine on Au surface, a novel method for developing an efficient and robust electrochemical sensing platform was established. The electrochemical sensor showed high sensitivity and simplicity for the detection of MDA.

It is important to point out that this polymeric sensor is suitable for detecting total serum MDA levels of both control (healthy) and case (patients) samples, because the normal level of total MDA in serum is in the range from 0·78 to 3·1 mM.[26,27]

Rapid, non-invasive analyses of EBC samples could be routinely performed in clinical laboratories with minimal stress to patients. Hence, monitoring of EBC can be applied to MDA with the aid of electrochemical methods. However, the difference between EBC and serum MDA levels is barely significant, and the normal level of total MDA in EBC is in the range from 0·83 to 3·1 mM. Therefore, the applicability of the proposed method for the determination of MDA in EBC was examined by measuring the peak current as functions of the bulk concentration of the biomarker in EBC samples using the PT/Au electrode. Using the proposed sensor, a linear calibration plot was obtained for 0·005–3 nM MDA in EBC samples with a slope of 0·033 μA μM^{-1}. The linear regression equation was I_p (μA)=0·033C_{MDA} (μM)+0·861, with a correlation coefficient of 0·990. A LOD of 0·995 nM and a LOQ of 3·31 nM were calculated according to 3 and 10 sb m^{-1} criteria respectively.

Since the EBC of healthy people is not free from MDA, in order to determine its MDA concentration, a standard addition method was used to obtain the intercept, and through this, the concentration of MDA in the EBC of healthy people is calculated. For this purpose, in a healthy EBC sample, three concentrations of MDA were applied by the standard addition method and obtained results are given in Table 4.

Table 3 Recoveries for analysis of MDA in spiked serum samples

Nominal concentration ($n=5$)/μM	Found concentration ($\pm\sigma$; $n=5$)/μM	Recovery/%
0·05	0·049 (±17·63)	98·35
0·3	0·36 (±17·92)	119·96
2	1·91 (±13·52)	95·48

Table 4 Obtained results for detection of MDA in EBC sample standard addition method

Concentration/nM	Signal *I* (μA)
50	13·17
15	12·23
10	12·05
Intercept (MDA concentration in EBC of healthy person)	11·79 nM

Conclusion

1. Polytaurine film was deposited electrochemically on the gold surface and tested for the electro-oxidation of MDA in aqueous solution.

2. The modified electrode was shown to be promising for MDA detection with many desirable properties, including high sensitivity and fast response time. The CV behaviour reveals that the mechanism in MDA electro-oxidation varies with pH in the same regions.

3. The results show that this nanopolymeric sensor is suitable for detecting MDA levels of both serum and EBC samples.

4. This work opens new horizons on the design of new nanopolymeric sensors for the detection of some disease biomarkers.

5. The proposed method is simple, precise, accurate and inexpensive with regard to reagent consumption and equipment involved.

6. Further developments are required to enhance the specificity of the nanopolymeric materials and to decrease the LOD.

Acknowledgement

The authors gratefully acknowledge the support of this work by the Drug Applied Research Center Tabriz University of Medical Sciences.

References

1. R. P. Wu, T. Hayashi, H. B. Cottam, G. Y. Jin, S. Y. Yao, C. C. N. Wu, M. D. Rosenbach, M. Corr, R. B. Schwab and D. A. Carson:

'Nrf2 responses and the therapeutic selectivity of electrophilic compounds in chronic lymphocytic leukemia', *Proc. Natl Acad. Sci. U.S.A.*, 2010, **107**, 7479–7484.
2. L. L. Yang, C. Latchoumycandane, M. R. McMullen, B. T. Pratt, R. L. Zhang, B. G. Papouchado, L. E. Nagy, A. E. Feldstein and T. M. McIntyre: 'Chronic Alcohol Exposure Increases Circulating Bioactive Oxidized Phospholipids', *J. Biol. Chem.*, 2010, **285**, 22211–22220.
3. J. F. Chao, Y. Leung, M. F. Wang and R. C. C. Chang: 'Nutraceuticals and their preventive or potential therapeutic value in Parkinson's disease', *Nutr. Rev.*, 2012, **70**, 373–386.
4. D. Del Rio, A. J. Steward and N. Pellegrini: 'A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress', *Nutr. Metab. Cardiovasc. Dis.*, 2005, **15**, 316–328.
5. M. Czauderna, J. Kowalczyk and M. Marounek: 'The simple and sensitive measurement of malondialdehyde in selected specimens of biological origin and some feed by reversed phase high performance liquid chromatography', *J. Chromatogr. B*, 2011, **879B**, 2251–2258.
6. J. C. Cooley and C. E. Lunte: 'Detection of malondialdehyde in vivo using microdialysis sampling with CE-fluorescence', *Electrophoresis*, 2011, **32**, 2994–2999.
7. M. Czauderna, J. Kowalczyk and M. Marounek: 'The simple and sensitive measurement of malondialdehyde in selected specimens of biological origin and some feed by reversed phase high performance liquid chromatography', *J. Chromatogr. B*, 2011, **879B**, 2251–2258.
8. J. L. Chen, Y. J. Huang, C. H. Pan, C. W. Hu and M. R. Chao: 'Determination of urinary malondialdehyde by isotope dilution LC-MS/MS with automated solid-phase extraction: a cautionary note on derivatization optimization', *Free Radic. Biol. Med.*, 2011, **51**, 1823–1829.
9. D. M. Zhang, R. Haputhanthri, S. M. Ansar, K. Vangala, H. I. De Silva, A. Sygula, S. Saebo and C. U. Pittman, Jr: 'Ultrasensitive detection of malondialdehyde with surface-enhanced Raman spectroscopy', *Anal. Bioanal. Chem.*, 2010, **398**, 3193–3201.
10. C. D. Stalikas and C. N. Konidari: 'Analysis of malondialdehyde in biological matrices by capillary gas chromatography with electron-capture detection and mass spectrometry', *Anal. Biochem.*, 2001, **290**, 108–115.
11. A. Zinellu, S. Sotgia, L. Deiana and C. Carru: 'Field-amplified online sample stacking capillary electrophoresis UV detection for plasma malondialdehyde measurement', *Electrophoresis*, 2011, **32**, 1893–1897.
12. L. Yuan, Y. Lan, M. Han, J. Bao and W. T. Z. Dai: 'Label-free and facile electrochemical biosensing using carbon nanotubes for malondialdehyde detection', *Analyst*, 2013, **138**, 3131–3134.
13. G. W. Cheng, H. L. Wu and Y. L. Huang: 'Simultaneous determination of malondialdehyde and ofloxacin in plasma using an isocratic high-performance liquid chromatography/fluorescence detection system', *Anal. Chim. Acta*, 2008, **616**, 230–234.
14. D. R. Janero: 'Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury', *Free Radic. Biol. Med.*, 1990, **9**, 515–540.
15. T. Sakai, A. Habiro and S. Kawahara: 'High-performance liquid chromatographic determination of 1,3-diethyl-2-thiobarbituric acid–malonaldehyde adduct in fish meat', *J. Chromatogr. B*, 1999, **726B**, 313–316.
16. J. P. Steghens, A. L. van Kappel, I. Denis and C. Collombel: 'Diaminonaphthalene, a new highly specific reagent for HPLC-UV measurement of total and free malondialdehyde in human plasma or serum', *Free Radic. Biol. Med.*, 2001, **31**, 242–349.
17. O. Korchazhkina and Y. Yang: 'Methyl malondialdehyde is not suitable as an internal standard for malondialdehyde detection in urine after derivatisation with 2,4-dinitrophenylhydrazine', *J. Chromatogr. B*, 2004, **806B**, 295–298.
18. G. Cighetti, P. Allevi, L. Anastasia, L. Bortone and R. Paroni: 'Use of methyl malondialdehyde as an internal standard for malondialdehyde detection: validation by isotope-dilution gas chromatography-mass spectrometry', *Clin. Chem.*, 2002, **48**, 2266–2269.
19. F. Karatas, M. Karatepe and A. Baysar: 'Determination of free malondialdehyde in human serum by high-performance liquid chromatography', *Anal. Biochem.*, 2002, **311**, 76–79.
20. E. M. Gioti, Y. C. Fiamegos, D. C. Skalkos and C. D. Stalikas: 'Improved method for the in vitro assessment of antioxidant activity of plant extracts by headspace solid-phase microextraction and gas chromatography-electron capture detection', *J. Chromatogr. A*, 2007, **1152A**, 150–155.
21. M. Hasanzadeh, M. H. Pournaghi-Azar, N. Shadjou and A. Jouyban: 'Electropolymerization of taurine on gold surface and its sensory application for determination of captopril in undiluted human serum', *Mater. Sci. Eng. C*, 2014, **C38**, 197–205.
22. M. G. Hosseini, M. M Momeni and M. Faraji: 'Preparation and electrocatalytic activity of gold nanoparticle embedded in highly ordered TiO_2 nanotube array electrode for electro-oxidation of galactose', *Surf. Eng.*, 2011, **27**, 784–789.
23. M. G. Hosseini, M. M. Momeni and S. Zeynali: 'Gold nanoparticles deposited on polyaniline nanofibres as for electro-oxidation of hydrazine', *Surf. Eng.*, 2013, **29**, 65–69.
24. M. G. Hosseini, S. A. S. Sajjadi and M. M. Momeni: 'Electrodeposition of platinum metal on titanium and anodised titanium from P salt: application to electro-oxidation of glycerol', *Surf. Eng.*, 2007, **23**, 419–424.
25. R. S. Nicholson and I. Shain: 'Theory and application of cyclic voltammetry for measurement of electrode reaction kinetics', *Anal. Chem.*, 1964, **36**, 706–711.
26. L. Lorente, M. M. Martìn, P. Abreu-Gonzalez, A. Dominguez-Rodrìguez, L. Labarta, C. Dìaz, J. Sole-Violan, J. Ferreres, J. M. Borreguero-Leon, A. Jimenez and A. Morera-Fumero: 'Prognostic value of malondialdehyde serum levels in severe sepsis: a multicenter study', *PLoS One*, 2013, **8**, 53741.
27. S. J. Mannan, M. A. K. Azad, M. A. Ullah, A. A. Maruf, M. I. Rayhan, M. S. Ahsan and A. Hasnat: 'Investigation of serum trace element, malondialdehyde and immune status in drug abuser patients undergoing detoxification', *Biol. Trace Elem. Res.*, 2011, **140**, 272–283.