

Ultrastable method for determination of folic acid based on polynuclear red and multiwalled carbon nanotubes composite nanofilm

Jianning Qu, Xueping Du, Tongfang Lou, Ying Dong, Lijuan Shi

Institute of Environmental and Analytical Sciences, College of Chemistry and Chemical Engineering, Henan University, Kaifeng, Henan 475004, People's Republic of China
E-mail: qjy405407@163.com

Published in Micro & Nano Letters; Received on 2nd November 2013; Revised on 8th January 2014; Accepted on 7th February 2014

By using the electrocatalytic property of the nanomaterials and the molecular recognition characteristic of neutral red (NR), a new modified electrode was fabricated by electropolymerising the NR onto the surface of the multiwalled carbon nanotubes modified glassy carbon electrode, which exhibited a good electrocatalytic activity for folic acid (FA). The results of experiments showed that a good linear relationship between the reductive peak current of FA and its concentration was in the range of 6.25×10^{-7} – 5.00×10^{-5} mol/l with a detection limit of 1.37×10^{-7} mol/l in the optimal conditions. The recovery ratio was in the range of 99.3 ~ 103.2%. The composite film modified electrode exhibited an excellent stability with a simple fabrication, which established a solid foundation for the practical applications.

1. Introduction: Folic acid (FA) is a water-soluble B group vitamin and is essential for cell growth and division and participates in lots of reactions in the body and mainly in the synthesis of nucleic acid and some important substances [1]. It exists in leafy green vegetables widely and promotes the synthesis of protein from amino acid, in the human body. The lack of FA gives rise to megaloblastic anaemia, leucopenia, cancer, increasing possibility of heart attack, some psychiatric disorders and neural tube defects in newborns [2, 3]. In recent years, FA has increasingly become one of the hot points in the research fields of medicine, biology and chemistry. Therefore, methods for the determination of FA such as high-performance liquid chromatography [4], spectrophotometry [5], flow injection chemiluminescence [6], microbiological assay [7], capillary electrophoresis [8] and electrochemical methods [1–3] have attracted much attention. Compared with the other methods, the electrochemical methods have the advantages of simplicity, low cost, accuracy and sensitivity, but stability is not better, especially for the modified electrodes. Owing to the particular characteristics, few electrochemical methods for detecting FA have been reported, and most of them did not exhibit a better stability [9, 10].

Multiwalled carbon nanotubes (MWCNTs) have been extensively used as a material for electrode preparation because of their unique physical, chemical and electrical properties, large specific surface area, subtle electronic properties and obvious catalytic activity [11], and the function of MWCNTs to promote electron transfer reactions has been documented in connection with important biomolecules [12].

Polymer films with π -conjugated structures are often used as the electron transfer mediators, which are widely applied in chemical sensors, biosensors and electrocatalysis [13, 14]. Owing to their conjugative parent ring structure, conjugated polymers containing an azine ring structure may be a promising block for building a high-quality conducting polymer [15]. Moreover, the polymer films are controllable, easy to prepare, and can be applied to an electrode surface of any thickness [16].

As a kind of phenazine dye, with an amino group located on a heteroaromatic phenazine ring, neutral red (NR) is often used as an electron transfer mediator in organic electrochemistry and bioelectrochemistry [17], and is electrochemically polymerised in acidic solutions. The poly(NR) film has been widely used in the field of electrochemistry for its better adsorption, homogeneity,

high stability, simple preparation and it exhibits an excellent electrocatalytic activity for the biological molecules.

In the work reported in this Letter, a new modified electrode was fabricated by electropolymerising the NR onto the surface of the MWCNTs modified glassy carbon (GC) electrode, which used the electrocatalytic property of the nanomaterials and the molecular recognition property of the NR. Compared with the MWCNTs [9, 10], the composite film showed an excellent catalytic behaviour for the reduction of FA. In addition, the modified electrode exhibited ultrastability with a low detection limit and a high sensitivity. This electrochemical sensor provided a more perfect method to detect FA, which showed the potential applications in practice.

2. Experimental

2.1. Materials and methods: Electrochemical experiments were performed with a CHI650 electrochemical workstation (USA, CHI). A conventional three-electrode system was used where GC (3 mm diameter) or chemically modified GC electrodes, a rotating platinum wire electrode and Ag/AgCl (saturated KCl) were used as the working, the counter and the reference electrodes, respectively. All the potentials in this Letter referred to this reference electrode.

A stock standard solution of FA was prepared in NaOH (0.1 mol/l) at a concentration of 5.0×10^{-4} mol/l and was stored in a refrigerator. The MWCNTs had a diameter of 10–15 nm, (University of Marburg, Germany, Department of Chemistry, Materials Science Center), Supporting electrolyte was a 0.1 mol/l phosphate buffer solution (PBS, pH = 6.5). The other reagents were of analytical grade, and the experimental water was redistilled.

2.2. Preparation of the modified electrodes: The GC electrode was polished with 1.0, 0.3 and 0.05 μ m alumina slurries, then rinsed ultrasonically with a 1:1 nitric acid solution, ethanol and doubly distilled water, and dried in air.

1 mg of the MWCNTs was dispersed in 5 ml of N,N-dimethyl-formamide (DMF) by using an ultrasonic agitation to obtain the MWCNTs suspension (0.2 mg/ml). 6 μ l of the MWCNTs suspension was dropped on the surface of the GC electrode, and dried in an infrared lamp to give an MWCNTs/GC electrode.

Electropolymerisation of the NR was then performed under a cyclic scanning at 100 mV/s. The monomer concentration of the

NR in the solution was 5.0×10^{-4} mol/l and the supporting electrolyte consisted of 0.025 mol/l PBS (pH 6.5) + 0.5 mol/l NaNO₃. The operation process initiated the polymerisation for ten cycles between -1.4 and 1.8 V, the potentials being controlled between -0.8 and 0.8 V, respectively, for the cyclic scan polymerisation to give the PNR/MWCNTs/GC electrode. The composite film modified electrode is formed and controlled by a consecutive cyclic voltammetry which produces a fine synthetic film.

All the modified electrodes were thoroughly washed with water before use.

2.3. Detection method: Electrochemical measurements were performed on a CHI650 electrochemistry workstation with the conventional three-electrode system mentioned above. The scan range of the electrochemical test is 1.0 ~ -0.9 V with a scan rate of 100 mV/s. All the electrochemical measurements were performed after being deoxygenised with nitrogen gas for 10 min.

3. Results and discussion

3.1. Comparison of the different electrodes: The cyclic voltammograms (CVs) responses at the bare GC (curve a), the MWCNTs/GC (curve b) and the PNR/MWCNTs/GC (curve c) electrodes, respectively, in a 0.1 mol/l PBS (pH 6.5) are shown in Fig. 1. Clearly, a couple of the redox peaks of the MWCNTs at about -0.05 V were found at the MWCNTs/GC electrode and for the PNR/MWCNTs/GC, two couples of the redox peaks at about 0.04 and -0.4 V appeared, which belonged to the MWCNTs and the NR, respectively. This indicated that the PNR film had been formed on the surface of the MWCNTs.

3.2. Electrocatalytic behaviour of the PNR/MWCNTs/GC electrode to the FA: As shown in Fig. 2, the electrochemical behaviour of a 4×10^{-5} mol/l FA in a 0.1 mol/l PBS (pH=6.5) at the bare GC (curve a), the PNR/GC (curve b), the MWCNTs/GC (curve c) and the PNR/MWCNTs/GC (curve d) electrodes, respectively, were investigated by a CV. In the FA solution, no obvious peak was found at the bare GC. In addition, a couple of the redox peaks of the MWCNTs at about 0 V, another couple of the redox peaks at about -0.4 V and an irreversible reduction peak at -0.64 V appeared at the MWCNTs/GC, which belonged to the FA; two couples of the redox peaks of the NR at about 0.1 and -0.4 V, and an irreversible reduction peak appeared at the PNR/GC (-0.655 V) and the PNR/MWCNTs/GC (-0.663 V), respectively. Obviously, a couple of the redox peaks of the FA also appeared at -0.4 V and the currents of a couple of the redox peaks and an irreversible reduction peak of the FA at the PNR/MWCNTs/GC were much higher, which showed that the composite film exhibited a good electrocatalytic activity for the FA. This might be attributed to an increase in the active surface of the electrode

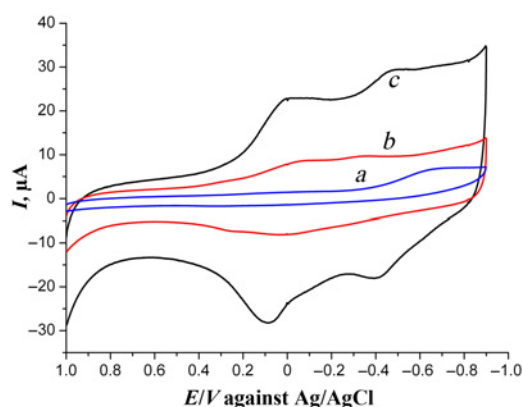


Figure 1 CVs at the bare GC (a), the MWCNTs/GC (b) and the PNR/MWCNTs/GC (c) electrode, respectively, in the 0.1 mol/l PBS (pH = 6.5)

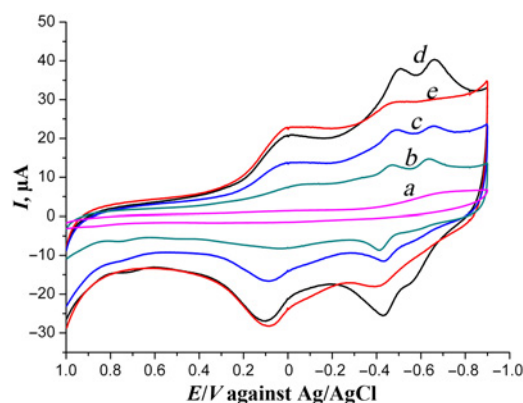


Figure 2 CVs at the bare GC (a), the PNR/GC (b), the MWCNTs/GC (c), and the PNR/MWCNTs/GC (d) in the 4×10^{-5} mol/l FA + 0.1 mol/l PBS (pH = 6.5) and the PNR/MWCNTs/GC (e) electrode in the 0.1 mol/l PBS (pH 6.5), respectively

when modified with a carbon nanotube film and the high density of the nanopolymer film with a π -electronic formed on the surface of the electrode.

3.3. Optimisation of the experimental conditions

3.3.1 Influence of the MWCNTs film thickness: The same concentration (0.2 mg/ml) of the MWCNTs with different volumes of 2, 4, 6, 8 and 10 μ l, was modified on the bare GC electrode. Then, the effect of the MWCNTs loading on the reduction peak current of the 4×10^{-5} mol/l FA was studied in the 0.1 mol/l PBS (pH = 6.5). The experiments showed that on improving the amount of the MWCNTs suspension from 2 to 6 μ l, the reduction peak currents of the FA were enhanced remarkably (shown in Fig. 3) and when the 6 μ l MWCNTs suspensions were used, the reduction peak current reached its maximum value. Hence, the 6 μ l MWCNTs suspension was chosen to prepare the sensor.

3.3.2 Influence of the polymerisation cycles: The influence of the number of the cycles on the reduction of the 4×10^{-5} mol/l FA was investigated in the 0.1 mol/l PBS (pH = 6.5). The results in Fig. 4 show that the higher reduction peak current of the FA was obtained with ten cycles. Electropolymerisation of the NR occurred at the high positive potentials, with the increase of the polymerisation cycles and with the thickness of the polymer film, the conduct or properties became worse. Therefore the optimum number of the polymerisation cycles was determined to be ten.

3.3.3 Types of the supporting electrolytes: The CV behaviours of the FA had been investigated in different electrolytic solutions,

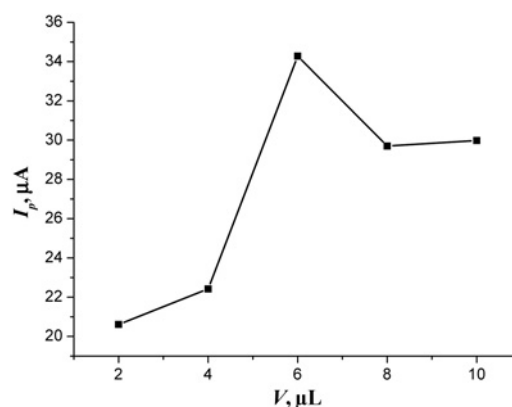


Figure 3 Dependence of the peak current on the dosage of the MWCNTs (0.2 mg/ml)

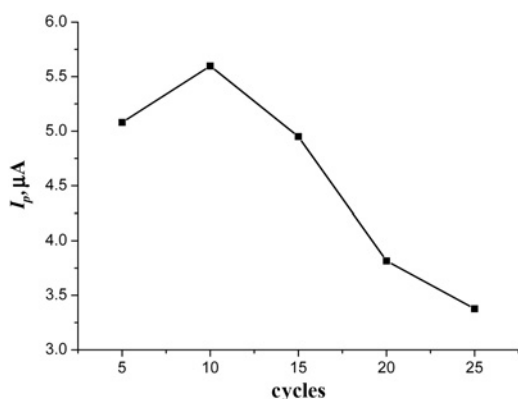


Figure 4 Dependence of the peak current on the polymerisation cycles

such as KCl, NaNO₃, KH₂PO₄-NaOH, citric acid-sodium citrate and the PBS buffer solutions, respectively. The results indicated that no peak of the FA appeared in KCl or NaNO₃, and a couple of the redox peaks and a reduction peak appeared in the KH₂PO₄-NaOH, the citric acid-sodium citrate buffer or the PBS buffer solutions, respectively, but a higher peak current and a better peak shape of the FA could be obtained in the PBS. Thus, the PBS was chosen as the supporting electrolyte.

3.3.4 Influence of pH: The effect of the pH value of the solution on the reduction of the 4×10^{-5} mol/l FA was also investigated in the range of 6.0–7.8 in the PBS (shown in Fig. 5). With the increase of pH, the peak current increased gradually until attaining a maximum at about pH 6.5, and then decreased. When the pH solution was lower than 6.5, the reduction peak current increased gradually with increasing pH. This result may be ascribed to the instability of the FA under the highly acidic conditions. When the pH solution is higher than 6.5, the reduction peak current decreases gradually, especially, the reduction peak current decreased sharply, when pH was >7.4. This result may be ascribed to the concentration of a hydrogen ion which is not only the reactant but also a counterion to the electrons, and the decrease of its concentration in the film will diminish the electrical conductivity and lower the reduction peak current lower [18]. Therefore a pH of 6.5 was selected for the determination of the FA.

3.4. Influence of the scan rate: The influence of the scan rate on the reduction of the FA at the PNR/MWCNTs/GC electrode was investigated in the range of 20–300 mV/s for the 4×10^{-5} mol/l FA in the 0.1 mol/l PBS (pH=6.5) by a linear sweep voltammetry (shown in Fig. 6). Clearly, with the increase of the scan rate, the reduction peak current increases and the reduction

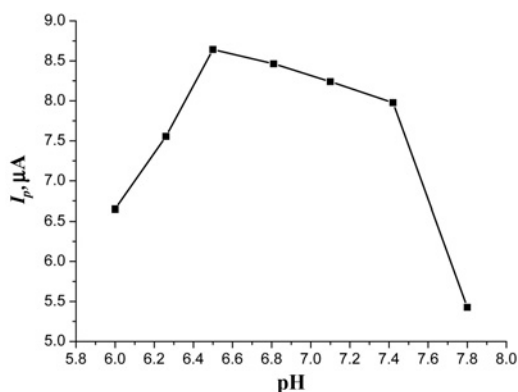


Figure 5 Dependence of the peak current on pH

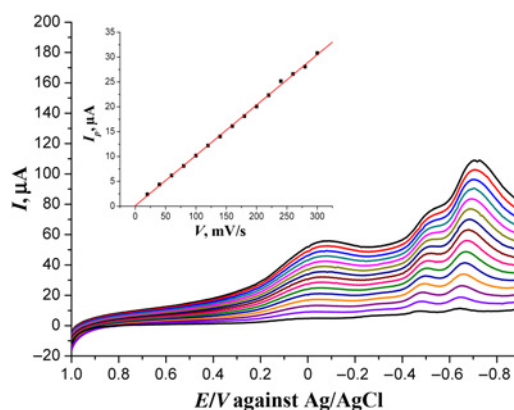


Figure 6 CVs of the PNR/MWCNTs/GC electrode at the different scan rates (from inner to outer: 20–300 mV/s, respectively) in the 4×10^{-5} mol/l FA + 0.1 mol/l PBS (pH = 6.5)

Inset chart: the relationship between the reduction peak current and the scan rate

current was found to be linear to the square root of the scan rate (Fig. 6, inset chart). The linear regression equation is I_p (μA) = $0.09988 + 0.10134v$ (mV/s) ($R = 0.9995$). These results indicate that the reduction reaction of the FA at the PNR/MWCNTs/GC electrode was adsorption controlled.

3.5. Stability and repeatability: The modified electrode was used for a determination of the FA by a continuous CV for 30 cycles; the reduction peak current remained almost the same, which demonstrated that the composite film modified electrode has an excellent stability.

The relative standard deviation for the five successive determinations is 3.82% in the 4×10^{-5} mol/l FA solution. These results indicate that the composite film modified electrode shows a good repeatability.

3.6. Calibration curve: The PNR/MWCNTs/GC electrode was utilised for the determination of the FA in the 0.1 mol/l PBS (pH=6.5). As shown in Fig. 7, the relationship between the reduction peak current and the FA concentration gives a good linear relationship from 6.25×10^{-7} to 5.00×10^{-5} mol/l, respectively. The regression equation is I_p (μA) = $0.36316 + 0.12706C$ (mmol/l) ($R = 0.9986$) with a detection limit of 1.37×10^{-7} mol/l.

3.7. Selectivity against interference: Under the same conditions, the influences of some inorganic ions and glucose, citric acid, ascorbic

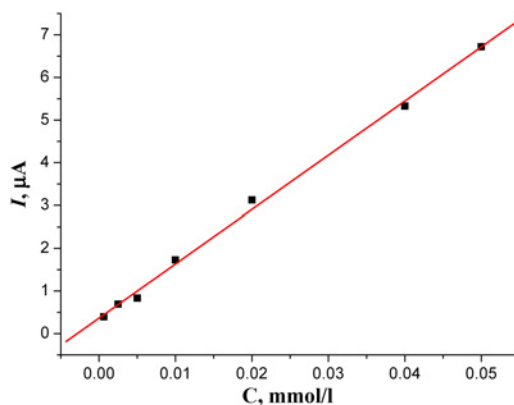


Figure 7 Standard curve for the different concentrations of the FA at the PNR/MWCNTs/GC electrode in the 0.1 mol/l PBS (pH = 6.5)

Table 1 Results of the test for the recovery ratio

content, mol	added, mol	observed, mol	recovery, %	average, %
1.00×10^{-5}	0.50×10^{-5}	1.49×10^{-5}	99.3	101.3
2.00×10^{-5}		2.58×10^{-5}	103.2	
4.00×10^{-5}		4.56×10^{-5}	101.3	

acid on the determination of the reduction peak current of the 4×10^{-5} mol/l FA were investigated in the 0.1 mol/l PBS (pH = 6.5). The experimental results showed that 100-fold of Na^+ , K^+ , Cl^- , NO_3^- , Zn^{2+} , 50-fold of glucose, citric acid and 20-fold of ascorbic acid almost have no interference on the determination of the FA, because its peak current changes below 5%. This demonstrated that the proposed method showed a good selectivity for the FA detection.

3.8. Recovery test: Under the optimal conditions, the PNR/MWCNTs/GC electrode was used for the detection of the 4×10^{-5} mol/l FA in the 0.1 mol/l PBS (pH = 6.5). The FA concentration was determined by a standard addition method to calculate the recoveries. As shown in Table 1, the recovery of the FA was 99.3–103.2% and the average recovery was 101.3%. It shows that the proposed method can be efficiently used for the determination of the FA.

4. Conclusion: In this work, the PNR/MWCNTs/GC modified electrode was fabricated, which exhibited the good electrocatalytic activity of the FA. The electrochemical process of the FA at this electrode is adsorption controlled. The reduction peak current of the FA changed linearly with its concentration in the range from 6.25×10^{-7} – 5.00×10^{-5} mol/l with a detection limit of 1.37×10^{-7} mol/l. The composite film modified electrode exhibited a good stability with a simple preparation, a low detection limit and a high sensitivity, which showed the potential applications in practice.

5 References

- [1] Mirmoghataie L., Ensafi A.A., Kadivar M., Norouzi P.: 'Highly selective electrochemical biosensor for the determination of folic acid based on DNA modified-pencil graphite electrode using response surface methodology', *Mater. Sci. Eng. C*, 2013, **33**, pp. 1753–1758
- [2] Manoj D., Kumar D.R., Santhanalakshmi J.: 'Impact of CuO nanoleaves on MWCNTs/GCE nanocomposite film modified electrode for the electrochemical oxidation of folic acid', *Appl. Nanosci.*, 2012, **2**, pp. 223–230
- [3] Kun Z., Ling Z., Yi H., *ET AL.*: 'Electrochemical behavior of folic acid in neutral solution on the modified glassy carbon electrode: platinum nanoparticles doped multi-walled carbon nanotubes with Nafion as adhesive', *J. Electroanal. Chem.*, 2012, **677**, pp. 105–112
- [4] Breithaupt D.E.: 'Determination of folic acid by ion-pair RP-HPLC in vitamin-fortified fruit juices after solid-phase extraction', *Food Chem.*, 2001, **74**, pp. 521–525
- [5] Shishehbore M.R., Sheibani A., Haghdost A.: 'Kinetic spectrophotometric method as a new strategy for the determination of vitamin B₉ in pharmaceutical and biological samples', *Spectrochim. Acta A, Mol. Biomol. Spectrosc.*, 2011, **81**, pp. 304–307
- [6] Zhang B.T., Zhao L.X., Lin J.M.: 'Determination of folic acid by chemiluminescence based on peroxomonosulfate-cobalt(II) system', *Talanta*, 2008, **74**, pp. 1154–1159
- [7] Han J.Y., Tyler R.T.: 'Determination of folate concentrations in pulses by a microbiological method employing trienzyme extraction', *J. Agric. Food Chem.*, 2002, **51**, pp. 5315–5318
- [8] Zhao S., Yuan H., Xie C., Xiao D.: 'Determination of folic acid by capillary electrophoresis with chemiluminescence detection', *J. Chromatogr. A*, 2006, **1107**, pp. 290–293
- [9] Chaochan R., Yugin S., Zuoning G.: 'Electrocatalytic oxidation of FA at multi-wall carbon nanotubes modified glassy carbon electrode and its electrochemical application', *J. Ningxia Univ. (Natural Science Edition)*, 2010, **31**, pp. 70–73
- [10] Jiang X.L., Li R., He X.Y., Li J.: 'Cyclic voltammetric behavior and electrochemic parameter determination of folic acid on multiwalled carbon nanotube modified electrode', *J. China West Normal Univ. (Natural Sciences)*, 2008, **29**, pp. 182–185
- [11] Nugent J.M., Santhanam K.S.V., Rubio A., Ajayan P.M.: 'Fast electron transfer kinetics on multiwalled carbon nanotube microbundle electrodes', *Nano Lett.*, 2001, **1**, pp. 87–91
- [12] Zeng B.Z., Wei S.H., Xiao F., Zhao F.Q.: 'Voltammetric behavior and determination of Rutin at a single-walled carbon nanotubes modified gold electrode', *Sens. Actuator B*, 2006, **115**, pp. 240–246
- [13] Lu B.P., Bai J., Bo X.J., Yang L., Guo L.P.: 'Electrosynthesis and efficient electrocatalytic performance of poly(neutral red)/ordered mesoporous carbon composite', *Electrochim. Acta*, 2010, **55**, pp. 4647–4652
- [14] Chen S.M., Lin K.C.: 'The electrocatalytic properties of polymerized neutral red film modified electrodes', *J. Electroanal. Chem.*, 2001, **511**, pp. 101–114
- [15] Chen C.X., Gao Y.H.: 'Electrosyntheses of poly(neutral red), a polyaniline derivative', *Electrochim. Acta*, 2007, **52**, pp. 3143–3148
- [16] Inzelt G., Csahok E.: 'Electrochemical quartz crystal microbalance studies of the formation and redox behavior of poly(neutral red) electrodes', *Electroanalysis*, 1999, **11**, pp. 10–11
- [17] Mažeikiene R., Balskus K., Eicher-Lorka O., Niaura G., Meškys R., Malinauskas A.: 'Raman spectroelectrochemical study of electrode processes at neutral red- and poly(neutral red) modified electrodes', *Vib. Spectrosc.*, 2009, **51**, pp. 238–247
- [18] Benito D., García-Jareño J.J., Navarro-Laboulais J., Vicente F.: 'Electrochemical behaviour of poly(neutral red) on an ITO electrode', *J. Electroanal. Chem.*, 1998, **446**, pp. 47–55