Carbon paste electrode incorporating multi-walled carbon nanotube/ferrocene as a sensor for the electroanalytical determination of *N*-acetyl-*L*-cysteine in the presence of tryptophan

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Abstract. The preparation and electrochemical performance of the carbon nanotube paste electrode modified with ferrocene (FCMCNPE) was investigated for electrocatalytic behaviour toward oxidation of *N*-acetyl-*L*-cysteine (NAC) in the presence of tryptophan (Trp) using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results showed an efficient electrocatalytic activity of FCMCNPE toward oxidation of NAC and Trp, as the electrooxidation of NAC and Trp together gave two well-defined anodic peaks, revealing the applicability of this modified electrode for simultaneous voltammetric detection of mentioned compounds in the same solution. The values of catalytic rate constant (k) and the apparent diffusion coefficient (D_{app}) were also calculated using chronoamperometry. The DPV method was applied as a sensitive method for the quantitative detection of trace amounts of NAC and Trp. A linear dynamic range from 1.0 to 18.0 μM for NAC and 2.0 to 150.0 μM for Trp was obtained using DPV method in pH 7.00 buffered solution and the detection limit (3σ) was determined as 0.49 μM and 0.54 μM for NAC and Trp, respectively. The proposed method was also applied for analysis of NAC tablet, investigating the applicability of the proposed voltammetric method for determination of NAC in real sample.

Keywords. *N*-acetyl-*L*-cysteine; tryptophan; ferrocene; electrocatalysis; carbon nanotube paste electrode.

1. Introduction

N-acetyl-*L*-cysteine (NAC), as an acetyl derivative and also one of the homologs of *L*-cysteine, is very important amino acid owing to its crucial roles in biological systems.[1] For example, it could be used as a prospective radiation protector, antitoxin, antioxidant and free radical and reactive oxygen species scavenger.[2] NAC has been efficient in the treatment of Sjogren's syndrome, influenza, smoking cessation, myoclonus epilepsy and hepatitis C,[3] cancer,[4] human immunodeficiency virus (HIV) infection,[5] cardiovascular and respiratory diseases,[6] acetaminophen toxicity,[7] neurodegenerative disorder[8] and the other diseases characterized by free radicals production and oxidative damage. Numerous chemical and instrumental techniques, such as chromatography,[9] spectrophotometry[10] and fluorimetry[11] were employed for the determination of this compound. However, these methods suffer

from some disadvantages such as high cost, long analysis time and requirement for sample pre-treatment that makes them unsuitable for routine analysis. Compared to these options, electro-analysis is more promising approach, due to simplicity, low expense and high sensitivity.[12] However, NAC oxidation at bare electrodes requires high overpotential and the corresponding voltammetric signals on the surface of bare electrodes are usually weak that makes its detection unsatisfactory. Tryptophan (2-amino-3-(1H-indol-3-yl)-propionic acid), Trp is essential amino acid for humans and a precursor for serotonin (a neurotransmitter), melatonin (a neurohormone) and niacin. This compound is sometimes added to dietary, food products, pharmaceutical formulas due to the scarcely presence in vegetables.[13] Tryptophan is known to undergo electrochemical oxidation and several polymeric/oligomeric products can be formed on the electrode surface.[14]

The electrochemical oxidation of NAC and Trp at bare electrode results highly overlapped current responses which make their discrimination very

difficult and leads to poor selectivity and reproducibility of the electrode. To our knowledge, several electrochemical methods based on the chemical modification of traditional electrode materials, have been developed for determination of NAC (e.g., carbon paste electrode modified with copper(II) hexacyanoferrate(III),[15] palladized aluminum electrode covered by prussian blue film[16] and nanocomposite containing naphthoquinone and nanogold modified glassy carbon electrode)[17] and Trp (e.g., nano-TiO$_2$/ferrocene carboxylic acid modified carbon paste electrode[18] and carbon nanotube paste electrode modified with cobalt salophen),[19] individually.

Ferrocene and its derivatives are the coordination compounds known as useful and suitable electrode modifier for their excellent electrocatalytic properties toward the detection of many important drugs and bio-subatrates.[20–22]

Application of carbon nanotubes (CNTs) as an important nano-structured material has also recently attracted a lot of attention in electrochemical researches for the determination of biomolecules due to their exceptional properties, such as strong electrocatalytic effect, fast electron transfer rate, high conductance, biocompatibility, high stability and high surface area.[23–31]

The integration of redox mediators and CNTs in the matrix of the modified electrodes is considered as a new and interesting area in the development of electrochemical sensors.[32–36] In the present work, we describe the preparation of a carbon nanotube paste electrode modified with ferrocene as a redox electron transfer mediator for improvement in the kinetic of the electrode process and sensitivity of the voltammetric measurements. We investigated the suitability of this modified electrode (FCMCNPE) for electrocatalysis of NAC in the presence of Trp. A complete resolution between DPV peaks of NAC and Trp (about 450 mV separation in peak potentials) provides a suitable and effective method for simultaneous determination of these two compounds at the surface of FCMCNPE.

2. Experimental

2.1 *Materials and apparatus*

The solvent used for the electrochemical studies was twice distilled water. Phosphate buffered solutions (PBS) 0.1 M for different pH values were prepared from orthophosphoric acid (H$_3$PO$_4$) and its salts (NaH$_2$PO$_4$, Na$_2$HPO$_4$ and Na$_3$PO$_4$). High viscosity paraffin (density = 0.88 g cm^{-3}) from Fluka was used as the pasting liquid for the carbon paste electrode. Graphite powder

(particle diameter = 0.1 mm) from Merck and multi-walled carbon nanotube (with purity OF >95%, diameter 54 nm, length 1–10 μm, number of walls 3–15, from Nanostartech. Co., Tehran, Iran) were used as the working electrode (WE) substrates. Ferrocene (FC), NAC and Trp were from Fluka. Acetylcysteine tablets (labelled value 600 mg, from Zambon Co., imported by Darman Yab Darou Co.) were purchased from a local drugstore. NAC and Trp solutions were prepared daily. All other chemicals used were of analytical reagent grade.

The electrochemical measurements were carried out using a potentiostat/galvanostat (SAMA 500; Electroanalysis System, Iran) connected to Pentium IV personal computer. The electrochemical experiments were performed at room temperature with a three electrode assembly including a carbon paste (unmodified or modified), platinum disk and Ag|AgCl|KCl (3 M) (from Azar Electrode Co., Iran) as a working electrode, auxiliary electrode and reference electrode, respectively. Also, a digital pH-meter (model 780, from Metrohm Co., Switzerland) was used to read the pH of the buffered solutions.

2.2 *Preparation of working electrode*

FCMCNPE was made by dissolving the mixture of 1% (w/w) ferrocene, 10% (w/w) multi-walled carbon nanotube and 89% (w/w) graphite powder in diethyl ether in a mortar. The mixture was stirred until the solvent was evaporated, completely. Then, the resulting composite and paraffin were blended by hand-mixing until a uniform paste was obtained. A portion of this paste was packed into the bottom of a glass tube (3.4 mm i.d.). Electrical contact was made by forcing a copper wire down into the tube. When necessary, a new surface was obtained by pushing out an excess of paste and polishing it on weighing paper. The ferrocene modified carbon paste electrode (FCMCPE) and carbon nanotube-paste electrode (CNPE) was prepared in the same way but without adding CNT and FC, respectively.

2.3 *Preparation of real sample*

For analysis of the NAC tablet, the tablet sample was finely powdered and homogenized. A weighted amount of this powdered sample was dissolved in 0.1 M phosphate buffered solution (pH 7.00). Then, a known amount of this aqueous sample of the drug was diluted to the volume of 10 mL and placed in a voltammetric cell. The standard addition method (n = 3) was

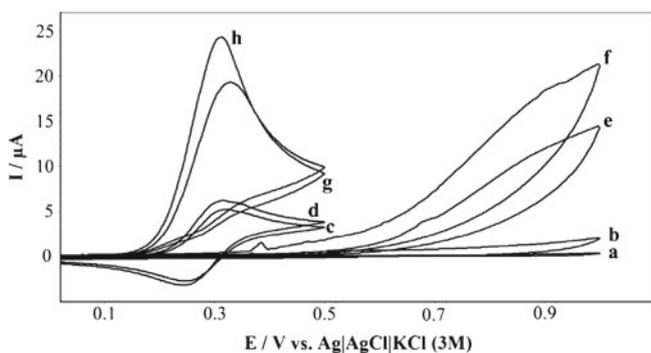employed for measuring NAC concentration in the sample.

3. Results and discussion

3.1 *Electrochemical behaviour of FCMCNPE and FCMCPE in the absence and presence of NAC*

We have previously shown that a carbon paste electrode spiked with ferrocene is constructed by the incorporation of ferrocene in a graphite powder/paraffin oil matrix.[37] The cyclic voltammograms of FCMCPE and FCMCNPE (figure 1c and d) exhibit one anodic and cathodic peak corresponding with redox system FC/FC^+, which show a quasi-reversible behaviour in an aqueous medium. The cyclic voltammograms of bare CPE and CNPE in pure supporting electrolyte show no anodic or cathodic peaks (figure 1a and b). Also, the comparison of FCMCNPE and CNPE with FCM-CPE and CPE electrochemical behaviour, respectively shows the presence of CNT with many specific advantages such as large surface area and high conductivity, can provide a suitable interface to promote the electron transfer reaction. Thus, the electrochemical response of FC will be greatly enhanced on FCMCNPE.

The kinetic parameters α and k for ferrocene were estimated using the model of Laviron:[38]

$$\begin{aligned} \log (k) \;=\;& \alpha \log (1 - \alpha) + (1 - \alpha) \log \alpha \\ &- \log (RT/nF\upsilon) \\ &- \alpha (1 - \alpha) \log (nF\Delta E_p/2.3RT), \end{aligned}$$

where α is the transfer coefficient, k is the heterogeneous electron-transfer rate constant, ΔE_p is the peak-to-peak separation, n is the number of electrons transferred in the rate-determining reaction, R is the gas constant, T is the absolute temperature and υ is the scan rate. A graph of ΔE_p versus the logarithm of the scan rate yielded a straight line. Based on this, the values of $\alpha = 0.64$ and $k = 3.47$ s^{-1} were calculated.
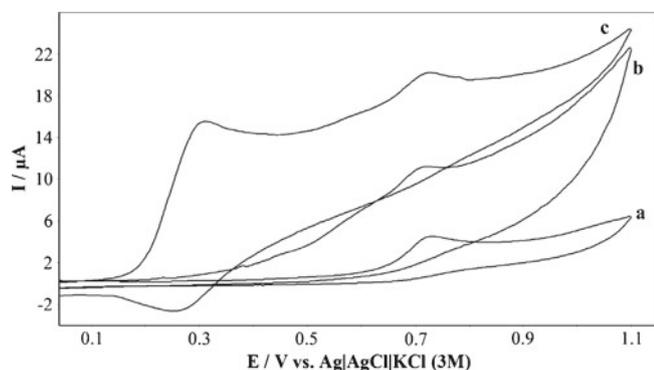
In order to study the electrocatalytic efficiency of the modified electrodes toward NAC oxidation, cyclic voltammetric responses of 1.0 mM NAC were recorded in 0.1 M phosphate buffered solution with pH 7.00 on the surface of modified and unmodified electrodes. As shown in figure 1, NAC oxidation occurs irreversibly with a broad peak at potential of about 850 mV at CPE (curve e) and CNPE (curve f), while at FCMCPE (curve g) and FCMCNPE (curve h), appears at potential of about 320 mV. These results confirm that the oxidation peak potential of NAC at FCMCPE and FCMCNPE is shifted about 530 mV toward less positive potential compared with that at bare CPE and CNPE. In the presence of NAC, the anodic peak current was increased greatly for FCMCPE and FCMCNPE, while the corresponding cathodic peak was disappeared on the reverse scan. All of these observations clearly show efficient electrocatalytic activity of FCMCNPE for lowering the anodic overpotential and improvement in the sensitivity of the voltammetric measurements.

3.2 *Electrochemical behaviour of NAC in the presence of Trp at FCMCNPE*

In order to study the simultaneous determination of NAC and Trp at the surface of FCMCNPE, cyclic voltammograms of FCMCNPE and CNPE in the buffer solutions containing 0.1 mM Trp and 0.5 mM NAC were recorded. As can be seen in figure 2 (curves a and b), the oxidation peak of NAC overlapped with that of Trp at CNPE, so electrochemical determination of them in their mixture solution is not possible. FC in the matrix of FCMCNPE can act as an efficient catalyst for lowering the anodic overpotential of NAC without any change in the peak potential of Trp (curve c). This effect makes a desirable separation between the oxidation peaks of NAC and Trp.

3.3 *The influence of pH on the electrochemical properties of NAC*

In order to optimize the electrochemical response of FCMCNPE to NAC oxidation, we investigated the effect of solution pH on the electrochemical behaviour of this compound in 1.0 mM solutions of NAC over a pH range between 4.0 and 9.0 at the surface of modified electrode using cyclic voltammetry. The results show that in the electrocatalytic oxidation process of NAC,

Figure 1. Cyclic voltammograms of (a) CPE, (b) CNPE, (c) FCMCPE and (d) FCMCNPE in the absence of NAC and (e) as (a), (f) as (b), (g) as (c) and (h) as (d) in the presence of 1.0 mM NAC in 0.1 M PBS (pH 7.00) at scan rate of potential 20 mV s^{-1}.

Figure 2. Cyclic voltammograms of (a) 0.1 mM Trp and (b) 0.1 mM Trp + 0.5 mM NAC in 0.1 M PBS (pH 7.00) at CNPE and (c) as (b) at FCMCNPE at scan rate of potential 20 mV s^{-1}.

the anodic peak current reaches to a maximum value in pH 7.00, indicating the maximum electrocatalytic efficiency of FCMCNPE for NAC electro-oxidation in this pH (not shown). Therefore, pH 7.00 was taken as the optimum pH for the determination of this compound at FCMCNPE.

3.4 *Effect of potential scan rate*

The effect of the potential scan rate (ν) on the electro-catalytic property of FCMCNPE toward electrooxidation of 1.0 mM NAC was studied by cyclic voltammetry (figure 3a). As can be seen in figure 3b, over the range of $\nu = 10$–300 mVs^{-1}, the anodic peak current (I_{pa}) increases linearly with the square root of the potential scan rate, indicating that the process is diffusion-controlled. In addition, with increasing the potential scan rate, the catalytic oxidation peak potential was shifted toward more positive potentials, suggesting a kinetic limitation in the reaction between the redox site of ferrocene in the FCMCNPE and NAC at higher scan rates.

In order to obtain information on the rate-determining step, a Tafel plot was drawn by using the rising part of the cyclic voltammograms of FCMCNPE in 0.1 M PBS containing 1.0 mM NAC at $\nu = 20$ mVs^{-1} (figure 3c). The resulted slope indicates a transfer coefficient (α) equal to 0.71 for one electron transfer process, which is rate-determining step. This value of α is in accordance with that reported data.[39]

3.5 *Chronoamperometry studies*

Double potential step chronoamperometry method was also employed for the investigation of electrode processes. Therefore, we studied the chronoamperometric behaviour of the modified electrode in 0.1 M phosphate buffered solution (pH 7.00) in the absence and presence of various concentrations of NAC by setting the
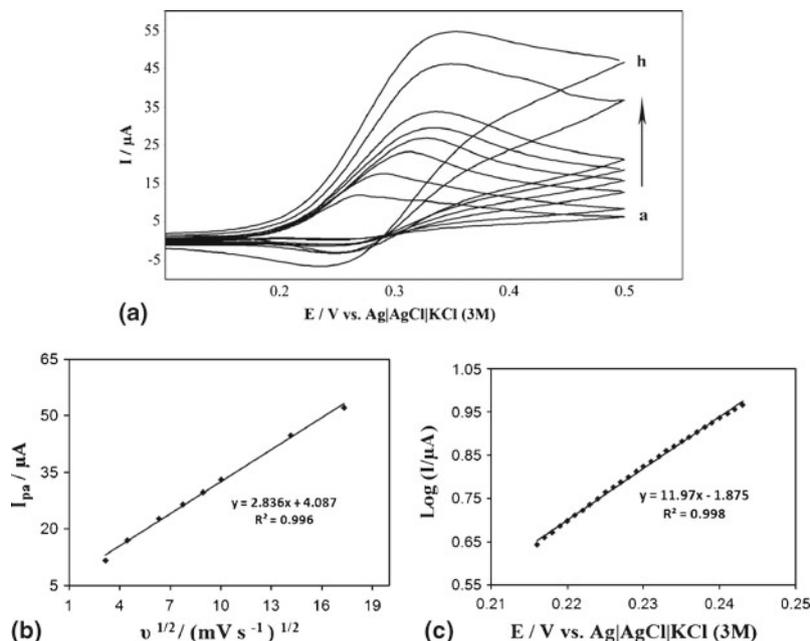
Figure 3. (**a**) Cyclic voltammograms of FCMCNPE in 0.1 M PBS (pH 7.00) containing 1.0 mM NAC at various potential scan rates (a) 10, (b) 20, (c) 40, (d) 60, (e) 80, (f) 100, (g) 200 and (h) 300 mVs^{-1}. (**b**) Plot of anodic peak current vs. $\nu^{1/2}$. (**c**) Tafel plot for FCMCNPE in the presence of 1.0 mM NAC in 0.1 M PBS (pH 7.00) at potential scan rate of 20 mV s^{-1}.
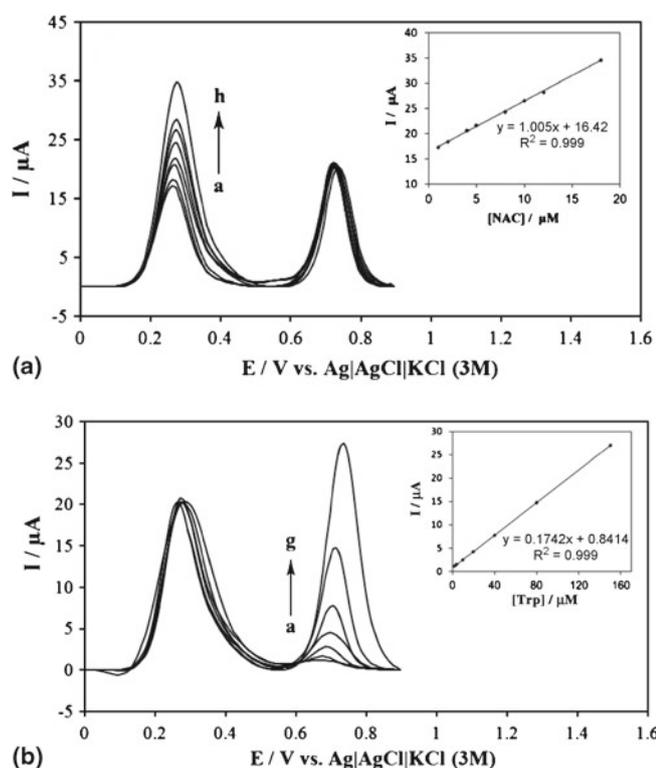
Figure 4. Differential pulse voltammograms of FCMC-NPE in 0.1 M PBS (pH 7.00) containing (**a**) 100 µM Trp and various concentrations of NAC: (a) 1, (b) 2, (c) 4, (d) 5, (e) 8, (f) 10, (g) 12 and (h) 18 µM; (**b**) 4 µM NAC and various concentrations of Trp: (a) 2, (b) 4, (c) 10, (d) 20, (e) 40, (f) 80, (g) 150 µM. scan rate and pulse amplitude were 20 mVs^{-1} and 50 mV, respectively. Insets **a** and **b** are calibration plots of anodic peak current vs. NAC and Trp concentrations, respectively.

working electrode potential at 0.4 V (first potential step) and 0.2 V (second potential step) (not shown). As can be seen, there is no net cathodic current corresponding to the reduction of FC$^+$ to FC in the presence of NAC, when the second potential step is employed. While the forward and backward potential steps chronoamperometry on the modified electrode in supporting electrolyte shows very symmetrical chronoamperogram with an equal charge consumed for the oxidation and reduction of redox system into modified electrode matrix. The current corresponding to the electrochemical reaction of an electroactive compound (under diffusion controlled) is described by Cottrell's equation: [40]

$$I = nFAD_{app}^{1/2}C_o\pi^{-1/2}t^{-1/2}, \qquad (1)$$

where D_{app} and C_o are the apparent diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. The plot of I versus $t^{-1/2}$ represents linear dependency and from the slopes of experimental plots for different concentrations of NAC, the mean value of D_{app} was found to be 9.92×10^{-6} cm^2 s^{-1}.

We have also used the chronoamperometric method to evaluate the heterogeneous catalytic rate constant according to the equation: [41]

$$I_C/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(k_hC_bt)^{1/2}, \qquad (2)$$

where I_C is the catalytic current of NAC at the modified electrode, I_L is the limited current in the absence of NAC, C_b is the bulk concentration of NAC, k_h and t are the catalytic rate constant (cm^3 mol^{-1} s^{-1}) and time

Table 1. Analytical data obtained for NAC detection at the surface of different modified electrodes.

Electrode	Modifier	Method	LDR[a] (µM)	LOD[b] (µM)	Reference
CPE[c]	CuHCF	LSV[d]	120–830	0.63	[15]
Pd–Al	Prussian blue	Amperometry	2–40	0.54	[16]
GCE[e]	Naphthoquinone / nanogold	DPV[f]	4.0–130	0.8	[17]
GCE	Acetylferrocene	CV[g]	600–6000	–	[39]
CPE	Fe$_2$O$_3$CoHCF nanoparticles	LSV	12.2–66.7	0.205	[42]
CPE	Fe$_2$O$_3$CoHCF nanoparticles	Amperometry	20–432	0.0209	[42]
CNPE	cobalt(III) salophen complexe E	DPV	0.1–100	0.05	[43]
CNPE	2,7-BF[h]	DPV	0.07–300	0.052	[44]
CNPE	Ferrocene	DPV	1.0–18.0	0.49	This work

[a]LDR: linear dynamic range
[b]LOD: limit of detection
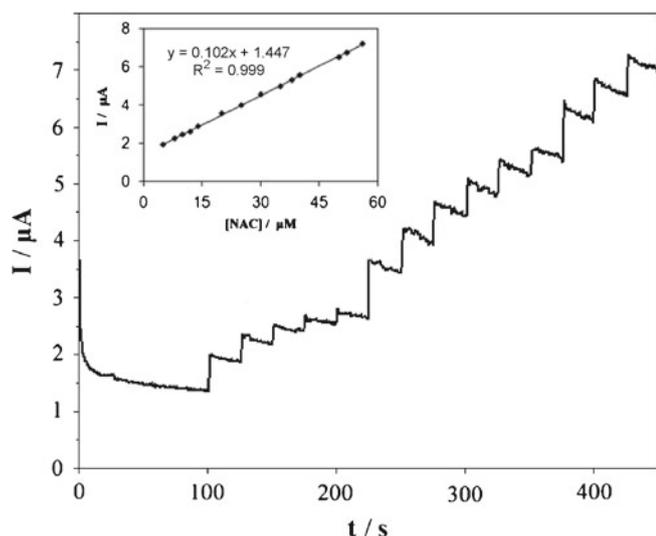[c]CPE: carbon paste electrode
[d]LSV: linear sweep voltammetry
[e]GCE: glassy carbon electrode
[f]DPV: Differential pulse voltammetry
[g]CV: cyclic voltammetry
[h]2,7-BF: 2,7-bis(ferrocenyl ethyl)fluoren-9-one

Figure 5. Hydrodynamic amperograms of FCMCNPE during the successive addition of NAC into PBS at constant electrode potential. Inset: The corresponding calibration plot for NAC.

elapsed (s). From the slope of I_C/I_L versus $t^{1/2}$ plot, the value of k_h was obtained as 3.07×10^5 cm^3 mol^{-1} s^{-1} for a given concentration of NAC.

3.6 *Analytical measurement*

In order to evaluate the performance of the modified electrode, as a sensor for sensitive determination of NAC in the presence of Trp, analytical experiments were carried out by varying the concentration of NAC in the presence of fixed amount of Trp (100 μM) and varying Trp concentration in the presence of fixed amount of NAC (4 μM) at pH 7.00 at the surface of FCMCNPE using differential pulse voltammetry method. The DPV results show two well-distinguished anodic peaks at 270 mV (NAC anodic peak potential) and 720 mV (Trp anodic peak potential) potentials (figure 4a and b). Thus, the response of the FCMCNPE to these two compounds was relatively independent. The calibration plots were linear within the concentration ranges of 1.0–18.0 μM (with the correlation coefficient of 0.999) for NAC (inset in figure 4a) and 2.0–150.0 μM (with the correlation coefficient of 0.999) for Trp (inset in figure 4b). The

obtained value of the detection limit (3σ) was to be 0.49 μM and 0.54 μM for the determination of NAC and Trp, respectively. As shown, we can use this method to measure Trp with high sensitivity too. The obtained analytical parameters for NAC determination at the surface of the modified electrode used in this research are comparable with the results reported by other research groups [15–17,39,42–44] (table 1).

In order to develop another simple and sensitive sensing procedure for the analysis of NAC, hydrodynamic amperometry technique at constant electrode potential was also employed. Typical amperometric responses of FCMCNPE to successive addition of NAC and corresponding calibration graph are depicted in figure 5 and its inset, respectively. In the optimized conditions, the electrode response was linear within the concentration range of 5.0–56.0 μM for NAC and the detection limit (3σ) was 1.23 μM.

3.7 *Interference study*

In this work, the interference effects of some biologically compounds, such as *L*-cysteine (*L*-cys), D-penicillamine (D-PA) and ascorbic acid (AA) on the electrode response to voltammetric determination of NAC were studied using DPV method. The results showed that *L*-cys and D-PA have interfering ability in voltammetric detection of NAC via increasing the sensor response. Although AA showed interfering effect on the electrode response to NAC in higher concentrations (about 1.0 mM), but in lower concentrations, it did not show interference in electroanalysis of NAC. Since AA content in red blood cells is not significant,[45] it would have no influence on the electrode response to voltammetric determination of NAC in human blood serum samples.

3.8 *Real sample analysis*

In order to investigate the applicability of the proposed voltammetric method for the electrocatalytic determination of NAC in real samples, we have used the acetylcysteine tablets. The standard addition method was employed for measuring NAC concentration in the sample. The results of NAC concentration determination in this sample and recovery experiment obtained using

Table 2. The obtained results from NAC determination in pharmaceutical formulation (n = 3).

Sample	Amount added (mM)	Amount found (mM)	Recovery (%)	RSD (%)	E_r (%)
Tablet	0.200	0.1959	97.95	3.005	−2.04

this procedure are represented in table 2, which demonstrate the accuracy of the proposed method and its efficiency for voltammetric determination of NAC in such samples.

4. Conclusion

In the present study, we have prepared a chemically modified electrode by incorporating FC in the matrix of CPE and CNPE. As it was found, FCMCNPE is compatible to FCMCPE because of the presence of CNT with many specific advantages such as large surface area and high conductivity; it can provide a suitable interface to promote the electron transfer reaction. The electrochemical behaviour of NAC in the presence of Trp at the surface of the prepared modified electrode was investigated by voltammetry. It is shown that The FCMCNPE is able to completely resolve the differential pulse voltammograms peak of NAC from Trp, revealing its good ability for simultaneous determination of these compounds in their mixture solution. The kinetic parameters such as transfer coefficient, catalytic rate constant, and diffusion coefficient for the oxidation of NAC were also determined. Also, the results of NAC concentration determination in tablet and the recovery experiment were obtained; demonstrate the accuracy of the proposed method and its efficiency for voltammetric determination of NAC in real samples.

References

1. Voet D and Voet J G 1995 *Biochemistry*, 2nd ed. (New York: John Wiley and Sons) p. 1263
2. De Viries N and De Flora S 1993 *J. Cell Biochem.* **53** 270
3. Czap K 2000 *Altern. Med. Rev.* **5** 467
4. Liu M, Wikonkal M and Brash D E 1999 *Carcinogenesis* **20** 1869
5. Valcour V and Shiramizu B 2004 *Mitochondrion* **4** 119
6. Dekhuijzen P N R 2004 *Eur. Respir. J.* **23** 629
7. Zafarullah M, Li W Q, Silvester J and Ahmad M 2003 *Cell. Mol. Life Sci.* **60** 6
8. Fu A L, Dong Z H and Sun M J 2006 *Brain Res.* **1109** 201
9. Tsikas D, Sandmann J, Ikic M, Fauler J, Stichtenoth D O and Frolich J C 1998 *J. Chromatogr. B: Biomed. Sci. Appl.* **708** 55
10. Suarez W T, Vieira H J and Fatibello-Filho O 2005 *Acta Chim. Slov.* **52** 164
11. Al-Ghannam S, El-Brashy A and Al-Farhan B 2002 *Farmaco* **57** 625
12. Chwatko G and Bald E 2000 *Talanta* **52** 509
13. Kochen W and Steinhart H 1994 *L-Tryptophan—Current prospects in medicine and drug safety* (Berlin: de Gruyter)
14. Nguyen N T, Wrona M Z and Dryhurst G 1986 *J. Electroanal. Chem.* **199** 101
15. Suarez W T, Marcolino L H and Fatibello-Filho O 2006 *Microchem. J.* **82** 163
16. Pournaghi-Azar M H and Ahour F 2008 *J. Electroanal. Chem.* **622** 22
17. Raoof J B, Ojani R, Chekin F, Jahanshahi M and Rashid-Nadimi S 2009 *Electroanalysis* **21** 2674
18. Raoof J B, Ojani R and Baghayeri M 2009 *Sens. Actuators B* **143** 261
19. Shahrokhian S and Fotouhi L 2007 *Sens. Actuators B* **123** 942
20. Fouladgar M 2011 *Int. J. Electrochem. Sci.* **6** 705
21. Raoof J B, Ojani R, Beitollahi H and Hossienzadeh R 2006 *Electroanalysis* **18** 1193
22. Ensafi A A, Khoddami E and Karimi-Maleh H 2011 *Int. J. Electrochem. Sci.* **6** 2596
23. Merkoci A 2006 *Microchim. Acta* **152** 157
24. Gooding J J 2005 *Electrochim. Acta* **50** 3049
25. Merkoci A 2007 *Electroanalysis* **19** 739
26. Wang J 2005 *Electroanalysis* **17** 7
27. Zhang H, Meng Z, Wang Q and Zheng J 2011 *Sens. Actuators B* **158** 23
28. Tasviri M, Rafiee-Pour H A, Ghourchian H and Gholami M R 2011 *J. Mol. Catal. B: Enzymatic* **68** 206
29. Gopalan A I, Lee K P, Ragupathy D, Lee S H and Lee J W 2009 *Biomaterials* **30** 5999
30. Raj C R and Chakraborty S 2006 *Biosens. Bioelectron.* **22** 700
31. Chakraborty S and Raj C R 2007 *Electrochem. Commun.* **9** 1323
32. Gong K, Dong Y, Xiong S, Chen Y and Mao L 2004 *Biosens. Bioelectron.* **20** 253
33. Wildgoose G G, Banks C E, Leventis H C and Campton R G 2006 *Microchim. Acta* **152** 187
34. Jin G P, He J B, Rui Z B and Meng F S 2006 *Electrochim. Acta* **51** 4341
35. Zhang M and Gorski W 2005 *Anal. Chem.* **77** 3960
36. Musameh M, Wang J, Merkoci A and Lin Y 2002 *Electrochem. Commun.* **4** 743
37. Raoof J B, Ojani R and Chekin F 2009 *J. Chem. Sci.* **121** 1083
38. Laviron E 1979 *J. Electroanal. Chem.* **101** 19
39. Gao Z N, Zhang J and Liu W Y 2005 *J. Electroanal. Chem.* **580** 9
40. Bard A J and Faulkner L R 2001 *Electrochemical methods: Fundamentals and applications* (New York: John Wiley and Sons)
41. Galus Z 1976 *Fundumentals of electrochemical analysis* (New York: Ellis Horwood)
42. Heli H, Majdi S and Sattarahmady N 2010 *Sens. Actuators B* **145** 185
43. Shahrokhian S, Kamalzadeh Z, Bezaatpour A and Boghaei D M 2008 *Sens. Actuators B* **133** 599
44. Beitollahi H, Raoof J B and Hosseinzadeh R 2011 *Talanta* **85** 2128
45. Westerman M P, Zhang Y, McConnell J P, Chezick P A, Neelam R, Freels S, Feldman L S, Allen S, Baridi R, Feldman L E and Fung L W M 2000 *Am. J. Hematol.* **65** 174