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Full Paper

Electrocatalytic Determination of Cysteamine in the Presence of Folic Acid Using a Modified Carbon Nanotube Paste Electrode

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Abstract- Electrocatalytic oxidation of cysteamine at a carbon paste electrode modified with benzoylferrocene has been studied. The modified electrode showed excellent electrocatalytic activity toward cysteamine oxidation in phosphate buffer solution (PBS) (pH 7.0) with an overpotential of about 225 mV lower than that of the bare electrode. Under the optimized experimental conditions, the proposed electrochemical cysteamine sensor exhibited a linear calibration plot ranged from 7.0×10^{-8} to 2.5×10^{-4} M with a detection limit of 5.0×10^{-8} M. Also, Square wave voltammetry (SWV) was used for simultaneous determination of cysteamine and folic acid at the modified electrode. Finally, the proposed method was applied to the determination of cysteamine and folic acid in urine.

Keywords- Cysteamine, Folic Acid, Voltammetry, Carbon Nanotube Paste Electrodes

1. INTRODUCTION

Carbon nanotubes (CNTs) have been the subject of intense research because of their high aspect ratio, electrical conductivity, chemical stability and high mechanical strength [1-6]. The ability of CNTs to promote the electron-transfer reactions of important molecules has made them attractive for several electrochemical and electroanalytical purposes, such as building of different kinds of modified electrodes [7-13].

Nephropathic cystinosis is a rare autosomal recessive disease characterized by an excessive intra lysosomal cystine accumulation due to a defect of its transport system in the lysosomal membrane [14]. This metabolic trouble leads to a multisystemic disease affecting all organ systems. Thus, within the first years of life, newborn children develop a renal Fanconi syndrome with polyuria, glucosuria, phosphaturia, and aminoaciduria. During their first decade of life, children are affected by growth impairment and progressive decline in renal function leading to hemodialysis or renal transplantation. In affected children, cystine accumulation is also responsible for delayed complications such as hypothyroidism, retinopathy, myopathy, pancreatic dysfunction, and dementia [15]. First is the in vitro experiment of the cysteine depleting effects of cystamine on cystinotic fibroblasts [16]. These results were confirmed on leukocytes in vivo [16]. The underlying mechanism of action was shown to be a disulphide exchange reaction between cysteamine and cystine. The product of the reaction, a mixed disulphide of cysteamine and cystine, is carried out of the lysosome by a lysine porter which is not defective in cystinosis patients [14,15]. Based on this biochemical evidence, cysteamine appears to be a promising treatment for cystinosis. Several long-term clinical trials have shown that cysteamine administration (as cysteamine hydrochloride) stabilize renal function, delays glomerular deterioration, and improve linear growth [14]. Therefore, determination of this compound is very important. Several methods have been reported for the determination of cysteamine in different samples including chromatography [17-20], electrophoresis [21,22], and electrochemical methods [23-27] using modified electrodes.

Folic acid and its derivates, are very important essential compounds, which belong to the group of vitamins B – vitamin B_9 (in some cases is denoted as vitamin M). Folic acid and folates (if not specified otherwise, it is not differentiated between “folic acid” and its salts – folates in this manuscript) are enzymatically transformed in the small intestine into their biologically active and very important form tetrahydrofolate (H4-folate) [28]. This reaction is catalyzed by enzyme tetrahydrofolate dehydrogenase (dihydrofolate reductase, EC 1.5.1.3) [28,29]. H4-folate participates in transferring one-carbon groups which are necessary for DNA and RNA biosynthesis, red blood cells formation and methylation processes in organism.

H4-folate also reduces homocysteine to cysteine [30]. Sufficient dietary intake of folic acid prevents neural tube defects (e.g., anencephaly or spina bifida) and certain types of

anemia [28]. The protective effect of folic acid was also observed for treatment of some diseases like stroke, ischaemic heart disease [31] or colorectal cancer [32]. The recommended daily intake of folic acid is 0.2 mg (0.453 μmol) for adults and 0.4 mg (0.906μmol) for pregnant women [33]. Many methods for the folic acid determination have been developed due to its biological significance. These methods include chromatography, capillary electrophoresis, spectrophotometry or electrochemical techniques [34-38].

To the best of our knowledge, most previously published electrochemical studies have dealt with individual determination of cysteamine or folic acid utilizing carbon paste electrodes or other kinds of modified electrode. Only one study has been reported on the simultaneous determination of cysteamine and folic acid using modified carbon nanotube paste electrodes [23], which is the focus of the present study. Therefore, in continuation of our recently studies concerning the preparation of modified electrodes [4,6,9,13,23 and 36], in the present work, we described the preparation of a new electrode composed of CNPE modified with benzoylferrocene (BFCNPE) and investigate its performance for the electrocatalytic determination of cysteamine in aqueous solutions. We also evaluated the analytical performance of the modified electrode for quantification of cysteamine in the presence of folic acid.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat(PGSTAT 12, Eco Chemie, The Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25±1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the BFCNPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. Cysteamine, folic acid and all other reagents were of analytical grade from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-11.0. Multiwalled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm, and tube length from 0.5 to 200 μm were prepared from Nanostructured & Amorphous Materials, Inc. Benzoylferrocene was synthesized in our laboratory as reported previously [13].

2.2. Preparation of the electrode

The BFCNPEs were prepared by hand mixing 0.01 g of BF with 0.89 g graphite powder and 0.1 g CNTs with a mortar and pestle. Then, ~ 0.7 mL of paraffin oil was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste

was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, BF modified carbon paste electrode (BF-CPE) without carbon nanotubes, carbon nanotubes paste electrode (CNPE) without BF, and unmodified carbon paste electrode (CPE) in the absence of both BF and carbon nanotubes were also prepared in the same way.

3. RESULT AND DISCUSSION

3.1. Electrochemical properties of BFCNPE

BFCNPEs were prepared and their electrochemical properties were studied in a 0.1 M phosphate buffer solution (PBS) (pH 7.0) using CV. The experimental results showed well-defined and reproducible anodic and cathodic peaks related to benzoylferrocene/ benzoylferricenium redox system, which show a quasi-reversible behavior in an aqueous medium. The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH 7.0 from five separately prepared BFCNPEs (Table 1).

Table 1. Cyclic voltammetric data obtained for constructed BFCNPE in 0.1 M PBS (pH 7.0) at 50 mV s^{-1}

E_{pa} (V)	E_{pc}(V)	$E_{1/2}$ (V)	ΔE_p (V)	I_{pa} (μA)	I_{pc} (μA)
0.645	0.540	0.592	0.105	3.42	-1.97

In addition, the longterm stability of the BFCNPE was tested over a three-week period. When CVs were recorded after the modified electrode was stored in atmosphere at room temperature, the peak potential for cysteamine oxidation was unchanged and the current signals showed less than 2.9% decrease relative to the initial response. The antifouling properties of the modified electrode toward cysteamine oxidation and its oxidation products were investigated by recording the cyclic voltammograms of the modified electrode before and after use in the presence of cysteamine. Cyclic voltammograms were recorded in the presence of cysteamine after having cycled the potential 13 times at a scan rate of 10 mV s^{-1}. The peak potentials were unchanged and the currents decreased by less than 2.8%. Therefore, at the surface of BFCNPE, not only the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decreases.

3.2. Electrocatalytic oxidation of cysteamine at a BFCNPE

Fig. 1 depicts the CV responses for the electrochemical oxidation of 100.0 µM cysteamineat unmodified CPE (curve c), CNPE (curve d), BFCPE (curve a) and BFCNPE (curve b). As it is seen, while the anodic peak potential for cysteamine oxidation at the CNPE, and unmodified CPE are 840 and 870 mV, respectively, the corresponding potential at BFCNPE and BFCPE is ~ 645 mV. These results indicate that the peak potential for cysteamine oxidation at the BFCNPE and BFCPE electrodes shift by ~ 195 and 225 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, BFCNPE shows much higher anodic peak current for the oxidation of cysteamine compared to BFCPE, indicating that the combination of CNTs and the mediator (BF) has significantly improved the performance of the electrode toward cysteamine oxidation (Table 2).

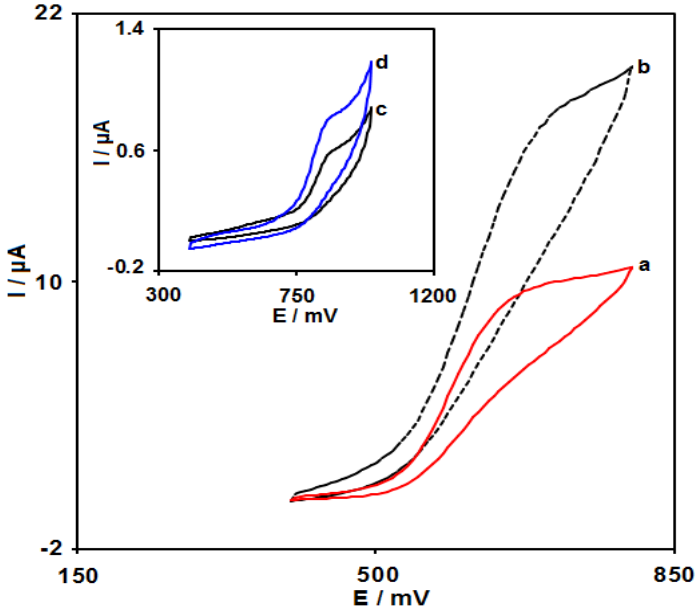

Fig. 1. CVs of BFCPE (a) and BFCNPE (b) in 0.1 M PBS (pH 7.0) containing 100.0 µM cysteamine; (c) and (d) are CVs of CPE and CNPE in 0.1 M PBS (pH 7.0) containing 100.0µM cysteamine, (e) CVs of CNPE in 0.1 M PBS (pH 7.0). In all cases scan rate is 10 mV s^{-1}

The effect of scan rate on the electrocatalytic oxidation of cysteamine at the BFCNPE was investigated by cyclic voltammetry (CV) (Fig. 2). As can be observed in Fig. 3, the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) *vs.* the square root of scan rate ($\nu^{1/2}$) was found to be linear in the range of 2-20 mV s^{-1}, suggesting that, at sufficient over-potential, the process is diffusion rather than surface controlled (Fig. 2A).

Table 2. Comparison of oxidation of 100.0 µM cysteamine on various electrode surfaces at pH 7.0.

Electrode	Anodic peak potential / mV	Anodic peak current / µA
BFCNPE	645	17.9
BFCPE	645	9.8
CNPE	840	0.9
CPE	870	0.6

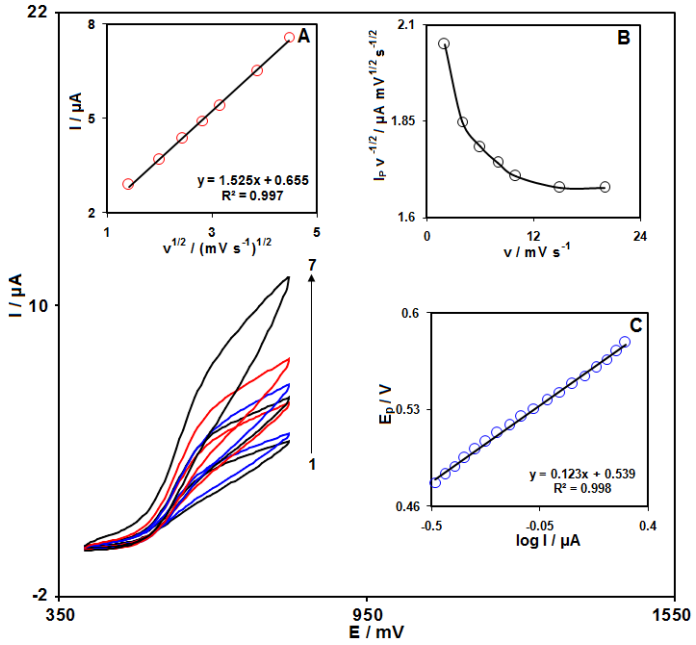

Fig. 2. CVs of BFCNPE in 0.1 M PBS (pH 7.0) containing 100.0 µM cysteamine at various scan rates; numbers 1-7 correspond to 2, 4, 6, 8, 10, 15 and 20 mV s^{-1}, respectively. Insets: Variation of **(A)** anodic peak current *vs.* $v^{1/2}$; **(B)** normalized current ($I_p/v^{1/2}$) *vs.* v; **(C)** Tafel plot derived from the CV at the scan rate of 4 mV s^{-1}

A plot of the scan rate-normalized current ($I_p/v^{1/2}$) *vs.* scan rate (Fig. 2B) exhibits the characteristic shape typical of an EC' process [39].

Fig. 2C shows the Tafel plot for the sharp rising part of the voltammogram at the scan rate of 4 mV s^{-1}. If deprotonation of cysteamine is a sufficiently fast step, the Tafel plot can be used to estimate the number of electrons involved in the rate determining step. A Tafel slope of 0.1232 V was obtained which agrees well with the involvement of one electron in the rate determining step of the electrode process [39], assuming a charge transfer coefficient, α of 0.52.

3.3. Chronoamperometric measurements

Chronoamperometric measurements of cysteamine at BFCNPE were carried out by setting the working electrode potential at 0.7 V (at the first potential step) and at 0.5 V (at second potential step) *vs.* Ag/AgCl/KCl (3.0 M) for the various concentration of cysteamine in PBS (pH 7.0) (Fig.3). For an electroactive material (cysteamine in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [39]. Experimental plots of I *vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of cysteamine (Fig. 3A). The slopes of the resulting straight lines were then plotted *vs.* cysteamine concentration (Fig. 3B). From the resulting slope and Cottrell equation the mean value of the D was found to be 4.9 $\times 10^{-5}$ cm^2/s.

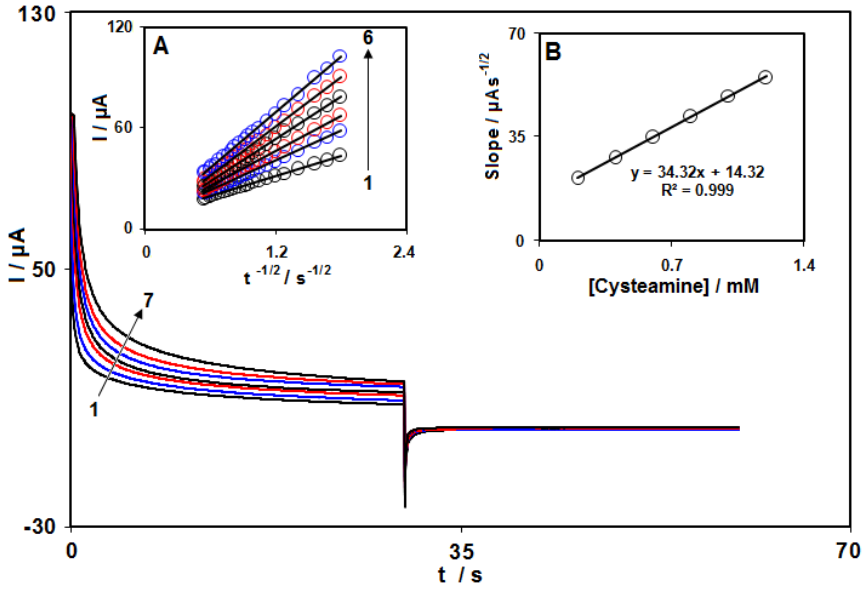

Fig. 3. Chronoamperograms obtained at BFCNPE in 0.1 M PBS (pH 7.0) for different concentration of cysteamine. The numbers 1–7 correspond to 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mM of cysteamine. Insets: **(A)** Plots of I *vs.* $t^{-1/2}$ obtained from chronoamperograms 2–7. **(B)** Plot of the slope of the straight lines against cysteamine concentration

3.4. Calibration plot and limit of detection

SWV method was used to determine the concentration of cysteamine (Fig. 4). The plot of peak current *vs*. cysteamine concentration consisted of two linear segments with slopes of 0.681 and 0.036 μA μM^{-1} in the concentration ranges of 0.07 to 10.0 μM and 10.0 to 250.0 μM, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation [39]. The detection limit (3σ) of cysteamine was found to be 0.05 μM. These values are compared with values reported by other research groups for electrocatalytic oxidation of cysteamine at the surface of chemically modified electrodes by other mediators (Table 3).

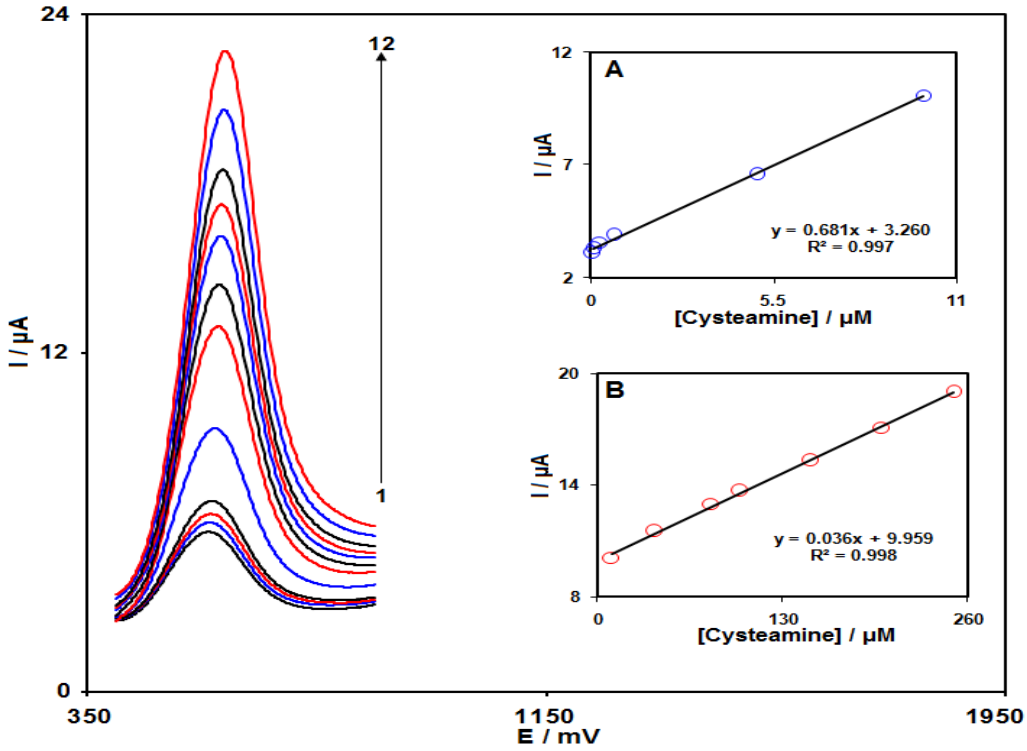

Fig. 4. SWVs of BFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of cysteamine; Numbers 1-12 correspond to 0.07, 0.1, 0.3, 0.7, 5.0, 10.0, 40.0, 80.0, 100.0, 150.0, 200.0 and 250.0 μM of cysteamine. Inset: **(A)** plots of the electrocatalytic peak current as a function of cysteamine concentration in the range of 0.07 to 10.0 μM and **(B)** 10.0 to 250.0 μM

Table 3. Comparison of the efficiency of some modified electrodes used in the electrocatalysis of cysteamine

Electrode	Modifier	pH	Peak potential shift (mV)	LOD (M)	LDR (M)	Ref.
Glassy carbon electrode	1,2-naphthoquinone-4-sulfonic acid sodium	6.0	710	3.0×10^{-6}	5.0×10^{-6}-2.7×10^{-4}	24
Carbon paste	Vinylferrocene	7.0	390	5.0×10^{-8}	9.0×10^{-8}-5.0×10^{-4}	25
Carbon paste	Poly-N,N-dimethylaniline/Ferro cyanide Film	7.0	-	7.97×10^{-5}	8.0×10^{-5}-1.14×10^{-2}	27
Carbon paste	Benzoylferrocene	7.0	225	5.0×10^{-8}	7.0×10^{-8}-2.5×10^{-4}	This work

3.5. Simultaneous determination of cysteamine and folic acid

To our knowledge, only one paper has used the modified electrode for simultaneous determination of cysteamine and folic acid [13] and this work is the second report for simultaneous determination of cysteamine and folic acid using modified carbon nanotube paste electrode. This was performed by simultaneously changing the concentrations of cysteamine and folic acid, and recording the SWVs. The voltammetric results showed well-defined anodic peaks at potentials of 605 and 800 mV, corresponding to the oxidation of cysteamine and folic acid, respectively, indicating that simultaneous determination of these compounds is feasible at the BFCNPE as shown in Fig. 5.

The sensitivity of the modified electrode towards the oxidation of cysteamine was found to be 0.038 $\mu A\ \mu M^{-1}$. This is very close to the value obtained in the absence of folic acid (0.036 $\mu A\ \mu M^{-1}$, see Section 3.4), indicating that the oxidation processes of these compounds at the BFCNPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

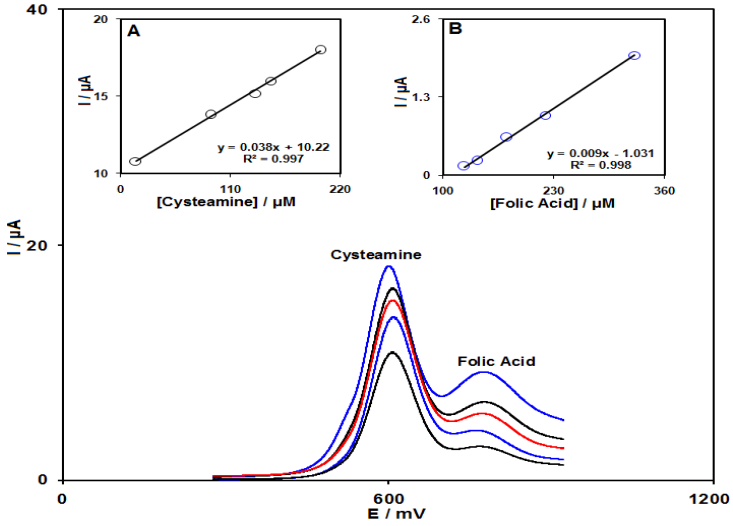

Fig. 5. SWVs of BFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of cysteamine + Folic acid in µM, from inner to outer: 15.0+125.0, 90.0+140.0, 135.0+175.0, 150.0+220.0 and 200.0+325.0 respectively. Insets **(A)** plots of I_p*vs.* cysteamine concentration; and **(B)** plots of I_p*vs.* folic acid concentrations

3.6. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of cysteamine and folic acid in urine sample. Based on the repeated square wave voltammetric responses (n=3) of the samples that were spiked with specified concentration of cysteamine and folic acid, measurements were made for determination of cysteamine and folic acid concentrations in the urine sample. The results are listed in Table 4.

Table 4. The application of BFCNPE for determination of cysteamine and folic acid in urine (n=3)

Added (µM)		**Found (µM)**		**Recovery (%)**	
Cysteamine	Folic acid	Cysteamine	Folic acid	Cysteamine	Folic acid
0	0	-	-	-	-
10.0	15.0	9.6	15.3	96.0	102.0
20.0	25.0	19.4	24.8	97.0	99.2
30.0	35.0	29.2	35.5	97.3	101.4
40.0	45.0	41.3	44.8	103.3	99.5
50.0	55.0	49.1	55.6	98.2	101.1

4. CONCLUSION

In this paper, a novel modified carbon nanotube paste electrode for the detection of cysteamine was constructed. The results of this study indicated that the electrode exhibited linear response from 0.07 to 250.0 μM with a detection limit of 0.05 μM. Importantly, the proposed electrode was successfully applied for simultaneous determination of cysteamine andfolic acid. Also, the constructed electrode was used for determination of cysteamine and folic acid inurine sample.

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