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NICKEL HEXACYANOFERRATE MODIFIED SCREEN-PRINTED CARBON ELECTRODE FOR SENSITIVE DETECTION OF ASCORBIC ACID AND HYDROGEN PEROXIDE

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TABLE OF CONTENTS

1. ABSTRACT

Electrochemically modified screen-printed carbon electrode (SPCE) has been prepared by electrodepositing nickel hexacyanoferrate(III) (NiHCF) onto the electrode surface using cyclic voltammetry (CV). The performance of NiHCF-SPCE sensor was characterized and optimized by controlling several operational parameters. The NiHCF film has been proven to remain stable after CV scanning from 0 to +1.0 V vs. Ag/AgCl in the pH range of 3 to 10 and is re-useable. The most favourable supporting electrolyte solution exhibiting the optimum electroanalytical performance of the NiHCF-SPCE sensor was found to be 0.2 mol/L sodium nitrate. The electrochemical response toward ascorbic acid (AA) and H_2O_2 in 0.2 mol/L sodium nitrate solution was studied by using CV and the results showed that both analytes were electrocatalytically oxidized at approximately +0.4 V, while H_2O_2 also revealed a reduction signal at -0.8 V vs. Ag/AgCl. The NiHCF-SPCE sensor exhibited highly linear response for AA and H_2O_2 in the examined concentration range from $5.0x10^{-5}$ to $1.5x10^{-3}$ mol/L and from $2.0x10^{-5}$ to $1.0x10^{-3}$ mol/L (at +0.4 V), with the correlation coefficients of 0.999 and 0.998, respectively. The reproducibility of the NiHCF-SPCE sensor was followed for the determination of AA by using four individual electrodes, and the relative standard deviation of CV peak currents varied between 0.9 % and 2.2 %. The proposed NiHCF-SPCE has been shown to be a very attractive electrochemical sensor for AA and H_2O_2, also in a view of inexpensive mass production of disposable single-use sensors. The NiHCF-SPCE sensor was tested by measuring AA in multivitamin tablets, with recoveries obtained between 94.4 % and 108.2 % (n=5).

2. INTRODUCTION

Screen-printing technology has been recognized as a simple and fast method for the mass production of disposable thick-film electrodes. One of the most attractive characteristics is its ability to produce inexpensive and reproducible substrate electrodes, which are convenient for further modifications. Most commercial electrochemical biosensors, in particular those for the detection of glucose, are screen-printed disposable sensors. These sensors have revolutionized the way of performing routine electroanalysis and can be viewed as disposable single-use sensors onto which a droplet of the sample is applied directly. This protocol can avoid time-consuming sample preparation associated with ubiquitous contamination problems and reduce the loss of response caused by the electrode fouling and/or denaturation of the bioactive molecules such as enzyme. These sensors can therefore successfully replace the traditional bulky electrodes and the conventional voltammetric cells. It has been demonstrated that screen-printed electrodes (SPEs) can be reliably manufactured (1,2) and applied for the determination of a great variety of biomolecules (3,4) and several inorganic species (5).

Since the unmodified SPEs usually exhibit relatively low sensitivity and selectivity, several bulk or surface modification routes have been adopted to improve their performance. These techniques include the electrochemical pre-treatment (6), coating of the electrode surface with a Nafion film (7), with redox mediators (8,9), cellulose acetate (10,11) and/or with enzymes (1,12). These modification techniques can convert relatively inexpensive

and disposable substrate electrodes into very specific electrocatalytically active and even re-useable electrochemical sensors.

Ascorbic acid (AA) is known as an electroactive molecule (vitamin C), with immense importance for humans and animals as a diet component, antioxidant, for neutralizing toxic peroxides and stabilizing free radicals (13,14). Its detection, based on the electrochemical oxidation, for example at a bare glassy carbon electrode, requires relatively high applied potential of about +0.5 V vs. Ag/AgCl (6). However, the overpotential for AA oxidation can be lowered at electrochemically modified electrodes (15,16). For example, at hexacyanoferrate(III) modified electrode (17) and at cobalt phthalocyanine modified graphite epoxy composite electrode (18), the electrocatalytic oxidation was shifted significantly towards lower potentials. In addition to these studies, determination of ascorbic acid through the modified SPEs have been reported by several authors. In this case, the successfully applied redox mediators include, e.g. ferricyanide (19) and 7,7,8,8-tetracyanoquinodimethane (20). These organo-metallic redox mediators exhibit good electrocatalytic activity toward the oxidation of ascorbic acid at significantly lower potentials, thus avoiding the adverse effect of some potentially present interferents.

H_2O_2 is the analyte of great (bio)importance in connection with many electrochemical biosensors, such as the biosensors for uric acid, glucose, cholesterol, etc. These sensors are based on the electrochemical detection of H_2O_2 generated through an enzyme activity. The continuous work on improving the sensors' performance towards the detection of H_2O_2 is of great interests and worthy of further investigation. In particular, the oxidation of H_2O_2 at conventional unmodified electrodes requires relatively high operating potential of about +1.0 V and hence, the detection suffers from poor selectivity and high background contribution. There are several reports suggesting the application of organo-metallic redox mediators such as Prussian Blue (21-23), which suffers from possible deterioration when detecting hydrogen peroxide at physiological pH, dimethylferrocene (24), Meldola Blue (25) and others (26). In addition, the manganese dioxide film modified SPE has been introduced to measure H_2O_2 (27), while J. Wang *et al* introduced a palladium-dispersed screen-printed carbon electrode to detect H_2O_2 at lower potentials in connection with a glucose sensor (28). Such modified electrodes had previously shown a great promise for the detection of several important biomolecules, and provided a platform for the preparation of a robust electroanalytical system with the application of disposable one-shot sensors.

Nickel hexacyanoferrate modified electrodes based on a glassy carbon electrode and on a platinum disk microelectrode have been already studied for measuring the thiosulfate (29) and dopamine (30). In addition, the modification of gold electrode surface by nickel hexacyanoferrate has been reported (31), and Giorgetti *et al* reported on a coated wire cation-selective electrode modified with nickel hexacyanoferrate for potentiometric sensing of several cations (32).

To the best of our knowledge, no report has been published on the application of nickel hexacyanoferrate as a redox mediator for the modification of a screen-printed carbon electrodes (SPCEs). In the present paper we described the preparation of the SPCE which was subsequently modified with the afore mentioned redox mediator. The method relies on the surface modification of a substrate SPCE supported by electrochemical reaction of nickel chloride and potassium hexacyanoferrate(III). The nickel hexacyanoferrate film was formed on the surface of a SPCE by employing cyclic voltammmetry, while the thickness of the film was controlled by changing the number of voltammetric cycles and the concentration of nickel chloride and/or potassium hexacyanoferrate in the modification solution. The influence of factors, such as supporting electrolyte composition and pH upon the response of NiHCF sensor towards the ascorbic acid and H_2O_2 are discussed. The solution of sodium nitrate (pH 7) was found to provide the most convenient detection for both analytes. Finally, we used NiHCF-SPCE sensor to measure the ascorbic acid in plain vitamin C tablets, Aspirin plus vitamin C tablets, and multivitamin tablets.

3. EXPERIMENTAL DESIGN

3.1. Apparatus

Cyclic voltammetry was performed using modular electrochemical system Autolab (Eco chemie, Utrecht, The Netherlands) equipped with PSTAT10 module and driven by a GPES software (Eco, Chemie), and a model 273 Potentiostat/Galvanostat with a model 270 electrochemical analysis software (PAR). Experiments were carried out in a Metrohm type electrochemical cell at room temperature. A three electrode configuration was used with the unmodified or modified SPCE as a working electrode, a Ag/AgCl/(satd. KCl) as a reference electrode and a platinum wire as a counter electrode. All potential in this work are referred to the Ag/AgCl/ (satd. KCl) as a reference. MA 5736 pH meter was used to measure the pH of electrolyte solution.

3.2. Reagents

All chemicals used were of analytical reagent grade, unless stated otherwise. Ascorbic acid, nickel chloride and potassium hexacyanoferrate(III) were obtained from Kemika (Zagreb, Croatia), hydrogen peroxide (30%) from Belinka (Ljubljana, Slovenia), Plivit C tablets (500 mg Vitamin C each) were obtained from Pliva Pharmaceutical works (Zagreb, Croatia), Aspirin Plus C tablets (240 mg vitamin C each) were obtained from Bayer Pharma (Ljubljana, Slovenia), Vitamin C tablets (350 mg vitamin C each) were from Krka (Novo Mesto, Slovenia), and Mutivitamin tablets (75 mg vitamin C each) were from Mebex International (Ljubljana, Slovenia). Standard solutions of ascorbic acid and H_2O_2 were prepared daily and wrapped in the aluminium foil to prevent photo and thermal degradation. The supporting electrolyte solution used throughout the experiments was 0.2 mol/L sodium nitrate (pH 7), unless stated otherwise. All solutions were prepared with water which was first deionized and then further purified via a Milli-Q unit (Millipore, Milford, MA) and degassed for 10 min with pure argon. Other chemical reagents were used as received.

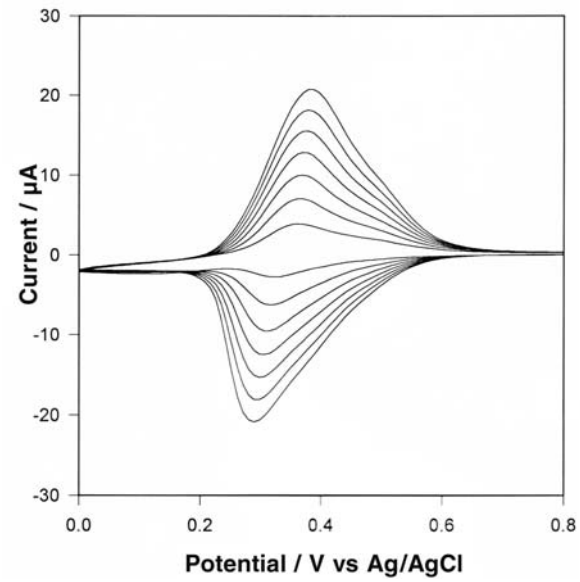

Figure 1. Cyclic voltammograms of NiHCF film electro-deposition on SPCE from a solution containing 0.05 mmol/L $NiCl_2$, 0.5 mmol/L $Fe(CN)_6^{3-}$ and 0.2 mol/L $NaNO_3$; scan rate: 50 mV/s.

3.3. Preparation of screen-printed carbon electrodes

The SPCEs were fabricated at the Northern Ireland Bio-engineering Center, UK. The carbon layer was printed through a polyester screen with a mesh size T90 onto the polyester substrate using an Avgon semi-auto screen-printer. The mesh size indicates the number of threads per centimetre. The screen-printed carbon electrode was left to cure in the oven at 80 ^oC for 20 minutes allowing the solvents to evaporate. The dried SPCE was subsequently covered by an insulation layer through screen-printing of the electrical insulating ink, leaving the active electrode surface and the contact pad exposed. The D14 carbon ink used for the preparation of SPCEs was obtained from Gwent Electronic Materials Ltd, UK, and the insulation ink was a commercial one from Acheson Colloids Company, UK. Finally, the electrodes were rinsed with double-distilled water and stored dry in a sealed opaque bag. Prior to electrochemical modification, the screen-printed carbon electrodes were cleaned by scanning the potential between -0.5 and +1.0 V in a 0.5 mol/L H_2SO_4 with the applied scan rate of 100 mV/s until the reproducible voltammograms were obtained.

3.4. Modification of screen-printed carbon electrodes with nickel hexacyanoferrate(III)

Before modification, the SPCE was pre-treated by CV from 0.0 to +1.0 V in a 0.2 mol/L sodium nitrate solution with the scan rate of 50 mV/s until the reproducible voltammmograms were obtained. Then the SPCE was placed in the modification solution containing 0.5 mmol/L nickel chloride, 0.5 mmol/L potassium hexacyanoferrate and 0.2 mol/L sodium nitrate followed by the electro-polymerization performed by CV in the range from 0.0 to +1.0 V by applying 10 voltammetric cycles at 50 mV/s. All modified electrodes exhibited very similar redox peak currents, achieved by controlling the number of voltammetric cycles. After modification, the electrode was thoroughly rinsed with water and transferred into a 0.2 mol/L sodium nitrate solution, where the potential was cycled repetitively until constant CV response was obtained.

3.5. Procedures

The measurements of ascorbic acid and H_2O_2 were performed by employing cyclic voltammetry. The examined concentrations used in both cases were over the range from 1.0 $x10^{-6}$ mol/L up to 1.0 x 10^{-3} mol/L for ascorbic acid and for H_2O_2 to determine the corresponding linear range and limits of detection. Standard addition method was subsequently employed by adding 100 µl of ascorbic acid or H_2O_2 standard solution of a known concentration into a 20 ml of supporting electrolyte solution (0.2 mol/L sodium nitrate, pH 7). Volume changes were taken into account when calculating the final concentrations of ascorbic acid and H_2O_2. The precision and reproducibility of NiHCF modified SPCEs were determined using four individual electrodes from 0 to 5.0 x 10^{-4} mol/L ascorbic acid.

3.6 Ascorbic acid sample preparation

Five tablets containing ascorbic acid were weighed and ground to powder. A weighed powder equivalent to about 352.3 mg was dissolved in water, diluted to 50 ml, and filtered. An appropriate volume of the filtered solution was diluted again to obtain the final solution containing an appropriate amount of ascorbic acid. The concentrations of ascorbic acid were obtained for all samples using the standard addition method.

4. RESULTS AND DISCUSSION

4.1. NiHCF modification of SPCEs

In order to obtain uniform and stable modification layer, cyclic voltammetric mode was chosen for the electrochemical deposition of NiHCF onto the surface of a substrate electrode. Figure 1 shows seven well-defined cyclic voltammograms presenting a nickel hexacyanoferrate growth on the surface of a SPCE. A redox couple revealed a cathodic peak at around +0.3 V and a corresponding anodic peak at around +0.4 V, with the peak currents gradually increasing with the number of CV scans. Thus, various thicknesses of the electrodeposited films can be obtained by simply controlling the number of voltammetric cycles.

To get more insights into the electrochemical behaviour of the newly prepared NiHCF-SPCE sensor, the cyclic voltammograms at different scan rates were followed in a 0.2 mol/L $NaNO_3$ solution (not shown). The ratio between anodic and cathodic peak currents of the redox couple remained practically unchanged with increasing scan rate, while a separation between anodic and cathodic peak potentials gradually increased, indicating quasi-reversible redox reaction. In addition, the peak currents increased linearly with the examined scan rate between 10 and 80 mV/s, reflecting the presence of adsorbed NiHCF on the surface of a SPCE. A negligible change in the peak current was observed when the NiHCF-SPCE sensor was

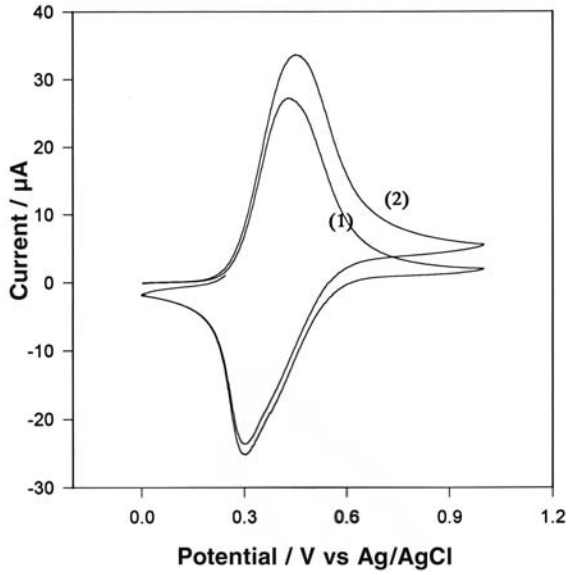

Figure 2. Cyclic voltammograms at NiHCF-SPCE in a 0.1 M PBS buffer(pH 7.4) solution (1), and after addition of 3.0 x 10^{-4} mol/L ascorbic acid (2); scan rate: 50 mV/s.

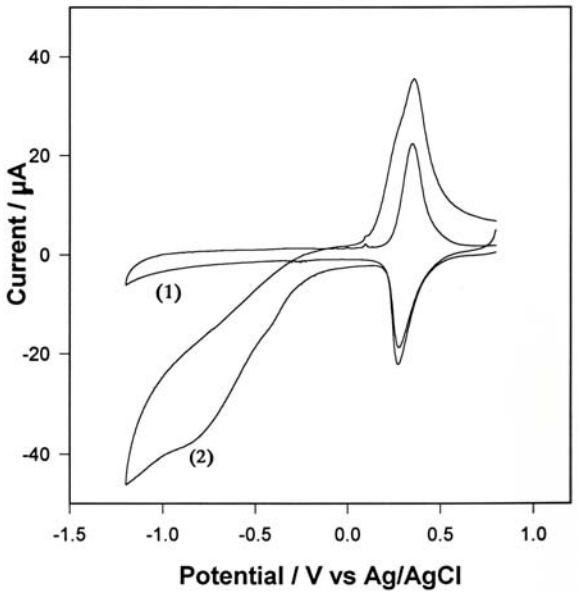

Figure 3. Cyclic voltammograms at NiHCF-SPCE in a 0.1 M PBS buffer(pH 7.4) solution (1), and after addition of 1.47 x 10^{-4} mol/L H_2O_2 (2); scan rate: 50 mV/s.

checked by cycling the potential with the scan rate of 50 mV/s for 30 cycles in a blank electrolyte solution. Keeping the modified electrode in the air for more then one month, the NiHCF film was still stable without noticeable loss of its activity, suggesting a favourable long term stability of the NiHCF modified SPCE.

4.2. Electrochemical reactions of ascorbic acid and H_2O_2 at SPCEs

Electrochemical oxidation of ascorbic acid was investigated at unmodified and at NiHCF modified SPCE. The oxidation peak potential for ascorbic acid was observed at approx. +0.4 V at unmodified electrode (not shown), while the current decreased with each consecutive voltammetric scan, e.g. after two cycles, the peak current decreased for approx. 45%. This phenomenon could be induced by the electrochemical polymerization of ascorbic acid onto the surface of an unmodified SPCE and consequently inhibiting the interfacial electron transfer at the electrode surface. Thus, it was inevitable to modify the electrode with aim to reduce the poisoning/blocking of the exposed electrode surface.

At NiHCF modified SPCE (Figure 2), the oxidation signal for ascorbic acid is observed also at approx. +0.4 V, superimposed onto the oxidation part of the NiHCF redox couple, associated with the peak current being approximately 1.2 times of that at the unmodified SPCE for the same concentration of ascorbic acid (3.0 x 10^{-4} mol/L). In addition, the peak current slightly increased when the potential was cycled for the first two times, probably reflecting the equilibration of the NiHCF film surface. The amplified oxidation current at the NiHCF modified SPCE in the presence of ascorbic acid is due to the fact that the ascorbic acid diffuses toward the modified electrode surface and reacts with $NaNiFe(CN)_6$ changing it into $Na_2NiFe(CN)_6$. Thus, the electrochemical process at the NiHCF film can be expressed as follows:

$$2NaNiFe(CN)_6 + 2Na^+ + AA(red) \longrightarrow 2Na_2NiFe(CN)_6 + AA(ox)$$

$$2Na_2NiFe(CN)_6 \longrightarrow 2NaNiFe(CN)_6 + 2e^-$$

These equations have already been reported in connection with the ascorbic acid detection using a microdisk platinum modified electrode.

The response toward H_2O_2 at the unmodified and modified NiHCF was also examined. After the addition of 1.47x10^{-4} mol/L H_2O_2, there was no corresponding signal observed upon cycling the potential in the range from -1.0V to +0.6V at the unmodified SPCE, only a small increase of the current at approx -0.8V, which could be due to the reduction of a small amount of H_2O_2 at the bare electrode surface. In contrast, Figure 3 shows two cyclic voltammograms for the blank solution (1) and for the same solution containing 1.47x10^{-4} mol/L H_2O_2 (2) at the NiHCF modified SPCE.

Using this sensor, H_2O_2 was found electroactive at both cathodic and anodic potentials with one oxidation peak at approx. +0.38 V superimposed onto the oxidation part of the NiHCF redox couple, and one reduction peak at approx. -0.8 V. The peak at +0.38 V is well-defined together with significantly increased current response as a consequence of the increased H_2O_2 concentration. The reduction current at -0.8V is also well-pronounced, with the peak current of about 30-fold larger than the one corresponding to the unmodified electrode (not shown). Both signals at +0.38 and at -0.8 V can be employed for the determination of H_2O_2. The oxidation and reduction reactions of H_2O_2 on the surface of NiHCF film can be presented by the following equations:

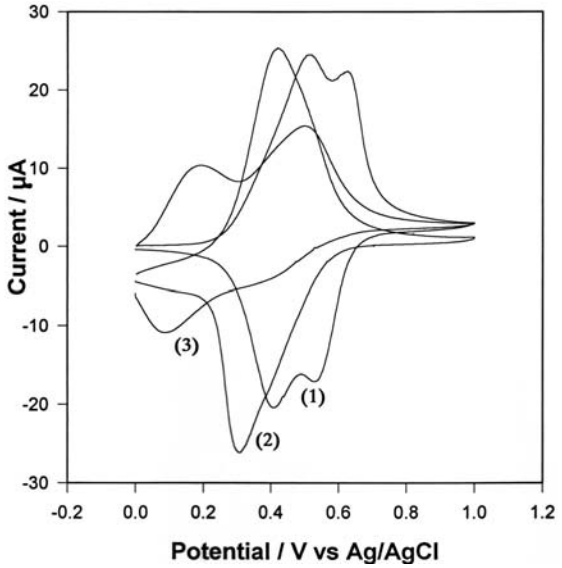

Figure 4. Cyclic voltammograms at NiHCF-SPCE of $2.0x10^{-4}$ mol/L ascorbic acid in different supporting electrolyte solutions: 0.2 mol/L KCl (1), 0.2 mol/L $NaClO_4$ (2), 0.2 mol/L LiCl (3); scan rate: 50 mV/s.

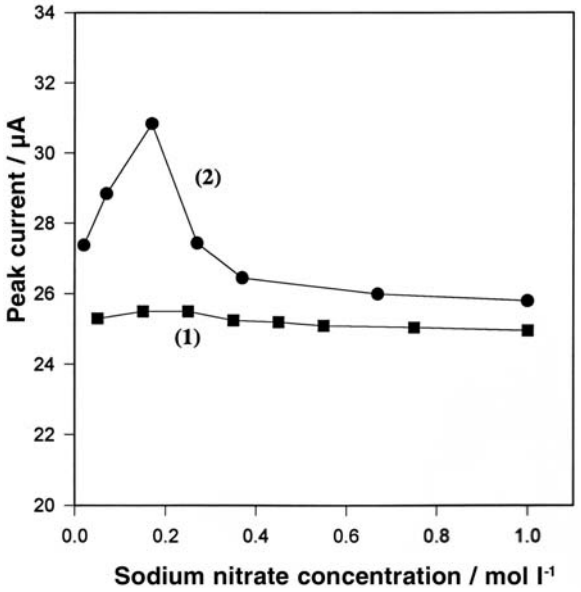

Figure 5. Effect of $NaNO_3$ concentration in the electrolyte solution upon the peak currents for $2.0x10^{-4}$ mol/L ascorbic acid (1) and $1.47x10^{-4}$ mol/L H_2O_2 (2).

$Na_2NiFe(CN)_6 + H_2O_2$ ----->$NaNiFe(CN)_6 + H_2O$
$NaNiFe(CN)_6 + H_2O_2$ -----> $Na_2NiFe(CN)_6 + O_2$

4.3. Effects of support electrolyte solutions

To expand the scope and application of a NiHCF-SPCE sensor, its cyclic voltammetric behaviour was studied regarding the response toward ascorbic acid in different supporting electrolyte solutions (0.2 mol/L KCl (1), $NaClO_4$ (2), LiCl (3)) presented in Figure 4 (the cyclic voltammogram of ascorbic acid in 0.2 mol/L $NaNO_3$ solution is presented in Figure 3). Two couples of redox peaks can be observed in 0.2 mol/L KCl solution (1), i.e. two anodic peaks (Epa1: +0.50 V; Epa2: +0.62 V) and two cathodic peaks (Epc1: +0.40 V; Epc2: +0.57 V), both relatively close to each other, respectively. In 0.2 mol/L LiCl solution (3), also two anodic peaks can be observed (Epa1: +0.18 V; Epa2: +0.5 V); and two cathodic peaks (Epc1: +0.1 V; Epc2: +0.4 V), but corresponding currents are significantly lower then those in the case of KCl, in particular at +0.4 V. In the case of a 0.2 mol/L $NaClO_4$ solution (2), the cyclic voltammogram was very similar to that of $NaNO_3$ solution in Figure 3, with one redox signal at approx. +0.3V and the other at +0.4 V.

The obtained results implies on the dependence of the sensor response toward ascorbic acid upon the composition of the electrolyte solution. It can be clearly seen, that the voltammograms obtained with the same cation in the electrolyte solution are very similar. Evidently, these cations affect the crystalline microstructures of the NiHCF film, and consequently they have a pronounced impact on the characteristics of voltammograms obtained for ascorbic acid. Furthermore, in the Na_2HPO_4/NaH_2PO_4 buffer solution (pH 7) the NiHCF film started to decompose and the corresponding voltammetric signal decreased gradually, since the PO_4^{3-} reacts with Ni^{2+} to $Ni_3(PO_4)_2$ salt. Thus, NiHCF-SPCE sensor can not be used in the solutions containing high concentrations of PO_4^{3-} anions. These electrolytes have very similar effect for the voltammetric behaviour towards H_2O_2 at the positive potential region, however, there were no effects observed in the negative potential region from 0 to -1.2 V. In order to facilitate the measurement of ascorbic acid and H_2O_2, 0.2 mol/L $NaNO_3$ solution was selected as the most convenient for all forthcoming studies.

In our further work, the effect of ionic strength of the electrolyte solution upon the CV response for both the ascorbic acid and H_2O_2 was investigated. Various concentrations of the supporting electrolyte solution (0.02 mol/L - 1.0 mol/L $NaNO_3$) were examined. Figure 5 shows that the changes in concentration of $NaNO_3$ have a profound effect upon the response toward H_2O_2 (2), which increased markably after rising the concentrations up to 0.2 mol/L. The highest signal was obtained in 0.2 mol/L $NaNO_3$ electrolyte solution, however when the concentration increased to 0.4 mol/L, the CV response levelled off. On the contrary, the CV response for ascorbic acid (1) was practically not affected by different concentrations of $NaNO_3$, with only a slight change at lower concentrations.

The effect of pH upon the peak current for ascorbic acid and H_2O_2 is demonstrated in Figure 6. NiHCF film is unstable in a strong alkaline solutions (pH>10), since it is influenced by the decomposition reaction and the formation of $Fe(OH)_3$. On the other hand, ascorbic acid can be easily oxidized in both strong acidic and alkaline solutions. Thus, the optimal pH range was chosen between 3-9. It can be seen, that the changes of pH between 3-9 have a relatively small effect upon the response to H_2O_2 (2). On the other hand, plot (1) shows that the peak currents belonging to the ascorbic acid increase slowly by rising the

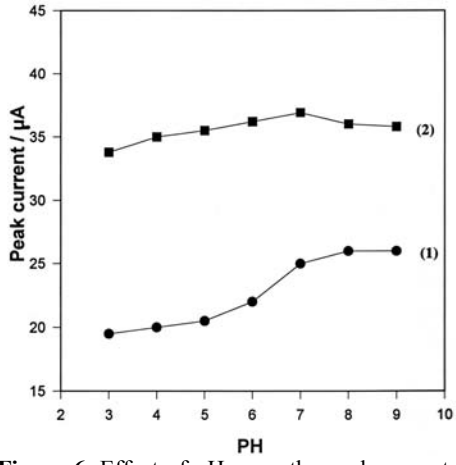

Figure 6. Effect of pH upon the peak current for $2.0x10^{-4}$ mol/L ascorbic acid (1) and: $1.47x10^{-4}$ mol/L of H_2O_2 (2).

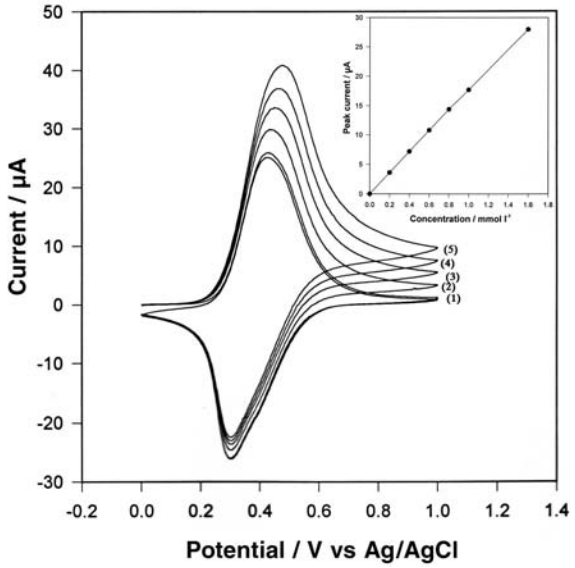

Figure 7. Cyclic voltammograms at NiHCF modified SPCE for increasing levels of ascorbic acid: 0 (1), $2.0x10^{-4}$ mol/L (2), $4.0x10^{-4}$ mol/L (3), $6.0x10^{-4}$ mol/L (4), and $8.0x10^{-4}$ mol/L (5); scan rate: 50 mV/s. The inset shows the corresponding calibration plot.

pH in the range between 3 and 5. The substantial increase is observed between pH 6 and 7.5, and when the pH is higher than 7.5, the response levels off, with the peak potential shifted to more positive potentials. To avoid any loss in the sensitivity and to ensure the stability of the sensor, a supporting electrolyte of pH 7 was chosen as an optimum.

4.4. Quantitative analysis of ascorbic acid and H_2O_2

The quantitative analysis of ascorbic acid and H_2O_2 was also investigated using cyclic voltammetry. Figure 7 shows some of the cyclic voltammograms for increasing levels of ascorbic acid obtained at NiHCF modified SPCE, and the inset displays corresponding calibration plot exhibiting highly linear behaviour in the examined concentration range from $5.0x10^{-5}$ mol/L up to $1.5x10^{-3}$ mol/L, with a correlation coefficient of 0.999, and the limit of detection (S/N=3) of $5.5x10^{-6}$ mol/L. A background signal obtained at the NiHCF modified SPCE electrode in a blank electrolyte solution have been subtracted from the signal in the presence of ascorbic acid. The two curves assigned as (1) in Figure 7 show two cyclic voltammograms at NiHCF modified SPCE in a blank solution (0.2 mol/L $NaNO_3$) before and after calibration. Only a slight attenuation of the signal could be observed (about 2.3 %) after 8 consecutive measurements of the ascorbic acid.

The determination of H_2O_2 was performed at the NiHCF modified SPCE employing the same experimental conditions as in the case of ascorbic acid. Figure 8 displays some of the cyclic voltammograms for increasing levels of H_2O_2 at the NiHCF modified SPCE. It is evident, that the CV signal increases with the increasing concentration of H_2O_2 at both potentials, i.e. in the anodic region at around +0.38V and in the cathodic region at around -0.80 V. At both potentials a good linear behaviour is revealed in the range from $2.0x10^{-5}$ to $1.0x10^{-3}$ mol/L (at +0.38 V) and from $5.0x10^{-5}$ to $6.0x10^{-4}$ mol/L (at -0.80 V), with the correlation coefficients of 0.998 and 0.996, respectively, and the limit of detection (S/N=3) of $1.20x10^{-6}$ mol/L (at +0.38 V).

In order to investigate the reliability and reproducibility of the NiHCF-SPCEs, four sensors were used to measure the ascorbic acid at different concentrations. The results are displays in Table 1. A good linear relationship was obtained between the average peak currents and concentrations. The correlation coefficient and the slope of the calibration plot were 0.999 and 18.42 μA/mmol L^{-1}, respectively. The RSD was in the range between 0.85 and 2.16% (n=4).

Finally, four kinds of vitamin C tablets were selected to measure the ascorbic acid with NiHCF-SPCE. The results are presented in Table 2, with the same experimental conditions as those mentioned above. The contents of ascorbic acid in the tablets were obtained using the standard addition method. Evidently, the other components (vitamins B1, B2, B6, E and Aspirin) existing in the tablets have hardly any effect to the determination of ascorbic acid. The obtained recoveries were: 102.5 %, 104.1 %, 108.2 %, and 94,4 %, with the RSDs of 1.67 %, 3.06 %, 3.47 %, and 2.33 %, respectively.

5. CONCLUSIONS

The electrochemically active nickel hexacyanoferrate(III) (NiHCF) film has been electrodeposited onto the surface of a substrate screen-printed carbon electrode (SPCE) using cyclic voltammetry from the modification solution containing 0.5 mmol/L nickel chloride, 0.5 mmol/L potassium hexacyanoferrate and 0.2 mol/L sodium nitrate. The NiHCF modified SPCE exhibited good stability, enhanced electrocatalytic activity towards ascorbic acid and H_2O_2 and can be used repeatedly or as a one-shot sensor.

Due to enhanced sensitivity and favourable reproducibility, the NiHCF-SPCE sensor offers convenient measurement of low concentration of ascorbic acid or

Table 1. Calibration for ascorbic acid measurements on four modified SPCEs [1]

Electrodes	Concentration($1.0x10^{-4}$ mol/l)/ peak current (μA)					
No.	1	2	3	4	5	6
1	0.0/0.0	1.0/1.9	2.0/3.75	3.0/5.6	4.0/74	5.0/9.35
2	0.0/0.0	1.0/1.8	2.0/3.65	3.0/5.5	4.0/7.2	5.0/9.13
3	0.0/0.0	1.0/1.82	2.0/3.68	3.0/5.5	4.0/7.36	5.0/9.20
4	0.0/0.0	1.0/1.84	2.0/3.74	3.0/5.52	4.0/7.35	5.0/9.21
Average	0.0/0.0	1.0/1.84	2.0/3.70	3.0/5.53	4.0/7.335	5.0/9.23
R.S.D%	0.0/0.0	2.16	1.14	0.853	1.17	0.996

[1] Slop = 18.42 μA/mmol/L, Intercept = 0.002 μA, R = 0.9994. The background currents were subtracted.

Table 2. Measurements of ascorbic acid in four kinds of vitamin C tablets using the standard addition method

Sample	Mean tablet weight(g)	Quoted content(mg) [1]	Determined content(mg)	R.S.D.%	Mean recovery(%) [2]
Plivit C	0.5900	500	515.35(n=5)	1.67	102.48
Aspirin plus	3.2066	240	249.96(n=5)	3.06	104.15
Vitamin C	1.282	350	378.70(n=5)	3.47	108.20
Multivitamin	4.4465	75	70.84(n=5)	2.33	94.45

[1] According to the value reported by manufactures, [2] Compare to quoted contents

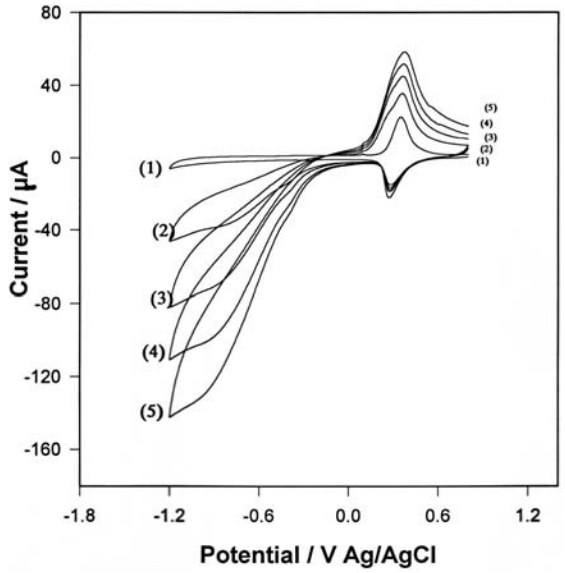

Figure 8. Cyclic voltammograms at NiHCF modified SPCE for increasing levels of H_2O_2: 0 (1), $1.47x10^{-4}$ mol/L (2), $2.84x10^{-4}$ mol/L (3), $4.41x10^{-4}$ mol/L (4), and $5.88x10^{-4}$ mol/L (5); scan rate: 50 mV/s.

H_2O_2. It imparts the possibility to facilitate the detection of those important biomolecules where the H_2O_2 is liberated by the chemical reaction in connection with the biorecognition elements, e.g. lactose, uric acid, cholesterol etc. In our future work, we will devote our efforts to fabricate the modified SPCEs by printing directly the mixture of the nickel hexacyanoferrate and carbon ink onto the polymer substrates. It is believed that the screen printing technique will be more convenient and effective for the mass production of the electrochemical sensors for determination of several important biomolecules.

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7. REFERENCES

1. Jager, A. & U. Bilitewski: Screen-printed electrode for the determination of lactose. *Analyst* 119 (Suppl. 6), 1251–1255 (1994)

2. Sprules, S.D., J.P. Hart, R. Pittson & S.A. Wring: Development of a disposable amperometric sensor for reduced nicotinamide adenine dinucleotide based on a chemically modified screen-printed carbon electrode. *Analyst* 119, 253 (1994)

3. Pemberton, R.M., J.P. Hart & T.T. Mottram: An assay for the enzyme N-acetyl-beta-D-glucosaminidase (NAGase) based on electrochemical detection using screen-printed carbon electrodes (SPCEs). *Analyst* 126(11) 1866-1871 (2001)

4. Dequaire, M. & A. Heller: Screen printing of nucleic acid detecting carbon electrodes. *Anal Chem* 74(17), 4370-4377 (2002)

5. Wang, J., J. Lu, S.B. Hocevar & B. Ogorevc: Bismuth-Coated Screen-Printed Electrodes for Stripping Voltammetric Measurements of Trace Lead. *Electroanalysis* 13, 13-16 (2001)

6. Wang, J., M. Pedrero, H. Sakslund, O. Hammerich & J. Pingarron: Electrochemical Activation of Screen-Printed Carbon Strips. *Analyst* 121, 345-350 (1996)

7. Dequaire, M., C. Degrand & B. Limoges: An immunomagnetic electrochemical sensor based on a perfluorosulfonate-coated screen-printed electrode for the determination of 2,4-dichlorophenoxyacetic acid. *Anal Chem* 71(13), 2571-2577 (1999)

8. Lupu, A., D. Compagnone & G. Palleschi : Screen-printed electrodes for the detection of marker analytes during winemaking. *Anal Chim Acta* 513, 67 (2004)

9. Ohfuji, K., N. Sato, N. Hamada-Sato, T. Kobayashi, C. Imada, H. Okuma & E. Watanabe: Construction of a glucose sensor based on a screen-printed electrode and a novel mediator pyocyanin from Pseudomonas aeruginosa. *Biosens Bioelectron* 19(10), 1237-1244 (2004)

10. Gilmartin, M.A.T., J.P. Hart & D.T. Patton: Prototype, solid-phase, glucose biosensor. *Analyst* 120, 1973-1981 (1995)

11. Gilmartin, M. A. T. & J. P. Hart: Novel, reagentless, amperometric biosensor for uric acid based on a chemically modified screen-printed carbon electrode coated with cellulose acetate and uricase. *Analyst* 119(5), 833-840 (1994)

12. Shumyantseva, V., G. Deluca, T. Bulko, S. Carrara, C. Nicolini, S.A. Usanov & A. Archakov: Cholesterol amperometric biosensor based on cytochrome P450scc. *Biosens Bioelectron* 19(9), 971-976 (2004)

13. Wheeler, G.L., M.A. Jones & N. Smirnoff: The biosynthetic pathway of vitamin C in higher plants. *Nature* 393, 365-369 (1998)

14. Padayatty, S.J., A. Katz, Y.H. Wang, P. Eck, O. Kwon, J.H. Lee, S.L. Chen, C. Corpe, A. Dutta, S.K. Dutta & M. Levine: Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J Am Coll Nutr* 22(1), 18-35 (2003)

15. Cai, C.X., K.H. Xue & S.M. Xu: Electrocatalytic activity of a cobalt hexacynoferrate modified glassy carbon electrode toward ascorbic acid oxidation. *J. Electroanal. Chem* 486, 111 (2000)

16. Pournaghi-Azar, M.H., H. Razmi-Nerbin & B. Hafezi: Amperometric Determination of Ascorbic Acid in Real Samples Using an Aluminum Electrode, Modified with Nickel Hexacyanoferrate Films by Simple Electroless Dipping Method. *Electroanalysis* 14, 206-212 (2002)

17. Mattos de, I.L. & L. Gorton: Metal-Hexacyanate films: A tool in analytical chemistry. *Quimica Nova* 24(2), 200-205 (2001)

18. Wring, S. A., J. P., Hart & B. J. Birch: Voltammetric behaviour of ascorbic acid at a graphite epoxy composite electrode chemically modified with cobalt phthalocyanine and its amperometric determination in multivitamine preparations. *Anal. Chim. Acta* 229, 63-70 (1990)

19. Zen, J.M., D.M. Tsai & A.S. Kumar: Flow injection analysis of ascorbic acid in real samples using a highly stable chemically modified screen-printed electrode. *Electroanalysis* 15(14), 1171-1176 (2003)

20. Kulys, J. & E. J. D'Costa: Printed electrochemical sensor for ascorbic acid determination. *Anal. Chim. Acta* 243, 173 (1991)

21. Zhang, X., J. Wang, B. Ogorevc & U. E. Spichiger: Glucose Nanosensor Based on Prussian-Blue Modified Carbon-Fiber Cone Nanoelectrode and an Integrated Reference Electrode. *Electroanalysis* 11, 945-949 (1999)

22. Deng, Q., B. Li & S. Dong: Self-gelatinizable copolymer immobilized glucose biosensor based on prussian blue modified graphite electrode. *Analyst* 123(10), 1995-1999 (1998)

23. O'Halloran, M. P., M. Pravda & G. G. Guilbault: Prussian blue bulk modified screen-printed electrodes for H_2O_2 detection and for biosensors. *Talanta* 55, 605-611 (2001)

24. Lukachova, L. V., A. A. Karyakin, Y. N. Ivanova, E. E. Karyakina & S.D. Varfolomeyev: Non-aqueous enzymology approach for improvement of reagentless mediator-based glucose biosensor. *Analyst* 123(10), 1981-1985 (1998)

25. Liu, H., H. Deng, K. Sun, D. Qi, J. Deng, Y. Liu & T. Yu: Structure and properties of porous composite membrane of regenerated silk fibroin and poly(vinyl alcohol) and biosensing of glucose via meldola blue dispersed in polyester ionomer as an electron transfer mediator. *Fresenius J Anal Chem* 357, 812 (1997)

26. Wang, J., L. Chen & J. Liu: Critical comparison of metallized and mediator-based carbon paste glucose biosensors. *Electroanalysis* 9, 298 (1997)

27. Schachl, K., H. Alemu, K. Kalcher, H. Moderegger, I. Svancara & K. Vytras: Amperometric Determination of Hydrogen Peroxide With a Manganese Dioxide Film-Modified Screen Printed Carbon Electrode. *Fresenius J. Anal. Chem* 368, 194-200 (1998)

28. Wang, J. & Q. Chen: Screen-printed glucose strip based on palladium-dispersed carbon ink. *Analyst* 119, 1849 (1994)

29. Zhou, X., S. Wang, Z. Wang & M. Jiang: Electrocatalitic oxidation of thiosulfate on a modified hexacyanoferrate film electrode. *Fresenius J. Anal. Chem* 345, 424 (1993)

30. Zhou, D. M., H.X. Ju & H. Y. Chen: Catalytica oxidation of dopamine at a microdisk platinum electrode modified by electrodeposition of nickel hexacyanoferrate and Nafion®. *J. Electroanal. Chem* 408, 219 (1996)

31. Zeng, B.Z., F. Zhao & X. Ding: Electrochemical characteristics of thin nickel hexacyanoferrate films formed on gold and thiol self-assembled monolayers modified gold electrodes. *Anal Sci* 17(2), 259-264 (2001)

32. Giorgetti, M., E. Scavetta, M. Berrettoni & D. Tonelli: Nickel hexacyanoferrate membrane as a coated wire cation-selective electrode. *Analyst* 126(12), 2168-2171 (2001)

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