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VOLTAMMETRIC DETERMINATION OF ASCORBIC ACID IN FOODSTUFFS USING MODIFIED CARBON PASTE ELECTRODES

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Application of differential pulse cathodic stripping voltammetry for determination of ascorbic acid in foodstuffs have been investigated using a carbon paste electrode modified by cobalt(II) phthalocyanine, which was found to facilitate its oxidation and to decrease the necessary overvoltage. The method showed a good linearity up to 70 ppm of ascorbic acid with a detection limit of 0.8 ppm. When applied to analyse the real samples (fruit juices), the results agreed well with those obtained by traditional titrimetric determination.

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Introduction

L-Ascorbic acid (AA, vitamin C) belongs among the most important vitamins. Its lack in living organisms manifested as scorbute is known from antiquity as well as the antiscorbutic influence of lemons, fruit and fresh vegetable but the existence of such antiscorbutic factor in green plants was not confirmed until 1912. AA is very much extended in nature; its high content can especially be found in some vegetables e.g., capsicum (paprika), tomato, potatoes, cabbage, broccoli, and some fruit such as hips, currants, oranges, lemons, etc. Microorganisms, plants and majority of animals are able to its autosynthesis but human organism is entirely dependent on its receipt with food. It should be mentioned that human body contains approx. 4–6 g AA which is not deposited in tissue but eliminated by urine. A daily need is 80–100 mg AA or even higher in case of infections, gravidity, or increased troubles [1]. This is why AA is often added to various food products such as, vitamin concentrates, fruit drinks, and even meat products where, besides its antiscorbutic function, it behaves as both an antioxidant and a pH regulator.

Stability of AA solutions decreases with decreasing concentration and increasing pH value. Its neutral solutions can quickly be oxidized by air oxygen, the oxidation being catalysed by ions of heavy metals, UV radiation, phenols and various enzymes. Other substances such as oxalic acid, amino acids, thiamine, proteins, saccharides, etc. stabilize the AA solutions.

With regard to the importance of AA, its determination in various matrices (drinks, food, medicaments) is frequently asked for. An official method [2] is based on oxidation of AA with iodine to dehydroascorbic acid which is then precipitated using 2,4-dinitrophenylhydrazine to produce a bis(2,4-dinitrophenyl-hydrazone). After separation by thin-layer chromatography and dissolution in acetic acid medium, the red-coloured derivative is determined spectrophoto-metrically at 500 nm. Besides other analytical techniques which are utilized to this purpose (e.g., spectrophotometry, liquid chromatography, electromigration methods), various electrochemical methods are also employed.

Kodíček and Wenig [3] were probably the first to apply polarography, and many other authors used this method to determine AA in various matrices, especially in drinks, fruit juices, fruit and vegetable [4]. In these determinations, both oxidation ability of AA near potentials of ca. 0 V as well as a reduction in ammonia buffers (pH 9.3) following its previous oxidation by oxygen were applied [5].

Polarographic or voltammetric determinations on mercury electrodes can be affected by the wave corresponding to oxidation of mercury as well as by compounds (e.g., some dyestuffs, biologically active compounds, etc.) giving their own signal near the half-wave potential of AA. In other cases, the use of the mercury electrode in flow systems or at *in vivo* measurements in living organisms is prejudicial. Some of these difficulties can be overcome by the use

of a solid electrode, whose properties can be influenced by a proper chemical modification of its electroactive surface. Electrodes based on different forms of carbon are most frequently used but in such a case, quite high overpotential values must be applied to achieve the AA oxidation. This, on the contrary, limits the selectivity of the determination, especially if measured in amperometric mode. Therefore, such modifiers are searched which could decrease the overpotential.

A number of modifiers were suggested for *in vivo* determination of AA in the presence of other electroactive substances present in body fluids. For example, a thin copper-heptacyanonitrosylferrate film on the carbon fiber catalyzed the electrochemical oxidation of AA and decreased the overpotential by several hundreds of millivolts [6]. Similar effects were achieved by modifying a carbon fibre using tetra-N-methyl-3,4-pyridoporphyrazinecobalt [7] or a glassy carbon fibre using some ferrocenes [8]. Also the uses of both a gold electrode coated with a layer of electrochemically polymerized poly-3-methyl-thiophene or polypyrrole, or polyaniline [9] and a platinum or glassy carbon electrode with a polypyrrole/dodecyl sulfate film [10] for *in vivo* measurements were reported.

To depress the high value of the oxidation overpotential of AA mentioned above, various paste electrodes modified with tetrathiafulvalene [11], methylene green [12], N,N,N',N'-tetramethyl-*p*-phenylenediamine [13], and a glassy carbon electrode modified with polyaniline [14] were suggested. Similar effect can also be achieved by using a paste modified with poly(4-vinylpyridine), whose sensitivity can be improved by electrostatically bound hexacyanoferrate(III) [15]. Other authors recommended paste electrodes made of metallized graphite powder [16], a platinum modified glassy carbon electrode [17], a gold electrode modified with hexacyanoferrate immobilized on 3,3'-thiodipropionic acid [18], or multilayer films containing phosphomolybdate [19]. The character of signal can also be influenced by proper electrochemical pretreatment of the electrode [20].

Adsorption voltammetry on glassy carbon electrode was applied to determine AA in fruit; the procedure involves an addition of iron(III) and 1,10-phenanthroline (tris-1,10-phenanthroline-ferrous ion is adsorbed) [21]. As regards the foodstuffs, both dropping [22-25] and stationary mercury electrodes [26] are still frequently applied.

Other methods of electroanalytical AA determination involve some flow systems [27] with amperometric [10,14,17] or voltammetric [28] detection. Amperometric biosensors based on a Clark oxygen electrode equipped with a membrane containing enyzme ascorbateoxidase were also suggested [29-31]. Direct potentiometric determination as well as titrations monitored amperometrically [33], coulometrically [24] and potentiometrically [34] were also recommended.

Experimental

Reagents

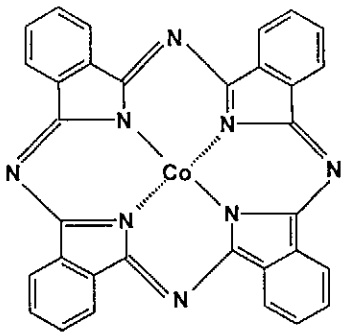
As a basic electrolyte, a 0.1M KCl solution whose pH was adjusted by additions of either 1M HCl, acetate buffer (made of 50 ml 99% acetic acid and 150 ml 0.2M sodium acetate in 250 ml), ammonia buffer (0.5M NH_4Cl plus 0.5M NH_3) or 1M NaOH was used. Since the pH value of 4.7 was found as the optimum, a combination of 0.1M KCl and acetate buffer (1+1) was applied. All chemicals were of analytical-reagent grade and were used without further purification. The supporting electrolyte was bubbled with argon for 15 min before each measurement. For carbon paste preparation, a RW-B spectroscopic graphite powder (Ringsdorf-Werke GmbH, Bonn) and paraffin oil (Uvasol, liquid paraffin for spectroscopy, Merck) were used; cobalt(II) phthalocyanine (purum, min. 97%, Fluka) served as the paste modifier. In titrimetric determinations, 2,6-dichlorophenol-indophenol titrant (a solution made of 50 mg of its sodium salt, a.c.s. reagent, Aldrich, plus 42 mg $NaHCO_3$ in 200 ml) and an HPO_3 extraction solution containing metaphosphoric acid (15 g, a.c.s. reagent, Aldrich) plus 40 ml acetic acid in 200 ml were used.

Apparatus

For voltammetric measurements, a PAR 174A polarograph (EG&G Princeton) was used in combination with an X-Y 1124 recorder (Siemens). The cell consisted of a glass conical vessel with teflon magnetic stirrer. All the measurements were carried out in the three-electrode system using a platinum plate and saturated Ag/AgCl as an auxiliary and reference electrodes, resp. Carbon paste electrode (CPE) was used as a working electrode. The electrode body was made of Teflon cylinder with a circular hollow of the 7 mm diameter. After filling with the paste, a platinum wire served as an electric contact.

Preparation of Carbon Paste Electrodes

The carbon paste was prepared by thorough mixing of 5 g carbon powder with 1.7 g liquid paraffin. For chemically modified carbon paste preparation, amounts of 0.1 g cobalt(II) phthalocyanine (CoPc), 4.9 g carbon powder and 1.7 g liquid paraffin were used. After perfect homogenization, a paste was stored in a small glass bottle for 24 hours. Prior to measurement, the cleaned hollow at the end of the electrode body was filled with the paste whose surface was levelled with soft paper, polished on a PVC desk and rinsed with redistilled water.



Practical Sample (Orange Juice) Treatment

A marketed 100%-orange juice (Hohes C, Eckes-Ybstaler Austria) was selected as a representative sample. As declared by the producer, it contained 35 mg of AA in 100 ml as a minimum value. The juice was neither sweetened nor chemically conserved.

Voltammetric determination. An aliquot of the stirred juice was filtered; the filtrate (2 ml) was mixed with 2 ml 1M KCl and 2 ml acetate buffer and completed to 20 ml with water, giving the pH value of 4.7. The AA determination was performed with both unmodified and modified (with CoPc) carbon paste electrodes. It should be mentioned that the modified CPE had to be activated before each measurement. To analyse the samples, a standard addition procedure was applied.

Reference titrimetric determination. For the reference titrimetric determination, an AOAC 967.21 method [35] was applied. First of all, a standard AA solution was titrated: 2 ml the solution (1 mg ml^{-1}) were mixed with 5 ml HPO_3 and titrated with the indophenol titrant to obtain a rose-violet colour for at least 5 s. Then the blank correction was made in the same way; the difference serving for the empirical amount of AA corresponding to 1 ml of the titrant. For AA determination in the sample, 2 ml of the fruit juice were taken, mixed with 5 ml HPO_3 solution and titrated until the rose-violet colouration.

Results

Unmodified electrode. A signal of AA on the cyclovoltammetric record (a scan from −1.2 to +1.2 V) appeared at 0.35 V as a wave or a deformed peak (in dependence on pH) on both the branches of the cyclic voltammogram. In the differential pulse mode (scan −0.4 to +1.1 V), the signal is a symmetric peak with a half-width of 220 mV and a maximum at ca. 0.35 V. The value of the

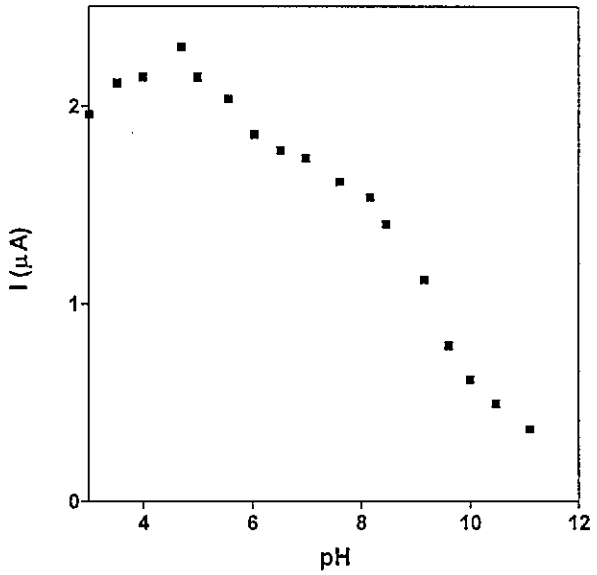


Fig. 1 Influence of pH on the peak current of ascorbic acid at the unmodified CPE. Measured for 50 ppm of ascorbic acid

signal observed depends strongly on the pH value. In the range studied (pH 3–11), the dependence had a falling sigmoidal shape (Fig. 1) with the minimum values between pH 3 and 5.

Subsequently, we tested the influence of the electrochemical pretreatment of the electrode surface on the current response magnitude of AA. For these experiments, the electrode surface was activated by the voltage of 1.2, 1.4, or 1.6 V for 20, 40, or 60 s. The highest current signal was observed in non-stirred solution when using the voltage of 1.2 V and the activation time of 20 s (sensitivity of ca. 0.03 mA ppm^{-1}). Having repeated the record under the activation before the first measurement, we found that the signal magnitude decreased but remained at about 70% of the first value. In case the activation was made before each measurement, the decrease of the signal was much smaller (about 90% of the first record when repeated for 15 times).

A calibration curve (Fig. 2) taken under optimum experimental conditions (scan -0.4 to +1.1 V, electrochemical pretreatment of the electrode surface at 1.2 V for 20 s without stirring) was linear up to 800 ppm, the detection limit being 13 ppm.

CPE modified with CoPc. A cyclic voltammogram showed a small irreversible wave at ca. 0.15 V. A record in the differential pulse regime (0.0 to +0.75 V) gave a marked peak at the same potential value with a peak of the half-width of ca. 150 mV; its value achieved a maximum when activating at 1.0

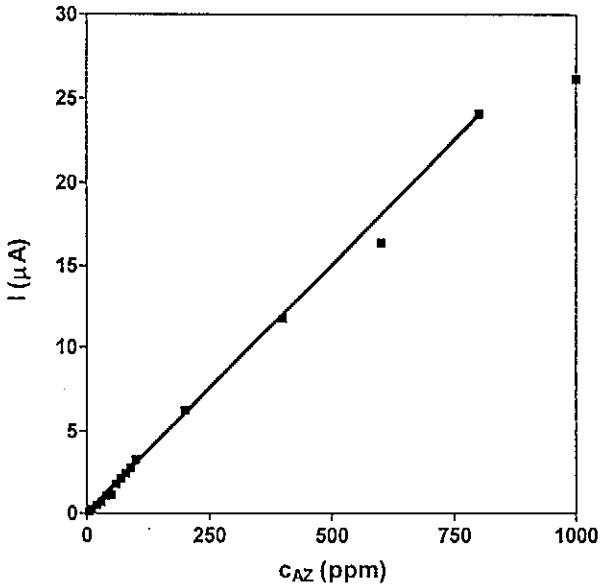


Fig. 2 Calibration graph for ascorbic acid at the unmodified CPE. Basic electrolyte, 0.1 M KCl plus acetate buffer, pH 4.7

V for 40 s in a medium of pH 4.7 (sensitivity 1.2 mA ppm^{-1}). Corresponding calibration curve taken under the optimum experimental conditions (scan 0.0 to +0.75 V, electrochemical activation of the electrode surface at +1.0 V for 40 s without stirring) was linear up to 70 ppm (Fig. 3). At higher AA concentrations, the calibration loses its linearity and the response remains practically constant from 100 mg l^{-1} AA. The detection limit found by statistical evaluation was 0.8 ppm.

It should be mentioned that the signal at the modified CPE, when compared to its unmodified version, is shifted by about 200 mV to more negative potential values and, at the same AA concentration, gives the value approximately 40 times higher (Fig. 4). When a modified electrode without any activation was employed, the AA signal achieved approx. one half of the value expected with the activated electrode and its potential was shifted from 0.28 V (for a rest time of 20 s) to 0.22 V (for a rest time of 60 s), the half-width being ca. 200 mV.

Measurements of the juice sample. The voltammogram recorded with an unmodified electrode shows two overlapping peaks, the first at 0.3, the second at 0.6 V (Fig. 5). However, for the second peak, the concentration dependence is much more significant (sensitivity 0.03 mA ppm^{-1}). The peak obtained with the modified CPE (Fig. 6) lies as far as 0.5 V (sensitivity 0.2 mA ppm^{-1}, half-width to 300 mV). The use of this electrode requires its activation before

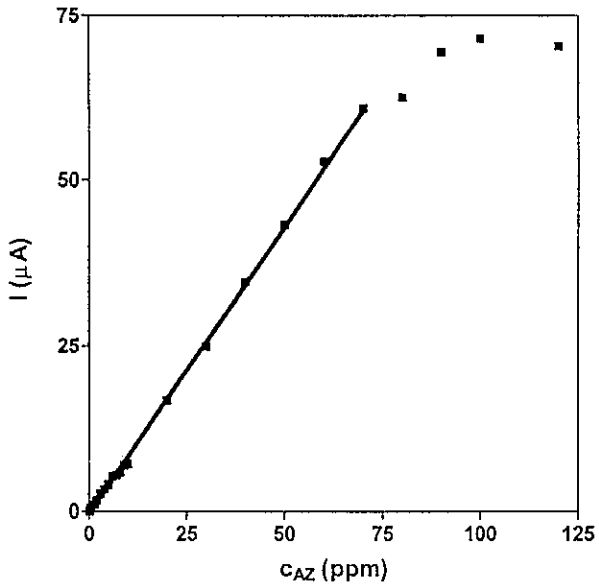


Fig. 3 Calibration graph for ascorbic acid at the CPE modified with cobalt(II) phthalocyanine. Basic electrolyte, 0.1 M KCl plus acetate buffer, pH 4.7

each measurement; if not done, the response of the second and further measurements achieved only one half of the first value and the peak was shifted to more positive values (the difference achieved 200–250 mV).

Having compared the results [36], the AA content in the orange juice sample was determined to give 48 mg/100 ml using an unmodified CPE, or 42 mg/100 ml using a CoPc-modified CPE. Titrimetrically, the content of 39 mg AA/100 ml was found. The manufacturer guarantees a minimum content of 35 mg AA/100 ml.

Discussion

It should be stated that using the unmodified electrode, the sensitivity for the AA determination in a juice sample was approximately the same as in a model electrolyte. With a modified CPE, the sensitivity in a juice was 6 times smaller than that in the pure electrolyte, but the detection limit for AA was much higher (7 times) than that obtained with an unmodified electrode; the signal being much better developed.

Electrochemical oxidation of AA at mercury electrodes represents an irreversible process. But the shape and the shift of the half-wave potential with increasing pH value corresponds to the thermodynamic presumptions. This behaviour can be explained by the fact that the above reaction proceeds in two

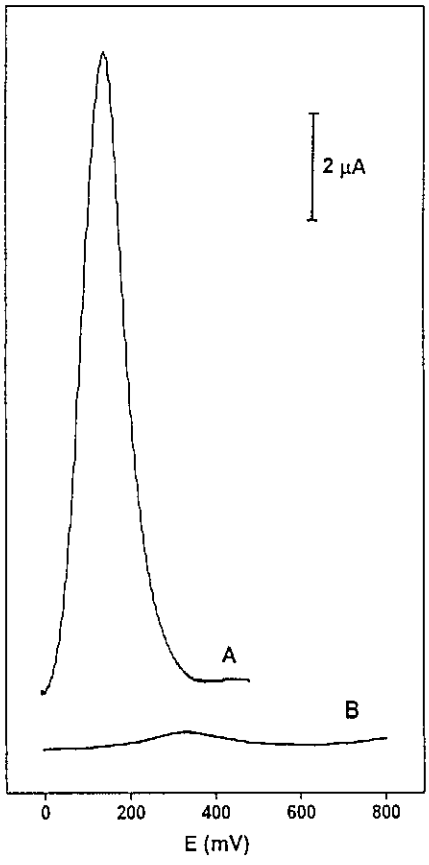
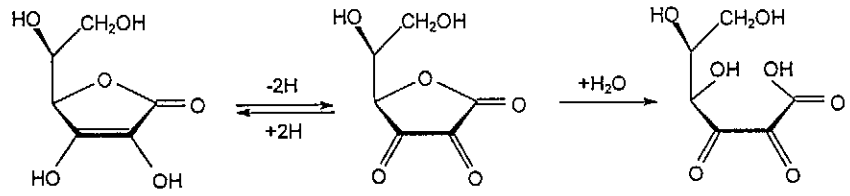


Fig. 4 Comparison of the current signal for 10 ppm of ascorbic acid recorded using (A) modified and (B) unmodified CPE

steps. In the first, reversible step AA changes to dehydroascorbic acid after splitting off two protons and two electrons (when the enediol group is oxidized to diketo group); in the second, irreversible step, dehydroascorbic acid is hydrolyzed to diketogulonic acid during which the lactone ring is split [4,37] (see Scheme I). The oxidation of AA at carbon-based electrodes is less reversible and usually connected with an overpotential reaching several tenth of volts.

The AA signal at the CPE modified by CoPc is probably influenced by two partially independent processes. The macrocyclic CoPc represents a planar complex structure whose central atom has four of its six coordinate positions occupied, and its electron density is lowered by the influence of four nitrogen atoms which do not coordinate directly. Then bonding the free position onto a donor atom increases the electron density of the central ion, resulting in decreasing its redox potential [38]. In dependence on such a potential shift, the



Ascorbic acid | Dehydroascorbic acid | Diketogulonic acid

Scheme I

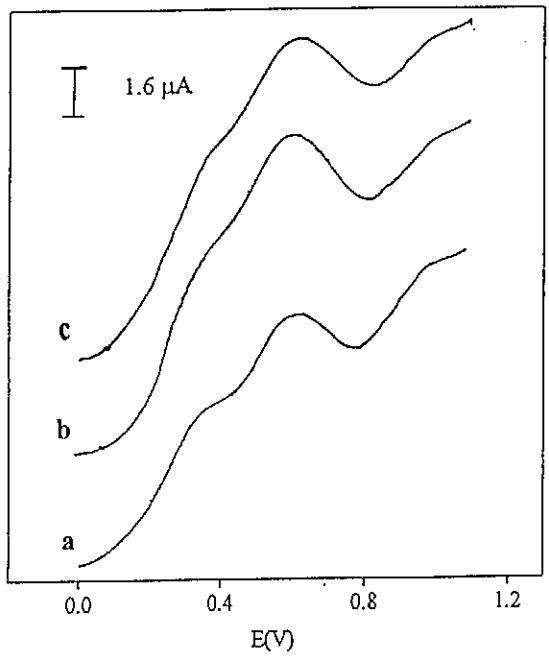


Fig. 5 Determination of ascorbic acid in the orange juice sample using a standard addition procedure at an unmodified CPE. (a) Sample, 2 ml; 1 M KCl, 2 ml; acetate pH 4.7 buffer, 2 ml; water to 20 ml; preliminary electrochemical activation at +1.2 V for 20 s; initial potential 0.0 V; final potential +1.1 V; scan rate 20 mV s^{-1}; pulse 25 mV. Standard additions: (b) plus 20 ppm AA, (c) plus 50 ppm AA

character of bond between the CoPc complex and the carbon substrate can be evaluated. In agreement with this presumption, the lowering of both the redox potential and sensitivity observed at the modified electrode without any activation achieved ca. one half of the values obtained with the electrode whose surface was partially oxidized during activation, i.e., where some kind of bonding through an oxygen atom between the electrode surface and a free coordination position of cobalt ion can be expected. Nevertheless, the measu-

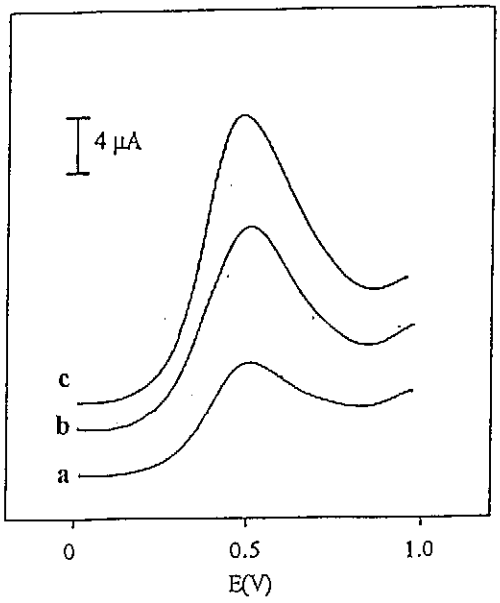


Fig. 6 Determination of ascorbic acid in the orange juice sample using a standard addition procedure at a modified CPE. (a) Sample, 2 ml; 1 M KCl, 2 ml; acetate pH 4.7 buffer, 2 ml; water to 20 ml; preliminary electrochemical activation at +1.0 V for 40 s; initial potential 0.0 V; final potential +1.0 V, scan rate 20 mV s^{-1}; pulse 25 mV. Standard additions: (b) plus 20 ppm AA, (c) plus 50 ppm AA

rement sensitivities with the inactivated but modified electrode were still much higher (15 times) than those with an unmodified electrode. Such a high sensitivity could be ascribed to the coordination of the AA anion onto the sixth coordination position of cobalt ion. In biological systems, this position is responsible for the catalytic effect of reactions connected with the transfer of hydrogen ion; this process is fully reversible [39].

The records of measurements taken in analyses of a juice sample show a significant influence of the matrix onto the electrooxidation of AA, especially at the modified electrode. This could probably be explained by the bonding of others nonreducible anions to CoPc, however, observing the significant increase of the overpotential and the necessity to repeat the electrode activation before each of the records, some destruction of the coordination between CoPc molecule and the carbon substrate could also be taken into account.

Conclusion

It was observed that the modification of the carbon paste with CoPc gives electrodes showing significantly lowered overpotential needed for the AA

electrooxidation, the signal of which in a free electrolyte was approx. 40 times higher. The calibration dependence was linear up to 70 ppm with a detection limit of 0.8 ppm. In real samples (fruit juices), sensitivity of the measurement significantly decreased but still remained higher (7 times) then with the unmodified electrode. Using a standard addition procedure, the CoPc-modified CPE can be recommended to determine AA voltammetrically, which will certainly be appreciated when analysing strongly coloured solutions where the traditional titrations fail.

Acknowledgements

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