

Direct Determination of Nitrite in Food Samples by Electrochemical Biosensor

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Porphyrinic microelectrode was applied for nitrite determination by differential pulse voltammetry (DPV) in some food samples. Described is the possibility of simplification of the experiment, minimization of the sample volume, and minimization of the time-consuming of experiment. Good precision and accuracy were obtained when using the simplified short procedure.

Nitrite and nitrate are commonly monitored for environmental protection purposes, in water, agriculture, and food control. The role of nitrite and nitrate ions in the formation of nitrosamines, which pose a human health hazard, has stimulated interest in the determination of these ions in environmental samples. The most conventional techniques for the determination of nitrite are based on the Griess method in which nitrite is used to diazotize an aromatic primary amine to form an azo dye, the absorbance of which is proportional to the amount of nitrite in the sample [1, 2].

Polák et al. [3] described a rapid determination of nitrite in waste and well waters based on nitrosation of diphenylamine with the nitrite present in the sample and subsequent measurement of the DPP peak height of the carcinogenic *N*-nitrosodiphenylamine formed. The limit of detection was 20–40 nmol dm⁻³ of nitrite. *Van den Berg and Li* [4] utilized the adsorption properties of the azo dye formed from nitrite *via* diazotization with sulfanilamide followed by coupling to 1-naphthylamine for the development of a very sensitive adsorptive cathodic stripping voltammetric method for the determination of nitrite, with a limit of detection of 0.3 nmol dm⁻³.

The determination of trace amounts of nitrite in aqueous solutions is possible in the presence of vitamin B₁₂ as coordinating agent *via* adsorptive accumulation of nitrite coordinated to vitamin B₁₂ at a stationary mercury drop electrode, followed by fast-scan differential pulse voltammetry. The method was applied to the determination of nitrite in drinking and river waters [5].

However, all these methods have disadvantages in long-time preparation of sample with possible contam-

ination. The aim of this work was to develop a simple, rapid, and sensitive method for the determination of nitrite in food samples. Our method for the determination of nitrite utilizes the modified carbon fibre microelectrode. The liquid sample is possible to determine without any preparation and solid sample requires some procedure of homogenization for the next determination. This is possible due to the important property of microelectrodes that the very low current results in the beneficial effect of very low ohmic potential loss with a resulting tolerance for sample of low ionic strength [6].

Recently, developing of microbiosensors brought the sensor for measuring of NO[•] [7]. The sensor is based on catalytic oxidation of NO[•] on polymerized layer of metalloporphyrin covered by Nafion. Sensor without coating by Nafion is very well sensitive also for nitrite.

EXPERIMENTAL

All chemicals used were of anal. grade or the highest purity available. Water used for the preparation of solutions was deionized and redistilled.

100 cm³ of stock nitrite solution ($\rho = 1 \text{ mg cm}^{-3}$) was prepared by dissolving 150 mg of dried (for 4 h at 105–110°C) sodium nitrite (Sigma) in doubly distilled water. A pellet of sodium hydroxide was added to prevent liberation of nitrous acid and 1 cm³ of spectroscopic grade chloroform to inhibit bacterial growth. The stock solution was kept in a refrigerator for preservation. Working standard solutions were freshly prepared by diluting the stock solution with 0.4 M-NH₄Cl.

Differential pulse voltammograms were measured using the EG&G PAR Potentiostat/Galvanostat

M273A (Princeton, NJ) with custom data acquisition electrochemical software made available by the manufacturer. A three-electrode arrangement was used with a nitrite sensor working electrode, saturated calomel reference electrode (SCE), and a platinum wire as a counter electrode.

The nitrite microsensor was produced by threading an array of seven micrometers (diameter) of carbon fibres (Amoco Performance Products, Inc.) through a pulled end of a glass capillary, with a 4 mm length of the fibres left protruding. A copper wire was inserted into the opposite end of the glass capillary, which was sealed with conductive silver epoxy (AI Technology). Then the tip of the glass capillary was sealed with bees wax. The polymeric film was obtained from monomeric tetrakis(3-methoxy-4-hydroxyphenyl)porphyrin (TMHPP), with nickel(II) as the central atom. We deposited poly-TMHPP-Ni from a solution of 0.1 mol dm^{-3} NaOH containing monomeric porphyrin by continuous scan cyclic voltammetry in the potential range -0.2 up to 1.2 V vs. SCE with a scan rate 100 mV s^{-1} by 15 cycles. The completed sensor was rinsed by distilled water and left to dry (5 min) and was stored in phosphate buffer at pH 7.4.

Juice and beer samples were taken to measurement without any adjustment. For fresh tomato, 2 g of sample were homogenized and then taken to measurement.

RESULTS AND DISCUSSION

In the procedures for electrochemical determination of nitrite the indicating electrode was always of conventional size (1 mm up to 1 cm). In this procedure the samples were adjusted by supporting electrolyte before measurement. A modified procedure for the determination of nitrite in food samples in this paper utilizes a modified microelectrode by metalloporphyrin. In the present work we tested two types of electrodes: carbon fibre microelectrode (CFM) and modified carbon fibre microelectrode (MCFM).

Differential pulse voltammetry was used in the potential range $+0.4$ to $+1.2 \text{ V vs. SCE}$ with amplitude 25 mV, pulse width 50 ms, and scan rate 20 mV s^{-1} . The standard solution of nitrite ($\rho = 1 \text{ mg dm}^{-3}$) was added to 4 cm^3 of phosphate buffer solution (pH 7.4) and signal of oxidation of nitrite was obtained. No interferences from nitrate were observed.

The potential of maximum current (peak) on CFM was about $+0.9 \text{ V vs. SCE}$. The obtained peak was asymmetric and of a very difficult shape for the next evaluation. The potential of peak oxidation of nitrite on MCFM was lower, at $+0.78 \text{ V vs. SCE}$ with symmetric peak which could be very well evaluated, as can be seen in Fig. 1.

From several additions of nitrite to standard solution in synthetic sample (phosphate buffer solution pH 7.4) calibration curves were constructed, as can be

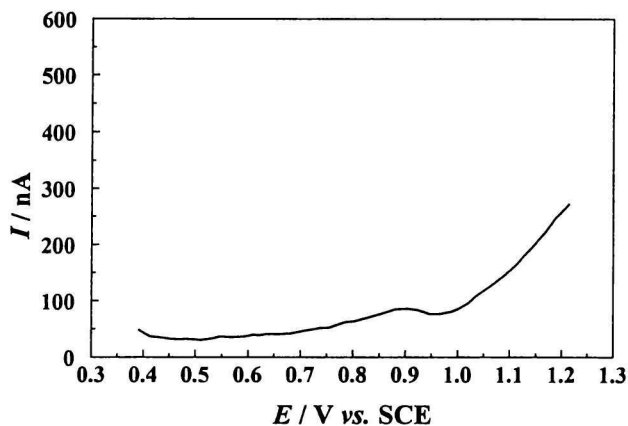


Fig. 1. Differential pulse voltammograms of nitrite ($\rho = 50 \text{ mg dm}^{-3}$) in phosphate buffer solution (pH 7.4) on: carbon fibre microelectrode (solid line) and modified carbon fibre microelectrode (dashed line).

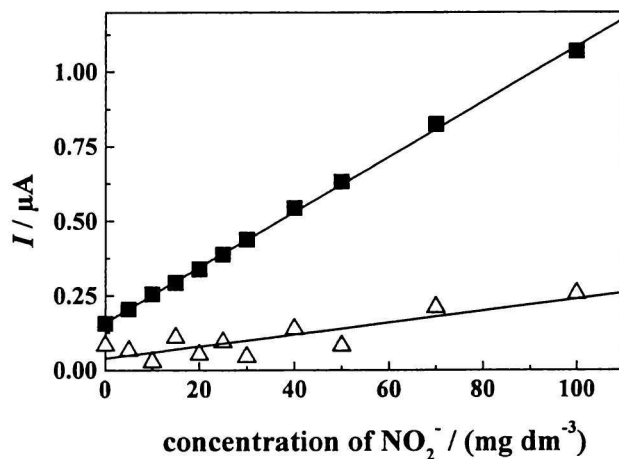


Fig. 2. Calibration curves of nitrite obtained from synthetic samples on carbon fibre microelectrode (■) and on modified carbon fibre microelectrode (Δ).

seen from Fig. 2. The concentration limits of detection were found from evaluation of calibration curves (calculated as 3σ), 1.8 mg dm^{-3} at MCFM and 38.5 mg dm^{-3} at CFM electrode, respectively. The correlation coefficients of calibration curves were 0.9974 for MCFM and 0.8941 for CFM, respectively. As can be seen from Fig. 2, the slope (sensitivity) of MCFM is significantly higher than at CFM. We can conclude that the modified metalloporphyrin layer has more specified properties of nitrite oxidation than carbon fibre itself.

We studied both types of electrodes on real sample of beer as a matrix. Nitrite standard solution was added to sample and differential pulse voltammograms were obtained. The calibration curve for MCFM electrode was constructed and correlation coefficient $r = 0.9824$ was calculated. The limit of detection in matrix of beer was calculated as 3σ with the value 2.2

Table 1. Comparison of the Nitrite Amount in Different Food Samples Measured by Two Independent Methods

Sample	Electrochemical biosensor		UV VIS spectroscopy	
	$L_{1,2}$	s_x	$L_{1,2}$	s_x
	$\mu\text{g cm}^{-3}$	$\mu\text{g cm}^{-3}$	$\mu\text{g cm}^{-3}$	$\mu\text{g cm}^{-3}$
Apple juice	4.1 ± 0.3	0.29	4.8 ± 1.3	1.24
Exotic juice	2.5 ± 0.6	0.57	3.7 ± 0.9	0.86
Multivitamin juice	3.4 ± 0.7	0.67	3.9 ± 1.2	1.14
Beer	29.6 ± 0.4	0.38	32.6 ± 3.1	2.95
Tomatoes	18.7 ± 0.7^a	0.65^a	21.7 ± 1.4^a	1.33

a) The results are given as $\mu\text{g g}^{-1}$.

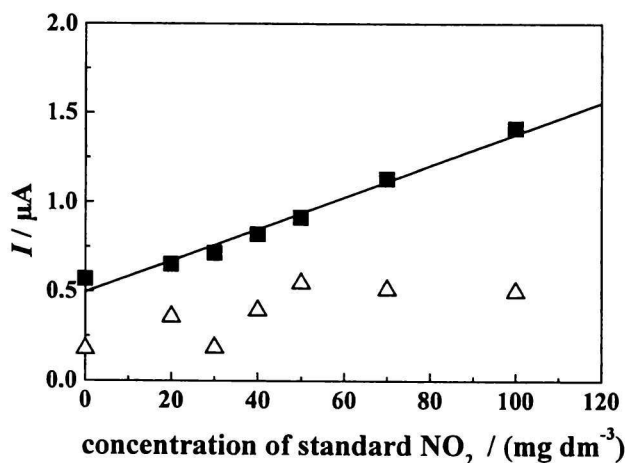


Fig. 3. Calibration curve of nitrite obtained from beer samples on modified carbon fibre microelectrode (■) and on carbon fibre microelectrode (Δ).

mg dm^{-3} , which is very close to the limit of detection obtained from synthetic sample (pH 7.4). Points (Δ) from Fig. 3 represent the peak current obtained on CFM electrode. After testing of linearity we found no dependence for determination of nitrite in real sample for this type of electrode.

From previous experiments we can conclude that the modified carbon fibre microelectrode can be successfully used for the determination of nitrite in real sample solution.

Finally, we determined nitrite on MCFM in different soft drinks, beer, and in the fresh tomatoes. The state regulation limit for nitrite in soft drinks is 10 mg dm^{-3} [8], in beer 50 mg dm^{-3} or 50 mg g^{-1} in vegetable [9]. As can be seen from Table 1, all analyzed samples are under state regulation limit. The results are given as average from six measurements

evaluated by standard addition with interval of confidence and compared to the Griess spectrophotometric method. The comparison of described procedures allows to conclude that the use of modified carbon fibre microelectrode by metalloporphyrin is advantageous since it enables to analyze small-volume sample without adding of supporting electrolyte and leaving out some electrodeposition steps.

The method of determination of nitrite in food samples described in this work simplifies the experimental conditions of analysis, eliminates possible influence of contamination by supporting electrolyte and enables the minimization of sample volume. The detection limit is about 5 times lower than the state regulation limit for soft drinks.

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