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SELECTIVE DETERMINATION OF NITRITE IN CURED MEAT PRODUCTS USING A NONCONDUCTIVE POLYMER FILM BASED SENSOR

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Abstract

For nitrite determination in cured meat, we have developed an original amperometric method based on a modified sensor surface using nonconductive polymers. The nonconductive films allow the target analyte to cross the sensor surface where an electrochemical reaction is taking place, albeit, restricting intereferences. Compared to a spectroscopic method, which requires a stringent pH control and the use of carcinogenic reagents, the amperometric method for nitrite determination is simple, rapid, and does not require dangerous reagents. The polymeric films were deposited onto the Pt and carbon paste electrodes, using cyclic voltammetry. The monomers used for nonconductive polymer film development were: 2,6-dihydroxynaphtalene (2,6-DHN), o-dianisidine (o-DIA) and 1,8-diaminonaphtalene (1,8-DAN). From all the permselective membrane that we have studied, the 2,6-DHN/Pt based sensor presents the sensibility and endurance required for developing the further studies for nitrite detection using a batch and a flow injection analyse system (FIA). Electrochemical tests have shown that 2,6-DHN/Pt is sensitive for sodium nitrite detection, it restricts potassium ferricyanide crossing, and acts as a barrier against ascorbic acid interference. Ascorbic acid is a major interferent in food products.The 2,6-DHN/Pt has a linear range (5-200μM), a correlation factor of 0.9994, and a low detection limit (2.5μM, S/N=3). Such a sensor has the ability to detect nitrite in various meat samples (e.g., hot-dogs, ham, italian salami, and canned beef).

Keywords*: nitrite, nonconductive polymers, modified electrodes*

INTRODUCTION

Sodium nitrate and sodium nitrite are chemicals that are being used in food, especially to preserve meat. These chemicals are also used in other foods as preservatives, antimicrobial agents, as well as flavouring agents (to confer them their characteristic colour, texture, taste, etc). Nitrates are considered compounds of lower toxicity, representing a danger only when ingested in excessive doses or when converted to nitrites (Pinho et al., 1998). This conversion occurs at high temperatures, such as when frying cured meat at high temperatures. It is well known that the nitrite's most toxic effect is methemoglobinemia.

Nitrites combining with amines form N-nitrosoamines, which are known to be potent carcinogen compounds. Nitrite is added to cured meat in concentrations of less than 150ppm for preventing the growth of *Clostridium botulinum,* which is responsible for food poisoning. Therefore, the level of nitrite in cured meat must be kept at a minimum level. For this reason a number of substitutes were tested and it became customary to add ascorbic acid in order to reduce the amount of nitrite added.

Ascorbic acid is added in higher concentrations than nitrite because of its preservative and antioxidant properties. As we have already reported in a previous work (Badea M. et al, 2004), nitrite can be oxidized at a potential of +0,9V at a platinum electrode using an Ag/AgCl reference electrode. The high potential required causes the direct oxidation of interfering species such as ascorbic acid.

In the presence of oxygen and in media of pH lower then 5.5, oxidation of ascorbic acid leads to the formation of dehydroascorbic acid and hydrogen peroxide (Taqui & Martell, 1967), without being involved in the electrode reaction. In order to determine the nitrite from cured meat samples, we proposed a selective amperometric procedure using three different size/charge exclusion membranes: 2,6-dihydroxynaphtalene (2,6-DHN), o-dianisidine (o-DIA) and 1,8-diaminonaphtalene (1,8-DAN).

The nonconductive film obtained through the electropolymerization of 2,6-DHN showed a better stability in time when introduced into a FIA system. The electrode developed showed a good response in respect to nitrite when we compare its signals with the ones obtained using the Griess spectroscopic method.

MATERIALS AND METHODS

All reagents were prepared from analytical reagent grade chemicals using bi-distilled water. Acetate buffer (Riedel de Haen) solution was prepared using 0.1M sodium acetate 0.5% methaphosphoric acid (Fluka) and 50µM EDTA (Sigma).

The 2,6-DHN (Fluka) was dissolved in 0.1M phosphate buffer pH7. A 0.5M stock L-ascorbic acid solution (Riedel de Haen) was performed in bi-distilled water and dilluted daily when needed. Hydrogen peroxide (Fluka) was prepared daily in acetate buffer. The titer of the hydrogen peroxide solution was verified through titration with $K_2Cr_2O_7$.

A potentiostat/galvanostat µ-AUTOLAB, type II (Ecochemie) was used for voltammetric studies. A Pt electrode (2mm) and an Ag/AgCl (3M KCl) Metrohm were used for all tests.

A four-channel Minipuls 3 Gilson peristaltic pump fitted with tygon tubing (1.52 mm id) was used for the propulsion of fluids. Also, the FIA system contains an injection valve (Rheodyne, 7725i model) and a wall jet flow cell which present a Ag/AgCl reference electrode and a gold counter electrode.

The valve loop volume was 100µL. Fittings and connectors were used to connect the different components of the manifold. The optimum flow rate was 0.6mL/minute. The detector was the same potentiostat/galvanostat used for voltammetric measurements. A diagram of the FIA manifold employed is shown in fig.1.

RESULTS AND DISCUSSIONS

2,6-DHN was prepared accordingly to 'Materials and Methods' section. The working electrode used was carrefully cleaned onto PSA microcloth (Buehler, UK) using micropolish II with different doses (1µm Al_2O_3, 0,3µm Al_2O_3 and 0,05 µm Al_2O_3). After mechanical cleaning the electrodes were immerse in distilled water and ultrasonicated for ten minutes.

The first voltammogram showed two oxidation peaks which corespond to the oxidation of amino groups, indicated by the formation of the non-conductive polymer film (figure 2).

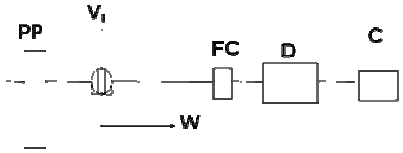


Figure 1. FIA system for nitrite determination of nitrite. PP-peristaltic pump, V_i - injection valve, W-waste, FC-flow cell, D-detector, C-computer

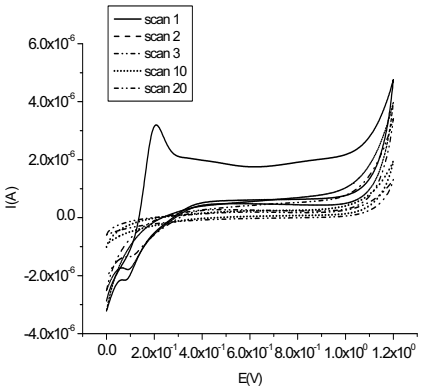


Figure 2. Electropolimerization of 2,6 - DHN onto the Pt (2mm) at a scan rate of 0.01V/s and a potential range between 0 – 1.2 V

In order to analyse the properties of the polymeric membrane, we studied its response to ascorbic acid interference (the main interferent of nitrite in canned meat) and also the size of the array formed (testing the electrode in potassium ferrycyanide). For the interference studies we investigated the voltammetric behaviour of the Pt 2mm electrode in acetate buffer pH4 and in ascorbic acid before and after the electropolymerization (figure 3).

As figures 3 and 4 show, the ascorbic acid gives an anodic peak at +0.6V when using the Pt electrode, which disappears when switching to the 2,6-DHN/Pt sensor. In conclusion, the polymer film is selective in respect to ascorbic acid. The results, demonstrate that the 2,6-DHN/Pt is selective to nitrite, restricts potassium ferrycyanide crossing, and acts like a barrier against ascorbic acid interference.

In order to study the nitrite optimum oxidation potential, we have characterized the 2,6-DHN/Pt electrochemical properties in DC amperometry. The maximum signals were

obtained via hydrodynamic voltammetry within a potential range 0.8-1V (figure 5).

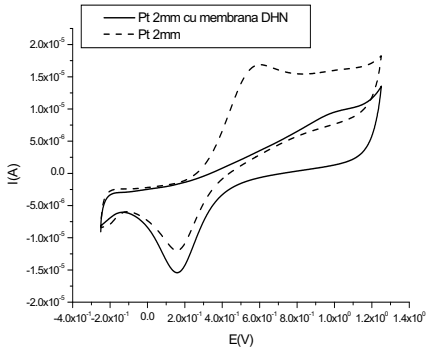


Figure 3. Influence of the interferences
a) cyclic voltammogram for Pt performed in 5mM ascorbic acid; b) cyclic voltammogram for 2,6-DHN/Pt performed in 5mM ascorbic acid. Working condition : 0.2-1.2V

The cathodic peak at 0.25V corresponds to the acetate buffer (figure 4).

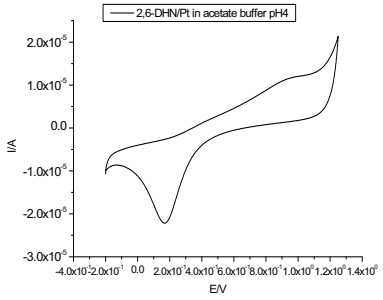


Figure 4. 2,6-DHN/Pt voltamogram obtained in acetate buffer pH4

The optimum potential is in the range of 0.9 – 0.95V.

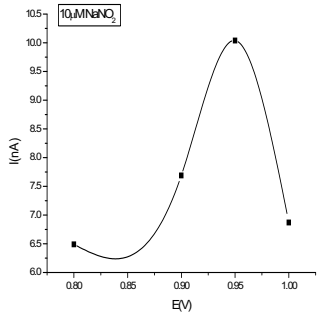
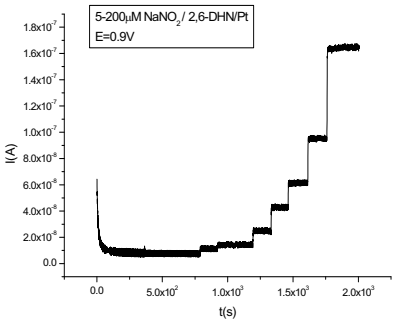


Figure 5. Hydrodynamic voltamogram for10μM nitrite. Working conditions: E=0.8 - 1V.

In figure 6 it is exemplified the aspect the DC amperogram obtained for various concentration of added nitrite at the applied potential of 0.9 V *vs* Ag/AgCl.

A



B

Figure 6. Amperograms obtained in a batch system for nitrite determination using 2,6-DHN/Pt (E=0.9V)

Batch Procedure

The 2,6-DHN/Pt electrode was immersed in a stirred buffer solution. After the base signal was stabilized, successive additions were performed using a stock solution of 5mM sodium nitrite in order to obtain final concentrations within the range 5-100μM nitrite. Based on the calibration graph (figure 7) obtained for the batch data, we have chosen 0.9V as the optimum potential for running further studies.

The equation $I(nA) = 1{,}15+0.19 \cdot C_{nitrite}$ (μM) is linear in the range of 5 - 200μM nitrite and has a correlation factor $r^2=0.9994$.

Flow injection analysis (FIA)

By using the optimal conditions mentioned, we have tested the 2,6-DHN/Pt in the FIA system presented in 'Materials and Methods' section.

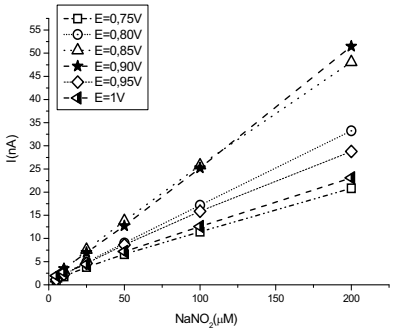


Figure 7. Calibration graphs at different applied potentials for nitrite determination in batch system.

The first studied parameter was the flow rate. In figure 8 are presented the FIA peaks recorded for flow rates varying between 0.36 and 0.67 mL/min. We have found an optimum flow rate equal to 0.6mL/min, varying the flow rate between 0.26-1.1mL/min (data not shown). FIA system showed an extended linear range of nitrite concentration up to 500 µM (Figure 9).

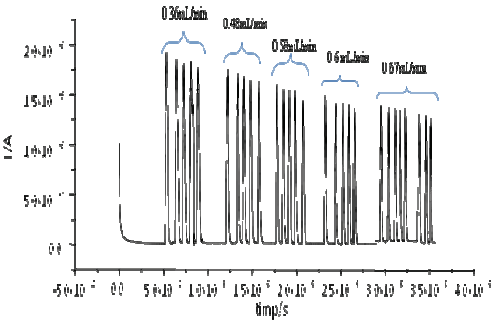


Figure 8. Influence of the flow rate on the FIA peaks recorded for injection of 100 µL of 50 µM nitrite

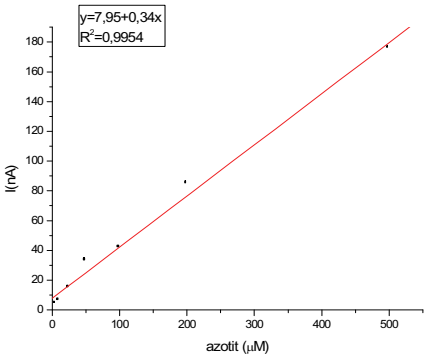


Figure 9. Calibration graph for nitrite determination using the DHN/Pt sensor in FIA system

Determination of nitrite in real samples

The extraction of nitrite from real meat samples was performed according to the methodology used by (Badea et al., 2004). The meat samples (hot-dogs, canned beef, Italian salami and ham) were crushed into small pieces and then blended until homogenous mixtures were obtained. The sample extracts were prepared by mixing 5g of homogenous mixture with 100mL acetate buffer. This mixture was stirred for 30minutes and then was filtered through a 150 µm filter. For further analysis, 15mL of the mixture was taken. A control was prepared similarly with the samples.

In order to evaluate the recovery of the electrochemical method, these samples were analyzed with and without spiking the meat samples with 100 ppm nitrite (table 1). The 2,6-DHN/Pt was immersed in 15mL acetate buffer and left for polarization for several minutes. Following addition of the 15mL sample, the signal was recorded.

Table 1. Recovery test for electrochemical method for nitrite determination

Sample	Electrochemical Method		
	nitrite (µg/g)	Spiked sample with 100 ppm nitrite (µg/g)	Recovery
Hot-dog	103,6	247,6	121%
Canned beef	91	199,5	104%
Italian Salami	250,5	333,2	95%
Ham	338	337,9	105%

CONCLUSIONS

In this work, we have proposed a nonconductive polymer film based sensor for determination of nitrite in cured meat samples. The interference study shows that the developed sensor presents the advantages of selectivity and reproducibility. Furthermore, introduced into a FIA system, it has great operational versatility, reduction of reagent consumption and automatic sample analysis.

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