ml and add a 10 ml aliquot containing not more than 15 μg of SO₂ through long stemmed funnel to 2 ml of KBrO₃, 7 ml of H₂SO₄ and 1 ml of DCF in a 25 ml standard flask. Make up to 25 ml. Extract with 5 ml of solvent mixture and reequilibrate the organic layer with 10 ml of acetate buffer and 1 ml of cetrimide. Separate the organic layer, add about 1 g of Na₂SO₄ and measure at 535 nm against a reagent blank. Prepare a calibration graph by taking 0–15 μg of SO₂ and following the above procedure.

Discussion

Interference studies. The positive interference of up to 50 μg of NO₂ was overcome by adding 1 ml of 4% sulphamic acid to the sampled solution. The interference of up to 7.5 μg of H₂S was overcome by precipitating it as ZnS, collecting it over Fe(OH)₃, and discarding the precipitate. There was no interference up to 40 μg of HCHO.

Evaluation of the trapping solution. The developed new trapping solution was evaluated by means of a permeation device [2]. It showed a collection efficiency of 99% at an optimum flow rate of 0.4 l/min for a period of 6 h. The sampled sulphite was stable for 22 days, however, freshly prepared sulphite in the trapping solution used for calibration was stable for 52 days if stored in a fridge. The developed trapping solution can serve as an alternative to the buffered formaldehyde trapping solutions [3, 4].

Applications. The method has been evaluated with low concentrations of SO₂ using the permeation devices developed by Balasubramanian et al. [2] and the results are compared with those of the pararosaniline method (Table 1).

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Determination of 2-amino-5-nitrothiazole by square-wave cathodic stripping voltammetry

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Abstract. An electrochemical method has been developed for the detection and determination of 2-amino-5-nitrothiazole (2,5-ANT) by adsorption square-wave voltammetric stripping. The best sensitivity/resolution ratio was obtained by adsorption at pH 8.0 using a phosphate buffer, an accumulation potential of ~10 mV (vs. Ag/AgCl 3 mol/l) and an accumulation time of 15 s. Under these conditions, the proposed method provides a linear electrode response over the 2,5-ANT concentration range 5–300 ng ml⁻¹, and a detection and determination limit of 4 and 7.5 ng ml⁻¹, respectively. The method was applied to the determination of 2,5-ANT in bacon.

Introduction

2-Amino-5-nitrothiazole (2,5-ANT), also called enheptin and entramin, is used as a tumor growth retardant in animals [1].

A wealth of literature references testifies to the antimicrobial effectiveness of this substance, as clearly demonstrated by in vivo tests [2, 3].

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Kolosova et al. showed 5-nitrothiazoles to inhibit the growth of Clostridium spores [4]. The activity of 2,5-ANT against Clostridium botulinum in both culture media and meat products prompted research on this compound as a potential substitute for nitrates added to cured meat products [5]. This was supported by the low toxicity of these substances in mice, turkeys, chickens and dogs [6], and the results of its permitted use in some veterinary treatments [7].

2,5-ANT has been identified by thin-layer chromatography [8] and quantified by UV-visible spectroscopy [9] and by high-voltage gel electrophoresis [10].

The electrochemical behaviour of 2,5-ANT was studied by d.c. polarography using a mercury electrode in an aqueous medium [11], as well as by cyclic voltammetry in non-protic solvents (DMF) [12], where it exhibits an irreversible, diffusion-controlled wave.

The determination of 2,5-ANT in animal feeds and food is usually only achieved by spectrophotometry [14]. In this paper we propose its determination by cathodic adsorptive stripping square wave voltammetry (casswv) at trace levels.

Experimental

Apparatus and reagents. A PAR 384B polarograph was used, equipped with a 303A multi-mode mercury stand Ag/AgCl 3 mol/l reference electrode, a platinum auxiliary electrode and a BAS 100B electrochemical analyzer, furnished with a mercury pool working electrode.

A 2,5-ANT stock solution containing 100 μg ml⁻¹ of the reagent (Aldrich) was made by dissolving in methanol, other diluted solutions by appropriate dilution with water. Tridistilled metal mercury was also employed. All chemicals used were of reagent grade.

Procedure. The solution containing the supporting electrolyte and 2,5-ANT at an appropriate concentration was placed into the polarographic cell, through which a nitrogen stream was passed for 300 s before the experiment and for a further 30 s
prior to each measurement. Then, the selected accumulation potential (Eac) was applied over the preset accumulation period (tac). The solution was kept under stirring in all these steps. After the accumulation time had elapsed, stirring was stopped and the selected accumulation potential was kept on the mercury drop for a rest time (tr), after which a potential scan was performed. The accumulation potential was scanned down to -1.0 V and square-wave voltammetry (SWV) was used as the measuring technique.

All experiments were carried out at room temperature.

Results and discussion

In order to determine the influence of the experimental variables, the effect of pH and the Eac on the analytical signals was studied first. For this purpose, variable Eac values were applied to solutions containing an analyte concentration of 0.3 µg ml⁻¹ for a tac of 30 s at the selected pH (adjusted by using an appropriate volume of Britton-Robinson buffer containing 0.04 mol l⁻¹ of each constituent acid). Under these conditions, 2,5-ANT gave one or two reduction waves, depending on the pH (Fig. 1).

As can be seen, at pH < 7 only the wave at less cathodic potential appears with lower intensity. The peak potential varies with the pH according to the following equations:

\[ E_{p1}(V) = -0.079 - 0.042 \text{pH} \quad (r = 0.992) \]
\[ E_{p2}(V) = -0.036 - 0.065 \text{pH} \quad (r = 0.997) \]

The variations of the peak current (Ip) with the pH and Eac, for the wave of the greater analytical interest (wave 2), shows which highest Ip value can be obtained at an accumulation potential of -10 mV and pH 8.0 (0.04 mol/l Britton-Robinson buffer). The variation of Ip with the square-wave frequency is in accordance with the following equation:

\[ I_p(A) = -8.93 \times 10^{-7} + 1.15 \times 10^{-7} f(\text{Hz}) \quad (r = 0.992). \]

The peak potential was found to depend linearly on the logarithm of the square wave frequency:

\[ E_p(V) = -0.459 - 0.027 \ln f(\text{Hz}) \quad (r = 0.991). \]

Using the slope of this straight line and exchanging the number of electrons determined coulometrically [4, 11], the transfer coefficient \( \alpha = 0.24 \) [13] was determined. The proportionality between \( E_p \) and lnf on one hand, and \( I_p \) and f on the other hand, revealed that the reduction of 2,5-ANT is a diffusion-controlled adsorptive process [13].

Other instrumental variables optimized are: \( f = 120 \text{ Hz}, \text{ scan rate} = 120 \text{ mV and 50 mV pulse amplitude} \).

The coverage of the surface was calculated to be \( 2.7 \times 10^{-10} \text{ mol cm}^{-2} \) (surface area of the mercury drop 0.0177 cm², concentration used 0.3 µg ml⁻¹ [13]).

The variation of Ip with the accumulation time in order to obtain an adequate sensitivity indicated a value of 15 s; a rest time of 60 s was selected as most suitable.

Use of an HBO₂/NaBO₂ buffer of any concentration at pH 8 as supporting electrolyte resulted in splitting of the voltammetric wave (Fig. 2); this was ascribed to the nitro group being reduced in two steps rather than a single one in this medium.

Under the optimal experimental conditions established, the electrode provides a linear response over the 2,5-ANT concentration range from 5 to 300 ng ml⁻¹, according to the following equation: \( I_p(\mu A) = 0.034 + 0.051 C \text{ ng ml}^{-1} \) (\( r = 0.9993 \)).

A statistical treatment of the results obtained from the determination of 2,5-ANT in five solutions containing four different analyte concentrations showed the proposed method to exhibit a maximum relative error of -4.7% and a maximum relative standard deviation of 5.9%.

The method was applied to the determination of 2,5-ANT in bacon by using the following procedure: an amount of 10 g of fresh bacon was sprinkled with 0.01 g of 2,5-ANT and allowed to stand for 72 h. Then the bacon was washed with water to remove excess solid and allowed to dry at room temperature for 7 days. A portion of 0.5000 g of the bacon was then brought into contact with 100 ml of methanol with stirring and gentle heating for 90 min, after which the liquid was filtered and made up to 100 ml with methanol. Finally, a 5-ml aliquot of the solution was mixed with an appropriate volume of Britton-Robin-