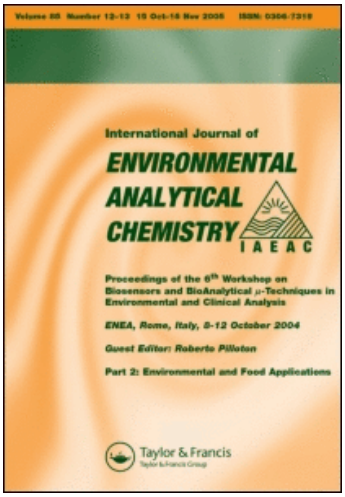

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Online publication date: 17 September 2010

To cite this Article Fang, Yanjun , Chen, Hui , Gao, Zhixian and Jing, Xinglong(2002) 'Flow Injection Determination of Nitrite in Food Samples by Dialysis Membrane Separation and Photometric Detection', International Journal of Environmental Analytical Chemistry, 82: 1, 1 — 6
To link to this Article: DOI: 10.1080/03067310290024030
URL: http://dx.doi.org/10.1080/03067310290024030

Intern. J. Environ. Anal. Chem., Vol. 82, No. 1, pp. 1–6

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FLOW INJECTION DETERMINATION OF NITRITE IN FOOD SAMPLES BY DIALYSIS MEMBRANE SEPARATION AND PHOTOMETRIC DETECTION

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(Received 15 January 2001; In final form 22 June 2001)

An on-line dialysis preconcentration determination of nitrite by injection method has been developed. In this paper, the polyetherimide-composed membranes (PEI) were used for the dialysing of the nitrite from the food samples matrix, because they showed a high dialysing yield and analytical signal, low blank signal and absence of membrane clogging. In the recipient (acceptor) stream, the nitrite which penetrated from the PEI membrane, is diazotised with sulphanilamide to form a diazonium action, which is subsequently coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a stable purple azo dye, the absorbance of which is measured at 525 nm. Various analytical parameters, such as effect of acidity (pH), flow rate, sample size, dialysing membranes, and interfering species were studied. The calibration curve was between 12.5 ng ml^{-1} and 3.2 μg ml^{-1}, and the detection limit was 4.5 ng ml^{-1}, and up to 15 samples can be analyzed per hour with a relative precision of ca.2.8%. The proposed method was successfully applied to the determination of nitrite in food samples with satisfactory results.

Keywords: Dialysis; Flow injection; Member separation; Nitrite

INTRODUCTION

The presence of nitrite in vegetables, drinking water, and other food products is a serious threat to human health[1]. The nitrite direct toxicity is low, but dangerous compounds (N-nitroso compounds) may be formed in the organism, and some of them are known to be carcinogenic, teratogenic and mutagentic[2, 3]. Most of the methods for the determination of the nitrite are based on the diazo-coupling reaction (Griess methods)[4–6], or catalytic kinetic methods[7, 8]. These methods are generally highly sensitive, but often have drawbacks of serious interferences, when carried out on the samples having turbidity, colour, and suspended matter.

However, dialysis flow injection analysis represents interesting methods for automation separation, Motomizu and Yoden[9] reported a tubular microporous polytetrafluoroethylene (PTFE) membrane that was applied to the permeation of chlorine,

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ISSN 0306-7319 print © 2002 Taylor & Francis Ltd
DOI: 10.1080/03067310290024030

bromine and iodine. By separation of the analyte from the samples and collecting it into a clean recipient stream, matrix problems can be avoided and the selectivity of the method can be enhanced.

The purpose of this study was to develop an automatic and rapid dialysis flow injection for the measurements of the nitrite in food samples, the transfer of the analyte from the donor to the acceptor phase was studied by using both standard solution and food samples. The use of the PEI membrane in on-line dialysis prior to the photometric determination of nitrite as in other previous studies[10, 11].

EXPERIMENTAL

Apparatus

The schematic of dialysis FIA is shown in Fig. 1. The system consists of LZ-2000 Flow injection apparatus (Shenyang, China). The detector used was an U-3400 Spectrophotometer (Hitachi, Japan) equipped with 10 mm path-length cell (18 μl), which was performed to measure absorbance at 525 nm. A digital pH-3C Meter with glass electrodes (Shanghai, China) was used for the pH adjustments. The dialysis diffusion cell consists of two pieces, each having curved grooves (245 × 5 × 0.2 mm), and the dialysis membranes were the standard dialysing membranes. The flow system used 0.7 mm i.d. Teflon tubing throughout.

Reagents

All chemicals used were of analytical-reagent grade and doubly distilled water and spectroscopic chloroform were used throughout.

A nitrite stock solution (1.0 mg ml^{-1}) was prepared by dissolving 492.8 mg dried (for 4 h at 105–110°C) sodium nitrite in doubly-distilled water, a pellet of sodium hydroxide was added to prevent liberation of nitrous acid and 1 ml of spectroscopic grade chloroform to inhibit bacterial growth. The stock solution was kept in a refrigerator for preservation. Working standard nitrite solutions (1.0 μg ml^{-1}) were freshly prepared by diluting the stock solution with 0.4 M NH_4Cl.

A 100 mg of N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) was dissolved in 100 ml of the reaction medium (0.4 M NH_4Cl), the solution was kept in a refrigerator for preservation.

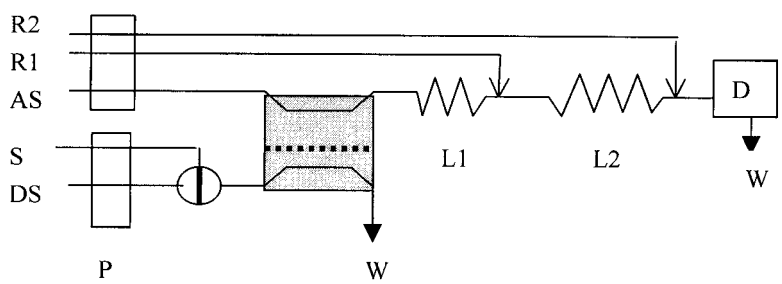

FIGURE 1 Schematic diagram of dialysis FIA system used to determine nitrite AS, acceptor stream; DS, donor stream; *S*, sample; R_1, SAN solution; R_2, NED solution; $L_{(1,2)}$, mixing coils; *P*, pump; *D*, detector.

Sulfanilamide (SAN) solution was prepared by dissolving 80 mg of Sulfanilamide in 100 ml of doubly distilled water, and the Sulfanilamide solution (0.08% w/v) was kept in a refrigerator.

A 100 ml stock solution of ammonium chloride solution (0.4 M) was prepared by dissolving 2.12 g of anhydrous ammonium chloride (dried at 105°C) in doubly-distilled water.

Solutions of a large number of inorganic ions were prepared from their analytical-reagent grade water-soluble salts, stock solutions, and the food samples were mixed with sand and homogenized in a mortar, the thoroughly mixed samples was then taken in a 100 ml beaker and digested carefully following the method recommended by the AOAC[12] and were kept in ploy(propylene) bottles containing 1 ml of spectroscopic grade chloroform.

Membranes

Different kinds of membranes were assayed. Ploytetrafluoroethylene (PTFE), cellulose acetate (CA), polyethersulfone (PES) and polyetherimide (PEI) membranes were previously conditioned by rinsing in distilled water before use. All the polymer membranes were cast as a thin film (245 × 5 mm) and sinking it in distilled water for 10 min in order to constitute the structure of the membrane. These membranes exhibited a molecular weight cut off (MWCO in Dalton) values between 2,000 and 10,000 Dalton.

Procedure

The dialysis flow injection system used for the determination of nitrite is schematically shown in Fig. 1. The sample (80 µl) was injected in donor stream and dialyzed, the non-dialyzed sample is led to the waste, whereas the dialyzed samples dragged by acceptor that carries it through the reaction coil (L_1) to be diazotised with the reagent SAN solution (R_1), and then coupled with reagent NED solution (R_2) in the reaction coil (L_2) to the highly colored azo dye. The reaction products monitored photometrically at 525 nm[13] and the peak height taken as the analytical signal.

RESULTS AND DISCUSSION

Optimization of the FI manifold

The sensitivity of the reaction will be influenced by the residence time in each segment of the reaction manifold. The optimum length of the mixing coils (L_1, L_2) was examined over the range 10–60 cm and 50–120 cm, respectively, so as to obtain a good sample rate. The length of coil L_1 has little effect on the coloration because the kinetics of the initial diazotation reaction is very rapid,[13] a minimal length of 10 cm is then chosen for L_1. The sensitivity is more dependent on the extent of the reaction between nitrite and NED reagent, the absorbance increase with the time of contact, thus, a length of 100 cm for L_2 was chosen, The volume of the sample injected has a significant effect in the coloration, the signal increase with increasing sample volume in the range of 25–100 µl, a compromise sample volume of 18 µl was used for subsequent experiments. Varying the flow-rates of SAN and NED reagent solution over the range

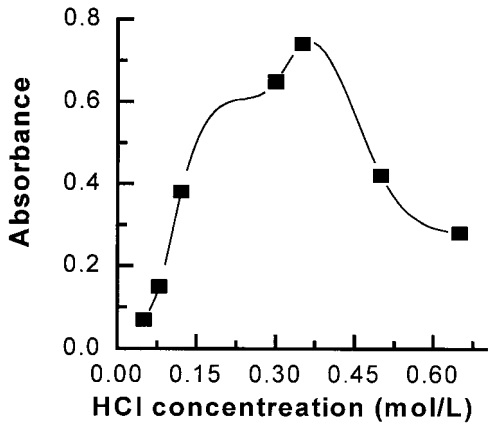

FIGURE 2 Effect of the HCl concentration on the sensitivity, (NO_2^{-1}: 1.0 μg ml^{-1}).

TABLE I Selected chemical and FIA parameters (nitrite: 1.0 μg ml^{-1})

Parameter	*Studied range*	*Selected value*
Size of sample loop (μl)	25–100	80
Overall flow rate (ml min^{-1})	0.2–1.2	0.30
Reaction coil L_1 (cm)	10–0	10
Reaction coil L_2 (cm)	50–120	100
pH	0.05–0.80	0.35

0.2–1.2 ml min^{-1}, the sensitivity is somewhat improved at lower flow-rates because of the increase in the reaction time. So a flow-rate of 30 ml min^{-1} were chosen for them respectively.

Various acids such as hydrochloric, sulfuric, nitric and phosphoric at the same concentration were tested. Phosphoric acid is a weaker acid and thus decreased the sensitivity, hydrochloric acid at the same concentration as sulfuric acid gives greater sensitivity. From the results, hydrochloric acid was found to be the best acid for the system. Different concentration of hydrochloric acid was tested in the range (0.05–0.80 M) (see Fig. 2), the results showed that the best sensitivity was achieved when 0.35 M hydrochloric acid was used. The results of the optimization of the FI manifold were shown in Table I.

Dialysing performance of the studied membranes

Once the system variables were optimised, the dialysing performance of the membranes was studied taking into account their composition as well as their physical and chemical features (see Table II). The following parameters were controlled: (a) the dialysing yield of the membrane (Y_d), (b) the blank signal (S_b), (c) the analytical signal ($S = S_s - S_b$) and (d) the trend of the membrane to be clogged when using food samples.

The dialysing yield of the membrane was measured using a standard solution of nitrite and the relationship $Y_d = 100 \times S_d/S_{nd}$,[14] where S_d was the signal with the dialysis unit, and S_{nd} without the dialysis unit. The clogging of the membrane caused a progressive increase in the pressure of the donor stream along with a decrease in

TABLE II Performance of the tested dialysing membranes (nitrite: 1.0 μg ml^{-1})

Composition	*MWCO* (Dalton)	Y_d (%)	*Clogging*
Celluioseacetate (CA)	5,000	15.5	Progressive
Polyethersulfone (PES)	10,000	19.3	Progressive
Polytetrafluroroethylene (PTFE)	7,500	34.5	No
Polyetherimide (PEI)	15,000	56.4	No

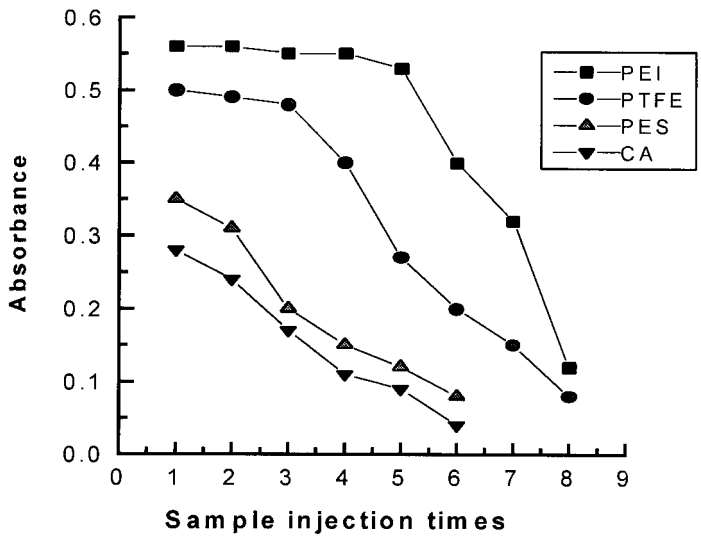

FIGURE 3 Clogging phenomenon observed in the dialysing membranes, 1.0 μg ml^{-1} of nitrite.

the analytical signal (see Fig. 3). In each case, the number of injections of each solution was the minimum to ensure the stability of the signal, usually 3–5 times.

Interference of foreign species

The interference of more than 25 foreign species on the determination of a solution containing 0.50 μg ml^{-1} of nitrite were examined by adding various amount of these foreign species, the amount at which the species caused an error of no more than 5% was taken as its tolerance limit. The results showed that large amounts of alkali and alkaline earth metal cations and anions normally could be found in the samples did not interfere, and a 1000-fold (μg μg^{-1}) amount of Pb^{2+}, NH_4^+, $C_2O_4^{2-}$; a 500-fold of Al^{3+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Ag^+, Cu^{2+}; a 200-fold of CO_3^{2-}, SO_4^{2-}, NO_3^-, F^-, Cl^-; and 50-fold of Fe^{2+}, Fe^{3+}, Ni^{2+} could be tolerated.

Application to the analysis of nitrite

The analysis of nitrite in food samples was carried out by FI with on-line dialysis. Under these conditions, a linear concentration range between 12.5 ng ml^{-1} and 3.2 μg ml^{-1} was obtained, the linear calibration cure was $H = 1.746 + 3.461\,C$ ($n = 5$, $r = 0.9994$), where H and C are the peak height (A.U.) and nitrite concentration (ng ml^{-1}).

TABLE III Analysis of nitrite in food samples by the optimised FI method

Sample	*Colour intensity*	*Turbidity*	*FI method** ($\mu g\,g^{-1}$)	*Standard method* ($\mu g\,g^{-1}$)	*RSD*
Sausage	Medium	High	1.251 ± 0.001	1.250 ± 0.002	0.97
Milk	High	Medium	0.268 ± 0.002	0.270 ± 0.001	1.20
Orange juice	Medium	No	0.105 ± 0.002	0.109 ± 0.003	2.52
Apple juice	Low	No	0.068 ± 0.003	0.065 ± 0.002	3.20

*Average of five determinations.

The detection limits was $4.5\,ng\,ml^{-1}$, which was calculated by multiplying the standard derivation of 15 blank measurements by three divided with the slop of the linear calibration curve. The relative standard deviation of 20 replicate measurements was 1.6% for the $1.0\,\mu g\,ml^{-1}$ of nitrite, and a samples frequency is $15\,h^{-1}$. The results are shown in Table III. As can be seen the results from the optimized FI method agree well with those obtained by the Griess standard method[12].

CONCLUSION

In this paper, the use of these membranes in on-line dialysis allows the removal of interference due to the matrix as colour, turbidity, high concentration of dissolved substance etc, that make the sample can be analyzed without tedious pretreatment. This method is very suitable for use in the flourometric and photometric detector, because it is necessary to measure the blank signal in both of them. So, the use of the dialysis membrane for the determination of nitrite in food samples is very versatile and useful especially for an intensely colored sample.

Acknowledgements

The authors are very grateful to Natural Foundation of the National (No. 39970644) and the Tianjin Natural Foundation (No. 00360811) for their financial supports.

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