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QUENCHED PHOSPHORESCENCE AS A DETECTION METHOD IN ION CHROMATOGRAPHY: THE DETERMINATION OF NITRITE AND SULPHITE

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SUMMARY

A detection method for the determination of nitrite and sulphite, based on the quenched room temperature phosphorescence of biacetyl, is described. The detector is placed directly after the high-performance liquid chromatographic anion-exchange column. By using electronic signal inversion, positive peaks and calibration graphs that are linear over at least two orders of magnitude of concentration are obtained. Absolute detection limits in water are one to two orders of magnitude better for nitrite (0.02 nmole) and about one order of magnitude better for sulphite (0.2 nmole) than those of other methods. Sulphite ions are detected indirectly via the thiosulphate ion, which is produced from sulphite by a simple reaction. The applicability of the method to real samples was explored: nitrite can be determined in processed meats without interferences; the determination of sulphite in wine is probably possible via the thiosulphate ion.

INTRODUCTION

In recent years the problem of detection in ion chromatography has received much attention[1]. For this class of liquid chromatography, the possibility of using a UV detector is limited. Monitoring of the electrical conductance to determine inorganic ions has been applied extensively[1]. In addition, electrochemical detectors[2], direct[1,3] and indirect[1,4] photometric detectors and indirect refractive index detectors[1,4] have been used.

We have developed a detection method for liquid chromatography based on the phenomenon of room temperature phosphorescence in liquids (RTPL) which requires the presence of a phosphorescent compound (*ca.* 10^{-4} *M*) in the eluent[5–8].

In this paper we show that nitrite (frequently used as a food preservative and known as a precursor of carcinogenic N-nitrosamines) can be sensitively determined in water by quenched RTPL of biacetyl with the detector placed directly after the separation step. Sulphite (the concentration of which is an important parameter for the quality of wines) cannot be detected analogously, because the rate constant of the quenching reaction is too low for this ion. Sensitive detection in water can still

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be achieved by utilizing the conversion of sulphite to thiosulphate.

The application of quenched RTPL of biacetyl to real samples may be restricted owing to interferences, as various inorganic and organic compounds cause depression of the RTPL signal. We have examined nitrite in meat samples and sulphite in wine. The determination of nitrite encountered no problems whereas the interferences encountered in the analysis of an inexpensive (rosé) wine were more serious.

THE QUENCHED PHOSPHORESCENCE DETECTION METHOD

Phosphorescence is, generally, not observable in liquid samples at room temperature. There are a few exceptions, for instance solutions of biacetyl. Intense phosphorescence can be observed for this compound in a wide variety of solvents, if they are thoroughly deoxygenated and purified[9]. Although these requirements seem to form a serious hindrance to basing a detection method on RTPL, we have shown that in practice the method can be readily applied, if a special eluent vessel is used[6,7].

In contrast to the sensitized RTPL method, where the analyte is excited[6], in the quenched RTPL method biacetyl (present in the eluent as a solute at about 10^{-4} M concentration) is excited with light of about 420 nm. The phosphorescence is recorded at about 515 nm. An analyte able to quench excited (triplet) biacetyl causes the phosphorescence signal intensity to be depressed from I to I' according to the Stern–Volmer expression[8]

$$I/I' = 1 + k_A \tau_0^B[A] \tag{1}$$

where k_A is the bimolecular rate constant (l mole^{-1} sec^{-1}) of the quenching reaction caused by the analyte, τ_0^B is the triplet state lifetime (seconds) of biacetyl in the absence of A and [A] is the analyte concentration (mole l^{-1}). The effect of A on the phosphorescence intensity depends only on the product $k_A \tau_0^B$, so that the success of the method is based on the long lifetime of the excited biacetyl. Under practical circumstances τ_0^B ranges from 10^{-4} to 10^{-3} sec in many eluents[9]. The sensitivity for a particular analyte is determined by the k_A value. In a previous paper[8] we estimated that the limit of detection (LOD) in mole l^{-1} for the azeotropic acetonitrile–water mixture using 10^{-4} M biacetyl is

$$\text{LOD} = 10k_A^{-1} \tag{2}$$

The depression of the phosphorescence signal by the analyte procedures a negative peak on the chromatogram. However, a positive signal is obtained if an electronic signal inverter is used so that $(I')^{-1}$ is registered. The latter signal intensity is proportional to [A] (eqn. 1).

Different reaction mechanisms may be responsible for the quenching of biacetyl. An important mechanism is energy transfer, which will give rise to diffusion-controlled rate constants if the triplet state of the analyte lies below the triplet state of biacetyl. Further, electron transfer can play a role, especially for readily oxidizable analytes, or for ligands that contain electron-donating groups. This mechanism will be efficient for strongly reducing species, which implies that some inorganic ions will be sensitively detectable by quenched RTPL of biacetyl. Therefore, this detection method has inherent selectivity.

EXPERIMENTAL

The experimental arrangement including the deoxygenation procedure for the batch experiments (performed to obtain the k_A values) and the chromatographic system are described in refs. 6–9. The eluent vessel described in ref. 7 was used. The role of oxygen in the sample is less important in the chromatographic system than in batch or flow injection experiments, because oxygen is usually separated on the column from the analytes to be detected. In aqueous solvents, biacetyl is partly hydrated; the hydrated form does not absorb light of 420 nm, so that it plays no role in the present detection method. The amounts of biacetyl added to the eluents used guarantee that the biacetyl concentration is between $1 \cdot 10^{-4}$ and $5 \cdot 10^{-4}$ *M*.

We used a home-made electronic signal inverter instead of the system described in ref. 9. This instrument, based on an AD 533 multiplier divider (Analog Devices), inverts linearly spectrometer outputs from 100 to 8 mV. Thirteen data points gave a regression coefficient of better than 0.999. in comparison with the older instrument with which linearity was obtained in the range 100–30 mV, this is an important improvement. As a consequence, the linear dynamic range of the quenched RTPL detector in the present arrangement is over two orders of magnitude.

The inorganic salts (Baker, analytical-reagent grade), dichloromethane (Baker, analytical-reagent grade) and Tris buffer (Sigma pH 7–9, biochemical buffer) were used as supplied.

Two chromatographic columns were used, *viz.*, a Varian Micropak SAX 10 column (30 cm × 4.6 mm I.D.) and a 10 cm × 4.6 mm I.D. column packed with ion exchanger AX 10, DMAE (Merck). In all experiments the injection volume was 20 μl. The chromatograms of nitrite and iodide were recorded under acidic conditions; the eluent used for thiosulphate was neutral as this ion is unstable at low pH values.

The conversion of sulphite to thiosulphate, prior to the chromatography, was performed with sulphur (Merck, analytical-reagent grade), recrystallized from xylene (Merck, mixture of isomers) and subsequently sublimed *in vacuo*. The reaction was carried out in a 10-ml glass tube closed with a glass stopper in which 2.5 ml of sample were mixed with 200 μl of Tris buffer (1 *M* buffer solution, adjusted with concentrated hydrochloric acid to pH 7.5) and 50–100 mg of sulphur (the excess amount of sulphur is not critical). The magnetically stirred mixture was heated in a water-bath at 87 ± 2°C for about 5 min. At this temperature longer reaction times did not give higher yields of thiosulphate. Higher temperatures had to be avoided because of boiling effects. Finally, the sample was cooled in a stream of tap water, deoxygenated by purging with nitrogen gas for 3 min and injected.

The processed meats were pre-treated as follows: 10 g finely ground sample and 30 ml water were thoroughly mixed in a Waring blender. The mixture was then centrifuged for 30 min at 4000 *g* and the (aqueous) supernatant decanted. To remove organic solutes such as fats and proteins from this solution, the decanted solution was extracted with dichloromethane in a separating funnel. Finally, the aqueous phase was diluted in most instances to 50 times its original volume before injection into the high-performance liquid chromatographic (HPLC) system.

The wine sample was diluted 20-fold. Subsequently, 50 ml of this diluted solution were passed through a preparative column (length about 6 cm, I.D. about 1 cm) filled with bonded-phase octadecyl C_{18} material (Baker). Finally, the reaction with sulphur was performed as described above.

TABLE I

BIMOLECULAR RATE CONSTANTS FOR THE QUENCHING OF BIACETYL PHOSPHORENCENCE IN AZEOTROPIC ACETONITRILE–WATER

All values (with exception of that for I^-) were taken from ref. 8.

Ion	k_A *(lmole^{-1} sec^{-1})*	*Ion*	k_A *(lmole^{-1} sec^{-1})*
NO_2^-	$1.3 \cdot 10^9$	SO_4^{2-}	$< 10^6$
NO_3^-	$< 10^6$	$S_2O_3^{2-}$	$6.6 \cdot 10^8$
SO_3^{2-}	$1 \cdot 10^7$	I^-	$3.2 \cdot 10^9$

RESULTS AND DISCUSSION

Quenching properties of nitrite and sulphite

The k_A values, measured in the azeotropic acetonitrile-water mixture, are summarized in Table I. The data suggest that nitrite can be detected sensitively even in the presence of large amounts of nitrate (see eqn. 1). However, the quenched RTPL detection will not be appropriate for sulphite. Indirect quantitation of sulphite via the following reactions:

$$SO_3^{2-} + I_2 + 3H_2O \rightarrow SO_4^{2-} + 2I^- + 2H_3O^+ \quad (3)$$

and

$$SO_3^{2-} + S \rightarrow S_2O_3^{2-} \quad (4)$$

which form the strongly quenching anions I^- and $S_2O_3^{2-}$, respectively, might be possible.

Fig. 1 shows chromatograms for nitrite, nitrite in the presence of nitrate, iodide and thiosulphate in water obtained by quenched phosphorescence detection after

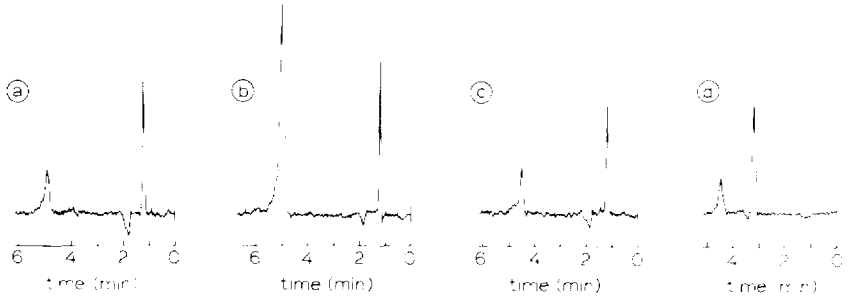

Fig. 1. Chromatograms of (a) 40 pmole of NO_2^-, (b) 200 pmole of NO_2^- in the presence of 200 nmole of NO_3^-, (c) 20 pmole of I^- and (d) 50 of pmole $S_2O_3^{2-}$ in water, obtained by quenched RTPL detection. The relatively intense positive peaks at 1.2 min (a, b and c) and 3.2 min (d) are attributed to oxygen; the weak negative peaks are probably due to fluorescent impurities in the sample solvent. Column: 30 cm × 4.6 mm I.D. Varian Micropak SAX 10. Eluent for (a), (b) and (c): acetonitrile–water (55:45, w/w) to which 10^{-2} *M* potassium phosphate buffer of pH 3.35 and $1.4 \cdot 10^{-3}$ *M* biacetyl were added; flow-rate, 2 ml min^{-1}. Eluent for (d): acetonitrile–water (10:90, w/w) to which were added $5 \cdot 10^{-3}$ *M* biacetyl, 0.02 *M* potassium phosphate buffer of pH 7.0 and 0.025 *M* potassium sulphate; flow-rate. 1 ml min^{-1}.

TABLE II

COMPARISON OF THE DETECTION LIMITS FOR NITRITE IN LIQUID CHROMATOGRAPHY OBTAINED WITH DIFFERENT DETECTORS

Detection method	*Ref.*	*LOD (nmole)*
Conductometric	10	0.4*
UV	4	0.2
Polarographic	3	4
Indirect UV	2	3.7
Indirect refractive index	2	1.2
Quenched RTPL	This work	0.02

* If 100 μl are injected and the detection limit is 0.3 ppm of sodium nitrite.

signal inversion. Incomplete deoxygenation of the sample is not a serious hindrance to the application of this detector because oxygen usually has a retention time different from that of the analyte. The approximate detection limits in water, based on a signal-to-noise ratio of three, are $2 \cdot 10^{-11}$ mole for nitrite, $1 \cdot 10^{-11}$ mole for iodide and $2 \cdot 10^{-11}$ mole for thiosulphate. The presence of nitrate does not affect the results. The linearity of the detection method was at least two orders of magnitude. The detection limit for nitrite compares favourably with those obtained with other detectors (see Table II).

The determination of sulphite via quenched RTPL detection of iodide (eqn. 3) is not possible. As expected, a strong signal generated by iodide is observed. Unfortunately, a weak signal is also present in the absence of sulphite. This weak signal interferes with the determination at low concentrations. However, the conversion of sulphite to thiosulphate (eqn. 4) at pH $\approx$ 7.5 can be applied. Many buffers prepared from inorganic salts of analytical-reagent grade gave a background signal after reaction with sulphur, but fortunately Tris buffer gave no problems.

The results for sulphite in water are depicted in Fig. 2. When the sample is not treated with sulphur, no thiosulphate signal is observed (Fig. 2b). If the reaction is

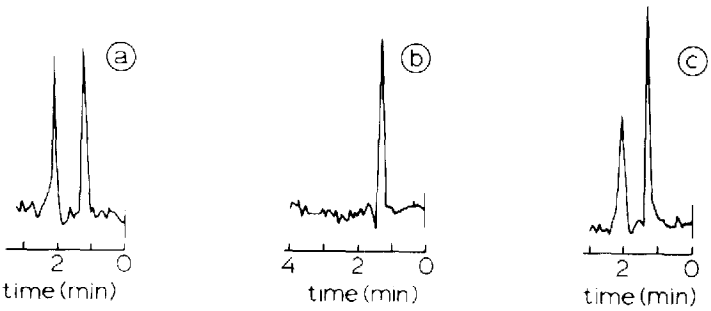

Fig. 2. Chromatograms of aqueous solutions of (a) $4 \cdot 10^{-5}$ *M* $S_2O_3^{2-}$, (b) $4 \cdot 10^{-5}$ *M* SO_3^{2-} without reaction with sulphur and (c) $4 \cdot 10^{-5}$ *M* SO_3^{2-} after reaction with sulphur; injection volume, 20 μl. The strong peak at 1.2 min is attributed to oxygen. Column: 10 cm × 4.6 mm I.D. packed with ion exchanger AX 10, DMAE (Merck). Eluent: acetonitrile to which was added $5 \cdot 10^{-3}$ *M* biacetyl, 0.02 *M* potassium phosphate buffer of pH 7.0 and 0.025 *M* potassium sulphate; flow-rate, 1 ml min^{-1}.

performed, a peak is observed which is attributed to thiosulphate (compare Fig. 2a and c). The recovery for sulphite is 70 + 2%. The detection limit is about 0.2 nmole, a value which compares favourably with literature data, *e.g.*, 1.3 nmole for indirect refractive index detection[4] and 1.5 nmole for indirect UV detection[4].

Application to real samples

It is interesting to examine whether the quenched RTPL detection method can be used for the determination of nitrite in processed meats and of sulphite in wines. These samples may contain many components in addition to nitrite or sulphite which might quench the phosphorescence of biacetyl. Both inorganic and organic compounds have been shown to be efficient quenchers[8].

In The Netherlands, processed meats can legally contain a maximum of 0.02% of sodium nitrite[11]. When 10 g of sample are mixed with 30 ml of water, this maximum corresponds to a nitrite concentration of about $1 \cdot 10^{-3}$ *M*. Hence, the high sensitivity of the quenched RTPL method for nitrite (the detection limit of 0.02 nmole in 20 μl corresponds to a concentration of $1.0 \cdot 10^{-6}$ *M*) allows dilution of the original sample solution and hence reduction of interferences from other constituents.

The pre-treatment of processed meats used to determine nitrite by the Griess reaction[12] (with a sulphanilic acid–α-naphthylamine solution, giving a strongly coloured dye) cannot be adopted for RTPL quenching as the mercury(II) chloride produces a strong background signal. However, the quenched phosphorescence detection mode permits a much simpler procedure to be followed, as outlined under Experimental.

The chromatograms recorded for two kinds of meat, *viz.*, liver sausage (LS) and filet americain (FA), are depicted in Fig. 3b and c, respectively. The retention time for nitrite is 4.9 min (see Fig. 3a); the peaks at 1.2 min are from oxygen, still present in the sample (apparently the deoxygenation of the FA solution was almost

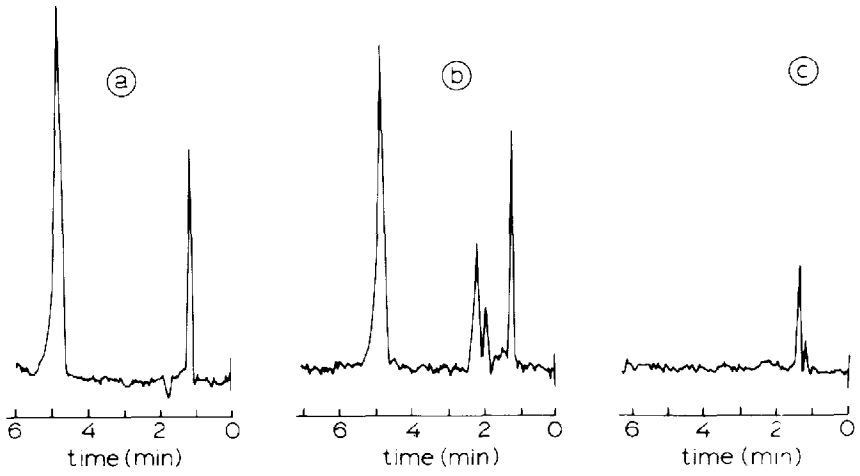

Fig. 3. Chromatograms of NO_2^-: (a) $1.0 \cdot 10^{-5}$ *M* in water, (b) 50-fold diluted extract from processed meat LS and (c) 20-fold diluted extract from processed meat FA. Oxygen peaks are observed at 1.2 min. The peaks at 1.9 and 2.2 min in (b) and the peak at 1.4 min in (c) are from unidentified quenchers. Chromatographic conditions as in Fig. 1a. Injection volume, 20 μl.

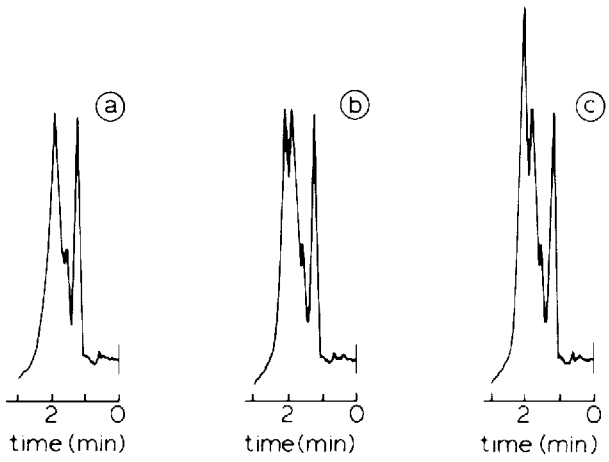

Fig. 4. Chromatograms of 20-fold diluted pre-treated rosé wine: (a) without reaction with sulphur, (b) without reaction with sulphur but containing $5 \cdot 10^{-5}$ *M* thiosulphate and (c) after reaction with sulphur. Chromatographic conditions as in Fig. 2; injection volume, 20 µl. The difference between the chromatograms in (c) and (a) is a measure of the amount of sulphite.

complete). The chromatogram of the LS sample clearly contains nitrite and oxygen peaks; the peaks at 1.9 and 2.2 min originate from unknown quenching compounds. In contrast, in the chromatogram of FA a nitrite peak cannot be detected, although the sample solution was diluted only by a factor of 20. In Fig. 3c, in addition to oxygen only one unidentified quenching component is found with a retention time of 1.4 min.

The procedure for the determination of nitrite in processed meats was not examined extensively with respect to errors possibly introduced by the sample pre-treatment. Based on calibrations with standard nitrite solutions in water (using peak heights), the LS sample contains 97 ± 7 mg kg^{-1} of sodium nitrite; for FA the content of sodium nitrite is below 2 mg kg^{-1}. Following the standard procedure with the Griess reaction, the sodium nitrite content of the LS sample was found to be 90 + 4 mg kg^{-1}, which approaches the result obtained by quenched RTPL within the limits of error. According to these results, the LS sample contains 50% of the allowed amount. The selectivity of quenched RTPL assures the interference-free analysis of processed meats for nitrite.

In view of the amount of sulphite allowed in rosé wine (225 mg l^{-1} as SO_2[13]) and the sensitivity of the quenched RTPL detector for sulphite (via thiosulphate), the wine sample was also diluted before analysis. The chromatograms are shown in Fig. 4. Similarly to Fig. 2, the sulphite peak is given by the difference between the chromatograms of the wine solution recorded before (Fig. 4a) and after reaction with sulphur (Fig. 4c). It is obvious that the wine under investigation contains sulphite: the reaction with sulphur causes a peak with a retention identical with that of the thiosulphate peak in Fig. 4b. However, for the reliable determination of sulphite in wine samples, further optimization of the chromatographic procedure is required.

CONCLUSIONS

Nitrite, thiosulphate and iodide ions are only a few of the inorganic ions that can be selectively detected by quenched RTPL[8]. Sulphite can be measured after reaction with sulphur to thiosulphate. We have shown that this mode of ion chromatographic detection can be simple and advantageous for the determination of nitrite in meat samples. The selectivity and sensitivity permit reliable and reproducible analysis with a minimum of sample handling. Using an electronic signal inverter, positive peaks and linear calibration graphs are obtained. The detection limits are one to two orders of magnitude better than for other known techniques. In wines, further improvement of the chromatographic procedure is needed for reliable quantitation of the thiosulphate peak. However, the example demonstrates the use of simple chemical reactions, to render a non-quenching species suitable for detection by quenched RTPL.

Further work along these lines and exploration of this method for the detection of, *e.g.*, iodide ions and platinum complexes in biological samples and chromium or tin in environmental samples is under way.

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