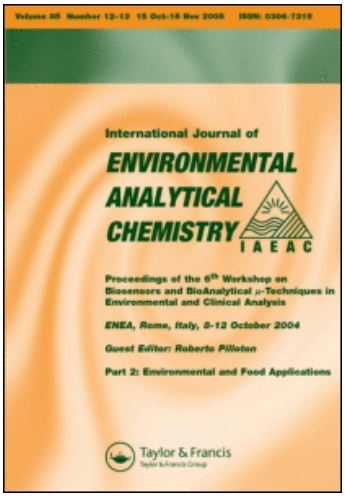

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A SIMPLE AND RAPID PHOSPHORIMETRIC METHOD FOR THE DETERMINATION OF α-NAPHTHALENEACETAMIDE IN FRUIT SAMPLES

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A simple and rapid phosphorimetric method for the determination of α-naphthaleneacetamide residues in fruit samples is presented. The method is based on a new, simple and sensitive methodology: Heavy Atom Induced Room Temperature Phosphorescence (HAI-RTP). The effect of different heavy atom salts and sodium sulfite concentration over the phosphorescence signals was investigated. The resultant HAI-RTP spectra were successfully applied for determining α-naphthaleneacetamide concentrations in the range of 7.8 to 100 $\mu g \cdot L^{-1}$, with relative standard deviations between 2.6 and 5.7%.

Keywords: α-Naphthaleneacetamide; heavy atom induced; room-temperature phosphorescence; fruit samples

INTRODUCTION

Phytohormones are used in agricultural practice to influence favorably the growth of cultivated plants and especially to prevent fruit fall. The control of these compounds in edible products according to the public regulations requires the development of analytical methods for their identification and determination. The methods most frequently used for this type of analysis include biological[1] and inmunological assays[2], as well as physical detection procedures.[3–6]

α-Naphthaleneacetamide is one of the most widely used phytohormones. It has been used to regulate the growth of numerous plants for more than 40 years. Its

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application, just after flowering, induces fruit clarification, preventing their picking 3 or 4 weeks before.[7,8]

As reported in the literature, the determination of different plant growth regulators including α-naphthaleneacetamide has been overtaken with different methodologies. The first one was proposed by Sigrist *et al.*[7] for the simultaneous determination of α-naphthaleneacetamide and 1-naphthaleacetic acid in apple samples using fluorimetry, yielding detection limits of 25 and 10 $\mu g \cdot L^{-1}$, respectively. Cochrane *et al.*[9] employed HPLC as separation technique with photometric and fluorimetric detection with a sensitivity of 100 and 10 $\mu g \cdot L^{-1}$, respectively. The sensitivity and selectivity obtained by these previous works were greatly improved by the application of modern instrumental methodologies, such as synchronous derivative.[10]

However, relatively few works have been devoted to the phosphorimetric study of plant growth regulators. In this sense, methodologies as micelle-stabilized room temperature phosphorescence (MS-RTP) or the use of cyclodextrins combined with sodium sulfite as oxygen scavenger may be employed.[11–16] An experimental design of the different factors that influence the determination of α-naphthaleneacetamide by micelle-stabilized room-temperature phosphorescence[17] has been stablished and has permitted the resolution of a mixture of two of these analytes (2-naphthoxyacetic acid and α-naphthaleneacetamide) employing, for the first time, derivative variable-angle synchronous scanning with RTP in a microemulsion medium.[18]

More recently, the authors have observed that it is possible to obtain phosphorescence signals in solution of naphthalene derivatives by using exclusively aqueous solutions of the analytes under the presence of heavy atom salts at high concentrations with sodium sulfite, as oxygen scavenger. This new methodology called Heavy Atom Induced Room Temperature Phosphorescence (HAI-RTP) has also been satisfactorily applied to other analytes[19–21]. In the present work, its applicability for the determination of α-naphthaleneacetamide in commercial food samples, has been investigated.

EXPERIMENTAL

Apparatus

All recordings of uncorrected luminescence spectra and measurements of HAI-RTP intensities were carried out with an Aminco Bowman series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp and thermostated

cell holder and controlled with a personal computer with the following characteristics: 40 MB hard disk, 4 MB RAM memory, 3.5-inch 1.44 MB floppy disk drive, VGA colour monitor with VGA graphics adapter card, serial 2-button mouse and working under DOS 6.0 and OS/2 2.0 operative systems. A GPIB(IEEE-524) interface card is used for computer instrument communication.

Reagents

Anhydrous sodium sulfite (Sigma Chemical Co.) and reagent grade potassium iodide (Sigma Chemical Co.) were used as received. Aqueous solutions were made with doubly distilled water. The sodium sulfite solutions were daily prepared and kept in tightly stopped containers. α-Naphthaleneacetamide (Sigma) was used without further purification. Stock solutions were prepared by dissolving 5 mg of α-naphthaleneacetamide in 250 ml of water.

Basic procedure

A 40 µl aliquot of the α-naphthaleneacetamide stock solution, 6 ml of 2 M potassium iodide and 150 µl of 0.1 M sodium sulfite were introduced into a 10 ml standard flask and made up to volume with water. Standard 10 mm fused silica cells were filled with this analyte solution. Reagent blanks lacking α-naphthaleneacetamide were prepared and measured following the same procedure. The relative phosphorescence intensities (RPI) of the samples and the corresponding blanks were measured at phosphorescence wavelength maxima $\lambda_{exc}/\lambda_{em}$ 292/492 nm. In order to establish a new phosphorimetric method, it is necessary to carry out an optimization of the instrumental parameters that influence the phosphorescence response. These are the measurement time, detector sensitivity, minimum period flash (m.p.f) or time between flashes, slits, delay time (t_d) and gate time (t_g). The following values were chosen as optima: 16 nm for the slits and for the excitation and emission monochromators, a scan speed of 2 $nm \cdot s^{-1}$, a delay time of 120 µs, a gate time 300 µs, a detector sensitivity of 1100 V and a minimum period flash of 5 ms. All these instrumental variables were kept constant for the rest of the experimental work.

Procedure for apple samples

The method was applied to two different commercial samples containing apple as raw material, such as apples, apple juice and cider.

About 250 g of apples was chopped in a food processor. A certain amount of α-naphthaleneacetamide was added to 15 g of the sample such that the final concentration was included in the range of the calibration graph. The mixture was transferred into a blender cup, 75 ml of water were added and subsequently blended for 20 min. The mixture was filtered using a 100 ml medium-porosity fritted-glass Büchner flask under suction, and the blender was washed three times with 5 ml of water, the washings being filtered. The filtrate was transferred to a 100 ml calibrated flask and diluted to volume with water.

For commercial samples, a certain quantity of α-naphthaleneacetamide was added to these samples in such a way that the final concentration was included in the range of the calibration graph. The samples were analyzed as described previously, without the need of previous separation procedures.

RESULTS AND DISCUSSION

Spectral characteristics

Figure 1 shows the three-dimensional phosphorescence spectra of α-naphthaleneacetamide in aqueous solution, obtained at 25°C in presence of KI and Na_2SO_3 as heavy atom and deoxygenation agent, respectively. The RTP spectra of α-naphthaleneacetamide show strong phosphorescence signals with maximum excitation and emission intensities at 292 and 492 nm, respectively. The triplet lifetime was determined in these conditions and established to be equal to 251 μs.

Effect of the heavy atom salt

The phosphorescence signals are obtained under the presence of heavy atom salts, since their concentrations might affect the intensity of HAI-RTP signal under certain conditions, as established in previous studies. [19–21]

As a consequence, the influence of heavy atom salt concentration has been proved in order to obtain maxima phosphorescence signals. The heavy atom salts selected were halogen of alkaline and alkaline earth and other salts used usually in phosphorimetry: KI, KBr, NaBr, NaI, RbCl, CsCl, KCl, $Cr(NO_3)_3$, $CeCl_3$, $InCl_3$, $Ca(NO_3)_2$, $Ca(NO_3)_3$, $TlNO_3$, $Pb(NO_3)_2$ and $AgNO_3$. $Pb(NO_3)_2$ and $AgNO_3$ precipitated under the presence of SO_3^{2-}, and thus were not used. The phosphorescence signal was not significant when KBr, $Cr(NO_3)_3$, $CeCl_3$, $InCl_3$, $Ca(NO_3)_2$, $La(NO_3)_3$, NaBr, NaI, RbCl, CsCl, KCl, and $TlNO_3$ were used. This

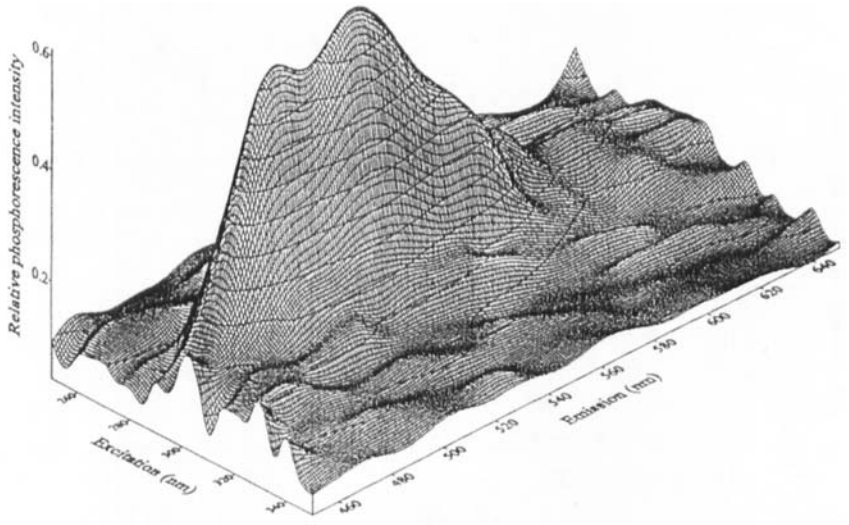

FIGURE 1 Projected three-dimensional spectrum of α-naphthaleneacetamide. [α-naphthaleneacetamide] = 80 $\mu g \cdot L^{-1}$, [KI] = 1.2 M, [Na_2SO_3] = 0.0015 M. Emission 450–700 nm, excitation 250–350 nm, $slits_{exc/em}$ 16/16 nm, t_d 120 μs, t_g 300 μs, detector sensitivity 1100 V and m.p.f. 5 ms

fact indicated that any of these heavy atoms do not significantly change the nature of the emitting specie or the radiative process. However, the concentration of KI could have a significant influence on the HAI-RTP signal.

Additionally, it has been proved that a non-phosphorescence response of the analyte is obtained in the total absence of a heavy atom and, in general, the HAI-RTP intensity increased with increasing heavy atom concentration. As a consequence, the determination of an optimum concentration range was necessary. The best results were obtained using KI in a concentration of 1.2 M since the intensity yield is maxima (see Figure 2).

Influence of sodium sulfite concentration

Besides the heavy atom salts needed to obtain a significant phosphorescence signals by HAI-RTP, it is necessary to use an efficient O_2 scavenger. N_2 purging can be used to remove O_2 from the solution in HAI-RTP. The use of sulfite ions yields an improvement of the technique. The concentration of SO_3^{2-} in the system is an important factor that affected the phosphorescence signal of α-naphthaleneacetamide.

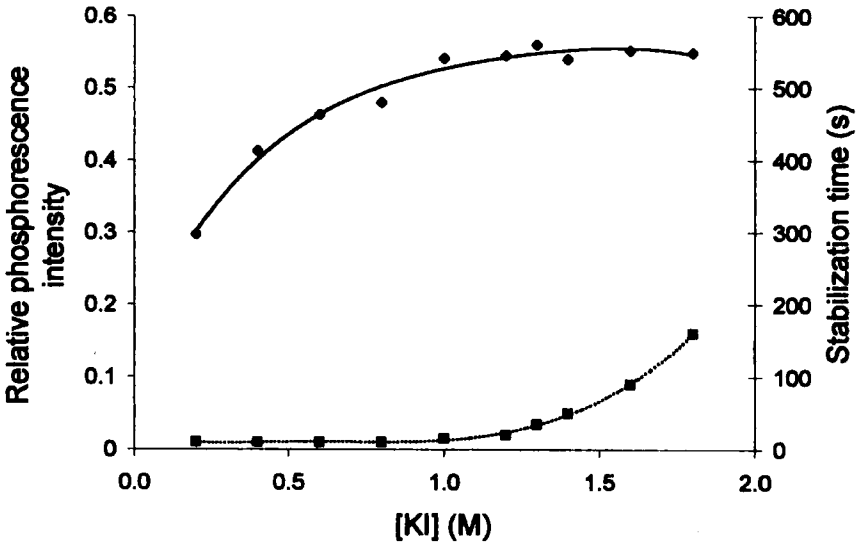

FIGURE 2 Effect of heavy atom concentration on RPI (—) and stabilization time (---). [α-naphthaleneacetamide] = 80 $\mu g \cdot L^{-1}$, $[Na_2SO_3] = 0.0015$ M, $\lambda_{exc/em}$ 292/492 nm, $slits_{exc/em}$ 16/16 nm, td 120 μs, tg 300 μs, detector sensitivity 1100 V and m.p.f. 5 ms

The method for sample deoxygenation is based on the redox reaction:[13]

$$2SO_3^{2-} + O_2 \leftrightarrow 2SO_4^{2-}$$

It should be taken into account that the analyte solutions were prepared in calibrated flasks in which the sulfite eliminated the oxygen; but subsequently the solutions were transferred into the phosphorimetric cells. Anyway, after some time, the sulfite remaining in the solutions was able to eliminate the oxygen in the cells.

The influence of sodium sulfite concentration was established by monitoring the RTP signal as a function of time until the HAI-RTP signal was stabilized for at least 5 min. Various amounts of sodium sulfite ranging from $5.0 \cdot 10^{-4}$ to $4.0 \cdot 10^{-3}$ M were added to a solution with a fixed amount of α-naphthaleneacetamide and heavy atom salt. The concentrations of α-naphthaleneacetamide and KI were 80 $\mu g \cdot L^{-1}$ and 1.2 M, respectively. They were transferred into a 10 ml flask, with appropriate amounts of a 0.1 M sodium sulfite stock solution in order to obtain the desired concentration. The development of the HAI-RTP emission was followed kinetically by monitoring at a wavelength of 492 nm with excitation at 292 nm.

Figure 3 shows the influence of the sodium sulfite concentration on the RTP emission of α-naphthaleneacetamide in presence of KI. As can be observed, an increase in the sodium sulfite concentration yields a gradual decrease of the phosphorescence signal of the system and an increase followed by a decrease in the stabilization time (t) or the period of time necessary for the RTP signal to reach stabilization. In view of these results a concentration of $1.5 \cdot 10^{-3}$ M was selected as best.

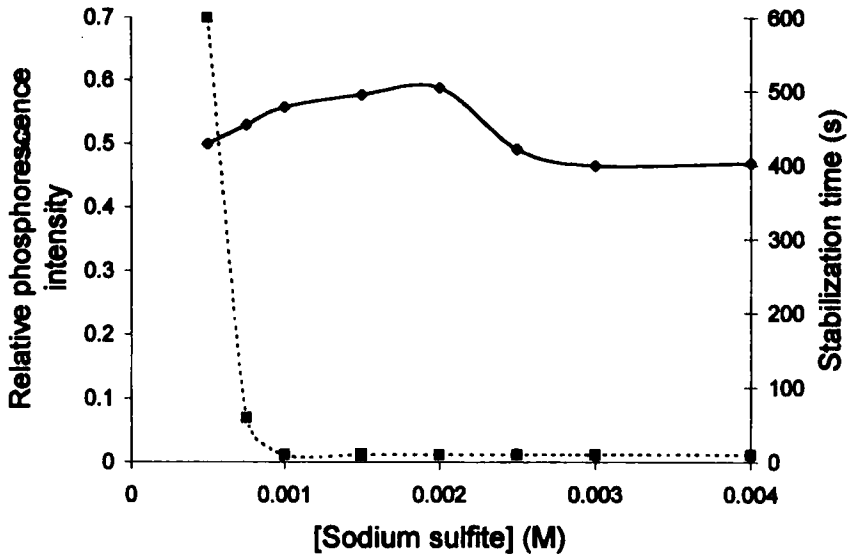

FIGURE 3 Effect of Na_2SO_3 concentration on RPI (—) and stabilization time (---). [α-naphthaleneacetamide] = 80 $\mu g \cdot L^{-1}$, [KI] = 1.2 M and instrumental conditions as Figure 3

Influence of temperature

Díaz García *et al.*[13] demonstrated that the temperature affects both the rate of oxidation of sulfite by the oxygen present in the solutions and the intensity of the phosphorescence signals. It was observed using naphthalene as the solute, that at 5°C the oxygen was not effectively eliminated from the solutions and as a consequence the phosphorescence signals were not reproducible, or even worse, the phosphorescence was quenched. It was then considered necessary to eliminate the oxygen from the solutions by reaction with sulfite at room temperature, measuring the phosphorescence signals at 25°C.

To obtain the HAI-RTP signals of this system, a detail study of temperature was carried out (see Figure 4). The HAI-RTP intensities decreased almost line-

arly with an increase in temperature. This effect is markedly appreciated and is mainly due to molecular motion and intermolecular energy conversion because of the collisional deactivation of the phosphors. A temperature of 25 ± 1°C was selected for the rest of the experimental work.

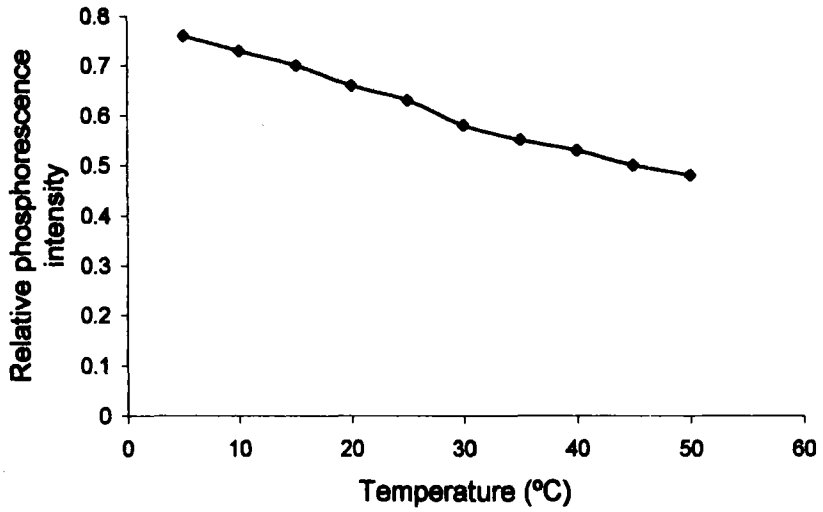

FIGURE 4 Effect of temperature on RPI. Experimental and instrumental conditions as Figure 1

Stability

The HAI-RPI signal of the system can reach stability instantaneously under the conditions of chemical deoxygenation, remaining stable for at least 1 h.

Validation of the method

The method was tested for linearity, precision, reproducibility and specificity. The calibration function (relation between phosphorescence signal and α-naphthaleneacetamide concentration) was determined, yielding the following regression equation:

$$RPI = 0.1612 + 0.064\ C$$

where C is the concentration of α-naphthaleneacetamide in $\mu g \cdot L^{-1}$. The correlation coefficient was > 0.999 (n=6), indicating a very good linearity from 0 to 100 $\mu g \cdot L^{-1}$. A detection limit[22] of 7.8 $\mu g \cdot L^{-1}$ was established.

When the method was applied to a series of six samples containing 20 and 100 $\mu g \cdot L^{-1}$ of the analyte, the relative standard deviations ranged between 2.6 and 5.7%, respectively.

Applications

The proposed method was applied satisfactorily to the determination of α-naphthaleneacetamide in apples and two different commercial products elaborated with apple, such as apple juice and cider. Mean recovery values of 90.6% for apples and of 91.4 and 85.8% for apple juice and cider, were obtained with a RSD equal to 5.4, 7.1 and 6.2%, respectively. As it can be seen, in all cases, a molecular deactivation by matrix effect causes a decrease in the obtained recoveries.

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