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Room temperature phosphorimetric determination of bromate in flour based on energy transfer

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ABSTRACT

Determination of bromate ions in contaminated flour samples by using a room temperature phosphorescence (RTP) optosensor is described. The optosensor is based on the non-radiative energy transfer from α-bromonaphthalene (a phosphorescent molecule insensitive to the presence of the analyte) acting as donor, to an energy acceptor bromate-sensitive molecule (trifluoperazine hydrochloride). The RTP emission of the selected donor greatly overlaps with the absorption spectrum of the acceptor, resulting in a decrease of the measured signal as the concentration of bromate ions increases.

A simple and general procedure is proposed to carry out the incorporation of both the donor and acceptor molecules in an appropriate solid material (sensing phase) through the co-immobilization of the species in a sol–gel inorganic matrix. The optimum amounts of the sol–gel precursors, including silica precursors, type of catalysis, and concentrations of donor and acceptor molecules, have been evaluated in order to obtain the best analytical features of the proposed optosensor for bromate determination. The highly stable developed sensing phase shows a selective and reversible response towards bromate even in presence of dissolved oxygen (a well-known quencher of the RTP). The calibration graphs were linear up to 200 mg L^{-1}, with a detection limit for bromate dissolved in aqueous medium of 0.2 mg L^{-1}. Sample throughput of the proposed optosensor was about 18 measurements h^{-1}. Application of the developed sensing phase was successfully proved for the detection of bromate ions in commercial flours, obtaining good recoveries.

1. Introduction

It is widely known that the bread making quality of freshly milled flour improves after two months storage. However, this process can be carried out faster through the addition of chemical substances called improvers [1]. Among these improvers, potassium bromate is commonly added because it is a slow-acting oxidizer that contributes to its functionality throughout the mixing, fermentation and proofing stages. Moreover, it presents an important residual action during the early stages of baking, resulting in strengthen dough and allowing for greater oven spring and higher rising in the oven [2].

However, there is a concern regarding the use of bromates in baking due to its possible relation to the development of tumors in laboratory animals, and therefore, European, American and Chinese regulations have limited its use. In this sense, the Code of Federal Regulations of the U.S. Food and Drug Administration has restricted the amount of potassium bromate added in a quantity not exceeding 50 parts to each million parts of the finished bromated flour, and is added only to flours whose baking qualities are improved by such addition [3]. For this reason, the development or improvement of analytical methods for the determination of bromate ions at such levels in flours is a matter of great interest. A large number of analytical methods have been published exploiting ion chromatography coupled with accelerated solvent extraction [4], high performance liquid chromatography-inductively coupled plasma mass spectrometry [5], or ion chromatography with a conductivity detector [6]. However, those methodologies present some disadvantages such as the need for extraction of the bromate ions at high temperatures [4], sophisticated instrumentation not affordable for many laboratories [5], or time consuming analytical strategies [6].

Room temperature phosphorescence (RTP) offers interesting advantages over fluorimetric-based methods that makes it attractive for analytical applications [7]. For instance, the phosphorescence signal is a low-noise emission because it is measured after any short-lived background luminescence or scattered light has ceased, allowing lower detection limits (DLs). Besides, the long emission wavelength of the phosphorescence phenomena facilitates

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the design of robust and inexpensive instrumentation based on decay time measurements [8]. Nevertheless, despite those benefits, there is a lack of known RTP emitters whose spectroscopic characteristics change when there are bromate ions present in the medium. This limitation might be overcome by resorting to energy transfer (ET) from a RTP emitter (donor) insensitive to bromate ions to a suitable acceptor which is sensitive to bromate. This methodology is based on the transfer of energy from the donor to the absorber and, then, the absorber dissipates this energy through nonradiative processes [9–12].

Moreover, in order to develop an ET-RTP sensor it is necessary to immobilize the appropriate reagents in solid supports to fabricate "active" solid phases [13]. In this sense, sol–gel processing has gained an increasing importance in the incorporation of luminescence indicators into a structured media. This is mainly due to the simplicity of its preparation, chemical inertness, tuneable porosity, optical transparency, mechanical stability and negligible swelling behavior [14]. Herein, donor and acceptor molecules are randomly mixed in close packed sol–gels. In this way, it is expected to demonstrate a very simple and general procedure for the co-immobilization of donor and acceptor molecules in a solid support, maintaining their optical properties and ensuring that they are close enough in proximity to allow the transfer phenomena to take place.

In this context, a simple, fast and selective ET-RTP sol–gel sensing phase for bromate determination in flour samples is here proposed. The method is based on the interaction of the donor molecule α-bromonaphtalene (BrN), which emits a very stable RTP that it is not affected by the presence of dissolved oxygen [12] nor by the presence of bromate ions, and it was matched with another molecule acting as acceptor whose spectroscopic characteristics change in the presence of bromate ions.

2. Experimental

2.1. Reagents

The reagents used for the preparation of the standards and solutions were of analytical reagent grade. All standards and solutions were prepared with ultrapure deionised Milli Q water (Milli-Q2 system, Milllipore, UK).

A stock solution of 1.00×10^{-4} M of α-bromonaphtalene (Riedel-deHäen, Seelze, Germany) was prepared in a 1.00×10^{-2} M solution of β-cyclodextrin (Aldrich, Milwaukee, WIS, USA) and stored in the darkness. The mixture is left to stand in an ultrasonic bath for 30 min before added to the sol–gel precursors mixture. Stock solutions 1.00×10^{-3} M of trifluoperazine hydrochloride (Sigma-Aldrich) or phenothiazine (Sigma-Aldrich) were prepared by dissolving the appropriate amount of reagent with deionized Milli-Q water. Sol–gel silicate precursors, tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMOS) were obtained from Fluka Chemie (Steinheim, Germany). A stock standard solution of 100 mg L^{-1} of bromate was prepared in milli-Q water from potassium bromate (Merck, Darmstadt, Germany), dried at 105 °C for 30 min. The stock solution is stable at least one week stored at 4 °C. Ethanol and hydrochloric acid were purchased from Prolabo (Spain). Sodium nitrate, sodium sulfate, sodium chloride, sodium acetate, sodium phosphate, potassium nitrate, magnesium sulfate, copper (II) sulfate, zinc sulfate heptahydrate, iron (III) chloride, sodium hydrogen carbonate and calcium chloride, (all of them from Sigma-Aldrich) were used for the study of the potential interferences.

For real sample applications, four wheat flours commercialized for different purposes (2 regular flours, 1 special type for frying and 1 special type for pastrymaking) have been used.

2.2. Instrumentation

Spectrophotometric measurements were performed with a Lambda 20 UV–vis spectrophotometer (Perkin Elmer, Norwalk, CT, USA) equipped with 10 mm quartz cells. Slits were fixed at 2 nm.

Phosphorescence measurements were performed on a Varian Cary Eclipse Luminescence spectrometer (Madrid, Spain), equipped with a discharge lamp. Unless stated otherwise, excitation and emission slits were fixed at 10 nm. A delay time of 0.1 ms and a gate time of 1.2 ms were typically used for RTP intensity measurements. The excitation and emission wavelengths were set at 220 nm and 540 nm, respectively, and the PMT voltage was adjusted to 775 V. In order to measure the solid sensing phase, sol–gel matrices were packed into a conventional Hellma luminescence flow-through cell made of quartz (Model 176.052-QS, Mullheim, Germany) of 1.5 mm of light path was employed in all the experiments. A peristaltic pump (model Minipuls 2, Scharlab, Barcelona, Spain) was used to generate the flow of the carrier solution through the system. 0.8 mm i.d. PTFE tubes were used throughout the flow system. The pH measurements were made with a pH-meter (Mod. MicropH 2000 from Crison, Barcelona, Spain). All the analytical measurements were carried out at room temperature (20 ± 3 °C).

2.3. Synthesis of the sensing phase

The preparation of the sol–gel sensing phase providing the best analytical features was as follows. Briefly, 2.5 mL of ethanol, 0.5 mL of stock solution of 10^{-4} M of BrN prepared in a 10^{-2} M solution of β-cyclodextrin, 0.05 mL of trifluoperazine hydrochloride 10^{-3} M and 0.25 mL of 0.1 M HCl, and 1.1 mL of Milli-Q H_2O were mixed and stirred. Afterwards, 0.6 mL of TMOS and 0.4 mL of MTMOS were added. After homogenization of the mixture, the polymerization process takes place and the mixture was left to dry for two weeks in the absence of light, until a constant weight is achieved. The dry xerogel was crunched and fragmented in an agate mortar and particle sizes of diameters between 80 and 160 μm were selected by sieving for further experiments.

2.4. General procedure

The two carriers employed in order to make the sample pass through the sensing phase were 3 M HCl and H_2O, at a flow rate of 1 mL min^{-1}. For the preparation of the calibration graphs, 1 mL of bromate standard (or sample) was injected into the flow system via an injection valve located in the H_2O carrier channel (see Fig. 1). After injection, the standard is mixed with 3 M HCl by using a "Y" type connector. Finally, the mixture passed through the

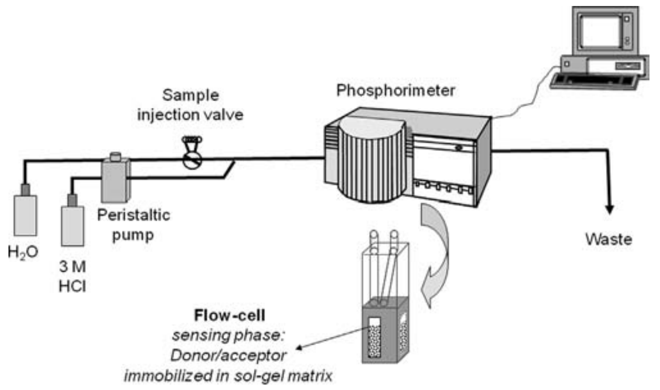

Fig. 1. Experimental set up used for the ET-RTP measurements.

measurement flow cell containing the sensing phase, giving rise to a transient signal as consequence of the energy transfer in presence of bromate ions. Reagent blanks were prepared and measured following the same procedure (in this case, it was introduced Milli-Q water instead of bromate standards).

2.5. *Real sample analysis*

The proposed ET-RTP method was evaluated for the determination of bromate ions in different type of commercial flours by following the general procedure after a pre-treatment step. First, 5 g of flour samples spiked with known amounts of potassium bromate were mixed with 15 g of Milli-Q water and let stand for 15 min to allow the solubilization of the present bromate ions in water [15]. Afterwards, the samples were filtrated in order to separate the solid from the supernatant, first through 0.45 μm filter device and then through a 0.22 μm filter device until the supernatant is clear. Finally, the clear supernatant was injected into the system following the general procedure.

3. Results and discussion

3.1. *Spectral characteristics of the energy donor/acceptor system*

The donor molecule selected for this system is BrN, which presents a rigid naphthalene molecule structure and a heavy atom. Moreover, based on previous studies made in our laboratory, in order to allow for an efficient protection of the phosphor towards oxygen quenching, *β*-cyclodextrin (CD) was added to the aqueous media [16]. After ultrasonic bath shaking, an enhancement of the rigidity of the microenvironment is achieved due to the inclusion of the BrN molecules in the cavity of the CD, resulting in strong RTP signals from the BrN that are detected even in the presence of dissolved oxygen. Fig. 2 shows the excitation and emission spectra of the donor. As it can be observed, BrN emits strong RTP emission in oxygenated aqueous media with two excitation peaks at 227 and 290 nm, respectively, and a wide emission band with two emission peaks at 500 and 525 nm.

The feasibility of the selected donor molecule for bromate determination based on the ET-RTP phenomenon was checked by proving that BrN is insensitive to the presence of bromate ions. For this purpose, the effect of the presence of increasing concentrations of bromate ions (up to 100 mg L^{-1}) on the RTP emission of the BrN was studied. The presence of bromates did not influence, at any bromate level tested, the RTP emission signals (neither the wavelengths nor the intensity signals) of the BrN donor.

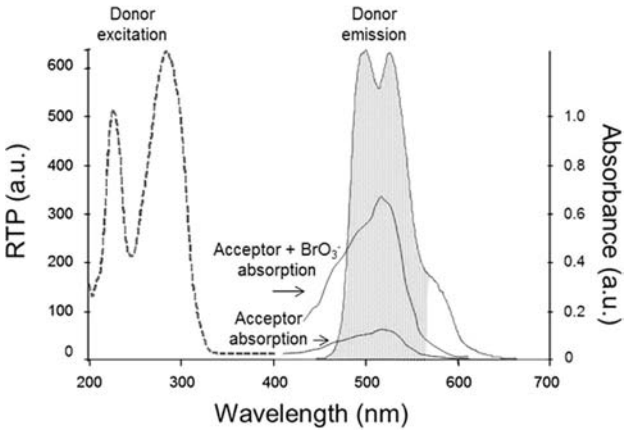

Fig. 2. RTP excitation and emission spectra of BrN in an aqueous *β*-cyclodextrin solution and absorbance spectra of trifluoroperazine hydrochloride before and after addition of bromate ions in aqueous solution.

Once the donor was selected, the following stage was to select an adequate acceptor that needs to fulfill two requirements: it has to be sensitive to the presence of bromate ions, and it has to possess an adequate absorption spectrum that overlaps with the RTP emission of the donor.

In this context, the acceptor system was selected from an article by Pacey and coworkers, which described a sensitive spectrophotometric method for the determination of bromate ions in aqueous media based on three different organic chromophores [17]. In such preliminary work, authors evaluated the use of trifluoperazine hydrochloride and phenothiazine for bromate spectrophotometric determination. They showed that phenothiazines form an intense color upon oxidation *via* a fast and reversible one-electron transfer to form radical cations. It was demonstrated that the presence of bromates produced an increase on the absorption spectra of trifluoperazine hydrochloride directly related to bromates concentration. Moreover, the increase on the absorption spectra of the trifluoperazine hydrochloride in the presence of bromated occurs at a wavelength range overlapping with the emission of the BrN (between 500 and 530 nm).

Fig. 2 shows the change on the absorption spectra of trifluoperazine hydrochloride in presence and absence of bromate ions, with a maximum at around 520 nm. Thus, Fig. 2 shows the excellent spectral overlap between the RTP emission spectra of BrN and the region of maximum absorbance of trifluoperazine hydrochloride in presence and absence of bromate ions. A good spectral overlap was also observed for phenothiazine. As a result of such suitable spectral characteristics, trifluoperazine hydrochloride and phenothiazine were selected as spectrophotometric bromate-selective indicators for the development of the ET-RTP method for bromate determination. Accordingly, a decrease in the measured RTP emission as the concentration of bromate increases could be expected, because the energy transfer would be higher. In other words, a decrease of the analytical signal (RTP intensity or RTP lifetime) with the presence of bromate should be expected and could be used as the analytical measurement principle of an optonsensor.

Since the aim of this work was to develop a sensing phase, the following section shows the studies carried out to co-immobilize both, donor and acceptor molecules, in a solid support.

3.2. *Synthesis of the sensing phase*

Sol–gel material is a type of solid support which exhibits an important number of advantages in terms of high rigidity, long-term stability, chemical inertness, optical transparency, low probability of leaching and low intrinsic luminescence [9,18]. Therefore, this type of matrix was selected as solid support for co-immobilization of the donor and acceptor molecules in order to develop a sensing phase based on ET-RTP. The sol–gel active phase developed in this work was prepared by trapping both donor and acceptor molecules inside the sol–gel silica matrix during the polymerization process, following modifications of a procedure previously described [19]. Thus, it is expected that donor and acceptor are in close proximity in order to allow good energy transfer efficiency.

3.2.1. *Parameters affecting the sol–gel matrix*

In order to obtain the final sensing phase, several experimental parameters have been evaluated, giving rise to a total of 92 different sol–gels synthesized and evaluated (details of the composition of each sol–gel can be found in the Supporting Information). The sol–gels evaluated have been prepared through two catalytic routes: acid and basic catalysis by adding HCl or NaOH respectively as catalysts of the polymerization process. Results proved that the acid catalysis (sol–gels 1–22; see Supporting information) showed better analytical performance than those synthesized through basic catalysis (sol–gels 23–44 and 81–92; see Supporting information), and therefore, this was selected for further experiments.

Another experimental parameter evaluated was the ratio of silica precursors employed, which affects to the final texture of the solid matrix. Different ratios of TMOS and MTMOS varying from 1:0 to 0:1 (v:v) respectively for the synthesis of the sol–gels were synthesized and evaluated (sol–gels 69–80; see Supporting information). All of these sol-gels showed a good behavior in terms of stability, since no lixiviation of the donor or acceptor molecules was observed for long periods. However, the highest change on the signal for the same bromate concentration was observed for a ratio of TMOS:MTMOS of 0.6:0.4, which was selected for further experiments.

3.2.2. Parameters affecting the response to bromate ions

The concentration of the donor/acceptor in the sol–gel is a critical feature that has to be carefully studied. In this sense, BrN concentration was evaluated from 1.8×10^{-6} M to 1.8×10^{-5} M (by adding 0.1–1 mL to the sol–gel precursors mixture) for the different concentrations of acceptor studied (sol–gels 45–68; see Supporting information). On the other hand, it is known that immobilization of an indicator dye into a solid support might modify its spectral characteristics or behavior in the presence of the analyte. For this purpose, two different molecules, trifluoperazine hydrochloride and phenothiazine, have been evaluated as energy acceptors of the system. They have been both added in a different range of concentrations from 9×10^{-7} M to 9×10^{-5} M (and in acid/basic catalysis as well).

In order to select the optimum sol–gel for the determination of bromate ions, the response of the different sol–gels to the presence of bromate was evaluated. For this purpose, the change on the luminescence observed for the different sol–gels when adding the same amount of bromate (200 mg L^{-1}) was recorded. Results showed that the sol–gels based on phenothiazine as acceptor do not respond to the presence of bromate at this level of concentration. However, several sol–gels synthesized with trifluoperazine hydrochloride produced a change on the RTP measured for the same concentration of bromate. Among these sol–gels, the one that produced a biggest change on the RTP was the sol–gel with a donor concentration of 9×10^{-6} M, and an acceptor concentration of 9.1×10^{-6} M. Thus, taking into account the results obtained in all these studies, this sol–gel was selected for further experiments.

After the evaluation of the performance characteristics of the 66 sol–gels under investigation, the preparation of the finally selected sol–gel was as follows. Briefly, 2.5 mL of ethanol, 0.5 mL of stock solution of 10^{-4} M of BrN prepared in a 10^{-2} M solution of β-cyclodextrin, 0.05 mL of trifluoperazine hydrochloride 10^{-3} M and 0.25 mL of 0.1 M HCl, and 1.1 mL of Milli-Q H_2O were mixed and stirred. Afterwards, 0.6 mL of TMOS and 0.4 mL of MTMOS were added. After homogenization of the mixture, the polymerization process takes place and the mixture was left to dry for two weeks in the absence of light, until a constant weight is achieved. The dry xerogel was crunched and fragmented in an agate mortar and particle sizes of diameters between 80 and 160 μm were selected by sieving for further experiments, which minimizes potential problems of overpressure inside the flow-cell.

3.3. Effect of the bromate concentration

Luminescence measurements were carried out in phosphorescent instrumental conditions, with a delay time of 0.1 ms and a gate time of 1.2 ms, and excitation and emission wavelengths at 220 and 540 nm respectively. The sol–gels obtained presented high phosphorescence signal in both, air and water flow and the main spectroscopic characteristics remained unaltered once donor and acceptor were entrapped inside the synthesized inorganic matrix.

Solutions with different concentrations of bromate ions were passed through the sensing phase containing both donor and acceptor molecules in order to investigate the effect of bromate concentration in the RTP emission signals measured under the selected conditions collected in the experimental section. As expected, it was observed that increasing concentrations of bromate ions gave rise to a decrease on the RTP emission signal detected, due to the changes on the energy transfer from the donor to the acceptor. Fig. 3 shows the RTP emission spectra from the BrN in the presence of the acceptor trifluoroperazine hydrochloride for different bromate concentrations. As can be seen, in the RTP emission spectral region between 490 and 550 nm (which corresponds with the spectral region which overlaps with the absorption of the acceptor) a good correlation between the bromate concentration and the intensity of the RTP emission was obtained.

It is well-known that dissolved molecular oxygen is a strong quencher of the molecular phosphorescence emission in liquid solutions. Hence analytical response of the RTP sensing phase was evaluated under different oxygen concentrations by addition of different concentrations of sodium sulfite (up to 20 mM), a well known and effective oxygen scavenger [20]. Unexpectedly no significant change on the RTP performance of the sensing material for water-dissolved bromated detection (in presence or absence of dissolved oxygen) was observed. This is of course an advantageous feature when compared with traditional phosphorescence optosensors where removal of oxygen is mandatory [21].

The following studies were aimed to design a simple experimental manifold in order to measure the signal of the solid sensing phase. Two carriers have been employed: a Milli-Q water flow was used to inject the sample in the system, and a HCl flow was employed to acidify the medium in order to develop the change on the acceptor spectroscopic characteristics in presence of bromate ions. At this stage, the concentration of HCl was found to be critical in order to produce a higher change on the RTP measured. Thus, concentration of the HCl used in the carrier was evaluated from 0.1 M up to 3 M (studies at higher HCl concentration were avoided in order to extend the life of the tubing employed in the system and to avoid sol–gel sensing material degradation). Results showed that, for the same bromate concentration of 200 mg L^{-1}, higher concentration of HCl produced a

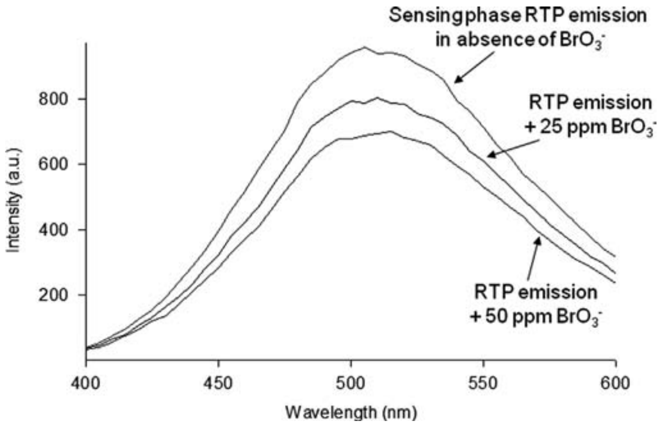

Fig. 3. Sensing phase RTP emission in absence and presence of increasing concentrations of bromate ions.

bigger change on the RTP measured. Therefore, 3 M HCl has been selected for further experiments.

Next the effect of inject increasing amounts of bromate ions on the RTP emission signal from the sensing material was investigated. As it can be observed in Fig. 4, as the concentration of water-dissolved bromate is higher, there is a bigger decrease on the luminescence monitored. The response of the active sensing phase is linear in a wide range of concentrations. Furthermore, an advantageous feature is that the emission signal is recovered to the base line without the addition of any regenerator, which makes the sensing phase highly suitable for the potential design of a sensor for bromate ions.

The possibility of using RTP lifetime measurements for bromate determination has also been investigated. Lifetimes were evaluated from the decay curves obtained at each bromate concentration. The decay curves obtained were fitted to an exponential curve ($r \geq 0.998$ in all the cases) and the lifetime (τ) was calculated. The lifetime for the sol–gel sensing material was 0.268 ms in the absence of bromates. As expected, the lifetimes measured from the sol–gel sensing material were lower in the presence of increasing concentrations of bromate (thus demonstrating that a dynamic energy transfer occurs). However, sensitivity achieved using triplet lifetime measurements was of about one order of magnitude lower as compared to direct intensity measurements. Therefore, RTP intensity emission signals were used as analytical signals for further experiments.

Another important key factor when designing a sensing phase is the stability of immobilization of the sensing elements in the solid matrix. In this sense, the stability of the signal was evaluated by recording the emission signal of the sensing phase during more than 8 h exposed to a continuous flow of an aqueous solution. Results showed that lixiviation of the donor or acceptor was not observed, since the RTP measured remained stable during continuous monitorization, being the same sensing phase reused for a large number of experiments (at least 200 consecutive measurements were carried out without an appreciable decrease on the sensing phase analytical performance characteristics). Moreover, the sensing phase was evaluated even one year after it was synthesized, and it was demonstrated to be stable when stored in the dark at room temperature.

Finally, it is worth to mention that we have evaluated a sensing material based on the immobilization of the bromate sensitive dye indicator (trifluoroperazine hydrochloride) on a sol–gel matrix and its spectrophotometric performance compared with the RTP-based sensing phase. It was observed that signal to noise ratio of the spectrophotometric based sensing material for bromate detection was, at least, two orders of magnitude worse than the obtained with the here proposed RTP optosensor.

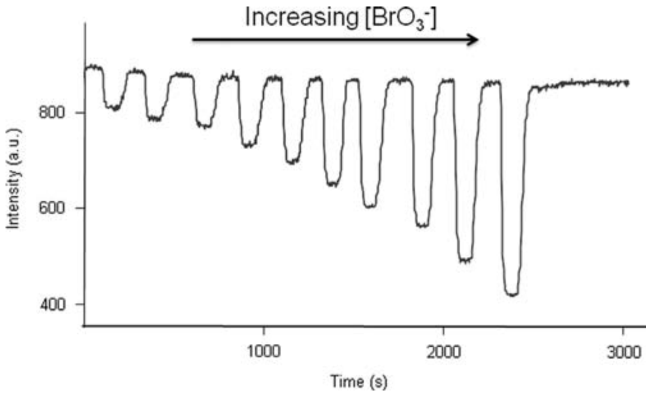

Fig. 4. Effect of increasing concentrations of bromate ions (from 0.5 up to 50 mg L^{-1} of dissolved bromated) injected in an aqueous matrix over the RTP emission of the donor/acceptor immobilized on a sol–gel support.

3.4. Analytical characteristics

The analytical characteristics of this reversible ET-RTP sensing phase were evaluated under the selected optimum conditions. Calibration graphs were plotted from the results of triplicate measurements of bromate standard solutions of increasing concentration, from 1 mg L^{-1} up to 200 mg L^{-1}. Results fit to the equation $I_{RTP}=13.0\ [BrO_3^-]+45.8$ with a regression coefficient, $r^2=0.998$. The DL, calculated as the bromate concentration which generated an analytical signal three times the standard deviation of the blank signal, turned out to be of 0.2 mg L^{-1}. To our knowledge this is the first sensing phase described in the literature for bromate determinations based on RTP measurements. Obtained detection limits compare favorably to other direct spectrophotometric methods for bromated determination described so far. The precision of the proposed methodology, evaluated as the relative standard deviation of five replicates of a sample containing 1 mg L^{-1} of bromate, was found to be ± 5%. Sample throughput was of about 18 samples h^{-1}.

Further potential application of the developed sensing phase for determination of bromate ions should ensure that it is unaffected by the presence of other species that might be present in real samples. For this purpose, following the proposed general procedure, the effect of foreign ions typically present in water (used as bromate extractant) or flours on the determination of bromate was studied. Potential interferences were added to a standard bromate solution, containing 5 mg L^{-1} of the analyte, and after injection in the system, their effect on the ET-RTP signal was evaluated. Cumulative results are summarized in Table 1. Briefly, cations including Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{3+} did not produced interference effects for concentrations up to 50 mg L^{-1}, since they do not modify the luminescent signal more than 5%. Anions such as Cl^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, CH_3COO^- neither produced any change on the phosphorescence measured even at the maximum concentration assayed of 50 mg L^{-1}. Only Cu^{2+} ions produced a significant change on the recorded emission for concentrations over 25 mg L^{-1}.

3.5. Real sample analysis

The proposed methodology was evaluated for the determination of bromate ions in different types of flours following the general procedure described before for real samples with the developed sensing phase. Briefly, the flour was spiked with known amounts of potassium bromate. For this purpose, 5 g of flour samples were spiked with different amounts of potassium bromate. Then, they

Table 1
Study of the potential interference effect of different species on the ET-RTP determination of 5 mg L^{-1} of bromate ions.

Species assayed	**Maximum contentration tolerated**[a] (mg L^{-1})
Chloride	50[b]
Nitrate	50[b]
Sulfate	50[1b]
Phosphate	50[b]
Carbonate	50[b]
Acetate	50[b]
Calcium	50[b]
Magnesium	50[b]
Zinc	50[b]
Sodium	50[b]
Iron	50[b]
Copper	25
Potassium	50[b]

[a] The presence of the potential interferent up to this concentration level results in deviations of the RTP signal lower than 5%.

[b] Maximum concentration assayed.

Table 2
Real sample analysis. Each result corresponds to the mean of three flour replicates that were previously spiked with known concentrations of potassium bromate.

Type of sample	$KBrO_3$ spiked (ppm)	$KBrO_3$ measured (ppm)	Recovery (%)
Flour 1	5.0	5.1 ± 0.9	102
Flour 2	10	9 ± 2	90
Flour 3	50	46 ± 2	92
Flour 4	50	49 ± 2	98

were mixed with 15 g of Milli-Q water, and let stand for 15 min to allow bromate solubilization [15]. Afterwards, these bromate enriched water aliquots were injected in the flow system, and the signal was recorded as the bromate ions passed through the sensing phase following the general procedure. Table 2 collects the results for the phosphorescent determination of bromate in flours. As it can be observed, good recoveries (between 90% and 102%) for the determination of bromate were obtained in all the samples, thus indicating the suitability of the proposed methodology for direct analysis of bromate in different types of wheat flour.

4. Conclusions

A novel flow-through room temperature phosphorescence optosensor for the determination of bromate ions in wheat flour samples has been here described. The method is based on the energy transfer of a phosphorescent donor (which does not respond to the presence of the analyte) to an acceptor whose spectroscopic characteristics change in the presence of bromate ions. For this purpose, donor and acceptor molecules have been entrapped inside a sol–gel matrix, and the deactivation of the RTP measured in the presence of the analyte was continuously monitored. Thus, a very easy and general procedure for the immobilization of donor and acceptor has been described, keeping the luminescent features of those components unaltered. Moreover, it should be noted that these synthesized sol–gels are stable for long time periods.

The proposed methodology is very simple, fast and selective, and its usefulness successfully tested by measuring, with appropriate sensitivity, bromate ions in real flour samples. Finally, it is worth to highlight the reversibility of this sensing phase, i.e. no reagents are needed to recover the emission signal of the sensing phase, this feature along with the improved sensitivity achieved (as compared to a spectrophotometric based sol–gel material) and the robustness of the sensing phase the potential applicability of the proposed system for routine analysis.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.05.019.

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