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A novel reaction for spectrophotometric determination of bromate in bread flour and water samples

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RESEARCH PAPER

Abstract

A sensitive and selective spectrophotometric method based on oxidation of Nile red with potassium bromate for determination of bromate in bread samples has been developed. The method allows the determination of bromate in the range 25.0-970.0 μg/ml (r^2=0.995) for 0.5-2 min. The relative standard deviation were 4.89, 3.88 and 4.63% for determination of 50.0, 250.0 and 750 μg/ml of bromate (n=5), respectively, and the limit of detection (3 S_b/m) was 0.0589 μg/ml. The precision, accuracy and selectivity of the method are discussed. The proposed method was applied successfully for the determination of bromate in spiked bread flour and water samples.

Keywords: bromate, flour, Nile red, spectrophotometry

1. Introduction

Bread is an important source of food in most countries in the world (Al-Dmoor, 2012). It is consumed extensively in homes, restaurants and hotels. Bread is made from low protein wheat. It usually contains several ingredients that would help improve the quality of the bread. Some of the basic identified ingredients, apart from flour are table salt, sugars, flavours and at least a flour improver, such as potassium bromate ($KBrO_3$) (Ekop *et al.*, 2008; Emeje *et al.*, 2010; Van der Kaaij *et al.*, 2009). Therefore, the quality and security of flour and related food are important for our health (Magazu and Migliardo, 2010; Rose *et al.*, 2009; Shahin *et al.*, 2010). $KBrO_3$, as an oxidant, can oxidise thiol groups to disulfide linkages, thus strengthening the protein network, so it is usually added to foods as an additive to make the flour stronger and more extensible. However, bromate is a kind of potential carcinogen, which has been proven by both the US Environmental Protection Agency and the World Health Organization. The International Agency for Research on Cancer has classified $KBrO_3$ as 2B (a possible human carcinogen) based on sufficient evidence that $KBrO_3$ induces cancer in experimental animals. Now, many countries have banned $KBrO_3$ as a flour ingredient (Campbell, 2006; El-Harti *et al.*, 2011; Fawell and Walker, 2006; Gradus *et al.*, 1984; Matsumoto *et al.*, 1980; Moore and Chen, 2006; Quick *et al.*, 1975; Shi *et al.*, 2006).

Many analytical methods have been developed for the determination of bromate, such as flow injection analysis (Alonso-Mateos *et al.*, 2008; Oliveira *et al.*, 2011), X- ray fluorescence (Perez and Leon, 2010), zone electrophoresis-isotachophoresis (Bodor *et al.*, 2002), capillary gas chromatography analysis with mass detector (Himata *et al.*, 1994), liquid chromatography (Himata *et al.*, 2000), colorimetric method (Boschserrat, 1997), inductively coupled plasma mass spectrometry (Akiyama *et al.*, 2002), high performance liquid chromatography (Kawasaki *et al.*, 2002), ion chromatography (Inoue *et al.*, 1997; Michalski, 2003), spectrophotometric (Afkhami *et al.*, 2001; Mitrakas, 2007) and spectrofluometric method (Gahr *et al.*, 1998). However, the methods mentioned above suffer as being time consuming procedures and requiring complicated instrumentation (Soylak *et al.*, 2011a,b). Kinetic methods are widely used for analysing industrial and natural samples because of their high selectivity and versatility and, in some cases, extremely high specificity. The advantage of kinetic determinations is the combination of very low determination limits with a simple and available experimental technique,

especially with photometric monitoring of the reaction rate (Soylak *et al.*, 2011a,b).

This paper describes a simple and sensitive kinetic spectrophotometric method for the determination of bromate at μg/ml and it was applied to the determination of bromate in spiked drinking water and bread flour samples. The method is based on the oxidation of Nile red with bromate in a nitric acid (HNO_3) medium. Different variables that effect the oxidation reaction, as well as a description of the procedures are presented below. The proposed method is highly selective and simple for the determination of bromate in real samples.

2. Materials and methods

Apparatus and reagents

Absorption measurements at λ_{max} 592 nm were performed using a Unicam UV 2 (Pye Unicam Ltd, Cambridge, UK) model spectrophotometer with a 1 cm quartz cell. Ultrasonication of flour samples was conducted with a Cole Parmer ultrasonic processor (Cole Parmer Instruments, Vernon Hills, IL, USA). All glassware and bottles were soaked in 5% HNO_3 overnight and washed with double distilled water.

All chemicals were of analytical grade. The cationic solutions used for interference study were prepared from their nitrate salt, and anionic solutions were from potassium or sodium salts (Merck, Darmstad, Germany). Stock Nile red (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) solutions were prepared by 0.0160 g of Nile red in 125 ml of absolute ethanol and diluted to 250 ml with double distilled water in a flask. The stock solution of Nile red was diluted to the desired concentration level before use. 0.10 M of $KBrO_3$ (Merck) was prepared by dissolving an appropriate amount of $KBrO_3$ in double distilled water. 1.0 M of HNO_3 (Merck) was prepared by diluting an appropriate amount of HNO_3 in double distilled water.

Recommended procedure for the determination of bromate

The catalytic reaction was monitored spectrophotometrically by measuring the change in absorbance of the reaction mixture at λ_{max} 592 nm. The fixed time method was used for 0.5-5 min.

An aliquot of real sample solution, an appropriate amount of 0.1 M $KBrO_3$, 0.5 ml of 1.0 M HNO_3, 1.5 ml of Nile red solution were transferred to a 5 ml flask, diluted to 5 ml with double distilled water. The zero time was taken as the moment at which the last drop of Nile red was added. The reaction mixture was mixed and immediately an appropriate amount of solution was transferred to the quartz cell and the reaction was monitored for 0.5-2 min at λ_{max} 592 nm. The measurements in the absence of bromate or sample solution were repeated to obtain data for uncatalysed reaction.

3. Results and discussion

Nile red is an oxazine type dye (Figure 1) and can be oxidised by bromate and HNO_3 at room temperature. Nile red is intensely coloured because of delocalization of Π electrons and it allows absorption of light in the visible region. The presence of both bromate and HNO_3 cause a synergic effect so the rate of oxidation reaction of Nile red accelerates. This causes a rapid decrease in the absorbance of Nile red at 592 nm. Thus determination of bromate can easily be done by monitoring the indicator reaction of Nile red at 592 nm.

There are many methods such as initial rate, variable time, fixed time and rate constant for kinetic measurements. Among these, the fixed time method is the simplest and the most conventional involving the measurements at a particular wavelength. Thus a fixed time method was chosen for the oxidation reaction of Nile red with bromate and HNO_3.

The effects of concentration of each reagent and temperature on the indicator reaction were investigated to find optimum reaction conditions to achieve sensitive and selective results for the determination bromate. The reaction condition was optimised by altering each variable in turn while the others were kept constant. ΔA was plotted versus reaction parameters to determine the optimum reaction conditions. ΔA is the absorbance difference for the studied time interval and is proportional with reaction rate. The optimum values taken were those giving the maximum sensitivity (net reaction rate; ΔΔA) and under conditions in which small variations in the variable concerned did not greatly affect the reaction rate.

Preliminary studies showed that the absorbance signal is independent of the order of mixing the components, i.e. the reagents can be added in any order.

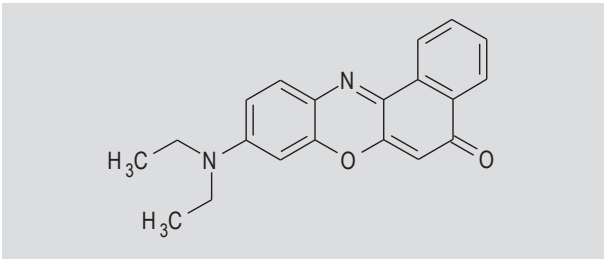

Figure 1. Molecular structure of Nile red.

Effect of nitric acid concentration

HNO_3 is a strong oxidizing agent and can oxidise a wide range of organic substances. In a Nile red and bromate system, the oxidative effect of bromate can be enhanced in the presence of HNO_3. In order to evaluate the effect of the concentration of HNO_3 on the oxidation of Nile red, the HNO_3 concentration in the range 0.01-0.13 M was studied. As can be seen from Figure 2, the sensitivity of the reaction increased with increasing HNO_3 concentration up to 0.1 M. The sensitivity of the reaction decreased above 0.1 M. Thus a concentration of 0.1 M was chosen as optimum HNO_3 concentration.

Effect of Nile red concentration

The effect of Nile red concentration on the reaction rate was studied over the range $0.5x10^{-5}$-$2.5x10^{-5}$ M (Figure 3). In the presence of bromate, the reaction rate increased gradually with increasing Nile red concentration up to $2.0x10^{-5}$ M but the repeatability of the results of absorbance differences decreased from $1.75x10^{-5}$ M of Nile red. On the other hand the reaction rate in the absence of bromate only slightly increased with increasing Nile red concentration. The overall reaction rate also increased up to $2.0x10^{-5}$ M. Thus, we chose a Nile red concentration of $1.50x10^{-5}$ M.

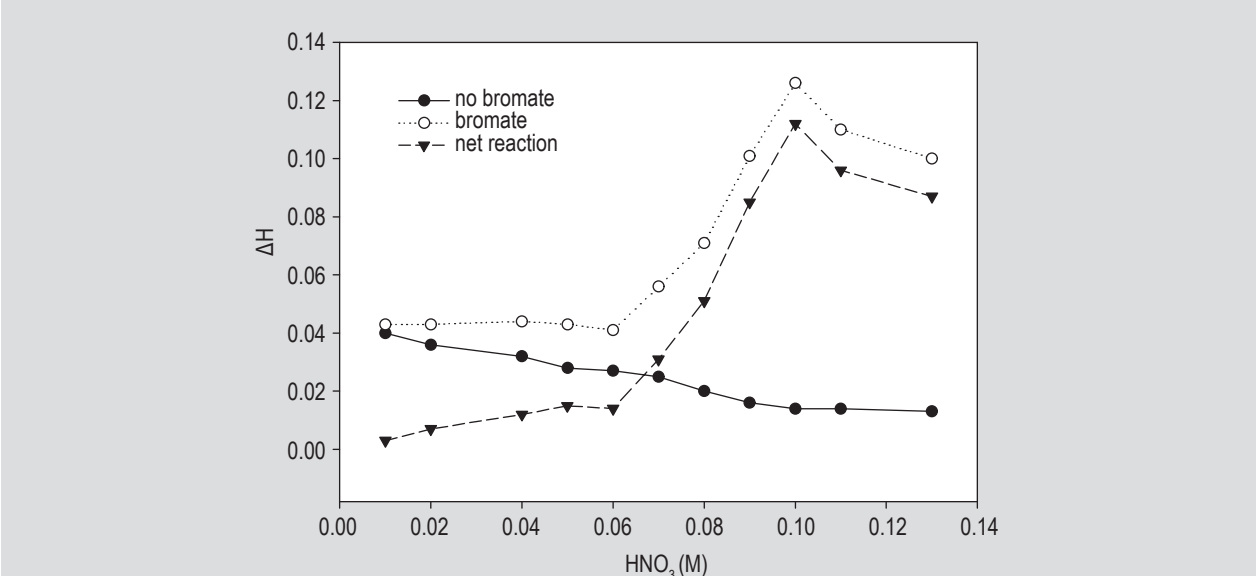

Figure 2. Effect of nitric acid (HNO_3) concentration on the absorbance difference (ΔA) (at 1.50×10^{-5} M Nile red, 25 °C).

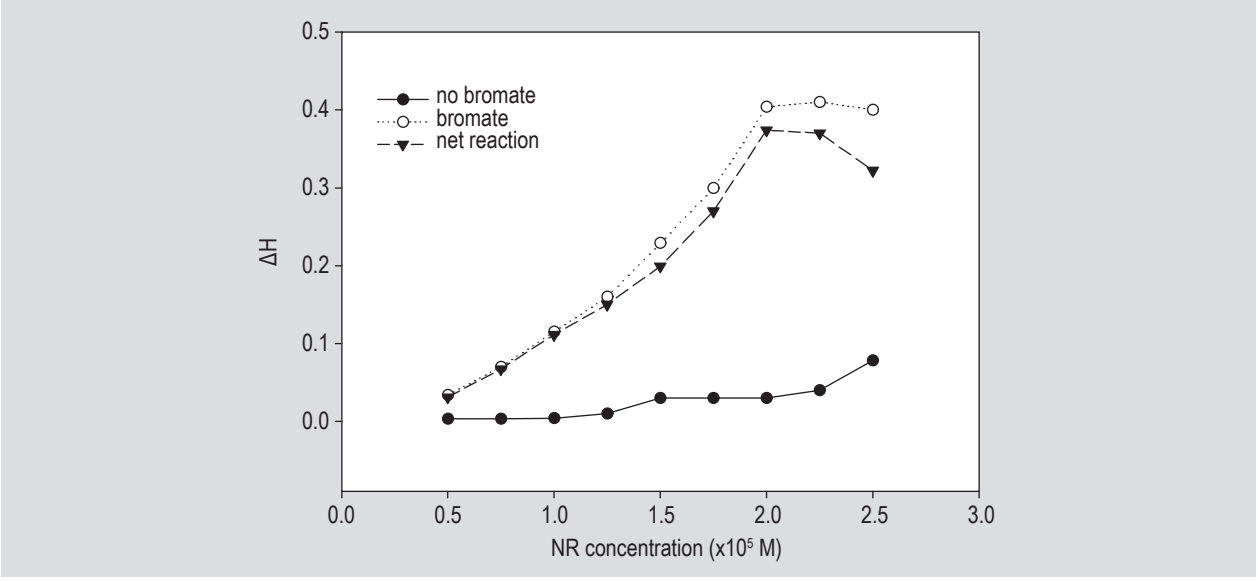

Figure 3. Effect of Nile red (NR) concentration on the absorbance difference (ΔA) (at 0.10 M HNO_3, 25 °C).

Effect of reaction temperature

Under the optimum reaction conditions the effect of temperature on reaction rate was investigated in the range 20.0-60.0 °C (Figure 4). It was observed that increasing temperature in the presence of bromate accompanied an increase in the rate of reaction up to 30.0 °C but from 30.0 °C the rate of reaction decreased so significantly that the sensitivity of the whole system decreased in the range 30.0-60.0 °C. The temperature of 30.0 °C gives relatively high sensitivity and stable absorbance data, but for simplicity of temperature control throughout the experiments a temperature of 25 °C was chosen.

Analytical parameters

The calibration graph was established under the optimum conditions by using the fixed time method for different time intervals. The results are given in Table 1 for the range 25.0-970 μg/ml.

Table 1. Calibration equation of the absorbance difference (ΔA) with respect to time intervals.

Time interval (s)	Calibration equation	r^2
30-120	$\Delta A = 0.422C_{bromate} - 0.00790$	0.995
30-180	$\Delta A = 0.731C_{bromate} - 0.0220$	0.979
30-240	$\Delta A = 1.118C_{bromate} - 0.0270$	0.977

$C_{bromate}$ = concentration of bromate (μg/ml); r^2 = correlation coefficient.

As can be seen from Table 1 for the determination of bromate the calibration curves were linear in the range 25.0-970.0 μg/ml with a correlation coefficient of 0.995 (30-120 s) where $C_{bromate}$ is the concentration of bromate in μg/ml and Δ(ΔA) is the difference of catalysed and uncatalysed reaction absorbance difference. The detection limit based on statically 3 Sb/m was 0.0589 μg/ml and 10 Sb/m was 0.1964 μg/ml where Sb is the standard deviation of the blank signal (n=20) and m is the slope of the calibration curve.

Selectivity of bromate-Nile red reaction

In order to establish the application of the proposed method to real samples, the selectivity of the method was evaluated by determining 500 μg/ml of BrO_3^- in the presence of varying amounts of different cations and anions. The tolerance limit was defined as the maximum concentration of the foreign ion causing a relative error of less than ±5% (Table 2).

Table 2. Effect of diverse ions on the determination of 500 μg/ml of BrO_3^-.

Foreign ion	Tolerance limit [interfering ion/BrO_3^-]
NO_3^-	1000
$H_2PO_4^{2-}$	500
IO_3^-	100
Cl^-	15
$Cr_2O_7^{2-}$	2.5
NO_2^-	0.1
Ca^{2+}, Mg^{2+},Zn^{2+},Sr^{2+},Pb^{2+},Ba^{2+},Al^{3+}	20
Fe^{3+},Cd^{2+},Mn^{2+},Mo^{2+}	10

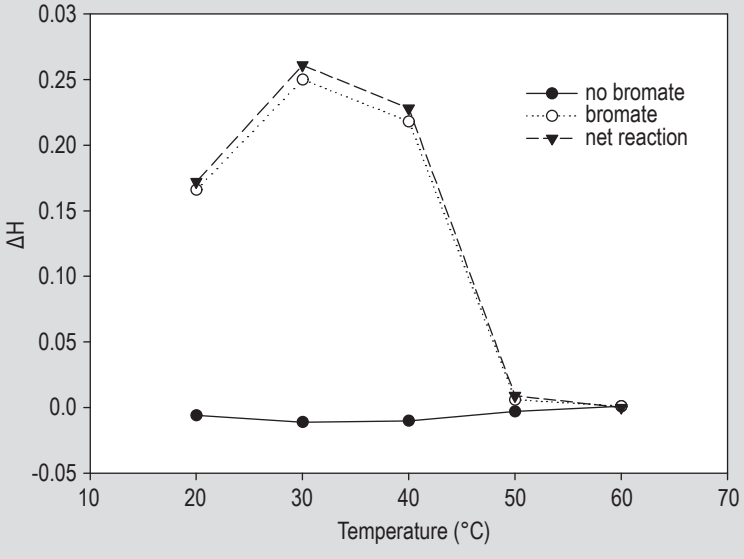

Figure 4. Effect of temperature on the absorbance difference (ΔA) (at 1.50×10^{-5} M Nile red, 0.10 M HNO_3).

The ions studied and their tolerance levels are given in Table 1. It was found that the rate of the catalyzed reaction was not affected by most of the cationic and anionic ions. On the other hand the selectivity of proposed method was greatly improved using cation exchange resin.

The accuracy and precision of the proposed method

In order to determine the accuracy and precision of the proposed method based on the oxidation of Nile red with bromate, seven independent experiments were carried out under optimum conditions and the concentration of bromate was calculated with a calibration curve method (Table 3).

Analytical applications

In order to evaluate the analytical applicability of the proposed method, it was applied for the determination of bromate in bread flour and water samples. The flour samples were collected from the local market in Sivas, Turkey. The sample was prepared for analysis according to Sanchez *et al.* (1989) with some modifications. Briefly, 5 g of flour samples were weighed and 100 ml of double distilled water and an appropriate amount of $KBrO_3$ were added. The mixture was subjected to ultrasonic extraction at intermittent mode (to avoid forming a dough) for 10 min followed by centrifugal separation. The extract was diluted with double distilled water in 250 ml in a calibrated flask. Aliquots of this solution were then treated the proposed method after passing through Dowex 50WX8-100 resin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The bromate contents of the samples were determined with the standard addition method (Table 4).

Tap water samples was collected from Sivas and filtered immediately. Bottled water was collected from a local market. The appropriate amount of bromate solution was spiked to water samples. The possible cationic interference accompanied with bromate was removed by passing ground water through Dowex 50WX8-100 resin. After removal of cationic species, the bromate content of ground waters was determined by the proposed method. The bromate contents of ground water samples were detected using the linear calibration curve (Table 5).

Table 3. Accuracy and precision of the proposed kinetic spectrophotometric method (n=5).

Present BrO_3^-) (µg/ml)	Found BrO_3^- ± SD (µg/ml)	Recovery (%)	%RSD
30.0	31.22±0.84	104.07	2.69
50.0	49.72±2.43	99.44	4.89
100.0	104.07±5.32	104.07	5.11
250.0	256.14±9.95	102.46	3.88
500.0	511.81±21.12	102.36	4.13
750.0	789.95±36.59	105.20	4.63
900.0	879.28±43.85	97.22	4.98

%RSD = percentage relative standard deviation.

4. Conclusion

A comparison between the proposed method based on its oxidative effect on Nile red with some existing methods for the kinetic determination of bromate indicates that this method provides an acceptable dynamic linear range and is free from most interference (Table 6). The proposed kinetic-spectrophotometric method is simple, easy and rapid to apply, inexpensive, employs available reagents, and provides adequate selectivity. Accuracy and precision of the proposed method are satisfactory. The procedure provides a wide linear dynamic range, 25.0-970 µg/ml. The method was successfully applied to the determination bromate in spiked bread flour samples and water samples.

Table 4. Determination of bromate in flour samples (n=5).

	Added BrO_3^- (mg/ml)	Found BrO_3^- ± SD (mg/ml)	Recovery (%)	%RSD
Flour sample I	0.0	BDL	-	-
	50.0	53.22±4.19	106.44	7.89
	250.0	259.18±14.70	103.67	5.67
	750.0	793.47±32.37	105.73	4.08
Flour sample II	0.0	BDL	-	-
	50.0	52.13±3.3	104.26	6.33
	250.0	238.23±11.21	95.29	4.70
	750.0	782.69±39.11	105.83	4.98

BDL = below detection limit; %RSD = percentage relative standard deviation.

Table 5. Determination of bromate (BrO_3^-) in water samples (n=5).

	Added BrO_3^- (mg/ml)	Found BrO_3^- ± SD (mg/ml)	Recovery (%)	%RSD
Tap water	0.0	BDL	-	-
	50.0	51.58±1.43	103.16	2.77
	250.0	242.49±4.68	97.0	1.93
	750.0	781.62±16.71	104.22	2.07
Bottled water	0.0	BDL	-	-
	50.0	52.24±2.27	104.48	4.34
	250.0	259.63±7.47	103.85	2.87
	750.0	754.12±24.32	100.55	3.22

BDL = below detection limit; %RSD = percentage relative standard deviation.

Table 6. Comparison of the proposed kinetic spectrophotometric method with some existing spectrophotometric methods.

Sample	Method and comments	Linear range	Reference
Bread	Spectrophotometric method based on redox reaction between bromate and promethazin in acidic medium	0.5-4.5 µg/ml	El-Harti *et al.* (2011)
Bread	Photometric and fluorimetric method based on oxidation of benzyl 2-pyridyl ketone 2-quinolylhydazone reaction between bromate and promethazin in acidic medium	0.25-6.5 µg/ml	Sanchez *et al.* (1989)
Bread	Spectrophotometric method based on the oxidation of Congo red dye in hydochloric acid medium	0.45-5.50 µg/g	Ojeka *et al.* (2006)
Bread	Spectrophotometric method based on the oxidation of crystal violet dye in hydochloric acid medium	0.78-4.80 µg/g	Ojeka *et al.* (2006)
Water	Spectrophotometric method based on the oxidation of phenothiazine in acidic medium	25-750 µg/l	Oliveira *et al.* (2011)
Waste effluents	Spectrophotometric method based on the reaction of bromate and 2-(5-bromo-2-pridiylazo)-5-diethylaminophenol (5-bromo-PADAP) with SCN^-	0.18-3.0 mg/l	Van Staden *et al.* (2004)
Flour and water samples	Spectrophotometric method based on the reaction of bromate and Nile red	25-970 µg/ml	current study

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