Simultaneous Stopped-Flow Determination of Butylated Hydroxyanisole and Propyl Gallate Using a T-Format Luminescence Spectrometer

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A simple and fast luminescent method is used for the first time to resolve a mixture of two synthetic antioxidants, propyl gallate (PG) and butylated hydroxyanisole (BHA), by the joint use of the stopped-flow mixing technique and a T-format luminescence spectrometer. The determination of these compounds involves two different and independent reactions. On the one hand, PG determination is based on an energy transfer process that involves the formation of a lanthanide chelate with terbium in the presence of Triton X-100 and tri-*n*-octylphosphine oxide. On the other hand, BHA is determined using a reaction between the oxidized form of Nile Blue and the antioxidant. Both systems are excited at the same excitation wavelength (310 nm), and the emission wavelengths are 545 and 665 nm for PG and BHA, respectively. The absence of overlap in the emission spectra makes it possible to measure separately the analytes in each channel of the instrument. Initial rate and equilibrium signal are used as analytical parameters and measured in 0.1 and 1 s for PG and BHA, respectively. Calibration graphs are linear over the range 0.09−3.5 μg mL^{-1} for PG and 0.3−15 μg mL^{-1} for BHA. The relative standard deviations of both systems are close to 2%. The proposed method is applied to the determination of these two antioxidants in several commercial food samples with recoveries ranging between 94.8 and 102.9% for PG and between 94.1 and 102.1% for BHA.

Keywords: *Luminescence; T-format spectrometer; antioxidants; food samples*

INTRODUCTION

The potential carcinogenic properties of phenolic antioxidants attract interest in the analytical control of the presence of these compounds in food, where they can be used individually or in mixtures. This last situation is becoming more frequent due to the fact that the antioxidant activity is more efficient in this case than when used alone. They are added to prevent oxidative rancidity in a great variety of commercial food samples that include liquid or solid fat in their composition.

Although chromatographic methods (Robards and Dilli, 1987; Page and Charbonneau, 1989; Saarlin and Cellerino, 1990; Rafecas et al., 1998) are usually chosen for the determination of antioxidants in food samples, nonchromatographic methods (Galeano et al., 1998; Diego et al., 1998; Aguilar-Caballos et al., 1997) have also shown their utility for this purpose. The last one reported the photometric simultaneous determination of butylated hydroxyanisole (BHA) and propyl gallate (PG) based on the different kinetic behaviors of these compounds when reacted with 3-methyl-2-benzothiazoline hydrazone (MBTH) in the presence of cerium(IV). Kinetic measurements were obtained at two wavelengths, by coupling a stopped-flow mixing technique and diode array detection, and the concentration of each analyte in the mixture was obtained by applying a chemometric method.

This paper reports a simpler new approach for the simultaneous determination of these antioxidants in foods which involves fluorescence measurements with an instrument in a T-format configuration, furnished with a single excitation monochromator and two emission paths symmetrically arranged on both sides of the sample compartment. The analytical utility of this equipment has been shown in the application of kinetic methodology for fluorescence polarization immunoassay (Pérez-Bendito et al., 1994) and in the determination of antibiotics in foods (Gala et al., 1997). Two independent chemical systems are used to obtain the corresponding luminescent derivatives of PG and BHA: the formation of the terbium(III)−PG chelate and the reaction of BHA with oxidized Nile Blue (NB).

The luminescence of the terbium(III)−PG chelate arises from the intramolecular energy transfer process from the excited triplet state of the ligand to the emitting level of the lanthanide ion. This process gives rise to a large Stokes shift and narrow and intense emission bands, which avoid or minimize selectivity limitations of luminescence methods. The formation of this chelate was previously applied to the determination of PG (Panadero et al., 1995) in a micellar medium containing sodium dodecyl sulfate (SDS) and Triton X-100. However, as mentioned below, the presence of SDS caused a notable increase on the blank signal of the NB−BHA system, which required a modification of the experimental conditions of the PG system.

The application of a long-wavelength dye as reagent for the fluorometric detection of BHA is described here for the first time. The spectral features of these dyes allow potential interferences from the sample matrix to be eliminated as they frequently occur at shorter wavelengths than that of the dye. An additional advan-

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10.1021/jf990886u CCC: $19.00
Published on Web 02/02/2000

tage of the analytical use of these compounds is their short fluorescence lifetime, which decreases the probability of nonradiative quenching processes. In addition to its application as redox indicator (Bishop, 1972), NB has been used as a reagent for the determination of metal ions (Cai and Xue, 1997; Chen et al., 1997) and nitrite (Ensafi and Keyvanfard, 1994; Ensafi and Kolagar, 1995) and as a label for albumin determination (Imasaka et al., 1989).

The luminescent reaction products of the PG and BHA systems used here for the simultaneous determination of these compounds are excited at the same excitation wavelength, by using the second-order grating effect for the BHA system, and show different emission wavelengths, fulfilling the spectral requirements of the dual-channel instrument used. Quantitative data for these antioxidants are obtained by measuring simultaneously the initial rate of the PG system and the equilibrium signal of the BHA system. A stopped-flow mixing technique has been used for this purpose, which provides a means for automation and rapid handling of reagents, both of which are highly desirable in food routine analyses. The absence of overlap in the emission maxima of these systems allows the signal corresponding to each analyte to be independently monitored, avoiding the use of mathematical methods.

EXPERIMENTAL PROCEDURES

Instrumentation. An AB2 SLM-Aminco (Urbana, IL) luminescence spectrometer, equipped with a 150 W continuous xenon lamp, was used. A 9050 visible and near-IR monochromator and a R636-10 Hamamatsu red sensitive photomultiplier tube, both supplied by Sciencetech (London, ON, Canada), were fitted to the T-format configuration of the instrument to obtain long-wavelength measurements. The instrument was furnished with a stopped-flow module fitted with an observation cell of 1 cm path length and controlled by the associated electronics. Excitation and emission slits were set to provide a 8 nm band-pass. The temperature of solutions in the stopped-flow module was kept constant at 25 °C with recirculating water from a thermostated tank.

Chemicals. All reagents used were of analytical grade. Stock solutions of PG and BHA (Sigma) of 1000 μg mL^{-1} were prepared weekly in a 70% ethanol solution and stored at 4 °C. More diluted solutions of 20 μg mL^{-1} of each analyte were daily prepared by dilution from the stock solutions. A 2×10^{-2} M terbium(III) solution using terbium(III) nitrate pentahydrate (Aldrich), 1% Triton X-100 (Fluka), and a 2.5×10^{-3} M solution of sodium nitrite (Merck) were prepared in distilled water. A 6×10^{-4} M solution of tri-*n*-octylphosphine oxide (TOPO) was prepared in a 50% ethanolic solution. A 2.8×10^{-5} M NB (Sigma) solution was prepared in 6% ethanol. A 0.7 M hexamine buffer solution was also prepared in distilled water, and the pH was adjusted to 6.3 using hydrochloric acid.

Preparation of Oxidized NB Form. The oxidation of NB was carried out by mixing the dye (1.4×10^{-5} M) with sodium nitrite (2.5×10^{-5} M) and hydrochloric acid (10^{-2} M). The oxidized NB was stable for 2 days.

Analytical Method. Two solutions were prepared to fill the two 2 mL drive syringes of the stopped-flow module. One of them contained terbium(III) (1.5×10^{-2} M), NB (8.0×10^{-6} M), TOPO (6×10^{-5} M), Triton X-100 (0.05%), sodium hydroxide (5×10^{-3} M), ethanol (2.5%), and hexamine buffer (7×10^{-2} M). The other solution contained Triton X-100 (0.05%), hexamine buffer (7×10^{-2} M), and standard or sample of BHA and/or PG at final concentrations of 0.3−15 and 0.09−3.5 μg mL^{-1}, respectively. In each run, 150 μL of each solution was mixed in the mixing chamber.The excitation wavelength used was 310 nm. The variation of the luminescence intensity with time due to PG system was monitored at 545 nm, and the equilibrium signal due to the BHA system was monitored at 665 nm. Kinetic data were obtained by applying the initial rate method to the values acquired from the detector, which were processed by the computer, furnished with a linear regression program for application of the initial rate method. The contribution of the blank signal in the kinetic measurements was found to be negligible. The initial rate was determined in ~0.1 s. Equilibrium measurements were acquired in only 1 s. It was necessary to subtract the blank signal in the determination of BHA. Each standard or sample was assayed in triplicate. All measurements were carried out at 25 °C.

Determination of BHA and PG in Several Food Samples. *Oil and Lard Samples.* The sample (10 g) was dissolved in 50 mL of purified petroleum ether, stirred for 10 min, and extracted three times for 3 min with 7-mL aliquots of a 72% ethanolic solution. The three portions were combined, filtered through 240 Albet paper, and diluted to 25 mL. A volume of 0.7 mL was treated as described under Procedure.

Solid Samples. Samples (7−15 g) were treated with 50 mL of petroleum ether, and the suspension was stirred for 10 min, filtered using 240 Albet paper, and treated as indicated above. The samples were then analyzed following the aforementioned procedure.

RESULTS AND DISCUSSION

Spectral and Kinetic Study of Chemical Systems. The chemical systems used for the simultaneous determination of PG and BHA by using a T-format spectrofluorometer must be chosen in such a way that the reaction products have similar excitation wavelengths, because the instrument has a single excitation monochromator, and different emission wavelengths. In the case that the reaction products show spectral emission overlap, the resolution of the mixture could be carried out by using an additional mathematical method. However, the determination is simpler in the absence of spectral emission overlap as the analytical signal corresponding to each analyte can be obtained independently. Also, it is desirable that the reaction products show emission bands more red-shifted than those of conventional derivative reagents to avoid potential spectral interferences from the sample matrix.

A series of assays were carried out to find a fluorescent chemical system suitable for the determination of BHA and compatible with the spectral features of the terbium(III)−PG system, which allows the sensitive and selective determination of PG (Panadero et al., 1995). Several long-wavelength dyes, namely, oxazine (Nile Blue, Cresyl Violet) and thiazine (Toluidine Blue, Azure A, Azure B) dyes, were assayed with this purpose. The excitation wavelength of these compounds is close to twice the excitation wavelength of the terbium(III)−PG system (310 nm), so that the use of this wavelength allows these compounds to be excited by the second-order grating effect. However, the spectral features of these compounds did not change in the presence of BHA. Additional assays, carried out by oxidizing previously these reagents in acid medium, showed that NB lost its fluorescence as a result of the redox process, but a fluorescent signal was obtained in the presence of BHA. The excitation wavelength of this reaction product was 620 nm, the same as that of NB alone, but the emission wavelength was 665 nm, slightly less than that of NB (675 nm). Similar results were obtained with the other oxazine dye assayed, Cresyl Violet, but its excitation wavelength (585 nm) is less compatible with the PG system than that of NB. Of the different oxidizing agents assayed, nitrite ion gave the best results, as it

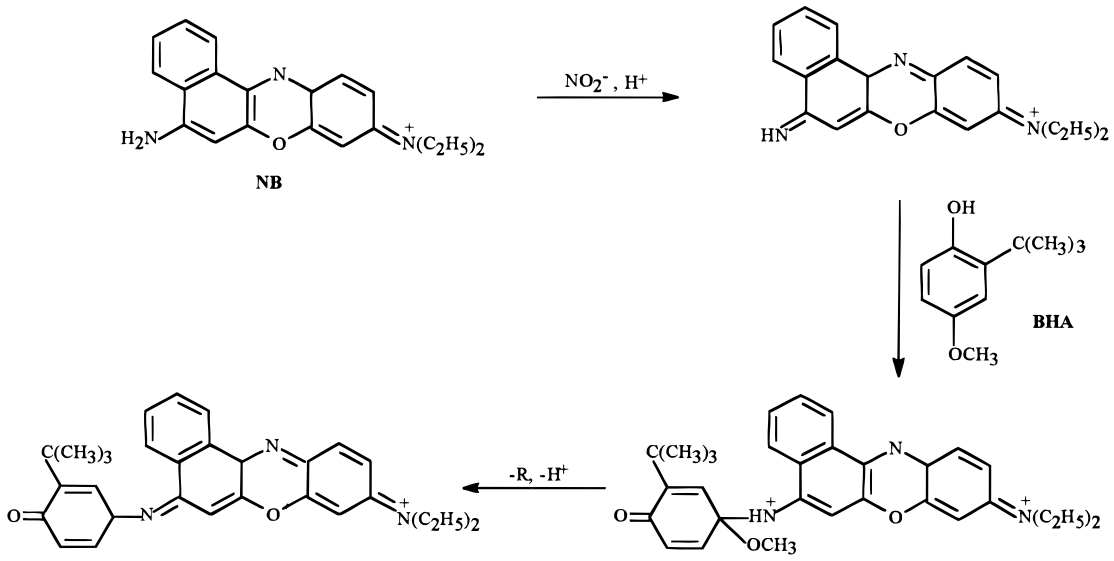

Figure 1. Reaction mechanism proposed for the BHA system (R = $-OCH_3$).

did not interfere with the PG system, unlike hydrogen peroxide or iron(III).

The fluorescent behavior of the nitrite−NB−BHA system could be explained according to the reaction scheme shown in Figure 1. The oxidation of NB by the nitrite ion involves the conversion of the nonsubstituted amino group to imino group, which would easily react with the phenol of BHA substituted in the para position, following a reaction mechanism similar to that proposed in the Gibb's reaction (Dacre, 1971; Josephy and Van Damme, 1984) for para-substituted phenols.

Regarding the experimental conditions, the simultaneous determination of PG and BHA requires that no interaction between the reagents involved in the chemical systems occurs in order to attain adequate development of both systems. However, the micellar medium obtained with SDS, which is used to favor the luminescent signal of the PG system (Panadero et al., 1995), caused a notable increase on the blank signal of the NB system, decreasing the signal corresponding to the analyte. The study of the effect of other alternative reagents in the PG system showed that TOPO caused an effect similar to that of SDS in the analytical signal corresponding to PG, whereas the behavior of the BHA system was independent of this reagent. TOPO is widely used as a synergistic agent in the analytical application of lanthanide chelates (Georges, 1993) because it eliminates water molecules in the coordination sphere of the lanthanide ion, which favors radiationless deactivation processes.

Figure 2 shows the emission spectra obtained for the PG and BHA systems by using 310 nm as the excitation wavelength. As can be seen, the separation between the emission maxima allows analytical data from each chemical system to be obtained independently by using the T-format instrument. The study of the kinetic behavior of both PG and BHA systems together by using the stopped-flow mixing technique showed that the equilibrium signal of the BHA system was instantaneously reached, and it was impossible to obtain the kinetic curve. Although the equilibrium signal corresponding to the PG system is also rapidly obtained, it has been proposed (Panadero et al., 1995) that kinetic measurements, which can be obtained in ~0.1 s, provide better selectivity than equilibrium measurements. According to these results, the determination of PG and BHA can be attained by obtaining, simultaneously, kinetic measurements for the PG system and equilibrium measurements for the BHA system, which is possible thanks to the availability of two emission channels and the independent behavior of the two chemical systems involved.

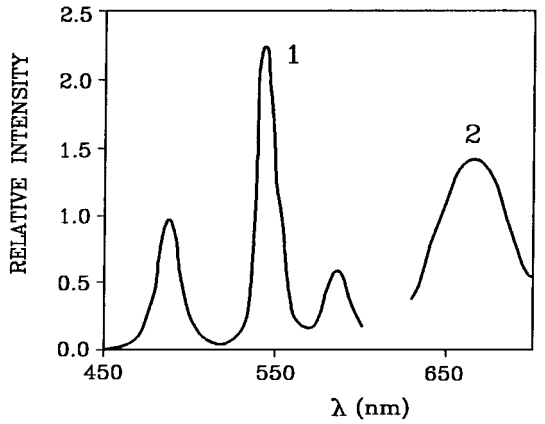

Figure 2. Emission spectra simultaneously obtained for PG and BHA systems by excitation at 310 nm. [terbium(III)] = 1.5×10^{-2} M; [NB] = 8.0×10^{-6} M; [TOPO] = 6×10^{-5} M; [Triton X-100] = 0.03%; [PG] = 0.3 μg mL^{-1}; [BHA] = 3 μg mL^{-1}; [nitrite] = 1.25×10^{-5} M; [ethanol] = 2.5%; [hexamine buffer] = 7×10^{-2}.

Optimization of Variables. The variables affecting the system were optimized by the univariate method. Each result was the average of three measurements. Values chosen as optimum were those yielding the minimum standard deviation for initial rate and equilibrium measurements.

The study of the PG system showed that the optimal experimental conditions are the same as those previously described (Panadero et al., 1995) and none of the reactants affected the BHA system except SDS, which notably increased the fluorescence of the oxidized NB, decreasing the net signal corresponding to the analyte. To avoid this shortcoming, the surfactant was replaced by TOPO, which is a common synergistic agent for lanthanide systems (Aihara et al., 1986; Peter et al., 1992). Figure 3a shows the effect of this variable on the initial rate of the PG system. As can be seen, this

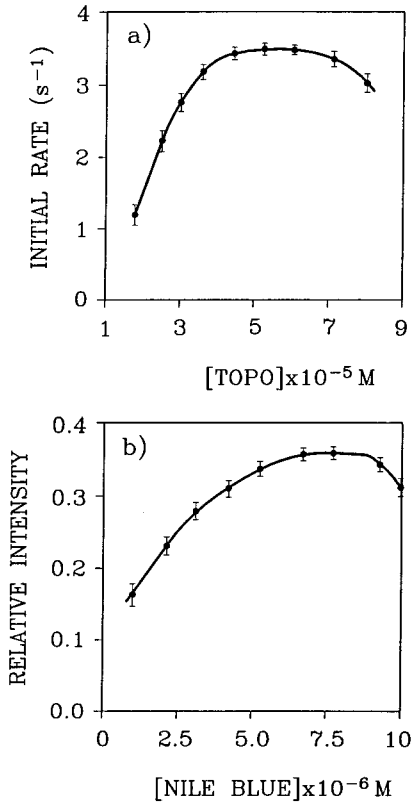

Figure 3. Influence of TOPO concentration on the initial rate of the PG system (a) and of NB on the fluorescence intensity of the BHA system (b). In (a) [terbium(III)] = 1.5×10^{-2} M; [NB] = 8.0×10^{-6} M; [TOPO] = 6×10^{-5} M; [Triton X-100] = 0.03%; [PG] = 0.5 μg mL^{-1}; [nitrite] = 1.25×10^{-5} M; [ethanol] = 2.5%; [hexamine buffer] = 7×10^{-2} M. In (b) [terbium(III)] = 1.5×10^{-2} M; [NB] = 8.0×10^{-6} M; [TOPO] = 6×10^{-5} M; [Triton X-100] = 0.05%; [BHA] = 1 μg mL^{-1}; [nitrite] = 1.25×10^{-5} M; [ethanol] = 2.5%; [hexamine buffer] = 7×10^{-2} M.

parameter was independent of the TOPO concentration in the range 4.2×10^{-5}–7.1×10^{-5} M. Also, the BHA system was not affected in the presence of this compound.

The optimum pH range for the development of the BHA system was 5.5–7.0, which is similar to that corresponding to the PG system. Hexamine buffer was chosen to adjust the pH of both systems, which were independent of the concentration of this buffer in the range 0.05–0.1 M. The study of the effect of Triton X-100 on both systems showed that the analytical parameters were independent of this variable when the Triton X-100 concentration in each syringe was in the range 4×10^{-2}–8×10^{-2}%.

As indicated above, the fluorescence of the BHA system is obtained by oxidizing previously the NB dye with sodium nitrite in acid medium. The study of the effect of the nitrite concentration in the system showed that the optimum range was 1×10^{-5}–3.8×10^{-5} M. The kinetic of the oxidation process was very dependent on the acid concentration. Thus, the oxidation was very slow when the concentration of the hydrochloric acid was $<10^{-3}$ M, whereas the stability of the oxidized NB notably decreased when the acid concentration was >0.1 M. A 10^{-2} M hydrochloric acid concentration allowed adequate oxidation of the reagent, which was stable for 2 days. This solution was neutralized in each assay by adding sodium hydroxide (5×10^{-3} M) to the drive syringe. Figure 3b shows the effect of NB on the fluorescence intensity of the BHA system; it can be seen that this signal is constant in the range 6.8×10^{-6}–9×10^{-6} M.

Table 1. Analytical Features of the Proposed Method

	BHA	PG
emission wavelength (nm)	665	545
linear range (μg mL^{-1})	0.3–15	0.09–3.5
slope[a]	$(4.6 \pm 0.2) \times 10^{-1}$	7.9 ± 0.3
intercept[b]	$(1.2 \pm 0.3) \times 10^{-2}$	$(9 \pm 3) \times 10^{-2}$
correlation coefficient (r)	0.992	0.998
detection limit (μg mL^{-1})	0.09	0.03
precision (% RSD)		
at 0.1 μg mL^{-1}		2.1
at 0.5 μg mL^{-1}	1.8	
at 1.5 μg mL^{-1}		2.3
at 2 μg mL^{-1}	2.1	

[a] Units: PG (s^{-1} μg^{-1} mL); BHA (μg^{-1} mL). [b] Units: s^{-1} for PG system.

Table 2. Effect of Foreign Substances over the Determination of 2 μg mL^{-1} of BHA and 2 μg mL^{-1} of PG

	max tolerated concn (μg mL^{-1})	
compound	BHA	PG
glucose	200[a]	200[a]
nordihydroguaiaretic acid	200[a]	200[a]
butylated hydroxytoluene	200[a]	40
gallic acid	200[a]	200[a]
octyl gallate	120	20
dodecyl gallate	160	50
tert-butylhydroquinone		200[a]

[a] Maximum concentration tested.

Because the TOPO and the analyte solutions were prepared in an ethanol–water medium, and ethanol was also used in the extraction step, the effect of this solvent was also studied. It was found that neither system was affected by the presence of ethanol up to a concentration of 8%. The equilibrium signal of the BHA system was less dependent on increasing temperatures than the initial rate of the PG system, which decreased when this variable increased. Thus, a temperature of 25 °C was chosen.

Analytical Features. Kinetic and equilibrium data for the PG and BHA systems at 545 and 665 nm, respectively, were obtained simultaneously by using the T-format luminescence spectrometer, by excitation at 310 nm. Kinetic curves were processed by using the initial rate method. Table 1 summarizes the calibration data obtained for both systems, which were attained in the presence of a sunflower oil matrix analyzed following the procedure described above. Under these conditions, the calibration graphs were linear over the ranges 0.3–15 μg mL^{-1} for BHA and 0.09–3.5 μg mL^{-1} for PG. The correlation coefficients suggest a good linearity in the calibrations. The detection limits obtained for BHA and PG in the presence of the sunflower oil sample, and calculated according to IUPAC recommendation (Long and Winefordner, 1983), were 0.09 and 0.03 μg mL^{-1}, respectively, which correspond to 3.0 and 1 mg kg^{-1} in the oil sample.

The precision of the method was evaluated at two concentrations of each antioxidant by assaying replicates of sunflower oil samples (n = 10) containing 17.9 and 71 mg kg^{-1} of BHA and 3.6 and 53.6 mg kg^{-1} of PG, so that the final concentrations were 0.5 and 2 μg mL^{-1} BHA and 0.1 and 1.5 μg mL^{-1} PG. As can be seen in Table 1, the relative standard deviation thus obtained was close to 2%.

Table 3. Resolution of BHA and PG Mixtures

BHA/PG mixture	BHA (μg mL^{-1})		PG (μg mL^{-1})	
	taken	found[a]	taken	found[a]
12:1	12	11.5 ± 0.5	1	0.96 ± 0.03
10:1	10	10.2 ± 0.4	1	0.98 ± 0.04
8:1	8	7.8 ± 0.1	1	1.04 ± 0.02
6:1	6	6.2 ± 0.2	1	0.99 ± 0.03
4:1	4	3.9 ± 0.3	1	1.01 ± 0.01
2:1	2	1.95 ± 0.06	1	0.97 ± 0.04
1:2	1	0.96 ± 0.02	2	2.06 ± 0.07
1:4	0.5	0.48 ± 0.02	2	1.9 ± 0.1
1:6	0.5	0.47 ± 0.01	3	2.9 ± 0.1

[a] Mean of three determinations ± SD.

The results obtained from the selectivity study are summarized in Table 2. Several potentially interfering compounds such as other antioxidants were assayed. A compound was considered not to interfere at a given concentration if the analytical signal in the presence of this substance was within one standard deviation of the value obtained with the analyte alone. The selectivity was quite good; only *tert*-butylhydroquinone (TBHQ) interfered at the same concentration level as BHA. These results confirm the mechanism of reaction suggested above for the BHA system. Thus, the lack of interference of gallic acid and gallate can be ascribed to the presence of a carboxylic group and ester group, respectively, in the para position, which confer electronic properties to the molecule different from those of BHA, hindering the reaction with the reagent. However, the TBHQ molecule, which has a hydroxy group in the para position, reacts in a similar way as BHA, as the methoxy group of this compound shows an electronic behavior similar to that of the hydroxy group. Thus, the compounds assayed followed a behavior similar to that reported (Josephy and Van Damme, 1984; Dacre, 1971) for the reaction of Gibbs reagent with para-substituted phenols. The interference caused by TBHQ in the BHA determination could be seen as a minor shortcoming in Canada and the European Community, where the use of this compound as an additive in foods is prohibited. However, it should be a limitation of the method in the United States, where TBHQ is permitted.

The simultaneous determination of BHA and PG can be applied to a wide range of concentration of each analyte thanks to the separation between the emission wavelengths of both systems and the lack of contribution of each analyte to the analytical signal of the other. This behavior allowed the direct resolution of the mixture by using the two emission channels of the instruments. Table 3 shows that the method can be applied to the resolution of BHA−PG mixtures in concentration ratios between 12:1 and 1:6 with an error of <6%. The fast development of both chemical systems is easily monitored by using a stopped-flow mixing technique, which is a useful alternative to routine determination of these antioxidants in foods.

Applications. To show the applicability of the proposed method to real samples, several foods, such as oil, chicken and vegetable soup extracts, lard, margarine, and mayonnaise were analyzed according to the proce-

Table 4. Determination of BHA and PG in Food Samples

	antioxidant content		antioxidant					
			BHA			PG		
sample	[BHA] (mg kg^{-1})	[PG] (mg kg^{-1})	added (mg kg^{-1})	found[a] (mg kg^{-1})	recovery (%)	added (mg kg^{-1})	found[a] (mg kg^{-1})	recovery (%)
chicken soup	30 ± 1	62 ± 1	70	71 ± 2	101.4	20	19.3 ± 0.5	96.6
			180	174 ± 3	96.6	40	39 ± 1	97.7
			250	233 ± 8	93.1	60	59 ± 1	98.3
vegetable soup	26 ± 1	65 ± 1	70	72 ± 2	102.9	20	20 ± 1	101.2
			180	179 ± 2	99.4	40	40.3 ± 0.9	100.8
			250	253 ± 5	101.2	60	59 ± 2	97.6
mushroom soup	87 ± 2		70	67 ± 3	95.7	20	19.0 ± 0.6	95.2
			180	182 ± 5	101.1	40	38 ± 2	96.3
			250	249 ± 9	99.6	60	59 ± 1	98.1
lard	78 ± 2		70	69 ± 3	98.6	20	20.0 ± 0.8	100.2
			180	170 ± 4	94.4	40	39 ± 1	97.6
			250	239 ± 10	95.6	60	57 ± 3	94.1
mayonnaise	82 ± 3		70	72 ± 2	102.9	20	20 ± 1	101.4
			180	178 ± 3	98.8	40	40 ± 1	100.7
			250	250 ± 9	100.1	60	59 ± 1	98.9
vegetable soup extract		48 ± 1	70	72 ± 1	102.3	20	19.8 ± 0.9	99.1
			180	176 ± 4	97.7	40	41 ± 1	101.8
			250	249 ± 3	99.6	60	59 ± 2	97.8
sunflower oil			70	68 ± 2	97.7	20	19.7 ± 0.5	98.4
			180	102 ± 6	101.4	40	40 ± 1	101.1
			250	236 ± 7	94.4	60	58 ± 1	96.5
corn oil			70	71 ± 1	102.4	20	20.1 ± 0.7	100.6
			180	181 ± 5	100.6	40	38 ± 1	95.8
			250	247 ± 8	98.8	60	57 ± 1	94.9
olive oil			70	69 ± 1	98.6	20	20.0 ± 0.4	99.8
			180	174 ± 4	96.7	40	41 ± 1	101.3
			250	245 ± 8	98.0	60	59 ± 1	98.7
margarine			70	71 ± 2	101.4	20	20.4 ± 0.8	102.1
			180	176 ± 5	97.8	40	41 ± 1	101.6
			250	246 ± 7	98.4	60	59 ± 2	97.9

[a] Mean of three determinations ± SD.

dure described above (Table 4). BHA and PG were found together in two of the samples analyzed, whereas BHA was present alone in three other samples and PG in the other one. Table 4 lists the analytical recoveries obtained by adding three different amounts of both antioxidants to each sample and subtracting the results obtained for similarly prepared unspiked samples. The values obtained ranged from 94.8 to 102.6% and from 94.1 to 102.1% for BHA and PG, respectively.

Conclusions. The study here presented is the first simultaneous luminescent determination of the antioxidants BHA and PG in food samples. The development of this simple, fast, and selective method is possible by the combination of long-wavelength measurements and the use of reactions having evolutions dependent on the functional group nature of each compound. This selectivity is obtained after the elimination of potential interferences from the sample matrix, which is a previous step common to any antioxidant determination. This study also shows that, although the excitation wavelength of NB is 620 nm, the use of the second-order grating effect allows both PG and BHA to be excited at 310 nm. Measurements corresponding to the PG and BHA systems are obtained rapidly by using a stopped-flow mixing technique: 0.1 and 1 s for initial rate and net equilibrium signal, respectively. The independent behavior of the chemical systems involved and the use of a T-format luminescence spectrometer simplify the method as the application of mathematical approaches is avoided. These features make the method very suitable for its use in routine analysis.

ABBREVIATIONS USED

NB, Nile Blue; SDS, sodium dodecyl sulfate; TBHQ, *tert*-butylhydroquinone; TOPO, tri-*n*-octylphosphine oxide; PG, propyl gallate; BHA, butylated hydroxyanisole; MBTH, 3-methyl-2-benzothiazoline hydrazone.

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Received for review August 6, 1999. Revised manuscript received December 7, 1999. Accepted December 7, 1999. We gratefully acknowledge financial support from the Spanish DGICYT (Grant PB96-0984).

JF990886U