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Flow-Injection Determination of Thiabendazole Fungicide in Water Samples Using a Diperiodatocuprate(III)–Sulfuric Acid–Chemiluminescence System

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Chemiluminescence (CL) with a flow-injection method is reported for the determination of thiabendazole (TBZ) fungicide based on its enhancement effect on diperiodatocuprate(III) (DPC)–sulfuric acid–CL system. The calibration graph was linear in the concentration range of 1 – 2000 μg L^{-1} (R^2 = 0.9999, n = 8) with a limit of detection (S/N = 3) of 0.3 μg L^{-1}. The injection throughput was 160 h^{-1} with relative standard deviations (RSD, n = 4) of 1.1 – 2.9% in the concentration range studied. The experimental variables *e.g.*, reagents concentrations, flow rates, sample volume, and PMT voltage were optimized, and the potential interferences were investigated individually. The method was successfully applied to the determination of TBZ in water samples showing good agreement and recovery in the range of 92 ± 2.2 – 108 ± 3% (n = 3) using dispersive liquid–liquid micro-extraction (DLLME). The possible CL reaction mechanism for DPC–sulfuric acid–TBZ is also discussed.

Keywords Flow injection analysis, DPC, chemiluminescence, thiabendazole, water samples

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Introduction

Benzimidazole fungicides are widely used pesticides in agriculture for pre- and post-harvest treatments for the control of a wide range of pathogens. They are either applied directly to the soil, or sprayed over crop fields. Most of these compounds persist in the environment for many years.[1] Thiabendazole [2-(4-thiazolyl)-1*H*-benzimidazole, TBZ] fungicide is used to control a variety of fruit and vegetable diseases, such as mold, blight, rot and stains, caused by various fungi.[2] It is also used as a food additive[3] to maintain freshness in vegetables and fruits,[4] as a pre- or post-harvest fungicide[5] and in industry as a mildew-proof agent. Human health risk assessment carried out by European Water Framework Directive has established a maximum concentration level (MCL) of 0.1 μg L^{-1} for most benzimidazole compounds present in natural water samples, and a total concentration of all pesticides of 0.5 μg L^{-1}.[6] The chemical structure of TBZ is given in Fig. 1.

Various analytical methods have been reported to determine TBZ in a variety of matrices, such as serum, plasma, urine, saliva, pharmaceutical preparations, pesticide residues, animal bodies, natural waters, soil, fruits, and vegetables. These are based on spectrophotometry,[7-9] high-performance liquid chromatography (HPLC) and mass spectrometry (MS),[10-14] gas chromatography (GC) with thermionic specific[15] and GC/MS,[16-18] capillary electrophoresis with electrospray-MS,[19] micellar electrokinetic chromatography,[20] thin-layer chromatography,[21] polarography,[22] enzyme linked immunosorbent assay,[23] fluorescence,[24] sequential injection analysis with fluorescence,[25] flow injection analysis with fluorescence[26,27] and room-temperature phosphorescence.[28]

In recent years, the developments of new oxidant reagents for the chemiluminescence (CL) reaction based on analytical approaches have been the subject of considerable interest. For example, transition metals, such as silver(III), copper(III) and nickel(IV), have been exploited in CL systems as oxidizing agents. They were stabilized by chelating with polydentate ligands. In general, emission is brought about by energy released in a redox reaction in which the oxidant and reductants species are a metal complex and a luminophore, which can be either an analyte or another compound, such as luminol. Copper complexes have occupied a major place in oxidation chemistry because of their abundance and relevance in biological chemistry.[29-33]

A limited number of FI-CL methods have been reported based on the use of the Cu(III) complex in aqueous phosphoric acid and sulfuric acid solutions for the determination of ofloxacin, levofloxacin and enrofloxacin in pharmaceutical preparations, urine and veterinary preparations with detection limits (S/N = 3)

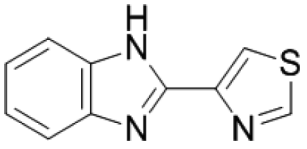

Fig. 1 Chemical structure of thiabendazole fungicide ($C_{10}H_7N_3S$, M_w: 201.25).

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Table 1 Comparison of analytical characteristic of various analytical methods for the determination of TBZ

Technique	Matrix	Linear range/μg L^{-1}	LOD/μg L^{-1}	Sample/h^{-1}	Ref.
Spectrophotometry	Fruits	178.3 - 2918	54.54	NR	7
Spectrophotometry	Tablets	0.2 - 1.2	0.2	NR	8
HPLC-UV	Tomatoes	5 - 200	0.24	03	10
HPLC-FL	River and underground water	2.5 - 3000	4×10^{-3}	03	11
HPLC-FL	Water and soil	5 - 1000	0.5 - 1.6	NR	12
HPLC-DAD	Juice, fruit, and vegetable	0 - 99.2	0.90	>6	13
GC-TSD	Lemons: peel and pulp	200 - 10000	200	NR	15
CE-ESI-MS	Fruits and vegetables	1000 - 10000	10	NR	19
FL-spectroscopy	Apple juice	5 - 50	2.2	NR	24
SIA-FL	Mushrooms	1600 - 40000	500	NR	25
FIA-FL	Water	2 - 242	0.7	60	26
FIA-FL	Water	8 - 120	2.8	14	27
FIA-RTP	Natural waters	12.9 - 110	4.5	14	28
FIA-CL	Natural waters	1 - 2000	0.3	160	This method

NR = not reported; HPLC-UV = high performance liquid chromatography–ultraviolet; DAD = diode array detector; FL = fluorescence; GC-TSD = gas chromatography–thermionic specific detection; CE-ESI-MS = capillary electrophoresis–electrospray ionization–mass spectrometry; SIA = sequential injection analysis; FIA-RTP = flow injection analysis–room temperature phosphorescence.

of 8.7, 9.2 and 15.4 μg L^{-1} respectively.[34,35] Several other methods have also been reported based on the luminol-DPC-CL reaction in an alkaline medium for the determination of chlortetracycline, cholesterol, ergometrine maleate, lincomycin, cefazoline, *N*-(4-aminobutyl)-*N*-ethylisoluminol and mitoxantrone[36-42] in pharmaceutical and biological fluids. Table 1 compares the analytical characteristic of various analytical methods for the determination of TBZ.

To our knowledge, there has been no work reported using the oxidation of DPC for TBZ by the CL method in an acidic medium. In this manuscript we report on an FI-CL method for the determination of TBZ, based on its enhancement effect on $[Cu(HIO_6)_2]^{5-}$–sulfuric acid-CL system. The method is simple, sensitive, high injection throughput with a limit of detection (S/N = 3) of 0.3 μg L^{-1} and relative standard deviations (RSD, n = 4) of 1.1 - 2.9% in the concentration range studied.

Experimental

Reagents and solutions

All chemicals were of analytical grade unless stated otherwise, and all solutions were prepared in ultra-high-purity (UHP) deionized water (0.067 μS cm^{-1}, Purelab Option, Elga, UK). Glassware used during the experiments and for the storage of reagents and standards were pre-cleaned with 10% hydrochloric acid for one week, thoroughly rinsed with UHP water and stored in plastic bags so as to avoid any contamination.

A stock solution (1 mol L^{-1}) of potassium hydroxide was prepared by dissolving 5.61 g potassium hydroxide in 100 mL UHP water. Working solutions were prepared by diluting the required volume in UHP water.

Stock solutions (1.0 mol L^{-1}) of sulfuric acid, phosphoric acid, hydrochloric acid, nitric acid and acetic acid were prepared by diluting the required volumes from commercial stock solutions in UHP water and working standard solutions (50 mmol L^{-1}) were prepared by diluting with UHP water.

Stock solutions (100 mg L^{-1}) of thiabendazole, carbophenothion, carbofuran, nabam, antu, permethrin, propanil, fubridazol, carbendazim, thiabencarb, aminocarb, aldicarb, asulam, fenarimol, benomyl, resmethrin, dodemorph, amitraz, paraquat, digoxim, ribavirin, aldrin, thiram, terbufos, diazinon, mancozeb, trimethoprim, atrazin, simetryn, tridemorph, gabapentin, phoxim, bendiocarb, molinate, maneb, malathion, and dianoseb (Dr. Ehrenstorfer, Augsburg, Germany) were prepared by dissolving the required amounts of each pesticide in absolute ethanol and stored in dark-brown bottles at –4°C. Working standard solutions were prepared daily by serial dilution of the stock solutions with ethanol (0.1%, v/v) as required for the interference study.

Stock solutions (1000 mg L^{-1}) of phenol, ascorbic acid, and humic acid, cation (Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+} and Mn^{2+}) and anion (NO_3^-, HCO_3^-, Cl^-, SO_4^{2-}, and PO_4^{3-}) were prepared in UHP water; subsequent standard solutions of each were prepared by serial dilution of the stock solutions with 0.1% ethanol, as required for the interference study.

DPC was prepared according to procedures reported previously.[43,44] Copper(II) sulfate·$5H_2O$ (1.56 g), sodium metaperiodate (2.67 g), potassium persulfate (1 g) and potassium hydroxide (8 g) were added in 100 mL UHP water. The mixture was heated to boiling for about 20 min on a hot plate with constant stirring. The boiled mixture turned intensely red, and the boiling was continued for another 20 min until completion of the reaction. The mixture was then cooled at room temperature, and filtered through a sintered crucible. The filtrate was then cooled in an ice bath, and again filtered. The resulting dark-brown filtrate was left standing to attain room temperature. In order to isolate the complex, a 50% sodium nitrate solution was added to the filtrate and left for crystallization. When the supernatant was colorless, the crystals were filtered and washed three times with UHP water (3 × 10 mL) until brown drops were formed under the sintered crucible. An alkaline solution of the complex was found fairly stable at room temperature for one month in dark. The complex was characterized by its UV/visible spectrum, which exhibits two absorption maxima at 263 and 415 nm. The DPC solutions were freshly prepared by dissolving an appropriate amount of the compound in a 5 mmol L^{-1} potassium hydroxide solution before use; the concentration was then determined by measuring the absorbance at 415 nm (molar absorbtivity ε = 6230 mol^{-1} L cm^{-1}) using a double-beam UV-Vis spectrophotometer (Shimadzu, Model UV-1700, Japan).

Instrumentation and procedures

Figure 2 shows the FI-CL manifold used for TBZ determination. A peristaltic pump (Ismatec Reglo 100, four

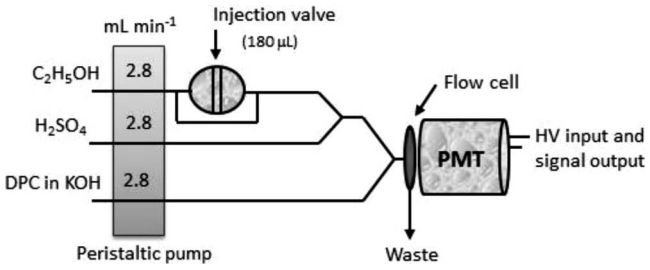

Fig. 2 Flow-injection chemiluminescence (FI-CL) manifold for the determination of TBZ.

channels, Switzerland) was used to propel the sample carrier and reagent solutions at a flow rate of 2.8 mL min^{-1}. A rotary injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to inject TBZ standards (180 µL) into an ethanol (0.1% v/v) stream and merged with a stream of sulfuric acid (50 mmol L^{-1}). This stream was then merged at a T-piece with an aqueous alkaline DPC reagent (0.25 mmol L^{-1} in potassium hydroxide, 5.0 mmol L^{-1}) stream. The merged streams were allowed to travel 2.0 cm before passing through a glass spiral flow cell (1.5 mm i.d., 18 mm dia) placed directly in front of an end window photomultiplier tube (PMT, 9798B, Electron Tubes Ltd., Ruislip, UK). The PMT, glass coil and T-piece were enclosed in a light-tight housing. The PMT was kept at 1250 V *via* a 2-kV power supply (Electron Tubes, PM30D, UK). The detector output was recorded using a strip chart recorder (BD 40, Kipp & Zonen, The Netherlands). The whole manifold was connected by Tygon tubing (1.02 mm, i.d.).

Results and Discussion

DPC–sulfuric acid–CL system

DPC produced weak CL directly with different acids *e.g.*, sulfuric acid, phosphoric acid, hydrochloric acid, nitric acid and acetic acid (50 mmol L^{-1}). The maximum CL intensity and better reproducibility were observed with sulfuric acid when compared with other acids. When TBZ was added to the DPC–sulfuric acid system, the CL signal was enhanced significantly. The pH value of the waste solution (DPC (0.25 mmol L^{-1} in 5 mmol L^{-1} potassium hydroxide,)-sulfuric acid (50 mmol L^{-1})–TBZ (100 µg L^{-1} in 0.1% ethanol)) system was 2.5, using a pH meter (3305, Jenway Ltd., Essex, UK).

Further, some compounds and ions, *e.g.*, rhodamine B, fluorescein, quinine sulfate, pyrogallol and sodium sulfite (0.1 mmol L^{-1} each) when added in DPC–sulfuric acid–TBZ system, resulted in a higher background and decrease in the CL response of the analyte. This is contrary to what had been being reported previously,[35] where sodium sulfite enhanced the CL intensity in Cu(III) complex-sulfuric acid system. Therefore, idea of using these compounds was abandoned for further study. In the absence of sulfuric acid, the DPC–potassium hydroxide–TBZ system gave no CL signal under the optimized conditions.

Kinetic profile and CL spectrum of DPC–sulfuric acid–TBZ

The kinetic behavior of the CL reaction was examined by registering the response curve (CL intensity *vs.* time, not shown) using the proposed FI-CL manifold under the optimum experimental conditions. The CL intensity was detected immediately after mixing the solutions and the intensity reached a maximum in 8 s. The CL intensity then became weaker, and was almost zero after 14 s. This indicated that the CL reaction

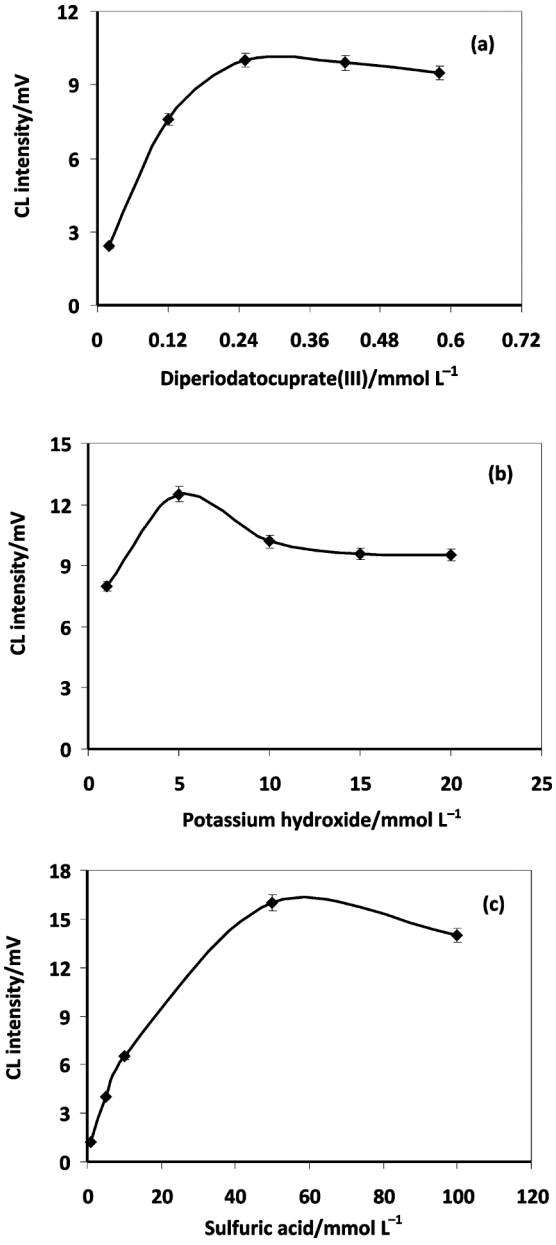

Fig. 3 Variation of the CL intensity with the concentration of (a) DPC; (b) potassium hydroxide; and (c) sulfuric acid. For optimizing each parameter the optimized conditions for all other parameters were used, *i.e.* flow rates of 2.8 mL min^{-1} for all three channels; sample volume 180 µL; PMT voltage 1250 ± 1.0 V.

was rapid and suitable for performing TBZ determination by controlling the flow rate.

Optimization of key chemical and physical variables

Various chemical and physical parameters were investigated in order to establish the optimized conditions for the determination of TBZ. The parameters optimized were DPC, potassium hydroxide, sulfuric acid and ethanol concentrations, flow rates of all channels, sample injection volume, and PMT voltage. The results are given in Fig. 3 and Table 2. All of these studies were performed with 100 µg L^{-1} TBZ, and all measurements were performed in triplicate.

In the CL system, DPC was used as an oxidant. DPC concentration not only changed sensitivity but also the linear range for the assay. This influence was investigated over the

Table 2 Effect of key physical parameters on the determination of TBZ (100 μg L^{-1}) using FI-CL manifold

Parameter	Range studied	Optimized value
Flow rate/mL min^{-1}	0.6 – 4.0	2.8
Sample volume/μL	60 – 300	180
PMT voltage/V	900 – 1300	1250

For optimizing each parameter the optimized conditions for all other parameters were used *i.e.*, flow rate 2.8 mL min^{-1} for all three channels; sample injection volume, 180 μL; PMT voltage, 1250 V.

range of 0.02 – 0.6 mmol L^{-1} on the CL intensity. The result showed that the CL intensity increased with an increase in the DPC concentration up to 0.25 mmol L^{-1}, above which the CL intensity leveled off (Fig. 3a). Therefore, a DPC concentration of 0.25 mmol L^{-1} was selected and used for subsequent experiments. The effect of the potassium hydroxide concentration on the CL reaction was investigated over the range of 1 – 20 mmol L^{-1}. Optimum and reproducible CL signals were observed at potassium hydroxide concentration of 5 mmol L^{-1} (Fig. 3b), and therefore, a potassium hydroxide concentration of 5 mmol L^{-1} was selected and used in further experiments.

The concentration of the acid used in the reaction has a very significant influence on the CL emission intensity. The effect of the sulfuric acid concentration on the CL emission intensity was further examined over the range of 1.0 – 100 mmol L^{-1}. The CL intensity increased as the concentration of sulfuric acid was increased up to 50 mmol L^{-1}; above this concentration, the CL intensity decreased (Fig. 3c). Therefore, 50 mmol L^{-1} sulfuric acid was selected and used in subsequent experiments.

The solubility of TBZ is comparatively higher in ethanol (2.1 g L^{-1}) than water (0.05 g L^{-1}) at 20°C.[45] Therefore, due to its high solubility in ethanol, the stock solution of TBZ was prepared in ethanol. It is also known that organic solvents in general influence the CL behavior; therefore, the effect of ethanol concentration was examined over the range 0 – 1.0% (v/v) based on signal to noise ratio. A small decrease in signal to noise ratio was observed from 0.01 – 0.1% ethanol, above which a considerable decrease in signal to noise ratio was examined. Therefore, an ethanol concentration of 0.1% (v/v) was selected and used as a sample carrier stream.

The effects of flow rate, sample injection volume and PMT voltage on the CL intensity were investigated in terms of sensitivity, speed and reagent consumption. The flow rates for each of three channels were examined over the range of 0.6 – 4.0 mL min^{-1}. The CL intensity reached the maximum at a flow rate of 2.8 mL min^{-1}, therefore, this flow rate was used by considering the sensitivity, reagents consumption and reproducibility. Similarly, the sample volume was examined over the range of 60 – 300 μL. The CL intensity of thiabendazole gradually increased with increasing sample injection volume. As a compromise between sensitivity of measurements and time of analysis, the sample injection volume of 180 μL was selected and used for all further experiments. The effect of PMT voltage was also examined over the range of 900 – 1300 V. The CL intensity increased gradually with the PMT voltage, however, 1250 V was used for all further experiments which gave steady baseline and reproducible CL signals.

Analytical figures of merit

Under the selected physical and chemical conditions, a series of TBZ standards were injected into the proposed FI-CL manifold and a linear calibration graph of CL intensity *vs.* TBZ concentration over the range of 1 – 2000 μg L^{-1} (r^2 = 0.9999; n = 8) was obtained, described by the equation $y = (3.33 \pm 0.1)x + (1.2 \pm 0.2)$, where y = CL intensity and x = concentration in μg L^{-1}. The injection throughput was 160 h^{-1} and the standard deviation (RSD, n = 4) was 1.1 – 2.9% over the range studied. The limit of detection (S/N = 3) (*i.e.* the concentration giving a mean response that was three times the peak-to-peak baseline noise) was 0.3 μg L^{-1} without applying any dispersive liquid–liquid micro-extraction (DLLME) method.

Interference study

The effect of major freshwater ions at an environmentally relevant concentration, and some organic compounds on the blank (in the absence of TBZ) and on the determination of TBZ (50 μg L^{-1}) was studies without applying the DLLME method. The tolerable foreign species were taken as a relative error not greater than ±5%. Calcium^{2+} 100000 μg L^{-1}; Mg^{2+} 30000 μg L^{-1}; Zn^{2+} 5500 μg L^{-1}; Fe^{2+} 300 μg L^{-1}; Cr^{3+}, Cd^{2+}, V^{4+}, Fe^{3+}, Co^{2+} and Mn^{2+} 500 μg L^{-1}; Pb^{2+} and Cu^{2+} 1000 μg L^{-1}; K^{+} 30000 μg L^{-1}; NH_4^{+} 25000 μg L^{-1}; Cl^{-} and SO_4^{2-} 250000 μg L^{-1}; HCO_3^{-} 100000 μg L^{-1}; NO_3^{-} 20000 μg L^{-1}; NO_2^{-} and PO_4^{3-} 1000 μg L^{-1}; ascorbic acid, phenol, and humic acid 1000 μg L^{-1} had no significant effect on the blank CL signal and on the determination of TBZ (50 μg L^{-1}).

Detection of fungicides, herbicides and insecticides

Under the selected optimum conditions given above, the CL responses for various fungicides, herbicides and insecticides were also examined on the blank (in the absence of TBZ) and on the determination of TBZ (50 μg L^{-1}) without applying the DLLME method. These include carbophenothion, carbofuran, and nabam 250 μg L^{-1}; antu, permethrin, propanil, fubridazol, carbendazim, thiabencarb, aminocarb, aldicarb, asulam, fenarimol, benomyl, resmethrin, dodemorph, amitraz, paraquat, digoxim, ribavirin, aldrin, thiram, terbufos, diazinon, mancozeb, trimethoprim, atrazin, simetryn, tridemorph, gabapentin, phoxim, bendiocarb, molinate, maneb, malathion, and dianoseb 2000 μg L^{-1}. No significant CL responses of these pesticides were observed on the blank as well as on the determination of TBZ.

Application to water samples

The proposed method was applied for analyzing TBZ in tap water, rain water, and irrigation water samples from various locations of Quetta valley were collected in acid washed (hydrochloric acid 10%), high density polyethylene bottles, filtered through cellulose membrane filter (pore size 0.45 mm, 47 mm diameter, Whatman, Maidstone, UK) to remove suspended particles and stored at 4°C. Recovery experiments were carried out with spiked water samples using 50 – 500 μg L^{-1} spikes for TBZ. The obtained results are given in Table 3. The recoveries were in the range of 92 – 108%. For the extraction of spiked TBZ, the DLLME procedure was adopted.[24] In brief, an aliquot of 10 mL water sample containing spiked TBZ was placed into a 15-mL screw-cap centrifuge tube. Subsequently, 1.2 mL of ethanol (as disperser solvent) containing 0.155 mL of chloroform (as extraction solvent) was injected rapidly into the sample solution. Then, the mixture was shaken for 1 min. A cloudy solution was formed that consisted of very fine droplets of chloroform dispersed into aqueous sample and the thiabendazole was extracted into the fine droplets. After centrifugation at 3000 rpm for 2 min at 5°C, the chloroform phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was transferred with the help of a microsyringe into another tube, and then evaporated to dryness under nitrogen stream. The residue was dissolved in

Table 3 Recovery study of TBZ in spiked water samples ($n = 3$)

Sample	Taken/μg L^{-1}	Found/μg L^{-1}	Recovery ± RDS, %
Tap water	50	48	96 ± 2.1
	250	230	92 ± 3.0
	500	520	104 ± 2.6
Rain water	50	47	94 ± 3.5
	250	260	104 ± 2.8
	500	530	106 ± 2.5
Irrigation water	50	46	92 ± 2.2
	250	270	108 ± 3.0
	500	480	96 ± 3.2

10 mL of ethanol (0.1% v/v) and then injected in the proposed FI-CL manifold for determination.

The effect of DLLME was also examined concerning the determination of TBZ. Under the optimum conditions, TBZ standards 50, 250 and 500 μg L^{-1}, prepared in tap water, were injected into the proposed FI-CL manifold, and the CL intensity was found to be inhibited up to 35 ± 5% compared with standards of TBZ prepared in UHP water. This was probably due to cationic and anionic interactions of ions in tap water with TBZ. To avoid the CL inhibition effect, the DLLME technique was adopted which almost recovered up to a 98 ± 3% CL response for these standards.

The proposed method was validated, for TBZ concentrations in three different mineral-water samples (purchased from local market). Each sample was spiked using 50, 100 and 150 μg L^{-1} TBZ and analyzed with proposed FI-CL method and reported fluorescence method[24] using a fluorescence spectrophotometer (KF-1501, Shimadzu, Japan), equipped with a 150-W continuous xenon lamp and a 1-cm quartz cell holder. The excitation and emission slits of the monochromators were both adjusted to 5 nm. The fluorescence intensity was measured at 345 nm with an excitation wavelength at 302 nm. There was no statistical difference between the two methods at the 95% confidence level ($t_{calc} = 1.20$; $t_{tab} = 2.31$).

Possible CL reaction mechanism

In order to study the possible reaction mechanism, the UV-Visible absorption spectra were recorded using a double beam UV-Vis spectrophotometer (Shimadzu, Model UV-1700, Japan). As shown in Fig. 4, it can be seen that DPC (0.25 mmol L^{-1}) in a potassium hydroxide solution (5.0 mmol L^{-1}) has two distinct absorption peaks at about 263 and 415 nm, as reported previously (curve a);[43,44] when aqueous sulfuric acid (50 mmol L^{-1}) was added in DPC solution, the absorption peaks of DPC disappeared (curve b), and when TBZ (10000 μg L^{-1}) was added to DPC and aqueous sulfuric acid mixture, a single absorption peak at about 301.4 nm (curve c) re-appeared.

The water-soluble copper(III) periodate complex is reported[46] to be $[Cu(HIO_6)_2]^{5-}$. Sun *et al.*[35] reported that $[Cu(HIO_6)_2]^{5-}$–sulfuric acid system gave CL emission at 490 nm suggested the production of $O_2^{-\bullet}$ in the CL reaction, a part of $O_2^{-\bullet}$ may recombine and generate energy rich precursors of excited molecule $(O_2)_2$* which decomposed to O_2 and emitting a bright luminescence at 491.6 nm. The CL emission produced from $[Cu(H_2IO_6)_2]^{3-}$–sulfuric acid–TBZ system was suggested *via* the intermolecular energy transfer from $(O_2)_2$* to TBZ then, the excited TBZ de-excited to its ground state, producing CL emission at 345 nm.[26] Based on the discussion above, a possible CL mechanism for the determination of TBZ can be described as follows:

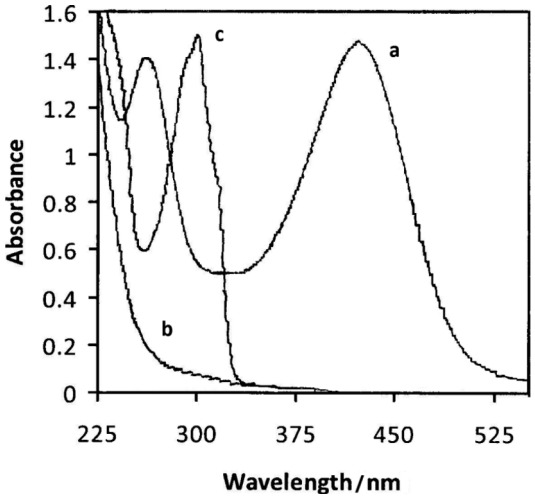

Fig. 4 UV-Vis absorption spectra. (a) DPC (0.25 mmol L^{-1} in potassium hydroxide (5 mmol L^{-1})); (b) DPC + sulfuric acid (50 mmol L^{-1}) and (c) DPC + sulfuric acid + TBZ (10000 μg L^{-1}).

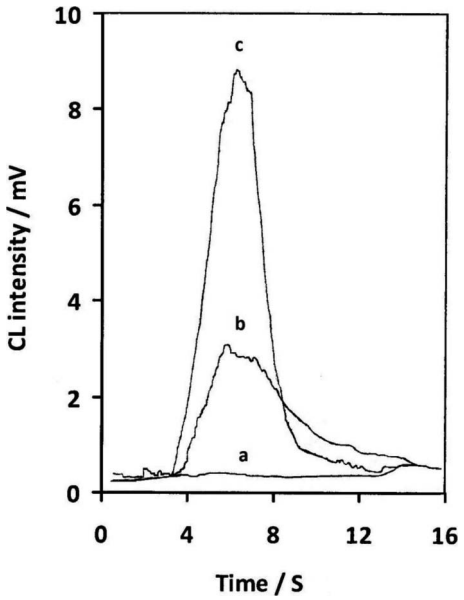

Fig. 5 The CL profile of DPC–sulfuric acid–TBZ system. (a) DPC: 0.25 mmol L^{-1} in potassium hydroxide 5.0 mmol L^{-1}; (b) DPC–sulfuric acid solution (50 mmol L^{-1}) and (c) DPC–sulfuric acid–TBZ: (25 μg L^{-1}); sample volume: 180 μL.

$$[Cu(H_2IO_6)(H_2O)] + H_3O^+ \longrightarrow Cu^{2+} + H_5IO_6 + O_2^{-\bullet}$$

$$O_2^{-\bullet} + O_2^{-\bullet} \longrightarrow (O_2)_2^*$$

$$(O_2)_2^* \longrightarrow 2O_2 + \text{light (491 nm)}$$

$$(O_2)_2^* + TBZ \longrightarrow 2O_2 + TBZ^*$$

$$TBZ^* \longrightarrow TBZ + \text{light (345 nm)}$$

The CL intensity profile of DPC–sulfuric acid reaction in the absence and presence of TBZ were also examined as shown in Fig. 5. The sample carrier, ethanol (0.1% v/v) was propelled *via* all three streams and the DPC solution (0.25 mmol L^{-1} in

potassium hydroxide 5.0 mmol L^{-1}) was injected in the sample carrier stream and no CL signal was observed (curve a). When a DPC solution was pumped in the third channel in place of the sample carrier an ethanol and sulfuric acid aqueous solution (50 mmol L^{-1}) was injected in the sample carrier stream in place of the DPC solution, a weak CL signal appeared (curve b). Then, a sulfuric acid aqueous solution was pumped in the second channel in place of the sample carrier ethanol, and when the TBZ solution (25 μg L^{-1}) was injected in the sample carrier stream, a remarkably increased CL signal was obtained (curve c). Therefore, it can be concluded that TBZ probably played the role of an enhancer in the DPC–sulfuric acid–CL reaction.

Conclusions

A simple CL emission system was developed for the determination of TBZ in water samples based on $[Cu(HIO_6)_2]^{5-}$–sulfuric acid–TBZ reaction system. The proposed FI-CL method is very simple, has a low limit of detection (0.3 μg L^{-1}) and high injection throughputs (160 h^{-1}). Common inorganic ions and some organic compounds present in water samples, and a number of pesticides had no effect on the determination of TBZ. The method was applied to the analysis of TBZ in water samples. It is better in terms of the detection limit, reagent consumption and sample throughputs compared with other flow-based methods. DLLME is based on the dispersion of tiny droplets of the extraction solvent within the aqueous solution,[47] and seems to be an environmentally friendly approach.[48] Transition metals in uncommon oxidation states, such as Ag(III), Cu(III) and Ni(IV), have been exploited in the CL systems as oxidizing agents in basic as well as in acidic conditions.

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