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FI-chemiluminometric study of thiazides by on-line photochemical reaction

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Abstract

The present manuscript deals with a simple and sensitive flow-injection method for the chemiluminescent determination of thiazides. The method is based on the on-line photodegradation and chemiluminescent determination of the resulting photo-fragments. The on-line photodegradation is performed in basic medium by using a photoreactor consisting of a 550 cm long × 0.8 mm ID piece of PTFE tubing helically coiled around an 8 W low-pressure mercury lamp. The determination of the photo-irradiated thiazides is performed by a chemiluminescent oxidative reaction with Ce(IV) in sulphuric acid medium. A heterogeneous group of thiazides (indapamide, metolazone, hydroflumethiazide, chlorthalidone and bendroflumethiazide) has been studied. Hydrochlorothiazide was selected as a test substance. The "on-line" photochemical reaction approach allows the sensitive chemiluminescent determination of thiazides which do not present native chemiluminescence in the absence of sensitizers such as Rhodamine 6G. Linear calibration graphs were typically over the range 0.5–12 $\mu g\,ml^{-1}$ (indapamide, metolazone, hydroflumethiazide and chlorthalidone); and over the range 0.5–5 $\mu g\,ml^{-1}$ (hydrochlorothiazide). Limits of detection ranged between 0.005 $\mu g\,ml^{-1}$ (hydrochlorothiazide) and 0.06 $\mu g\,ml^{-1}$ (bendroflumethiazide). The relative standard deviation for the test substance was 2.0% for 2 $\mu g\,l^{-1}$ of the drug (n = 11) and the throughput was 65 h^{-1} in all cases. The assessment of the photodegradation step on the molecular structure of thiazides was established by recording UV and fluorimetric spectra. The viability of the on-line photoinduced fluorescent determination of hydroflumethiazide and bendroflumethiazide was confirmed. The method was also applied to the determination of hydrochlorothiazide in commercially available formulation.

Keywords: Chemiluminescence; Photochemical reaction; Hydrochlorothiazide; Thiazides; Pharmaceuticals; FIA

1. Introduction

Thiazides are the most widely used group of diuretics and are effective in milder forms of high blood pressure and heart failure. Although they lower blood pressure initially by increasing salt loss through the kidneys, they also have a relaxing effect on artery walls. This is probably the main way in which they work. Occasionally they may cause attacks of gout and raise the level of glucose in the blood, which could lead to diabetes in susceptible people. A wide range of thiazides is used with only minor differences among them (see Fig. 1).

Hydrochlorothiazide (HCT) is a member of the thiazides family which has been extensively used since 1957 as a powerful diuretic and as antihypertensive agent. Some quantitative determination methods of hydrochlorothiazide have been developed by using different techniques. The British Pharmacopoeia [1] proposes the determination of HCT tablets by the direct measurement of the absorbance in the UV region from the solution of the drug in NaOH medium. The USP [2] also recommends this method for the reserpine and HCT tablets, but when the last drug is mixed with others substances, the chromatographic separation is recommended. Others phar-

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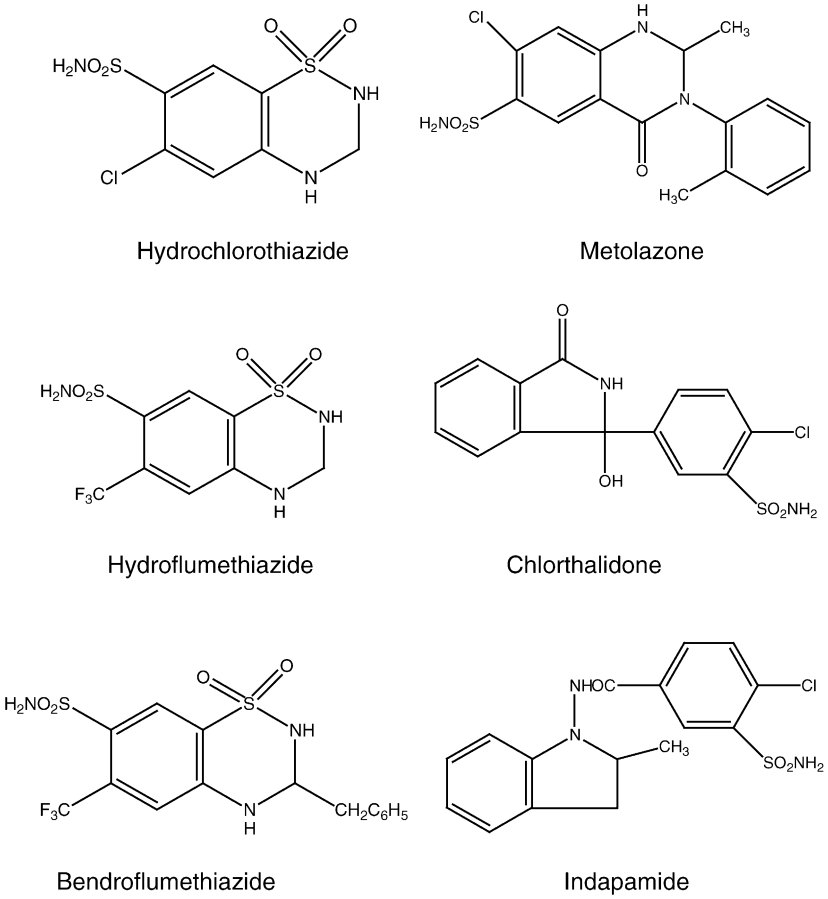

Fig. 1. Chemical structures of thiazides.

macopoeias [1,3] recommended a non-aqueous titration with potentiometric end-point.

Most of the methods developed in the analytical literature are spectrophotometric; ethanolic solutions of chlorothiazide, hydrochlorothiazide and thrichloromethiazide have been analysed by first derivative UV-spectrometry and HPLC in the presence of their photo-decomposition products [4]. Thiazides have been determined in urine using electroanalytical techniques [5], liquid chromatography with fluorimetric [6] and mass spectrometry [7] detection. The tandem gas chromatography–mass spectrometry has been also applied to the determination of thiazide diuretics in human urine [8].

Fluorimetric [9,10] and electrochemical detection [11,12] has been also applied. However, as HCT often is combined with other drugs, methods of separation (chromatography [13,14] and electrophoresis [15,16]) have been suggested for its determination, just as multicomponent analysis based on derivative or multiwavelength spectrophotometry [17–19]. Methods devoted to obtain the dissolution profiles of hydrochlorothiazide [20] or the simultaneous dissolution profiles of this drug and captopril [21], have been also presented.

Analytical procedures involving chemiluminescence (CL) measurements have been proposed. In the first [22], a CL reaction occurs between thiazide compounds and tris-(2,2′-bipyridine)ruthenium(III). However, a complicated instrumental set-up is necessary for the electro-generation of CL, and the system shows important interferences from organic amines. A second CL system developed by Ouyang et al. [23–26], was based on the reaction of HCT with Ce(IV) in sulphuric acid, sensitized by Rhodamine 6G.

On the other hand, it has been established that the irradiation of photoreactive analytes leads to the formation of species that can be detected by CL [27–29]. Bibliographic information dealing on irradiation of thiazides [8,30–32] is scarce and most of it is dealing on the photo-stability or UV–vis determination of these drugs rather than on the photo-fragments.

The purpose of the present investigation was to develop a sensitive, rapid and a simpler assay for determination of thiazides using a FI system coupled to photochemically induced CL. The method uses the photochemical decomposition of the drugs, and the product generated is determined by CL, with the aid (for the first time) of Ce(IV) in acid medium.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-grade reagents in deionized water (18 MΩ cm) from a

Sybron/Barnstead Nanopure II water purification system provided with a fiber filter of 0.2 μm pore-size. Thiazides (Guinama), $Ce(NH_4)_2(NO_3)_6$ (Probus), NaOH and H_2SO_4 (both from J.T. Baker) were used. Other reagents were $K_3Fe(CN)_6$, $KMnO_4$, H_3PO_4, HNO_3, NaCl, acetone, Triton X-100, 1,10-phenanthroline-1-hydrate, formic acid, acetylsalicylic acid, lactose 1-hydrate and NH_4Cl, $Na_2B_4O_7{\cdot}10H_2O$ (all from Panreac); Na_2EDTA, NH_3 and sodium acetate (all from Probus); KIO_4 and $AgNO_3$ (from Prolabo); $K_2S_2O_8$, hydrogen peroxide 30%, acetonitrile, starch soluble and acetic acid (Merck); HCl and KH_2PO_4 (both from J.T. Baker); sodium dodecyl sulphate and hexadecylpyridinium chloride (Fluka); glycine (Scharlau); formamide (Roche); quinine sulphate, magnesium stearate, talc, calcium pantothenate, sucrose, wheat starch, captopril and ethanol (all from Guinama); Rhodamine B and Rhodamine 6G (Sigma).

2.2. Flow-injection procedure

The FIA system used is depicted in Fig. 2. Connections between the different parts of the flow assembly were effected with a PTFE coil of 0.8 mm ID. A Gilson (Worthington, OH, USA) Minipuls 2 peristaltic pump, provided with tygon pump tubes from Omnifit, was used for carrier delivering and flow regulation. A laboratory-made photo-reactor included PTFE tubing (0.5 mm ID with variable lengths, 550 cm in the proposed system) tightly coiled around an 8 W low-pressure mercury lamp (Zalux) for germicidal use. The analyte was prepared in 0.05 mol l^{-1} NaOH, and flowed at 1.7 ml min^{-1} through the photo-reactor. The 421 μl of the photodegradated sample was injected into the carrier stream (water at 6 ml min^{-1}) using a rotatory injection valve (Rheodyne, Model 5041, Cotati, CA, USA). Then the inserted solution merged with an oxidant solution (5×10^{-3} mol l^{-1} $Ce(NH_4)_2(NO_3)_6$ in 0.1 mol l^{-1} sulphuric acid) flowing at

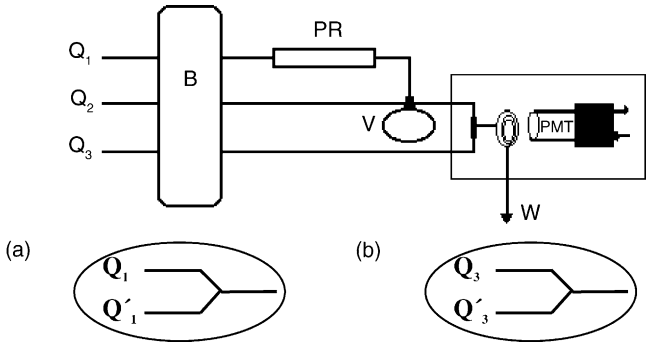

Fig. 2. Optimized flow assembly. Q_1: sample solution in the suitable medium for photodegradation; Q_2: carrier solution (water); Q_3: oxidant solution and medium of oxidation; V: volume of photodegradated injected sample; B: peristaltic pump; PR: photoreactor; PMT: photomultiplier tube; W: waste. For preliminary assays, the manifold was modified adding a confluence: (a) channel 1 was replaced for one confluence to mix sample and photodegradation medium before the lamp; (b) channel 3 was replaced by a confluence to mix oxidant and medium for the chemiluminescent reaction.

1.5 ml min^{-1}, and the mixture was led to the detector flow cell. The flow cell was a flat-spiral quartz tube of 1 mm ID and 3 cm total diameter backed by a mirror for maximum light collection. The photodetector package was a P30CWAD5F-29 Type 9125 photomultiplier tube (PMT) supplied by Electron Tubes operating at 1280 V and located in a laboratory-made light-tight box. The output was fed to a computer equipped with a counter-timer, also supplied by Electron Tubes.

2.3. Preparation of sample

Adelfán-Esidex (*Novartis Farmacéutica, S.A., Barcelona, Spain*). Ten tablets were weighed and crushed. After a proper homogenization, an amount (higher than 0.11 g) was powered, shakened in 100 ml of 0.05 mol l^{-1} NaOH and filtered through a glass mesh of three pore numbers. After filtering the resulting solution was made up to 250 ml with 0.05 mol l^{-1} NaOH. Then this solution was used to obtain a final concentration in the range of application of the method. The process was achieved in triplicate.

3. Results and discussion

3.1. Preliminary assays

Preliminary tests were performed with a FI manifold (Fig. 2 modified with a confluence to replace channel 1 (a)). The study was performed employing as oxidant $KMnO_4$ in H_2SO_4 (Q_3), and nine different media for the photodegradation (Q'_1), namely: 10^{-3} mol l^{-1} H_2SO_4, H_2O, HAcO/NaAcO buffer at pH 4.6, KH_2PO_4/K_2HPO_4 buffer at pH 7.1, glycine buffer at pH 8.6, borax buffer at pH 9.0 and NH_4^+/NH_3 buffer at pH 10.0 (all buffers with concentration 0.02 mol l^{-1}), 10^{-3} and 0.5 mol l^{-1} NaOH. The hydrochlorothiazide (5×10^{-4} mol l^{-1}) was prepared in water and run by Q_1 at the same flow rate as Q'_1 (0.8 ml min^{-1}). The carrier was water and the photoreactor consisting of 690 cm × 0.5 mm PTFE tubing helically coiled around an 8 W low-pressure mercury lamp.

The signal obtained with 0.5 mol l^{-1} NaOH as medium for the photodegradation was 10 times higher than that obtained with the rest of media, and not peaks were obtained when the lamp was OFF. Strong basic media was necessary for the photodegradation of hydrochlorothiazide (see Fig. 3).

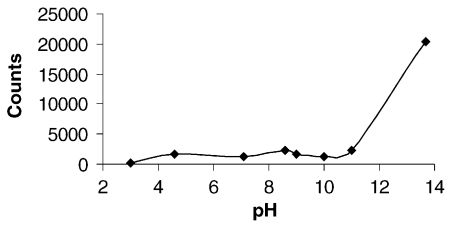

Fig. 3. Influence of pH on the photodegradation.

3.2. Optimization of the flow-injection manifold

The FI manifold (Fig. 2) was optimized by using the univariate method. Chemical parameters affecting photochemical and chemiluminescent indicator reaction were optimized before the FI variables, and the most significant variables were re-optimized after establishing the FI conditions.

3.3. Chemiluminescent reaction

The first step was to establish the best oxidant system for the direct determination of the photodegradated hydrochlorothiazide. The following oxidant systems were tested: $KMnO_4$, Ce(IV), KIO_4, $K_2S_2O_8$ (with Ag(I) 2×10^{-5} mol l^{-1}) all of them in H_2SO_4, $K_3Fe(CN)_6$ and H_2O_2 both in NaOH. The FI assembly depicted in Fig. 2 was employed, with a confluence to mix the oxidant (2×10^{-3} mol l^{-1}) and the medium for the oxidation (3 mol l^{-1} H_2SO_4 or NaOH). The hydrodynamic conditions were as follows: Q_1, 0.7 ml min^{-1} sample solution (5×10^{-4} mol l^{-1} sample); Q'_1, 0.7 ml min^{-1} medium for photodegradation (0.5 mol l^{-1} NaOH); Q_2, 6.0 ml min^{-1} carrier solution (water); Q_3, 0.7 ml min^{-1} oxidant; Q'_3, 0.7 ml min^{-1} medium for the oxidant; V, 330 μl; and photoreactor, 550 cm × 0.5 mm PTFE tubing helically coiled around 8 W low-pressure mercury lamp.

As shown in Table 1, the best results were found with $KMnO_4$ and Ce(IV) as oxidants. Oxidant concentration is a critical variable in direct chemiluminescence, so before to chose an oxidant system, different concentrations of both, $KMnO_4$ and Ce(IV), were tested. The studied intervals covered from 2×10^{-4} to 2×10^{-3} mol l^{-1} for $KMnO_4$, and from 10^{-3} to 2×10^{-2} mol l^{-1} for Ce(IV). The maximum height of the peak obtained with the first system (7.5×10^{-4} mol l^{-1} $KMnO_4$) was half of the signal supplied with 2×10^{-2} mol l^{-1} for Ce(IV), so this last oxidant was selected for further work.

The best medium for the oxidant reaction was also examined, including H_2SO_4, HCl, $HClO_4$, H_3PO_4 and HNO_3, all at a 3 mol l^{-1} concentration. The highest outputs were given for sulphuric acid, which concentration was then optimized over the range 0.1–4.0 mol l^{-1}. A plateau was observed between 1.0 and 2.0 mol l^{-1} of H_2SO_4, and a concentration of 1.5 was pre-selected.

Table 1
Oxidants tested for the chemiluminescent emission of the photodegradated hydrochlorothiazide

Oxidant	ON		OFF	
	Counts	RSD (%)	Counts	RSD (%)
$KMnO_4$	95378.0	8.8	–	–
$Ce(NH_4)_2(NO_3)_6$	210894.3	3.3	–	–
KIO_4	702.4	14.5	26.5	4.7
$K_2S_2O_8$/Ag(I)	1362.2	6.3	10.3	15.1
H_2O_2	33.5	7.5	–	–
$K_3Fe(CN)_6$	776.2	2.7	–	–

3.4. Study of the photodegradation reaction

It has been established that the chloride substituent in HCT is replaced by H, OH or OCH_3 in aqueous or methanolic solutions on photolysis [33,34], as well as the hydrolysis of the thiamine ring. On the other hand, the alkaline hydrolysis of hydrochlorothiazide occurs resulting in a *m*-disulphonamide and formaldehyde [35]. Other authors [30–32] established the photo-decomposition appears to proceed according to the first-order kinetics and the main products resulting from irradiation are formed after dechlorination of the parent molecules (hydrochlorothiazide and trichloromethiazide), while the aromatic $-SO_2NH_2$ (chlorothiazide) group is replaced by a hydrogen atom.

The effect of three buffers (glycine, phosphate and borax buffer) at different pH (9.5–12.5) and of some concentrations of NaOH was tested. None of the buffers provided signals comparables to those obtained when NaOH solutions. After a re-optimization between 0.2 and 0.6 mol l^{-1}, NaOH 0.5 mol l^{-1} was fixed as medium of photodegradation.

The kinetic of the photodegradation was studied by varying simultaneously the flow rate of sample and medium of photodegradation (Q_1 and Q'_1). The curve signal versus time of irradiation were characterised by a continuous increase in the chemiluminescence, up to 104 s where a plateau was obtained up to 138 s of irradiation; and after it a diminution of the signal was observed. The 104 s was selected in order to have higher throughput ($Q_1 = Q'_1 = 0.8$ ml min^{-1}).

The influence of different potential photolysis and chemiluminescence sensitizers was studied. Selected compounds and results are summarised in Table 2. By Q_1 flowed the sample in the 0.5 mol l^{-1} NaOH medium and by Q'_1 the photosensitizer. Only formic acid and Rhodamine 6G seemed to provide a significant increase. The growth with quinine was small and the blank output was very high. For other substances an inhibition of the signal was observed.

Table 2
Influence of surfactants and sensitizers on the emission intensity

Sensitizer[a]	Signal (blank)	Blank	Increase[b] (%)
Acetonitrile, 20%	43075.0	40	−83.5
Quinine, 5×10^{-4} mol l^{-1}	301954.3	15425	15.9
Acetone, 0.5%	19130.7	562	−92.7
Dimethylformamide, 5%	25198.5	0	−90.3
Ethanol, 5%	32798.7	0	−87.4
Formic acid, 1%	438872.5	42	68.5
Hexadecylpyridinium, 0.09%	22381.8	2393	−91.4
Triton X-100, 0.03%	42565.8	112	−83.7
SDS, 0.55%	143897.2	115	−44.8
Acetone, 0.5% + acetonitrile, 20%	24600.0	2226	−90.6
Rhodamine 6G, 5×10^{-4} mol l^{-1}	682636.8	12908	162.0
Rhodamine B, 5×10^{-4} mol l^{-1}	66916.8	37183	−74.3

[a] All the % are v/v, except the surfactants (SDS, Triton X-100 and hexadecylpyridinium) in w/v.

[b] Increase over the reference signal obtained without sensitizer or surfactant.

A new study with several concentrations of formic acid and Rhodamine 6G was performed over the range 0.5–2.0% and 10^{-4} to 7.5×10^{-4} mol l^{-1}, respectively. Best results (increase of 100% respect the signal without photosensitizer) were obtained for (2.5×10^{-4} mol l^{-1}) Rhodamine 6G. However, the blank obtained was very high. A new configuration was studied to avoid the blank signal, and also to obtain information about the influence of Rhodamine 6G on the photodegradation and the chemiluminescent reaction. The Ce(IV) was prepared in sulphuric acid and inserted into the system through Q_3. The Rhodamine flowed through Q_3' at the reported concentrations. High base line was obtained, which can result in negative peaks. Beside inhibition of the signals were obtained in all the concentrations. Due to the high blanks and in order to have a simple and clean system, the use of Rhodamine 6G was discarded.

The FI system was simplified by removing both confluences, namely sample and medium of photodegradation, and oxidant and medium of oxidation ((a) and (b) in Fig. 2) were prepared off-line. The final system is like the one depicted in Fig. 2: Q_1, 1.5 ml min^{-1} (sample in 0.25 mol l^{-1} NaOH); Q_2, 6.0 ml min^{-1} (water); and Q_3, 1.5 ml min^{-1} (10^{-2} mol l^{-1} Ce(IV) in 0.75 mol l^{-1} H_2SO_4).

The temperature can have a double influence on the system. It can influence the photodegradation step in different ways: either accelerating the kinetic process or determining the final products of the photolysis, which in turns can influence the chemiluminescent response. A continuous output decrease was observed when the sample solution was heated by immersing it into a water bath over the range 20–85 °C. On the other hand, the temperature can also influence the chemiluminescent reaction. In general the increase of temperature damages the luminescent response due to the deactivation by not emissive mechanisms. However, a positive influence can be noticed when it affects to the kinetic of the oxidative reaction. The study of the chemiluminescent reaction was performed by heating carrier and oxidant solutions in a water bath over the interval 20–85 °C. Very small increases (under 16%) were obtained. Due to that, room temperature was selected.

Once established the chemical conditions for the photodegradation, a re-optimization of the NaOH concentration (medium for photodegradation) and the time of photodegradation was carried out. Higher signals were obtained for sample in 0.05 mol l^{-1} NaOH and irradiate during 97 s with the UV light.

3.5. FI variables

Two hydrodynamic conditions of the flow system affecting the chemiluminescent reaction, injected volume and flow rate, were studied.

The signal increased with the injected volume (over the range 260–444 μl) and 421 μl was chosen, to obtain the best compromise between the height and the base-width of the peak.

To establish the kinetic of the chemiluminescent reaction is very important to guarantee that the maximum emission occurs inside the flow cell. Keeping constant the relation flow-carrier/flow-oxidant (Q_1/Q_2), the global flow ($Q_1 + Q_2$) was varied from 2.5 to 10 ml min^{-1}. However, the signal increased in the whole interval was less pronounced from 5 ml min^{-1}. The 7.5 ml min^{-1} was selected to avoid overpressure problems and to minimise the consumption of reagents.

4. Photodegradation and fluorimetric/chemiluminometric detection of thiazides

The assessment of the photodegradation step on the molecular structure of thiazides was established by recording UV and fluorimetric spectra. UV and fluorimetric spectra were obtained by passing through the photoreactor and under optimal conditions of irradiation time a 50 μg ml^{-1} solution of thiazide in 0.05 mol l^{-1} NaOH. Spectra were recorded for the emerging solutions. UV and fluorimetric spectra were obtained with the lamp ON and OFF (see Fig. 4 and Table 3). The experiment allowed checking both the viability of the proposed method for this group of drugs and the relation between photodegradation, photoinduced fluorescence and chemiluminescent behaviour after UV-irradiation.

According to the fluorimetric response, thiazides can be divided into three groups: (a) The viability of the on-line photoinduced fluorescent determination of hydroflumethiazide

Table 3
Influence of photodegradation on the fluorimetric behaviour of thiazides; [thiazide] = 50 μg ml^{-1} in 0.05 mol l^{-1} NaOH

Thiazide	Lamp OFF			Lamp ON		
	λ_{ex}	λ_{em}	I	λ_{ex}	λ_{em}	I
Hydrochlorothiazide	–	–	–	266/356	455	437/614
Hydroflumethiazide	–	–	–	295	435	1400[a]
				394	445	1710[a]
Chlorthalidone	–	–	–	300	420	524
Bendroflumethiazide	–	–	–	240/379	445	400[a]/335[a]
Indapamide	–	–	–	266	363	500
Metolazone	274/344	444	2084[a]/1487[a]	250–350/379	492	486/933

[a] Intensity at 700 V; rest at 900 V. $Slit_{ex}$ and $slit_{em}$ = 5 nm.

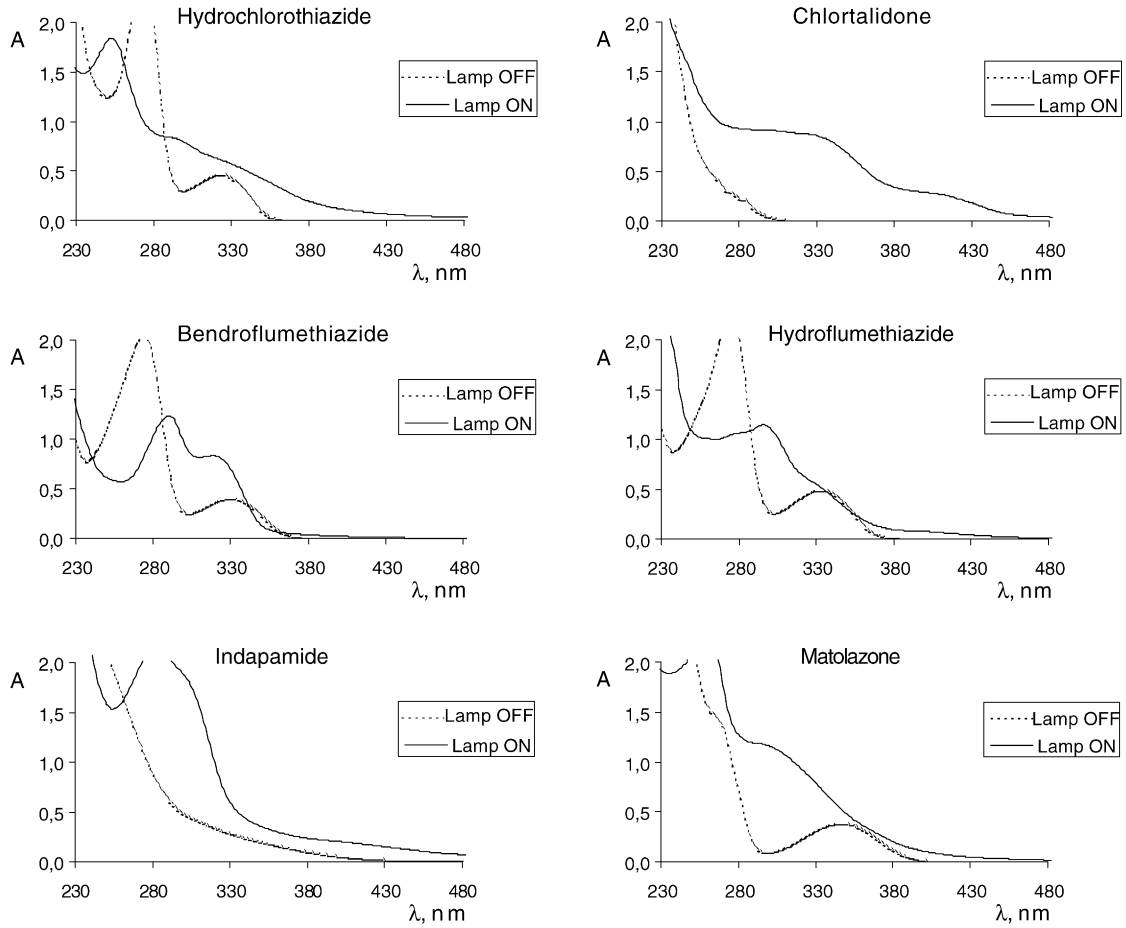

Fig. 4. UV–vis spectra of thiazides with lamp ON and OFF (50 μg ml^{-1} for all thiazides in 0.05 M NaOH).

and bendroflumethiazide was confirmed. These thiazides exhibit a very weak fluorescence with lamp OFF, but they turn into strongly fluorescent compounds after UV-irradiation. (b) Indapamide, chlorthalidone and hydrochlorothiazide presents null native fluorescence. UV-irradiation causes the photoinduced fluorescence of these thiazides. (c) Metolazone presents a strongly fluorescent behaviour with lamp OFF (λ_{ex} = 274 nm and 344 nm; λ_{em} = 444 nm), and a significant decrease of the fluorescent intensity and changes of the excitation and emission wavelength with lamp ON.

There is not a clear relation between changes in the UV spectra after UV-irradiation, photo-induced fluorimetric and chemiluminometric response for the tested thiazides. All drugs presented dramatic changes of UV and/or fluorimetric spectra. Hydrochlorothiazide, hydroflumethiazide and bendroflumethiazide presenting similar ring's structure and UV-OFF/ON spectra are rather different attending the fluorimetric and chemiluminometric response. Hydrochlorothiazide and bendroflumethiazide differ in the Cl/CF_3 group bonded to the aromatic ring. Nevertheless, bendroflumethiazide turns into a strongly fluorimetric thiazide and the weakest chemiluminescent drug after UV-irradiation (see calibration slopes in Table 4).

5. Analytical applications

The calibration graph was linear over the range 0.2–5 μg ml^{-1} and fitted the equation I = (450 000 ±

Table 4
Influence of photodegradation on the chemiluminometric response of thiazides

Thiazide	Linear range (μg ml^{-1})	Limit of detection (μg ml^{-1})	Equation
Hydrochlorothiazide	0.2–5.0	0.005	I = (450 000 ± 30 000)C − (200 000 ± 80 000); r^2 = 0.994
Hydroflumethiazide	0.5–12.0	0.025	I = 2242.2C − 1237.1; r^2 = 0.997
Chlorthalidone	0.5–12.0	0.020	I = 2294.1C − 82.4; r^2 = 0.998
Bendroflumethiazide	0.5–20.0	0.060	I = 683.7C − 127.3; r^2 = 0.998
Indapamide	0.5–14.0	0.020	I = 1054.1C + 1135.9; r^2 = 0.992
Metolazone	0.5–12.0	0.010	I = 9308.1C − 2102.6; r^2 = 0.998

30 000)C − (200 000 ± 80 000) with a correlation coefficient of 0.997, where I is the chemiluminescent emission and C the concentration of hydrochlorothiazide in $\mu g\,ml^{-1}$. The limit of detection (0.005 $\mu g\,ml^{-1}$) was defined as three times the background average and was established by decreasing the concentration of injected chloramphenicol until this relationship was reached. The inter-day reproducibility of the proposed method was estimated by running calibrations with solutions of the reagents freshly prepared each day. The RSD of the slopes of four different calibration graphs was 6.7% for hydrochlorothiazide. The RSD for a series of 11 injections of a 2 $\mu g\,ml^{-1}$ solution of chloramphenicol was 2.0%. The throughput, calculated using the same series, was 65 samples h^{-1}.

Indapamide, metolazone, hydroflumethiazide, chlorthalidone and bendroflumethiazide were tested under the optimal conditions obtained for hydrochlorothiazide (see Table 4). The limits of detection were at least two-fold higher (10 $ng\,ml^{-1}$ for metolazone) than that obtained for hydrochlorothiazide. The sensitivity in terms of the slope of the calibration graph was not comparable; it ranged (slope HCT/slope thiazide) from 48 (metolazone) to 658 (bendroflumethiazide). Linear ranges compare favourable with hydrochlorothiazide due to the lost in sensitivity. None chemiluminometric response was obtained (lamp OFF) for a solution containing 50 $\mu g\,ml^{-1}$ metolazone, hydroflumethiazide or bendroflumethiazide. In the case of indapamide and chlorthalidone, 50 $\mu g\,ml^{-1}$ of thiazide provided an analytical signal (lamp OFF) 3.3- and 2.9-fold lower than that obtained (lamp ON) for a solution 0.5 $\mu g\,ml^{-1}$ of these thiazides.

The robustness of the system concerning flowing and chemical variables was studied. The effect of a variation of the relation flow-carrier/flow-oxidant (Q_1/Q_2), equivalent to change the concentration of oxidant, was studied. The previous flow rates, 6 $ml\,min^{-1}$ for the carrier solution and 1.5 $ml\,min^{-1}$ for the oxidant solution, were kept, and the chemical conditions were re-optimized.

A plateau was obtained when the concentration of Ce(IV) was varied over the range 0.0025–0.0075 $mol\,l^{-1}$, followed by a signal decrease when an excess of oxidant was present. A 0.005 $mol\,l^{-1}$ concentration of Ce(IV) was selected as optimum. The concentration of sulphuric acid was also re-optimized (studied interval: 0.05–1.25 $mol\,l^{-1}$). A slight acid medium was necessary to obtain the oxidation and CL emission of HCT. The optimal concentration of sulphuric acid was established in 0.10 $mol\,l^{-1}$.

Interferences were sought among the more frequents substances accompanying the hydrochlorothiazide in the commercially available formulations. The potential interferents were added to 3.25 $mg\,l^{-1}$ of HCT in the basic medium. The relative errors were obtained by comparing the output with the observed with a pure HCT solution and are depicted in Table 5. Captopril shows chemiluminescent response by itself, which presumed an important interference when the method needs to be applied to the determination of hydrochlorothiazide in the presence of captopril.

Table 5
Influence of foreign substances (for 3.2 $mg\,l^{-1}$ of hydrochlorothiazide)

Interferent	Concentration ($mg\,l^{-1}$)	Error (%)
Lactose	25	−4.8
Magnesium stearate	8	−4.7
Talc	40	−4.5
Calcium phosphate	100	−1.8
Starch soluble	100	−3.4
Wheat starch	10.8	−5.6
Reserpine	2.6	−3.0
Na_2-EDTA	50	−0.4
Sucrose	15	−4.8
Mannitol	6	−2.6
Captopril	0.9	−1.6

To investigate the applicability of the proposed method to real samples, hydrochlorothiazide was determined in a pharmaceutical preparation (Adelfan-Esidrex). Good concordance was found with figures provided from the manufacturer; calculated error versus label claim was 3.0%.

6. Conclusions

The proposed method for the determination of hydrochlorothiazide combines the advantages of photochemical reaction (such as cleanliness, reproducibility and easy manipulation) and the chemiluminescent detection (such as sensitivity, low limit of detection and selectivity).

The photodegradation step allows the determination of hydrochlorothiazide employing the chemiluminescent reaction with Ce(IV) in acid medium by avoiding the use of sensitizers (Rhodamine 6G) as required in former works [23,25]. Moreover, the proposed method presents lower detection limits than previously reported methods concerning chemiluminometric determination of hydrochlorothiazide (e.g. 59 $\mu g\,l^{-1}$ [23] and 44 $\mu g\,l^{-1}$ [25]).

From author's knowledge, no previous works have been reported related to the chemiluminometric behaviour of thiazide diuretics such as indapamide, metolazone, hydroflumethiazide, chlorthalidone and bendroflumethiazide.

The viability of an on-line photochemical reaction–chemiluminometric determination of these thiazides was confirmed.

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