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A tandem-flow assembly for the chemiluminometric determination of hydroquinone

B. Gómez-Taylor Corominas [a], M. Catalá Icardo [a], L. Lahuerta Zamora [b], J.V. García Mateo [b], J. Martínez Calatayud [a,*]

[a] *Department of Analytical Chemistry, University of Valencia, Valencia, Spain*
[b] *Department of Chemistry, Biochemistry and Molecular Biology, University Cardenal Herrera-CEU, Moncada, Valencia, Spain*

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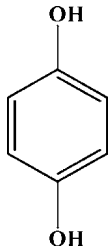
Abstract

A direct chemiluminescent procedure for determination of hydroquinone based on the emergent flow methodology known as multicommutation or tandem-flow is presented for first time. The manifold was based on a set of three channels and three solenoid valves; and, the determination was performed at 60 °C and at flow-rate of 7.5 ml min^{-1}. The complete cycle lasted 35 s, which resulted in a sample flow trough of 103 h^{-1}. The chemical process was the hydroquinone oxidation with the system sulphuric acid-potassium permanganate; and the light emission was clearly enhanced by the presence of quinine sulphate and benzalkonium chloride reaching a detection limit of 30 μg l^{-1}. The dynamic interval was over the range 0.1–15.0 mg l^{-1} and a large list of interferents were assayed; the chemical robustness was also tested. The method was applied to different type of samples: namely, pharmaceutical formulations, a photographic solution and irrigation and residual superficial waters.

Keywords: Hydroquinone; Chemiluminescence; Tandem-flow; Pharmaceuticals; Environmental samples

1. Introduction

Hydroquinone [1] is a hydroxy derivative of benzene with the empirical formula $C_6H_6O_2$ and the following molecular structure:



It occurs [2,3] as colourless, odourless and it is soluble in water, ethanol, ether, chloroform, glycerine and also, sparingly, in benzene. Hydroquinone occurs naturally in wheat products, coffee, and tea, fruits such as the cranberry, various vegetables, red wine and some beers. Discovered in 1880, it can be synthesised by oxidation of aniline, reduction of quinone and oxidation of phenol with persulphate [1].

Hydroquinone is primarily used [4] as a photographic developer and as a skin lightener in cosmetics—because it inhibits melanocyte metabolism and the action of the enzyme tyrosinase, which converts tyrosine into melanin, it reduces cell oxidation, facilitates the removal of free radicals and strengthens the immune system. Hydroquinone-based [5] skin lighteners can cause itch, dermatitis and erythema—in individuals with an especially sensitive skin—, as well as changes in skin colour upon prolonged treatment with high doses of the chemical. For all these reasons, the European Union [6] issued directive 84/415 to restrict the maximum allowed amount of hydroquinone in cosmetic products.

Hydroquinone [7] can be determined by titration with cerium sulphate in the presence of diphenylamine as indicator, as well [8] as with 1,3-dibromo-5-ethylmethylhydantoin (an oxidising derivative of hydantoin) and Eriochrome Black as indicator.

* Corresponding author. Tel.: +34-96-354-4062; fax: +34-96-354-4062.
E-mail address: jose.martinez@uv.es (J. Martínez Calatayud).
URLs: http://www.uv.es/~martinej/, http://www.uv.es/FlowAnalysis.

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There are various spectrophotometric procedures [6,8] for the determination of hydroquinone in creams by its native absorbance using ethanol or methanol medium. Thus, He et al. [9] determined hydroquinone in the presence of phenol by using derivative spectrophotometry, and Afkhami et al. [10] developed a kinetic method for pharmaceutical formulations based on the reaction with nitrite. Other spectrophotometric methods [11,12] allowed the determination of hydroquinone traces in wastewater.

Hydroquinone in cosmetic products, urine, photographic developers and tobacco, among other products, has been determined by thin-layer and high-performance liquid chromatographies. Hydroquinone and some of its ether derivatives in cosmetics have been quantified by micellar electrokinetic chromatography and capillary electrochromatography [13–15].

As regards electrochemical methods, Wang [16] used a carbon paste electrode in combination with differential pulse voltammetry to determine hydroquinone. Zhao et al. [17] examined its electrochemical behaviour in cyclic voltammetry. In addition, preconcentration–stripping voltammetry was successfully applied to the simultaneous determination of metal traces and hydroquinone in wastewater.

Regarding FIA methods, Nakagama [18] conducted a scan of various organic chemicals including hydroquinone, using different oxidising systems with a view to obtaining a chemiluminescent signal. Du et al. [19] developed a chemiluminescence-based FIA method for determining hydroquinone and other phenol compounds in which the catalytic effect of ferricyanide and ferrocyanide ions enhances the chemiluminescence produced in the reaction of these compounds with luminol.

Zhou and Wang [20] reported a method involving amperometric detection. The sample, carried by a stream consisting of phosphate buffer containing methanol and EDTA, was detected at +0.1 V in an amperometric cell comprising a glassy carbon electrode coated with CuCl crystals, an Ag/AgCl reference electrode and a Pt auxiliary electrode.

Satake et al. [21] determined hydroquinone, catechol, resorcinol and pyrogallol with FIA and amperometric detection. To this end, they inserted the sample into a water stream that was subsequently merged with a solution of KIO_3 and KBr in H_2SO_4. The resulting decrease in KIO_3 concentration was detected at +0.65 V with a platinum electrode.

In this work, the chemiluminometric determination of hydroquinone is performed in a continuous-flow assembly of the type known as multicommutation or tandem-flow and based on a set of solenoid valves [22–24]. The manifold consisted in three channels and three solenoid valves. The chemical process was the hydroquinone oxidation with potassium permanganate in a sulphuric medium and presence of a light-emission enhancer and a surfactant, quinine sulphate and benzalkonium chloride.

2. Experimental

2.1. Reagents and apparatus

All reagents were of analytical grade unless stated otherwise. Aqueous solutions were prepared in pure (reverse osmosis) and de-ionised water (system Sybron Barnstead Nan pure II provided with a filter 0.2 μm pore size). Hydroquinone was from two different manufactures (Panreac and Merck). Reagents were also used from different manufacturers for the study of chemical robustness; H_2SO_4 (Panreac and Merck), $KMnO_4$ (Panreac and Probus), quinine sulphate (Guinama) and benzalkonium chloride (Fluke).

2.2. Flow assembly

Fig. 1 depicts the flow manifold finally proposed for the chemiluminescent determination of hydroquinone. This assembly comprised a PTFE tubing of 0.8 mm i.d., a peristaltic pump Minipuls 2 from Gilson; and three-way solenoid valves NResearch, model 161T031, for the flow manifold. The measurements of the chemiluminescent outputs were obtained by means of a homemade flow-cell which consisted in a flat spiral-coiled quartz tube of 1.0 mm i.d., 3 cm total diameter and without gaps between loops. The flow-cell was about 2 mm from the photomultiplier tube window (model 9125 from Electron Tubes Limited) and backed by a mirror for maximum light collection. Flow-cell and photomultiplier tube were placed inside a homemade light-tight box. The photomultiplier package was operated at 1273 V supplied by a PHV-40 programmable high voltage power supply (from Acton Research Cop.). The output was fed to a computer equipped with a counter time, (home made) and the peak heights were measured in KHz.

2.3. Optimisation of the flow assembly; chemical and hydrodynamic parameters

The optimisation of the chemical and flow variables was performed by a sequential series of experiments. First were tested the chemical parameters and temperature; then with the selected chemical values we optimised the hydrodynamic and chemical variables by using the multiparametric method known as modified simplex method (MSM). As the optimisation steps resulted in increased analytical outputs, each step comprised a new calibration graph to select the suitable hydroquinone concentration (decreasing) for the following assays.

The initial simplex was selected according to Yarbro and Deming [25] and the variable region was standardised by following the modification of Morgan and Deming [26]. The different simplex vertices were obtained with the aid of software based on the method of Nelder and Mead [27] with the target flow variables and the value (as KHz) corresponding to each combination of such variables provided by the simplex inputs. The program is written to optimise the height

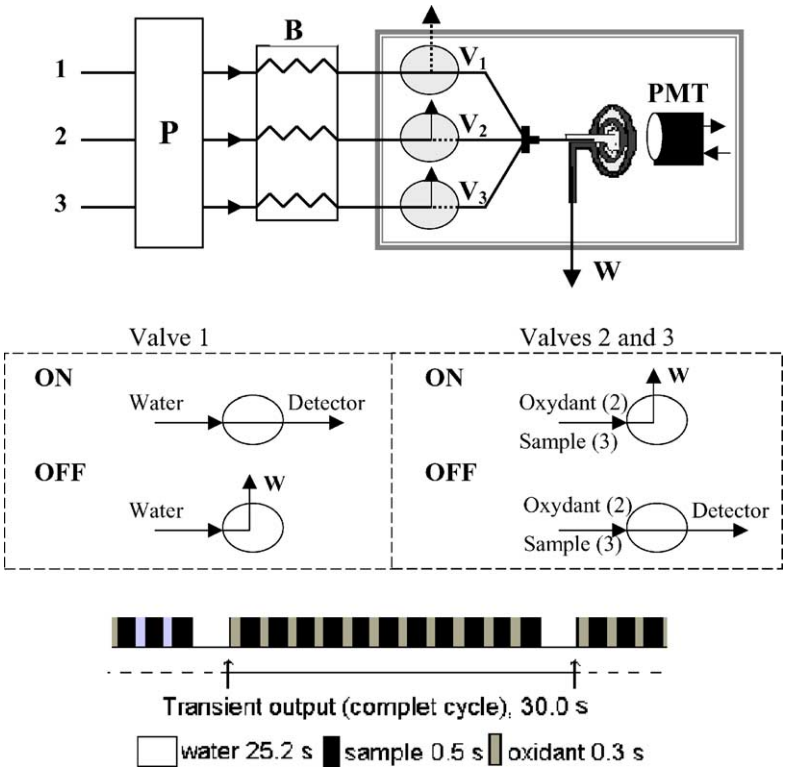

Fig. 1. Proposed multicommutation manifold for the chemiluminometric determination of hydroquinone. *Top*: optimised manifold for the determination of hydroquinone. *Centre*: run sequence of the three parallel valves (V_1, V_2 and V_3). *Bottom*: definitive sequence to obtain the analytical outputs. (1) H_2O through V_1. (2) $KMnO_4$ 1×10^{-3} mol l^{-1} in H_2SO_4 1.0 mol l^{-1} through V_2. Number of segments 11 of 0.3 s. (3) Hydroquinone in H_2SO_4 1.000 mol l^{-1} + quinine 5×10^{-4} mol l^{-1} + BAC 10 mmol l^{-1} through V_3. Number of segments 11 of 0.5 s. Flow-rate (any channel) 7.5 ml min^{-1}. (B) water-bath at 60 °C; P, peristaltic pump; W, waste; PMT, photomultiplier tube; and, V, solenoid valve.

of the output. Two consecutive simplex were performed, the interval for each variable in the second being restricted to the zone that gave the best results in the first. For the last optimisation step, we select some of the higher vertices for a new comparative study to choose the output resulting in the best compromise: sensitivity (peak height)–sample throughput (peak-base width)–repeativity (R.S.D., %); or when calibration graphs were obtained peak height was substituted by linear slope.

2.4. Stability of aqueous solutions of hydroquinone

Hydroquinone crystals are stable; at room temperature, however, they must be stored in air- and light-tight containers. Hydroquinone solutions are oxidised to a brown colour by air and light, the process being accelerated by alkaline media, where the compound is somewhat unstable.

The stability of aqueous solutions of hydroquinone was studied in our laboratory by periodically recording UV-Vis spectra ($n = 8$) for 17 days. To this end, the absorbances of three 10.0 ppm hydroquinone solutions exposed to ambient light, stored in the dark and at 3 °C in a refrigerator, respectively, were measured over the stated period. Spectra were recorded over the UV-Vis wavelength range and invariably exhibited an absorbance band at 222 nm. Based on the obtained spectra and visual observations—all spectra were virtually identical—aqueous solutions of hydroquinone can be assumed to remain stable for at least 15 days under the three different types of storage conditions studied.

3. Results and conclusions

3.1. Preliminary flow-assays

Preliminary assays were performed with the aid of a flow manifold in which the hydroquinone solution or pure water as blank solution, merged with the solution for adjusting the medium solution; then this mixture merged with the oxidative system formed by the mixture of the oxidant and the same solution used to adjust the sample. All solutions were flowing at 2.8 ml min^{-1} and the concentration of hydroquinone was 200 mg l^{-1}. Different oxidants in 0.02 mol l^{-1} concentration in different 1.0 mol l^{-1} media (NaOH or H_2SO_4) were tested; namely, KIO_4, $KMnO_4$, $Ce(NH_4)_2(NO_3)_6$, H_2O_2, $K_3Fe(CN)_6$ and *N*-bromosuccinimide; only potassium permanganate in acidic media resulted in luminescent emission. Very small outputs (almost null) were also observed for

N-bromosuccinimide, hydrogen peroxyde and potassium ferricyanide.

The influence of the concentration of potassium permanganate demonstrated to be so critical as expected bearing in mind its usual behaviour [28]; the influence was tested over the interval 1.0×10^{-5} to 5.0×10^{-2} mol l^{-1} with concentrations of hydroquinone ranging from 1 to 200 mg l^{-1} (1, 20, 50, 100, 120, 150 and 200). Highest outputs were observed on 5.0×10^{-3} mol l^{-1} at any tested concentration. Further assays tested the influence of different acidic media: hydrochloride, perchloric, nitric and sulphuric; all of them in different concentrations. These assays were performed with concentrations of hydroquinone ranging from 1 to 150 mg l^{-1}. Sulphuric acid always resulted in higher outputs and was pre-selected as the suitable. The influence of concentration of sulphuric acid (over the range 0.05–2.00 mol l^{-1}) revealed higher outputs when the acidic concentration was increased; up to 0.5 mol l^{-1}; beyond this the differences were not relevant. Trying to avoid empirical problems with a high sulphuric acid concentrations, was selected 0.6 mol l^{-1}. Some preliminary assays tested the influence of the temperature (from 22 to 80 °C at 50 and 100 mg l^{-1} of hydroquinone) by immersing 2 m of PTFE tubing into a water-bath; slightly higher outputs were observed for room temperature.

3.2. Design of a multicommutation manifold

Up to four different flow-manifold configurations were prepared and studied by testing the influence of the flow parameters. Studied configurations included a set of solenoid valves in parallel of in linear range; by performing zone-confluence or only sequentially segmented solutions, etc. The study to select the suitable configuration consisted in testing the influence of the following parameters: flow-rates, sample and reagents volume and the number of successive segments sample-reagent. The individual study for each assembly was based on the multiparametric modified simplex method (MSM). Tested ranges were as follows: flow-rates (in ml min^{-1}) 2.8–7.7; size of the oxidative and sample segments (in s) 0.06–1.0; number of sample and oxidant segments in the sequential insertion, 2–16. Chemical parameters were kept constant: 50 mg l^{-1} of hydroquinone; 2.5×10^{-3} mol l^{-1} for potassium permanganate; and, for oxidant and sample solutions the medium was 0.3 mol l^{-1} sulphuric acid. Once finished the MSM; the peaks from each assembly (total tested vertices, 8) showing the best compromise sensitivity (peak height)–reproducibility (R.S.D. in %) and sample throughput (base peak width) were pre-selected for a new test based on checking the repeativity of a calibration graph.

Further work with the pre-selected manifolds was leaded to minimise the consumption of strong oxidant solution per sample unit on the basis of either economic and environmental protection reasons. A new valve was added to flow-assemblies to circulate a water stream working as a "carrier" and "cleaning" solution between samples; in this form potassium permanganate was inserted into the manifold only during the sample insertion.

The selection of the depicted manifold in Fig. 1 finally resulted from a large series of assays in which the behaviour of calibration graphs were compared. The tested range of hydroquinone concentrations (in mg l^{-1} from 5.0 to 50.0 and 15 replicates of each of the four tested concentrations) revealed similar calculated relative standard deviations, comprised between 4.2 and 2.7%; however peak-heights and linear slopes were higher with the finally selected.

The finally selected flow assembly, comprised three parallel channels with a solenoid valve (see Fig. 1) and a single merging point; this confluence was nesting as close as possible to the flow-cell to minimise sample dispersion. The resulting manifold resulted in a series of alternated segments oxidant-sample to generate the analytical outputs and separated by a long segment of pure water. Parameters: flow-rate, 5.7 ml min^{-1}; total number of segments, 26 (formed by alternated sample-carrier segments, 13 and 13) and all segments 0.8 s length.

3.3. Influence of the organised media and sensitizers

The influence of tensoactive presence should be critical in emission procedures mainly due to protection of the fluorophore molecules from the environment and minimising the non-fluorescence decay [29]. The inserted sample solutions contained the tensoactive in the indicated concentration (see Table 1) plus 5.0 mg l^{-1} of hydroquinone.

The results presented in Table 1 compared the output from the pure hydroquinone with the obtained with hydroquinone and tensoactive. The hexadecylpiridinium chloride presented an output increase of 573% and, benzalkonium chloride of 757%, both compared versus the reference output. Then, to test the influence of their concentration resulted mandatory and it was studied by varying their concentration around the micellar critical concentration. After preparing calibration graphs for different concentrations of tensoactives, benzalkonium chloride was the selected.

On the other hand, relevance increase in the emission outputs can be obtained with the presence of sensitizers; compounds able to be excited by receiving the energy from the previously excited analyte [30]. Tested sensitizers can be seen in the Table 1, the assays were performed by preparing 5 mg l^{-1} of hydroquinone with the sensitizer. Several fluorophores were discarded: (a) by presenting a high blank signal: oxine, acridine orange, Rhodamine and Rhodamine B. (b) by inhibiting the analytical signal: dimethylformamide, acetonitrile and the mixture acetone/acetonitrile.

Best increases (in %) were presented by ethanol, 432; formic acid, 236; quinine sulphate 466; and sodium sulphite 110.

Calibration graphs from 1.0 to 15.0 mg l^{-1} of hydroquinone were obtained with the presence of a sensitizer. The obtained slopes were: 48.76 (ethanol 20%); 25.40 (5%

Table 1
Influence of the organised media and sensitizers

	Blank (KHz)	Output (KHz)	Output-blank (KHz)
Tensoactive			
Reference (no tensoactive)	0.0	26.7	26.7
Sodium dodecyl sulphate, 16 (mmol l^{-1})	0.3	35.2	34.9
Cethyl trimethylammonium bromide, 1.84 (mmol l^{-1})	0.4	69.7	69.3
Hexadecylpiridimium chloride, 2 (mmol l^{-1})[a]	1.4	181.2	179.8
Triton X-100, 0.48 (mmol l^{-1})	0.4	37.8	37.4
Benzalkonium chloride, 12 (mmol l^{-1})[a]	3.5	232.4	228.9
Tween 80, 0.02%	2.9	29.0	26.1
β-Ciclodextrine, 2 (mmol l^{-1})	0.3	37.9	37.6
Sensitizer			
Reference (no sensitizer)	0.0	26.6	26.6
Acetone, 0.5%	0.0	28.0	28.0
Acetone, 0.5% + acetonitrile, 20%[b]	0.1	1.9	1.8
Acetonitrile, 20%[b]	0.0	2.1	2.1
Ethanol, 20%	10.0	151.4	141.4
Dioxane, 5%	1.9	28.7	26.8
Sodium sulphite, 1.0×10^{-3} (mmol l^{-1})	0.0	55.9	55.9
Formic acid, 5%	0.9	90.3	89.4
Quinine sulphate, 5.0×10^{-4} (mmol l^{-1})	12.5	163.0	150.5
Oxine 5.0×10^{-4}, (mmol l^{-1})[a]	622.9	811.9	189.0
Dimethylformamide, 5%[b]	0.84	19.8	18.9
Acridine orange, 5×10^{-4} (mmol l^{-1})[a]	169.3	288.3	119.0
Rhodamine 6G, 5×10^{-4} (mmol l^{-1})[b]	1668.4	1555.2	−113.3
Rhodamine B, 5×10^{-4} (mmol l^{-1})[a]	2437.1	2675.6	2437.1

[a] Sensitizers showing high blank signals.
[b] Its presence resulted in inhibiting the chemiluminescence emmission.

formic acid); 35.39 (5.0×10^{-4} mol l^{-1} quinine sulphate); and, 17.52 (1.0×10^{-3} mol l^{-1} sodium sulphite). Further selection was based on the influence of the concentration of ethanol and quinine sulphate by performing the calibration graph (1.0–15.0 mg l^{-1} of hydroquinone) with the presence of one of these sensitizers; and, then with the sensitizer and benzalkonium chloride. Ethanol was assayed in concentrations 10, 20 and 30%; and, the quinine in 1×10^{-5}, 1×10^{-4} and 5×10^{-5} mol l^{-1}. Sensitivity (higher slope) was slightly better for ethanol (especially at 20%) assays but it generates some bubbles; it should be worse for higher temperature in further studies. Finally, the mixture quinine sulphate and benzalkonium chloride was selected; as the quinine concentration increased both outputs, the analytical and the blank, a last test was performed and it revealed the 5×10^{-4} mol l^{-1} quinine sulphate and 10 mmol l^{-1} benzalkonium chloride as the suitable concentrations.

3.4. Study on the related influence flow-rate and temperature

The influence of temperature is very important due to the double action, on the chemiluminescence emission and on the kinetics of the oxidation reaction [31]. The last action is critical; the light should be completely emitted into the flow-cell, which in turn means the relevance of the flow-rate to avoid the chemiluminescence reaction before or after the flow-cell according to variation of the reaction speed with the variation of the temperature. Preliminary experiments resulted in an increase of the outputs at higher temperatures (about 12% increase at 60 °C from room temperature) however, further temperature increases showed very small output increase. The close relation temperature-flow rate is illustrate with the example at 60 °C (flow-rate in ml min^{-1}, peak height in Hz and R.S.D. in %): 1.7, 345.4, 3.7; 3.7, 740.0, 2.0; 5.6, 1139.2, 2.7; and, 7.8, 1577.7, 2.1.

3.5. Final optimisation of empirical parameters

As a final step of the sequential optimisation process the MSM was applied. The studied parameters and corresponding intervals were as follows: flow rates of the water-carrier, sample and oxidant, 3.0–7.8 ml min^{-1}; potassium permanganate and sample segment lengths, 0.1–0.8 s; number of the sample and oxidant segments, 5–16 s; concentration of quinine sulphate, 0.001–1.0 mmol l^{-1}; concentration of benzalkonium chloride, 6.0–14.0 mmol l^{-1}; sulphuric acid concentration, 0.1–1.5 mol l^{-1}; potassium permanganate concentration, 0.01–10 mmol l^{-1}; and, the temperature, 20–80 °C.

According to reported optimisation methodology (see procedures) several simplex were performed by restricting the new tested ranges to the apparently most suitable range. Further re-optimisation research on the variation of influ-

Table 2
Optimal parameters for the FIA-chemiluminometric determination of hydroquinone

Parameter	Studied interval	Selected value
Flow-rate (ml min^{-1})	3.0–7.8	7.5
Oxidant segment (s)	0.1–0.8	0.3
Sample segment (s)	0.1–0.8	0.5
Number of oxidant segments	7–15	11
Number of sample segments	7–15	11
Concentration of quinine (mmol l^{-1})	0.1–1.0	0.5
Concentration of benzalkonium (mmol l^{-1})	7.0–14.0	10.0
Concentration of sulphuric acid (mmol l^{-1})	0.5–1.5	1.0
Concentration of permanganate (mmol l^{-1})	0.5–10.0	1.0
Temperature (°C)	40.0–80.0	60.0

ence of the pair flow-rates/temperature, demonstrate it was possible to obtain an interesting increase of the peak height (over 20%) and the slope of the calibration graph. Two calibration graphs at 60 °C and flow-rates 7.5 and 9.4 ml min^{-1}, resulted in slopes 204.15 and 223.16, respectively. To avoid overpressures in the routinely work, the flow-rate proposed but not the maximum tested (9.4 ml min^{-1}), was 7.5 ml min^{-1}. Final parameters are summarised in Table 2.

3.6. Analytical figures of merit

With the optimised manifold, the linear dynamic ranged from 0.1 to 15 mg l^{-1}; fitting the equation, $I = 192x - 11.75$; with correlation coefficient of $r^2 = 0.9989$; x meaning the drug concentration in mg l^{-1} and I the emission intensity in KHz units. The limit of detection, 0.03 mg l^{-1}, was experimentally confirmed as the concentration yielding a signal higher than the corresponding to the blank plus two times the standard deviation of the blank (n 30).

Sample throughput (h^{-1}) and repeativity (% R.S.D.) resulted in 103–2.60% and 103–1.98%, for 0.5 and 10 mg l^{-1} of the drug, respectively. The reproducibility obtained by preparing six independent calibration graphs resulted in an average slope of 192 and its reproducibility was (as R.S.D. in %) of 2.9.

The chemical robustness of the method is relevant in chemiluminescence due to influence of small amount of impurities present in reagents. Reagent solutions were prepared from two different manufacturers (one for benzalkonium chloride) obtaining 16 different combinations. The reproducibility studies were performed on 2.0 and 10.0 mg l^{-1} of hydroquinone (15 replicates each) and the obtained peak-heights were compared with the average of

Table 3
Influence of foreign compounds: Prp., precipitate

Interferent	mg l^{-1}	%	Interferent	mg l^{-1}	%
β-Ciclodextrine	102.0[a]	3.6	Acetate (CH_3COONa)	1000.2[a]	0.5
Acetone 15%	150.000[a]	2.7	Carbonate (Na_2CO_3)	502.2	3.1
Ascorbic acid	5.0	3.7	Chloride (NaCl)	999.8[a]	1.4
Citric acid	1.0	3.2	Phosphate (H_2PO_4K)	999.9[a]	−2.0
Glycolic acid	1.0	3.5	Nitrate ($NaNO_3$)	1199.4[a]	−2.7
Retinoic acid (Vitamin A)	10.1	0.5	Nitrite ($NaNO_2$)	0.5	−1.6
Aloe vera	121.8[a]	0.2	Sulphate (Na_2SO_4)	499.9	3.1
EDTA$(Na)_2$	1.0	3.1	Sulphite (Na_2SO_3)	0.5	3.9
Ethanol	50.0	2.0	Al ($Al_3(NO)_3{\cdot}9H_2O$)	49.9	−1.9
Glicerine	5.0	−0.2	NH_3 (NH_4Cl)	60.0	2.9
Lauryl sodium sulphate	100.0[a]	0.7	Ba ($Ba(NO_3)_2$	100.0	Prp.
Sodium metabisulphite	0.1	0.2	Cd ($Cd(NO_3)_2$)	50.0	−2.7
Methylparaben	0.1	−0.0	Ca ($Ca(NO_3)_2{\cdot}3H_2O$)	503.0[a]	2.9
NaCl	102.0[a]	2.0	Co ($Co(NO_3)_2{\cdot}6H_2O$)	50.1	−1.1
NH_4OH	100.0[a]	−2.1	Cu ($Cu(NO_3)_2{\cdot}3H_2O$)	100[a]	0.8
Propylenglicol	5.0	1.1	Cr ($Cr(NO_3)_3{\cdot}9H_2O$)	100.9[a]	2.6
Propylparaben	0.1	0.5	Fe ($Fe(NO_3)_3{\cdot}9H_2O$)	20.0	−0.7
Sodium sulphite	0.5	3.9	Mg ($Mg(NO_3)_2{\cdot}6H_2O$)	200.3[a]	−0.3
Sulisobenzone	0.1	3.4	Mn ($Mn(NO_3)_2{\cdot}6H_2O$)	55.1	3.7
Trietanolammine 99%	101.4[a]	2.5	Hg ($Hg(NO_3)_2{\cdot}H_2O$)	99.4[a]	−1.9
Urea	100.1[a]	0.0	Ni ($Ni(NO_3)_2{\cdot}6H_2O$)	98.6[a]	0.4
Potassium bromide	11.5	−0.9	Ag ($AgNO_3$)	100.0	Prp.
Sodium metaborate	48.7[a]	−1.0	Pb ($Pb(NO_3)_2$)	100.0	Prp.
Na_2CO_3	502.2	3.1	K (KNO_3)	315.4[a]	3.0
NaOH	500[a]	2.2	Na ($NaNO_3$)	444.7[a]	−2.7
Metol	0.05	5.7	Zn ($Zn(NO_3)_2{\cdot}6H_2O$)	99.3[a]	3.3
Sodium sulphite (2)	52.1[a]	0.87			
Sodium tetraborate	55.7[a]	−2.3			

Concentration of hydroquinone 1 mg l^{-1}.

[a] Maximum assayed concentration; Interference of methol (polyphenol) due to its own chemiluminescence methol. Sulphite interference eliminated through heating in acid media resulted in errors under 1.0% for concentration higher than 50.0 ppm. Prp., precipitate.

Table 4
Analysis of different type of samples

Pharmaceutical formulation	Flow-method (R.S.D. %)	Reference method (R.S.D. %)	Label claim	Deviation vs reference method (%)	Deviation vs label claim (%)
Licostrata[a] Gel (g/100 g)	2.13 (0.9)	2.09 (1.0)	2.00	1.9	6.5
Nadona[a] Crema (g/100 g)	2.02 (4.3)	2.13 (5.4)	2.00	−5.1	1.0
Sample	Amount of hydroquinone (sample content or added) ($mg\,l^{-1}$)	Flow-method (R.S.D., %) ($mg\,l^{-1}$)	Deviation (%)		
Photographic solution D8[a]	4.5	4.7 (1.0)	4.4		
Irrigation water channel (Puig)	5.5	5.3 (3.5)	−3.6		
Irrigation water channel (Alboraya)	5.5	5.4 (0.9)	−1.8		

[a] Commercially available product.

the obtained with the solutions used in the work and calculating the "relative error" in %. These "errors" ranged from 0.4 to 4.6, and 0.2–5.1 for 2.0 and 10 $mg\,l^{-1}$ of hydroquinone, respectively; two exceptions were found out of the reported ranges, with values 11.2 and 9.8%.

Interferences were sought among some of the usual excipients and active principles in pharmaceutical formulations containing hydroquinone or usual ingredients of the other tested samples. Results are shown in Table 3.

The determination of hydroquinone was performed on different type of samples; namely, pharmaceutical formulations, a photographic developer solution and irrigation and residual superficial waters; the last by spiking the drug in the sample. See Table 4. The results of the pharmaceutical formulations were compared with the obtained from the reference method recommended by the USP [7]; this method is based on the absorbance measurements at 293 nm in methanol medium. The presence of sodium sulphite in the photographic developer solution resulted in a serious increase of the chemiluminescence output. The sulphite was previously eliminated by heating in acidic medium.

The method was also applied to six polyphenols with pharmacological activity by using the same manifold optimised for the hydroquinone as a tests substance. The assayed pharmaceuticals were (name, activity, average slope (R.S.D., %) and limit of detection).

- Resorcinol, anti seborrheic and keratolitic: 237.99, (1.15), and 10.0 $\mu g\,l^{-1}$.
- Floroglucinol, anti spasmodic, 160.45, (2.20) and 10 $\mu g\,l^{-1}$.
- Catechol, antiseptic; 101.58, (3.41) and 40 $\mu g\,l^{-1}$.
- 4-Hydroxibenzoic acid, anti bacterial and anti fungic; 91.56, (1.00) and 20 $\mu g\,l^{-1}$.
- Quercetine, hair protector, 108.98, (1.91) and 20 $\mu g\,l^{-1}$.
- Eugenol, dental analgesic and anti spasmodic, 49.71, (2.46), and 80 $\mu g\,l^{-1}$.

4. Conclusions

The flow-chemiluminometric determination of hydroquinone is described for first time. The method is quick, 103 h^{-1} and shows competitive sensitivity and detection limit, 30 $mg\,l^{-}$As it is based on the tandem-flow methodology is widely automated. The method has been applied to other six polyphenols showing pharmaceutical activity. Finally, the large series testing the influence of foreign compounds demonstrated an interesting selectivity. The method has been applied to different types of samples.

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