Video approach to chemiluminescence detection using a low cost complementary metal-oxide-semiconductor (CMOS) based camera: determination of paracetamol in pharmaceutical formulations.

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Abstract

A new system for continuous flow chemiluminescence detection, based on the use of a simple and low-priced lens-free digital camera (with Complementary Metal Oxide Semiconductors technology) as detector, is proposed for the quantitative determination of paracetamol in commercial pharmaceutical formulations. Through the camera software, AVI video files of the chemiluminescence emission are captured and then, using friendly ImageJ public domain software (from National Institutes for Health), properly processed in order to extract the analytical information. The calibration graph was found to be linear over the range 0.01-0.10 mg L^{-1} and over the range 1.0-100.0 mg L^{-1} of paracetamol, being the limit of detection of 10 μg L^{-1}. No significative interferences were found. Paracetamol was determined in three different pharmaceutical formulations: Termalgin®, Efferalgan® and Gelocatil®. The obtained results compared well with those declared on the formulation label and with those obtained through the official analytical method of British Pharmacopoeia.

Keywords Flow chemiluminescence, CMOS camera, ImageJ, Pharmaceutical analysis.

Introduction

Chemiluminescence (CL) is usually defined as the production of electromagnetic radiation as a result of a chemical reaction. One of the reaction products is in an excited state and emits light on returning to its ground state. The process by which chemiluminescence is generated is the same as that for photoluminescence (fluorescence and phosphorescence) except that the former requires no light excitation source. The analytical interest of CL lies in the ability to produce molecules that emit light without prior irradiation, which avoids problems arising from stray light, unselective excitation and instability of the light source. Moreover, the advantages of CL analysis include low limits of detection and a wide linear range. Analytical applications of liquid-phase chemiluminescence have increased considerably over the last three decades and, up to date, the analytical literature published a number of applications for the determination of both, inorganic and organic compounds in a variety of industrial, clinical, biotechnological and environmental matrices. Chemiluminescence methods have been also frequently used in recent years for the analysis of pharmaceutical compounds [1-7].

Chemiluminescence is usually measured either in (a) static mode or (b) continuous-flow mode. In the first one, a syringe is employed to deliver portions of sample and chemiluminescent reagent(s), which are mixed in a cell housed in a luminometer chamber located in front of the detector window. From the obtained CL curve (variation of the chemiluminescence intensity with the reaction time), different parameters (height, area, profile width, decay rate) can be related to the analyte concentration. In the second mode, analyte and chemiluminescent reagent(s) are continuously flowing through different channels before merging (Fig. 1). Chemiluminescence intensity is then measured at a fixed position beyond the merging point, where a flow cell is placed in front of the detector window. The flow rates and the volume between the merging point and the flow cell position are the parameters which determine the reaction time and so the arising CL intensity [2].

Thus, the essential components of a flow chemiluminescence detection system are the reagent mixing chamber, the flow-cell, the photodetector (frequently a photomultiplier tube or PMT) and an electronic data acquisition and processing software. Many instruments designed for other purposes include all these components and can thus be used for chemiluminescence measurements. In fact and in order to measure CL emission intensity, it has been a common practice in analytical chemistry to use suitably modified ordinary instruments, such as a spectrofluorimeter, where the emission monochromator has been replaced with an appropriate device (a parabolic mirror) to reflect the whole emitted light by the CL reaction through the PMT. Moreover, CL measures can also be generally achieved with simple, robust and often home-made PMT based instrumentation [2, 6, 7].

Besides photomultiplier tubes, which are relatively expensive devices, other usually employed detection systems for CL are silicon photodiodes and in the last decades, the increasing technical improvement and affordability of digital image-based systems hardware and software, have promoted the use of charge-coupled devices (CCD) and complementary metal oxide semiconductors (CMOS) as suitable detectors in CL analytical methods as well as in other kinds of quantitative chemical analyses [8-12].

Digital cameras capture images by means of CCD or more frequently CMOS sensors. Each cell (pixel or photosite) in a CCD or CMOS acts as a light-sensitive individual element providing an electrical response that can be digitized to build an optical image. A black and white (B&W) sensor consisting of 8-bit pixels can respond to $2^8 = 256$ levels of grey ranging from 0 (black) to 255 (white). This allows each pixel in a captured image to be assigned a numerical value from 0 to 255 (grey level or analytical signal) which can be subsequently used for analytical calibration. As a result, a digital image-based system can be used as a suitable analytical detector, since each captured image provides a vast amount of information [8-12].

Both technologies (CCD and CMOS) are intended for taking images digitally with several similarities and differences in the signal processing, performance and cost. Both are pixelated metal oxide semiconductors which convert light into electrical charge and then to electrical signals. The main difference is that each pixel of a CCD transfers charge as an analog signal to be converted to voltage outside the pixel, so it needs an external analog-to-digital converter, while a CMOS has its own charge-to-voltage conversion system integrated within each pixel, being its output a digital signal. There has been debate among pros and cons of the two systems and until recent years CCD has been more widely adapted for use as detectors, perhaps owing to its earlier and more mature development. Nevertheless, although theoretically CCD images are superior, CMOS works faster and can be produced at cheaper rate. In fact, owing to its advantages over CCD, CMOS are being nowadays massively employed as detectors in all kind of digital image-based systems [9, 10].

There is an extensive market offer of such technology at very affordable prices and different types of commercial CCD or CMOS digital image-based systems such as digital cameras, mobile phone cameras, desktop scanners or even webcams have been described for use as an alternative detector unit for chemical or biochemical analysis systems, especially when analysis are based on colour changes [10]. The digital image-based detectors can replace the more expensive UV-VIS, fluorescence and chemiluminescence spectrophotometers. For instance, a webcam is a very common low cost CMOS based device broadly used in computer/internet applications and its application for immunoassay microplate reading or fluorescence detection has been yet reported, though so far the use of a webcam as an analytical detector is still infrequent [13, 14].

The analytical applications of the liquid-phase chemiluminescence have been [2] classified into (a) direct methods, those where the light emission arouses from the analyte oxidation and (b) indirect methods, those where the analyte usually playing a different role in the CL emission (e.g. reagent, catalyst, quencher, enhancer) and which involve well known chemiluminescence emitters, being luminol, acridinium salts, oxalate esters, dioxetanes and tris(2,2´-bipyridyl)ruthenium(II) the most usually employed reagents in this kind of analytical applications [1-7].

In liquid phase (aqueous solutions), luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) is the most usually employed CL reagent [15]. Luminol reacts in alkaline solution with a strong oxidizing agent such as hydrogen peroxide, hexacyanoferrate (III), permanganate, N-bromosuccinimide or periodate in the presence of a catalyst (usually a metal or metal-containing compound or an enzyme). The redox reaction yields 3-aminophthalate in an excited electronic state, which returns to the ground state with the emission of visible light (CL) around 425nm. Luminol-based systems [1-7, 15] have been broadly exploited in the

quantitative analysis of numerous compounds (including pharmaceuticals), because of its relatively high quantum efficiency (quantum yield) and susceptibility of its CL mechanism to a vast number of compounds. In fact, in luminol-based procedures, the analyte itself usually plays either as inhibitor or enhancer of the CL emission, making possible to correlate the analyte concentration vs the intensity of CL emission (analytical signal).

Here we propose the development of new system of continuous-flow CL detection, for the quantitative determination of paracetamol in pharmaceutical formulations, based on the use of a simple and low-priced CMOS lens-free digital black and white camera.

As far as the authors know, this is the first attempt to employ a lens-free imaging (CMOS) camera to monitor a chemiluminescent signal in a flow system by recording video to obtain the analytical signal and applying it to the quantitative determination of a drug substance (paracetamol) in commercial pharmaceutical formulations.

Experimental

Materials

All reagents used were analytically pure. $K_3[Fe(CN)_6]$, H_2O_2 (30% w/v; 100 vol.), NaOH, NH_4Cl, NH_3, Na_2HPO_4, Na_2CO_3, borax, ascorbic acid, citric acid anhydrous, sodium benzoate, sorbitol, sulphuric acid, hydrochloric acid, ammonium cerium sulphate and ferroin from Panreac (Castellar del Vallés, Barcelona, Spain). Acetylsalicylic acid, caffeine, hydroxypropyl methylcellulose, magnesium stearate, methocarbamol, microcrystalline cellulose, polyvinylpyrrolidone, saccharin, silicon dioxide, stearic acid, talc and wheat starch from Guinama (La Pobla de Vallbona, Valencia, Spain). Starch from Merck (Darmstadt, Germany). Codeine phosphate from Uquifa (Barcelona, Spain). Luminol from Fluka (nowadays Sigma-Aldrich: St. Louis, Missouri, USA).

Solutions were prepared from water purified by reverse osmosis, de-ionized to 18 MΩ·cm with a Sybron/Barnstead Nanopure II water purification system furnished with a fibre filter of 0.2 μm pore size.

The signal (AVI file) was taken with a Luna-QHY 5 lens-free digital B&W camera (equipped with a monochrome CMOS MT9M001 from Micron: Manassas, Virginia, USA), usually intended for astronomical purposes, from Lunático Astronomía: Torrelodones, Madrid, Spain (http://tienda.lunatico.es/). An integration time of 1000 ms and a gain of 50 were employed. The obtained CL signal was processed with the public domain software ImageJ (v 1.43g), Windows version, developed by the National Institutes for Health and available for free download at http://rsbweb.nih.gov/ij.

The finally proposed flow manifold (Fig. 2) consisted of a peristaltic pump (Gilson Minipuls 2; Middleton, WI, USA) which pumped paracetamol (Flow-rate 1, 1.2 mL min^{-1} of paracetamol aqueous solution), hexacyanoferrate(III) (Flow-rate 2, 1.2 mL min^{-1} of 1.4 x 10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ aqueous solution), hydrogen peroxide (Flow-rate 3, 2.0 mL min^{-1} of 1.0 x 10^{-2} mol L^{-1} H_2O_2 aqueous solution) and luminol (Flow-rate 4, 4.3 mL min^{-1} of 1.0 x 10^{-3} mol L^{-1} luminol and 0.1 mol L^{-1} NaOH solution) through PTFE tubes (0.8 mm internal diameter). Finally, all channels merged in a T shaped piece positioned 2 cm before entering the flow cell, which consists on a flat spiral-coiled quartz tube (1.0 mm internal diameter,

3 cm total diameter of the flow cell, without gaps between loops). The flow cell was backed by a mirror for maximum light collection and placed 2 mm from the CMOS. The T-piece, flow cell and camera were placed in a home-made, absolutely light-tight box. The output was fed to a computer equipped with the specific software (QGSoftware) also supplied by Lunático Astronomía, Spain (http://tienda.lunatico.es/).

Methods

Each session work, a video file of the generated chemiluminescence was recorded through the CCD software and saved as a VideoFile.avi.

All the obtained video files were processed with the software ImageJ. The processing sequence (4 steps) was as follows (operation A>B>C means select command B on menu A and then select subcommand C in command B):

1: File>Open>VideoFile.avi

2: Edit>Selection>Select All

3: Plugins>Stacks-T-functions>Intensity v Time Monitor>List

4: File>Save as>Plot Values.xls

The generated data (Plot Values.xls file) was finally imported into Microsoft Excel for statistical analysis. Bearing on mid the 8 bits nature of the CMOS, the signal was always comprised and so expressed between 0 (total inhibition) and 255 (maximum CL emission: no inhibition) arbitrary units (a.u.).

The results were compared with those obtained through the official methods of British Pharmacopoeia [17]. The official procedure is based on the drug solution in sulphuric acid (1 mol L^{-1}) followed by boiling (reflux) for 1 hour and a final titration (in HCl, 2 mol L^{-1}) with ammonium cerium sulphate with ferroin as indicator.

Results and discussion

The main goal of the experimental work was the development of new system of continuous-flow CL detection, for the quantitative determination of paracetamol in pharmaceutical formulations, based on the use of a simple and low-priced CMOS lens-free digital black and white camera (initially intended to obtain telescopic astronomical images), which has the advantage of being even more robust and simpler than a webcam because it do not have optical systems in front of CMOS sensor, allowing to easily place the spiral CL flow cell in front of the detector. Through the camera software, a number of images per second are captured when the reagents reach the flow cell and the CL emission is produced and then, the obtained AVI video file is properly processed in order to extract the analytical information by using ImageJ, public domain software, designed and freely distributed by NIH, which yields the intensities (mean grey level) of each image (frame) in the captured video sequence. The data generated by means of ImageJ are then imported into Microsoft Excel (Microsoft, Redmond, WA) becoming the register of the CL analytical signal.

The presented procedure is based on procedure published by one of the authors [16], where the chemiluminescence arising from the well-known chemiluminescent system luminol-H_2O_2-$Fe(CN)_6^{3-}$, is employed. The method is based on the oxidation of paracetamol by hexacyanoferrate (III) and their subsequent inhibitory effect on the reaction between luminol and hydrogen peroxide (produced by de consumption of the hexacyanoferrate (III)), which allows relating the CL intensity (analytical signal) with the paracetamol (analyte) concentration.

Bearing in mind that CL signal intensity is time-depending, all the work was performed, instead of in a batch mode, by means of a continuous flow manifold, where the time of measurement after mixing of solutions is accurately controlled by the flow rates.

Instrumental parameters (Luna-QHY 5 CMOS based camera) were initially maintained in its native value, namely: Integration time, 1000 ms and Gain, 50.

All experiments were conducted in the four-channel configuration of Fig. 2. This was the simplest continuous flow manifold that allowed individual solutions of the three reagents and the analyte to be used. Also, it ensured that the chemiluminescent reaction started after the last T-junction. In order to establish the conditions required to obtain nearly but not completely saturated chemiluminescence (maximum empirical intensity near but below 255 a.u. to be able to use a wide enough inhibition range on the y-axis), the study and optimization of chemical and continuous flow variables was performed by using a univariate method.

Optimization of flow, chemical and CMOS parameters

First and pumping deionized water by channel 1, we studied the effect of the concentrations of the three reagents (luminol, hexacyanoferrate (III) and hydrogen peroxide). Bearing in mind the former work [16], the concentrations were studied in the next intervals: luminol, 1.0×10^{-4} – 1.0×10^{-2} mol L^{-1} (in NaOH 0.05 M); hexacyanoferrate (III), 1.0×10^{-3} – 1.0×10^{-1} mol L^{-1} and hydrogen peroxide, 1.0×10^{-4} – 1.0×10^{-2} mol L^{-1}. The concentrations adopted were: 1.0×10^{-3} mol L^{-1} luminol (in NaOH 0.05 M); 1.0×10^{-2} mol L^{-1} of hydrogen peroxide (see Fig. 3a) and, in order to obtain nearly but not completely saturated chemiluminescence, 1.4×10^{-3} mol L^{-1} hexacyanoferrate (III) (see Fig. 3b).

The influence of the pH and the nature of alkaline media employed to dissolve luminol was then studied between pH 8.91 and 12.35 by using NH_4Cl/NH_3 (pH from 8.91 to 10.05), Borax/NaOH (pH from 9.52 to 11.20), Na_2HPO_4/NaOH (pH from 11.25 to 11.72) and NaOH (pH 12.35), being NaOH the medium which yield the maximum signal and thus selected for further work (Fig. 4).

The NaOH concentration was then optimized by studying values comprised over the range 0.05 – 0.25 mol L^{-1} (measured pH between 11.51 and 12.71, respectively). The highest useful analytical signal (higher concentrations saturated the detector response with analytical signal over 255 a.u.) was obtained at pH 12.50 (NaOH 0.1 mol L^{-1}), which was chosen for subsequent experiments.

Then flow rates were optimized by studying values of the variables over the range 1 – 4.5 mL min^{-1}. Attending to the maximum analytical signal, the chosen conditions were $Q_1 = Q_2 = 1.2$ mL min^{-1}; $Q_3 = 2.0$ mL min^{-1}; $Q_4 = 4.3$ mL min^{-1} which yield a signal of 251 a.u.

Next, the influence of paracetamol concentration over the CL signal (inhibition) was tested at concentrations ranging from 0.01 to 100 mg L^{-1}. The first one showed an inhibition of 4 a.u. whereas 100 mg L^{-1} showed an inhibition of 235 a.u.

The influence of the reactor lengths on the paracetamol oxidation rate (See Fig. 2, L_1 and L_2) were then studied over the range = 10 – 150 cm. Testing different paracetamol concentrations ranging from 0.01 to 100 mg L^{-1}, no differences were observed, showing that at the previously established flow rates, the reactor lengths have no influence over the reaction rate. So, initial values of L_1= L_2 = 50 cm were maintained.

Once continuous flow variables were optimized, chemical variables were re-optimized. Significant differences were not observed so chemical variables were maintained in the previously optimized values.

Finally, the influence of instrumental parameters was also studied. Different values of the integration time and gain of the CMOS camera were tested in the ranges 0 – 5000 ms and 0 – 100, respectively. Obviously, to obtain the same value of CL signal, high gain values needed shorter integration times; however, the signal had higher noise, being 3% the RSD of the blank signal. On the other hand, longer integration times needed lower gain, and the noise was lower (RSD of the blank signal, 0.1%); nevertheless, at lower gain values, low concentrations of paracetamol (below 0.1 mg L^{-1}) were not detected. Consequently, an integration time of 1000 ms and a gain of 55 were finally selected as the best compromise between signal noise (RSD of the blank signal, 0.3%) and a lower limit of detection.

Analytical figures of merit.

Limit of Detection

Considering the inhibition of the CL emission (Ih, in arbitrary units, a.u.) as the analytical signal, the limit of detection was empirically determined by testing diluted solutions of paracetamol until 10 μg L^{-1}, which was experimentally confirmed as the concentration yielding a signal (2.8 a.u.) higher than the corresponding to the blank (0.0 a.u.) more three times the standard deviation of the blank (0.8 a.u.). The obtained limit of detection (10 μg L^{-1}) shows that it is possible to detect paracetamol even in very diluted samples.

Calibration graph

The calibration graph was found to be linear over the range 0.01 – 0.10 mg L^{-1} and over the range 1.0 – 100.0 mg L^{-1} of paracetamol concentration (Fig. 5). For the first range, the regression equation was Log (Ih) = 1.82 + 0.690 Log(C) (where C is the paracetamol concentration in mg L^{-1}) and the correlation coefficient (N=5) was 0.9999. For the second range, the regression equation was Log (Ih) = 1.70 + 0.270 Log(C) (where C is the paracetamol concentration in mg L^{-1}) and the correlation coefficient (N=5) was 0.9995. Double logarithmic scale was employed in order to obtain a linear relation between Ih and C. The obtained correlation coefficients (both higher than 0.999) show a good linear behaviour in both studied paracetamol concentration ranges.

Reproducibility

The between day reproducibility study was performed working each day (5 days) with freshly prepared solutions, being the paracetamol concentrations (five values for each range) comprised in the range 0.01 – 0.10 mg L^{-1} and the range 1.0 – 100.0 mg L^{-1}. For the first range, the mean (N=5) slope obtained was 0.686, with an RSD of 4.1% and mean correlation coefficient of 0.9970 (RSD of 0.8%). For the second range, the mean (N=5) slope obtained was 0.282, with an RSD of 2.2% and mean correlation coefficient of 0.9993 (RSD of 0.3%).

Foreign compounds

The influence of foreign compounds and excipients that may be found in pharmaceutical formulations containing paracetamol was also studied. Synthetic solutions containing 1.0 mg L^{-1} of the analyte and different amounts of foreign substances (up to 100 mg L^{-1} or saturated solution) were measured. The relative errors (%) were calculated by comparing the analytical signal with that obtained by testing a solution of pure paracetamol at the same concentration (1.0 mg L^{-1}). The obtained results are depicted in Table 1. The obtained data shows no significant influence of the studied foreign compounds; therefore,

the proposed method shows a relevant selectivity and can be used for the determination of paracetamol in samples containing those compounds, without previous separation steps.

Assay of real samples

Paracetamol was determined in three different pharmaceutical formulations:

Termalgin® (tablets, from NOVARTIS): paracetamol 500 mg.
Efferalgan® (tablets, from BRYSTOL-MYERS SQUIBB): paracetamol 500 mg.
Gelocatil® (tablets, from GELOS): paracetamol 650 mg.

The results (average of three determinations) were compared with those declared on the formulation label and with those obtained through the official methods of British Pharmacopoeia [17]. The official procedure is based on the drug titration with ammonium cerium sulphate with ferroin as indicator. Table 2 shows the obtained results (average recovery ± 95 % CI and % RSD); it is possible to affirm that the proposed method provides good results, perfectly comparable with those declared by the manufacturers and with those obtained with the official method (the proposed method yields even better results than the official one, especially for the Gelocatil formulation).

Conclusions

A low-priced device like CMOS lens-free digital B&W camera, specifically intended for astronomical imaging purpose, shows to be useful to detect and to measure CL emission intensity. The proposed camera has the advantage of being more robust and simpler than other kind of digital devices, because it do not have optical systems in front of CMOS sensor, allowing to easily place the spiral CL flow cell in front of the detector. The fundamental handicap could be the relatively low sensitivity of the CMOS sensor (versus PMTs or cooled CCDs), which only could be used to measure CL arising from high quantum yield systems. Nevertheless, bearing in mind the vast number of applications of such systems [1-7, 15] there are a lot of potential uses of CMOS sensors in chemiluminescence detection.

Moreover, in this case, the performance of the new proposed method compares very well with the previous one [16] bearing on mind that there, the calibration graph inhibition-concentration was found to be linear over the range 2.5-12.5 mgL^{-1} of paracetamol (correlation coefficient of 0.997, N=6) and the limit of detection was 2.1 mgL^{-1} of analyte.

Through the camera software, AVI video files of the CL emission are captured and then, using friendly ImageJ public domain software (by NIH), properly processed in order to extract the analytical information. The data generated by means of ImageJ are then imported into Microsoft Excel (Microsoft, Redmond, WA) becoming the register of the CL analytical signal.

In addition to competitive accuracy, precision and sensitivity, the new proposed procedure shows a relevant selectivity which allows analysing the proposed samples without separation steps of the chromatography-based methods. The obtained results for the tested pharmaceuticals, compares well with

those declared by the manufacturer and with the obtained by the official (BP) method and it could be emphasized than the proposed method yields better results than the official one, especially for the Gelocatil formulation. On the other hand, the new procedure, opposite to the titrimetric official procedure, has all the advantages arising from continuous flow methodology and could be easily exported to other continuous flow analytical methodologies like FIA, SIA or HPLC.

Finally, it could be emphasized that the proposed method can be especially advantageous for scenarios where available resources for analytical equipment investments are scant.

Competing interests

The authors declare that they have no competing interests.

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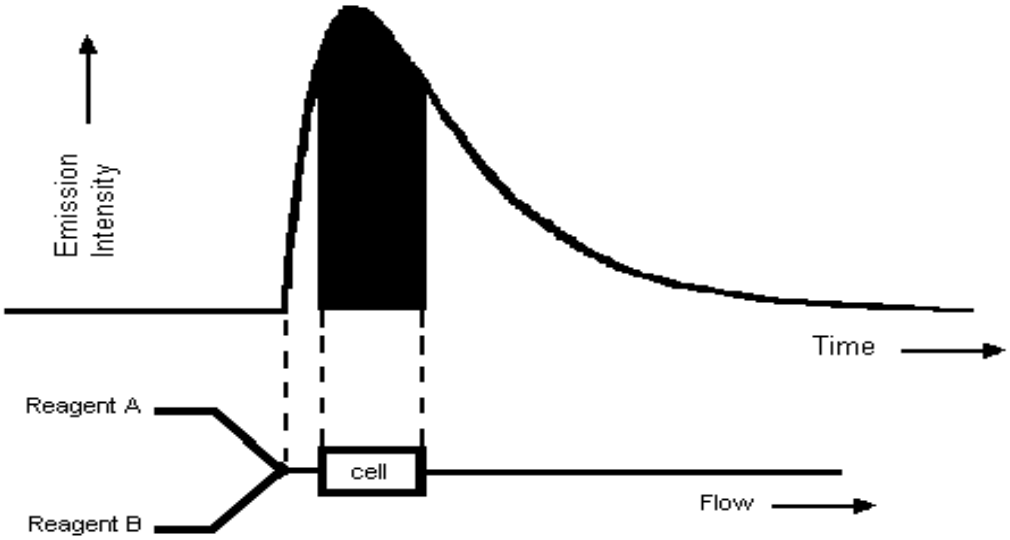

Fig. 1 Position of observation window relative to emission-time curve for flow-through chemiluminescence measurements. Hatched region under the curve represents the detected portion of the emission.

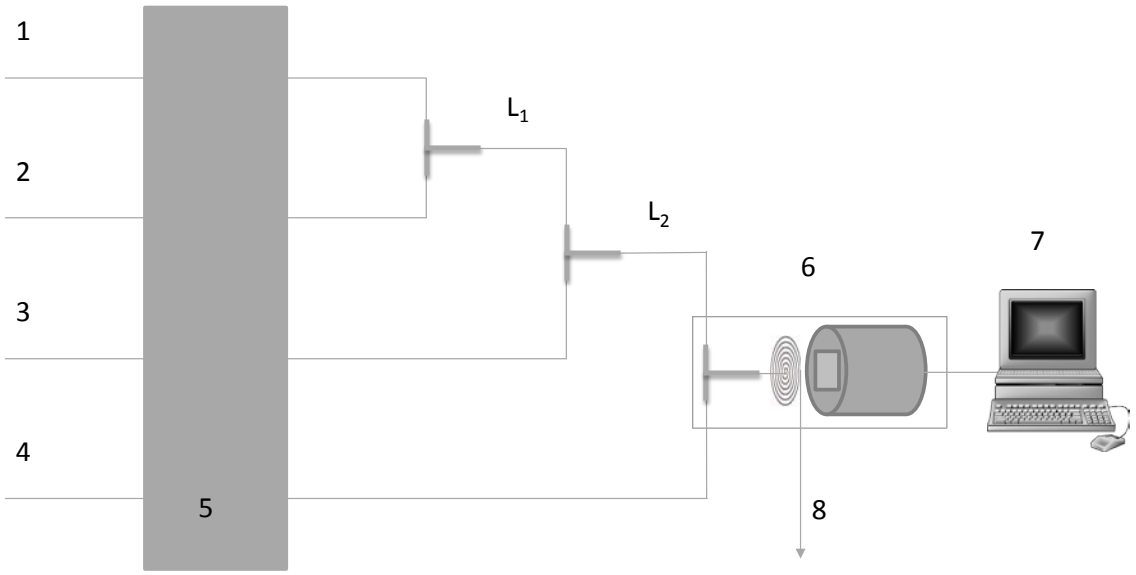

Fig. 2 Proposed continuous flow assembly for paracetamol determination. (See text). 1, paracetamol (1.2 mL min^{-1} of paracetamol aqueous solution); 2, hexacyanoferrate (III) (1.2 mL min^{-1} of 1.4 x 10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ aqueous solution); 3, hydrogen peroxide (2.0 mL min^{-1} of 1.0 x 10^{-2} mol L^{-1} H_2O_2 aqueous solution); 4, luminol (4.3 mL min^{-1} of 1.0 x 10^{-3} mol L^{-1} luminol and 0.1 mol L^{-1} NaOH solution); 5, peristaltic pump; 6, light-tight box containing the spiral flow cell and the CMOS camera; 7, computer and 8, waste.

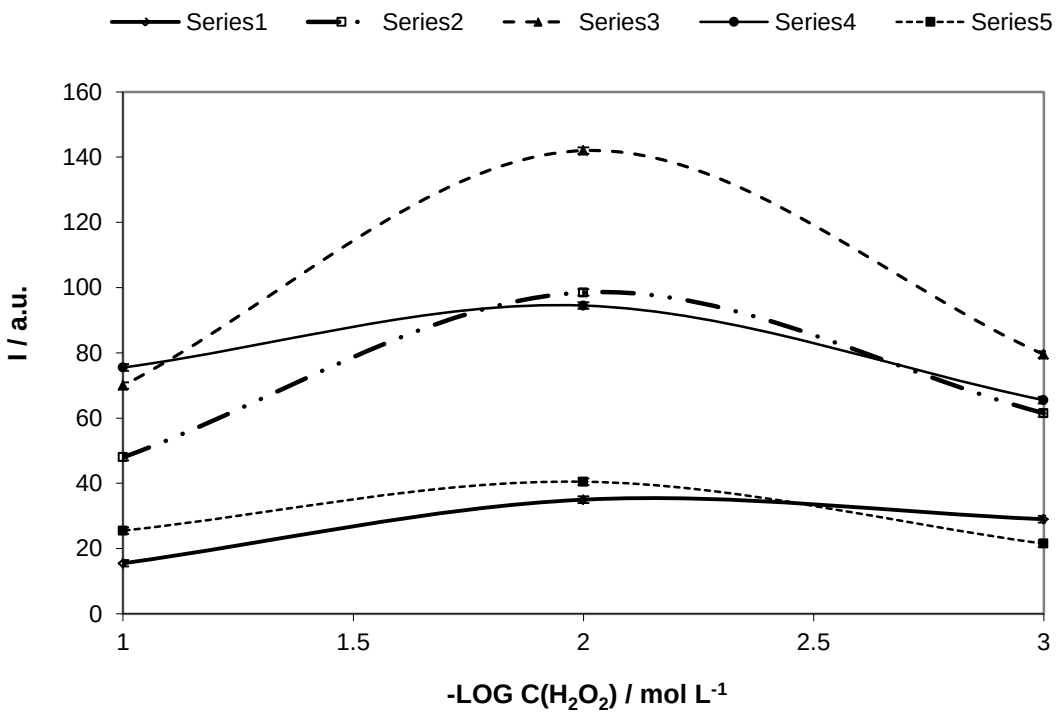

Fig. 3a Influence of luminol and hydrogen peroxide concentrations. Luminol: Series1, 0.0001 mol L^{-1}; Series2, 0.0005 mol L^{-1}; Series3, 0.001 mol L^{-1}; Series4, 0.005 mol L^{-1}; Series5, 0.01 mol L^{-1}. Hydrogen peroxide concentrations: 0.1 mol L^{-1}, 0.01 mol L^{-1} and 0.001 mol L^{-1}. Hexacyanoferrate (III) concentration was maintained at 0.001 mol L^{-1} in the five series.

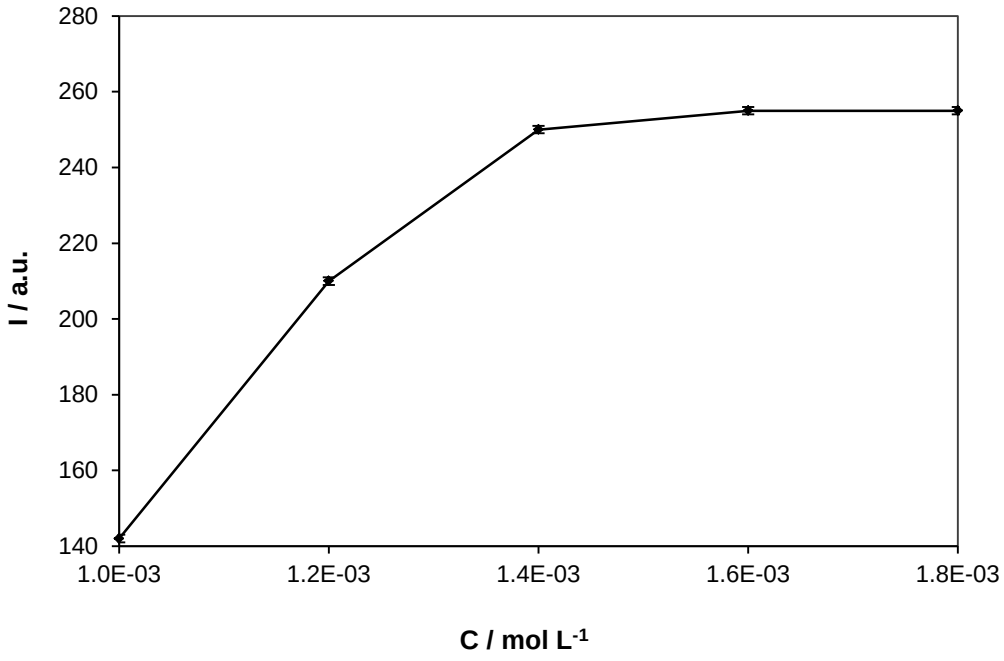

Fig. 3b Influence of hexacyanoferrate(III) concentration. Luminol 0.001 mol L^{-1}; Hydrogen peroxide 0.01 mol L^{-1}.

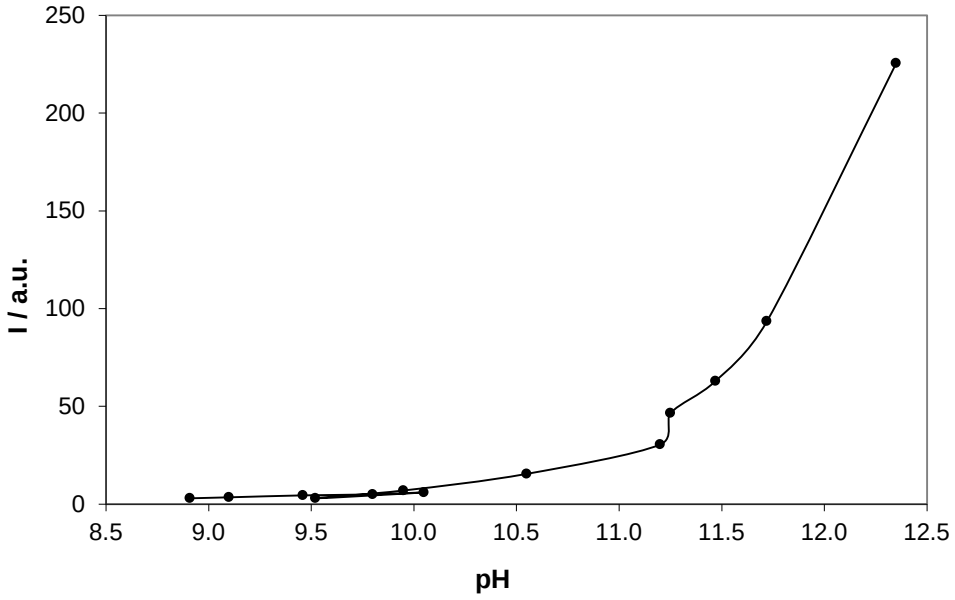

Fig. 4 Influence of pH.

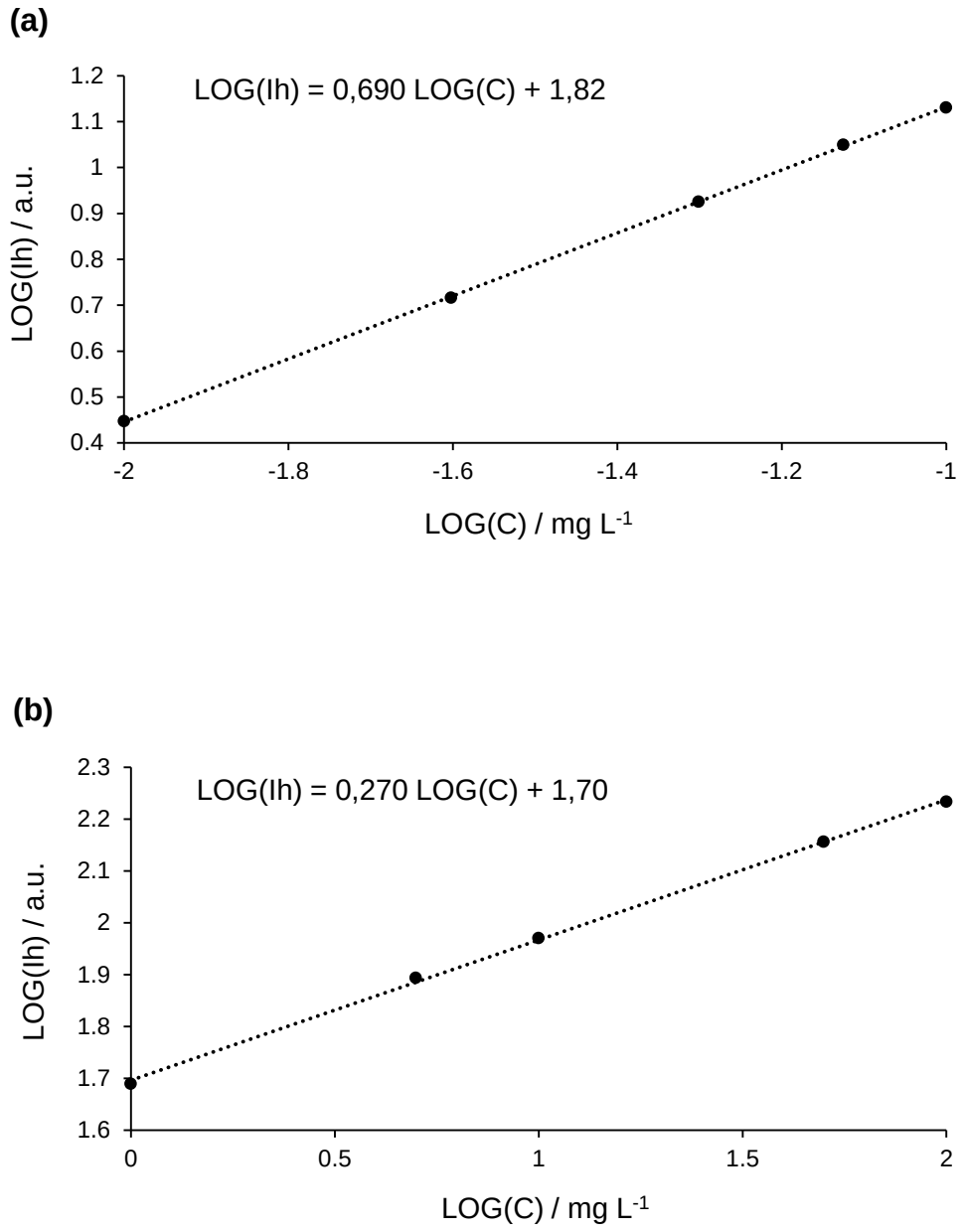

Fig. 5 Calibration graphs over the range (a) 0.01 – 0.10 mg L^{-1} and (b) 1.0 – 100.0 mg L^{-1} of paracetamol concentration.

Table 1 Influence of foreign compounds. All solutions containing 1.0 mg L^{-1} of paracetamol.

Foreign compound	**C / mg L^{-1}**	**E_r / %**
Acetylsalicylic acid	100	0.0
Ascorbic acid	5.0	-3.5
Caffeine	100	-1.3
Citric acid anhydrous	100	0.1
Codeine Phosphate	100	0.0
Hydroxypropyl methylcellulose	Saturated solution	0.0
Magnesium stearate	Saturated solution	0.0
Methocarbamol	100	-0.6
Microcrystalline cellulose	Saturated solution	0.0
Polyvinylpyrrolidone	100	-0.2
Saccharin	100	1.5
Silicon dioxide	Saturated solution	0.0
Sodium benzoate	100	0.4
Sodium carbonate	100	0.0
Sorbitol	100	0.3
Starch	Saturated solution	0.0
Stearic acid	Saturated solution	0.1
Talc	Saturated solution	0.0
Wheat starch	Saturated solution	0.2

Table 2 Tested pharmaceutical formulations.

*Average recovery (of three determinations) ± 95 % CI and precision (% RSD).

Pharmaceutical Formulation	**Label claim per tablet / mg**	**Official BP method found amount* per tablet / mg**	**Proposed method found amount* per tablet / mg**
Termalgin®	500	504 ± 5 (95 % CI) (0.4 % RSD)	500 ± 5 (95 % CI) (0.4 % RSD)
Efferalgan®	500	510 ± 5 (95 % CI) (0.4 % RSD)	503 ± 5 (95 % CI) (0.4 % RSD)
Gelocatil®	650	679 ± 7 (95 % CI) (0.4 % RSD)	658 ± 7 (95 % CI) (0.4 % RSD)