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Indirect determination of paracetamol in pharmaceutical formulations by inhibition of the system luminol–H_2O_2–$Fe(CN)_6^{3-}$ chemiluminescence

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Abstract

After a large drug scanning, the system Luminol–H_2O_2–$Fe(CN)_6^{3-}$ is proposed for first time for the indirect determination of paracetamol. The method is based on the oxidation of paracetamol by hexacyanoferrate (III) and the subsequent inhibitory effect on the reaction between luminol and hydrogen peroxide. The procedure resulted in a linear calibration graph over the range 2.5–12.5 μg ml^{-1} of paracetamol with a sample throughput of 87 samples h^{-1}. The influence of foreign compounds was studied and, the method was applied to determination of the drug in three different pharmaceutical formulations.

Keywords: Chemiluminescence; Flow injection; Paracetamol; Pharmaceutical analysis

1. Introduction

Paracetamol or acetaminophen is an analgesic and antipyretic drug which either replace or is formulated together with acetylsalicylic acid in pharmaceutical formulations; in some cases a third active drug (e.g. caffeine or codeine phosphate) is also present.

Data about chemical, physical and biopharmaceutical properties of paracetamol are easily available [1,2]. Several types of analytical procedures have been proposed, mainly gravimetric, titrimetric, polarographic, UV–vis absorption, chromatographic and even automatic determinations. A review [3] of broad scope points to the analytical interest on paracetamol and its further update [4] has been also published.

In recent years, a certain number of pharmaceuticals have been determined through continuous-flow chemiluminescence procedures [6], which has been also reviewed in several papers [7–9] and at present it still shows a growing trend. As far as we know, only one FIA–chemiluminescence (CL) procedure [5] has been proposed for measuring

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paracetamol concentration in pharmaceutical formulations.

The oxidation of luminol in basic solution (pH 10–11) [8] is catalyzed by a number of metal ions (among others) and this fact has been exploited in order to develop indirect determinations of several drugs which complexes them (e.g. amino acids [10] and thiol-containing drugs [11]). The same well known CL reaction has been used in a similar way to determine thiol-containing drugs which react with hypochlorite (luminol oxidant) and yield the subsequent CL-inhibition [12].

Hexacyanoferrate (III) is an oxidant with an almost constant redox potential between pH 4 and 13, and its oxidant behaviour has been exploited in drug analysis in a number of analytical procedures: titrimetric (direct [13] and indirect [14]), spectrophotometric [15,16], fluorimetric [17,18], amperometric [19] and even chemiluminescent [20–22]. On other hand, this species acts as catalyst-cooxidant on the luminol–hydrogen peroxyde CL reaction. There is only one paper [23] which exploit the chemiluminescent reaction between luminol and hexacyanoferrate (III) in order to developing an indirect chemiluminescent procedure for the determination of ascorbic acid, nevertheless in the mentioned paper it is not related the existence of drugs that can exalt the chemiluminescence of luminol when reacts with hydrogen peroxyde in presence of hexacyanoferrate (III).

2. Experimental

2.1. Reagents

Luminol (Merck), hydrogen peroxide (Scharlau, PA), potassium hexacyanoferrate (III) (Panreac, PA), paracetamol (Guinama), potassium hydroxide (Panreac, PA), glycine (UCB, PA), boric acid (Probus, PA), potassium bicarbonate (Panreac, PA), ascorbic acid (Merck) *N*-acetylcysteine (Guinama), phenylbutazone (Sigma), doxycycline (Pfizer), terramycin (Pfizer), thiamine (Guinama), nicotinic acid (UCB, PA), L-dopa (Guinama), dipyrone (Guinama), adrenaline (Guinama), ephedrine (Guinama) chlorpromazine (Guinama), promethazine (Guinama) and thioridazine (Guinama). All solutions were prepared with de-ionized water. Hydrogen peroxide concentration was determined by titration with $KMnO_4$ in 5% H_2SO_4.

2.2. Continuous-flow assembly and apparatus

Fig. 1a depicts the continuous-flow manifold proposed for paracetamol determination. Aliquots of sample (504 μl) and 1.9×10^{-3} mol l^{-1} luminol (504 μl) solutions were simultaneously injected into two different streams, 1.7×10^{-2} mol l^{-1} H_2O_2 and 0.9×10^{-3} mol l^{-1} $K_3[Fe(CN)_6]$, respectively, both at the same flow rate of 2.9 ml/min. Both inserted solutions merge at a T-piece placed just before the flow-cell. Chemiluminescence measurements were performed by means of a Luminescence Spectrometer from Perkin Elmer, mod. LS50B (Bioluminiscence mode, PTM 900 V, em.slit 20 nm, ex.slit 0 nm, $l_{ex} = l_{em} = 0$ nm.), provided with a spiral-flow cell, placed in front of the emission window. The pH was measured with a pHmeter CRISON, mod.

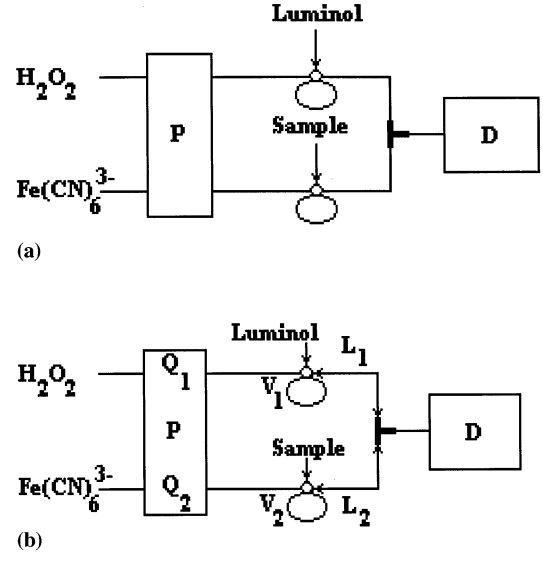

Fig. 1. (a) Proposed FIA assembly for determination of paracetamol. (luminol, 1.9×10^{-3} mol l^{-1}; potassium hexacyanoferrate (III), 0.9×10^{-3} mol l^{-1} and hydrogen peroxide, 1.7×10^{-2} mol l^{-1}). P, pump; D, detector and W, waste. (b) Assembly used to carry out the FIA-parameters optimization.

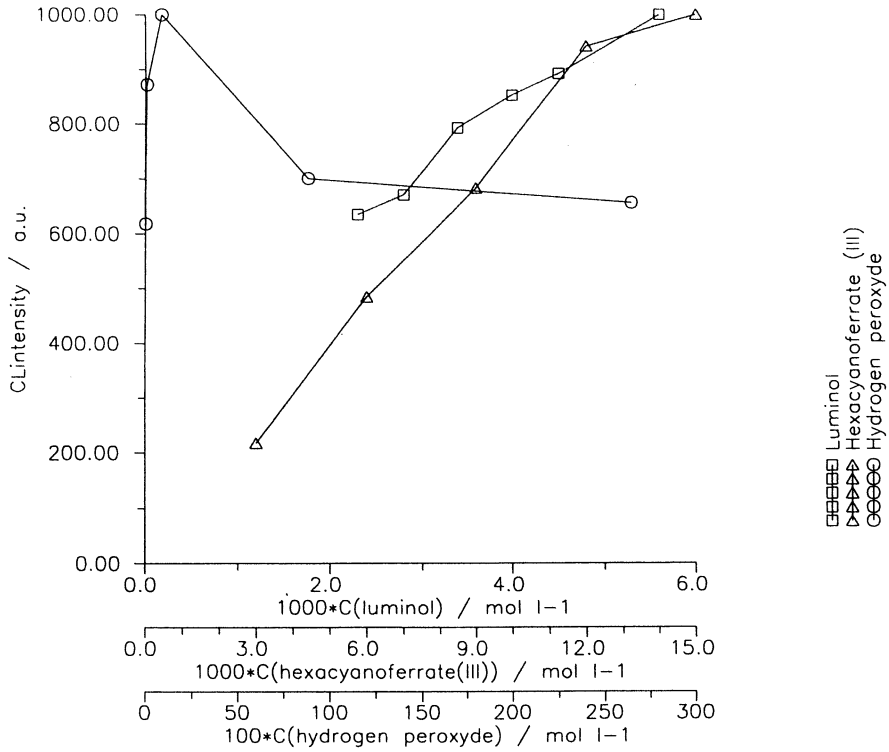

Fig. 2. Preliminary tests. Influence of the concentration of luminol, potassium hexacyanoferrate (III) and hydrogen peroxide on the chemiluminescence emission.

micropH 20R. A Rheodyne Model 5041 sample injector and a Gilson Minipuls 2 pump were used. The internal diameters of the PTFE tubing for the manifold were 0.8 mm i.d.

3. Results and discussion

3.1. Preliminary tests

Preliminary experiments were conducted in the two-channel FIA configuration of Fig. 1a (with the position of the hexacyanoferrate (III) and sample solutions switched); the manifold included two valves that were simultaneously operated. This was the simplest possible FIA assembly that allowed individual solutions of the three reagents and the analyte to be used. Also, it ensured that the chemiluminescent reaction started after the T-junction.

First, we studied the effect of the concentrations of the three reagents (luminol, hexacyanoferrate (III) and hydrogen peroxide) in order to establish the starting conditions required to obtain nearly but not completely saturated chemiluminescence (maximum empirical intensity) in order to be able to use a wide enough inhibition range on the y-axis. De-ionized water was injected through the second injection valve. The results are shown in Fig. 2. The concentrations adopted were: 3.4×10^{-3} mol l^{-1} luminol (a compromise between adequate CL intensity and consumption of luminol, which is an expensive reagent) buffered with a mixture of 0.44 M boric acid and 0.39 M KOH (pH 10.8); 6.1×10^{-3} mol l^{-1} hexacyanoferrate (III) (higher concentrations required larger amounts of analyte for CL inhibition to be observed) and 8.8×10^{-2} mol l^{-1} of hydrogen peroxide.

Table 1
Study of buffer solution composition

Buffer solution	Concentration (mol l^{-1})	$pH_{experimental}$	Linear equation	r ($n=5$)
Boric acid/KOH	0.40/0.43	10.8	$Ih^a = 26.4 + 19.9C^b$	0.97
Glycine/KOH	0.40/0.36	10.8	$Ih^a = 2.7 + 12.7C^b$	0.99
$NaHCO_3$/KOH	0.40/0.32	10.8	$Ih^a = 23.1 + 13.4C^b$	0.95

[a] Ih, inhibition in arbitrary units.
[b] C, concentration of paracetamol in μg ml^{-1}.

Next, we performed a scan for various active principles that might react with potassium hexacyanoferrate (III) according to the literature cited in the introduction or their reactivity to oxidants. Each principle was tested at concentrations from 1 to 200 μg ml^{-1} in the manifold of Fig. 1a with the positions of the hexacyanoferrate (III) and sample solutions switched. The active principles studied included paracetamol, ascorbic acid, cysteine, *N*-acetylcysteine, phenylbutazone, doxycycline, terramycin, thiamine, nicotinic acid, paracetamol, L-dopa, dipyrone, adrenaline, ephedrine, chlorpromazine, promethazine and thiorhidazine. The analytical signal was calculated as the difference between the FIA signal obtained by inserting hexacyanoferrate (III) into de-ionized water (the blank) and that provided by the oxidant inserted into the channel through which drug was propelled. All the drugs tested altered the blank signal proportionally to their concentrations.

Phenothiazines (promethazine, chlorpromazine and thiorhidazine) increased the signal (exaltation) while the other drugs decreased it (inhibition). From the corresponding calibration curves, paracetamol was chosen because it exhibited one of the strongest inhibitory effects and presented the most favourable linear range and calibration slope.

3.2. Influence of the chemical parameters

We assayed every possible reactant addition sequence in the FIA assembly of Fig. 1a. Each sequence was tested with aqueous solutions of paracetamol at concentrations from 1 to 10 μg ml^{-1}. Of the 12 sequences tested, that shown in Fig. 1a was adopted because it led to the best compromise between the slope of the calibration curve and width of the linear range.

Subsequently, the analytical signal was obtained as the difference between the FIA signal for de-ionized water and that for the drug (inhibited CL).

The influence of the nature of the buffer used to dissolve luminol and hexacyanoferrate (III) was studied by using boric acid/KOH, glycine/KOH and Na_2CO_3/KOH. The buffer solutions contained similar concentrations of their components and had a pH of 10.8 (which was selected according to the results obtained in a preliminary study) that was adjusted by dropwise addition of KOH. Each buffer was tested with different concentrations of analyte in the range 1–10 μg ml^{-1}. Table 1 shows the solution compositions tested and the results obtained. We chose the glycine/KOH buffer because it resulted in the widest linear range.

The pH of the selected buffer was optimized by studying its effect on injections of an aqueous solution of 7.5 μg ml^{-1} paracetamol over the range 10.3–12.0. The highest analytical signal was obtained at pH 11.6 (Fig. 3), which was chosen for subsequent experiments.

The influence of the buffer concentration was examined at ratios between $C/8$ and $2C$, where C is the concentration of a 0.40 M glycine/0.36 M KOH buffer. The analytical signal obtained by injecting aqueous solutions of 7.5 μg ml^{-1} paracetamol increased with increasing buffer concentration up to $0.5C$, beyond which no significant differences were observed. A concentration C was thus adopted for subsequent work in order to prevent small changes in the buffer concentration from altering the analytical signal.

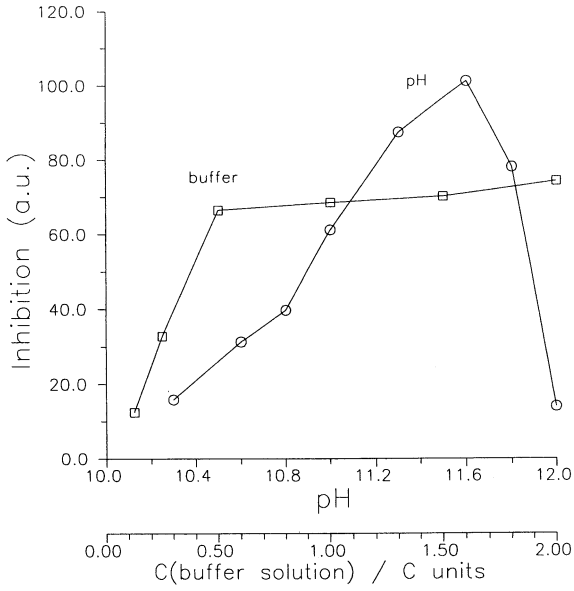

Fig. 3. Influence of the buffer pH and concentration on the chemiluminescence inhibition.

3.3. Optimization of FIA variables

FIA variables were optimized by using the manifold of Fig. 1b (insertions of 7.5 μg ml^{-1} of paracetamol) and values of the variables over the following ranges: luminol and sample volumes, $V_1 = V_2 = 117–620$ μl; reactor lengths, $L_1 = L_2 =$ 30–100 cm and flow-rate, $Q_1 = Q_2 = 1–4.5$ ml min^{-1}. All these variables were optimized by using the modified simplex method [24,25]. Of the 29 apices tested, the three that resulted in maximum inhibition were chosen (see Table 2); a study involving recording 20 FIA signals for each apex was then carried out in order to select that leading to the best compromise between analytical signal, peak–base width and repeatability. The conditions corresponding to the first apex were chosen, namely: $Q_1 = Q_2 = 2.9$ ml min^{-1}, $L_1 = L_2 = 78.3$ cm and $V_1 = V_2 = 504$ μl.

3.4. Re-optimization of chemical variables

After FIA variables were optimized, chemical variables were re-optimized. First, the luminol, hydrogen peroxide and potassium hexacyanoferrate (III) were readjusted to the following optimum values: 1.9×10^{-3} mol l^{-1} luminol, 0.9×10^{-3} mol l^{-1} potassium hexacyanoferrate (III) and 1.7×10^{-2} mol l^{-1} hydrogen peroxide (titrated against a potassium permanganate standard). We also studied the concentration and pH of the glicine/potassium hydroxide buffer used to prepare the luminol solution. Significant differences were not observed.

3.5. Analytical figures of merit

The calibration graph inhibition–concentration of paracetamol was found to be linear over the range 2.5–12.5 μg ml^{-1} of analyte. For this range the regression equation was $Ih = 2.5 + 15.3C$ (Where Ih is the inhibition in arbitrary units and C is the paracetamol concentration in μg ml^{-1}) and the correlation coefficient ($n = 6$) was 0.997. The calculated limit of detection, defined as the concentration that cause an inhibition equal to twice the SD of the blank peaks was 2.1 ppm.

The RSD of the method (peak height) calculated from 25 replicate injections (7.5 μg ml^{-1} of paracetamol) was found to be 2.2% and the calculated injection frequency was 87 h^{-1}.

Table 2
Simplex; chosen apices (See text)

Apex	$Q_1 = Q_2$ (ml min^{-1})	$L_1 = L_2$ (cm)	$V_1 = V_2$ (μl)	Peak width(s)	Inhibition (au[a])	RSD% ($n = 20$)
A	2.9	78.3	504	43.6	136	2.1
B	2.1	57.1	504	57.2	112	2.2
C	2.2	78.3	327	51.4	97	1.7

[a] au, arbitrary units.

Table 3
Influence of foreign compounds[a]

Foreign compound	C (μg ml^{-1})	E_r (%)
Sucrose	150	3.1
Glucose	100	1.4
Saccharin sodium	200	1.9
Codeine phosphate	30	2.2
Caffeine	100	1.4
Ascorbic acid	7.5	1.3

[a] All solutions containing 7.5 μg ml^{-1} of paracetamol.

The influence of foreing compounds and excipients that can be found in pharmaceutical formulations containing paracetamol was also studied. Synthetic solutions containing 7.5 μg ml^{-1} of the analyte and different amounts of foreing substances were measured. The errors were calculated by comparing the peak height with that obtained by injecting solution of pure paracetamol at the same concentration. The obtained results are depicted in Table 3.

Paracetamol was determined in three different pharmaceutical formulations:

1. Apiretal (drops, from ERN); paracetamol, 100.0 mg ml^{-1}; saccharin, 5.0 mg ml^{-1}.
2. Efferalgan (drops, from UPSA MEDICA): paracetamol, 120.0 mg ml^{-1}; saccharin, 7.5 mg ml^{-1}; sucrose, 2.0 mg ml^{-1}.
3. Efferalgan (tablets, from UPSA MEDICA): paracetamol, 500 mg ml^{-1}.

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 [26]. The official procedure is based on the drug titration with ammonium cerium sulphate with ferroin as indicator. Table 4 shows the obtained results.

Also, the rate of dissolution or in vitro availability of Efferalgan tablets was determined, The sample vessel consisted of a 1-l beaker containing 500 ml of 0.1 mol l^{-1} HCl (pH 1) at 37°C where one tablet was immersed inside a platinum basket fitted to a shaking arm rotated at 180 rpm. The time (average of three determinations) needed for complete dissolution of the tablet was computed from that required to obtain a minimum of 12 peaks, which was 8 min.

4. Conclusions

The inhibition effect of paracetamol over the ferricyanide–hydrogen peroxide–luminol CL reaction is the basis of this new procedure. As far as we know, this is a non-exploited CL system to determination of pharmaceuticals.

An important advantage over others direct-CL procedures which allow to determine the drug (and which use a luminometer) is the employed detector; an spectrofluorimeter, more commonly present in any laboratory of pharmaceutical analysis than luminometer.

The CL measurement was automated in a Flow-Injection system with good precision and sample throughput.

In addition to competitive precision and sensitivity, the new proposed procedure shows a relevant selectivity that allows to analyze the proposed samples without separation steps of the chromatography-based [27–30] methods. On the other hand, the new procedure presents, opposite

Table 4
Tested pharmaceutical formulations

Pharmaceutical formulation	Declared concentration (label) (mg/ml)	Found concentration[a] (proposed method) (mg/ml)	Found concentration[a] (official method) (mg/ml)
Apiretal (drops)	100	98.7	98.0
Efferalgan (drops)	24	24.1	23.7
Efferalgan (tablets)	500 mg/tablet	496 mg/tablet	487.2 mg/tablet

[a] Average of three determinations.

to the titrimetric official procedure, all the advantages arising from FIA [6] methodology.

The obtained results for the tested pharmaceuticals, compares well with those declared by the manufacturer and with the obtained by the official (BP) method.

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