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Talanta 69 (2006) 976–983
Talanta
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Flow injection chemiluminescence determination of paracetamol

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Received 4 October 2005; received in revised form 29 November 2005; accepted 29 November 2005
Available online 10 January 2006

Abstract

A simple chemiluminometric method using flow injection has been developed for the determination of paracetamol (acetaminophen), based on the chemiluminescence produced by the reduction of tris(2,2′-bipyridyl)ruthenium(III). The latter is obtained by oxidation of tris(2,2′-bipyridyl)ruthenium(II) by potassium permanganate in dilute sulphuric acid in the presence of paracetamol. A standard or sample solution was injected into the ruthenium(II) stream (flow rate 1.5 ml min^{-1}) which was then merged with potassium permanganate in dilute sulphuric acid stream (flow rate 0.5 ml min^{-1}). The chemiluminescence intensity is enhanced by the presence of manganese(II) ions. Under the optimum conditions, a linear calibration graph was obtained over the range of 0.3–50.0 μg ml^{-1} and the detection limit was 0.2 μg ml^{-1} (s/n = 3). The relative standard deviation of the proposed method calculated from 20 replicate injections of 5.0 μg ml^{-1} paracetamol was 1.1%. The sample throughput was 90 h^{-1}. The method was successfully applied to the determination of paracetamol in commercial pharmaceutical formulations.

Keywords: Chemiluminescence; Paracetamol; Flow injection; Ruthenium(II)

1. Introduction

Paracetamol (acetaminophen, *N*-acetyl-*p*-aminophenol, 4-acetamidophenol) is a widely used minor analgesic. The structure of paracetamol is shown in Fig. 1. Although it has some cyclo-oxygenase inhibiting properties this action is very weak in the peripheral tissues and it has practically no anti-inflammatory action. It is also known that large doses will damage the liver and kidneys [1,2]. Numerous methods have been used for the determination of paracetamol in pharmaceutical formulations and biological fluids including titrimetry [3,4], UV–Vis spectrophotometry [5–8], spectrofluorimetry [9], near infrared transmittance spectroscopy [10], electrochemical methods [11,12] and chromatography [8,13–17]. Recently, several flow injection (FI) methods for the determination of paracetamol have been proposed using an UV–Vis spectrophotometer [18–20], fluorimeter [21], multioptosensor [22], or Fourier transform infrared spectrophotometer [23] as a detector. However, some of these methods are less convenient for the determination of paracetamol in pharmaceutical formulations because the methods are based on the hydrolysis of paracetamol sample to 4-aminophenol, which then produced a coloured complex compound by an appropriate reaction which are time-consuming. The more rapid FI method is therefore sought. Flow-injection (FI) with chemiluminescense (CL) seems promising to serve this purpose.

Chemiluminescence methods provide many advantages for pharmaceutical determinations such as high sensitivity, high selectivity, small amount of chemical consumption, cost effectiveness, simple sample preparation and instrumentation [24–26]. Only three procedures have been reported in the literature for the determination of paracetamol in pharmaceutical formulations or biological fluids by using flow injection with chemiluminescence detection [27–29]. The procedures are based on the oxidation of paracetamol with cerium(IV) [27] and the inhibition of a luminol–H_2O_2–$Fe(CN)_6^{3-}$ or luminol–permanganate system [28,29].

Tris(2,2′-bipyridyl)ruthenium(II) has been used as the basis of CL detection of a wide range of compounds after oxidation to the ruthenium(III) complex [30]. The analyte interacts with the ruthenium(III) complex reducing it to the ruthe-

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doi:10.1016/j.talanta.2005.11.050

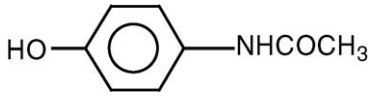

Fig. 1. The structure of paracetamol.

nium(II) complex in an excited state, which then emits CL as it returns to the ground state. In the present study, a FI procedure for paracetamol determination with CL detection was proposed in which ruthenium(II) was oxidised on-line by mixing with potassium permanganate solution. Subsequent reaction with paracetamol produces chemiluminescence. The intensity is enhanced by the presence of manganese(II) ions. Similar procedures have been applied to tetracyclines [31] and cephalosporins [32]. The CL emission intensity depended on the concentration of the analyte in the FI system. This work describes a relatively sensitive, rapid and reproducible flow injection chemiluminescense (FI-CL) method for paracetamol determination based on tris(2,2′-bipyridyl)ruthenium(II) without sample hydrolysis process. Application of this method to paracetamol determination in commercial pharmaceutical formulations is performed which will be useful for drug quality control.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent grade and were used without further purification. All solutions were prepared with distilled deionised water.

Paracetamol was purchased from Sigma (Poole, Dorset, UK). The stock standard solution of paracetamol (500 μg ml^{-1}) was prepared by dissolving 0.5000 g of paracetamol in water and diluting to 1000 ml with water. Solutions of the desired concentrations were obtained by diluting the stock solution to volume with water.

The solution of $Ru(bpy)_3^{2+}$ (6.4×10^{-4} mol l^{-1}) was obtained by dissolving 0.2500 g of tris(2,2′-bipyridyl)ruthenium(II) (Fluka, Gillingham, Dorset, UK) in water and diluting to 500 ml. Potassium permanganate stock solution was prepared by dissolving an appropriate amount of $KMnO_4$ (Riedel-de Häen, Gillingham, Dorset, UK) in 0.1 mol l^{-1} sulphuric acid (Fisher, Loughborough, UK). A 1.25×10^{-2} mol l^{-1} manganese(II) solution was prepared from 0.2473 g of $MnCl_2 \cdot 4H_2O$ (Sigma, Poole, Dorset, UK) dissolved in water and adjusted to 100 ml.

Solutions (2%) of Tween-20, Tween-40, Tween-60, Tween-80 or Triton X-100 (Lancaster, UK) were prepared by dissolving 2.0 g in water and diluting with water to 100 ml; and 1.25×10^{-2} mol l^{-1} cetylpyridinium bromide (CPB, Sigma), hexadecyltrimethylammonium bromide (HTAB) and dodecylbenzenesulphonic acid (DBS) (both Fluka) were prepared by dissolving 0.4805, 0.4556 and 0.4356 g, respectively, in water and diluting with water to 100 ml.

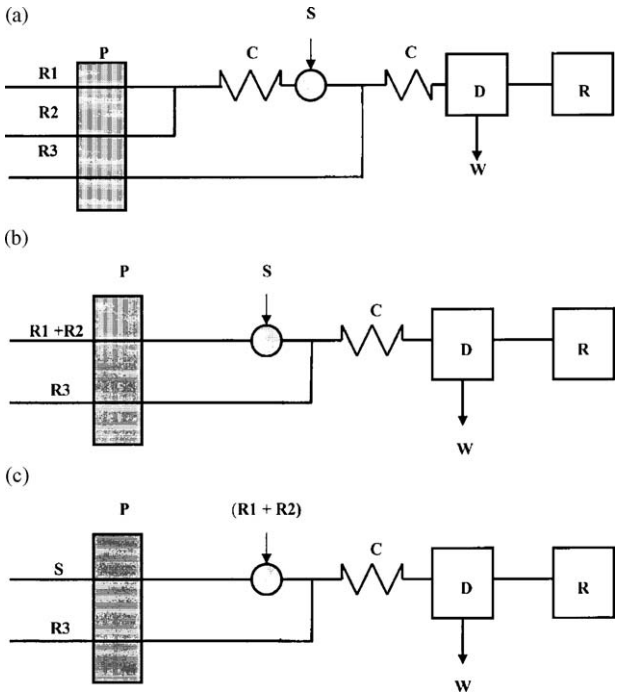

Fig. 2. Various types of the FI manifold: R1, $Ru(bpy)_3^{2+}$; R2, Mn(II); R3, $KMnO_4$ in H_2SO_4; P, peristaltic pump; S, sample; C, 160 cm reaction coil; D, chemiluminescence detector; R, recorder; and W, waste.

2.2. Flow manifold

The flow injection manifold (Fig. 2) consisted of a peristaltic pump (Gilson® Minipuls 3, Villiers-le-Bel, France), and the sample or standard solution was injected via a four way PTFE rotary valve with a 100 μl sample loop (Rheodyne® model 5020, Cotati, CA). PVC tubing (Elkay, Galway, Ireland) with 2.0 and 1.6 mm i.d. was used as flow lines for the chemiluminescence reagent, $Ru(bpy)_3^{2+}$ and potassium permanganate solutions, respectively, and a T-shaped connector was used for merging the reagent streams. A mixing coil was made from PTFE tubing, 0.5 mm i.d. and 160 cm in length. All other tubings in the system were 0.5 mm i.d. PTFE. The FI peaks were acquired by using a chemiluminescence detector (Camspec®, Cambridge, UK), coupled with a chart recorder (Chessell®, Kipp and Zonen, The Netherlands).

Initially, three FI manifolds were designed as shown in Fig. 2. The first designed FI manifold was a three channel FI manifold (Fig. 2(a)) in which the three solutions (tris(2,2′-bipyridyl)ruthenium(II) (R1), manganese(II) (R2) and acidic potassium permanganate (R3) solutions were propelled into the three flow lines. R1 and R2 were merged and passed through a mixing coil to permit effective mixing. Sample solution was injected into the merged streams of R1 and R2 which was then merged with R3 followed by passing through another mixing coil where the chemical reaction took place and reached the detector flow cell where measurements were made.

The second designed FI manifold was a double channel FI manifold (Fig. 2(b)) in which (tris(2,2′-bipyridyl)ruthenium(II) (R1) and manganese(II) (R2) were premixed in the reagent reser-

voir prior to propelling into the flow system. R1 + R2 solution and acid potassium permanganate (R3) solution were propelled into the flow lines. Sample solution was injected into the flow R1 + R2 stream which was then merged with R3 followed by passing through a mixing coil where the chemical reaction took place which was then reached the flow cell of the detector where the CL intensity was measured.

The third designed was a double channel FI manifold (Fig. 2(c)) similar to the second designed manifold but the experimental procedure was different. This manifold was designed for reverse FIA (rFIA) in which sample solution (S) and acid potassium permanganate (R3) solution were propelled into the flow lines, a portion of mixed reagents containing tris(2,2′-bipyridyl)ruthenium(II) (R1) and manganese(II) (R2) was injected into the sample stream which was then merged with the R3 stream followed by passing through a mixing coil where the chemical reaction occurred which was then reached the detector where the CL intensity was measured.

2.3. Experimental procedure

2.3.1. Sample preparation

A total of 20 tablets or caplets of paracetamol were accurately weighed individually, then ground and mixed well. An appropriate amount of paracetamol equivalent to one tablet or caplet (500 mg of paracetamol) was accurately weighed and dissolved in water by sonication in a 250 ml volumetric flask and diluting with water to 250 ml. The dissolved sample was filtered through Whatman No. 1 filter paper and diluted with water to volume to obtain the appropriate concentration for analysis.

2.3.2. Flow injection procedure

Using the two-channel manifold shown in Fig. 2(b), a 100 μl sample or standard solution containing paracetamol was injected into the reagent stream (R1 + R2) consisting of 6.4×10^{-4} mol l^{-1} $Ru(bpy)_3^{2+}$ and 1.25×10^{-2} mol l^{-1} manganese(II) in the ratio 100:20 (v/v) at the optimum flow rate of 1.5 ml min^{-1} which was then merged with the oxidant stream (R3) (7.0×10^{-4} mol l^{-1} $KMnO_4$ in 0.1 mol l^{-1} H_2SO_4) with an optimum flow rate of 0.5 ml min^{-1}. Subsequently, the sample zone was carried through the reaction coil where the CL reaction took place. The CL emission was monitored by the CL detector and the FI signal was displayed by the chart recorder.

3. Results and discussions

3.1. Optimisation of the flow injection system

3.1.1. Manifold design

The performance of the three FI-CL manifolds were tested for paracetamol determination based on CL signal produced by the reduction of $Ru(bpy)_3^{3+}$ by the drug. It is found that the FI manifold as shown in Fig. 2(b) is suitable because it exhibits the greatest CL emission intensity (21.64 mV) which is above five and four times as much as those obtained by the first (Fig. 2(a)) and the third (Fig. 2(c)) manifolds, respectively. It is possible that the configuration of the second FI manifold (Fig. 2(b)) is optimum to provide effective oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ with a faster reaction rate than the FI manifold in Fig. 2(a), (4.35 mV). However, the CL intensity produced using the reverse FI manifold in Fig. 2(c) was very low (6.04 mV) probably owing to the limitation of the injection volume of the reagent, $Ru(bpy)_3^{2+}$. Therefore, the FI-CL manifold as shown in Fig. 2(b) was selected for subsequent experiment.

3.1.2. Effect of different oxidants

Common oxidants such as concentrated nitric acid, lead oxide, cerium(IV), chlorine and potassium permanganate have been used to efficiently produce CL emission with $Ru(bpy)_3^{2+}$ [30]. In this study, six soluble oxidants were tested; 3.0×10^{-4} mol l^{-1} solutions of the following oxidants were prepared in 0.1 mol l^{-1} H_2SO_4: $KMnO_4$, $Ce(SO_4)_2$, $K_2S_2O_8$, $KBrO_3$ and KIO_3, and $K_3Fe(CN)_6$ was tested in 0.1 mol l^{-1} NaOH. Potassium permanganate was the only oxidant that produced a chemiluminescence signal when an aliquot of paracetamol solution was injected into the FI system, thus potassium permanganate was selected for further use. Paracetamol did not give any chemiluminescence emission when added to $KMnO_4$ solution alone.

3.1.3. Effect of different acids

The influence of acid media was studied by using 3.0×10^{-4} mol l^{-1} $KMnO_4$ in 0.1 mol l^{-1} of each of the following acids: H_2SO_4, HCl, HNO_3, H_3PO_4 and polyphosphoric acid. The relative CL were 100%, 89%, 58%, 36% and 5%, respectively. It can be seen that sulphuric acid was the acid that gave the highest CL. Therefore, sulphuric acid was chosen for subsequent studies, probably because $KMnO_4$ in sulphuric gives sufficient oxidising power to oxidise $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ which permits the CL reaction to be favoured. In addition, acidic permanganate especially in sulphuric acid can lead to the stabilisation of an intermediate species such as manganese(II) in paracetamol determination. According to Agater and Jewsbury [33] who examined the influences of various mineral acids on the CL emission. They found that sulphuric acid provided the greatest CL intensity which was chosen for their experimentals based on acidic permanganate with chemiluminescent detection. This contrasts somewhat with some other systems, where polyphophoric acid gave the most intense emission. In this instance, a polyphosphoric acid system gives a very low intensity chemiluminescence.

3.1.4. Effect of sensitizers and metals

This study investigated the CL emission when paracetamol was injected into a CL reagent stream consisting of 3.2×10^{-4} mol l^{-1} $Ru(bpy)_3^{2+}$ and potential enhancers in a volume ratio of 100:5 (v/v) which was merged with 3.0×10^{-4} mol l^{-1} $KMnO_4$ in 0.1 mol l^{-1} H_2SO_4. Two percent solutions of Tween-20, Tween-40, Tween-60, Tween-80 and Triton X-100 and 1.25×10^{-2} mol l^{-1} HTAB, DBS, CPB, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Fe^{2+}, $Fe^{3+}$and Pb^{2+} were tested. The results obtained are shown in Table 1. These show that most metal ions and non-ionic surfactants had little

Table 1
Effect of various sensitizers and metals on relative CL intensity (100 μl of 20 μg ml^{-1} paracetamol with 3.0×10^{-4} mol l^{-1} $KMnO_4$, 3.2×10^{-4} mol l^{-1} $Ru(bpy)_3^{2+}$)

Metal ion	Relative CL	Sensitizers	Relative CL
Mn^{2+}	100.0	Tween-20	36.4
Co^{2+}	35.7	Tween-40	31.8
Ni^{2+}	36.2	Tween-60	36.4
Zn^{2+}	38.1	Tween-80	49.5
Cu^{2+}	33.2	Triton X-100	33.2
Mg^{2+}	39.3	HTAB[a]	12.4
Ca^{2+}	38.7	DBS[b]	12.2
Al^{3+}	38.7	CPB[c]	19.5
Fe^{3+}	34.1		
Fe^{2+}	77.1	None	31.0
Pb^{2+}	34.3		

[a] Hexadecyltrimethylammonium bromide.
[b] Dodecylbenzene sulfonic acid.
[c] Cetylpyridinium bromide.

or no effect, but Tween 80 < Fe^{2+} < Mn^{2+} gave some enhancement. All three ionic surfactants tested decreased the emission intensity.

3.1.5. Effect of manganese(II) concentration

As manganese(II) gave appreciable enhancement, as noted in the tetracycline system [31], its effect on the determination of paracetamol was studied in more detail. The effect of the volume ratio of 6.4×10^{-4} mol l^{-1} $Ru(bpy)_3^{2+}$: 1.25×10^{-2} mol l^{-1} Mn^{2+} showed a maximum at a ratio of 100:20, and was used for subsequent experiments. The effect of manganese(II) concentration on the CL intensity can be seen by comparing the results (Table 2).

This study investigated the effect of $Ru(bpy)_3^{2+}$ concentration on emission intensity for various paracetamol concentrations in the presence or absence of manganese(II). The highest intensity in each instance was obtained with the highest $Ru(bpy)_3^{2+}$ concentration tested, 12.8×10^{-4} mol l^{-1} (Table 2), but the concentration selected for further study was 6.4×10^{-4} mol l^{-1} because it gave a reasonable intensity but decreased the consumption of the expensive ruthenium salt. A comparison of the results in Table 2 showed that the presence of 1.25×10^{-2} mol l^{-1} manganese(II) increased the intensity for all concentrations of ruthenium and paracetamol tested. Further experiment showed that intensity increases with manganese(II) concentration from 2.0×10^{-5} mol l^{-1} until reaching a maximum value at 1.25×10^{-2} mol l^{-1}, above which the chemiluminescence intensity decreased. Therefore, 1.25×10^{-2} mol l^{-1} manganese(II) was selected as optimum.

3.1.6. Effect of H_2SO_4 concentration

The effect of sulphuric acid concentration on the CL emission was investigated in the range of 0.01–0.50 mol l^{-1}. It was found that the CL emission intensity increased with increasing sulphuric acid concentration and reached a maximum value at 0.10 mol l^{-1}, above which the chemiluminescence intensity decreased gradually. The optimum concentration of sulphuric acid was thus found to be 0.10 mol l^{-1}. This might be due to

Table 2
Effect of ruthenium complex concentration on the CL intensity of paracetamol (without Mn^{2+} and with Mn^{2+}; 1.25×10^{-2} mol l^{-1})

Concentration of $[Ru(bpy)_3^{2+}]$ (mol l^{-1})	CL intensity without paracetamol (mV)		CL intensity with paracetamol (mV)									
			0.5 μg μl^{-1}		1.0 μg μl^{-1}		5.0 μg μl^{-1}		10.0 μg μl^{-1}		20.0 μg μl^{-1}	
	No Mn^{2+}	With Mn^{2+}	No Mn^{2+}	With Mn^{2+}	No Mn^{2+}	With Mn^{2+}	No Mn^{2+}	With Mn^{2+}	No Mn^{2+}	With Mn^{2+}	No Mn^{2+}	With Mn^{2+}
0	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.28	0.23	0.62	0.53
0.8×10^{-4}	0.00	0.00	0.05	0.34	0.09	0.65	0.35	4.68	0.60	11.3	0.99	27.1
1.6×10^{-4}	0.05	0.44	0.08	0.59	0.16	1.23	0.80	9.69	1.65	24.6	3.40	62.1
3.2×10^{-4}	0.05	0.66	0.08	1.08	0.25	2.56	1.70	17.0	3.85	42.5	8.10	100.8
6.4×10^{-4}	0.08	0.96	0.10	1.99	0.32	3.86	3.40	24.1	8.20	58.8	18.5	123.6
12.8×10^{-4}	0.12	1.28	0.30	2.06	0.53	5.41	7.35	34.6	16.5	73.1	40.3	155.7

the fact that the sulphuric acid concentration present in acid permanganate solution should be high enough to provide effective oxidizing power to oxidise $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$. Excess of H^+ and MnO_4^- solution leading to decrease in oxidizing power of the acid permanganate solution.

3.1.7. Effect of $KMnO_4$ concentration

The effect of $KMnO_4$ concentration on the CL emission intensity was studied from 1.0×10^{-4} to 1.2×10^{-3} mol l^{-1}. The CL intensity was found to increase with the increasing concentration of $KMnO_4$ from 1.0×10^{-4} to 7.0×10^{-4} mol l^{-1}, above which the CL intensity decreased slightly. The concentration of MnO_4^- in acid permanganate solution should be sufficient to oxidize $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ which was then reduced by the analyte to generate CL emitting species. Therefore, 7.0×10^{-4} mol l^{-1} $KMnO_4$ was chosen as the optimum concentration.

3.1.8. Effect of ruthenium complex concentration

This study investigated the effect of $Ru(bpy)_3^{2+}$ concentration on the emission intensity for various paracetamol concentrations. The highest intensity in each instance was obtained with the highest $Ru(bpy)_3^{2+}$ concentration tested, 12.8×10^{-4} mol l^{-1} (Table 2). The linear equations obtained at the two highest $Ru(bpy)_3^{2+}$ concentrations (6.4×10^{-4} and 12.8×10^{-4} mol l^{-1}), where $I = 6.30C - 3.48$ ($r^2 = 0.998$) and $I = 7.88C - 3.36$ ($r^2 = 0.999$), respectively, where I is peak height (mV) and C the paracetamol concentration (μg ml^{-1}). The sensitivities (slopes of the regression equations) were 6.30 and 7.88, respectively. The reagent consumption under the latter condition was twice that of the former, therefore, as a compromise between sensitivity, linear range and expensive reagent consumption, the 6.4×10^{-4} mol l^{-1} $Ru(bpy)_3^{2+}$ solution was chosen for further use.

3.1.9. Effect of mixing tubing internal diameter, length of mixing coil and injection loop volume

The mixing tubing used as flow lines and the mixing reactor plays important role on the FI signal because it depends on the resident time of the sample zone in the tubing with an appropriate inner diameter and the length of the tubings used. The inner diameter of the mixing tubing has to optimize because the dispersion of sample zone increases with mixing tubing diameter and the band broadening eventually results in loss of sensitivity and lowers the sampling rate. The effects of inner diameter of the mixing tubing on the CL intensity were examined over the range of 0.5–1.3 mm i.d. and the 0.5 mm i.d. tubing was chosen as optimum because it provided high CL signal. Tubings with smaller inner diameter than 0.5 mm i.d. were not tested because the 0.5 mm i.d. tubing is the smallest one which was available.

The effect of various mixing coil tubing lengths between 40 and 400 cm, injection loop volumes between 50 and 500 μl on the reaction produced by 20 μg ml^{-1} of paracetamol. It was found that the peak height increased with the mixing coil length up to 200 cm, and began to decrease gradually up to 400 cm. The mixing coil lengths of 40, 80, 120, 160, 200, 240, 280, 320, 360 and 400 cm provided the peak height of 28.13, 45.05, 50.71, 56.26, 56.70, 54.78, 51.37, 48.85, 43.68, and 34.78 mV, respectively.

It is necessary to optimize the injection volume to achieve the desired sensitivity. Since the amounts of sample injected into the FI system should be sufficient to permit effective CL reaction. The influence of the sample/standard volume on the CL-signal was investigated by injecting the standard solution with varying volumes in the range of 50–500 μl of 20 μg ml^{-1} paracetamol. It was shown that peak height increased from 48.90 to 51.98 mV on increasing the injection volume from 50 to 500 μl. It was found that the peak height increased with the injection volume up to 100 μl, and the injection volume of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 μl produced the peak heights of 48.90, 57.20, 55.22, 51.04, 51.37, 53.51, 53.79, 52.03, 52.09 and 51.98 mV, respectively. The most suitable mixing coil inner diameter and length together with the injection loop volume values at the reasonable sample throughput rate for further use were 0.5 mm i.d., 160 cm and 100 μl, respectively.

3.1.10. Effect of reagent flow rate

The effect of flow rate of 7.0×10^{-4} mol l^{-1} $KMnO_4$ (at 1.5 ml min^{-1} $Ru(bpy)_3^{2+}$) and 6.4×10^{-4} mol l^{-1} $Ru(bpy)_3^{2+}$ (at 0.5 ml min^{-1} $KMnO_4$) were investigated for paracetamol determination (20 μg ml^{-1}). The intensity increased with increasing flow rate of each reagent flow rate up to a $KMnO_4$ flow rate of 0.5 ml min^{-1} and up to a $Ru(bpy)_3^{2+}$ of 1.5 ml min^{-1} above which the intensity decreased. Thus, 0.5 ml min^{-1} $KMnO_4$ and 1.5 ml min^{-1} $Ru(bpy)_3^{2+}$ were regarded as the optimum flow rates (Fig. 3).

3.1.11. Selected optimum conditions

The univariate optimisation method used above to study the effect of variables on CL emission intensity gave the optimum conditions shown in Table 3.

3.2. Analytical application

3.2.1. Analytical figures of merit

Under the optimum conditions for the determination of paracetamol given in Table 3, the calibration graph was found to be linear over the range of 0.3–50.0 μg ml^{-1}. The regression plot obtained fitted the equation: $I = 5.89C - 0.72$ ($r = 0.998$, $n = 7$),

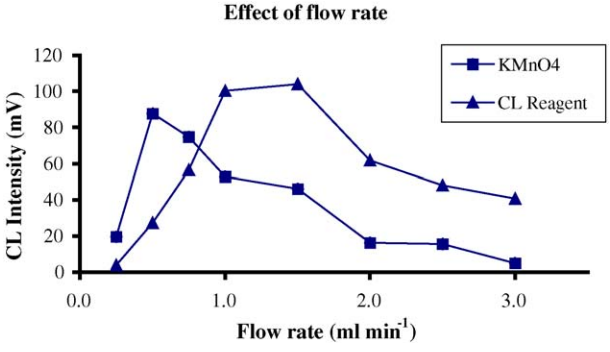

Fig. 3. Effect flow rate of $KMnO_4$ and CL reagent; [$Ru(bpy)_3^{2+}$] (20 μg ml^{-1} paracetamol).

Table 3
The optimum conditions for determination of paracetamol

Parameters studied	Range	Optimum
Flow rate $Ru(bpy)_3^{2+}$ (ml min^{-1})	0.25–3.0	1.5
Flow rate $KMnO_4$ (ml min^{-1})	0.25–3.0	0.5
Mixing tubing (mm i.d.)	0.5–1.3	0.5
Injection volume (μl)	50–500	100
Length of reaction coil (cm)	40–400	160
$Ru(bpy)_3^{2+}$:Mn(II) (v/v)	100:0–100:100	100:20
Mn(II) conc. (mol l^{-1})	2.0×10^{-5} to 3.12×10^{-1}	1.25×10^{-2}
Types of acid media	HNO_3, H_2SO_4, HCl, H_3PO_4, polyphosphoric acid	H_2SO_4
H_2SO_4 conc. (mol l^{-1})	0.01–0.50	0.10
$KMnO_4$ conc. (mol l^{-1})	1.0×10^{-4} to 1.2×10^{-3}	7.0×10^{-4}
$Ru(bpy)_3^{2+}$ conc. (mol l^{-1})	0.8×10^{-4} to 12.8×10^{-4}	6.4×10^{-4}

where I is the peak height (mV) and C the concentration of paracetamol (μg ml^{-1}). The limit of detection (0.2 μg ml^{-1} or 1.3×10^{-6} mol l^{-1}) was defined as the lowest concentration yielding a signal three times the corresponding blank signal (s/n = 3). The detection limit obtained by the proposed method (0.2 μg ml^{-1}) was in excellent agreement with that reported (0.2 μg ml^{-1}) by Criado et al. [20]. But it (0.2 μg ml^{-1}) was lower than those reported flow injection chemiluminescence based on cerium(IV) or the luminol–H_2O_2–$Fe(CN)_6^{-3}$ reaction [27,28] which were 1.0 μg ml^{-1} [27] and 2.5 μg ml^{-1} [28]. In comparison with the results from other methods, for examples, spectrofluorimetry [9] the detection limit was 0.01 μg ml^{-1}; IC with a detection limit of 0.06 μg ml^{-1} [16]; FI-fluorimetry [21] based reaction with a detection limit of 0.1 μg ml^{-1}; FI-CL by luminol–permanganate based reaction [29] with a detection limit of 1.0×10^{-8} mol l^{-1} or 0.001 μg ml^{-1}; FI spectrophotometry [34] with a detection limit of 0.01 μg ml^{-1}; and FI using inhibited luminol–dimethylsulfoxide–NaOH–EDTA chemiluminescence [35] with a detection limit of 1.9×10^{-10} g ml^{-1} which are far more lower than that obtained by the proposed FI-CL procedure. Although the proposed method was not as sensitive as the above cited methods the proposed method was simple and rapid. The advantage of the proposed FI system over the current ones is no need to hydrolysis the sample prior to determination of paracetamol. The aim of this research is to develop a fairly sensitive and rapid FI system for determining paracetamol in commercial pharmaceutical formulations only which will be adopted to use for drug (paracetamol) quality control. Therefore, in this circumstance, high sensitivity is not needed as provided by other methods [9,16,21,29,34,35]. Further development of the FI-CL system to achieve the high sensitivity for this drug determination and application to biological samples will be performed. The relative standard deviation of the proposed method (peak height in mV) calculated from 20 replicate injections of 5.0 μg ml^{-1} paracetamol was 1.1% with a sample throughput rate of 90 h^{-1}.

3.2.2. Recovery study

A standard addition procedure was carried out with real samples in order to test the recovery of the proposed method. Thus, paracetamol concentrations of 50.0 and 200.0 μg ml^{-1} were added to known samples (50 μg ml^{-1}) of tablets and caplets. After measurement of the CL intensity, the recovery of each spiked standard was calculated. The results are shown in Table 4. The percentage recoveries were found to be between 99.2% and 101.6%. The mean recoveries (±S.D.) for paracetamol tablets and caplets were found to be 100.5 ± 1.6% and 101.2 ± 0.9%, respectively. It can be seen that the proposed method provided accurate results.

3.2.3. Interference study

The effects of some possible excipients (glucose, sucrose, lactose, citric acid, starch and sorbitol) were investigated, together with these of ascorbic acid, saccharin sodium, salicylic acid and caffeine. Synthetic sample solutions containing 5.0 μg ml^{-1} paracetamol and different concentrations of the other compounds were tested, and the peak heights obtained were measured. Table 5 shows the maximum tolerable concentrations of the various compounds. It can be seen that glucose, sucrose, lactose, caffeine, saccharin sodium and starch have no significant effect on the determination of paracetamol even when they are present up to 50 to 1000 times the weight ratio of paracetamol. Sorbitol, citric acid and ascorbic acid interfered at concentrations above 20, 7.5 and 2.0 μg ml^{-1}, respectively. The most serious interference was from salicylic acid which gave a response greater than paracetamol.

It was found that citric acid and sorbitol depressed CL intensity. The structures of citric acid and sorbitol include the oxygen

Table 4
Recovery of paracetamol in pharmaceutical samples (n = 5)

Pharmaceutical formulations	Concentration of paracetamol added (μg ml^{-1})	Concentration of paracetamol found (μg ml^{-1})	Recovery[a] (%)
Tablets	0	50.5	101.0
	50.0	99.6	99.2
	200.0	252.5	101.2
Mean[b] (±S.D., %)			100.5 ± 1.6
Caplets	0	50.5	101.0
	50.0	100.8	101.6
	200.0	251.9	101.0
Mean[b] (±S.D., %)			101.2 ± 0.9

[a] Average from five determinations.
[b] Average from 15 determinations.

Table 5
Maximum tolerable concentration of some excipients

Excipients	Maximum tolerable concentration ($\mu g\,ml^{-1}$)	Error (%)
Starch	5000	−0.8
Caffeine	1500	−0.7
Saccharin sodium	1000	1.6
Glucose	250	−2.9
Sucrose	250	−2.6
Lactose	250	−2.6
Sorbitol	20	−2.8
Citric acid	7.5	−2.2
	(10.0)	(−8.0)
	(20.0)	(−18.1)
Ascorbic acid	2.0	3.0
	(3.0)	(10.1)
	(10.0)	(24.3)
Salicylic acid	0.05	3.9
	(0.075)	(9.2)
	(0.10)	(12.5)

Tested solutions containing 5.0 $\mu g\,ml^{-1}$ paracetamol, $n = 5$.

groups and lacking of aromatic or certain multiple conjugated double bond which might lead to quenching effect. Quenching of the luminescence intensity is a possible type of interference [36].

Ascorbic acid and salicylic acid increased CL intensity. Luminescence was expected to increase in molecules that contained multiple conjugated double bond portions with a high degree of resonance stability. The substituents that delocalize the π-electron, such as –OH, –NH and $-OCH_3$ groups, often enhance luminescence. They tend to increase the transition probability between the lowest excited singlet state and the ground state [37]. In addition, ascorbic acid and salicylic acid may involve in the direct oxidation with potassium permangante in dilute sulphuric acid and the resulting CL emission can be obtained from this proposed reaction. These compounds increased the reduction efficiency of $[Ru(bpy)_3{}^{3+}]$ to $[Ru(bpy)_3{}^{2+}]^*$ and followed with the increasing of the CL intensity. This feature led to the method development for determination of ascorbic acid and salicylic acid with the direct oxidation with acidic potassium permanganate [33,38].

3.2.4. Application

The proposed method has been applied to the determination of paracetamol in commercial pharmaceutical formulations. Paracetamol was determined in two different pharmaceutical formulations ($n = 5$):

1. Paracetamol (Tablets, The Boots Company, Nottingham, UK), paracetamol: 500 mg/tablet.
2. Paracetamol Plus (Caplets, Wrafton Laboratory Ltd., Braunton, Devon, UK), paracetamol: 500 mg/caplet and caffeine 65 mg/caplet.

The results were compared with those declared on the formulation labels and with those obtained by using the official method of the British Pharmacopoeia [39] (titration of sample with ammonium cerium(IV) sulphate, ferroin indicator). The drug contents were found to be 497.7 ± 2.5 and 496.1 ± 2.0 mg/tablet by using this proposed method and the official method, respectively. The results for the caplets were 498.9 ± 2.6 and 496.9 ± 1.7 mg/caplet, respectively.

4. Conclusions

The proposed FI chemiluminescence detection method has proved to be simple, rapid and sensitive for paracetamol determination. The linearity of the calibration graph is in the useful concentration range for quatitation of the drug in pharmaceutical preparations. The detection limit of the method was lower than those reported for batch wise absorption spectrophotometry [6,7], chromatography [10,13–15], flow injection analysis [22] and flow injection chemiluminescence based on inhibition of the luminol–H_2O_2–$Fe(CN)_6{}^{3-}$ reaction [28]. The method developed is fast and reasonably cost effective, providing a good sample frequency of 90 h^{-1}, and should be useful for routine analysis for paracetamol in pharmaceutical formulations. However, the detection limit of the proposed method is poorer than a number of previously reported methods [9,16,21,29,34,35], the main advantage of this method do not require the use of hydrolysis. For pharmaceutical product control, the high sensitivity is not needed.

Acknowledgements

The authors gratefully acknowledge The Thailand Research Fund that supported the research assistantship under the Royal Golden Jubilee (RGJ) Ph.D. programme to W. Ruengsitagoon. Special thanks are also expressed to the Postgraduate Education and Research Programme in Chemistry (PERCH), the Graduate School and the Chemistry Department of Chiang Mai University together with the Chemistry Department, The University of Hull, Hull, UK, for partial support.

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