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Flow-injection determination of cinnarizine using surfactant-enhanced permanganate chemiluminesence

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Abstract

Chemiluminescence (CL) of permanganate system in the presence of polyphosphoric acid (PPA), ethanol and Tween 60 was investigated and implemented using flow-injection analysis (FIA) to determine cinnarizine (C) in pharmaceutical preparations. Five hundred microliter samples were injected and the sample throughput was $130\,h^{-1}$. Preliminary experiments identified Tween 60 as the surfactant of choice, improving the detection limit of the CL system 20-fold. Optimum CL signals were given using $7.5 \times 10^{-4}\,mol\,l^{-1}$ potassium permanganate in $0.02\,mol\,l^{-1}$ polyphosphoric acid as the oxidant stream and a carrier stream of 10% (v/v) of ethanol in aqueous $1.5 \times 10^{-3}\,mol\,l^{-1}$ Tween 60 with a total flow rate of $7.6\,ml\,min^{-1}$. The calibration graph was linear over the range 0.5–6.0 $\mu g\,ml^{-1}$ ($r = 0.9963$, $n = 7$). The detection limit (3σ) was $18\,ng\,ml^{-1}$ and the percentage recovery of spiked tablet solutions was 98.4–100.2.
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Keywords: Cinnarizine; Chemiluminescence; Flow-injection analysis; Permanganate; Surfactants

1. Introduction

Cinnarizine (C), (*E*)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine (structure in Fig. 1), is a piperazine derivative with antihistamine, sedative and calcium-channel blocking activity. It is used for the symptomatic treatment of nausea and vertigo caused by Menière's disease and other vestibular disorders and for the prevention and treatment of motion sickness. It is also used in the management of various peripheral and cerebral vascular disorders [1].

Although clinical experience of the use and prescription of cinnarizine goes back over a period of two decades, no pharmacopoeia method is available for its assay in preparations. Only bulk cinnarizine appears in the *British Pharmacopoeia* [2] and *European Pharmacopoeia* [3]; assay is by non-aqueous titration. A number of articles have been published concerning quantification of cinnarizine in pharmaceutical preparations and biological fluids by different techniques such as liquid chromatography [4–7], thin layer chromatography [4], gas chromatography [8], spectrophotometry [9–12] and potentiometry with an ion selective electrode [13,14]. However, chromatography requires a sophisticated instrument, high cost and long analysis time whereas potentiometric membrane sensors,

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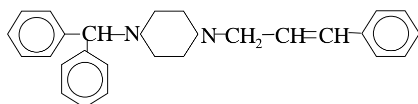

Fig. 1. Chemical structure of cinnarizine.

ion selective electrodes and spectrophotometry are not applicable to determine low amounts of the drug.

Flow injection (FI) coupled with chemiluminescence (CL) has grown up rapidly bringing together the advantages of these two techniques in simple and rapid instrumentation and improvement sensitivity, selectivity and precision. Potassium permanganate is a common oxidant for generating CL; Hindson and Barnett [15] have reviewed its CL application for inorganic and organic substances and have also discussed the mechanism of such emission [16]. There are several FI–CL procedures that use the acidic potassium permanganate system in pharmaceutical determinations such as those of buprenorphine hydrochloride [17], benzodiazepine loprazolam [18], perphenazine [19], salicylamide [20], thioridazine hydrochloride [21], trimetazidine [22], cefadroxil monohydrate [23], ranitidine and salbutamol [24].

Due to various unique and advantageous properties of surfactants, which should better facilitate analytical CL measurement, there are a number of reports [25–51] of the application of various kinds of surfactant in CL measurement, set out chronologically in Table 1. In almost all these cases, micelles are used to enhance the CL signal. However, the work of Zhang et al. [36], Safavi and Karimi [49], Li et al. [50] and Ilyina and Hermandez [51] employed the inhibition effect of micelles to improve measurement performance. Only a few reports [25,28,32,45] achieved the advantage of micelles enhancement in the permanganate CL system. We have therefore investigated the effects of surfactants on emission intensity in permanganate CL system and report significant and useful improvements in both signal strength and S/N ratio applied to determining cinnarizine in pharmaceutical preparations.

2. Mechanisms of micellar enhancement

Well-defined mechanistic principles emerge from the previous work on micellar enhancement of CL. The review of Lin and Yamada [52] focuses on how micelles may be used to improve CL measurement by changes in microenvironment (i.e. polarity, viscosity and/or acidity, etc.); in the chemical and photophysical pathway and rate; and in solubilization, concentration and organization of the solute/reactant that affect the reaction rate. Although we shall now use these principles as a framework for discussing some of the earlier work, it will become clear both in this section and in Section 4.2 that they are highly inter-related rather than mutually exclusive.

2.1. Changes in the microenvironment

(1) Huang et al. [35] investigated the interaction of the sulfite group in drugs with dissolved oxygen in presence of acidic Rhodamine 6G. Tween 60 enhanced CL by 200% and they suggested that the microenvironment of this medium leads to an increase in CL quantum yield and prevents oxygen-based quenching.
(2) Zhang and Chen [42] suggested possible ways to sensitize CL in micellar media by (i) solubilization, (ii) electrostatic effects, (iii) altering the microenvironment of the CL reaction and (iv)altering the pH of the microenvironment. However, from their discussion in their own work, the CL system of iodate anion and hydrogen peroxide in the presence of various surfactants and at various concentrations, the most reasonable seems to be explanation (iii).
(3) Hadjianestic and Nikokavouras [55] reported the CL reaction of luminol with hypochlorite in CTAC micelles and concluded that the light reaction in micellar media results in chemiexcitation yields which are higher than those in the corresponding homogeneous aqueous media due to the less polar microenvironment of the micellae stern region. However, the actual CL quantum yields are lower due to quenching, both chemical and photophysical.

2.2. Changes in the chemical and photophysical pathway and rate

(1) Ingvarsson et al. [27] studied the effects of two cationic surfactants, CTAOH and CTAB, to the CL system of lucigenin-biological reductants (i.e. fructose, glucose, ascorbic acid and uric acid) and they proposed that CTAOH micelles could

Table 1
Some applications of micelles in CL

Year	Analyte	CL system	Surfactants	LOD	Reference
1984	SO_2	$KMnO_4/SO_2$/FMN	Tween 85	3 ppb	Kato et al. [25]
1987	Copper	β-Nitrobenzene/NaOH/copper/ fluorescein	CTAB	0.1 ng 20 μl^{-1}	Yamada and Suzuki [26]
1988	Biological reductants	Lucigenin/biol reductants	CTAOH CTAC	2.3 mg l^{-1} 7.0 mg l^{-1} (fructose)	Ingvarsson et al. [27]
1997	H_2O_2	$KMnO_4/H_2O_2$	OP	6×10^{-9} mol dm^{-3}	Feng et al. [28]
1998	Hydrochloro-thiazide	Ce(IV)/H_2SO_4/Rhodamine 6G	Brij 35 (not used)	0.15 µmol l^{-1}	Ouyang et al. [29]
1998	Co(II)	Cl-PF/H_2O_2/Co(II)	CTAB	0.07 ng ml^{-1}	Xie et al. [30]
1998	Pyrogallol	*N*-Bromosuccinate/pyrogallol/ hydroxylamine	CTAB 300	2×10^{-7} mol l^{-1}	Safavi and Baezzat [31]
1998	Uric acid	$KMnO_4$/PPA	OP	0.055 µg ml^{-1}	Li et al. [32]
1998	Iron(II) Total iron	Luminol/iron(II)/H_2O_2/citric acid	TTAB	2×10^{-9} mol dm^{-3} 1×10^{-9} mol dm^{-3}	Saitoh et al. [33]
1999	Ergonovine maleate (EM)	EM/$[Fe(CN)_6]^{3-}$/NaOH	CPC	70 ppt	Fuster et al. [34]
1999	Menadione sodium bisulphite/analgin	Sulfite/O_2/acidic Rhodamine 6G	Tween 80 ≈200	0.01 µg ml^{-1} 0.003 µg ml^{-1}	Huang et al. [35]
1999	Dopamine	Iron(II)/lucigenin	Brij 35	2×10^{-9} mol dm^{-3}	Zhang et al. [36]
1999	Zinc	1,10-Phenanthroline/H_2O_2/NaOH	TSAC	2.3×10^{-8} mol l^{-1}	Watanabe et al. [37]
1999	Sulfite	Sulfite/Rhodamine 6G	Tween 80	0.03 mg l^{-1}	Huang et al. [38]
1999	Phenothiazines	Fe(II)/luminol	PVA	–	Perez-Ruiz et al. [39]
2000	Cobalt	Pyrogallol/H_2O_2/MeOH	CTAB	5×10^{-12} mol l^{-1}	Cannizzaro et al. [40]
2000	Phosphorus	HPA/alkaline luminol	Cationic surfactant	0.02 µg l^{-1}	Zui and Birks [41]
2000	SDBS	Sodium periodate/H_2O_2/cyclohexane	SDBS	3.2×10^{-8} g ml^{-1}	Zhang and Chen [42]
2000	Codeine	$(Ru(bipy)_3)^{3+}$/codiene	Triton X-45	8.3×10^{-7} mol l^{-1}	Greenway et al. [43]
2001	H_2O_2	DTMC/H_2O_2	CDAC	4×10^{-8} mol l^{-1}	Ma et al. [44]
2001	Maltol	$KMnO_4/H_2SO_4$/formic acid	CPC	10 mg l^{-1}	Alonso et al. [45]
2001	Cobalt	Luminol/alkaline	CTAB, CTAC	(3–4) $\times 10^{-11}$ mol l^{-1}	Nelstrop et al. [46]
2001	Dichlorvos pesticide	Luminol/H_2O_2	CTAB	0.008 µg ml^{-1}	Wang et al. [47]
2002	Gold	Luminol/8-hydroxyquinoline	Surfactant	–	Li and Li [48]
2002	DTAB, CTAB, CPC	Luminol/NaOH/NBS or NBC	DTAB, CTAB, CPC		Safavi and Karimi [49]
2003	Procaine HCl	Luminol/H_2O_2	β-Cyclodextrin	0.08 µg ml^{-1}	Li et al. [50]
2003	Phenol	Luminol/HRP	AOT	0.0005–0.003 mg ml^{-1}	Ilyina and Hernandez [51]

Cl-PF: 2,6,7-trihydroxy-9-(4′-chlorophenyl)-3-fluorone; CTAB: cetyltrimethylammonium bromide; TTAB: tetra decyltrimethylammonium bromide; DTMC: 7-(4,6-dichloro-1,3,5-triazinylamino)-4-methylcoumarin; CDAC: cetyldimethylammoniumchloride; $(Ru(bipy)_3)^{3+}$: (2,2′-bipyridyl)ruthenium(III); PVA: poly(vinylalcohol); PPA: polyphosphoric acid; OP: octylphenyl polyglycol ether; FMN: flavin mononucleotide; CPC: cetylpyridinium chloride; DTAB: dodecyltrimethylammonium bromide; NBS: *N*-bromosuccinamide; NBC: *N*-chlorosuccinamide; HRP: horseradish peroxidase; AOT: aerosol OT or Na bis(2-ethylhexyl)sulfosuccinate; TSAC: trimethylstearylammonium chloride; HPA: vanadomolybdophosphoric acid; SDBS: sodium dodecylbenzene sulfonate.

increase this CL intensity better than CTAB due to the superior enhancement of CTAOH in micellar catalysis of the rate-limiting step of the lucigenin-reductant CL reaction.

(2) Li et al. [32] described permanganate CL in the presence of octylphenyl polyglycol ether for determination of uric acid. They referred to an alteration in the local microenvironment allowing the solute to associate with a micellar system, affecting various photophysical rate processes. The net result was that micelles provided a protective environment for the excited singlet state, explaining the observation of enhanced CL.

(3) Li and Li [48] proposed that a small amount of surfactant added to CL system of luminol–Au(III)–hydroxyquinoline, could stabilize Au(III) in aqueous solution, accelerate the reaction rate and increase CL intensity.

(4) Tuncay et al. [58], studied the kinetics of the reaction between colloidal manganese oxide (MnO_2) and formic acid in perchloric acid and surfactants (CTAB, SDS and Triton X-100). It was found that only Triton X-100 could accelerate this CL reaction.

2.3. Changes in the solubilization, concentration and solute/reactant organization

(1) Saitoh et al. [33] determined iron(II) and total iron by catalytic effect of iron(II) on the CL system of luminol and hydrogen peroxide enhanced by TTAB in presence of citric acid. They proposed that an anion complex of iron(II)–citric acid is formed and concentrated at the surface of the cationic micelle and then reacts with hydrogen peroxide at this surface; this increases the rate of the CL reaction.

(2) The work of Yamada and Suzuki [53] employed a cationic surfactant in the copper-catalyzed CL of 1,10-phenanthroline with hydrogen peroxide and gives a model of this mechanism: 1,10-phenanthroline concentrates in the center of the micelle, superoxide anionic radicals are attracted on the surface and the induced reaction occurs more easily.

(3) Boyatzis and Nikokavouras [56] studied the effect of CTAB and SDS on CL of lophine and its derivative in alkali in term of spectroscopic behavior and CL.

2.4. Facilitating energy transfer

(1) Greenway et al. [43] mentioned that a non-ionic surfactant helps to overcome the pH imbalance between codeine (in acetate buffer) and $Ru(bipy)_3{}^{3+}$ (in sulfuric acid and Triton X-100). The reacting species is enclosed within a micelle and enabled easier energy transfer.

(2) Lasovsky and Grambal [54] studied the effect of micellar complexes on energy transfer in the CL system of luminol-hydrogen peroxide, enhancing the signal in the presence of CTAB and fluorescein. They gave an expectation that the donors (aminophthalate anion) and the acceptors (fluorescein anion) of the energy will be located at distances approximately corresponding to the diameter of the micelle (1–3 nm). Since the transfer of electron excitation energy in solutions can be realized up to a distance of 10 nm (Perrin–Forster mechanism), the dynamic concentration of both species in the stern zone of micelle is very effective for energy transfer.

(3) Lasovsky et al. [57] focussed on CL of luminol and its related compounds in the presence of CTAC, enhanced by intramicellar transfer of electronic excitation energy. The effectiveness of enhancement can be explained on the same assumptions as their previous work [54]. They concluded that intramicellar processes of energy transfer can easily be modified by altering concentration and optimized in order to reach maximum conversion of chemical energy. An increase in CL intensity by the effect of intramicellar complexes is a generally applicable procedure, weakly dependent on the choice of CL reaction, acceptors of electron excitation energy, catalysts and enhancer applications.

3. Experimental

3.1. Instrument

A simple two line flow injection (FI) manifold was employed which consisted of a peristaltic pump (Gilson® Minipuls 3, Villers-le-Bel, France), 0.056 in. i.d. PVC pump tubing (Elkay, Hampshire, England), a low-pressure sample injection valve (Omnifit, Cambridge, England) and 0.8 mm i.d. PTFE tubing.

Chemiluminescence was monitored by a home-made luminometer consisting of a PTFE T-piece for mixing the two reagents, a flat spiral glass flow cell placed in a vertical plane, the flat shape facing the window of the photomultiplier tube (Electron Tubes Ltd., model 9789QB, Ruislip, London); the luminometer was housed in a light-tight aluminium cylinder. The signal was recorded by a chart recorder(Chessell®, Kipp and Zonen, The Netherlands). In all experiments, five replicate injections were performed for each measurement.

3.2. Reagents

All solutions were prepared daily from analytical reagent grade materials. De-ionized water obtained by reverse osmosis to a resistivity of >5 MΩ cm (Elgastat option 4, Elga, High Wycombe, UK) was used throughout. A solution of 1.5×10^{-3} mol l^{-1} Tween 60 was prepared by dissolving 1.9676 g of Tween 60 (polyoxyethylene sorbitan monostearate 60, Fluka Chemika, Buchs, Switzerland) in 1000.0 ml water. The carrier stream consisted of 10% ethanol (purchased in bulk from Fisher Scientific, Loughborough, Leicestershire, UK and rebottled on site) in 1.5×10^{-3} mol l^{-1} Tween 60. A stock solution of 0.10 mol l^{-1} potassium permanganate in water was prepared by dissolving 0.4110 g of potassium permanganate (Fisher Scientific UK, Loughborough, Leicestershire, UK), in 50.0 ml of water. An oxidant stream consisted of 7.5×10^{-4} mol l^{-1} of potassium permanganate and 0.02 mol l^{-1} polyphosphoric acid (PPA, Merck, Poole, Dorset, UK).

Cinnarizine (Sigma, Poole, Dorset, UK) stock solution I, 1000 μg ml^{-1}, was prepared by dissolving 100 mg of cinnarizine in 100.0 ml of ethanol. Stock (II) 100 μg ml^{-1} of cinnarizine was prepared by diluting 10.0 ml of stock I to 100.0 ml by 1.5×10^{-3} mol l^{-1} Tween 60. The carrier solution was used as a diluent to prepare the final solution of cinnarizine.

4. Results and discussion

4.1. Preliminary study

Initially cinnarizine solution gave a weak CL signal with potassium permanganate in acidic medium. The reason is that cinnarizine is a derivative of piperazine, which is a secondary aliphatic amine; there have been previously reported cases in the review of Hindson and Barnett [15] and, in addition, other piperazine derivatives such as trimetazidine [22], in which the oxidation of amines by potassium permanganate is accompanied by chemiluminescence. Various acids such as hydrochloric, sulphuric, perchloric, polyphosphoric, acetic, propionic and formic acid at the same concentration, 0.5 mol l^{-1}, were tested as carrier stream to maximize the signal using the two channel manifold consisting of an oxidant stream of potassium permanganate (1.65 ml min^{-1}, 5×10^{-4} mol l^{-1} in water) and acid carrier stream (1.65 ml min^{-1}, 0.5 mol l^{-1}). Because cinnarizine is only slightly soluble in water but soluble in alcohol and acid, the cinnarizine solutions at 50 μg ml^{-1} were prepared in two solvent systems, 5% (v/v) ethanol in 0.1 mol l^{-1} HCl (solvent I), and 80% (v/v) ethanol in water (solvent II). 125-μl samples were injected into the acid carrier stream. It was found that only polyphosphoric acid produced the excellent signal, an 18-fold (solvent I) and 14-fold (solvent II) increase on hydrochloric acid. Polyphosphoric acid, as has been found in a number of other permanganate CL reactions [59–62], may give enhanced light emission by stabilization of the reaction intermediates. Therefore, polyphosphoric acid was accompanied with permanganate as oxidant stream for further study and cinnarizine in solvent I was selected because it gave a higher signal and better precision than that obtained by use of solvent II. Cinnarizine in 80% ethanol gave a fluctuating signal due to bubble formation in line, which may be eliminated by degasifying the sample solutions, e.g. by bubbling nitrogen through the solution [63].

In an attempt to improve further the sensitivity of the chemiluminescence signal, common enhancers such as formaldehyde, acetaldehyde, glutaraldehyde, Rhodamine 6G, fluorescein or riboflavin were included in the carrier stream, when the oxidant was potassium permanganate (5×10^{-4} mol l^{-1}) in 0.1 mol l^{-1} polyphosphoric acid. All of these substances diminished signal of 10 μg ml^{-1} cinnarizine, except formaldehyde that slightly increased the signal (about 1.2 times). Therefore, none of these enhancers was employed for further studies. Formaldehyde has been previously used as an enhancer in permanganate CL [64–69].

4.2. Study of effect of various kinds of surfactant

The effect of surfactant media including a cationic surfactant (cetyltrimethylammoniumbromide (CTAB)), an anionic surfactant (sodium dodecyl sulphate (SDS)) and three non-ionic surfactants (Tween 20, Tween 60 and Triton X-100), each mixed to 5% ethanol, were investigated comparing to 5% ethanol in 0.1 $mol\,l^{-1}$ HCl. Each concentration of surfactant was prepared at higher concentration than the critical micelle concentration (CMC). The oxidant stream was 5×10^{-4} $mol\,l^{-1}$ potassium permanganate in 0.1 $mol\,l^{-1}$ polyphosphoric acid and carrier was water. The results are set out in Table 2. All blank signals of surfactants are lower than the signal from 0.1 $mol\,l^{-1}$ hydrochloric acid. The anionic surfactant, SDS, diminished the analyte signal whereas cationic and non-ionic surfactants (except Triton X-100) could enhance the CL emission. Micelle-enhanced CL intensity was observed in CTAB, Tween 20 and Tween 60. Although CTAB was the most effective enhancer in this study, a precipitate occurred after mixing with the oxidant stream indicating that the reaction product does not dissolve in this micelle system. Surprisingly, no blank signal was observed in the presence of SDS and CTAB even though it was mixed with 5% ethanol whereas one was present in non-ionic surfactants. Tween 20 and Tween 60 promoted similar enhancement but the blank signal in Tween 60 media is lower and the precision (both blank and analyte signal) is better than those in Tween 20. The limits of detection (LOD) in the absence and presence of surfactants were estimated by 3σ of blank signal calculated on single point calibration, and the results were 2.14, 0.32 and 0.11 $\mu mol\,l^{-1}$ in the system without surfactant, and with Tween 20 and Tween 60, respectively. At this pre-optimization stage, the LOD in the CL system in the presence of Tween 60 is approximately 20-fold lower than in the system without surfactant. Therefore, Tween 60 was selected to mix with ethanol in the carrier for further FIA system.

Table 2
Effect of surfactants on 10 $\mu g\,ml^{-1}$ cinnarizine CL

Solvent[a]	Concentration ($mol\,l^{-1}$)	CL intensity[b] (mV)	
		Blank	Cinnarizine
HCl	0.1	1.71 ± 0.09	5.14 ± 0.06
SDS	4.1×10^{-2}	0	0.77 ± 0.03
CTAB	4.6×10^{-3}	0	7.91 ± 0.13
Tween 20	1.5×10^{-3}	0.33 ± 0.03	7.76 ± 0.11
Tween 60	5.0×10^{-4}	0.15 ± 0.01	7.60 ± 0.04
Triton X-100	3.0×10^{-4}	0	2.71 ± 0.02

[a] Each solvent mixed with 5% ethanol.
[b] CL intensity ± S.D.

The principles of micellar enhancement have already been discussed (Section 2) and including solubilization and solute organization [33,53,56], altering the local microenvironment [35,42,55] and changes to the light-emitting pathways that affect the quantum yield and reaction rate [27,32,48,58]. In this work, cinnarizine, being quite a non-polar compound, should concentrate in the center of the micelle and it is supposed that permanganate anion could concentrate at the surface of non-ionic micelle and react with the cinnarizine. This is reasonable because the CL signal in the presence of SDS, an anionic surfactant, decreased perhaps because the permanganate anion could not get close to the concentrated cinnarizine in the micelle; on the other hand, in the presence of cationic and non-ionic micelles, the CL increased. In previous work [25,28,32,45] on surfactant-enhanced acidic permanganate CL, anionic surfactants were not used to enhance the CL signal. However, using Triton X-100, the signal is decreased when an increase might have been expected. Surfactants often work by bringing the analyte in to better contact with the chemiluminescence reagent; this is achieved in this case partly by assisting the water solubility of cinnarizine. The least water-soluble intermediate of permanganate reduction is manganese(IV), which forms a colloid, but this unlikely to cause significantly light scattering at the low concentration of permanganate employed in this work. However, the permanganate must be reduced in a stepwise manner via Mn(IV), even though in acid solution, it is too rapid to observe each intermediate. If Mn(IV) → Mn(III) is the rate determining step, the surfactant might speed the reaction of Mn(IV) with the analyte by promoting better contact and hence enhance CL that way.

4.3. Optimization of chemical and physical variables

The FI conditions chosen for further univariate optimization of chemical variables consisted of an oxidant stream of 5×10^{-4} $mol\,l^{-1}$ potassium permanganate in 0.1 $mol\,l^{-1}$ polyphosphoric acid and a carrier stream

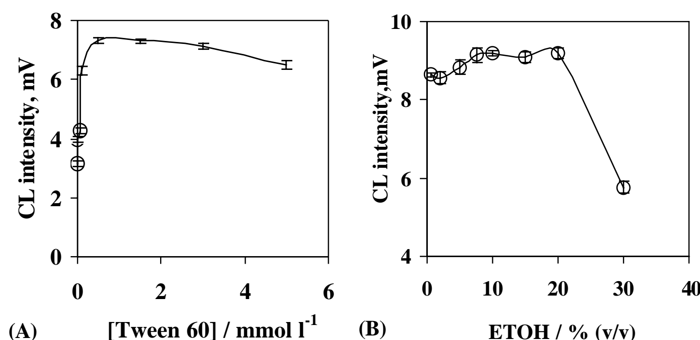

Fig. 2. Effect of chemical variables on CL signal of cinnarizine (10 μg ml^{-1}), each point of plotted by mean value with S.D. ($n = 5$). (A) Concentration of Tween 60, and (B) percentage (v/v) of ethanol.

of 5% (v/v) of ethanol in aqueous 5.0×10^{-4} mol l^{-1} Tween 60 with a total flow rate of 3.3 ml min^{-1}.

The effect of Tween 60 concentration in the carrier stream was investigated at concentrations higher than the CMC (CMC = 0.0027 mmol l^{-1}), at 0.005–5 mmol l^{-1}. The effect is shown in Fig. 2(A). Increasing the amount of surfactant sharply increases the signal up to 0.5×10^{-3} mol l^{-1}, above which it slightly decreased. Because of the risk of error in the preparation of solutions of optimum concentration, a concentration of 1.5×10^{-3} mol l^{-1} was selected for further study.

The above (Section 4.1) comparison of two systems using as solvent containing 5 and 80% (v/v) of ethanol demonstrated that the higher concentration of alcohol decreased the signal and the precision. Thus, the amount of ethanol affected the CL signal and the solubility of cinnarizine. Therefore, the effect of ethanol in the range 0.5–30% (v/v) mixed with Tween 60 was optimized; the results are presented in Fig. 2(B). No significant differences of signal were obtained up to 20% (v/v) ethanol, above which there was diminished intensity. The decreased signal present at >20% (v/v) ethanol was more severe than in the absence of surfactant. It was supposed that the higher ethanol level led to the disaggregation of micelles. Therefore, 10% (v/v) ethanol with 1.5×10^{-3} mol l^{-1} Tween 60 was selected as the carrier and solvent because of the greater precision and the satisfactory signal obtained.

Various concentrations of polyphosphoric acid were studied over the range 0.01–0.5 mol l^{-1}. The highest intensity was obtained at 0.02 mol l^{-1} acid with the intensity decreasing sharply at higher or lower concentration (Table 3). Next, the effect of potassium permanganate concentration was investigated in the range 1×10^{-4} to 2×10^{-3} mol l^{-1} in

Table 3
Effect of various concentration of potassium permanganate and polyphosphoric acid (PPA) on CL of cinnarizine (C)

[PPA] (mol l^{-1})	CL intensity[a] (mV ± S.D.)	[$KMnO_4$] (10^{-4} mol l^{-1})	CL intensity[b] (mV ± S.D.)	
			1 μg ml^{-1} C	10 μg ml^{-1} C
0.01	10.4 ± 0.19	1.0	0.90 ± 0.04	6.34 ± 0.11
0.02	11.8 ± 0.19	2.5	1.53 ± 0.07	9.84 ± 0.11
0.03	11.0 ± 0.19	3.5	1.80 ± 0.04	12.4 ± 0.08
0.04	10.7 ± 0.11	5.0	2.09 ± 0.09	13.2 ± 0.40
0.05	10.4 ± 0.18	7.5	2.36 ± 0.11	13.7 ± 0.15
0.10	9.30 ± 0.13	10	2.08 ± 0.08	11.9 ± 0.14
0.20	8.11 ± 0.00	15	2.32 ± 0.08	11.7 ± 0.15
0.50	7.13 ± 0.07	20	1.91 ± 0.10	10.0 ± 0.21

[a] CL intensity of cinnarizine 10 μg ml^{-1}.
[b] CL intensity of two concentrations.

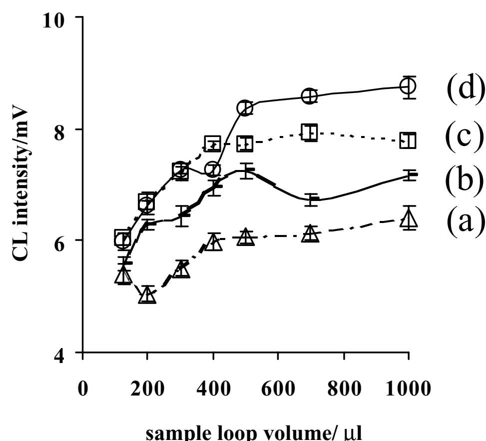

Fig. 3. Effect of flow rate and sample injection volume on CL intensity of cinnarizine 5 µg ml^{-1} at flow rate 3.2, 4.8, 6.3 and 7.6 ml min^{-1} (a–d, respectively).

0.02 mol l^{-1} polyphosphoric acid at two concentrations of cinnarizine (1 and 10 µg ml^{-1} or 2.7×10^{-6} to 2.7×10^{-5} mol l^{-1}). For both drug solutions, the highest CL intensity was obtained at 7.5×10^{-4} mol l^{-1} potassium permanganate (Table 3) and this concentration was applied for subsequent studies.

The influence of total flow rate and injected sample volume were studied simultaneously. Sample loops of 125–1000 µl were investigated and flow rates of 3.3, 4.8, 6.3 and 7.6 ml min^{-1}were tested for each size of loop. Cinnarizine solution (5 µg ml^{-1}) was injected through the sample loop. The results are shown in Fig. 3. In general, the higher the flow rate used, the greater the intensity obtained, although for the smaller sample (100–300 µl), 6.3 and 7.6 ml min^{-1} gave the same responses. There was little or no change intensity at sample volume >500 µl at any flow rate studied. CL increases with flow rate because the reaction is rapid and at high flow rate, more of it takes place in the flow cell. The selected flow rate and sample loop, therefore, were 7.6 ml min^{-1} and 500 µl that gave high sensitivity, good precision and reasonable sample throughput rate (130 h^{-1}).

The optimized conditions consisted of 7.5×10^{-4} mol l^{-1} potassium permanganate in 0.02 mol l^{-1} polyphosphoric acid as the oxidant stream and a carrier stream of 10% (v/v) of ethanol in aqueous 1.5×10^{-3} mol l^{-1} Tween 60 with a total flow rate of 7.6 ml min^{-1} for 500 µl sample injected.

4.4. Method validation

A calibration graph obtained by the use of the optimized system was linear over the range 0.5–6.0 µg ml^{-1} cinnarizine (seven concentrations, 5-replicate injection for each, $y = 2.27x + 0.36$ and $r = 0.9963$). The detection limit (3σ) was 18 ng ml^{-1} (4.9×10^{-8} mol l^{-1}). The precision of the system was studied by injecting cinnarizine solutions at 2.5 and 5 µg ml^{-1}; the %R.S.D. was 1.6 ($n = 10$) and 1.0 ($n = 11$), respectively. The average sample injection rate at 2.5 µg ml^{-1} was 130 h^{-1}.

The possible interferences of common excipients in tablets were investigated. This was done by preparing mixed solutions of foreign compounds and cinnarizine (2.5 µg ml^{-1}) which were tested against a pure solution of cinnarizine at the same concentration. The maximum tolerable concentrations are shown in Table 4. A substance was considered not to interfere if it caused a relative error of <5%. At higher concentrations, all of these substances gave negative interferences.

The recovery of this drug measured by the proposed method, after spiking five amounts of cinnarizine in the sample (tablet A), was in the range 98.4–100.2%. Three samples of commercial cinnarizine tablets (tablets A and B were purchased in Thailand and C in England) were analyzed by preparing the final solution of cinnarizine, 2 µg ml^{-1} in carrier (10% (v/v) ethanol and 1.5×10^{-3} mol l^{-1} Tween 60) thus eliminating the blank signal. The results are presented in Table 5 in comparison with those obtained by a reference method (UV-Vis spectrophotometry [10] using bromocresol green to form ion pairs in chloroform solvent). There was no significant difference (t-test) between the mean values obtained by both methods at 95% confidence. Moreover, reproducibility was studied by seven determinations of each sample. The R.S.D. ($n = 7$) of each was less than 3.0%.

Table 4
Maximum tolerable concentration for the determination of 2.5 µg ml^{-1} cinnarizine

Excipient	Tolerance (µg ml^{-1})
Starch	200
Saccharin sodium	40
Sucrose	200
Glucose	200
Sorbitol	40

Table 5
Determination of cinnarizine in commercial tablets

Sample	Labeled (mg per tablet)	Found ± S.D.[a] (mg per tablet)		t-value[c]
		Proposed method	Reference method[b]	
Tablet A	25	25.4 ± 0.5	25.2 ± 0.4	0.58
Tablet B	25	24.9 ± 0.7	24.9 ± 0.3	1.02
Tablet C	15	14.1 ± 0.3	14.3 ± 0.2	0.06

[a] Standard deviation from seven determinations for proposed method and three determinations for reference method.
[b] Spectrophotometric method [10].
[c] t-critical = 2.45 [71] for $P < 0.05$.

A preliminary evaluation of the determination of the drug in urine was undertaken. Unfortunately, when a blank urine sample (100 × dilution) was injected, there was intense chemiluminescence. This may be from uric acid in urine, for this has been assayed by Li et al. [32] using acidic permanganate CL enhanced with non-ionic surfactant. It would be possible to use HPLC with this CL system post-column for analysis of urine samples. It is also worth investigating whether the use of suitable short columns [70] could remove the interfering substances from the urine sample or let the urine interferences go through while the analyte is retained.

The figures of merit of this proposed method are compared with other methods to determine cinnarizine in Table 6. Although the chromatographic method [4–8] provided a low detection limit and is better to use for biological samples, sophisticated instrumentation, high cost and time consumption are required. The best advantage in this proposed technique beyond other techniques is the high sample throughput and low cost consumption both in reagent and equipment. Although potentiometry with ion selective membrane electrode [13] also is a rapid technique, it needs time and experience to prepare the electrode with the same quality every time, and moreover, its detection limit is less satisfactory and it is difficult to study the dissolution profile. Spectrophotometric techniques [9,10,12]

Table 6
Various methods for determination of cinnarizine

No.	Method	Sample	Linearity range (μg ml^{-1})	LOD (ng ml^{-1})	Sample through-put (h^{-1})	Reference
1	Non-aqueous titration	Raw material	–	–	–	British Pharmacopoeia/ European Pharmacopoeia [2,3]
2	TLC HPLC	Tablet and serum	4–30 ($r = 0.9997$) 0.3–10 ($r = 0.9996$)	16 10	≈1.5 ≈11–12	Hassan et al. [4]
3	HPLC	Plasma	0.020–0.200 ($r > 0.999$)	2	≈6–7	Hund et al. [6]
4	HPLC	Tablet (CIN + DOM)	4–1000 ($r = 0.9998$)	–	≈12[a]	Argekar and Shah [5]
5	HPLC	Tablet (CIN + DOM)	40–120	–	≈12[a]	Zarapkar et al. [7]
6	GC	Biological samples	0.0005–2 ($r = 0.9999$)	0.5	≈12	Woestenborghs et al. [8]
7	Spectrometry (TCNQ)	Tablets	2–18 ($r = 0.9996$)	–	<6	Zhoa et al. [9]
8	Spectrometry (BCG, BCP, BPB)	Tablets and capsules	1–10 ($r > 0.999$)	290–680	–	Abdine et al. [10]
9	Derivative spectrophotometry/ CLS	Tablet CIN + DOM	2.5–25 ($r = 0.9998$)	–	–	Salem et al. [12]
10	Potentiometric PVC membrane sensors	Tablets	1×10^{-2} to 1×10^{-6} M ($r = 0.979$–0.998)	6.2×10^{-6} to 5.1×10^{-5} M	90–180	Hassan et al. [13]
11	FI–CL	Tablets	0.5–6.0 ($r = 0.9963$)	18	130	This proposed method

CIN: cinnarizine; DOM: domperidone; TCNQ: 7,7,8,8-tetracyanoquinodimethane; BCG: bromocresol green; BCP: bromocresol purple; BPB: bromophenol blue; CLS: classical least squares model.
[a] For cinnarizine analysis only.

need organic solvents such as chloroform, methanol or acetone, creating environmental problems; batch implementation takes time and it is not easy to develop in FIA due to the organic solvent use. This FI–CL, the proposed method, is best suited for quality control purposes of pharmaceutical formulations.

5. Conclusions

This proposed FI–CL technique is simple, rapid and economic, which gave good linearity, accuracy, precision and detection limit for the determination of cinnarizine. It is a suitable method for routine analysis and possibly to investigate for automation and in the study of dissolution profile. Moreover, common reagents were used in small amounts, none of which was a serious problem to the environment, especially compared to other methods.

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