Lansoprazole Determination in Pharmaceutical Formulations by Flow Injection Coupled with Acidic KMnO₄–Quinine Chemiluminescence System

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A simple and rapid flow injection chemiluminescence (FI-CL) method based on the reaction of potassium permanganate ($KMnO_4$) and quinine was established for the determination of lansoprazole in pharmaceutical formulations. A linear calibration curve was achieved over the range from 0.01 to 20.0 mg L^{-1} LNP ($R^2 = 0.9997$ ($n = 8$); RSD = 1.1 – 3.7% ($n = 4$)) with a limit of detection of 3.0×10^{-3} mg L^{-1} ($S/N = 3$) and injection throughput of 150 h^{-1}. By applying the Student *t*-test (calculated *t*-test value: $t = 1.059907664$, and tabulated *t*-distributed (95%) = 2.200985) it was found that the proposed method and reported spectrophotometric method were not significantly different. The LNP was efficiently extracted and the recovery of LNP from the spiked pharmaceutical formulations was in the range of 91.0 – 105.9% (%RSD = 1.6 – 3.6, $n = 4$). No significant interference activity was detected from the excipients commonly found in the drug samples analyzed. The possible chemiluminescence emission mechanism is discussed briefly.

Keywords Flow injection analysis, chemiluminescence, lansoprazole, pharmaceutical formulations

Introduction

Lansoprazole (LNP: $C_{16}H_{14}F_3N_3O_2S$, Mw: 369.363 g mol^{-1}; IUPAC, 2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]-methylsulfinyl]-1*H*-benzimidazole) is a type of proton pump inhibitor (PPI) commonly employed for the treatment of gastroesophageal reflux disease where esophagus injury and heart burn are caused by the backward flow of stomach acid. LNP is effective as an inhibitory agent of gastric acid, increases intragastric pH, for the short- and long-term treatment and healing of duodenal ulcer, gastric ulcer as well as erosive reflux esophagitis. It is also used in the treatment of Zollinger–Ellison syndrome, which is a pathological hypersecretory condition, and for the eradication of infection caused by *H. pylori* during dual- and triple-therapy.[1-3] LNP is a substituted benzimidazole, an antisecretory compound that suppresses the secretion of gastric juice. This compound inhibits the enzyme system of (H^+, K^+)-ATPase + + in gastric parietal cell's secretory surface. LNP is considered to act as the inhibitor of gastric acid pump by blocking the acid production's final step and the enzyme is thought to act as an acid (proton) pump.[4-6] The chemical structure of LNP is shown in Fig. 1.

During the past few years, various analytical methodologies have been established for the determination of LNP in diverse samples. These include liquid chromatography with tandem mass spectrometry (LC-MS/MS),[7-10] chiral LC-MS,[11] reverse phase ultra-fast liquid chromatography (RP-UFLC),[12] RP-high performance-LC,[13] LC-diode array detector (LC-DAD),[14] LC-chemometric techniques,[15] LC-UV and LC-MS,[16] potentiometry,[17] capillary zone electrophoresis,[18] colorimetric,[19] Fourier transform infrared spectrometry (FTIR),[20] spectro-photometric and potentiometric methods,[21] and UV-visible spectrophotometric determination.[22-24] In addition, El-Kommos *et al.*[25] and Patel *et al.*[26] reported comprehensive reviews covering most of the chromatographic and electrophoretic methods used for the assay of PPIs including LNP in pharmaceutical and biological samples.

Chemiluminescence (CL) is the production of light in the infrared, visible or ultraviolet regions of electromagnetic spectrum resulting from a chemical reaction.[27] In flow injection analysis (FIA), a small fixed volume of a liquid sample is injected into a moving carrier liquid, flowing through a fine bore tube. The injected sample forms a zone, to produce reactive or detectable species because of chemical reaction that can be measured by any one of a variety of flow-through
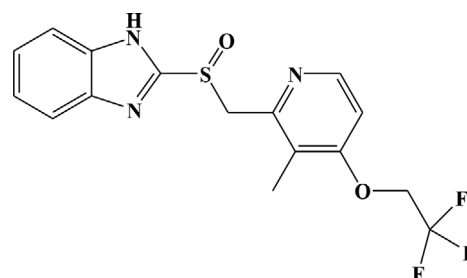
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Fig. 1   Chemical structure of LNP ($C_{16}H_{14}F_3N_3O_2S$, Mw: 369.363 g mol^{-1}).

detectors such as a photomultiplier tube (PMT). This technique is accompanied by several advantages, including high sensitivity and sample throughput, controllable emission rate and safety.[28] Numerous analytical applications of flow injection (FI) techniques with CL detection have been reported for the determination of environmentally, pharmaceutically and biologically important analytes[29-32] using a number of oxidants.[33,34]

Potassium permanganate ($KMnO_4$) as a CL reagent has been extensively used since 1975, when sulfur dioxide (SO_2) was determined in atmospheric samples by developing an analytical method in which $KMnO_4$ was employed as a CL reagent.[35] Review articles show the potential analytical applications of $KMnO_4$ as a CL reagent in different fields of science.[36-38] Recently, a direct CL method using $KMnO_4$ as a CL reagent has been proposed for the determination and on site monitoring of chemical oxygen demand of water samples.[39]   Ma *et al.*[40] developed a FI-CL method for LNP analysis in human serum and tablets based on the enhancement of AgNPs-luminol-$K_3Fe(CN)_6$ CL reaction. The limit of detection (LOD), linear range and relative standard deviation (RSD) have been achieved as 2.0×10^{-4} mg L^{-1} (3σ), 3.0×10^{-3} – 1.5 mg L^{-1} and 1.5% ($n = 11$) for 3.0×10^{-2} mg L^{-1}, respectively, with the recovery in the range 98.0 – 102.5%.

In this work, a FI-CL method is proposed to determine LNP in pharmaceutical formulations based on its enhancement effect on basic DPA-acidic Rh-6G CL reaction with good linearity and a detection limit ($S/N = 3$) of 3.0×10^{-3} mg L^{-1}.   LNP from formulations was extracted with the mixture of propanol:acetic acid:water with satisfactory results. The proposed CL emission mechanism has been thoroughly discussed.

Experimental

Reagents and solution

All chemicals were analytical grade reagents used throughout without further purification, and ultra-high purity (UHP) water (Elga, Purelab Option, High Wycombe, Bucks, UK) was used for cleaning and solution preparations. Glass and plasticware used during experimental works were washed with commercially available surfactant, followed by rinsing with UHP water, then by soaking in 20% (v/v) aqueous hydrochloric acid for almost a week and again rinsing with UHP water several times.

LNP stock solution (1000 mg L^{-1}) was prepared by dissolving the required quantity (10 mg) from its bulk in methanol (10 mL) and stored at 4°C. Serial stock's aliquots were diluted with the solution used as the optimum carrier stream of aqueous methanol and propanol mixture (7.5 and 2.0% v/v respectively) for preparing working standard solutions. Sulfuric acid (H_2SO_4) stock solution (5.0 M) was prepared from commercially available stock solution (18 M) by diluting an appropriate volume with UHP water, and working standard solutions were prepared by serial dilution of stock solution with UHP water. $KMnO_4$ stock solution (0.001 M) was prepared by dissolving 0.0158 g of compound in H_2SO_4 (100 mL, 0.2 M) and fresh working standard solutions were arranged from this stock solution by diluting required aliquots with H_2SO_4 (0.2 M). Quinine sulfate stock solution (0.1 M) was prepared by dissolving 0.782 g of quinine sulfate in H_2SO_4 (10 mL, 0.5 M) and working standards were prepared from this stock solution by serial dilution in H_2SO_4 (0.50 M).

Different surfactant stock solutions (1.0% w/v) were prepared by dissolving 0.1 g sodium dodecyl sulfate (SDS), Triton-X-100, polyoxyethylene sorbitan monolaurate (Tween-20), Tween-80,
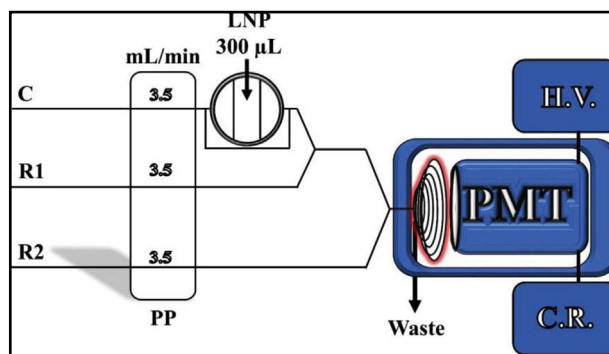

Fig. 2   FI-CL manifold for the determination of LNP. C = Carrier (aqueous methanol and propanol mixture solution, 7.5 and 2% v/v), R1 = quinine (5.0 μM in H_2SO_4 0.5 M), R2 = $KMnO_4$ (250 μM in H_2SO_4 0.2 M), PP = peristaltic pump, H.V. = high voltage, C.R. = chart recorder, PMT = photomultiplier tube.

polyoxyethylene lauryl ether (Brij-35) and cetyltrimethyl-ammonium bromide (CTAB; MP Biomedicals, Solon, OH) separately in 10 mL UHP water and stored at room temperature. Working standards of these stock solutions were prepared and used as a carrier stream containing methanol and propanol, (0.75 and 2.0% v/v) respectively.

Organic compound stock solutions (100 mg L^{-1}), including for sucrose, starch, lactose, fructose, folic acid, riboflavin, polyethylene glycol, pantothenic, and tartaric acid (Merck, Darmstadt, Germany), were prepared by dissolving the required quantity of each compound in UHP water and their working standards were arranged by diluting the required aliquots in the mixture of solutions used as a carrier stream. Anion and cation stock solutions (500 mg L^{-1}), including for chloride (Cl^-), bicarbonate (HCO_3^-), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}) and nitrate (NO_3^-), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), cupric (Cu^{2+}), zinc (Zn^{2+}), ferric (Fe^{3+}), ferrous (Fe^{2+}), manganous (Mn^{2+}) and cobaltous (Co^{2+}) (BDH chemicals Ltd., Poole, UK), were prepared by dissolving an appropriate quantity of each from their respective salts in UHP water.   Working standards were prepared from their stock solutions in methanol and propanol mixture (7.5 and 2.0% v/v) and used for interference study.

Flow injection manifold

The proposed FI-CL manifold used for LNP assay is shown in Fig. 2.   PTFE (polytetrafluoroethylene) tubes (0.8 mm i.d., Fischer Scientific Loughborough, UK) were used for making an injection loop and as connectors or junctions for connecting all the FIA parts. A peristaltic pump (Ismatec, Glattburgg-zueich, Switzerland) was used to deliver all the solutions at a flow rate of 3.5 mL min^{-1}. LNP standard/sample solutions (300 μL) were injected *via* Rheodyne 5020 injection valve (Anachem, Luton, UK) into methanol and propanol mixture (7.5 and 2% v/v) sample carrier stream (C), which was connected and combined at a T-piece with quinine solution (R1, 5.0 μM in H_2SO_4 0.5 M). The mixed stream was then merged with $KMnO_4$ CL reagent (R2, 250 μM in H_2SO_4 0.2 M) and allowed to travel and permitted to pass *via* the spring like glass flow cell (1.5 i.d., 18 mm dia) fitted directly infront of the end window photomultiplier tube (PMT, electron tubes Ruislip, UK) attached with 2 kV PM20SN power supply (electron tubes) and operated at 1250 V. The CL-intensity was recorded on strips of chart recorder (BD40, Kipp & Zonen, delft, Holland).

Sample preparation

The commercially available LNP capsules were purchased from the local market. The contents of five capsules were mixed and thoroughly ground. The weighed quantity of LNP tablet was dissolved in a 50-mL mixture of propanol, acetic acid (17.5 M) and water (9.3:0.25:0.5 v/v/v), respectively, which gave an LNP solution of 12.8 mg L⁻¹. This solution was vigorously shaken for almost 10 min to ensure complete dissolution. A series of aliquots of sample mixture was diluted with aqueous methanol and propanol mixture (7.5 and 2.0% v/v) for a matrix match and analyzed by the proposed FI-CL method and a reported spectrophotometric method[23] by standard addition method for recovery experiments.

Results and Discussion

Optimization studies

To achieve low LOD and limit of quantification (LOQ), high injection throughput and wide dynamic linear range, the effect of different chemical parameters including KMnO$_4$, quinine, H$_2$SO$_4$ and methanol concentrations were checked and optimized. The physical parameters such as sample loop volume, flow rate and PMT voltage were investigated. All optimization studies were performed with LNP standard solution (0.25 mg L⁻¹) and all the measurements were made in triplicate.

Kinetic studies

For kinetic studies, a quartz cuvette (3.0 mL) attached with an injection valve, for the introduction of reagents followed by washing, was fitted in front of an end window PMT connected with a 2-kV power supply and a chart recorder. Initially, 1.0 mL KMnO$_4$ solution (1.0×10^{-4} M in H$_2$SO$_4$ 0.1 M) was injected into the cuvette followed by introducing 1.5 mL quinine solution (2.4 µM in H$_2$SO$_4$ 0.5 M) containing LNP (1.0 mg L⁻¹). The reaction started promptly after mixing the reagents, a maximum CL signal was observed within 6 s and declined to baseline after 30 s (Fig. 3). The kinetic curve indicated the CL method is sensitive enough and suitable to perform the determination of LNP.

Optimization of KMnO$_4$ concentration

KMnO$_4$ is a strong oxidizing and CL reagent in acidic conditions with a standard reduction potential of +1.52 V at 25°C and can acquire 5.0 mol electrons from a reducing agent per mol KMnO$_4$ during a redox reaction, and its CL applications have been thoroughly reviewed.[34] The concentration of KMnO$_4$ considerably affected the CL emission intensity during the proposed CL reaction making its optimization essential. Therefore, KMnO$_4$ concentration was optimized from 1.0 to 500 µM and the highest and most reproducible CL signals were obtained when its concentration reached 250 µM as shown in Fig. 4(A). Further increase in KMnO$_4$ concentration resulted in a decrease in CL intensity and, therefore 250 µM was selected as the optimum to be employed in subsequent experiments.

Optimization of quinine concentration

A number of sensitizers such as rhodamine 6-G, rhodamine-B, pyrogallol, quinine, rose bengal and fluorescein were checked in the second channel as sensitizers, but quinine was found to be the most suitable because it produced the highest enhancement of CL signals for LNP. This is possibly due to the lack of self-absorption by quinine because its solution is almost colorless in comparison to the other mentioned sensitizers. Secondly, quinine has shown strong fluorescence emission in acidic medium especially in H$_2$SO$_4$ and the proposed CL reaction takes
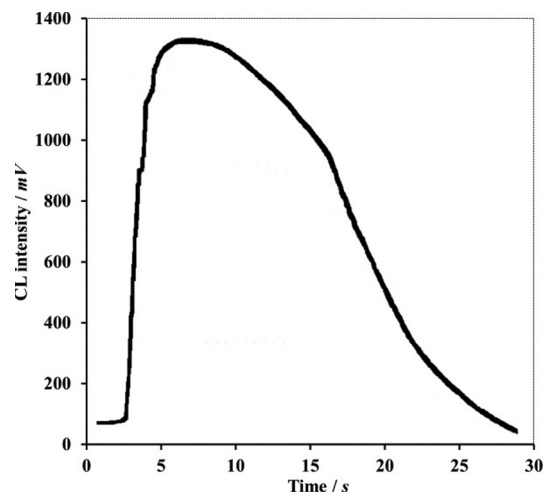

Fig. 3   Kinetic transient curve for KMnO$_4$-quinine-LNP CL reaction. Conditions: 1.0 mL KMnO$_4$ (0.1 mM in H$_2$SO$_4$ 0.1 M), 1.5 mL quinine (2.4 µM in H$_2$SO$_4$ 0.5 M) containing LNP (1.0 mg L⁻¹), PMT voltage 1000 V, chart recorder speed 0.5 mm s⁻¹.

place in H$_2$SO$_4$ acidic medium. The concentration of quinine was optimized from 1.0 up to 25 µM. LNP CL signal intensity was increased up to 5.0 µM and was selected as the optimum. The intensity of CL signal intensity decreased when quinine concentration was increased further, as shown in Fig. 4(B).

Optimization of H$_2$SO$_4$ concentration of KMnO$_4$ stream

KMnO$_4$ gives CL emission in an acidic environment because it acts as a stronger oxidant in acidic medium than basic or neutral medium. Therefore, various acids were compared, such as HCl, HNO$_3$, HClO$_4$, H$_2$SO$_4$ and H$_3$PO$_4$. The most suitable acid was found to be H$_2$SO$_4$ due to the appearance of the highest and most reproducible CL signals. H$_2$SO$_4$ concentration was optimized from 10 to 300 mM and the highest and most reproducible CL response was observed at 200 mM H$_2$SO$_4$, as shown in Fig. 4(C), and was selected as the optimum for further studies.

Optimization of H$_2$SO$_4$ concentration of quinine stream

Quinine in the proposed CL reaction acts as a sensitizer, which is freely soluble in H$_2$SO$_4$ and gives enhanced CL signals in an acidic environment. Therefore, H$_2$SO$_4$ concentration was optimized from 0 to 600 mM and the highest and most reproducible CL signals were observed when its concentration reached up to 500 mM, as shown in Fig. 4(D), and this was selected as the optimum for further studies. Beyond this concentration, the emission intensity declined.

Optimization of methanol concentration

During the experimental work, it was observed that LNP is more soluble in organic solvents such as methanol, ethanol, chloroform, propanol, butanol, *etc.* than water. Therefore, the effect of methanol concentration, acting as the carrier stream for a matrix match, was checked from 0.1 to 10% (v/v). Figure 4(E) shows the increase in CL signal intensity with the increase in methanol percentage up to 7.5% (v/v) due to an increase in solubility of LNP and was selected as the optimum for subsequent experiments. Further increase in methanol concentration resulted in quenching of CL emission. In addition, various surfactants such as Brij-35, Triton X-100, Tween-20, Tween-80, SDS and CTAB (each 0.1% in 7.5% v/v aqueous
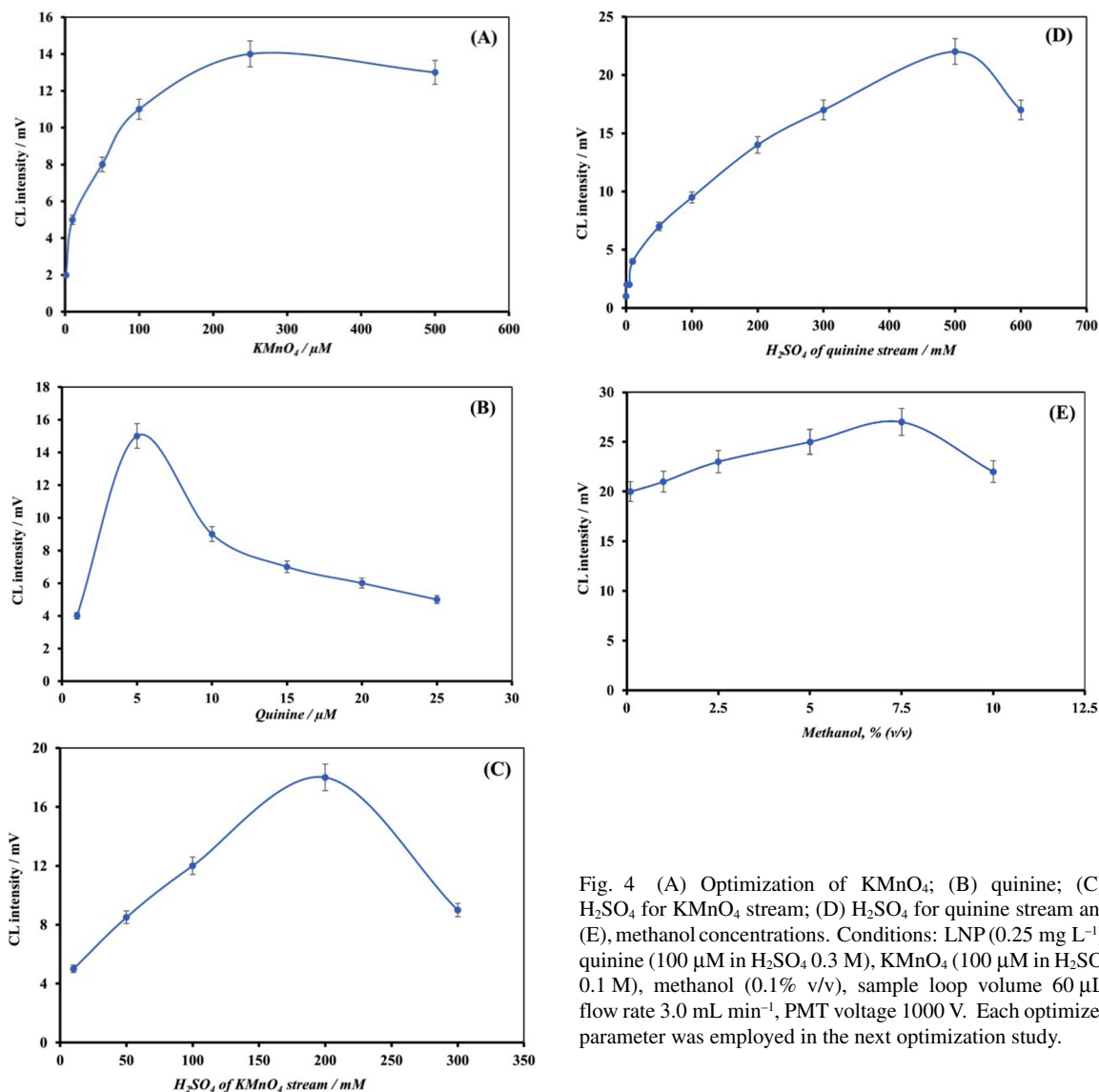
Fig. 4   (A) Optimization of KMnO$_4$; (B) quinine; (C), H$_2$SO$_4$ for KMnO$_4$ stream; (D) H$_2$SO$_4$ for quinine stream and (E), methanol concentrations.   Conditions: LNP (0.25 mg L^{-1}), quinine (100 μM in H$_2$SO$_4$ 0.3 M), KMnO$_4$ (100 μM in H$_2$SO$_4$ 0.1 M), methanol (0.1% v/v), sample loop volume 60 μL, flow rate 3.0 mL min^{-1}, PMT voltage 1000 V.   Each optimized parameter was employed in the next optimization study.

methanol) were propelled as carrier streams, but none of them increased CL signals for LNP appreciably and, therefore the idea of using surfactants in the carrier stream was abandoned. Similarly, the effect of n-propanol concentration was checked from 0.1 – 10% (v/v).   This experiment was performed due to a matrix effect because sample preparation needs propanol, which was found to enhance the CL intensity slightly.   The most appropriate propanol concentration was found to be 2.0% (v/v) and selected as optimum.   The effect of acetic acid concentration from 1.0×10^{-4} to 0.1 M was also examined in carrier stream, but the intensity of CL signals was neither enhanced nor quenched.   Therefore, acetic acid was not used further in the carrier stream.

Optimization of physical parameters

   Table 1 shows the optimization of physical parameters such as flow rate, sample injection volume and PMT voltage.   As kinetic studies showed that the kinetics of the proposed reaction is fast, the effect of flow rate was checked from 0.5 to 4.0 mL min^{-1}. The CL emission intensity was increased as the flow rate was increased up to 3.5 mL min^{-1} and was selected as the optimum. Sample injection volume was checked from 60 to 420 μL; the emission intensity increased with the increase in sample volume linearly, but 300 μL was selected for subsequent experiments

Table 1   Optimization of physical parameters under the optimized chemical parameters; each optimized parameter was employed in the experimental studies

S. No.	Physical parameter	Range studied	Optimum
1	Flow rate/mL min^{-1}	0.5 – 4.0	3.5
2	Sample volume/μL	60 – 420	300
3	PMT voltage/V	800 – 1300	1250

due to economy of sample consumption.   Signal emission intensity increased in a curve linear fashion with the increase in PMT voltage from 800 to 1300 V, but 1250 V was selected as an optimum to avoid any PMT damage.

Analytical figures of merit and application

   Under optimized parameters, a calibration curve was obtained between the concentration of LNP (mg L^{-1}, taken at abscissa) and CL intensity (mV, taken on ordinate).   Figure 5 shows the chart recorder traces under optimized parameters and the inset shows the LNP calibration curve (range 0.01 – 20.0 mg L^{-1}). The LNP quantity enhanced the CL intensity linearly over a dynamic range 0.01 – 20 mg L^{-1} with a regression equation

$y = 89.797x + 13.954$, coefficient of determination (R^2) 0.9997 (where y is the CL intensity in mV and x is LNP concentration in mg L^{-1}) and LOD of 3.0×10^{-3} mg L^{-1}, which was calculated as the amount of LNP required to yield CL signals three times the standard deviation of the blank signal (3σ of the blank). RSDs for blank, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15 and 20 mg L^{-1} were 1.2, 3.7, 3.32, 3.06, 2.63, 2.51, 1.21, 1.19, 1.18 and 1.10%, respectively. The sample throughput was 150 h^{-1}.

Table 2 reports the comparison of analytical characteristics of the proposed method with previously reported methods for the determination of LNP in different samples. Although some of
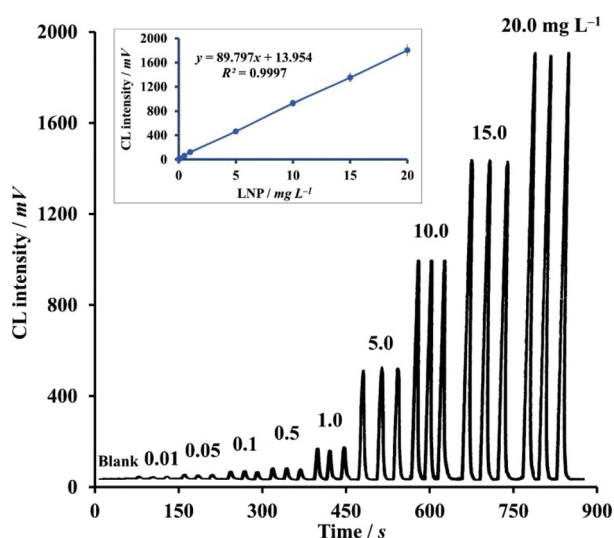
these methods are sensitive, selective, accurate, and have good efficiency of separation, they suffer from drawbacks such as expensive equipment, time-consuming procedures, large consumption of reagents, temperature sensitivity, and low sample throughput. However, the proposed CL system provides comparatively satisfactory linearity with high sample throughput.[40]

Interference study

Different metal ions, anions and organic compounds may be present as excipients in pharmaceutical formulations of LNP and were checked on the blank (in the absence of LNP) and on the LNP determination. The concentrations (0.005, 0.05, 0.5 and 10 mg L^{-1}) of the possible interferent chemical species were injected into the proposed manifold in blank and with LNP standard 0.05 mg L^{-1}. The tolerance limit of the foreign chemical species was taken as the concentration that caused the relative error of less than ±5% in the CL intensity. Chemical species such as Cl$^-$, HCO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$, NO$_3^-$, Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, Mn^{2+}, Co^{2+}, starch, folic acid, polyethylene glycol, tartaric acid, lactose, fructose, riboflavin, tween-80 and pantothenic acid at 200 fold and Fe^{2+}, phenol, sucrose and glucose up to 100 fold did not show any interferent activity as shown in Table 3. Therefore, the method is suitable for the quantitative analysis of LNP in pharmaceutical formulations in the presence of the above chemical species. In addition, different standards over the range of 0.1 – 5 mg L^{-1} of another PPI, namely omeprazole, were injected into the proposed FI-CL manifold in blank and on the determination of 0.05 mg L^{-1} LNP. As a result, no interference effect was observed due to omeprazole.

Validity

Table 4 shows the %recoveries for the proposed FI-CL method and a reported spectrophotometric method,[23] which were

Fig. 5   Chart recorder traces under optimized parameters; inset is the LNP calibration curve (range 0.01 – 20.0 mg L^{-1}).

Table 2   Comparison of analytical performance of different analytical methodologies for the determination of LNP

Technique	Matrix	LOD/mg L^{-1}	LOQ/mg L^{-1}	Linear range/mg L^{-1}	R^2	Sample/h^{-1}	Ref.
LC-MS/MS	Plasma	—	0.001	0.001 – 0.500	0.9938	—	7
LC-MS/MS	Plasma	—	0.00303	0.02 – 5.0	0.9917	—	8
LC-MS/MS	Plasma	—	0.003	0.003 – 0.8	0.999	06	9
LC-MS/MS	Plasma	0.004	0.0046	0.0045 – 2.8	0.999	—	10
LC-MS/MS	Plasma	—	0.005	0.005 – 3.0	0.9994	07	11
RP-UFLC-PDA	Tablet	0.25	1.0	1.0 – 300	0.999	—	12
RP-HPLC	Synthetic mixtures	0.2716	0.8450	2.0 – 10	0.998	09	13
LC-DAD	Plasma	—	0.2	0.2 – 2.0	0.9910	06	14
LC-Chemom	Drug	—	—	5.0 – 25	0.9984 – 1.0000	—	15
LC-UV	Plasma	0.0015	0.005	0.0688 – 2.2	>0.999	—	16
LC-MS		—	7.3×10^{-7}	2.5×10^{-6}	2.2×10^{-5} – 0.011	>0.989	
Pot.	Drug	5.8	—	7.4 – 739	0.9999	—	17
CZE	Capsules	0.0028	0.008	0.008 – 0.4	0.9992	18	18
Colorimetry	Standards	0.093	0.98	0.8 – 8.8	R = 0.998	—	19
FTIR	Drug	7.0	20	20 – 800	0.9973	—	20
Spec and	Drug	—	—	15 – 200	0.9998	—	21
Pot		—	—	15 – 100	—		
Spec-I	Capsules	0.62	1.89	2.0 – 32	0.999	—	22
Spec-II		—	0.08	0.23	0.8 – 12.0	0.999	
Spec.	Drug	0.033	0.101	0.250 – 20.00	0.9999	—	23
Spec.	Synthetic mixtures	0.059	0.179	5.0 – 25	>0.996	—	24
FI-CL	Serum and tablets	2.0×10^{-4}	—	0.003 – 1.5	0.9992	—	40
FI-CL	Drug	0.003	0.01	0.01 – 20	0.9995	150	This method

LC-MS, Liquid chromatography–mass spectrometry; RP-UFLC-PDA, reverse-phase ultrafast liquid chromatography–photo diode array; DAD, diode array detector; Chemom, chemometrics; UV, ultraviolet; Pot, potentiometry; CZE, capillary zone electrophoresis; FTIR, Fourier transform infrared; Spec, spectrophotometry.

91 – 105.9% (%RSD = 1.6 – 3.6, $n = 4$), and 90.5 – 109.1% (%RSD = 1.5 – 4.7, $n = 4$) respectively. For 0.5, 5.0 and 15.0 mg L^{-1} LNP, the intraday %RSDs were 3.7, 1.21 and 1.18, and the interday %RSDs were 4.562, 2.531 and 2.1. Furthermore, Table 4 reports the results obtained for the determination of LNP in un-spiked and spiked pharmaceutical samples both by the proposed FI-CL method and the reported spectrophotometric method.[23] Paired Student t-test was applied to the results of both methods and $t_{calc.}$ was obtained as 1.059907664, which is lower than $t_{tab.}$ 2.200985 at 95% confidence level. These results show that the results of both methods were not significantly different from each other.

Possible CL reaction mechanism

The KMnO$_4$ CL reaction mechanism is one of the most explored in literature, and generalized reaction schemes and light producing pathways have been reported.[42] According to the literature, an analyte reduces Mn(VII) in acidic medium with simultaneous generation of Mn^{3+} and intermediate radicals of the analyte in a multistep redox reaction (Reaction 1). In the next step (Reaction 2), the intermediate radicals of the analyte generated in the previous step reduce Mn^{3+} into electronically excited Mn^{2+},[42] which acts as a CL emitter. This electronically excited Mn^{2+} comes to a ground state by emitting red-light (Reaction 3) with $\lambda_{max.}$ of 734 ± 5 nm.[43] The quinine is a very good fluorescent substance, having an emission maximum at about 450 nm.[44] The excited Mn^{2+} may transfer its energy to the quinine molecule through intermolecular collision to the quinine molecule (Reaction 4). The excited quinine molecule undergoes de-excitation and emits intense light centered at about 450 nm (Reaction 5), which is indirectly related to LNP concentration.

Keeping the above discussion in consideration, the most possible CL reaction mechanism can be written as follows.

$$Mn^{7+} + LNP \longrightarrow Mn^{3+} + \text{intermediate radicals} \qquad (1)$$

$$Mn^{3+} + \text{intermediate radicals} \longrightarrow Mn^{2+*} + \text{other products} \quad (2)$$

$$Mn^{2+*} \longrightarrow Mn^{2+} + hv \ (734 \pm 5 \ nm) \qquad (3)$$

$$Mn^{2+*} + \text{quinine} \longrightarrow Mn^{2+} + \text{quinine*} \qquad (4)$$

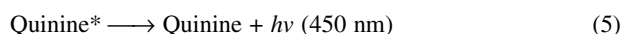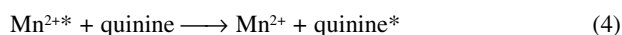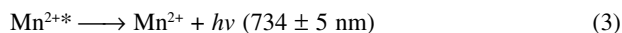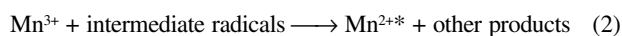$$\text{Quinine*} \longrightarrow \text{Quinine} + hv \ (450 \ nm) \qquad (5)$$

Figure 6 shows the transient peaks for the CL reaction among LNP-KMnO$_4$-quinine in flow mode. The curve-A was obtained when aqueous methanol and propanol mixture solution (7.5 and 2% v/v) was propelled in three channels and KMnO$_4$ (300 μL,
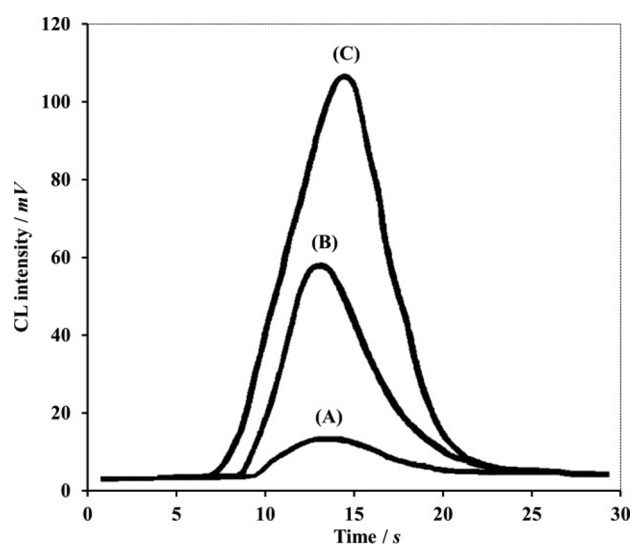

Fig. 6　Transient peaks for the CL reaction of LNP-KMnO$_4$-quinine under flow-mode.   Curve-A = KMnO$_4$ (240 μM in H$_2$SO$_4$ 0.2 M), Curve-B = KMnO$_4$ (240 μM in H$_2$SO$_4$ 0.2 M) and LNP (1.0 mg L^{-1}) and Curve-C = KMnO$_4$ (240 μM in H$_2$SO$_4$ 0.2 M), LNP (1.0 mg L^{-1}) and quinine (5.0 μM in H$_2$SO$_4$ 0.5 M).   Physical parameter: PMT voltage 1250 V, chart recorder speed 1 mm s^{-1}, flow rate 3.5 mL min^{-1} and sample volume 300 μL.

Table 3　Tolerable concentration (fold) of foreign species on LNP 0.05 mg L^{-1}

Chemical species	Tolerable concentration (fold)
Cl$^-$, HCO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$, NO$_3^-$, Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, Mn^{2+}, Co^{2+}, starch, folic acid, polyethylene glycol, tartaric acid, lactose, fructose, riboflavin, tween-80 and pantothenic acid	200
Fe^{2+}, phenol, sucrose, glucose	100

Table 4　LNP recovery from pharmaceutical capsules and its comparison with a reported method

Sample matrix	Spiked/ mg L^{-1}	Proposed FI-CL method			Spectrophotometric method[23]		
		Found/mg L^{-1}	Recovery, %	RSD, % ($n = 4$)	Found/mg L^{-1}	Recovery, %	RSD, % ($n = 4$)
Capsule-I	0.00	0.26	—	2.1	0.24	—	1.5
	0.25	0.47	92.2	2.2	0.52	106.1	3.4
	0.50	0.71	93.4	2.5	0.67	90.5	1.6
	0.75	0.96	95.0	1.6	1.08	109.1	2.3
Capsule-II	0.00	0.26	—	3.1	0.23	—	2.2
	0.25	0.52	101.9	2.3	0.46	95.8	1.8
	0.50	0.78	102.6	2.3	0.74	101.4	3.1
	0.75	1.07	105.9	3.4	0.93	94.9	4.7
Capsule-III	0.00	0.28	—	3.6	0.26	—	4.5
	0.25	0.56	105.7	2.5	0.48	94.1	3.3
	0.50	0.71	91.0	1.9	0.73	96.1	2.3
	0.75	0.98	95.2	1.8	0.98	97.0	3.8

Student t-test value: t = 1.059907664, t-distributed (95%) = 2.200985.

240 μM in H_2SO_4 0.5 M) was injected into the carrier stream, which shows that the $KMnO_4$ in an acidic condition emits weak CL in the absence of either LNP or quinine. The curve-B was obtained when $KMnO_4$ (240 μM in H_2SO_4 0.5 M) was propelled in the stream as shown in the proposed manifold, and LNP (1.0 mg L^{-1}) was injected into the carrier stream that has shown a strong CL emission due to the reaction of LNP and $KMnO_4$. The curve-C was obtained when $KMnO_4$ and quinine (5.0 μM in H_2SO_4 0.1 M) were propelled in their respective streams and LNP (1.0 mg L^{-1}) standard solution was injected, and as a result, an enhanced transient CL peak was obtained. It can be therefore concluded that the CL is emitted due to the redox reaction of LNP and $KMnO_4$, which is further enhanced by quinine.

Conclusions

In this work, a CL reaction based on the reduction of acidic $KMnO_4$ in the presence of quinine by LNP has been reported. LNP possibly acted as a reducing agent and the highest and intense CL emission was detected for it during the redox reaction. The linear dynamic range was obtained for LNP as 0.01 – 20 mg L^{-1} ($y = 89.797x + 13.954$, $R^2 = 0.9997$)   with LOD of 3.0×10^{-3} mg L^{-1} and %RSD of 1.2 – 3.6% over the range studied with sample throughput of 150 h^{-1}. LNP was determined in pharmaceutical formulations showing the % recoveries as 91 – 105.9% (%RSD = 1.6 – 3.6, $n = 4$) and validated by a reported spectrophotometric method by applying the paired Student t-test ($t = 1.059907664$, t-distributed (95%) = 2.200985). No interference activity was found from the commonly found excipients in LNP formulations. The probable CL mechanism has been thoroughly studied and written.

Acknowledgements

References

1. R. J. Brummer, B. J. Geerling, and R. W. Stockbrugger, *Digest. Dis. Sci.*, **1997**, *42*, 2132.
2. K. G. Tolman, S. W. Sanders, K. N. Buchi, M. D. Karol, D. E. Jennings, and G. L. Ringham, *J. Clin. Gastroenterol.*, **1997**, *24*, 65.
3. D. S. Threlkeld, "Gastrointestinal Drugs, Proton Pump Inhibitors", In Facts and Comparisons Drug Information, **1998**, St. Louis, MO, Facts and Comparisons, 305.
4. A. Fitton and L. Wiseman, *Drugs*, **1996**, *51*, 460.
5. A. J. Matheson and B. Jarvis, *Drugs*, **2001**, *61*, 1801.
6. R. L. Bown, *Int. J. Clin. Pract.*, **2002**, *56*, 132.
7. L. Luo, X. Wen, Y. Du, Z. Jiang, and X. Guo, *Biomed. Chromatogr.*, **2018**, *32*, e4345.
8. E. F. Elkady, M. A. Fouad, and B. M. Jaadan, *J. Chromatogr. B*, **2018**, *1076*, 61.
9. H. Wang, Y. Sun, X. Meng, B. Yang, J. Wang, Y. Yang, and J. Gu, *J. Sep. Sci.*, **2015**, *38*, 2960.
10. R. N. Kachave, M. Kale, and R. D. Wagh, *Open Anal. Chem. J.*, **2015**, *8*, 7.
11. L. Sun, Y. Cao, H. Jiao, Y. Fang, Z. Yang, M. Bian, H. Zhang, X. Gong, and Y. Wang, *J. Sep. Sci.*, **2015**, *38*, 3696.
12. S. S. Panda, V. V. R. K. Bera, S. Beg, and O. Mandal, *J. Liq. Chromatogr. Relat. Technol.*, **2017**, *40*, 479.
13. F. J. Chodvadiya, K. C. Thula, and D. G. Maheshwari, *Int. J. Rec. Sci. Res.*, **2015**, *6*, 3385.
14. R. P. Horta, B. do Amaral, P. G. Peralta-Zamora, and B. J. G. Silva, *J. Chromatogr. Sci.*, **2018**, *56*, 564.
15. A. H. Aktas and A. M. Sarıdag, *J. Chromatogr. Sci.*, **2017**, *55*, 798.
16. K. Kostolanska, O. Pes, O. Zendulka, J. Machal, and J. Jurica, *Chem. Pap.*, **2019**, 1.
17. N. Rahman and S. Khan, *Ind. Eng. Chem. Res.*, **2018**, *57*, 9351.
18. H. K. Chung, Q. K. Truong, X. L. Mai, Y. Choi, J. S. Kang, W. Mar, and K. H. Kim, *Arch. Pharmacal Res.*, **2017**, *40*, 962.
19. M. Y. Nassar, E. A. El-Moety, and M. F. El-Shahat, *RSC Adv.*, **2017a**, *7*, 43798.
20. P. Y. Khashaba, H. R. H. Ali, and M. M. El-Wekil, *Spectrochim. Acta, Part A*, **2018**, *190*, 10.
21. M. Y. Nassar, M. F. El-Shahat, S. M. Khalil, and E. A. El-Moety, *Indian J. Pharm. Sci.*, **2017b**, *79*, 420.
22. S. A. Abdulrahman, O. Z. Devi, K. Basavaiah, and K. B. Vinay, *J. Taib. Uni. Sci.*, **2016**, *10*, 80.
23. H. Mandil, A. A. Sakur, and A. A. Allabban, *Res. J. Pharm. Technol.*, **2017**, *10*, 1417.
24. A. Bhim, F. Buchiy, H. Raj, and V. Jain, *PharmaTutor*, **2015**, *3*, 38.
25. M. E. El-Kommos, P. Y. Khashaba, H. R. H. Ali, and M. M. El-Wekil, *J. Liq. Chromatogr. Relat. Technol.*, **2015**, *38*, 1639.
26. M. M. Patel, S. D. Bhuva, and M. M. Patel, *Rev. Anal. Chem.*, **2015**, *34*, 29.
27. A. M. Garcia-Campana and F. J. Lara, *Anal. Bioanal. Chem.*, **2007**, *387*, 165.
28. J. Ruzicka and E. H. Hansen, "*Flow Injection Analysis*" **1988**, Vol. 62, John Wiley and Sons.
29. I. I. Timofeeva, C. S. Vakh, A. V. Bulatov, and P. J. Worsfold, *Talanta*, **2018**, *179*, 246.
30. T. H. Hasanin and T. Fujiwara, *Anal. Sci.*, **2018**, *34*, 777.
31. M. Ahmed, M. Asghar, M. Yaqoob, N. Munawar, F. Shahid, M. Asad, and A. Nabi, *Anal. Sci.*, **2017**, *33*, 1259.
32. A. Miyamoto, S. Nakano, K. Nagai, N. Kishikawa, K. Ohyama, T. Aoyama, Y. Matsumoto, and N. Kuroda, *Anal. Sci.*, **2017**, *33*, 697.
33. B. Li, Z. Zhang, and M. Wu, *Talanta*, **2000**, *51*, 515.
34. M. Su, P. Chen, and H. Sun, *TrAC, Trends Anal. Chem.*, **2018**, *100*, 36.
35. J. Stauff and W. Jaeschke, *Atmos. Environ.*, **1975**, *9*, 1038.
36. J. L. Adcock, N. W. Barnett, C. J. Barrow, and P. S. Francis, *Anal. Chim. Acta*, **2014**, *807*, 9.
37. B. J. Hindson and N. W. Barnett, *Anal. Chim. Acta*, **2001**, *445*, 1.
38. J. L. Adcock, P. S. Francis, and N. W. Barnett, *Anal. Chim. Acta*, **2007**, *601*, 36.
39. T. K. Do, S. Hashimoto, H. Nishikawa, Y. Maeda, and N. Takenaka, *Anal. Sci.*, **2017**, *33*, 931.
40. Y. Ma, Y. T. Zhang, H. M. Liu, H. Liu, and H. J. Shuang, *Chin. J. Anal. Lab.*, **2015**, *4*, 17.
41. T. Slezak, Z. M. Smith, J. L. Adcock, C. M. Hindson, N. W. Barnett, P. N. Nesterenko, and P. S. Francis, *Anal. Chim. Acta*, **2011**, *707*, 121.
42. C. M. Hindson, P. S. Francis, G. R. Hanson, J. L. Adcock, and N. W. Barnett, *Anal. Chem.*, **2010**, *82*, 4174.
43. J. L. Adcock, P. S. Francis, T. A. Smith, and N. W. Barnett, *Analyst*, **2008**, *133*, 49.
44. J. R. Lakowicz, "*Principles of Fluorescence Spectroscopy*", 2nd ed., **1999**, Kluwer Academic Plenum Press, New York, 53.