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ORIGINAL PAPER

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Investigating the post-chemiluminescence behavior of phenothiazine medications in the luminol–potassium ferricyanide system: molecular imprinting–post-chemiluminescence method for the determination of chlorpromazine hydrochloride

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Abstract A new post-chemiluminescence (PCL) phenomenon was observed when phenothiazine medications were injected into the reaction mixture after the chemiluminescence (CL) reaction of luminol and potassium ferricyanide had finished. A possible reaction mechanism was proposed based on studies of the kinetic characteristics of the CL, CL spectra, fluorescence spectra, and on other experiments. The feasibility of determining various phenothiazine medications by utilizing these PCL reactions was examined. A molecular imprinting–post-chemiluminescence (MI-PCL) method was established for the determination of chlorpromazine hydrochloride using a chlorpromazine hydrochloride-imprinted polymer (MIP) as the recognition material. The method displayed high selectivity and high sensitivity. The linear range of the method was 1.0×10^{-8} ~1.0×10^{-6}, with a linear correlation coefficient of 0.9985. The detection limit was 3×10^{-9} g/ml chlorpromazine hydrochloride, and the relative standard deviation for a 1.0×10^{-7} g/ml chlorpromazine hydrochloride solution was 4.0% (n=11). The method has been applied to the determination of chlorpromazine hydrochloride in urine and animal drinking water with satisfactory results.

Keywords Post-chemiluminescence (PCL) · Molecular imprinted polymer (MIP) · Phenothiazine medications · Chlorpromazine hydrochloride

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Introduction

The reaction between luminol and potassium ferricyanide is a chemiluminescence (CL) reaction. A peculiar CL (PCL) phenomenon has been reported to occur when some metal ions are present in the luminol–potassium ferricyanide system [1], and it has been predicted that there are other substances that will cause PCL reactions in this system.

Phenothiazine medications such as chlorpromazine hydrochloride, fluphenazine hydrochloride, perphenazine, trifluoperazine hydrochloride, thioridazine hydrochloride and imipramine hydrochloride exhibit PCL behavior when present in the luminol–potassium ferricyanide system; in other words, a new CL reaction is initiated when these phenothiazine medications are injected into the reaction mixture after the CL reaction between alkaline luminol and potassium ferricyanide is finished. One possible mechanism for this reaction was proposed based on studies of the kinetic characteristics of the CL, CL spectra, fluorescence spectra, and on other experiments.

In this work, the feasibility of determining various phenothiazine medications by utilizing their relevant PCL reactions was examined. Because most phenothiazine medications initiate PCL reactions when present in the luminol–potassium ferricyanide system, this PCL-based method of determining phenothiazine medications didn't exhibit very good selectivity, and therefore couldn't be applied to the direct determination of phenothiazine medications in complicated samples.

In order to improve the selectivity of the CL method, MIPs capable of recognizing the target molecule have also been used in CL analysis [2]. Chlorpromazine hydrochloride was selected as a representative phenothiazine medication and a MI-PCL-based method of determining this substance was established that used a chlorpromazine hydrochloride MIP as the recognition material. This method displayed a high selectivity and was applied to

the determination of chlorpromazine hydrochloride in urine and animal drinking water with satisfactory results.

Dollowing on from this work, highly selective MI-PCL methods could potentially be used to determine other phenothiazine medications in complicated samples.

Experimental

Apparatus

Schematic diagrams of the FI-PCL and MI-PCL flow systems used in this work are shown in Figs. 1 and 2, respectively. The reagent solutions and sample solutions were delivered using two peristaltic pumps. PTFE tubes (0.8 mm i.d.) were used to connect all of the components in the flow system. CL measurements were performed using an IFFL-D flow injection CL analyzer (Remax Electronic High-Tech Ltd., Xi'an, China). Data acquisition and data treatment were both performed with the IFFL-D flow-injection CL data processing software (Remax Electronic High-Tech Ltd.). CL spectra were obtained using a refitted RF-540 fluorescence spectrophotometer (Shimadzu, Kyoto, Japan). Absorbance was measured using a TU-1901 UV-visible spectrophotometer (Beijing Currency Instrumental Ltd., China). A 970CRT fluorescence spectrophotometer (General Analytical Instrument Factory, Shanghai, China) was used to extract spectra. An ultrasonator (Ultrasonic Instruments Ltd., Kunshan, China) was used to promote mixing.

Reagents

Chlorpromazine hydrochloride was purchased from the Funing Shengda Medicine Chemical Co., Ltd. (Jiangsu, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma (St. Louis, MO, USA). Methacrylic acid (MAA) and 2,2′-azobis(2-methylpropionitrile) (AIBN) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Other reagents were purchased from Xi'an Chemical Reagent Factory (Xi'an, China). All of the reagents used were of analytical reagent grade except for AIBN, which was chemical purity grade.

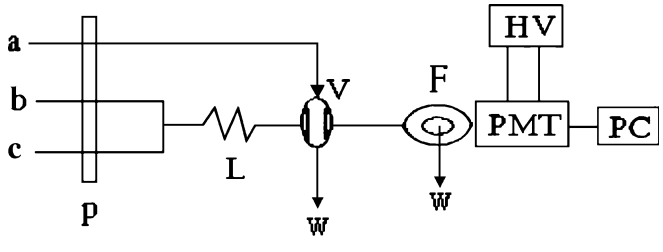

Fig. 1 Schematic diagram of the FI-PCL flow system: (*a*) sample solution; (*b*) luminol solution; (*c*) potassium ferricyanide solution; (*P*) peristaltic pump; (*L*) mixing tube; (*V*) injection valve; (*F*) flow cell; (*HV*) high voltage; (*PMT*) photomultiplier tube; (*PC*) personal computer; (*W*) waste solution

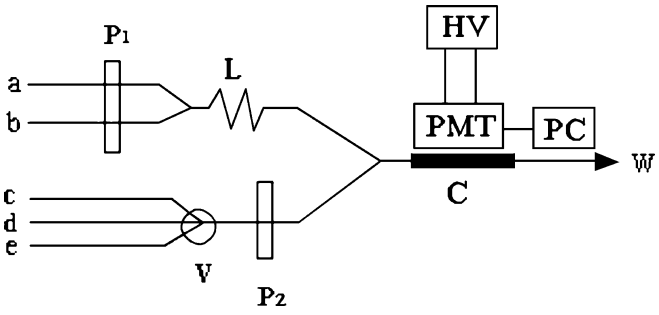

Fig. 2 Schematic diagram of the MI-PCL flow system: (*a*) potassium ferricyanide solution; (*b*) luminol solution; (*c*) dioxane solution; (*d*) sample solution; (*e*) ethanol; (P_1, P_2) peristaltic pumps; (*L*) mixing tube; (*V*) switch valve; (*C*) MIP column; (*PMT*) photomultiplier tube; (*HV*) high voltage (*PC*) computer; (*W*) waste solution

EGDMA and MAA were redistilled and AIBN was recrystallized prior to use.

A stock standard solution of chlorpromazine hydrochloride (1.00×10^{-4} g/ml) was prepared by dissolving 0.0100 g of chlorpromazine hydrochloride in 100 ml of doubly distilled water. Working standard solutions of chlorpromazine hydrochloride were prepared by diluting the stock solution with doubly distilled water. The chlorpromazine hydrochloride stock solutions were stored in the refrigerator at 4 °C and protected from light. Potassium ferricyanide stock solution (5.0×10^{-2} mol/l) was prepared with doubly distilled water. Luminol stock solution (1.0×10^{-2} mol/L^{-1}) was prepared by dissolving 1.771 g of luminol (synthesized by the Institute of Analytical Science of Shaanxi Normal University, China, purity >95%) in 1000 ml of 0.01 mol/l NaOH. Doubly distilled water was used throughout the experiments.

Synthesis of chlorpromazine hydrochloride MIP and preparation of the MIP column

The chlorpromazine hydrochloride MIP was synthesized and packed into a column according to the following method [3]. One mmol (0.3553 g) of chlorpromazine hydrochloride, 6 ml of chloroform and 4 mmol of MAA were added to a 50 ml flask. The mixture was oscillated in an ultrasonic bath for 4 h to allow the MAA to mix with the chlorpromazine hydrochloride molecules sufficiently. Then 20 mmol of EGDMA and 50 mg of AIBN were added, and the solution was purged with nitrogen for 15 min and sealed under vacuum. The polymerization reaction was carried out at 60 °C in a water bath for 24 h. The polymers obtained were crushed, ground and sieved to collect the particles of size 74~105 μm for subsequent experiments.

A nonimprinted polymer (NIP) was synthesized in the same way, only without the template molecule.

Before the adsorption experiments, the chlorpromazine hydrochloride molecules in the MIP were removed by washing with a mixture of carbinol/acetic acid (9:1, v/v) until the adsorbance peak (λ=254 nm) due to chlorpromazine hydrochloride was no longer detected in the elution

solution. The polymer was dried to a constant weight at 60 °C under vacuum.

A portion of the polymer particles (20 mg) collected as described above was packed into a colorless glass tube (4 mm i.d.×25 mm length) and plugged with a small amount of glass wool at both ends. This MIP column was connected to the CL flow system and placed in front of the window of the photomultiplier tube.

Experimental procedure

FI-PCL method

The analytical parameters for the FI-PCL were obtained using the flow system shown in Fig. 1. The potassium ferricyanide stream was merged with the luminol stream using a Y-piece to give a stable baseline. Then the sample solution of phenothiazine medications was injected into the merged stream. The CL intensity (peak height) was used for calibration.

MI-PCL method

The chlorpromazine hydrochloride was determined using the flow system shown in Fig. 2.

First, the valve was switched so that it connected to the sample solution, and pump 2 pushed chlorpromazine hydrochloride solution through the MIP column for 120 s (or 500 s) in order to adsorb the chlorpromazine hydrochloride molecules onto the polymer. Then, the valve was switched to connect with the dioxane solution, and pump 2 pushed dioxane solution through the MIP column for 120 s in order to remove any other substances aside from chlorpromazine hydrochloride in the MIP column. Next, pump 2 was stopped and pump 1 was started, and the merged stream containing potassium ferricyanide solution and luminol solution flowed through the MIP column for 110 s, reacting with the chlorpromazine hydrochloride molecules on the MIP and thus producing CL. Finally, pump 1 was stopped and the valve was switched so that it was connected with ethanol, and pump 2 was started so that ethanol was pushed through the MIP column in order to clean the MIP for 80 s before the next determination.

Results and discussion

PCL behavior of phenothiazine medications in the luminol–potassium ferricyanide system

The PCL behavior of chlorpromazine hydrochloride in the luminol–potassium ferricyanide system was examined using the static measuring system of the IFFL-D multifunction CL analyzer. The CL dynamic curve is shown in Fig. 3. When 1.0 ml of a 1.0×10^{-4} mol/l potassium ferricyanide solution was added to 1.0 ml of a 2.0×10^{-5} mol/l alkaline luminol solution, a CL reaction occurred immediately (peak 1). About 90s later, the CL reaction terminated and the CL signal declined to baseline. Subsequently, by injecting 1.0 ml of 1.0×10^{-4} g/ml chlorpromazine hydrochloride into the above reaction mixture, a new CL reaction was initiated (peak 2). The CL reaction then finished and the CL signal declined to baseline again after ~80 s.

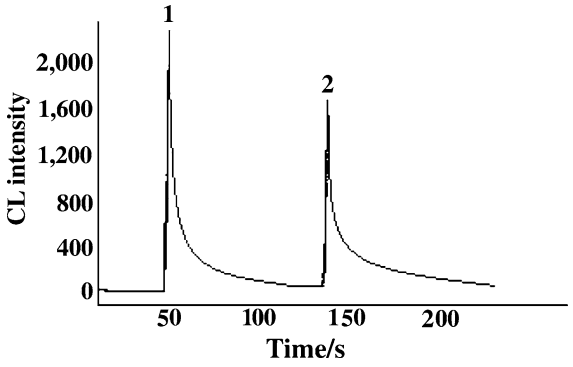

Fig. 3 CL intensity–time curve: (1) potassium ferricyanide (1.0×10^{-4} mol/l)–luminol (2.0×10^{-5} mol/l); (2) (potassium ferricyanide (1.0×10^{-4} mol/l)–luminol (2.0×10^{-5} mol/l))–chlorpromazine hydrochloride (1.0×10^{-4} g/ml)

Under the same conditions, no CL signal was detected using the blank solution instead of the chlorpromazine hydrochloride solution. So the new CL reaction was a PCL reaction arising from the chlorpromazine hydrochloride in the luminol–potassium ferricyanide system.

In order to explore the mechanism of the PCL reaction, the following experiments were performed, and their results are discussed below.

The CL spectra for the potassium ferricyanide–luminol CL reaction and the PCL reaction were obtained using a refitted RF-540 fluorescence spectrophotometer (Fig. 4). It can be seen from Fig. 4 that the maximum emission wavelengths (λ_{max}) of the two CL spectra were both 425 nm, which indicates that the PCL reaction and the CL reaction have the same illuminant: the excited state of the 3-aminophthalate ion (3-AP*) [4].

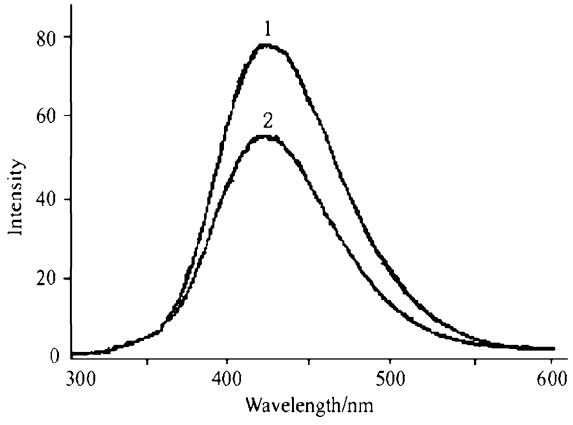

Fig. 4 CL spectra for the reactions: (1) potassium ferricyanide (1.0×10^{-4} mol/l)–luminol (2.0×10^{-5} mol/l); (2) (potassium ferricyanide (1.0×10^{-4} mol/l)–luminol (2.0×10^{-5} mol/l))–chlorpromazine hydrochloride (1.0×10^{-5} g/ml)

It has been reported that potassium ferricyanide is the catalyzer in the luminol–potassium ferricyanide CL system; this catalyzes the reaction between dissolved oxygen and luminol that produces the CL [5]. Therefore, there must be some potassium ferricyanide in the solution after the CL reaction. In order to confirm this, Fe^{2+} solution (1.0×10^{-2} mol/l) was added to the solution after alkaline luminol (2.0×10^{-4} mol/l) had reacted with the potassium ferricyanide (5.0×10^{-5} mol/l) for 4 h. A blue precipitate (Turnbull's blue) appeared, which indicated that potassium ferricyanide was still present in the solution. It was therefore proposed that potassium ferricyanide may be the reactant in the PCL reaction.

Solutions I, II and III were then prepared. Solution I was alkaline potassium ferricyanide (5.0×10^{-5} mol/l), II was a mixed solution of alkaline potassium ferricyanide (5.0×10^{-5} mol/l)–chlorpromazine hydrochloride (1.0×10^{-4} mol/l), and III was a mixed solution of (luminol (5.0×10^{-5} mol/l)–potassium ferricyanide (5.0×10^{-5} mol/l)–chlorpromazine hydrochloride (1.0×10^{-4} mol/l). After 4 h, Fe^{2+} solution (1.0×10^{-2} mol/l) was added to solutions I, II and III, respectively. A blue precipitate (Turnbull's blue) appeared in solution I immediately, but there was no blue precipitate in solutions II or III. This indicated that all of the potassium ferricyanide had reacted and been consumed in solutions II and III.

Solutions A, B and C were then prepared. Solution A was chlorpromazine hydrochloride solution (1.0×10^{-4} mol/l), solution B was a mixed solution of potassium ferricyanide (3.0×10^{-4} mol/l)–chlorpromazine hydrochloride (1.0×10^{-4} mol/l), and solution C was a mixed solution of (luminol (5.0×10^{-5} mol/l)–potassium ferricyanide (3.0×10^{-4} mol/l))–chlorpromazine hydrochloride (1.0×10^{-4} mol/l). The absorption spectra of solutions A, B and C were obtained, as shown in Fig. 5. The absorption peak from solution A was initially at 254 nm, but this disappeared and a new absorption peak with a wavelength of maximum absorption at 277 nm emerged in the absorption spectra of solutions B and C, which was the absorption peak from the oxidation product of chlorpromazine hydrochloride [6]. This suggested that chlorpromazine hydrochloride was oxidized by potassium ferricyanide in the PCL reaction.

The fluorescence spectra of solutions A and B were also obtained (Fig. 6). It can be seen from Fig. 6 that the wavelength of maximum emission for chlorpromazine hydrochloride was 460 nm and the fluorescence peaks for solution B appeared at 366 nm and 470 nm, which should be the fluorescence peak from the oxidization product of chlorpromazine hydrochloride. Since the wavelength of maximum excitation for the illuminant in the PCL reaction (3-AP) is 315 nm [5], which is shorter than 366 nm, it is impossible for the oxidization product to transfer the energy to 3-AP in the PCL reaction. This suggests that 3-AP absorbs the energy from the reaction of potassium ferricyanide and chlorpromazine hydrochloride, and then changes into 3-AP*.

Based on the experimental results described above, a possible mechanism for the PCL reaction of chlorpromazine hydrochloride in the luminol–potassium ferricyanide system may be as follows. The dissolved oxygen oxidizes luminol, a reaction which is catalyzed by potassium ferricyanide, and this produces CL in an alkaline medium. After the reaction has finished, both potassium ferricyanide and 3-AP are present in the solution. When chlorpromazine hydrochloride is injected into the above solution, it reacts with the potassium ferricyanide and some energy is released. 3-AP absorbs the energy and changes into 3-AP*. When 3-AP * drops back down to the ground state, PCL is produced.

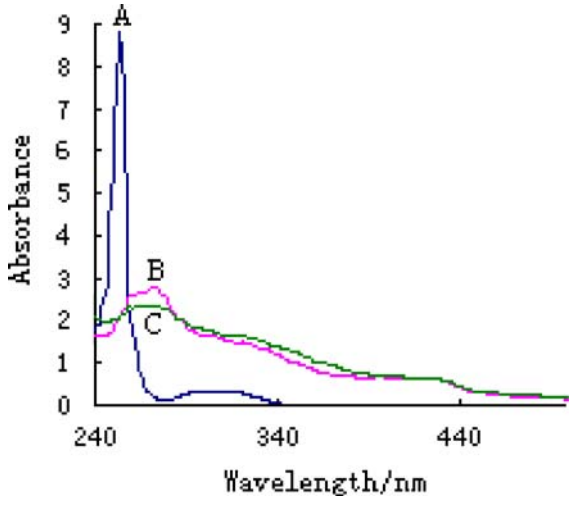

Fig. 5 UV-visible spectra: (*A*) chlorpromazine hydrochloride (1.0×10^{-4} mol/l); (*B*) potassium ferricyanide (3.0×10^{-4}mol/l)–chlorpromazine hydrochloride (1.0×10^{-4} mol/l); (*C*) [potassium ferricyanide (3.0×10^{-4} mol/l)–luminol (5.0×10^{-5} mol/l)]–chlorpromazine hydrochloride (1.0×10^{-4} mol/l)

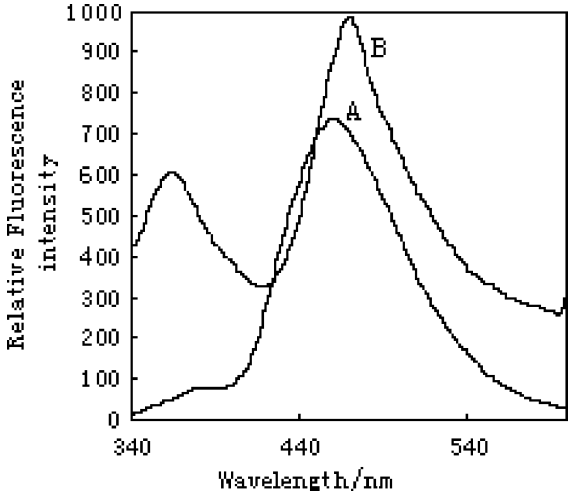

Fig. 6 Fluorescence spectra: (*A*) chlorpromazine hydrochloride (1.0×10^{-4} mol/l); (*B*) potassium ferricyanide (3.0×10^{-4} mol/l)–chlorpromazine hydrochloride(1.0×10^{-4} mol/l)

This possible mechanism for the PCL reaction can be simply expressed as follows:

$$\text{Luminol} + O_2 + OH^- \xrightarrow{K_3Fe(CN)_6} 3\text{-AP}^*$$
$$3\text{-AP}^* \rightarrow 3\text{-AP} + h\nu(\lambda_{max} = 425\,\text{nm})$$
$$\text{Chlorpromazine hydrochloride} + \text{Potassium ferricyanide} \rightarrow \text{products} + \text{Energy(E)}$$
$$3\text{-AP} + E \rightarrow 3\text{-AP}^*$$
$$3\text{-AP}^* \rightarrow 3\text{-AP} + h\nu(\lambda_{max} = 425\,\text{nm})$$

The PCL reaction mechanisms that occur when other phenothiazine medications are present in the luminol–potassium ferricyanide system should be similar to the mechanism above.

Analytical parameters for the PCL from phenothiazine medications

Since we are studying the PCL reaction, it is important that the CL reaction between luminol and potassium ferricyanide has completed before initiating the PCL reaction. To check this, a mixing tube (L) (0.8 mm i.d.) was connected between the Y-piece and the injection valve. If the mixing tube is too short, luminol and potassium ferricyanide do not react adequately, so the baseline is higher and the signal-to-noise ratio (SNR) is lower; on the other hand, if the mixing tube is too long, the PCL intensity is lower. Therefore, in order to maximize the sensitivity, the length of the mixing tube was examined in the range 20~200 cm at a fixed flow rate of 1.4 ml/min. Meanwhile, the concentrations of the sodium hydroxide in luminol solution, the luminol solution itself and the potassium ferricyanide solution were optimized by varying them over the ranges 0.005~0.5 mol/l, 5.0×10^{-6}~5.0×10^{-4} mol/l and 5.0×10^{-6}~1.0×10^{-3} mol/l, respectively, and studying the results. The optimal values found for these parameters for each phenothiazine medication tested are listed in Table 1.

The analytical parameters of the various phenothiazine medications, derived under these optimal experimental conditions, are shown in Table 2.

From Tables 1 and 2, it is clear that these PCL-based methods provide an adequate approach to determining phenothiazine medications.

MI-PCL-based method of determining chlorpromazine hydrochloride

Chlorpromazine hydrochloride is a phenothiazine medication that is often used to treat schizophrenia and manic disease, and is used as an antiemetic, in hypothermic anesthesia, and so on. However, it can lead to unwanted effects such as acute poisoning and even death when an excessive dose is used. Indeed, the addition of chlorpromazine hydrochloride to feed and animal drinking water is forbidden by the government [7]. Therefore, it is important to be able to determine chlorpromazine hydrochloride. A wide variety of analytical techniques have been used for this purpose, such as photometry [8, 9], chemiluminescence [10, 11], electron spin resonance [12], voltammetry [13–15], enzyme inhibition assays [16], liquid chromatography [17–19], capillary electrophoresis [20] and liquid chromatography/mass spectrometry (LC/MS) [21]. However such methods either require expensive instruments or give low sensitivity or poor selectivity towards the substance. In this work, a MI-PCL method was established for directly determining chlorpromazine hydrochloride in complicated samples. It also provides an example of how to establish other selective CL methods that could be used to determine other phenothiazine medications.

Washing agent

MIP can adsorb both target molecules and coexisting substances, but the adsorption mechanisms are quite different. The adsorption of target molecules to the MIP is mainly a specific type of binding based on molecular recognition, while the adsorption of coexisting substances occurs via nonspecific binding based on the hydrophobic interaction. The adsorption based on specific binding is stronger than that due to nonspecific binding. Therefore,

Table 1 Optimal experimental conditions for PCL reactions related to phenothiazine medications

Species	*L* (cm)	Luminol alkalinity (mol/l NaOH)	Luminol (mol/l)	Potassium ferricyanide (mol/l)
Fluphenazine hydrochloride	75	0.01	1.0×10^{-5}	5.0×10^{-4}
Perphenazine	85	0.10	8.0×10^{-6}	2.0×10^{-4}
Trifluoperazine hydrochloride	75	0.01	2.0×10^{-4}	6.0×10^{-6}
Thioridazine hydrochloride	75	0.10	2.0×10^{-5}	1.0×10^{-5}
Chlorpromazine hydrochloride	110	0.01	1.0×10^{-5}	1.0×10^{-5}
Imipramine hydrochloride	90	0.10	4.0×10^{-5}	1.0×10^{-5}

Table 2 Analytical parameters for the PCL method applied to phenothiazine medications

Species	Linear range (g/ml)	Regression Equation(μg/ml)	Correlation coefficient (r)	Detection limits(g/ml)
Fluphenazine hydrochloride	1.0×10^{-6}~1.0×10^{-4}	I=12.6C+46.7	0.9991	3×10^{-7}
	5.0×10^{-8}~2.0×10^{-6}	I=1.04C+20.8	0.9980	
Perphenazine	2.0×10^{-6}~2.0×10^{-5}	I=0.25C+160	0.9948	2×10^{-8}
	1.0×10^{-6}~2.0×10^{-5}	I=10.2C+16.4	0.9996	
Trifluoperazine hydrochloride	2.0×10^{-5}~1.0×10^{-4}	I=4.44C+140	0.9990	3×10^{-7}
Thioridazine hydrochloride	2.0×10^{-8}~8.0×10^{-6}	I=7.14C+180	0.9986	7×10^{-9}
Chlorpromazine hydrochloride	1.0×10^{-7}~1.0×10^{-5}	I=11.0C+154	0.9985	3×10^{-8}
Imipramine hydrochloride	1.0×10^{-7}~1.0×10^{-5}	I=10.8C+34.3	0.9974	3×10^{-8}

coexisting substances can be washed out by applying a suitable washing agent, whereas the target molecule is still retained in the MIP. In order to select a suitable washing agent, the effects of some washing agents, such as sodium sulfite, sodium hyposulfite, dioxane, formaldehyde (3%), benzene, toluene, acetonitrile, THF, chloroform and carbon tetrachloride were examined. The experimental results showed that dioxane/water (1:3, V:V) gave the best results, so this was selected as the washing agent.

Optimizing the analytical conditions used for the MI-PCL method

Using the schematic diagram shown in Fig. 2, a series of experiments were conducted to optimize the experimental conditions used for the determination of chlorpromazine hydrochloride.

Adsorption time The adsorption time is the period of time that the standard solution or sample solution flows through the MIP column. It determines the amount of chlorpromazine hydrochloride adsorbed in the MIP column, and so it influences the detection sensitivity, the linear range of the method and the analytical efficiency. Given that it affects these three important parameters, the adsorption time was varied and studied using 2.0×10^{-6} g/ml and 1.0×10^{-7} g/ml chlorpromazine hydrochloride solutions, respectively. The experimental results indicated that 120 s was the best adsorption time for the high-concentration sample. For the sample with low concentration, the adsorption time had to be increased in order to improve the detection sensitivity. In this case, if the adsorption time chosen is 500 s, the detection limit can reach down to the 10^{-9} g/ml level.

Washing time Following the adsorption step, it is necessary to wash the MIP column to remove any other substances adsorbed via nonspecific interactions. In order to select the best washing time, fluphenazine hydrochloride (which is similar in structure to chlorpromazine hydrochloride and can also trigger a PCL reaction in the potassium ferricyanide–luminol system) was added to the chlorpromazine hydrochloride standard solution in order to highlight any interference (chlorpromazine hydrochloride 2.0×10^{-6} g/ml, fluphenazine hydrochloride 2.0×10^{-6} g/ml). Using dioxane solution as the washing agent, the effect of the washing time was examined in the range 10~200 s. The experimental results revealed that fluphenazine hydrochloride could be removed effectively when the washing time was 120 s. The PCL intensity of the mixed chlorpromazine/fluphenazine hydrochloride solution was no different to that of a chlorpromazine hydrochloride standard solution (2.0×10^{-6} g/ml) that did not contain fluphenazine hydrochloride. Therefore, 120 s was selected as the washing time.

PCL reaction time When the combined stream of CL reagents flowed through the MIP column, they reacted with the chlorpromazine hydrochloride adsorbed on the polymer, producing PCL. The experiments showed that 110 s was enough time for a complete reaction.

Cleaning time During the PCL reaction, the molecular structure of the chlorpromazine hydrochloride on the polymer was altered and the chlorpromazine hydrochloride was desorbed from the MIP. However, it was very difficult to remove the remaining chlorpromazine hydrochloride from the MIP column with water. Because the chlorpromazine hydrochloride can be redissolved in ethanol [22], ethanol was used as the cleaning agent instead, and it exhibited no obvious influence on the next determination. The effect of the cleaning time over the range 10~150 s was examined by alternately measuring the blank signal and the PCL signal from a 2.0×10^{-6} g/ml chlorpromazine hydrochloride solution. When the cleaning time was not more than 80 s, both the blank signals and the PCL signal from the 2.0×10^{-6} g/ml chlorpromazine hydrochloride solution showed good repeatability. Therefore 80 s was selected as the cleaning time.

Conditions used in the PCL reaction The conditions used in the PCL reaction were optimized. The most suitable length of the mixing tube L was 110 cm and the optimal concentrations of potassium ferricyanide and luminol were 5.0×10^{-4} mol/l and 2.0×10^{-5} mol/l, respectively. The concentration of sodium hydroxide used in the luminol solution was 0.03 mol/l.

Table 3 Tolerable ratios of interfering species

Species	MI-PCL method	FI-PCL method
Glucose	1000	20
Alanine	100	10
Tyrosine	1000	100
Tryptophan	50	1
Urea	500	25
Uric acid	100	0.005
Ascorbic acid	10	0.1
Epinephrine	1	0.01
Fluphenazine hydrochloride	5	0.5
Fe^{2+}	1	0.01
Mg^{2+}	20	2
Cr^{3+}	100	5
Ca^{2+}	50	10

Analytical parameters

The relationship between the PCL intensity and the chlorpromazine hydrochloride concentration was examined under optimal experimental conditions. When the adsorption time was 120 s, the PCL intensity was found to be linearly related to the concentration of chlorpromazine hydrochloride over the range 1.0×10^{-7}~1.0×10^{-5} g/ml, with a linear regression equation of I=17.9C+39.6 (n=5, r=0.9985), where I is the PCL intensity (relative unit) and C is the concentration of chlorpromazine hydrochloride (10^{-7} g/ml); when the adsorption time is 500 s, the PCL intensity is linearly related to the concentration of chlorpromazine hydrochloride over the range 1.0×10^{-8}~1.0×10^{-6} g/ml, with a linear regression equation of I=8.89C+19.5 (n=5, r=0.9980), where C is in 10^{-8} g/ml. The relative standard deviation is 4.0% (1.0×10^{-7} g/ml chlorpromazine hydrochloride solution, n=11) and the detection limit is 3×10^{-9} g/ml.

Selectivity

In order to examine the selectivity of the method, the interference from foreign species during the determination of 2.0×10^{-6} g/ml chlorpromazine hydrochloride was investigated using the MI-PCL method and the FI-PCL method, respectively. The foreign species selected are substances normally present in urine and animal drinking water, and substances that show CL behavior in the potassium ferricyanide and luminol PCL system. The tolerable ratio of the foreign species to chlorpromazine hydrochloride is taken to be that ratio that gives a relative error of less than ±5%. The results in Table 3 show that the MI-PCL method exhibits excellent selectivity compared to the FI-CL method.

Table 4 Determination of chlorpromazine hydrochloride in animal drinking water

Sample	This method* ($\times10^{-6}$ g/ml)	R.S.D.	Pharmacopoeia method* [22] ($\times10^{-6}$ g/ml)
1	0.78	3.6%	0.81
2	0.97	3.2%	0.99
3	5.11	3.3%	5.00

*Average of three measurements

Table 5 Results of determination of the blank urine samples

Method	Doubly distilled water	Sample 1	Sample 2	Sample 3
FI-PCL	−9	−35	−33	−35
MI-PCL	85	85	83	83

The data shown in the table denote the relative CL intensity

Applications

Determination of chlorpromazine hydrochloride in animal drinking water Three samples of animal drinking water were obtained from three troughs from a hoggery. Each sample was centrifuged at 3000 r/min for 15 min, and 10 ml of the supernatant from the sample was transferred to a 100 ml volumetric flask and diluted with water to the mark. The solutions obtained in this way were used as sample solutions and determined using the MI-PCL method. Control experiments were also performed, based on the method given in the Chinese Pharmacopoeia. The results are shown in Table 4. The t-test assumes that there is no significant difference between the MI-PCL method and the Pharmacopoeia method at a confidence level of 95%.

Determination of chlorpromazine hydrochloride in urine samples Blank urine samples were collected from three healthy volunteers; the urine samples were obtained from three mental patients, who had taken chlorpromazine hydrochloride for 8 h before the detection. The urine samples were centrifuged at 3000 r/min for 15 min. For each sample, one ml of the supernatant was transferred to a 100 ml volumetric flask and diluted with water to the mark, and this solution was used as a sample solution.

A sample solution of blank urine was also determined by both the MI-PCL and the FI-PCL methods. The results are shown in Table 5. Table 5 shows that the CL signals

Table 6 Determination results of chlorpromazine hydrochloride in urine samples

Sample	Found * ($\times10^{-6}$ g/ml)	R.S.D. (%)	Added ($\times10^{-6}$ g/ml)	Found * ($\times10^{-6}$ g/ml)	Recovery (%)
1	4.42	3.2	1.00	5.40	98
			5.00	9.65	105
2	0.50	3.1	0.80	1.26	95
			1.00	1.45	95
3	0.71	2.9	0.80	1.48	96
			1.00	1.73	102

* Average of three measurements

from different blank urine samples were not significantly different to the signa from doubly distilled water during the MI-PCL determination. These results suggest that the other species present in the urine were removed effectively and didn't interfere with the determination of chlorpromazine hydrochloride in the MI-PCL method. Therefore, the recovery of chlorpromazine hydrochloride could be used to evaluate the accuracy of this method.

The levels of chlorpromazine hydrochloride in the urine samples were determined with the MI-PCL method, and the recovery was evaluated (Table 6). The results in Table 6 show that the recoveries of chlorpromazine hydrochloride were quantitative, and *t*-test suggested that there was no significant difference between any of the recovery efficiencies and 100% at a confidence level of 95%.

Conclusion

A PCL phenomenon exhibited by phenothiazine medications present in the luminol–potassium ferricyanide system has been discovered, which adds to the usefulness of the PCL reaction. Under optimal experimental conditions, the analytical parameters of PCL-based methods of determining various phenothiazine medications were obtained, and these analytical parameters indicated the feasibility using these PCL reactions to determine these medications. A possible PCL reaction mechanism was proposed which provides interesting and useful insight into the PCL reaction. A new MI-PCL method was developed for determining chlorpromazine hydrochloride, and the method was applied to the determination of chlorpromazine hydrochloride in urine samples and animal drinking water with satisfactory results. It is hoped that this will lead to the application of similar MI-PCL methods to determine other phenothiazine medications in complicated samples.

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