Luminol–K₃Fe(CN)₆ chemiluminescence system for the determination of glipizide

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Abstract A rapid and sensitive flow-injection chemiluminescence (CL) method for the determination of glipizide was developed on the basis of finding that glipizide can enhance the CL intensity of the luminol–K₃Fe(CN)₆ system. In optimum condition, the increased CL intensity was directly proportional to the concentration of glipizide in the range from 4.0×10⁻⁶ g/mL to 1.0×10⁻⁴ g/mL and the detection limit was 1.0×10⁻⁸ g/mL glipizide. The relative standard deviation (RSD) of the developed method was 2.1% with 11 repeated measurements of 1.0×10⁻⁷ g/mL glipizide. The developed method has been successfully applied to the analysis of glipizide in its pharmaceutical preparations.

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1. Introduction

Glipizide (GP), 1-cyclohexyl-3-[[p-2-(5-methylpyrazine-carboxamido)ethyl]phenyl]sulfonyl]urea (as shown in Scheme 1), is a second generation sulfonylurea hypoglycemic agent and is typically used in the treatment of non-insulin dependent diabetes mellitus [1,2], which lowers the blood glucose level in humans by stimulating the release of insulin from the pancreas and helping the body to use insulin efficiently [3]. Because its effect is indeed quick and well tolerated, more importantly for a long-term use, GP has been widely used, as an ideal anti-diabetic drug with the more commonly-known name Glucotrol, in Europe as well as other regions worldwide [4]. Therefore, it is quite necessary to develop a new method for the analysis of GP.

In the past few years, several methods were reported for the determination of GP, which involved different high performance liquid chromatographies (HPLCs) [5–13], liquid chromatography–mass spectrometry (LC–MS) [14–17], ultraviolet spectrophotometry [18], thin-layer chromatography (TLC) [19] and an electrochemical method [20]. Most of these methods are tedious, inconvenient and suffer from low sensitivity and narrow linear range.

Recently, extremely sensitive analytical techniques based on chemiluminescence (CL) systems (such as NBS–H₂O₂–
nitrofurazone, KMnO₄–Na₂SO₃–atenolol, luminol–potassium peroxydisulfate–bisphenol, etc. [21,22]), have been paid considerable attention, which had been applied in determining trace and ultra-trace concentrations of inorganic and organic species (like drugs or pesticides) in a variety of industrial, clinical and environmental matrices [23]. The CL method had the advantages such as high sensitivity, relatively inexpensive apparatus, short analysis time, wide working range, low detection limit and no background scattering light interference. Coupled with flow injection analysis (FIA), the reproducibility and selectivity of CL analysis can be improved significantly. However, up to now, there is no report on the use of FI-CL method for determination of GP in pharmaceutical preparations. In this work, it was found that GP could obviously enhance the CL of the luminol–K₃Fe(CN)₆ reaction in NaOH solution and the enhanced CL was proportional to the concentration of GP. So, a new flow-injection CL method was proposed and the mechanism of luminol–K₃Fe(CN)₆–GP was also discussed briefly.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared from analytical reagent grade chemicals combined with double distilled water.

A stock solution of GP (1.0 × 10⁻³ g/mL) was prepared by dissolving 0.1000 g of GP (National Institute of Pharmaceutical and Biological Authentication of China, Xi’an, China) in alcohol and then diluting to 100 mL.

Luminol stock solution (1.0 × 10⁻² M) was obtained by dissolving 0.1771 g of luminol (Aldrich, Sigma-Aldrich Química) in 10 mL 0.1 M NaOH and diluting to 100 mL with water.

Potassium ferricyanide stock (1.0 × 10⁻² M) was prepared by dissolving 0.1150 g K₃Fe(CN)₆ (Xi’an Chemical Reagent Factory, Xi’an, China) in distilled water and then diluting to 100 mL with water.

The stock solutions were stored at 4 °C in a refrigerator and protected from light. The working solutions were prepared by diluting stock solutions freshly into appropriate concentration with water before being used.

2.2. Apparatus and procedures

The CL measurement was conducted on an IFFM-E flow injection CL analyzer (Xi’an Remax Electronic Science-Tech. Co. Ltd., Xi’an, China), which includes a model IFFM-E flow injection system and a model IFFS-A luminometer. A schematic representation of the proposed FI-CL system for the detection of GP is illustrated in Fig. 1. Two peristaltic pumps, labeled as P₁ and P₂, were used to deliver the respective components to the flow cell at a flow rate of 2.65 mL/min. The peristaltic pumps, labeled as P₁ and P₂, were used to deliver the respective components to the flow cell at a flow rate of 2.65 mL/min. The light output from the CL reaction was detected and amplified with a photomultiplier tube and luminometer. Data acquisition and processing were performed using the IFFM-E software running under Windows XP. The determination of GP was accomplished based on the increase in the CL intensity, calculated as $D_I = I/I_0$, where $I$ denotes the CL signal in the presence of GP and $I_0$ is the CL intensity corresponding to baseline.

3. Results and discussion

3.1. Kinetic characteristic of the CL reaction

The kinetic curves of the CL reaction of luminol–K₃Fe(CN)₆–GP system were taken on an IFFS-A multipurpose chemiluminescence detector (shown in Fig. 2). It could be seen that the oxidation of luminol with K₃Fe(CN)₆ was comparatively fast (2–3 s) and the CL intensity was relatively strong. The CL signal (3200) in the presence of GP (1.0 × 10⁻⁷ g/mL) is stronger than that (5350) in the absence of GP. The results indicated that GP could sensitize the CL emission of luminol–K₃Fe(CN)₆ system significantly.
3.2. Selection of oxidants

The characteristics of several oxidants, including KMnO\textsubscript{4}, K\textsubscript{3}Fe(CN)\textsubscript{6}, KIO\textsubscript{4} and H\textsubscript{2}O\textsubscript{2} of the same concentration reacting with luminol CL systems in the presence of GP, were evaluated. It was found that GP had more effective enhancement in luminol–K\textsubscript{3}Fe(CN)\textsubscript{6} system, compared with other systems. Therefore, K\textsubscript{3}Fe(CN)\textsubscript{6} was selected as an oxidant for the subsequent study.

3.3. Effect of sample injection sequences

In this work, different injection sequences were tested to optimize conditions. The strongest relative CL intensity was found when K\textsubscript{3}Fe(CN)\textsubscript{6} solution was premixed with luminol and then with sample solution (as described in Fig. 1).

3.4. Optimization of experimental conditions

A series of experiments had been performed for optimizing the chemical and instrumental parameters affecting the analytical response, which included the component concentration, flow rate, the length of mixing tube, etc. 1.0 \times 10^{-7} g/mL standard solution of GP was applied to optimizing the experimental conditions.

3.4.1. Effect of chemical variables

The chemical variables that need to be assessed include luminol, potassium ferricyanide and the medium. The effect of luminol concentration (the medium was 0.1 M NaOH) was investigated in the range of 8 \times 10^{-6}–2 \times 10^{-4} M. As shown in Fig. 3, increasing the luminol resulted in a sharp increase in the relative CL signal up to 8 \times 10^{-3} M; after that, 1 \times 10^{-6} g/mL glipizide could not be detected (the CL signal had reached beyond the maximum system detection) and S/N decreased although CL still increased. Considering the stability of the CL signal, a luminol concentration of 8 \times 10^{-5} M was selected for the subsequent experiment.

The dependence of the increased CL intensity (ΔI) on the concentrations of potassium ferricyanide and the medium. The effect of luminol concentration (the medium was 0.1 M NaOH) was investigated in the range from 1.0 \times 10^{-5} M to 1.0 \times 10^{-3} M. The result related to 1.0 \times 10^{-3} M–10 \times 10^{-5} M is shown in Fig. 4. A general improvement in the CL intensity sharply increases with an increase of the concentration of potassium ferricyanide from 1.0 \times 10^{-5} M to 4.0 \times 10^{-5} M, and then slowly increases. 6.0 \times 10^{-5} M potassium ferricyanide was selected to further study since a higher signal to noise ratio was obtained in this concentration solution.

The CL signal was tested in several alkaline solutions, which included NaOH, Na\textsubscript{2}CO\textsubscript{3}, NaHCO\textsubscript{3}, Na\textsubscript{3}PO\textsubscript{4} and Na\textsubscript{2}HPO\textsubscript{4}. We observed that the CL signal was stronger when adding appropriate concentration of NaOH in the reaction system. In this work, over the range 0.005–0.2 M, the effect of the concentration of NaOH was studied and the results are shown in Fig. 5. Maximum CL response was obtained at a NaOH concentration of 0.1 M, over which the relative CL intensity signal did not increase remarkably any more. So, 0.1 M NaOH was regarded as the optimized working concentration for the subsequent work.

3.4.2. Effect of instrumental parameters

The effect of flow rate on the CL response was calibrated in terms of sensitivity, reagent consumption and speed. Flow rate \( V \) was studied over the range 1.55–4.50 mL/min each stream. The results showed that the CL response continued to increase with increasing flow rate up to 2.65 mL/min, at which maximum CL intensity was observed, and then began to drop. Thus, a flow rate of 2.65 mL/min was chosen as the suitable rate with a steady baseline and reproducible peak height.

The length of the mixing tubing (\( L \)) was also adjusted to yield maximum light emission in the cell. Because CL reaction is very fast, if the length of mixing tubing to the flow cell is too long or too short, the CL signal cannot be detected. In this work, the effect of \( L \) was investigated over the range from 3 to 15 cm. It was found that a 10 cm mixing tubing afforded the best results with good reproducibility and sensitivity, which was selected for further work.

To sum up, the optimized conditions consisted of luminol (8 \times 10^{-5} M) in NaOH (0.1 M) as the stream with a flow rate
of 2.65 mL/min and potassium ferricyanide (6.0 × 10⁻⁵ M) introduced to the carrier by a six-way valve selected for the purpose of optimization.

3.5. Analytical performance—calibration curve, detection limit and precision

A series of working standard solutions of GP with different concentrations (1.0 × 10⁻⁸ g/mL–1.0 × 10⁻⁴ g/mL) were prepared by diluting freshly respective stock standard solutions with water. Under the selected experimental conditions described above, AI, the difference in CL intensity in the absence and presence of GP, possessed a linear relationship with GP concentration ranging from 4.0 × 10⁻⁷ g/mL to 1.0 × 10⁻⁶ g/mL. The linear regression equation was expressed as ΔI=19.738c+1102.9 (r=0.9901, n=5). The detection limit (3σ) of the method was estimated to be 1.0 × 10⁻⁸ g/mL and the relative standard deviation (RSD) for determination of 1.0 × 10⁻⁶ g/mL GP was 2.1% (n=11), which was indicative of repeatability and reproducibility of the assay.

3.6. Interference

In order to assess the selectivity of the developed CL method, varying amounts of possible interfering substances were added to the determination of a standard GP solution (1.0 × 10⁻⁷ g/mL). The obtained signals were tested against a pure solution of GP at the same concentration. The tolerance of foreign species was taken as the largest concentration yielding a relative error less than 5% in CL signal. In all the mixed media, the determined GP concentration was in good agreement with that in the pure GP solution. The above results indicated that the common accessories found in the pharmaceutical preparations, including lactose, starch, glucose, saccharin, sucrose and magnesium stearate, had no obvious effects on the determination of GP. And the existence of charin, sucrose and magnesium stearate, had no obvious in pharmaceutical preparations, including lactose, starch, glucose, saccharin, sucrose and magnesium stearate, had no obvious effects on the determination of GP. The determinations of the calculated GP concentration was in good agreement with the Chinese pharmacopoeia reference method (4.8 mg per tablet, n=5) and the nominal content of GP (5.0 mg per tablet), the RSD being less than 2%. The recoveries were determined by adding 3 GP solutions (1.5 × 10⁻⁷, 3.0 × 10⁻⁷, 5.0 × 10⁻⁷ g/mL) respectively to the pharmaceutical preparation (2.0 × 10⁻¹ g/mL). The determination results (n=9) were 3.46 × 10⁻⁷, 4.94 × 10⁻⁷, 6.95 × 10⁻⁷ g/mL, correspondingly, the average recovery being 98.1%, which was in the acceptable range.

3.9. Discussion of possible CL mechanism

In order to obtain the CL reaction mechanism, the fluorescence spectra of luminol, luminol–K₃Fe(CN)₆, and luminol–K₃Fe(CN)₆–GP reaction were scanned in the range of 280–650 nm, using an RF 5301 fluorescence spectrophotometer. It had been reported that 3-aminoephthalate ion (3-AP), an oxidized product of luminol, peaking at 425 nm was known as the emitter in the luminol–K₃Fe(CN)₆ system [24]. The results obtained were all found to have the same maximum emission appearing at 425 nm; the luminophor was confirmed to be 3-AP. This indicated that the luminant in the luminol–K₃Fe(CN)₆–GP system was still 3-AP.

On the other hand, when GP standard solution, luminol solution and K₃Fe(CN)₆ solution were purged with nitrogen for 5 min and the dissolved oxygen was removed from all solutions, it was found that the CL intensity decreased by about 30%. The result indicated that the dissolved oxygen is required in the CL reaction.

The superoxide radical had been confirmed to oxidize luminol to produce CL in alkaline solution [25]. The increasing agents could increase dissolved oxygen to intermediate superoxide radical in alkaline solution. The produced superoxide radical reacts with luminol to yield an unstable endoperoxide leading to an excited aminophalate. The CL reaction of luminol with superoxide radical can be catalyzed by potassium ferricyanide in alkaline solution.

Based on the discussion described above, the possible CL reaction mechanism was considered as follows:

luminol + K₃Fe(CN)₆→ 3-aminoephthalate ion* (3-AP*)

3-AP*→ 3-AP + hν (λₘₐₓ = 425 nm)

GP + dissolved oxygen + NaOH→ superoxide radical + other reaction product

Superoxide radical + luminol + K₃Fe(CN)₆→ 3-AP*

3-AP*→ 3-AP + hν (λₘₐₓ = 425 nm)

4. Conclusion

A sensitive FI-CL method for the determination of anti-diabetic drug—GP based on luminol and potassium ferricyanide in NaOH was proposed for the first time in the present work. It had been proved that this method is sensitive, simple and rapid to analyze GP in pharmaceutical preparation based on its sensitizing effect. The result obtained was in reasonable agreement with that achievable by the Chinese pharmacopoeia.
method. If combined with separation techniques, the system may be adopted to detect GP in other samples.

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