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Flow-injection chemiluminescence determination of chrysin and baicalein assisted by theoretical prediction of chemiluminescence behavior of chrysin and baicalein

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Abstract

In this paper, the molecular connectivity indices were applied to theoretically predict the direct chemiluminescence (CL) behavior of chrysin and baicalein with our recently proposed discriminant function. Then, combined with flow-injection analysis, a new CL system for determination of chrysin and baicalein was proposed. The method was based on the oxidation of chrysin and baicalein by acidic KMnO4 in the presence of formaldehyde to produce strong CL emission. The present paper suggested a new model to discover new CL analytical system: first, to theoretical predict the CL behavior, and the second, to suggest analytical system.

Keywords: Chemiluminescence; Flow injection; Chrysin; Baicalein; Molecular connectivity indices

1. Introduction

Chemiluminescence (CL) is defined as the emission of electromagnetic radiation (usually in the visible or near-infrared region) produced by a chemical reaction. Since the appearance of the highly sensitive photomultiplier tube in the 1950s, CL has been known as a powerful and important analytical technique. Some advantages of CL over spectrophotometry and fluorescence, such as higher sensitivity, lower detection limits and wider linear ranges, can be achieved with simpler instrument (no excitation light source and no spectroscope). In the last 20 years, the CL phenomenon in liquid phase has been widely applied in pharmaceutical, biomedical, environmental and food analysis [1,2]. One main direction of the current research in CL is to discover new CL reactions. Research on new CL reactions is very important since it opens new horizons for the technique. It is well known that the CL emission is frequently accompanied by an oxidation–reduction reaction involving large free energy change (130–340 kJ/mol). The present trend is to obtain and exploit on the so-called “direct CL determinations”, which involves CL emission from the oxidation of the analyte. Many oxidants, such as H2O2, Br2, ClO−, KMnO4, Ce4+, KBrO3, Ru(bpy)33+, Fe(CN)63−, IO4−, Co3+, Ag2+, Mn3+, PbO2, NaBiO3, MnO2 and N-bromosuccinimide, have been used for testing direct CL reactions in liquid phase [3]. When searching for a new direct CL method, preliminary work is to examine an analyte with a wide range of oxidants in many different media (such as H2SO4, HCl, H3PO4, NaCO3 and NaOH). This usually involves the trial-and-error screening procedures, which is tedious and time-consuming, or even expensive.

The “ideal” way to find compounds showing the direct CL properties requires tools for predicting the behavior with a reasonable degree of reliability. In this way, quantitative structure–property relationship (QSPR) provides a promising method for the estimation of the compounds’ behavior based on the descriptors derived solely from the molecular structure to fit experimental data [4–6]. The advantage of this approach lies in the fact that it requires only the knowledge of chemical structure and is not dependent on the experiment properties. In QSPR studies, molecular connectivity is a topological method capable of describing the structure of a molecular by means of numbers named indices. In 2001, Calatayud and his co-workers [7] firstly applied molecular
connectivity calculation to predict the direct CL behavior of substances in liquid phase, and later they applied the CL discriminant function to theoretically predict the CL behavior of phenols and polyphenols [8], ergot alkaloids [9] and pesticides [10]. In our recently published work [11], the new discriminant function of direct CL was suggested with cosine coefficient as a descriptor, and the discriminant function was applied to predicting the direct CL behavior of 101 compounds with a success of 95.05%.

Chrysin (5,7-dihydroxyflavone) and baicalein (5,6,7-trihydroxyflavone) are two active flavonoid compounds, and their structures have been presented in Fig. 1. Chrysin and baicalein exist ubiquitously in herbal medicines. For example, chrysin and baicalein are the main active components of *Oroxylum indicum* [12], which is one herbal medicine commonly used in China and other East Asian countries, and has been officially listed in the Chinese Pharmacopoeia for a long time [13]. Chrysin possesses the potent anti-inflammation, anti-oxidation properties and cancer-preventative effects [14], and it is able to simulate nitric oxide released from endothelial cells, leading to vascular cGMP accumulation and subsequent endothelium dependent vascular relaxation. Baicalein has function to restrain the bacteria and reduce the permeability of the capillary vessel and the inflammation, so it is useful for cleaning the uterus and decreasing the degree of advanced menstruation, discharge and menorrhagia, and it has anti-HIV [15], anti-tumor [16] and anti-SARS coronavirus effects [17]. Quantitative analysis of chrysin and baicalein with known therapeutic activity could provide useful information for the quality evaluation of the herbal medicines. There are several reported methods for the determination of chrysin and baicalein. The main methods are HPLC with mass spectrometric detection [18] or ultraviolet detection [19].

In this article, we chose chrysin and baicalein as the model compound to validate and apply our recently proposed CL discriminant function [11]. The study was performed in two different steps: first, to predict the CL behavior of chrysin and baicalein by our recently proposed CL discriminant function [11]; and, the second to develop a flow-injection CL method for determination of chrysin and baicalein. The goal of the present article was to study the CL behavior of chrysin and baicalein to propose analytical procedure.

2. Experimental

2.1. Chemical and reagents

All reagents were of analytical grade unless stated otherwise, and the water used was deionized and double distilled. Chrysin and baicalein (98.5%, purity) were from Professor Zongting Zhang (Shaanxi Normal University, China). Other chemicals (sensitizers, strong inorganic acids and alkalis, oxidants as KMnO₄, Ce(NH₄)₂(NO₃)₆, H₂O₂, K₃Fe(CN)₆) were from Xi’an Reagent Plant (Xi’an, China).

The stock solution of potassium permanganate (5.0×10⁻³ mol l⁻¹) was prepared by dissolving 0.0790 g potassium permanganate into a 100 ml flask with water, and the solution was protected from light. A formaldehyde stock solution (10%, v/v) and a hydrochloric acid solution (2.0 mol l⁻¹) were also prepared. A standard solution of baicalein (6×10⁻³ mol l⁻¹) was prepared by dissolving 0.1620 g baicalein in 3 ml 2 mol l⁻¹ NaOH and diluting to 100 ml with water. A standard solution of chrysin (4×10⁻³ mol l⁻¹) was prepared by dissolving 0.1016 g chrysin in 3 ml 2 mol l⁻¹ NaOH and diluting to 100 ml with water. These standard solutions were stored in the refrigerator (4°C), and the working standard solutions were prepared daily from the stock solution by appropriate dilution immediately before use.

2.2. Apparatus and manifold

The flow system used in this work is shown in Fig. 2. There are two peristaltic pumps: one was used to deliver the flow streams of sample (chrysin or baicalein) and carrier at a flow rate of 2.0 ml min⁻¹ (per tuber), and another was used to deliver...
the oxidant (KMnO₄) stream and media (HCl + formaldehyde) stream at a flow rate of 4 ml min⁻¹ (per tuber). PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. The flow cell is a flat spiral-coiled colorless glass tube (i.d. 1.0 mm; total diameter of the flow cell, 3 cm, without gaps between loops) and placed close to the window of the photomultiplier tube (PMT). A sample solution (150 μl) was injected by a six-way valve into the carrier stream (water), and then merged with the stream of acidic KMnO₄ just prior to reaching a flow cell. The CL signal produced in the flow cell was collected with a CR-105 PMT (Hamamatsu, Tokyo, operated at −800 V) of the IFFL-D Chemiluminescence Analyzer (Xi’an Ruike Electronic Science Tech. Co. Ltd., China). The signal was recorded using an IBM-compatible computer, equipped with a data acquisition interface. Data acquisition and treatment were performed with IFFL-D software running under Windows 2000. The concentration of analyte was quantified by the peak height of the CL intensity.

3. Results and discussion

3.1. Theoretical prediction

Molecular topology has demonstrated clearly its ability to design and find new compounds showing the desired chemical properties [20]. It is able to easily and quickly characterize molecular structures after defining the so-called topological indices or topological descriptors. In this way, the molecule is assimilated to a graph, representing by segments the bonds (edges) and by points, vertices, atoms; and, in this way, the resulting graphs can be represented by matrices to derive one single topological descriptors or a set of them, resulting in a unique characterization of the molecular structure. Furthermore, they can be correlated with many physical, chemical or biological properties of molecules, so obtaining the QSPR model, which can be used to find new compounds with pre-determined properties or behaviors.

As our recently published work [11], the five molecular topology indices \( \chi \), \( \chi' \), \( J_3 \), \( J_4 \) and \( S_{\text{CH}} \) were used to describe the structure of some classical active compounds, which could react with common oxidant in liquid phase to produce CL emission, and some inactive compounds. In the training group, 15 well-known classical chemiluminescent compounds (such as luminol, luciferin, acridinium ester and lophine) and 25 non-chemiluminescent compounds, respectively.

\[ \mu_1 = \frac{\sum \alpha(i)}{15}, \quad \mu_2 = \frac{\sum \beta(i)}{25} \]  

The cosine of the angle between two vectors is an indication of vector similarity and is equal to the dot-product of the vectors normalized by the product of the vector length. \( X \) was the vector of the tested compound.

\[ \cos(X, \mu_1) = \frac{(X, \mu_1)}{||X|| | |\mu_1||} \]  

\[ \cos(X, \mu_2) = \frac{(X, \mu_2)}{||X|| | |\mu_2||} \]

Thus, cosine coefficient was used as a descriptor and the discriminant function (DF) of direct CL was obtained.

\[ \text{DF} = \cos(X, \mu_1) - \cos(X, \mu_2) \]

The discriminant function was used to classify the CL compounds as active if DF > 0 and inactive if DF < 0. In our recently published work [11], the discriminant function was applied to predicting the direct CL behavior of 101 compounds with a success of 95.05%.

In this work, we used Kier and Hall connectivity indices up seventh order \( \chi \), \( \chi' \) [21], topological charge indices \( J_3 \) and \( J_4 \) [22] and atom-type e-state indices \( S_{\text{CH}} \) [23]. Based on the molecular structure of chrysin and baicalein (Fig. 1), the five molecular topology indices of chrysin and baicalein were calculated, and the results are shown in Table 1. Based on the CL discriminant function in our recently published work [11], the DF of chrysin is 1.1703 and the DF of baicalein is 1.1987. Chrysin and baicalein are tested through the discriminant function, yielded DF > 0. So chrysin and baicalein are predicted as direct chemiluminescent compounds.

3.2. Influence of the oxidant on the chemiluminescence emission

Based on the above theoretical prediction, chrysin and baicalein could produce CL emission when react with common oxidants in liquid phase. We used the batch mode of CL to study the influence of the oxidant on the CL emission. The tested oxidants included KMnO₄, Ce(IV) and N-bromosuccinimide in \( H_2SO_4 \) media; and in NaOH media, the studied oxidants were \( H_2O_2 \), \( K_3Fe(CN)_6 \) and N-bromosuccinimide. The experimental results (Table 2) showed that the acidic KMnO₄ performed the strongest CL emission.

The next test was intended to expose the influence of the oxidant concentration, which was examined over the range of 0.01–1 mmol l⁻¹ KMnO₄. The maximum CL intensity was

| Compound | \( \chi \) | \( \chi' \) | \( J_3 \) | \( J_4 \) | \( S_{\text{CH}} \) |
|----------|--------|--------|--------|--------|--------|
| Chrysin  | 0.40038 | 0.08284 | 0.5606 | 0.2229 | 12.8987 |
| Baicalein| 0.41226 | 0.08588 | 0.1451 | 0.1224 | 11.2858 |
Table 2
Characteristics of oxidants for the CL system

| Oxidant   | KMnO₄ | Ce(IV) | NBS | H₂O₂ | K₃Fe(CN)₆ | NBS |
|-----------|-------|--------|-----|------|-----------|-----|
| Media     | H₂SO₄| H₂SO₄ | H₂SO₄| NaOH | NaOH | NaOH |
| Relative CL intensity | 700  | 90  | 60  | 50  | 200  | 50  |

* Condition: 1 × 10⁻³ mol⁻¹ oxidant, 2 mol⁻¹ H₂SO₄, 1 mol⁻¹ NaOH, 1 × 10⁻⁵ mol⁻¹ baicalein.

Table 3
Characteristics of media for the CL system

| Media (1 mol⁻¹) | HCl | H₂SO₄ | HNO₃ | H₃PO₄ | H₆P₄O₁₃ |
|----------------|-----|-------|------|-------|---------|
| Relative CL intensity | 1800 | 1160 | 250  | 160  | 100  |

* Condition: 5 × 10⁻⁴ mol⁻¹ KMnO₄, 3% formaldehyde, 8 × 10⁻⁶ mol⁻¹ baicalein.

observed at 0.5 mmol⁻¹ KMnO₄. So, 0.5 mmol⁻¹ KMnO₄ was thus chosen for subsequent tests. Notice that in the experiments described below only baicalein was used to optimize the experimental variables because the behavior of chrysin and baicalein were almost same.

3.3. Choose sensitizer and cofactor for the CL system

Although chrysin and baicalein reacted with acidic KMnO₄ to produce CL emission, the intensity is not sufficient for analytical purposes. It is thus necessary to use cofactors or sensitizers. According to the literature [24], some possible enhancers on the present CL reaction were examined. The sensitizers, including radamine 6G, rodamine B, quinine, fluorescein, Tween-20, Tween-80, Tritxon-100, β-cyclodextrin and sodium dodecylsulphate, were studied. On the other hand, the cofactors, including formic acid, glutaraldehyde, formaldehyde, acetaldehyde, n-butanal and benzaldehyde, were selected. No useful enhancing effect was noticed with most sensitizers and cofactors, and only formaldehyde showed excellent enhancing effect. When 3% formaldehyde was used in the CL system, the ratio of signal to blank of CL intensity could be increased 26-fold.

In the present CL system, formaldehyde was used as enhancer. The effect of formaldehyde concentration on CL intensity was investigated in the range of 1–7% (v/v). The result (Fig. 3) showed that the highest CL intensity was at 5% formaldehyde concentration. Therefore, the formaldehyde concentration of 5% was chosen for further studies.

3.4. Effect of reaction media on CL intensity

Bearing in mind that potassium permanganate can act as oxidant in acidic as well as in alkaline media. Preliminary experiment showed that only in strong acidic media potassium permanganate could oxidize baicalein to produce CL emission. The effect of the type of acid was investigated by using sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid and polyphosphate acid as the reaction media. The experimental results (Table 3) showed that baicalein gave the strongest CL signal in hydrochloric acid. For 1 mol⁻¹ HCl and 1 mol⁻¹ H₂SO₄, the concentration of H⁺ is almost same because the Ka₂ of H₂SO₄ is rather small (Ka₂ = 0.01), but the CL signal in 1 mol⁻¹ HCl was higher than it in 1 mol⁻¹ H₂SO₄. In order to explain this phenomenon, we added the 1 mol⁻¹ KCl into 1 mol⁻¹ H₂SO₄. It was found that the CL signal in 1 mol⁻¹ HCl was higher than it in 1 mol⁻¹ H₂SO₄. On the other hand, a small amount of Mn²⁺ (less than 4 × 10⁻⁵ mol⁻¹) was directly added into 1 mol⁻¹ H₂SO₄, and the CL signal was enhanced. So, the reason was that in HCl media, KMnO₄ could oxidize Cl⁻ to produce Mn²⁺, and Mn²⁺ could enhance the CL emission of KMnO₄ system [25].

We also investigated the effect of HCl concentration on the CL intensity in the range from 0 to 2 mol⁻¹. The experimental result is shown in Fig. 4. The CL intensity increased with increasing concentration of HCl in the range of 0–1 mol⁻¹, however above the concentration of 1 mol⁻¹, the CL intensity probably because of decreasing KMnO₄ concentration (HCl could react with KMnO₄). Then 1 mol⁻¹ HCl was chosen as the reaction media.
3.5. Effect of length of mixing tube of KMnO₄ and HCl

When HCl was directly added into KMnO₄ solution, the obtained CL signal was unstable. This was the reason why HCl was not used as the media of KMnO₄ CL system though the higher CL signal in HCl media was observed in the previous study [26]. So, we prepared the HCl solution and KMnO₄ solution, respectively, and then the two solutions were mixed on-line in the flow system (Fig. 2). It was found that the mixing tube length of the two solutions also influenced the CL intensity. Fig. 4 showed the effect of mixing tube length on CL intensity. In the flow system (Fig. 2), the reaction degree performed in 1 min. complete analysis, including sampling and washing, could be on-line in the flow system (Fig. 2). It was found that the mixing tube length of 5–78 cm, probably due to producing more Mn⁵⁺ (Mn⁵⁺ could enhance the CL emission of KMnO₄ system [25]); when the length of mixing tube was more than 78 cm, the CL intensity decreased probably because of greatly decreasing KMnO₄ concentration. So, 78 cm mixing tube of HCl and KMnO₄ was used in this flow system at 4.0 ml min⁻¹ flow rate (per tube).

3.6. Performance of the flow system for chrysin and baicalein

Under the optimum conditions described above and using the flow-injection system described in Fig. 2, the calibration graph of emission intensity (I, mV) versus chrysin concentration was linear in the range 3 × 10⁻⁷–9 × 10⁻⁵ mol l⁻¹ with the detection limit of 1 × 10⁻⁷ mol l⁻¹, and the regression equation was 

\[ I = 96.3 + 30.7C \]  

(\(C\): chrysin concentration, μmol l⁻¹) with a correlation coefficient of 0.9993 (\(n = 7\)). The calibration graph of emission intensity (I, mV) versus baicalein concentration was linear in the range 1 × 10⁻⁶–7 × 10⁻⁵ mol l⁻¹ with the detection limit of 4 × 10⁻⁷ mol l⁻¹, and the regression equation was 

\[ I = 425 + 94.1C \]  

(\(C\): baicalein concentration, μmol l⁻¹) with a correlation coefficient of 0.9991 (\(n = 7\)). The relative standard deviation for 11 measurements of chrysin and baicalein were 4.5% and 3.0%, respectively. Two typical recording outputs of the proposed CL system for measurements of different concentrations of chrysin and baicalein are shown in Figs. 5 and 6. A complete analysis, including sampling and washing, could be performed in 1 min.

4. Conclusions

In this paper, we used the molecular connectivity to predict the CL behavior of chrysin and baicalein, and then suggested a flow-injection CL method for chrysin and baicalein. So, we proposed a new model to discover new CL analytical system: first, to theoretical predict the CL behavior, and the second, to suggest analytical system. Compare with the “trail-and-error” screening model for new CL system, the new model is easily and timesaving. On the other hand, this work applied the sensitive and simple CL method for determination of chrysin and baicalein, and the CL system will be quite promising for post column detector of HPLC and capillary electrophoresis for the simultaneous determination of chrysin and baicalein in herbal medicines.

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