Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 422–426
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Chemiluminescent determination of chlorogenic acid in fruits

Xiaoxia Wang [a,1], Jingwu Wang [a,*], Nianjun Yang [b,*,2]

[a] Department of Chemistry, Nanchang University, Nanchang, Jiangxi 330047, P.R. China
[b] Graduate School of Engineering, University of Fukui, Fukui-shi 910-8507, Japan

Received 26 August 2005; received in revised form 27 February 2006; accepted 4 March 2006

Abstract

Chlorogenic acid has been detected by a new flow injection chemiluminescent (FI-CL) method, based on the CL reaction of the acidic potassium permanganate with chlorogenic acid in the presence of formaldehyde as an enhancer. The CL intensity difference of the acidic potassium permanganate and formaldehyde in the presence of chlorogenic acid from the CL intensity without chlorogenic acid was linear with the concentration of chlorogenic acid in the range from 5.0×10^{-8} to 5.0×10^{-5} g ml^{-1} with a detection limit of 5.7×10^{-9} g ml^{-1} when the sampling rate was 150 injections h^{-1}. The method was applied successfully to the determination of chlorogenic acid in fruits with recoveries in the range of $100 \pm 6\%$.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Chemiluminescence; Flow injection analysis; Chlorogenic acid; Potassium permanganate; Formaldehyde

1. Introduction

Chlorogenic acid (1,3,4,5-tetrahydroxycyclohexane carboxylic acid 3-(3,4-dihydroxycinna-mate)), of which chemical structure is shown in Fig. 1, is a kind of polyphenol derivative which widely exists in higher plants like fruits, vegetables, black teas, and some traditional Chinese medicines (Delage, Bohuon, Baron, & Drilleau, 1991; Zhang, Li, & Jiang, 1996). It not only has the functions of antioxidation, of inhibiting hypertension, and of stimulating the flowering of plants, but also affects the activity of trypsin, amylase, and other enzymes (Zhu & Xiao, 1991). Moreover, chlorogenic acid is the main component of producing the bitter taste in crude coffee and thus deliberative elimination of chlorogenic acid into the instant coffee has been adopted extensively to improve the taste of various kinds of coffees. The contents of chlorogenic acid in different areas and in various foods are also quite different. Therefore, it is very important to establish some quantitive methods to monitor the concentration of chlorogenic acid in all kinds of real samples.

The reported analytical methods for chlorogenic acid include infra-red spectrometry (Fabian, Izvekov, Salgo, & Orsi, 1994; Oleszek, Lee, & Price, 1989), thin layer chromatography (Liang, Zhang, Li, & Zheng, 1995), spectrophotometry (Tono, 1987), ^{1}H NMR spectroscopy (Berregi, Santos, del Campo, Miranda, & Aizpurua, 2003), liquid chromatography (Bicchi, Binello, Pellegrino, & Vanni, 1995; Rehwald, Meier, & Sticher, 1994; Snookm, 1982), gas chromatography (Sakaki, 1982), and chemiluminescence (Jiang, He, Zhao, & Hu, 2004; Sakaki, 1982). However, spectroscopic techniques are time-consuming and laborious; chromatographic techniques are slow and expensive and the complicated instruments are also required. These drawbacks prevent the previously reported methods from being utilized as an official way to monitor the content of chlorogenic acid in the real sample analysis.

However, the connection of chemiluminescence with flow injection analysis (FI-CL) has overcome these shortcomings and exhibited high sensitivity, wide linear range, and simple instrumentation required. FI-CL is also

* Corresponding authors. Tel.: +1 505 646 2996.
E-mail addresses: nucaw0104@sina.com (J. Wang), nianjun@nmsu.edu (N. Yang).
[1] Present address: Graduate School of Engineering, University of Fukui, Fukui-shi 910-8507, Japan.
[2] Present address: Department of Chemistry & Biochemistry, New Mexico State University, Las Cruses 88003-8001, NM, USA.

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.03.002

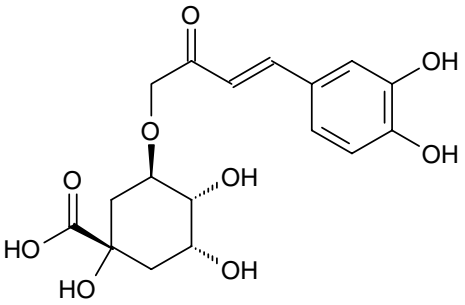

Fig. 1. The chemical structure of chlorogenic acid.

possible to mix the samples and the reagents rapidly with a high degree of reproducibility. It has been applied successfully to the detection of DNA, amino acid, pharmaceutical, and polyphenol compounds (Barnet, Rolfe, & Paton, 1993; Barnett, Hindson, & Lewis, 1998; Barnett, Hindson, Jones, & Smith, 2002; Hindson & Barnett, 2001). Although the CL detection of chlorogenic acid has been reported (Jiang et al., 2004; Sakaki, 1982), the connection of capillary electrophoresis (Jiang et al., 2004) or the utilization of the expensive, unstable, and harmful chemicals (Sakaki, 1982) limits their practical applications for the real samples. Here, we propose a novelly improved FI-CL method for the determination of chlorogenic acid using the acidic potassium permanganate as the CL reagent for the oxidation of chlorogenic acid. The optimization of the experimental conditions of this FI-CL method for the detection of chlorogenic acid in fruits is also presented.

2. Experimental

2.1. Apparatus and chemicals

The IFFL-D FI-CL system (Xi'an Ruike Electronic Ltd. Corporation, China), illustrated in Fig. 2, consists of two peristaltic pumps and an eight-way injection valve. PTFE tube (0.75 mm i.d.) is used to connect all components in the flow system. The sample is injected into a carrier stream via the eight-way injection valve. Two Y-shaped mixing elements are used for mixing the streams, positioned just before the eight-way valve. The CL intensity, amplified by a sensitive photomultiplier tube (PMT) operated at 750 or 850 V, is measured with a detector under the control

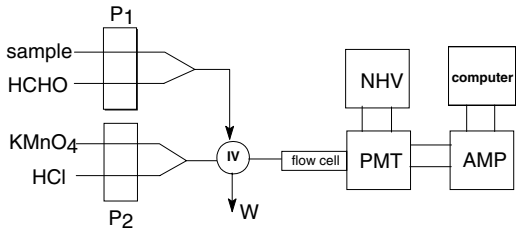

Fig. 2. FI manifold for the CL determination of chlorogenic acid: P_1, P_2, peristaltic pump; IV, injection valve; PMT, photomultiplier tube; AMP, amplifier; NHV, negative high voltage; and W, waste liquid.

of a computer. A KQ-50 ultrasonic bath (Kunsan ultrasonic instrument Ltd. Corporation, China) was used to prepare the samples from the fruits extracts.

Chlorogenic acid (Sigma), potassium permanganate, and formaldehyde (Shanghai Chem. Co., China) were used as received. All other chemicals are of analytical reagent grade. The stock solution of potassium permanganate (5.0 mM) was prepared by dissolving 0.0790 g potassium permanganate into a 100 ml flask with twice distilled water. The stock solution of chlorogenic acid (0.1 mg ml^{-1}) was prepared by dissolving 5 mg chlorogenic acid into 20 ml hot twice distilled water, and then diluted into 50 ml. A formaldehyde stock solution (3%, v/v) and a hydrochloric acid solution (2.0 M) were also prepared.

2.2. Procedure

The solutions of chlorogenic acid, formaldehyde, potassium permanganate, hydrochloric acid were pumped continuously at 1.0, 0.8, 1.2, 1.0 ml min^{-1} into the mixing element by the peristaltic pump, respectively, as shown in the schematic diagram in Fig. 2. The mixture of chlorogenic acid with formaldehyde was then merged into the mixed stream of potassium permanganate and hydrochloric acid by an 80 μl valve injector. The final stream was introduced into the flow CL cell. The full CL intensity vs. time curve was then recorded.

2.3. Preparation of fruit extracts

The sample powder (1 g), grounded from the frozen and dried fruits flesh with a mortar, was extracted with a 60 ml 80% (v/v) methanol aqueous solution for 30 min in an ultrasonic bath. The extract was concentrated to dryness under vacuum at 35 °C. The product was dissolved again in 30 ml 80% methanol as the same way as previously described and the extract was diluted to 100 ml with methanol for paper chromatography in order to separate chlorogenic acid from other polyphenols. The extract solution (100 μl) was developed by using the mixture of ethyl acetate:formic acid:water with the volume ratio of 7:2.5:2.5. A 2.0 M NaOH aqueous solution was used as the coloring medium to determine the position of chromatographic spots. Chlorogenic acid was obtained lastly by washing the paper with 10 ml 0.2 M hydrochloric acid. The resulted solution was diluted 10 times and used for the detection of the concentration of chlorogenic acid.

3. Results and discussion

Fig. 3 shows the CL intensity of the acidic potassium permanganate and chlorogenic acid with respect to time (curve (a)). The CL intensity in curve (a) was detected after 2 s, reached the maximum intensity after 6 s, then became weaker and was almost zero after 18 s. The CL signal of potassium permanganate and chlorogenic acid was detectable but very weak. While after adding formaldehyde as an

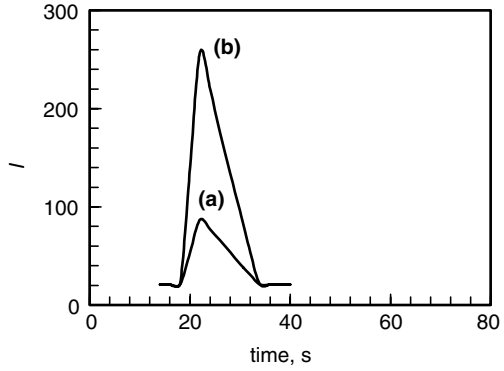

Fig. 3. Variations of FI-CL intensity, I, of the acidic potassium permanganate and 1.0 μg ml^{-1} chlorogenic acid (a) in the absence and (b) in the presence of 5% (v/v) formaldehyde with time. The concentration of potassium permanganate and hydrochloric acid were 0.5 mM and 2.0 M, respectively.

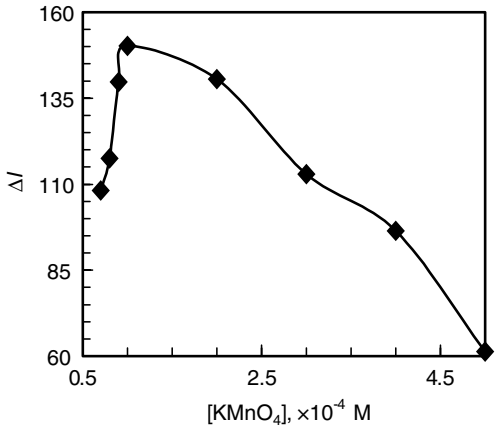

Fig. 4. Concentration dependence of potassium permanganate, [$KMnO_4$] on the difference of the CL intensity, ΔI. The concentration of chlorogenic acid, hydrochloric acid, and formaldehyde were 1.0 μg ml^{-1}, 2.0 M, and 5% (v/v), respectively.

enhancer, the CL intensity of the acidic potassium permanganate and chlorogenic acid was enhanced remarkably, as shown in curve (b). The increase in the concentration of chlorogenic acid also resulted in the increase of this CL intensity. It should be noted here that potassium permanganate did not react with hydrochloric acid in our case and no effect of hydrochloric acid on the CL intensity of the acidic potassium permanganate and chlorogenic acid was observed within its concentration investigated. Subsequently, the FI-CL system of the acidic potassium permanganate and formaldehyde can be used for the quantitive detection of chlorogenic acid.

Since the emission of CL in this system was caused by the oxidation reaction of chlorogenic acid with the acidic potassium permanganate solution containing formaldehyde, the subtraction of the CL intensity, ΔI, can be utilized for the quantitive detection of chlorogenic acid. ΔI was obtained through the difference of the CL intensity of the sample solution from that of the blank solution which included only acidic potassium permanganate and formaldehyde.

A series of experiments were conducted to establish the optimum experimental conditions, including chemical variables and physical variables, e.g. the concentrations of the reagents used, total flow rate, and sample loop volume.

The effect of the type and concentration of oxidant reagents on the CL intensities of chlorogenic acid was investigated. Potassium dichromate, sodium persulphate, potassium iodate, cerium(IV) sulphate, potassium permanganate were tested in acidic and basic media. The CL emission of chlorogenic acid was obtained only by use of the acidic potassium permanganate. Fig. 4 shows the variation of the CL intensity with the concentration of potassium permanganate in the range from 0.07 to 0.5 mM. ΔI reached the maximum value when the concentration of potassium permanganate solution was 0.1 mM, over which the CL intensity decreased. The decreased signal may be due to the absorption of the emitted light by the reagent itself. Therefore, the concentration of potassium permanganate was selected as 0.1 mM.

The effect of the type and concentration of the acidic media, HCl buffer, H_2SO_4 buffer, HNO_3 buffer, H_3PO_4 buffer, on the CL intensity was investigated. Higher ΔI was obtained in HCl buffer. Fig. 5 shows the concentration effect of HCl buffer on the CL intensity in the range from 0.5 to 2.5 M. The increase in the concentration of HCl in the range of 0.5–2.0 M led to enhancement of ΔI. When the concentration of HCl buffer was larger than 2.0 M, the CL intensity decreased. Therefore, 2.0 M HCl buffer was chosen.

Cetyltrimethylammonium bromide, sodium dodecylbenzenesulfonate, Triton-100, hydrogen peroxide, acetaldehyde, formaldehyde were tested as enhancers in our CL system. Different enhancers led to different enhancement of the CL intensity of the acidic potassium permanganate-chlorogenic acid system. Formaldehyde enhanced the CL intensity stronger than other enhancers. The concentration effect of formaldehyde on the CL intensity was then studied and shown in Fig. 6. The increase in the con-

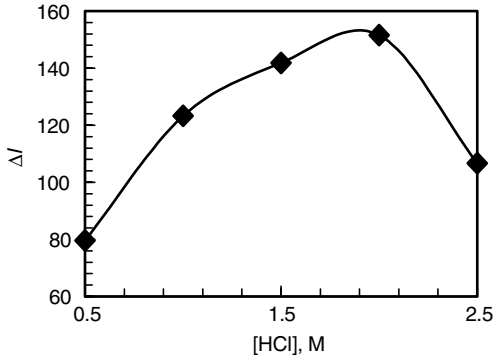

Fig. 5. Concentration variation of hydrochloric acid, [HCl], with the difference of CL net intensity, ΔI. The concentration of chlorogenic acid, potassium permanganate, and formaldehyde were 1.0 μg ml^{-1}, 0.5 mM, and 5% (v/v), respectively.

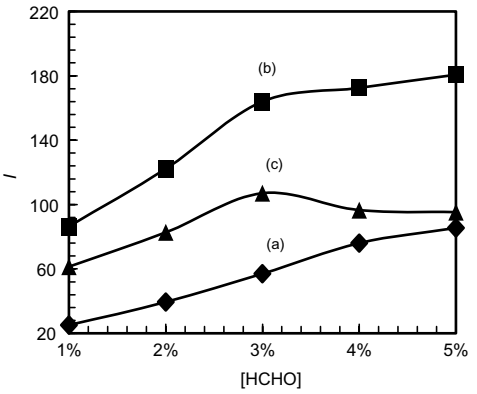

Fig. 6. Concentration effect of formaldehyde [HCHO], on the net CL intensity, I, of the acidic potassium permanganate and formaldehyde in the absence of chlorogenic acid (a) and in the presence of 1.0 μg ml^{-1} chlorogenic acid (b). Curve (c) is the difference of the CL intensity of curve (a) from that of curve (b). The concentration of chlorogenic acid, potassium permanganate, and hydrochloric acid were 1.0 μg ml^{-1}, 0.5 mM, and 2.0 M, respectively.

centration of formaldehyde resulted in the enhancement of the CL intensity for both the blank solution and the sample solution. The optimum ΔI was reached as the concentration of formaldehyde was 3% (v/v).

The instrumental parameters like the flow rate and the volume of the sample loop were studied using the selected reaction conditions discussed above. The total flow rate was divided into the four flow streams. The CL signal increased rapidly with increasing total flow rate up to 4.0 ml min^{-1} and increased at a slower rate up to 7.2 ml min^{-1}. However, the noise was excessive at total flow rate higher than 4.6 ml min^{-1}. A total flow rate of 4.0 ml min^{-1} was selected as a compromise of signal to noise ratio, sensitivity, and consumption of reagents. In the range from 30 to 200 μl, the CL intensity increased with an increase in the loop volumes to 80 μl, and then the signal became unstable and decreased with larger loop volumes. Thus a loop volume of 80 μl was adopted.

Under the optimum experimental conditions mentioned above, the working curve for the detection of chlorogenic acid was plotted by use of ΔI as a function of the concentration of chlorogenic acid. ΔI varied linearly with the concentration of chlorogenic acid, c, in the range from 5.0×10^{-8} to 5.0×10^{-5} g ml^{-1} with a regression equation of $\Delta I = 4.6c - 0.03$. The detection limit was 5.7×10^{-9} g ml^{-1}, calculated from the IUPAC recommendations (3σ). The relative standard deviations (RSD) for 11 injections with 0.5 μg ml^{-1} chlorogenic acid was 2.1%. The sample throughput was 150 injections h^{-1}. The reproducibility was studied by analyzing 10 identical solutions of chlorogenic acid solution (0.5 μg ml^{-1}) on the sequential days and each day five injections were detected. The RSD was 2.3%.

The effect of foreign species on the determination of chlorogenic acid was studied. No effect was noticed when the mass concentration ratios of the foreign species to the sample (0.5 μg ml^{-1} chlorogenic acid) were more than 200 for K^+, Na^+, Zn^{2+}, NH_4^+, Ca^{2+}, Ba^{2+}, Cl^-, SO_4^{2-}, EDTA, tartaric acid, alcohol, acetone, 100 for Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, citric acid, 5 for sodium benzoate, Cr^{3+}, 1 for Fe^{2+}, Cu^+, respectively. The interferes of Fe^{2+}, Cu^+ on the determination of chlorogenic acid can be eliminated by adding 0.5 ml 0.2 mM EDTA as a masking reagent.

The proposed method was utilized for the determination of chlorogenic acid in three kinds of fruits. The results are shown in Table 1. The RSD for these real samples in the range of 1.1–2.1% and the recovery in the range of 94.0–105.2% indicate that this new FI-CL method is sensitive and accurate for the concentration detection in these fruit samples. The comparison of this method with other methods (Berregi et al., 2003; Bicchi et al., 1995; Fabian et al., 1994; Jiang et al., 2004; Liang et al., 1995; Oleszek et al., 1989; Rehwald et al., 1994; Sakaki, 1982; Snookm, 1982; Tono, 1987) also shows that this method is more favorable and more economic for the content monitor of chlorogenic acid in the real samples.

4. Conclusion

An improved FI-CL method for the determination of chlorogenic acid in fruits has been proposed by use of the acidic potassium permanganate as oxidant reagent. The introduction of formaldehyde enhanced the CL intensity remarkably. The difference of the CL intensity for the chlorogenic acid solution from that of the blank solution was linear with the chlorogenic acid concentration in the range of 5.0×10^{-8} to 5.0×10^{-5} g ml^{-1}. The detection limit was 5.7×10^{-9} g ml^{-1}. The proposed method was applied successfully to detect the content of chlorogenic acid in fruits. Compared with the reported CL methods, the big advantage of the proposed method is to utilize a cheap, stable, unharmful oxidant reagent and to avoid using expensive instrumentals. The developed FI-CL

Table 1
The determination results of chlorogenic acid in fruits samples ($n = 5$)

Samples	Sample/10^{-7} g ml^{-1}	RSD/%	Added/10^{-7} g ml^{-1}	Found/10^{-7} g ml^{-1}	Recovery/%
Fuji apple	1.47	1.1	1.50	1.42	94.7
Delicious apple	1.89	2.1	2.00	1.88	94.0
Qinguan apple	1.53	1.9	1.50	1.45	96.7
Grape	1.36	1.5	1.50	1.51	100.7
Hawthorn	2.43	1.7	2.50	2.63	105.2

method may be adopted for officially quantitive detection of chlorogenic acid in medicine, fruits, and foods in future.

References

Barnet, N. W., Rolfe, D. G., & Paton, T. A. (1993). *Analytica Chimica Acta, 282*, 551.

Barnett, N. W., Hindson, B. J., & Lewis, S. W. (1998). *Analytica Chimica Acta, 362*, 131.

Barnett, N. W., Hindson, B. J., Jones, P., & Smith, T. A. (2002). *Analytica Chimica Acta, 451*, 181.

Berregi, I., Santos, J. I., del Campo, G., Miranda, J. I., & Aizpurua, J. M. (2003). *Analytica Chimica Acta, 486*, 269.

Bicchi, C. P., Binello, A. E., Pellegrino, G. M., & Vanni, A. C. (1995). *Journal of Agricultural and Food Chemistry, 14*, 1549.

Delage, E., Bohuon, G., Baron, A., & Drilleau, J. F. (1991). *Journal of Chromatography, 555*, 125.

Fabian, Z., Izvekov, V., Salgo, A., & Orsi, F. (1994). *Analytical Proceedings, 31*, 261.

Hindson, B. J., & Barnett, N. W. (2001). *Analytica Chimica Acta, 445*, 1.

Jiang, H. L., He, Y. Z., Zhao, H. Z., & Hu, Y. Y. (2004). *Analytica Chimica Acta, 512*, 111.

Liang, S., Zhang, G., Li, Y., & Zheng, X. (1995). *Yaowu Fenxi Zazhi., 15*, 24.

Oleszek, W., Lee, C. Y., & Price, K. R. (1989). *Acta Societatis Botanicorum Poloniae, 58*, 273.

Rehwald, A., Meier, B., & Sticher, O. (1994). *Journal of Chromatography A, 677*, 25.

Sakaki, T. (1982). *Kenkyu Hokoku-Nippon Senbai Kosha Chuo Kenkyusho, 124*, 33.

Snookm, M. E. (1982). *Tobacco International, 184*, 111.

Tono, T. (1987). *Tetsuzo Nippon Nogei Kagaku Kaishi, 61*, 1441.

Zhang, D., Li, Z., & Jiang, Y. (1996). *Chinese Journal of Pharmceutical Analysis, 16*, 83.

Zhu, M., & Xiao, P. (1991). *Phytotherapy Research, 5*, 239.