Electrochemical determination of emodin in acidic media by high-performance liquid chromatography and its application to Polygoni Multiflori Radix samples

Aya Shiozawa1 · Yusuke Kojima1 · Akira Kotani1 · Koichi Machida1 · Kazuhiro Yamamoto1 · Hideki Hakamata1

Received: 4 July 2022 / Accepted: 3 August 2022 / Published online: 22 August 2022 © The Author(s), under exclusive licence to The Japan Society for Analytical Chemistry 2022

Abstract
Electrochemical reduction of emodin under acidic media occurs at a less negative potential when compared with that under neutral media. When emodin is electrochemically detected at a less negative potential, a decrease in background noise and improvement in specificity benefit the development of high-performance liquid chromatography with electrochemical detection (HPLC-ECD) for its determination. HPLC-ECD was performed using an octadecyl silica column, acetonitrile–water (60:40, v/v) containing 5 mmol L⁻¹ hydrochloric acid and 10 mmol L⁻¹ lithium perchlorate, as a mobile phase, and an applied potential at −0.4 V vs. Ag/AgCl. Under these optimal HPLC-ECD conditions, the detection limit (signal-to-noise ratio, S/N = 3) of emodin was 0.61 μg L⁻¹. When this HPLC-ECD system was applied to the determination of emodin in Polygoni Multiflori Radix (PMR) samples, other peaks did not appear close to the emodin peak on a chromatogram. The emodin contents in PMR samples were determined with relative standard deviations (RSDs, n = 6) of less than 3.9%, and their recoveries ranged from 92 to 106%. We have shown that our HPLC-ECD system performed an accurate, precise, and specific determination of emodin in PMR samples.

Keywords Emodin · Electrochemical reduction · Polygoni Multiflori Radix · HPLC

Introduction
Polygoni Multiflori Radix (PMR), which is the root of Polygonum multiflorum Thunb., has been widely used as a tonic and purgative in traditional Chinese medicine and Kampo medicine [1, 2]. In the 18th edition of the Japanese Pharmacopoeia, PMR is identified by its morphological properties using a microscope and thin layer chromatography using a crude drug of PMR [3]. Because the content of various medicinal components in herbal medicines are affected by growth environment and geographical origin [4, 5], quantitative analysis for medicinal components as indicators should be applied to provide a stable supply of PMR with consistent quality.

In PMR, emodin (1,3,8-trihydroxy-6-methylanthraquinone, Fig. S1), which has anti-inflammatory, antioxidant, and liver protection effects [6], is one of the main medicinal components with electrochemical activity [7, 8]. Thus, we propose an electroanalytical method with selectivity and sensitivity for determining emodin in PMR. Furthermore, our method will also be useful to assess the quality of PMR using the content of emodin as an indicator. In addition, some electrochemical studies have reported on the mechanism of electrochemical reduction for emodin and this reaction has been applied to the development of electrochemical sensors using emodin as a mediator [9–11]. In the previous studies, various liquid chromatographic methods using high-performance liquid chromatography with ultra-violet detection (HPLC-UV), HPLC with photo-diode array detection (HPLC-PDA), LC-mass spectrometry (LC-MS), and LC-tandem mass spectrometry (LC-MS/MS) have been reported to determine emodin in PMR [12–16]. However, to the best of our knowledge, electroanalytical methods including HPLC with electrochemical detection (HPLC-ECD) have not been attempted for the determination of emodin in PMR. In this study, for
the development of a selective and sensitive HPLC-ECD method to determine emodin in PMR, we proposed that the detection of emodin at a less negative potential would improve the specificity and decrease the background noise in an HPLC-ECD analysis.

The presence of a small amount of acid in an unbuffered electrolyte containing quinone, such as vitamin K₃, has been found to result in a new cathodic peak (termed a prepeak) at a less negative potential than the original cathodic peak of the quinone itself [17], and we have proposed HPLC-ECD methods for determining various acid compounds based on the current measurement of prepeak caused by an acid compound [18, 19]. In the present study, the electrochemical reduction behavior of quinone in the presence of acid was adopted to detect emodin at a less negative potential. Further, we have developed an HPLC-ECD method using an acidic mobile phase for determining emodin, and we have applied the present HPLC-ECD method to the determination of emodin content in PMR.

### Experimental

#### Materials and reagents

All chemicals and solvents were reagent grade. Emodin and 2-methyl-6-nitrobenzoic anhydride (Fig. S1) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Pure water was prepared using an ultrapure water system (RFU666HA, ADVANTEC, Tokyo, Japan). PMR samples were gathered from the Guizhou (two lots), Sichuan (two lots), and Shaanxi (two lots) provinces, China. A Japanese Pharmacopoeia PMR sample, which was produced in Guizhou province, was purchased from Uchida Wakanyaku (Tokyo, Japan).

#### Linear sweep voltammetry

An electrochemical analyzer (HZ-7000, Hokuto Denko, Tokyo, Japan) was used to measure a linear sweep voltammogram. A glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum auxiliary electrode were inserted through the lid in the electrochemical cell. A mixture of the acetonitrile–water mixture (90:10, v/v) containing 5 mmol L⁻¹ hydrochloric acid and 50 mmol L⁻¹ lithium perchlorate was used as the mobile phase at a flow rate of 15 μL min⁻¹. Injection volume and column temperature were set at 5 μL and 50 °C, respectively. The applied potential in the electrochemical detector was set at −0.4 V vs. Ag/AgCl.

#### Sample preparation procedures

A pulverized PMR sample (0.1 g) was dissolved in 20 mL of an acetonitrile–water mixture (60:40, v/v), and extraction into the acetonitrile–water mixture was performed by ultrasonication for 30 min. This solution was centrifuged for 5 min at 4000 r.p.m., and then 5 mL of supernatant and 0.4 mL of 0.35 g L⁻¹ 2-methyl-6-nitrobenzoic anhydride (internal standard, IS) were diluted to 10 mL with an acetonitrile–water mixture (60:40, v/v). This solution was passed through a membrane filter (pore size 0.45 μm, Chromatodisc 4N, Kurabo, Osaka, Japan). A 5 μL sample of the filtrate was injected into the HPLC-ECD system. When a recovery test was performed for a PMR sample, an appropriate volume of the emodin standard solution (2.7 g L⁻¹ in an acetonitrile–water mixture (60:40, v/v)) was directly spiked in the pulverized PMR sample before it was dissolved in the acetonitrile–water mixture.

#### Results and discussion

##### Electrochemical reduction behaviors of emodin under acidic media

A potential sweep was started at +0.2 V vs. Ag/AgCl and proceeded to the negative direction as shown in Fig. 1a. In the linear sweep voltammogram of emodin (0.5 mmol L⁻¹), a well-defined cathodic peak of emodin was observed at −0.68 V vs. Ag/AgCl as shown in Fig. 1a. A similar voltammogram of emodin was performed in the presence of 5 mmol L⁻¹ hydrochloric acid, which was significantly higher than the emodin concentration in the test solution. As shown in
Electrochemical determination of emodin in acidic media by high-performance liquid…

Fig. 1b (solid line), a well-defined cathodic peak of emodin was observed at −0.28 V vs. Ag/AgCl. When the linear sweep voltammogram was measured without emodin as background (Fig. 1b, dashed line), an ill-defined curve appeared at more than −0.5 V vs. Ag/AgCl. However, no peaks appeared at around −0.28 V vs. Ag/AgCl. Linear sweep voltammograms of emodin (0.5 mmol L⁻¹) in the presence of 5 mmol L⁻¹ hydrochloric acid were obtained at various sweep rates \( (v = 20 - 75 \text{ mV s}^{-1}) \). As shown in Fig. S2, the values of the cathodic peak current height of emodin \( (I_{pc}) \) show a linear relationship with the square root of the sweep rate \( (v^{1/2}) \): \( I_{pc} = 2.53 v^{1/2} - 1.24 \) \( (r = 0.991) \). From these results, we conclude that the electrochemical reduction of emodin in the acetonitrile–water (90:10, v/v) with 5 mmol L⁻¹ hydrochloric acid was diffusion-controlled.

Electrochemical reduction of emodin is known to progress via \( 2\text{H}^+/2\text{e}^- \), and emodin hydroquinone \((\text{emodin-H}_2) \) is formed as an electrochemical product \([8, 9]\). The reduction potential for Eq. (1) is expressed by the Nernst equation as follows:

\[
E = E^0 + \frac{RT}{2F} \ln \frac{[\text{emodin}]_0}{[\text{emodin} - \text{H}_2]_0} + \frac{RT}{F} \ln [\text{H}^+]_0 + \text{const}.
\]

where \([\text{emodin}]_0\), \([\text{emodin-H}_2]_0\), and \([\text{H}^+]_0\) are concentrations of emodin, emodin hydroquinone, and the proton on a working electrode surface, respectively. At \([\text{emodin}]_0 = [\text{emodin-H}_2]_0\), the half peak potential \((E_{p/2})\) may be represented simply as follows: \([20, 21]\)

\[
E_{p/2} = \frac{RT}{F} \ln [\text{H}^+]_0 + \text{const} = 0.0592 \log [\text{H}^+]_0 + \text{const}.
\]

In the present study, to examine the effects of hydrochloric acid concentration on the cathodic peak potential of emodin, linear sweep voltammograms of emodin were measured as shown in Fig. S3A. When the concentration of hydrochloric acid in the electrolyte solution is approximated as the concentration of proton on the electrode surface, the regression equation between \(E_{p/2}\) of the cathodic peak of emodin and the pH was \(E_{p/2} = -0.0481 \text{ pH} - 0.0462 \) \( (r = 0.974) \) as shown in Fig. S3B. The shift of a cathodic peak potential of emodin for the positive direction induced by the presence of hydrochloric acid would be explained by the Nernst equation for the electrochemical reduction of emodin. A cathodic peak caused by dissolved oxygen in the test solution appeared at a more negative potential than −0.6 V vs. Ag/AgCl under the present conditions. The utilization of voltammetric detection under acidic media would benefit the improvement of specificity and decrease of baseline noises in the determination of emodin by HPLC-ECD analysis.

HPLC-ECD for determining emodin

A typical chromatogram of emodin (2.7 mg L⁻¹) and an IS (14 mg L⁻¹) is shown in Fig. 2. Chromatographic peaks of emodin and IS were observed at 34 and 40 min, respectively. In addition, some system peaks appeared at about 7, 15, and 20 min. These peak heights were remarkably decreased when the mobile phase of the same lot was injected into the HPLC-ECD system. Moreover, using another HPLC-PDA system with the same separation conditions as the HPLC-ECD, the chromatogram of emodin (2.7 mg L⁻¹) and the IS (14 mg L⁻¹) was measured. As shown in Fig. S4, no peaks appeared around 20 min. Thus, the appearance of these peaks was caused by the injection of sample solutions with different compositions from the mobile phase in this HPLC-ECD system. The optimal ratio of water and
acetonitrile in the mobile phase was examined, and thus an acetonitrile–water ratio at 60:40 (v/v) was selected to provide sufficient separation between emodin and IS peaks within a short measurement time. The resolution between the chromatographic peaks of emodin and the IS was 2.0, indicating that this HPLC-ECD can be applied to the quantitative analysis of emodin using an IS method. Moreover, the optimal applied potential was selected by the result of a hydrodynamic voltammogram for emodin. As shown in Fig. 3, emodin was reduced at potentials more negative than −0.3 V. For potentials more negative than −0.5 V, peak current height was decreased, because of the increase in the background current, which is derived from an ill-defined cathodic current shown in Fig. 1b (dashed line). As such, the applied potential was set at −0.4 V to determine emodin with high sensitivity.

Chromatographic peak height was found to be linearly related to emodin concentration in the standard solution from 2.02 μg L⁻¹ to 20.2 mg L⁻¹ (r=0.999), and the calibration curve of emodin is shown in Fig. S5. Repetitive measurements were performed to evaluate the repeatability of this HPLC-ECD. The relative standard deviations (RSDs, n=6) of the chromatographic peak height ratios of emodin at 135 μg L⁻¹ and 2.7 mg L⁻¹ to that of the IS were 2.6% and 1.7%, respectively. The detection limit (signal-to-noise ratio, S/N = 3) of the emodin concentration was 0.61 μg L⁻¹. The detection limits of emodin by HPLC-PDA, LC-ESI-MS, and LC-MS/MS have been reported to be 71 μg L⁻¹ [12], 70 μg L⁻¹ [13], and 10 ng L⁻¹ [14], respectively. This HPLC-ECD was more sensitive than the HPLC-PDA and LC-ESI-MS, but less sensitive than the LC-MS/MS. There are economic and environmental advantages in this HPLC-ECD because an LC-MS/MS system requires high power consumption and expensive instrumentation including running costs in comparison with an HPLC-ECD system [22–24].

Determination of emodin in PMR samples

A typical chromatogram obtained from a PMR sample is shown in Fig. 4. No peaks appeared close to the peak of emodin and the IS. In Fig. S6, the chromatogram obtained from the PMR sample by the HPLC-PDA is shown. It is confirmed that any components that have UV-visible absorbing groups derived from a PMR sample are not eluted close to the retention times of emodin and the IS. The emodin content in the PMR from different origins were determined using the IS method. According to the sample preparation described in the Experimental section, this HPLC-ECD method can be applied to the determination of emodin in

---

**Fig. 2** Chromatogram obtained from emodin standard by HPLC-ECD. HPLC conditions: column, InertSustain C18 (250×1.0 mm i.d., 3 μm); column temperature, 50 °C; mobile phase, acetonitrile–water mixture (60:40, v/v) containing 5 mmol L⁻¹ hydrochloric acid and 10 mmol L⁻¹ lithium perchlorate; flow rate, 15 μL min⁻¹; applied potential, −0.4 V vs. Ag/AgCl. Peaks: a emodin; b 2-methyl-6-nitrobenzoic anhydride (IS)

**Fig. 3** Hydrodynamic voltammogram of emodin. HPLC conditions used were the same as in Fig. 2 except for the applied potential

**Fig. 4** Chromatogram obtained from Polygoni Multiflori Radix by HPLC-ECD. HPLC conditions used were the same as in Fig. 2
Electrochemical determination of emodin in acidic media by high-performance liquid chromatography-electrochemical detection (HPLC-ECD) is a method commonly used for the analysis of medicinal compounds. In this study, we have employed this technique to evaluate the emodin content in Polygoni Multiflori Radix (PMR) samples.

Table 1: Emoodin content in Polygoni Multiflori Radix (PMR) samples and the recovery of emodin from PMR spiked with a standard

| PMR  | Origin | Repeatability | Recovery | RSD, % | Added amount/mg g⁻¹ | Recovery, % | RSD, % |
|------|--------|---------------|----------|--------|---------------------|------------|--------|
| CHNb | Guizhou| 0.51          | 2.89     | 0.51   | 105.5               | 4.44       |
|      | Guizhou| 0.60          | 1.28     | 0.60   | 92.3                | 1.48       |
|      | Sichuan| 0.50          | 1.50     | 0.50   | 101.5               | 2.35       |
|      | Sichuan| 0.45          | 1.29     | 0.45   | 99.5                | 1.68       |
|      | Shaanxi| 0.68          | 2.60     | 0.68   | 101.8               | 5.05       |
|      | Shaanxi| 0.52          | 1.22     | 0.52   | 102.5               | 0.65       |
| JPc  | Guizhou| 0.55          | 3.93     | 0.55   | 100.4               | 1.41       |

Notes:

*a* n=6

*Commercial sources of herbal medicines from Chinese markets*

*Commercial product adopted by the Japanese Pharmacopoeia*

A PMR sample ranging from 0.808 μg g⁻¹ to 8.08 mg g⁻¹ because the linear range for emodin was from 2.02 μg L⁻¹ to 20.2 mg L⁻¹ in this HPLC-ECD. As shown in Table 1, the contents of emodin in the PMR studied ranged from 0.45 to 0.68 mg g⁻¹ and the RSDs were less than 3.93%. In previous studies, the contents of emodin in PMR samples were reported to range from 0.07 to 3.27 mg g⁻¹ [13, 14] and thus their quantitative values from the PMR studied by HPLC-ECD were within the reported values. The emodin content in the commercial PMR sample adopted from the Japanese Pharmacopoeia was 0.55 mg g⁻¹ and this value was also within the reported values and the results of other commercial sources of PMR samples obtained from Chinese markets. The recoveries of emodin from the spiked PMR sample ranged from 92.3 to 105.5% and their RSDs were less than 5.05%. These results demonstrate that this HPLC-ECD method provides accurate and precise determinations of emodin in PMR samples. To the best of our knowledge, this was the first study describing an HPLC-ECD method for determining emodin in PMR samples using a simple sample preparation.

In this study, the present HPLC-ECD for the determination of emodin will surely contribute to the quality control of PMR since quantitative analysis for a main medicinal component such as emodin has not yet been proposed in the current Japanese Pharmacopoeia.

**Conclusion**

On a linear sweep voltammogram, it was shown that the cathodic peak of emodin under acidic media appeared at a less negative potential when compared with that under neutral media. Using this electrochemical behavior of emodin, an HPLC-ECD method using an acidic eluate was developed for determining emodin in PMR samples. In real sample analysis using PMR samples, we demonstrated that accurate, precise, and specific determination of emodin in PMR samples was performed by the HPLC-ECD method. In short, the HPLC-ECD method was shown to have the potential for assessing the quality of PMR.

**Supplementary Information** The online version contains supplementary material available at https:// doi.org/10.1007/s44211-022-00177-5.

**Acknowledgements** This work was supported in part by JSPS KAKENHI Grant number JP22K05176.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**References**

1. C. Li, M. Niu, Z. Bai, C. Zhang, Y. Zhao, R. Li, C. Tu, H. Li, J. Jing, Y. Meng, Z. Ma, W. Feng, J. Tang, Y. Zhu, J. Li, X. Shang, Z. Zou, X. Xiao, J. Wang, Front Med. 11, 253 (2017).
2. Y. Liu, Q. Wang, J. Yang, X. Guo, W. Liu, S. Ma, S. Li, Front. Pharmacol. 9, 364 (2018).
3. The Japanese Pharmacopoeia, 18th edn. (Ministry of Health, Labour and Welfare, 2021).
4. T. Tsujimoto, R. Arai, T. Yoshitomi, Y. Yamamoto, Y. Ozeki, T. Hakamatsuka, N. Uchiyama, Chem. Pharm. Bull. 69, 741 (2021).
5. Y. Li, G. Zhao, R. Zhang, Y. Wei, Z. Yao, S. Su, Z. Li, Anal. Sci. 38, 1083 (2022).
6. X. Dong, J. Fu, X. Yin, S. Cao, X. Li, L. Lin, M. Huyiligeqi, J. Ni, Phytother. Res. 30, 1207 (2016).
7. M.H. Lee, L. Kao, C. Lin, J. Agric. Food Chem. 59, 9135 (2011).
8. Z.H. Yin, Y. Xu, X. Yu, Y. Tu, Q.J. Zou, J.H. Yu, Y.D. Zhao, Bioelectrochemistry 72, 155 (2008).
9. L. Wang, Z. Zhang, B. Ye, Electrochim. Acta 51, 5961 (2006).
10. H. Gao, G. Liu, Y. Zhu, Z. Wen, X. Liu, G. Wang, F. Li, Green Chem. Eng. (2022). https://doi.org/10.1016/j.gece.2022.06.002
11. L. Hou, C. Kong, Z. Hu, Y. Han, B. Wu, J. Electroanal. Chem. 895, 115402 (2021).
12. Y. Xu, X. Yu, J. Gui, Y. Wan, J. Chen, T. Tan, F. Liu, L. Guo, Foods 11, 386 (2022).
13. J. Feng, H. Ren, Q. Gou, L. Zhu, H. Ji, T. Yi, Anal. Methods 8, 1557 (2016).
14. T.-H. Wang, J. Zhang, X.-H. Qiu, J.-Q. Bai, Y.-H. Gao, W. Xu, Molecules 21, 40 (2016).
15. T. Yi, K.S.Y. Leung, G.H. Lu, H. Zhang, K. Chan, Phytochem. Anal. 18, 181 (2007).
16. F. Zhang, W. Chen, L. Sun, Chromatographia 67, 869 (2008).
17. A. Kotani, F. Kusu, K. Takamura, H. Hakamata, J. Electroanal. Chem. Soc. 167, 037517 (2020).
18. A. Kotani, M. Watanabe, K. Yamamato, F. Kusu, H. Hakamata, Anal. Sci. 32, 1011 (2016).
19. A. Kotani, Y. Miyaguchi, N. Miyashita, F. Kusu, K. Takamura, H. Hakamata, Bunseki Kagaku 70, 415 (2021).
20. K. Takamura, Y. Hayakawa, J. Electroanal. Chem. 31, 225–232 (1971).
21. J. Lindquist, Analyst 100, 339–348 (1975).
22. R. Jiang, J. Yang, S. Mei, Z. Zhao, Anal. Sci. 38, 1009 (2022).
23. Y. Sun, A. Kotani, K. Machida, K. Yamamoto, H. Hakamata, Chem. Pharm. Bull 70, 43 (2022).
24. Y. Liu, Q. Xue, C. Chang, R. Wang, Z. Liu, L. He, Anal. Sci. 38, 55 (2022).

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.