Study on Electrochemical Fingerprints of *Radix Paeoniae Alba*

Jian Zhong Jin* and Chao Shen
College of Biological and Environmental Engineering,Zhejiang Shuren University,Hangzhou 310015，China.
hzjjz@sohu.com*

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Summary: The electrochemical fingerprints of *Radix Paeoniae Alba* were studied by chemical oscillation system of H^+-Mn^{2+}-CH_3COCH_3-BrO_3^-. Influences of variety of *Radix Paeoniae Alba* for the chemical oscillation system were studied. Finally, 43 ℃ temperature, 500 rpm stirring rate, 1000 mg *Radix Paeoniae Alba*, 2.0 mol·L^{-1} hydrogen ion concentration, 0.084 g $MnSO_4$, 0.835 g $KBrO_3$, 1mL acetone and 100 mL total volume were chosen as the optimal experimental conditions. The results indicated that the electrochemical fingerprints of *Radix Paeoniae Alba* from different regions showed significantly different characteristics, so the electrochemical fingerprint is a efficacious method to identify the regions and variety of *Radix Paeoniae Alba*.

Keyword: Radix Paeoniae Alba, Electrochemical fingerprint, Oscillation system.

Introduction

Radix Paeoniae Alba (also known as Paeonia sterniana), which can be divided into Hang-Radix Paeoniae Alba, Hao-Radix Paeoniae Alba and Chuan-Radix Paeoniae Alba, is the dry root of Paeonia Lactiflora Pall and is a commonly-used traditional Chinese medicinal material in China [1]. According to the modern pharmacological studies, Radix Paeoniae Alba has beneficial effect on anti-inflammation, anti-virus and anti-convulsion, etc [2].

The chemical components of *Radix Paeoniae Alba* are complex, the mainly compounds in them include terpenoids, flavonoids and tannins, etc. At present, most of the quality control methods of *Radix Paeoniae Alba* use the paeoniflorin as the examining index [3], but it can't demonstrate the quality of *Radix Paeoniae Alba* obviously by testing a single component. Fingerprint has been widely used in identifying and evaluating the quality of traditional Chinese medicine because of its superiority in representation of chemical components. As a hot spot of current research, a large number of achievements about fingerprint of traditional Chinese medicine have been reported, such as chromatography fingerprint (ME-TLC [4], HPLC [5], CE [6], etc) and spectrum fingerprint (UV [7], IR [8], NMR [9], etc). These methods have high sensitivity and good selectivity, but the pre-treatment of sample is not only very difficult, but also time-consuming and power-wasting. Electrochemical fingerprint uses the technology of chemical oscillation with the traditional Chinese medicine as the substrate of oscillation reaction, determines the change value of potential in the system by electrochemical workstation, obtains the distinctive E-t curves [10]. Electrochemical fingerprint has been widely used in identifying traditional Chinese medicine in recent years because the equipments are cheap and the operation is simple.

In this study, the H^+-Mn^{2+}-CH_3COCH_3-BrO_3^- was used as the chemical oscillation system to investigate the influences of electrochemical fingerprints of *Radix Paeoniae Alba* in terms of temperature, stirring rate, dosage, hydrogen ion concentration, etc. The electrochemical fingerprints were established, and the considerable difference of electrochemical fingerprints of *Radix Paeoniae Alba* from different regions was determined under the same conditions of oscillation reaction. According to this study, we demonstrated that electrochemical fingerprint is a efficacious method to identify the producing area and variety of *Radix Paeoniae Alba.*

Experimental

Materials

H_2SO_4, $MnSO_4$, $KBrO_3$, CH_3COCH_3 (analytical grade; Hangzhou Chemical Reagent Co., Ltd); *Hang-Radix Paeoniae Alba*(from Zhejiang Province China), *Hao-Radix Paeoniae Alba*(from Anhui Province China), both of the *Radix Paeoniae Alba* were bought from Hangzhou Chinese medicine factory and identified by deputy researcher Z. M. Tao from subtropical crops research institute in zhejiang

***To whom all correspondence should be addressed.**

province. The *Radix Paeoniae Alba* used in the single factor experiments and verification experiments was *Hang-Radix Paeoniae Alba*.

Instruments

Oscillation experiments were performed in a glass container with a magnetic stirring apparatus (model: RCT basic; IKA, Germany). The reaction solution temperature was kept at 37.0±0.1℃ by a electronic contact temperature control meter (model: ETS-D5; IKA, Germany). Changes in potential were detected by a platinum electrode (model: 213; Shanghai precision scientific instrument Co., Ltd, China) as working electrode and a saturated calomel electrode (model: 217; Shanghai precision scientific instrument Co., Ltd, China) as reference electrode. Potentials (E) of the electrode as a function of time (t) were recorded by a electrochemical workstation (model: CHI620E; Shanghai Chenhua Instruments Co., Ltd,China) to obtain kinetic curves (E–t) of the oscillation reaction.

Experimental

1000 mg *Radix Paeoniae Alba* powders were put in the reactor, then 0.084 g $MnSO_4$, 1 mL acetone, 0.835 g $KBrO_3$ were added successively while keeping the temperature 43 ℃, stirring rate 500 rpm, hydrogen ion concentration 2.0 mol·L^{-1} and total volume 100 mL. The saturated calomel electrode was used as the reference electrode and the platinum electrode as the indicator electrode. In order to record the electrochemical potential of oscillation system over time and draw the E-t curve, we started to time after adding the $KBrO_3$.

Results and Discussion

Influence of Temperature to Electrochemical Fingerprint of Radix Paeoniae Alba

1000 mg *Radix Paeoniae Alba* powders，0.084 g $MnSO_4$, 1 mL acetone and 0.835 g $KBrO_3$ were added successively under the conditions of stirring rate 500 rpm and hydrogen ion concentration 2.0 mol·L^{-1} while keeping the total volume 100 mL. The influence of temperature to electrochemical fingerprints of *Radix Paeoniae Alba* was studied from 35 to 50 °C. The results were shown in Fig. 1 and Table-1.

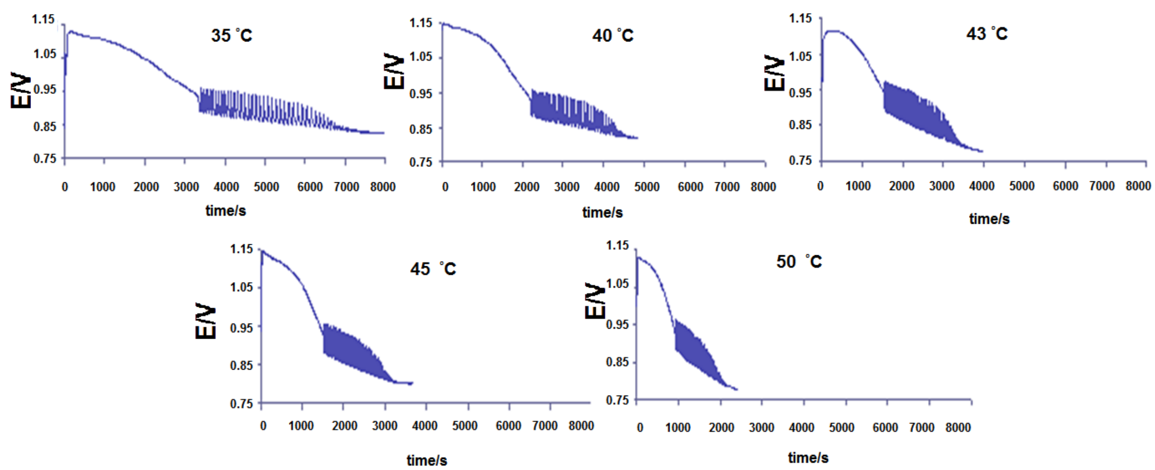

Fig. 1: Electrochemical fingerprints of *Radix Paeoniae Alba* on different temperatures.

Table-1: Charateristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* on different temperatures.

Temperature/°C	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/mV
35	3280	3917	0.06
40	2168	2500	0.07
43	1509	2056	0.09
45	1496	1808	0.09
50	909	1272	0.09

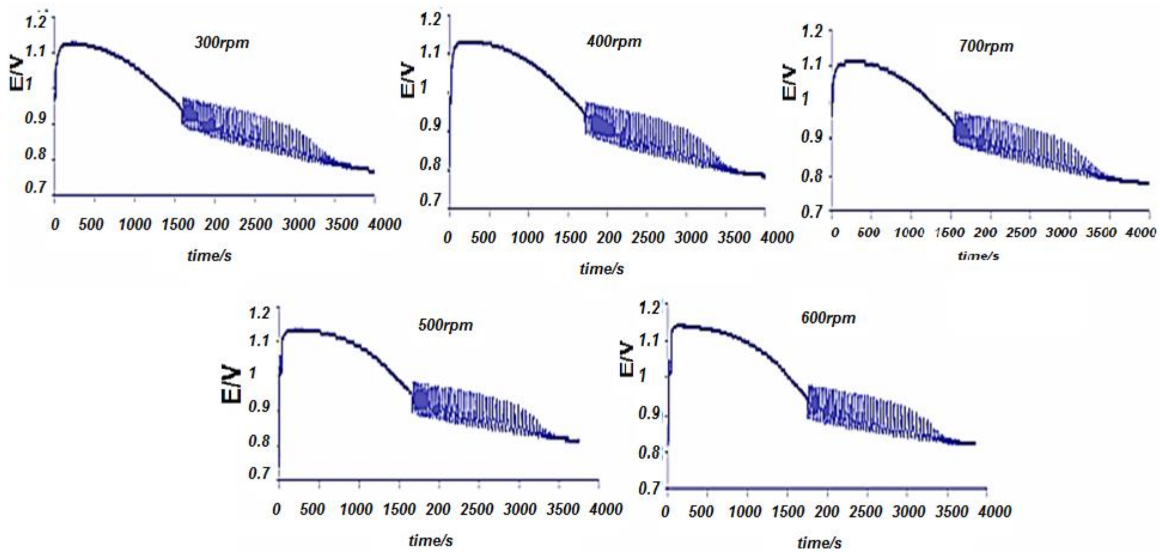

Fig. 2: Electrochemical fingerprints of *Radix Paeoniae Alba* on different stirring rate.

According to Fig. 1 and Table-1, we could find that both of the induction time and the oscillation lifetime were reduced with the increasing of temperature, but the maximum oscillation amplitude was enhanced. In order to find the optimal experimental conditions of the electrochemical fingerprints of *Radix Paeoniae Alba*, we had better choose the shorter induction time, longer oscillation lifetime and bigger oscillation amplitude. Finally, 43℃ as the optimum condition of temperature was chosen according to the principles above and the experimental data from Fig. 1 and Table-1.

Influence of stirring rate to electrochemical fingerprints of Radix Paeoniae Alba

The stirring rate was varied from 300 to 700 rpm under other identical conditions that temperature 43 °C, *Radix Paeoniae Alba* powders 1000 mg, hydrogen ion concentration 2.0 mol·L^{-1}, $MnSO_4$ dosage 0.084 g, acetone dosage 1mL, $KBrO_3$ dosage 0.835 g and the total volume 100 mL to investigate the influence of stirring rate to electrochemical fingerprints of *Radix Paeoniae Alba*. The results were shown in Fig. 2 and Table-2.

The increasing of stirring rate conduced to enhance the speed of dissolving out components from traditional Chinese medicine and shorten the induction time, but the oscillation system would be instability if the stirring rate was excessively fast. Finally, 500rpm as the optimum condition of stirring rate was chosen according to the principles above and the experimental data from Fig. 2 and Table-2.

Table-2: Charateristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* on different stirring rates.

Stirring rate/rpm	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/mV
300	1583	2207	0.08
400	1685	2044	0.08
500	1509	2056	0.09
600	1723	1952	0.08
700	1862	1980	0.08

Influence of dosage to electrochemical fingerprints of Radix Paeoniae Alba

The influence of dosage to electrochemical fingerprints of *Radix Paeoniae Alba* was given in Fig. 3 and Table-3 under other identical conditions. The dosage was varied from 0.6 to 1.4 g while keeping temperature 43 °C, stirring rates 500 rpm, hydrogen ion concentration 2.0 mol·L^{-1}, $MnSO_4$ dosage 0.084 g, acetone dosage 1mL, $KBrO_3$ dosage 0.835 g and the total volume 100 mL.

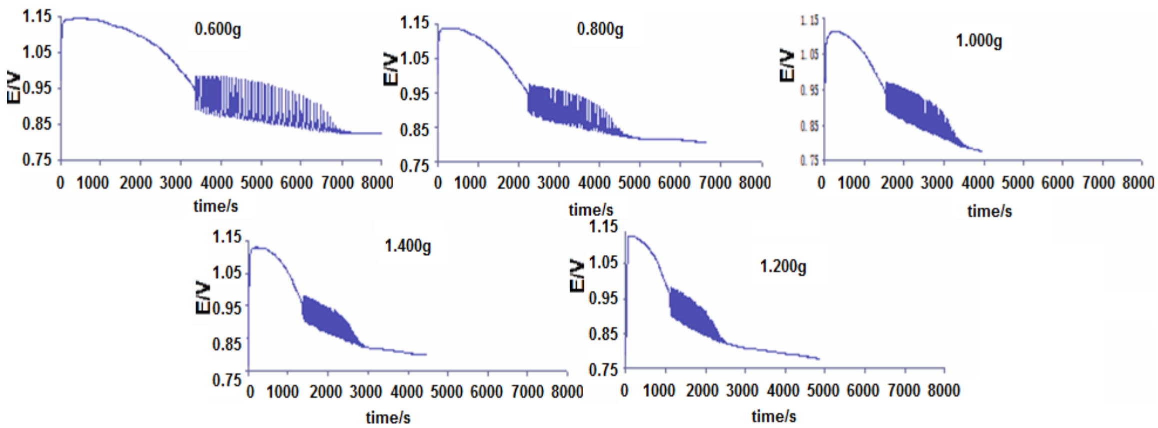

Fig. 3: Electrochemical fingerprints of *Radix Paeoniae Alba* on different dosage.

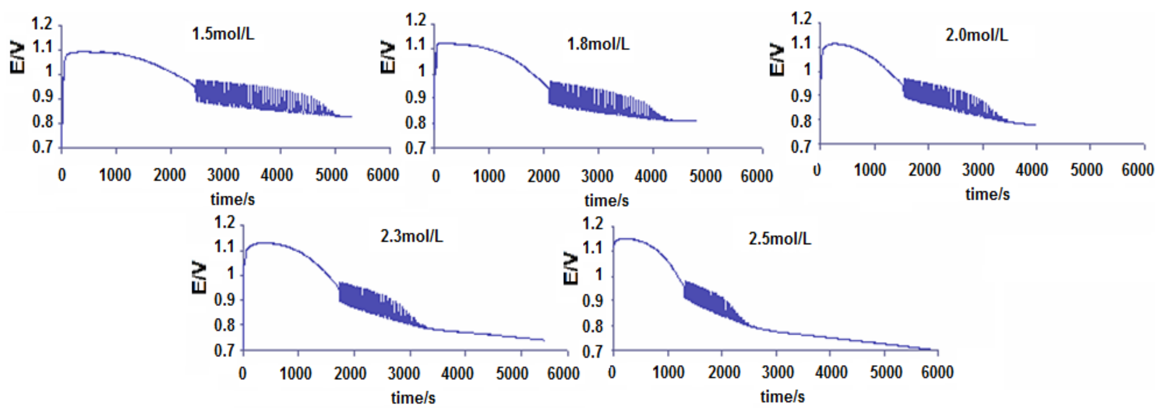

Fig. 4: Electrochemical fingerprints of *Radix Paeoniae Alba* on different hydrogen ion concentrations.

Table-3: Charateristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* on different dosage.

Radix Paeoniae Alba dosage/mg	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/mV
600	3303	3711	0.10
800	2202	2453	0.09
1000	1509	2056	0.09
1200	1337	1537	0.09
1400	1096	1407	0.08

The dosage was an important factor affecting electrochemical fingerprints of *Radix Paeoniae Alba*. According to Fig. 3 and Table-3, we could find that the induction time, oscillation lifetime and maximum oscillation amplitude were all decreased with the increasing of dosage. Finally, 1000 mg as the optimum condition of dosage was chosen according to the principles above and the experimental data from Fig. 3 and Table-3.

Influence of hydrogen ion concentration to electrochemical fingerprints of Radix Paeoniae Alba

The influence of hydrogen ion concentration to electrochemical fingerprints of *Radix Paeoniae Alba* was demonstrated in Fig. 4 and Table-4. Hydrogen ion concentration was varied from 1.5 to 2.5 mol·L^{-1} while keeping temperature 43 °C, stirring rates 500 rpm, *Radix Paeoniae Alba* powders 1000 mg, $MnSO_4$ dosage 0.084 g, acetone dosage 1mL, $KBrO_3$ dosage 0.835 g and the total volume 100 mL.

According to Fig. 4 and Table-4, we could find that the maximum oscillation amplitudes were quite similar with the increasing of hydrogen ion concentration, but both of the induction time and oscillation lifetime were decreased. Finally, 2 mol·L^{-1} as the optimum condition of hydrogen ion concentration was chosen according to the principles above and the experimental data from Fig. 4 and Table-4.

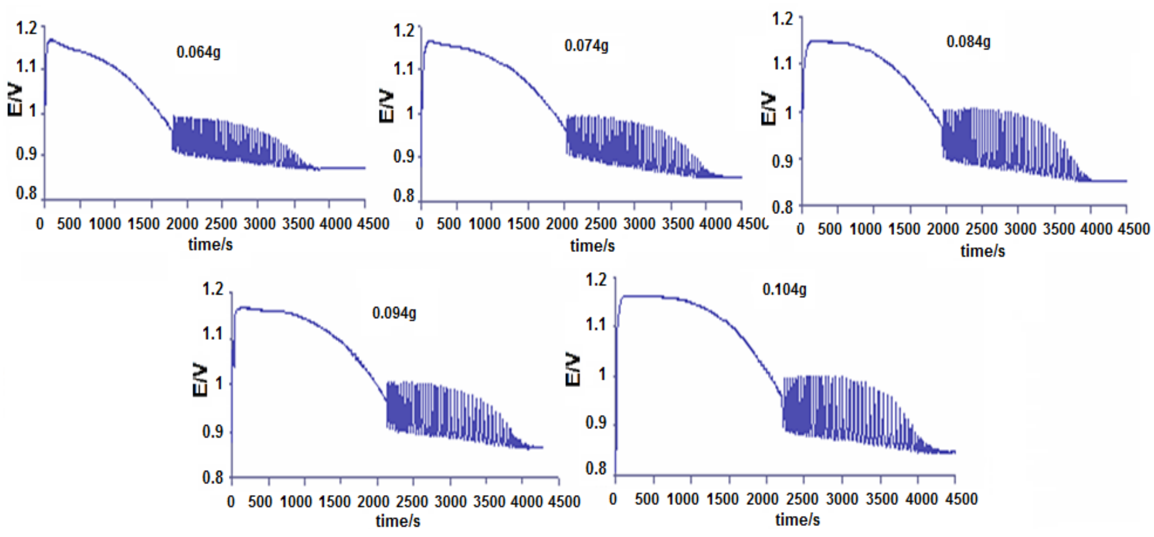

Fig. 5: Electrochemical fingerprints of *Radix Paeoniae Alba* on different manganese sulfate dosage.

Table-4: Charateristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* on different hydrogen ion concentrations

$[H^+]$/mol·L^{-1}	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/mV
1.5	2363	2645	0.09
1.8	2061	2253	0.09
2.0	1509	2056	0.09
2.3	1483	1564	0.09
2.5	1299	1175	0.08

Influence of manganese sulfate dosage to electrochemical fingerprints of Radix Paeoniae Alba

The manganese sulfate dosage was varied from 0.064 to 0.104 g under other identical conditions that temperature 43 °C, stirring rates 500 rpm, *Radix Paeoniae Alba* powders 1000 mg, hydrogen ion concentration 2.0 mol·L^{-1}, acetone dosage 1mL, $KBrO_3$ dosage 0.835 g and the total volume 100 mL to investigate the influence of manganese sulfate dosage to electrochemical fingerprints of *Radix Paeoniae Alba*. The results were shown in Fig. 5 and Table-5.

According to Fig. 5 and Table-5, we could find that the maximum oscillation amplitude was biggest with the manganese sulfate dosage at 0.084 and 0.104 g, while the induction time was shorter with the manganese sulfate dosage at 0.084 g. Finally, 0.084 g as the optimum condition of manganese sulfate dosage was chosen according to the principles above and the experimental data from Fig. 5 and Table-5.

Table-5: Charateristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* on different manganese sulfate dosage

Manganese sulfate dosage/g	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/mV
0.064	1750	2065	0.08
0.074	2003	2176	0.10
0.084	1918	2090	0.12
0.094	2078	2021	0.11
0.104	2184	2112	0.12

Influence of potassium bromate dosage to electrochemical fingerprints of Radix Paeoniae Alba

1000 mg *Radix Paeoniae Alba* powders，0.084 g $MnSO_4$, and 1 mL acetone were added successively under the conditions of temperature 43 °C, stirring rate 500 rpm and hydrogen ion concentration 2.0 mol·L^{-1} while keeping the total volume 100 mL. The influence of potassium bromate dosage to electrochemical fingerprints of *Radix Paeoniae Alba* was studied from 0.635 to 1.035 g. The results were shown in Fig. 6 and Table-6.

According to Fig. 6 and Table-6, we could find that both of the induction time and oscillation lifetime were enhanced with the increasing of potassium bromate dosage and all the maximum oscillation amplitudes were the same. Finally, 0.835 g as the optimum condition of potassium bromate dosage was chosen according to the principles above and the experimental data from Fig. 6 and Table-6

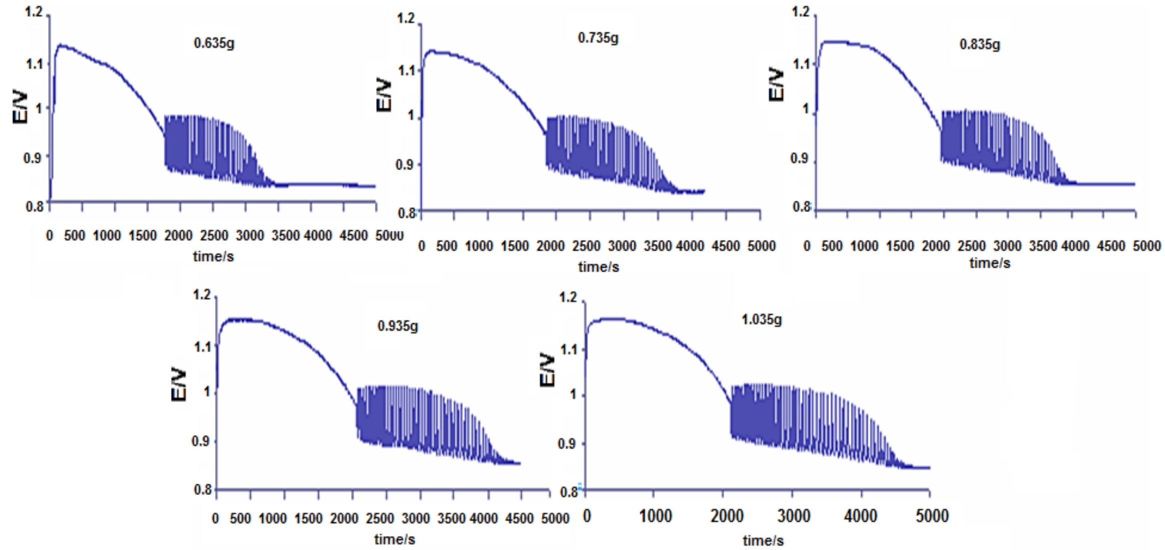

Fig. 6: Electrochemical fingerprints of *Radix Paeoniae Alba* on different potassium bromate dosage.

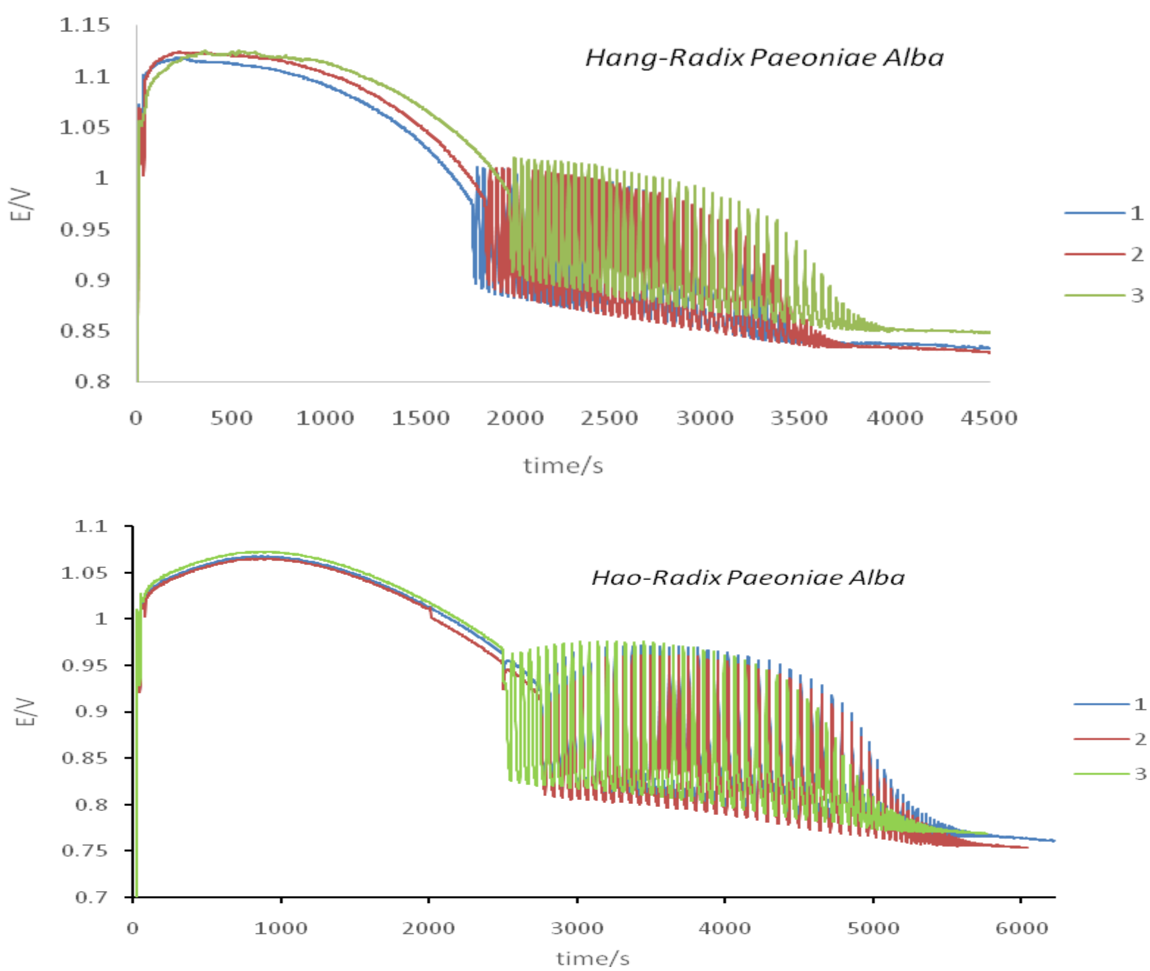

Fig. 7: Electrochemical fingerprints of *Radix Paeoniae Alba*.

Table-6: Charateristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* on different potassium bromate dosage

Potassium bromate dosage/g	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/mV
0.635	1743	1700	0.12
0.735	1801	1957	0.12
0.835	1918	2090	0.12
0.935	2037	2215	0.12
1.035	2074	2497	0.12

Verification Experiments and Contrast experiments

According to the single factor experiments above, the optimum determining conditions of electrochemical fingerprints of *Radix Paeoniae Alba* were established with temperature 43 ℃, stirring rate 500 rpm, *Radix Paeoniae Alba* powders 1000 mg, hydrogen ion concentration 2.0 mol·L^{-1}, $MnSO_4$ dosage 0.084 g, acetone dosage 1mL, $KBrO_3$ dosage 0.835 g and the total volume 100 mL. 1000 mg *Hang-Radix Paeoniae Alba and Hao-Radix Paeoniae Alba* from different regions was took respectively, the electrochemical fingerprints of them were determined for three times parallelly under the optimal determining conditions. The results were shown in Fig. 7 and Table 7.

According to Fig. 7 and Table-7, we could find that both of the maximum oscillation amplitude and maximum potential kept the same under the optimum determining conditions, the RSD of induction time and the oscillation lifetime were 1.7-5.1% and 0.8-3.4%. It demonstratesd that the reproducibility of electrochemical fingerprints of *Radix Paeoniae Alba* was excellent under the optimal conditions.

The electrochemical fingerprints of *Hang-Radix Paeoniae Alba and Hao-Radix Paeoniae Alba* were totally different under the same conditions according to Fig. 8 and Table-7. It demonstrated that the electrochemical fingerprint can be used for identifying *Radix Paeoniae Alba* from different regions and it was a efficacious way to identify the producing area and variety of *Radix Paeoniae Alba*.

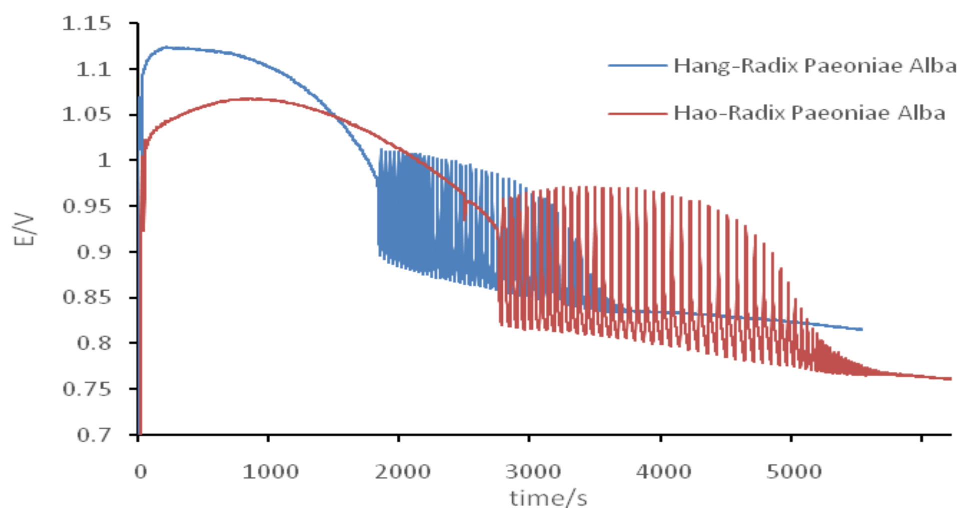

Fig. 8: Electrochemical fingerprints of *Radix Paeoniae Alba* from different regions.

Table-7: Characteristic parameters of electrochemical fingerprints of *Radix Paeoniae Alba* from different regions.

Variety	Group	Induction time/s	Oscillation lifetime/s	Maximum oscillation amplitude/V	Maximum potential/V
	1	1748	1831	0.12	1.12
Hang-Radix Paeoniae Alba	*2*	1806	1916	0.12	1.12
	3	1931	1966	0.12	1.12
Average		1828	1904	0.12	1.12
RSD%		5.1	3.4	0	0
	1	2726	2978	0.16	1.07
Hao-Radix Paeoniae Alba	*2*	2642	3021	0.16	1.07
	3	2562	2905	0.16	1.07
Average		2643	2968	0.16	1.07
RDS%		1.7	0.8	0	0

Conclusion

The electrochemical fingerprints of *Radix Paeoniae Alba* were studied by single factor experiments. Finally, 43 ℃ temperature, 500 rpm stirring rate, 1000 mg *Radix Paeoniae Alba*, 2.0 mol·L^{-1} hydrogen ion concentration, 0.084 g $MnSO_4$, 0.835 g $KBrO_3$, 1mL acetone and 100 mL total volume were chosen as the optimal experimental conditions. The electrochemical fingerprints of *Radix Paeoniae Alba* were determined for three times parallelly under the optimal conditions, which demonstrated that the reproducibility of electrochemical fingerprints of *Radix Paeoniae Alba* was excellent. Then the electrochemical fingerprints of *Hang-Radix Paeoniae Alba* and *Hao-Radix Paeoniae Alba* were contrasted under the optimal conditions, which indicated the electrochemical fingerprint was a efficacious method to identify the regions and variety of *Radix Paeoniae Alba*.

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