Study on Electrochemical Fingerprints of *Rhizoma Atractylodis Macrocephalae*

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Summary: The electrochemical fingerprints of *Rhizome atractylodis macrocephalae* were studied by B–Z (Belousov-Zhabotinski) oscillation system of H^+-Mn^{2+}-CH_3COCH_3-BrO_3^-. The influences of temperature, stirring rate, hydrogen ion concentration, *Rhizome atractylodis macrocephalae* dosage, etc, to electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* were investigated. The optimum conditions were established with total volume of 100 mL, temperature of 37□, stirring rate of 400 rpm, hydrogen ion concentration of 1.980 mol·L^{-1}, *Rhizoma atractylodis macrocephalae* dosage of 1.000 g, $KBrO_3$ concentration of 0.05000 mol·L^{-1}, $MnSO_4$ concentration of 0.005600 mol·L^{-1} and CH_3COCH_3 of 1.00 mL by comparing difference of the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from two choosen regions. The results indicated that the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from different origins showed significantly different characteristics, so the electrochemical fingerprint is a simple method to identify the origins and variety of *Rhizoma atractylodis macrocephalae*.

Keyword: *Rhizoma atractylodis macrocephalae*; Electrochemical fingerprint; B–Z oscillation.

Introduction

Rhizoma atractylodis macrocephalae, the dry rhizome of feverfew *Atractylodes macrocephala* Koidz., has delicate fragrance and bitter taste [1]. It is mainly used to treat abdominal distension, phlegm retention disease, edema, spontaneous perspiration and threatened abortion [2]. *Rhizoma atractylodis macrocephalae* is one of well-known traditional Chinese medicine and produced mostly in Zhejiang, also in Anhui, Henan and Hubei etc [3]. The quality of Rhizoma atractylodis macrocephalae from different regions is different obviously. In order to insure the safety, effectiveness, stabilization and controllability of traditional Chinese medicine, it is necessary to establish a reliable and convenient analytical method to identify the regions of origin of *Rhizoma atractylodis acrocephalae* [4].

At present, the identification method of the traditional Chinese medicinal materials mainly includes traditional identification method (such as morphological characters identification [5]) and modern identification method (such as microscopic identification [6], thin layer chromatography (TLC) [7], HPLC [8] and infrared spectroscopy [9], etc.). These methods have high sensitivity and good selectivity, but the pre-treatment of sample is not only very difficult, but also time- and energy-consuming. However, the Belousov–Zhabotinskii (B–Z) oscillation system will be stable compared with unvaried medicine materials. It shows that the B–Z oscillation system is objective and has good repeatability. The application of oscillation system for the identification of traditional Chinese medicine has gained interest from some scientists [10, 11].

In this study, the H^+-Mn^{2+}-CH_3COCH_3-BrO_3^- was used as the B-Z oscillation system with *Rhizoma atractylodis macrocephalae* as the reaction substrate to measure electric potential in the system with the electrochemical workstation, and the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* were established. The results indicated that the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from different regions show significantly different characteristics, and can be used to identify the regions of *Rhizoma atractylodis macrocephalae*.

Experimental

Materials

H_2SO_4， $KBrO_3$， CH_3COCH_3， $MnSO_4$ (analytical grade; Hangzhou Chemical Reagent Co., Ltd); *Rhizoma atractylodis macrocephalae* (from Bozhou, Anhui; Xianju, Zhejiang; Panan, Zhejiang) all above were bought from Hangzhou Chinese medicine factory.

Instruments

Electrochemical workstation (model:

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CHI620E; Shanghai Chenhua Instruments Co., Ltd); magnetic stirring apparatus (model: RCT basic; IKA); electronic contact temperature control meter (model: ETS-D5; IKA); saturated calomel electrode (model: 217; Shanghai precision scientific instrument Co., Ltd); platinum electrode (model: 213; Shanghai precision scientific instrument Co., Ltd).

Experimental Method

1.000 g *Rhizoma atractylodis macrocephalae* powders were put in the reactor, then 23.00 mL 4.304 mol·L^{-1} H_2SO_4, 1.00 mL 0.5600 mol·L^{-1} $MnSO_4$, 1.00 mL CH_3COCH_3, 20.00 mL 0.2500 mol·L^{-1} $KBrO_3$ were added respectively while keeping the total volume 100 mL by adding deionized water. Next the reactor was put in magnetic stirrer, while controlling temperature of 37ºC, stirring rate of 400 rpm. 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

According to the experimental method above, the temperature was controlled at 35℃, 37℃, 39℃, 41℃ and 43℃ to record the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju respectively under other identical conditions that [H^+]=2.000 mol·L^{-1}, [$MnSO_4$]=0.005600 mol·L^{-1}, acetone 1.00 mL, [$KBrO_3$]=0.05000 mol·L^{-1}, *Rhizoma atractylodis macrocephalae* powder dosage 1.000 g, stirring rate 400 rpm and total volume 100 mL. The results are shown in Fig. 1 and Table-1.

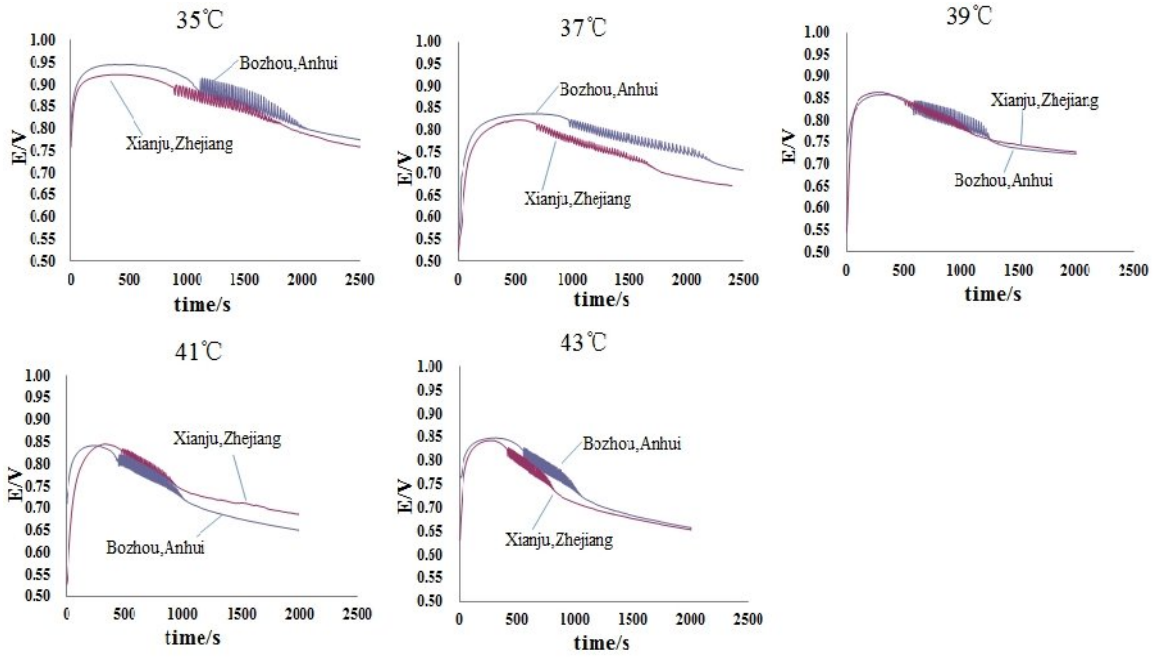

Fig. 1: Electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju on different temperatures.

Table-1: Differences in the parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju at different temperatures.

Temperature /℃	Difference of induction time/s	Difference of maximum oscillation amplitude /V	Difference of oscillation lifetime /s
35	29	0.01	3
37	299	0.00	245
39	72	0.01	75
41	-35	0.01	124
43	133	0.01	101

Temperature would influence the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae*. The results are shown in Fig. 1 and Table-1. In this study, the differences of the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from different regions were chosen as the criterion to determine the optimum determining conditions, and the bigger difference the better. From Table-1, we find that the difference of induction time and oscillation lifetime was the biggest at 37℃, so we chose 37ºC as the optimum reaction temperature.

Influence of Hydrogen Ion Concentration to Electrochemical Fingerprint

According to the experimental method above, the volume of 4.304 $mol \cdot L^{-1} H_2SO_4$ was controlled at 20.00 mL, 22.00 mL, 23.00 mL, 24.00 mL and 26.00 mL to determine the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju respectively under otherwise identical conditions that $[MnSO_4]$=0.005600 $mol \cdot L^{-1}$, acetone 1.00 mL, $[KBrO_3]$=0.05000 $mol \cdot L^{-1}$, *Rhizoma atractylodis macrocephalae* powder dosage 1.000 g , temperature 37℃, stirring rate 400 rpm and total volume 100 mL. The results are shown in Fig. 2 and Table-2

According Table-2, we found that the biggest difference of induction time and oscillation lifetime were in $[H^+]$=1.980 $mol \cdot L^{-1}$, so chose $[H^+]$=1.980 $mol \cdot L^{-1}$ as the optimum reaction hydrogen ion concentration.

Influence of $KBrO_3$ Concentration to Electrochemical Fingerprint

The volume of 0.2500 $mol \cdot L^{-1}$ $KBrO_3$ solution was varied from 17.00 mL to 23.00 mL while keeping stirring rate 400 rpm, temperature 37℃, *Rhizoma atractylodis macrocephalae* powder dosage 1.000 g, $[H^+]$=1.980 $mol \cdot L^{-1}$, 0.5600 $mol \cdot L^{-1} MnSO_4$ 1.00 mL, acetone 1.00 mL, and the total volume 100.00 mL to investigate the influence of $KBrO_3$ concentration to electrochemical fingerprints of *Rhizoma atractylodis macrocephalae*. The results are shown in Fig. 3 and Table-3. The optimum $KBrO_3$ concentration was $[KBrO_3]$=0.05000 $mol \cdot L^{-1}$ on the basis of Table-3.

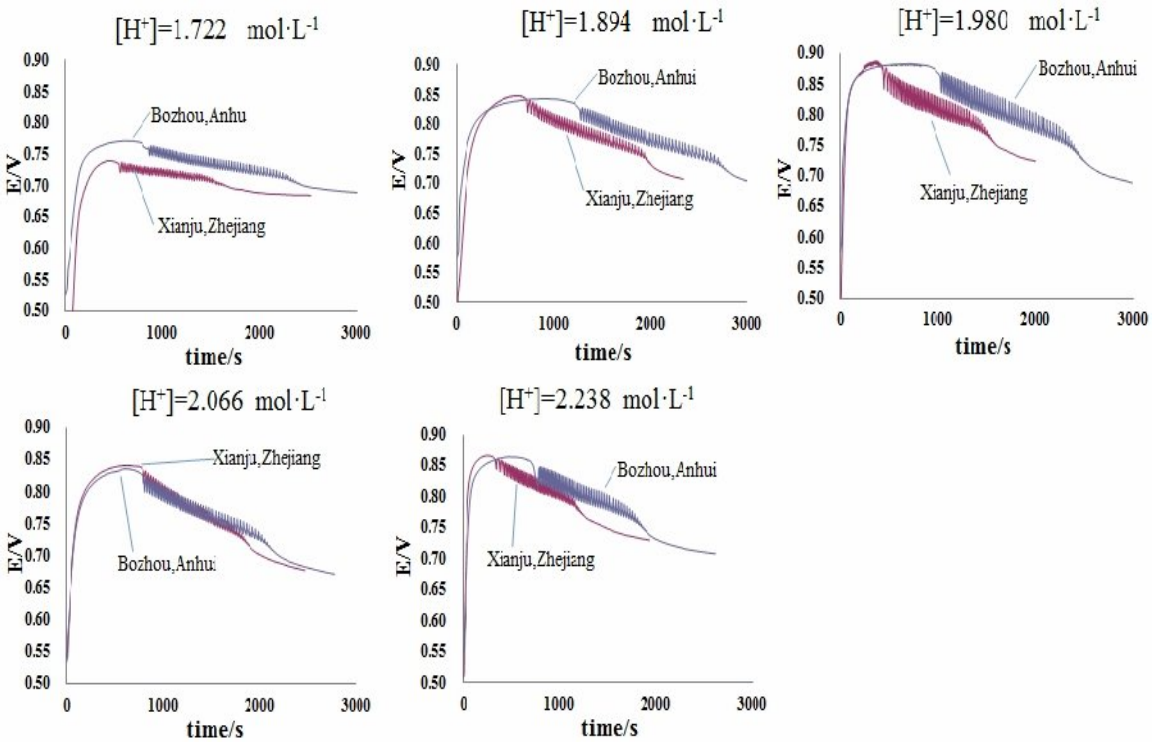

Fig. 2: Electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou, Anhui and Xianju, Zhejiang on different hydrogen ion concentration.

Table-2: Differences in the parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju at different hydrogen ion concentration.

Hydrogen ion concentration /mol·L^{-1}	Difference of induction time/s	Difference of maximum oscillation amplitude /V	Difference of oscillation lifetime /s
1.722	277	0.01	481
1.894	571	0	221
1.980	607	0.01	307
2.066	13	0	256
2.238	377	0.01	273

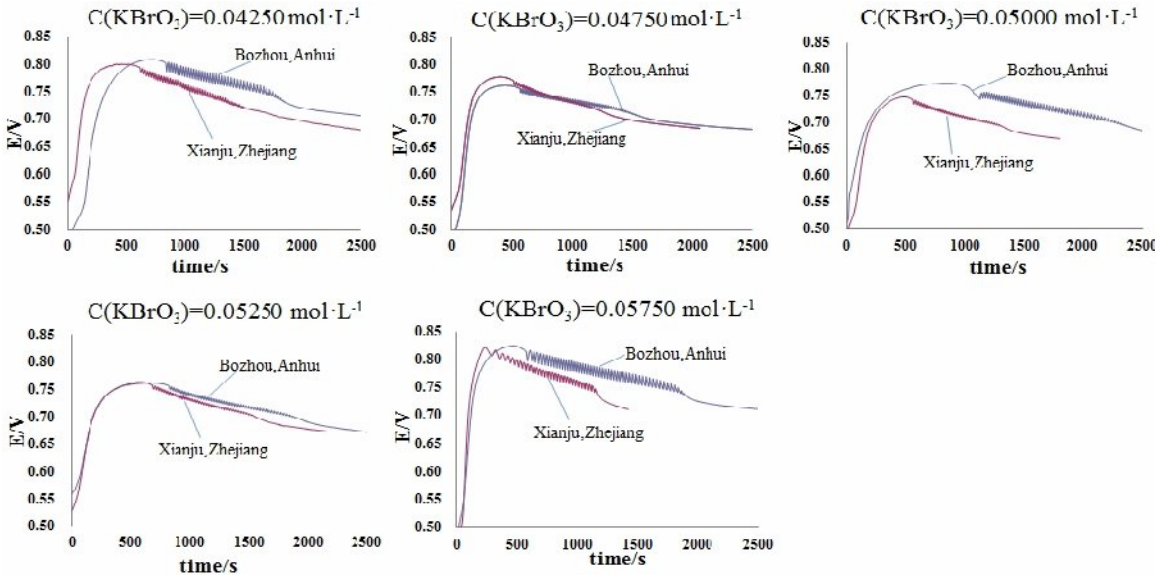

Fig. 3: Electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju on different $KBrO_3$ concentration.

Table-3 Differences in the parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju at different $KBrO_3$ concentration.

$KBrO_3$ concentration / mol·L^{-1}	Difference of induction time/s	Difference of maximum oscillation amplitude /V	Dfference of oscillation lifetime /s
0.04250	217	0	136
0.04750	51	0	235
0.05000	571	0	494
0.05250	141	0	252
0.05750	342	-0.01	400

Influence of $MnSO_4$ Concentration to Electrochemical Fingerprint

According to the experimental method above, the volume of 0.5600 mol·L^{-1}$MnSO_4$ solution was varied from 0.60 mL to 1.40 mL while keeping stirring rate 400 rpm, temperature 37℃, *Rhizoma atractylodis macrocephalae* powder dosage 1.000 g, $[H^+]$=1.980 mol·L^{-1}, $[KBrO_3]$=0.05000 mol·L^{-1}, acetone 1.00 mL and the total volume 100.00 mL to investigate the influence of $MnSO_4$ concentration to electrochemical fingerprints of *Rhizoma atractylodis macrocephalae*. The results are shown in Fig. 4 and Table-4.

According Fig. 4 and Table-4, when $MnSO_4$ concentration was 0.005600 mol·L^{-1}, the difference of induction time and oscillation lifetime were biggest. So we chose $[MnSO_4]$=0.005600 mol·L^{-1} as the optimum $MnSO_4$ concentration.

Influence of Stirring Rate to Electrochemical Fingerprint

The stirring rate was controled from 300 rpm to 500 rpm to determine the electrochemical

fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju respectively under otherwise identical conditions that $[H^+]$=1.980 mol·L^{-1}, $[MnSO_4]$=0.005600 mol·L^{-1}, acetone 1.00 mL, $[KBrO_3]$=0.05000 mol·L^{-1}, *Rhizoma atractylodis macrocephalae* powder dosage 1.000 g, temperature 37℃ and the total volume 100.00 mL. The results are shown in Fig. 5 and Table-5. According Fig. 5 and Table-5, we chose 400 rpm as the optimum reaction stirring rate from overall consideration.

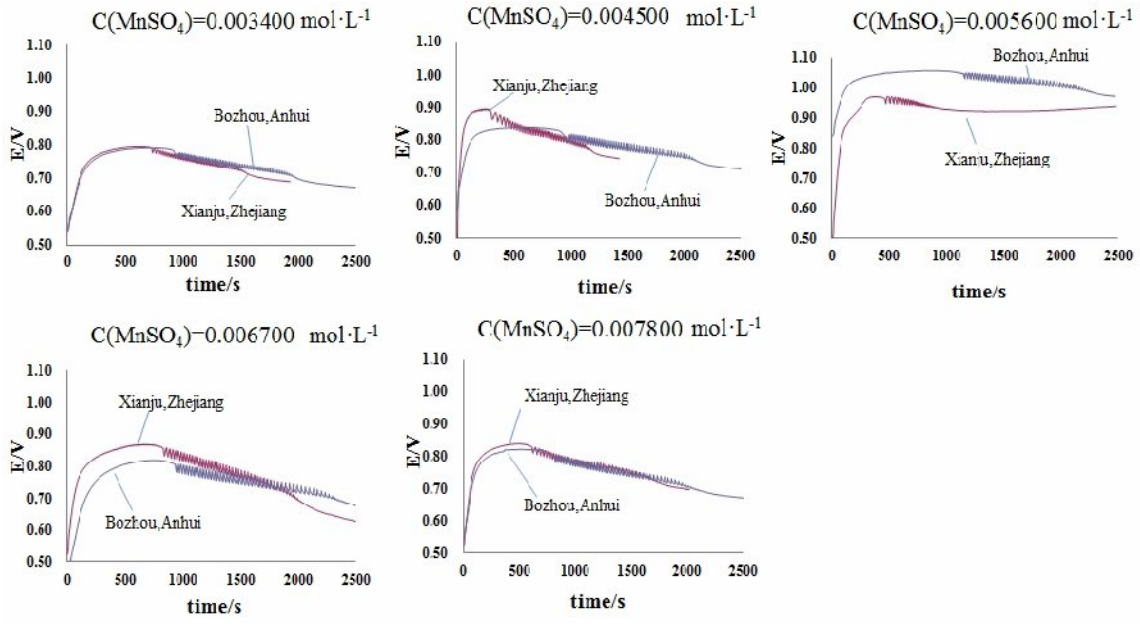

Fig. 4: Electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju on different $MnSO_4$ concentration.

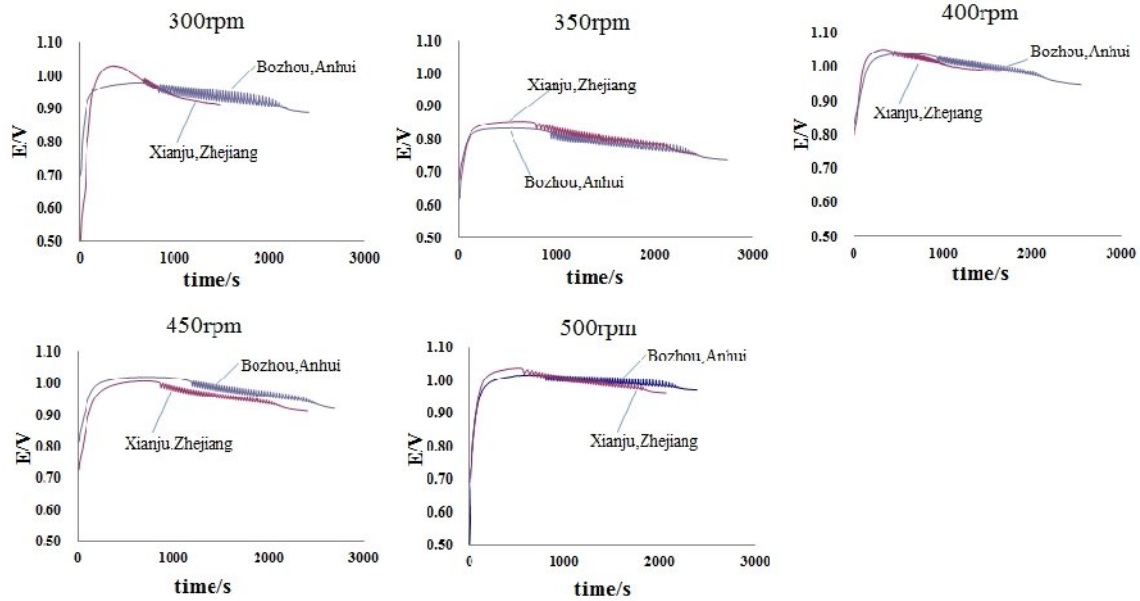

Fig. 5: Electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou, Anhui and Xianju , Zhejiang on different stirring rate.

Table-4: Differences in the parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju at different $MnSO_4$ concentration.

$MnSO_4$ concentration / mol·L^{-1}	Difference of induction time/s	Difference of maximum oscillation amplitude /V	Difference of oscillation lifetime /s
0.003400	205	0	242
0.004500	659	0	282
0.005600	698	0.01	632
0.006700	102	0	333
0.007800	201	0	181

Table-5: Differences in the parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju at different stirring rate.

Stirring rate /rpm	Difference of Induction time/s	Difference of maximum oscillation amplitude /V	Difference of oscillation lifetime /s
300	161	0.01	997
350	173	0.01	153
400	505	0.00	601
450	347	0.01	82
500	239	0.00	96

Table-6: Differences in the parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju at different *Rhizoma atractylodis macrocephalae* powder dosage.

Rhizoma atractylodis acrocephalae powder dosage /g	Difference of induction time/s	Difference of maximum oscillation amplitude /V	Difference of oscillation lifetime /s
0.6000	380	0.01	491
0.8000	237	0	215
1.000	404	0.01	196
1.200	59	0.02	-268

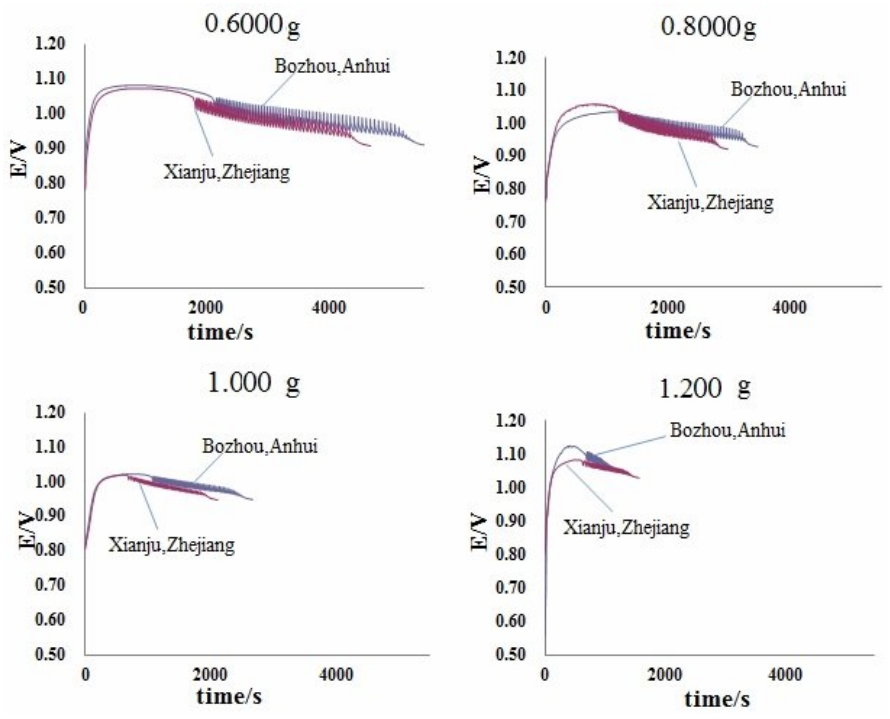

Fig. 6: Electrochemical fingerprints of *Rhizoma Atractylodis Macrocephalae* from Bozhou and Xianju on different *Rhizoma atractylodis macrocephalae* powder dosage.

Influence of Rhizoma Atractylodis Macrocephalae Powder Dosage to Electrochemical Fingerprint

The influence of *Rhizoma atractylodis macrocephalae* powder dosage on electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju is demonstrated in Fig. 6 and Table-6. *Rhizoma atractylodis macrocephalae* powder dosage was varied from 0.6000 to 1.200 g while keeping temperature at 37 °C, stirring rates 400 rpm, $[H^+]$=1.980 mol·L^{-1}, $[MnSO_4]$=0.005600 mol·L^{-1}, acetone 1.00 mL, $[KBrO_3]$=0.05000 mol·L^{-1} and the total volume 100.00 mL.

According Table-6, we found when *Rhizoma atractylodis macrocephalae* powder dosage was 0.6000 g, the differences of induction time and oscillation lifetime of *Rhizoma atractylodis macrocephalae* from Bozhou and Xianju were biggest, but the response time was too long and the efficiency of operations too lower, so chose 1.000 g as the optimum dosage of *Rhizoma atractylodis macrocephalae* powder.

Verification Experiments

The electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Panan, Xianju and Bozhou were determined for three times under the optimal conditions $[H^+]$=1.980 mol·L^{-1}, $[MnSO_4]$=0.005600 mol·L^{-1}, acetone dosage 1.00 mL, $[KBrO_3]$=0.05000 mol·L^{-1}, *Rhizoma atractylodis macrocephalae* powder dosage 1.000 g, temperature 37°C, stirring rates 400 rpm. The results are shown in Fig. 7 and Table-7. The reproducibility of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* was excellent under the optimal conditions.

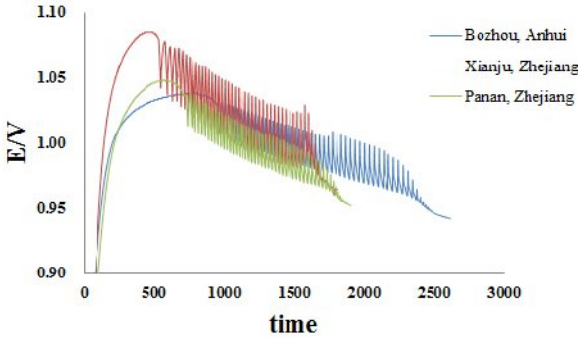

Fig. 7: Electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou, Panan and Xianju.

Fig. 7 and Table-7 show that the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou, Xianju and Panan were obviously different. Because Panan borders on Xianju in geographic position, and their climates were similar, their difference of electrochemical fingerprints was smaller than with Bozhou. The results indicated that the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from different origins showed significantly different characteristics, so the electrochemical fingerprint is a simple method to identify the origins and variety of *Rhizoma atractylodis macrocephalae*.

Table-7: Characteristic parameters of electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from Bozhou, Panan and Xianju

Producing area	Group	Induction time/s	Oscillation lifetime /s	Maximum oscillation amplitude /V
Bozhou, Anhui	1	951	1423	0.1
	2	917	1452	0.06
	3	970	1470	0.04
Average		946	1448	0.07
RSD%		2.8	1.6	3.1
Xianju, Zhejiang	1	540	1132	0.05
	2	545	1146	0.05
	3	537	1180	0.05
Average		541	1153	0.05
RSD%		0.8	2.1	0.0
Panan, Zhejiang	1	758	1169	0.05
	2	745	1149	0.03
	3	748	1191	0.02
Average		750	1170	0.03
RSD%		0.9	1.8	1.6

Conclusion

The B–Z oscillation system has been used to analyze and compare the different regions of *Rhizoma atractylodis macrocephalae* and demonstrated the electrochemical fingerprints of *Rhizoma atractylodis macrocephalae* from different origins showed significantly different characteristics. We can identify the different regions of *Rhizoma atractylodis macrocephalae* by adopting the electrochemical fingerprint characters. Using B–Z oscillation system as an analytical method to distinguish the traditional Chinese medicines is more objective, reliable and effective.

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