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Original Article

Determination of L-arginine content in Radix isatidis by a composite fluorescent probe of Pd (II)

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ABSTRACT

In the presence of Britton-Robinson buffer solution (pH = 9.5) and surfactant of Tween-80, fluorescence intensity of calcein was quenched by Pd^{2+}. However, the fluorescence intensity can be enhanced after adding a certain concentration of L-arginine, and the rate of the enhancement showed a good liner relationship with the added amount of L-arginine. We then established a fluorescence spectrometry for the determination of L-arginine. In addition, the linear range, along with detection limit, was different when the slit width changed. Thus, we could use a different slit width to meet our requirements according to the samples we treated. By testing actual samples and the reliability of our method, we found that our method was reliable for determining the content of L-arginine in Radix isatidis.

1. Introduction

L-arginine is not an essential amino acid for adults. However, the human body cannot maintain a positive nitrogen balance and the normal physiological function without it in some cases. Without adequate L-arginine, patients' ammonia levels will be high enough to cause a coma, and infants who have congenital absence of the urea cycle enzymes are unable to maintain normal growth and development. The important metabolic function of L-arginine is that it helped wound healing and accelerated the synthesis of collagen around the wound. We could observe that activity of L-arginine was increased in the wound fluid secretion, which suggested that substantial amounts of L-arginine are required near the wound. In general, L-arginine is of great significance for human health [1–3].

Currently limited methods exist to detect the content of L-arginine, and mainly include high-performance liquid chromatography, but this method is associated with disadvantages such as complex pretreatment of the sample, long determination time, and expense. Therefore, it is necessary to establish fast, simple, and accurate methods for the determination of L-arginine. In our experiment, We first studied the methods of detecting L-arginine in the references [4–11]. Then we made use of a complex formed by Pd^{2+} and calcein as the fluorescence probe to establish a new method for the determination of the content of L-arginine. There were many advantages of this method, such as speed, less amount of the sample, simple operation, and low cost.

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2. Experimental methods

2.1. Reagents

The following reagents were used: $PdCl_2$ solution (1.0×10^{-3} mol/L; Shanghai Reagent Factory, Shanghai, China); acridine red solution (2.5×10^{-3} mol/L; Shang Hai SSS Reagent Co., Ltd., Shanghai, China); Pd^{2+}-acridine red solution (1.0×10^{-4} mol/L); cationic surfactant cetyltrimethylammonium bromide (CTMAB; 1.0×10^{-2} mol/L; Shanghai Reagent Factory); cetylpyridinium bromide (CPB; 1.0×10^{-3} mol/L; Shanghai Reagent Factory); anionic surfactant sodium dodecyl sulfate (SDS; 1.0×10^{-3} mol/L; Shanghai Reagent Factory); sodium lauryl sulfate (SLS; 1.0×10^{-3} mol/L; Shanghai Reagent Factory); non-ionic surfactant Tween-80 (1.0×10^{-2} mol/L; Qingming Chemical Co., Ltd., Wenzhou, Zhejiang, China); OP solution (1 g/L; Qingming Chemical Co., Ltd.); series of Britton-Robinson (B-R) buffer solution (pH = 7.0–10.0; H_3PO_4–CH_3COOH–H_3BO_3–NaOH mixed solution; all three from Luoyang Chemical Reagent Company, Luoyang, Henan, China); NaCl aqueous solution (0.1 mol/L; Tianjin Kemiou Chemical Reagent Co., Ltd., Tianjin, China); methanol, ethanol, acetone, and acetonitrile (Beijing Chemical Works, Beijing, China); 732# sodium cation exchange resin (Tianjin Kemiou Chemical Reagent Co., Ltd.); perchloric acid ($HClO_4$, 11.1 mmol/L; Sinopharm Chemical Reagent Co., Ltd., Beijing, China); ammonia (3 mol/L, Luoyang Chemical Reagent Company); potassium hydrogen phthalate (Tianjin Chemical Reagent Factory).

Reagents involved were at least analytical grade; doubly distilled water was used throughout.

2.2. Apparatus

FP-6500 fluorescence spectrometer (JASCO Corporation, Japan); TU-1900-double beam UV-visible spectrophotometer (Beijing Analysis Apparatus Factory); PHS-3C digital pH meter (Hangzhou Dongxing Instrument Equipment Factory); KQ-218 ultrasonic cleaner (Kun Shan Ultrasonic Instruments Co., Ltd.); thermostatic bath (Shanghai Heng Ping Scientific Instrument Co.).

2.3. Methods

We added 1.4 mL Pd^{2+}-calcein solution (1.0×10^{-4} mol/L), 1.7 mL Tween-80 solution (1.0×10^{-3} mol/L), 1.8 mL B-R buffer solution (pH = 9.5) to a series of 10 mL volumetric flasks; one of the bottles was used as a blank. Then we added different amounts of L-arginine solution (100 mg/L) to the rest flasks, and shook for a few minutes to make them fully reacted, leaving for 90 minutes. Next, the flasks were irradiated with exciting light whose wavelength was 492 nm. The fluorescence intensity (F) at 522 nm was recorded, along with the blank's fluorescence intensity (F_0) under the same condition, and he difference obtained (ΔF, $\Delta F = F - F_0$).

2.4. Sample treatment

Different Chinese herbal medicine-Radix isatidis were purchased from two different supermarkets in Xinxiang City, China. The herbal medicine-Radix was grated and passed through a 40 mesh sieve. Then, 0.500 g of the powder was placed in a 100-mL conical flask with a stopper. Next, 50.0 mL 40% ethanol was added and the weight was recorded. The sample was placed under ultrasonic treatment for 30 minutes, then cooled to room temperature, and the weight recorded. The loss of weight was compensated with 40% ethanol, the sample shaken well and filtered; the filtrate went through a G4 glass core funnel and the original sample solution was obtained. In order to eliminate the interference of coexisting amino acids, the original solution of 10.00 mL to 732# cation exchange resin was eluted with 3 mol/L aqueous ammonia under the flow rate of 5 drops/min, and 90–115 mL of eluent was collected. In addition, 1.00 mL eluent was poured it into a 100-mL volumetric flask. The pH of the solution was adjusted with 1:3 sulfuric acids, and made to stay consistent with the original solution of L-arginine. The solution was diluted with redistilled water to the mark and for testing [12].

2.5. National standard methods (National standard of China (GB) 28306—2012)

The original sample solution was diluted 100-fold, 5.00 mL was placed in a 100-mL Erlenmeyer flask, 15.0 mL of redistilled water added, and the sample solution was titrated by using perchloric acid ($HClO_4$, 11.1 mmol/L), which was calibrated by potassium hydrogen phthalate. The value of the pH was recorded each 0.05 mL, and the titration end point was determined with the graphing method [13].

3. Results

3.1. Fluorescence spectrum

As shown in Fig. 1, in the B-R (pH = 9.5) buffer solution, the maximum excitation wavelength (λex) and the maximum emission wavelength (λem) of Pd^{2+}-calcein was 492 nm and 522 nm (curve 1). Fluorescence intensity increases with the increasing of L-arginine (curves 2 and 3). The rate of the enhancement showed a good linear relationship along with the increasing of L-arginine in a certain range. Therefore, the system could be used for the determination of L-arginine.

3.2. Optimization of the reaction system and the reaction conditions

3.2.1. Optimization of the reaction system

Different types of surfactants (cationic surfactant CTMAB and CPB, anionic surfactant SLS and SDS, nonionic surfactant OP and Tween-80) were selected, and the systems of Pd^{2+}-calcein-CTMAB, Pd^{2+}-calcein-CPB, Pd^{2+}-calcein-SDBS, Pd^{2+}-calcein-SDS, Pd^{2+}-calcein-OP, and Pd^{2+}-calcein-Tween-80 were formed for comparison. The results showed that: Tween-80, SLS, SDS, and OP had a sensitizing effect for the Pd^{2+}-calcein system, and these types of sensitizing effects had little effect on the system. This study selected the Pd^{2+}-calcein-Tween-80 system.

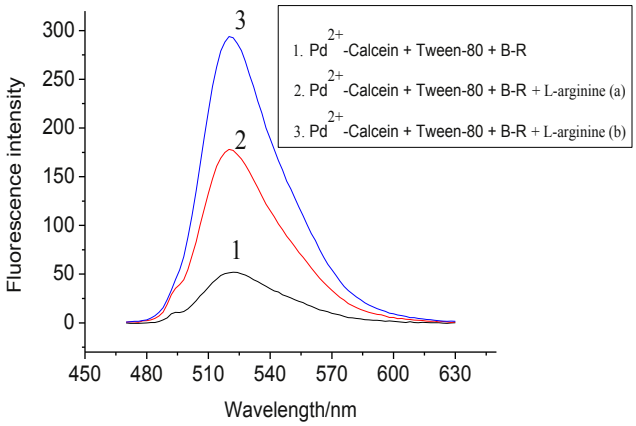

Fig. 1 – System of fluorescence spectra diagram for Pd^{2+}-calcein and Pd^{2+}-calcein-L-arginine. [Pd^{2+}-calcein]: 1.4×10^{-5} mol/L, pH = 9.5; [L-arginine]: (a) 10 mg/L, (b) 20 mg/L; [B-R]: 1.8×10^{-5} mol/L.

3.2.2. *Optimization of buffer solution and pH*

Pd^{2+}-calcein showed strong fluorescence in an alkaline condition; therefore, we chose a series of buffer solutions (pH = 9.5; Michaelis, B-R, Kolthoff, Sørensen, Clark–Lubs), and it showed that value of ΔF reaches its maximum in the B-R buffer solution. Therefore, we selected the B-R buffer solution in our test. Next, we chose a series of B-R buffer solution with the pH range from 8.0 to 9.8 for the experiment. The results in Supporting Information 1 showed that values of ΔF increased slightly at first and then reduced with the increasing pH. ΔF reached its maximum when pH = 9.6, but ΔF showed a sharp decline when pH was a little higher than 9.6. To avoid this occurrence, we chose pH = 9.5 in this study.

3.2.3. *Optimization of the concentration of fluorescence probe*

In the selected buffer solution (pH = 9.5), fluorescence intensity of the system increased gradually and finally became steady with the increasing of Pd^{2+}-calcein (Supporting Information 2). When concentration of Pd^{2+}-calcein was 1.4×10^{-5} mol/L, ΔF reached its maximum. Therefore, we chose 1.4×10^{-5} mol/L of Pd^{2+}-calcein.

3.2.4. *Optimization of the concentration of Surfactant-Tween-80*

It was shown that with Supporting Information 3, the value of ΔF increased gradually with the increasing of Tween-80, but ΔF reduced when its amount exceeded 1.7 mL. Therefore, we chose 1.7 mL of Tween-80 in this study.

3.2.5. *Optimization of the dosage of the buffer solution*

In the optimized dosage of Tween-80, Pd^{2+}-calcein, L-arginine, and pH, we could see the effect of the dosage of buffer solution on the system. As was shown in Supporting Information 4, value of ΔF increased gradually and reached the maximum and finally remained stable with the increasing of buffer solution. When the amount of buffer solution was 1.8 mL, the value of ΔF reached its maximum; therefore we chose 1.8 mL as the optimal dosage of buffer solution in this study.

3.2.6. *Optimization of the order of addition of the reagents*

In accordance with the principles of permutations and combinations, we investigated the influences of the 24 different orders on addition of the four different reagents on the system. The results showed that value of ΔF had its maximum when the reagent was added in the following order: Pd^{2+}-calcein→Tween-80→B-R→L-arginine and B-R→L-arginine→Tween-80→Pd^{2+}-calcein. We chose the former order in this research.

3.2.7. *Optimization of reaction time*

In the other optimized conditions, we investigated the effect of the reaction time on the system. The results showed that value of ΔF stayed stable after 85 minutes and it had little change within 2 hours. Therefore, the fluorescence intensity was measured after 90 minutes in our study.

3.2.8. *Influence of temperature*

We investigated the effect of temperature influences on the system in the optimized conditions. The results showed that with increasing temperature, the value of ΔF increased gradually, and when the temperature was higher than 90°C,the value of ΔF had a sharp increase, but our laboratory had no strict temperature control device, and it had been able to meet our needs at room temperature, so this experiment was done at room temperature.

3.2.9. *Influence of ionic intensity*

We examined the effect of ionic intensity on the system by adding an aqueous solution of NaCl (0.1 mol/L). As was shown in Supporting Information 5, the value of ΔF decreased gradually with the increasing NaCl solution. Therefore, we did not add NaCl solution, and did not change the ionic intensity.

3.2.10. *Influence of organic solvents on the system*

We examined the effects of some organic solvents on the system (Supporting Information 6) by adding several organic solvents (methanol, ethanol, acetone, and acetonitrile). The results showed that the value of ΔF had little change with the addition of methanol (Supporting Information 6a) and ethanol (Supporting Information 6b), but the system became unstable. When acetone was added to the system, the value of ΔF decreased slightly (Supporting Information 6c). The value of ΔF enhanced slightly at first and then reduced with the increase in acetonitrile (Supporting Information 6d). Therefore, we should not minimize the use of organic solvents in this study.

3.3. *Working curve*

Under the optimal experimental conditions, we achieved the working curve according to the experimental method. When the excitation and emission slit widths were all 5 nm, the results obtained were as follows: the linear regression equation: F = −4.007 + 10.58 C (mg/L); correlation coefficient: R = 0.9991; linear range: 0.012–80 mg/L; detection limit: 0.01165 mg/L. The content of 10 mg/L of standard solution was measured 11 times by our method, and the average value was 9.92 mg/L; the relative standard deviation was 4.1%.

When the excitation slit width was 5 nm, the emission slit width was 10 nm, and the following results were obtained: the

Table 1 – Influence of coexistences.

Coexisting substance	Concentration (mg/L)	Relative error (%)	Coexisting substance	Concentration (mg/L)	Relative error (%)
Cl^-	0.468	+4.1	NH_4^+	34	−4.4
Na^+	0.468	+4.1	Sucrose	960	5.6
K^+	0.746	−4.2	Lactose	230	+4.8
Ca^{2+}	0.123	−4.4	L-proline	0.0015	+4.7
Mg^{2+}	0.7	+4.7	Glucose	275	+4.8
Zn^{2+}	3220	−4.6	Maltose	75	+4.2
Fe^{3+}	1.7	−4.5	Valine	0.1	+4.9
Cu^{2+}	0.06	+4.4	Glutamate	0.6	+4.1
L-leucine	0.016	+5.2	D/L-methionine	0.008	−5.3

linear regression equation: F = 194.0 + 267.8 C (mg/L); correlation coefficient: R = 0.9981; linear range: 0.00050–25 mg/L; detection limit: 0.00050 mg/L.

3.4. *Influence of coexistences*

We investigated the effect of some common coexisting ions and compounds when determined determining the content of L-arginine in Radix. Relative error was controlled within ± 5% and the concentration of L-arginine was 10 mg/L. The results in Table 1 showed that the coexisting substances were less than the maximum allowed in Radix. So Therefore, the samples passed through a cation exchange column to avoid interference.

3.5. *Ratio of Pd^{2+} and calcein*

Reaction between calcein and Pd^{2+} formed a complex finally, and the fluorescence intensity of the complex was very weak. When a series of calcein with different ratios of the two was prepared, it was found that fluorescence intensity of calcein decreased gradually with an increase of Pd^{2+}, whereas the curve turning when the number of moles of Pd^{2+} and calcein was equal, so the formation of complex compound mole ratio of 1:1 was formed [14]. The reaction between the two is shown in Fig. 2A.

3.6. *Study of mechanism*

We speculated on the reason why the fluorescence intensity increases with an increase of L-arginine (Fig. 2B): Pd^{2+} can quench the fluorescence of calcein, but calcein dissociated from the complex after adding L-arginine, so the fluorescence intensity was enhanced. The effect of temperature on the system can also indicate this point. The reason was that formation of the complex becomes more and more unstable with the increase in temperature, and the free state of calcein was also increasing, which fully illustrates this mechanism was reasonable in another way.

Further, we calculated the average reaction rate of the reaction from the dates of the influence of reaction time on the system (Fig. 3).

What can be seen from Fig. 3 is that the rate of dissociating of the complex was very slow, which explained why the system became stable after 85 minutes.

3.7. *Application in actual samples*

We determined the content of L-arginine in the Radix isatidis in the optimal conditions in accordance with experimental method. Each sample was measured four times. The results

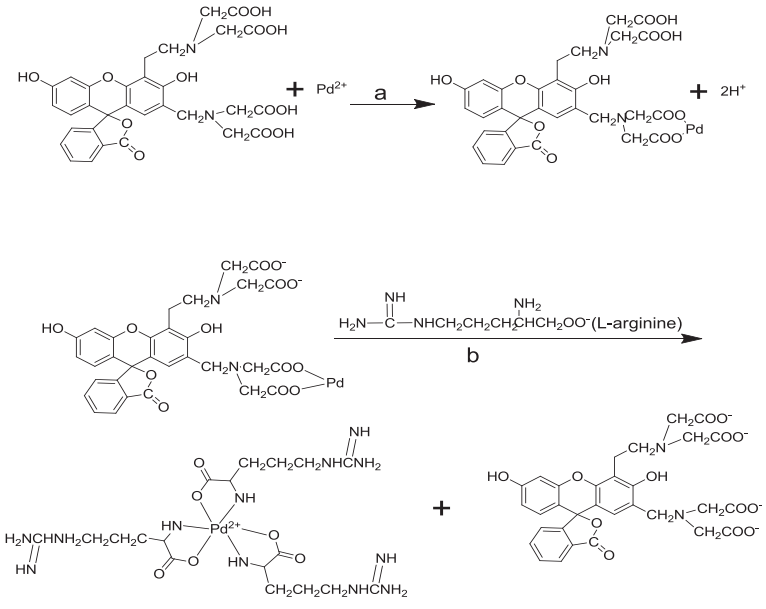

Fig. 2 – The reaction between calcein and Pd^{2+}.

$$\bar{r} = \frac{\Delta c}{\Delta t} = 6.75 \times 10^{-7}\ \text{mol/L min} = 0.1176\ \text{mg/L min}$$

Fig. 3 – Reaction rate.

Table 2 – Results of sample determination using our method and GB.

	Sample 1	Sample 2
Measured values using our method (mg/mL)	22.525, 22.325, 22.320, 22.530	22.575, 22.925, 22.637, 22.863
Average value using our method (mg/mL)	22.425	22.75
Average value using method of GB (mg/mL)	22.237	22.523
RSD (%)	0.798	1.295

GB = National standards of China; RSD = relative standard deviation.

are shown in Table 2, and the results obtained by our method were compared with the national standard method at the same time, which showed that our method is reliable.

4. Discussion

The Student *t* test between our method and GB showed that method used in this article had no significant difference with GB. In addition, the relative standard deviation was allowable, which showed that we have established a new method for testing the content of L-arginine in Radix, and it was reliable.

5. Conclusion

This study established a new fluorescence spectroscopy for the determination of the content of L-arginine in Radix. In case the slit width of excitation and emission were all 5 nm, the linear range was 0.012~80 mg/L; the detection limit was 0.012 mg/L. In case the slit width of excitation was 5 nm, emission slit width was 10 nm; the linear range was 0.00050~25 mg/L; the detection limit was 0.00050 mg/L. This method had the following advantages: simple sample handling; high sensitivity; fast detection; less amount of the sample; low cost; and a simple operation. We tested the actual samples successfully, and the measurement results were compared with GB. It showed that our method exist no significant difference with GB by the Student *t* test. Therefore, we provided a rapid, simple, sensitive, accurate, and reliable new method for the determination of L-arginine in Radix.

Conflicts of interest

We declare that we have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2014.04.006.

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