Preparation and process optimization of tea polysaccharide-nano-selenium

Bailin Xia, Su Xu*, Yuguo Liu, Junbin Li, Huanxing Li, Yalin Zhang

School of Environmental Sciences and Engineering, Xiamen University of Technology, Xiamen 361005, China

*Corresponding author e-mail: xusu@xmut.edu.cn

Abstract. Response Surface Methodology (RSM) was used to study the effects of reaction temperature, the mass fraction of tea polysaccharide and the VC/Na$_2$SeO$_3$ ratio (M/M) on the particle size of composite. The complex was characterized by UV, Zeta potential, XRD and FT-IR. When the mass fraction of tea polysaccharide is 4‰ and the VC/Na$_2$SeO$_3$ ratio (M/M) is about 5:1 at 50°C, the particle size of the complex can be less than 70 nm.

Keywords: Optimization of preparation conditions tea polysaccharide nano-selenium, response surface methodology (RSM), particle size.

1. Introduction
Selenium is one of the essential micronutrients of the body, which participates in Glutathione peroxidase reactions, protects the body from free radicals and radiation, stimulates the immune response, and maintains cellular respiration. [1] The range between nutritional dose and toxic dose is narrow, and it is easy to cause side effects such as anemia, cirrhosis and so on because of the overdose. Compared with inorganic selenium, nano-selenium has the advantages of large specific surface area, low toxicity and easy biological absorption. [2] Selenium-rich biological preparations were prepared by using tea polysaccharide as biological template, which can effectively and safely increase the selenium content of each product, and can control the absorbed dose, obtain the selenium source with safe, stable output and good absorption.

2. Materials and methods

2.1. Chemicals and reagents
Disodium selenite (Na$_2$SeO$_3$) was purchased from Sigma-Aldrich (St Louis, MO, USA). Ascorbic acid (Vc) was purchased from Aladdin Chemicals (Shanghai, China). Tea polysaccharide was purchased from China National Pharmaceutical Group Corporation (Beijing, China).

2.2. Instrumentation
A Nanobrook Omni BI-ZTU Zeta potentiometer was used to measure the particle size of the complex. An IKA C-MAG HS 4 S25 Hot plate was used to control the reaction temperature. A NICOLET 380 FT-IR was used to measure the infrared spectra of the sample. A lichen LC-10N-50A desktop laboratory
vacuum freeze dryer was used to prepare the freeze-dried powder. A Shimadzu UV-2006 Ultraviolet photometer was used to obtain the full-wave scan of samples.

2.3. Experimental design
In this experiment, the tea polysaccharide-nano-selenium was constructed by using sodium selenite as selenium source, tea polysaccharide as template and VC as reductant. The complex can avoid the adhesion of selenium simple substance and form scattered and stable nano-selenium particles by wrapping the template of tea polysaccharide.

Several beakers were prepared with 1% tea polysaccharide solution, 4.29 g/L VC solution and 2.17 g/L Disodium Selenite solution respectively. Take a certain amount of tea polysaccharide solution (2 ml, 3 ml, 4 ml, 5 ml, 6 ml) in a beaker, add 4 ml disodium selenite solution, rest for a moment, slowly add a certain amount of VC solution (2 ml, 3 ml, 4 ml, 5 ml, 6 ml), add deionized water to form 100 ml reaction solution, stir and shake, at a certain temperature, let the reaction stand for 2 hours, prepare to be measured. After the reaction, the solution was stirred slightly with a stirring rod, and then the sample was sampled and repeated the measurements three times with Zeta potentialmeter.[4]

2.4. Characterization
The properties of tea polysaccharide-nano-selenium crystals were characterized by XRD.[6] And the infrared spectra of tea polysaccharide-nano-selenium, tea polysaccharide-VC complex, tea polysaccharide-disodium selenite complex were measured by Fourier transform infrared spectroscopy (FT-IR) with NICOLET 380 in the wavelength range of 0~4000 cm\(^{-1}\), to analyze the interaction between tea polysaccharide and sodium selenite [5]. The spectra of tea polysaccharide solution, VC solution, tea polysaccharide and VC compound solution, disodium selenite and VC compound solution, nano-Se complex of tea polysaccharide were observed by full-wave scanning with Shimadzu UV-2006 ultraviolet photometer in the wavelength range of 190nm~600nm, the composition of each solution was analyzed according to its peak wave band [5]. The Z-average particle size of tea polysaccharide-nano-selenium solution was measured by NanoBrook Omni BI-ZTU Zeta potentiometer, the average value of three measurements is taken as the valid data [6].

3. Results and discussion

3.1. Qualitative analysis
The results of UV spectrum scanning were consistent with that of reference [5], the results of FT-IR spectroscopy were consistent with that of reference [5], and the results of XRD spectra were consistent with that of reference [6].

3.2. Optimization of experimental data analysis
According to the results of previous studies and experiments, the particle size of tea polysaccharide-nano-selenium did not change obviously when the reaction temperature was above 40°C, and the optimum mass fraction of tea polysaccharide is about 4‰. when the VC/Na\(_2\)SeO\(_3\) ratio (M/M) is more than 4:1, the change is not obvious. Therefore, the reaction temperature was 20°C~80°C, the mass fraction of tea polysaccharide was 0.2‰~0.6‰, and the ratio of VC to disodium selenite was 2:1~6:1[4].

Using Design-Expert software to design response surface method (RSM) BBD experiment scheme, totally 17 groups, involving 3 factors and 3 levels, repeat the experiment three times, the results are as follows:
### Table 1. Analysis of variance

| Source   | Sum of Squares | df | Mean Square | F Value | P-value | Prob>F |
|----------|----------------|----|-------------|---------|---------|--------|
| Model    | 1.741E+005     | 10 | 17410.93    | 454.14  | <0.0001 |        |
| A        | 4468.92        | 1  | 4468.92     | 454.14  | <0.0001 | significant |
| B        | 7276.09        | 1  | 7276.09     | 189.79  | <0.0001 |        |
| C        | 20675.61       | 1  | 20675.61    | 539.3   | <0.0001 |        |
| AB       | 1764.00        | 1  | 1764.00     | 46.01   | 0.0005  |        |
| AC       | 5090.82        | 1  | 5090.82     | 132.79  | <0.0001 |        |
| BC       | 4264.09        | 1  | 4264.09     | 111.22  | <0.0001 |        |
| A²       | 22438.62       | 1  | 22438.62    | 585.28  | <0.0001 |        |
| B²       | 18433.82       | 1  | 18433.82    | 480.82  | <0.0001 |        |
| A'B      | 21445.21       | 1  | 21445.21    | 559.37  | <0.0001 |        |
| AB²      | 296.46         | 1  | 296.46      | 7.73    | 0.0320  |        |
| Residual | 230.03         | 6  | 30.34       |         |         |        |
| Lack of Fit | 40.76    | 2  | 20.38       | 0.43    | 0.6770  | not significant |
| Pure Error | 189.27    | 4  | 47.32       |         |         |        |
| Cor Total| 1.743E+005     | 16 |             |         |         |        |

Note: A: the mass fraction of tea polysaccharide; B: the VC /Na2SeO3 ratio (M/M); C: the reaction temperature.

The Model F-value of 454.14 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Lack of Fit F-value" of 0.43 implies the Lack of Fit is not significant relative to the pure error. There is a 67.70% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit means that the model is fit. The "Pred R-Squared" of 0.9885 is in reasonable agreement with the "Adj R-Squared" of 0.9965."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 67.240 indicates an adequate signal. It shows that the experimental operation is reliable and has certain reference value.

#### 3.3. Effect of reaction condition change on experiment

Selenium has the characteristics of easy adhesion and polymerization, in order to obtain nano-selenium particles, a layer of "Sugar coating", i.e. the template of tea polysaccharide, should be coated on the surface of selenium simple substance to achieve the goal of isolating selenium. Therefore, the combination of tea polysaccharide template and inorganic selenium they can be reflected by the particle size of the complex.

As can be seen from Fig. 1, when the mass fraction of tea polysaccharide is suitable, the interaction between the VC /Na2SeO3 ratio (M/M) and reaction temperature is not obvious. As can be seen from Fig. 2, there is a certain interaction between the reaction temperature and the mass fraction of tea polysaccharide. When the mass fraction of tea polysaccharide is about 4‰, the temperature change has the least influence on the particle size. Anything else, the influence of temperature on the experiment is obvious, and the particle size increases with the decrease of temperature. As can be seen from Fig. 3, there is a significant interaction between the VC /Na2SeO3 ratio (M/M) and the mass fraction of tea polysaccharide. When VC content is low, the effect of tea polysaccharide content on particle size is significant, when the content of VC is high, the content of tea polysaccharide has no significant effect on the experimental results.
In combination with Fig. 1 and Fig. 2, it can be seen that the temperature has little effect on the size of the nano-selenium particles when other conditions are suitable, but the size of the nano-selenium particles decreases gradually with the increase of temperature when other conditions are bad. As can be seen from Fig. 1 and Fig. 3, the particle size decreases with the increase of VC /Na2SeO3 ratio (M/M). The optimum VC /Na2SeO3 ratio (M/M) is about 5:1, the particle size does not change significantly above 5:1. As can be seen from the combination of Fig. 2 and Fig. 3, the optimum mass fraction of tea polysaccharide is about 4‰, too much or too little can affect the results.

4. **Conclusion**

The results of the project show that heating, selecting the appropriate concentration of tea polysaccharide, increasing the ratio of Vc to disodium selenite can make the size of nano-selenium particles smaller. The optimum reaction conditions are that the reaction temperature is 50 °C, the mass fraction of tea polysaccharide is 4‰, the VC /Na2SeO3 ratio (M/M) is about 5:1. With the advent of new crown disease and the improvement of cognition level, more and more people will pay attention to health problems. The research about optimization of preparation conditions of biological nano-selenium complex will provide the possibility for the stable production of safe selenium source, it has practical value of scientific research and market application.

**Acknowledgments**

The authors wish to thanks for innovative training program for college students of Xiamen University of Technology (201711062227); Natural of Science Foundation of Fujian.
(202010025) and Research project of Xiamen University of Technology (XPDKQ2008, YKJ2014R)

References
[1] Kim Y. Y., Mahan D.C., Biological aspects of selenium in farm animals. Asian-Australas, J. Anin. Sci. 16 (2003) 435 - 444.

[2] Abed Abbas, Dhyaa Effect of in ovo - injection with Nano-Selenium on hatchability and posthatch biological parameters in quail, J. Earth and Environmental Science. 553 (2020).

[3] Jia, X, Liu, Q, Zou, S, Xu, X & Zhang, L, Construction of selenium nanoparticles/β-glucan composites for enhancement of the antitumor activity, J. Carbohydrate Polymers. 117 (2015) 434 – 442.

[4] Mu JJ, Ye XG, Chen ZZ, Zhang YY, Lin XR, Li B, Preparation and Characterization of Complexes of Tea Polysaccharide-selenium Nanoparticles, J. Modern Food Science and Technology. 35 (2019) 225 - 231+144.

[5] Wang HY, Zhang SY, Liu MZ, Gao HW, Tian YP, Synthesis of Selenium Nanoparticles in the Presence of Chitosan Template, J. Chinese Journal of Applied Chemistry. 21 (2004) 788 - 792.

[6] Wang HY, Zhang L, Zhang SY, Shi HW, Wang C, Synthesis and Characterization of Selenium Nanoparticles, J. Journal of Huaibei Coal Industry Teachers College. 25 (2004) 36 - 39.

[7] Wang YY, Qiu WY, Sun L, Ding ZC, Yan JK, Preparation, characterization, and antioxidant capacities of selenium nanoparticles stabilized using polysaccharide–protein complexes from Corbicula fluminea, J. Food BioscienceVolume. 26 (2018) 177 - 184.