REPORT

Optimization study of the preparation factors for argan oil microcapsule based on hybrid-level orthogonal array design via SPSS modeling

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Abstract: To optimize the preparation factors for argan oil microcapsule using complex coacervation of chitosan cross-linked with gelatin based on hybrid-level orthogonal array design via SPSS modeling. Eight relatively significant factors were firstly investigated and selected as calculative factors for the orthogonal array design from the total of ten factors effecting the preparation of argan oil microcapsule by utilizing the single factor variable method. The modeling of hybrid-level orthogonal array design was built in these eight factors with the relevant levels (9, 9, 9, 9, 7, 6, 2 and 2 respectively). The preparation factors for argan oil microcapsule were investigated and optimized according to the results of hybrid-level orthogonal array design. The priorities order and relevant optimum levels of preparation factors standard to base on the percentage of microcapsule with the diameter of 30~40μm via SPSS. Experimental data showed that the optimum factors were controlling the chitosan/gelatin ratio, the systemic concentration and the core/shell ratio at 1:2, 1.5% and 1:7 respectively, presetting complex coacervation pH at 6.4, setting cross-linking time and complex coacervation at 75 min and 30 min, using the glucose-delta lactone as the type of cross-linking agent, and selecting chitosan with the molecular weight of 2000 ~ 3000.

Keywords: argan oil microcapsule, hybrid-level orthogonal array, complex coacervation, SPSS modeling

INTRODUCTION

The argan tree (Argania spinosa L. Skeels), an endemic tree in Morocco, is the most remarkable species in North Africa, due to its botanical and bioecologic interest as well as its social value. Argan oil is traditionally well known for its cardioprotective (Guillaume D *et al*, 2013). Microencapsulated substances have been utilized for sustained drug release, electro rheological fluids, intumescent fire retarding powders, preservation of flavours, electro phoretic display applications, textiles, biotechnology and inorganic metal salt catalyst, etc (Suryanarayana C *et al*, 2008).

Generally, factor completely designs are able to synthetically investigate simple effects of each single factor, main effects and interaction effects between factors. However, with the increasing of parameters and their relevant levels, factor completely designs not only augments experimental workloads but wastes a large amount of time and material (Sabzi S *et al*, 2013).

The single parameters involving in the optimization process for the preparation of argan oil microcapsule were more complex and complicated. Therefore, in this study, we firstly optimized the preparation parameters for argan oil microcapsule via using the hybrid-level orthogonal array design combing with single factor and multilevel orthogonal designs based on the SPSS modeling. This method integrated advantages of these two different designs, making the experiments more efficient and scientific.

MATERIAL AND METHODS

Materials, reagents and instruments

Chitosan (molecular weight: 2000 ~ 3000), chitosan (molecular weight: 3000 ~ 6000), gelatin, argan oil, HAc (purity ≧ 99%), NaOH (purity ≧90%), Tween-80 and formaldehyde, glucose - delta lactone. UV-2450 PC UV-VIS spectrophotometer (Shimadzu Co. Ltd., Kyoto, Japan), DHG-9023 Electro- thermostatic blast oven (Qi Xing instrument of science and technology Co. Ltd., Shanghai, CHN), HZK-210 Electronic balance (You ZHong Heng electronic Co. Ltd., Shanghai, CHN), HH S11-4 Electric-heated thermostatic water bath plot (HuiEr

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instruments and equipment Co. Ltd., Hangzhou, CHN), Bx 53 microscope study level (Olympus, Japan), Screen type PH adjustment control instrument (HengAoDe science and technology Co. Ltd., Beijing, CHN), ERS2000 High shear emulsifying agent (YiKen machine Co. Ltd., Shanghai, CHN) and OMEC LS900 laser particle size analyzer(OMEC instrument Co., Ltd., Zhuhai, CHN).

The technological process for argan oil microcapsule

In order to prepare for the emulsion, argan oil, distilled water and Tween 80 were choosed as the oil phase, as the water phase and as emulsifier, respectively. A suitable amount of glycerol was placed in the beaker, and a suitable amount of Tween 80 surfactant was added, and the mixture was dissolved completely in 40□ water bath. When the temperature reached equilibrium, the distilled water was poured into argan oil, and the shearing machine

Table 1: Single factor experiment table

Gelatin/ chitosan ratio	Systemic concentration /%	Core/ shell ratio	Complex coacervation pH	Temperature of complex coacervation /□	Time of complex coacervation /min	Cross-linking time /min	Type of cross-linking agent	Volume of type of cross-linking agent /mL	Molecular weight of chitosan
1:1	4.5	1:1	5.4	30	10	45	formaldehyde	0.5	2000 ~ 3000
1:5	3.0	1:5	6.6	40	15	90	glucose - delta lactone	1	3000 ~ 6000
1:9	0.5	1:9	8.0	50	30	120		1.5	

Table 2: Hybrid orthogonal array table

Level\factor	Gelatin/chitosan ratio	Systemic concentration /%	Core/shell ratio	Complex coacervation pH	Time of complex coacervation /min	Cross-linking time /min	Type of cross-linking agent	Molecular weight of chitosan
1	1:1	4.5	1:1	5.4	10	45	formaldehyde	2000 ~ 3000
2	1:2	4.0	1:2	5.8	15	60	glucose - delta lactone	3000 ~ 6000
3	1:3	3.5	1:3	6.0	20	75		/
4	1:4	3.0	1:4	6.2	25	90		/
5	1:5	2.5	1:5	6.4	30	105	/	/
6	1:6	2.0	1:6	6.6	35	120	/	/
7	1:7	1.5	1:7	6.8	40	/	/	/
8	1:8	1.0	1:8	7.0	/	/	/	/
9	1:9	0.5	1:9	8.0	/	/	/	/

Table 3: Variance results of SPSS analysis

Source of variance	sum of squares	df	Mean squares	F	Sig.
Gelatin/chitosan ratio	3178.600	8	397.325	1.116	.377
Systemic concentration	5968.212	8	746.027	2.095	.063
Core/shell ratio	2588.224	8	323.528	.908	.521
Complex coacervation pH	4198.107	8	524.763	1.473	.202
Temperature of complex coacervation	1763.012	6	293.835	.825	.558
Time of complex coacervation	3371.583	5	674.317	1.893	.121
Type of cross-linking agent	188.288	1	188.288	.529	.472
Molecular weight of chitosan	1194.193	1	1194.193	3.353	.076

Mean value can be used as the best choice of indicators.

Table 4: Gelatin/chitosan ratio

	1	2	3	4	5	6	7	8	9
Level	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
Mean value	23.17	30.25	19.66	18.97	9.80	11.27	27.36	20.52	21.74

speed was adjusted in A file for 3 min. A suitable amount of gelatin was swelled completely with a suitable amount of distilled water at 50□ constant temperature water bath by stirring constantly. Chitosan was dissolved in 2.5% HAc solution and diluted with distilled water for the preparation of chitosan solution. A suitable amount of chitosan solution and gelatin solution were poured into emulsion, and the emulsion was stirred constantly until smooth. And then the pH of the emulsion was adjusted with 10% NaOH solution. The microcapsule solution was cooled to 4□ approximately in the frozen layer of the refrigerator. 36% curing agent was added into the microcapsule solution and the mixture was stirred for 15min, and then the pH of the mixture was adjusted to 8.0 ~ 8.5 with 10% NaOH solution The microcapsule solution was stirred until smooth, and a suitable amount of microcapsule solution was drawn in slides that covered with glass and observed under bx53 microscope.The particle size of the microcapsule solution was measured by a laser granulometer (Wang B *et al*, 2014).

Single factor optimization experiment

Ten conditions that effected the preparation of the microcapsule were screened by the analysis of the literature, and three levels were selected from each condition for experiment. 1. gelatin/chitosan ratio:1:1, 1:5 and 1:9; 2. systemic concentration: 4.5%, 3.0% and 0.5%; 3. core/shell ratio: 1:1, 1:5 and 1:9; 4. complex coacervation pH: 5.4, 6.6 and 8.0; 5. temperature of complex coacervation: 30□, 40□ and 50□; 6. Time of complex coacervation: 10min, 15min and 30min; 7. cross-linking time: 45min, 90min and 120min; 8. type of cross-linking agent: formaldehyde and glucose-delta lactone; 9. volume of type of cross-linking agent: 0.5mL, 1.0mL and 1.5mL; 10. molecular weight of chitosan: 2000 ~ 3000 and 3000 ~ 6000.

As shown in fig. 1-18, the levels of the conditions that effected the experiment results were screened for orthogonal test with microscopic examination as the evaluation index.

Hybrid-levels orthogonal array design

Eight factors were determined through this experiment, they were as follows: gelatin/chitosan ratio, systemic concentration, core/shell ratio, complex coacervation pH, temperature of complex coacervation, time of complex coacervation, cross-linking time, type of cross-linking agent, volume of type of cross-linking agent and molecular weight of chitosan (Liu RJ *et al*, 2010). and the levels were 9, 9, 9, 9, 7, 6, 2, 2 respectively. The results were shown as follows: 1. gelatin/chitosan ratio: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9; 2. systemic concentration: 4.5%, 4.0%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.0%, 0.5%: 3. core/shell ratio: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8,

Table 5: Systemic concentration

	1	2	3	4	5	6	7	8	9
Level	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Mean value	26.41	32.66	15.29	11.91	14.43	17.51	35.20	9.68	19.64

Table 6: Core/shell ratio

	1	2	3	4	5	6	7	8	9
Level	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
Mean value	26.16	17.52	12.76	25.21	9.74	18.99	26.30	23.61	22.42

Table 7: Complex coacervation pH

	1	2	3	4	5	6	7	8	9
Level	5.4	5.8	6.0	6.2	6.4	6.6	6.8	7.0	8.0
Mean value	19.66	14.39	10.19	14.46	33.58	23.40	17.52	30.30	19.23

Table 8: Time of complex coacervation

	1	2	3	4	5	6	7
Level	10	15	20	25	30	35	40
Mean value	17.11	18.10	13.94	15.83	29.36	24.40	23.40

Table 9: Cross-linking time

	1	2	3	4	5	6
Level	45	60	75	90	105	120
Mean value	26.54	18.27	29.91	21.350	10.26	15.49

1:9:4. complex coacervation pH: 5.4, 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 8.0: 5. time of complex coacervation: 10min, 15min, 20min, 25min, 30min, 35min, 40min ; 6. cross-linking time: 45min, 60min, 75min, 90min, 105min, 120min; 7. type of cross-linking agent: formaldehyde and glucose-delta lactone; 8: molecular weight of chitosan: 2000 ~ 3000 and 3000 ~ 6000. A hybrid orthogonal array table that contained 81 tests was generated by the hybrid-level orthogonal array design via SPSS modeling (Wu ZF *et al*, 2006)

RESULTS

Analysis results of SPSS

Mean square value is an important indicator for evaluating the effects of the priorities of factors. It is known that the effect of each factor is proportional to their mean square value. In the other hand, SIG also has impacts on experimental results. A smaller SIG value of a factor indicates that this factor has a more significant effect on the results (Deng ZW *et al*, 2009).

Table 10: Type of cross-linking agent

	1	2
Level	Formaldehyde	Glucose - delta lactone
Mean value	18.686	21.920

As shown in the following table, a total of eight factors including: the gelatin/chitosan ratio, the systemic concentration, the core/shell ratio, complex coacervation pH, cross-linking time, complex coacervation, type of the type of cross-linking agent, and the molecular weight of chitosan had effects in different levels on the results. In this study, our data demonstrated that the relative importance of factors, from most significant to least significant: the molecular weight of chitosan, the systemic concentration, cross-linking time, complex coacervation pH, the gelatin/chitosan ratio, the core/shell ratio, complex coacervation, and type of the type of cross-linking agent.

Table 11: Molecular weight of chitosan

	1	2
Level	2000 ~ 3000	3000 ~ 6000
Mean value	24.376	16.230

Based on the criterion about the average diameter of argan oil microcapsule, our experimental data showed that the optimum factors for preparation using complex coacervation of chitosan cross-linked with gelatin were that controlling the ratio of chitosan to gelatin, the systemic concentration and the core/shell ratio at 1:2, 4.0 % and 1:1 respectively, presetting complex coacervation pH at 7.0, setting cross-linking time and complex coacervation at 45 min and 40 min, respectively, using the glucose-delta lactone as the cross-linking agent, and selecting chitosan with the molecular weight of 2000 ~ 3000.

DISCUSSION

As shown in table 5, the group of 1:2 possessed the largest average value (30.250), therefore, the optimum chitosan/gelatin ratio was 1:2. Similarly, the other optimum factors were that controlling the systemic concentration and the core/shell ratio at 1.5 % (35.200) and 1:7 (26.300) respectively, presetting complex coacervation pH at 6.4 (33.580), setting cross-linking time and complex coacervation at 75 min (29.910) and 30 min (29.360), respectively, using the glucose-delta lactone as the cross-linking agent (21.920), and selecting chitosan with the molecular weight of 2000 ~ 3000 (24.376). We put forward this study, which from hybrid-level orthogonal via SPSS modeling perspective to investigates Argan oil microcapsule for the first time. This is probably a way of simplifying the fundamental microcapsule process. However, It should be noted that this study will always be room for improvement in establishing new SPSS modeling.

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