Application of fuzzy analytical method in the optimization of liposome preparation by orthogonal experiments

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Abstract: A new statistical method, the fuzzy analytical method, was introduced in the optimization processes of liposome preparation. It took the full advantage of the information from orthogonal experiments to obtain the optimal liposome preparation conditions by considering all the evaluation indexes. Liposomes were made by the modified reverse-phase evaporation method and the properties of liposomes including size, encapsulation efficiency and physical stability were selected as the evaluation indexes to indicate the quality of liposomes. The experimental data of these properties were analyzed by three different methods including direct observation, variance analysis and fuzzy analytical method. The optimal preparation conditions obtained from these methods were validated with further experiments. The results of all possible combinations of levels for all factors in orthogonal experiments were acquired by the fuzzy analytical method. All evaluation indexes were taken into account and the optimal preparation condition was obtained. The optimal preparation conditions from direct observation and fuzzy analytical method were different and further validation studies indicated that the optimal conditions obtained from the fuzzy analytical method were in agreement with that from traditional statistical analysis. Fuzzy analytical method avoided the problem resulted from the traditional method, in which different levels of the same factor were obtained when considering different evaluation indexes. More information could be obtained from the fuzzy analytical method and the blind area within the experimental range was eliminated. As a result, fuzzy analytical method can be applied in the optimization processes of liposome preparation.

Keywords: Orthogonal experiment; Liposome preparation; Fuzzy analytical method

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1. Introduction

As one of the most important drug delivery systems in pharmaceutics, liposomes has been extensively investigated and experiments show that preparation factors can directly influence the quality of liposomes. Orthogonal experiment is usually used to make an integrated analysis in order to obtain the optimal conditions of liposome preparation[1].

The results of orthogonal experiment are generally analyzed by the methods of direct observation and variance analysis. The analysis of the range by direct observation can provide the optimal preparation parameters for each evaluation index, while the variance analysis can generate the validity information of the factors[2].

Nevertheless, it is difficult to analyze the data from more complicated orthogonal experiments concerning multiple indexes, and the optimal preparation conditions can only be obtained separately for single index. Often, different levels are acquired for the same factor and it is difficult to decide which one to choose[3]. Some studies brought in general scores to avoid this problem, but it was subjective[4]. Moreover, optimal preparation conditions are obtained only in part of the level combinations by orthogonal experiments. Some of the experimental possibilities are ignored and the information obtained from the orthogonal experiment cannot be utilized sufficiently by the conventional analytical methods[5].

Fuzzy analytical method has been used in orthogonal experiments of chemical or biological studies to gain the optimal combinations of different factor levels since fuzzy mathematics was proposed by Zadeh[6–8]. In this paper, three evaluation indexes of liposome preparation including size, encapsulation efficiency and physical stability were selected to optimize liposome preparation conditions by fuzzy analytical method in comparison with the traditional methods.

2. Materials and methods

2.1. Materials and instruments

Lecithin, calcein, and cholesterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were commercially available. Dynamic light

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scattering apparatus was from Malvern Instruments Ltd. (Herefordshire and Worcestershire, UK). Fluorospectrophotometer was from Shimadzu Corp. (Kyoto, Japan). Rotary vacuum evaporator was from Senco Technology Co. Ltd. (Shanghai, China). Vortex mixer was from Kylin-bell Lab Instruments Co. Ltd. (Jiangsu, China).

2.2. Preparation of liposomes

The modified reverse-phase evaporation method was used to prepare liposomes. Calcein was selected as a model drug and the calcein-encapsulated liposomes were separated from free calcein through gel-filtration chromatography[9].

2.3. Size and encapsulation efficiency

The size of the calcein-encapsulated liposomes was determined by dynamic light scattering. Encapsulation efficiency was calculated according to the equation: encapsulation efficiency = $(W_{total} - W_{free})/W_{total} \times 100\%$, where W_{total} was the total calcein in liposomes while W_{free} represented the free drug in the dispersed system[10]. The calcein concentration was determined by the intensity of its fluorescence[11].

2.4. Physical stability

Calcein-encapsulated liposome suspensions were centrifuged at 5000 r/min for 30 min. The relative stability was calculated according to the equation: physical stability = $(A_0 - A_t)/A_0$, where A_t and A_0 represent the absorption value of liposomes at 600 nm at room temperature after and before the centrifugation, respectively[12].

2.5. Orthogonal experiment designs

Size, encapsulation efficiency and physical stability were selected as three main indexes to evaluate the quality of liposomes. According to earlier experiments, lecithin-cholesterol proportion (A), oil-water proportion (B), vibration time (C), ultrasonic time (D), vacuum evaporation temperature (E), ultrasonic time for hydration (F), refrigeration time (G) and drug-lipid proportion (H), which had significant influence on the three indexes, served as eight factors, and each factor had three levels. Thus, orthogonal L_{27} (3^{13}) experiments were performed and the detailed conditions for preparation of liposomes were shown in Table 1.

Table 1. Factors and levels for orthogonal experiments

Factors	Levels		
	1	2	3
A Lecithin-cholesterol proportion (mol/mol)	5:1	8:1	10:1
B Oil-water proportion (mL/66 μmoL)	2:1	8:3	4:1
C Vibration time (min)	5	10	15
D Ultrasonic time (min)	5	10	15
E Vacuum evaporation temperature (°C)	40	50	60
F Ultrasonic time for hydration (min)	5	10	15
G Refrigeration time (min)	45	90	180
H Drug-lipid proportion (μmoL/66 μmoL)	3	5	7

2.6. Data analysis and statistics

The experimental arrangements and results were obtained and three different statistical methods were carried out in this study through software Microsoft Office Excel® (2003 for windows) and the SPSS® software (13.0 for windows). A direct observation was carried out to compare the effect of each factor and generate the optimal preparation conditions for liposomes; analysis of variance (ANOVA) was used to determine the statistical validity of the factors[13]. In addition to the two traditional analytical methods, fuzzy analytical method was conducted to predict all possible combinations of factor levels. Both direct observation and fuzzy analytical method were used to obtain the optimal preparation conditions, and validation studies were conducted accordingly.

With the method of fuzzy mathematics, results were analyzed after the multiple indexes had been transformed into a single index to obtain the optimal experimental conditions. The detailed procedure was as follows:

2.6.1. Fuzzy transformation of the index

The experimental results (y_i) of each evaluation index (y) are expressed as a series of scores ($r_i \in [0,1]$), which represents the membership degree of a good experiment[14]. If the score increases when the result increases, r_i is expressed by equation (1). Conversely, r_i is expressed by equation (2):

$$r_i = (y_i - y_{min})/(y_{max} - y_{min}) \quad (1)$$

$$r_i = (y_{max} - y_i)/(y_{max} - y_{min}) \quad (2)$$

2.6.2. Transformation of multiple indexes into single index

The importance of each evaluation index is represented by the weights, which are a_1, a_2, …, a_n ($\sum_{k=1}^{n} a_k = 1$), respectively. A new fuzzy vector is consisted of the weights, A = (a_1, a_2, …, a_n). All the scores of the evaluation indexes compose the fuzzy relation matrix R of order n (representing evaluation indexes) by m (representing experiment points in orthogonal experiments)[15]:

$$R = \begin{bmatrix} r_{11} & r_{21} & \cdots & r_{m1} \\ r_{12} & r_{22} & \cdots & r_{m2} \\ \cdots & \cdots & \cdots & \cdots \\ r_{1n} & r_{2n} & \cdots & r_{mn} \end{bmatrix}$$

Then the comprehensive scores (b_j) for each experiment were obtained through multiplying the matrix A by R mentioned above:

$$B = A \cdot R = (a_1, a_2, \ldots, a_n) \begin{bmatrix} r_{11} & r_{21} & \cdots & r_{m1} \\ r_{12} & r_{22} & \cdots & r_{m2} \\ \cdots & \cdots & \cdots & \cdots \\ r_{1n} & r_{2n} & \cdots & r_{mn} \end{bmatrix} = (b_1, b_2, \ldots, b_m)$$

$$b_j = \sum_{k=1}^{n} a_k r_{kj} \quad (j = 1, 2, \ldots, m)$$

2.6.3. Establishment of fuzzy sets of factors and levels

After the direct observation results of comprehensive scores were obtained, all levels of each factor could specify the domain I = (i_1, i_2, i_3) = (I_i/k_i, II_i/k_i, III_i/k_i) (I_i, II_i and III_i represent the sum of comprehensive scores at level I, II and III where i represents factor A, B, C, D, E, F, G or H; k_i represents the number of experiments of each level in each factor). To establish the fuzzy sets in domain I, each set represented the combination of different level of each factor (A_i, B_j, C_k, D_l, E_m, F_n, G_s, H_t) (i, j, k, l, m, n, s, t = 1, 2, 3).

2.6.4. Cartesian product analysis of fuzzy sets

All possible experiments were calculated. In order to evaluate the effect of every possible combination of levels, the membership degree of each combination was simulated with the method of cartesian product[16]. The cartesian product of A, B, C, D, E, F, G and H was:

$$A \times B \times C \times D \times E \times F \times G \times H = \int_{X_A \times X_B \times X_C \times X_D \times X_E \times X_F \times X_G \times X_H} \frac{\min(a_i, b_j, c_k, d_l, e_m, f_n, g_s, h_t)}{(A_i, B_j, C_k, D_l, E_m, F_n, G_s, H_t)}$$

the degree of membership of (A_i, B_j, C_k, D_l, E_m, F_n, G_s, H_t) was the corresponding minimum value of a_i, b_j, c_k, d_l, e_m, f_n, g_s and h_t in order to achieve a high reliability, which represented by

$$a_i \wedge b_j \wedge c_k \wedge d_l \wedge e_m \wedge f_n \wedge g_s \wedge h_t = \min(a_i, b_j, c_k, d_l, e_m, f_n, g_s, h_t)$$

3. Results

3.1. Direct observation

As shown in Table 2, the effects of the factors on the size of liposomes were in the order of E>C>B>D>H>G>F>A. As for encapsulation efficiency and physical stability, the effects of the factors were in the order of E>H>A>B>G>C>D>F and G>E>F>B>C>A>H>D, respectively. In terms of size, encapsulation efficiency and physical stability, the three optimal conditions were $A_3 B_2 C_3 D_2 E_2 F_3 G_3 H_2$, $A_3 B_2 C_3 D_3 E_2 F_1 G_2 H_1$ and $A_3 B_2 C_1 D_1 E_2 F_3 G_3 H_3$.

Table 2. Direct observation results on size, encapsulation efficiency and physical stability

Evaluation indexes		Factors (i)							
		A	B	C	D	E	F	G	H
Size (nm)	R_i	8.8	31.5	54.3	29.5	57.7	13.8	14.3	26.2
Encapsulation efficiency	R_i	0.139	0.096	0.061	0.054	0.239	0.012	0.073	0.213
Physical stability	R_i	0.040	0.073	0.056	0.010	0.102	0.076	0.111	0.014

R_i represented the range of size, encapsulation efficiency, or physical stability between there levels; i represented factor A, B, C, D, E, F, G or H.

3.2. ANOVA

To further investigate the significance of all the factors, ANOVA was carried out with software SPSS 13.0 for windows. The significance of the factors for size was in the following order of B>D>C>E>H>G>F>A, which was in good agreement with the direct observation method. Yet, all of the *F*-values were less than $F_{0.1}(2, 10) = 2.92$ and all the factors were rejected. The significance orders of the factors for encapsulation efficiency and physical stability were E>H>A>B>G>C>D>F and G>E>F>B>C>H>A>D, respectively. ANOVA demonstrated that none of the eight factors played a significant role in influencing the encapsulation efficiency and physical stability of the liposomes prepared.

3.3. Fuzzy analytical method

The degrees of membership for evaluation indexes including size, encapsulation efficiency and physical stability of the orthogonal L_{27} (3^{13}) experiment results mentioned above were listed in Table 3.

The weights of size, encapsulation efficiency and physical stability were 0.4, 0.4 and 0.2 respectively, thus A = (0.4, 0.4, 0.2). All the scores of the three evaluation indexes composed the fuzzy relation matrix R of order 3 by 27. B = A·R = (0.038, 0.404, 0.482, 0.088, 0.525, 0.164, 0.028, 0.137, 0.090, 0.305, 0.460, 0.970, 0.189, 0.082, 0.357, 0.157, 0.137, 0.173, 0.637, 0.317, 0.528, 0.633, 0.092, 0.256, 0.396, 0.523, 0.202).

The results of the direct observation of the comprehensive scores listed in Table 3 indicated that the order of influence of all the factors to the synthetic mark was E>B>A>H>D>C=F>G. To establish fuzzy sets in the domains I, the sets of all possible experiments were listed in Table 4.

All possible experiments for three levels of eight factors were calculated and the results of maximum degree of membership were listed in Table 5.

Table 3. Membership degrees of each evaluation index and the direct observation results of comprehensive scores

No.	Factors								Degrees of membership			Comprehensive scores
	A	B	C	D	E	F	G	H	Size (nm)	Encapsulation efficiency	Physical stability	
1	1	1	1	1	1	1	1	1	0.478	0.411	0.166	0.308
2	1	1	1	2	2	2	2	2	0.602	0.436	0.333	0.404
3	1	1	1	3	3	3	3	3	0.521	0.000	1.000	0.482
4	1	2	2	1	1	2	2	3	0.214	0.103	0.049	0.088
5	1	2	2	2	2	3	3	1	0.598	0.560	0.474	0.525
6	1	2	2	3	3	1	1	2	0.392	0.251	0.027	0.164
7	1	3	3	1	1	3	3	2	0.251	0.016	0.000	0.028
8	1	3	3	2	2	1	1	3	0.545	0.190	0.004	0.137
9	1	3	3	3	3	2	2	1	0.269	0.100	0.047	0.090
10	2	2	3	2	3	1	3	3	0.525	0.198	0.383	0.305
11	2	2	3	3	1	2	1	1	0.624	0.763	0.098	0.460
12	2	2	3	1	2	3	2	2	1.000	1.000	0.933	0.970
13	2	3	1	2	3	2	1	2	0.415	0.193	0.143	0.189
14	2	3	1	3	1	3	2	3	0.425	0.094	0.007	0.082
15	2	3	1	1	2	1	3	1	0.367	0.385	0.324	0.357
16	2	1	2	2	3	3	2	1	0.316	0.064	0.230	0.157
17	2	1	2	3	1	1	3	2	0.612	0.104	0.087	0.137
18	2	1	2	1	2	2	1	3	0.000	0.343	0.018	0.173
19	3	3	2	3	2	1	2	2	0.466	0.878	0.405	0.637
20	3	3	2	1	3	2	3	3	0.483	0.152	0.465	0.317
21	3	3	2	2	1	3	1	1	0.285	0.861	0.208	0.528
22	3	1	3	3	2	2	3	1	0.374	0.932	0.353	0.633
23	3	1	3	1	3	3	1	2	0.291	0.065	0.085	0.092
24	3	1	3	2	1	1	2	3	0.682	0.163	0.280	0.256
25	3	2	1	3	2	3	1	3	0.764	0.366	0.362	0.396
26	3	2	1	1	3	1	2	1	0.186	0.614	0.485	0.523
27	3	2	1	2	1	2	3	2	0.779	0.048	0.264	0.202
I_i	2.794	3.068	3.450	3.048	2.997	3.410	3.116	3.752				
II_i	3.416	4.209	3.065	3.448	4.564	3.086	3.599	3.575				
III_i	3.937	2.871	3.632	3.651	2.587	3.650	3.432	2.820				
k_i	9	9	9	9	9	9	9	9				
I_i/k_i	0.310	0.341	0.383	0.339	0.333	0.379	0.346	0.417				
II_i/k_i	0.380	0.468	0.341	0.383	0.507	0.343	0.400	0.397				
III_i/k_i	0.437	0.319	0.404	0.406	0.287	0.406	0.381	0.313				
R_i	0.127	0.149	0.063	0.067	0.220	0.063	0.054	0.104				

I_i, II_i and III_i represent the sum of comprehensive scores at level I, II and III where i represents factor A, B, C, D, E, F, G or H; k_i represents the amount of experiments of each level in each factor; R_i represents the range of comprehensive scores between level I, II and III in the case of factor (i).

Table 4. All fuzzy sets of possible combinations of level

No.	$(a_i, b_j, c_k, d_l, e_m, f_n, g_s, h_t)$	No.	$(a_i, b_j, c_k, d_l, e_m, f_n, g_s, h_t)$
1	(1, 1, 1, 1, 1, 1, 1, 1)	2	(1, 1, 1, 1, 1, 1, 1, 2)
3	(1, 1, 1, 1, 1, 1, 1, 3)	4	(1, 1, 1, 1, 1, 1, 2, 1)
5	(1, 1, 1, 1, 1, 1, 2, 2)	6	(1, 1, 1, 1, 1, 1, 2, 3)
7	(1, 1, 1, 1, 1, 1, 3, 1)	8	(1, 1, 1, 1, 1, 1, 3, 2)
9	(1, 1, 1, 1, 1, 1, 3, 3)	10	(1, 1, 1, 1, 1, 2, 1, 1)
…	…	…	…
6556	(3, 3, 3, 3, 3, 3, 2, 1)	6557	(3, 3, 3, 3, 3, 3, 2, 2)
6558	(3, 3, 3, 3, 3, 3, 2, 3)	6559	(3, 3, 3, 3, 3, 3, 3, 1)
6560	(3, 3, 3, 3, 3, 3, 3, 2)	6561	(3, 3, 3, 3, 3, 3, 3, 3)

Table 5. Experiment No. with maximum degree of membership

No.	Degrees of membership	Factors (i)							
		A	B	C	D	E	F	G	H
5800	0.400	3	2	3	3	2	3	2	1
5801	0.397	3	2	3	3	2	3	2	2
3046	0.380	2	2	1	2	2	3	2	1
3047	0.380	2	2	1	2	2	3	2	2
3049	0.380	2	2	1	2	2	3	3	1
3050	0.380	2	2	1	2	2	3	3	2
3127	0.380	2	2	1	3	2	3	2	1
3128	0.380	2	2	1	3	2	3	2	2
3130	0.380	2	2	1	3	2	3	3	1
3131	0.380	2	2	1	3	2	3	3	2

The number represents each combination of levels of eight factors in 3^8 = 36561 possible experiment points.

The fuzzy sets with high degrees of membership represented the combinations of levels for eight factors and the experiment numbers with maximum degrees of membership were shown in Table 6.

3.4. Experimental validations of the optimal conditions

Because none of the factors was more significant than the others in the ANOVA, the optimal preparation conditions for size by direct observation and the best two with high membership degrees by fuzzy analytical method displayed in Table 6 were selected. The validation experiments were conducted based on the predicted optimal levels and the results were listed in Table 7. The optimal conditions suggested by the fuzzy analytical method offered more information and the new method was superior to the traditional one.

4. Discussion

4.1. Fuzzy analytical method

A conventional experimental procedure involves altering one factor at a time while keeping all the other factors constant, and it is costly and time-consuming to obtain the optimal experimental conditions. The orthogonal design is utilized as a process-optimization approach in which only a few of the total possible number of experiments are conducted with certain levels of each factor. It is usually the first method of choice to avoid the problems of the conventional procedure. Both direct observation and variance analysis are usually used in the process of experimental data analysis. However, the direct observation can only provide different optimal parameters for each evaluation index and the variance analysis can just determine the statistical validity of the factors. The optimal parameters are obtained in parts of all possible experiments by orthogonal designs. But with the aid of fuzzy analytical method, the experimental result of each possible combination of levels can be predicted. More information can be obtained and the blind area within the experimental range is eliminated. Furthermore, the optimal preparation condition was obtained under the comprehensive consideration of the multiple indexes.

4.2. Analysis results

The ANOVA results revealed that no factor made a remarkable influence on size, encapsulation efficiency and physical stability. According to the conventional method, the good result was not got from the current data because of the neglect of some area in the orthogonal experiments. But the optimal preparation conditions were identified by the fuzzy method, which was the best among the three different analytical methods.

Table 6. Optimal preparation conditions

Factors (i)	Preparation conditions				
	1	2	3	4	5
	Direct observation (size (nm))	Direct observation (encapsulation efficiency)	Direct observation (physical stability)	Fuzzy analytical method 5800	Fuzzy analytical method 5801
A	10:1	10:1	10:1	10:1	10:1
B	8:3	8:3	8:3	8:3	8:3
C (min)	15	15	5	15	15
D (min)	10	15	5	15	15
E (°C)	50	50	50	50	50
F (min)	180	45	180	180	180
G (min)	15	10	15	10	10
H	5	3	7	3	5

The best preparing conditions for each method are labeled with number 1, 2, 3, 4 and 5, respectively.

Table 7. Validation experimental results

Evaluation indexes	1			4			5		
	Results	Average	RSD	Results	Average	RSD	Results	Average	RSD
Size (nm)	271.8 267.2 278.6	272.53	0.021	200.6 191.2 212.9	201.57	0.054	233.3 225.7 237.2	232.07	0.025
Encapsulation efficiency	0.524 0.478 0.600	53.4%	0.116	0.565 0.573 0.547	56.2%	0.024	0.563 0.583 0.557	56.8%	0.024
Physical stability	0.665 0.779 0.876	0.773	0.137	0.779 0.568 0.781	0.709	0.173	0.857 0.809 0.930	0.865	0.071

No. 1, 4 and 5 represent the best preparation conditions of direct observation (size (nm)), fuzzy analytical 5800 and 5801, respectively.

As shown in Table 7, the average size of liposomes acquired from the three optimal preparation conditions were (272.53±2.1) nm, (201.57±5.4) nm and (232.07±2.5) nm, respectively. The liposome sizes of validation experiments 4 and 5 for the fuzzy method had obvious advantage over the one for the traditional method.

In the ANOVA, none of the factors played a marked role in the encapsulation efficiency. It means that none of the chosen eight factors was significant for encapsulation efficiency. The possible reason is that the significant factors affecting the encapsulation efficiency may be determined by the characteristics of drugs and the method involved. However, the results of the validation experiments of encapsulation efficiency using fuzzy method were slightly higher than that of the direct observation method. The result derived from validated experiment 4 was 0.709±0.173, which was better than that of the other two experiments. The fuzzy method was obviously better than the traditional one.

5. Conclusion

We used the fuzzy analytical method to determine the optimal preparation conditions of liposomes. The fuzzy method has three advantages over the traditional methods. Firstly, it can identify the optimal preparation conditions under the consideration of the three evaluation indexes; secondly, through bringing in the weights of evaluation indexes, the results are more accurate and reliable; lastly, points beyond experimental design can be gained by the fuzzy analytical method and errors resulted from the chosen points in the experimental range are eliminated. However, more complicated computation is needed in the process. Further research should be done to apply the fuzzy analytical method in liposome preparation.

References

[1] Song, J.C.; Huang, L.; Chen, J.L. *Chin. Pharm. J*. **2008**, *43*, 1564–1568.

[2] Mei, C.L.; Zhou, J.L. *Practical Statistical Method*. Beijing: Science Press. **2002**.

[3] Zhou, X.Z.; Fan, Z.X.; Zhan, J.J. *Application of Fuzzy Mathematics in Chemistry*. Hunan Changsha: National University of Defense Technology Press. **2002**.

[4] Huang, H.B.; Liu, T.; Xu, Y.H.; Lin, Z.C.; Zhong, J.T.; Liu, Q.; Zheng, Y.Y.; Ruan, H.S. *China Pharm. J*. **2007**, *42*, 1872–1876.

[5] Deng, M.L.; Sun, G.L. *China Ceramic Industry*. **2003**, *10*, 38–41.

[6] Liu, Y.B.; Pu, W.F.; Ni, X.Y.; Li, S.H.; Shi, Y. *Chem. Ind. Engineering*. **2005**, *22*, 193–196.

[7] Zhao, S.J. *Forest Inventory Planning*. **2005**, *30*, 24–26.

[8] Zadeh, L.A. *Inf. Control*. **1965**, *8*, 338–353.

[9] Xian, R.Q.; Maitani, Y.; Nagai, T. *Int. J. Pharm*. **1995**, *114*, 33–41.

[10] Wang, L.; Zhou, H.; Zhang, S.Q.; Gu, Y.; Fang, K.Q.; Zhou, M.; Xi, M.M.; Lian, J.F. *J. Fourth Mil. Med. Univ*. **2005**, *26*, 1902–1905.

[11] Zhang, J.M.; Yang, H.; Hou, X.P. *Chin. Biochem. J*. **1995**, *11*, 615–617.

[12] Zhang, J.; Yang, J.; Wu, J.L.; Zhan, C.; Zhang, L. *Chin. Hosp. Pharm. J*. **2005**, *25*, 1046–1049.

[13] Li, X.Z.; Liu, S.; Lei, P.; Xu, L.M. *China Med. Engineering*. **2004**, *12*, 21–23.

[14] Li, Z.B.; Ye, G.B.; Xu, C. *Hydrogeology Engineering Gedogy*. **2005**, 117–119.

[15] Wang, Z.W.; Li, G.Z.; Liu, J.C.; Mu, J.H.; Liao, L.L. *China Surfactant Detergent Cosmetics*. **2001**, *3*, 14–16.

[16] Su, H.H.; Yao, Z.J. *J. Nanjing Univ. Aeronaut. Astronaut*. **2004**, *36*, 29–33.

模糊分析法在脂质体正交试验中的应用

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摘要: 为了更好的利用正交实验的信息, 模糊分析法作为新的统计方法应用于优化脂质体的制备, 对评价指标综合考虑获得最优制备条件。设计正交实验通过逆相蒸发法制备脂质体, 选择粒径、包封率和物理稳定性作为脂质体质量的评价指标, 通过直观分析、方差分析和模糊分析对结果进行处理, 由直观分析和模糊分析获得最佳制备条件, 再由验证实验比较各方案的优劣。通过模糊分析法, 得到了正交实验中所有因素不同水平可能组合实验的全部结果, 在综合考虑各个评价指标的情况下得到最佳处方。直观分析和模糊分析的结果并不相同, 通过验证实验结果表明模糊分析法更具有优势。与传统方法相比, 模糊分析方法预测了各因素与水平全部搭配实验的结果, 借助计算机提供比直观分析法与方差分析方法更多的信息, 消除了实验范围内的盲区, 对处方的优化和筛选具有更好的指导性。

关键词: 正交试验; 脂质体制备; 模糊分析法