Optimization of the Crystallization Process for Ceftriaxone Sodium, a Third-Generation Cephalosporin, Utilizing Response Surface Methodology

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Abstract [**Objectives**] To optimize the crystallization process of ceftriaxone sodium using response surface methodology (RSM) for enhancing both the crystallization rate and the quality of the final product. [**Methods**] Four key factors, including crystallization temperature, stirring speed, solvent drop rate, and seed crystal content, were employed as independent variables, while the crystallization rate served as the response variable. The Box-Behnken response surface method was utilized for the optimization design. [**Results**] The optimal parameters for the crystallization process, determined through optimization, were as follows: a temperature of 10.6 ℃, a stirring rate of 150 rpm, a solvent drop rate of 1.50 mL/min, and a seed crystal content of 0.12 g. Validation tests conducted under these conditions yielded an average crystallization rate of 94.38% for the refined product. [**Conclusions**] The crystallization efficiency of ceftriaxone sodium is markedly enhanced, thereby offering substantial support for its industrial production and clinical application.

Key words Ceftriaxone sodium, Response surface methodology (RSM), Crystallization process, Process optimization

1 Introduction

Ceftriaxone sodium is classified as a third-generation cephalosporin antibiotic. It was first introduced to the Swiss market in 1982 and received formal approval from the U.S. Food and Drug Administration (FDA) in 1984. Since then, it has emerged as one of the most widely utilized pharmaceuticals globally, attributed to its broad antimicrobial spectrum, potent antimicrobial activity, extended half-life, low toxicity, and high efficacy[1-3]. Ceftriaxone sodium is a widely utilized antibacterial agent in clinical practice in China, with extensive application across various medical fields[4]. Following the expiration of the patent protection period for the innovator drug, both domestic and international markets for generic drugs have experienced rapid development. Consequently, enhancing product quality and optimizing production processes have emerged as primary concerns within the industry[5].

The crystallization process of ceftriaxone sodium plays a pivotal role in the overall production process, as it directly influences the product's purity, crystal shape, particle size distribution, and stability[3]. In the preparation of ceftriaxone sodium, the instability of its crystalline form and the low rate of refined crystallization have been significant challenges that hinder the enhancement of its quality[6]. In recent years, numerous scholars have undertaken comprehensive studies on the crystallization process of third-generation cephalosporin antibiotics, with the objective of optimizing process conditions and enhancing product quality. Wang Jing *et al.*[7] conducted a systematic investigation into the effects of yield, crystal habit, and particle size distribution during the crystallization process of ceftazidime. This study addressed the issues of aggregation and suboptimal crystal habit commonly associated with traditional crystalline products. Xie Chenxin *et al.*[8] conducted an investigation into the effects of various parameters, including the pH of the crystallization process, the concentration of the raw solution, and the residence time, on the yield of the product and the distribution of particle size during the crystallization of cefradine. Wang Hairong *et al.*[9] indicated that the incorporation of an appropriate quantity of seed crystals, along with the regulation of the crystal growth duration to a maximum of 20 min, facilitates the refining process of ceftriaxone sodium, resulting in a product characterized by a larger average particle size. Although the crystallization technology for ceftriaxone sodium has yielded some positive outcomes, it continues to encounter numerous challenges, including the thermal instability of ceftriaxone sodium[9]. Consequently, enhancing production efficiency and reducing production costs, while maintaining product quality, has emerged as a pressing issue for the industry[10].

The objective of this study is to employ response surface methodology (RSM) for the optimization of the crystallization process of ceftriaxone sodium. This approach aims to achieve a substantial enhancement in product quality and an optimization of production efficiency through the precise control of various process parameters. The findings of this research are intended to offer an innovative perspective and methodology for the manufacturing process of ceftriaxone sodium, thereby contributing to the sustained growth of its generic drug market.

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2 Materials and methods

2.1 Materials The crude product of ceftriaxone sodium was produced by Guangxi Kelun Pharmaceutical Co., Ltd. Acetone, of analytical grade, was sourced from Xilong Science Co., Ltd. Activated carbon, also of analytical grade, was obtained from Fuchen Chemical Reagent Co., Ltd. The refined product of ceftriaxone sodium, specifically in the form of seed crystals, was manufactured by Guangxi Kelun Pharmaceutical Co., Ltd.

2.2 Instruments The instruments utilized in this study included the ME204E electronic balance, manufactured by Shanghai Yaoxin Electronic Technology Co., Ltd.; the DF-101SA collective thermostatic heating magnetic stirrer, produced by Shanghai Mani Instrument Co., Ltd.; the DZF-250 vacuum drying oven, supplied by Zhengzhou Greatwall Scientific Industrial & Trade Co., Ltd.; the HH-S1 digital thermostatic water bath, provided by Changzhou Huaao Instrument Manufacturing Co., Ltd.; and a circulating water multi-purpose vacuum pump, sourced from Xi'an Taikang Biotechnology Co., Ltd.

2.3 Methods

2.3.1 Preparation process of refined ceftriaxone sodium. 10.0 mL of purified water and 5.0 mL of acetone solution were loaded into a 50 mL conical flask. Subsequently, 5 g of crude ceftriaxone sodium was added, and the mixture was magnetically stirred at room temperature for 10 – 15 min to facilitate dissolution. Following this, activated charcoal, equivalent to 10% of the mass of the crude product, was incorporated into the mixture and stirred magnetically at room temperature for 30 min to achieve decolorization and depyrogenation. Subsequently, the mixture underwent vacuum filtration, during which the carbon cake and filter flask were rinsed with a 2∶1 mixture of acetone and purified water. The resulting filtrate was then transferred to a three-necked flask maintained at a predetermined temperature. The filtrate was gradually treated with 50.0 mL of acetone over a period of 30 min, at which point the flow was halted. Subsequently, the weighed seed crystals were introduced to facilitate crystal growth for 20 – 30 min. Following this, an additional 94.0 mL of acetone solvent was added. Once the solvent addition was complete, the crystals were allowed to grow for approximately 30 min. The resulting crystal slurry underwent vacuum filtration, and the filter cake was washed twice with acetone. The wet powder was then dried under vacuum at a temperature of 40 – 45 ℃ for 3 – 4 h, resulting in the final product, ceftriaxone sodium.

2.3.2 Single-factor test. The normal stirring temperature was maintained at 20 ℃, while the drying conditions were set to vacuum drying at 43 ℃ for 3.5 h. A single-factor test was conducted at a crystallization temperature of 12 ℃, with a stirring speed of 165 rpm, a solvent drop rate of 2.0 mL/min, and a seed crystal content of 0.15 g. The study further investigated the effects of crystallization temperature (A), stirring speed (B), solvent drop rate (C), and seed crystal content (D) on the crystallization rate of ceftriaxone sodium crude product (Y).

2.3.3 Response surface test. Four key factors were identified as independent variables in this study: crystallization temperature (A), stirring speed (B), solvent drop rate (C), and seed crystal content (D). The crystallization rate (Y) was designated as the response variable. A Box-Behnken response surface design was implemented following a single-factor test. The coding levels for the experimental factors are presented in Table 1.

Table 1 RSM levels for the optimization of the crystallization process of ceftriaxone sodium

Level	Factor			
	Crystallization temperature (A)//℃	Stirring speed (B)//rpm	Solvent drop rate (C)//mL/min	Seed crystal content (D)//g
−1	10.0	110	1.5	0.10
0	12.0	165	2.0	0.15
1	14.0	220	2.5	0.20

2.4 Data analysis Design-Expert software was employed for data analysis, and mathematical modeling was developed. Additionally, an analysis of variance (ANOVA) was performed to assess the extent of influence of each factor on the crystallization rate and to identify the optimal process conditions.

3 Results and analysis

3.1 Single-factor test

3.1.1 Crystallization temperature. The results indicated that the crystallization rate of ceftriaxone sodium increased with rising temperature, reaching its peak at 12 ℃. However, when the temperature exceeded 12 ℃, a significant increase in solubility occurred, which could potentially reduce the nucleation rate, thereby resulting in a decrease in crystallization yield. While reducing the temperature may enhance the final yield of the product, it concurrently results in a smaller particle size[11]. Additionally, creating lower temperatures in industrial production necessitates increased energy consumption, thereby elevating production costs. Consequently, crystallization temperatures of 10, 12, and 14 ℃ were selected for the RSM test. This approach not only ensured higher yields but also improved the primary particle size of the product, contributing to energy savings.

3.1.2 Stirring speed. The findings indicated that the crystallization rate of ceftriaxone sodium was maximized at a stirring speed of 165 rpm. Initially, the crystallization rate of ceftriaxone sodium exhibited a gradual increase with the enhancement of the stirring rate. This phenomenon can be attributed to the fact that stirring facilitates the collision and aggregation of solute molecules, thereby enhancing the rate of nucleation. However, when the stirring speed exceeds 165 rpm, an excessive number of crystal nuclei may be generated. This can lead to the formation of fine crystal particles and an increased specific volume. Furthermore, elevated stirring speeds may lead to the fragmentation of crystals, thereby com-

promising their structural integrity and negatively impacting the crystallization rate, which ultimately results in a decrease in the overall crystallization rate[12]. Consequently, stirring speeds of 110, 165, and 220 rpm were selected for the RSM test. Within this range, the resulting crystal product exhibited a uniform particle size distribution, reduced specific volume, enhanced flowability, and an increased crystallization rate.

3.1.3 Solvent drop rate. The findings indicated that the crystallization rate of ceftriaxone sodium was maximized at a solvent drop rate of 2.0 mL/min, resulting in the formation of crystals characterized by a regular shape and uniform particle size. Initially, the crystallization rate of ceftriaxone sodium exhibited a gradual increase with the acceleration of the solvent drop rate. An optimal solvent drop rate facilitates the crystallization of the solution within the metastable zone, thereby preventing spontaneous nucleation. This process ultimately results in the production of a crystal product characterized by uniform particle size and high yield. However, an excessively rapid solvent drop rate may result in a high local concentration of the solution, which can lead to the instantaneous formation of a significant number of primary nuclei. These nuclei may collide and aggregate during the growth process, ultimately resulting in the formation of crystals with non-uniform particle sizes[13]. Conversely, an excessively slow solvent drop rate may result in an inconsistent crystal growth rate and a broader distribution of particle sizes. Consequently, solvent drop rates of 1.5, 2.0, and 2.5 mL/min were chosen for the RSM test.

3.1.4 Seed crystal content. The results indicated that ceftriaxone sodium exhibited the highest crystallization rate when the seed crystal content was set at 0.15 g. The introduction of seed crystals resulted in an initial gradual increase in the crystallization rate of ceftriaxone sodium. This phenomenon can be attributed to the fact that the addition of seed crystals mitigates the excessive formation of nuclei during the early stages of crystallization, enhances the stability of the solvent-out crystallization process, and establishes favorable conditions for the regulation of crystallization[14]. However, the crystallization rate of ceftriaxone sodium was observed to decrease when an excessive amount of seed crystals was introduced. This phenomenon occurs because the number of growth units in the crystallization system becomes limited in relation to the growth points, leading to the formation of smaller crystal sizes. Furthermore, the incorporation of large seed crystals is economically disadvantageous for the generic manufacturing sector, as it elevates raw material costs and may diminish production efficiency. Consequently, seed crystal contents of 0.10, 0.15, and 0.20 g were selected for the RSM test.

3.2 RSM test The results of the RSM test are presented in Table 2, while the ANOVA results are displayed in Table 3. The quadratic multinomial regression equation derived from the analysis of the response values in relation to the independent variables, utilizing RSM software, is expressed as follows: $Y = 83.116\,1 + 3.527\,8A + 0.007\,9B - 7.658\,0C - 27.643\,3D + 0.005\,4AB + 1.010\,0AC + 0.125\,0AD - 0.001\,1BC + 0.237\,3BD + 72.400\,0CD - 0.276\,0A^2 - 0.000\,3B^2 - 3.981\,3C^2 - 514.133\,3D^2$.

Table 2 Results of the RSM test for the optimization of the crystallization process of ceftriaxone sodium

No.	Crystallization temperature (*A*)	Stirring speed (*B*)	Solvent drop rate (*C*)	Seed crystal content (*D*)	Crystallization rate//%
1	10	110	2	0.15	93.02
2	14	110	2	0.15	90.80
3	10	220	2	0.15	92.41
4	14	220	2	0.15	92.57
5	12	165	1.5	0.1	93.68
6	12	165	2.5	0.1	89.81
7	12	165	1.5	0.2	90.45
8	12	165	2.5	0.2	93.82
9	10	165	2	0.1	91.79
10	14	165	2	0.1	91.66
11	10	165	2	0.2	92.24
12	14	165	2	0.2	92.16
13	12	110	1.5	0.15	92.55
14	12	220	1.5	0.15	93.88
15	12	110	2.5	0.15	91.06
16	12	220	2.5	0.15	92.27
17	10	165	1.5	0.15	93.68
18	14	165	1.5	0.15	90.81
19	10	165	2.5	0.15	91.11
20	14	165	2.5	0.15	92.28
21	12	110	2	0.1	92.31
22	12	220	2	0.1	91.22
23	12	110	2	0.2	91.21
24	12	220	2	0.2	92.73
25	12	165	2	0.15	94.73
26	12	165	2	0.15	94.24
27	12	165	2	0.15	93.98
28	12	165	2	0.15	94.09
29	12	165	2	0.15	94.03

The results of the study indicated that the model employed yielded a *P*-value of 0.000, which is less than the significance threshold of 0.001, thereby demonstrating statistical significance. Conversely, the lack of fit exhibited a *P*-value of 0.193 0, exceeding the 0.05 threshold, indicating that it was not statistically significant. Both findings met the requisite criteria for model adequacy, thereby affirming the model's validity. Furthermore, the correlation coefficient ($R^2 = 0.943\,2$) suggested a strong fit of the model, while the adjusted correlation coefficient ($R^2_{Adj} = 0.886\,4$) indicated an 88.64% probability that the variation in the crystallinity of the response value was attributable to the factors of temperature, stirring speed, drop rate, and seed crystal content. In conclusion, the model is capable of predicting the influence of temperature, stirring speed, drop rate, and seed crystal content on the crystallization rate. The *P*-values associated with the primary term *C*, the interaction terms *AC* and *CD*, as well as the secondary

terms A^2, B^2, C^2, and D^2, were all found to be less than 0.01, signifying that their effects on crystallization are highly significant. Conversely, the *P*-values for the primary terms *A* and *B*, along with the interaction terms *AB* and *BD*, were below 0.05, indicating that their effects on the response values are statistically significant. In contrast, the *P*-values for the remaining terms exceeded 0.05, suggesting that they do not exert a significant influence on the response values.

Table 3 RSM analysis

Source	Sum of squares	Degree of freedom	Mean square	*F*	*P*	Significance
Model	45.017 1	14	3.215 5	16.607 8	0.000 0	* *
Crystallization temperature (*A*)	1.313 4	1	1.313 4	6.783 6	0.020 8	*
Stirring speed (*B*)	1.421 4	1	1.421 4	7.341 5	0.016 9	*
Solvent drop rate (*C*)	1.840 8	1	1.840 8	9.507 8	0.008 1	* *
Seed crystal content (*D*)	0.381 6	1	0.381 6	1.971 1	0.182 1	
AB	1.416 1	1	1.416 1	7.314 0	0.017 1	*
AC	4.080 4	1	4.080 4	21.074 9	0.000 4	* *
AD	0.000 6	1	0.000 6	0.003 2	0.955 5	
BC	0.003 6	1	0.003 6	0.018 6	0.893 5	
BD	1.703 0	1	1.703 0	8.796 0	0.010 2	*
CD	13.104 4	1	13.104 4	67.683 1	0.000 0	* *
A^2	7.907 0	1	7.907 0	40.839 1	0.000 0	* *
B^2	5.449 5	1	5.449 5	28.146 0	0.000 1	* *
C^2	6.426 1	1	6.426 1	33.190 2	0.000 0	* *
D^2	10.716 2	1	10.716 2	55.348 3	0.000 0	* *
Residual	2.710 6	14	0.193 6			
Lack of fit	2.339 7	10	0.234 0	2.523 1	0.193 0	
Error	0.370 9	4	0.092 7			
Total	47.727 7	28				
	R^2 = 0.943 2; R^2_{Adj} = 0.886 4					

The regression analysis table indicated that the magnitude of the *F*-values for the three factors followed the order $C > B > A > D$. This hierarchy suggests that a larger *F*-value corresponds to a more significant influence of the respective factor on the response value. Fig. 1 illustrates the 3D response surface plot derived from the optimization of the extraction scheme in the RSM test.

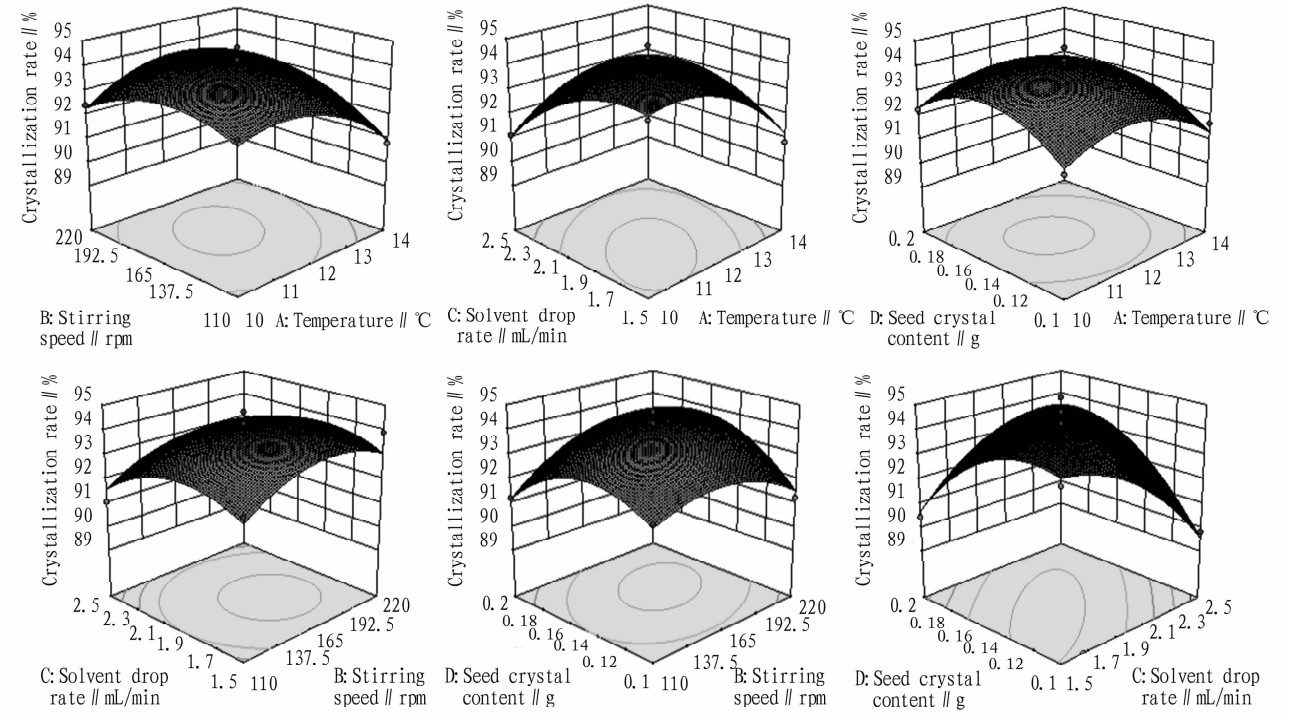

Fig. 1 3D response surface and contour plots for the optimization of the extraction scheme in RSM test

Overall, the crystallinity exhibited a gradual increase followed by a subsequent decrease in response to variations in temperature, stirring speed, drop rate, and seed crystal content. The 3D surface plot revealed that the slopes of the interaction terms *AC* and *CD* were the most pronounced, while the interaction terms *AB* and *BD* demonstrated the second highest slopes. This indicates that the interaction terms *AC* and *CD* exert a highly significant influence on crystallinity, whereas the interaction terms *AB* and *BD* have a significant, albeit lesser, effect on crystallinity. These findings are consistent with the results obtained from the regression analysis.

3.3 Verification test The optimal process conditions for the crystallization of ceftriaxone sodium were determined using RSM as follows: temperature of 10.631 ℃, stirring rate of 150.168 rpm, solvent drop rate of 1.500 mL/min, and seed crystal content of 0.115 g. The predicted crystallization rate was found to be 94.595%. To enhance the operational efficiency, the process parameters were modified as follows: a crystallization temperature of 10.6 ℃, a stirring rate of 150 rpm, and a solvent drop rate of 1.50 mL/min; additionally, a seed crystal content of 0.12 g was incorporated. Three repetitions of the experiment resulted in a crystallization rate of 94.38% for crude ceftriaxone sodium, which closely approximated the predicted value. This outcome suggests that the model is both reliable and practical.

4 Conclusions

The crystallization process of ceftriaxone sodium was successfully optimized using RSM. The optimal process conditions were determined through the established mathematical model, which indicated a temperature of 10.6 ℃, a stirring rate of 150 rpm, a solvent drop rate of 1.50 mL/min, and the incorporation of seed crystal content at 0.12 g. The validation test was conducted under the specified conditions, resulting in a crystallization rate of 94.38% for the refined product. The experimental results indicated a significant increase in the crystallization rate of ceftriaxone sodium when subjected to optimal process conditions. The final product was characterized as a white crystalline powder, devoid of odor, exhibiting uniform particle size and stable quality.

Through the development of a mathematical model, the optimal conditions for the crystallization process were identified, resulting in significant enhancements in both the crystallization efficiency and the quality of ceftriaxone sodium. The influence of various critical crystallization temperature adjustment points on the crystallization rate is substantial, as it directly impacts the solubility, nucleation rate, and crystal growth of sodium ceftriaxone. An increase in temperature facilitates a greater dissolution of sodium ceftriaxone in the solvent, thereby diminishing the driving force for crystallization[14-16]. According to the Arrhenius Equation, $k = Ae^{-\frac{E_a}{RT}}$, an increase in temperature enhances the thermal motion of molecules, thereby accelerating the rate of nucleation and promoting more rapid crystallization. Several critical temperature control parameters are outlined as follows: the initial crystallization temperature should be maintained within the range of 18 – 22 ℃; the cooling rate should be regulated between 0.2 – 0.5 ℃/min; and the endpoint temperature should be set between 8 – 12 ℃. Consequently, it is imperative to rigorously monitor and control the temperature regulation at each stage of the production process.

The optimized process offers several advantages, including ease of operation and economic feasibility, thereby providing substantial support for the industrial production and clinical application of ceftriaxone sodium. This system demonstrates significant potential for clinical application and is anticipated to further enhance the market competitiveness of ceftriaxone sodium.

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