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APPLICATION OF THE BEHNKEN DESIGN BOX TO OPTIMIZE THE PRODUCTION OF DEXIBUPROFEN NANOCRYSTALS USING THE TOP-DOWN METHOD

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Abstract

Background: Dexibuprofen (DXI) is the S (+) enantiomer form of Ibuprofen [(±)-α-methyl-4-(2-methylpropyl) benzeneacetic acid]. DXI exhibits poor physical solubility, measuring less than 1 mg/mL, which results in low bioavailability in the gastrointestinal tract. One approach to enhance the solubility of DXI involves reducing its particle size to form nanocrystals. **Objective**: This study aims at synthesizing stable dexibuprofen (DXI) nanocrystals using the top-down method. Additionally, it aims to optimize the synthesis process using the Box-Behnken method. **Method**: The study aims to optimize the concentrations of DXI (50-150 mg), PVP K90 (1-3%), and Decyl Glucoside (DG) (1-3%) to achieve optimal conditions to produce DXI nanocrystals using the top-down method. Furthermore, the study aims to develop a mathematical model for the preparation of DXI nanocrystals, considering the concentrations of DXI, PVP K90, and Tween 80. The optimization of DXI nanocrystal production conditions employs the Response Surface Method, specifically the Box-Behnken Design, with three factors and three levels. **Results**: Within the framework of the Box-Behnken design, this study encompassed a total of 15 trials. The analysis outcomes revealed that the optimal concentration for DXI in the production of DXI nanocrystals was determined to be 150 mg, while the optimal concentrations for PVP K90 and DG were found to be 1% each. The Response Surface Regression analysis yielded a constant value of 1.22. Consequently, an equation was derived as follows: Y = 1.22 - 0.654DXI + 5.338PVPK90 + 1.400DG. **Conclusion**: The optimized DXI nanocrystals were successfully synthesized using an anti-solvent deposition method, incorporating the condition optimization results obtained from the Box-Behnken design.

INTRODUCTION

Dexibuprofen has demonstrated efficacy comparable to diclofenac, naproxen, and celecoxib, along with good tolerability [1, 2]. Studies have shown that pain treatment with dexibuprofen in a specialized crystal form is a safe and effective option [2, 3]. However, this particular form of DXI falls under the very slightly soluble category in water, measuring less than 1 mg/mL [4]. One approach to improve the physical properties of active substances is to reduce the particle size. Reducing the drug size to the nanoscale increases the surface area-to-volume ratio, thereby enhancing the solubility, dissolution rate, and in vivo performance of poorly soluble drugs [5, 6].

This study aims to prepare dexibuprofen (DXI) nanocrystal formulations and assess the influence of polymers on the production of stable DXI nanocrystals with enhanced therapeutic potential. The main challenge in nanocrystal preparation lies in achieving stability. During manufacturing or storage, nanocrystals have a propensity to agglomerate, resulting in the

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formation of agglomerates through physical interactions [7–9]. The strength of hydrogen bonds and Van der Waals attractions surpasses hydrophobic forces. The significance of these forces varies depending on the surface properties of the active agent and the particle size variation. The addition of a stabilizer to the surface of nanocrystals alters the balance between repulsive and attractive forces among the particles themselves [5]. Therefore, the incorporation of a stabilizer is necessary to maintain the stability of nanocrystal formulations. Polyvinylpyrrolidone (PVP) polymer was chosen as the stabilizer for its well-established use in various research studies on nanoparticle formulations [10]. PVP is recognized for its ability to enhance solubility [11]. Additionally, Decyl Glucoside (DG) is included as a surfactant. To optimize the DXI nanocrystal formulation, the addition of PVP K90 and DG to the DXI solution in ethanol employed a statistical approach known as the Design of Experiment (DoE), which incorporates factorial design and response surface methods (RSM). This approach was employed to yield more effective and efficient results.

This study utilized Minitab ver. 19 software to implement the response surface method (RSM) for mathematical and statistical modeling of experimental data [12–14]. The experimental data in this study were analyzed using mathematical equations based on the response surface method (RSM), providing an overview of the variables or factors' influence on the desired response. To identify the influential variables, the study employed the Design of Experiment (DOE) approach, which involved selecting an initial design, such as a full or half-full 3-level factorial design, determining the minimum and maximum response ranges, estimating function parameters, conducting experiments to observe responses, and exploring the response surface. In this study, the Box-Behnken design was employed in the RSM method [12, 15]. The Box-Behnken design in RSM experiments offers advantages over 3-level full factorial designs and central composite designs (CCD). It was observed to be more efficient, requiring fewer experiments, thus reducing testing costs [9]. The optimization of response analysis using the Box-Behnken design shall yield the optimal conditions for the study.

MATERIALS AND METHODS

Materials

DXI (Batch Number: A 001-202001004) was purchased from Beijing Mesochem Technology, Co., Ltd., Beijing. Polyvinyl pyrrolidone (PVP) K-90 (Batch number: 08297052G0) and Decyl Glycoside were procured for use in the study. Minitab Software 19 trial version was utilized for data analysis.

Methods

Preparation of DXI

This study utilized experimental analysis methods to determine the appropriate operating parameter values for the synthesis of DXI nanocrystals, aiming to achieve optimal conditions. The obtained experimental data were then subjected to surface response analysis using the Behnken Design Box.

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The initial step involved the use of Design of Experiments (DOE) to determine the formulation of DXI nanocrystals, with various concentrations of DXI (50-150 mg), PVP K90 (1-3%), and DG (1-3%) as stabilizers for the nanocrystal preparations. This step was crucial to ensure the stability of the DXI nanocrystals in liquid form.

To manufacture the DXI nanocrystals using the top-down method, a supersaturated DXI solution was prepared. This involved dissolving 50 mg, 100 mg, and 150 mg of DXI in 100 ml of water, with the addition of PVP K90 and Tween 80 in concentrations determined by the Box-Behnken Design. The DXI solution in water, containing PVP K90 and Tween 80, was subjected to ultrasonic treatment using an Ultrasonic Cell Disrupter (BSD-250W) from Prosperity Biotech (Shandong) Co., Ltd. The ultrasonic treatment was performed at a power ratio of 75% and a temperature of 40°C to break down the DXI material and facilitate nanocrystal formation.

Table 1: Design of Experiment Box Behnken with Three Factors and Three Levels

Factor	Name	Range & Level		
		-1	0	+1
A	DXI (mg)	50	100	150
B	PVP K90 (%)	1	2	3
C	DG (%)	1	2	3

Table 2: Run Order Testing from Box-Behnken Design

Run Order	Rotation Speed (RPM)	PVP K90 (%)	Tween 80 (%)
1	50	1	2
2	150	1	2
3	50	3	2
4	150	3	2
5	50	2	1
6	150	2	1
7	50	2	3
8	150	2	3
9	100	1	1
10	100	3	1
11	100	1	3
12	100	3	3
13	100	2	2
14	100	2	2
15	100	2	2

FINDINGS AND DISCUSSIONS

DXI Nanocrystals Manufacture

The manufacturing process of DXI nanocrystals was conducted using the top-down method, which is a physical process for producing materials at the nano scale. This method involves breaking down larger materials into nanometer-sized particles. It can also involve the combination of small materials, such as clusters, to form nanometer-sized particles while

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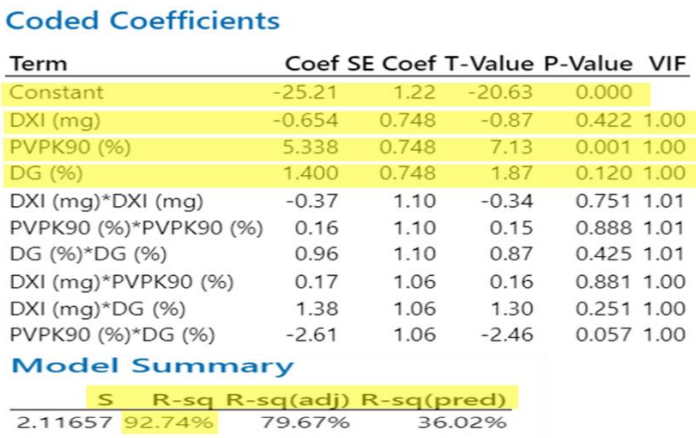
preserving the properties of the material [16]. The proper composition of the active substance and stabilizer is crucial in the production of DXI nanocrystals, as it relates to the super saturation conditions of the solution. The optimal combination yielded DXI nanocrystals with particle sizes below 1000nm, as shown in Table 3. Further, Figure 1 illustrates the results of the DXI nanocrystal production using the Box-Behnken design.

Table 3: Results of DXI Nanocrystals Manufacture Using Box-Behnken Design

Run Order	DXI (mg)	PVP K90 (%)	DG (%)	Particle Size	Polydispersity Index	Zeta Potential
1	50	1	2	781.70	0.364	-29.13
2	150	1	2	57.33	0.315	-31.13
3	50	3	2	546.70	0.380	-20.03
4	150	3	2	137.10	0.337	-21.37
5	50	2	1	562.73	0.420	-25.13
6	150	2	1	88.83	0.367	-28.83
7	50	2	3	14.33	0.462	-23.17
8	150	2	3	146.90	0.236	-21.37
9	100	1	1	53.13	0.322	-33.10
10	100	3	1	136.93	0.441	-15.97
11	100	1	3	20.93	0.567	-27.00
12	100	3	3	532.57	0.431	-20.30
13	100	2	2	126.20	0.346	-27.70
14	100	2	2	118.60	0.256	-22.87
15	100	2	2	118.43	0.291	-25.07



Coded Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-25.21	1.22	-20.63	0.000	
DXI (mg)	-0.654	0.748	-0.87	0.422	1.00
PVPK90 (%)	5.338	0.748	7.13	0.001	1.00
DG (%)	1.400	0.748	1.87	0.120	1.00
DXI (mg)*DXI (mg)	-0.37	1.10	-0.34	0.751	1.01
PVPK90 (%)*PVPK90 (%)	0.16	1.10	0.15	0.888	1.01
DG (%)*DG (%)	0.96	1.10	0.87	0.425	1.01
DXI (mg)*PVPK90 (%)	0.17	1.06	0.16	0.881	1.00
DXI (mg)*DG (%)	1.38	1.06	1.30	0.251	1.00
PVPK90 (%)*DG (%)	-2.61	1.06	-2.46	0.057	1.00

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.11657	92.74%	79.67%	36.02%

Figure 1: Response Surface Regression

Among the three independent variables in the DXI nanocrystal manufacturing process, the concentration of PVP K90 was found to have a significant effect on the zeta potential of the formed nanocrystals, with a P-Value of 0.001. On the other hand, the concentrations of DXI and DG did not have a statistically significant effect on the production of DXI nanocrystals, as indicated by the P-Values obtained from the Box-Behnken experimental design. In the Box-Behnken design, if the P-Value is less than α (≤ 0.05), the null hypothesis H0 is accepted,

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indicating that the independent variable has an impact on the dependent variable. Conversely, if the P-Value is ≥ α (0.05), the null hypothesis H0 is rejected, suggesting that the independent variable does not have a significant effect on the dependent variable [17]. Based on the analysis, the coefficient value (Coef) in Figure 1 demonstrates a constant value of 1.22. Therefore, the equation can be formulated as follows:

$$Y = 1.22 – 0.654DXI+5.338PVPK90+1.400DG$$

If the concentration of PVP K90 increases by one point while the other independent variables remain constant, the potential zeta value is expected to increase by 5.338%. To ensure the adequacy of the model, a lack-of-fit test and a coefficient of determination test (R2) were conducted. The lack-of-fit test examines whether the first-order linear model adequately fits the data. If the lack-of-fit test results are not significant, it indicates that the first-order linear model is appropriate. The coefficient of determination (R2) is used to assess how well the first-order linear model predicts the obtained data. A higher R2 value indicates a better fit of the first-order linear model to the data [18]. The ANOVA results from the Box-Behnken experimental design are presented in Figure 2.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	286.058	31.784	7.09	0.022
Linear	3	247.015	82.338	18.38	0.004
DXI (mg)	1	3.423	3.423	0.76	0.422
PVPK90 (%)	1	227.911	227.911	50.87	0.001
DG (%)	1	15.680	15.680	3.50	0.120
Square	3	4.156	1.385	0.31	0.819
DXI (mg)*DXI (mg)	1	0.504	0.504	0.11	0.751
PVPK90 (%)*PVPK90 (%)	1	0.099	0.099	0.02	0.888
DG (%)*DG (%)	1	3.371	3.371	0.75	0.425
2-Way Interaction	3	34.887	11.629	2.60	0.165
DXI (mg)*PVPK90 (%)	1	0.111	0.111	0.02	0.881
DXI (mg)*DG (%)	1	7.563	7.563	1.69	0.251
PVPK90 (%)*DG (%)	1	27.214	27.214	6.07	0.057
Error	5	22.399	4.480		
Lack-of-Fit	3	10.687	3.562	0.61	0.670
Pure Error	2	11.712	5.856		
Total	14	308.457			

Figure 2: ANOVA Data from the Box-Behnken Experimental Design

The model test results reveal a lack-of-fit value of 0.670, indicating some degree of inaccuracy in the first-order linear model. However, if the P-Value in the regression model for variation I is less than α = 0.05, then the lack of fit is considered not significant. In this case, the data model presented in demonstrates a P-Value of 0.022 in the first-order regression model, which is smaller than α (0.05). Consequently, it can be concluded that the lack of fit is not significant, and the first-order linear model is deemed acceptable [12, 17]. The coefficient of determination (R2) test evaluates the goodness of fit of the model to the data, with values ranging from 0 to 100. A higher R2 value indicates a better fit of the model [17]. Generally, a regression model

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with an R2 value of ≥70% is considered good enough [18]. In the case of the machining process of Magnesium AZ31, the percentage of total model variation (R2) is determined to be 92.74% (Figure 1). This high value signifies a significant contribution of the independent variable to the response of the dependent variable (Ra).

The Box-Behnken experimental design data also provides a contour response plot, illustrating that a zeta potential below -35 can be achieved in the preparation of nanocrystals using a composition of 150 mg DXI, 1% PVP K90, and 1% DG. Figure 3 presents a visual representation of this contour response.

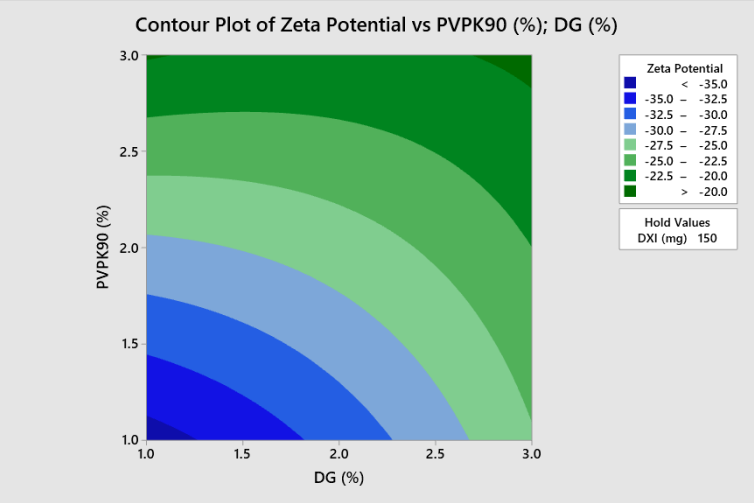

Figure 3: Contour Plot Response Potential Zeta Value of the Box-Behnken Experimental Design

Variable	Setting
DXI (mg)	150
PVPK90 (%)	1
DG (%)	1

Response	Fit	SE Fit	95% CI	95% PI
Zeta Potential	-36.00	2.50	(-42.43; -29.57)	(-44.42; -27.58)

Figure 4: Response Optimization of the Box-Behnken Experimental Design

The P-value of the data serves as an indicator of the significance of factors on the response variable, indicating the approximate agreement between the results of the Pareto analysis and the obtained responses. A lack-of-fit value of 0.670 indicates an error factor > 0.05, suggesting that the model does not significantly differ from the experimental results [19]. The optimal formula was determined based on the potential zeta response, which reached -36.00 with the composition of 150 mg DXI, 1% PVP K-90, and 1% DG. The optimal formula is depicted in Figure 4.

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CONCLUSION

The study on DXI nanocrystals using the top-down method reveals that the concentration of PVP K90 significantly influences the zeta potential value in the formation of DXI nanocrystals, as indicated by the surface regression design data obtained from the Box-Behnken experiment with a P-Value of 0.001, satisfying the α (0.05) significance level. The mathematical model for predicting the zeta potential values in the DXI nanocrystal production process, considering different concentrations of DXI, PVP K90, and DG, is as follows

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