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COMMUNICATION

Formulation Design and Optimization of Modified-Release Microspheres of Diclofenac Sodium

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ABSTRACT

The present study deals with the preparation of microspheres of diclofenac sodium using cross-linked poly(vinyl alcohol) (PVA). A central composite design consisting of a two-level full factorial design superimposed on a star design was employed for developing the microspheres. The PVA to the drug ratio X_1 *and amount of glutaraldehyde cross-linking agent* X_2 *were chosen as the independent variables. The time required for 50% drug dissolution* t_{50} *in phosphate buffer (pH 7.2) was selected as the dependent variable. An optimum polynomial equation was generated for the prediction of the response variable* t_{50}*. Based on the results of multiple linear regression analysis and* F *statistics, it may be concluded that sustained action can be obtained when* X_1 *and* X_2 *are kept at high levels. The* X_1X_2 *interaction was found to be statistically significant. A response surface plot is presented to show the effects of* X_1 *and* X_2 *on* t_{50}*. The drug release pattern fit the Higuchi model well. A model was validated for accurate prediction of the drug dissolution profile with constraints on the percentage drug release in the first, fifth, and seventh hours. The data of a selected batch were subjected to an optimization study, and an optimal formulation was fabricated. Good agreement was observed between the predicted and the observed dissolution profiles of the optimal formulation.*

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INTRODUCTION

Diclofenac sodium is used in the long-term treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Its biological half-life has been reported as 1–2 hr (1). Gastrointestinal side effects are commonly seen (2). Due to the short biological half-life and associated adverse effects, it is considered an ideal candidate for controlled drug delivery.

Natural and synthetic polymers have been tried by researchers for the preparation of microspheres of drugs. Among them, poly(vinyl alcohol) (PVA) has interesting characteristics (3–5). At body temperature, dry PVA remains in its glassy state. On contact with an aqueous medium, a glassy to rubbery state transition occurs that can greatly modify the rate of drug release. The cross-linked form of PVA forms a three-dimensional network that serves as a drug delivery device. The swelling and the drug release rate are dependent on the degree of cross-linking. The objective of the present study was to investigate systematically the impact of cross-linking conditions on modulation of drug release. The advantages of cross-linking PVA with glutaraldehyde are well documented in literature (6–8).

The applications of central composite Box and Wilson experimental design for developing pharmaceutical dosage forms are well documented (9–14). The PVA-to-drug ratio X_1 and the amount of glutaraldehyde X_2 were chosen as independent variables. Eleven experiments were performed according to the design matrix system shown in Table 1, which includes two additional replicates of the center point for estimation of the error involved in the manufacturing and testing of microspheres. The time for 50% of drug dissolution t_{50} was selected as the dependent variable.

EXPERIMENTAL

Materials

Diclofenac sodium (JP) was received as a gift sample from Sharda Drugs, India. The PVA (molecular weight 1000 and degree of hydrolysis 88.35%) was a gift sample from Shin-Etsu Chemical Company, Limited, Japan. Glutaraldehyde and dioctylsulfosuccinate sodium (DOSS) were obtained from Loba Chemie Pvt. Limited, India, and Aldrich Chemical Company, United States, respectively. All the other solvents and chemicals were analytical grade and were used without further purification.

Methods

Preparation of Poly(Vinyl Alcohol) Microspheres

For preparation of the PVA microspheres, 10 ml of 20% w/v aqueous solution of PVA was mixed with the required amount of the drug. The dispersion was heated at 70°C for 10 min, and then glutaraldehyde was added in the required amount. The semiliquid mass was dispersed slowly in 50 ml of liquid paraffin blend (3:1 ratio mixture of light to heavy liquid paraffin containing 40 mg DOSS). The dispersion was stirred for 5–7 min using a propeller stirrer (1000 rpm) to obtain a water-in-oil (w/o) emulsion. The cross-linking of PVA was favored by using an acid catalyst (1N HCl, 4 ml). The dispersion was stirred for 15 min. The hardened microspheres were separated by filtration and washed several times with petroleum ether. After removing traces of petroleum ether, the microspheres were washed two times with 15% w/v aqueous solution of sodium bisulfite and water to remove the traces of unreacted glutaraldehyde. The microspheres were finally dried under vacuum. The particle size analysis was carried out using a mechanical sieve shaker. The formulations are depicted in Table 1.

Dissolution Study

Hard gelatin capsules were filled with microspheres equivalent to 100 mg of diclofenac sodium and were evaluated for in vitro dissolution studies. The study was carried out in a USP XXII basket apparatus at a rotational speed of 50 rpm at 37°C in 900 ml phosphate buffer (pH 7.2). Samples (10 ml) were withdrawn at regular time intervals and filtered through a 0.45-μm membrane filter. The drug content was determined in the filtrate either directly or after appropriate dilution with the dissolution medium. The absorbance measurements were done on a Hitachi double beam ultraviolet/visible (UV/Vis) spectrophotometer at 276 nm (15). Corresponding concentrations in the samples were calculated from the standard plot generated by fitting a weighted linear regression model (Absorbance = 0.029 ∗ Concentration − 0.0062) to the data obtained in triplicate (16). The time required for 50% of the drug to dissolve t_{50} was calculated for each formulation using the Higuchi model. The average values of t_{50} and percentage drug released in the first, fifth, and seventh hr are depicted in Table 1.

RESULTS AND DISCUSSION

An interactive statistical second-order complete model (Eq. 1) was generated to evaluate the selected response.

Table 1

Design Layout of Central Composite Design and Summary of Experimental Results

Batch No.	Variable Levels in Coded Form		Average Response	Percentage Drug Released		
	X_1	X_2	t_{50} (min)	Y_{60} (min)	Y_{300} (min)	Y_{420} (min)
1	−1	−1	272	19.73	54.29	66.91
2	−1	1	418	19.11	41.63	53.34
3	1	−1	537	18.78	36.81	45.20
4	1	1	886	15.67	29.45	36.69
5	−1.41	0	267	22.32	49.06	65.78
6	1.41	0	939	19.97	29.57	34.49
7	0	−1.41	293	21.98	49.44	61.55
8	0	1.41	939	13.49	28.45	34.45
9	0	0	586	15.58	35.66	40.36
10	0	0	610	14.46	33.73	41.30
11	0	0	600	14.57	35.63	43.92

Coded Values	Actual Values	
	X_1	X_2
−1.41	0.41:1	0.118
−1	0.5:1	0.2
0	1:1	0.4
1	2:1	0.6
1.41	2.41:1	0.682

X_1 = PVA-to-drug ratio.
X_2 = Amount of glutaraldehyde (ml).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

where Y is the predicted response, b_0 is the arithmetic mean response of 11 runs (Table 1), and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low value to its high value. The interaction (X_1X_2) shows how the t_{50} value changes when two factors are simultaneously changed, and the exponential terms (X_1^2 and X_2^2) represent curvature. The fitted equation relating the response (t_{50}) to the transformed factors is shown in Eq. 2.

$$t_{50} = 599.2 + 210.6\ X_1 + 176.3\ X_2 + 50.5\ X_1X_2 - 18.5\ X_1^2 - 11.8\ X_2^2 \quad (2)$$

The t_{50} values for the 11 batches show a wide variation, that is, the response ranged from a minimum of 267 min to a maximum of 939 min. The data clearly indicate that the t_{50} value is strongly dependent on the independent factors selected in the study. The value of the correlation coefficient r^2 was found to be .9366, indicating a good fit. Batches 9, 10, and 11 showed an average t_{50} of 598.95 ± 11.76. The results reveal good reproducibility of the system. Equation 2 can be used to draw conclusions after considering the magnitude of the coefficient and the mathematical sign it carries (i.e., positive or negative). It may be concluded that the high levels of X_1 (PVA-to-drug ratio) and X_2 (amount of glutaraldehyde) appear to favor the preparation of sustained-release microspheres of diclofenac sodium.

The significance test for regression coefficients was carried out by applying Student's t test. A coefficient is significant if the calculated value of t is greater than the critical (table) value of t. The significance levels of coefficients b_{11} and b_{22} were found to be .65 and .77, respectively; hence, they were omitted to evolve the reduced model (Eq. 3).

$$t_{50} = 577.2 + 210.6\ X_1 + 176.3\ X_2 + 50.5\ X_1X_2 \quad (3)$$

This argument is further investigated by testing the model in portions (17). The computation steps for calculating the value of F are shown in Table 2. The calculated value

Table 2

Summary of Results of Regression Analysis and ANOVA for Measured Response (t_{50})

Response (t_{50})	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
Full model (FM)	599.2	210.6	176.3	50.5	−18.5	−11.8	0.9366
Reduced model (RM)	577.2	210.6	176.3	50.5	—	—	0.9333

Sample Calculation for Testing the Model in Portions

	DF	SS	MS	*F*
Regression				
FM	5	614,462.1	122,892.4	14.77
RM	3	612,284.5	204,094.8	32.64
Error				
FM	5	41,583.08	8316.616	
RM	7	43,760.7	6251.529	

SSE1 − SSE2 = 43,760.7 − 41,583.08 = 2177.62.
No. parameters omitted = 2.
MS of error (full model) = 8316.616.
F = (2177.62/2)/8316.616 = 0.13092.

of F was found to be 0.131. The critical value of F for $\propto = 0.05$, v1 = 2, and v2 = 5 is 5.79. Since the calculated value of F is lower than the critical value, we can conclude that the polynomial terms do not significantly contribute for the prediction of t_{50}. The results of the full and the reduced models are depicted in Table 2.

The results of t_{50} are in agreement with the general theory of microspheres. At high level of PVA-to-drug ratio, greater resistance is offered to the dissolution fluid; hence, the drug diffuses out at a slower rate. The percentage drug release also decreases when a higher level of glutaraldehyde was used, probably because of reduced porosity. The coefficient of factor X_1 is greater in magnitude than that of factor X_2. Hence, it can be concluded that the effect of the PVA-to-drug ratio is more significant in increasing the t_{50} value than the amount of glutaraldehyde. The value of the coefficient of the interaction X_1X_2 is relatively lower than that of the main effects; thus, it seems to exhibit very little effect on the t_{50} value. The relationship between the PVA-to-drug ratio X_1 and the amount of glutaraldehyde X_2 and t_{50} is presented in a response surface plot (Fig. 1). The plot is drawn using the interpolate function of Sigmaplot®.

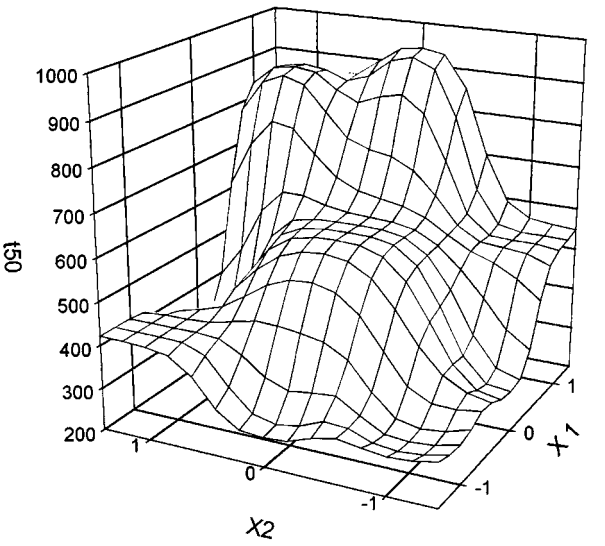

Figure 1. Response surface plot for time for 50% drug dissolution (t_{50}).

The purpose of this study was to develop a product that provides a loading dose in the first hour and then controlled drug release for up to 12 hr. For carrying out preliminary screening, it was decided to identify the batches that exhibited t_{50} between 4 and 5 hr. Batches 1, 5, and 7 met this selection criterion. The following additional constraints were chosen for the selection of batches: 20% $< Y_{60} <$ 40%; 40% $< Y_{300} <$ 55%; 55% $< Y_{420} <$ 70%. Batch 1 could not fulfill the criteria. Batch 7 showed a higher value of t_{50} (293 min) compared to that of batch 5 (267 min). The dissolution data of batch 7 were used for deciding the kinetics of drug release. The drug incorporation efficiency of batch 7 was found to be 70.44%. About 70% of the microspheres of batch 7 were found to be in the size range 355 to 500 μm.

The goodness-of-fit test proposed by Bamba and coworkers (18) was applied to the dissolution profile of batch 7 to determine the kinetics of drug release. The release profile fitted best to the Higuchi equation ($F = 3.32$), showing the least residual sum of square as compared with the Korsemeyer and Peppas equation ($F = 6.01$) or the Weibull equation ($F = 9.65$). This superiority is, however, statistically insignificant, as shown by F ratio test. The values of the correlation coefficient were found to be .9925, .9716, and .9583 for the Higuchi, Korsemeyer and Peppas, and Weibull models, respectively.

Peck, Johnson, and Anderson (19) derived a mathematical relationship for the expression of the entire dissolution profile from matrix tablets. An effort was made in the present investigation to derive a similar type of relationship. A linear interactive model was generated using data of percentage drug released at 60, 180, 300, 360, and 420 min from all the 11 batches. The Higuchi model showed an excellent fit to the data set, and hence the square root of time was chosen as an additional independent variable for carrying out multiple linear regression analysis using the actual values. The equation describing the dissolution pattern is as follows:

$$Y = 42.766 - 37.71\,X_1 - 74.21\,X_2 + 7.08\,X_1X_2 + 9.88\,X_1^2 + 48.34\,X_2^2 + 2.33\,\sqrt{t} \quad (4)$$

where Y is the percentage drug dissolved at time t. The R^2 was found to be .9089, indicating a good fit. The F test was found to be significant at $P < .05$. The derived equation may be used for calculating percentage drug release from different batches within the factor space. The data of batch 7 were subjected to an optimization study in Excel®. The results of the optimization study indicated that, if X_1 and X_2 are kept at 0.89:1 and 0.14 ml (actual values), respectively, then all the constraints are satisfied. The batch was fabricated and evaluated. The actual release profile of the optimized batch compared quite well with the predicted release profile obtained from the mathematical equation.

CONCLUSION

This investigation demonstrates the effectiveness of central composite design in the optimization of the preparation of sustained-release diclofenac sodium microspheres. The critical variables are identified and tailored to produce a formulation with desirable characteristics.

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