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COMMUNICATION

Characterization of Polyvinylalcohol Microspheres of Diclofenac Sodium: Application of Statistical Design

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ABSTRACT

Microspheres of polyvinylalcohol (PVA) containing diclofenac sodium were prepared by an emulsion–chemical cross-linking method. A statistical design was used to study the variables that affect the preparation of microspheres and to study the release profile of diclofenac from the microspheres. To account for the drug content, a mass balance study of the process was performed. A high concentration of polyvinylalcohol, a high stirring speed, and a low level of glutaraldehyde were found to be important to obtain spherical and discrete microspheres. The concentration of polyvinylalcohol and the amount of heavy liquid paraffin were found to be critical factors in influencing the t_{50} *value. Almost 98% of the total diclofenac sodium added was accounted for in mass balance studies*.

Key Words: *Diclofenac; Factorial design; Mass balance; Microspheres; Polyvinylalcohol.*

INTRODUCTION

Diclofenac sodium is a potent nonsteroidal anti-inflammatory drug (NSAID) with a plasma half-life of 1.1 $\pm$ 0.2 hr (1). It acts by inhibiting the enzyme cycloxygenase and thereby the synthesis of prostaglandins and thromboxanes. Besides anti-inflammatory action, this results in gastrointestinal disturbances, peptic ulcer, and bleeding in about 20% of patients (2). As microspheres are widely distributed throughout the gastrointestinal tract, a localized high concentration of diclofenac sodium at a particular point may be avoided (3).

To design a new formulation in the field of pharmaceutical dosage forms, it is very important to identify the parameters and variables in the preparation method that may affect the properties of the new dosage form. Statis-

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tical designs can be used for analyzing the influence of different factors on the properties of the system being studied (4). The present study was carried out to explore the potential of statistical design in optimizing the variables affecting the preparation of microspheres and the release profile of diclofenac sodium from polyvinylalcohol microspheres.

MATERIALS

Diclofenac sodium IP (Crystal Pharmaceuticals, Ambala, India), glutaraldehyde (SD Fine Chemicals, Boisar, India), polyvinylalcohol (medium viscosity, 98–99 mol% hydrolysis), and dioctylsodium sulfosuccinate (DOS; Loba Chemie, Bombay, India) were used. All other chemicals were analytical grade.

METHODS

Preparation of Microspheres

A solution of polyvinylalcohol (5–15% w/v) was prepared in distilled water. DOS (0-5% w/v), and glutaraldehyde (2–8 ml of 25% v/v solution) were stirred (500–1500 rpm) in a dispersion medium using a four-blade propeller stirrer. The dispersion medium consisted of light liquid paraffin (20–40 ml), heavy liquid paraffin (0–20 ml), glacial acetic acid (1 ml), and sulfuric acid (1 ml of 10% v/v solution). Polyvinylalcohol solution was dispersed in the agitating dispersion medium, and stirring continued (0.5–2 hr) to obtain wet microspheres. Then, the microspheres were separated by filtration. These microspheres were washed with petroleum ether, followed by triple washing with cold water to remove adhering liquid paraffin and unreacted glutaraldehyde. The wet microspheres were finally dried at 40°C and sieved through a no. 44 sieve to obtain uniform product. The flow diagram of the process is shown in Fig. 1.

Drug Content

Microspheres (200 mg) were treated with methanol overnight. The solution was filtered to remove exhausted microspheres, and the filtrate was analyzed at 283 nm. The drug content was determined using the regression equation: Concentration = (Absorbance − 0.012)/0.035 (r^2 = .997, n = 21).

In Vitro Dissolution Test

Microspheres of the selected batches containing diclofenac sodium (equivalent to 100 mg) were used to fill hard gelatin capsules. A USP basket apparatus (50 rpm) was used to study the release profile of microspheres in phosphate buffer (900 ml, pH 6.2, 37°C). The aliquots withdrawn (5 ml) were filtered, and the concentration of diclofenac sodium was determined spectrophotometrically at 275 nm directly or after appropriate dilution with phosphate buffer.

Accelerated Stability Testing

The microspheres of the best batch were subjected to accelerated stability testing at 45°C and ambient humidity for 6 weeks. The samples were evaluated at the end of 6 weeks for changes in physical characteristics, diclofenac content, and diclofenac release profile.

Statistical Design

Most formulation studies involve the variation of one factor at a time, keeping other factors constant (5). Such an empirical method is acceptable only when factors are independent of one another (6). The factorial designs allow all factors to be varied simultaneously, thus en-

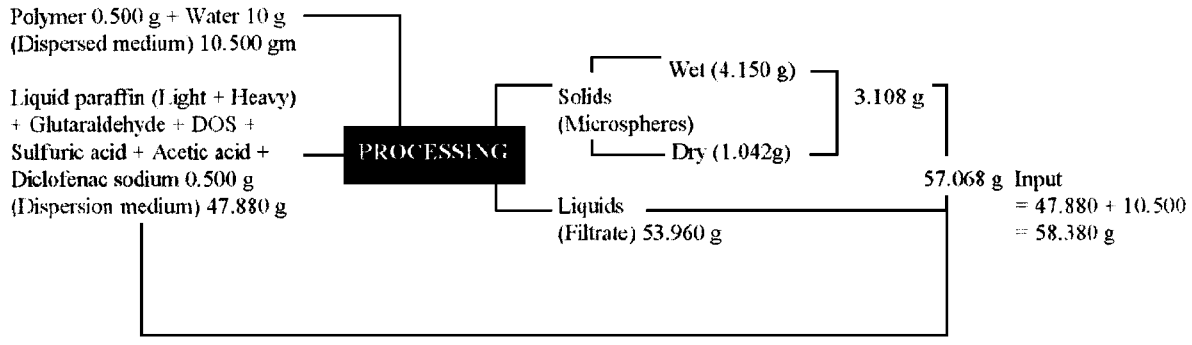

Figure 1. Mass balance of the process for diclofenac-loaded microspheres (batch 2H).

Table 1

Formulation of Different Batches for Preliminary Studies

Batch	Concentration of PVA (%w/v) X1	Stirring Speed X2	Glutaraldehyde (ml) X3
1	5	500	2
2	5	500	8
3	5	1500	2
4	5	1500	8
5	15	500	2
6	15	500	8
7	15	1500	2
8	15	1500	8

abling evaluation of effects of each variable at each level and showing interrelationship among them (7–9).

In the present investigation, a 2^3 factorial design was used sequentially to study the effect of independent process/formulation variables on the preparation of polyvinylalcohol microspheres and to study the effect on the release profile of diclofenac sodium from these microspheres.

Preliminary studies were carried out to identify the process/formulation variables. A 2^3 randomized factorial design was used to study the effect of concentration of polymer, stirring speed, and amount of cross-linking agent on aggregation, shape, and size of microspheres. At each point of these eight batches processed, a 2^3 factorial design was further used to study the effect of cross-linking time, concentration of DOS, and amount of heavy liquid paraffin on the t_{50} value. The formulation of these microspheres is shown in Tables 1 and 2.

Table 2

Formulation of Different Batches for Further Studies

Batch	Cross-Linking Time (hr) X4	Concentration of DOS (%w/v) X5	Heavy Liquid Paraffin (ml) X6
A	0.5	0	0
B	0.5	0	20
C	0.5	5	0
D	0.5	5	20
E	2	0	0
F	2	0	20
G	2	5	0
H	2	5	20

Mass Balance of the Process

To account for the diclofenac content, a mass balance study was undertaken (replicate of batch 2H). Tarred beaker was used for processing, and all the inputs were weighed. The weight of total contents in the beaker was recorded after stirring to account for the loss during processing. The microspheres obtained after stirring were separated by filtration, and the weight of wet microspheres and the filtrate was recorded. Finally, the wet microspheres were dried to a constant weight. Mass balance of the diclofenac-loaded microspheres of batch 2H is depicted in Fig. 1.

RESULTS AND DISCUSSION

The microspheres were prepared by an emulsification–chemical cross-linking method because it is easy and requires simple equipment. Preliminary batches were prepared to obtain discrete microspheres. At high polymer concentration, relatively large microspheres (batch 6) were produced, whereas small irregular microspheres (batch 3) were produced at high stirring speed and low polymer concentration. An increase in the amount of glutaraldehyde did not affect the shape, but increased the size of the microspheres (batch 3 vs. batch 4, batch 7 vs. batch 8). The aggregation of microspheres was reduced at high polymer concentration and high speed (batch 1 vs. batch 8). Large spherical microspheres (batch 8) were obtained at high polymer concentration and large amount of cross-linking agent. A high level of polyvinylalcohol at a high stirring speed and a low level of glutaraldehyde gave discrete spherical microspheres (Table 3).

Table 3

Physical Characteristics of Microspheres

Batch	Aggregation	Shape	Size
1	++++	Spherical	++
2	+++	Spherical	++
3	+++	Not defined	+
4	+++	Not defined	++
5	+++	Spherical	++
6	++	Spherical	+++
7	+	Spherical	++
8	++	spherical	+++

Size parameters: +, up to 100 μm; ++, 100–600 μm; +++, more than 600 μm.

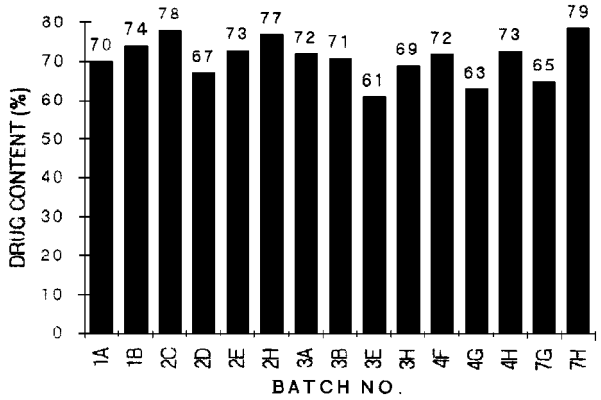

Figure 2. Drug content of selected batches.

At each point of the preliminary studies, a 2^3 factorial design was used to study further the effect of cross-linking time, concentration of DOS, and amount of heavy liquid paraffin on t_{50} values of diclofenac sodium–loaded polyvinylalcohol microspheres. Some of the batches (1D; 4B–4D; 5A–5D, 5F; 6A, 6B, 6D, 6F–H; 7A–7D, 7F; 8A–8D, 8F–8H) could not be obtained. This can be attributed to insufficient cross-linking time. The release profiles of those batches (1C, 1E–1H; 2A, 2B, 2F, 2G; 3C, 3D, 3F, 3G; 4A, 4E; 5E, 5G, 5H; 6C, 6E; 7E; 8E) having diclofenac content less than 60% were not studied because the bulk of microspheres equivalent to 100 mg of diclofenac sodium was too high to be filled in one capsule. The drug content of different batches is shown in Fig. 2.

To determine the mechanism of diclofenac release from polyvinylalcohol microspheres, the dissolution data were fitted to zero-order, first-order, Higuchi, and Hixson-Crowell models (Table 4). The sum of squares of residuals was found to be minimum in the case of the first-order model. Therefore, it was concluded that release of diclofenac sodium from polyvinylalcohol microspheres can be explained by a first-order equation. To determine the magnitude of contribution of different factors on t_{50} values, multiple linear regression analysis was carried out. The values of the factors were coded to facilitate independence of results and to simplify calculations. The results show that the maximum contribution toward t_{50} value is by the concentration of polyvinylalcohol and the amount of heavy liquid paraffin in the dispersion medium. A long cross-linking time (2E, 4G) appears to utilize more hydroxyl groups of polyvinylalcohol for the cross-linking reaction by acetal formation (Table 5). As a result, the penetration of dissolution medium into microspheres decreases, which leads to an increase in the t_{50} value.

The microspheres of batch 7H exhibited maximum drug content and percentage of diclofenac released during the dissolution test. Hence, capsules containing microspheres of batch 7H were compared with market product and were subjected to an accelerated stability test. The shells of the capsules became brittle and were

Table 4

Model Fitting by Method of Least Squares (Batch 7H)

		Zero Order		First Order		Higuchi		Hixson-Crowell	
Time (min)	Observed Drug Released	CCPR	Residual Square	CCPR	Residual Square	CCPR	Residual Square	CCPR	Residual Square
30	0.826	2.184	1.843	2.041	0.0001	0.326	0.250	2.083	0.0004
60	1.647	3.903	5.088	3.908	0.0005	3.630	3.933	3.899	0.0012
90	6.888	5.662	1.603	5.739	0.0002	6.166	0.521	5.692	0.0004
120	8.793	7.314	2.109	7.535	0.0002	8.304	0.239	7.462	0.0005
180	12.604	10.778	3.332	11.026	0.0003	11.980	0.509	10.936	0.0008
240	15.113	14.216	0.803	14.386	7.5×10^{-5}	14.913	0.039	14.323	0.0002
300	17.623	17.654	0.001	17.618	3.6×10^{-8}	17.577	0.002	17.622	4.4×10^{-10}
360	19.293	21.092	3.225	20.728	0.0003	19.985	0.474	20.835	0.0008
ssr/*df*			3.001		0.0003		0.995		0.0007

CCPR = calculated cumulative percentage release; ssr = sum of squares of residuals; *df* = degrees of freedom.
Zero order: $Q = Kt$; $Q = 0.057 * t + 0.465$.
First order: $\ln Q = -Kt + \ln Qo$; $\ln Q = -0.0006 * t + 4.604$.
Higuchi: $Q = K(t)^{0.5}$; $Q = 1.457 * (t)^{0.5} - 7.652$.
Hixson-Crowell: $Q^{1/3} = -Kt + m_o^{1/3}$; $Q^{1/3} = -0.0009 * t + 100^{1/3}$.

Table 5

Multiple Linear Regression of Selected Batches

Batch	X1	X2	X3	X4	X5	X6	t_{50}
1A	−1	−1	−1	−1	−1	−1	1342
1B	−1	−1	−1	−1	−1	1	1364
2C	−1	−1	1	−1	1	−1	3445
2D	−1	−1	1	−1	1	1	1687
2E	−1	−1	1	1	−1	−1	6820
2H	−1	−1	1	1	1	1	3435
3A	−1	1	−1	−1	−1	−1	2270
3B	−1	1	−1	−1	−1	1	3370
3E	−1	1	−1	1	−1	−1	3495
3H	−1	1	−1	1	1	1	3335
4F	−1	1	1	1	−1	1	3415
4G	−1	1	1	1	1	−1	6630
4H	−1	1	1	1	1	1	2113
7H	−1	1	1	1	1	1	1153
Regression output *X* coefficients	650.175	299.584	−683.613	627.843	−294.486	−645.126	Constant = 2454
$r = .772$							
No. of observations = 14	$df = 7$						

X1 = polymer concentration; X2 = stirring speed; X3 = amount of glutaraldehyde; X4 = cross-linking time; X5 = concentration of surfactant; X6 = amount of heavy liquid paraffin.

slightly deformed compared to the capsules stored at room temperature. The diclofenac content of microspheres fell by a marginal 1.43%. There was no significant difference in the release profile of fresh sample and aged sample (Fig. 3). Therefore, it can be concluded that the capsules containing batch 7H are not largely affected by accelerated stability test conditions.

A mass balance study of the process was carried out by random selection of batch 2H to account for the drug content. It was observed that, of the total diclofenac added in batch 2H, 79.8% was present in microspheres, whereas the filtrate contained 18.4% of the diclofenac. Thus, 98.2% of the total diclofenac added was accounted for and 1.8% diclofenac loss was found to be inevitable during the preparation of microspheres.

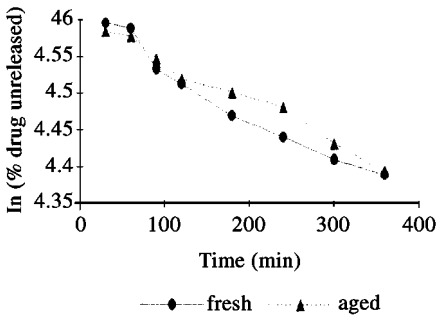

Figure 3. In vitro release profile of batch 7H (fresh and aged).

It can be concluded that, during the preparation of microspheres by an emulsion–chemical cross-linking method, about 2% of the drug is lost. High concentration of polymer, high stirring speed, and low level of cross-linking agent are desirable to obtain microspheres. The release profile of a drug depends on formulation/process variables such as concentration of the polymer and composition of the dispersion medium.

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