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Research Article

Formulation and Optimization of Celecoxib-Loaded Microspheres Using Response Surface Methodology

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Abstract

Purpose: *To employ response surface methodology (RSM) for statistical optimization of formulation factors in the preparation of celecoxib-loaded microspheres.*
Methods: *Celecoxib microspheres were prepared by solvent evaporation method. Biodegradable/biocompatible polymers, Eudragit L-100 and polyvinyl pyrrolidone, were used in the encapsulation procedure. A central composite design employing Stat-Ease design Expert®, version 7.0.3 having a unit value of α was used according to reference protocols to assess the influence of two independent variables (i.e., the concentration of the two polymers used) on four dependent variables (i.e., recovery, encapsulation efficiency and % drug released). The polymers used were Eudragit-L100 (X_1) and polyvinyl pyrrolidone (X_2). The microspheres were characterized for size, shape, recovery (%), entrapment efficiency and drug release.*
Results: *The recovered total weight of microspheres ranged between 49.4 ± 3.1 and 91.1 ± 4.8 %, and it decreased with increase in the concentration of PVP. Entrapment efficiency was in the range of 54.1 ± 2.9 to 95.6 ± 3.7 %, and was also dependent on polymer concentration. The release of celecoxib increased with decrease in Eudragit L-100 concentration and increase in PVP concentration. Higuchi model was the best-fit drug release from all the formulations. Korsemeyer-Peppas release exponent (n) indicates that drug release pattern was non-Fickian diffusion.*
Conclusion: *Using RSM, it is possible to optimize the drug release properties of celecoxib-loaded microspheres. A celecoxib-loaded microsphere formulation with optimum recovery, entrapment efficiency and release behavior was proposed.*

Keywords: *Celecoxib, Eudragit L-100, Polyvinyl pyrrolidone, Response surface methodology, Microspheres.*

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INTRODUCTION

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for the modeling and analysis of problems in which a response of interest is influenced by variables and the objective is to optimize this response [1]. It has been applied to pharmaceutical systems and particulate carriers such as gelispheres, matrices and microspheres. It is necessary to have a clear understanding of how preparation conditions and inherent characteristics of excipients employed in pharmaceutical preparations are influenced by potential interaction between various factors in order to optimize a formulation [2,3].

Formulations based on microspheres provide optimum control of kinetics of drug release from the dosage form [4]. Controlled release preparations are preferred as these produce maximum therapeutic effect with a low risk of adverse effects [5]. Biodegradable polymers degrade within the body as a result of natural biological processes, and therefore, there is no need to eliminate the delivery system after its function is over [6]. Hence, microspheres made with biodegradable polymers, are useful as controlled release (CR) systems. Several methods are being used for the preparation of microspheres both from natural and synthetic polymers [7].

Celecoxib is used for pain, osteoarthritis, rheumatoid arthritis, painful menstruation and to reduce the number of colon and rectal polyps in patients. It is approximately 10 - 20 times more selective for COX-2 inhibition than COX-1 [8-10].

The aim of this study was to prepare an optimized microparticulate formulation of celecoxib employing solvent evaporation technique to elaborate the influence of specific combinations of two polymers, i.e. Eudragit L-100 and polyvinyl pyrrolidone, on the release behavior of the drug. Since RSM with polynomial equations is helpful in the rapid development of an optimum formulation with minimum number of experiments for the investigation of the influence of the independent variables on results, this approach was employed in this study to obtain an optimized microparticulate formulation of celecoxib as well as to assess the effect of specific combinations of two polymers.

EXPRIMENTAL

Materials

Celecoxib (99.8 %) and Eudragit L-100 were donated by IRZA Pharmaceutical, Lahore, Pakistan. Polyvinyl pyrrolidone (PVP) was a gift from Pharma-wise, Lahore, Pakistan, respectively. All other chemicals used - light liquid paraffin (BDH, UK), acetone and n-hexane (Merck, Germany), and Tween 80 (Sigma, USA) - were of analytical grade.

Preparation of microspheres

Microspheres were prepared by solvent evaporation technique. Different ratios of Eudragit L-100/polyvinyl pyrrolidone (PVP) were dissolved in 40 ml acetone using a magnetic stirrer. Celecoxib was dispersed in the polymer solution and stirred for 15 min. The resulting dispersion was mechanically added in a thin stream to a mixture of 360 ml light liquid paraffin, 0.5% Tween-80 and 40 ml n-hexane contained in a 500 ml beaker. Stirring at 700 rpm was continued for 3 h, until the acetone evaporated completely. The microspheres formed were collected by filtration on Whatman filter paper no.1 and washed 4 - 5 times with n-hexane [11]. The product was then air-dried at room temperature for 12 h. Formulations were coded as F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F1, F12 and F13, respectively, as per Table 1. All the batches were prepared in triplicate (n = 3). The experiments were performed in random order.

Table 1: Factor combination, based on experimental design, for microsphere formulations

Coded factor level		Formulation code	Drug/Eudragit/ PVP ratio
X_1	X_2		
-2	0	F1	2:2:5
-1	-1	F2	2:4:3
-1	1	F3	2:4:7
0	-2	F4	2:6:1
0	0	F5	2:6:5
0	2	F6	2:6:9
1	-1	F7	2:8:3
1	1	F8	2:8:7
2	0	F9	2:10:5
0	0	F10	2:6:5
0	0	F11	2:6:5
0	0	F12	2:6:5
0	0	F13	2:6:5

Table 2: Translation of coded levels in actual units

Coded levels	-2	-1	0	1	2
X_1 : Eudragit-L100	2	4	6	8	10
X_2 : PVP	1	3	5	7	9

Experimental design

A central composite design having a unit value of α was applied according to reference protocols [12] to assess the influence of two independent variables (i.e., the concentration of the two polymers used) on three dependent variables [i.e., recovery (Y_1), encapsulation efficiency (Y_2) and percent drug released (Y_3)]. The polymers used were Eudragit-L100 (X_1) and polyvinyl pyrrolidone (X_2). All other formulation and process parameters were kept constant during the study. Stat-Ease design Expert®, version 7.0.3, was employed to generate and evaluate the statistical experimental design and construction of a design matrix with 13 experimental trials. The response variables were evaluated by following second order polynomial model, as in Eq 1

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 \ldots\ldots (1)$$

where, β_o – β_5 represents regression coefficients, X_1 –X_2 the studied variables and Y the measured response with each factor level combination (Table 1). Variable combinations selected on the basis of the experimental design and translation of coded levels in actual units are given in Tables 1 and 2, respectively.

Morphology and size of microspheres

Optical microscope (Erma, Japan) fitted with a digital camera (Yashica, Japan) was used to measure directly the size of 200 microspheres by taking their photographs and then evaluating the mean diameter [13]. Scanning electron microscope (Hitachi, Japan) was employed to determine external morphology by mounting the microspheres on the stubs, applying thin coating of gold and taking the photographs (operating voltage 15 KV and working distance 20 nm) [13].

Recovery of microspheres

After preparation, the microspheres were recovered and dried overnight at room temperature. Recovery is the ratio of the weight of microspheres recovered to the total weight of solid ingredients charged at the beginning of the process, expressed as a percentage [13].

Drug loading

The dried microspheres (50 mg) were dispersed in 10 ml phosphate buffer (pH 6.8); it was placed in an ultrasonic bath for 15 min and then filtered through a 0.22 mm millipore filter (Suzhou Hyford Machinery Company, China). The absorbance of the filtrate was taken at 251 nm using a UV/Vis spectrophotometer (model 1601, Shimadzu, Japan) to determine the amount of celecoxib present in the microspheres. Drug loading was determined as the ratio of the amount of drug in the microspheres to the weight of the microspheres, expressed as a percentage [13].

Entrapment efficiency

Entrapment efficiency can be determined as the ratio of actual drug loading to the theoretical drug loading, expressed as a percentage.

In vitro drug release study

In vitro release studies on the drug-loaded microspheres were carried out both at gastric and intestinal pH, i.e., pH 1.2 and 7.4, respectively [14] using United States Pharmacopeial (USP) paddle method. The microspheres (700 mg) were placed in 900 mL dissolution medium and tested first in HCl buffer (pH 1.2) for 2 h and then in phosphate buffer (pH 7.4) for 12 h. The temperature was maintained at 37.0 ± 0.5 °C and the stirring speed at 100 rpm. Samples (3 mL) were withdrawn and replaced with an equal volume of fresh dissolution medium at regular time intervals, filtered through 0.45 µm nylon disc filter (Suzhou Hyford Machinery Company, China) and analyzed spectrophotometrically (model 1601, Shmadzu, Japan) at 254 nm [14]. The concentration of celecoxib in the samples was determined from a standard calibration) curve. The dissolution studies were carried out in triplicate.

Drug release kinetic analysis

Drug release kinetics indicates the mechanism of drug release from drug delivery systems. Four kinetic models - zero order, first order, Higuchi and Korsemeyer-Peppas represented as Eqs 2 – 5, respectively - were applied to analyze the *in vitro* drug release data to determine the best-fit release model [13].

$$F_t = K_o t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where "F_t" is the fraction of drug released in time "t" and "K_o" is the zero-order release constant.

$$\ln (1 - F) = -K_1 t \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

where "F" is the fraction of drug released in time "t" and "K_1" is the first-order release constant.

$$F_H = K_2 t^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

where "F_H" is the fraction of drug released in time "t" and "K_2" is the Higuchi constant.

$$M_t / M_\infty = K_3 t^n \quad \ldots\ldots\ldots\ldots \quad (5)$$

where "M_t" and "M_∞" are the amount of drug released at time "t" and "∞", respectively, and "n" is the diffusional coefficient. In spherical matrices, if n <0 .43, diffusion is Fickian, 0.45 < n < 0.89, diffusion is non-Fickian, and n > 0.85, case-II (zero order) drug release mechanism dominates [13].

Statistical analysis

One-way ANOVA was applied to determine the significant difference between various values using SPSS version 15.0. The level of significance was set at $p < 0.05$.

RESULTS

Appearance of microspheres

The microspheres were spherical in shape with size range of 10.73 ± 0.97 – 31.29 ± 1.34 µm. They were white in color and free flowing.

Recovery and drug entrapment

The recovered total weight of microspheres ranged between 49.4 ± 3.1 and 91.1 ± 4.8 % and it decreased with increase in PVP content of the formulations (Table 3). Entrapment efficiency was in the range 54.1 ± 2.9 and 95.6 ± 3.7 % and was dependent on the concentration of the polymer. RSM results for response Y_1 (% recovery) is given in Figure 2. The resultant equation for response Y_1 is shown in Eq 6.

$$Y_1 = 85.6481 + 2.1358\ X_1 - 2.4108\ X_2 - 0.2312\ X_1X_2 - 0.0246\ X_1^2 - 0.2454\ X_2^2 \quad \ldots. \quad (6)$$

RSM results for response Y_2 (entrapment efficiency) is given in Figure 2. The resultant equation for response Y_2 is given in Eq 7.

$Y_2 = 37.0947 + 1.7348\ X_1 + 13.2304\ X_2 + 0.0375\ X_1X_2 – 0.1240\ X_1^2 – 0.9022\ X_2^2$ …… (7)

In vitro drug release

Drug release from the microspheres depended mainly on the type and concentration of the polymers used in the formulation. Plots of drug release from the microspheres against time are sown in Figure 1. All the formulations displayed sustained release pattern with 18 – 40 % released at gastric pH in the first 2 h and almost 42 to 93 % released in the next 12 h. The microspheres (F3) containing the highest concentration of PVP showed the fastest drug release with $t_{50\%}$ = 5.37 h, while the slowest release of celecoxib was observed for F4 ($t_{50\%}$ = 10.43 h) which contained a higher level of Eudragit-L100 than PVP. RSM results for response Y_3 (% drug release) is given in Figure 2. The resultant equation for response Y_2 is presented in Eq 8.

$Y_3 = 5.0553 + 0.4851\ X_1 + 2.3141\ X_2 + 0.1531\ X_1X_2 – 1.3954 \times 10^{-2}\ X_1^2 – 0.0302\ X_2^2$ … (8)

Table 3: Recovery (%) and entrapment efficiency (%) data for microsphere formulations

Formulation code	Recovery (%)	Entrapment efficiency (%)
F1	71.1±2.6	81.2±3.7
F2	82.2±3.4	78.4±4.9
F3	50.7±4.3	95.6±5.4
F4	91.1±2.2	54.1±2.5
F5	73.8±5.5	86.7±3.7
F6	49.4±3.6	89.8±4.9
F7	86.9±15.7	76.9±5.3
F8	51.7±3.9	94.7±2.5
F9	76.4±2.3	87.6±3.7
F10	73.0±6.5	87.7±3.8
F11	73.8±3.2	90.0±2.9
F12	73.2±5.4	88.1±5.2
F13	71.9±4.6	84.7±4.3

For all the formulations, i*n vitro* drug release was best fitted to Higuchi model (Table 3) as the highest linear regression was obtained from this model. Korsemeyer-Peppas release exponent (n) data indicate that drug release mechanism was non-Fickian diffusion.

RSM data for various responses (Y_1, Y_2, and Y_3) are given in Figure 2.

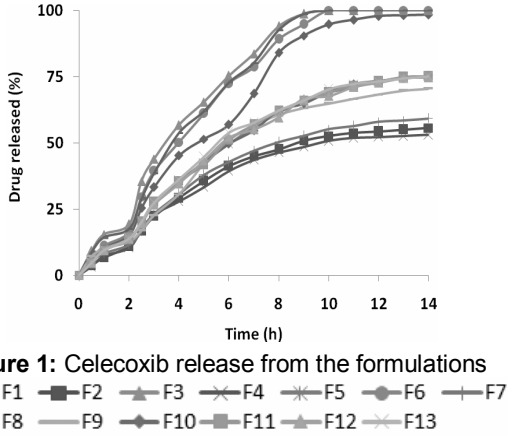

Figure 1: Celecoxib release from the formulations

DISCUSSION

The method used for the preparation of the microspheres successful entrapped celecoxib. Surfactant (Tween 80) used at a concentration of 0.5 %w/v was sufficient to facilitate the production of satisfactory microspheres. Attempts made in preliminary studies to use lower surfactant concentrations failed to yield microspheres but rather an aggregated mass was formed. Stirring rate also influenced the recovery (%). The stirring speed of 700 rpm produced the microspheres of optimum size. By increasing stirring speed above 700 rpm, there was low recovery. This low recovery could be due to the formation of smaller microspheres which were lost during washings process. Decreasing the stirring speed promotes aggregation of the microspheres; and causes materials to adhere to the walls of beaker, thus resulting in low recovery. Similar observations have been reported by Giannola et al [15] and Varshosaz and Keihanfar [16]. Increase in Eudragit L-100 content of the microspheres resulted in the significant ($p <$

Table 4: Release kinetics of microspheres

Formulations	$t_{50\%}$ (h)	Zero order		First order		Higuchi		Korsmeyer–Peppas	
		R^2	K_0	R^2	K_1	R^2	K_H	R^2	N
F1	5.81	0.928	8.60	0.943	0.177	0.909	26.376	0.967	0.748
F2	10.23	0.855	4.986	0.959	0.073	0.945	15.490	0.958	0.637
F3	5.37	0.813	9.310	0.957	0.230	0.933	29.052	0.947	0.602
F4	10.43	0.847	4.793	0.953	0.069	0.939	14.912	0.959	0.626
F5	7.66	0.913	6.524	0.992	0.110	0.934	20.121	0.975	0.697
F6	5.50	0.875	9.099	0.948	0.208	0.920	28.153	0.953	0.672
F7	9.51	0.863	5.260	0.968	0.079	0.939	16.334	0.963	0.640
F8	5.44	0.854	9.193	0.946	0.215	0.922	28.519	0.948	0.649
F9	8.07	0.888	6.534	0.977	0.102	0.919	19.128	0.957	0.686
F10	7.65	0.913	6.937	0.992	0.111	0.934	20.149	0.976	0.698
F11	7.62	0.907	6.560	0.991	0.112	0.934	20.247	0.972	0.691
F12	7.70	0.909	6.494	0.990	0.110	0.932	20.033	0.973	0.696
F13	7.57	0.892	6.604	0.988	0.114	0.932	20.416	0.967	0.677

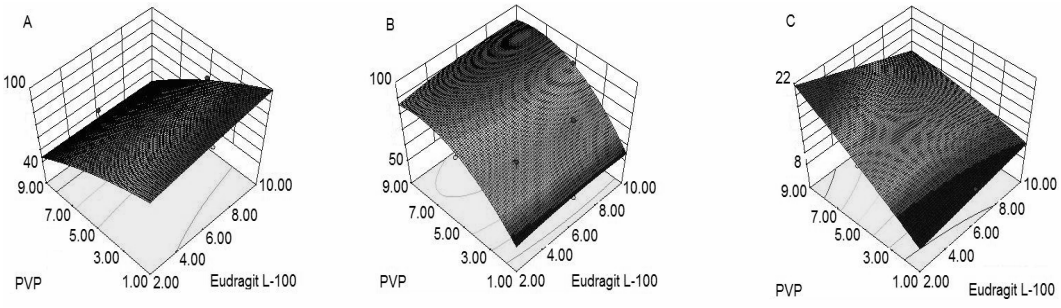

Figure 2: Response surface plots illustrating the influence of polymer concentrations on % recovery (A), entrapment efficiency (B) and drug release (C)

0.05) decrease in entrapment efficiency. This might be as a result of the low drug carrying capacity of the polymer.

Drug release from the microspheres depended mainly on the type and concentration of polymer used in the formulation. Formulation batches F3, F6 and F8 which had a higher concentration of PVP relative to that of Eudragit L-100 showed complete (100 %) release of drug over the 14 h period of the release study. A similar result was observed by Babu *et al* [17] who prepared microspheres using cellulose acetate butyrate and polyvinyl pyrrolidone. PVP is an amorphous polymer and a water-soluble tertiary amine which swiftly dissolves in aqueous environment [17]. It behaves as a strong Lewis base. Being biocompatible, it is widely employed in the fabrication of drug delivery systems. Due to its polar groups, it is an efficient proton acceptor; therefore, it can easily undergo hydrogen bonding with suitable compounds, especially polymers that behave as proton donors. The application of PVP as polymer matrix has been confirmed to possess significant outcomes, including enhancement of drug stability [17]. Thus, celecoxib release was fastest from formulation F3 which contained which contained a relatively higher level of PVP.

The microspheres with the highest concentration of Eudragit L-100 showed slower (10.4 % in the first 2 h) drug release in acidic medium (0.1N HCl); however faster

release (17.5% in the next 2 h) occurred in phosphate buffer (pH 7.4). This implies that Eudragit L-100 provided protection for the microspheres in acidic conditions. Eudragit L-100 is a neutral copolymer of polyethyl-acrylate, methylmethacrylate and trimethyl-ammoniumethylmethacrylate chloride [18]. It is inert to the content of digestive tract, thus does not adversely affect the mucosal lining and is commonly use for the development of oral drug delivery systems [19]. Its degradation is pH dependent, being sparingly soluble in acidic medium due to the presence of acidic groups such as methacrylic acid [18], but soluble in solution medium of pH > 6.0. Therefore, it is widely used for targeted delivery of drugs to the colon [19]. It exhibits excellent swelling as a result of which it can retard drug release and is therefore used to achieve prolonged drug release. This probably accounts for the very slow release of celecoxib from formulation F4 which contained a relatively high concentration of Eudragit L-100.

Higuchi was best-fit model for all the dissolution data indicating that drug was diffusion-controlled. Based on the Korsemeyer-Peppas release exponent (n), drug release was non-Fickian diffusion. Non-Fickian release, also termed as anomalous release, is a combination of two mechanisms, i.e., erosion and diffusion operating simultaneously [20].

The outcome of the optimization studies indicate that the optimum formulation (F10) is the one containing Eudragit-L100/PVP in a ratio of 6:5. Its recovery and entrapment efficiency (%) were 73.0 ± 6.5 and 87.7 ± 3.8 %, respectively. It also showed prolonged release of drug (approx. 75 % in 14 h).

Furthermore, regression equation was a unique model for the description of responses of the experimental parameters employed in the production of the microcapsules.

CONCLUSION

The results of this study demonstrate that RSM is a valuable optimization tool for selecting suitable variables. Using RSM, an ptimized formulation of celecoxib-loaded microspheres was achieved in terms of % recovery, entrapment efficiency and release characteristics.

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