PREPARATION AND IN VITRO EVALUATION OF DICLOFENAC SODIUM LOADED SUSTAINED RELEASE PELLETS

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ABSTRACT

The objective of this investigation was to prepare sustained release (SR) pellets of Diclofenac Sodium (DFS), a widely used non-steroidal anti inflammatory drug (NSAID) using Taguchi robust design. Drug layered pellets were prepared using solution layering technique in conventional coating pan. Ethylcellulose (EC) and Hydroxy Propyl Methyl Cellulose (HPMC) were used for sustained release polymer coating on drug layered pellets. Polymer coating was applied in a fluid bed coater. The effect of three independent variables used in fluid bed coating viz., EC: HPMC ratio, atomizing air pressure, and spray rate on the response variable (percentage drug released in 120 minutes from pellets-Y_{120}) was studied using Taguchi orthogonal array (L_4) design with an intention to rank their effect on the response variable, standard deviations, and signal to noise ratios. The results indicated that among the three variables studied, EC: HPMC is the most important (and principal) factor influencing the Y_{120}, standard deviations, and signal to noise ratios. In vitro release studies indicated that drug release could be prolonged up to 20 hours and zero order release approximation might be achieved by pelletization. The data obtained in release rate studies was fitting well to nonlinear kinetic models as well. The shelf life of selected pellet formulation F4 was predicted to be 3.0 years.

KEY WORDS : Sustained release, Diclofenac Sodium, Taguchi robust design, Independent variables, Response variable, Signal to noise ratios.

1.INTRODUCTION

The single most important factor responsible for proliferation of pelletized products is the popularity of controlled-release technology. Controlled-release pellets are useful to sustain the action of drugs over an extended period of time (Ghebre,1989). Diclofenac Sodium (DFS), like majority of NSAIDs is ulcerogenic. Also, its short biological half-life of 1–2 h necessitates multiple dosing for maintaining therapeutic effect throughout the day. Due to these adverse effects and its short biological half life, DFS is an ideal candidate for prolonged release preparations (Bertocchi,2005).

Research and development in the academic or industrial context makes extensive use of experimentation to gain a better understanding of a process or system under study. The methodology of Design of Experiments (DoE) provides proven strategies and methods of experimental design for performing and analyzing test series in a systematic and efficient way. All experimental parameters are varied in an intelligent and balanced fashion so that a maximum of information is gained from the analysis of the experimental results. In most cases, the time and money spent on the experimental investigation will be greatly reduced (Montgomery,1976).

Conventional statistical experimental design can determine the optimum condition on the basis of the measured values of the characteristic properties; while Taguchi's experimental design (also known as a robust parameter design) does this on the basis of the variability of characteristic properties. In other words, the Taguchi method can determine the experimental condition having the least variability as the optimum condition (Taguchi,1993).

In the present investigation, DFS layered pellets were prepared in a coating pan and polymer coating was applied on the layered pellets in a fluid bed coater to sustain the release of the drug. In this process the effect of three independent variables viz., EC: HPMC ratio, atomizing air pressure, and spray rate on the response variable (percentage drug released in 120 minutes from pellets-Y_{120}) was studied using Taguchi orthogonal array (L_4) design with an intention to rank their effect on the response variable, standard deviations, and signal to noise ratios.

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2.MATERIALS AND METHODS

Materials

Diclofenac Sodium (gift sample from Amoli Organics, Ahmadabad), Ethylcellulose (EC) (MERCK), Hydroxy Propyl Methyl Cellulose (HPMC) (MERCK), Povidone (MERCK), Stearic acid (Loba-Chemie), Isopropyl alcohol (Amoli Organics), Methylene chloride (Amoli Organics), Talc , IPA were used. All other chemicals and reagents used were of analytical grade.

Preparation of DFS Layered Pellets

DFS layering on non-pareil seeds was done in conventional coating pan (18″-Jhansi Laxmi Enterprises, Hyderabad). The formula for drug loading on pellets is shown in Table1. And the operating conditions employed for coating are shown in Table 2.

Table 1. Formula for Drug Loading

Ingredients	Size	Purpose in Formula
Non-pariel seeds (Celphere 507)	# 20/30	Starter seeds
Povidone solution in Isopropyl alcohol	--	Binder solution
DFS	#200	Active
Talc	#200	Anti-tacking agent

Table 2. Operating Conditions for Pan Coating

Item	Conditions
Machine	Conventional coating pan
Speed	40 rpm
Charge	1000 g
Spray gun	Art master
Gun position	15 cm from the pellet bed surface
Spray rate	1 $ml.min^{-1}$
Pressure	40 psi
Supply air temperature	70-75° C
Pellet bed temperature	40-45° C

A weighed quantity (1000 g) of non-pareil seeds (NPS) of approximately 20 /30 mesh was charged into coating pan and DFS solution (20% w/v in 95% isopropyl alcohol) containing 2% w/v povidone as binder was sprayed over the cascading NPS. Hot air was blown onto the cascading pellets to evaporate the solvent. Drug-loaded pellets were dried in an oven at 50°C for 24 hr.

Polymer Coating on Drug Layered Pellets

Polymer coating (Table 3) was applied on the prepared drug containing (layered) pellets.

Table 3. Formula for Polymer Coating on Drug Layered Pellets (Batch size: 250 g)

Ingredients	Quantity	Purpose in Formula
Hypromellose	10 g (level 1); 15 g (level 2)	Coating material
Ethylcellulose	10 g (level 1); 15g (level 2)	Coating material
Stearic acid	10g	Plasticizer
Isopropyl alcohol	QS	Solvent
Methylene chloride	QS	Solvent

The coating process was carried out using the fluid bed coater. In fluid bed coater, process variables include inlet air temperature (°C), outlet air temperature (°C), inlet air flow rate ($m^3.min^{-1}$), feed rate ($g.min^{-1}$), spray air flow rate ($l.min^{-1}$), and atomizing air pressure (bar). In the present investigation, the effect of two process variables: atomizing air pressure and feed rate and one formulation variable: EC: HPMC ratio on the quality of SR pellets was studied. For this purpose, Taguchi robust design was employed selecting EC: HPMC ratio, atomizing air pressure, and feed rate as independent variables (predictors) and percentage drug released in 120 minutes (Y_{120}) as dependent variable (response).

The coded and actual values of the independent variables selected in Taguchi design are shown in the Table 4.

Table 4. Coded and Actual Values of the Independent Variables

Variable	Level 1	Level 2
A: EC: HPMC ratio	1:1	2:1
B: Atomizing air pressure (bar)	1	2
C: Feed rate ($g.min^{-1}$)	1.0	2.0

Taguchi orthogonal array (L_4) design was used to select the parameters (predictors) having the most principal influence on the Y_{120} of DFS loaded pellets. L and subscript 4 mean Latin square and the number of experiments, respectively. The purpose was to identify and rank the parameters (predictors), using signal to noise ratio as smaller the better option (Taguchi,1993) in the design. Signal to noise ratio is a measure of robustness which can be used to identify control factor settings that minimize effect of noise on the response. As the effect of noise is reduced on product or process

variability the quality of product is improved. The experimental design matrix is shown in Table 5.

Table 5. L_4 Taguchi Experimental Design Matrix (4 runs)

Run Order	Formulation	EC: HPMC ratio	Atomizing Air Pressure	Feed Rate
1	F1	1	1	1
2	F2	1	2	2
3	F3	2	1	2
4	F4	2	2	1

* **1** means level 1 and **2** means level 2 (see Table 4)

A mass of 250 g of pellets was placed in the fluid bed coater. Processing parameters, inlet air temperature of 50°C, outlet air temperature of 22°C, inlet air flow rate of 0.28 $m^3.min^{-1}$, and spray air flow rate of 60 $l.min^{-1}$ were kept constant. These values were chosen after preliminary testing. The variables shown in Table 5 were varied according to the Taguchi Experimental Design Matrix. The polymer solution was sprayed on to the pellets. The coated pellets were collected.

Characterization of Pellets

Drug Content: The accurately weighed thoroughly ground DFS loaded pellets were taken into 100 ml volumetric flask; 5 ml of methanol was added and mixed thoroughly to dissolve the coat. To this 15 ml of pH 6.8 buffer was added and the resulting solution was heated on water bath to evaporate the methanol. The solution was made up to volume and was assayed after appropriate dilution by measuring the absorbance at 276 nm in Shimadzu UV- 2550 UV/Visible Spectrophotometer. The drug content was estimated in triplicate.

Loading Efficiency: Loading efficiency was calculated using the following formula:

Loading efficiency = (estimated percentage drug content/theoretical percentage drug content) × 100.

Yield: The percentage yield of pellets was calculated using the following formula:

% yield= weight of pellets (g)/initial weight of DFS (g) + initial weight of excipients (g) × 100

Morphology and Size Distribution: The morphology of pellets was evaluated by the scanning electron microscopy (SEM) (JSM-6510LV).

Particle size range and distribution of microspheres were determined using US standard sieves.

Measurement of Micromeritic and Pharmacotechnical Properties of Pellets

The angle of repose of pellets was determined by the fixed- funnel and free-standing cone method. Hausner ratio and Carr's Index were determined from bulk density measurements (Amareshwar,2010).

Friability: Friability was measured by a Friability tester USP 23 (Electrolab, India). Briefly, about 10 gram of pellets were accurately weighed, placed in the drum of the friability tester, and rotated 100 times. Thus, they were recovered, and reweighed after having removed from the friability tester. The friability was expressed as percentage of mass loss with respect to the initial mass.

Sphericity: Ten pellets were observed by optical microscopy, fitted with a camera lucida connected to a grabbing board. The size and shape descriptors characterizing each particle as in its silhouette were calculated. Two parameters, the silhouette breadth (B, smallest dimension) and length (L, largest dimension), were noted, and the degree of sphericity (Xinde,2007) ø is defined as: $ø = \frac{L}{B}$

Release Rate Studies

Pellets equivalent to 100 mg of DFS were filled in hard gelatin capsules and were evaluated for in-vitro dissolution studies. The study was carried out in USP XXII basket apparatus at a rotational speed of 50 rpm at 37 ± 0.5°C in 900 ml phosphate buffer (pH 6.8). Samples (10 ml) were withdrawn at regular time intervals and filtered through 0.45 µm membrane filter. The drug content was determined in the filtrate at 276nm either directly or after appropriate dilution with the dissolution medium.

To find out the kinetics and mechanism of drug release, data obtained from release rate studies was fitted to both linear and non-linear kinetic models (Zhang,2010) (Table 6).

Table 6. Fitting of Data from Release Rate Studies to Linear and Non-Linear Models

Linear Models		Non- linear Models	
Zero-order	$F = k_0.t$	Gompertz	$F = F_{max}.e^{-e^{-k.(t-\gamma)}}$
First-order	$F = 100.(1-e^{-k_1.t})$	Logistic	$F = F_{max}.\frac{1}{1+e^{-k.(t-\gamma)}}$
Higuchi	$F = k_H.t^{0.5}$	Quadratic	$F = 100.(k_1.t^2 + k_2.t)$
Korsmeyer-Peppas	$F = k_{KP}.t^n$	Weibull	$F = F_{max}.(1-e^{-\frac{t^\beta}{a}})$

F denotes fraction of the drug released at time t; k stands for release rate constant in respective models; n indicates the exponent- If the n value is 0.5 or less, the release mechanism follows Fickian diffusion, and the values $0.5 < n < 1$ indicate a non-Fickian release (anomalous/zero order release). The drug release follows zero-order and case-II transport if the n value is 1. For the values of n higher than 1, the mechanism of drug release is regarded as super case-II transport. F_{max} is the maximum fraction of the drug released at infinite time; k stands for release rate constant in respective models; á, â, and ã denote scale factors in respective models. The criteria considered for selecting the best fit model were: the adjusted coefficient of determination ($R^2_{adjusted}$) and standard error of mean (SEM) (Costa and Sousa,2001).

To characterizing drug release curve, area under the release curve (AUC), mean residence time of the drug substance molecules in the dosage form (MRT), mean dissolution time (MDT), and dissolution efficiency up to 120 minutes (DE_{120}) were also calculated (Zhang,2010).

Further, data obtained from release rate studies was fitted for the following probability distributions using Anderson-Darling test statistic (De,2006) as the criterion: normal, log-normal, exponential, Weibull, logistic, and log logistic.

Shelf Life Prediction

For the purpose of shelf life prediction, the selected formulation (F4) was tested for potency (drug content) by testing at 0, 6, 12, and 18 months. The resultant data was subjected to linear regression and linear regression line was drawn with the potency data. The lower and upper 95% confidence lines were also graphed. The accepted definition of shelf life time is the x-axis coordinate for the intersection of the lower 95% confidence line with 90% drug activity (Guidance for submitting,1987).

3.RESULTS AND DISCUSSION

For pelletization a powder layering technique was used. Initially drug layering on non-pareil seeds was done in a coating pan. On the resulting drug layered pellets, polymer coating of EC and HPMC was applied in a fluid bed coater. Taguchi orthogonal array (L_4) design was used to select the parameters (predictors) having the most principal influence on the Y_{120} of DFS loaded pellets. Figs. 1-3 show main effects plots of means of response variable, signal to noise (SN) ratios, and standard deviations respectively. Response Tables for signal to noise ratios, means of Y_{120}, and Standard Deviations are summarized in Table 7.

Figs. 1-3 reveal that changing the three variables from level 1 to level 2 decrease Y_{120} values, SN ratios, and standard deviations. Therefore it may be concluded that in order to get optimized pellet formulation all the three variables may be fixed at level 2.

Further, Table 7 reveals the following:

- The three variables influence the SN ratios in the order:
  EC: HPMC ratio > Atomizing air pressure > Feed rate
- The three variables influence the Y_{120} values in the order:
  EC: HPMC ratio > Atomizing air pressure > Feed rate
- The three variables influence the standard deviations in the order:
  EC: HPMC ratio > Atomizing air pressure = Feed rate

On the whole, it may be concluded that, among the three variables selected in the present investigation, EC: HPMC ratio is the most important (and principal) variable influencing the Y_{120} (and hence, quality) of pellets.

Four formulations F1-F4 were prepared (Table 5). To characterize the pellets, three parameters were calculated: the drug content, the loading efficiency, and the weight yield. Low s.d values in the mean percent drug content ensured uniformity of drug content in each batch of pellets. Also, loading efficiency and weight yield are satisfactory (Table 8). SEM picture of the selected formulation F4 is shown in Fig.4 which indicates spherical nature and uniform coating of the pellets.

Angle of repose, Hausner ratio and Carr's Index values indicate good flow characteristics of pellets (Amareshwar,2010) (Table 9). Low friability values (Table 9) indicate that pellets have sufficient mechanical strength. High sphericity values (Table 9) indicate that pellets are of uniform coat thickness and have good flow properties. The size of pellets ranged from 704 to 794μm.

The release profile of pellet formulations is shown in Fig.5. The differences in DFS release rate from pellets may be attributed to varying coat thickness. The

calculated values of Dissolution Efficiency upto 120 minutes (DE_{120}), Mean Dissolution Time (MDT), Mean Residence Time (MRT), Area under the Release Curve (AUC), and T_{50} values were calculated and shown in Table 10. The differences of these parameters may be attributed to varying coat thickness.

When the data from release rate studies was fitted to linear models (Table 11), zero order model was found to be the best fit model. When the data was fitted to non-linear models (Table 11), it was observed that they could also be used to fit the data. This is in agreement with the literature report (Costa and Sousa,2001). Among the probability distributions verified, it was observed that normal distribution fitted well (based on high value of Anderson-Darling test statistic for normal distribution).

For expiry dating the graph between time (months) and potency (%) was plotted (Fig.6). It was found that the shelf life of F4 was 35.19 months (~ 3.0 years) which is satisfactory.

4.CONCLUSIONS

SR pellets of DFS were successfully prepared using Taguchi experimental design. It was observed that EC: HPMC ratio is the most important (and principal) variable influencing the Y_{120} (and hence, quality) of pellets. The data obtained from release rate studies followed normal distribution. Drug release from pellets could be extended up to 20 hours. All the pellet formulations have satisfactory drug content and possess satisfactory pharmacotechnical properties. Both linear and non-linear models are suitable to fit the data obtained from release rate studies. The calculated shelf life of selected pellet formulation F4 was approximately 3 years.

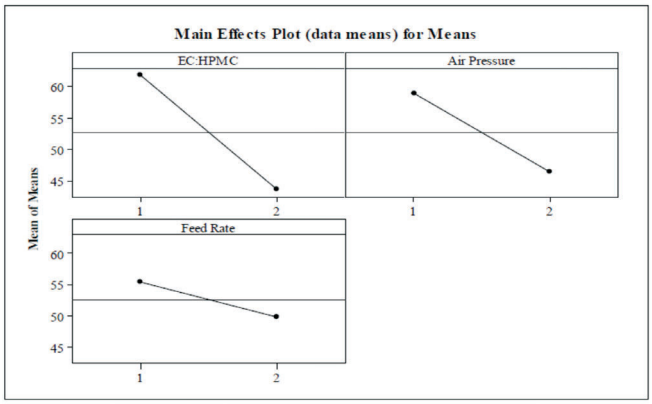

Fig.1. Graphical Representation of Main Effects (Y_{120}) in Taguchi DoE

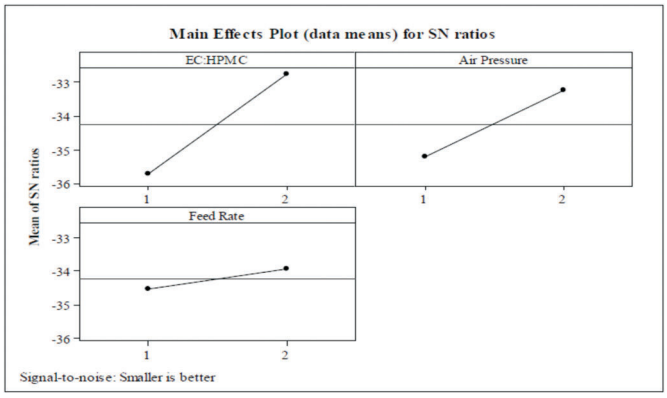

Fig.2. Graphical Representation of Main Effects (SN Ratios) in Taguchi DoE

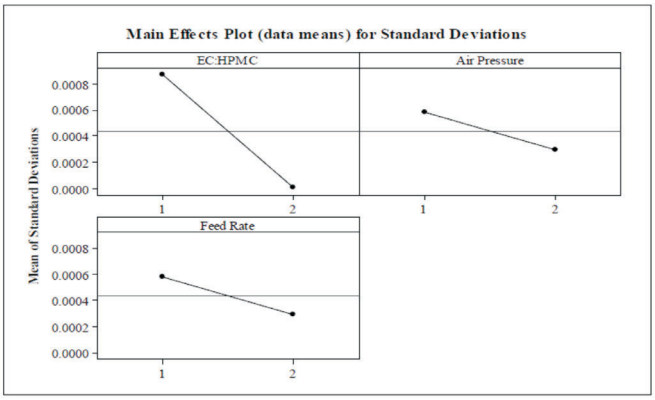

Fig.3. Graphical Representation of Main Effects (Standard Deviations) in Taguchi DoE

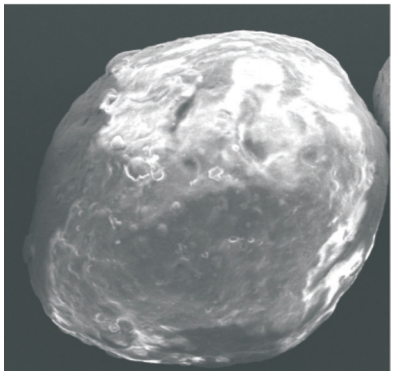

Fig.4. SEM picture of the selected formulation F4

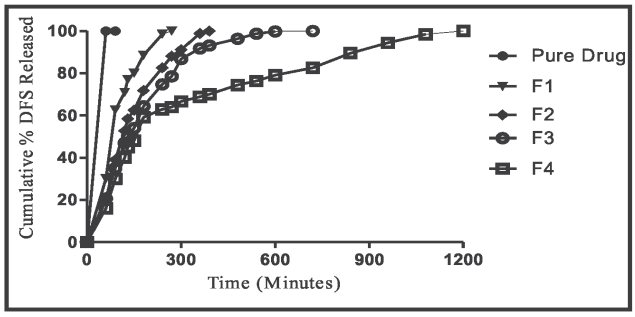

Fig.5. Release Rate Profiles of DFS from Pellets

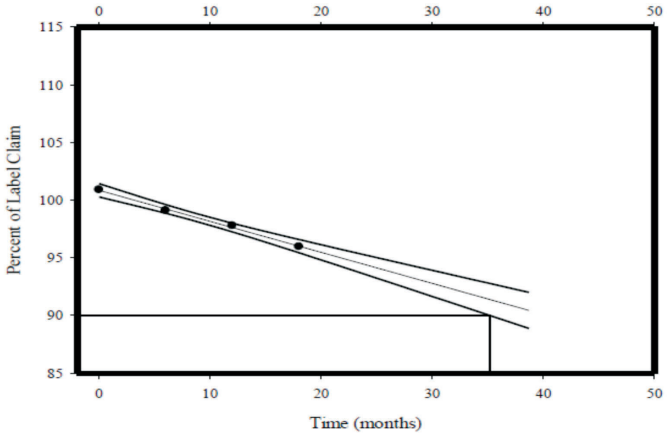

Fig.6. Prediction of Shelf Life of Pellet Formulation F4

Table 7. Response Tables for signal to noise ratios, means of Y_{120}, and Standard Deviations

Response Table for Signal to Noise Ratios
Smaller is better

Level	EC:HPMC	Air Pressure	Feed Rate
1	-35.73	-35.23	-34.53
2	-32.74	-33.24	-33.94
Delta	2.99	1.99	0.59
Rank	1	2	3

Response Table for Means

Level	EC:HPMC	Air Pressure	Feed Rate
1	61.83	58.97	55.47
2	43.50	46.36	49.86
Delta	18.33	12.61	5.61
Rank	1	2	3

Response Table for Standard Deviations

Level	EC:HPMC	Air Pressure	Feed Rate
1	0.000866	0.000577	0.000577
2	0.000000	0.000289	0.000289
Delta	0.000866	0.000289	0.000289
Rank	1	2.5	2.5

Table 8. Drug Content, Loading Efficiency, and Yield of Pellets

Formulation	Drug Content		Loading Efficiency (%)	Weight Yield (%)
	Theoretical	Estimated		
F1	100.00	98.23 (0.48)	98.23	95.13
F2	100.00	99.15 (0.34)	99.15	95.34
F3	100.00	99.46 (0.76)	99.46	97.23
F4	100.00	99.45 (0.23)	99.45	97.45

*Values in parentheses indicate standard deviation values

Table 9. Flow, Micromeritic and Pharmacotechnical Properties of Pellets

Formulation	Angle of Repose	Hausner Ratio	Carr's Index	Size µm	Friability %	Sphericity
F1	20.15° (0.12)	1.07 (0.34)	06.37 (0.15)	704 (0.16)	0.07 (0.17)	0.96 (0.15)
F2	21.43° (0.25)	1.07 (0.26)	06.12 (0.18)	737 (0.45)	0.04 (0.19)	0.97 (0.64)
F3	20.45° (0.27)	1.07 (0.29)	06.78 (0.31)	773 (0.41)	0.03 (0.27)	0.96 (0.51)
F4	21.29° (0.11)	1.06 (0.49)	06.05 (0.39)	794 (0.17)	0.09 (0.29)	0.98 (0.63)

* Values in parentheses indicate standard deviations values

Table 10. Dissolution Parameters of Pellets

Formulation	DE_{120} (%)	MDT (min)	MRT (min)	AUC (%.min)	T_{50} (min)
F1	58.4 (0.19)	50.10 (0.40)	25.25 (0.17)	8000 (0.66)	53.98 (0.56)
F2	50.4 (0.25)	62.45 (0.73)	38.89 (0.34)	11755 (0.61)	64.13 (0.19)
F3	35.8 (0.81)	92.79 (0.17)	63.38 (0.79)	17722 (0.48)	89.27 (0.91)
F4	23.9 (0.27)	137.88 (0.47)	99.03 (0.75)	25212 (0.32)	135.48 (0.77)

*Values in parentheses indicate standard deviation values

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