

Preparation and Evaluation of Sustained Release Colon Targeted Micropellets of Lornoxicam

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ABSTRACT

The aim of the present work was to develop and evaluate sustained release colon targeted micropellets of lornoxicam in order to achieve release of the drug at colon which could result in enhanced local absorption and thereby improved bio-availability. The present worker prepared micropellets of lornoxicam in nine batches using pure drug, DCP, PVPK-30 and different ratios of HPMCK-4M taking into account the direct pelletization technique. Resulted micropellets were filled in capsules and coated with eudragit S-100 to achieve colon targeted release. Compatibility studies were by using FTIR and TLC methods. Particle size determination of micronized lornoxicam was performed by SEM which revealed that the mean particle diameter was in the range of 104-263 μ m while percentage yield was in the range of 88.4-98.4%. Various pre-formulation physiochemical parameters of formulation blends were evaluated such as bulk density, tapped density, carr's index, hausner's ratio and angle of repose and coating durability test for coated capsules (passed). The optimized batch was compared for its release profile in pH 7.4 phosphate buffers and simulated colonic fluid,

which were found to be 94.64% and 90.58% respectively. The actual responses were in accordance with the predicted values which showed validity of the model. There were no physical and chemical changes occurred in accelerated stability of tablets during three months study.

Key words: Lornoxicam, Sustained drug delivery system, Colon targeting, Micropellets, HPMCK-4M

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INTRODUCTION

The most convenient route for drug administration is the oral and important method for systemic effects. Availability of the oral drug delivery system in the market is about 90% having more advantages due to ease of administration and higher patient acceptance.¹ In chronic therapies, where repeated administration is required this is the preferred route for drug administration. In addition, greater convenience, less pain, higher compliance, reduced risk of cross infection and needle stick injuries are the added advantages for oral delivery over other routes of administration. Hence, oral drug delivery systems continue to dominate more than fifty percent market share.²

During the few years there has been interest in developing site specific formulations for targeting drug delivery to the colon. In colon both local and systemic drug delivery takes place. Local drug delivery system contains topical treatment of disease associated with the colon such as ulcerative colitis, amoebiasis, colon cancer and crohn's disease. Treatment might be more effective if the drug substances were targeted directly on the site of action in the colon. Lower doses might be adequate and, if so, systemic side effects might be reduced.³ Colon drug delivery is not only beneficial for the oral delivery of proteins and peptide drugs which are degraded by digestive enzymes of stomach and small intestine also for the delivery of low molecular weight compounds used to treat diseases associated with the colon or large intestine. The colon has a long retention time and highly beneficial to agents that enhance the absorption of poorly absorbed drugs. Drugs specific targeting to the colon has several therapeutic advantages.⁴ Controlled release drug delivery system can be a major advance towards solving problems concerning targeting of drug to specific organ or tissue and controlling the rate of drug delivery to the targeted tissue. Colon is associated with a number of inflammatory bowel diseases (IBD) which consists of a group of diseases in which the

intestine becomes inflamed.⁵

The present worker prepared and evaluated sustained release colon targeted micropellets of lornoxicam for enhance absorption of the drug from the colonic site and produce sustained pharmacological activity with reduced dosing frequency and increase bioavailability. The statistical analysis for different models were performed by ANOVA. Full and reduced models for particle size and % drug release were obtained. The finally optimized batch of formulation (F7) was subjected to release profile studies in pH 7.4 phosphate buffer and simulated colonic fluid. The actual responses showed validity of the model. The optimized formulation was evaluated for stability studies which showed the dosage form are stable with enhanced bioavailability pertaining to inference drawn on *in-vitro* drug release profile.

MATERIALS AND METHODS

Materials

Lornoxicam purchased from Hetero Pharmaceuticals Ltd, India. Various excipients like HPMC-K4M, PVPK-30 were obtained from Rankem India whereas eudragits-100 from Evonik Industry, India and DCP from R.K. Enterprises, India. Deionized distilled water was used in this entire experiment. Others chemicals of analytical grade were used.

METHOD

Drug and Polymer Compatibility Studies

UV Spectrophotometric analysis of lornoxicam and determination of λ_{max} of the drug

Phosphate buffer (pH 6.8) was selected as solvent for analysis (Labtronics LT2900, India). Phosphate buffer (pH 6.8) containing different concentrations i.e. 5, 10, 15, 20 and 25 µg/ml, of the drug was taken and the overlain spectra (absorbance Vs concentration) was obtained for determining the reproducibility of λ max and validation of the process.⁶⁻⁷

Fourier-transformed infrared (FTIR) spectroscopy

The drug-polymer compatibility study was performed by FTIR (Shimadzu IR Affinity-1) spectrophotometer. In mortar pestle drug with different additives and potassium bromide (KBr) was ground into a fine powder. Samples were prepared in KBr discs (2 mg sample in 200 mg KBr) then compressed into a KBr disc with a hydraulic press at a pressure of 75 Kg/cm². Each KBr disc was scanned 45 times at a resolution of 2 cm⁻¹ and characteristic peaks were recorded⁸⁻⁹ IR spectra of drug polymer combinations with DCP, PVPK-30 and HPMC-K4M were obtained. The compatibility was studied by comparing peaks of the drug alone with that obtained in different combinations (with polymers).

Thin layer chromatography (TLC)

The drug polymer compatibility study was also performed by densitometric TLC evaluation using UV chamber at 265 nm wavelength. The spots of drug and different polymers were obtained in the pre-coated silica gel 60 F₂₅₄. Toluene: Methanolic NaOH: Glacial acetic acid in the ratio of (7:2:1.5 V/V) were used as mobile phase.¹⁰

Preparation of Lornoxicam Micropellets

Powdered drug of appropriate quantity was mixed and moistened with the binder solution in IPA. The powder bed was set into centrifugal motion at different rotational speed (100, 200 and 300 rpm) using disc pelletizer. This results in the formation of round agglomerates to produce uniform and dense pellets. The moist pellets were subsequently dried in the tray dryer and collected.¹¹ The formulation was divided into nine batches prepared with different ratios of suitably chosen polymers as depicted in the Table 1.

Evaluation of Micropellets

The physical properties of prepared micropellets was evaluated for particle size analysis, percentage yield, bulk density, tapped density, carr's index, hausner's ratio, angle of repose.

Scanning Electron Microscopy (SEM)

Particle morphology of the micronized lornoxicam was determined by using a scanning electron microscopy (SEM), Model Quanta FEI 200F.

Content Uniformity Test

Content of 10 capsules of known amount were taken into ten separate 50 mL volumetric flask and 20 mL of methanol was added to each. The mixture was shaken for about 30 min. Flasks were kept in hot water bath to dissolve the lornoxicam and methanol was added to make up the

volume and mixed well. The solution was filtered through syringe filter #0.22 µm. 1 ml was filtrate of this solution was pipetted out and diluted with 10 ml of phosphate buffer (pH 6.8) solution. The resulting solution from each sample was measured at 376 nm and the drug content was determined.¹²

Coating of Capsules

The coating of capsule was performed by dip coating method. A polymer solution containing 10% w/v eudragit S100 in Isopropyl alcohol (IPA) and PEG-400 as a plasticizer (10% w/w based on the polymer) used for the coating. The nine coated batches were named as F1 - F9.

Coating Durability Test

Coating durability of coated formulations was performed in the pH 1.2 and 6.8 buffers and simulated gastrointestinal fluids. The test was done for 2 hours in pH 1.2 hydrochloric acid buffer, then pH 6.8 phosphate buffer for 4 hours and finally examined in pH 7.4 phosphate buffer.

In vitro dissolution study

The dissolution process was carried out by using USP II rotating Basket type dissolution apparatus Electrolab, Mumbai (USP-TDT 06L). The micropellets loaded drug (equivalent to 40 mg of lornoxicam) were put into the basket rotated at a constant speed at 75 rpm and the temperature of the dissolution medium maintained at 37 ± 0.5 °C. The release profile of lornoxicam from micropellets was examined in three different buffer solutions resembling with various physiological environment of GI-tract. The dissolution test was performed using 750 ml of 0.1 N HCl (pH 1.2) for 2 h at 37 ± 0.5 °C and then 250 ml of 0.2 M tri sodium phosphate (Na₃PO₄.12H₂O) was added and pH adjusted to 6.8 as described in the USP. Dissolution test was carried out for a period of 12 hr in pH 7.4 phosphate buffers. 5 ml of the sample was withdrawn at regular intervals and replaced with the same volume of fresh dissolution medium. The withdrawn samples were filtered through a 0.22 µm membrane filter and after appropriate dilution, absorbances were recorded at 376nm spectrophotometrically (Labtronics-2900). Finally, corresponding drug contents in the samples were calculated from the calibration curve to determine the drug release pattern.¹³⁻¹⁴

Drug Release Kinetic Studies

To analyze the *in-vitro* drug release, the obtained data were fitted in to the zero order, first order, higuchi and korsmeyer-peppas model. For each plot, the regression coefficients were calculated.¹⁵⁻¹⁶

Full Factorial Design

A 3³ randomized full factorial design was used to optimize the variables in the present study. In this design 2 factors were evaluated, each at 3 levels and experimental trials were performed for all 9 possible combinations. The amounts (5.00, 7.50 & 10.00 mg) of polymer (X₁), and rotating speeds (100, 200 and 300 rpm) of pelletizer (X₂) was selected as independent variables. The particle size and percentage of drug release were selected as dependent variables.

Table 1: Formulation design of micropellets (quantities in gm)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lornoxicam	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DCP	14.75	12.25	9.75	14.75	12.25	9.75	14.75	12.25	9.75
PVPK-30	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HPMC-K4M	5.00	7.50	10.00	5.00	7.50	10.00	5.00	7.50	10.00
IPA-99%	qs								

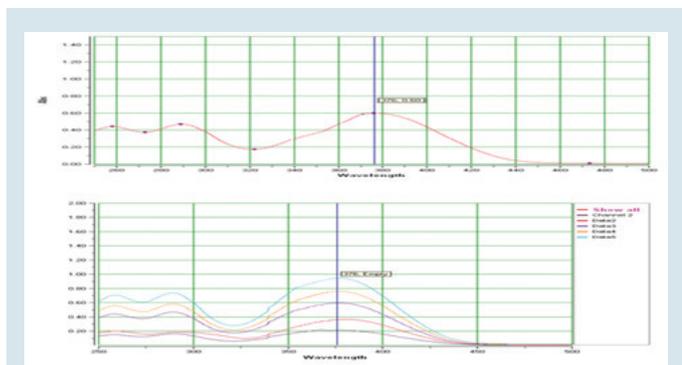


Figure 1: U.V. Scan

A: Overlain spectra of lornoxicam in pH 6.8 Phosphate buffer, B: Lornoxicam

Stability Studies

The selected (optimized) formulations were subjected to accelerated stability studies as per the ICH guidelines. It was stored at $40 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}/75 \% \pm 5 \% \text{ RH}$ for three months and evaluated for changes in various physicochemical parameters like weight variation and % drug release of pellets.¹³

Response surface graph

The prediction points for particle size and % drug release were determined. % Drug release was affected both by rotating speed of pelletizing machine and amount of polymer in the response graph. % release was affected both due to rotating speed and amount of the polymer (HPMC-K4M).

RESULTS AND DISCUSSION

UV scanning of the drug, 376 nm was the wavelength maxima selected for the estimation of lornoxicam. Various samples with different concentrations of the Sample of drug of different concentration were loaded on the UV spectrophotometer and respective absorbance were obtained at the λ_{max} 376 nm. A graph was plotted (concentration vs absorbance) which resulted straight line concluding that the drug followed Beer's Lambert's Law at the concentration range of 5-30 $\mu\text{g}/\text{ml}$.

The regression analysis was carried out on these experimental data and Y and r^2 values were calculated. The obtained values were: $Y = 0.037x$, $r^2 = 0.997$ in 6.8pH phosphate buffer; $Y = 0.035x$, $r^2 = 0.996$ in simulated intestinal fluid; $Y = 0.037x$, $r^2 = 0.996$ in 7.4 pH phosphate buffer and $Y = 0.035x$, $r^2 = 0.997$ in simulated colonic fluid as shown in Figure 1.

Samples containing different concentrations 5, 10, 15, 20 and 25 $\mu\text{g}/\text{ml}$ of the drug, were run and overlain spectra describing the reproducibility of the λ_{max} was obtained that confirmed and validated the process. The retention of characteristic peaks of the pure drug, in its combination with different polymers, confirmed that it was compatible with all other excipients incorporated in the formulations as in Figure 2.

The R_f values of lornoxicamin was 0.62 and approximately nearly similar values were obtained with its combination with different excipients thus confirmed compatibility between drug and different excipients used.

Micromeritic parameters like bulk density, tapped density, carr's index, angle of repose and hausner's ratio for formulations (F1-F9) were determined and found in the ranges of $(0.55 \pm 0.01$ to $0.57 \pm 0.01 \text{ g/cc})$, $(0.60 \pm 0.01$ to $0.66 \pm 0.01 \text{ g/cc})$, $(8.29 \pm 2.86$ to $14.28 \pm 2.70 \%)$, $(17.08 \pm 0.75$ to $17.64 \pm 0.31)$ and $(1.07 \pm 0.045$ to $1.14 \pm 0.041)$ respectively. The analysis was performed for all nine batches by photomicroscope using micrometric

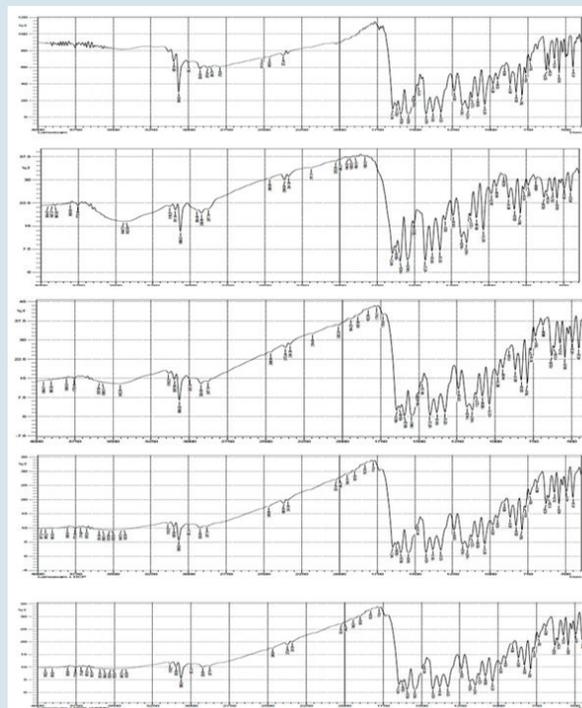


Figure 2: FTIR Spectra of Pure drug and Formulations

A: Drug with DCP and HPMC-K4 M, B: Drug with DCP, C: Drug with HPMC-K4 M, D: Drug with PVP, E: Lornoxicam

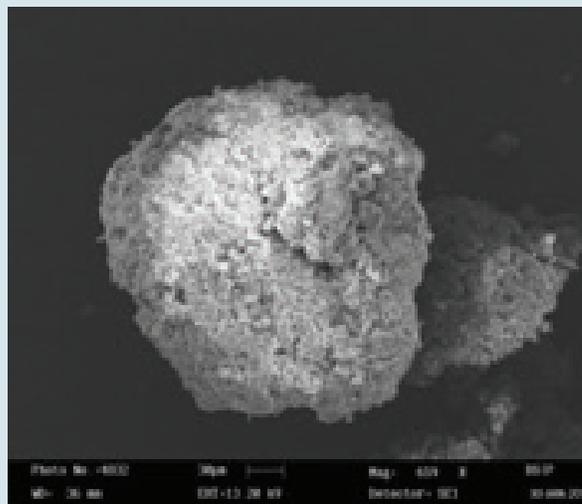


Figure 3: SEM image of optimized formulation F7.

tools. The results were shown in Table 2.

The mean diameters of micropellet for all batches were found in the range of 104.6-263.8 μm .

The particle size determination by SEM revealed that the mean particle diameter was in the range of 104-263 μm and Percentage yield (88.4-98.4 %) was calculated. The characteristic IR peaks of the pure drug and R_f value obtained with thin layer chromatographs were compared with

Table 2: Evaluation for physical properties of pellets

Code	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉
Bulk Density (gm/cm ³)	0.57	0.56	0.60 ±	0.56 ±	0.56	0.56	0.55	0.56	0.57
	± 0.01	±	0.01	0.01	±	±	±	±	±
Tapped Density (gm/cm ³)	0.65	0.66	0.66 ±	0.65 ±	0.63	0.63	0.60	0.61	0.63
	±	±	0.01	0.01	±	±	±	±	±
Carr's Index (%)	12.32	14.28	08.95±	13.03±	11.91	10.52	08.29	08.33	09.58
	±	±	3.24	3.13	±	±	±	±	±
Hausner's Ratio	2.44	2.70	2.32	1.95	2.86	2.35	1.36	1.10	1.10
	0.03	0.03	0.03	0.04	0.03	0.02	0.04	0.02	0.01
Angle of Repose	17.64 ±	17.41	17.60±	17.42±	17.13	17.32	17.08	17.62	17.36
	0.31	±	0.38	0.70	±	±	±	±	±

Table 3: 32 Full factorial design layout.

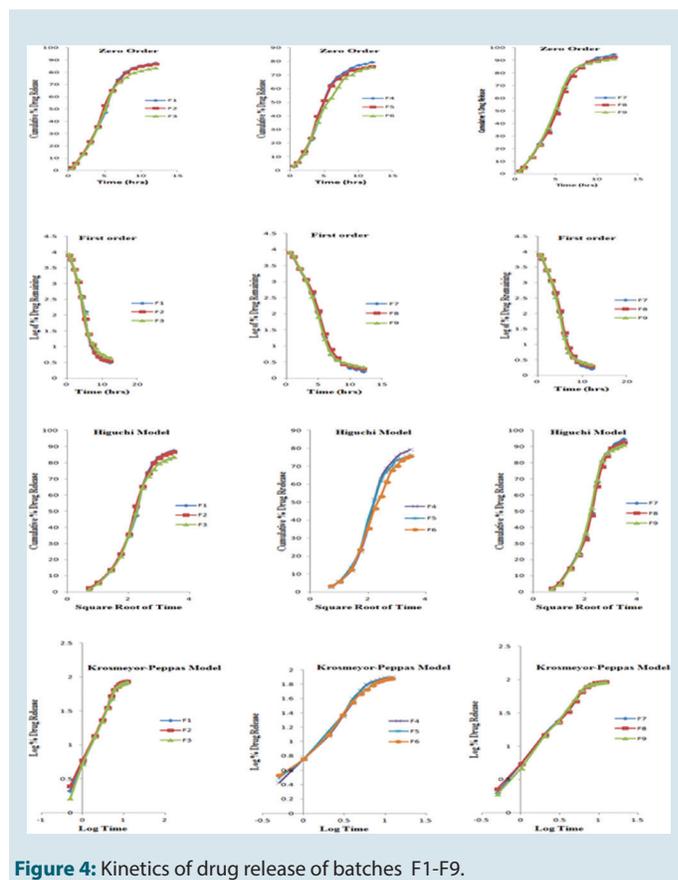
Batch code	Variable levels in coded form		Particle size Size in (µm)	Dissolution 12 h %Drug release
	X ₁ (mg)	X ₂ (rpm)		
F ₁	-1	-1	154.1	87.37
F ₂	0	-1	173.4	86.67
F ₃	1	-1	189.0	83.50
F ₄	-1	0	221.9	79.33
F ₅	0	0	249.2	76.20
F ₆	1	0	263.8	75.60
F ₇	-1	-1	104.6	94.64
F ₈	0	-1	128.7	92.60
F ₉	1	-1	140.5	91.22

Coded values	Actual values	
	X ₁ (gm)	X ₂ (rpm)
-1	5.0	100
0	7.5	200
1	10	300

combination of drug and individual excipients which remained nearly same as in Figure 3.

Post-formulation parameters of coated capsules were drug content (95.91 - 98.78 %), coating durability test (passed) and *in-vitro* drug release in pH 7.4 phosphate buffer solution ranged from (75.60% - 94.64%) during the period of 12 h as shown.

Finally, the optimized batch (F7) was screened out and comparative % drug released data in pH 7.4 phosphate buffer and in simulated colonic fluid was calculated which was found to be 94.64% and 90.58% respectively. The stability study was performed with the optimized formulation (F7) as per ICH guidelines under officially prescribed conditions which showed that the formulations were stable and thus complied with dose conformity criterion. The present worker suggested that such a formulation design had enhanced the aqueous solubility including bioavailability

**Figure 4: Kinetics of drug release of batches F1-F9.**

through colon targeted drug delivery and minimized the local and systemic toxicity.

All the release data were fitted into various kinetic models like Zero order, First order, Higuchi and Korsmeyer-peppas in order to find out the mechanism of drug release from lornoxicam micropellets in pH 7.4 phosphate buffer. The release kinetics of different formulations slope (y) and r² values were calculated. All formulation batches (F₁ - F₉) showed r² values nearer to one thus followed korsmeyer-peppas case II transport

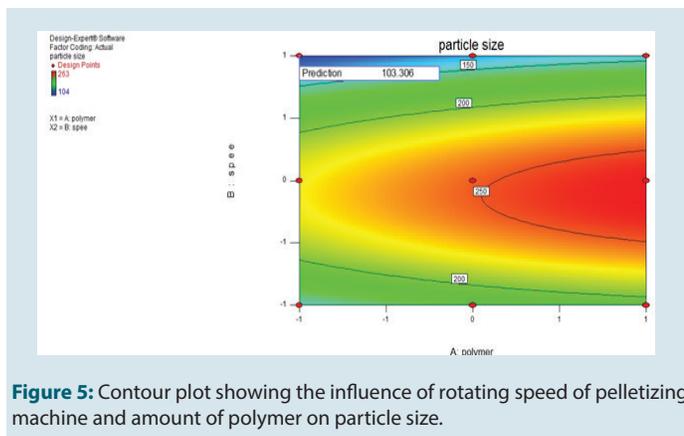


Figure 5: Contour plot showing the influence of rotating speed of pelletizing machine and amount of polymer on particle size.

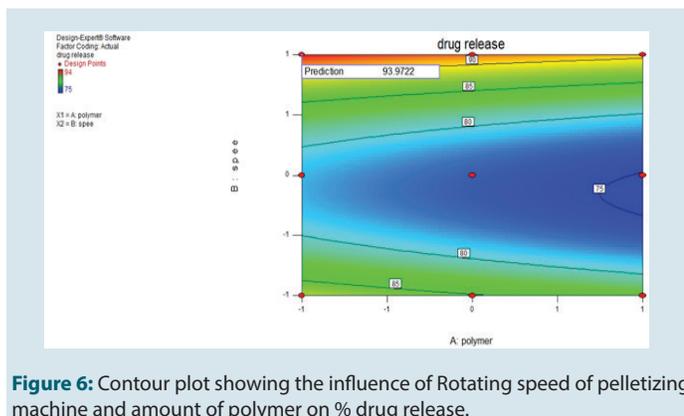


Figure 6: Contour plot showing the influence of Rotating speed of pelletizing machine and amount of polymer on % drug release.

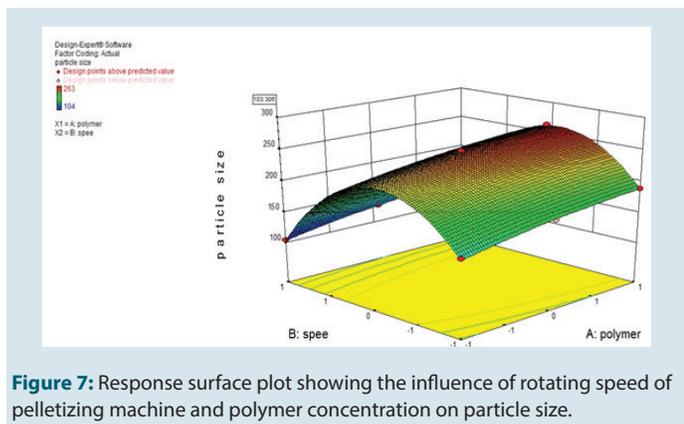


Figure 7: Response surface plot showing the influence of rotating speed of pelletizing machine and polymer concentration on particle size.

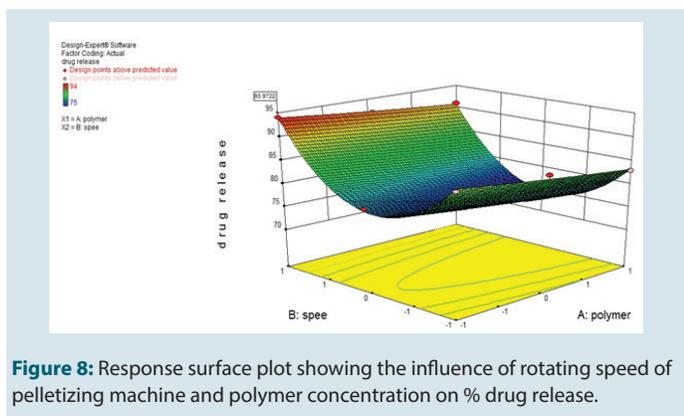


Figure 8: Response surface plot showing the influence of rotating speed of pelletizing machine and polymer concentration on % drug release.

mechanism as shown in Figure 4.

The statistical analysis on different models was performed by ANOVA. Full and reduced models for particle size and % drug release were obtained. The finally optimized batch of formulation (F7) was subjected to release profile studies in pH 7.4 phosphate buffer and simulated colonic fluid. The actual responses were in accordance with the predicted values which showed validity of the model. The optimized formulation was subjected to stability studies which revealed the dosage form as stable with probable enhanced bioavailability pertaining to inference drawn on *in-vitro* drug release profile as shown in Table 3.

Particle size and % drug release increased from blue to red region in contour graph shown in Figure 5 and Figure 6.

% Drug release was affected both by rotating speed of pelletizing machine and amount of polymer in the response graph. Particle size decreased from blue to orange region in contour graph, the prediction points was determined as 103.30 and 93.97 as in Figure 7.

Particle size increased from blue to orange owing to increasing rotating speed in the response surface graph. % release was affected both due to rotating speed and amount of the polymer (HPMC-K4M) as in Figure 8.

CONCLUSION

The stability study was performed with the optimized formulation (F7) as per ICH guidelines under officially prescribed conditions which showed that the formulations were stable and thus complied with dose conformity criterion. The present worker suggested that such a formulation design had enhanced the aqueous solubility including bioavailability through colon targeted drug delivery and minimized the local and systemic toxicity.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

Authors have no conflict of interest.

ABBREVIATION USED

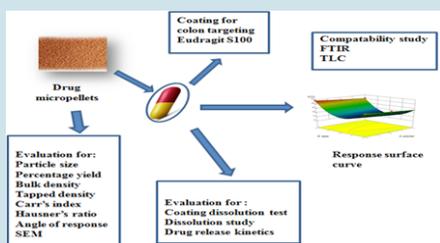
DCP: Di Calcium Phosphate; **PVPK:** Poly Vinyl Pyrrolidone; **HPMC:** Hydroxy Propyl Methyl Cellulose ; **PEG:** Polyethylene Glycol.

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PICTORIAL ABSTRACT



SUMMARY

- Micropellets of Lornoxicam were prepared using DCP, PVPK-30 and different ratios of HPMCK-4m.
- Drug and Polymer compatibility studies revealed that there is no any physical and chemical changes occurred between drug and polymer under study.
- Retention of FTIR peaks confirmed that the drug was compatible with selected polymers.
- Accelerated stability results showed that there were no physical and chemical changes in the tablets during three month study.

ABOUT AUTHORS



Dr Preeti Rawat: Is a Ph.D. scholar and working as a senior research fellow in CSIR- National Botanical Research Institute, Lucknow.



Mr Neetishwar Saroj: Is working as an officer in Quality Assurance, Department in Multi-National Company. His work of interest is formulation development of different dosage form.