Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011, 3(3):443-452
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Formulation and statistical optimization of controlled release pellets of Cetrizine Dihydrochloride

D. M. Mathure*[1], M. R. Bhalekar[1], D. M. Mathure[1], R. R. Padalkar[1], B. S. Dawane[2] and S. S. Chobe[2]

[1]*Department of Pharmaceutics, AISSMS College of Pharmacy, Pune (M.S) INDIA*
[2]*School of Chemical Sciences, S.R.T.M. University, Nanded, (M.S) INDIA*

ABSTRACT

The objective of this study was to formulate controlled release pellets of cetrizine dihydrochloride and optimize the effect of formulation variables i.e. concentration of Eudragit RLPO and ethyl cellulose. The experimental design selected was 3^2 full factorial design using these two variables. Drug release after 24 hr and t50% were evaluated as response parameters. Polymers were coated onto the drug loaded pellets using pan coater. It was observed that the concentrations of polymers directly affected the drug release profile. Eudragit RLPO and ethyl cellulose showed effects opposite to each other on drug release. Mathematical models were generated for each response parameters to predict their values at selected levels of formulation variables. The effect of the variables and behavior of the system was studied using response surface plots. The optimized formulation showed drug release of 96.22% in 24 hr and t50% of 11hr 54 min. The results of this study revealed that the pellets of cetrizine dihydrochloride coated with 1:5 ratio of Eudragit RLPO: Ethyl cellulose showed optimum controlled release.

Keywords: Cetrizine dihydrochloride**,** ethyl cellulose, Eudragit RLPO, polymer coating, controlled release pellets, optimization.

INTRODUCTION

Cetrizine dihydrochloride is an H1 receptor antagonist. Cetrizine is administered in daily dose of 5 to 10 mg as antiallergic. In certain severe allergic conditions this dose is administered twice a day and maintenance of blood levels throughout 24 hr is required. A conventional tablet may not be able to maintain the blood levels to a steady state throughout 24 hr. Hence in such cases, it is necessary to develop a controlled release formulation.

Multiple unit dosage forms have several advantages compared with single-unit dosage forms demonstrated as flexibility during formulation development and therapeutic benefits for the patients. These include increased bioavailability, reduced risk of systemic toxicity arising from dose dumping observed with other formulations, reduced risk of local irritation and importantly, more flexible modification can be applied to obtain the ideal in vitro release profile compared with the single-unit dosage forms [1-4].

MATERIALS AND METHODS

Materials:

Cetrizine dihydrochloride (Iatros Pharmaceuticals Pvt.Ltd), non pareils (Murli Krishna Pharmaceuticals), Eudragit RLPO (Degussa), (Pharmaceutical Coating Pvt Ltd) and potassium dihydrogen orthophosphate (S.D. Fine Chem. Ltd; India) were received as gift samples. All other chemicals and reagents were of analytical grade.

Coating of Pellets:

Cetrizine dihydrochloride-loaded pellets were prepared by layering a drug-binder solution onto non-pareil beads using a pan coater. Coating suspension was prepared from polymers (mixture of Eudragit RLPO and Ethyl Cellulose), talc, plasticizer and solvents. Talc was previously dispersed in mixture of acetone and isopropyl alcohol (IPA). Dispersions of Eudragit RLPO and ethyl cellulose in acetone: isopropyl alcohol (1:1) were mixed in the desired ratio based on the experimental design. The polymer content of the mixture was then adjusted by dilution with acetone and isopropyl alcohol. With gentle stirring, suspension of talc was added to the prepared polymeric dispersion. The polymer dispersion was plasticized with dibutylpthalate. Coating dispersion was blended for 1 hr.

30 gm of drug loaded pellets were coated in pan coater. Coating solution composition and coating conditions are listed in Table2 and Table 3.

TABLE 1: Independent variables and their selected levels for pellet formulations

Coded Factors	Level	Factor 1 Eudragit RLPO(X1)	Factor 2 Ethyl Celllose(X2)
-1	Low	1	1
0	Medium	3	3
+1	High	5	5

TABLE 2: Composition of coating solution for nonpareils

Sr.no.	Ingrdients	Quantity								
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1	Eudragit RLPO (gm)	1	1	1	3	3	3	5	5	5
2	Ethyl Cellolose (gm)	1	3	5	1	3	5	1	3	5
3	Talc (gm)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
4	Glycerin (ml)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
5	Dibutyl Phthalate (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
6	Acetone:IPA-1:1 q.s. (ml)	100	100	100	100	100	100	100	100	100

TABLE 3: Coating parameters for nonpareils

Nonpareils	30.00gm
Spray rate	0.8ml/min
Atomizing air pressure	2lb/inch^2
Nonpareil bed temperature	65^{0}C
Pan speed	25rpm
Preheating of pan	15 min

Experimental design and statistical analysis:
In this study, a 3^2 full factorial experimental design was employed to optimize the formulation of pellets. In order to optimize formulations, the amounts of Eudragit RLPO (X1) and the amount of the ethyl cellulose (X2), were chosen as independent variables. Eudragit RLPO, being hydrophilic is more permeable to water so it promotes release of drug. Ethyl cellulose is hydrophobic and retards drug release being less permeable to water. Hence the combination of a release promoting and retarding polymers was used to obtain controlled drug release. Levels of these formulation variables are shown in Table 1. Selection of response variables was crucial. The target was to maintain the drug release throughout 24 hr but simultaneously to achieve maximum release at the end of this time period. Therefore the response variables selected for evaluation of controlled release were percent of drug release in 24hr and time required for release of 50% of the drug (t50%). The t50% should be as close to 12 hr as possible as it would be the more realistic measure of maintenance of controlled release.

Evaluation of pellets:
Drug Content:
100 mg of drug loaded pellets were accurately weighed, powdered and transferred to 100 ml volumetric flask containing distilled water [5].The flask was shaken on vortex mixer for 10 min and sonicated for 15 min. The concentration of cetrizine dihydrochloride was analyzed by UV-Vis spectrophotometer at 230 nm. All assays were carried out in triplicate and the mean value was reported.

Scanning electron microscopy:
The external surface morphology of coated pellets was observed by scanning electron microscopy. Samples were mounted on to stubs. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The stub containing the coated samples was placed in the scanning electron microscope (JSM- 6360A; JEOL, Tokyo, Japan) chamber. The samples were then randomly scanned and photographs were taken.

In vitro drug release studies:
Dissolution studies were carried out using USP dissolution test apparatus I (Veego DA-6D USP Standard) in 900 ml medium at 37^{0}C at a rotation speed of 100 rpm. Accurately weighed pellets containing the equivalent of 10 mg of cetrizine dihydrochloride were transferred to the dissolution medium. Considering the gastric transit time of 2hr dissolution tests were carried out in media 0.1N HCl (pH 1.2) for 2 h followed by phosphate buffer pH 6.8 until 24 h [6,7]

Drug release Models:

To describe the kinetics of the drug release from the sustained release pellets, various mathematical models such as zero-order, first-order, Higuchi, Hixon-crowell, Koresmeyer-peppas models were used. The drug release data was evaluated by model-dependent (curve fitting) method using PCP Disso ver. 2.08 software.

Analysis of data:

The data obtained by experimental design was processed using Design Expert 7.1.4 software. 3-D response surface curves were constructed to study the effect of two independent variables alone and in combination on percent drug release and t50 % [8-10]. All the responses observed were simultaneously fitted to quadratic-models and were evaluated in terms of statistical parameters. Grid search was conducted over the experimental domain to find the compositions of the optimized formulations. The five optimized checkpoint formulations were prepared and evaluated. The resultant experimental values of the responses were quantitatively compared with that of the predicted values by calculating residuals and linear plots.

TABLE 4: A 3^2 Full factorial experimental design layout, values of % release and T50% for cetrizine dihydrochloride pellets prepared as per full factorial design

Formulation code	Drug content %	% Release 24 hrs	t50% hr
F1	95.12±0.23	87.9±0.2494	13.8
F2	94.63±0.15	82.14±0.16	15.4
F3	94.55±0.24	78.12±0.2124	15.9
F4	96.92±0.59	92.54±0.1635	12.9
F5	98.00±0.42	86.89±0.2451	14.1
F6	97.25±0.13	83.79±0.2060	15.0
F7	98.92±0.20	96.22±0.1633	11.9
F8	96.33±0.52	89.97±0.2451	13.5
F9	98.13±0.22	85.46±0.2062	14.6

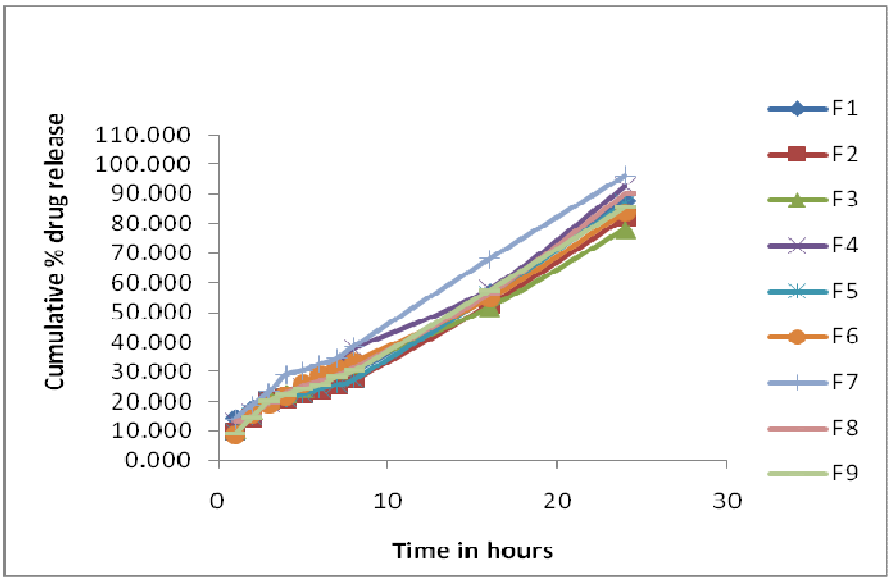
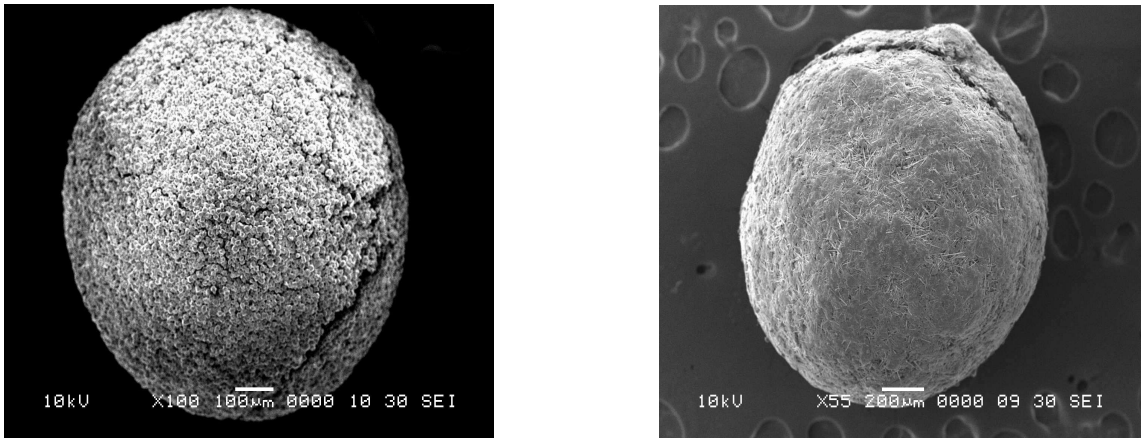


Figure 1: Comparison of cumulative percent release of cetrizine from coated pellets formulations F1-F9.

RESULTS AND DISCUSSION

In vitro Drug release studies:
The data of % release in 24 hr and t 50% is shown in Table 4. The dissolution profiles are shown in Figure 1. Dissolution study shows that as the concentration of ethyl cellulose increased the % cumulative drug release of cetrizine dihydrochloride decreased. This was due to hydrophobic nature of ethyl cellulose which results in increase diffusion path length and consequent retardation of drug release [11-16]. On the other hand, increase in concentration of hydrophilic polymer Eudragit RLPO showed diffusion controlled mechanism for drug release [17-18].

Scanning electron microscopy (SEM):
Morphology of non pareils was examined by scanning electron microscopy [19]. Coated pellets showed smooth and porous surface. The polymers were distributed on to the pellet which allows penetration of dissolution media and passage of drug (figure 2).



[A] [B]

Figure 2: SEM of (A) Nonpareils (B) Coated pellets

Drug Content:
Drug content of pellet formulations (F1 to F9) was found out to be 94.55±0.24 to98.92±0.20 which reveals drug content was within the limits prescribed by I.P. Drug contents from all formulations are shown in table 4.

Experimental design and statistical analysis [20-23]:
Most formulation studies involve variation of one factor at a time, keeping other factors constant. Factorial design enables all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. Hence factorial design was selected as design of experiment.

The experiments were designed to study effect of two independent variables at three levels on response variables such as % drug release 24 hr and t50%.The coefficients of the polynomial equations (Eq.1 and 2)generated for % release and t50% are given below.

Statistical Models [24]:

$$\% \text{ release} = 87.07+3.91*A-4.88*B-0.24*A*B-1.10*A^2+1.01*B^2 \quad \text{.....(1)}$$

$$t50\% = 14.21-0.85*A+1.15*B+0.15*A*B+0.18*A^2-0.32*B^2 \text{..... (2)}$$

For % release response, the Model F-value was found 170.56 which imply the model is significant. P value which is 0.001 was less than 0.0500 indicates model terms are significant. Adeq. Precision shows signal to noise ratio. The ratio of 40.647 indicates an adequate signal thus the proposed model can be used to navigate the design space.

For t50% response, the Model F-value was 86.32 which imply the model is significant. P value 0.001 was less than 0.0500 indicate model terms are significant. The signal to noise ratio of 28.640 indicates an adequate signal. Thus the proposed model can be used to navigate the design space. Since the values of r^2 are quite high for both the responses, i.e., 0.9965 for % release and 0.9931 for t50%, the polynomial equations form excellent fits to the experimental data and are highly statistically valid.

TABLE 5: Analysis of variance (ANOVA) of dependent variables.

Response model	F value	P value	R square	Adeq. Precision
% release	170.56	0.0007	0.9587	40.647
t50%	86.32	0.0001	0.9262	28.640

Response-surface analysis [25]:
It was observed that % release and t50% were both dependent on concentration of polymers. The response surface and respective contour maps are shown for both the responses in Figure 3.

It was observed that ethyl cellulose and Eudragit RLPO were having effect opposite to each other on both the responses. As the concentration of Eudragit RLPO is increased, % release in 24hr gets increased while t50% decreased. This indicates that Eudragit RLPO enhanced the release of drug from coated pellets. However ethyl cellulose showed decrease in % release in 24 hr and increase in t50% indicating retarded release.

This suggests that in order to obtain controlled release of cetrizine these two polymers must be used in combination. Thus there was a need of searching for optimum composition of polymers that controls the release upto 24 hr.

Search for optimum formulations [26-27]:
The search for optimum formulations was carried out using a grid (Table 6) which was further explored by intensive grid search to find the region having optimum concentration of both the polymers (Table 7). The selection criteria were maximum release in 24 hr (> 90%) and t50% near to 12hr. It was observed from the grid search that favorable controlled drug delivery (93.08 - 96.01 % drug release in 24 hr and t50% of 12.04-12-42 hr) was achieved when ethyl cellulose was used in the range of coded levels -0.8 to -1 and Eudragit RLPO in the range of coded levels 0.2 to 1. The actual levels obtained after transforming the coded levels are 1 to 1.4%

w/v of ethyl cellulose and 3.4 to 5% of Eudragit RLPO. The optimum formulation was the one containing ethyl cellulose and Eudragit RLPO in concentration of 1% and 5% respectively.

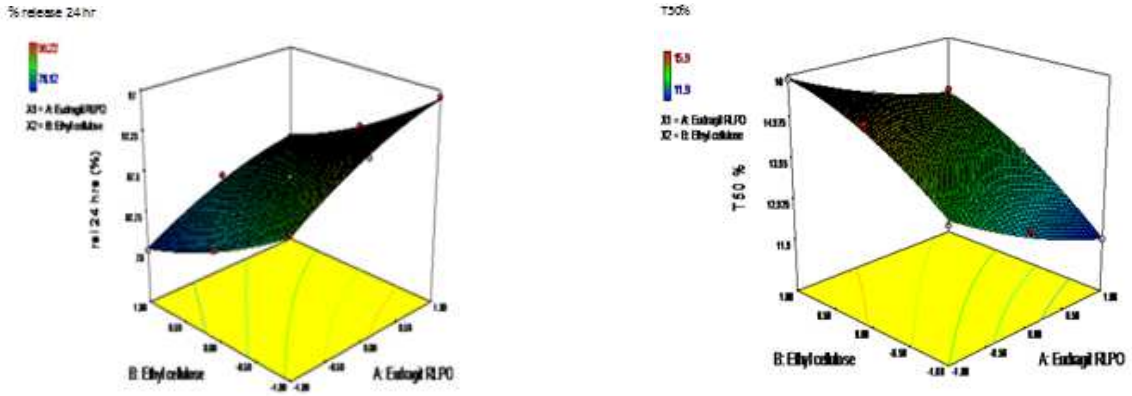


FIGURE 3A: Response surface plot for % release and T50%.

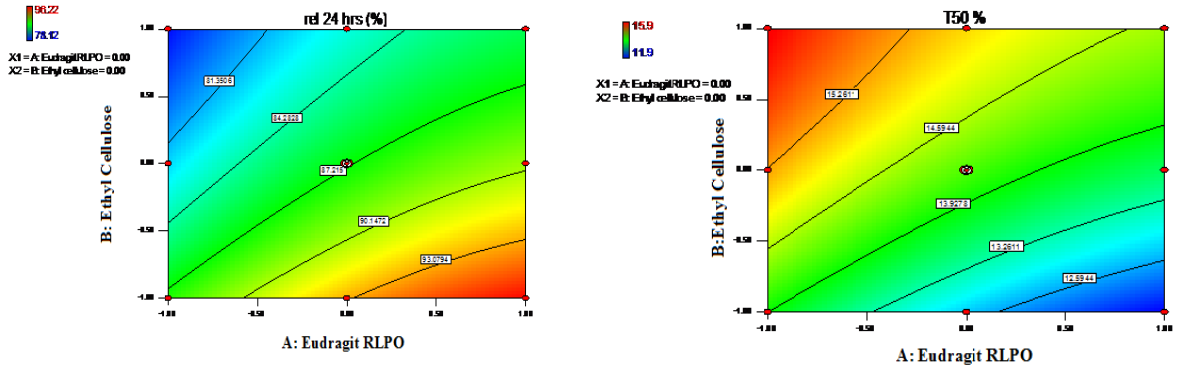


FIGURE 3B: Contour plot for %release and T50%

TABLE 6: Grid search for locating a region of suitable formulation for eudragit rlpo and ethyl cellulose (Highlighted Cells Show Selected Region).

	% rel_{24h}										
X1† / X2*	**-1**	**-0.8**	**-0.6**	**-0.4**	**-0.2**	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
-1	87.71	86.41	85.20	84.07	83.02	82.06	81.17	80.36	79.63	78.99	78.43
-0.8	88.93	87.63	86.41	85.27	84.21	83.23	82.34	81.52	80.78	80.13	79.56
-0.6	90.07	88.76	87.53	86.38	85.31	84.32	83.42	82.59	81.85	81.18	80.60
-0.4	91.12	89.80	88.56	87.40	86.32	85.33	84.41	83.57	82.82	82.14	81.55
-0.2	92.08	90.75	89.50	88.33	87.25	86.24	85.31	84.47	83.70	83.02	82.42
0	92.96	91.62	90.36	89.18	88.08	87.07	86.13	85.27	84.50	83.81	83.21
0.2	93.74	92.39	91.12	89.94	88.83	87.80	83.86	85.99	85.21	84.51	83.69
0.4	94.44	93.08	91.80	90.61	89.49	88.45	87.50	86.62	85.83	85.12	84.49
0.6	95.05	93.68	92.39	91.19	90.06	89.02	88.05	87.17	86.36	85.64	85.00
0.8	95.57	94.19	92.90	91.68	90.54	89.49	88.52	87.62	86.81	86.08	83.40
1	96.01	94.62	93.31	92.08	90.94	89.88	88.89	87.99	87.17	86.43	85.77
	T50%										
	-1	**-0.8**	**-0.6**	**-0.4**	**-0.2**	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
-1	13.77	14.11	14.43	14.72	14.99	15.24	15.45	15.64	15.81	15.95	16.07
-0.8	13.53	13.88	14.2	14.49	14.76	15.00	15.22	15.41	15.58	15.72	15.83
-0.6	13.31	13.66	13.97	14.27	14.54	14.78	15.00	15.19	15.35	15.5	15.61
-0.4	13.10	13.45	13.77	14.06	14.33	14.57	14.79	14.98	15.15	15.29	15.40
-0.2	12.91	13.26	13.58	13.87	14.14	14.38	14.60	14.79	14.96	15.10	15.21

0	12.74	13.88	13.40	13.69	13.96	14.21	14.42	14.61	14.78	14.92	15.04
0.2	12.57	12.92	13.24	13.53	13.65	14.04	14.26	14.45	14.62	14.76	14.87
0.4	12.42	12.77	13.09	13.38	13.52	13.89	14.11	14.30	14.47	14.61	14.72
0.6	12.29	12.64	12.95	13.25	13.52	13.76	13.98	15.17	14.33	14.48	14.59
0.8	12.17	12.52	12.84	13.13	13.54	13.64	13.86	14.05	14.22	14.36	14.47
1	12.04	12.4	12.73	13.02	13.29	13.54	13.75	13.94	14.11	14.25	14.37

Coded amounts of †Eudragit RLPO and *Ethyl cellulose

TABLE 7: intensive grid search for locating optimum formulation for % RELEASE AND T50 % (Highlighted Cells Show Checkpoint Formulation Used For Validation Of Mathematical Models)

% rel_{24h}

X1† / X2*	**-1**	**-0.98**	**-0.96**	**-0.94**	**-0.92**	**-0.9**	**-0.88**	**-0.86**	**-0.84**	**-0.82**	**-0.8**
0.4	94.44	94.30	94.16	94.02	93.89	93.75	93.61	93.48	93.35	93.21	93.08
0.46	94.63	94.49	94.35	94.21	94.08	93.94	93.80	93.67	93.54	93.40	93.27
0.52	94.82	94.68	94.54	94.40	94.26	94.12	93.99	93.85	93.72	93.58	93.45
0.58	94.99	94.85	94.71	94.57	94.44	94.30	94.16	94.03	93.89	93.76	93.62
0.64	95.16	95.0	94.88	94.74	94.60	94.47	94.33	94.19	94.06	93.92	93.79
0.7	95.32	95.18	95.04	94.90	94.76	94.62	94.49	94.35	94.22	94.08	93.95
0.76	95.47	95.33	95.19	95.05	94.91	94.78	94.64	94.50	94.37	94.23	94.10
0.82	95.62	95.48	95.34	95.20	95.06	94.92	94.78	94.64	94.51	94.37	94.24
0.88	95.76	95.61	95.47	95.33	95.19	95.05	94.92	94.78	94.64	94.51	94.37
0.94	95.88	95.74	95.60	95.46	95.32	95.18	95.04	94.91	94.77	94.63	94.50
1	96.01	95.86	95.72	95.58	95.44	95.30	95.16	95.03	94.89	94.75	94.62

T50%

	-1	**-0.98**	**-0.96**	**-0.94**	**-0.92**	**-0.9**	**-0.88**	**-0.86**	**-0.84**	**-0.82**	**-0.8**
0.4	12.42	12.46	12.49	12.53	12.56	12.60	12.63	12.67	12.70	12.74	12.77
0.46	12.38	12.42	12.45	12.49	12.52	12.56	12.59	12.63	12.67	12.69	12.73
0.52	12.34	12.38	12.41	12.45	12.48	12.52	12.55	12.59	12.62	12.65	12.69
0.58	12.30	12.34	12.37	12.41	12.44	12.48	12.51	12.55	12.58	12.61	12.65
0.64	12.26	12.30	12.34	12.37	12.41	12.44	12.47	12.51	12.54	12.58	12.61
0.7	12.23	12.26	12.30	12.33	12.37	12.40	12.44	12.47	12.51	12.54	12.57
0.76	12.19	12.23	12.26	12.30	12.33	12.37	12.40	12.44	12.47	12.50	12.54
0.82	12.16	12.19	12.23	12.27	12.30	12.33	12.37	12.40	12.44	12.47	12.50
0.88	12.13	12.16	12.20	12.23	12.27	12.30	12.34	12.37	12.40	12.44	12.47
0.94	12.10	12.13	12.17	12.20	12.24	12.27	12.31	12.34	12.37	12.41	12.44
1	10.07	12.10	12.14	12.17	12.21	12.24	12.28	12.31	12.34	12.38	12.41

Coded amounts of †Eudragit RLPO and *Ethyl cellulose

Validation of optimum formulation:

The five checkpoint formulations were prepared and evaluated for % release and t50%. The observed values were found to be in close agreement with the predicted values. This was confirmed by low values of residuals (Table 8) and linear plots (Figure 4). From these results and ANOVA, it was found that the mathematical models generated were statistically significant and valid for predicting values of response parameters at selected levels of formulation variables.

TABLE 8: Comparison of experimental results with predicted responses

Formulation Code	Response	Predicted value	Experimental value	Residuals
S1	% Release	94.44	93.12	-1.32
	t50%	12.42	12.76	0.34
S2	% Release	96.01	96.97	0.96
	t50%	12.07	11.96	-0.11
S3	% Release	93.08	91.25	-1.83
	t50%	12.77	13.23	0.46
S4	% Release	94.62	93.72	-0.9
	t50%	12.40	12.72	0.32
S5	% Release	94.62	94.10	-0.52
	t50%	12.41	12.70	0.29

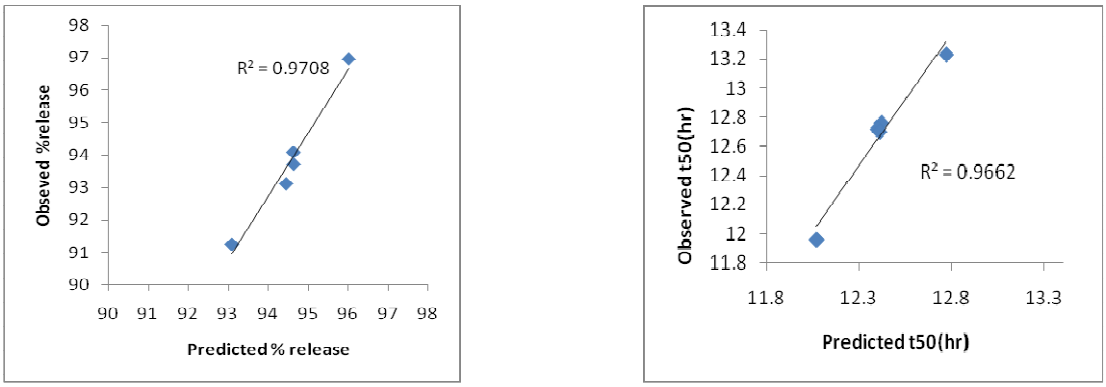


FIGURE 4: Comparison of predicted and experimental values for % release and T50%.

CONCLUSION

It was observed that Eudragit RLPO enhanced the release of cetrizine from drug loaded pellets whereas ethyl cellulose retarded the same. In order to obtain a controlled delivery, a combination of these polymers in different concentration was tried. It was concluded that a combination of Eudragit RLPO and ethyl cellulose in 5% w/v and 1% w/v respectively showed optimum controlled release (96.22 % in 24 hr and t50% of 11.9 hr i.e. 11 hr 54 min). The mathematical models generated during this optimization process were found to be valid on the basis of ANOVA and practically observed values.

Acknowledgements
The authors are thankful to University of Pune for providing facilities for SEM studies and all the suppliers for gift samples And also sincere thanks to Director, School of chemical sciences,Nanded.

REFERENCES

[1] Kramar A, Turk S, and Vrecer F: *Int. J. Pharm* **2003**; 43–52.
[2] Ghebre-Sellassie I. (Eds): Pharmaceutical Pelletization Technology. Marcel Dekker Inc, New York **1989;** 1-13.
[3] Follonier N, Doelker E: *STP Pharm. Sci*.**1992**; 141–158.
[4] Xiong Zhang, Xing Tang, and Rui Yang: *Drug. Dev. Ind. Pharm*. **2009;** 35:26–33.
[5] Fatemeh Sadeghi, James L Ford, Michael H. Rubinstein and Ali R.Rajabai-Siahboomi, *Drug. Dev. Ind. Pharm*. **2000;** 26:6,651-660.
[6] The United States Pharmacopoeia-27/ National Formulary-22., Asia Edition. Rockville MD, **2000**; 417-418.
[7] Mukesh Patel, Sanjay Patel, and Natavarlal Patel: Controlled release condurum **2007**; 23-24.
[8] Min –Soo K, Jeong-Soo K and Yeon-Hee Y: *Int. J. Pharm*. **2007**; 341 (1-2), 97-100
[9] Banker G, Rhodes L: Optimization techniques in Pharmaceutical formulation and processing, Modern Pharmaceutics. 3rd Edition, Marcel Dekker, New York, **1996**: 727-752.
[10] Bolton S. In: Pharmaceutical Statistics. 2nd Edition, Marcel Decker Inc, New York, **1990**; 532-34.

[11] Akhgari A, Afrasiabi Garekani H, Sadeghi F and Azimaie M: *Int. J. Pharm*. **2005**; 305: 22–30.
[12] Handa A, Bhalla H: *Ind. J. Pharm. Sci* **2000;** 147-149.
[13] Lippold B, Gunder W, Lippold B: *Eur. J. Pharm. Biopharm* **1999**; 47, 27–32.
[14] Wesseling M, Bodmeier R: *Eur. J. Pharm. Biopharm* **1999;** 47:33–38.
[15] Ozturk A, Ozture S, Palsson B, Wheatley T, and Dressman J, *J. Controlled release* **1990**; 14,203-213
[16] Bianchini R, Bruni G, Gazzaniga A, and Vecchio C, *Drug. Dev. Ind. Pharm*. **1993;**19, 2021-2041
[17] Abbaspour M, Sadeghi F, Garkani H: *Int. J. Pharm*. 303:88-94.
[18] Fukumori Y. Coating of multiparticulates using polymeric dispersions. In: Multiparticulate Oral Drug Delivery-Drugs and pharmaceutical science. Marcel Dekker Inc, New York, Vol 65, **1994;** 79-117.
[19] GovenderT, Dangor C, Chetty D: *Drug.Dev.Ind.Pharm* **1995**; 21.1303-1322
[20] Schwartz J, O'Connor R: Optimization techniques in pharmaceutical formulation and processing. In: Banker GS, Rhodes CT. (Eds.), Modern Pharmaceutics, 3rd Edition, Marcel Dekker, New York, **1997**:727–752.
[21] Sam T, Rubinstein M: *Pharm. Int.* **1985;** 6, 265–266.
[22] Gohel M, Amin A: *J. Controlled. Release* **1998;** 51, 115–122.
[23] Bodea A, Leucuta S: *Int. J. Pharm***. 1997;** 154, 49–57.
[24] Madgulkar A, Bhalekar M, Padalkar R: *AAPS Pharm SciTech*, **2009:**10(2):574-81.
[25] Singh B and Ahuja N: Response surface optimization of drug delivery system. In: Jain N K (eds) Progress in controlled and novel drug delivery systems.CBS Publishers and Distributors, First Edition, New Delhi, **2004**;470-509
[26] Singh B and Ahuja N: *Drug. Dev. Ind. Pharm***. 2002**; 28:433-444.
[27] Gohel M, Patel M, Agarwal R , Amin A, Dave R, Bariya N: *AAPS Pharm SciTech*, **2004;** 5(3): 36.