Journal of Pharmaceutical Research Vol. 9, No. 3, July 2010 : 141-147.

DESIGN, EVALUATION AND STATISTICAL OPTIMIZATION OF DIAZEPAM LOADED CONTROLLED RELEASE MICROPELLETS

Chandra G[1], Bhattacharyya S*[2], Ray S[3], Ghosh LK[1], Gupta BK[1]
[1]Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata - 700 032, India.
[2]Oxford College of Pharmacy, 6/9, 1st Cross, Begur Road, Hongasandra, Bangalore - 560 068, India.
[3]Krupanidhi College of Pharmacy, #12/1, Chikkabellandur, Carmelram Post, Bangalore - 560 035, India.

Received on : 17.04.2010 Revised : 17.07.10 Accepted : 27.07.10

ABSTRACT
This work aims at designing and evaluating a Extended release dosage form of Diazepam using Alginate-Eudragit RS 30D as the matrix-forming polymer employing the Ionotropic Gelation technique of micropellletization. The product was characterized by physicochemical parameters such as yield, drug entrapment efficiency, particle size, surface topography, granulometric study, loose surface crystal study, drug-excipient compatibility and *in vitro* release. The controlled release profile was optimized using a factorial design for achieving the correct blend of microparticles that closely matches the target release profile. The study illustrates the utility and advantage of designed experimentation in controlled drug delivery research.

Keywords: *Diazepam; Extended release; micropellets; factorial design; optimization.*

INTRODUCTION

The success of any therapeutic device of drugs not only depends upon its technical feasibility and marketability to capture a giant share, but more so on product acceptability by users due to a series of advantages based on maintenance of steady-state plasma levels of drugs, reduced side-effects and effective immediate termination of therapy if need do arise, besides other considerations. While addressing this objective one obviously looks forward to controlled release drug delivery which is a vast field of research and among the various biopharmaceutically important dosage forms oral controlled release systems occupy the pivotal position. This provides phasing of drug administration to needs of individual patient.

Thus, the success of drug therapy is highly dependent on the dosage regimen design. A properly designed dosage form delivery system aims to achieve an optimum drug concentration at the receptor site to produce an optimal therapeutic effect with minimum adverse reactions. Pharmacokinetic and Biopharmaceutical studies of drugs and the formulations are very useful to understand the relationship between the physicochemical properties of the product and its clinical effect. Biopharmaceutics embraces factors that influence the release of drug from the product, the rate of dissolution of the drug and the eventual bioavailability of the drug.

Diazepam is rapidly absorbed and delivered to highly perfused tissues including the brain, where a rapid psychotropic effect is produced. The drug is then redistributed to less well-perfused tissues. Thus, diazepam has a rapid onset and relatively brief duration of action after a single dose due to redistribution out of the brain, even though the elimination half-life is long. Moreover, while correlations between plasma concentration of benzodiazepines (BZDs) and clinical effects are limited, concentrations in plasma only twice those usually considered to be effective are associated with undesirable degrees of sedation. For this reason, BZDs are neither effectively nor safely given once a day in a conventional dosage form; even those with relatively long elimination half-lives are best given in 2 to 4 portions for the treatment of daytime anxiety to avoid early intoxication and latter reemergence of anxiety symptoms or mild withdrawal[1-3].

In the present study, an attempt has been made to control the release of diazepam from the dosage form[4,5], so that as the drug gets redistributed out of the brain, a constant supply into that region could be achieved. This will also minimize the early intoxication and latter reemergence of anxiety symptoms. The incidence of tolerance development would also be reduced to some extent. In a nutshell, better control over the therapy would be achieved by controlling the release rate of diazepam from the delivery system and thus controlling the plasma concentration[6].

Diazepam is a very good anti-anxiety agent having an early onset but short duration of action in spite of its relatively long elimination half-life. This is primarily due to its very short retention in the target organ, i.e., the brain. Therefore, if a constant supply of Diazepam can be given to the brain over a prolonged period, one can effectively eliminate the problem of early intoxication

***Correspondence :** sayanibh@hotmail.com

and reemergence or withdrawal symptoms of anxiety[7,8]. This can be best achieved with an oral extended release drug delivery system, keeping in view the patient compliance and better control over the plasma drug-level[9].

USP XXII has specified one protocol for tolerance limit of the release of diazepam with time, which should be followed for extended release diazepam capsules[10].

USP XXII PROTOCOL

Tolerances for Extended Release Diazepam Capsules

TIME (HR)	AMOUNT DISSOLVED
0.042D	15% - 27%
0.167D	49% - 66%
0.333D	76% - 96%
0.500D	85% - 115%

D = Dosing Interval

In the present study, attempt has been made to design an aqueous solvent based micropelletization procedure for diazepam (which is slightly soluble in water) for better therapeutic management of anxiety.

EXPERIMENTAL

Materials

(1) Sodium Alginate (Loba Chemie, Bombay)
(2) Calcium chloride (Qualigens, Glaxo chemicals, Bombay)
(3) Eudragit RS 30D (Degussa, Germany)
(4) Triethyl citrate (Citroflex 2, CBC (EUROPE) Ltd.)
(5) Ethanol (E. Merck, Germany)
(6) Diazepam

All the other chemicals were of analytical grades and used without further purification.

Method of preparation of alginate eudragit micropellets

For all the nine batches, total amount of water was 25 ml and accordingly amounts of all the ingredients were calculated on the basis of 25 ml of water present in the formulation.

Required amount of sodium alginate was soaked in distilled water. It was then stirred by electrical stirrer at 500rpm for 30 min to get a clear solution. Required amount of eudragit RS 30D was then given by means of a pipette and the dispersion was mixed thoroughly at the same speed for another 30 min. Drug was incorporated into this homogenous dispersion, and the resulting mixture was homogenized for another 1 hr at the same speed. The dispersion was then taken into a glass syringe and extruded through a 20G needle. Meanwhile, 6 gm of calcium chloride was dissolved in 100 ml of distilled water to make 6% calcium chloride solution. The dispersion that was extruded through the needle was collected in the form of small pellets in the 6% calcium chloride solution. The pellets were formed instantaneously, got solidified, and attained spherical shape gradually. No agglomeration between the particles was observed. After 30 minutes, pellets were filtered, washed 3 times with equal volumes of distilled water and spread evenly on tissue paper bed. It was then dried in hot air oven at 60°C for 5 hrs.

The micropellets from different batches were found to be spherical in shape and off white in color. For higher concentrations of the polymers, occasionally minute tails appeared, but this problem was overcome by careful manufacture of the pellets (Table -1).

Table 1. *3^2 Factorial Design for Alginate Eudrajit Micropellets*

Batch no.	Levels of Sod. Alginate (X1)	Levels of Eudragit RS30D (X2)
DE-a	-1	-1
DE-b	-1	0
DE-c	-1	+1
DE-d	0	-1
DE-e	0	0
DE-f	0	+1
DE-g	+1	-1
DE-h	+1	0
DE-i	+1	-1

Translation of coded levels in actual units:

Levels (X1)	Conc.
-1	4%
0	5%
+1	6%

Levels (X2)	Conc.
-1	6%
0	8%
+1	10%

Physicochemical study of the micropellets

The prepared micropellets of diazepam were assessed for drug content by crushing a definite portion of the micropellets in a mortar, extracting the drug from an aliquot of the powder by 5 ml of ethanol for 2 hrs and the volume was made up to 100 ml by simulated gastric fluid pH 1.2. Two ml of the above solution was diluted to 25ml by the same fluid and this solution was analyzed spectrophotometrically at 240 nm against the blank prepared similarly without the powdered micropellets. The micropellets were also assayed by employing Indian Pharmacopoeia assay procedure, which yielded similar results.

Flowability Study

Angle of repose method was employed to assess the flow property of the micropellets. These were allowed to fall freely through a funnel fixed at 1 cm above the horizontal flat surface, until the conical pile just touches

the tip of the funnel. The angle of repose (θ) was determined by the following formula:

$$\theta = \tan^{-1} (H/R)$$

Where, H = cone height of the micropellets,
R = Radius of the circular base formed by the micropellets on the ground.

Granulometric Study

Particle size analysis of the micropellets was very important to know the size distribution pattern of the different batches of micropellets. The particles contained in the sieve of maximum retention were used for content determination and *in vitro* dissolution study.

To determine the percentage frequency distribution of particle size, sieve analysis was done using standard ASTM (American Society of Testing and Materials) sieves. The total amount of micropellets retained by each sieve was measured and percent retention vs. mess size was plotted in bar diagram.

Loose Surface Crystal Study

This was conducted to know the amount of drug present on the surface of the micropellets, which would be released immediately after placing the drug in dissolution media. In this study, 100 mg of the accurately weighed micropellets (#12 size) were suspended in dissolution medium and the drug leached out from the surface was analyzed spectrophotometrically at 240 nm wavelength.

Drug-Polymer Compatibility Study

The incompatibility between drug and polymers was assessed by subjecting them to Infra-Red Spectroscopic studies. The primary objective of the IR study was to detect any potential incompatibility or interaction between drug and polymers. Micropellets containing 2% sodium alginate, 6% eudragit RS 30D aqueous dispersion and 200mg of drug were grinded and passed through ASTM # 80 mesh sieve. Both the powder and drug were subjected to IR study using KBr pellets.

Atomic Absorption Spectroscopy (AAS)

AAS of some of the batches of micropellets (DE-c, DE-f & DE-i) were performed to find the residual sodium content in the micropellets. As during the formation of micropellets, sodium alginate is converted to calcium alginate, so the per cent of sodium present in the micropellets will represent the extent of completeness of reaction.

Accurately weighed 10 mg micropellets were demineralized by heating in a boiling water bath for 30 min after addition of 10 ml of conc. HNO_3 (Merck Pro analysis grade). The solutions were filtered by Whatmann filter paper No. 1. After suitable dilution with deionized water, the samples were analyzed by using Atomic Absorption Spectrophotometer against control.

***In-vitro* release study**

The micropellets of different batches were subjected to in-vitro dissolution study as per USP protocol (Table 2)

Table 2. *USP XXII Dissolution protocol for Diazepam Extended Release Capsules*

	Parameters	Specifications
1.	Dissolution medium	Simulated gastric fluid TS, prepared without enzyme, pH 1.2
2.	Volume of the medium	900ml
3.	Dissolution apparatus	Apparatus 1 (Basket type)
4.	RPM	100
5.	Temperature of the water bath	37 ± 1°C
6.	Time of sampling (hours)	0.0420; 0.167 0; 3.330; 0.5000. [D = Dosing Interval = 12hrs (for this study)]

In addition to the specified time of sampling, some other points of sampling were also chosen to observe the drug release pattern from the micropellets. At these time intervals, a fixed volume of sample (5ml) were taken out from the dissolution medium and substituted by equal volumes of fresh medium. The withdrawn samples were diluted considerably (to 25ml) and the drug contents in the samples were determined by using a Beckman DU64 spectrophotometer at 240nm.

RESULTS AND DISCUSSIONS

Physicochemical study of the micropellets

Table 3 shows the drug content of different batches of the micropellets:

Table 3. *Formulation Characteristics*

Batch code	Amount of sodium alginate (mg)	Amount of Eudragit RS 30D (mg)	Drug (mg)	Expected drug content (% of total wt)	Actual drug content (% of total wt)	Drug entrapment efficiency (%)
DE-a	1000	1500	200	7.407	6.563	88.60
DE-b	1000	2000	200	6.250	5.580	89.28
DE-c	1000	2500	200	5.405	5.309	98.21
DE-d	1250	1500	200	6.799	5.400	79.66
DE-e	1250	2000	200	5.797	5.151	88.85
DE-f	1250	2500	200	5.033	4.376	86.43
DE-g	1500	1500	200	6.250	5.016	80.26
DE-h	1500	2000	200	5.405	4.44	82.15
DE-i	1500	2500	200	4.760	4.16	87.39

Flowability Study

In the above batches, the angle of repose was less than 15°, which indicated that the micropellets were highly free flowing.

Granulometric Study

The maximum yield for most of the batches occurred at ASTM #12 to #16 mesh sieves. When high concentrations of polymers were used, the particle diameter consequently increased presumably due to higher viscosity.

Loose surface Crystal Study

The Loose Surface Crystal study is an important parameter to know the bursting effect of the micropellets when it comes in contact with the GI fluid. From Table 4, it can be concluded that for a particular concentration of sodium alginate, as the concentration of eudragit RS 30D increased, the amount of drug present on the surface of the micropellet decreased.

Table 4. *Losse Surface Crystal quantification*

Batch code	Amount of drug released after 5 mins (mg)	Drug content (mg)	LSC (%)
DE-a	0.6215	6.563	9.47
DE-b	0.4649	5.619	8.27
DE-c	0.2705	5.454	4.96
DE-d	0.7835	5.351	14.64
DE-e	0.4271	5.451	7.84
DE-f	0.2921	4.520	6.46
DE-g	0.5729	5.036	11.38
DE-h	0.4757	4.555	10.44
DE-i	0.2381	4.171	5.71

Drug-Polymer Compatibility

The major peaks obtained from the spectra of the pure drug were retained in the spectra of the alginate-eudragit-drug formulation also, indicating the absence of any interaction between the drug and the polymers (Data not shown). Hence, no potential drug-polymer interactions were revealed in the study.

Atomic Absorption Spectroscopy (AAS)

The residual sodium content of the micropellets was found to be <1% indicating completeness of the calcium alginate formation.

In-vitro **release study**

From the Coefficients of determination (R^2) values of the drug release profiles of different batches, it is obvious that release profile follows Higuchi release kinetics. So, in the graphical representation, cumulative percent release was plotted against square root of time. Both the concentrations of sodium alginate and Eudragit RS 30D have significant effect on the drug release rate from the micropellets. From the graphs (Fig; 1,2,3), it is obvious that for a particular concentration of sodium alginate, as the concentration of Eudragit RS 30D was increased, the cumulative percentage of drug release at a particular time decreased. This was obvious, as Eudragit RS 30D is a rate-controlling polymer. Consistently with all batches, the release kinetics obtained was following Higuchi square root equation as evident from the sigmoidal nature of the release profiles, as well as the highest coefficient of determination values obtained from regression analysis of release data of all formulations (Data not shown). When the USP Protocol limits were compared with the release data obtained, the formulations were found to be compliant at several time points as shown in Tables 5 - 7.

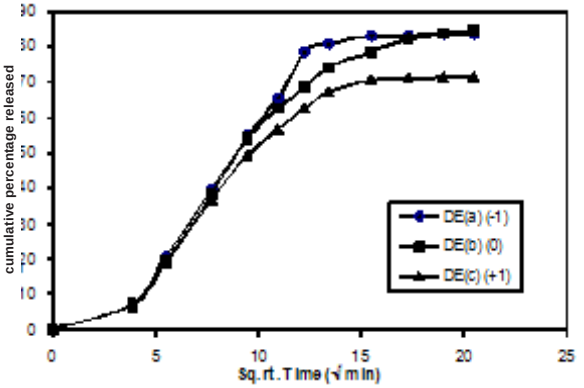

Fig.1. *Release profile of diazepam from micropellets with low alginate level*

Table 5. *USP compliance matrix at low alginate level*

Time (min)	Percent cumulative release			USP compliance matrix		
	DE(a) (-1)	DE(b) (0)	DE(c) (+1)	DE(a) (-1)	DE(b) (0)	DE(c) (+1)
0	0	0	0			
15	7.07	6.99	6.81			
30	20.43	19.2	18.66	C	C	C
60	39.75	38.38	36.66			
90	54.65	54.12	49.38			
120	66.48	62.46	66.66	C	C	C
150	78.5	68.89	62.96			
180	80.79	74.27	67.1			
240	88.02	78.66	70.73	C	C	X
300	83.16	82.15	70.95			
360	83.4	83.9	71.2	X	X	X
420	83.6	84.6	71.5			

C = Complied; X = did not Comply

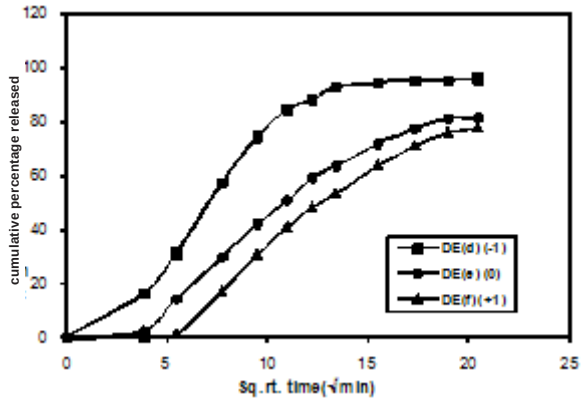

Fig. 2. *Release profile of diazepam from micropellets with medium alginate level*

Table 6. *USP compliance matrix at medium alginate level*

Time (min)	Percent cumulative release			USP compliance matrix		
	DE(d) (-1)	DE(e) (0)	DE(f) (+1)	DE(d) (-1)	DE(e) (0)	DE(f) (+1)
0	0	0	0			
15	16.48	27.2	0.9			
30	31.17	14.86	1.71	X	C	C
60	57.27	29.15	17.43			
90	74.3	42.19	30.95			
120	84.43	60.76	41.11	X	C	X
150	88.14	59.28	48.44			
180	92.73	63.79	53.52			
240	94.88	71.85	64.19	C	X	X
300	95.04	77.35	70.97			
360	95.19	81.31	76.05	C	X	X
420	95.8	81.4	77.76			

C = Complied; X = did not Comply

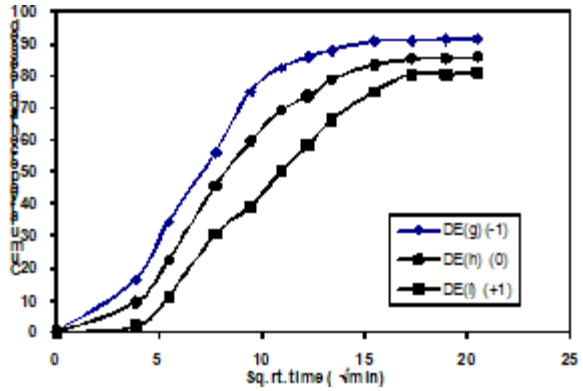

Fig. 3. *Release profile of diazepam from micropellets with high alginate level*

Table 7. *USP compliance matrix at high alginate level*

Time (min)	Percent cumulative release			USP compliance matrix		
	DE(g) (-1)	DE(h) (0)	DE(i) (+1)	DE(g) (-1)	DE(h) (0)	DE(i) (+1)
0	0	0	0			
15	16.52	9.35	1.81			
30	34.53	22.62	11.13	X	C	X
60	58.94	45.55	30.4			
90	74.95	59.89	39.24			
120	82.38	69.26	50.86	X	X	C
150	85.75	73.69	58.46			
180	87.71	78.63	66.18			
240	90.68	83.68	74.95	C	C	X
300	90.82	85.25	80.24			
360	91.23	86.46	80.27	C	C	X
420	91.48	85.69	80.89			

C = Complied; X = did not Comply

Optimization[11]

The selected response for study was the release rate constant (K) of Higuchi model. Suitable first order and second order models were used to fit the data after analysis of variance study and test of significance of regression using computer programs. Two way ANOVA study for determining the significance of linear and quadratic interaction terms were also carried out separately.

Table 8. *Rate of Release of drug from micropellets (K_{HC})*

Sod. Alginate		Eudragit RS30D			$Y_{i.}$
		-1	0	+1	
-1	R1	4.7655	4.7191	3.988	
	R2	4.8740	4.9693	4.3524	
	Total	9.6395	9.6884	8.3404	27.6683
0	R1	5.0448	4.6683	4.6162	
	R2	5.0999	4.5922	4.2724	
	Total	10.1447	9.2605	8.8886	28.2938
+1	R1	4.7214	4.7207	4.7846	
	R2	4.8933	4.9313	5.2038	
	Total	9.6147	9.6520	9.9884	29.2551
$Y_{.j}$		29.3989	28.6009	27.2174	$Y_{...}$ = 85.2172

$SS_T = \sum\sum Y_{ij}^2 - [Y_{...}^2/abn] = 1.5480$

Table 9. *Polynomial effects of Sodium Alginate (A) and Eudragit(E)*

Treatment totals		Orthogonal Contrast Coefficients		Totals
Sod. Alginate ($Y_{i.}$)	Eudragit RS30D ($Y_{.j}$)	Linear	Quadratic	
27.6683	29.3989	-1	+1	
28.2938	28.6009	0	-2	
29.2551	27.2174	+1	+1	
Effects:		A_L = 1.5868	A_Q = 0.3358	
		E_L = -2.1815	E_Q = -0.3855	
Sum of Squares:		SS_{AL} = 0.2098	SS_{AQ} = 0.0031	0.2129
		SS_{EL} = 0.3966	SS_{EQ} = 0.0095	0.4061

$SS_{AL} = (A_L)^2/an\sum C_i^2$ $SS_{AQ} = (A_Q)^2/an\sum C_i^2$ $SS_{EL} = (E_L)^2/bn\sum C_i^2$ $SS_{EQ} = (E_Q)^2/bn\sum C_i^2$

Table 10. *Two way ANOVA for Alginate Eudragit Micropellets by 3^2 factorial design*

Source of variation	Sum of Square	D.F.	Mean Square	F_0	$F_{0.05}$	P-Value
Sod. Alginate, A	0.21293	2	0.106465	3.28320952	4.256492	0.084965
A_L	0.2098	1	0.2098	6.48888488	5.117357	0.031824
A_Q	0.0031	1	0.0031	0.09589902	5.117357	0.764214
Eudragit RS30D, E	0.4061	2	0.20305	6.26178672	4.256492	0.019769
E_L	0.3966	1	0.3966	12.2306067	5.117357	0.006763
E_Q	0.0095	1	0.0095	0.29296473	5.117357	0.601466
AE Interaction	0.637121	4	0.15928	4.91188928	3.63309	0.022811
AE_{LL}	0.34978	1	0.34978	10.7886831	5.117357	0.008464
AE_{LQ}	0.11985	1	0.11985	3.69598141	5.117357	0.086709
AE_{QL}	0.1049	1	0.1049	3.23494744	5.117357	0.109525
AE_{QQ}	0.0525	1	0.0525	1.62139957	5.117357	0.198442
Error	0.291844	9	0.032427 11			
Total	1.5480	17				

$R^2 = SS_{model}/SS_{Total} = 0.8115$

$R_{adj}^2 = [(n-1)/(np)](1-R^2) = 0.70865$

The two-way ANOVA study, incorporating the effect of individual polymers and their linear, quadratic and interaction terms (Tables 8 - 10), shows that both the linear effects of the polymers are significantly affecting the release rate as evident from their calculated F0 values and they are found to be significant at $p< 0.05$. It was further found that the overall and linear interactions between the polymers have significant contributions to the modulation of drug release. Regarding the other terms, due to lack of high degrees of freedom associated with them, the predictability of the statistical model incorporating them can only be ascertained after rigorous multiple regression analysis.

Table 11. *Data-table for full polynomial model*

X1	X2	Ya	Yc	Ea	E(%)
-1	-1	4.7655	4.81975	-0.05425	-1.13839
-1	-1	4.874	4.81975	0.05425	1.113049
-1	0	4.7191	4.8442	-0.1251	-2.65093
-1	0	4.9693	4.8442	0.1251	2.517457
-1	1	3.988	4.1702	-0.1822	-4.56871
-1	1	4.3524	4.1702	0.1822	4.186196
0	-1	5.0448	5.07235	-0.02755	-0.54611
0	-1	5.0999	5.07235	0.02755	0.540207
0	0	4.6683	4.63025	0.03805	0.815072
0	0	4.5922	4.63025	-0.03805	-0.82858
0	1	4.6162	4.4443	0.1719	3.723842
0	1	4.2724	4.4443	-0.1719	-4.0235
1	-1	4.7214	4.80735	-0.08595	-1.82043
1	-1	4.8933	4.80735	0.08595	1.756483
1	0	4.7207	4.826	-0.1053	-2.2306
1	0	4.9313	4.826	0.1053	2.13534
1	1	4.7846	4.9942	-0.2096	-4.38072
1	1	5.2038	4.9942	0.2096	4.027826

[Key: X1 = Alginate level; X2= Eudragit RS30D level;
Ya= Actual response (release rate constant)
Yc= Calculated response (from model); Ea= Absolute error (= Ya- Yc);
E(%) = per cent error]
Full Polynomial model:
$(a+b*x1+c*x2+d*x1^2+e*x2^2+f*x1^2*x2+g*x1*x2^2+h*x1^2*x2^2)$

Therefore, we carried out multiple regression analysis on the release rate kinetics data for ascertaining and building a mathematical model describing the behavior of the Alginate-Eudragit system for modulating the drug –diffusion within our design space (-1 to +1 in coded terms). We had enough design points and thus degrees

of freedom for generating the full quadratic polynomial response model involving the linear, quadratic, linear-quadratic as well as biquadratic terms (Table 11). Within the stipulated non-linear iteration limit of 250 iterations and at a residual tolerance of 10^{-10}, the data were analyzed by a commercial statistical program.

The full polynomial response model thus obtained (in coded terms) was:

$$Y = 4.63025 - 0.0091*\mathbf{x1} - 0.31402*\mathbf{x2} + 0.20485*\mathbf{x1}^2 + 0.128075*\mathbf{x2}^2 + 0.19835*\mathbf{x1}^2*\mathbf{x2} + 0.212*\mathbf{x1}*\mathbf{x2}^2 + 0.2091*\mathbf{x1}*\mathbf{x2} - 0.2653*\mathbf{x1}^2*\mathbf{x2}^2$$

$F_0 = 4.841229201$
Standard Error of the Estimate (SE)= 0.1800950243
Coefficient of Multiple Determination (R^2)
= 0.811438689
Adjusted coefficient of multiple determination (Ra^2)
= 0.6438286347

The t-test on individual regression coefficients led us to discard a few terms which had high p-values and which showed large individual variations for all of the confidence intervals. The F_0 ratio from the variance analysis of the model was significant at $p<0.015$ which indicated further scope of refinement on the model. The associated contour plot gives a graphical representation of the model (Figure 4).

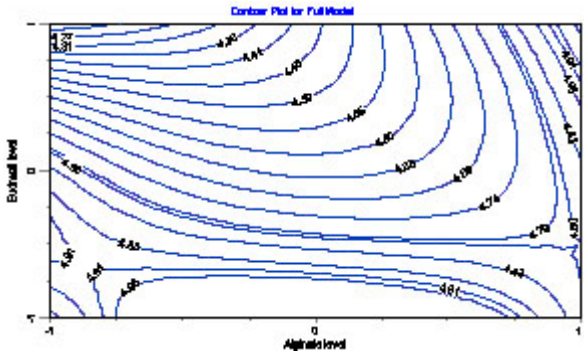

Fig. 4. *Contour Diagram of release rate response vs. polymer levels*

CONCLUSION

The objective of the present study was to develop an aqueous solvent based technology for manufacturing multiparticulate oral drug delivery system of a potent anti-anxiety drug, Diazepam, using a hydrophilic swellable natural polymer, Sodium Alginate and a polymethylmethacrylate copolymer, Eudragit RS30D.

In the present study, it was found that at high alginate and Eudragit levels (+1,+1) the release conforms to the USP guidelines, though the other formulations also follow the same in varying degrees.

The authors further studied the statistical properties of the release kinetics and developed mathematical models describing the system behavior within the preset boundary limits of polymer levels. The model was validated and optimized to ascertain the best possible combinations of the two polymers to be used to arrive at the desired formulation.

Therefore, this study has shown the potentiality of an aqueous solvent based technology in the development of extended release drug delivery system for effective clinical management of anxiety using the combination of natural and synthetic polymers. This particular method avoids the use of organic solvents and thus provides safe, reproducible and less time consuming methods for the production of micropellets and is also industrially feasible. Due to the inherent mucoadhesive nature of alginate, it is expected that these micropellets would be retained over a longer time-period in the stomach, which is the predominant absorption site of diazepam, thus further extending their utility. There is an urgent need for development of more such eco-friendly technologies keeping in view the stringent environmental regulations.

REFERENCES

1. Montgomery AE, *Anxiety and Depression*, Wrightson Biomedical publishing Ltd., 1990, pp 23-30, 75-84.
2. Baldessarini RJ, Drugs and the treatment of psyachiatric disorders, in *The Pharmacological Basis of Therapeutics* (Goodman Gilman A., Rall T.W., Nies A.S. and Taylor P. Eds); 8th Ed., Pergamon Press, NY, USA.
3. Riss J, Cloyd J, Gates J, Collins S, Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. Acta Neurol Scand Epub 2008 Mar31. Review 2008 Aug;118(2):69-86.
4. Montandon A, Skreta M, Riggenbach H, Ward J, Comparison of controlled-release diazepam capsules and placebo in patients in general practice. Curr Med Res Opin. 1986;10(1):10-6.
5. Abdelbary G, Fahmy RH. Diazepam-loaded solid lipid nanoparticles: design and characterization. AAPS PharmSciTech. 2009;10(1):211-9.
6. Wahlgren M, Christensen KL, Jørgensen EV, Svensson A, Ulvenlund S. Oral-based controlled release formulations using poly(acrylic acid) microgels. Drug Dev Ind Pharm. 2009 Aug;35(8):922-9.
7. Silvestri TM, Wills RJ. Pharmacokinetics of diazepam during multiple dosing of a 6-mg controlled-release capsule once daily. Ther Drug Monit. 1988;10(1):64-8.

8. Abed KK, Hussein AA, Ghareeb MM, Abdulrasool AA. Formulation and optimization of orodispersible tablets of diazepam. AAPS PharmSciTech. 2010 Mar;11(1):356-61. Epub 2010 Mar 16.

9. Wills RJ. Pharmacokinetics of diazepam from a controlled release capsule in healthy elderly volunteers. Biopharm Drug Dispos. 1984 Jul-Sep;5(3):241-9.

10. United States Pharmacopoeia XXII, 22rd rev., United States Pharmacopoeial Convention Inc., Rockville, MD, 1995.

11. Kempthorne O, Experiments involving factors with three levels, In "*The Design and Analysis of Experiments*", John Wiley & Sons, Inc., NY, USA, 1951, pp 300-305.