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Research Article

OPTIMIZATION OF FORMULATION OF MULTIPARTICLES CONTAINING CALCIUM ION INFLUX INHIBITOR

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ABSTRACT
The current investigation was aimed to reduce dosing frequency and improve patient compliance by designing and systematically evaluating sustained release multi particles containing Verapamil hydrochloride (VH), a BCS class I calcium channel antagonist. Frequent administration and variable low bioavailability (40-60%) after oral administration are problems of conventional dosage forms of VH, which can be attenuated by designing it in the form of multi particles which would prolong the residence time at the absorption site, to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability. VH loaded multi particles were prepared by double emulsion solvent evaporation technique using Gelatin and Eudragit RS 100 as rate controlling polymers. The formulation was characterized by SEM, DSC, XRD, Entrapment efficiency, *In-Vitro* dissolution study. The SEM indicated that the multi particles were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state, which was further confirmed by x-ray diffraction analysis. The *in-vitro* dissolution showed wash-off was faster at simulated intestinal fluid (phosphate buffer, pH 7.4) than that at simulated gastric fluid (0.1 M HCl, pH 1.2). The *in-vitro* drug release mechanism was fickian type controlled by swelling and relaxation of polymer. There was no significant change in drug content and cumulative drug release of drug-loaded microspheres stored at different storage condition after 6 months of study.

Keywords: *Calcium Blocker, Emulsion, Release, Kinetics*

INTRODUCTION
Modified release formulation technologies offer an effective means to optimize the bioavailability and resulting blood concentration time profile of drugs (Padhee *et al.*, 2011; Charman and Charman, 2003). Drug release from the delivery devices can be sustained up to 24 h for many drugs using current release technologies. However, the real issue in the development of oral controlled release dosage forms is to prolong the residence time of the dosage form in the stomach or upper gastrointestinal tract until the drug is completely released (Baumgartner *et al.*, 2000). The transit of drug or formulation through gastrointestinal tract will determine how long a compound will be in contact with its preferred absorptive site (Jain *et al.*, 2004). Prolonged gastric retention improves bioavailability, reduces drug waste and improves solubility for drugs that are less soluble in a high pH environment. It has also applicable for local drug delivery to the stomach and proximal small intestine (Cuna *et al.*, 2001). Several approaches are currently used to retain the dosage form in the stomach.
Verapamil hydrochloride is a phenyl alkylamine calcium channel blocker (acts on L-type calcium channels in the heart causes a reduction in ionotropy and chronotropy, thus, reducing heart rate and blood pressure) (Sweetman, 2009). Approximately about 90% of Verapamil is absorbed from GIT, but is subjected to very considerable first-pass metabolism in the liver and the bioavailability is only about 20%. Verapamil exhibits bi-or-tri-phasic (Sean, 2011; Dollery, 1999) elimination kinetics and is reported to have a terminal plasma half-life of 2 to 8 hrs following a single oral dose or after intravenous administration. After repeated oral doses this increases to 4.5 to12 hrs (Sichelbaum and Somogyi, 1984) (Aboutaleb *et al.*, 2013). It acts within 5 mins of intravenous administration and in 1 to 2 hrs after an oral

dose. There is considerable inter individual variation in plasma concentrations (Thummel and Shen, 2001; Passerini *et al.,* 2003; Vidhyadara *et al.,* 2014; Tanwar *et al.,* 2007). Thus, there is a strong clinical need and market potential for a dosage form that will deliver Verapamil hydrochloride in a controlled manner to a patient needing this therapy, thereby resulting in better patient compliance.

The physico-chemical properties of Verapamil and is shorter half-life make its suitable molecule for preparation of floating microspheres. The objective of the present study is to develop suitable gastroretentive floating microspheres of Verapamil HCL and to study release kinetics of drug with a view to reduce the dose frequency and to achieve a controlled drug release with improved bioavailability.

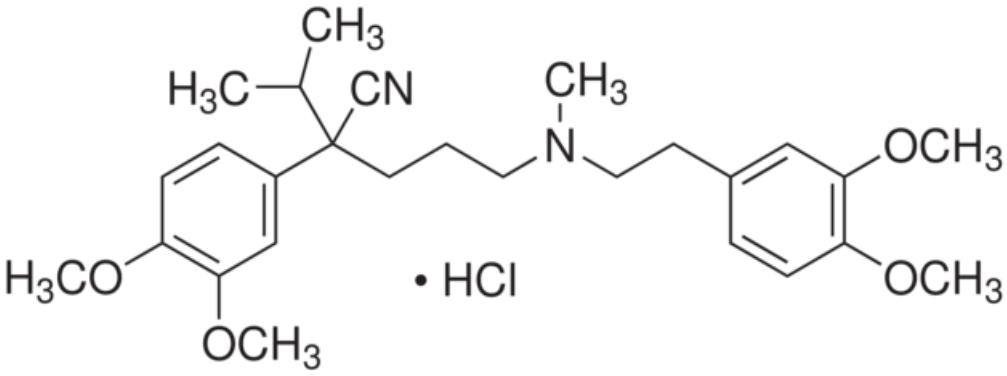

Figure 1: Structure of Verapamil Hydrochloride (VH)

MATERIALS AND METHODS

Materials

Verapamil hydrochloride was obtained as a gift sample from M/s Apotex labs Bangalore. Eudragit RS 100 was obtained as gift sample from Watson labs. Gelatin was obtained as a gift sample from M/s Mylan labs, Hyderabad. Other chemicals such DMSO (dimethyl sulfoxide), Tween 80, Span 80, Liquid Paraffin, Glutaraldehyde used were of laboratory grade.

Methods

Preparation of Standard Calibration Curve of Verapamil HCl

Verapamil HCl exhibits peak absorbance at 278 nm in distilled water. 100 mg of Verapamil HCl was accurately weighed and dissolved in sufficient amount of distilled water taken in 100 ml volumetric flask, finally the volume was made up to 100ml with the same (1000 µg/ml) which is a primary stock solution. 10 ml of the above solution was pipette out into second 100ml volumetric flask; volume was made up with distilled water to get a concentration of 100µg/ml, which is the Secondary stock solution. Aliquots of 0.5ml, 1ml, 1.5 ml, 2ml, 2.5 ml, 3 ml, 3.5ml, 4ml, 4.5ml, 5ml, 5.5ml, 6ml, 6.5ml, 7ml, and 7.5ml were pipette out from the working standard solution and transferred into 10 ml volumetric flask. Then, the volume was made up with distilled water to get a concentration which falls within the Beer's range 5 – 75µg/ml. The absorbance of these solutions was measured at 278 nm against reagent blank.

Formulation of Verapamil HCl Multi Particles

Multi particles were prepared by adopting o/w/o double emulsion-solvent evaporation technique. Gelatin was dissolved in hot water followed by addition of appropriate amount of drug (external aqueous phase). To this tween 80 was added. Weighed quantity of polymers was dissolved in Dimethylsufoxide (internal non aqueous phase). Internal non aqueous phase was slowly added to aqueous phase with vigorous stirring at 3000 to form primary o/w emulsion. The above solution is stirred for 10 minutes. The primary emulsion formed was then added to external non aqueous phase containing span 80 and liquid paraffin to form o/w/o emulsion. Stirring was continued, allowed the DMSO to get evaporated. Further glutaraldehyde was used as cross linking agent and added to the emulsion. Then, the multiple emulsion was kept for overnight stirring. The formed multi particles of various batches (Table 1) were filtered and washed with acetone and petroleum ether to remove oil and kept overnight drying in hot air oven at 40^0C. The dried multi particles were stored in a desicator till further analysis.

Table 1: Formulation of Various Batches of Verapamil Hydrochloride (VH) Multi Particles

Ingredients	F1	F2	F3	F4	F5
Drug	100	100	100	100	100
Gelatin (%)	15	15	15	15	15
Gluteraldehyde (%)	5	5	5	5	5
Cellulose acetate	5	--	--	--	--
Cellulose acetate phthalate (%)	--	5	--	--	--
Xanthan gum (%)	--	--	5	--	--
Eudragit RS100 (%)	--	--	--	5	10
Tween 80 (%)	4	4	4	4	8
Span 60 (%)	2	2	2	2	2
Dichloromethane (ml)	10	10	10	10	10
Water (ml)	20	20	20	20	20
Liquid paraffin (ml)	60	60	60	60	60
Parameters Evaluated					
Sphericity	Irregular	Flakes	Coagulation	Spherical	Spherical
EE %	20	--	--	71.34	71.26
Appearance	Light brown	--	--	Light brown	Dark brown

Characterization Studies of Multi Particles

SEM studies

The surface morphology of multi particles was examined by using scanning electron microscope. The samples were mounted on a cleaned brass specimen studs using double sided tape and then gold coated in vacuum by sputter coater. Then, the samples were observed at excitation voltage of 20 KV in the Joel 840A scanning electron microscope, Jeol- Japan with JFC – 1100E ion sputtering device.

XRD studies

The X-ray diffractograms for the pure drug and the optimized multi particulate formulation were obtained using on X-ray diffraction instrument (Bruker D8 Advance Powder XRD) with Ni-filtered, Cu radiation as anode target at $1.5406A^0$ at a voltage of 40 kV and current of 30 mA. The scan was performed in the range of 3^0 2Ɵ to 45^0 2Ɵ with 15 sec as time per step and step size is 0.01^0 2Ɵ.

DSC studies

The DSC of pure Verapamil hydrochloride and the optimized multi particles preparation was carried out by using DSC apparatus Metler Toledo STAR SW model with a scan range from 30 to 300°C with a ramp (heating rate) of 10°C per minute. The sample holder was Standard 40µl Aluminium crucible (pin holed). About 3 to 6 mg of sample powder was taken into a standard aluminium crucible and crimped with a cap. Crucible was pin holed before putting into analysis. Data was collected from 30° C to 300°C with ramp of 10°C per minute. The position and intensities of thermograms were considered for the identification and comparison of drug or formulation.

Entrapment efficiency

Multi particles were washed with cold water and filtered and the residue was used for determination of the entrapment efficiency. Multi particles equivalent to 10mg of drug were accurately weighed and transferred to 100ml volumetric flask. To this flask, sufficient amount of distilled water was added to extract the drug from the multi particles. Then, volume of flask was made up to the mark with same solvent. From this, further suitable dilutions were made and drug content were analysed by UV spectrophotometrically at 278nm. It can be calculated by using the formula:

$$\textbf{Entrapment efficiency} = \frac{\textbf{Drug content after washing}}{\textbf{Drug loading}} \times 100$$

Particle size determination

The multi particles were evaluated for particle size. The multi particles sizes (*n*= 50) were taken for particle size analysis and average particle size was determined. Particle size of the multi particles was measured using a LYNX (Lawrence & Mayo) microscope fitted with stage micrometer.

In-Vitro dissolution studies (Venkateshwara and Navaneetha, 2015)

The dissolution studies were carried out according to USP XXIII apparatus 2 (rotating paddle method - model TDT-08L, M/s. Electrolab). 900ml of 0.1N HCl for 2 hours and 900ml of 7.4 pH phosphate buffer for 6 hours was used as the dissolution medium, which was maintained at $37^0C \pm 0.5^0C$ and stirred at 75rpm. At appropriate time intervals, 2ml samples were withdrawn and volume made upto 10ml. The concentration of Verapamil Hydrochloride was determined by UV spectroscopy at 278nm using blank solution. All experiments were performed in triplicate.

Curve fitting analysis

Release data of formulations TD1 to TD9 were fitted to various mathematical models for describing the release mechanism from the prepared multi particle formulations. Korsmeyer-Peppas (Equation (1)), Zero-order (Equation (2)), Higuchi release models (Equation (3)) and First-order (Equation (4)).

$$\frac{M_t}{M_\infty} = k_{KP} t^n \qquad (1)$$

M_t/M_∞ = fraction of drug released at time't', k_{KP} is the release rate constant, and n = diffusion coefficient.

$$M_t = M_0 + k_0 t \qquad (2)$$

M_t = amount of drug released at time't', M_0 = concentration of drug in the solution at t = 0, k_0 = zero-order release constant.

$$M_t = k_H t^{1/2} \qquad (3)$$

M_t = amount of drug release at time '$\sqrt{t}$', k_H = Higuchi release constant.

$$LogM_t = LogM_0 - k_1 t / 2.303 \qquad (4)$$

M_t = amount of drug released at time't', Log M_0 = initial concentration of drug in the solution at t = 0, k_1 = first-order release constant.

All curve fitting, simulation and plotting was carried out by using commercially available Sigma Plot R version 9 (Systat Software, Inc.).

Stability studies

Stability studies were conducted on the optimized formulation. The optimal formulations were packed in capsule as dosage form in a screw capped ambered coloured glass container with closure secured tightly on the container. The containers were then exposed to 40°C ± 2°C/ 75% ± 5% RH in a stability chamber (396LAG, Remi Instruments division, India) as per ICH guidelines for a period of 6 months. At predetermined time intervals, samples were taken out and the multi particles were subjected to various physico-chemical parameters viz. percent entrapment efficiency and particle size. At the end of stability studies, the formulation was also studied for *in vitro* drug release profile. To confirm the similarity of the drug release profiles before and after stability studies, a model independent statistical tool for comparison of dissolution profile Similarity Factor (f_2) was used.

$$f_2 = 50 \cdot \log\{[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} * 100\}$$

Design of experiment

A Taguchi design was adapted (Design expert version 6.05) to study the effect of four independent variables on the formulation of Verapamil hydrochloride multi particles system as per Table 2.

Table 2: Taguchi Design for the Formulation of Verapamil HCl Multi Particulateate System

Factor	Independent Variables	Type	Lower Level	Higher Level
X_1	Conc. of Gelatin	Numeric	10	20
X_2	Conc.of Eudragit RS100 polymer %	Numeric	5	10
X_3	Ratio of Non-Aqueous to Aqueous phase volume	Numeric	2	6
X_4	Conc. of Tween 80 %	Numeric	4	12
Other parameters				
Conc. of drug mg		100		
Stirring Speed rpm		1200		
Conc. of Span 80 %		4		
Volume of external phase ml		80		
Conc. of Glutaraldehyde %		10		
Response variables				
Y_1		% Entrapment Efficiency (%EE)		
Y_2		% Yield		
Y_3		Particle Size µm		

RESULTS AND DISCUSSION

Preparation of Standard Calibration Curve of Verapamil Hydrochloride

The standard calibration curve for BCS Class I drug Verapamil hydrochloride was carried out in distilled water. The results are presented in Table 5. The linear regression was done on absorbance data points. The slope was 0.011 with a correlation coefficient of 0.9996 (Figure 5). A straight-line equation (Y = mx + c) was generated to facilitate the calculation of amount of drug. The equation Absorbance = 0.011 x Concentration will further be used for all further calculations for drug content, entrapment efficiency and *in vitro* release profile.

Table 3: Standard calibration curve of Verapamil hydrochloride in distilled water

Sl. No	Concentration (µg/ml)	Average Absorbance	SEM (±) n=5
1	5	0.067	0.0107
2	10	0.111	0.0049
3	15	0.167	0.0048
4	20	0.219	0.0032
5	25	0.271	0.0030
6	30	0.328	0.0076
7	35	0.382	0.0051
8	40	0.441	0.0046
9	45	0.491	0.0042
10	50	0.551	0.0009
11	55	0.609	0.0062
12	60	0.652	0.0012
13	65	0.718	0.0020
14	70	0.761	0.0041
15	75	0.823	0.0074

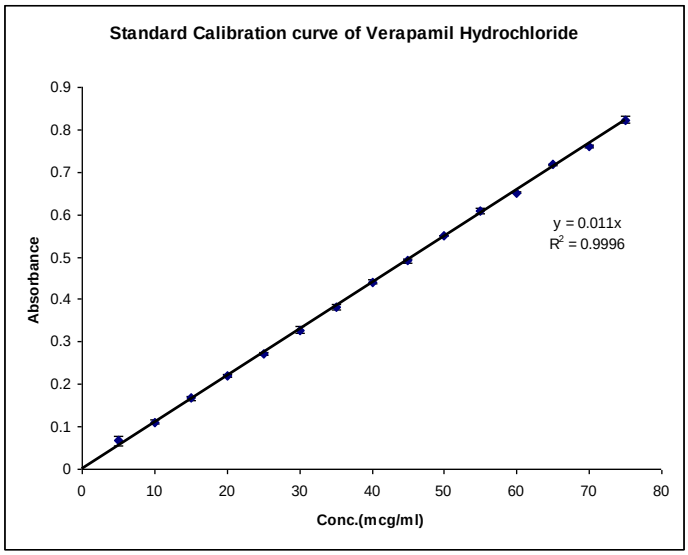

Figure 2: Standard Calibration Curve of Verapamil Hydrochloride

Linear Regression Analysis:
The linear regression was done on absorbance data points. The results are presented as follows.
For standard curve of Verapamil HCl:
The slope = 0.011
The correlation coefficient = 0.9996
A straight-line equation (Y = mx + c) was generated to facilitate the calculation of amount of drug. The equation is as follows:
Absorbance = 0.011 x Concentration

Formulation of Multi Particles

Verapamil Hydrochloride micro particulates were successfully prepared by adopting o/w/o double emulsion solvent evaporation technique using Gelatin and Eudragit RS 100 as rate controlling polymers.

Characterization Studies of Multi Particles

SEM Studies:

Characterization studies viz., scanning electron microscopy, X-Ray diffraction and differential scanning colorimetry were carried out. The result of the optimized formulation shows that the experimental particle size was found to be 229.92± 12.27µm. This is confirmed by obtaining the SEM of the formulation (Figure 3).

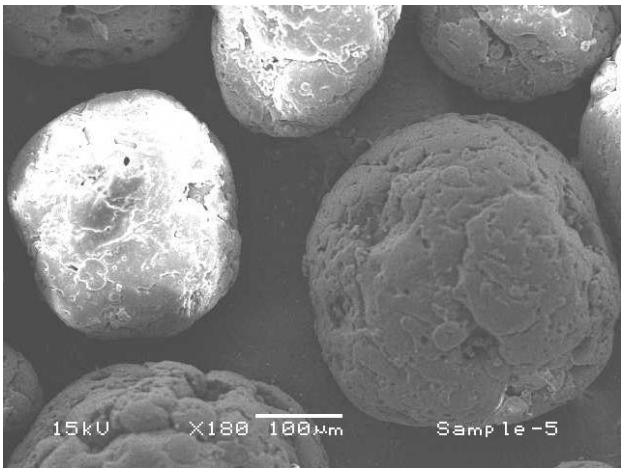

Figure 3: SEM Photograph of Optimized Formulation of Verapamil Hydrochloride

XRD studies

The diffraction peaks of Verapamil Hydrochloride and the optimized formulation are given in Figure 4 and 5 for comparison purposes. The diffractogram of pure drug reveals its highly crystalline nature, as indicated by the numerous distinctive peaks. The peak position (angle of diffraction) is an indication of crystal structure in which, peak height is the measure of sample crystallinity (Figure 4). A lack of numerous intense peaks (Figure 5) signifies that the drug is distributed homogenously in an amorphous state within the multi particulate formulation without any interaction. The crystallinity of the drug is lost.

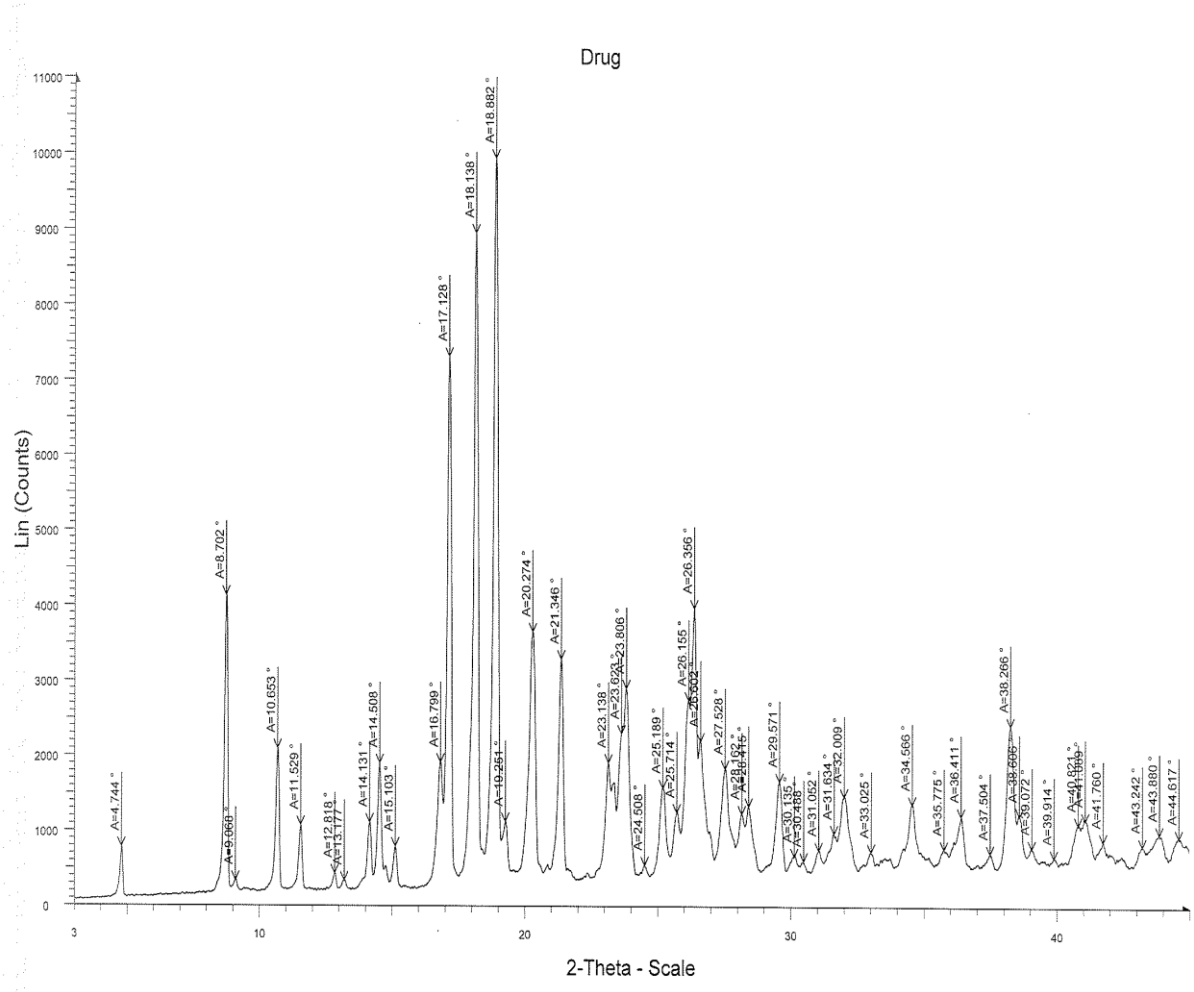

Figure 4: XRD of Pure Verapamil Hydrochloride

DSC studies

Differential Scanning Calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e. endothermic and exothermic phase transformations). The DSC thermogram of Verapamil Hydrochloride is shown in Figure 6 and 7.

A single endothermic peak corresponding to the melting point of pure Verapamil Hydrochloride was observed at 146.57^0C. The onset of melting point was observed at 145.54ºC. The pure Verapamil Hydrochloride peak disappears completely in the optimized formulae, thereby confirming the multi particulate formation.

In-Vitro Dissolution studies:

The *in-vitro* drug release profiles of the optimized multi particulate formulation before and after stability studies are presented in Table 4. The release profiles appear to be super imposable and the calculated f_2 value was found to be 66.26 (Figure 8 and 9).

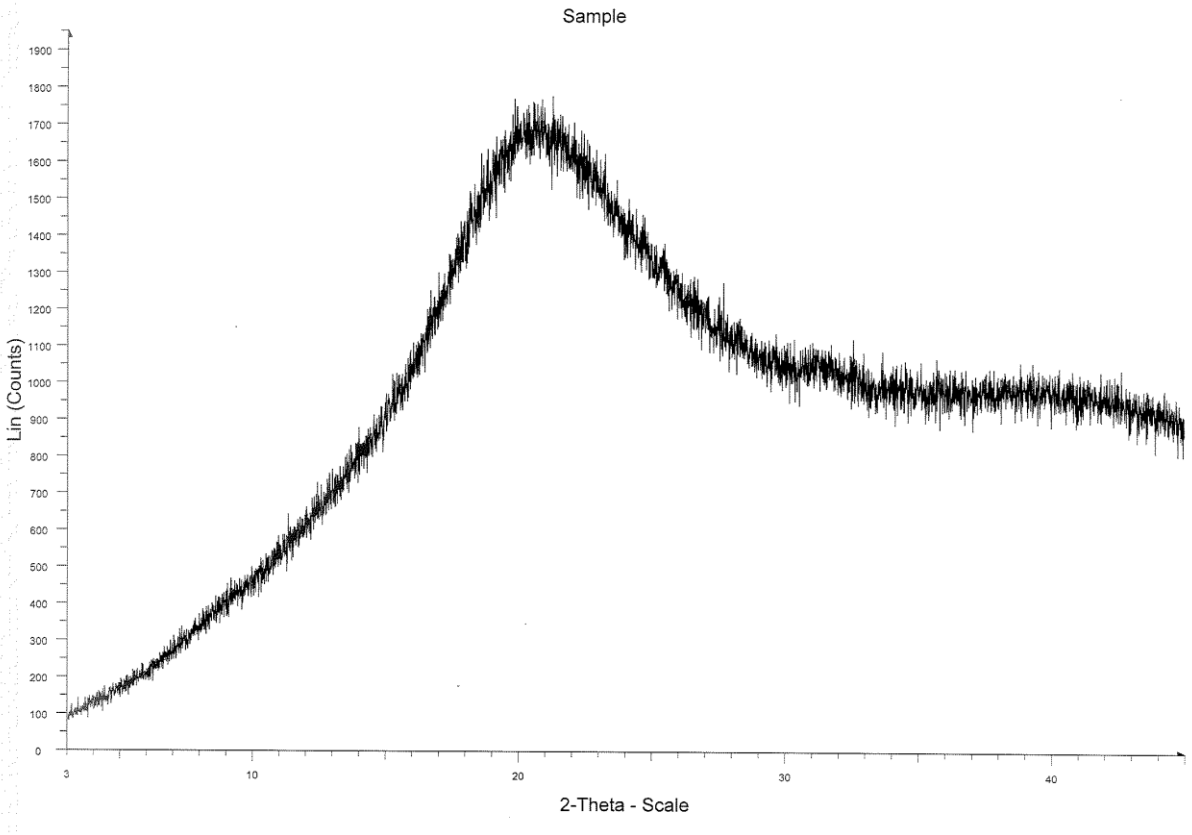

Figure 5: XRD of Optimized Verapamil Hydrochloride Multi Particulate Formulation

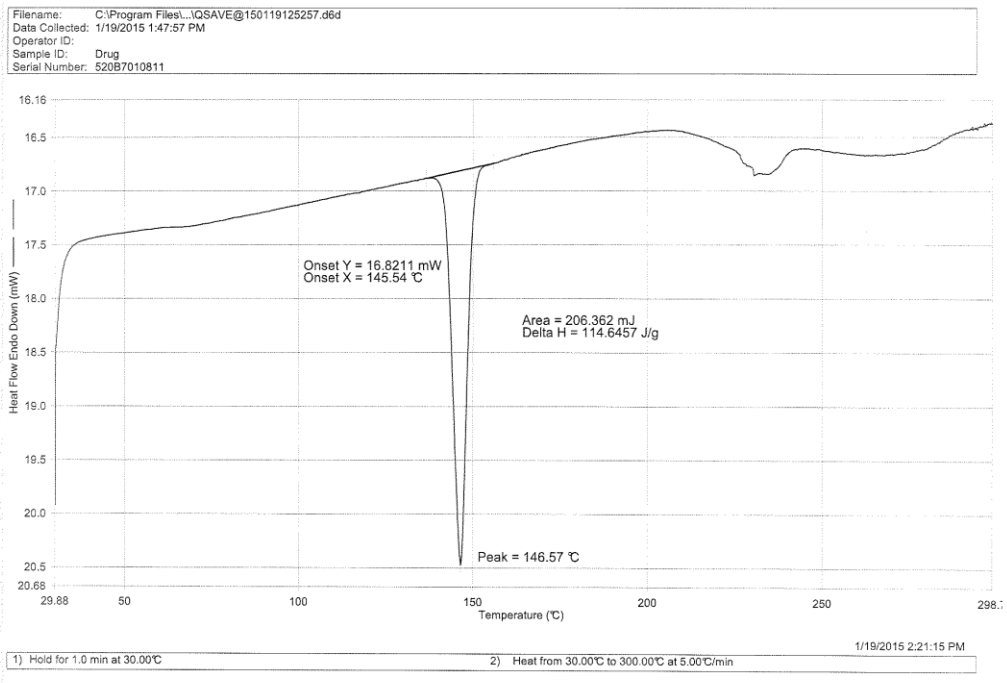

Figure 6: DSC of Pure Verapamil Hydrochloride

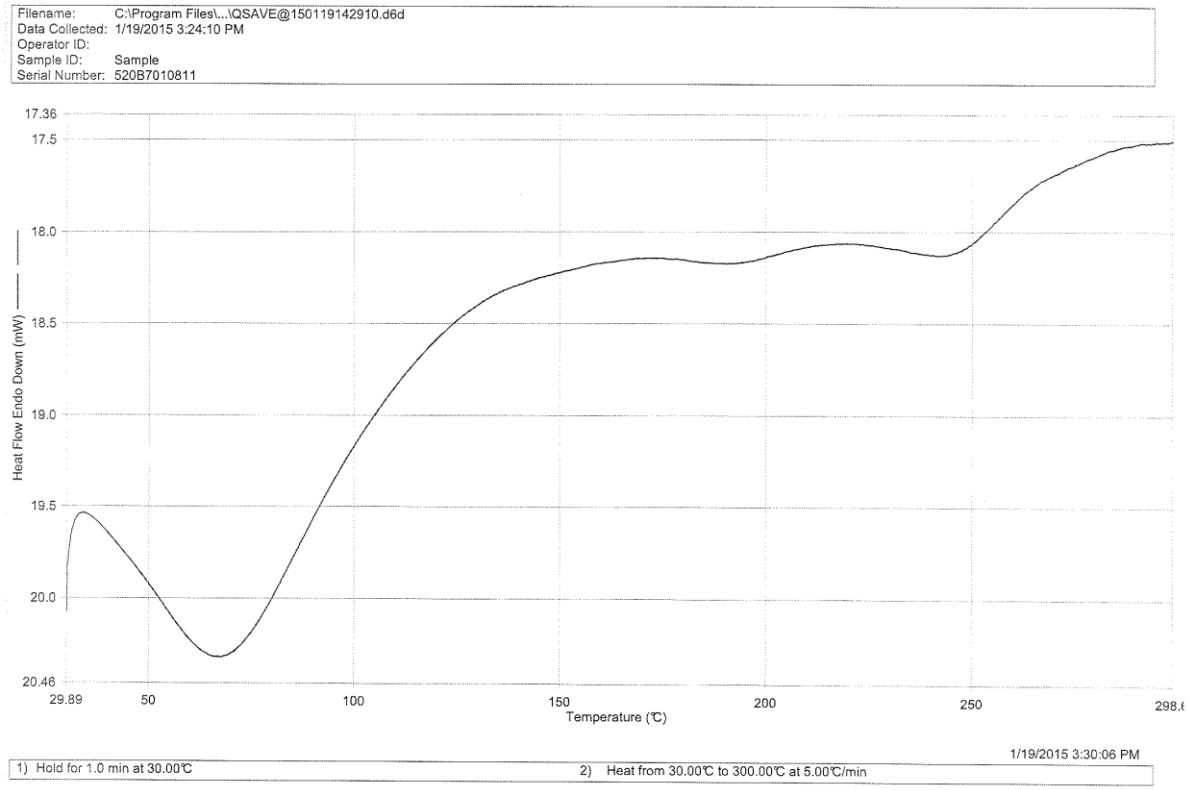

Figure 7: DSC of Optimized Verapamil Hydrochloride Multi Particulate Formulation

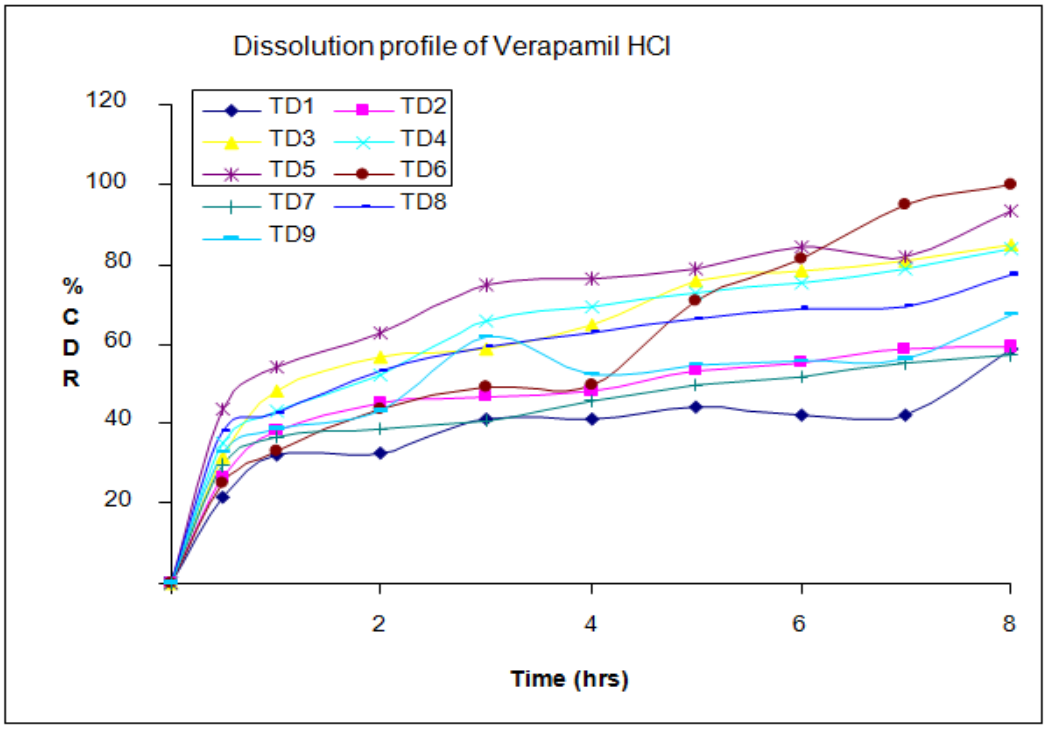

Figure 8: Dissolution Profile of the Prepared Verapamil Hydrochloride Formulations

Table 4: Dissolution Profile of the Optimized Formulation

Sl no.	Time (hr)	%CDR
1	0.5	22.32 ± 1.12
2	1	33.27 ± 2.09
3	2	42.33 ± 2.25
4	3	46.27 ± 1.44
5	4	53.87 ± 1.25
6	5	62.62 ± 2.42
7	6	68.82 ± 2.14
8	7	74.46 ± 1.98
9	8	79.76 ± 2.64
10	9	82.90 ± 1.03
11	10	88.98 ± 1.52
12	11	95.75 ± 1.89
13	12	102.93 ± 2.98

*n=3

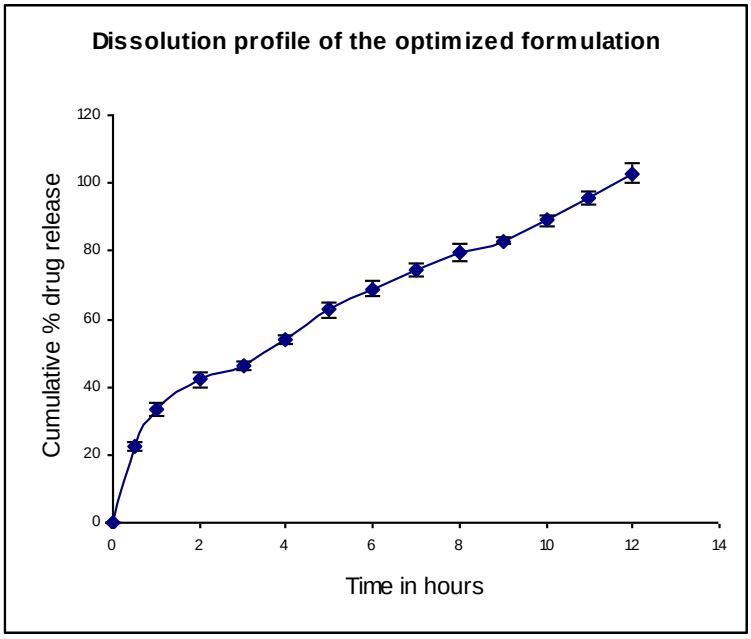

Figure 9: Dissolution Profile of the Optimized Formulation

Curve Fitting Analysis

In order to understand the mechanism of drug release from the optimized formulation, the *in vitro* drug release data was fitted to Korsmeyer –Peppas, Higuchi equation, zero order and first order equations (Table 5). The n value ranged from 0.22 ± 0.04 to 0.32 ± 0.022 for all the formulations except TD6 which has a value of 0.61 ± 0.06 indicating anomalous (non- Fickian) release mechanism from the dosage form. Hence, all other formulation follows a Fickian release indicating diffusion is the main mechanism for drug release. The criteria to choose the "best model" to study drug release phenomena use the coefficient of determination R^2 to assess the fit of a model equation. The best model would be the one with the highest adjusted coefficient of determination. This concludes that the formulation follows the First order kinetics for the release.

Table 5: Results of Curve Fitting Analysis

Formulation Code	Korsmeyer Peppas			Higuchi	
	K_{kp} (h^{-n})	n	R^2	K_H ($h^{-1/2}$)	R^2
TD1	28.44 ± 2.43	0.27 ± 0.053	0.9894	19.93 ± 1.15	0.9710
TD2	35.60 ± 1.09	0.25 ± 0.019	0.9985	23.95 ± 1.30	0.9740
TD3	43.53 ± 1.57	0.32 ± 0.022	0.9983	32.76 ± 1.25	0.9870
TD4	43.96 ± 1.03	0.31 ± 0.015	0.9993	32.53 ± 1.28	0.9862
TD5	53.64 ± 1.50	0.25 ± 0.018	0.9988	36.16 ± 1.94	0.9747
TD6	27.21 ± 3.26	0.61 ± 0.069	0.9913	32.55 ± 1.17	0.9886
TD7	34.00 ± 1.06	0.24 ± 0.019	0.9984	22.43 ± 1.26	0.9725
TD8	44.33 ± 0.92	0.25 ± 0.013	0.9993	29.89 ± 1.57	0.9756
TD9	39.47 ± 2.73	0.22 ± 0.044	0.9919	25.51 ± 1.70	0.9614

The same analysis was extrapolated to study the release mechanism with the optimized formulation. The results in Table 6 and 7 confirm the above findings. The n value was found to be 0.48 ± 0.01 indicating Fickian release mechanism. The R^2 value of the optimized formula for first order release profile is 0.99 confirming that the multi particulate formulation follows first order release kinetics. This data is well supported by Higuchi indicating a R^2 value of 0.9987. The optimized formulation shows a burst release of 22.32% drug in the first half an hour and then the release were found to be sustained for 12 hours which shows 102.93% release.

Table 6: Results of Curve Fitting Analysis

Formulation Code	Zero Order		First Order	
	K_0(h^{-1})	R^2	K_1(h^{-1})	R^2
TD1	6.07 ± 0.01	0.6744	0.12 ± 0.016	0.9166
TD2	7.28 ± 0.02	0.6276	0.16 ± 0.024	0.9262
TD3	9.99 ± 0.01	0.7940	0.32 ± 0.040	0.9734
TD4	9.90 ± 0.03	0.7666	0.32 ± 0.041	0.9734
TD5	10.99 ± 0.01	0.6302	0.48 ± 0.079	0.9733
TD6	10.04 ± 0.02	0.9693	0.27 ± 0.029	0.9796
TD7	6.83± 0.01	0.6235	0.14 ± 0.021	0.9193
TD8	9.09 ± 0.03	0.6397	0.26 ± 0.040	0.9482
TD9	7.74 ± 0.02	0.5142	0.18 ± 0.031	0.9153

Table 7: Results of Curve Fitting Analysis for the Optimized Formulation

	Korsmeyer Peppas			Higuchi	
	K_{kp} (h^{-n})	N	R^2	K_H ($h^{-1/2}$)	R^2
	28.56 ± 1.16	0.48 ± 0.01	0.9987	28.44 ± 0.283	0.9987
Optimized Formulation	**Zero Order**		**First Order**		
	K_0(h^{-1})	R^2	K_1(h^{-1})		R^2
	9.678± 1.33	0.7678	0.219 ± 0.014		0.9907

Stability studies

Stability studies were performed for the optimized multi particulate formulation containing Verapamil hydrochloride as per ICH guidelines. The entrapment efficiency and particle size were evaluated before and after 6 months of stability studies and results are shown in Table 4. The percent entrapment efficiency was found to be 65.25 $\pm$ 1.85 % on 0 day and 63.79 $\pm$ 1.59 % at the end of the studies. The particle size was 232 $\pm$ 12.88µm initially and 249.45 $\pm$ 11.61 µm at the end of six months study. The results thus

obtained were subjected to statistical analysis by using paired t-test and based on the p value obtained as 0.1406 and 0.3405 respectively, it was concluded that no significant differences were observed before and after stability studies. This confirms that the formulation was stable during the study period.

Table 8: Results of Stability Studies (40^0C/75%RH)

Days	Entrapment Efficiency (%)	Particle Size (µm)
0 Day*	65.25 ± 1.85	232 ± 12.88
I Month	65.18 ± 1.32	235 ± 10.69
2 Month	65.11± 1.65	237 ± 11.98
3 Month	64.89 ± 1.50	241 ± 09.64
4 Month	64.35 ± 1.23	244 ± 10.25
5 Month	64.08 ± 1.66	246 ± 12.03
6 Month*	63.79 ± 1.59	249 ± 11.61
Paired t test		
P value	0.1406	0.3405
P Value summary	ns	ns

- t-test: 0 day v/s 6 months, ns = non-significant, *n=3

Design of Experiment
The data obtained by using Design expert (version 6.05) are as follows:

Table 9: Result of Response Variables for Various Formulations

F. Code	Y_1(%EE)	Y_2(% Yield)	Y_3(Particle Size)
TD1	34.4 ± 2.48	72.0 ± 3.33	229.16 ± 10.58
TD 2	39.6 ± 1.65	77.9 ± 2.85	255.83 ± 12.66
TD 3	40.0 ± 3.36	94.5 ± 3.05	316.66 ± 11.65
TD 4	60.4 ± 3.85	94.3 ± 2.22	251.66 ± 12.87
TD 5	60.3 ± 3.98	90.6 ± 2.65	261.66 ± 13.03
TD 6	35.9 ± 2.45	75.6 ± 1.65	170.00 ± 11.87
TD 7	68.1 ± 3.87	97.3 ± 1.08	271.66 ± 13.44
TD 8	47.7 ± 3.15	91.7 ± 2.62	135.30 ± 10.89
TD 9	45.5 ± 3.68	87.0 ± 2.58	157.52 ± 11.98

Table 10: ANOVA for Selected Factorial Model for Response % EE

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	1083.28	3	361.09	18.41	0.0039
X_1	372.88	1	372.88	19.02	0.0073
X_2	287.04	1	287.04	14.64	0.0123
X_3	423.36	1	423.36	21.59	0.0056
Residual	98.05	5	19.61		
Cor Total	1181.33	8			

Table 11: Statistical Parameters

Std. Dev.	4.4282	R-Squared	0.9170
Mean	47.9888	Adj R-Squared	0.8672
C.V.	9.2285	Pred R-Squared	0.6970
PRESS	357.966	Adeq Precision	13.047

Polynomial equation for Response Y_1: $47.98 + 7.88\ X_1 - 6.92\ X_2 + 8.4\ X_3$

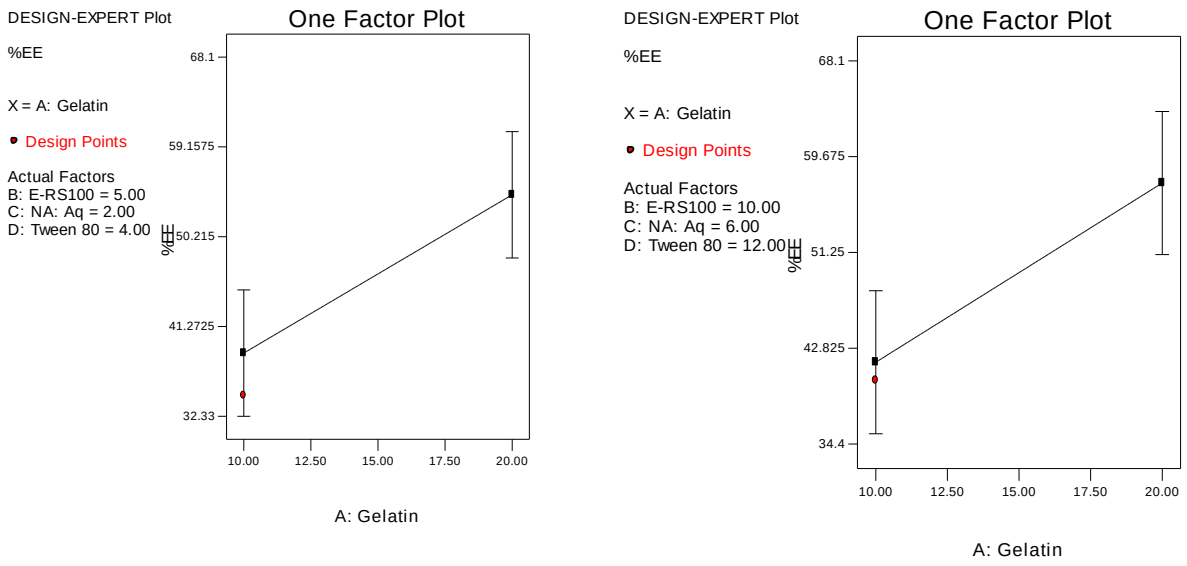

Figure 10: Effect of Factor X_1 on Y_1 by Keeping at Low and High Level

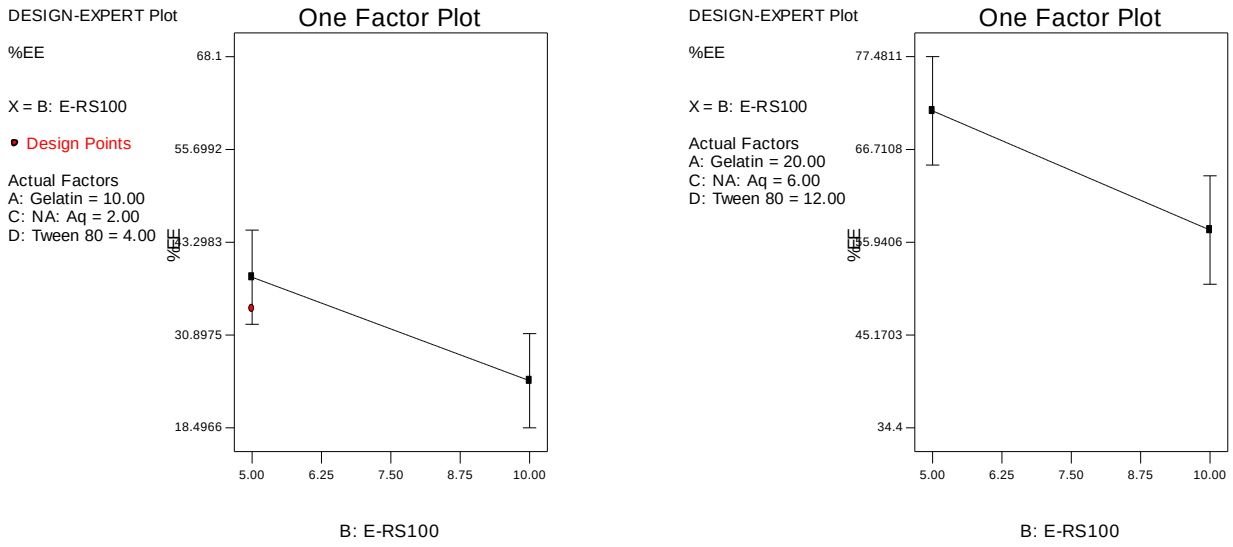

Figure 11: Effect of Factor X_2 on Y_1 by Keeping at Low and High Levels

Table 12: ANOVA for Selected Model for Response for Percentage Yield

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	633.25	3	211.08	20.16	0.0032
X_1	165.59	1	165.59	15.82	0.0106
X_3	309.46	1	309.46	29.56	0.0029
X_4	158.21	1	158.21	15.11	0.0116
Residual	52.34	5	10.47		
Cor Total	685.59	8			

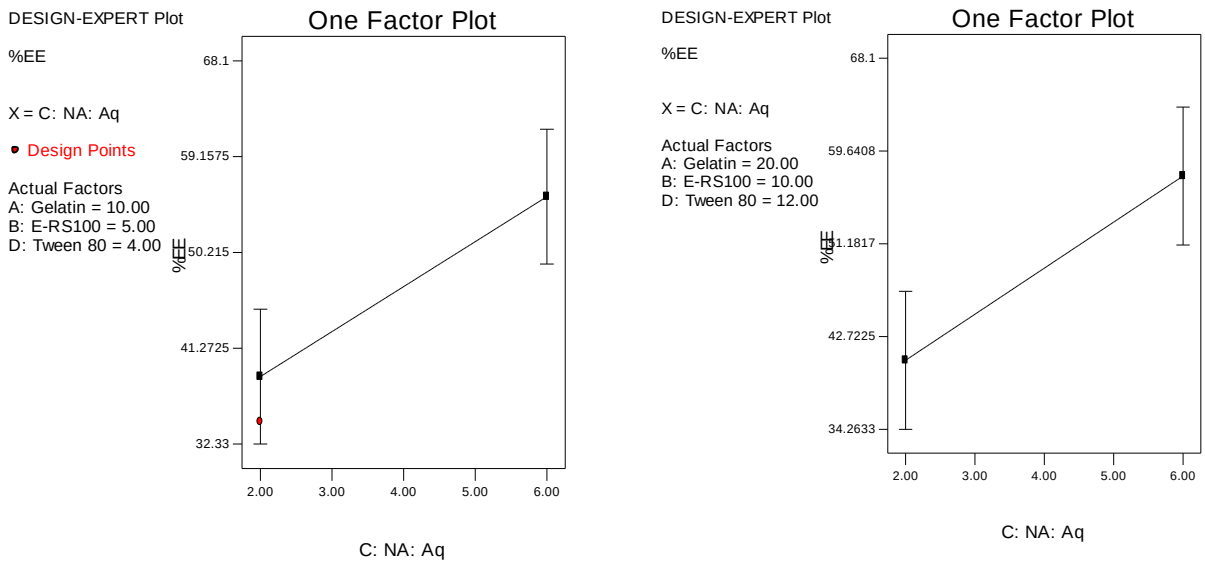

Figure 12: Effect of Factor X_3 on Y_1 by Keeping at Low and High Levels

Table 13: Statistical Parameters

Std. Dev.	3.2354	R-Squared	0.9237
Mean	86.766	Adj R-Squared	0.8779
C.V.	3.7294	Pred R-Squared	0.7628
PRESS	162.606	Adeq Precision	13.911

Polynomial equation for Response Y_2: $86.77 + 5.25\ X_1 + 7.18\ X_3 + 5.14\ X_4$

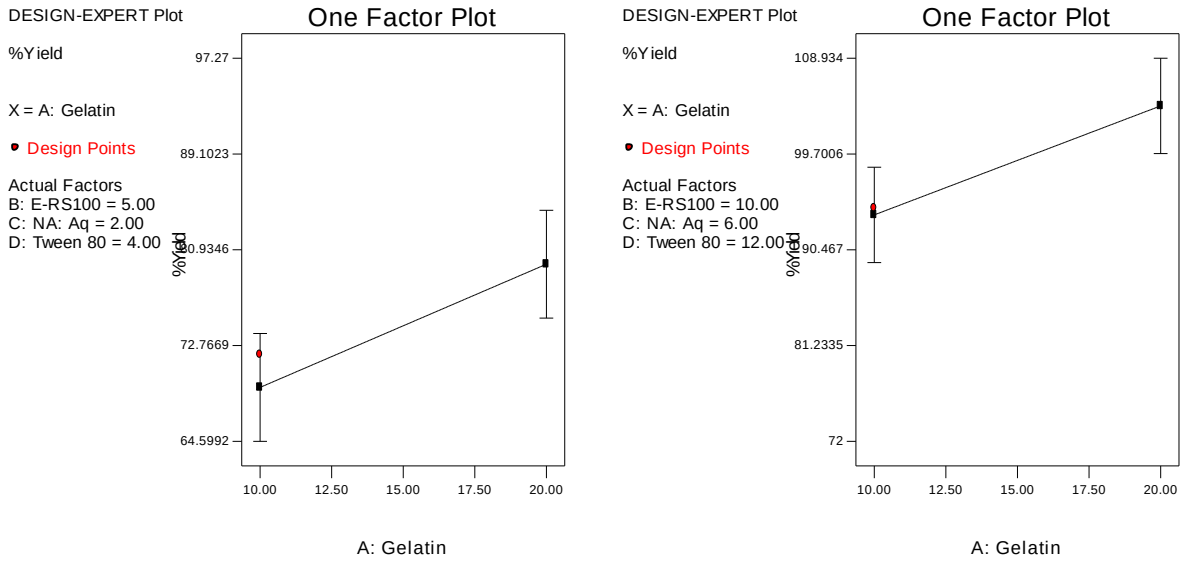

Figure 13: Effect of Factor X_1 on Y_2 by Keeping at Low and High Levels

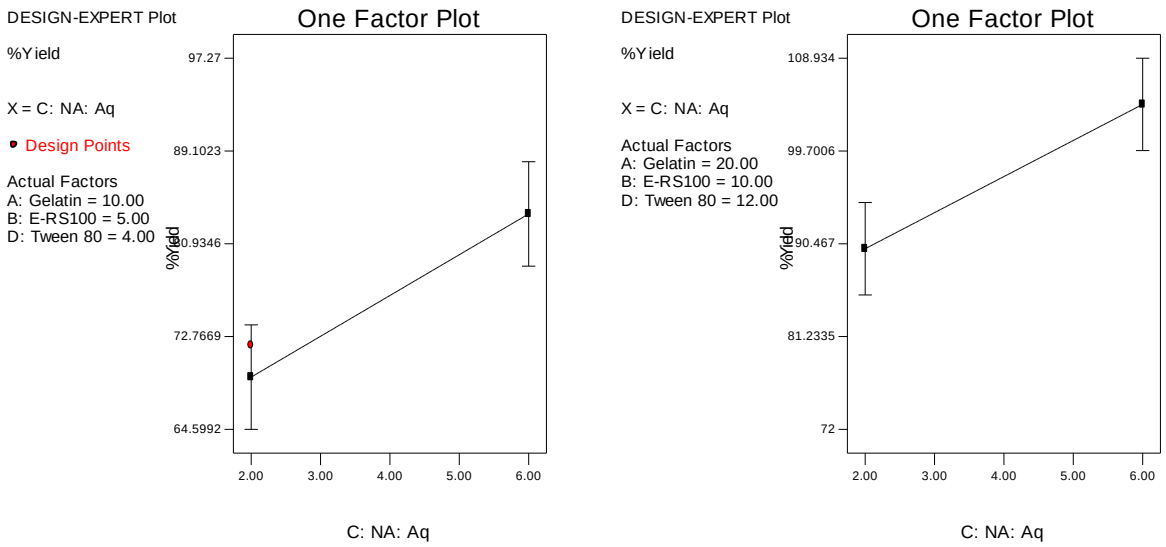

Figure 14: Effect of Factor X_3 on Y_2 by Keeping at Low and High Levels

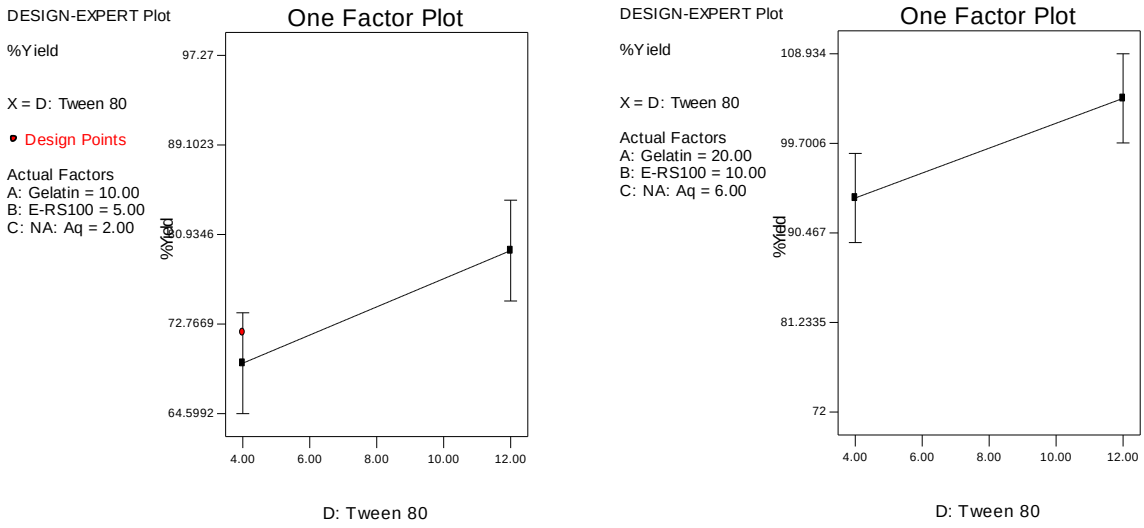

Figure 15: Effect of Factor X_4 on Y_2 by Keeping at Low and High Levels

Table 14: ANOVA for Selected Factorial Model for Response for Particle Size

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	27979.5	3	9326.5	37.66	0.0007
X_1	9400.25	1	9400.25	37.95	0.0016
X_2	1955.54	1	1955.54	7.90	0.0375
X_3	16623.71	1	16623.71	67.12	0.004
Residual	1238.27	5	247.654		
Cor Total	29217.77	8			

Table 15: Statistical Parameters

Std. Dev.	15.7370	R-Squared	0.9576
Mean	227.681	Adj R-Squared	0.9322
C.V.	6.9119	Pred R-Squared	0.8401
PRESS	4672.58	Adeq Precision	15.8591

Polynomial equation for Response Y_3: $227.68 – 39.58\ X_1 - 18.05\ X_2 + 52.64\ X_3$

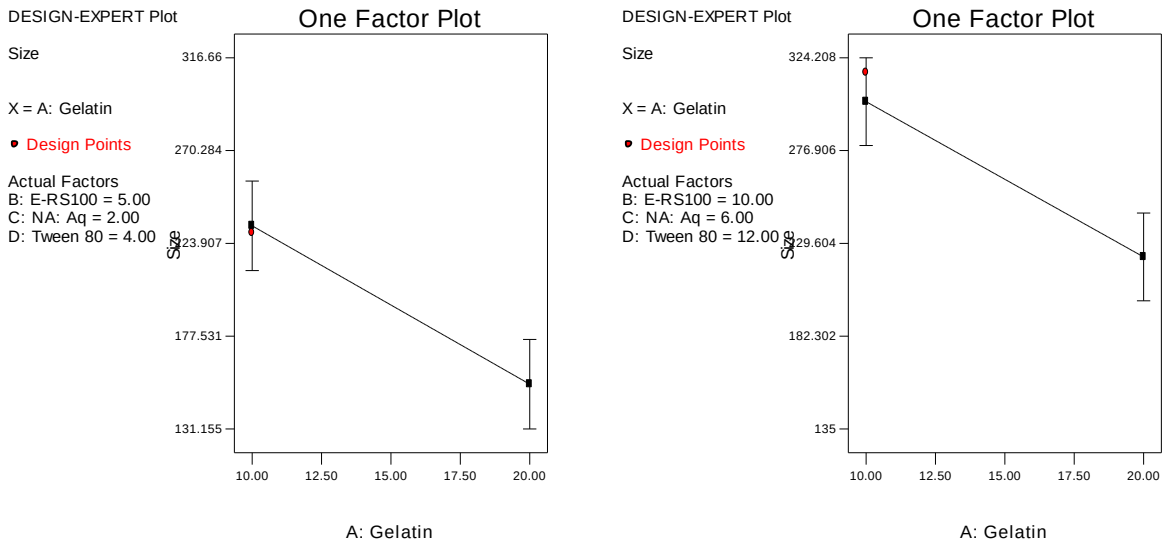

Figure 16: Effect of Factor X_1 on Y_3 by Keeping at Low and High Levels

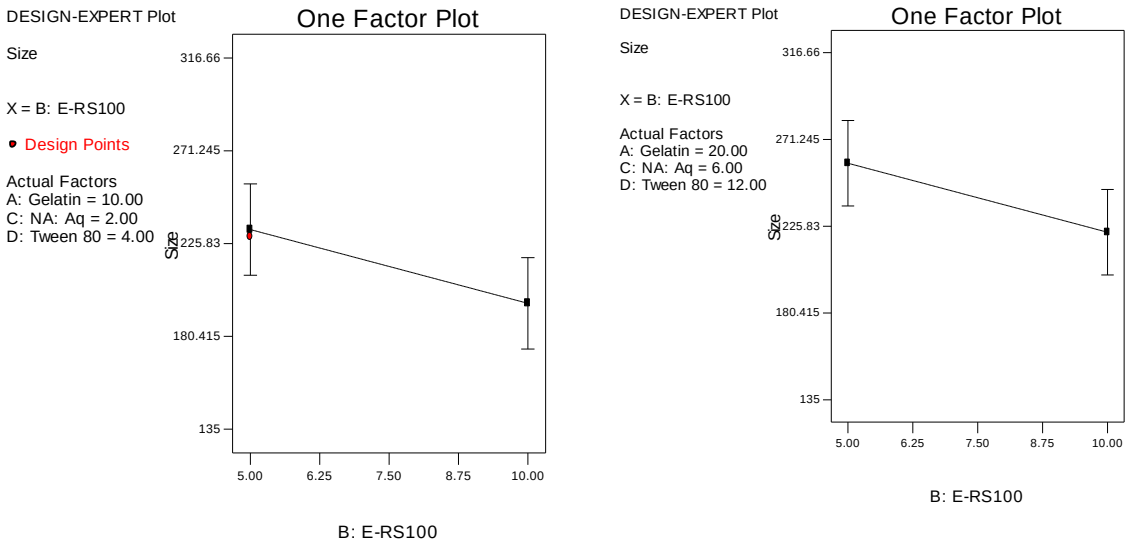

Figure 17: Effect of Factor X_2 on Y_3 by Keeping at Low and High Levels

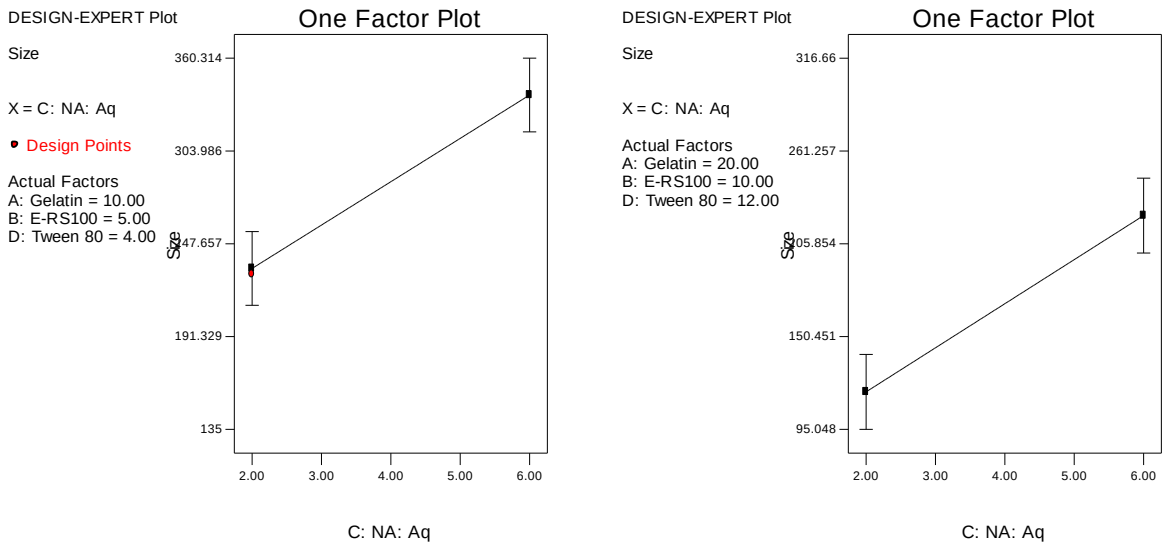

Figure 18: Effect of Factor X_3 on Y_3 by Keeping at Low and High Levels

Table 16: Constraint Table

Factor Name	Goal	Lower Limit	Upper Limit
X_1	is in range	10	20
X_2	is in range	5	10
X_3	is in range	2	6
X_4	is in range	4	12
Y_1	is in range	65	68.1
Y_2	is in range	95	97.27
Y_3	is in range	200	230

Table 17: Optimized Formula for Verapamil Hydrochloride Multi Particulate Formulation

Sl. No.	Formula Composition	Solution 1
1	Conc. of Gelatin %	19.91
2	Conc. of Eudragit RS100 %	5.58
3	Ratio of Non-Aqueous to Aqueous phase volume	5.04
4	Conc. of Tween 80 %	8.74
5	Conc. of drug mg	100
6	Conc. of Glutaraldehyde	10
7	Conc. of Span 80 %	4
8	External Phase volume ml	80
9	RPM	1200

Conclusion

The optimized formulation of Verapamil Hydrochloride multi particles could be successfully prepared by adopting o/w/o double emulsion solvent evaporation technique using Gelatin and Eudragit RS 100 as rate controlling polymers. The experimental results are found to be concurrent with the predictive solution. The curve fitting analysis reveals Fickian release mechanism indicating diffusion release. The formulation follows first order release kinetics. The prepared multi particles were stable throughout the study period and the degradation was insignificant.

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