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DESIGN AND OPTIMIZATION OF FAMOTIDINE MULTIPARTICULATE SYSTEM THROUGH MIXTURE EXPERIMENTAL DESIGN – A NOVEL APPROACH

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ABSTRACT
The objective of this study was to develop a multiparticulate dosage form and optimise its release profile through a novel approach employing simplex mixture design. Microparticles of Famotidine were prepared with Eudragitâ RS100, RL100 and Ethyl cellulose separately using 1:4 drug polymer ratios by emulsification solvent evaporation technique. The three master formulations were characterised for different physicochemical properties and their release profile was studied. Statistical modelling and numerical optimisation was done to predict blends of component microparticles of three polymers, which gave the desired release profile. Experimental validation of the response parameters showed only around 5% error in prediction. Thus, an accurate, economical and time saving methodology could be devised for easy and reproducible development and optimisation of multiparticulate sustained release product.

Keywords: *Microparticles; Famotidine; Release optimization; Mixture Design.*

INTRODUCTION

Microparticles have proved to be a successful delivery system industrially and clinically for variety of drugs for a multitude of reasons[1]. Various methodologies have been established for production, all of which ensures that the formulation developed should perform as programmed. However, for each drug to be formulated as microparticles, extensive preformulation and formulation design studies need to be undertaken leading to increased production cost and time. Various aspects of dosage form including drug polymer ratio, ideal level of ingredients, plasticizer etc. needs to be ascertained for arriving at the desired formulation particularly with respect to its release profile. These studies are performed predominantly on trial and error basis or may be designed. Mixture design has been used for such purpose of optimising the different component levels required for successful multiple particulate system[2], however, such reports are rare.

In this paper we put forward a novel application of mixture design to optimise the desired release characteristics of microparticles with a view to reduce product development time and resources utilisation to arrive at a successful product. Following the proposed technique, release profile as well as other desirable characteristics of the formulation can be easily optimised to arrive at the final product within a short time frame.

The model drug selected for the study was Famotidine, a H_2 receptor blocker having a short half life of 2.5 to 3.5 hrs; freely soluble in 0.1 N HCl. Famotidine has no anti-androgenic action because of low affinity for cytochrome P450 and requires a low dose. Being a poorly bioavailable drug (40 – 50%) due to reasons unrelated to hepatic metabolism, it is ideally suited to be formulated as a floating drug delivery system to improve its PK/PD profile[3,4]. Three polymers, namely, Eudragitâ RS100, RL100 and Ethyl cellulose (EC) were investigated to develop the multiparticulate formulation following a {3,3} simplex lattice design[5]. Microparticles were prepared through non-aqueous emulsification-solvent evaporation procedure[1] and its release characteristics studied by USP XXVI methodology[6].

From *in vitro* dissolution study it was found that release of drug from microparticles followed Higuchi kinetics. By optimising release period for 12 hrs and 24 hrs, the blend of microparticles was predicted for the two duration of release. The prediction was experimentally verified and results were found to be within acceptable limits.

MATERIALS AND METHOD

Materials

Famotidine was a gift sample from Micro Labs, Bangalore, India. Eudragitâ (RS100 & RL100; Degussa, Germany) was gift samples from a local industry. Ethyl cellulose (EC) (Loba Chemie, Mumbai, India), Acetone (Merck), Light liquid paraffin (Rankem, Mumbai, India), Polyisobutylene [PIB] (National Chemicals, Baroda), Trichloroethylene (SD Fine chemicals Limited, India) and Petroleum Ether (40^0-

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60^0C grade) (Rankem, Mumbai, India) were procured commercially. All other chemicals were of AR grade and used as such without further purification. Instruments used were Spectrophotometer (Shimadzu UV-1700 PharmaSpec), FTIR spectroscope (Perkin Elmer RX1), Scanning Electron Microscope (JEOL JSM-840A), and Dissolution apparatus (Disso 2000, Labindia, Chennai, India).

Method of preparation of microparticles

Required amount of PIB (plasticizer) and polymer were dissolved in trichloroethylene and acetone by magnetic stirrer until total polymer dissolved. Required amount of drug was incorporated into this polymer solution to get homogeneous dispersion. The stirring was continued until a desired consistency was achieved. The dispersion was poured into light liquid paraffin being stirred by electrical stirrer at about 600 rpm. Stirring was continued for 2 hrs. Initial speed was kept high for emulsification and reduced during solvent evaporation stage. Time was allowed for rigidisation of surface of microparticles. Finally, microparticles were washed thrice with equal volume of petroleum ether (40° – 60°C grade) and spread evenly on tissue paper bed for air-drying. The optimised parameters for preparation of microparticles are shown in Table 1.

Table 1. *Optimised parameters for preparation of microparticles*

Parameters	Optimised level
Drug : polymer ratio	1:4
Volume of light liquid paraffin	30 ml
Volume of PIB	One drop
Volume of trichloroethylene	3 ml
Volume of acetone	5 – 10 ml

Formulation study on drug polymer ratio

The prepared microparticles were analysed for size distribution and the particles were passed through ASTM sieve and those retained on # 30 mesh with an average diameter[7] of approx. 420 m was chosen for further study. By using drug polymer ratio 1:2 and employing USP XXVI dissolution protocol, it was found that after 3-hrs study, 80% drug was released in case of all polymers used for coating. This provides insignificant retardation of release. It was found that moderate to high sustenance of drug release could be achieved with 1:4 drug polymer ratios. Therefore, the microparticles with drug polymer ratio 1:4 was selected for further study employing {3, 3} simplex mixture design.

The microparticles were subjected to evaluation for appearance, yield, granulometric study, drug-polymer compatibility study by Infra-Red spectroscopy, drug content and entrapment efficiency and *in vitro* dissolution study.

Drug entrapment efficiency

The prepared microparticles of Famotidine were assessed for drug content by crushing a small portion of microparticles in a mortar. Then equivalent weight of drug powder was dissolved in 100ml of 0.1N HCl. One ml of the above solution was diluted to 10 ml by methanol-water mixture and this solution was analysed spectrophotometrically at 266.2 nm against suitable blank.

Mixture Design

Mixture experimental design is applied here to obtain optimum proportion of three different components of a mixture where factors are components of a mixture and their levels are not independent, e.g., if x1, x2, x3, …, xp denotes proportion of p components of a mixture then x1 + x2 + x3 + … + xp = 1 i.e (100%).

The standard form of the mixture model applied in the study is a Quadratic model:

$E(y) = \text{“}\square\hat{a}iXi + \square\square\hat{a}ijXiXj$

Where E(y) is the response parameter; in this case it is the cumulative release % of the drug at various time points of dissolution, âi & âij are the coefficients for the dependent and interaction terms respectively.

Although mixture designs have been widely used in optimising component levels of particularly tablet formulations[8-11], its use in microparticle development is rare[12].

In the present investigation, the cumulative release percentages at different time points of dissolution have been chosen as the response parameters and the proportion of individual microparticle types in a mixture are components of the system. Regression analysis followed by ANOVA study and numerical optimisation have been followed to arrive at an optimum mixture satisfying the desired objective (release pattern).

RESULTS AND DISCUSSION

Various physicochemical tests were carried out with the formulated microparticles –

Appearance of the microparticles

The microparticles prepared were found to be spherical in shape and off white in colour, which showed good flow property.

Yield

The yield of microparticles was calculated from the equation-

% yield = (observed yield / theoretical yield) *100

Where the observed yield is weight of microparticles produced and theoretical yield is the total weight of drug and polymer taken for preparation of microparticles by taking core : wall ratio = 1:4 (Table 2).

Table 2. *% yield of microparticles and drug content of different formulations*

Batches	Core: wall ratio	% Yield	Wt.Taken (mg)	Expected drug content (% of total wt.)	Actual drug content (% of total wt.)	Drug entrapment efficiency (%)
EC	1:4	63.99	20.59	20	20-97	107
RS100	1:4	64.91	20.14	20	16.44	82
RL100	1:4	54.50	20.17	20	16.85	84

Granulometric study

The size distribution of microparticles was evaluated using a mechanical sieve shaker. A series of standard ASTM sieves were arranged in the decreasing order of aperture size. Maximum % of microparticles was retained at ASTM #30-mesh sieve.

Drug Entrapment Efficiency

The drug content of the microparticles showed some variation and the drug entrapment efficiency varied from 81% to 104% of the theoretical drug load (Table 2).

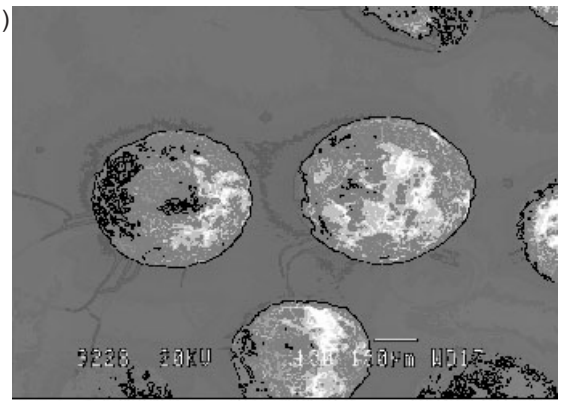
Drug polymer compatibility

The drug and the polymers did not show any appreciable shift/appearance/disappearance of peaks in the crushed microparticle FTIR profile when compared to the pure drug spectrum (data not shown). Hence, it was concluded that no potential interaction exist between the drug and the individual polymers.

Scanning electron microscopy (SEM)

The photomicrographs of microparticles containing Ethyl cellulose showed presence of rough surface and some cavities on the particles indicating air entrapment during micro encapsulation. It may be due to increase in viscosity of slurry of Ethyl cellulose compared to Eudragitsâ. On the other hand, surface of microparticles containing Eudragitâ RS100 & RL100 polymers increase size of particles and smooth surface compared to Ethyl cellulose microparticles due to better consolidation. The smooth surface before dissolution (Fig.1) and appearance of distinct pores on surface after dissolution (Fig.2) implies diffusion kinetics may be operative during release. The topographical study at high-resolution photography reveals increase in surface roughness indicating loss of drug from microparticles after dissolution.

(a)



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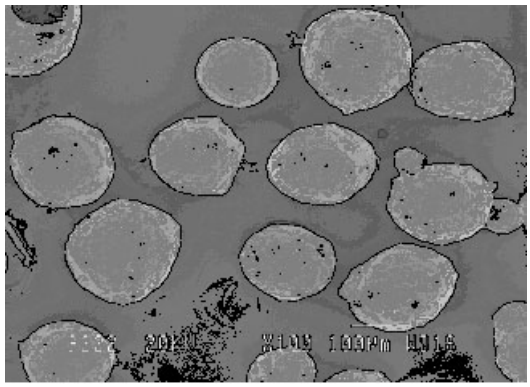

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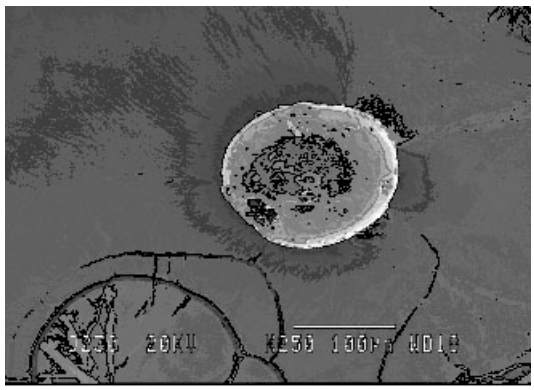
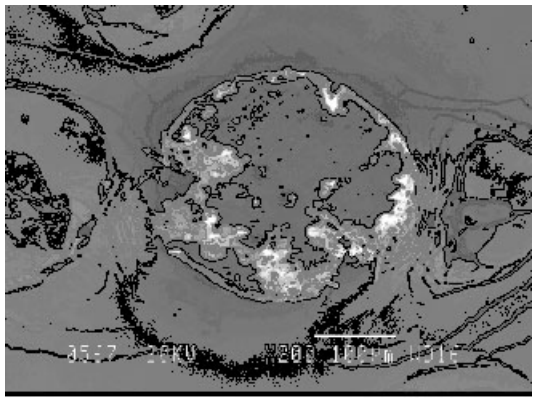


Fig.1: *Photomicrograph of microparticles before dissolution.*

(a)



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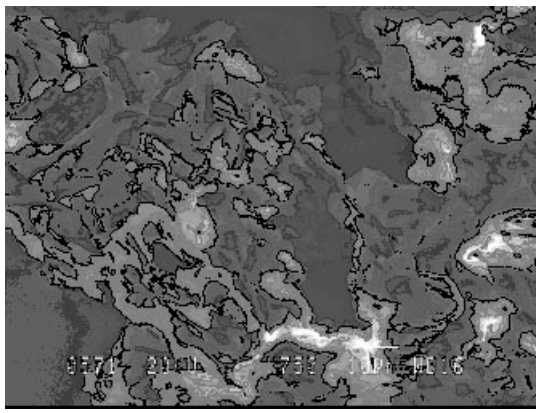

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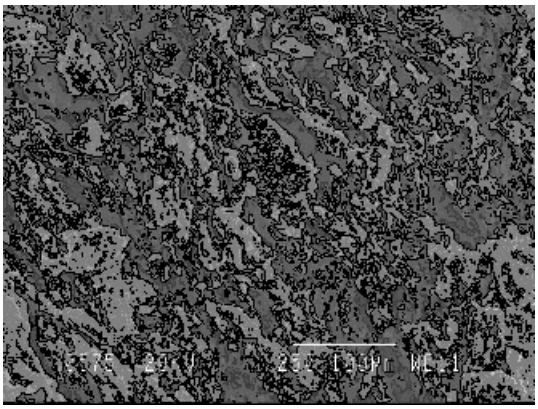


Fig.2: *Photomicrograph of microparticles after dissolution.*

In vitro dissolution study

Having characterised the microparticles prepared separately with three different polymers, the master formulations were mixed according to {3,3} simplex mixture design and subjected to *in vitro* dissolution study as per USP XXVI protocol (USP XXVI, 2003) using 600 ml 0.1N HCl as dissolution medium maintained at 37° ± 0.5 °C at 50 rpm, employing basket type dissolution apparatus. At predetermined time intervals, a fixed volume of sample was withdrawn from dissolution medium and substituted by equal volume of fresh medium to maintain sink condition. The withdrawn samples were suitably diluted and the drug contents in the sample were determined using a Shimadzu UV 1700 spectrophotometer at 266.2 nm against suitable blank and with respect to a calibration curve prepared earlier. Table 3 shows the drug release pattern of the different batches of mixtures of microparticles. The formulations followed Higuchi square root kinetics (diffusion dependent release) as ascertained from the R^2-values for different kinetics models.

Table 3. *Release rate of microparticle mixtures according to {3, 3} simplex design.*

Batch no.	Component 1 A:RS 100	Component 2 B:RL 100	Component 3 C:EC	Coefficient of determination (R^2 values) [Higuchi Model]	Release rate constant (K_H) [mg% $hr^{-1/2}$]
1	1.0000	0.0000	0.0000	0.9553	2.5461
2	0.5000	0.5000	0.0000	0.9375	2.4553
3	0.5000	0.0000	0.5000	0.9230	2.9982
4	0.0000	1.0000	0.0000	0.9633	2.1161
5	0.0000	0.5000	0.5000	0.8486	2.8574
6	0.0000	0.0000	1.0000	0.9148	3.1426
7	0.6667	0.1667	0.1667	0.9212	2.8393
8	0.1667	0.6667	0.1667	0.8911	2.5216
9	0.1667	0.1667	0.6667	0.9381	3.0418
10	0.3333	0.3333	0.3333	0.9067	2.8138
11	1.0000	0.0000	0.0000	0.9303	2.4483
12	0.0000	0.0000	1.0000	0.8800	3.2667
13	0.0000	1.0000	0.0000	0.9706	1.8548
14	0.5000	0.5000	0.0000	0.9182	2.3927

[A+B+C = 1(100%); each batch contain 120 mg of microparticles]

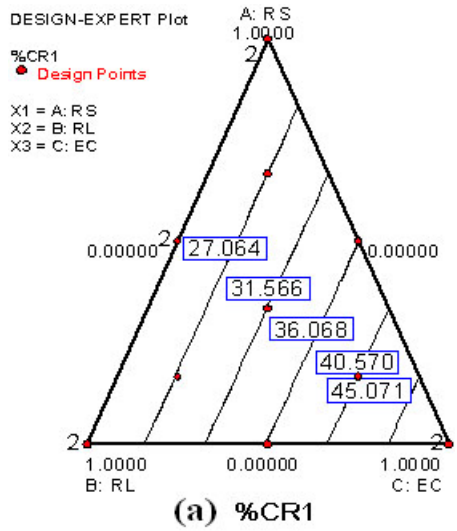
Statistical analysis & optimization of release profile

Recent updates in different pharmacopoeia, particularly USP, have incorporated stringent requirements for extended release formulations to follow certain stipulated release % range at specific time points of dissolution to qualify as an extended release product. Therefore, four representative cumulative release (CR) points at 1hr, 2hr, 5hr and 8hr for effective modelling of Famotidine release pattern from microparticles were taken. They were represented as %CR1, %CR2, %CR5, and %CR8 respectively.

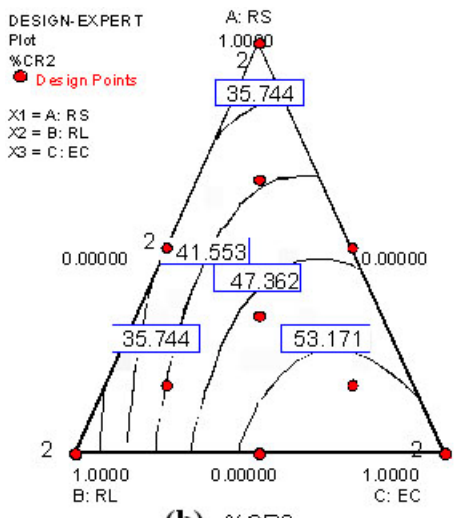
Four representative polynomial models (model nos. 1 - 4) relating the components of master formulation and the % cumulative release at different time points were generated through linear regression. The relevant numerical data is shown in Table 4 and the graphical representation is shown in the form of contour plots[13] for the ternary models, are shown in Fig1.

Table 4. *Model equations and diagnostic statistics.*

Response Parameter	Model obtained [n = 14]	F_0	p < F	SM. Dev.	R^2_{adj}	PRESS	Model No
%CR1 =	22.56*RS+ 22.82*RL+ 49.57*EC	9.63	0.003	8.1469	0.5705	1226.9	1
%CR2 =	31.05*RS+ 24.01*RL+ 54.87*EC +35.26*RS*RL+ 12.68*RS*EC+ 62.88*RL*EC	1108	0.001	5.5144	0.7949	758.46	2
%CR5 =	42.43 * RS+ 33.25 * RL+ 63.37 * EC + 34.50 * RS*RL * RL+ 25.14 * RS * EC + 68.79* RL *EC	419.2	0.0001	2.674	0.9446	137.37	3
%CR8 =	48.44* RS+ 40.43 *RL+ 72.89*EC+ 21.20*RS*RL+ 21.66*RS * EC + 37.64* RL * EC	293.3	0.0001	3.4007	0.9159	332.82	4



(a) %CR1



(b) %CR2

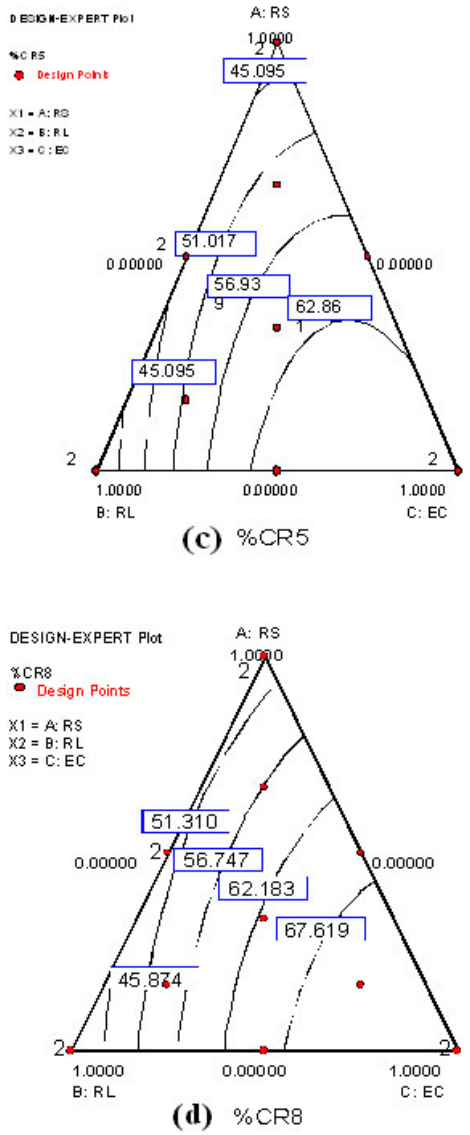


Fig.3: *Contour plot for responses: (a) % CR1, (b) % CR2, (c) % CR5, (d) % CR8.*

The models were validated through lack of fit (LOC) statistics, R^2 values, and PRESS statistics (Table 4) and were found to be highly satisfactory. Subsequently, numerical optimisation of the four parametric models predicted microparticle blends of the three master formulations, of which we considered two predictions based on highest desirability values for experimental validation and error calculations.

From these predicted data, the amount of microparticles of different components were calculated on basis of 120 mg (100%) of microparticles considering total drug release duration of 12 hrs (Blend A) & 24 hrs (Blend B) and diffusion dependent drug release kinetics. From the observed %CR values after respective time points average % error was calculated. The resultant experimental values and % error (compared to the predicted release at the specific time points) for 12 hrs and 24 hrs dosing intervals are shown in Table 5 and Table 6 respectively.

Table 5 : *Optimised release study for Blend A (12 hr projected release).*

Parameters	mg % drug released			Predicted value (mg %)	Average % error*
	Replication 1	Replication 2	average		
%CR1	27.5	28.37	27.93	28.87	3.221
%CR2	40.77	41.93	41.35	40.82	1.273
%CR5	52.69	52.69	52.69	53.47	1.458
%CR8	57.04	56.75	56.89	58.71	3.082
R²value	0.9693	0.9634			
K_{HIG}	3.0730	2.9879			

Table 6 : *Optimised release study for Blend B (24 hrs projected release)*

Parameters	mg % drug released			Predicted value (mg %)	Average % error*
	Replication 1	Replication 2	Average		
%CR1	25.25	25.82	25.53	24.53	4.11
%CR2	39.19	39.42	39.30	33.64	16.82
%CR5	47.77	50.38	49.07	45.65	7.49
%CR8	54.14	53.13	53.63	51.67	3.79
R²value	0.9648	0.9623			
K_{HIG}	2.8866	2.8995			

**Average % error = ∑(predicted values – observed values)/predicted values∑*100*

The error % for Blend A was 3% maximum indicating a very good predictive capacity of model. However, Blend B performed well for the overall period studied and had an error of only 3% after 8 hrs release, though at 2nd and 5th hr the error was more than 5% which may be due to the fact that we assumed a linear profile where as sigmoid release may be more realistic. However, the absolute difference in % cumulative release was only 4.5 – 5.5%, which in practical terms may be allowed in routine works.

Therefore, three formulations and their 14 different blends could predict the desired blend of master formulations that could be utilised for developing a twice daily or once daily Famotidine sustained release multiparticulate products. This saves a lot of time and conservation of resources in pursuit of the desired release characteristics with the help of computer supported mathematical modelling.

CONCLUSION

Mixture experimental design offers a unique method in predicting the optimum blend of microparticles that can give desired release profile within the experimental data range obtained through designed experimentation by systematic formulation approach in shortest possible time. The same set of master formulations was shown to be useful for developing either a once daily or twice daily formulation, which usually requires two complete formulation development endeavours. Thus, this method is expected to cut down cost of R&D and help in economic pricing of dosage forms. The approach is expected to be helpful particularly in matching the release profile desired for bioequivalent dosage forms. Therefore, it can be concluded that a novel method for preparation of a predictable and reproducible multi-

component system of Famotidine could be achieved quickly and efficiently through mixture design and computer supported mathematical optimisation. This methodology is not only suitable for the particular drugs but can be adopted for a wide variety of systems for economic formulation development. We intend to expand this postulate and methodology for other drug delivery systems for robust modelling of release kinetics.

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