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Statistical Optimization and Development of Gastro Retentive System of an Antiretroviral Drug using a 3^2 Factorial Design

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Abstract

The goal of the present study was to develop a gastric floating drug delivery system (GFDDS) of Zidovudine (AZT), an antiretroviral drug. The tablets of AZT were prepared by direct compression technique using HPMC K15M as a polymer with other standard excipients. The mixture of sodium bicarbonate and citric acid was used as gas generating agent. Preliminary studies demonstrated that the, tablets remained buoyant for over 15 hr in the release medium(pH 1.2). 3^2 factorial design was applied to systematically optimize the drug release profile. The proportion of retardant material HPMC K15 M (X_1) and gas generating agent (X_2) were selected as independent variables. The time required for 50% drug release $t_{50\%}$ (Y_1) was selected as dependent variable. The optimization was carried out using softwares like statplus. The derived polynomial equations for $t_{50\%}$ were verified by two check point formulations, which showed that factor X_1 and X_2 significantly affects the studied dependent variable. The non Fickian diffusion release transport was confirmed as the release mechanism for the optimized formulation. The predicated values agreed well with the experimental values and the results demonstrated feasibility of the model in the development of GFDDS.

Key words: *3^2 factorial design, Zidovudine, HPMC, floating system, Non Fickians diffusion.*

INTRODUCTION

Oral drug delivery system represents one of the frontier areas of controlled drug delivery system; such dosage forms are having a major advantage of patient compliance. Floating drug delivery system belongs to oral controlled drug delivery system group that are capable of floating in the stomach by bypassing the gastric transit. These dosage forms are also defined as gastric floating drug delivery system (GFDDS), which can float in the contents of the stomach and release the drug in a controlled manner for prolonged periods of time. The release rate will be controlled depending upon the type and concentration of the polymer that swells, leads to diffusion and erosion of the drug.[1]

Acquired Immunodeficiency Syndrome (AIDS), which threatens to cause a great plague in the present generation. In reality AIDS is not a disease but a collection of seventy or more conditions which result from the damage done to the immune system and other parts of the body as a result of infection by HIV. It is crucial for the success of AIDS therapy to maintain the systemic drug concentration consistently above its target antiretroviral concentration throughout the course of the treatment. There are number of drugs that have been considered as to be anti HIV. The drug like Zidovudine appears most promising because it crosses the blood brain barrier and can be taken orally and in treaties they do not cause serious side effects[2,3].

Zidovudine (AZT) is the first approved compound for the treatment of AIDS; however the main limitation to therapeutic effectiveness of AZT is its dose-dependent toxicity, short biological half-life and poor bioavailability. This limitation can be overcomed by formulating gastroretentive drug delivery systems which retained in the stomach and help in continuously releasing the drug, thus ensuring optimal bioavailability[4]. Treatment of AIDS using conventional formulations of AZT is found to have many drawbacks such as adverse side effects due to accumulation of drug in multi-dose therapy, poor patient compliance,and high cost. So, sustain release once daily formulations of AZT can overcome some of these problems.

Reports were found on the extended release of AZT from matrix tablets prepared using combination of hydrophilic

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and hydrophobic polymers[4]. However, no literature has been found on GFDDS of AZT . F Feleke and coworkers are the first who design the GFDDS of antiretroviral drug stavudine[5]. The purpose of this study is to obtain a sustain release of AZT using HPMC as the retarding polymer by the help of GFDDS.

In a full factorial deign, all the factors were studied at all the possible combinations, by considering it is most efficient in estimating the influence of individual variables and their interactions, using minimum experimentation. In the present study, fitting a 3^2 factorial design (FD) was considered as the concentrations of polymer and mixture of gas generating agents were taken as two factors at the three levels of its concentration.

Two factors were evaluated each at three levels and experimental trials were performed at all possible nine combinations. In the present investigation, amounts of HPMC K15 M (X_1) and amount of mixture of gas generating agent (X_2) were selected as independent variables. The time required for $t_{50\%}$ (Y_1) and was selected as dependent variable. The experimental design with corresponding formulations is outlined in (Table- 1). A statistical model incorporating interactive and polynomial terms was utilized to evaluate the response.

MATERIALS AND METHODS

Zidovudine was received as a gift sample from Cipla Ltd, India. HPMC K15M was kindly supplied by Colorcon Asia Pvt Ltd. (Goa, India). Microcrystalline cellulose, sodium bicarbonate and citric acid anhydrous (here after referred as citric acid), magnesium stearate and talc were purchased from Qualigens fine chemicals, Mumbai, India. All other ingredients were of laboratory grade.

A) Experimental design

In the preliminary studies, nine formulations with different drug polymer ratios were formulated based on 3^2 factorial design. The design includes 2 factors evaluated each at 3 levels. The amounts of HPMC K15 M (X_1) and amount of mixture of gas generating agent (X_2) were selected as independent variables. The time required for $t_{50\%}$ (Y_1) was selected as dependent variable[6,7].

B) Preparation of matrix tablet

The formulations were fabricated according to 3^2 factorial design(Table-1). Zidovudine, polymer, microcrystallinecellulose, sodium bicarbonate and citric acid were passed through sieve no.40 separately. The drug was mixed with the polymer and the other ingredients for 10 minutes. The powder blend was then lubricated with magnesium stearate (presifted through 40#), talc (2%w/w) and this lubricated blend was evaluated for granular properties such as angle of repose, bulk density and compressibility index before compression. Then the powder blend was compressed into tablet using 12.5mm flat-face tooling on a tablet compression machine (Rimek minis press)[8].

C) Evaluation

1. Pre compression parameters

a) Angle of repose

Flow properties of the granules were evaluated by determining the angle of repose and the compressibility index. Static angle of repose was measured according to the fixed funnel and free standing cone method. Angle of repose was calculated by using the equation[9]

$\tan \theta = h/r$

Where θ is the angle of repose.

b) Bulk density

Both loose bulk density (LBD) and tapped bulk density (TBD) of the powder blend were determined using the following formula[10].

LBD= weight of the powder/ volume of the packing

TBD= weight of the powder/ tapped volume of the Packing

c) Compressibility index:

Compressibility index of the powder blend was determined by Carr's compressibility index[10].

Carr's index (%) = [(TBD-LBD) X 100] / TBD

2. Evaluation of floating tablet

a) Floating property study[11,12]

The floating lag time and the total floating duration was determined by placing the tablets in a 100 ml flask containing pH 1.2solution. The time needed to go upward and float on the surface of the liquid and total floating duration was determined.

The time required for dosage form to emerge on surface of medium is called total floating lag time. The duration of time by which the dosage forms constantly emerge on surface of medium called is total floating time.

b) In-vitro release studies

In-vitro release study was carried out according to USP XXIII dissolution type II apparatus (Electro Lab.DTD –

06P) using paddles. 0.1 N HCl solution was selected as a dissolution medium. The study was conducted by keeping 100 rpm paddle rotation at, 37 ± 0.5 ^{0}C. The samples were withdrawn at predetermined time interval and same volume of fresh medium was replaced. The withdrawn samples were suitably diluted and the amount of drug release was estimated using UV spectrophotometer (Shimadzu-1700) at 267 nm. All the studies were conducted in triplicate.

c) Statistical analysis[13].

The results from statistical analysis of the factorial design batches were performed by multiple regression analysis using Microsoft Excel®. To evaluate contribution of each factors with different levels on responses, two way analysis of variance (ANOVA) was performed using Statplus software. To graphically demonstrate the influence of each factor on responses, the response surface plots were generated using Table Curve 3D V4 (Sigma Plot Software 8.0, SPSS, USA). The $P < 0.05$ was considered to be significant.

RESULT AND DISCUSSION

The powder blend exhibited good flow properties and compressibility index (Table-2). The angle of repose and compressibility index (%) was found in the range 25 to 35 and 10 to 18, respectively. These values are considered to be acceptable. Lower the angle of repose, lower the fictional forces existing within the particulate mass and hence better is the flow properties. The results of LBD and TBD ranged from 0.658 to 0.780 and 0.542 to 0.660, respectively. The bulk densities of blend were found to be quite high which indicates that there is no excessive air voids and hence the granules mass shows sufficient compressibility. The floating lag time for the compressed tablet (table-1) was found in the range of 12 to 28 min. Floating lag time decreased with increase in concentration of gas generating agent.

3^2 factorial design was adopted to optimize the formulation variables. In this design 2 factors were evaluated at three different levels to study the effect of the amount of HPMC K15M and amount of mixture of gas generating agent on the drug release from floating tablet.

The dependent variable chosen was time required for 50% drug release. The fitting of an empirical polynomial equation to the experimental results facilitates the optimization procedure. The general polynomial equation is as follows:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 —$$

Where, Y is the response, X_1, X_2, X_3 is the levels (concentration) of the factors

B_1, B_2, B_3, B_{12}, B_{13}, B_{23}, are the polynomial coefficient and B_0 is the intercept (which represents the response when the level of all factors is Low) i.e. arithmetic mean response of the 9 runs). X_i(X_1,X_2, X_1X_2, $X_{12}$and X_{22}), which represents the average result of changing 1 factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when 2 factors are simultaneously changed. The polynomial terms ($X_1 2_{and} X_2 2$) are included to investigate nonlinearity. The $t_{50\%}$ for the 9 formulations (F1-F9) showed a wide variation the responses (Table 1). The data clearly indicates that the $t_{50\%}$ values are strongly dependent on the selected independent variables. The fitted equations relating to the response $t_{50\%}$ to the transformed factor are shown in Equation 1 and Equation 2, respectively.

Final Equation in Terms of Coded Factors:

$$t_{50\%}(Y_1) = 7.503 + 0.520\,X_1 + 0.7033\,X_2 \quad \ldots\ldots 1$$

Final Equation in Terms of Coded Factors:

$t_{50\%}(Y_1)$ = 7.503+0.520 HPMC K15M+0.7033 mixture of gas generating agent..... 2

Equation 1 and 2 suggested that, factors were significant to the responses. positive sign for the coefficient, suggested that, as the polymer concentration increases the value of $t_{50\%}$ also increases.

Validity of the above equations was verified by designing two check point formulations (C1 and C2).The dissolution parameters predicted from the equations derived and those observed from experimental results are summarized (Table 3). The closeness of predicated and observed values for $t_{50\%}$ indicates validity of derived equations for dependent variables.

Figure1 and 2 illustrates the release profiles of the first 5 factorial design formulations and second 4 factorial design formulations respectively. It is clear from the figure1 that formulations 1, 2, and 3, showed a linear pattern of drug release, at least in their initial phase, indicating the appropriate choice of the range of the formulation variables. However, these formulations have low HPMC loading, which contributed to the much faster

release of Zidovudine from the delivery system. Where as the formulations 6 to 9 have highest loading of HPMC found to be worst in terms of controlling the release of AZT. However, the formulations 4 and 5 emerged as a best formulation as they shown drug release which is more than 90 % within 24 hrs. Formulation F5 emerged as the best i.e. optimal formulation showing the 98.64% drug release with in 24 hrs.

Analysis of variance

Table 4 shows ANOVA for dependent variables. The $t_{50\%}$ is the only significant term of the model is retained in the table. The coefficients of $X_1$and X_2 were found to be significant at P.G< 1, hence they were retained in the reduced model. Increasing the concentration of HPMC K4M (X_1) resulted in reduction of drug release. However, its interaction terms had a retardation influence on the release of AZT. Graphical presentation of the data using response surface plots shows the relationship between response and independent variables. The results of response surface plot are similar to that obtained by statistical analysis of mathematical equations (Figure 3). From the graph it is evident that as the amount of the polymer increased the drug release from the system decreased, where as the amount of the gas generating agent in formulation is also believed to be played a very important role as far as the drug release is concerned. Besides its buoyancy effect due to the liberation of CO_2 after interacting with simulated gastric fluid, the release of drug increased due to the formation of pores by CO_2 and subsequent entry of water through pores increased the wetting rate of polymers as well as the alkalizing effect of sodium bicarbonate contributed the solubility of drug better in all the formulations. The polymers used were of low density, highly swellable in shortest time and which upon contact with water, a hydrogel layer is formed to act as a gel would be gel boundary for the release of drug. Use of microcrystallinecellulose gave good gastro retentive property and avoids the matrix from erosion in the dissolution medium. Mixture of citric acid and sodium bicarbonate was incorporated in the formulation in such a way that when it contact with the acidic gastric contents, CO_2 is liberated and gets entrapped in swollen hydrocolloids, which provides buoyancy to the dosage form. Where as the amount of the gas generating agent in formulation is also played a very important role as far as the drug release is concerned.

From the kinetic data of all factorial formulations the n value of korsmeyer-peppas model was in the range of 0.5 to 0.78(Table-1). Therefore, the most probable mechanism that the release patterns of all formulations followed was non fickian diffusion.[14] Since no lag time was observed in the dissolution of the formulations it can be concluded that HPMC was not able to turn into gel immediately in contact with dissolution fluid in these cases. But as the time increased the gel barrier established around the tablet is rate imitating factor on behalf of the drug release.

CONCLUSION

From the results it can be concluded that factors X_1 and X_2 significantly affect the studied dependent variable. Hence the prepared floating matrix tablet appears to be a promising for the delivery of drug release for a period of 24 hrs, to achieve gastric retention.

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Table 1 (a): Formulation and Dissolution characteristics for 3^2 full factorial designs

Batch code	F1	F2	F3	F4	F5	F6	F7	F8	F9	C1	C2
X_1 Variable level in Coded form	-1	-1	-1	0	0	0	+1	+1	+1	-0.5	+0.5
X_2	-1	0	+1	-1	0	+1	-1	0	+1	-0.5	+0.5
t 50% (hrs)	3.45	5.26	7.33	6.21	7.19	7.24	7.35	8.56	8.44	7.18	7.68
Diffusion Coefficient (n)	0.53	0.55	0.56	0.56	0.70	0.56	0.56	0.54	0.52	0.56	0.55
Floating time(hr)	<15	<18	<21	23	24	24	>24	>25	>25	<22	=22

*All batches contains 300 mg of Zidovudine, 10% microcrystallinecellulose, 2% talc and 2% magnesium stearate:
X_1 is the amount HPMC K15 M and(X_2)is amount of gas generating agent (X_2)
C1 and C2 check point batches.

Table 1 (b): Coded values of the variables

Coded Values	Amount Values in mg	
	X_1	X_2
-1	40	10
0	80	25
1	120	40
-0.5	20	5
+0.5	60	20

Table 2: Evaluations of granules containing Zidovudine and HPMC K15 M

Formulations	Loose Bulk Density(LBD) (g/ml)	Tapped Bulk Density(TBD) (g/ml)	Carr's Compressibility Index(%)	Angle of Repose(Ø)
F1	0.780	0.562	14	26.86
F2	0.766	0.614	11.67	29.48
F3	0.731	0.542	10.43	27.60
F4	0.764	0.660	13.61	25.86
F5	0.747	0.634	13.43	32.43
F6	0.711	0.576	12.44	28.49
F7	0.717	0.573	12.68	29.23
F8	0.7	0.56	12.75	30.22
F9	0.675	0.547	11.74	30.58
C1	0.697	0.656	12.70 3	0.41
C2	0.658	0.589	13.01	28.64

Table 3: Dissolution parameters of check point formulations

Formulation	Observed values (hrs)	Predicted Values
	$t_{50\%}$	$t_{50\%}$
C1	6.58	6.50
C2	7. 52	7.17

Table 4 : Analysis of Variance

Parameters	d,f	SS	MS	F	Significance F
For t50%					
Regression	2	2.61	1.30	1.90	0.22
Residual	6	4.1	0.68		
Total	8	6.72			
Diffusion coefficient (n)					
Regression	2	0.0059	0.0029	0.204	0.820
Residual	6	0.1158	0.0193		
Total	8	0.1217			

*df: degree of freedom; SS: sum of square; MS: mean sum of square; F: Fischer's ratio.

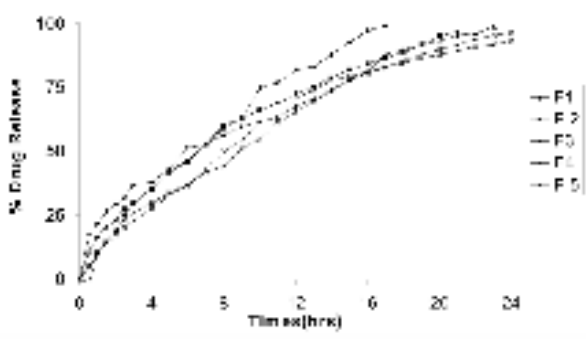

Fig. 1: In vitro drug release profiles of the F1-F5 formulation

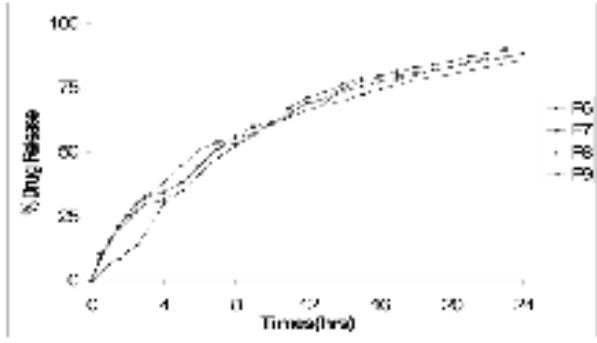

Fig. 2 In vitro drug release profiles of the F6-F9 formulation

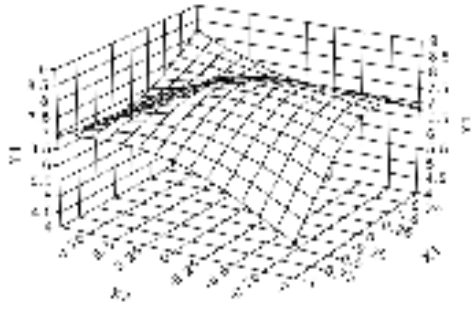

Fig. 3: Response Surface plot showing effect of factorial variables on $t_{50\%}$

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