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FORMULATION AND EVALUATION OF CONTROLLED RELEASE TABLETS OF LAMIVUDINE BY 2^2 FACTORIAL DESIGN

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ABSTRACT

Lamivudine is a potent antiviral agent used in the treatment of AIDS. Conventional oral formulations of lamivudine are administered multiple times a day because of its moderate biological half-life ($t_{1/2}$) of 5-7h. Treatment of AIDS using conventional formulations of lamivudine is found to have many drawbacks, such as adverse side effects resulting from accumulation of drug in multidose therapy, poor patient compliance, and high cost. Controlled release formulation of lamivudine can overcome some of these problems. The objective of the present study is development of oral controlled release tablets of Lamivudine employing a combination of hydrophilic polymer, HPMC K 100M and hydrophobic polymer, ethyl cellulose using 2^2 factorial design. Matrix tablets each containing 120 mg of Lamivudine were formulated employing HPMC K100M and ethyl cellulose as per 2^2 factorial design and were prepared by wet granulation method and were evaluated. All the CR tablets of lamivudine prepared were of good quality with regard to drug content, hardness, friability, disintegration and were suitable for controlled release. Lamivudine release from the CR tablets prepared was slow and spread over 10 h and depended on the composition of the tablets. The order of increasing release rate (K_0) from various formulations was $F_{ab} < F_1 < F_a < F_b$. ANOVA of release rate (K_0) values indicated that individual and combined effects of the two factors are highly significant ($P < 0.01$) in influencing the release rate of drug from the CR matrix tablets. Non-Fickian diffusion was the release mechanism from formulations F_1, F_a and F_{ab} which gave relatively slow drug release. Fickian diffusion was the release mechanism in the case of formulation F_b, that gave rapid release of drug. The polynomial equation describing the relationship between the response, Y (percent drug released in 4h) and the variables, X_1 (concentration of HPMC K100M) and X_2 (concentration of ethyl cellulose) based on the observed data was found to be

Y = 83.75 - 6.75 (X_1) + 5.75 (X_2) – 3.75 (X_1 X_2). As per the above polynomial equation factor A (HPMC K100M) has greater effect in influencing the drug release from the matrix tablets, followed by factor B (ethyl cellulose) and the combined effect of the two factors (AB) is least. Among all formulation F_{ab} provided lamivudine slowly over 8-10h at a release rate (K_0) of 13.50 mg/h which is nearer to the desired release rate (12.5 mg/h) based on its pharmacokinetics and hence it is considered as the best controlled release formulation of lamivudine for b.i.d administration.

KEYWORDS: Lamivudine, Controlled release tablets, HPMC K100M, Ethyl cellulose, Factorial design

INTRODUCTION

The last 40 years have seen the development of several antiviral drugs with therapeutic value in treating life-threatening or debilitating diseases such as those caused by HIV, hepatitis B virus, herpes viruses (such as herpes simplex virus and varicella zoster virus) and influenza virus. These developments are due to technical breakthroughs in the cultivation of viruses in the laboratory, identification of viral enzymes and, more recently, their molecular biology. Antiretroviral drugs[1] are active against human immunodeficiency virus (HIV) which is a retrovirus. They are useful in prolonging and improving the quality of life and postponing complications of acquired immunodeficiency syndrome (AIDS) or AIDS related complex (ARC), but do not cure the infection. The clinical efficacy of antiretrovirus drugs is monitored primarily by plasma HIV-RNA assays and CD4 lymphocytes count carried out at regular intervals. The first antiretroviral (ARV) drug, zidovudine was developed in 1987.Over the past 20 years, more than 20 drugs belonging to the following three classes have been introduced and a large number of others are under development.

The oral route is the route most often used for administration of drugs. Tablets are the most popular oral formulations available in the market and are preferred by patients and physicians alike. In long-term therapy for the treatment of chronic disease conditions, conventional formulations are required to be administered in multiple doses and therefore have several disadvantages.[2] Controlled release (CR) tablet formulations are preferred for such therapy because they offer better patient compliance, maintain uniform drug levels, reduce dose and side effects, and increase the safety margin for high-potency drugs.[3]

Lamivudine is a potent antiviral agent used in the treatment of AIDS. It is phosphorylated intracellularly and inhibits HIV reverse transcriptase as well as hepatitis B virus (HBV) DNA polymerase. Its incorporation into DNA results in chain termination. Lamivudine is used in combination with other anti-HIV drugs, and appears to be as effective as AZT. It is also frequently used for chronic hepatitis B. Conventional oral formulations of lamivudine are administered multiple times a day because of its moderate biological half-life ($t_{1/2}$) of 5-7h.[4-5] Treatment of AIDS using conventional formulations of lamivudine is found to have many drawbacks, such as adverse side effects resulting from accumulation of drug in multidose therapy, poor patient compliance, and high cost. Controlled release formulation of lamivudine can overcome some of these problems.

The objective of the present study is development of oral controlled release tablets of Lamivudine employing a combination of hydrophilic polymer, HPMC K 100M and hydrophobic polymer, ethyl cellulose using 2^2 factorial design. The individual and combined (interaction) effects of the two polymers on drug release from the CR matrix tablets was evaluated. The drug release data were fitted into a polynomial equation to establish the relationship between the response, percent drug release (Y) and concentration of the two polymers, HPMC K100M (X_1) and ethyl cellulose (X_2).

EXPERIMENTAL

MATERIALS

Lamivudine was a gift sample from M/s Natco Pharma Ltd.,Hyderabad. Hydroxypropyl methyl cellulose (HPMC K 100M), ethyl cellulose (18-22 cps), talc and magnesium stearate were procured from commercial sources. All other materials used were of pharmacopoeial grade.

METHODS

Estimation of Lamivudine

An UV Spectrophotometric method based on the measurement of absorbance at 270 nm in 0.1N hydrochloric acid was used for the estimation of lamivudine. The method was validated for linearity, accuracy, precision and interference. The method obeyed Beer's law in the concentration range of 1 – 10 µg/ ml. When a standard drug solution was repeatedly assayed (n=6), the relative error and coefficient of variance were found to be 0.85% and 1.25% respectively. No interference by the excipients used in the study was observed.

Formulation of CR Tablets of Lamivudine

Matrix tablets each containing 120 mg of lamivudine were formulated employing HPMC K100M and ethyl cellulose. Lamivudine CR tablets were formulated as per 2^2 factorial design. The two factors involved in the 2^2 factorial design are HPMC K100M (Factor A) and ethyl cellulose (Factor B). The two levels of HPMC K100M (Factor A) are 80 % and 150 % of drug content and the two levels of ethyl cellulose (Factor B) are 20% and 40% of drug content. Four lamivudine CR tablet formulations were prepared employing selected combinations of the levels of the two factors as per 2^2 factorial design. The CR tablets of lamivudine were prepared by wet granulation method as per the formula given in Table 1.

Method of Preparation of CR Tablets of Lamivudine

The required quantities of lamivudine, HPMC K100M and ethyl cellulose were thoroughly mixed in a dry mortar by following geometric dilution technique. The granulating fluid, water- alcohol (1:1) solution was added and mixed thoroughly to form a dough mass. The mass was pressed through mesh No. 16 to obtain wet granules. The wet granules were dried at 65^0C for 1h. The dried granules formed were passed through mesh No. 16 to break the aggregates. The lubricants talc and magnesium stearate were passed through mesh No. 60 on to the dry granules and blended in a closed polyethylene bag. The tablet granules were then compressed into tablets on a 8-station tablet punching machine (Karnavathi Rimek Minipress II) to a hardness of 4-5 Kg/cm^2.

Evaluation of CR Tablets of Lamivudine

Hardness of the tablets was tested using a Monsanto hardness tester.

Friability of the tablets was determined in a Roche friabilator.

Disintegration time of the tablets was determined using a Paramount tablet disintegration test machine using water, 0.1N HCl and phosphate buffer of pH 7.4 as the test fluids.

Drug Release Study

Drug release from the CR tablets of lamivudine prepared was studied using 8-station dissolution rate test apparatus (Labindia, DS 8000) employing a paddle stirrer at 50 rpm and at a temperature of 37±0.5^{0}C. Hydrochloric acid, 0.1N (900 mL) was used as dissolution fluid. A 5mL aliquot of dissolution medium was withdrawn through a filter (0.45µm) at different time intervals and assayed spectrophotometrically by measuring absorbance at 270 nm. All drug release experiments were conducted in triplicate (n=3).

Data Analysis

Drug release data were analysed as per zero order, first order, Higuichi[6] and Korsemeyer – Peppas[7] equation models to assess drug release kinetics and mechanism from the floating tablets prepared. Drug release rates (K_0) were subjected to ANOVA to find out the significance of the individual and combined effects of the two factors involved on the drug release from the CR matrix tablets prepared.

RESULTS AND DISCUSSION

CR tablets of lamivudine were designed employing combination of a hydrophilic polymer (HPMC K100M) and a lipophilic polymer (ethyl cellulose). Matrix tablets each containing 120 mg of Lamivudine were formulated employing HPMC K100M and ethyl cellulose. Lamivudine CR tablets were formulated as per 2^2 factorial design. The two factors involved in the 2^2 factorial study are HPMC K100M (Factor A) and ethyl cellulose (Factor B). The two levels of HPMC K100M (Factor A) are 80% and 150 % of drug content and the two levels of ethyl cellulose (Factor B) are 20% and 40% of drug content. Four Lamivudine CR tablet formulations were prepared employing selected combinations of the levels of the two factors as per 2^2 factorial design. The CR tablets were prepared by wet granulation method as per the formula given in Table 1. All the CR tablets prepared were evaluated for drug content, hardness, friability, disintegration and drug release characteristics.

Hardness of the CR tablets prepared was in the range 4.0-5.0 Kg/cm^2.Weight loss in the friability test was less than 0.85% in all the cases. All the tablets prepared contained Lamivudine within 100±2% of the labelled claim. All the CR tablets prepared were found to be non-disintegrating in water and aqueous acidic (pH 1.2) and alkaline (pH 7.4) fluids. As such the prepared CR tablets were of good quality with regard to drug content, hardness, friability, disintegration and were suitable for controlled release.

Lamivudine release from the CR tablets prepared was studied in 0.1N hydrochloric acid. The drug release profiles of CR tablets prepared are shown in Fig.1. Drug release parameters of the CR tablets prepared are summarized in Table 3. Lamivudine release from the CR tablets prepared was slow and spread over 10 h and depended on the composition of the tablets. The order of increasing drug release rate (K_0) from various formulations was $F_{ab} < F_1 < F_a < F_b$.

The release data were analysed as per zero order, first order, Higuchi and Korsemeyer - Peppas kinetic models. The coefficients of determination (R^2) values in the analysis of release

data as per various kinetic models are given in Table 2. The R^2 values in all the models with all the products were greater than 0.914 indicating that the release data equally obeyed all the four kinetic models. ANOVA of release rate (K_0) values (Table 4) indicated that individual and combined effects of the two factors are highly significant ($P < 0.01$) in influencing the release rate of drug from the CR matrix tablets.

Drug release from all the CR tablets prepared was diffusion controlled as indicated by the linear Higuchi plots. When the release data were analysed as per Korsemeyer- Peppas equation, the release exponent 'n' was found to be in the range 0.467-0.574 in all the cases except formulation F_b indicating 'non-Fickian diffusion' as the release mechanism from these CR tablets. In the case of formulation F_b, that gave rapid release of drug, the release exponent 'n' was found to be 0.410 indicating fickian diffusion as the drug release mechanism.

The drug release data were fitted into a polynomial equation to establish the relationship between the response, percent drug released (Y) and concentration of the two polymers, HPMC K100M (X_1) and ethyl cellulose (X_2). For this purpose percent drug released in 4h was taken as response (Y) and the concentration of HPMC K100M as (X_1) and the concentration of ethyl cellulose as (X_2). The polynomial equation describing the relationship between the response, Y and the variables, X_1 and X_2 based on the observed data was found to be $\mathbf{Y = 83.75 - 6.75 (X_1) + 5.75 (X_2) – 3.75 (X_1 X_2).}$ The coefficients in above polynomial equation indicate the magnitude of the effect of the factors involved. The increasing order of the coefficients of the two factors was 6.75 (X_1) > 5.75 (X_2) > 3.75 (X_1 X_2). Hence the factor A (HPMC K100M) has greater effect in influencing the drug release from the matrix tablets, followed by factor B (ethyl cellulose) and the combined effect of the two factors (AB) is least.

Based on the pharmacokinetics[8], lamivudine CR tablets for b. i. d administration shall provide the drug at a desired rate of 12.5 mg/h. Among the lamivudine CR tablets prepared formulation F_{ab} provided lamivudine slowly over 8-10h at a release rate (K_0) of 13.50 mg/h which is nearer to the desired release rate. Hence formulation $F_{ab,}$ which is formulated employing HPMC K100M at a strength of 150 % of drug content and ethyl cellulose at a strength of 40% of drug content is considered as the best controlled release formulation of lamivudine for b.i.d administration.

Table 1: Formulae of CR Tablets of Lamivudine Prepared Employing HPMC K100M and Ethyl Cellulose as per 2^2 Factorial Design

Ingredient (mg/tablet)	Formulation			
	F_1	F_a	F_b	F_{ab}
Lamivudine	120	120	120	120
HPMC K100M	96	180	96	180
Ethyl Cellulose	24	24	48	48
Talc	5	6.5	5	6.5
Magnesium Stearate	5	6.5	5	6.5
Total Weight (mg)	250	337	274	361

Table 2: Coefficient of Determination (R^2) Values in the Analysis of Release Data as Per Various Kinetic Models

Formulation	R^2 Values			
	Zero Order	First Order	Higuchi Model	Korsemayer Peppas
F_1	0.995	0.994	0.995	0.989
F_a	0.975	0.960	0.993	0.995
F_b	0.935	0.914	0.991	0.949
F_{ab}	0.947	0.988	0.996	0.996

Table 3: Drug Release Parameters of Various CR Tablets of Lamivudine Prepared

Formulation	Percent Drug Released in 4 h	Release Rate		n' in Korsemayer Peppas
		K_0 (mg/h)	K_1 (h^{-1})	
F_1	81	14.22	0.410	0.542
F_a	75	15.27	0.485	0.574
F_b	100	23.14	1.45	0.410
F_{ab}	79	13.50	0.416	0.467

Table 4: ANOVA of Release Rates (K_0) of CR Tablets of Lamivudine Prepared

Source of Variation	d.f	Sum of Squares (ss)	Mean Sum of Squares (mss)	F- ratio	Critical F- ratio
Total	11	293.9267	26.72061	---	
Treatment	3	282.1467	94.04889	63.87021	$F_{0.01}$ (3, 8) = 7.59
Error	8	11.78	1.4725	---	
F_a	1	87.48	87.48	59.40917	$F_{0.01}$ (1, 8) = 11.3
F_b	1	33.33333	33.33333	22.63724	
F_{ab}	1	161.3333	161.3333	109.5642	

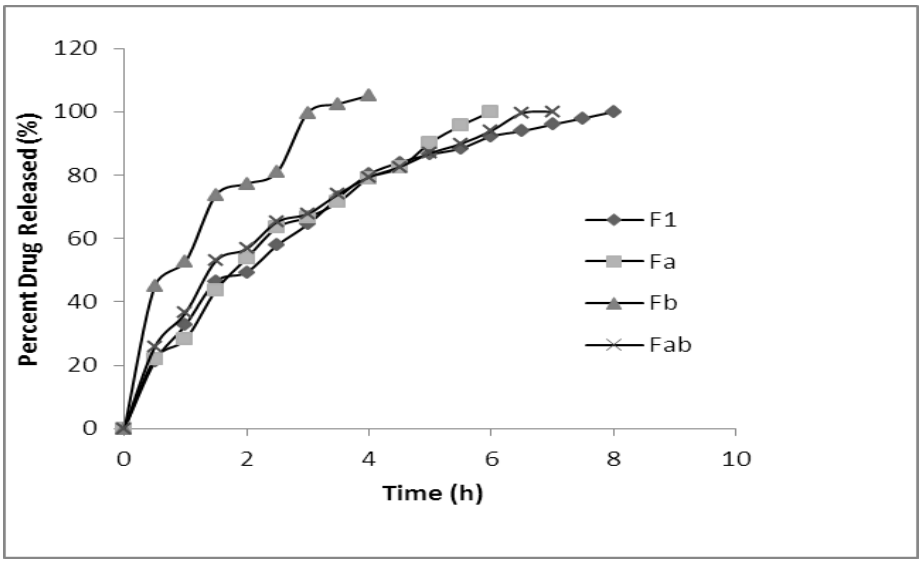

Fig.1: Drug Release Profiles of Various CR Tablets of Lamivudine Prepared

CONCLUSIONS

1. All the CR tablets of lamivudine prepared were of good quality with regard to drug content, hardness, friability, disintegration and were suitable for controlled release.

2. Lamivudine release from the CR tablets prepared was slow and spread over 10 h and depended on the composition of the tablets.

3. The order of increasing release rate (K_0) from various formulations was $F_{ab} < F_1 < F_a < F_b$.

4. ANOVA of release rate (K_0) values indicated that individual and combined effects of the two factors are highly significant ($P < 0.01$) in influencing the release rate of drug from the CR matrix tablets.

5. Non-Fickian diffusion was the release mechanism from formulations F_1, F_a and F_{ab} which gave relatively slow drug release. Fickian diffusion was the release mechanism in the case of formulation F_b, that gave rapid release of drug.

6. The polynomial equation describing the relationship between the response, Y (percent drug released in 4h) and the variables, X_1 (concentration of HPMC K100M) and X_2 (concentration of ethyl cellulose) based on the observed data was found to be $\mathbf{Y = 83.75 - 6.75 (X_1) + 5.75 (X_2) – 3.75 (X_1 X_2).}$

7.As per the above polynomial equation factor A (HPMC K100M) has greater effect in influencing the drug release from the matrix tablets, followed by factor B (ethyl cellulose) and the combined effect of the two factors (AB) is least.

8.Among all formulation F_{ab} provided lamivudine slowly over 8-10h at a release rate (K_0) of 13.50 mg/h which is nearer to the desired release rate (12.5 mg/h) based on its

pharmacokinetics and hence it is considered as the best controlled release formulation of lamivudine for b.i.d administration

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