

Formulation and *In-Vitro* Evaluation of Oral Sustained Release Drug Delivery System for Lamivudine Matrix Tablets Using Methocel K15M CR Polymer

Mofizur Rahman^{1*}, Qamrul Ahsan², Abul Bashar Ripon Khalipha¹, A. K. Azad¹, Nazneen Ahmeda Sultana³, Sophia Hossain¹, & Sanjida Haque¹

¹Department of Pharmacy, Bangladesh University, Dhaka, Bangladesh,

²Department of Pharmacy, Southern University, Bangladesh,

³Pharmacy Discipline, Khulna University, Bangladesh,

*Corresponding author: Md. Mofizur Rahman, Assistant Professor, Dept. of Pharmacy, Bangladesh University, Bangladesh Tel: +88-01911605139, E-mail: rmfi02@yahoo.com

ABSTRACT

The present work reports the study of different proportion of Lamivudine: Methocel K15M formulations, in order to investigate the effect of polymer proportion and diluent on the drug release mechanism. Lamivudine, an anti-HIV agent, was used as a model drug to evaluate its release characteristics from different matrices. Matrix tablets of Lamivudine were prepared by direct compression process using methocel K15M CR polymer. In vitro release studies were performed using US Pharmacopeia type 1 apparatus (basket method) in 900 mL of pH 6.8 phosphate buffer at 100 rpm for 8 hours (Initial 2 hours in simulated gastric fluid (pH 1.2)). Scanning Electron Microscopy (SEM) was used to evaluate and surface properties of the matrices. Drug release was analyzed according to their kinetic models. A one-way analysis of variance (ANOVA) was used to interpret the results. Statistically significant differences were found among the drug release profile from different formulations. Higher proportion of polymeric content (25 to 30% of the total tablet weight) in the matrix, release was extended > 8 hours due to increased tortuosity and decreased porosity. At lower proportion of polymeric content (10% of the total tablet weight), the rate of drug release was elevated. Two formulations showed drug release is more controlled. The release mechanism was explored and explained with zero order, first order, Higuchi and Korsmeyer and Hixon-Crowell equations. The results generated in this study showed that the profile and kinetics of drug release were functions of polymer type, polymer level and physico-chemical properties of the drug. By suitable modulation could be developed controlled delivery of such type of drug.

KEY WORDS: Lamivudine, methocel K15M CR, matrix tablets, sustained release

INTRODUCTION

AIDS is considered to be an epidemic, and according to estimates from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) AIDS Epidemic Update 2005, 38 million adults and 2.3 million children were living with the human immunodeficiency virus (HIV) at the end of 2005. The annual number of AIDS deaths can be expected to increase for many years to come, unless more effective and patient-compliant antiretroviral medications are available at affordable prices^[1]. The major drawbacks of antiretroviral drugs for the treatment of AIDS are their adverse side effects during long-term therapy, poor patient compliance, and their huge cost^[2, 3].

Lamivudine is a synthetic nucleoside analog that is being increasingly used as the core of an antiretroviral regimen for the treatment of HIV infection^[4, 5]. In vivo, nucleoside analogs are phosphorylated intracellularly by endogenous kinases to putatively active 5'- triphosphate (3TC-TP) derivatives that prevent HIV replication by competitively inhibiting viral reverse transcriptase and terminating proviral DNA chain extension^[6, 7, 8]. Lamivudine is rapidly absorbed after oral administration with an absolute bioavailability of 86% ± 16%, peak serum concentration of lamivudine (C_{max}) of 1.5 ± 0.5 mcg/mL and mean elimination half-life (t_{1/2}) of 5 to 7 hours, thus necessitating frequent administration to maintain constant therapeutic drug levels^[9].

Therefore, the objective of the present work is to provide a long acting pharmaceutical composition containing lamivudine in a sustained release matrix formulation, to maintain the blood levels of the active ingredient for a prolonged period of time.

Oral sustained release dosage form by direct compression technique is a simple approach of drug delivery systems that proved to be rational in the pharmaceutical arena for its ease, compliance, faster production, avoid hydrolytic or oxidative reactions occurred during processing of dosage forms^[10]. Sustained or controlled drug delivery occurs while embedded within a polymer that may be natural or semi-synthetic or synthetic in nature. The polymer is judiciously combined with the drug or other active ingredients in such a way that the active agent is released from the material in a predetermined fashion and at constant rate for desired time period^[11].

The matrix tablet by direct compression has attracted much attention due to its technological simplicity in comparison with other controlled release systems. It required fewer unit operations, less machinery, reduced number of personnel and processing time, increased product stability and production rate^[12]. A wide array of polymers has been employed as drug retarding agents each of which presents a different approach to the matrix concept. Polymers that primarily forming insoluble or skeleton matrices are considered as the first category of retarding materials and are classified as plastic matrix systems. The second class represents hydrophobic and water-insoluble materials which are potentially erodable and the third group exhibits hydrophilic properties. There are three primary mechanisms by which active agents can be released from a delivery system: diffusion, degradation and swelling followed by diffusion. The release of drug from the tablet matrix depends on the nature of polymer. Methocel K15M CR, used in this study is hydrophilic polymer that becomes hydrated, swollen and facilitates to diffuse the drug^[13].

Matrix based CR tablet formulations are the most popular and easiest to formulate on a commercial scale. The matrix can be prepared via wet granulation or by direct compression^[14].

MATERIALS AND METHODS

Materials

Lamivudine was obtained as gift sample from Beximco Pharmaceuticals Ltd. Tongi, Bangladesh. HPMC (Methocel K15M CR) was a gift sample received from Colorcon Asia Pvt.Limited. Avicel pH 102 was purchased from Ming Tai Chemical Co.Ltd., (Taiwan). Magnesium stearate and talc were procured from Hanua Chemicals Limited, (Japan).

Preparation of matrix tablets

Tablets were prepared by direct compression process. In all cases, the amount of the active ingredient was 200 mg and the total weight of the tablet was 400 mg (Table-1).

Table 1. Formulation of Lamivudine sustained release matrix tablets

Formulation Code	Weight (mg) / Tablet					
	Methocel K15M CR	Lamivudine mg	Avicel PH 102	Talc	Mg - stearate	Total mg
LAMF1	40	200	144	12	4	400
LAMF2	60	200	124	12	4	400
LAMF3	80	200	104	12	4	400
LAMF4	100	200	84	12	4	400
LAMF5	120	200	64	12	4	400

During granulation process matrix-forming agents, talc, methocel, magnesium stearate, avicel pH 102 and the active ingredient were weighed properly. Firstly active ingredient, talc and Methocel were mixed for 10 minutes properly. Dried granules were sieved through 20 mesh SS screen to get compressible particle. Lubricants are added during blending part. During blending total mass was taken in a container and blended in a laboratory designed small drum blender machine for about 30 minutes. Particular attention was given to ensure thorough mixing and phase homogenization. Finally, the prepared granules were compressed using Pressima D type 4-station compression machine with a 10.00 mm punch. Before compression, the surfaces of the die and punch were lubricated with purified talc. All the preparations were stored in airtight containers at room temperature for further study.

Control tests for matrix tablets

The tablets of the proposed formulations (LAMF1 to LAMF5) were evaluated for hardness, weight variation, thickness, friability and drug content. Hardness of 10 matrix tablets from each formulation was measured using Hardness tester (Erweka GMBH, 300H model, Germany). Friability of the tablets was determined by testing 10 tablets in a Roche friabilator (Campbell Electronics, Mumbai) for 4 minutes at 25 rpm. A slide calipers was used to measure the thickness for 5 tablets. Weight variation test was performed by taking 10 tablets using an electric balance (OHAUS LS 200, Switzerland) according to the official method. Drug content for Lamivudine was carried out by measuring the absorbance of the sample at 271 nm using Shimadzu 1240 UV visible spectrophotometer, Japan and comparing the content from a calibration curve prepared with standard Lamivudine in the same medium by taking 20 tablets were taken, weighed and finely powdered. An accurately weighed quantity of this powder was taken, suitably dissolved in pH 6.8 phosphate buffer, making dilution and analyzed and carried out in triplicate and mean was taken.

In vitro dissolution study of tablets

Dissolution studies were conducted using a tablet dissolution tester (Dissolution Tester [US Pharmacopeia] VEEGO VDA 8 DR, Germany), type 1 (basket method), in 900 mL of pH 6.8 phosphate buffer at $37.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The stirring speed was set at 100 rpm. At predetermined time intervals, a 10-mL sample was withdrawn and replaced with fresh dissolution medium. After filtration and appropriate dilution, the sample solution was analyzed at 271 nm for Lamivudine by UV spectrophotometer (Shimadzu 1240, UV visible spectrophotometer, Japan). The amounts of drug present in the samples were calculated with the help of straight-line equation obtained from the calibration curves for respective drug. The mean of six tablets from each formulation was used in data analysis. The dissolution study was continued for 8 hours (Initial 2 hours in simulated gastric fluid (pH 1.2) and next 6 hours in phosphate buffer of pH 6.8) to get a simulated picture of the drug release in the in-vivo condition and drug dissolved at specified time periods was plotted as percent release versus time (hours) curve. This drug release profile was fitted into several mathematical models to get an idea of the release mechanism.

Kinetic modeling of the drug release

Different kinetic models (zero-order, first-order, Higuchi's , korsmeyer's and Hixon Crowell) were applied to interpret the release profile from matrix system. To study the mechanism of drug release from the matrix tablets, the release data were fitted to zero-order, first-order, and Higuchi equation. However, two factors diminish the applicability of Higuchi's equation to matrix systems. This model fails to allow the influence of swelling of the matrix (upon hydration) and gradual erosion of the matrix. Therefore, the dissolution data were also fitted according to the well-known exponential equation (Korsmeyer equation), Eq. (1), which is often used to describe the drug release behavior from polymeric systems^[15].

$$\text{Log} (M_t / M_f) = \text{Log} k + n \text{Log} t \quad (1)$$

Where, M_t is the amount of drug release at time t ; M_f is the amount of drug release after infinite time; k is a release rate constant incorporating structural and geometric characteristics of the tablet; and n is the diffusional exponent indicative of the mechanism of drug release. To clarify the release exponent for different batches of matrix tablets, the log value of percentage drug dissolved was plotted against log time for each batch according to the equation 1. A value of $n = 0.45$ indicates Fickian (case I) release; >0.45 but <0.89 for non-Fickian (anomalous) release; and >0.89 indicates super case II type of release. Case II generally refers to the erosion of the polymeric chain and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled-drug release^[16]. The Hixon - Crowell cube root equation is:

$$M^{1/3} = M_0^{1/3} - Kct \quad (2)$$

Where, Kc is the cube root dissolution rate constant. Cube roots of percent releases (Cube root of initial drug load minus cube root of % drug remaining) are plotted against time (hour) to demonstrate the Hixson Crowell plot.

Mean dissolution time (MDT) was calculated from dissolution data using the following equation^[17]

$$\text{MDT} = (n / n + 1) . K^{-1/n} \quad (3)$$

Scanning Electron Microscopy

Then tablets were position into sample holder to the microscope and visualized under scanning electron microscope (SEM), (HITACHI, Model: S-3400N). Tablets were taken using different magnifications. The magnifications used for taking tablets were 100-10000 (SE-Secondary Electron).

Statistical Analysis:

A one way analysis of variance (ANOVA) was used to analyze the dissolution data obtained for each batch of formulation to compare the drug release rate and comparison of mean dissolution time (MDT) of all formulations. A confidence limit of $P < .05$ was fixed and the theoretical calculated values of F (F_{crit} and F_{cal}) were compared for the interpretation of results. ANOVA was determined using SPSS software (Version 12, SPSS Inc., USA).

RESULTS

Physical Evaluation of Lamivudine matrix tablets

The tablets of the proposed formulations (LAMF1 to LAMF5) were evaluated for hardness, weight variation, thickness, friability and drug content. The thickness (mean \pm SD, n=5) of the tablets were (4.71 \pm 0.01, 4.72 \pm 0.02, 4.72 \pm 0.03, 4.71 \pm 0.02, 4.750 \pm 0.04 respectively) ranged from 4.71 to 4.75 mm. The hardness (mean \pm SD, n=10) and percentage friability (< 1%) of the tablets of all batches (6.05 \pm 0.27, 6.10 \pm 0.27, 6.15 \pm 0.27, 6.20 \pm 0.27 and 6.25 \pm 0.28 respectively) ranged from 6.05 to 6.25 kg/cm² and 0.75 to 0.85 %, respectively. The average percentage weight deviation of 10 tablets of each formula was less than \pm 5%. Drug content (mean value \pm SD within 0.9) among different batches of tablets ranged from 200.6501mg to 200.6505mg.

Effect of methocel K15M CR on release pattern of Lamivudine

Matrix tablets of Lamivudine were formulated using direct compression technique. Different methocel K15M CR matrix tablet containing Lamivudine as active ingredient having methocel K15M CR polymer 10%, 15%, 20%, 25% and 30% respectively of total tablet weight (i.e. 20%, 30%, 40%, 50% & 60% respectively of total drug) in the matrix tablet with the formulation code LAMF1, LAMF2, LAMF3, LAMF4, LAMF5 were prepared to evaluate the effect of this polymer. After preparation according to formulation shown in the table 1, their dissolution studies were carried out in basket method at 100 rpm in 900ml , phosphate buffer ph 6.8 medium at 37 0C (\pm 0.50C). Six tablets from each formulation were used in dissolution study. The release profile of Lamivudine was monitored up to 8 hours (Initial 2 hours in simulated gastric fluid (pH 1.2) and next 6 hours in phosphate buffer of pH 6.8). The average release pattern is shown in figure 1.

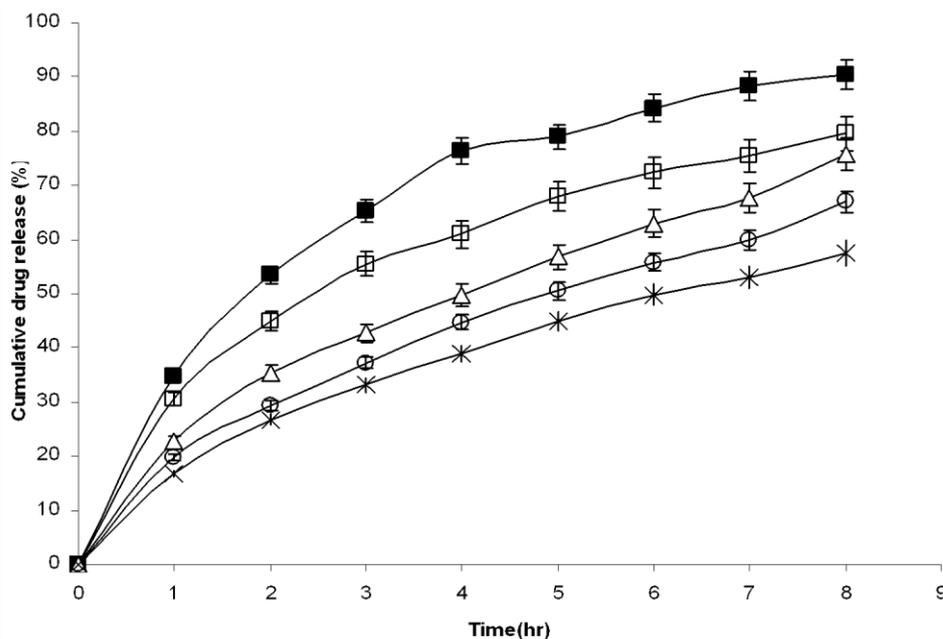


Figure 1: Zero order plot of release (mean \pm SD) of Lamivudine matrix tablets from LAMF1(■), LAMF2(□), LAMF3(Δ), LAMF4(○) and LAMF5(×) formulations.

A release profile of Lamivudine containing methocel K15M CR matrix tablet of five formulations was obtained from the graphs. The total % of Lamivudine release (mean value \pm SD within 0.9, n = 6) from the formulation LAMF1, LAMF2, LAMF3, LAMF4, LAMF5 was 90.52%, 79.59%, 75.64%, 66.96%, and 57.48% respectively. It has been observed that the release rate has been extended with the increase of polymer % and with the decrease of avicel percent. The highest percent of drug release within 8 hours is obtained from LAMF1 where polymer content is 10% of total tablet weight and that of avicel was 36 %. But in LAMF5, the polymer content is 30% of total tablet weight and avicel is 16%, the release of drug is controlled with 57.48 % within 8 hours.

The rate of drug release was found to be inversely related to the amount of methocel K15 M CR present in the matrix structure, i.e. the drug release increased with decrease in the polymer content of the matrix tablet. The release rate was significantly dependent on the proportion of polymer. A statistically significant decrease ($P < .05$, $F_{crit}(4, 25) = 2.76$ and $F_{cal} = 405.95$) at the end of first hour, ($P < .05$, $F_{crit}(4, 25) = 2.76$ and $F_{cal} = 2423.87$) at the end four hours, ($P < .05$, $F_{crit}(4, 25) = 2.76$ and $F_{cal} = 884.61$) at the end of eight hours, was observed % drug release in the formulation LAMF1 to LAMF5 as the polymer proportion increase from 10% to 30% of the total drug weight.

The release kinetics data has been mention in the table 2.

Table 2: Release kinetics of formulated Lamivudine from methocel K15M based matrices

Code	Zero order		First order		Higuchi		Korsmeyer		Hixon Crowell	
	R ²	K ₀ % h ⁻¹	R ²	K ₁ % h ⁻¹	R ²	K _h % h ^{-0.5}	R ²	n	R ²	K _c % h ⁻¹
LAMF1	0.834	9.957	0.989	-0.124	0.979	32.899	0.970	0.455	0.543	0.384
LAMF2	0.859	8.660	0.976	-0.081	0.991	28.345	0.989	0.453	0.551	0.366
LAMF3	0.941	8.449	0.989	-0.072	0.996	26.497	0.998	0.561	0.619	0.373
LAMF4	0.948	7.577	0.991	-0.056	0.995	23.665	0.999	0.583	0.629	0.362
LAMF5	0.939	6.608	0.985	-0.044	0.996	20.738	0.999	0.586	0.625	0.346

R², Correlation coefficients, K₀, K₁, K_h, K_c Release rate constant for zero order, first order, Higuchi, and Hixon Crowell release equation, respectively, n, diffusional exponent, indicative of release mechanism in Korsmeyer equation. LAMF1-LAMF2 = Fickian Release (Case I), LAMF3-LAMF5 = Non-Fickian (Anomalous) Release

T_{25%}, T_{50%}, T_{80%} and MDT values of the designed tablets are also shown in figure 2.

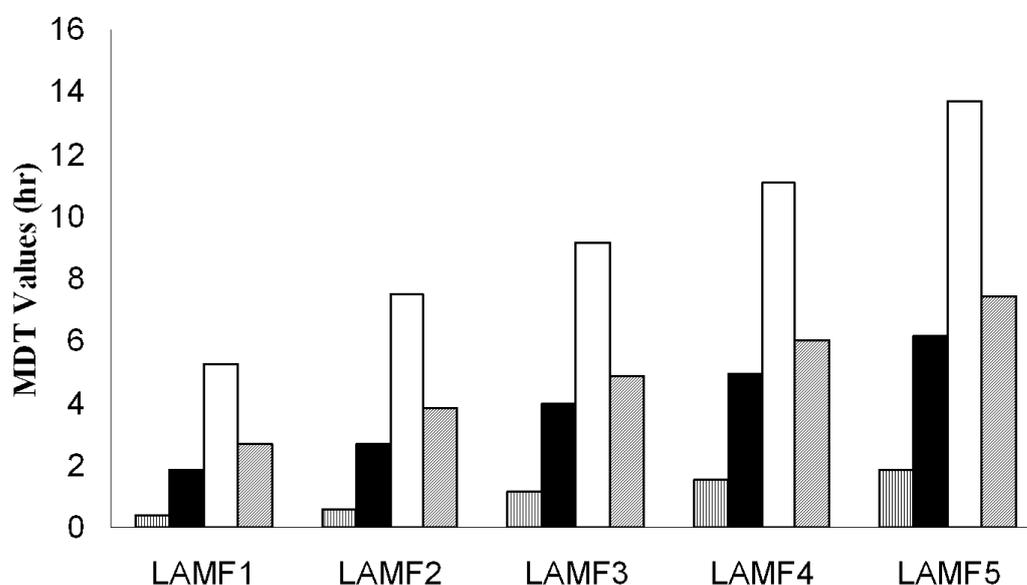


Figure 2: Successive dissolution time [T_{25%}(▨), T_{50%}(■), T_{80%}(□), MDT Value(▩)] of Lamivudine containing Methocel K15M matrices

In case HPMC 15000 cps (methocel K15) containing formulations, formulation LAMF1 showed lowest MDT (mean dissolution time) values of all as it increased the release rate. But, as the concentration of HPMC 15000 cps increased in the latter formulations (LAMF2, LAMF3, LAMF4, LAMF5), MDT values were increased i.e increase polymer load increase MDT value. Formulation LAMF5 showed highest MDT (7.4 hr) value. The MDT values increased significantly $P < .05$, $F_{crit}(4, 25) = 2.76$ and $F_{cal} = 701.21$) as polymer load was increased 10% to 30% of the total drug weight.

Scanning electron microscope analysis

Matrix tablets were prepared at different concentration of methocel K15M CR polymer and different exepients like avicel PH102, talc, magnesium stearate. Morphology and surface properties of the tablets were examined with a Scanning Electron Microscope “SEM” (Fig.-3) (HITACHI, Model: S-3400N). Tablets were taken using different magnifications.

The magnifications used for taking tablets were 100-10000 (SE-Secondary Electron). Morphology and surface properties of the tablets were found to be affected by the polymer concentration.

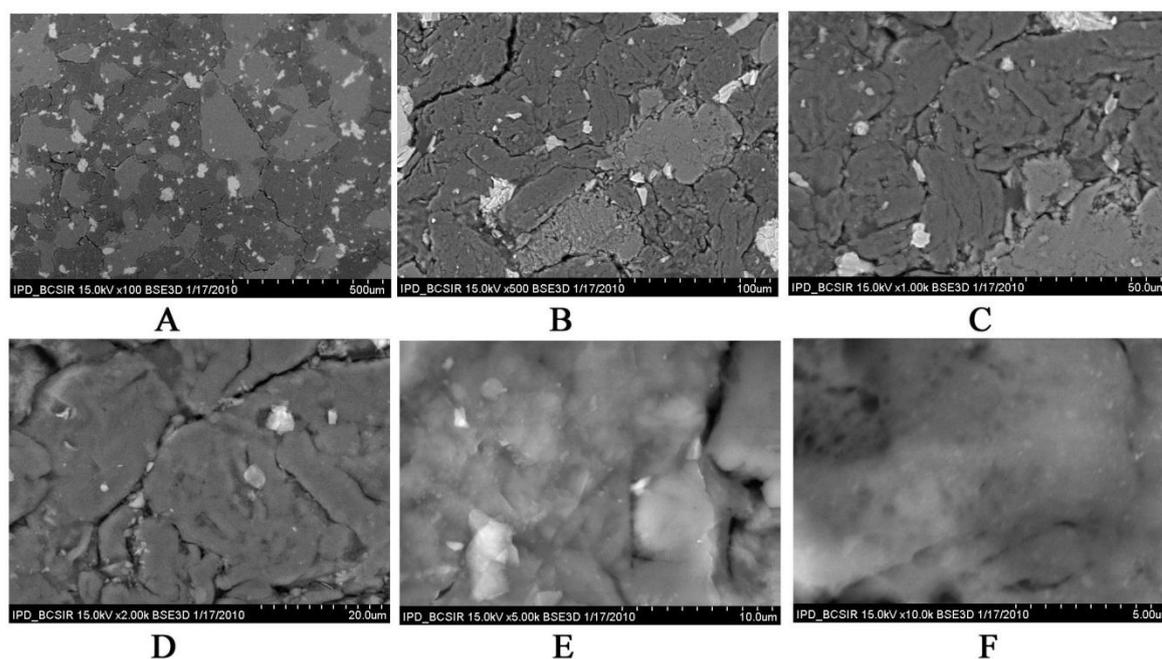


Figure 3: SEM of Methocel K15M CR on Lamivudine sustained release Tablets

- | | |
|-----------------------------------|------------------------------------|
| A. 100 SE(Magnification x 100) | D. 2.00k SE(Magnification x 2000) |
| B. 500 SE(Magnification x 500) | E. 5.00k SE(Magnification x 5000) |
| C. 1.00k SE(Magnification x 1000) | F. 10.0k SE(Magnification x 10000) |

DISCUSSION

Physical Evaluation of Lamivudine matrix tablets

In vitro dissolution study of tablets

The present study was carried out to formulate oral sustained release drug delivery system for Lamivudine as Matrix Tablets. The drug content of all formulations was between 100.32 and 100.33 %, indicating the presence of an acceptable amount of drug in the formulations. Furthermore, all the formulations showed acceptable hardness and friability.

The formulations, LAMF1 LAMF2 showed initial burst release within the 2-hour dissolution test period in pH 1.2 buffer. This phenomenon may be attributed to surface erosion or initial disaggregation of the matrix tablet prior to gel layer formation around the tablet core^[18].

However the later formulations LAMF3 to LAMF5 containing higher proportion of methocel K15 polymer no burst release was observed within two hours probably to the low solubility of the drug at pH 1.2 and retained their shape throughout the 8 hour dissolution period. they showed slow drug release from 0 to 2 hours followed by faster but controlled release from 3rd to 8th hour.

The release rate decreased and the drug release prolonged as the polymer proportion was increase. Such increase in polymer content results in a decrease in the drug release rate due to a decrease in the total porosity i.e. release is extended to long period. Avicel causes a decreased tortuosity of the path of the drug due to its preferential solubility than methocel K15 M CR, by its swelling effect, additionally weakened the integrity of the matrix.

For the formulation LAMF5 containing highest proportion of polymer drug release is more controlled both pH 1.2 (less than 20% in acidic media) and phosphate buffer pH 6.8 (extended more than 8 hours). This may be owing to a more rigid complex formed by presence of higher proportion of HPMC which helped in retaining the drug in matrix and did not allow rapid diffusion of drug from the matrix.

Among these formulations (LAMF1 to LAMF5), the rate and extent of drug release was decreased with increasing the amount of Methocel K15M CR. This polymer has been well known to retard the drug release by swelling in aqueous media. A polymer's ability to retard the drug release rate is related to its viscosity. Processing factors including particle size, hardness, porosity and compressibility index etc. also can affect the release rate of drug from tablets. The hydration rate of HPMC depends on the nature of the substituents like hydroxypropyl group content. Hence, Methocel K15M CR was used because it forms a strong viscous gel in contact with aqueous media, which may be useful in controlled delivery of drug^[19].

Kinetic modeling of the drug release

The drug release data obtained were extrapolated by Zero order, First order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell equations to know the mechanism of drug release from these formulations. In this experiment, the in vitro release profiles of drug from all these formulations could be best expressed by Higuchi's equation as the plots showed highest linearity (R^2 : 0.98 to 0.99). To confirm the diffusion mechanism, the data were fitted into Korsmeyer-Peppas equation (15). The formulations showed good linearity (R^2 :0.97 to 0.99) with slope (n) values ranging from 0.4527 to 0.5856 indicating that diffusion was the predominant mechanism of drug release from these formulations indicating that the release mechanism was non-Fickian or anomalous release ($0.45 < n < 0.89$). It can be inferred that the release was dependent on both drug diffusion and polymer relaxation, which appears to indicate a coupling of diffusion and erosion mechanisms-so called anomalous diffusion^[20], when plotted according to Korsmeyer-Peppas equation, the formulations LAMF4 to LAMF5 showed highest linearity (R^2 : 0.999). The poor correlation coefficients (R^2 values ranged from 0.83 to 0.94) observed for the kinetic parameters based on the zero-order model equation were mainly due to the drug release mechanism. First order plot for all formulation showed good linearity. The release profile of Lamivudine from all these formulations displayed very poor fitting with Hixson-Crowell cube root model of drug release, which were related, with the method of manufacture followed.

Scanning electron microscope analysis (SEM)

Study further confirmed both diffusion and erosion mechanisms to be operative during drug release from the surface of matrix tablet. Morphology and surface properties of the tablets were found to be affected by the polymer concentration.

CONCLUSION

From the study, it is possible to conclude that the selected excipients are likely to be suitable for the preparation of tablet formulations direct compression method. According to the release studies, formulations containing low level of polymer are not appropriate for the preparation of modified hydrophilic matrix tablets while higher level may be advantageous as release rate of the drug from matrix tablets was dependent on the proportion and type of the polymer used. The results of release studies indicated the possibility of achieving a suitable modulation of Lamivudine release rate by opportunely varying the methocel K15M CR in the matrix tablet.

ACKNOWLEDGEMENT

The authors are thankful to Colorcon Asia Pvt. Ltd., Bangladesh for their generous donation of Methocel K15M CR. The authors are also thankful to The University of Asia Pacific (UAP), Bangladesh University, Primeasia University and the University of Dhaka for their supports and co-operations.

REFERENCES

1. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). "AIDS epidemic update 2005," Geneva: UNAIDS. Available at: http://www.unaids.org/epi/2005/doc/EPIupdate2005_pdf_en/epiupdate2005_en.pdf. Accessed 10 December, 2006.
2. Zhou J, Paton NI, Ditangco R, et al. Experience with the use of a first-line regimen of stavudine, lamivudine and nevirapine in patients in the TREAT Asia HIV Observational Database. *HIV Med.* 8 (2007) 8 -16.
3. Castillo SA, Hernandez JE, Brothers CH. Long-term safety and tolerability of the lamivudine/abacavir combination as components of highly active antiretroviral therapy. *Drug Saf.* 29 (2006) 811- 826.
4. Katlama C, Valantin MA, Matheron S, Coutellier A, Calvez V, D Descamps D, Longuet C, Bonmarchand M, Tubiana R, De Sa M, Lancar R, Agut H, Brun-Vezinet F, and Costagliola D. Efficacy and tolerability of stavudine plus lamivudine in treatment-naive and treatment experienced patients with HIV-1 infection. *Ann Intern Med.* 129 (1998) 525- 531.
5. Merrill DP, Moonis M, Chou TC, Hirsch MS. Lamivudine or stavudine in two- and three-drug combinations against human immunodeficiency virus type 1 replication in vitro. *J Infect Dis.* 173 (1996) 355- 364.
6. Furman PA, Fyfe JA, St Clair MH, Richards CA, Spector T. Phosphorylation of 3' - azido- 3' - eoxythymidinend selective interaction of the 5'-triphosphate with human immunodeficiency virus reverseranscriptase. *Proc Natl Acad Sci USA.* 83 (1986) 8333- 8337.
7. Katy H P Moore; Jo E Barrett; Shuching Shaw; Gary E Pakes; Richard Churchus; Anu Kapoor; Judy Lloyd; Michael G Barry; David Back. The pharmacokinetics

- of lamivudine hosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. *AIDS*. 13 (1999) 2239- 2250.
8. Becher F; Landman R; Mboup S; NdeyeToure Kane C; Canestri A; Liegeois F; Vray M; Prevot M H; Leleu G; Benech H. Monitoring of didanosine and stavudine intracellular trisphosphorylated anabolite concentrations in HIV-infected patients. *AIDS* 18 (2004)181-187.
 9. Himadri Sen, Surva Kumar J, inventors. Long acting composition containing zidovudine and Lamivudine. US patent publication US 20050175694A1 (2005) August 11.
 10. Amidon, GL and R Löbenberg. Modern Bioavailability, Bioequivalence and Biopharmaceutics Classification system. *New Scientific Approaches to International Regulatory Standards. Eur. J. Pharm. Biopharm* 50 (2000) 3–12.
 11. Ford, JL, Rubinstein, MH, Hogan, JE., Formulation of sustained-release promethazine hydrochloride tablets using hydroxypropyl methyl cellulose matrixes. *Int. J. Pharm* 24 (1985) 327- 338.
 12. Reddy RK, Mutalik S, Reddy S. Once daily sustained release matrix tablets of Nicorandil: formulation and in vitro evaluation, *AAPS. Pharm Sci Tech.* 4 (2003) 25-29.
 13. Vazquez MJ, Perez-Marco, Gomez- Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A Influence of technological variables on drug release of drug from hydrophilic matrices. *Drug Dev Ind Pharm.* 18 (1992) 1355-1375.
 14. Vargas CI, Ghaly ES. Kinetic release of theophylline from hydrophilic swellable matrices. *Drug Dev Ind Pharm.* 25 (1999) 1045-1050.
 15. Korsmeyer RW, Gurny R, Docler E, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. *Int J Pharm.* 15 (1983) 25- 35.
 16. Shato H, Miyagawa, Y, Okabe T, Miyajima M and Sunada H. Dissolution mechanism of diclofenac sodium from wax matrix granules. *J. Pharm. Sci.* 86 (1997) 929- 934.
 17. Mockel JE, Lippold BC. Zero order release from hydrocolloid matrices. *Pharm Res.* 10 (1993) 1066- 1070.
 18. Ebube NK, Hikal A, Wyandt CM, Beer DC, Miller LG, Jones AB. Sustained release of acetaminophen from heterogeneous matrix tablets, influence of polymer ratio, polymer loading and coactive on drugrelease. *Pharm Dev Technol.* 2 (1997) 161-170.
 19. Hogan J. Hydroxypropylmethylcellulose sustained release technology. *Drug Dev IndPharm.* 15 (1989) 975-999.
 20. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm Acta. Hel.* 60 (1985) 110-111.