

Research Article

Preparation and characterization of lamivudine microcapsules using various cellulose polymers

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Abstract

Purpose: The objective of the present study was to prepare and evaluate microcapsules for the controlled release of lamivudine using various cellulose polymers

Methods: The microcapsules were prepared by the solvent evaporation method. The prepared microcapsules were characterized for the percent drug content, entrapment efficiency, FTIR, DSC, scanning electron microscopy (SEM) and in vitro dissolution studies. Accelerated stability studies were also carried out.

Results: The microcapsules were spherical and free flowing. The entrapment efficiency was 76-86%. The release of drug from the microcapsules extended up to 8 to 12 hours. FTIR and DSC thermograms showed the stable character of lamivudine in the microcapsules. SEM revealed that the microcapsules were porous in nature. The release kinetics study revealed that the prepared microcapsules were best fitted to the zero order for F-2, F-4 and F-5 formulations and Higuchi model, for F-1 and F-3 microcapsules

Conclusion: The release kinetics data and characteristic studies indicate that drug release from microcapsules was diffusion – controlled and that the microcapsules were stable..

Keywords: Lamivudine, cellulose polymers, microcapsules, controlled release, stability.

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS), which is now a plague in several countries, was first identified in California in 1981. It is a disease in which the body's immune system breaks down and is unable to fight off infections caused by human immunodeficiency virus (HIV). HIV infects human cells and uses the energy and nutrients provided by those cells to grow and reproduce and it is often necessary to take several medicines for prolonged periods. This problem is compounded especially for drugs having a shorter biological half life which have to be administered frequently. It is crucial for the success of AIDS therapy to maintain systemic drug levels consistently above its target antiretroviral concentration throughout the course of the treatment^{1, 2}. This can be achieved prolonging release.

Lamivudine is a synthetic nucleoside analog that is being increasingly used as the core of an antiretroviral regimen for the treatment of HIV infection.^{3,4} *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.⁵⁻⁷ 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.⁸ Therefore, the objective of the present work is to provide a long acting pharmaceutical composition containing

lamivudine in a modified release matrix formulation, to maintain the blood levels of the active ingredient for a prolonged period of time.

Only a limited study lamivudine extended release formulations has been carried out using ethyl cellulose⁹, Matrix tablets of anti-HIV drug didanosine¹⁰ have also been formulated.

MATERIALS AND METHODS

Materials

Lamivudine was obtained as a gift from Alkem laboratories Ltd (Mumbai, India). Cellulose acetate phthalate (CAP) was obtained from GM Chemicals, India, cellulose acetate butyrate (CAB) was obtained from Eastman chemical company, USA, ethyl cellulose (EC) was obtained from The DOW chemical company, USA and hydroxy propyl methyl cellulose acetate phthalate (HPMCP) was obtained from SHIN-Etsu, Japan. All other chemicals and reagents used in the study were of analytical grade.

Methods

Preparation of microcapsules

Lamivudine microcapsules were prepared by the solvent evaporation method. The drug and polymer (1:1 ration) were dissolved or dispersed in acetone and added to heavy liquid paraffin with stirring. Microcapsules were recovered by treating with n-hexane. The micro capsules prepared with CAP, CAB, EC, HPMCP and combination of CAP : CAB were coded as F-1, F-2, F-3, F-4 and F-5 respectively.

Table 1: Physical characteristics of microcapsules

| Formulation code | F-1 | F-2 | F-3 | F-4 | F-5 |
|-----------------------------------|-------------------------|-----------|-----------|-----------|-----------|
| Particle size distribution | Percent retained | | | | |
| 10/20 (1242 μ) | 9 ± 0.25 | 6 ± 0.29 | 9 ± 0.22 | 12 ± 0.21 | 5 ± 0.11 |
| 20/30 (666.5 μ) | 77 ± 1.96 | 72 ± 2.33 | 78 ± 1.78 | 65 ± 1.23 | 81 ± 2.63 |
| 30/40 (445 μ) | 7 ± 0.23 | 2 ± 0.19 | 3 ± 0.21 | 9 ± 0.21 | 6 ± 0.12 |
| 60/80 (225 μ) | 7 ± 0.22 | 20 ± 0.11 | 11 ± 0.37 | 14 ± 0.18 | 8 ± 0.22 |

Table 2: Accelerated stability data for lamivudine microcapsules at 40°C/75 % RH

| Formulation code | F-1 | F-2 | F-3 | F-4 | F-5 |
|------------------|-------------------------|-------|-------|-------|-------|
| | Drug content (%) | | | | |
| 0 Month | 37.22 | 34.72 | 41.01 | 35.90 | 42.12 |
| 1 Month | 36.33 | 34.22 | 40.12 | 34.99 | 41.22 |
| 2 Months | 36.02 | 33.12 | 40.36 | 35.22 | 41.01 |
| 3 Months | 35.22 | 33.34 | 40.21 | 35.12 | 40.12 |

Characterization of microcapsules**Encapsulation efficiency (EE)**¹¹

Drug loaded microcapsules (100 mg) were powdered and suspended in water and then sonicated (Power sonic 505, HWASHIN technology co) for about 20 minutes. It was shaken for another (ORBITEX, Scigenics biotech) 20 minutes for the complete extraction of drug from the microcapsules. The mixture was filtered through a 0.45 μm membrane filter (MILLIPORE). Drug content was determined by UV- visible spectrophotometer (Schimadzu, UV-1700 E 23) at 271 nm. The percent entrapment was calculated using the Eq (1).

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \dots(1)$$

Particle size distribution

Particle size analysis¹² of the microcapsules was done by sieving method using Indian Standard Sieves # 16, #20, #30, #40, #60 and #80. The

results of particle size distribution are given in the Table1.

Fourier Transforms Infrared Spectroscopy (FT-IR)¹³ and Differential scanning calorimeter (DSC)

The FT-IR spectra acquired were taken from dried samples. An FT-IR (Thermo Nicolet 670) spectrometer was used for the analysis in the frequency range between 4000 and 400 cm⁻¹, and 4 cm⁻¹ resolution. The results are the means of 16 determinations. A quantity equivalent to 2 mg of pure drug and drug loaded microcapsules were used.

Differential scanning calorimetry (DSC) study of drug loaded microcapsules was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipient compatibility study. The analysis was performed at a rate 5 °C min⁻¹ from 50 °C to 200 °C temperature range under nitrogen flow of 25 ml min⁻¹.

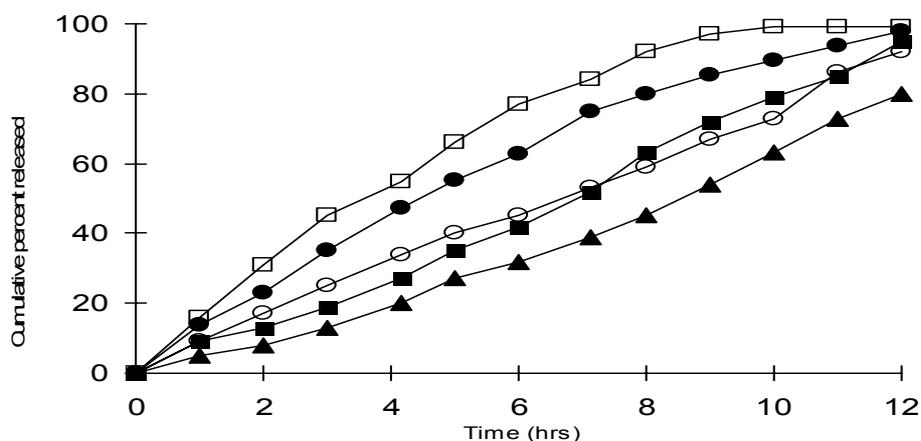


Fig 1: Cumulative % release vs time plots of lamivudine microcapsules prepared with (□-) with CAP, (●-) with CAB, (○-) with EC, (■-) with HPMCP and combination of (▲-) CAP: CAB

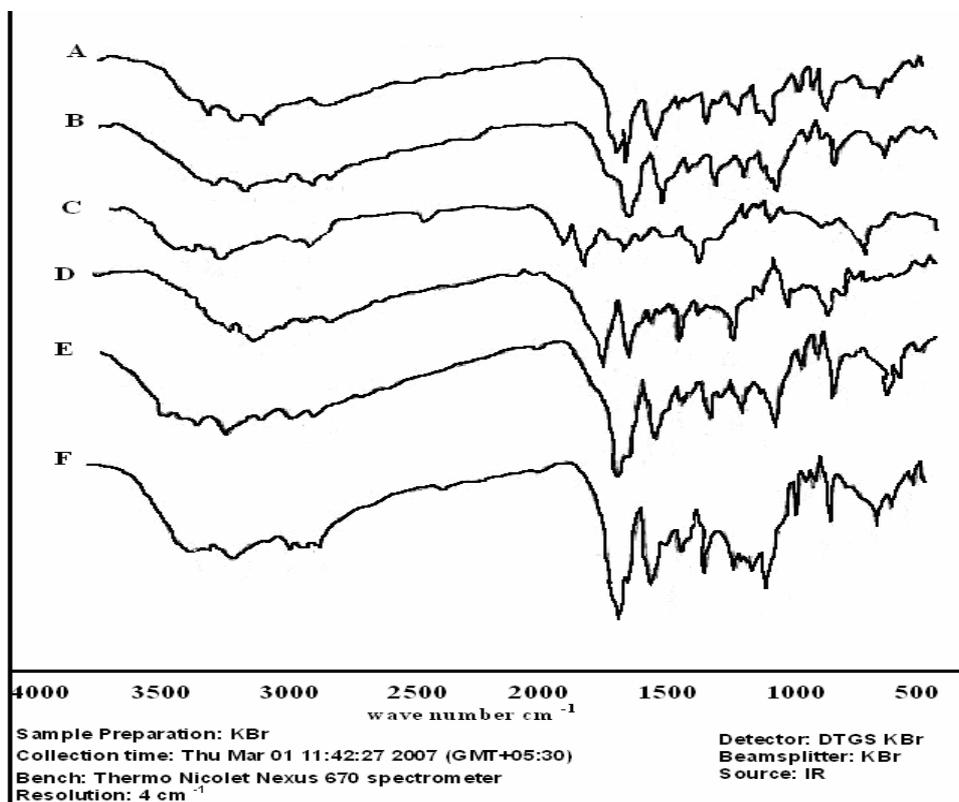


Fig 2: FT IR Spectra of pure (A) lamivudine, (B) CAP microcapsules (F-2), (C) CAB microcapsules (F-1), (D) EC microcapsule (F-3), (E) HPMCP microcapsule (F-4) and (F) combination of CAP:CAB (F-5)

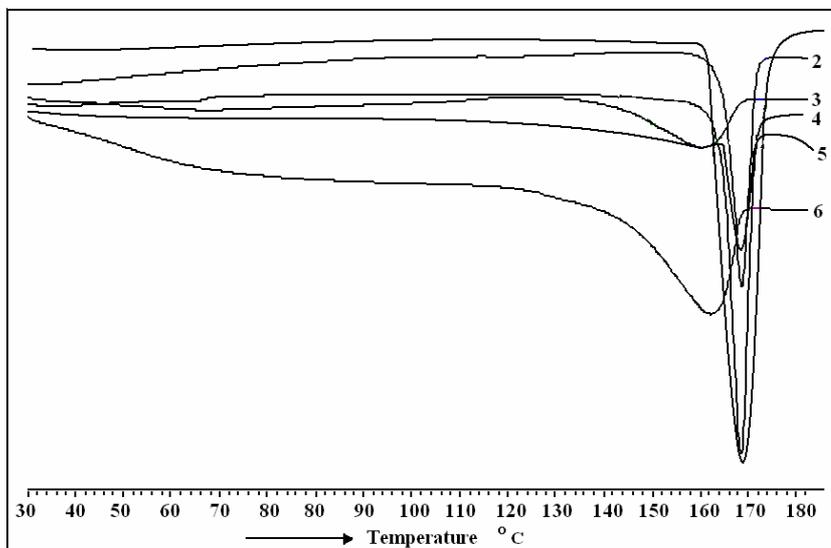


Fig 3: DSC thermo grams of lamivudine and lamivudine microcapsules with different polymers (1) Pure lamivudine (2) CAP (F-1), (3) CAB (F-2), (4) EC (F-3), (5) HPMCP (F-4) and (6) combination of CAP: CAB (F-5).

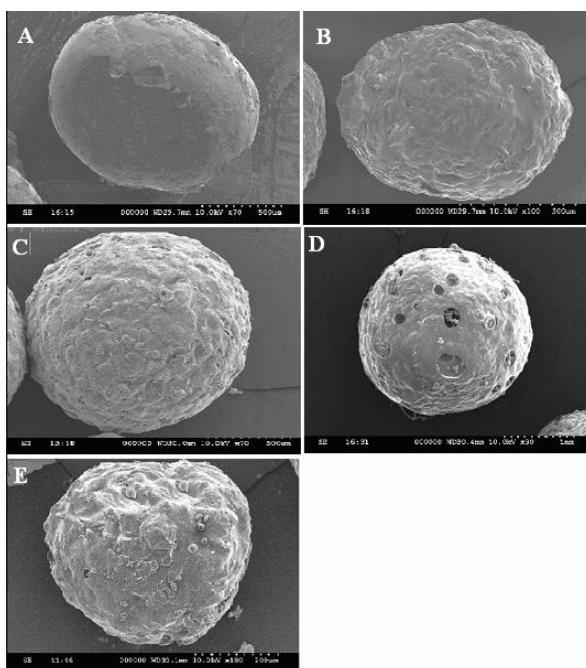


Fig 4: SEM photographs of (A) Cellulose acetate phthalate microcapsules (B) Cellulose acetate butyrate microcapsule (C) Ethyl cellulose microcapsules (D) Hydroxy propyl methyl cellulose phthalate microcapsules (E) Combination of Cellulose acetate Phthalate and Cellulose acetate butyrate

Scanning electron microscopy (SEM)

Morphological characterization of the microcapsules was carried out scanning electron microscopy (JEOL JSM -5200). The samples were coated to 200Å thickness with gold-palladium using prior to microscopy.

In Vitro Drug Release Studies

In vitro dissolution studies were performed using USP type I dissolution apparatus (LABINDIA, DISSO-2000, Mumbai, India) at 75 rpm. The micro capsules were weighed and filled in the empty capsule shells and placed in the basket. The dissolution medium (900ml) consisted of 0.1M hydrochloric acid for the first 2 hours and then changed to phosphate buffer pH 7.4 from 3rd to 12th hour, Temperature was maintained at 37°C ± 5°C. An aliquot (5 mL) was withdrawn at specific time intervals and rephenished with an equivalent volume of dissolution fluid. Drug content was determined by UV - visible spectrophotometer (Schimadzu, UV-1700 E 23) at 271 nm. The release studies were conducted in triplicate and the results are showed in Fig1.

Determination of stability of the microcapsules

The microcapsules prepared in the present study were filled in the hard gelatin capsules and stored in HDPE containers at 40°C/75% RH for 3 months as per ICH guidelines. The samples were then characterized for % drug content. The results are summarized in the Table 2.

RESULTS

Maximum release of lamivudine from the various formulations was achieved in 12 hrs or longer, Figure1. The release mechanism of the lamivudine formulations was determined by comparing their respective correlation coefficients given in Table 1. Drug release from formulations F-1 and F-3 followed the Higuchi model but for F-2, F-4 and F-5 formulations, the release was best fitted to the zero order model. FTIR studies indicate four bands present in the lamivudine spectrum, namely; N-H, O-H, C=O, C=N linkages respectively. The same bands were also found in the spectra of the formulations, showing that no drug-polymer interaction occurred (see Figure2). DSC

thermogram of pure lamivudine showed a sharp endothermic peak at 180°C. The thermograms of the formulations F-1 to F-5 also showed a similar the same endothermic peak at 180°C. This further confirms that there was no drug polymer interaction. Scanning electron microscopy (SEM) results (Figure4) show that the microcapsules were spherical and that the microcapsules prepared with cellulose acetate phthalate (CAP) had a formed smooth surface. The results of all the formulations were found good. High entrapment efficiency was observed in the microcapsules prepared with ethyl cellulose and combination of cellulose acetate phthalate and cellulose acetate butyrate. The results of accelerated stability study on the microcapsules (shown in Table 1) revealed a good correlation between the original and the aged samples.

DISCUSSION

The formulations either followed zero order release or the Higuchi release model. Thus drug release was diffusion controlled. The release mainly deepened on the type of polymer and viscosity. The results indicate that F-5, formulations showed the slowest release rate while FTIR indicated that there was no drug-polymer interaction. This was further confirmed by the DSC results. The results of accelerated stability study showed the stable nature of the drug. A good entrapment efficiency was observed. SEM demonstrated the spherical nature of the microcapsules and the presence of drug particles on their surface.

CONCLUSION

The lamivudine microcapsules prolonged drug release for 12 hours or longer. Would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional lamivudine tablets. No drug polymer interaction was found and lamivudine remained stable over a long period of time.

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