**Formulation and Evaluation of Mucoadhesive Microspheres of Lamivudine**

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**ABSTRACT**

The aim of the present work was to prepare and evaluate mucoadhesive microspheres of Lamivudine. Microspheres were formulated using sodium alginate (5%) with mucoadhesive polymer (Chitosan 1%) and copolymer Sodium CMC HPMC, Xanthan gum (XG) in concentration of 1% (Chitosan1% + HPMC1%) (1%) retarding agents and 10% of Calcium chloride (CaCl₂). Aluminium sulphate (Al₂O₃) as cross linking agents by employing Ionic Gelation Technique. The particle size was characterized for by scanning electron microscopy (SEM) and drug excipients compatibility was determined by FT-IR spectroscopy. Percentage drug content, Entrapment efficiency and in-vitro dissolution studies were also carried out. Among the prepared microspheres (F8) formulation in which Al₂O₃ was used as cross linking agent, portray better sustained release for more than 12hrs. The dissolution profile followed the near zero order profile and Hixon-Crowell as “best fit” model. SEM shows that prepared microspheres were of spherical in shape and free flowing. FT-IR results showed compatibility of Lamivudine with excipients used.

**Keywords:** Microsphere, Hixon-Crowell, FT-IR Spectroscopy, SEM analysis

**INTRODUCTION**

New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard novel drug delivery systems (NDDS) have many benefits, which includes improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce unwanted adverse effects. [1-2] Micro particulate drug delivery posses many advantages such as high bioavailability, rapid kinetic of absorption as well as avoidance of hepatic first pass effect and improvement of patient compliance. [3-4] The purpose of designing microsphere dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behaviour due to unit to unit variation, change in gastro-luminal pH and enzyme population. Lamivudine is an active anti-retroviral drug belonging to non-nucleosides reverse transcriptase inhibitor. Lamivudine treatment has gained immense popularity in the AIDS treatment in the present era. [5-6] Dosage and duration of lamivudine therapy should be individualized according to requirement and response of the patient. The daily-recommended dose is 150 mg b.i.d. [7] The oral administration of lamivudine exhibits side effects in GIT as well as in CNS. Thrombocytopenia, paraesthesia, anorexia, nausea, abdominal cramps, depressive disorders, cough and skin rashes etc have been reported as possible adverse reactions. [8] Controlled release (CR) preparations helps to achieve maximum therapeutic effect with simultaneous minimization of adverse effects. Lamivudine is anti-retroviral drug, freely soluble in water and has a short life (5 to 7hrs), Lamivudine is the (-) enantiomer of 2',3'-dideoxy-3'-thiacytidine, is a nucleoside analog that exhibits HIV reverse transcriptase. [9] The use of natural polymers in dosage form design has received considerable attention, especially from the viewpoint of safety. Among these polymers, chitosan, Hydroxy propyl methyl cellulose, Sodium CMC, Xanthan gum and sodium alginate are very interesting biomaterials for multiparticulate oral drug delivery. Microspheres formulation is based upon the interaction between the polymer and crosslinking agent. Sodium alginate (SA) is an anionic polymer, which can be easily cross linked with CaCl₂ and Al₂O₃. The complexation between Ca²⁺ or Al³⁺ ions with SA leads to retard the release of the drug. This provides an opportunity of developing once daily (OD) controlled release

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formulation, by employing Calcium chloride (CaCl₂) and Aluminum sulphate (Al₂(SO₄)₃) were used as cross linking agents.

MATERIALS AND METHODS

Lamivudine was obtained as gift sample from Aurobindo Pharma, Hyderabad, India. Xanthan gum from Raj enterprises Mumbai, India, Sodium Carboxy Methyl Cellulose (SCMC) (high viscosity grade) from Reliance Cellulose Product, Hyderabad, India, was used. All other materials were of analytical or reagents grade.

Preparation of Lamivudine Loaded Microspheres

Lamivudine loaded microsphere formulations were prepared by using Hydroxy propyl methyl cellulose (HPMC), Guar gum (GG), Sodium carboxy methyl cellulose (SCMC) as mucoadhesive polymer along with sodium alginate solution. Ionic Gelation technique was employed for the preparation of microspheres. The interaction between Sodium Alginate (SA) and Calcium chloride was used to prepare calcium alginate microspheres. Lamivudine (1%) was dispersed in the SA (5%) along with (1%) of mucoadhesive polymer i.e HPMC, GG, SCMC. The formulations are coded as F1, F2, F3, F4, F5 and F6 is shown in Table 1. The drug to polymer ratio is 1:1. This solution was mixed thoroughly with a stirrer to form viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (10% w/v) solution through a syringe with a needle of size no 24G. The added droplets were retained in calcium chloride solution for 15 minutes to complete the curing reaction and to produce rigid microspheres. The microspheres were collected by decantation and then washed thoroughly with distilled water and dried at 45°C for 12 hours. Similarly Lamivudine loaded microspheres were formulated by using Aluminium Sulphate (Al₂(SO₄)₃) as cross linking agent. The formulations are coded and shown in Table 1.

Characterization of Microsphere

Particle Size Determination

The size of the prepared microspheres was measured by the optical microscopy method using a pre-calibrated stage micrometer. Particle size was calculated by using equation

\[ X_g = 10 \times \left( \frac{\frac{1}{n_i} \times \log X_i}{N} \right) \]

\( X_g \) is geometric mean diameter, \( n_i \) is number of particle in range, \( X_i \) is the midpoint of range and \( N \) is the total number of particles. All the experimental units were analyzed in triplicate (n=3).

Drug Encapsulation Efficiency

About 100mg of microspheres was taken and triturated with phosphate buffer pH 6.8 and transferred to 100 mL volumetric flask. The volume was made up to 100mL and mixed well. The solution was then kept aside for 12 hours. It was sonicated in ultrasonicator and then filtered through membrane filter 0.45μm and estimated for drug content by measuring the absorbance at 270nm. The drug entrapment efficiency was calculated using the formula.\[^{[10]}\]

\[ \text{Drug Encapsulation Efficiency} = \left( \frac{\text{Estimated } \% \text{ drug content}}{\text{Theoretical } \% \text{ drug content}} \right) \times 100 \]

Degree of Swelling\[^{[12]}\]

The swelling ability of microspheres in physiological media was determined by swelling them in the Phosphate buffer pH 6.8. Microspheres were suspended in 5 mL of phosphate buffer pH 6.8, the increase in particle size of microspheres was noted up to 10 hours and the swelling index was calculated. The degree of swelling was calculated using following formula:

\[ \alpha = \frac{(W_s - W_o)}{W_o} \]

\( \alpha \) is the degree of swelling; \( W_s \) is the particle size of microspheres before swelling; \( W_s \) is the particle size of microspheres after swelling.

In-vitro wash off test for microspheres\[^{[13]}\]

The mucoadhesive properties of the microspheres are evaluated by in vitro wash off test reported by Lehr et al. A 1cm² piece of sheep mucosa was tied on a glass slide using thread. About 100 microspheres were spread on to the wet rinsed tissue specimen and the prepared slide was hung onto one of the grooves of a USP tablet disintegration apparatus. The USP disintegration apparatus is operated such that the tissue specimen is given regular up and down movements in a beaker containing 800 mL of phosphate buffer pH 6.8. At the end of 30 min, 1 hour and hourly intervals up to 10 hours the number of microspheres still adhering to the tissue was counted.

In-vitro Dissolution Study

The USP rotating-paddle dissolution rate apparatus (USP XXII type II apparatus (Lab India Disso 2000 system, India) is used to study drug release from the 100 mg microspheres. The dissolution parameters were 37±2°C, 50 rpm, 900 ml of phosphate buffer pH 6.8 were maintained for all the formulations. About 5 ml of aliquot samples were withdrawn at specified intervals and after suitable dilution were assayed by using UV-Visible spectrophotometer at 270nm.\[^{[13]}\]

Characterization of Release Data

The description of dissolution profiles has been attempted using different release models. The data were evaluated according to the following equations.\[^{[14]}\]

- Zero order: \( M_t = M_s + K_o t \)
- First order: \( \ln M_t = \ln M_s + K_o t \)
- Higuchi model: \( M_t = K_h \sqrt{t} \)
- Korsmeyer-Peppas model: \( M_t/M_s = K_t^n \)

Hixson-Crowell cube root law: \( Q_t^{1/3} - Q_0^{1/3} = K_q C_t \)

Where \( M_t \) is the amount of drug dissolved in time \( t \), \( M_s \) is the initial amount of drug, \( K_o \) is the first order release constant, \( K_h \) is the zero order release constant, \( K_t \) is the Higuchi rate constant, \( K_q \) is the release constant and \( n \) is the diffusion release exponent indicative of the operating release mechanism. The correlation coefficient \((r^2)\) was used as an indicator of the best fitting, for each of the models considered.

Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles Where, \( Q_t \) is the remaining amount of drug in the dosage form at time \( t \), \( Q_0 \) is the initial amount of the drug in tablet and KHC is the rate constant for Hixon-Crowell rate equation. A graphical representation of the cube root of the amount remaining versus time will be linear if the equilibrium conditions are not reached and if the geometrical shape of the dosage form diminishes proportionally overtime (Cube root of initial drug load minus cube root of % drug remaining are plotted against time to demonstrate the Hixon Crowell plot.\[^{[15]}\] This model is used by assuming that release rate is limited by the drug particles dissolution rate.
The dissolution parameters used for comparing the different formulations was MDT and $D_E\%$. The following equation was used to calculate the mean dissolution time (MDT) from the mean dissolution data.

$$MDT = \frac{\sum_{i=1}^{n} \Delta M}{\sum_{i=1}^{n} t_{md}}$$

Where i is the dissolution sample number, n is the number of dissolution sample time, $t_{md}$ is the time at the midpoint between i and i-1 and $\Delta M$ is the additional amount of drug dissolved between i and i-1. \[1\] MDT, which is calculated from the amount of drug released to the total cumulative drug. MDT is a measure of the rate of the dissolution process: the higher the MDT, the slower the release rate.

Dissolution efficiency (DE) after 8hr of release test was used to compare the results of dissolution tests of different formulations. \[17\]

$$DE\% = \frac{\int_0^{t} y dt}{y_{100}} \times 100$$

FT-IR spectroscopy
Infrared spectrum was taken (FT-IR, Spectrum RX1, Perkin Elmer Ltd, Switzerland) by scanning the sample in Potassium bromide discs. The samples of pure drug and formulated microsphere (M3C3) were scanned individually.

Scanning Electron Microscopy
Shape and surface morphology of formulated microspheres were studied using scanning electron microscopy (SEM-JEOL-JSM-6510, Japan). The microspheres were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument.

Stability Studies\[18\]
Stability studies were conducted for the microspheres of formulation (M3C3) to assess their stability with respect to their physical appearance, drug content and drug release characteristics after storing at 40±2°C/75±5% RH for 6 months was seen.

Statistical analysis
In-vitro release data of Lamivudine release from the microspheres formulations (M1C3) and formulation (M3C3) were subjected to the 1-way analysis of variance (ANOVA) at different time intervals of drug released up to 12h, by
Newman-Keuls multiple comparison test Graph pad prism version 5 (Graph pad prism Software, Inc).

RESULTS AND DISCUSSION
The mucoadhesive microspheres of lamivudine was prepared by Ionic gelation technique, as this method is most simple, easy, cost effective and extensively used to prepare microspheres. Calcium chloride (CaCl₂) and Aluminum sulphate Al₃(SO₄)₂ were used as cross linking agents to prepare microspheres. The interaction between Sodium Alginate (SA)-CaCl₂ and SA-Al₃(SO₄)₂ results in the preparation of microspheres, this is because the Ca²⁺ and Al³⁺ ions are bound to carboxyl residues of both mannuronic acid and glucuronic acid, which are the components of SA. Here it is the interaction of Ca²⁺ or Al³⁺ with glucuronic acid that contributes to the complexation mechanism. This complexation leads to controlled release of drugs. Modified Alginate microspheres were prepared by adding or coating with HPMCK15M, GG and SCMC as mucoadhesive polymers to retard the drug release. The drug and polymer were taken in (1:1) in ratio F1 to F6 formulations. The composition was shown in Table 1. The formulations were prepared to reduce the dosing frequency thereby improving the effectiveness of the drug.\(^{[19]}\)

Evaluation of Mucoadhesive Microspheres

Production Yield
The production yield of microspheres prepared by Ionic Gelation method was found to be 84.60% to 86.31% is shown in Table 2. It was found that production yield of microspheres prepared by SCMC was less than HPMC. The probable reason behind this may be the high viscosity of the solution, which decreased its syringeability resulting in blocking of needle and wastage of the drug-polymer solution, which ultimately decreased the production yield of microspheres. Another reason for that may be the agglomeration and sticking of polymer to stirrer as well as to the sides of the beaker during preparation, it relative decrease in production yield of the SCMC formulations (F3 and F6).

Drug Entrapment Efficiency
Drug entrapment efficiency was found to be 77.98±0.01% to 89.99±0.03%. The results obtained are given in Table 2. The drug entrapment efficiency from M3C3 formulation was found to be 89.99±0.03%. The drug entrapment efficiency from M3C3 formulation was found to be 89.99±0.03%. The higher concentration of the polymer increases the viscosity of the medium as well as greater availability of Calcium and Aluminum binding sites in the polymeric chains, as a result cross linking agent is increased, and larger droplets were formed entrapping a greater amount of drug. The entrapment efficiencies were higher for microspheres prepared with Al₃(SO₄)₂, it may be attributed to the amount of cross linking agent and also the higher density of Al₃(SO₄)₂, when compared to CaCl₂. Thus it is inferred that there was a proper distribution of lamivudine in the microspheres.

Particle size analysis
Mean particle size of all formulations are in the range of 528.87±0.02μm to 828.0±0.08μm. The results were given in Table 2. From the results obtained it was observed that higher concentration of polymer increases the viscosity of the medium, which increases the particle size of the microspheres. The viscosity of SCMC>G>HPMC, thus particle size of SCMC was larger compared to HPMC.
The concentration of SCMC was increased in range of 1-2% which increased the particle size from $618\pm0.05\mu m$ to $652\pm0.03\mu m$. Thus Polymer concentration seemed to affect the values of particle size.

**Flow Property of Microspheres**
The flow property of microspheres was checked by using the angle of repose method. Acceptable range of angle of repose was found to be $22.20^\circ$ to $26.20^\circ$, which shows all formulations exhibit good flow property. The results were shown in the Table 2.

**Degree of Swelling**
The degree of swelling of all the formulations was shown in Table 2. The results revealed that all formulations showed rapid swelling, when immersed in PBS pH 6.8. The adhesive and cohesive properties are generally affected by their swelling behavior. The degree of swelling of formulations F1 to F3 was found to be $0.83\pm0.08$ to $0.78\pm0.01$, where as in case of F4 to F6 it was $0.80\pm0.04$ to $0.69\pm0.01$ and for formulations M1C1 to M3C1 it was $0.67\pm0.01$ to $0.73\pm0.03$. It was found that with increase in polymer concentration,
swelling of microspheres was found to be increased; this could be due to higher ionization of carboxymethyl groups of side chains of SCMC at pH 6.8. The presence of charges develops repulsive forces between polymer chains of the network causing its expansion.

**In-vitro Wash off Test for Microspheres**

*In-vitro* wash off test was carried out to ensure the adhesion of the formulation to the mucosa for prolonged period of time at the absorption site, indicates *in-vitro* percentage mucoadhesion after 1 hr it reveals that the microspheres poses good mucoadhesive properties. The combination of the SA-SCMC increases the viscosity of the microsphere produce more viscous gel, which leads to increase in adhesion to the intestinal mucosa. The prepared microspheres M3C3 showed 96% mucoadhesion after 1hr. Hence it shows that the drug released from the microspheres is in a controlled manner before being eroded off. It was found that the percentage mucoadhesion is increased with increase in concentration of mucoadhesive polymer. The results were shown in Table 2.

**In-vitro Drug Release Studies**

The affect of various polymers and its concentration along with the cross linking agents were studied for the release profile of prepared microspheres of lamivudine. The release mainly deepened on the type of polymer, its concentration and viscosity. The results were shown in Table 3. The results indicate that the drug released from formulation (F6) is in the range of 84.31±0.12% to 85.11±0.11 up to 12 hours. Hence formulation (F6) was chosen as a better formulation to retard the release for the high soluble drug lamivudine. The release was dependent on amount of polymer added and it is also affected with cross linking agent. The amount of polymer (SCMC) and cross linking agent Al2(SO4)3 was selected for further retarding the release of the lamivudine. The *in-vitro* release profile for all the prepared microspheres is shown in Figure 1 and 2.

The formulation M1C1 shows the drug release in the range of 84.91±0.02% to 85.71±0.01% up to 12 hours where as M2C1 formulation shows the drug release in the range of 76.53±0.12% to 76.93±0.22% up to 12 hours and M3C1 shows release of 68.±0.07% to 69.9±0.05% up to 12 hours, there is a marked difference in the release profile of M1C1 to M3C1, the difference is 7.1±0.13% it is due to 1:2 ratio of SCMC in the formulation of M3C1. The cross linking agent (Al2(SO4)3) has interaction with SA, Al3+ ions are bound to carbohydrate residues of both mannuronic acid and glucuronic acid of SA which enables to retard the release from microspheres.

Similarly the M1C2 shows the amount of drug release of 83.3±0.11% in 12 hours, M2C2 formulation release the drug 74.5±0.03% in 12 hours and M3C2 shows release of 68.±0.05% in 12 hours, there is a marked difference in the release profile of M1C2 to M3C2, the difference is 14.9±0.06% and the M1C3 shows drug release of 80.7±0.02% in 12 hours, M2C3 formulation release the drug 72.6±0.14% in 12 hours and M3C3 showed the amount of drug release of 65.3±0.12% in 12 hours, there is a marked difference in the release profile of M1C3 to M3C3 is 15.3±0.9%. The *in-vitro* release profile of microspheres shows controlled release of lamivudine, among the formulation M3C3 showed slowest release rate of 65.3±0.12% in zero order fashion.

**Release Kinetics of mucoadhesive microspheres formulations**

The release mechanism of the lamivudine formulations was determined by comparing their respective correlation coefficients ($r^2$) is shown in Table 3. According to the results obtained the coefficient of determination ($r^2$) for all the formulations revealed a higher correlation coefficient in the range 0.955 to 0.997, for zero order release. korsmeyer–Peppas model shows the release exponent value ($n$) ranged from 0.637 to 0.790, hence all the formulation followed anomalous non-Fickian diffusion mechanism. A combined release mechanism of drug diffusion and spheres erosion would be appropriate. The correlation coefficient ($r^2$=0.997) value for the formulation M3C3, was higher when compared to other formulation for zero order kinetics and at the same time when compared to first order kinetics, which reveals that it was best fitted to the zero order kinetics and better control release of the lamivudine microspheres.

Formulation F1 to F6 and M1C1 to M3C3 followed zero order kinetics, due to their higher correlation coefficient in the range 0.978 to 0.997, when compared to first order kinetics. All the formulations followed Higuchi equations proving that the release is by diffusion mechanism. The Hixson-Crowell cube root law describes the release from system where there is a change in surface area and diameter of the particles of the microspheres. For studying the mechanism of drug release from the microspheres, the dissolution data was fit into korsmeyer’s and peppas equation. Formulations F1 to F6 shows values greater than ($n$) 0.5 and they follow Non-Fickian diffusion, is shown in Table 3. The diffusional exponent values ($n$) of microspheres have values greater than 0.5 and less than 1 and they follow Non-Fickian diffusion. Non-Fickian diffusion is also called as anomalous transport, where diffusion and relaxation occur at comparable rates and thus interacting complex fashion. The formulation M3C3 slightly eroded till the end of the 12h of dissolution study. From this study, we may infer that SCMC provided better release to achieve zero-order profile than Guar Gum & HPMC K15M with better mucoadhesive property. The analysis of the dissolution kinetic data for the microspheres prepared this study show that it follows the near zero-order kinetics, and the release process involves erosion/diffusion and an alteration in the surface area and diameter of the swellable microspheres as a matrix system as well as in the diffusion path length from the cross linked microspheres with the drug load during the dissolution process. The correlation coefficient for Hixon-Crowell cube root law was found to be higher $r^2$= 0.998 for the formulation M3C3 when compared to other formulations, it indicates the drug release is with diffusion with prolonging release with spherical shape. This relation is best described by the use of both the Higuchi equation and Hixon-Crowell cube root law as shown in Table 3.

The calculated values of MDT revealed that, MDT for the formulation M3C3 is higher than formulation M1C1, is shown in Table 3. It indicates that MDT is increased, while D.E. % decreased, while increasing the amount of SCMC from 1 to 2%. MDT and D.E. % values of M3C3 formulation were found to be 16.14 hours and 67.68% respectively, indicating that the release of drug is slower from M3C3 formulation.

**FT-IR Studies**
The FT-IR study is shown in Figure 3. The interaction study between the drug (lamivudine) and polymer (GG, HPMC, SCMC) in different formulations was evaluated using FT-IR spectrophotometer. Four bands present in Lamivudine spectrum at 1404.87, 1610.90, 3241.81, 3630.09 cm⁻¹, due to the formation of C=O, C=N, N-H, O-H linkage respectively, was also detected and identified in the spectrum of the formulations M3C3 indicating that no chemical interaction, occurred between the drug and the excipients used in the study.

**Scanning Electron Microscopy**

The surface morphology of lamivudine microspheres were studied by using SEM analysis. SEM photographs of formulations M3C3 at different time intervals were shown in Figure 4. This indicated that the microspheres were discrete, uniform and spherical with a smooth textural surface.

**Stability Studies**

The stability study of the formulation (M3C3) was performed after 3 months and the effect on the various parameters was studied the microspheres (M3C3) after 3 months showed good physical appearance, drug encapsulation efficiency is same as that of initial. After 3 months the formulation M3C3 under stability study was assayed and found to be the same. The In-vitro drug release profile studies were performed after storage for 3 months at 40±2°C/75±5%RH, In-vitro release studies showed that there was no much difference in the drug release of formulation and it is stable. The result obtained is shown in Figure 5.

**Statistical analysis**

Analysis of variance (single factor ANOVA) showed a significant differences (P<0.01) for the amount of Lamivudine released from the microspheres formulations (M1C3) and formulations (M3C3).

![Fig. 5: Comparison of In-vitro drug release of formulation (M3C3) Initially and After 3 months storage](image)

Lamivudine release from the microspheres was influenced by the cross-linking agents and with the modified (SA) of different retarding polymers. Formulation F6 containing was found to give a maximum entrapment efficiency of 94.65% and an optimum drug release of 85.1% in 12 hours. Sodium CMC showed higher mucoadhesion and degree of swelling than other polymers. The data obtained are fitting to various kinetic models indicated that the drug release followed near zero order kinetics, This relation is best described by the use of both the Higuchi equation and Hixon-Crowell cube root law for the formulation M3C3. Thus, the formulated microspheres seem to be a potential candidate as controlled drug delivery system for symptomatic therapy of HIV/AIDS. Therefore, M3C3 formulation may be used for reducing the dosing frequency thereby improving the effectiveness of the drug.

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