Research Article

Gastroretentive sustained release beads of Lamivudine

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Abstract

The objective of the investigation described is to develop gastroretentive sustained release beads of Lamivudine using sodium alginate and Eudragit S100 through the ionotropic gelation method. The resulting solution was extruded into a cross-linking solution containing calcium chloride (1% w/v) and acetic acid (10% v/v) using a 22 gauge syringe needleThe in vitro release profiles of the Lamivudine beads were assessed using 0.01N HCl as the dissolution medium. The oral bioavailability of the optimized microsphere dosage forms was evaluated through a single-dose study, revealing significant differences in parameters such as Cmax (maximum plasma concentration), Tmax (time to reach Cmax), T1/2 (elimination half-life), Ka (absorption rate constant), Ke (elimination rate constant), MRT (mean residence time), MDT (mean dissolution time), and AUC (area under the concentration-time curve) when compared to conventional tablets. Furthermore, a linear relationship was observed between the percentages of dissolved and absorbed Lamivudine, suggesting the possibility of predicting in vivo absorption by measuring in vitro dissolution.To determine the significance of the data, one-way ANOVA followed by the Tukey test was employed. The results indicated that the F3 formulation exhibited the best in vivo performance, demonstrating a controlled release profile that correlated well with the in vitro release profile of Lamivudine from the microspheres. This optimized formulation holds promise for the improved management of AIDS.

Keywords : Dissolution rate, in vivo parameters, in vivo-in vitro correlations, release kinetics.

Introduction

The use of novel drug delivery technologies is revolutionizing drug discovery and development, leading to advancements in the pharmaceutical industry. Novel drug delivery systems (NDDS) offer several benefits, including improved therapy through increased drug efficacy and duration of action, enhanced patient compliance by reducing dosing frequency and offering convenient routes of administration, and targeted delivery to specific sites to minimize adverse effects[1,2]. Microsphere-based drug delivery systems have advantages such as high bioavailability, rapid absorption kinetics, and avoidance of first-pass hepatic metabolism, thereby improving patient compliance[3,4]The objective of designing microsphere dosage forms is to develop a reliable formulation that combines the advantages of single-unit formulations while minimizing the risks associated with variations in drug release profiles and formulation behavior due to unit-to-unit variation, changes in gastrointestinal pH, and enzyme populations. Lamivudine, a non-nucleoside reverse transcriptase inhibitor, is an active antiretroviral drug widely used in AIDS treatment[5,6]. The dosage and duration of lamivudine therapy should be individualized based on patient requirements and response, with the recommended daily dose being 150 mg twice daily[7].Oral administration of lamivudine can lead to gastrointestinal and central nervous system side effects, including thrombocytopenia, paresthesias, anorexia, nausea, abdominal cramps, depressive disorders, cough, and skin rashes[8].

Controlled release (CR) preparations can help achieve maximum therapeutic effects while minimizing adverse effects. Lamivudine is freely soluble in water and has a short half-life of 5 to 7 hours.

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It acts as an HIV reverse transcriptase inhibitor[9]The use of natural polymers in the design of dosage forms has garnered significant attention due to safety considerations. Among these polymers, chitosan, hydroxypropyl methylcellulose, sodium carboxymethylcellulose (CMC), xanthan gum, and sodium alginate are interesting biomaterials for multiparticulate oral drug delivery. Microsphere formulations are based on the interaction between the polymer and crosslinking agent. Sodium alginate (SA) is an anionic polymer that can be easily crosslinked with calcium chloride (CaCl2) and aluminum sulfate (AlSO4). The complexation between Ca2+ or Al3+ ions and SA retards drug release, allowing for the development of once-daily controlled release formulations by employing $CaCl_2$ and $AlSO_4$ as crosslinking agents. It is important to note that the information provided is a continuation of the previous discussion and is not based on specific findings or results from the investigation[10]

Preparation of lamivudine microspheres

Sodium alginate powder was stirred in deionized water for 30 minutes to prepare a 100 ml solution of sodium alginate.Eudragit S100, another polymer, was also added to the solution.Accurately weighed Lamivudine was added to the solution to achieve a homogenous dispersion. The drug dispersion (Lamivudine with SA and Eudragit S100 solution) was added dropwise into a 100 ml cross-linking solution.A 10 ml hypodermic syringe fitted with a 20 gauge needle was used for the addition.The mixture was stirred at 500 rpm during this process.The formed beads were cured for different time intervals, allowing them to solidify and undergo gelation.After the specified curing period, the solution of the cross-linking agent was decanted.The alginate beads were washed three times with 50 ml of deionized water to remove any residual components or impurities.The washed beads were then dried at 60°C for 2 hours in a hot air oven to remove any remaining moisture. Throughout the process, various variables were investigated, including the concentration of sodium alginate, concentration of Eudragit S100, concentration of the cross-linking agent, and different curing time intervals.

Table1: Formulation of lamivudine Microspheres

Formulation	Drug(mg)	Eudragit S100	Sodim Alginate	Cross linking type	Cross linking %w/v
F1	100	-	1%	$CaCl_2$	5
F2	100	-	2%	$CaCl_2$	5
F3	100	1%	1%	$CaCl_2$	5
F4	100	1%	2%	$CaCl_2$	5
F5	100	2%	1%	$CaCl_2$	5

Determination of drug encapsulation efficiency

50 mg of beads from each formulationwere weighed and crushed in a mortar and pastel and the crushed material was dissolved in 100 ml of phosphate buffer at ph 7.4.This solution was mechanically agitated on shaker at 200 rpm for 2hours. The resultant dispersions were filtered and analyzed at 276 nm using UV spectrophotometer (JASCO-V500, Kyoto, Japan). The encapsulation efficiency was determined by the followingformula[11,12]

Table 2: Correlation coefficients according to different kinetic equations

Formulation Code	Mathematical Model (Kinetics)						Best fit Model
	Korsmeyer Peppas		Higuchi	Hixson Crowell	First order	Zero order	
	R^2	n	R^2	R^2	R^2	R^2	
F1	0.9352	0.4480	0.9687	0.9636	0.8979	0.9834	Zero Order
F2	0.9239	0.4408	0.9499	0.9308	0.8678	0.9552	Zero Order
F3	0.9349	0.4231	0.9681	0.9422	0.8874	0.9636	Zero Order
F4	0.967	0.4460	0.9908	0.9899	0.9304	0.9961	Zero Order

Table3: Average particle size of Lamivudine Microspheres

Formulation code	Yield (%) (X± S.D)	Particle size (µm)(X ± S.D)	Drug Entrapment efficiency (%) (X ± S.D)
F1	96.00 ± 0.014	940±11.28	95.36 ± 0.11
F2	97.10 ± 0.019	456±12.42	96.66 ± 0.22
F3	98.00 ± 0.017	743±12.24	99.01 ± 0.12
F4	95.56 ± 0.027	650±8.69	97.01 ± 0.19

Encapsulation efficiency = (AQ/TQ) X 100

where AQ is the actual drug content of beads and

TQis the theoretical quantity of drug present in bead[13].

FT-IR study.

The FT-IR spectrum of the Lamivudine pure drug was found to be similar to the standard spectrum of Lamivudine as in I.P. The individual FT-IR spectra of the pure drug Lamivudine, as well as the combination spectra of the drug and polymers are same. All the characteristic of peaks of Lamivudine were present in spectrum of drug and polymers, indicating compatibility between drug and polymers.

Scanning electron microscopy (SEM).:The surfaces and cross-section morphologies of the beads were observed using a scanning electron microscope(SEM) (JSM-6490 LA, JEOL, Tokyo, Japan)operated at an acceleration voltage of 25 kV. The beads were made conductive by sputtering thin coat of platinum under vacuum using Jeol JFC-1600 autofine coater and then the images were recorded at different magnifications[14].

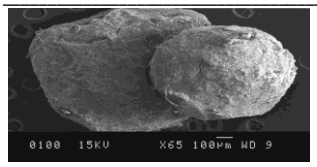

Fig1(a) : SEM images of F4 microsphere

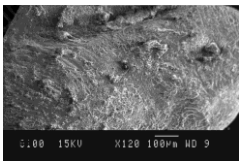

Fig 1(b) : Sem image of F4 microsphere

Figure 1 shows the surface and cross-sectional SEM pictures of the beads. The surface of the dried beads of Formulation 3 were rough and porous (Fig. 4A and 4B). The cross-sectional morphologies of floating beads were also examined with SEM Many large hollow pores or multiple small hollow pockets were observed in the alginate matrix. The number of observed pores appears to be directly related to the amount of incorporated gas-forming agent. The precipitated drug crystals can be seen embedded in the matrix15.

In vitro dissolution studies.

The USP rotating – paddle Dissolution Rate apparatus (Veego, Mumbai, India) was used to study drug release from the microspheres. The dissolution parameters [100 mg microsphere; 37 ± 2°C ; 50 rpm ; 900 ml of 0.01N HCl (n=3); coefficient of variation < 0.05] were maintained for all the four formulations. About 3 ml of aliquot samples were withdrawn at specified intervals and after suitable dilution were assayed by using UV-Visible spectro photometer at 270 nm. The data for percent drug release was fitted for zero order, first order and Higuchi matrix equation.[16-18]

Table 4: In vitro drug release profile of different microsphere formulation

	Formulation Code			
Time (h)	F1	F2	F3	F4
1.	60.22	25.35	19.36	23.47
2.	70.47	21.30	15.73	22.63
3.	74.91	19.57	20.03	15.59
4.	72.09	21.27	17.94	21.31
5.	74.80	21.28	19.38	10.37
6.	72.09	18.41	24.02	10.89
7.	76.29	19.31	22.60	15.01
8.	72.93	21.06	24.71	18.83
9.	71.16	21.41	17.38	23.12

All release data are expressed in cumulative percentage drug release

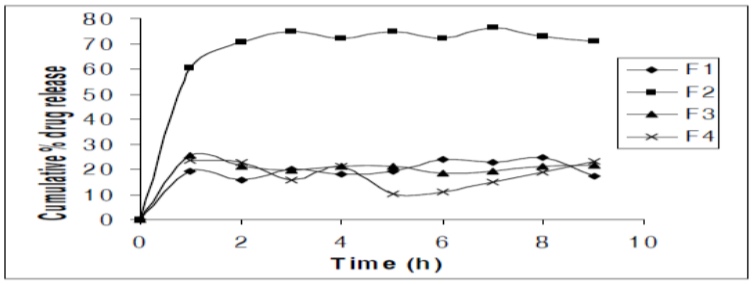

Fig 2:Comparative in Vitro Dissolution Profile of Lamivudine Microspheres

Stability of the microspheres

The formulations showing the best performance, with respect to invitro release, from each set of formulations, microspheres were stored at 4°C, room temperature and 45°C for a month. In an interval of every week, samples were withdrawn and were assayed by using UV-Visible spectrophotometer at 270 nm using distilled water as blank[15]

Table 5:Stability studies

Week	Temp. (° C)	Formulation Code			
		F1	F2	F3	F4
		Potency of formulations in Percentage			
Initial	Room Temp.	99.42	99.56	99.24	99.88
1.	RT	99.01	99.66	99.20	99.78
	37 ± 1	98.89	99.05	98.53	98.55
	60 ± 1	98.88	99.00	98.23	96.36
2.	RT	99.0	99.68	99.21	99.68
	37 ± 1	98.87	98.97	98.65	98.58
	60 ± 1	98.56	98.91	98.22	97.01
3.	RT	98.97	98.99	98.92	98.90
	37 ± 1	98.79	98.87	98.56	98.53
	60 ± 1	98.50	98.82	98.12	97.21
4.	RT	98.83	98.91	98.97	98.80
	37 ± 1	98.69	98.78	98.62	98.41
	60 ± 1	98.32	98.45	98.09	97.0

Results and discussion

Determination of drug encapsulation efficiency.

The microspheres obtained from the formulation process were found to be spherical without any aggregation. The mean geometric particle size ranged from 456±12.42 to 940±11.28 µm, as summarized in Table 3. Smaller particle sizes are desirable as they enhance absorption properties of the formulation. The percentage yield of all formulations was satisfactory, and each formulation exhibited high drug entrapment efficiency (DEE), as presented in Table 3. Among all the formulations, F3 showed the highest DEE.

In vitro drug release profiles for all batches were summarized in Table 4. The F3 formulation displayed the slowest release rate profile among all formulations. The in vitro drug release profiles were depicted in Figure2 . To determine the kinetics of drug release from microspheres, the release data was analyzed using various kinetic models. Table 2 showed that the drug release fromF1,F2 and F3 formulations followed zero-order kinetics, while the F4 formulation fit best with the Higuchi square root model.

Statistical analysis using one-way ANOVA confirmed the significance of the drug release data for the F formulations ($p<0.05$). Stability studies of the prepared formulations were conducted and recorded in Table4.

The best correlation (Level A correlation) was observed with the release profile of the F3 formulation, as presented in Table 2. The results were further validated using one-way ANOVA followed by the Scheff's test, and they were found to be significant at a 5% level of significance ($p<0.05$). The results suggest that the developed in vitro model of the F3 formulation allows for better prediction of the in vivo performance of the 3TC microspheres and can be used for further development of oral sustained-release 3TC formulations for effective management of AIDS.

In vitro release kinetics.

Dissolution studies on all the nine formulations of Lamivudine microspheres were carried out using a USP dissolution apparatus Type II. 0.1N HCl (pH 1.2) and pH 6.8 was used as the dissolution medium. . The cumulative percent drug release after 12 hours was found to be in the range of 81.723, 79.038,76.389 and 71.558% for the formulations F1, F2, F3 and F4 respectively whereas cumulative percent drug release after 9 hours was 71.16, 21.41, 17.38, 23.12% for formulations F1 to F4 respectively. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release

Conclusion

Sustained release formulation of Diclofenac Sodium was successfully prepared using sodium alginate in combination with Eudragit S100 by Ionotropic Gelation Technique. The in vitro dissolution data showed sustained release of the formulation up to 12 hours. The microspheres were prepared without the use of organic solvents. Microspheres of Diclofenac sodium decrease the incidence of side effects and also improve patient compliance by reducing the number of dosings and by reducing the fluctuations of drug in the blood.

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