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Research

Sustained drug delivery of microspheres containing entecavir by Ionotropic gelation technique

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Abstract

In our current research work,we primarily focused on the designing of microspheres containing Entecavirusing PLGA (Poly lactic-co-glycolic acid)and Chitosanas polymers to deliver Entecavir via oral route of adminstration. The results of this investigation indicate that Inotropic gelation technique can be successfully employed to fabricate Entecavir microspheres. In this work an effort has been made toformulate microsphere of Entecavirby using different polymers.Prepared formulations are evaluated for bulk density, tapped density, precentmucoadhesion, Percent compressibility, hausners ration, percentage yield, sizeand interaction study by FTIRand *in vitro* drug release. Formulation has shown significant results in all the evaluation parametersand considered as best formulation of Entecavir. The formulationsF1, F2, and F3 containing PLGA showed a maximum release of97.58% at 10 hours, 98.12% 11 hours, 99.88% 12 hours respectively.The present study reveals that Entecavir microsphere formulation by ionic gelation method could bea conducive method which delivers an excellent bioavailability to the fabricated drug.

Keywords: Entecavir, PLGA, Chitosan andMicrospheres, Ionotropic gelation technique

INTRODUCTION

One of the most challenging areas of research in pharmaceuticals is the development of novel delivery systems for the controlled release of drugs and their delivery at the targeted site in the body to minimize the side effects and enhance the therapeutic efficacy ofdrugs[2,3]. The basic principle behind the controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamics properties of drug in such a way that its efficacy is maximized by reducing side effects, dose frequency and cure the disease in short time by using low amount of drug administered with the most suitable route.

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats, and waxes.Microspheres are small and have large surface to volume ratios. At the lower end of their size range, they have colloidal properties[1-7]. The interfacial properties of microspheres are extremely important, often dictating their activity.

As for as the preparation of microspheres is concern, various methods are available, however current method of ionic gelation method has some Ionic gelation method has some advantages. The synthesis of Microspheres, based on an electrostatic interaction between opposite charge types that contains at least one polymer under mechanical stirring conditions.In Ionotropic gelation technique, the preparation of drug containing microparticles is based on the principle of coalescence of colloidal polymer particles. Ionotropic gelation of the anionic polysaccharide sodium alginate with oppositely charged calcium ions forms microparticles. Subsequent curing step induces the fusion of colloidal polymer particles into a homogenous matrix[8-11]. During the coating and drying process, the colloidal polymer particles coalesce and fuse into a homogenous film. It is a cost-effective technique that establishes intimate contact of the drug with the release retardant.

MATERIALS AND METHODS

The materials exploited in this current research work are procured from reliable sources of Entecavir, Free gift sample from Hetero Pharma limited Hyderabad,PLGA (Poly lactic-co-glycolic acid), Chitosan, Sodiumalginate, CalciumChloride are procured from Merk specialties Pvt Limited. All other reagents used I this research work are procured from most reliable vendors.

Spectroscopic studies

The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 255nm against reference solution 0.1N HCl (pH 1.2). The procedure repeated to pH 6.8 phosphate buffer and pH 7.4 phosphate buffer.

Method of preparation- Ionotropic gelation method

The microspheres were prepared by the Ionotropic gelationtechnique. The sodium alginate solution was prepared bydispersing the sodium alginate in de-ionized water undercontinuous stirring for 30 minutes. The weighed amount ofthe drug was thoroughly mixed with sodium alginatedispersion. By following the same procedure, the alginatebeads of different ratios of drug: polymer were prepared.The resulted homogeneous dispersion was extruded in tothe 5% calcium chloride solution through hypodermicsyringe with flat tip needle (20G) and stirred for 15minutes at 100rpm using magnetic stirrer. The formed microbeads were allowed to cure for 30 minutes in the calciumchloride solution to complete the gelation reaction. Themicrospheres were then filtered and dried in hot air oven at60ºC for 3 hr[12-16]. The formulation of microspheres with the ingredients and their ratios are mentioned in the table 1.

Table 1: Prepared formulation of Microspheres

Ingredients (mg)	Formulation codes					
	F1	F2	F3	F4	F5	F6
Entecavir	0.5	0.5	0.5	0.5	0.5	0.5
PLGA	20	40	60	-	-	-
Chitosan	-	-	-	20	40	60
Sodiumalginate(w/v)	3%	3%	3%	3%	3%	3%
CalciumChloride (w/v)	5%	5%	5%	5%	5%	5%

Micrometric properties

The micromeritics properties are evaluated fo r the prepared formulation of Particle size analysis, bulk density, tapped density, Hausner's ratio, drug entrapment efficiency and swelling study.

Compatibility studies

A proper design and formulation of a dosage form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Compatibility must be established between the active ingredient and other excipients to produce a stable, efficacious, attractive and safe product. If the excipient(s) are new and if no previous literature regarding the use of that particular excipient with an active ingredient is available, then compatibility studies are of paramount importance[16-22]. Hence, before producing the actual formulation, compa-tibility of Entecavir with different polymers and other excipients was tested using the Fourier Transform Infrared Spectroscopy (FT-IR) technique.

Fourier Transform Infrared Spectroscopy (FTIR)

In order to check the integrity (Compatibility) of drug in the formulation,FT-IR spectra of the formulations along with the drug and other excipients were obtained and compared using Shimadzu FT-IR 8400 spectrophotometer. In the present study, Potassium bromide(KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded[23-25].The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug and the polymers.

RESULTS AND DISCUSSION

The calibration curve data of Entecavir in simulated gastric fluid pH 1.2 at 255 nm Fig.8.1 shows the standard calibration curve with a regression value of 0.999, slope of 0.033 and intercept of 0.009 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25µg/ml. Claibration of Entecavir in simulated gastric fluid pH 1.2 and pH 7.4 phosphate buffer has been shown in the figure 1&2.

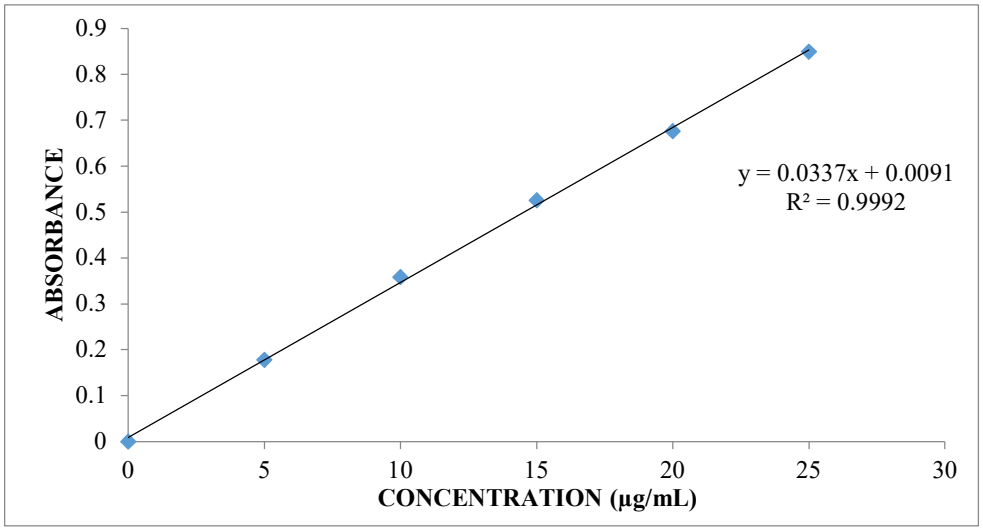

Fig 1: Standard graph of Entecavir in simulated gastric fluid pH 1.2

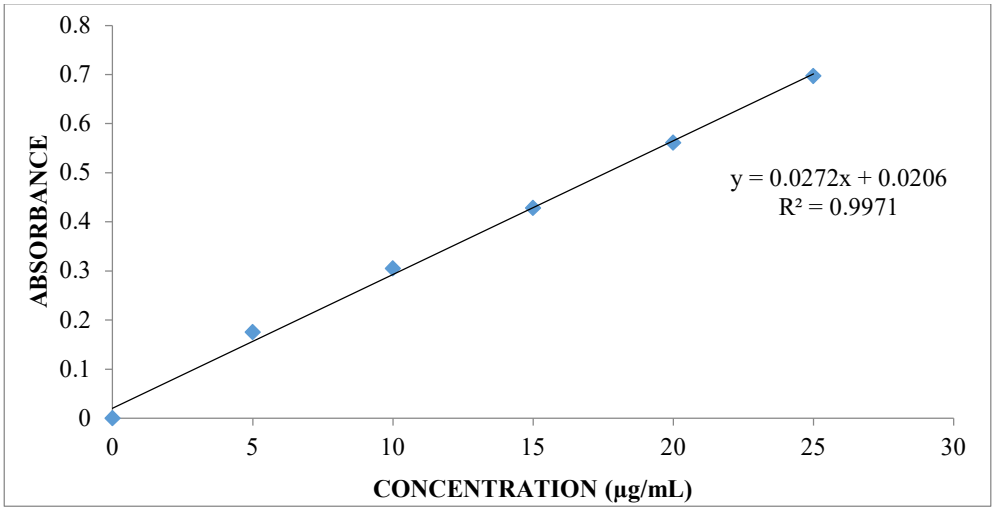

Fig 2: Standard graph of Entecavir in pH 7.4 phosphate buffer

Evaluation and characterization of microspheres

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres

containing PLGA as a polymer had a size range of 418.41µm to 452.14µm. Microspheres containing Chitosan as polymer exhibited a size range between 421.65µm to 460.15µm.
The bulk density of formulation F1 to F6 containing PLGAand Chitosan formulation was in the range of 0.277 ± 0.2± 0.2 to 0.625 ± 0.1 gm./cm^3 (as shown in table 8.3), tapped density 0.312 ± 0.2 to 0.833 ± 0.1 and Hausners ratio 1.095 to 1.333. Characterization of microspheres are mentioned in the table 2.

Table 2: Micromeritic property of microspheres of Entecavir

Formulation code	Mean particle size	Bulk density ((gm./cm^3))	Tapped density (gm./cm^3)	Hausener's ratio	Carr's index	Angle of repose
F1	452.14	0.434 ± 0.2	0.476 ± 0.3	1.095	8.695	23.2 ± 0.2
F2	441.95	0.277 ± 0.2	0.312 ± 0.2	1.133	11.11	25.2 ± 0.1
F3	418.41	0.588 ± 0.3	0.666 ± 0.4	1.333	11.76	27.1 ± 0.1
F4	460.15	0.521 ± 0.3	0.631 ± 0.3	1.121	17.39	24.4 ± 0.4
F5	430.96	0.625 ± 0.1	0.833 ± 0.1	1.333	25.00	28.3 ± 0.4
F6	421.65	0.476 ± 0.3	0.526 ± 0.2	1.105	9.52	25.1 ± 0.1

Drug Entrapment Efficiency

Percentage Drug entrapment efficiency of Entecavir ranged from 84.45 to 91.72 % for microspheres containing PLGA and HPMC K4M polymer, the drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers as mentioned in the table 3.

Table 3: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

S.No.	Formulation code	% Yield	Drug Content (mg)	% Drug entrapment efficiency
1	F1	89.31	96.14	86.14
2	F2	91.12	98.65	88.91
3	F3	96.08	99.76	91.72
4	F4	90.74	98.14	75.58
5	F5	96.91	96.52	84.45
6	F6	98.24	100.04	89.87

Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swell ability is animportant characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. The effect of drug to polymer ratio on percentage swelling is displayed in Figure 3&4. Percentage swelling of the prepared microspheres. The percentage swelling of Entecavir microspheres containing PLGA & Chitosan in the figure 3&4 respectively.

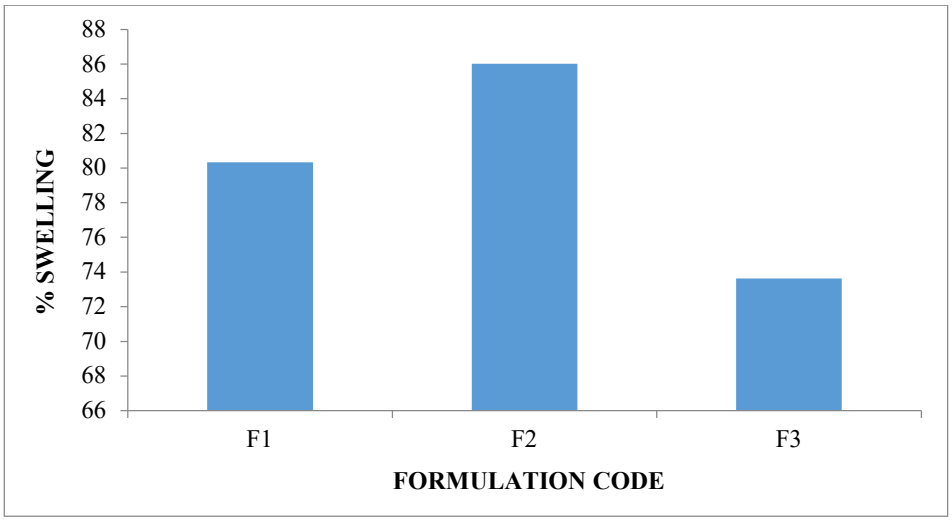

Fig 3: Percentage swelling of microspheres containing PLGA

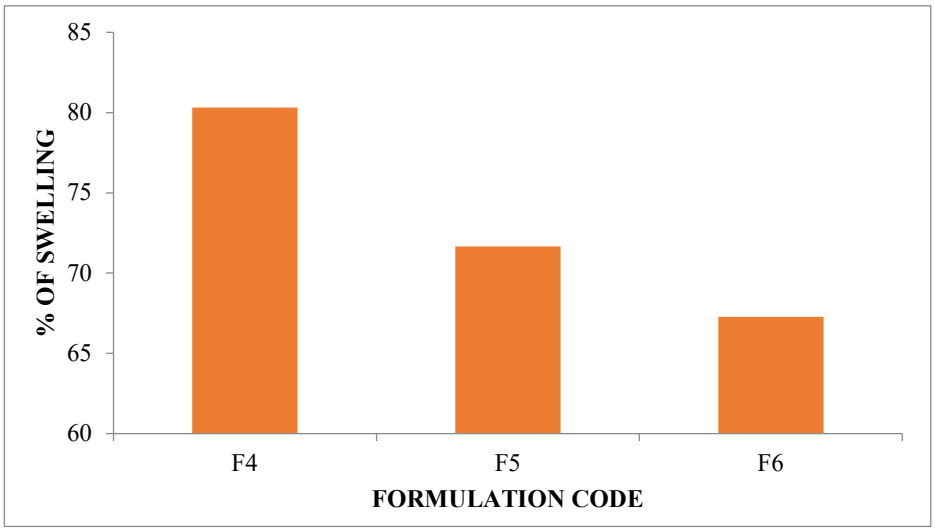

Fig 4: Percentage swelling of microspheres containing Chitosan

***Invitro* mucoadhesion test**

As the polymer to drug ratio increased, microspheres containing PLGA exhibited % mucoadhesion ranging from 61 to 70%, microspheres containing Chitosan exhibited % mucoadhesion ranging from 75 to 95%. The results of *in-vitro* mucoadhesion test are compiled in Table 8.6. Effect of polymer proportion on % mucoadhesion is depicted in Figures and comparative depiction of % mucoadhesion were represented in Figure 5&6 Percentage mucoadhesion of the prepared microspheres. The percentage mucoadhesion of Entecavir microspheres containing PLGA & Chitosan in the figure 5&6 respectively.

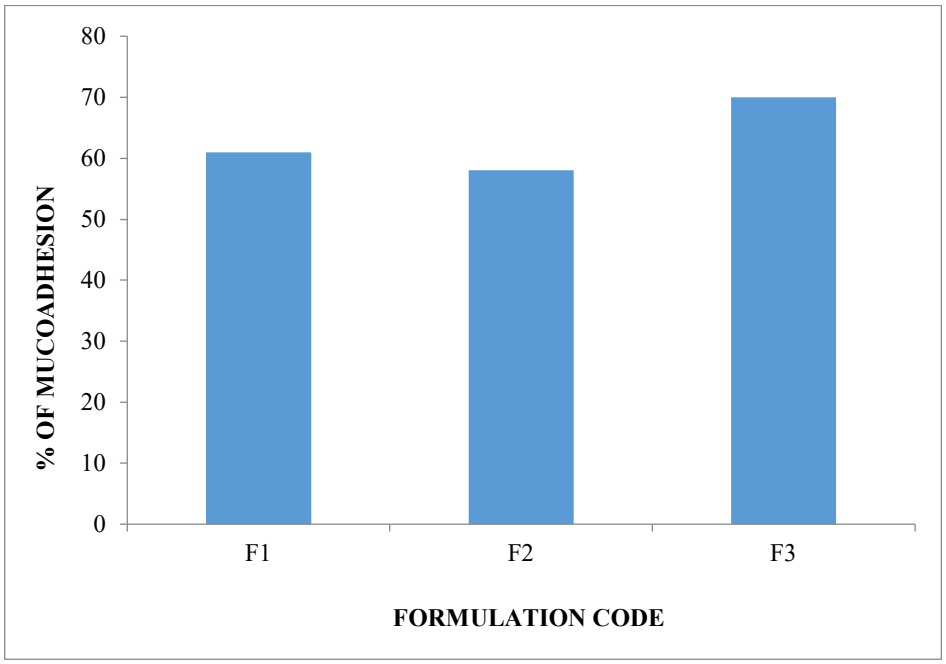

Fig 5: Percentage mucoadhesion of microspheres containing PLGA

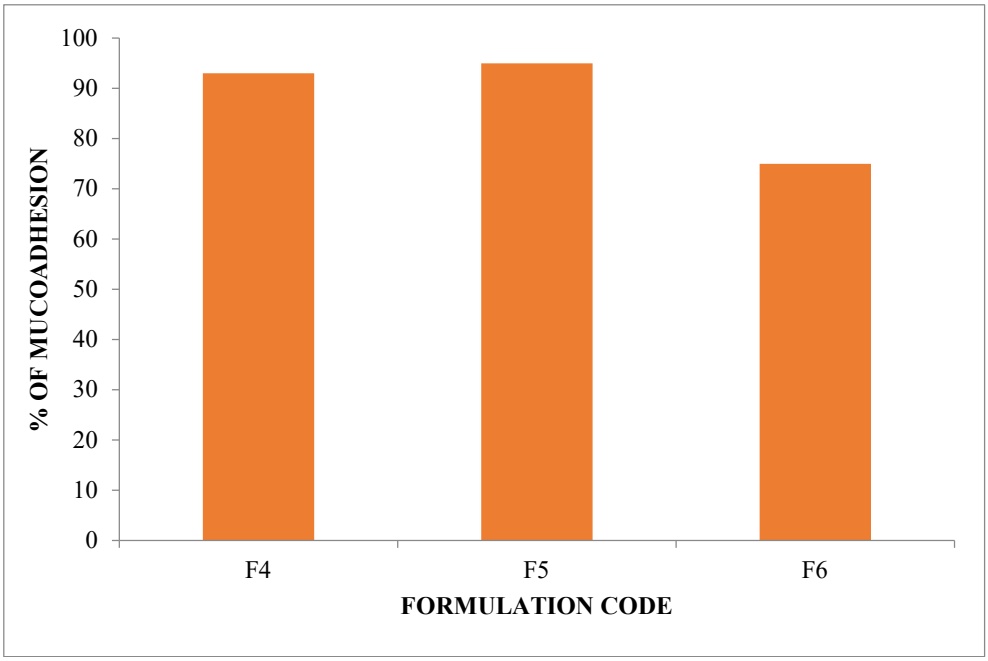

Fig 6: Percentage mucoadhesion of microspheres containing Chitosan

***Invitro* Drug Release Studies**

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the *in-vitro* dissolution studies of formulations F1 to F6 are shown in table 8.7. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations F1 to F3, figure for formulations F4 to F6.The formulationsF1, F2, and F3 containing PLGA showed a maximum release of97.58% at 10 hours, 98.12% 11 hours, 99.88% 12 hours respectively.The formulationsF4,F5and F6 containing Chitosanpolymer showed a maximum release of 97.14% 10 hours, 97.35% 12 hours, 91.17% 12 hours respectively. The invitro release profiles of Entecavir microspheres containing PLGA & Chitosan in the figure 7&8 respectively.

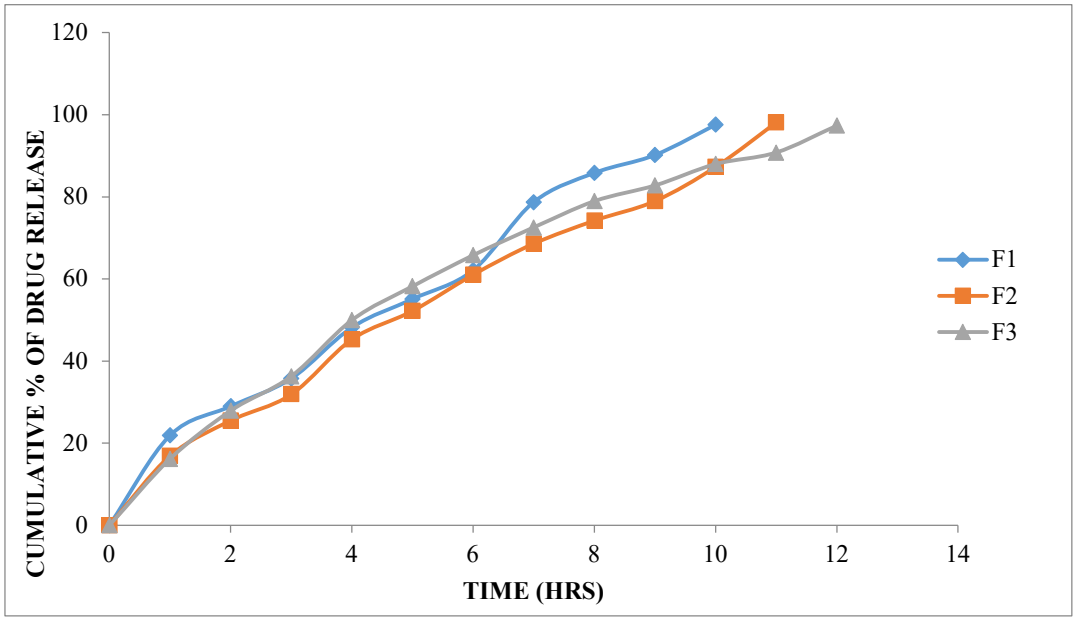

Fig 7: *Invitro* drug release profile of Entecavir microspheres containing PLGA

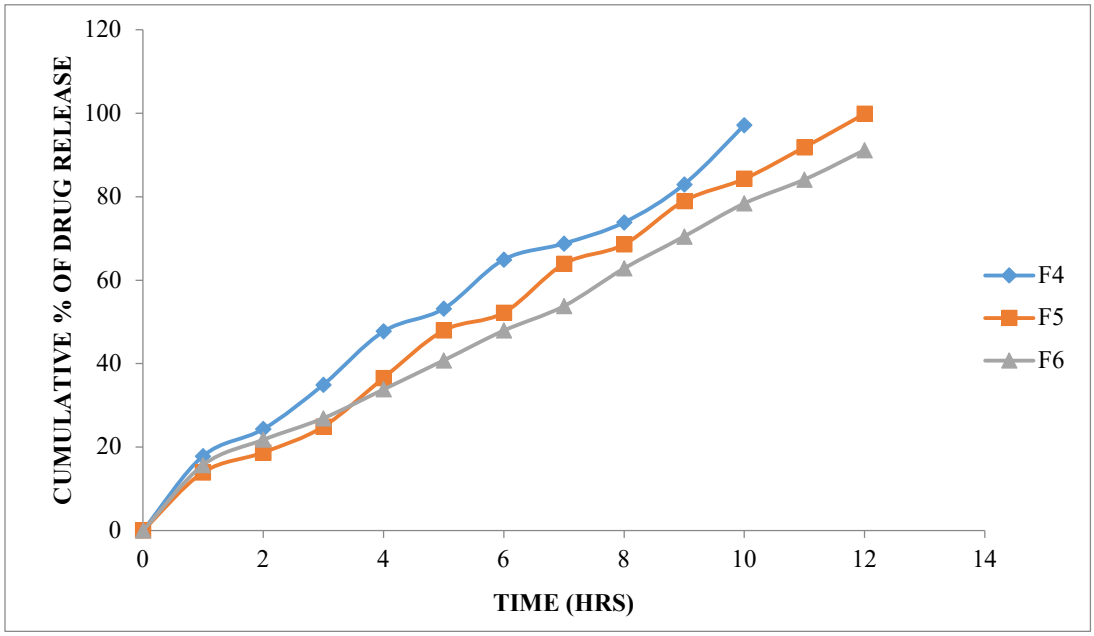

Fig 8: *Invitro* drug release profile of Entecavir microspherescontaining Chitosan

***Invitro* Drug Release Kinetics**

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the in-vitro drug dissolution data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, and Krosmeyer-Peppas model. From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows zero order release kinetics along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion as shown in the figure 9.

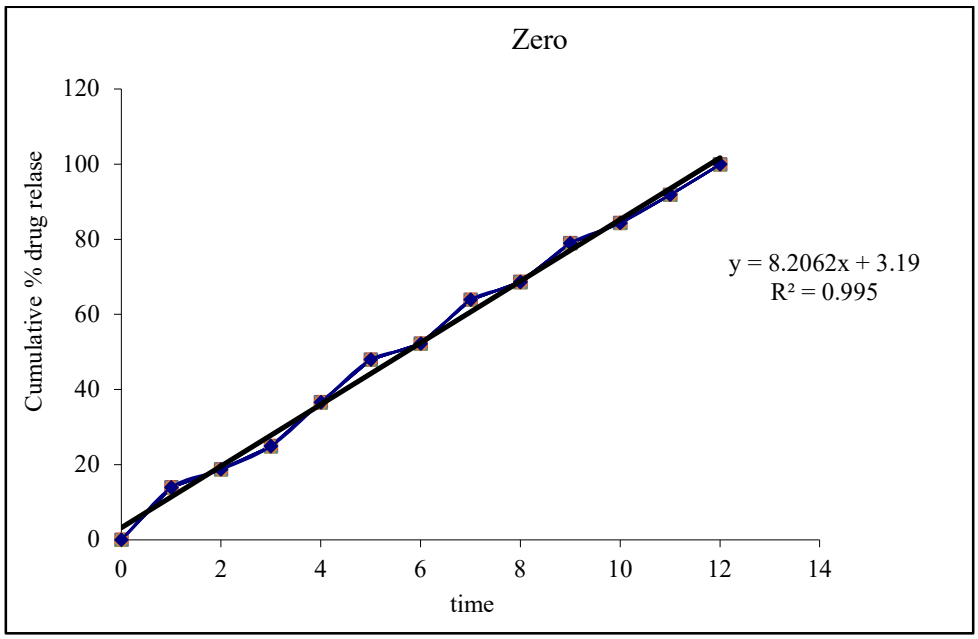

Fig 9: Graph of zero order release kinetics of optimized formula

Compatibility studies

Drug polymer compatibility studies were carried out using Fourier Transform Infra-Red spectroscopy to establish any possible interaction of Drug with the polymers used in the formulation. The FT-IR spectra of the

formulations were compared with the FTIR spectra of the pure drug. The FTIR results are as shown in the figure 10&11 as there no interaction between the drug and the other ingredients of the formulation. The SEM images are shown in the figure 12.

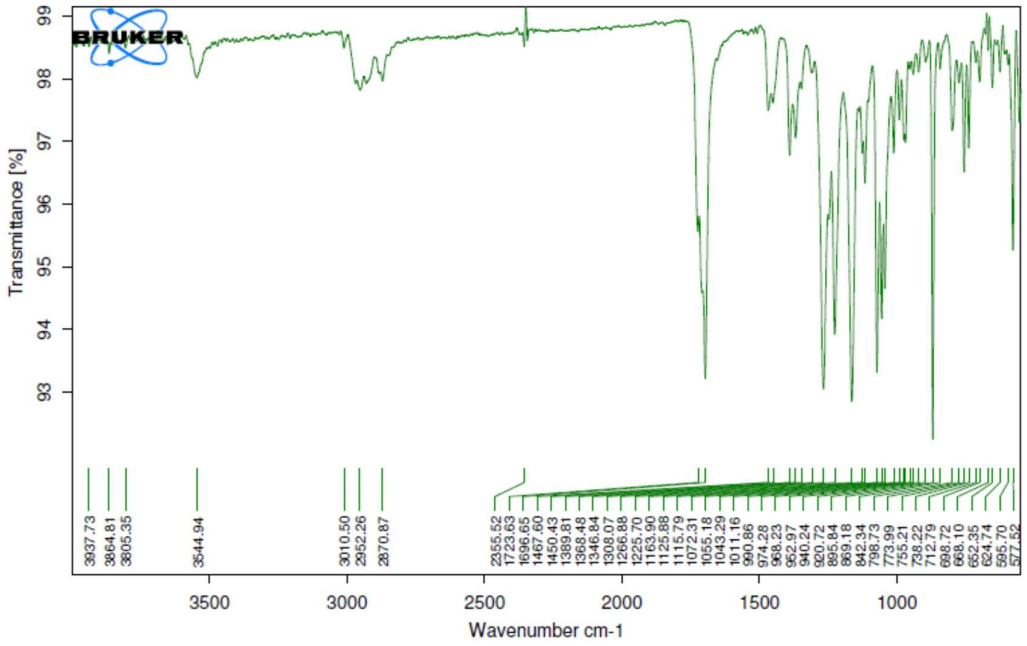

Fig 10: FT-IR spectra of Pure drug

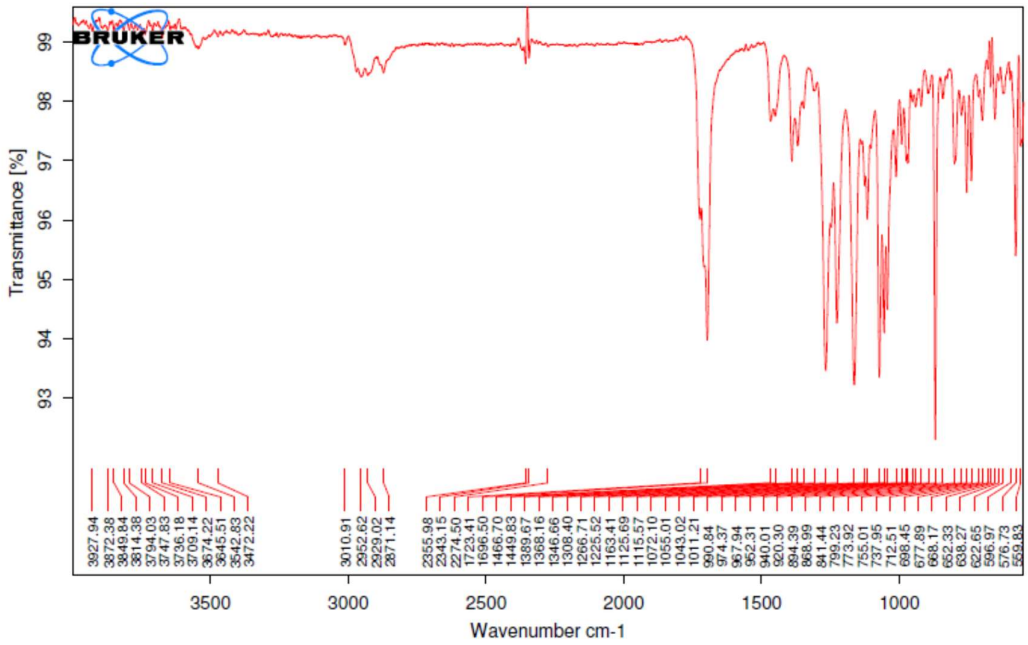

Fig 11: FT-IR spectra of Optimised formulation

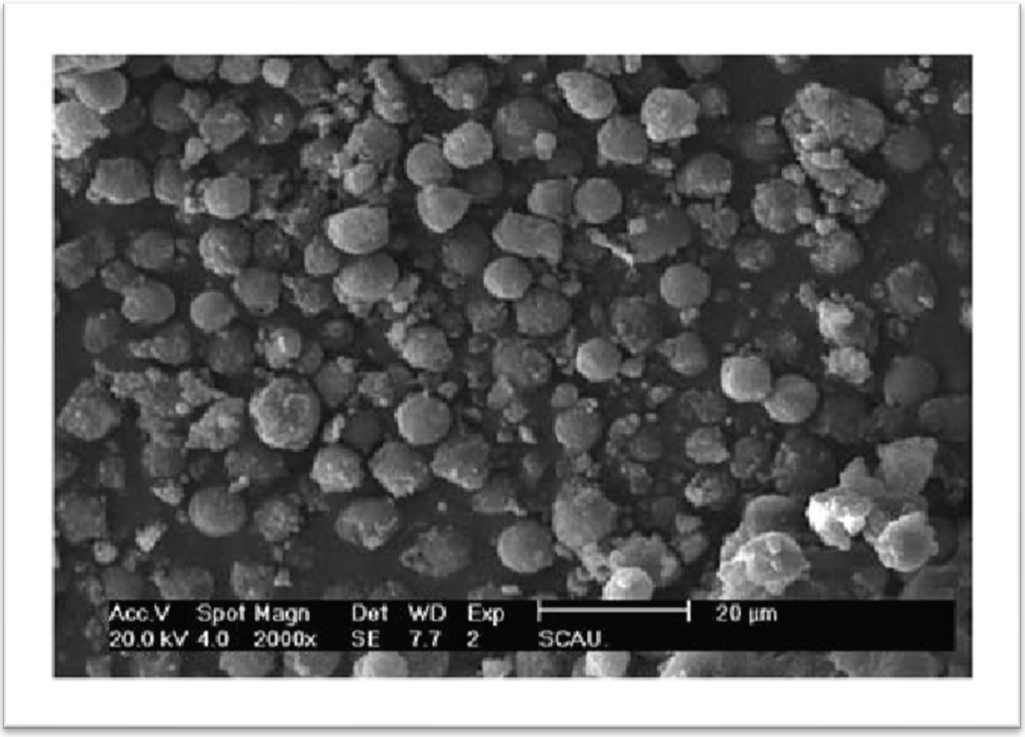

Fig 12: Scanning Electron Microscopyof optimised formulation (F3)

CONCLUSION

In this context, the Microspheres were prepared with PLGA and Chitosan successfully bythe Ionotropic gelation technique. Microspheres of Entecavir showed excellent mucoadhesivity,% yield, Drug Content, % Drug entrapment efficiency and prolonged drug releaseup to 12 hours. Microspheres of different size and drug content could be obtained by varying the formulation variables.Thus the prepared microspheres may prove to be potential candidates for oral delivery devices. Formulation Batch F3 showed best appropriate balance between mucoadhesivity and drug release rate, which can be considered as a bestfit for microspheres. The polymer ratio (PLGA) of 1:3 were selected as bestformulation, The formulated system showed sustained release up to 12 hours and the system has been potentially useful to overcomepoor bioavailability problems associated with Entecavir. Analysis of drug release mechanism showed that the drug release from the formulations the best fit model was found to be zero order release kinetics.Hence it can be concluded that Entecavir loaded PLGA Microsphere may be useful to achieve sustained drug release profile suitable for oral administration prepared by ionic gelation method.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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