International Journal of Pharmacology and Clinical Research (IJPCR)

ISSN: 2521-2206

IJPCR |Volume 7 | Issue 2 | Apr - Jun - 2023
www.ijpcr.net

Research article ***Clinical research***

Formulation And Evaluation Of Microsphere Of Entecavir

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Published on: 13.05.2023

ABSTRACT

In the present work, Microspheres of Entecavir using PLGA and Chitosan as polymers were formulated to deliver Entecavir via oral route. The results of this investigation indicate that Ionotropic gelation technique can be successfully employed to fabricate Entecavir microspheres. In this work an effort was made to formulate microsphere of Entecavir by using different polymers. Prepared formulations are evaluated for bulk density, tapped density, precent mucoadhesion, Percent compressibility, hausners ration, percentage yield, size and interaction study by FTIR and *in vitro* drug release. Formulation which passed all the evaluation parameters was considered as best formulation of Entecavir. The present study conclusively that Entecavir microsphere could be prepared successfully and formulation F3 was shows satisfactory result.

Keywords: Entecavir, PLGA, Chitosan and Microspheres.

INTRODUCTION

Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half-life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site-specific manner.

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 of drugs[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 [4,5, 6,7].

In 1997, first time microspheres were prepared for the sustained action of the drug. Since then, microparticles have proved to be good candidates for sustained and controlled release of drug and become an alternative of conventional or immediate release formulations. These particles are also a beneficial to deliver the active pharmaceutical ingredients which are pharmacologically active but are difficult to deliver due to limited solubility in water. In such type drugs, the attainment of required therapeutic concentrations of drug in the blood is problematic enabling to attain higher C_{max}, $T_{max}$and area under curve. Microsphere – based formulations can release a constant amount of drug in the blood or to target drugs to specific site in the body [8,9].

For many decades, medication of an acute disease or a chronic disease has been accomplished by delivering drugs to the

patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories as carriers. To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. This factor as well as other factors such as repetitive dosing and unpredictable absorption leads to the concept of controlled drug delivery systems. The word new or novel in the relation to drug delivery system is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be major advance toward solving the problem associated with the existing drug delivery system.

The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while Temporal delivery refers to controlling the rate of drug delivery to the target tissue.

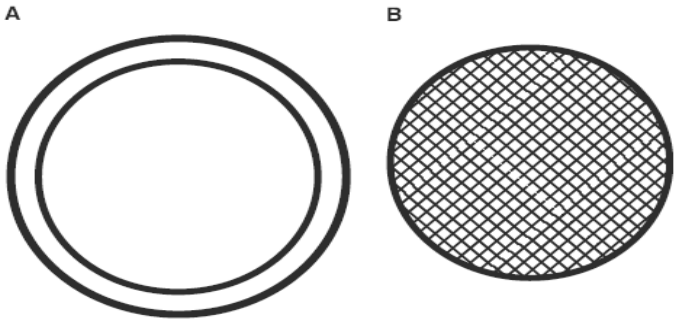
Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. However, this approach is be dilled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.[10,11]

The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e release from the dosage form should follow zero-order kinetics.[12]

Definition And General Description

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. The natural polymers include albumin and gelatin9-10 the synthetic polymers include polylactic acid and polyglycolic acid. Fig. 1.2 shows two types of microspheres: Microcapsules, where the entrapped substance is completely surrounded by a distinct capsule wall, and micromatrices, where the entrapped substance is dispersed throughout the microsphere matrix.

Microspheres are small and have large surface to volume ratios. At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often dictating their activity.



(A) Microcapsule consisting of an encapsulated core particle and
(B) micromatrix consisting of homogeneous dispersion of active ingredient in particle.

Fig 1: Schematic diagram illustrating microspheres.

The potential use of microspheres in the pharmaceutical industry has been considered since the 1960s for the following applications:

Taste and odor masking Conversion of oils and other liquids to solids for ease of handling Protection of drugs against the environment (moisture,light, heat, and/or oxidation) and vice versa (prevention of pain on injection) Delay of volatilization Separation of incompatible materials (other drugs or excipients such as buffers) Improvement of flow of powders Safe handling of toxic substances Aid in dispersion of water-insoluble substances in aqueous media,[13] and Production of sustained-release, controlled-release, and targeted medications Reduced dose dumping potential compared to large implantable devices Microencapsulation has also been used medically for the encapsulation of live cells and vaccines. Biocompatibility can be improved by the encapsulation of artificial cells and biomolecules such as peptides, proteins, and hormones, which can prevent unwanted immunological reactions that would lead to inactivation or rejection. Microspheres are used for isolating materials until their activity is needed. The biotechnology industry employs microspheres to contain organisms and their recombinant products to aid in the isolation of these products.[14]

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[15]. Microspheres have varied applications and are prepared using assorted polymers. However; the success of these microspheres is limited owing to their short residence time at the site of absorption. So, various attempt have been made to increase the bioavailability as well as prolong the gastric residence time of dosage form in the stomach resulted in development of bio adhesive drug delivery system which will provide an intimate contact of the drug delivery system with the absorbing membranes[16]. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[17]. Gastric mucoadhesive drug delivery offers a number of applications for drugs having poor bioavailability because of narrow absorption window in the upper part of gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability.

Advantages of microspheres [18]

- They provide protection before after administration for unstable drug.
- They reduced concentration of drug at site other than the tissue or the target organ.
- Decrease dose and toxicity.
- Particle size reduction for enhancing solubility of poorly soluble drugs.
- Provide constant and prolonged therapeutic effect.

Limitation [19]

Some of the disadvantages were found to be as follows

- The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulations.
- The fate of polymer matrix and its effect on the environment.
- The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
- Reproducibility is less.
- Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation may influence the stability of core particles to be encapsulated.
- The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents.

MATERIALS

Entecavir-Procured from Hetero Pharma limited Hyd, provided by SURA LABS, Dilsukhnagar, Hyderabad, PLGA- Merk specialiities Pvt Limited, Chitosan- Merk specialiities Pvt Limited, Sodium alginate (w/v)-Merk specialiities Pvt Limited, Calcium Chloride (w/v)-Merk specialiities Pvt Limited.

METHODOLOGY

Preformulation Studies

Spectroscopic Studies

PREPARATION OF 0.1N HCl (pH 1.2): Take 8.5ml of HCl in a 1000ml volumetric flask and make up the volume with distilled water.

DETERMINATION OF λ_{MAX}: Weigh 10mg of Entecavir and transferred into 10ml volumetric flask and dissolved in 10ml methanol (stock-I) to get concentration of 1000 µg/ml. From the stock-I take 1ml solution and make up 10ml with 0.1N HCL. From the second stock take 1ml solution and make up to 10ml with 0.1N HCL to get 10 µg/ml. Then scan from 200-400nm.

Preparation of Standard Calibration Curve of Entecavir

1. 10 mg of Entecavir was accurately weighed and dissolved in 10ml of methanol (Stock Solution – I) to get a concentration of 1000 µg/ml.
2. From the stock solution- I, 1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution-II) to get concentrations of 100µg/ml.
3. From the stock solution- II, aliquots were taken and suitably diluted with 0.1N HCl (pH 1.2) to get concentrations in the range of 2 to 10µg/ml. 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 gelation technique. The sodium alginate solution was prepared by dispersing the sodium alginate in de-ionized water under continuous stirring for 30 minutes. The weighed amount of the drug was thoroughly mixed with sodium alginate dispersion. By following the same procedure the alginate beads of different ratios of drug: polymer were prepared. The resulted homogeneous dispersion was extruded in to the 5% calcium chloride solution through hypodermic syringe with flat tip needle (20G) and stirred for 15 minutes at 100rpm using magnetic stirrer. The formed micro beads were allowed to cure for 30 minutes in the calcium chloride solution to complete the gelation reaction. The microspheres were then filtered and dried in hot air oven at 60ºC for 3 hr.

Characterization Of Microspheres

Table 1: Prepared formulation of Microspheres

INGREDIENTS	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
Sodium alginate (w/v)	3%	3%	3%	3%	3%	3%
Calcium Chloride (w/v)	5%	5%	5%	5%	5%	5%

RESULT AND DISCUSSION

Preformulation Studies

Spectroscopic Studies

*Determination of λ_{max}:*A solution of 10µg/ml of Entecavir was scanned in the range of 200 to 400nm. The drug exhibited a λ_{max} at 255 nm in simulated gastric fluid pH 1.2 and pH 7.4 phosphate buffer respectively.

*Calibration curve of Entecavir in pH 7.4 phosphate buffer:*Tableshows the calibration curve data of Entecavir in pH 7.4 phosphate buffer at 256nm. Fig. 8.2 shows the standard calibration curve with a regression value of 0.997, slope of 0.027 and intercept of 0.020 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25µg/ml.

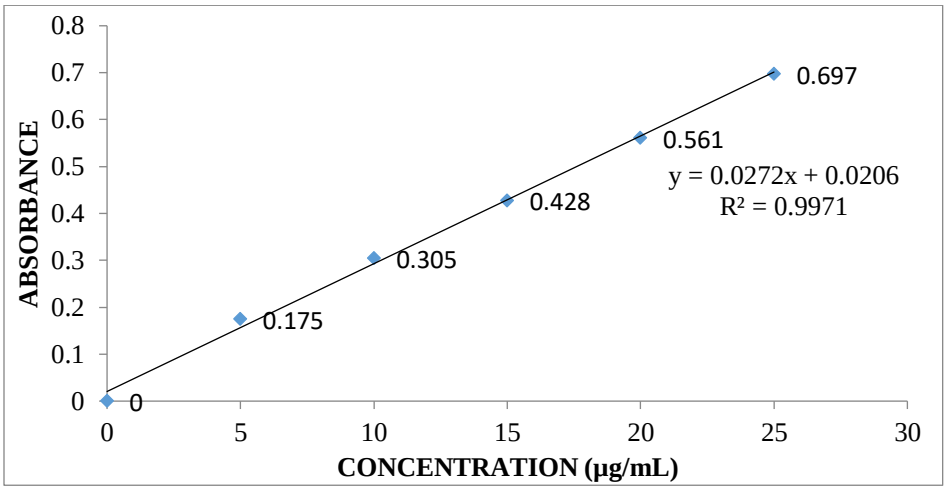


Fig2: Standard graph of Entecavir in pH 7.4 phosphate buffer

In vitro 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 is depicted in Fig. Table Percentage mucoadhesion of the prepared microspheres.

Table 2:*In Vitro* Mucoadhesion Test of all Formulations

S.NO.	FORMULATION CODE	No. OF MICROSPHERES		PERCENTAGE MUCOADHESION
		INITIAL	FINAL	
1	F1	20	15.48	61
2	F2	20	11.85	58
3	F3	20	15.14	70
4	F4	20	17.96	93
5	F5	20	20.71	95
6	F6	20	16.17	75

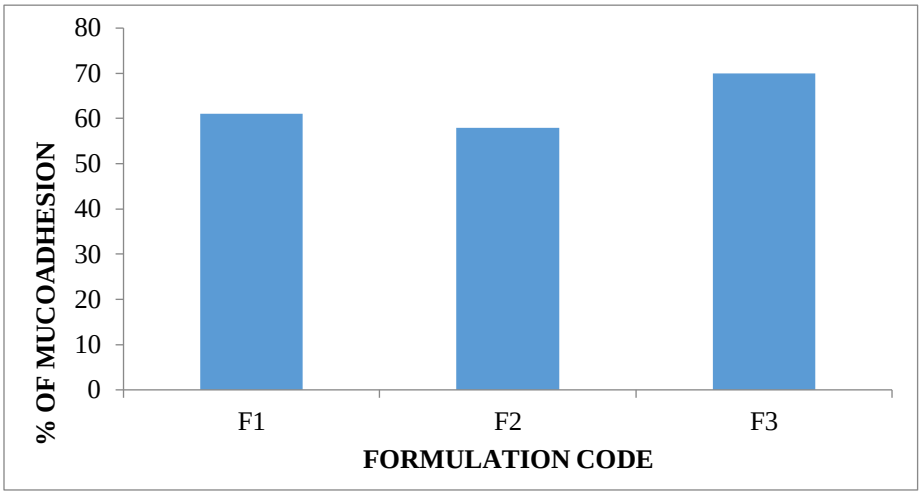


Fig 3 :Percentage mucoadhesion of microspheres containing PLGA

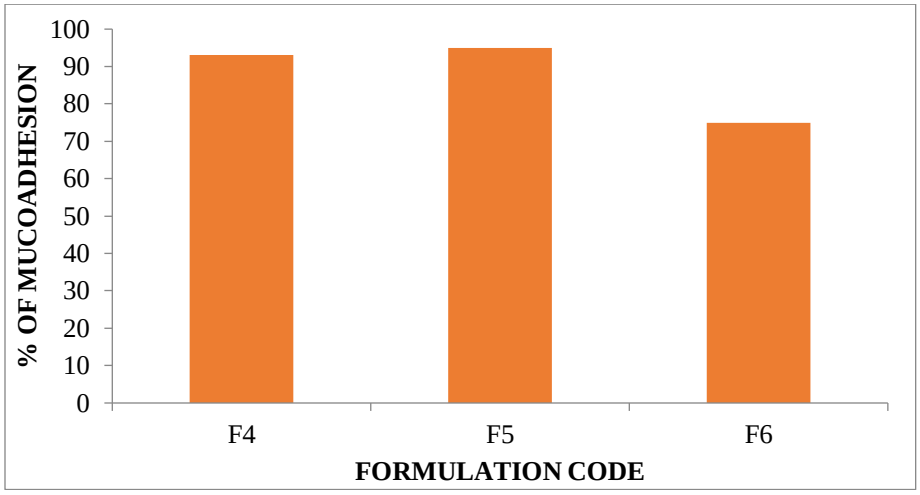


Fig 4: Percentage mucoadhesion of microspheres containing Chitosan

IN-VITRO 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 formulations F1, F2, and F3 containing PLGA showed a maximum release of97.58% at 10 hours, 98.12% 11 hours, 99.88% 12 hours respectively.The formulations F4, F5 and F6 containing Chitosanpolymershowed a maximum release of97.14% 10 hours, 97.35% 12 hours, 91.17% 12 hours respectively.Thisshowsthat more sustained release was observed withthe increase in percentageof polymers.As the polymer to drug ratio was increased the extent of drug releaseincreased. A significant increase in the rate and extent of drug release is attributed to the increase in density of polymer matrix that results in increased diffusion path length which the drug molecules have to traverse. The release of the drug has been controlled by swelling control release mechanism. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in slower release.

Table 3: *In-vitro* drug release data of Entecavir microspheres

TIME (H)	Cumulative percentage of drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	21.89	16.87	16.18	17.82	13.91	15.67

2	28.96	25.50	27.92	24.31	18.68	21.75
3	35.75	31.89	36.27	34.93	24.90	26.90
4	48.18	45.23	49.96	47.72	36.53	33.83
5	55.09	52.19	58.19	53.15	47.95	40.76
6	62.10	60.97	65.76	64.91	52.18	47.92
7	78.67	68.57	72.51	68.75	63.87	53.76
8	85.79	74.21	78.93	73.81	68.56	62.81
9	90.14	78.92	82.74	82.94	78.97	70.47
10	97.58	87.28	87.94	97.14	84.28	78.38
11		98.12	90.75		91.84	84.10
12			99.88		97.35	91.17

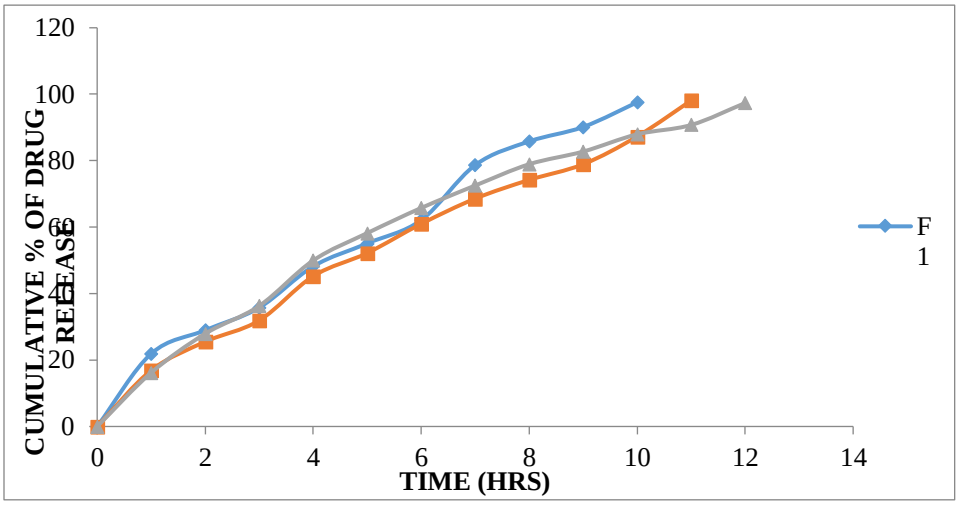


Fig 5: *In-Vitro* drug release profile of Entecavir microspheres containing PLGA

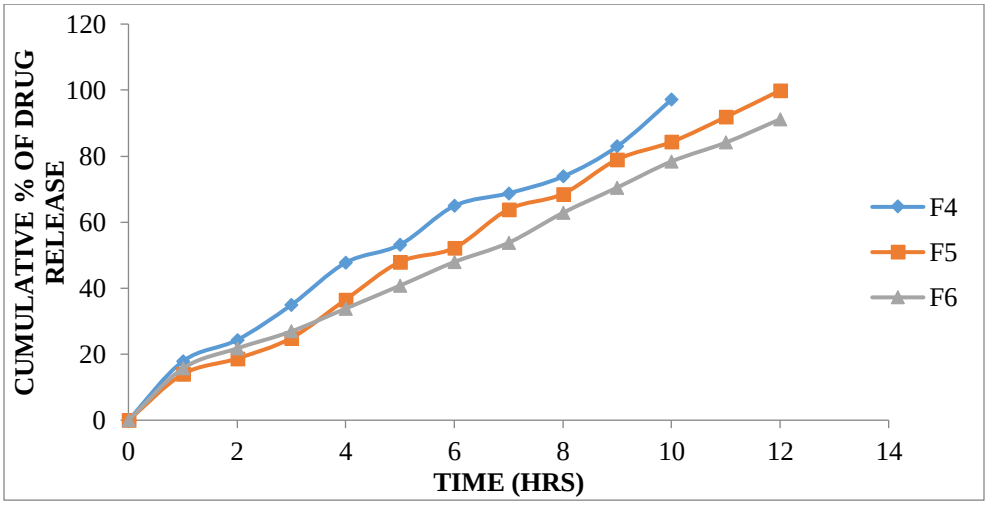


Fig 6: *In-Vitro* drug release profile of Entecavir microspheres containing Chitosan

In vitro drug release from all the formulation was found to be slow and sustained over the period of 12 hours, among other formulation F3 showed better sustained release pattern and the cumulative percentage release at the end of 12 hours was found to be 99.88%.

IN-VITRO *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. The values are compiled in Table 8.10. The coefficient of determination (R2) was used as an indicator of the best fitting for each of the models considered.

The kinetic data analysis of all the formulations reached higher coefficient of determination with the zero order release kinetics whereas release exponent value (n) ranged from 0.992. 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.

Table 4: Release kinetics studies of the optimized formulation (F3)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
13.91	1	1.000	1.143	0.000	1.935	13.910	0.0719	-0.857	86.09	4.642	4.416	0.226
18.68	2	1.414	1.271	0.301	1.910	9.340	0.0535	-0.729	81.32	4.642	4.332	0.309
24.9	3	1.732	1.396	0.477	1.876	8.300	0.0402	-0.604	75.1	4.642	4.219	0.423
36.53	4	2.000	1.563	0.602	1.803	9.133	0.0274	-0.437	63.47	4.642	3.989	0.653
47.95	5	2.236	1.681	0.699	1.716	9.590	0.0209	-0.319	52.05	4.642	3.734	0.908
52.18	6	2.449	1.718	0.778	1.680	8.697	0.0192	-0.282	47.82	4.642	3.630	1.012
63.87	7	2.646	1.805	0.845	1.558	9.124	0.0157	-0.195	36.13	4.642	3.306	1.336
68.56	8	2.828	1.836	0.903	1.497	8.570	0.0146	-0.164	31.44	4.642	3.156	1.485
78.97	9	3.000	1.897	0.954	1.323	8.774	0.0127	-0.103	21.03	4.642	2.760	1.881
84.28	10	3.162	1.926	1.000	1.196	8.428	0.0119	-0.074	15.72	4.642	2.505	2.137
91.84	11	3.317	1.963	1.041	0.912	8.349	0.0109	-0.037	8.16	4.642	2.013	2.628
99.88	12	3.464	1.999	1.079	-0.921	8.323	0.0100	-0.001	0.12	4.642	0.493	4.148

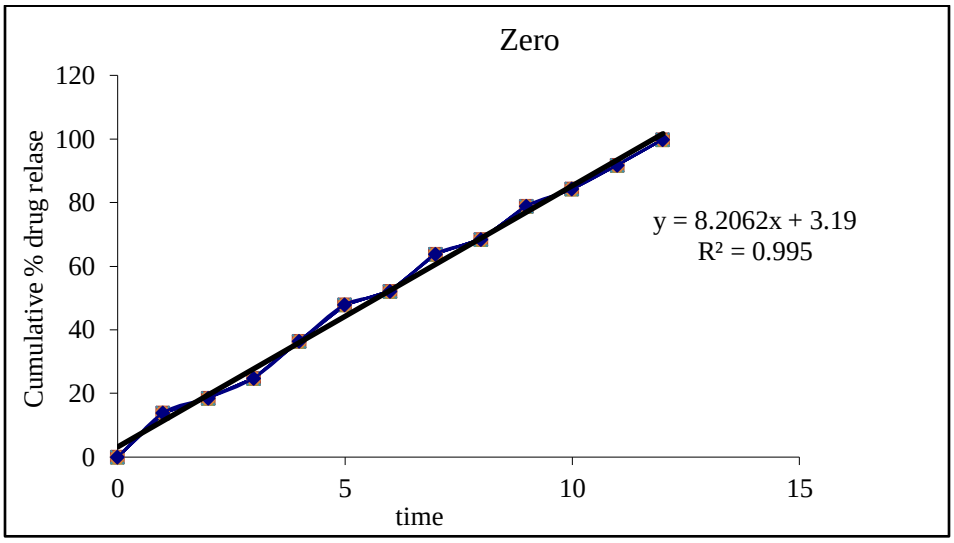


Fig 7: Graph of zero order release kinetics of optimized formula

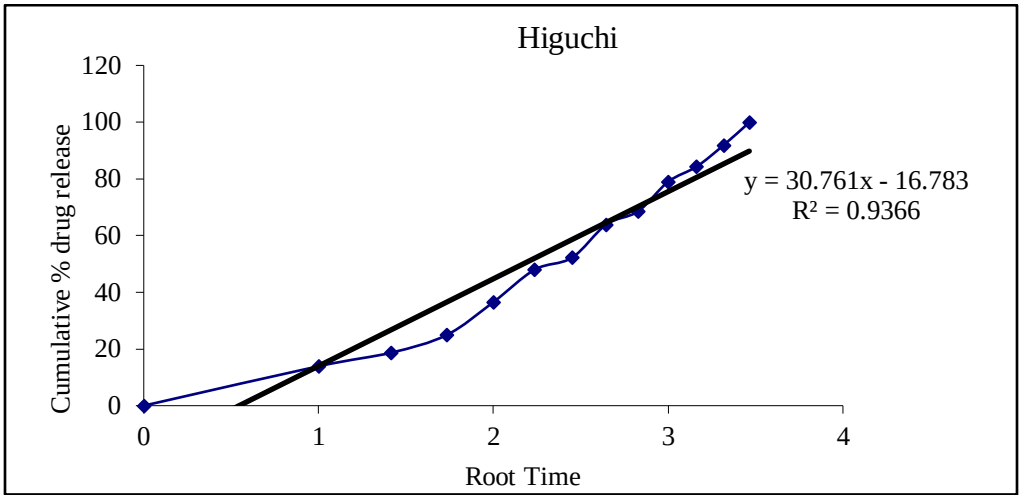


Fig 8: Graph of Higuchi release kinetics of optimized formula

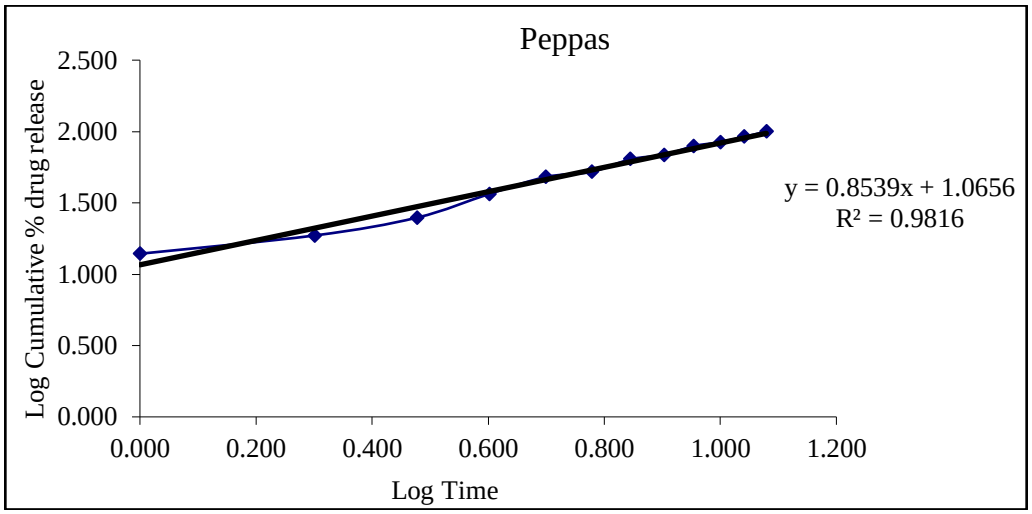


Fig 9: Graph of Peppas drug release kinetics of optimized formula

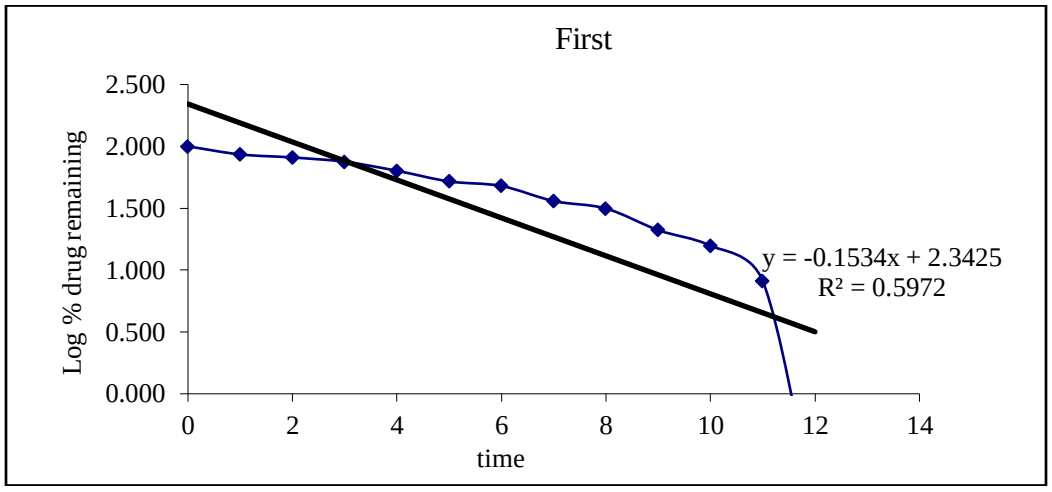


Fig 10: Graph of first order release kinetics of optimized formula

Optimised formulation F3 was kept for release kinetic studies. From the above graphs it was evident that the formulation F3 was followed zero order release kinetics.

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.

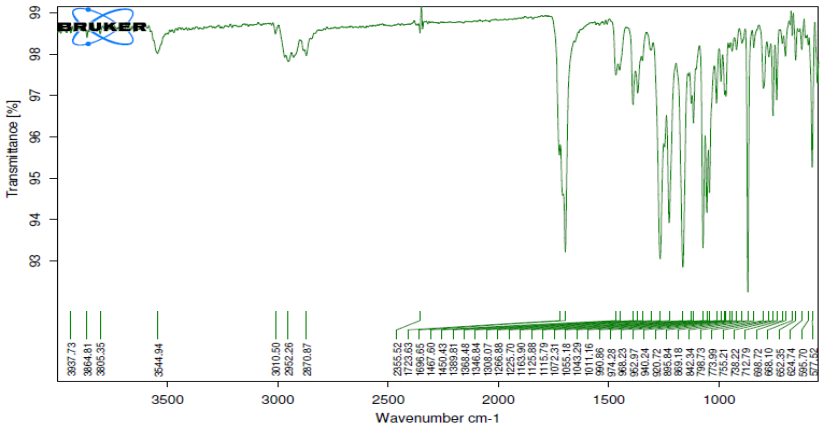


Fig 11 : FT-IR spectra of Pure drug

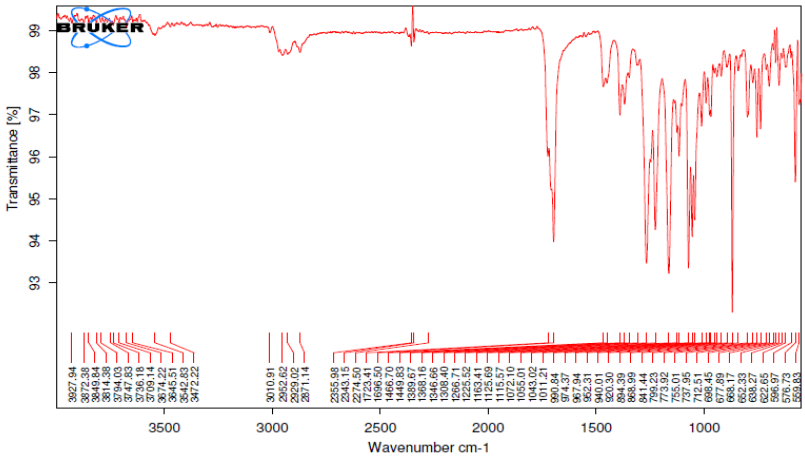


Fig 12: FT-IR spectra of Optimised formulation

SEM

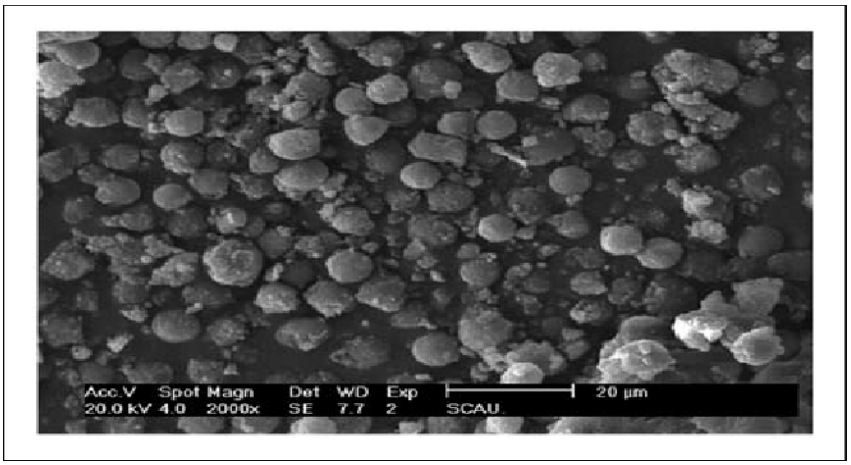


Fig 13 : SEM of Optimised formulation

CONCLUSION

Microspheres are prepared with PLGA and Chitosan successfully by the Ionotropic gelation technique. Microspheres of Entecavir showed excellent mucoadhesivity,% yield, Drug Content, % Drug entrapment efficiency and prolonged drug release up 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 best fit for microspheres. The polymer ratio (PLGA) of 1:3 were selected as best formulation, The formulated system showed sustained release up to 12 h and the system is potentially useful to overcome poor 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.

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