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Formulation Development and InVitro Characterisation of Entecavir Microspheres

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A B S T R A C T

The objective of present study was to encapsulate the anti-retroviral drug in various polymers in order to provide the sustained release and to minimize or reduce the dose dependent side effects as well as to improve the patient compliance. Entecavir sulphate microspheres were prepared by w/o emulsification solvent evaporation method using different polymers viz. sodium alginate and gelatin. The prepared microspheres were characterized for drug entrapment efficiency, muco-adhesion test, particle size analysis, surface morphology and in-vitro drug release study. The in-vitro release studies were performed using pH 1.2 Hcl and pH 7.4 phosphate buffer and drug release is evaluated. Morphology of microspheres was characterized by using Scanning Electron Microscopy (SEM). The prepared microspheres were small, discrete, free flowing and spherical in shape. The mean diameter of micro- spheres was between 21.00±1.96 to 32.43±2.19 µm for different formulations. The drug loaded microspheres showed 40-65% and 45-50% of entrapment for sodium alginate and gelatin microspheres respectively. Fourier Transform- Infra Red (FT-IR) was performed to evaluate interaction between drug and polymer. The prepared microspheres exhibited prolonged drug release (10h) as the concentration of sodium alginate increased, the muco- adhesion is also increased as the sodium alginate concentration increases and the drug release rate was decreased at higher concentration of gelatin.

Keywords: Entecavir; microspheres; solvent evaporation method; SEM.

1. Introduction

Controlled drug delivery systems can include the maintenance of drug levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. While these advantages can be significant, the poten- tial disadvantages cannot be ignored like the possible toxicity or non-biocompatibility of the materials used, undesirable by-products of degradation, any surgery required to implant or remove the system, the chance of patient discomfort from the delivery device, and the higher cost of controlled-release systems compared with traditional pharmaceutical formulations. The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, ca- pable of achieving high drug loading, safe from acci- dental release, simple to administer and remove, and easy to fabricate and sterilize. (Jain.N.K, 2008) The goal of many of the original controlled-release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. With traditional drug delivery systems, the drug level intheblood follows the in which the level rises after each administration of the drug and then decreases until the next administration. The key point with traditional drug administration is that the blood level of the agent should remain between a maximum value, which may represent a toxic level, and a minimum value, below which the drug is no longer effective (Debjit Bhowmik 2012).

Microsphere is a term used for small spherical parti- cles, with diameters in the micrometer range (typically 1µm to 1000µm (1mm). Microspheres are sometimes often referred to as micro particles (Microsphere, 2015). Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm^3 (Kataria Sahil, 2011).

2. Solvent Evaporation Method

Entecavirmicrospheres were prepared using PLGA, Ethyl cellulose andHPMC K4M and distilled water as continuous phase by solvent evaporation technique. Initially dichloromethane (DCM) and methanol was mixed uniformly at room temperature, then PLGA, Ethyl cellulose and HPMC K4M in various proportions was dissolved in the above solution. To this mixture, a drug solution corresponding was added and mixed thoroughly and injected drop wise in to the continuous phase consisting of 100mL of 0.2% (w/v) SLS (sodium lauryl sulphate) at 250 rpm. The microspheres obtained was washed for 2-3 times with distilled water and dried at room temperature. Different concentrations and ratios of polymers used in the formulation of microspheres are mentioned in Table.

Table 1.Characterization of Microspheres

INGREDIENTS (MG)	FORMULATIONS								
	E1	E2	E3	E4	E5	E6	E7	E8	E9
Entecavir	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PLGA	5	10	15	-	-	-	-	-	-
Ethyl cellulose	-	-	-	5	10	15	-	-	-
HPMC K4M	-	-	-	-	-	-	5	10	15
Dichloromethane (mL)	10	10	10	10	10	10	10	10	10
Methanol(mL)	30	30	30	30	30	30	30	30	30
Sodium lauryl sulphate (mg)	20	20	20	20	20	20	20	20	20

2.1 Micromeritic properties

The microspheres were characterized by their micromeritic properties such as Particle size, Bulk density, Tapped density, Compressibility index, Hausners ratio and Angle of repose.

2.2 Bulk density

In this method floating microspheres are transferred to a measuring cylinder and is tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Bulk volume}}$$

2.3 Tapped density

In this method floating microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density floating microspheres.

Percent Compressibility index was determined by using the formula,

Carr's Index = (tapped density – bulk density) x 100 / tapped density

2.4 Hausners ratio

Hausners ratio of microspheres was determined by comparing tapped density to bulk density using the equation

Hausner ratio = tapped density / bulk density

2.5 Angle of repose

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method4. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and angle of repose was calculated using the following equation.

$\theta = \tan^{-1} h / r$

Here,

θ - Angle of repose

h - Height of granules above the flat surface

r - Radius of the circle formed by the granule heap.

2.6 Percentage yield

The percentage of production yield was calculated from the weight of dried microsphe-res recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \times 100$$

2.7 Drug entrapment efficiency

Weighed amount of microspheres (100 mg) with phosphate buffer pH 7.4 (10 ml) was added in a vial. The solution was stirred vigorously for 24 hours with mechanical stirrer. Supernatent was collected by centrifugation and drug content in supernatent was determined by using UV spectrophotometer at wavelength 260nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

2.8 Swelling study

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2 .The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{(Wt - W0)}{(W0)} \times 100$$

Where W0 & Wt are initial weight and Final weight of microspheres respectively

2.9 In vitro drug release study

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^0C, 50 rpm) using the USP type – I rotating basket method in simulated gastric fluid pH 1.2 (900ml) for 2 hours then replace the media with pH 6.8 phosphate buffer for 3 hours, then replace the media with pH 7.4 Phosphate buffer. A quantity of accurately weighed microspheres equivalent to 100mg Sumatriptan Succinate each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 270nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment.

***2.10 In Vitro* drug release kinetics**

The release data obtained was fitted into various mathematical models.The parameters 'n' and time component 'k',the release rate constant and 'R',the regression coefficient were determined by Korsmeyer-Peppas equation to understand the release mechanism.

To examine the release mechanism of Sumatriptan Succinate from the microspheres, the release data was fitted into Peppa's equation,

$$Mt / M\infty = Ktn$$

Where, Mt / M∞ is the fractional release of drug, 't' denotes the release time, 'K' repr-esents a constant incorporating structural and geometrical characteristics of the device, 'n' is the diffusional exponent and characterize the type of release mechanism during the release process

3. Results and Discussion

3.1 Preformulation Studies -Evaluation and characterisation of microspheres

Micrometric Properties - 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 312.14µm to 3.32.41µm. microspheres containing Ethyl cellulose as polymer exhibited a size range between 310.15µm to 341.65µm. Microspheres containing HPMC K4M as copolymer had a size range of 309.54µm to 325.14µm.

The particle size data is presented in Tables 8.3 and displayed in Figures. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.

The bulk density of formulation E1 to E9 containing PLGA, Ethyl cellulose and HPMC K4M formulation was in the range of 0.277 ± 0.2to 0.625 ± 0.1gm./cm^3 (as shown in table 8.3), tapped density 0.312 ± 0.2to 0.833 ± 0.1and Hausners ratio 1.095to 1.333.

The carr's index of formulation E1 to E9 containing different grades of PLGA, Ethyl cellulose and HPMC K4M 8.695to 25.00respectively. The angle of repose of formulation E1 to E9 containing PLGA, Ethyl cellulose and HPMC K4M formulation was in the range <28.3 respectively(as shown in table 8.3) The values of carr's index and angle of repose indicate good flow properties.

Table 2: Micromeritic property of floating microspheres of Entecavir

Formulation code	Mean partical size	Bulk density ((gm./cm^3))	Tapped density (gm./cm^3)	Hauseners ratio	Carr's index	Angle of repose
E1	312.14	0.434 ± 0.2	0.476 ± 0.3	1.095	8.695	23.2 ± 0.2
E2	3.25.95	0.277 ± 0.2	0.312 ± 0.2	1.133	11.11	25.2 ± 0.1
E3	3.32.41	0.588 ± 0.3	0.666 ± 0.4	1.333	11.76	27.1 ± 0.1
E4	310.15	0.521 ± 0.3	0.631 ± 0.3	1.121	17.39	24.4 ± 0.4
E5	320.96	0.625 ± 0.1	0.833 ± 0.1	1.333	25.00	28.3 ± 0.4
E6	341.65	0.476 ± 0.3	0.526 ± 0.2	1.105	9.52	25.1 ± 0.1
E7	325.14	0.416 ± 0.2	0.476 ± 0.3	1.142	12.50	26.7 ± 0.4
E8	310.69	0.384 ± 0.4	0.434 ± 0.3	1.130	11.53	26.0 ± 0.3
E9	309.54	0.555 ± 0.1	0.714 ± 0.1	1.285	22.22	26.6 ± 0.2

3.2 Percentage Yield

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range.

3.3 Drug Entrapment Efficiency

Percentage Drug entrapment efficiency of Entecavir ranged from 95.24to 99.76% for microspheres containing PLGA, Ethyl cellulose and HPMC K4M polymer, The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared microspheres is displayed in Table 8.4, and displayed in Figures.

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	E1	90.56	97.14	73.14
2	E2	93.91	98.65	86.91
3	E3	95.21	99.76	90.72
4	E4	92.47	98.14	96.58
5	E5	96.14	96.52	98.45
6	E6	97.35	99.34	91.87
7	E7	95.41	95.24	89.72
8	E8	93.11	97.53	91.51
9	E9	90.48	99.21	95.82

3.4 Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swell ability is an important 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. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 8.5 that with an increase in polymer concentration, the percentage of swelling also increases. Thus we can say that amount of polymer directly affects the swelling ratio. As the polymer to drug ratio increased, the percentage of swelling increased from 29 to 60% for microspheres containing sodium alginate as polymer, 59 to 73% for microspheres containing Chitosanas polymer.The percentage of swelling of the prepared microspheres is displayed in Figures.Containing Eudragit as polymer.The percentage of swelling of the prepared microspheres is displayed in Figures.The effect of drug to polymer ratio on percentage swelling is displayed in Figure Table 8.5: Percentage swelling of the prepared microspheres.

Table 4 : Swelling studies

S.NO.	FORMULATION CODE	INITIAL (Wt)	FINAL (Wt)	PERCENTAGE SWELLING
1	E1	10	12.45	62
2	E2	10	11.62	70
3	E3	10	13.58	78
4	E4	10	12.45	75
5	E5	10	13.95	79
6	E6	10	14.86	83
7	E7	10	10.59	60
8	E8	10	11.75	65
9	E9	10	12.96	76

3.5 In Vitro Mucoadhesion Test

As the polymer to drug ratio increased, microspheres containing sodium alginate exhibited % mucoadhesion ranging from 69 to 91%, microspheres containing Chitosan exhibited % mucoadhesion ranging from 76 to 91% and microspheres containing Eudragit exhibited % mucoadhesion ranging from 58 to 79%. The results of in-vitro mucoadhesion test are compiled in Table 8.6. Effect of polymer proportion on % mucoadhesion is depicted in Figures 8.6 to 8.8 and comparative depiction of % mucoadhesion is depicted in Fig. 8.6.Table 8.6: Percentage mucoadhesion of the prepared microspheres.

Table 5:*In Vitro* Mucoadhesion Test of all Formulations

S.NO.	FORMULATION CODE	No. OF MICROSPHERES		PERCENTAGE MUCOADHESION
		INITIAL	FINAL	
1	E1	20	15.48	61
2	E2	20	11.85	58
3	E3	20	15.14	70
4	E4	20	17.96	93
5	E5	20	20.71	95
6	E6	20	16.17	39
7	E7	20	16.80	93
8	E8	20	11.58	86
9	E9	20	17.21	78

3.6 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 E1 to T9 are shown in table 8.7. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations E1 to E3, figure for formulations E4 to 46 and E7 to E9. The formulationsE1, E2, and E3 containing PLGA showed a maximum release of97.58% at 10 hours, 98.12% 11 hours, 97.35% 12 hours respectively.

The formulationsE4, E5 and E6 containing Ethyl cellulosepolymershowed a maximum release of97.14% 10 hours, 99.88% 12 hours, 91.17% 12 hours respectively. The formulationsE7, E8 and E9 containing HPMC K4M showed a maximum release of84.78% 12 hours, 90.53% 12 hours, 98.14% 10hours respectively.

This shows that more sustained release was observed withthe increase in percentage of 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 releaseof 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 6: *In-vitro* drug release data of Entecavir microspheres

TIME (H)	Cumulative percentage of drug release								
	E1	E2	E3	E4	E5	E6	E7	E8	E9
0	0	0	0	0	0	0	0	0	0
1	21.89	16.87	16.18	17.82	13.91	15.67	18.90	20.15	26.39
2	28.96	25.50	27.92	24.31	18.68	21.75	23.36	27.96	35.52
3	35.75	31.89	36.27	34.93	24.90	26.90	30.21	26.82	42.80
4	48.18	45.23	49.96	47.72	36.53	33.83	38.89	37.56	59.93
5	55.09	52.19	58.19	53.15	47.95	40.76	47.23	41.29	65.28
6	62.10	60.97	65.76	64.91	52.18	47.92	50.15	48.75	70.23
7	78.67	68.57	72.51	68.75	63.87	53.76	56.82	56.51	78.06
8	85.79	74.21	78.93	73.81	68.56	62.81	64.97	60.18	82.16
9	90.14	78.92	82.74	82.94	78.97	70.47	68.56	74.32	87.47
10	97.58	87.28	87.94	97.14	84.28	78.38	72.10	78.69	98.14
11		98.12	90.75		91.84	84.10	79.64	86.82	
12			97.35		99.88	91.17	84.78	90.53	

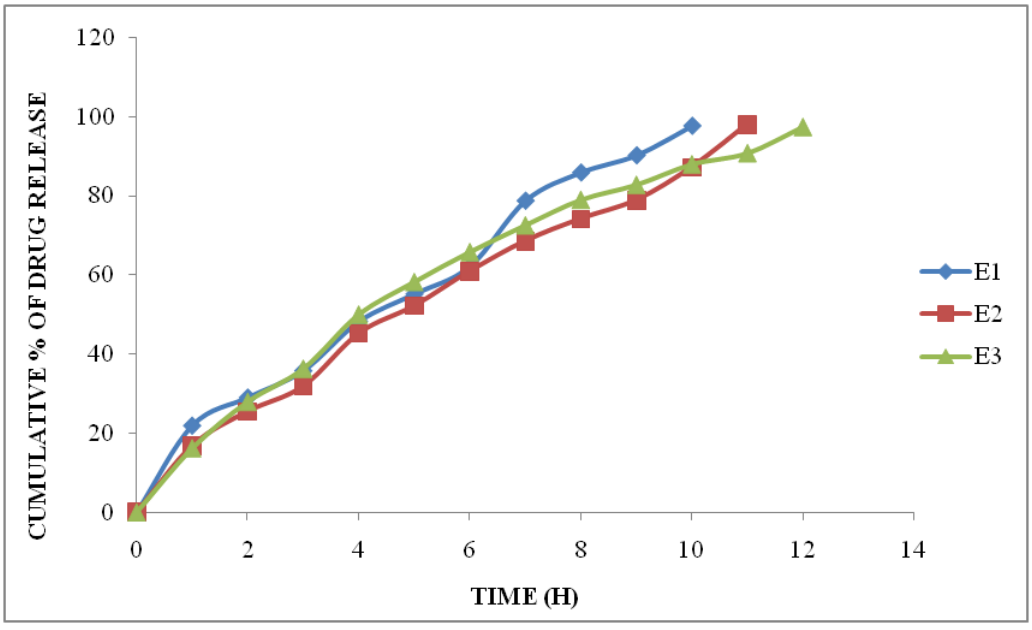

Figure 1: *In-Vitro* drug release profile of Entecavir microspheres containing PLGA

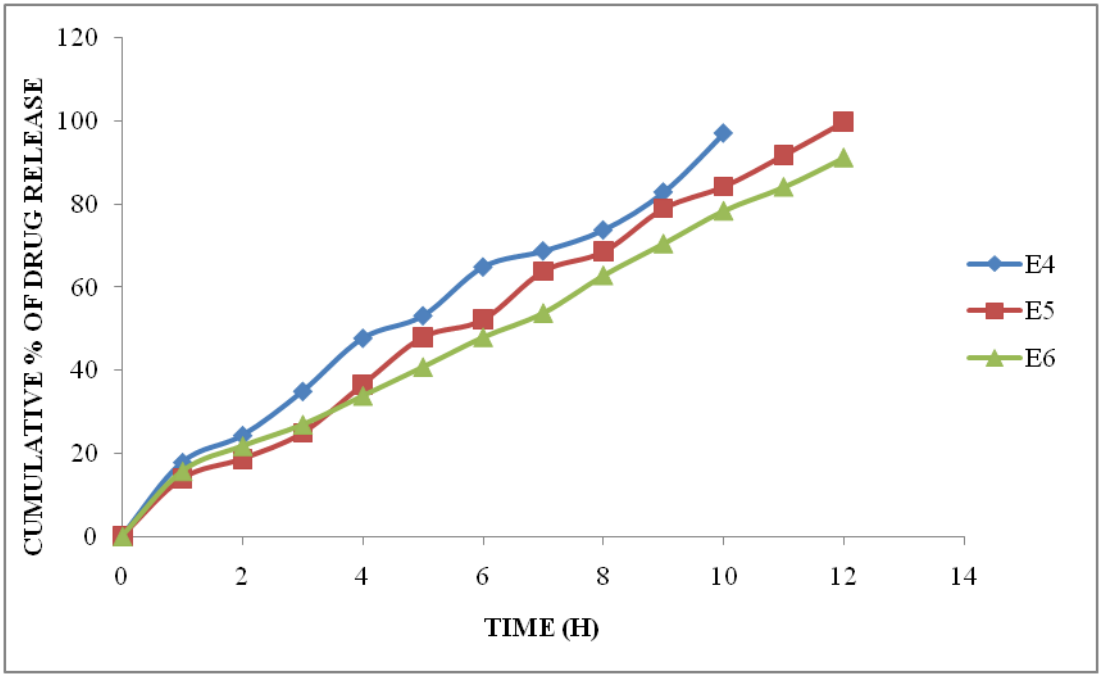

Figure 2. ***In-Vitro*** **drug release profile of Entecavir microspherescontaining Ethyl cellulose**

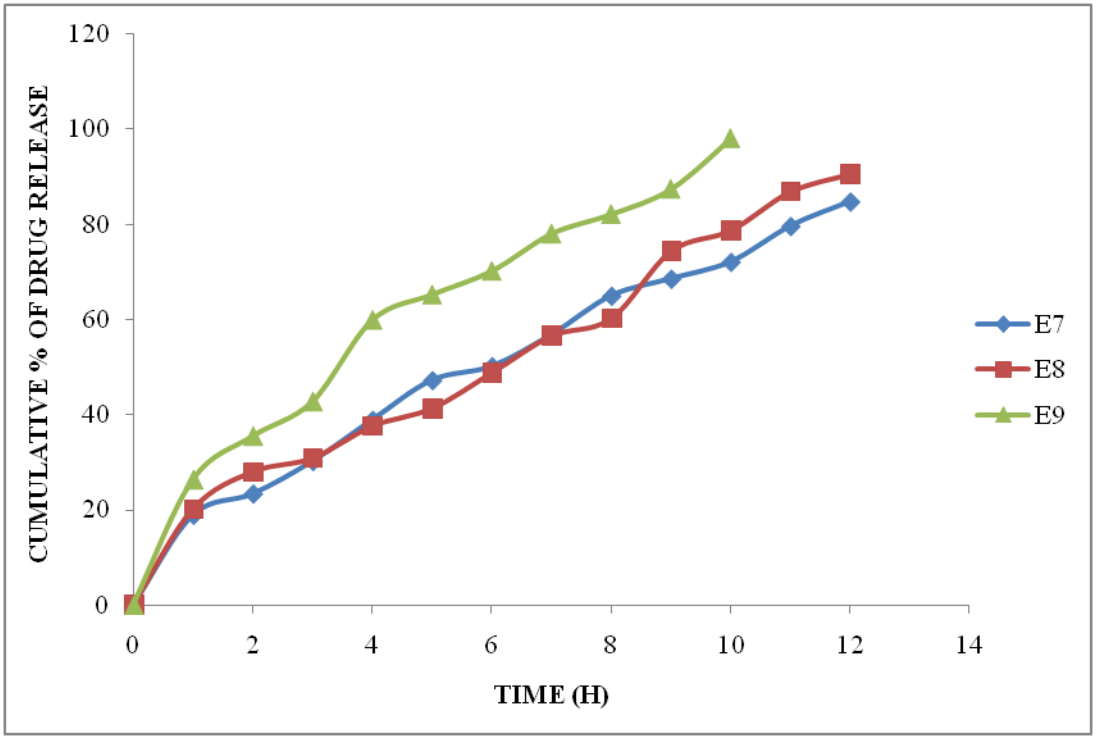

Figure 3.*In-Vitro* drug release profile of Entecavir microspheres containing HPMC K4M

Invitro drug release from all the formulation was found to be slow and sustained over the period of 12 hours, among other formulation E5 showed better sustained release pattern and the cumulative percentage release at the end of 12 hours was found to be 99.88%.

3.7 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 7: Release kinetics studies of the optimized formulation (E5)

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

3.8 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.

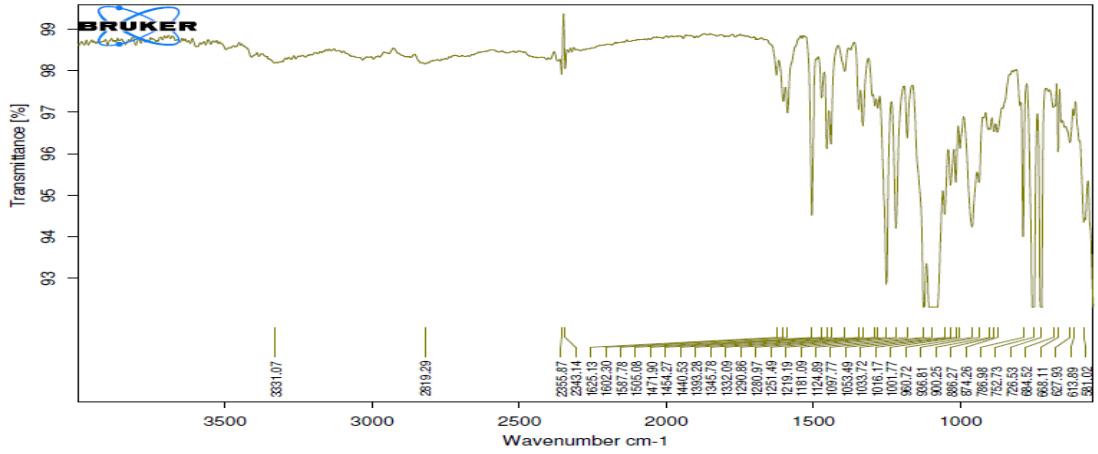

Figure 4: FT-IR spectra of Pure drug

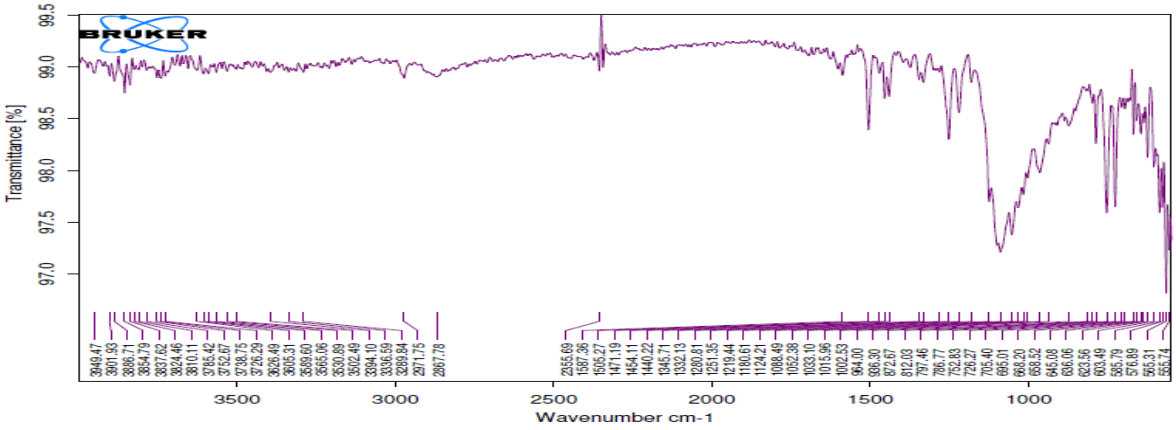

Figure 5: FT-IR spectra of Optimised formulation

SEM :

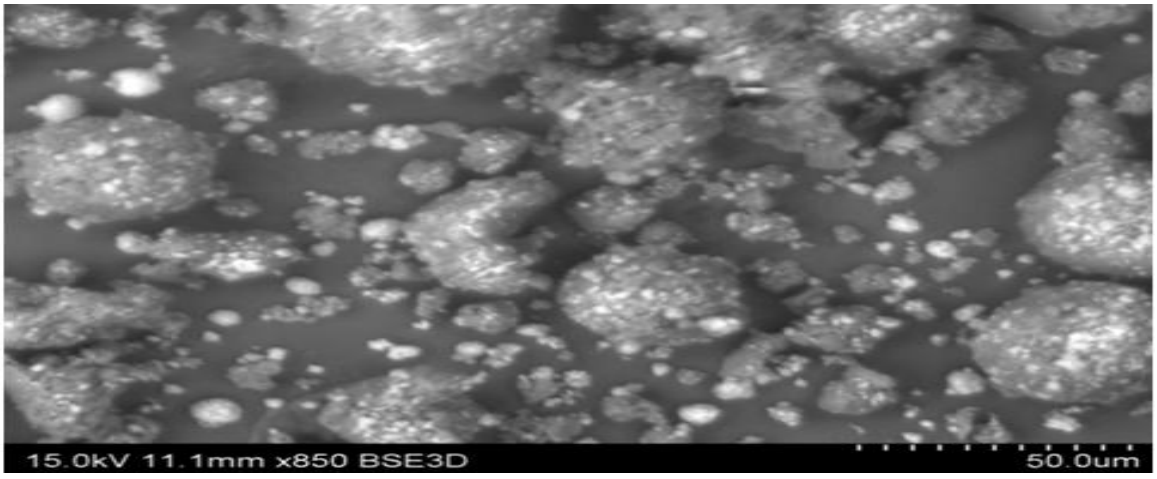

Figure 6: SEM of Optimised formulation

4. Conclusion

Microspheres are prepared with PLGA, Ethyl cellulose and HPMC K4M successfully bythe solvent evaporation 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 E5 showed best appropriate balance between mucoadhesivity and drug release rate, which can be considered as a bestfit for microspheres. The polymer ratio (Ethyl cellulose) of 1:2 were selected as bestformulation, The formulated system showed sustained release up to 12 h and the system is 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 Ethyl cellulose Microsphere may be useful to achieve sustained drug release profile suitable for oral administration.

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