FORMULATION AND EVALUATION RALTEGRAVIR LOADED MICROSPHERES

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ABSTRACT:

The objective of the present study was prepared and evaluate Raltegravir microspheres by using emulsion solvent diffusion technique. Raltegravir is loaded with ethyl cellulose & HPMC microspheres and it was prepared by emulsion solvent diffusion method. The results of FTIR indicated the stable character of Raltegravir microspheres loaded with ethyl cellulose microspheres and also absence of drug polymer interaction. The compatability studies like FTIR & DSC were used to investigate there is no incompatability in the formulation. The Morphological particle size of Raltegravir microspheres is carried out by SEM. The Microspheres were evaluated for total formulation codes is RGV 1 to RGV 9. The Percentage Yield was found to be 83.44% to 85.61%. Drug content was 64.7 to 95.3%. The Particle size of microspheres 16 µm to 213 µm, Drug entrapment efficiency was 87.3% to 53.5 %, The drug loading capacity was 94.6% to 55.5%. Thw swellability studies was 0.7 sec to 1.6 sec. Invitro dissolution studies of best formulation RGV8 was found to be 63.87%. The invitro drug dissolution data obtained was fitted to various mathematical models such as zero order, first order, Higuchi matrix & Korsmeyer peppas model. The Raltegravir microspheres follows model having R2 value was 0.937, 0.399, 0.899, 0.785 & m Value was 1593, 0.061, 11409, 2.560. The release of drug from the microspheres extended up to 45 Mints. The Raltegravir loaded with ethyl cellulose & HPMC microspheres were prepared under optimised conditions that shows good release characteristics.

Key words: *Raltegravir, Microspheres, Invitro dissolution studies, FTIR & DSC.*

INTRODUCTION: Microencapsulation is a process by which thin coatings are applied to the small particles of solids or droplets of liquids and dispersions. The particle size is ranging from several 10^{th} of a micron to 5000 micron. The Contents of capsules are contained within the wall until it is released that serve to break, crush, melt, dissolve, rupture or remove the shell, or the internal phase diffuses through the capsule wall. The first research is the development of micro encapsulation procedures for Pharmaceuticals. The development of the new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. Micro particles can be used in the controlled release of drugs, vaccines, antibiotics, and hormones. For example, by taking an advantage of the characteristics of microspheres, beyond the basic benefits, the microspheres could provide a larger surface area and possess an easier estimation of the diffusion and mass transfer behaviour, and it is also encapsulated the small molecules that could diffuse out of the barrier with precise

kinetics modelling and control-release of drugs to the body fluid.

METHODOLOGY

Pre-formulation Studies

Compatibility studies:

IR studies: In the preparation of drug and polymer may interact with each other, It leads to the instability of drug preformulation studies regarding the drug and polymer interaction. They are very critical in appropriate polymer. FTIR Spectroscopy was employed to ascertain the compatibility between Raltegravir and the cellulose polymer. (Perkin Elmer Jasco FTIR- 401, Japan).

Differential scanning calorimetry: The output of a DSC is a plot of heat flux (rate) versus temperature at a specified temperature rate. It provides the information about physical properties of the sample. It is in crystalline or amorphous in nature. In formulations it demonstrates a possible interaction between the drug and polymers, according to the thermograms.

MORPHOLOGY OF THE PARTICLES:

The following methods are used to determine the Particle size, size distribution, and morphology of the Microspheres.

Scanning Electron Microscopy:

Scanning Electron Microscopy is a technique (SEM). It is very useful in ascetaing the overall shape and morphology of the Microspheres. The morphology and surface appearance of both coated microspheres and Ethyl cellulose microcapsules were found by Scanning Electron Microscopy (SEM). The particles were freeze dried, and coated with gold palladium to achieve a film of 20nm thickness (Sputter coater, Balzers SCD 004, Liechtenstein) and observed microscopically (SEM, JSM-6400, Tokyo, Japan).

Preparation of Raltegravir Microspheres:

The Raltegravir microspheres were obtained by the Emulsion solvent Diffusion method by using distilled water as an external phase. The internal phase consists of a good solvent ethanol including Raltegravir with concentration of polymers like HPMC& Ethyl cellulose.

The drug and polymers were co-dissolved in an organic solvent mixture with polymers with different ratio. The drug solution was slowly injected via syringe in to the external water phase under agitating. The system was stirred at 800 rpm continuously for about I hr. Along with the good solvent diffusing in to the poor solvent. The droplets gradually solidified & formed microspheres. The system was filtered to separate the microspheres from the preparation system. The resultant product was washed with distilled water& dried. The whole process was carried out at room temperature. The ratio of drug& polymers were showed.

Table 1: FORMULATIONS OF RALTEGRAVIR MICROSPHERES

Ingredients	RGV1	RGV 2	RGV 3	RGV 4	RGV 5	RGV 6	RGV 7	RGV 8	RGV 9
Ralte	2.0	2	1.0	3	3.0	3	4	3.0	4

gravir									
Ethyl Cellulose	2	2.0	2	2.0	3	3.0	3	3	3.0
HPMC	1.0	1.0	1.0	2	2.0	2	1.0	2	2.0
Ethanol	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml
Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

EVALUATION OF MICROSPHERES:

Percentage production yield (PY)

$$PY(\%) = \frac{\text{Practical mass of microspheres}}{\text{Theoretical mass}} \times 100$$

Each formulation was carried out in triplicate and the PY (%) was calculated.

Entrapment efficiency: The Microspheres was prepared by Emulsion solvent diffusion technique. It was centrifuged at 14,000 rpm for 40 min at 10°C. The amount of Raltegravir is encapsulated into the Ethyl cellulose & HPMC. It was the difference between the total amounts that are used to prepare the Microspheres and the amount was found in the supernatant. The amount of free Raltegravir in the supernatant was analyzed by UV-spectrophotometer at 328 nm. It is Calculated by the following equation

$$\%EE = \left(\frac{M_{\text{Initial drug}} - M_{\text{Free drug}}}{X\ 100} \right)$$

$M_{\text{Initial drug}}$

Drug loading efficiency: Drug loading efficiency was removed and the remaining sediments (precipitations) were washed by distilled water. It is dispersed in a mixture of chloroform: acetone (2.5:2.5, v/v) in a 10 mL volumetric flask. It is used to ensure the complete extraction of drug from Microspheres, then it was sonicated for 30 min. The volume was made-up to 10 ml with chloroform. The resulting solution was centrifuged at 14,000 rpm at 10°C for 30 min and supernatants were obtained and analyzed in triplicate for the loaded drug by UV spectrophotometer at 328 nm.

Particle size determination: Particle size of Microspheres was determined by using an optical microscopy method. Approximately 100 microspheres were counted for particle size. The distribution of particle size was measured by suspending in water.

Equilibrium swelling studies of microspheres: A preweighed amount of microspheres was placed in Phosphate

buffer (pH 7.4).It is allowed to swell at a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured. The degree of swelling (a) was calculated by the following formula.

α=wg-wo/wo

Drug content determination: 50mg of Raltegravir microspheres was crushed and suspended in water to extract the drug from the microspheres. After 24 h, the filtrate was assayed spectrophotometrically at 328 nm for drug content against water as blank.

MICROMERITIC PROPERITIES OF RALTEGRAVIR MICROSPHERES:

Bulk Density:

The powder whose bulk density is to be determined is passed through sieve no 20. About 20gm is weighed accurately and carefully introduced in to a 100ml graduated measuring cylinder. The cylinder is dropped at 2seconds interval on to a hard surface 3 times from a height of 1 inch. The volume of powder is noted.

Bulk density = mass of powder/Bulk volume of powder

Angle of Repose:

The powder is filled in to an open ended cylinder with a bottom resting on a horizontal surface. The cylinder is then lifted vertically allowing the powder to form a heap on the horizontal. The diameter on the base of the concentration is determined by measuring the same in more than one direction. The height of the heap is also measured.

Angle of repose=tanϴ=h/r

Carr's Index:

It is the determination of tapped density and poured density.

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A fixed quantity of powder is poured in to an measuring cylinder and the volume is noted. The tapped density is then determined as described under bulk density determination.

Carr's index(%)=Tapped density- poured density/Tapped density*100

True density:

It is the ratio between mass of powder and its true volume.

True density=weight of powder/True volume

Hausner's Ratio:

Hausner's Ratio = Tapped density/Bulk density

Tapped density:

Tapped density=M/V_Y

***In-vitro* drug release studies:**

In-vitro drug release studies were carried out by using USP XXIV dissolution apparatus type II, with 500 ml of dissolution medium. It is maintained at 37±0.5 °C for 45 Mits, at 50 rpm, and pH 7.4 ± 0.2 phosphate buffer as dissolution medium.Results of *In-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows:

1. Log cumulative percent drug remaining versus time (first order kinetic model)
2. Cumulative percent drug release versus square root of time (Higuchi model)
3. Cumulative percent drug remaining versus time (zero order kinetic model)

4. Log cumulative Percent Drug released versus log time (korsmeyer's peppas model)

DATA ANALYSIS: To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained and it was fitted in to Zero order, First order, Higuchi matrix and Korsmeyer and Peppas model. Comparing the r-values are obtained, the best-fit model was selected[76].

1. **Zero order kinetics:** Drug dissolution from pharmaceutical dosage forms that does not disaggregateand the drug will be released slowly, assuming that the area does not change and no equilibrium conditions are obtained. It can be represented by the following equation

$$Q_t = Q_o + K_o t$$

2. First order kinetics: To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\text{Log } Q_t = \log Q_o + K_1 t / 2.303$$

3. Higuchi model: This model is developed by several theoretical models. To study the release of water-soluble and low soluble drugs.They are incorporated in to semisolids and or solid matrices, the equation is

$$Q_t = K_H . t^{1/2}$$

4. Korsmeyer and Peppas release model:

To study this model the release rate data are fitted to the following equation

$$M_t / M_\infty = K.t^n$$

Stability Conditions:

Stability study of microspheres containing Raltegravir was performed at following temperatures for one month and three months.

1. Long term testing : 25oC/ 60%RH (1Month) (3Month)
2. Accelerated testing : 40oC/75% RH (1Month) (3Month)

Parameters estimated: drug content

RESULTS AND DISCUSSION

Computability studies

IR studies

The IR spectrum of the pure Raltegravir sample is recorded by FTIR. This is compared with standard functional group frequencies of Raltegravir as shown in Table 2. FTIR spectrum of formulation shown in Figure 1 to 5.

Table 2: IR Interpretations for Pure

Functional groups	Raltegravir	Drug + Ethyl Cellulose	Drug + Ethyl Cellulose + HPMC
O-H Stretch (Carboxylic acids)	2513.25	3566.38	3520.09
N-H Stretch (1° &2° amines)	3342.64	3419.79	3442.94
C-H Stretch (Aromatics)	2850.79	2974.23	3358.07
C-C Stretch (Alkynes)	1618.28	1348.24	1230.58
C-O (Alcohols and Ether)	1521.84	1274.95	1165.00

drug ad polymer

COMPARISON OF FT-IR SPECTRA OF RALTEGRAVIR AND FORMULAE

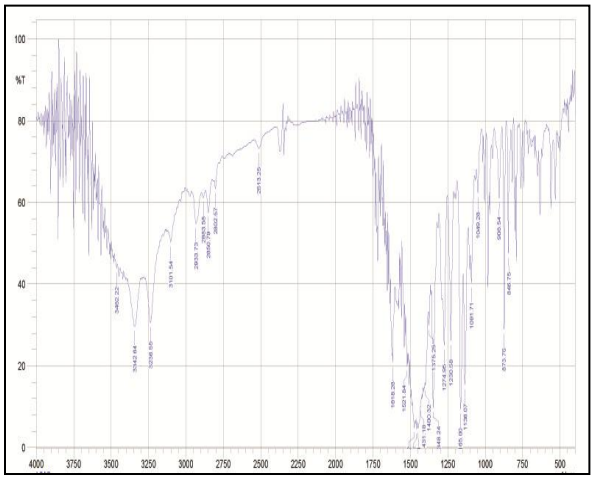


Figure 1: FTIR Spectrum of Pure drug (Raltegravir)

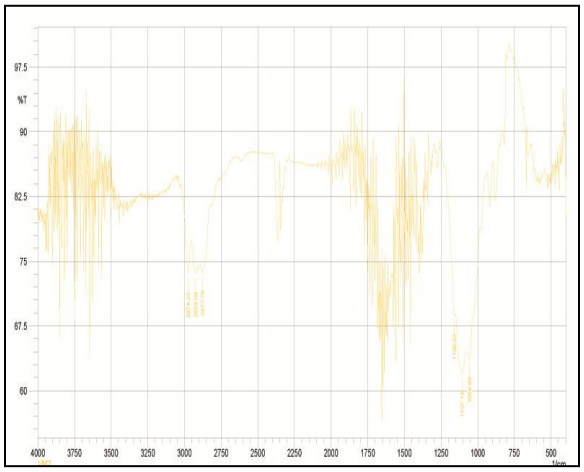


Figure 2: FTIR Spectrum of Ethyl cellulose

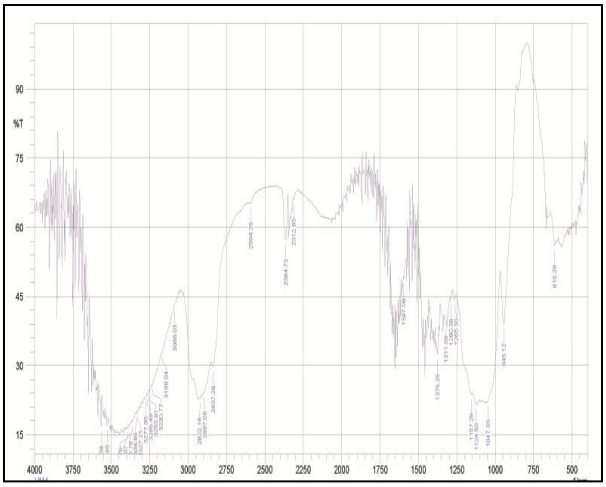


Figure 3: FTIR Spectrum of HPMC

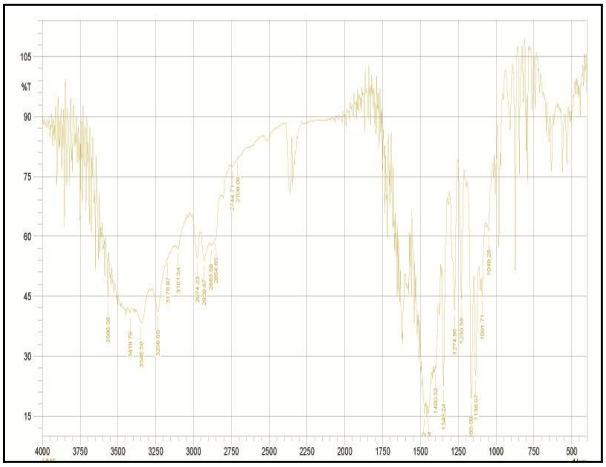


Figure 4: FTIR Spectrum of Drug + Ethyl cellulose

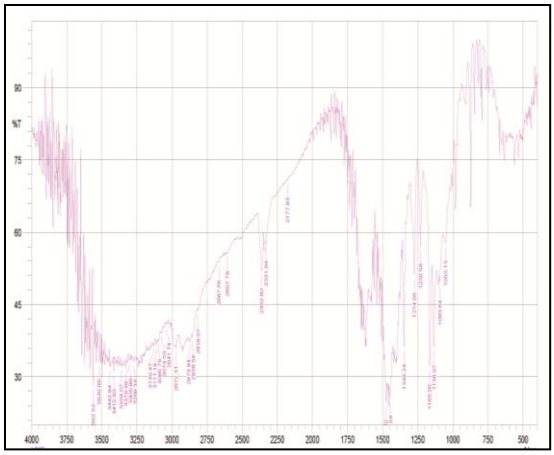


Figure 5: FTIR Spectrum of Drug + EC + HPMC

SI. No	Drug & Excipients	Exothermic peak	Endothermic peak
1	Raltegravir(RGV1)	122.5°c & -10.65 mw	----------
2	Drug+ethyl cellulose+Hpmc (RGV2)	121.9°c & -9.29 mw	286.2°C & -7.50 mW

Differential scanning calorimetry:

The pure drug of DSC sample of spectra the Exothermic peak is 122.5^{0}c and -10.65 mw. The mixture sample contain Drug (Raltegravir), Ethyl cellulose, HPMC the exothermic peak is 121.9^{0}c &-9.29 mw, the endothermic peak of the mixture 286.2 ^{0}c & -7.50 mw ,the compatibility of drug& excipients shows there is no compatibility in the formulation as shown in Table 3**.** It is suitable for performing formulation of microspheres.

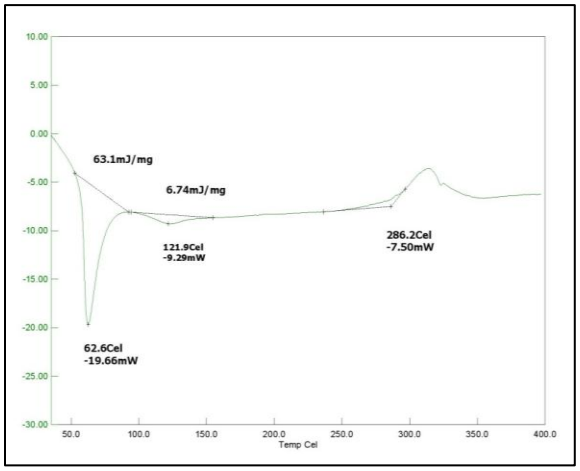


Figure 6: DSC spectrum of pure drug Raltegravir

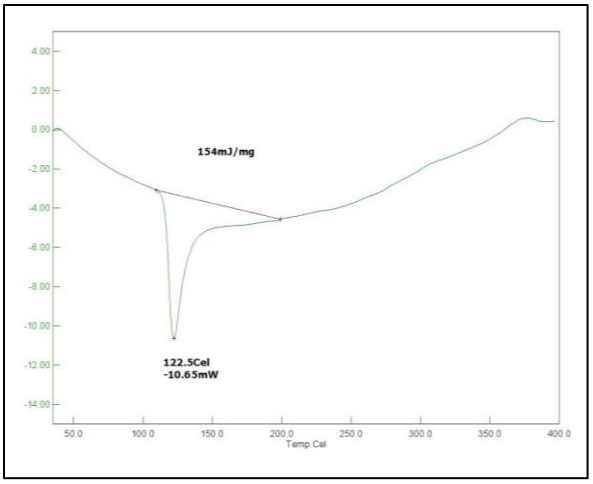


Figure 7: DSC spectrum of Mixtures (Drug + EC + HPMC)

Table 3: Interpretation of DSC Spectrum

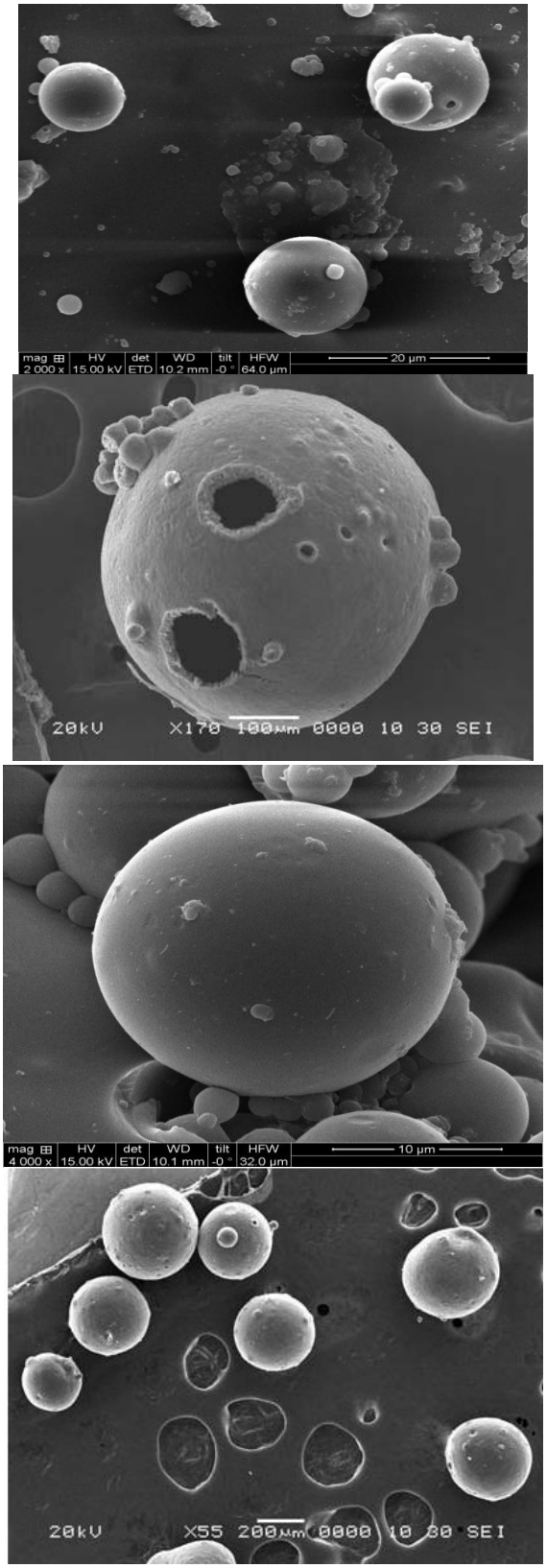
MORPHOLOGY OF THE PARTICLES:

The following methods are used to determine the particle size, size distribution, and morphology of Raltegravir microspheres.

SEM

Morphology and structure of Microspheres were determined by using scanning electron microscopy (SEM) and photomicrographs were taken at suitable magnifications. The photographs of the optimized formulation are taken by Scanning electron microscopy are shown in the Figure 8.

SHAPE AND SURFACE MORPHOLOGY



Figures 8: SEM Samples of Best formulations of RGV 8

EVALUATION OF RALTEGRAVIR MICROSPHERES

PERCENTAGE YIELD

The production yield of microspheres of Raltegravir using HPMC & Ethyl cellulose results as shown in Table 4 and Figure 9.

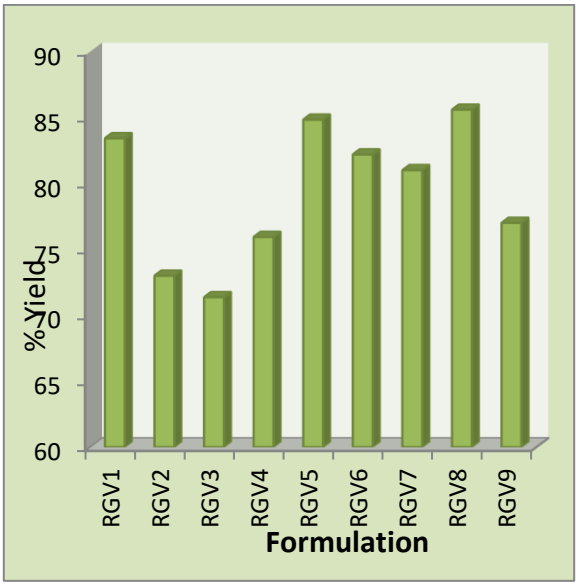


Figure 9: Percentage yield of Raltegravir microspheres

Encapsulation efficiency

Drug entrapment efficiency (%EE)

Percentage entrapment efficiency of RGV 1- 88.2%, RGV2- 84.2%, RGV3- 75.3%, RGV 4 – 64.2%, RGV 5- 61.8%, RGV 5 – 61.8%, RGV 6- 62.9%, RGV 7- 56.8, RGV 8-54.6, RGV 9- 53.9%, The RGV 8 shows the good formulation & high efficiency. Results as shown in Table 4 and Figure 10.

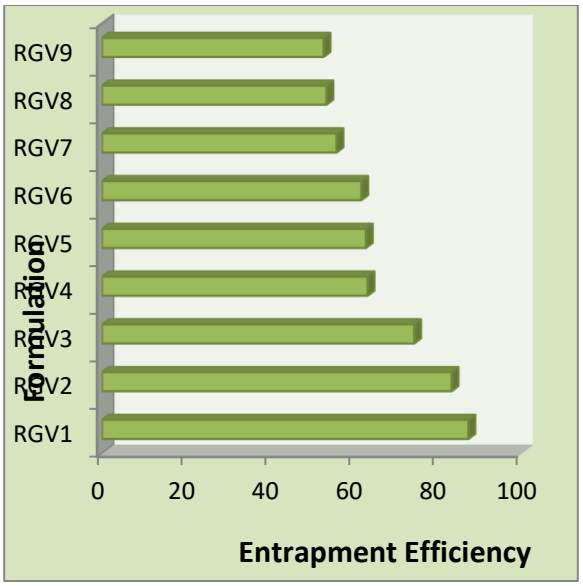


Figure 10: Entrapment efficiency of Raltegravir Microspheres

Entrapment Loading (%EL)

Percentage entrapment loading of RGV 1- 95.7%, RGV2- 85.8, RGV3- 82.4, RGV 4 – 68.8, RGV 5- 67.3, RGV 6- 66.7, RGV 7- 64.6, RGV 8- 56.6, RGV 9- 54.7%, The RGV 8 shows the good formulation & high efficiency. Results as shown in Table 4 and Figure 11.

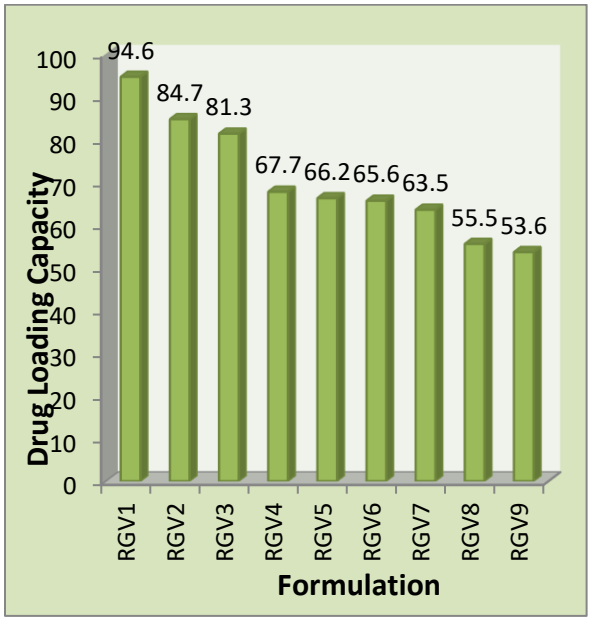


Figure 11: Drug Loading Capacity of Raltegravir microspheres

Particle size

Particle size distribution of Microspheres represented by RGV 1 (212µm), RGV 2 (60 µm), RGV3 (115 µm), RGV4 (55 µm), RGV 5 (455 µm), RGV 6 (315 µm), RGV 7 (265 µm), RGV 8 (15 µm), RGV 9 (115 µm).

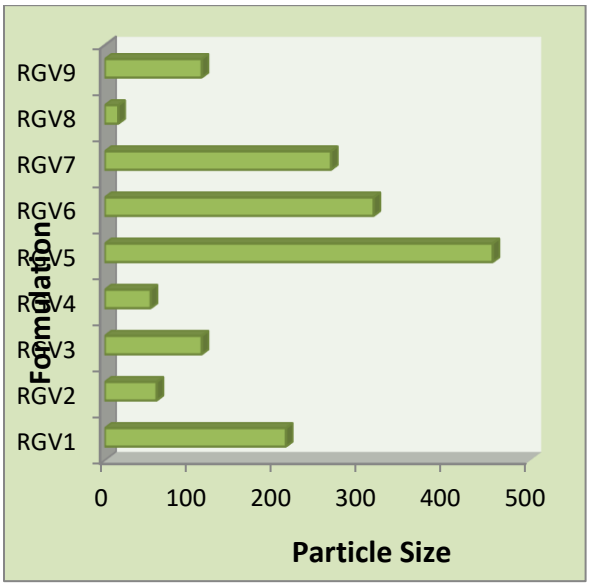


Figure 12: Particle Size of Raltegravir microspheres

Equilibrium swelling studies of microspheres.

A pre weighed amount (100 mg) of microspheres was placed in Phosphate buffer (pH7.4) and allowed to swell to a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured results as shown in Table 4 and Figure 13.

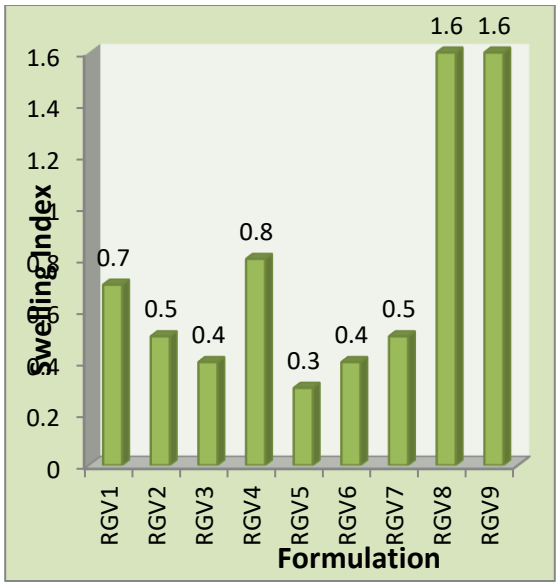


Figure 13: Swelling index of Raltegravir microspheres.

Percentage drug content Determination:

Drug content distribution of Microspheres represented it indicated that drug content is RGV 1 (65.8%), RGV 2 (67.3 %), RGV3 (88.4%), RGV4 (91.6%), RGV 5 (75.3%), RGV 6 (85.3 %), RGV 7 (86.6%), RGV 8 (96.3%), RGV 9 (72.5%), Formula as shown in given Table 4 and Figure 14.

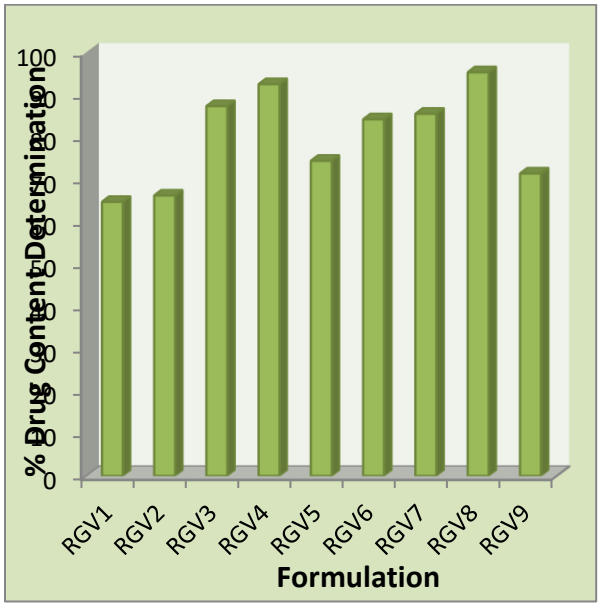


Figure 14: Percentage drug content of Raltegravir microspheres.

Table 4: Characterizaton of Raltegravir microspheres

Formulation code	% yield (%)	Drug Content	Entrapment Efficiency (%)	Drug Loading capacity (%)	Particle size (µm)	Swellability Studies (Sec)
RGV1	83.44	64.7	87.3	94.6	213	0.7 Sec
RGV2	73.01	66.2	83.3	84.7	61	0.5 Sec
RGV3	71.38	87.3	74.4	81.3	114	0.4 Sec
RGV4	75.95	92.5	63.3	67.7	54	0.8 Sec
RGV5	84.85	74.4	62.9	66.2	456	0.3 Sec
RGV6	82.21	84.2	61.8	65.6	316	0.4 Sec
RGV7	81.02	85.5	55.9	63.5	266	0.5 Sec
RGV8	85.61	95.3	53.5	55.5	16	1.6 Sec
RGV9	77.02	71.4	52.8	53.6	114	1.6 Sec

***In vitro* dissolution Studies:**

For understanding the mechanism of drug release rate kinetics of the drug from dosage forms, the *invitro* drug dissolution data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, and Korsmeyer Peppas model. The values are complied in Table 5. The % drug release with data to various kinetic models for different microspheres formulations is presented in figure 15.

Table 5: *In Vitro* dissolution Studies:

S. No	Time	% of Drug release								
		RGV1	RGV2	RGV3	RGV4	RGV5	RGV6	RGV7	RGV8	RGV9
1	5	2.52	5.08	6.94	8.68	9.58	13.16	13.62	14.44	13.58
2	10	4.63	8.94	9.24	14.36	15.42	18.95	18.32	27.08	16.21
3	15	6.94	8.44	13.59	16.62	17.32	25.16	22.16	34.83	23.09
4	20	7.66	13.58	14.64	19.32	21.16	26.94	26.72	38.66	22.95
5	25	13.56	15.42	19.32	25.16	25.06	36.32	36.86	46.38	33.93
6	30	15.42	27.06	28.05	33.32	35.08	38.64	41.48	52.24	27.85
7	3	27	32	32	36	37	42	44	54	42
	5	.06	.06	.06	.64	.43	.96	.62	.14	.24
8	40	29.07	42.46	53.07	52.92	62.64	73.64	67.64	63.88	63.87
9	45	38.62	48.22	54.12	63.86	68.26	74.54	76.29	88.94	69.62

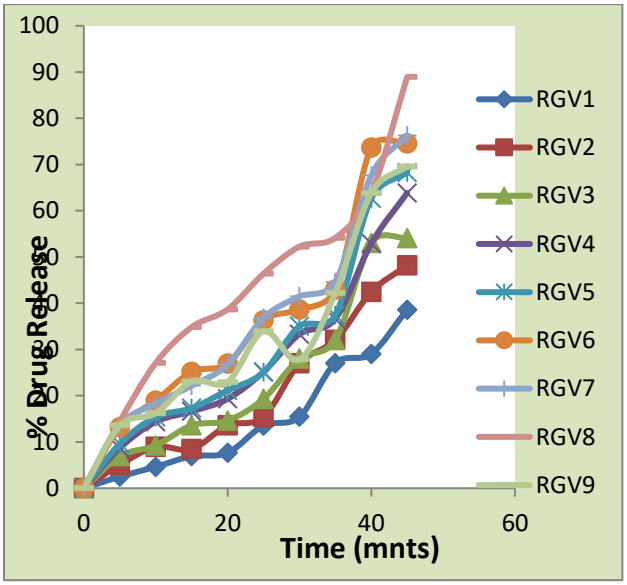


Figure 15*: In vitro* dissolution studies of Raltegravir microspheres

RELEASE ORDER KINETICS OF RALTEGRAVIR MICROSPHERES:

Table 6: Release order kinetics of zero order kinetics

sl. no	Time	zero order kinetics	first order kinetics	korsmeyer peppas	Higuchi
1	0	0	0	0	0
2	5	15420	4.188	4.188	15420
3	10	27060	4.432	4.432	27060
4	15	34800	4.541	4.541	34800
5	20	38640	4.587	4.587	38640
6	25	46380	4.666	4.666	46380
7	30	50220	4.7	4.7	50220
8	35	54210	4.733	4.733	54120
9	40	61860	4.791	4.791	61860
10	45	88920	Log cumulative % drug release	4.948	88920

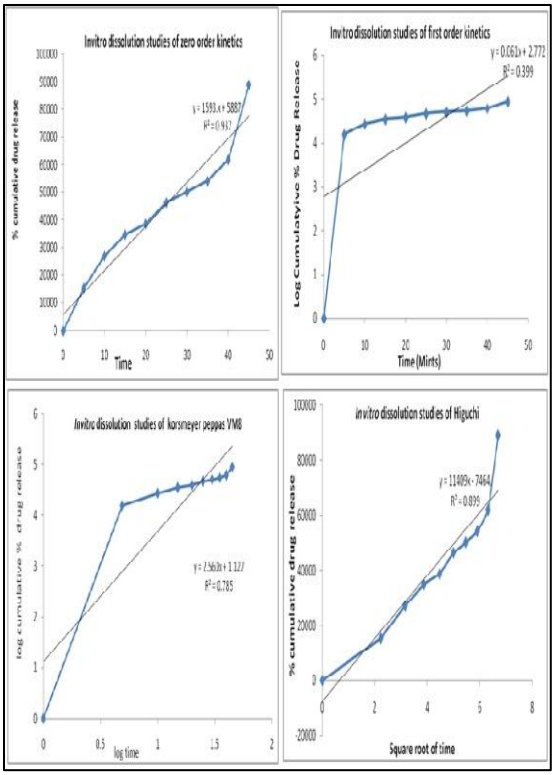


Figure 16: RGV 8 *In vitro* dissolution studies

Table 7: Release kinetics of Raltegravir Microspheres

Model	Equation	RGV 1		RGV 2		RGV 3		RGV 4	
		R^2	m	R^2	m	R^2	M	R^2	m
Zero order	Mo - Mt =kt	0.6555	69.44	0.9939	11239	0.0007	15.993	0.2028	72.888
First order	In M= In Mo	0.4944	0.0061	0.5406	0.0067	0.0257	0.0038	0.3522	0.0044
Higuchi's	$M_0 - M_t =$	0.511	4500	0.7676	7422	0.002	2122.	0.118	5155.

Matrix	$kt1/2$	6	8	7	0	3	0	9	5
Kosmeyer Peppas	log (M_0 - M_t) = log k	0.8354	2.3544	0.8844	2.5445	0.5729	1.7099	0.6633	1.8133

Model	Equation	RGV 5		RGV 6		RGV 7		RGV 8		RGV 9	
		R^2	M	R^2	m	R^2	m	R^2	M	R^2	M
Zero order	Mo-Mt=kt	0.9288	1414	0.9177	15.49	0.9499	15.54	0.9373	1593	0.9945	1399.7
First order	InM=InMo	0.4388	0.062	0.4811	0.052	0.4655	0.051	0.3999	0.061	0.4778	0.051
Higuchi's Matrix	$M_0 - M_t = kt1/2$	0.8038	96188	0.7988	10577	0.8488	10677	0.8999	11409	0.8366	960.9
Kosmeyer Pepp	log (M_0-M_t)=lo	0.8066	2.5177	0.8355	2.0322	0.8827	2.0333	0.7855	2.5600	0.8388	2.018
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STABILITY STUDY: Optimized RGV 8 was subjected to stability studies for 1 to 3 months and the Microspheres were tested for drug content. The results obtained were as in the following table 8.

Table 8: Stability studies of the optimized formulation RGV 8

Time in hrs	Drug Content		
	RGV 8	After 1 Month	After 3 Month
1	75.43	75.41	74.31
2	65.43	65.31	64.31
3	79.22	79.14	80.38
4	82.24	82.43	81.61
5	89.41	89.45	90.43
6	87.28	87.14	86.46
7	90.27	90.32	91.65
8	95.67	94.34	94.61

CONCLUSION

The purpose of present work was to develop microspheres of Raltegravir for sustained drug delivery system. From the results it seem that formulation RGV 8 was found to be the excellent Morphological properties, % yield of microsphere of best formulation was found to be RGV 8 (85.61%), Entrapment efficiency of best formulation was found to be (53.5 %) , Drug loading efficiency of best formulation was found to be (55.5%), Swelling index best formulation was found to be (1.6 sec), Particle size of best formulation was found to be (16 µm) , Drug content determination of best formulation was found to be (95.3) and *in vitro* drug release was fitted with various Release kinetic studies of a sustained

manner with constant fashion over extended period of time for 45 Mints. It was observed that concentration of Ethyl cellulose affected all the evaluation parameter significantly. Hence the prepared microspheres of Raltegravir may prove to be potential candidate for safe and effective sustained drug delivery.

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