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Formulation and Evaluation of Mucoadhesive Microspheres of an Anti-Viral Drug for Sustained Release and Improved Bioavailability

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ABSTRACT:
The main objective of the present research work was to develop and to evaluate an anti-viral drug loaded microspheres using natural and synthetic polymers for sustained release. Microspheres of Acyclovir were prepared by emulsion solvent evaporation techniques by using Eudragit RS-100 and HPMC as polymers. The prepared Acyclovir microspheres were subjected to IR, SEM, particle size and size distribution, DSC, %yield, drug content, entrapment efficiency, in vitro dissolution studies, and release kinetics. In vitro dissolution studies revealed that, increase in the polymer content resulted in delayed release of Acyclovir from microspheres.. A maximum of 85.39% drug entrapment efficiency was obtained in the Acyclovir microspheres. The Invitro performance of Acyclovir microspheres showed that sustained release was dependent upon the polymer concentration. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. The developed Acyclovir microspheres may be used for clinicals for prolonged drug release for at least 12hrs, which improves the bioavailability and patient compliance.

KEYWORDS: Sustained release, Acyclovir, Eudragit RS100, HPMC, Microspheres, Bioavailability

I. INTRODUCTION:

Sustained release drug delivery system are the “Drug Delivery” which covers a very extensive range of techniques used to deliver therapeutic agents into the human body(1). Sustained release, sustained action, prolonged action controlled release, extended release, depot release, these are the various terms used to identify during delivery system that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over a long period of time after administration of a single dose of drug(2). Microspheres are the solid spherical particles ranging from 1- 1000µm in size(3). The microspheres are two types ; microcapsules and micro matrices Microspheres are manufactured from both natural and synthetic materials. Microcapsules are those in which entrapped substances is distinctly surrounded by distinct capsule wall and Micro matrices in which entrapped substance is dispersed throughout the matrix (4). Microsencapsulation technique is used to slow down the drug release and to reduce or to remove the gastrointestinal tract inflammation. It is evenly distributed in theGIT. A low microsphere scale that increases drug absorption and also minimizes side effects including gastric irritation. (5). There are various methods to prepare microspheres that are Emulsion solvent evaporation technique, Spray drying method, Emulsion solvent diffusion technique etc.(6,7, 8, 9). The Microspheres were used in pharma industry as Ophthalmic drug delivery, Oral drug delivery, Genedelivery(10,11,12). Viruses are the ultimate expression of parasitism. They not only take nutrition from host cell but also direct its metabolic machinery to synthesize new virus particles. Anti-viral drugs are a class of drugs which target virus specific steps like cell penetration, uncoating, reverse transcription, virus assembly, maturation, etc. the drugs are Acyclovir, Valcyclovir, Idoxuridine, Tenofovir (13,14).

II. MATERIALSANDMETHODS:

Acyclovir was received as a gift sample from Medopharm.Pvt.Ltd, EudragitRS100 and HPMC from Leo Chemicals and IsopropylAlcohol, n-hexane, Petroleumether, LiquidParaffin , MethyleneChloride and Span-80 from ThomasBakers pvt ltd.

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PREPARATION OF ACYCLOVIR MICROSPHERES (15):

Microspheres of acyclovir were prepared by the emulsion- solvent evaporation method using Eudragit RS 100 and HPMC polymer at variable concentrations of drug and polymer. Dissolved 1g of polymer in a mixture of 10ml of each of ethanol, iso propyl alcohol and dichloromethane (1:1:1) and sonicated for 5min till completely miscible. A solution of Acyclovir 1g was dissolved in 10ml HCl and was added to polymer solution and futher sonicated for 20min till clear solution was obtained. This formed the aqueous phase. This was then added to 200ml liquid paraffin containing 1% w/v span 80 using 20 No. needle and stirred about 75-85^{0}C for 3h at 1000rpm till complete evaporation of solvent and microspheres solidified. Then 10ml of n-hexane was added to harden the spheres and stirring continued for 1h. this was then filtered and washed with petroleum ether to remove excess oil and dried at 50^{0}C for 30min. Six batches from F-1 to F-6 were prepared with different proportions of core to coat materials. Where F-1 to F-3 contains the drug with polymers Eudragit RS 100, F-4 to F-6 contains the drug with polymer HPMC.

TABLE 1:FORMULATION DESIGN:

SL.NO	INGREDIENTS	FORMULATION CODE					
		F1	F2	F3	F4	F5	F6
1	Drug (mg)	200	200	200	200	200	200
2	Eudragit RS100(mg)	200	400	600	-	-	-
3	HPMC (mg)	-	-	-	200	400	600
4	Ethanol (ml)	2	2	2	2	2	2
5	Iso propylalcohol(ml)	2	2	2	2	2	2
6	Dichloromethane (ml)	2	2	2	2	2	2
7	Span- 80 (ml)	0.5	0.5	0.5	0.5	0.5	0.5
8	n- hexane (ml)	2	2	2	2	2	2
9	Liquid paraffin (ml)	40	40	40	40	40	40
10	HCl (ml)	2	2	2	2	2	2

EVALUATION OF MICROSPHERES (16)

1. Particle size and Surface morphology

Determination of average particle size of Acyclovir microspheres was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microspheres was spread on a clean glass slide and average size of 300 microspheres was determined in each batch. Scanning Electron Microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large part to simplicity of sample preparation and ease of operation. The studies were carried out by using JEOL JSM T-330 a Scanning microscope (Japan). Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres were taken by random scanning of the stub.

2. Percentage yield

The percent yield of each of the sample was calculated from the expression:

$$\% \text{ Yield} = \frac{\text{weight of micro particles}}{\text{weight of solid starting materials}} \text{ X } 100$$

3. **Drug content**

Accurately weighed 100 mg microspheres were crushed in glass mortar and pestle, powder microspheres were suspended in 100 ml of suitable solvent. After 12 hours the solution was filtered and the filtrate was analysed for the drug content using UV-Visible spectrophotometer.

4. **Determination of Percentage Drug Entrappment (PDE)**

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula

$$\text{PDE} = \frac{\text{Practical drug content}}{\text{theoretical drug content}} \text{ X } 100$$

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5. **In-vitro dissolution studies**

In-vitro drug release study was carried out using USP type-II dissolution test apparatus. The dissolution medium 900ml of 7.4 phosphate buffer was maintained at 37 ± 1^0C and stirred at 50rpm. Aliquots of samples (5ml) at an interval of 1 hour were withdrawn and filtered through whatmann filter paper. The samples were analyzed for Acyclovir content by UV-Visible spectrophotometer at 252nm.

Data obtained was also subjected to kinetic treatment to obtain the order of release and release mechanism.

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q_0-Q) v/s t], Higuchi's square root of time (Q v/s t1/2) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t.

III. RESULTS AND DISCUSSION:

1. **Scanning electron microscopy (SEM)** :Scanning electron microscopy was performed to characterize the surface of the formed microspheres. Particles from F1, F3 and F5 were rough surfaced but spherical, whereas F2, F4 and F6 are found to be spherical, smooth and discrete.

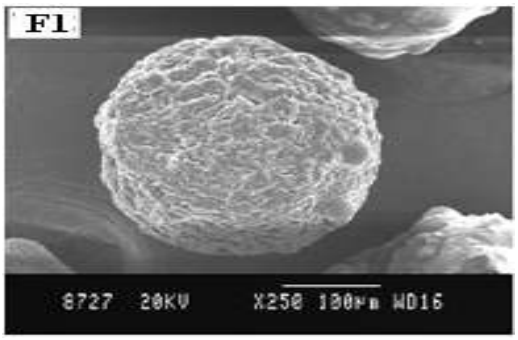

Fig 1: SEM for F1

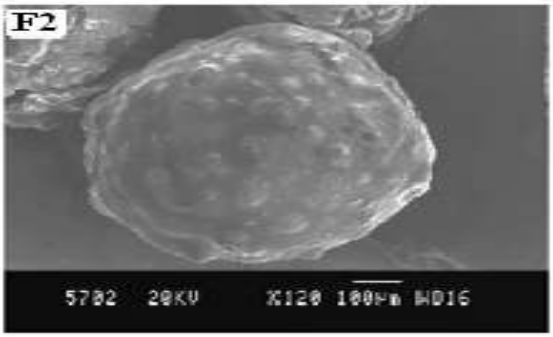

Fig 2: SEM for F2

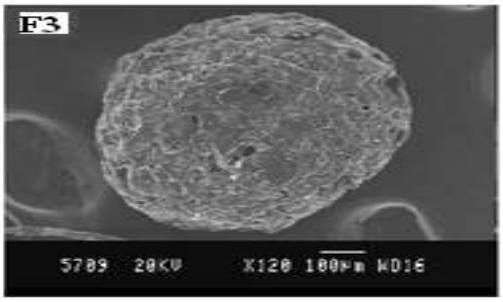

Fig 3: SEM for F3

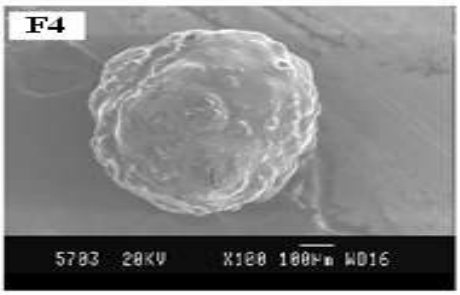

Fig 4: SEM for F4

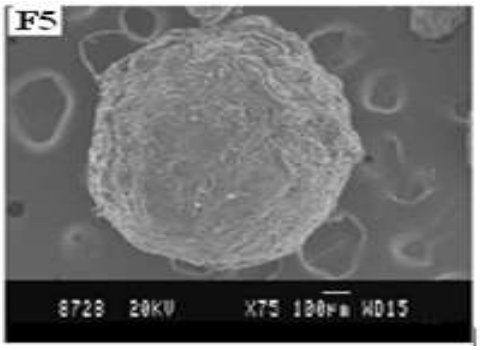

Fig 5: SEM for F5

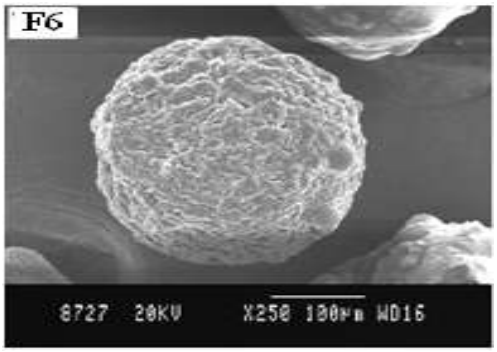

Fig 6: SEM for F6

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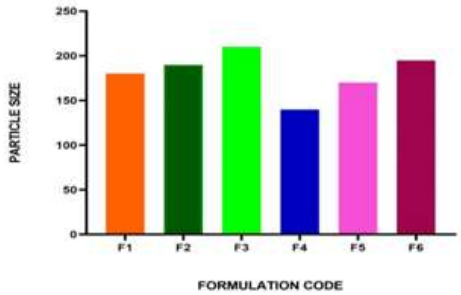
2. Determination of particle size

The arithmetic mean size of the formulations was determined by the optical microscope, fitted with an ocular micrometer and stage micrometer. The average mean particle sizes of the microcapsules were found to be µm for formulations F-1, F-2, F-3, F-4, F-5 and F-6 respectively.

Table 2: Average Diameter of Acyclovir Microspheres

Sl no	Formulation code	Average size (µm)
1	F 1	180±2
2	F 2	190±4
3	F 3	210±6
4	F 4	140±3
5	F 5	170±7
6	F 6	195±8

SD= Standard Deviation (n=3), Particle size (µm)



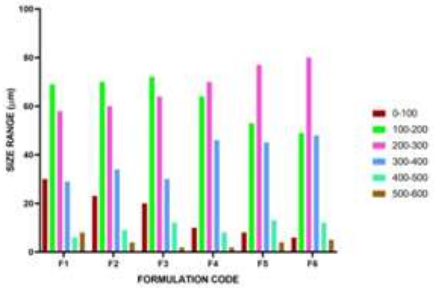


Fig 7: Average particle size of Acyclovir microspheres Fig8: Frequency distribution of Acyclovir microspheres

3. Frequency Distribution Analysis

Table 3: Frequency Distribution Analysis

Size Range(µm)	Number of Particles					
	F1	F2	F3	F4	F5	F6
0-100	30	23	20	10	8	6
100-200	69	70	72	64	53	49
200-300	58	60	64	70	77	80
300-400	29	34	30	46	45	48
400-500	6	9	12	8	13	12
500-600	8	4	2	2	4	5

4. Percentage drug entrapment efficiency

Table 4: Drug entrapment efficiency of Acyclovir Microspheres

Formulation	% Yield	% Drug Content	Entrapment Efficiency (%)
F1	78.96±0.04	80.09±0.04	76.04±0.03
F2	85.03±0.10	85.05±0.06	82.36±0.02
F3	92.60±0.08	90.45±0.02	89.45±0.10
F4	72.09±0.02	79.56±0.03	72.06±0.06
F5	79.45±0.03	82.35±0.08	77.09±0.01
F6	88.32±0.06	88.79±0.02	83.04±0.03

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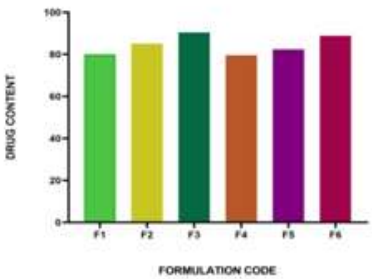
SD= Standard Deviation (n=3)



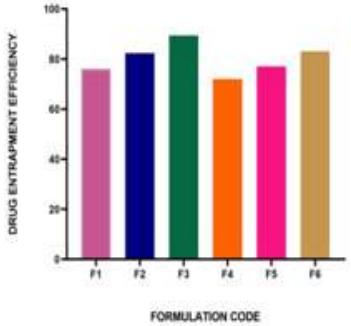


Fig9 : Drug content of Acyclovir microspheres **Fig 10: Percentage drug entrapment of Acyclovir microspheres**

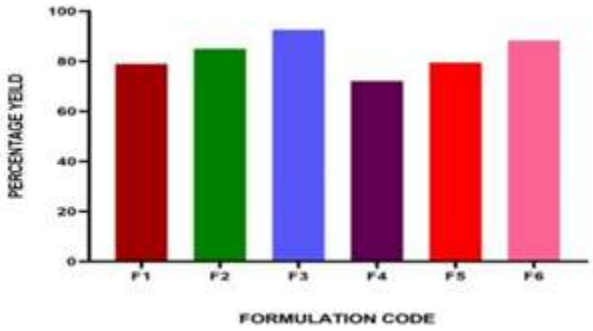


Fig 11:% Yield of Acylcovir Microspheres

IN-VITRO DISSOLUTION STUDIES

In-vitro release studies were carried out using USP-XXIII dissolution. Cumulative % drug release after 12 hrs was for F1, F2, F3, F4, F5 and F6 respectively. The results of in-vitro release studies were plotted into four models of data treatment as follows:

- Cumulative % drug release Vs Time (Zero order rate kinetics).
- Log cum. % drug retained Vs Time (First order rate kinetics).
- Higuchi's classical diffusion equation (Higuchi Matrix) in which cumulative % release was plotted against √T (root time).
- Log of cumulative % drug released Vs log Time (Peppas exponential equation).

The release data obtained for all the six formulations were tabulated in Table5. and depicted in Fig 12 respectively. Plots of zero order, first order, Higuchi matrix, Peppas depicted in Table 6 to 9 and Fig 13 to 15.

Table 5: In-vitro release data of Acyclovir Microspheres

TIME (hrs)	% Cummulative drug release					
	F1±SD	F2±SD	F3±SD	F4±SD	F5±SD	F6±SD
0	0	0	0	0	0	0
1	18.60±0.23	18.70±0.77	18.05±0.43	18.26±0.30	17.20±0.45	17.55±0.66
2	25.15±0.88	26.22±0.47	25.78±0.53	26.50±0.66	27.85±0.39	25.67±0.56
3	35.88±0.66	31.05±0.33	32.25±0.36	34.65±0.71	39.90±0.57	33.76±0.78

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4	40.05±0.48	37.24±0.65	37.75±0.29	38.53±0.49	48.40±0.67	44.15±0.64
5	56.26±0.79	44.25±0.53	48.05±0.75	44.95±0.59	54.90±0.57	49.23±0.81
6	59.54±0.81	52.60±0.45	55.60±0.32	51.40±0.37	58.10±0.81	55.97±0.67
7	64.31±0.66	60.22±0.53	64.85±0.36	56.95±0.66	63.65±0.72	60.94±0.39
8	69.33±0.56	65.35±0.89	70.25±0.52	62.10±0.59	69.45±0.67	65.09±0.81
9	70.30±0.55	71.95±0.58	75.30±0.54	67.70±0.81	74.06±0.59	70.85±0.66
10	73.41±0.89	78.26±0.66	81.25±0.45	73.25±0.36	78.07±0.64	74.83±0.54
11	77.95±0.66	85.24±0.39	90.30±0.70	77.30±0.53	82.53±0.63	79.58±0.67
12	87.70±0.67	91.21±0.58	93.60±0.54	81.80±0.66	88.15±0.59	88.48±0.55

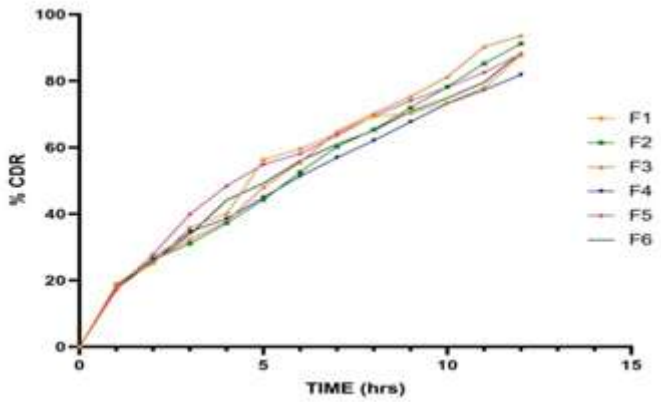

Fig 12: Invitro release profile of Acyclovir microspheres

RELEASE KINETICS OF ACYCLOVIR MICROSPHERES

Table 6 :Zero Order release kinetics data of Acyclovir microcapsules

TIME (hrs)	**% Cummulative drug release**					
	F1±SD	**F2±SD**	**F3±SD**	**F4±SD**	**F5±SD**	**F6±SD**
0	0	0	0	0	0	0
1	18.60±0.23	18.70±0.77	18.05±0.43	18.26±0.30	17.20±0.45	17.55±0.66
2	25.15±0.88	26.22±0.47	25.78±0.53	26.50±0.66	27.85±0.39	25.67±0.56
3	35.88±0.66	31.05±0.33	32.25±0.36	34.65±0.71	39.90±0.57	33.76±0.78
4	40.05±0.48	37.24±0.65	37.75±0.29	38.53±0.49	48.40±0.67	44.15±0.64
5	56.26±0.79	44.25±0.53	48.05±0.75	44.95±0.59	54.90±0.57	49.23±0.81
6	59.54±0.81	52.60±0.45	55.60±0.32	51.40±0.37	58.10±0.81	55.97±0.67

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7	64.31±0.66	60.22±0.53	64.85±0.36	56.95±0.66	63.65±0.72	60.94±0.39
8	69.33±0.56	65.35±0.89	70.25±0.52	62.10±0.59	69.45±0.67	65.09±0.81
9	70.30±0.55	71.95±0.58	75.30±0.54	67.70±0.81	74.06±0.59	70.85±0.66
10	73.41±0.89	78.26±0.66	81.25±0.45	73.25±0.36	78.07±0.64	74.83±0.54
11	77.95±0.66	85.24±0.39	90.30±0.70	77.30±0.53	82.53±0.63	79.58±0.67
12	87.70±0.67	91.21±0.58	93.60±0.54	81.80±0.66	88.15±0.59	88.48±0.55

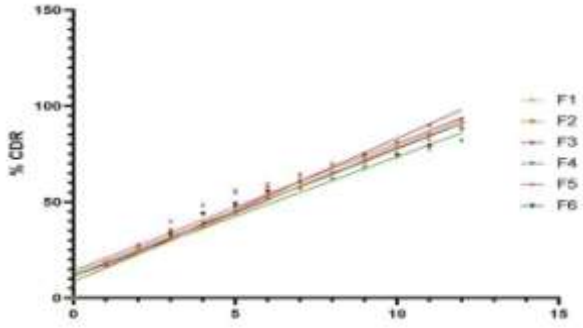

Fig 13: Zero order release kinetics profile of Acyclovir microspheres

Table 7 : First Order release kinetics data of Acyclovir Microspheres

TIME (hrs)	**% Cummulative drug release**					
	F1±SD	**F2±SD**	**F3±SD**	**F4±SD**	**F5±SD**	**F6±SD**
0	2.000±0.000	2.000±0.000	2.000±0.000	2.000±0.000	2.000±0.000	2.000±0.000
1	1.900±0.004	1.895±0.009	1.905±0.005	1.905±0.006	1.902±0.006	1.904±0.008
2	1.850±0.020	1.855±0.012	1.850±0.008	1.855±0.007	1.823±0.017	1.859±0.004
3	1.755±0.019	1.820±0.009	1.820±0.012	1.795±0.012	1.764±0.008	1.802±0.018
4	1.701±0.011	1.785±0.016	1.784±0.008	1.779±0.019	1.695±0.012	1.736±0.008
5	1.675±0.015	1.725±0.012	1.701±0.016	1.730±0.009	1.658±0.009	1.684±0.014
6	1.621±0.018	1.656±0.004	1.635±0.006	1.669±0.006	1.602±0.016	1.629±0.011
7	1.545±0.009	1.550±0.008	1.542±0.009	1.614±0.013	1.524±0.009	1.578±0.008
8	1.520±0.008	1.515±0.023	1.452±0.012	1.544±0.019	1.464±0.003	1.522±0.012
9	1.425±0.023	1.430±0.036	1.364±0.020	1.482±0.031	1.389±0.016	1.459±0.020
10	1.395±0.016	1.310±0.032	1.248±0.013	1.401±0.023	1.315±0.008	1.376±0.008
11	1.305±0.007	1.125±0.026	0.980±0.019	1.328±0.019	1.209±0.024	1.275±0.016
12	1.050±0.019	0.885±0.006	0.710±0.013	1.216±0.031	1.015±0.016	1.004±0.021

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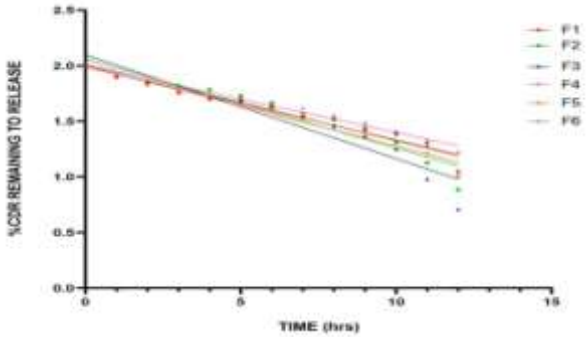

Fig 14 : First order release kinetics profile of Acyclovir microspheres

Table 8: Higuchi Matrix release kinetics data of Acyclovir Microspheres

SQUARE ROOT TIME (hrs)	% Cummulative drug release					
	F1±SD	F2±SD	F3±SD	F4±SD	F5±SD	F6±SD
0	0	0	0	0	0	0
1	18.60±0.23	18.70±0.77	18.05±0.43	18.26±0.30	17.20±0.45	17.55±0.66
1.414	25.15±0.88	26.22±0.47	25.78±0.53	26.50±0.66	27.85±0.39	25.67±0.56
1.732	35.88±0.66	31.05±0.33	32.25±0.36	34.65±0.71	39.90±0.57	33.76±0.78
2	40.05±0.48	37.24±0.65	37.75±0.29	38.53±0.49	48.40±0.67	44.15±0.64
2.236	56.26±0.79	44.25±0.53	48.05±0.75	44.95±0.59	54.90±0.57	49.23±0.81
2.449	59.54±0.81	52.60±0.45	55.60±0.32	51.40±0.37	58.10±0.81	55.97±0.67
2.645	64.31±0.66	60.22±0.53	64.85±0.36	56.95±0.66	63.65±0.72	60.94±0.39
2.828	69.33±0.56	65.35±0.89	70.25±0.52	62.10±0.59	69.45±0.67	65.09±0.81
3	70.30±0.55	71.95±0.58	75.30±0.54	67.70±0.81	74.06±0.59	70.85±0.66
3.162	73.41±0.89	78.26±0.66	81.25±0.45	73.25±0.36	78.07±0.64	74.83±0.54
3.316	77.95±0.66	85.24±0.39	90.30±0.70	77.30±0.53	82.53±0.63	79.58±0.67
3.464	87.70±0.67	91.21±0.58	93.60±0.54	81.80±0.66	88.15±0.59	88.48±0.55

SD= Standard Deviation (n=3)

Table 9:Peppas release kinetics data of Acyclovir Microspheres

LOG TIME (hrs)	% Cummulative drug release					
	F1±SD	F2±SD	F3±SD	F4±SD	F5±SD	F6±SD
0	1.265±0.009	1.285±0.011	1.228±0.006	1.250±0.013	1.224±0.009	1.216±0.008
0.301	1.4165±0.013	1.3349±0.018	1.3278±0.013	1.3398±0.006	1.3609±0.016	1.3260±0.013
0.477	1.4668±0.029	1.4054±0.023	1.4225±0.016	1.4317±0.026	1.5119±0.008	1.4411±0.015

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0.602	1.5128±0.037	1.4825±0.029	1.4884±0.008	1.4969±0.019	1.5937±0.013	1.5451±0.021
0.698	1.5637±0.006	1.5553±0.024	1.5914±0.015	1.5627±0.026	1.6317±0.019	1.6010±0.001
0.778	1.6479±0.032	1.6293±0.021	1.6529±0.019	1.6192±0.034	1.6722±0.014	1.6556±0.004
0.845	1.6820±0.013	1.6869±0.009	1.7169±0.009	1.6631±0.028	1.7108±0.017	1.6920±0.010
0.903	1.7150±0.016	1.7216±0.005	1.7528±0.019	1.7004±0.029	1.7479±0.006	1.7201±0.024
0.954	1.7471±0.031	1.7578±0.016	1.7825±0.009	1.7375±0.036	1.7754±0.019	1.7513±0.015
1	1.7716±0.026	1.7990±0.007	1.8150±0.016	1.7700±0.031	1.7980±0.021	1.7798±0.019
1.041	1.7973±0.019	1.8357±0.016	1.8361±0.013	1.7940±0.021	1.8218±0.007	1.8062±0.026
1.079	1.8489±0.016	1.8648±0.009	1.8763±0.021	1.8180±0.029	1.8501±0.014	1.8517±0.019

SD= Standard Deviation (n=3)

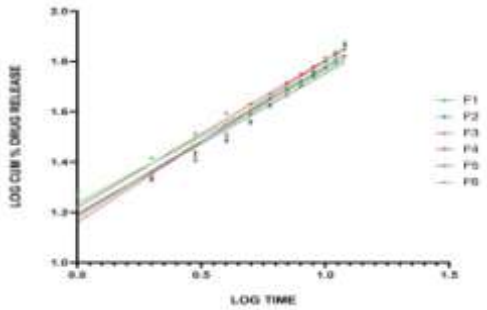

Fig 15: Peppas release kinetics profile of Acyclovir microspheres

Table 10 : Regression co-efficient (r^2) values of different kinetic models and diffusion exponent (n) of Peppas model for Acyclovir Microspheres.

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				r^2 value	n value
F1	0.9669	0.9449	0.9932	0.9932	0.6245
F2	0.9854	0.9169	0.9650	0.9650	0.6439
F3	0.9848	0.9116	0.9683	0.9983	0.6232
F4	0.9829	0.9683	0.9951	0.9951	0.5945
F5	0.9782	0.9492	0.9926	0.9926	0.6232
F6	0.9658	0.9500	0.9974	0.9974	0.642

IV. CONCLUSION:

In the present study, an attempt was made to develop and evaluate Acyclovir microspheres containing HPMC and Eudragit RS 100 polymers to treat viral infectious diseases. Formulation as sustained release drug delivery enhances the better release of drug for a longer period of time and prevents the effect of viral infections. Acyclovir and Eudragit RS 100 in the ratio 1:3 are suitable for preparation of microsphere. As the drug to polymer

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ratio was increased, the mean particle size of Acyclovir microspheres was also increased. Acyclovir microspheres with normal frequency distribution were obtained. Entrapment efficiency increases with increase in the polymer concentration from the result it can be inferred that there was a proper distribution of Acyclovir in the microspheres and the deviation was within the acceptable limits. Increase in amount of polymer increased the particle size of microspheres. The increasing order of drug content was found in formulation F3>F2>F1 and F6>F5>F4. On the basis of drug content, particle size and morphology, invitro release studies and its kinetic data, F3 (formulation with Eudragit RS100) was selected as optimized formulation for designing pulsatile device. Hence, based on the above study it can be concluded that Sustained release microspheres of Acyclovir can be successfully developed on a lab scale.

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