ORIGINAL ARTICLE

Formulation and Evaluation of Controlled Release Microspheres of Acyclovir for Antiviral Therapy

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Abstract

Aim: Controlled drug delivery system (CDDS) provides the continuous oral drug delivery at predictable and reproducible kinetics for a predetermined period throughout the course of Gastro intestinal transit and emerges various routes of administration to achieve oral controlled drug delivery. Aim of this study was to formulate and evaluate controlled release microspheres of Antiviral drug, Acyclovir. Polymethacrylates (Eudragits) used as release retardant polymer material. **Material & Methods:** Microspheres prepared by non-aqueous solvent evaporation method using alcohol/light liquid paraffin. Magnesium stearate act as a droplet stabilizer and n-hexane is to harden the microspheres. The microspheres evaluated for their compatibility study Fourier transform infra-red(FT-IR), differential scanning calorimeter(DSC), flow & micromeritics properties, particle size, drug content, entrapment efficiency, In vitro dissolution study in both 1.2 and 6.8 pH buffer, Scanning Electron Microscope(SEM) and accelerated stability study. **Results & Discussion:** The prepared microspheres were free flowing and spherical shape. The actual drug content in microspheres showed entrapment and release controlled till 12h the rate of drug release was found to decrease with increasing sphere size. The compatibility studies showed no interactions in formulations. Scanning electron microscope study of microspheres were Spherical and porous in nature. The best fit release kinetics achieved was Hixon-crowell plot. **Conclusion:** The formulation and evaluation of Controlled Release Acyclovir microspheres is influenced by drug to polymer ratio and particle size and was found to be dissolution controlled. The formulation enhanced bioavailability upto 24hrs.

Key words: Acyclovir, controlled drug delivery system, controlled release, eudragits, *in vitro*, microspheres

INTRODUCTION

Oral controlled-release drug delivery system (CDDS) that provides continuous oral delivery of drugs at a predictable and reproducible kinetics at a predetermined period throughout the course of GI transit time. Microspheres are monolithic spheres distributed throughout the matrix either as a molecular dispersion of particles. Among the various methods prepared for formulation of microspheres, the non-aqueous solvent evaporation method has a great attention due to its ease of fabrication without compromising the activity of drug. In the present study, polymethacrylates (Eudragits RSPO, RLPO) were used as a retardant for controlled release for microspheres. This is due to its biocompatibility, good stability, and low cost. The drug of choice, acyclovir is a guanosine analog antiviral drug used for the treatment of herpes simplex virus infections, as well as in the treatment of varicella zoster (chicken pox) and herpes zoster (shingles).[1-3]

MATERIALS AND METHODS

Materials

Acyclovir was received as a gift from AUROBINDO Pharma Ltd., Hyderabad, India. Eudragit RSPO and RLPO

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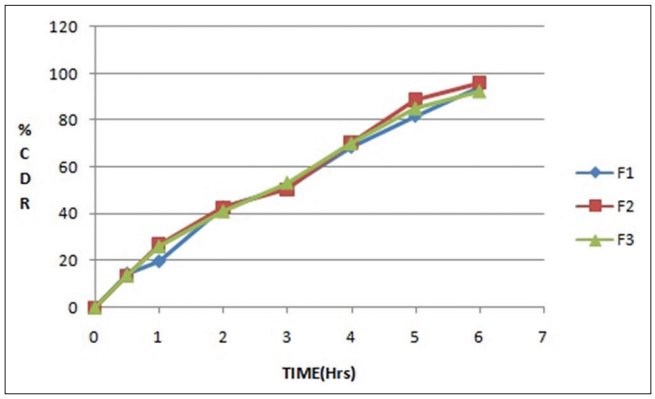
were obtained from Evonik Rohm Pharma, Germany. All other reagents and solvents used were of pharmaceutical or analytical grade.

Methods

Controlled release microspheres of acyclovir were prepared by non-aqueous solvent evaporation method using polymethacrylates (Eudragits) polymers. Take 100 ml of beaker add 10 ml of alcohol; place magnetic beads on a magnetic stirrer at 400 rpm–500 rpm. Following the solvent mix, add polymer and the drug as core material to dissolve in it. Finally, magnesium stearate is added as a stabilizer in the mixture of solvents for 15 min. Then add the above-prepared solution to the beaker undergoing homogenization process containing 90 ml of light liquid paraffin and 10 ml of n-hexane with mechanical stirring at 700 rpm. The spheres are formed, and the solvent is removed from the spheres for 3–4 h then the spheres are filtered and washed with 50 ml of n-hexane for 3–4 times to get microspheres hardened and then kept 24 h for drying at room temperature and the formulation table is done in Table 1 and Graph 1 shows formulation design.[4,5]

Compatibility studies



Graph 1: *In vitro* drug release profile of acyclovir matrix tablets of formulation F1–F3

Fourier transforms-infrared (FT-IR) spectroscopy

The drug and excipients must be compatible with one another to produce a product that is efficacious and safe. This can be confirmed by visual inspection and FT-IR spectroscopy. Pure drugs were mixed with the polymers in the ratio of 1:1 and filled in vials, labeled, and stored. The samples were subjected to visual observation and FT-IR studies.[6]

Differential scanning calorimeter (DSC)

Thermograms were obtained using a differential scanning calorimeter at a higher rate of 10°C/min over a temperature range of 0–400°C. The sample was hermetically sealed in the aluminum crucible.[7]

Flow properties and micromeritic properties

Flow characteristics such as angle of repose (Θ) of the microspheres, which measures the resistance to particle flow was determined by the fixed funnel method using the following equation.

$$\text{Angle of repose } \Theta = \tan^{-1}(h/r)$$

Where "h" is the height of the pile, "r" is the radius of the base pile on the graph paper. The prepared microspheres were characterized for their micromeritic properties such as bulk density, tapped density, and % Carr's index. The tapping method was used to calculate tapped densities and % Carr's Index.[8,9]

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}}$$

$$\%\ \text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Particle size analysis

The size of the microspheres was determined by two steps, sieve analysis method and optical microscopy method. In sieve analysis, the mesh size of sieve no 22, 44, and 60 was taken and arranged in ascending order from top to bottom.

Table 1: Formulation design

Formulation no	Drug (g)	Eudragit RSPO (g)	Eudragit RLPO (g)	Magnesium stearate (g)
F1	1	1	-	0.1
F2	1	1.5	-	0.1
F3	1	2	-	0.1
F4	1	-	1	0.1
F5	1	-	1.5	0.1
F6	1	-	2	0.1
F7	1	0.5	0.5	0.1
F8	1	0.75	0.75	0.1
F9	1	1	1	0.1

5 g of microspheres were added on a sieve and are shaken for 5 min to separate the microspheres. Then, different sizes of microsphere were collected and further evaluated in optical microscopy. From the above-obtained microspheres of different size were examined under an optical microscope to know there the actual size of microspheres and mean of five microspheres of each sieve were taken. Average of all the three mean particle sizes was calculated.[10,11]

Percentage yield (%)

The prepared microsphere was collected and weighed. Total weight obtained after preparation divided by total amount of drug and polymer taken for the preparation of microspheres.[12]

$$\text{Percentage yield} = \frac{\text{Total weight obtained from preparation of microspheres}}{\text{Total weight of drug + Polymer for preparation of microspheres}}$$

Drug content and entrapment efficiency

Accurately weighed 50 mg of microspheres are added to 50 ml of hydrochloric acid buffer (1.2 pH). The resulting mixture was agitated on a rotary flask shaker for 48 h. The solution was withdrawn, and the actual drug content was measured at 255 nm using UV-visible spectrophotometer. Moreover, theoretical drug content was calculated using absorbance and withdrawal volume using the intercept values.[13-15]

$$\text{Percentage entrapment efficiency } (\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Drug release behavior

The *in vitro* dissolution studies were carried out in 900 ml of pH 1.2 for 2 h and later at phosphate buffer pH 6.8 maintained at 37 ± 0.5° and 100 rpm using United States Pharmacopoeia basket type dissolution test apparatus (Electrolab TDT-08L Servewell Instruments Pvt. Ltd, Bengaluru). Under sink conditions. Accurately weighed samples of the microspheres were added to the dissolution medium and at preset time intervals, 0.9 ml aliquots were withdrawn and replaced with an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectrophotometrically at 255nm. The concentration of acyclovir in test samples was corrected and calculated using a regression equation of the calibration curve. The dissolution studies were carried out, and values were plotted as percentage cumulative release versus time.[16,17]

Selection of optimized formulation based on drug release

The drug release profiles are dependent on the size of microspheres; the particle size was separated by two steps by sieve analysis and optical microscope. In which the different particles held for dissolution and finally the entrapment efficiency and drug release profile graph were plotted.

Scanning electron microscope (SEM)

SEM (S-3700N, Hitachi) was used to characterize the shape and surface topography of the microspheres. Before examination, samples were gold sputter-coated to render them electrically conductive.[18]

Release kinetics

Data obtained from *in vitro* release studies were fit to various kinetic equations to find out the mechanism of drug release from microspheres. The kinetic models used were zero-order, first-order, Higuchi, and Hixson-Crowell models. The rate constants were also calculated for the respective models.[19]

Accelerated stability studies for the optimized formulation

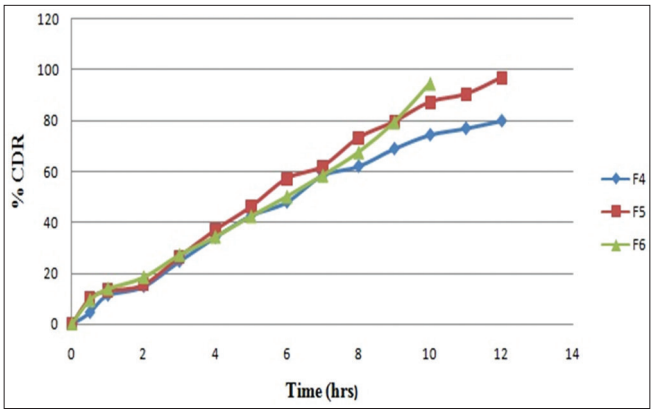
Accelerated stability studies were carried out as per the ICH guidelines, at 40°C ± 65° RH and 40°C ± 75° RH for a period of 1 month and carried out drug content, entrapment efficiency, and drug release profile of microspheres.[20]

RESULTS

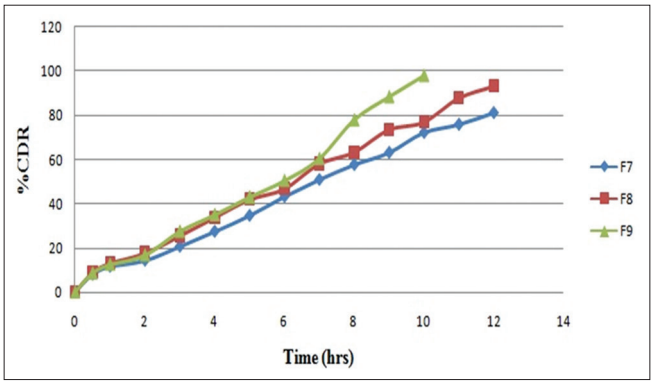
The resulting microspheres formulated by non-aqueous solvent evaporation method were spherical and free flowing in nature. The average particle size of microspheres ranged from 480 µm to 530 µm. It was noticed that mean particle size increased with an increase in polymer concentration in the optimized formulation. The entrapment efficiencies ranged from 64.47% to 92.48%. The entrapment efficiency was also found to be dependent on the nature of the polymer used in the formulation. From the *in vitro* dissolution studies, it was found that the controlled effect of microspheres depended on the polymer concentration and particle size of the microspheres.

DISCUSSION

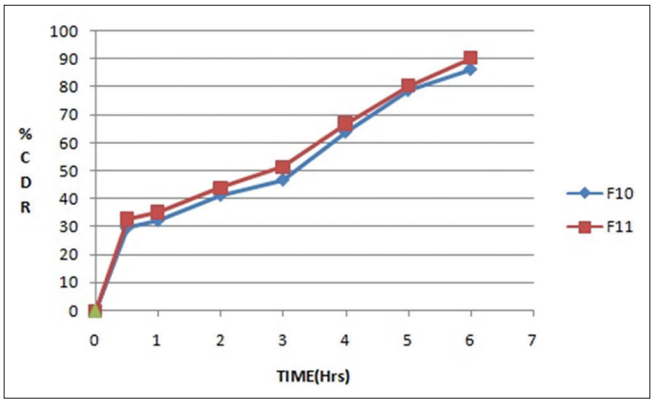
Oral CDDS has been known for decades as the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs through various pharmaceutical products of different dosage forms. Compatibility of the drug with various polymers was accessed by IR and DSC spectra. The DSC and IR spectra of the drug, polymers, and their combinations were compared with the spectra of pure drug and individual polymers and combinations where the principal peaks in IR and endothermic peak obtained for the combinations had no variations in the peaks. Hence, there was no interaction between the drug and the polymers shown in the spectra [Graphs 1-6].



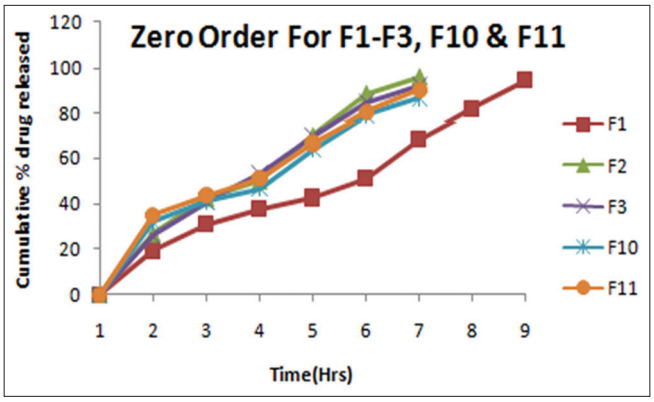
Graph 2: *In vitro* drug release profile of acyclovir matrix tablets of formulation F4–F6



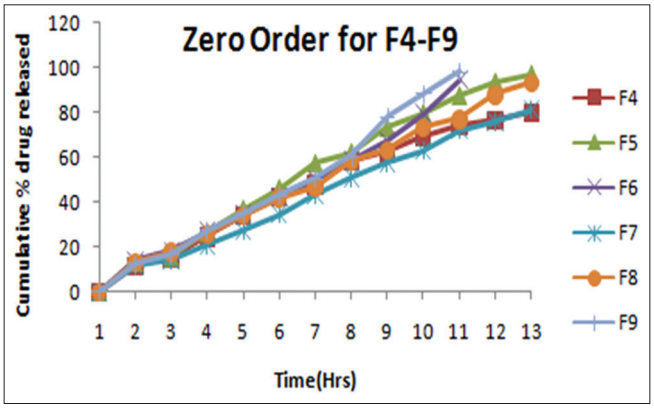
Graph 3: *In vitro* drug release profile of acyclovir matrix tablets of formulation F7–F9



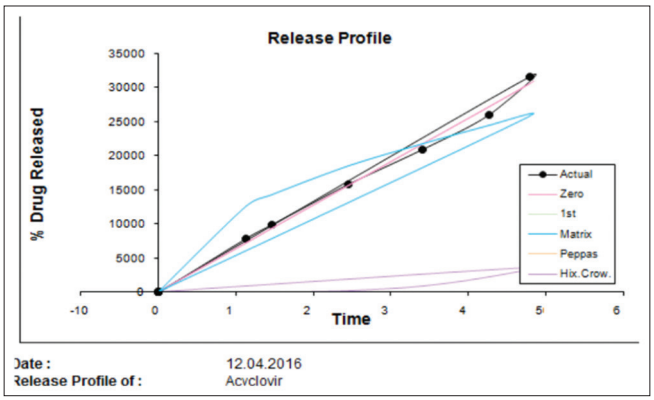
Graph 4: *In vitro* drug release profile of acyclovir matrix tablets of formulation F10–F11



Graph 5: Kinetic data of various models for release study of acyclovir matrix tablet



Graph 6: Kinetic data of various models for release study of acyclovir matrix tablet



Graph 7: Curve fitting data of the release rate profile of formulations

The flow property and micromeritic studies of the prepared formulation showed good flow properties and micromeritic studies with their tan Θ and % Carr's index values within the permissible limits. Graph 7 shows the formulation design.

The microspheres size in all the formulations ranged from 250 to 800 µm was separated by sieve analysis method. Microspheres were observed in the optical microscope, the mean value of particles and the average particle size was calculated and taken for the drug release profile. Formulation 1 has the average particle size about 504.8 µm.

The percentage yield for each formulation was based on the ratio of drug:polymer ratio. The percentage yield obtained for all the formulations ranged from 68% to 83%.

The drug content of 50 mg microspheres, all the formulations were in the range 10.72 mg–23.14 mg. The drug entrapment efficiency of all the formulations was in the range

Table 2: Evaluation parameters of formulation F1–F9 of acyclovir microspheres

Formulation code	Percentage yield (%)	Actual drug content (mg)	Theoretical drug content (mg)	Entrapment efficiency (%)	Average particle size (µm)	Carr's index %	Angle of repose (Θ)	Bulk density g/ml	Tapped density g/ml
F1	77.58	21.28	25	84.83	504.8	14.76	$24^0.14'$	0.71	0.833
F2	75.26	14.92	20	74.65	492.73	20.30	$27^0.40'$	0.526	0.66
F3	76.62	10.72	16.66	64.47	509.86	6.025	$25^0.70'$	0.714	0.76
F4	82.13	23.14	25	92.48	489.06	12.4	$28^0.44'$	0.625	0.714
F5	76.89	16.24	20	81.26	494.6	17.7	$30^0.25'$	0.625	0.76
F6	68.37	11.61	16.66	69.73	487.73	14.76	$30^0.57'$	0.71	0.833
F7	77.55	22.15	25	88.67	508.46	13.15	$32^0.27'$	0.66	0.76
F8	76.05	15.58	20	77.97	528.66	26.33	$31^0.75'$	0.526	0.714
F9	74.61	11.17	16.66	67.06	501.8	6.57	$29^0.74'$	0.71	0.76

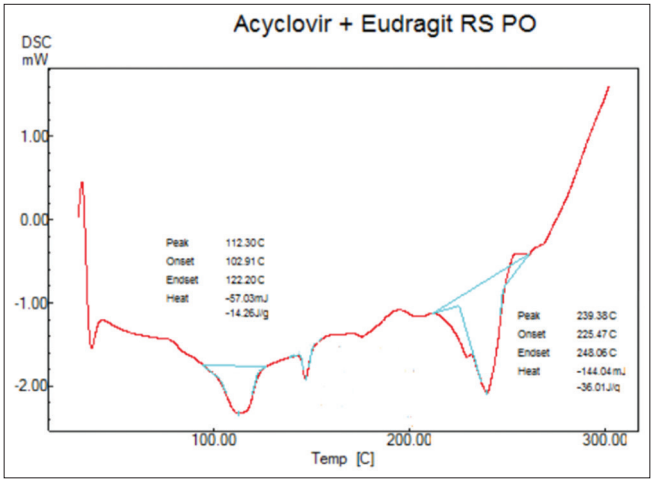
64.47%–92.48%. Formulation containing ratios 1:1 shows an increased entrapment compared to other formulations. The results are shown in Table 2.

Dissolution studies of all the formulations were carried out using dissolution test apparatus USP XXIII-basket Type I. The dissolution studies were conducted using dissolution media pH 1.2 and pH 6.8. The formulations F1, F4, and F7 containing 1:1 ratio showed the maximum release 98.4%, 98.2%, and 97.20%, respectively, compared to other formulations at 12 h. This indicates that the amount of drug release decreases with an increase in polymer concentration. Further, these drug releases were subjected for mathematical analysis to check which type of release kinetics the optimized formulation followed. The values of coefficient of correlation were found to be the best fit in Hixson-Crowell model followed by zero-order and first-order. Graphs 1-8 show the formulation graph of drug release profile and Tables 3 and 4 show drug release kinetics.

Selection of optimized formulation was based on dissolution method and the release optimized formula. Further, they were evaluated for different particle size by sieve analysis method and optical microscopy following separation of the microspheres. The selected microspheres average particle size (µm) is 509.8, 450.8, 313.8 were assessed for drug entrapment and dissolution were resulted as 85.69%, 96.27%, and 98.05% respectively. The rate of the drug release was found to decrease with increasing sphere size. Graph 1 shows the graph for drug release based on particle size.

Morphology of the microparticles investigated by SEM showed the particle size in the range 500 µm–600 µm. The Figures 1 and 2 show SEM of microspheres.

Optimized formulation F1 was subjected to a stability study at 40°C±2°C and 75% ± 5% RH for 30 days. The samples



Graph 8: Digital scanning calorimeter spectra of optimized formulation F5

Table 3: Drug release kinetics of acyclovir microspheres

Formulation code	Zero-order kinetics		First-order kinetics		Higuchi		Peppas			Hixson			Best fit model
	R	K	R	K	R	K	R	K	N	R	K	N	
F1	0.9893	3.4539	0.9978	2.0011	0.9607	5.1867	0.9539	6.0944	62.614	0.9987	0.0204	0.1894	Hixson-Crowell
F2	0.985	1.7886	0.9775	1.9993	0.9267	3.723	0.9067	3.99	41.738	0.9806	0.0124	0.1174	Zero-order
F3	0.9938	0.7864	0.9841	2.0073	0.8783	4.4313	0.9346	0.7671	32.453	0.9881	0.0208	0.0852	Zero-order
F4	0.9932	2.46	0.9922	2.0189	0.9447	7.5203	0.9661	5.5159	75.983	0.9985	0.0208	0.2446	Hixson-Crowell
F5	0.9887	1.2064	0.9775	0.20016	0.9191	4.0244	0.9123	3.3383	40.848	0.9829	0.0039	0.1139	Zero-order
F6	0.9942	0.3579	0.9871	2.0027	0.9086	3.7941	0.9243	2.0673	33.867	0.9903	0.0038	0.906	Zero-order
F7	0.9843	5.3721	0.9917	2.0085	0.9692	5.5388	0.9441	8.6951	76.594	0.9975	0.0217	0.2574	Hixson-Crowell
F8	0.9783	2.6582	0.9712	1.9961	0.9369	3.3022	0.8947	5.0109	43.349	0.9759	0.0248	0.1241	Zero-order
F9	0.9905	1.0232	0.9841	2.0008	0.9191	3.6934	0.9134	2.959	36.974	0.9873	0.0044	0.1011	Zero-order

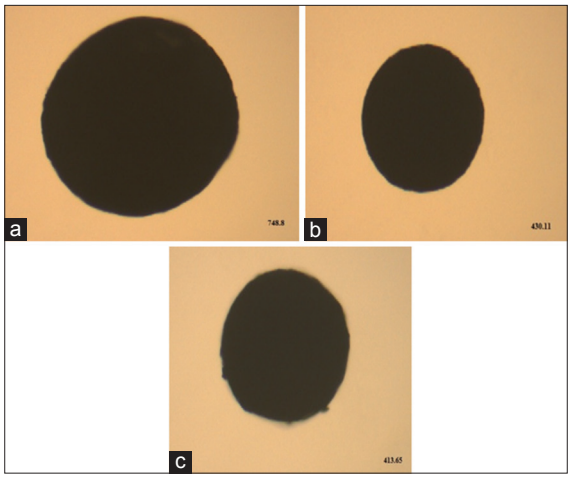


Figure 1: Particles sizes using electronic microscope. (a) Particle size in sieve no-22, (b) Particle size in sieve no-44, (c) Particle size in sieve no-60

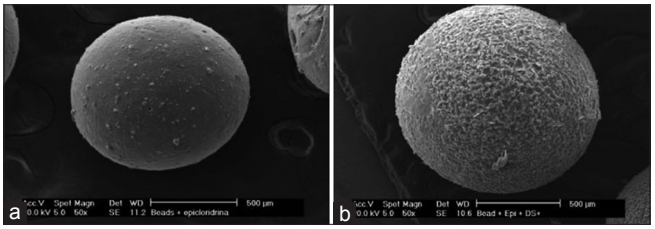


Figure 2: Scanning electron microscope (SEM) of microsphere. (a) SEM before dissolution, (b) SEM after dissolution

were analyzed for drug content, and drug release study carried out at different time intervals such as 15 days and 30 days. There was a very slight change in drug content as well as drug release.

CONCLUSION

The drug acyclovir was selected for the study, due to its poor bioavailability, proved activity, and better clinical applications. The compatibility study of FTIR and DSC thermogram revealed that there was no interaction between the drug and the polymer Eudragit RSPO and Eudragit RLPO. Oral controlled release microspheres of acyclovir can be prepared by a non-aqueous solvent evaporation method using polymers polymethacrylates (Eudragits). The flow property and micromeritic studies of the prepared formulation were evaluated and found to show good flow properties and micromeritic studies as per the standard rate flow properties. The particle size was in the range <1000µm in which the selection of optimized formulation was taken based on drug release where the rate of the drug release was found to decrease with increasing sphere size. The *in vitro* drug release study also indicates that the amount of drug release decreases with an increase in polymer concentration. The drug showed good entrapment with the different

Table 4: Selection of optimized formulation based on drug release

Sieve no	Mean particle size	Drug content (mg)	Entrapment efficiency	Withdrawal volume (ml)	Amount of microspheres for dissolution
22	749.8	20.9	83.6%	0.4 ml	478.46
44	450.8	23.025	92.1%	0.4 ml	430.107
60	313.8	24.175	96.7%	0.4 ml	413.65

ratios of polymer concentrations. The release of drug from microspheres was studied using release kinetics were the study indicates a good fit of Hixson-Crowell Model. The accelerated stability of the microspheres was studied after 30 days in which it showed no interaction based on temperature, humidity, and light.

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