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Formulation and Evaluation of Mucoadhesive Microspheres of Acyclovir: Optimization Of Process Parameter.

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ABSTRACT

The aim of the present work was to prepare and evaluate mucoadhesive microspheres of acyclovir. The objective of the present work was to increase bioavailability, drug entrapment efficiency and drug stability. Acyclovir has low bioavailability that is 15% to 20%. Microspheres were formulated using sodium alginate (2%) with mucoadhesive polymercarbopol940 andco-polymer HEC, retarding agents and 2% of Calcium chloride (CaCl2) as cross linking agents by employing Ionic Gelation Technique. The surface morphology was characterized for by scanning electron microscopy (SEM) and drug excipients compatibility was determined by FT-IR spectroscopy. The particle size was characterized by optical microscopy. Percentage drug content, Entrapment efficiency, Swelling index, In-vitro wash off test and in-vitro dissolution studies were also carried out. Among the prepared microspheres (F9) formulation in which calcium chloride was used as cross linking agent, portray better sustained release for more than 9hrs. SEM shows that prepared microspheres were of spherical in shape and free flowing. FT-IR results showed compatibility of acyclovir with excipients used, X- ray diffraction showed crystalline nature of acyclovir, DSC showed melting point of pure drug.

Keywords: Microsphere, FT-IR Spectroscopy, SEM analysis, X-ray diffraction, DSC analysis

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INTRODUCTION

The focus of pharmaceutical research is being steadily shifted from the development of new chemical entities to the development of novel drug delivery system (NDDS) of existing drug molecule to maximize their effective in terms of therapeutic action and patent protection. The development of NDDS has been made possible by the various compatible polymers to modify the release pattern of drug. In the recent years the interest is growing to develop a drug delivery system with the use of a mucoadhesive polymer that will attach to related tissue or to the surface coating of the tissue for the targeting various absorptive mucosa such as a ocular, pulmonary, buccal, vaginal etc. This system of drug delivery is called as mucoadhesive drug delivery system. Mucoadhesive is defined as the ability of a material (mucoadhesive polymer) to adhere to the mucosal layer. They are held together for an extended period of time by interfacial forces

Mucoadhesion is a complex process and numerous theories have been proposed to explain the mechanisms involved. These theories include Wetting theories, Diffusion theory, Fracture theory, Electronic theory, Adsorption theory.

The mechanism of mucoadhesion is generally divided into two steps: the contact stage and the consolidation stage. The first stage is characterized by the contact between the mucoadhesive and the mucus membrane, with spreading and swelling of the formulation, initiating its deep contact with the mucus layer. In the consolidation step, the mucoadhesive materials are activated by the presence of moisture[1, 15].

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MATERIALS AND METHODS

Materials:

Acyclovir was purchase from Sava pharmaceutical's private limited, Pune. Sodium alginate (Low viscosity), Carbopol 940, Hydroxy ethyl cellulose, Calcium chloride, Sodium hydroxide, Hydrochloric acid, Disodium hydrogen phosphate, Potassium dihydrogen phosphate and Sodium chloridewere purchased from Loba Chemie Pvt. Ltd., Mumbai.

Method for preparation of mucoadhesive microspheres:

Ionotropic external gelation technique:

The drug is added to 0.1N Sodium hydroxide solution of sodium alginate, HEC, Carbopol940 then final volume fill up to 100ml. In order to get the complete solution stirring is continued at 120 rpm in magnetic stirrer.After that the drug polymers solution added to 2% aqueous solution of calcium chlorideby using 24 gauze needles by continuous stirring at 120 rpm. After that filter the solution by using whatman filter paper and microspheres dry at 37^0C, composition of formulation as shown in table 1 [4-7].

Table 1: Composition of formulation by using 3*2 factorial designs (F1-F9)

Ingredients Used	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug(mg)	200	200	200	200	200	200	200	200	200
Sodium alginate(mg)	2000	2000	2000	2000	2000	2000	2000	2000	2000
Carbopol940	500	900	500	900	500	100	100	100	900
HEC(mg)	500	500	900	900	100	100	500	900	100
Calcium chloride (%)	2	2	2	2	2	2	2	2	2

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RESULTS AND DISCUSSION

Formulation and optimization using central composite design (CCD)

The experimental results of CCD are reported in table 2. All the data were computed by design expert software (Version 7.1.3).The four responses were fitted to quadratic second-order polynomial model. The model, which shown a lesser P values (≤ 0.05) and greater F values was identified as the fitting model. This finding has supported that the formulation factors had significant effect on the responses.

Table 2: Experimental runs with results of response

Formulation code	Carbopol940(mg) X_1	HEC(mg) X_2	Entrapment Efficiency(%) Y1	Swelling index (%) Y2	Particle size (µm) Y3
F1	500	500	89.89	0.77	45.23
F2	900	500	92.34	0.94	43.31
F3	500	900	82.76	0.92	60.32
F4	900	900	93.67	0.87	42.54
F5	500	100	87.78	0.89	38.36
F6	100	100	80.89	0.72	23.25
F7	100	500	72.23	0.81	32.23
F8	100	900	77.56	0.87	32.45
F9	900	100	91.78	0.95	53.87

The polynomial equation obtained for response Entrapment efficiency (Y_1), Swelling index (Y_2), Particle size(Y_3) as follows,

$Y_1 = +0.79+0.060X_1-0.017X2-0.058X_1X_2+ 0.029X_1^2-0.059X_2^2$(1)
$Y_2=+ 78.24+0.27X_1-4.46X_2– 0.082X_1X_2+10.13X_12-2.745X_2^2$(2)
$Y_3= +45.76+8.63X_1+3.30X_2–5.13X_1X_2-9.32X_1+2.25X_2^2$(3)

Keywords- X_1= Concentration of carbopol (Independent variable)
X_2= Concentration of HEC (Independent variable)
Y_1, Y2, Y3= Dependent variables

For assessment of mucoadhesive microspheres, entrapment efficiency and particle size plays critical role. The increase in a carbopol concentration a larger interfacial area for drug absorption and also percentage of mucoadhesion increases.

Table 3 shows the results of the analysis of variance (ANOVA), which were used to generate mathematical models. The high values of correlation coefficient for percentage drug entrapment efficiency, swelling index and particle size indicate a good fit that is good agreement between the dependent and independent variables. The equations may be used to obtain estimates of the response as a small error of variance was noticed in the replicates. The model F-value of 4.81 for percentage drug entrapment efficiency,4.45 for swelling index and 5.61 for particle size suggest that these models are significant. There is only 1.14% and 2.45% chances that a 'Model F-value' this large could occur due to noise. Values of 'Prob>F' less than 0.0500 indicate model terms are significant. In this case all the models generated response parameters are significant. PRESS (Predicted Residual Sum of Squares) is a measure of how well the model fits each point in the design. Smaller the PRESS statistic, the better the model fits the data points. Small values for the same in these models show a good fit of the data points [8, 9, 10].

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Table 3: ANOVA results of measured responses

ANOVA RESULT OF THE MEASURED RESPONSE				
		MUCOADHESIVE MICROSPHERES		
ANOVA	Coefficient	Y_1	Y_2	Y_3
	F value	4.81	4.45	5.61
	P value (Prob > F)	0.031	0.038	0.0215

Response surface plots

Eq. no.1 indicates that X_1 (Concentration of Carbopol 940) has positive effect, - sign of X_2 indicates that factor X_2 (concentration of HEC) has negative effect on Y_1 (% Drug entrapment efficiency). This indicates that the percentage drug entrapment increases with increase in Carbopol 940 Concentration.F6, F7, F8 formulation contains lowest amount of carbopol (100 mg) shown lowest drug entrapment as compare to other(80.89%, 72.23%, 77.56%) whereas F2, F4, F9 formulation shows highest amount of carbopol (900 mg) shown higher Value for percentage drug entrapment (92.34%, 93.67%, 91.78%). This is because carbopol 940 is a polymer which is known to form viscous colloidal solution which causes more entrapment of drug in the formulation. (Figure 1)

Eq. no.2 indicates that factor X_1 (Concentration of carbopol 940) has positive effect,- sign of X_2 indicates that factor X_2 (concentration of HEC) has negative effect on Y_2 (Swelling index).This indicates that the Swelling index increases with increase in increase in carbopol concentration(Figure 2).

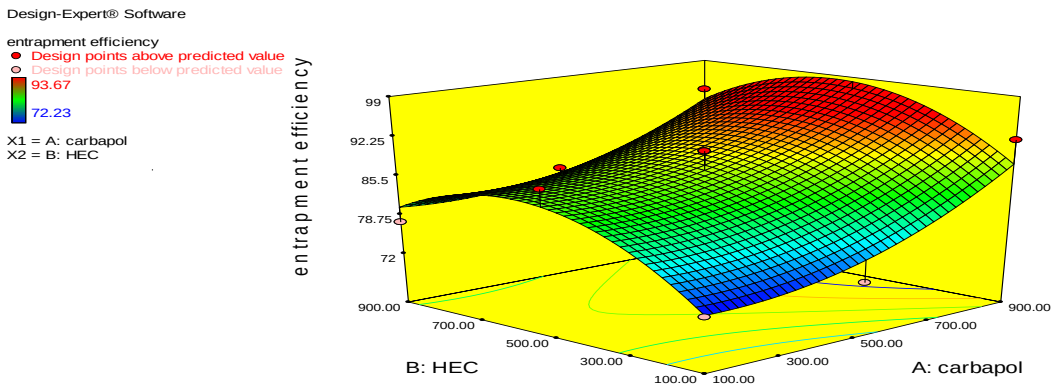

Figure 1: Response surface plot for entrapment efficiency

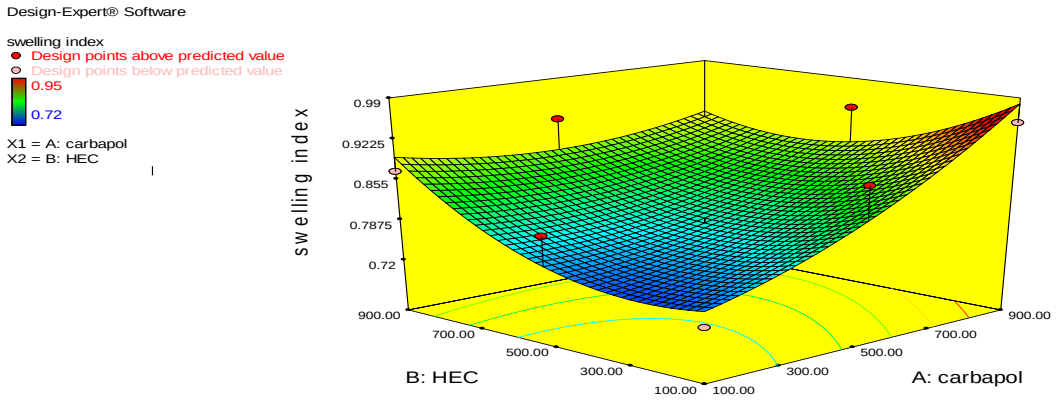

Figure 2: Response surface plot for swelling index

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Eq. no.3 indicates that factor X_1 (Concentration of carbopol 940) has positive effect,- sign of X_2 indicates that factor X_2 (concentration of HEC) has negative effect on Y_3 (Particle size).This indicates that the particle size increases with increase in increase in carbopol 940 concentration and decreases with increase in HEC concentration (Figure 3) [11].

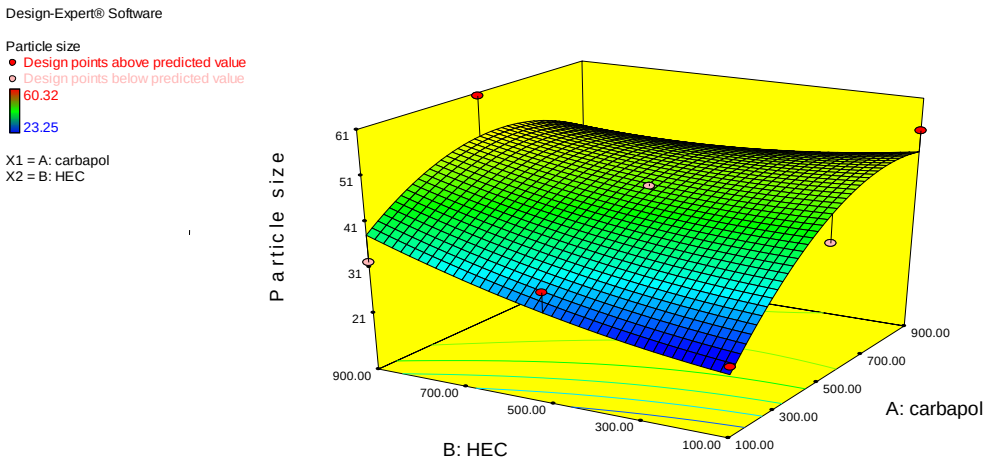

Figure 3: Response surface plot for particle size

Validation of central composite factorial design

Developed central composite factorial designs were validated by setting targets for drug entrapment efficiency, swelling index and particle size. The experimental values and predicted values of each response are shown in table 4. The percentage relative error of each response was calculated using the following equation:
Percentage Relative Error = (| Predicted value – Experimental value | / Predicted value) ×100

Table 4: Validation of central composite factorial design (Acyclovir)

Batch	Carbopol 940	HEC	%Entrapment efficiency		Swelling index	
			Predicted value	Experimental value	Predicted value	Experimental value
F9	900	131.47	93.01%	91.78%	0.93	0.95

Batch	Carbopol 940	HEC	Particle size(µm)	
			Predicted value	Experimental value
F9	900	131.47	47.31	53.87

Differential Scanning Calorometry analysis

DSC analysis of Acyclovirshowed the endothermic peak at its melting point that at 257°C (Figure 4) Enthalpy of peak is 15.35mw. Thus it can be proved that the endothermic peak obtained is melting peak which is sharp and it indicates purity of drug.

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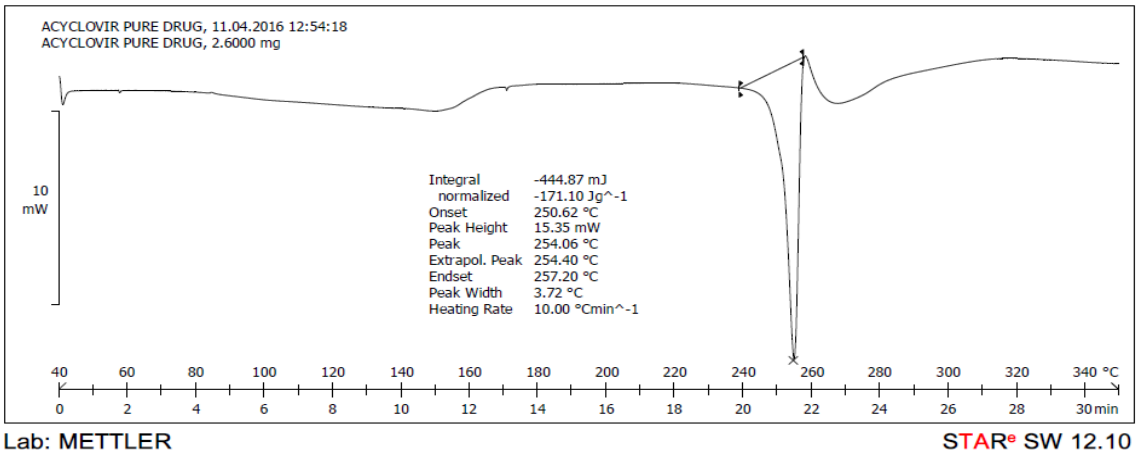

Figure 4: DSC thermogram pure acyclovir

DSC thermo grams of pure drug and formulation revealed that there is no considerable change observed in melting endotherm of acyclovir pure drug (257.9^{0}C) and polymer in optimized formulation (180.45^{0}C) which is shown in figure 5. It indicates that the drug has transformed from crystalline to amorphous state and completely solubilized into the formulation.Our result shows the shift of endothermic peak of the drug in thermo gram of mucoadhesive microspheres supports the presence of acyclovir in an amorphous form in formulation.

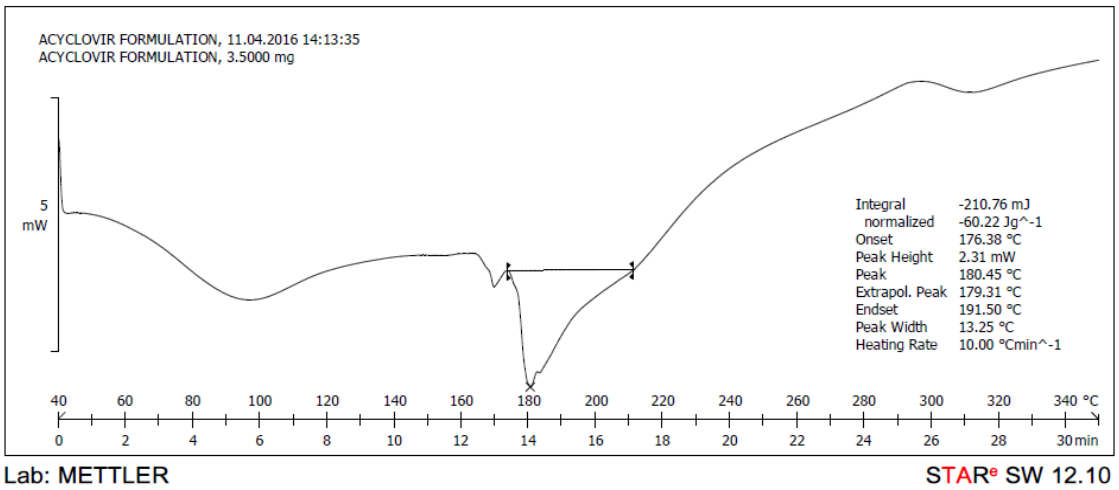

Figure 5: DSC thermogram of Acyclovir formulation

X-ray diffraction study

Powder X-ray diffraction study reveals information about the crystallographic structure and composition of materials and used to study the peaks which indicate purity of the material. In below figures 6 and 7 x-ray diffraction physical mixture and formulation (F9) is carried out. The sharp peaks indicate purity of the drug and the physical mixture. The Bragg reflection angle, (2Θ), along with the inter-polar spacing d, and the relative intensity of the peaks were calculated. PXRD graph were shown in figure 27 andfigure 6 it had been confirmed polymer mixture are crystalline nature and formulation (F9) (Figure 7) were in amorphous state.

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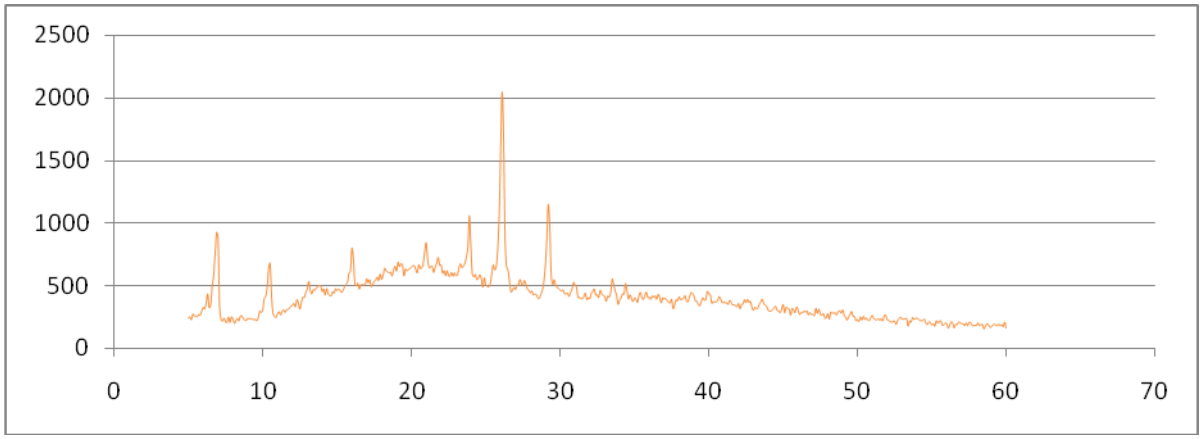

Figure 6: XRD spectra of drug-polymers mixtures

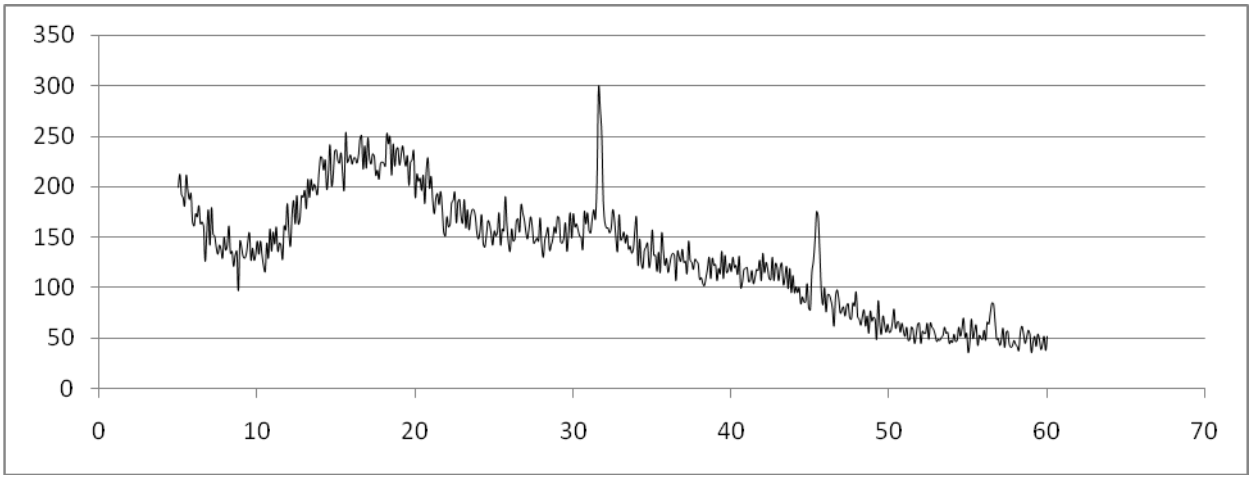

Figure 7: XRD spectra of formulation (F9)

Percentage drug content determination

The percentage drug content in each batch of mucoadhesive microspheres were analyzed spectrophotometrically. Increase in concentration of polymer in the internal phase leads to increase in size of microspheres and also increases drug content.Highest percentage drug content was observed in F9 formulation as shown in table 6.

Table 5: Percentage relative error

Sr.No.	Dependent variables	Percentage relative error
1	% Entrapment efficiency	1
2	Swelling index	-2
4	Particle size	1.85

Table 6: Percentage drug content, Percentage entrapment efficiency, Average swelling index, Particle size.

Formulation Code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Percentage drug content*	87.59± 0.27	87.36± 0.20	85.56± 0.23	82.68± 0.19	90.66± 0.24	88.63± 0.28	85.48± 0.35	81.40± 0.26	92.44± 0.23
Percentage entrapment efficiency*	89.89± 0.26	92.34± 0.26	82.76± 0.16	93.67± 0.030	87.78± 0.015	80.89± 0.035	72.23± 0.25	77.56± 0.28	91.78± 0.070

Average swelling index*	0.77± 0.02	0.94± 0.01	0.92± 0.01	0.87± 0.032	0.89± 0.03	0.72± 0.01	0.81± 0.03	0.87± 0.01	0.95± 0.01
Particle size*(µm)	45.23± 0.32	43.31± 0.30	60.32± 0.28	42.54± 0.29	38.36± 0.29	23.25± 0.35	32.23± 0.32	32.45± 0.34	53.87± 0.32

*** Indicates Average ± SD (n = 3)**

Percentage drug entrapment efficiency

Drug entrapment efficiency in different formulations was estimated by the U V Spectrophotometric method. Percent drug loading efficiency ofmicrospheres was found in the range of 72.23 to 93.67% (Table 6). Formulation F2, F4 and F9 containing blend of sodium alginate and Carbopol showed maximum % drug loading about 92.34%, 93.67%, 91.78 respectively. Because these microspheres has larger size as compared to other formulations. Whereas formulation F7, F8, containing low carbopol concentration showed the minimum % drug loading about 72.34%, 77.56 because these microspheres are small in size, which results more loss of drug from surface during washing of microspheres. Increase in polymer concentration of internal phase also increases in drug entrapment of microspheres. Higher concentration of the polymer increases the viscosity of the medium as well as greater availability of Calcium and polymeric chains, as a result cross linking agent is increased, and larger droplets were formed entrapping a greater amount of drug. The entrapment efficiencies were higher for microspheres prepared with calcium chloride; it may be attributed to the amount of cross linking agent and also the higher density of calcium chloride. Thus it is inferred that there was a proper distribution of Acyclovir in the microspheres [13].

Swelling index

The results revealed that all microsphere formulations swelled rapidly when immersed in phosphate buffer (pH 6.8). The liquid enter the particles through pores and bind to large molecule, breaking the hydrogen bond and resulting in swelling of particles. It isreported that adhesive properties and cohesiveness of mucoadhesive polymers are generally affected by their swelling behaviour.The percent swelling of different microsphere formulation was found in the range0.72 to 0.95. It was observed that carbopol microspheres swelled slowly and produced higher mucoadhesive strength. This is perhaps because slow swelling avoids the formation of over hydrated structure that loses its mucoadhesive properties before reaching the target. On the other hand, the highest swelling observed in microspheres of Carbopol 940 could be due to its high ionization at pH 6.8, which is capable of absorbing a high amount of water.The degree of swelling of all the formulations was shown in table6. The results revealed that all formulations showed rapid swelling, when immersed in PBS pH 6.8. The adhesive and cohesive properties are generally affected by their swelling behavior. The degree of swelling of optimized formulations F9 was found to be 0.95±0.01. It was found that with increase in carbopol concentration,swelling index of microspheres was found to be increased [13, 14].

Particle size analysis

From the results obtained it was observed that higher concentration of polymer increases the viscosity of the medium, which increases the particle size of the microspheres.Particle size analysis of different formulations was done by optical microscopy. The average particle size was found to be in the range of 23.25±0.35µm to 53.87±0.32µm. The mean particle size was significantly varied according to type of polymer used for the preparation of microspheres; this may be due to the fact that difference in the viscosity of the polymer solution. Since high viscosity of the polymer solution requires high shearing energy for breaking of droplets of the emulsion. Microspheres containing carbopol concentration high are larger as compared to other microspheres because carbopol solution has more viscosity at the same concentration. Particle size decreased with increase in volume of continuous phase due to the fact that increased in continuous phase, more efficiently utilized the energy produced by stirring, which leads to further decrease in droplet size of internal phase. Increase in concentration of polymer in the internal phase leads to increase in size of microspheres because at a higher concentration polymer solution have more viscosity which requires more energy to breaking the droplets of dispersed phase. The particle size decreases with increase in stirring speed that is 120rpm speed produced desired size of microspheres. Results of particle size analysis are shown in table 6 [13].

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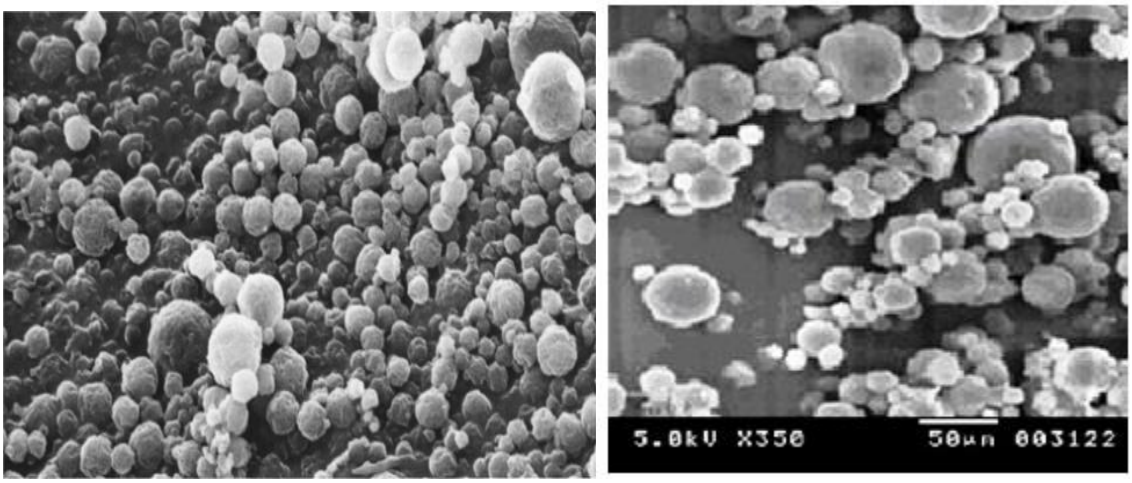
Morphological study by Scanning Electron Microscopy

The scanning electron micrographs of the mucoadhesive microspheres prepared with carbapol 940, HEC and sodium alginate appeared as spherical smooth surfaced particles (Figure8) [13].



A B

Figure 8: SEM image of mucoadhesive microsphere

In-vitro wash-off test for mucoadhesive microspheres

In-vitro wash off test was carried out to ensure the adhesion of the formulation to the mucosa for prolonged period of time at the absorption site, indicates in-vitro percentage mucoadhesion after 1hr it reveals that the microspheres possess good mucoadhesive properties. The combination of the Carbopol-HEC-Sodium alginate increases the viscosity of the microsphere produce more viscous gel, which leads to increase in adhesion to the intestinal mucosa. The prepared microspheres (F9) showed 91.23%. Mucoadhesion after 1hr then it was decreases up to 8 hr that is 23.12%. Hence it shows that the drug released from the microspheres is in a controlled manner before being eroded off. It was found that the percentage mucoadhesion is increased with increase in concentration of mucoadhesive polymer that is carbopol. The results were shown in table 7 [13, 14].

Table 7: Percent of microspheres adhering to tissue at different times Interval in (hrs.)

Formulation code	% Mucoadhesion (hrs)*				
	1 hr	2 hr	4 hr	6 hr	8 hr
F1	84.34±0.9	67.47±2.8	41.13±1.4	23.04±0.2	9.28±1.23
F2	90.23±1.2	74.32±0.9	58.25±1.23	43.23±0.23	18.02±2.12
F3	76.76±1.18	52.62±0.7	30.12±1.56	15.12±1.3	2.23±0.81
F4	83.67±0.7	69.23±1.5	48.58±1.1	29.10±2.4	14.23±0.27
F5	80.34±2.3	64.87±1.1	39.23±0.8	23.12±3.1	9.67±0.89
F6	60.67±2.4	41.10±1.2	21.12±0.45	12.58±0.21	1.89±0.1
F7	68.33±1.16	46.67±0.8	23.12±0.23	13.12±1.12	1.23±0.2
F8	62.23±1.27	39.12±2.4	18.81±0.89	9.56±1.23	1.78±0.11
F9	91.23±0.9	82.76±3.1	59.12±2.12	41.23±1.55	23.12±0.8

*** Indicates Average ± SD (n = 3)**

In –vitro drug release study

To understand the characteristics of drug release from mucoadhesive microspheres, an in vitro release study was carried out.The effect of various polymers and its concentration along with the cross linking agents were studied for the release profile of prepared microspheres of acyclovir. The release mainly depends on the type of polymer, its concentration and viscosity. The results were shown in Table 8. The results indicate that

the drug released from formulation (F9) is in the range of 87.121±0.12% to 97.12±0.11 up to 9 hours. Hence formulation (F9) was chosen as a better formulation to retard the release for the high soluble drug acyclovir. The release was dependent on amount of polymer added and it is also affected with cross linking agent. The amount of polymer sodium alginate and cross linking agent calcium chloride was selected for further retarding the release of the acyclovir. The in-vitro release profile for all the prepared microspheres is shown in Figure 9. The cross linking agent calcium chloride has interaction with $Ca2^{+}$,Cl^{-} ions are bound to Na ion of sodium alginate which enables to retard the release from microspheres. Similarly the F2 shows the amount of drug release of 90.02±0.11% in 9 hours [7,8,9].

Table 8: In-Vitro Drug release

Time (Min.)	F1	F2	F3	F4	F5	F6	F7	F8	F9
5	0.823	5.536	3.23	4.34	4.78	3.23	2.34	5.87	6.45
10	1.65	9.44	7.34	8.21	12.78	6.26	7.89	13.56	8.34
15	3.526	13.67	10.34	11.56	13.98	10.23	11.76	13.76	14.89
30	6.69	16.28	13.14	14.78	18.23	13.32	11.24	19.34	18.67
45	9.265	18.89	14.23	15.23	21.56	12.37	12.37	21.34	20.33
90	11.98	25.72	23.12	24.23	25.78	21.37	19.89	26.46	27.34
120	15.16	34.12	33.23	32.12	26.33	30.45	27.45	27.43	36.46
180	19.74	42.23	40.12	39.48	31.98	35.54	32.18	33.45	43.34
240	22.56	48.34	45.67	44.23	42.23	42.22	39.78	45.78	50.89
300	27.5	57.34	52.34	54.12	49.89	55.43	52.23	51.34	61.23
360	34.53	72.32	67.34	68.32	61.23	69.67	65.78	64.34	72.45
420	42.23	83.23	72.23	73.45	72.12	78.89	73.45	75.43	87.12
480	38.23	90.02	73.89	72.23	82.25	83.56	79.45	82.23	92.12
540	34.23	87.23	70.22	71.89	76.34	79.45	76.23	79.45	89.34

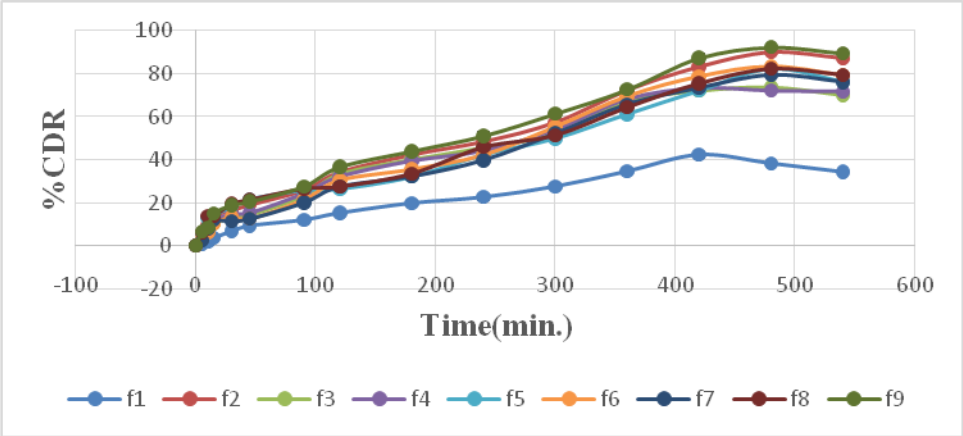


Figure 9: In-vitro Drug release

Accelerated stability study

Accelerated stability study of formulation F9 formulation as shown in table 9.The stability study of the formulation (F9) was performed after 3 months and the effect on thevarious parameters was studied the microspheres (F9) after 3 months showed good physical appearance, drug encapsulation efficiency is same as that of initial. After 3 months the formulation (F9) under stability study was assayed and found to be the same. The In-vitro drug release profile studies were performed after storage for 3 months at 40±2°C/75±5%RH, In-vitro release studies showed that there was no much difference in the drug release of formulation and it is stable. The result obtained is shown in figure 10 [6, 7, 8].

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Table 9: Accelerated stability study

Month	Swelling index	Drug content	Drug entrapment efficiency
0 Month	0.95 ±0.01	92.44±0.23	91.78±0.070
1 Month	0.96 ±0.05	92.45±1.151	91.67±0.87
2 Month	0.96±0.02	91.17±1.039	90.45±1.02
3 Month	0.95±0.03	92.39±0.632	91.43±1.04

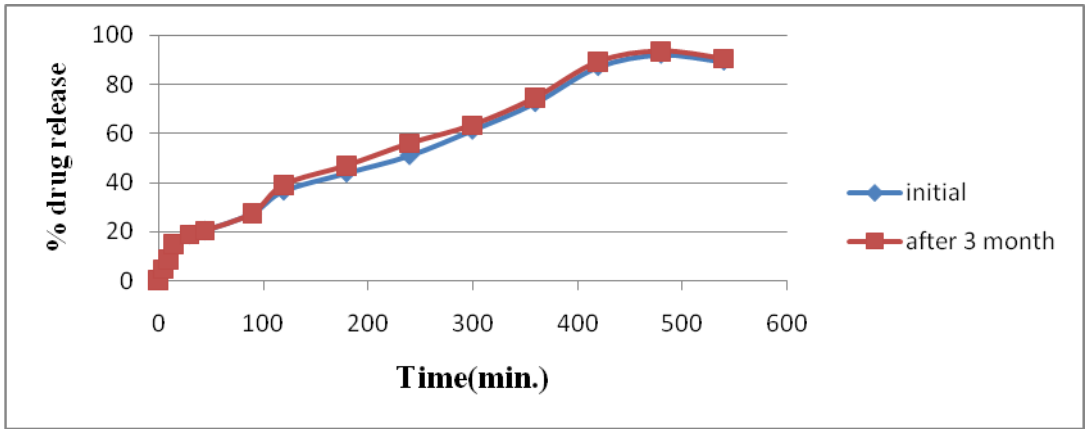

Figure 10: Comparison of In-vitro drug release of formulation (F9) initially and after 3 months storage.

CONCLUSION

- In this study, the Mucoadhesive microspheres of acyclovir were successfully formulated in an attempt to increase its bioavailability, stability and dissolution rate. The central composite designs (CCD) were successfully employed as optimized tool to optimize mucoadhesive microspheres.
- Ionotropic external gelation method was found to be useful technique for formulation of mucoadhesive microspheres which produced uniform and free flowing microspheres of acyclovir mucoadhesive microspheres.
- The SEM analysis, DSC measurement and X-ray diffraction analysis suggested that acyclovir is present in the dissolved state in mucoadhesive microspheres formulation.
- In-vitro wash-off test suggested that acyclovir stay longer time contact to intestinal mucosa to increase drug bioavailability and half-life.
- In-vitro dissolution test showed that the mucoadhesive microspheres had a higher in-vitro release rate than drug powder. A significant improvement in dissolution is obtained for the drug. Mucoadhesive microspheres for acyclovir holds promise to be developed as useful oral dosage form.
- Hence above studies can be further supported with animal studies.

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