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Design, Development and Evaluation of Antiviral Drug Loaded Microcapsules Prepared by Microencapsulation Technique

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ABSTRACT

The aim of the present research work is to develop microcapsules of an antiviral drug acyclovir. Acyclovir microcapsules with a coat consisting of sodium alginate and polymers such as HPMC E15 and HPMC E50 were prepared by an ionotropic gelation technique, where gelation was achieved with oppositely charged counter ions to form microcapsules. The percentage yield, drug entrapment and drug content of all formulations were good. The microencapsulation efficiency of all the formulations was in the range of 58.91 to 98.74%. The swelling indexes of microcapsules were found satisfactory. The formulations released acyclovir in a controlled manner for a prolonged period over 8 to 10 hours. It was observed that microcapsules prepared using HPMC K15 in 1:3 ratio exhibited the best release profile and able to sustain the drug release for 10 h. The selected formulations were subjected to stability studies at different temperature and humidity conditions as per ICH guidelines. The drug release data was evaluated for release kinetics using zero order and first order kinetic equations and the data was also fitted into mathematical models such as Higuchi, Korsmeyer-Peppas to determine the mechanism of the drug release and it was found to be by non-Fickian diffusion.

Keywords: Acyclovir; Hydroxy propyl methyl cellulose; Ionotropic gelation technique; Microencapsulation; Sodium alginate

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INTRODUCTION

In spite of its popularity, the oral route has some inherent problems that limit its use for administration of sustained release systems. Most critical among these is the total residence time of the dosage form in the GIT. Considering the gastric emptying rate and the transit times in the small and large intestines, the maximum duration in the GIT is approximately 24 hours. Moreover, there are large differences among patients in respect of gastric emptying rates, pH effects, enzyme activity and intestinal motility. Another complicating factor is the preferential absorption of some drugs from certain segments of the GIT. If the sustained release system releases its active ingredient after passage through these areas, absorption of the drug may be seriously impaired. Furthermore, if the gut wall and/or the first-pass liver metabolism are significant, the release rate of the dosage form can have strong effect on the amount of drug that reaches the peripheral circulation unchanged. Because of the above limitations, at present, the residence time of sustained release dosage forms cannot be extended beyond 10 to 12 hours. Nevertheless, the oral route is still the most convenient and common mode of administration of sustained release system[4].

Oral dosage forms of antiviral drugs like acyclovir having short half-life are required to be administered around four to five times a day during the course of treatment. In addition, long term administration of this drug is required in some clinical conditions like relapsing herpes simplex infection. Frequent dosing is required to maintain therapeutically effective blood concentration. This results in pill burden and consequently leads to patient non-compliance. It is well known that patient compliance is better when the drug dosing is only once or twice daily. It has been reported that, as the number of doses per day increases, there is a greater risk that the patient will either forget or neglect to take every dose. The available oral dosage forms of acyclovir include tablets, capsules and suspensions. The disadvantages associated with these conventional preparations are low bioavailability, inconvenient frequent dosing regimen (4-5 times) because of short half-life (less than four hours) which results in poor patient compliance and increased chances of missing the dose of a drug.

Hence there is a need to formulate acyclovir loaded microcapsules for prolonged release. This can be a promising aspect to improve patient compliance towards oral therapy of acyclovir. Prolonged drug release can be achieved in the form of microcapsules that can reduce the frequency of dosing and also increase the bioavailability. Microcapsules are essentially discontinuous microspheres where the active core material is completely covered with a non-active surface coating. The coating thickness may be varied depending on the characteristics desired in the final product. With the vast array of encapsulation techniques currently available, nearly any active agent can be successfully incorporated into a microparticulate formulation [5-7].

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MATERIALS AND METHODS

Reagents and chemicals

Acyclovir was procured from Yarrow Chem Products, Mumbai, India. Sodium alginate and hydroxyl propyl methyl cellulose (HPMC-E15 and HPMC E-50) were purchased from Loba Chemie, Mumbai, India. All other reagents used were of analytical grade.

Preparation of acyclovir loaded microcapsules

In the ionotropic gelation method, coating material sodium alginate (alone and also in combination with HPMC-E15 or HPMC E-50 polymers) was dispersed in distilled water under continuous stirring for 30 minutes to form a homogenous solution. The weighed amount of the core material acyclovir was added to the sodium alginate solution and mixed thoroughly to form a viscous dispersion. The dispersion was allowed to stand for few hours to remove air bubbles. The resulting dispersion was added drop wise into 250ml calcium chloride solution (10%w/v) through a syringe fitted with a needle of 21 gauge and stirred for 15 minutes. The added droplets were retained in the calcium chloride solution for 3 hours to complete the curing reaction and to produce spherical rigid microcapsules. The microcapsules were collected by filtration and the product thus produced was washed repeatedly with water and dried at 45^0C for 8 hours in hot air oven. Microcapsules in the ratio 1:1, 1:2 and 1:3 were prepared and subsequently stored in a dessicator over fused $CaCl_2$ until further study[3]. The composition of various formulations is given in table 1.

Table 1: Composition of various formulations

INGREDIENTS (mg)	CODE RATIO	F1 1:1	F2 1:1	F3 1:1	F4 1:2	F5 1:2	F6 1:2	F7 1:3	F8 1:3	F9 1:3
Acyclovir		100	100	100	100	100	100	100	100	100
Sodium alginate		100	50	50	200	150	150	300	200	200
HPMC E15		-	50	-	-	50	-	-	100	-
HPMC E50		-	-	50	-	-	50	-	-	100

Table 2: Characterization of formulated acyclovir microcapsules by different techniques

Formulation code	Particle size (µm)	Angle of repose (θ)	Carr's index (%)	Hausner ratio	% yield	% Entrapment efficiency	Drug content (mg)
F1	791.90	24.30	9.30	1.10	91.45	74.86	37.43
F2	828.59	24.78	10.23	1.11	87.56	64.42	32.21
F3	889.89	25.38	10.66	1.12	90.33	58.91	29.46
F4	796.78	22.61	8.20	1.09	97.45	98.74	32.91
F5	834.97	28.88	9.91	1.11	81.34	66.85	22.28
F6	917.64	29.47	10.78	1.12	82.49	59.08	19.69
F7	812.83	26.38	11.97	1.14	80.67	72.17	18.04
F8	866.31	21.32	7.84	1.09	95.76	97.49	24.37
F9	918.34	22.22	8.21	1.09	96.45	94.67	23.67

Evaluation of microcapsules

Particle size determination

Particle size analysis was done by sieving method using Indian standard sieves ≠ 10, 12, 16, 22, 28, 44. Weighed quantity of the microcapsules was shifted into the sieve shaker. The machine was run for five minutes after which all the meshes were taken out and retained microcapsules were collected by respective mesh and the % retention of microcapsules by that mesh was calculated. Average particle size was calculated using the formula

$$davg = \frac{\Sigma dn}{\Sigma n}$$

where n is frequency weight and d is the mean diameter. The results obtained are given in table 2[8,10]

Rheology properties

Angle of repose, Carr's index and Hausner ratio were determined to assess the flow ability of the prepared microcapsules. The results obtained are given in table 2.

Percentage yield and drug entrapment study

The prepared microcapsules were collected and weighed. The measured weight was divided by the total weight of all the components used for the preparation of microcapsules.

$$\%\ yield = \frac{Weight\ of\ microcapsules\ obtained}{Total\ weight\ of\ excipients\ and\ drug} \times 100$$

The drug entrapment efficiency (DEE) of the microcapsules was calculated by the equation.

$$DEE = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100$$

The results obtained are recorded in table 2[9].

Drug content estimation

Drug loaded microcapsules (100 mg) were powdered and suspended in 100 ml 0.1N HCl solution and kept for 24hr. It was stirred for 5 minutes and filtered through Whatman filter paper. 0.5ml of filterate was then diluted to 10ml with 0.1N HCl to give a concentration of 50µg/ml and the acyclovir content in the filtrate was determined spectrophotometrically (UV-visible spectrophotometer) at 256nm against appropriate blank. The results are recorded in table 2.

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Determination of swelling properties:

The dynamic swelling property of microcapsules in the dissolution medium was determined. Microcapsules of known weight (100mg) were placed in dissolution solution (0.1N HCl) for 6 hr. The swollen microcapsules were collected and weighed[2]. The percentage of swelling of microcapsules in the dissolution media was then calculated by using equation:

$$Swelling\ Index = \frac{(Weight\ of\ swollen\ microcapsules - Dry\ weight)}{Dry\ weight} \times 100$$

The results obtained are given in table 3.

Table 3: Swelling index of formulations

Formulation	Initial weight(mg)	Final weight(mg)	% swelling
F1	100	109	9
F2	100	132	32
F3	100	166	66
F4	100	111	11
F5	100	143	43
F6	100	169	69
F7	100	115	15
F8	100	165	65
F9	100	175	75

Scanning electron microscopy (SEM)

Scanning electron microscopy has been used to determine surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM was carried out by Diya Labs, Mumbai, India. The scanning electron micrographs are shown in figures 1, 2 and 3.

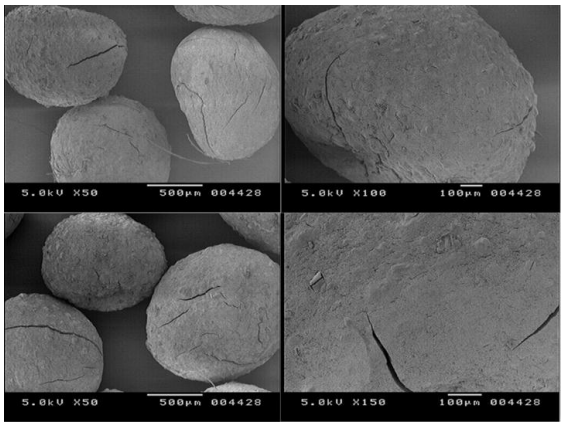

Figure 1: Scanning electron micrograph showing the shape and surface morphology of acyclovir loaded microcapsules of formulation F4

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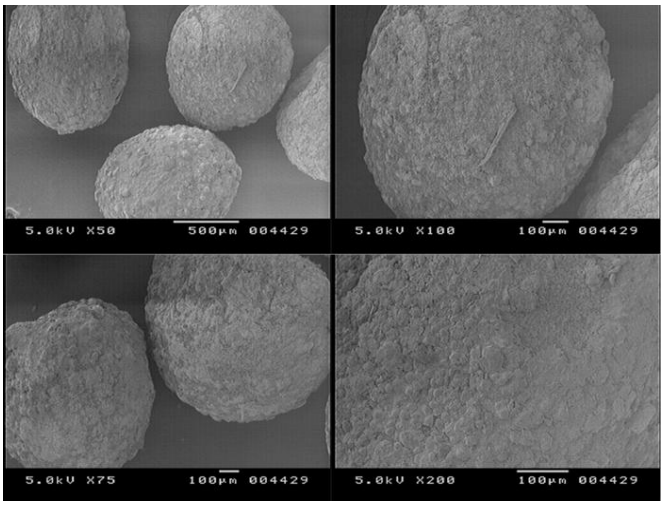

Figure 2: Scanning electron micrograph showing the shape and surface morphology of acyclovir loaded microcapsules of formulation F8

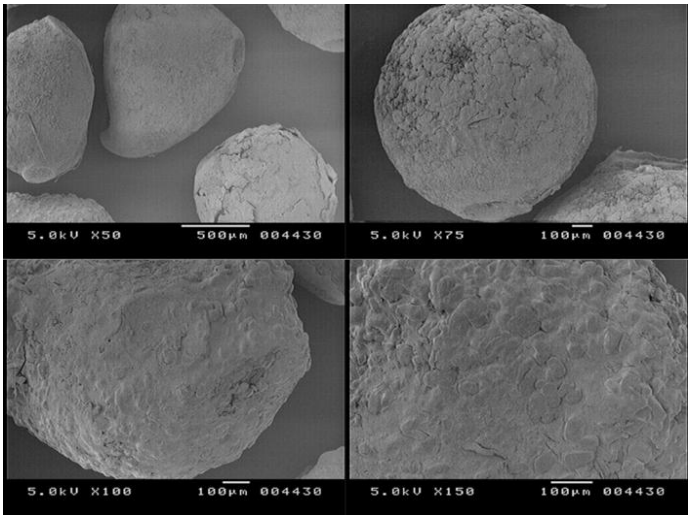

Figure 3: Scanning electron micrograph showing the shape and surface morphology of acyclovir loaded microcapsules of formulation F9

In vitro drug release study

In vitro release studies were carried out for the formulations in dissolution test apparatus USP type I. The medium used was 900ml 0.1N HCl. Microcapsules equivalent to 100mg of the pure drug were used. The tests were carried out for 10hrs and at 50rpm at 37° ± 0.5 °C. 5ml of the aliquots were withdrawn at different predetermined time intervals and filtered. The required dilutions were made with dissolution medium and the solution was analysed for the drug content spectrophotometrically at 256nm against suitable blank. 5ml of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. Three trials were carried out for all the formulations. From this % drug release was calculated and this was plotted against time to study the pattern of drug release. The release

data obtained were fitted to zero order, first order, Higuchi and Korsemeyer Peppas equations to determine the corresponding release rate and mechanism of drug release from the microcapsules [1].

RESULTS AND DISCUSSION

Microcapsules with good properties were obtained by the ionotropic gelation method. The concentration of the polymers is very important in the preparation of microcapsules. As all the microcapsules were formulated from swellable hydrophilic polymers, concentrations exceeding those indicated in the formulas could not be used because the resulting solutions were too viscous for handling and adding drop-wise through syringe. Preparation of microcapsules by dropping the prepared solution into calcium chloride solution should be done after complete deaeration otherwise it will cause imperfection in the microcapsules. All formulated microcapsules were found to be discrete, free flowing, spherical to near spherical and without aggregation.

The average particle size of all the formulations was found to be within the range of 791.90 to 918.34µm. The particle size of formulations F8 and F9 prepared using HPMC E15 and HPMC E50 as co-polymers was found to be 866.31 and 918.34µm respectively which is slightly bigger as compared to the formulation F4 prepared using sodium alginate alone i.e. 796.78µm. This may be attributed to the increase in the viscosity of the polymeric dispersions, after the inclusion of copolymers, which eventually leads to the formation of larger size of microcapsules. It was found that with increase in the concentration of co-polymers HPMC E15 and HPMC E50, the mean particle size of the microcapsules was also increased. In case of formulations F2, F5 and F8 prepared using HPMC E15, the particle size was found to be 828.59, 834.97 and 866.31µm respectively. In case of formulations F3, F6 and F9 prepared using HPMC E50, the particle size was found to be 889.89, 917.64 and 918.34µm respectively. In case of formulations F1, F4 and F7 prepared using sodium alginate alone the particle size was found to be 791.90, 796.78 and 812.83µm respectively.

The rheology study of microcapsules reflected that microcapsules were having satisfactory flow properties. Carr's compressibility index and Hausner ratio were determined to be less than 15% and 1.15 for all formulations respectively, which indicates that the prepared microcapsules of all the formulations have good flow property. The results of angle of repose indicated that the microcapsules of all the formulations are freely flowable. The better flow property indicates that the microcapsules produced are non-aggregated.

The percentage yield for all the formulations was determined as per methodology and was found to be good, within the range of 80.67 to 96.45%. The entrapment efficiency for all the formulations was determined as per methodology and was found to be good, within the range of 58.91 to 98.74%. The entrapment efficiency of formulations F8 and F9 prepared using HPMC E15 and HPMC E50 as co polymers was found to be 97.49 and 94.67% respectively, which is slightly lower as compared to formulation F4 prepared using sodium alginate alone i.e. 98.74%, which may be attributed to the lesser availability of sodium alginate to encapsulate the

drug. Alginate microcapsules are formed due to the intermolecular crosslinking between the divalent calcium ions present in $CaCl_2$ solution and negatively charged carboxyl groups of sodium alginate. So, with the addition of co-polymers the concentration of sodium alginate is decreased leading to a decrease in the availability of carboxyl groups of sodium alginate for crosslinking resulting in lesser entrapment efficiency. But formulation F8 prepared using HPMC E15 as co-polymer showed better entrapment efficiency of 97.49% as compared to other formulations. It was found that with increase in the concentration of co-polymers the entrapment efficiency of the microcapsules was increased. In case of formulations F2, F5 and F8 prepared using HPMC E15, the entrapment efficiency was found to be 64.42, 66.85 and 97.49% respectively. In case of formulations F3, F6 and F9 prepared using HPMC E50, the entrapment efficiency was found to be 58.91, 59.08 and 94.67% respectively. In case of formulations F1, F4 and F7 prepared using sodium alginate alone the entrapment efficiency was found to 74.86, 91.74 and 72.17% respectively. Drug content for all the formulations was determined as per methodology and was found to be good, within the range of 18.04 to 37.43mg per 100 mg of microcapsules.

Swelling of all the formulations was found to be in the range of 9 to 75%. The swelling ratio of formulation F1, F4 and F7 prepared using sodium alginate alone was found to be 9, 11 and 15% in 6 h, indicating that sodium alginate alone has very low ability to swell in 0.1N HCl. In solutions of low pH, the ionization of carboxylate groups on sodium alginate might be repressed and there is less water incorporation into interchain entanglements due to closer network structure of hydrogel i.e. less swellable microcapsules. The formulations F2, F3, F5, F6, F7 and F8 prepared using co-polymers HPMC E15 and HPMC E50 showed an increase in swelling. Also there was an increase in %swelling with the increase in polymer concentration. In case of formulations F2, F5 and F8 prepared using HPMC E15 the %swelling was found to be 32, 43 and 65% respectively. In case of formulations F3, F6 and F9 prepared using HPMC E50 the %swelling was found to be 66, 69 and 75% respectively.

The SEM photographs of formulations F4, F8 and F9 revealed that the microcapsules were almost spherical with rough surface which may be due to the drug that is uniformly dispersed at molecular level in the calcium-alginate matrices. Surface morphology also revealed presence of cracks and deposits of the fine drug crystals on the surface. Formulation F4 prepared with sodium alginate alone showed the presence of cracks on the surface. Formulation F8 prepared with a combination of sodium alginate and HPMC E15 showed almost spherical microcapsules with rough surface but without the presence of cracks. Formulation F9 prepared with a combination of sodium alginate and HPMC E50 showed a few irregularly shaped microcapsules with rough surface.

The percentage drug release from various formulations was found in range of 76.79% to 98.15 % within 10 hours. By comparing the drug release results of all the formulations it was found that the formulations F4, F8 and F9 showed better and sustained drug release.

From dissolution studies it was observed that the release rate from the microcapsules of acyclovir prepared from different polymers in different ratios varied as follows:

With sodium alginate: F7 > F1 > F4
With HPMC E15: F8 > F5 > F2
With HPMC E50: F9 > F6 > F3

Drug released from all the formulations followed the Korsemeyer-Peppas and it was the best fit model as per the data obtained. Korsemeyer-Peppas model release mechanism is not well known, since more than one type of release phenomenon could be involved. However the slope of equation 'n' value could be used to characterize different release mechanisms as shown in Table 5. It is seen that, value is less than 1 and suggests that the formulation follows non-Fickian diffusion. Drug released from the formulations F4, F8, F9 followed the Korsemeyer-Peppas as the best fit model. The slope of equation 'n' value could be used to characterize different release mechanisms as shown in Table 5. It is seen that, value is less than 1 and suggests that the formulation follows non-Fickian diffusion. If the value of 'n' in Korsmeyer-Peppas is 0.5 or less, then the release mechanism follows Fickian diffusion or if 'n' value is greater than 0.5 then the mechanism of drug release follows Non–Fickian diffusion. From the above kinetic study, the value of diffusional exponent 'n' obtained from the slope of Korsmeyer-Peppas model , remained in the range of 0.5767-0.7279, suggested that the drug releasing mechanism was Non-Fickian diffusion (anomalous transport).

Table 4: *In vitro* drug release profiles of microcapsule formulations

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	32.772	14.185	8.967	15.815	9.130	10.924	40.598	11.250	8.315
2	35.380	25.109	11.902	26.413	24.293	12.228	49.402	24.620	10.109
3	43.696	37.174	14.185	41.739	37.826	13.207	70.435	38.478	11.413
4	65.054	45.815	28.043	59.348	51.033	28.043	75.489	53.967	24.946
5	74.837	68.967	35.217	75.489	69.130	36.359	85.435	69.620	38.967
6	86.087	75.326	48.261	89.674	80.870	54.783	88.533	83.315	53.967
7	97.989	85.272	59.837	94.239	90.652	60.000	97.011	93.587	69.946
8	98.478	88.696	76.957	96.033	94.728	70.272	98.641	95.054	83.315
9	--	91.467	77.446	--	96.033	82.337	--	97.011	92.120
10	--	95.380	78.098	--	96.196	83.641	--	98.804	93.098

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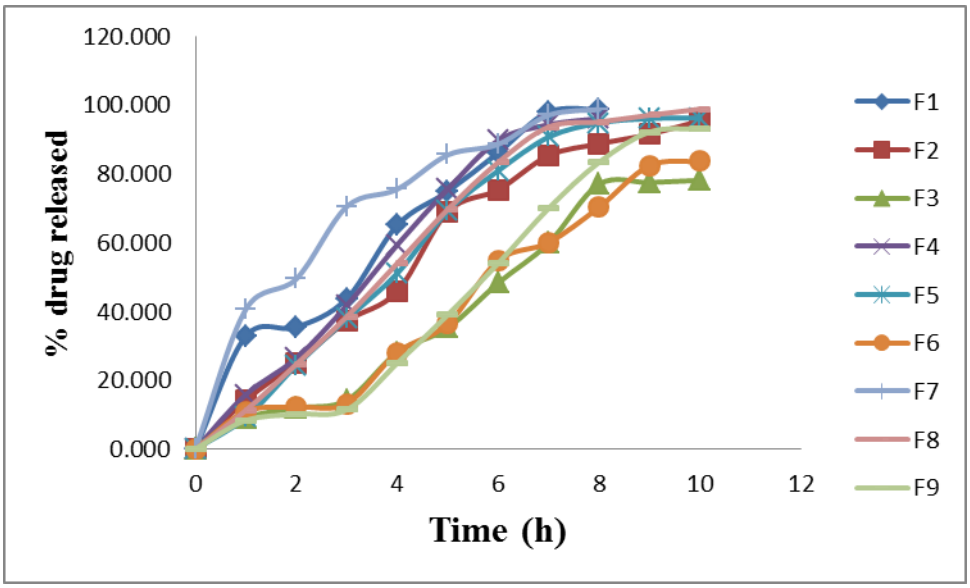

Figure 4: Dissolution profiles of acyclovir microcapsule formulations

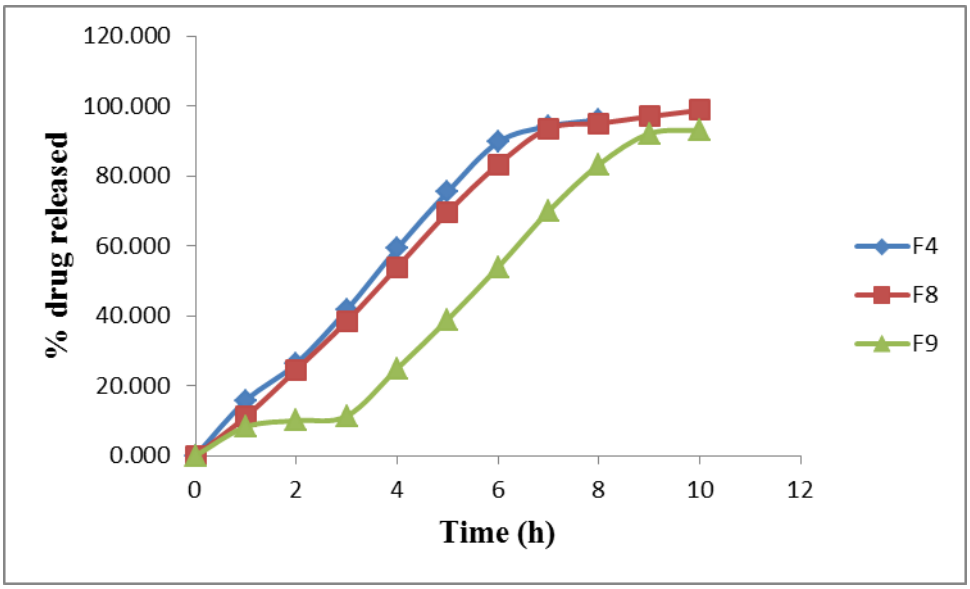

Figure 5: Dissolution profiles of formulations F4, F8, F9

Table 5: Drug release kinetics for acyclovir microcapsules

Formulation	Zero Order (r^2)	First Order (r^2)	Higuchi Model (r^2)	Korsemeyer-Peppas Model (r^2)	n Values
F4	0.9727	0.9389	0.9288	0.9829	0.7279
F8	0.9424	0.9404	0.9368	0.9925	0.7129
F9	0.9624	0.8687	0.8209	0.9854	0.7234

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CONCLUSION

The results of the studies reveal that the choice of combination of polymers instead of single polymer may be an effective strategy for the designing and development of acyclovir loaded microcapsules for easy, reproducible and effective oral controlled drug delivery. Here the combination of sodium alginate with HPMC E15 was found to be more effective.

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