Research Article

DESIGN AND EVALUATION OF ACYCLOVIR MUCOADHESIVE MICROCAPSULES

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ABSTRACT

Acyclovir microcapsules with a coat consisting of alginate and mucoadhesive polymers such as sodium carboxy methyl cellulose and methyl cellulose were prepared by an ionotropic gelation technique. The microcapsules were prepared and found to be discrete, free flowing, spherical to near spherical and without aggregation. The microencapsulation efficiency was found to be in between 38.60% to 70.35%. The percent yield, drug entrapment and drug content in all formulations were good. The average particle size was found to be in the range of 409.25 to 725µm. A percentage of moisture loss was calculated for all the prepared acyclovir microcapsules and was found to be within limit. The swelling indices of all the formulation were enhanced with the increased alginate concentration. Microcapsules prepared with sodium carboxy methyl cellulose with alginate (FS1) exhibited good mucoadhesive property in the in-vitro wash off test. Acyclovir release from these mucoadhesive microcapsules was slow and extended over a period of 8 hour and depends upon the concentration of alginate. All formulations were followed first order kinetics with diffusion mechanism. In conclusion, alginate-sodium CMC mucoadhesive microcapsules could be promising vehicle for the controlled release of Acyclovir.

Keywords: Acyclovir, ionotropic gelation, *in-vitro* wash off test, microcapsulation efficiency, swelling index.

INTRODUCTION

Controlled release dosage form are becoming increasingly important, either to achieve the desired level of therapeutic activity required for a new drug entity or to extend life cycle of an existing drug through improved performance or patient compliance[1]. Microencapsulation by various polymers and their applications are well known[2,3]. Microencapsulation and resulting microcapsules have gained good acceptance as a process to achieve controlled-release drug targeting. Mucoadhesion is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of the drug[4]. There is always significant interest in the development of drug delivery system via oral route due to patient compliance and acceptability. These dosage forms are swallowed so that the pharmaceutically active substance can be absorbed via gastrointestinal tract (GIT). The traditional oral delivery system has certain disadvantages that needed to be overcome such as the short retention time in GIT. The major absorption zone (stomach or upper part of the intestine), can result in incomplete drug release from the drug delivery system leading to diminished efficacy of administrative dose. Therefore restraining a drug delivery system in specific region of the GIT due to its mucoadhesiveness increases the intimacy and duration of contact between a drug containing polymer and a mucous surface. Such drug delivery systems offer numerous advantages, especially for drugs exhibiting an absorption window or for drugs with stability problem. The objective of this study was to develop, characterize, and evaluate mucoadhesive microcapsules of Acyclovir using various mucoadhesive polymers. Thus the microcapsules were prepared by using ionotropic gelation technique. This study describes the development and evaluation of Mucoadhesive microcapsule of drug for oral controlled release[5,6,7,8,9].

The primary goal of Mucoadhesive controlled drug delivery system is to localize a delivery device with the body to enhance the drug absorption process in a specific manner and to facilitate intimate contact of the dosage form with underlying absorption surface to improve and enhance the bioavailability of drugs. An Attempt shall be made in this study to increase the bioavailability and short half life of Acyclovir as it is only 10–20% (oral) and 2.2–3 hr respectively in conventional dosage form.

MATERIALS AND METHODS

Acyclovir was a gift sample from Alpha drug laboratory, Indore. Sodium alginate, Sodium Carboxy Methyl Cellulose and Methyl Cellulose were purchased from Loba chemicals, Mumbai. All other reagents used were of analytical grade.

Methods for preparation of microcapsules

In the ionotropic gelation method, coating material (sodium alginate) and mucoadhesive polymer were dissolved in distilled water (32 ml) to form a homogenous polymer solution. The core material, acyclovir was added to the polymer solution and mixed thoroughly to form a viscous dispersion. 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. The added droplets were retained in the calcium chloride solution for 3 h to complete the curing reaction and to produce spherical rigid microcapsule. The microcapsules were collected by decantation and the product thus produced was washed repeatedly with water and dried at 45^0C for 8 h in hot air oven.

Evaluation of Mucoadhesive microcapsules

Particle size measurement study [10]

Particle size analysis was done by sieving method using Indian standard sieves ≠ 10, 12, 16, 20, 22, 40, 44. Average particle size was calculated using the formula-

$$d_{avg} = \sum dn \,/\, \sum n$$

Where n is frequency weight and d is the mean diameter

Rheology properties

Angle of repose, Carr's index, Bulk density and Hausner's ratio were determined to assess the flow ability of the prepared microcapsules.

Drug content estimation [11]

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 minute and filtered by whatman filter paper 41 sizes. Acyclovir content in the filtrate was determined spectrophotometrically (UV-visible-sl 164, double beam spectrophotometer Elico) at 254 nm using a regression derived from the standard graph (r^2=0.9995).

Drug Entrapment Study [11]

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

EE = (Pc / Tc) X 100

Pc is practical content, Tc is the theoretical content.

Loose surface crystals study [12]

From each batch, 100mg of microcapsules was shaken in 20 ml of double distilled water for 5 minute and then filtered through whatman filter paper 41. The amount of drug lost in filtrate was determined spectroscopically and calculated as a percentage of total drug content.

Determination of swelling properties[13]

The dynamic swelling property of microcapsules in the dissolution medium was determined. Microcapsules of known weight were placed in dissolution solution for 6 hr and the swollen microcapsules were collected by a centrifuge and the wet weight of the swollen microcapsules was determined by first blotting the particles with filter paper to remove absorbed water on surface and then weighing immediately on an electronic balance. The percentage of swelling of microcapsules in the dissolution media was then calculated by using equation.

$$S_w = (W_t - W_o) / W_o \text{ X } 100$$

Where S_w = percentage of swelling of microcapsules, Wt = weight of the microcapsules at time t, W_O = initial weight of the microcapsules

Determination of Percentage of moisture loss [14]

The Acyclovir loaded microcapsules was evaluated for % of moisture loss which sharing an idea about its hydrophilic nature. The microcapsules weighed initially kept in desiccator containing calcium chloride at 37°C for 24 hour. The final weight was noted when no further change in weight of sample.

$$\% \text{ of moisture loss} = \frac{\text{Initial weight- Final weight}}{\text{Final weight}} \text{ X } 100$$

***In-vitro* drug release study** [15]

In vitro drug release study was carried out in USP/IP/BP std peddle type dissolution test apparatus using 0.1 N HCl as dissolution medium. Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1°C throughout study. Peddle speed was adjusted to 50 rpm. An interval of 1 hr, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for Acyclovir content by UV-Visible spectrophotometer at 254nm.

***In vitro* drug release kinetic study**

In order to study the exact mechanism of drug release from microcapsules, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer-Peppas model.

Mucoadhesion testing by *In Vitro* Wash-off test [10,16]

The mucoadhesive property of microcapsule was evaluated by an *in vitro* adhesion testing method known as the wash-off test. Freshly excised pieces of intestinal mucosa from sheep were mounted onto glass slide. About100 microcapsules were spread onto wet rinsed tissue specimen and immediately thereafter the slides were hung onto the arm of a tablet disintegrating machine. Then the machine was operated. The tissue specimen was given a slow, regular up and down movement in the test fluid at about 37°C contained in a1 l vessel of the machine. At the end of 1, 2, 3, 4, 5, 6, 7 and 8hrs the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed at 0.1N hydrochloric acid solution.

Scanning electron microscopy (SEM) [16]

Scanning electron microscopy (Stereo scan S250 MK III, Cambridge, UK) was carried out to study the morphological characteristics of Acyclovir microcapsule. The dried microcapsules were coated with gold (100 Å) under an argon atmosphere in a gold coating unit and Scanning electron micrographs of both higher and lower resolutions were observed. The scanning electron microscopy was held at Birbal Sahini Institute of Palaeobotany, Lucknow (U.P.)

RESULTS AND DISCUSSION

Preparation of Acyclovir Microcapsules

The microcapsules were prepared by ionotropic gelatin method by using different drug: polymer ratio which is indicated in table-01.

Table 01: Formulation of Acyclovir microcapsules

Batch code	Coat : core ratio	Coat composition
FMC1	1:1	Na alg : MC
FS1	1:1	Na alg : SCMC
FMC2	2:1	Na alg : MC
FS2	2:1	Na alg : SCMC
FMC3	3:1	Na alg : MC
FS3	3:1	Na alg : SCMC

Percent Yield, Drug Content and Encapsulation Efficiency of Acyclovir Loaded Microcapsules

The percent yield, drug entrapment and drug content in all formulations were determined. The results are summarized in table-02. The microencapsulation efficiency of all the formulations were in the range of 51.30 to 91.25%. The microencapsulation efficiency was relatively high with alginate-Sodium CMC (FS1>FS2>FS3) and gradually decreases to alginate- MC (FMC1>FMC2>FMC3).

Particle size measurement of Acyclovir microcapsule formulations

The average particle size was found to be in range of 402.85 to 821.03µm. The average particle size of microcapsules increased as the concentration of the polymer increased. The results are shown in table-03.

Table 03: Particle size measurement of Acyclovir microcapsules

Formulation	Particle Size (µm)
FMC1	402.85
FS1	475.39
FMC2	539.27
FS2	558.39
FMC3	789.54
FS3	821.03

Rheology determination of microcapsules

The rheology study of microcapsules reflected those microcapsules were having satisfactory flow properties. The results are shown in table-04. Particle size of the microcapsules were large, angle of repose were increased as the amount of the polymer is increased. However angle of repose indicates that the microcapsules have better flow property. The better flow property indicates that the microcapsules produced are non aggregated. All the formulations show excellent flowability as expressed in term of angle of repose (<25).

Table 02: Percent Yield, Drug Content and Encapsulation Efficiency of Acyclovir Loaded Microcapsules

Formulation	Yield (%)	Theoretical Drug Content (mg)	Practical Drug Content (mg)	Encapsulation Efficiency
FMC1	79.04	20	12.00	60.00
FS1	81.20	20	18.25	91.25
FMC2	81.64	20	11.32	56.60
FS2	76.52	20	17.75	88.75
FMC3	80.15	20	10.26	51.30
FS3	83.06	20	15.00	75.00

Table 04: Rheology determination of microcapsules

Formulation	Carr's index	Hausner's ratio	Angle of repose	Comment
FMC1	11.98	1.02	20.9^{o}	Excellent
FS1	13.39	1.09	23.7^{o}	Excellent
FMC2	8.16	1.01	21.3^{o}	Excellent
FS2	8.94	1.02	22.7^{o}	Excellent
FMC3	9.34	1.26	22.8^{o}	Excellent
FS3	15.33	1.03	26.1^{o}	Good

Table 05: Swelling Index

Formulation	Initial Weight(mg)	Final Weight(mg)	% Swelling
FMC1	100	151	51
FS1	100	158	58
FMC2	100	156	56
FS2	100	157	57
FMC3	100	163	63
FS3	100	180	80

Table 06: Loose Surface Crystal Studies of Acyclovir Encapsulated Microcapsules

Formulation	Drug content In filtrate	Loaded drug Content	% drug content in surface
FMC1	0.747	12.00	6.23
FS1	0.961	18.25	5.27
FMC2	0.816	11.32	7.21
FS2	1.584	17.75	8.92
FMC3	1.127	10.26	10.98
FS3	1.445	15.00	9.63

Table 07: *In-vitro* drug release kinetic studies of microcapsules

Formulation	Zero order (r)	First order (r)	Higuchi square root (r)
FMC1	0.956	0.996	0.991
FS1	0.970	0.992	0.996
FMC2	0.937	0.986	0.983
FS2	0.933	0.971	0.988
FMC3	0.993	0.995	0.985
FS3	0.944	0.987	0.982

Table 10: Determination of percentage of moisture loss of Acyclovir Encapsulated microcapsules

Formulation	Initial weight(mg)	Final weight(mg)	Moisture loss	% Moisture loss
FMC1	200	185.28	14.72	7.36
FS1	200	181.24	18.76	9.88
FMC2	200	173.46	26.54	13.26
FS2	200	193.62	6.38	3.19
FMC3	200	192.41	7.59	3.80
FS3	200	192.48	8.92	4.46

Swelling Index

The swelling indexes of microcapsules were found satisfactory and results are shown in table-05**.** The results indicate that, swelling index increases as the concentration of polymers increases. The swelling indices were found to be in the range of 80% to 51%.

Loose Surface Crystal Studies of Acyclovir Encapsulated Microcapsules

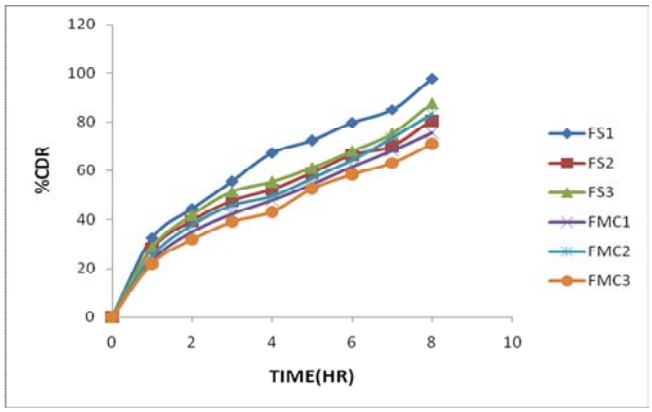
The loose surface crystal studies lend a hand to estimate the excess amount of drug attached on the surface of microcapsules after a successful drug entrapment. The study was executed with various prepared formulations and the results were tabularized in table-06. The

percentage of drug content in surface was found to be 10.98% to 5.27%.

In-vitro drug release study

The *in vitro* release profile of acyclovir microcapsules were conducted in 0.1N HCL. All the formulations were found to be release Acyclovir in a controlled manner for a prolonged period of 8 hour. The percentage of drug release from the formulations FS1, FS2 and FS3 were found to be 97.65%, 87.65% and 83.25% respectively. The percentage of drug release from the formulations FMC1, FMC2 and FMC3 were found to be 80.45%, 75.50% and 70.87% respectively. The percentage of drug release from the formulations were decreased as the concentration polymers increased. So acyclovir release from alginate-Sodium CMC formulation (FS1) was found to be 97.65% in slow and extended over a period of 8 hours. The results are shown in fig-01.

Figure 01*: In-vitro comparative* drug release profile of FMC1, FS1, FMC2, FS2, FMC3 and FS3.



In-vitro drug release kinetic studies of microcapsules

The release data was analyzed according to different kinetic equation. All formulations followed first order kinetics. And formulations seems to be fit in Higuchi square root kinetic model and formulations have diffusion controlled release pattern which is dependent on concentration of release retarding polymer with process variables epitomized in table-07**.**

Characterizations of release mechanism from microcapsules

To examine the release mechanism of acyclovir from the microcapsules the result were analyzed according to the Korsmeyer-Peppas equation.

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

Where M_t / M_∞ is the fractional drug release at time t, k is a kinetic constant incorporating structural and geometric characteristic of the drug / polymer system [device], n is the diffusional exponent that characterizes the mechanism of drug release. In this formulation the value of n which is greater than 0.5, in this formulation the release is non Fickian that is not depend upon the concentration gradient. If value of n is less than 0.5 so this release is the Fickian table-08.

Table 08: *In-vitro* drug release kinetic mechanism studies of microcapsules

Formulation	Korsmeyer-Peppas
FMC1	0.55
FS1	0.60
FMC2	0.50
FS2	0.51
FMC3	0.54
FS3	0.52

In vitro wash-off test of microcapsules

The mucoadhesion of the selected microcapsules were studied by *in vitro* wash off test. The microcapsules for the test were selected on the basis of their *in vitro* drug release profile. Formulations FS1, FS2 and FS3 were selected for this test in 0.1N HCL solution.

The result of the wash off test and adhesion number is reported in table-09 and fig-02, which indicates fairly good Mucoadhesive property of the microcapsule prepared from sodium alginate and a Mucoadhesive polymer in acidic medium. This is increase with increase the concentration of Mucoadhesive polymer.

Table 09: *In vitro* wash-off test of microcapsules

TIME	Number of Microcapsules Adhering		
	FS1	FS2	FS3
1	88	84	80
2	82	76	71
3	76	70	65
4	71	64	61
5	63	58	52
6	55	44	43
7	42	39	38
8	38	33	31

Figure 02: *In vitro* wash-off test of microcapsules

Determination of percentage of moisture loss of Acyclovir Microcapsules

The percentage of moisture loss was tabularized in Table-10 and found in a range 3.19% to 13.26% ensure the presence of diminutive water content which can be due to the involvement of water in process method and hydrophilic property of Mucoadhesive polymers. But the lessen proportion of water obtained indicates its proper drying and instant hardening of microcapsule due to quick gelation occurred between calcium chloride and sodium alginate facilitate the storage behaviour of the formulations**.**

Scanning electron microscopy (SEM)

The microcapsules prepared were found to be spherical to near spherical and without aggregation, (as revealed in SEM studies), discrete and free flowing, which are shown in fig-03 and fig-04. The scanning electron microscopy was held at Birbal Sahini Institute of Palaeobotany, Lucknow (U.P).

Figure 03: SEM photograph of Sodium CMC–sodium alginate coated mucoadhesive microcapsule (Magnification: x 30), formulation (FS1)

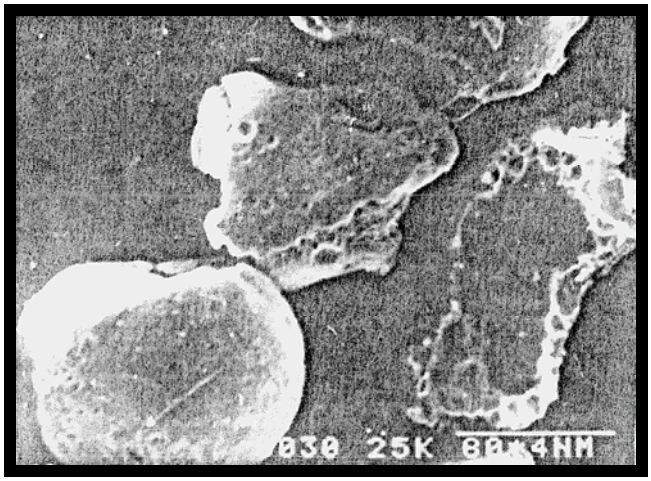
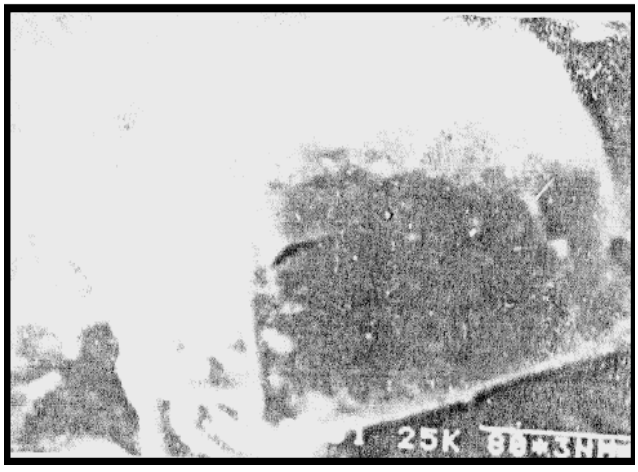


Figure 04: SEM photograph of Sodium CMC–sodium alginate coated mucoadhesive microcapsule (Magnification: x 300), formulation (FS1)



CONCLUSION

Controlled release Mucoadhesive Acyclovir microcapsules could be formulated by using sodium alginate as a release retardant by ionotropic gelation technique. The microcapsules of all the formulated batches were spherical, discrete and free flowing. The drug content was found to be uniform in a batch of microcapsules. Increasing the polymer concentration in microcapsule formulation decreases the rate of drug release dramatically. Further, an elaborate *in vivo* study is to be carried out for the formulated microcapsule using a suitable animal model.

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