ISSN: 0975-766X
Research Article
Available Online through
www.ijptonline.com

PREPARATION AND *IN-VITRO* EVALUATION OF MUCOADHESIVE MICROCAPSULES OF ACYCLOVIR

Ishwar Chandra Giri*, A.K.Sharma, S.B.Bhanja, P. Ellaiah, K.V.R Murthy, D. Das
1. Department of Pharmaceutics, Jeypore College of Pharmacy, Jeypore (K), Odisha.
2. Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam.
3. HI-Tech College of Pharmacy, Bhubaneswar.
4. School of Pharmaceutical Sciences, SOA University, Bhubaneswar.
E-mail: satyabrata_bhanja@rediffmail.com

Received on 20-09-2010 *Accepted on 11-10-2010*

ABSTRACT

Acyclovir microcapsules with a coat consisting of alginate and a mucoadhesive polymer such as carbopol 934P and hydroxypropyl methyl cellulose E 15 V were prepared by an ionotropic gelation technique, where gelation was achieved with oppositely charged counter ions to form microcapsules. The microcapsule prepared were found to be spherical to near spherical and without aggregation discrete and free flowing. The percent yield, drug entrapment and drug content in all formulations were good. The microencapsulation efficiency of all the formulations were in the range of 38.60 to 70.35%. The average particle size was found to be in the range of 409.25 to 725µm. All the formulations show excellent flowability as expressed in term of angle of repose (<25) and the formulation FC1 show good flowability. A percentage of moisture loss was calculated for all the prepared acyclovir microcapsules and was found to be within limit. The swelling indexes of microcapsules were found satisfactory. All the formulations were found to release Acyclovir in a controlled manner for a prolonged period over 8 hour. All formulations were followed first order kinetics and formulations have diffusion controlled release pattern. The mucoadhesion of the selected microcapsules were studied by *in vitro wash off* test according to their *in vitro* drug release profile. The result of the *in vitro wash off* test fairly showed good mucoadhesive property of the microcapsules prepared from sodium alginate. The percentage of moisture loss was found in a range 2.24 to 8.81%.

KEYWORDS: Acyclovir, *in-vitro* drug release, *in-vitro* wash off test, microcapsulation efficiency, Swelling index.

INTRODUCTION

Microencapsulation has been accepted as a process to achieve controlled release and drug targeting. It is the novel design of an oral controlled drug delivery system should primarily be aimed to achieving more predictable and increased bioavailability of drug. There is always significant interest in the development of drug delivery system via oral rout 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 .Thus the microcapsule were prepared by using ionotropic gelation technique. This study describes the development and evaluation of Mucoadhesive microcapsule of drug for oral controlled release.[1, 2,3,4,5]

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, Carbopol 934p and Hydroxy Propyl Methyl Cellulose(HPMC-E15V) were purchased from Loba chemicals, Mumbai. All other reagents used were of analytical grade.

Methods for preparation of microcapsules

The Mucoadhesive microcapsules were prepared by an ionotropic gelation technique. Because other encapsulation techniques normally involve the polymer as carrier which require large quantities of organic solvent for their solubilization except ionic gelation method. As a result, the doses becomes vulnerable to safety hazard, toxicity and increase the cost of production making the techniques non-reproducible, economically and unsuccessful at industrial scale. These concerns demand a technique free from any organic solvent. Thus the microcapsules were prepared by using ionotropic gelation method.

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 microcapsules. 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.

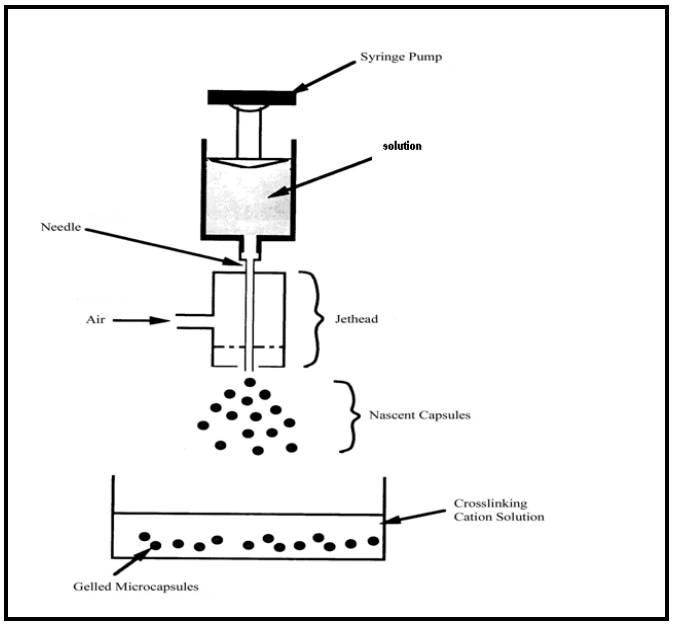

Figure- 1: Set-up for Microcapsule Preparation by Ionotropic Gelation.

Evaluation of Mucoadhesive microcapsules

Particle size measurement study [6]

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 [7]

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 [7]

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 [8]

The Acyclovir loaded microcapsules prepared by ionotropic gelation technique were evaluated by loose surface crystal study to observe the excess drug present on the surface of microcapsules. 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 [9]

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.

$$S_w = (W_t - W_{o)} / W_o \times 100$$

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 [10]

The Acyclovir loaded microcapsules was evaluated for percentage 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.

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

***In-vitro* drug release study** [11]

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.

Drug interaction study [12]

The Fourier Transform Infrared Radiation measurement (FTIR) spectral measurements were taken at ambient temperature using IR spectrophotometer (shimadzu, model 840, Japan).the FTIR study was held at school of pharmacy DAVV, Indore (M.P.)

Mucoadhesion testing by *In Vitro* Wash-off test [6,13]

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) [12]

Scanning electron microscopy (Stereo scan S250 MK III, Cambridge, UK) was carried out to study the morphological characteristics of Acyclovir microcapsules. The dried microcapsules were coated with gold (100 A°) 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
FC1	1:1	Na alg : Car 934
FH1	1:1	Na alg : HPMC
FC2	2:1	Na alg : Car 934
FH2	2:1	Na alg : HPMC
FC3	3:1	Na alg : Car 934
FH3	3:1	Na alg : HPMC

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 38.60 to 70.35%. The microencapsulation efficiency was relatively high with alginate-carbopol(FC1>FC2>FC3)andgraduallydecreases to alginate- HPMC(FH1>FH2>FH3).

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
FC1	83.30	20	14.07	70.35
FH1	77.63	20	11.08	55.40
FC2	84.07	20	12.81	64.04
FH2	79.26	20	09.16	45.80
FC3	86.05	20	12.45	62.25
FH3	78.40	20	07.72	38.60

Particle size measurement of Acyclovir microcapsule formulations

The average particle size were found to be range of 409.25to 723µm. The average particle size of microcapsules increased as the concentration of the polymer increased(table-3).

Table-03: Particle size measurement of Acyclovir microcapsules.

Formulation	Particle Size (µm)
FC1	409.25
FH1	440.45
FC2	521.98
FH2	472.16
FC3	674.36
FH3	723.76

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-04: Rheology determination of microcapsules.

Formulation	Carr's index	Hausner's ratio	Angle of repose	Comment
FC1	09.34	1.03	20.1^{o}	Excellent
FH1	9.18	1.04	21.7^{o}	Excellent
FC2	09.34	1.26	22.3^{o}	Excellent
FH2	09.18	1.04	21.7^{o}	Excellent
FC3	10.11	1.09	24.8^{o}	Excellent
FH3	12.24	1.02	23.1^{o}	Excellent

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 87% to 62%.

Table-05: Swelling Index.

FORMULATION	INTIAL WEIGHT(mg)	FINAL WEIGHT(mg)	% SWELLING
FC1	100	162	62
FH1	100	168	68
FC2	100	178	78
FH2	100	171	71
FC3	100	183	83
FH3	100	187	87

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 were found to be 11.61% to 4.72%.

Table-06: Loose Surface Crystal Studies of Acyclovir Encapsulated Microcapsules.

Formulation	Drug content In filtrate	Loaded drug Content	% drug content in surface
FC1	1.155	14.07	08.20
FH1	1.149	11.08	10.37
FC2	0.605	12.81	4.72
FH2	0.853	09.16	09.31
FC3	0.889	12.45	07.14
FH3	0.901	07.72	11.61

***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 FC1, FC2 and FC3 were found to be 98.5%,78.01% and 75.87% respectively. The percentage of drug release from the formulations FH1, FH2 and FH3 were found to be 74.45%, 69.72% and 62.72% respectively. The percentage of drug release from the formulations were decreased as the concentration polymers increased. So acyclovir release from alginate-carbopol formulation (FCI) was found to be 98.5% in slow and extended over a period of 8 hours. The results are shown in table 07 and fig 01.

Table-07: Drug Release Profile of FH1, FC1, FH2, FC2, FH3, FC3.

Sl. No.	TIME HOURS	FH1 %C.D.R.	FC1 %C.D.R	FH2 %C.D.R	FC2 %C.D.R	FH3 %C.D.R.	FC3 %C.D.R
1	1	24.25	29.54	19.32	26.81	14.43	25.34
2	2	36.76	41.32	30.43	41.65	24.56	34.89
3	3	47.32	52.65	39.05	49.95	34.13	44.63
4	4	58.43	65.12	46.03	57.43	44.91	53.54
5	5	66.34	74.78	52.01	63.67	51.65	56.89
6	6	70.03	80.45	56.51	68.05	56.78	65.32
7	7	72.12	87.61	61.02	73.09	59.96	68.02
8	8	74.45	98.5	69.72	78.01	62.72	75.87

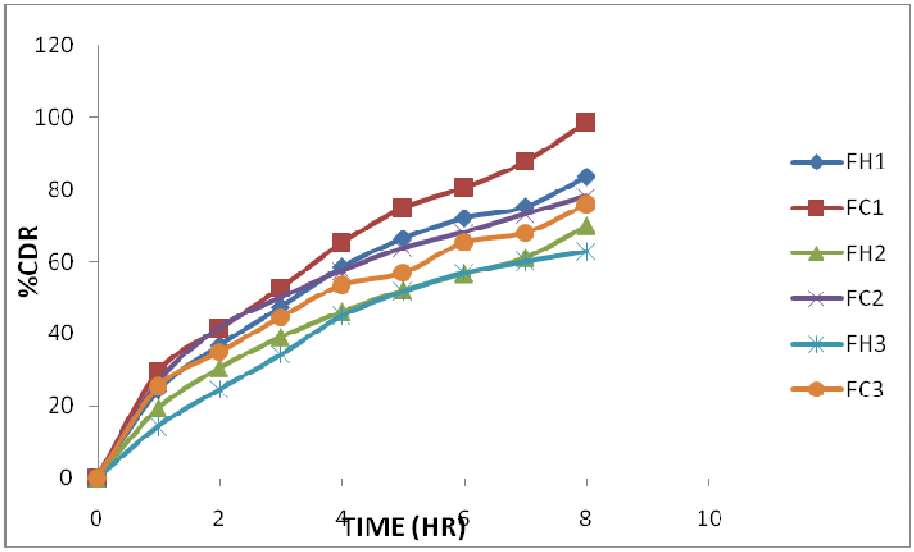

Figure-01*: In-vitro comparative* drug release profile of FH1, FC1, FH2, FC2, FH3, FC3

***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 08**.**

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

Formulation	Zero order (r)	First order (r)	Higuchi square root (r)
FC1	0.942	0.987	0.985
FH1	0.966	0.991	0.992
FC2	0.964	0.994	0.997
FH2	0.957	0.996	0.994
FC3	0.953	0.985	0.993
FH3	0.980	0.995	0.994

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 .5 so this release is the Fickian table 9.

Table-09: *In-vitro* drug release kinetic mechanism studies of microcapsules.

Formulation	Korsmeyer-Peppas model (n)
FC1	0.58
FH1	0.57
FC2	0.57
FH2	0.58
FC3	0.50
FH3	0.73

***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 FC1, FC2 and FC3 were selected for this test in 0.1N HCL solution.

The result of the wash off test and adhesion number is reported in table 10 that indicates fairly good Mucoadhesive property of the microcapsules prepared from sodium alginate and a Mucoadhesive polymer in acidic medium. This is increase with increase the concentration of Mucoadhesive polymer.

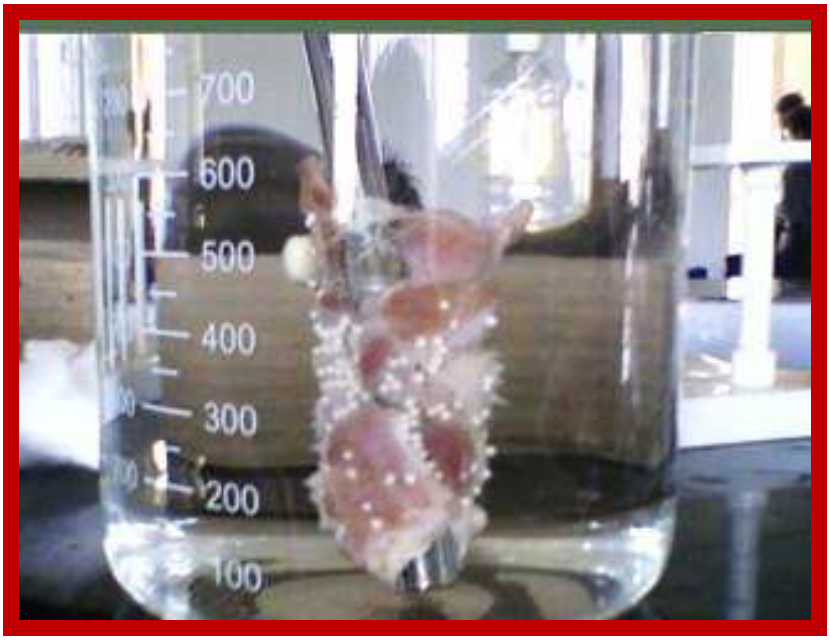

Figure - 02: *In vitro* wash-off test of microcapsules.

Table-10: *In vitro* wash-off test of microcapsules

TIME	Number of Microcapsules Adhering		
	FC1	FC2	FC3
1	80	84	88
2	71	76	82
3	65	70	76
4	61	64	71
5	52	58	63
6	43	44	55
7	38	39	42
8	31	33	38

Determination of percentage of moisture loss of Acyclovir Microcapsules

The percentage of moisture loss was tabularized in Table 11 and found in a range 2.24 to 8.81 % 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 lesser proportion of water obtained indicates its proper drying and instant hardening of microcapsules due to quick gelation occurred between calcium chloride and sodium alginate facilitate the storage behaviour of the formulations**.**

Table-11: Determination of percentage of moisture loss of Acyclovir Encapsulated microcapsules.

Formulation	Initial weight(mg)	Final weight(mg)	Moisture loss	% Moisture loss
FC1	200	191.78	8.22	4.11
FH1	200	189.62	10.38	5.19
FC2	200	183.38	16.62	8.81
FH2	200	195.52	4.48	2.24
FC3	200	195.06	4.94	2.47
FH3	200	194.52	5.48	2.74

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. The scanning electron microscopy was held at Birbal Sahini Institute of Palaeobotany, Lucknow (U.P.)

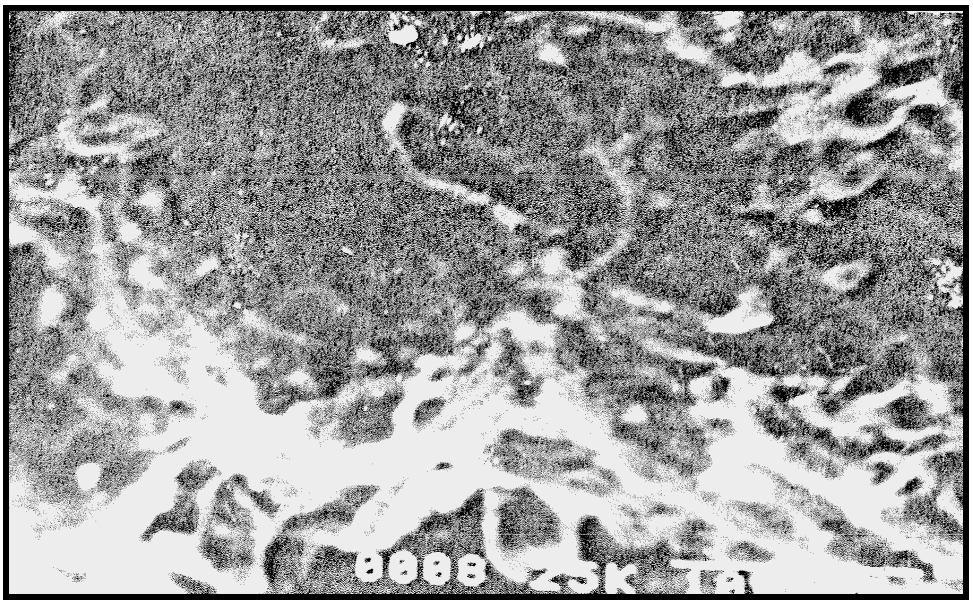

Figure-03: SEM photograph of carbopol–sodium alginate coated mucoadhesive microcapsule (Magnification: x 30), formulation (FC1)

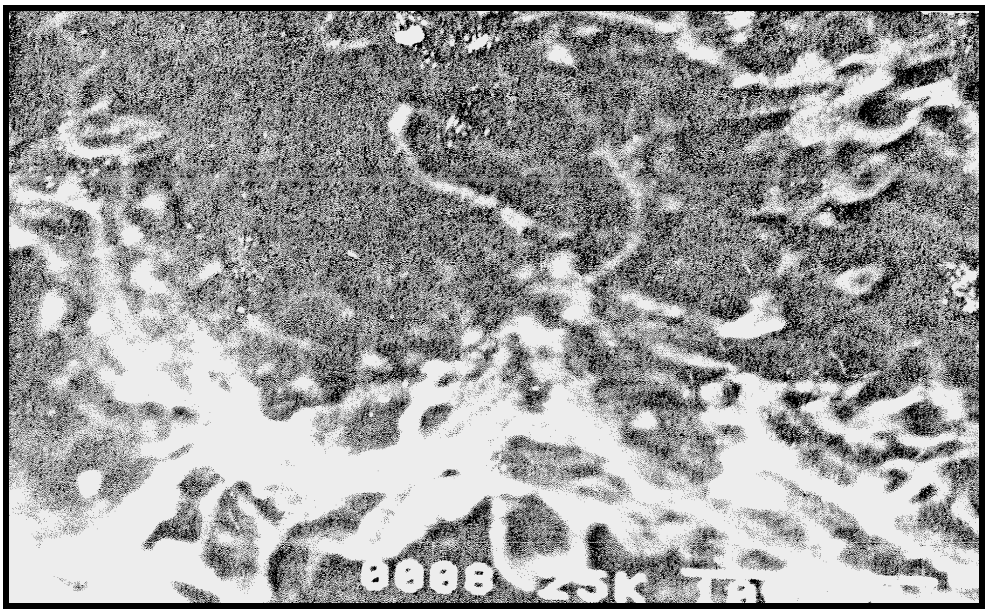

Figure-04: SEM photograph of carbopol–sodium alginate coated mucoadhesive microcapsule (Magnification: x 300), formulation (FC1)

CONCLUSIONS

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 microcapsules using a suitable animal model.

ACKNOWLEDGEMENTS

Authors wish to give thanks to Jeypore College of Pharmacy, Jeypore, Orissa, authority for providing suitable research laboratory to carry out this project work and also my deep greatness to M/S Alpha Drug Laboratory, Indore, for providing Acyclovir as gift sample.

REFERENCES

1. Ikeda K, Murata K, Kobayashi monody K. Enhancement off bioavailability of dopamine via nasal route in beagledogs,Chem pharm bull.1992,vol 40,pp 2155-2158.
2. Nagai T, Nishimoto Y, Nambu N. Powar dosage form of insulin for nasal administration, J control release,1992,vol1,pp15-22.
3. Kondo A, Ed microcapsule processing and technology, New yark, NY marcel Dekker, 1979.
4. Chowdary K.P.R, Srinivas L. Mucoadhesive drug delivery system: Status of Current review, Indian drug ,2000,vol 37,pp400-40.
5. Bahadur S, Chanda R, and Roy A, Preparation and evaluation of mucoadhesive microcapsules of captopril for oral controlled release, Research J. pharm. And tech, 2008, vol1(2),pp 100-105.
6. Shovarani K.N. and Goundalkar A.G., Preparation and evaluation of microsphere of diclofenac sodium, Indian J. Pharm. Sci., 1994,vol 56(4), pp 45-50.
7. Shabaraya A.R,Narayanacharyulu R, Design and evaluation of chitosan microspheres of metoprolol tartrate for sustained release, Indian J. Pharm. Sci, 2003, vol65(3),pp 250-252.

8. Kulkarni G.T, Gowthamarajan K, and Suresh B, Stability testing of pharmaceutical products: an overview, Indian J. Pharm. Educ. Res, 2004,vol 38(11),pp 194-202.
9. Desai K.G, Park H.J, Study of gamma-irradiation effects on chitosan microparticles, Drug delivery, 2006,vol 13,pp 39-50.
10. Abu-Izza K, Garcia L.C, Robert D, Preparation and evaluation of zidovudine loaded sustain release microspheres: optimization of multiple response variables, J. Pharm. Sci, 1996,vol 85(6),pp 572-574.
11. Bhumkar D.R, Maheshwari M, Patil V.B, Pokharkar V.B, Studies on effect of variabilities by response surface methodology for naproxane microspheres, Indian Drugs, 2003, vol40 (8),pp 455-461.
12. Gohel M.C, Parik R.K, Amin A.F. and Surati A.K, Preparation and formulation optimization of sugar crosslinking gelatin microspheres of diclofenac sodium, Indian J. Pharm Sci, 2005,vol 67 (8),pp 575-581.
13. Chowdary K.P.R, Rao Y.S, mucoadhesive microcapsules of glipizide: characterization, in vitro and in vivo evaluation, Indian J. Pharm. Sci., 2003,vol 65(3), pp279-284.

Corresponding author:

Ishwar Chandra Giri*

Email: *icgiripharma@gmail.com*