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PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1421-1428, Oct-Dec 2009

USE OF CARBOMERS TO DESIGN MUCOADHESIVE MICROSPHERESFOR AN ANTI- H. PYLORI DRUG, CLARITHROMYCIN

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ABSTRACT: The aim of present work was to prepare mucoadhesive microspheres containing Clarithromycin, an anti- *H. pylori* agent, used for treatment of peptic ulcers. Mucoadhesive microspheres were prepared by "Calcium induced ionotropic gelation" method. Sodium alginate was used as encapsulating agent and carbopol 934 P & polycarbophil were used as bioadhesive polymer. A 3^2- full factorial design was employed taking concentration of carbomer and sodium alginate as independent variables. The evaluation parameters included encapsulation efficiency, in vitro mucoadhesion, swelling study, in vitro release study. Microspheres were discrete, spherical and free flowing. Swelling index was ranging from 1.2 to 2, indicating excellent swelling property. The microspheres exhibited excellent mucoadhesive property and good drug entrapment. Formulations showed polymer concentration dependent drug release over a period of 12 hrs. Microspheres were having good mucoadhesive property with good encapsulation capacity and a sustained release property.
KEYWORDS: Carbopol, Clarithromycin, Ionotropic Gelation Microspheres, Mucoadhesion.

INTRODUCTION

Helicobacter pylori is a causative organism of peptic ulcer. Investigations have shown that the bacteria have multiple polar flagellae, tail-like structures with which it propel through the mucus layer lining the stomach until they can attach to the cells at the bottom of the lining. Beneath the mucosal surface, bacteria are protected from direct contact with gastric acid. They create microenvironment. This environment has balance of acidity and alkalinity[1].

One reason for incomplete eradication of *H. pylori* is probably due to the short residence time of antimicrobial agents in the stomach. So the effective antimicrobial drug concentration cannot be achieved for a required time period at gastric mucosa or epithelial lining, where the causative organism exists.

Literatures also reveal that the costing of the anti-*H. pylori* treatment is too high due to less eradication rate. Hence, it is necessary to design a drug delivery device that can target the epithelial site where bacteria reside for longer period of time. Also it is important to make a cost effective treatment through controlling the release pattern.

Clarithromycin is a macrolide antibiotic. The pharmacokinetics of the drug shows that it is rapidly absorbed from GIT but oral bioavailability is about 50% due to first pass metabolism. Food delays absorption of clarithromycin[2].

The mucoadhesive drug delivery can be used for improving the contact time of the drug delivery system with the gastric mucosa [3, 4, 5]. Micro particulate drug delivery system can increase the effective surface area[6]. Also it is known that the microparticulate carriers can be tailored as mucoadhesive device. This combination is useful to provide a greater surface area. Thus the mucoadhesive microspheres will provide greater area more contact time as well as control the drug release[7].

Mucoadhesive microspheres provide good contact of drugs with mucus. Thus the drugs can penetrate the microenvironment created by the bacteria. Secondly, microspheres provide more contact area, thus a bigger surface area in the stomach can be targeted. The most important thing is that the controlled release of drug can be achieved to make the therapy convenient and improved. Hence this drug delivery system can enhance the efficiency of anti-*H. pylori* drug.[8, 9,10, 11]

The prime objective of the work was to formulate a Mucoadhesive Microsphere System for Clarithromycin for better eradication of *H.pylori.* Thus reduced period of therapy will make it cost-effective.

EXPERIMENTAL

Materials:

Clarithromycin was supplied by Glenmark Pharmaceuticals. Ltd., Mumbai. Polycarbophil was obtained as gift sample from Noveon Ltd, Mumbai.

Pluronic F-68 was gift sample by BASF Ltd, Mumbai. Sodium alginate, Calcium chloride dihydrate and other chemicals were of laboratory grade.

Methods:

Preparation of Mucoadhesive Microspheres: [12, 13]
Microspheres containing Clarithromycin were prepared using sodium alginate in combination with carbomers as mucoadhesive polymers. An orifice-ionic gelation process that has been extensively used to prepare large alginate beads was employed to prepare the microspheres.

Orifice-Ionic Gelation Method:

Microspheres were prepared by orifice-ionic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate was used as encapsulating material. Carbopol 934 P and polycarbophil were used as mucoadhesive polymers. Pluronic F 68 was stabilizing and suspending agent.

Sodium alginate and the mucoadhesive polymer were dissolved in purified water (50 ml) to form a homogeneous polymer solution. Clarithromycin (750 mg) and Pluronic F 68 (0.5%w/v) were added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a lab-scale developed spray device with an air compressor into calcium chloride (10% w/v) solution. The addition was done with continuous stirring. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water to remove excess calcium impurity and air dried. The microspheres prepared along with their matrix composition are listed in Table No.1 & 2. Batches were prepared for two bioadhesive polymers. Polymers were coded as Polycarbophil (A) & Carbopol- 934 P (B).

Factorial Design[14]**:**

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta 2X2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

Where, Y is the dependent variable, β_0 is the arithmetic mean response of the nine runs, and β_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average results of changing on factor at a time from low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity.

Results of each evaluation test were obtained in triplicates.

Evaluation of Mucoadhesive Microspheres:

a) Estimation of Clarithromycin [15]

Clarithromycin content in microspheres was estimated by UV-VIS spectrophotometric method based on the measurement of intensity of blue colour at 760 nm in 0.1n HCl. 2 ml of Follin Catechu Reagent and 2 ml of 10% Sodium Carbonate solution were added to each test tube. Final volume was made up to 10 ml with 0.1 N HCl. The solution was allowed to stand for 15 minutes for complete colour development. Absorbance of blue colour was measured using the Shimadzu 1700 UV- VIS spectrophotometer.

b) Microencapsulation Efficiency

Microspheres were crushed in a glass mortar and the powdered equivalent to 50 mg of clarithromycin were suspended in 50 ml 0.1 N HCl and stirred well. After 24 hours the solution was filtered and filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated as per following formula:

% Encapsulation Efficiency = Practical drug content/Theoretical drug content x 100.

c) Mucoadhesion studies

Falling Film Technique[16]*:*

This method is one of the suitable methods for testing mucoadhesion strength of mucoadhesive particulate systems like mucoadhesive microspheres, suspension. System consists of effluent reservoir, single drive syringe pump, supporting platform for mucosal segment, adjustable jack and collector for fluid effluent. In weight percent method, a fixed weight of microsphere sample was added (50 mg) over an intestinal segment mounted on a tilted slide with an angle of 45^0. The effluent (0.1 HCl) was run over the segment with a rate of 4-5 ml per minute. The effluent was collected in a Whattman filter paper and weight of detached particle was determined. Percentage of bioadhesion was determined by following formula:

% Mucoadhesion= (wt of added sample - wt of detached particles)/ wt of sample x 100

d) Swelling Index[17]**:**

Swelling of microspheres was determined by soaking 0.5 ml of microsphere bed in 5 ml 0.1 N HCl in 10 ml measuring cylinder. Volume of microspheres was determined after 12 hrs. Swelling index was calculated by using following formula:

Swelling Index = Volume after 12 hrs/ original volume

e) Scanning Electron Microscopy

The microspheres were observed under Scanning Electron Microscope (SEM-Jeol Instruments, Japan JSM-6360). They were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with platinum film. Scanning Electron photographs were taken at an accelerating voltage of 20 KV, chamber pressure of 0.6 mm Hg.

f) Particle size Determination:

Particle size and particle size distribution of microspheres were determined by using optical microscopic method. The eyepiece micrometer was calibrated using the stage micrometer. Around 200 particles of each batch were evaluated for particle size determination.

g) Drug Release Study

Release of clarithromycin from the microspheres was studied in 0.1 N HCl (900 ml) using a United States Pharmacopoeia (USP) XXIII 3-station Dissolution Rate Test Apparatus with a rotating paddle stirrer at 50 rpm and 37° ± 1°C as prescribed for Clarithromycin tablets in USP XXIV. A sample of microspheres equivalent to 500 mg of Clarithromycin was used in each test. Samples were withdrawn through a filter at different time intervals and were assayed at 760 nm for Clarithromycin content by UV VIS spectrophotometry using Shimadzu 1700 UV-VIS spectrophotometer.

RESULT AND DISCUSSION

a) Encapsulation Efficiency:

The factorial equation for encapsulation efficiency in Polycarbophil microspheres (Equation 1) and Carbopol 934 P (Equation 2) are

$Y = 81.635 + 11.720\ X_1 + 6.213\ X_2 - 10.885\ X_1X_2 - 6.45\ X_1^2 - 9.652\ X_2^2$ ……. (1)

$Y = 69.647 + 5.084\ X_1 + 0.287\ X_2 - 4.251\ X_1X_2 - 24.66\ X_1^2 - 2.212\ X_2^2$……. (2)

Encapsulation Efficiency of Polycarbophil Microspheres and Carbopol 934 P varied from 44 % to 95 % and 35 % to 86 % respectively as shown in Fig. 1 & 2. Relative standard deviation was varying from 0.491 to 4.623 %. The encapsulation efficiency is a combined effect of both the factors but significantly dependent on concentration of sodium alginate than that of carbomers. Drug encapsulation increased with increasing concentration of sodium alginate, but later on it decreased with increasing concentration of carbomer. This may be due to possible competition for space between drug and swelled carbomer chains[18].

b) In vitro Mucoadhesion:

Equation 3 and Equation 4 shows the factorial analysis for % Mucoadhesion of Polycarbophil and Carbopol 934 P microspheres respectively.

$Y = 96.98 + 1.667X_1 + 2.154\ X_2 - 0.512\ X_1X_2 + 0.025\ X_1^2 + 0.075\ X_2^2$ ……. (3)

$Y = 94.68 + 2.833X_1 + 3.929\ X_2 - 0.737\ X_1X_2 - 0.025\ X_1^2 + 0.425\ X_2^2$……. (4)

Mucoadhesion of microspheres varied from 88 % to 100 % (Fig.2 & 3), indicating excellent mucoadhesive property of microspheres. Although both factors showed positive effect, the effect of carbomer concentration was much dominant than sodium alginate, which may be due to higher capacity of carbomer to adhere to gastric mucosa[18, 19] and secondly due to gelation of sodium alginate due to reaction with calcium that may reduce the mucoadhesive bond formation by sodium alginate.

c) Swelling study:

Both factors show positive effect on swelling index (Fig.3 & 4). But the factorial analysis shows that swelling property was significantly dependent on concentration of carbomers. The reason for this may be the higher water sorption capacity of carbomers than sodium alginate[18].

The factorial equations for swelling index in Polycarbophil (Equation 5) and Carbopol 934 P (Equation 6) microspheres are-

$Y = 1.373 + 0.072\ X_1 + 0.317\ X_2 + 0.105\ X_1X_2 + 0.04\ X_1^2 + 0.07\ X_2^2$……. (5)

$Y = 1.713 + 0.0517\ X_1 + 0.533\ X_2 - 0.015\ X_1X_2 + 0.03\ X_1^2 + 0.09\ X_2^2$…… (6)

d) Scanning Electron Microscopy:

SEM studies (Fig. 4) indicate that microspheres are spherical in shape with a rough surface due to presence of carbomer coat. Drug is seen as adsorbed or embedded particles on the surface of microspheres (Fig. 4a, 4b, 4c). Cut view of blank microsphere shows empty cavity which may be created in the process of gelation and curing of calcium alginate (Fig. 4d). Due to presence of cavity accommodation of drug in microsphere is easier.

e) Particle size distribution:

Microspheres with higher polymer concentration were found to be more spherical. Average particle size varies from 200 to 400 µm, increases with increasing polymer concentration. Increased viscosity affects the performance of spray, forming larger droplets while spraying.

f) Dissolution study:

Drug dissolution profiles are shown in Fig. No.5 & 6. Sustained release of drug from polymer matrix is a combined effect of cross-linked polymer network of sodium alginate and swelled carbomer chains. The release was retarded up to 9-10 hours with higher carbomer concentration batches. $T_{50\%}$ (Time required for 50% dissolution) was found to be lengthened as the concentration of carbomer and sodium alginate increased. The 'n' values are less than 0.5, which indicate the Fickian release with initial rapid release followed by tailing off overtime (Table No.3). The best fit dissolution models with most of the combinations were Peppas type but it was matrix type with higher polymer concentration batches (A7-A9 and B6, B8 and B9). The initial fast release may be due to easy drug diffusion from the surface of microspheres as observed in SEM images.

Dissolution retardation is dependent on both the factors. It may be due to the hydrogel structure of cross-linked calcium alginate network, which encapsulates the drug and swelling of mucoadhesive polymers. In the dry state, the drug is entrapped in the glassy core. As the external surface of microspheres is hydrated, it forms a gelatinous layer, which is responsible for increased path length for drug diffusion[19].

The factorial equation for $T_{50\%}$ in Polycarbophil (Equation 7) and Carbopol 934 P (Equation 8) microspheres are as follows:

$Y = 1.513 + 0.252\ X_1 + 0.167\ X_2 + 0.0850\ X_1X_2 + 0.03\ X_1^2 - 0.01\ X_2^2$ ... (7)

$Y = 1.478 + 0.250\ X_1 + 0.133\ X_2 + 0.0838\ X_1X_2 + 0.0325\ X_1^2 - 0.0025\ X_2^2$ …(8)

Carbomer concentration was found to be slightly less significant than that of sodium alginate because of the fact that carbopol particles have a high concentration of ionic groups inside, causing large influx of water by osmosis, swelling the particles until the cross-links are strained. This will lead to rapid diffusion of drug out of the polymer[18].

CONCLUSION

The results of a 3^2 full factorial design revealed that sodium alginate and mucoadhesive polymer concentration significantly affected the dependent variables like mucoadhesion, dissolution profile drug entrapment efficiency and swelling index.

Table 1: Factorial Design for Preparation of Batches

Batch Code	Variable levels in Coded form	
	X_1	X_2
1	-1	-1
2	-1	0
3	-1	+1
4	0	-1
5	0	0
6	0	+1
7	+1	-1
8	+1	0
9	+1	+1

Table 2: Translation of coded levels in actual units:

Variable levels	Low (-1)	Medium (0)	High (+1)
X_1= Concentration of sodium Alginate (%w/v)	1	1.5	2
X_2= Concentration of Carbomer (%w/v)	1	1.5	2

Table No. 3: Evaluation of Mucoadhesive microspheres

Batch Code	% Encapsulation	% Mucoadhesion	Swelling index	Drug Dissolution		Average Particle Size µm
				$T_{50\%}$ (hr)	'n' value	
A1	57.969 ± 3.177	93	1.2	1.2	0.4181	208.5
A2	81.55 ± 3.004	94	1.4	1.3	0.4442	244.5
A3	95 ± 2.835	96	1.7	1.5	0.4479	288.5
A4	73.81 ± 3.636	95	1.1	1.3	0.4241	340.5
A5	75.328 ± 1.523	97	1.4	1.3	0.4329	319.5
A6	82.867 ± 2.595	99	1.7	1.6	0.4539	259.5
A7	53.365 ± 2.705	97	1.2	1.6	0.4431	288.75
A8	56.606 ± 2.704	99	1.6	1.7	0.4680	292.5
A9	44.559 ± 4.528	100	2.0	1.9	0.4981	380.25
B1	48.512 ± 1.095	88	1.1	1.2	0.4195	214.5
B2	62.25 ± 2.634	90	1.3	1.3	0.4425	258.5
B3	51.352 ± 1.106	92	1.5	1.5	0.4593	312.25
B4	38.423 ± 0.491	91	1.3	1.4	0.4711	380.75
B5	85.945 ± 2.256	94	1.4	1.5	0.4698	408.5
B6	35.249 ± 4.623	99	1.7	1.6	0.4886	388.5
B7	38.656 ± 0.965	95	1.6	1.7	0.4698	340.5
B8	50.03 ± 1.374	99	1.9	1.8	0.5034	380.25
B9	40.714 ± 1.504	100	2.1	2.0	0.5320	319.5

Fig. 1: Contour plot for Encapsulation efficiency of Polycarbophil Microspheres:

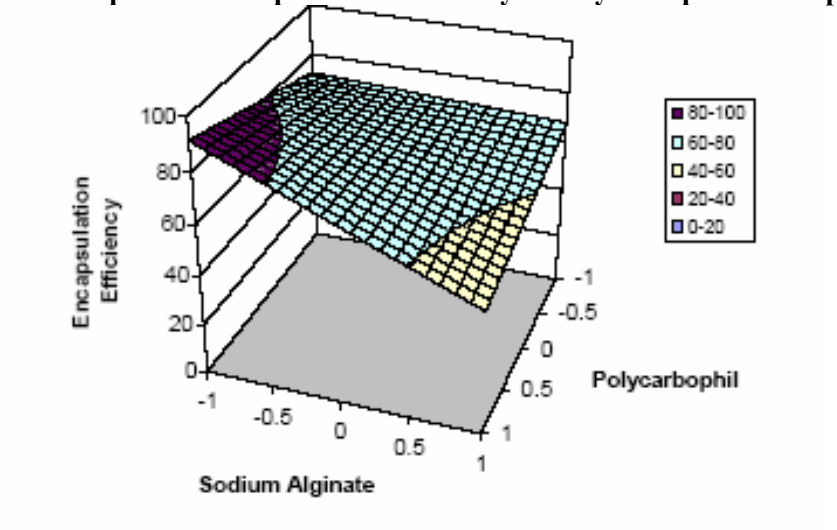


Fig. 2: Contour plot for Encapsulation efficiency of Carbopol 934P Microspheres:

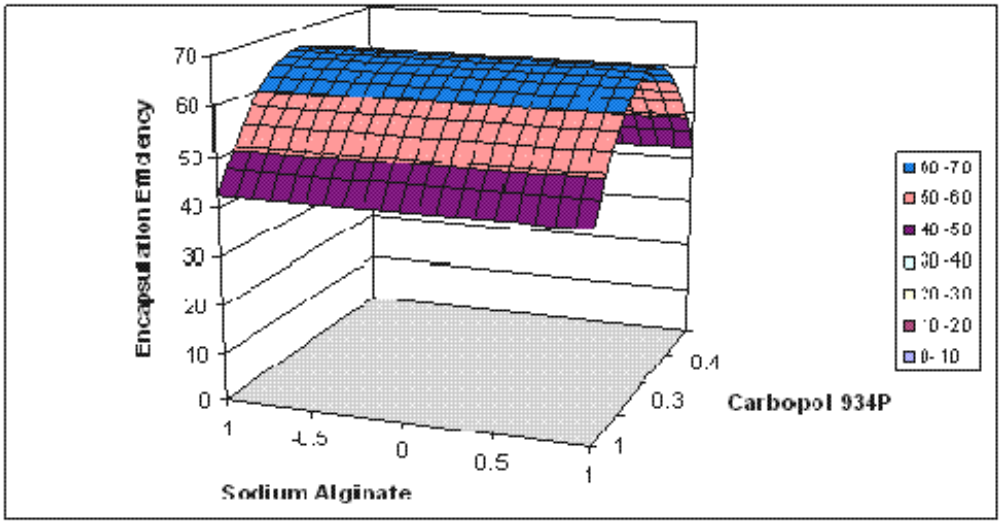


Fig.3: Contour plot for Bioadhesion study of Polycarbophil microspheres

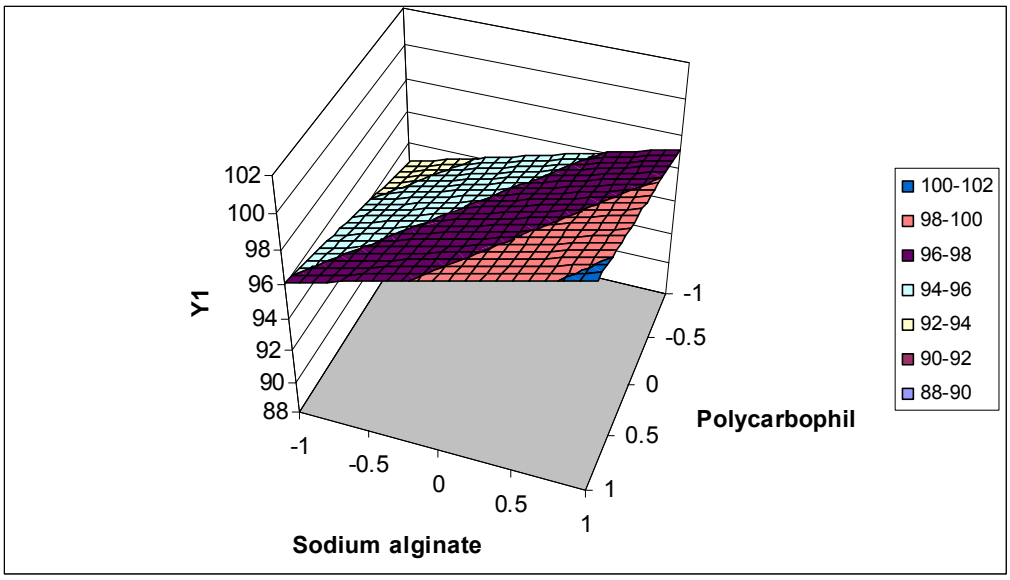


Fig.4: Contour plot for Bioadhesion study of Carbopol 934P microspheres

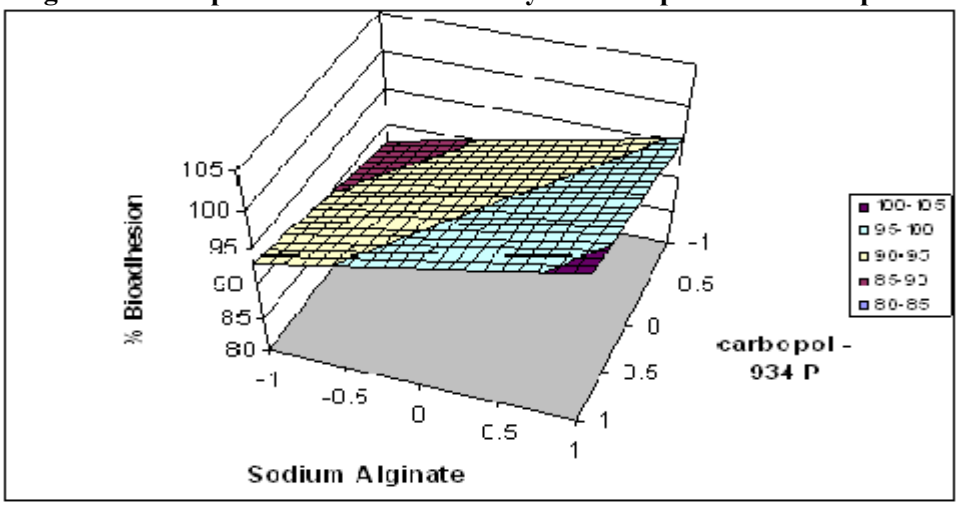


Fig.5: Contour plot for swelling study of Polycarbophil microspheres

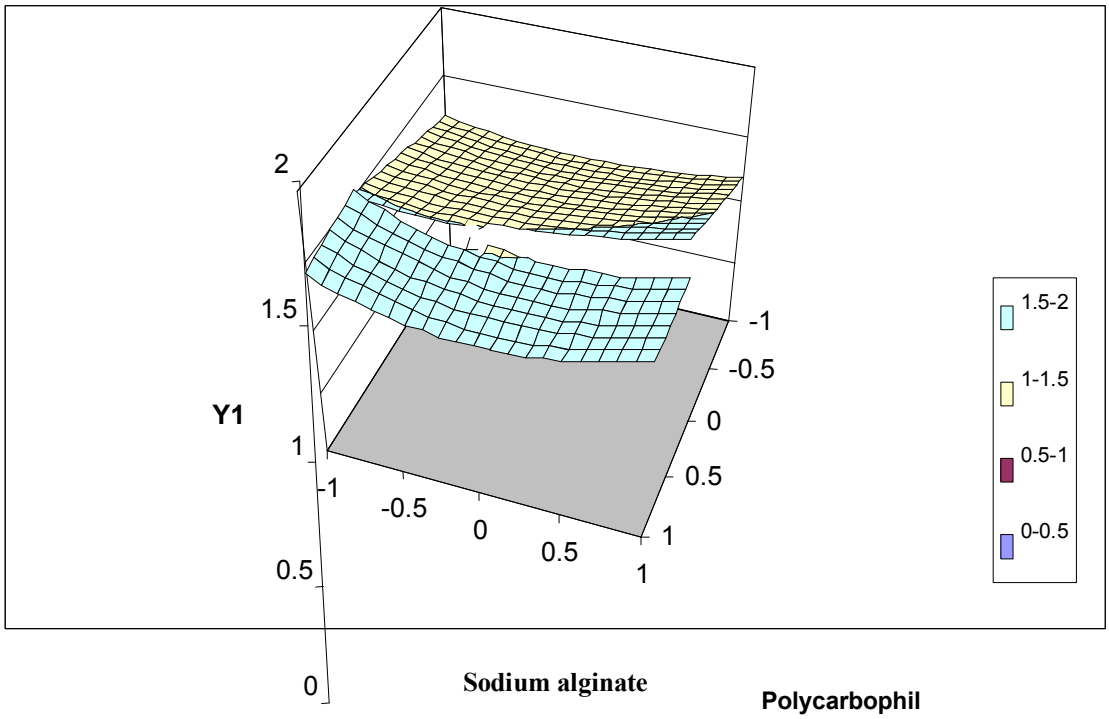


Fig.6: Contour plot for swelling study of Carbopol 934P microspheres

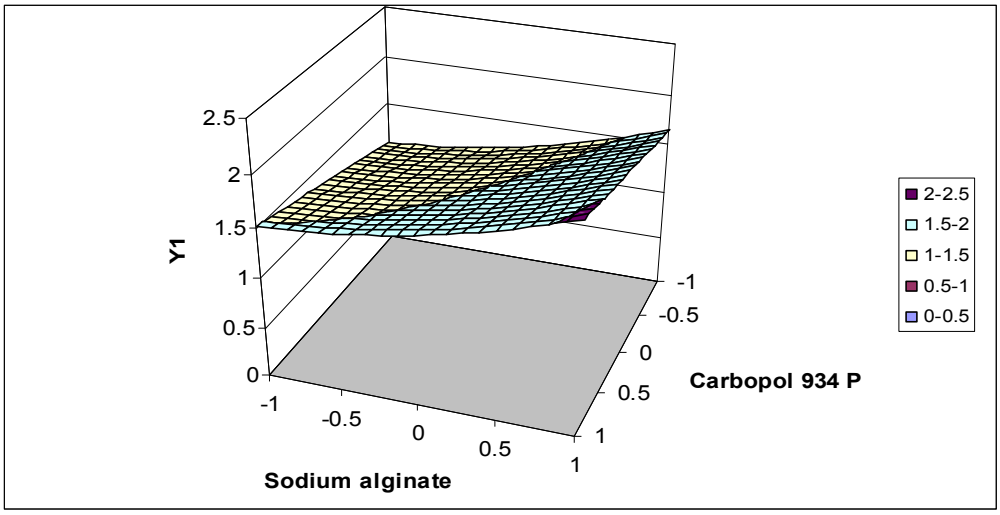
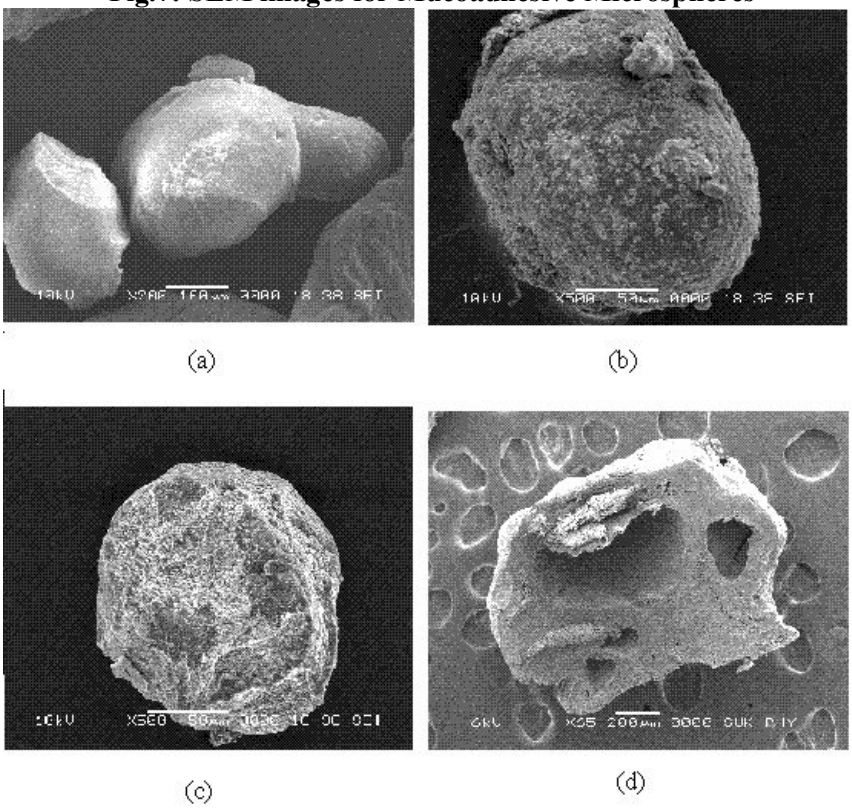


Fig.7: SEM images for Mucoadhesive Microspheres



a- A2 b- A7 c- B7 d- Cut view of blank microsphere

Fig. 8: Drug Dissolution study of Polycarbophil microspheres

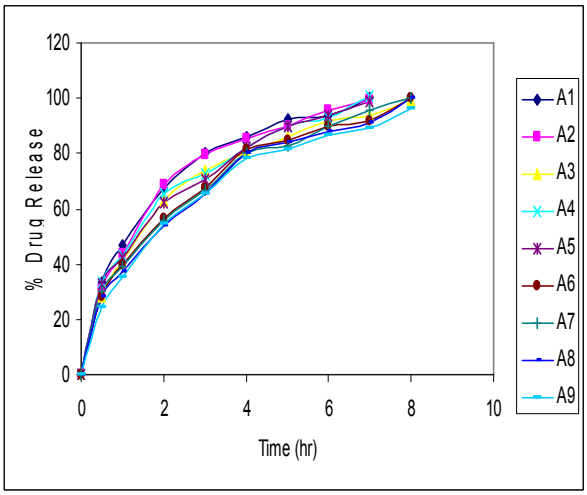
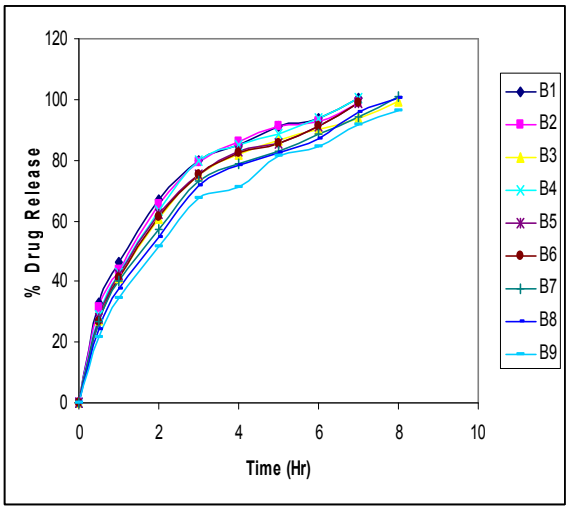


Fig. 9: Drug Dissolution study of Carbopol 934P microspheres



REFERENCES

1. Ananthnarayan, Panikar. **Textbook of Microbiology**. Orient Longman, 7th Edition, 2005, 407-8.
2. Bennet P.N, Brown M.J. **Clinical Pharmacology**, Churchill Livingstone 9th Edition, 2003,631-32.
3. Jasti D.B., Xiaoling L.I, Cleary G. Recent Advances in Mucoadhesive Drug Delivery System. **Business Briefing Pharmatech,** 2003, 194-196.
4. Jin W.L.et al; Bioadhesive-based dosage forms: The Next Generation; Mini Review; **J Pharm. Sci.**, 2000, 89(7), 850-866.

5. Jochen H., Claus M. L. Development in the area of bioadhesive drug delivery systems; **Expert Opin. Biol. Ther.** 2002, 2(3), 287-98.
6. Patil V.B., Pokharkar V.B. Preparation and Evaluation of Sustained release Nimsulide Microspheres prepared from Sodium Alginate. **Indian J. Pharm. Sci**. 2001, 63 (1), 15-19.
7. Chowdary K.P.R., Rao S. Mucoadhesive Microspheres and Microcapsules: Current Status; **Indian J.Pharm. Sci.**, 2005, 67(2), 141-50.
8. Nagahara N., Akiyama Y., Nakao M., Tada M., Kitano M., Ogawa Y. Mucoadhesive Microspheres Containing Amoxycillin for Clearance of Helicobacter pylori; **Antimicrobial Agents and Chemotherapy**, 1998, 42(10), 2492-94.
9. Liu Z., Lu W., Qian L, In vitro and in vivo Studies on Mucoadhesive Microspheres of amoxycillin; **J. Control Release**. 2005, 102 (1), 135-44.
10. Shah S., Quqish R., Patel V., Amiji M. Evaluation of the factors influencing Stomach-Specific Delivery of Antibacterial Agents for Helicobacter pylori infection; **J.Pharm. Pharmacol**, 1999, 51, 667-72.
11. Umamaheshwari R.B., Ramteke S, Jain N.K Anti–*helicobacter pylori* effect of mucoadhesive nanoparticles bearing amoxicillin in experimental gerbils model. **AAPSPharmscitech**. 2004; 5(2), article 32.
12. Chowdary K.P.R., Rao Y.S. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: a technical note; **AAPSPharmscitech** 2003; 4(3), article 39
13. Chowdary K.P.R., Rao Y.S. Preparation and evaluation of mucoadhesive microcapsules of Indomethacin**, Saudi Pharm. J.**, 2003, 11 (3), 97-103.
14. Patel J.K., Patel R. P., Amin A.F., Patel M.M. Formulation and Evaluation of Mucoadhesive Glipizide Microspheres**; AAPSPharmScitech**, 2004, 12, 24-9.
15. Kuchekar B.S., Patil R., Doke S. Development of Spectrophotometric method of estimation for azithromycin and Clarithromycin in pharmaceutical dosage forms, **Indian Drugs,** 2003, 40(1), 44-46.
16. Alessia de ascentiis A,B, Janet L. Degrazia C, Christopher N. Bowman C, Paolo colombo B, Nikolaos A., Peppas A. Mucoadhesion of poly (2-hydroxyethyl methacrylate) is improved when linear poly (ethylene oxide) chains are added to the polymer network. **J. Control Rel**, 1995, 33, 197-201
17. Baloglu E., Hýzarcýoglu S. Y., Karavana H. A. An alternative Evaluation method for swelling studies of Bioadhesive tablet formulations Pharmaceutical development and technology 9, (3), pp. 233–237, 2004
18. Hosmani A.H., Thorat Y.S., Kasture P.V. Carbopol and its Pharmaceutical Significance: A Review, 2004, http://www.pharmainfo.net/reviews/carbopol-and-its-pharmaceutical- significance-review
19. Product Regulatory Guide. **Noveon Pharmaceutical Polymers,** Bulletin 2: 1-8, www.pharma.noveoninc.com.