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Mucoadhesive microcapsules of amoxicillin trihydrate for effective treatment of *H. pylori*

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ABSTARCT

The aim of present research work was to develop amoxicillin trihydratemucoadhesive microcapsules to prolong residence time of dosage form in stomach and to achieve controlled drug release for more effective H. pylori eradication. Microcapsules containing sodium alginate and mucoadhesive polymer (carbopol 934 P, Hydroxy Propyl Methyl Cellulose (HPMC), Sodium Carboxy Methyl Cellulose (SCMC), and Methyl Cellulose (MC))were prepared by orifice ionic gelation method and evaluated for morphology, particle size analysis, percentage yield, drug content, drug entrapment efficiency, swelling ratio, mucoadhesive properties and in vitro drug release. Prepared microcapsules were spherical, free flowing and exhibited good mucoadhesive property. Average particle size for microcapsules was in the range of 300 to 550 µm and encapsulation efficiency was in the range of 51–69%. Formulations containing alginate-carbopol 934P as showed more mucoadhesion, swelling and drug entrapment efficiency than alginate-HPMC, alginate-SCMC and alginate-MC. As per pictures of scanning electron microscopy, optimized batch F9 (containing one part of carbopol and nine parts of alginate) proved that microcapsules were spherical having size range of 500-550µm and uniformly covered with the coat polymer. The optimised batch showed zero order drug release up to 10 - 12 h which suggested that the optimised mucoadhesive microcapsules are promising for controlled drug delivery of amoxicillin trihydrate for effective treatment.

Key words: Mucoadhesive, Amoxicilline, Microcapsule, controlled release

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INTRODUCTION

Microcapsule as a part of carrier system accepted as an approach for controlled release and drug targeting[1].Controlled release drug delivery systems are designed for controlling the rate of drug delivery, sustaining the duration of therapeutic activity and/or targeting the delivery of the drug to a specific tissue[2].When compared with single unit sustained release tablets, multiunit controlled release dosage forms like microcapsules and microspheres release drugs more uniformly and prevent dose dumping[3].

Helicobacter pylori have become recognized as a major gastric pathogen with worldwide distribution. *H. pylori* are a spiral-shaped bacterium found in the stomach, which (along with acid secretion) damages stomach and duodenal tissue, causing inflammation and peptic ulcers[4,5]. Amoxicillin (a-amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, board-spectrum antibiotic which is widely used in the standard eradication treatment of gastric and duodenal ulcers, which are associated with *H. pylori* infection combined with a second antibiotics and an acid-suppressing agent. However, some reports and clinical trials indicate that the therapies cannot bring out compete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation [6]. One of the reasons for failure of complete eradication of *H. pylori* with conventional dosage forms of amoxicillin includes shorted residence time in the stomach [7,8].

Yellanki et al prepared microsphere of amoxicillin trihydrates by emulsification/ evaoparation method using organic solvent.[3].Microsphere is a homogeneous structure made of a continuous phase of one or more miscible polymers

in which particulate drug is dispersed throughout the matrix, at either the macroscopic (particulates) or molecular (dissolution) level. Microcapsule is a reservoir-type system with regular or irregular shapes that contains a welldefined core and envelope. The core can be solid, liquid, or gas and the envelope are made of a continuous, porous or nonporous, polymeric phase created by one or more polymers[9].Microcapsules are very small droplets or particles of liquid or solid material, coated with a continuous film of polymeric material. However, the success of these microcapsules is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microcapsules and developing bioadhesive microcapsules[10]. Bioadhesive microcapsules shows efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site[11].

In the present study, mucoadhesive microcapsules of amoxicillin trihydrate were prepared to prolong residence time of the formulation in stomach and to achieve controlled drug release for more effective treatment of *H. pylori* eradication.

MATERIALS AND METHODS

Amoxicillin trihydratewas procured as a gift sample from Tuton Pharmaceuticals, (Ahmedabad, India). Sodium alginate, potassiumdihydrogen phosphate and calcium chloride were purchased from S.D. Fine chem. Ltd.(Mumbai, India). Carbopol, HPMC E 50LV, sodium carboxymethylcellulose and methyl cellulose were purchased from Corel Pharma Chem (Ahmadabad, India), Yerrow chem. Pvt. Ltd(Mumbai, India), Chemport private limited (Mumbai, India) and Suvidhinath laboratories(Baroda, India); respectively. All other chemicals used were of analytical grade.

2.1 Identification of drug

The identification of amoxicillin trihydrate was carried out using Fourier transform Infrared spectroscopy (FTIR). Samples were prepared in KBr disks by means of a hydrostatic press. FTIR spectrum was recordedin the range of 3500 to 500 cm^{-1} using a Shimadzu FTIR spectrometer and compared with the reference spectrum of amoxicillin trihydrate present in Indian Pharmacopoeia 2007.

2.2Preparation of amoxicillin trihydratemucoadhesivemicrocapsules

Microcapsules of amoxicillin trihydrate were prepared by an orifice- ion gelation method. Compositions of amoxicillin trihydratemucoadhesive microcapsules are mentioned in table 1. Sodium alginate and mucoadhesive polymer (carbopol 934 P / Hydroxy Propyl Methyl Cellulose (HPMC)/ Sodium Carboxy Methyl Cellulose (SCMC)/ Methyl Cellulose (MC)) were dissolved in15 ml purified water to form a homogeneous polymer solution. Amoxicillin trihydratewas dissolved in 5 ml purified water and added to the polymer solution. The resulting mixture was mixed thoroughly to form a smooth viscous dispersion and was added dropwise into 40 ml calcium chloride (10% w/v) solution through a syringe with a needle of no 23. The added droplets were retained in the calcium chloride solution for 15 minutes for completing the curing reaction to produce microcapsules. The microcapsules were collected by decantation; washed repeatedly with water and dried at 45^0C for 12 h. The prepared microcapsules were stored in a desiccator for further studies.

Table 1: Compositions of amoxicillin trihydratemucoadhesive microcapsules

Batch no.	Amoxicillin trihydrate (mg)	Sodium alginate (mg)	Carbopol 934P (mg)	HPMC (mg)	SCMC (mg)	MC (mg)
F1	100	300	100	-	-	-
F2	100	300	-	100	-	-
F3	100	300	-	-	100	-
F4	100	300	-	-	-	100
F5	100	700	100	-	-	-
F6	100	700	-	100	-	-
F7	100	700	-	-	100	-
F8	100	700	-	-	-	100
F9	100	900	100	-	-	-
F10	100	900	-	100	-	-
F11	100	900	-	-	100	-
F12	100	900	-	-	-	100

2.3 Evaluations of amoxicillin trihydratemucoadhesive microcapsules

The prepared amoxicillin trihydratemucoadhesive microcapsules were evaluated for morphology, particle size and shape, yield, drug content, microencapsulation efficiency, swelling ratio, mucoadhesive properties, *in vitro*drug release.

1.3.1.Particle size and shape of microcapsules

The particle size and shape of 100 microcapsules wasanalyzed by an optical microscopy.

1.3.2.Flow propertyof microcapsules

Flow property of microcapsules was indicated by angle of repose which was estimated using fixed height funnel method.

1.3.3.Yields of production

The yields of production of microcapsules of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microcapsules. Percentage yield was calculated as per equation 1.

$$\% \text{ Yield} = \frac{\text{Practical mass (microcapsules)}}{\text{Theoretical mass (drug + polymers)}} X\ 100 \quad (1)$$

1.3.4.Swelling study

50 mg of microcapsules were placed in a glass vial containing 10 ml of distilled water at 37±0.5ºC in an incubator with occasional shaking. The microcapsules were periodically removed, blotted with filter paper, and their changes in weights were measured during the swelling until equilibrium was attained. Weight of the swollen microcapsules was recorded after a period of 3 h and swelling ratio was calculated using equation 2.

$$Swellingratio = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} X\ 100 \quad (2)$$

Whereas, W_t = Equilibrium weight of microcapsules after swelling and Wo = Initial weight of microcapsules

1.3.5.Microencapsulation efficiency

Microcapsules equivalent to 100 mg drug were crushed in mortar and pestle; added to 100 ml 0.1 N HCland shaken for 1 hr at 37±0.5^0 C in sonicator. The samples were filtered to obtain a clear solution and analysed for the drug content using UV spectrophotometer (UV-1800shimadzu) at 272 nm.

Percentage encapsulation efficiency was calculated using equation 3.

$$\%\ \text{encapsulation efficiency} = \frac{\text{Calculated \% drug content}}{\text{Theoretical \% drug content}} X\ 100 \quad (3)$$

1.3.6.*In vitro* mucoadhesion study

The mucoadhesive property of microcapsules was evaluated by an *in vitro* adhesion testing method known as wash off method. A 2 cm wide and 2 cm long (2×2) piece of rat intestinal mucosa was tied onto a glass slide (3 in. long and 1 in. wide) using thread. About 50 microcapsules were spread onto wet tissue specimen and allowed to hydrate for 30 s. The prepared slide was hung onto one of the grooves of a USP 24 tablet disintegrating test apparatus and given regular up and down movements in the test fluidat 37 °C. 0.1N HCl and pH 7.4 phosphate buffer was used as the test fluid to check mucoadhesion of microcapsules in acidic as well as alkaline pH. At the end of each 1 hr interval up to 8 hr, the apparatus was stopped and number of microcapsules still adhering to the tissue was counted.

1.3.7.*In- vitro* drug release studies

In vitro drug release studies of microcapsules were carried out using USP XXIII Eight-station dissolution rate test apparatus Type-II with a paddle stirrer (TDT-06T, Electro Lab) at 100 rpm. Microcapsules containing 100 mg of drug were placed in the vessel containing 900 ml 0.1 N HCl maintained at 37±0.5^{o}C. 10 ml of samples were withdrawn at regular interval of 1 h up to 12 h and replaced with fresh medium maintain at same temperature. Samples were filtered through 0.45 micrometer Whatman filter paper and absorbance of the filtrates was measured after suitable dilution using UV visible double beam spectrophotometer (UV -1800, Shimadzu) at the wavelength of 272 nm. The cumulative percentage drug release was calculated and plotted as a function of time. Drug release from microcapsule of optimized batch was also studied in pH 7.4 phosphate buffer.

Kinetics modelling of drug dissolution profiles

The dissolution profile of all the batches was fitted to Zero order (equation 4), First order (equation 5) Higuchi (equation 6) and Korsmeyer-Peppas (equation 7) model to ascertain mechanism of drug release.

Zero order release model:

$$m = k * t \quad [4]$$

Where, k is zero-order constant, m is the % drug unreleased and t is the time. The plot of % drug released versus time is the linear.

First order release model:

$$m = ea * e\text{-}bt \quad [5]$$

Where a is the intercept and b is the slop. It assumes that the drug molecules, diffuses out through a gel like layer formed around the drug during the dissolution process. A plot of log % drug release versus time is the linear.

Higuchi Model:

$$m = 100 - q * \text{square root of time} \quad [6]$$

Where q is the Higuchi constant(% per square root of time), in Higuchi model, a plot of % drug unreleased (released) versus square root of time is linear.

Korsmeyer Peppas Model:

$$Mt/M\infty = kt^n \quad [7]$$

Where, $Mt/M\infty$ is the fraction of drug released at time t, k the kinetic constant and n is the release exponent that characterizes the mechanism of drug release. if n= 0.45 indicates drug release mechanism by Fickian diffusion, and if $0.45 < n < 0.89$, then it is non Fickian or anomalous diffusion.

1.3.8.Scanning electron microscopy (SEM) study of microcapsules

Particle size, shape and surface morphology of microcapsules of optimized batch were examined by scanning electron microscopy (SEM) study (Eacn edex-xl-30, Sicard). Microcapsules were mounted onto the sample stub using double sided sticking carbon tape and coated with platinum film. Scanning Electron photographs were taken at an accelerating voltage of 30 kV, chamber pressure of 0.8 mm Hg.

1.3.9.Stability studies

Amoxicillin trihydrate mucoadhesive microcapsules were weighed; wrapped in abutter paper and subjected at two different temperature conditions, at room temp (25 ± 2^0C) and at elevated temperature (45 ± 2^0C), for 3 months. Stored microcapsules were analyzed for physical changes such as colour, texture, mucoadhesion, drug entrapment and *in-vitro* drug release at interval of 1 month up to 3 months.

RESULTS AND DISCUSSION

3.1. Identification of drug

All principle peaks were present in the IR spectra of drug when compared with the reference spectrum of amoxicillin trihydrate given in Indian Pharmacopoeia 2007(Figure 1). Peaks at wave number 1773.39, 1685.62, 1575.42 and 1518.53 cm^{-1} indicated vibrations of β-lactam, Amide C=O stretching, COO- asymmetric stretching and aromatic ring, respectively.

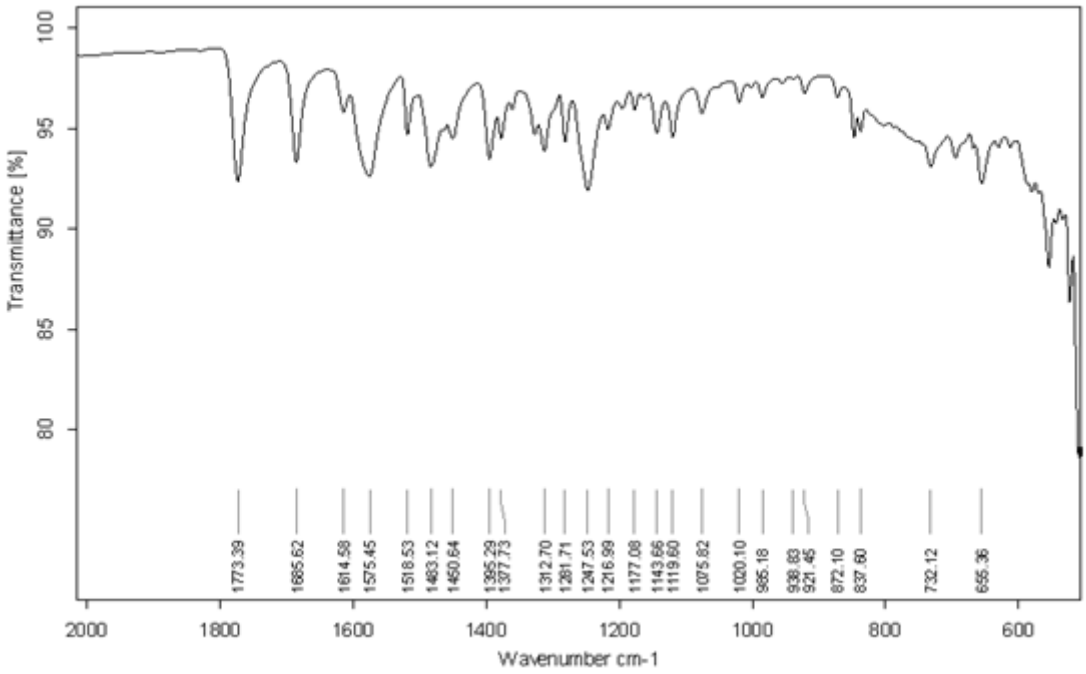

Figure 1:FTIR Spectrum of amoxicillin trihydrate

3.2. Particle size and shape of microcapsules

The drug loaded microcapsules of batch F1-F12 were yellowish white in appearance. They were found to be discrete, large and spherical. Particle size of microcapsules was in the range of 300 to 550µm (Table 2). Sodium alginate had a great influence on size and shape of microcapsules. Particle size of microcapsule was increased as increased in alginate concentration might be due to more viscous nature of polymer solution. Microcapsules containing higher amount of alginate (Batch F9-F12) were more spherical and regular as compared to that of microcapsules having lower percent of alginate (Batch F1-F4). Although, it was observed that as increasing the concentration of sodium alginate beyond 900 mg produced non–spherical microcapsules.

Table 2: Results of particle size, % yield and encapsulation efficiency of microcapsules

Batch no,	Particle size (µm)	% yield	% Entrapment efficiency
F1	300-340	95.12	56.82
F2	305-345	99.03	51.87
F3	305-340	98.63	53.79
F4	307-345	81.75	54.68
F5	400-445	93.70	62.48
F6	405-450	99.80	57.92
F7	404-445	95.10	58.28
F8	405-445	98.30	60.13
F9	500-550	**99.13**	**69.76**
F10	505-545	91.73	61.45
F11	505-540	96.55	63.43
F12	504-550	96.19	67.82

3.3. Flow property

Angle of repose of prepared microcapsules of each batch was less than 20 which indicated excellent flow property.

3.4. Percentage yield

The production yields of prepared formulations were in the range of 81.75 to 99.13% (table 2). Maximum yield was observed for batch F9 having alginate-carbopol 934 P whereas, minimum yield was observed for batch F4 having alginate-methyl cellulose.

3.5. Swelling study

Swelling of microcapsules increased with time which could explained by the weight gain of microcapsules. The graph of swelling index v/s time is shown in figure 2. Maximum swelling of microcapsules was observed within 8 h, after which polymer started eroding slowly. The swelling index showed that formulation containing higher ratio of alginate having maximum swelling index. The microcapsules of A9 batch containing carbopol 934 P showed maximum swelling property as compared to HPMC, SCMC, MC because Carbopol 934 P has viscosity more compared to HPMC, SCMC, and MC resulting in low binding forces between the molecules. Linearity in swelling index indicated the sustained release of drug.

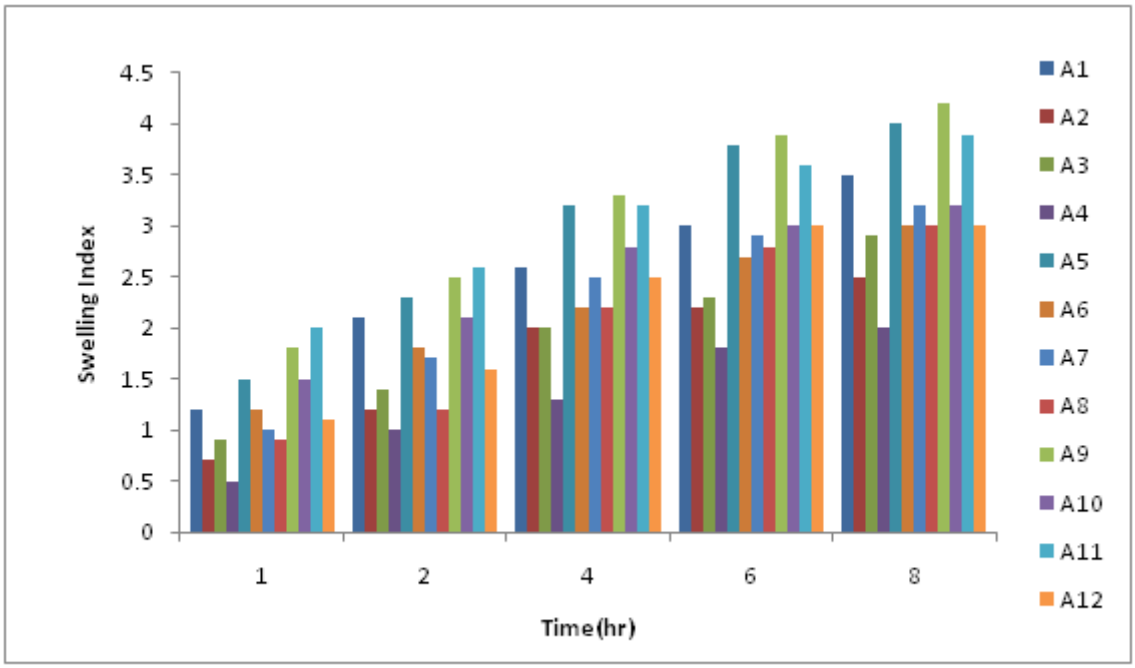

Figure 2: Swelling Index of microcapsules (Batch F1-F12)

3.6 Microencapsulation efficiency

The entrapment efficiency of microcapsules of batch F1 to F12 was in the range of 51 to 69% (Table 2). Encapsulation efficiency of the microcapsules was dependent mainly on the concentration of sodium alginate. As concentration of sodium alginate increased, the encapsulation efficiency of the microcapsules increased. Microencapsulation efficiency for sodium alginate–carbopol 934P was found higher compared to sodium alginate–HPMC/ SCMC/MC.Batch F9 having maximum concentration of alginate and carbopol 934 P showed maximum microencapsulation efficiency.

3.7. *In vitr o*mucoadhesion study

Percentage of microcapsules adhering to mucosa in 0.1 N HCl and phosphate buffer till 8 h is shown in table 3 and 4, respectively. No significant differences were obtained ($p>0.05$) in percentage mucoadhesion in 0.1 N HCl and phosphate buffer at 1, 2, 4, 6 and 8 h. The mucoadhesion study showed that formulations containing higher ratio of alginate showed higher mucoadhesion (Batch F9-F12) compared to that of microcapsules having lower percent of alginate (Batch F1-F4). Formulations containing alginate-carbopol 934P as showed more mucoadhesion than alginate-HPMC/SCMC/MC. The greater mucoadhesive property of alginate- carbopol 934P is due tothe presence of a certain amount of unionized carboxyl groups present within carbopol934P which forms a strong gel network with the mucus glycoprotein network of the intestinal mucosa.

Table 3:Results of *In-vitro* wash off test of microcapsules in 0.1 N HCl

Batch no.	Percent of microcapsules adhering to mucosa at different time				
	1 h	2 h	4 h	6 h	8 h
F1	92	80	56	44	10
F2	70	58	34	22	-
F3	80	74	44	38	8
F4	74	66	48	32	-
F5	94	84	64	44	18
F6	78	52	42	28	-
F7	84	70	54	48	16
F8	86	58	44	40	8
F9	**96**	**86**	**60**	**52**	**30**
F10	80	63	41	38	12
F11	90	78	62	50	19
F12	84	64	42	48	16

Table 4:Results of *In-vitro* wash off test of microcapsules in pH 7.4 phosphate buffer

Batch no	Percent of microcapsules adhering at different times (hr)				
	1 h	2 h	4 h	6 h	8 h
F1	86	74	48	34 (1)	6
F2	64	50	26	20 (1)	-
F3	76	70	40	38 (6.33)	4
F4	70	62	44	30 (4)	-
F5	88	78	54	42 (1)	12
F6	70	54	32	24 (1)	-
F7	78	76	50	44 (2.33)	9
F8	76	66	44	36 (1)	7
F9	**90**	**84**	**56**	**48 (1)**	**14**
F10	78	56	36	32	8
F11	84	74	52	46	12
F12	80	56	36	42	10

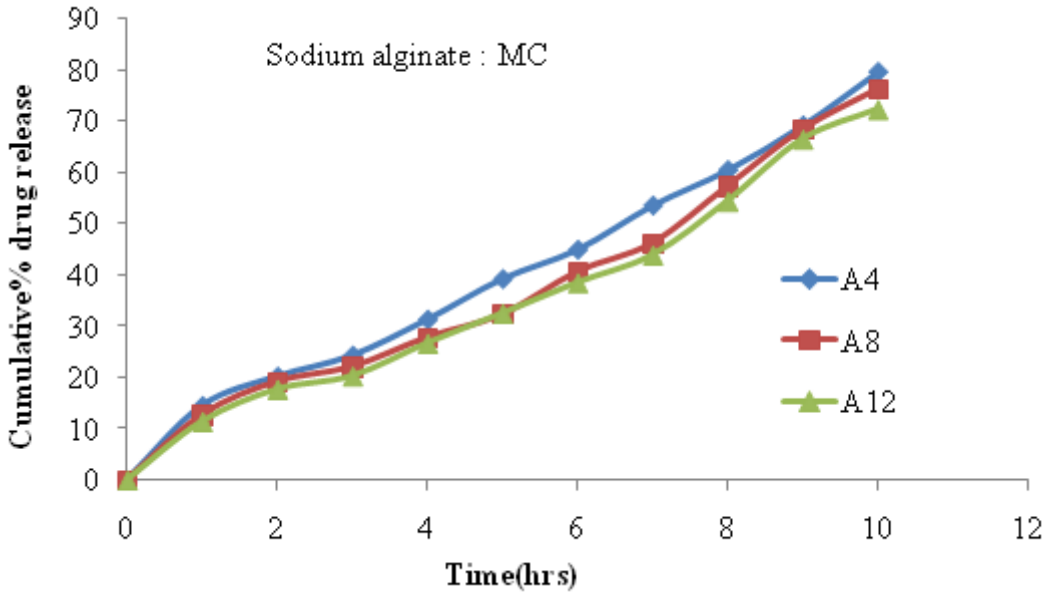

Figure 3:Cumulative % drug release of microcapsules contain alginate-carbopol 934 P

3.7 *In-vitro* drug release studies

All polymers (SCMC< MC< HPMC<Carbopol) were retarded drug release up to 10 hrs (Figure 3, 4, 5 and 6). The order of increasing drug release rate observed with various microcapsules was alginate-SCMC < MC <Carbopol< HPMC. The difference in the drug release characteristics of various microcapsules was due to the difference in solubility of coat material (Carbopol 934-P, SCMC, MC, HPMC) in dissolution medium. Optimized batch F9, containing one part of carbopol and nine parts of alginate, showed slow and extended release up to 10 to 12 h. This may be due to the hydrogel structure of calcium alginate (which encapsulated the drug in its network) and the swelling of carbopol 934P.Batch F9 was considered as an optimised batch based on results of percentage yield, *in vitro* mucoadhesion, microencapsulation efficiency, swelling and *in vitro* drug release. Results of *In-vitro*drug release

study of the batch F9 in 0.1 N HCl and phosphate buffer pH-7.4 were subjected to one way ANOVA (Table 5). The compositions of dissolution medium showed no significant effect on release rate of drug as calculated F value was less than tabulated F value. Although, drug release of batch F9 in 0.1 N HCl was fast as compared to pH7.4phosphate buffer (Figure 7).

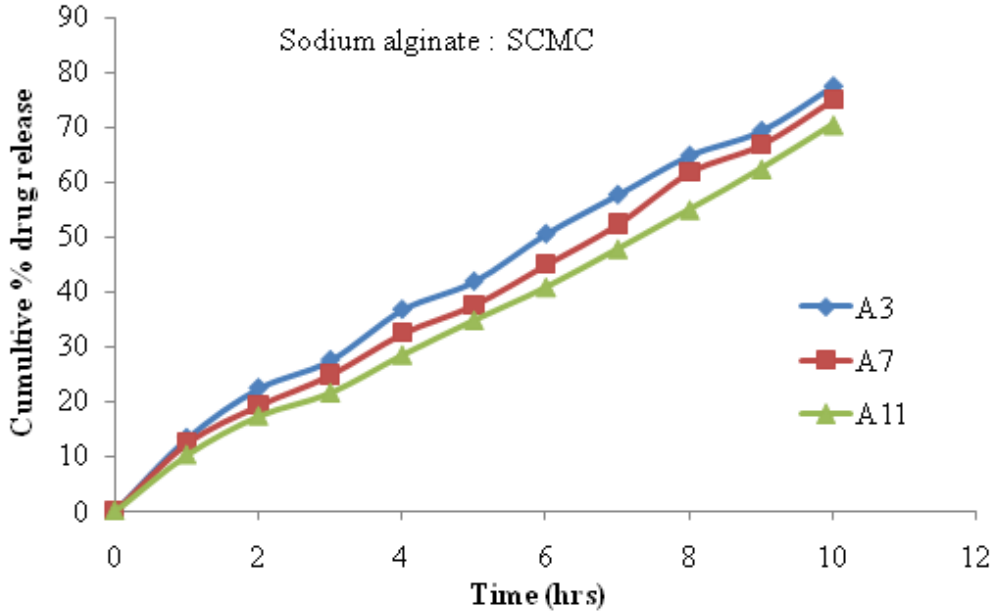

Figure 4: Cumulative % drug release of microcapsules contain alginate-HPMC

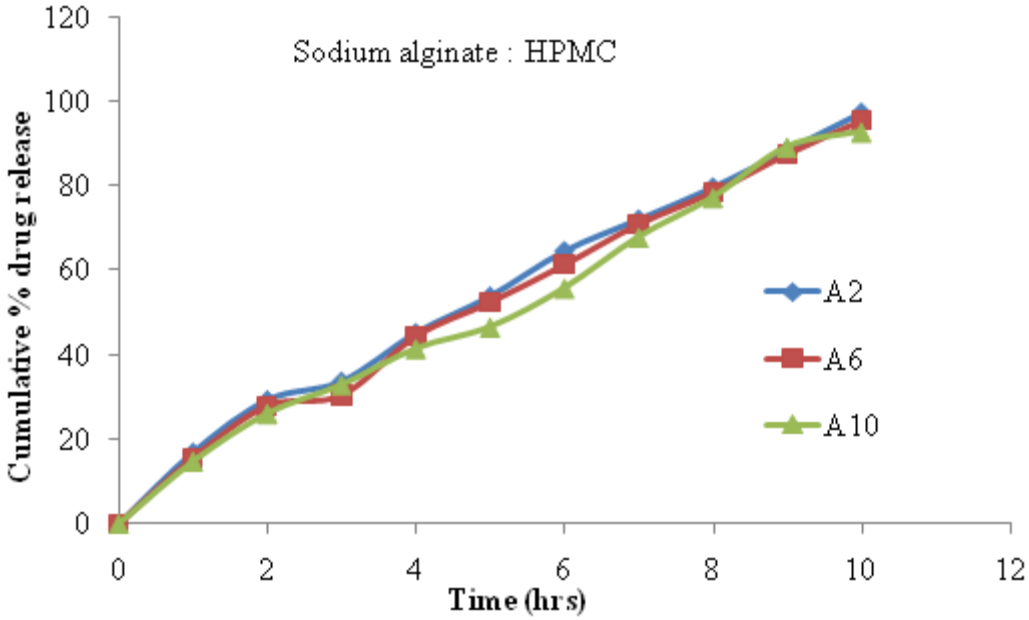

Figure 5: Cumulative % drug release of microcapsules contain alginate-SCMC

Table 5: Results of ANOVA test for comparison of *In-vitro* drug release of Batch A9 in 0.1 N HCl and pH 7.4phosphate buffer

Source of Variation	Sum of Squares	Degree of freedom	Mean of Squares	F calculated	P-value	F tabulated
Between Group	66.6125	1	6.6125	0.099437	0.756133	4.413873
Within Groups	12058.13	18	669.8963	-	-	-

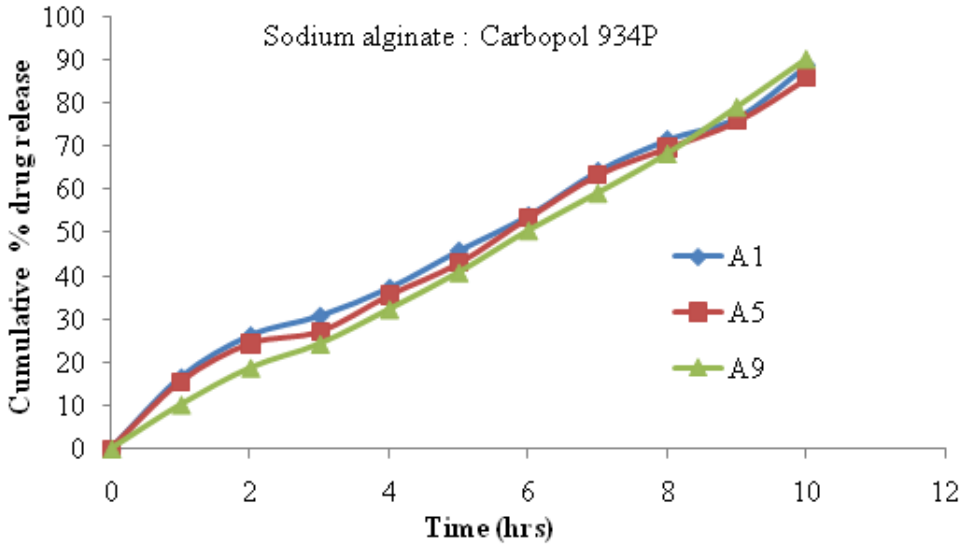

Figure 6: Cumulative % drug release of microcapsules contain alginate-MC

Kinetic model study

Plots of cumulative percent drug release versus time were found to be linear with all the microcapsules indicating that the drug release from these microcapsules was followed zero order kinetics. The regression co-efficient (r^2) values of different model study and the values of Korsmeyer- Peppas co-efficient (n) of all formulations are shown in Table 6. In case of batch F9, regression co-efficient value of zero order model showed more linearity compare to first order, Higuchi, Hixson Crowell and Korsmeyer-Peppas model. n value of Krosmeyer-peppas model was found to be 0.879 which indicated drug release by non Fickian or anomalous diffusion. There is no significant difference of release profile of optimized batch at 0.1 N Hcl and 7.4 pH(figure 7).

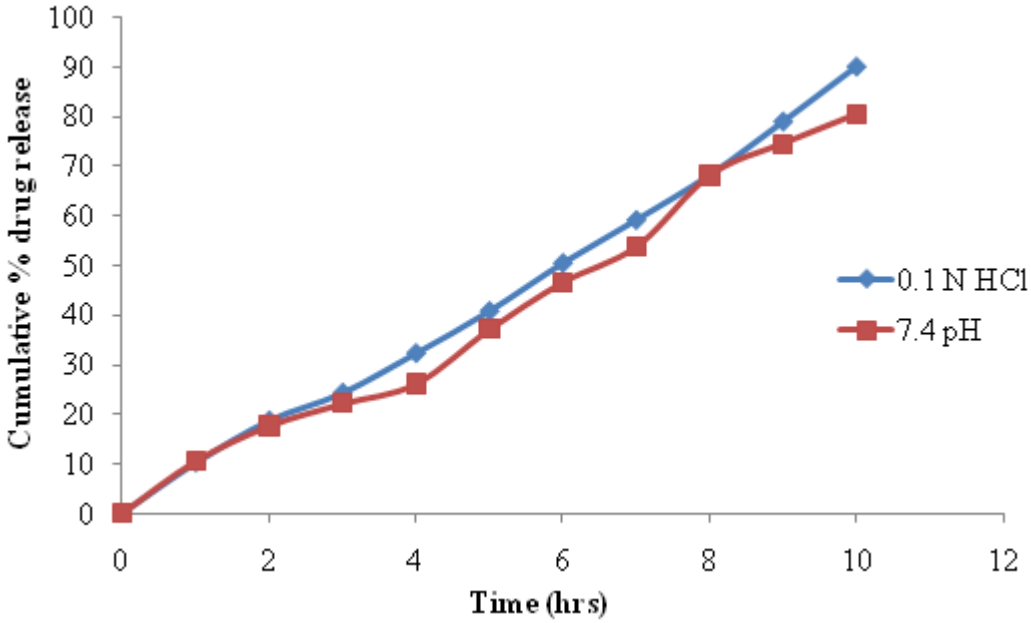

Figure 7: Cumulative % drug release of Batch A9 in 0.1 N HCl and pH 7.4 phosphate buffer

Scanning electron microscopy (SEM) study of optimised microcapsules

SEM image of optimised batch F9 indicated that the microcapsules were spherical having size range of 500-550µm and uniformly covered with the coat polymer (Figure8).

Table 6: Results of kinetic model study of microcapsules

Batch no	Regression coefficient (r^2)					Release Exponent
	Zero order	First order	Higuchi	Hixoncrowell	K-peppas	n
F1	0.9947	0.959	0.9627	0.9504	0.982	0.636
F2	0.9974	0.931	0.9814	0.9321	0.992	0.700
F3	0.9975	0.929	0.9825	0.9885	0.995	0.745
F4	0.9907	0.979	0.9445	0.9510	0.968	0.696
F5	0.9936	0.961	0.9602	0.9643	0.975	0.656
F6	0.9959	0.928	0.9775	0.9489	0.988	0.728
F7	0.9973	0.953	0.9661	0.9773	0.989	0.737
F8	0.9721	0.986	0.9106	0.9274	0.952	0.693
F9	**0.9976**	**0.932**	**0.9684**	**0.9700**	**0.993**	**0.879**
F10	0.9929	0.940	0.9637	0.9415	0.990	0.722
F11	0.9888	0.957	0.9459	0.9496	0.986	0.799
F12	0.9748	0.981	0.9167	0.9365	0.960	0.728

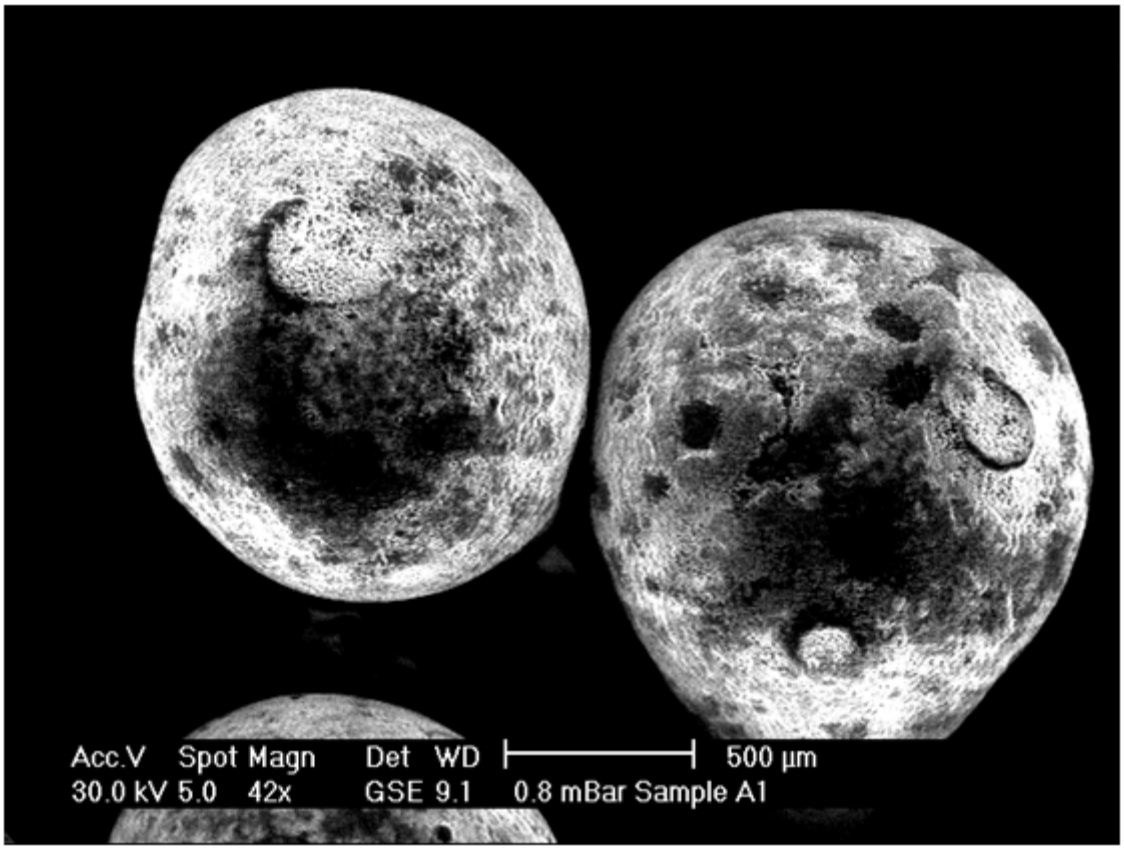

Figure 8: Scanning electron microscopy of optimized batch

Stability study

No significant changes were observed in appearance, texture, shape, mucoadhesion, microencapsulation efficiency and *in vitro* drug release of Batch F9 stored at room temperature and at 45ºC/ 75% RH up to 3 months, which proved stability of optimized batch.

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