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Formulation and development of Mucoadhesive microcapsule for delivery of Clarithromycin and Omeprazole used against Helicobacter pylori infection.

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Abstract

The basic objective of this study was to prepare and evaluate Mucoadhesive capsule enclosing Microspheres of drugs Clarithromycin and Omeprazole to treat Helicobacter pylori Infection. Mucoadhesive Microcapsule remains in vicinity of absorption site for prolonged period of time, so residence time of drug at absorption site is increases. No marketed preparation of drug combination Clarithromycin and Omeprazole is available to treat Helicobacter pylori Infection. Preformulation study of drugs was done. Ultraviolet and infrared spectroscopic study of drugs is carried out to check authenticity of drug and interaction between drug and excipient. Evaluation of Microsphere were done including micromeritics studies, percentage recovery of microsphere, drug entrapment study, In Vitro drug release, swelling and adhesion property were evaluated separately. Optimization of the batch of microsphere was done. Scanning electron microscopy studies of microapsheres were done to study surface topography of the uncoated and coated (optimized) microsphere. Optimization of Microcapsule batch was done. Evaluation of Microcapsule was done. In Vitro drug release study (Dissolution study) of optimized batch of Microcapsule was done. Therefore, by the formulation of Mucoadhesive capsule enclosing Microspheres of drugs Clarithromycin and Omeprazole, drugs residence time at absorption site is increases, so drug remains in vicinity of absorption site for prolonged period of time which is most beneficial to treat Helicobacter Pylori infection.

Keywords-Helicobacter Pylori, Clarithromycin, Omeprazole, Mucoadhesive, Microcapsule.

INTRODUCTION

Helicobacter pylori are a gram negative bacteria. It is spirally shaped bacterium having 0.5-0.9 µm in width and 2-4 µm long. Helicobacteria pylori is a highly adapted organism that lives only on gastric mucosa that lives only on gastric mucosa.[1] Helicobacter pylori cause chronic infection, antral gastritis, atropic gastritis, gastric ulcer, duodenal ulcer, atrophic gastritis, intestinal metaplasia and dyspepsia, gastric carcinoma, nonatropic pangastritis and gastric mucosa associated lymphoid tissue lymphoma (MALT lymphoma).[2]

Drug biodisponibility is a crucial facet in therapeutic effectiveness. One of the essential factors is the residence time of the drug at the absorption site. Over the last two decades, numerous gastroretentive dosage forms have been designed to prolong gastric residence time. They may be broadly classified into: high-density (sinking) systems, low-density (floating) systems, expandable systems, superporous hydrogel systems, mucoadhesive systems and magnetic systems [3]

Mucoadhesive drug delivery systems are delivery systems, which utilized the property of bioadhesion of certain polymers, which become adhesive on hydration, and hence can be used for targeting a drug to a particular region of the body for extended periods of time. Bioadhesion is an interfacial phenomenon in which the two materials, at least one of which is biological, are held together by means of interfacial forces. The attachment could be between an artificial material and biological substrate, such as adhesion between a polymer and a biological membrane. In the case of polymer attached to mucin layer of a mucosal tissue, the term "Mucoadhesion'' is used. [4] They enable

oral therapy by drugs with a narrow absorption window in the upper part of the gastrointestinal tract or drugs with a poor stability in the colon. Furthermore, the drug can act locally within the stomach and prolonged intimate contact with the absorbing membrane increases efficacy. This is especially important in treatment of microorganisms, which colonize the stomach because the three main factors reducing luminal delivery of drugs to them are gastric emptying, gastric acidity and the epithelial mucus layer. In particular, Helicobacter pylori lives deep within the gastric mucus layer and prolonged local application of drug is needed for sufficient to diffuse to the bacteria. Moreover, efficacy of topical application of antibiotics can sometimes be enhanced by absorbed by the gastric wall, followed by re-secretion into the lumen. [3]

Clarithromycin is an antimicrobial, it inhibit the Helicobacter pylori. Omeprazole is proton pump inhibitor, it inhibit acid secretion in stomach. This combination used to treat Helicobacter pylori infection. No marketed preparation of Clarithromycin and Omeprazole drug combination available.

Conventional drug delivery system has little or no control over the drug release, and effective concentration at the target site. This kind of dosing pattern may results in constantly changing, unpredictable plasma concentrations. The main objective of proposed work is to formulate mucoadhesive microcapsules containing selected Active Pharmaceutical Ingredients. The Microsphere of drugs Clarithromycin and Omeprazole with excipient were prepared, finally microsphere enclosed in mucoadhesive microcapsule. Optimization was done and optimized batch was evaluated for in vitro drug release. Number of approaches is done to increase the drug residence time, absorption at the particular site in the gastrointestinal tract. Apart from that mucoadhesive drug delivery system utilizes the principle of mucoadhesion to the mucosa of the GI tract to increase the drug residence time at particular site and ultimately increases its absorption. In present investigation microcapsules are preferred over the tablet dosage form as they provide effective utilization of drug and to avoid dose dumping.

The main objective of proposed work is to formulate the dosage form for drug combination Clarithromycin and Omeprazole that will remain in vicinity of absorption site for prolonged period of time to treat infection of Helicobacter pylori.

MATERIALS AND METHODS

Materials

Clarithromycin was obtained from Micro lab Private Limited, Bangluru, India. Omeprazole was obtained from Smilax lab Limited, Hyderabad, India. Carbopol 971p was obtained from Lubrizol Private Limited, Mumbai, India. Petroleum ether was obtained from GTPL Limited Aurangabad, India. Ethanol, Liquid Paraffin, Dichloromethane were obtained as a gift sample from Concept Pharmaceutical, Aurangabad.

Methods

Preformulation Studies of Selected drug

Organoleptic properties

Clarithromycin and Omeprazole were tested for organoleptic properties such as appearance, colour, odour, taste, etc. [5]

Melting point determination

Melting point of the drug was determined by taking a small amount of drug in a capillary tube closed at one end and it was placed in melting point apparatus and the temperature at which the drug melts was noted. Average of triplicate readings was taken.

Solubility determination

The Solubility of the selected drug was determined in distilled water and in polymeric solution by the following procedure:

Excess amount of drug was taken and dissolved in a measured amount of 0.1 N HCL solution of each polymer in a volumetric flask to get a saturated solution. The solution was shaken intermittently to assist the attainment of equilibrium with the undissolved drug particles. After 24 Hour the solution was sonicate for 30 min & the resultant solution was centrifuged to separate supernatant then withdrawn the supernatant and successively diluted and analyzed concentration of Clarithromycin by U. V. (Ultraviolet) Spectrophotometer at 282nm and Omeprazole at 305nm.

Bulk density

Bulk density was determined by Weight of powder / Volume of powder before tapping.

Tapped density

Tapped density was determine by Weight of powder / Volume of powder after tapping

Spectroscopic Studies:

A) UV Spectroscopy (Determination of λmax)

Stock solution (100µg/ml) of Clarithromycin was prepared in 0.1N HCL solution. The solution was kept in a fused silica cuvette 10 mm. The UV spectrum was recorded in the range of 200-400 nm on Thermo UV-visible spectrophotometer at 1 cm, slit width. [6, 7]

B) IR (Infrared) spectrum interpretation

The infrared absorption spectrum of pure Clarithromycin and Omeprazole the physical mixture of CARBOPOL 971P & HPMC (Hydroxy propyl methyl cellulose) sample was recorded on FT-IR spectrophotometer (Model: IRAFFINITY-1 Make: Shimadzu) and the spectrum analysis was done for functional groups & drug excipient compatibility. [6, 7]

Standard calibration curve of Clarithromycin and Omeprazole

Preparation of calibration curve in 0.1 N HCl [8]

Method of preparation of 0.1NHCl

Dissolve 85 ml of 0.1 N HCl in sufficient carbon dioxide-free water to produce 1000 ml. Accurately weighed drugs (100mg) were dissolved in 100 ml of 0.1 N HCl . Dilutions were made in the range of 5-50µg/ml. The absorbance values at 282nm for Clarithromycin & for Omeprazole at 305nm corresponding to each concentration was then statistically evaluated and plotted taking absorbance on Y-axis and concentration on the X-axis. [8]

Preparation of microspheres:

The microspheres were prepared by non-aqueous emulsification solvent evaporation method. Briefly, Drug and Polymer i.e. Clarithromycin and Carbopol 971P & HPMC (Hydroxy propyl methyl cellulose) K100 M were mixed in Ethanol & Dichloromethane. The slurry was introduced in to 200 ml of Liquid Paraffin while being stirred at 1000 rpm by mechanical stirrer for 45 min to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed repeatedly with Petroleum ether 40 –60 ^{o}C until free from oil. The collected microspheres were dried for 1hr at room temperature and subsequently stored in desiccator over

fused calcium. The same procedure was followed for Omeprazole. [9, 10, 11]Firstly the six trial batches were prarared by using drug and polymer in 1:1 concentration but vary in solvents ethanol & dichloromethane and liquid Paraffin temperature batch to batch. The fourth batch formulation was selected. (Table 1) Then various batches of microsphere for Clarithromycin and Omeprazole were prepared by using drug and polymers combination. (Table 2,3)

Table 1. Trial batches

Sr. No	Drug (mg) Clarithromycin /Omeprazole	Carbopol974/ HPMC K4M/ HPMCK100M	Solvent Ethanol & Dichloromethane (ml)	Liquid Paraffin Temp (°C)	Rejected(R)/ Selected (s)
1	**100**	**100**	**15+15**	**40**	**R**
2	**100**	**100**	**14+14**	**37**	**R**
3	**100**	**100**	**13+13**	**40**	**R**
4	**100**	**100**	**12+12**	**37**	**S**
5	**100**	**100**	**11+11**	**40**	**R**
6	**100**	**100**	**10+10**	**37**	**R**

***Drug: Polymer ratio is 1:1 concentration**

Table 2. Formulation Table of drug: polymer batches. (Formulation table containing drug Clarithromycin)

Batches Variables	CF1	CF2	CF3	CF4	CF5	CF6
Carbopol 971p	300	300	150	150	150	150
HPMCK 4M	---	---	150	150	---	---
HPMC K100M	----	----	----	----	150	150
RPM	1000	750	1000	750	1000	750
TIME	45	60	45	60	45	60
DRUG	300	300	300	300	300	300

Table 3. Formulation Table of drug: polymer batches. (Formulation table containing Drug and Polymer combination in solvent mixture of Ethanol and Dichloromethane for drug Omeprazole)

Batches Variables	OF1	OF2	OF3	OF4	OF5	OF6
Carbopol 974p	300	300	150	150	150	150
HPMCK4M	---	---	150	150	---	---
HPMC K100M	----	----	---	----	150	150
RPM	1000	750	1000	750	1000	750
TIME	45	60	45	60	45	60
DRUG	300	300	300	300	300	300

Evaluation of microspheres

Micromeritics Studies of Microspheres

Particle size determination

Particle size was determined by using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 50-100 particles with the help of a calibrated ocular micrometer. [10, 11]

Bulk Density

The bulk density was obtained by dividing the mass of a powder by the bulk volume in cm^3. The sample of about 10 cm^3 of powder was carefully introduced into a 25 ml graduated cylinder. The cylinder was dropped at 2-second intervals onto a hard wood surface three times from a height of 1 inch. The bulk density of each formulation was then obtained by dividing the weight of sample in grams by the final volume in cm^3 of the sample contained in the cylinder. [10, 11] It was calculated by using equation given below

$$D_f = M / Vp \quad (1)$$

Where D_f is bulk density, M is weight of samples in grams and Vp is final volumes of granules in cm^3. [10, 11]

Tapped Density

The tapped density was obtained by dividing the mass of a powder by the tapped volume in cm^3. The sample of about 10 cm^3 of powder is carefully introduced into a 25 ml graduated cylinder. The cylinder was dropped at 2-second intervals onto a hard wood surface 100 times from a height of 1 inch. The tapped density of each formulation was then obtained by dividing the weight of sample in grams by the final tapped volume in cm^3 of the sample contained in the cylinder. [10, 11] It was calculated by using equation given below

$$D_o = M / Vp \quad (2)$$

Where D_o is bulk density, M is weight of samples in grams and Vp is final tapped volumes of granules in cm^3. [10, 11]

Carr's Index

The percentage compressibility of microspheres was calculated according to equation given below. [10,11].

$$\% \text{ Compressibility} = \frac{D_o - D_f}{D_o} \times 100 \quad (3)$$

Where D_f is bulk density and Do is Tapped density.[10,11]

Hausner ratio

The Hausner ratio of a microsphere was calculated according to equation given below. [10, 11]

$$\text{Hausner ratio} = D_o / D_f \quad (4)$$

Where D_o is Tapped density and D_f is bulk density. [10, 11]

The Angle of repose

The Angle of repose (θ) i.e. Flow property of the microspheres, which measures the resistance to particle flow, was calculated as

$$\tan \theta = 2H / D \quad (5)$$

Where, 2H / D is the surface area of the free standing height of the microspheres heap that is formed after making the microspheres flow from the glass funnel. [10, 11].

Percentage yield (i.e. recovery) of microspheres formed

The percentage yield of microsphere determined by weighing after drying. The prepared microspheres with a size ranging from 250µm to 325µm. The measured weight of prepared microspheres was divided by the total amount of all the non–volatile components used for the preparation of the microspheres, which gave the total percentage yield of microspheres. [12, 13]

$$\% \text{ Yield} = (\text{Actual weight of product / Total weight of excipient and drug}) \times 100 \quad (6)$$

Drug Content determination

Microspheres equivalent to 20 mg of the drug Clarithromycin were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1 N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1 N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 282 nm against 0.1 N HCl as a blank. The amount of drug entrapped in the microspheres was calculated by the following formula. (Table 13 Fig 8) The same procedure is repeated for drug Omeprazole and absorbance was measured at 305nm. The drug content was calculated from standard curve. [13]

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100 \quad (7)$$

Placebo microspheres were used as reference. Theoretical drug concentration = 20 mg

Swelling study of Microsphere

A known weight (100mg) of various mucoadhesive microspheres was placed in 500ml of 0.1 N HCL and allowed to swelled for the required period of time, at 37± 0.5^0C using USP (United state Pharmacopoeia) dissolution apparatus with the dissolution basket assembly at 100rpm.The microparticles were periodically removed ,blotted with filter paper and their changes in weight were measured during the swelling until equilibrium was obtained. [14] Finally, weight of the swollen microparticles was recorded after a time period of 4 hrs and swelling ratio (SR) was calculated from the formula

$$SR = (We - W0)/W0 \quad (8)$$

Where W0 is the initial weight of the dry microparticles and We is the weight of swollen microparticles at equilibrium swelling in the media.[14]

Adhesion property

A freshly cut of 5 cm long piece of pig intestine obtained from a local abattoir within 1 h of killing the animal was cleaned by washing with isotonic saline solution. An accurate weight of microspheres was placed on mucosal surface which was attached over a polyethylene plate that fixed in an angle of 40^0 relative to the horizontal plane, and 0.1 N HCl warmed at 37^0C was peristaltically pumped at a rate of 5 ml/min over the tissue. The duration for completely washing of microspheres from pig intestine was recorded and averaged from five determinations. [15]

Dissolution test (in-vitro drug release) of microspheres

The use of several methods has been described in the literature, but in the present study, the standard six stations USP (United state Pharmacopoeia) basket (apparatus I) method was used. The microspheres were placed in a basket having mesh size lower than microsphere to avoid the escape of any microspheres. [15, 16]

The release rate of drug Clarithromycin and Omeprazole from microsphere was determined using USP Dissolution Testing Apparatus I (Basket type). The dissolution test was performed using 900 ml of 0.1 N HCl, at 37 ± 0.5^0C and 100 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at various sampling time 0 min, 1hr, 2hr, 3hr, 4hr&so on), and the samples were replaced with fresh dissolution medium to avoid sink condition. The samples were filtered through Whatman filter paper no. 41. Absorbance of these solutions was measured at 282nm for Clarithromycin and 305.0nm for Omeprazole. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.[16, 17]

The difference of dissolution profile of the prepared formulations was compared with that of the intrinsic formulation to predict the enhancement of dissolution of drug as that of pure drug formulation.

Surface morphology

Surface morphology was studied by using stereomicroscope to know the external morphology of the prepared microspheres (Intel play)

Optimization

On the basis of evaluation parameters such as entrapment efficiency, percent yield and dissolution characteristics the batch that has shown the best results was optimized and selected for formulation of final dosage form (capsule) and advanced studies such as drug polymer interaction, scanning electron microscopy (SEM) studies etc.

Advanced studies on optimized batch of microspheres

a) Morphological Study using SEM (Scanning electron microscopy)

The surface topography of the uncoated and coated (optimized) microsphere examined under a FEI-Philips XL-30 Analytical Electron microscope (VNJT, Nagpur, India). The sample was loaded on copper sample holder and sputter coated with platinum.

B) Infrared Spectroscopy Interpretation for interaction between drug and polymer in Blend of Microsphere
Fourier transforms infrared spectroscopy (FTIR) spectra of the pure Clarithromycin and Omeprazole drug and the polymer blends of microsphere were produced using KBr disk method. Powder microsphere and the ingredients used in drug loading were subjected to FTIR with a, Model: IR affinity-1Make: Shimadzu) FTIR. Background spectrum was collected before running each sample. The samples were analyzed between wave numbers 4000 and 400 cm-1.

Formulation of capsule of optimized for the study

Capsules size was selected according to the volume of microsphere needed to deliver the drug dose. The contents were filled in the tared capsule, which were then reweighed. Care was taken to fill the capsule completely in order to maintain a uniformity of weight. Evaluation study is to be conducted to know uniformity of weight, content uniformity, drug released and stability.

$$\text{Capsule volume} = \frac{\text{Capsules fill weight of formulation}}{\text{Tapped bulk density}} \quad (10)$$

Evaluation of Capsule

The optimized batch of microsphere filled into capsule containing Clarithromycin and Omeprazole was evaluated for the following parameter

a. General appearance

b. Weight variation

This test was conducted on 20 capsules. Each capsule was individually weighed to find out weight variation. Average weight was calculated. [17]

c. Content uniformity

10 capsules were accurately weighed, remove the hard gelatin shells and powder equivalent to 20mg of drug Clarithromycin and dissolved it in 1N NaOH. Drug content was calculated by measuring absorbance at wavelength 282.00 nm. The results are given in Table same procedure is followed for Omeprazole at wavelength 305nm. [17]

Dissolution Studies

In-vitro release studies of batch of capsules containing microspheres with the drug loaded were conducted in 0.1 N HCl in Six station USP (United state Pharmacopoeia) Dissolution apparatus I for 08 Hrs.

RESULT AND DISCUSSION

Preformulation results of selected Drugs

The preformulation studies for drugs Clarithromycin and Omeprazole were done. The results were illustrated in table 4(a),4(b).

Table 4(a). Preformulation study of Clarithromycin (S.D. n=3)

Sr. No.	Characters	Inference
1	.Nature	A white to off–white crystalline powder
2.	Colour	White
3.	Odour	Odorless
4.	Taste	Slightly Bitter taste
5.	Melting point	222° to 225° C
6.	Solubility-	
	In water	Practically Insoluble
	In acetone	Soluble
	In methanol	Soluble
	Dichloromethane	Soluble
7.	Bulk density	0.454 gm/cm^3
8.	Tapped density	0.555 gm/cm^3

Table 4(b). Preformulation of Omeprazole (S.D. n=3)

Sr. No.	Characters	Inference
1	Nature	A white or almost white powder
2.	Color	White hygroscopic powder
3.	Odor	Odorless
4.	Taste	Slightly Bitter taste
5.	Melting point	156°C
6.	Solubility-	
	In water	Soluble
	In acetone	Soluble
	In methanol	Soluble
	Dichloromethane	Slightly Soluble
7.	Bulk density	0.454 (0.34) gm/cm^3
8.	Tapped density	0.555 (0.43) gm/cm^3

Spectroscopic Studies

a) UV Spectroscopy (Determination of λmax)

It showed a λmax at 282nm for Clarithromycin and for Omeprazole at 305nm. (Figure 1,2).

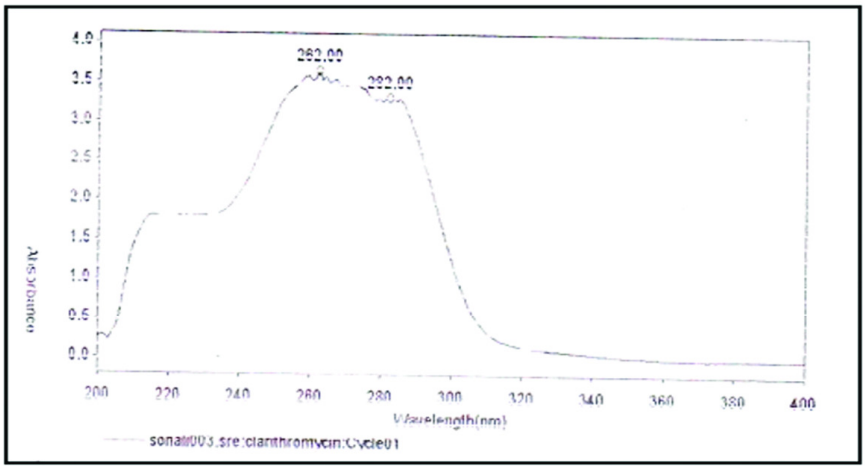

Figure No. (1).UV Spctra of Clarithromycin in 0.1N HCl

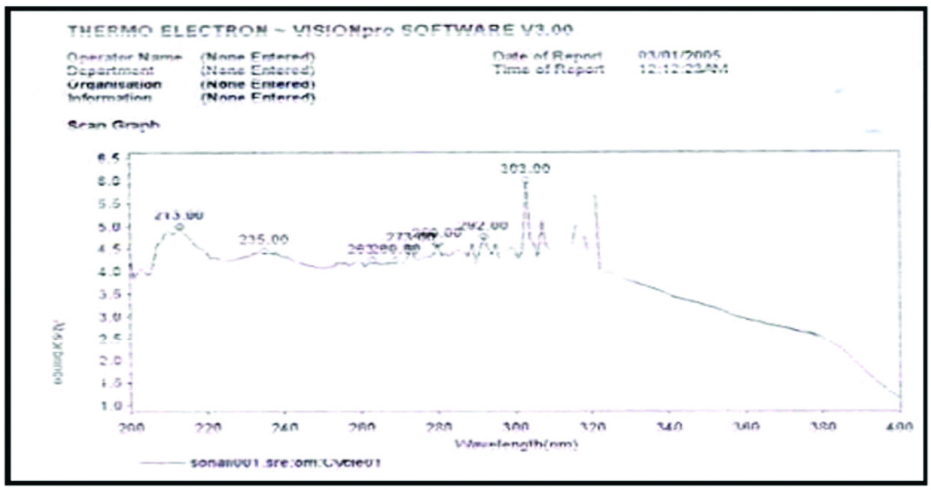

Figure No.(2). UV Spctra of Omeprazole in 0.1N HCl

b)

IR spectrum interpretation

IR spectra of pure drug samples of Clarithromycin and Omeprazole were recorded for determination of various functional groups. (Figure 3, 4) Interpretation of spectra of Clarithromycin and Omeprazole were done.

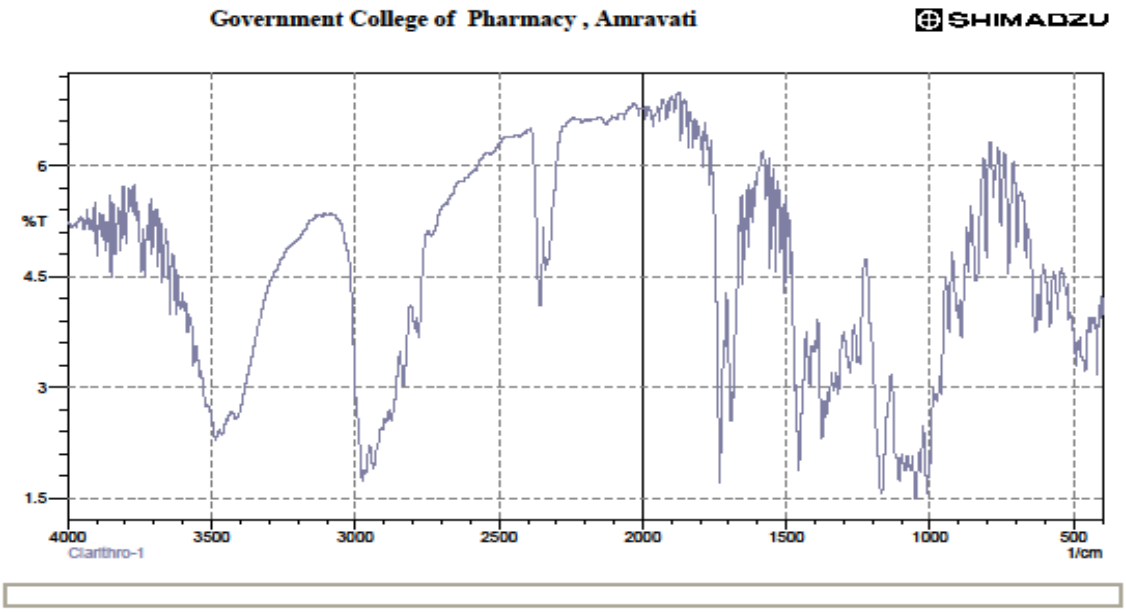

Figure 3 IR Interpretation of Pure drug Clarithromycin

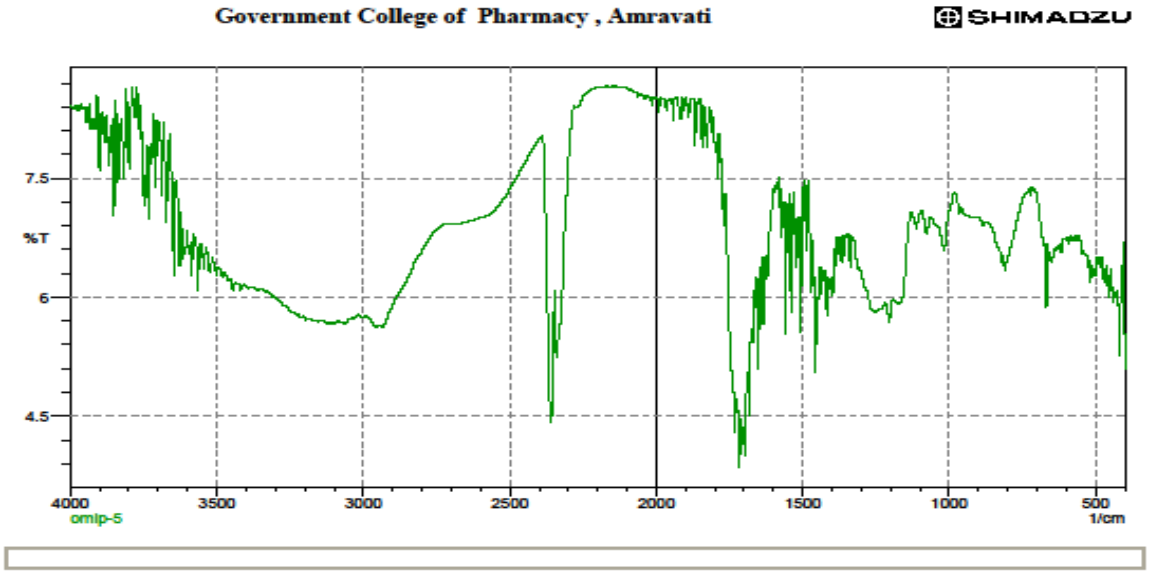

Figure 4.IR Interpretation of pure drug Omeprazole

Standard calibration curve of Clarithromycin and Omeprazole

The standard calibration curve of Clarithromycin and Omeprazole in 0.1NHCl were established, the curves were follows Beer's Lambert law. (Figure 5,6) So it confirmed that samples of the drugs Clarithromycin and Omeprazole were authentic one.Results were illustrated in table 5(a) and 5(b).

Table5(a).Calibration curve for Clarithromycin in 0.1 N HCl(S.D n=3)

S. No	Conc.(µg)*	Absorbance
01	**0**	**0**
02	**5**	**0.138** (0.45)
03	**10**	**0.276** (0.60)
04	**15**	**0.411** (0.23)
05	**20**	**0.541** (0.12)
06	**25**	**0.687** (0.33)
07	**30**	**0.817** (0.53)
08	**35**	**0.923** (0.19)
09	**40**	**1.086** (1.5)
10	**45**	**1.223** (0.83)
11	**50**	**1.362** (0.52)

***Conc. Means Concentration**

Table 5(b).Calibration curve for Omeprazole in 0.1 N HCl (S.D n=3)

S.N	Conc.(µg) *	Absorbance
01	0	0
02	5	0.278 (0.66)
03	10	0.520 (0.33)
04	15	0.719 (0.43)
05	20	0.949 (0.77)
06	25	1.210 (1.3)
07	30	1.429 (0.63)
08	35	1.742 (0.44)
09	40	1.896 (0.23)
10	45	2.139 (0.19)
11	50	2.332(0.45)

***Conc. Means Concentration**

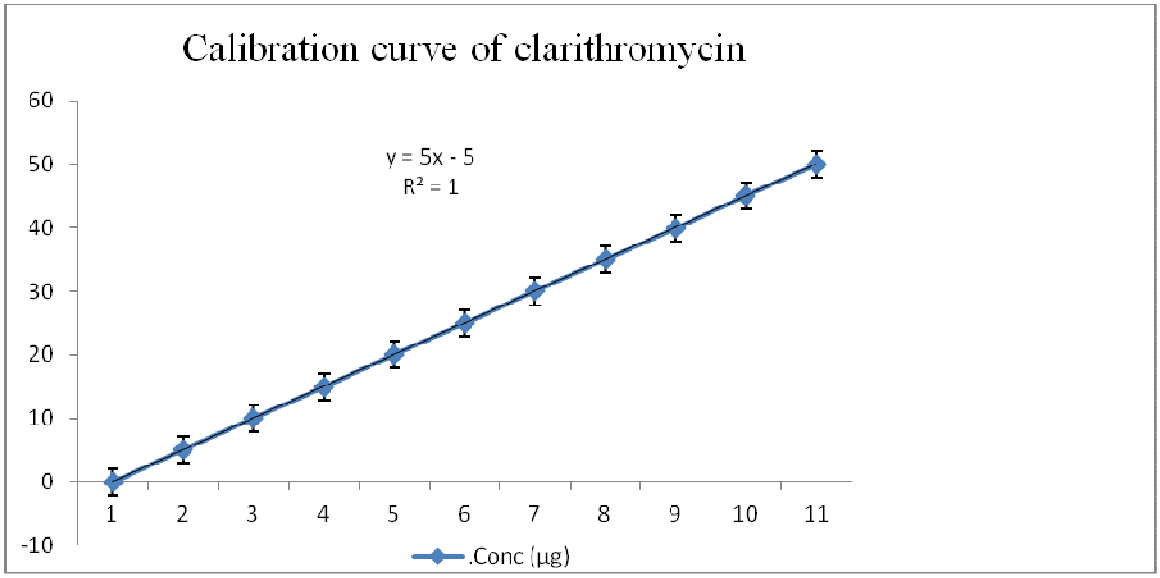

Figure 5. Calibration Curve of Clarithromycin, Bars indicates SD (n=3)

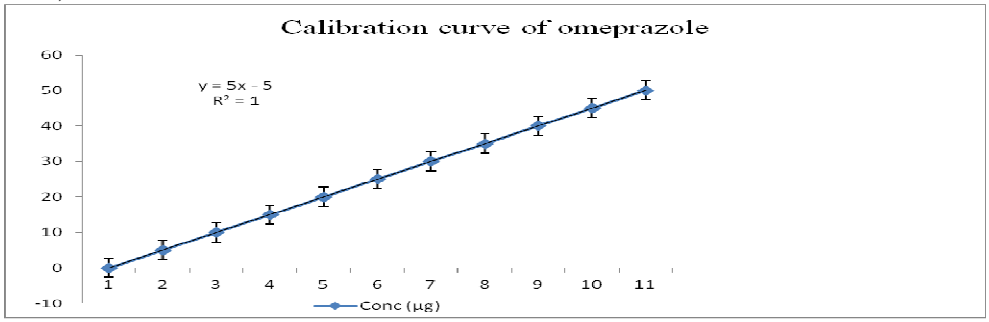

Figure 6. Calibration curve of Omeprazole in 0.1 N HCl , Bars indicates SD (n=3)

Evaluation for microsphere

Micromeritics Studies of Microspheres

The various batches have the average particle size in the range of 250µm to 325µm. The particle size was increased as the stirring time and stirring speed was decreased. The tapped density value ranged from 0.4-0.5, bulk density in between 0.37-0.45, Carr's index in between 8-16% and Hausner ratio within 1.1-1.2. All formulation showed excellent flowability as expressed in terms of angle of repose was found within the range of 25^0 to 35^0, which is an appreciable limit for microspheres to show flow property while formulating in the dosage form. The average diameter of the microparticles at various agitation speed i.e., 750, 1000 were found in decreasing order, i.e., 312.054 to 250.16µm and 321.89 to 281.46 µm.

This suggests that the size of the droplets formed during microcapsulation is closely related to the size of final microcapsules, which increased by decreasing the stirring speed. A faster stirring speed 1000rpm gave much smaller but cohered particles. At lower speed (750rpm), the mean particle diameter and size distribution of the microparticles increased considerably. The irregularity in shape of microparticles occurred at higher speed (1000 rpm) but discret and uniform size of microparticles were obtained at750 rpm. The average diameter of the microparticles decreases from 369.13 ± 11.2µm to 252.16 ± 11.4 for drug Clarithromycin and for drug Omeprazole it decreases from 369.13 ± 11.2 to 252.16 ± 11.4 on increasing stirring times. The short time (45min) resulted in aggregates and less discreet microparticles where at high stirring time (60 min) resulted smaller microparticles with increased size distribution. The discreet and uniform size of the microparticles was found at 45 min stirring time. The results were presented in table 6(a) and 6(b).

Table 6(a). Micromeritics studies of drug Clarithromycin (S.D. n=3)

Parameters / *Batces*	Average Particle of size (µm) S.D n=3	Tapped (density) (g/cm^3)	Bulk density (g/cm^3)	*%Compress-ability* index	Hausner ratio	Angle of repose
CF1	**369.13** (11.2)	**47** (0.08)	**41** (0.0016)	**12.76**	**1.14**	**30 º 2**
CF2	**324.65** (15.6)	**48** (0.008)	**43** (0.003)	**10.41**	**1.11**	**29 º 74**
CF3	**312.05** (11.4)	**48** (0.004)	**41** (0.004)	**14.58**	**1.17**	**29 º 74**
CF4	**281.46** (7.8)	**47** (0.008)	**43** (0.003)	**8.51**	**1.09**	**27 º 21**
CF5	**256.16** (8.4)	**49** (0.008)	**44** (0.008)	**10.20**	**1.11**	**30 º 21**
CF6	**252.16** (11.4)	**49** (0.004)	**44** (0.004)	**10.20**	**1.11**	**30 º 12**

Table 6(b). Micromeritics studies of Omeprazole (S.D. n=3)

Param-eters / *Batces*	Average Particle size (μm) S.D n=3	Tapped (density) (g/cm^3)	Bulk density (g/cm^3)	*%Compress-ibility* Index	Hausner ratio	Angle of repose (θ)
OF1	**325.65** (15.4)	**0.4** (0.008)	**44** (0.008)	**10.20**	**1.11**	**30 o 21**
OF2	**312.05** (11.4)	**0.47** (0.008)	**43** (0.003)	**8.51**	**1.09**	**27 o 21**
OF3	**312.13** (8.46)	**0.48** (0.004)	**41** (0.001)	**14.58**	**1.17**	**29 o 74**
OF4	**270.81** (14.4)	**0.47** (0.004)	**41** (0.004)	**12.76**	**1.14**	**28 o 76**
OF5	**281.46** (7.8)	**0.46** (0.003)	**44** (0. 008)	**11.10**	**1.04**	**26 o 21**
OF6	**256.16** (8.8)	**0.48** (0.004)	**44** (0.004)	**10.33**	**1.091**	**27 o 21**

Percentage recovery (i.e. Yield) of microspheres formed

The prepared microsphere gives good percentage yield. The percentage yield of microsphere determined by weighing after drying. The maximum percentage yield was found of f5 batch and was noted to be 83.33% for drug Clarithromycin and for Omeprazole it was found 81.40 as among all the batches. It was found that average percentage yield of microsphere was greater than 78 % for all. Results were given in Table 7(a) and7(b).

Table7(a).Percentage yield of Clarithromycin. (S.D. n=3)

Batch. No	Percentage Yield
CF1	**50.0**(2.1)
CF2	**58.33**(2.5)
CF3	**66.6(**0.60)
CF4	**71.66**(1.2)
CF5	**83.33**(0.70)
CF6	**75.00**(2.3)

Table7(b).Percentage yield of Omeprazole (S.D n=3)

Batch. No	Percentage yield
OF1	**63.0**(0.50)
OF2	**67.3**(0.90)
OF3	**68.4**(1.20
OF4	**73.9**(2.3)
OF5	**81.40**(1.4)
OF6	**78.0(**0.60)

Drug entrapment:

The prepared microsphere was evaluated for drug entrapment study. The percent drug entrapment of Clarithromycin in all formulation was found to be good i.e. above 60%%. The microsphere of batch F5 formulation showed an entrapment of 82.0% while the other formulations showed lesser entrapment than this formulation. This can be attributed to the stirring speed and stirring time, that could facilitate the diffusion of a part of entrapped drug to the surrounding medium during preparation of microspheres. (Table 8(a)) Similarly, for the drug Omeprazole, the microsphere of batch F5 formulation showed an entrapment 96.26%.(Table 8(b)) While the other formulations showed lesser entrapment than this formulation.The lower entrapment efficiency at 500 rpm agitation speed may be due to inadequately stirring of droplets, which leads to increased chances of coalescence. But at 1000 rpm, no

sticking of polymeric material around the walls of flask was visually observed which may leads to improved drug: polymer contact time resulting highest drug entrapment efficiency.

The data of entrapment efficiency indicates that approx 10% of the drug appears to leach out to the external phase (liquid paraffin) during the process and it is confirmed by estimation of residual drug content in external phase. So, microparticles which prepared under optimized conditions i.e., agitation speed 1000 rpm and stirring time 45 min were found to be spherical in shape, uniform in size and exhibited a smooth surface. The uniform size and smooth surface of microparticles was due to the tightening of the polymeric net work leading to shrinkage of the microcapsules as the time of evaporation of solvent increased. The optimum size of microcapsules were obtained with 45 min stirring time because larger time 60 min gave smaller microparticles.

Table 8(a). Drug entrapment efficiency of Clarithromycin (S.D. n=3)

Batch No.	Drug entrapment efficiency	
	Drug Content	% Drug entrapment Of Clarithromycin
CF1	40.9 (0.40)	68.0
CF2	41.8 (0.52)	69.8
CF3	43.8(1.1)	73.0
CF4	45. 0 (0.7)	75.0
CF5	54.66(1.5)	82.0
CF6	46.8 (2.0)	78.0

Table 8(b). Drug entrapment efficiency of Omeprazole (S.D. n=3)

Batch No.	Drug entrapment efficiency	
	Drug Content	% Drug entrapment Of Omeprazole
OF1	49.5 (0.60)	51.0
OF2	50.5 (1.3)	65.0
OF3	52.8 (0.45)	70.0
OF4	55.3(0.60)	73.0
OF5	89.12 (1.4)	96.26
OF6	57.0 (1.6)	75.0

Swelling study

Swelling Index was calculated with respect to time. Swelling Index increased with weight gain by the microsphere was increased proportionality with the rate of hydration. The swelling ratio of batches Cf1 and Cf2 was high as compared to other batches because of high viscosity of Carbopol as compared to HPMC.(Table.9 (a)) The Swelling ratio of batch Cf5 was less as compared to other batches and hence it is optimized one. Similar results were obtained for the drug Omeprazole and batch Of5 is optimized.(Table9(b))

Table 9(a).Swelling study of Clarithromycin: (S.D. n=3)

Time in hrs	CF1	CF2	CF3	CF4	CF5	CF6
0	0	0	0	0	0	0
1	0.68 (0.15)	0.84 (0.13)	0.76 (0.31)	0.74 (0.33)	0.67 (0.30)	0.63 (0.51)
2	0.86 (0.21)	0.92 (0.710)	0.84 (0.60)	0.85 (0.72)	0.74 (0.39)	0.73 (0.430)
4	1.36 (1.2)	1.23 (1.0)	0.92 (0.500)	0.96 (1.25)	0.84 (0.46)	0.83 (0.82)
6	1.61 (0.50)	1.3 (2.3)	1.2 (0.45)	1.19 (2.5)	0.94 (0.55)	0.93 (1.5)
8	1.74 (0.33)	1.56 (0.40)	1.42 (0.30)	1.28 (0.900)	1.2 (0.65)	1.21 (0.19)

Table 9(b). Swelling study of Omeprazole (S.D. n=3)

Time in hrs	OF1	OF2	OF3	OF4	OF5	OF6
0	**0**	**0**	**0**	**0**	**0**	**0**
1	**0.72** (0.52)	**0.7** (0.150)	**0.75** (0.21)	**0.73** (0.51)	**0.68** (0.400)	**0.69** (0.39)
2	**0.87** (0.93)	**0.9** (0.35)	**0.83** (1.30)	**0.86** (0.46)	**0.76** (0.71)	**0.77** (0.71)
4	**1.56** (1.5)	**1.3** (0.70)	**0.92** (2.2)	**0.94** (0.810)	**0.83** (0.53)	**0.85** (0.830)
6	**1.6** (1.25)	**1.42** (0.17)	**1.25** (0.93)	**1.2** (0.92)	**0.92** (0.19)	**0.94** (0.6)
8	**1.72** (0.90)	**1.54** (0.92)	**1.45** (0.80)	**1.28** (1.5)	**1.23** (1.4)	**1.25** (1.2)

Adhesion property of Microsphere

In the adhesive time study, the Cf5 batch is optimized one because it was having more adhesion time as compared to other batches.(Table No.10(a)) For the batches containing drug and Carbopol concentration having 1:1 ratio, was found less adhesion time because of high viscosity of Carbopol 971p as compare to HPMC. Similar results were obtained in case of drug Omeprazole. OF5 is optimized one. (Table no.10(b))

Table 10(a). Adhesive Time Study of Clarithromycin

Sr. no	Batch Code	Adhesive Time in hrs
1	**CF1**	**6**
2	**CF2**	**5.4**
3	**CF3**	**6.5**
4	**CF4**	**6.45**
5	**CF5**	**8**
6	**CF6**	**7.35**

Table 10(b). Adhesive Time Study of Omeprazole

Sr. no	Batch Code	Adhesive Time in hours
1	**OF1**	**6.1**
2	**OF2**	**5.45**
3	**OF3**	**6.45**
4	**OF4**	**6.55**
5	**OF5**	**7.55**
6	**OF6**	**7.2**

Dissolution (In-vitro Drug release) studies

The triplicate study *in-vitro* dissolution was carried out on all the batches in 0.1 N HCl. The release of drug from microsphere of batches F1-F6 was containing Clarithromycin as model drug and Carbopol 971p& HPMCK100M in ratio 1:1. Here the variable among the formulation is the stirring speed. The drug release was almost linear with time. As the time goes, release rate also goes. As the stirring speed increased the size of microsphere decreases and increases the release rate. Release of batches CF1-CF6 was 54.90%, 59.94, 72.82, 76.96, 91.35, 84.34 % respectively. The batch CF5 shows highest release of drug among the all batches. (Figure No.7) The released profile of these batches shown in Table 11(a)

Similarly, release profile for Omeprazole is shown below containing drug Omeprazole and Carbopol 974p & HPMCK100M in ratio 1:1. Here the variable among the formulation is the stirring speed. The drug release was almost linear with time.. As the stirring speed increased the size of microsphere decreases and increases the release rate. Release of batches OF1-OF6 was 48.46 %, 59.94%, 67.63%, 73.53% ,95.69%, 84.34% respectively. The batch OF5 shows highest release of drug among the all batches (Figure No.8). The released profile of these batches

shown in Table 11(b). The batches of both drugs were prepared for the study of effect of stirring time on the release of microsphere as 45 min and 60 min respectively by keeping stirring speed constant (1000 rpm and 750rpm)Here also The release of the batch CF5 and Of5 (1000 rpm & 45 min) shows the highest result of release. These batches were also compared with the intrinsic. Various batches of Clarithromycin microspheres were prepared by applying the process variables such as stirring speed and stirring time using two different polymers as Carbopol971p and HPMCK100M. These batches were subjected to the percent yield, drug entrapment and in vitro dissolution studies. However, based on the percent yield, drug entrapment and release rate studies of the formulations, it could concluded that the formulations containing 300mg Clarithromycin and Carbopol 971p and HPMCK100M batch F5 released approximately 91% drug. And also shows much enhanced rate and extend of release of drug as compared to other formulations which is taken as standard, that's why it was chosen as the optimized formulation.

Similarly, the formulation containing Omeprazole and Carbopol 971p and HPMCK100M batch F5 release approximately 95% drug And also shows much enhanced rate and extend of release of drug as compared to other formulations which is taken as standard, that's why it was chosen as the optimized formulation.

Table 11(a). Drug release pattern of batches CF1-CF4

Time	Percentage cumulative release Of Drug Clarithromycin					
	CF1	CF2	CF3	CF4	CF5	CF6
0	00.00	00.00	00.00	00.00	00.00	00.00
1	**10.90**±0.2	**2.11**±0.3	**16.33**±0.15	**14.73**±0.57	**24.01**±0.28	**22.98**±0.33
2	**12.29**±0.29	**6.92**±0.4	**20.81**±0.18	**22.003**±0.55	**42.23**±0.38	**37.87**±0.42
3	**17.46**±0.17	**11.71**±0.10	**25.22**±0.26	**25.76**±0.43	**51.42**±0.48	**42.88**±0.32
4	**18.42**±0.31	**16.39**±0.31	**39.86**±0.34	**34.43**±0.30	**58.92**±0.50	**51.68**±0.49
5	**31.73**±0.30	**17.090**±0.28	**50.60**±0.45	**51.24**±0.39	**59.33**±0.52	**56.38**±0.43
6	**31.93**±0.35	**33.98**±0.34	**57.84**±0.53	**65.29**±0.47	**66.41**±0.63	**75.16**±0.63
7	**5.24**±0.38	**46.98**±0.28	**65.46**±0.59	**68.43**±0.61	**69. 6**±0.49	**79.39**±0.72
8	**54.90**±0.40	**59.94**±0.45	**72.82**±0.71	**76.96**±0.70	**91.35**±0.53	**84.34**±0.82

* Each sample was analyzed in triplicate **(S.D. n=3)**

Table 11(b). Drug release pattern of batches OF1to OF6

Time (hrs)	Percentage cumulative release Of drug Omeprazole					
1	OF1	0F2	0F3	0F4	OF5	OF6
2	00.00	00.00	00.00	00.00	00.00	00.00
3	**13.05**±0.20	**21.57**±0.18	**24.41**±0.29	**29.48**±0.23	**34.31**±0.27	**31.24**±0.2
4	**21.65**±0.25	**24.53**±0.26	**29.61**±0.26	**32.25**±0.17	**43.87**±0.46	**36.79**±0.2
5	**24.57**±0.30	**29.61**±0.33	**32.33**±0.45	**35.11**±0.28	**52.82**±0.48	**35.13**±0.4
6	**29.61**±0.35	**35.48**±0.44	**43.17**±0.49	**44.33**±0.35	**58.09**±0.53	**47.56**±0.38
7	**32.33**±0.40	**43.18**±0.0.58	**50.90**±0.54	**51.21**±0.45	**65.80**±0.63	**58.06**±0.53
8	**43.17**±0.53	**43.23**±0.38	**52.25**±0.58	**59. 9**±0.53	**81.80**±0.75	**68.56**±0.6
9	**45.61**±0.23	**53.20**±0.43	**56.17**±0.63	**68.03**±0.55	**91.87**±0.85	**80.98**±0.7
10	**48.46**±0.42	**59.94**±0.63	**67.63**±0.67	**73.53**±0.83	**95.69**±0.93	**84.34**±0.81

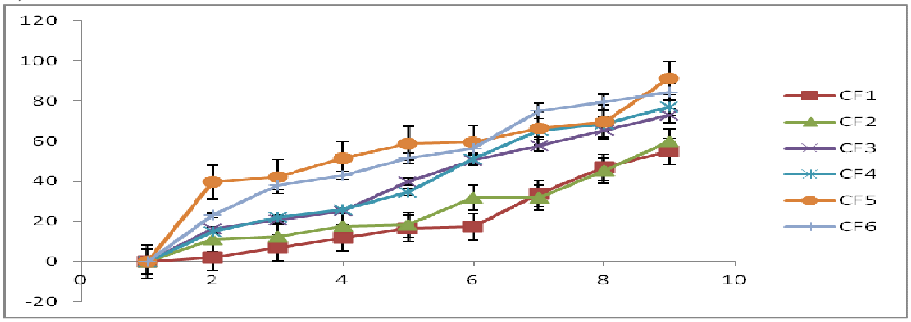

Figure 7.Drug release pattern of batches CF1 to CF6

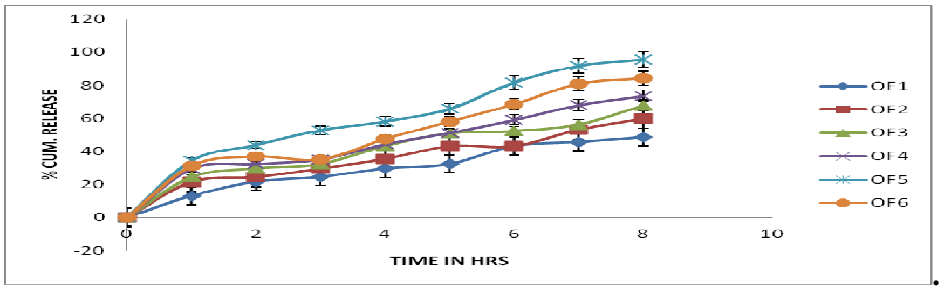

Figure 8.Drug release pattern of batches F1toF6 of Omeprazole

Studiesof optimized batch of microspheres

Morphological results with Scanning Electron Microscopy (SEM)

Morphology of microspheres was examined by scanning electron microscopy. The smooth surface of such microspheres as seen by SEM might be due to this complete homogeneity of drug and polymers. The outer surface of the microspheres was smooth and dense, while the internal surface was porous. The shell of the microspheres also showed some porous structure.(Figure No.9,10) It may be caused by the evaporation of the solvent entrapped within the shell of microspheres after forming a smooth and dense skin layer.

The surface topography revealed a spherical surface for all the formulations and a round cavity enclosed by an outer shell composed of the drug and polymer. They appeared to be hollow presumably because of the rapid escape of volatile solvent from the polymer matrix. This hollow nature was also responsible for the microspheres to entrap the simulated gastric fluids and enhance the surface area for solubility.

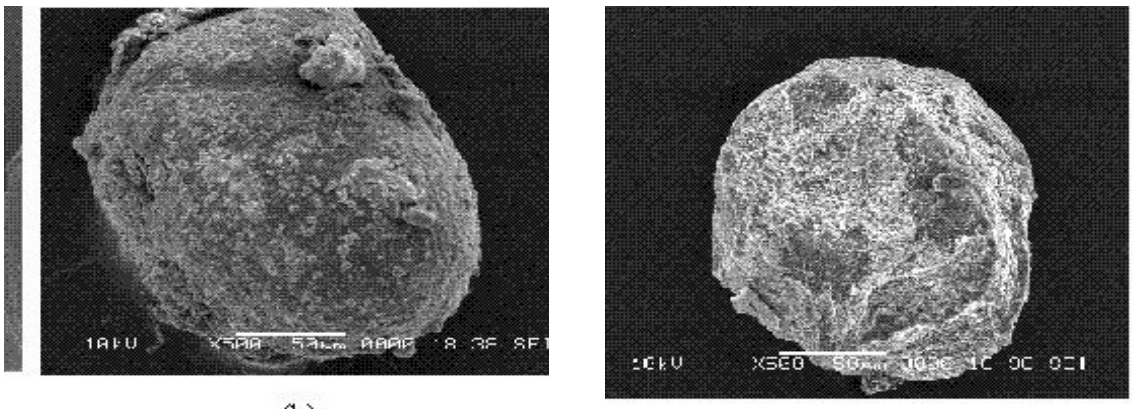

Figure 9.SEM of microspheres of optimized batch of drug Clarithromycin

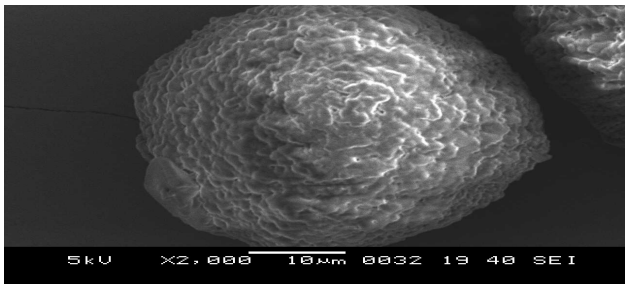

Figure 10. SEM of microspheres of optimized batch of drug Omeprazole.

IR interpretations for drug polymer interaction in formulation of Clarithromycin;

IR Spectra of pure drugs sample of Clarithromycin and Omeprazole (Figure No.3,4) were compared with IR spectra of drugs (Clarithromycin and Omeprazole) with polymer(Figure No.11,12) used in microsphere, as there was no significant change in the pattern of peaks of pure drug and drugs with polymer in microsphere. So there was no incompatibility seen in between drug Clarithromycin/Omeprazole and polymers.

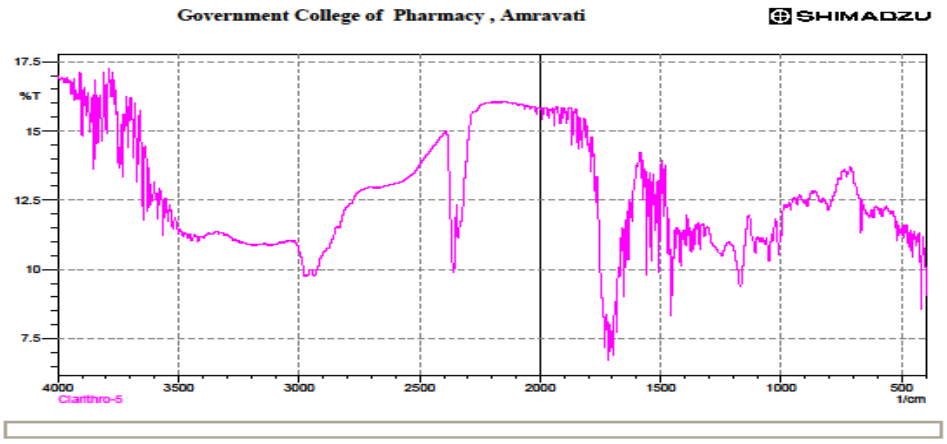

Figure 11.IR interpretations for pure drug Clarithromycin & physical mixture containing microspheres.

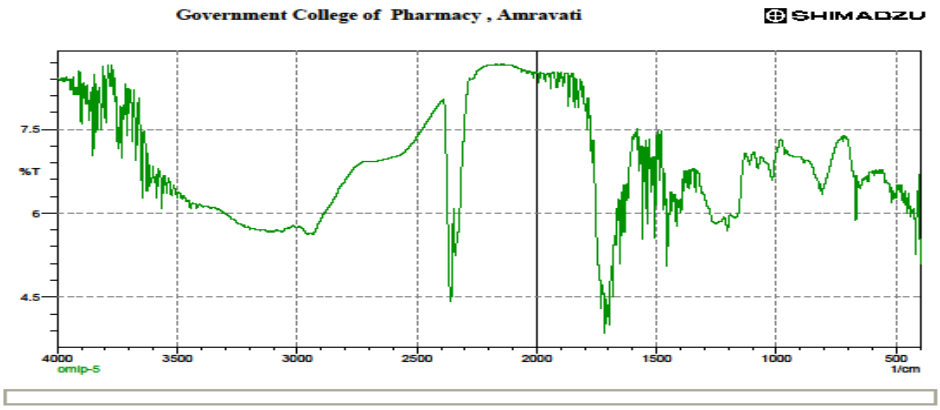

Figure 12.IR interpretations for pure drug Omeprazole& physical mixture containing microspheres IR interpretations for pure drug Omeprazole& physical mixture containing microspheres

Formulated capsule dosage form of optimized batch

The capsules were successfully prepared of only optimized batch of microsphere by filling the microspheres and were subjected to evaluations.(Table 12)

Table 12.Formulation of Capsule single unit dosage form of optimized batch: (S.D.n=3)

Batches of Micro-balloons	Drug content (mg)	Entrapment efficiency	Microsphere equivalent to drug dose(mg)	Capsule
CF5	**89.65**	**96.26**(1.8)	**20**	**0**
OF5	**92.6**	**82.0**(2.0)	**20**	**0**

* Each sample was analyzed in triplicate **(S.D. n=3)**

Evaluation of Capsules

General appearance

The texture of Capsule was found fine and smooth. No pinhole was found in Capsule. No deformity was seen in Capsule. The appearance of capsule was satisfactory.

Weight variation

This test was conducted on 20 capsules. Each capsule was individually weighed to find out weight variation. Average weight was calculated.(Table 13) The results are presented in Table 13.

Table 13 .Weight variation test on prepared capsule (S.D. n=3)

Average weight (mg)	Permitted limit (mg)	Range of all capsule (mg)	Permitted limit of variation	Pass/ fail
650.43(1.25)	682.95-617.91	631.43-660.45	±.5%	Pass

Content uniformity test

10 capsules were accurately weighed, remove the hard gelatin shells and powder equivalent to 20mg of drug Clarithromycin and dissolved it in 1N NaOH. Drug content was calculated by measuring absorbance at wavelength 282.00 nm. The results are given in Table. Same procedure is followed for Omeprazole at wavelength 305nm.(Table 14)

Table no. 14. Content uniformity test on prepared capsule:

Batches.	Amount of drug (mg) (n= 10)	% Drug content
CF5	224.13(2.2)	89.65
OF5	18.52(1.3)	92.6

Dissolution study of Optimized batch of Capsule

In vitro drug release of Optimized batch of Capsule of drugs Clarithromycin and Omeprazole were done.(Figure No.19) Results were illustrated in table 15.

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Table no. 15. In–vitro evaluation of drug release from capsule of optimized batch {Cumulative Release of Batch F5 Batches of Capsule (S.D. n=3)}

T Time (hrs)	% Cumulative Release of Batch F5 Batches of Capsule	
	Capsule of CF5 batch	**Capsule of OF5 batch**
0	**00.00**	**00.00**
1	**24.01**(0.28)	**34.31**(0.190
2	**42.23**(0.26)	**43.87**(0.37)
3	**51.42** (0.42)	**52.829**(0.51)
4	**58.92** (0.30)	**58.09**(0.42)
5	**59.33** (0.44)	**65.8**(0.58)
6	**66.42** (0.53)	**81.89**(0.73)
7	**69.06** (0.63)	**91.87**(0.82)
8	**91.36** (0.83)	**95.69**(0.93)

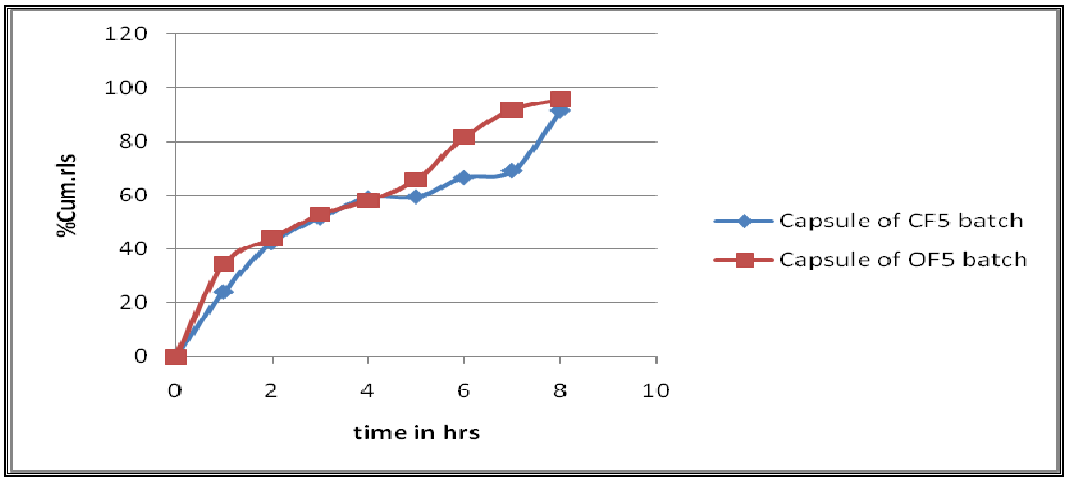

Figure13. Drug release of Optimized batch of Capsule

CONCLUSION

The present study was carried out to develop Mucoadhesive drug delivery System in the form of microsphere dosage form and combination of Clarithromycin and Omeprazole used for treatment of Helicobacter Pylori infection using Carbopol971p and HPMC K100M and thereafter formulating the formulation. From the study it is observed that formulation act as prolonged dosage form. As the stirring speed increased the size of microsphere decreases and increases the released rate drug. The prepared microsphere of Clarithromycin and Omeprazole also gave good Micromeritics result, percent yield, drug entrapment and in-vitro release. In dissolution study of all formulations it was observed that change in process variables during the formulation of microspheres like stirring speed (RPM) and stirring time significantly affect the release rate of drug. The microspheres of F5 batch were found to be satisfactory in terms of percent yield, percent drug entrapment and in-vitro release; Surface morphology

by stereomicroscope gives smooth and spherical shape of all batches. IR study show positive result there was no interaction in drug and polymer. SEM also show good spherical surface. The unit dosage form such as capsule also gave good satisfactory result.

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