DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF CLARITHROMYCIN MICROSPHERES

A Sneha*[1], V Kavitha[2], Shafiya begum[3], Samreen begum[4], J Bikshapahi[5], B Krishnaveni[6]

[1]*Assistant Professor, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

[2]*Student, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

[2]*Student, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

[3]*Student, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

[4]*Student, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

[5]*Student, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

[6]*Student, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda (V), Gajwel (M), Siddipet (D), Telangana (state) 50231, India*

ABSTARCT

In the present work, bioadhesive microspheres of Clarithromycin using Sodium alginate along with HPMCK100M, HPMCK15M, as copolymers were formulated to deliver Clarithromycin via oral route. The results of this investigation indicate that ionic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Clarithromycin microspheres. The technique provides characteristic advantage over conventional microsphere method, which involves an "all-aqueous" system, avoids residual solvents in microspheres. Other methods utilize larger volume of organic solvents, which are costly and hazardous because of the possible explosion, air pollution, toxicity and difficult to remove traces of organic solvent completely. Micromeritic studies revealed that the mean particle size of the prepared microspheres was in the size range of 512-903µm and are suitable for bioadhesive microspheres for oral administration.Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion.The in-vitro mucoadhesive study demonstrated that microspheres of Clarithromycin using sodium alginate along with HPMCK15M as copolymer adhered to the mucus to a greater extent than the microspheres of Clarithromycin using sodium alginate along with HPMCK100M as copolymer.

KEYWAORDS : *Clarithromycin ,HPMC ,Microspheres, Bio adhesive*

1.INTRODUCTION

Microspheres are one of the particulate delivery systems used to achieve sustained or controlled drug delivery, improve bioavailability , stability and target drug to specific sites. Microspheres also offer advantages such as limiting fluctuation within a therapeutic range, reduction in side effects, decreased dose frequency and hence improved patient compliance[1,2]. The popular method for the encapsulation of drugs within water-insoluble polymers is the emulsion solvent evaporation method. This technique offers several advantages and is preferable to other preparation methods such as spray drying, sonication and homogenization because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the drugs.

Most of the conventional drug delivery system for treating various disorders and diseases such as inflammatory bowel disease, colon cancer and intestinal amoebiosis have failed as drugs in its intact form as they do not reach the site of action in appropriate concentration. Microspheres drug administration offers a number of advantages in therapeutics, where the controlled releases of drug delivery as well as the predictable and reproducible drug release kinetics are important features of them in colon drug delivery system.

Clarithromycin is a semi-synthetic macrolide antibiotic. Chemically, it is 6-0 methylerythromycin.Its molecular formula is C38H69NO13. Clarithromycin exerts its antibacterial action by binding to the 50S ribosomal subunit of susceptible microorganisms resulting in inhibition of protein synthesis.The dosage of clarithromycin is 500mg [3]. The purpose of this study was to design mucoadhesive microspheres containing clarithromycin as an anti-H. pylori agent and to evaluate the effectiveness of the mucoadhesive microspheres for H. pylori eradication therapy. Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000 µm in diameter and consist either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in stomach, thus offering the possibilities of localized as well as systemic controlled release of drugs. The application of mucoadhesive microspheres to the mucosal tissues of gastric epithelium is used for administration of drugs for localized action. Mucoadhesive microspheres are widely used because they release the drug for prolong period, reduce frequency of drug administration and improve the patient compliance

2. Materials and Methods

Clarithromycin, (drug)Natco LABS ,,.HPMCK4M, (Polymer) Signet chemical corporation,Mumbai, india..HPMC K15M,(Polymer)Merck specalistsPvtltd,Mumbai, India. HPMCK100M,(polymer)r Manufacture of Merck specailistsPvt Ltd Mumbai, India..Water(,Diluent).Na.Alginate,(Binder) Merck specailistsPvt Ltd Mumbai, India. Calcium Chloride,(Coating agent) Merck specailistsPvt Ltd Mumbai, India.Na.Alginate,(Binder) Merck specailistsPvt Ltd Mumbai, India.

2.1 PREFORMULATION STUDIES

Preparation of Standard Calibration Curve Of Clarithromycin [4]:

10 mg of Clarithromycin was accurately weighed and dissolved in 10ml of methanol (Stock Solution – I) to get a concentration of 1000 µg/ml.From the stock solution- I,1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution-II) to get concentrations of 100µg/ml.From the stock solution-II,aliquots were taken and suitably diluted with 0.1N HCl (pH 1.2) to get concentrations in the range of 2 to 10µg/ml.The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 252nm against reference solution 0.1N HCl (pH 1.2).

The Linear Regression Analysis:

The linear regression analysis was done on Absorance points. The standard calibration curve obtained had a Correlation Coefficient of 0.998 with of slope of 0.028 and intercept of 0.004.

2.2 METHOD OF PREPARATION

IONOTROPIC GELATION METHOD [5]:

Batches of microspheres listed in table 1 were prepared by ionotropic gelation method which involved reaction between sodium alginate and Polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API, Clarithromycin (100 mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried shown in figure 1.

Figure- 1 Photograph of prepared microspheres

Table 1: Prepared formulation of Biooadhesive Microspheres

S.No.	FORMULATION CODE	DRUG:POLYMER RATIO	POLYMER RATIO
1	**T_1**	**1:2**	Na alginate : HPMC K100M(1.1)
2	**T_2**	**1:3**	Na alginate : HPMC K100M(2:1)
3	**T_3**	**1:4**	Na alginate : HPMC K100M(3:1)
4	**T_4**	**1:2**	Na alginate : HPMC K15M(1:1)
5	**T_5**	**1:3**	Na alginate: HPMC K15M(2:1)
6	**T_6**	**1:4**	Na alginate: HPMC K15M(3:1)

2.3 CHARACTERIZATION OF MICROSPHERES [6-9]:

Percentage yield

The percentage of production yield was calculated from the weight of dried microsphe-res recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

Practical mass (Microspheres)

% Yield= pratical mass (Microspheres)/Theoritical mass (Polymer and drug) X100

Drug entrapment efficiency:

Microspheres equivalent to 100 mg of the drug Clarithromycin were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100 ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using simulated gastric fluid pH 1.2 After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectrophotometrically at 252 nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Particle size analysis:

Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Microparticles sizes were calculated under 45x magnification.

The average particle size was determined by using the Edmondson's equation:

$$D_{mean} = \frac{nd}{n}$$

Where,

n – Number of microspheres observed

d – Mean size range

Swelling study: Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2 .The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship

Swelling ratio = (W1-W0/W0) 100

Where W0 & Wt are initial weight and Final weight of microspheres respectively.

2.4 EVALUATION

In vitro drug release study:

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^0C, 50 rpm) using the USP type – I rotating basket method in simulated gastric fluid pH 1.2 (900ml). A quantity of accurately weighed microspheres equivalent to 100mg Clarithromycin each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 252nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment.

If $n < 0.5$, the polymer relaxation does not affect the molecular transport, hence diffus-ion is Fickian.

If $n > 0.5$, the solid transport will be non-fickian and will be relaxation controlled

3. RESULTS AND DISCUSSION

3.1 PREFORMULATION STUDIES

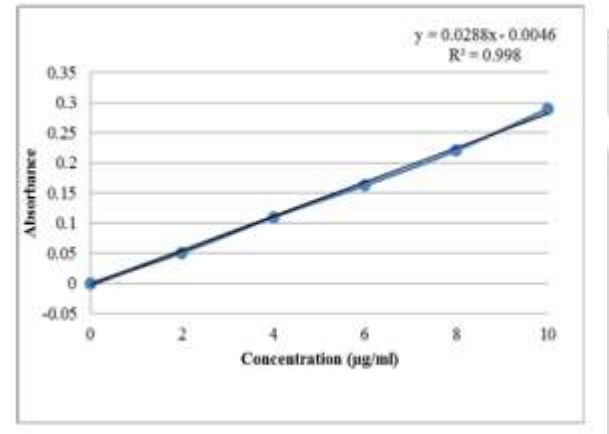
Calibration curve of Clarithromycin in simulated gastric fluid pH 1.2:

The data was shown in Table and presented as graph in figure 2

Table:2 Calibration curve data for Clarithromycin in simulated gastric fluid pH 1.2

CONCENTRATION (µg/ml)	ABSORBANCE
2	0.051
4	0.110
6	0.163
8	0.221
10	0.290

Figure:2 Standard graph of clarithromycin in simulatedgastric fluid PH 1.2



3.2 EVALUATION AND CHARACTERIZATION OF MICROSPHERES

PERCENTAGE YIELD

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug-polymer solution, addition of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range of 80-88% for microspheres containing sodium alginate along with HPMCK100M as co polymer, 62.22 – 87% microspheres containing sodium alginate along with HPMC K15M as copolymer.

DRUG ENTRAPMENT EFFICIENCY

Percentage Drug entrapment efficiency of Clarithromycin ranged from 53.2 to 81.66% for microspheres containing sodium alginate along with HPMC K100M as copolymer and 84.66 to 87.55% for microspheres containing sodium alginate along with HPMC K15M as copolymer. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The % drug entrapment efficiency of the prepared microspheres is displayed in Table 3.

Table 3 Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

S.No.	Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency
1	T_1	80	8.55	53.22
2	T_2	85	8.88	74.4
3	T_3	88	12.40	81.66
4	T_4	87	12.88	84.66
5	T_5	86	12.40	85.88
6	T_6	62.2	13.44	87.55

PARTICLE SIZE ANALYSIS

Microspheres containing sodium alginate along with HPMC K100M as copolymer had a size range of 512µm to 826µm, microspheres containing sodium alginate along with HPMC K15M as copolymer exhibited a size range between 517µm to 834µm. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration is displayed in Table 4

Table 4 Particle size analysis

	PARTICLE SIZE RANGE (µm)	FREQUENCY	AVERAGE PARTICLE SIZE (µm)
T1	200-400 400-800	28 22	512
T2	200-400 400-800	19 41	617
T3	200-400 400-800	22 27	826
T4	200-400 400-800	25 25	517
T5	200-400 400-800	18 32	642
T6	200-400 400-800	20 30	834

SWELLING STUDY

As drug ratio increased, the percentage of swelling increased from 28 to 49% for microspheres containing sodium alginate along with HPMC K100M as copolymer, 55 to 85% for microspheres containing sodium alginate along with HPMC K15M as copolymer. The percentage of swelling of the prepared microspheres are displayed in table5

Table 5 Percentage swelling of the prepared microspheres

S.NO.	FORMULATION CODE	INITIAL (Wt)	FINAL (Wt)	PERCENTAGE SWELLING
1	T_1	10	12.8	28
2	T_2	10	13.9	39
3	T_3	10	14.9	49
4	T_4	10	15.5	55
5	T_5	10	17.8	78
6	T_6	10	18.5	85

***IN-VITRO* DRUG RELEASE STUDIES**

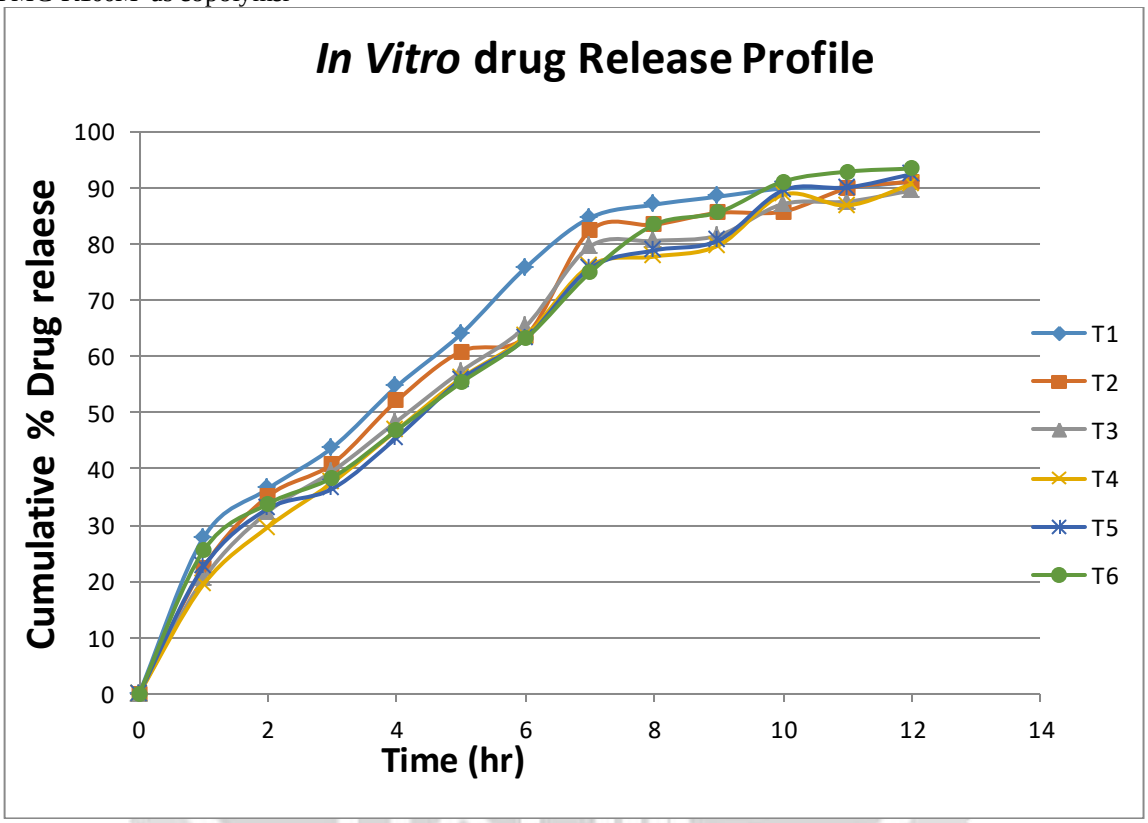
Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the in-vitro dissolution studies of formulations T_1 to T_6 to be shown in table6, Figure 3 shows the comparison of % CDR for formulations T_1 to T_6, The formulations T_1 to T_6 containing Sodium alginate along with HPMC K15M as copolymer showed a maximum release of 93.44% for 12 hours respectively.

Table -6 *In-Vitro* drug release data of Clarithromycin microspheres containing sodium alginate along with HPMC K100M as copolymer

Time (h)	**cumulative percentage of drug released**					
T	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
0	0	0	0	0	0	0
1	27.77	22.44	20.44	19.44	22.55	25.44
2	36.44	35.11	32.33	29.64	32.88	33.77
3	43.77	40.88	39.55	37.55	36.44	38.44
4	54.66	52.11	48.44	46.88	45.55	46.88
5	64.01	60.88	57.33	56.11	55.88	55.33
6	75.77	63.55	65.33	63.55	63.11	63.11

7	84.65	82.33	79.55	76.11	75.66	74.88
8	87	83.44	80.55	77.77	78.88	83.44
9	88.44	85.55	81.55	79.77	80.66	85.66
10	89.88	85.77	87.11	88.77	89.55	91.11
11	90.06	89.88	87.55	86.88	90.06	92.88
12	91.11	91.11	89.55	90.66	92.44	93.44

Figure 3 Comparison of *In-Vitro* drug release profile of Clarithromycin microspheres containing sodium alginate along with HPMC K100M as copolymer



4. SUMMARY AND CONCLUSION

In the present work, bioadhesive microspheres of clarithromycin using sodium alginate along with HPMC K100M, HPMC K15M as copolymers were formulatd to deliver clarithromycin via oral route.The results of this investigation indicates that ionic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Clarithromycin microshheres. Micrometric studies revealed that the mean particle size of the prepared microspheres was in the size range of 512-834µm and suitable for bio adhesive microspheres for oral administration. Increase in the polymer concentration led to increase in % yield, % Drug entrapment efficiency, particle size, % swelling and % mucoadhesion. The *in vitro* mucoadhesive study demonstrated that microspheres of clarithromycin using sodium alginate along with HPMC K15M as copolymer adhered to the mucus to a greater extent than the microspheres of clarithromycin using sodium alginate along with HPMC K100 as copolymer. Based on the results of evaluation tests formulation coded T6 was concluded as best formulation.

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