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DESIGN, FORMULATION AND CHARACTERIZATION OF TASTE MASKING DOSAGE FORM OF MACROLIDES ANTIBIOTIC

Pharma

Aniruddha Pare Department of Pharmaceutics, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India.

Sunil Kumar Shah* Department of Pharmaceutics, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India. *Corresponding Author

C. K. Tyagi Department of Pharmacology, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India.

Shubham Pare Assistant Manager, Cipla Ltd, SEZ, Indore Madhya Pradesh, India.

ABSTRACT

Microencapsulation have been considering for a wide range of application both in soluble and insoluble forms. In the present study Microencapsulation techniques were used for masking the taste of Clarithromycin, based on microencapsulation and formation of insoluble complex. The aim of present study was to develop a formulation which should be tasteless upon administration and not release drug at pH of saliva but should release the drug rapidly in to the stomach (pH 0.9-1). Hence for masking the taste of Clarithromycin and materials were used on the basis of formulation design. The Microencapsulation of Clarithromycin (CLM) was prepared by Chitosan microspheres by emulsification. Chitosan was used to prepare microcapsule because it is a natural and biocompatible cationic polymer. Chitosan have film forming property and pH dependent solubility. It is insoluble at pH 6.5 to 7.0 and dissolves rapidly in acetic condition. Initial trails carried on the formulation of microcapsule revealed that stirring speed and D: P ratio had profound effect on microcapsule, EE of the microcapsule. Hence these parameters were studied for optimizing the formulation. The D: P ratios were varied between 1:1 to 1:5 and stirring speed varied between 500 rpm to 2000 rpm and the result of experiments carried out for optimization is illustrated in Table 4.5. Assay of the optimized drug formulation was found 53%. Formulation CMF15 having 1:4 D: P ratio and stirring speed of 1500 rpm was optimized. The results indicate that the chitosan microcapsule can be prepared by microencapsulation by emulsification technique for taste masking of water soluble bitter drug clarithromycin. But this method showed draw-back of releasing negligible amount of drug (12.3%) in acidic pH while, it showed sustained release in pH 7.4 Hydrochloride buffer.

KEYWORDS

Clarithromycin (CLM), Chitosan, Microencapsulation, by emulsification method

INTRODUCTION

Although a variety of delivery systems are being developed for different routes of administration like the oral, parenteral, nasal, and transdermal, the oral route remains attractive for drug delivery[1,2] because this mode of administration is an easy, convenient, noninvasive and familiar method of drug delivery[3]. The common oral dosage forms include: liquid mixtures like solutions, suspensions, solid dosage forms like tablets and capsules and liquid filled capsules etc. However, patients at the extremes of age, such as children and the elderly[4], often experience difficulty in swallowing solid oral dosage forms. For these patients the drugs are mostly provided in liquid dosage forms such as emulsions and suspensions. These dosage forms usually lead to perceptible exposure of the active drug ingredient to taste buds and this is a very serious problem when the drug has an extremely unpleasant or bitter taste.

The bitter taste of the drugs, which are orally administered, is disadvantageous in several aspects[5]. Taste is an important parameter governing the compliance. **"***The worse the taste of the medication, the better the cure"* was once the prevailing attitude. A change in patient attitude and development of taste masking technique has reversed this opinion [6]. Patients now expect and demand formulations that are pleasantly, or at least tolerably, flavoured. The disagreeable taste of drugs causes difficulties in swallowing (dysphagia) or causes patients to avoid their medication thereby resulting in low compliance of patients[7]. Conventional taste masking techniques such as use of sweetener, amino acids, flavouring agents are often unsuccessful in masking the taste of the highly bitter drugs like quinine, barberin, antibiotics like levofloxacin, ofloxacin, sparfloxacin, ciprofloxacin, cefuroxime axetil, erythromycin [8]. Thus taste masking technologies are considered important and developed by many researchers.

In the current study, clarithromycin is used as model drug. It is a semi-synthetic macrolide antibiotic which inhibits bacterial protein synthesis by binding to the bacterial 50S ribosomal subunit[9]. In this work BCS Class II drug Clarithromycin is used as a model drug, which is having poor solubility but high permeability. It is also clinically active against bacteria responsible for exacerbations of chronic bronchitis and the atypical pathogens that cause respiratory tract infections. Clarithromycin (CLM) was stable in the gastric acid and well absorbed, but it has a very bitter taste, this make it inconvenient when taken orally[10]. The objective of this study was to formulate the taste mask clarithromycin.

MATERIAL AND METHOD

Clarithromycin was procured from Cipla Dewas Madhya Pradesh. Excipients used for preparation of microsphere using chitosan as polymeric matrix were also procured from Merck Company Mumbai.

Standard calibration curve of Clarithromycin

Clarithromycin was found to be soluble in organic solvents such as methanol[11]. HPLC method of estimation was carried out in methanol ranging from 10-100 mcg/ml solutions at 220 nm against the blank. The standard graph obtained was linear, with regression coefficient 0.999.

Techniques used in the present study for taste masking of bitter drug

In the present study microencapsulation techniques were used for masking the taste of Clarithromycin, based on microencapsulation and formation of insoluble complex [13]. The aim of present study was to develop a formulation which should be tasteless upon administration and not release drug at pH of saliva but should release the drug rapidly in to the stomach (pH 0.9-1). Hence for masking the taste of Clarithromycin various techniques and materials were used on the basis of formulation design[14].

Chitosan microspheres by emulsification:

A natural cationic, polysaccharide chitosan, has many applications in pharmaceutical and biomedical formulations [15]. Since 1980s the processing techniques for preparation of chitosan microsphere have been extensively developed. The chitosan microsphere are used as controlled drug delivery systems for conventional drug, DNA and protein drugs and has attracted increasing attention since beginning of the past decade [16]. The techniques proposed are, Simple or complex coacervation, Ionotropic gellation with an oppositely charged, emulsification/solvent evaporation and, recently spray drying etc[17]. The bitterness of the preparation leads to lack of patient agreement in paediatric and geriatric formulation. Clarithromycin is a bitter in taste. Chitosan has film forming property. In the present study, we have made

attempts to mask the bitterness of clarithromycin by formulating it as microsphere using chitosan as polymeric matrix prepared by emulsification method[18-19]. Formulation and process parameters such as drug: polymer (D:P) ratio and stirring speed were optimized to get maximum entrapment, controlled size distribution and eventually good taste masking. The efficiency of the coating was assessed through In Vitro dissolution studies.

6.3.1.1: Method of Preparation:

Microspheres were prepared by emulsification method [19]. Drug was added to 2% chitosan solution in acetic acid and added to continuous phase comprising of light and heavy liquid paraffin (1:1) and Span 80 under stirring. On formation of Emulsion, Glutaraldehyde (1ml) was added drop wise to form microspheres. The microspheres were washed under vacuum using n-hexane. Finally washed with water to remove glutaraldehyde and dried. The various variables such as D:P ratio, stirring speed, were altered to optimize the microsphere formulation and are described in Table 3**.** Various parameters were studied in the formulation of microspheres such as stirring speed, D: P ratio, taste evaluation, EE, particle size, and finally In-vitro release.

Characterization[20]

Taste Evaluation Study

The microspheres were evaluated for taste by human volunteers. Taste evaluation was performed by 10 human volunteers at different places, the prepared microspheres, equivalent to 10mg of drug were taken orally by the volunteers and swallowed in mouth for 10 sec, and the results were recorded in Table 3 with taste score.

Entrapment Efficiency (EE)

The formulations were analyzed for EE, the result of all batches are shown in Table 3. The dried microspheres were crushed and dispersed under stirring in 25ml 0.1 N HCl for 24 hrs[21]. Resultant mixture was filtered and filtrate was titrated with 0.004M Sodium dodecyl sulphate (SLS).

Entrapped drug Percent entrapment efficiency (PEE) = X 100 Wt. of drug taken

Particle Size:

Particle size of the microspheres was determined by optical microscopy. Measurements were conducted for 70 microspheres of each batch and average was calculated.

In vitro Release Studies:

In-vitro release studies were carried out in three different Media. Initially the release was studied in pH 6.8 Hydrochloride buffer for 5 minute, and then in 0.1N HCl for 2 hrs and finally in pH 7.4 Hydrochloride buffer for 12 Hrs. Microsphere equivalent to 100 mg of DH was dispersed in 25 ml of dissolution media in a 50 ml beaker with mild stirring using magnetic stirrer, 5 ml sample was withdrawn at time interval and centrifuged at 4000 rpm for 10 min on Remi centrifuge apparatus[22]. The supernatant thus obtained was analyzed titrimetrically using 0.004M SLS. The sedimented pellet obtained on centrifugation was redispersed in fresh Media (5ml) and added to the dissolution Media. The in-vitro release studies in 0.1N HCl was carried for 2 hrs. similarly. The release study in pH 7.4 buffer was continued till 12 hrs.

E. Assay:

Accurately weighed (100mg) dried microspheres were crushed and dispersed under stirring in 25ml 0.1 N HCl for 24 hrs. Resultant mixture was filtered and filtrate was titrated with 0.004M Sodium dodecyl sulphate (SLS) using dimethyl yellow as indicator.

RESULT AND DISCUSSION

Standard calibration curve of Clarithromycin

Clarithromycin was found to be soluble in organic solvents such as methanol. HPLC method of estimation was carried out in methanol ranging from 10-100 mcg/ml solutions at 220 nm against the blank. The standard graph obtained was linear[23], with regression coefficient 0.999as indicated in Figure 1.

Stock solution preparation:

From stock solution accurately measured 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml,1.2ml,1.4ml,1.6ml,1.8ml and 2.0ml quantity of stock solution into a series of 10ml volumetric flask and diluted to about 7ml with distilled water. A volume of 1.2 ml $4x10^{-3}$ M Eosin Y solutions was added to each flask and the solution were mixed well before addition of 1 ml of 0.4M Acetate buffer (pH 3).The mixtures were diluted to 10ml with the distilled water to get the concentration of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40 µg/ml[24]. The absorbance of each solution was measured at 547 nm using the blank prepared simultaneously by same method without stock solution.

Table 1: Absorbance of Macrolide antibiotics in 0.1N HCl at specific range.

S.No.	Clarithromycin(λmax =547nm)	
1	Conc.(µg/ml)	Absorbance (nm)
2	0	0
3	4	0.180
4	8	0.450
5	12	0.605
6	16	0.802
7	20	0.935
8	24	1.190
9	28	1.394
10	32	1.610
11	36	1.805
12	40	1.975

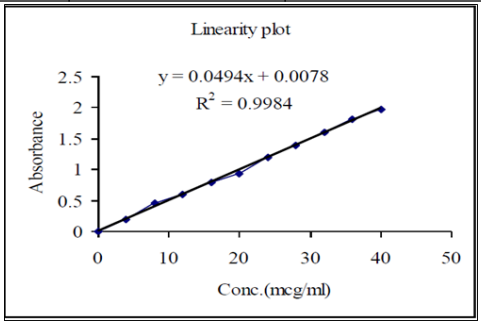

Figure 5.1: Linearity curve of Clarithromycin in 0.1N HCl.

Chitosan was used to prepare microcapsule because it is a natural and biocompatible cationic polymer. Chitosan have film forming property and pH dependent solubility. It is insoluble at pH 6.5 to 7.0 and dissolves rapidly in acetic condition[26]. Therefore the aim of this study was, to mask the bitter taste of Clarithromycin by chitosan microcapsule which remain un-dissolve in oral cavity on administration but dissolve rapidly in acidic condition to release drug in to stomach. Initial trails carried on the formulation of microcapsule revealed that stirring speed and D: P ratio had profound effect on microcapsule, EE of the microcapsule. Hence these parameters were studied for optimizing the formulation. The D: P ratios were varied between 1:1 to 1:5 and stirring speed varied between 500 rpm to 2000 rpm and the result of experiments carried out[27] for optimization is illustrated in Table 3. Assay of the optimized drug formulation was found 53%.

Table 3: optimization of various parameters of formulation

Formulation code	Drug: polymer ratio (w/w)	Stirring speed (rpm)	Mean particle size(µm)	Entrapment Efficiency (%)
CMF1	1:1	500	85.2	21.8
CMF 2	1:1	1000	73.2	22.3
CMF3	1:1	1500	65.6	23.5
CMF4	1:1	2000	66.2	22.3
CMF5	1:2	500	85.4	35.2
CMF6	1:2	1000	76.3	35.3
CMF7	1:2	1500	64.3	40.5
CMF8	1:2	2000	67.4	42.6
CMF9	1:3	500	87.8	48.5
CMF10	1:3	1000	76.7	48.6
CMF11	1:3	1500	62.5	49.4
CMF12	1:3	2000	65.4	49.6
CMF13	1:4	500	84.2	48.9
CMF14	1:4	1000	76.8	57.4
CMF15	1:4	1500	66.5	59.8
CMF16	1:4	2000	86.4	59.3
CMF17	1:5	500	75.8	54.4

CMF18	1:5	1000	65.2	56.2
CMF19	1:5	1500	66.7	59.4
CMF20	1:5	2000	66.5	59.1

Stirring Speed:
The stirring speed of formulations was optimized on the basis of EE. The stirring speed varied from 500 rpm to 2000 rpm, and the optimum EE was found at stirring speed of 1500 rpm (CMF15). But at 2000 rpm there was no significant change in EE shown in Table 3

Drug: Polymer Ratio:
The drug to polymer (w/w) ratio was varied from 1:1 to 1:5 as shown in Table 3As the drug to polymer ratio was increased the EE was also increased. The optimum EE was obtained at D: P ratio 1: 4 and at stirring rate of 1500 rpm (CMF15).

Taste Evaluation Study:
The optimized batch CMF15 was found to be tasteless, reported by 100% volunteer shown in table 4.

Table 4: Taste Evaluation Report of Optimized Batch of Clarithromycin Chitosan Microspheres in Human Volunteers.

Volunteers	2sec	4sec	6sec	8sec	10sec
1	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++
3	+++	+++	+++	+++	+++
4	+++	+++	+++	+++	+++
5	+++	+++	+++	+++	+++
6	+++	+++	+++	+++	+++
7	+++	+++	+++	+++	+++
8	+++	+++	+++	+++	+++
9	+++	+++	+++	+++	+++
10	+++	+++	+++	+++	+++

+ (bitter)
++ (litter bitter)
+++ (tasteless)

Entrapment Efficiency (EE):
The optimum EE (59.8) was observed at drug to polymer ratio 1: 4, at stirring speed of 1500 rpm. Increase in D:P ratio up to 1:4 increase %EE of microsphere. Further increase in D:P ratio did not showed significant effect on EE. Hence the batch has D: P ratio of 1:4 was found to be optimized (CMF15), as further increase in D: P ratio up to 1:5 showed no change in % EE. Increase in stirring speed caused decrease in mean particle size of microparticle, but negligible increase in %EE. Hence Optimum %EE (59.8) was found at stirring speed 1500 rpm.

Particle Size:
The particle size of optimized batch (CMF15) was found to be 66.5µm. Increase in stirring speed caused decrease in the mean particle size of microparticle, but negligible increase in %EE. Optimum %EE (59.8) was found at stirring speed 1500 rpm with mean particle size 66.5 µm. Further increase in stirring speed (2000 rpm) shows no effect on particle size. Results are shown in Table 3.

In Vitro Release Studies:
The formulation was designed using the approach of reducing the contact of drug with taste receptor through microencapsulation[28]. To ascertain that the formulation does not release the drug in oral cavity at pH 6.8 and release the drug in 0.1 N HCl (pH of stomach).The In-Vitro release study was carried out at pH 6.8, at pH 1.2 and finally at pH 7.4 buffer. Samples withdrawal up to 5 min did not shown any release of drug in pH 6.8 buffer[29]. This may be attributed to the fact that the microspheres does not erode at pH 6.8 .The release study carried out in 0.1N HCl showed, 12% of drug released after 2 hrs. But 75.5 % drug was released in pH 7.4 buffer at the end of 12 Hrs. the results are shown in Table 5.

Table 5: In Vitro release study in different medium

Time in min.	%Drug release at pH 6.8 buffer	%Drug release in 0.1N HCl	%Drug release at pH 7.4 buffer
-5	0%	-	-
15	-	-	-
30	-	Not found	-
60	-	Not found	-
90	-	4.8±3.2	-
120	-	12.3±2.6	-
180	-	-	36.5±3.6
240	-	-	48.6±3.2
360	-	-	61.2±2.2
480	-	-	69.6±3.4
600	-	-	74.2±4.5
720	-	-	76.2±2.5

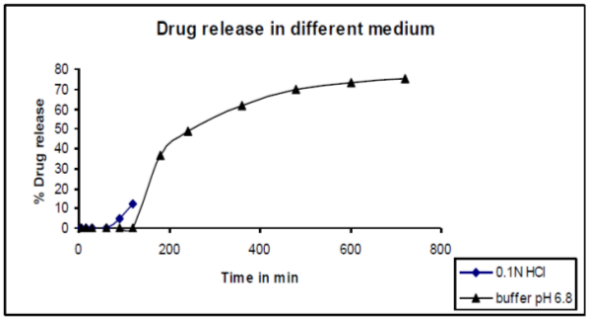

Figure 3: Drug release study

Summary and Conclusion
From the above studies, formulation (CMF15) having 1:4 D: P ratio and stirring speed of 1500 rpm was optimized[30]. The results indicate that the chitosan microcapsule can be prepared by microencapsulation by emulsification technique for taste masking of water soluble bitter drug (clarithromycin). But this method showed draw-back of releasing negligible amount of drug (12.3%) in acidic pH while, it showed sustained release in pH 7.4 Hydrochloride buffer.

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