Original Research Paper

Volume - 10 | Issue - 9 | September - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pharma

DESIGN, FORMULATION AND CHARACTERIZATION OF TASTE MASKING OF CLARITHROMYCIN

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ABSTRACT Microencapsulation techniques were used for masking the taste of Clarithromycin, microencapsulation of chitosan colloidal suspension containing clarithromycin which was prepared by precipitation method. The objective was to over-come bitterness of clarithromycin-chitosan colloidal suspension. Microencapsulation of Clarithromycin (CLM) was prepared by Drug-Chitosan Colloidal Suspension Coated by Eudragit E (by Precipitation). Initial trials carried on the formulation of Eudragit microparticles revealed that D:P(Drug :Polymer) (Eudragit E and chitosan) ratio and pH had profound effect on taste, free drug concentration, EE, taste and formation of the microparticles. Hence these parameters were studied for optimizing the formulation. The D:P (Eudragit E and chitosan) ratios were varied between (1: 0.5: 0) to (1: 1.5: 0.3) and pH varied between acidic to alkaline (approximately pH 8.0). In this study it was observed that as the drug to polymer ratio was increased from 1: 0.5 to 1: 1.5, the free drug concentration in the supernatant was decreased leading to change of taste from bitter to tasteless. The optimum taste masked formulation was obtained at D: P ratio DCF4 1: 1.5, at pH 7.5-8. But the taste less product was sticky gummy and could not be recovered in dried form. Hence, chitosan was used with Eudragit E. The concentration of chitosan was varied between 0.1 to 0.3%, It was observed that the gummy product changed in to isolated microparticles at chitosan concentration batch DCF4 0.3%, at pH 7.5-8.0. Optimized taste masked product batch DFC7 (Eudragit coated particle) was obtained at pH 7.5- 8.5, at D: P ratio 1: 1.5: 0.3 (drug: Eudragit: chitosan). *In Vitro* drug release study at pH 6.8 buffer showed, no drug release in 5 min but 98.4 % drug released in 30 min in 0.1 N HCl

KEYWORDS : Clarithromycin (CLM), Eudragit E, precipitation method

INTRODUCTION

Antibiotics are frequently used today for the therapy of infectious diseases both in human and veterinary medicine[1]. In human medicine, antibiotics pose the third biggest group among all pharmaceuticals and in veterinary medicine, more than 70% of all consumed pharmaceuticals are antibiotic agents[2]. So, antibiotics are designed to act very effectively even at low doses and, after a short time of residence, 50% to 95% of administered antibiotics are excreted from the body[3-4]. 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[10]. 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. The objective of this study was to formulate the taste mask clarithromycin.

MATERIALAND METHOD

Clarithromycin, Eudragit L100 was procured from Cipla dewas, Madhya Pradesh. Excipients used for preparation of suspension are Sucralose, Aerosil, citric acid, sugar, xanthan gum and colourquinidine yellow 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.

Drug and Excipient Compatibility Studies

Fourier transforms infrared spectroscopy (FTIR) analysis

Drug and polymer were mixed in ratio of 1:1 and mixtures were placed in sealed vials for 3 months at room temperature. FTIR measurement of drug and individual polymer and drug polymer mixture were obtained on Simadzu FTIR[12]. Samples were prepared with Kbr and placing in sample holder. The spectra were scanned over the wave number range of 4000-400 cm−1 at the ambient temperature.

Drug-Chitosan Colloidal Suspension Coated by Eudragit E (by Precipitation):

This method was based on the microencapsulation of chitosan colloidal suspension containing clarithromycin which was prepared by precipitation method[13]. The objective was to over-come bitterness of clarithromycin-chitosan colloidal suspension. Eudragit was used to coat the chitosan-drug particle[14]. Like chitosan, Eudragit E also has pH dependent solubility, at acidic pH Eudragit E is soluble, with the sol-gel transition occurring at slightly alkaline pH. Eudragit E is cationic polymer based on dimethylaminoethyl methacrylate and other neutral methacylic acid esters. It is soluble in gastric fluid as well as in weakly acidic buffer solutions. During co-precipitation, chitosan participates first at pH 6 to 7. Further increase in pH causes precipitation of Eudragit E, which coats the previously precipitated chitosan-drug particle to form Eudragit E coated particle.

Method or Preparation:

Drug clarithromycin, Eudragit E and chitosan were dissolved in 0.1 N HCl on magnetic stirrer at slow stirring speed[15]. 1N NaOH solution was added drop wise up to pH 6-7 to precipitate chitosan. Addition of

1N NaOH was continued up to pH 7.8 to 8.0 until the Eudragit coated particles were obtained. The particles formed were kept over-night undisturbed. Supernatant was siphoned off. The separated particles were re-suspended in fresh DM water. Various variables such as D:P(Drug:Polymer) ratio and pH were altered to optimize the formulation.

Characterization:
Eudragit microparticles are soluble in acidic medium, insoluble in alkaline medium; on drying they were sticky in nature. Various parameters were studied during optimization such as taste, free drug concentration, drug entrapment, particle size, DSC, microscopy and finally *In-vitro* release.

Taste Evaluation:
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 second[16].

Free Drug Analysis:
The formulations were analyzed for free drug concentration. Supernatant obtained was analyzed for free drug titrimetrically using 0.004M SLS.

Entrapment Efficiency:
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. Resultant mixture was filtered and filtrate was titrated with 0.004M Sodium dodecyl sulphate (SLS).

Percent entrapment efficiency (%EE) = Wt. of drug taken - free drug/ Wt. of drug taken X 100

In Vitro Release Studies:
In Vitro release studies were carried out in two different Media. Initially the release was studied in pH 6.8 Hydrochloride buffer for 5 minute and then in 0.1N HCl for 20 min[17]. 5.4 ml of optimized formulation of clarithromycin was dispersed in 25 ml of dissolution Media in a 50ml beaker with stirring using magnetic stirrer, 5 ml sample was withdrawn every 5 min and centrifuged at 4000 rpm for 10 min on Remi Centrifuge Apparatus. The supernatant thus obtained was analyzed titrimetrically using 0.004M SLS. The sediment if any, obtained on centrifugation was redispersed in fresh Media (5ml) and added to the dissolution Media. The results are shown in table 4.

Assay:
1 ml of suspension was taken and assayed titrimetrically.

Microscopic Analysis:
Shape and surface morphology observed using optical microscope. The suspension of microparticle was spread on glass slide and sealed by cover slip. The slide of microparticle observed under microscope at 10 X and 40 X. result are shown in figure 5 and 6.

Particle Size:
Particle size was analyzed by Malvern Master Sizer. Particle size distribution of optimized batch is shown in figure 7

DSC analysis:
The DSC is a very useful investigation in the study of micro particles[17]. Thermograms of samples were carried out using differential scanning calorimeter (Shimadzu DSC, Japan). The samples was placed in to aluminum pans and then sealed. The thermograms were obtained at a scanning rate 100C/minute over a temperature range of 40 to 3000C. In the present investigation, DSC Thermogram of pure drug, physical mixture of drug and polymers (Eudragit E and chitosan), Thermogram of Eudragit coated micro particles were performed. Results of DSC thermogram recorded in figure 8, 9 and 10.

RESULT AND DISCUSSION

Standard calibration curve of Clarithromycin
Clarithromycin was found to be soluble in organic solvents such as methanol [18]. 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 0.999as 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 [19]. 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. The absorbance of each solution was measured at 547 nm using the blank prepared simultaneously by same method without stock solution[20].

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

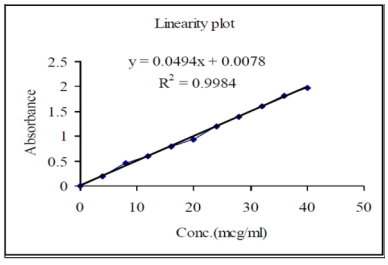

Figure 1: Linearity curve of Clarithromycin in 0.1N Hcl.

Drug and Excipient Compatibility Studies
When IR (Infrared) of Clarithromycin figure 2 was correlated with physical mixture of drug and excipients Figure 2 and 3 the region of 3410 cm-1 was found due to the N-H (aromatic) stretching[21]. However other peaks related to C-H, C-O and carbonyl stretching remain unchanged Table 2. This indicates that overall symmetry of the molecule might not be significantly changed; therefore the FTIR study revealed that there are no interactions taking place between Clarithromycin and Eudragit L100.

Table 2: FT-IR study of Clarithromycin and Physical mixture of Clarithromycin -Eudragit E

Material	Functional group	FT-IR signaling(cm^{-1})
Clarithromycin	Tertiary –N stretching Carbonyl stretching Aliphatic-CH stretching Hydroxyl (OH) stretching	3468 1734 1172 2941
Clarithromycin and Excipients	Tertiary –N stretching Carbonyl stretching Aliphatic-CH stretching Hydroxyl (OH) stretching	3410 1734 1172 2941

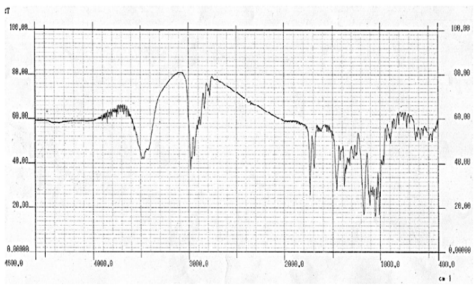

Figure2. : IR (Infrared) Spectrum of Clarithromycin.

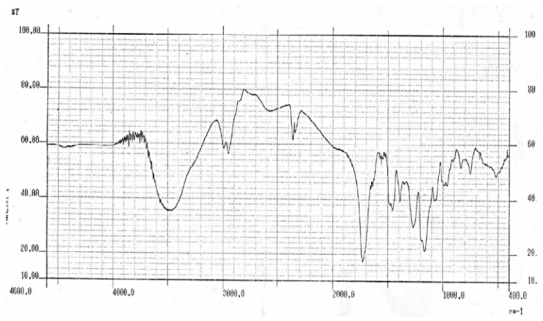

Figure 3: IR (Infrared) Spectrum of Microcapsules

Drug-Chitosan Colloidal Suspension Coated by Eudragit E (by Precipitation)

Eudragit E was used in this technique because it has pH dependent solubility and film forming property. The objective was to over-come sourness of clarithromycin-chitosan colloidal suspension formed in previous method. Eudragit E used to coat the chitosan-drug particles. Initial trials carried on the formulation[22] of Eudragit microparticles revealed that D: P (Eudragit E and chitosan) ratio and pH had profound effect on taste, free drug concentration, EE[23], taste and formation of the microparticles. Hence these parameters were studied for optimizing the formulation. The D:P (Eudragit E and chitosan) ratios were varied between (1: 0.5: 0) to (1: 1.5: 0.3) and pH varied between acidic to alkaline (approximately pH 8.0). The results of the experiments carried out for optimization is illustrated in table 3 Assay of optimized formulation was found 92%.

Table 3: Optimization Parameter of Formulation

Batch Code	Drug: Polymer (Eudragit E)	Chitosan w/w	pH	Taste	Free Drug %	Entrapped Drug %	Observation (product obtained)
DCF1	1:0.5	-	7.5-8.0	+++	26.8	73.2	Sticky gummy product
DCF2	1:1	-	7.5	++	8.0	13.7	Less sticky gummy
DCF3	1:1.5	-	7.5	+	8.2	9.4	Less sticky gummy
DCF4	1:1.5	1%	7.5-8.0	+	9.5	90.5	Sticky gummy product
DCF5	1:1.5	0.2%	7.5-8.0	+	9.0	92.5	Less sticky gummy
DCF6	1:1.5	0.3%	6.0-7.0	+++	24.6	74.4	Colloidal suspension
DCF7	1:1.5	.03%	7.5-8.0	+	7.6	93.6	Micro particle

***+++ Bitter, ++ Less Bitter, + Taste Less**

Drug: Polymer (Eudragit E) r=Ratio:

The drug to Eudragit E ratio was varied from 1:0.5 to 1:1.5 as shown in Table 3 In this study it was observed that as the drug to polymer ratio was increased from 1: 0.5 to 1: 1.5, the free drug concentration in the supernatant was decreased leading to change of taste from bitter to tasteless[24]. The optimum taste masked formulation was obtained at D: P ratio DCF4 1: 1.5, at pH 7.5-8. But the taste less product was sticky gummy and could not be recovered in dried form. Hence, chitosan was used with Eudragit E. The concentration of chitosan was varied between 0.1 to 0.3%, It was observed that the gummy product changed in to isolated microparticles at chitosan concentration batch DCF4 0.3%, at pH 7.5-8.0.

pH:

The pH of suspension has significant effect on, free drug concentration, drug entrapment, formation of particles isolated and the taste. This is the formulation containing two polymers, both soluble in acidic medium and insoluble in alkaline medium[25]. Hence As the pH increased to 7.0 the chitosan precipitated out first and the drug adsorbed on the surface of chitosan. Further increase in pH up to 7.5-8.0 caused precipitation of Eudragit E which in turn coats the chitosan particles. As pH increases, the free drug concentration decreases and finally a taste masked product was obtained batch DCF5 at pH 7.5-8.0 with drug entrapment 92.5% shown in Table 3.

Taste Evaluation:

Preliminary taste evaluation was performed on three volunteer. The optimized formulation was found taste less at D: P ratio 1: 1.5: 0.3 (clarithromycin: Eudragit E: chitosan) at pH 7.5-8.0. Therefore the optimized batch was taken for final formulation development. The results are shown in table 3.

Free Drug Analysis:

It was observed that minimum free drug batch DCF7 7.6% was found in batch prepared with D: P ratio of 1: 1.5: 0.3 at pH 7.5 to 8.0 shown in Table 3.

Entrapment Efficiency:

The formulations were analyzed for entrapment efficiency and the results of all batches are shown in table 3. Optimum drug entrapment batch DCF7 93.6% was observed at drug: Eudragit: Chitosan ratio 1: 1.5: 0.3, at pH 7.5-8.0

In Vitro Release Studies:

The results of *In vitro* drug release study in two different Media are recorded in table 4. Release study of batch DCF7 at pH 6.8 showed , no drug release in 5 min but 98.2 % drug was release at the end of 30 min in 0.1 N HCl.

Table 4: In -vitro release in Two Different Media

Drug Release	5 min	10 min	15 min	20 min	30 min
At pH 6.8	0% release	-	-	-	-
0.1 N HCl	63.5 ±4.6%	92.0 ±3.5%	92.5 ±2.8%	92.5 ±3.9%	98.2 ±2.5%

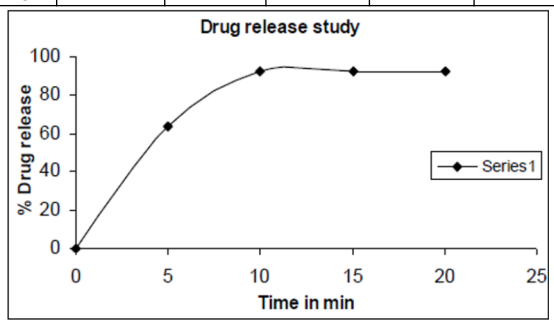

Figure 4: In-vitro drug release of Clarithromycin in 01. N HCL

Microscopic Analysis:

The shape and surface characteristic of microparticles observed by optical microscope at 10 X and 40 X. The results are shown in Figure 5 and 6.

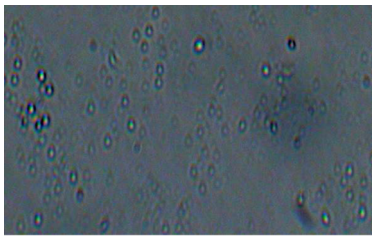

Figure 5: Shape and surface characteristic of microparticles of clarithromycin microparticles at 10 X.

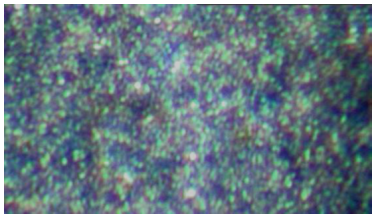

Figure 6: Shape and surface characteristic of microparticles of clarithromycin microparticles at Microparticle at 40 X

Particle Size:

By Malvern master Sizer, the 90 % particles of optimized batch DCF7 were to be found in range of size of optimized batch were 100-1034 µm. As shown in figure 7.

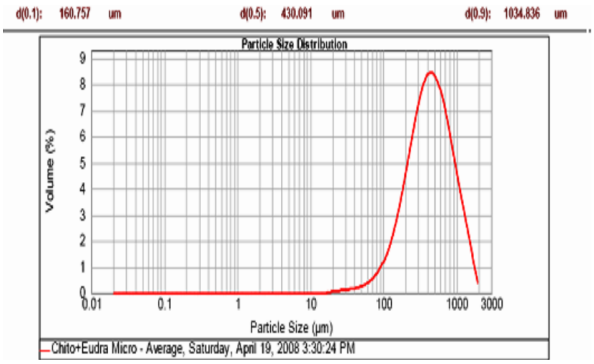

Figure 7: Particle Size Distribution of clarithromycin optimized formulation DCF7

DSC Analysis:

The DSC is very useful investigation in the study of micro particle[25]. The results are shown in Figure 8, 9 and 10. Thermogram of pure drug shows a sharp endothermic peak at 1760C. Thermogram of physical mixture of drug and polymers (Eudragit E and chitosan). Thermogram of Eudragit coated micro particle; there is no sharp endothermic peak of drug at 1760C which indicate the formation of drug loaded microparticle.

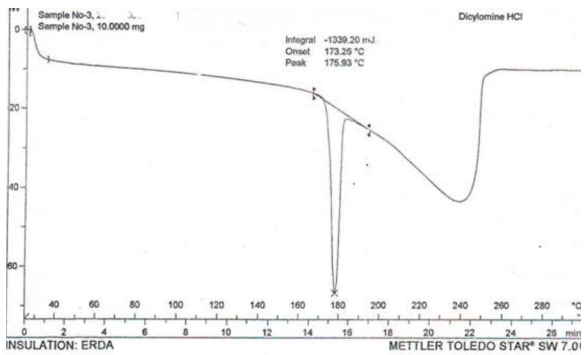

Figure 8: Thermogram of Pure Drug of clarithromycin

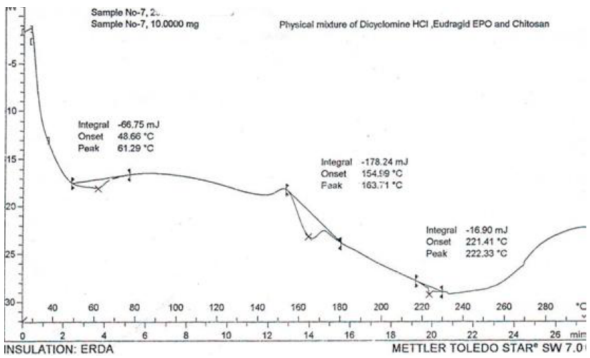

Figure 9: Thermogram of Physical Mixture

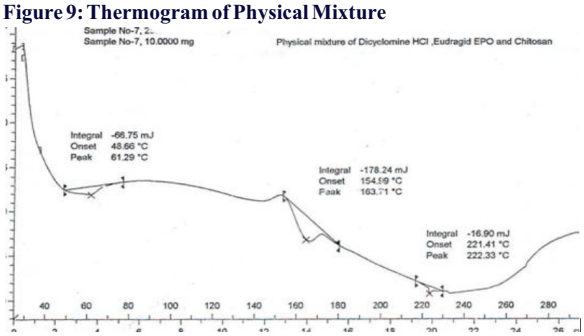

Figure 10: Thermogram of Eudragit Coated Micro Particle

CONCLUSION:

Optimized taste masked product batch DFC7 (Eudragit coated particle) was obtained at pH 7.5- 8.5, at D: P ratio 1: 1.5: 0.3 (drug: Eudragit: chitosan). In Vitro drug release study at pH 6.8 buffer showed, no drug release in 5 min but 98.4 % drug released in 30 min in 0.1 N HCl. Therefore the optimized batch was as per our original design therefore this batch was taken for development of final formulation development, and further study.

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