Research Article

Development and *In-Vitro* Evaluation of Pectino-Eudragit NE 30D Based Sustained Release Gastroretentive Microspheric Drug Delivery System of Ciprofloxacin Hydrochloride

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ABSTRACT

The sole intention of this research was to develop and evaluate gastro retentive micro particulate based drug delivery system for deliverance of ciprofloxacin hydrochloride owing to its narrow absorption window, stomach specific absorption, low bioavailability and short biological half-life. The design approach of this carrier system was focused on coupling few factors like floating, prolonged gastro retention and sustaining the drug action over an extended period of time. The various batches of ciprofloxacin loaded multi particulate system were formulated by ionic gelation technique. Quite a number of parameters like entrapment efficiency, particle size, *in-vitro* buoyancy and *in-vitro* drug release were investigated in relation to polymer concentrations. Particulate flow behavior study indicated that aerodynamic characteristics and compressibility of pure drug could be ameliorated by micro particulate formulations. From the experiment it was found that polymer concentration played a significant role in increasing of the particle size with every increment of it in the formulated batches. Similarly there was increment in the values of entrapment efficiency with every rise in polymer concentration. FTIR and DSC study indicated that there was no interaction between drug and polymer. All the formulations exhibited excellent buoyancy properties with total floating duration for more than 12 hours. *In-vitro* drug release study depicted that the prepared micro particulates could able to sustain the drug release for prolong period of time. Kinetics of drug release study suggested that drug release from the micro particulates followed first order kinetics to release drug from the carrier system. So, from the present study it is conclude that ciprofloxacin loaded pectino-eudragit carrier system can be utilized to deliver the drug for a prolonged period with good sustainability of drug action along with reduction if dosage frequency.

Keywords: Ciprofloxacin hydrochloride, gastro retentive microspheres, sustain release, pectin, Eudragit NE 30D, *in-vitro* buoyancy.

INTRODUCTION

Past 3 decades have been dedicated towards developing a good sustained drug delivery system with a focus on gastro retentive devices along with few others. The purpose of focusing on gastro retention devices is mostly because of narrow absorption window in the upper part of the gastrointestinal tract (i.e. stomach and small intestine) or less soluble or unstable in the alkaline pH or absorbed from the upper gastric region only[1]. All of which become contributing reason towards lesser bioavailability of the active drug[2]. Quite a large number of drug categories have been identified and lot of research works are carried out in order to delivery them either to achieve controlled release or deliver them at required site of action. One of such system is active principle encapsulated micro particulate drug carriers. These devices owing to their size help resolve many delivery associated problems. To add upon this if these micron sized devices are coupled with gastro retention properties then the chances of better delivery of drug can be achieved with improved patient compliance as because these devices unlike single unit systems will not cause dumping of dose.Ciprofloxacin hydrochloride, a broad-spectrum fluoroquinolone antibiotic is absorbed from the stomach and the proximal part of the small intestine[3]. This drug has potential therapeutic consideration in various disease states like diarrhoea, mycobacterial infections, urinary tract infections (UTI)[4], chronic bacterial prostatitis[5] and is also the drug of choice for the treatment of *H.pylori* infection which is considered as a risk factor of gastric cancer and duodenal ulcer[6]. Though Ciprofloxacin hydrochloride is a potential active principle but it has short biological half-life (about 4 h), low oral bioavailability (about 70%) and upper gastro tract absorption which make it an ideal candidate for being contrived in to gastro retentive sustained release drug delivery system[7]. Thus, the aim of present research work was to develop gastroretentive floating microparticulate delivery systems for ciprofloxacin hydrochloride and investigates the effect of polymer on *in-vitro* drug release with involvement of simple processing methods, non utility of organic solvents and cost effectiveness.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride was a gift sample from Spansules Pharmatech Pvt. Ltd., Hyderabad, India. Low methoxy (LM) pectin (degree of esterification 36%) was obtained as a gift sample from CP Kelco, Lille Skensved, Denmark. Sodium bicarbonate was purchased from S.D.

Fine Chemical Pvt. Ltd., Mumbai, India. Barium chloride was purchased from Loba Chem., India. Other chemicals and solvents used were of AR grade.

Method

Fabrication of Ciprofloxacin hydrochloride Floating Polymeric Micro particulates

Ciprofloxacin hydrochloride loaded pectin - Eudragit NE 30D based floating micro particulates were prepared by ionic gelation method (Table 1). The polymeric blend solution of pectin (low methoxylated) and Eudragit NE 30D was prepared by dissolving pectin in sufficient amount of water followed by addition of Eudragit NE 30D under gentle agitation. Then the drug was added slowly to the polymeric blended solution and the mixture was stirred at 4000 rpm to obtain a homogenized mixture of drug with polymeric blends. Then to the prepared drug-polymer mixture, sodium bi-carbonate ($NaHCO_3$) was added and mixed at 8000 rpm for 2 min. after this followed the step of dropping the drug containing polymeric solution through a 24 G needle to a 5 % (w/v) barium chloride solution with continuous agitation of 1000 rpm. The process was continued for 30 minutes for complete reaction. After 30 minutes, the formulated micro particulates were collected, washed with distilled water and dried overnight at room temperature and were kept in desiccators for future use.

Characterization of Microspheres

Physicochemical Characterization of* pectino- Eudragit NE 30D *Floating Microspheres

% Yield

The formula below depicted was used to calculate the percentage yield for each batch of prepared micro particulates based on the weight of final product (microspheres) after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres (Table 2).

% Yield = W_f / W_i

Wf is the final weight of the dried microspheres; *Wi* is the initial total weight of drug and polymer.

Particle Size Analysis

As described by the sieve analysis method[8] the particle size analysis was carried out for each batch of prepared micro particulates (Table 2). The prepared micro particles were separated into various size fractions (% weight fraction) by sieving for 5 min. with help of standard sieves having nominal mesh apertures of 1.4, 1.2, 1.0, 0.85 and 0.71 mm. The study was conducted in triplicate and mean particle size of microspheres was calculated using the following formula:

$$\text{Mean particle size} = \frac{\Sigma (\text{mean particle size of the fraction} \times \text{weight fraction})}{\Sigma \text{ weight fraction}}$$

Entrapment Efficiency

The encapsulation efficiency of each batches of formulated micro particles were determined as per the procedure described below. Approximately (100 mg) of micro particulates was weighed and placed in 100 ml of 0.1 N HCl (pH 1.2) with vigorous stirring for 4 h. The entrapment efficiency of the prepared pectin floating micro particulates was determined as per the reported method Chakraborty[9]. The resulted dispersion was sonicated at 125 W for 30 min (Imeco Sonifier, Imeco Ultrasonics, India) and filtered by help of Whatman filter paper (0.45mm). Then, the polymeric debris was washed with fresh solvent (0.1 N HCl) to extract any adhering drug. The drug content of the filtrate so obtained was determined spectrophotometrically at 271 nm (UV-2450, Shimadzu, Japan). Each determination was made in triplicate and entrapment efficiency values (Table 2) were calculated using the following formula:

$$Entrapmentefficiency(\%) = {}^{Totalamountofdruginmicrospheres}/_{Totalamountofdrugaddedinitially} X\ 100$$

In-vitro Floatability Studies

In-vitro floating duration studies of the formulated system were carried out in 0.1 N HCl using USP apparatus II at 37 ± 0.5°C with a rotation speed of 50 rpm. 0.02 % v/v tween 80 was used as a surfactant. The time taken by the micro particulatesto rise to the solvent surface (FLT i.e. floating lag time) and the total time up to which the formulations remained in afloat condition (TFT i.e. total floating time) were determined and shown in Table 3.

In-vitro Release Study

In-vitro drug release study of ciprofloxacin hydrochloride from the prepared pectino- Eudragit NE 30Dfloating micro particulates was studied using USP dissolution rate test apparatus II (DISSO 2000, LABINDIA, India) at 37 ± 0.5 °C with a rotational speed of 50 rpm. 100mg of accurately weighed quantity of micro particulates (equivalent to 100 mg of pure ciprofloxacin hydrochloride) was placed in dissolution flask containing 900 ml of 0.1 N HCl (pH 1.2). At predetermined time intervals, 5 ml samples were withdrawn and replaced by an equal volume of fresh pre warmed 0.1 N HCl. After suitable dilutions, the samples were filtered and analyzed spectrophotometrically (UV-2450 Shimadzu, Japan) at 271 nm. The drug release studies were conducted in triplicate shown in Figure 1.

Analysis of Release Profiles

The analysis of release kinetic studies were carried out by utilising various kinetic modeling in order to find out the coefficient of determination values and release exponent (n)value, that describes the drug release mechanism. The kinetic data of formulations CPE1 shows best fit in first order kinetics followed by fickian method while for CPE2 to CPE6the best fit was with zero order kinetics followed by non-Fickian diffusion mechanism (n = 0.795 to0.945).

All the kinetics data are summarized in Table 4. The rate and mechanism of release of ciprofloxacin hydrochloride from the prepared microspheres were analyzed by fitting the dissolution data into the zero-order equation,

$$Q = k_0 t$$

Where, Qis the amount of drug released at time tandk_0 is the zero order release rate constant.

First order equation,

$$\ln(100 - Q) = \ln 100 - k_1 t$$

Where k_1 is the first order release rate constant.

The dissolution data was fitted to the Higuchi's equation[10],

$$Q = k_H t^{1/2}$$

Where, Qis the percentage of drug released at time t, k_H is the Higuchi release rate constant.

Peppas equation[11],

$$\frac{M_t}{M_\infty} = kt^n$$

Where, n is the release exponent; indicative of the mechanism of release, $M_t/M_\infty$is the fraction of the drug at time t, K is the release rate constant. The criteria for selecting the most appropriate model were based on the highest values of the coefficient of determination (r^2).

Surface Topographical Analysis

The surface texture analysis of the optimized micro particulate formulation was studied by scanning electron microscope imaging instrument (SEM). The samples of the micron sized particles was spread on stub and coated for 120s with a layer of gold using a sputter coater. Then the coated particles were placed in the scanning electron microscope(S 3700 VP FE-SEM, Hitachi High-Technologies, Europe) chamber at the acceleration voltage of 20 kV and chamber pressure of 0.6 mm Hg. SEM photographs were taken at different magnifications and shown in Figure 2.

Differential Scanning Calorimetric Analysis

Thermal analysis study of pure ciprofloxacin hydrochloride (PD) and drug-loaded optimized microparticulates optimised formulation (OF)was performed using a differential scanning calorimeter (DSC) (Diamond DSC, PYRIS, Perkin Elmer, USA). The samples were heated at constant rate of 10 °C/min between 30 and 300 °C. The system was purged with nitrogen gas to maintain the inert atmosphere. The DSC thermograms were shown in Figure 3.

Stability Studies

From all the prepared formulations, optimized formulation of each polymeric blend was tested for stability studies following ICH guidelines. Test formulation (micro particulates) were divided into 6 sample sets, sealed in tubes and stored at room temperature (25 ± 2 °C and 60 ± 5 % RH) and accelerated condition (40 ± 2 °C and 75 ± 5 % RH). After each 30 days interval, one tube was used to evaluate % drug content, shelf life and physical appearance of the samples. The study was carried out for six months.(Table 5a and Table 5b).

RESULTS AND DISCUSSION

Physicochemical Characterization of* pectino- Eudragit NE 30D *Floating Microspheres

The % yield of the prepared pectino- Eudragit NE 30D floating micro particulates was found within the range of 72.15 ± 1.18 % to 94.27 ± 3.02 % (Table 2). The percentage entrapment efficiency and particle size of all the formulations were found within the range of 73.49 ± 2.68 % to 91.57 ± 1.83 and 659 ± 15.49µm to 1013 ± 14.59µmrespectively (Table 2). It was observed that there was a predominant increase in the percentage entrapment efficiency and particle size values with increase in polymer concentration which may be attributed to the fact that at higher concentration the polymer may produce a viscous dispersion which might have formed larger micro particles and entrapped greater amount of drug[12].

In-vitro Floatability Studies

It was found from the study of*In vitro* floatability that the floating lag time of all the formulations was in the range of 84 **±** 11seconds to 131 ± 13seconds while the total floating time was more than 12 hours (Table 3). The change in floatability of formulations may be attributed to the fact that there might be change in overall density of the polymer composite. This may have contributed to such an effect on total floating time.

In-vitro Drug Release Behavior

The *In- vitro* release profiles of ciprofloxcin hydrochloride from the prepared formulations CPE1 to CPE6 are shown in Figure 1. Formulation CPE1 and CPE2 (pectin: eudragit NE 30D ratio 1:1 and 1:2 respectively) were able to sustain the drug released for 9 and 11 hours respectively. But subsequently as there was increase the concentration of eudragit NE 30D polymer in formulation CPE3 the release of active principle also got prolonged up to 12 hours since eudragit NE 30D might have formed a rigid coat that might have attributed in delaying the drug release. On increasing the polymeric ratio in formulations CPE5 and CPE6, it was observed that there was tremendous lowering of release of active principle and only75.68±1.44and69.15±2.46% drug was released at the end of 12 hours respectively. It was observed that at low polymeric concentration drug release was faster as compared to high polymeric concentration. This might be due to the fact that a higher polymeric amount may have increased the tortuosity and gel strength of the polymer there by forming a viscous gelatinous layer. As a result the impregnation of the dissolution media inside the matrix was delayed thereby suppressing the diffusion of the drug from the formulated micro particulates. Higher

polymeric concentration also increases the diffusional path length around the matrix which might be an acting force towards retarding the drug diffusion from the micro particulate formulations.

Kinetic Modeling

The *in vitro* dissolution data was subjected to different kinetic models in order to find out the coefficient of determination values and release exponent (n) value, which describes release mechanism of the drug from the carrier system. The kinetic data of formulations CPE1 and shows best fit in Higuchi model followed by non-Fickian diffusion mechanism (n = 0.853) whereas formulations CPE3 to CPE6 shows best fit in zero order kinetics followed by non-Fickian diffusion mechanism (n = 0.795 to 0.945). All the kinetics data are summarized in Table 4.

Topographical Analysis

The surface texture analysis of the optimized formulation (CPE3) was studied with help of SEM (Figure 2) suggested that prepared micro particulates were rigid, spherical in shape with smooth outer surface.

Thermal Property Studies

In the present investigation, DSC thermograms of pure drug, pure polymer and optimized microspheres (formulation CPE3) were taken and shown in Figure-3. It was observed from the DSC thermogram that ciprofloxacin hydrochloride shows a typical crystallinity with a sharp peak at 155.09°C corresponding to its melting and decomposition whereas optimized micro particle formulation (CPE3) shows a melting endotherm at 137.26°C for ciprofloxacin hydrochloride demonstrating that there is no considerable change in melting endotherm of the drug.

Stability Studies

Optimized microsphere formulation (CPE3) was tested for % drug content, shelf life ($t_{90\%}$) and physical appearance with stability studies as per ICH guidelines for solids. It was observed that the shelf life of the optimized batch of CPE3 at room temperature (25 ºC / 60 % RH) was 1133 days years whereas in accelerated condition (40 ºC / 75 % RH) the shelf life was found to be 757 days only. It was also observed that was no significant changes in the physical appearance and % drug content of the optimized formulation at room temperature as well as in accelerated condition. The drug content in accelerated condition was reduced to 97.67±1.83 % within 180 days whereas in case of room temperature good stability was observed (98.41±1.48 % within 180 days), table-5(a) and 5 (b).

CONCLUSION

Thus, the authors of the above research work conclude from the experiment that pectin-eudragit NE 30D carrier system prepared by ionic gelation technique can be utilized for prolonged duration to deliver Ciprofloxacin Hydrochloride. It can be also help in improving patient compliance and reduce the dosing frequency by enhancing the systemic absorption of ciprofloxacin hydrochloride in upper GIT.

Table 1: Formula of Different Ciprofloxacin Hydrochloride loaded Pectino-Eudragit Floating Microspheres.

Formulation code	Polymer level (% w/w)	Drug level (g)	Sodium bicarbonate (mg)	Rigidizing solution (% w/v)	Stirring Speed (rpm)
CPE1	Pectin, 1% + Eudragit, 1%	1	100	$BaCl_2$, 5%	1000
CPE2	Pectin, 1% + Eudragit, 2%	1	100	$BaCl_2$, 5%	1000
CPE3	Pectin, 1% + Eudragit, 3%	1	100	$BaCl_2$, 5%	1000
CPE4	Pectin, 1% + Eudragit, 4%	1	100	$BaCl_2$, 5%	1000
CPE5	Pectin, 1% + Eudragit, 5%	1	100	$BaCl_2$, 5%	1000
CPE6	Pectin, 1% + Eudragit, 6%	1	100	$BaCl_2$, 5%	1000

CPL: Ciprofloxacin hydrochloride loaded pectino-eudragitfloating microspheres, Eudragit: Eudragit NE 30 D.

Table 2: Physicochemical Characterizations of Pectino-Eudragit Microspheres.

Formulation	Yield (%)	Particle size (µm)	Entrapment efficiency (%)
CPE1	72.15 ± 1.18	659 ± 15.49	73.49 ± 2.68
CPE2	76.63 ± 2.31	794 ± 17.03	78.84 ± 3.47
CPE3	85.20 ± 3.11	881 ± 17.85	86.94 ± 2.97
CPE4	90.19 ± 1.76	928 ± 10.21	88.74 ± 3.36
CPE5	94.27 ± 3.02	973 ± 7.14	89.58 ± 3.05
CPE6	92.83 ± 2.56	1013 ± 14.59	91.57 ± 1.83

Mean ± SD, n = 3. CPE: Pectino-Eudragit floating microspheres.

Table 3: *In-Vitro* Buoyancy test of Pectino-Eudragit Microspheres.

Formulation	FLT (sec.)	TFT (h.)
CPE1	84 ± 11	> 12 hr
CPE2	93 ± 14	> 12 hr
CPE3	96 ± 09	> 12 hr
CPE4	108 ± 10	> 12 hr
CPE5	118 ± 08	> 12 hr
CPE6	131 ± 13	> 12 hr

Mean ± SD, n = 3; FLT: Floating lag time; TFT: Total floating time.

Table 4: *In-Vitro* Drug Release Kinetics of Pure Drug (Ciprofloxacin Hydrochloride) and Prepared Pectino-Eudragit Microspheres*

Formulations	Drug release kinetics, Coefficient of determination (r^2)					Release exponent (n)	$t_{50\%}$ (hour)
	Zero Order	First Order	Higuchi Model	Hixson-Crowell Model	Korsmeyer Model		
PD	0.821	0.991	0.758	0.987	0.793	1.146	0.45
CPE1	0.885	0.996	0.987	0.747	0.963	0.412	2.38
CPE2	0.994	0.919	0.991	0.599	0.970	0.853	3.13
CPE3	0.997	0.934	0.993	0.618	0.979	0.795	3.85
CPE4	0.995	0.948	0.992	0.683	0.974	0.945	4.65
CPE5	0.994	0.952	0.989	0.674	0.976	0.933	5.66
CPE6	0.996	0.963	0.991	0.710	0.986	0.851	6.76

*Analyzed by regression coefficient method.

Table 5 (a): Stability Study for Optimized Pectino-Eudragit Microsphere Formulation (CPE3) at Room Temperature.

Parameters	25 ºC / 60 % RH					
Sampling Interval (days)	30	60	90	120	150	180
Drug Content (%)	100±1.86	100±1.17	99.81±1.04	99.18±1.83	98.83±2.81	98.41±1.48
Physical appearance	+	+	+	+	+	+
Slope	-4.128×10^{-5}					
K ($days^{-1}$)	0.951×10^{-4}					
t_{90} (days)	1133					

+ No changes in physical appearance; ++ Changes in physical appearance.

Table 5 (b): Stability Study for Optimized Pectino-Eudragit Microsphere Formulation (CPE3) in Accelerated Condition.

Parameters	40 ºC / 75 % RH					
Sampling Interval (days)	30	60	90	120	150	180
Drug Content (%)	100±1.06	99.76±1.36	99.35±2.27	98.81±2.04	98.06±1.67	97.67±1.83
Physical appearance	+	+	+	+	+	+
Slope	-6.177×10^{-5}					
K ($days^{-1}$)	1.42×10^{-4}					
t_{90} (days)	757					

+ No changes in physical appearance; ++ Changes in physical appearance.

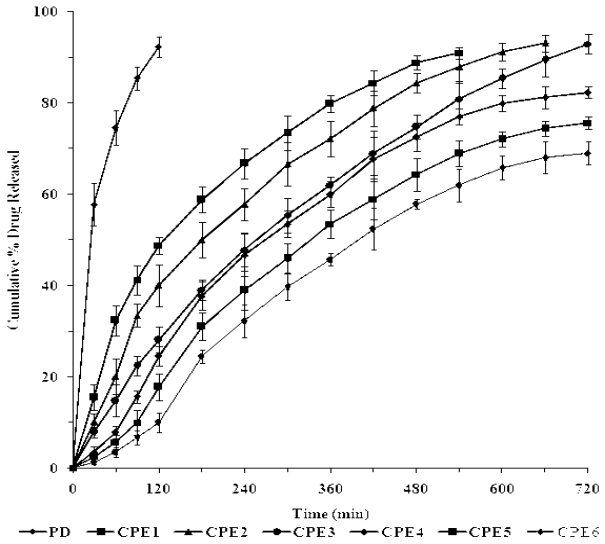

Figure 1: *In-Vitro* Drug Release Profile of Pure Drug (PD) and Pectino-Eudragit Floating Microsphere Formulations.

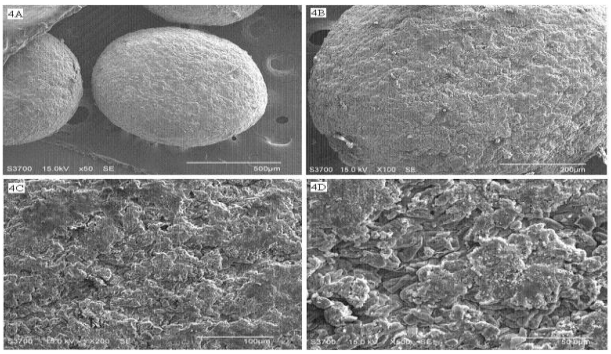

Figure-2: Scanning electron photomicrographs of optimized pectin-eudragit NE 30 D microparticulates system of optimized of formulation CPE3.

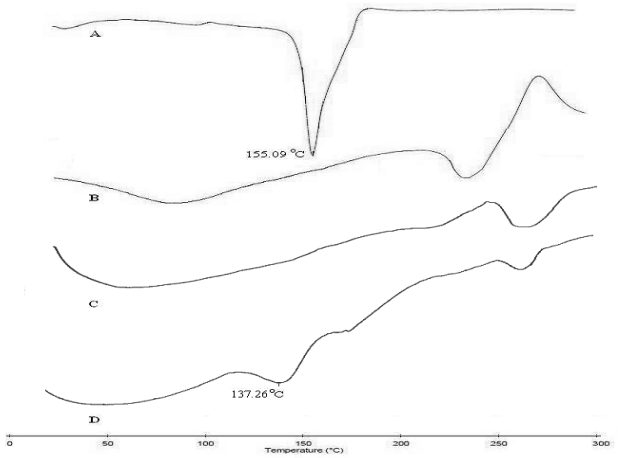

Figure 3: DSC thermodram of A) Ciprofloxacin hydrochloride (CP), B) Pectin (P), C) Eudragit NE 30 D and D) Optimized micro particulate formulation (CPE3).

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