Original Article

# DEVELOPMENT OF COLON-SPECIFIC MULTI PARTICULATE DRUG DELIVERY SYSTEM OF FENOVERINE

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## ABSTRACT

**Objective**: The main objective of this study was to formulate and evaluate colon-specific multi- particulate drug delivery system of fenoverine, using Eudragit S 100 as enteric coating polymer.

**Methods**: Microparticles were prepared by oil/water emulsion solvent evaporation technique and characterized for micromeritic properties, encapsulation efficiency, percentage yield and particle size. Various analytical techniques such as FTIR, DSC, PXRD, and SEM were used to characterize microparticles. *In vitro* release studies were performed in 900 ml of enzyme free SGF (pH 1.2) for 2 h followed by enzyme free SIF (pH 7.4) containing 2% Tween 80 till the end of test using USP paddle apparatus. Drug release data was subjected to release kinetic studies and effect of stirring rate on particle size and percent entrapment of microparticles was also studied.

**Results**: *In vitro* release studies showed that microparticles were able to release the drug in a controlled manner with zero order rate kinetics; 'n' values obtained from the Korsmeyer Peppas model showed that the release mechanism was non-fickian type. SEM studies revealed that microparticles were smooth and spherical in shape. The DSC and PXRD studies of drug loaded microparticles showed amorphous state of the drug in the microparticles. Mean particle size was reduced with increase in stirring rate but percent drug entrapment of was found to be high 72.12 ± 1.63.

**Conclusion**: Colon specific microparticles prevent premature drug release in simulated upper GIT and maximize the drug release in simulated intestinal environment at controlled rate over a period of 24h.

**Keywords**: Fenoverine, Multiparticulate system, Colon specific system, Eudragit S 100.

## INTRODUCTION

Although oral delivery has become a widely accepted route of administration of therapeutic drugs, gastro intestinal tract presents several formidable barriers to drug delivery. For example, oral administration of peptide and protein drugs is limited due to instability of the drugs in acidic condition and hydrolytic enzymes from stomach and small intestine, respectively [1]. The site specific delivery of a drug to the colon can provide major advantages [2], if inflammatory bowel diseases are to be treated locally, or protein drugs are to be administered orally with the aim to be absorbed into the systemic circulation [3]. In the first case, conventional dosage forms lead to a rapid and complete drug release within the stomach and generally to subsequent absorption into the blood stream. Consequently, the systemic drug concentrations and related undesired side effects can be considerable. At the same time, the resulting drug concentrations at the site of action (the inflamed colon) are low, resulting in poor therapeutic efficacies [4]. For protein drugs a premature release within the upper gastro intestinal tract (GIT) results in the rapid loss of their biological activity due to denaturation at low pH and enzymatic degradation. Thus, in both cases, an ideal dosage form should effectively suppress drug release/protect the drug in the stomach and small intestine. But once the colon is reached, drug release should set on and be time-controlled (including – if desired – rapid and complete release). In the case of proteins, the drugs should subsequently be absorbed into the blood stream, but in inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), the drug should be released at its target site, providing optimal therapeutic effects and minimized undesired side effects [5, 6].

In the present study it has been aimed at developing a multiparticulate drug delivery system of fenoverine with a view of minimizing the drug release in the physiological environment of stomach and small intestine and to ensure maximum drug release in the physiological environment of colon with an improved patient compliance, least side effects and better drug therapy.

This was developed by using Eudragit S 100 as pH sensitive polymer in the preparation of colon specific multiparticulate drug delivery system of fenoverine, which is used for the management of irritable bowel syndrome. Fenoverine, is a non-anticholinergic synchonizer of smooth muscle motility [7]. It effectively inhibits the asynchronous spasmodic contractions without appreciable interferences with the physiological synchronous motility of the intestine.

## MATERIALS AND METHODS

Fenoverine and Eudragit S 100 was gift sample from Dr. Reddy's laboratories, Hyderabad, India, Polyvinyl alcohol was purchased from Himedia laboratories Pvt. Ltd. Mumbai, India. Dichloromethane, potassium dihydrogen orthophosphate, Tween 80, sodium hydroxide, ethanol, sodium chloride was purchased from S.D. Fine Chemicals, Mumbai, India. All the chemicals used were of analytical grade.

## Methods

### Preparation of fenoverine microparticulates

Fenoverine loaded Eudragit S-100 microparticles were prepared by the emulsion solvent evaporation method [8, 9]. Eudragit S-100 was dissolved in a solvent mixture of dichloromethane and absolute ethanol (1:1) together with fenoverine. This resulting dispersion was added drop wise into a vessel containing 100 mL of 1% w/v polyvinyl alcohol (PVA) solution with continuous stirring to form o/w emulsion at 500 rpm for 3h, until complete solvent mixture was evaporated. After decantation, the microparticles obtained were collected by vacuum filtration, washed 4-5 times with 50 mL water each and dried at room temperature for 24 h and stored in desiccator for further use.

Stirring rate is a significant parameter which affects the formation of microparticles therefore effect of stirring rate on formation of microparticles, particle size and percent entrapment was studied on best formulation. Formulation F10 and F11 were formulated using

formula of best batch on two different stirring rate ie. 300 rpm (low speed) and 700 rpm (high speed).

**Table 1: Various formulations of fenoverine microparticles**

| Formulation Code | Drug $X_1$ (mg) | Eudragit S 100 $X_2$ (mg) | Concentration of PVA (%) | RPM |
|---|---|---|---|---|
| F1 | 100 | 100 | 1 | 500 |
| F2 | 100 | 300 | 1 | 500 |
| F3 | 100 | 500 | 1 | 500 |
| F4 | 150 | 100 | 1 | 500 |
| F5 | 150 | 300 | 1 | 500 |
| F6 | 150 | 500 | 1 | 500 |
| F7 | 200 | 100 | 1 | 500 |
| F8 | 200 | 300 | 1 | 500 |
| F9 | 200 | 500 | 1 | 500 |
| F10* | 100 | 500 | 1 | 300 |
| F11* | 100 | 500 | 1 | 700 |

Note: *Effect of stirring performed on formulation F3

**Characterization**

**Percent drug entrapment**

The entrapment efficiency was carried out as a measure of calculating the amount of the drug that is entrapped in the microparticles and the drug, which is adsorbed on the surface of the polymer. This study carries vital importance in surface studies since this is indicative of the measure of drug that goes in forming the microparticles, present in an adsorbed state on the surface of the polymer or as free drug and as a fraction which is found entrapped within the polymeric matrix [10, 11]. Percent drug entrapment was calculated using formula given below,

$$\text{Percent Percent drug entrapment} = \frac{\text{Parctical drug content}}{\text{Theoretical drug content}} \times 100$$

**Percentage yield**

The percentage yield was determined as the weight percentage of final product after drying, with respect to the initial total amount of drug polymer and other materials used for the preparation of microparticles [10, 11].

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

**Particle size measurement**

Determination of average particle size of fenoverine microparticles was carried out by optical microscopy in which stage micrometer was employed. Microparticles were uniformly spread on a slide. The particle size of the microparticles was measured, along the longest axis and the shortest axis (cross shaped measurement). Average of these two readings was given as mean diameter of the particle. The diameter of a minimum number of 100 microparticles in each batch was calculated [11, 12].

**Micromeritic properties**

Microparicles were evaluated for micromeritic properties like, angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio to determine its flow characteristics [12].

**Fourier Transform Infrared Spectroscopy (FTIR)**

Drug loaded microparticles were subjected to FTIR studies to find out the possible interaction between the drug and the polymer during the time of preparation. FTIR analysis of the fenoverine, eudragit S 100, physical mixture and the optimized formulation F3 were carried out using an FTIR spectrophotometer (PERKIN ELMER FT-I Insf. USA) [11]. Samples were prepared in KBr disks (1:5 ratio samples: KBr). FTIR spectra were recorded for each sample within the scanning range was 4000 to 400cm$^{-1}$ and the resolution was 2cm$^{-1}$.

**Differential scanning calorimetry (DSC)**

This was performed to find out possible interaction between the drug and polymer. Studies were done on pure drug, polymer, physical mixture, placebo and drug loaded microparticles using a NETZSCH DSC 204 thermal analyzer Gmbh, Germany [11]. The instrument was calibrated using purified Indium (99.99%). The sample was sealed in a flat bottomed aluminum pan and the pan was placed in the DSC instrument and scanned between -20°C and 200°C at a rate of 10°C/min.

**Powder X-ray diffraction studies (PXRD)**

Fenoverine, eudragit S 100, physical mixture, placebo and optimized formulation F3 were analyzed using Bruker AXS D8 advance diffractometer with a copper X-ray source (λ=1.5406 A°, 40KV, 35mA). The detection pattern was measured with a step size of 0.020° and a dwell time of 31.2s at each step between 3 and 80 2θ at ambient temperature. The powdered sample was smeared over an amorphous substrate and subjected to X-ray analysis [11].

**Scanning electron microscopy (SEM)**

Examining the surface of a polymeric drug delivery system can provide vital information on the porosity and microstructure of these systems. The distribution and morphology of the surface and the encapsulated matrix can also be directly observed. The most common technique used for characterizing the surface morphology of drug delivery systems is scanning electron microscopy. Prior to loading the samples for taking the photomicrograph, samples are coated (20- 30 nm in thickness) with electron-dense coating materials like gold to render them electrically conductive and examine under the microscope [12,13].

***In vitro* release study**

Dissolution studies of fenoverine microparticles were carried out in triplicate using USP dissolution test apparatus II (Paddle apparatus, 50 rpm, 37±0.5 °C). Accurately weighed amount of microparticles equivalent to dose were packed in muslin cloth bag and tied to paddle of the dissolution apparatus, the pH changes were performed starting with 900 mL of enzyme free simulated gastric fluid (SGF) pH 1.2 for 2 h, followed by enzyme free simulated intestinal fluid (SIF) pH 7.4 containing 2% Tween 80 till the end of test [14, 15, 16]. Aliquots of the dissolution medium were at 1h interval for a period of 24 h and the sampled volume of buffer maintained at the same temperature. The samples withdrawn every hour were assayed spectro photometrically at 261 nm. The equal volume of fresh release medium was replaced at the same time intervals. The dissolution data was analyzed for calculating the amount of drug released and percentage cumulative drug released at different time intervals.

**Kinetic analysis of dissolution data**

In order to describe the kinetics of the release process of drug in different formulations, models were fitted to the dissolution data of optimized formulation using linear regression analysis [17]. Drug release rates and mechanism of release was determined by fitting data to various mathematical models like zero order, first order, Higuchi kinetics and Korsmeyer Peppa's.

**RESULTS AND DISCUSSION**

**Evaluation of the formulated microparticles**

**Physicochemical characterization of formulated microparticles**

The granules of different formulations were evaluated for angle of repose, bulk density, tapped density, Carr's index (compressibility index) and Hausner's ratio and their values are shown in Table 2. The results of angle of repose and Carr's index suggested that all formulations have good flow properties and compressibility.

Formulated microparticles (F1 – F11) were characterized for various physicochemical parameters like mean particle size, percentage yield and percent drug entrapment data is given in Table 3.

As indicated in Table 3, the mean particle size of the formulation F1 to F3 found to increase from 22.80 ± 0.77 to 32.50 ± 0.91μm when

drug to polymer ratio was increased from 1:1 to 1:5 respectively. The particle size was increased with increase in amount of eudragit S 100. With increase in polymer concentration, percent drug entrapment was also increased from 52.78±2.96 to 71.61±1.85%.This could be due to increase in viscosity of organic phase by increase in the eudragit S 100 concentration. Higher the amount of polymer concentration more amount will be available for encapsulating which finally leads to more drug entrapment. On the basis of percentage entrapment values formulation F3 which showed highest percent entrapment of 71.61±1.85 was considered to be the best batch. Effect of stirring speed on particle size and percentage entrapment was studied on F3 formulation. It can be observed from the data (F10 and F11) formulation that when stirring speed was 300 rpm the mean particle size of the

microparticles was increased and they appeared to be large and aggregated. When the stirring speed was increased to 700 rpm, mean particle size of the microparticles was decreased to 28.26±0.99 µm showing high percentage entrapment of 72.12 ± 1.63.It may be due to the fact that, at low speed uniform polymer dispersion is not obtained which results in aggregation and lump formation. At high speed uniform dispersion of polymer would be there which helps in encapsulating the drug uniformly; it also results in uniform size microparticles. If the stirring rate was further increased from 700 rpm to 900 rpm solvent gets evaporated quickly which leads to precipitation of polymer and lump formation. So the optimum stirring speed was found to be 700 rpm. The percentage yield of all the batches of the formulations F1 to F11 ranged between 61.85 ± 3.89 to 74.56 ± 1.99, as indicated in Table 3

**Table 2: Micrometric data of drug loaded microparticles**

| Formulation Code | Angle of repose (θ) | Bulk density (gm/cc) | Tapped density (gm/cc) | Carr's index (%) | Hausner's ratio |
|---|---|---|---|---|---|
| F1 | 28.28± 1.24 | 0.32± 0.04 | 0.402±0.05 | 20.149 | 1.25 |
| F2 | 27.90± 1.32 | 0.33± 0.34 | 0.415± 0.25 | 19.417 | 1.24 |
| F3 | 27.35± 1.27 | 0.321± 0.02 | 0.386±1.01 | 19.170 | 1.20 |
| F4 | 31.02±1.46 | 0.323±0.83 | 0.398±0.99 | 18.844 | 1.23 |
| F5 | 29.96±1.13 | 0.325±0.83 | 0.402±1.02 | 19.753 | 1.24 |
| F6 | 30.35±1.35 | 0.365±0.41 | 0.469±0.96 | 22.174 | 1.22 |
| F7 | 28.12±1.84 | 0.344±0.19 | 0.436±0.32 | 21.100 | 1.22 |
| F8 | 30.65±1.35 | 0.332±1.20 | 0.412±1.09 | 19.417 | 1.24 |
| F9 | 28.62±1.86 | 0.331±1.21 | 0.412±1.09 | 21.641 | 1.27 |
| F10 | 30.12±1.23 | 0.312±0.43 | 0.384±0.46 | 18.750 | 1.23 |
| F11 | 26.85±1.55 | 0.316±0.05 | 0.391±0.92 | 19.181 | 1.24 |

*Each value represents the mean±SD; n=3

**Table 3: Characterization of the formulated fenoverine microparticles**

| Formulation Code | Particle size (µm) | Percentage yield (%) | Drug Entrapment (%) |
|---|---|---|---|
| F1 | 22.80±0.77 | 62.70±3.08 | 52.78±2.96 |
| F2 | 25.44±1.08 | 67.19±2.91 | 63.43±0.82 |
| F3 | 32.50±0.91 | 72.88±2.56 | 71.61±1.85 |
| F4 | 24.48±1.11 | 68.67±3.52 | 52.01±1.49 |
| F5 | 29.70±1.27 | 65.74±2.50 | 55.91±0.87 |
| F6 | 38.50±1.69 | 71.07±1.47 | 64.87±2.77 |
| F7 | 23.49±0.56 | 61.85±3.89 | 49.04±1.79 |
| F8 | 31.44±1.08 | 63.91±2.33 | 58.31±0.96 |
| F9 | 39.80±0.91 | 74.56±1.99 | 63.01±2.44 |
| F10 | 43.13±1.36 | 68.92±2.23 | 62.12±1.63 |
| F11 | 28.26±0.99 | 72.58±2.85 | 72.78±0.98 |

*Each value represents the mean±SD; n=3

### *In vitro* release study

From figure 1 it is evident that formulations F1-F9 released about 90% of drug in 16 to 20 h as compared to F3 formulation which showed controlled drug release up to 24 h. *In vitro* release data reveals that formulation F10 and F11 released 50.81±2.03 and 83.00±0.16 of drug at the end of 24h respectively. Slower drug release was observed from microparticles prepared at lower speed as compared to higher speed. Formulation F7 F8 and F9 showed no significant change in drug release even though drug concentration was increased.

### *In vitro* release kinetics

The mechanism and kinetics of drug release of fenoverine was determined by fitting *in vitro* release data to various kinetics models such as Zero order, First order, and Higuchi and Korsmeyer- Peppas kinetics. All formulations F1 to F9 follows zero order release with $r^2$ values of 0.9456, 0.9389, 0.9885, 0.9615, 0.9207, 0.9233, 0.9455 and 0.9571 respectively. The mechanisms of drug release are non-fickian diffusion, since they fitted well with Korsmeyer-Peppas models with

"n" value above 0.5. This indicates that the drug release depends on relaxation and erosion of polymer with zero order release kinetics.
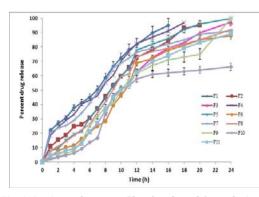


**Fig. 1: *In vitro* release profiles developed formulations**

**Fourier Transform Infrared Spectroscopy**

The FTIR spectras (Figure 2) obtained for fenoverine, eudragit S 100, physical mixture of fenoverine and eudragit S 100, optimized formulation F3 showed distinguished peaks of drug at respective wave numbers 1042, 1238, 1672 and 2810 cm-1 for C–N stretch (aliphatic amines) C–N stretch (aromatic amines), C=O stretch, H–C=O: C–H stretch respectively showing no interaction between the drug and excipients.
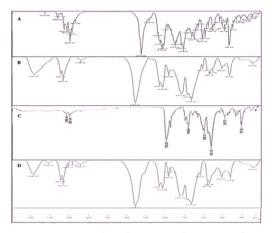


**Fig. 2: FTIR spectra of A = drug; B = polymer; C = physical mixture; and D = optimized formulation**

**Differential scanning calorimetry**

DSC thermograms of pure fenoverine, pure polymer, drug and polymer physical mixture, placebo and optimized formulation (F3) can be seen in the (Figure 3). Prominent softening points of pure fenoverine was found at 142°C, drug loaded Eudragit S 100 microparticles showed broad very small peak, at 5.1°C with no endothermic peak corresponding to fenoverine indicates the drug completely present in the form of molecular dispersion state in the final Eudragit S 100 microparticles.
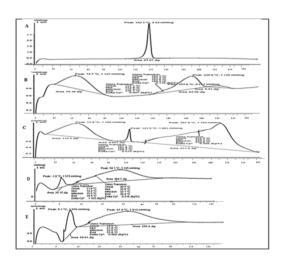


**Fig. 3: DSC thermograms of A = drug; B = polymer; C = physical mixture; D = placebo; and E = optimized formulation**

**Powder X- ray diffraction studies**

X-ray diffractograms (Figure 4) of Fenoverine indicated the presence of a crystalline material with principal peaks at 12.411° and 18.304° 2θ, whereas the polymer Eudragit S 100 was found to be amorphous. Although the diffractogram of drug demonstrated the presence of crystalline material, the diffractogram of Eudragit S 100 coated microparticles showed an amorphous material devoid of any crystallinity. This could be attributed to a dilution effect by the amorphous polymer.
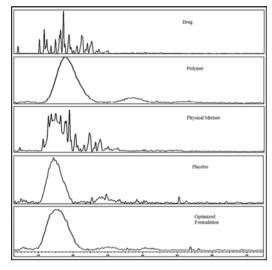


**Fig. 4: Powder XRD patterns**

**Scanning electron microscopy**

The surface morphology and shape of various formulations was observed by using SEM. An increase in the amount of drug result an increase in surface roughness similarly decrease in polymer concentration also decrease the surface smoothness of microparticles (Figure 5 a). Proportionally increment in surface smoothness was observed by increasing the amount of Eudragit S 100 (Figure 5 b). Higher the polymer concentration more uniform encapsulation would be as compared to less polymer concentration. Higher smoothness and spherosity was observed for F3 (Figure 5b) having drug to polymer ratio 1:5. Scanning electron microscopy of the optimized formulation revealed that Eudragit S 100 microparticles were mostly spherical in shape with smooth outer surface.
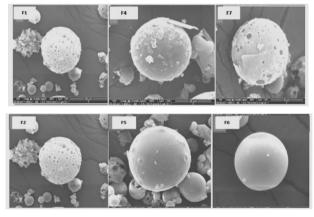


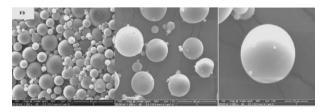**Fig. 5a: SEM photomicrographs of formulation F1, F2, F4, F5, F6 and F7**



**Fig. 5b: SEM photomicrograph of best formulation F3 at different magnification**

## CONCLUSIONS

In present study, an attempt was made to develop pH sensitive microparticles for colon specific delivery of fenoverine by emulsion solvent evaporation technique. The method was able to produce free flowing spherical particles with uniform size. The resulting microparticles were suitable for colon specific drug delivery with initial lag phase in upper gastrointestinal tract and finally releasing drug in colon at controlled rate for a period of 24 h. Hence they may reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug.

## ACKNOWLEDGEMENTS

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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