Development and Characterization of Colon Targeting Curcumin Nanoparticles

Shailja Gour*, B. K. Dubey, Brijesh Mishra

Technocrats Institute of Technology-Pharmacy, Bhopal (Madhya Pradesh)

*Corresponding Author's E mail: shailjagour@gmail.com

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ABSTRACT

Inflammatory Bowel Disease (IBD) cannot be controlled effortlessly and the reappearance is the most challenging issue for the physicians. There are various controlled and colon targeted drug delivery systems available for the treatment with limited success rate. Nanoparticles prepared by using the colon targeted polymers such as chitosan may improve the IBD due to their smaller size, unique physico chemical properties and targeting potential. The results of measurement of mean particle size of optimized formulation F2 nanoparticles were found 86.23 nm. When the regression coefficient values of were compared, it was observed that 'r' values of Pappas was maximum i.e. 0.949 hence indicating drug release from formulations was found to follow Pappas kinetics. . Eudragit-coated chitosan nanoparticles can be a promising carrier for colon-targeted delivery of Curcumin found to have high encapsulation efficiency and predetermined *in vitro* release profile.

Keywords: Inflammatory Bowel Disease, Curcumin, Chitosan, Eudragit-coated.

INTRODUCTION

The phytopharmaceutical product Curcumin, a hydrophobic polyphenol, is derived from the rhizome of the herb *Curcuma Longa* and has a wide spectrum of biological and pharmacological activities including anti-oxidant, anti-inflammatory, anti-hyperlipidemic, anti-carcinogenic effects and antiangiogenic effects¹⁻⁶. Furthermore, various animal models and human studies have proven that curcumin is extremely safe even at high doses of 12 g/day⁷⁻¹².

Curcumin is classified as a biopharmaceutical classification system (BCS) class IV molecule on the basis of its poor aqueous solubility (11 ng/ml in aqueous buffer pH 5) and permeability through intestinal

epithelial cells¹³⁻¹⁴. Chemically, curcumin is a bis- α,β -unsaturated β diketone commonly called diferuloylmethane, and exhibits keto-enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium.

The oral route of drug administration constitutes the most convenient and hence the preferred means of drug delivery to systemic circulation of body. However, oral administration of most drugs in conventional dosage forms has limitations due to their inability to restrain and localize the drug delivery system at GIT. Because of smaller size and efficient carrier capacity, Nanotechnology is now becoming an important area of research due to its wide range of benefits in pharmaceutical industry and applied sciences¹⁵⁻¹⁶. Recent research reports revealed the opportunities of nanomedicine in the treatment of IBD. Nanomedicine may replace the conventional dosage forms in the treatment of IBD¹⁷. Present treatment of IBD lacks cure (only remission). Nanoparticles are helpful in improving the function of current existing drugs by targeting action, solubility improvement and dose reduction¹⁸. Various study reports revealed the advantage of nano formulations for IBD at minimum doses¹⁹. Chitosan nanoparticles have gained significant importance since chitosan is the natural colon targeted polymer²⁰. The formation of chitosan nanoparticles include simple and non toxic method called as ionic gelation method. This method includes cations formation of chitosan dissolved in the aqueous acid solution²¹⁻²³. This formed solution is then added to the aqueous TPP solution under stirring conditions. In this research work, Curcumin nanoparticles were prepared by high result oriented ionic gelation method with slight modification in the method. In this research work, chitosan was used along with STPP. The formulations are developed with a scope of better therapeutic efficacy, solubility and penetration at the inflamed site.

MATERIALS AND METHODS

Materials

Curcumin was obtained as a gift sample from Phyto drug Pvt. Ltd (India). Chitosan, Eudragit S-100 was obtained from HiMedia Laboratories Ltd, Mumbai, India. STPP, acetic acid, ethanol, Span 80, acetone, dichloromethane and light liquid paraffin were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Formulation of Curcumin nanoparticles by ionic gelation technique

Chitosan nanoparticles were prepared by ionotropic gelation method. Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature²⁴. The drug (2-5 mg) was dissolved in chitosan solution. 1% Sodium tripolyphosphate solution was prepared in water. Sodium tripolyphosphate solution was added drop wise with a syringe to chitosan solution while stirring. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water.

Nanoparticles were obtained which was air dried for 24 hours followed by oven drying for six hours at 40°C. The composition of chitosan nanoparticles given in table 1.

Coating of prepared nanoparticles

Nanoparticles were coated with Eudragil S-100 (ES) using solvent evaporation method. Nanoparticles (50 mg) were dispersed in 10 mL of coating solution prepared by dissolving 500 mg of ES-100 in ethanol: acetone (2:1) to give 5:1 (coat: core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% w/v Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. The coated nanoparticles thus obtained were filtered, washed with n-hexane, and dried in desiccators.

Table 1: Formulations of chitosan nanoparticles

Sr. No	Formulation Code	Curcumin (mg)	Chitosan (mg)	STPP (mg)
1.	F1	5	250	250
2.	F2	5	250	500
3.	F3	5	250	750
4.	F4	5	500	250
5.	F5	5	500	500
6.	F6	5	500	750

Evaluation of nanoparticles

Percentage Yield

The prepared nanoparticles F1-F6 were collected and weighed from each formulation. The percentage yield (%) was calculated using formula given below:

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 100$$

Entrapment Efficiency

10 mg of chitosan nanoparticles from each batch were accurately weighed. The powder of chitosan nanoparticles were dissolved in 10 ml 7.2 pH Phosphate Buffer and centrifuge at 1000 rpm. This supernatant solution is then filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.2 pH Phosphate Buffer. The supernatant was analyzed for drug content by measuring the absorbance at 428nm²⁵.

$$\% \text{ EE} = \frac{\text{Total drug content} - \text{Free drug}}{\text{Total drug content}} \times 100$$

Measurement of mean particle size

The mean particle size of the nanoparticle was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern particle size analyser) at a scattering angle of 90°. A sample (0.5mg) of the nanoparticle suspended in 5 ml of distilled water was used for the measurement ²⁶.

Determination of zeta potential

The zeta potential of the drug-loaded nanoparticless was measured on a zeta sizer (Malvern particle size analyser) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate ²⁷.

In-vitro Release Studies

In vitro drug release in gastrointestinal fluids of different pH

The prepared nanoparticles were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus ²⁸. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows:

- 1st hour: Simulated gastric fluid (SGF) of pH 1.2.
- 2nd and 3rd hour: Mixture of simulated gastric and Intestinal fluid of pH 4.5.
- 4th to 5th hour: Simulated intestinal fluid (SIF) of pH 6.8.
- 6th hour and onward: SIF pH 7.5

A weighed quantity of formulation (equivalent to 30mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 428 nm for percent of release Curcumin using UV visible spectrophotometer. The release of Curcumin was calculated with the help of Standard curve of Curcumin.

RESULTS AND DISCUSSIONS

Percentage yield of different formulation was determined by weighing the nanoparticles after drying. The percentage yield of different formulation was in range of 62.23– 72.25% (Table 2).

The drug entrapment of different formulations was in range of 65.45-73.23% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Curcumin nanoparticles (Table 2).

The maximum percentage yield and entrapment efficiency was found formulation F2. The optimized formulation among other batches subjected to further studies. The mean size of the nanoparticles was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Particle Size Analyzer from Malvern) at a scattering angle of 90°C. A sample (0.5mg) of the nanoparticles suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F2 nanoparticles were found 86.23 nm. The zeta potential of the drug-loaded nanoparticles was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. A result of zeta potential of optimized formulation F2 nanoparticles was found to be -30.5 mV. Shape and surface characteristic of Curcumin nanoparticles examined by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnifications 55X which illustrate the smooth surface of Nanoparticles. When the regression coefficient values of were compared, it was observed that 'r' values of Pappas was maximum i.e. 0.949 hence indicating drug release from formulations was found to follow Pappas kinetics (Table 3).

Table 2: Results of evaluation of prepared nanoparticles of curcumin

Formulation	Percentage Yield* (%)	% Entrapment Efficiency*
F1	68.85±0.45	65.56±0.32
F2	76.65±0.56	73.23±0.45
F3	70.12±0.85	70.12±0.45
F4	62.23±0.65	68.85±0.65
F5	65.56±0.63	65.45±0.56
F6	69.98±0.41	65.56±0.41

* Average of three determinations

Table 3: *In Vitro* drug release data for coated formulation

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release $\pm$ SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	1	1	0	3.12	0.494	96.88	1.986
2	2	1.414	0.301	4.56	0.659	95.44	1.980
3	3	1.732	0.477	8.15	0.911	91.85	1.963
4	4	2	0.602	12.32	1.091	87.68	1.943
5	5	2.236	0.699	25.56	1.408	74.44	1.872
6	6	2.449	0.778	45.56	1.659	54.44	1.736
7	7	2.646	0.845	59.98	1.778	40.02	1.602
8	8	2.828	0.903	69.98	1.845	30.02	1.477
9	9	3	0.954	75.56	1.878	24.44	1.388
10	10	3.162	1	83.32	1.921	16.68	1.222
11	12	3.464	1.079	96.65	1.985	3.35	0.525

* Average of three determinations

Table 4: Regression analysis data of nanoparticle formulation

Formulation	Zero order	First order	Pappas plot
F2	$y = 9.819x - 15.73$ $R^2 = 0.963$	$y = -0.118x + 2.331$ $R^2 = 0.854$	$y = 1.614x + 0.299$ $R^2 = 0.949$

CONCLUSION

The developed formulation was found to be spherical with tiny pores on the surface. Eudragit S-100 was used as a pH sensitive polymer having threshold pH value above 6, which bypassed the upper GIT and showed targeted and controlled release at colonic pH as revealed by *in vitro* drug release study. The *in vitro* studies also revealed better therapeutic outcomes as compared to free curcumin. Thus the results suggested that curcumin loaded nanoparticles may be considered as a promising drug delivery system for treating ulcerative colitis and colonic cancer.

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