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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF MICROPARTICLES CONTAINING CURCUMIN FOR COLORECTAL CANCER

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ABSTRACT:

The aim of this present investigation was to formulate and evaluate pH sensitive polymer coated micro particles containing Curcumin for colon targeting. pH sensitive polymer such as Eudragit S 100 were selected as a model encapsulation material. The micro particles were prepared by solvent evaporation technique using different ratio of curcumin and Eudragit (1:1 to 1:3). The prepared microspheres were subjected to various evaluation parameters such as angle of repose, drug loading, encapsulation efficiency, scanning electron microscopy, differential scanning colorimeter, in-vitro release studies, release kinetics and stability studies. Scanning electron microscopy revealed spherical nature and smooth surface morphology of the microspheres. The size of the microspheres was between 29.21-31.27 μ m. The encapsulation efficiency was between 21.73% - 43.75 % and drug content was between 14.83% - 18.36 %. The FT-IR and DSC thermograms confirmed the absence of drug polymer interaction. The micro particles prepared were filled in hard gelatin capsules which were enteric coated with cellulose acetate phthalate (CAP) which prevent the burst effect of capsule in acidic pH of stomach. The *in vitro* release study showed that there was no drug release in pH 1.2 acidic buffer, drug release was between 66.12% - 71.87% for 10 hrs in phosphate buffer and controlled by Fickian diffusion mechanism. It is concluded from the present investigation that Eudragit microspheres are promising controlled release carriers for colon-targeted delivery of curcumin.

Key words: Curcumin, eudragit S 100, solvent evaporation method, microspheres, pH dependent, colon targeting.

1. INTRODUCTION:

Colorectal cancer (CRC) is unfortunately an all-too common lethal disease and is a major health concern that affects men and women equally. Worldwide it accounts for approximately 1 million new cancers and one-half million deaths, equating 10 percent of all cancer deaths annually.¹ Colon cancer arises from mucosal colonic polyps. Colon cancer commonly known as bowel cancer, is a cancer caused by uncontrolled cell growth (neoplasia), in the colon, rectum, or veriform appendix. Colorectal cancers start in the lining of the bowel. It can grow into the muscle layers underneath and then through the bowel wall (If left untreated).

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The curcuminoids are polyphenols and are responsible for the yellow color of turmeric.²

Curcumin is a potential anticancer drug it shows its effect through Molecular Targets in Colon Cancer. Accumulating evidence suggests that curcumin has a diverse range of molecular targets. This polyphenol modulates various targets through direct interaction or modulation of gene expression. Curcumin physically binds to as many as 33 different proteins, including thioredoxin reductase, cyclooxygenase-2 (COX-2), protein kinase C (PKC), 5-lipoxygenase (5-LOX), and tubulin. Molecular targets modulated by this agent are transcription factors, growth factors and their receptors, cytokines, enzymes, and genes that regulate cell proliferation and apoptosis.³

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to

target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance.¹

2. MATERIALS AND METHODS:

Curcumin is a gift sample from Natural Remedies, Bangalore. Eudragit S 100, Propan-1-ol and Chloroform were also used in study. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

2.1 Preparation of microspheres

Curcumin microspheres were prepared by solvent evaporation technique⁵. Eudragit S 100 was dissolved in a mixture of Propan-1-ol and Chloroform (1:2) at room temperature. Curcumin was added to the above solution stirred in a magnetic stirrer to form a homogeneous solution. Then the above solution poured into 100ml of water containing 0.02% of Sodium lauryl sulfate maintained at room temperature and stirred at 1000 rpm for 90 minutes, the resultant mixture was filtered then washed with distilled water. The microspheres formed were then dried at room temperature. Three different formulations with different drug: polymer ratios (1:1, 1:2, 1:3) were prepared.

2.2. Encapsulation efficiency of the microspheres⁶

About 50mg of microspheres were weighed which was dissolved in Ethanol and made up to 100ml with distilled water shaken in mechanical shaker for 24h. Then solution

was filtered and made suitable dilution and analyzed spectrophotometrically at 427 nm.

2.3. Scanning electron microscope analysis

Shapes and surface characteristics of the microspheres were investigated and photographed using scanning electron microscope (SEM; JEOL JSM T-330A, Japan).

2.4 Particle size analysis

Measurements of the particle size distribution of microspheres were carried out with an optical microscope. Stage micrometer was used to calculate calibration factor. The particle size was calculated by multiplying the number of division of the ocular disc occupied by the particle with calibration factor. Hundred randomly chosen spheres were taken to measure their individual size.

2.5 Differential Scanning Calorimetry

The physical state of Curcumin in the microspheres was analyzed by Differential Scanning Calorimeter (Mettler-Toledo star 822 system, Switzerland). The thermo grams of the Curcumin, physical mixture of Curcumin and polymer, and Curcumin microspheres were obtained at a scanning rate of 10°C/min conducted over a temperature range of 170–250°C, respectively.

2.6 Drug polymer interaction (FTIR) study

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 3 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm⁻¹. FT-IR study was carried on Curcumin, physical mixture of Curcumin, and Curcumin microspheres.

2.7 Coating of Hard Gelatin Capsules^{7,8}

Hard gelatin capsules of 'size 0' were chosen for coating. The bodies and caps separated manually. Curcumin microspheres equivalent to 5 mg of curcumin were accurately weighed and filled into the bodies by hand filling and the cap was replaced. The enteric coating solution was prepared by using the formula which is given in table 1. The capsules were completely coated with 5% CAP by dip coating method. The capsules were alternatively dipped in 5% CAP solution and dried. Coating was repeated until an expected weight gain 8-12% was obtained and capsule resists disintegration in 0.1N HCl for minimum of 2 hrs. Percentage weight gain of the capsules before and after coating was determined.

Table: 1 Composition of coating solution

Sl.no	Ingredients	Qty(%w/w)
1	CAP	6
2	Titanium dioxide	2.6
3	Diethyl phthalate	2.2
4	Acetone	59.4
5	Isopropyl alcohol	30.8

2.7 In vitro dissolution studies⁹

Dissolution studies were carried out by using USP type II dissolution test apparatus by rotating basket method in stimulated gastric fluid pH 1.2 for 2 h, pH 6.8 for 3 h and pH 7.4 for next 5 h. The dissolution media were maintained at a temperature of 37 ± 5°C. The speed of rotation of basket maintained was 100 rpm.

Enteric coated capsules which were previously filled with Curcumin microspheres were placed in basket in each dissolution vessel and covered with nylon cloth to prevent floating. 1 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed and the amount of Curcumin released was determined by UV absorption spectroscopy at 427 nm.

3. RESULTS AND DISCUSSION:

Microspheres of Curcumin were prepared by solvent evaporation techniques by using polymer like Eudragit S 100. The prepared Curcumin microspheres were then subjected to FT-IR, SEM, particle size, size distribution, % yield, drug content, entrapment efficiency, *in vitro* dissolution, release kinetics, and DSC.

First, trials were made to prepare microspheres by using a solvent evaporation technique in the chloroform/ liquid paraffin system was used but no spherical particles could be obtained. Then water phase is used and various formulations with different polymer and drug ratios were tried, stirring speed was also changed to obtain spherical particles. These microsphere formulations are shown in table 2.

Loading efficiency of the drug depended on solubility of drug in solvents and continuous phase and physicochemical properties of drug and polymer¹⁰. High encapsulation efficiency was observed in different formulation of Curcumin. Encapsulation efficiency is given in table 3.

The mean particle size of Curcumin microspheres was in range of 29.21-31.27 μm. As it was evident from the results that particle size increases as polymer concentration was increased. The data showing particle size analysis is given in table 4.

Table: 2 Formulation details of Eudragit S 100 microspheres of Curcumin

Ingredients	Formulation codes		
	A-1	A-2	A-3
Curcumin (mg)	50	50	50
Eudragit S 100(mg)	50	100	150
Propan-1-ol and Chloroform (1:2) in ml	12	12	12
Sodium lauryl sulfate	0.02 %	0.02 %	0.02 %
Water (ml)	100	100	100

Table: 3 Drug loading and % Entrapment efficiency

Formulation	Percentage yield	Drug content (%)	Entrapment Efficiency (%)
A1	57.5	15.81	21.73
A2	45.33	18.6	36.73
A3	56.25	14.83	43.75

Table: 4 Particle size analysis of Curcumin microspheres

Formulation	Average size (μm)
A1	29.21 \pm 7.91
A2	30.41 \pm 11.4
A3	31.27 \pm 5.56

From Scanning electron microscopy, it was observed that particles were spherical. The surface of the drug-loaded Eudragit S 100 microspheres shown in Figure: 1. Eudragit S 100 microspheres showed smooth surface. All the microspheres had small pores on their surfaces, which will be responsible for release.

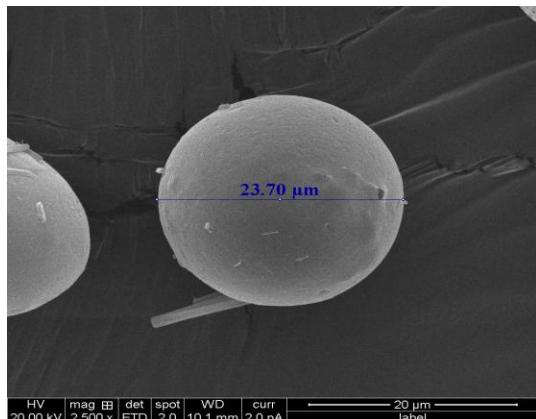


Figure: 1 SEM images of Eudragit L 100microspheres of Curcumin

Drug polymer interaction can be studied by FT-IR analysis. Figure: 2 show the IR spectra of pure Curcumin, drug loaded microspheres and physical mixture. The same peaks were also reported in all drug loaded microspheres and in physical mixture. There was no change or shifting of characteristic peaks in drug loaded microspheres suggested that there was no significant drug polymer interaction which indicates the stable nature of drug in all formulations.

Any possible drug polymer interaction can also be studied by thermal analysis. DSC studies were performed on pure drug and drug-loaded microspheres. DSC thermograms of pure Curcumin (figure: 3) showed a sharp endothermic peak at 176.77°C. The thermograms of formulation also showed a similar endothermic peak at 180.24°C. This further confirms that there is no drug polymer interaction.

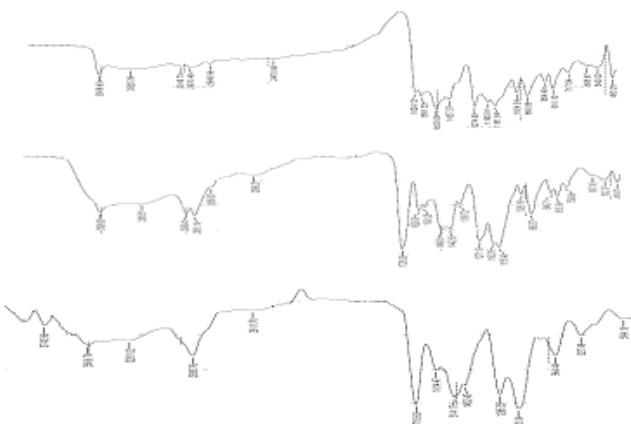


Figure: 2 FT IR spectra of pure Curcumin(A) physical mixture(B) Eudragit S 100microspheres(C)

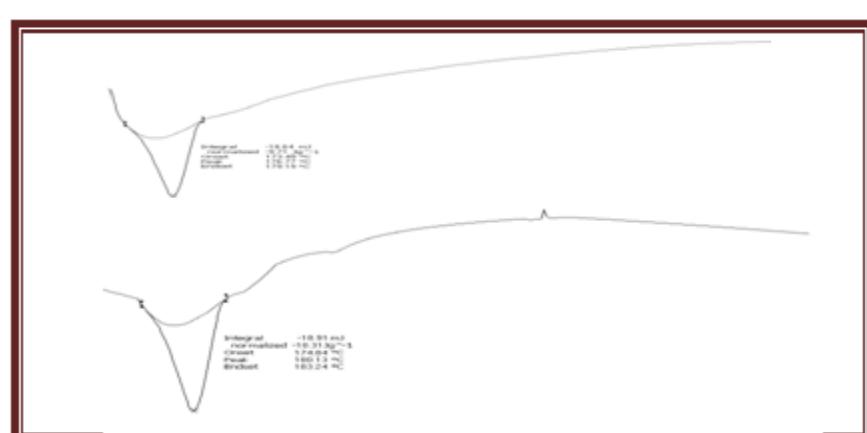


Figure: 3 DSC thermograms of Curcumin (A) Curcumin microspheres (B)

Drug release from microspheres was sustained up to 10 hours. Different formulation showed different degree of release (figure: 4). the cumulative release of Curcumin significantly decreased with increasing Eudragit S 100concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusion path length¹¹. After 10 hours drug release from different formulation was in range of 66.12 to 71.87 % .

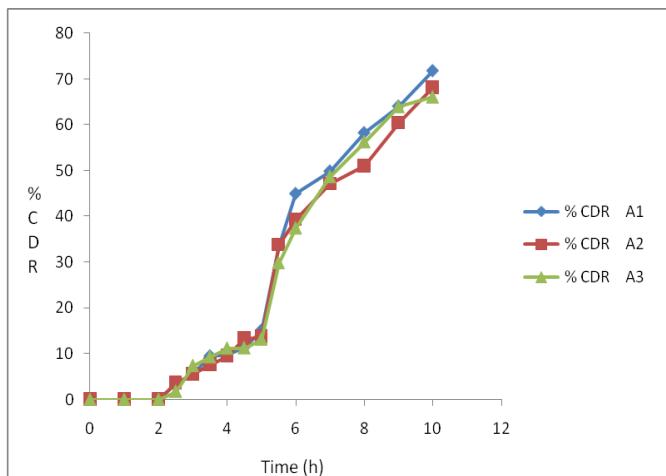


Figure: 4 Cumulative percentage drug release profile of Curcumin microspheres (A1, A2 &A3)

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The in-vitro release data was fitted to various kinetic models in order to find out the drug release mechanism. The 'n' value obtained from Korsmeyer- Peppas equation for Eudragit L 100was in range of 0.365 to 0.371 suggested that the drug release from microspheres followed fickian diffusion mechanism¹².

4. CONCLUSION:

The Curcumin loaded microspheres were prepared by solvent evaporation method using Eudragit S 100. Polymer: drug ratio and stirring speed of the system were important to obtain spherical particles with smooth surfaces. The yields of preparation and encapsulation efficiencies were very high for all microspheres obtained. The prepared microspheres were free flowing and discrete. The FT-IR and DSC suggested no drug polymer interaction during encapsulation process. The drug release was studied up to 10 hours and results indicate that release of drug from microspheres followed fickian diffusion mechanism.

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