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FORMULATION AND EVALUATION OF CURCUMIN-LOADED MICROSPHERE FOR TARGETED THERAPY OF COLON CANCER

Sayali H. Dherange[1], Anil B. Panchal[2]*, Sampat D. Navale[3]

[1]Research Scholar, Department of Pharmaceutics, Delight College of Pharmacy, Koregaon Bhima, Pune, Maharashtra, India-412216.

[2]Assistant Professor, Department of Pharmaceutics, Delight College of Pharmacy, Koregaon Bhima, Pune, Maharashtra, India-412216.

[3]Principal (Professor) Department of Pharmacognosy, Delight College of Pharmacy, Koregaon Bhima, Pune, Maharashtra, India-412216.

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***Corresponding Author**

Anil B. Panchal

Assistant Professor, Department of Pharmaceutics, Delight College of Pharmacy, Koregaon Bhima, Pune, Maharashtra, India-412216.

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ABSTRACT

Curcumin, the bioactive compound found in turmeric, a natural polyphenol, has shown promise in colon cancer treatment due to its anti-proliferative and anti-inflammatory properties. To overcome these challenges, curcumin microspheres have been developed as an innovative drug delivery system aimed at enhancing its therapeutic potential. The microspheres, formulated using polymers such as ethyl cellulose , provide a controlled and sustained release of curcumin, ensuring prolonged therapeutic effects. An "emulsion solvent evaporation" technique was used in the preparation of microspheres. The optimized formulation was chosen based on the preparation of microspheres with a 1:2 drug-to-polymer ratio, a stirring speed of 1000 rpm, and a 1%w/v concentration of emulsifying agent. The microspheres were evaluated for particle size, surface morphology, encapsulation efficiency, and drug loading capacity. In vitro release studies demonstrated a gradual release of curcumin over time, and stability studies confirmed the protection of curcumin from degradation. These findings highlight that curcumin-loaded microspheres are a promising approach to improving curcumin's bioavailability and therapeutic effectiveness, especially for conditions requiring long-term treatment or sustained drug delivery.

KEYWORDS: Curcumin, Microspheres, Colon cancer, Ethyl Cellulose, Polyvinyl Alcohol.

INTRODUCTION

The high rates of morbidity and death, colon cancer, commonly referred to as colorectal cancer (CRC), is one of the most common cancers in the world and a significant public health problem. It is one of the leading causes of cancer-related mortality, especially in both developed and developing nations.[1-2] The unchecked growth of epithelial cells lining the colon or rectum is the disease's defining feature. It frequently progresses through phases from benign adenomatous polyps to malignant cancer.[3-4] Genetic susceptibility, inactive lifestyles, dietary habits (high intake of processed foods and low fibre consumption), obesity, smoking, and chronic inflammatory bowel illnesses including Crohn's disease and ulcerative colitis are risk factors for colon cancer.[5-6]

Colon cancer care is still difficult despite improvements in therapeutic approaches and diagnostic methods. Radiation, chemotherapy, and surgery are examples of conventional therapeutic approaches.[7] Chemotherapeutic drugs like oxaliplatin, irinotecan, and 5-fluorouracil are commonly used, but they have serious drawbacks, such as systemic toxicity, non-specific distribution, drug resistance, and unfavourable side effects like nausea, immunosuppression, and gastrointestinal problems. These disadvantages underscore the pressing need for more efficient and focused drug delivery methods that can minimise systemic exposure while delivering therapeutic compounds straight to the site of action.[9]

A promising strategy to get around the drawbacks of traditional treatments is targeted drug delivery systems (TDDS).[10] For the treatment of colon cancer, colon-targeted medication delivery devices are particularly intriguing. Longer transit times, a neutral to slightly alkaline pH environment, and decreased enzymatic activity in the upper gastrointestinal tract are just a few benefits of using the colon as a medication delivery location. These features enable the creation of dosage forms that release the medication precisely in the colon while shielding it from breakdown in the stomach and small intestine.[11-12]

Curcumin, a naturally occurring polyphenolic compound derived from the rhizome of Curcuma longa (turmeric), has drawn a lot of attention lately because of its many pharmacological properties, which include immunomodulatory, anticancer, anti-inflammatory, antioxidant, and antimicrobial effects. Curcumin has been demonstrated to

block many signalling pathways involved in the genesis, development, and advancement of colon cancer.[13]

One of the most extensively researched drug delivery methods for attaining targeted and regulated medication release is microspheres. They are spherical particles made of synthetic or biodegradable polymers that encapsulate the medicine within their matrix. Their sizes generally range from 1 to 1000 micrometres.[14] Microspheres provide a number of benefits, such as better bioavailability, controlled and prolonged release, protection against drug degradation, and less frequent dosage. Additionally, microspheres may be made to release the medication in response to particular physiological parameters, such pH or enzyme activity, by choosing the right polymers.[15]

The current work uses Ethyl cellulose is biocompatible, hydrophobic polymer derived from cellulose. In colon-targeted microspheres, ethyl cellulose acts as a sustained-release polymer that protects the drug from premature release in the stomach and small intestine. Its hydrophobic nature reduces water penetration into the microsphere matrix, thereby controlling drug diffusion and prolonging drug release. Polyvinyl Alcohol is a synthetic, water-soluble polymer commonly used as a stabilizer, emulsifying agent, and surfactant in microsphere preparation. In colon-targeted microsphere formulations, PVA mainly functions as a stabilizing agent during the emulsification process, preventing aggregation of particles and ensuring uniform microsphere size distribution.[17]

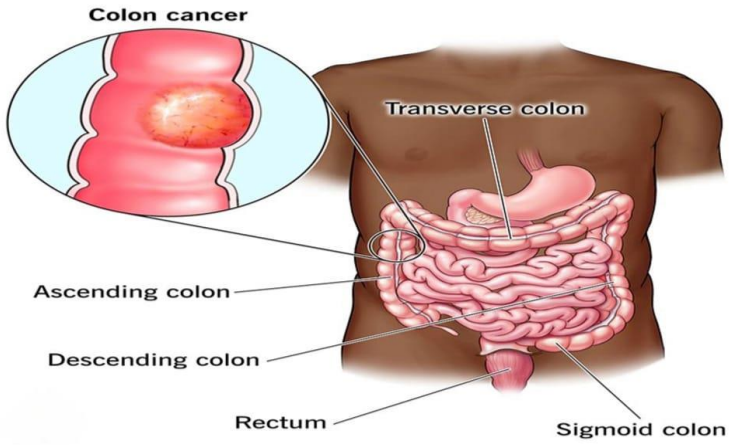

Figure 1: Colon Cancer.

Ionotropic gelation and solvent evaporation are two methods that can be used to create microspheres.[18] Particle size, medication loading, and release properties may all be precisely controlled with these techniques.[19]

MECHANISM OF ACTION

1. Suppression of Cell Growth-By altering cell cycle regulatory proteins such cyclins and cyclin-dependent kinases (CDKs), curcumin inhibits the growth of cancer cells.
2. Apoptosis Induction-By up regulating pro-apoptotic proteins (Bax) and down regulating anti-apoptotic proteins (Bcl-2), as well as activating caspases, it initiates pathways leading to programmed cell death.
3. NF-κB Pathway Inhibition-The nuclear factor-kappa B (NF-κB) signalling system, which is essential for inflammation, cancer growth, and cell viability, is inhibited by curcumin.
4. Reduction of Inflammation-It lowers the expression of inflammatory mediators linked to colon carcinogenesis, including TNF-α, IL-6, and COX-2.[20]
5. Activity of Antioxidants-Curcumin prevents oxidative DNA damage by scavenging free radicals and increasing the activity of antioxidant enzymes.
6. Angiogenesis Inhibition-By inhibiting the production of new blood vessels, it limits the development of tumours by down regulating vascular endothelial growth factor (VEGF).
7. Prevention of Metastasis-By blocking matrix metalloproteinase (MMPs), curcumin decreases the invasion and spread of cancer cells.
8. Signalling Pathway Modification-It controls several signalling pathways that contribute to the development of colon cancer, including Wnt/β-catenin, PI3K/Akt, and MAPK pathways.[20]

PLANT PROFILE

Table 1: Plant Profile.[18-19]

Parameter	Details
Scientific Name	*Curcuma longa*
Family	Zingiberaceae
Common Name	Turmeric
Synonyms	Indian saffron, Haldi
Biological Source	Dried rhizomes of *Curcuma longa*
Geographical Source	India, China, Southeast Asia
Part Used	Rhizome
Plant Description	A perennial herb with underground rhizomes, broad leaves, and yellow-orange colored roots
Macroscopic Characters	Rhizomes are cylindrical, aromatic, yellow to orange in color, with rough surface
Microscopic Characters	Presence of starch grains, oleoresin cells, and vascular bundles
Chemical Constituents	Curcuminoids (Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin), volatile oils (turmerone, atlantone, zingiberene)

Active Constituent	Curcumin
IUPAC Name of Curcumin	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione
Molecular Formula	$C_{21}H_{20}O_6$
Molecular Weight	368.38 g/mol
Solubility	Poorly soluble in water; soluble in ethanol, acetone, and oils

Figure 2: Curcumin.

MATERIALS

Table 2: Formulation of Curcumin Loaded Microspheres.

Sr. No.	Ingredient	Quantity	Purpose
1	Curcumin	1.2mL	Active herbal ingredient
2	Ethyl Cellulose	2.5g	Polymer for encapsulation
3	Acetone	q.s. (up to 20mL)	Organic solvent for drug-polymer solution
4	Polyvinyl Alcohol	0.25g	Emulsifier for aqueous phase
5	Distilled Water	q.s. (up to 10ml)	Continuous phase (external)

METHOD OF PREPARATION

Step 1: Turmeric Root Extraction

Method: Soxhlet Extraction Method

1. Clean and dry turmeric roots and grind into a fine powder using a grinder.
2. Place 15g turmeric powder in a filter paper thimble.
3. Add 150mL ethanol to the round-bottom flask and set up the Soxhlet apparatus.
4. Heat the solvent to evaporate, condense, and repeatedly wash the turmeric powder.
5. Continue extraction for 2.5 hours.

6. Evaporate the solvent to obtain the concentrated extract.

Step 2: Preparation of Internal Phase

1. Dissolve 2.5g ethyl cellulose and 1.2mL curcumin in acetone under constant stirring.

Step 3: Formation of Emulsion

1. Prepare the external aqueous phase by dissolving 0.25g polyvinyl alcohol (PVA) in distilled water.
2. Slowly add the organic phase (curcumin and polymer solution) into the aqueous phase under high-speed stirring to form an oil-in-water (O/W) emulsion.

Figure 3: Organic Phase and Aqueous Phase.

Step 4: Solvent Evaporation

1. Continue stirring the emulsion for 4–6 hours to allow the evaporation of dichloromethane. This leads to the solidification of microspheres.

Step 5: Washing

1. Wash the microspheres with distilled water to remove excess PVA and unencapsulated drug.

Step 6: Drying

1. Dry the collected microspheres at room temperature or in a vacuum oven at 40°C for 24 hours.

EVALUATION PARAMETERS

1. Fourier Transform Infrared Spectroscopy (FTIR)

a. Test Method: FTIR

b. Purpose: To identify any chemical interactions between curcumin and the polymer (ethyl cellulose).

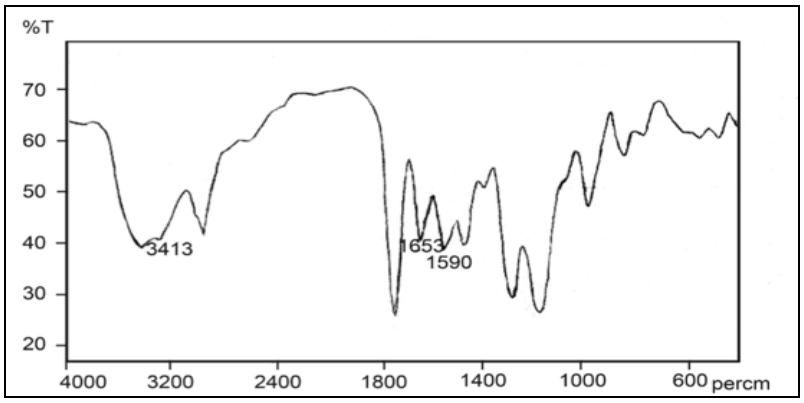

Figure 4: Simulated FTIR Spectrum Curcumin Microspheres (A).

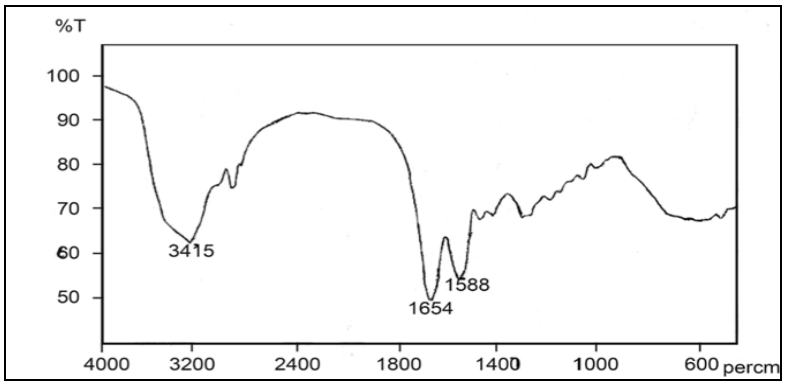

Figure 5: Simulated FTIR Spectrum of Curcumin (B).

Panchal

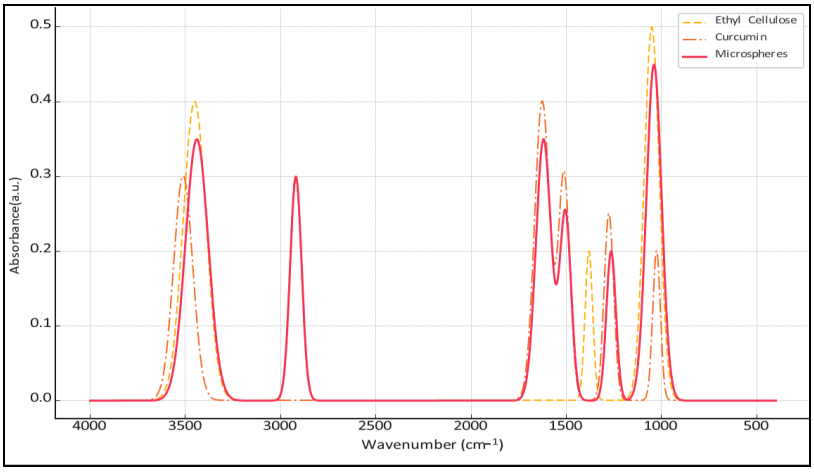

Figure 6: Simulated FTIR Spectrum of Ethyl Cellulose + Curcumin + Curcumin Microspheres.

2. Size of Particles-One important factor affecting medication release, stability, and bioavailability in microspheres is particle size. Optical microscopy or scanning electron microscopy (SEM) are typically used to determine it. An optimal particle size range of 10–200 µm is thought to be appropriate for microsphere formulations as it guarantees appropriate drug encapsulation and regulated release behaviour.

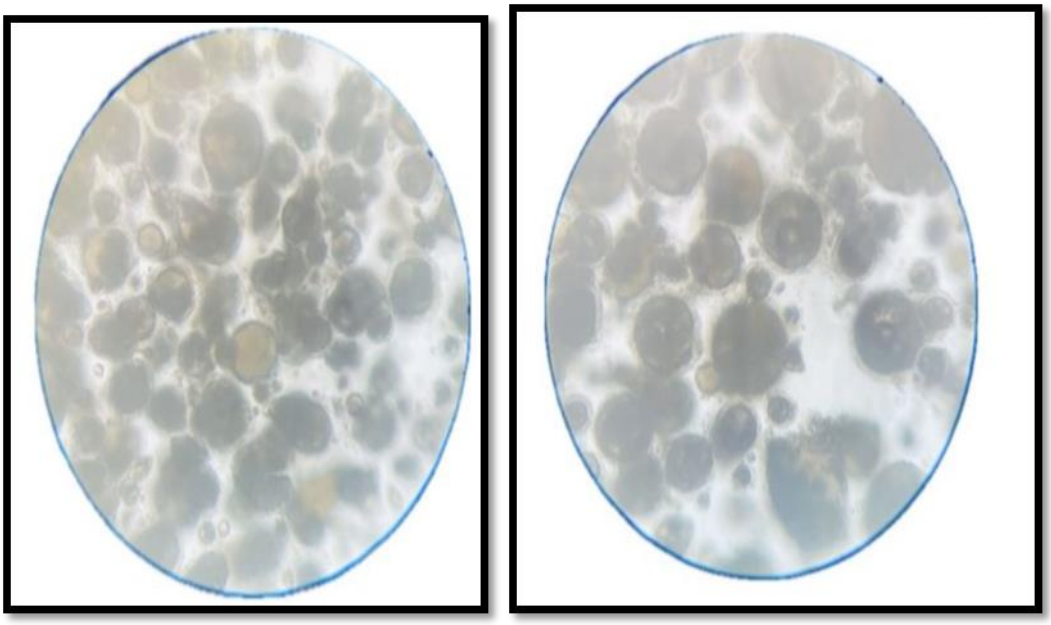

Figure 7: Optical microscopy image of curcumin-loaded microspheres showing particle size and surface morphology.

3. Drug Content (%)-UV-visible spectroscopy is used to measure drug content, which is the quantity of drug contained in the microspheres. A drug content range of 85–100% denotes low loss during formulation and homogenous drug distribution inside the polymer matrix.

4. Efficiency of Entrapment (%)-The proportion of drug that is effectively encapsulated within the microspheres in relation to the total amount of medication utilised is known as entrapment efficiency. Spectrophotometric techniques are used to calculate it. Effective drug loading and formulation stability are indicated by a number between 70 and 95%, which is regarded as satisfactory.

5. Yield Percentage (%)-The effectiveness of the preparation process is assessed using percentage yield. It is computed by comparing the total weight of the medicine and polymer utilised with the weight of the microspheres that were produced. A 70–90% yield indicates a repeatable and effective formulation procedure.

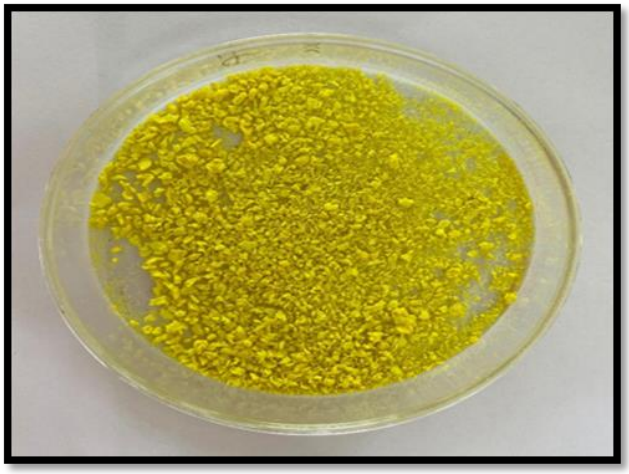

Figure 8: Prepared curcumin-loaded microspheres collected after formulation.

6. Index of Swelling-Adequate swelling behaviour is indicated by a swelling index between 1.5 and 3.5, which is crucial for colon targeting and regulated medication release.

7. Repose Angle-The funnel technique is used to determine the angle of repose, which is utilised to assess the flow characteristics of microspheres. Good flow characteristics are indicated by an angle less than 30°, and passable flow qualities—which are crucial for handling and processing are suggested by values between 30 and 40°.

8. Density of bulk -A density apparatus is used to calculate bulk density, which is defined as the mass of microspheres per unit bulk volume. The microspheres' appropriate packing and flow properties are indicated by values between 0.3 and 0.6 g/cm^3.

9. Density Tapped -The density that results from mechanically tapping a measuring cylinder filled with microspheres is known as "tapped density." It sheds light on the formulation's capacity to pack. The usual acceptable range is between 0.4 and 0.7 g/cm^3.

10. Carr's Index (%) -Compressibility and flowability of microspheres are measured using Carr's index, which is computed using bulk and tapped density measurements. Excellent flow characteristics are indicated by a number between 5 and 15%, while high flowability is indicated by a value between 15 and 25%.

11. The Hausner Ratio-The Hausner ratio, which is computed as the ratio of tapped density to bulk density, is another metric used to evaluate flow characteristics. Good flow behaviour, which is necessary for further processing like capsule filling, is indicated by a number between 1.00 and 1.25.

12. In-vitro Drug Release Studies

a. Test Method: **USP dissolution apparatus** in simulated gastrointestinal fluids (e.g., pH 1.2, pH 6.8).

b. Purpose: To evaluate the **release profile** of curcumin over time.

c. Results

i. **Initial Burst Release**: A small percentage (e.g., **10–20%)** of curcumin is released rapidly.

ii. **Sustained Release**: The remaining curcumin is gradually released, with up to **80%** of curcumin released over 24-48 hours for controlled release formulations.

CONCLUSION

The formulation of curcumin-loaded microspheres represents a promising approach for the targeted therapy of colon cancer. The developed microsphere system successfully enhances the therapeutic potential of curcumin by improving its stability, bioavailability, and site-specific delivery to the colon. The use of suitable polymers such as Ethyl cellulose and Polyvinyl Alcohol contributed to the formation of stable microspheres with satisfactory particle size, drug entrapment efficiency, and controlled drug release characteristics.

Evaluation studies demonstrated that the microspheres were capable of protecting curcumin from premature release in the upper gastrointestinal tract and providing sustained release in the colonic region. This targeted delivery system may reduce systemic side effects, enhance local drug concentration at the tumor site, and improve anticancer efficacy against colon cancer cells.

The microsphere formulation exhibited acceptable physicochemical properties, good stability, and reproducible performance, indicating its suitability for oral colon-targeted drug delivery. Overall, curcumin-loaded microspheres offer a safe, effective, and patient-friendly strategy for colon cancer therapy and hold significant potential for future pharmaceutical and clinical applications.

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