Preparation and Characterization of Colon-Targeted Metronidazole-Loaded Microspheres by Ionic Gelation Technique

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***Abstract*—Colon-targeted drug delivery systems (CTDDS) have emerged as a promising approach for the site-specific delivery of therapeutic agents to the large intestine, improving treatment efficacy while minimizing systemic side effects. The present study aimed to formulate and evaluate colon-targeted microspheres of Metronidazole using natural polymers for the effective treatment of colonic infections such as amoebiasis and anaerobic bacterial infections. Metronidazole-loaded alginate-chitosan microspheres were prepared by the ionic gelation technique using sodium alginate as the primary polymer, calcium chloride as the cross-linking agent, and chitosan as a coating material.**

***Index Terms*—Colon-Targeted Drug Delivery System (CTDDS), Metronidazole, Microspheres**

I. Introduction

In recent years, targeted drug delivery systems have gained significant attention in the pharmaceutical field for improving the efficacy, safety, and patient compliance of therapeutic agents. Among the various approaches, colon-targeted drug delivery systems (CTDDS) have emerged as an important strategy for delivering drugs specifically to the large intestine. The colon provides unique advantages as a drug delivery site due to its near-neutral pH, long transit time, low enzymatic activity, and the presence of dense microflora capable of degrading certain biodegradable polymers. These physiological characteristics make the colon an ideal site for both local and systemic drug delivery.

Oral route is most widely used for controlled delivery of drugs. It offers several advantages such as prevention of peak and valley fluctuation, reduced dosing frequency, reduced dose, improved patient compliances, and nearly constant level at the site of action. In past decades, via oral route colon has been exhaustively investigated as a specific site for delivery and absorption of drugs.

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome and constipation but also for the systemic delivery of proteins, therapeutic peptides, anti-asthmatic drugs, antihypertensive drugs and anti-diabetic agents. Because of near neutral pH and longer transit time colon offer several therapeutic advantages as a site of drug delivery.[1]

- **Why Colon Targeted Drug Delivery Needed?**

To ensure direct treatment at the disease site, lower dosing and fewer systemic side effects. Colon specific Formulation could also be used to prolong the drug delivery. It should be considered as beneficial in the Treatment of colon diseases. The colon is a site where both local or systemic drug delivery could be achieved. Topical treatment of inflammatory bowel disease, e.g. ulcerative colitis or Crohn's Disease. Such Inflammatory conditions are usually treated with glucocorticoids and Sulphasalazine. A number of others Serious Diseases of the colon, e.g. colorectal cancer, might also be capable of being treated more effectively if Drugs were targeted to the colon. Formulations for colonic delivery are also suitable for delivery of drugs Which polar and/or susceptible to chemical and enzymatic degradation in the upper GI tract, highly affected by hepatic metabolism in particular, therapeutic proteins and peptides.

- **Need For Colon-Targeted Metronidazole Delivery:**

Metronidazole is a nitroimidazole derivative that exhibits potent activity against anaerobic bacteria and protozoa, including Entamoeba histolytica. It is widely used in the treatment of intestinal and extraintestinal amoebiasis, bacterial vaginosis, anaerobic infections, and amoebic colitis. However, conventional oral metronidazole formulations face several limitations:

1. Rapid absorption from the stomach and small intestine
2. Poor availability of the drug at the colon (site of infection)
3. High systemic concentrations leading to side effects such as nausea, metallic taste, and neurotoxicity
4. Need for frequent dosing due to its short half-life.[2]

Colon Physiology and Drug Absorption

The colon plays an important role in drug absorption due to its unique anatomy, pH, long transit time, and rich microbial flora. These physiological characteristics influence drug release, stability, and absorption, making them essential considerations in the design of colon-targeted drug delivery systems.

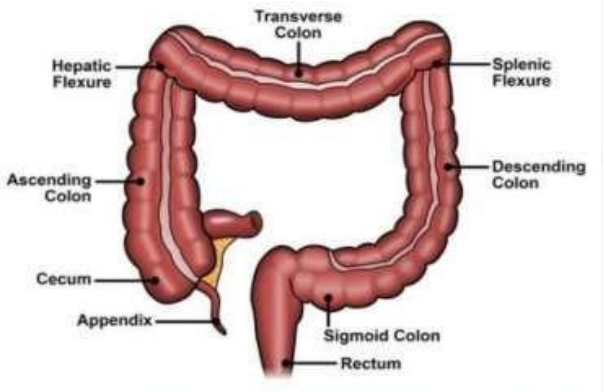

Figure 1: Parts of colon

1. Colon Anatomy

The large intestine is about 1.5 m long and consists of the caecum, appendix, colon, and rectum. Its main functions are the absorption of water, electrolytes, and nutrients, storage of fecal matter, and providing a suitable environment for colonic microflora.

2. Transit Time and Motility

Colonic transit time is longer than in other parts of the gastrointestinal tract, allowing prolonged contact between the dosage form and the mucosal surface. Motility is influenced by diet, peristalsis, and dosage form, which affects drug absorption.

2. Colonic Secretions and Enzymes

The colon secretes mucus that protects the mucosal surface and influences drug diffusion. Colonic bacteria produce enzymes that can metabolize drugs, affecting their release, absorption, and therapeutic efficacy.

3. pH Variations in the Colon

The pH of the colon ranges from slightly acidic in the proximal region to nearly neutral in the distal colon. These pH variations influence drug solubility, stability, and release, especially for pH-sensitive formulations.

1. Water Absorption and Drug Concentration

The colon absorbs water and electrolytes, increasing the local concentration of drugs. This concentration effect can enhance drug This concentration effect can enhance drug availability and improve the performance of colon-targeted formulations.

2. Drug Absorption in the Colon

Although the colon has a smaller absorptive surface area than the small intestine, it can effectively absorb drugs with suitable molecular weight, lipophilicity, and stability. Drug absorption mainly occurs through passive diffusion.

3. Influence of Disease States on Drug Absorption

Diseases such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome can alter colonic physiology, affecting drug absorption, residence time, and therapeutic efficacy. Therefore, disease conditions should be considered when designing colon-targeted drug delivery systems.[4]

- **MICROSPHERES**

Microspheres are small spherical particles, also referred to as microparticles, with diameters typically ranging from 1 μm to 1000 μm. They can be fabricated from a wide range of natural and synthetic materials and are commercially available as glass, polymer, or ceramic microspheres. Depending on their structure, microspheres can be solid or hollow, with hollow microspheres often used to reduce material density, while solid microspheres serve diverse applications based on their composition and size. Among polymers, polyethylene and polystyrene microspheres are the most commonly used. Polystyrene microspheres are widely employed in biomedical research for applications such as immunoprecipitation, cell sorting, and laboratory diagnostics, as their surfaces allow for easy and permanent adsorption of proteins and ligands. Polyethylene microspheres are utilized as temporary or permanent fillers and can form porous structures due to their low melting point. Their high sphericity and availability in coloured or fluorescent forms make them useful for fluid flow studies, microscopy, and various research applications. Additionally, charged polyethylene microspheres find applications in electronic paper and display technologies. Glass microspheres are primarily used as fillers, retro-reflectors, and cosmetic additives, while ceramic microspheres are often employed as grinding media.[5]

- **Types Of Microspheres**

1. Bioadhesive Microsphere
2. Magnetic Microsphere
3. Radioactive Microsphere
4. Floating microspheres
5. Polymeric microspheres
6. Biodegradable polymeric microspheres
7. Synthetic polymeric microsphere

1. Bioadhesive Microsphere: Bioadhesive microspheres utilize the ability of water-soluble polymers to adhere to biological membranes, including buccal, ocular, rectal, and nasal surfaces. This adhesion prolongs the residence time of the drug at the application site, allowing closer contact with the absorption surface and enhancing therapeutic efficacy.

2. Magnetic Microsphere: Magnetic microspheres are small, spherical particles, typically ranging from 1–1000 μm, made from proteins or synthetic polymers that are biodegradable. They offer a controlled and targeted method for delivering therapeutic agents to specific sites

3.Radioactive Microsphere: Radioactive microspheres are designed for radioembolization therapy. Typically, larger than capillary diameters (10–30 μm), they are introduced into the arteries feeding a tumour,

delivering targeted radiation while sparing surrounding healthy tissues. Unlike conventional drug delivery, these microspheres do not release medication but exert their effect through radiation. Radioactive microspheres can be classified based on the type of emission: alpha (α), beta (β), and gamma (γ) emitters.

4. Floating Microspheres: Floating microspheres have a lower density than gastric fluids, enabling them to remain buoyant in the stomach without altering gastric emptying. This prolonged gastric residence allows gradual drug release at the desired rate, maintaining steady plasma concentrations and reducing the risk of dose dumping. Floating microspheres also enhance patient compliance by providing sustained therapeutic effects and reducing dosing frequency.

5. Polymeric Microspheres

Polymeric microspheres are classified into biodegradable and synthetic types:

Biodegradable Polymeric Microspheres: These microspheres are biocompatible, bio adhesive, and often composed of natural polymers like starch. Their strong swelling properties allow prolonged contact with mucosal surfaces.

Synthetic Polymeric Microspheres: These microspheres are generally safe and biocompatible and are used as drug carriers, fillers, embolic agents, and bulking materials.[6]

- **Advantages of Colon-Targeted Microspheres**

1. Site-specific drug delivery
2. Reduced side effects
3. Improved drug stability
4. Controlled and sustained drug release
5. Enhanced bioavailability
6. Lower dosing frequency
7. Effective treatment of colonic diseases
8. Protection of sensitive drugs from degradation
9. Improved patient compliance[11]

- **Disadvantages of Colon-Targeted Microspheres**

1. Complex formulation process
2. High manufacturing cost
3. Variable drug release
4. Difficult scale-up for manufacturing
5. Low drug loading capacity
6. Stability problems during storage

7. Risk of dose dumping
8. Dependence on colonic conditions
9. Limited suitability for certain drugs[12]

- **Applications of Colon-Targeted Microspheres**

1. Treatment of inflammatory bowel diseases (IBD)
2. Management of colorectal cancer
3. Treatment of amoebiasis and colonic infections
4. Delivery of peptides and proteins
5. Treatment of irritable bowel syndrome (IBS)
6. Chronotherapy
7. Sustained and controlled drug release[13]

II. DRUG PROFILE

Metronidazole

- Drug category: Antiprotozoal and antibacterial
- Molecular formula: $C_6H_9N_3O_3$
- Molecular weight: 171.15 g/mol
- Appearance: White crystalline powder
- Melting point: 159–163°C
- Half-life: 6–8 hours
- BCS Class: I
- Dose: 500 mg every 8 hours
- Role: Active drug used for colon-targeted delivery.

Chitosan

- Polymer used as an encapsulating agent
- Molecular formula: $(C_6H_{11}NO_4)$□
- Appearance: Off-white powder
- Molecular weight: 10,000–1,000,000 g/mol
- Melting point: 220–230°C
- pKa: 6.2–6.5
- Dose: 0.5–5% w/v
- Role: Forms microspheres and controls drug release

III. MATERIALS, METHODS AND EQUIPMENTS

Materials:

- Metronidazole – Active Pharmaceutical Ingredient (API)
- Chitosan – Polymer
- Sodium Alginate – Encapsulating agent
- Calcium Chloride – Cross-linking agent
- Acetic Acid – Solvent
- Distilled Water – Solvent

Equipments:

- Magnetic Stirrer – Continuous stirring.
- Hot Plate – Heating the formulation.
- Mechanical Stirrer – Uniform mixing.
- Syringe with Needle – Forming microspheres.
- Beaker – Mixing solutions.
- Analytical Balance – Accurate weighing.
- FTIR Spectrophotometer – Drug–polymer compatibility study.
- Hot Air Oven – Drying microspheres.
- Petri Dish – Drying and storage.
- pH Meter – Measuring pH.[10]

Method of Preparation:

Formulation of Metronidazole Microspheres by Ionic Gelation Method

Procedure:

The Ionic Gelation Method is a simple and widely used technique for preparing drug-loaded microspheres, especially using natural polymers like Sodium alginate. In this method, polymer droplets react with Multivalent ions such as Calcium chloride to form gelled microspheres. The method is based on ionic cross-linking between negatively charged polymer molecules and positively Charged calcium ions. When sodium alginate solution containing drug is added into calcium chloride solution, Calcium ions replace sodium ions and form calcium alginate gel microspheres.[9]

Figure 2: Ion Gelation Method

Figure 3: Microspheres

Step 1: Prepare alginate-drug solution

1. Dissolve 1.5 g sodium alginate in 75 mL distilled water. Stir 4-6 hrs until clear, no lumps. Let sit overnight To degas.

2. Dissolve 250 mg metronidazole in 10 mL warm water -40°C.

3. Mix metronidazole solution into alginate solution. Stir 30 min. Final volume ~85 mL = ~1.75% w/v alginate.

Step 2: Prepare crosslinking + coating solutions

1. CaCl2 bath: 2 g CaCl2 in 100 mL water = 2% w/v. Put in 250 mL beaker with magnetic stirrer.

2. Chitosan solution: 0.5 g chitosan in 100 mL 1% acetic acid. Stir overnight, filter. Final = 0.5% w/v, pH 4.8-5.2. Adjust with 0.1N NaOH if needed.

Step 3: Form calcium alginate beads

1. Fill 10 mL syringe with alginate-metronidazole solution. Use 24G-26G needle.

2. Drop into CaCl2 bath from 6-8 cm height while stirring bath at 200 rpm.

3. Beads form instantly. Cure for 15 min in CaCl2.

4. Decant CaCl2, wash beads 2x with distilled water. You now have uncoated metronidazole-alginate beads.

Step 4: Chitosan coating

1. Transfer washed beads into chitosan solution.

2. Stir gently 100 rpm for 30 min. Chitosan binds to surface COO- groups of alginates.

3. Filter or decant. Wash beads 1x with water to remove excess chitosan.

Step 5: Drying

1. Air drying: Spread on filter paper, 25°C, 24 hrs. Gives shrunken, dense beads -300-800 µm.[7]

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IV. RESULTS

- **Organoleptic Properties**

The prepared metronidazole-loaded colon-targeted microspheres were off-white, odourless, spherical, free-flowing, and non-sticky with a smooth surface. These characteristics indicate good physical quality, uniformity, and stability of the formulation.

1. Solubility Study

Metronidazole was found to be slightly soluble in water and soluble in methanol, ethanol, and phosphate buffer (pH 7.4). The solubility profile supports effective formulation and controlled drug release.

2. Determination of Melting Point

The melting point of metronidazole was found to be 159–163°C, which is in agreement with the reported standard value, confirming the purity and identity of the drug.

- **Standard Calibration Curve of Metronidazole**

The standard calibration curve of metronidazole showed a linear relationship between concentration (2–12 µg/mL) and absorbance at 320 nm. This confirms compliance with Beer–Lambert's law and indicates that the method is suitable for accurate drug estimation.[8]

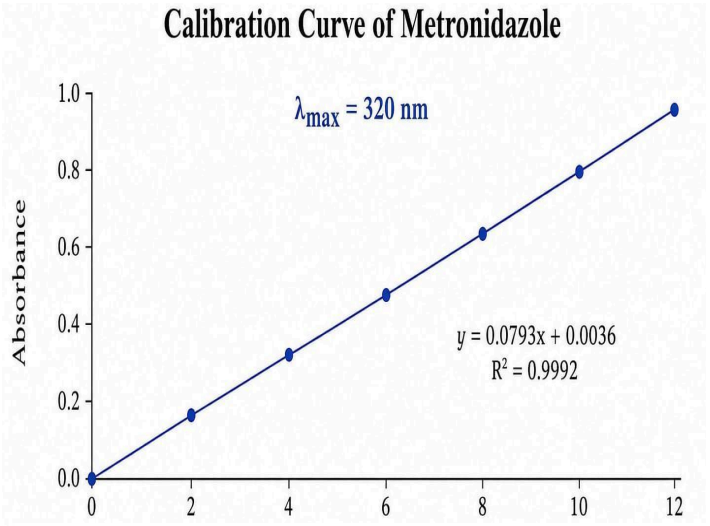

Figure 4: Standard Calibration Curve of Metronidazole

Fourier Transform Infrared Spectroscopy (FTIR)

Pure Metronidazole

The FTIR spectrum Metronidazole showed characteristics peaks at

- 3929–3383 cm^{-1}---O–H stretching---Hydroxyl group
- 3212 cm^{-1}---N–H stretching---Imidazole ring
- 3098 cm^{-1}---=C–H stretching---Aromatic/heterocyclic ring
- 2953, 2922 & 2868 cm^{-1}---C–H stretching---Aliphatic CH stretching
- 1808 cm^{-1}---C=O stretching---Carbonyl group
- 1688 cm^{-1}---C=N stretching---Imidazole ring

These peaks confirm the presence of functional groups characteristic of Metronidazole.

chitosan exhibited characteristic peaks corresponding to hydroxyl, amino, carbonyl, and amide groups, confirming the identity and purity of the polymer. No significant changes were observed, indicating good compatibility with the drug.

Pure chitosan

The FTIR spectrum Chitosan showed characteristics peaks at

- 3834 & 3726 cm^{-1}---Free O–H stretching--- hydroxyl groups
- 3452 cm^{-1}---O–H and N–H stretching---amino groups
- 2859 cm^{-1}---C–H stretching---Aliphatic CH stretching
- 1800 & 1739 cm^{-1}---C=O stretching---Carbonyl group
- 1688 cm^{-1}---Amide I band---N-acetyl groups
- 1627 cm^{-1}---N–H bending--- amino group
- 1586 cm^{-1}---Amide II band---N–H bending

These peaks confirm the presence of functional groups characteristic of Chitosan.

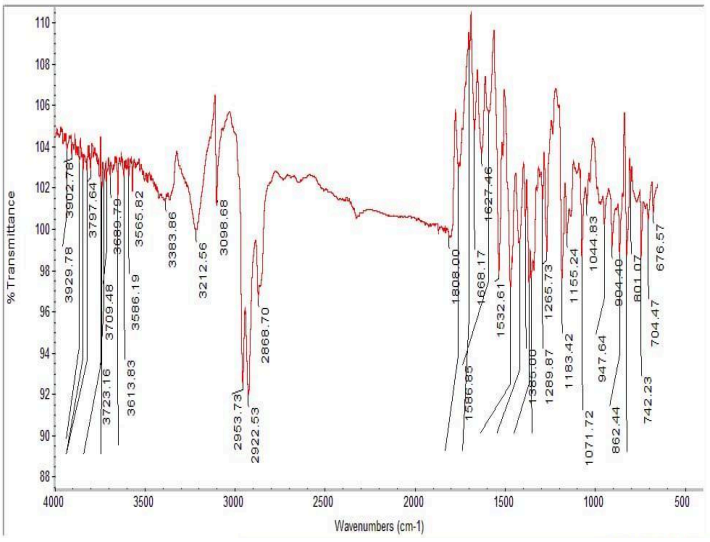

Figure 5: IR Spectra of Pure Drug Metronidazole.

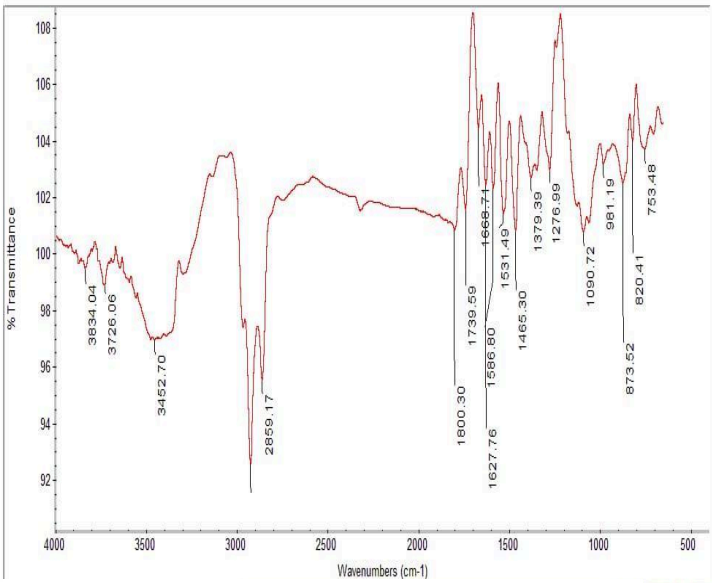

Figure 6: IR Spectra of Pure Chitosan

- **Micromeritic Properties of Microspheres**

The prepared metronidazole-loaded alginate-chitosan microspheres showed good micromeritic properties suitable for formulation and capsule filling.

1. **Particle Size:** Ranged from 742 ± 18 µm to 912 ± 24 µm with a uniform size distribution. Larger particles showed higher drug entrapment and slower drug release.
2. **Angle of Repose:** 24°–29°, indicating good to excellent flowability. The optimized batch (F3) showed the best flow (24.6° ± 0.8°).
3. **Bulk & Tapped Density:** Bulk density ranged from 0.42–0.51 g/cm³, while tapped density ranged from 0.49–0.59 g/cm³, indicating a porous structure suitable for capsule filling.
4. **Carr's Index & Hausner's Ratio:** Carr's index (12.4–16.1%) and Hausner's ratio (1.13–1.19) confirmed good compressibility and flow properties. Batch F3 showed the best values.
5. **Porosity & True Density:** Porosity ranged from 42–48%, and true density from 0.81–0.92 g/cm³, indicating a lightweight, porous structure that supports swelling and controlled drug release.

- **Physicochemical Evaluation**

1. **Drug Entrapment Efficiency:** Drug entrapment efficiency ranged from 68.4% to 84.7%, with higher polymer concentration improving drug entrapment. Drug content was uniform, confirming good reproducibility of the formulation.
2. **Surface Morphology and Shape:** The microspheres were spherical, discrete, and smooth, with a uniform chitosan coating. Their shape supports good flow properties and controlled drug release.

3. **Percentage Yield:** The percentage yield ranged from 68.5% to 82.3% and increased with higher polymer concentration, indicating efficient microsphere preparation by the ion gelation method.
4. **Swelling Index:** The swelling index increased with increasing polymer concentration. The microspheres showed pH-dependent swelling, with minimal swelling in acidic medium and higher swelling in phosphate buffer, demonstrating their suitability for colon-targeted controlled drug release.
5. **In-vitro Drug Release Studies:** The prepared microspheres showed minimal drug release in gastric fluid, limited release in intestinal fluid, and maximum sustained drug release in colonic fluid (pH 7.4). The optimized formulation (F3) released 96.4% of the drug within 12 hours, demonstrating effective colon-targeted and controlled drug delivery.
6. **Release in Intestinal Fluid pH 6.8:** The microspheres released only 28–35% of the drug in intestinal fluid over 5 hours, indicating good protection against premature drug release before reaching the colon.
7. **Release in Colonic Fluid pH 7.4:** A significant increase in drug release was observed in colonic fluid, with the optimized batch (F3) releasing 96.4% of the drug in 12 hours. This confirms enzyme-triggered and sustained drug release in the colon.
8. **Colon Targeting Efficiency:** The optimized formulation showed a colon targeting efficiency of 68.8% with minimal drug release in the stomach, confirming successful and efficient colon-specific drug delivery.

V. CONCLUSION

The present study concludes that colon-targeted microspheres are an effective and promising drug delivery system for delivering Metronidazole directly to the colon. Microsphere-based delivery systems successfully overcome the limitations associated with conventional oral dosage forms by protecting the drug from premature release and degradation in the upper gastrointestinal tract. Colon-targeted delivery improves local drug concentration at the site of infection or inflammation while minimizing systemic exposure and adverse effects.

The use of natural polymers such as sodium alginate, chitosan, guar gum, and pectin along with synthetic polymers like Eudragit and ethyl cellulose provides controlled, sustained, and site-specific drug release. Microspheres offer several advantages including improved bioavailability, prolonged drug release, reduced dosing frequency, better patient compliance, and enhanced therapeutic efficacy in the treatment of colonic diseases. Evaluation parameters such as particle size, swelling index, entrapment efficiency, morphology, and in vitro drug release studies confirmed the suitability of microspheres as colon-targeted carriers.

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