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Formulation and Evaluation of Tinidazole Calcium Alginate Beads for Peptic Ulcers by Using the Emulsion Gelation Technique

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Abstract

Introduction: *Peptic ulcer is a persistent ailment impacting approximately 10% of the worldwide populace, primarily caused by Helicobacter pylori (H. pylori) infection. This research aims to develop and assess Tinidazole-loaded calcium alginate beads through the emulsion gelation method to improve drug delivery for sustained release in the stomach.*

Research Methodology: *Tinidazole-loaded calcium alginate beads were prepared using the emulsion gelation technique. These beads underwent thorough evaluation of their physical and chemical properties, as well as drug release behavior under simulated gastric conditions.*

Results and Discussion: *Tinidazole-loaded beads demonstrated effective gastro-retentive properties, maintaining therapeutic drug levels at the ulcer site with reduced dosing frequency.*

This delivery system shows potential for improved treatment of peptic ulcers, enhancing patient outcomes and compliance.

Keywords: Tinidazole, Calcium Alginate Beads, Peptic Ulcers, Helicobacter pylori

Introduction

Peptic ulcer disease affects up to 10% of the world's population, primarily causing lesions in the inner lining of the gastrointestinal tract, especially in the duodenum and stomach. While traditional treatments aimed to neutralize stomach acid, recent research identifies Helicobacter pylori (H. pylori) infection as a primary cause. This study investigates the use of Tinidazole-loaded calcium alginate beads created through the emulsion gelation method to improve gastro-retentive drug delivery for treating peptic ulcers [1-3].

Peptic ulcer disease, affecting up to 10% of the global population, has a prevalence rate of 11.22% and affects about 4.72% of individuals. It commonly manifests in men as duodenal and gastric ulcers with an equal ratio between the two types. Prevalence decreases with age, peaking at 28.8% in individuals in their fifties. Mortality rates from peptic ulcers have decreased to about 1 death per 100,000 patients, with a fatality rate of approximately 5% for ulcer hemorrhage in duodenal ulcer patients. Excess stomach acid or pepsin leads to the development of peptic ulcers, which penetrate through the gastric epithelium's muscularis propria layer, affecting the proximal duodenum and stomach, and occasionally the distal duodenum, lower esophagus, or jejunum. Stomach ulcers typically cause epigastric pain 15-30 minutes after eating, whereas duodenal ulcers cause discomfort 2-3 hours post-meal [1, 4].

In recent years, calcium-induced alginate gel beads have emerged as an innovative drug delivery system. These beads rapidly form when alginic acid reacts with calcium ions, creating a gel matrix that can incorporate drugs and polysaccharides. They have been used in the gastrointestinal tract for sustained

drug release and bile acid adsorption, with research focusing on improving gastro retention to control drug release and facilitate site-specific delivery.

Calcium alginate beads are produced by the reaction between alginic acid and calcium ions, forming a gel matrix that can incorporate drugs. These beads offer sustained drug release, enhanced bioavailability, and reduced dosing frequency, leading to better patient compliance. Tinidazole, chosen for its effectiveness against H. pylori and its extended-release capabilities, is evaluated for its physical and chemical properties, drug release profiles, and in vitro performance in simulated gastric conditions [5-7].

The findings suggest that Tinidazole-loaded calcium alginate beads are a promising gastro-retentive drug delivery system, maintaining therapeutic drug levels at the ulcer site while reducing systemic exposure. This innovative approach could significantly improve peptic ulcer treatment, enhancing patient outcomes and adherence to therapy [8, 9].

Material & Methods

Tinidazole I.P purchase from Sai Mirra Inno Pharm Private Limited India, and Castor Oil Rama Chemicals, Saharanpur U.P India, HCl purchase from Nice chemicals Pvt. Ltd. Kochi Kerala, India. Black seed oil purchase online Indus Cosmeceuticals Shimla, H.P. India, Sodium Alginate purchase from Nice Chemicals Pvt. Ltd. Ernakulam Kerala, India.

Preformulation: Tinidazole, the subject of this research, was accurately identified and characterized according to the identification tests prescribed in the official monograph. Its physical properties and melting point were consistent with those noted in official records. Further validation of Tinidazole's identity was achieved through infrared (IR) spectroscopy, which displayed characteristic peaks that matched those in the standard spectra detailed in the official monograph [10].

Preparation of Calcium Alginate Beads Loaded with Tinidazole

Tinidazole-loaded calcium alginate beads were created using the emulsion gelation technique. Initially, a pre-gelation mixture was prepared in distilled, demineralized water with constant stirring, containing sodium alginate at concentrations of 1%, 2%, and 3% w/w. Black seed oil or castor oil was then added to the polymer solution at concentrations of 10%, 20%, and 30% w/w, forming 100-g mixtures. Tinidazole was then added to the emulsion at different drug-to-polymer ratios (1:1, 2:1, and 3:1 w/w). To stabilize the emulsion, the mixtures were homogenized at 10,000 rpm for 10 minutes using a homogenizer. The bubble-free emulsion was then extruded through a 23G syringe needle into 250 ml of a gently stirred calcium chloride solution (1%, 2%, and 3% w/w) at room temperature. After a 20-minute incubation, the emulsion gel beads were collected, washed three times with 100 ml of distilled water, and air-dried at room temperature [11]. Step by step procedure illustrated in flow chart Figure 1.

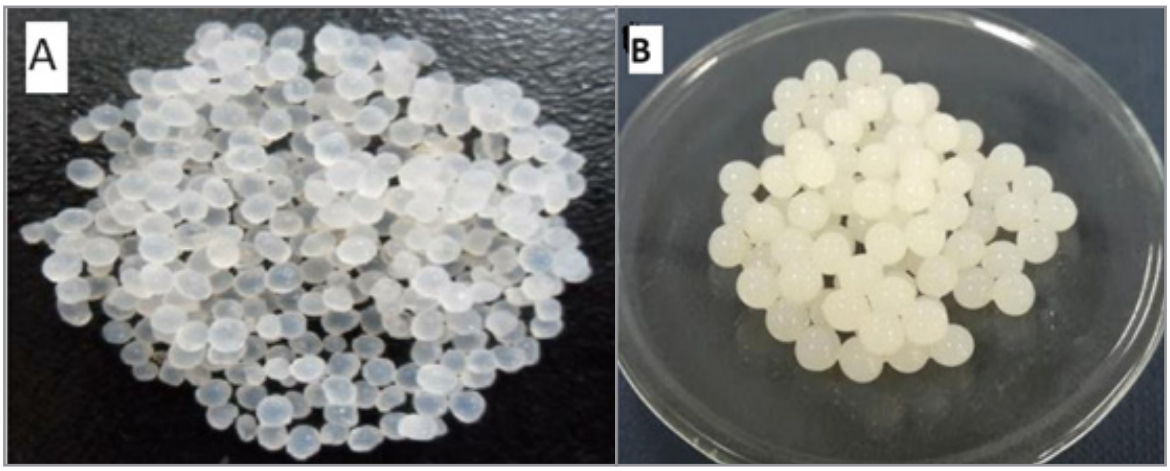

Figure 5.2: Representative Digital Photographs of Tinidazole-containing Alginate Beads: (a) Before Drying; (b) After Drying.

Preparation of pre-gelation mixture in distilled demineralized water

Add sodium alginate 1%, 2%, and 3% w/w concentrations

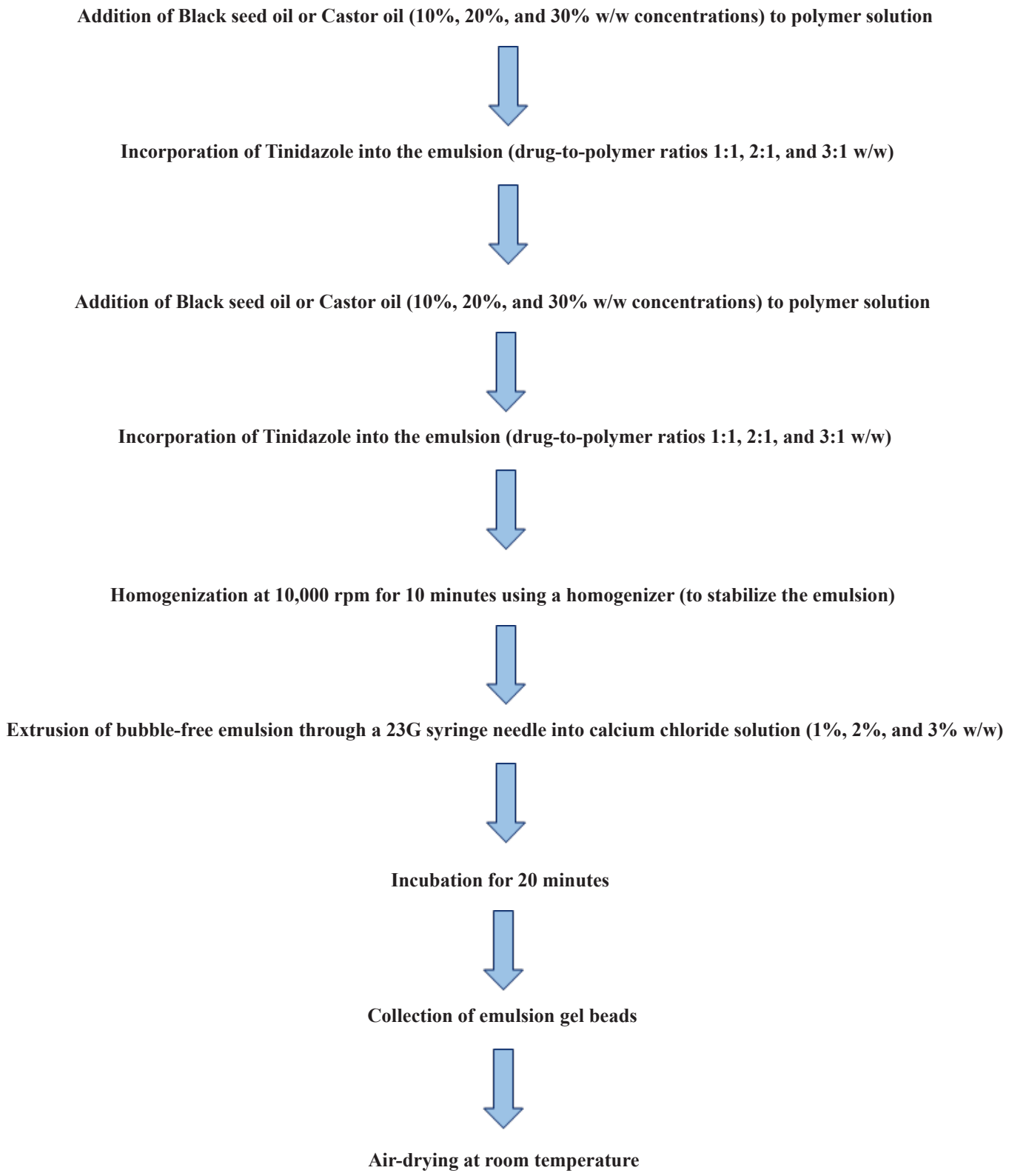

Figure 1: Flowchart for Formulation of Tinidazole Calcium Alginate Beads

Preliminary Investigations for Enhancing Floating Hydrogel Beads

This study focused on identifying three primary factors to enhance floating hydrogel beads: the concentration of sodium alginate (1% to 4%), the concentration of calcium chloride used as a cross-linking agent (1% to 4%), and the oil content percentage. These were treated as independent variables. The dependent variables considered during the optimization of these factors included bead size, morphology, buoyancy, drug encapsulation efficiency, density, flow properties, and the prevention of oil leakage. These dependent variables were crucial for refining the process parameters (independent variables) in the production of floating calcium alginate beads [11].

Table 1: Optimization of Calcium Chloride ratio and Sodium Alginate

S. No	Formulation Code	Calcium chloride	Sodium alginate
1.	F 1	1	1
2.	F 2	2	1
3.	F 3	3	1
4.	F 4	4	1
5.	F 5	1	2
6.	F 6	2	2
7.	F 7	3	2
8.	F 8	4	2
9.	F 9	1	3
10.	F 10	2	3
11.	F 11	3	3
12.	F 12	4	3
13.	F 13	1	4
14.	F 14	2	4
15.	F 15	3	4
16.	F 16	4	4

*Black seed oil concentration remains constant (20%) in above formulation

Evaluation and Characterization of Tinidazole Loaded Alginate Beads

Analysis of Bead Homogeneity and Uniformity

To produce beads with uniform size and density, it is essential to maintain consistent synthesis parameters such as viscosity, drop falling rate, stirring speed, and the distance between the syringe and medium throughout the bead formation process. Deviations in these factors can result in irregular and non-uniform beads, which can significantly impact the final results. Emulsion homogenization is crucial for achieving uniform bead size by ensuring a fine dispersion of oil and water. Inadequate homogenization can cause oil to separate from the solution, leading to uneven bead sizes. To assess product uniformity, the diameters of 20 dried calcium alginate beads were measured with a caliper, and the results were reported as the mean diameter (mm) ± standard deviation. [11].

Determination of Density

The average weight and diameter of the beads were measured to determine the densities of the spherical calcium alginate beads using the formulas below:

$$\rho = \frac{m}{v}$$

$$v = 4/3\pi r^3$$

Where,

P = Density of the beads,

m = Weight of the beads,

v = Volume of the beads,

r = Radius of the beads.

Determination of Angle of Repose

The flow properties of the beads were evaluated by placing 1 gram of beads into a rotating cylinder within a laboratory fabricator. The cylinder was set to rotate at 25 rpm for 5 minutes, during which the angle formed by the bulk of the beads against the horizontal plane was measured. This dynamic angle of repose was then calculated. To ensure accuracy, measurements were taken in triplicate [12].

Beads Buoyancy Evaluation

The buoyancy of the beads was assessed using the United States Pharmacopeia dissolution test apparatus II with the paddle method. Fifty beads were placed into the vessels, and the paddles were rotated at 50 rpm in 500 ml of 0.1 N HCl adjusted to pH 1.2, maintained at 37 ± 0.5 °C. The ability of the beads to float was visually observed, and the percentage of floating beads was averaged from three trials. Buoyancy was confirmed if all beads floated immediately or within a lag time not exceeding 2 minutes [11].

Measurements of Drug Entrapment Efficiency (DEE)

To quantify the amount of Tinidazole encapsulated within the calcium alginate beads, 100 mg of Tinidazole-loaded beads were dissolved in 250 ml of 0.1 N HCl at pH 1.2 and stirred for 6 hours using a magnetic stirrer. The resulting solution was filtered through a 0.45 µm Millipore filter, and the Tinidazole content was measured spectrophotometrically at 317 nm [11, 13]. Measurements were conducted in triplicate, and the DEE was calculated using the formula:

$$\text{Percentage of DEE} = \frac{\text{Actual drug content}}{\text{Theoretical quantity of drug content}} X\ 100$$

Scanning Electron Microscopy (SEM)

A scanning electron microscope equipped with a secondary electron detector was utilized to examine the surface and internal structure of the dried calcium alginate beads. The microscope operated at an accelerating voltage of 10 kilo Volts, and samples were coated with a thin gold layer using a vacuum evaporator. The internal structure was further investigated by cutting the beads with a steel blade [14, 15].

In-vitro Tinidazole Release Studies

In vitro release studies were conducted using a United States Pharmacopeia rotating basket apparatus. Baskets containing beads equivalent to 250 mg of Tinidazole were rotated at 50 rpm in 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5 °C. At set intervals, 5 ml aliquots were withdrawn and replaced with fresh dissolution medium. The Tinidazole content in the samples was determined spectrophotometrically at 317 nm. No interference from the bead formulation ingredients was observed. Results represent the average of three experiments [16].

Stability Studies

Pharmaceutical dosage forms consist of active drug substances and additives that enhance product properties. These formulations are susceptible to chemical and physical degradation over time. Stability studies aim to optimize formulation and determine shelf life, ensuring the product remains effective and safe under specific storage and packaging conditions. Stability encompasses maintaining physical, chemical, microbiological, therapeutic, and toxicological properties throughout shelf life. Factors influencing stability include environmental conditions (temperature, humidity, light), properties of active substances and additives, dosage form composition, manufacturing processes, container closures, and packaging materials. Understanding stability is critical for patient safety, regulatory compliance, and preventing economic losses due to unstable products [17, 18].

Biodegradability Studies of Gel Beads

Biodegradability studies were performed using the USP rotating basket apparatus. Beads weighing 200 mg were placed in the baskets, which rotated at 50 rpm in 900 ml of buffer solutions with different pH levels (5.0, 6.8, 8.0) maintained at 37 ± 0.5 °C [11, 19].

Physical Characteristics

Parameters such as appearance, diameter, and floating time were assessed in simulated gastric fluid (pH 1.2) [20, 21].

Residual Drug Content (Assayed by U.V. Spectrophotometry)

A precise amount of 300 mg of tinidazole-loaded calcium alginate beads was dissolved in 300 ml of 0.1N HCl (pH 1.2) by stirring for 6 hours using a magnetic stirrer. The resulting solution was then filtered through a 0.45 µm Millipore filter. One milliliter of this solution was diluted to 100 ml with 0.1N HCl (pH 1.2) and analysed spectrophotometrically at the predetermined wavelength of 317 nm to determine the drug concentration. All measurements were performed in triplicate [22].

In-vitro Dissolution Studies in Different Time Period

The optimized formulations were stored in small glass bottles with screw caps at room temperature and in a stability, chamber set at 40±1ºC with 75% relative humidity. The samples were analysed for physical appearance, residual drug content, floating time, and in vitro release after 15, 30, 45, 60, 75, and 90 days. The initial drug content for each formulation was considered 100%.

Result and Discussion

Optimization of Sodium Alginate and Calcium Chloride Ratio

Table 2 illustrates that the Drug Entrapment Efficiency (DEE) increased with the sodium alginate concentration rising from 1% to 3% w/v. However, concentrations above 3% w/v caused the alginate solution's viscosity to increase so much that drop formation was significantly hindered. Therefore, a concentration of 3% w/v sodium alginate was used for all subsequent formulations. Further analysis (Table 2) indicated an unexpected decrease in drug loading as calcium chloride concentration increased from 1% to 4% w/v, with drug loading percentages of 61.88%±0.57, 53.35%± 0.82, 47.72%±1.08, and 38.15%±0.65, respectively. Consequently, a calcium chloride concentration of 1% w/v was maintained for all future formulations. The formula with 61.88%±0.57 drug loading, consisting of 3% w/v sodium alginate and 1% w/v calcium chloride, was chosen for further studies.

Table 1: Optimization of Calcium Chloride ratio and Sodium Alginate

S. No	Preparation Code	$CaCl_2$	$NaC_6H_7O_6$	Drug Entrapment Efficiency (mean ± SD)	Floating property (0.1N HCL)	Spherical Shape	Free flowing
1	F1	1	1	48.72%±1.56	> 12hr	yes	Yes
2	F2	2	1	42.63%±0.87	> 12hr	yes	Yes
3	F3	3	1	38.54%±1.30	> 12hr	yes	Yes
4	F4	4	1	30.12%±0.91	> 12hr	yes	Yes
5	F5	1	2	55.31%±1.23	> 12hr	yes	Yes
6	F6	2	2	40.72%±0.82	> 12hr	yes	Yes
7	F7	3	2	36.37%±1.09	> 12hr	yes	Yes
8	F8	4	2	31.27%±1.71	> 12hr	yes	Yes
9	F9	1	3	61.88%±0.57	> 12hr	yes	Yes
10	F10	2	3	53.35%±0.82	> 12hr	yes	Yes
11	F11	3	3	47.72%±1.08	>12hr	yes	Yes
12	F12	4	3	38.15%±0.65	> 12hr	yes	Yes
13	F13	1	4	Sticky beads	> 12hr	No	No
14	F14	2	4	Sticky beads	> 12hr	No	No
15	F15	3	4	Sticky beads	>12hr	No	No
16	F16	4	4	Sticky beads	> 12hr	No	No

*Black seed oil concentration remains constant (20%) in above formulation

Optimization of Oil and Its Concentration

The creation of calcium alginate gel beads using various oils is a straightforward and quick process. The oil was incorporated into the drug-alginate solution both with and without homogenization. Without homogenization, the oil began to separate, resulting in beads of uneven sizes. Increasing the homogenization time led to smaller and more uniformly sized beads. While the drug and polymer concentrations were kept constant throughout the study, the concentrations of Black seed oil and castor oil were varied, as shown in Table 3. The mean diameters of the oil-entrapped formulations were 1.79 mm (10% Black seed oil), 1.99 mm (20% Black seed oil), 2.21 mm (30% Black seed oil), 1.96 mm (10% castor oil), 2.19 mm (20% castor oil), and 2.40 mm (30% castor oil). Higher oil concentrations resulted in larger and more spherical beads, likely due to differences in density and volatility. The formula with 20% Black seed oil, which floated immediately without oil leakage, was chosen for further studies.

Table 3: Optimization of Oil and Its Concentration

S. No	Formulation Code	Oil concentration (%W/W)	Mean Diameter (mm± SD)	Density (g/cm3)	Drug Entrapment Efficiency (% ±SD)	Buoyancy	Oil leakage
1	F9Aa	10	1.79±0.21	0.1112	38.77%±1.82	S	No
2	F9Ba	20	1.99±0.20	0.1059	61.58%±0.77	S→F	No
3	F9Ca	30	2.21±0.22	0.0905	56.23%±1.89	F	Yes
4	F9Ab	10	1.96±0.18	0.1311	53.32%±1.07	S	No
5	F9Bb	20	2.19±0.23	0.1221	41.65%±0.78	S	Yes
6	F9Cb	30	2.40±0.18	0.0975	37.65%±1.98	F	Yes

*Drug Polymer Ratio (1:1) remain constant in all above formulation

Abbreviations: S = sink,
F = float (Start immediately, and continue for at least 24 hours),
S→F = Sink immediately and then gradually start float,
a = Black seed oil induced calcium alginate beads,
b = castor oil induced calcium alginate beads

Optimization of Drug and Polymer Ratio

Table 4 shows that the Drug Entrapment Efficiency increased from 49.89% to 93.27% as the Tinidazole to sodium alginate ratio increased from 1:1 to 3:1. Given the statistically insignificant difference in DEE between the 2:1 and 3:1 Tinidazole to sodium alginate ratios, the 2:1 ratio was selected for all subsequent formulations. The formulation that showed 93.27% drug loading, containing 3% w/v sodium alginate and 1% w/v calcium chloride at a 2:1 drug to sodium alginate ratio, was chosen for further studies.

Table 4: Optimization of Drug and Polymer Ratio

S.No	Formulation Code	Drug: Polymer Ratio (%W/W)	Drug Entrapment Efficiency (% ±SD)	Mean Diameterm (mm ±SD)	Buoyancy
1	F9Ba1	1:1	49.89%±1.64	1.79±0.88	F
2	F9Ba2	2:1	93.27%±0.87	2.15±1.06	S-F
3	F9Ba3	3:1	89.75%±1.22	2.40±0.78	S-F

*Optimized oil (Black seed oil) 20% remain constant in all above formulation

Characterization of Tinidazole Loaded Alginate Beads

Study of Beads Homogeneity and Beads Uniformity

The average diameters of the Tinidazole-loaded calcium alginate beads, have low standard deviation values, indicated high process uniformity and low variability in processing conditions. The mean diameter of the calcium alginate beads with Black seed oil ranged from 1.79 to 2.21 mm, while those with castor oil were slightly larger, ranging from 1.96 to 2.40 mm. Regardless of the oil used, increasing both the oil concentration and the drug-to-polymer (D) Ratio led to larger bead sizes, likely due to increased droplet viscosity from higher oil content or drug concentrations. Lower viscosity droplets were more efficiently stirred, reducing emulsion droplet size and resulting in smaller beads. Visual inspection confirmed that the dried calcium alginate beads were spherical.

Density Measurement

The diameter and weight of the calcium alginate beads were measured to calculate their density. As shown in Table 3, all the prepared beads had densities lower than 0.1 N HCl (1.004 g/cm³), allowing them to float. The densities ranged from 0.0905 to 0.1112 g/cm³ for beads containing Black seed oil, and from 0.0975 to 0.1311 g/cm³ for beads containing castor oil. It was observed that formulations with Black seed oil had lower density values compared to those with castor oil.

Determination of Dynamic Angle of Repose

The dynamic angle of repose was measured using a lab-fabricated rotating cylinder. The flow properties of optimized gel beads are reported in the table 5. The dynamic angle of repose, which closely mimics manufacturing conditions, showed good flow properties with an angle of repose less than 30°. The flow properties of gel beads can be significantly affected by changes in bead size, density, shape, and adsorbed moisture, which may arise from processing or formulation. Therefore, proper storage is recommended to maintain these properties [23].

Table 5: Dynamic Angle of Repose Result

S.No	Parameters	F9B2 (Optimized Batch) (mean ±SD)
1	Dynamic Angle of Repose	280±10

Determination of the Bead's Buoyancy

Table 3 illustrates the effect of oil content on the buoyancy of alginate beads. Beads without oil failed the buoyancy test, with many sinking immediately upon contact with simulated gastric fluid (SGF) or shortly after agitation began. In contrast, all oil-containing samples remained buoyant for the entire 24-hour test period, indicating that air bubbles within the alginate gel matrix were neither reliable nor consistent for buoyancy. The table also shows that the buoyancy of drug-loaded beads decreased for beads with less oil or higher drug content. When Tinidazole was added to traditional calcium alginate beads, those with 10% Black seed oil or 20% castor oil did not float immediately, likely due to increased bead density. However, they floated after the drug was released, suggesting a need for more oil to maintain flotation. Tinidazole-loaded calcium alginate beads with 20% Black seed oil or 30% castor oil floated immediately in SGF. The formulation with 20% Black seed oil, which floated immediately without oil leakage, was chosen for further studies [24].

Determination of Drug Entrapment Efficiency (DEE)

Table 3, shows that increasing the sodium alginate concentration from 1% to 3% w/v improved DEE. Concentrations above 3% w/v increased the viscosity of the alginate solution to the point where drop formation was significantly hindered, so 3% w/v sodium alginate was used in all subsequent formulations. Further analysis revealed that increasing the calcium chloride concentration from 1% to 4% w/v unexpectedly decreased DEE in the beads (61.88%, 53.35%, 47.72%, and 38.15%, respectively). Consequently, a 1% w/v calcium chloride concentration was maintained in all future formulations. Table 6.2 indicates that for calcium alginate beads, keeping the drug-to-polymer (D) ratio constant, DEE reached its maximum by increasing oil concentration up to 20%. The DEE of calcium alginate beads with 20% Black seed oil ranged from 49.89% to 93.27%, as shown in Table 4. Further analysis indicated that at a given oil concentration (20%), increasing the initial drug loading significantly increased DEE up to a 2:1 D ratio. A higher D ratio (3:1) did not significantly affect DEE. These findings suggest that a 2:1 D ratio with 20% Black seed oil concentration in calcium alginate beads provides optimal DEE.

Scanning Electron Microscopy (SEM)

SEM was used to analyze the external and cross-sectional structures of calcium alginate beads containing 20% Black seed oil with a 2:1 D ratio. The external surface of these spherical beads appeared rough, independent of the polymer type, as depicted in Figure 2 (A and B). This rough surface featured irregular clusters of Tinidazole crystals, likely formed through water migration and subsequent leaching during drying and shrinkage. Cross-sectional examination revealed a sponge-like structure resembling an egg crate, where oil droplets were trapped. Numerous oil globules were found embedded in the matrix pores of the beads, which characterize their buoyant properties as floating calcium alginate beads.

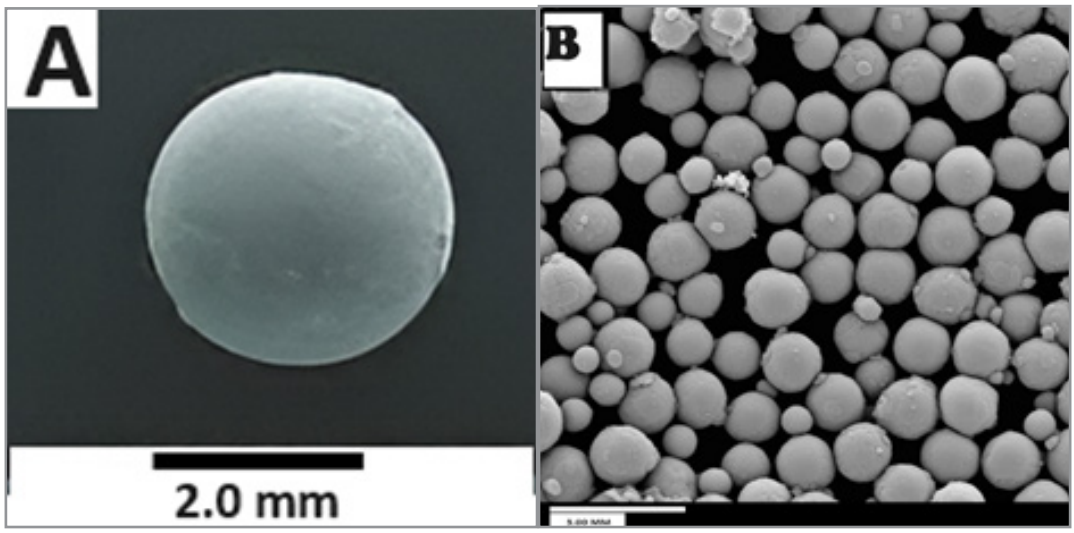

Figure 2: Scanning Electron Micrographs of Optimized Formulation.

In-Vitro Tinidazole Release Studies

The release profiles of Tinidazole-loaded calcium alginate beads are shown in Table 6. The release of Tinidazole from these beads was significantly slower compared to the dissolution of a marketed conventional tablet, which completed within 2 hours. The cumulative drug release from formulations (F9Aa, F9Ba, F9Ca, F9Ab, F9Bb, F9Cb, F9B1, F9B2, and F9B3) was 83.03%, 80.95%, 76.21%, 79.88%, 74.92%, 71.77%, 82.05%, 88.93%, and 82.75% respectively, over 8 hours. Notably, beads containing Black seed oil exhibited a faster drug release compared to those with castor oil, which showed a slower release likely due to the higher specific gravity and viscosity of castor oil and

greater drug partitioning into castor oil compared to Black seed oil. Increasing the oil concentration led to a decrease in the Tinidazole release rate. This can be explained by the hypothesis that Tinidazole, being insoluble in mineral oil but freely soluble in 0.1 N HCL, diffuses easily out of the beads, increasing the rate of penetration into the beads. Therefore, for basic Tinidazole, the hydrophobic barrier created by oil inclusion was crucial for slowing drug release. This effect was compounded by the compact and dense nature of the calcium alginate beads.

The results indicated that formulations F9Ca, F9Bb, and F9Cb had issues with oil leakage, low drug entrapment efficiency, and reduced drug release. Formulations F9Aa, F9Ba, and F9Ab experienced sinking (non-floating) problems, low DEE, and less drug release, while F9B1 and F9B3 had good floating properties but showed lower DEE and drug release. The F9B2 formulation was selected as the optimized batch because it exhibited the highest drug release percentage, excellent floating ability, and no oil leakage.

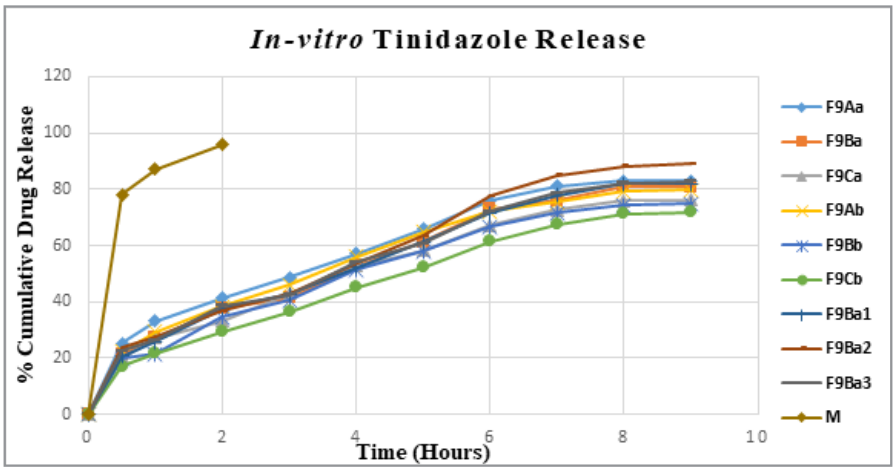

Figure 3: Drug Release Profiles of Formulations in Simulated Gastric Fluid (Mean± SD, n = 3).

Table 6: In-Vitro Tinidazole Release Studies:

F.code. /Time (hr)	F9Aa	F9Ba	F9Ca	F9Ab	F9Bb	F9Cb	F9Ba1	F9Ba2	F9Ba3	M
0.5	25.23±1.34	21.07±1.78	20.11±1.25	22.24±0.71	19.84±0.57	17.07±0.68	20.62±1.42	23.67±0.88	22.45±0.43	78.057±0.62
1	33.17±0.87	27.14±1.22	27.21±1.62	29.32±0.92	21.32±0.45	21.78±0.91	25.84±0.63	27.55±0.65	26.25±1.31	87.22±0.88
2	41.25±0.62	37.86±0.85	33.17±0.45	38.23±0.88	34.42±1.53	29.31±0.73	37.50±0.45	36.748±0.92	38.54±0.45	95.87±0.92
3	48.78±0.86	41.45±0.48	42.87±0.67	45.93±1.55	40.52±0.83	36.45±0.88	42.83±1.38	42.75±0.91	42.52±0.31	
4	57.21±1.17	52.88±1.71	52.77±1.35	55.97±0.82	51.56±0.71	45.21±0.58	52.16±1.22	53.77±1.42	54.26±1.25	
5	65.81±0.55	61.13±0.85	58.22±0.86	64.67±0.54	57.89±1.21	52.24±0.85	61.14±0.85	63.38±0.75	60.72±1.43	
6	75.92±0.62	72.98±0.76	67.15±0.39	72.17±1.34	66.82±0.65	61.58±1.21	71.73±1.28	77.67±1.05	72.17±0.84	
7	81.06±0.78	76.03±1.35	72.56±0.86	75.44±0.88	71.64±0.82	67.54±0.67	77.93±1.25	84.92±0.32	78.56±0.68	
8	82.95±0.92	80.78±0.64	75.85±1.21	79.21±0.67	74.31±0.94	71.32±0.36	81.88±1.67	88.32±0.86	82.32±1.12	
9	83.03±0.54	80.95±0.32	76.21±0.51	79.88±0.78	74.92±0.82	71.77±0.76	82.05±0.81	88.93±0.92	82.75±0.82	

- F9Aa (10%Black seed oil, 1:1 D: P ratio)
- F9Cb (30%castor oil, 1:1 D: P ratio)
- F9Ba (20%Black seed oil, 1:1 D: P ratio)
- F9Ba 1(20%Black seed oil, 1:1 D: P ratio)
- M (marketed formulation)
- F9Ca (30%Black seed oil, 1:1 D: P ratio)
- F9Ba2 (20%Black seed oil, 2:1 D: P ratio)
- F9Bb (20%castor oil, 1:1 D: P ratio)
- F9Ab (10% castor oil, 1:1 D: P ratio)
- F9Ba3 (20%Black seed oil, 3:1 D: P ratio)

Biodegradability Studies of Gel Beads

Research on the biodegradability of alginate beads, specifically the F9B2 formulation, revealed that these beads disintegrated and dissolved within 3 hours at intestinal pH (Figure 4). The F9B2 beads fully biodegraded in intestinal fluid, with the pH of the medium playing a key role in their gradual dissolution. This process shows that once the beads pass through the stomach, they begin to erode and eventually degrade due to the intestinal conditions. As a result, alginate beads are suitable for gastro-retentive dosage forms, maintaining a rigid structure in gastric pH but completely degrading in phosphate buffer pH 8.0.

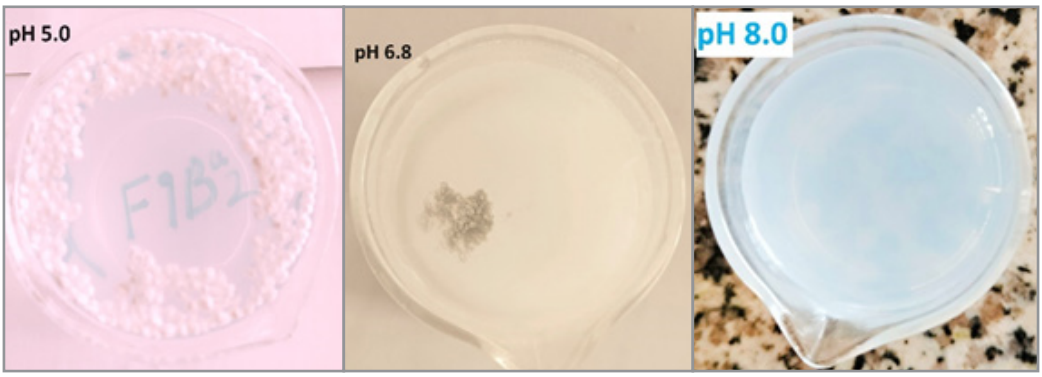

Figure 4: Images of complete biodegradation of F9B2 alginate beads after 3 hours

Physical Parameters of the Optimized Beads

The physical parameters recorded on the 15th, 30th, 60th, and 90th days are presented in the table. All physical parameters remained within acceptable limits, indicating that the formulation remained stable over the 90-day period.

Table 7: Effect of aging on Physical Parameters

S.No.	Physical Parameters	0 days	15thdays	30thdays	60thdays	90thdays
1	Appearance	+	+	+	+	+
2	Diameter(mm)	+	+	+	+	+
3	Floating time	+	+	+	+	+

+ No change,

Analysis of Residual Drug Content for Stability Study

The initial drug content of the formulations was 99.63%. The drug content at the end of the 15th, 30th, 60th, and 90th days is provided in the table 8. The drug content remained within permissible limits throughout the study period.

Table 8: Effect of aging on residual drug content at room temperature & 40±20C/ 75±5%RH

Sampling interval (days)	% Residual Drug Content Mean ± S.D. (n=3)	
	Mean ± S.D. (n=3)	At 40±20C/75±5% RH
0	99.62±0.07	99.62±0.07
15	99.38±0.11	99.15±0.09
30	98.92±0.13	98.73±0.12
45	98.22±0.06	98.12±0.07
60	97.93±0.15	97.45±0.08
75	97.54±0.08	97.06±0.09
90	97.13±0.12	96.31±0.10

In-vitro Dissolution Studies in Different Time Period

In-vitro dissolution studies were carried out using simulated gastric fluid (pH 1.2) as described earlier. The recorded observations are presented in Table 9.

Table 9: Effect of Aging on % Cumulative Drug Release at Room Temperature &40±20C/75±5%RH

Time Interval (Days)	% Cumulative Drug Release in 9 Hours ±SD	
	(Days)	40±20C/75±5% RH
0	88.93±0.92	88.93±0.92
15	86.83±0.74	85.67±0.72
30	84.77±0.61	83.95±0.68
60	82.26±1.54	80.54±1.45
90	80.97±0.93	78.88±0.67

The dissolution behavior of samples taken at different intervals remained consistent, with negligible differences in the dissolution patterns of samples stored under the two different conditions. The percentage of residual drug content was measured, and plotting the logarithm of the percent residual drug content against time (t) showed an almost linear relationship.

The log percentage of residual drug content verses time graph was also plotted in order to evaluate shelf-life and half-life of formulations. Shelf-life was evaluated by the following equation:

$T_{10\%} = 0.104/K$

Half-life was evaluated by the following equation:

$T_{1/2} = 0.693/K$

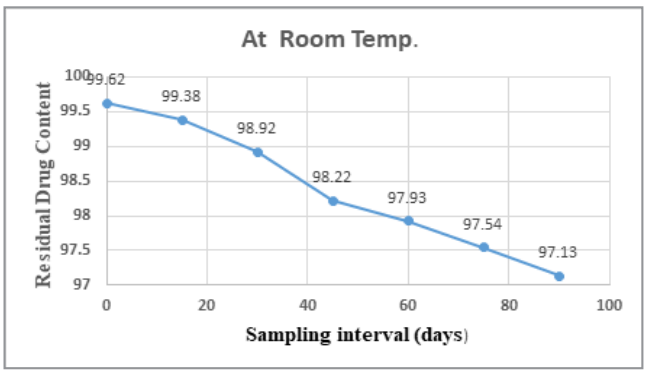

Figure 5: Effect of aging on Residual Drug Content at Room Temperature

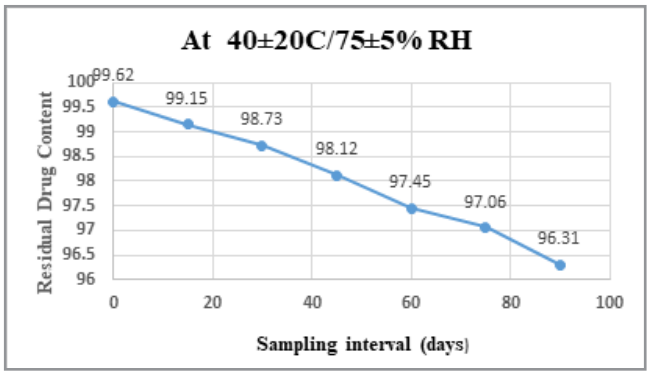

Figure 6: Effect of Aging on Residual Drug Content at 40±2ºC/75±5%RH

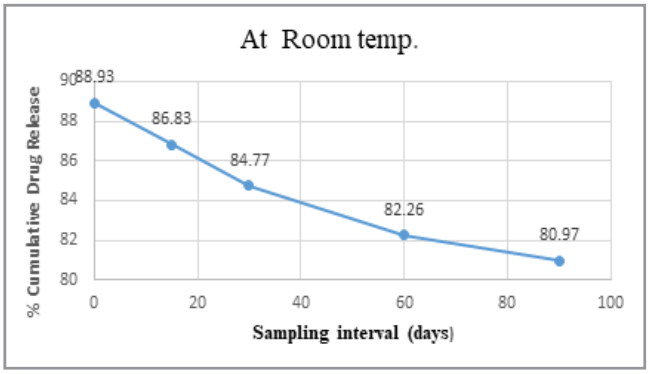

Figure 7: Effect of aging on % Cumulative Drug Release Vs time at Room Temp.

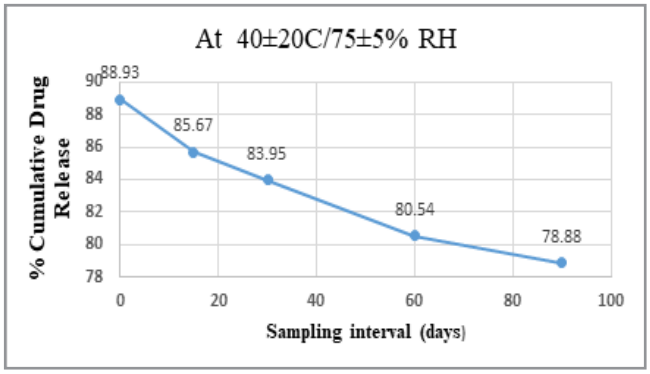

Figure 8: Effect of Aging on % Cumulative Drug Release Vs time at 40±2ºC/75±5%RH

Table 9: Shelf life of Optimized Formulation

S.No.	Storage condition	K (day-1)	T10% (days)	t1/2 (days)
1	40±2ºC/75±5%RH	3.831 x 10-4	274.17	184.67
2	Room temperature	2.312 x 10-4	448.62	3010.08

Conclusion

Tinidazole floating alginate beads were prepared using the emulsion gelation method. The study examined how process parameters influence bead size, flow properties, density, buoyancy, and drug entrapment efficiency. The developed system maintains a constant drug concentration over extended periods, reducing administration frequency and side effects, thus improving patient compliance. The optimized beads measured 1.79 to 2.40 mm, with entrapment efficiency between 49.89% and 93.27%, a dynamic angle of repose of 280±10, and bead density from 0.0905 to 0.1311 gm/cm³.

Stability studies involved storing the beads at room temperature and in a stability chamber (40±2ºC and 75±5% RH) for three months, showing no significant physical changes. The degradation rate constant (K) and time for 10% degradation (T10%) were calculated. Beads stored at higher temperatures/humidity degraded faster (T10% = 274.17 days) than those at room temperature (T10% = 448.62 days).

To ensure a long shelf life, the beads should be stored in cool, dry conditions. The study concludes that the sustained-release

floating alginate beads of Tinidazole, designed for gastro-retentive delivery, represent a significant advancement in treating H. pylori-related ulcers.

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- **Consent for Publication:** Not applicable.
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- **Data Availability:** The authors affirm that the data supporting the conclusions of this study are accessible within the article [and/or] its supplementary materials.
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