Heliyon 10 (2024) e29246

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Obliteration of *H. pylori* infection through the development of a novel thyme oil laden nanoporous gastric floating microsponge

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ARTICLE INFO

Keywords:
Microencapsulation
Stability
Thyme oil
Anti-microbial
H. pylori

ABSTRACT

Thyme oil (TO) is a valuable essential oil believed to possess a variety of bioactivities, including antibacterial, anticancer, and antioxidant properties. These attributes grant TO the excellent capability to treat a wide range of diseases, particularly the effective eradication of *Helicobacter pylori* infection in the stomach. However, its practical use is limited by its low stability under atmospheric conditions. Our current research aims to encapsulate TO in eudragit (EGT) microsponges to enhance its stability and improve its effectiveness against *H. pylori*. The TO microsponges were prepared using EGT as a polymer, polysorbate 80 as a stabilizer, and dichloromethane (DCM) as a solvent via the quasi-emulsion solvent evaporation method. The product yield, particle size, surface morphology, entrapment efficiency, drug-polymer interaction, in-vitro floating, and in-vitro drug release of the microsponges were evaluated. The most promising microsponge was tested against *H. pylori* ATCC 43504 strains. The results showed that the microsponges exhibited a high product yield (ranging from 41 % ± 0.75–81.27 % ± 1.13), excellent entrapment efficiency (ranging from 63.01 % ± 0.79–88.64 % ± 0.98), prolonged in-vitro floating time (more than 12 h) and sustained in-vitro drug release for 18 h (81.53 %). Scanning electron microscopy results indicated that the microsponges were spherical in shape with a spongy surface. The average particle size of the selected microsponges was determined to be 49.79 ± 1.4 μm, and their average pore size was measured to be 0.81 ± 0.14 μm. DSC study results revealed that TO was physically entrapped in the microsponges. In-vitro *anti-H. pylori* activity studies demonstrated that TO in microsponge was more effective against *H. pylori* than pure TO. In conclusion, the developed microsponges containing thyme oil provide a promising alternative for the efficient targeting and eradication of *H. Pylori* infection.

1. Introduction

Peptic ulcer disease (PUD) is a disorder that affects the stomach and duodenum and is widespread, particularly among individuals aged 65 years and older [1]. In the stomach, PUD affects the body and antrum regions. The causes of PUD include several factors such

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https://doi.org/10.1016/j.heliyon.2024.e29246
Received 26 August 2023; Received in revised form 1 April 2024; Accepted 3 April 2024
Available online 8 April 2024

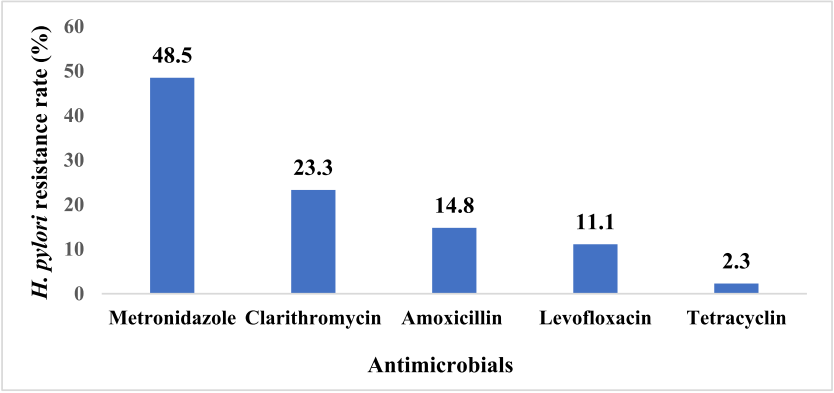

Fig. 1. *H. pylori* resistance rate of antimicrobials in Saudi Arabia.

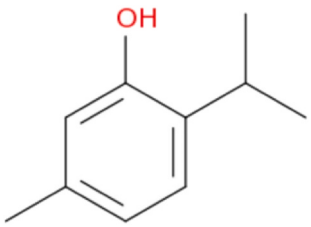

Fig. 2. Chemical structure of thymol.

as *Helicobacter pylori* infection, non-steroidal anti-inflammatory drugs, the imbalance between aggressiveness and protective factors, and stress [2]. Among these factors, *H. pylori* is noteworthy, being a gram-negative spiral-shaped bacterium that colonizes between the mucus layer and gastric epithelium in the body and/or antrum, leading to genetic alterations with virulence factors. *H. pylori* infects more than 50 % of the population worldwide without signs or symptoms [3]. However, not everyone infected with *H. pylori* will develop PUD; only 10–20 % could develop the disease. Antibiotic regimens used in managing PUD-associated *H. pylori* include macrolides (clarithromycin and azithromycin), amoxicillin, tetracycline, and metronidazole. A major concern with antibacterial medications is the resistance rate, with 4.95 million deaths globally associated with bacterial resistance in 2019 [4]. In the U.S., over 2.8 million cases of antimicrobial resistance occur each year (CDC, Oct 2022). Two core regimens are used in *H. pylori* treatment, one of which includes a drug metronidazole with a bacterial resistance rate of 48.5 % in Saudi Arabia [5] (Fig. 1).

Point mutation resistance is notably high, especially with metronidazole and clarithromycin. Additionally, adverse drug effects significantly impact the quality of life. Tetracycline may cause photosensitivity, a metallic taste with metronidazole, and Bismuth salts may lead to darkening of the stool and tongue, resulting in poor medication compliance and decreased efficacy. Furthermore, non-antimicrobial treatments such as proton pump inhibitors and antihistamines can result in adverse drug reactions, including hip fracture, vitamin B12 deficiency, confusion, acute kidney injury (AKI), and an increased risk of falls, especially in pregnant and elderly individuals, raising concerns about clinical significance in the field of therapeutics.

Given these challenges, there is an urgent need to find an adjuvant or alternative to conventional therapy. Therefore, the development of a targeted drug delivery system is proposed to reduce bacterial escape mechanisms. Phytotherapy, derived from plants, presents a potential candidate against *H. pylori* strains [6], offering a vast scope for research on its effects against bacteria. Essential oils (EOs) have been observed to exhibit interesting antibacterial and antioxidant properties, providing a safe alternative to antibiotics. Recently, numerous studies have explored the potential of EOs as adjuvants or alternatives to antibiotics for targeting and eradicating *H. pylori.* Some EOs are effective against specific bacteria, while others are particularly investigated for their efficacy against *H. pylori.*

Thyme oil (TO), derived from various species within the Thymus genus, is a member of the mint family, Lamiaceae, and has a rich historical background in traditional medicine for treating diverse ailments. Recognized for its therapeutic properties, TO encompasses anti-inflammatory, antioxidant, and antibacterial attributes [7]. Particularly noteworthy is its potent bactericidal activity against *H. pylori*, setting it apart from other essential oils [8]. However, one notable challenge is its relatively low stability towards environmental factors like heat, and light [9]. Thymol, the primary bioactive compound in TO, chemically identified as 5-methyl-2-propanyl phenol (Fig. 2), possesses a molecular weight (MW) of 150.2 g/mol and typically melts at around 229 °C [10]. Its mechanism of action involves interaction with bacterial membranes via the hydroxyl group, thereby augmenting permeability to protons and potassium ions [11]. Furthermore, thymol addresses bacterial resistance by targeting efflux pumps [12]. To date, there is no documented evidence of bacterial resistance to thyme oil, including strains of *H. pylori.*

TO itself cannot be directed to the site of action with a prolonged release manner. Current oral drug delivery systems in conventional medicines face challenges such as low gastric retention time, poor drug release, and low bioavailability. Despite these

Table 1
Composition of thyme oil laden microsponge formulations.

Composition	Formulations		
	F-1	F-2	F-3
Thyme oil (gm)	1	1	1
Eudragit RS 100 (gm)	1	2	3
Dichloromethane (mL)	6	6	6
Polysorbate 80 (mL)	0.6	0.6	0.6
Distilled Water (mL Up to)	100	100	100

challenges, oral dosage forms are preferred for ease of administration, increased compliance, and cost-effectiveness. Therefore, the need for a novel oral drug delivery system with defined characteristics to overcome these disadvantages is crucial. The microsponge-based drug delivery system (MDS) is one such innovative carrier. It is a polymeric novel carrier with a spherical, porous, and spongy-like structure, ranging in size from 5 to 300 μm and having a pore size of 0.25 μm [13]. Microsponges are a type of microparticulate drug delivery system, along with microspheres and microcapsules. Microsponges excel over other microparticulate systems because of their entrapment efficiency without dose dumping, sustained release of drugs, targeted site of action, stability over a wide pH range [1–11], stability at high temperatures, high dose entrapment (50%–60 %), improved adherence, and cost-effectiveness [14]. Encapsulating TO in a microsponge addresses its low stability; additionally, due to the low density, TO-laden microsponges can float on gastric fluid for an extended period, releasing the oil in a sustained release pattern at the site of action. This ensures that the microsponges would stay longer in the stomach, leading to a remarkable activity against *H. pylori*. For future work, microsponges can be formulated into tablets, capsules, pills, or granules. The literature survey indicates that essential oil-encapsulated microsponges significantly enhance oil stability and safety [15–17]. There are two mechanisms of *H. pylori* inhibition: firstly, the hydroxyl group of TO interacts with the membrane, leading to an increased permeability of proton and potassium flux; secondly, by targeting efflux pumps, which plays a vital role in bacterial resistance [12]. This would result in the depletion of *H. pylori* load and its resistance development. Moreover, the formation of biofilms is also reduced in the presence of TO Ref. [18]. The polymer Eudragit (EGT), containing acrylic acid and methacrylic acid esters, is used in the drug delivery system to target the gastrointestinal system. EGT is stable and insoluble at physiological pH, i.e., in both acidic and alkaline media. There are two different classes of EGT: RS 100 and LS 100. Eudragit RS 100 contains a lower content of the ammonium group (4.5%–6.8 %) compared to Eudragit LS 100 (8.8%–12 %). As a result, EGTRS 100 will float on the surface of gastric fluid, providing prolonged release of oil in higher amounts compared to EGTLS 100 [19]. EGTs have characteristics such as low toxicity and lower cost [20]. Currently, no studies have been performed on thyme oil-loaded microsponges for targeting *H. pylori*. Therefore, the objective of the current study was to develop the first thyme oil-loaded microsponge using EGTRS 100 for a local gastric system, intending to target *H. pylori* and prolong gastric residence time for the effective obliteration of *H. pylori* infection.

2. Materials and methods

2.1. Materials

We purchased thyme oil from Sigma Aldrich, and Eudragit RS100 from UFC Biotechnology in Amherst, New York. USA. The following items were purchased from SD Fine Chemicals Pvt. Ltd. In Mumbai, India: Tween-80, dichloromethane, and hydrochloric acid. The study made use of the reference strain *H. pylori* ATCC43504 from the American Type Culture Collection in Manassas, Virginia, USA. Bovine serum albumin, brain heart infusion broth, and agar media were purchased from Sigma (St. Louis, MO, USA).

2.2. Methods

2.2.1. Method of preparation of TO laden gastric floating microsponge

Thyme oil microsponges were prepared by the widely used Quasi emulsion solvent diffusion method. In this method, in the first step both the thyme oil and the eudragit RS100 in 1:1, 1:2, and 1:3 drug to polymer weight ratios had been dissolved in DCM (Table 1) in 3 different 100 ml capacity beakers, and these solutions were served as the organic internal phase. In the second step the organic internal phase was gradually added to an aqueous solution containing an emulsifying agent, polysorbate 80, in quantities of 0.6 mL in three different 250 ml capacity beakers. These emulsions were then stirred using an overhead stirrer for 90 min at a speed of 1500 rpm (Eurostar 20, IKA, Staufen, Germany). Microsponges were subsequently produced because of the evaporation of DCM from the emulsion system. The formed microsponges were now filtered, cleaned with distilled water, and allowed to dry at room temperature. The quantities of DCM and distilled water used for each formulation were 6 mL and 100 mL, respectively.

2.2.2. Physical evaluation of TO laden gastric floating microsponge

2.2.2.1. Product yield. The product yield of the microsponge formulation was calculated to assess the effectiveness of the method employed to prepare microsponge formulations. The individual ingredients of each microsponge formulation i.e., both TO and EGT, were weighed separately on a digital weighing balance, and the weights of these ingredients were recorded, and denoted as the

theoretical weights. Similarly, the weights of the developed microsponge formulations were recorded and these weights were referred to as the practical weights of the microsponge formulations. The product yield was then determined using the following formula:

$$\text{Product Yield } (\%) = \frac{\text{Practical weight}}{\text{Theoretical weight } (\text{TO} + \text{EGT})} \times 100$$

2.2.2.2. In-vitro floating study. According to the published report [20], the flotation ability of TO-laden gastric floating microsponge formulations was assessed. The instrument employed for evaluating the floating ability of these microsponges was a paddle-type dissolution test apparatus (Electrolab USP XXIV 8 basket dissolution test station, Electrolab Pvt. Ltd, India). Each microsponge formulation containing 10 mg of TO was weighed accurately and subsequently transferred to each basket of the dissolution instrument, which contained 900 mL of gastric pH 1.2 dissolution fluid. The paddles were rotated in the basket at a speed of 50 rpm, and the dissolution fluids were maintained at a temperature of 37 ± 0.5 °C. A stopwatch was utilized to monitor the floating lag time—the time it took for the microsponges to float on the media—and the overall floating log time (the duration of floating).

2.2.3. Drug content estimation and entrapment efficiency

Each TO-laden microsponge formulation, containing 10 mg of TO, was accurately weighed and crushed in a separate clean and dry glass mortar using a clean and dry glass pestle. Subsequently, 5 ml of methanol was added to each mortar to dissolve the microsponge formulations. The resulting methanolic solutions of the microsponges were then transferred to separate10 ml Eppendorf tubes, and then the tubes were capped. All three tubes were vortexed for 2–3 min, followed by centrifugation in a centrifuge at 2000 rpm for 10 min. After centrifugation, the solutions were appropriately diluted with a pH 1.2 medium in three separate volumetric flasks, and the absorbance of each solution was measured at 293 nm using a double-beam UV–Visible spectrophotometer (Shimadzu 1700, Kyoto, Japan).

Finally, the percent drug content and percent entrapment efficiency of each TO-laden microsponge formulation were calculated utilizing the following equations:

$$\%\ Drug\ content = \frac{Actual\ amount\ of\ TO\ in\ microsponge}{Weighed\ amount\ of\ microsponges} \times 100$$

$$\%\ Entrapment\ efficiency = \frac{Actual\ amount\ of\ TO\ in\ microsponge}{Theoretical\ amount\ of\ TO\ in\ microponge} \times 100$$

2.2.4. SEM assessment of TO laden gastric floating microsponge

Scanning electron microscopy (TESCAN VEGA3, Czech Republic) was employed for the analysis of the prepared microsponge formulations to determine the surface roughness, surface texture, particle size and shape. Samples were affixed to a metal stud using double-faced adhesive tape, coated with gold-palladium under vacuum, and then examined using a secondary electron detector at a 20 kV electric voltage. SEM photomicrographs were captured at various magnification powers to study the surface topography of the microsponge formulations. The size of the microsponges were analyzed by the SEM's Essence software from the top view of SEM photomicrographs. Data from two distant image fields for each sample was used for the analysis of microsponges sizes whereas approximately 12 microsponges from each image field was counted by the software. Additionally, the pore sizes of the developed microsponges were manually calculated using open-sourced ImageJ/FIJI software from SEM photomicrographs taken at 30× magnification, and the average diameter of the pores in micrometers was noted.

2.2.5. Drug-polymer interaction study using DSC

Differential Scanning calorimeter (DSC 214 Polyma NETZSCH, Germany) was utilized to obtain thermograms of TO, EGT, TO-EGT physical mixture and TO-laden gastric floating microsponge (F 2 formulation). A small aluminum pan exclusively designed for DSC measurements was weighed with 4–8 mg of the substance, and the pan was then tightly sealed. An empty pan served as reference was also weighed and sealed. The sealed pans were transferred to the DSC instrument to obtain thermograms of the specified test samples. During the measurement of the sample's DSC thermogram, key factors such as a nitrogen gas environment (40 and 60 ml per minute flow rate), a temperature range (50 °C–300 °C), and a constant heating rate of 10 °C per minute were maintained consistently throughout the measurements.

2.2.6. In-vitro drug release study

The release of TO from the prepared TO-laden gastric floating microsponge was investigated using an automated LOGAN 8 basket USP-compliant type-2 dissolution test instrument (LOGAN Instruments Inc., Somerset, NJ, USA). A cellophane membrane (Himedia Pvt. Ltd. Mumbai, India), soaked in pH 1.2 solution overnight, was wrapped around the gastric floating microsponges containing 5 mg of TO. This assembly was then tied to the paddle of the dissolution instrument, and the paddles were immersed and stirred in 900 ml of acidic medium (pH 1.2). To prevent damage to the microsponge structure during prolonged stirring, the paddle was stirred at a speed of not more than 50 rpm, while maintaining a temperature of the medium 37 ± 0.5 °C constant throughout the study. At various time intervals, 5 ml of samples were withdrawn, filtered, and examined for the presence of TO using a spectrophotometer at a maximum wavelength of 293 nm. The cumulative percentage of TO released at each time interval was then calculated. The dissolution experiments were repeated three times. The mechanism of TO released from the microsponge formulations was studied using PCP Disso V3,

Table 2
Physicochemical evaluation of thyme oil laden microsponge formulations.

[a]Parameters	Formulations		
	F-1	F-2	F-3
Physical appearance	White solid	White solid	White solid
Product Yield (%)	41 ± 0.75	55.25 ± 1.1	81.27 ± 1.13
Drug content (%)	33.25 ± 0.94	33.25 ± 0.39	15.75 ± 1.53
Entrapment efficiency (%)	66.5 ± 0.86	88.64 ± 0.98	63.01 ± 0.79
In-vitro floating time (h)	>12	>12	>12
Particle size (μm)	24.66 ± 0.82	49.79 ± 1.4	66.56 ± 1.23

[a] Each experiment was done in triplicate, and the findings were presented as mean SD (n = 3).

an Excel-based application developed in India by Pune College of Pharmacy, Maharashtra.

2.2.7. In-vitro anti H. pylori activity

2.2.7.1. Evaluation of minimum inhibitory concentration. The minimum inhibitory concentrations (MICs) of test compounds (thyme oil and thyme oil microsponge) were determined against *Helicobacter pylori* ATCC43504 using broth microdilution method. The solutions of test compounds were made in 1 % DMSO. The strain under study was cultivated on Brain heart Infusion broth (with 7 % bovine serum albumin) and incubated for 3 days under microaerophilic condition. Whereas two-fold serial dilution of compounds under study were made in 96 well microtiter plate. Briefly, to the 100 μl of Mueller-Hinton broth having test compounds in a concentration ranging from 0.125 μg/ml to 16 μg/ml, 0.1 μl of *H. pylori* cells (10^7 cfu/ml) prepared in phosphate buffer saline was added. Further, plates were incubated for 5 days at CO_2 10 %, O_2 5 %, N_2 85 %, 37 °C. The MICs of test compounds were recorded as the lowest concentration at which bacterial growth is prevented as observed for the turbidity.

2.2.7.2. Determination of duration of growth inhibition. To determine the duration of action of test compounds, bacterial cells (10^7 cfu/ml) in 200 μl of Mueller-Hinton broth was treated with the 2 × MIC of test antibiotics and incubated at 37 °C, 96 h under microaerophilic environment (CO_2 10 %, O_2 5 %, N_2 85 %). The period of action of compounds were noted as the duration after which bacterial growth started appearing.

2.2.8. Stability study

The stability of the prepared thyme essential oil loaded microsponges was evaluated over a three-month period at 40 ± 2 °C and 75 ± 5 % RH. Samples were withdrawn at intervals of 30, 60, and 90 days, and these samples were examined for their physical condition and drug content. Additionally, DSC (Differential Scanning Calorimetry) was performed to assess the stability of the formulations.

2.2.9. Statistical analysis

For this study, we performed a one-way analysis of variance test (ANOVA) to assess the acquired experimental data. Additionally, to compare the standard TO with the test samples of the microsponge formulation, a student's t-test was employed, and the significance levels were denoted at a p-value of <0.005.

3. Results and discussion

3.1. Physical evaluation of the TO laden gastric floating microsponge

For the TO-loaded gastroretentive microsponge formulations, the product yield values obtained were ranged from 41 ± 0.75 to 81.27 ± 1.13 (Table 2). The relatively low product yield of these microsponge formulations could be attributed to the aggregation and adhesion of polymers to various surfaces, including glass rods, beaker walls, stirrer blades, etc. Another factor influencing the product yield could be the choice of polymer; EGT polymer was reported to yield a higher product yield for microsponges compared to other polymers like ethyl cellulose [20] the reason attributed to this was that the less adhesion of EGT to the surfaces than the ethyl cellulose. Overall, the low product yield might be due to EGT entering the continuous phase and forming thick agglomerates, with the polymer adhering to various surfaces.

In the in vitro floating test, the floating lag time recorded for all microsponge formulations was zero i.e., the TO-loaded microsponges after transferring to the acidic medium (pH 1.2) they did not sink into medium, but they remained at the surface of the medium, indicating their excellent floating ability. The floating log time of the microsponges exceeded 12 h overall **(**Table 2**)**, demonstrating their prolonged floating capability, attributed to their low density and internal hollow spaces.

3.2. Drug content estimation and entrapment efficiency

Formulation F1 exhibited the second-highest entrapment efficiency (66.5 ± 0.86), with Formulation F2 showing the first highest entrapment efficiency (88.64 ± 0.98) **(**Table 2**)**. As the amount of EGT increased, more polymer concentration became available for

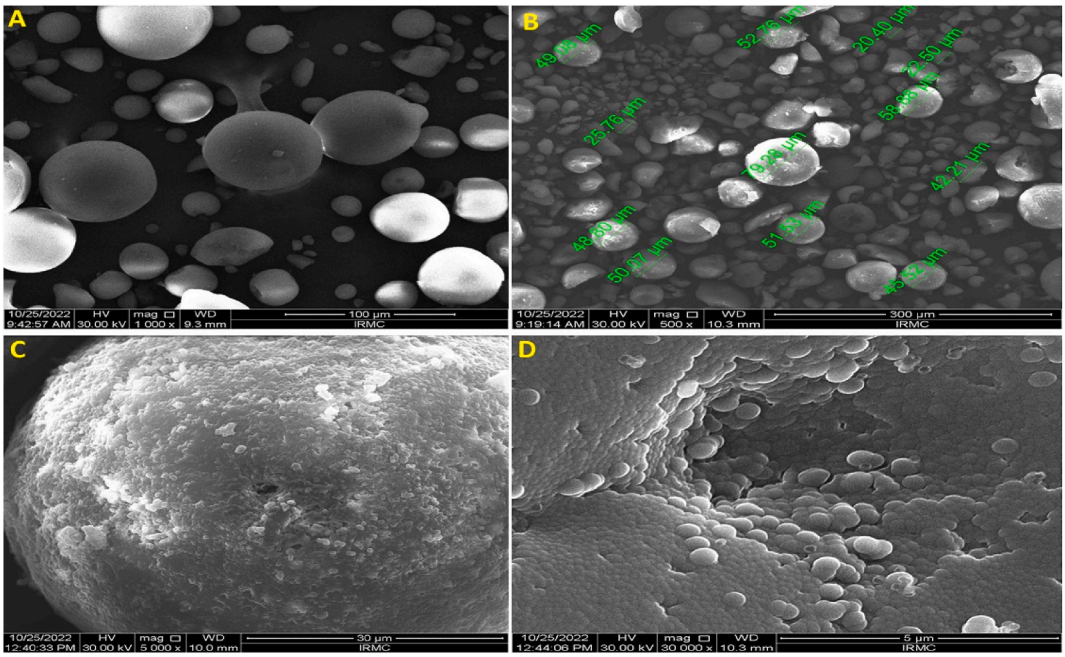

Fig. 3. SEM images illustrating **A)** Eudragit, **B)** TO laden microsponge formulation (F2) highlighting marked particle sizes, **C)** Porous surface structure of F-2 formulation, and **D)** Close-up view of the surface of the of microsponge formulation (F2) revealing interconnected channels.

each microsponge to encapsulate the TO. The intramolecular pressure and internal phase viscosity increased with the polymer content [13,15], leading to the formation of larger droplets during the emulsification process and, consequently, larger microsponges. Larger microsponge particles can entrap a higher amount of essential oil. According to Pawar and his co-researchers, a higher amount of polymer in the microsponge formulation reduces the diffusion of the oil solution into the external phase during the emulsification process, allowing more time for droplet formation and resulting in high entrapment efficiency [21].

However, Formulation F3 exhibited an out-of-trend drug entrapment efficiency, i.e., despite the increase in the amount of EGT in F3, the drug entrapment efficiency decreased compared to the F2 formulation. This result is consistent with the published reports [22, 23]. The porosity of microsponges was also linked to higher levels of entrapment efficiency [24]. According to Zaki Rizkalla et al. [25], the presence of numerous pores in the microsponge structure provides a large surface area for more drug entrapment. Furthermore, in systems where the amount of DCM is constant, the same size pores are present in the microsponge structures and are uniformly distributed throughout the colloidal carrier. This intimate interaction in the pores facilitates the loading of essential oil in microsponges [26–28].

3.3. SEM assessment of TO laden gastric floating microsponge

Scanning electron microscopy (SEM) analysis of the developed thyme oil-loaded microsponges was conducted to investigate surface topography and morphology. Fig. 3 illustrates the SEM photomicrographs. The uniform spherical shaped EGT powder can be seen in Fig. 3A. The resulting microsponges were predominantly spherical in shape (Fig. 3B), and their surfaces appeared highly porous (Fig. 3C). It is also clear from Fig. 3D that the developed microsponges possess a distinctive internal structure comprised of spherical chambers enclosing essential oil within the polymer used. Thus, the SEM images clearly indicate that the formed colloidal particles exhibit all the morphological characteristics typical of microsponges, with numerous pores in their inner structure. According to Kumar and Ghosh [29], the formation of pores in the microsponge structure was primarily due to the diffusion of DCM from the surface of the microsponges during the drying process. Additionally, porous, and spherical Eudragit microsponges have been reported by Jafar et al. [30]. In consistent with the reported literature, microsponges are expected to have a mean particle size between 5 µm and 300 µm [31,32]. The developed TO-loaded microsponges exhibited average particle sizes ranging from 24.66 ± 0.82 µm to 66.56 ± 1.23 µm **(**Table 2**)** and their average pore size was found to be 0.81 ± 0.14 µm. In each microsponge formulation, approximately 45 % of the particles selected by the software in SEM photomicrographs showed the sizes nearly the same size as their average sizes, indicating that the microsponges were homogeneous and had a narrow size distribution. The incorporation of EGT was found to increase the particle size in the following order of F3>F2>F1. Formulation F3, with highest concentrations of EGT, exhibited the maximum particle size of 66.56 ± 1.23 µm. The larger particle size observed in F 3 could be attributed to the formation of a viscous organic phase at higher EGT concentrations, resulting in larger emulsion droplets and, consequently, larger colloidal particles with a microporous structure were formed [26]. Similar outcomes were reported for prednisolone microsponges prepared using the same polymer EGT [33]. Thus, scanning electron microscopy revealed the spongy structure of the microsponges with minute pores while maintaining structural

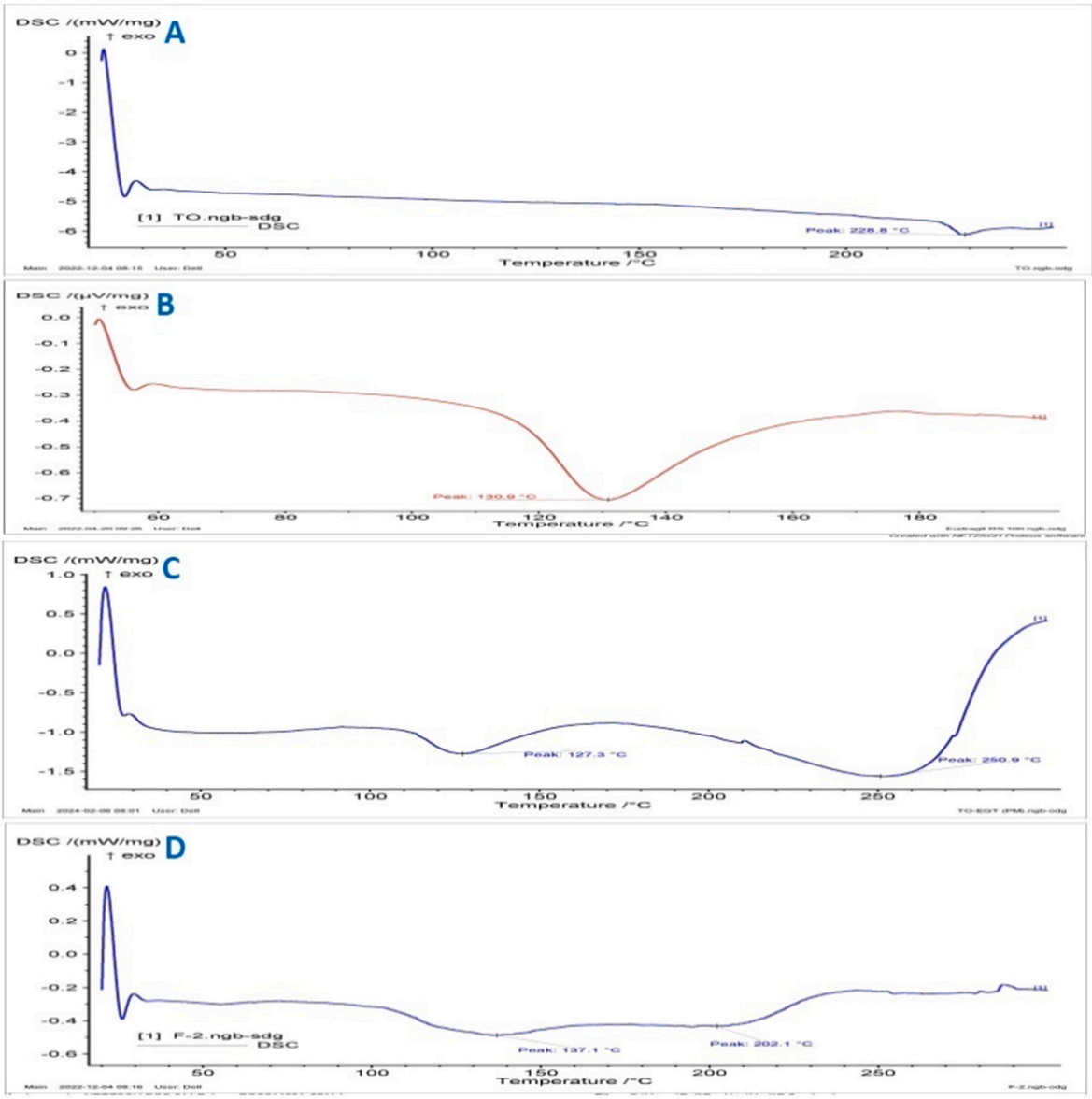

Fig. 4. DSC thermograms of **A)** Thyme oil **B)** Eudragit **C)** TO-EGT Physical mixture **D)** Thyme oil laden microsponge formulation (F-2).

integrity, affirming the successful formation of a desirable drug carrier system.

3.4. Drug-polymer interaction study using DSC

When guest molecules are entrapped in the porous cavities of microsponges, differential scanning calorimetry can be employed to identify them, as reported in the literature (34). During this process, the physical properties of the guest molecule, such as melting point, boiling point, and sublimation points, are typically shifted to different temperatures or might disappear. Fig. 4 displays the thermograms of TO, EGT, TO-EGT physical mixture and TO-loaded microsponges. A prominent exothermic peak of TO in the thermogram (Fig. 4A) appeared around 228.8 °C in the graph depicts the relationship between heat flow and temperature, corresponding to the process of oil evaporation. While the broad endothermic peak observed at 130.9^0 C in the DSC thermogram of EGT (Fig. 4B) mainly arises from the dehydration process, indicating the amorphous nature of the polymer. The DSC thermogram of physical mixture (Fig. 4C) signifies that thyme oil had no interaction with EGT as no new peak appeared in it. However, slight shift in temperatures and slight broadening of peaks of both components in the physical mixture compared to individual components indicates high miscibility between the drug and the polymer used. The thyme oil peak was found to be diminished with a broadening of the curve in TO-incorporated microsponge formulation (Fig. 4D), indicates that TO had been successfully trapped inside the microsponges.

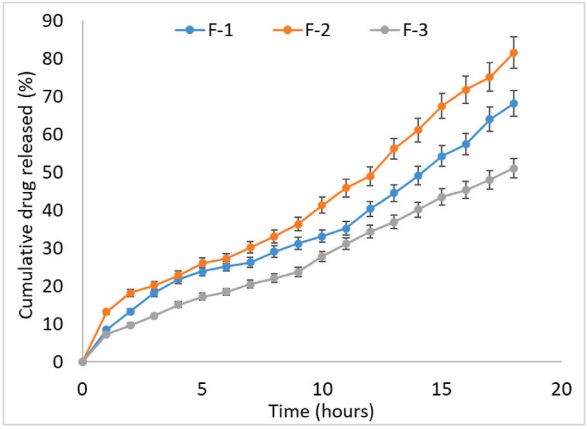

Fig. 5. Cumulative % drug release Vs time profile of thyme oil loaded microsponge formulations.

Table 3
MICs of thyme oils alone and its microsponge formulation and their duration of action against the strain of *H. pylori* ATCC 43504.

Test compounds	MIC (μg/ml)	Duration of action (At 2 × MIC) (h)
Thyme oil	16	48 ± 2
Thyme oil laden microsponge	32	96 ± 2

Furthermore, it can be deduced from the acquired thermograms that the thermal characteristics of TO remained intact when formulated into microsponges. Similar results have been reported in the literature ([15,34]).

3.5. In-vitro drug release study

Fig. 5 illustrates the in vitro drug release characteristics of the TO laden microsponges in 0.1 N HCl (pH 1.2). At the end of 18 h, F 2 exhibited the highest in vitro cumulative drug release (81.53 %), followed by F1 (68.07 %), and then F3 (51.02 %).

According to Chinna et al. [35], an increase in the amount of EGT leads to a decrease in the amount of drug near the surface of microporous particles, while the amount of drug entrapped in the polymer matrix increases. Consequently, the release rate of drugs from the microsponges was slowed down. F 2, developed with a lower quantity of EGT compared to F 3, resulted in small microsponges with a larger effective surface area. When this large effective surface area encounters the dissolution medium, the drug release rate was increased. Moreover, the improved drug release rate in small-sized microsponges could be attributed to the shorter length for the acidic medium to traverse within the microsponge, facilitating the release of the drug outside the microsponge carrier.

Reduction in the release rate was reported with an increasing amount of EGT in the microsponge [20,33]; in other words, a higher amount of EGT used in the microsponge formulation results in a larger microsponge size, thereby reducing drug release. The rapid entry of the acidic medium into the microsponges may be due to the release of TO from the microsponges to the pores, promoting the breakdown of the drug and its diffusion through the polymeric matrix of the microsponge [36]. The higher concentration of EGT, as per Abdelmalak and El-Menshawe [37], leads to the formation of thick layers, resulting in a prolonged release of the drug.

In summary, the observed pattern in drug release can be attributed to the interplay of EGT concentration, microsponge size, effective surface area, and the resulting impact on drug diffusion within the polymeric matrix. These factors collectively contribute to the distinctive release profiles exhibited by the different microsponge formulations.

3.6. In-vitro anti H. pylori activity

3.6.1. Minimum inhibitory concentration and duration of action of thyme oil and its formulation

As shown in Table 3, the MIC of thyme oil is found to be 16 μg/ml against the test strain *H. pylori* ATCC43504. Whereas thyme oil-microspomge exhibited MIC of 32 μg/ml. However, the inhibition activity of thyme oil-microsponge formulation lasting until 96 h which is higher than thyme oil alone (48 h). The difference in lasting activity could be attributed to the presence of EGT as additional compound in thyme oil-microsponge formulation that is an indication that our developed formulation can hold oil and results in sustained release of it and hence showed long lasting activity compared to the thyme oil alone.

In this experiment, the thyme oil loaded microsponge exhibited antimicrobial activity like thyme oil alone against the test strain (Table 3). The raise in MIC of thyme oil loaded microsponge compared to thyme oil alone could be as reason of the presence of other inert chemicals like EGT in the developed formulations. However, the targeted organism is found to be susceptible to thyme oil loaded microsponge at a quite reduced concentration for a significantly delayed period. The study demonstrated that microsponge thyme oil has a powerful and sustained *anti-H. pylori* activity. Consequently, it could be a valuable alternative therapeutic approach of using

Table 4
Stability data of TO laden gastric floating microsponge (F 2).

Sl. No.	Duration (Months)	Physical appearance	[a]Drug content (%)
1	0	–	33.25 ± 0.39
2	1	No change	32.62 ± 1.21
3	2	No change	31.95 ± 0.98
4	3	No change	30.03 ± 0.69

[a] The drug content experiment was done in triplicate, and the findings were presented as mean SD (n = 3).

microsponge formulation of thyme oil exhibiting enhanced bioavailability to be exploited against *H. pylori* stimulated gastric ulcers.

3.7. Stability study

The results of the short-term stability study for the microsponge (F-2 formulation) indicated no significant change in physical appearance, and the drug content remained consistent throughout the study ($p > 0.005$) (Table 4**).** Additionally, the DSC spectra demonstrated the stability of the formed microsponge formulations and showed no signs of oil degradation. Therefore, it is suggested that the use of microsponges has significantly improved the stability of TO, addressing a critical limitation of its low stability in atmospheric conditions [38].

4. Conclusion

Thyme oil-laden microsponges were formulated with the aim of achieving intragastric drug delivery to eradicate *H. pylori* infection in the stomach mucosa. Although microsponges have been minimally explored for their potential to encapsulate essential oils, they prove to be effective carriers for these bioactive oils. The study successfully employed the quasi-emulsion solvent evaporation method for loading thyme oil (TO) into Eudragit microsponges, showcasing an effective and reliable encapsulation technique. Apart from handling advantages, favorable floating characteristics, and improved stability of thyme oil, the encapsulation of TO in microsponges yielded a substantial payload and facilitated delayed release, indicating the potential for a controlled and sustained drug delivery system. Scanning electron microscopy revealed the spongy structure of the microsponges with tiny pores and regulated integrity. The selected thyme oil-laden microsponge demonstrated significant *anti-H. pylori* activity. Consequently, encapsulating thyme oil in microsponges proved to be a stable and effective delivery method. Finally, the current study sets the stage for future pharmacokinetic and pharmacodynamic investigations of our optimized F-2 microsponge formulation containing 1:2 wt ratio of thyme oil: eudragit, paving the way for a more comprehensive understanding of its potential applications in eradicating *H. pylori* infection.

Data Availability

Sharing research data helps other researchers evaluate your findings, build on your work and increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.

Has data associated with your study been deposited into a publicly available repository?

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as follow-up to "**Data Availability**.

Sharing research data helps other researchers evaluate your findings, build on your work and increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.

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Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Mohammed Jafar: Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Conceptualization. **Mohd Sajjad Ahmad Khan:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **Mohammad Jamal Akbar:** Writing – original draft, Visualization, Validation, Software, Resources, Formal analysis, Data curation. **Hadi Saleem AlSaihaty:** Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Sultan Saad Alasmari:** Writing – original draft, Software, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

The College of Clinical Pharmacy and Centre for Research and Medical Consultation at Imam Abdulrahman bin Faisal University in Dammam, Saudi Arabia, generously provided the resources we required to complete this study. We would like to express our sincere gratitude to Dr. Rajesh Ajesh Arruri, Assistant Professor, Department of English language (DEL), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia, for his invaluable contribution to the improvement of our manuscript through English language correction. His expertise has significantly enhanced the clarity and quality of the content.

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Abbreviations

TO –: Thyme oil
EGT –: Eudragit
H. pylori –: Helicobacter pylori
USP –: United States Pharmacopoiea
MIC –: Minimum inhibitory concentration
DSC –: Differential scaning calorimetry
SEM –: Scanning electron microscopy
DCM –: Dichloromethane