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RESEARCH ARTICLE

Eudragit S100-based Floating Microballoons of Antitubercular Drug: Design, Preparation, and *In Vitro* Characterization

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SUMMARY

The present study aimed to develop and characterise floating microspheres of Eudragit S100 containing rifampicin (RFN) to enhance bioavailability, control drug release, and achieve site-specific delivery. Rifampicin is an antitubercular drug commonly used in the treatment of tuberculosis. Floating microspheres were prepared using the solvent evaporation method with varying drug-to-polymer ratios (1:1–1:5), stirring speeds (300, 400, and 500 rpm), and stirring times (1, 1.5, and 2 hours). The developed formulations were evaluated for particle size, floating lag time, percentage buoyancy, surface morphology (via scanning electron microscopy), swelling index, drug content, entrapment efficiency, and in vitro drug release. The microspheres were spherical with smooth surfaces, and all formulations were found to float for up to 24 hours. Particle size and percentage buoyancy increased with rising polymer concentration. In vitro studies revealed that the drug was released from the floating microspheres over 24 hours and followed the Higuchi kinetic model. Among all formulations, FES4 (1:4 drug-to-Eudragit S100 ratio) exhibited the lowest floating lag time and the highest percentage buoyancy. Therefore, FES4 was selected as the optimised formulation based on its floating behaviour and other in vitro parameters. Further, antimicrobial activity studies demonstrated a clear zone of inhibition as compared to the control. The FES4 formulation remained stable under storage conditions. Overall, the optimised formulation (FES4) displayed excellent floating properties and antimicrobial activity, indicating the potential of rifampicin-loaded microspheres as an effective system for tuberculosis treatment.

Keywords: *Floating microspheres, eudragit S 100, rifampicin, in vitro characterization, anti-microbial activity.*

Eudragit S100-bazlı Antitüberküloz İlaç İçeren Yüzen Mikroküreler: Tasarım, Hazırlık ve İn Vitro Karakterizasyon

ÖZ

Bu çalışmanın amacı, biyoyararlanımı artırmak, kontrollü ilaç salımını sağlamak ve hedefe yönelik ilaç iletimini gerçekleştirmek amacıyla rifampisin (RFN) içeren Eudragit S100'den yapılmış yüzen mikroküreler geliştirmek ve karakterize etmektir. Rifampisin, tüberküloz tedavisinde yaygın olarak kullanılan bir antitüberküloz ilaçtır. Yüzen mikroküreler, çözücü buharlaştırma yöntemi kullanılarak farklı ilaç-polimer oranlarında (1:1–1:5), karıştırma hızlarında (300, 400 ve 500 rpm) ve karıştırma sürelerinde (1, 1,5 ve 2 saat) hazırlanmıştır. Geliştirilen formülasyonlar; parçacık boyutu, yüzme gecikme süresi, yüzdürme yüzdesi, yüzey morfolojisi (taramalı elektron mikroskobu ile), şişme indeksi, ilaç içeriği, tutma verimi ve in vitro ilaç salımı açısından değerlendirilmiştir. Mikrokürelerin küresel ve düzgün yüzeyli olduğu gözlenmiştir ve tüm formülasyonlar 24 saat boyunca yüzebilir durumda kalmıştır. Polimer konsantrasyonunun artmasıyla birlikte parçacık boyutu ve yüzdürme yüzdesi de artmıştır. In vitro çalışmalar, ilacın yüzen mikrokürelerden 24 saat boyunca salındığını ve Higuchi kinetik modeline uyduğunu göstermiştir. Tüm formülasyonlar arasında, FES4 (1:4 ilaç-Eudragit S100 oranı) en düşük yüzme gecikme süresini ve en yüksek yüzdürme yüzdesini göstermiştir. Bu nedenle, FES4 formülasyonu, yüzdürme davranışı ve diğer in vitro parametreler temelinde optimize edilmiş formülasyon olarak seçilmiştir. Ayrıca, antimikrobiyal aktivite çalışmaları, kontrol grubuna kıyasla belirgin bir inhibisyon zonu göstermiştir. FES4 formülasyonu depolama koşullarında stabil kalmıştır. Genel olarak, optimize edilmiş FES4 formülasyonu mükemmel yüzdürme özellikleri ve antimikrobiyal aktivite sergilemiş olup, rifampisin yüklü mikrokürelerin tüberküloz tedavisinde etkili bir sistem olma potansiyelini göstermektedir.

Anahtar Kelimeler: *Yüzen mikroküreler, eudragit s100, rifampisin, in vitro karakterizasyon, anti-mikrobiyal aktivite.*

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INTRODUCTION

Oral administration has garnered the greatest attention because it is the most convenient method of drug delivery and offers significant flexibility in dosage form design. However, limited bioavailability and short gastric retention time remain major drawbacks of conventional drug delivery systems. The gastrointestinal tract (GIT) still poses several challenges with standard drug delivery methods, such as insufficient drug release, reduced dose efficacy, and the need for frequent dosing. The inability of conventional systems to retain drugs in the stomach has led to the development of gastro-retentive drug delivery systems (GRDDS). These systems improve drug absorption and prolong gastric retention time. Gastric retention can be achieved through various approaches, including raft-forming systems, high-density systems, magnetic systems, super-porous hydrogels, low-density systems, expandable systems, and mucoadhesive or bio-adhesive systems (Mandal et al., 2016).

It is anticipated that floating drug delivery systems will remain buoyant upon reaching the stomach, thereby increasing the bioavailability of the drug. Several types of buoyant formulations exist, including hollow microspheres, granules, tablets, capsules, pills, laminated films, and powders. Among these, floating microspheres are being extensively studied due to their ability to deliver drugs directly to the stomach. They allow the continuous release of the drug while remaining afloat on the gastric contents for extended periods. Since these systems do not adversely affect the gastrointestinal tract (GIT) motility, they have been the focus of considerable research (Pawar et al., 2011).

Rifampicin (RFN) is a first-line antitubercular drug used to treat tuberculosis caused by *Mycobacterium tuberculosis (M. tuberculosis).* It has a short half-life of 1.5 to 4.5 hours and belongs to BCS Class II. RFN exhibits gastric permeability, and site-specific sustained drug delivery in the stomach may be beneficial for enhancing its bioavailability. Because RFN is less soluble at higher pH values (pH 7.4; 1 in 100 of phosphate buffer at 37°C) and more soluble at lower pH values (pH 1.5; 1 in 5 of 0.1 M HCl at 37°C), it is a suitable candidate for gastro-retentive drug delivery system (GRDDS) formulation. The development of a gastro-retentive controlled-release (CR) formulation of RFN is required to improve its biopharmaceutical properties, owing to its high solubility in acidic pH and optimal absorption window in the stomach. Eudragit S100, a methacrylic acid and methyl methacrylate anionic copolymer, is widely used as a sustained-release polymer (Patra et al., 2017). This polymer is soluble in intestinal fluid at pH values greater than 7 and has a low density (0.83–0.85 g/cm^3). These properties make it suitable for use in sustained-release formulations, ensuring that the drug remains intact until it reaches a specific pH environment within the GIT. When the formulation reaches the duodenum or stomach, the drug is released and absorbed from the polymer matrix at these sites.

Floating microspheres containing Eudragit S100 have been reported for a wide range of drugs, such as itopride hydrochloride (Bansal et al., 2016), curcumin (Kumar et al., 2016), acyclovir (Junyaprasert & Pornsuwannapha, 2008), repaglinide (Sivaprasad et al., 2021), metformin hydrochloride (Verma et al., 2020), amoxicillin and rabeprazole sodium (Choudhary et al., 2016), and famotidine (Gupta et al., 2014). RFN has also been formulated using various technologies, including microspheres (Bhadouriya et al., 2012), microparticles (Goyal et al., 2011), biodegradable microcapsules (Maurya et al., 2012), solid lipid nanoparticles (Sarfaraz et al., 2010), and niosomes (Singh et al., 2013). Although several studies have explored rifampicin-loaded floating microspheres using polymers such as ethyl cellulose, hydroxypropyl methylcellulose (HPMC), and Eudragit RLPO (Bhise et al., 2010; Goyal et al., 2011; Pingale & Amrutkar, 2021), the use of Eudragit S100 to enhance gastric retention remains largely under-investigated (Kallepu et al., 2016; Pallam & Maruvajala, 2024; Raveendra et al., 2012). Traditionally, Eudragit S100 has been employed for colon-targeted drug delivery due to its pH-dependent

solubility—being soluble only at pH values above 7—making its application in gastric-floating systems both unconventional and novel.

In the present study, Eudragit S100 was innovatively employed to develop buoyant microspheres with prolonged gastric retention. This strategy was designed to optimize drug release at the primary absorption sites—the stomach and upper small intestine—thereby enhancing the bioavailability of rifampicin, a drug characterized by limited solubility and a short half-life.

Unlike previous formulations—such as those by Patel et al. (2006), which incorporated ethyl cellulose and HPMC, or Pingale & Amrutkar (2021), who utilized Eudragit RL/RS for rifampicin microspheres—our approach uniquely capitalizes on the gas-trapping capacity and low density of Eudragit S100. Despite its pH-dependent solubility, Eudragit S100 exhibits a remarkable ability to entrap air during the solvent evaporation process, enabling the microspheres to remain buoyant under gastric conditions.

This formulation strategy not only extends gastric residence time but also provides a controlled and sustained drug release profile, which is particularly advantageous for rifampicin due to its narrow absorption window and degradation in acidic media.

To the best of our knowledge, Eudragit S100 has not been previously explored in the development of floating microspheres for rifampicin delivery, underscoring the novelty and potential impact of this research in the field of gastro-retentive drug delivery systems.

The present study focused on the design and formulation of Eudragit S100-based floating microspheres for the delivery of RFN. The developed microspheres were characterized using various *in vitro* parameters, including particle size, floating lag time, total buoyancy time, scanning electron microscopy (SEM), *in vitro* drug release, and antimicrobial activity.

MATERIALS AND METHODS

Materials

Rifampicin was purchased from Yarrow Chemicals, Mumbai. Eudragit polymer was obtained as a gift sample from Evonik, Mumbai. All other chemicals, like ethanol, isopropanol, dichloromethane, and polyvinyl alcohol, were obtained from SD Fine Chemicals, Hyderabad.

Methods

Preparation of Rifampicin Floating Microspheres

Eudragit microspheres were prepared using various formulation variables, such as the drug-to-polymer ratio (1:1 to 1:5), and process variables, including stirring speed (300, 400, and 500 rpm) and stirring time (1, 1.5, and 2 hours), as shown in Table 1. The microspheres were fabricated by the solvent evaporation method, as reported earlier, with slight modifications. RFN (200 mg) and the required quantity of polymers, as mentioned in Table 1, were dissolved in a solvent mixture of dichloromethane (DCM), ethanol, and isopropanol in a 5:8:2 ratio. This organic phase was poured dropwise over 3–4 minutes into 1000 mL of an aqueous phase containing 0.6% polyvinyl alcohol (PVA) and stirred at room temperature using a mechanical stirrer for 1 hour at speeds of 300, 400, or 500 rpm. The resulting microspheres were then filtered and dried (Pallam & Maruvajala, 2024; Phutane et al., 2010).

Table 1. Formulation composition of floating microspheres using Eudragit S 100

Formulation codes	Drug (mg)	Polymer (mg)	Stirring speed (RPM)	Stirring time (Hrs)
Effect of polymer concentration				
FES1	200	200	300	1
FES2	200	400	300	1
FES3	200	600	300	1
FES4	200	800	300	1
FES5	200	1000	300	1
Effect of stirring speed				
FES6	200	800	400	1
FES7	200	800	500	1
Effect of stirring time				
FES8	200	800	300	1.5
FES9	200	800	300	2

Characterization of rifampicin floating microspheres

Fourier transformed infrared spectroscopy (FT-IR) analysis

An FTIR spectrophotometer was used to record the FTIR spectra of the drug and drug–polymer mixture using a KBr disc over the range of 4000–400 cm^{-1}.

Shape of floating microspheres

The shape and surface morphology of the floating microspheres were examined by spreading the samples on a clean glass slide and observing them under an optical microscope.

Production yield of microspheres

The total weight of the dried microspheres was measured, and the production yield was determined (Khan et al., 2020) by comparing the weight of the dried microspheres from each formulation with the initial dry weight of the drug and polymer combined, as calculated using the following equation:

$$Yield(\%) = \frac{Weight\ of\ micro\ spheres}{Weight\ of\ drug + Polymer\ use\ is\ formulation} \times 100$$

Particle size

Microspheres were carefully examined using a calibrated optical microscope to estimate their particle size. The diameters of a minimum of 100 microspheres from each batch were measured, and the results were recorded.

Drug content

To determine the drug content of the floating microspheres, 50 mg of a precisely weighed formulation was dissolved in 10 mL of ethanol and stirred with a magnetic stirrer to break down the polymer. The drug was then extracted and filtered. The concentration of the drug in the ethanol phase was measured using UV spectrophotometry at 475 nm (Nila et al., 2014).

$$Drug\ content(\%) = \frac{Calculated\ amount\ of\ drug}{Total\ weight\ of\ floating\ microspheres} \times 100$$

Entrapment efficiency

Entrapment efficiency was assessed using a previously reported method with slight modifications (Bakshi, 2016). Briefly, microspheres containing 10 mg of RFN were ground using a glass mortar and pestle, and the resulting powder was suspended in 10 mL of methanol. After an appropriate dilution, the drug content was analyzed at 475 nm using a UV spectrophotometer.

$$Entrapment\ efficiency(\%) = \frac{Calculated\ drug\ concentration}{Theoritical\ Drug\ Concentration} \times 100$$

***In vitro* buoyancy studies**

A 50 ml beaker was filled with 50 mg of the microspheres. Then, 20 mL of 0.1N HCl containing 0.02% Tween 20 was added, and the mixture was stirred using a magnetic stirrer in a water bath maintained at 37 ± 0.5 °C. The time taken for the microspheres to float to the surface, referred to as the floating lag

time, was recorded. After 24 hours, the floating microspheres were collected.

$$Buoyancy(\%) = \frac{Weight\ of\ Floating\ Microspheres}{Initial\ weight\ of\ hollow\ microspheres} \times 100$$

Swelling index

With minor modifications, the swelling index was determined using the methodology described in the literature (Mukund et al., 2012). Precisely weighed microspheres (100 mg) were allowed to swell in 0.1N HCl for 24 hours. After excess surface-attached liquid was gently blotted away, the swollen microspheres were weighed using a microbalance. The swelling index was calculated using the following formula:

$$Swelling\ index(\%) = \frac{Final\ weight - Initial\ weight}{Final\ weight} \times 100$$

Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical comparisons between different formulations (e.g., entrapment efficiency, particle size, buoyancy, drug release) were conducted using one-way analysis of variance (ANOVA). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All data were analyzed using GraphPad Prism 10. (GraphPad Software, Version 10.4.1).

Scanning electron microscopy

Scanning electron microscopy (S-3700N) was used to examine the surface characteristics of the microspheres. The dried microspheres were mounted on brass stubs using double-sided adhesive tape and then coated with gold. The samples were scanned at a voltage of 10 kV, with a magnification range of 100–2000X.

Differential scanning calorimetry (DSC)

DSC (Perkin-Elmer DSC 7, USA) was used to assess the thermal characteristics of the microspheres. Approximately 3–5 mg of each sample was placed in an aluminium pan and heated at a rate of 10 °C per minute up to 300 °C in a nitrogen atmosphere with a flow rate of 20 mL/min. Thermograms of the microspheres were recorded over the temperature range of 50–300 °C.

***In vitro* drug release studies**

The drug release of RFN microspheres was evaluated using a USP Type I (basket) apparatus, following the method described by Devi et al. (2016). The apparatus was filled with 900 mL of simulated gastric fluid (SGF, pH 1.2) and maintained at 37 ± 0.5 °C with a rotation speed of 100 ± 2 rpm. Microspheres containing 50 mg of RFN were accurately weighed and placed in the basket. At predetermined intervals, 5 mL samples were withdrawn and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. SGF (pH 1.2) was used as a blank, and the drug concentration in the samples was measured spectrophotometrically at 475 nm (Jain et al., 2015).

Release kinetic studies

To understand the kinetics of drug release, various release kinetic models were applied to analyze the drug release data. The best-fit model was selected by comparing the R^2 values obtained from the different kinetic models.

Antimicrobial activity/Antitubercular activity

Staphylococcus aureus (S. aureus) was used as a model strain due to its well-characterized nature and ease of handling under standard biosafety conditions. Testing against *M. tuberculosis* requires BSL-3 containment, which was not available during this study.

The antibacterial activity of RIF-loaded microspheres was evaluated against *S. aureus.* Bacterial spores were initially added to 5 mL of Mueller-Hinton broth and incubated for 12 hours. Subsequently, 0.1 mL of the culture was transferred into another 5 mL of Mueller-Hinton broth to achieve a final concentration of approximately 3×10^8 colony-forming units (cfu)/mL.

Meanwhile, molten Mueller-Hinton agar was poured into sterile Petri dishes and allowed to solidify. Sterile filter discs with a diameter of 1 cm were immersed in PBS containing the pure drug, while PBS containing RIF released from the RIF-loaded microspheres was placed at the centre of the plates. All samples were incubated at 37 °C for 18 hours, and the

results were compared with RIF-free microspheres. The diameter of the zone of inhibition was measured. All antibacterial activity experiments were performed in triplicate (Jain et al., 2008).

Stability study

Stability testing of the formulation was conducted under different storage conditions: 25 °C ± 2 °C /60% RH ± 5% RH and refrigerated conditions (2–8 °C) for a period of three months, following ICH guidelines. The formulation was stored in aluminium foil throughout the study. At predetermined intervals, the samples were withdrawn and evaluated for physical appearance, buoyancy, and drug content. Drug content and buoyancy were measured on days 30, 60, and 90.

RESULTS AND DISCUSSIONS

Drug compatibility studies

By UV spectrophotometry

Drug–excipient compatibility was evaluated using UV spectrophotometry by scanning solutions of the drug, the excipient, and a physical mixture of the drug and excipient separately, over the wavelength range of 400–800 nm. The spectra were shown in Figure 1. The results reveal that the UV absorption maxima of the physical mixture overlapped with those of the drug, indicating no interaction between the drug and the polymer. Thus, the drug was found to be compatible with the excipient.

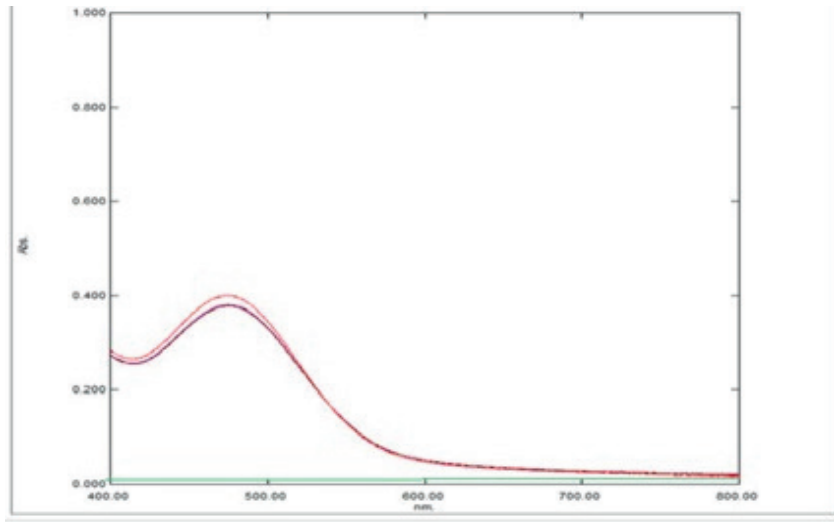
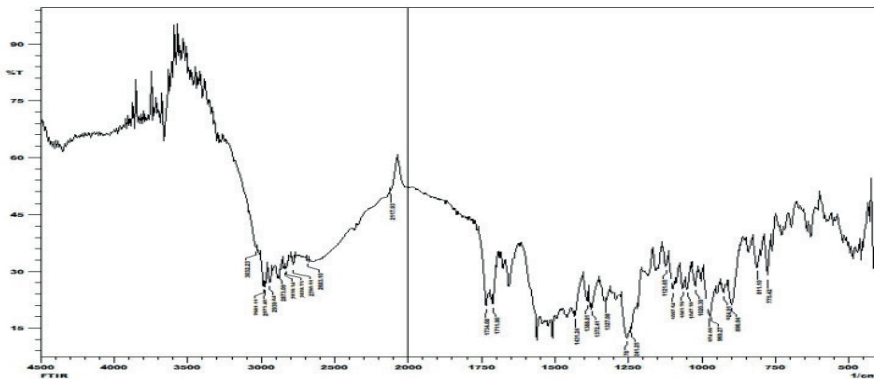


Figure 1. Compatibility study of Rifampicin and Eudragit S100 by using the UV spectrophotometric method

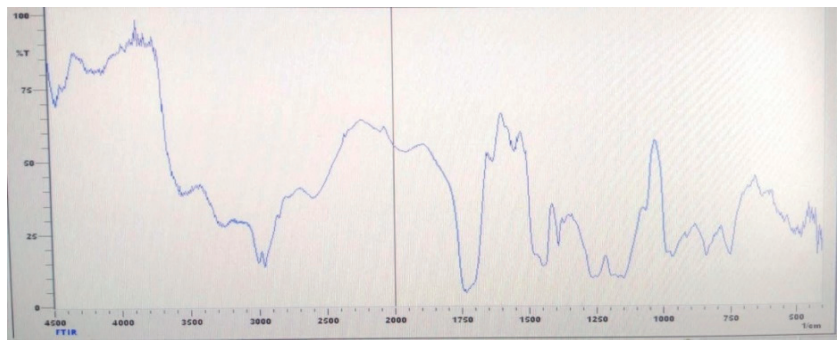
By fourier-transformed infrared spectroscopy (FT-IR)

The drug was identified using FTIR, and the spectra are shown in Figure 2 (A–D). The characteristic peaks for all functional groups of RFN—C=O acetyl stretching, CH_3O symmetric stretching, $-CH_3$ stretching, and –C=N asymmetric bending—were observed at 1734.05, 2828.73, 2934.04, and 1654.03 cm^{-1}, respectively. Peaks corresponding to the functional groups C=O (1749 cm^{-1}) and OH (1248 cm^{-1}) were observed in the FTIR spectrum of Eudragit S100 (Figure 2B).

All the functional group peaks of the drug appeared in the FTIR spectra of the physical mixture of drug and polymer, indicating that there was no interaction between the drug and the polymer (Figure 2C and 2D). Similar findings of drug–polymer compatibility via FTIR have been reported for rifampicin microspheres (Goyal et al., 2011) and for other drugs in Eudragit-based systems (Verma et al., 2020).



(A)



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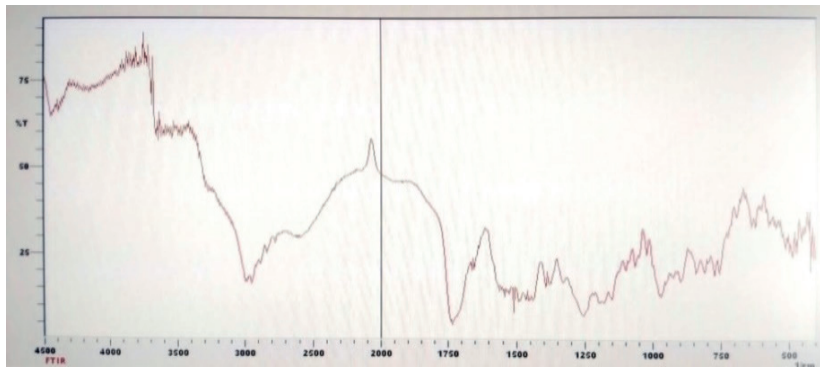


(C)

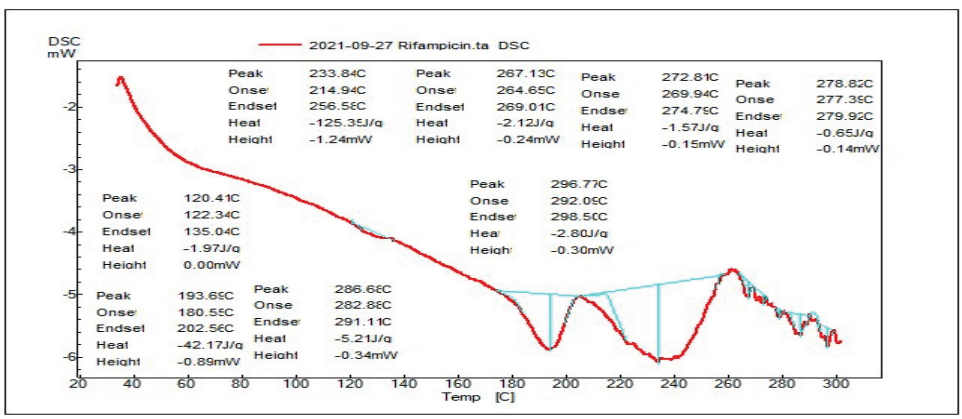
(D)

Figure 2. FTIR spectrum of (A) RFN, (B) Eudragit S100, (C) Physical mixture of Drug and Eudragit S100, (D) Overlay FTIR spectra of RFN, Eudragit S100, and Physical mixture of Drug and Eudragit S100

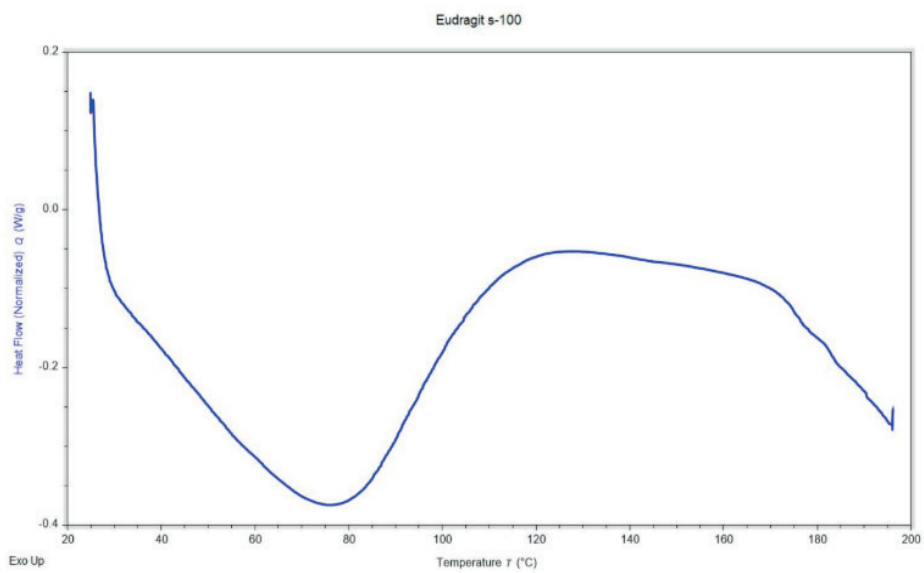
By differential scanning colourimetry (DSC)

RFN can exist in two polymorphic forms: form I and form II, consistent with previously reported transitions (Patel et al., 2006). Form I of RFN is less chemically stable at higher temperatures and decomposes before melting at 258 °C. The DSC thermogram of pure RFN displayed an endothermic peak at 193.69 °C, corresponding to the melting of form II, followed by an exothermic peak at 205.62 °C, indicating the crystallization of form I. An endothermic peak identified the decomposition of RFN at 233.84 °C (Figure 3A).

The DSC thermogram of Eudragit S100 displayed a broad endothermic peak at 77.49 °C, as shown in Figure 3B. In the physical mixture of drug and polymer, a single endothermic peak was observed at 195.7 °C, similar to the DSC thermogram of the pure drug, indicating no interaction between the drug and polymer (Figure 3C and 3D). These results suggest that the polymer is compatible with RFN for the design of microspheres. The absence of additional or missing peaks further supports the conclusion that no thermal degradation or interaction occurred, consistent with the findings of Maurya et al. (2012) for RFN-loaded microcapsules.



(A)



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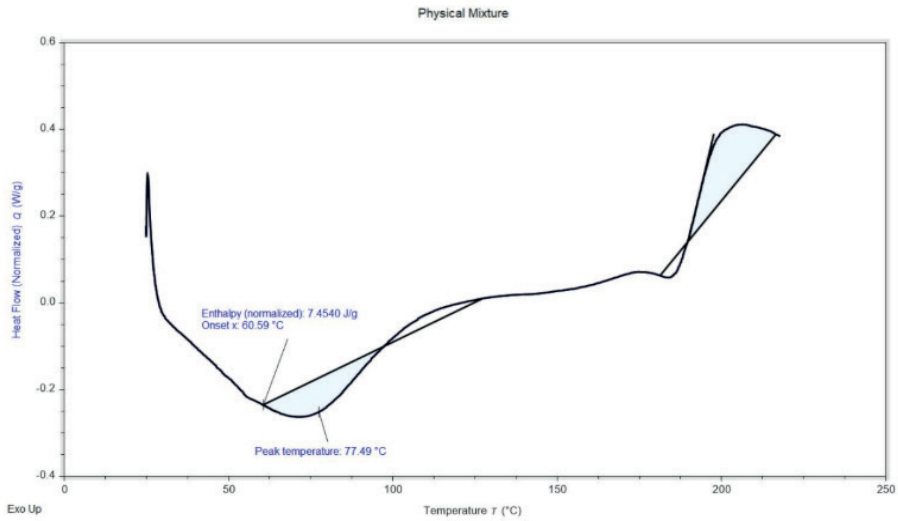

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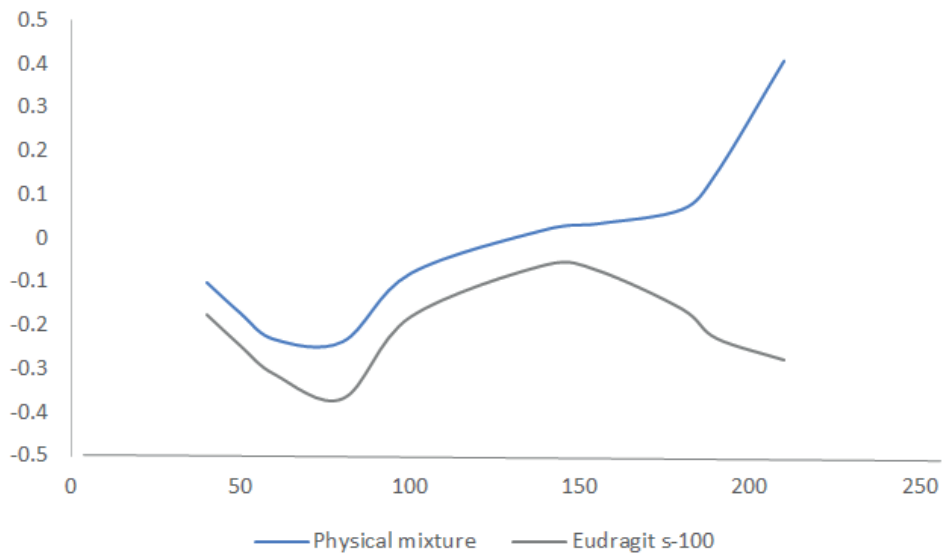


(D)

Figure 3. DSC thermogram of (A) Rifampicin, (B) Eudragit S 100, (C) Physical mixture of Drug and Eudragit S 100, (D) Overlay DSC thermogram of Eudragit S100, and Physical mixture of Drug and Eudragit S100

Formulation of Rifampicin floating microspheres

RFN microspheres were successfully prepared using the solvent evaporation technique by varying formulation variables, such as polymer concentration (Eudragit S100), and process variables, including stirring speed (300, 400, and 500 rpm) and stirring time (1, 1.5, and 2 hours), while keeping the quantity of RFN constant.

Evaluation of rifampicin-loaded floating microspheres

Shape of floating microspheres

All floating microspheres were observed to be spherical solid particles under an optical microscope (Figure 4A) and appeared as free-flowing powder (Figure 4B).

(A) (B)

Figure 4. (A) Microscopic image of RFN floating microspheres (FES4), (B) Final floating microspheres (FES4)

Production yield of microspheres

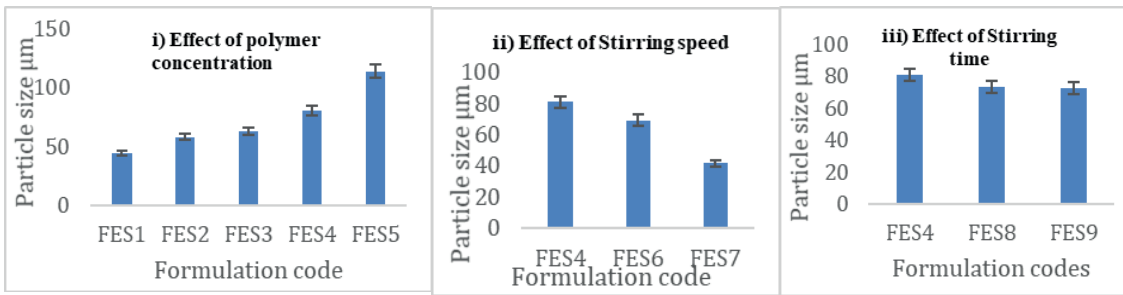
All floating microsphere formulations exhibited yield percentages ranging from 71.87 ± 0.04% to 96.1 ± 0.07%, indicating efficient encapsulation using the solvent evaporation technique. Such high yields are favourable for scalability and are comparable to those reported in similar studies on floating microspheres (Bakshi, 2016; Phutane et al., 2010)

Particle size

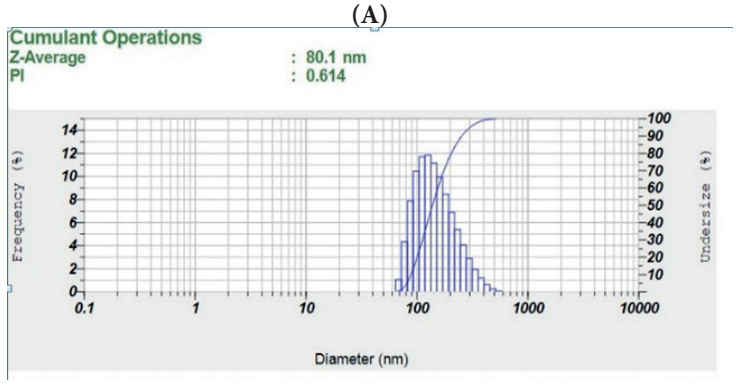
As the microspheres are administered orally, particle size plays a significant role in achieving prolonged gastric retention of the drug formulation and, consequently, its therapeutic performance. The particle size of the floating microspheres increased from 41.66 ± 1.77 µm to 114.16 ± 2.88 µm as the ratio of Eudragit S100 increased from 1:1 to 1:5 (Figure 5Ai and B), due to the increased viscosity of the medium and resistance to shear during emulsification. At higher viscosities, enhanced interfacial tension and reduced shearing efficiency result in the formation of larger particles (FES1–FES5). This trend is consistent with the findings of Agarwal et al. (2022) and Gupta et al. (2014), where polymer content significantly influenced microsphere diameter and flotation potential. A statistically significant difference in particle size was observed ($p < 0.05$).

To prevent foaming on the emulsion surface—which could affect the rate of dichloromethane evaporation and, in turn, the formation of floating microspheres—a minimum stirring speed of 300 rpm and a stirring time of one hour were employed. Among the first five formulations, FES4 exhibited the smallest particle size and was selected for further optimization by modifying parameters such as stirring speed and stirring time.

When the propeller rotation speed was increased from 300 to 500 rpm, the average particle size significantly decreased from 80.83 ± 1.40 µm to 41.66 ± 1.44 µm (Figure 5Aii), possibly due to foam formation and breakage of microspheres at high speed. Increasing the stirring time from 1 to 2 hours resulted in a slight decrease in particle size, from 80.83 ± 1.40 µm to 72.73 ± 1.41 µm (Figure 5Aiii), likely due to irregular microsphere formation.



(A)



(B)

Figure 5. (A) Effect of (i) polymer concentration, (ii) stirring speed, and (iii) stirring time on particle size of RFN floating microspheres (n = 3) (B) Particle size analysis of final floating microspheres (FES4)

Drug content and entrapment efficiency

As the polymer ratio increased from 1:1 to 1:5, the drug content increased up to the FES4 formulation and then decreased, likely due to polymer hardening, as reported previously (Nila et al., 2014). When the rotation speed was increased from 300 to 500 rpm, a slight decrease in drug content was observed. A modest increase in entrapment efficiency was noted with increasing polymer ratio, ranging from 88.21 ± 0.10% to 93.16 ± 0.45% (Figure 6). The differences between the floating microspheres were statistically significant ($p < 0.05$) based on ANOVA analysis.

Compared with previously reported gastro-retentive microsphere systems, the optimized FES4 formulation demonstrated superior performance in terms of entrapment efficiency, floating behavior, and sustained drug release. Goyal et al. (2011) reported entrapment efficiencies of approximately 85% for rifampicin-loaded microspheres using ethyl cellulose and HPMC, whereas the present study achieved up to 93.16% with Eudragit S100. The improvement in entrapment efficiency can be attributed to increased matrix density, consistent with earlier work by Bhadouriya et al. (2012).

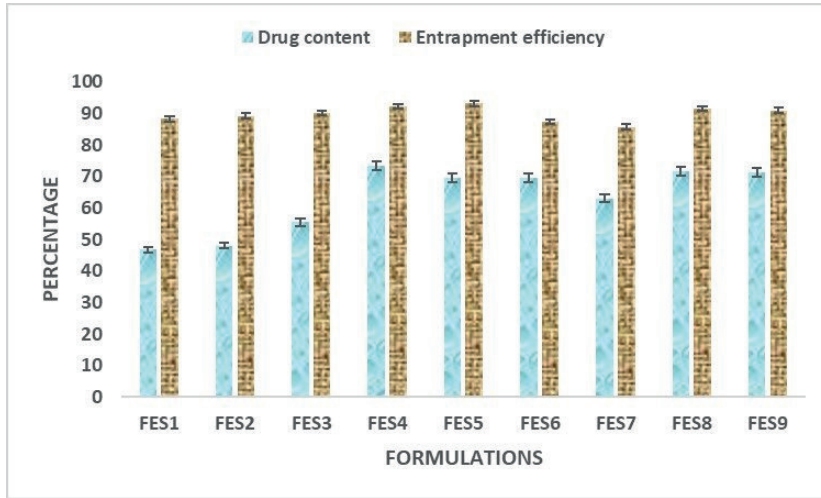
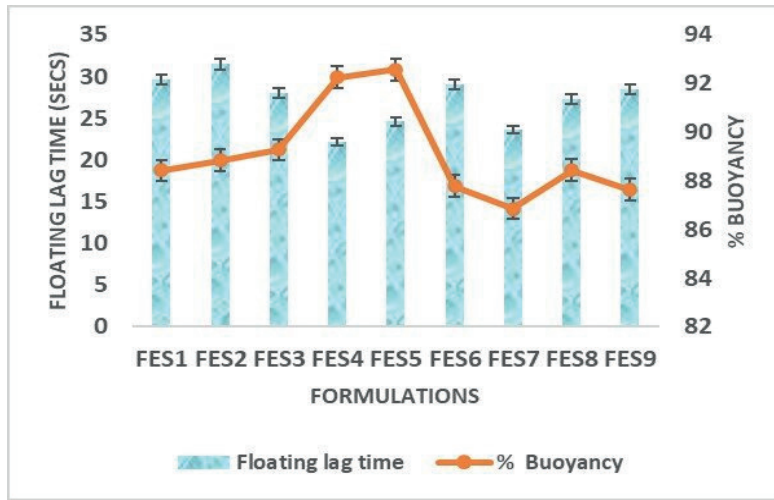


Figure 6. Effect of polymer concentration, stirring speed, and stirring time on drug content and entrapment efficiency of various RFN floating microspheres (n = 3)

Floating lag time and *in vitro* buoyancy

The improved buoyancy observed with higher polymer concentrations is consistent with the findings of Gupta et al. (2014), who reported a similar trend in Eudragit-based microspheres. A higher percentage of buoyancy is essential for the drug formulation to achieve prolonged gastric residence, as noted in previous studies (Ammar et al., 2016). The *in vitro* floating behavior of all the prepared microspheres was evaluated. According to the literature (Agarwal et al., 2022; Pingale & Amrutkar, 2021), floating ability increases with particle size. All formulations floated for 24 hours, as shown in Figure 7A. The percent buoyancy for all developed formulations exceeded 86%, with the FES4 formulation exhibiting the highest buoyancy at 92.56% (Figure 7B). Similarly, Gupta et al. (2014) reported Eudragit-based floating microspheres of famotidine with buoyancy exceeding 90%, comparable to the 92.56% buoyancy observed for FES4 in the present study.

(A)



(B)

Figure 7. (A) Photograph showing the floatability of the developed formulation up to 24 hours.

(B) Effect of polymer concentration, stirring speed, and stirring time on floating lag time and % buoyancy of various RFN microspheres (n=3)

Swelling index of microspheres

The swelling index increased from 48 ± 0.04% to 73 ± 0.09% with increasing polymer concentration, as the water-absorbing capacity of the microspheres depends on the polymer content. In contrast, variations in rotation speed and stirring time did not produce any significant changes in the swelling index, as shown in Figure 8. This observation is consistent with previous studies by Kawashima et al. (1992), Mukund et al. (2012), and Pingale & Amrutkar (2021) which identified polymer concentration as a key determinant of swelling behaviour.

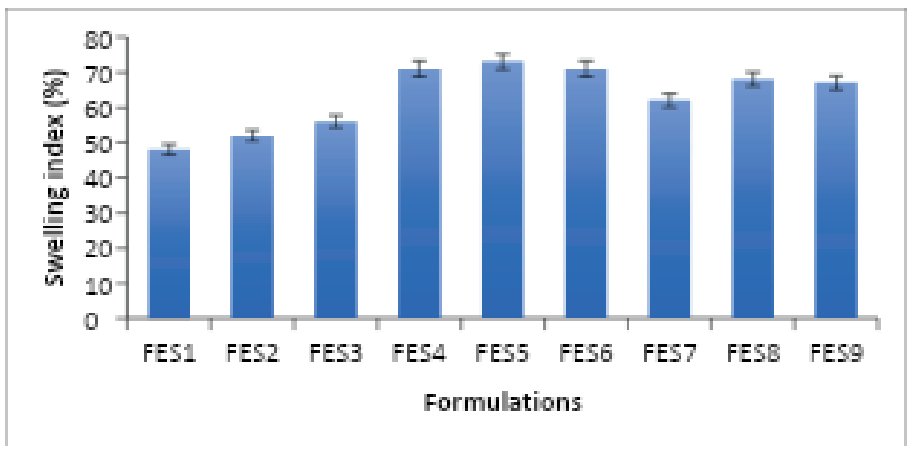


Figure 8. Effect of polymer concentration, stirring speed, and stirring time on swelling index of RFN microspheres (n=3)

Scanning electron microscopy

SEM was used to evaluate the surface characteristics of the floating microspheres, and the results are presented in Figure 9. The microspheres were spherical with smooth surfaces and a hollow inner cavity. They did not exhibit agglomeration, as evident from the photomicrograph. This structure supports prolonged buoyancy and controlled drug release, consistent with observations reported in floating systems by Bhadouriya et al. (2012) and Kawashima et al. (1992).

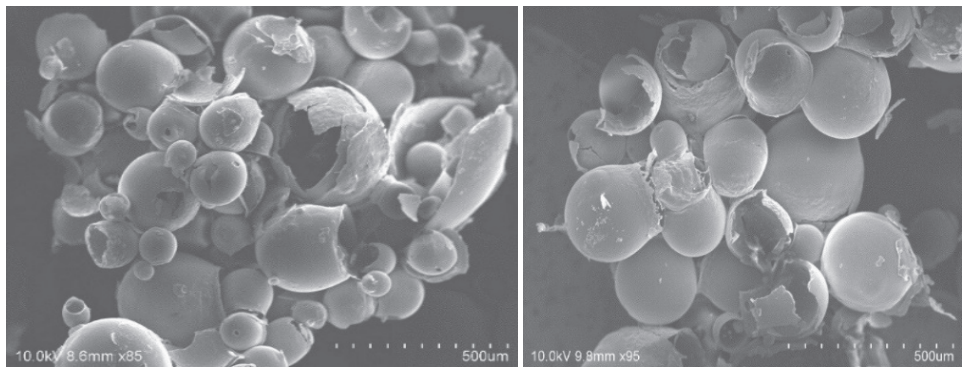


Figure 9. SEM images of RFN FES4 microspheres

***In vitro* drug release studies**

The results suggest that all formulations exhibited a noticeable initial burst release during the first 2 h, followed by a sustained release profile over the next 24 h, as shown in Figure 10, which presents the quantitative dissolution data. The initial burst release may be attributed to the drug present on the surface of the microspheres, a pattern also observed by Kumar, Jat & Kumar (2019), Kumar & Srivastava (2021), and Pingale & Amrutkar (2021).

An increase in rotation speed from 300 to 500 rpm resulted in enhanced drug release, likely due to a reduction in microsphere particle size. The smaller particle size increases the surface area, leading to faster dissolution. Drug release from the formulations at 24 h ranged from 67.8 ± 2.3% to 91.4 ± 1.9%, with the FES4 formulation exhibiting significantly higher release compared to the others ($p < 0.01$, ANOVA).

The release profile of FES4 is consistent with non-Fickian diffusion behaviour (n = 0.367) and follows the Higuchi model ($R^2 = 0.984$), indicating that drug release is governed by both diffusion and polymer matrix erosion.

Similar kinetics have been reported in Eudragit-based sustained-release systems (Devi et al., 2016; Verma et al., 2020). The Higuchi model behaviour of FES4 aligns with findings from Maurya et al. (2012) and Verma et al. (2020), confirming diffusion-controlled release.

Release kinetics studies

The release kinetics of the floating microspheres were analysed and are presented in Table 2. The results indicate that the Higuchi model provided the best fit, exhibiting the highest R^2 value (0.9840). The n value was 0.367, suggesting a non-Fickian transport mechanism.

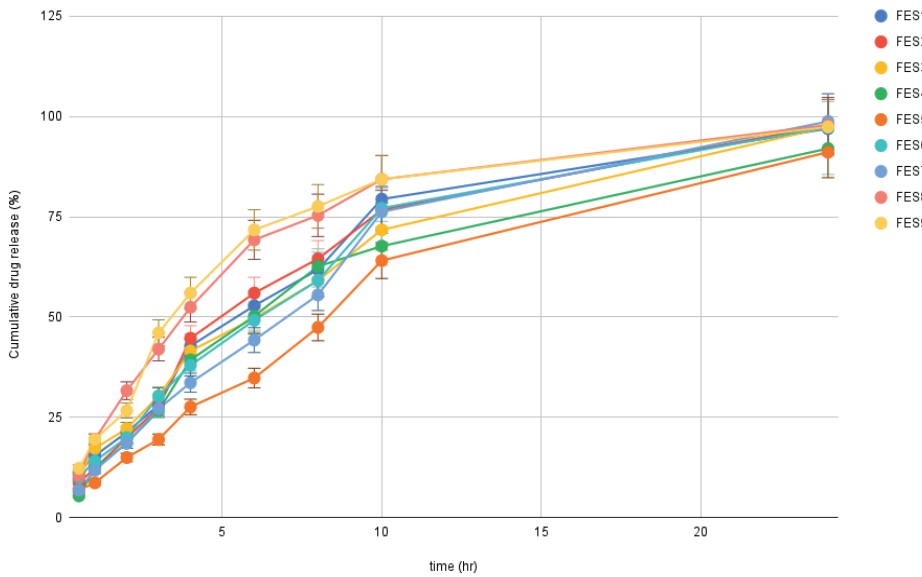


Figure 10. Cumulative percentage drug release from various Eudragit S-100-based floating microspheres

Table 2. Drug release kinetics of FES4 formulation

Formulation code	Zero order	First order	Higuchi	Hixson-crowell	Korsmeyer peppas	Best fit model
R	0.9736	0.9807	0.984	0.8832	0.949	Higuchi
K value	3.595	0.107	21.610	0.031	37.388	

Anti-microbial activity

The antibacterial efficacy of rifampicin (RFN)-loaded floating microspheres was evaluated against *S. aureus*. A clear zone of inhibition was observed, as shown in Figure 11A and 11B. The mean zone of inhibition measured 21.32 ± 0.21 mm for the pure drug and 15.25 ± 0.10 mm for the optimised formulation (FES4), while no inhibition was observed for the blank microspheres. This confirms the sterility of the formulation and indicates the absence of residual antimicrobial effects from the excipients (Goyal et al., 2011; Jain et al., 2008; Pingale & Amrutkar, 2021).

S. aureus was selected as a model organism for preliminary antimicrobial testing due to its classification as a biosafety level-2 (BSL-2) pathogen, allowing evaluation under standard laboratory conditions. In contrast, *M. tuberculosis,* the primary causative agent of tuberculosis and the main therapeutic target of rifampicin, is a BSL-3 organism that requires specialised high-containment facilities, which were not available for the present study.

The primary objective of this study was to develop and systematically characterise rifampicin-loaded floating microspheres designed for enhanced gastric retention and sustained drug release. Within this framework, *S. aureus* was strategically selected as a safe and practical model organism to serve multiple purposes. It enabled assessment of whether the microencapsulation process effectively preserved the antimicrobial activity of rifampicin, facilitated preliminary evaluation of the formulation's antibacterial potential, and supported iterative optimization of critical formulation parameters—such as polymer composition, drug entrapment efficiency, and release kinetics. Importantly, the use of *S. aureus* allowed these evaluations to be conducted under standard laboratory conditions, thereby circumventing the stringent biosafety requirements associated with *M. tuberculosis* (a BSL-3 pathogen), thus streamlining early-stage formulation development.

S. aureus is extensively used in pharmaceutical research as a surrogate organism for preliminary screen-

ing of antibacterial activity. Its application in this study enabled rapid and efficient evaluation of drug integrity following encapsulation, as well as verification of the formulation's sterility. This approach aligns with standard practices in formulation research, where initial antimicrobial validation is typically performed using non-mycobacterial strains before advancing to more complex, pathogen-specific assays (Bhise et al., 2010).

Although *S. aureus* is not etiologically linked to tuberculosis, its use in this context is scientifically justified and widely accepted for preliminary antimicrobial evaluation. Further investigations using *M. tuberculosis* or suitable non-pathogenic surrogates such as *Mycobacterium smegmatis* are warranted to directly confirm the anti-tubercular potential of the developed system under appropriate biosafety conditions.

Moreover, rifampicin exhibits broad-spectrum antibacterial activity, including notable efficacy against *S. aureus,* particularly in complex clinical scenarios. Owing to its lipophilic nature, rifampicin can effectively penetrate biofilms and intracellular compartments, making it especially valuable in the treatment of persistent *S. aureus* infections such as prosthetic joint infections and infective endocarditis. Additionally, rifampicin is commonly employed in combination with other antibiotics to enhance therapeutic outcomes and prevent the rapid emergence of resistance—a significant concern when the drug is used as monotherapy. In clinical practice, rifampicin has also been utilised to eradicate *S. aureus* nasal carriage in healthcare settings to reduce the risk of infection transmission, although its application in this context is limited due to resistance development when used alone.

Evaluating the antibacterial activity of rifampicin-loaded floating microspheres against *S. aureus* is a justified and effective approach for confirming the preservation of drug bioactivity post-encapsulation and for supporting the formulation's antimicrobial potential. While not specific to tuberculosis, the inclusion of *S. aureus* as a model organism provides valuable insights during the early development phase. Future studies are planned to validate the anti-tubercular efficacy of the formulation against *M. tuberculosis* or appropriate mycobacterial surrogate models.

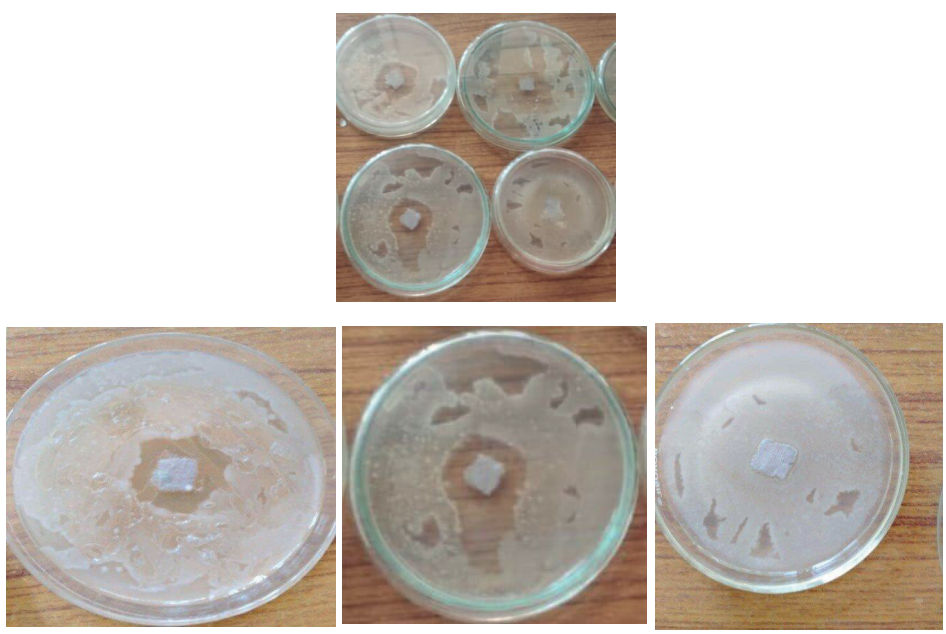


Figure 11. (A) Zone of Inhibition of FES4, formulation, Drug and Blank against *S. aureus* after 18 h incubation at 37°C

Stability studies

The stability evaluation of FES4 was carried out over three months in compliance with ICH guidelines, during which the percentage of buoyancy and drug content was assessed. The findings, as shown in Figure 12, indicate negligible variation in both drug content and buoyancy. This suggests that the developed formulation maintains its performance during prolonged storage—an essential criterion for regulatory approval and marketability, as highlighted in stability studies by Khan et al. (2020).

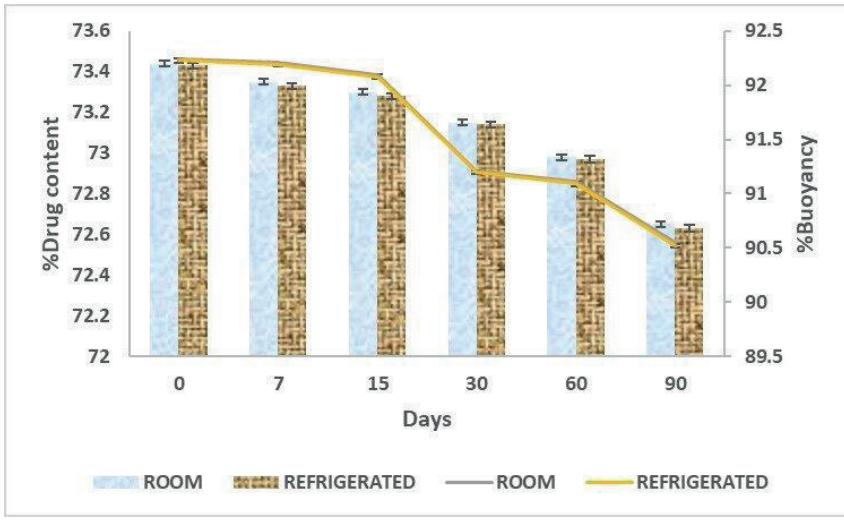


Figure 12. Stability studies of FES4 for 90 days in refrigerated temperature and 25°C±2°C/60% RH ± 5% RH

CONCLUSION

In conclusion, the development of gastro-retentive floating microspheres containing rifampicin (RFN) yielded promising results as a controlled drug delivery system, effectively addressing the challenges associated with RFN's short half-life and limited gastric residence. Drug–excipient compatibility was confirmed through UV, FTIR, and DSC analyses, indicating no significant interactions between rifampicin and the formulation excipients.

Floating microspheres were successfully prepared using Eudragit S100 via the solvent evaporation technique, and the influence of key formulation parameters—including stirring speed and polymer concentration—was systematically evaluated. These variables significantly influenced entrapment efficiency, buoyancy, and particle size.

Among all batches, formulation FES4 (drug-to-polymer ratio of 1:4) was identified as the optimized formulation, exhibiting excellent buoyancy (92.24%), high entrapment efficiency (88.21–93.16%), and prolonged drug release. The drug release profile of FES4 followed the Higuchi kinetic model and was governed by non-Fickian diffusion, suggesting a combination of diffusion- and erosion-controlled mechanisms.

Stability studies conducted under ambient and room temperature conditions for 90 days confirmed the robustness of the formulation, showing no significant changes in key parameters and indicating good shelf-life stability.

Compared with previously reported gastro-retentive microsphere systems, the optimized formulation (FES4) demonstrated superior performance in terms of entrapment efficiency, floating behavior, and sustained drug release. Notably, the innovative use of Eudragit S100—a polymer typically reserved for colon-targeted delivery—within a gas-trapping floating system represents a novel approach to enhance gastric

retention. This unconventional application, in combination with solvent evaporation and gas-trapping techniques, resulted in improved performance over systems employing ethyl cellulose, HPMC, or Eudragit RL/RS and Eudragit RLPO (Bhise et al., 2010; Patel et al., 2012; Pingale & Amrutkar, 2021).

These findings collectively highlight the originality, efficacy, and potential clinical relevance of the Collectively, these findings underscore the originality, efficacy, and potential clinical relevance of the developed Eudragit S100-based floating microsphere system for rifampicin delivery.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed to data collection, processing, writing, revision of the draft, reading, and approval of the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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