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Original Research Article

Formulation and characterization of sustained-release rifampicin-loaded beeswax microparticles

Yousra Bahron[1]*, Mahmud Treki[2]
[1]Department of Pharmaceutics, Faculty of Pharmacy, University of Zawia, [2]University of Tripoli, Libya

For correspondence:* ***Email:* *Y.bahron@zu.edu.ly;* ***Tel:*** *00218927236541;* ***ORCID:*** *0009-0003-5524-0546*

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Abstract

Purpose: *To develop rifampicin-loaded microparticles as a polymer-based delivery system capable of providing sustained drug release.*
Methods: *A meltable dispersion and cooling technique was used to create the microparticles. Various ratios of beeswax were employed as a release-controlling matrix in an effort to improve the formed microparticles' effectiveness, additional excipients, specifically cetyl alcohol (CA) and polyvinylpyrrolidone (PVP), were added to the formulations. The micromeritic characteristics, drug content, drug-polymer compatibility, antimicrobial activity, and in vitro drug release profile of the microparticles were all described. Additionally, the impact of the excipients' incorporation on drug release was examined.*
Results: *The microparticles had good flow characteristics and were distinct granules. The drug content ranged from 65.1 to 112.9 %, with a yield value of up to 98.9 %. The medication and the additional polymers did not interact, according to a Fourier transform infrared analysis. The granules possessed antibacterial qualities just as potent as the free medication, according to the antimicrobial investigation. Depending on the concentration of beeswax, in vitro release experiments showed that the drug release was maintained for up to 6 h; however, the addition of the excipients had no significant impact on drug release ($p > 0.05$).*
Conclusion: *A sustained-release dosage form of rifampicin has been produced by a simple and reproducible method.*

Keywords: *Beeswax, Microparticles, Rifampicin, Sustained release*

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INTRODUCTION

Tuberculosis is a chronic infectious disease caused by acid-fast bacterium *Mycobacterium tuberculosis,* affecting millions of people worldwide. Rifampicin, in particular, is considered to be one of the first-line oral treatments for tuberculosis. Rifampicin possesses physicochemical and biopharmaceutical characteristics that are important for its formulation and drug-release behavior. It is a zwitterionic compound with pH-dependent solubility, and its dissolution behavior may affect its oral absorption [1]. In addition, rifampicin has a

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relatively short half-life of approximately 2.5 h, which may decrease upon repeated administration due to autoinduction of its metabolism [2]. These characteristics make rifampicin a suitable model drug for investigating sustained-release drug delivery systems. However, treatment of tuberculosis now includes multiple drug regimens for long periods, which are linked to unwanted side effects and poor patient compliance [3]. Extensive efforts have been made to develop sustained-release drug delivery systems to improve patient compliance and reduce fluctuations in drug plasma concentrations [4]. Matrix systems are widely used for sustained-release drug delivery because they provide a relatively simple approach for prolonging drug release; the drug is dispersed within a polymeric or lipid-based matrix, and drug release may occur through diffusion, dissolution, or erosion mechanisms. Hydrophobic and waxy materials can retard drug release by limiting fluid penetration and controlling drug diffusion through the pores of the matrix [5].

Beeswax is a natural lipid excipient with hydrophobic and thermoplastic properties that has been used in pharmaceutical formulations as a matrix-forming and release-modifying material. Its hydrophobic nature can retard drug diffusion and dissolution, making it useful in controlled- and sustained-release drug delivery systems. Beeswax has been investigated in various drug delivery systems, including microspheres and other lipid-based formulations [6,7]. In the present study, sustained-release microparticles were prepared using the melt dispersion method.

EXPERIMENTAL

Materials

Rifampicin (purity > 95 %, Solarbio, China), beeswax and cetyl alcohol (BDH Laboratory Reagents, England), polyvinylpyrrolidone (PVP, Merck, Germany), ethanol (99.6 %, Riedel-de Haen, Germany), methanol (Sigma, Iceland), chloroform (Sigma, Iceland), distilled water.

Preparation of rifampicin microparticles

Rifampicin-loaded microparticles were prepared by a technique known as the melt dispersion technique [8]. The formulations prepared by this method are described in Table 1. Beeswax was employed as a release-controlling polymer to prepare microparticles with various drug-to-polymer ratios. For formulation F1, 1 g of beeswax was melted in a 50 mL beaker heated in a water bath at 60 °C. The amounts of beeswax used for the other formulations were adjusted according to the compositions presented in Table 1.

Ten milliliters of ethanol was added to the molten wax to make it thinner, and the predetermined amount of rifampicin was suspended in the melt. The melt was removed from the water bath and gradually added to a 150 mL pH 4.5 phosphate buffer solution, which had previously been heated to 5 °C above the melting point of beeswax, in order to reduce the solubility of the drug. A magnetic stirrer was used to mechanically stir the whole mixture at 900 rpm as it cooled to room temperature. The microparticles were obtained by filtration through Whatman filter paper, air-dried at room temperature for 48 h and kept in a desiccator for further studies [7].

Formulation F2 was modified by the incorporation of cetyl alcohol (CA) in different ratios and given the code (F7-F9), while formulation F5 was modified by the addition of PVP in an effort to increase the obtained microparticles' efficiency and given the code (F6). Thus, F2, F7, F8, and F9 had the same rifampicin content, allowing comparison of the effect of increasing CA concentration, while F5 and F6 had the same rifampicin content, allowing assessment of the effect of PVP incorporation (Table 1). For each formulation, the amount of microparticles equivalent to 50 mg of rifampicin was filled into each capsule to ensure a uniform rifampicin dose across all formulations.

Characterization of rifampicin-loaded microparticles

Microparticle yield

Equation 1 was used to determine the microparticle yield, which was stated as a percentage of the total weight of dried microparticles produced relative to the total weight of medication and polymer utilized [9].

Percent yield =

$$\frac{total\ weight\ dried\ microparticles\ (g)}{total\ weight\ of\ drug\ and\ polymer\ used(g)} \times 100 \quad (1)$$

Table 1: Composition of rifampicin-loaded microparticles

Ingredient	Formulations								
	F1 %(w/w)	*F2 %(w/w)*	*F3 %(w/w)*	*F4 %(w/w)*	*F5 %(w/w)*	*F6 %(w/w)*	*F7 %(w/w)*	*F8 %(w/w)*	*F9 %(w/w)*
Rifampicin	50	40	33.3	25	16.6	16.6	40	40	40
Beeswax	50	60	66.6	75	83.4	73.4	55	50	45
CA	-	-	-	-	-	-	5	10	15
PVP	-	-	-	-	-	10	-	-	-

Determination of bulk and tapped density

The bulk density (ρ_b) and the tapped density (ρ_t) were measured using a 5 mL dry graduated cylinder. The bulk density is the ratio of the predetermined amount of microparticles to the bulk volume (V_0), while in measuring tapped density, the cylinder was given 100 taps and the resulting tapped volume (V_t) was determined [9]. Equations 2 and 3 were used to calculate the bulk and tapped density. Carr's index (CI) and Hausner ratio (HR) were calculated using Eq 4 and 5.

$$\rho_b = \frac{w}{V0} \quad (2)$$

$$\rho_t = \frac{w}{Vt} \quad (3)$$

$$CI = (\rho_t - \rho_b/\rho_t)100 \quad (4)$$

$$HR = \rho_t/\rho_b \quad (5)$$

where w is the microparticle weight in g; V_0 is the apparent bulk volume in mL; V_t is the ultimate tapped volume in mL; and ρ_b and ρ_t are the bulk density and tapped density in g/mL.

Microparticles' flow characteristics

An easy way to assess a powder's flowability is using the angle of repose. The fixed funnel method, which involves pouring microparticles from a funnel at a specific height onto a chosen base with known roughness qualities, is the most often used technique for calculating the angle of repose. The angle of repose was calculated using equation 6 once the material pouring had stopped and the heap had reached a predefined height [10].

$$\theta = \tan^{-1}\frac{h}{r} \quad (6)$$

where (θ) is the angle of repose and (h) and (r) are the height and radius of the heap formed, respectively.

Particle size analysis

The size distribution analysis of the microparticles was performed using a sieve analyzer [7]. Five sieves ranging in size from 125 to 710 µm were employed. The frequency distribution was calculated for every range of microparticle size.

Morphological study

An optical microscope (Leica DM1000, Leica Microsystems, Wetzlar, Germany) was used to examine the external morphology and typical shape of microparticles [11].

Drug content uniformity

A UV spectrophotometer was used to estimate the amount of rifampicin present in the microparticles. For each formulation, 50 mg of the drug's microparticles were added to a conical flask, and 25 mL of methanol was added. To ensure complete drug release from the microparticles, the flask was shaken for 24 h at room temperature. Whatman filter paper was then used to filter the suspension. After diluting two milliliters of the filtrate with 50 mL of methanol, the drug content was measured using spectrophotometry at 334 nm and computed using a pre-constructed calibration curve. The determinations were carried out in triplicate [12].

Drug-polymer compatibility studies

Fourier transform infrared (FTIR) spectroscopy (Shimadzu, IR Prestige-21, Kyoto, Japan) was used to determine drug-polymer compatibility. To extract rifampicin from the prepared microparticles, 0.3 g of microparticles were dissolved in 10 mL of chloroform and filtered. The filtrate was then evaporated to dryness, and the obtained residue was examined by FTIR spectroscopy over a wavenumber range of 400-4000 cm^{-1} using the potassium bromide pellet method. The spectrum was compared with that of pure rifampicin [13].

Antimicrobial study

The antimicrobial activity of formulated rifampicin microparticles was determined using the agar gel diffusion method. The Zawia Medical Research Center provided the cultured microorganism *Staphylococcus aureus*, which was then spread

out on the nutrient medium. After adding the prepared suspension (equivalent doses) of selected microparticles (F2) and pure drug to the well, it was refrigerated at 4 °C for pre-diffusion for 20 minutes. After a 24-hour incubation period at 37 °C, the Petri plates were examined to determine the zone of inhibition [12].

In vitro release studies

Rifampicin-loaded microparticles equivalent to the drug dose (50 mg) were placed inside a colourless transparent hard gelatin capsule (size "0"). The USP dissolution apparatus, type I basket method (Pharma Test PTWS 1420, Germany), was used to conduct dissolution studies. These capsules were then placed in the dissolution basket, wrapped in surgical gauze to prevent small particles from escaping into the dissolution medium, and immersed in 900 mL of distilled water at 37 ± 0.5 °C. The stirring rate was kept at 100 rpm throughout the release studies. Throughout the release study, sink conditions were maintained. Five milliliters of samples were taken out at appropriate intervals, filtered, and then examined at 334 nm using a UV spectrophotometer following the correct dilution. The dissolution volume was immediately maintained by adding an equivalent volume of new medium. Dissolution studies were carried out for up to 6 h. Triplicate determination of the drug release was carried out. The content of the drug was determined by a standard rifampicin calibration curve [12]. Several mathematical models were used to analyse the data and calculate the release constant and correlation coefficient (r^2; Equations 7 - 10) [14].

Zero order model $Q = Q_0 + k_0 t$ (7)

First order model $\ln Q = \ln Q_0 - K_1 t$ (8)

Higuchi model $Q = K_H t^{0.5}$ (9)

Korsmeyer-Peppas model $Q = K_p t^n$ (10)

where Q is the amount of drug released at time t, Q_0 is the value of Q at time zero, n is the transport exponent, and K is the rate constant.

Statistical analysis

Following a normality test, the IBM SPSS Statistics software program (version 23) was used to evaluate all data, which were given as mean ± standard deviation (SD). T-tests or one-way analysis of variance (ANOVA) followed by Tukey's test were performed, as appropriate.

RESULTS

Characterization of rifampicin-loaded microparticles

The percent yield and micromeritic properties of rifampicin microparticle formulations are shown in Table 2. The total yield percentage varied from 80.5 to 98.9 %. The bulk density of the formulations ranged from 0.20 to 0.33 g/mL, and the tapped density ranged from 0.30 to 0.42 g/mL. Carr's index ranged from 10.96 to 35.04 %, indicating stable packing, and the values of Hausner ratio > 1.34 indicate poor flow (F1, F7 and F9) [15]. The angle of repose ranged from 27.7 to 33.6 °.

Particle size analysis

The frequency distribution for each size range of the produced microparticles is shown in Figure 1. The amount of smaller particle-size microparticles decreased as the concentration of the polymer increased up to formulation F5, which has the highest wax concentration. The inclusion of CA in formulations F7-F9 has resulted in a higher amount of smaller particle-size microparticles compared to F2.

Morphological study

The microparticles exhibited a rough surface and an irregular shape.

Drug content uniformity

All batches had drug contents ranging from 65.1 % to 112.9 %, according to Table 3.

Drug-polymer compatibility studies

Figure 2A represents the FTIR spectrum of pure rifampicin, which has three principal peaks at 3472, 2894, and 1629 cm^{-1}, representing N-H, C-H, and keto group C=O vibrational stretch, respectively [16]. Figure 2B, the FTIR spectrum of extracted rifampicin did not show the presence of any additional peaks, and the prominent peaks were not affected. Therefore, drug-polymer compatibility was established.

Antimicrobial study

The diameter of the zone of inhibition was 3.85 ± 0.21 cm for the pure drug and 4.00 cm for formulation F2 against *Staphylococcus aureus*.

Table 2: The yield and micromeritic properties of rifampicin microparticle formulations

Formulation code	Yield (%w/w)	Bulk density (g/mL)	Tapped density (g/mL)	Angle of repose (θ°)	Carr's index (%)	Hausner ratio
F1	91.66	0.29±0.01	0.42±0.00	27.8±0.4	29.19	1.41
F2	95.58	0.26±0.02	0.33±0.01	33.6±1.8	22.48	1.29
F3	91.33	0.28±0.01	0.37±0.01	31.6±0.6	22.90	1.29
F4	97.60	0.29±0.03	0.39±0.01	30.0±1.3	24.70	1.32
F5	90.83	0.33±0.00	0.37±0.05	27.7±1.5	10.96	1.12
F6	80.50	0.27±0.03	0.34±0.01	29.9±3.0	19.64	1.20
F7	98.90	0.20±0.00	0.30±0.00	29.7±0.5	34.20	1.52
F8	97.16	0.20±0.01	0.31±0.02	28.4±0.1	35.04	1.53
F9	95.98	0.26±0.00	0.36±0.00	28.5±3.1	29.61	1.42

Mean of triplicate determinations ± standard deviation (SD)

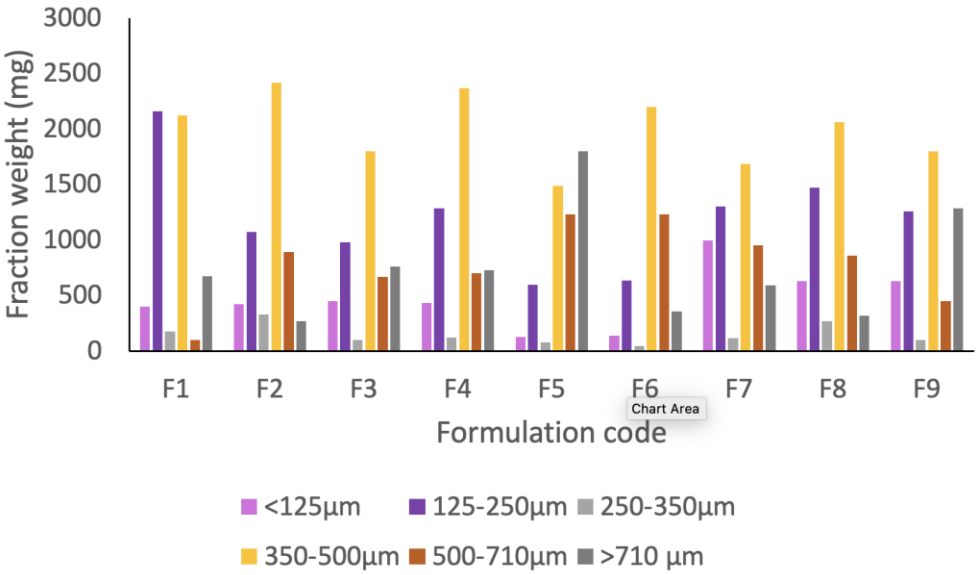


Figure 1: Frequency distribution for each size range of microparticle formulations

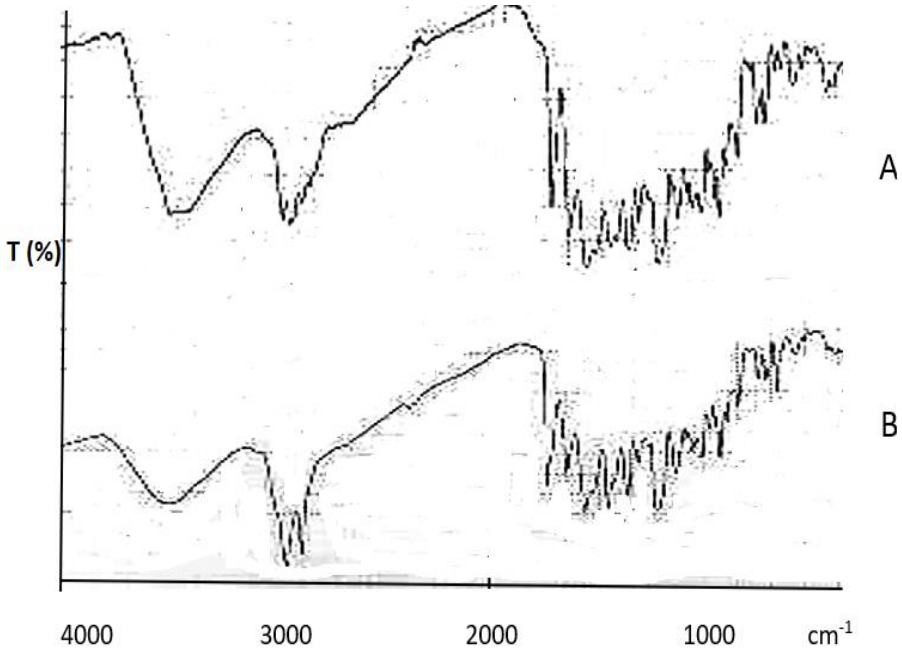


Figure 2: FTIR spectrum of (A) pure rifampicin, (B) extracted rifampicin

Table 3: The percentage of drug content uniformity of formulations F1-F9

Formulation	Concentration (mg/mL±SD)	Drug content (%)
F1	1.917±0.2	95.80
F2	2.179±0.1	108.90
F3	2.171±0.2	108.50
F4	1.883±0.3	94.20
F5	1.301±0.1	65.10
F6	1.477±0.1	73.80
F7	2.258±0.4	112.90
F8	2.050±0.1	102.50
F9	2.120±0.2	106.30

In vitro release studies

The impact of beeswax on rifampicin release from formulations F1-F5 is shown in Figure 3 A. The percentage of rifampicin release decreased from 101 % in F1 to 26.2 % in F5 after 6 h. This reduction may be related to the increased proportion of beeswax in the formulations, which could form a more hydrophobic matrix and retard drug diffusion, although differences in drug loading among the formulations should also be considered. The effect of incorporation of PVP is demonstrated in Figure 3 B. An independent sample t-test was run, and the results showed that there was no statistically significant difference ($p > 0.05$) in the percentage of drug release compared to F5. In comparison to microparticles composed of beeswax alone, particles formed from a combination of CA and beeswax did not further sustain the drug release (Figure 3 C); the difference was not statistically significant among the formulations ($p > 0.05$) based on a one-way ANOVA test.

DISCUSSION

Rifampicin was selected as a model drug in the present study because of its relevant physicochemical and biopharmaceutical characteristics, which make it suitable for investigating sustained-release drug delivery systems. In the present study, almost all formulations had a high yield percentage, up to 98.9 % w/w, which indicated that the simple method of preparation was efficient under the conditions employed. In consideration of the micromeritic properties, the bulk and tapped densities did not differ significantly ($p > 0.05$), indicating that the shape of microparticles was uniformly arranged [17]. The angle of repose showed free-flowing behavior, with degrees less than 33.6 ± 1.8 ° [7]. This means that handling the prepared microparticles during processing will be easy.

Particle size is an important parameter to be assessed; it influences the surface area and therefore the dissolution rate [18]. It was noticed in this study that by increasing the polymer concentration in the formulation, there was a corresponding increase in the viscosity, which led to mass hardening and prevented a further reduction in the particle size during preparation [14]. The prepared microparticles were formed as granules. According to the British Pharmacopoeia (BP), the content uniformity limit is 92.5-107.5 % [19]. The drug content of the majority of formulations was close to and within the BP range. The low percentage of drug content in formulations F5 (65.1 %) and F6 (73.8 %) may be attributed to their lower initial drug content and the low percentage yield for these formulations. The drug content depends on the percentage yield of the granules [9].

Regarding the peak intensity in the FTIR spectrum, no noticeable changes were observed between pure rifampicin and the extracted rifampicin, indicating that there was no significant alteration in the characteristic peaks following formulation. The antimicrobial study shows the efficacy of the formulations in terms of antibacterial capability, which might be used for further medical application. The prepared granules exhibited an initial burst effect followed by controlled release. The initial in vitro burst release is probably due to surface-accumulated drug on wax granules. The controlled release is probably due to the hydrophobic coating of beeswax around the drug particles, which hinders the penetration of dissolution fluid, leading to diminished drug release for a long time [20]. Because PVP is a hydrophilic polymer that is highly soluble in water, it might have remained in the aqueous phase during the microparticle preparation process and not been completely incorporated into the formulation, which explains its negligible impact on drug release when compared to F5. This finding may also explain the decrease in the percentage yield for this formulation (80.5 %) compared to F5 (90.83 %). Although CA had no significant impact on the drug release profile, it had a good effect on particle size, which affects other physicochemical properties.

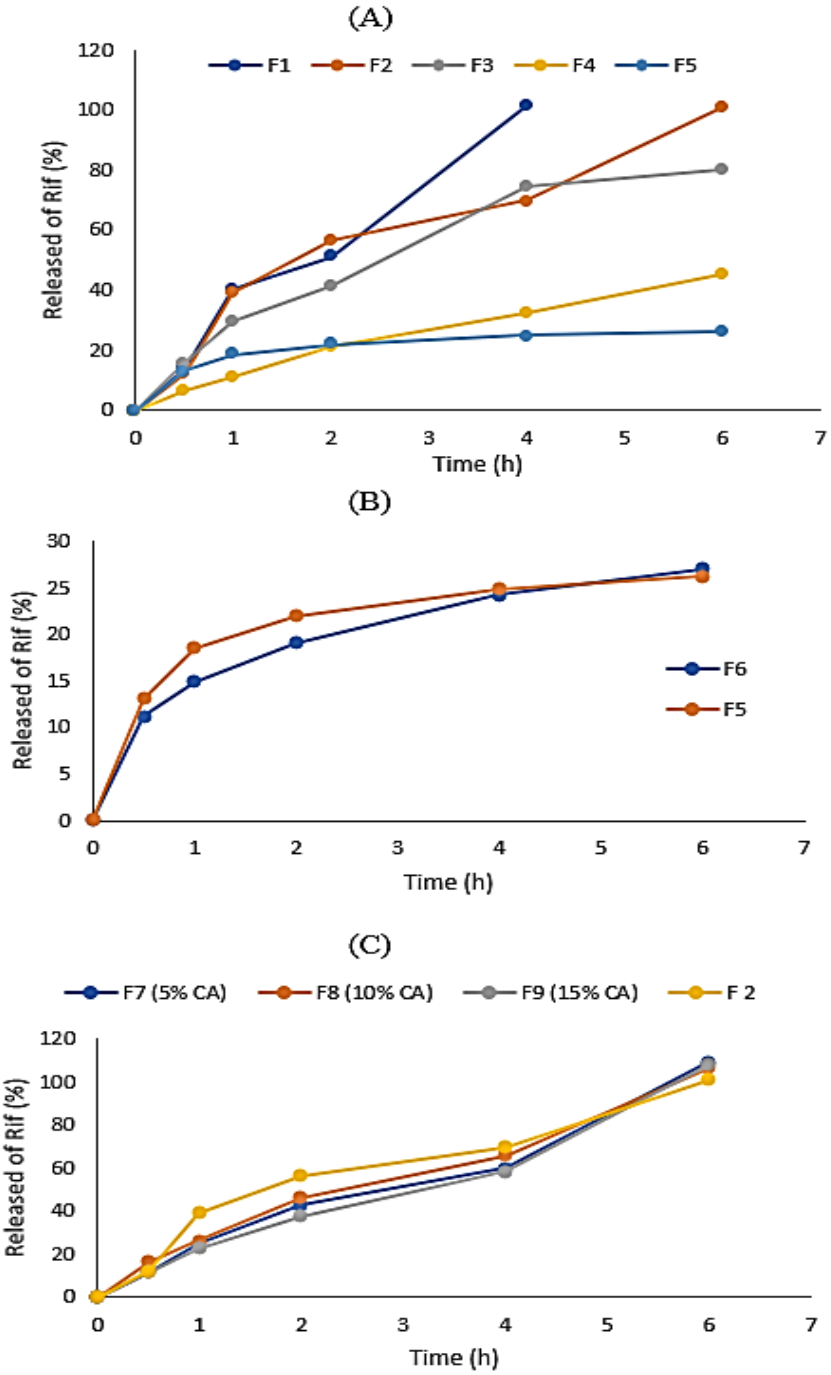


Figure 3: (A) In vitro release of rifampicin from microparticles of formulations F1-F5. (B) Formulation F6 prepared with PVP compared to F5. (C) Formulations F7-F9 prepared with different ratios of CA compared to F2

CONCLUSION

Rifampicin microparticles employing the meltable dispersion and cooling technique with beeswax as the primary polymer have been prepared. Reduced rifampicin release with initial burst release was noticed with increasing beeswax concentration in the formulation, and the inclusion of other polymers did not significantly affect the release rate of the drug. The prepared microparticles were found to be irregular in shape, with satisfactory levels regarding antibacterial effectiveness, drug content, polymer compatibility, and particle size. More research is needed to create a system that can sustain the drug release for up to 24 h, as well as to carry out in vivo trials to verify the efficacy and safety of the system.

DECLARATIONS

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Conflict of interest

No conflict of interest associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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