University of Tripoli
Faculty of pharmacy
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Preparation and Evaluation of Beeswax Microparticles Loaded with Rifampicin for Sustained Effect

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Sustained release dosage forms

One of advanced drug delivery systems that designed to release the drug molecules over an extended period of time.

Offer advantages over conventional DDS, such as:

- Reducing dosing frequency.
- Improving patient compliance.
- Minimizing fluctuations in drug conc.
- Reducing side effects.

Rifampicin

- ✓ Reddish brown crystalline powder.
- ✓ Semisynthetic antibiotic used in treatment of tuberculosis.
- ✓ The treatment of TB requires multiple drug regimens for a Long time, 4-6 months.
- ✓ Its half life varies from 2-4 hours.

The aim of the study

- To develop a new drug delivery system in the form of microparticles to sustain the release of Rifampicin for the ideal treatment of tuberculosis.
- The formulated microparticles are going to be characterized physiochemically in terms of shape, size, drug content uniformity, drug polymer compatibility…

03 Experimental part

1 Calibration curve of Rifampicin in deionized water

1 mg/ml stock solution of Rif. was prepared

Series concentrations ranging from 5-30µg/ml were prepared

The Absorbance was measured by UV/VIS spectrophotometer at 334nm

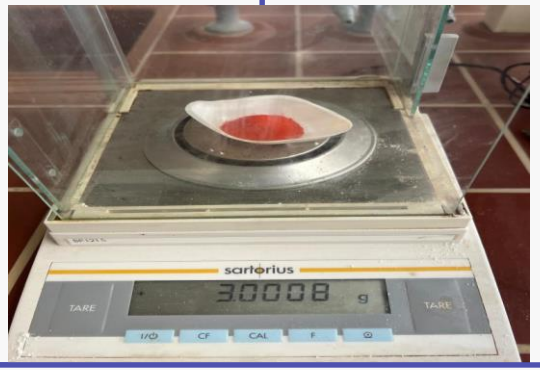
Calibration curve was constructed

2

Preparation of the wax microparticles

meltable dispersion and cooling process

Known amount of Beeswax melted in a water path at 62°C



Predetermined amount of Rif was suspended in the molten wax

2 Preparation of the wax microparticles

The melt left out of water path and poured slowly into previously heated buffer pH4.5 under stirring

Left to cool down to room temp. while stirring

2 Preparation of the wax microparticles

The microparticles obtained by filtration, air dried for 48 hr

Collected and kept in desiccator for further studies

2

Preparation of the wax microparticles

Ingredients	Formulations								
% w/w	F1	F2	F3	F4	F5	F6	F7	F8	F9
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
Cetyl alcohol	/	/	/	/	/	/	5	10	15
Polyvinylpyrrolidone	/	/	/	/	/	10	/	/	/

1 Physicochemical characterization of the microparticles

A. Percentage yield :

$$\% \text{ Yield} = \frac{\text{Actual weight}}{\text{Theoretical weight}} * 100$$

B. Determination of density:

1) Bulk density.
2) Tapped density.
3) Porosity.

1 Physicochemical characterization of the microparticles

C. Determination of flow properties:

1) Angle of repose:
By fixed funnel method
$\theta = \tan^{-1} h/r$

2) Compressibility: the ability of drug powder to decrease in volume under pressure and also considered as indirect measure of the flow properties of the powder.

- % Carr's index = $(\rho t - \rho b)/\rho t * 100$
- Hausner ratio = $\rho t/\rho b$

2 Particle size analysis

- ✓ By sieve analyzer, 5 standard sieves were used ranged of 125-710 µm.
- ✓ The frequency distribution for each size range was measured.

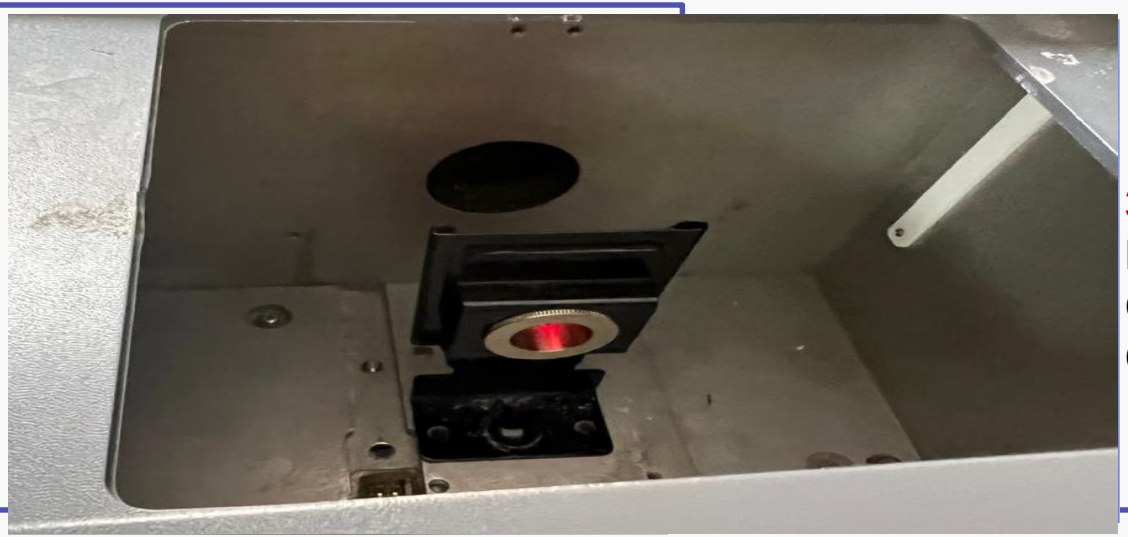
3 Particle shape and surface morphology

- ✓ The external morphology and shape were investigated by an optical microscope.

4 Drug content uniformity

- ✓ It was estimated by UV/VIS spectrophotometer.
- ✓ A quantity of microparticles equivalent to 50 mg Rifampicin of each formulation was transferred into a conical flask, 25 ml of methanol was added to it, shaken for 24hr, filtered ,diluted sufficiently, assayed and calculated using preconstructed calibration curve.

5 Drug : polymer compatibility



3 principal peaks:

N-H > 3496 cm $^{-1}$

O-H > 2984 cm $^{-1}$

C=O > 1694 cm $^{-1}$

- ✓ **It was performed by FTIR using potassium bromide pellet method to identify any chemical changes in the functional group of Rifampicin.**
- ✓ **The extracted Rifampicin was examined at wavelength ranges from 400-4000cm^{-1} and compared with FTIR spectrum of pure Rifampicin**

6 Antimicrobial study

- ✓ It was carried out using Agar gel diffusion method.
- ✓ Cultured *S. aureus* was spread on the nutrient medium.
- ✓ The prepared suspension of equivalent dose of microparticles F2 and pure drug were poured into the well.
- ✓ The petri plates were incubated for 24hr and observed to calculate zone of inhibition

7 In vitro release study

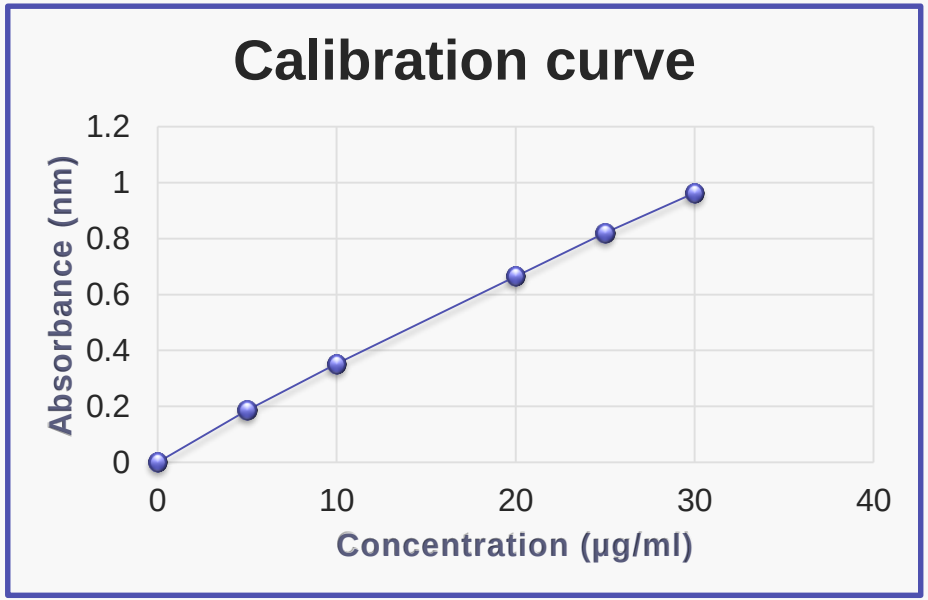
- ✓ carried out using USP dissolution apparatus, type I basket method.
- ✓ microparticles equivalent to the drug dose (50 mg) were filled in a (size “0”) colorless transparent hard gelatin capsules.
- ✓ Perfect sink condition was maintained throughout the release study.

04

Results and discussion

1 Calibration curve of Rifampicin in deionized water

Conc. (µg/ml)	Absorbance (nm)
5	0.186
10	0.352
20	0.665
25	0.770
30	0.963



r = 0.999

2 Physicochemical characterization of the microparticles

A. Percentage yield :

Formulation code	% yield
F1	91.66
F2	95.58
F3	91.33
F4	97.60
F5	90.83
F6	80.50

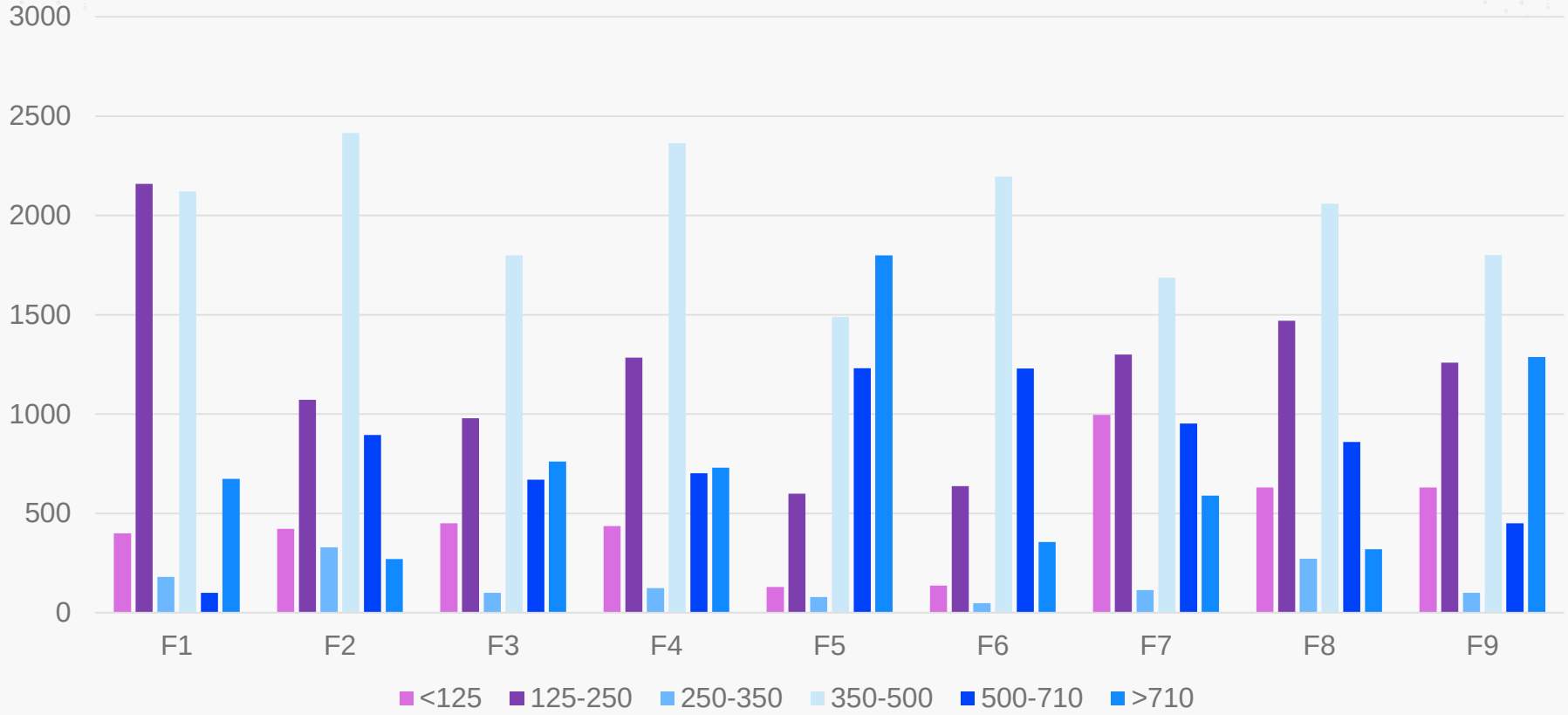
Formulation code	% yield
F7	98.90
F8	97.16
F9	95.98

B. Micromeritic properties:

	ρB	ρT	(ε)	θ	Carr's index	Hausner ratio
F1	0.29±0000	0.42±0000	0.29	27.8±0.4	29.19	1.41
F2	0.26±0.002	0.33±0.009	0.22	33.6±1.8	22.48	1.29
F3	0.28±0.010	0.37±				1.29
F4	0.29±0.003	0.39±				1.32
F5	0.33±0000	0.37±				1.12
F6	0.27±0.007	0.34±				1.20
F7	0.20±0000	0.30				1.52
F8	0.20±0.010	0.31±0.009	0.35	28.4±0.1	35.04	1.53
F9	0.26±0000	0.36±0000	0.29	28.5±3.1	29.61	1.42

This study suggests that the microparticles can be easily handled during processing

3 Particle size analysis



4 Particles shape and surface morphology

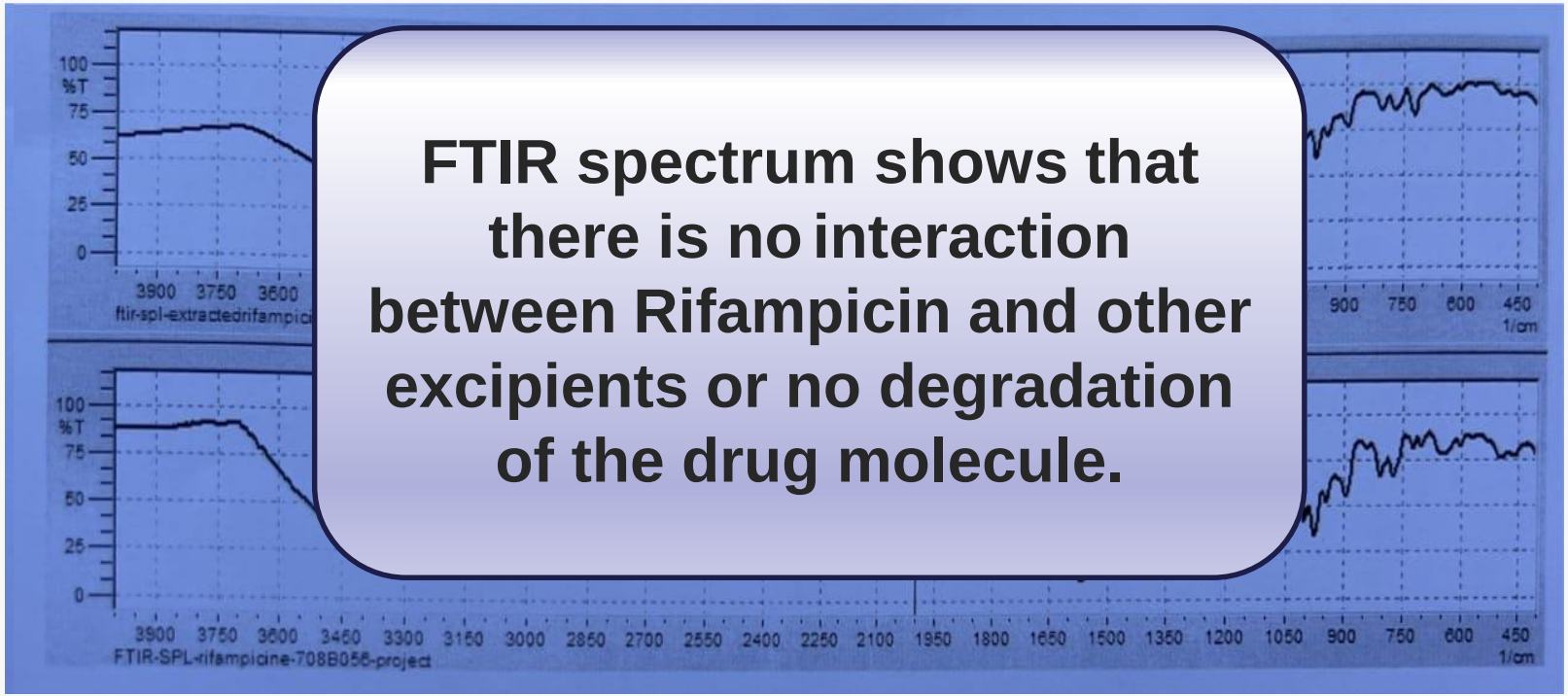
The particles are granules with an irregular shape and rough surface

5 Drug content uniformity

	Conc.(mg/ml)	% 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

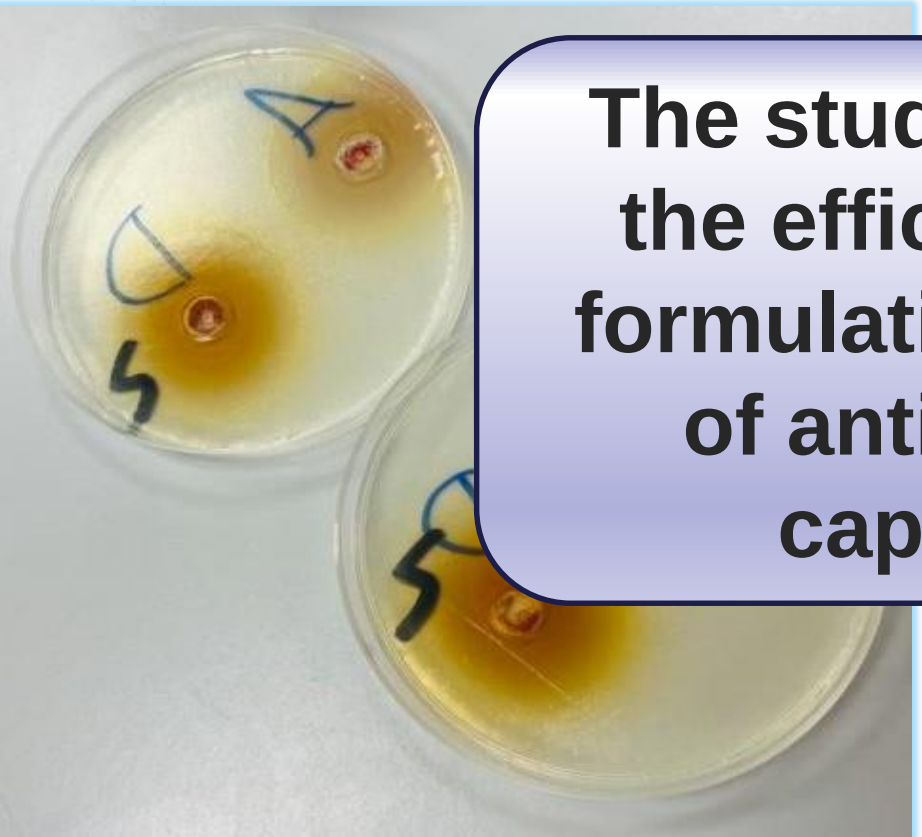
Most formulations show drug content near and within the range stated in the British Pharmacopeia 2008, which is 92.5-107.5% of the stated amount.

6 Drug : polymer compatibility



FTIR spectrum shows that there is no interaction between Rifampicin and other excipients or no degradation of the drug molecule.

7 Antimicrobial study



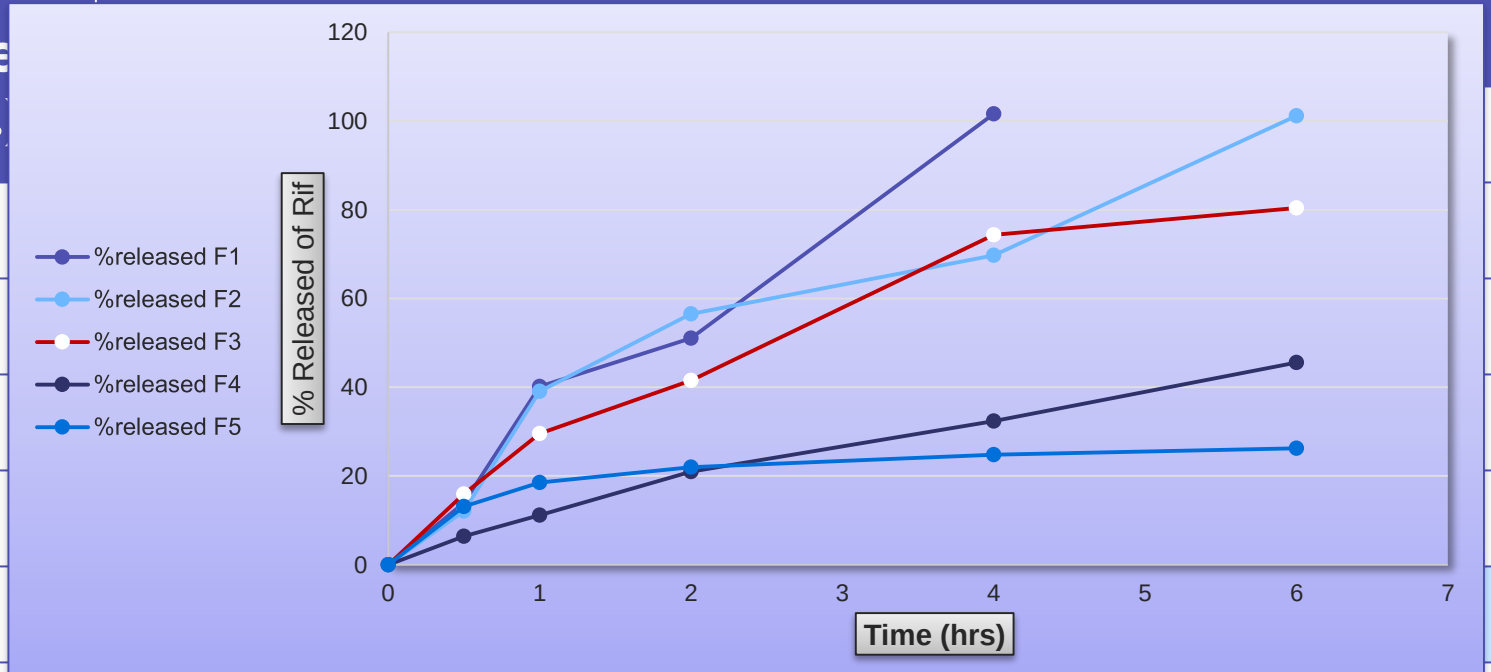
The study indicates the efficacy of our formulations in term of antibacterial capability.

er (cm) of the of inhibition	
g	Drug formulation F2
1	4 ± 000

8 In vitro release studies

A. The effect of Rif-Beeswax ratio on the drug release from the granules (size 125-250µm) of formulations F1-F5:

Time (hrs.)	F5
0.5	13.12
1	18.54
2	21.98
4	24.82
6	26.20



In vitro release studies

C. The effect of incorporation of PVP on drug release from formulation F6:

Independent sample t-test

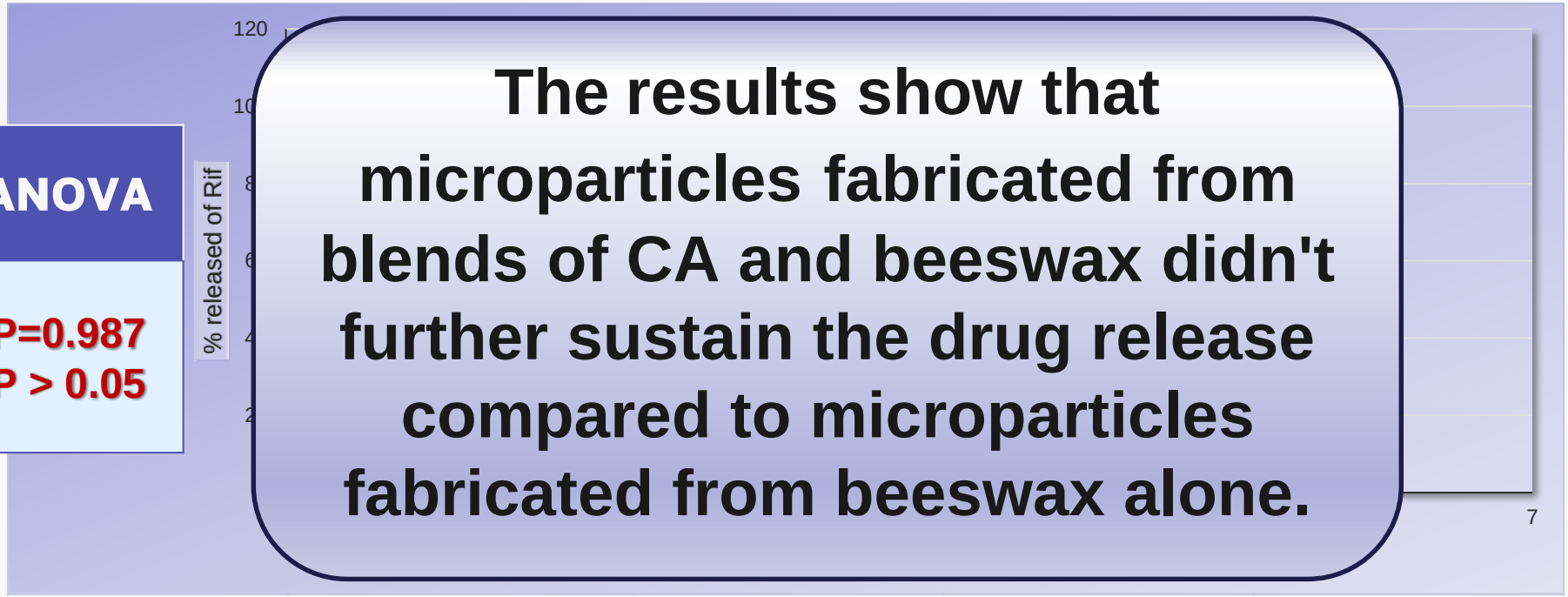
P=0.816
P > 0.05

It appears that, PVP does not have considerable effect (< 1%) on the release of the drug in comparison to F5. This may be attributed to the fact that PVP is hydrophilic, which might have remained in the aqueous phase during the microparticles preparation process

Time (hrs)

In vitro release studies

C. The effect of incorporation of CA on drug release from formulations F7-F9:

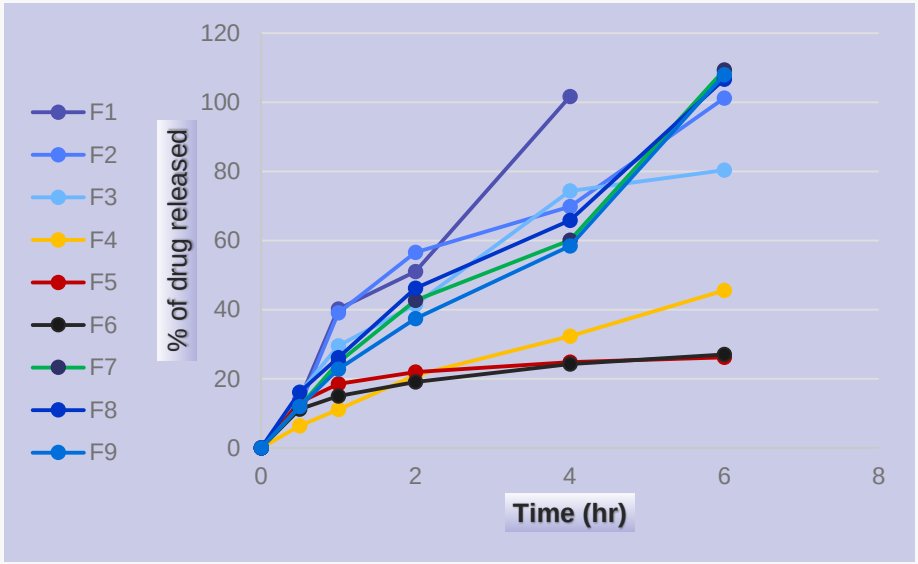


ANOVA

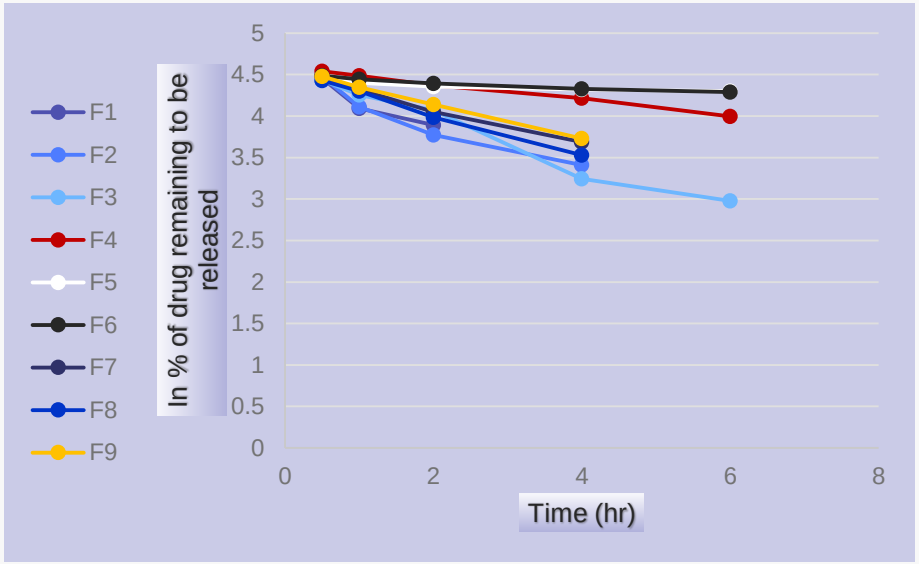
P=0.987
P > 0.05

The results show that microparticles fabricated from blends of CA and beeswax didn't further sustain the drug release compared to microparticles fabricated from beeswax alone.

9 Mechanism of drug release

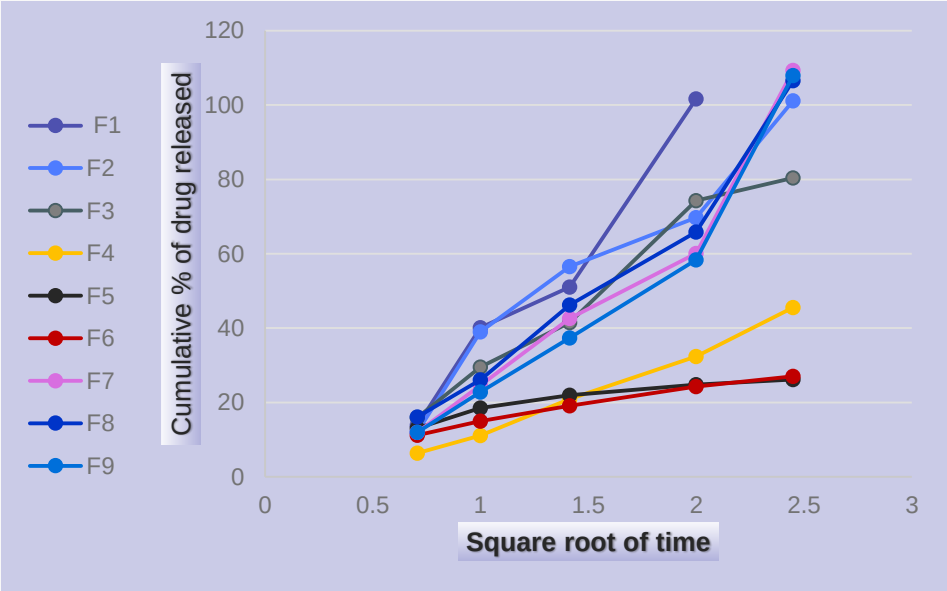


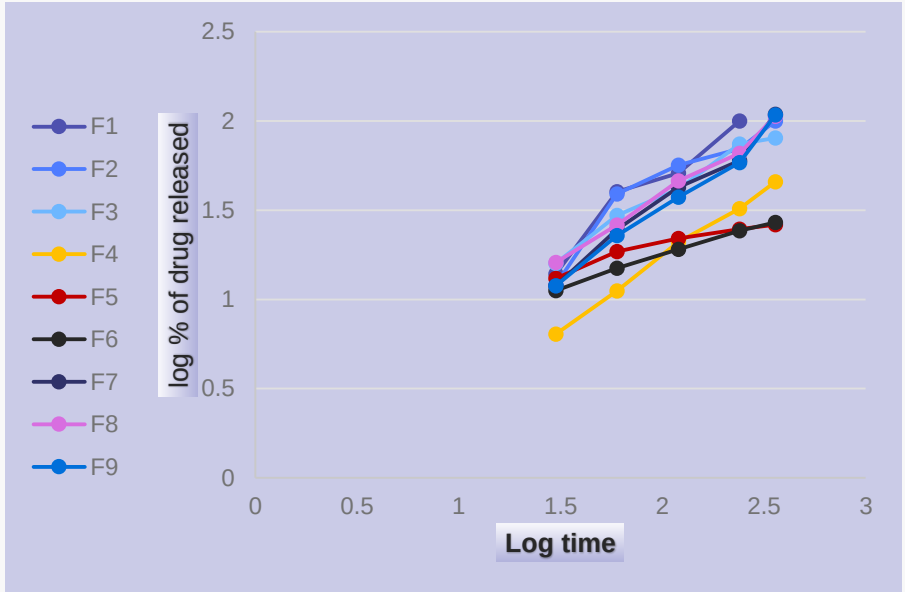
A. zero-order plot



B. First-order plot

9 Mechanism of drug release





C. Higuchi equation plot

D. Korsmeyer-Peppas plot

	R^2 for Zero-order equation	R^2 for First order equation	R^2 for Higuchi equation	R^2 for peppas equation	n for peppas equation
F1	0.974	0.880	0.965	0.943	0.889
F2	0.922	0.920	0.956	0.899	0.762
F3	0.926	0.969	0.975	0.982	0.661
F4	0.984	0.996	0.993	0.997	0.785
F5	0.634	0.818	0.900	0.935	0.267
F6	0.787	0.945	0.987	0.996	0.355
F7	0.975	0.985	0.937	0.981	0.830
F8	0.981	0.994	0.963	0.991	0.735
F9	0.978	0.999	0.928	0.985	0.832

The current work represented a satisfactory attempt to formulate a sustained release dosage form of Rifampicin as an effective model drug by a simple and reproducible method of preparation which is the meltable dispersion and cooling process.

Recommendation

Further research to develop a system that can sustain the drug release for up to 12-24 hours is warranted in addition to the establishment of safety and efficacy of this system by carrying out animal studies.

Thank you

Any question?