**Pharmacophore**

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

# QUERCETIN LOADED RIFAMPICIN-FLOATING MICROSPHERES FOR IMPROVED STABILITY AND *IN-VITRO* DRUG RELEASE

**Prashant Lakshaman Pingale[1*], Sunil Vishwanath Amrutkar[2]**

1. ***Department of Pharmaceutics, GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik-422005, India.***
2. ***Department of Pharmaceutical Chemistry, GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik-422005, India.***

**ARTICLE INFO**

**Received:**
30 Mar 2021
**Received** in revised form:
05 Jun 2021
**Accepted:**
10 Jun 2021
**Available online:**
28 Jun 2021

***Keywords:*** Quercetin, Rifampicin, Floating microspheres, Bioavailability, Drug release

**ABSTRACT**

After HIV, tuberculosis (TB) is the world's second most frequent disease. MTB (Mycobacterium tuberculosis) is a major infectious disease that poses a considerable public health issue. Fixed-dose drug combination microspheres appear to be a better option for long-term, regulated medication therapy. The drugs could be given orally once a week to encourage patient compliance. For long-term pharmaceutical therapy, fixed-dose drug combination microspheres appear to be a superior option. Oral administration is the most common and favored mode of pharmaceutical administration. Drug release is modulated throughout the GI tract with oral controlled-release (CR) formulations. Swelling and expanding systems, floating systems, forms of the mucoadhesive systems of high-density dose, and magnetic systems have all been employed. The goal of this study is to develop rifampicin-floating microspheres that will increase gastric retention time. The influence of quercetin on in-vitro drug release has been looked. The efficiency of entrapment was determined to be 76.50 percent. After 8 hours, the percentage buoyancy was observed at 61.50. In gastric media, the microspheres produced displayed extended drug release, indicating that they could be employed for long-term anti-tubercular medicine delivery.

*This is an **open-access** article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non commercially, as long as the author is credited and the new creations are licensed under the identical terms.*

**To Cite This Article:** Pingale PL, Amrutkar SV. Quercetin Loaded Rifampicin-Floating Microspheres for Improved Stability and In-vitro Drug Release. Pharmacophore. 2021;12(3):95-9. https://doi.org/10.51847/yBXnl2bSUH

## Introduction

MTB (Mycobacterium tuberculosis) is an infectious disease that poses a considerable public health problem and infects hundreds of millions of old people around the world. MTB treatment, including MDR-TB (multidrug-resistant tuberculosis), is a major concern. Microsphere-based medication delivery can increase drug bioavailability and minimize dose frequency [1].

Microspheres have been investigated in the treatment of tuberculosis and HIV for decades. Fixed-dose drug combination microspheres appear to be a better option for long-term, regulated medication therapy, as well as being more cost-effective and boosting compliance. The drug in the form of microparticles is released for 3–5 days in plasma and up to 9 days in organs. The drugs could be given orally once a week to encourage patient compliance [2].

Oral administration is the most common and favored mode of pharmaceutical administration. This could be due to the ease, with which it is administered, as well as patient compliance and formulation flexibility. It does, however, have limitations due to the wide diversity of biochemical and physiological conditions found in the gastrointestinal system. Furthermore, the development of oral dosage forms has been hampered by first-pass drug metabolism [3].

Oral controlled-release (CR) formulations, which enable regulated drug release throughout the GI tract, constant drug concentration maintenance in the serum for prolonged periods, bioavailability improvement, effectiveness of therapeutic, and decrease dose allowance, can help with these concerns. Longer gastric retention aids in the controlled release drug delivery system predictable stomach retention for an extended period. Floating systems, dosage forms of mucoadhesive, systems of high-density and super porous hydrogel have all been used. Traditional dosage forms have fewer design choices than these technologies [3, 4].

**Corresponding Author:** Prashant Lakshaman Pingale; Department of Pharmaceutics, GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik-422005, India. E-mail: prashant.pingale@gmail.com.

Gastro-retentive preparations are a sort of formulations that floats in gastric juice for more duration. They allow pharmaceuticals to be dispensed in a controlled and predictable manner. Single and multiple unit variants of floating drug delivery systems (FDDS) are available [5].

Many herbal treatments and herbal extracts have limited or no in-vivo efficacy due to insufficient lipid solubility or unsuitable molecular size. Novel drug delivery systems have now opened the way to the development of herbal drugs with higher bioavailability. Liposomes, microspheres, transferases, and other new carriers have been described for the successful modified delivery of numerous herbal drugs [6].

With gastroretentive floating microspheres, drug bioavailability and controlled distribution could be improved. The effect of quercetin on drug release in vitro was investigated.

## Materials and Methods

Lupin Pharmaceutical Aurangabad, India gifted a sample of Rifampicin. Quercetin was procured from Yucca Enterprises, Mumbai, India. Preparation of microsphere required by the Polymers such as ethylcellulose hydroxypropyl and methylcellulose were procured from Colorcon Asia Ltd., Goa, India. Analytical grade chemicals were used.

*Microspheres Preparation*

The solvent evaporation approach was used to make quercetin-loaded rifampicin floating microspheres. In a 1:1 mixture of ethanol and dichloromethane, ethylcellulose, hydroxypropyl methylcellulose, rifampicin, and quercetin were dissolved. This solution was gently placed in a percentage of $H_2O$ containing 0.01, a mixture of Tween 80 and stirred together for 40minsandletthe dissipation of volatile solvent. The microspheres were filtered, water-washed, and vacuum-dried [7, 8]. Micromeritic characteristics of quercetin-loaded rifampicin microspheres are shown in **Table 1**.

**Table 1.** Micromeritic characteristics of quercetin loaded rifampicin microspheres

| Characteristic | Value (±SD) |
|---|---|
| Angle of repose ($^0$) | $40.17^0$ ±0.97 |
| Density of Bulk (g/cm$^3$) | 0.151 ±1.27 |
| Density of Tapped (g/cm$^3$) | 0.197 ±0.77 |
| Index of Carr (%) | 23.35 |
| Ratio of Hausner | 1.30 |
| Index of % Compressibility | 30.46 |

*Microspheres Characterization*

*Fourier Transform Infra-Red Analysis (FT-IR):* The physical drug combination, formulation, extract of herbal, and polymerwith the use of Fourier Transform Infrared (FT-IR) spectroscopy were all examined. The polymers and other excipients in the drug if compatible and evaluated by FTIR [9].

*Differential Scanning Calorimeter (DSC) Study:* aluminium samples of 5-10 mg pans were weighed, and below stationary air at a heating rate of 10°C/min scanned over a temperature range of 250° to 30°C [10].

*Percentage Yield:* The overall microspheres weight produced was compared to the overall polymer weight, medication, and bio-enhancers utilized in the formulation to determine the product of microspheres [11, 12].

The yield percentage of the microsphere was calculated utilizing the ensuing formula:

$$Percentage\ yield = \frac{Weight\ of\ microspheres\ obtained}{Total\ weight\ of\ drug\ polymer\ used\ in\ formulation} \times 100 \quad (1)$$

*Particle Size Analysis:* A microscope analysis was used to assess the size of quercetin-loaded rifampicin microspheres. As illustrated in **Figure 1**, no major visible surface flaws are formulated in microspheres withnearly spherical. Under a Motic microscope at 40 X magnification, photomicroscopic images illustrate the size of individual particles [12].

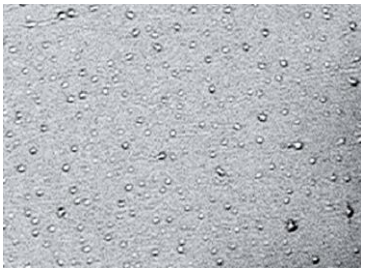

**Figure 1.** Magnification of 40 X photomicrograph of the developed formulation

Percentage of buoyancy *In-vitro*: (50 mg) of Hollow microspheres were agitated at 100 rpm in HCI (100 ml) of 0.1 N. Filtration was used to separate the microspheres. The desiccator is used to dry microspheres overnight. The formula below was used to compute the percent buoyancy [12]. Floating ability at different time intervals is shown in **Figure 2**.

$$\%\ buoyancy = \frac{weight\ of\ floating\ microspheres}{weight\ of\ floating\ microspheres + weight\ of\ settled\ microspheres} \times 100 \quad (2)$$

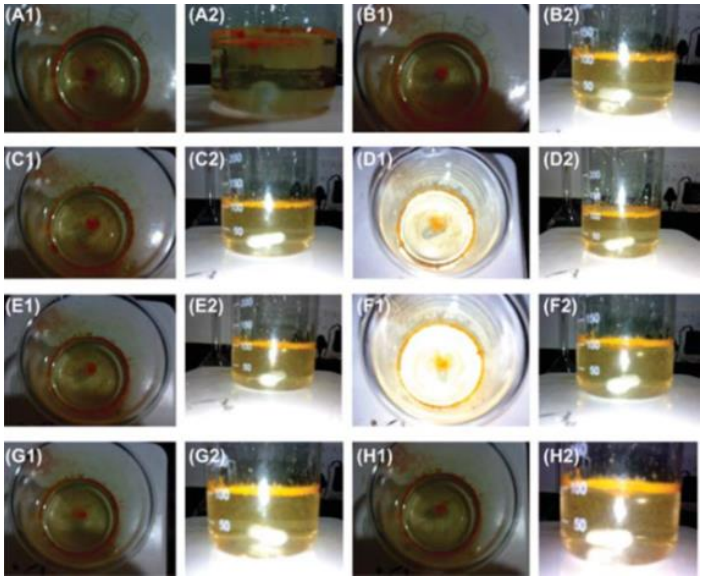


**Figure 2.** The floating ability of quercetin loaded rifampicin microsphere at different time intervals, upper view and side view at 1 to 8 hours (A to H)

*Percent Drug-Entrapment Efficiency:* In 10 mL methanol, quercetin-loaded rifampicin microspheres (10 mg) were shaken for 24 hours. Shimadzu 1800 UV-Vis spectrophotometer were used to measure the resulting solution from the extracted drug, and the concentration (Wd) of the drug entrapped in the microsphere was calculated using a calibration curve. Each technique was repeated three times in total [13]. The following formulae were used to calculate the drug-loading ratio and entrapment efficiency percentages:

$$Drug - loading\ ratio\ (\%) = \frac{W_d}{W_m} \times 100\% \quad (3)$$

$$Entrapment\ efficiency\ (\%) = \frac{Drug - loading\ ratio}{Theoretical\ drug - loading\ ratio} \times 100\% \quad (4)$$

Where,
Wd is the calculated weight of microsphere (g),
Wm is the weight of drug-loaded microspheres (g)

*Measurement of Bio-Adhesion:* Microspheres were distributed throughout the small intestine of an albino rat (area 2 $cm^2$) and kept in a humidity temperature-controlled cabinet. The mucosal lumen was thoroughly cleansed with pH 6.8 phosphate-buffered saline and dried in a hot air oven at $70^0$C as shown in **Figure 3** [14].

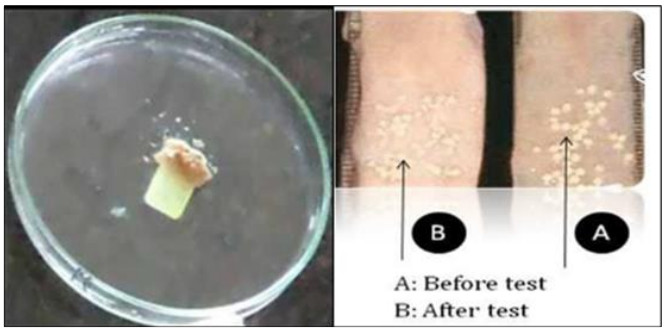


**Figure 3.** Bioadhesion study of Rifampicin Microspheres

The following formula was used to calculate the percentage of bio-adhesion:

$$Percentage\ bioadhesion = \frac{Weight\ of\ adhered\ microspheres}{Weight\ of\ applied\ microspheres} \times 100 \quad (5)$$

**Table 2.** Particle size, percent yield, percent entrapment of drug, percent bio-adhesion, percent drug release, andzeta potential of quercetin rifampicin microspheres

| Characteristic | Value for microsphere (±SD) |
|---|---|
| Particle size (ɱ) | 100-110 ±1.11 |
| Percentage yield | 72.48 ±0.89 |
| Drug Entrapment (%) | 76.50 ±1.19 |
| Bioadhesion (%) | 81.12 ±1.43 |
| Buoyancy (%) | 61.50 ±1.04 |
| *In-vitro* drug release (%) | 89.11 ±1.27 |
| Zeta Potential (mV) | 14.50 ±0.72 |

*Surface Charge:* The zeta potential of microparticle formulations was also measured since, as is well known, it affects particle stability. Positive or negative zeta potential values that are more apparent in principle likely to stabilize particle suspension. The spheres do not aggregate due to electrostatic repulsion between particles with the same electric charge. **Table 2** shows that the microspheres generated by the solvent evaporation process were negatively charged [10].

*Drug release In-vitro:* The discharge of drug quercetin-loaded rifampicin microspheres was tested using a USP XXXI basket-type dissolution apparatus. A weighted quantity of equal microspheres was placed to 50 mg of drug in the basket. Replicated gastric juice was utilized as the dissolution medium, which was held at 37°C and 100 rpm. Sample of a 5 mL was taken once a 30-minute interlude and processed for 1 hour by a membrane filter. For the next 8 hours, further samples were obtained every hour, and to maintain the preliminary dimensions of the dissolving fluid, a fresh dissolving fluid of 5 ml was supplementary after each withdrawal. The samples were spectrophotometrically examined at 475 nm to regulate the amount of drug in the dissolving fluid. Triplicate has carried out the drug release study. Drug release from quercetin loaded microspheres is shown in **Figure 4** [12, 15, 16].

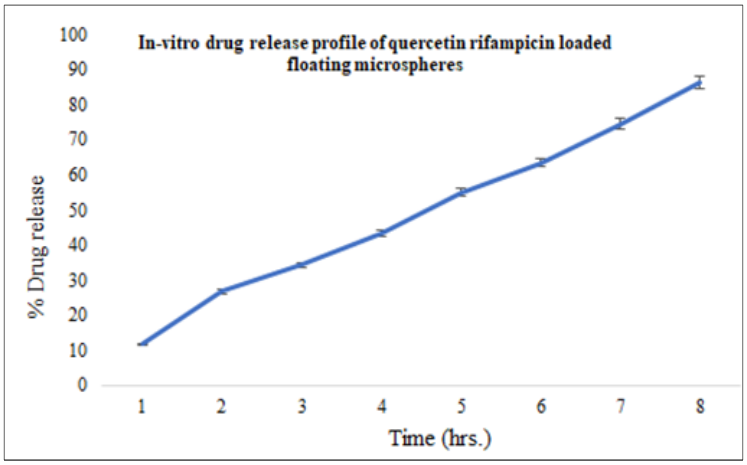


**Figure 4.** Quercetin loaded rifampicin microspheres to drug release (In-vitro)

*Drug Release Kinetics:* The solution of the *in vitro* dissolution investigation of microspheres were fixed with countless equalities of kinetic, such as zero-order (release percentage versus time), first-order (log percentage of cumulative drug remaining versus time), and, to comprehend the mechanism and kinetics release of drug by Higuchi's model (square root of time versus release of drug percentage). Regression analysis of the above plotscreated bythe linear curves, the correlation coefficient (R2) values were determined. *In vitro*, quercetin-loaded rifampicin microspheres release medicine as seen in **Figure 4**.

**Results and Discussion**

Hollow microspheres were produced utilizing the method of solvent evaporation with a 1:6 ratio of HPMC and EC using quercetin as a herbal bioenhancer in this investigation. 76.50 percent of the medications were discovered to be entangled. The hollow microsphere's lag time was discovered as zero. At a 1:6 ratio, HPMC and EC displayed good buoyancy time with no lag time. In the simulated gastric fluid, about 15% of the drug was evacuated within an hour.

**Conclusion**

We attempted to construct floating quercetin-loaded rifampicin microspheres of HPMC and EC to improve tuberculosis management and preserve rifampicin release in the stomach. Better trapping and longer release patterns were observed in the research. Furthermore, the new system is stable.

It is well established that when the solubility of a medication diminishes, so does the amount of time available for dissolution, and therefore the time of conveyance becomes a critical factor affecting drug immersion. This may perhaps assist to boost rifampicin bioavailability, which is crucial for tuberculosis treatment. For reconstitution, the microspheres possibly will be compressed into tablets, packed into capsules, or molded into oral solutions.

Furthermore, the formulation's stability was compared to that of microspheres without quercetin, and it was observed that even after 6 months of stability testing, the quercetin-loaded microspheres showed no change.

**Acknowledgments:** None

**Conflict of interest:** None

**Financial support:** None

**Ethics statement:** None

**References**

1. Sandhu GK. Tuberculosis: current situation, challenges and overview of its control programs in India. J Glob Infect Dis. 2011;3(2):143.
2. Albanna AS, Smith BM, Cowan D, Menzies D. Fixed-dose combination antituberculosis therapy: a systematic review and meta-analysis. Eur Respir J. 2013;42(3):721-32.
3. Homayun B, Lin X, Choi HJ. Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. Pharmaceutics. 2019;11(3):129.
4. Vargason AM, Anselmo AC, Mitragotri S. The evolution of commercial drug delivery technologies. Nat Biomed Eng. 2021 5(9):951-67.
5. Tripathi J, Thapa P, Maharjan R, Jeong SH. Current state and future perspectives on gastroretentive drug delivery systems. Pharmaceutics. 2019;11(4):193.
6. Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: An overview. Asian Pac J Trop Biomed. 2013;3(4):253-66.
7. Subedi G, Shrestha AK, Shakya S. Study of effect of different factors in formulation of micro and nanospheres with solvent evaporation technique. Open Pharm Sci J. 2016;3(1).
8. Raut NS, Somvanshi S, Jumde AB, Khandelwal HM, Umekar MJ, Kotagale NR. Ethyl cellulose and hydroxypropyl methyl cellulose buoyant microspheres of metoprolol succinate: Influence of pH modifiers. Int J Pharm Investig. 2013;3(3):163.
9. Goyal P, Gill S, Gupta UD, Rath G, Narang RK, Goyal AK. Development and characterization of rifampicin loaded floating microspheres. Artif Cells, Blood Substit, Biotechnol. 2011;39(5):330-4.
10. Ravindra RP. Effect of Piper Nigrum on In-Vitro Release of Rifampicin Microspheres. Asian J Pharm Clin Res. 2013:79-83.
11. Ahmed TA, Aljaeid BM. Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug Des Devel Ther. 2016;10:483.
12. Pingale PL, Ravindra RP. Comparative Study of Herbal Extract of Piper Nigrum, Piper Album and Piper Longum on Various Characteristics of Pyrazinamide and Ethambutol Microspheres. J Drug Deliv Ther. 2019;9(4-A):72-8.
13. Feng R, Zhang Z, Li Z, Huang G. Preparation and in vitro evaluation of etoposide-loaded PLGA microspheres for pulmonary drug delivery. Drug Deliv. 2014;21(3):185-92.
14. Jain SK, Jangdey MS. Lectin conjugated gastroretentive multiparticulate delivery system of clarithromycin for the effective treatment of Helicobacter pylori. Mol Pharm. 2009;6(1):295-304.
15. Jain SK, Kumar A, Kumar A, Pandey AN, Rajpoot K. Development and in vitro characterization of a multiparticulate delivery system for acyclovir-resinate complex. Artif Cells Nanomed Biotechnol. 2016;44(5):1266-75.
16. Mandal U, Pal TK. Formulation and in vitro studies of a fixed-dose combination of a bilayer matrix tablet containing metformin HCl as sustained release and glipizide as immediate release. Drug Dev Ind Pharm. 2008;34(3):305-13.