Erciyes Med J 2013 35(1): 1-5 • DOI: 10.5152/etd.2013.01

ORIGINAL INVESTIGATION
*ÖZGÜN ARAŞTIRMA*

# Formulation and Characterization of Solid Lipid Nanoparticles of Rifampicin

## *Katı Solid Rifampisinin Nano-Parçacıklarının Formülasyon ve Karakterizasyonu*

Durgaramani Sivadasan, Bindu Madavan, Shalini Devi Penmatsa, Sree Tejaswi Bathini

ABSTRACT
*ÖZET*

**Objective:** In this study, solid lipid nanoparticles of rifampicin were prepared by the emulsion solvent diffusion method using stearic acid to retard release and achieve the required release profile for the treatment of tuberculosis.

**Materials and Methods:** The polymer DL-lactide/glycolide copolymer (PLGA) and lipid were dissolved in the organic phase separately. Stearic acid and rifampicin were dissolved in a mixture of chloroform and methanol. The resulting organic solution was poured into polyvinyl alcohol and homogenized. The organic solvents were removed using a rotary flash evaporator. The SLNs were recovered by centrifugation and lyophilized.

**Results:** The twelve prepared formulations showed that the highest encapsulation efficiency was 78.79±0.1%. In vitro release studies were performed in which the particle stability was found to be enhanced by poly vinyl alcohol (PVA), which forms a barrier to the release of the incorporated drug. The stability study conducted for three months revealed that formulations stored at room temperature were prone to fungal growth whereas formulations stored in the refrigerator were stable.

**Conclusion:** The ultimate goal of controlled drug release is to maximize therapeutic activity while minimizing the negative side effects of the drug. In this regard, solid lipid nanoparticles have emerged as a novel drug carrier system for the hydrophobic anti-tubercular drug, rifampicin.

**Amaç:** Bu çalışmada, salımı geciktirmek ve tüberküloz tedavisinde gerekli olan serbest bırakma profiline ulaşmak için, katı lipid rifampisin nanopartikülleri stearik asit kullanarak, emülsiyon çözücü difüzyon yöntem ile hazırlandı.

**Gereç ve Yöntemler:** Polimer DL-laktid/glikolid kopolimeri (PLGA) ve lipid ayrı ayrı organik faz içinde çözüldü. Stearik asit ve rifampisin, kloroform ve metanol karışımı içinde çözüldü. Elde edilen organik solüsyon poli vinil alkol içine döküldü ve homojenize edildi. Organik çözücüler döner flaş buharlaştırıcı ile uzaklaştırıldı. Oluşan SLNs santrifüjleme ile elde edildi ve liyofilize edildi.

**Bulgular:** Hazırlanan on iki formülasyon, %78,79±0,1'luk en yüksek kapsülleme etkinliğinin elde edildiğini gösterdi. in vitro salınım çalışmaları sırasında, inkorpore edilen ilacın serbest bırakılması için bir bariyer oluşturan PVA ile kullanılarak yapıldı. Üç ay boyunca yürütülen çalışmalar, soğutma altında saklanan formülasyonların stabil olduğunu ancak oda sıcaklığında saklanan formülasyonlarda mantar üreme eğilimli olduğunu ortaya koydu.

**Sonuç:** Kontrollü ilaç salım için nihai hedef, ilacın olumsuz yan etkileri en aza indirirken terapötik etkinliğini maksimize etmektir. Bu bağlamda, katı lipid nanopartiküller, hidrofobik Antitüberküloz ilaçlardan rifampisin için yeni ilaç taşıyıcı sistem olabilir.

**Key words:** Rifampicin, nanoparticles, stearic acid, tuberculosis

**Anahtar kelimeler:** Rifampisin, nanopartiküller, stearik asitler, tüberküloz

Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, India

**Available Online Date**
**Çevrimiçi Yayın Tarihi**
15.02.2013

**Submitted/Geliş Tarihi**
09.05.2011

**Accepted/Kabul Tarihi**
14.11.2012

**Correspondance/Yazışma**
Dr. Durgaramani Sivadasan,
Department of Pharmaceutics,
PSG College of Pharmacy,
Peelamedu, Coimbatore
641 004, Tamil Nadu, India
Phone: +91 9605890202
e.mail:
durgimural@rediffmail.com



## Introduction

Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers (50-1000 nm) which are composed of a physiological lipid, dispersed in water or in an aqueous surfactant solution. Nanoparticles made from solid lipids have attracted major attention as novel colloidal drug carriers as they have been proposed as an alternative particulate carrier system (1). SLNs are a nanocrystalline suspension in water, prepared from lipids, which are solid at room temperature. Nanoparticles hold promise as therapeutic drug carriers, and SLNs are a new form of particulate carriers in addition to the more conventional ones such as liposomes, lipid emulsions and polymeric nanoparticles (2). SLNs possess good tolerability (due to their derivation from physiological lipids), scaling up feasibility, the ability to incorporate hydrophobic/hydrophilic drugs, and an enhanced stability of incorporated drugs. In addition, SLNs provide improved possibilities for drug release profile amelioration (3). Thus, SLNs are unique in the sense that they combine the virtues of traditional nanoparticles while eliminating some of their problems. SLNs are composed of biodegradable and well-tolerated lipid compounds and emulsifying agents. Some of the most frequently used lipids are stearic acid, cocoa butter, and cholesterol while surfactants such as soybean lecithin, poloxomer 188, and sorbitan esters are used for the preparation of SLN (4).

Tuberculosis continues to be a leading killer disease worldwide. Although an effective treatment regimen against TB is available, the fact that multiple anti-tubercular drugs (ATDs) need to be administered continuously for at

least six months is responsible for patient non-compliance as well as for the emergence of multi-drug resistant TB (MDR TB). Treatment for MDR TB is even more prolonged, costlier, and often leads to drug-related side effects (5). The application of encapsulation technology is likely to play a role in the formulation of ATDs into a sustained release system (6).

Although liposomes and polymeric nanoparticles have proven to be successful ATD carriers in experimental tuberculosis, it was only recently that SLNs have been explored as an inhalable ATD carrier. However, since the oral route is preferable over all other routes of drug delivery, the route of drug administration cannot be overemphasized. Oral administration of SLN is possible as an aqueous dispersion or, alternatively, after transformation into a traditional dosage form, i.e. tablets, pellets, capsules, or powders in sachets. Therefore, the present study was planned to incorporate the first line drug rifampicin into SLNs and to evaluate its efficacy by oral administration.

## Materials and Methods

Rifampicin, stearic acid, polyvinyl alcohol, chloroform, methanol, hydrochloric acid, and dialysis membranes were used in this study. Rifampicin (100 µg/mL) was prepared and scanned at a wavelength of 200 to 800 nm using UV spectroscopy. The peak with the highest absorbance was taken as the lambda max of rifampicin for further studies.

**Formulation of Solid Lipid Nanoparticles of Rifampicin**

The concept of the preparation method is based on the "emulsion solvent diffusion method in water", in which a polymer such as DL-lactide/glycolide copolymer (PLGA) is generally dissolved in the organic phase. However, the lipid used in this study could not be completely dissolved in the organic phase at room temperature (6, 7). Rather, the lipid was dissolved in the organic phase in a water bath at 50°C. The weighed amount of stearic acid and rifampicin were dissolved completely in a mixture of chloroform and methanol in a water bath at 50°C. The resulting organic solution was poured into polyvinyl alcohol at 4-8°C and homogenized for 15 minutes. The organic solvents were removed using a rotary flash evaporator at 50°C for 15 min. The SLNs were recovered by centrifugation at 13,000 rpm for 30 min at 4°C. The recovered SLNs were washed thrice with distilled water to remove any excess poly vinyl alcohol (PVA) remaining within the formulation and then lyophilized. Twelve formulations were prepared by this method; the various parameters considered for optimization are shown in Table 1.

**Determination of Particle Size**

The particle size distribution of the white nanoparticle powder was determined by the laser diffraction method using a Microtrac particle size analyzer (Bluewave model S4521). The samples were dispersed in a volume of water and the size of the nanoparticles was scanned by the refractive index using a 30 s run time.

**Standard Graph of Rifampicin**

Accurately, 10 mg of rifampicin were weighed and dissolved in 5 mL of methanol and the volume was made up to 10 mL with distilled water (stock solution). Next, 1 mL of the stock solution was pipetted out and the volume was made up to 10 mL with distilled water (solution A). Then, 5 mL of solution A was removed and made up to 25 mL with distilled water (solution B). The required concentrations, i.e. 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL were prepared by pipetting out 1 mL, 2 mL, 3 mL, 4 mL, 5 mL of solution B, respectively, and then making up the volume to 10 mL with distilled water. The absorbance was determined through UV spectroscopy at 333 nm. A graph was prepared by plotting the concentration of the solution on the X axis and absorbance on the Y axis (8).

**Determination of Entrapment Efficiency**

The entrapment efficiency of the drug was determined by measuring the concentration of the free drug present in the supernatant after centrifugation at 13,000 rpm for 30 min at 4°C (9). The concentration of the drug present in the supernatant was determined

**Table 1. Formulation optimization of solid lipid nanoparticles of rifampicin**

| Formulation code | Formulation ratio rifampicin: stearic acid | Concentration of PVA (%) |
|---|---|---|
| F1 | 1:1 | 1 |
| F2 | 1:2 | 1 |
| F3 | 1:3 | 1 |
| F4 | 1:5 | 1 |
| F5 | 1:1 | 2 |
| F6 | 1:2 | 2 |
| F7 | 1:3 | 2 |
| F8 | 1:5 | 2 |
| F9 | 1:1 | 3 |
| F10 | 1:2 | 3 |
| F11 | 1:3 | 3 |
| F12 | 1:5 | 3 |

**Table 2. Percentage entrapment of formulations prepared with 1%, 2%, and 3% PVA**

| Formulation no | Drug/Lipid ratio | Concentration of PVA (%) | % EP |
|---|---|---|---|
| F1 | 1:1 | 1 | 29.5±0.8 |
| F2 | 1:2 | 1 | 28.07±0.3 |
| F3 | 1:3 | 1 | 34.15±0.5 |
| F4 | 1:5 | 1 | 20.45±0.7 |
| F5 | 1:1 | 2 | 46.01±0.5 |
| F6 | 1:2 | 2 | 78.79±0.1 |
| F7 | 1:3 | 2 | 55.6±0.9 |
| F8 | 1:5 | 2 | 35.5±0.3 |
| F9 | 1:1 | 3 | 34.64±0.2 |
| F10 | 1:2 | 3 | 50±0.4 |
| F11 | 1:3 | 3 | 71.6±0.5 |
| F12 | 1:5 | 3 | 43.3±0.8 |

%EP= 100 x (amount of drug added initially-amount of drug present in the supernatant)/amount of drug added initially

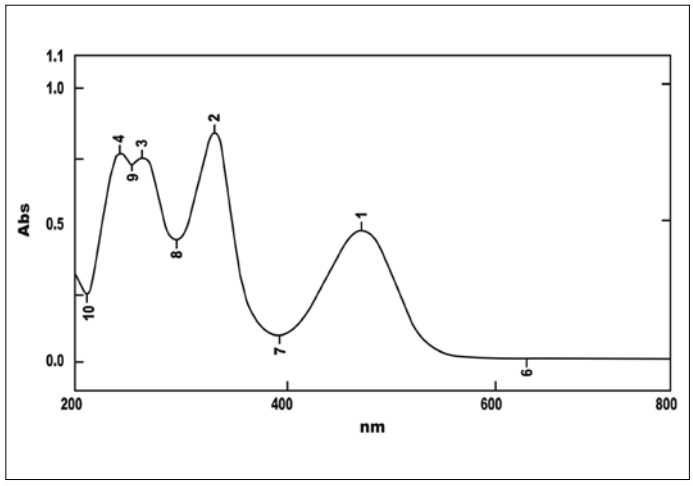


**Figure 1. UV spectrum of rifampicin**

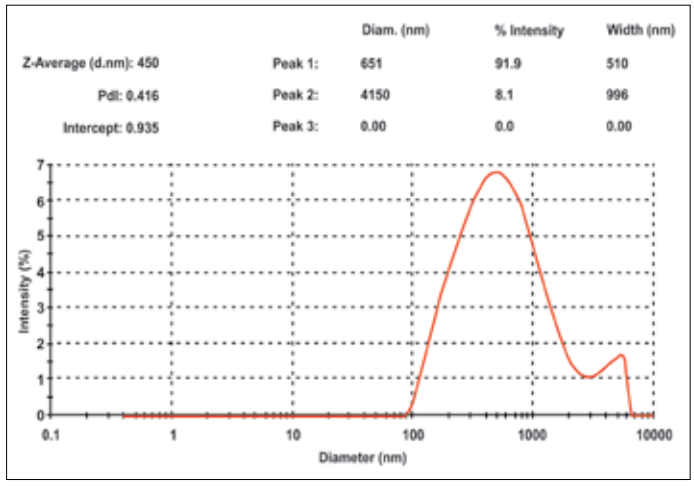


**Figure 2. Particle size analysis**

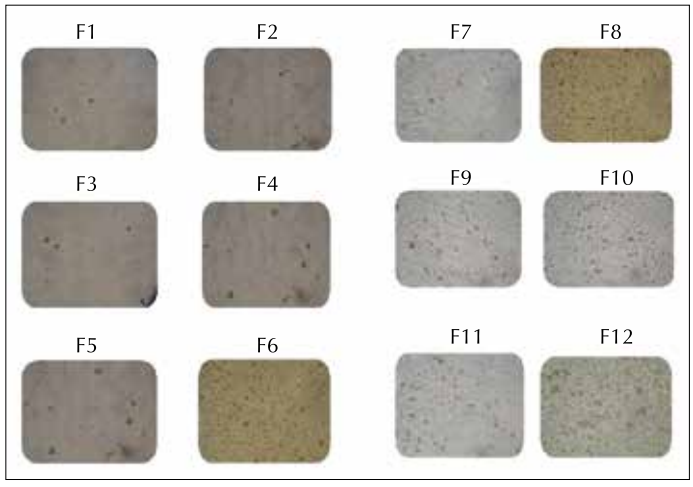


**Figure 3. Images of rifampicin-loaded SLNs**

using a UV spectrophotometer (Shimadzu 1650, Japan) at 333 nm. The entrapment percentage (EP) of the drug was determined by the following formula:

%EP= 100 x (amount of drug added initially- amount of drug present in the supernatant)/amount of drug added initially

### Stability Studies

The physical and chemical stability of rifampicin-loaded solid lipid nanoparticles were evaluated by storing the formulations at room temperature (28°C) and at refrigeration temperature (4°C) (9). Samples were withdrawn at one-month time intervals and were checked for clarity and the percentage of encapsulation.

### *In Vitro* Drug Release Studies

*In vitro* release studies were performed using a Hi-Media Dialysis membrane with a pore size of 2.5 nm and a molecular weight cut-off of 12,00 to 14,000 Da. The membrane was soaked in double distilled water for 12 hours before use. The formulation (1 mL) was placed inside the dialysis membrane and put into 50 mL of simulated gastric fluid (SGF; 0.1 M HCl, pH 1.2). At various time points, 5 mL aliquots were drawn for drug analysis and replaced with an equal volume of buffer (10).

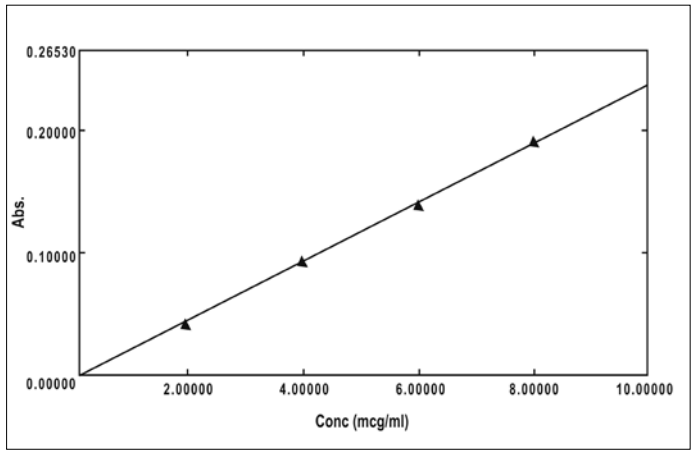


**Figure 4. Standard curve of rifampicin. Y=0.0242X-0.00233; $r^2$=0.99964**

## Results

### Determination of λ Max

The peaks of rifampicin (100 µg/mL) are shown in Figure 1. From the spectrum report, it is evident that the λ max of rifampicin is 333 nm.

### Particle Size Analysis

The particle size distribution by intensity is shown in Figure 2. The particle size was found to be 450 nm. Images of the rifampicin-loaded SLN formulations F1 to F12 are shown in Figure 3.

### Standard Graph of Rifampicin

The absorbance values vs. the concentrations 2, 4, 6, 8, and 10 µg/mL are shown in Figure 4. The correlation coefficient ($r^2$) was 0.99964.

### Entrapment Efficiency

The drug entrapment efficiency of the 12 formulations with 1%, 2%, and 3% PVA are shown in Table 2.

### Stability Studies

The formulations were evaluated for their clarity and encapsulation efficiency at one-month intervals. The results obtained after one month, two months and three months are shown in Table 3.

**Table 3. Clarity and encapsulation efficiency of the formulations sampled at one-month intervals**

| | EP (%) | | | | | | Clarity | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Storage conditions | | | | | | Storage conditions | | | | | |
| | Room temperature (28°C) | | | Refrigeration temperature (4°C) | | | Room temperature (28°C) | | | Refrigeration temperature (4°C) | | |
| Formulation number | After 1 mo | After 2 mo* | After 3 mo* | After 1 mo | After 2 mo | After 3Mo | After 1 mo | After 2 mo | After 3 mo | After 1 mo | After 2 mo | After 3Mo |
| F1 | 29.5 | | | 29.4 | 29.2 | 28.1 | Clear emulsion and nanoparticles were visible under the microscope | Mild fungal growth was observed | Fungal growth increased and the color of the formulation had changed | Clear emulsion and the nanoparticles were visible under the microscope | Emulsion was clear and the particles were still visible under the microscope | Emulsion was stable and the nanoparticles were visible under the microscope |
| F2 | 27.2 | | | 28.07 | 27.6 | 26.8 | | | | | | |
| F3 | 34.1 | | | 34.15 | 33.12 | 32.0 | | | | | | |
| F4 | 20.3 | | | 20.3 | 18.2 | 17.8 | | | | | | |
| F5 | 45.2 | | | 45.3 | 43.2 | 42.1 | | | | | | |
| F6 | 77.6 | | | 78.54 | 77.5 | 76.5 | | | | | | |
| F7 | 55.4 | | | 55.23 | 52.23 | 51.3 | | | | | | |
| F8 | 35.2 | | | 35.2 | 34.1 | 33.1 | | | | | | |
| F9 | 34.6 | | | 34.7 | 33.2 | 32.8 | | | | | | |
| F10 | 49.2 | | | 49.2 | 47.4 | 46.3 | | | | | | |
| F11 | 70.6 | | | 71.4 | 70.4 | 69.2 | | | | | | |
| F12 | 43.3 | | | 42.3 | 41.3 | 40.2 | | | | | | |

*percentage entrapment could not be determined due to fungal growth. %EP = 100 x (amount of drug added initially-amount of drug present in the supernatant)/ amount of drug added initially

***In Vitro* Drug Release Studies**

The percentage drug release of the formulations prepared with 1%, 2%, and 3% PVA are shown in Figure 4.

The *in vitro* drug release profile of the formulations prepared with 1%, 2%, and 3% PVA are shown in Figures 5a-c, respectively.

## Discussion

The results show that the highest encapsulation efficiency of 78.79±0.1% was obtained in the formulation with a drug:lipid ratio of 1:2 with a polyvinyl alcohol concentration of 2%. An encapsulation efficiency of 71.6±0.5% was obtained with the drug:lipid ratio of 1:3 with a polyvinyl alcohol concentration of 3%. The encapsulation efficiency of the formulations prepared with 1% PVA was very low when compared with the formulations prepared with 2% and 3% PVA. On comparing the encapsulation efficiency of all the formulations, it was revealed that an increase in the concentration of PVA enhanced the encapsulation efficiency (11).

The stability study conducted over three months revealed that the formulations stored at room temperature were prone to fungal growth, whereas the formulations stored in the refrigerator were stable. The encapsulation efficiency determined for the formulations stored under refrigeration conditions were decreased by a small fraction. Transitions of dispersed lipid from metastable forms to stable forms might occur slowly during storage due to the small particle size and the presence of the emulsifier, which might lead to drug expulsion from SLNs. Therefore, the lower entrapment efficiency observed after storage may be due to drug expulsion during lipid modification (9).

*In vitro* release studies were performed in which the stability of the particles was indicated by slow drug release. Particle stability is known to be enhanced by PVA, which forms a barrier to the release of the incorporated drug. PVA is the most commonly used emulsifier to stabilize emulsions, since it forms particles of relatively small size and uniform size distribution. A fraction of PVA was associated with the nanoparticles despite repeated washing because PVA forms an inter-connected network at the interface (11). Since residual PVA associated with the nanoparticle surface could be up to 13% w/w of the nanoparticles, we hypothesized that it could influence various physical properties of the nanoparticles (11).

## Conclusion

The ultimate goal of controlled drug release is to maximize therapeutic activity while minimizing the negative side effects of the drug. In this regard, SLNs have emerged as a novel drug carrier system for the hydrophobic anti-tubercular drug, rifampicin. We have shown that rifampicin as in SLNs is a promising nanomedicine with improved stability and more sustained release. Lipophilic drugs such as rifampicin can be successfully loaded in stearic acid. The *in vitro* release study of rifampicin demonstrated that this system is suitable for improving the oral delivery of rifampicin in the treatment of tuberculosis.

**Conflict of interest**

No conflicts of interest were declared by the authors.

**Authors' contributions:** Conceived of and designed the experiments: DS. Experiments were performed and the data were analyzed at PSG College of Pharmacy, Coimbatore, TN, India by all authors. Wrote the paper: DS. All authors read and approved the final manuscript.

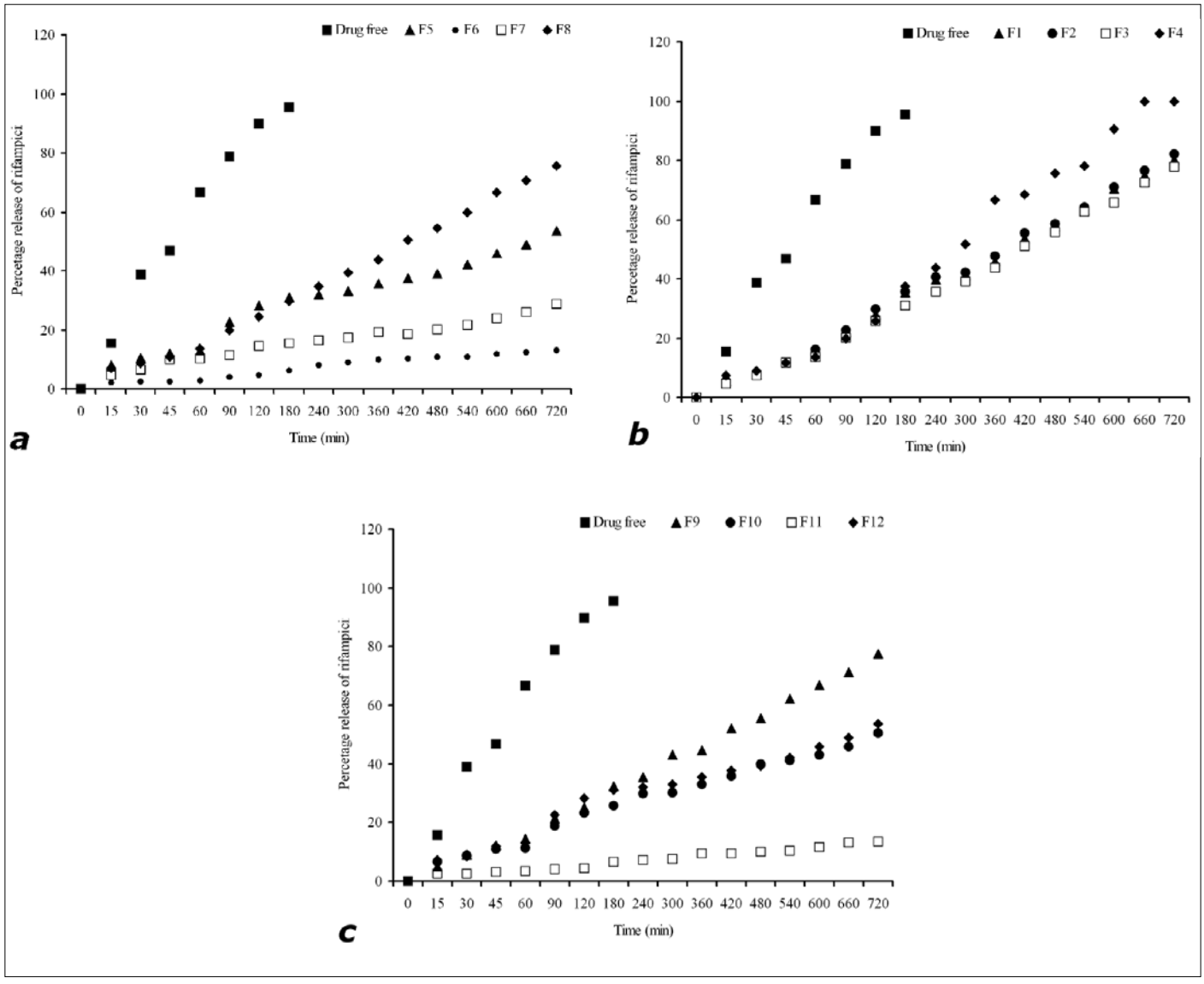


**Figure 5. Percentage drug release of formulations prepared with 1% PVA (a), 2% PVA (b) and 3% PVA (c) n=3**

## References


1. Eliana B. Souto, Rainer H. Müller, Lipid nanoparticles (SLN and NLC) for drug delivery, In: Domb AJ, Tabata Y, Ravi Kumar MNV, Farber S, Editors. Nanoparticles for Pharmaceutical Applications. Los Angeles, California: American Scientific Publishers; 2007.p.103-22.
2. Vyas SP, Khar RK. Targeted and Controlled Drug Delivery: Novel Carrier Systems. 1st ed. New Delphi: CBS Publishers; 2002.
3. Tabatt K, Kneuer C, Sameti M, Olbrich C, Müller RH, Lehr CM, et al. Transfection with different colloidal systems: comparison of solid lipid nanoparticles and liposomes. J Control Release 2004; 97(2): 321-32. [CrossRef]
4. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev 2001; 47(2-3): 165-96 [CrossRef]
5. Quenelle DC, Winchester GA, Staas JK, Barrow EL, Barrow WW. Treatment of tuberculosis using a combination of sustained-release rifampin-loaded microspheres and oral dosing with isoniazid. Antimicrob Agents Chemother 2001; 45(6): 1637-44. [CrossRef]
6. Pandey R, Khuller GK. Antitubercular inhaled therapy: opportunities, progress and challenges. J Antimicrob Chemother 2005; 55(4): 430-5. [CrossRef]
7. Manjunath K, Venkateswarlu V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J Control Release 2005; 107(2): 215-28. [CrossRef]
8. Vijaya Raghavan C. Experimental Biopharmaceutics and Pharmacokinetics. 1st ed. India: New Century. 2006.
9. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release 2004; 95(3): 627-38. [CrossRef]
10. Pandey R, Sharma S, Khuller GK. Oral solid lipid nanoparticle-based antitubercular chemotherapy. Tuberculosis (Edinb) 2005; 85(5-6): 415-20. [CrossRef]
11. Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release 2002; 82(1): 105-14. [CrossRef]