International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 5, Issue 1, pp: (163-172), Month: April - September 2017, Available at: www.researchpublish.com

Levofloxacin Loaded Nano-Niosomes for Effective Treatment of Tuberculosis

Ranjini D S[1], Parthiban S[2], Senthil kumar G P[3], Tamizh Mani T[4]

Department of Pharmaceutics, Bharathi College of Pharmacy,Bharathinagar, Maddur Taluk, Mandya District, Karnataka, India

Abstract: **The delivery of drugs by "vesicular drug delivery system" such as nano-niosomes provides several important advantages over conventional drug therapy. Levofloxacin was selected as a suitable drug for the present study because it is a potent second generation fluoroquinolone active against a broad range of gram positive and gram-negative aerobic and anaerobic bacteria. Levofloxacin loaded nano-niosomal formulations were prepared by using different ratio of surfactant (Span 60, span 80) and cholesterol by thin film hydration followed by sonication method and was evaluated for entrapment efficiency, particle size, zeta potential, surface morphology and *in-vitro* drug release. Particle size and zeta potential of the F3 and F6 formulation were found to be 303.5 and 305.8 nm and -49.2 and -42.6 mV respectively. Highest entrapment efficiency was observed in F3 94% and F6 92%. The percent drug release from F1-F6 was observed as follows F1- 86.89%, F2- 74.62%, F3- 59.44%, F4- 80.59%, F5- 72.46% , and F6- 55.8% which follows zero order drug release and Non- Fickian diffusion mechanism.**

Keywords: **Levofloxacin, nano-niosomes, tuberculosis, thin film hydration method, *in-vitro* study, stability study.**

1. INTRODUCTION

The field of nano chemistry research has shown a great progress in the developing of novel nanocarriers as potential drug delivery systems. Rapid progress in the application of nanotechnology for therapy and diagnosis has made a new field called "nanomedicine" and related subfields such as "pharmaceutical nanocarriers".[1]

Reducing the size into nanoscale in drug carriers offer many advantages such as: improving pharmacokinetics and biodistribution of therapeutic agents due to higher ratio of surface area to volume; diminishing toxicity by their preferential accumulation at the target site, facilitating intracellular delivery and prolonging their retention time either inside the cell which improves therapeutic potential of drugs or in blood circulation limitations.[2]

Tuberculosis (TB) is an important public health problem; about two billion people (one third of the world's population) were infected with TB. Multidrug-resistant (MDR) tuberculosis (TB) is a form of TB that is resistant to some of the first-line drugs used for the treatment of the disease. It is associated both with a higher incidence of treatment failures and of disease recurrence, as well as with higher mortality than forms of TB sensitive to first-line drugs.[3]

It is usually initiated by the entry of the Mycobacterium into the respiratory system as aerosol droplet. Bacteria are non-specifically phagocytosed by alveolar macrophages that process the bacterial antigens and present them to lymphocytes. Then, the number of pathogens increases exponentially by killing host cells and spreading locally to regional lymph nodes in the lungs by lymphatic circulation 3-8 weeks after infection. Later on, spreading of the bacilli from the infected lungs to distant highly irritated organs takes place within 3 months after infection. At this stage, acute TB meningitis or disseminated TB can sometimes result in death.[4]

Fluoroquinolones are among the most promising antibiotic drugs used in the treatment of tuberculosis (TB) for drug-sensitive patients who are intolerant to first-line antituberculous agents or who are infected with drug-resistant organisms.[5, 6]

Levofloxacin is one of newer fluoroquinolones with high bactericidal activity against M.Tuberculosis and is the pure (-)-(S)- enantiomer of the racemic drug substance oflaxacin and it has recently become available for therapy. The mechanism of action relies on the DNA-DNA-gyrase complex by inhibiting DNA gyrase (topoisomerase II) mainly in Gram-negative bacteria, and topoisomerase IV mainly in Gram-positive bacteria.

Levofloxacin has low resistance, good activity levels and high respiratory penetration and it is well tolerated with good adherence. In addition, it is particularly well suited for shorter courses of therapy at higher doses; it may reduce the emergence of resistant strains, decrease the impact on endogenous flora, offer high cure rates and avoidance of adverse effects, enhanced patient and healthcare convenience.[7, 8]

2. MATERIALS AND METHODS

Levofloxacin is a gift sample from the Micro Laboratories ltd., Bangalore. Span 60, span-80, cholesterol, chloroform and methanol were purchased from SD fine chemicals ltd, (Mumbai, India). Phosphate Buffer Saline pH 7.4 (PBS pH 7.4) were prepared as described in the Indian Pharmacopoeia (1996).

METHODS:

Preparation of nano-niosomes:

Thin film hydration method:

Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform – methanol mixture (1:1 v/v) in 100 ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60 ℃ to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of residual solvent can be ensured by applying vaccum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60 ℃ for a period of 1 hr until the formation of niosomes and was subjected to bath sonication, maintained the temperature at 60 ℃ for 3 min to produce small and uniform size nano-niosomes.[9]

Table1: composition of surfactant and cholesterol for preparation of niosomes.

SI. No	Code	Surfactant	Drug: Surfactant: Cholesterol	Weight taken (mg)		
				Drug	Surfactant	Cholesterol
1	F1	Span 60	1:1:1	100	100	100
2	F2		1:2:1	100	200	100
3	F3		1:1:2	100	100	200
4	F4	Span 80	1:1:1	100	100	100
5	F5		1:2:1	100	200	100
6	F6		1:1:2	100	100	200

EVALUATION PARAMETER FOR LEVOFLOXACIN NANO-NIOSOMES [10-16]

The evaluation parameters of Levofloxacin loaded nano-niosomes are; Drug-Excipient compatibility studies by FT-IR, Surface morphology, Particle size analysis, Entrapment efficiency, Zeta potential, *In-vitro* drug release, Stability studies.

***In-vitro* drug release:**

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. Levofloxacin niosomal suspension equivalent to 10 mg was taken in dialysis bag and the bag was placed in a beaker containing 100 ml of pH 7.4 Phosphate buffer. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37±0.5 ℃. 1 ml sample were withdrawn periodically and were replaced by fresh buffer. The samples were assayed by UV Spectrophotometer at 288 nm using phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and plotted against time.

Stability studies as per ICH guidelines:

To confirm the stability of nano-niosomal formulation, intermediate stability testing studies was performed for 6 months. The optimized formulation was kept at 30±2 ℃ and 65±5% ℃ RH and 4±2 ℃ in stability chamber. Drug particle size, entrapment and drug release were fixed as physical parameters for stability testing.

3. RESULT AND DISCUSSION

Drug-excipients compatibility studies were carried out using FT-IR. The spectra of pure drug (Levofloxacin) were compared with physical mixture of drug: span60: cholesterol and drug: span 80: cholesterol. Characteristic peaks of pure drug are also found in physical mixture indicated that there was no interaction between drug and excipients. The results are shown in Fig 1-3. The formulation F3 and F6 was studied by SEM analysis in which the nano-niosome was appeared with irregular surface due to the presence of unentrapped drug. The result of SEM was shown in fig 4, 5. Particle size of the nano-niosomes was analyzed by using Malvern particle size analyzer for the formulations F3 and F6. The mean particle size of formulation F3 and F6 was found to be 303.5 nm and 305.8 nm with particle size distribution less than 600 nm. The results are shown in the fig 6, 7. The percentage entrapment efficiency of loaded drug in different nano-niosomal formulations with different surfactant and cholesterol ratio was determined spectrophotometrically. The results were shown in Table 1 and Figure 8, 9. Highest entrapment efficiency was observed in F3 94% for the formulation containing span 60 and in F6 92 % for the formulation containing span 80 .The high drug entrapments may be observed due to increase in the cholesterol ratio. On comparison for the different formulation the niosomes containing span 60 with higher cholesterol ratio have higher entrapment efficiency than the niosomes containing span 80. Zeta potential is a key factor for evaluation of the stability of colloidal dispersion. It was currently admitted that zeta potentials above -30 mV were required for full electrostatic stabilization. The zeta potential was measured for the Formulations F3 and F6. The values of zeta potential of Levofloxacin loaded niosomal formulation F3 and F6 were found to be -42.6mV and -49.2mV which are shown in fig 10, 11. From the results it was observed that the formulation were stable sufficiently. *In-vitro* release study of Levofloxacin from various formulations was conducted for 12 hrs by using dialysis membrane. Cumulative % drug release was plotted against time (t). The percent drug release from F1-F3 was observed as follows F1- 86.89%, F2- 74.62%, F3- 59.44%, after 12 hrs and for the formulation F4-F6 it was observed as F4- 80.51%, F5- 72.46%, F6- 55.8% at the end of 12 hrs. The increase in surfactant and cholesterol ratio causes decrease in the drug release. Compare to the formulation containing span-60 (F1-F3), the span-80 containing formulation (F4-F6) shows higher drug release due to lesser particle size. The percent drug release of F3 and F6 showed the decrease in the drug release due increasing the ratio of cholesterol, the release was more controlled by increasing the cholesterol ratio. All the formulations released the drug in a controlled manner. The *in-vitro* release data were shown in Table 2, 3 and fig 12, 13. The intermediate stability study for F3 was performed for 6 months according to the ICH guide lines. Drug entrapment and drug release were fixed as physical parameters for stability testing and stability studies of selected formulation F3 showed that negligible changes in entrapment efficiency and drug release. This revealed that the formulation stable on storage at 4±2 ℃, 30±2 ℃ and 65±5% ℃ RH and the result were given in the table 4 and 5.

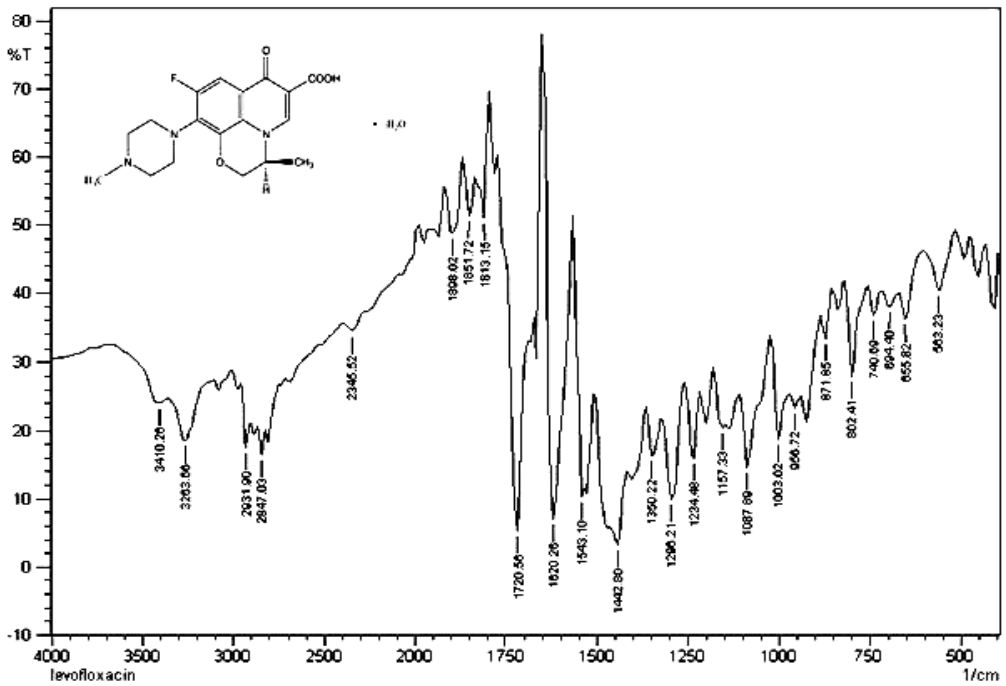

Fig 1-: FTIR spectrum of Levofloxacin

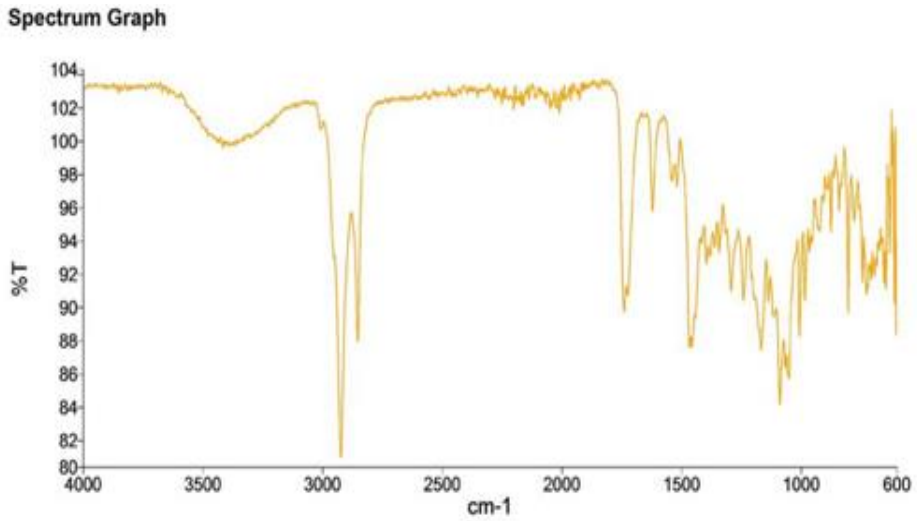

Fig-: 2 FTIR spectra of physical mixture of Levofloxacin, cholesterol and span-60

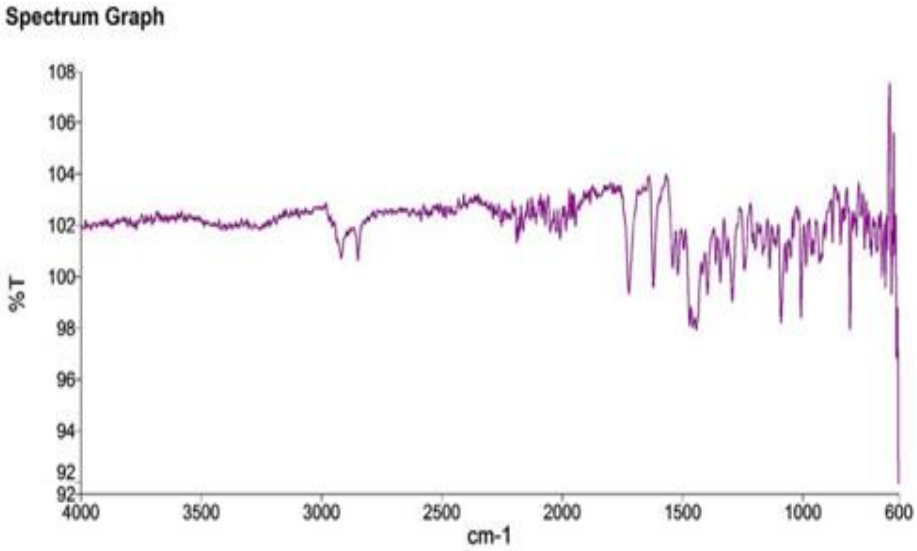

Fig-:3 FTIR spectra of physical mixture of Levofloxacin, cholesterol and span-80

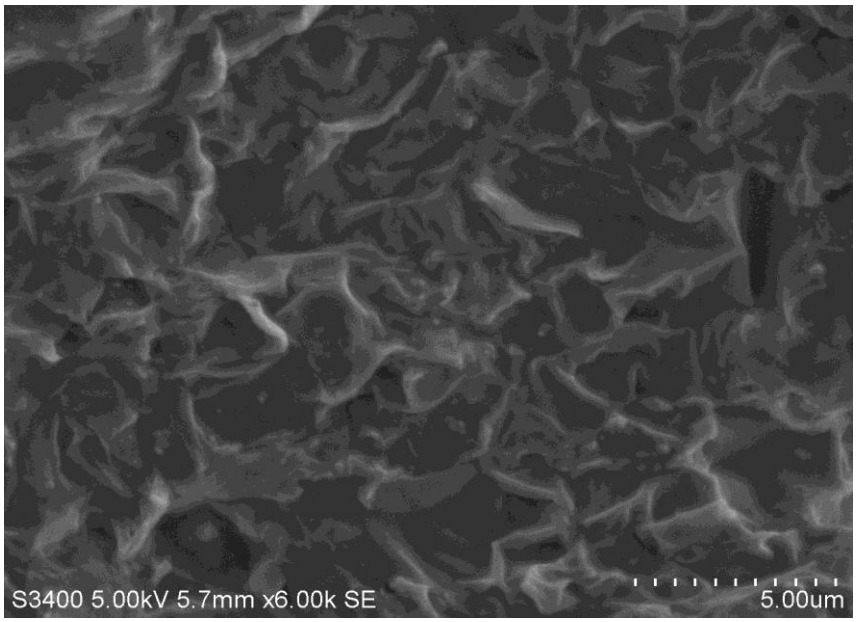

Fig- :4 SEM of the optimized formulation F3

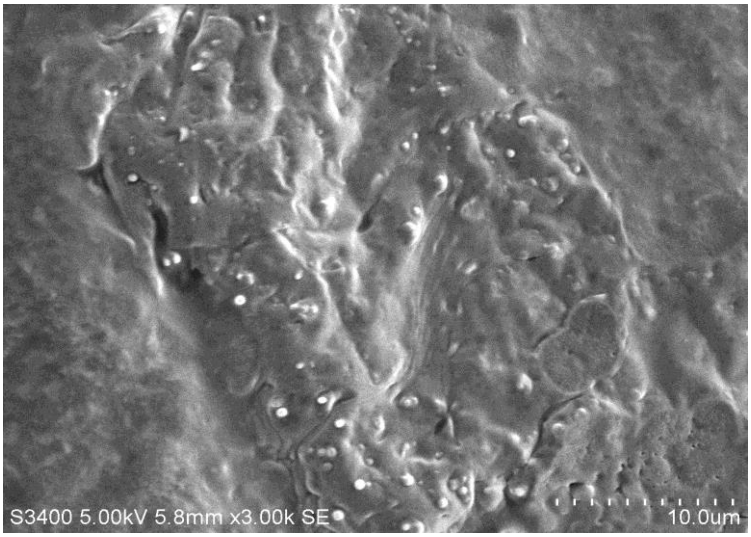

Fig- :5 SEM of the optimized formulation F6

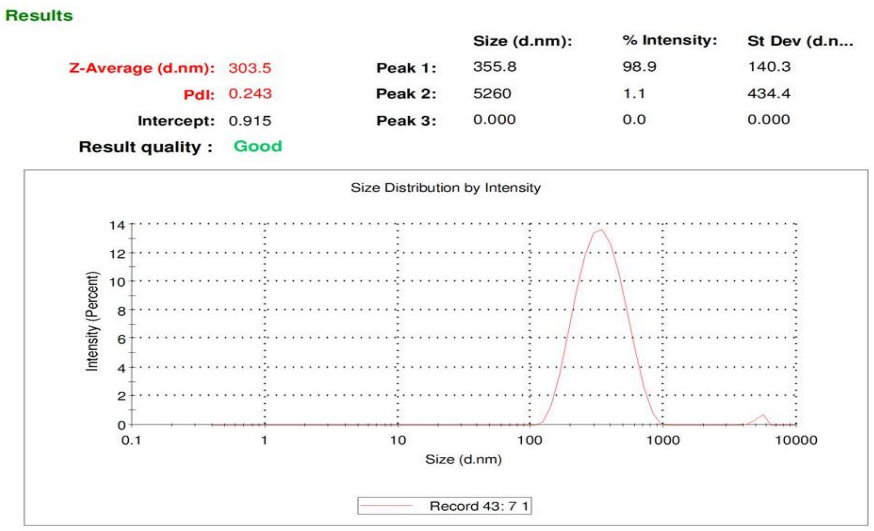

Fig -:6 Particle size distribution analysis of formulation F3

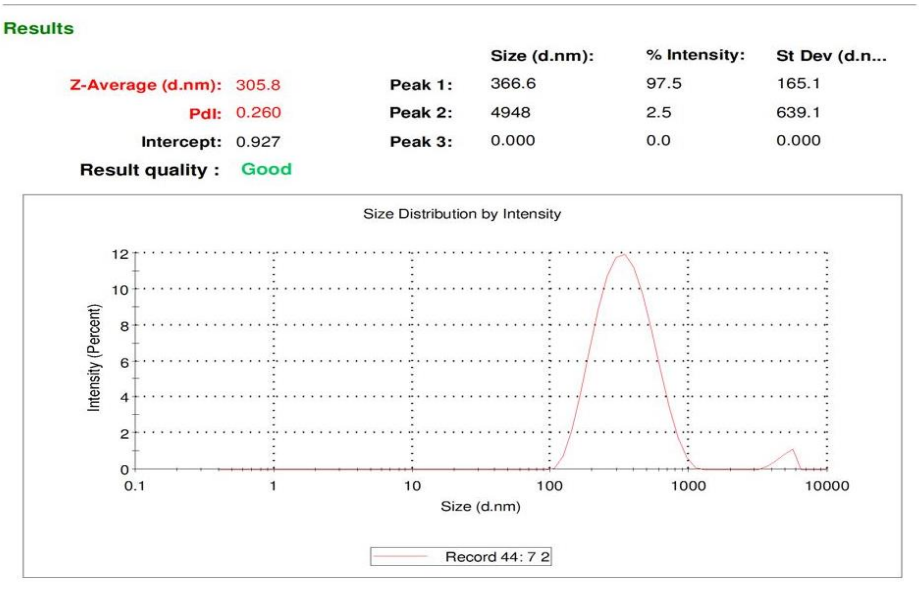

Fig -:7 Particle size distribution analysis of formulation F6

Table: 1 entrapment efficiency

Sl. No.	Formulation code	% Entrapment Efficiency
1	F1	87
2	F2	90
3	F3	94
4	F4	85
5	F5	89
6	F6	92

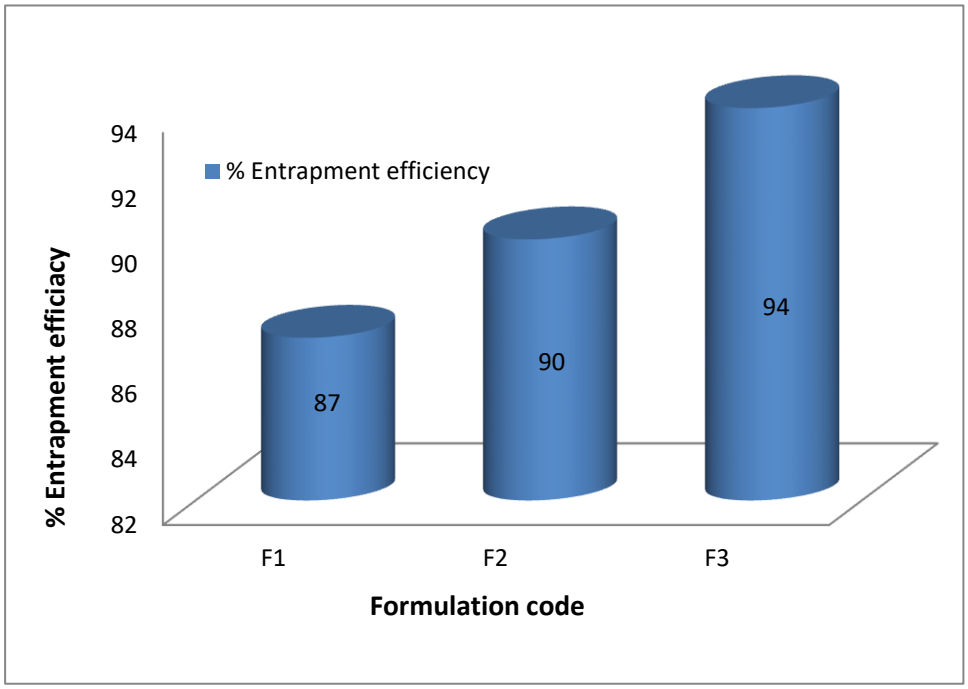

Fig- :8 Entrapment efficiency of formulations F1-F3

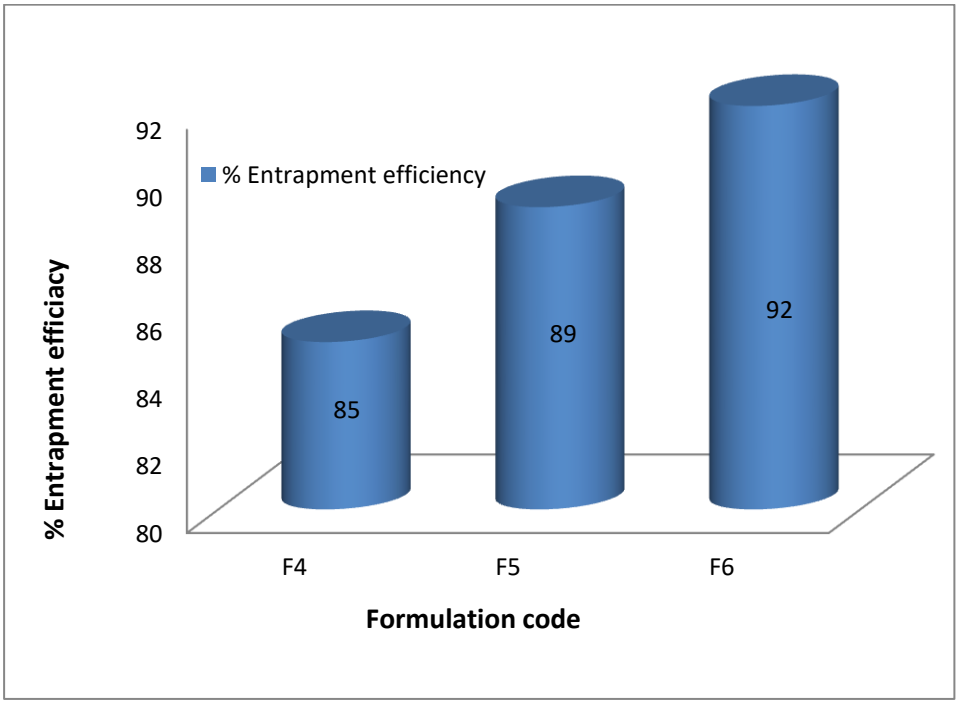

Fig- :9 Entrapment efficiency of formulations F4- F6

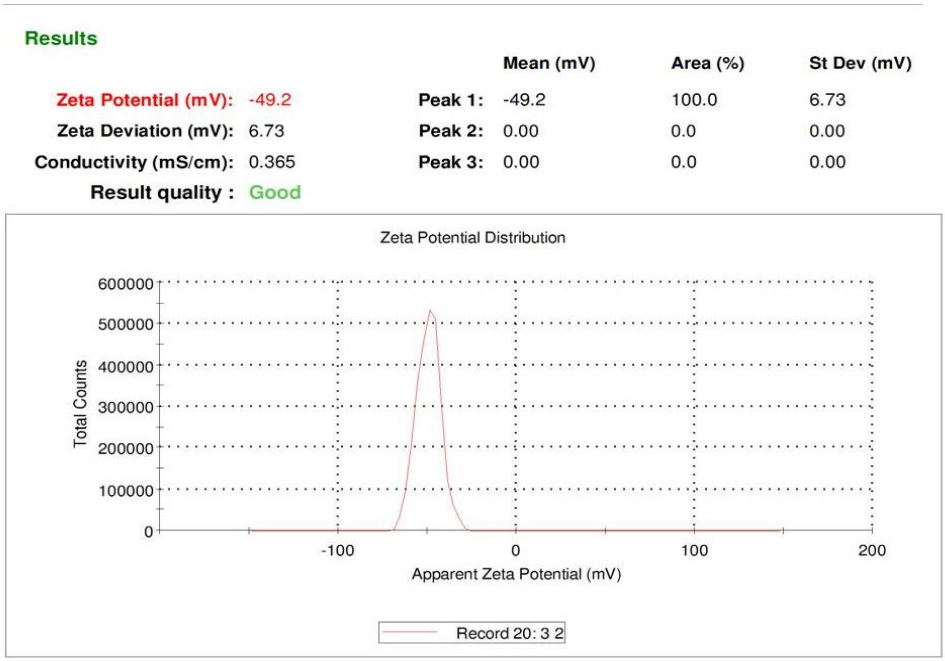

Fig-:10 Zeta potential analysis of formulation F3

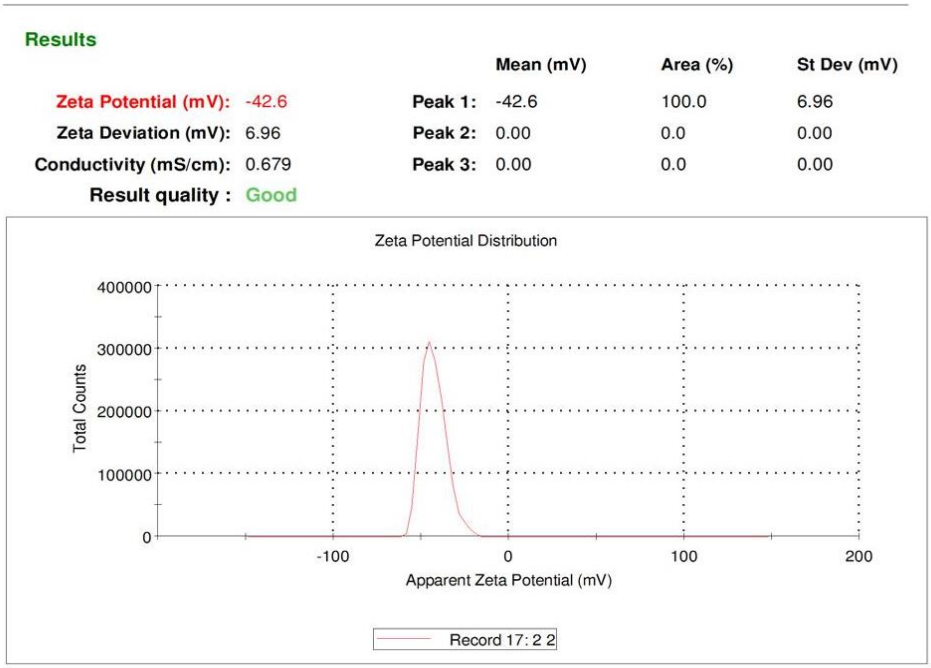

Fig-:11 Zeta potential analysis of formulation F5

Table: 2 *In-vitro drug release* profile of F1-F3

Time (hrs)	% Cumulative Drug Release		
	F1	F2	F3
0	0	0	0
1	8.34	6.1	4.25
2	16.18	13.27	9.11
4	32.84	26.59	18.23
6	48.02	41.51	29.69
8	64.16	52.84	40.53
12	86.89	74.62	59.44

Table: 3 *In-vitro drug release* profile of F4-F6

Time (hrs)	% Cumulative Drug Release		
	F4	F5	F6
0	0	0	0
1	7.07	5.34	3.05
2	14.58	12.83	7.76
4	30.96	25.89	15.41
6	44.15	39.34	27.67
8	58.98	50.9	39.22
12	80.51	72.46	55.8

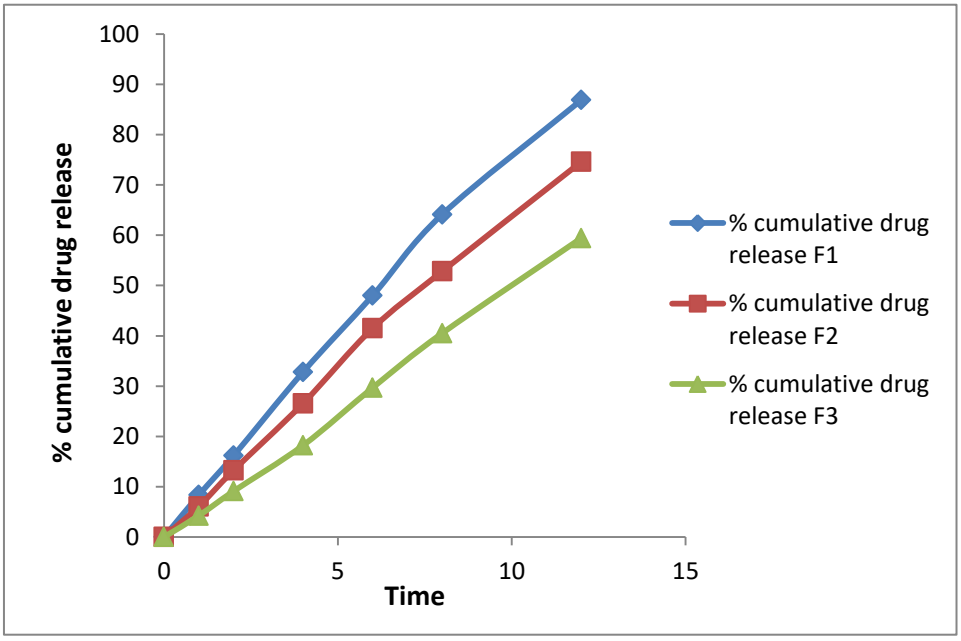

Fig-:12 *In-vitro* release profile of F1-F3 formulation

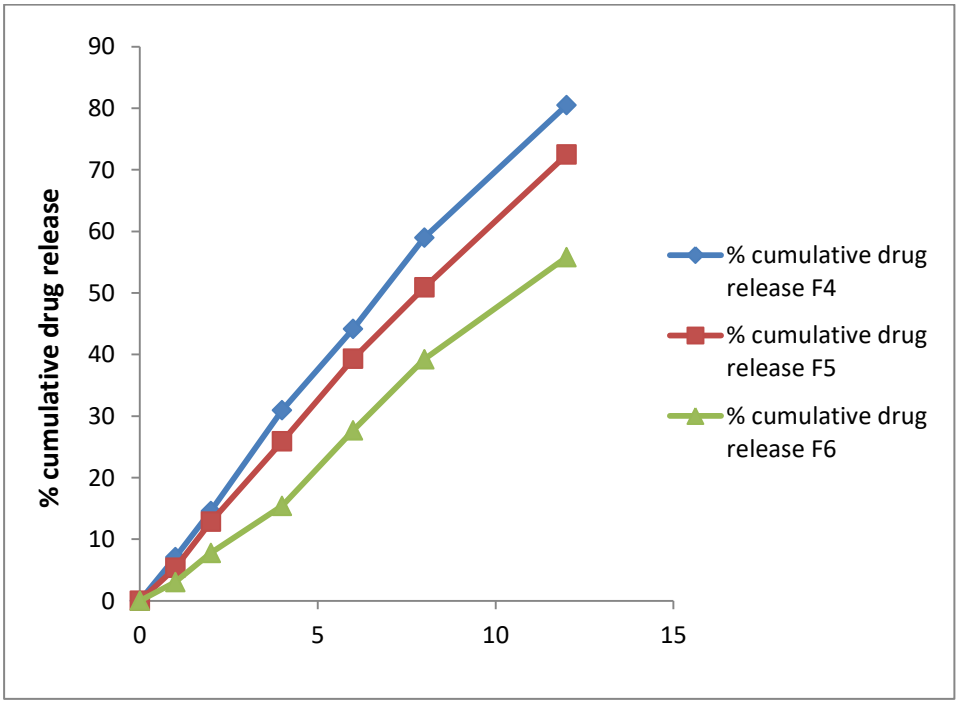

Fig-:13 *In-vitro* release profile of F4-F6 formulation

Table 4: Effect of storage condition on the stability of the optimized formulation F3 at 30±2 ℃ and 65±5% ℃ RH

Parameters	Duration in Months			
	0	1	3	6
Entrapment efficiency (%)	94%	93.04%	91.75%	89.84%
% Drug release	59%	58.21%	56.53%	55.05%

Table 5: Effect of storage condition on the stability of the optimized formulation F3 at 4±2 ℃ RH

Parameters	Duration in Months			
	0	1	3	6
Entrapment efficiency (%)	94%	93.88%	92.53%	91.02%
% Drug release	59%	58.92%	57.67	56.55%

4. CONCLUSION

In the recent years, attentions have been attracted toward vesicular drug delivery systems such as nano-niosomes. It is obvious that, nano-niosomes appears to be a well preferred drug delivery and present a convenient, prolonged, targeted and effective drug delivery system with the ability of loading both hydrophilic and lipophillic drugs. The increase in surfactant and cholesterol ratio causes decrease in the drug release. Compare to the formulation containing span-60, the span-80 containing formulation shows higher drug release due to lesser particle size. The percent drug release of F3 and F6 showed the decrease in the drug release due increasing the ratio of cholesterol, the release was more controlled by increasing the cholesterol ratio.

REFERENCES

[1] Boulaiz H, Alvarez PJ, Ramirez A, Marchal JA, Prados J, Rodríguez-Serrano F *et al*., Nanomedicine: application areas and development prospects. Int J Mol Sci. 2011 May 19;12(5):3303-21.

[2] Hughes GA. Nanostructure-mediated drug delivery. Nanomed nanotech biol med. 2005 Mar 31;1(1):22-30.

[3] Richeldi L *et al*. Clinical use of levofloxacin in the long term treatment of drug resistant tuberculosis. Monaldi Arch Chest Dis. 2002 Feb;57(1):39-43.

[4] Hari BV, Chitra KP, Bhimavarapu R, Karunakaran P, Muthukrishnan N, Rani BS. Novel technologies: A weapon against tuberculosis. Indian J Pharmacol. 2010 Dec;42(6):338-44.

[5] Ahmad V, Sidiq Z, Vashishtha H, Hanif M, Shrivastsava D. Additional Resistance to Moxifloxacin and Levofloxacin among MDR-TB Patients with Base Line Resistance to Ofloxacin at a Reference Laboratory. J Biotechnol Biomater. 2016;6(3):1-3.

[6] Marra F *et al*. Levofloxacin treatment of active tuberculosis and the risk of adverse events. Chest Journal. 2005 Sep 1;128(3):1406-13.

[7] Morrissey I, Hoshino K, Sato K, Yoshida A, Hayakawa I, Bures MG, Shen LL. Mechanism of differential activities of ofloxacin enantiomers. Antimicrob Agents Chemother. 1996 Aug 1;40(8):1775-84.

[8] Chi-Huei Chiang, Levofloxacin for the treatment of respiratory tract infections based on treatment guidlines. 44-51.

[9] Moghassemi S, Hadjizadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. J Control Release. 2014 Jul 10;185:22-36.

[10] Javad MP, Reza MS, Ahmed ES, Bahram B, Azadeh H. Preparation and evaluation of the antiviral activity of acyclovir loaded nano-niosomes against herpes simplex virus type. Pharmacophore. 2014;5(4):483-93.

[11] Malay K Das and Ranjeet Kumar.Development of Curcumin nanoniosomes for skin cancer chemoprevention. Int.J. ChemTech Res.2014-2015;7(2):747-754.

[12] Shilakari Asthana G, Sharma PK, Asthana A. In Vitro and In Vivo Evaluation of Niosomal Formulation for Controlled Delivery of Clarithromycin. Scientifica. 2016 May 16;2016:1-10.

[13] Sundaresan P, Sravanthi C, Gowtham T. Evaluation of aceclofenac niosomes prepared by various techniques. Int J Pharm Sci Rev Res. 2012;16(1):75-78.

[14] Mahajan HS, Shah NN, Nerkar PP, Kulkarni A, Shirpur MS. Niosomes encapsulated with Gatiflaxacin for ocular drug delivery. Recent Advances in Pharmaceutical Science Research. 2012;1(1): 28-39.

[15] Navya MN, Parthiban S, Neasalin JA, Vikneswari A. Advanced Pharmaceutics. A. Formulation and evaluation of niosomal drug delivery system for Flutamide for the treatment of prostate cancer. Int J Adv Pharm. 2015;5(1):9-14.

[16] Srinivas S, Kumar YA, Hemanth A, Anitha M. Preparation and evaluation of niosomes containing aceclofenac. Dig J Nanomater Bios. 2010 Mar 1;5(1):249-54.