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# Formulation of Ofloxacin Loaded Lipospheres with Improved Oral Bioavailability

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**Abstract:** The aim of this research was to formulate the ofloxacin loaded lipospheres as a drug delivery system to improve the oral bioavailability, reduce toxicity and achieve better patient compliance. The ofloxacin loaded lipospheres were formulated by melt dispersion technique using cetyl alcohol; poly vinyl alcohol (0.1%w/v) and pectin (1%w/v) act as lipid carrier, surfactant and co-surfactant respectively. The *in vitro* release kinetic studies were carried out for lipospheres loaded with ofloxacin and the value of $R^2$ in Higuchi model is greater than 0.99 and release exponent (*n),* was found to be more than 0.5 that is Non-Fickian type. The *in vitro* release kinetic followed dissolution and then Korsmeyer–Peppas models. The bioavailability of ofloxacin loaded lipospheres was performed in rabbits after oral administration was studied. The plasma drug concentration was estimated by using a simple, accurate and precise high performance thin layer chromatographic technique. The pharmacokinetics studies demonstrated that the liposphere system enhance the bioavailability of ofloxacin by 2.45 fold after oral administration. Based on these results, we concluded that lipospheres might be a promising lipid based colloidal carrier system to enhance the bioavailability of ofloxacin.

**Keywords:** Bioavailability, Lipospheres, Lipids, Melt dispersion, Ofloxacin, Release kinetics, Surfactants.

## INTRODUCTION

Ofloxacin is a synthetic fluorinated carboxy quinolone that against both gram$^{(-)}$ and gram$^{(+)}$ bacteria [1-4] This drug has been effectively used to treat *Mycobacterium* tuberculosis and also treat a variety of bacterial infections, including those of the respiratory tract, skin, bone, gastrointestinal tract, and urinary tract, and bacterial prostatitis, sexually transmitted diseases, and wound and surgical infections. Though the drug having wider application is associated with numerous adverse reactions, such as tendon damage and hepatotoxicity. The poor aqueous solubility of ofloxacin gives rise to difficulties in the design of pharmaceutical formulations and leads to variable bioavailability [5-6]. In addition, almost all of the oral ofloxacin formulations are available only as conventional, immediate-release tablets that require twice daily administration for consecutive days or weeks [7-9]. The repeated oral doses of ofloxacin over long time could result in nervous system and gastrointestinal system disorders [10-11]. Efforts have been made to develop alternative formulations of ofloxacin to improve therapeutic efficacy, and reduce the drug induced toxicity and frequency of administration [12-15].

The unique properties of lipids viz., their physiochemical diversity, biocompatibility, and proven ability to enhance oral bioavailability of poorly soluble and lipophilic drugs through selective lymphatic uptake have made them very attractive candidates as carriers for oral delivery. With the above promises, the emerging field of lipospheres as a drug delivery system (LS) has attracted considerable academic and research attention in recent decades.

The objectives of this research were to formulate the ofloxacin loaded LS by melt dispersion technique, and characterize the pharmacokinetics, oral bioavailability of OFX-LS in rabbit models. The bioavailability of OFX-LS in plasma samples was determined by using HPTLC technique because these formulations contain various lipophilic excipients that are not soluble in commonly used organic solvents used in HPLC methods. Further, extraction of drug from such lipophilic excipients may not be achieved easily, and such excipients may get adsorbed on stationary phase. Hence, analysis of OFX, particularly from lipid-based delivery systems, would be difficult with respect to identification of suitable solvents and stationary phase [16].

## MATERIALS AND METHODS

### Materials

Ofloxacin was gifted from Microlabs (Bangalore, India). Cetyl alcohol and pectin were purchased from Lobachemi (Mumbai, India), Poly vinyl alcohol (PVA) was obtained from SD Fine- Chemicals (Mumbai, India). All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

### Formulation of Optimized OFX Loaded Lipospheres

The ofloxacin loaded lipospheres were formulated by melt dispersion technique. The accurately weighed quantity of cetyl alcohol (500mg) was melted at 70°C and ofloxacin (100 mg) was dissolved in to molten lipid then the lipidic mixture was emulsified into hot aqueous phase containing

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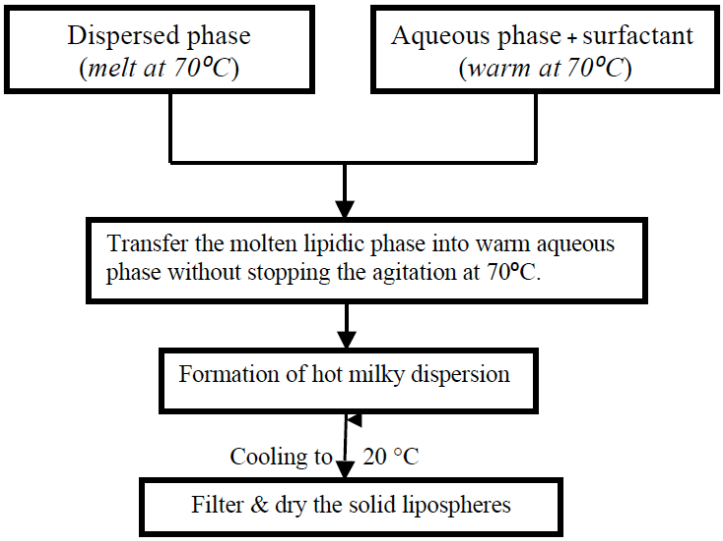


**Fig. (1).** Schematic presentation of formulation of OFX loaded lipospheres by melt dispersion technique.

PVA (0.1%w/v) and pectin (1%w/v). This o/w emulsion was stirred by mechanical stirrer (Remi, India) equipped with three-blade rotor at 750 rpm. Afterwards, the emulsion was heated to the same temperature as the melted lipidic phase. The hot milky dispersion was then rapidly cooled to about 20°C by immersing the formulation flask in ice bath without stopping the agitation to yield a uniform dispersion of LS. The obtained solid LS was then washed with water and isolated by filtration through a paper filter (Fig. **1**).

The characterization of Ofloxacin loaded lipospheres such as yield, entrapment efficiency, morphological character, crystallinity, and *in vitro* release of OFX loaded LS and tableted OFX loaded LS were published in our previous publication. The drug release kinetics, *in vivo* and stability studies were discussed in this article.

### *In vitro* Drug Release of OFX Loaded Lipospheres

To obtain quantitative and qualitative information on drug release from the LS, and possibly to correlate the experimental data with the release mechanism, the complete release profile of LS encapsulated drugs was determined by placing a drug loaded LS in a buffer solution under USP dissolution test apparatus XII. The release rate of OFX from LS was measured in phosphate buffer medium at pH 7.4 containing 0.5%w/v SLS as wetting agent. Accurately weighed quantity of LS (100mg equivalent weight of drug) was suspended in 900ml buffer solution maintained at 37°C± 0.5°C and rotating at 50rpm. At predetermined time intervals (0-24 hr), the specified volume of samples was withdrawn from the dissolution basket and the same volume of fresh medium was added in the meantime. The samples were centrifuged at 2000 rpm for 5 min and collect the supernatant was taken for quantitative analysis measured by using UV spectroscope at 294nm.

### Pharmacokinetic Studies of OFX loaded Lipospheres

The controlled release formulations bring formulators to work with the common aim of improving efficacy of the bioactives to realize more and more effective products. In order to study the exact mechanism of drug release from lipospheres, drug release data was analyzed according to Zero Order, First Order, Higuchi square root, Hixon Crowell, Koresmeyer model. The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration.

$$C = k_o t \quad (1)$$

Where, $K_0$ is zero-order rate constant expressed in units of concentration/time and t is the time.

The first order Eq. (2) describes the release from system where release rate is concentration dependent.

$$LogC = LogC_o - kt / 2.303 \quad (2)$$

Where, C0 is the initial concentration of drug and K is first order constant.

Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (3).

$$Q = Kt^{1/2} \quad (3)$$

Where, K is the constant reflecting the design variables of the system.

The Hixson-Crowell cube root law Eq. (4) describes the release from systems where there is a change in surface area and diameter of particles or tablets (Hixson and Crowell, 1931 Higuchi, 1961; Higuchi, 1963; Korsmeyer and Peppas, 1981).

$$Q_0^{1/3} - Qt^{1/3} = K_{HC}\, t \quad (4)$$

Where, $Q_t$ is the amount of drug released in time t, $Q_0$ is the initial amount of the drug in tablet and KHC is the rate constant for Hixson-Crowell rate equation.

### Mechanism of Drug Release

Korsmeyer derived a simple relationship which described drug release from a polymeric system Eq. (5). To find out the

mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model:

$$Mt / / M_{\infty} = Kt^{n} \quad (5)$$

Where $Mt / M_{\infty}$ are fraction of drug released at time t, k is the rate constant and n is the release exponent.

| Diffusion Exponent (n) | Overall Solute Diffusion Mechanism |
|---|---|
| 0.45 | Fickian diffusion |
| 0.45 < n < 0.89 | Anomalous (non-Fickian) diffusion |
| 0.89 | Case-II transport |
| n > 0.89 | Super case-II transport |

## Calibration of OFX in Plasma by HPTLC Method

### *Preparation of Standard Stock Solutions*

Standard stock solution of OFX was prepared by dissolving 5 mg of OFX in 10 mL of phosphate buffer pH 7.4 separately to get concentration of 0.5 mg/mL from which 1 mL was further diluted to get stock solution ranging from100-500 ng/µL of drug concentration.

### *Calibration of Ofloxacin*

The final stock solutions of OFX at various concentrations were applied by over spotting on TLC plate in range 10 µL with the help of CAMAG 100 µL sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in three replicates was analyzed and peak areas were recorded. The calibration curves of OFX were plotted against the peak area versus concentration.

The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec were employed. The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using mobile phase n-butanol: Methanol: Ammonia (6:1:3 v/v/v). The optimized chamber saturation time for mobile phase was 10 min. The length of chromatogram run was ~9 cm and development time was approximately 20 min. The TLC plates were dried in a current of air drier. Densitometric scanning was performed at 366nm on CAMAG thin layer chromatography scanner operated by WINCATS software version 1.4.2.

## *In vivo* Bioavailability of Optimized OFX-LS

### *Animal Preparation and Treatment*

The optimized batch Ofloxacin loaded liposphere formulation was considered for *in vivo* studies using rabbit model either sex 1.5 ± 0.2 kg. The animals were acclimatized for at least 1-2 weeks before experimentation, fed with standard diet and allowed for water. The animals were kept on fasting prior to treatment but had free access to tap water. None subject was receiving any other drug at least two weeks before commencement of the study and no other drug was permitted throughout the duration of the study. They were randomly assigned to 4 groups (3 animals per group). The first two groups of rabbits were administered with suspensions of pure OFX with 0.3% carboxy methyl cellulose and OFX lipospheres in the dose of 1mg/ kg were administered orally through an intra-gastric tube. The third group had received placebo LS and the fourth group was considered as control. All experiments adhered to the institutional animal's ethics committee (*KU/IAEC/PhD/ 067*). Then, blood samples (1ml) were withdrawn into heparinized tubes from the marginal ear vein at predetermined time intervals for up to 24hr. Samples were thoroughly mixed, centrifuged and the plasma was stored at 2-8$^{o}$C till the analysis.

### *Instruments and Chromatographic Conditions*

In view of this, high-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis (Bharat *et al.*, 2011). Major advantage of HPTLC method is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. This method also facilitates repeated detection of chromatogram with same or different parameters. Furthermore, in case of HPTLC, there are no restrictions on the choice of solvents and mobile phases; drug and lipophilic excipients can be dissolved in a suitable solvent that would evaporate during spotting on precoated TLC plate, leaving behind analyte as a thin band. Therefore, for such methods, extraction procedure is not required always however could be developed for analyzing drug without any interference from excipients (Rashmin *et al.*, 2009).

### *Preparation of Plasma Samples*

The ofloxacin was extracted from plasma samples obtained from study subjects by addition of acetonitrile to precipitate plasma proteins, which were then vortex mixed for 3 min and centrifugation at 2500 rpm for 20 min for separation. The supernatant was then analysed by HPTLC (CAMAG LINOMET, Japan) method. The densitometric scanning at wavelength 366 nm was done and the concentration of OFX in each sample was calculated using the areas obtained by densitometric scanning. The concentration of ofloxacin in plasma was calculated using the calibration curve prepared.

The samples were spotted on Merck TLC aluminium plates, precoated with silica gel 60F254 (10 cm by 20 cm with 250 µm layer thickness) using a Camag Linomat V applicator (Camag, Switzerland). The samples were applied onto the plates in the form of narrow bands of 5 mm width with a Camag 100µl sample syringe (Camag, Switzerland) under a nitrogen atmosphere. The mobile phase consist of n-butanol: Methanol: Ammonia (6:1:3 v/v/v) was used. The length of the chromatographic run was 9 cm and the time required for each run was approximately 25 min. Linear ascending development was carried out in a twin trough glass chamber (10 cm × 10 cm). Densitometric scanning of the developed plates was performed using Camag TLC scanner III, operated with winCATS software (Version 1.4.2, Camag) in the absorbance mode at 366 nm. Scanning speed was kept at 4 mm/s.

### *Stability Studies on Optimized Batch LS*

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with the time under the influence of a variety of environmental conditions such as temperature, humidity and light. The stability studies of optimized lipospheres were performed for 90 days period. All the stability study samples were prepared in triplicate and were kept at amber glass bottle at refrigerator condition (2-8°C) and 25°C/60% RH; upto 90 days. Particle sizing was performed for the evaluation of the physical stability of the formulation. For establishing the stability of the drug in the lipospheres, drug content was evaluated using UV analysis. At each stability time point, LS samples were evaluated for particle size and drug content.

## RESULTS AND DISCUSSION

The LS was formulated by melt dispersion technique using cetyl alcohol (500 mg) with PVA (0.1%w/v) and pectin (1.0%w/v) as surfactant and co-surfactant respectively. The stirring speed was fixed at 750 rpm for the optimized formulation process. The formulated lipospheres were characterized the *in vitro* kinetic & *in vivo* bioavailability of optimized batch of LS. The morphological character was investigated by using SEM, the results revealed that the particles were spherical in shape with wrinkled surface, (Fig. **2**). The SEM report, particle size of lipospheres was found to be ~35µm, this observation resembles with the observation of particle size by optical microscopy.

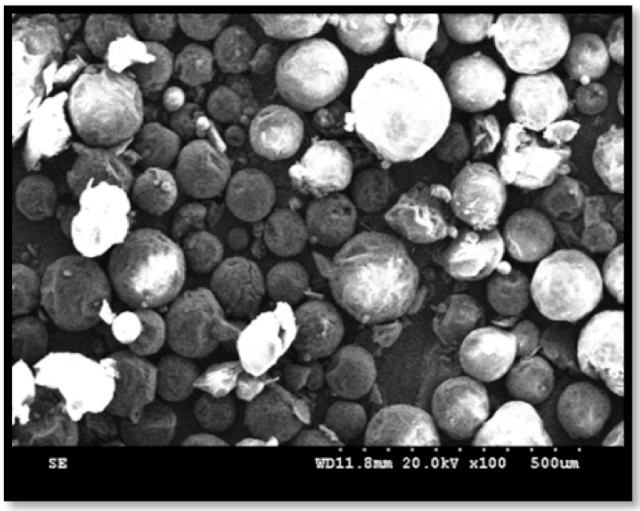


**Fig. (2).** Scanning electron microscopic images showing the morphology of OFX loaded LS (batch- OFX 34) at 100x magnification.

### *In vitro Drug Release Studies of Optimized LS Batches*

The complete release profile of LS encapsulated drugs was determined by using phosphate buffer at pH 7.4 (0.5%w/v SLS) for 24 hr. The *in vitro* release studies of OFX LS 34, was investigated and found that there is an initial rapid removal of the drug possibly by the drug associated loosely on the surface of the lipid matrix. This initial release was rapid (<20%), achieved at 2 hr and is termed as burst release and at 12th hr time interval the drug release rate was achieved at nearly 60% respectively. *In vitro* drug release curve of LS showed the rapid and sustained phase which indicates the *in vitro* release of LS exhibited biphasic phase. The total cumulative drug release was observed ~80% in this study which is shown in (Fig. **3**).

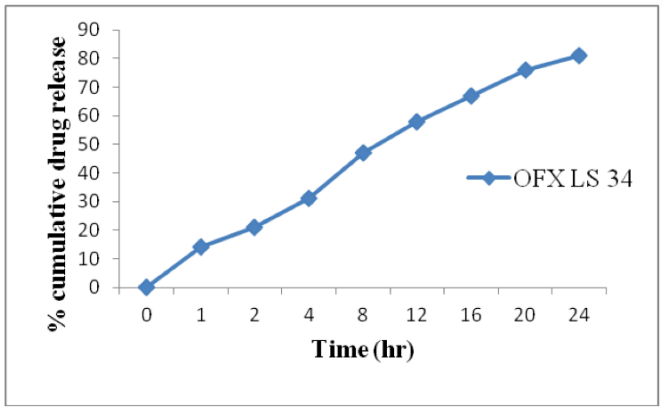


**Fig. (3).** The *in vitro* drug release pattern of OFX Loaded LS (batch code- OFX LS 34).

### *In vitro Drug Release Kinetic Studies of OFX Loaded LS*

The results of *in vitro* drug release studies of LS were fit to various kinetic equations. The Values of $r^2$ and $k$ were calculated for the linear curve obtained by regression analysis of the above plots. The mathematical expression that best describes drug release from these lipospheres was the Korsmeyer–Peppas release model in which the resultant $R^2$ values were greater than 0.99. The Korsmeyer–Peppas release exponent (*n)*, was found to be more than 0.5 that is Non-Fickian type which conforms that the erosion followed by diffusion is the controlling factor for drug release.

The dissolution kinetic studies were carried out for lipospheres loaded with ofloxacin, and the value of $R^2$ in Higuchi model is greater than 0.99 and release exponent (*n)*, was found to be more than 0.5 that is Non-Fickian type. Thus, we concluded that, the OFX LS 34 followed Korsmeyer–Peppas order kinetics (Table **1**).

## Pharmacokinetics Studies of OFX Loaded LS

### *Calibration of OFX in Plasma by HPTLC Method*

Linearity of HPTLC method was constructed by analysis of six solutions containing different concentration of ofloxacin. The linearity of the detector response was tested by spotting standards in triplicate for each concentration ranging between 100 to 500 ng. The data were best fitted by a linear equation mx+ b= y. The recovery of ofloxacin from plasma was determined by comparing peak areas obtained from plasma spiked with Ofloxacin at concentrations of 100, 200, 300, 400 and 500 ng/spot with the peak areas obtained from standards. The peak area was observed to be dependent on the amount of the standard, Ofloxacin and a linear relationship ($r^2$=0.996) was observed between the peak areas of ofloxacin at various concentrations over the range 100–500 ng (Fig. **4**). The solvent system used for the development of the plates produced no interference peaks in the area under the curve, and all other compounds were distinctly separated.

**Table 1.** ***In vitro*** **Drug Release Kinetic Studies of OFX Loaded LS.**

| Model Fitting | OFX LS | |
|---|---|---|
| | $R^2$ | *k* |
| Zero order | 0.9160 | 0.0040 |
| T-test | 6.043 | passes |
| 1st order | 0.9161 | 0.000 |
| T-test | 6.046 | passes |
| Matrix | 0.9984 | 0.0166 |
| T-test | 46.017 | Passes |
| Peppas | 0.9991 | 0.0142 |
| T-test | 62.237 | Passes |
| Hix. crow | 0.9161 | 0.000 |
| T-test | 6.045 | passes |
| **Best fit model** | **Peppas** | |
| **n** | **0.5605** | |
| **K** | **0.0142** | |

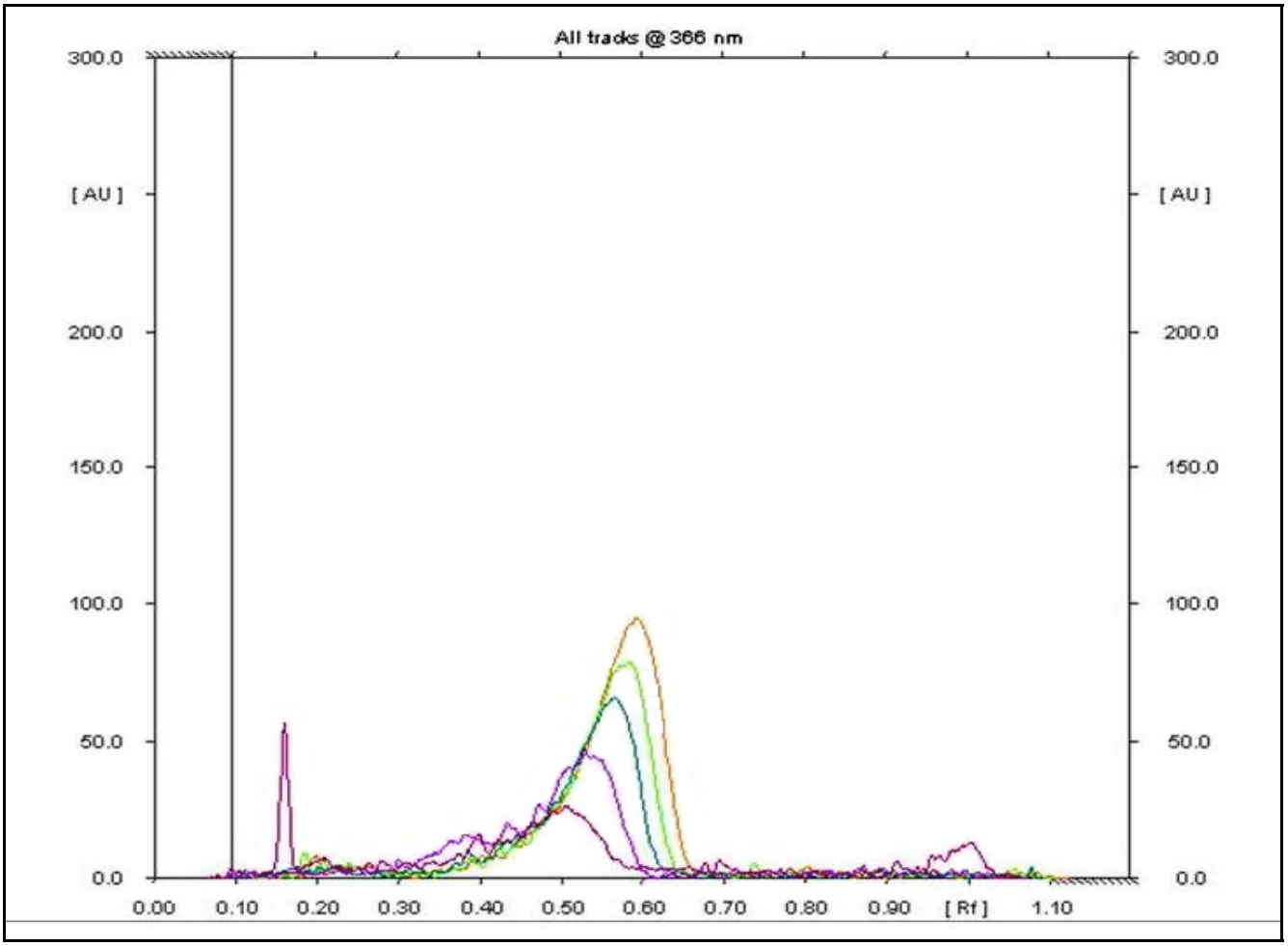


**Fig. (4).** Calibration curve of Ofloxacin in plasma using HPTLC technique.

The RF value of Ofloxacin under the conditions used was found to be 0.45±0.05 and spots were quantified at a wavelength of 366 nm.

### HPTLC Assay

After oral administration of ofloxacin suspension, plasma drug concentration reached a peak level of 1.4µg/mL at 1 hr, and then decreased slowly (Fig. **5.a-5e** & Fig. **6**). The plasma drug level dropped to 0.12 µg/ ml by 12 hr and was undetectable after 16 hrs. In the OFX-34 groups, ofloxacin reached a significantly higher peak concentration of 2.64µg/ ml at 1 hr, and then decreased to 0.16 µg/ ml at 12 hr, but the concentration was maintained 0.12 µg/ mL for up to 24 hr. The $AUC_{0-\infty}$ value of OFX-loaded LS was ~2.45-fold higher than those obtained with the ofloxacin solution. The elimination half-life ($T_{1/2}$el) and the mean residence time (MRT) were also enhanced significantly compared with the ofloxacin solution.

### Stability Studies on Optimized Batch Lipospheres

During long-term storage, triglycerides undergo degradation to fatty acids and mono- and di-glycerides, which could compete with formulation surfactants for positioning on the surface. Fatty acids and monoglycerides can form mixed micelles that might enhance the partitioning of hydrophobic drug out of the nanoparticle. Therefore, the concentration of excipients and possible degradation products need to be determined to understand the stability of nanoparticle.

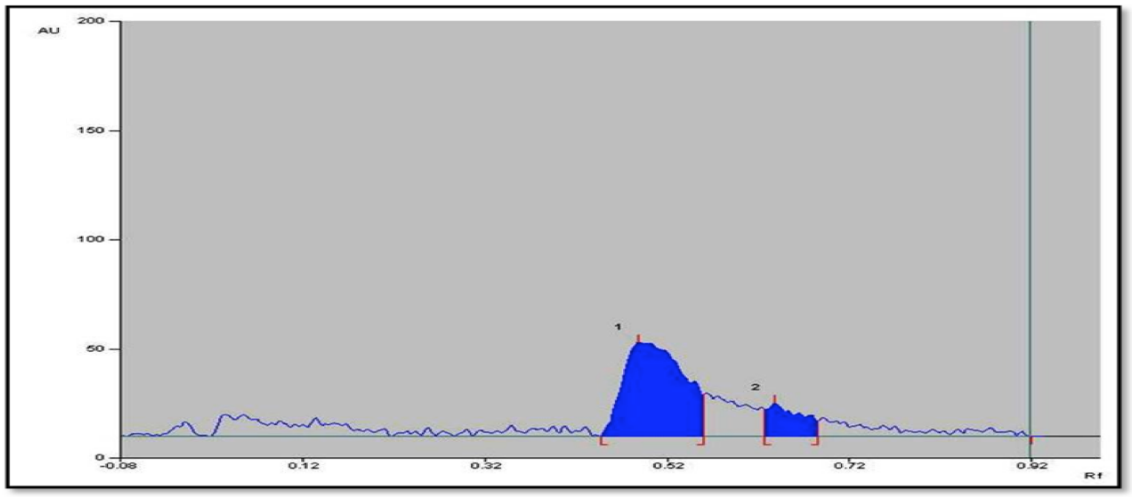


**Fig. (5a).** HPTLC chromatogram of plasma containing OFX- 34 LS at 2h in rabbit model.

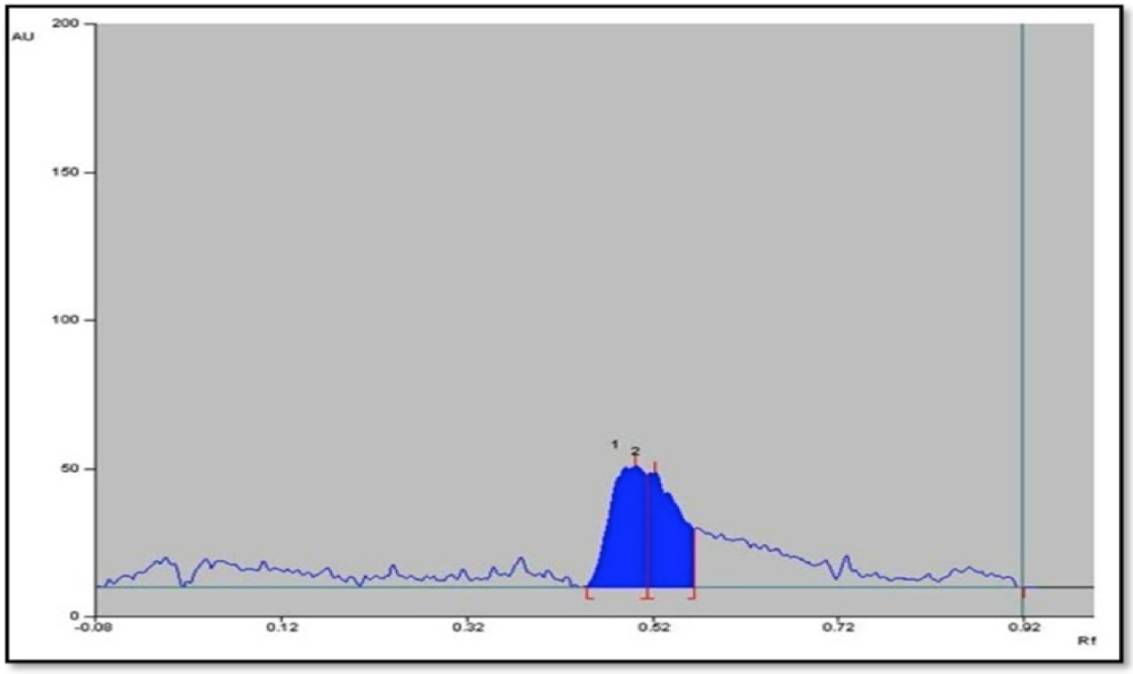


**Fig. (5b).** HPTLC chromatogram of plasma containing OFX- 34 LS at 4h in rabbit model.

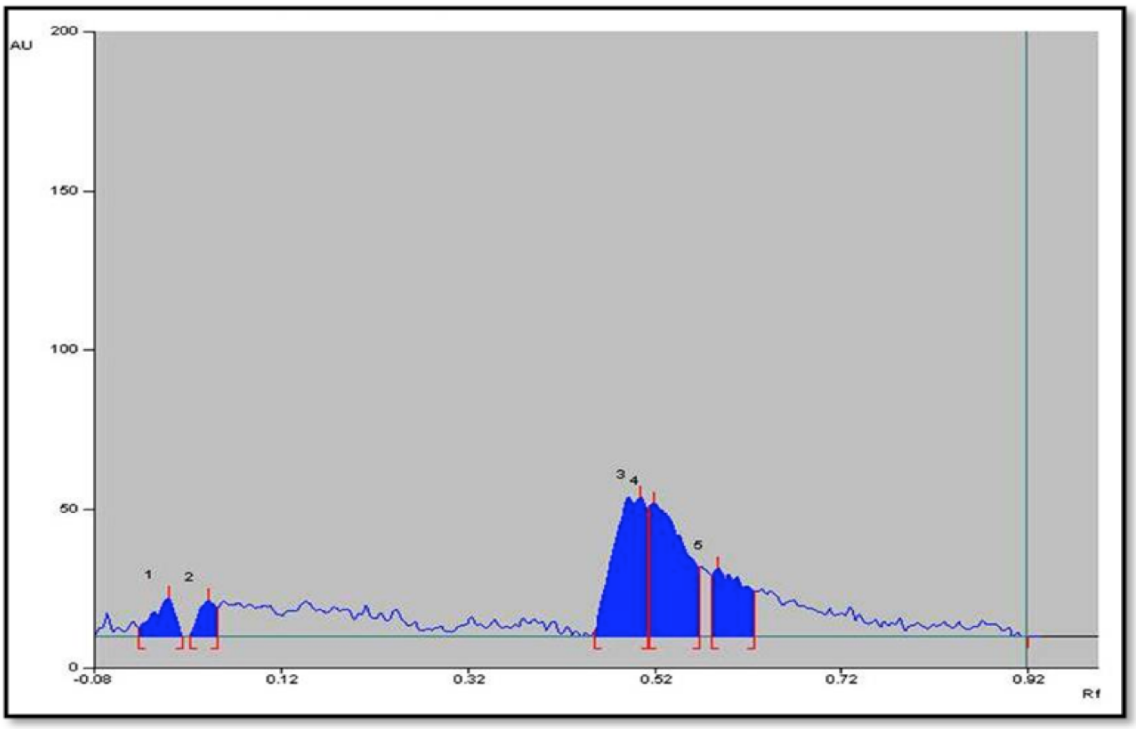


**Fig. (5c).** HPTLC chromatogram of plasma containing OFX- 34 LS at 8h in rabbit model.

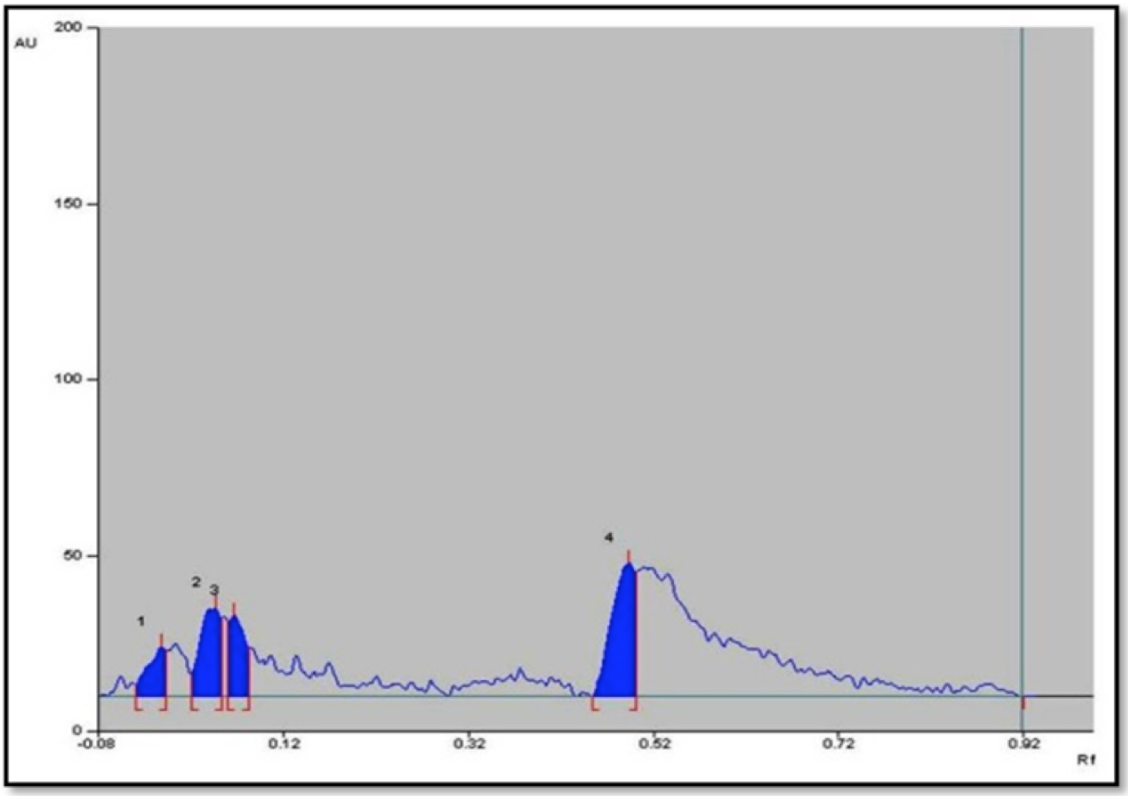


**Fig. (5d).** HPTLC chromatogram of plasma containing OFX- 34 LS at 12h in rabbit model.

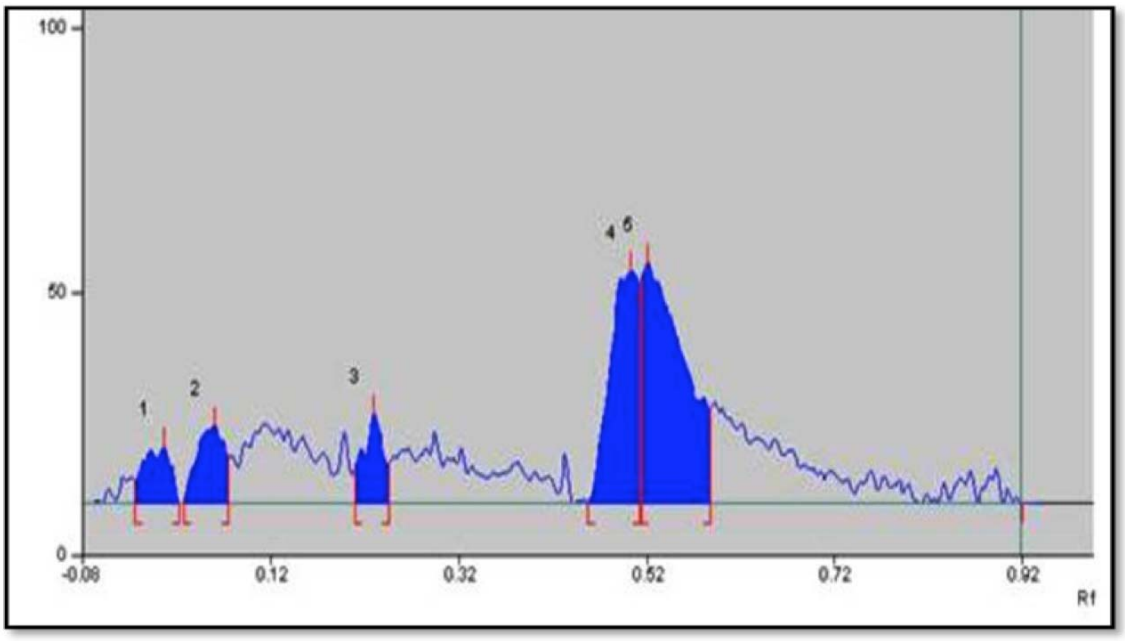


**Fig. (5e).** HPTLC chromatogram of plasma containing OFX- 34 LS at 24h in rabbit model.

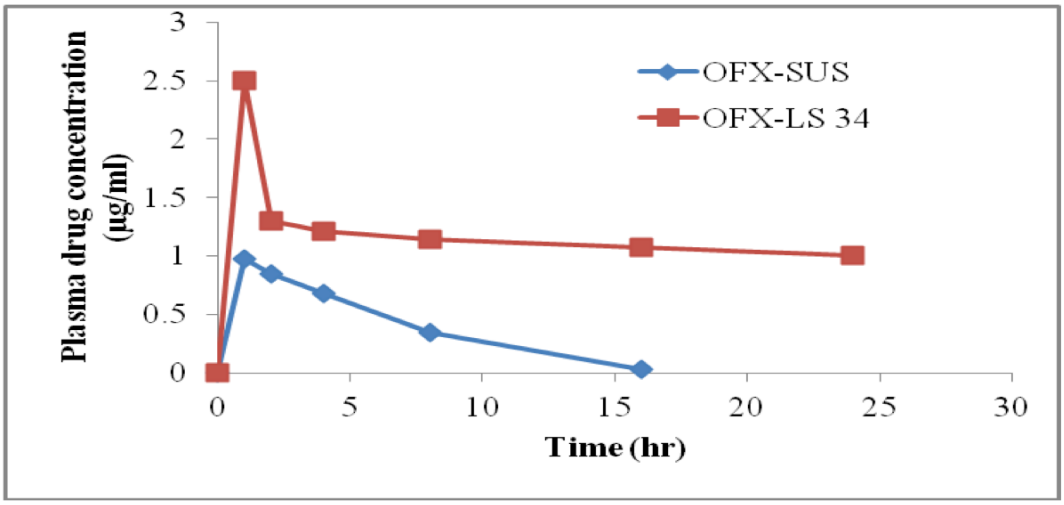


**Fig. (6).** Plasma drug concentration–time curves after oral administration of OFX-LS and Ofloxacin suspension in rabbit (mean ±SD, n=3).

All the formulations are stored in amber colored bottles at room temperature and refrigerator temperature. The formulations were analysed for particle size, and drug content after stored at room temperature and refrigerator temperature for the Day 30, 60 and 90. OFX-LS34, batch was analysed for particle mean diameter and drug content at different time intervals (day 30, 60 and 90) after stored at room temperature. The effect of duration on storage and storage condition on particle size, drug content is shown in (Fig. **7-9** and Table **2**). Stability studies revealed that after 90 days of storage at 25°C the mean diameter and drug content of LS remain practically the same, which emphasizes the physical stability of these lipospheres.

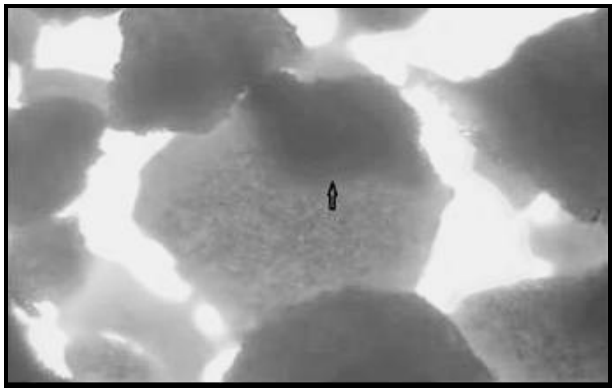

**Fig. (7).** Photomicroscopic image (10X magnification) of OFXLS-34 at 1st day.

**Table 2. Stability Studies on Optimized OFX LS 34 Liposphere.**

| Stability Condition | Study Period | Mean Diameter (µm) | Drug Content (%) |
|---|---|---|---|
| 2-8 °C | 30 | 35±0.531 | 99.87±1.023 |
| | 60 | 34±0.832 | 99.86±0.739 |
| | 90 | 34±0.34 | 99.14±0.994 |
| 25 °C/ 60% RH | 30 | 36±0.03 | 98.87±1.04 |
| | 60 | 36±0.529 | 97.86±1.012 |
| | 90 | 35±0.671 | 95.84±0.899 |

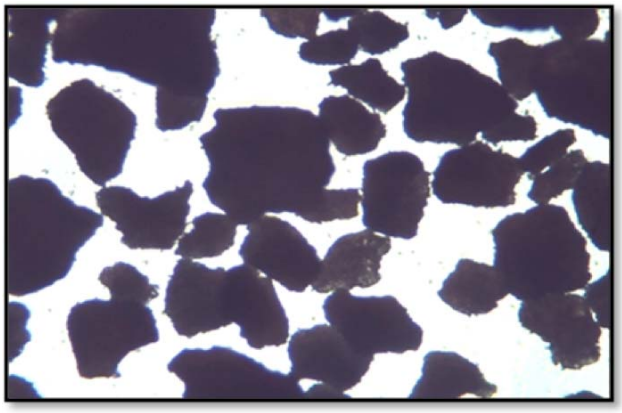

**Fig. (8).** Photomicroscopic image (10X magnification) of OFXLS-34 stored at 2-8$^0$C after 90 days period.

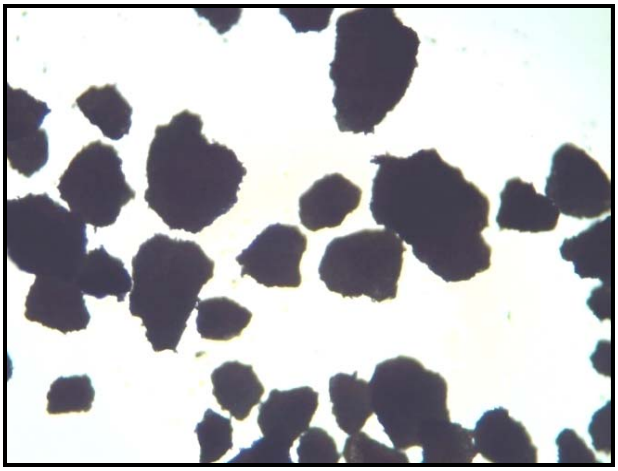

**Fig. (9).** Photomicroscopic image (10X magnification) of OFXLS-34 stored at 25$^0$C / 60% RH after 90 days period.

## CONCLUSION

Ofloxacin loaded lipospheres were successfully incorporated in to cetyl alcohol by melt dispersion technique and investigated on *in vitro* release *in vitro* release kinetics and *in vivo* release of lipospheres. The proposed ofloxacin loaded lipospheres illustrates an effective way to deliver the drug in controlled manner. The *in vivo* results obtained after oral administration of lipospheres to healthy rabbits were found to be satisfactory. The ofloxacin loaded lipospheres were formulated with cetyl alcohol enhanced the bioavailability of ofloxacin to 2.45 fold on oral administration, when compared with ofloxacin suspension. This liposphere formulation approach can be used to improve the pharmacological activity of ofloxacin. The approach of controlled drug delivery of Ofloxacin loaded lipospheres especially for treating tuberculosis might be more beneficial, which allows a significant reduction of the total treatment duration and may be improve the patient compliance and cost of treatment.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

## ACKNOWLEDGEMENTS

Declared none.

## PATIENT CONSENT

Declared none.

## HUMAN/ANIMAL RIGHTS

Declared none.

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