

## Novel Nanoformulation of levofloxacin and its antimicrobial efficacy

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### Abstract

In this study, new levofloxacin loaded mesoporous silica nanoparticles were prepared by sol-gel technique using tetraethyl orthosilicate (TEOS) as silica precursor and cetyltrimethylammonium bromide (CTAB) as pore generating agent. The synthesis conditions were tailored by varying the molar ratio of water, NaOH and amount of CTAB used. The synthesized silica carriers were characterized by Scanning Electron Microscope (SEM) which showed that spherical particles with an average size between 80-87 nm were prepared. UV and Infra Red Spectroscopy (FTIR) confirmed the formation of levofloxacin-nano particulate system and showed the participation of carboxylic group in the synthetic process. It is envisaged that nano based drug delivery of levofloxacin would serve as a promising and innovative tool against resistant bacterial infection.

**Keywords:** Levofloxacin, Nanoparticles, cetyltrimethylammonium bromide, sol-gel

### 1. Introduction

Levofloxacin, a broad spectrum antibiotic belongs to class fluoroquinolones. It is racemic isomer of ofloxacin designed for more potent antimicrobial activities treating acute bronchitis acute maxillary sinusitis, pneumonia and UTI (urinary tract infections) [Rubinstein, 2011; Davis & Bryson, 1994]. It acts by inhibiting two enzymes involved in bacterial DNA synthesis, both of which are DNA topoisomerases [Wang, 1996].

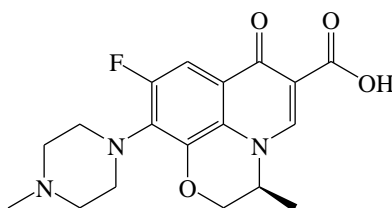


Figure 1: Chemical structure of levofloxacin

Sustained release of drug at the target site is one of the modes of efficient drug delivery [Balaji et.al. 015] with particle size playing an important role in bio-distribution and bioavailability [Mohanraj et.al. 2006]. A variety of designs have been established for the efficient and economically valued drug delivery. In this regard capsules [Mouzam et.al. 2011], filament [Mack et.al. 2009], microsphere [Lu et.al., 2014; Yu et.al., 2014; Jin et.al., 2016] and nanoparticles [Cheow & Hadinoto, 2011; Cheow & Hadinoto, 2010] have been developed. Sol-gel technique is considered to be one of the simplest methods employing less toxic compounds for the designing of nano-particles [Thangaraja et.al., 2010]. The efficiency of nano-particles may further be raised by constructing their mesopores [Kwon et.al., 2013]. Such exhibited good biocompatibility and chemical stability having high surface areas, uniform and tailor-made morphologies [Song et.al., 2015; Huh et.al., 2003; Radu et.al., 2004]. The ability of Nano-particles to circulate and get uniformly distributed

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throughout the body without tangling with even very small blood vessels, has enabled its use as a medium to deliver drugs to the targeted sites which includes; foreign genes and tumor cells [Waterston, 2003; Dave et.al., 1994]. Hence the aim of our study was to fabricate the mesoporous silica Nano carriers for sustained release of levofloxacin with the aim to raise the rate of delivery and absorption of drug at the target site (bacterial cell).

## **2. Experimental**

### **2.1 Materials and equipments**

Reference standard of Levofloxacin was a kind gift from Getz Pharma Pakistan Ltd. Dimethyl sulfoxide (DMSO), tetraethyl orthosilicate (TEOS) and ammonia (NH<sub>3</sub>) of analytical grade were purchased from Merck, Damstadt, Germany. CTAB was purchased from Sigma chemicals, Perth WA. Freshly prepared doubly distilled deionized water was used throughout experiment.

UV-Visible spectrophotometer (Shimadzu 2600), 1cm rectangular quartz cells, FT-IR spectrophotometer (Thermonicolet Avatar 330 FTIR) Omni software was used for coupling with FTIR, scanning electron microscope (SEM) (Jeol, Japan), centrifuge, electrical balance, (Shimadzu, AUW 220) [max 220G d  $\pm$ 0.1 mg]; pH meter [Jenway3510]; STEDECE CSW-300 deionizer (Stedec Pvt. Ltd., Karachi, Pakistan), magnetic stirrer and Elma Ultrasonic LC 30 H sonicator (Elmer NY) were used in our study.

### **2.2 Synthesis of mesoporous silica nanoparticle**

Homogenous mixture of CTAB, NaOH and water having a pH 10 was heated constantly to 80°C under stirring for 15 minutes. TEOS was added drop by drop to this clear solution under continuous stirring for 2 h at 80°C (table 1). A white precipitate was immediately formed indicating the start of reaction. The product was then isolated by filtration and washed with water and methanol. To remove surfactant from the synthesized nanoparticles, acid extraction was performed using mixture of methanol and concentrated HCl. Resulting product was washed with methanol and water using the centrifugation and sonication method. The washing was carried out until sample was clear of all surfactant and particles were dried at room temperature. Dried powder of silica mesoporous nanoparticles (SNP) was obtained.

### **2.3 Drug Loading on Mesoporous Silica Nano-particles**

100 mg of the drug levofloxacin was dissolved in DMSO (5mL). 134 mg of SNP was added to the clear solution of the drug. The sample was stirred for 24 hours at room temperature.

### **2.4 Characterization**

The mesoporous silica nanoparticles and drug loaded silica nanoparticles were characterized by UV and FT-IR spectroscopic studies and SEM morphological pattern.

#### **2.4.1 FT-IR studies**

Evidence of formation of mesoporous of silica nanoparticles was given by FT-IR spectral analyses of dried silica particles, recorded by FT-IR system. Pinch of samples of CTAB coated SNP, alone SNP, drug loaded SNP and alone

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drug were taken individually on the sensitive glass of FT-IR spectrophotometer. The sensitive needle of instrument was placed over the sample and spectra were recorded. Spectra were analyzed for changes in functional groups.

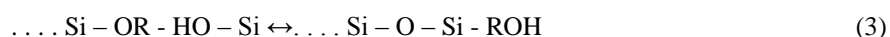
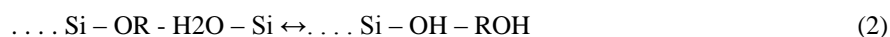
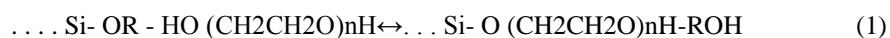
#### 2.4.2 SEM imaging

To prepare the samples for SEM studies, dried CTAB coated silica nanoparticles, alone SNP, drug loaded SNP and alone drug were dispersed into water and the resultant suspension was sonicated for 1-2 hours. A drop of the nanoparticle suspension on a piece of micro glass slide was dried gradually at room temperature. The sample was then visualized with a Jeol XL30FEG SEM to assess the particle size and shape.

### 3. Results and discussions

Microorganisms have gained resistance against many antibiotics due to their extensive use against infections. Currently different nano-strategies are used to overcome multi-drug bacterial resistance. Therefore, the aim of this study was to design nano-particles of Levofloxacin; antibacterial agent to improve its activity against resistant bacteria's using sol gel technique.

In Solgel technique solution is gradually converted into gel of small sized particles in homogenous system of liquid and solid from an alkoxide precursor. The solgel contains polymeric chain of precursor [Monton et.al., 2012, Toledano, & Mandler, 2010]. Werner Stober employed TEOS i.e., (Si(OC<sub>2</sub>H<sub>5</sub>)<sub>4</sub>) as alkoxide to get solgel in the presence of ammonia as catalyst in which hydrolysis of TEOS and condensation reaction was carried out. In the reaction siloxane (Si-O-Si) polymeric structure was formed in solgel state. The size of particles was in the range of 50-2000 nm [Xie et.al., 2010]. In fact, alkoxide readily reacts with water and upon hydrolysis OH gets attached to silicon of TEOS. Complete hydrolysis requires a lot of water and catalyst. The mechanism of silica network is as under:



The development of mesoporous nano-particles for drug delivery has increased recently as hollow spaces in them can efficiently hold and carry the drugs [Slowing et.al., 2008; Sobhani et.al., 2017; Kandpal et.al., 2007; Lodha et.al., 2012]. In our study cationic surfactant CTAB has been used for homogenous distribution of molecules within the mesoporous silica nano-particle structure. Ionic interaction also exists between the cationic surfactant and siloxane molecule giving it a uniform morphology.

For synthesis different volumes of water and CTAB were assessed. Adjusting the synthesis conditions, nanoparticles with different characteristics can be produced [Arayne et.al., 2007]. CTAB forms micelles upon which the silica condensates to form the nanoparticle structure. The particle exhibited homogenous spherical shape with average diameter of 83nm. Once the nanoparticle was formed, the surfactant was removed by solvent extraction (HCl in methanol) to generate the pores.

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**Table 1: Composition of reaction mixture**

Exp. No	CTAB (g)	NaOH (mL)	Water (mL)	TEOS (g)	Temperature (°C)
1	0.5	1.75	115	2.335	80
2	1	1.75	120	2.335	80
3	1.5	1.75	150	2.335	80
4	1.5	1.75	200	2.335	80

### 3.1 Characterization of Compounds

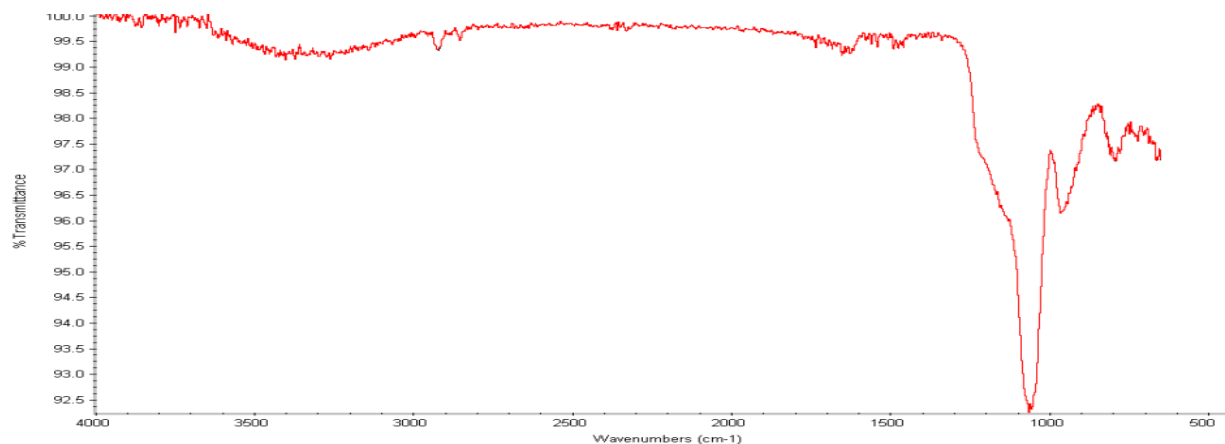
#### 3.1.1 UV spectral study

UV result showed CTAB exhibited absorption at 258nm, while spectra of TEOS gave absorbance at 650nm (absorbance 0.003). Absorption of silica nanoparticles in the presence of CTAB surfactant appeared at 284nm (absorbance 0.321) and 254nm (absorbance 1.650) (figure 2). 284nm is characteristics peak of silica Nano-particles. After removal of surfactant the peak of 254nm was not observed in the spectrum of silica Nano particle which is indicative of complete removal of surfactant. Constant observation of drug loading with UV-absorbance suggests complete attachment of levofloxacin into Nano carriers.

UV spectrum of levofloxacin showed characteristic peaks at 292nm and 238nm (figure 3). In the spectra of levofloxacin loaded silica nanoparticles none of these peaks were observed instead a new peak was found at 278 nm (figure 4) which arises due to the presence of ionic interaction between silica and levofloxacin facilitating drug loading.

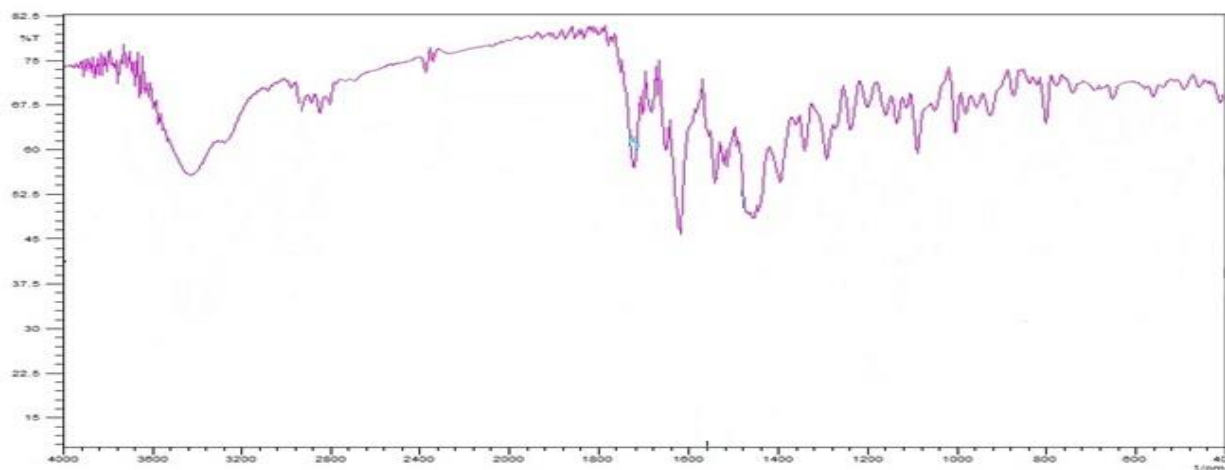
#### 3.1.2 IR spectral study

FT-IR analysis of silica nanoparticles showed prominent IR resonance at 1094 and 954  $\text{cm}^{-1}$  which is due to the vibration of Si-O-Si and Si-OH bands respectively. High intensity of these bands is the confirmation of presence of SiO<sub>2</sub> network. The broad band observed at 3438 and 3419  $\text{cm}^{-1}$  in pure silica nanoparticles indicated the existence of free Si-OH groups (figure 4).



**Figure 6: FT-IR spectrum of pure silica nano-particles**

FT-IR spectrum of levofloxacin (chemical structure shown in figure 1) showed characteristics peak of carboxylic OH (-COOH) at  $3500\text{ cm}^{-1}$  (figure 6). In the spectrum of levofloxacin loaded silica Nano carriers, sharp peak of COOH group of levofloxacin was broadened indicating hydrogen bonding between the -COOH group of levofloxacin and OH group of silica nano-particle assembly (figure 7). Moreover, the intensity of aromatic C=CH absorption near  $3000\text{ cm}^{-1}$  in the spectra of levofloxacin alone was considerably increased in the spectra levofloxacin loaded silica nano-particle owing to the interaction of the drug with silica. On comparing the spectrum with that of silica nano-particle (figure 5) high intensity absorption bands near  $1094$  and  $954\text{ cm}^{-1}$  was observed in the spectra of levofloxacin loaded silica nano-particle due to the presence of Si-O-Si and Si-OH bands which was not well defined in the spectra of levofloxacin.



**Figure 7: FT-IR spectrum of levofloxacin**

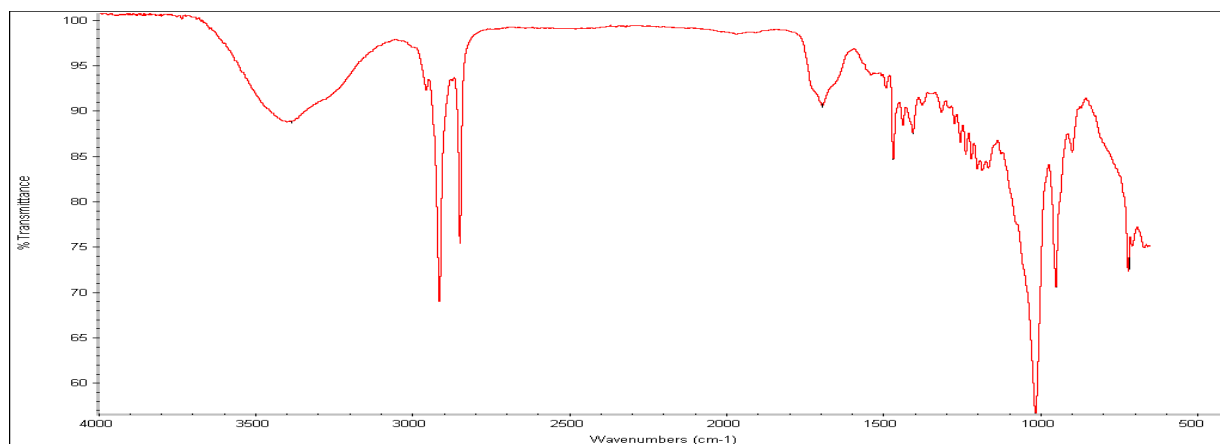


Figure 8: FT-IR spectrum of levofloxacin loaded silica nano-particles

UV and IR spectral studies suggest the formation of nanoparticles and drug loading into nanoparticles.

### 3.1.3 SEM Imaging

SEM imaging helps in morphological studies of the compounds [Liu et.al., 2010]. Mesoporous silica nanoparticles are well controlled particles with well-defined porosity. The SEM images of silica nanoparticles showed nano-porous particles of approximately 80-87 nm. In case of drug loaded nanoparticles, no change in shape and size of Nano carriers was found. The nanoparticles were spherical in size. The uniform size also suggested no agglomeration in nanoparticles.

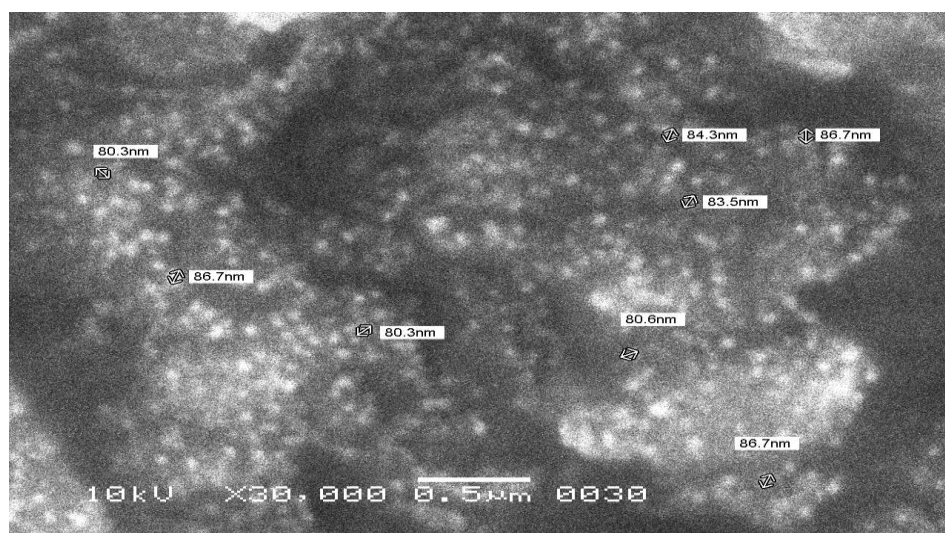


Figure 9 SEM images of levofloxacin loaded silica nano-particles

Enhancing the efficacy of antimicrobial agents loaded into the polymeric nanoparticles are related to many factors, including: easy penetration of drug into the microorganism cell, improved delivery of the drug to its site of action, and the increased stability of the encapsulated drug loaded nanoparticles [Sobhani et.al., 2017]. In our study the

synthesized levofloxacin loaded silica nanoparticles could be used to enhance the bioavailability and effectiveness of the parent antibiotic. Hence, increasing the potency of levofloxacin by using silica nanoparticles could produce promising result for clinical studies.

#### 4. Conclusions

Mesoporous Silica Nano-particles with larger surface area and pore volume seems to hold promise for the achievement to permit alterations in the bioavailability of drugs and improve the pharmacokinetic profile of numerous drugs with biomedical purposes. Strong bonding also helps in efficient drug delivery, by which activity of drug is enhanced. Our results confirmed that silica nano-particles were synthesized and that the amorphous nature of levofloxacin was not affected during the drug loading procedure as silica nanoparticles did not affect the chemical structure of compound. Increasing the potency of levofloxacin by using silica nanoparticles could be a promising tool to combat infections especially caused by resistant pathogens.

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