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Preparation of Silica-Gold Nanoshell Incorporated Levefloxacin for the Improved Anti-Bacterial Activity and Controlled Release.

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ABSTRACT

Among the various nanomaterials with distinct application in various field of science and technology, silica-gold core shell based drug delivery system is highly attractive because of its improved efficacy and less toxicity.The present investigation was aimed to prepare silica-gold nano shell loaded levofloxacin and evaluate drug release profile, improved anti bacterial activity against human pathogenic bacterial strains. Prepared drug loaded nano shell showed distinct nano structure with 70-120nm confirmed by transmission electron microscopy (TEM) and specific interaction pattern of drug with nano shell by fourier transform infrared (FTIR) spectroscopy studies. Nano shell drug formulation showed effective drug loading efficiency and drug release profile revealed 87.5 and 99.0 % respectively. Anti-bacterial activity studied against tested bacterial strains exhibited effective inhibition.

Keywords: Nano Shell, Levofloxacin, Drug Release, Antibacterial Activity

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INTRODUCTION

Nanomaterial is matter at dimensions of roughly 1–100 nm, where a unique phenoenon offers novel applications. The major advantages of nanoparticles as a delivery system are in controlling particle size, surface properties, and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Although nanoparticles offer many advantages as drug carrier systems, there are still many limitations to besolved such as poor oral bioavailability, instability in circulation, inadequate tissue distribution, and toxicity [1]. During the past few decades there has been an increasing interest in the development of biodegradable nanoparticles for effective drug, peptide, protein, and DNA delivery . Incorporation of the drug into a particulate carrier can protect the active substance against degradation in vivo and in vitro, improve therapeutic effect, prolong biological activity, control drug release rate, and decrease administration frequency [2].

In the recent past there has been considerable interest in nanosystems such as Gold nanoparticles, carbon nanotubes, etc. for drug delivery applications. To date, Gold nanoshells offer the added features of high biocompatibility and bioconjugation to antibodies via long-standing protocols adapted from Gold colloid bioconjugate chemistry, their near-infrared optical properties make them an ideal, if not unique, vehicle for a whole blood immunoassay[3]. Nanoshells made of oxides such as Silica and Titania find application in the field of drug delivery. The outer surface of these shells can be used for attaching antibodies so that the Silica shell-antibody complex can be used for targeted drug delivery in biological systems [4].Silica-Gold core nanoshell in drug delivery system may offer plenty of advantages over conventional dosage forms, which improves efficacy, reduced toxicity, enhanced biodistribution and reduces patient's compliance. The particles have the ability to conjugate the molecules without affecting the core and also can be used to encapsulate the drugs [5]. In the present study,silica gold shell incorporated levofloxacin was prepared for the improved anti bacterial activity and controlled drug release under in vitro condition.

MATERIALS AND METHODS

Preparation of silica-gold core shell loaded levofloxacin

Sol-gel method was employed for the synthesis of silica and gold nanoparticles separately.Silica nanoparticles was synthesized by hydrolysis of of tetraethoxysilane in ethanol (1.5ml; 15 ml ratio) and ammonium hydroxide [6] followed by functionalization with 3-aminopropyltriethoxysilane (APS).The mixture was kept under stirring under magnetic stirrer.To remove non funcationalized silica nanoparticles,the mixture was centrifuged at 3000rpm for 30 minutes followed by dissolving in ethanol. Citrate reduction method was carried out to synthesis gold nanoparticles [7]. Synthesized silica nanoparticles attached to the gold nanoparticles to form core shell by the method of Osterloh [8] which involves mixing of 1.0 ml of functionalized silica nanoparticles dispersed in ethanol and 20 ml of gold nanoparticles suspension, the reaction mixture was kept under stirring, the suspension was centrifuged at 3000 rpm, the collected red soild was was hed with distilled water and used for further studies .Levofloxacin loading was done by modified method of Kefalides [9].In this method,5 ml of levofloxacin (mg/ml) was stirred with 25 ml of nanoshell suspension for 12 hours.After stirring, the drug loaded nanoshell mixture was freeze dried.

Characterization

Levifloxacin loaded silica –gold nanoshell was characterized by FTIR to find out specific interaction of drug with nano core shell and scanning electron microscopy. Freeze dried samples were palletized with KBr for FTIR measurements. The spectrum was recorded in the range of 4000–500 cm_1 using Bruker OpticGmbH Tensor 27. Particle morphology was observed by transmission electron microscopy (TEM), Transmission electron microscopy (TEM) images were obtained by aJEM-2000EX microscope at an accelerating voltage of 120.0 kV. The samples for TEM studies were prepared by drying a drop of the aqueous suspension of films on carbon coated copper grid under ambient conditions. Prior to the TEM measurements, the samples were ground into small pieces at liquid nitrogen temperature to improve the depth of resolution.

Drug Loading Efficiency

The nanosuspension with known amount of levofloxacin (10mg/20ml) was prepared with purified water. The suspension was then ultrasonicated for 30 minutes for disruption and then filtered through a memberane. The drug content in the suspension was then detected by UV visible spectrophotometer 295nm to calculate the drug in the conjugate and total weight of the nanoshell- Drug conjugate.

***In vitro* drug release study**

In vitro drug release profile was studied by dissolving the freeze dried drug loaded nanoshells in 5ml of phosphate buffered saline at 37oC. Concentration of levofloxacin released in the aqueous solution was observed at defined time interval.

Anti-bacterial activity

Bacterial strains

Antibacterial activity of nano drug conjugate was tested against *Staphylococcus aureus*, *Streptococcus pyogens*, *Pseudomonas aeruginosa* and *Klebshiella pneumonia* were obtained from Microbial Type Culture Collection, Chandigurh, India. Respective bacterial strain was maintained on trypticase soy agar (TSA) slant at 4^{o}C.

Inoculum preparation

A loopful of respective bacterial culture was inoculated from the TSA slant into trypticas soy broth, incubated overnight on a rotary shaker (200 rpm) at 35°C. The inoculums were prepared by diluting the overnight cultures with 0.9% sterile saline to a 0.5 McFarland units standard.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibition concentration (MIC) was determined by a turbidimetric method [10].Bacterial inocula prepared in tryptic soy broth as earlier was used in this study. In this method, a series of 5 ml of screwcap vial (Borosil) each containing 2mL broth medium was prepared. 2mL of Nanoparticles suspension with different concentration were prepared and added into the first vial.After mixing, 2 mL of mixture from this vial were transferred to the next vial, and a similar procedure was repeated for the subsequent vials. The bacteria inocula was added to the vial to achieve a final bacterial concentration of 10^5 cells/mL Inoculated vials were incubated under shaking condition (150 rpm/min) at 37°C for 20 hours. The MIC was determined as the minimum concentration at which there is no visible change in the turbidity of the medium. The minimum bactericidal concentration (MBC), defined as the lowest concentration of sample that kills 99.9% or more of the initial inoculum, was determined in those test samples after the MIC test showed no growth. The assay was carried out by counting the number of colonies after the bacteria were seeded overnight on agar plates. The MIC and MBC were determined in a similar fashion. Triplicates were maintained in each treatment.

Well diffusion assay

Anti bacterial activity of the tested bacterial strains was studied by well diffusion assay. Inocula of the respective bacterial culture thus prepared was uniformly spread with sterile cotton swabs on sterile Mueller Hinton (MH)Agar Media (Hi-media, India). The wells were made using cork borer and aliquots of silver nanoparticles (aliquots of 25, 50, 75 and 100 µg/ml were prepared from concentrated nanoparticles) was loaded into the wells. The plates were incubated at 37ºC for 24 hours. After the incubation period, the plates were observed for zone of inhibition. Three replicates were maintained.

RESULT AND DISCUSSION

The design and controlled fabrication of core-shell nanomaterials and nanostructure are important in many research areas, including the developments of novel catalysis, the potential applications as chemical sensors, and some advanced photonic researches such as surface-enhance Raman scattering (SERS),photonic

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crystal, and nanoengineering of optical resonance. In particular, the fabrication and study of metallic nanoshells with dielectric cores have attracted wide interests because of their good optical and chemical properties for biomedical imaging and therapeutic applications.[11-13].In the present study,silica gold nano shell loaded levofloxacin has been prepared and the prepared drug conjugate was evaluated against in vitro drug release profile and anti bacterial activity.

Silica gold nano shell clusters prepared funcationalization by 3-aminopropyltriethoxysilane (APS) silica spheres with citrate reduced gold nanoparticles at acidic pH and loading of levofloxacin carried out by modified method of Kefalides [9] .Characterization of the core shell –drug conjugate was primarily studied by FTIR analysis of freeze dried sample palletized with KBr using Bruker OpticGmbH Tensor 27.FTIR is used to identify types of chemical bonds (functional groups) between the atoms or molecules. The wavelength of light absorbed is characteristic of the chemical bond present in the chemicals. By interpreting the infrared absorption spectrum; the chemical bonds in a molecule can be determined. FTIR spectra of pure compounds are generally so unique that they are like a molecular fingerprint [14].Figure 2 shows FTIR spectra of drug loaded nano shell revealed functional groups of functionalized silica, gold and levofloxacin. Further characterization with TEM showed defined spherical shape with the size range of 70-120 nm (Figure 3).

Table 1: Minimum inhibition concentration (MIC) and minimum bacteriacidal concentration of nano drug against tested bacteria

Tested Bacteria	MIC ((mg/ml)	MBC (mg/ml)
P.aeruginosa	0.43	0.51
Staph.aureus	0.61	0.70
Strep.pyogenes	0.72	0.75
K.pneumoniae	0.32	0.44

Mean value of three replication

Table 2. Zone of inhibition (mm) against tested bacteria

Tested Bacteria	Zone of inhibition (mm)
P.aeruginosa	24.0
Staph.aureus	19.5
Strep.pyogenes	22.0
K.pneumoniae	26.2

Mean value of three replication

Figure 1.FTIR spectra of silica gold nanoshell loaded levofloxacin

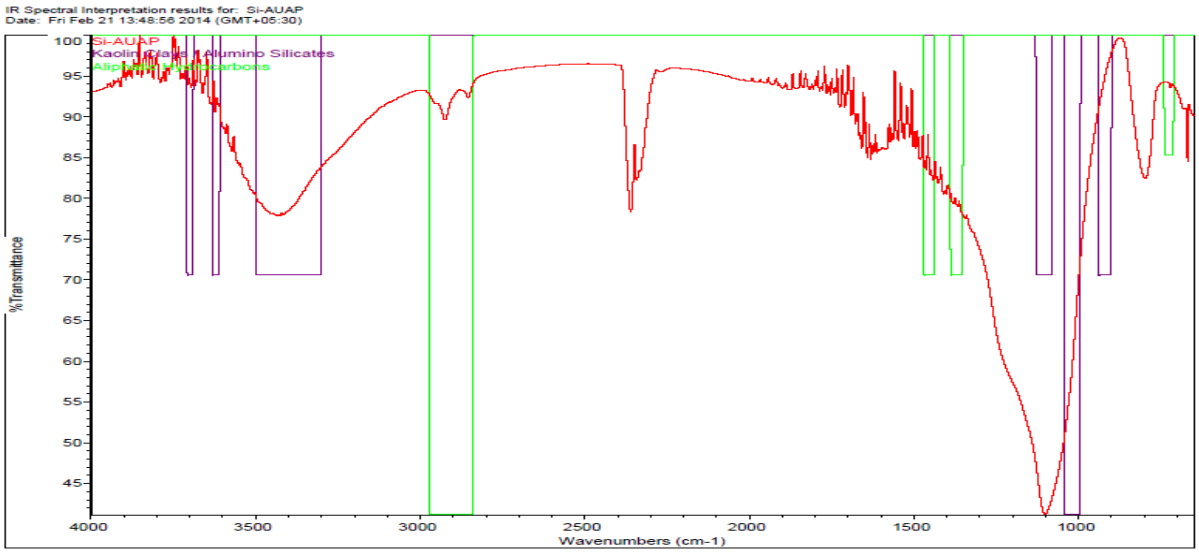

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Figure 2: TEM image of nanoshell loaded levofloxacin

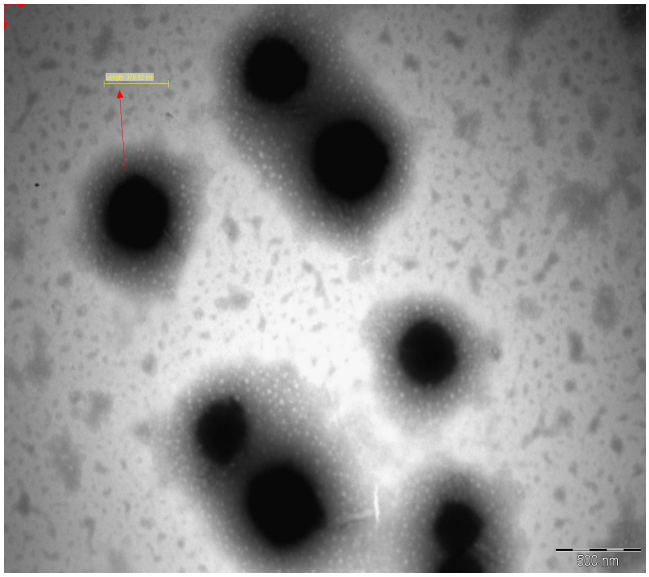
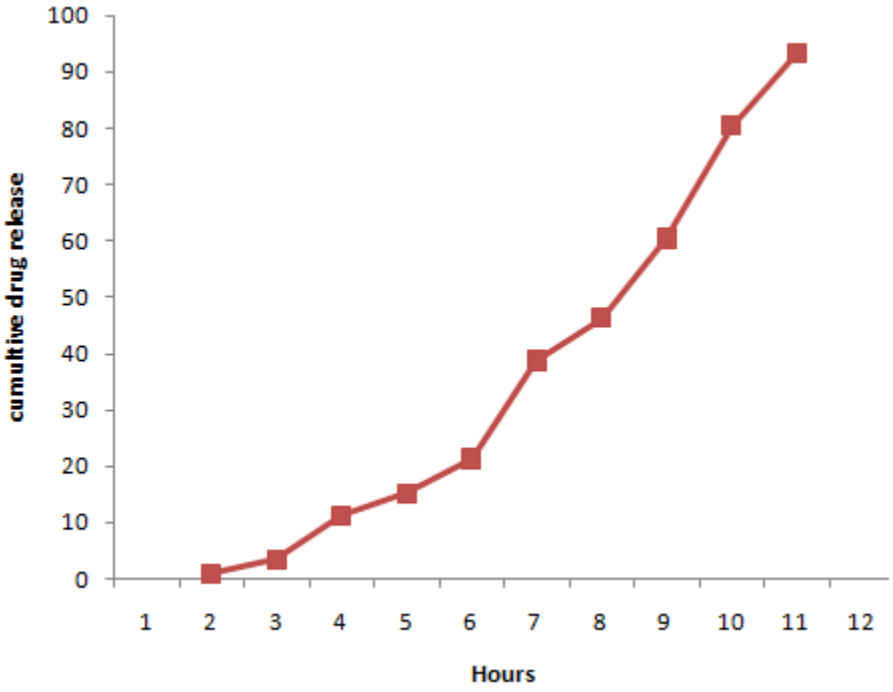


Figure 3: Drug release profile (%) of levofloxacin from nanoshell



The loading efficiency of the drug on to the core shell is found by the spectrophotometric analysis of the drug-nano core shell conjugate suspension. The unbound concentration was found by correlating the absorbance of the supernatant after the centrifugation with the standard absorbance concentration ratio. The drug loading efficiency was found to be 87.5 %. *In vitro* drug release of the drug was studied using 1% PBS. The sample was taken at regular intervals and analysed spectrometrically. The release percentage was calculated using the intial drug concentration and the release at specified time. The drug release was calculated for 24 hours. There was a burst release of drug in the early hours and a total release of about 99.0 % was observed. An initial burst of 41.0% in the first 5 hours can be observed. In the following 5 hours, cumulative release reached 80.6 %, in a sustained manner, which provides the possibility to fight continually against bacterial cells, resulting in decreased cell viability. Cumulative release reached almost 99.0% after 24 hours and showed an almost released ability of the nanoparticle formulation (figure 3).The generally sustained and controlled release profile of drug facilitates the application of nanoparticles for the delivery of drugs [15]. Previous

reports on controlled release pattern of various drugs formulated with nanoparticles also exhibited a steady state release of drug from the nanoformulation. Anti-bacterial activity of nano shell-drug studied against human pathogenic bacteria .Initially, minimum inhibition concentration was done by turbidity method. The MIC values of free silica nanoparticles, gold nanoparticles, free levofloxacin and nano shell-levofloxacin conjugate against tested bacterial strains was presented in Table 1which can be seen that nano shell-levofloxacin conjugate showed higher anti bacterial activity than free silica, gold nanoparticles and free levofloxacin. Well diffusion assay also showed maximum antibacterial activity by recording higher zone of inhibition than free nanoparticles and drug against all the tested bacteria (Table 2).The present study revealed high stable functionalized silica-gold nano core shell-levofloxacin formulation and its distinct steady or controlled release pattern, improved anti-bacterial activity would suggest possible utilization in chemotherapy against fatal infectious diseases.

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