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Starch Based Coating Influence On Antibacterial Activity And *In-vitro* Drug Release Profile Of Silver Nanoparticles Loaded Levofloxacin Nano Drug Conjugate (AgNp-LF).

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ABSTRACT

Among the various metallic nanoparticles ,silver nanoparticles extensively used in nanomedicine without toxic effect to human and effective against bacteria, viruses, and other eukaryotic microorganisms and their unique ability of synergistic effect with the various antibiotics and polymers would suggest an effective antibacterial agents.In the present study,silver nanoparticles synthesized from cold tolerant strain of *Spirullina platensis* levofloxacin nano drug conjugate coated with starch was prepared and the prepared nano drug conjugate was evaluated against pathogenic bacterial strain adopting well diffusion method and control release study was done under *in vitro* condition. Synthesized nano drug conjugate was characterized by scanning electron microscopy (SEM) and fourier transform infrared spectroscopy (FTIR) which revealed spherical nanoparticles with the size range of 80-90nm and the presence of functional groups confirmed effective coating of polymer with the nano drug conjugate.Improved antibacterial activity was not recorded in polymer coated nano drug conjugate against human pathogenic bacterial strains. Cumulative release of levofloxacin revealed a steady state of release with 55 %.

Keywords; silver nanoparticles, levofloxacin, starch, antibacterial, control release

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INTRODUCTION

Nanotechnology is being increasingly explored in science and industry for widely different applications. Nanotechnology and polymers have captivated a tremendous interest in many areas such as the pharmaceutical industry and therapeutic innovation among others. Natural and synthetic polymers have been used as promising tool for nanoscale drug carrier systems, especially in oral administration of poorly absorbed therapeutic drugs [1]. In recent years, great developments have been made in the field of muco adhesive polymer systems in formulations .The rapid expansion of nanotechnology promises to have great benefits for society that increase the residence time of drugs on mucosal membranes and subsequently enhance the bioavailability of drugs with poor oral absorption [2-5].

A tremendous effort has been and is currently being devoted to the research in the field of pharmaceutical nanotechnology. Several peculiar properties of gelled polymeric nanosize(<1µm) particulate systems have been reported, among which the ability to encapsulate molecular weight or macromolecular active principles in mild conditions and protect them from degradation by the harsh pH conditions or enzymes they may encounter in the organism, promote transport of actives across mucosal barriers, undergo internalization by cells thereby carrying actives into them [6]. Coating of nanoparticles is necessary for their stability, functionality, and biocompatibility. For biomedical applications, of AgNPs is essential in order to target them to specific disease areas and allow them to selectively interact with cells or biological molecules. In general,coating can be performed by either using chemical functional groups or biological molecules [7-9]. In the present study, biogenic silver nanoparticles loaded levofloxacin coated with starch mediated effect on the antibacterial activity against human pathogenic bacteria and drug release profile has been carried out.

MATERIALS AND METHODS

Synthesis of silver nanoparticles

Silver nanoparticles used in the present study was synthesized from cold resistance strain of *Spirullina platensis* biomass as described in our previous work [10]. Synthesized and purified particles were lyophilized and used for further studies.

Coating of silver nanoparticles loaded levofloxacin

In the present study, starch was used for coating. In a typical procedure of starch coated nano drug conjugate synthesis, 2.5ml of silver nano suspension prepared from original stock and equal volumes of 0.01% of starch and 0.01% of levofloxicin were suspended in 100ml of deionised water and kept under magnetic – stirring for three hours at 30^{o}C. Slurry thus obtained was lyophilized and stored in screw capped vial. Characterization carried out with fourier transform infrared spectroscopy (FT-IR) and Scanning electron microscope. FT-IR was carried out with KBr palletized dried sample in the range of 4000–500 cm_1 using Bruker OpticGmbH Tensor 27.Particle morphology (Shape and size) and elemental composition was studied by field emission scanning electron microscopy equipped with energy dispersive X-ray analysis (FESEM–EDAX) was performed by SUPRA 55-CARL ZEISS ,Germany.

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.

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Determination of minimum inhibition concentration (MIC)

Micro dilution colorimetric liquid broth assay using chromogenic reagent 3-(4, 5-dimethyl thiazol-2-yl)-2-5-dephenyl tetrazolium bromide (MTT) carried out by the modified method of Abe,and Matsuki [11] was done to determine minimum inhibition concentration (MIC).

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 100 µl of nano drug conjugate (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.

***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.

RESULT AND DISCUSSION

In the present study, silver nanoparticles synthesized from *S.platensis* has been formulated with starch coated levofloxacin nano drug conjugate and the prepared nano drug conjugate was evaluated against human pathogenic bacterial strains was studied. Silver nanoparticles were synthesized from cold resistance strain of *S. platensis* used in the present study was characterized by various techniques as described earlier. Plasmon absorption maxima by UV visible spectrophotometer, particles morphology by SEM ,elemental composition by EDAX, crystallinity and the lattice properties by XRD, functional groups determination by FTIR revealed the sysnthesized particles were nano dimensional uniform monodispersive particles Polymer coated levofloxacin-silver nanoparticles conjugate was primarily confirmed by colour change of the reaction mixture from dark brown to pale yellow, scanning electron microscopy analysis and FT-IR The scanning electron microscopy study reveals starch coated levofloxacin-silver nano drug conjugate as spherical particles with the size range of 80-90 nm (Figure 1). Such size distribution analysis of antibiotic nanoparticle conjugates confirms that the particles are well dispersed. FT-IR analysis helps to detect the functional groups, structure of a compound and purity of the sample in a given environment in terms of frequencies of radiation present in the nanoparticles which showed characteristic pattern of absorption peaks indicates the functional groups of the polymer (Figure 2). When the FTIR spectra of control and nano drug conjugate were compared, it is clear that all the absorbed peaks were changed upon nano drug conjugate coating.

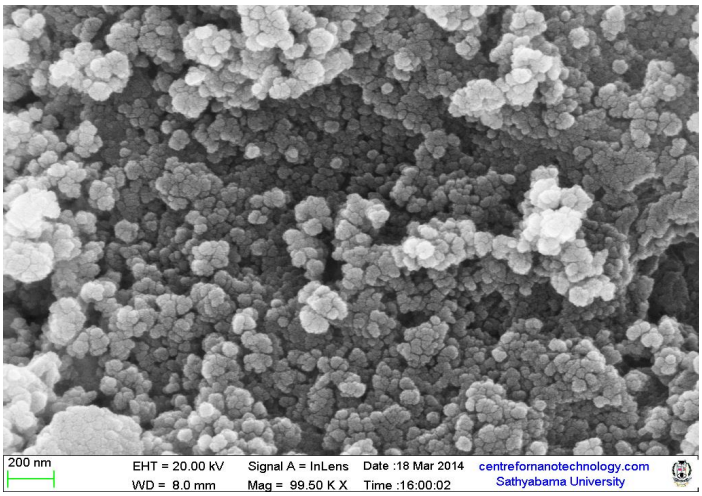

Figure 1.SEM image of starch stabilized AgNp-LF

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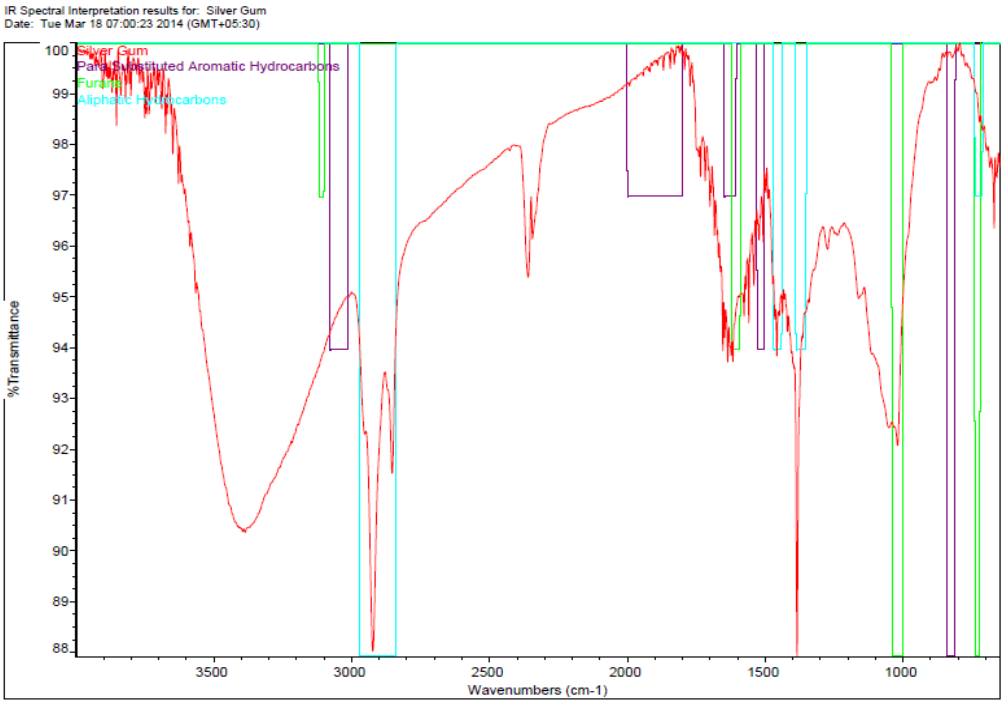

Figure 2.FTIR spectra of starch stabilized AgNp-LF

Table 1. MIC(mg/ml) and MLC (mg/ml) of nano drug conjugate against the tested bacterial strains

Treatment	Tested bacteria	MIC	MLC
Free AgNps	*Staphylococcus aureus*	0.7	0.6
Free levofloxacin		0.7	0.9
Free starch		0.0	0.0
Nano drug conjugate		0.6	0.6 [a]
Free AgNps	*Streptococcus pyogenes*	4.3	5.4
Free levofloxacin		2.3	2.4
Free starch		0.0	0.0
Nano drug conjugate		1.0 [a]	0.98 [a]
Free AgNps	*P.aeruginosa*	4.8	5.2
Free levofloxacin		4.0	5.0
Free starch		0.0	0.0
Nano drug conjugate		2.2 [a]	2.3 [a]
Free AgNps	*K.pneumoniae*	9.2	8.0
Free levofloxacin		4.1	4.0
Free starch		0.0	0.0
Nano drug conjugate		6.4 [a]	7.0 [a]

In column, mean carrying the alphabet is statistically significant at 5 % level by DMRT

Table 2.Zone of inhibition (mm) of nano drug conjugate against tested human pathogenic bacteria

Tested bacteria	Zone of inhibition (mm)
Staph.aureus	*22.0*
Strep.pyogenes	21.0
P.aeruginosa	14.0
K.pneumoniae	13.4

Mean values are not statistically significant by DMRT

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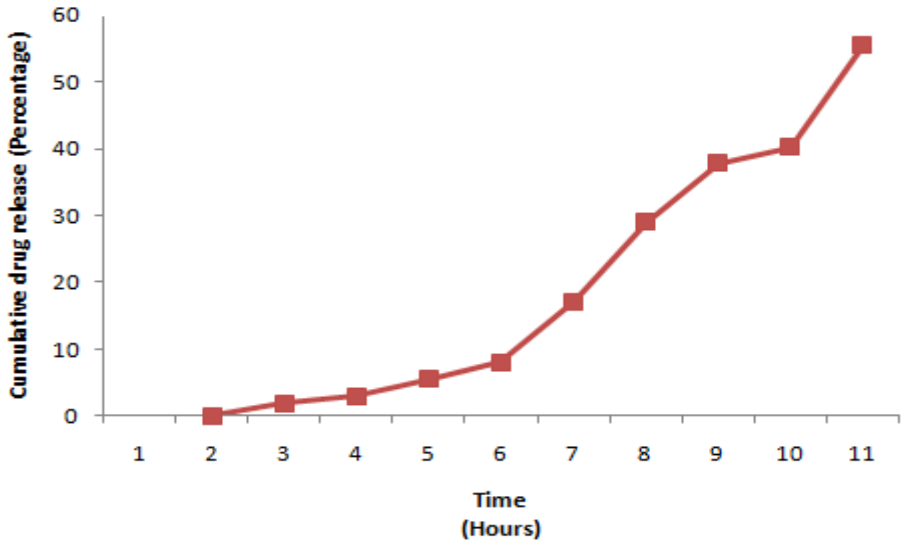

Figure 3.In vitro drug release profile of starch coated AgNp-LF

Anti bacterial activity of the nano drug conjugate was studied by determination of minimum inhibitory concentration (MIC) using MTT assay.MIC of the nanoparticles against the tested bacterial strains was studied by broth dilution method. MIC values of starch coated nano drug conjugate against all the tested bacterial strains was found be lesser than free antibiotic . It can be seen that polymer coated nano drug conjugate showed high antibacterial efficacy (Table 1).Agar diffusion assay was done to evaluate anti bacterial activity . All the tested bacteria were found to be susceptible to polymer coated nano drug w .As in free nano drug conjugate,starch coating revealed similar pattern of zone of inhibition Starch coating did not cause improved activity against the all tested bacterial strains (Table 2).Effect of gum acacia- a natural polymer coated silver nanoparticles and chitosan coated ovalbumin nanoparticles on the antibacterial activity has been reported [12,13]. Effect of starch coating on *in vitro* drug release of levofloxacin from the prepared nano drug conjugate was presented in figure 3 which shows that there is a steady state release pattern. Maximum release was recorded at 16 hours time interval with 55.4 % release. A study of Karthick Raja Namasivayam et al [13] on *in vitro* drug release profile of levofloxacin from chitosan coated ovalbumin nano drug conjugate revealed steady state release at 12 hours with the maximum release % of 90. Control release percentage of levofloxacin was also found to be steady and maximum in silica gold nanoshell loaded levofloxacin nano conjugate [14].But, starch coating of the prepared nnao drug conjugate recorded lesser release percentage than our previous studies.Further study will helpful to improve the activity and release profile of antibacterial nano drug conjugates coated with starch.

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