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Systematic Review

The characteristics of silver – doped zein nanoparticles coated with lipopolysaccharides

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To investigate the interaction between nanoparticles and bacterial lipopolysaccharides, researches and experiments made showed promising results when synthetized zein nanoparticles were dropt with silver and mixed with coatedlipopolysaccharides.

Keywords: bacterial, nanoparticles, lipopolysaccharides.

INTRODUCTION

Lipopolysaccharides (LPS) is a gram-negative bacterium with immune - stimulatory effects. The Lipopolysaccharides at the level of external membrane are complex molecules that contain in their structure both lipids and carbohydrates, being formed of three components, namely: Lipid A, polysaccharide core and polysaccharide O. when gram negative bacteria die, they release the lipid A in the environment, this one have the role of exotoxin. This A lipid is the one responsible for the infections associated with gram negative bacteria (such as fever, septic shock, blood clots). The core polysaccharide is attached to a lipid, and contains unusual sugars. Its role is structural, i.e. it provides stability.

A polysaccharide extending outwardly from the core polysaccharide and is composed of carbohydrate molecules. The O polysaccharide acts like an antigen and is very useful for distinguishing between species of gram negative bacteria.(Tortora și colab., 2013).

Infections caused by Gram negative bacteria are particularly dangerous because after treatment with antibiotics cells die and release into the environment lipopolysaccharide. Their accumulation has particularly harmful effects on the body and may induce several diseases.

In human IgM is a pentameric structure, but an amount of about 5% or less of serum IgM is a hexameric form. This molecule contains 6 monomeric units but apparently lacks the snare J. IgM hexamer activate complement of up to 20 times more efficiently than a normal pentameric form. It has been suggested that the lipopolysaccharide of Gram negative bacteria can stimulate B cells to produce IgM hexamer with J chain. (Prescott și colab., 2002).

Lipopolysaccharide is a powerful innate immunity with immune-stimulatory effects. LPS activates B cells and induces production of interleukin-1 and interleukin-6 in dendritic cells and macrophages. With the Shwartzman reaction (disseminated intravascular coagulation) it results in the release of large amounts of exotoxins in the bloodstream. (Murray și colab., 2013).

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Previous experiments made by using a sensitive test to identify the presence of endotoxin in drugs, medical devices and body fluids is particularly important. The materials may contain endotoxins were sterilize even if they are not contaminated with bacteria. A method which can detect even trace amounts of endotoxin by the LAL assay is the (Limulus amebocyte lysate assay). The hemolymph horseshoe crab, Limulus Polyphemus, containing white blood cells called amebocytes. They show large amounts of a protein that causes clotting. In the presence of exotoxins, amebocytes releases its clotting protein; If the resulting precipitate when the reaction is positive. The reaction is measured using a spectrophotometer (Tortora și colab., 2013).

To test the reaction of gram negative bacteria (lipopolysaccharides) in our experiments we have introduced as well zein nanoparticles, silver and lecithin.

Zein is the major storage protein from corn with strong hydrophobicity and unique solubility and has been considered as a versatile food biopolymer. Due to the special tertiary structures, zein can self-assemble to form micro- and nano-particles through liquid–liquid dispersion or solvent evaporation approaches. Zein-based delivery systems have been particularly investigated for hydrophobic drugs and nutrients. Recently, increasing attention has been drawn to fabricate zein-based advanced drug delivery systems for various applications. In this review, the molecular models of zein tertiary structure and possible mechanisms involved in zein self-assembly micro- and nano-particles are briefly introduced. Then, a state-of-the-art introduction and discussion are given in terms of preparation, characterization, and application of zein-based particles as delivery systems in the fields of food science, pharmaceutics, and biomedicine. (2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 2014, 131, 4069).

Zein is well-known for its film forming properties and has been widely used for coating and packaging in foodand pharmaceutical industries. The major structure of zein is a helical wheel conformation in which nine homologous repeating units are arranged in an anti-parallel form stabilized by hydrogen bonds. The high proportion of non-polar amino acid residues in zein controls its solubility. Zein is extremely soluble in alcohols; however, a water-ethanol mixture has been shown to be the best solvent for zein (Optimum Conditions for the Fabrication of Zein/Ag, Composite Nanoparticles from Ethanol/H2O, Co-Solvents Using Electrospinning, Academic Editor: Thomas Nann, Published: 1 December 2016).

Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. But due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably. Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize, which drastically changes the chemical, physical and optical properties of metals. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as a potential antimicrobial agent. The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, silver nanoparticles have emerged up with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nanogels, nanolotions. (Silver nanoparticles as a new generation of antimicrobials – ResearchPaper - Department of Biotechnology, SGB Amravati University, Mahendra Rai, Alka Yadav, Aniket Gade),

Lecithin, a kind of natural small molecular surfactant, is widely used in food and pharmaceutical industries.

Zein, silver, lecithin are the base materials were used in our experiments.

The **materials** used for nanoparticles (NP) preparation were: Zein with a protein content of 91.3% (w/w),

Soy lecithin (S-100, 94% phosphatidylcholine), Absolute ethanol (99.9%), Silver nitrate 1ml/100ml.

NP were **synthesized** following the method described as per below, using a 5:1 zein to soy lecithin ratio:1.0 g zein was dissolved in 100 mL 70% (v/v) ethanol-water solution with magnetic stirring at 600 rpm for 3 h. The corresponding quantity of soy lecithin was added to the zein solution, under continuous stirring (600 rpm) for 30 min.

The dispersions were stored in the refrigerator at 4^0C for 48 hours.

The base solution of **Zein+ Absolute ethanol +Lecithin** - was kept 3 hours with magnetic stirring at 600 rpm. From this base solution, we prepared two derivate solutions: 1. Zein + Absolute ethanol+ Lecithin + **Water**, 2. Zein + Absolute ethanol+ Lecithin + **Silver Nitrate ($AgNO_3$)**

NP were coated with LPS molecules by mixing 1 mL from each solution with the following LPS concentrations: 300 mg/mL (LPS1), 450 mg/mL (LPS2), 600 mg/mL (LPS3) and 750 mg/mL (LPS4).

The measurements were made with both **Terahertz (THz) spectroscopy and Fourier transformed infrared (FTIR) spectroscopy**.

Terahertz (THz) spectroscopymeasurements were performed using a Tera View TPS-Spectra 3000 spectrometer purchased from Tera View. THz spectra of NP – LPS mixtures were recorded in attenuated total reflection (**ATR)** configuration, with a resolution of 4 cm^{-1}final spectra represent the average of 900 measurements with a scan frequency of 30 scans / second resulting spectra were normalized to their value @ 0.5 THz.

Fourier transformed infrared (FTIR) spectroscopy measurements were performed using a Bruker Tensor 27 spectrometer. FTIR spectra of NP – LPS mixtures were recorded in ATR configuration, with 4 cm-1 resolution, during a collection time of 1 minute.

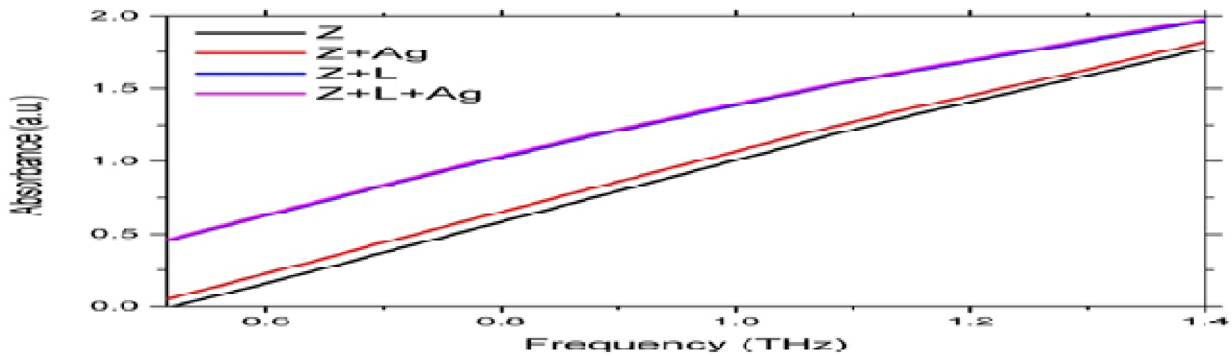

Figure 1 THz spectra of solutions comprising zein, zein with $AgNO_3$ (Z+Ag), zein with lecithin (Z+L) and zein with lecithin and $AgNO_3$ (Z+L+Ag)

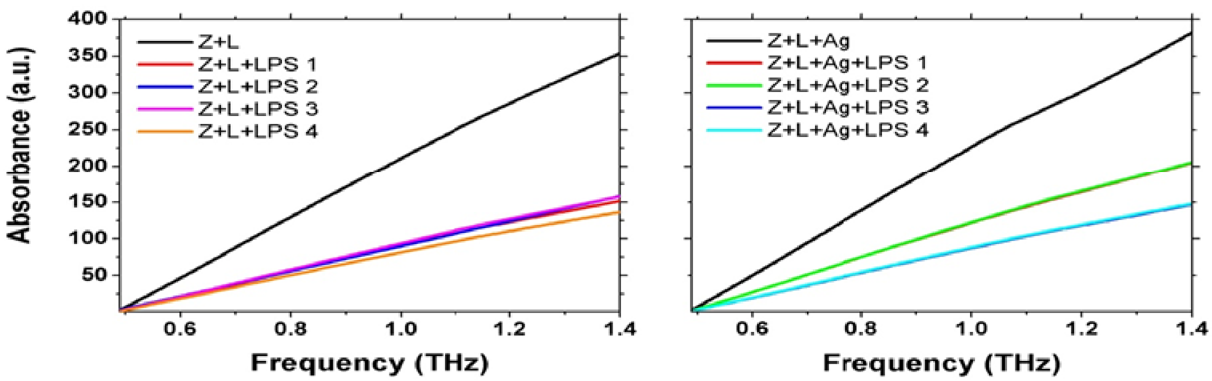

Figure. 2 THz spectra of solutions comprising zein, zein with $AgNO_3$ (Z+Ag), zein with lecithin (Z+L) and zein with lecithin and $AgNO_3$ (Z+L+Ag) with LPS molecules normalized to the absorption @ 0.5 THz.

Results and discussion based on terahertz spectroscopy evidenced the following:

In Figure. 1is presented the THz spectra of solutions comprising zein, zein with silver, zein with lecithin and zein with lecithin and silver.

Zein with and without silver nitrate has a similar absorption

The nanoparticles of zein with lecithin, with and without silver nitrate shows differences in Figure. 2, all absorptions increase linearly.

For a better comparison, we fitted the spectra with lines and compared the slopes of the lines that fit the spectra (Figure. 2).

The slopes of the lines presented in Figure 3 suggest that: zein with lecithin and zein with lecithin and silver present the highest THz absorption; zein with lecithin and LPS and zein with lecithin, silver and LPS present different THz absorptions, the NP doped with silver having a higher absorption.

Results suggest an event occurring at LPS concentrations higher than LPS3 in the case of zein and lecithin nanoparticles. In the case of zein with lecithin nanoparticles dropped with silver, the event occurs after the LPS2 concentration.

Results and discussion based on Fourier transformed infrared (FTIR) spectroscopyevidenced the following:

The FTIR spectra of pure lecithin and LPS molecules in the frequency domain 2000 – 800 cm^{-1} are presented in Figure. 4. It can be seen that both molecules have several distinct absorption peaks.

The spectrum of zein features the amide I and amide II bands (Figure. 5). Amide II band is absent from the spectra of solutions with zein NPs.

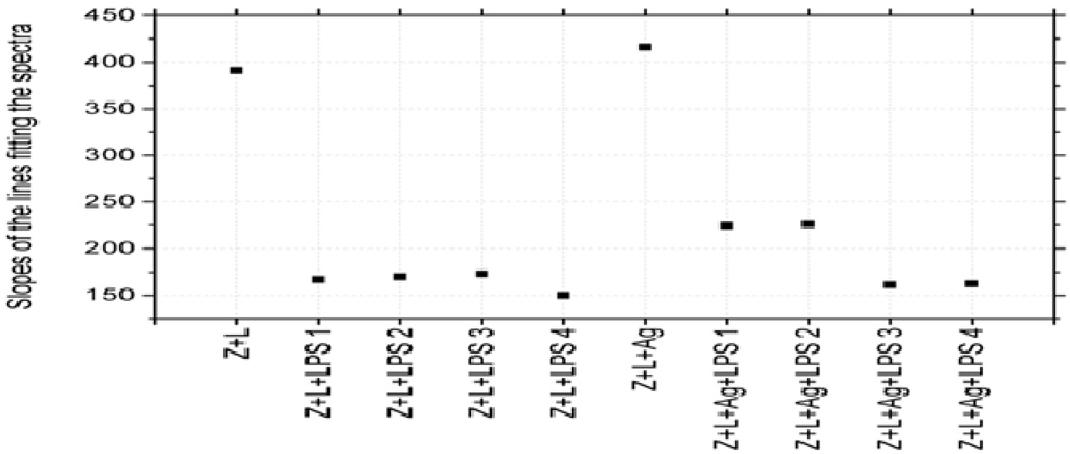

Figure. 3 Slopes of the lines that fit the experimental THz spectra.

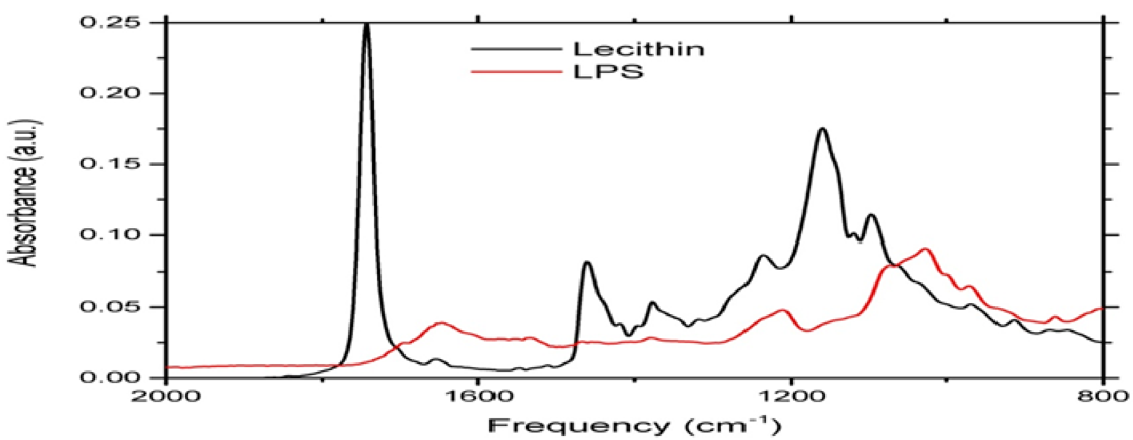

Figure 4. FTIR spectra of pure lecithin and LPS.

The FTIR spectra of pure zein, of zein – lecithin np and of np dopped with silver and coated with LPS molecules are presented in Fig.5.

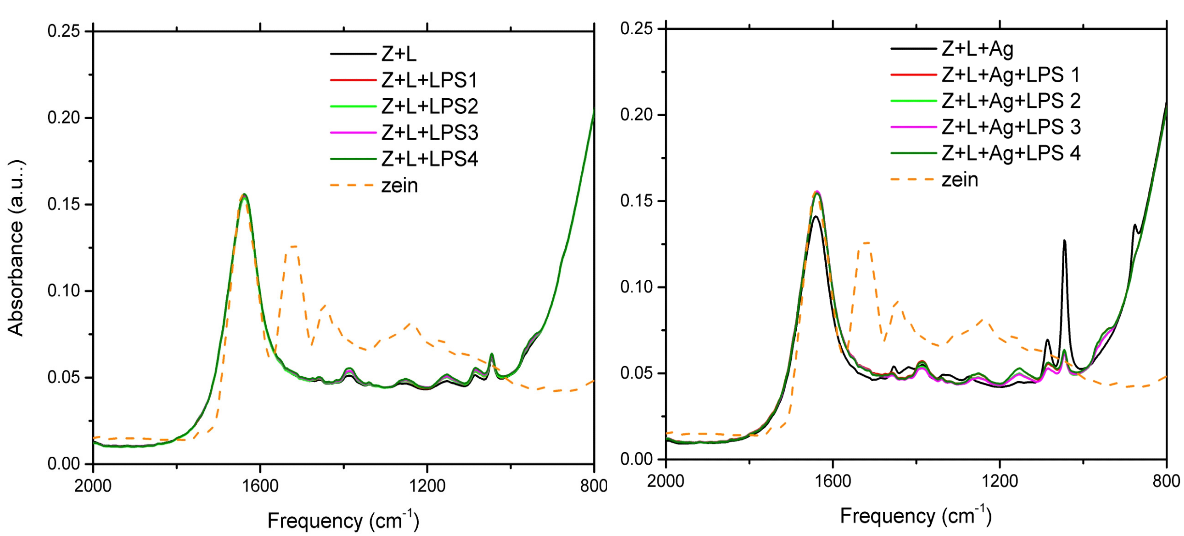

Figure. 5 FTIR spectra of zein, zein with lecithin and zein with lecithin dopped silver np covered with LPS molecules.

In the case of zein – lecithin NPs without silver, there are no significant differences between spectra on NPs and NPs coated with LPS.

In the case of zein-lecithin NPs dopped with silver, we notice the absorption peaks specific to nitrate group (1087 and 1044 cm^{-1}). These are absent from the spectra of NPs doped with silver and covered in LPS.

Only slight differenced can be seen between NPs with silver and NPs with silver covered with LPS.

Conclusion of these experiment evidence that the spectroscopic study of zein-lecithin NP s with and without silver ions shows absorbance differences probably due to the interactions between protein, silver ions and LPS molecules. The THz spectroscopy results show a complex interaction between LPS molecules and the zein-lecithin NPs, that is influenced by the presence and absence of silver ions.

Very important are also the perspectives that these experiments have opened such as the future study the effect of doping the zein NPs with different ions (CuII, ZnII, etc) also the interest in studding the dopping of zein NPs with Ag^{o} in comparation with Ag^{+} and the biological effect of synthesized NPs on Gram-negative and Gram-positive bacteria.

Is also intended to diversify the techniques (TEM, SEM, AFM) to study of the surface of NPs and LPS-coated NPs.

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