Effect of Green Synthesized Silver Nanoparticles Incorporated with Gelatin for Sustained Drug Release

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Abstract: Synthesized silver nanoparticles using *Erythrina indica* leaf extract incorporated gelatin was used in this study to analyze the sustained drug release capacity. In this study gelatin was used as a drug binding and sustained drug release medium. The biomolecules (alkaloids, flavonoids, carbohydrates and amino acids) present in the nanoparticles was acting as a capping agent to enhance the medicinal properties of the AgNPs. Silver nanoparticle incorporated into gelatin was loaded with Atorvastatin calcium and characterized by UV, FT-IR and TGA for sustained drug release. When compared without incorporating AgNPs into gelatin-ATVC the complete release was extended for AgNPs-gelatin with ATVC. From this study, AgNPs was found to be effective drug carrier for delivering drugs.

Keywords: Silver nanoparticle, atorvastatin calcium, drug delivery.

Introduction

Nanotechnology is a study of manipulating matter on an atomic and molecular scale. Generally nanotechnology deals with materials, devices or other structures processing at least one dimension sized from 1 to 100 nanometers. Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis. The main two approaches are used in nanotechnology. In the “bottom-up” approach, materials and devices are built from molecular components. In the “top-down” approach, nano objects are obtained from larger entities without atomic level modification. Many silver nanoparticles have been synthesized from plants. Particularly synthesis of silver nanoparticle from *Erythrina indica* which is having more biomolecules such as alkaloids, flavonoids, carbohydrates, tannins and amino acids have been synthesized by Kalainila et al.

Nanoparticles present a higher surface to volume ratio with their decreasing size. As the specific surface area of nanoparticle increases, their biological effectiveness also increases due to the increase in surface energy. Currently most of the applications of silver nanoparticles are in antibacterial/antifungal agents in biotechnology, textile engineering, bioengineering, water treatment and silver-based consumer products. Silver nanoparticles have unique properties such as optical, conductivity and antibacterial properties which help in several medical procedures.

Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be incorporated into biosensor materials, antimicrobial applications, cryogenic superconducting materials, composite fibers, electronic components and cosmetic
products. Silver metal particle in nanometer range have attracted considerable interest in recent years. They have tremendous applications in area of biomedical and catalysis due to their unique size dependent properties.

A wide range of AgNPs applications has emerged in consumer products ranging from disinfecting medical devices and home appliances to water treatment. Furthermore, their unique Plasmon-resonance optical scattering properties allow AgNPs in bio-sensing and imaging applications. In recent years, the green synthesis of metal nanoparticles has become a major focus of scientists. It has attracted researchers to probe on mechanisms of metal ions bio reduction by plants and to understand the possible mechanism of metal nanoparticle formation using plant compounds.

The importance of controlled drug delivery systems that release drug over an extended period of time has long been recognized in the pharmaceutical field. Oral route is the most popular route of drug administration because of its ease of administration and patient compliance. Nowadays, the application of nanotechnology to healthcare holds great promise in medical field in areas, such as tissue regeneration, drug delivery, faster diagnosis and imaging as well as the development of new therapeutics.

The development of green synthesis of nanoparticles is evolving into an important approach in nanotechnology. Plants have been reported to be used for synthesis of metal Nano particles of gold, silver, copper and their alloys. The silver nanoparticles are also reported to be nontoxic to human with low concentration and toxic to animals with high concentration. The application of silver nano particle is the dramatic increase in the specific surface area for particles with a size in the range of a few nanometers. Synthesis of nanoparticles using microorganisms consume more time for the maintenance of microorganisms whereas the plant extract mediated methods require less processing time.

Drug delivery can be defined as the process of releasing bioactive agent at a specific rate and at a specific site but in the current scenario, targeted drug delivery must be overcome to exploit thousands of new therapeutics that are limited by a safe and effective drug delivery system. Drug delivery technologies play a vital role in pharmaceutical industry. The marketability and efficiency of drugs depends on the mode of delivery. Recently, industries are moving to new delivery systems to effectively and safely deliver novel products and formulations. Delivery technologies represent an important part of science, which engages multidisciplinary medical improvement. Usually, nanoparticles are used as a carrier. In nanoparticle based delivery systems, the active compound entrapped in the carrier could be adsorbed or attached to the nanoparticle.

The emergence of nanotechnology is likely to have a significant impact on the drug delivery sector, with many potential applications in clinical and medicinal research.

The applications of nanotechnology in various disciplines and specifically in healthcare are becoming increasingly common. Nanotechnology focuses on formulating therapeutic agents in biocompatible nanocarriers, such as NPs, nano-capsules, micelles systems and dendrimers. Moreover, one of the major advantages that nanotechnology offers is targeted drug delivery to the site of disease. Nano-medical approaches to drug delivery are on rise because nanoscale particles help to improve drug bioavailability. Bioavailability is defined as presence of drug molecules in an active form where they are needed in the body, which focuses on maximizing bioavailability at specific places in the body over a period of time.

Several nano-sized protein based delivery systems are available. Albumin, gelatin, gliadin and legumin are the protein based nanoparticles which play a vital role in drug delivery. To formulate sustained release of nanoparticles, there are many biocompatible polymers available in market. Among these proteins, gelatin is one of the most constructive polymers used to provides sustained release of hydrophilic and hydrophobic drugs. Gelatin is translucent, brittle biodegradable, biocompatible, non-toxic, non-irritant, polymer and flavorless solid substance. Gelatin is a natural polymer derived from collagen recognized as a biodegradable and biocompatible material. The natural source of gelatin is obtained from animals. It is obtained mainly by thermal or enzymatic degradation of the collagen. Only limited work was already established using the gelatin with silver nanoparticles for the sustained drug delivery study.

In the present investigation, the newly green synthesized silver nanoparticles from novel plant along with gelatin were used in sustained drug delivery study as first time. The AgNPs incorporated with gelatin and drug was characterized using various analyses such as UV-Vis spectroscopy, FTIR and TGA. The characterized AgNPs and gelatin with drug was taken for invitro drug release study.
2. Materials and methods

Materials

The green synthesized AgNPs prepared in our laboratory was used in this study. All chemicals used for the synthesis of AgNPs were purchased from Sigma-Aldrich, India. Double distilled water was used throughout the reaction. All glass wares were washed well and dried using hot air oven. Gelatin and Atorvastatin calcium was purchased from Hi-media Laboratories, Mumbai, Ltd.

2.3. Preparation of AgNPs-gelatin

100 mg of gelatin was dissolved in 100 ml of double distilled water then 10µg of AgNPs was added into it. Then it was made as a film by pouring it into petri plate and used for further process.

2.4. Preparation of AgNPs-gelatin-ATVC and experimental procedure

For drug release study, egg membrane is used as dialysis membrane. Egg was immersed in diluted acetic acid for 5 to 6 hrs. Then the membrane was separated. 100 mg of AgNPs-gel was taken along with 10mg of ATVC in 2 ml methanol. Then the sample (AgNPs gelatin with drug) was packed in egg membrane and immersed in PBS solution (200 ml) with magnetic stirrer at 37°C in dark condition throughout the process (Fig 1). 4 ml of sample was collected and replaced by fresh PBS after 1st, 2nd, 3rd, 4th, 5th, 6th, 12th, 18th, 24th, and 48th hrs. The samples were analyzed using UV–visible spectrophotometer at 246 nm.

Fig.1 Apparatus setup for drug release

Characterization of prepared samples

The UV-visible spectrum of the reaction medium was analyzed with the range between 300-700. The UV-visible spectra were recorded on a UV-visible JascoV-550 spectrophotometer. FT-IR measurements were used to determine functional groups present AgNPs incorporated in the gelatin with drug. The samples were dried and ground with KBr to form a pellet and analyzed on Jusco 5300 model with the range of 400 – 4000cm⁻¹.

Results and Discussion

3.1. UV-Visible Spectroscopy

(a)
Fig. 2. (a) UV-Visible spectrum of ATVC, (b) AgNPs with gelatin, (c) AgNPs-gelatin-ATVC

UV–Visible spectroscopic studies were performed to monitor the ATVC loading on AgNPs – gelatin. From the UV–visible results, there is no Plasmon resonance in free atorvastatin calcium Fig. 2(a). In Fig. 2(b), a new absorption peaks showed at 262 nm leads to the presence of AgNPs and gelatin. In contrast, the peak was disappeared in Fig. 2(c), due to the addition of ATVC. The change in the UV–visible spectrum suggests the AgNPs was loaded with atorvastatin calcium. The same type of result was previously reported by Afifa Bathool et al.31.

3.2. FT-IR analysis

Fig. 2. FT- IR Spectrum of ATVC-gel, AgNPs-gelatin-drug
To understand the interaction between atorvastatin and AgNPs, the FTIR analysis was carried out. The FTIR spectra of AgNPs – gelatin and AgNPs – gelatin-drug are shown in Fig. 3. In Fig. 3, for AgNPs-gelatin two bands at 3348 cm\(^{-1}\) and 3496 cm\(^{-1}\) were attributed to the NH/OH group. Peak at 1620 cm\(^{-1}\) was corresponding to stretching vibration of C=C bond. The peaks at 1463 cm\(^{-1}\) and 1251 cm\(^{-1}\) represented the stretching vibrations of the C–C or C–H bonds. In contrast to AgNPs-gelatin, the FTIR spectrum of the AgNPs-gel-ATVC exhibited peak at 2605 cm\(^{-1}\) and it represents N-H group in Atorvastatin\(^{32}\). The N-H stretching peak of AgNPs-gelatin-ATVC shifted towards a higher wavenumber (3618 cm\(^{-1}\)) in comparison to that of the AgNPs-gelatin (3450 cm\(^{-1}\)). Peak at 1444 cm\(^{-1}\) assigned to C=C vibration. Peak at 975 cm\(^{-1}\) indicated C–C group and peak at 1768 cm\(^{-1}\) indicated aldehyde or ketone group (C=O or CHO)\(^{32}\). The nature of the interactions between AgNPs-gelatin and AgNPs-gelatin-ATVC was confirmed by comparing their FTIR spectra.

3.3. Thermo gravimetric analysis

![Fig. 3. TGA curve of AgNPs with gelatin](image)

Thermogram of AgNPs-gelatin is shown in Fig. 3. Thermal stability of material was measured using TGA in nitrogen atmosphere. The TGA plot of AgNPs with gelatin normally displays three thermal stages such as loss of water (15%) between 25 and 100 °C, gelatin decomposition between 250 and 450 °C and finally combustion of the remaining material between 450 and 700 °C. The actual decomposition temperature depends on the structure, molecular weight and conformation of the polymer. This shows that the thermal stability of the polymer was improved due to presence of AgNPs. The similar type of result was previously reported by HoneihEtamad et al [33].

3.4. Drug release

![Fig. 4. Drug release graph](image)

The percentage of atorvastatin release at various time intervals is shown in Fig. 4. The in vitro drug release showed an initial release of about 10-50% within 1-7 hrs followed by the slow release of 75% release was reached at 24 hrs. The 98% release was reached at 72 hrs. The sustained drug release capacity of AgNPs with gelatin-ATVC was found to be more (72 hrs) when compared with without incorporating AgNPs into gelatin-ATVC (24 hrs). The initial burst release might be due to the adsorption of drugs on the outer surface of the
samples. Increase in crystallinity, crystalline size and surface area may play main role for the sustained drug release of the samples. The sustained drug release capacity was found to be increased with increasing gelatin [34]. From the present study, it was concluded that Atorvastatin calcium loaded silver nanoparticles was an effective carrier for the controlled drug delivery of Atorvastatin calcium. The similar trend was already established by Daniele et al., [35, 36].

3.5. Cumulative release

![Cumulative release graph](image)

**Fig.5. Cumulative release**

**Percentage drug loading**

The loading percentage is calculated using the eq. (1)

\[
\% \text{ drug loading} = \frac{\text{Total amount of drug} - \text{Amount of drug in the supernatant}}{\text{Total amount of drug}} \times 100 \quad (1)
\]

The loading percentage of ATVC on AgNPs-gelatin was determined using the above equation as 91.2%

**Percentage drug release**

The release percentage is calculated using the following eq. (2)

\[
\% \text{ drug release} = \frac{\text{Released ATVC from AgNPs-gel}}{\text{Total amount of ATVC in AgNPs-gel}} \times 100 \quad (2)
\]

The percentage drug release was achieved as 98%

**Conclusion**

In this study, a green approach for the synthesis of AgNPs using *Erythrina indica* leaf extract was used which is the simplest and efficient step to obtain AgNPs without engaging any harmful chemicals as reducing and dispersing agent. The biomolecules (alkaloids, flavonoids, carbohydrates and amino acids) present in the AgNPs was responsible for the medicinal value. Silver nanoparticle incorporated into gelatin along with Atorvastatin calcium was characterized by UV, FT-IR, and TGA. The sustained drug release capacity of AgNPs-gelatin with ATVC was found to be more when compared with gelatin-ATVC without AgNPs. From this study, AgNPs was found to be an effective drug carrier for sustained delivery of drugs. The AgNPs with gelatin can be formulated along with the drug for the sustained drug delivery.

**References**


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