Journal of Pharmaceutical Research International

32(18): 21-27, 2020; Article no.JPRI.59770
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Biogenic Synthesis of Gold Nanoparticles from Aspartic Acid - A Preliminary Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KAK, IN and SR designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SR managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1830685
Editor(s):
(1) R. Deveswaran, University of Applied Sciences, India.
Reviewers:
(1) Fatima Javeria, University of Agriculture Multan, Pakistan.
(2) Kruti Dangar, Saurashtra University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59770

Original Research Article

Received 23 May 2020
Accepted 30 July 2020
Published 25 August 2020

ABSTRACT

A nanoparticle is an ultra-fine particle with at least one dimension between 1-100 nanometers (nm). Metallic nanoparticles are considered as most promising as they contain remarkable antibacterial properties. Gold nanoparticles are of high importance in research. Aspartic acid is an alpha amino acid and contains one amino group and one carboxylic group. The aim of the current study was to bio synthesize gold nanoparticles using aspartic acid. Gold Chloride (AuCl3) and Aspartic acid ($C_4H_7NO_4$) were used for the study. AuCL3 solution (0.266 M) was slowly added to 250 aspartic acid with stirring at 45°C. The mixture of the solutions was kept in a long-necked borosilicate flask and continuously stirred on a magnetic stirrer. The formation of gold nanoparticles was confirmed by the change of the colorless solution to a reddish hue. Characterization of the newly formed nanoparticles was then done. After approximately 9 hour incubation and intermittent stirring with a magnetic stirrer the solution color changed from colorless to a reddish hue, which indicated the formation of AuNPs. The spectrometric reading was recorded at a scanning range of 400–700 nm.

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AuNPs nanoparticles at 24 hours showed an increased intensity and a Surface Plasmon Resonance (SPR) band at 575 nm. The gold nanoparticles by Transmission electron microscopy were confirmed to be spherical in shape and of 20 nm. A simple and efficient method for the synthesis of AuNPs from the aspartic acid was demonstrated. Nano particles were formed in approx. 9 hours with peak absorbance at 24 hours at 575nm. The synthesized nanoparticles were spherical in shape, with an average size of 20 nm. The synthesized nanoparticles showed excellent plasmon resonance and optical properties.

Keywords: Aspartic acid; bio synthesis; gold nanoparticles; transmission electron microscopy spectrometric analysis.

1. INTRODUCTION

Nanotechnology is an emerging area of research in the modern era [1]. It has been continuously expanding and has gained immense popularity in a wide range of applications. It has been popular in various fronts like biomedical, pharmaceutical, localized drug delivery and targeted drug delivery and several other antimicrobial applications [2]. The change in the physio-chemical properties of these nanoparticles contribute to their range of superior properties. Apart from the medical field nanoparticles are also being widely studied for their prospective uses in various areas such as the food industry, environmental, space industry and optical devices [3].

Among the various nanoparticles being researched, metallic nanoparticles are considered as most promising as they contain remarkable antibacterial properties [4]. This is due to their large surface area to volume ratio. Due to the increasing evidence on microbial resistance being reported in recent times [5] and also the development of antibiotic resistant strains, there is remarkable interest for researchers towards newer antimicrobial agents. Nanoparticles have been successful in keeping hope for such a potent alternative. Several metallic nanoparticles which have gained widespread popularity in recent times. These include noble metal nanoparticles such as silver, gold, platinum, titanium, palladium etc. Gold nanoparticles are of high importance in research due to its long history of medicinal use due to their biocompatibility. These nanoparticles have been used for various biomedical applications including biosensor, bioimaging, photothermal therapy and targeted drug delivery. The biosynthesis of gold nanoparticles has been reported using plant tissues, bacteria, fungi etc [6]

Generally, there are two approaches which are involved in the synthesis of nanoparticles. These may be classified as either from “top to bottom” approach or a “bottom to top” approach. In bottom to top approach nanoparticles are synthesized using chemical reduction and by various other biological methods by self-assembling atoms to new nuclei which grow into a particle of nanoscale [7]. However, in top to bottom approach suitable bulk material breaks down into fine particles by size reduction by various techniques such as grinding, milling, sputtering, thermal/laser ablation, etc. are preferred. These methods used for synthesis of nanoparticles are very hazardous due to use of toxic chemicals that are responsible for various biological risks [8]. It also is very expensive. The development of ecofriendly syntheses of nanoparticles is evolving rapidly.

In the process of lab synthesis of a nanoparticle an additional or second agent is often used as a reducing agent. The reducing agent or capping agent plays an important role in breaking down the bulk material into finer nano sized particles that are desired for the intended application. Aspartic acid is an alpha amino acid and is used by the body for the biosynthesis of proteins. Aspartic acid like most other amino acids contains one amino group and one carboxylic group. The carboxylic group is the free end of the amino acid and can form bonds with other atoms or elements. We have numerous highly cited publications on well-designed clinical trials and lab studies using similar biomaterials, applications and innovative techniques [9-24]. This provided the right platforms for us to pursue the current study. The aim of the current study was to bio synthesize gold nanoparticles using aspartic acid.

2. MATERIALS AND METHODS

2.1 Source of Chemicals

Gold Chloride ($AuCl_3$) and Aspartic acid ($C_4H_7NO_4$) were procured from Sisco Research

Laboratories (Mumbai, India). All chemicals utilized in this study were of analytical grade.

2.2 Synthesis of AuNPs

$AuCL_3$ solution (0.266 M) was slowly added to 250 ml of aspartic acid with stirring at 45°C. The mixture of the solutions was kept in a long-necked borosilicate flask and continuously stirred on a magnetic stirrer. The formation of gold nanoparticles was confirmed by the change of the colorless solution to a reddish hue. The solution was stirred for approx 9 hours intermittently. The synthesized gold nanoparticles were then purified by centrifugation (10,000 rpm; 30 min) at 4°C. The nanoparticles collected were thoroughly washed with deionized water and re-dispersed in Millipore water.

2.3 Characterization

The formation of the AuNPs were confirmed by UV–vis spectrophotometry (UV-1800 spectrophotometer, Shimadzu, Japan). The sample was diluted with deionized water and the UV–vis spectrum was recorded using a quartz cuvette with deionized water as the reference. The spectrometric reading was recorded at a scanning range of 400–700 nm. The size and morphological characteristics of the AuNPs were examined by Transmission electron microscopy (TEM: Hitachi S3000 H).

3. RESULTS AND DISCUSSION

3.1 UV Characterization of AuNPs

UV-visible spectroscopy is an important technique for characterization. Initially, the synthesis of AuNPs was confirmed by color changes in the solution. The reaction started within a few minutes of AuCl3 addition into the solution of aspartic acid. After approx. 9-hour incubation and intermittent stirring with a magnetic stirrer the solution color changed from colorless to a reddish hue, which indicated the formation of AuNPs. The process of incubation and continuous stirring was continued for up to 24 hours, to achieve complete saturation of the newly formed gold nanoparticles. The Color changes occurred due to the formation of nanoparticles and the subsequent coherent oscillation of electrons at the surface of nanoparticles resulted in surface plasmon resonance (SPR). The excitation of the surface plasmon resonance of AuNPs could be responsible for the color change in the reaction mixture. The AuNPs showed prominent peaks of absorbance at 550 nm and 575 nm at 12 and 24 hours, respectively. However, the AuNPs nanoparticles at 24 hours showed an increased intensity and an SPR band at 575 nm. The results indicate that nanoparticle formation was directly proportional to the intensity of absorbance and the incubation time [Fig. 1].

3.2 Microscopic Analysis of AuNPs

Transmission electron microscopy (TEM) demonstrated the formation of nanocrystalline gold particles. Majority of the synthesized AuNPs were spherical in configuration and well separated with mild agglomeration. The average size of AuNPs was approximately 20 nm [Fig. 2].

The mechanism of synthesis of gold nanoparticles using different biological agents is still unclear [25]. Different chemical entities present in biological compounds may act as reducing agents reacting with metal ions leading to their reduction and thereby synthesis of metal nanoparticles. Recent studies showed that biomolecules such as protein, phenol, flavonoids, etc. play an important role in the reduction of metals ions and capping of the nano particles [26]. In comparison to chemically synthesized gold nanoparticles, gold nanoparticles obtained from biological sources are free from toxic contamination of by products that become attached to the nanoparticles during chemical synthesis which limits the use of resulting gold nanoparticles in various applications. This is because conventionally synthesized nanoparticles present possible dangers, both medically and environmentally. Most of these are due to the high surface to volume ratio. This can make the particles very reactive or catalytic. They are also able to pass through cell membranes in organisms, and their interactions with biological systems are relatively unknown [27-28].

Biocompatibility is required for gold nanoparticles for their biomedical applications [29]. The biological synthesis of gold nanoparticles have several advantages like simple, single step, environmental friendly, cost effective and biocompatible nature of synthesized gold nanoparticles. Additionally, there is no need to add any external stabilizing agents because the biologic agents themselves act as stabilizing as well as capping agents [30]. A shorter time is required for biosynthesis of gold nanoparticles in comparison to chemical synthesis of gold

nanoparticles. Another advantage of biologically synthesized nanoparticles is that it can reduce the number of steps including the attachment of some functional groups to gold nanoparticles surface to make them biologically active, a step which is required in chemical synthesis [31]. There are nearly numerous applications of gold nanoparticles, such that they have been used in targeted drugs delivery, gene delivery, antitumor, cancer therapy, antimicrobial, bio-imaging, catalytic, bioassay, antioxidant, sensing, antagonistic activity against bacteria and fungi, etc. Aspartic acid like most other amino acids contains one amino group and one carboxylic group. The carboxylic group is the free end of the amino acid and can form bonds with other atoms or elements [32]. Hence, aspartic acid was chosen as the biological entity of choice in this study.

Currently research on biosynthesis of gold nanoparticles is still in the discovery phase. Several studies still are performed to understand the effect of time, temperature, light, and several other factors on the formation of gold nanoparticles still require optimization and also on the control of size and shape of the nanoparticles. Lack of knowledge of chemical components responsible and mechanism for the reduction and stabilization of biosynthesized gold nanoparticles are still challenges for researchers. The gold nanoparticles prepared in the current

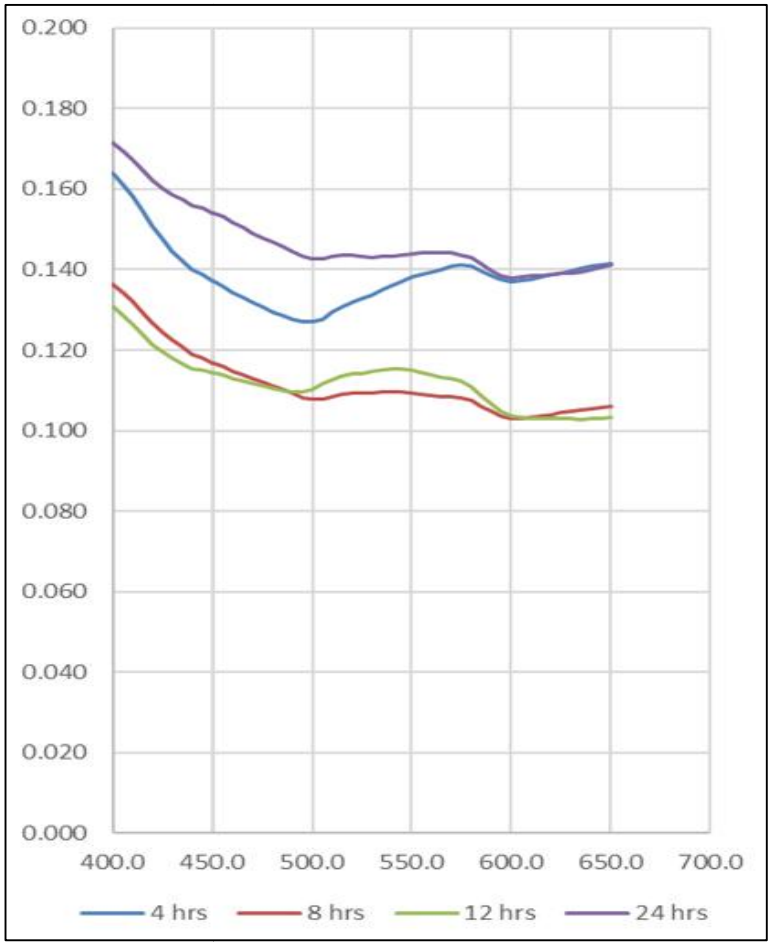

Fig. 1. Figure showing the absorbance of the solution in test at various time intervals under U.V visible spectrometric analysis. X axis represents the scanning range of wavelengths in nanometers and the Y axis represents absorbance levels. The peak absorbance was achieved at 24 hours at 575nm wavelength and is represented by the purple line graph. This indicated the complete formation of the Gold nanoparticles (AuNPs)

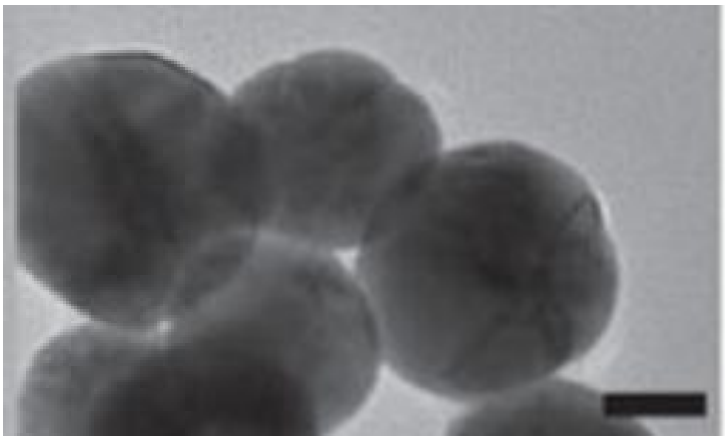

Fig. 2. Transmission electron microscopy showing configuration of the newly formed AuNPs. The gold nanoparticles formed are spherical in nature with minimal agglomerations. They were approximately 20nm in diameter

study will be further analyzed and used in future research to explore its potential applications in the field of dentistry.

4. CONCLUSION

A simple and efficient method for the synthesis of AuNPs from the aspartic acid was demonstrated. Nano particles were formed in approx. 9 hours with peak absorbance at 24 hours at 575nm. The synthesized nanoparticles were spherical in shape, with an average size of 20 nm. The synthesized nanoparticles showed excellent plasmon resonance and optical properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Pharmacology and Nanotechnology Laboratory of the institution for providing all the necessary facilities and guidance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59770