EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 4
100-107
Year: 2018

GREEN SYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES (AUNPS) USING FENUGREEK SEEDS EXTRACT (*TRIGONELLA FOENUM-GRAECUM*)

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Article Received on 08/02/2018 **Article Revised on 28/02/2018** **Article Accepted on 20/03/2018**

ABSTRACT
In the present research, cost effective and environmentally friendly gold nano particles (AuNPs) were synthesized. Biosynthesis of AuNPs was carried out using the aqueous extract of fenugreek seeds (*Trigonellafoenum-graecum*) as reducing and capping agent. The principle is based on the reduction of $Aucl_4$ by the extract of fenugreek seeds. Gold nanoparticles having different sizes in the range from 15 to 25 nm were obtained by controlling the synthesis parameters. Physico-chemical characteristics were carried out by UV-Vis spectra, fluorescent activity, dynamic light scattering and transmission electron microscopy. UV-Vis spectra showed the maximum absorbance of GNP at 535 nm. Transmission electron microscopy (SEM) showed that the shape of GNP was nearly spherical with 5–20 nm size. After addition of plant extract in the gold salt solution, the color changed from pale yellow to ruby red and deep purple indicating the formation of AuNPs. This is due to the synthesis of AuNPs with the molecular assistance of biological reducing agents represented by the fenugreek seeds extract. The appearance of the color varied drastically with the temperatures, and the amount of extract at 50ºC, more time was required for the complete reduction; whereas at 100ºC the color appeared in few mints. The clear colloidal suspension of AuNPs was stable for more than two months at 4ºC. The corresponding gold nanoparticles solutions contained in glass showed the variations in color of AuNPs due to excitation of surface Plasmon vibrations. The AuNPs is intended for further investigations in the area of drugs in regard to toxicity.

KEYWORDS: Biosynthesis, Gold nanoparticles, Plant Extract, Surface Plasmon.

1. INTRODUCTION
Nanotechnology is getting developed at several levels: materials, devices and systems. The nonmaterial level is the most advancing at present, both in scientific knowledge and in commercial applications. Nanomaterials (NMs) are commonly described as the materials with atoms arranged in nano sized 10-100. Clusters which includes liposome, dendrimers, carbon nanorods, carbon nanotubes, fullerenes, graphene derivatives, titanium oxides, gadolinium nitride nanowires, silver NPs, gold NPs, platinum NPs, magnetic NPs and quantum dots.[1,2,3,4,5] It becomes the building blocks of the material.

In recent years nanomaterials, specifically metal nanoparticles, have received particular interest in diverse field of applied science ranging from material science to biotechnology.[6,7,8] Nanoparticles are less than a few 100 nm. Small size and high surface volume ratio of nanoparticles, the physicochemical properties of nanoparticles containing materials are quite different to those of the bulk materials.[9,10,11,12] Most of these changes are related to the appearance of quantum effects as the size decreases, and are the origin of phenomena such as the superparamagnetism, surface Plasmon resonance.[13,14]

Gold nanoparticles (AuNPs) have unique optical properties in the visible region, due to Surface Plasmon Resonance i.e., the interaction of electromagnetic waves (visible light) and the electrons in the conduction band around the nanoparticles they transmit different colors.[15] AuNPs have interesting properties include their stability, bio-compatibility, characteristic optical properties and surface Plasmon resonance (SPR) behavior. These provide the potential for unique catalytic and biological applications.[16] The colloidal gold nanoparticles were used to change the color of the glass in the Roman Lycurgus Cup, which looks opaque green but turns red when light shines from the inside. Many sources credit Johann Kunckel (1638)[4], with developing the first systematic procedures for incorporating gold

into molten silica, thus producing the well-known "ruby glass".
Currently, Gold nanoparticles can be synthesized via chemical and physical methods which are basically based on aggressive agents such as sodium borohydride, hydrazinium hydroxide, under high temperature and pressure, as well as harm solvents. To pursue a clean synthetic approach using the concept of "green chemistry" to obtain nanomaterials targeted for different applications, especially in biomedical fields. Green chemistry, aims to reduce or eliminate hazardous of the substances to both human and the environment. Accordingly, the development and implementation of chemical processes and products based-on green chemistry becomes more and more important.

Recently, Nanotechnology has been applied widely to fight and prevent disease by using the atomic scale tailoring of the materials. Production of nontoxic nanoparticles under safe and green conditions has an extreme importance to address growing concerns about toxicity of nanoparticles for medical applications.

To circumvent the effect of toxic chemicals in the synthesis of nanoparticles, researcher and scientists have developed benign and harmless methods for the fabrication of nanoparticles using plant extracts due to its eco-friendly nature. Plant parts such as leaf, root, latex, seed, and stem are being used for metal nanoparticles synthesis.[3] Gold nanoparticles are the most attractive member of metallic nanoparticles due to their huge applications in fields such as catalysis[16], drug delivery[7], imaging, bio-sensing, gene expression, and disease diagnosis.[17,18,19,20,21] Extracts of several plants (leaf, seed, fruits etc), have been reported to demonstrate their potential in reducing Au (III) ions into gold nanoparticles. Shankar et al, reported the formation of gold nanoparticles employing the leaf extract of Neem (*Azadirachtaindica*).[22]

Similarly, other workers were obtained gold nanoparticles using different plants extract such as *Magnolia kobus* and *Diopyros kaki* leaf extracts[23], Banana peel extract[24], fruit peel extract of *Momordicacharantia*[25], onion (*Allium cepa*) extract as the reducing agent[26], seed extract of *Nigella sativa*[18], citrus fruits juice extract as the reducing and stabilizing agent[21], *Solanuslycopersicum* (Tomato) aqueous extract[26,27], In addition, gold nanoparticles were prepared using four different plant extracts as reducing and stabilizing agents. The extracts were obtained from the following plants: *Salvia officinalis*, *Lippiacitriodora*, *Pelargonium graveolens* and *Punicagranatum*.[28] In the present day AuNPs seem to be more interest than macro counterparts, at least to world of research.

In this study gold nanoparticles was carried out from the aqueous seeds extract of Fenugreek (*Trigonellafoenum-graecum*). The reason for selecting plant for biosynthesis is to avoid problems of employing hazardous substances and toxic reducing agents. To avoid the problem of agglomeration of Au NPs in solution, it was suspended in high salt concentration for clinical uses such as drug delivery.

2. Materials

The fenugreek seeds were purchased from a local market in Sudan, Hydrogen tetrachloroaurate trihydrate (HAuCl4.3H2O) purchased from Lab Course Trading Enterprises, Khartoum Sudan and used without further purification.

Methods

Preparation of plant seed extract

The fenugreek seeds extract was done at cold and hot conditions as follow:

1- 8gram of cleaned and dried fenugreek seeds was added to 60ml distilled water in 150 ml conical flask, the mixture was maintained at room temperature for 24h. After the incubation period, the extract was poured on centrifuge tubes and centrifuged at 1500 rpm for 15 min. Then the supernatant was decanted and stored at 4 °C.
2- 8gram of cleaned and dried fenugreek seeds was added to 60 ml distilled water in 150 ml conical flask, the mixture was placed in hotplate and temperature increased till boiling. After boiling the mixture was cooled down to room temperature and centrifuged at 1500 rpm for 15 min. The supernatant was decanted and stored at 4°C.

Green synthesis (Biosynthesis) of Gold Nanoparticles

Gold Nanoparticles were synthesized using the following procedure:
50 ml of light yellow (1.0 mM) $HAuCl_4$was placed in 250 ml conical flask. The temperature of solution was increased to boiling temperature using hotplate. To the boiling solution, a 5 ml of fenugreek seeds extract was added to carefully under magnetic stirring, immediately the solution color changed from light yellow to ruby red and to purple. Then after, the solution was gradually cooled to room temperature and transferred into sterile plain container (5ml) and stored at 4°C for further analysis and applications. Table 1 described the five samples were prepared by change in some parameters.[29,30,31,32]

Table 1: The five samples were prepared by changing Some parameters.

$HAucl_4$	Samples ID	Fenugreek seeds extract	Tempe rature	Reaction time(min)
50 ml of 1.0 Mm	A	24h incubated	50°C	10 min
	B	24h incubated	100°C	10min
	C	Boiled	50°C	10 min
	D	Boiled	100°C	10 min
	E	Boiled	50°C	10min

3. RESULTS

2. Concept of Reduction

The current research work, biosynthesis of AuNPs was carried out using the aqueous extract of fenugreek seeds *(Trigonellafoenum -graecum*) as reducing and capping agent. The principle is based on the reduction of $Aucl_4$ by the extract of fenugreek seeds. In previous reports, the synthesis of gold nanoparticles through fenugreek (*Trigonellafoenum-graecum*) seeds extract as reducing and protecting agent was reported by Aswathy Aromal et al,[33] [34] The gold nanoparticles having different sizes in the range from 15 to 25 nm were obtained by controlling the synthesis parameters.

3.1 Visual Observations

After addition the plant extract in the gold salt solution, the color changed from pale yellow to ruby red and deep purple indicating the formation of AuNPs. This is due to the synthesis of AuNPs with the molecular assistance of biological reducing agents represent by the fenugreek seeds extract. The appearance of the color varied drastically with the temperatures, and the amount of extract. At 50ºC, more time was required for the complete reduction; whereas at 100ºC the color appeared in few mints. The clear colloidal suspension of AuNPs was stable for more than two months at 4ºC. The corresponding gold nanoparticles solutions containing glass vials labeled as (A-E) werepresented asfigure (1); the image showed the variations in color of AuNPs due to excitation of surface Plasmon vibrations.

Fig. 1: The obtained samples (A- E), Fenugreek seeds extract and gold salt.

3.2 Stability of Gold Nanoparticles Synthesized by Fenugreekseeds Extract in Vitro

In this experiment used strong ionic solutions (0.9%Nacl) to induce the aggregation of gold nanoparticles and observed the change. In the previous experiment the observed change is the solution to turn deep blue and solution appears clear with big precipitate forms. The images below described the phases of the present experiment (figure 2-a) the image presented the first adding of ionic solution to AuNPs samples (A- E) 1ml+0.5ml NaCl, (figure 2-b) the image presented the first dilution samples (A- E) 1ml+0.5ml NaCl, (figure 2-c) the image showed the second dilution, (figure 2-d) the image presented the third dilution and (figure 2-e) the image presented the end point of reaction.

Figure2-a: The first adding of ionic solution to AuNPs samples.

Figure 2-b: The first dilution samples.

Fig. 2-c: The second dilution.

Fig. 2-d: The third dilution.

Figure 2-e: The end point of reaction.

The color did not change, in the first adding may be due to strong reduction of $HAuCl_4$ by fenugreek seeds extract, the greater amount of the carboxylate group present in proteins can act as surfactant to attach on the surface of gold NPs and it stabilizes gold NPs through electrostatic stabilization, but sample C has less amount of carboxylate group, because the sample C has been synthesis by addition of (2ml) of reducing agents1.

The FTIR analysis supported these results. Second change in sample A may be due to seeds extract preparation. At the end point of reaction for samples B, E and D were obtained by the same dilution, indicate that they have the same amount of functional group.

The stability of both gold nanoparticles synthesized at 100ºC and seeds extract prepared by boiling were reluctant for coagulation even after addition of several (0.9%w/v Sodium Chloride) solution. This exceptional stability of biogenic nanoparticles can be attributed to protection of gold nanoparticles by intelligent capping proteins. Under optimal ionic strength of the solution these proteins avoid the columbic attraction between the nanoparticles by maintaining suitable surface potentials. In The current work carried out using plant seeds extract, is different from the previous experimental reported that usined the citrate acting as a weak reducing agent and as a stabilizer.[35] Thus, Colorimetric gold nanoparticle experiment represented good indicator that fenugreek seeds extract has ability to perform dual functions of reducing and stabilizer agents.

Microscopic techniques

The scanning electron microscopy (SEM) image of the nanoparticles presented the topography of the particle was showed in figure (3). SEM photograph of gold nanoparticles clearly indicates that synthesized gold nanoparticles have average size less ~200 nm, with spherical and cubic shape.

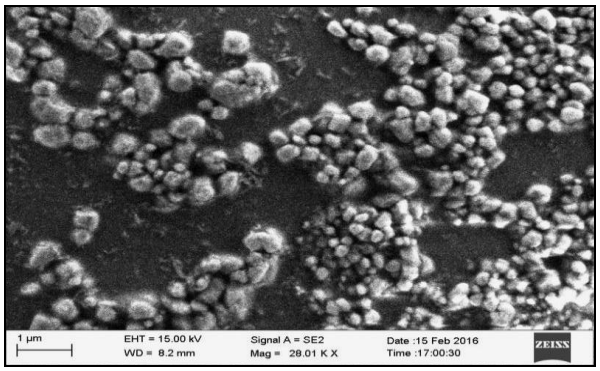

Fig. 3: SEM image of sample.

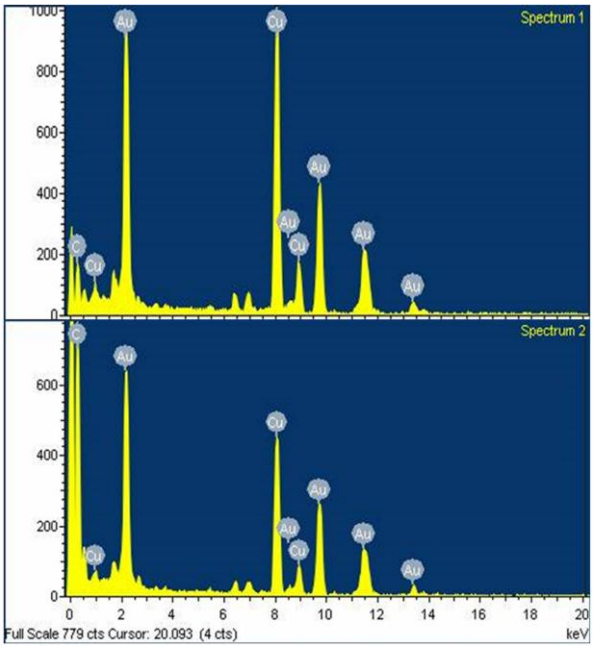

Fig. 4: EDS of samples synthesized with incubated extract (spectrum 1) and boiled extract (spectrum 2).

The elemental compositions of various particles observed in the colloidal samples were analyzed by Energy Dispersive X. Ray Spectroscopy (EDS) and were positively confirmed the presence of Au (0) in solution presented as figures (4). Peaks of copper and carbon can be rejected because samples were applied to carbon-coated copper grids and allowed to air-dry (in the dark) prior to TEM analysis.

In addition, High resolution transmission electron microscopy (HR.TEM) at PSG Research Department of Microbiology, PSG CAS, Coimbatore-14, India; was used to view the shapes of the Au NPs. TEM images of synthesized nanoparticles using Fenugreek seeds extract as reduction agent are shown in figures 5(A & B).The particles morphologies appear to be anisotropic in nature, triangular, truncated triangular, hexagonal plates, rounded and rod particles. Also overlapping particles can be seen indicating that these particles are only a few nanometers thick.

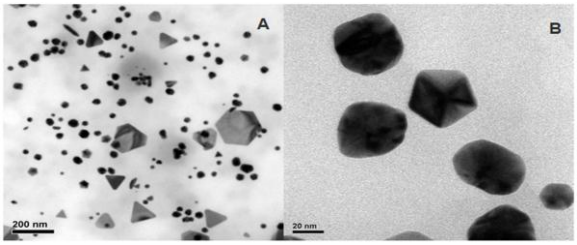

Figure 5: TEM for samples synthesized with incubated extract (A) and boiled extract (B).

UV-Vis Spectra

An absorption spectrum was a plot of absorbance of electromagnetic radiation passing through a sample vs. wavelength (or energy). The range of electromagnetic

radiation chosen to analyze a sample depends upon theenergy required to cause transitions within the absorbing species. Colored solutions contain species that absorb in the visible region of the electromagnetic spectrum (400 nm – 700 nm). The UV-Vis absorption spectra of gold nanoparticles of obtained samples were presented in (figures 6). The wavelength with maximum absorbance λ_{max} was found out to increase from 534 nm to 564 nm, as the size of Au NPs increases, the λ_{max} increases. A strong resonance of absorbance around 540 nm is clearly seen and arises due to the excitation of vibrations in the gold nanoparticles. The presence of the multiple peaks in these samples, attributed to the nanoplates at the longer wavelengths as well as the peak around 550nm attributed to the smaller rounded nanoparticles red –shifts of absorbance peak indicate the presence of NPs with a larger average particle size and vice versa for blue-shifts.

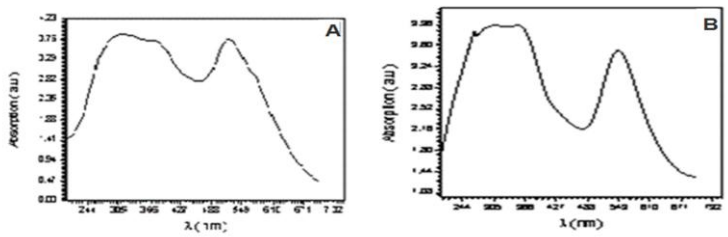

Fig. 6: UV/Vis spectrophotometer samples (A-E).

Zeta potential and Intensity Distribution

Dynamic light scattering was used to determine the Zeta potential**,** or surface charge of nanoparticles. For Intensity distribution study, peak number and peak area gives important explanation. The peak mean gives the mean diameter of particles and area gives the percentage of mean diameter according to intensity. The negative Zeta potential values of sample (D) as Table (2) showed, which provide stabilization of the particles in the form of electrostatic repulsion from each other. Z-Average diameter measurement reveal mean diameters of 54.6, 72.1, 73.0 for samples (E, A, D) respectively. The polydispersity index (PI) of nanoparticles increases with the temperature of preparation nanoparticles decreasing (table 2). The results also presented as figures (7a, 7b, and 7d); the graphs were plotted using the means of all peaks mean diameter and the intensity of peak area for samples (A, E, D) respectively.

Table2: Z-Average diameter based on light scattering intensity.

Sample ID	Temperature of reactions	Z-Average diameter(nm)	Zeta potential(mv)	PI
A	50°C	72.1		0.497
D	100°C	73.0	-55.04	0.226
E	50°C	54.6		0.684

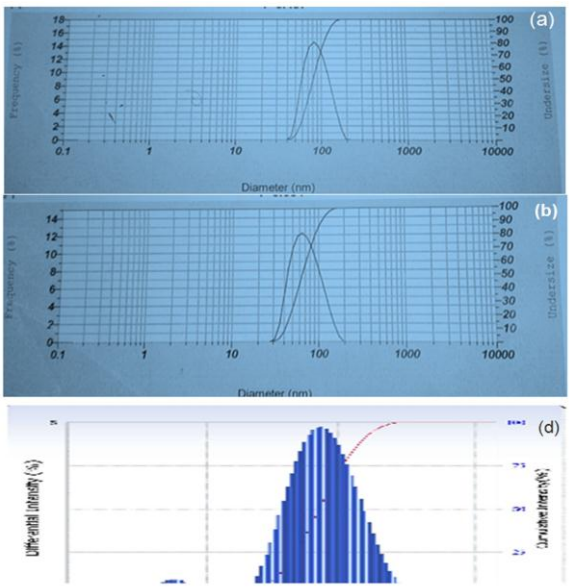

Fig. 7: Zeta Size distribution of synthesized gold nanoparticles with incubate (7-a), boiled extracts(7-b) and Size distribution by intensity(7-d).

Fourier Transform Infrared (FT-IR) spectroscopy

Phytochemical analysis of the dried seed extract of fenugreek has been reported to show the presence of proteins, vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin, saponin and plant sterol.[32] The FTIR analysis was carried out to identify the possible biomolecules present in dispersion of AuNPs. In the spectrum of seeds extracts, incubated and boiled as showed in figure 8 (A) and figure 8(B), respectively. Both the spectrum of seeds extracts as showed strong broad band at 3404 cm^{-1} indicates the presence of O-H stretch. addition to these peaks, other were obtained at 2927.7 cm^{-1} medium band O-H and the functional group is carboxylic acids. And the N–H stretch of firstly amides at 1610.45 cm^{-1}, and strong symmetric stretch N-O at 1515.94 cm^{-1} functional group nitro compounds. In addition, all bands at 1320-1000 were strong and have C-O stretch and the functional group are alcohols, carboxylic acids esters, ethers.

Figure 8 (C & D) showed the FTIR spectrum of synthesized Au NPs for sample D. The spectrum showed

bands at 1074.3cm^{-1}, 1236.3cm^{-1}, 1359.7cm^{-1}, 1382.09cm^{-1}, 1508cm^{-1}, 1620cm^{-1}, 1647cm^{-1} and 1726.17cm^{-1} these bands are characteristic carbonyl stretching vibration in ketones aldehyde, andcarboxylic acid. The broad intense band at about 3406.5cm^{-1} and 3380.98cm^{-1} represent O-H stretch and H-bond. Medium band at 2931.6 cm^{-1} represent O-H stretch, the functional group is carboxylic acids. Thus, indicates that gold NPs are possibly bound to proteins through carboxylate group. They were agreements between the two results of sample(D), large value of Zeta (-55.04) suggested a large localized surface charge density due to accumulation of negative electrons of the C=O group on the surface of AuNPs.

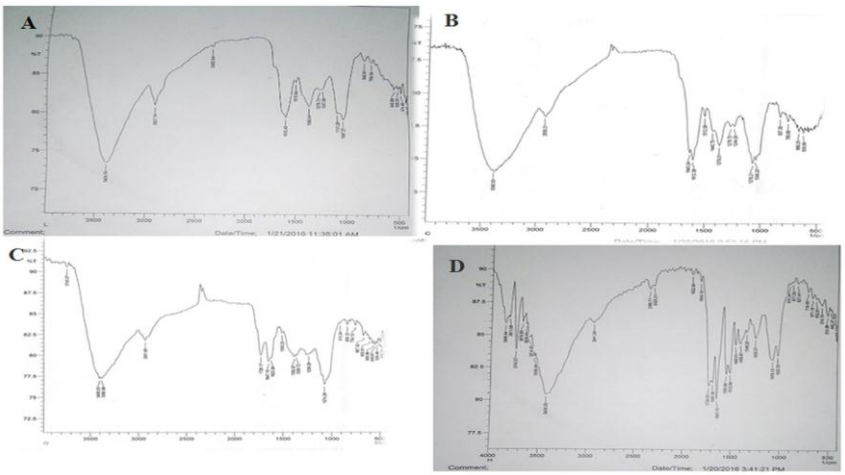

Fig. 8: FTIR spectrums of seeds incubated extract(A), boiled extract (B) and synthesized gold nanoparticles with incubated extract(C), boiled extract (D).

Atomic Absorption Spectrometry (AAS)

Table (4.3) showed the results of Atomic Absorption Spectrometry (AAS) analysis for synthesis Fenugreek gold nanoparticles.

Table 4.3: The results of Atomic Absorption Spectroscopy (AAS-7000Shimadzu).

Sample ID	Actual Au Concentration	Concentration Unit
A	59.9	ppm
B	83.02	ppm
C	57	ppm
D	83.97	ppm
E	76.13	ppm

4. CONCLUSION

In the present work, green synthetic method was a low-cost approach and capable of synthesizing gold nanoparticles at low temperature as well as high. The size and structure of obtained gold nanoparticles were characterized by SEM, TEM, UV-Vis absorption, FTIR and Zeta potential. Results have shown that the plant seeds extract were easy prepared, economic and eco-friendly way to synthesize metallic nanoparticles. Moreover, this plant mediated synthesis method represents a considerable improvement in the preparation of gold nanoparticles because of various advantages such as reduced reaction time, no need of capping agent, and better control over shape and size is achieved through careful experimental conditions including the specific reducing and capping agent, reaction time, temperature. Seeds extract has ability to perform dual functions of reduction and stabilization of Au NPs. The stability and biocompatibility of the gold nanoparticles synthesized using biological protocols was found to be extremely high than the chemically synthesized gold nanoparticles when tested using normal saline (0.9% w/v Sodium Chloride).

Also triangular gold nanoprisms can synthesize in high yield at low temperature (50ºC, 100ºC). Nanotriangle is a candidate that can be used for cancer cell treatment.[36,37,38,39,40] The larger near infrared absorption of the NPs has potential applications in hyperthermia treatment of cancer cells. The flat nature of the Nanotriangle would facilitate thermal contact; thereby letting reduce the exposure times. Nanotriangle can also be used to target specific delivery to the cancer cells, resulting in low dosage and thus reducing the metal toxicity.[41,42,43,44]

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