Synthesis and characterization of Gold nanoparticles using Sigmoidin B Flavonoid for Biological Applications.

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Abstract- Research has turned focus to exploring nanotechnology for valuable applications. The main concern however has been on the efficient synthesis of metallic nanoparticles. This study focuses on the green synthesis of Gold nanoparticles using Sigmoidin B flavonoid. Reduction of Au^{3+} to Au^{0} was monitored colorimetrically and spectrophotometrically at 350 to 800 nm. Synthesized nanoparticles were characterized by ultraviolet-visible spectrophotometry which confirmed nanoparticles with maximum absorbance at 595 nm. Transmission electron microscopy (TEM) coupled with Energy-dispersive x-ray (EDX) was used to obtain the morphology and size of the nanoparticles. Average particle size and particle surface charge were acquired with a zeta-sizer by dynamic light scattering (DLS). The nanoparticles were evaluated for their cytotoxicity against colorectal cancer cell lines by MTS assay and real-time xCELLigence.

Index Terms- Gold nanoparticle, Flavonoids, MTS assay and Cancer.

I. INTRODUCTION

Synthesis of metallic nanoparticles has been widely explored in recent years [1], [2]. Nanoparticles contain some properties that are more efficient as compared to their bulk material form. Gold nanoparticles (Au NPs) have become the highlight of metallic synthesized nanoparticles due to their novel properties and because their physical properties such as surface charge, particles size and shape can be controlled. Their attractive properties include amenability to chemical stability, amenability to preferred dispersion of particle size, electrical conductivity, good optical properties and antimicrobial activity [3]. These properties have allowed applications of these nanoparticles for use in the medical field as theranostic agents for various
threatening diseases such as cirrhosis, tuberculosis, cardiovascular diseases, Alzheimer's , cancer and HIV/AIDS [4].

Conventional methods of Au NPs synthesis have been criticized due to the reported toxicity.

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Synthesis by natural compounds has advantages such as that the process is environmentally friendly, it's simple yet efficient, it is cost effective for industrial production, requires less power and pressure for synthesis, requires use of a few compounds since the compound may play role as a reducing agent and capping agent and the process may easily be scaled up [4], [5].

These advantages have allowed for continuous and successful use of the method for synthesis of more metallic nanoparticles other than Au NPs.

Flavonoids are polyphenolic ubiquitous natural compounds presents in almost all plant species. These are secondary metabolites which are known to constitute pigmentation on different plant parts which include the flower, fruits, fruit peels, the roots and the leaves though it may differ with different plants. These natural compounds have been in the human diet for as long as humanity relied on plant fruits, vegetables and beverages such as tea, coffee and wine as food [6]. Consumption of these natural compounds has been recommended due to their reported benefits which include reduction of certain chronic diseases which include cancer, cardio vascular diseases and neurodegenerative disease such as Alzheimer's disease and Parkinson's disease [7].

Polyphenol compounds are natural antioxidants which should control oxidative stress by inducing an expression of required genes to relieve the immune system from stress. They fulfil this activity by acting as free radical scavengers and reducing agents against a buildup of free radicals as result of oxidative reactions which cause stress within the body [8]. Flavonoids have beneficial properties to human kind which include antitumor, antiproliferative, antiallergic, anti-inflammatory and antiviral properties hence the intense research and applications of these compounds in the health and medical fields [6].

The chemistry of flavonoids contributes to their continuous applications. The basic skeletal structure of flavonoids is a flavan nucleus with two aromatic rings called rings A and B which are linked by a heterocyclic three-carbon structure called ring C [8]. Different groups of flavonoids emerge from modification of the basic C6-C3-C6 skeleton by methylation, alylation, rearrangement, methoxylation, hydroxylation, glycosylation and oxidation. Modification has allowed for classification of flavonoids according to their unique functional groups which brought forth three classes which include isoflavonoids, flavonoids and neofalvonoids. These classes are further divided into various subgroups such as flavones, flavans, Dihydroflavonols, flavonols, and flavan-3-ols depending on their saturation and oxidation levels [6].

Sigmoidin B is one of the naturally occurring flavonoids contained in selected plants such as those of *Erythrina* and *Glycyrrhiza* species. This study aims to synthesize Au NPs using Sigmoidin B as a reducing and capping agent with a subsequent application of the nanoparticles against colorectal cancer cells [9].

II. MATERIALS AND METHODS

A. Materials and reagents:

Ultra-pure (type 1) deionized water was used to prepare all stock solutions. Chloroauric acid ($HAuCl_4$) (Sigma-Aldrich (St. Louis, MO, USA) was used to prepare gold solutions for synthesis of Au NPs. Methanol (anhydrous, 99.8%) (Sigma-Adrich, South Africa) was used to prepare flavone solutions for Au NPs synthesis. Sigmoidin B flavone was extracted and isolated from *Erythrina abyssinica* obtained from Makerere University in the Department of Botany, Uganda [10]. Cell viability was determined by MTS assay using UV plate reader (Thermo Scientific Multiscan Go) and by xCELLigence (Real-Time Cell Analysis (RTCA) system, multi-plate (MP) system from ACEA Biosciences).

B. Instrumentation

The Surface Plasmon Resonance profiles of Au NPs were determined using the UV-2400 PC series. Hydrodynamic particle sizes and zeta potential charges of Au NPs were measured by the Malvern Zetasizer, Nano series (Malvern Instruments, Malvern, UK). The morphology, nano structure and particle size of the material were examined using the transmission electron microscopy (TEM, JEM-2100, JEOL, Tokyo, Japan) coupled with energy dispersive x-ray spectroscopy (EDS) for elemental composition analysis at an accelerating voltage of 20kV Cell viability of the MTS assay was confirmed by UV-2400 PC series.

C. Synthesis of Sigmoidin B-Au NPs

Synthesis of Sigmoidin B-Au NPs was done by a modified Turkevich citrate method. A flask containing 50 ml of deionized water was boiled then 1mM of Chlororauric acid solution was added to the boiling water (100 ºC) while stirring at 350 rpm. An amount of 20 ml of 1mM Sigmoidin B was added to the boiling flask which gave a rapid color change. The reaction was stopped when no further color change was observed [11].

Application of Sigmoidin B-Au NPs

D. MTS assay

The BHK 21 (Normal Kidney Fibroblasts) and HCT 116 (Colorectal Carcinoma) cells were grown using normal tissue culture techniques. The BHK 21 (6 x 104 cells/ml) and HCT 116 (2 x 105 cells/ml) cells were incubated in 96 well plates at 37°C overnight, with the subsequent addition of the compounds to be tested.

Initial concentration ranges were tested to investigate the toxicity of the Sigmoidin B compound and its nanoparticle complex. In all cases, the cells were left to incubate for 4 days after compound addition, whereupon MTS (5 µl) was added to the cells. The absorbance values were measured at 490 nm after 1h, 2h and 4 hour incubation periods. Sigmoidin B and its nanoparticle complex were run in (100, 50.0, 25.0, 12.5, 6.25, 3.13 and 0.00 µM) concentration ranges.

E. xCELLigence assay

The BHK 21 (Normal Kidney Fibroblasts) and HCT 116 (Colorectal Carcinoma) cells were grown using normal tissue culture techniques. The BHK 21 (6 x 104 cells/ml) and HCT 116 (2 x 105 cells/ml) cells were incubated at 37°C overnight, in a gold electrode coated 96 well E-plate, with the subsequent addition of the Sigmoidin B-Au NPs sample, in concentrations of (100, 50.0, 25.0, 12.5, 6.25, 3.13 and 0.00 µM). The cells were left to incubate for a minimum of 4 days, with impedance measurements taken at various time points during the course of the incubation period. The data was retrieved and a graphic representation of the toxicity constructed.

F. Results and Discussion

Characterization of Sigmoidin B-Au NPs

G. UV-Vis spectroscopy

UV-Vis spectrometry was used to confirm the formation of gold nanoparticles in addition to the visual observation of color change. Color change has been reported as confirmation of nanoparticle formation however it's necessary to confirm this by appropriate techniques. UV-Vis spectrometry is a technique that takes advantage of the surface plasmon resonance (SPR) of metallic nanoparticles to determine their maximum wavelengths of absorbance. SPR is a property of metallic nanoparticles which is defined as a collection of oscillation of conducting band electrons around the nanoparticle which allows for absorption of light at the UV-Vis region of the light spectra [12]. This optical property of gold nanoparticles was used to confirm nanoparticle formation as observed when color changed from golden yellow to pink and purple colors. Color change and the maximum absorbance peaks of Sigmoidin B-Au NPs at 554.5 nm and methanol at 564 nm as shown in Fig. 1 and Fig. 2 illustrated the conversion of Au^{3+} ions to gold nanoparticles in the presence of Sigmoidin B as the reducing and capping agent. Methanol was part of the analysis with the aim to understand the effect of dissolving the flavonoid compound in it for the synthesis and as the spectrum showed, methanol was also able to synthesize nanoparticles. The formation of nanoparticles at the wavelength range of 554.5nm and 564nm is proved to be possible for gold colloidal nanoparticles by previous research which reported absorption of light by gold nanoparticles at a range of 513 nm as the minimum to a maximum of 570 nm [13].

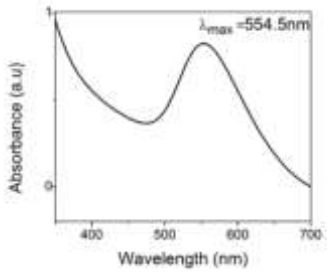

Fig 1: UV-Vis spectrum of Sigmoidin B-Au NPs after reduction of chloroauric salts by Sigmodin B.

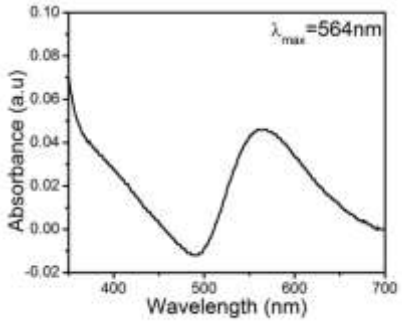

Fig 2: UV-Vis spectrum of Methanol-Au NPs after reduction of chloroauric salts by Methanol

H. Dynamic Light scattering analysis

A confirmation of synthesized Sigmoidin B-Au NPs was determined by the obtained DLS data. The Data showed the presence of dispersed nanoparticles by giving a polydispersity index (PdI) of 0.303. Hydrodynamic particle size of the synthesized nanoparticles found to be 8.6-97.3 nm with an average of 34.4 nm was obtained under the assumption of the presence of spherical nanoparticles in dispersion since DLS assumes the presence of non-agglomerated spherical nanoparticles [14]. The presence and the size of the Sigmoidin B-Au NPs were further confirmed by TEM because DLS can only determine a size bigger than the actual particle size due to the possible attachment of molecules and ions within the dispersion [14], [15]. The zeta potential of the Au NPs reported was -23.7 mV which indicated stability of Sigmoidin B-Au NPs and hence great potential for biological.

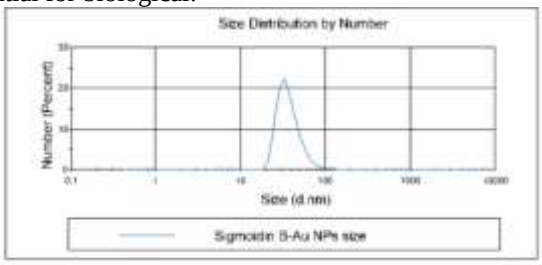

Fig 3: Hydrodynamic particle size (d.nm) of Sigmoidin B-Au NPs.

I. TEM and EDX analysis

Morphology and size of the Sigmoidin B-Au NPs were determined by TEM measurements. Fig 5 shows the obtained data which clearly indicated the dispersion of nanoparticles without agglomeration. This data confirms the reports made by DLS which showed a good PdI value from which non-agglomeration and a uniform dispersion of nanoparticles could be assumed. Fig. 3 shows the presence of spherical and triangular nanoparticles of different sizes with an average particle size of 27nm which is slightly lower than that of DLS data. These uniformly dispersed Sigmoidin B-Au NPs of different morphologies could be used for various applications with further analysis. Fig. 4 shows the elemental percentage which confirms the successful synthesis of Sigmoidin B-Au NPs with no impurities [16].

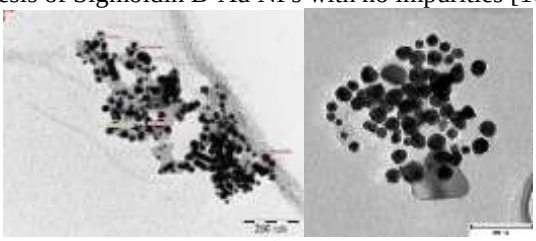

Fig 3: Particle morphology and size of Sigmoidin B-Au NPs obtained after reduction of chloroauric salts by Sigmoidin B represented by TEM

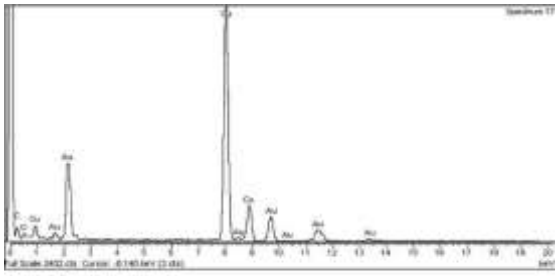

Figure 4: A confirmation of formation of Au NPs by a representative elemental composition of Sigmoidin B-Au NPs by EDX.

J. MTS assay analysis

Sigmoidin B and its nanoparticles were run in both a normal and cancerous cell lines to compare the activities of the compounds between the cell lines and also between the natural product and its respective nanoparticles.

Figure 5 illustrates the difference in Sigmoidin B toxicity between the two different cell lines. It is clear from the graphs that the natural compound seemed to be more toxic to the cancerous HCT 116 cell line. This observation is appreciable since toxicity is high for cancerous cells shown in Fig. 5 (b) which indicates a potential for colorectal cancer treatment. It's notable that toxicity is directly proportional to the concentration and it would be easily optimized and scalable for industrial production. The toxicity properties were examined by application of respective Sigmoidin B-Au NPs and major toxicity was noted against the cancer cell line. Fig. 5 (d) showed a cell viability of more than a 100% for various concentrations. An increase in cell viability is mode of defense of cancer cell lines upon treatment with Sigmoidin B-Au NPs [17].

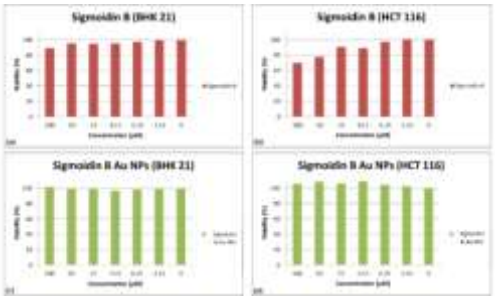

Fig 5: MTS assay graphical representation of Sigmoidin B against Normal Kidney Fibroblasts (BHK 21) (a) cell line and cell line (b). Respective Au NPs toxicity against Normal Kidney Fibroblasts (BHK 21) (c) and the Colorectal Carcinoma (HCT 116) cell line (d).

K. xCELLigence assay analysis

Figure 7 below shows the toxicity profile for the Sigmoidin B natural compound and it's Au NPs against the BHK 21 cell line and HCT 116 cancer cell line. The normal cellular growth pattern is seen, in all cases, as the light grey-blue line, shown at a concentration of 0.00 μM. This illustrates the unhindered cells and the 100% viability curvature. As seen in the MTS assay, some toxicity was expected for the compound and its nanoparticles against BHK 21 cell line but more for HCT 116.

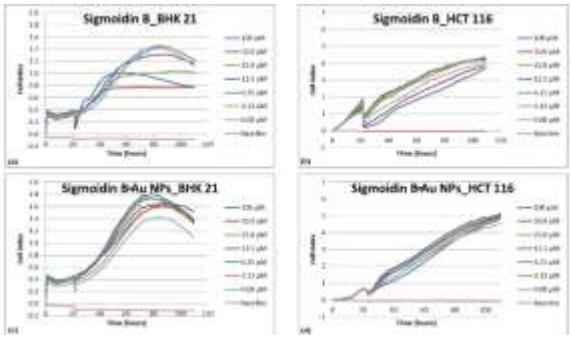

Fig 6: xCELLigence graphical growth curve representation of Sigmoidin B against Normal Kidney Fibroblasts (BHK 21) (a) cell line and cell line (b). Respective Au NPs toxicity against Normal Kidney Fibroblasts (BHK 21) (c) and the Colorectal Carcinoma (HCT 116) cell line (d).

III. CONCLUSION

Gold nanoparticles were successfully synthesized by Sigmoidin B and were employed as cytotoxic agents against HCT 116 colorectal cell line. TEM was used to determine the morphologies and multiple shapes were discovered. Spherical shapes had been evaluated for applications but combining them with triangular shapes gave a notable report. These nanoparticles were effective against both normal and cancer lines, however optimization of the methods could yield more effective results, preferably to make them less toxic on normal cell lines. The method was effective, less costly, and simple and is amenable to optimization.

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