Effect of Gold (III) chloride ($AuCl_3$) concentrations on the crystallite and morphology of bio-nano gold synthesized using *Caulerpa sertularioides*

Narjes H. Dashti*, Nedaa Y. Ali Al-Sarraf

Department of Biological Sciences, College of Science, Kuwait University

ABSTRACT

The current study aim is to carry out green synthesis of gold nanoparticles (AuNPs) using an aqueous extract from a native marine macroalgae (*Caulerpa sertularioides*), as well as to assess the impact of different concentrations of AuCl3 on the morphology and crystallite form of AuNPs. The reaction mixture was prepared by combing a 1:1 ratio (v/v) of aqueous pure algal extract (PAE, 5% w/v) with different $AuCl_3$ concentrations (0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0mM), and kept in dark condition under room temperature for 18-24 hr. Several approaches were used to study the influence of $AuCl_3$ concentrations on the crystallite and morphology of biosynthesized AuNPs. The Fourier-transform infrared spectroscopy (FTIR) spectrum revealed the presence of amine, hydroxyl and carbonyl groups that could have served as NP capping agents, possibly reducing Au(3+) ions to Au(0) during the AuNP synthesis reaction. The UV-visible NIR spectroscopy of the reaction mixtures produced prominent localized surface plasmon resonance (LSPR) absorbance band within the visible spectrum range 535-554nm, indicated by the formation of AuNPs. The negative zeta potential values between -10 and -30 mV indicated a stable dispersion of the AuNPs. Transmission Electron Microscopy (TEM), and Field Emission Scanning Electron Microscope (FESEM) images of the AuNPs synthesized using various concentrations of $AuCl_3$ revealed a shift from smaller spherical/ellipsoidal particles to larger triangle, tetragonal, and hexagonal particles, with mean diameters ranging from 17.14 (d. nm) to 0.435 (d. µm) and 15.07 (d. nm) to 0.519 (d. µm). The topography, roughness and size (3D) of the AuNPs samples were analyzed using Atomic Force Microscopy (AFM). X-ray diffraction (XRD) analysis revealed the crystallite nature of AuNPs, which manifested a cubic form in all AuNPs produced using $AuCl_3$ concentrations ranging from 0.25 to 5.0mM. In conclusion, the size of the AuNPs had a discernible impact with increasing $AuCl_3$ concentrations, where the particle size grew in tandem with the higher concentrations (TEM, and FESEM). In addition, when $AuCl_3$ concentrations increased, hexagonal, triangular, and truncated shapes predominated, and the proportion of spherical particles were reduced (2mM–5mM). However, the crystallite form of the AuNPs persisted as cubic crystals regardless of the tested $AuCl_3$ concentration (XRD).

INTRODUCTION

The most enticing metal nanoparticles are AuNPs, which have several uses in the fields of agriculture, biology, biomedical science, food industry, cosmetics, catalysis, gene expression, nonlinear optics, nanoelectronics, and disease diagnosis[1]. Since, nanoparticles (NPs) are frequently utilized in industries that interact with humans, such as agriculture and medicine, it is crucial to develop synthesis processes that don't use hazardous materials [2-3]. Biological or green synthesis of nanoparticles (NPs), which use natural reducing, capping, and stabilizing agents (algae, bacteria, fungi or plants) without the use of hazardous, expensive chemicals or high-power consumption, is the focus of current research. The preparation of size controlled AuNPs is an essential step for the medical applications and nanopriming technologies. To attain this objective, it is crucial to optimize the parameters that affects the morphology of the biosynthesized AuNPs. With this in view, the current study aimed to assess the effect of different $AuCl_3$ concentrations on the morphology of bio-AuNPs using the pure algal extract of a native marine green algae (*Caulerpa sertularioides*) as a reducing agent.

OBJECTIVES

- To collect and identify the native marine macroalgae from the Sea shore in Kuwait.
- To prepare the pure algal extract.
- To biosynthesize the AuNPs using different $AuCl_3$ concentrations (0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0mM) with PAE as reducing agent.
- To study the impact of different $AuCl_3$ concentrations on the crystallite and morphology of bio-nano gold using various techniques.

METHODOLOGY

- PAE preparation (5%, w/v) – Extraction followed by filtration [4]
- UV-Vis NIR spectroscopy- (Agilent CARY) Absorbance at 200 and 800 nm [4]
- FTIR–(Jasco FT/IR-6300) average of 16 scans (scan range of 500-4000 cm^{-1}) [4]
- XRD- Smear dry method using 3 × 3 cm glass slide (Rigaku Smart-Lab X-ray Diffraction system (ƛ=1.54Å) and crystallite size calculation using Scherrer Equation [4,5]
- TEM – Drop dry method using 150Mesh Copper grid (JEOL JEM-1200EX II)
- FESEM–(JSM-7001F, Japan) with EDS (INCAx-act, Oxford instruments, UK)
- DLS & Zeta potential –(Nano ZS , Malvern, USA) 632.8 nm laser [6]

RESULTS

By observing key morphological characteristics, the collected native marine green macroalgae samples were identified as *Caulerpa sertularioides* (S.G. Gmelin 1768), M.A. Howe 1905.[7] **(Fig. 1A)**. *C. sertularioides, a*queous pure algal extract (5%,w/v) prepared from the dry algae powder (**Fig. 1B, 1C**) was incubated with various concentrations of $AuCl_3$ (0, 0.25, 05, 1.0, 2.0, 3.0, 4.0, and 5.0 mM) for 18-24 hrs. The accelerated color change observed as $AuCl_3$ concentrations increased. (**Fig.2 inset**).

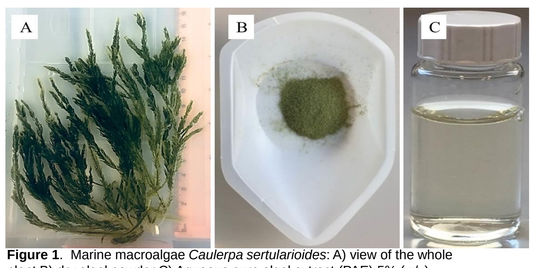

Figure 1. Marine macroalgae *Caulerpa sertularioides*: A) view of the whole plant B) dry algal powder C) Aqueous pure algal extract (PAE) 5% (w/v).

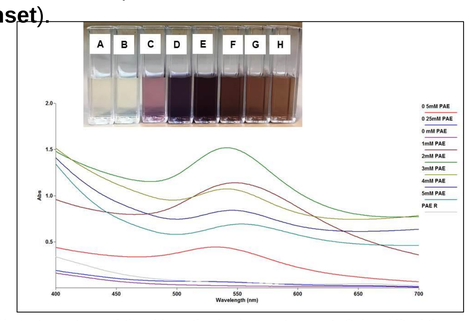

Figure 2. UV Vis NIR spectrum of the biosynthesized AuNPs. **Inset**: Color shift of the reaction mixtures after 18-24 hr. of incubation. PAE: $AuCl_3$ 1:1 (v/v) mixture; control: 0 mM (A), 0.25 mM (B); 0.5 mM (C); 1 mM (D), 2 mM (E); 3 mM (F), 4 mM (G), and 5 mM (H).

According to UV-Vis spectroscopy, the localized surface plasmon resonance (LSPR) of gold nanoparticles, causes a prominent absorbance band in the visible spectrum between 535-554 8nm confirming the synthesis of AuNPs. The absorbance peak reached its maximum at a concentration of 2.0mM $AuCl_3$, 1.520 a.u., and was then followed by peaks of 1.140 a.u. at 1mM and 1.074 a.u. at 3mM, respectively (**Fig.2**). FTIR spectrum showed almost similar pattern among the AuNPs synthesized from $AuCl_3$ at 0.25mM to 5mM. A comparison of the FTIR analyses of PAE (black) and AuNP(1mM) (red) was displayed in **Fig.3**. The spectra of PAE-AuNP (1mM) and PAE (*C.sertularioides*) revealed significant bands at 3324 and 3388 cm^{-1},1644 and1656 cm^{-1}, 1408 and 1416 cm^{-1}, 1234 and 1256 cm^{-1}, 1156 and 1150 cm^{-1}, 1077 and 1076 cm^{-1}, 1025 and 1028 cm^{-1}, and 579 and 580 cm^{-1}.

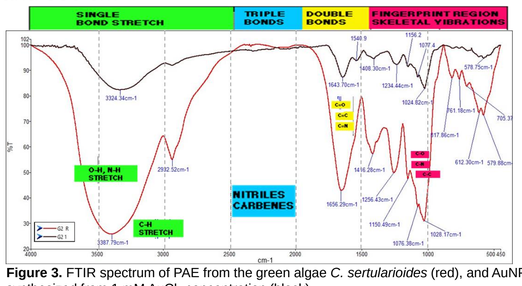

Figure 3. FTIR spectrum of PAE from the green algae *C. sertularioides* (red), and AuNPs synthesized from 1 mM $AuCl_3$ concentration (black)

Six distinct peaks were visible in the XRD peak profile, all of which were contained in the usual Bragg reflections of the face center cubic (fcc) lattice (peaks 111, 200, 220, 311, 222, and 400), indicating that all of the biosynthesized AuNPs were in the cubic crystal form of Au (0.25mM – 5.0mM) (**Fig.4**). Using Scherrer's equation, the average crystallite size for the AuNPs was determined to be between 7.40 ± 1.50 to 24.97 ± 2.74 nm (0.25mM – 5.0mM) (**Table.1**).

TEM images of PAE-AuNPs revealed a variety of sizes and shapes (**Fig.5**). Most PAE-AuNPs produced at lower $AuCl_3$ concentrations of 0.25 mM to 1.0 mM were spherical or ellipsoidal in shapes with the diameters range of 10.248-23.592 (d. nm), 5.882- 27.848 (d. nm), and 11.157-34.728 (d. nm), respectively (**Fig 5.a-c**). With increasing concentrations, especially between 3 mM and 5 mM, large counts of PAE-AuNPs in their hexagonal form are seen. Larger, less uniform particles that frequently form agglomerations are produced at higher $AuCl_3$ concentrations (3 mM–5 mM) (**Fig.5 d, f, g**).

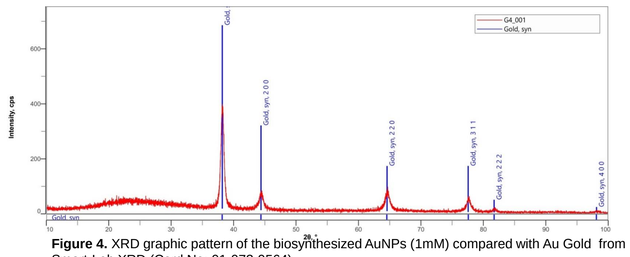

Figure 4. XRD graphic pattern of the biosynthesized AuNPs (1mM) compared with Au Gold from Smart-Lab XRD (Card No: 01-073-9564).

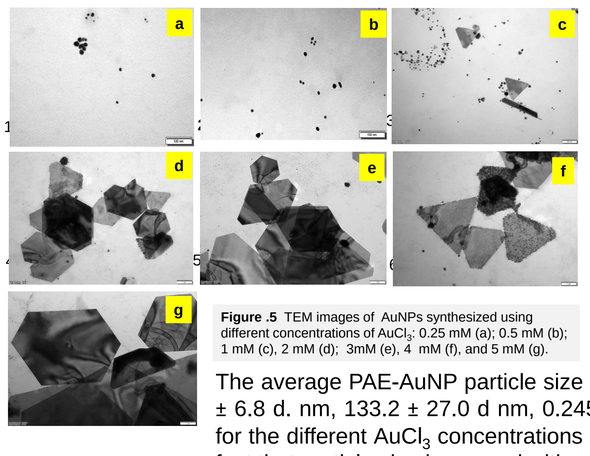

Figure .5 TEM images of AuNPs synthesized using different concentrations of $AuCl_3$: 0.25 mM (a); 0.5 mM (b); 1 mM (c), 2 mM (d); 3mM (e), 4 mM (f), and 5 mM (g).

Table 1. XRD peak FWHM (Full Width at Half Maximum) and crystallite size Dp (nm) for the AuNPs. Mean particle size ±SD in highlighted cells

No.	2θ, °	AuNPs (0.5mM)		AuNPs (1.0mM)		AuNPs (2.0mM)		AuNPs (3.0mM)		AuNPs (4.0mM)		AuNPs (5.0mM)	
		FWHM, °	Dp (nm)	FWHM, °	Dp (nm)	FWHM, °	Dp (nm)	FWHM, °	Dp (nm)	FWHM, °	Dp (nm)	FWHM, °	Dp (nm)
1	38.18	1.121	7.83	1.073	8.18	0.596	14.73	0.582	15.08	0.377	23.28	0.351	25.01
2	44.39	1.590	5.64	1.650	5.43	0.840	10.67	0.730	12.27	0.387	23.15	0.394	22.74
3	64.59	1.380	7.12	1.190	8.26	0.900	10.91	0.820	11.97	0.420	23.36	0.412	23.81
4	77.55	1.100	9.68	1.100	9.68	0.820	12.97	0.680	15.64	0.490	21.71	0.450	23.64
5	81.80	1.630	6.73	1.800	6.09	0.950	11.55	0.390	28.12	0.390	28.14	0.370	29.65
			7.40±1.50		7.53±1.74		12.16±1.69		16.62±6.64		23.93±2.45		24.97±2.74

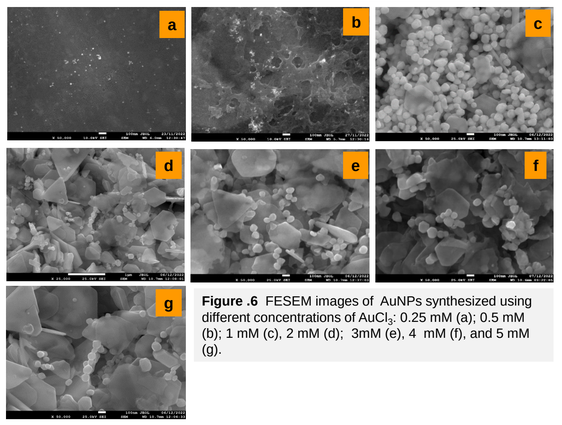

Figure .6 FESEM images of AuNPs synthesized using different concentrations of $AuCl_3$: 0.25 mM (a); 0.5 mM (b); 1 mM (c), 2 mM (d); 3mM (e), 4 mM (f), and 5 mM (g).

The average PAE-AuNP particle size was measured to be 15.1 ± 5.1 d. nm, 19.1 ± 4.8 d. nm, 27.7 ± 6.8 d. nm, 133.2 ± 27.0 d nm, 0.245 ± 0.12 d µm, 0.325 ± and 0.519 ± 0.20 d. µm, respectively, for the different $AuCl_3$ concentrations (0.25 mM to 5.0 mM) investigated. Additionally notable is the fact that particle size increased with escalating $AuCl_3$ concentrations (**Fig.6**).

Particle size distribution of the nanostructured system is shown by PDI values (**Table.2**), which range from 0.0 (perfect homogeneity) to 1.0. (high heterogeneity). The PDI values of less than 0.5 signify a confined particle size distribution . The stability of colloidal dispersions is indicated by zeta potential (ZP), which is the potential difference between the electric charges on the particle surface and the dispersant phase .

Table 2. Zeta potential measurements of colloidal AuNPs synthesized using various concentrations of $AuCl_3$

$AuCl_3$ conc. (mM)	Zeta Potential (mV)	Zeta Deviation (mV)	Z-Average (d. nm)	PdI
0.25	-20.1	9.03	140	0.470
0.5	-25.9	5.18	40.94	0.596
1.0	-18.1	4.07	89.99	0.48
2.0	-13.5	30.6	113.5	0.442
3.0	-19.1	6.78	172.4	0.607
4.0	-21.3	4.22	87.64	0.349
5.0	-24.8	5.47	213.7	0.478

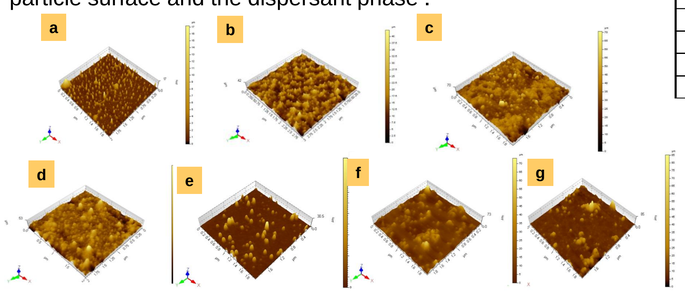

Figure 7. AFM -Three-dimensional images of the AuNPs (2 µm × 2 µm) synthesized using different concentrations of $AuCl_3$: 0.25 mM (a); 0.5 mM (b); 1 mM (c), 2 mM (d); 3mM (e), 4 mM (f), and 5 mM (g).

Table.3 AFM -Height parameter analysis of AuNPs using Fig.7 (3D 2 µm × 2 µm)

Measurement	PAE-AuNPs 0.25 mM	PAE-AuNPs 0.5 mM	PAE-AuNPs 1.0 mM	PAE-AuNPs 2.0 mM	PAE-AuNPs 3.0 mM	PAE-AuNPs 4.0 mM	PAE-AuNPs 5.0 mM
Max.peak height (µm)	15.4	27.5	28.5	37.2	34.9	59.8	74.7
Max.pit height (µm)	1.76	14.9	2.17	33.4	18.3	13.4	10.4
Max.height (µm)	17.2	42.4	30.6	70.5	53.1	73.2	85.1
Mean height (µm)	0.894	2.93	1.64	4.36	3.43	5.54	3.57
Root means square height (µm)	1.48	3.79	3.07	5.60	4.85	7.67	6.28
Skewness	3.70	0.889	3.88	0.347	1.26	1.95	4.36
Kurtosis	21.8	5.08	19.8	5.76	8.27	9.07	31.9

AFM facilitates in the evaluation of surface topography (**Fig.7a-g**) or roughness measures such as amplitude or height parameters, functional or statistical parameters, and spatial parameters (**Table 3**), revealing that the PAE-AuNPs particle size increased significantly across the tested $AuCl_3$ concentrations.

CONCLUSIONS

- AuNPs were successfully synthesized using *C.sertularioides* pure algal extract as a reducing agent.
- The amine, hydroxyl, and carbonyl groups could be the functional groups responsible for reducing $Au3^+$ to Au0, according to FTIR analysis.
- The XRD peak profile showed six distinct peaks, all of which were contained in the face center cubic (fcc) lattice's typical Bragg reflections (111, 200, 220, 311, 222, and 400), suggesting that all of the biosynthesized AuNPs were in the cubic crystal form of Au (0.25mM – 5.0mM). The mean crystallite size for the AuNPs was calculated using the Scherrer's equation and ranged from 7.40±1.50 nm to 24.97±2.74 nm (0.25mM – 5.0mM).
- TEM and FESEM images of the AuNPs synthesized at different $AuCl_3$ concentrations demonstrated a diverse in shapes from smaller spherical/ellipsoidal particles to larger triangle, tetragonal, and hexagonal particles with mean diameters ranging from 17.14 (d. nm) to 0.435 (d. µm) and 15.07 (d. nm) to 0.519 (d. µm), respectively.
- The crystallite shape of AuNPs was not changed across the tested $AuCl_3$ concentration (XRD). However, the size of the AuNPs had a noticeable effect with increasing $AuCl_3$ concentrations, where the particle size was increased along with the increasing concentrations (TEM, and FESEM). In addition, the proportion of spherical particles decreased, and triangular, truncated, triangular, and hexagonal forms took precedence as $AuCl_3$ concentrations raised (2mM – 5mM).

REFERENCES

[1] Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P., & Ahmad, F. (2022). Green Nanotechnology: Plant-Mediated Nanoparticle Synthesis and Application. Nanomaterials, 12(4), 673.
[2] Mahakham, W., Sarmah, A. K., Maensiri, S., & Theerakulpisut, P. (2017). Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. Scientific reports, 7(1), 1-21.
[3] Cai, W., Gao, T., Hong, H. and Sun, J., 2008. Applications of gold nanoparticles in cancer nanotechnology. Nanotechnology, science and applications, 1, p.17.
[4] Montasser, M. S., Hegazi, M. M., Younes, A. M., Dashti, N. H., & El-Sharkawey, A. E. (2017). Effect of gold chloride concentration and volume on size and shape of biological synthesized gold nanoparticles (AuNPs) using red algae (Laurencia papillosa). Science of Advanced Materials, 9(7), 1105-1113
[5] Ingham, B., & Toney, M. F. (2014). X-ray diffraction for characterizing metallic films. In Metallic Films for Electronic, Optical and Magnetic Applications (pp. 3-38). Woodhead Publishing.
[6] Clayton, K. N., Salameh, J. W., Wereley, S. T., & Kinzer-Ursem, T. L. (2016). Physical characterization of nanoparticle size and surface modification using particle scattering diffusometry. Biomicrofluidics, 10(5), 054107.
[7] Al-Yamani, F. Y., Polikarpov, I., Al-Ghunaim, A., & Mikhaylova, T. (2014). Field guide of marine macroalgae (Chlorophyta, Rhodophyta, Phaeophyceae) of Kuwait. Kuwait Institute for Scientific Research, Kuwait.Arabian Journal of Chemistry, 10, S3029 - S3039

ACKNOWLEDGEMENT

The authors would like to thank Research Administration of Kuwait University (KU) for funding the Research Project (Project No: SL03/16). The authors would also like to thank Research Sector Projects Unit (RSPU) for grants GS01/01, GS01/03, GS03/01, and GS03/05, Electron Microscopy Unit, Faculty of Medicine as well as the Nanotechnology Research Facility (KUNRF).