Optimal Synthesis of Organic@Inorganic (Algae@Cu) Hybrid Nanoflowers and Their Fenton-like Catalysis and Antioxidant Properties

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Abstract

Organic@inorganic hybrid nanoflowers (hNFs), which are widely used in enzyme purification and catalytic activity applications, consist of organic and inorganic components. Herein, hNFs was synthesized by the coordination of *Ascoseria mirabilis* extract and Cu in PBS, depending on concentration and medium pH, instead of expensive and difficult to obtain molecules (enzymes, DNA etc). According to the FE-SEM images, the morphology of hNFs depend to the pH of the PBS (synthesis did not occur at pH 5) and the volume of extract. The presence of Cu and other components was detailed with EDX mapping. The presence of functional groups playing key role in the synthesis was discussed with FT-IR peaks. Cu hNFs exhibited peroxidase like catalytic activity against guaiacol, and antioxidant activity. It is thought that this study will shed light on hNFs synthesis and catalytic activity application studies with bioextracts instead of biomolecules obtained by expensive and complex processes.

1 Introduction

Nanomaterials synthesized by physical, chemical and biological methods, and each method has advantages and disadvantages relative to each other [1]. Flower-shaped organic-inorganic hybrid nanoflowers (hNFs) exhibit the properties of accelerating reaction kinetics and carrier immobility due to their large surface area (surface to volume ratio) [1]. In addition, the 3D structure of NFs provides the ability to increase the efficiency of the surface reaction [2].

hNFs which are the interesting forms of nanomaterials, have attracted the attention of researchers due to potential applications of catalytic activity, optoelectronic, biosensors, solar cells, drug delivery, antioxidant, antimicrobial, purification, miscellaneous and enzyme immobilization [2,3,4,5]. Liu et al., (2021) demonstrated the catalytic activity of Cu-based NFs synthesized with the thermophilic lipase (from *Alcaligenes sp.*) enzyme by hydrolysis of p-nitrophenyl caprylate (p-NPC) [6]. They also reported that the immobilized enzyme could be reused (8 cycles). It has been reported that Cu NFs synthesized by the coordination of graphene oxide and laccase enzymes are effective in the removal of Crystal Violet and Neutral Red dyes from water [7]. The researchers based this result on increasing the efficiency by immobilizing the laccase enzyme. In another study, it has been suggested that NFs synthesized by α -chymotrypsin enzyme and calcium coordination can be used as an enzyme reactor for high efficiency digestion of proteins [4]. It was determined that NFs obtained by the coordination of Bovine Serum Albumin (BSA) and Zn ion in PBS medium can absorb Cu ions [8]. Tran et al., (2021) suggested that DNA-based Cu NFs could be used as sensors for the detection of phenolic compounds [9]. They also reported that the obtained DNA-NFs could catalyze the neutral red dye.

However, the use of expensive bio-molecules such as enzymes, and DNA as organic components for the synthesis of NFs limits their applications due to both cost and limited supply. In recent years, as an alternative to these molecules, current studies have been developing to obtain low-cost NFs as a result of the coordination of bioextracts and various metal ions and to determine their potential applications. Guven et al., (2021), reported that Cu NFs synthesized with cherry stalk extract exhibit antimicrobial

activity against *Pseudomonas auroginosa*, *Listeria monogonanta*, *Escherichia coli*, and *Enterococcus faecalis* strains [5]. Researchers have noted that NFs have catalytic and antioxidant activity. Koca et al., (2020) determined that Cu-based NFs synthesized with allicin extract exhibit peroxidase-like activity against guaiacol [10]. Demirbas (2021) reported that Cu-based NF synthesized with orange peel extract effectively exhibited antimicrobial activity against *Yersinia ruckeri* strain [11]. The antimicrobial effect of lemon peel extract coordinated Cu NFs against *Candida albicans*, *Staphylococcus aureus*, and *E. coli* strains has been demonstrated [12]. Photocatalytic activities of silver (Ag) NFs synthesized by using *Kalanchoe daigremontiana* extract against methylene blue dye; antimicrobial activities have been demonstrated against *S. aureus*, and *E. coli* strains. [13]. Kumar et al., (2021) emphasized the catalytic activities of gold (Au)-*Nephelium lappaceum* extract coordinated Au hNFs by reducing 4-nitrophenol to 4-aminophenol [14].

In this study, Cu hNFs were synthesized by using *Ascoseria mirabilis* extract alternative to enzymes and DNA. The characterization of NFs detailed by FE-SEM, EDX and FT-IR analysis. It was observed that the obtained NFs exhibited antioxidant and catalytic activity. The study shows that for the synthesis of organic@inorganic hybrid NFs, they can be synthesized cheaply and effectively with the use of bio-extracts such as plants, algae and fungi. It is thought to be a guide for nanotechnology and multidisciplinary study areas related to this field in terms of developing eco-friendly and low cost NFs synthesis and potential applications.

2 Materials And Methods

2.1 Synthesis of NFs

Cu NFs were synthesized with extract and their antimicrobial activities were evaluated. 5 g of dried algae sample and infused in 50 ml distilled water and at 80 °C for 1 h, and the resulting extract was filtered with Whatman No 1 filter paper, then centrifuged (10.000 rpm, 10 min). For the synthesis of organic-inorganic hNFs, algae extracts at 1 mg/L (NFs) with 8×10^{-4} M Cu (copper sulfate 5 aqueous) in 10 mM PBS buffer (pH 7.4). The reaction was ensured by vortexing, then incubated at 4 °C for 3 days. The precipitates formed at the bottom of the tubes were centrifuged (10000 rpm, 10 min) and then washed with distilled water [10]. The characterization of the obtained nanostructures was evaluated by FE-SEM, EDX mapping, and FT-IR analysis.

2.2 Antioxidant Activity of NFs

In the test based on DPPH oxidation [5], Cu hNFs reacted with DPPH (0.1 mM) prepared at different concentrations (0.15625, 0.3125, 0.625, 1.25, 2.5, 5 and 10 mg/ml) to determine the antioxidant activity of Cu hNF. After the mixture was incubated (30 min, in the dark), the samples whose color change was observed (purple to orange) were read at 517 nm wavelength. DPPH activity was determined by the following formula;

Scavenging activity (%) = $\frac{([\text{Absorbance of control} - (\text{Absorbance of sample} - \text{Absorbance of blank})]}{\text{Absorbance of control}} \times 100$

where the absorbance of control was sample replaced by an equivalent volume of distilled water, and the absorbance of blank was the same volume of 99.5% ethanol replacing DPPH solution.

2.3 Catalytic Activity of NFs

The catalytic activity of Cu NF was tested by the method based on the oxidation of guaiacol [10]. The oxidation of guaiacol, which occurred by the reaction of Cu NFs (3 mg), H₂O₂ (1 ml, 22.5 mM) and guaiacol (1 ml, 45 mM) in PBS buffer (10 mM, pH 6.8, 50 ml), was recorded with spectrophotometer readings at 570 nm. The mixture without hNFs was used as the blank solution under the same conditions.

3 Results And Discussions

The morphologies and diameters of Cu hNFs synthesized with algae extract were detailed by FE-SEM analysis, elemental composition of NFs by EDX mapping results, and functional groups were detailed by FT-IR analysis. The peroxidase-like catalytic activities of hNFs have been explained by a Fenton-like mechanism.

3.1 Characterization of hNFs

According to the characterization test results, the diameters of hNFs and petals synthesized as a result of the reaction of 1 ml *A. mirabilis* extract and 8×10^{-4} M Cu ion in 10 mM PBS buffer (pH: 7.4) were 31 μm (Figure 1a), and 27 nm (mean), respectively. (Figure 1b). With the change in the concentration of the plant extract, and the pH of the PBS medium, disruption in the morphological structures of the synthesized hNFs and differences in the size distribution were determined (Figure 2a-d). It was observed that no blue precipitate was formed and synthesis did not occur in the tubes (at all plant concentrations) under pH 5 conditions of PBS. The formation mechanism of NFs has been detailed in the previous literature [5,10,11]. In the mechanism mainly consisting of nucleation, growth and finish phase, the process that starts with the formation of primary phosphate crystals as a result of the reaction of Cu ions and amide, hydroxyl and diol groups of the bioextract (nucleation phase) is completed with the arrangement of the petals [5,10,11,16,17]. Baldemir et al., 2020 determined the diameters of hNFs synthesized with *Artemisia absinthium*, *A. vulgaris*, and *A. ludoviciana* extracts in the range of 2-10 μm [16]. In addition, it has been reported that while hNF is synthesized with the use of 0.1 mg/ml bioextract in the reaction, hNF formation was not observed with the use of 0.5 mg/ml bioextract. In their studies, the researchers showed that the bioextract content and concentration as an organic component had a significant effect on the formation and size of hNF. In another study, NF synthesis did not occur at 0.02, 0.03, and 0.05 mg/ml concentrations of *Trigonella foenum-graecum* extract; in addition, it was emphasized that the encapsulation yields of NFs increased by increasing the concentration of the extract from 0.1 mg/ml to 0.5 mg/ml [15]. Guven et al., (2021) reported that Cu-based hNFs synthesized with cheery stalk extract

were synthesized in the pH 6-9 range of PBS buffer, while NFs were not synthesized in other pH conditions [5]. In a study in which urease-based NF was synthesized in PBS conditions in the pH range of 6-9, this was explained by the effect of medium pH on the binding affinity of urease molecules and Cu ion [18]. Although an interesting study reported that chance is an important factor in the growth of NF [19], on the contrary, we claim that concentration of extracts, and pH of PBS significantly affects the size, morphology and formation of NFs based on the consistency of our findings and literature reviews.

Inorganic and organic components of hNFs determined by using EDX (Figure 3) and FT-IR (Figure 4) analysis, respectively. The presence of Cu and other components in the structure of NFs is demonstrated by EDX spectrum (Figure 3a) and EDX mapping (Fig. 3b-f). The weight % of Cu was determined at %15.45 in hNF. The distribution of four key elements including C (turquoise color), O (green color), P (yellow color), and Cu (red color) in NF was proved with EDX mapping (Figure 3b). The elements of C (Figure 3c), O (Figure 3d), P (Figure 3e), and Cu (Figure 3f) analyzed with mapping by representing different color in NF. The functional groups were determined by FT-IR analysis. The presence of C-H (alkane groups) were reveal at 2916 cm^{-1} , 2848 cm^{-1} , and 1453 cm^{-1} wavenumber. The peaks at 1652 cm^{-1} , and 1143 cm^{-1} correspond to amine (-NH), aliphatic ether (C-O), respectively. Primary phosphate crystals formed in PBS buffer were associated with peaks at 1039 cm^{-1} , 987 cm^{-1} , 717 cm^{-1} , 623 cm^{-1} , and 558 cm^{-1} [5,10,20]. Characterization peaks confirmed the formation of organic/inorganic hybrid NFs in PBS buffer along with their morphology.

3.2 Catalytic Activiy of NFs

The peroxidase-like catalytic activity of hNFs were determined by spectrophotometry readings of the oxidation of guaiacol (Figure 5a). The conversion of guaiacol to 3,3-dimethoxy-4,4-diphenoquinone as a result of the reaction is explained by Fenton's mechanism (Figure 5b) [5,20].

The free radicals formed by the reaction of Cu^{+1} with H_2O_2 , formed by the reaction of H_2O_2 and Cu^{+2} (contained of Cu hNFs) in the reaction medium, provide the oxidation of the substrate (Fig. 5b). By this mechanism, 3,3-dimethoxy-4,4-diphenoquinone was formed by the oxidation of guaiacol [20]. Similar to our study, the peroxidase-like catalytic activities of cherry stalk, thymol, allicin, *Viburnum opulus*, and *Laurocerasus officinalis*-based Cu NFs against guaiacol were explained by a Fenton-like mechanism [5,10,20,21,22]. Mei et al. (2022) reported that tetracycline degradation caused by CeO_2 hNFs is mediated by radicals formed as a result of Fenton's mechanism [23]. Dadi et al. (2020) reported that peroxidase activities of gallic acid@Cu NFs depend on reaction time, substrate concentration and NF morphology [24]. Jiang et al. (2021) explained the peroxidase activity that provides the oxidation of 3,3',5,5'-tetramethylbenzidine of amino acid-based Cu hNFs with the Fenton mechanism [25]. In the light of this information, we attribute the peroxidase-like catalytic activity of hNFs synthesized with the cooperation of algae extract and Cu to the decomposition of guaiacol by free radicals formed as a result of Fenton's mechanism.

3.3 Antioxidant activity of NFs

Studies to determine the antioxidant activities of nanoparticles and hNFs synthesized by biological method have an important position [5]. Free radicals that occur as a result of various bioreactions and cause oxidative damage are detoxified by antioxidants that prevent the oxidation of molecules [4,25]. The DPPH scavenging activity with the concentration increase of Cu hNFs is given in Fig. 6.

In this study, antioxidant activity of algae@Cu hNFs determined as 50% inhibitory concentration (IC₅₀) were calculated at 2.07 mg/ml. In previous studies, it was noted that the free radical scavenging activity increased with the increase in the concentration synthesized by the biosynthesized nanomaterials [26,27,28,29,30]. Guven et al., (2022) reported that NFs exhibit enhanced antioxidant activity against DPPH with increasing concentration (IC₅₀: 1.35 mg/ml) [5]. Consistent with previous studies, according to our findings, Cu hNFs showed antioxidant properties by exhibiting DPPH scavenging activity with increasing concentration.

4 Conclusion

Cu NFs were synthesized under pH 7.4 and 9 conditions with the coordination of Cu and *Ascoseria mirabilis* extract at different concentrations; hNFs with a complete flower morphology were synthesized under optimum pH 7.4, and 1 ml extract conditions. Alg@Cu hybrid hNFs have been characterized by FE-SEM, EDX mapping and FT-IR analyzes, and synthesized by using algae extract cheaply, effectively and in a wide pH and concentration range, instead of expensive and difficult-to-obtain molecules. It was determined that the obtained hNFs had antioxidant activity against DPPH, and catalytic activity against guaiacol. It is predicted that the findings of the study will be a guide in nanotechnology, biotechnology, biomedical and environmental applications.

Declarations

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

FE-SEM analysis of hNFS synthesized PBS (pH 7.4, 1 ml extract), (a) diameter of hNFs, (b) Diameters of petals.

Figure 2

FE-SEM analysis of hNFS synthesized different pH of PBS, (a) pH 7.4, 0.65 ml extract, (b) pH 7.4, 1 ml extract, (c) pH 7.4, 1.65 ml extract, (d) pH 9, 0.65 ml extract, (e) pH 9, 1 ml extract, (f) pH 9, 1.65 ml extract

Figure 3

EDX mapping of Cu hNFs (pH 7.4, 1 ml extract), (a) EDX spectrum, (b) distribution of key elements, (c) presence of C, (d) presence of O, (e) presence of P, (f) presence of Cu

Figure 4

FT-IR analysis of Cu hNFs (pH 7.4, 1 ml extract).

Figure 5

Catalytic activity of Cu hNFs (pH 7.4, 1 ml extract). A. Absorbance change of guaiacol, B. Fenton reaction

Figure 6

Antioxidant activity of Cu hNFs (pH 7.4, 1 ml extract)

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