Synthesis and antifungal activity of soluble starch and sodium alginate capped copper nanoparticles

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Abstract
In this work copper nanoparticles (Cu NPs) were prepared by using biopolymers soluble starch and sodium alginate (NaAlg) with its abundance of hydroxyl groups and its biocompatibility as the capping agents. Soluble starch capped Cu NPs (CuS NPs) and sodium alginate capped Cu NPs (CuA NPs) were first prepared by microwave assisted chemical reduction method using hydrazine, copper sulfate, soluble starch and sodium alginate as the reduction, oxidation and capping agents, respectively. The synthesized nanoparticles were characterized by x-ray diffraction (XRD), scanning electron microscopy (SEM), dynamic light scattering analysis (DLS), thermogravimetric analysis (TGA). The antifungal activities of the Cu NPs against Candida albicans and Candida krusei were determined depending on the stabilizing agents. The results showed that CuS NPs have better antifungal activity and stability under the open environment atmosphere than CuA NPs. For this reason, CuS NPs are thought to have the potential to be used in areas such as biotechnology and food packaging.

1. Introduction

Metallic nanoparticles (MNPs) have outstanding chemical, physical, thermal and optical properties due to the large amount of high-energy surface atoms relative to bulk solid and they have been used in various applications such as engineering photovoltaic technology, electronic, information storage, catalytic, chemical, environmental technology, biosensors, medicine and biomedical fields [1, 2]. While most of these applications prefer noble metals such as gold (Au) and silver (Ag), the high cost of these metals is a major limitation in their large-scale production. Preparation of copper nanoparticles (Cu NPs) has become a thrust area in material investigations, as they are thought to be a possible exchange of Au NPs and Ag NPs in various potential applications such as catalysts, diagnostic, conductive inks and therapeutic applications [2, 3].

Copper is a fundamental component encountered in plant and animal tissues. The human body has a copper metabolism active in proteins and other biomolecules that have key catalytic and basic roles. According to certain limits, there are mechanisms in the human body that provide protection against copper toxicity at cellular, tissue and organ levels. Cu NPs have been reported to have antibacterial effects comparable to Ag NPs in single E. coli, B. subtilis strains Staphylococcus species and similar antifungal properties [4, 5]. Upon the achievement of stable Cu NPs as suitable biomaterials part, they can be anticipated in biotechnology and medicine [6].

Cu NPs have been synthesized by various recognized techniques such as thermal decomposition, microwave heating, radiation methods, micro-emulsion techniques, reverse micelles, electrode discharge, photochemical reduction, gamma radiolysis, laser irradiation, electrochemical techniques, sonochemical reduction, the polyol process, and chemical reduction [1, 7–10]. Within these techniques, the chemical reduction strategy is the most conventional and practical way to synthesize Cu nanoparticles due to its predominant properties such as simple processing, high yield, high quality products, basic and finite equipment and straightforward control method.
The major drawback with their preparation and protection is their immediate oxidation to CuO or Cu₂O when exposed to the air [1, 11]. In order to avoid oxidation, these processes are usually carried out in non-aqueous medium, the reaction solutions were carefully deoxygenated and all processes were performed under rigorous protection of an inert environment or in presence of reducing, capping or protecting agents [1, 10, 11]. In this regard, dispersants and modifiers are used as surfactants in the synthesis of copper nanoparticles. However the processes performed with surfactants require organic media and metallic precursors decompose at elevated temperatures [2]. For this reason, cetyltrimethylammonium bromide (CTAB), polyethylene glycol 8000 (PEG8000), sodium dodecyl sulfate (SDS), and polysorbate 20 (Tween 20) were reported as the soluble surfactants in the copper nanoparticle synthesis [2, 12]. Furthermore, the use of natural and synthetic polymer surfactants and ligands as stabilizers in aqueous media also prevents agglomeration by producing well-dispersed metal nanoparticles with high stability [13]. It is also possible to synthesize copper nanoparticles in aqueous medium by leaf extracts and fruit extracts due to metabolites (flavonoids, proteins, terpenoids, tannins and polyphenols) in their structures [1, 14].

The interaction of nanoparticles with proteins and enzymes within mammalian cells inhibits the antioxidant defense mechanism. Eventually apoptosis (programmed cell death) or necrosis happens. Toxicity of nanoparticles is regulated by using capping agents [15]. Uncapped nanoparticles are very sensitive to environmental factors (e.g., pH, temperature, electrolytes and solvent) and aggregate easily [16]. To increase the in vivo stability, circulation lifetime and cellular uptake of nanoparticles, particles may be incorporated into or on the surface of liposomes or capped with non-toxic biocompatible macromolecules like polysaccharides or proteins [15]. The studies carried with nanoparticles have pointed that the nontoxic or toxic nature of nanoparticles depend on the capping agent and dosage [16]. For example, while chitosan loaded copper nanoparticles and Ag-Dendrimer nanocomposites show toxicity, Ag-polysaccharide nanocomposites and copper–starch conjugates have non-toxic nature [15, 16]. Due to having biocompatibility, stability and biodegradability, renewable polymers such as polysaccharides, cellulose, starch, and chitosan can be used in various formulations depending on their superior properties [4, 17].

Furthermore, the resistance of microorganisms increases as a result of misuse of antibiotics. So therapeutic solutions of fungal infections need to be presented. Among all fungal infections, Candida species are both common and highly effective microorganisms on human health [18]. In the case of immune system efficiency occurs, these pathogenic microorganisms cause a disease known as candidiasis [19, 20]. Similarly, Candida species attack and colonize in medical devices such as peripheral and central vascular catheters utilized in the treatment of patients under chemotherapy, hemodialysis and parenteral nutrition [19]. Cu is a broad-spectrum biocide that successfully prevents the growth of bacteria, fungi and algae [21]. Aqueous ions based on copper ions, complex copper species or copper-containing polymers have been proposed and used as effective antifungal agents [22]. In addition, Cu NPs, previously used as antifungal agents, have been found to be effective against various fungi species such as Saccharomyces cerevisiae, Stachybotrys chartarum, Candida albicans, Rhizoctonia solani, and Candida tropicalis [5, 22–26].

There have been limited studies on the production of Cu NPs with biopolymers. In the literature, Cu NPs synthesis has been carried out especially by chemical reduction using hydrazine. When these studies were evaluated, it was observed that the low copper precursor’s concentrations changing from 1 mM to 60 mM were used and copper reduction was carried out for long time periods (between 6 h to 324 h) or at elevated temperatures (between 80 °C–120 °C) [4, 6, 9, 13, 17, 27–34]. For this reason, in this study, it was aimed to produce Cu NPs in a shorter time by using a high copper salt solution. Therefore, microwave assisted reduction method was used to reduce the long reaction times. As biopolymers soluble starch and sodium alginate were selected due to the availability of their hydroxyl groups to stabilize Cu NPs, biodegradability, biocompatibility, cheapness and renewability [9, 17]. There have been studies based on the production of Cu NPs by microwave method using starch and sodium alginate [16, 35–37], but in this study, Cu NPs synthesis was performed in the soluble starch and sodium alginate solutions by combining microwave radiation and chemical reduction by hydrazine at highly concentrated copper salt solution (0.32 M) for the first time. It was also aimed to broaden the knowledge of antifungal properties of Cu NPs capped with polysaccharides (soluble starch and sodium alginate) against Candida krusei and Candida albicans.

2. Materials and methods

2.1. Materials

CuSO₄·5H₂O (CAS-No: 7758–99–8), hydrazine hydrate (64%–65%, reagent grade, CAS-No: 7803–57–8) and sodium alginate ([C₃₅H₇₇O₂₉⁺Na⁺]·H₂O, alginic acid sodium salt from brown algae, CAS-No: 9005–38–3, W201502) were obtained from Sigma Aldrich. Soluble starch ([C₆H₁₀O₅]ₓ)·H₂O, CAS-No: 9005–84–9) ammonia solution (25%, Catalogue number: 105432), pepton (CAS-No: 91079–38–8), D(+) glucose monohydrate (CAS-No: 14431–43–
concentrations changing from 4 mg ml\(^{-1}\) medium, respectively. As positive controls, aliquots of suspensions containing microorganisms in culture after microwave irradiation which was the very early stage of Cu NPs synthesis, it was noticed that the color of the solutions changed to pink, deep red when irradiated by microwave [32].

2.2. Synthesis of copper nanoparticles

The synthesis of Cu NPs was carried out on the basis of the work of Tantubay et al [26]. Firstly, 0.5% (wt/v) soluble starch solution was prepared by stirring until the solution became clear. The sodium alginate solution was also obtained after stirring for 3 h with deionized water at the same concentration. The pH value of these solutions were adjusted to 9 by addition of ammonia solution and 0.32 M CuSO\(_4\).5H\(_2\)O solution was added into these solutions. Subsequently, 5 ml of hydrazine hydrate was added to the resulting mixtures and the obtained brown mixtures were then exposed to microwave irradiation for 2 min at 600 W in a domestic microwave oven. The obtained wine-red colored Cu nanoparticle solutions were centrifuged at 22000 rpm for 40 min. The Cu NPs separated from the solution were dispersed in deionized water and then centrifuged. This process was repeated two times, after which the washed Cu NPs were dried overnight in a vacuum oven at 40 °C at a pressure of 20 mbar.

2.3. Characterization of copper nanoparticles

Dynamic light scattering (DLS) analyzer (Nanolplex particle size analyzer) and scanning electron microscope (SEM, Jeol JSM-5410) were used to measure the nanoparticle size and determine the nanoparticle morphology. DLS measurements were performed by dispersing the nanoparticles into deionized water. The average particle size was analyzed with the aid of an image visualization software (Image-J, National Institute of Health, USA) from about 100 random measurements of the nanoparticles. The success of the nanoparticle synthesis process was determined by x-ray diffraction (XRD, Bruker™ D8 Advance) with Cu–K\(_\alpha\) radiation. XRD patterns were acquired over a 2\(\theta\) range from 5° to 80° with a step size of 0.01°. Fourier transform infrared spectroscopy (FTIR, Spectrum 100, Perkin Elmer) with 4 cm\(^{-1}\) resolution in transmittance mode in the mid-IR region (4000–650 cm\(^{-1}\)) was used to determine interactions between Cu NPs and polysaccharides (soluble starch and sodium alginate). KBr pellet method was used to perform FTIR measurements of Cu NPs. Cu NPs/KBr pellets were prepared at weight ratio of 1 wt%.

2.4. Antifungal activity of copper nanoparticles

The antifungal activities of Cu NPs against Candida albicans (ATCC 10231) and Candida krusei (KUEN 1001) were analyzed by broth microdilution method according to Tantubay et al. [26]. Microorganisms were obtained by cultivating in the Sabouraud dextrose broth (SDB) medium and then concentrations were adjusted at about 10\(^6\) colony forming units (CFU) ml\(^{-1}\). Aliquots of suspensions containing microorganisms in culture medium (150 \(\mu\)l) were added to aqueous dispersions of 150 \(\mu\)l of Cu NPs at concentrations changing from 4 mg ml\(^{-1}\) to 7.8 \(\mu\)g ml\(^{-1}\) in 96 well plates, respectively. The inhibition of growth was determined by measuring the absorbance with a microplate reader (BioTeK Synergy HT) at 540 nm for each concentration after an incubation at 37 °C for 16 h. Microbial growth inhibition was calculated using the following formula:

\[
\text{Inhibition} \% = 100 - \left( \frac{A_2}{A_1} \times 100 \right)
\]

where \(A_1\) and \(A_2\) refer to the absorbance of fungal cells in the control and the absorbance of fungal cells in the test medium, respectively. As positive controls, aliquots of suspensions containing microorganisms in culture medium (150 \(\mu\)l) were added to 150 \(\mu\)l of SDB medium. Aqueous dispersions of 150 \(\mu\)l of Cu NPs at concentrations changing from 4 mg ml\(^{-1}\) to 7.8 \(\mu\)g ml\(^{-1}\) were mixed with 150 \(\mu\)l of SDB medium to prepare negative controls.

To determine minimum fungicidal concentration (MFC), broths from the wells (included NPs and fungi) after the measurement of optical density were cultured onto Sabouraud dextrose nutrient agar (SDA) plates and incubated for 24 h at 37 °C. MFC was the lowest fungicidal concentration of the tested NPs under defined condition.

3. Results and discussion

At the very early stage of Cu NPs synthesis, it was noticed that the color of the solutions changed to pink, deep red after microwave irradiation which was the first sign of Cu NPs formation [38]. When an aqueous Cu\(^{2+}\) solution reacted with hydrazine, the starting blue color of the CuSO\(_4\) solution turned to pale yellow, yellowish black, light purple-brown, light brown and finally dark brown, and the color of these solutions changed to pink and dark red when irradiated by microwave [32].
3.1. Phase analysis of copper nanoparticles

XRD analysis was performed to investigate the phase purity and crystallinity of both CuS NPs and CuA NPs. In the XRD pattern of CuS NPs given in figure 1(a) sharp peaks were determined at 44.27°, 51.39°, and 75.06°, and in the XRD pattern of CuA NPs given in figure 1(b) sharp peaks were determined at 43.55°, 50.70°, and 74.41°. These peaks show that the synthesized structures are metallic copper with cubic (FCC) structure with Miller indices corresponding to (111), (200) and (220) crystal planes [9, 10, 14, 32, 34]. Also, other phases such as Cu2O and CuO were not observed [10]. It shows that pure Cu-NPs were obtained under microwave conditions.

3.2. Morphology of copper nanoparticles

SEM analyses revealed the pseudospherical morphology of CuA and CuS NPs (figures 2(a), (d)) [2, 37]. The mean particle size distributions of the Cu NPs were measured randomly using the Image-J software, and the obtained data were represented by the histograms given in figures 2(b), (e). CuA NPs and CuS NPs have mean particle size diameters of 57 ± 10 nm with a size distribution changing from 32 nm to 85 and 67 ± 28 nm with a size distribution changing from 32 nm to 292 nm, respectively. Based on SEM images, it was seen that the size distribution of CuA NPs was more homogeneous than the size distribution of CuS NPs. In this case, it can be considered that the biopolymer type plays an important role as a polymeric capping agent and possibly as a size controller [2].

Figure 1. XRD analysis of CuA NPs (a) and CuS NPs (b).

Figure 2. SEM images of CuA NPs (a) and CuS NPs (d); Particle size distribution histograms of CuA NPs (b) and CuS NPs (e); DLS analysis of CuA NPs (c) and CuS NPs (f).
Starch, in aqueous solutions, has the right-hand helix structure, which may facilitate the complexation of a large number of hydroxyl groups with the molecular matrix of the metal ions. It consists of a linear component, amylose and amylopectin, a branched component. Branching results from 1,6 acetal bonds in amylopectin that are not present in amylose. As a result of the bond angles in the alpha acetol bond, it is assumed that the amylose forms a spiral structure that helps stabilize [39]. In addition, starch acts as a dispersing agent for separating metal ions from one another and provides size control of the nanoparticles [40]. The amylose in starch has a wide size range changing from 3 μm to 200 μm [32]. Therefore, it was thought that the size distribution of CuS NPs obtained in this study depends on the size range of amylose content of starch. It was concluded that carboxylate and hydroxyl groups of sodium alginate selectively trap Cu NPs by providing inter and intramolecular interstices [35].

In addition, DLS analyzes of these nanoparticles were carried out and results with a large size distribution were obtained. This was because DLS showed the hydrodynamic diameter, while SEM showed the actual nanoparticle diameter [23]. As the hydrodynamic volume of NPs is measured in the DLS analysis, the polymer chain area surrounding the NPs is included [41, 42]. When the DLS results of CuA NPs (figure 2(c)) were examined, it was observed that there were two peaks in the size distribution. In the particle size distribution of CuA NPs, 82.9% of the total volume had an average particle size of 263.8 nm and 17.1% of the total volume had an average particle size of 67.5 nm. The occurrence of this distribution with particle size was based on the fact that the particles were capped with alginate. The distribution of CuS NPs had a mean particle size of 253.3 nm for the entire volume to form a single peak (figure 2(f)). This result indicates that the starch capped on the particles. Furthermore, it was also supported by other studies in the literature that copper nanoparticles tend to accumulate and aggregate when dispersed in deionized water [43–45].

It has been reported in the literature that nanoparticles can more easily pass through cell membranes than bulk materials. Smaller nanoparticles have high toxic effects due to their high surface area to volume ratio [46]. Therefore, Auffan et al stated that nanoparticles under 20–30 nm have thermodynamic instability and this critical dimensions should be considered for nanoparticles used in nanotoxicogical studies [47]. Chen et al reported that, nanoparticle-sized copper nanoparticles (23.5 nm) had acute toxicity comparing with micron-sized copper nanoparticles (17 μm) in mice in vivo [48]. Prabhu et al investigated the effect of copper nanoparticles on dorsal root ganglion (DRG) in rats. In their study, they exposed these neurons to copper nanoparticles at increasing concentrations (10–100 μM) and sizes (40, 60 and 80 nm) for 24 h, and found that the smallest copper nanoparticles had toxic behaviour [43]. Alizadeh et al investigated the in vitro wound healing promotion of copper nanoparticles at specific sizes (20 nm, 40 nm, 80 nm) and different concentrations. They found that copper nanoparticles (40 nm/10 mM) have proliferation effect in endothelial, keratinocyte and fibroblast cells, and also copper nanoparticles (80 nm/1 mM) support collagen expression [46]. When the dimensions of Cu NPs obtained in this study are examined, it is thought that these nanoparticles have a potential to be used in biotechnological applications at appropriate concentrations.

3.3. Thermal analysis of copper nanoparticles

The differential thermogravimetric analysis (DTG) and differential thermal analysis (DTA) curves of sodium alginate, soluble starch, CuA NPs and CuS NPs given in figures 3(a)–(d) were obtained under N2 atmosphere. Based on these curves, the onset and ending temperatures of the weight losses occurring in the structures, the weight loss percentages in these temperature ranges and the temperatures with the maximum weight losses were summarized in table 1. Initial weight losses occurring from room temperature (RT) to 100 °C–130 °C range refer to the removal of the adsorbed water from the structure. The subsequent weight losses of the biopolymers ended at about 600 °C. At these intervals, the polymers are degraded and the mass losses at temperatures higher than 600 °C can be neglected. As the N2 medium was studied, the Cu NPs remained intact without any oxidation [14]. The weight loss of CuS NPs between 130.7 °C–408.6 °C was 6.87% and the weight loss of CuA NPs between 124.3 °C–467.9 °C was 7.37%. These weight losses can be attributed to the biodegradation of the polymers which have the role as the biocapping materials.

The largest exothermic transition peak recorded in the DTA curve of the soluble starch starts at 215 °C and continues up to 300 °C with a maximum decomposition temperature at the DTG curve. This range can be interpreted as the temperature at which the amylase molecules are thermally decomposed [32]. When the DTA curve of starch-capped Cu NPs was examined, an exothermic peak with a maximum value of 250 °C with the starting temperature of 211 °C was observed similar to that of starch. These results show that soluble starch and Cu NPs interact with each other. The sodium alginate biopolymer has also shown an exothermic peak starting at 184 °C and reaching a maximum value of 240 °C. In the case of CuA NPs, two exothermic peaks were observed at higher temperatures than 240 °C. As a result, it can be decided that the structure breaks down between 240 °C–535 °C. This range is similar to the decomposition temperature range of alginate [32].
Thermogravimetric analysis (TGA) was carried out to investigate the thermal changes of CuS NPs and CuA NPs under air environment given in figure 4. According to these graphs, the initial weight changes from room temperature to 135 °C–150 °C range is due to the release of moisture in the structure [21]. The next thermal events starting from the 140–150 °C range were caused by sodium alginate and soluble starch degradation. When the thermal analysis curves of CuS NPs and CuA NPs were examined, weight increases were observed after 379.5 °C and 366.9 °C, respectively due to the copper oxidation. Thermal changes occurring up to these values were given in table 2. Weight losses between 140 °C–150 °C range to 380 °C–420 °C range of both Cu NPs are very fast and these losses can be interpreted to be related to biopolymer degradation because they are similar to weight losses temperatures under nitrogen atmosphere [35]. The weight gain of CuS NPs to 800 °C is found as 10.95%. And also, this value for CuA NPs was determined as 15.74%. The DTG curve presented in figure 4(b) shows that the approximate weight changes for CuS NPs occur at 165, 190, 246 and 303 °C, while the associated

![Figure 3. DTG and DTA curves of soluble starch (a), sodium alginate (b), CuS NPs (c) and CuA NPs under N2 atmosphere.](image)

| Table 1. Thermal analysis of soluble starch, CuS NPs, sodium alginate (NaAlg) and CuA NPs under N2 atmosphere. |
| --- |
| **Sample** | **T$_{onset}$ (°C)** | **T$_{max}$ (°C)** | **T$_{end}$ (°C)** | **Weight loss (%)** | **T$_{onset}$ (°C)** | **T$_{max}$ (°C)** | **T$_{end}$ (°C)** | **Weight loss (%)** |
| Starch | RT | 69.2 | 133.3 | 8.2 | 215.2 | 299.5 | 613.3 | 74.1 |
| CuS NPs | RT | 56.4 | 130.7 | 1.2 | 130.7 | 165.5 | 408.6 | 6.9 |
| NaAlg | RT | 62.7 | 103.7 | 11.7 | 103.7 | 239.8 | 535.9 | 47.7 |
| CuA NPs | RT | 44.7 | 71.5 | 0.4 | 124.3 | 167.8 | 467.9 | 7.4 |

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differential thermal analysis (DTA) curve shows a multi-step exotherm. These changes in sodium alginate are also seen in the DTG and DTA curves of CuA NPs at 165.39 °C, 205 °C, 243 °C, 290.92 °C, 338 °C and 362 °C. The resulting peaks may be associated with the combined effect of the decomposition of starch and sodium alginate, oxidation of Cu and the burning of any carbonaceous residue (starch and sodium alginate) [26]. Tantubay et al [26] have determined the exothermic peaks for Cu nanoparticles synthesized by carboxymethyl chitosan (CMC) at 158 °C, 205 °C, 243 °C and 304 °C. They proposed that the exothermic peak at 158 °C is related to the degradation of CMC and deconvenient thermal effects associated with the oxidation of Cu to Cu2O [26]. In this study, the decomposition temperature of the biopolymers in the structure of CuS NPs and CuA NPs and the oxidation temperature of copper were determined as 165.1 °C and 170.6 °C, respectively.

Tantubay et al [26] compared the observed peaks at 205, 243 and 304 °C with three oxidation steps of Cu including surface oxidation of Cu2O to CuO, oxidation of the cores of Cu NPs to Cu2O and oxidation of Cu2O cores to CuO. Considering these data; it can be interpreted that copper oxidation at CuS NPs takes place at temperatures of 190.1 °C, 243.8 °C and 303.1 °C. In addition to this, the copper oxidation temperatures of CuA NPs were found to be 201.2 °C, 242.6 °C, 302.1 °C, 351.2 °C and 362.3 °C.

3.4. FTIR analysis of copper nanoparticles

FTIR spectra of soluble starch, sodium alginate, CuS NPs and CuA NPs were recorded to investigate the interaction of functional groups involved in the reduction of CuSO4·5H2O and subsequent stabilization of Cu NPs. Figures 5(a), (b) show the FTIR spectra of soluble starch and sodium alginate biopolymers. The main peaks of the soluble starch were observed at 3000–3600, 2926, 2163, 1979, 1681, 1584, 1487, 1409, 1364, 1325, 1294, 1236, 1148, 1104, 1077, 1013, 925, 832, 763, 691 and 660 cm⁻¹. The large absorption band between 3000–3600 cm⁻¹ and the small peaks at 2163 cm⁻¹, 1325 cm⁻¹, 1294 cm⁻¹ and 1980 cm⁻¹ are attributed to the vibration mode of O–H bonds and the combination of O–H stretching vibrations that represent the degree of hydrogen bonding in soluble starch, respectively [6, 8, 14, 16, 32, 49, 50]. Peaks at 2926 cm⁻¹, 1681 cm⁻¹, 1584 cm⁻¹, 1487 cm⁻¹, 1409 cm⁻¹, 1236 cm⁻¹ refer to asymmetric C–H stretching of CH2, C–H bending vibration, asymmetric stretch of carboxylate, plane bending at CO–H symmetric carboxylate and acetyl group stretching [1, 6, 8, 32]. The peaks at 1149 and 1013 cm⁻¹ are associated with C–O stretching vibration of ether and alcohol groups [6, 16]. The peaks at 925, 832, 763, 691 and 660 cm⁻¹ correspond to the pyranose ring [6]. Figure 5(c) depicts the FTIR spectrum of CuS NPs showing characteristic frequencies at 2915, 1647, 1566, 1519,
Small peaks similar to starch-like peaks mean that both hydroxyl and carboxyl groups were involved in the synthesis and stabilization of Cu NPs. The major peaks in the FTIR spectrum of sodium alginate were observed at 3298, 2162, 1980, 1682, 1588, 1407, 1300, 1218, 1011, 949, 878, 816 and 664 cm$^{-1}$. The absorption band at 3298 cm$^{-1}$ and the small peaks at 2162 cm$^{-1}$ and 1980 cm$^{-1}$ are attributed to the vibration mode of O-H bonds and the combination of O-H stretching vibrations, respectively [6, 8, 14, 16, 32]. The peaks at 1682 cm$^{-1}$, 1588 cm$^{-1}$, 1407 cm$^{-1}$, 1218 cm$^{-1}$ correspond to C-H bending vibration, carboxylate asymmetric stretching (COO$^{-}$), symmetric carboxylate and acetyl group stretching [1, 8, 32]. The peak at 1013 cm$^{-1}$ is associated with C–O stretching vibration of ether groups [16]. The peaks at 949, 878, 816 and 664 cm$^{-1}$ correspond to the out-of-plane deformation of the glycosidic bond on the pyranose rings coupled with other deformation vibrations (C–H of the β-mannuronic acid residues and C–H of the α-gluluronic acid residues) [6, 35]. Figure 5(d) shows the FTIR spectrum of alginate capped Cu NPs indicating characteristic frequencies at 1418, 1051, 875, and 801 cm$^{-1}$. Small peaks similar to sodium alginate mean that hydroxyl groups were involved in the synthesis and capping of Cu NPs.

Interactions in carboxyl and hydroxyl groups were observed in the FTIR spectrum which mean that these structures were effective in the synthesis of Cu nanoparticles. The reason for this was the formation of a chemical bond between the oxygen atoms of these groups and the Cu$^{2+}$ and Cu atoms. The positively charged Cu$^{2+}$ surfaces form ion-pairs with O–H groups. Thus, the reduction of the Cu$^{2+}$ to Cu$^{0}$ occurs in the amylose chain in soluble starch and the soluble starch serves a role as a capping agent that controls the morphology of the nanomaterials instead of being stabilizer [32]. The local bridging interaction process between Cu$^{0}$ and the oxygen of the –C=O group has influence upon the vibrations of the O–C–O$^{-}$ group, the C–OH deformation, –O–C–O$^{-}$ symmetrical stretching and C–C stretching of mannuronic and gluluronic acid depending on the strength and spatial range of the –C–O–Cu interaction due to the high electron density of copper [35]. Based on the findings from XRD, FTIR and literature studies, chemical reduction reactions of CuSO$_4$·5H$_2$O salt using hydrazine, soluble starch or sodium alginate and ammonia solution can be anticipated as the following equations [26, 31, 32, 34]:

\[
\text{CuSO}_4(\text{aq}) + 4\text{NH}_4\text{OH}(\text{aq}) \rightarrow \text{Cu(NH}_3)_4\text{SO}_4 \cdot \text{H}_2\text{O(s)} + 3\text{H}_2\text{O} \tag{2}
\]

\[
\text{Cu(NH}_3)_2^{2+} + \text{Starch or Alginate} \rightarrow \text{Starch} - \text{Cu(NH}_3)_2^{2+} + \text{Alginate} - \text{Cu(NH}_3)_2^{2+} \tag{3}
\]

\[
2\text{Starch} - \text{Cu(NH}_3)_2^{2+} + \text{N}_2\text{H}_4 + 4\text{OH}^- \rightarrow 2\text{Starch} - \text{Cu} + \text{N}_2 + 4\text{NH}_4\text{OH} \tag{4}
\]

\[
2\text{Alginate} - \text{Cu(NH}_3)_2^{2+} + \text{N}_2\text{H}_4 + 4\text{OH}^- \rightarrow 2\text{Alginate} - \text{Cu} + \text{N}_2 + 4\text{NH}_4\text{OH} \tag{5}
\]

In the light of these information, hydrazine instantly reduces copper ions. Thus, the possibility of occurrence of impurities such as oxide phase Cu$_2$O and Cu(OH)$_2$ are eliminated. In addition, during the reduction process the sustained release of N$_2$ provides an inert atmosphere, so that no external inert gas source is required during
synthesis [26, 31]. Because of the high concentration of Cu$^{2+}$ solution, this procedure has good reproducibility which can be used for large-scale synthesis of Cu-NPs [26].

3.5. Stability of copper nanoparticles

In order to determine the stability of CuA NPs and CuS NPs at the ambient conditions, nanoparticles were kept in open air conditions for 6 weeks and the changes in their structures were determined by the XRD analysis given in figure 6. Extra peaks at 36.63° and 61.68° were detected for CuS NPs which correspond to the (111) and (220) crystal planes of crystalline Cu$_2$O. In CuA NPs, similar to CuS NPs the (110), (111), (220) crystal planes of copper oxide were found at 29.91, 36.65 and 61.72 [51–53]. However, when these two XRD graphs were compared, it was seen that the Cu$_2$O peaks of CuA NPs were more pronounced, indicating that CuA NPs were more sensitive to oxidation. It was also important to note that no Cu$_2$O was observed in the XRD pattern of the copper nanoparticles obtained after the synthesis of CuA NPs (figure 1).

Similar results were also obtained by Murtaza et al. To verify the oxidation stability, they determined the presence of copper oxide (Cu$_2$O) in the copper nanoparticles synthesized with polyvinylpyrrolidone (PVP) by using XRD patterns. According to the XRD pattern they obtained, they found that copper nanoparticles synthesized without PVP have Cu$_2$O in the structure even one hour after the synthesis. This showed that uncapped copper nanoparticles were very sensitive to oxidation. The XRD pattern of the PVP capped copper nanoparticles after one week showed that PVP prevented oxidation [54].

3.6. Antifungal activity of copper nanoparticles

Cu NPs have a great activity for the removal of bacterial and fungal contaminants, which may be useful for surface disinfection, inside paints, biomedical and pharmaceutical purposes. Primary action modes associated with the antibacterial effect of Cu NPs are unclear [55]. Most likely the dynamic structure of the fungal cell wall allows the passage of NPs. Subsequently, Cu NPs (as reported for AgNPs) probably result in disruption of the plasma membrane integrity and inhibition of the fungal budding process [23].

Cu NPs synthesized with soluble starch and sodium alginate were further tested for antifungal activities against C. albicans (ATCC 10231) and C. krusei (KUEN 1001), which are the major causative agents of fungal diseases in humans [18]. According to our literature survey, there was no report on antifungal activity of Cu NPs against C. krusei. The results were presented in figure 7. Both of the nanoparticles tested against these fungi showed inhibitory activity after 16 h and the inhibitory activity was increased with the increase in the concentration of Cu NPs.

After the microdilution test, samples taken from the post-test medium were spread on SDA and they incubated for 24 h at 37 °C to determine whether there was any growth of the microorganisms (figure 8).

To compare the efficiency of the synthesized Cu NPs, the minimum fungicidal concentrations (MFC) were determined. Similarly to the microdilution test, MFC values for C. krusei were determined as 1 mg CuA NPs ml$^{-1}$ and 0.5 mg CuS NPs ml$^{-1}$. The MFC values for C. albicans were found to be as 1 mg ml$^{-1}$ for CuA NPs and 0.5 mg ml$^{-1}$ for CuS NPs. Based on the MFC values it was concluded that CuS NPs had more effectiveness against both microorganisms than CuA NPs.

The antifungal properties of Cu NPs against C. albicans have been reported in various studies and the minimum inhibitory concentration (MIC) values and MFC values differ from each other. For example; Rasool et al. found the MFC of the Cu NPs synthesized by plant extract (Gelidium sp.) as 5 mg/ ml [56]. Beltrán-Partida et al. synthesized ascorbic acid capped copper nanoparticles and they found their MFC value as 500 µg ml$^{-1}$ [57]. Kruk et al. determined the minimum inhibition concentration of Cu NPs synthesized by...
Figure 7. Percentage inhibition of C. albicans (a) and C. krusei (b) by Cu NPs.

Figure 8. Growth of C. krusei and C. albicans on Agar medium loaded with CuA NPs and CuS NPs microdilution test samples at different concentrations (4, 2, 1, 0.5, 0.25 mg ml$^{-1}$).
sodium dodecyl sulfate (SDS) as 3.75 μg ml⁻¹ and Bogdanović et al. found that the MFC value of copper/polyaniline nanocomposite was 1 μg ml⁻¹ [58, 59]. Although there are few studies based on the efficacy of Cu nanoparticles against C. albicans, it seems that the stabilizing agent and particle size of Cu NPs are the important factors on the antifungal activity against C. albicans [56].

4. Conclusion

In this study, the effects of polymeric capping agents (soluble starch and sodium alginate) on Cu NPs synthesized by microwave assisted chemical reduction method were compared. Nanoparticle size distributions of these particles were determined using SEM and DLS analyzes. In the DLS analysis of CuA NPs, the size distribution showed two peaks. In contrast, CuS NPs had a more uniform distribution. Thermal analysis results showed that both polymers covered Cu NPs. In FTIR analysis, the determination of soluble starch and sodium alginate peaks in the FTIR spectra of the nanoparticles pointed the capping of the polymers on the nanoparticles which confirmed the results of the thermal analysis. The behavior of the nanoparticles at the ambient conditions determined by XRD indicated the relatively high intensity of the Cu₂O,0 peaks at CuA NPs and the stability of the CuS NPs. The antifungal tests against C. krusei and C. albicans showed that CuS NPs had more inhibitory activity than CuA NPs. Since no published information regarding the antifungal activity of Cu NPs against C. krusei has been found in the literature, this study was the first report introducing the antifungal activity of biopolymer capped Cu NPs against this fungus. Furthermore, this study demonstrated the potential of starch capped Cu NPs in various antifungal applications including biotechnological and food packaging applications.

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