Received: 30 June 2023 | Revised: 1 December 2023 | Accepted: 4 December 2023

DOI: 10.1002/pat.6272

RESEARCH ARTICLE

polymers advanced technologies WILEY

Production of flower-shaped nanobiocatalysts from green tea and investigation of their peroxidase mimicking activity on the polymerization of phenol derivatives

Berkant Kalayci[1] | Naime Kaplan[1] | Seyma Dadi[2] | Ismail Ocsoy[3] | Ersen Gokturk[1]

[1]Department of Chemistry, Hatay Mustafa Kemal University, Hatay, Turkey

[2]Department of Nanotechnology Engineering, Abdullah Gül University, Kayseri, Turkey

[3]Department of Analytical Chemistry, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey

Correspondence
Ersen Gokturk, Department of Chemistry, Hatay Mustafa Kemal University, 31001 Hatay, Turkey.
Email: ersengokturk@gmail.com

Funding information
Mustafa Kemal Üniversitesi, Grant/Award Number: 20.M.048

Abstract

Enzyme catalyzed reactions are known to be environmental friendly and easy method for many applications. However, utilization of enzymes in a variety of reactions is strictly limited due to their high cost, instability in aqueous solutions, denaturation in organic solvents and high temperatures. For this reason, it is important to discover new generation catalyst systems indicating enzyme-like catalytic activity. Here, we report hybrid organic–inorganic flower-shaped green tea-Cu^{2+} nanobiocatalyst synthesized from green tea extract as an organic component and copper (II) ions (Cu^{2+}) as inorganic component. The effect of the peroxidase-mimicking activity of green tea-Cu^{2+} nanobiocatalyst was investigated on the polymerization of phenol and derivatives (guaiacol and salicylic acid) through Fenton-like reaction mechanism. Obtained successful outcomes showed that the synthesized nanobiocatalyst showed very high catalytic activity upon polymerization of phenol and guaiacol. The slight solubility of salicylic acid in water limited to achieve its polymerization under-performed reaction conditions. The yields and molecular weights of the obtained polymers were found to be quite high. While free peroxidase enzymes like horseradish peroxidase (HRP) enzyme loses its catalytic activity at 60°C and above temperatures, green tea-Cu^{2+} nanobiocatalyst exhibited very high catalytic activity upon polymerization reactions even at 60°C reaction temperature. This outcome provides significant advantages in some reactions requiring high temperatures. In order to understand the origin of the catalytic activity of the green tea-Cu^{2+} nanoflowers, similar biocatalysts were also synthesized from caffeine and catechin alkaloids which are the active components of green tea. Caffeine-Cu^{2+} and catechine-Cu^{2+} nanobiocatalysts also exhibited quite high catalytic activity toward polymerization of phenol and derivatives. We suggest that green tea-Cu^{2+} and similar types of nanobiocatalysts may expand their

utilization in polymer chemistry as promising catalytic agents for radicalic polymerizations.

KEYWORDS
enzymatic polymerization, green tea extract, organic–inorganic hybrid nanoflowers, peroxidase, phenol derivatives

1 | INTRODUCTION

Enzyme catalyzed reactions are known to be environmental friendly and simple methods for many applications and have been extensively studied by many research groups.[1–4] Various enzymes have active sites containing metal ions, for example peroxidases and superoxide dismutases.[5] Hemoproteins like horseradish peroxidase (HRP) is mainly used to achieve radicalic polymerizations of some aromatic (phenol, aniline, etc.) and vinyl-based monomers.[6–8] These catalysts transfer an electron from hydrogen peroxide to the substrate to generate radicals for the initiation of the polymerization.[9,10] Many polymeric materials can be successfully synthesized through enzyme catalyzed radicalic polymerizations.[11]

Flower-shaped hybrid organic–inorganic nanobiocatalysts were first discovered by Ge et al.[12] Organic–inorganic hybrid nanoflowers were mainly prepared using different proteins as organic components and Cu(II) ions as inorganic component. Formation of complexes between Cu(II) ions and protein molecules generates micrometer-sized flower-shaped particles. Although these particles are micrometer-sized, they are termed as nanoflowers due to their nanoscale properties.[12] Utilization of enzymes such as laccase enzyme as an organic component for the formation of hybrid nanoflowers, obtained laccase-Cu^{2+} nanobiocatalyst was reported to show 2.5 times higher catalytic activity than that of the free-laccase enzyme on the oxidation reactions. Observation of such a high catalytic activity and stability for the synthesized protein-Cu(II) nanoflowers was explained as follows: (1) high surface area of the nanoflowers; (2) less mass-transfer limitation; (3) presence of the enzyme in the nanoflower in the appropriate conformation; and (4) interactions between Cu(II) ions and protein molecules.

Flower-shaped organic–inorganic hybrid nanoflowers have potential to be used in many applications including biosensors, bioanalytical devices, bio-fuel cells, and industrial biocatalysts.[13–16] After this important discovery by Ge et al,[12] numerous studies have been carried out on the synthesis and characterization of hybrid organic–inorganic nanostructured materials. Especially, non-protein or non-enzymes incorporated nanobiocatalysts have attracted attention due to their enzyme-like activities.[17–20] Complexation of green tea extract as an organic component and Cu^{2+} ions as an inorganic component generates flower-shaped nanobiocatalyst, and peroxidase-mimicking activity of green tea-Cu^{2+} nanoflowers toward guaiacol was measured spectrophotometrically recorded at 470 nm. The obtained green tea-Cu^{2+} nanoflowers was reported to show peroxidase-mimicking activity through Fenton-like mechanism.[21]

Here, we report catalytic activity and stability of green tea-Cu^{2+} hybrid nanoflowers toward the polymerization of phenol and derivatives (guaiacol and salicylic acid). Guaiacol and salicylic acid are natural phenolic compounds, and have respectively electron releasing methoxy (—OMe) and electron withdrawing carboxylic acid (—COOH) groups in addition to the phenolic hydroxyl (—OH) group. Therefore, these monomers will provide better insight to detect the effect of electron releasing and withdrawing groups on the polymerization of phenolic monomers.

It was observed that the synthesized green tea-Cu^{2+} nanobiocatalyst showed very high catalytic activity upon polymerization of phenol and guaiacol. The slight solubility of salicylic acid in water limited to achieve its polymerization under-performed reaction conditions. The yields and molecular weights of the obtained polymers were found to be quite high. In order to understand the origin of the catalytic activity of green tea-Cu^{2+}, similar nanoflowers from caffeine and catechin alkaloids, which are the main components of green tea, were also synthesized. Catalytic activities of the synthesized caffeine-Cu^{2+} and catechine-Cu^{2+} nanobiocatalysts toward the polymerization of phenol and derivatives were also investigated. According to the obtained outcomes, caffeine-Cu^{2+} and catechine-Cu^{2+} nanobiocatalysts exhibited quite high catalytic activity toward polymerization of phenol similar to that of the green tea-Cu^{2+} hybrid nanoflowers.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Methanol (Isolab, catalog# 947046), phenol (Merck, catalog# 1002061000), guaiacol (Sigma-Aldrich, catalog# W253200-1KG-K), salicylic acid (Sigma-Aldrich, catalog# 8187311000), pH 7.4 phosphate-buffered saline (PBS, MP biomedicals, catalog# 2810305), hydrogen peroxide (H_2O_2, Merck, catalog# 1.08597), and coppersulfate pentahydrate ($CuSO_4.5H_2O$, Sigma-Aldrich, catalog#18304) were purchased and used without further purification during the synthesis of catalysts and polymerization experiments.

2.2 | Instrumentation

^1H and ^{13}C nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded on Bruker-Instruments-NMR (DPX-400) 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm)

downfield relative to tetramethylsilane (TMS, 0.0 ppm) or residual protons in the specified solvent. FT-IR spectra of the polymers were recorded on Shimadzu IRAffinity-1S spectrometer. Thermogravimetric analyses were performed under nitrogen (N_2) atmosphere with Mettler-Toledo TGA/DSC 1 Star system instrument. About 5–10 mg of each sample was heated at 10°C/min from RT to 1000°C. Differential scanning calorimetry (DSC) thermograms were obtained with a Mettler-Toledo DSC 1 Star system instrument. Thermal history was established by a heat cycle at 10°C/min from 0 to 500°C under nitrogen atmosphere. Gel permeation chromatography (GPC) was performed at 40°C using a Shimadzu LC-20 AD instrument with an internal differential refractive index detector, and an Agilent PLgel mixed-B column using HPLC grade N,N'-dimethylformamide (DMF) as the mobile phase at a flow rate of 1 mL/min. Calibration was performed with narrow polydispersity polystyrene (PS) standards.

2.3 | Preparation of green tea extract

Twenty grams of dried green tea was pulverized into powder and kept in distilled water at 40°C for a day. The obtained green tea extract was filtered, and water was evaporated under vacuum until dryness. The dried extract was stored in the refrigerator at +4°C for the synthesis of nanobiocatalyst.[21]

2.4 | Preparation of green tea-Cu^{2+} nanoflowers

A volume of 0.1 mg/mL of green tea extract prepared in ultrapure water was added to the mixture containing 50 mL of 10 mM pH 7.4 PBS buffer and 0.8 mM $CuSO_4.5H_2O$ solution. The resulting mixture was vortexed for 30 s and incubated for 3 days at +4°C. The obtained light blue colored precipitate was then collected by centrifugation and washed several times with water to remove unreacted residues. The obtained precipitate was dried in an oven at 40°C and stored in the refrigerator at +4°C for the polymerization experiments[21]

2.5 | Preparation of caffeine-Cu^{2+} nanoflowers

Twenty milligram of caffeine was dissolved in 100 mL of pH 7.4 PBS buffer. In a separate beaker, 1.5 g of $CuSO_4.5H_2O$ was dissolved in 50 mL of pH 7.4 PBS buffer. The obtained copper sulfate solution was poured into the caffeine solution, and the final mixture was vortexed for 30 s and incubated at +4°C for 3 days. The light blue precipitate was centrifuged, washed with water several times and dried in an oven at 40°C until dryness.[21]

2.6 | Preparation of catechin-Cu^{2+} nanoflowers

Twenty milligram of catechin was dissolved in 100 mL of pH 7.4 PBS buffer. In a separate beaker, 1.5 g of $CuSO_4.5H_2O$ was dissolved in 50 mL of pH 7.4 PBS buffer. The obtained Cu^{2+} solution was poured into the catechin solution and the final mixture was vortexed for 30 s and incubated at +4°C for 3 days. The obtained light blue precipitate was centrifuged at 5000 rpm for 10 min, washed with water several times and dried in an oven at 40°C until dryness.[21]

2.7 | Representative polymerization procedure

Hundred milligram of a phenol derivative and the nanoflower (green tea-Cu^{2+}, caffeine-Cu^{2+}, or catechin-Cu^{2+}) were mixed in 5.0 mL of a buffer solution (pH 7.0, 7.4, or 8.0). During the polymerization of salicylic acid, 0.2 mL of THF was also added to the reaction mixture in order to dissolve it. Obtained mixture was adjusted to the desired reaction temperature. Then, 70 µL of hydrogen peroxide (H_2O_2, 34.5%–36.5%) was added to the mixture 15 times at every 10 min to initiate/propagate the polymerization. At the end of the polymerization, obtained black colored precipitate was filtered, washed with water and methanol and dried in an oven at 60°C (Figure 1).[22]

Polyphenol formation (Table 1; entry 6) = FT-IR (ATR): 3400 cm^{-1} (O—H stretch), 1720 cm^{-1} (C═C aromatic stretch), 1620 cm^{-1} (C═C aromatic stretch), 1195 cm^{-1} (C—O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 6.90 (bs, 2H), 7.50 (s, 1H), 7.80 (s, 1H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 100, 116, 145.

Polyguaiacol formation (Table 1; entry 10) = FT-IR (ATR): 3500 cm^{-1} (O—H stretch), 1712 cm^{-1} (C═C aromatic stretch), 1604 cm^{-1} (C═C aromatic stretch), 1195 cm^{-1} (C—O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 3.82 (s, 3H, —OMe), 6.59 (m, 2H), 9.0 (bs, 1H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 56, 100, 116, 120, 145.

Polysalicylic acid formation (Table 1; entry 11) = FT-IR (ATR): 3500 cm^{-1} (O—H stretch), 1705 cm^{-1} (C═O carboxylic acid), 1160 cm^{-1} (C—O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 6.55, 9.30, 12.5 (s, 1H, —COOH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 100, 117, 119, 131, 136, 162.

3 | RESULTS AND DISCUSSION

Green tea-Cu^{2+} nanobiocatalyst obtained from green tea extract and Cu^{2+} ions showed spherical morphology according to the SEM image (Figure 2). The size of these nanoflowers is measured to be around 10 µm, and they mainly show close size distribution. Even though these particles are micrometer-sized according to the SEM image, they are termed as nanobiocatalyst due to their nano-scale properties.[12] Elemental composition of the nanoflowers was previously analyzed by Ocsoy and coworkers.[21] The presence of copper atom in the hybrid nanoflowers was proven using EDX and FT-IR analyses in the related report.

In order to detect the optimum polymerization condition of phenol derivatives, polymerization of phenol in the presence of green tea-Cu^{2+} nanobiocatalyst was first investigated. All polymerization reactions were achieved in buffer solutions, and variable parameters

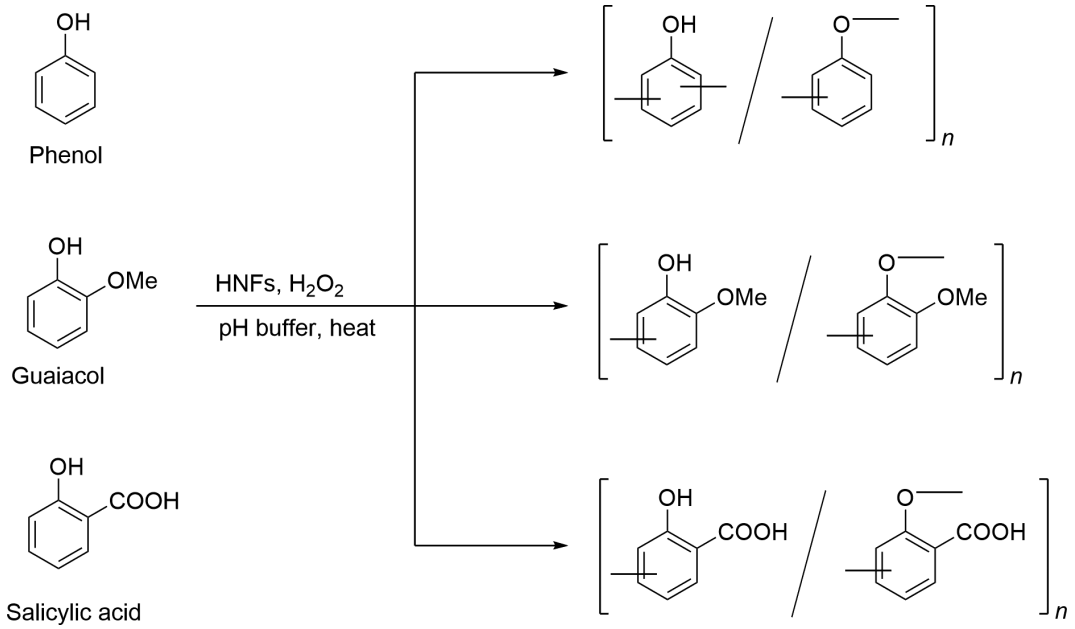

FIGURE 1 Polymerization of phenol derivatives by HNFs and H_2O_2, and the possible polymeric structures. HNFs: hibrit nanoflowers (green tea-Cu^{2+}, caffeine-Cu^{2+}, or catechin-Cu^{2+}).

TABLE 1 Polymerization of phenol and derivatives (guaiacol and salicylic acid) by green tea-Cu^{2+} nanobiocatalyst in the presence of H_2O_2.

Entry	Wt% of green tea-Cu^{2+}	pH	T_p (°C)	Yield (%)	T_{50} (°C)	Residue (%) at 1000°C	M_n (Da)	$Đ$
1[a]	2.5	7.4	30	27	590	20.6	53,000	2.86
2[a]	5.0	7.4	30	48	507	22.1	58,000	2.74
3[a]	7.5	7.4	30	59	473	3.3	60,000	2.70
4[a]	10.0	7.4	30	63	464	1.3	53,000	2.64
5[a]	7.5	7.4	40	72	600	22.0	89,000	2.88
6[a]	7.5	7.4	50	82	624	36.7	285,000	2.30
7[a]	7.5	7.4	60	81	583	23.2	131,000	2.77
8[a]	7.5	7.0	50	66	675	15.1	91,000	2.21
9[a]	7.5	8.0	50	72	401	1.7	57,000	2.65
10[b]	7.5	7.4	50	69	608	27.3	92,000	1.16
11[c]	7.5	7.4	50	9	367	0	52,000	2.60

Abbreviations: $Đ$, heterogeneity index; T_{50}, temperature at 50% residue; T_p, reaction temperature; M_n, the number average molecular weight.
[a]All polymerizations were carried out with 100 mg of phenol in different pH buffers in the presence of H_2O_2.
[b]Polymerization was carried out with 100 mg of guaiacol in the presence of H_2O_2.
[c]Polymerization was carried out with 100 mg of salicylic acid in 4.8 mL of pH 7.4 buffer and 0.2 mL of THF solvent mixture in the presence of H_2O_2.

including reaction temperature, pH, and the amount of the nanoflowers were optimized for the polymerization of phenol. During the polymerization, the amount of the green tea-Cu^{2+} nanoflowers was first determined (entries 1, 2, 3, and 4 in Table 1). The optimum catalyst loading in pH 7.4 buffer at 30°C was found to be 7.5 wt% of the phenol, the obtained yield was 59% (entry 3 in Table 1). Although utilization of 10% of the catalyst during the polymerization resulted in 63% of yield (entry 4 in Table 1), the molecular weight and thermal stability of the obtained polymer was found to be lower compared to that of the product obtained from entry 3 condition in Table 1. Therefore, optimum green tea-Cu^{2+} amount was decided to be 7.5 wt% of the phenol.

After establishing the amount of green tea-Cu^{2+}, the polymerization temperature was then optimized. During the polymerization of phenol, four different reaction temperatures, 30, 40, 50, and 60°C (entries 3, 5–7 in Table 1) were investigated. It was observed that polymerization carried out at 50°C reaction temperature resulted in the highest polymer yield (82%), the number average molecular weight (M_n = 285,000 Da) and thermal stability (entry 6 in Table 1). Interestingly, green tea-Cu^{2+} catalyst showed extremely high catalytic activity at 60°C (entry 7 in Table 1), and 81% of polymer yield was observed under this reaction condition. But, the molecular weight and thermal stability of the obtained polyphenol was found to be lower

compared to the condition carried out at 50°C. While free peroxidase enzymes like HRP enzyme loses its catalytic activity at 60°C and above temperatures,[23] green tea-Cu^{2+} nanobiocatalyst exhibited very high catalytic activity even at 60°C.

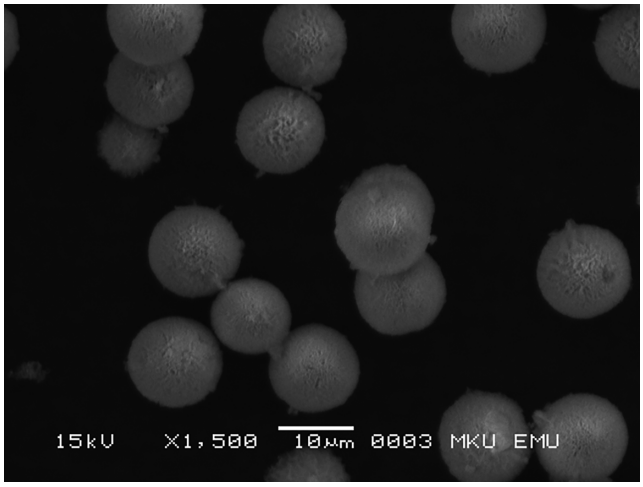

FIGURE 2 The morphology of green tea-Cu^{2+} nanobiocatalyst.

Finally, the effect of reaction pH on the polymerization of phenol as a last optimization parameter was investigated, and three different pH media (pH 7.0, 7.4, and 8.0) were explored at 50°C with 7.5 wt% of the green tea-Cu^{2+} catalyst loading (entries 6, 8, and 9 in Table 1). The efficient polymerization of phenol with 82% of yield was observed to be accomplished under pH 7.4 condition at 50°C with 7.5 wt% of the green tea-Cu^{2+} catalyst loading (entry 6 in Table 1). Polymerization attempts under pH 7.0 (entry 8 in Table 1) and pH 8.0 (entry 9 in Table 1) conditions were also successful, and resulted in polyphenol with quite high yields and molecular weights. However, polymerization yields and the number average molecular weights of the obtained polymers from these media were lower compared to the polymerization carried out under pH 7.4 condition.

Structural characterization of the synthesized polymers was confirmed by FT-IR, ^{1}H and ^{13}C NMR analyses. According to the ^{1}H NMR spectrum of the polyphenol (entry 6 in Table 1), resonances of aromatic C—H protons were detected between 6.90 and 7.80 ppm (Figure 3). Broadening of the spectrum can be attributed to the existence of polymeric structure.[24] The structure of the polyphenol was also confirmed by ^{13}C NMR analysis, and the aromatic carbon peaks in the structure were observed around 100–145 ppm (Figure 4). FT-IR

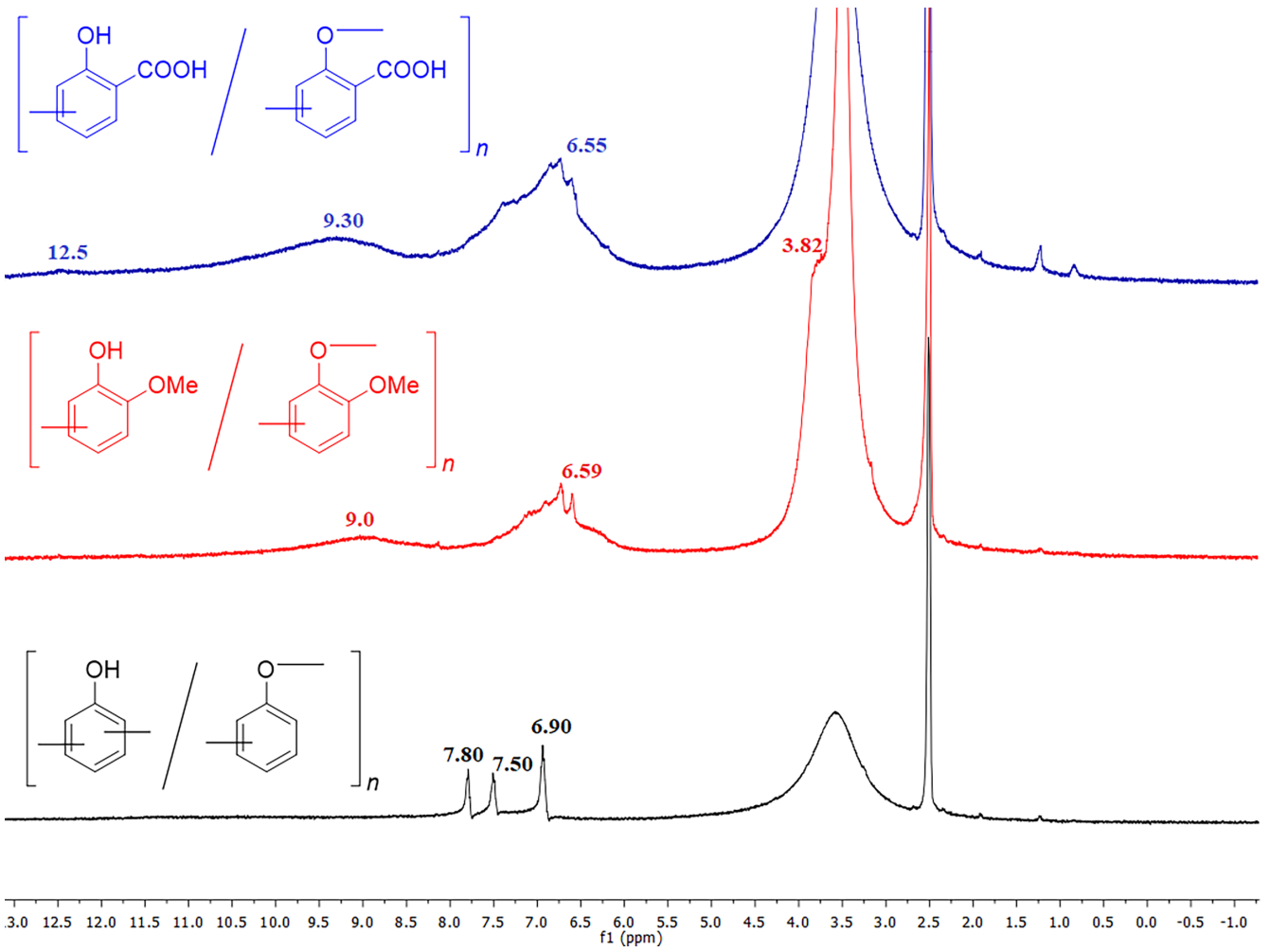

FIGURE 3 ^{1}H NMR results of the obtained polymers from entries 6 (black line), 10 (red line), and 11 (blue line) in Table 1.

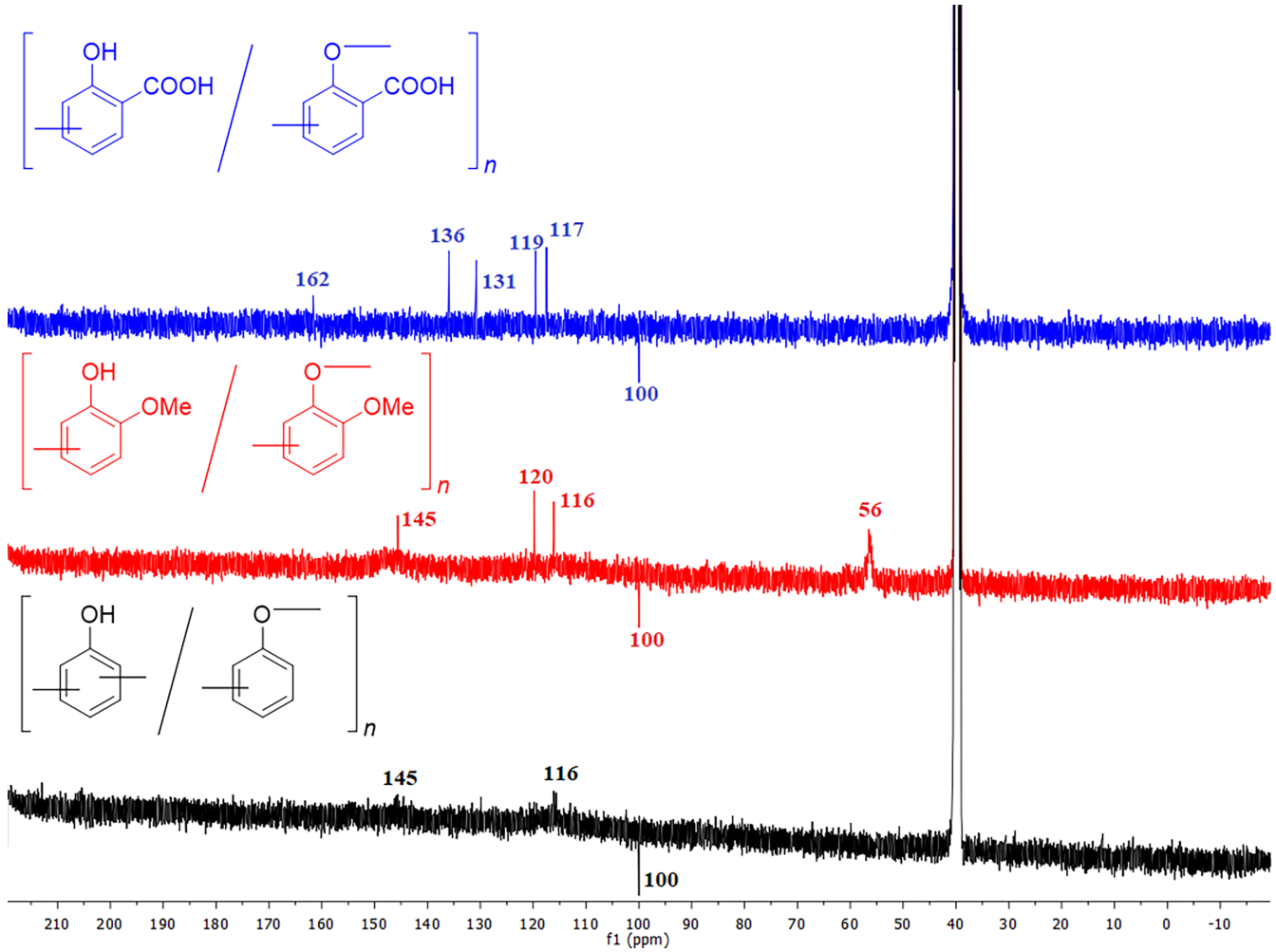

FIGURE 4 ^{13}C NMR results of the obtained polymers from entries 6 (black line), 10 (red line), and 11 (blue line) in Table 1.

spectra results provided extra information about the structure of polyphenol. The stretching vibration band of the phenolic hydroxyl (—OH) functional group appeared as a broad peak at 3400 cm^{-1} (Figure S1). Absorption bands appeared at 1720 and 1620 cm^{-1} were attributed to the aromatic (—C═C—) stretching vibrations. C—O vibration band also appeared around 1195 cm^{-1}.[25] Thermal stability of the obtained polymer was also performed using thermogravimetric analysis (TGA). The polymer lost 50% of its weight at 624°C. Pyrolysis residue of the polymer was found to be 36.7% at 1000°C under nitrogen atmosphere (Figure S4). DSC thermogram of the polyphenol (entry 6 in Table 1) showed a broad peak around 110°C which indicates thermoset (crosslinking) properties on the polymer (Figure S12).[22]

After the polymerization of phenol using green tea-Cu^{2+} nanoflowers was successfully achieved, polymerizations of other phenol derivatives, guaiacol and salicylic acid, were also attempted under entry 6 condition in Table 1 (pH 7.4, at 50°C and 7.5 wt% green tea-Cu^{2+} loading). Polymerization of guaiacol was achieved with 69% of yield (entry 10 in Table 1). In our previous report, polymerization of guaiacol by free-HRP enzyme in the presence of H_2O_2 was accomplished in pH 7.4 phosphate buffered saline (PBS) solution at 30°C, and the optimum polymerization yield was reported to be 51%.[26] In the current study, the highest yield for the polymerization of guaiacol using green tea-Cu^{2+} nanobiocatalyst in pH 7.4 buffer at 50°C was 69%. According to these observations, green tea-Cu^{2+} nanoflowers demonstrated slightly higher catalytic activity toward polymerization of guaiacol when compared with the free-HRP enzyme. The number average molecular weight of the obtained polyguaiacol in the current study was found to be 92,000 Da (Figure S15). Obtained polymer also showed quite high thermal stability, and 50% weight loss of polyguaiacol was detected at 608°C under N_2 atmosphere (Figure S5). Carbonaceous residue of the polyguaiacol at 1000°C was found to be 27.3% (entry 10 in Table 1). According to the DSC thermogram of the polyguaiacol, an endothermic peak appeared around 105°C indicates thermosetting properties of the polymer. Exothermic peak seen at 395°C is related to the decomposition temperature of the product (Figure S13).

From the ^1H NMR spectrum of the polyguaiacol (entry 10 in Table 1), the peak at 3.82 ppm can be recognized as the methyl protons (—OCH_3) of guaiacol repeating units (Figure 3). Chemical shifts appeared between 6.59 and 9.00 ppm confirms the existence of

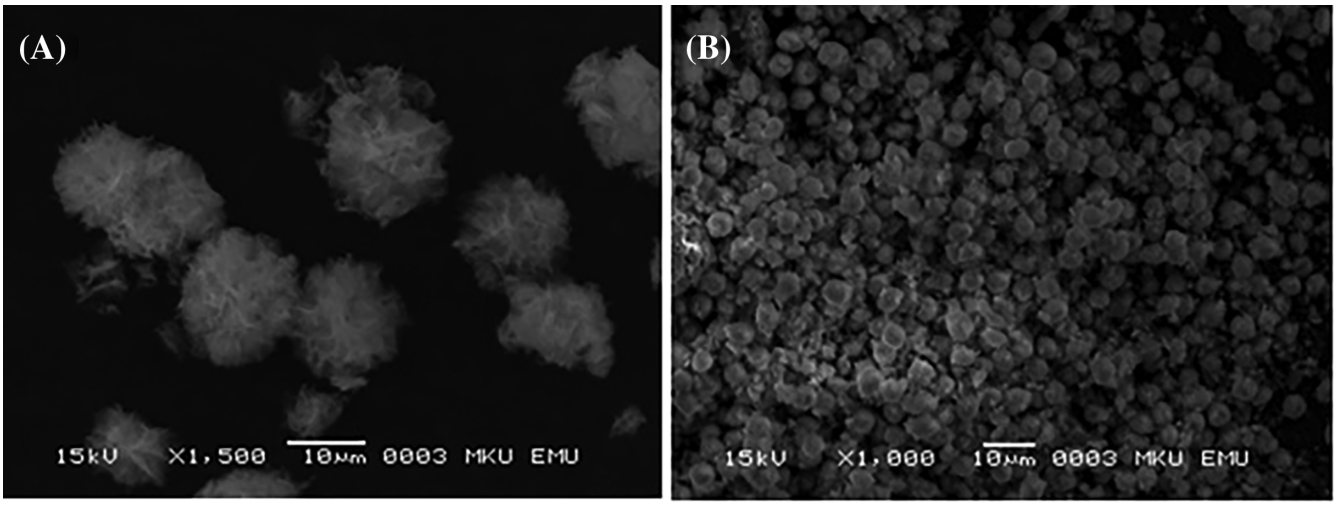

FIGURE 5 SEM images of caffeine-Cu^{2+} (A) and catechin-Cu^{2+} (B) nanobiocatalysts.

aromatic protons in the structure. The presence of methyl ^{13}C peak due to the methoxy group (—OCH_3) was also recognized at 56 ppm in the ^{13}C NMR spectrum of the product (Figure 4). The peaks observed at 100–145 ppm can be assigned to the aromatic carbon peaks of the product. FT-IR spectrum of the product also confirmed the polyguaiacol structure. Broad absorption band appeared at 3500 cm^{-1} can be assigned to the stretching vibration band of the phenolic hydroxyl (—OH) functional group (Figure S2). Absorption bands appeared at 1712 and 1604 cm^{-1} were attributed to the aromatic (—C═C—) stretching vibrations. Vibration band at 1195 cm^{-1} can be recognized to the C—O functional group.[26]

Polymerization attempts of salicylic acid under entry 6 condition in Table 1 were unsuccessful due to the insolubility of salicylic acid in pH 7.4 buffer. Therefore, polymerization of salicylic acid was accomplished in the mixture of 4.8 mL of pH 7.4 buffer and 0.2 mL of tetrahydrofuran (THF) solvent (entry 11 in Table 1). Addition of THF in the reaction mixture enabled to dissolve salicylic acid, and thus, polymerization was achieved. However, only 9% of yield was observed for the polymerization of salicylic acid at 50°C with 7.5 wt% of the green tea-Cu^{2+} catalyst loading. The reason for obtaining such a low polymerization yield is probably due to the addition of THF to the reaction medium, and perhaps, green tea-Cu^{2+} biocatalyst was deactivated after addition of THF. The number average molecular weight of this polymer was found to be 52,000 Da (entry 11 in Table 1). The 50% weight loss of the obtained polysalicylic acid was 367°C under N_2 atmosphere, and it was observed that the polymer was completely decomposed at 1000°C (Figure S6). Endothermic peak around 80°C observed from the DSC thermogram of the polysalicylic acid represents evaporative loss of water molecules. Another endothermic peak appeared at 280°C is considered to be the melting temperature of the polymer (Figure S14).

Proton signal belonging to the carboxylic acid (—COOH) group of the polysalicylic acid product (entry 11 in Table 1) was observed at 12.50 ppm in the ^1H NMR spectrum. Chemical shifts appeared between 6.55 and 9.30 ppm was assigned as aromatic protons (Figure 3). ^{13}C NMR spectrum of the polymer 11 (Table 1) also confirmed the existence of the salicylic acid repeating unit in the product. Carbonyl carbon regarding to the carboxylic acid functional group in the structure was observed at 162 ppm. ^{13}C peaks appeared around 100–136 ppm can be recognized as aromatic carbons of the product (Figure 4). Figure S3 displays FT-IR spectrum of the polysalicylic acid product (entry 11 in Table 1). Hydroxyl (—OH) stretching vibration band was recognized at 3500 cm^{-1}. Stretching vibration band of the carbonyl group (C═O) belonging to the carboxylic acid appeared at 1705 cm^{-1}. C—O absorption band was also observed at 1160 cm^{-1}.

In order to understand the origin of the catalytic activity of green tea-Cu^{2+} nanoflowers, similar kinds of nanoflowers from caffeine and catechin alkaloids, which are the active components of green tea,[21] were used as organic components to synthesize caffeine-Cu^{2+} and catechin-Cu^{2+} nanobiocatalysts. Caffeine alkaloid contains amine and amide functional groups in its structure. Catechin has resorcinol and catechol rings in its structure. Interaction between these functional groups of caffeine and catechin with Cu^{2+} ions resulted in the formation of flower shaped caffeine-Cu^{2+} and catechin-Cu^{2+} nanobiocatalysts resembling to the green tea-Cu^{2+} nanoflowers.

Figure 5 shows SEM images of (a) caffeine-Cu^{2+} and (b) catechin-Cu^{2+} nanobiocatalysts. The obtained caffeine-Cu^{2+} nanoflowers possessed flower-shaped morphology (Figure 5A) as reported in the literature,[21] whereas catechin-Cu^{2+} nanobiocatalyst was observed as spherical morphology similar to the green tea-Cu^{2+} nanoflowers (Figure 5B). The average particle sizes of caffeine-Cu^{2+} and catechin-Cu^{2+} nanoflowers were measured as approximately 15 and 5 µm, respectively. Both nanoflowers show uniform particle size distributions. Although obtained particles have micrometer-sized according to the SEM images, they show nanoscale properties. Therefore, caffeine-Cu^{2+} and catechin-Cu^{2+} nanobiocatalysts were named as nanoflowers.[12]

After successful syntheses of these nanoflowers, the effect of caffeine-Cu^{2+} biocatalyst on the polymerization of phenol was first investigated (Table 2). Solution pH for the polymerization of phenol was initially optimized. When polymerizations were carried out under

TABLE 2 Polymerization of phenol and derivatives by caffeine-Cu^{2+} nanobiocatalyst in the presence of H_2O_2.

Entry	Wt% of caffeine-Cu^{2+}	pH	T_p (°C)	Yield (%)	T_{50} (°C)	Residue (%) at 1000°C	M_n (Da)	Đ
12[a]	2.5	7.0	50	94.4	630	35.3	123,000	2.75
13[a]	2.5	7.4	50	64.1	580	25.3	93,000	2.77
14[a]	2.5	8.0	50	59.8	598	15.2	80,000	3.00
15[a]	2.5	7.0	40	58.4	555	20.4	72,000	2.95
16[a]	2.5	7.0	60	79.0	625	23.5	112,000	2.89
17[a]	5.0	7.0	50	65.6	500	12.2	89,000	2.89
18[a]	7.5	7.0	50	71.1	424	0	71,000	2.94
19[b]	2.5	7.0	50	29.3	644	27.7	75,000	3.24
20[c]	2.5	7.0	50	nd	-	-	-	-

Abbreviations: Đ, heterogeneity index; T_{50}, temperature at 50% residue; T_p, reaction temperature; M_n, the number average molecular weight.
[a]All polymerizations were carried out with 100 mg of phenol in different pH buffers in the presence of H_2O_2.
[b]Polymerization was carried out with 100 mg of guaiacol in the presence of H_2O_2.
[c]Polymerization was carried out with 100 mg of salicylic acid in 4.8 mL of pH 7.0 buffer and 0.2 mL of THF solvent mixture in the presence of H_2O_2.

pH 7.4 (entry 13 in Table 2) and pH 8.0 (entry 14 in Table 2) conditions at 50°C, obtained polyphenol yields were found to be quite low compared to that of the polymerization performed under pH 7.0 condition (entry 12 in Table 2). In order to optimize reaction temperature, polymerization of phenol was carried out at three different temperatures 40, 50, and 60°C under pH 7.0 (entries 12, 15, and 16 in Table 2). The reaction carried out at 50°C was discovered to be the optimum polymerization temperature (entry 12 in Table 2). The polymerization yields decreased at 60°C (79.0%) and 40°C (59.4%) reaction temperatures. Finally, optimization of the caffeine-Cu^{2+} catalyst loading was accomplished. 5.0 wt% (entry 17 in Table 2) and 7.5 wt% (entry 18 in Table 2) of the caffeine-Cu^{2+} catalyst loading in the polymerization of phenol resulted in decreasing yields and molecular weights for the obtained polymers. Therefore, optimum polymerization of phenol using caffeine-Cu^{2+} nanobiocatalyst was determined to be entry 12 condition in Table 2. Polymerization of phenol under entry 12 condition was achieved with 94.4% of yield. The number average molecular weight (M_n) of the obtained polyphenol was 123,000 Da (Figure S16), and the 50% mass loss of this polymer occurred at 630°C. The carbonaceous residue of the obtained polyphenol at 1000°C under nitrogen atmosphere was found to be 35.3% (Figure S7).

Phenol derivatives, guaiacol and salicylic acid, were also polymerized under entry 12 reaction condition (Table 2). Polymerization of guaiacol in the presence of caffeine-Cu^{2+} nanobiocatalyst was achieved with 29.3% of yield (entry 19 in Table 2), and M_n of the obtained polyguaiacol was 75,000 Da Figure S17). 50% mass loss of this polymer was observed at 644°C under N_2 atmosphere. Carbonaceous residue at 1000°C was 27.7% (Figure S8). Polymerization of salicylic acid in the presence of caffeine-Cu^{2+} catalyst (entry 20 in Table 2) was unfortunately unsuccessful. Caffeine-Cu^{2+} nanobiocatalyst probably lost its catalytic activity due to the addition of THF in the reaction medium.

Polymerization results of phenol and derivatives using catechin-Cu^{2+} catalyst were summarized in Table 3. Polymerization of phenol under pH 7.0 condition at 50°C with 2.5 wt% of catechin-Cu^{2+} loading resulted in 66% of yield, and the molecular weight of the polymer was 79,000 Da (entry 21 in Table 3). pH 7.4 (entry 22 in Table 3) and pH 8.0 (entry 23 in Table 3) media resulted in polyphenols with lower yields and molecular weights compared to that of carried out under pH 7.0 condition. After optimization of solution pH, temperature optimization was accomplished. 50°C reaction temperature gave the highest yielded polymer product (entry 21 in Table 3). The polymerization yield was lower in reactions performed at 40°C (entry 24 in Table 3) and 60°C (entry 25 in Table 3). However, the number average molecular weight of the polyphenol obtained at 60°C was higher compared to that of the polymer synthesized at 50°C reaction temperature. Catalyst loading as a last optimization parameter showed that 7.5 wt% of catalyst loading under pH 7.0 medium at 50°C gave 75% of yield, and the molecular weight of the polymer was 114,000 Da (entry 27 in Table 3). The 50% mass loss of the obtained polymer under N_2 atmosphere was observed at 649°C, and the carbonaceous residue of the obtained polyphenol at 1000°C was 33.7% (Figure S9). Although the highest polymerization yield was achieved under 10 wt% of catalyst loading (entry 28 in Table 3), obtained polymer showed lower thermal stability and molecular weight compared to those of the polymer observed in entry 27 condition. Therefore, entry 27 condition was determined to be optimum polymerization condition for phenol.

The polymerization of guaiacol using catechin-Cu^{2+} catalyst was carried out under optimized entry 27 condition in Table 3, and polyguaiacol was observed with 51% of yield (entry 29 in Table 3). M_n of the polyguaiacol was found to be 89,000 Da (Figure S18). 50% of weight loss of this polymer occurred at 634°C, and the carbonaceous residue at 1000°C was 23.6% under nitrogen atmosphere (Figure S10). Polymerization of salicylic acid carried out under entry 27 condition in Table 3 was partially successful with catechin-Cu^{2+} catalyst in contrast with those of the experiments using green tea-Cu^{2+} (entry 11 in Table 1) and caffeine-Cu^{2+} (entry 20 in Table 2) nanobiocatalysts. The yield of the polysalicylic acid was 25%, and M_n was found to be 66,000 Da (entry 30 in Table 3). 50% mass loss of

TABLE 3 Polymerization of phenol and derivatives by catechine-Cu^{2+} nanobiocatalyst in the presence of H_2O_2.

Entry	Wt% of catechine-Cu^{2+}	pH	T_p (°C)	Yield (%)	T_{50} (°C)	Residue (%) at 1000°C	M_n (Da)	Đ
21[a]	2.5	7.0	50	66	542	12.8	79,000	2.97
22[a]	2.5	7.4	50	63	536	17.5	77,000	3.44
23[a]	2.5	8.0	50	65	532	15.5	73,000	3.16
24[a]	2.5	7.0	40	49	502	8.2	78,000	4.95
25[a]	2.5	7.0	60	65	662	20.0	86,000	3.12
26[a]	5.0	7.0	50	68	624	17.3	87,000	3.40
27[a]	7.5	7.0	50	75	649	33.7	114,000	2.91
28[a]	10.0	7.0	50	76	672	33.1	105,000	2.79
29[b]	7.5	7.0	50	51	634	23.6	89,000	3.65
30[c]	7.5	7.0	50	25	430	0	66,000	2.93

Abbreviations: Đ, heterogeneity index; T_{50}, temperature at 50% residue; T_p, reaction temperature; M_n, the number average molecular weight.
[a]All polymerizations were carried out with 100 mg of phenol in different pH buffers in the presence of H_2O_2.
[b]Polymerization was carried out with 100 mg of guaiacol in the presence of H_2O_2.
[c]Polymerization was carried out with 100 mg of salicylic acid in 4.8 mL of pH 7.0 buffer and 0.2 mL of THF solvent mixture in the presence of H_2O_2.

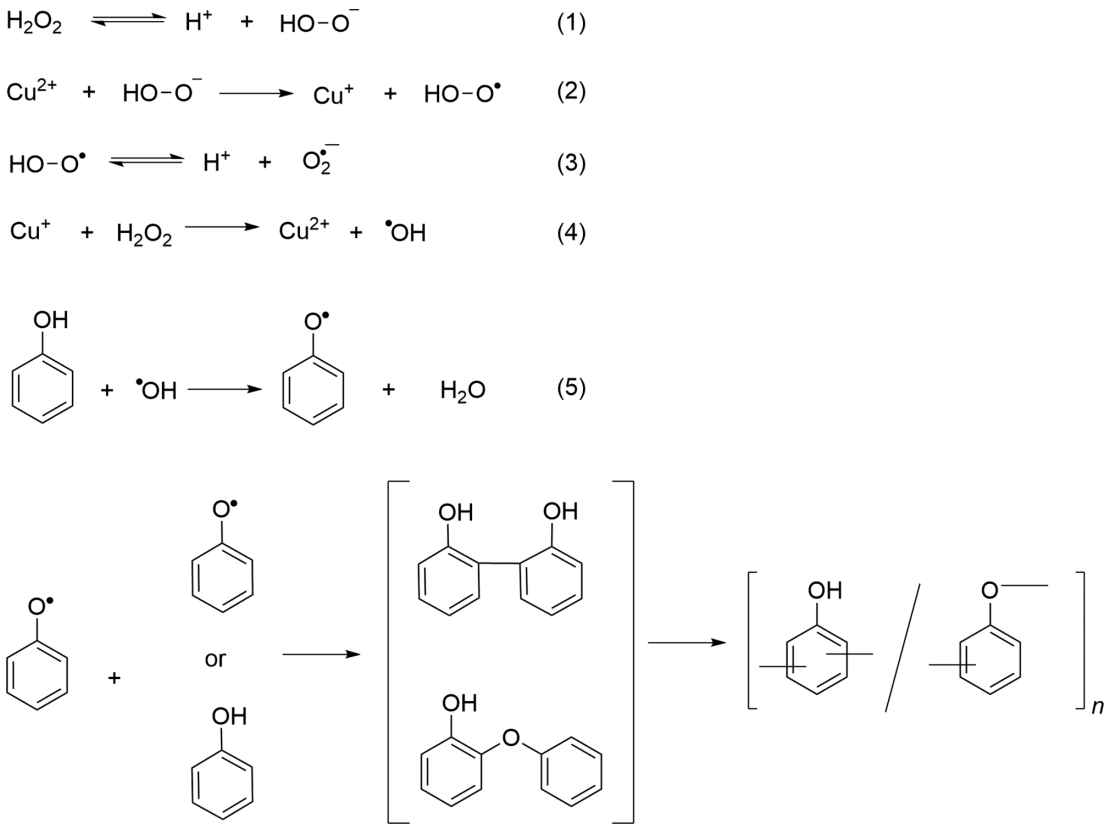

FIGURE 6 The polymerization mechanism of phenol.

this polymer occurred at 430°C, and no carbonaceous residue was also detected at 1000°C under nitrogen atmosphere (Figure S11).

Overall results showed that green tea-Cu^{2+}, caffeine-Cu^{2+}, and catechine-Cu^{2+} nanoflowers exhibited very high catalytic activity toward the polymerization of phenol derivatives. Among these nanoflowers, caffeine-Cu^{2+} nanobiocatalyst with only 2.5 wt% of catalyst loading provided the highest polyphenol and polyguaiacol yields. Green tea-Cu^{2+} nanoflowers mainly showed an average catalytic activity when compared with caffeine-Cu^{2+} and catechine-Cu^{2+} nanoflowers. One of the interesting results is that, while catechine-Cu^{2+} nanobiocatalyst showed the lowest catalytic activity toward the polymerization of phenol and guaiacol, the highest polymerization yield for salicylic acid was achieved using catechine-Cu^{2+} nanoflowers among these three nanobiocatalysts.

As previously mentioned, one of the aims of this study was to investigate the effects of electron releasing methoxy (—OMe) and electron withdrawing carboxylic acid (—COOH) groups on the polymerization of phenol and derivatives. When combined the results from the performed polymerizations, it can be concluded that phenol is far more reactive toward the polymerization compared with guaiacol and salicylic acid. Electron releasing and withdrawing groups on the phenol does not positively affect polymerization yield. This could be a reason of steric hindrance since both groups attached on ortho position of the phenol.

The proposed mechanism for the polymerization of phenol by obtained nanoflowers in the presence of H_2O_2 was illustrated in Figure 6. According to the proposed mechanism, Cu^{2+} ions in the presence of H_2O_2 first generate Cu^{+} ions. Subsequent reaction between Cu(I) ion and H_2O_2 results in the formation of hydroxyl radicals (HO•) via Fenton-like reaction.[27] Hydroxyl radical then reacts with phenol, and phenoxy radicals are formed. Formation of phenoxy radical propagates the polymerization through C—C (ortho-ortho, ortho-para or para-para) and C—O (oxy-ortho or oxy-para) couplings with phenol monomers.

4 | CONCLUSION

In conclusion, the effects of hybrid green tea-Cu^{2+} nanoflowers on the polymerization of phenol and derivatives (guaiacol and salicylic acid) were investigated. The obtained successful outcomes showed that green tea-Cu^{2+} hybrid nanobiocatalyst showed peroxidase-mimicking catalytic activity through Fenton-like reaction mechanism, and polymerizations of phenol and derivatives were successfully achieved with quite high yields. Obtained polymers showed very high molecular weights and thermal stabilities. One of the interesting result is that polymerization of phenol using green tea-Cu^{2+} hybrid nanoflowers was achieved even at 60°C in which peroxidase enzymes lose their catalytic activities at this temperature. Polymerization of phenol at 60°C was accomplished with very high yields without decomposition of green tea-Cu^{2+} nanobiocatalyst. Utilization of hybrid nanoflowers provides a great advantage for especially polymerizations requiring high reaction temperatures like 60°C. In order to understand the origin of the catalytic activity of the green tea-Cu^{2+} nanoflowers, similar biocatalysts were also synthesized from caffeine and catechin alkaloids which are the main components of green tea. Caffeine-Cu^{2+} and catechine-Cu^{2+} nanobiocatalysts also exhibited quite high catalytic activity toward polymerization of phenol and derivatives. We suggest that green tea-Cu^{2+}, and similar kinds of nanobiocatalysts may expand their utilization in polymer chemistry as promising catalytic agents for radicalic polymerizations, and can be considered to be used as an alternative catalysts to enzymes.

ACKNOWLEDGMENT

This work was supported by Hatay Mustafa Kemal University Coordinatorship of Scientific Research Projects (project # 20.M.048).

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Ersen Gokturk https://orcid.org/0000-0001-6742-2847

REFERENCES

1. Ahmad R, Sardar M. Enzyme immobilization: an overview on nanoparticles as ImmobilizationMatrix. *Biochem Anal Biochem*. 2015;2015:1-8.
2. Ansari SA, Husain Q. Potential applications of enzymes immobilized on/in nano materials: a review. *Biotechnol Adv*. 2012;30(3):512-523.
3. Altinkaynak C, Tavlasoglu S, Ocsoy I. A new generation approach in enzyme immobilization: organic-inorganic hybrid nanoflowers with enhanced catalytic activity and stability. *Enzyme Microb Technol*. 2016;93:105-112.
4. Ocsoy I, Dogru E, Usta S. A new generation of flowerlike horseradish peroxides as a nanobiocatalyst for superior enzymatic activity. *Enzyme Microb Technol*. 2015;75:25-29.
5. Hollmann F, Arends IWCE. Enzyme initiated radical polymerizations. *Polymers*. 2012;4(1):759-793.
6. Dordick JS, Marletta MA, Klibanov AM. Polymerization of phenols catalyzed by peroxidase in nonaqueous media. *Biotechnol Bioeng*. 1987;30(1):31-36.
7. Isci I, Gokturk E, Turac E, Sahmetlioglu E. Chemoenzymatic polymerization of hydrazone functionalized phenol. *Polym Sci Ser B*. 2016;58(4):411-420.
8. Topal Y, Tapan S, Gokturk E, Sahmetlioglu E. Horseradish peroxidase-catalyzed polymerization of ortho-imino-phenol: synthesis, characterization, thermal stability and electrochemical properties. *J Saudi Chem Soc*. 2017;21(6):731-740.
9. Uyama H, Kobayashi S. Enzyme-catalyzed polymerization to functional polymers. *J Mol Catal B Enzym*. 2002;19-20:117-127.
10. Nabid MR, Entezami AA. Enzymatic synthesis and characterization of a water-soluble, conducting poly(o-toluidine). *Eur Polym J*. 2003;39(6):1169-1175.
11. Yildirim P, Gokturk E, Turac E, Demir HO, Sahmetlioglu E. Chemoenzymatic polycondensation of para-benzylamino phenol. *Chem Pap*. 2016;70(5):610-619.
12. Ge J, Lei J, Zare RN. Protein–inorganic hybrid nanoflowers. *Nat Nanotechnol*. 2012;7(7):428-432.
13. Dadi S, Celik C, Ocsoy I. Gallic acid nanoflower immobilized membrane with peroxidase-like activity for m-cresol detection. *Sci Rep*. 2020;10(1):16765.
14. Dadi S, Temur N, Gul OT, Yilmaz V, Ocsoy I. In situ synthesis of horseradish peroxidase nanoflower@carbon nanotube hybrid Nanobiocatalysts with greatly enhanced catalytic activity. *Langmuir*. 2023;39(13):4819-4828.
15. Vo TN, Tran TD, Nguyen HK, Kong DY, Kim MI, Kim IT. In situ growth of hybrid nanoflowers on activated carbon fibers as electrodes for mediatorless enzymatic biofuel cells. *Mater Lett*. 2020;281:128662.
16. Zhong L, Jiao X, Hu H, et al. Activated magnetic lipase-inorganic hybrid nanoflowers: a highly active and recyclable nanobiocatalyst for biodiesel production. *Renew Energy*. 2021;171:825-832.
17. Celik C, Ildiz N, Ocsoy I. Building block and rapid synthesis of catecholamines-inorganic nanoflowers with their peroxidase-mimicking and antimicrobial activities. *Sci Rep*. 2020;10(1):2903.
18. Yilmaz SG, Demirbas A, Karaagac Z, et al. Synthesis of taurine-$Cu_3(PO_4)_2$ hybrid nanoflower and their peroxidase-mimic and antimicrobial properties. *J Biotechnol*. 2022;343:96-101.
19. Koca FD, Demirezen Yilmaz D, Ertas Onmaz N, Ocsoy I. Peroxidase-like activity and antimicrobial properties of curcumin-inorganic hybrid nanostructure. *Saudi J Biol Sci*. 2020;27(10):2574-2579.

20. Wu Z-F, Wang Z, Zhang Y, et al. Amino acids-incorporated nanoflowers with an intrinsic peroxidase-like activity. *Sci Rep*. 2016;6(1):22412.
21. Baldemir A, Köse NB, Ildız N, et al. Synthesis and characterization of green tea (Camellia sinensis (L.) Kuntze) extract and its major components-based nanoflowers: a new strategy to enhance antimicrobial activity. *RSC Adv*. 2017;7(70):44303-44308.
22. Gokturk E, Ocsoy I, Turac E, Sahmetlioglu E. Horseradish peroxidase-based hybrid nanoflowers with enhanced catalytical activities for polymerization reactions of phenol derivatives. *Polym Adv Technol*. 2020;31(10):2371-2377.
23. Kumbul A, Gokturk E, Turac E, Sahmetlioglu E. Enzymatic oxidative polymerization of Para-imine functionalized phenol catalyzed by horseradish peroxidase. *Polym Adv Technol*. 2015;26(9):1123-1129.
24. Kocak A, Kumbul A, Gokturk E, Sahmetlioglu E. Synthesis and characterization of imine-functionalized polyphenol via enzymatic oxidative polycondensation of a bisphenol derivative. *Polym Bull*. 2016;73(1):163-177.
25. Kumbul A, Gokturk E, Sahmetlioglu E. Synthesis, characterization, thermal stability and electrochemical properties of ortho-imine-functionalized oligophenol via enzymatic oxidative polycondensation. *J Polym Res*. 2016;23(3):52.
26. Gokturk E. Flowerlike hybrid horseradish peroxidase nanobiocatalyst for the polymerization of guaiacol. *Turk J Chem*. 2020;44(5):1285-1292.
27. Pham AN, Xing G, Miller CJ, Waite TD. Fenton-like copper redox chemistry revisited: hydrogen peroxide and superoxide mediation of copper-catalyzed oxidant production. *J Catal*. 2013;301:54-64.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kalayci B, Kaplan N, Dadi S, Ocsoy I, Gokturk E. Production of flower-shaped nanobiocatalysts from green tea and investigation of their peroxidase mimicking activity on the polymerization of phenol derivatives. *Polym Adv Technol*. 2024;35(1):e6272. doi:10.1002/pat.6272