

PILOT PREPARATION STUDY OF GOLD NANOPARTICLE-CHITOSAN COMPOSITE CATALYST

Monika MUCHOVÁ*, Alžběta DŮBRAVOVÁ, Lenka LOVECKÁ, Ivo KUŘITKA, Jan VÍCHA,
Lukáš MÜNSTER

Centre of Polymer Systems, Tomas Bata University in Zlín, Zlín, Czech Republic, EU, m_muchova@utb.cz

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Abstract

The wide range of applications of AuNPs is related to their different shapes and sizes, which are dependent on the synthesis process and reaction parameters. Therefore, the optimization of a novel and highly efficient approach to chitosan substrate decoration by AuNPs via covalent bonding using dialdehyde cellulose (DAC) is presented here. The covalent binding of AuDAC to chitosan nanofibers is mediated by a Schiff base reaction. This improves particle binding compared to conventional methods such as simple dip coating where AuNPs bind to the substrate only via weak interaction. In this work, initial attempts for the preparation of a composite material based on mixing the AuDAC solution with a chitosan solution were conducted. However, this process was found to be inefficient due to the inhomogeneous morphology of the resulting material, i.e., the formation of irregular chitosan micro-nanofibers and sheets sparsely decorated with AuDAC. Therefore, an optimized approach based on the binding of pre-made AuDAC nanoparticles to electrospun chitosan nanofibers was utilized. This method showed high binding efficiency and very dense decoration of nanofibers with AuDAC opposed to method involving mixing of chitosan and AuDAC solutions. Subsequently, the ability of the composite material in the form of a thin filtration layer to catalytically reduce 4-nitrophenol (4NP) to 4-aminophenol (4AP) was demonstrated. The efficacy of binding AuNPs to nanofibers was also compared to nanoparticles synthesized using another type of polysaccharide, specifically hyaluronic acid dialdehyde (DAH).

Keywords: Dialdehyde cellulose, gold nanoparticles, chitosan nanofibers, catalysis

1. INTRODUCTION

Gold nanoparticles (AuNPs) have expanded to many fields, including medicine [1], the food industry [2], and the chemical industry [3]. The wide range of applications of AuNPs is linked to their various shapes and sizes, which depend on the parameters of the synthesis process. The selection of a suitable preparation method and reaction conditions, such as pH, temperature, reaction time, and the concentration of reagents, determines the shape, surface morphology, and size of the resulting AuNPs [4, 5]. Recently, the need for green syntheses of AuNPs arose from the growing demand for gold nanoparticles in the biomedical sector and in environmental applications. For this reason, polysaccharides, biocompatible, biodegradable, non-toxic, and renewable substances, are one of the best candidates for green synthesis of AuNPs [6].

Polysaccharides allow AuNPs synthesis without the use of hazardous solvents and reducing/capping agents. They contain various functional groups such as aldehydes, hydroxyls, carboxyls, etc., which enable the reduction of gold salts and the stabilization of the resulting AuNPs. Furthermore, polysaccharide-reduced and stabilized AuNPs offer various benefits, primarily in the fields of biomedicine or theranostics [7–9]. However, there are challenges associated with use of polysaccharides for AuNPs preparation, including limited density of reducing ends and variable count of these ends depending on molecular weight. Therefore, the use of selectively oxidized polysaccharides has been proposed in previous studies to at least partially mitigate these limitations [10,11]. Some polysaccharides, i.e., chitosan or cellulose, can additionally serve as substrates for

preparation of composites with AuNPs due to their natural tendency to form fibrous structures [12]. One such example is chitosan, which is a linear polysaccharide exhibiting antimicrobial, antioxidative, analgesic, and hemostatic properties [11,13]. These properties can be potentially utilized in the field of biomedicine [13]. Additionally, chitosan is only soluble at low pH and has a stable nature which makes it a suitable AuNPs support [14].

This pilot study focuses on the synthesis of AuNPs using dialdehyde cellulose (DAC) and dialdehyde hyaluronate (DAH) and their incorporation on chitosan substrate. The first approach was based on a combination of pre-formed AuNPs with a diluted chitosan solution, followed by lyophilization of such mixture with the aim of achieving fibrous structures in which these AuNPs were incorporated. The second approach encompassed the preparation of composite material by combining pre-formed electrospun chitosan nanofibers and pre-formed AuNPs. The second optimized approach resulted in densely decorated chitosan nanofibers by AuNPs, i.e., CHIT-AuDAH and CHIT-AuDAC. CHIT-AuDAC was subsequently tested as a catalytically active thin filter for the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP).

2. EXPERIMENTAL

2.1 Materials

Cellulose SigmaCell type 20 (Sigma Aldrich Co.), sodium hyaluronate ($M_w = 1.5$ MDa, $DP = 3740$, $PDI = 4.3$; Contipro Ltd., Czech Republic), sodium periodate (NaIO_4 ; VWR, Czech Republic), ethylene glycol (Et-Gly; Penta, Czech Republic), gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$; Sigma Aldrich Co.), sodium hydroxide (NaOH ; Lachner, Czech Republic), hydrochloric acid (HCl ; Penta, Czech Republic), medium molecular weight chitosan (75–85% deacetylated, viscosity-average molecular weight $M_v = 190$ – 310 kDa, Sigma Aldrich Co.), polyethylene oxide (PEO; $M_w = 600$ kDa, Sigma Aldrich Co.), acetic acid (CH_3COOH ; Penta, Czech Republic), sodium triacetoxyborohydride (STAB; Sigma Aldrich Co.), sodium borohydride (NaBH_4) and 4-nitrophenol (4-NP) from Sigma Aldrich Co. All used compounds were of analytical purity (p.a.).

2.2 Preparation of DAC/DAH-reduced AuNPs

DAC and DAH were prepared in advance according to established procedures [13]. The DAC and DAH were then dissolved (0.6 wt.%) overnight in 9.4 mL of water at 40°C using an incubating orbital shaker (VWR, Czech Republic). Then, the pH of the resulting DAC/DAH solutions was then set to 7 by NaOH solution. Subsequently, the solutions were transferred into reaction vials and heated to 90°C for (15 min). Immediately after heating, $100\ \mu\text{L}$ of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ solution ($0.5\ \text{mg/mL}$) was added to the hot DAC and DAH solutions. Then $500\ \mu\text{L}$ of NaOH (0.1 M) solution was added to each reaction mixture. Finally, AuDAC and AuDAH solutions were immediately cooled in the freezer and dialyzed against pH 3.5 HCl solution using 50 kDa MWCO tubing.

2.3 Preparation of composite from solution

A 0.1 g of chitosan was dissolved in 10 mL of 0.1 M acetic acid and subsequently heated to 65°C for 24 h. Next, a given amount of AuDAC solution was added to this mixture so the final concentration of Au was $1.25\ \text{mg/mL}$. Then, STAB was added ($1.2 \times$ molar amount of the aldehyde groups of DAC) and left to react for several hours. The mixture was dialyzed using a 3.5 kDa MWCO membrane for 24 h. Finally, the sample was diluted $50 \times$ by H_2O , lyophilized and analysed.

2.4 Preparation of composite AuDAC/AuDAH-decorated chitosan nanofibers

Chitosan nanofibers were prepared by electrospinning using following procedure. Chitosan solution ($20\ \text{mg/mL}$) was prepared by dissolving chitosan in 70 % acetic acid. Then PEO was added in a 5:1 ratio of chitosan:PEO and mixed for 24 h. Next, the mixture was filtered, degassed, and electrospun using SpinLine 40 ($0.15\ \text{mL/min}$, spinning voltage $60\ \text{kV}$). Chitosan $2 \times 2\ \text{cm}$ specimens were cut from electrospun material

and washed. Subsequently, these specimens were added to prepared AuDAC and AuDAH solutions (pH 7) for 48 h. Next, the specimens were washed in UPW (1 h) and then placed in STAB solution 1.2 × molar amount of the aldehyde groups of DAC) for several hours. After reduction, they were washed again in UPW (1 h) and gently dried at laboratory temperature. CHIT-AuDAC sample was used for further catalytic experiments.

2.5 Catalysis

The catalytic activity of the prepared CHIT-AuDAC was investigated using catalytic reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of NaBH₄. Catalytic mixture composed of: 2 mL of NaBH₄ (7.5 mg/mL), 3.8 mL of H₂O, 0.2 mL of 4-NP (0.71 mg/mL). Catalysis was performed using AuCHIT composite, which was placed between two layers of PET film with a 1.1 cm diameter cut out to allow flow of the medium. The prepared catalytic mixture was then filtered through catalytically active filter (CHIT-AuDAC) and the collected filtrate was measured using double-beam UV-Vis spectrometer Lambda 1050 (PerkinElmer, USA). The 4-NP to 4-AP reaction was monitored by the decrease of characteristic absorption band of 4-NP (400 nm). The filtration of catalytic mixture was cycled until there was no observable peak of 4-NP at 400 nm.

2.6 Characterization methods

UV-Vis (ultraviolet-visible spectroscopy): A double-beam UV-Vis spectrometer Lambda 1050 (PerkinElmer, USA) was used to estimate the LSPR (local surface plasmon resonance) of AuNPs samples over the wavelength range of 250 to 800 nm.

SEM (Scanning electron microscopy): Nova NanoSEM 450 microscope (FEI, Czech Republic) operated at 5 kV accelerating voltage was used to acquire micrographs CHIT-AuDAC.

TEM (Transmission electron microscopy): Transmission electron microscope JEM-2100 (JEOL, Japan) operated at acceleration voltage of 160 kV was employed in imaging of CHIT-AuDAC samples.

DLS (Dynamic light scattering): The Zetasizer Nano ZS90 (Malvern Instruments, UK) was used to determine the hydrodynamic radius (r_h) of diluted AuDAC and AuDAH solutions. On a DTS1070 cell, the temperature was set to 25 °C using the Smoluchowski model.

3. RESULTS AND DISCUSSION

First, an analysis of chitosan-AuDAC composite prepared from AuDAC and chitosan lyophilized solution was performed. The results of the SEM analysis showed significant shape inhomogeneity of the prepared sample, i.e. presence of irregular nano-microfibers and large sheets, see **Figure 1** (bottom-left side). However, the TEM analysis confirmed the presence of AuDAC within chitosan domains. Therefore, we decided to optimize the preparation procedure and prepare the composite from pre-made chitosan nanofibers and pre-made AuNPs (AuDAC and AuDAH).

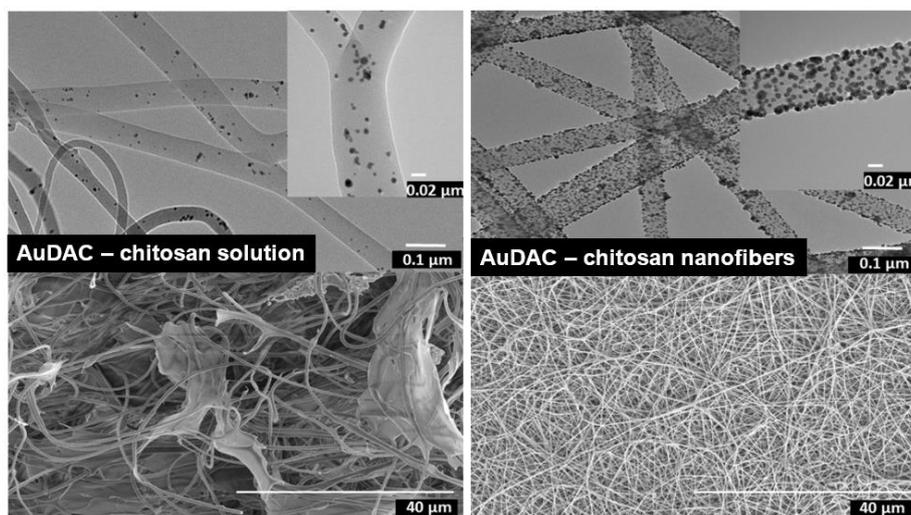


Figure 1 Representative TEM (top) and SEM (bottom) images of the combination of AuDAC with chitosan solution (left side) and AuDAC with chitosan nanofibers (right side).

The chitosan nanofibers were prepared as described in the method section. Subsequently, specimens of chitosan nanofibers were soaked in pre-made AuDAC and AuDAH solutions. These nanoparticles were attached to the substrate via a reaction of aldehyde-rich shell of AuDAC/AuDAH and amine groups of chitosan. The homogeneity and coverage of AuNPs were significantly better compared to the first approach, see SEM and TEM image **Figure 1** (right side). Subsequently, the binding efficacy between CHIT-AuDAC and CHIT-AuDAH was compared by UV-VIS spectral analysis of AuDAC or AuDAH solutions used before and after binding to the nanofibers, see **Figure 2**. The graphs in **Figure 2** show that more than 94 % of AuDAC and 75 % of AuDAH particles were bound to the chitosan substrate. Thus, the efficacy of binding to chitosan nanofibers is greater for the AuDAC sample than for the AuDAH. This is probably due to fewer $-CHO$ reactive groups present in DAH which are capable of conjugation with the chitosan substrate via the Schiff base reaction. The size of the prepared AuNPs was also analysed by DLS analysis, see inset table in **Figure 2**. The AuDAC and AuDAH sample solutions contained particles with a hydrodynamic radius of 65.9 ± 0.1 nm and 32.3 ± 0.6 nm, respectively. These values are higher than the diameter of AuDAC and AuDAH nanoparticles estimated by TEM analysis (AuDAC = 6.4 ± 2.1 nm, AuDAH = 7.3 ± 3.1 nm) because the values obtained in DLS analysis also account the ionic/polymeric corona around the particles.

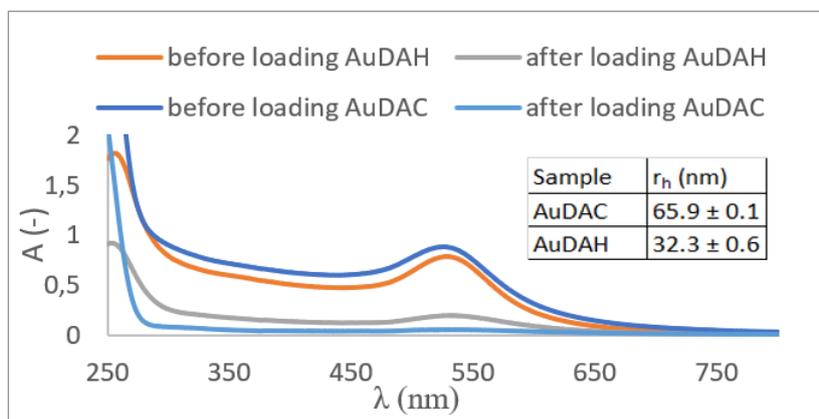


Figure 2 The UV-Vis spectra of the AuDAC and AuDAH before and after the loading and their DLS analysis.

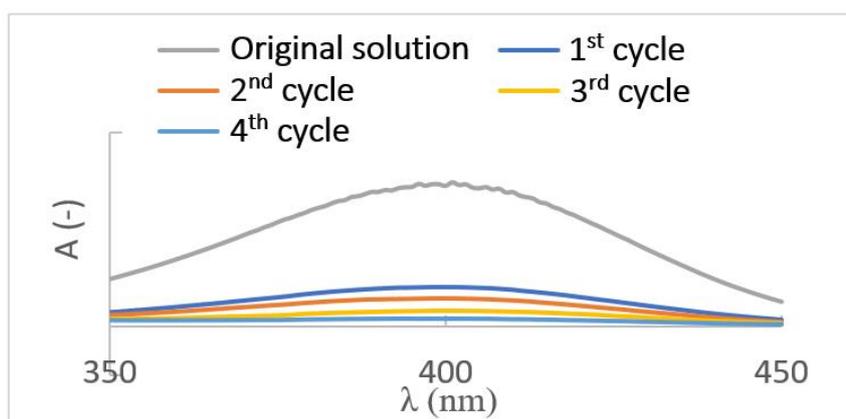


Figure 3 UV-Vis analysis of reduction of 4-NP to 4-AP showing absorption of original 4-NP solution and absorptions of solutions after each pass (cycle) through catalytic filter (left), and filter apparatus for catalysis (right).

The catalytic efficacy was evaluated for the CHIT-AuDAC sample. The catalysis was performed using a simple filtration setup as depicted in **Figure 3** (right side). The reduction of 4-NP to 4-AP was monitored by UV-VIS spectroscopy, see **Figure 3** (left side). UV-VIS analysis of the filtrates obtained after each cycle showed a

90 % reduction after 31 min and 17 s. Subsequently, the TOF (turnover frequency, which indicates the number of moles of reactant converted by a given amount of catalyst per minute) value was estimated to be 0.20 min^{-1} [15]. The rate constant (k) was 0.072 min^{-1} . It should be stressed, that the overall catalytic efficacy was limited due to the slow flow rate during filtration. Therefore, further optimization of the catalytic setup is needed to adjust the flow rate as well as increase the catalytic performance. Our recent study focuses on this topic [16].

4. CONCLUSION

This pilot study showed the possibility of preparation of AuNPs composite with chitosan. The initial experiment of simple mixing of chitosan solution with AuDAC resulted in limited decoration of chitosan substrate by AuNPs. Thus, the optimized preparation of the composite material was introduced to form chitosan specimens densely decorated with AuDAC and AuDAH. The binding of AuNPs to the chitosan nanofibers was found to be more effective for the CHIT- AuDAC sample, with up to 94 % binding efficacy compared to the CHIT-AuDAH sample, in which only 75 % of the nanoparticles were attached. The catalytic activity for the reduction of 4-NP to 4-AP was also confirmed for the catalytically active filter from CHIT-AuDAC, with a conversion of 90 % after 31 min and 17 s.

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