Development of Nanofibers Containing Rhodamine B as Colorimetric Chemosensor for \( \text{Cu}^{2+} \) Determination

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Abstract

In this study, nanofibers were prepared from cellulose acetate (CA) fibers doped with rhodamine B (L1) by electrospinning. The obtained nanofibers were characterized using scanning electron microscopy (SEM). SEM results revealed that the prepared nanofibers had an average diameter of 92 ± 24 nanometers. The prepared nanofibers were used as a colorimetric sensor for \( \text{Cu}^{2+} \) ions. The color of the nanofibers changed from pink to purple upon the addition of \( \text{Cu}^{2+} \) ions, indicating the formation of a complex \( \text{CuL}1 \) in a suitable pH range. The detection limit for \( \text{Cu}^{2+} \) ions was 1.62 mg L\(^{-1}\), which is lower than the maximum allowable concentration for drinking water in Thailand.
Abstract

In this work, a visual colorimetric sensor based on cellulose acetate nanofibers incorporated with rhodamine B hydrazone derivative L1 was successfully prepared via electrospinning technology. Morphology of the nanofibrous sensor was characterized by SEM, which showed that the uniform nanofibers having diameter of 92 ± 24 nm and formed a non-woven mat. The prepared colorimetric nanofibers showed high sensitivity towards Cu²⁺ due to the color change from pale pink to intense pink which confirmed the formation of CuL₁ complex ion in L₁-CA nanofibers. Upon the optimal conditions of amount of L₁ at 25 mg, pH solution at 5.0 and response time of 20 min, the detection limit for Cu²⁺ with L₁-CA nanofibers was found to be 1.62 mg L⁻¹.

Keywords: nanofibers, cellulose acetate, copper(II)ion, rhodamine B, electrospinning

Introduction

Copper is the third-most abundant transition metal in the human body and also has been used as catalytic cofactors for various types of metalloproteins for example superoxide dismutase, cytochrome c oxidase and tyrosinase (O'Halloran & Culotta, 2000; Crichton et al., 2008; Nunes et al., 2015). Accumulated Cu²⁺ ions in the body in high amount through drinking water can be harmful and toxic to our biological system (Ahuja et al., 2015; Miotto et al., 2014; Strausak et al., 2001; Telianidis et al., 2013; Jensen et al., 1999). According to WHO, the limit of concentration of Cu²⁺ ions in drinking water is 2 mg/L (WHO., 2017). Because of these potential hazards, it is essential to control and measure the Cu²⁺ ions for protection of our environment and health.

Several analytical techniques such as atomic absorption spectrometry (AAS) (Pourreza & Hoveizavi, 2005), inductively coupled plasma atomic emission spectrometry (ICP-AES) (Liu et al., 2005), electrochemistry (Flores et al., 2017) and colorimetric methods have employed for determination of Cu²⁺. Among them, a colorimetric method shows simplicity, convenience, low-cost and easy way to detect Cu²⁺ by naked-eye without the use of any sophisticated instrument (Piriya et al., 2017; McDonagh et al., 2008).

As already known, rhodamine derivatives are non-fluorescent and colorless, whereas ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color. Due to their outstanding optical characteristics, rhodamine derivatives have been often employed and exploited as fluorescent and colorimetric chemosensors for metal ions and anions (Kim et al., 2008; Chen et al., 2012). It should be noted that all of these synthetic sensing molecules are insoluble in water due to their hydrophobic in nature, however in real samples most of analysis target samples dissolves in water. Therefore, it would be a challenge to fabricate the sensing materials by incorporation of rhodamine derivatives into suitable solid support materials and apply as naked-eye sensor for selective sensing of interested target analytes.
Various types of sensing materials from rhodamine and its derivatives have been fabricated. For example, Felpin and co-workers have modified cellulose paper by anchoring rhodamine B derivative and applied this device for detection of hydrogen sulfate anions (Felpin et al., 2016). Ozay and co-workers have developed rhodamine based hydrogel for sensing Fe$^{3+}$ (Ozay & Ozay, 2013). Xie and co-workers have immobilized rhodamine 6G on surface of SiO$_2$ nanoparticles which exhibited the high sensitivity and selectivity for Hg$^{2+}$ (Liu et al., 2013). Li and co-workers have modified graphene oxide sheets and dispersed into rhodamine B solution. It was found that this material showed the highly selective sensing for Fe$^{3+}$ (Zhang et al., 2018).

Nowadays, nanofibrous membrane became a more attractive attention due to their high surface area to volume ratio and high porosity material. Various methods for nanofiber production are reported for example drawing-processing, template-assisted synthesis, self-assembly, solvent casting and phase separation (Terra et al., 2017). However, one of the most cost-effective techniques is electrospinning. Not only provides non-wovens few nanometers fibrous membrane, but also electrospinning could produce the thin fibers in large scale production. In order to provide nanofibrous sensors, various types of support polymers for their immobilization have been employed. Remarkably, several colorimetric sensors from electrospun nanofibers with various types of synthetic polymers and biopolymers support have been reported (Chen et al., 2015; Wu & Lai, 2016; Saithongdee et al., 2014; Hu et al., 2017).

Tong and co-workers reported that the rhodamine B derivative L1 could be used as colorimetric sensor for Cu$^{2+}$ in 50% (v/v) water/CH$_3$CN at 10 mM Tris-HCl pH 7.0 (Xiang et al., 2006). It was found that addition of Cu$^{2+}$ to the solution of L1 resulted in the ring-opening of the spirolactam moiety giving CuL1 as complex adduct concomitantly to the color change from colorless to pink color. The change in chemical structure of Cu$^{2+}$ sensing by L1 was depicted in Figure 1. Therefore, this research work aimed to fabricate colorimetric naked-eye sensor for Cu$^{2+}$ in aqueous solution from cellulose acetate (CA) electrospun nanofiber using the rhodamine B hydrazone derivative L1 as a chemical sensor.

![Figure 1 Change in chemical structure of Cu$^{2+}$ by L1.](image)
Methods

Chemicals

Cellulose acetate (CA, Mn ∼ 30,000) was purchased from SIGMA-ALDRICH. N,N-Dimethylacetamide (DMAc,) and acetone (AR) were ordered from LABSCAN. All other chemicals are analytical grade and used without further purification.

Synthesis of the rhodamine dye L1

The rhodamine dye L1 was facilely synthesized from rhodamine B by a two-step reaction according to the method reported in the literature (Xiang et al., 2006).

Preparation of L1-CA colorimetric nanofibers

Before electrospinning, the CA polymer (20 %wt) solution and L1 (15 – 35 mg) was stirred together in a mixture solvent of acetone: DMAc (3:2, v/v) at room temperature for 15 h. Next, the polymer mixture was loaded in a 5 mL syringe which was placed onto a syringe pump (NE-1000, ProSense B.V.). The mixture was spun under an electrical field of 14 kV by a high voltage power supply (EQ-30P1-L(230V), Matsusada Precesion Inc). The distance between the needle and the collector was 14 cm and the flow rate used was 0.2 mL/h. Electrospinning in this work was performed in horizontal alignment having a grounded aluminum foil which serves as a collector.

Characterization methods

The morphology and diameter of nanofibers were determined by SEM (Model LEO 1450VP). The SEM image was recorded at the magnifications of 15,000. The average diameter and its standard deviation (n = 40) of the electrospun fibers were determined by image J soft-ware. (National Institute of Health, USA). To investigate the functional groups presented on the nonofibers, an attenuated total reflectance Fourier transform (ATR-FTIR) spectrometer (PerkinElmer-Frontier) was used to record the IR absorption in the range of wavenumber between 4000–400 cm⁻¹ and a resolution of 4 cm⁻¹.

Water contact angles were measured with a sessile drop method by a DCA 20 contact angle meter (Data Physics Co., Ltd., Germany). The angles between the water droplet (2 µL) and the surface were measured. The measurement used distilled water as the reference liquid and it was automatically dropped on the electrospun scaffolds. To contact angle was measured in triplicates from different positions and an average value was calculated by a statistical method.

For sensing studies of nanofibrous sensor, all of experiments have been performed in 5 replicates. Before color analysis for metal ion sensing, the nanofibers were air dried for 30 minutes to allow the formation of a stable color and complete drying. Thereafter, the image of nanofibers was measured using a CanoScan LiDE 25 scanner and analyzed the intensity by Image J program (National Institute of Health, USA).
Results and Discussion

Sensing abilities of L1 with metal ions

The sensing abilities of L1 towards metal ions were investigated by UV-vis spectrophotometry in CH₃CN/H₂O mixture (1:1, v/v). As already known, metal ions could induce ring opening of the spirolactam moiety in rhodamine structure resulted in the color change from colorless to deep pink or purple color. Therefore, after addition of various metal ions to the solution of L1 (10 µM), only Cu²⁺ changed the color of L1 solution from colorless to intense pink color whereas other metal ions such as Cr³⁺, Fe³⁺, Ni²⁺, Zn²⁺ and Hg²⁺ showed a negligible change in color, as seen in Figure 2(a). These results are agreed with the UV-Vis spectrum of L1 in the presence of various metal ions as shown in Figure 2(b). The addition of Cu²⁺ to the solution of L1 obviously causes the appearance of a new peak at 558 nm assigned to the internal charge transfer process. Therefore, L1 could not sense Cu²⁺ selectively in a mixture of organic/aqueous medium.

Figure 2 (a) The photograph and (b) the UV-Vis spectra of L1 (10) µM in the presence of various metal ions (10 eq.) in 50%(v/v) H₂O/CH₃CN.
Characterization of the L1-CA nanofibers

A typical SEM image of CA and L1-CA nanofibers are shown in Figure 3. The average diameter (D) of nanofibers was estimated by the equation (1):

\[ D = \frac{1}{n} \sum_{i=1}^{n} X_i \frac{B}{L} \]  

where \( n \) stands for the number of the nanofibers in SEM images, \( X \) stands for the diameter of each nanofiber, \( B \) is the scale bar, and \( L \) refers to the length of the scale bar (Wang et al., 2011). In Figure 3, the morphology of both nanofibers showed satisfactory fiber mats observed by smooth and straight fibers. The average diameter of electrospun CA nanofibers and L1-CA nanofibers were 87 ± 15 nm and 92 ± 24 nm (mean ± S.D., \( n = 40 \)), respectively.

\[ \text{Diameter} = 87 \pm 15 \text{ nm} \]

\[ \text{Diameter} = 92 \pm 24 \text{ nm} \]

Figure 3 SEM images and diameter distribution diagram of (a) CA nanofibers and (b) L1-CA (L1 = 25 mg) nanofibers.
ATR-FTIR spectra of L1, CA, and L1-CA nanofibers are presented in Figure 4. CA showed the vibrational peak at 1743 cm$^{-1}$ and 1426 cm$^{-1}$ corresponded to C=O stretching of acetyl group and the asymmetric vibration of CH$_3$ group in CA Figure 4(a). The L1 powder showed the several characteristic medium or strong peaks at 2932, 1720 and 1613 cm$^{-1}$ vibration referred to CH$_3$ or CH$_2$, C=O and C=N vibrations, Figure 4(b) (Wang et al., 2017). The ATR-FTIR spectrum of the electrospun L1-CA membrane (Figure 4(c)) exhibited very similar characteristic absorption to those of CA nanofibers. This indicated that in the presence of L1 the electrospinning process did not alter the structure of CA in L1-CA membrane. It should be noted that the characteristic absorption bands of L1 were not observed probably because it was loaded into the membrane at a low ratio of 1.25 %w/w of CA. However, the membrane showed a pale-pink color of L1 which was significantly different from the free CA membrane.

Figure 4  ATR-FTIR spectra of (a) CA nanofibers, (b) L1 powder and (c) L1-CA nanofibers.

The elemental composition on the surface of modified fibers was further investigated by energy dispersive X-ray spectroscopy (EDX). It was found that increasing amount of nitrogen in the spectrum of L1-CA nanofibers compared to CA nanofibers could confirm the participation of L1 in L1-CA nanofibers (data not shown).
Optimization of Cu$^{2+}$ sensing by L1-CA nanofibers

The optimization of conditions for sensing Cu$^{2+}$ by L1-CA nanofibers are performed by the study of amount of L1, optimum pH and response time. The composition of the nanofibers with respect to L1 was optimized by preparing the samples having different amounts of L1 from 15, 20, 25, 30 and 35 mg in 20 wt% CA solution. After immersion of fabricated L1-CA nanofibers to Cu$^{2+}$ solution, it was found that by increasing amount of L1 the intensity of pink color on nanofibers increased up to 25 mg, and then the color intensity was insignificantly decreased as shown in Figure 5(a). It could be rationalized that the decreasing of intensity might be related to the large amount of hydrophobic L1 molecule in CA nanofibers resulting in minimization of Cu$^{2+}$ exchange from solution. Therefore, the amount of the L1 in the nanofibers was fixed to 25 mg for further studies. Changing in color of L1-CA nanofibers from colorless to pink confirmed that Cu$^{2+}$ could induce the formation of the ring opening of the spirolactam moiety in L1 incorporated in L1-CA nanofibers. SEM image showed that the mean diameter of L1-CA nanofibers after immersion to Cu$^{2+}$ solution was found to be 96 ± 10 nm, Figure 5(b). The presence of CuL1 complex was confirmed by EDX spectra (Figure 5(c)).
Figure 5  (a) The optimum of L1 amount in L1-CA nanofibers,  (b) SEM image and diameter distribution diagram of L1-CA nanofibers (L1 = 25 mg) after immersed into Cu^{2+} 200 ppm solution and (c) EDX spectra for L1-CA nanofibers after immersed into Cu^{2+} 200 ppm solution.
To investigate the surface properties of nanofibers, water contact angles were measured and shown in Figure 6. The bare CA nanofibrous membrane showed an angle around 121° (Figure 6(a)) indicating that CA nanofibrous scaffolds were hydrophobic. The water contact angle of L1-CA nanofibers increased to 124° (Figure 6(b)) indicating that the hydrophobic property of L1-CA nanofibers are increased due to the presence of hydrophobic L1 molecule. Interestingly, after the immersion of L1-CA nanofiber to Cu²⁺ solution the contact angles decreased to 12.7° (Figure 6(c)), implying that the nanofibers transformed to be hydrophilic. This observation could reveal the formation of CuL1 complex fromed in nanofibers that led to increasing of the membrane hydrophilicity.

![Contact angle images of (a) 20% wt CA nanofibers, (b) L1-CA nanofibers and (c) L1-CA nanofibers (L1 = 25 mg) after immersion to Cu²⁺ solution.](image)

In order to select the optimum pH value for Cu²⁺ naked-eye sensing, the influence of the pH of the medium was studied over the pH range of 3.0 - 5.0. Figure 7 shows the influence of pH on the intensity of the L1-CA nanofibers after immersion to Cu²⁺ solution. It was found that the intensity of pink color increased when pH increased from 3 to 5 and then it became decrease. The decreasing in the intensity at higher pH values can be attributed to the formation of stable copper hydroxide in the solution. Therefore, the pH of the solutions at 5 was chosen in the rest of experiments.
Figure 7 Effect of pH for detection Cu$^{2+}$ (200 ppm) by L1-CA nanofibers with corresponding color changed in nanofiber image.

Response time for the colorimetric detection of Cu$^{2+}$ by L1-CA nanofibers was studies by immersion of L1-CA nanofibers in Cu$^{2+}$ solution at 200 ppm with various times. It can be seen in Figure 8, that at 20 minutes the highest intensity was observed. However, after 20 minutes the intensity was decreased probably due to the leaching of hydrophilic CuL1 species from nanofibers. These results are agreed well with the results from contact angle experiments.

Figure 8 Response time for the colorimetric detection of Cu$^{2+}$ by L1-CA nanofibers.
The selectivity of the L1-CA nanofiber as metal ion sensor was evaluated with various metal ions such as Mn$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ at concentration of 200 ppm and pH 5. Figure 9 shows that only Cu$^{2+}$ could change the color of L1-CA from colorless to pink. Visually, no significant color changes were promoted by other studied metal ions. Therefore, L1-CA nanofibers could be an excellent naked-eye sensor for the detection Cu$^{2+}$ in water at pH 5.

**Figure 9** Selectivity of L1-CA nanofibers in the presence of various metal ions (200 ppm) at pH 5.

L1-CA nanofibers were tested for colorimetric response with Cu$^{2+}$ in different concentrations. The nanofibers were immersed into various concentrations of Cu$^{2+}$ at pH 5 for 20 minutes and kept for observation at room temperature for 30 minutes. Figure 10(a), the color of L1-CA nanofibers were changed from colorless to pink with increasing amount of Cu$^{2+}$ from 1 – 300 ppm. Notably, the intensity of pink color on L1-CA nanofibers were found to increase as a good linear relationship with the concentration of Cu$^{2+}$ between 1 – 10 ppm, $R^2 = 0.9958$. The result suggested that L1-CA nanofibers was potentially applicable for quantitative analysis of Cu$^{2+}$ ion in water.
Figure 10  (a) The color changes of L1-CA nanofibers when increased concentration of Cu$^{2+}$ from 1 – 300 ppm at pH 5, (b) the responses curve between intensity and different concentration of Cu$^{2+}$ from 1 - 300 ppm at pH 5 and (c) the linear dependence concentration between intensity and different concentration of Cu$^{2+}$ from 1 - 10 ppm at pH 5.

The limit of detection (LOD) determines the sensitivity of the method. LOD is the lowest concentration of analyte in a sample which can be detected. LOD was calculated by equation (2):

$$LOD = \frac{3\sigma}{s}$$

where $\sigma$ is the standard deviation and $s$ is the slope of calibration (Busaranon et al., 2006). The standard deviation was calculated from the slope of the calibration curve. The limit of detection (LOD) of the proposed method was found to be 1.62 mg L$^{-1}$. The method was found to have a detection limit lower than the WHO guideline limit (2 mg L$^{-1}$) for Cu$^{2+}$ in drinking water.
The prepared nanofibrous sensing materials were applied to determine Cu$^{2+}$ in synthetic waste water from our laboratory and the results are shown in Table 1. As can be seen from the table, L1-CA nanofibers could be applied to detect Cu$^{2+}$ in water samples.

**Table 1** Analysis of Cu$^{2+}$ in waste water from our laboratory.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration added (ppm)</th>
<th>Concentration from AA (ppm)</th>
<th>This work (ppm)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample -1</td>
<td>3.0</td>
<td>2.68 ± 0.01</td>
<td>3.12 ± 0.40</td>
<td>104.39</td>
</tr>
<tr>
<td>Sample -2</td>
<td>5.0</td>
<td>5.04 ± 0.01</td>
<td>5.20 ± 0.55</td>
<td>107.93</td>
</tr>
<tr>
<td>Sample -3</td>
<td>7.0</td>
<td>6.93 ± 0.01</td>
<td>7.21 ± 1.26</td>
<td>96.09</td>
</tr>
</tbody>
</table>

Conclusions

The nanofibers containing cellulose acetate as the supporting natural polymer and rhodamine hydrazone derivative L1 as the coloring agent, were successfully fabricated by electrospinning and subsequently utilized for Cu$^{2+}$ sensing in aqueous samples by naked-eye detection. The color of the membrane changed from pale-pink to intense pink color as a result of the formation of CuL1 product. The effects of amount of L1, pH and response time were investigated. The nanofibrous sensor can detect Cu$^{2+}$ in water samples displayed good sensitivity, selectivity and the detection limit for Cu$^{2+}$ with L1-CA nanofibers was found to be 1.62 mg L$^{-1}$.

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References


