GREEN-SYNTHESIZED GOLD NANOPARTICLES SUPPORTED ON CELLULOSE NANOWHISKERS FOR EASY-TO-INTERPRET COLORIMETRIC DETECTION OF CADMIUM (II)

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In the present study, we report on a simple and practical optical sensor based on cellulose nanowhiskers functionalized with gold nanoparticles (CNW/AuNP), which was applied as a colorimetric sensor for detecting Cd (II). Our results revealed that a simple and fast synthesis was capable to produce small and well-controlled CNW/AuNP nanocomposites. These nanocomposites can bind to Cd (II), owing to the citrate anions present on the AuNP surface, and this process is followed by an optical change in the surface plasmon resonance (SPR) band of AuNPs. As a consequence, a linear decrease of absorbance was verified as the Cd (II) concentration increased in the range 500-2000 nM, yielding a detection limit of 60 nM. Our sensor also proved to be suitable for real samples analyses, even in the presence of other interfering substances, indicating its potential for Cd (II) colorimetric sensing applications.

Keywords: cellulose nanowhiskers, gold nanoparticles, cadmium, heavy metal monitoring, optical sensor, colorimetric sensor

INTRODUCTION

Cadmium (Cd (II)) is considered one of the most toxic metals, it is non-biodegradable and has a long half-life in the biosphere.[1] The sources of cadmium pollution arise from non-ferrous metal manufacturing, and effluents released from electroplating and fertilizers.[2] This metal can be extremely harmful to human health, causing, for instance, diabetes, lung cancers, hypertension, osteoporosis, among other diseases, and, therefore, its detection and monitoring are of great importance.[3-5] Although some methods have been employed for the detection of heavy metals, including electrochemical measurements, fluorescent chemosensors, surface plasmon resonance (SPR) and colorimetric sensors, easy-to-interpret and expedite analytical methods to detect Cadmium in real water samples are still

scarce and pose a great challenge to overcome, which was the main task of this work.[6-9]

Colorimetric sensors present remarkable advantages compared to other analytical techniques, including high sensitivity and selectivity, low detection limit and simplicity, allowing the observation of the color change with the naked eye.[10-11] Noble metal nanomaterials are of outmost importance in several fields of nanotechnology,[12-13] and among them, gold nanoparticles (AuNPs) have received a lot of attention due to their excellent characteristics, such as strong optical absorption, scattering and fluorescence quenching properties, combined with exciting chemical and biological properties and low or no toxicity.[13-17] AuNPs can be a potential material for cadmium colorimetric detection, due

to the capability to induce aggregation of AuNPs, which causes a strong overlap between the plasmon fields of the nearby nanoparticles, resulting in visual color changes.[18]

Several well-established methods have been employed for AuNP synthesis, but more recently, alternative green methodologies have gained importance,[16,19] once they avoid the use of toxic and harmful reactants and materials. Additionally, such syntheses allow the formation of smaller nanoparticles with a lower coalescence level.[20-22] Small AuNPs are highly needed for colorimetric cadmium detection, once the mechanism involves the redshift of SPR bands as a consequence of AuNP aggregation. In this context, the use of cellulose in green synthesis is advantageous due to its non-toxicity and biodegradability, as well as to the possibility to obtain it from diverse natural sources, including crop wastes.[23-24] Polysaccharides such as cellulose present in their chemical composition a large number of hydroxyl groups, a hemicetal reducing end and other functionalities that enable their application in green synthesis of metal nanoparticles.[21,25-27]

In this context, here we developed an optically active nanoplatform able to work as an easy-to-interpret colorimetric sensor for Cd (II). The nanoplatform was based on a hybrid system composed of cellulose nanowhiskers and gold nanoparticles (CNW/AuNP), which were prepared by *in situ* chemical reduction using a sodium citrate solution. The reaction strategy employed here guaranteed the dispersion of small AuNPs and allowed exploring the interesting properties of the nanoplatform for colorimetric detection of Cd (II) in water samples. Moreover, the use of colloidal suspension excludes additional steps required to produce gels or films, which enables the hybrid system to be directly employed as a colorimetric Cd (II) sensor after completion of a fast and simple green synthesis employing cellulose.

EXPERIMENTAL
Reagents
White cotton was obtained from Apolo – Brazil. Sulphuric acid, sodium citrate, cadmium (II) nitrate, mercury (II) acetate, lead (II) nitrate, copper (II) nitrate, aluminum nitrate (III), chromium oxide (III), silver sulfate, iron sulphate (II) and nickel (II) nitrate were all purchased from LabSynth Chemical (Brazil). Dialysis membrane (D9402) and gold (III) chloride hydrate were purchased from Sigma-Aldrich.

Synthesis of cotton cellulose nanowhiskers (CNW)
CNW were obtained *via* acid hydrolysis of natural fibers, following the methodology previously described.[21] In this experiment, 5.0 g of commercial white cotton was added to 100 mL of 60.0 wt% H_2SO_4 aqueous solution previously heated at 45 °C. The hydrolysis was performed under constant stirring during 75 minutes, and cold water (500 mL) was added to the reaction medium in order to stop the reaction, which was then washed by centrifugation at 10,000 rpm for 10 minutes. Dialysis against distilled water was performed in order to neutralize the CNW suspension, which was then ultrasonicated during 5 minutes using 20% of amplitude and freeze-dried in a Thermo Fisher Scientific, SuperModulyo 220.

CNW/AuNP synthesis
CNW/AuNP was produced by adding CNW in AuNP synthesis, following the Turkevich method,[28] in which gold (III) chloride hydrate was used as precursor and sodium citrate was used as reducing and stabilizing agent. The synthesis was performed in a round-bottom flask connected to a reflux system. This reaction medium was composed of 200 mL of gold (III) aqueous solution (3.7×10^{-4} mol L^{-1}) and 20 mL of aqueous CNW suspension (50 mg mL^{-1}). Once it reached the boiling point, 4 mL of a sodium citrate aqueous solution (2.4×10^{-3} mol L^{-1}) was added to the solution. The reaction was performed during 40 minutes and stored in the fridge in a glass flask, and protected from light.

CNW:Au characterization
The morphologies of CNW and CNW:AuNP were investigated by Field Emission Scanning Electron Microscopy (FESEM), using a PHILLIPS-XL30 FEG-SEM microscope. Diluted suspensions (0.5 mg mL^{-1}) of CNW and CNW:AuNP were stained with 100 µL of uranyl acetate (1.5 wt%). Next, 1.5 µL of each stained suspension was dripped on a hot silicon board, and left to dry in a desiccator at room temperature. The dimensions of CNW and AuNPs were estimated using Image J software, and 100 measurements were used for calculations.

The optical properties of the CNW/AuNP nanocomposite were evaluated by UV-Vis absorption spectroscopy, monitoring the typical AuNP band at 525 nm, using a Shimadzu UV-16000 spectrometer, software UV Probe 2.31. Samples were placed in a 1 cm optical path quartz cell and ultrapure water (Millipore system) was used as blank for the measurements. The mean sizes of AuNPs before and after the analyte addition were evaluated (in triplicate) by Dynamic Light Scattering (DLS), using a Malvern Zetasizer Nano ZS.

Cadmium detection essay
Experiments to detect Cd (II) were performed directly using the CNW/AuNP aqueous suspension. Cd

(II) solutions were prepared by varying the metal concentration in the range from 500 to 2000 nM. For this purpose, 1 mL of Cd (II) solution was added to 4 mL of CNW/AuNP solution. The incubation time was optimized, in the range 10-60 minutes, and the band at 525 nm was monitored.

In order to evaluate the sensor selectivity, heavy metal interferents (Ni^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Al^+, Cr^{3+}, Ag^+ and Fe^{2+}) were tested using solutions of the same concentration of Cd (II) solution, and the absorbance peak at 525 nm was monitored by UV-Vis spectroscopy. The sensor performance was also evaluated employing real samples (tap water and river water).

RESULTS AND DISCUSSION
CNW/AuNP characterization

FESEM micrographies of representative regions of CNW and CNW:AuNP samples are depicted in Figure 1 (a) and (b), respectively. CNW presents typical needle-like structures, indicating the efficiency of the acid hydrolysis procedure to extract CNW from cotton fibers.[29] The dimensions of 15 nm for diameter and 185 nm for length are typical of CNW.[21] Figure 1 (b) reveals the presence of small spherical structures related to AuNPs. Histograms of size distribution of AuNP diameters are depicted in Figure 1 (c), yielding an average diameter 15 ± 2 nm. The

synergism between AuNPs and CNW occurs due to electrostatic interactions between gold ions and the negatively charged groups (hydroxyl and sulphate) present on the CNW surface during the synthesis. The inclusion of CNW in Turkevich synthesis can lead to a lower coalescence level and consequently, the formation of smaller nanoparticles, once CNW also acts as a stabilizing agent.[21,30-31]

The chemical reduction of gold ions to metallic gold in CNW/AuNPs was confirmed by the presence of a well-defined absorption band at 525 nm, as displayed in Figure 2. This typical band is a consequence of the surface plasmon resonance (SPR) effect, which occurs due to a collective oscillation of conductive electrons from metallic gold nanostructures when interacting with electromagnetic radiation.[32]

Optical sensing of Cd (II) ions using CNW/AuNP nanocomposite

In order to evaluate the effect of Cd (II) on the CNW/AuNP nanocomposite, different concentrations of Cd (II) ions, varying from 500 to 2000 nM, were examined in order to determine the sensitivity of the colorimetric assay.
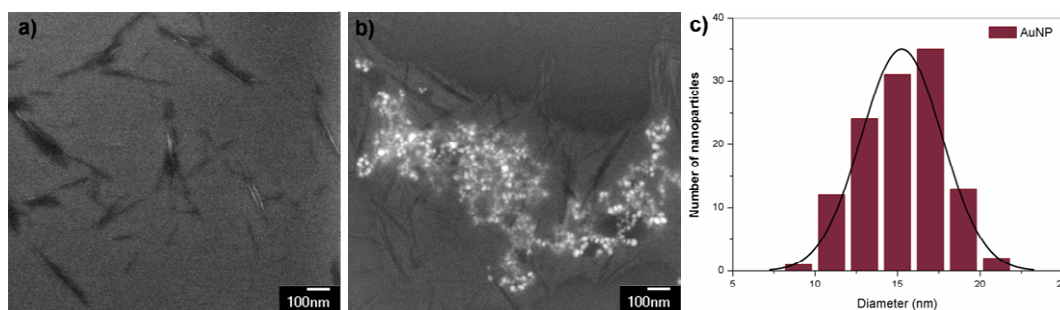

Figure 1: FESEM micrographs of CNW (a) and CNW/AuNPs (b), and histogram of AuNP size distribution (c)
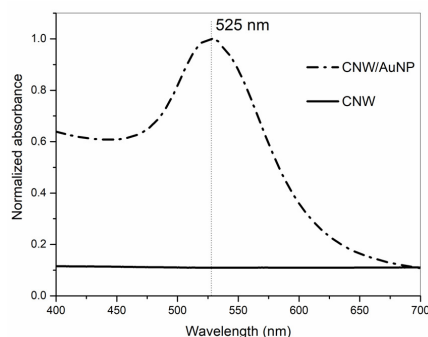

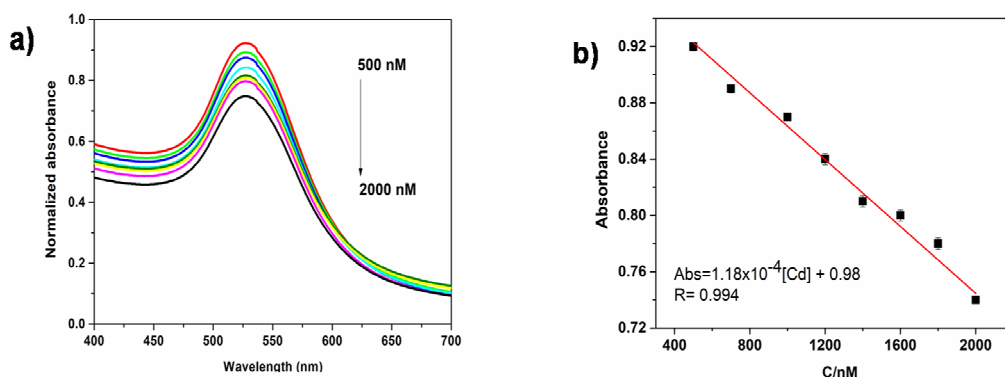Figure 2: UV-Vis absorption spectra of CNW and CNW/AuNP

Figure 3: Optical detection of Cd (II) using CNW/AuNP nanoplatform; (a) UV-Vis absorption spectra of CNW/AuNPs in the presence of varied concentrations of Cd (II), and (b) Linear response of the colorimetric assay as a function of Cd (II) concentrations

Table 1
Performance of CNW/AuNP nanocomposite in comparison with other Au NPs probes
for detection of Cd (II) ions

Sensor probe	Size (nm)	D.L. (M)	Detection method	References
Au NPs	10-30	0.07×10^{-3}	UV–visible	34
Au NPs	15	63×10^{-9}	UV–visible	35
Au NPs	19	4.6×10^{-9}	UV–visible	36
Au NPs	7.5	21×10^{-9}	UV–visible	1
Au NPs	15	60×10^{-9}	UV–visible	This work

The corresponding changes in absorbance at 525 nm, the typical band of AuNPs, were recorded using UV-Vis absorption spectroscopy (Fig. 3). In all the cases, a decrease in the absorbance at 525 nm was verified.

The calculation of the detection limit (D.L.) of the analyte was based on the standard deviation of the response and the slope of curve, according to D.L. = 3.3 σ/S,[33] in which σ corresponds to the standard deviation of the absorbance measurement (five replicates) at 525 nm, and S corresponds to the calibration curve slope (Fig. 3 (b)). Hence, the D.L. determined for Cd (II) using our colorimetric assay was 60 nM for the concentration range of 500-2000 nM. A comparison of sensor performance regarding our proposed Cd (II) sensor and previous results available in the literature is displayed in Table 1. Our results indicate that the easily synthesized CNW/AuNP nanocomposite sensor is suitable as a nanoplatform for optical detection of Cd (II).[1,33-36]

Sensor selectivity

The sensor selectivity for Cd (II) against common interferents was also evaluated. For this purpose, the detection of Cd (II) was evaluated in the presence of some heavy metals using solutions of the same Cd (II) concentration (1000 nM), and eventual changes in the absorbance peak at 525 nm were monitored using UV-Vis spectroscopy. The results displayed in Figure 4 indicate that the absorbance value at 525 nm for Cd (II) presents a change (increase of absorbance) of 18% when Nickel (Ni^{2+}) is present in the solution. On the other hand, other common heavy metals (Pb^{2+}, Hg^{2+}, Cu^{2+}, Al^+, Cr^{3+}, Ag^+ and Fe^{2+}) had a negligible effect on the absorbance peak at 525 nm, and consequently, did not induce remarkable solution color changes. The higher selectivity of the developed sensor towards Cd (II) relies on the fact that Cd (II) ions at neutral pH show the highest chelating ability with oxygenated functions available at citrate groups. As a consequence, the sensor becomes more selective

to Cd (II), whereas the other metals ions here investigated show low or negligible interference in the sensor response.

Analysis of Cd (II) in river and tap water

To test the usefulness of this method, the standard addition method was used for determination of Cd (II) in river and tap water samples.[37] River water was collected from Monjolinho River (located in São Carlos – São Paulo, Brazil) and filtered using a paper filter (J Prolab JP42). The experiments were carried out by adding different concentrations of Cd (II) ions, varying from 500 to 2000 nM, to river and tap water, and the recovery percentage was calculated as varying from 96.0% to 104.2% for river and tap water samples, respectively, as shown in Table 2. The results suggest that the developed assay is suitable for the accurate determination of Cd (II) in the analysis of real samples.
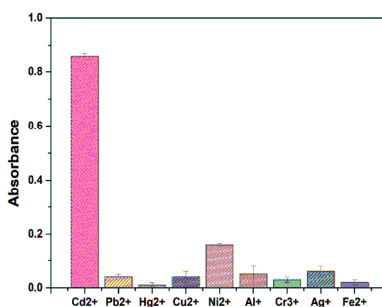

Figure 4: Selectivity of the colorimetric sensor for Cd^{2+} detection in the presence of common interferents

Table 2
Recovery for the determination of Cd (II) in tap water and river water samples

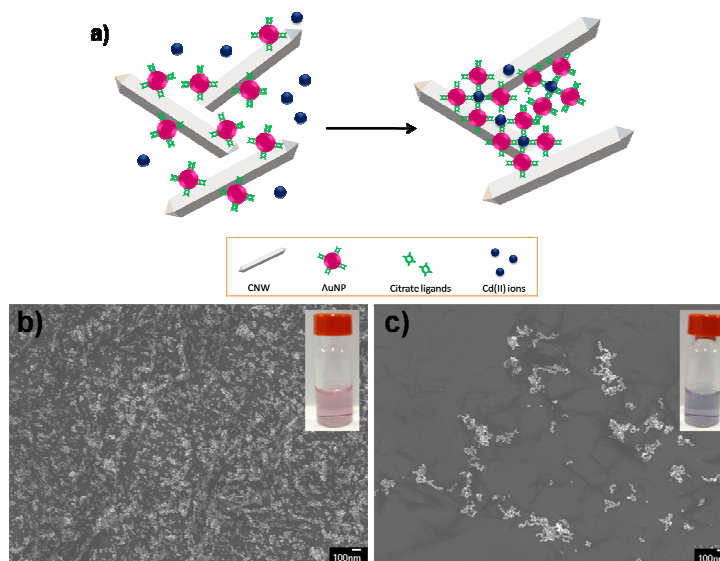| Sample Cd (II) 1600 nM	% Recovery	% RSD
River water	93	2.1
Tap water	90	1.3

Figure 5: (a) Schematic representation of AuNP aggregation induced by the presence of Cd (II); FESEM images of CNW/AuNP system (b) before and (c) after addition of 1 mM of Cd (II)

Mechanism of Cd (II) detection

The optical monitoring of Cd (II) by the CNW/AuNP nanocomposite occurs by changes in the SPR absorbance peak of AuNPs, as a consequence of metal induced particle aggregation.[38] Cd (II) ions coordinate with the citrate present on the AuNP surface, causing the formation of clusters of gold nanoparticles, which results in a shift of the plasmonic band to longer wavelengths, changing the color from light pink to light purple (Fig. 5 (a)). This change can be also confirmed by FESEM microscopy (Fig. 5 (b-c)). Figure 5 (b) displays a great amount of well-dispersed AuNPs in a CNW matrix, while Figure 5 (c) shows clusters of AuNPs formed after addition of 1 mM de Cd (II). Measurements of Dynamic Light Scattering (DLS) related to particle size distribution revealed that before contact with Cd (II), 50.7% of particles present hydrodynamic diameter at around 35 nm. On the other hand, after adding 1.0 mM of Cd (II) to the CNW/AuNP suspension, the analysis revealed the presence of 91.5% of clusters with hydrodynamic diameter of ~70 nm.

CONCLUSION

A hybrid nanoplatform composed of cellulose nanowhiskers and gold nanoparticles was developed and employed as a colorimetric sensor of Cd (II) in water samples. Such a platform was obtained by a simple, affordable and green synthesis route, which resulted in AuNPs with suitable optical properties for the colorimetric detection of cadmium. The mechanism of detection was based on the cluster formation tendency of AuNPs (in the presence of Cd (II)), which caused a redshift of the AuNP SPR band. The results showed that the developed sensor for detecting Cd (II) presented a low detection limit of 60 nM (concentration range of 500-2000 nM). Furthermore, the sensing platform showed good sensitivity and selectivity for detecting Cd (II) in real samples and in the presence of other interfering heavy metals. Thus, the developed sensor can be considered a potential approach for monitoring Cd (II) in aquatic environments with high sensitivity and selectivity. Futhermore, the method employed is simple and expedite, allowing the use of the Cd (II) colorimetric sensor immediately after completion of chemical synthesis.

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