

Rapid capture and visual detection of copper ions in aqueous solutions and biofluids using a novel cellulose-Schiff base

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Abstract In this work, a novel colorimetric sensor was prepared via a cellulose-polyethylenimine-based Schiff base (cellulose-Schiff base) for capturing and detecting copper ions (Cu^{2+}) in aquatic environments and life systems. The cellulose-Schiff base displayed continuous color changes from inherent pale yellow to deep green due to the formation of a cellulose-Schiff base- Cu^{2+} charge transfer complex within 30 s, indicating excellent “naked-eye” detection capability for Cu^{2+} ions in aqueous solution. The limit of detection (LOD) was 1.054 ppm, lower than the U.S. EPA guidelines for drinking water of 1.3 ppm. Moreover, the cellulose-Schiff base colorimetric

sensor was successfully used to capture and detect exogenous Cu^{2+} ions in biofluids (simulated urine and bovine serum). The proposed sensing mechanism of the cellulose-Schiff base for Cu^{2+} ions is that N atoms (C=N and N-H) in the sensor capture and concentrate Cu^{2+} ions to form sensor- Cu^{2+} complexes, then intramolecular charge transfer occurs as a consequence. In addition, test strips based on cellulose-Schiff base were developed, and provide a convenient, cheap, sensitive, and reliable monitoring platform for on-site semi-quantitative analysis of Cu^{2+} ions in real samples.

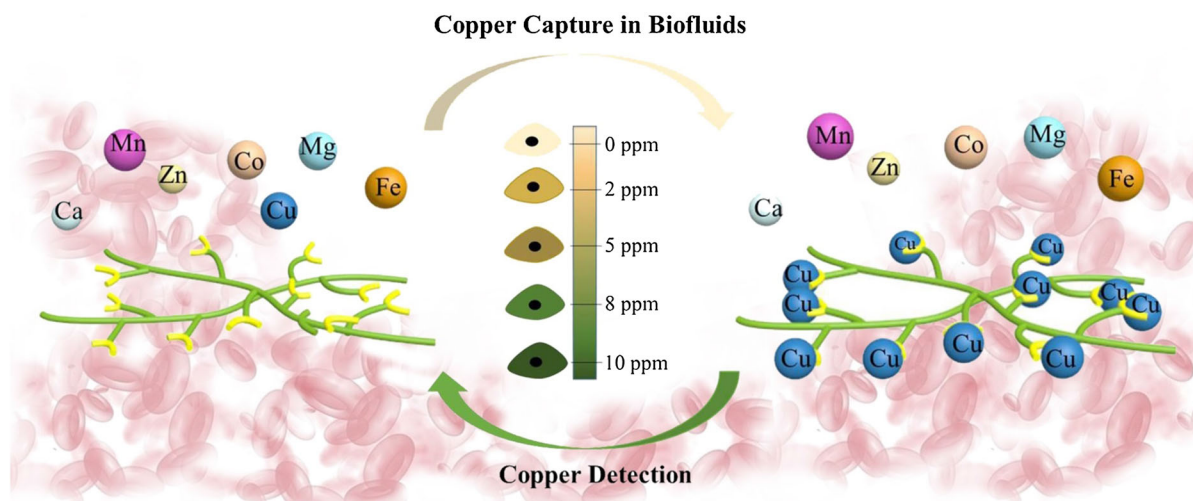
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Graphical abstract



Keywords Schiff base · Capture · Visual detection · Biofluids · Chelation · Copper ions

Introduction

Heavy metal ion pollution problems have had a terrible influence on human health and the environment. Therefore, the monitoring of heavy metal ions is of great practical significance. Copper ion (Cu^{2+}) is an essential trace metal element for human health and plays a cofactor role in various enzyme functions, redox processes and pigment synthesis in all living organisms (Quintanar et al. 2016; Bian et al. 2017; Giampietro et al. 2018). However, deregulation of copper homeostasis can cause liver and kidney damage, many types of cancers, several neurodegenerative diseases such as Parkinson's, Alzheimer's and Wilson's disease (Pall et al. 1987; Kaler 2011; Singh et al. 2013; Bandmann et al. 2015a, b; Barnham and Bush 2014). Cu^{2+} ion is produced and accumulates in the environment and the food chain with the rapid development of industry and agriculture. The daily allowance of copper intake as suggested by the National Research Council is 1.5–3.0 mg for adults, 1.5–2.5 mg for children, and 0.4–0.6 mg for infants (Jung et al. 2009; Udousoro et al. 2017). Additionally, the U.S. Environmental Protection Agency (EPA) ruled that the limit of Cu^{2+} ion is 1.3 ppm in drinking water (Richardson and Ternes 2011). Therefore, there

is an urgent need to develop a convenient, real-time, and sensitive sensor for Cu^{2+} detection in the environment and life systems.

So far, a variety of detection technologies for Cu^{2+} ions have been exploited, such as atomic absorption/emission spectroscopy (AAS/AES), inductively coupled plasma mass spectrometry (ICP-MS), surface plasmon resonance (SPR), and high-performance liquid chromatography (HPLC) (Lin and Huang 2001; Zhao et al. 2012; Zhang et al. 2014a, b; Loegel et al. 2017; Pereira et al. 2018). These methods have greatly enhanced the trace detection for Cu^{2+} ions in different samples. However, these traditional methods usually require expensive equipment, complex sample preparation, and professional operators. To address the above issues, various fluorometric and colorimetric chemosensors were studied due to their potential for naked-eye detection in organic media (Jin and Han 2014; Ndokoye et al. 2014; Liu et al. 2017; Fang et al. 2017; Joo et al. 2017; Miao et al. 2017; Zhao et al. 2017; Awual et al. 2014). For example, Joo et al. synthesized a chemosensor 1 by a combination of N-aminophthalimide and 8-hydroxyjulolidine-9-carboxaldehyde for colorimetric detection of Cu^{2+} ions in a buffer/DMF solution (3/2, v/v, 10 mM bis-tris, pH 7.0), with a detection limit of 0.14 μM (Joo et al. 2017). Jin and Han designed hexadecyl trimethylammonium bromide (CTAB) modified CdSe/ZnS quantum dots (QDs), presenting ultrasensitive and selective fluorimetric detection of Cu^{2+} ions in the presence of thiosulfate (Jin and Han 2014). These methods

developed highly sensitive sensors for copper detection. However, the ability to detect trace amounts of Cu^{2+} ions in aquatic environments and life systems remains a challenge.

Schiff bases derived from the condensation of aldehyde/ketone and amine can coordinate metal ions via azomethine nitrogen ($-\text{C}=\text{N}$), which are important materials in the fluorescent analysis of metal ions (Cheng et al. 2013; Horak et al. 2018; Awual et al. 2015). In our previous work, cellulose-Schiff bases were synthesized based on branched polyethylenimine (PEI) and displayed excellent selective adsorption and separation abilities for dyes (Zhu et al. 2016). Cellulose is an abundant, biocompatible, biodegradable, and renewable biomacromolecule that can be chemically modified to be various sensors (Kumari and Chauhan 2014; Schyrr et al. 2014; Nawaz et al. 2018). Compared with activated small molecule probes and nanoparticle probes, cellulose-based sensors are more stable in surrounding changes in solution. This inspired an assumption for simultaneous capture and detection of Cu^{2+} ions in aqueous solution and biofluid using cellulose-Schiff base as visual sensor.

Therefore, in this work, we designed an alternative technology that would capture Cu^{2+} ions efficiently and selectively, while simultaneously enabling copper detection and readout of corresponding levels directly from aqueous solution and biofluids (urine and bovine serum) in a colorimetric analysis. This strategy depends on the utilization of cellulose-polyethylenimine-based Schiff base with abundant amino receptors ($-\text{NH}$). These receptors exhibit a high affinity for Cu^{2+} capture and concentration and simultaneous detection and evaluation of copper levels by intramolecular charge transfer (ICT). Further, more convenient test strips have also been developed for semi-quantitative analysis of Cu^{2+} ions from aqueous solution as well as biofluids. This work presents a promising viability of cellulose-polyethylenimine-based Schiff base for convenient, real-time, highly selective and sensitive, probe-free visual detection of Cu^{2+} ions in the environment and life systems.

Experimental

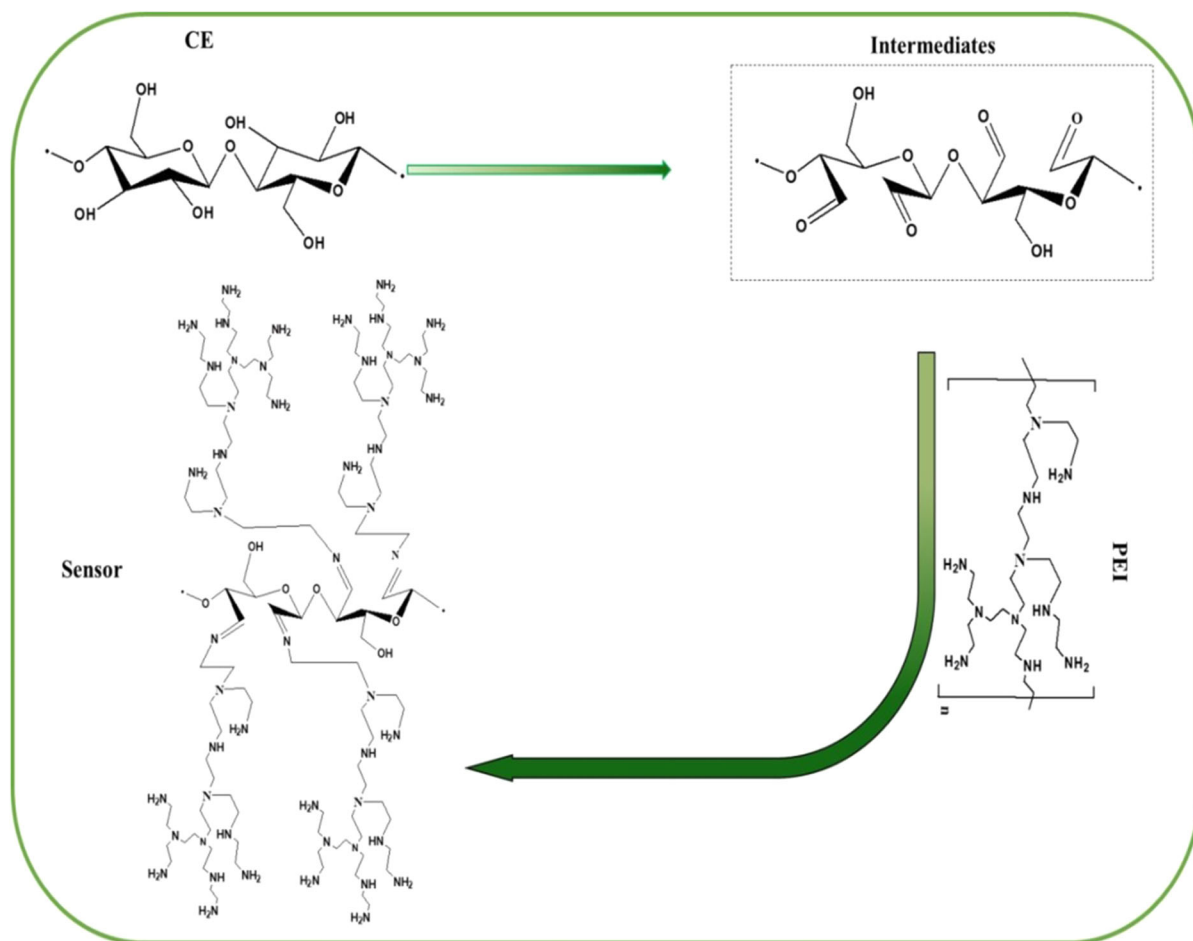
Reagents

Branched polyethylenimine (PEI, Mw = 600, 99%), cellulose (Mw = 20,000), NaOH, urea, NaIO_4 , NaCl, NH_4Cl , $\text{Cu}(\text{CH}_3\text{COO})_2$, $\text{Mn}(\text{CH}_3\text{COO})_2$, $\text{Co}(\text{CH}_3\text{COO})_2$, $\text{Ca}(\text{CH}_3\text{COO})_2$, $\text{Mg}(\text{CH}_3\text{COO})_2$, FeCl_3 , ZnCl_2 , hydrogen nitrate, creatinine, sodium orthophosphate disodium, potassium orthophosphate, anhydrous sodium sulfite, ethylene glycol, ethanol, bovine serum, and filter paper were all purchased from Shanghai Aladdin Chemical Reagent Co, Ltd., and all of the chemicals were used without further purification.

Synthesis and characterization of cellulose-Schiff base

Cellulose-polyethylenimine based Schiff base (named as cellulose-Schiff base) were prepared through sodium periodate-mediated oxidation and then PEI grafting. The synthetic process of cellulose-Schiff base is shown in Scheme 1. Briefly, 2 g of cellulose powders and 2 g of NaIO_4 were dissolved into 60 mL of 7 wt% NaOH/12 wt% urea solution. The mixed solution covered using foil paper to prevent photo-induced NaIO_4 decomposition, was adjusted by 1 M HCl to pH 4, then reacted for 4 h under stirring at 60 °C. 2 g of PEI was then added into aforementioned reaction system. After 3 h reaction, the mixture was centrifuged with 8000 rpm for 5 min to harvest the supernatant. Subsequently, the supernatant was extracted using anhydrous ethanol to harvest pale yellow precipitate i.e. cellulose-Schiff base, which was washed three times with anhydrous ethanol and dried at 60 °C in a vacuum oven.

The chemical structures of the samples were characterized by Fourier transform infrared spectroscopy (FTIR, Nicolet 5700, Thermo Electron Corp., USA) ranging from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} . The crystal phases of samples were detected on an ARL X'RA diffractometer (XRD, Thermo Electron Corp.) using monochromatic $\text{Cu K}\alpha$ radiation ($k = 1.54056 \text{ \AA}$), operating at 40 kV and 30 mA. The elemental analysis of cellulose and cellulose-Schiff base were performed using Elemental analyzer (Vario MICRO cube, Elementar Co., Germany). ^{13}C Nuclear magnetic resonance (NMR) was



Scheme 1 Synthetic process of cellulose-Schiff base sensor

recorded on a Bruker 300 MHz solid-state NMR spectrometer. The surface chemical composition of cellulose-Schiff base before and after Cu^{2+} capture was recorded by X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific, USA) using a monochromatic Al $K\alpha$ radiation.

Capture and detection of Cu^{2+} ions in aqueous solution

Selective

20 mg of cellulose-Schiff bases were incubated with different metal ions (Cu^{2+} , Mn^{2+} , Co^{2+} , Fe^{3+} , Zn^{2+} , Ca^{2+} , and Mg^{2+} ions) in 20 mL aqueous solution for 1 min with/without the coexistence of Cu^{2+} ions, respectively. Residual concentration of metal ions in aqueous solution were measured by Atomic

absorption spectroscopy (AAS, Spectr AA-220, USA). The capture efficiency and capture capacity was calculated on the following Eqs. 1 and 2:

$$\text{Capture efficiency (\%)} = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

$$\text{Capture capacity (mg/g)} = \frac{(C_0 - C_t)V}{m} \quad (2)$$

where C_0 and C_t are the initial concentrations (mg/L) and residual concentrations (mg/L) of metal ions in solution at t time, respectively. V is the volume of the aqueous phase (L), and m is the weight of dry sample (g).

Sensitivity

The relationship between sensitivity and condition parameters, including sensor dosage, solution pH, reaction time, Cu^{2+} ion concentration was investigated. Desired amount of cellulose-Schiff base (5, 10, 15, 20 and 25 mg) as sensor were added into 20 mL Cu^{2+} ions aqueous solution, the color changes of sensor- Cu^{2+} complex were observed by naked-eye. Here, effects of solution pH (2.0, 3.0, 4.0, 5.0, 6.0 and 7.0), reaction time (10–90 min), and Cu^{2+} ion concentration (10, 15, 20, 25, 50, 100, 150, 200, 250 and 300 ppm) on sensitivity of sensor for Cu^{2+} ions were investigated. In addition, the capture efficiency and capture capacity of sensor for Cu^{2+} ions were calculated by Eqs. 1 and 2, respectively.

UV–Vis titration

2 mg of cellulose-Schiff bases were added into 2 mL stock solution of Cu^{2+} ions (0–100 ppm). UV–Vis spectra of mixed solution were recorded at room temperature (UV, U-3900 spectrophotometer, Japan).

Job plot measurement

Cellulose-Schiff base sensors were added into Cu^{2+} solution to obtain a series of mixed solutions with a total concentration of 1.0×10^{-4} M, in which the molar ration of $[\text{Cu}^{2+}]/[\text{Sensor}] + [\text{Cu}^{2+}]$ was 0.1–1.0. The absorbance of each solution is measured at room temperature.

Capture and detection of Cu^{2+} ions in biofluids

Simulated urine and bovine serum were added with different concentrations of Cu^{2+} ions to evaluate the practicability of sensor for real samples. Simulated urine was made according to the U.S. patent #7109035 (Lock et al. 2013) listed in Table S1 to avoid component interference in individual differences. Briefly, 20 mg of cellulose-Schiff bases were added into 20 mL simulated urine, while 10 mg of cellulose-Schiff bases for 10 ml bovine serum. The color changes were recorded and the capture capacity was calculated by Eq. 2.

Preparation of test strips and semi-quantitative detection

As described in *Synthesis and characterization of cellulose-Schiff base* section, the harvested supernatant was dialyzed using dialysis bag (MWCO 8000) for 48 h to obtain cellulose-Schiff base solution. Commercial filter paper was immersed into cellulose-Schiff base solution for 2 min, then dried at 25 °C. The dipping process was repeated 50, 100, and 150 times to prepare test strip. Finally, the detecting ability of test strip for Cu^{2+} visualization was performed in aqueous solution, simulated urine and serum with exogenous copper (0, 2, 4, 6, 8, 10 ppm).

Results and discussion

Synthesis and characterization of cellulose-Schiff base

Briefly, cellulose was oxidized at the $\text{C}_2\text{-OH}$ and $\text{C}_3\text{-OH}$ positions with NaIO_4 to 2,3-dialdehyde cellulose, followed by treatment with polyethylenimine to produce the corresponding Schiff base (Scheme 1). FTIR spectrum confirmed the successful synthesis of the cellulose-Schiff base according to the appearance of the characteristic peaks at 1460 cm^{-1} (C–N stretching vibration) and 1670 cm^{-1} (C=N stretching vibration), as well as the peak of the N–H stretching vibration at 3490 cm^{-1} (Fig. 1a) (Park et al. 2014; Lindh et al. 2016; Ruan et al. 2016; Hu et al. 2018). In addition, the crystal phase structure of the resultant cellulose-Schiff base was determined by XRD (Fig. 1b). For raw cellulose, the diffraction peaks at 15.5° , 22.7° and 34.5° corresponding to (110), (200) and (004) planes are indexed to the cellulose I crystal (Luis et al. 2016; Yang et al. 2016). After PEI modification, the diffraction peaks at $2\theta = 15.5^\circ$ and 34.5° in cellulose-Schiff base obviously weakened, while the diffraction peaks at $2\theta = 22.7^\circ$ became broader, which was ascribed to the introduction of PEI resulting in the destruction of (110) and (004) crystal planes for cellulose. Further, the solid-state ^{13}C CP/MAS NMR spectrum monitored the signals of C2/3/5 in cellulose-Schiff bases, which have an obvious shift compared to pure cellulose. In addition, broad signals of PEI at 38.53–58.49 ppm appeared, revealing successful grafting of PEI to the $\text{C}_2/\text{C}_3\text{-OH}$ positions in

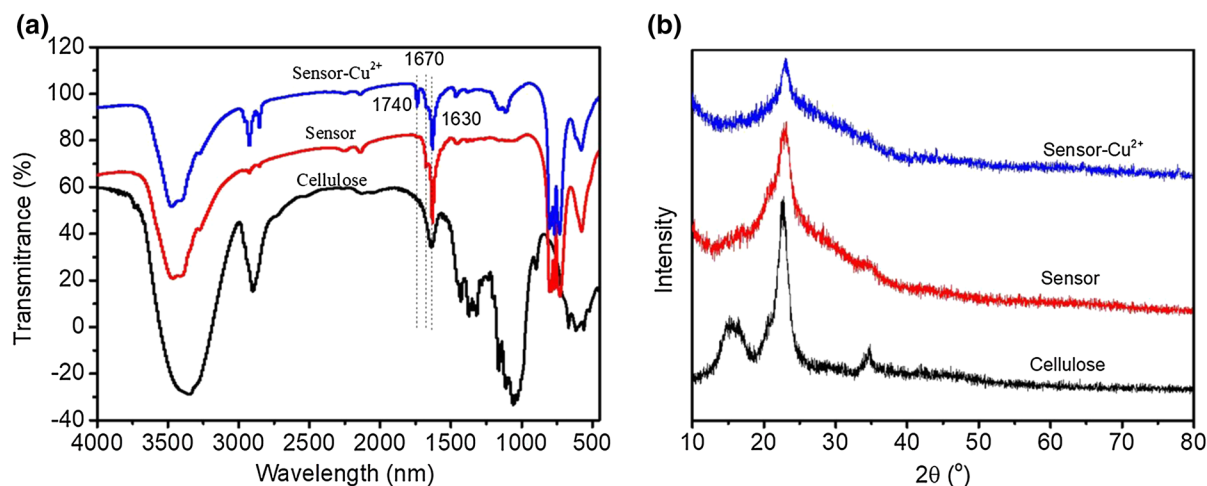


Fig. 1 **a** FTIR spectra and **b** XRD patterns of cellulose and cellulose-Schiff base before and after Cu^{2+} ion detection

cellulose (Fig. S1) (Harpe et al. 2000; Kim et al. 2005). Finally, the elemental analysis presented N element content with 9.18% in cellulose-Schiff base (Table 1), providing further evidence of successful grafting of amino groups.

Capture and detection of Cu^{2+} ions in aqueous solutions

To verify the potential of the proposed approach for detecting Cu^{2+} and to investigate its recognition and capture capability, 20 mg of cellulose-Schiff bases were dispersed in 10 ppm aqueous solutions containing different metal ions (Cu^{2+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Zn^{2+} , Co^{2+} and Mn^{2+} ions). As shown in Fig. 2a, cellulose-Schiff base showed high selectivity for Cu^{2+} ions because the color of cellulose-Schiff base changed from inherent pale yellow to green only in the presence of Cu^{2+} ions within < 30 s. Meanwhile, it was found that 94.2% of Cu^{2+} ions could be captured, a much greater amount than other metal ions. It is well known that the coexistence of various metal ions with Cu^{2+} ions may interfere with Cu^{2+} detection. Thus,

the Cu^{2+} selectivity of cellulose-Schiff base was investigated by competitive response in mixed solution. As shown in Fig. 2b, despite the concentration of Cu^{2+} ions was as low as 5 times than that of other metal ions, the color of cellulose-Schiff base could still turn green within < 30 s, simultaneously the capture efficiency of Cu^{2+} ions reached 93.7%. UV–Vis spectra in Fig. 3 also revealed the high selectivity of cellulose-Schiff base for Cu^{2+} ions. Compared to Fe^{3+} , Mg^{2+} , Ca^{2+} , Zn^{2+} , Co^{2+} and Mn^{2+} ions, the initial absorption peak of cellulose-Schiff base at 320 nm shifted to 275 nm once adding Cu^{2+} ions. Also, a specific absorption peak at 635 nm appeared (inset in Fig. 3), indicating the formation of complexes between cellulose-Schiff base and Cu^{2+} ions. All the results suggested great potential of cellulose-Schiff base as a colorimetric sensor for selective capture and detection of Cu^{2+} ions derived from the formation of cellulose-Schiff base- Cu^{2+} (called the sensor- Cu^{2+}) charge transfer complex (Kumari and Chauhan 2014; Joo et al. 2017).

To understand the relationship between the sensitivity and the condition parameters, the effects of sensor dosage, solution pH, and reaction time, Cu^{2+} concentration on the capture of Cu^{2+} ions were investigated. First, the dependence of the sensitivity for Cu^{2+} detection on sensor dosage is shown in Fig. 4a. After adding 5 mg of sensor into 20 mL of 10 ppm Cu^{2+} ion aqueous solutions, no obvious color change was observed. Once the sensor dosage exceeded 5 mg, green sensor- Cu^{2+} complexes were

Table 1 C, H, O and N contents in cellulose and cellulose-Schiff base sensor

Sample	C (%)	H (%)	O (%)	N (%)
Cellulose	44.82	8.172	47.008	–
Sensor	38.69	7.114	44.188	9.81

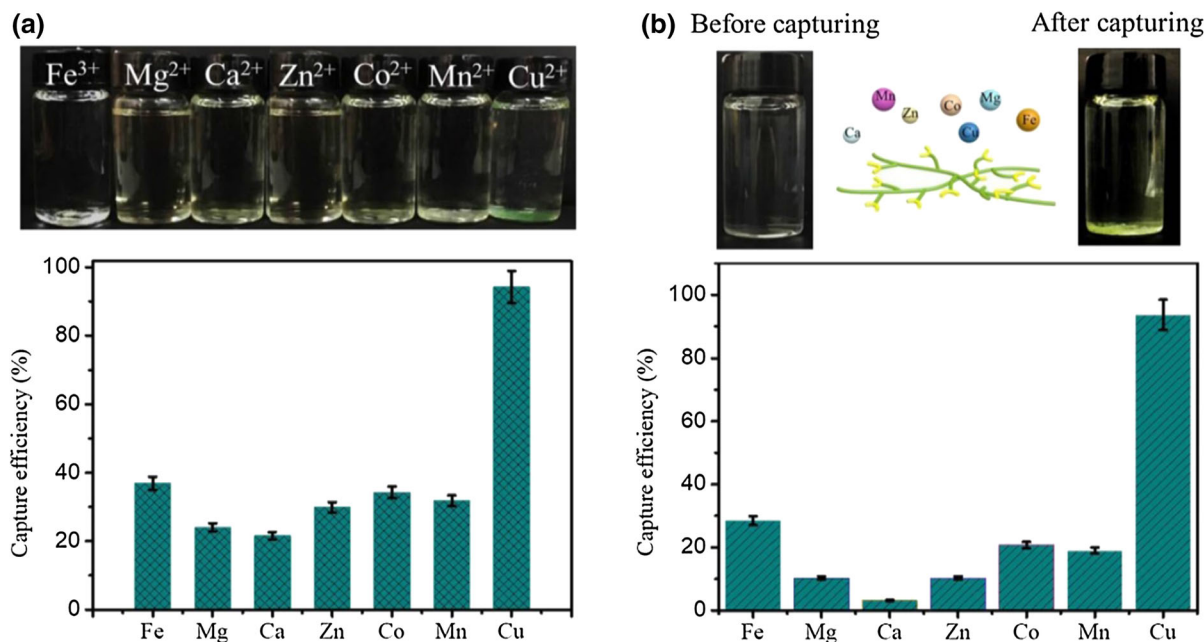


Fig. 2 The color changes and capture efficiencies of cellulose-Schiff base for **a** various metal ions (10 ppm) and **b** co-existed ions solution (the Cu²⁺ ions constitute 10 ppm while other metal ions constitute 50 ppm in each)

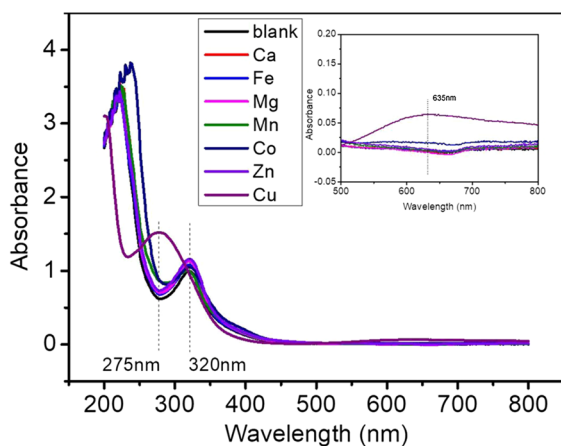


Fig. 3 UV-Vis spectra of cellulose-Schiff base sensor upon addition of different metal ions. Inset: magnification curves at 635 nm

observed within 30 s because of the increase in active recognition sites (Sayed et al. 2017; Gholam et al. 2017). In addition, the capture efficiency values as a function of sensor dosage presented an equilibrium with a sensor dosage of 15 mg, indicating excellent performance in the recognition and capture of Cu²⁺ ions from aqueous solutions. The effect of pH on Cu²⁺ detection was performed in the pH range of 2.0–7.0,

and the result is shown in Fig. 4b. As seen from the optical picture, light green sensor-Cu²⁺ complexes were found at pH 2.0 within 1 min, while obvious green complexes were observed within 30 s when increasing the pH from 3.0 to 7.0. Additionally, the increased capture ability indicated that cellulose-Schiff base could be used as a “naked-eye” colorimetric sensor for detecting Cu²⁺ ions under physiological conditions.

Further, the sensor-Cu²⁺ capture kinetics were investigated and presented in Fig. S2 and Table S2. A time-dependence in the capture process was found. When the sensor was added to the Cu²⁺ solution, the capture capacity (q_t) sharply increased in the first 10 min because of the fast complex reaction and then reached saturation gradually. The chemical capture mechanism was confirmed based on the analysis of a pseudo-second-order kinetics model with a high correlation coefficient R^2 of 0.99758 and a rate constant k_2 of 0.03924 g/g/min. Thus, the N atom of the amino group and/or the O atom of free hydroxyl group of the cellulose-Schiff base bound to Cu²⁺ ions from aqueous solution to form a cellulose-Schiff base-Cu²⁺ complex, consequently exhibiting a strong response for Cu²⁺ ions. The overall capacity of the sensor for Cu²⁺ ions was further determined by fitting

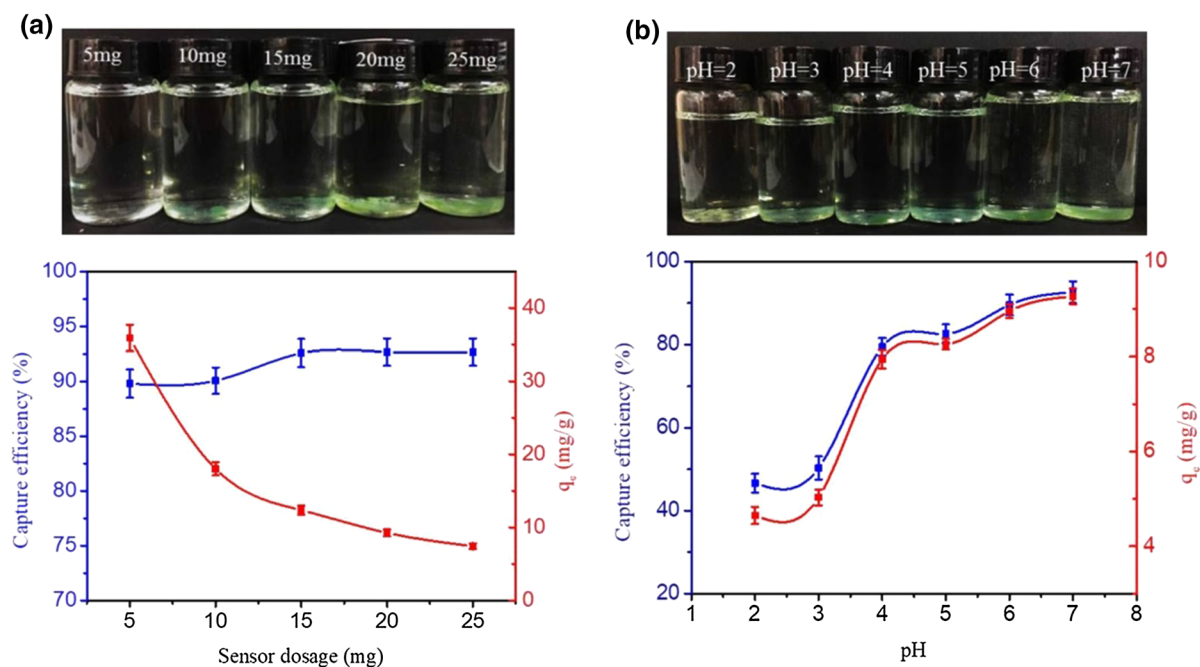


Fig. 4 The color changes and capture efficiencies of cellulose-Schiff base for Cu^{2+} ions **a** with different dosages and **b** under different solution pH values

capture isotherms with a broad range of copper equilibrium concentration (0–110 ppm). The fitting results are summarized in Fig. S3 and Table S3. It is clear that the capture capacity of Cu^{2+} ions increased with the increase of Cu^{2+} equilibrium concentration, and the maximum capture capacity (q_{max}) reached 191 mg/g. In comparison to other capture agents listed in Table 2 (Zhang et al. 2013; Yu et al. 2013; Gonzalez et al. 2014), cellulose-Schiff base displays more higher capture capacity for Cu^{2+} ions due to its abundant amino group contents. Comparing the correlation coefficient (R^2), the Freundlich model can better predict the capture behaviors of cellulose-Schiff

base with a multi-molecular layer capture, indicating a strong complexation between the cellulose-Schiff base and Cu^{2+} ions in a wide concentration range of Cu^{2+} ions.

To further assess the detection performance of cellulose-Schiff base, UV–Vis titration of the sensor with Cu^{2+} ions was carried out. After adding 2 mg of sensor into 2 mL of Cu^{2+} ion solution (0–100 ppm), the specific absorption peaks at 275 and 635 nm appeared and increased gradually with the increase in Cu^{2+} ion concentrations (Fig. 5a) revealing the sensor- Cu^{2+} complex formation and increase in amount. In addition, Fig. 5b shows a linear correlation between

Table 2 Comparison of Cu^{2+} ions capture capacities with different capture agents

Used functional materials	Maximum capture capacity for Cu^{2+} ions (mg/g)	References
Silica based mesoporous adsorbent	182.40	Awual et al. (2014)
Schiff base ligand based adsorbent	173.62	Awual et al. (2015)
$\text{NH}_2\text{-MnO}_2$	97.98	Zhang et al. (2013)
Graphene oxide-chitosan	25.4	Yu et al. (2013)
Layered double hydroxide-humate hybrid	47.63	Gonzalez et al. (2014)
Cellulose-Schiff base	191.00	This study

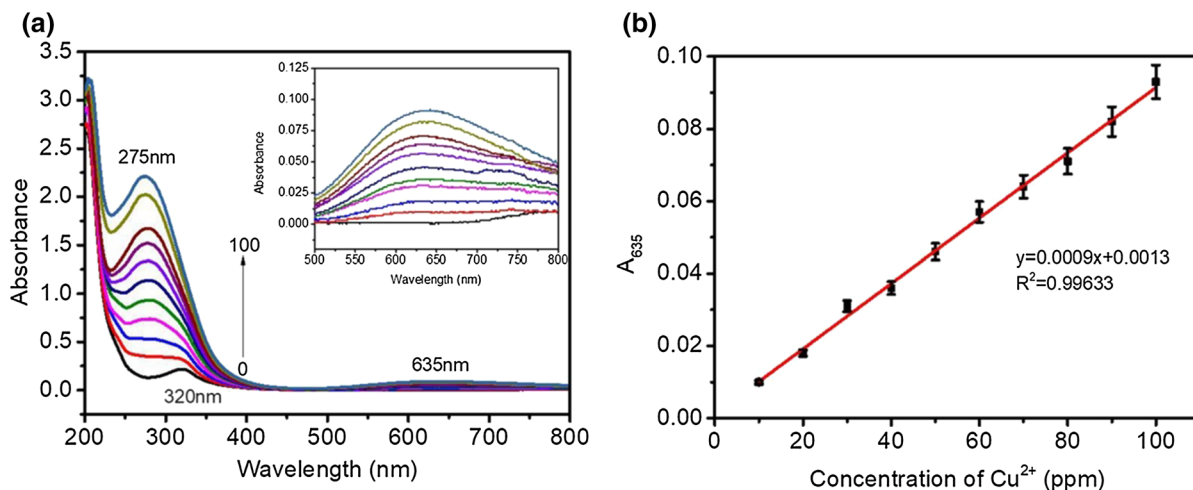


Fig. 5 **a** UV-Vis spectral changes of cellulose-Schiff base sensor upon addition of different concentrations of Cu^{2+} ions. Inset: Magnification curves at 635 nm. **b** The linear correlation between absorption intensity and Cu^{2+} ions concentration at 635 nm

absorption intensity and Cu^{2+} ion concentration at 635 nm. The limit of detection (LOD) was calculated to be 1.054 ppm according to the calculation formula $\text{LOD} = 3\sigma/k$ (where σ is the standard deviation of blank measurement, k is the slope between the absorption intensity versus Cu^{2+} ion concentration). It is clearly seen that the detection limit of the cellulose-Schiff base for Cu^{2+} is lower than the limit of Cu^{2+} of 1.3 ppm in drinking water and approaches the blood copper levels of 0.94–1.31 ppm in healthy individuals as defined by the U.S. EPA (Richardson and Ternes 2011; Zhang et al. 2014a, b; Lee et al. 2016), indicating excellent sensitivity to Cu^{2+} ions in solution systems.

To evaluate the binding model of the sensor and Cu^{2+} ion, a Job plot model was employed to determine the stoichiometric ratio between sensor and Cu^{2+} ions. The total concentration of Cu^{2+} ions and sensor was 1.0×10^{-4} M. The absorption intensities at 635 nm were recorded and plotted against the molar fraction of the Cu^{2+} ions in solution. It is clearly seen in Fig. 6 that the break point appeared at a molar ratio of 0.48 $[\text{Cu}^{2+}]/([\text{Sensor}] + [\text{Cu}^{2+}])$, indicating that the cellulose-Schiff base forms a 1:1 stoichiometric complex with Cu^{2+} ion.

To further investigate the mechanism of cellulose-Schiff base chelating Cu^{2+} ions, X-ray photoelectron spectra (XPS) was performed and confirmed the successful capture of Cu^{2+} ions, owing to the appearance of a $\text{Cu}2p_{3/2}$ characteristic peak at 933.1 eV

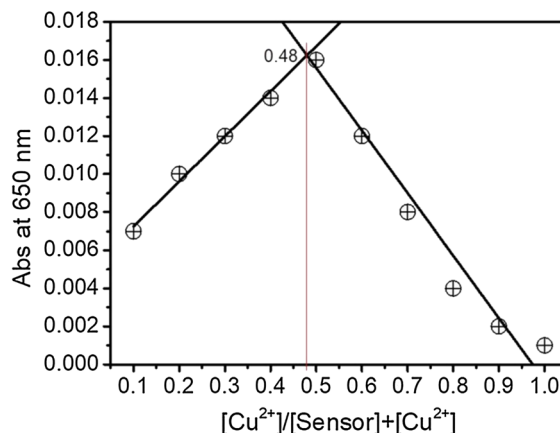


Fig. 6 Job plot for the binding between cellulose-Schiff base sensor and Cu^{2+} ions

(Fig. 7a) (Xiong et al. 2016). For C1 s, the characteristic peaks of binding energy at 284.7, 286.6, 285.9 and 288.5 eV in the cellulose-Schiff base were assigned to C–C, C–O, C–N and C–N, respectively (Sui et al. 2013; Qiu et al. 2014). After capture of Cu^{2+} ions, the proportion of C=N peaks was reduced, revealing the Schiff base structure involved in the capture of Cu^{2+} ions (Fig. 7b). This was further confirmed in N1 s XPS spectra. As shown in Fig. 7c, the N1 s spectra in the cellulose-Schiff base can be deconstructed into three major peaks with binding energies at 398.7, 399.5 and 401.7 eV, corresponding to C=N, N–C and N–H bonds, respectively (Sui et al. 2013; Qiu et al. 2014; Deng et al. 2016). After binding

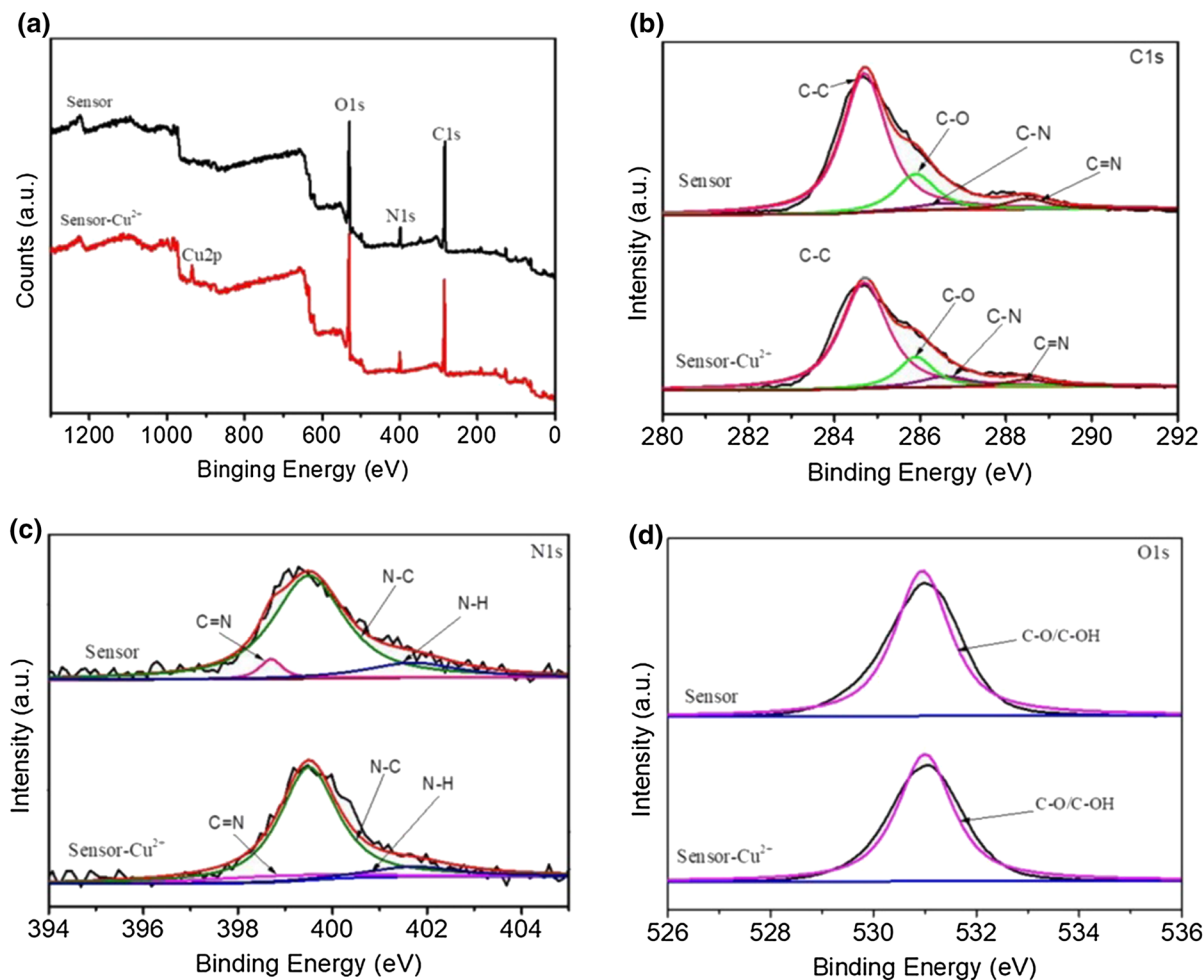


Fig. 7 XPS spectra of cellulose-Schiff base sensor and sensor-Cu²⁺ complex (a), and high-resolution XPS spectra of **b** C1s, **c** N1s and **d** O1s

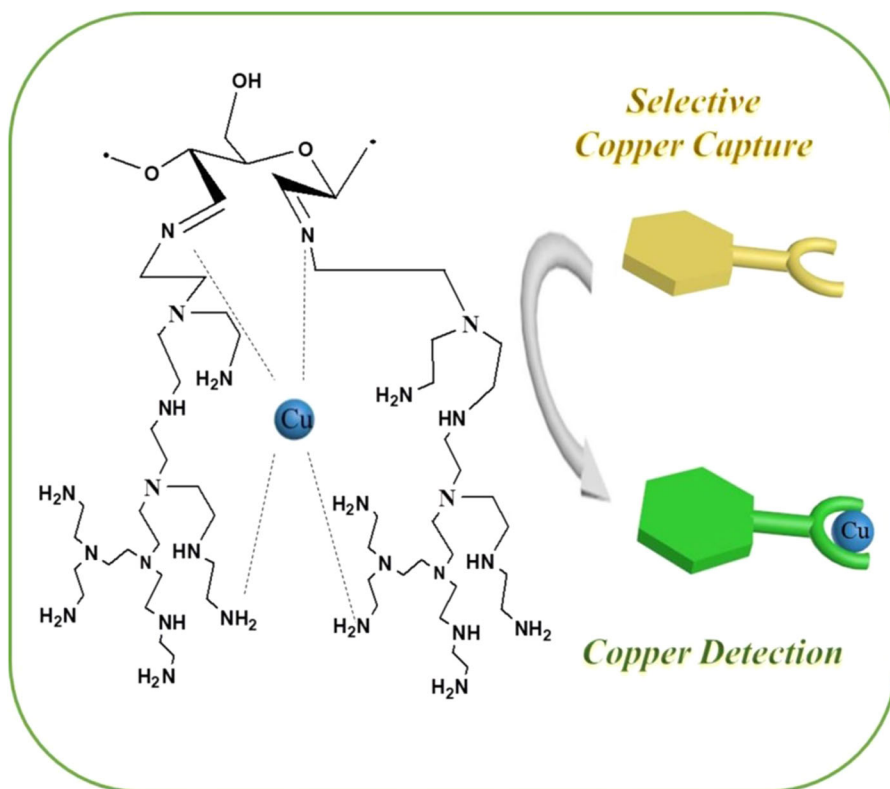
with Cu²⁺ ions, the intensity of the C=N and N-H peaks was weakened. However, there was no obvious change in binding energy peak of the C-O/C-OH at 531.0 eV, suggesting that hydroxyl group of cellulose-Schiff base did not take part in capturing Cu²⁺ ions. Thus, it can be proposed that both N atoms of the Schiff base (C=N) and the amino group (N-H) of the cellulose-Schiff base participated in chelation with Cu²⁺ ions. This consists with the results of FTIR spectra and XRD pattern in Fig. 1. After Cu²⁺ ion capture, the characteristic peak at 1670 cm⁻¹ (C=N stretching vibration) in sensor-Cu²⁺ disappeared obviously, while a new characteristic peak at 1740 cm⁻¹ appeared and the N-H stretching vibration peak became narrower compared to these of cellulose-Schiff base. No significant change was found in XRD

pattern of sensor-Cu²⁺, suggesting that Cu²⁺ capture can not influence the crystal structure of cellulose-Schiff base. On the basis of the Job plot, XPS and FTIR analysis, we speculate that the binding mode between cellulose-Schiff base and Cu²⁺ ions is as shown in Scheme 2.

Capture and detection of Cu²⁺ ions in biofluids

The applicability of the cellulose-Schiff base for Cu²⁺ ion detection in real samples was further assessed after verifying the ability for copper capture with good selectivity and sensitivity from aqueous solution. Generally, patients suffering from Wilson's disease display high urinary copper (> 100 mg/day) and serum free copper (> 25 µg/dL) levels compared to

Scheme 2 The binding mode between cellulose-Schiff base and Cu^{2+} ions



healthy individuals, who have 20–40 mg/day and 11–25 $\mu\text{g}/\text{dL}$ (Kaler 2011). Hence, bovine serum and urine were chosen to evaluate the recognition and capture ability of the cellulose-Schiff base by adding 2–10 ppm of exogenous copper. After adding the sensor into urinary copper solutions, the green sensor- Cu^{2+} complexes appeared within 60 s, and the color of the sensor- Cu^{2+} complexes became darker and deeper with the increase of urinary copper concentration. Moreover, the capture capacity and efficiency of the sensor for Cu^{2+} ions increased when exogenous copper amounts were increased to 10 ppm. Consequently, the maximum values reached 9.58 mg/g and 95.8%, respectively (Fig. 8a). The results confirm that the cellulose-Schiff base has a strong recognition and capture ability in complex simulate urine.

Finally, the capture and detection performances of the cellulose-Schiff base for Cu^{2+} ions were evaluated in serum, and the results are shown in Fig. 8b. Sensor- Cu^{2+} complexes formed within 5 min. The sensor presented dosage-dependent copper capture in the range of 0–10 ppm, and the color of the complexes gradually deepened from inherent pale yellow to light

green to dark green. The capture efficiency of the sensor was 51.6% when the Cu^{2+} amount was increased to 10 ppm. It is known that serum is a more complex biofluid than urine, and it contains more iron, approximately 5 times more than copper (Lee et al. 2016). Although there is iron interference, as shown in Fig. 2, the cellulose-Schiff base still exhibits potential as a colorimetric sensor for direct copper capture from serum. The reduction of iron interference will be done by adding a highly specific affinity iron chelator in our further work to improve the sensitivity of the sensor. Even so, the cellulose-Schiff base provides a promising prospect for Cu^{2+} -related disease diagnosis.

Test strips and semi-quantitative detection

Paper-based sensors are a convenient, cheap, and stable monitoring platform for naked-eye detection. Here, the test strips were obtained after being dipped 100 times, which displayed the strongest sensitivity for Cu^{2+} ion visualization. The detecting ability of test strip was shown in Fig. S4. Then, the relationships between sensitivity of the test strips and Cu^{2+} ion

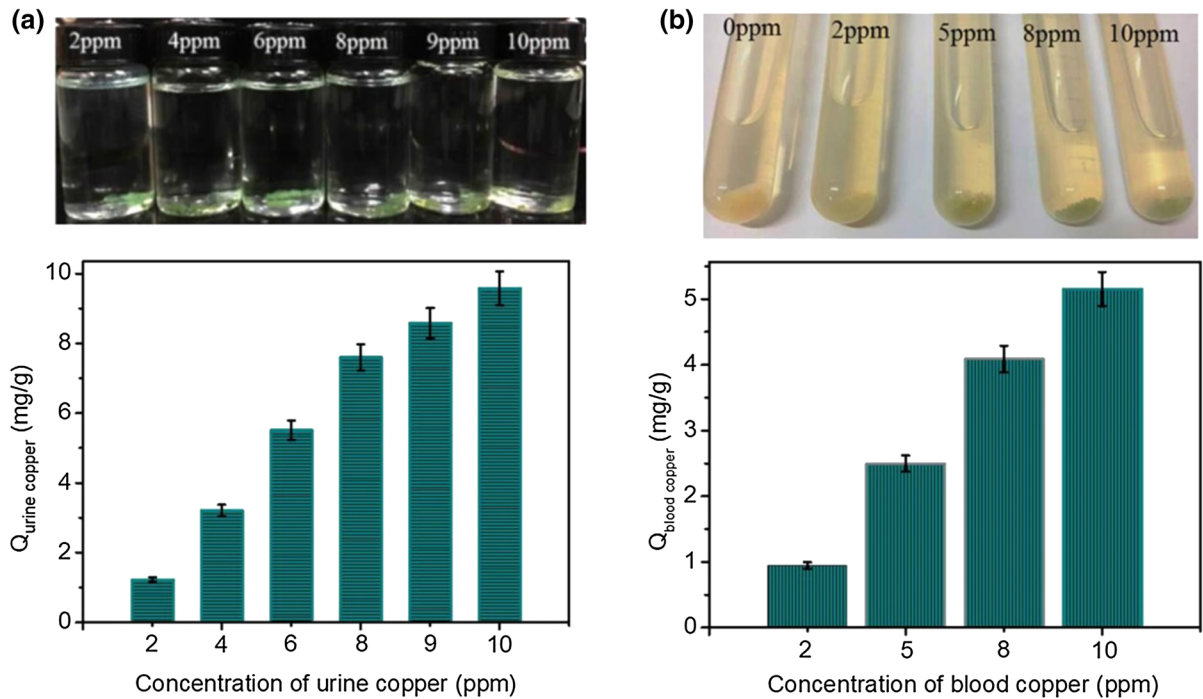
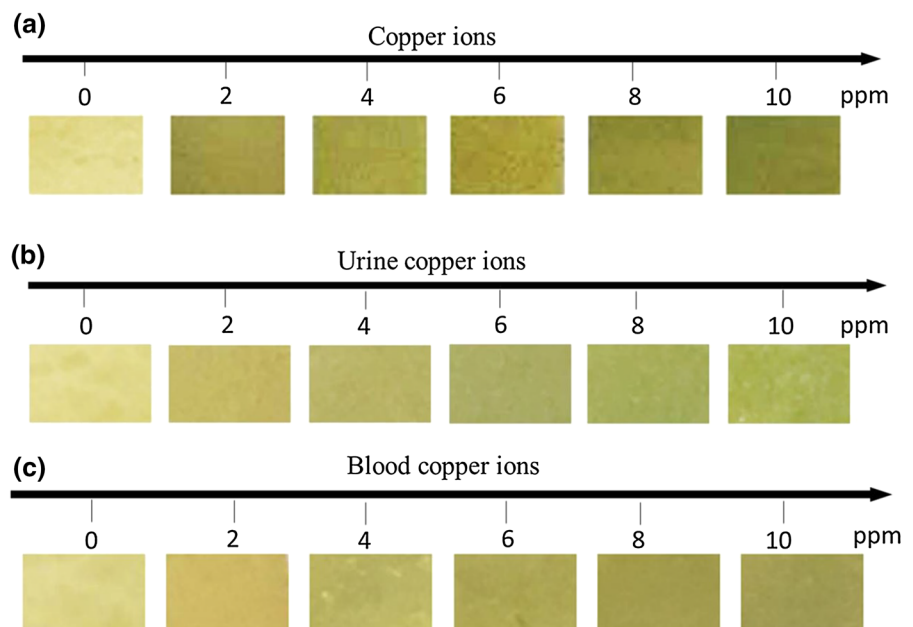


Fig. 8 The color changes and capture capacities of cellulose-Schiff base for **a** urine copper and **b** blood copper

amount were evaluated, and semi-quantitative colorimetric cards were prepared. As seen from Fig. 9, with the increase of Cu^{2+} ions in aqueous media including a simulated urine and serum sample, the test strips displayed color changes from pale yellow to green for

aqueous solution within 30 s (Fig. 9a), to light green for urine sample within 100 s (Fig. 9b), and to yellow-green for serum sample within 5 min (Fig. 9c). Even when the amount of Cu^{2+} ions was lowered to 2 ppm, light green strips were clearly observed,

Fig. 9 Colorimetric cards upon visual detection of Cu^{2+} ions from **a** aqueous solution, **b** simulated urine and **c** serum. (Color figure online)



demonstrating a high sensitivity of the prepared test strips. The results demonstrated that the cellulose-Schiff base as a sensor has promising applications in on-site visual detection of Cu^{2+} ions for real samples without the need for complex and expensive instruments.

Conclusions

The cellulose-polyethylenimine-based Schiff base was fabricated for capture and detection of Cu^{2+} ions in aqueous solution, complex simulated urine as well as bovine serum. The resultant cellulose-Schiff base exhibited an obvious color change from inherent pale yellow to deep green in the presence of Cu^{2+} ions, which derived from the formation of cellulose-Schiff base- Cu^{2+} charge transfer complexes with a 1:1 stoichiometric ratio. The sensing mechanism of cellulose-Schiff base for Cu^{2+} ions was proposed to be such that N atoms (C=N and N-H) in the sensor capture and concentrate Cu^{2+} ions to form sensor- Cu^{2+} complexes, with intramolecular charge transfer occurring as a consequence. On the basis of sensor- Cu^{2+} chelation-induced color-change, semi-quantitative test strips based on the cellulose-Schiff base were developed and exhibited sensitive and visual detection capability for Cu^{2+} ions in aqueous solution, simulated urine and serum. Thus, the cellulose-Schiff base has potential as a convenient, cheap, sensitive, and reliable monitoring platform and can be used in environmental and biomedical applications, including on-site naked-eye detection and disease diagnosis.

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