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ARTICLE TYPE

A visual volumetric hydrogel sensor enables quantitative and sensitive detection of copper ions†

Rui Wu,^{a,b} Shenghai Zhang,^a Jitong Lyu,^c Fang Lu,^a Xuanfeng Yue^{a,*} and Jiagen Lv^{a,*}

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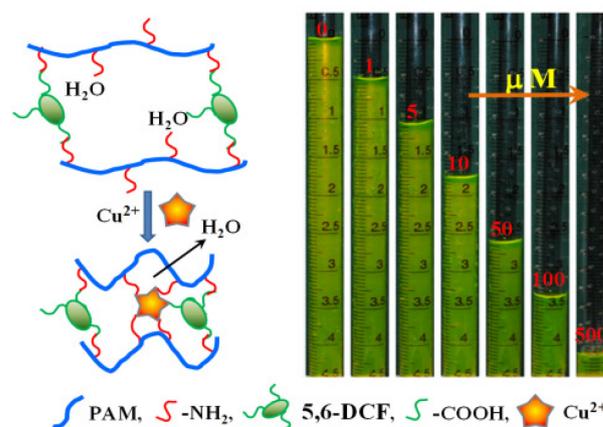
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We propose a visual volumetric sensor with 5,6-dicarboxylic fluorescein cross-linked amine-functionalized polyacrylamide hydrogel. The sensor undergoes volume response to Cu^{2+} ions at μM level, which enables naked-eye quantitative detection by reading graduation on a pipette.

Because of such advantages as being instrument-free and perhaps also in line with the human habits of “seeing is believing”, naked-eye readout chemical sensors are drawing increasing attraction in many areas and disciplines.¹ Most of the reported visual sensors are based on the notable changes in color to indicate the presence of a specific chemical species. Such imaginative and sensible colorimetric reagents as nanoparticles,² dyes,³ and polymers,⁴ have been introduced into the designs of analyte triggered visual sensors. Stimuli-responsive hydrogels have also attracted particular attention for their “smart” phase-transition in response to chemical or physical changes. Recent years have seen remarkable efforts made to develop visual hydrogel sensors for many important analytes, including nucleotide,⁵ lectin concanavalin A,⁶ glucose,⁷ nitric oxide,⁸ melamine⁹ and metal ion,¹⁰ etc. These sensors with straightforward designs are directly working devices which provide simple, portable and sensitive detection without requirement of instrument or power source. However, in contrast to the widely available qualitative and semi-quantitative detection, visual hydrogel sensors for quantitative detection remain significant challenges.

Compared with the variation in optical properties, the variation in volume of liquid or fluid can be straightforwardly measured by reading the graduations on a proper container, for example, a thermometer. Being aware of this, Qin’s group and Yang’s group successively reported an elegant bar-chart chip design for visual quantitative sensing.¹¹ Once the analyte triggered catalytic decomposition of hydrogen peroxide, large amount of oxygen is produced and collected to move an ink column, where the analyte’s contents is translated into volumetric signal. Beyond the great potential for point of care diagnostics for visual sensitive analysis of ions, molecules and even cells,¹¹ their works proved the concept and feasibility of developing volumetric sensors for naked-eye quantitative detection. Regarded as one of the most proper materials for chemical and bio-chemical sensing, responsive swelling/shrinking of hydrogel to various analytes has been well acknowledged.^{12a,b} Most of quantitative (or potentially quantitative) hydrogel sensing designs are developed by characterizing the analyte stimulated variation in physical

properties of color and light absorbance, light emission, resonance frequency, light diffraction and refraction rather than of volume, while in many cases the responsive volume changes to analyte have been noticed.¹² For example, Lin et al. reported a color visualization copper ions sensor with the DNzyme and Substrate incorporated hydrogel, where the proportional relationship between the size change of the hydrogel and the copper concentration was observed.^{12c} All these works imply the potential of developing volume readout hydrogel sensors for quantitative sensing. The challenge is the difficulty of measuring hydrogel volume changes with sufficient precision.¹³ And one step further, if the hydrogel volume changes can be visually measured with high resolution, quantitative volumetric sensor can be expected. Inspired by such potential, we proposed a volumetric sensor design for metal ion sensing. By filling Cu^{2+} stimuli-responsive hydrogel particles into a graduated pipette and subsequently reading the volume response of hydrogel, quantitative detection of Cu^{2+} at μM level is readily accomplished.



Scheme 1. A, the working principle of 5,6-dicarboxylic fluorescein (5,6-DCF) cross-linked amine-functionalized polyacrylamide (PAM) hydrogel for volumetric response to Cu^{2+} . B, photograph images of hydrogel sensor and its responses to Cu^{2+} ranging from 0 to 500 μM (figures in red) which can be quantitatively distinguished by naked-eye. Borate saline buffer of 2 mM pH=7.4 was used to control the acidity as well as the ion strength.

The working principle of our hydrogel sensor is summarized in **Scheme 1A**. Via Hoffman degradation, 19.4 percent of amide groups in linear PAM was converted into amine groups. 5,6-DCF was used as cross-linker as well as the dye to form the bright yellow-green 3D network through dehydration between the amine

group and carboxylic group (S2, ESI†). The resultant hydrogel is abundant in amine and carboxylic groups (S3, ESI†). The hydrogel swelling capacity is of significance to achieving the high resolution of volume readout, as well as the sufficient precision and sensitivity. To meet the need of the following sensor construction, the hydrogel swelling capacity was determined as the apparent swelling rate by measuring the accumulation volumes of dried particles and fully swelled particles, respectively. Experiment results found that the dried hydrogel particles (ground into 80-120 mesh) may swell in apparent volume by about 70 times and 67 times with respect to pure water and 2 mM borate buffer (S4, ESI†). In our primary sensor construction, hydrogel particles were thoroughly immersed in borate saline buffer, and the fully swelled hydrogel particles were filled into a 5 mL graduated pipette with the minimum scale of 50 μ L. Experiments found that if this hydrogel particles loaded pipette was directly applied to 10 μ M Cu^{2+} solution, it took about 2 h to achieve the steady volume readout. This time consuming response time is attributed to the diffusion of Cu^{2+} from sample solution into the 3D network of hydrogel particles throughout the pipette. To shorten the response time for each detection, about 80 diffusion holes (0.8 mm in diameter) were uniformly engraved in the pipette wall with a laser beam (Fig. S3 ESI†). In order to examine the effect of particle size on sensitivity, three size range particles of 40-60, 60-80, and 80-120 mesh (dried hydrogel) were applied to 1.0 μ M Cu^{2+} standard. Experiment results showed in Fig. S4 (see ESI†) indicate that the 80-120 mesh particles performed the best volume response to Cu^{2+} . Further reduction of hydrogel particle size requires the size reduction of diffusion holes to prevent the leakage of particles from the pipette. Restricted by the capability of the available laser instrument, 80-120 mesh particles were chosen to construct the sensor. For such constructed device, the visual readout accuracy of volume variation is estimated to be ± 20 μ L. That is, for any volume response exceeding 400 μ L, the readout error is theoretically less than 5%. Such precision is generally acceptable for quantitative detections. Copper ions, at as low as μ M concentration level, are found triggering distinct and proportional volume responses (Scheme 1B). According to literature,¹⁴ it is supposed that Cu^{2+} ions form the heteroligand chelates with amine and carboxylic groups in hydrogel, which brings about microscopic interchain aggregation and consequent macroscopic volume shrinkage coupled with water drainage.

To verify the assumption above, TEM and SEM investigations on the hydrogel micromorphology in the absence and presence of Cu^{2+} were conducted. A less 5,6-DCF cross-linked hydrogel was specifically synthesized for TEM investigation. As showed in Fig. 1A, the water swelled and freeze-dried hydrogel exhibits a dendrimer-like structure. In contrast, upon addition of 1.0 μ M Cu^{2+} , the disappearance of dendrimer branches and the appearance of cube-like blocks suggests the aggregation of the adjacent PAM chains (Fig. 1a). As for the SEM investigation, the water swelled and freeze-dried hydrogel displays irregular pore shape (with variable sizes ranging from 15 μ m to 50 μ m) and interconnected 3D network structure (Fig. 1B). Upon addition of 0.1 mM Cu^{2+} , the hydrogel dramatically shrinks its porous network and ends in a structure with smooth outer surface (Fig. 1b). Macroscopically, the hydrogel exhibits drastic shrinkage in

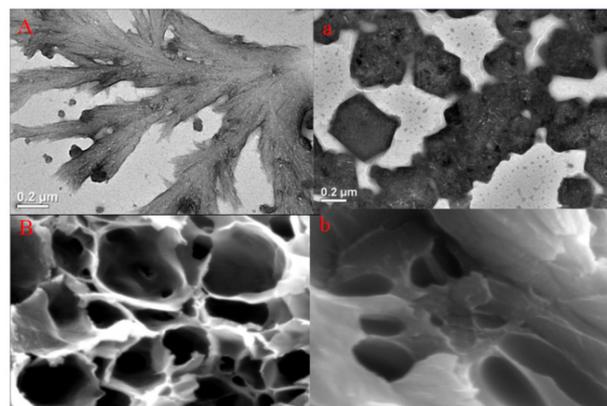


Fig. 1 TEM microphotographs of the freeze-dried less cross-linked hydrogel (A) in absence and (a) in presence of 1.0 μ M Cu^{2+} ; and SEM microphotographs of the freeze-dried hydrogel (B) in absence and (b) presence of 0.1 mM Cu^{2+} .

Cu^{2+} presence. Such a SEM result is in consistent with what we have observed in TEM investigation. In addition, TEM and SEM results obtained also explain the phenomenon that smaller hydrogel particles perform better sensitivity to Cu^{2+} . For bigger hydrogel particles, the initial chelation taking place on their surface leads to the formation of dense shells, thus, the disappearance of micro-channels kinetically prevents Cu^{2+} from diffusing into the inner network. In our sensor, each particle is an independent sensing element; the visually observed volume response is virtually the integration of shrinkages of many particles. Consequently, the signal amplification and highly sensitive detection can be expected.

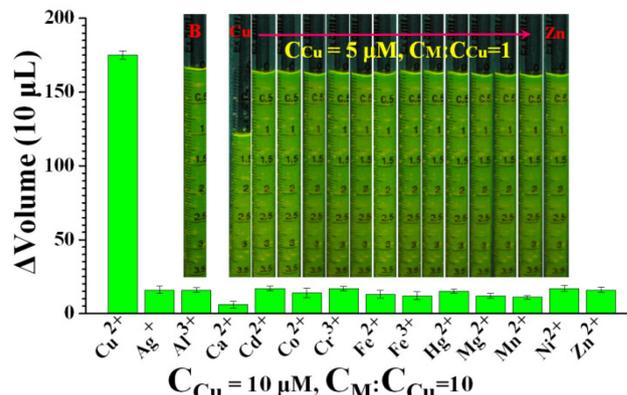


Fig. 2 Selectivity of the volumetric sensor. The heights of the vertical bars represent the volume responses corresponding to 10 μ M Cu^{2+} and other 13 metal ions at a 10 fold concentration, respectively. The inset shows a photograph of the sensor for the same investigation, with the difference that 5.0 μ M Cu^{2+} and 5.0 μ M other metal ions were used; Red B stands for the blank.

By monitoring the volume variation along with response time, kinetics of Cu^{2+} responsive shrinkage was studied, as is showed in Fig. S5 (see ESI†). Graduated pipettes (5.0 mL) loaded with 4.5 mL hydrogel particles were immersed in 1.0, 5.0, and 12.0 μ M Cu^{2+} solutions, respectively; their volume responses were recorded every 2 min. It was found that gradual shrinkage of the hydrogel over elapsing time came to an end in 10 min for all three concentrations. As it is, the sensor can respond rapidly to Cu^{2+} . It is noticed that the 0.51 mL volume response,

corresponding to 1.0 μM Cu^{2+} , is significant enough for quantitative readout. This result suggests the potential of sensor for quantitative detection of Cu^{2+} at μM levels. In comparison of our hydrogel with the reported hydrogel,^{12c} both of them exhibit the sensitive response toward copper ions in spite of their difference in hydrogel composition, sensing principle, response time, and the signal for quantification. While the reported hydrogel performed the observable proportional volume response to Cu^{2+} concentrations, our design is specified to approach the precise visual readout of volume variations for quantitative detection. In addition, by removing the accumulated Cu^{2+} with proper chelating reagents, our hydrogel is expected to be recoverable rather than disposable.

In order to evaluate the selectivity of our volumetric sensor toward Cu^{2+} when it is used for water samples, other metals that possibly coexisting in natural water were investigated in place of 5.0 and 10 μM Cu^{2+} with 1 and 10 fold higher concentrations under identical conditions, as is showed in Fig. 2. In comparison with the clear volume response to Cu^{2+} , other tested metals including Ag^+ , Al^{3+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} and Zn^{2+} barely cause slight or negligible volume responses. Such selectivity toward Cu^{2+} matches that of reported selective chelation-based sensors.¹⁵ The developed sensor enables differentiation of Cu^{2+} from other common metal ions in natural waters with naked-eye observation.

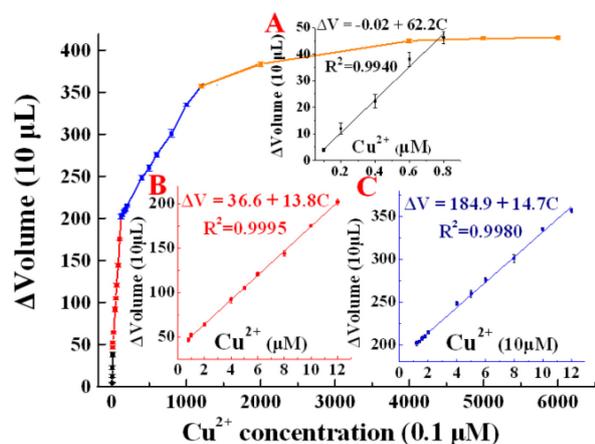


Fig. 3 Sensor calibration curve response to Cu^{2+} concentrations from 0.1 to 500 μM . Insets of A, B, and C, the linear plots of the volume response vs. Cu^{2+} concentration in range of 0.1 to 0.8 μM , 0.8 to 12 μM , and 12 to 120 μM , respectively.

In addition to the investigation on selectivity of proposed visual volumetric sensor, its capability for quantitative detection of Cu^{2+} was evaluated. As aforementioned in Scheme 1B, our sensor exhibits the visually measurable volume responses proportional to Cu^{2+} concentration in a wide range. That is, the sensor is potentially able to detect Cu^{2+} without the requirement for any external electrical device or power source. Fig. 3 offers the panorama of the sensor's response curve to Cu^{2+} ranging from 0.1 to 500 μM . A close observation of the trend of sensor response with Cu^{2+} concentration increase, a leveling off point corresponding to about 160 μM Cu^{2+} is found. As demonstrated in S3 (see ESI[†]), the ratio of amine to carboxylic group in

hydrogel is about 4:1. According to literature,^{14a,b} it is assumed that a Cu^{2+} ion chelates four amine and one carboxylic groups to form a highly stable structure as showed in Fig. S6 (see ESI[†]). The range over which the signal response linearly behaved with analyte concentration is important for a sensor. In our case, the responsive volume varies linearly with Cu^{2+} concentration over the range 0.1 - 0.8 μM , 0.8 - 12 μM , and 12 - 120 μM (Fig. 3 inset), respectively. 0.8 μM Cu^{2+} is noticed to produce about 0.47 mL volume response; a Cu^{2+} solution lower than this concentration may cause a volume readout error exceeding 5%. Therefore, 0.8 μM is set as the initial concentration for quantitative detection, and the first linear range 0.1 - 0.8 μM permits semi-quantitative detection while the other two for quantitative detection. To examine the sensor reproducibility, replicate detections with respect to 0.8, 6.0 and 12 μM Cu^{2+} were carried out. As we can see in Fig. S7 (see ESI[†]), the sensor shows less than 5% relative standard deviations for all three cases. Such reproducibility is normally acceptable for quantitative detections. According to the 1.3 mg/L limit for Cu^{2+} in drinking water issued by the US Environmental Protection Agency, our sensor is capable for quantitative detection of Cu^{2+} in drinking waters. To examine the sensor capability for real samples, Cu^{2+} in swimming pool water and tap water were determined. As summarized in Tab S1 and Tab S2 (see ESI[†]), the agreements with ICP-MS method and the recovery test results for spiking samples provide evidence for the accuracy and feasibility of our sensor for detection of copper ions in water.

Convenience, and even smartness, remains perennial pursuit in sensor development. It will be welcome if we can directly read the sensor in Cu^{2+} concentration instead of the volume. For this purpose, a scale bar was prepared by pre-interpreting the volume graduations into Cu^{2+} concentrations as showed in Fig. S8 (see ESI[†]). With the aid of scale bar, naked-eye direct quantitative detection of Cu^{2+} was realized.

Whether a sensor is recoverable or not determines whether it is an environmentally friendly platform.¹⁶ For our sensor recovery, EDTA was selected as the extraction agent to remove Cu^{2+} from hydrogel. Fig. S9A-B (see ESI[†]) shows the success of sensor recovery within 3 hours by immersing Cu^{2+} accumulative sensors into 0.1 mM EDTA and water alternatively, suggesting the effectiveness of EDTA and recoverability of hydrogel. For the purpose of saving time, hydrogel particles from many used sensors were collected and recovered together with the established procedure. In Fig. S9C (see ESI[†]), no significant difference in response to μM level Cu^{2+} is found between fresh hydrogel and recovered hydrogel constructed sensors. Therefore, our sensor could serve as a recoverable tool for visual Cu^{2+} detection.

In summary, we proposed a visual volumetric sensor design for quantitative detection. A Cu^{2+} sensor was developed by filling hydrogel particles, of which highly porous 5,6-DCF crosslinked hydrogel 3D network presents sensitive and proportional volume response toward Cu^{2+} , into an elaborately altered graduated pipette. The experiment demonstrated analytical characteristics of acceptable reproducibility, wide linear range, and high sensitivity show the capability of our sensor. The results of the sensor for Cu^{2+} detection in real water samples agreed well with the ICP-MS results, establishing the sensor accuracy and reliability. Our

design stands out in direct visual quantification of analytes to the best of its credit without the aid of instrument and power source. The proved hydrogel recovery renders the sensor being environmentally friendly. By employing other analyte stimuli-responsive hydrogels, together with the platform established here, new portable visual volumetric sensors can be expected for substances of interest in agriculture, environment, and food safety.

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^a Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, 710119, P. R. China. fax: +86 29 81530727, lvjiagen@snnu.edu.cn and xfyue@snnu.edu.cn.

^b College of Chemical and Environment Science, Shaanxi University of Technology, Hanzhong, 723001, P. R. China.

^c College of Chemical Engineering, Beijing University of Chemical Technology, Beijing 100029, P. R. China.

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