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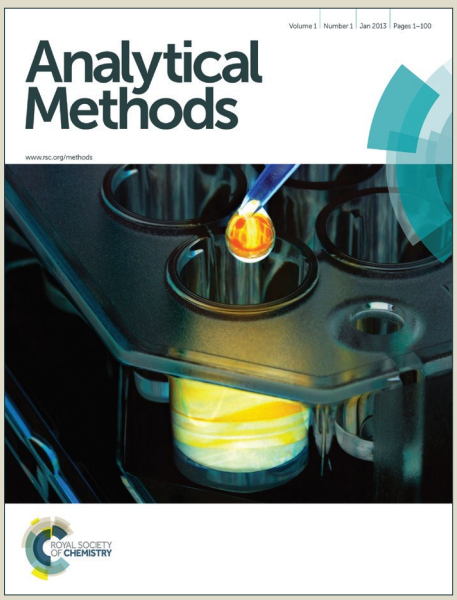

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An Ultrasensitive and Selective Turn-Off Fluorescent Nanoprobe for the Detection of Copper Ions

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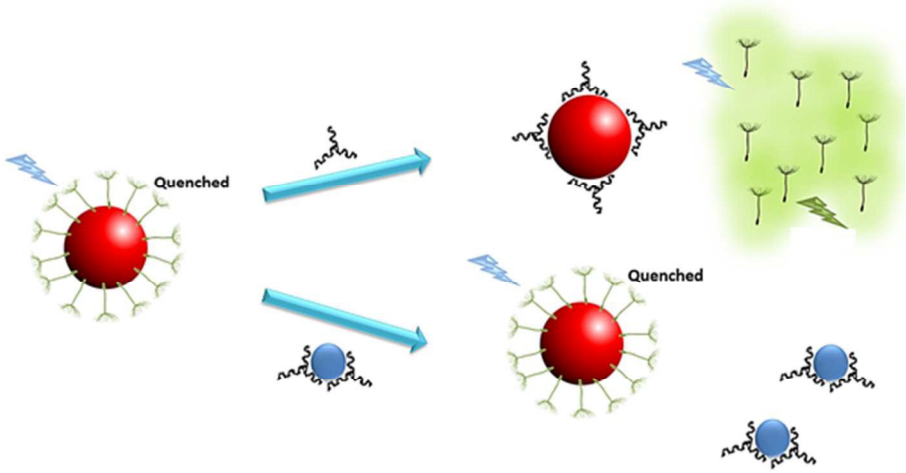
Abstract

In this study, a novel approach for sensitive and extremely selective detection of copper ions has been developed based on fluorescence resonance energy transfer (FRET) between AuNPs as an acceptor and Fluorescein isothiocyanate (FITC) as a donor. Initially, the fluorescence intensity of FITC molecule turns-off due to adsorption to the AuNPs surface. However, the fluorescence intensity of FITC-AuNPs system switched to turn-on by adding D-Pencillamine (D-PC) to the solution mixtureas FITC molecules are released upon displacement by D-PC on NPs surface. The higher affinity of D-PC toward the surface of AuNPs was further evidenced by controlling the fluorescence intensity enhancement of the released FITC in presence of copper ions. In this scenario, the chelating agent of the D-PC strongly interacts with copper ions, thereby becoming less competitive for adsorption the AuNPs surface. This methodology was implemented for determination of copper ions, where under the optimum experimental conditions(pH 8, [FITC]=1.0µM, [D-PC]=6.25µM), two linear calibration curves were obtained within the range of 1-9nM and 10-40nM with detection limit of 0.3nM. Ultimately, the designed nanoprobe was successfully employed for detection of copper ions in food and water samples.



Key words: Fluorescence resonance energy transfer (FRET),Gold nanoparticles (AuNPs), Fluorescein isothiocyanate (FITC), 3-Mercapto-D-valin (D-Penicillamine), Copper ions

Introduction

Recently, development of novel fluorescent and colorimetric nanoprobes for sensing of biologically active metal ions have received considerable attention in the quest for ultra-sensitive chemical analysis, medical diagnostics and biotechnology applications. Copper is one of the trace elements in human body that plays crucial roles in various biological processes [1]. For example, Copper-dependent enzymes are the essential catalysts for the transformation of melanin to the skin's pigment. This provides the required energy for biochemical reactions, such as maintaining and repairing connective tissues by assisting formation of cross links in collagen and elastin [2-4]. The human body obtains copper through daily diet. Also, large amount of mechanisms exist to maintain the copper balance in the body [5]. A healthy adult human body contains 1.4-2.1 mg copper per kilogram of body weight [6]. However, if copper exists in slightly larger amounts, it can also be toxic to the living cells. In the other way, the copper ions can react with molecular oxygen to form reactive oxygen species (ROS), that subsequently, causes damage to proteins, nucleic acids and lipids [7-8]. According to accumulated evidences, long time exposure to copper ions might potentially decelerates the physical and neurological degenerative processes that consequently lead to serious diseases, such as Menkes and Wilson disease, Alzheimer's disease and Parkinson's disease [9].By virtue of this fact, development of simple analytical techniques for detection of copper ions is crucial.

In this regard, Inductively Coupled Plasma (ICP), Atomic Absorption (AA)and Atomic Fluorescence Spectrometry (AFS)are examples of practical methods that have been employed for the detection of copper ions [10-11]. However, these techniques have limitations, such as sophisticated instrumentation, high operation cost, cumbersome sample preparation, time consuming processes and a number of parameters that have to be controlled. Therefore, in recent years, copper selective chemosensors are being developed based on colorimetric detection [12-16], photoinduced electron transfer (PET) [17-18], internal charge transfer (ICT)[19-21], quantum photoelectric effect[22] and fluorescence resonance energy transfer (FRET)

mechanisms [23-31]. Among the above mentioned methods, the fluorescent chemosensors are well-known for their selectivity, sensitivity and fast response time for trace analysis. However, design and development of the functional biocompatible sensors in the complex matrixes is a big challenge. Several copper-selective chemosensors have been reported based on fluorescence quenching mechanism,although, they rarely provide sensitivity at nanomolar levels. In addition, AuNPs have been widely used for FRET based chemosensors [32-33] owing to their extremely high extinction coefficient and tunable optical properties that can be exploited to quench the fluorescence emissions of the fluorophores attached to the surface of the NPs. This happens due to the fluorescence resonance energy transfer (FRET); a process which is inversely proportional to the sixth power of the distance between fluorophore and quencher [34].]. Based on this mechanism, our group recently developed a highly sensitive probe for detection of captopril based on energy transfer between fluorescein isothiocyanate (FITC) and gold nanoparticles [27].
In this work, we extended our previous effort on developing FRET-based chemosensors by designing a novel turn-off copper specific, biocompatible and extremely sensitive method based on FRET between AuNPs as acceptor and FITC. The fluorescence emission intensity of FITC is significantly quenched due to overlapping of its emission spectra and absorption spectra of AuNPs at plasmon resonance wavelength. A rise in fluorescence intensity is expected upon adding D-PC that compete with FITC for AuNPs surface, mainly due to high affinity of thiol group in D-PC to Au surface. The intensity of recovered fluorescence spectra is controllable through formation of selective complex between D-PC and copper ions, while providing an ultra-sensitive and cost-effective method for detection of copper ions.

Experimental

Reagents. Hydrogen tetrachloroaurate ($HAuCl_4.3H_2O$, 99.9%), Trisodium citrate, FITC (Fluorescein isothiocyanate), 3-Mercapto-D-valin (D-Penicillamine), $Cu(NO_3)_2$, NaH_2PO_4 and Boric acid were purchased from Merck. All solutions were prepared using deionized water (18.2 MΩ).

Apparatus. The fluorescence spectroscopy (excitation wavelength was λex=490 nm and emission wavelength was λem=518 nm) was performed using Cary Eclipse fluorescence spectrometer. Water used for the preparation of all samples, was purified with cartridges from Millipore (Direct-Q3) to a resistivity of 18.2 MΩ. Measurements of pH were made with a Denver Instrument Model of 270 pH meter equipped with a Metrohm glass electrode. A Varian SpectrAA Model 220 atomic absorption spectrometer, equipped with copper hollow cathode lamp as radiation source, was used for obtaining AAS data. Transmission electron microscopy (TEM) image was recorded with a PHILIPS MC 10TH microscope (USA) at an accelerating voltage of 80 kV. Size distributions of the nanoparticles were obtained using Malvern Zetasizer Nan ZS90.

Preparation of Gold Nanoparticles. Gold nanoparticles with average size around8.0 nm were prepared based on the presented method reported by J. Turkevich et al. [35-36].Accordingly; a 50 mL solution containing $HAuCl_4$ with the concentration of 1mM was prepared and heated to the boiling point under reflux. Then, 5mL of 38.8mM trisodium citrate was added under vigorous stirring and reflux process was continued. The color of the mixture changed to deep red indicating the formation of gold nanoparticles. After 30 min, heating was stopped and the prepared AuNPs solution was cooled to the room temperature and was stored at 4 °C for further use. The concentration of AuNPs solutions was estimated to be 15nM according to Beer's law and the extinction coefficient (ε) of 8.0nm AuNPs at 520 nm [37-38].Fig. 1 displays representative typical TEM image and DLS size distribution.

Detection of Copper Ions using FRET Probe. The FRET sensor was prepared in a 5ml volumetric flask including 0.5ml of citrate-capped AuNPs, 20 µl of 2.5×10^{-4}MFITC and 2ml of 0.2M pH 8 Britton-Robinson buffer (B-R buffer) and made up to the volume. Different concentrations of copper ions were added to a 5ml flask containing 200µl of 1.0×10^{-3}M D-Penicillamine and made up to the volume. 1ml of the resent solution was added to the FRET probe. After 25 min of incubation, the fluorescence intensity was measured in 1.0 cm quartz cell.

Results and Discussions

As shown in Fig. 2, the FITC emission spectrum and the extinction spectrum of AuNPs significantly overlap that allow for a FRET process in which FITC and AuNPs act as the donor and the acceptor, respectively. This results substantial quench of the fluorescence intensity of the FITC at 518 nm. However, in the presence of D-penicillamine, the fluorescence intensity of FITC is restored. This is mainly due to the thiol functional group in D-PC that provides a stronger affinity than that of FITC for adsorption to the AuNPs surface. Besides, D-PC is a well-known copper selective chelator that can form complex with copper ions in different stoichiometry [39-43]. Therefore, in the presence of copper ions, D-PC coordinates to Cu^{2+} instead of performing a releasing agent role for FITC. Hence, an increase in the fluorescence intensity would be less likely expected (Scheme. 1).

Optimization of FRET-based Gold Nanoprobe. In order to explore the extreme detection capability of the prepared FRET-based sensor, we investigated and optimized the effect of critical parameters, including FITC and D-PC concentrations, pH and incubation time for each step. Initially, the effect of FITC concentration was truly investigated in the range of 0-1.75μM on the fluorescence intensity of AuNPs-FITC in the presence and absence of D-PC(Fig. 3 and Fig. S 1).The optimal concentration of FITC is critical for saturation of the AuNPs surface, whileremaining almost no free FITC in the solution. A FITC concentration of 1.25 μM was found to be optimum for saturation of AuNPs surface as the fluorescence intensity rises dramatically at higher concentrations of FITC that can be explained by increases in non-adsorbed FITC. Additionally, the fluorescence intensity was significantly quenched at concentrations below to the 1μM, indicating all of FITC molecules were loaded on AuNPs surface. Thus, the concentration of1μM was chosen as the optimum concentration of FITC for future experiments. In the next step, the optimum concentration of D-PC was estimated. The effect of this parameter was investigated by the evaluation of fluorescence intensity of FITCs connected to AuNPs at different concentrations of D-PC (0-18.75 μM) in the absence of copper ions. As shown in Fig. 4, the fluorescence intensity remain almost constant with the increase in the concentrations of D-PC above the 6.25μM. we inferred that 6.25 μM of D-PC is the minimum required concentration to release almost all of the adsorbed FITC molecules at experiment condition.

We found that pH of solution has strong influence on the fluorescence signal intensity of the released FITC molecules due to the presence of pH sensitive functional groups in D-PC (carboxyl and amine) and FITC (carboxyl).This is demonstrated in Fig.5, where the difference between fluorescence intensity of the released FITC in presence (F) and adsorbed FITC in absence(F_0)of copper ion is plotted versus the pH buffered in the range of 6 to 10. The pH range was chosen based on the fact that in acidic pH values, the two carboxylic acid groups of FITC are in their acidic forms, thereby the sensor stays turn-off. Also, at alkaline pH values (pH>9), the copper ions exist in a hydroxide form, $Cu(OH)_2$.In Fig. 5, the larger values of F-F_0 refers to higher sensitivity of the sensor for detection of copper ions and this was achieved in optimum pH 8.0 B-R buffer.

As the last parameter, the variation of fluorescence intensity of FITC vs. time for each step was estimated. The first step is the adsorption of FITC to the NPs surface. Based on decay of fluorescence intensity of FITC in Fig. S 2 (A), this process is usually completed within 5 min after incubation and afterward the sensor is ready to use. The next step is FITC displacement by free D-PC that is almost completed within 25 min according to Fig. S 2 (B). Therefore, the developed methodology is relatively fast as whole detection process is accomplished within 30 min.

Analytical figures of merit. As demonstrated above, the restored fluorescence intensity of the released FITC in presence of D-PC is declined upon addition of copper ion. Based on this approach, we have employed the developed AuNPs/D-PC/FITC sensor for the trace detection of copper ion under optimized condition. As illustrated in Fig. 6, the fluorescence intensity decreases along with increasing the concentration of copper ion in the range of 1−40 nM. There is a linear relationship between fluorescence intensity and Cu^{2+} concentration in two ranges of 1−9 and 10-40 nM. The limit of detection (LOD) for this probe was calculated (3σ) to be about 0.3 nM. In order to study the precision, three independent experiments were made and the results revealed relative standard deviations (RSD%) of 0.34% and 0.24% for 1 nM and 40 nM of Cu^{2+}, respectively.

Interference Study. As Fig. 7 shows, the specificity of the FRET sensor towards copper ions was investigated in the presence of several abundant metal ions. Each metal ion was added to the probe at the concentration of one hundred times greater than copper ions.

Real samples analysis. The concentration of copper ions in several samples such as mining water and biological tissues was tested to assay the effect of matrix complicity on the proposed FRET-based sensor. Appropriate digestion procedures were applied prior to copper detection. Briefly, certain amounts of concentrated nitric acid and Hydrogen peroxide were added to the sample followed by heating up to the 150 ℃ on a hotplate for 2 h [44]. Then, the digests were filtered and brought to the specified volume. The results demonstrated in Table 2, show great potential and feasibility of the developed method for the determination of copper in real samples.

Conclusion

In conclusion, a simple FRET-based nanoprobe for the detection of trace amount of copper ions has been developed. The proposed nanoprobe showed good sensitivity toward the target species (Cu^{2+}) in complex matrix. The system offered a practical potential for trace determination of Cu^{2+}in the presence of a large excess of other metal ions, without the need for any pre-concentration or separation steps. The developed methodology could achieve quantification limit at low level, good linearity accompanied with acceptable accuracy, and reproducibility. In addition, the principle of this assay could also be extended to other metal ions.

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Legend of Figures:

Scheme 1. Schematic illustration of the FRET mechanism for AuNP-FITC in the presence and absence of D-Penicillamine and copper ions

Figure 1. (A)TEM image and (B) DLS size distribution of AuNPs.

Figure2. Overlap of the emission and absorption spectra of FITC and AuNPs.

Figure 3. Effect of FITC concentration on the fluorescence intensity in the presence of 1.5 nM AuNPs at pH=8. At the absence of D-PC (a) and in the presence of 8.5 μM D-PC (b)

Figure 4. Effect of D-PC on the fluorescence intensity in the presence of 1.5 nM AuNPs and 1μMFITC at pH=8.

Figure 5. Effect of pH on the fluorescence intensity of FITC (1μM)-AuNPs (1.5 nM) in the presenceof 6.25μM D-PC (F_0), 6.25 μM D-PC and 1μM FITC (F).

Figure 6. Fluorescence emission spectra of FITC-AuNPs upon addition of 6.25 μM D-PC and different concentrations of Cu^{2+}; in the range of 1-40 nM (insets are calibration curves for copper) at optimum conditions.

Figure 7. (Top) Color changes of probe (FITC (1 μM)-AuNPs (1.5 nM)) upon addition of different metal ions under UV excitation (365 nm) and (Down) fluorescence intensity changes of probe in the presence of D-PC (6.25 μM) at pH=8.0. The blue bars represent the fluorescence response of probe after addition ofvarious metal cations (1μM).The red bars represent the change of the emission that occurs upon subsequent addition of Cu^{2+} (10 nM) to the above solution.

Scheme 1

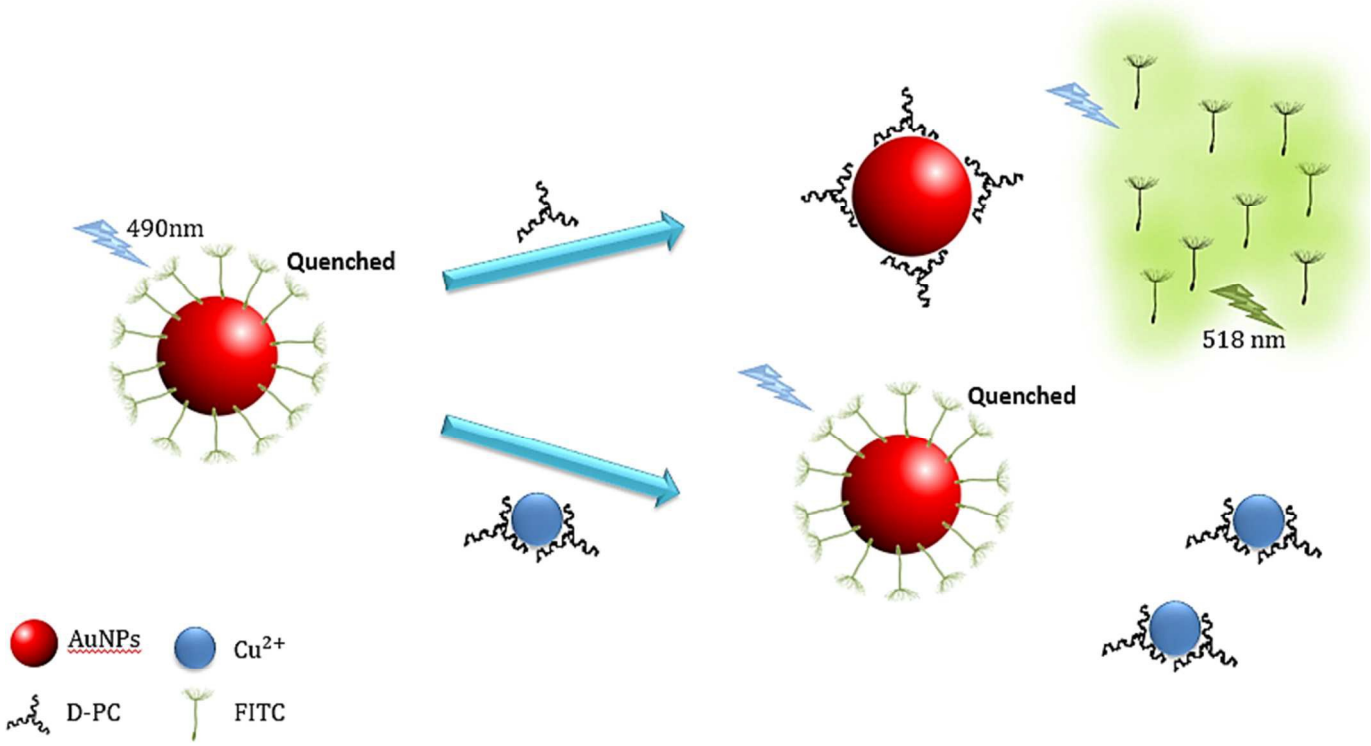
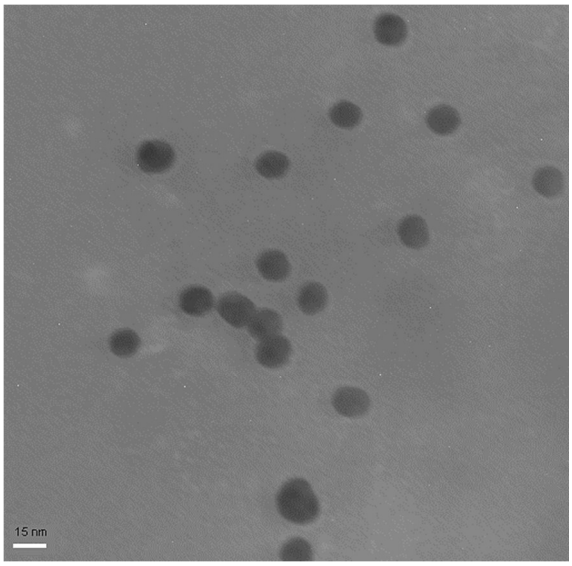


Figure 1

(A)



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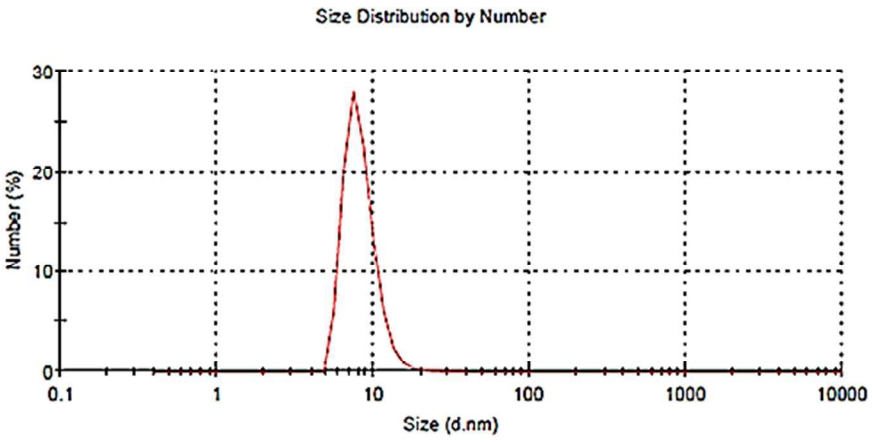

Figure 2

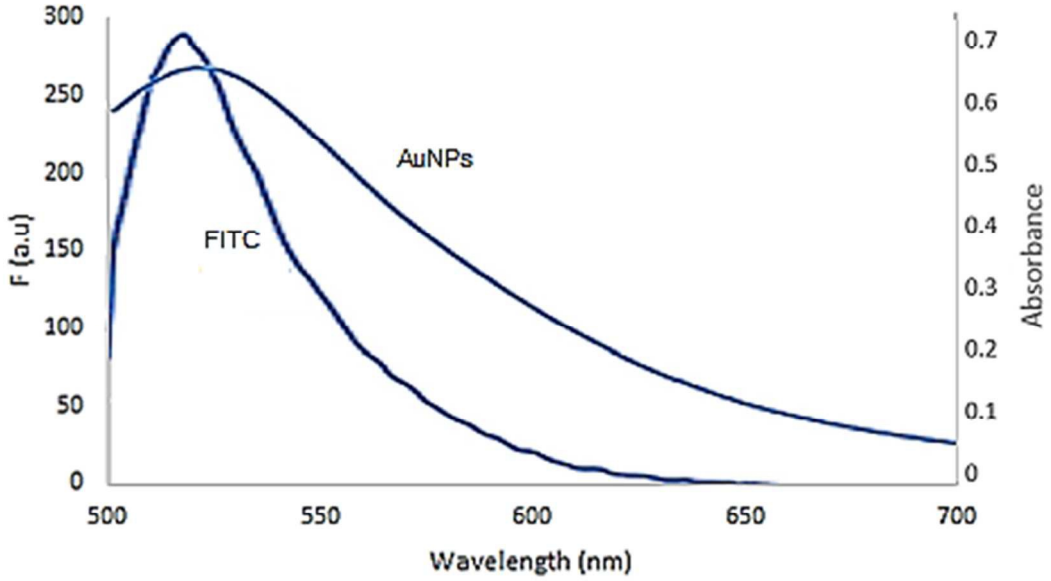

Figure 3

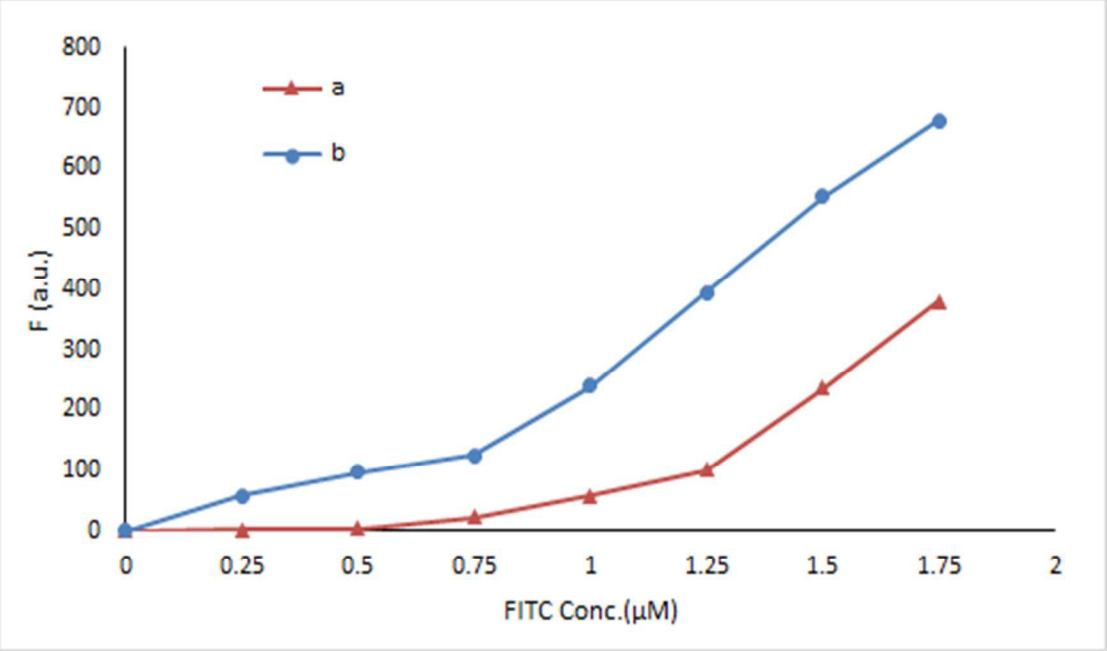

Figure 4

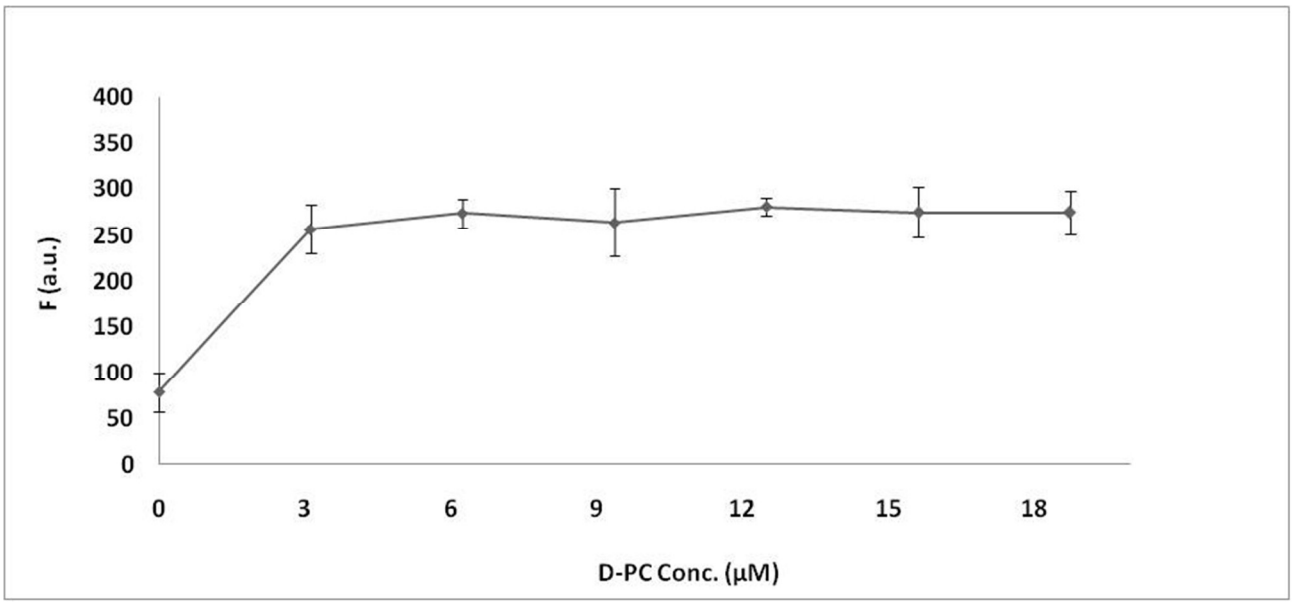

Figure 5

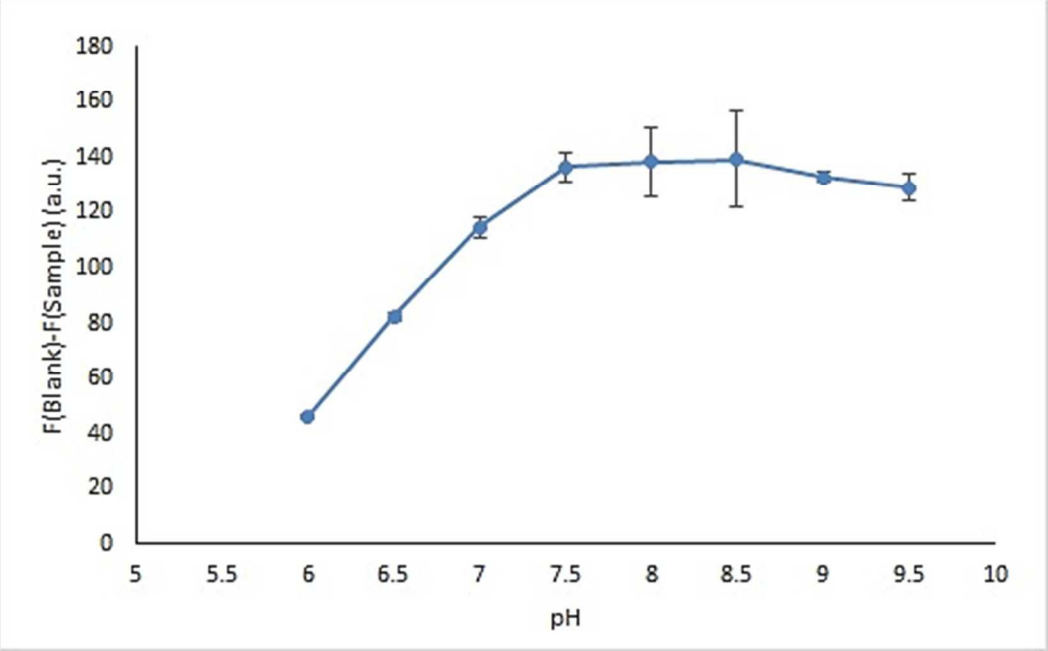

Figure 6

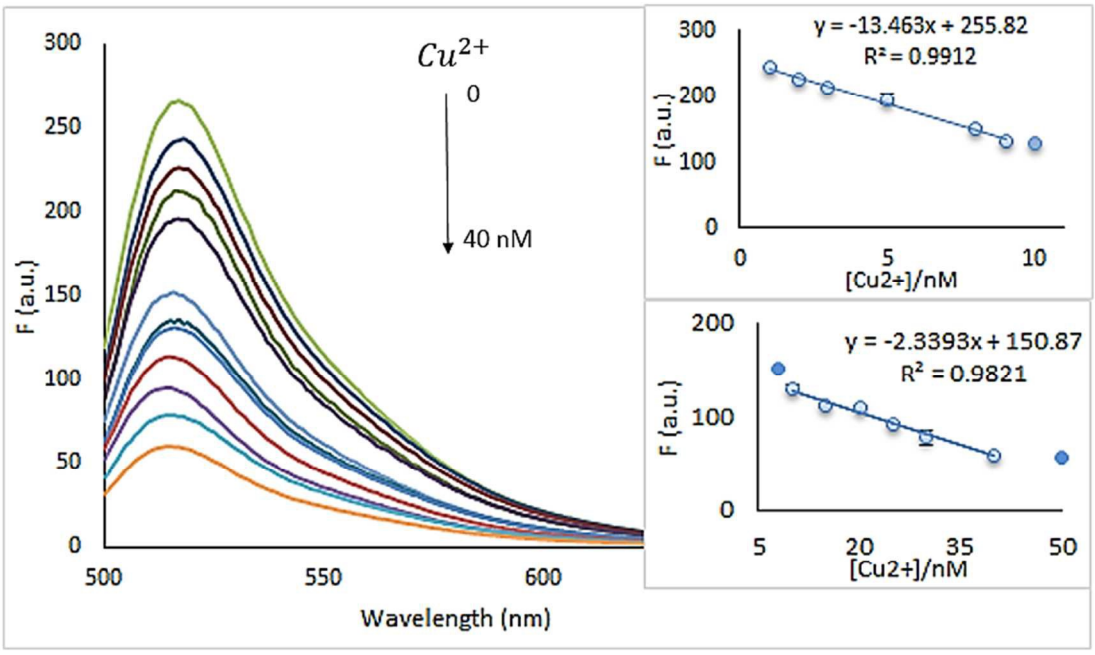

Figure 7

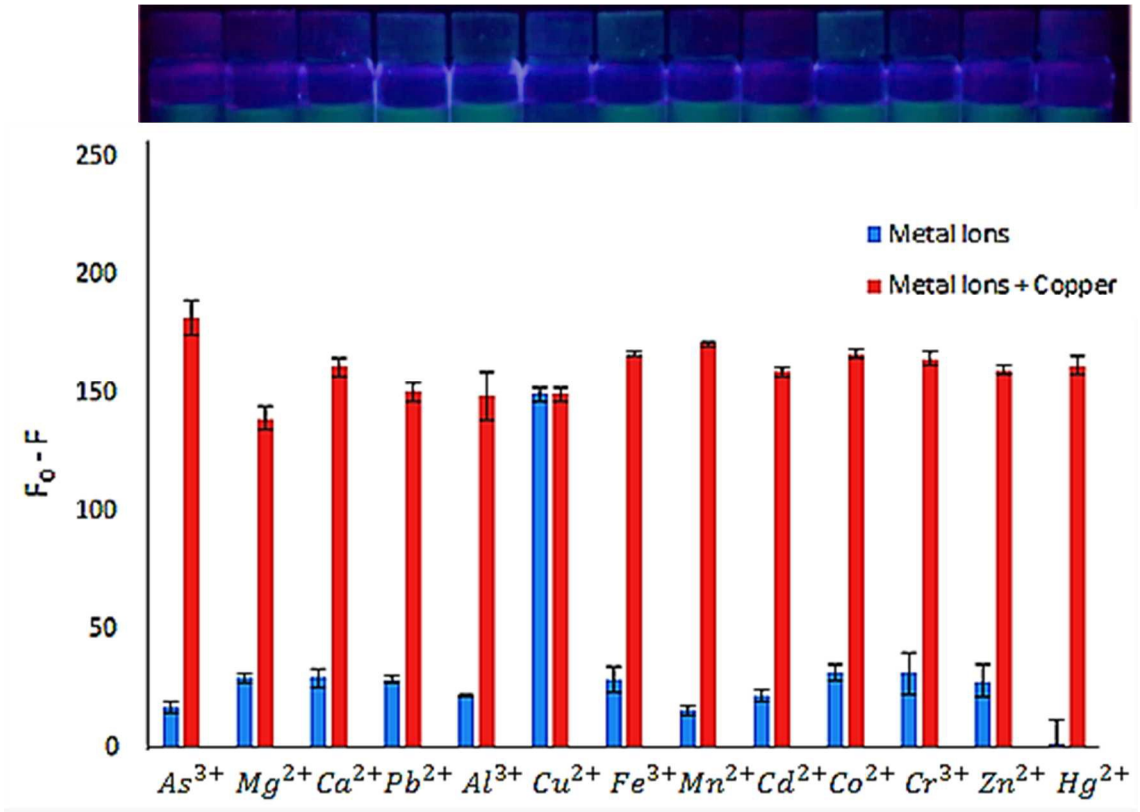

Table 1. Comparison between the developed method and AAS method for application in mining water, liver, sesame and mushroom**.**

Sample	AAS (µM)	Proposed Method (µM)	RSD%	Relative Error%
Mine Water(Sarcheshme)	0.5	0.5	3.0	8.3
Liver	53.0	54.3	4.3	0.6
Sesame	4.8	4.9	5.9	3.8
Mushroom	11.1	12.2	6.2	9.9