

# A Chelation-Enhanced Fluorescence Assay Using Thiourea Capped Carbonaceous Fluorescent Nanoparticles For As (III) Detection In Water Samples

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## Research Article

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# Abstract

Herein, we designed a sensitive and selective “Turn-On” fluorescence nanosensor using water-soluble carbonaceous fluorescent nanomaterials (CFNs) functionalized with thiourea (CFNs-thiourea) for efficient detection of trace amounts of arsenic (III) in aqueous samples. The CFNs and CFNs-Thiourea were characterized by transmission electron microscopy (TEM), UV–Visible spectroscopy (UV-vis) and Fourier transformed infrared spectroscopy (FTIR). The emission peak intensity of proposed nanosensor at 425 nm was gradually enhanced on arsenite addition in wide detection range (3.3–828.5  $\mu\text{g L}^{-1}$ ) attributed to the binding of arsenite species with sulfur groups of CFNs-thiourea. The limit of detection (LOD) was 0.48  $\mu\text{g L}^{-1}$  being much lower than the World Health Organization (WHO) recommended threshold value of 10  $\mu\text{g L}^{-1}$ . Furthermore, the as prepared thiourea -CFNs exhibited an superb selectivity for As (III) compared various cations and anions such as;  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{F}^-$ ,  $\text{Ni}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Mn}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Al}^{3+}$  and As (V) at 100 folds concentration of As (III). The turn on fluorescence nanosensor was successfully exploited for quantification of arsenic in spiked water samples with acceptable efficiencies.

## Introduction

Today, water are getting contaminated in an alarming rate by waste from factories which has converted a major global challenge. Not only does it pose a serious threat to human health but it also endangers ecosystem [1]. One of the most significant causes of water contamination is nothing but heavy metals like arsenic. There is a definite link between long-term exposure to arsenic and a wide variety of health related physical problems such as skin and respiratory diseases and various cancers [2]. According to World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA), permissible levels of total arsenic in drinking water have been reported below 10 ppm [3]. Generally, arsenic exists in several oxidation states including As (III), As (V), As (0) in the environment. Inorganic arsenic species (arsenite As (III) and arsenate As (V)) are more toxic than organic ones (monomethylarsonic acid and dimethylarsinic acid). Furthermore, arsenite is 60 times much more toxic than As (V), via binding to the sulfhydryl unit of proteins, interfering with the reaction of enzymes and other proteins in human metabolism [4, 5]. Since the pollution of water sources with arsenic has irreversible effects on the ecosystem, the design of highly sensitive, efficient and reliable analytical methods for measuring arsenic is imperative [1]. Until now, several reliable conventional techniques have been reported for arsenic detection in water, such as Atomic Absorption Spectroscopy (AAS) [6], Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) [7], high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) [8], ion chromatography (IC) [9], ultraviolet visible spectroscopy (UV-vis) [10], Capillary Electrophoresis (CE) [11], Surface Enhanced Raman Spectroscopy (SERS) [12] and electrochemical methods [13–15], etc. Although these analytical methods are sensitive and accurate, most of them are costly and require complex pretreatment steps, yet they are unsuitable for fast on-site detection [3, 16]. To overcome these limitations of conventional techniques, optical methods (fluorometric sensors) have been extensively developed for simple, sensitive, selective, cost-effective and fast analysis of arsenic [17].

Given that, As (III) compounds have represented a strong affinity for sulfur associated biomolecules such as cysteine and glutathione which has led to the use of sulfur compounds in sensors to arsenic detection [18]. In particular, due to the great catalytic performance, large surface area, high surface reactivity and robust adsorption capability of nanomaterials-based sensors, they have attracted a great amount of attention in the quantification of toxic metals. Numerous of fluorescent nanomaterials such as carbon-based nanomaterials, metallic nanoparticles (NP) and silicon-based materials have been broadly utilized in a wide variety of fields, such as bioimaging, heavy metal detection, nanomedicine, drug delivery, and the fabrication of these kinds of sensors. Among these, carbonaceous fluorescent nanoparticles (CFNs), as a member of the carbon family, have small size, superior aqueous solubility, high photostability and great biocompatibility. Possibility of utilizing a great number of materials in their synthesis is one of the considerable features of CDs. In addition, because of the feasibility of doping different elements on their surface, they offer the multicolor emission [19, 20]. Therefore, CFNs are used in various areas, including fluorescent carbon inks, selective detection of metal ions, and biological labeling [21].

In the present study, we have used thiourea possessing free sulfur groups for functionalization of CFNs surface. Because CFNs have various polar functional groups on their surface, they have a strong tendency to bind to different ligands. A number of sulfur-containing ligands such as cysteine, cysteamine, p-toluenesulfonamide and thiourea was investigated for modification CFNs in order to efficient selective sensing of As (III). The as prepared thiourea -CFNs exhibited good selectivity for As (III) detection in the presence of various common anions and cations at 100 fold concentration of As (III). The application of the proposed assay for As detection in water samples at low ppb level concentration was evaluated and the satisfactory results showed high performance of the developed fluoremetric method.

## Experimental

### Materials

As (III), As (V) stock solution (1000 ppm), L-Histidine, and thiourea were purchased from Merck Company. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS), and dialysis bags (molecular weight cut-off =1000 Da) was bought from Sigma-Aldrich. Triethylenetetramine (TETA) were ordered from Riedel-de Hean. The tap water samples were collected from the Baneh, Sanandaj and Ghorveh, Iran. All solutions were prepared in DI water.

### Apparatus

The surface morphology of the CFNs was characterized by transmission electron microscope (TEM), H600 TEM (Hitachi, Japan). Optical characteristics of synthesized CFNs were measured by fluorescence, LS 55 Perkin Elmer spectrophotometer (Germany). The functionalization of CFNs with thiourea was confirmed using a Fourier transform infrared (FTIR), Bruker-Vector 22 spectrometer (Germany). UV-vis absorption spectra of CFNs and CFNs-Thiourea were obtained from an UV-vis spectrometer, SPECTROD 250 Analytik Jena model from the U.S.A.

## Synthesis of CFNs

CFNs were synthesized using hydrothermal method (Scheme 1). In brief, a mixture of L-Histidine (0.08 g) and triethylenetetramine (74 $\mu$ L) was diluted to 10 mL by deionized water. The mixture was transferred to a poly (tetrafluoroethylene) (Teflon)-lined autoclave (50 mL), and heated at 200 °C for 10 h, resulting in a brown solution, afterwards centrifuged at 4000 rpm for 10 min to remove high-weight carbon aggregates. Then the collected CFNs were dialyzed through a dialysis membrane for 24 h. The obtained brown solution stored for further functionalization and characterization experiments.

## Functionalization of CFNs with thiourea ligand (Thiourea-CFNs)

EDC/NHS is used to activate the carboxylic acid functional groups present at the CFNs surface. Firstly, 0.002 g of EDC and 0.001 g of NHS were added to 500  $\mu$ L of the synthesized CFNs solution. After 60 min of keeping at 4°C, 0.005 g of thiourea was added to the solution. After adding 500  $\mu$ L of 0.01 M phosphate buffer solution (pH 7.4, PBS), the mixture was incubated by thiourea at 4°C for overnight to obtain functionalized CFNs (CFNs-Thiourea). The resulting solution was stored in the refrigerator for subsequent tests for As (III) quantification. Scheme 1 shows how to functionalize CFNs with thiourea ligand.

## As (III) sensing procedure

Briefly, 290  $\mu$ L of 0.01 M PBS (pH 7.4) was added to 10  $\mu$ L of Thiourea-CFNs. Then fluorescence (FL) spectra were recorded at different excitation wavelengths. Next, 1  $\mu$ L of As (III) in different concentration (3.3 to 828.5 ppb) was added to the solution inside the cuvette and FL spectra are recorded and FL intensity changes ( $\Delta F = F - F_0$ ) are calculated at the emission wavelength of 425 nm. As displayed in scheme 1, the fluorescence intensity of Thiourea-CFNs has increased dramatically.

# Results & Discussion

## Characterization of CFNs and CFNs-Thiourea

UV - vis absorption and FL spectra were employed for verifying the optical properties of CFNs and modified CFNs. Figure 1a, clearly shows the UV-vis spectra of the synthesized CFNs and the CFNs modified with the thiourea (CFNs-Thiourea), thiourea, As (III) and CFNs-Thiourea in the presence of As (III). UV - vis absorption spectra in Fig. 1a displayed the strong optical absorption in the UV region (may be corresponding to the  $\pi \rightarrow \pi^*$  transitions) and a tail covering the visible region (may be due to the  $n \rightarrow \pi^*$  transitions of C = O) [22] for CFNs which have changed and shifted after the modification of the CFNs with thiourea ligand verifying the successful modification of CFNs with aforementioned ligand and the covalent bond formation among thiourea and carboxylic acid groups on the surface of CFNs. FT-IR spectra were utilized to identify functional groups on the surface of CFNs and Thiourea-CFNs being in line with suggested molecular structure. The FT-IR spectrum of CFNs exhibits the presence of functional groups such as -COOH, -OH, and -NH<sub>2</sub> on the surface of the CFNs. Therefore, in order to modify the

surface of CFNs, EDC and NHS were employed to activate carbonyl groups and create amide covalent bonds with chemical materials containing  $\text{-NH}_2$  groups such as thiourea [23]. According to the FT-IR spectrum of Thiourea-CFNs, the peak around  $1620\text{ Cm}^{-1}$  is due to  $\text{-C=O}$  stretching vibrations of the carbonyl amide group on the Thiourea-CFNs [24]. The absorption peaks at  $3200\text{ Cm}^{-1}$  and  $3400\text{ Cm}^{-1}$  correspond to the  $\text{-NH}_2$  and  $\text{O-H}$  stretching vibrations. Two bands observed at  $1420\text{ Cm}^{-1}$  and  $1092\text{ Cm}^{-1}$  correspond to the stretching vibration of  $\text{C=S}$  [25]. TEM image was applied to investigate the morphology and size of the synthesized CFNs. According to Fig. 2a, it can be seen that the synthesized CFNs are approximately spherical with an average diameter of  $23.0 \pm 2.7\text{ nm}$  having a relatively uniform distribution. Figure 2b exhibits characteristic excitation and emission spectra of the synthesized CFNs. As seen, the fluorescence emission spectrum of CFNs was recorded at  $425\text{ nm}$  when excited wavelength was set at  $280\text{ nm}$ .

## The Turn on mechanism of arsenite detection

The presence of mercapto groups in addition to amine, hydroxy and carboxyl groups at the nanomaterial surface may result in improving the sensitivity and selectivity of inorganic arsenic quantification [26]. Recently, a various mercaptan ligands like cysteine, glutathione, mercapto-acetic acid, dithioreitol, mercapto-propionic acid, etc., have been exploited for the arsenic determination through stable  $\text{As-S}$  bond formation [15]. Hence, a vast number of sulfur containing ligands (cysteine, cysteamine, p-toluenesulfonamide and thiourea) were investigated in order to construct high sensitive assay for  $\text{As(III)}$  (Fig. 3a). The highest fluorescence signal was obtained using Thiourea-CFNs. According to Scheme 1, the turn on mechanism for detecting  $\text{As(III)}$  by the proposed assay is clearly observed. In the presence of  $\text{As(III)}$ , the fluorescence intensity of modified CFNs is significantly increased due to the selective interaction and coordination of arsenic (III) with sulfur groups of thiourea. According to the hard-soft-acid-base (HSAB) theory,  $\text{As(III)}$ , known as a “soft” acid similar to  $\text{Hg}^{2+}$  [27], preferentially interacted with “soft” base groups existing on the surface of CFNs. The sulfur atom onto the Thiourea-CFNs surface bound to  $\text{As(III)}$ , leading to a stable  $\text{As(III)-Thiourea-CFNs}$  complex by presenting lone pairs into unfilled orbitals of  $\text{As(III)}$ . Usually, Metal-improved fluorescence mechanism is because of surface plasmon enhanced fluorescence (SPEF) or chelation enhanced fluorescence (CHEF). Since, according to Fig. 1a, no obvious change in the modified CFNs adsorption spectra was observed in the absence and presence of  $\text{As(III)}$ , the CHEF mechanism is proved [28]. Furthermore, the formation of a stable complex of arsenic (III) and a thiourea ligand causes increasing molecule hardness and enhancing fluorescence signal.

## Optimization of experimental condition

In order to improve the detection efficiency of the proposed sensor for the  $\text{As(III)}$  detection, some experimental conditions including the effect of different ligands, various excitation wavelength, the concentration of ligand, different pH at the range of 3–10, and also stability of Thiourea-CFNs in the absence and presence of  $\text{As(III)}$  in 60 min were investigated. One of the interesting features of CFNs is

the dependence of their emission spectra position on the excitation wavelength, which has a red shift emission wavelength as the excitation wavelength increases. This property can be because of the broad size distribution of dots (quantum effects) and surface chemistry, various kinds of emissive traps (solvation effect) [22]. As shown in Fig. 3b, when the excitation wavelength is altered from 240 to 400 nm, a red shift of the maximum emission wavelength of CFNs was observed from 410 to 490 nm. As can be seen, the maximum emission response ( $\lambda_{em} = 425$  nm) of the sensor was obtained when the excitation wavelength was 280 nm (Fig. 3b). Moreover, the effect of different concentrations of thiourea ligand (0.5, 1, 5 and 10 mg mL<sup>-1</sup>) were examined for improving performance proposed assay, and the best fluorescence response was achieved for concentration of 5 mg mL<sup>-1</sup> (Fig. 3c).

The pH value of the sample solution plays a crucial role on the fluorescence intensities and is an effective parameter in obtaining a sensitive arsenic sensor. Hence, the fluorescence intensity changes of CFNs-Thiourea in the presence and absence of arsenic (III) at varying pH (3.0–10.0) were calculated. As shown in the Fig. 3d, the designed sensor performance at acidic and basic pH is weaker than neutral pH. At low pH, the amine and thiole groups on the CFNs-Thiourea surface that can interact with metal ions become protonated. There is a definite link between protonating functional groups of ligands and lose their capability to complex heavy metal ions. At high pH values, most of As (III) species are present in the form of metal hydroxides [29]. However, in a highly acidic (<5) or basic medium (> 8), the fluorescence signal change reduced considerably. The highest fluorescence intensity change was observed at the mid-pH range of 6–8 [30]. Therefore, pH = 7 was selected as the optimal pH for As (III) determination in the subsequent tests. Moreover, it is crystal clear that investigating the stability of fluorimetric sensor is a significant feature affecting the reproducibility and accuracy of fluorescence signal. Thus the stability of aqueous solution of CFNs-Thiourea and upon addition of As (III) to present system was examined (Fig. 3e and f). The fluorescence intensities were recorded throughout 60 min that the results exhibited the signal remained relatively stable.

## Analytical performance of the CFNs-Thiourea probe towards As (III)

To prove the applicability of this fluorescence assay, we assessed the response of the CFNs-Thiourea probe towards different amounts of As (III) in the range from 3.3 to 828.5 ppb under optimal conditions. According to Fig. 4a, the fluorescence signal of CFNs-Thioureae was gradually increased as the concentration of As (III) changed from 3.3 to 828.5 ppb. The fluorescence signal changes ( $\Delta F = (F - F_0)$ ) as a function of the As (III) concentration investigated, which F and F<sub>0</sub> are the fluorescence intensities of the CFNs-Thiourea probe in the presence and absence of various concentrations of As (III), respectively. A good linear relationship in the range of 3.3 to 828.5 ppb with regression equation of  $\Delta F = 0.5056CA_s + 22.234$  and a correlation coefficient ( $R^2 = 0.9914$ ) was obtained (Fig. 4b). Meanwhile, the detection limit for As (III) was measured to be 0.48 ppb ( $3\sigma/s$ ), which lower than the World Health Organization (WHO)'s limit for arsenic in drinking water (10  $\mu\text{g L}^{-1}$ ) ( $\sigma$  shows the standard deviation of ten blank measurements,

and  $s$  is the slope of the regression equation). Table 1 displays the performance of the present methodology assessed by comparing with recent reported procedures for measuring of As (III). As presented in Table 1, the sensitivity and linear range of the designed sensor were comparable or more sensitive than the reported methods without the need for complex apparatus signifying that the CFNs-based fluorescent probe has great potential for determination of As (III).

Table 1

Comparison of the performance of presented sensor with different reported sensors for Arsenic detection

Detection system	Nanomaterials	Linear range	LOD	Ref.
Electrochemical	gold nanotextured electrode (Au/GNE)	0.1 to 9 $\mu\text{gL}^{-1}$	0.1 $\mu\text{gL}^{-1}$	1
Fluorescence	Magnetic bead	10 pM to 1 $\mu\text{M}$	2 pM	2
Fluorescence	Carbon quantum dots	5 to 100 $\mu\text{gL}^{-1}$	0.086 $\mu\text{gL}^{-1}$	15
Ratiometric fluorescence	Reduced carbon dot/graphene quantum dot nanohybrid	0.5 to 100.0 $\mu\text{gL}^{-1}$	<0.5 $\mu\text{gL}^{-1}$	30
Electrochemiluminescence	Polydopamine nanospheres	$2 \cdot 10^{-3}$ to $2 \cdot 10^3$ $\mu\text{gL}^{-1}$	$1.2 \cdot 10^{-3}$ $\mu\text{gL}^{-1}$	31
Electrochemical	carbon nanotubes and graphene	1 to 10 $\mu\text{gL}^{-1}$	20 $\mu\text{gL}^{-1}$	32
Colorimetric and smartphone	gold nanoparticles	10 to 800 $\mu\text{gL}^{-1}$	4 $\mu\text{gL}^{-1}$	33
Fluorescence	CFNs-Thiourea probe	3.3 to 828.5 $\mu\text{gL}^{-1}$	0.48 $\mu\text{gL}^{-1}$	This work

## Interference and Selectivity study

To study further the potential efficacy of the sensor, fluorescence intensity changes in the presence of  $7 \times 10^{-6}$  M As (III) and  $7 \times 10^{-4}$  M different interferers ( metal ions -  $\text{Ni}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Al}^{3+}$  and As (V) and also anions -  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ) were measured. As exhibited in Fig. 5, the CFNs-Thiourea probe response was selective towards As (III) compared to other chemical species at a 100-fold concentration of As (III) and interfere species did not cause any significant changes in the fluorescence intensity of CFNs-Thiourea.

## Real samples analysis

To estimate the applicability of the suggested fluorescent sensor, this assay was applied to the detection of As (III) in spiked water samples. The collected water samples from different cities (Baneh, Sanandaj and Ghorveh, Iran) were filtered through a 0.7  $\mu\text{m}$  membrane to eliminate particles. Various amounts (6.32 to 118.01  $\mu\text{g L}^{-1}$ ) of As (III) were spiked on tap water samples and the As (III) ion was measured by the aforementioned method. As revealed in Table 2, the analytical recovery of As (III) in spiked tap water samples were acquired in the range of 90.67–102.0%, with relative standard deviation (RSD %) < 7.0%, demonstrating a reliable procedure for sensing of As (III) ion.

Table 2  
Recovery experiments using the proposed sensor for As (III) determination in the water samples

Samples	Added concentration ( $\mu\text{g L}^{-1}$ )	Found concentration ( $\mu\text{g L}^{-1}$ )	Recovery%	RSD% (n = 3)
Baneh tap water	0	1.08	-	2.3
	10	11.11	100.3	6.09
	30	31.1	100.06	3.7
	60	62.02	101.6	2.8
	80	81.02	99.92	4.75
	100	99.58	98.5	3.68
	120	118.01	97.44	4.2
Qorveh tap water	0	0.56	-	3.2
	20	19.25	93.45	6.79
	30	29.23	95.60	5.42
	40	41.36	102.00	0.67
Sanandaj tap water	0	0.52	-	3.67
	6	6.32	96.67	2.80
	9	9.58	100.67	3.09
	12	11.4	90.67	6.83

## Conclusions

In conclusion, we have introduced a simple, cost-effective, highly sensitive and selective “Turn-On” fluorescence method using thiourea-functionalized CFNs for detection of As (III) in tap water samples of Kurdistan province. More importantly, the fluorescent signal of Thiourea-CFNs assay was considerably increased with adding of As (III) ion, demonstrating the selective and robust interaction of thiol groups



with arsenic ion, which enables to design “turn-on” fluorescence assay through CHEF mechanism. Moreover, the aforementioned assay displays great selectivity to detect As (III) ion at 7  $\mu\text{M}$  over other competing heavy metals. Thus, Thiourea-CFNs assay was successfully employed for the quantitative detection of As (III) in tap water samples and good recoveries (90.67–102.00%) were demonstrated the validity of the recommended nanoprobe for analysis of arsenic in real samples.

## Declarations

### Author Declarations

The authors declare that they have no conflict of interest to the publication of this article.

### Data Availability

Data has been included as electronic supplementary material.

### Code Availability

Not Applicable.

### Authors' contributions

Susan Mohammadi: Carried out the experiment, wrote - original draft, Somayeh Mohammadi: Carried out the experiment, Writing - review & editing. Abdollah Salimi: Supervision, Project administration, Funding acquisition. Rezgar Ahmadi: Supervision; All authors read and approved the final manuscript

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## Figures

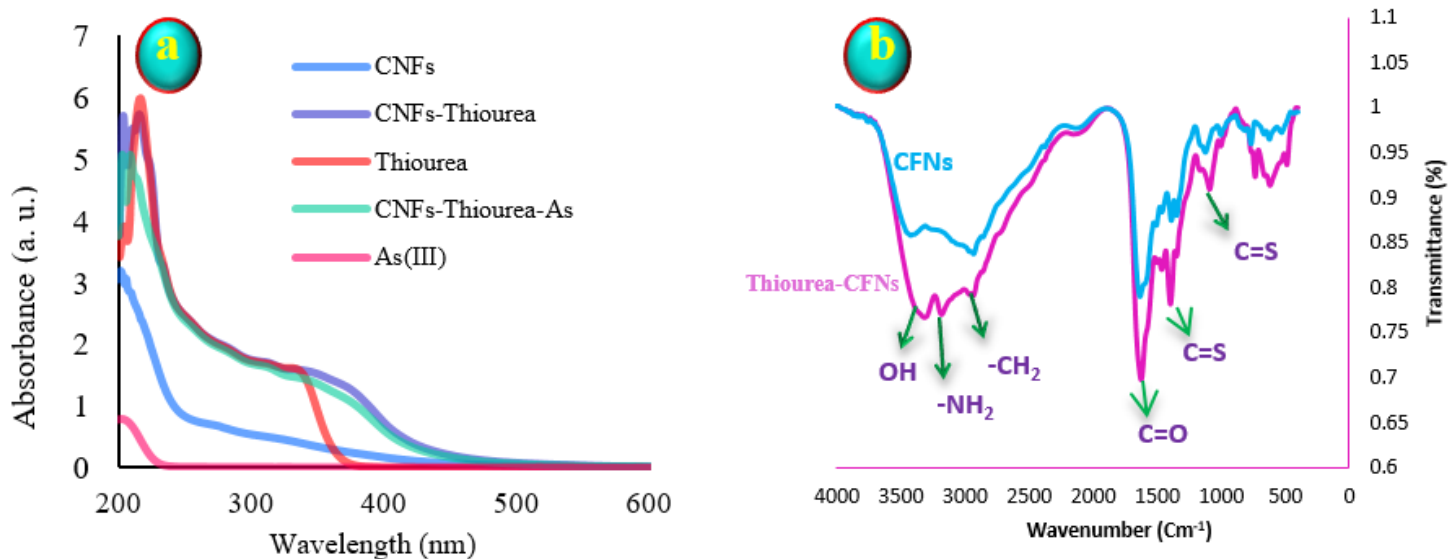


Figure 1

(a) UV-vis absorption spectra of CNFs, Thiourea, CNFs-Thiourea, CNFs-Thiourea-As (III) and As (III); (b) FT-IR spectra of CNFs and Thiourea-CFNs;

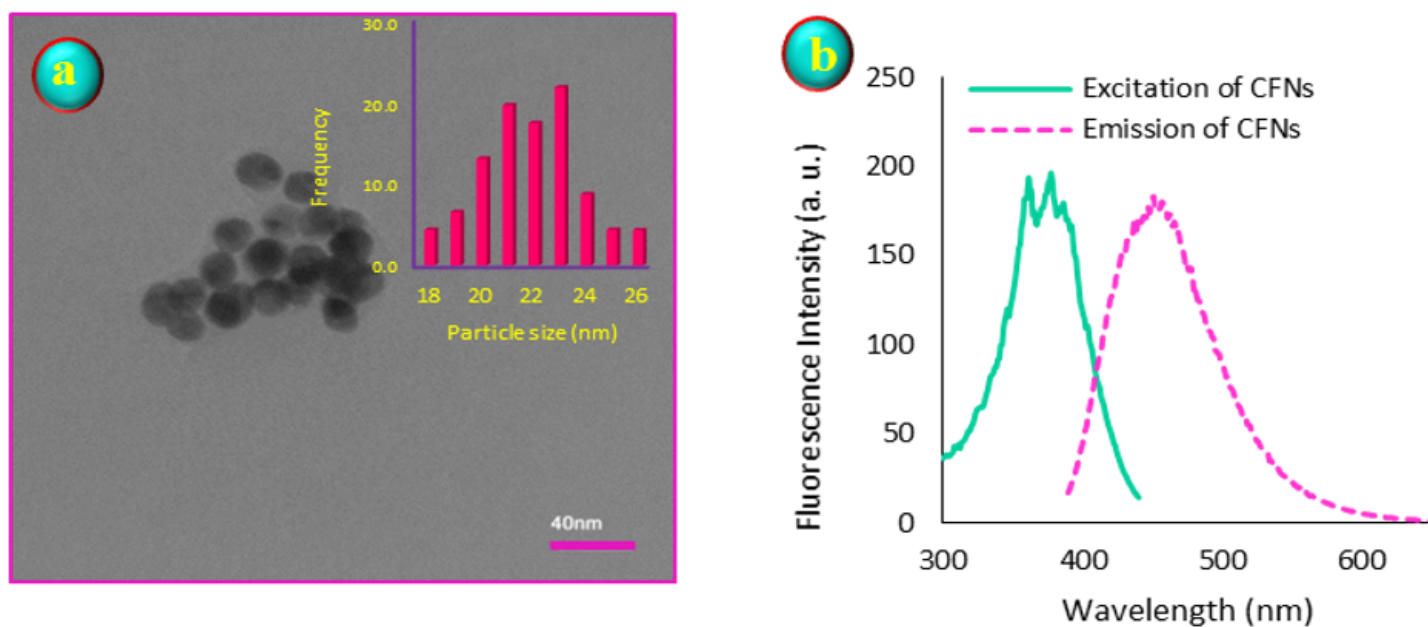
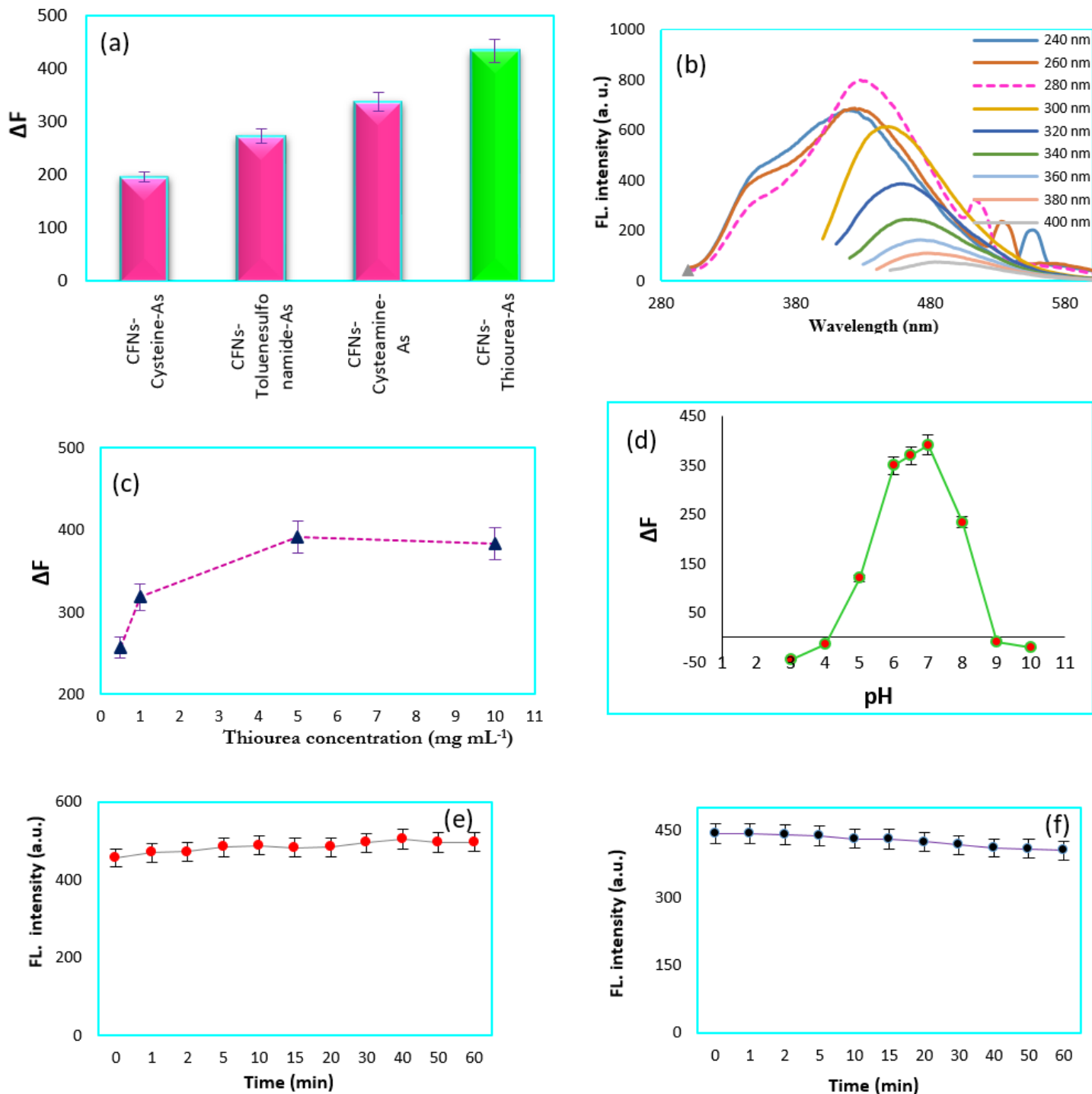


Figure 2

(a) Representative TEM image of the synthesized CFNs and their related particle size distribution histogram was inserted; (b) The typical absorption, excitation and emission of the obtained CFNs.



**Figure 3**

(a) Investigating the effect of functionalization of CFNs with different ligands for arsenic (III) determination; (b) The Fluorescence spectra of CFNs-Thiourea scanned at different excitation wavelengths; (c) The effect of various concentrations of thiourea used to modify CFNs on fluorescence intensity changes; (d) Effect of different pH (range 3-10) on the stability and performance of CFNs-

Thiourea; (e) Effect of time on the stability of CFNs-Thiourea; (f) Effect of time on the stability of CFNs-Thiourea after adding As (III).

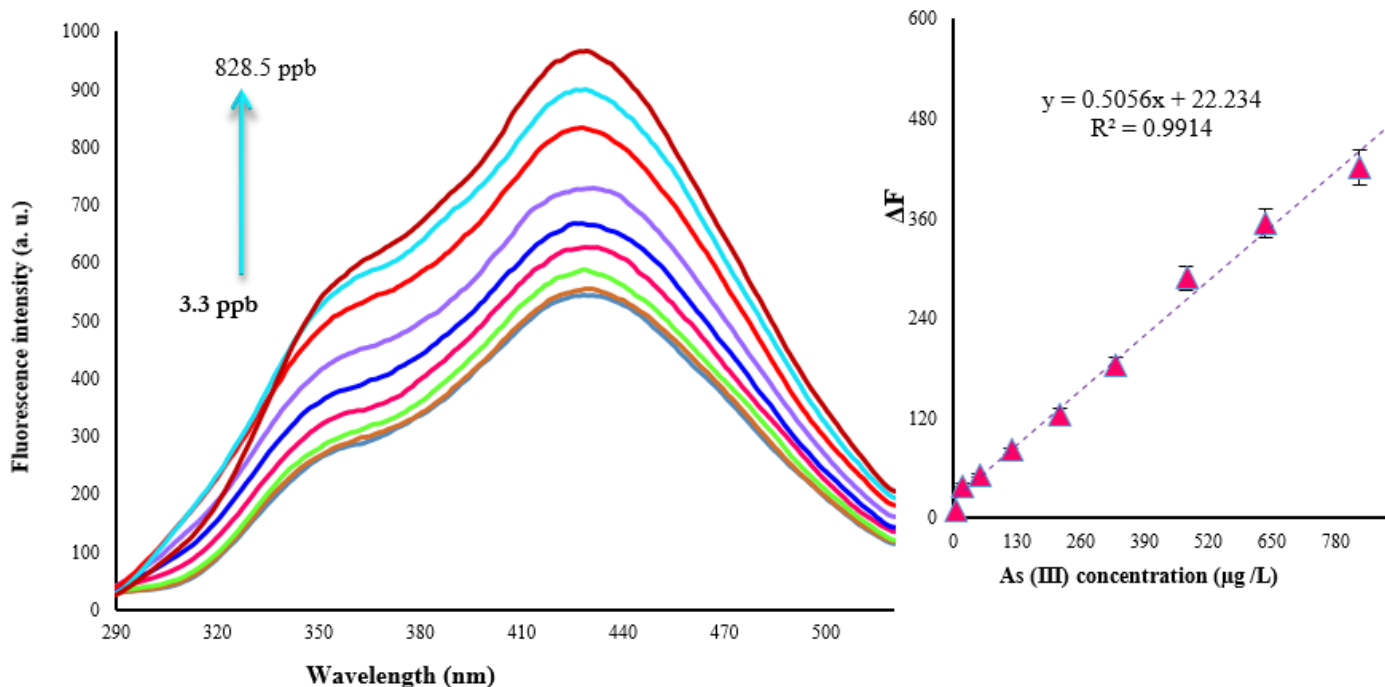


Figure 4

Emission spectra of CFNs-Thioureae probe toward the increasing varied concentrations of As (III) (3.3, 19.9, 52.8, 118.4, 216.4, 330.6, 475.6, 636, 828.5  $\mu\text{g L}^{-1}$ ) and corresponding calibration plot.

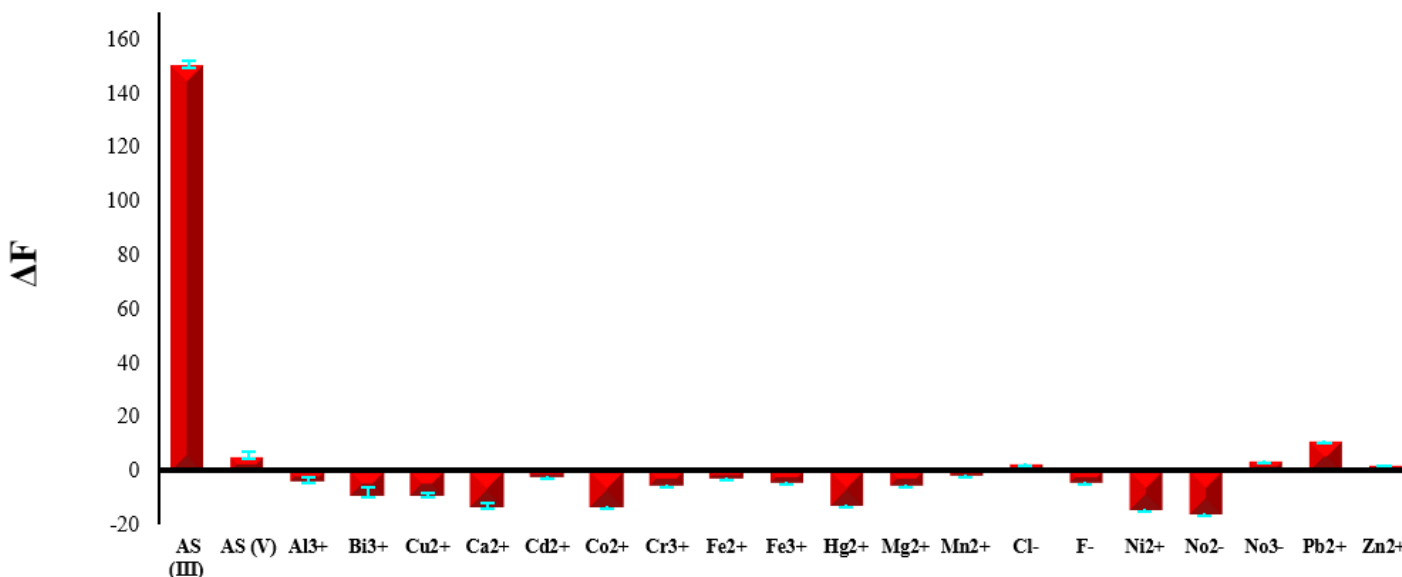


Figure 5

Selectivity investigation of the CFNs-Thioureae probe for As<sup>3+</sup> detection against other metal ions as interfering agents. As<sup>3+</sup>,  $7 \times 10^{-6}$  M; other ions,  $7 \times 10^{-4}$  M. All measurements were managed in triplicate.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)
- [Supplementarymaterial.docx](#)