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RESEARCH ARTICLE

NOVEL METHOD USING O-HYDROXYPHENYLTHIOUREA FOR CADMIUM SENSING IN WATER SAMPLES †

Sunil, A.* and Jagadeeswara Rao, S.

Department of Chemistry, Sri Sathya Sai Institute of Higher Learning (Deemed to be University), Prasanthi Nilayam, Puttaparthi -515134, Andhra Pradesh, India

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ABSTRACT

Cadmium(II) catalyses dimerisation of o-Hydroxyphenylthiourea(OHPTU) to a yellow coloured disulphide, 1,1'-dithio-bis-N-(o-Hydroxyphenyl)formamide. Rate of formation of the coloured dimer increases with the concentration of cadmium(II) ions. Photometric and fluorometric methods were employed to study this reaction at pH 9.0 and the range of determination was determined to be 600ng.mL^{-1} - 30.0ng.mL^{-1} with a detection limit of 20.0**n**g.mL^{-1} using fixed time approach. The absorbance measurements were taken at λ_{max}=416nm and the quenching effect of cadmium(II) on the fluorescence intensity of OHPTU was studied at λ_{Em}=520nm using Stern-Volmer plots. Effects of variation in analytical parameters and interference of associated metal ions in the catalysed oxidation of OHPTU were investigated. This method is fairly selective, sensitive, low in cost and free from pre-concentration procedures. The developed method was successfully applied to the determination of cadmium(II) present in stream water, ground water, fountain water, tap water and sludge water samples.

INTRODUCTION

The anthropogenic emission of cadmium into the environment is rapidly increasing due to its usage in detergents, cosmetics, batteries, electroplating of steel, etc. As a result, the cadmium levels in water, soil and air; particularly in the more populated and industrial areas are rising which is a major concern. Many analytical reagents have been developed for cadmium determination in wide range of environmental and biological samples but, their synthesis involves costly chemicals and multistep procedures. Thioureas and substituted thioureas are used as potential reagents in analytical determinations of metals present in trace levels. These compounds are characterized by potential donor atoms which interact with metal ions in various possible ways. So, based on this nature, many methods have been developed (Fertier *et al.*, 2009; Kato., 2001; Lin *et al.*, 2010; Otazo-Sánchez *et al.*, 2002; Velu *et al.*, 2011) considering their properties in association with metals such as absorbance, fluorescence, electrical potentials etc., utilizing equipments such as UV/VIS spectrophotometer, Fluorescencemeter. This paper presents for the first time the study of o-hydroxyphenylthiourea(OHPTU) system for the determination of cadmium(II). OHPTU undergoes auto-oxidation and dimerises to form an yellow coloured disulphide, 1,1'-dithio-bis-N-(o-hydroxyphenyl)formamide in presence of cadmium(II) ions. It has been observed experimentally that there is a relation between the formation of disulphide and the concentration of cadmium(II). This reaction system has many advantages over many other methods in general. Synthesis of OHPTU is very simple, cost effective and doesn't involve any multistep procedures. There is no need for any oxidizing agent in the reaction system. Normal laboratory conditions are adequate without the need for any pre-concentration procedures. The method is highly sensitive and pH selective. Various activators and surfactants were used in the study to enhance the sensitivity range.Many sophisticated techniques such as electroanalytical (Gupta *et al.*, 2008; Soares *et al.*, 1999; Zhang *et al.*, 1993), inductively coupled plasma-optical emission spectroscopy (ICP-OES)(Moss *et al.*, 2010; Silva *et al.*, 2009; Suleiman *et al.*, 2008), flow injection analysis(Alpízar *et al.*, 1997; Mulaudzi *et al.*, 2002) , atomic absorption spectrometry (AAS), cold vapour AAS or flame AAS–ETA (electrothermal atomisation) (Fries and Getrost, 1977; Gupta *et al.*, 2007; Haase *et al.*, 1998)etc., have been developed in the last two decades for the determination of trace metals in wide range of sample matrices. While the above mentioned methods have some pros and cons, spectrophotometry and spectrofluorimetry are two methods which are convenient, simple and user friendly for trace metal determinations(Ivaska and Kubiak, 1997; Lakovich, 1986; Potts, 1987; Stephenson and Lester, 1987; Tao *et al.*, 1999; Van Staden and Botha, 1999). Usage of photometry and fluorometry may lead to portable hand held devices for onsite monitoring. Affordable instrumentation and cost effectiveness in terms of reagent preparation are added advantages. Hence, this work is the devoted effort towards the development of more effective, sensitive and simple method for the determination of cadmium (II) in environmental and biological water samples.

***Corresponding author:** asunil@sssihl.edu.in;

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Experimental

Reagents, standards and buffers

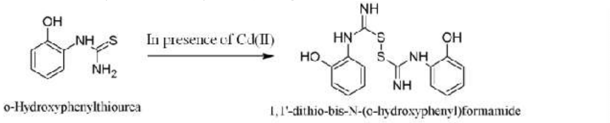
All the chemicals and solvents used here were of analytical reagent grade unless otherwise mentioned. o-aminophenol, potassium thiocyanate, 2-amino pyridine, bathophenanthroline, 1,10-phenanthroline, 2,2'-bipyridyl, pyridine and cadmium chloride hydrate (99.995% trace metal basis) were purchased from Sigma-Aldrich. Solvents (HPLC grade) such as acetonitrile, methanol, ethanol, nitric acid, perchloric acid, hydrofluoric acid were purchased from MERCK. Distilled water from Millipore Mill-Q plus System has only been used in the study. Various buffers were prepared as per the standard procedure prescribed by Vogel(Vogel and Jeffery, 1989). Stream water, underground water, pond water, laboratory tap water and sludge water samples were collected for analysis in the current study.

Instrumentation

The absorbance readings were recorded using UV-VIS spectrophotometer (HITACHI U-2001) with a 10mm path length Hellma quartz cuvette. The fluorescencemeter (PERKIN ELMER LS-55) was used for recording fluorescence measurements with a Perkin Elmer quartz cuvette of 10mm path length. The quantification of cadmium(II) in the unknown samples was also done using atomic absorption spectrometer (VARIAN) and pH meter (MICROPRO) was utilised for pH measurements of buffer solutions in the study.

METHODOLOGY

The reagent, o-Hydroxyphenylthiourea (OHPTU) was prepared as per the standard procedure(Beilstein, 1943). The purity was confirmed by TLC, melting point, UV-VIS and FTIR spectral evidence. OHPTU undergoes auto-oxidation to form a dimer which is yellow coloured disulphide, 1,1'-dithiobis-N-(o-Hydroxyphenyl)formamidine, which was confirmed by the analysis (Feigl, 1966).



The reaction is very slow and the rate of the reaction increases significantly in the presence of trace quantities of cadmium(II). The uniqueness of this reaction is that the monomer to dimer conversion is catalysed by the cadmium(II). The quantification of cadmium(II) was achieved by utilizing this reaction system using spectrophotometric and spectrofluorimetric techniques.

Spectrophotometric method

The λ_{max} of fresh OHPTU and its oxidized product was recorded using UV-VIS spectrophotometer and the spectrum showed a peak at 416 nm which corresponds to OHPTU and its oxidized product respectively. In the current study, the determination of the cadmium(II) was done by monitoring the absorbance at 416nm, the λ_{max} of the oxidized product. It was evident that absorbance at 416nm is proportional to the amount of cadmium(II)(Fig.1).

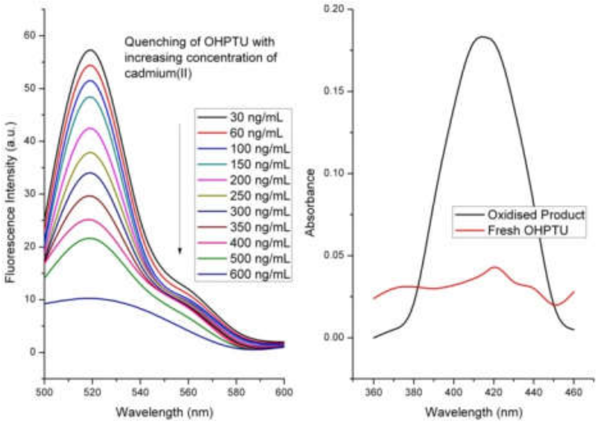


Fig. 1. Fluorescence and UV-Vis spectrum of cadmium(II) catalysed oxidation of OHPTU

Spectrofluorimetric method

The reagent, OHPTU is fluorogenic in nature and the fluorogenic property of OHPTU was studied using fluorescencemeter. OHPTU emits a fluorescence peak at 520nm upon excitation at 416nm. Hence, the emission studies were done at λ_{Em}=520nm for quantitative determination of cadmium(II). OHPTU loses the fluorogenic property upon formation of its oxidized product. As a result the quenching of the fluorescence intensity peak was observed and the fluorescence quenching is proportional to the concentration of cadmium(II) present(Fig.1).

Application: Analysis of cadmium in water samples

Water samples were collected from five different sources and they are stream water, ground water, fountain water, tap water and sludge water. These samples were filtered using whatman filter paper (50:2.7nm) to eliminate any particulate matter and finally digested with concentrated nitric acid to remove the coexisting organic substances. The solution was adjusted to the required pH~9.0 and analyzed, following the recommended procedure for cadmium (II) determination.

RESULTS AND DISCUSSION

Influence of analytical parameters

Effect of pH

One of the most important factors that control the rate of reaction is the pH of solution. An aliquot of cadmium(II) solution (600ng of Cd(II)/mL) was transferred into a 10-mL volumetric flask and to it were added: 1.0mL of buffer, 1.0mL of reagent solution (1mg/mL) and made up to the mark with distilled water. The absorbance of the resulting solution was measured at 416nm (30th minute). The reaction was studied under different varying pH and it was observed that the absorbance was found to be maximum at pH 9.0 (Fig. 2). The inference from this observation is that the catalysed oxidation of OHPTU by cadmium(II) ions takes place at pH 9.0. The reaction is sufficiently fast and the solution was clear when pH of the solution was adjusted to 9.0 with 0.05M borax buffer. Therefore, the further studies were carried out at pH 9.0.

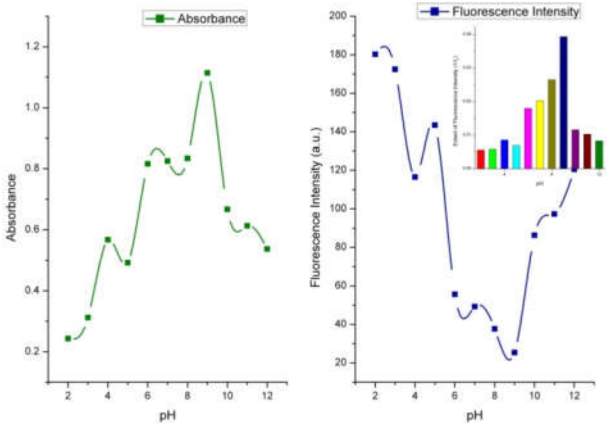


Fig. 2. The behavior of the reaction system [OHPTU (1mg/mL) and 600ng of Cd(II)/mL] at different pHs.

Similar procedure was followed to record the effect of pH on the fluorescence intensity of OHPTU in the presence of cadmium(II) ions (600ng of Cd(II)/mL). Maximum quenching by the cadmium(II) ions is evident from the drop of fluorescence intensity at pH 9.0 (Fig. 2). This infers that the oxidized product of OHPTU catalysed by cadmium(II) was formed at pH 9.0. Hence, further studies were done by maintaining the pH of medium at 9.0.

Effect of temperature and time

It was observed that there was no considerable change in fluorescence intensity and absorbance when the reaction was studied by warming the mixture from 20^0C to 60^0C. Therefore, the reaction was carried out at normal room temperature (25^0C±5^0C). At 30^{th} minute, the absorbance and fluorescence intensity values were stable and remained constant for next three hours. Hence, all the experimental observations were recorded at 30^{th} minute.

Effect of OHPTU concentration

The effect of OHPTU concentration on the catalysed oxidation by cadmium (II) was studied to know the optimum amount of OHPTU required in the study. In the reaction mixture, the rate of auto-oxidation of OHPTU varies with its concentration. Hence, fixing its concentration in the investigation is required. So, the quantity of OHPTU was varied from 0.2-10.0 mg in 10.0 mL of the solution under investigation and the experiment was reiterated as described above.

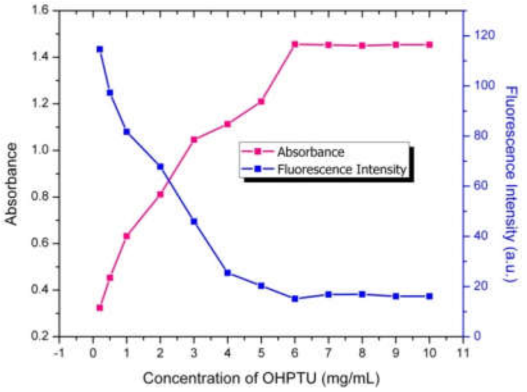


Fig. 3. The effect of OHPTU concentration on the reaction system [600ng of Cd(II)/mL and 1.0mL of pH~9.0 solution]

The concentration of reagent was fixed as 6.0mg in the investigation, as there was no significant increase in the rate of oxidation when the concentration of the reagent is 6.0mg and above, as evident from both the absorption and fluorescence spectra (Fig. 3).

Effect of activators

In certain catalysed oxidation reactions, the rate of reaction is further enhanced by activators there by increasing the sensitivity. Hence the reaction was studied by addition of different activators like 2-aminopyridine, pyridine, nicotinic acid, diethylenetriaminepentaaceticacid (DTPA), bathophenanthroline, 2,2'-bipyridyl, quinoline, 2-hydroxyquinoline and 1,10-phenanthroline.

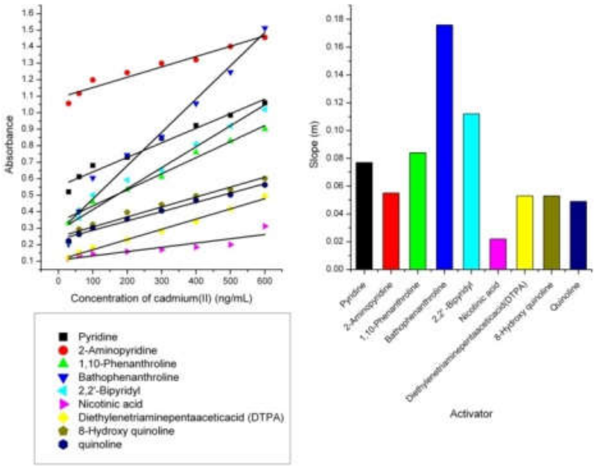


Fig. 4(a). The absorbance study showing the effect of activators on the reaction system [600ng of Cd(II)/mL, 1.0mL of pH~9.0 solution and 1.0mL of OHPTU(6mg/mL)]

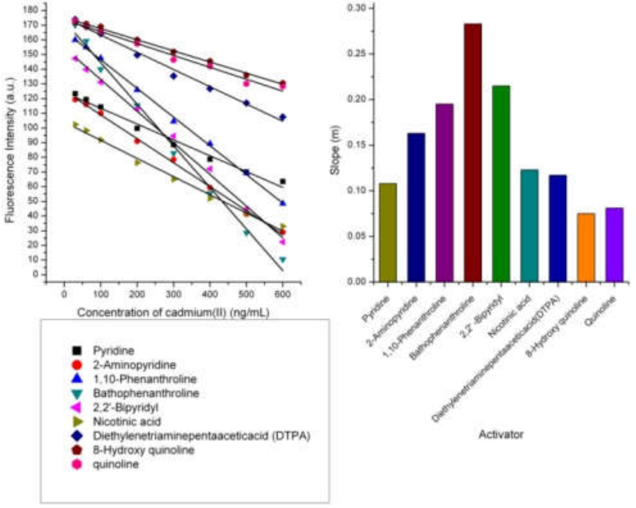


Fig. 4(b). The fluorescence emission study showing the effect of activators on the reaction system [600ng of Cd(II)/mL, 1.0mL of pH~9.0 solution and 1.0mL of OHPTU(6mg/mL)]

These activators were used to promote the catalysed oxidation of OHPTU by cadmium(II). Among these, 1% bathophenanthroline showed a considerable increase in absorption and a maximum quenching effect, than other activators on the rate of reaction, as evident from the slope values (Fig. 4(a) and Fig. 4(b)) of each activator system on the catalysed oxidation of OHPTU by cadmium(II) ions.

Effect of surfactants

One might observe an increase in absorption and quenching effects due to the availability of more stable conditions in presence of micellar medium. Hence, various surfactants like sodium dodecylsulphate(SDS) (anionic), Tween-80 (non-ionic), Triton X-100 (non-ionic) and cetyltrimethylammoniumbromide(CTAB)(cationic) were used in the investigation.

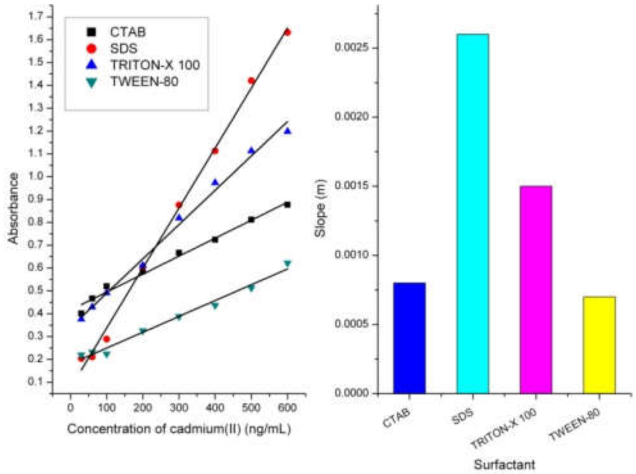


Fig. 5(a). The absorbance study showing the effect of surfactants on the reaction system [600ng of Cd(II)/mL, 1.0mL of pH~9.0 solution, 1.0mL of OHPTU(6mg/mL), and 1.0mL of 1% bathophenanthroline]

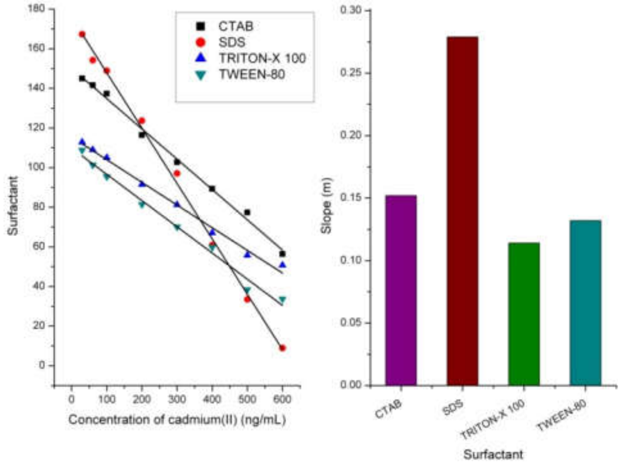


Fig. 5(b). The absorbance study showing the effect of surfactants on the reaction system [600ng of Cd(II)/mL, 1.0mL of pH~9.0 solution, 1.0mL of OHPTU(6mg/mL), and 1.0mL of 1% bathophenanthroline]

A maximum increase in absorption and quenching effects were observed in the case of 1% sodium dodecylsulphate. The slope values for each surfactant system were also recorded (Fig. 5(a) and (Fig. 5(b)). The concentration of sodium dodecylsulphate was varied from 0.5% to 2.0% but, the absorbance and the fluorescence intensity values showed no change. Therefore, it was proposed to use 1% sodium dodecylsulphate as a suitable surfactant system in the current investigation.

Effect of cadmium(II) concentration

Into 10mL volumetric flasks an aliquot of cadmium(II) solutions (800ng/mL-10ng/mL) were transferred. To each flask, 1mL of 1%bathophenanthroline, and 1mL of 1% sodium dodecylsulphate were added and pH was adjusted to 9.0 with 0.05M borax buffer using pH meter. 1mL of OHPTU solution (1mg/mL) was added to each of this mixture in the flask and made up to the mark using millipore water. The study was done by following fixed-time approach, so the flasks were left aside for 30 minutes. In the fixed-time method, the absorbance and the fluorescence intensity of each solution was measured at 30th minute and the observations were shown in figure 6. The calibration curves were plotted for both photometric and fluorometric methods at optimum experimental conditions. A linear response was observed in the range of 600ng-30.65ng of cadmium(II) per mL. The correlation coefficients of the calibration curves, 0.997 & 0.998, for both photometric and fluorometric methods infer a good linearity.

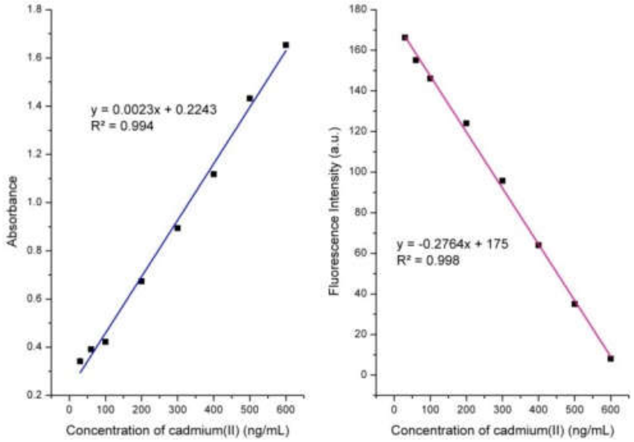


Fig. 6. Calibration plots for the cadmium(II) determination using spectrophotometry and spectrofluorimetry

Quenching by cadmium(II) ions and Stern-Volmer plots

The efficiency of quenching of a fluorescent species by a quencher follows the Stern-Volmer relationship (Wolfbeis, 1993). If the fluorophore and quencher concentrations are in appropriate range, the Stern-Volmer equation is given by $I_0/I = 1+K_{sv}[Q]$ where I_0 and I are fluorescence intensities in the absence and presence of quencher respectively. K_{sv} is Stern-Volmer quenching constant and $[Q]$ corresponds to concentration of the quencher.

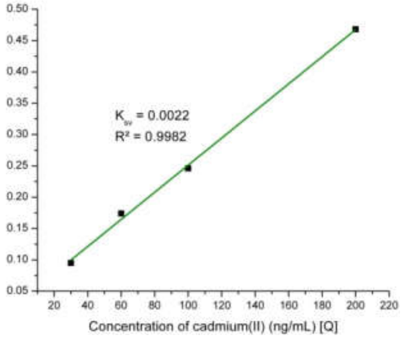


Figure 7: Stern-Volmer plot for the fluorescence quenching of OHPTU by cadmium(II) ions

If a system obeys the Stern - Volmer equation, a plot of (I_0/I)-1 versus *[Q]* will give a straight line with a slope K_{sv}. The S-V plots obtained experimentally determined values of I_0 and I showed that (I_0/I)-1 term linearly increased with increasing concentration of cadmium(II) in the range 200ng - 30ng of cadmium(II) per mL, obeying the S-V relation.

Interfering studies of various cations and anions

The rate of the reaction is enhanced, decreased or inhibited by the presence of certain foreign ions, especially closely associated metal ions. The effect of foreign ions (anions &cations) on the determination of cadmium(II) were studied. Different known amounts of foreign ion solutions were added to 10-mL volumetric flasks each containing 100 ng of cadmium(II), 1.0mL of borax buffer of pH 9.0, 1mL of the OHPTU solution (1mg/mL), 1mL of 1% bathophenanthroline, and 1mL of 1% sodium dodecylsulphate. The absorbance values were recorded at 30th minute. A change of 0.01 units in absorbance value is considered as tolerance limit for interference. In the present method, metal ions like nickel(II), mercury(II), vanadium(V), chromium(III), magnesium(II), aluminium(III), arsenic(III), lead(II) do not interfere even up to 1000 fold excess. manganese(II), cobalt(II), copper(II), iron(II), zinc(II) interfere. Among these, cobalt(II), zinc(II) interfere to the negligible extent. The interference due to copper(II), iron(II), is up to 20 folds. At pH 9.0 the other metals of the group (mercury and zinc) do not interfere. The interference of iron(II) and copper(II) were eliminated by masking with fluoride and thiosulphate respectively. The anions such as chloride, sulphate, oxalate, carbonate, acetate, and nitrate do not interfere in the determination.

Analytical applications: Analysis of stream water, ground water, fountain water, tap water and sludge water samples

The applicability of the proposed photometric and fluorometric methods have been evaluated by determining the trace levels of cadmium(II) in various water sample matrices(Tab. 1) and compared with AAS method. The statistical treatment for the obtained data was done by following student's t-test (at *P*=95%) to show that there is no difference between the proposed methods and AAS method. Also, analysis of variance(ANOVA) (at *P*=95%) was done to show that the variation between the three methods; photometric, fluorometric and AAS is not significant.

Table 1: Analysis of stream water, ground water, fountain water, tap water and sludge water samples

samples	Spiked (ng/mL)	Photometric*	Fluorometric*	AAS*
Stream water 1	3.00	3.03	3.06	3.05
Stream water 2	6.00	6.04	6.01	6.01
Stream water 3	9.00	9.03	9.01	8.99
Ground water 1	3.00	2.98	3.01	3.03
Ground water 2	6.00	6.04	6.01	6.02
Ground water 3	9.00	9.03	9.02	9.04
Fountain water 1	3.00	3.08	3.11	3.10
Fountain water 2	6.00	6.05	6.10	6.09
Fountain water 3	9.00	9.13	9.15	9.11
Tap water 1	3.00	3.03	3.01	3.01
Tap water 2	6.00	6.01	6.01	6.00
Tap water 3	9.00	9.03	9.02	9.02
Sludge water 1	3.00	3.17	3.22	3.19
Sludge water 2	6.00	6.18	6.14	6.15
Sludge water 3	9.00	9.16	9.13	9.12

*Mean of three determinations

The student's t-test analysis show that the *t*-value (0.54) is very small when compared with one-sided critical value (1.72) and thereby we can say that there is no difference between the proposed photometric method and standard AAS method. Similarly in the case of fluorometric and AAS methods, *t*-value (1.22) is small when compared with one-sided critical value (1.72), which lies within the acceptance region. Therefore, we can say that the proposed fluorometric method is also equally reliable with respect to standard AAS method using student's *t*-test. The Pearson correlation value (0.999) infers that there is a high degree of linear relationship between the observations pertaining to the proposed and the AAS methods. The results of ANOVA show that the variation between the proposed analytical methods is not significant because the *F*-value (7.23E-06) is smaller than the *F*-critical value (3.16). To add to this, the *P*-value is larger than 0.05 and therefore, the proposed methods has no effect on the samples taken. Hence, the null hypothesis is accepted and can be concluded that the three methods are equally reliable.

Conclusion

A novel chemosensor for the determination of trace amounts of cadmium (II) in water samples was proposed. OHPTU was used to determine cadmium (II) in very trace levels in the current study using absorption and fluorescence quenching studies. The activators and surfactants used in the study enhanced the sensitivity of the developed method. The Stern-Volmer plots indicate efficient fluorescence quenching and the statistical analysis proves that there is close concordance between the proposed and the standard AAS methods. Therefore, the method is simple, fairly selective with good sensitivity and was applied to determine cadmium in wide variety of samples. Application studies of the chemosensor to various other metals ions are in progress along with a portable hand held device for onsite monitoring.

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