



Eco-friendly Approach for a Facile Synthesis of *o*-Hydroxyphenylthiourea and its Property as an Analytical Reagent in Sensing Mercury (II)

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Available online at: www.isca.in

(Received 9th January 2012, revised 13th January 2012, accepted 17th January 2012)

Abstract

An efficient, eco-friendly synthesis of *o*-Hydroxyphenylthiourea (OHPTU) using microwave and ultrasound assisted methods are described. Green reaction conditions, uniform heating, short reaction time, high yield, high efficiency and convenient work up conditions are advantages of these methods. These merits are in congruence with the green chemistry requirements of energy saving, environmental safety aspects and using nonclassical energy forms. OHPTU undergoes auto-oxidation to form a yellow coloured disulphide, 1,1'-dithio-bis-*N*-(*o*-Hydroxyphenyl) formamide. The reaction is very slow and rate of formation of the coloured dimer increases with concentration of mercury(II) ions. Photometric and fluorometric methods were employed to study this reaction at pH 5.0 and the range of determination was determined to be 800nM–15nM using fixed time method. The absorbance measurements were taken at $\lambda_{max}=416nm$ and the quenching effect of mercury(II) on the fluorescence intensity of OHPTU was studied at $\lambda_{Em}=520nm$ using Stern-Volmer plots. Effects of variation in pH, temperature, reagent concentration; role of activators and surfactants; and interference of associated metal ions in the catalysed oxidation of OHPTU are reported. This method is cost effective, fairly selective, sensitive and there is no need for any pre-concentration procedure. The developed method was successfully applied to the determination of mercury(II) present in stream water, ground water, fountain water, tap water and sludge water samples.

Key words: Green chemistry, *o*-Hydroxyphenylthiourea, microwave, ultrasound, mercury (II), fluorescence quenching, stern-volmer plots

Introduction

Thiourea derivatives are sulfur-containing compounds that have high industrial potential. These are used as powerful reductive bleaching agents in the textile industry, as corrosion inhibitors in industrial equipment such as boilers, sometimes used as a complexing agent for removing scales from water baths also¹. Besides this, several thiourea derivatives have various agricultural and analytical sensing applications which include applications in rubber industries as accelerators², in photography as fixing agents and to remove stains from negatives³, and in agriculture as fungicides, herbicides, and rodenticides⁴. The use of an aqueous solution of thiourea as a leaching agent for gold⁵ has been widely reported in the literature. Thiourea derivatives are also used as spectrophotometric reagents for determination of several metals⁶. Mercury is one of the very toxic elements that are widely spread in the atmosphere, lithosphere, and surface water. The anthropogenic emission of mercury into the environment is rapidly increasing due to its usage in cosmetics, dentistry, batteries, thermometers, sphygmomanometer, etc. The improper disposal of mercury containing products is the main reason for the increase of the mercury levels in water, soil and air; particularly in the more populated and industrial areas, which is a major concern. The toxicity of mercury poses serious problems to human health, as bioaccumulation of mercury in various parts of human

body ultimately leads to neurological diseases. To control mercury pollution and reduce mercury damage to human health, sensitive determination of mercury is important to be aware of its levels.

Many analytical reagents have been developed for mercury determination in wide range of environmental and biological samples but, their synthesis involves costly chemicals and multistep procedures. Novel methods of kinetic activation, which reduces the energy input by optimizing reaction conditions, are currently in need for obtaining environmental friendly processes along with cost effectiveness and saving time. The 'greening' of conventional reactions is done to meet the ever increasing demands of selectivity in modern synthesis⁷. Microwave and sonochemical methods of synthesis use non classical forms of energy to modify the time duration and product yield by avoiding the undesired side products^{8,9}. Microwave heating and sonochemical methods have emerged as a powerful energy and time saving techniques to promote a variety of chemical reactions¹⁰⁻¹⁴. These reaction methods, under solvent-free conditions are eco-friendly by reducing pollution and offer low cost, facile, safe and reproducible experimental procedures¹⁵. Therefore, this paper presents an eco-friendly, facile, cost-effective, one-pot, solvent-free synthesis of *o*-hydroxyphenylthiourea and its analytical property to sense mercury(II) ions present in water samples.

Material and methods

Chemicals and instruments used: All the chemicals used in the current study are Analytical grade purchased from Sigma-Aldrich chemicals Ltd. and all the solvents used here are HPLC grade purchased from Merck chemicals Ltd. The absorbance readings were recorded using UV-VIS spectrophotometer (HITACHI U-2001) with a Hellma quartz cuvette of 10mm path length. The fluorescence measurements were taken using a fluorescence meter (PERKIN ELMER LS-55) with a Perkin Elmer quartz cuvette of 10mm path length and pH meter (MICROPRO) was utilised for pH measurements in the study.

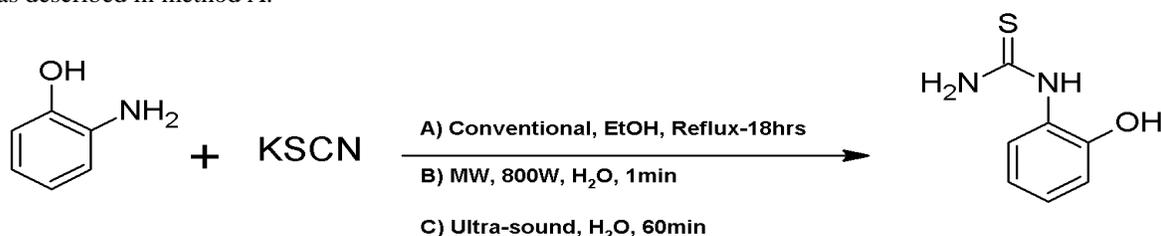
Conventional solution phase synthesis (Method A): o-Hydroxyphenylthiourea (720mg, 47%, white solid) was prepared (scheme-1) by taking 0.017 moles of o-aminophenol in 15ml of ethanol was stirred at room temperature while 2.14mL of 37.4% concentrated hydrochloric acid was added dropwise. The suspension formed was heated to reflux. After all the suspension had dissolved, 0.0255 moles of potassium thiocyanate in 5mL of ethanol was added to the reaction mixture and was stirred at reflux for 18 hours. The precipitate formed upon cooling was dried under vacuum and recrystallized from ethanol.

Microwave assisted solvent-free synthesis (Method B): 0.017 moles of o-aminophenol was taken in an Erlenmeyer flask and few drops of concentrated hydrochloric acid was added and mixed thoroughly (scheme-1). Then, 0.0255 moles of potassium thiocyanate was added to the reaction mixture along with 25mL of water. The contents were subjected to microwave irradiation for 1min at 800W and worked-up as described in method A.

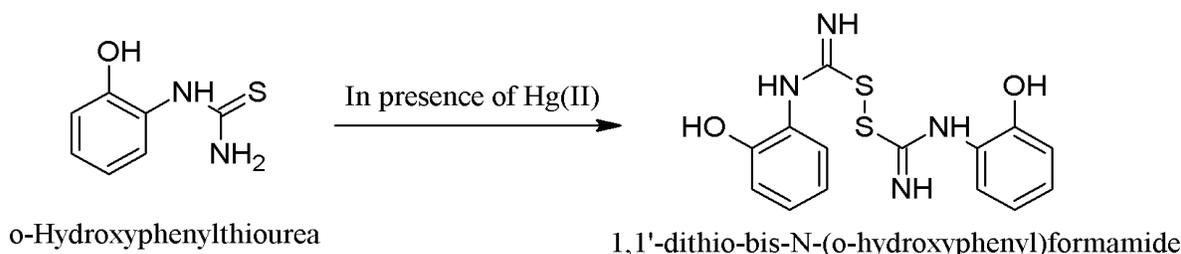
Ultrasound assisted solvent-free synthesis (Method C): 0.017 moles of o-aminophenol was taken in an Erlenmeyer flask and the contents were mixed thoroughly after few drops of concentrated hydrochloric acid were added (scheme-1). Then, 0.0255 moles of potassium thiocyanate was added to the reaction mixture along with 25mL of water. The contents were subjected to sonication for 60min at a temperature of 60°C with 57 Hz frequency and worked-up as described in Method A.

Analytical property of o-Hydroxyphenylthiourea (OHPTU): The reagent, OHPTU has potentialities as an analytical reagent, but it has not been extensively studied. It undergoes auto-oxidation with the formation of a yellow coloured disulphide, 1,1'-dithio-bis-N-(o-hydroxyphenyl)formamide, which is confirmed by the analysis¹⁶.

The reaction is very slow and the rate of the reaction increases significantly in the presence of trace quantities of mercury(II). The uniqueness of this reaction is that the conversion is catalysed by mercury(II) (scheme-2). OHPTU was used as an analytical reagent for the quantification of cobalt(II)¹⁷, copper(II)¹⁸, cadmium(II)¹⁹, etc. and its potential biological activity is also being studied. The greatest advantage of using this reagent as an analytical reagent is its auto-oxidation property and in the process there is no need for any other oxidizing agent. The quantification of mercury(II) was achieved by utilizing OHPTU reaction system using spectrophotometric and spectrofluorimetric techniques.



Scheme-1
Synthesis of o-Hydroxyphenylthiourea



Scheme-2
Catalysed oxidation of OHPTU by Mercury (II) ions

Results and Discussion

The conventional synthesis of OHPTU, which has been reported in the literature²⁰, takes 18 hours for the reaction to yield the product, but using the MWI, the reaction time was reduced to 1 minute from 18 hours with increase in yield from 47% to 92%, which is a markable achievement. The process of sonochemical activation also reduced the reaction time from 18hours to 60 minutes with 71% yield (table-1).

Table-1
Physical data of o-Hydroxyphenylthiourea

Method	Yield(%)	Reaction Time
A	47	18hrs
B	92	1min
C	71	60min

Spectral characteristics: The compound (OHPTU) prepared, was analysed using TLC, UV-VIS, IR and ¹H NMR spectral data. Thin layer chromatogram showed a single spot that shows purity of the sample, UV-VIS spectrum of OHPTU shows a peak at 280nm, which was reported in the literature¹⁶ and FTIR spectra of OHPTU was also examined. This compound showed N—H stretching vibrations in the 3350–3100 cm⁻¹ range. Characteristic C—N stretching vibrations were calculated in the frequency range of 1554–1250 cm⁻¹. The C=S stretching vibrations were discovered in the range of 1190–1150 cm⁻¹, which is in agreement with literature data. Some of the C=S vibration bands overlap with the N—C—N bands. Characteristic C_{ar}—H stretching vibrations of substituted benzenes were observed in the range of 3000–3100 cm⁻¹, and C=C bands were calculated in the frequency range of 1800–2000 cm⁻¹. These bands prove the presence of benzene. ¹H NMR spectra was recorded in DMSO at room temperature in a 400.00 MHz instrument. The chemical shift for N-H proton was found at δ 9.044 and for H-N-H at 4.413. Protons attached to the phenyl ring were observed in the range δ 6.3-6.6.

Absorbance and fluorescence studies: The λ_{max} of fresh OHPTU and OHPTU in presence of mercury(II) was recorded using UV-VIS spectrophotometer and the spectrum showed an increase in absorbance at 416 nm in the case where mercury(II) was added to OHPTU (figure-1), inferring the conversion of OHPTU to its oxidized product.

Hence, in the current study, the determination of the mercury(II) was done by monitoring the absorbance at 416nm, the λ_{max} of the oxidized product. It was observed that absorbance at 416nm is proportional to the amount of mercury(II) present. In addition to this, the reagent, OHPTU is fluorogenic in nature and it emits two fluorescence peaks at 485nm and 520nm upon excitation at 416nm.

Upon addition of mercury(II), the peaks at 485nm and 520nm get quenched with increase in concentration of

mercury (II) ions resulting in mercury (II) catalysed conversion of OHPTU to its oxidized product (figure-2). Using this particular property of OHPTU, the quantification of mercury was done. Hence, the ability to sense mercury by OHPTU has been established in this paper. The fluorescence emission studies were carried out at λ_{Em}=520nm for quantitative determination of mercury (II).

Effect of pH: The pH of solution is one of the most important factors which control the rate of reaction. An aliquot of mercury (II) solution (300nM of Hg(II)) was transferred into a 10-mL volumetric flask and to it were added: 1.0mL of buffer, 1.0mL of reagent solution (5.94mM) and made up to the mark with distilled water. The absorbance of the solution was measured at 416nm (30th minute). The reaction was studied photometrically under different varying pH and mercury(II) catalysed oxidation of OHPTU was found to be more effective at pH 5.0 (figure-3). The reaction was sufficiently fast and the solution is clear when the pH of the solution is adjusted to 5.0 with acetate buffer.

The fluorescence emission studies also show that the catalytic activity of mercury (II) on OHPTU oxidation is more prominent at pH 5.0. Maximum quenching was observed at this pH (figure-3), which shows that the conversion of OHPTU to its disulphide by mercury (II) ions is complete at this pH. Hence, further investigations were done by maintaining the pH at 5.0 using acetate buffer.

Effect of temperature and time: The effect of temperature was studied by warming the reaction mixture. As there was no considerable change in the absorbance and the fluorescence intensity with temperature, the reaction was carried out at normal room temperature (25^oC). The absorbance and the fluorescence intensity values stabilized at 30th minute and remained constant for next three hours of time. Hence, all the experimental observations were recorded at 30th minute.

Effect of OHPTU concentration: The rate of auto-oxidation of OHPTU varies with its concentration in the reaction mixture. Hence, there is a need to fix its concentration in the investigation to know the optimum amount of the OHPTU required for the determination of mercury(II). The amount of OHPTU was varied from 0.5mM-59.4mM in the investigation and the experiment was repeated as described above.

There was no increase in the rate of oxidation when the concentration of the reagent is 5.94mM and above, as evident from both the absorption and fluorescence spectra (figure-4). Hence, the concentration of reagent was fixed as 5.94mM in the investigation.

Role of activators: The rate of the reaction is further enhanced by activators in certain catalysed oxidation reactions, thereby increasing the sensitivity. Hence the reaction was studied by addition of different activators like pyridine, 2-aminopyridine, nicotinic acid, diethylenetriaminepentaacetic acid (DTPA), 2,2'-bipyridyl, bathophenanthroline, quinoline, 2-hydroxyquinoline and 1,10-phenanthroline. Among these, 1% 2,2'-bipyridyl showed a considerable increase in absorption and a maximum quenching effect, than the other activators on the rate of reaction (figure-5).

Effect of surfactants: Due to the availability of more stable conditions in the presence of micellar medium, one might observe an increase in the absorption and quenching effects in the mercury catalysed oxidation of OHPTU. Hence, various surfactants like sodium dodecylsulphate (anionic), cetyltrimethylammonium bromide (cationic), Tween-80 (non-ionic), Triton X-100 (non-ionic) were used in the investigation. A maximum increase in the absorption and quenching effects were observed in the case of 1% Triton X-100 (figure-6). Therefore, it was proposed to use 1% Triton X-100 as a suitable surfactant system in the current investigation.

Effect of mercury(II) concentration on absorbance: An aliquot of mercury(II) solutions (600nM – 15nM) were transferred into a 10-mL volumetric flasks. To each flask, 1mL of 1% 2,2'-bipyridyl, and 1mL of 1% Triton X-100

were added and the pH was adjusted to 5.0 with acetate buffer using pH meter. To each of this mixture in the flask, was added 1mL of the OHPTU solution (5.94mM) and made up to the mark using millipore water.

The flasks were left aside for 30 minutes. The study was done by following fixed-time approach. In the fixed-time method, the absorbance of each solution was measured at 30th minute. A plot of absorbance versus the catalytic action of mercury(II) on OHPTU gives a straight line with a correlation coefficient 0.983 inferring a good linearity (figure-7). The linear response was observed in the range of 600nM – 20nM of mercury(II).

Quenching by mercury(II) ions and Stern-Volmer plots: The quenching efficiency of a fluorescent species by a quencher follows the Stern-Volmer relationship²¹. If the fluorophore and quencher concentrations are in the appropriate range, the Stern-Volmer equation is given by $I_0/I_f = 1 + K_{sv}[Q]$ where I_0 and I_f are the fluorescence intensities in the absence and presence of the quencher respectively. K_{sv} is the Stern-Volmer quenching constant and $[Q]$ is the concentration of the quencher.

Figure 8 shows the stern-volmer plot, the quenching of OHPTU by mercury(II) ions. The plots obtained by experimentally determined values of I_0 and I_f was found to be linear in the range 100nM – 15nM of mercury(II) obeying the stern-volmer relation (figure-8).

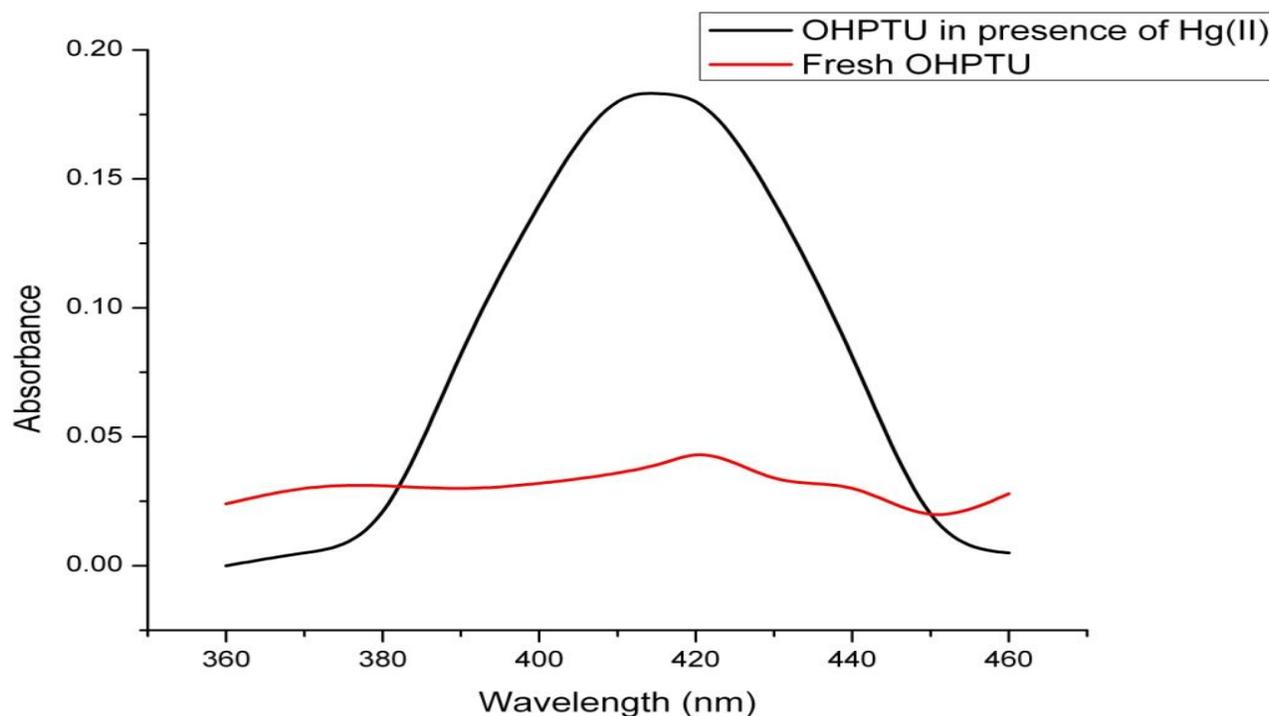


Figure-1
UV-Vis spectrum of OHPTU and its oxidized product

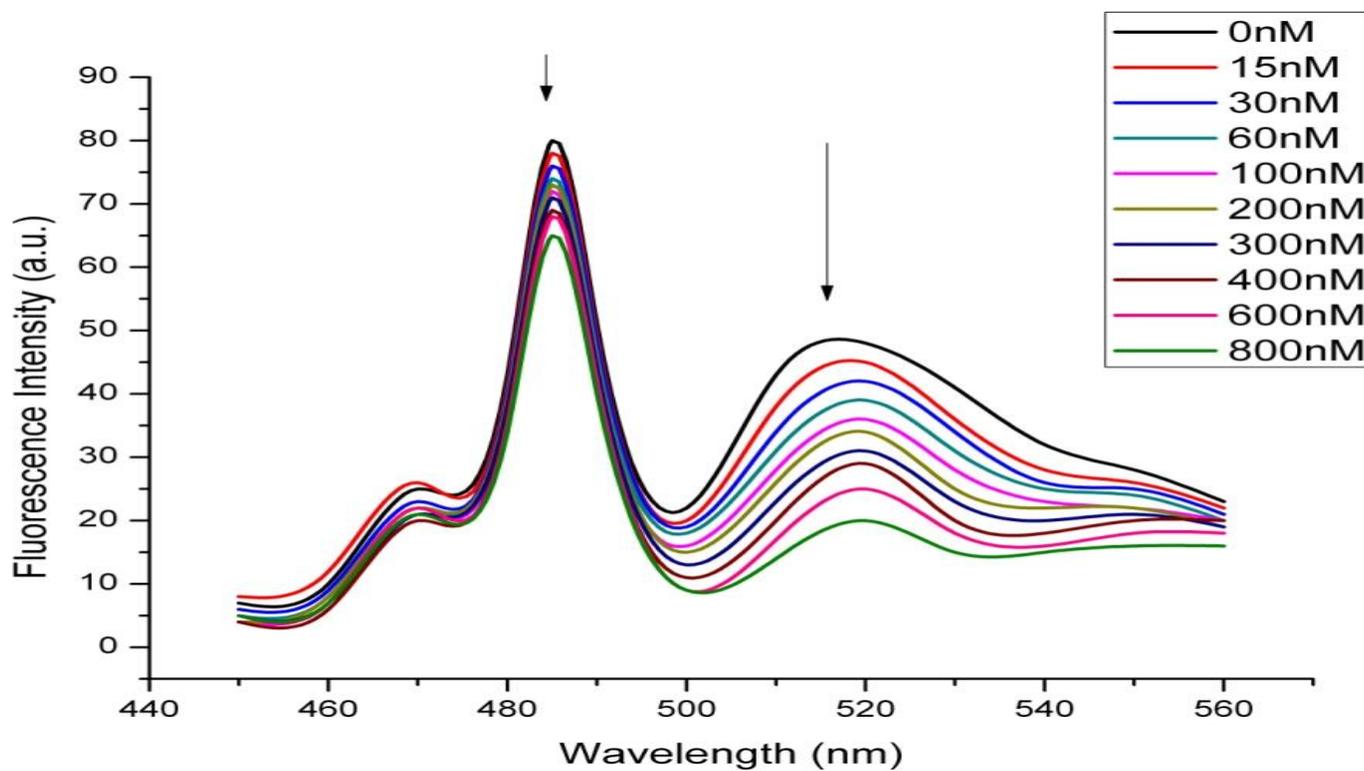


Figure-2
 Fluorescence spectrum of OHPTU quenching by mercury(II) ions

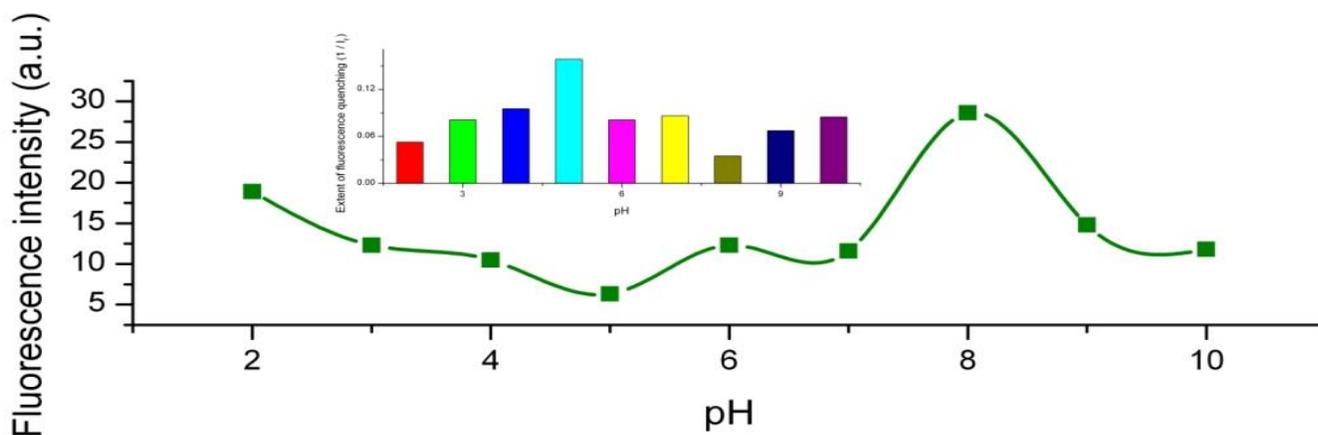
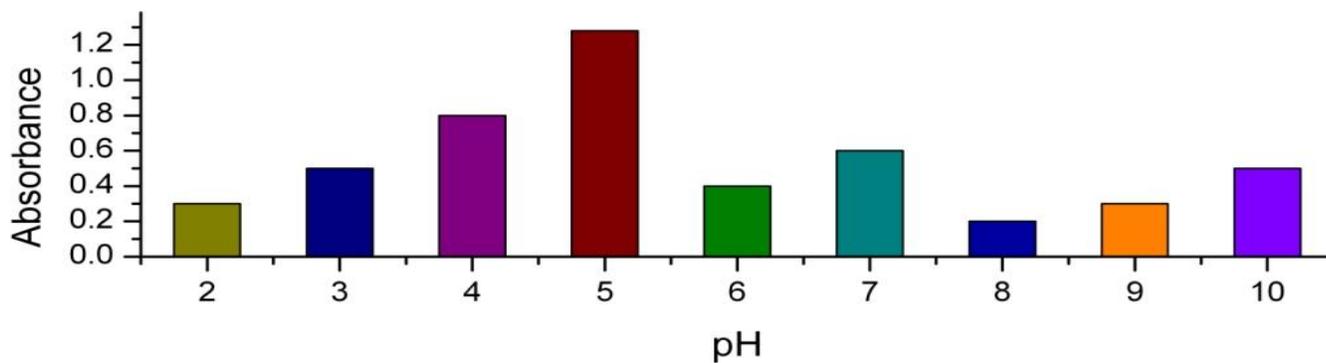


Figure-3
 Effect of pH on catalysed oxidation of OHPTU by mercury(II) ions

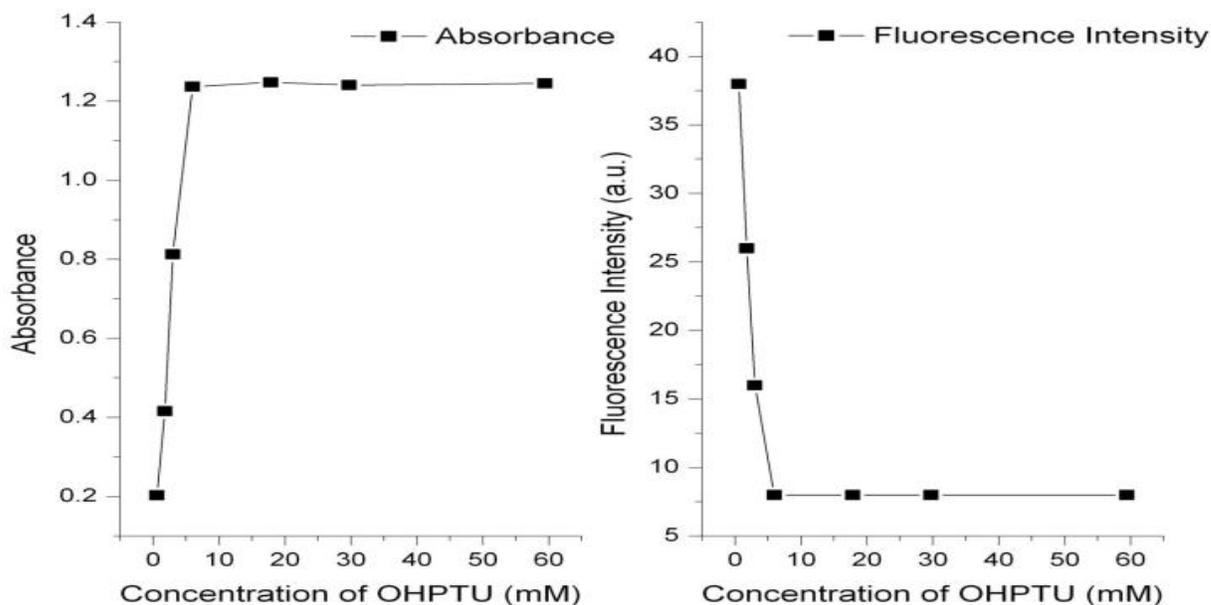


Figure-4
 Plot depicting the optimum concentration of OHPTU required in the investigation

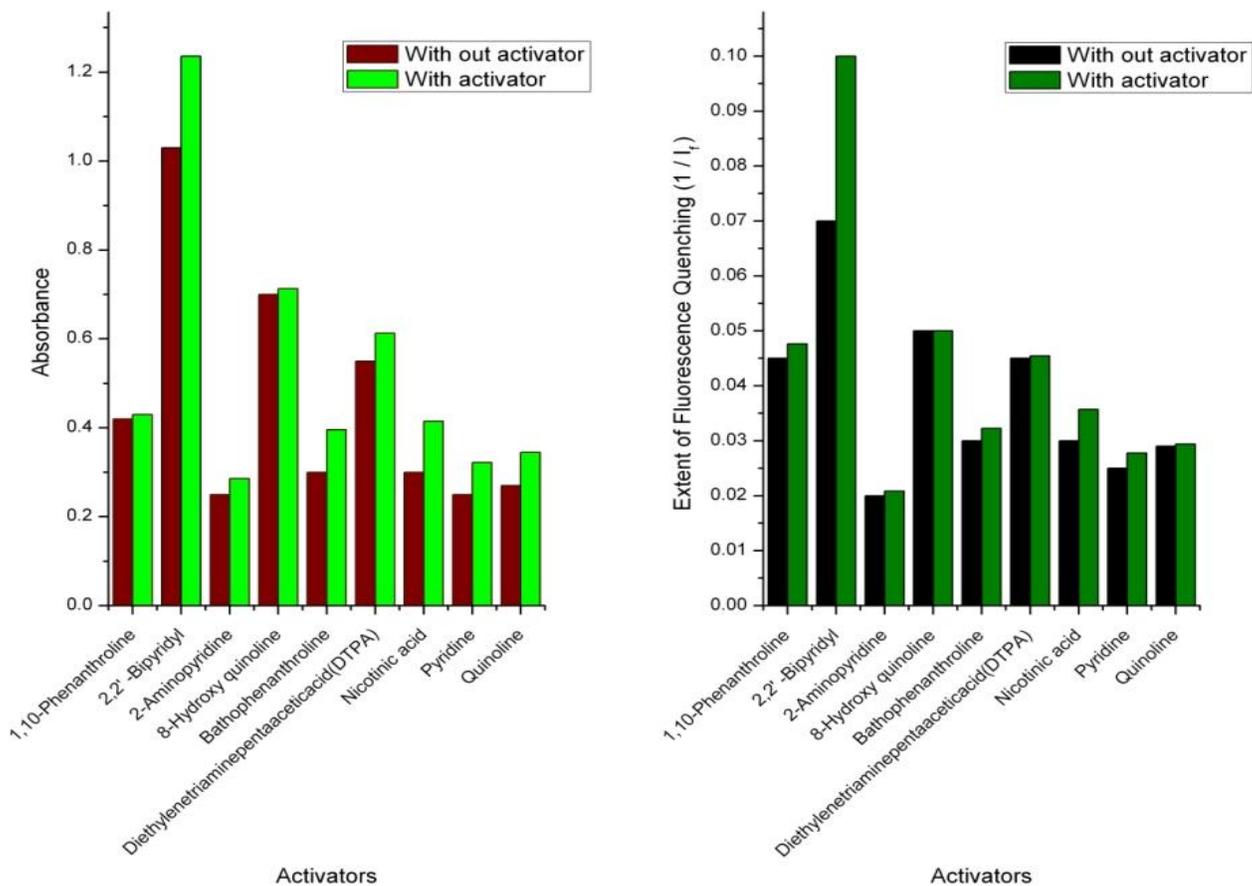


Figure-5
 Effect of activators on catalysed oxidation of OHPTU by mercury(II) ions

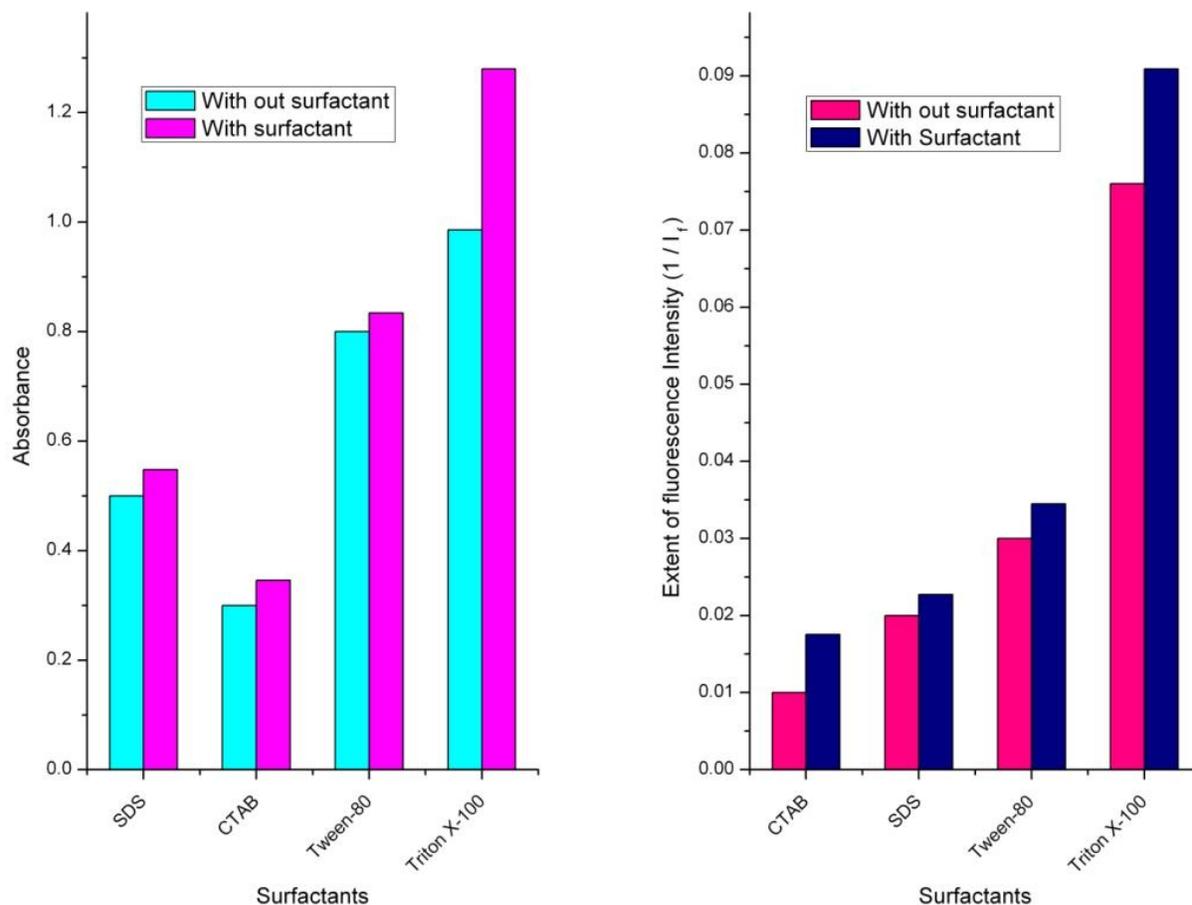


Figure-6
 Effect of surfactants on catalysed oxidation of OHPTU by mercury(II) ions

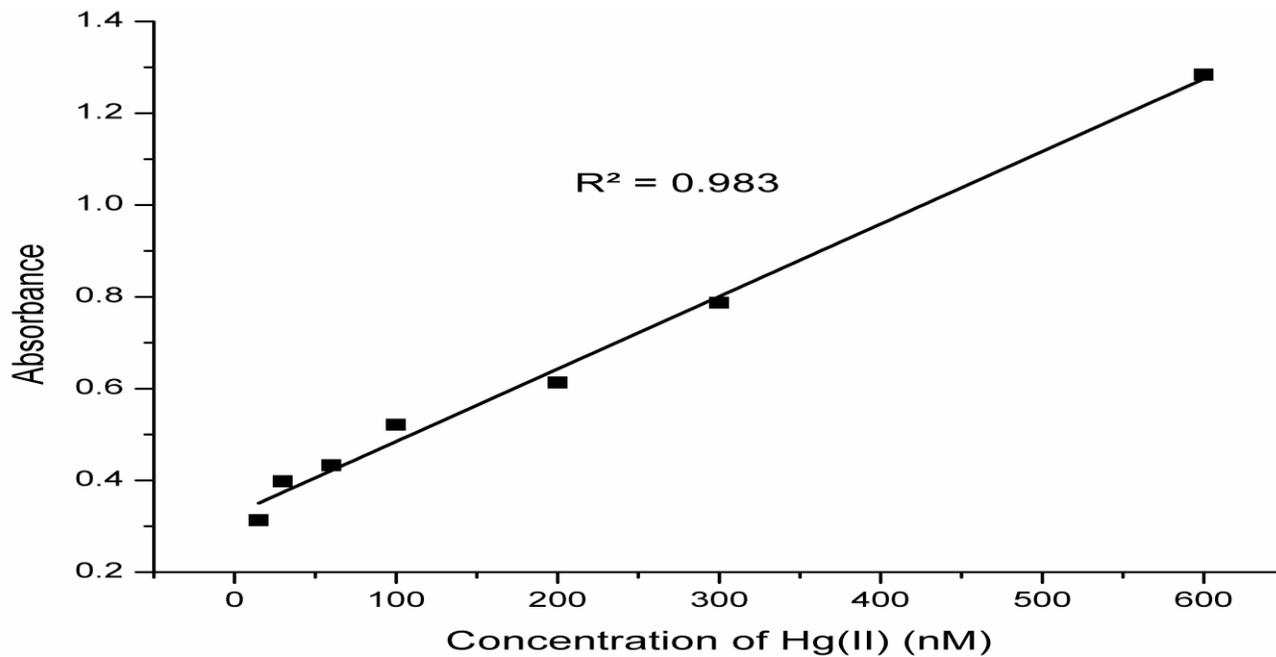


Figure-7
 Plot of Absorbance Vs catalytic oxidation of OHPTU by mercury(II) ions

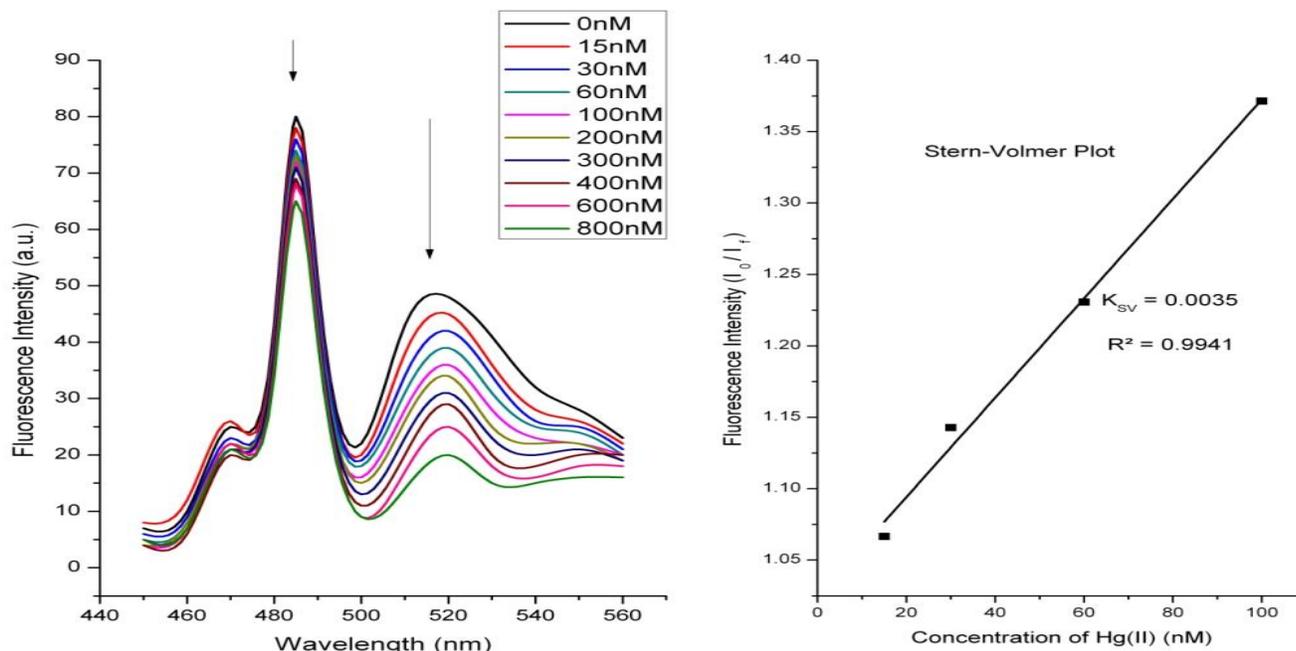


Figure-8
Fluorescence quenching plot of OHPTU by mercury(II) ions at $\lambda_{Em}=520nm$

Interference of 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 and cations) on the determination of mercury (II) were studied. Different known amounts of foreign ion solutions were added to 10-mL volumetric flasks each containing 100 nM of mercury(II), 1.0mL of acetate buffer of pH 5.0, 1mL of the OHPTU solution (5.94mM), 1mL of 1% 2, 2'-bipyridyl and 1mL of 1% Triton X-100. 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), cadmium(II), zinc(II), 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) interfere. Among these, cobalt (II) doesn't interfere upto 800 fold excess. The interference due to copper (II), iron(II), is up to 20 folds. The interference of iron (II) and copper(II) was eliminated by the addition of fluoride and thiosulphate respectively. The anions such as chloride, sulphate, carbonate, acetate, and nitrate do not interfere in the determination.

Determination of mercury in water samples: The developed absorbance and fluorescence quenching analytical methods have been applied to determine mercury(II) present in stream water, ground water, fountain water, tap water and sludge water samples (table-2). The obtained results were statistically treated using student's *t*-test and ANOVA to show that the both methods have no significant difference.

Table-2
Analysis of mercury in water samples

Samples	Spiked(n M)	Photometric Method*	Fluorometric Method*
Stream water	150.0	150.03	150.02
Ground water	180.0	180.01	180.00
Fountain water	90.0	90.06	90.08
Tap water	120.0	120.03	120.02
Sludge water	50.0	51.30	51.50

*Mean of three determinations

Table-3
Statistical analysis of mercury determination in water samples using both proposed methods

Statistical treatment of the data showing NO significant difference between photometric and fluorometric methods	
Student's <i>t</i> -test	- $t_{Stat} (0.92) < t_{Crit} (2.13)$ - Pearson Correlation = 0.99
ANOVA	- $F_{Ratio} < 1$ - p -value (0.99) > 0.05

The student's *t*-test for both the developed methods shows that the statistical *t*-value (0.928) is very small when compared with the one-sided critical value (2.13) (table-3). This proves that the difference between the two methods is not significant. The Pearson correlation value (0.999) infers

that there is a high degree of linear relationship between the observations pertaining to the proposed methods.

The ANOVA: Single Factor analysis shows that the *F*-ratio (1.43E-06) is smaller than 1, which implies that there is no significant difference between the two proposed methods. The *p*-value (0.999) is larger than 0.05 (table-3) and hence we can say that the proposed method has no significant effect on type of water sample chosen. Hence, the developed analytical methods are reliable.

Conclusion

Novel and innovative synthetic techniques that lead to milder operating conditions, shorter reaction times, higher yields and reducing solvent usage or using solvent free conditions are of current interest to many chemists. Focusing all these criteria, a facile one-pot synthesis of OHPTU was achieved using greener techniques by avoiding volatile or toxic solvents and external use of bases at shorter reaction time. Thus, we can say that microwave-assisted organic synthesis has become a boon for a synthetic chemist in designing a rapid as well as controlled, ecofriendly and solvent free synthesis²². Transition metals have played an important role in industrial development and technological advances but unfortunately most metals are accumulating at an accelerated pace in the environment, due to the ever-growing demands of modern society²³.

OHPTU proved its potentiality as an analytical reagent in determination of mercury up to 15nM detection limit. The method was successfully developed by studying various analytical parameters and has been applied to determine mercury present in wide range of water samples.

Acknowledgement

The authors dedicate this endeavour to the Revered Founder Chancellor, Bhagawan Sri Sathya Sai Baba, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam for His constant support and inspiration.

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