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Spectrometric Microfluidic Technique for Determination of Mercury (II) in Water Using Dithizone as Chromogenic Reagent

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Abstract

Mercury is an extremely toxic metal that causes many health problems for Organisms even if it is present in small concentrations. There for It is necessary to detect its levels in water, manage and control mercury levels in water with a simple and fast analytical device there for this paper present a class of microfluidic devices, which is locally manufactured. Microfluidic chip made of PMMA emerged with dithizon,e which was used as a chromogenic agent with high sensitivity for low concentration of the metal ion, the method enhanced with colorimetric analysis using image J software.

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1. Introduction

Mercury is ubiquitous in the environment and exists in three forms: elemental, inorganic and organic mercury. All forms of mercury induce toxic effects in mammalian species [1]. The natural dissolved phase of water contains three primary forms of mercury: elemental mercury (Hg0), organic mercury (monomethyl mercury and dimethylmercury), and inorganic mercury (Hg^{2+} and its compounds). The biogeochemical Hg cycle in Fig. 1 illustrates how mercury is extremely exchangeable for all of these organisms [2]. Mercury sources can be categorized into two types: natural and anthropogenic. Volcanoes and forest fires are naturally occurring sources of mercury. Anthropogenic sources, such as the incineration of municipal and medical wastes and emissions from coal-using power plants, significantly elevate the amounts of mercury found in the environment [3]. Mercury can be introduced into the surrounding environment through both natural processes and human activities, particularly those related to industry. These activities include industrial mineral extraction. Given a comprehensive list of sources that contribute to mercury exposure, also take a look at Table 1 [4]. Various analytical procedures are employed to detect Mercury (II). The techniques used include inductively coupled plasma mass spectrometry (ICP-MS), colorimetry, chemiluminescence, atomic absorption spectroscopy, X-ray fluorescence spectrometry, high-performance liquid chromatography, neutron activation analysis (NAA), and enzyme-linked immune sorbent assay (ELISA) [5]. In the past few years, there has been a growing trend in the development of affordable sensors for detecting mercury on-site, utilizing various techniques [6]. Various analytical approaches have been developed in recent years to precisely determine Hg speciation using enzyme-based sensors. These sensors offer an alternative approach to detecting mercury ions directly [7]. The prevailing laboratory techniques, employing cold vapor atomic fluorescence spectrometry or atomic absorption spectrometry, are highly sensitive and precise. However, they necessitate the use of specialized facilities and equipment [8]. However, a study focusing on the latest developments in mercury analysis in water, particularly after 2010 as indicated in table 2 [9]. There is a need to improve water quality with rapid and fast analytical technique there for a generation of microfluidic devices appear to meet this need. Additionally Microfluidic systems are distinguished by their capacity to manipulate small quantities of analyte through channels that have diameters ranging from tens to hundreds of micrometers. These gadgets emerged using optical detection and electrochemical detection technologies [10]. Microfluidic devices enable precise manipulation of small amounts of fluids, usually a few microliters or less, facilitating chemical or biological analysis on a single chip [10]. LOCs are a subclass of microfluidic devices, LOC abbreviation of Lab-on-chip [11]. Which are defined as miniaturized laboratories that process many specific analysis [16] they are consolidating all the features of traditional laboratory operations onto a single platform, encompassing everything from preparing samples to analysis [12]. The substance that is most widely favored is silica, sometimes known as glass. Glass exhibits optical transparency and electrical insulation properties. On the other hand, silicon is not transparent and forms vertical walls on the sides of its channels in its crystalline form. Polymer-based chips were introduced some years after introduction of silicon/glass circuits [13, 14]. No single material can fulfill all these needs simultaneously. Therefore, typically, a material is chosen based on the desired application. Plastics are inexpensive, pliable, and easily manageable, making them appear suited for a wide range of applications. Nevertheless, the photolithography process employed by Sensors is costly and incompatible with powerful solvent chemicals, hence restricting their range of applications. Conversely, silicon demonstrates favorable chemical and thermal compatibility, but it may be costly, delicate, and impervious to visible and ultraviolet radiation. Consequently, its application in optical-based contexts is restricted [15]. In this study microfluidic chip manufactured locally with PVC using a laser-CO2 machine where the chip was etched. The design has been changed several times to make the injection process easy and efficient also the chip was effective and accurate. However, there is a need for further development in this field [16].

2. Experiment

2.1. Apparatus: The following are apparatuses are used in this study UV/Visible Spectrophotometer (Model Shimadzu UV- 1800 UV/Visible, Japan) double beam, Analytical balance (Sartorius Entries, China).

2.2. Chemicals: All compounds are of the highest quality for analytical reagents, all compounds are of the highest quality for analytical reagents. Stock mercury (II) solution, 1000 ppm prepared by dissolving 1.354 g of mercuric chloride in 1,000 ml of 1 N sulfuric acid. One milliliter of this solution contains 1 mg of mercury. Standard mercury (II) solution prepared by pipetting 5.00 ml of stock mercury solution to 500 ml with 1 N sulfuric acid. Each milliliter of this solution contains 10 /μg of mercury. Working mercury (II) solution dilute 5 ml of standard mercury solution to 500 ml with mercury-free distilled water (1 ml - 0.lμg of Hg). Dithizone, 0.1 percent dissolve 0.500 g of dithizone in reagent-grade chloroform and dilute to 500 ml. Refrigerate. Dithizone, 0.001 percent dilute 5 ml of 0.1 percent dithizone solution to 500 ml with reagent-grade chloroform. Prepare fresh daily. Sulfuric acid, concentrated, reagent grade. Acetic acid, 6 N. Chloroform, ACS grade, bottle

2.3. Producer:

A series of $HgCl_2$ solutions were dissolved in 500 ml of 1N H_2SO_4 to prepare (0.04-4.5 mg/L). For each solution of Hg (II) add 14 ml concentrated sulfuric acid, mix, and cool to 20 °C to 28 °C. To the cooled solution, add 40 ml of 6 N acetic acid and 5 ml of chloroform and Transfer to a 1000 ml separatory funnel. Add10.0 ml of 0.01% (w/v) dithizone solution and shake vigorously again for 1 min Allow the layers to read at 488 nm against a reagent blank.

2.4. Device design and fabrication:

The Microfluidic chip was manufactured locally. The material of the chip from poly methyl methacrylate (PMMA) designed by using a program Adobe Illustrator 2023. The device was intended to give commands to the thunder laser (Model: Hightech HT1390E). The Micro Chip consists of two pieces they are fixed with 4 screws, a base on which the map is engraved with a width of 2 mm and a depth of 2.5 mm and a width of 1mm engraving, and a cover that contains three openings as shown in figure 2A, two of them which are for the sample and the chromogenic agent to enter, and the third is for them to exit together as shown in figure 2B. The Microchip covered with wax in order to overcome the reaction between chemicals and material of microchip. In addition, the detection zone filled with powdered sillicagel to absorb the aqueous layer. Optimization conditions for injection of mercury and sample with reaction time, in order to optimize the volumes both samples and metal solution that give results can see with naked eyes, series of concentrations were experimented. The limit of detection when applied on microchip starting with 0.02 mg/|L of $HgCl_2$. Injection presses applied by filling the tow blue small receivers with sample and reagent by micro pipette and withdraw manually by medical syringe 3 cc. All process Appears in figure 3 A , B , C and D respectively. The processing at 25 °C by utilizing a 100 µL of metal ion and a dithizone volume of 9 µL. For qualitative examination, the device can be visually examined within reaction times 30 seconds, although it required more than 30 second to completely dry. An entirely devoid of moisture apparatus is required to conduct a quantitative analysis scan. Thus, a 5-minute duration was chosen to optimize additional variables, given that the chip had fully dried.

2.5. Volume of metal ion:

It was found that the optimal response time is 30 sec after it was previously tuned using a sample volume of 100 µL this allow to the metal ion solution to completely penetrate the detection zone. As a result, a 100 µL sample volume of the metal ion solution was chosen for further research that may be used to any metal.

2.6. Complexing agent volume:

For the metal ion volume and reaction time, the complexing agent volumes were optimized to 9 µL which give a suitable color with 30 sec as shown in table 3. In light of the detection zone's dimensions, many smaller aliquots were added to obtain the optimal volume of complexing agents. Due to the limited amount of complexing agent, the reaction between the complexing agent and the metal ion was not efficient, resulting in a weaker color intensity. Nonetheless, the detecting zone's color intensity decreased due to an overabundance of complexing agent. This resulted from certain complexing agents in the detection zone having a high hydrophobicity and a high background color intensity. Below the table, 3 are the volumes of the injection mercury (II) solution and dithizone, as well as the time it takes for the color of the Hg-dithizone complex to appear.

2.7. Injection process:

First step was injection with dithizone solution (0.01%) loading in inlet 1 as shown in figure 3 A and injection of mercury (II) solution with interference ions and masking agent solutions with inlet 2 as shown in figure 3 after that the solutions were withdrawn with medical syringe as show in figure 3C, the injection process was slowly and in batches in order to give time for the reaction to complete and the color to appear as shown in figure 3D.

2.8. Image analysis:

Once the appropriate response time was established, the microchip device underwent colorimetric examination and the results were stored in JPEG format. The color intensity of the detecting zones was quantified by analyzing the pictures using Image J software.

3. Result and discussion

Figure 3A displays the UV-visible spectra of Hg-dithizone complexes at various concentrations. The band corresponding to the Hg-dithizone combination is observed at approximately 488 nm as shown in figure 3 . Given that the spectral data range from 190 nm to 800 nm, encompassing a substantial amount of data to be analyzed, the correlation coefficient is utilized to determine the important bands. As a result, the computational workload is decreased without sacrificing any information, thereby achieving the goal of rapid and effective detection.

3.1. Factors effect on the absorbance

i. Effect of pH

Sulfuric acid H_2SO_4 was included in the suggested protocol for the spectrophotometric analysis of mercury in aqueous solution with dithizone, which was previously determined to be the most appropriate acid for mercury determination. The pH of the solution before color development was varied (all with 1 mg/l of Hg^{+2} from 2 to10 Maximum absorptivity was obtained at pH 0.3(1N in sulfuric acid). The absorbance decrease with increasing in pH as show in figure 5.

ii. Effect of Solvent

Chloroform, benzene, and 1,4-dioxane were tested as solvents for the system. Chloroform was the ideal solvent. All samples of Hg (II) were 1 mg/L tested with dithzone dissolved in these three solvents. absorbance was observed in the organic phase with the 1,4 –dioxane , benzene and chloroform as shown in figures 6-8.The change in peak position of the absorption band of dithizone in different solvents is an indication of the interaction of the probe molecule with the solvent with a change in solvent polarity, the absorption band of dithizone was shifted. As the figures show some of bands shifted to higher wave length and the absorbance intensity increased when solvent changed due to changing in polarity. The most polar solvent 1,4-dioxane more polar than benzene but chloroform is the most polarity from three solvents.

iii. Effect of time

The response exhibits rapid kinetics, reaching a steady absorbance within 30 minutes, which remains unchanged for a duration of 2 hours as shown in figure 9.

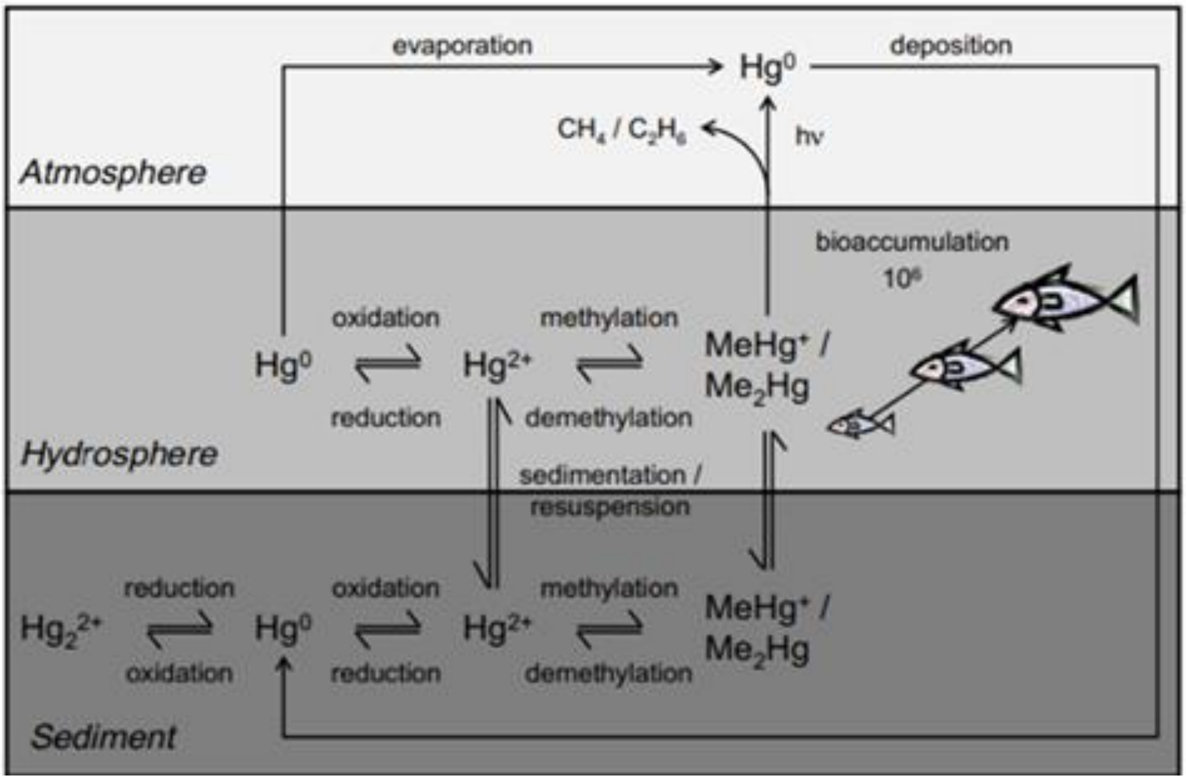
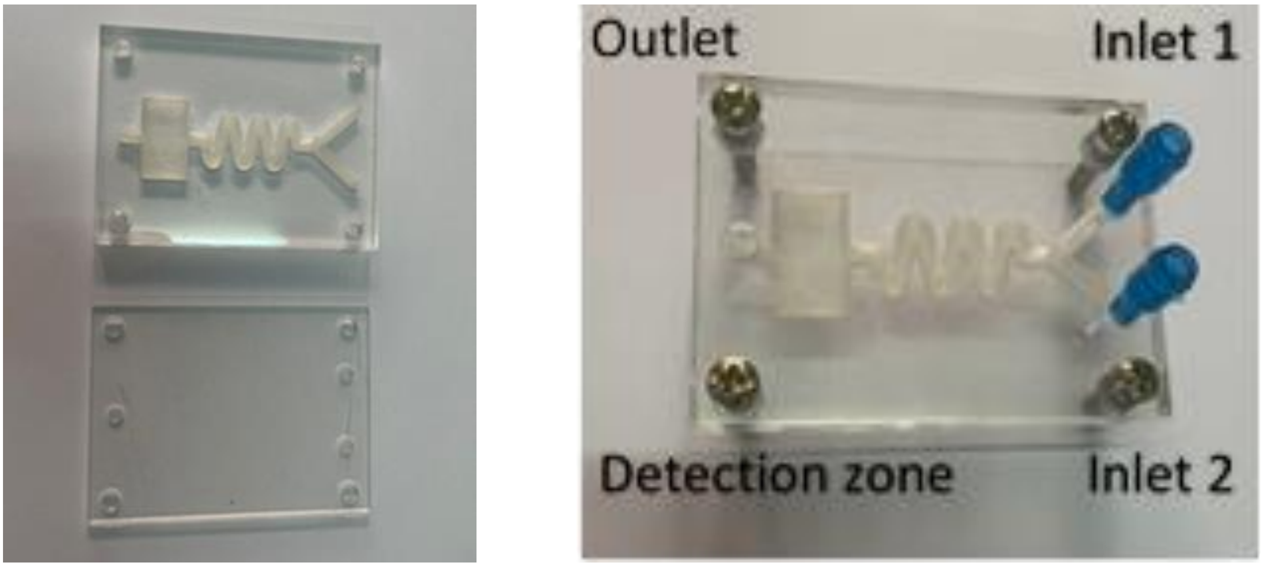


Figure 1. Biochemical mercury cycle.



(A)
(B)

Figure 2. (A) (Microfluidic chip with the cover. (B) Microfluidic chip and the cover fixed with four screws

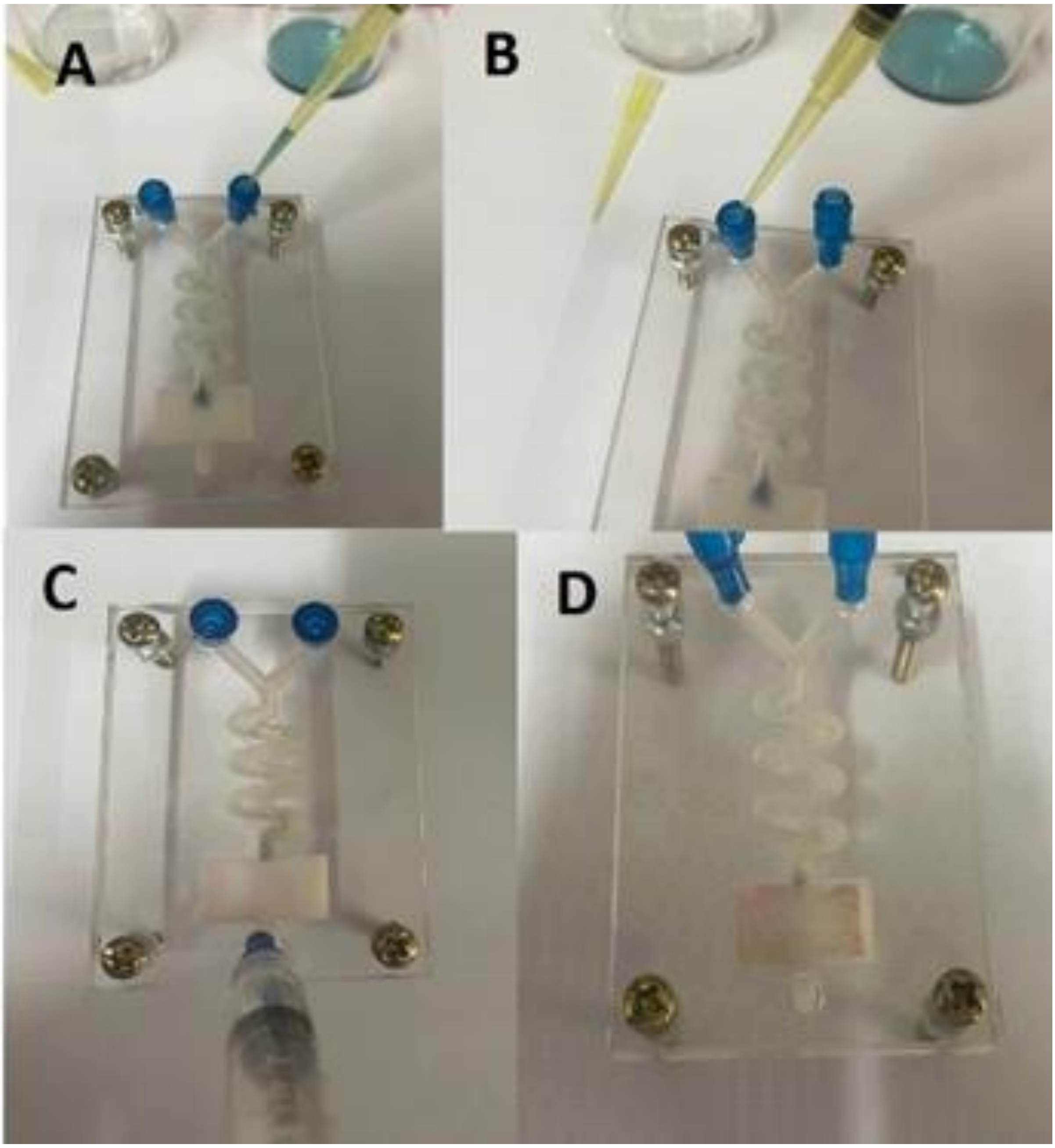


Figure 3. Steps of injection with dithizone solution.

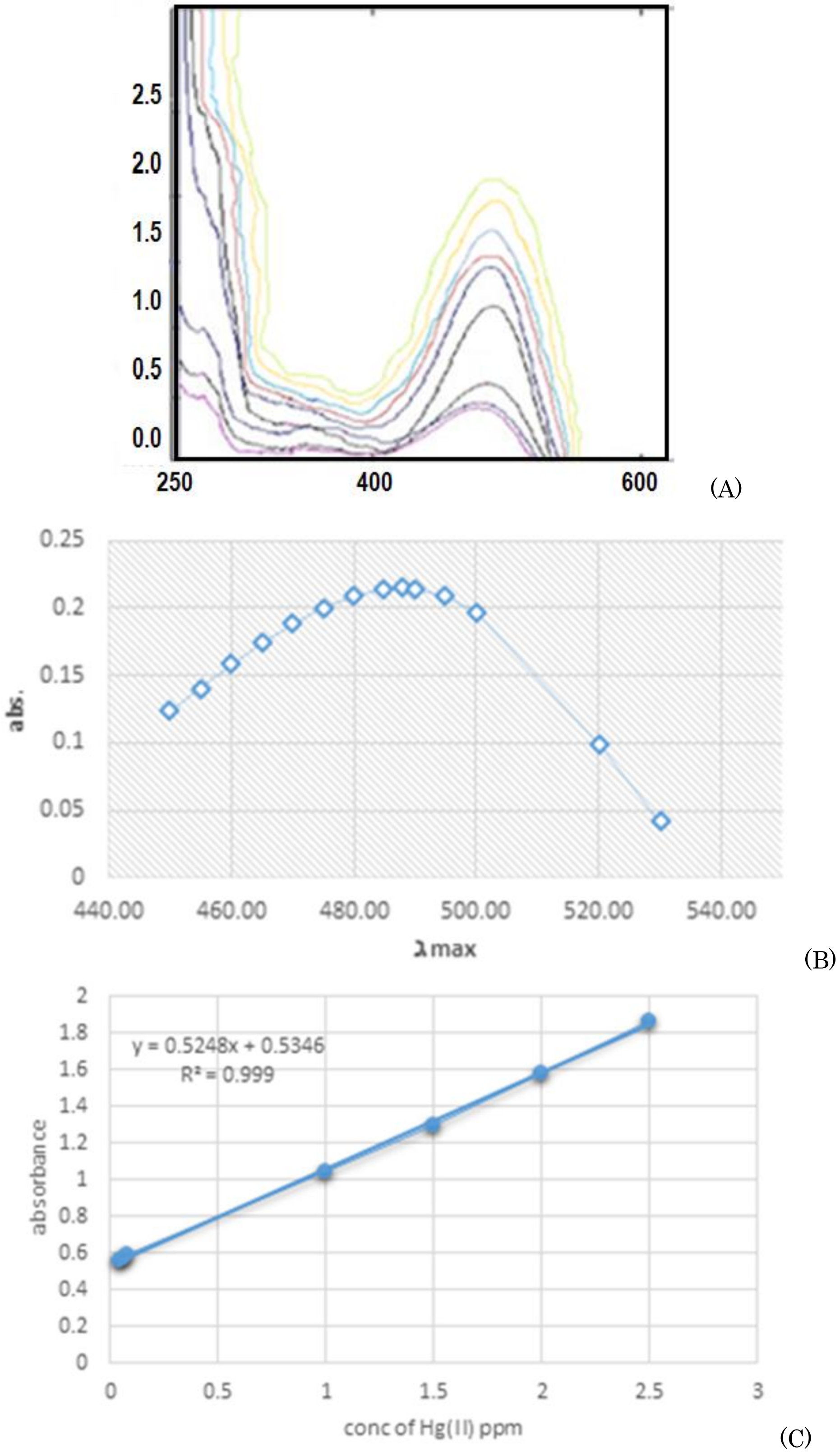


Figure 4. (A) Spectrum of Hg-dithizone with different concentrations of Hg (II). (B) Absorbance against λmax of Hg-dithizone. (C) Calibration curve of Hg-dithizone.

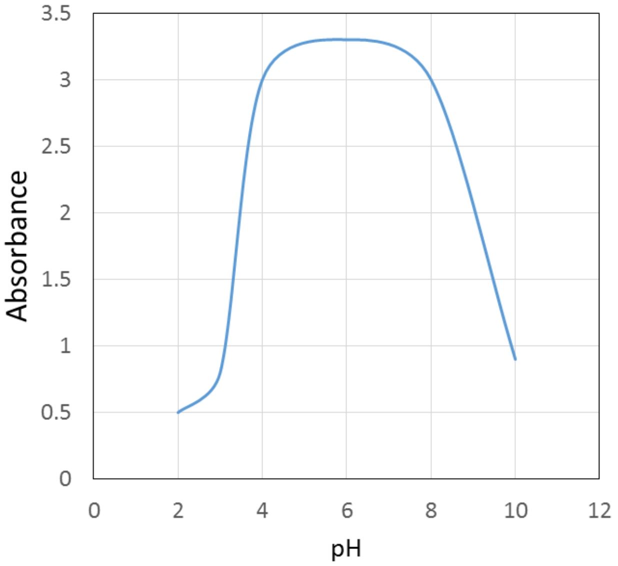


Figure 5. Effect of pH on absorbance of Mercury (II).

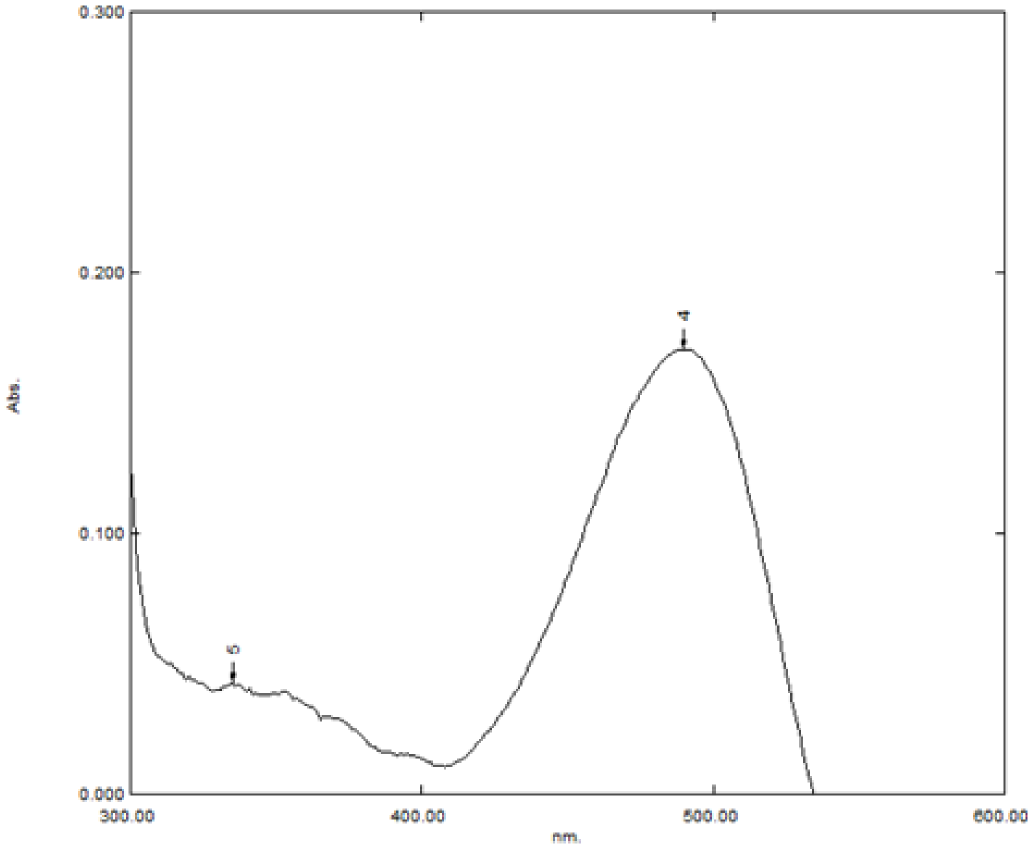


Figure 6. Hg(II)-dithizone complex absorbance dissolved in chloroform.

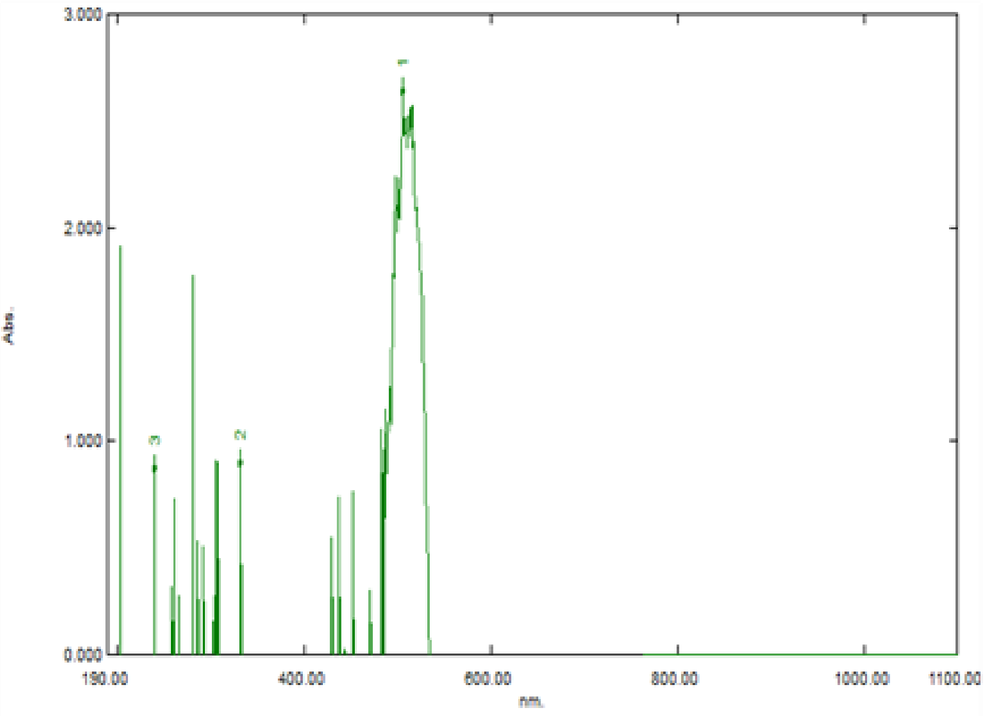


Figure 7. Hg(II)-dithizone complex absorbance dissolved in Benzene.

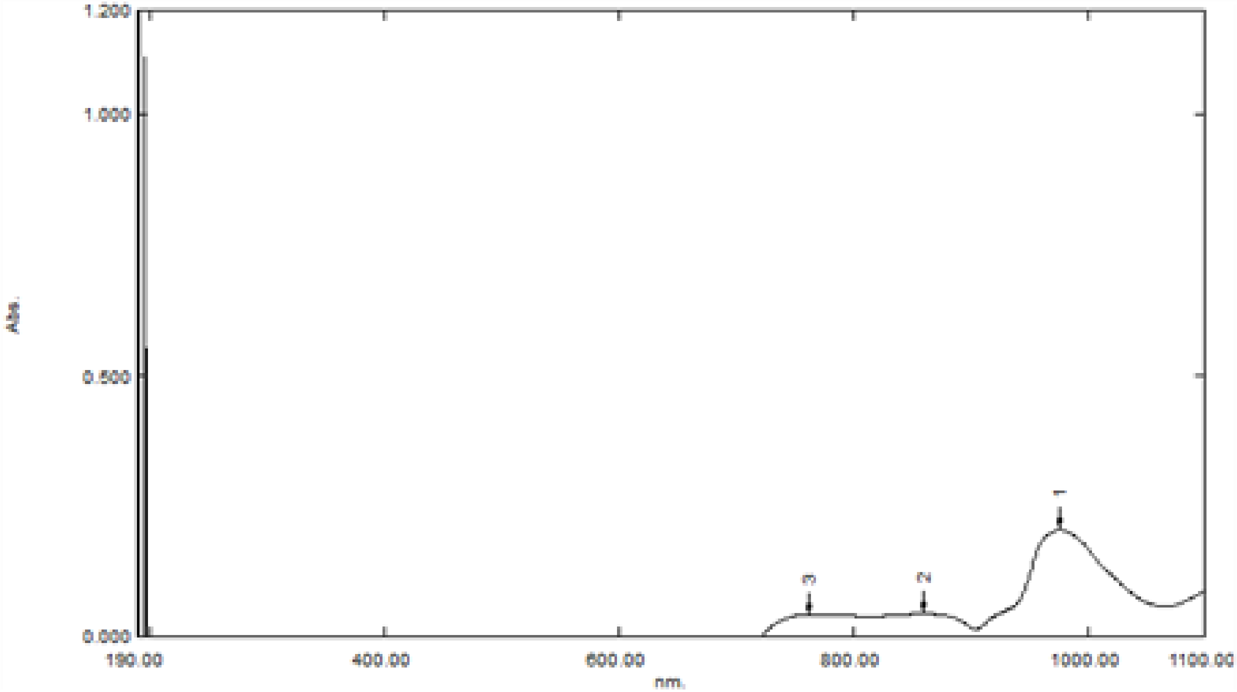


Figure 8. Hg(II)-dithizone complex absorbance dissolved in 1,4-dioxane.

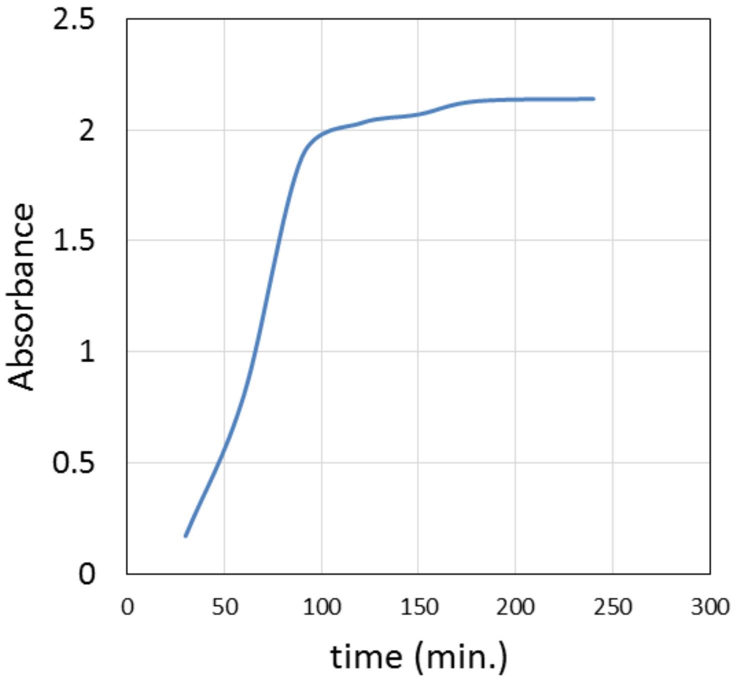


Figure 9. Effect of the time on absorbance of Mercury.

Table 1. Mercury (Hg) exposure Sources [4].

Specific exposure groups	Source of exposure
Mercury (atmosphere) Unintentional mercury spills or intentional misuse.	The utilization of Hg in industrial operations encompasses various activities such as the combustion of coal as well as other fossil fuels in facilities. Additionally, Hg is employed in gold and other metal mining, as well as in the production of chemicals like chlor-alkali and vinyl chloride monomer, which is a key constituent of PVC. The emission of mercury (Hg) is amplified as a result of climate change, specifically due to the rise in wildfires or the reduction of sea ice.
Mercury in the hydrosphere refers to the presence of mercury that have been contaminated with mercury.	Water contamination with mercury can occur due to different industrial procedures. Additionally, discharges of water from industries that use mercury or mine drainage can also contribute to water contamination. Human activity, such as soil erosion and runoff from watersheds, results in the release of Hg into freshwater habitats, including rivers and lakes.
Mercury in diet manufacturing.	The ingestion of fish, shellfish, and marine mammals, as well as poultry and rice, that have been contaminated with mercury.
Mercury in cosmetic manufacturing.	Mercury is present in teething powders, skin whitening treatments, and detergents.
Mercury in medical manufacturing.	Mercury is present in pediatric immunizations, namely in the form of the preservative thimerosal.

Table 2. This table provides a summary of the most recent scientific publications regarding the analysis of Hg (II) in water samples. It focuses on the analytical techniques used and the minimum concentration that was successfully detected.

Analytical method	**Hg(II) detection limit (ng mL-1)**
HPLC-ICP-MS	0.000016
UV–Vis spectrometry	1
Microfluidic Immunoassay	0.13
Stripping voltammetric sensor	0.6
Stripping voltammetry	0.006
HPLC	0.32
Flow injection	2.4
Near-IR spectrometry	4300
XRF	0.064

Table 3. Volumes of the injection mercury (II) solution and dithizone.

Conc. of Hg $^{+2}$ (ppm)	Vol of Hg (μL)	Vol. of dithizone(0.01%) (μL)
0.02	100	5
		7
		9

4. Conclusions

In summary, this method is a fast, efficient, inexpensive, and highly sensitive colorimetric technique for detecting Hg^{+2} in water-based solutions. Using spectral data obtained from a microchip and the Image J program, the approximate intensity was measured and compared to the intensities of known sample concentrations, Unknown sample wasn't used in this method because the spectrometric analysis used only to enhance results and compare with intensities. The promise to development this line of analysis which has limit cost and very little consumption of materials it's like a small laboratory.

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Conflicts of Interest: The authors declare no conflict of interest.

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