SAFEGUARDING WATER RESOURCES: A NOVEL PRECONCENTRATION-BASED COLORIMETRIC APPROACH FOR DETECTING HEAVY METALS

SAFEGUARDING WATER RESOURCES: A NOVEL PRECONCENTRATION-BASED COLORIMETRIC APPROACH FOR DETECTING HEAVY METALS

By MOHAMED FATHALLA, B.Sc.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Doctor of Philosophy

McMaster University

DOCTOR OF PHILOSOPHY (2023)

Graduate Program in School of Mechanical Engineering

McMaster University, Hamilton, Ontario, Canada.

Title:	Safeguarding Water Resources: A Novel Preconcentration-based Colorimetric Approach for Detecting Heavy Metals
Author:	Mohamed Fathalla B.Sc. Mechanical Engineering, Alexandria University
Supervisor:	Dr. P. Ravi Selvaganapathy
Pages:	XVIII, 138

LAY ABSTRACT

Heavy metals can be found naturally and are needed in small amounts for our bodies to function properly. However, many heavy metals are toxic and can cause serious health problems even at very low concentrations. These metals can contaminate water sources through activities like mining and improper waste disposal. Currently, detecting heavy metals in water requires expensive equipment and skilled experts in a laboratory setting. This process is time-consuming and not easily portable for on-site testing. The existing methods also have limitations such as low sensitivity or the need for complex procedures.

This thesis aims to improve the way we detect harmful heavy metals in water. The goal of this thesis is to develop a simpler and more sensitive method for detecting heavy metals in water. The focus is on using color-changing dyes that react to the presence of heavy metal ions. However, these dyes often have detection limits higher than what is considered safe, so the thesis also explores ways to concentrate the samples to improve sensitivity. By addressing these challenges, the thesis aims to contribute to the development of a reliable and easy-to-use method for testing heavy metal concentrations in drinking and surface waters, helping to protect public health and identify potential sources of contamination.

ABSTRACT

Heavy metals, despite their essential roles as minerals in biological systems, pose a significant threat to human health and the environment due to their toxic properties. Even at low concentrations, heavy metals such as lead, mercury, arsenic, and cadmium can cause adverse effects on humans and animals. Consequently, stringent regulations have been established to limit heavy metal concentrations in water resources. However, existing laboratory-based analytical methods for heavy metal detection are time-consuming, expensive, and require skilled personnel. The current detection limit required by several health organizations around the globe is below 10 ppb for Lead, Mercury, Chromium, and Arsenic. The current state of the art which can accomplish low levels of detection is either expensive to operate or incapable of achieving the required trace level sensing. This thesis aims to address the need for a simple, cost-effective, and portable method for detecting heavy metals in water.

The thesis begins by reviewing the current state-of-the-art heavy metal sensing methods, highlighting their limitations and the requirement for sample preconcentration. Various preconcentration techniques are discussed, emphasizing their performance parameters and advancements in trace-level detection. Furthermore, the thesis identifies the gaps in current technology, particularly in the context of developing a reliable and user-friendly method for testing heavy metal concentrations in drinking and surface waters.

The primary objective of this thesis is to develop a preconcentration-based colorimetric method for detecting heavy metals in water. This method aims to overcome the limitations of existing techniques by offering high sensitivity and a limit of detection below regulatory ranges without the need for complex equipment or extensive sample preparation. The thesis contributes to the advancement of the state-of-the-art by providing a simplified, portable, and efficient solution for in-line detection of heavy metal contamination in water resources. This has been achieved through the design and deployment of sensor utilizing a novel architecture, measuring heavy metal ions down to the sub ppb level. we were able to

detect ions such as copper and Lead at concentrations below 0.5 ppb with a limit of detection (LOD) of 0.14 ppb.

Overall, this thesis combines knowledge from the fields of analytical chemistry, sensor technology, and environmental science to address the pressing need for a practical and accessible method for monitoring heavy metal concentrations in water. By achieving this goal, the research will contribute to safeguarding public health and promoting sustainable water resource management.

ACKNOWLEDGEMENT

In the face of the arduous challenges presented by the COVID-19 pandemic, my journey as an international student pursuing a PhD has been nothing close to easy, and was undeniably demanding. Were it not for the unwavering support and enduring affection bestowed upon me by my family, this undertaking would have proved overwhelmingly daunting.

Throughout this eventful expedition, I extend my heartfelt gratitude and recognition to my esteemed supervisor, Dr. Ravi, who consistently made time for engaging discussions, collaborative idea generation, and the cultivation of innovative solutions. Furthermore, I am indebted to him for nurturing my aspirations, encouraging both my personal growth and scientific advancement. I am also grateful to Dr. Ching Chan and Dr. Chang-qing Xu for their recommendations. Their remarkable understanding of the fundamental aspects of my research helped me venturing in various directions. I would also like to extend my appreciation to my brilliant manager, Allison Van, who afforded me the opportunity to evolve into a comprehensive thinker of complex systems and a polymath.

Gratitude permeates not only the moments of joy but also the trials encountered along my PhD journey. Despite being diagnosed with MS, Depression, and Generalized Anxiety Disorder during this time, I find solace in the fact that these challenges are currently managed. I cannot overstate the depth of my gratitude for the lessons acquired from enduring hardships, as well as the days of blossoming success.

Furthermore, I wish to express my sincere appreciation for the boundless support provided by my parents, Amal and Alaa, and my siblings Ahmed and Nada. This accomplishment would not have been as gratifying without the unwavering presence and inspiration of my wife, Nada, who consistently inspires me to transcend my limits and be the best version of myself. It is worth mentioning that both my sister and my wife bear the name Nada. I extend my deepest gratitude to my grandfather, Gaber, as well as my in-laws Zain and Sahar for their unwavering support throughout this journey. I would also add my dear family members Hanan, Maram and Mayada. I would also like to express my gratitude to my dear friends Mahmoud, Maisara, Wessam, Jehan, and Ahmed Fayez. These people helped me shape the way I think and perceive life. I wouldn't forget to mention the love and support I have received from all my friends here, in Canada, since day one, I have been lucky to meet people who continuously stimulated my growth and supported me in countless ways. Thanks, Ahmed Wael, Yassen, Mohamed Gamal, Waraky, Ahmed and Mohamed Ali, in addition to Mohamed Gamal (Manchi), Mohamed Helal, Sahar, and Nour.

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CE	Counter Electrode
CMOS	Complementary metal–oxide–semiconductor
COVID	Corona Virus
CPAHPD	5-(4′-chlorophenylazo)-6-hydroxypyrimidine-2,4-dione
CPE	Cloud point extraction
DI	Deionized
DNA	deoxyribonucleic acid
DSLR	Digital Single Lens Reflex
HMI	Heavy Metal Ions
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ISO	The International Organization for Standardization
LED	Light Emitting Diode
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LPR	liquid-phase polymer-based retention
MEMS	Micro Electro Mechanical Systems
MPA	3-mercaptopropionic acid
MS	Multiple Sclerosis
NP	Nano Particles
OD	Optical Density
PAA	Poly(acrylic acid)
PH	Potential of Hydrogen
RE	Reference Electrode
RGB	Red, Green, Blue
SDS	Safety Data Sheet
SPA	Sodium Polyacrylate
SPE	Solid Phase Extraction
SPR	surface plasmon resonance
SRP	Solvent removal preconcentration
SSD	Solid State Dye

TA	Thioctic acid
WE	Working Electrode

DECLARATION OF ACADEMIC ACHIEVEMENT

Mohamed Fathalla contributed to the ideation, literature search, writing, experimental design, performing experiments, data analysis, results interpretations and creating figures for all the chapters of this thesis.

P. Ravi Selvaganapathy contributed to experimental design, data interpretation and revision of all the chapters of the thesis.

Introduction

Motivation

The prevalence of heavy metal contamination in water resources has become a pressing concern, as even trace amounts of these toxic elements can have detrimental effects on human and animal health. Traditional laboratory-based methods of heavy metal sensing are expensive and time-consuming and require skilled personnel for operation. Furthermore, some of these methods have limited sensitivity, requiring pre-concentration of samples, which can be complicated and inefficient. Colorimetric sensing using dyes is a simple and low-cost method, but its sensitivity often falls short of regulatory limits. Pre-concentration can enhance the sensitivity of colorimetric sensing, but current pre-concentration methods are not without their limitations. Therefore, new pre-concentration methods are needed to enable effective heavy metal sensing with colorimetric dyes. Currently, pre-concentration methods require skilled operation, time control, and aliquoting, making them inconvenient and ineffective. The challenge of pre-concentration is that most methods require sample recovery after liquid removal or reduction. A method that preconcentrates samples one thousand or more times enables a transducer with a LOD of 100 µg/l to sense 100 ng/l of the measurand. Building a platform that enables preconcentration and measurement of solutions using a solid-state format is highly challenging because the offered solutions in the literature are limited.

This thesis introduces a solution that utilizes the high adsorption capacity of sodium polyacrylate hydrogels for heavy metals in preconcentrating them thousands of orders of magnitude which in turn allows for the detection of ng/l quantities of heavy metals. The use the hydrogels as a solid-state medium for the colorimetric dye delivery allows for the timely passive release of the dye. These contributions enable the construction of a system that is easy to use, cost effective, doesn’t require experienced operators, and has a limit of detection similar to that of ICP-MS.

Objectives of the thesis

The aim of this extensive work is to develop a unique solution that utilizes the versatility of colorimetric sensing of heavy metals and the high adsorption capacity of hydrogels. This solution would be capable of the detection of trace level concentrations of Copper, Iron, and Lead. The solution could also maintain an easy-to-use interface and be cost effective for it to be deployed in remote setting and regions with no access to water quality monitoring tools. The study would focus on the following objectives:

Objective 1: Colorimetric Detection of Heavy Metal Ions Using Superabsorptive Hydrogels and Evaporative Concentration for Water Quality Monitoring.

Objective 2: Sub ppb sensing of trace heavy metals using pre-concentration from large sample-volumes on solid state hydrogels.

Objective 3: Semi automation of solid-state reagent delivery and Colorimetric Sensing of Heavy Metals.

Thesis Outline

This is a sandwich format thesis with first and last chapters being the introduction and conclusion. In total, the thesis contains five chapters where the three other chapters are three journal papers. One of those papers was published in ACS ES&T Water while the others are currently under preparation. The chapters outline and summary are as follows:

Chapter 1: This chapter, detailed the literature review and fundamental theory of heavy metals sensing and concentration techniques focusing on the use of sodium polyacrylate hydrogels in heavy metals preconcentration.

Chapter 2: This chapter describes a novel method of sensing metal ions at low concentrations using hydrogels for pre-concentration and selective colorimetric indicator dyes. It describes the basic principle of ultra preconcentration where a mix of solid phase extraction and solvent removal reconcentration was used in reducing the detection limit of commercially available dyes. The detection of Copper and Iron at trace level concentrations with limit of detection of 5.4 ppb was then reported. The total time required for Copper sensing was estimated to be 38 minutes. The process was compared to the measurements of the gold standard method (ICP-MS) achieving an error of less than 3%.

Chapter 3: In this chapter, the previously, described method was improved to detect even lower concentrations below the 1 ppb limit for the method to have a linear response in the low ppb range (0.5 - 10) ppb. This was possible by the utilization of large volume preconcentration on SPA hydrogels and performing a solvent removal strategy based on the flow of large volumes of samples over the adsorptive material (SPA Hydrogel). This, unlike the approach developed in the previous chapter, enables the preconcentration of ng/l quantities of Copper and Lead into the SPA Hydrogel which in turn enables the commercial dye to detect the ions at lower concentrations. This approach yields an ultra-low trace-level detection technique capable of detecting Copper and lead at 1.4 ng/l.

Chapter 4: This chapter describes the efforts made design a method suited for eventual automation. Here, the dye is loaded onto SPA Hydrogels and dehydrated to be delivered in a solid- state format into the sample solution enabled the automation of the process. This is performed after the adsorption of the metal ions on another SPA Hydrogel bead. Delivering the dye in a solid-state format and conduct the measurements using a platform designed specifically to measure the colorimetric response of solid-state samples with a small footprint of a few centimetres and cost-effective building blocks. Using LEGOs as the building blocks, and an RGB sensor in connection with Arduino UNO measurement of the emittance of the solid-state hydrogel took place. This solution is the first of its kind to use an RGB sensor in measuring the emittance of solid-state beads. This moves the process one step forwards enabling the development of an on-line sensors and multi analyte platforms.

Chapter 5: In this chapter summarizes the main contributions of the this thesis to the state of the art knowledge. Then a highlight on future trends and the potential new applications of the currently used technologies was presented.

Contributions

In this thesis, a first of its kind platform for the detection and quantification of trace level toxic heavy metals was developed. The super absorptive behaviour of sodium polyacrylate hydrogels in addition to their capacity to adsorb metal ions in the development of sub-ppb detection platform for toxic heavy metal ions in aqueous media was utilized in a unique approach towards the preconcentration and sensing. This can be considered a first of its kind achievement reaching a detection limit of 140 nanograms per litre of Copper and Lead in water samples using extremely cost-effective methods and off shelf solutions. Comparing the platform with the gold standard technique (ICP-MS) it has been confirmed that it is feasible to automate a process that is cost effective, selective, specific, and has a very low limit of detection.

Chapter 2 Literature review

2.1 Heavy metals sensing methods

Sensing is a process through which a quantity of a measurand is identified. A chemical sensor can quantify the presence and amount of a particular chemical species in a sample solution. The process requires two stages where initially the chemical properties of species is recognized/ detected, then, these properties are transduced into a physically quantifiable signal. Typically a number of steps are required to detect a measurand, namely: (i) analyte conditioning, where the analyte goes through processes such as pre-concentration, separation, PH conditioning, and control of media parameters; (ii) recognition/detection of the desired chemical property in a specific and sensitive manner; (iii) proportional transduction of the signal producing a quantifiable signal which can be electrical, optical, mechanical signal or other types; (iv) quantification where a specific value in relevance to a control value is identified and characterized

Heavy metal sensing methods can be classified into two main categories: (i) electrochemical, and (ii) optical, which include the colorimetric techniques as well [20]. In both optical, and electrochemical methods, ion selective materials are usually used for specific responses to ions. These transducers selectively generate a characteristic response when exposed to their specific target. Then, the sensing target concentration can be determined by calibrating their responses and correlating the response intensity with the species concentration. Ion selective electrochemical sensors are devices which utilize a specialized membrane that selectively change their potential in response to the concentration of the analyte and reduce any disturbances from the surrounding environment. These sensors operate by establishing a balanced, complex reaction between the substance being analyzed and the sensor probe [21]. On the other hand, optical based ion selective sensing utilizes variety of optical characteristics which can be intrinsic of the liquid sample or can develop after the interaction between the liquid sample and a specific colorimetric reagent/ probe. Hence, they only provide response at certain wavelengths that the optical instrumentation can detect.

Although, these well-established methods can be automated, their high cost and short lifetime (due to fouling) are limitations that prevent their implementation in remote locations and on -line sensing applications. Colorimetric methods on the other hand are simple to use and don't require skilled personnel for operation[22]. They rely on adding selective indicators into samples where these indicators develop colorimetric response when bound with selective targets [23]. The color intensity depends on the concentration of species in the solution which can be measured using a spectrometer or a camera making them more accessible and cheaper to manufacture. Nevertheless, they still require some sample processing which has not been automated and have low sensitivity that must be improved.

The next section discusses the various techniques used to quantify heavy metals, highlighting the basic principles of effectively and reliably identifying the trace level compounds. The performance parameters of these techniques and the sensors made using them are discussed. The advantages of the methods and their limitations are detailed and the ability for these methods to be adapted for in-field monitoring is discussed.

2.2 Types of sensors and transducers for heavy metals

Several types of transducers can interact with heavy metals providing qualitative signal. The main parameters that can be characterized to assess a sensor performance are its selectivity, accuracy, and precision (including repeatability, resolution), sensitivity, drift, and response time in addition to limit of detection (LOD) and dynamic range. Additional performance parameters such as operational range and robustness to overdose in addition to power consumption and suitability for automation can inform the decision making in selecting the proper technology for a certain application. Specificity is defined as the ability of a sensor to detect a certain analyte (measurand) with no interference of any other substance. This enables a sensor to only provide response when it gets in contact with its own specific the analyte [24].

As discussed earlier, optical, and electrical transducers can be used as chemical sensors. Optical transduction methods such as changes in emittance, absorbance, scattering, and plasmons in response to an analyte are all used widely in microsystems and large analytical equipment. Electrical transduction methods can be categorized based on changes in electrode potential, generated current, conductance/ resistance, or impedance. In the upcoming section a discussion of each method is presented, and the performance parameters of each method are furtherly investigated.

2.2.1 Electrochemical

Electrochemical sensors rely on the changes in electrochemical properties at an electrode due to the presence of the analyte of interest. The electrochemical sensing of heavy metals is typically conducted using an electrochemical cell that includes basic configuration of an electrochemical sensor composed of an ionic

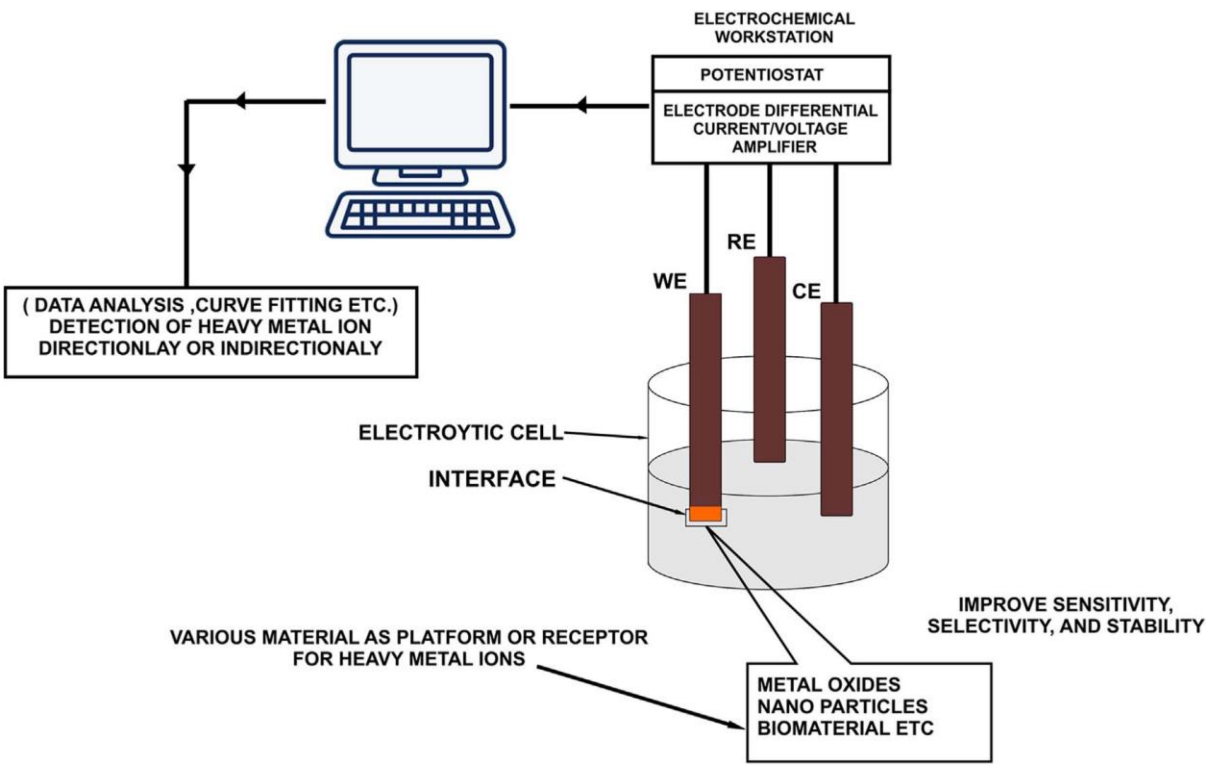

Figure 2-1 The working principle of electrochemical sensing. Adapted from [26]

conducting medium (electrolyte) and two or three electrodes. The electrodes are usually referred to as working electrode (WE- where the detection takes place), a reference electrode (RE - that serves as the reference potential on the solution side), and a counter electrode (CE - that completes the circuit) as shown in Figure 1-1 . The heavy metal ion of interest is typically present in an electrolytic solution, and the working electrode is designed to specifically detect [25] and respond to the heavy metal ions in the solution. Both amperometric and potentiometric modes can be used for sensing of analytes in the electrolyte. Potentiometry relies on measuring the changes in the electric potential of the working electrode due to reaction or influence of analyte present in the solution. Alternatively, amperometry relies on the measurement of current flowing through the working electrode when it is polarized at a certain potential to initiate a reaction of the analytes on it.

In potentiometric methods the cell potential is measured at the interface between the electrolyte and the electrode giving indication on the electrochemical reactions and potentials occurring at that interface. Different types of half reactions occur at the interfaces where one of those halves is the reaction of interest taking place at the working electrode. The electrochemical system relies on an external power source, usually, to supply and excitation signal and to measure the response. The working electrode can be modified with various interface materials to serve as a platform for the detection of heavy metal ions. Potential is measured using a high input impedance device to prevent current flow from the RE. The electrodes are connected to an electrochemical workstation that provides excitation signals to the electrode setup and measures the response signals. The workstation is linked to a computer with software for data analysis. In the case of solutions with low solution resistance, a two-electrode cell configuration can be employed, consisting of the WE and RE to measure the electrode potential[26]. Several examples of potentiometric sensing of heavy metals are highlighted in Table 1-1. For example a group was able to detect Mercury in aqueous solutions at a limit of detection of 1.0×10^{-10} M [27]. Another group was able to achieve a response time of 9 seconds sensing Lead with a limit of detection of 6.7×10^{-7} mole dm^{-3} [28].

In amperometry, a fixed potential at which a particular metal ion of interest reduces is applied to the working electrode and the current generated by the electrochemical reactions at the interface measured. The current is proportional to the concentration of the ionic species present in the sample. Various heavy metal species have been measured through amperometry methods as shown in Table 1-1. For example, amperometry based sensor developed by Arduini et al was reported to have LOD of 5 nM for the detection of Mercury. Another one developed by Mohammadi et al was able to achieve 10 nM LOD for the detection of Mercury as well. For Lead, some of the sensors were reported to be in the sub micromolar range at 1 nM [29].

Sensitivity and limit of detection are often improved by using advanced techniques such as stripping voltammetry[30], the use of enzyme-modified electrodes[31], or by including a preconcentration step[32]. For example, the LOD of heavy metals such as Pb, Cd, and Cu can be as low as few ppb or even ppt level with electrochemical sensors[26]. In general, for heavy metals, the LODs can be in low ppb to high ppt level. Some other parameters may have influenced the final readings, such as, interferences, sample preparation, etc. To achieve a better performance, different techniques can be used to improve the sensitivity of the sensor, such as using a modified working electrode [33] or incorporating a chemical amplification mechanism into the sensor[26].

Electrochemical sensors have been constructed in different configurations and using fabrication techniques such as (i) microband-based electrochemical sensors[34], use a microband of carbon or metallic materials as the working electrode, which is integrated into a microfluidic chip. (ii) microfabricated electrochemical sensors [25], these sensors use a microfabricated working electrode, which is typically made of carbon or metallic materials, integrated into a microfluidic chip. (iii) inkjet-printed electrochemical sensors[35], these sensors use inkjet printing to deposit the working electrode onto a microfluidic chip. (iv) electro-spun nanofiber-based sensors [36] where these sensors use electrospun nanofibers as a support for the working electrode and (v) ionic liquid-based electrochemical sensors [37]as these sensors use ionic liquids as the electrolyte. These sensors have been used for the detection of trace elements such as Lead, Cadmium, and Mercury. The limits of detection of these sensors are typically in the range of low-nanomolar to sub-nanomolar levels.

Electrochemical methods are an attractive solution as they can be miniaturized while retaining their sensitivity and selectivity, leading to minimal performance loss as they are scaled down. They also offer the advantage of being a universal platform as they can be electrical based. A miniaturised system can be equipped with simple electrodes to measure a chemical signal, offering a quick, low-powered, and affordable detection, while still being sensitive. However, one of the limitations of electrochemical method is that they require reference electrodes that need to be maintained over time. They also suffer from drift and in some cases different metal ions may have overlapping reduction potentials that make them not selective. Finally, the instrumentation needed such as a potentiostat which is also relatively more expensive as compared with colorimetric methods.

Colorimetric methods that will be introduced in the next section are more versatile and adaptable technique for the detection of multiple heavy metals. As those devices can use a general purpose imager as the electronic transducer for the analysis of several ions[38].

Table 1-1 electrochemical sensors in the literature for heavy metal sensing

Elicit was used to identify literature for this table.

	Ref	LoD	Measurement	Detection range	Response Time	Sample Type	Electrode material
Mercury	[39]	3×10^{-10} M	Voltammetry	1.5×10^{-6} M to 3×10^{-10} M,	30 minutes	Water/ Natural water	2,2′ (ethane-1,2-diylbis((2-(azulen-2-ylamino)-2-oxoethyl)azanediyl))diacetic acid
	[40]	1 fM	Voltammetry	From 1 fM to 1 micro-M	10 minutes	Aqueous solutions	gold SPR sensing surface
	[27]	1.0×10^{-10} M	Potentiometry	1.0×10^{-9} to 1.0×10^{-2} M	5 seconds	Aqueous solutions	Palm shell activated carbon modified with trioctylmethylammonium thiosalicylate (TOMATS)
	[41]	0.004 aM	Voltammetry	0.1 aM to 0.1 nM	60 minutees	Aqueous solutions	DNA/poly l-methionine gold nanoparticles/pencil graphite electrode
	[42]	9.5×10^{-9} M	Voltammetry	0.25 to 0.81 M	-	spiked wastewater	Gold
	[43]	0.01 nM	Voltammetry	0.05 to 350 nM	10 minutes	Municipal wastewater and river water	Gold nanoclusters
	[44]	100 nM	Amperometry	250–500 nM	-	Aqueous solutions	Mercaptopropionic acid modified gold nanoparticles
	[45]	141 nM	Voltammetry	2.0×10^{-7} to 2.1×10^{-4} M	5 Minutes	Tap water and industry wastewater samples	Glassy carbon, modified with hydroxyapatite nanoparticles that were biosynthesized using aloe vera plant extract
Lead	[46]	Lower than 1 ppm	Voltammetry	1-50 ppm	10 seconds	seawater samples	Silver, Ag (Electrodag PF 410) and carbon, C

[47]	1.5 μg	Voltammetry	0.9 -1.5 μg l^{-1}	-	Aqueous solutions	Screen printed electrodes (SPES)
[48]	5.0×10^{-9}	Voltammetry	1.0×10^{-8}-5.0×10^{-7} M	300 seconds	tap water samples & lake water samples spiked at different concentrations	NH2–Cu3(BTC)2 Amino functionalized metal organic frameworks
[28]	6.7 x 10^{-7} mole dm^{-3}	Potentiometry	$1x10^{-6}$ - $1x10^{-1}$ mol dm^{-3}	9 Seconds	groundwater samples	A PVC membrane based on 1, 5-diphenylthiocarbazone (dithizone)
[49]	21 nm or 4.4 ppb	Voltammetry	21 nM	-	-	Copper
[29]	1 nM	Voltammetry	1 nm to 1.9 nM	-	Milli-Q water	A gold electrode substrate with self assembled monolayers (SAMS) of 3-mercaptopropionic acid (MPA) or Thioctic acid (TA) followed by covalent attachment of a Lead binding peptide

2.2.2 Optical – spectroscopic

Optical sensors are devices that are designed to detect and measure light. These sensors can convert the energy of photons into an electrical signal, which can be used to analyze or monitor different physical phenomena, such as temperature, pressure, humidity, and others. In this section, the focus is on optical sensors that are designed specifically for analyzing liquid samples containing heavy metal ions. These sensors can be composed of various components, including channels that transport or contain the fluid/ material, waveguides positioned adjacent to these channels that guide the light, and tunable gratings defined on these waveguides that variably define cavities for selecting specific wavelengths of radiation. All these components work together to achieve accurate analysis results of liquids under examination[50].

Optical responses vary depending on whether the measurand is an intrinsic property of the analyte or a response that is induced due to binding with a ligand. Changes in the optical properties of a sensitive material including absorption, reflection, or luminescence can be used for sensing. The sensitive material can be in the form of disposable test strips, or optical waveguides such as optical fibers, integrated on chip optics, or capillary-format devices.

In contrast to the electrochemical systems, optical sensor eliminates the need for physical contact between the reagent phase and the optoelectronic system. This allows for the use of irreversible reactions for some of the assays. As a sensor can be designed out of several micro probes with a single optical instrument enabling multiparametric analysis. Colorimetric and fluorescent sensors can also be integrated with several optical setups and usually can be designed and fabricated to be more cost effective than the other methods as they require less expensive optics and most of the time require simple configurations. Optical sensors have been classified into various groups based on their functional principle and the requirements have been detailed [51].

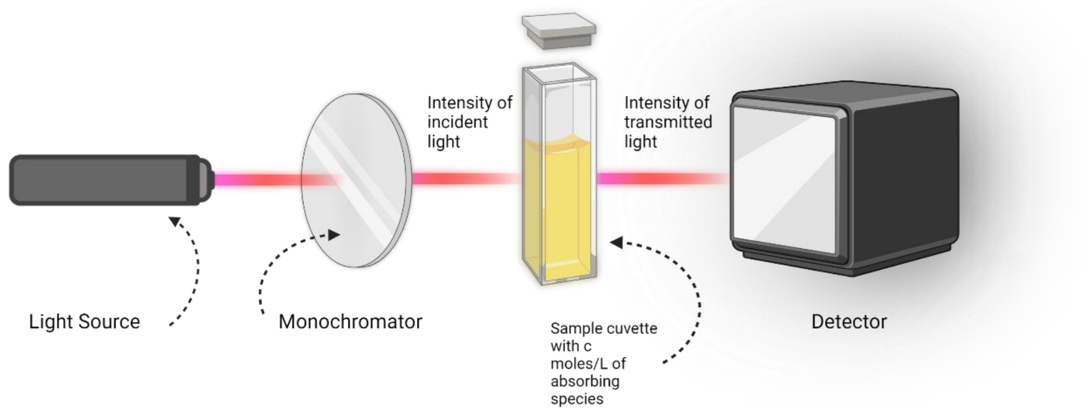

Figure 2-2 Absorption Based Optical Sensor Basic configuration. Created with BioRender.com

Optical detection techniques are some of the most versatile, inexpensive, practical, and widely used method of sensing. The small volumes used in microfluidic devices can be a challenge in optical measurements due to the requirement to sense at very low concentration limits. Recent advances in microsystems have led to the development of a new generation of optical sensors that incorporate preconcentration and extraction methods to enable lower limits of detection of samples of trace concentrations. Optical sensors can be a single use measurement probe or a continuous monitoring platform. This literature review presents the different optical sensing techniques and recent technological advances with a focus on their detection limits, selectivity, dynamic range, and scale of use.

Spectrophotometry is a scientific technique used to quantify the amount of light absorbed by an object or material and is one of the most widely used optical sensing method. The method involves transmitting a light beam through a sample and measuring the amount of energy that is absorbed [52]. This information can then be used to determine various properties of the sample, such as its concentration, purity, and composition. The technique relies on the use of two main components: a spectrometer, that provides a fixed illumination of the sample at defined wavelength over the spectrum, and a photodetector, which detects intensity, or the light absorbed or emitted by the sample at that wavelength. Optical sensors are microscale devices that can respond to the presence of analytes sensitively and selectively producing optical information. If these responses are reversible, that results in a sensor; otherwise, it can be identified as a probe [51]. These optical responses can be measured using conventional methods absorption, reflection, or luminescence spectroscopy methods.

A typical optical sensor or probe for heavy metal ions (HMIs) is based on an indicator-loaded thin membrane or film, being referred to as the sensing element. This film can be polymer based or a deposition of solid-state material over a substrate. The system is based on two elements that constitute a transducer. The first element is the reagent that converts chemical information to optical signal and interacts directly with the sample. The other element is the optical instrument that doesn’t have to be in direct contact with the liquid sample and therefore can be reused for multiple testing. This modular design of the transduction enables a frequent and rapid change of the reagent phases and faster and multiplexed sensing capabilities. Test strips are an example for semiquantitative optical quantification for several HMIs which have been around for decades. And they offer cost-effective solution for versatile detection of ions. Combining the ease of use of optical methods and their selectivity with the sample preconcentration can enable low detection limits to be characterized.

2.2.2.1 Optical sensing using intrinsic optical properties of fluids.

Before exploring indicator-mediated sensing methods, it is essential to illustrate the sensing strategies for HMIs that do not rely on indicators and alternatively rely on the inherent optical characteristics of HMIs. Quantification is performed by measurement of absorption (from UV to near infrared) or luminescence. Copper, cobalt, nickel, the lanthanide ions europium and terbium, and the radionuclides uranium and plutonium are all suitable candidates[53]. Plain fiber sensors are one of the most widely used sensors for intrinsic measurements. The optical fiber serves as a waveguide to transmit the optical properties of a sample to an optical detector. Limits of detection are typically in the higher end of the spectrum of mg/l. This method is reported to be unsuitable for trace metal analysis [53]. Such sensors are prone to interference since they solely rely on the natural optical properties of the ions of interest. These tests are most useful when the ions are present with known background that does not present any interference.

2.2.2.2 Optical sensing using reagents.

This method enables specific and selective sensing of variety of elements with high sensitivity and low interference. The method relies on the optical properties of the products of an interaction between the metal of interest and a specific indicator dye that is tailored specifically to respond with high specificity to the ion of interest. Absorbance, fluorescence, and chemiluminescence are all feasible optical characteristics that a sensor can rely on.

Fluorescence-based sensors [54] use the fluorescence properties of specific dyes or nanoparticles to detect the presence of heavy metal ions in a sample. The fluorescence intensity changes when the dyes or nanoparticles bind to the heavy metal ions, and accordingly induce a change that can be used to quantify the concentration of the heavy metal ions in the sample. These sensors have shown high sensitivity and selectivity for heavy metal ions, with limits of detection in the low micromolar range. An interesting approach has been developed where the weak fluorescence exhibited by albumin has been used to detect

Lead. [55] Albumin exhibits weak fluorescence at 430 nm and the intensity was found to be affected by the presence of Lead ions in the solution. LOD of 10 pM was identified using such technique.

Another type of optical sensing is the Surface-Enhanced Raman spectroscopy (SERS)[56]. These sensors use the Raman scattering properties which is a characteristic property of certain molecules to rule out the presence of heavy metal ions in a sample. Some of these sensors were reported to detect Lead at trace level [56].

Optical fiber sensors[57] have been developed where the fiber delivers the light to the sample and from the sample to the detector. Measurement of absorption, fluorescence, or scattering of light delivered by the optical fiber and its changes is the main working principle of such method. They can have multiple configurations and can provide significantly low limits of detections. For example, A sensor developed by F. H. Suhailin et al. [58] was able to detect Lead in aqueous solutions using $\gamma - Fe2O3$ nanoparticles on the surface of an optical fibre. The researchers have successfully deposited a nanocomposite of maghemite and reduced graphene oxide onto the surface of a gold-coated D-shaped optical fiber. This combination of materials has shown an enhancement of the resonance field and sensitivity of the fiber-based plasmonic sensor. A shift in the surface plasmon resonance (SPR) wavelength from 610 to 615 nm in the wavelength is noticed when the Lead is present in the water sample at 1 ppm concentration. They reported a limit of detection of 0.001 ppm and operated in a range between 0.001 and 15 ppm. The response time was inversely proportional to the Lead concentration in the solution where at 15 ppm the response time was 25 seconds while at 0.001 ppm the response time was almost 500 seconds. The response was noted to change at a rate of 2.8 nm per mg/L. this result suggest that the method is capable of the detection of trace amounts of Lead in drinking water samples. The major drawback of such approach is the use of spectroscopy and optical fibers for signal collection which is an expensive hardware to build and require delicate handling.

Colorimetric sensors are the final category to be discussed here and these sensors use the color change properties of certain dyes or nanoparticles to detect the presence of heavy metal ions in a sample. When these reagents bind to the heavy metal ions, the color of the dyes or nanoparticles changes and in turn the emitted signal changes. The colorimetric sensing methods offer a unique advantage over the other approaches as they utilize the low-cost CMOS sensors (such as DSLR and mobile phone cameras) as the colorimetric their detectors. This enables the development of low-cost platforms that are versatile and adaptive in addition to being tailored to the specific analyte of interest. They are of particular interest when the analysis only requires a few elements to be measured unlike the electrochemical methods that can potentially measure several ions using the same electrode. This advantage is shared between most of the optical methods yet, the colorimetric methods offer the most cost effective and versatile solutions. In the next subsection, the colorimetric methods state of the art is discussed and latest advancements of such technique are presented.

2.2.3 Optical - Colorimetric sensors

Colorimetric sensors are designed to detect and quantify analytes in a sample by measuring changes in their color in the visible spectrum. These sensors use the color change or absorbance properties of certain dyes or nanoparticles to detect the presence of heavy metal ions in a sample. The color and absorbance of the dyes or nanoparticles changes when they bind to the heavy metal ions, and this change can be used to quantify the concentration of the heavy metal ions in the sample. These sensors have been reported to have limits of detection in the low micromolar range, depending on the type of heavy metal ions and the specific sensor design.

Developing colorimetric sensors that are both sensitive and selective is a task that requires several parameters to be considered put to check. The sensor must be designed to interact with the target analyte, triggering a chemical or physical response that leads to a visible color change that is unique to that element and proportionally increase with the increase of concentration in a quantifiable fashion. This requires the development of chemical reagents or functional materials that can selectively bind to the target analyte while not reacting to other chemicals or being affected by sample conditions (temperature, pH, interfering Ion's concentration). For instance, a sensor developed by Marc R Knecht et al. used gold nanoparticles for the colorimetric detection of Lead and Mercury. Despite the successful detection of trace level ions of 1.2 nM, and operating within a linear range 10 nM to 1 mM, the method required acid digestion for sample preparation and saline water samples such as sea water presented some difficulties causing matrix interference and insufficient precision[59]. In other instances, the colorimetric dye requires preservation at certain temperature until used and require pH conditioning of the sample in order for the ligand to emit response[60]. Table 1-2 shows the various efforts to develop a Lead sensor using colorimetric methods. Specific examples are highlighted in the description below.

A group of researchers developed a sensor that have a limit of detection in the low micromolar range at 4.95 µmol/l while utilizing plasmonic nano particle l [61]. They utilize the change in absorbance due to the complex forming with presence the metal ions. The plasmon resonance band was observed to be at a wavelength of 410 nm. This method enables trace level detection but require high end instruments to measure the resonance response.

In another example, [62] 5-(4′-chlorophenylazo)-6-hydroxypyrimidine-2,4-dione (CPAHPD) was used to determine Lead in vegetables, biological, and soil samples with a limit of detection of 15 ng/mL and with high selectivity. The CPAHPD molecule forms a complex with Lead in solution and is concentrated using solid phase extraction. This can be eluted into Isopropyl Alcohol for detection. The molar absorptivity of the Pb(II)-CPAHPD complex was reported to be 113×10^6 L/mol cm at 647 nm in isopropyl alcohol. This method despite specificity and low detection limit, require the use of recovery techniques for the Lead to be tested in Isopropyl Alcohol. Additionally, it includes many precise, and volume sensitive processes which requires highly skilled and trained personnel to operate which makes it suited for lab environments.

Similarly, Firdaus et.al. [63] found that a colorimetric sensor made of silver nanoparticles can detect Mercury (II) in aqueous solution with a detection limit of 0.86 ppb. This method utilized silver nanoparticles to react with Mercury producing a yellow/brown complex due to a redox reaction. This method used a smartphone camera to image and analyze the colorimetric response which makes the most viable solution for in-situ usage and can be used by the public. Nevertheless, nano-silver can be potentially toxic to cells and handling of reagents must be carefully controlled in this case. The use of a more sustainable approach that imposes less risk on the public users would enable a wide spread of these analytical tools[64].

These findings incentivise the investigation of colorimetric technique that are stable under different conditions of temperature, pH, and fluid flow. These requirements are crucial to achieve the in-field implementation. A small form factor of the system and compactness also are also essential. This thesis discusses and develops methods for the use of colorimetric sensing technology for the detection of trace elements such as Copper, Iron, and Lead which also can be extended to other heavy metal ions.

Table 1-2 colorimetric sensor advancements Elicit was used to collect this information.

Measurand	Reference	Reagent	LoD	Detection range	Sample Type
Lead	[65]	Glutathione (GSH) and label-free gold nanoparticles (AuNPs)	6.0 ppb	6.0 ppb to 500 ppb	Water
	[66]	Colloidal gold nanoparticles	1.5 µM	520 ng/ml to 13 ug/ml	Water
	[67]	Cyclodextrins, calixarene, and crown ethers	2.0×10-7 mol l-1	10-6 to 10-2 mol l-1	Water
	[68]	Alginate	1 µM	1 – 10 µM	Water
	[69]	iminodiacetic acid functionalized nanoparticles (IDA-Ag NPs)	13 nM	0.4 to 8.0 µm	Tap Water and urea
	[70]	Gold Nanoparticles	1.2 µM	30 – 110 µM	Water
Mercury	[63]	Silver Nanoparticles	0.86 ppb	1 – 4 ppb	Water
	[71]	Dithizone	3 ppm	3 – 10 ppm	Water
	[72]	Modified dibenzo-18-crown-6-ether (DB18C6)	1.25 x 10^{-8} M.	25 – 325 x 10^{-8} M	Water

2.2.4 In-line devices and automation of sensing

Inline or periodic monitoring is of importance in water quality assessment as there can be daily or seasonal fluctuations because of weather, human, or industrial activity. Several autonomous and semi-autonomous systems have been developed for periodic monitoring. Some systems are semi-autonomous, requiring the operator to manually load the sample and operate the analysis with some level of autonomy in data analysis. Yet, this is not the goal, as the main objective of the development of on-line sensing and analysis is to, mostly, remove the human factor either to prevent errors or to enable the analysis to be conducted continuously or remotely. As discussed previously, analyzers and sensors can rely on several operating principles. Optical, or electrochemical methods can be automated, and some can manage various analytes. Colorimetric methods provide the cheapest and most diverse option available as it provides specificity. Using different reagents, the same platform can conduct colorimetric analysis of various metal ion species. Electrochemical methods are the most popular method for on-line systems as it requires no reagents supply and depends only on electrical approaches. Yet, the electrodes can suffer from hysteresis, and performance degradation is expected leading to errors and hindering the performance increasing the maintenance frequency of such system. Several commercially available systems can monitor water quality and toxicity like automated trace metal monitoring - Ova7000, topaz from Seres environment (France), and HMA-TCR of Toadkk. Their main disadvantages are that they are either bulky and require large rooms for installation or require frequent maintenance and change of electrodes. Other systems are semi-autonomous, yet they require substantial amounts of samples and produce large volumes of chemical waste.

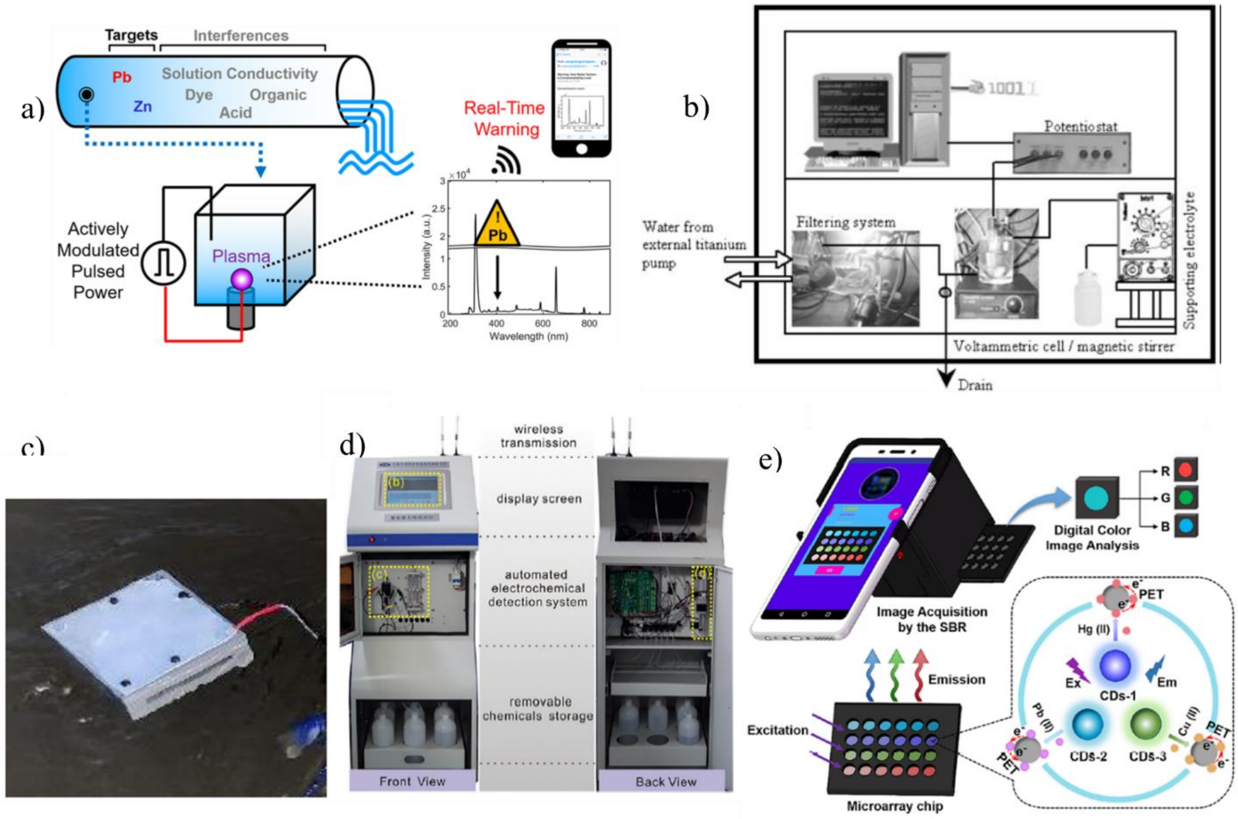

Figure 2-3 a) Actively modulated pulsed power Plasma device that can detect lead and zinc [73]. b) Voltammetry system for zinc, copper, Iron and lead [74]. c) Microbial fuel cell biosensor for copper sensing [75]. d) Electrochemical measuring equipment using voltammetry [76]. e) Smartphone-based device using carbon nanodots- based microarrays cabable of detecting mercury, lead, and copper [78]. Each figure was adapted from the corresponding publication.

The classification of in-situ devices can be divided into (i) semi autonomous devices including a) mobile phone-based devices and b) handheld analyzers, and (ii) fully autonomous devices which include the on-line monitoring devices. In Table 1-3, different on-line devices are presented along their limitations. The proposed system then eliminates the chemical liquid waste as it produces solid state confined hydrogels that can be handled mechanically and stored in smaller volumes. It also provides passive aliquoting, preconcentration and sensing enabling compact design and efficient operation in terms of the use of reagents and sample as well.

Table 1-3 The different devices used for heavy metals sensing.

	System description	Transducer	Analysed species	Detection limit	Limitations	Fig.	Ref.
Autonomous	Actively modulated pulsed power plasma	Spectrometer	Pb Zn	50 mg/l	High detection limit, and high voltage required. Energy consumption is high. Limited number of analytes.	Figure 1-3(a)	[73]
	Voltammetry system	Potentiometric electrode	Zn Cu Fe Pb	200 µg/l	High detection limit. Bulky system	Figure 1-3(b)	[74]
	Microbial fuel cell biosensors	Daphnia toxicity assay	Cu	35-40 µg/l	High detection limit.	Figure 1-3(c)	[75]
	Electrochemical measuring equipment using voltammetry	Three-electrode system	Cu Pb Cd	75 µg/l	Large instrument. Interference is expected when several metals are present.	Figure 1-3 (d)	[76]
Semi- autonomous	Smartphone-based	Carbon nanodots-Based microarrays	Hg Pb Cu	0.5 µM	Requires accessory connections and human operation.		[77]
	Smartphone-based Fluorescence device	Doped carbon quantum dots,	Hg Fe Cu	3, 0.5, and 30 nM	Requires manual sample quantification and handling.	Figure 1-3(e)	[78]
	LEGO-based platform utilizing RGB sensor and Arduino	Colorimetric	Cu^{+2} , Pb^{+2}	5.4 ppb 0.14 ppb	Require preconcentration of liquid samples	-	This thesis

2.3 Preconcentration and phase extraction

To make the detection methods more sensitive it is a common procedure to preconcentrate the sample rather than using a sensor with a lower detection limit. Sample enrichment and preconcentration is considered the most compatible approach with most of the analytical methods to obtain the sensitivity limits required. Analytical methods such as UV-Vis, ICP-MS, and ICP-OES offer viable solutions for in lab analysis but require sample handling between different steps such as enrichment and separation leading to added complexity and consequently not being suitable for autonomous systems. A desirable solution then is the integration of all the analytical process different steps starting from simple sample introduction methods to the detection in addition to the sample preparation and preconcentration. On-line sample preparation and sensing techniques can be considered a reliable solution as it saves time and allow autonomy.

Various preconcentration methods can be employed for the selective extraction and concentration of heavy metal ions from a sample solution. Many of the methods involve various forms of chemical interaction between a reagent (solid or liquid) and the ion of interest. These can be classified into (i) solid-phase extraction (SPE); (ii) cloud point extraction (CPE); (iii) and liquid-liquid extraction (LLE).

Solid-phase extraction (SPE)

SPE utilizes a solid-phase material to selectively bind and concentrate heavy metal ions from the sample. For example, M. Ghaedi et al. used silica-gel that has been chemically altered with a substance known as 2-((3-silylpropylimino) methyl)-5-bromophenol as the SPE matrix to bind and concentration trace amounts of metal ions (Fe3+, Pb2+, Cu2+, Ni2+, Co2+, and Zn2+). Subsequently, the concentration ions were eluted into a small volume using nitric acid and then analysed by flame atomic absorption spectrometry. This method had the advantage of High sorption preconcentration efficiency even in the presence of interfering ions, applicable for real samples analysis with recoveries in the range of 95 to

105%. They were able to achieve detection limit between 1.4 and 2.8 µg/L. The main drawback of such method is the need for species recovery from the Solid-Phase material using harsh solvents [79].

Cloud point extraction (CPE)

CPE involves the addition of a surfactant to the sample solution, which forms a cloud of micelles. Heavy metal ions are concentrated in the micelle phase, which can then be separated from the sample. This method can achieve concentration factors up to 300 while achieving limits of detection as low as 2.0 ng/L. Despite the low detection limit of such method, it requires the use of a liquid organic phase through which the ions can be concentrated. The analysis of those samples is typically conducted using ICP-MS which is a high tech and costly method[80].

Liquid-liquid extraction (LLE)

LLE works by separating two liquid phases that are not miscible together based on the variances in their solubility and distribution coefficients[81]. An extractant is used to move the solute from one liquid phase to another by selectively dissolving the solute in the first phase and transferring it to the second. Typically, the extractant is a solvent with a strong affinity for the solute and a weak attraction to the other liquid phase. The solvent used, the solute concentration, the temperature, and the pH of the system are some of the variables that affect how effectively an extraction is carried out. This method is able to achieve concentration factors of anywhere between 30 to 800 [82]. These techniques similar to the previously mentioned preconcentration approaches require the use of ICP-MS or similar analytical technique to measure or quantify the analytes.

There are also other methods of preconcentration such as electrochemical and solvent removal methods. Electrochemical deposition involves depositing heavy metal ions onto an electrode surface by applying an electric potential over a period resulting in their concentration on the electrode. Solvent removal preconcentration (SRP) is another widely employed technique in analytical chemistry to concentrate analytes in a sample by eliminating the solvent in which they are dissolved. The process involves the initial dissolution of the sample in an appropriate solvent, which is then subjected to a technique that eliminates the solvent and generates a concentrated sample. Evaporation, centrifugation, and freeze-drying are some of the techniques used for solvent removal preconcentration. This technique is often used in combination with other analytical techniques such as chromatography or spectroscopy to enhance the sensitivity and accuracy of the analysis. It is a widely accepted method for the analysis of complex mixtures such as biological or environmental samples. Solvent removal techniques are considered the most versatile as they can handle different species with diverse characteristics and properties while other methods would be applicable to specific analytes[19], [83].

Each method has its limitations and requires careful optimization to achieve accurate and efficient separation. For instance, the selection of a suitable surfactant for CPE can be challenging, and there can be interferences with the surfactant affecting the analysis accuracy. The selectivity of the precipitating agent in co-precipitation can be limited, leading to the precipitation of unwanted species, and reducing the efficiency of the separation. Similarly, the choice of the organic solvent for LLE can affect the selectivity and efficiency of extraction, and the method can be time-consuming. The selectivity of the membrane in membrane filtration can be limited, and fouling can affect the reproducibility and accuracy of the analysis. Additionally, the selectivity of the electrode in electrochemical deposition can be limited, and the efficiency of the deposition can be affected by the concentration and speciation of the analyte, requiring careful optimization. Unlike solvent removal methods, the Isotachophoresis method requires electric field just like ion concentration polarisation where both are considered to be electrophoretic and electro kinetic methods depending on voltage to concentrate charged species in a specific location[84],[85],[86],[87].

Solvent removal methods are considered versatile because the solvent is selectively removed from the sample leading to enrichment of all the solute present [19]. One solvent removal technique that can be used to preconcentrate samples is evaporative concentration [88]. The main issue with such a technique is that it requires large initial volumes to reach the final concentrated sample. Also, analyte recovery procedures have always been a significant issue for the evaporative preconcentration. Direct evaporation and membrane assisted evaporation are the two most popular techniques for microfluidic sample enrichment and solvent removal[19]. The first is based on direct evaporation of liquid sample with direct exposure to airflow [88][89][90], while the other technique includes a membrane that separates the air and liquid domains[91].

A good recent review on all these methods for different analytes was published by Fornells et al. [19]. A comprehensive review of the work done on heavy metals preconcentration will be presented in the next section with some discussion on the methods that have not been applied for heavy metals but have the potential to be used. However, the solvent removal technique is not without limitations. Firstly, it requires a relatively large sample volume, which can be challenging when dealing with limited or precious samples. Secondly, some analytes may be volatile or unstable and can be lost during the solvent removal process, thereby affecting the accuracy of the analysis. Additionally, this method may not be suitable for all types of samples, particularly those with complex matrices or high salt content, which can interfere with the solvent removal process or affect the stability of the analytes. Finally, the process can be time-consuming and may require specialized equipment or expertise, which may limit its practicality in certain settings.

The selection of a suitable approach for preconcentration is then an essential step in the design of on-line systems. Some of the most popular methods are specific for charged molecules such as ion concentration polarization and Isotactophoresis where both rely on the use of an electric field [92]. The working principle of it is the use of electric potential to migrate and preconcentrate charged analytes in a certain region. Some other approaches are solid-phase extraction (SPE) or partitioning the analyte in immiscible liquids using liquid-liquid extraction (LLE). SPE relies on the preferential affinity of the analyte to the surface while preferential solubility in a certain liquid phase is the working principle in LLE.

2.3.1 Solvent removal preconcentration

Solvent removal preconcentration (SRP) is an important technique in analytical chemistry used to increase the sensitivity of analysis by concentrating analytes in a sample. It can be classified into three types, namely: 1) Direct evaporation, 2) Membrane-assisted evaporation and 3) Droplet methods.

Direct evaporation involves heating the sample solution in an open container to evaporate the solvent and concentrate the analytes. This technique is simple and low cost. However, direct evaporation can result in the loss of volatile or thermally sensitive analytes and can also be affected by environmental factors such as humidity and temperature. As shown in Figure 1-4, direct evaporation can be further classified into a) open environment direct evaporation which does not require any confinement and can be applied in flow-based configuration and static based configuration and b) closed channel evaporation that requires confined channels with multiphase flow using either (i) virtual walls, (ii) drop trapping (iii) annular flow, or (iv) micropillars induced annular flow.

Direct evaporation: This process as shown in Figure 1-4a is usually used with non-confined chambers where the sample is in direct exposure to the ambient[93]Sample pre-treatment procedures implementing evaporation by gas flow are well established analytical methods that for decades was implemented for SPE and LEE methods by many researchers as they lead to the evaporation of the volatile solvents. The rate of concentration in this method is dependent on many ambient conditions such as air temperature, humidity, and pressure. Handling microfluidic volumes with such technique has its own challenges as well. Some of the limitations of the open channel format (Figure 1-4a) is the risk of sample contamination and the need for sample recovery as the concentrated volume usually end up on a substrate or filter material. An alternative method is the enclosed evaporation as shown in Figure 1-4b, makes use of closed microfluidic channels with multiphase flow providing a liquid/gas evaporative interface. As the parameters to be controlled are limited due to the use of controlled streams and flow rates. This method provides higher precision and better process controls in addition to evaporation monitoring. The enclosed channel method (Figure 1-4b) offers better flexibility in terms of gas/liquid interface but require tuning and precise controlled flows hence making the interface vulnerable to changes. Direct evaporation is simple and cost-effective but may result in analyte loss and can be affected by environmental factors.

Membrane-assisted evaporation: This method as shown in Figure 1-4c, involves passing the sample solution through a membrane with a high surface area to facilitate evaporation of the solvent. Vapour permeable membranes are implemented in the design of these devices separating the liquid and gaseous phases. The membrane supports two main functions, providing a porous media for the gas phase to migrate through, and acts as a non-wetted barrier for the liquid phase meaning that the liquid can not travel through. The partial vapour pressure difference between the two confinements is considered the driving force in this case which can be significantly enhanced with the use of a temperature difference[94]. The membrane is typically made of a hydrophobic material that allows the passage of vapor but not liquid. This method can achieve concentration factor between 3 and 27 within 60 minutes [95]. The method also can maintain a relatively simple configuration and easy to control interface. Membrane-assisted evaporation allows for better control and reduces the risk of analyte loss but requires specialized equipment and can be more expensive. However, they can be affected by eventual membrane wetting due to fouling or nonspecific adsorption and have limited lifetime. They also suffer from analyte binding to the membrane and small particles tend to block the pores.

Droplet-Based methods: The emulsion-based droplet shrinkage and solvent removal is a preconcentration method that creates an emulsion from the sample solution, immiscible organic solvent, and surfactant. Droplet shrinkage occurs when the emulsion is subjected to a temperature gradient, causing the droplets to shrink and release the solvent. The solvent is then removed from the system, leaving behind a concentrated sample. The droplet emulsion preconcentration relies on the preconcentration of the species within the droplet by heating in an emulsion phase such as soybean oil. If the water phase contains any solute it shrinks to a constant minimal volume while if the water encountered absence of ions the water vanishes into nano droplets and eventually disappear. The use of droplets in microfluidics as shown in Figure 1-4d introduces several advantages where it introduces smaller volumes with higher surface to volume ratios leading to faster heat transfer, evaporation, and chemical reactions. A droplet can undergo multi-step operations and allow for the integration of the preconcentrating steps with microdroplet based systems for total analysis.[19]. This method can preconcentrate samples with small volumes and the use of simple and inexpensive equipment. It’s been reported that this technique can achieve a concentration factor of 4 – 10. The operation temperature is usually around 35 ^{o}C [95]. However, the efficiency of droplet shrinkage and solvent removal can be impacted by the choice of surfactant and organic solvent, and the technique may not be suitable for samples with high salt content. There is also a risk of losing volatile or thermally sensitive analytes during the droplet shrinkage process, which requires careful optimization. Finally, these methods are difficult to integrated with flow-based systems [95]. .

Since all the preconcentration methods discussed above enable sample enrichment, they vary in performance depending on the operation conditions. For instance, the open channel (Figure 1-4a) encounters a risk of sample contamination and require sample recovery as the concentrated volume usually end up on a substrate or filter material. The enclosed channel (Figure 1-4b) offers higher flexibility in terms of gas/liquid interface but require tuning and precise controlled flows hence making the interface vulnerable to changes. The membrane assisted methods (Figure 1-4c) offer better and stable interface but can encounter membrane wetting and have limited lifetime. Finally, the emulsion method (Figure 1-4d) provides a reasonable preconcentration limit to the droplet-based systems but is hardly integrated with flow-based systems [95].

Table 1-4 Presents the different preconcentration methods and their performance characteristics. [33]

	Interface/ membrane	Mode	Application	Concentration Factor	Time	Solvent	Ref
Evaporation	Paper based	Static	Metals in ethanol fuel	250	-	Water/ ethanol	[89]
		Dynamic- fed evaporation	Lipoarabinomannan (lam)	20	10 min	Aqueous	[90]
			Dyes	100	180 min	Aqueous	[96]
	Annular flow	Dynamic	Distillation	-	NN/a	Water/ acetonitrile	[97]
	Droplet induced flow microfluidic channel	Dynamic	Fluorescent spheres and FITC-labeled BSA		28 min	Aqueous	[88]
Adsorption	Chemically treated filter paper	Dynamic	Lead Pb (ii)	-	13 min	Aqueous	[98]
	Empore chelation disks	Dynamic	Copper Cu	-	35 min	Aqueous	[99]
Electrophoresis	Electrophoresis on a paper chip	Dynamic	Cr^{3+},Cu^{2+}, fe^{3+}	160	6 min	Aqueous	[100]
Hybrid	Adsorption and Evaporation	Static/ Dynamic	Cu^{+2} , Fe^{+2} , Pb^{+2}	300 - 3000	10 minutes – Several days	Aqueous	This thesis

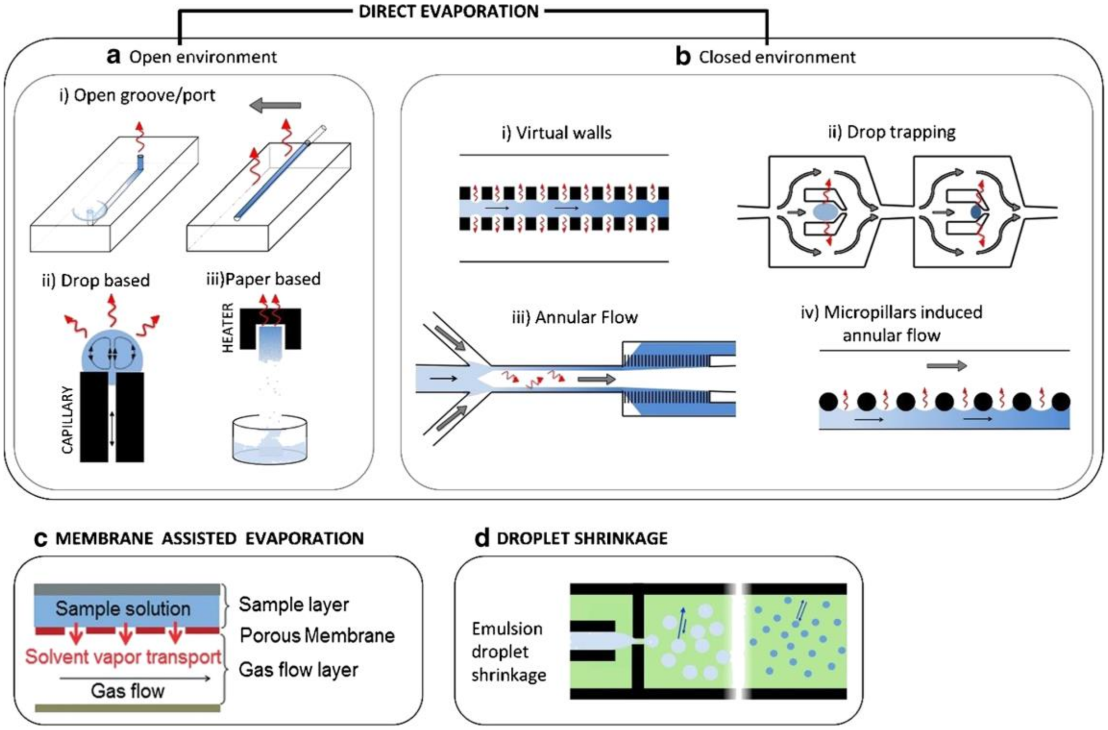

Figure 2-4 Schematic of different approaches of solvent removal preconcentration.

Adapted from [19]

The above discussed preconcentration methods suffer form one major disadvantage which is that they are affected by changes in operation conditions such as flow rate, temperature, and humidity leading to lower accuracy and reliability. The reported techniques provide a wide collection of approaches that can be integrated with on-line systems and total analysis devices since they concentrate the entire sample without any selectivity. They have wide applicability with aqueous or other solvent media. By evaluating these parameters, the performance of solvent removal preconcentration methods can be optimized for specific analytical applications. A good solvent removal preconcentration method should have high recovery rate typically higher than 80%, precision, and selectivity with low interference, low detection limit and cost, and be able to handle a reasonable sample volume in a short amount of time [19].

2.3.2 Preconcentration media

2.3.2.1 Hydrogels as a media for pre-concentration

Hydrogels are defined as "*a group of polymeric materials, the hydrophilic structure of which renders them capable of holding large amounts of water in their three-dimensional networks*." [101]. The applications of these hydrogels include diapers and sanitation products, agricultural applications, biomedical applications, selective drug release and delivery, in addition to environmental heavy metals remediation. Synthetic hydrogels gradually replaced natural ones over the course of years due to their higher water absorptivity and various control methods over their material properties leading to longer service life. Significant amount of work has been carried out the recent decades on the engineering of synthetic hydrogels for applications in several areas such as drug delivery [102], wound dressing [103], hygienic products [104], pharmaceuticals [105], and biosensor [106]. This versatile nature of hydrophilic polymers enabled a leap in the integration of solid polymers in liquid media.

Hydrogels have significant affinity to water and passively swell them. Swelling and diffusion are the main contributing phenomena in mass transport within the hydrogel. The hydrogel elastic behaviour directly impacts the swelling rates and quantities. The hydrogel composition impacts the adsorption behaviour as well. Stimulus-responsive polymer gels can expand and shrink in response to changes in the solvent's composition, pH, temperature, electric field, or light irradiation[101]. These functional polymers have sparked much interest in sectors including chemistry, biochemistry, agriculture, medicine, and engineering, and different applications have been investigated.

Sodium polyacrylate (SPA) hydrogel is a type of polymer hydrogel that has been extensively studied as a heavy metal adsorption medium[107]. It is a water-swollen polymer that is composed of repeating units of acrylamide and acrylic acid. These repeating units are chemically linked together through crosslinking agents, creating a three-dimensional network of polymer chains that can adsorb heavy metal ions from solution. The adsorption of heavy metals by SPA hydrogel is typically accomplished through a combination of physical and chemical interactions. The large surface area and porous nature of the hydrogel allows for a high degree of physical adsorption of the heavy metal ions, while the presence of functional groups such as carboxyl and amine groups on the polymer chains can also facilitate chemical adsorption.

One of the key advantages of SPA hydrogel as a heavy metals' adsorption medium is its high adsorption capacity for a wide range of heavy metals. This is primarily due to the high surface area and high degree of porosity of the hydrogel. Additionally, SPA hydrogel also has a high degree of selectivity for certain heavy metals over others, which makes it useful for removing specific contaminants from solution. For example, SPA hydrogels have been shown to chelate with heavy metals such as lead, Mercury, copper, and cadmium, among others[108] [107]. Another advantage of SPA hydrogel as a heavy metal's adsorption medium is its ability to be easily regenerated and reused. This allows the hydrogel to be reused multiple times without a significant loss of adsorption capacity. The efficiency of the SPA hydrogel in heavy metals adsorption is highly dependent on the preparation method, pH) and the presence of other competing ions or dissolved species in the solution. Also, SPA hydrogel is inexpensive and readily available which makes it an attractive adsorbent material. [108]

As an example, SPA hydrogel-based beads have been used for the removal of trivalent chromium ions from aqueous solutions [109]. The removal efficiency was found to be 90% at an initial concentration of 10.4 ppm while reaching equilibrium after 60 minutes. They also tested the adsorption up to 150 ppm and showed that the initial concentration significantly affects the adsorption process. They did not study the effect of sample volume nor flow over the adsorption. They also reported that higher temperatures lead to faster adsorption. Another study has reported adsorption of metal ions such as Cu and Fe in sodium

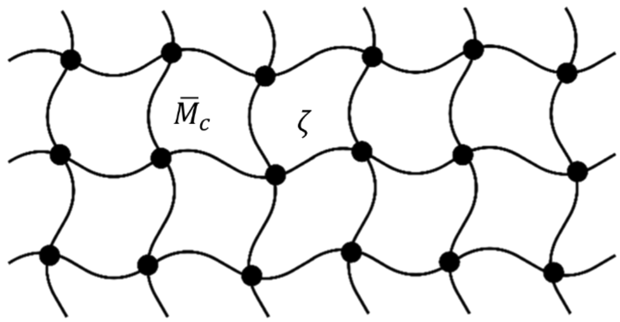

Figure 2-5 The figure depicts the building structure of a hydrogel network. Mc is the molecular weight of the polymer chains between cross-links, and ζ is the molecular mesh size. Reproduced from [110]

polyacrylate gel. They characterized the swelling and adsorption behaviours under variety of pH conditions and concentrations. A mathematical model was developed to explain the adsorption of divalent and trivalent ions under various pH conditions [107].

Another study on the mechanism of metal ion adsorption on SPA hydrogels and its interaction was conducted using Copper sulfate as a model sample solution [44]. They investigated the relationship between the polymer composition and swelling. They also derived a correlation between the crosslinking density of the hydrogel and its ability to adsorb divalent ions. They concluded that the degree of swelling of the hydrogels has dependency on the number of crosslinks only at concentrations below 0.025 M while when the concentration was higher the swelling was identical. They also found out that the penetration of the Copper ions within the polymer network was responsible for the generation of additional cross-linking leading to the formation of an in-soluble Copper acrylate compound.

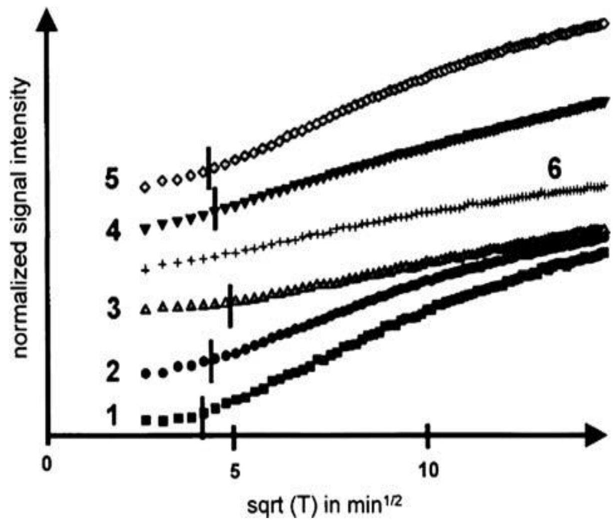

Figure 2-6 The transition from glassy state to fully swollen state can take any or all of the three intermediate transient states. The glassy-rubbery transition can take place. Adapted from [110]

These studies demonstrate the high capacity of SPA hydrogel-based beads for a wide range of heavy metals adsorption suggesting that the polymer can be a great platform for adsorption and preconcentration of heavy metals since it is transparent and swells to be more than 99% water in mass.

2.4 Transport within Hydrogels

2.4.1 Swelling of a hydrogel network

The theory of hydrogel polymer network swelling is based on diffusion of solvent molecules within the network. Several books and reviews have discussed their underlying fundamentals [110][111]. This section briefly discusses the swelling phenomenon of hydrogels and the parameters affecting it.

Hydroxylic (-OH), carboxylic (-COOH), amidic (-CONH-), primary amidic (-$CONH_2$), sulphonic (-SO_3H), and other chemical groups existing in the hydrogel structure and polymer backbone give it the hydrophilic characteristics. Several important parameters impact the equilibrium state of hydrogels. The swelling of hydrogels and their dimensional change are determined by factors such as the degree of crosslinking and the balance between hydrophobic and hydrophilic groups present in its composition.

These factors affect the equilibrium between the forces related to osmosis and the mechanical response of the hydrogel network. As shown in Figure 1-5, the effective molecular weight between cross-linking points, M_c, and the correlation distance between two adjacent cross-links, ζ and the polymer volume fraction in the swollen state, $v_{2,s}$ are considered the main parameters affecting the structure and properties of a swollen hydrogel. Two theories have been used to describe the swelling of a hydrogel using these parameters which are the rubber elasticity theory and equilibrium swelling theory [112]. Rubber elasticity theory claims that polymeric substances, made up of long molecular chains, exhibit elasticity and can stretch significantly and then return to their original shape. The equilibrium swelling theory states that the state of the system is due to the balance of entropy gain from mixing of the polymer with water and entropy loss from polymer chain extension and reduced conformations. The two theories are used to describe the relationship between those three parameters.

Based on the theories, the relationship between the polymer volume (V_p) and the swollen gel volume (V_g) can be represented using $v_{2,s}$. The volumetric swelling ratio (Q) is the inverse of ($v_{2,s}$) that can be defined in terms of the densities of the solvent (ρ_1), polymer (ρ_2) and the mass swollen ratio (Q_m) [110]:

$$v_{2,s} = {}^{V_p}\!/_{V_g} = Q^{-1} = \frac{\frac{1}{\rho_2}}{\frac{Q_m}{\rho_1} + \frac{1}{\rho_2}} \qquad (1)$$

The degree of crosslinking (X) can be described in terms of the effective molecular weight of the polymer chain[110]:

$$X = \frac{M_0}{2\bar{M}_c} \qquad (2)$$

where, M_0 represents the estimate of the repeating unit's molecular weight. ζ represents the estimated available space between macromolecular chains for solvent diffusion and can be calculated using this equation[110]:

$$\zeta = v_{2,s}^{-1/3}.l.\left(\frac{C_n.2\bar{M}_c}{M_r}\right)^{1/2} \quad (3)$$

for a polymer-solvent system, C_n is Flory characteristic ratio which is a constant defining the stiffness for a given polymer-solvent system. It affects how much deformation a polymer can physically undergo. While, l is the length of the carbon-carbon bond and M_r is the weight of the repeating units of the polymeric chain [110]. The swelling process occurs by the diffusion of different species within the

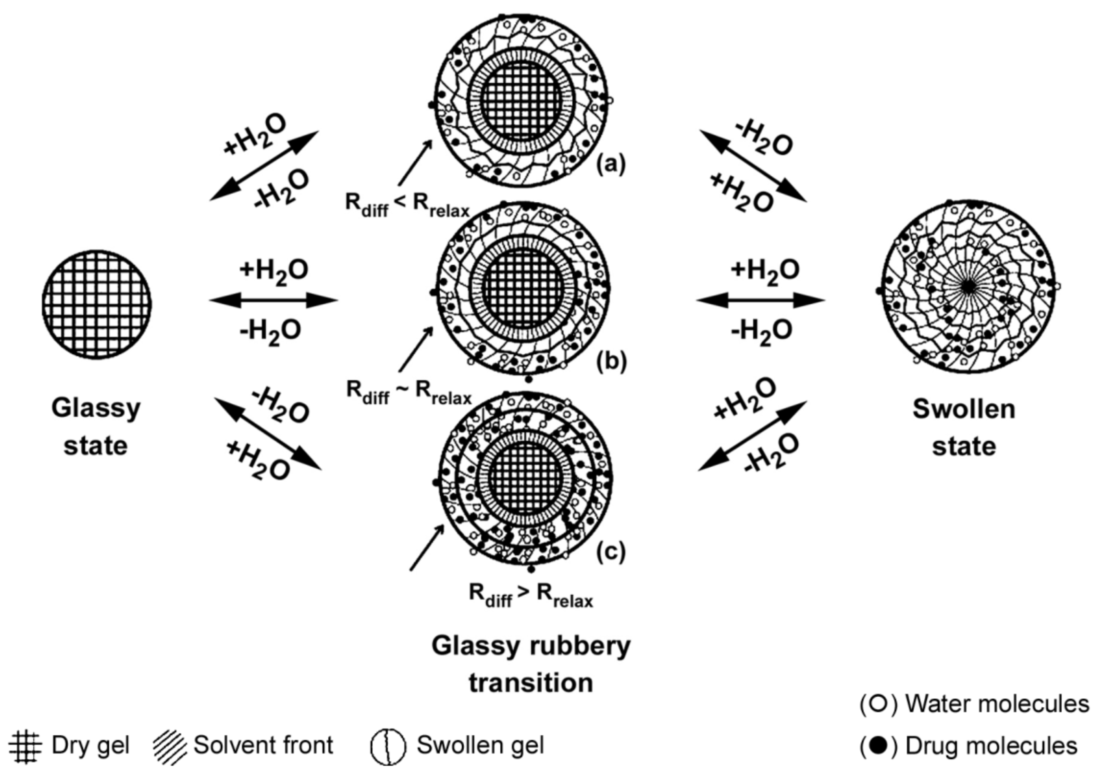

Figure 2-7 The transition from glassy state to fully swollen state can take any or all of the three intermediate transient states. The glassy-rubbery transition can take place. Adapted from [110]

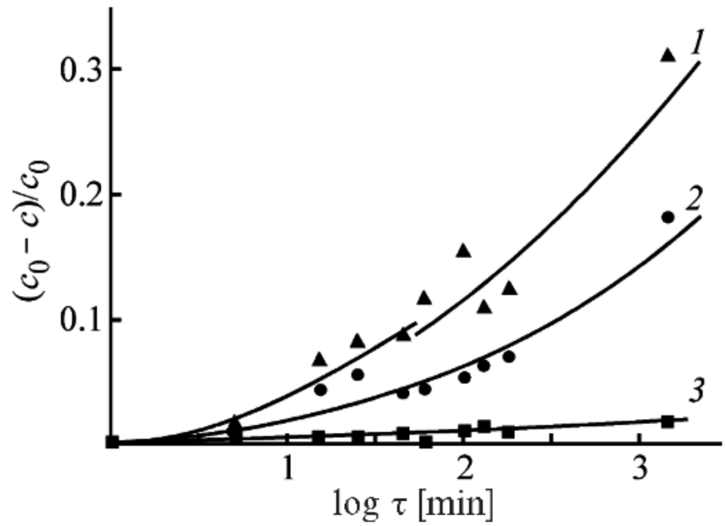

Figure 2-8 Kinetic curves of copper sorption to sodium polyacrylate hydrogel. concentrations of samples were (1) 0.005, (2) 0.01, and (3) 0.1 M. C0, and C are the initial and final concentration of ions within the liquid sample. Adapted from [113]

polymeric network. The polymer chains change their position, expanding, as a response to the diffusion of the swelling agent (the solvent). Hence, the degree of swelling (Q) is time dependent and its relation to time relies on the relative rate of penetrant diffusion and the relaxation of the polymeric chain. As shown in Figure 1-7, Q then can be related to three different rates (i) $t^{1/2}$ for Fickian diffusion where the diffusion is significantly slower than the polymer chain relaxation (Figure 1-7a), (ii) t^{1} for case ii transport when the rate of penetration diffusion is higher than the relaxation rate (Figure 1-7b), and (iii) $t^{n-1}$where 0.5 < n <1 for anomalous transport when the rates are comparable (Figure 1-7c). The transition between different states is shown in where the vertical line in the graph marks the transition from the first case Fickian diffusion to the second case [110].

2.4.2 Adsorption properties of heavy metals in hydrogels

Adsorption is a process in which a substance accumulates on the surface of a material through intermolecular forces, regardless of its state, and the accumulating substance is called the adsorbate.

Physical and chemical adsorption mechanisms are two key types of adsorptions with the former existing due to weak van der Waals forces of interaction and the latter produced by strong chemical bonds. The specific adsorption mechanism explored in this section involves metal ions, such as divalent and trivalent metals, adsorbing to the surface and bulk of sodium polyacrylate gel through a combination of physical and chemical adsorption mechanisms, depending on the pH buffer solution used. This process is essential in the field of heavy metals remediation, as it enables the removal of harmful metal ions from contaminated environments by trapping them onto an appropriate adsorbent material[113] .

The adsorption characteristics of divalent and trivalent metal ions onto sodium polyacrylate gel have been examined in detail in the past few decades [107]. It is assumed that carboxylic acid groups in the gel stoichiometrically interact with metal ions based on their valency, and the adsorption mechanism is theoretically investigated using the dissociation equilibrium for carboxylic acid groups. Furthermore, it has been discovered that the adsorption efficiency factor, which is inherent to metal ion species, plays a crucial role in determining adsorption equilibrium.

The time dependency of the chelation process makes the control of treatment time essential. The metal uptake is proportional to the remediation time so the increased immersion time would directly lead to increase of the adsorbed quantity. A maximum value would eventually be attained as the adsorption process slows down after a while. The main cause of adsorption reduction is that in the beginning all the free sites are available and the ions can travel through the gel boundary. After a while as the ions build up on the gel boundary, repulsion accurs and the ions encounter resistance preventing them from reaching the inner free sites [114]. As shown in Figure 1-8 the curves represent the adsorption of Copperonto the sodium polyacrylate gel. The kinetics indicate that the concentration of Copperin the sample solution decreases exponentially over the course of the two hours experiment. These measurements were conducted at 0.0005, 0.01, and 0.1 M of concentration. The sorbtion rate was found to increase with the increase in initial sample concentration. Yet, as seen in the graph, the higher concentration also results in longer duration of time to reach equilibrium. It was also noted that for all the tested concentrations, the sorbtion took place during and after full swelling. This indicates that the adsorption and swelling are indeed separate phenomenon. The pH of the solution has a significant effect on the adsorption kinetics of the gels. For example, the optimal conditions for Cd (ii) and Pb (ii) adsorption were found to be at pH 5.0 and the smallest at Ph of 2. This is due to preferential adsorption of hydrogen ions at low pH [114].

In summary, The carboxylic acid groups in the SPA gel interact with metal ions based on their charge number, and the adsorption mechanism is investigated using the dissociation equilibrium for carboxylic acid groups. The adsorption efficiency factor plays a crucial role in determining adsorption equilibrium. The control of treatment time is essential, and the metal uptake is proportional to the remediation time.

2.4.2.1.1 Dissociation equilibrium in the gel

The concentration of metal complexes per unit mass of the dry gel can be described as follows [107]:

$$q_{R_nM} = \frac{(C_{i,M}.V_i - C_M.V)}{m_i} \tag{4}$$

Where $C_{i,M}$ and C_M are the initial and equilibrium concentrations of the metal ions in the liquid solution respectively. V_i *and* V are the initial and equilibrium volume of the sample solution, and m_i is the mass of dry gel prior to the swelling. This is a defining parameter that can give indication on the concentration of the adsorbed metal ions per unit mass of the dry hydrogel. It is unique to each hydrogel complex and pH and is influenced by, temperature, and synthesis conditions of the hydrogel.

Carboxylic acid groups are the functional units in the sodium polyacrylate hydrogel and associate with with Sodium Na^+ ions at a ratio of 1:1. The representation of the disassociation equilibrium of carboxyl acids is shown below:

$$RCOONa \rightarrow RCCO^- + Na^+ \quad (5)$$

Where R represents a polymer chain –(C$H_2 - CH) -$. When the pH of the sample is changed to lower values, the carboxylic groups in the gel assosciate with the hydrogen ions changing into polyacrylic acid. The equilibrium relation is then expressed as:

$$R^- + H^+ \rightleftarrows RH \quad (6)$$

Where RCOO is represented by R^- and RCOOH by RH. Then the dissociation constant (K_d) can be expressed as:

$$K_d = \frac{q_R . C_H^*}{q_{RH}} \quad (7)$$

Where, q_R or q_{RH} shows the concentration of disassociated carboxyl groups and the undisasociated carboxyl acids respectively. C_H^* is the hydrogen ion's concentration in the gel that stays in equilibrium with the outside solution which cannot be directly measured but can be expressed as a function of the C_H which is the concentration of hydrogen ions in aqueous phase [mol·dm^{-3}]

$$C_H^* = K . C_H \quad (8)$$

Where K which is the Donan ratio is assumed to be constant in the range of 4< pH <7. The sum of q_{RH} and q_R is defined as:

$$q_{1,0} = q_R + q_{RH} \quad (9)$$

The concentration of hydrogen and sodium are assumed to be at equilibrium at all times, and since $q_R = q_H$, then:

$$q_R = q_{Na} \tag{10}$$

The relationship between the degree of disassociation in the gel and the hydrogen concentration in liquid solution can be expessed as a straight line:

$$\frac{1}{q_R} = \frac{C_H}{(K_d/K)q_{1,0}} + \frac{1}{q_{1,0}} \tag{11}$$

Experimental data were collected verifying the hypothesis and correlating the sodium and hydrogen concentrations [108]. As expressed in equation (10) the concentration of the fixed carboxyl groups in the gel is equivalent to that of the Na^+ ions.

2.4.3 Adsorption of divalent metal ions

Applying the fundamental theory of separation using chelate resin developed by (Blasius and Blonzio, 1967) [115] , the adsorption equilibrium of the divalent metal ions onto the hydrogel can be precisely described. Carboxyl groups that are fixed in the gel bind with the divalant metal ions. The combination ratio is 2:1 of the groups to metal ions stoichiometrically. The equilibrium can be expressed as:

$$2R^- + M^{2+} \rightleftarrows R_2M \tag{12}$$

$$K_m = \frac{q_{R_2M}}{q_R^2 . C_M} \tag{13}$$

Where q_R is the concentration of carboxylic groups in the gel, K_m is the formation constant of the divalent metal complexes. Equations (6) and (12) indicates that the fixed carboxyl group species coexist as RH, R^-, and R_2M. Subsequently, when the metal ions are adsorbed from an acidic solution the mass balance can be expressed as:

$$q_{2,0} = q_{RH}/2 + q_{R_2M} \tag{14}$$

Where $q_{2,0}$ is equivalent to the half of fixed carboxyl groups in the gel matrix. Combining equations (7), (8), (13) and (14) results in the following equation:

$$\frac{C_M\left(q_{2,0}-q_{R_2M}\right)^2}{q_{R_2M}} = \frac{C_H^2}{4(K_d/K)^2K_M} \tag{15}$$

Equation (15) determines the relationship between the metal complexes in the gel q_{R_2M}, the hydrogen ions in the aqueous solution C_H, and the metal ions present in th aquous solution C_M. The q_{R_2M} depends on the maximum concentration in the solution $q_{R_2M,Max}$, and hypothetical total concentration of them $q_{R_2M,tot}$.

2.5 References

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Chapter 3 Colorimetric Detection of Heavy Metal Ions Using Super Absorptive Hydrogels and Evaporative Concentration for Water Quality Monitoring

Mohamed A. Fathalla[1], P. Ravi Selvaganapathy[1,2]

[1] Department of Mechanical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada

[2] School of Biomedical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada

Corresponding Author

P. Ravi Selvaganapathy – Department of Mechanical Engineering, McMaster University, Hamilton, ON, L8S 4K1, Canada; https://orcid.org/0000-0003-2041-7180

* selvaga@mcmaster.ca

Status: published

Full citation

Fathalla, M., & Selvaganapathy, P. R. (2022). Colorimetric Detection of Heavy Metal Ions Using Superabsorptive Hydrogels and Evaporative Concentration for Water Quality Monitoring. ACS ES&T Water, 2(4), 658-666.

KEYWORDS: Super absorbent polymers; Hydrogel, Heavy metals; preconcentration; colorimetric detection; solid state

3.1 Introduction:

Metal ions are potentially significant contaminants in diverse environments, from urban watersheds to remote mining or mineral processing operations. Even small concentrations at the level of parts per billion (ppb) of heavy metals such as Lead, Mercury, Arsenic and Cadmium can cause human and animal toxicity when water containing them is consumed [1]. Apart from death, morbidities including brain damage [2], kidney damage [3], development of cancer [4], and DNA damage [5] are all directly linked to exposure to heavy metals. Even elements such as Iron and Copper [6] have regulatory limits at the level of sub-parts per million (ppm) due to aesthetic considerations and taste [7]. Heavy metals are toxic due to their tendency to be rapidly bio-absorbed and their interaction with proteins and other biomolecules through complexation as well as bioaccumulation [8].

Several well-established methods such as mass spectroscopy, optical emission spectroscopy and anodic stripping voltammetry exist to determine metal ion concentrations in sample at low concentrations, precisely. Some of these methods, in particular mass spectroscopy and optical emission spectroscopy [9], are laboratory-based as they require sophisticated equipment, skilled operating personnel and extensive sample preparation. Other methods, such as anodic stripping voltammetry elegantly combine preconcentration with detection but still requires the analyte to be electroactive within the electrochemical window of the solvent, which in the case of environmental monitoring is water. Furthermore, it has other limitations such as interference from dissolved oxygen, formation of intermetallic, influence of other species at high concentration on measurements apart from hysteretic effect and electrode degradation [10][11]. Due to these factors, many of these methods are difficult to adapt to field monitoring or may not cover the entire spectrum of analysis that is required.

An alternate approach is to use colorimetric dyes that change color when they complex with the appropriate metal ion of interest. These dyes are specific and do not suffer from significant interferences.

Furthermore, since they provide signal in the visible range, simple measurement of the presence of the ions of interest can often be made using widely available cell phone cameras for detection. Nevertheless, many of the colorimetric sensing methods that have been developed are not sensitive enough to detect at extremely low detection limits (in ppb range) and will require some form of pre-concentration [12], [13].

Several methods of pre-concentration have been developed both for analytical purposes [14] as well as in environmental remediation [15]. These methods concentrate the solute of interest from a large volume of solvent and provide a viable and versatile way to increase the concentration of the analyte of interest to reach the detection range of the colorimetric dyes. These preconcentration methods can range from solvent removal methods [14], [16], electrokinetic methods [17],[18],[19],[20] and evaporative methods [21][22][23] [24]. All these pre-concentration methods require accurate aliquots of the sample fluid to be deposited on them to provide a repeatable and accurate determination of the original concentration of the analyte of interest in the sample. Typically, either a pipetting step that provides repeatable sample volume or control over the time of exposure to the sample are used, both of which are active methods requiring human intervention or some degree of automation. A simple method that can accurately aliquot a pre-determined amount of the sample, pre-concentrate it for measurement using colorimetric methods in a passive manner would be invaluable to make accurate measurements at low concentrations for environmental monitoring.

Here, we describe such a passive method that combines accurate sample aliquoting with pre-concentration using solid state superabsorbent materials. Superabsorbent materials are commercially available and can take up 300-1000 times their mass in water, providing the necessary motive force to pre-concentrate. Their size and shape determine the volume of the sample that can be absorbed when placed in excess sample solution, thereby providing a simple and robust way to aliquot samples. Finally, the drying of the swollen superabsorbent materials can retain the ions while simultaneously pre-concentrate them due to volumetric shrinkage of the material as a result of the removal of solvent, making them ideally suited for preconcentration and detection. We demonstrate that by using superabsorbent beads where heavy metals such as Copper and Iron can be pre-concentrated significantly and the detection limit of standard colorimetric dyes for them can be reduced by two orders of magnitude from 1 ppm to <10 ppb. This passive approach enables the sample processing for these assays to be performed without any user expertise and hence is useful for field diagnostics.

3.2 Working principle

Superabsorbent polymers such as sodium acrylate can absorb hundreds of times their own weight in water. As they absorb water, dissolved ions that are present in the water will also be absorbed. If such superabsorbent polymers are cast in a specific shape such as a bead, the size of the bead determines the volume of the liquid that it can absorb. Therefore, by defining the size of the superabsorbent bead one can determine the amount of sample that it will absorb, precisely. Furthermore, the dehydration of the bead will remove the solvent (water) selectively and lead to concentration of the ions present into a smaller volume which can be used for pre-concentration. Finally, by adding an ion-selective colorimetric dye that exhibits color upon binding to the ion of interest, this concentrated ionic species can be measured and corelated to the original concentration in the sample solution. This sequence of operations is shown in Figure 2-1.a. The original size of dried hydrogel bead is shown in Figure 2-1bi. When this bead is placed in excess sample, it swells significantly as shown in Figure 2-1bii. The degree of swelling and absorption of the water can be determined by the volumetric ratios which can be calculated by measuring the diameters of the dry and the fully hydrated beads. Commercially available sodium polyacrylate super absorbent beads that are 2.7 mm in diameter in their dried state swell to 18 mm when fully hydrated which corresponds to a ~297 times volumetric increase due to absorption. When such fully absorbed beads are dehydrated, they shrink back reversibly to their original size while retaining all the absorbed analyte content which can result in a significant concentration of the species of interest.

3.3 Materials and methods

Sodium acrylate superabsorbent beads of diameter 2.7 mm (Water Beads Clear) and 1.8 mm (eboot 10000 Pieces Gel Soil Water Crystal Beads) were purchased from Water Beads Canada and Amazon eboot respectively. Lamotte 6446-E Copper reagent 1% Diethylcarbamodithioic acid) was used as an indication dye for Copper tested in samples of Copper sulfate. The dye changes to a brown color upon binding with the Cu (II) ion and the color develops instantly. Lamotte 2776-E reagent acid phenanthroline indicator consisting of phenanthroline (1,10) monohydrate 2.5% wt and Sulfuric acid 3-5% wt was used as an indicator dye for Iron tested on samples of Iron sulfate. The dye changes to a red color upon binding and is fully developed after 5 minutes. A control experiment was performed where the standard colorimetric assay using the Copper sensing dye was used. Following the prescribed procedure, 125 µl of the indicator dye solution was added to 10 ml of the sample solution and measured using Smart 3 colorimeter.

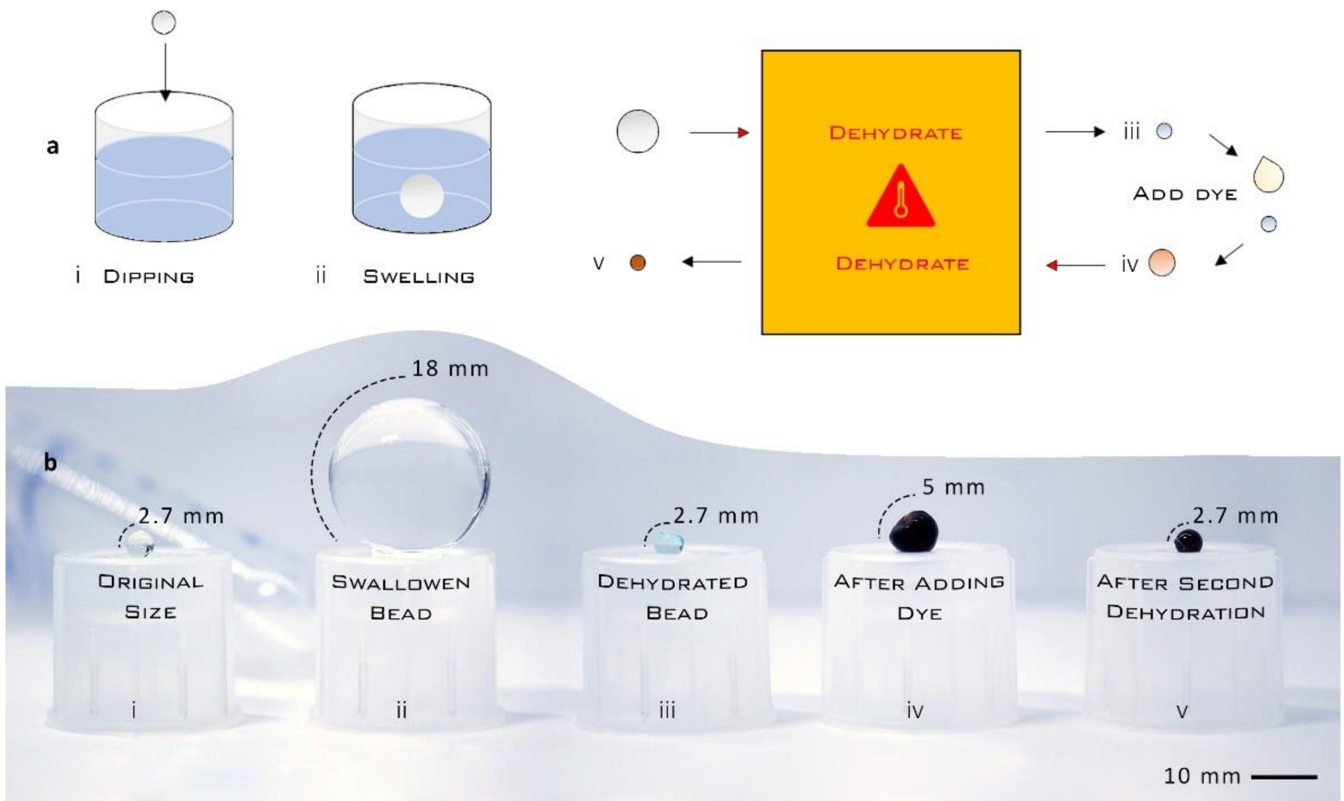

Figure 3-1 The swelling and dehydration of spherical superabsorbent beads. a) The schematic of the process sequence starting with (i) dipping the bead in test solution; (ii) allowing it to swell to saturation for aliquoting the sample, (iii) draining the excess water and dehydrating it in the oven, (iv) addition of the indicator dye (v) final dehydration. b) Corresponding images of the beads after each step. (i) The original size bead, (ii) fully swollen to a size of 1.8 cm in 5 ppm copper solution, (iii) dehydration of the bead which shows a significant blue color due to concentration of copper, (iv) addition of the dye to the dehydrated bead which swells it again partially turning into the dark brown color (almost black), (v) dehydration of the bead for the second time which intensifies the color further.

Two different protocols were used to study the effect of swelling and mass transport on the sensitivity and colorimetric response. The first one was to directly immerse the initial dehydrated bead into the sample and to saturate them before dehydration. The second one, conceived to reduce the initial absorption time, was to pre-soak the beads in a control solution (DI water) so that it is fully swollen followed by a shorter duration exposure to the sample solution. Each experiment consisted of 10 beads soaked in 40 ml of sample with a range of metal ion concentration from 10 ppb to 10 ppm. The larger beads (2.7 mm) took up to six hours to swell completely after which the sample was drained, and the beads were placed in an oven with enclosed circulating airflow at constant temperature of 80^{o} C for four hours. Subsequently, ~50 µl of the colorimetric dye solution was added to the dehydrated bead which quickly absorbs it and swells it slightly. This swollen bead is further dehydrated completely. A similar procedure was followed for the smaller beads (1.8 mm diameter) but with a swelling time of four hours, dehydration time of three hours and an addition of 25 µl of the colorimetric dye.

In some of the experiments, the shape of the superabsorbent material was modified from spherical to cylindrical to obtain structures which are smaller and with different form factors, both for characterizing the method as well as to reduce the analysis time. Superabsorbent materials of cylindrical form were made by using a biopsy punch to core the spherical beads and form a smaller structure with cylindrical shape. A 3.5 mm biopsy punch was used to core fully swollen beads in DI water. The cores dimensions were 3.5 mm in diameter x 18 mm in length when fully grown and 600 µm diameter x 2.7 mm length when dehydrated. Half cores were formed by cutting the cylindrical cores along their length. Dehydration was performed using an oven at 80^{o} C which provides precise control over the process time and temperature distribution. The cylinders were swollen for 10 minutes, then dehydrated for 20 minutes, after that they were swollen in 5 µl of indication dye for one minute, and finally dehydrated again for 7 minutes.

The imaging of the beads were then performed using a NIKON DSLR FX with 105 mm lens that was placed inside a black box to isolate it from any light interference. An LED light source within the black box was used to reliably reproduce the illumination. Image capture settings of 1/60 seconds shutter speed, f - stop of 9.0, and ISO of 400 were used. RGB analysis of the obtained images was performed using open-source ImageJ software. The area of interest in these images were chosen to contain only the beads. The intensity of each of the components (R, G, B) increased with concentration. R, G increased gradually and linearly over the range of measurement while that of B changed rapidly at low concentrations and did not change at higher concentrations. Therefore, an average of the R,G,B intensities was chosen to represent the change and optical density was calculated from it. $OD=-\log(I_s/I_b)$, where I_s the average of the pixel intensities in the region of interest, and I_b is the background intensity. [25]. Since this corresponds with grey scale intensity, grey scale cameras can also be used for greater sensitivity in imaging measurements.

To assess real water samples, three tap water samples were obtained from McMaster University. Two samples were obtained directly from the tap and represents city drinking water supply. They were found to contain Copper concentration of 167 and 98.5 ppb respectively as measured by mass spectrometry. Another sample of water was collected from a drinking water fountain which filters the drinking water supply, and this was found to have 92.5 ppb of copper. The samples contained 30, 20 and <10 ppb of iron, respectively; around 34 ppm of Ca for all samples,14.3, 14.8, 14.9 ppm of sodium, 9.1, 9.4 and 9.4 ppm of magnesium, respectively. This analysis was performed using ICP-MS method by Activation labs,

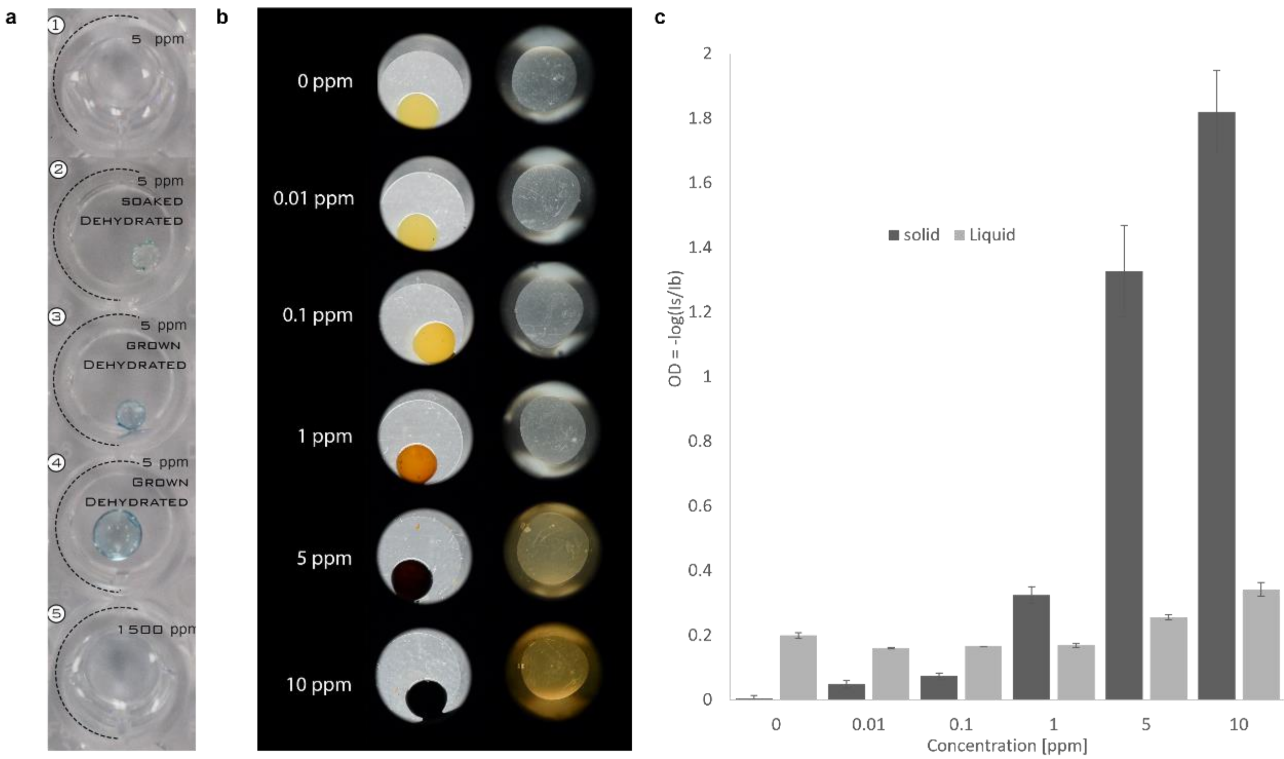

Figure 3-2 Measurement of preconcentration using superabsorbent beads. a) Demonstration of direct preconcentration of copper ions from water samples. a1) Liquid sample (control) containing 5 ppm of copper ions, a2) A 1.8 mm diameter bead that was initially swollen in DI water fully and then soaked in 5 ppm sample for 5 minutes followed by dehydration, a3) A 1.8 mm bead fully swollen in 5 ppm sample then dehydrated a4) A 2.7 mm bead fully swollen in 5 ppm sample and dehydrated, and a5) Liquid sample (control) of 1500 ppm copper ions. b) Images of the beads and the liquid samples obtained using a DSLR camera which show the beads demonstrate a higher sensitivity than equivalent liquid samples c) Optical density measurement of the beads and liquid samples from image measured using ImageJ software. (n=4)

Hamilton, Canada. Briefly, Fused samples with sodium peroxide undergo an acid dissolution. ICP-MS is then used to analyze the samples. Five synthetic calibration standards were used for calibration and with every batch of samples, a set of (10-20) fused certified reference material is run for quality control and calibration. Fused duplicates are run every 10 samples.

3.4 Results

3.4.1 Preconcentration Using Superabsorbent Beads

The ability of the superabsorbent polymers in the form of beads to preconcentrate metal ions for further analysis was demonstrated by absorbing water samples with Copper concentrations of 5 ppm and dehydrating them as shown in Figure 2-1 The protocols are described in the methods section. Both the small (1.8 mm diameter) and the large (2.7 mm) beads were immersed in excess volume (~40 ml) of 5 ppm Copper solution. Ten beads were immersed in the sample in each test. Swollen beads were placed in separate Falcon tubes then dehydrated. The swelling of the beads automatically aliquots ~ 3 ml of the sample solution for the 2.7 mm bead and ~ 1 ml for the 1.8 mm which is subsequently concentrated upon dehydration. In addition, 1.8 mm beads were also fully swollen in DI water and then dipped into excess volume of 5 ppm Copper sample for 5 minutes followed by dehydration. No colorimetric reagent to detect Copper was added. In addition, the two control samples of 0.25 ml Copper solution at 5 ppm

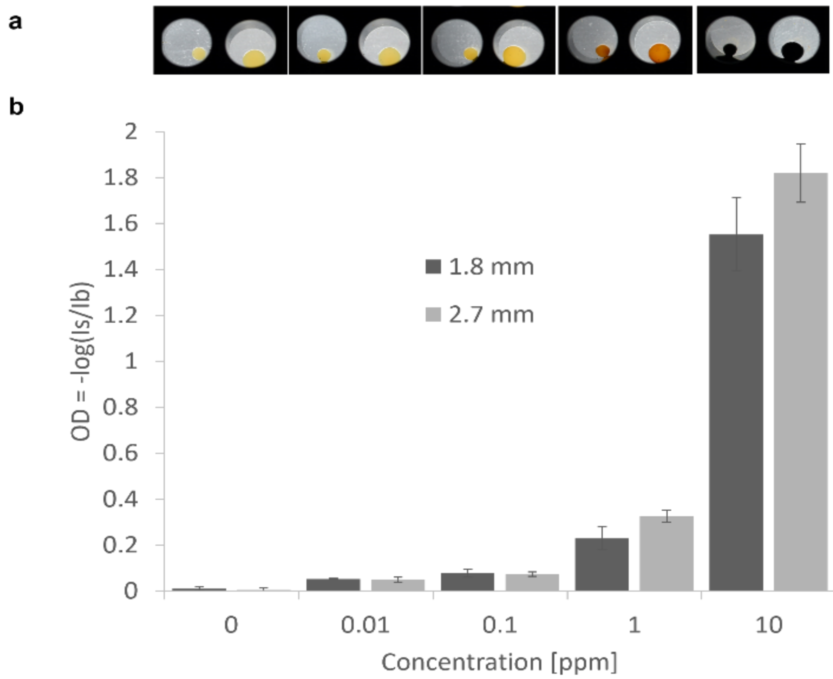

Figure 3-3 Effect of bead size on the colorimetric response to various copper ion concentrations in water samples. a) Images of 1.8 mm and 2.7 mm beads used to aliquot and preconcentrate copper ions from water samples. b) The measured optical density obtained from these images at various sample concentrations (n=4).

and 1500 ppm corresponding to the original sample concentration and the final one that would be achieved after pre-concentration were also prepared. The results as shown in Figure 2-2(a) demonstrate that while the sample solution at 5 ppm has no associated color, both the dehydrated beads that concentrate the sample show a blue tinge that is the natural color of Copper sulfate. The bead which was swollen entirely in the sample solution shows a greater OD related to its blue color while that which was pre-swollen in DI water and then soaked for a few minutes in the sample showed a reduction in the OD. This method could be used to concentrate and detect metal ions which have a natural color at high concentrations in a label free manner.

To measure the amount of Copper at the low level of 10 ppb in water samples, the addition of dye is necessary. One droplet (25 µl) of colorimetric dye was added to both the 250 ml liquid samples and the beads. Figure 2-2(b) shows the beads in comparison to liquid samples of the same concentration with the dye added to both beads and liquid. The results are evident to the naked eye and show that the beads have higher OD than the liquid. The optical density obtained from the beads and the liquid samples is presented in Figure 2-2(c). The liquid samples show no significant difference for samples less than 1 ppm concentration, hence cannot be used for the trace level sensing.

On the other hand, the pre-concentrated beads show linear increase in OD between 0 and 100 ppb and can further be used to detect changes till 10 ppm. The color of the bead changes from light yellow at 0 ppm to completely black at 10 ppm. The optical density was also found to be uniform across the diameter of an individual bead as shown in Figure S1. Also, the beads become significantly less transparent with the increase in concentration of Copper ions in the solution. Notably, there was a significant difference between 0 and 10 ppb solid state bead samples demonstrating its capability to identify presence of Cu ions at this low level. The limit of detection (LOD) was found to be 5.4 ppb for the 1.8 mm beads. The sensitivity was found to be 0.29/ppm change in OD for 2.7 mm beads at the lower concentrations where the response is linear. This compares favorably to the insignificant response of the liquid samples at these lower concentrations as well as the 0.017/ ppm change in OD at higher concentrations (> 1ppm). It was notices that when the beads were loaded, dried, and sectioned across its cross section to stain with the colorimetric dye, the rims of the beads closer to its surface was stained while the interior was clear (Figure

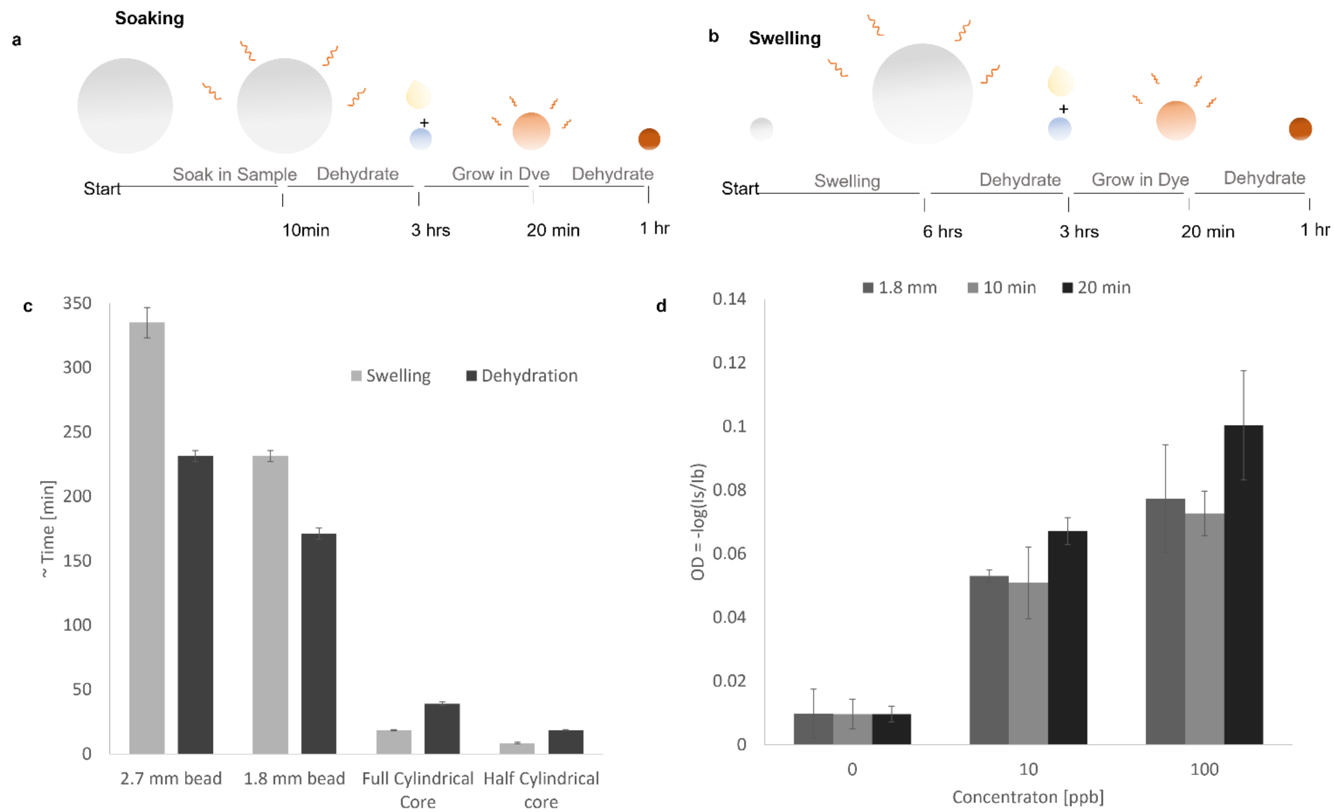

Figure 3-4 Comparison of different processes of preconcentration. a) Growing (swelling) the bead in the sample solution then dehydrating it, growing it in the dye then eventually dehydrating it again, b) Replacing the first step with a previously DI water swollen bead soaking it in the test solution for several minutes rather than swelling it completely in the solution. c) The change in swelling and dehydration time with the change in hydrogel size and formfactor (n=10). d) Comparison of OD change between fully swollen beads and those that were soaked for 10 and 20 minutes.

S2). This may be related to the accumulation of the ions at these sites during the swelling and adsorption process.

Different sizes of the beads were tested to show versatility and investigate the dependence of the technique on the bead size. Beads of 1.8 mm were used to aliquot, pre-concentrate and measure various test solutions with different Cu concentrations in a manner identical to the test process used for the large 2.7 mm beads. Four beads were evaluated and tested for each sample. The smaller beads had similar changes in OD as the larger beads as shown in Figure 2-3 .The small differences between the widely different sized beads indicates that the measurement of concentration using this approach is robust and independent of small variations in sizes of the beads that could be introduced by the manufacturing process. Also, the significance of results indicates that the results are reliable and of significant measurable values at both sizes. Deconstructing the changes in color to their R,G,B values (Figure S3) shows that there is a linear change in R and G values as the concentration is increased. The change in B values on the other hand are more rapid below 200 pb and plateau above that concentration. Therefore, the combination of all of these components can provide a reasonable sensitivity over the entire range of measurements from low concentration (where contribution from B dominates) to higher concentration.

3.4.2 Process time reduction

Although the beads demonstrate the use of superabsorbent materials for aliquoting, preconcentration and detection in a simple format, its form factor requires considerable amount of time to absorb the sample and to dehydrate it as well. For instance, a bead of 1.8 mm requires ~4 hrs to completely absorb the sample to and a further ~3 hrs to dehydrate. To reduce the process time, two different approaches were investigated. The first was the use of DI water pre-swollen beads to decrease the time associated with the absorption of the sample Figure 2-4(a). Since most of the OD measured is associated with the surface of the bead this method was expected to provide similar measurement response while considerably reducing the time for measurement. Instead of swelling the dehydrated beads in the sample directly as shown in Figure 2-5(b) for several hours, the beads (n= 10) as shown in Figure 2-5 (c) were pre-soaked in DI water to swell fully and only exposed to the samples (0, 0.01, and 0.1 ppm) for 10 or 20 minutes. The rest of the procedure for their dehydration, dye addition and imaging were the same as previously described.

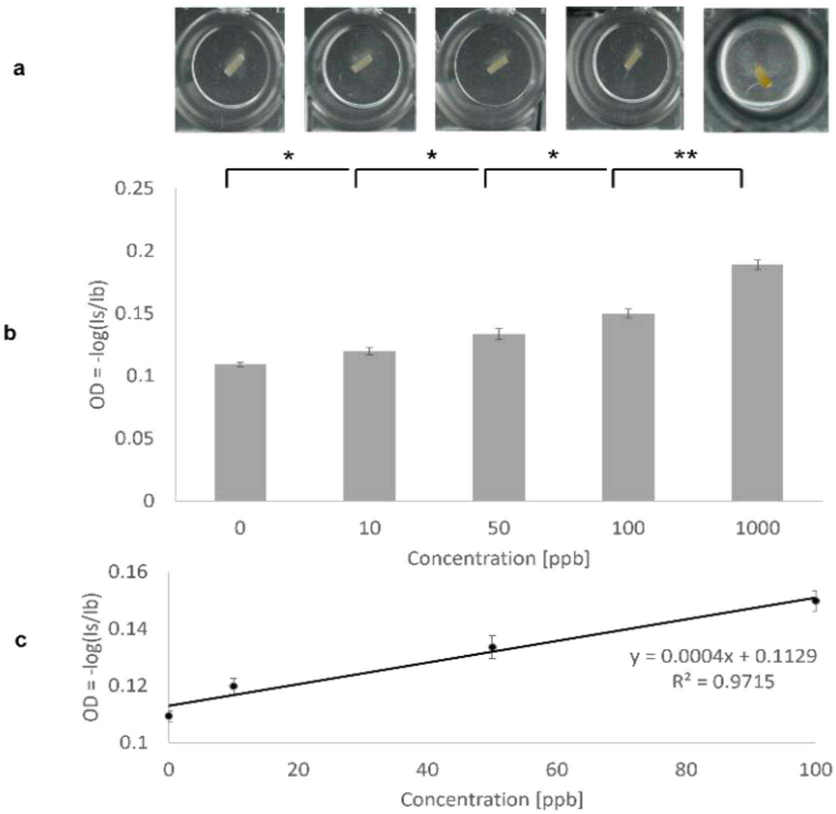

Figure 3-5 a) Images of half cores loaded with samples of concentrations 0 to 1 ppm b) Corresponding measured optical density obtained after pre-concentration process using half-core cylinders. c) Graph shows the linear response region between 0 – 100 ppb. (n=10)

The change in OD, shown in Figure 2-5 (d), demonstrate that there was no statistically significant difference between the pre-swollen beads and dehydrated ones. The pre-soaked beads show less intense blue color compared to the fully swollen beads in the same sample. They also show slightly reduced change in OD after the dye addition as well. However, the pre-soaked beads still showed change in OD between the 0, 10 and 100 ppb solutions of Copper which indicates that the new modified procedure with a considerably reduced time (from ~620 min to ~265 min) can be used effectively to determine trace amounts of metal ions colorimetrically. When the pre-soaked beads were exposed to the sample solution for 5 min or under, there was no observable color change at the trace level.

The other strategy was to reduce the size of the superabsorbent material and to increase its surface to volume ratio. For this purpose, beads of two different sizes (2.7 mm and 1.8 mm) as well as cored cylinders from the swollen beads were used. The cored cylinders were prepared by using a tissue biopsy punch of 3.5 mm size to punch through the fully swollen 18 mm beads resulting in cylinders of 3.5 mm in diameter and 18 mm height. The dehydrated sizes of these “full cylindrical cores” were ~ 0.6 mm in diameter and 1.8 mm in height. The full cylindrical cores where also cut in half to produce “half cylindrical cores” that were ~ 0.6 mm in diameter and 0.9 mm in height. All these superabsorbent shapes (dehydrated) (n=10) where then soaked in sample solutions containing 1 ppm of Copper ion and the process time to fully swell and dehydrate was determined. The resulting time for swelling and dehydration is shown in Figure 2-5 (c). Both the swelling and the dehydration times reduce as the size of the beads become smaller indicating that miniaturization can reduce the process time. Additionally, increasing the surface to volume ratio by 4 times by using cylindrical cores instead of beads has a dramatic effect on both the swelling and dehydration time. Remarkably, the total process time for detection was reduced from more than 8 hours using a 2.7 mm bead to ~ 38 min minutes using the half - cylindrical core. The ratio of the swelling and dehydration times invert between the spherical beads and the cylindrical cores. It was noticed during experiments that while the spherical beads swelled and dehydrated uniformly, the process was non-uniform for the cylinders. The edges at the tip of the cylinders were able to swell and dehydrate faster than the bulk and this led to dramatic transformation in shape during the process while it still achieved the final shape that was uniform. The reversal of the ratio between swelling and dehydration times is attributed to the presence of the edges. Similar non uniform swelling and dehydration have been observed previously [28], [29] which has been attributed to the increased contact area of the gel with the solution at the edges. Figure 2-5(d) show the different responses for different conditions and methods. It can be

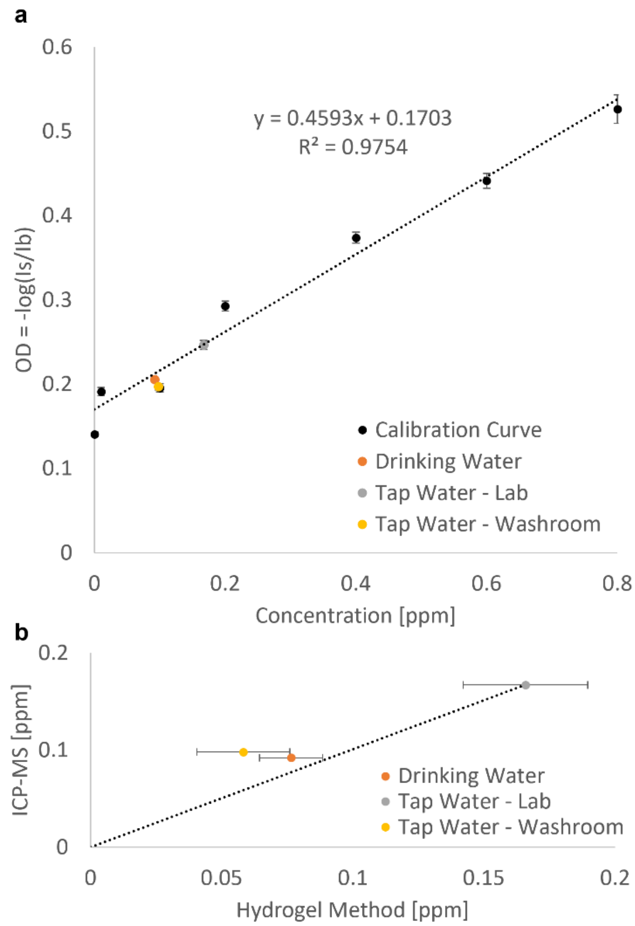

Figure 3-6 a) Measurement of drinking water samples obtained from the tap and from a drinking water fountain in comparison with reference samples prepared in the lab for calibration purposes. (n=10). b) Shows the values measured by the hydrogel method in contrast to the measured values using the standard method (ICP-MS)

noted that all methods show similar response and were able to distinguish 10 ppb samples from those that did not contain copper. The 1.8 mm beads had a limit of detection of 5.4 ppb while it was 6.8 ppb for the beads soaked for 10 minutes and 6.2 ppb for those soaked for 20 minutes. We also tested whether loading the beads multiple times in fresh samples would increase the loading of ions on the beads. As seen in Figure S4 there was increase in optical density obtained at each concentration after the second loading. This effect was small bellow 200 ppb and became significant above that concentration.

The cylindrical half-core was the optimal geometry that facilitated fast process time for pre-concentration and detection of Copper ions calorimetrically. The dehydrated half cores 0.6 mm in diameter and 0.9 mm length, fully swell in ~ 7 min and can be completely dehydrated in ~ 20 min. Subsequent addition of 5 µl of the dye followed by 10 min of further dehydration completes the process for a total process time of ~ 38 min. A calibration curve developed based on this process for various concentrations of Copper ions in the sample solution is shown in Figure 2-5. The limit of detection was found to be 10.9 ppb for the half cores which is slightly higher than the 5.4 ppb for 1.8 mm beads but still at the trace level.

3.4.3 Tap Water measurements

Although the use of superabsorbent polymers functions effectively in lab prepared sample solutions, interfering species such as other cations that may be present in the field samples may have an influence on the measurement accuracy. Since the need for measurement of heavy metal ions in drinking water is the most pertinent, water samples from three sources. The first was unfiltered tap water (Sample 1) which had a Copper ion concentration of 167 ppb confirmed by ICP-MS measurement. The next was washroom water that had a concentration of 98.5 ppb. The final sample was drinking fountain water that had filtering capability and which was found to have 92.5 ppb of Copper in it. All samples were obtained from McMaster University in Hamilton. The three water samples contained 30, 20 and <10 ppb of iron, respectively. The Ca concentration was around 34 ppm for all samples, while they had 14.3, 14., 14.9 ppm of Sodium, 9.1, 9.4 and 9.4 ppm of Magnesium, respectively. Beads were used of 1.8 mm initial diameter. Soaking approach was used for this experiment for 20 minutes in the liquid samples of 40 ml per 10 beads then dehydration was performed for 3 hrs. 25 µl of dye was then added to each bead and allowed to swell for few minutes. Finally, dehydration for 20 minutes took place, and then the analysis was performed. RGB measurements were performed using ImageJ as described in the methods section previously.

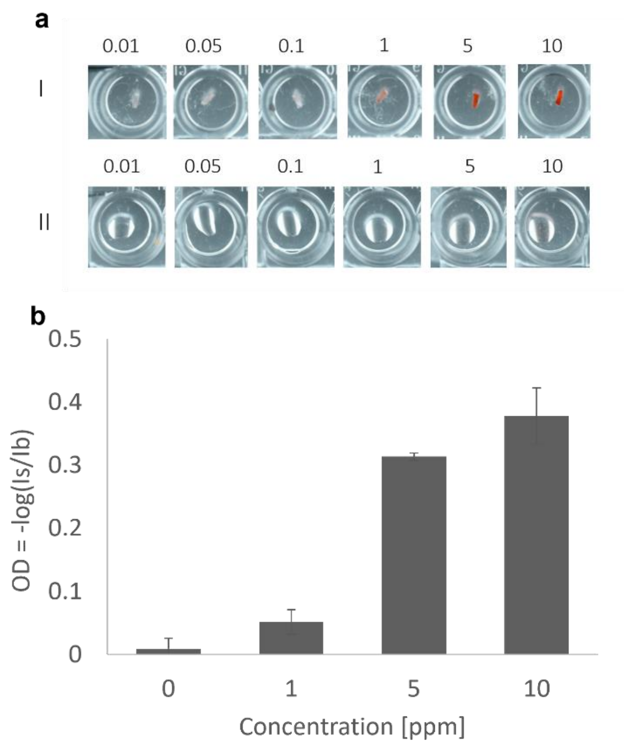

Figure 3-7 Concentration of Iron on half core cylinders and their measurement. a) Comparative images of the half cores with equivalent liquid samples. The half cores (I) concentrate the ions and are clearly responsive at 1 ppm while the liquid samples (II) do not show a meaningful response b) Optical density of the half core cylinder to various concentrations of Iron ions in sample solution (n=10).

The results of the measurements from the drinking water samples in reference to the calibration curve from laboratory prepared samples in DI water in shown in Figure 2-6. It shows that samples of drinking water align closely with the calibration curve and deviations of < 5% was observed. These experiments were performed with a higher intensity LED source that provided greater illumination of the sample. These measurements show the promise that the pre-concentration process is not significantly affected by the presence of other cations in the solution. It is known that high ionic strength and large variation in pH can influence the swelling behavior of these superabsorbent materials [30]. However, the narrow range of pH and ionic concentration encountered in drinking water negate any significant effect of these parameters in these measurements. Care must be taken if this measurement approach is used for samples with widely varying pH or ionic concentrations such as in wastewater or ground water samples.

3.4.4 Measurement of other cations

To test the versatility of the developed method, sample solutions containing Iron sulfate were tested using the same approach. Sample solutions were prepared with concentrations from 0-10 ppm. Half-cores (n=10) were used to measure the concentration in these solutions and compared with equivalent liquid sample measurement. The half cores were fully soaked in the sample solution to absorb it completely, dehydrated and then 5 µl of Iron sensitive dye were added to them. Finally, the cores were dehydrated completely, and the images were analyzed to obtain the optical density. The liquid control samples were prepared by adding the same volume of dye to 60 µl of sample (equivalent to the volume of the half cores) and obtaining images.

The Iron species were pre-concentrated successfully as shown in Figure 2-7 (a-i) and b. The equivalent volume of liquid samples shown in Figure 2-7 (a-ii) do not show significant color difference especially at low concentrations demonstrating that the solid-state concentration provided by the cylindrical half cores is able to effectively increase the local concentration and achieve higher sensitivity of the same dye. It was observed that the colorimetric indicator solution for Iron was not fully absorbed by the concentrated and dehydrated half cores. This could be due to the pH of the indicator dye or due to the interaction between the indicator and the hydrogel. Due to this reason, detection of concentrations lower than 1 ppm wasn't possible. We also tested the effect of co-presence of Iron in the sample with Copper will affect

Copper sensing. Measurements of Copper concentration shown in Figure S5 demonstrate that the optical density obtained does not significantly change (<3%).

3.5 Conclusion

The use of super absorbent polymer beads to automatically aliquot, pre-concentrate and quantitatively measure concentrations of heavy metal ions such as Cu and Fe has been demonstrated. This method is passive and does not require complex sample processing required for other colorimetric methods and can enhance the sensitivity to lower concentrations. The process time for this method was substantially reduced by increasing the surface to volume ratio of the polymer shape and miniaturizing it. Drinking water samples with other potential interfering species were tested and the response was found to within 5% deviation to the calibration curves generated from spiked DI water standardized samples. The technique is shown to measure Cu concentrations with limit of detection below 10 ppb. Due to its passive nature and simple instrumentation, this method has significant potential for use in resource poor settings.

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Chapter 4 Sub ppb sensing of trace heavy metals using pre-concentration from large sample-volumes on solid state hydrogels

Mohamed A. Fathalla[1], P. Ravi Selvaganapathy[1,2]

[1] Department of Mechanical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada

[2] School of Biomedical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada

Corresponding Author

P. Ravi Selvaganapathy – Department of Mechanical Engineering, McMaster University, Hamilton, ON, L8S 4K1, Canada; https://orcid.org/0000-0003-2041-7180

* selvaga@mcmaster.ca

Status:

Pending Submission

KEYWORDS: Super absorbent polymers; Hydrogel, Heavy metals; preconcentration; colorimetric detection; solid state

4.1 Abstract:

Environmental monitoring is becoming increasingly important as human activities such as mining increase that can potentially introduce toxic elements such as Lead, Mercury and Arsenic into water bodies used for human consumption. Yet, monitoring the environmental waters for these contaminants at the level that they cause harm is not always easy, cost-effective, or simple for the population that is affected by it. In this paper, we present a method that enables cost effective monitoring of heavy metal ions in water at the sub ppb level. We demonstrate that using widely available hydrogels for preconcentration and colorimetric dyes for visualization, it is possible to measure concentrations as low as 500 ng/l of lead and Copper without the use of any sophisticated equipment. By preconcentrating analytes from a large volume of sample of 500 ml to 2 liters by 5 orders of magnitude onto a solid-state hydrogel we enable the detection of sub ppb amounts of Copper when combined with commercially available low sensitivity colorimetric dyes. Detection of low levels of Lead was also demonstrated. Our approach provides an off the shelf solution that can be easily implemented and replicated without the need for skilled personnel to perform the analysis in resource poor settings.

Introduction:

Over the last century and more, resource extraction and rapid intensification of industrial activity has lead to a significant contamination of water resources at several locations around the world [1]. This resulted in legislation of several regulations to limit the exposure of the water resources to the most toxic contaminants to monitor these vital resources [2][3][4]. Heavy metals are toxic at even the trace level, but are generally low in many of water bodies[5][6][7][8]. However, industrial and mining activities can, in some instances, lead to higher concentrations. Even old infrastructure such as water distribution pipes made of Lead can leach them into drinking water affecting millions of people in cities. Measuring heavy metals in water, soil, and industrial waste is then a crucial aspect to protect the human and ecosystem health [9][10]. Highly sensitive measurement methods such as mass spectrometry typically require samples to be collected from the field and sent to specialized laboratories that have skilled personnel to perform the measurements [11]. The alternative methods would be using biosensors [12] and ultrasensitive reagents [13] such as DNA-zymes [14] or aptamers [15] that are also highly sensitive and selective but very expensive and may require certain preservation methods such as low temperature and dark environment for them not to lose efficacy or deteriorate [16]. Finally, electrochemical sensors using anodic stripping voltammetry method are available for some heavy metals that have higher detection limits. However, they are only suitable for certain electroactive elements that do not have overlapping reduction potentials as well as can suffer from fouling[17].

The challenge of heavy metal sensing in drinking and natural waters is the requirement to detect concentrations at extremely trace levels due to their toxicity. Some elements such as Lead (Pb) are regulated at as low as 3 μg/L (ppb). The methods used to detect such low concentrations are lab based and usually only available in large, centralized labs to which the field samples have to be carried. Interference from other ionic species present as well as other organic and inorganic materials also pose challenges to measurement. In this context, species preconcentration is effective in accumulating the analyte of interest to a level that can enable simple and less sensitive detection modalities that maybe more suitable for field deployment to be applied to sensing trace level contamination of heavy metals [18].

Several methods currently exist where some or all the sample is removed, and the species are either extracted in a reduced quantity of the solution, a different solution, absorbed or deposited on a surface or within the bulk of a material [19]. Nevertheless, many of these methods either required complicated device fabrication approaches or limited amount of concentration or require further sample transfer for detection. We have previously, developed a novel method of using hydrogel beads both as a sample concentration device as well as an effective substrate to perform detection [20]. This approach does not need any complicated fabrication process, provides high degree of concentration and samples thus concentrated can be directly measured using simple colorimetric techniques. However, this method based on evaporative pre-concentration was able to detect only down to ~5 ppb concentration which was suitable for some heavy metals such as Cu and Fe but not for the most toxic ones such as Pb.

Here, we combine the previous evaporative pre-concentration method with specific chelation of heavy metal ions to acrylate hydrogels to demonstrate ultra-sensitive sub ppb level detection. It is based on our finding that the hydrogel beads have a large capacity to chelate with metal ions and a flow system that can expose the sample multiple times or over larger volume can accumulate through the chelation process a much higher quantity than possible over shorter exposure times or smaller sample volumes. Through this dual approach we were able to overcome the limits of evaporative pre-concentration and achieve a five orders of magnitude increase in concentration. We then use selective colorimetric dyes that are sensitive only at higher concentrations to determine sub ppb levels of the heavy metal ions in water. We first characterize the mechanism using Cu ions and then demonstrate its implementation to detect Pb in drinking water samples. This approach requires much simpler instrumentation and is capable of implementation in the field or by the stakeholders in the community while providing highly sensitive detection of important toxic elements in drinking water.

4.2 Working principle:

Sodium polyacrylate (PAA) hydrogels are well known for their large capacity of metal ion adsorption [21]. They are three-dimensional crosslinked polymer networks with hydrophilic characteristics that allow them to swell in water and retain them at large water to polymer weight ratios exceeding three orders of magnitude. They are structured from polymeric networks with -COOH/ -COONa functional groups. These materials show preferential binding to divalent metal ions (M^{2+}) over monovalent ones as the

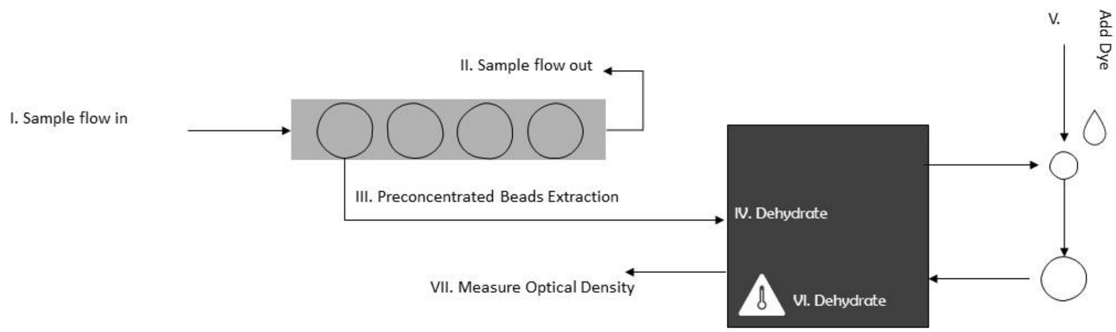

Figure 4-1 the process flow starts with the sample flow inside a flow cell containing the hydrogel beads; i -ii) after the exposure time interval is over, the beads are extracted; iii) then dehydrated; iv) after that, the colorimetric dye is added which slightly swells the bead; v) and the dehydration process is repeated; vi) the final step is measuring the optical density (od) by imaging using a DSLR camera and analysis using ImageJ.

adjacent COO- groups associate with the metal ions to stabilize the bonding [21]. Binding affinities of various divalent metal ions have been determined for PAA and it has been shown that ions such as Pb^{2+} and Cu^{2+} have much greater affinity as compared with others such as Cd^{2+}, Ni^{2+}, or Zn^{2+} ions [22]. Ions with a bigger ionic radius have a greater binding affinity as complexation becomes more favorable sterically. Therefore, these weak polyelectrolyte gels have a greater affinity to binding and extract heavy metal ions over more commonly prevalent mono and divalent ions such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}. The pH of the solution also plays an important role in the complexation process and therefore its binding affinity. However, environmental water has a narrow range of pH values it is not expected to play a critical role in the uptake of the ions from the sample making the ion concentration and the time as critical parameters. We hypothesize that at low sample concentrations, the capacity of the hydrogel network to adsorb all the ions that are present in the sample is not saturated in a brief exposed period and therefore exposing a larger volume of the sample over a longer period can facilitate adsorption of a greater proportion of the metal ions from the sample onto the bead that can enhance the limit of detection.

Our device as shown in Figure 3-1 consists of a flow cell (channel) that can be perfused in a continuous or periodic manner from a reservoir containing a finite volume of the sample. The sample (up to two litres) is recirculated through this flow through device over several days (7 days) at a flow rate of ~30 mL/min providing 150 circulations of the entire volume over the beads in that period, to preconcentrate a significant fraction of the ionic content of the sample onto the beads (0.9 µL each). Subsequently, the pre-concentrated beads are dehydrated in an oven (commercial food dehydrator at 75^{o}C for 4 hours) where they lose the solvent (water) while retaining the adsorbent (metal ions). Then a specific colorimetric dye (25 µL) that changes color based on the presence of the analyte of interest is added which quickly absorbs into the beads and partially swells the bead. The bead is then dehydrated further to concentrate the color generated and measured. Although recirculation was used to pass over the sample several times over the beads, our approach can be used also for inline monitoring where 2L of fresh sample is flowed over the beads over the same time. In this mode it serves to measure the average concentration of the metal ion of interest over the period that the device is perfused.

Sets of 5 beads each soaked in 50 ml to 1000 ml of sample with a range of metal ion concentration from 10 ppb to 800 ppb (or 0.5 – 5 ppb for some experiments) were used to characterize the adsorption behaviour of the hydrogel. The beads of diameter (1.2 mm) could swell completely in four hours. For the specific duration associated with equilibrium of that sample volume each experiment was performed for 1, 3, 6, 7 days. Then the sample was drained, and the beads were placed in a dehydrator with circulating airflow at a set temperature of 75 ^{o}C for four hours. Subsequently, 25 µl of the colorimetric dye solution was added to the dehydrated bead which quickly absorbs it and swells it completely. This swollen bead is furtherly dehydrated completely.

To provide selectivity for detection of Pb, we use reaction of sulphur with Pb to produce dark insoluble precipitates that can be easily visualized. This reaction is highly selective to Pb as compared with other alkali and alkaline-earth metal ions that are commonly found in environmental waters. This selectively combined with the pre-concentration approach allows for detection in the 10's of ppb range which was not possible before.

4.3 Materials and methods:

4.3.1 Materials and Regents

Sodium acrylate superabsorbent beads of diameter 2.7 mm (Water Beads Clear) and 1.8 mm (eboot 10000 Pieces Gel Soil Water Crystal Beads) were purchased from Water Beads Canada and Amazon eboot respectively. Lamotte 6446-E Copper reagent 1% Diethylcarbamodithioic acid) obtained from Cole-Parmer was used as an indication dye for Copper tested in samples containing Copper ions. The dye changes to a brown color upon binding with the Cu ion and the color develops instantly. Sodium sulfide obtained from Sigma-Aldrich is used as an indicator dye for Lead ions. The solution turns into black due to precipitates when mixed with Lead containing solution. A molarity of 0.4 M of sodium sulfide was utilized for all experimental procedures. A control experiment was performed where the standard colorimetric assay using the Copper sensing dye was used. Following the prescribed procedure, 125 µl of the indicator dye solution was added to 10 ml of the sample solution and measured using smart 3 colorimeter. For lead, the standard solution of sodium sulfide was mixed with samples of 10 ml ranging between 10 and 50 mg/l.

In some of the experiments, powdered acrylate gels (obtained from Flynn Scientific) were used to obtain a higher surface to volume ratio that can be useful in reducing the time for adsorption of the amount of metal ions present in the sample. In this experiment the powdered gel was stirred in a one litre sample for the specified duration of the experiment and then collected by filter paper. The powder is then dehydrated in a food dehydrator and the dye is then added to the powder, allowed to swell fully, and dehydrated again.

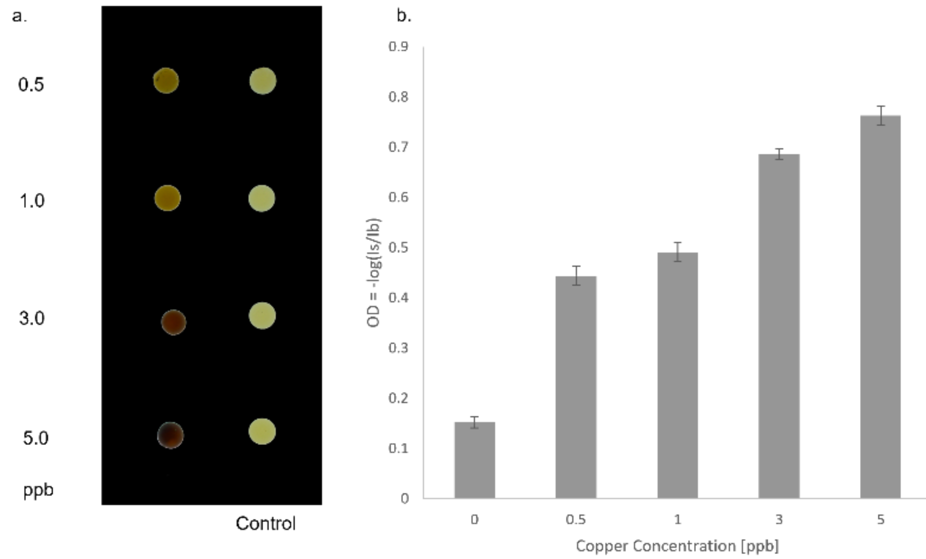

Figure 4-2 a) Presents the colorimetric response of the beads swollen in copper samples of 2L volume each for 7 days. b) shows the OD of the measured colorimetric responses. (n=5)

4.3.2 Dehydration Methods

Two different protocols were used to study the effect of swelling and mass transport on the sensitivity and colorimetric response. (i) The first was thermal dehydration where the bead was placed in the oven to remove the solvent. (ii) The second one was using the property that these gels exclude water at basic pH. Placing the swollen beads in a 2M KOH solution rapidly excludes water from the gel and leads to its shrinking and can be used as an alternative to thermal dehydration in a lab setting.

4.3.3 Imaging Setup

The imaging measurements of the beads were then performed using a NIKON DSLR FX with 105 mm lens that was placed inside a black box to isolate it from any light interference. An LED light source within the black box was used to reliably reproduce the illumination. Image capture settings of 1/125 seconds shutter speed, f - stop of f/45, and ISO Speed of ISO – 150 were used. RGB analysis of the obtained images was performed using open-source ImageJ software. The area of interest in these images were chosen to contain only the beads. The optical density was calculated as (OD=-log (I_s/I_b), where I_s is the average of the pixel intensities in the region of interest, and I_b is the background intensity. The Red (R), Green (G) and Blue (B) channels showed an equivalent contribution to the increase in OD despite the R, and G values being the main components of the mean value with the B being of highest OD. The value optical density (OD) found in all the reported data is of the average of R, G, and B measurements.

4.3.4 Simulated Water Sample

To simulate measurement in samples that contain other ions that could potentially interfere, simulated samples were prepared based on the composition of tap water samples obtained from McMaster University. Two samples were obtained directly from the tap and represents city drinking water supply. Analysis of the samples using ICP-MS performed at Activation laboratories, Hamilton, Canada revealed that it contained 59.2 ppb of Copper and 0.52 ppb of Lead ions. The tap water also contained around 39 ppm of Calcium, 14.9 ppm of Sodium, 9.4 ppm of Magnesium ions, respectively. The simulated water were prepared by adding similar concentrations of Mg, Ca, and Na salts to DI water and used in all experiments.

4.4 Results and Discussion:

4.4.1 Sub ppb detection of heavy metals

The ability of the superabsorbent polymers in the form of beads to preconcentrate metal ions for sub ppb analysis was demonstrated by adsorbing from water samples containing Copper ions at concentrations of 0.5 to 5 ppb and dehydrating them. The simulated water samples (2L) prepared with different concentrations of Copper sulfate were flowed over the beads placed in a flow cell at 30 mL/min over 7 days. Upon dehydration, 25 µl of Copper colorimetric indicator dye was added and the OD of the beads was measured. Five beads were tested per sample.

Figure 3-2a shows that the color of the beads was light yellow when the concentration of Cu ions was 0 ppb (control sample) and increasingly became darker as the concentration of the Cu ions in the sample was increased. The color reached saturation becoming completely black when the concentration in the sample was 10 ppb (result not shown). The optical density (OD) of the beads is shown in Figure 3-2b and increases with the increase in the concentration of Copper ions in the sample as it increases from 0.5 ppb to 5 ppb. Concentrations as small as 0.5 ppb can be visually distinguished from control samples that don't contain Copper ions. On the other hand, measuring liquid samples of 10 ml using the same colorimetric dye with a spectrophotometer show no difference in color for samples less than 100 ppb hence cannot be used for the trace level sensing. The dynamic range of sensing for this exposure condition of 2L sample exposed over 7 days was between 0 and 10 ppb. The change in OD was a second order function that increased rapidly at lower concentrations and saturated at higher concentrations. The saturation may be because the chelating sites that are present on the beads may be saturated with the ions leading to minimal change in the amount adsorbed further [23]. Also, since the measurement is based on the transmittance of light through the bead, formation of a opaque and dense layer at the surface can lead to less sensitivity and saturation at higher concentrations[20]. Limit of detection was calculated based on the blank value. Three standard deviations were added to the value of the blank leading to the determination of LOD for all techniques. LOD was found to be 0.14 ppb.

4.4.2 Effect of the sample volume and exposure duration on sensing performance

The exposure conditions such as the sample volume used as well as the duration of the exposure can have a significant effect on the sensitivity, limit of detection and dynamic range of this technique. To understand the effect of the exposure conditions, experiments were conducted using sample volumes between 50 - 500 ml and exposure duration between 1-3 days as well as with larger samples of 250 – 2000 ml over a period of 3-7 days. As before, five beads were exposed in each of the experiment to simulated samples with concentrations between 0 and 400 ppb of Cu and after exposure, dehydrated, dye added and further dehydrated to be measured for OD.

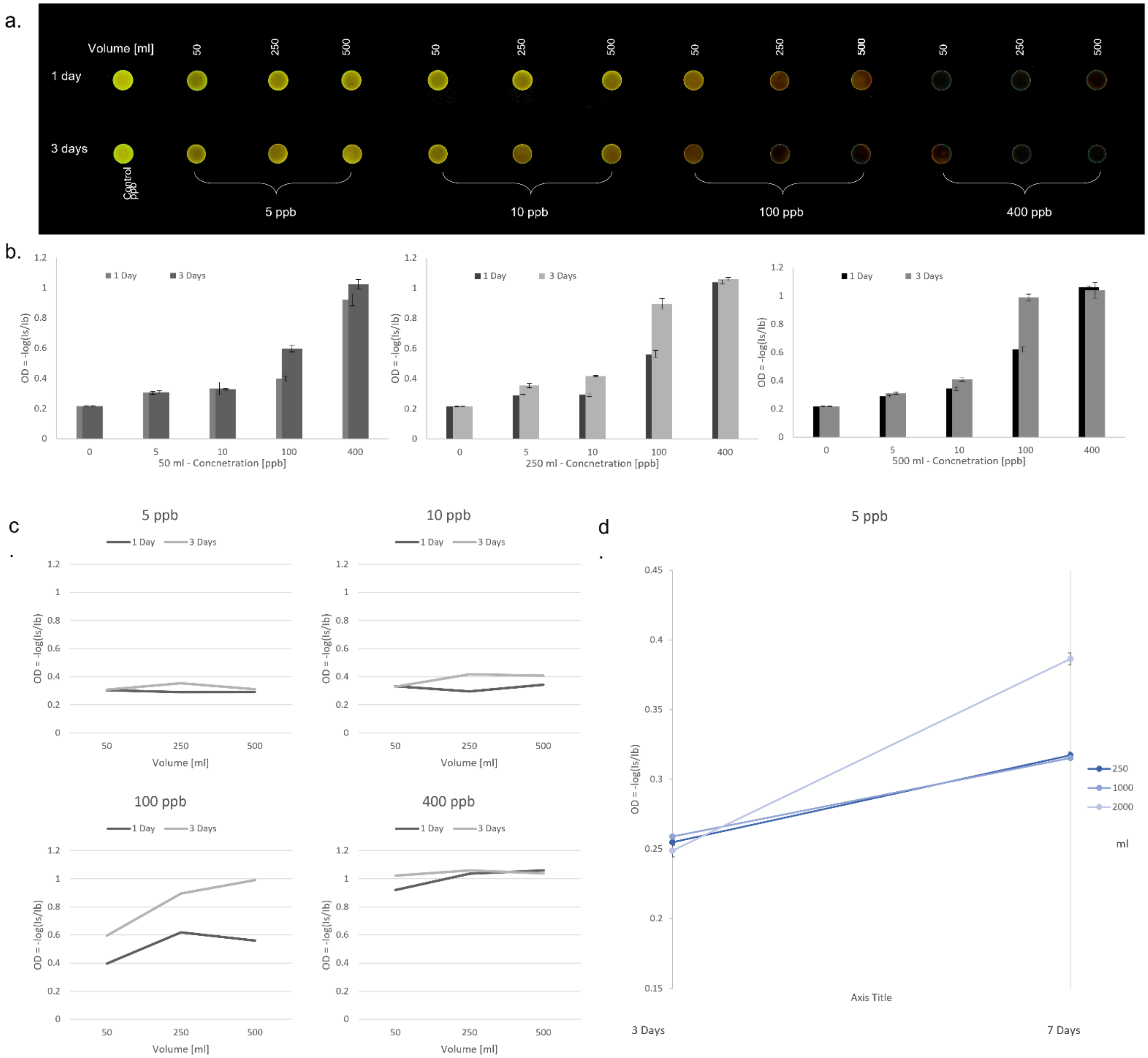

Figure 4-3 a) The images of the preconcentrated beads showing the change in color with the changes of both sample volume and time of exposure. The graphs in b) shows the quantitative change in OD with the change of the sample concentration. C) The comparison of the OD between the two different exposure duration over different exposure volumes show that at 100 ppb concentration a 3-day exposure can produce a significant difference over one day exposure which not much difference is observed in lower or higher concentrations. d) Same experiment performed at lower concentration of 5 ppb in sample volumes between 250 – 2000 mL shows that while the OD is similar at 3 days, it become significantly different at 7 days.

Figure 4-4 (I) 20 ml vial has 400 ppm solution of copper persulfate. (II) has spherical 2.7 mm hydrogel bead in the same solution where some of the Cu ions have adsorbed and (III) 20 mg Powder format hydrogel after 90 seconds of adsorption in the same solution.

The colorimetric response of the beads thus exposed are shown in Figure 3-3 indicate that the colorimetric change increases with concentration as expected. The increase in sample volume also increased the color change observed even at the same concentration of the sample. For example, the same duration of 1 day the 5 and 10 ppb samples encountered significant increase in optical density in the 500 ml compared to the 50 ml which did not undergo similar increase. The larger volume allows for lower detection limits to be achieved. Finally, in all instances the higher time of exposure resulted in higher degree of color change. Since most of the adsorption sites available on the hydrogel bead are still vacant, further exposure by recirculating larger sample volumes over longer duration of time can lead to great uptake [24]. The quantitative measurement of OD at these various conditions are shown in Figure 3-3b which support the visual observations. It shows both the effect of increase in concentration of the sample on the OD of the beads as well the effect of time of exposure. At low concentrations and low volumes, most of the ions present in the sample are adsorbed within the first day and hence there is insignificant difference in the colorimetric response between one and three days of exposure as shown in Figure 3-3c. Interestingly, there is a significant difference between the OD of the beads exposed to 100 ppb sample for one and three days. However, at even higher concentrations, that difference is insignificant. This observation suggests that there may exist an optimal exposure time for specific concentrations to be detected and that lower concentration range may require longer exposure for more sensitive detection. To confirm this hypothesis, we conducted experiments using 5 ppb samples where volumes of 250, 1000 and 2500 mL were exposed to the beads for 3 and 7 days. The OD measured is shown in Figure 3-3d and demonstrates that for 5 ppb samples significant difference in OD was observed at 7 days for a volume of 2000 mL while the 1000 mL and 250 mL samples were similar. The LoD for the 7 day exposure was calculated to be 0.14 ppb while that for the 3 day exposure was calculated to be ~ 1 ppb. This indicates a seven day exposure is optimal to detect concentrations in the 1-5 ppb range with high sensitivity.

4.4.3 Effect of the surface to volume ratio on the sensor response

Higher surface area of the chelating gel can lead to faster chelation and more rapid uptake of the material of interest. To investigate this effect, the bead format was compared with the powder format for their uptake of the ions and the ensuing colorimetric change. For effective comparison, the mass of the material used in each format was kept constant. The powdered particles had a specific gravity of 0.4 – 0.7 g/ml [25] while the beads were 2.7 mm in diameter [20]. A preliminary experiment was conducted where a 50 mL of 400 ppm Cu Figure 3-4 sample was exposed to a 2.7 mm bead and the equivalent 20 mg of gel in powder format for 90 sec. Figure 3-4 shows the pre (I) and post (II), (III) adsorption samples with the powder in liquid sample in (III). These samples are 15 ml of the total 50 ml sample initially used for the analysis. As observed, the powder format provides a much more rapid adsorption of ions from the solution

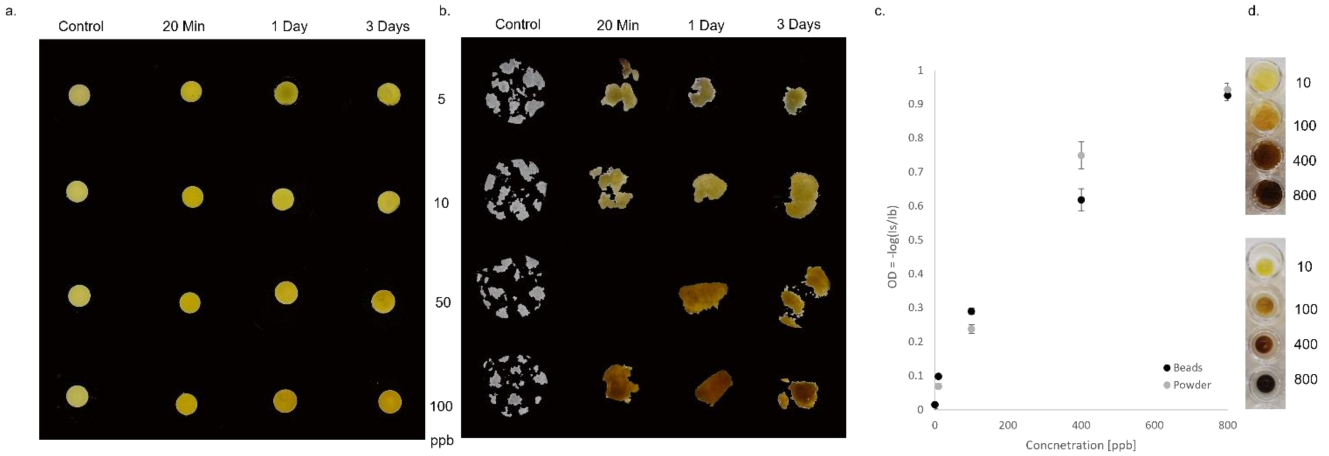

Figure 4-5 a) beads after adsorption in 50 ml simulated water samples with copper concentrations 5 – 100 ppb. b) powder after adsorption in similar concentrations. c) Presents the OD of both powder format samples shown in (d) (top right) in contrast to beads in (d) (bottom right) concentrations are 10 - 100 - 400 – 800 ppb top to bottom.

as compared with the beads. It shows that higher surface to volume of the chelating gel can lead to rapid assaying. As can be seen in Figure 3-4 II, the bead does not show much of change in color in 90 sec indicating adsorption over a minimal area of the bead's surface while the powder shows a dramatic color change due to rapid adsorption of Cu ions from the solution.

This phenomenon was tested further by evaluating the adsorption from 50 mL samples containing 5-100 ppb of Copper ions prepared as described in the methods section. Adsorption at 20 min, 1 day and 3 days were measured to understand the time course of the color change. Figure 3-5 presents the colorimetric response of the powder format (Figure 3-5b) in contrast to the beads (Figure 3-5a). Powder of 20 mg (equivalent to weight of 5 beads) was added to a 50 ml sample and dehydrated. The beads and powder were then collected, and the samples drained. After dehydration, 25 µl of the dye was then added, which swelled the powder followed by dehydration. Then 1/5th of this powder was used as a comparison to the being then divided adsorption on a single bead of 1.8 mm diameter. The powder format can differentiate all the concentrations from 5-100 ppb within 20 min from control samples. This in contrast with the bead samples where the differentiation is less obvious at 20 min. The contrast between concentrations increases for the beads as time progress and at 3 days it is also able to clearly distinguish between concentrations visually. This result show that the powder format can allow a faster way to perform detection especially at low concentrations. However, quantitative measurement of the dried powder proved to be a challenge as the shape was not uniform for the different samples that were dried which can lead to challenges in quantification in the field.

In a separate experiment sample volumes of 500 mL were also used to develop calibration curves for the two formats which were exposed for 1 day and they are shown in Figure 3-5c and d. In these experiments that powder was imaged in a slightly hydrated state so that it assumes the shape of the microwell that it is loaded in and therefore can be quantified. These experiments show that at long duration of time (1 day) the OD of the bead and the powder sample are similar which indicates that they adsorb similar amounts of the ions eventually. Nevertheless, the powder format is one that can be considered in the future to reduce the detection time by packing the dried powder into a compact and repeatable format.

4.4.4 pH response and intensification optical density

One of the potential limitations of this method is the need for dehydration, as it requires the extraction of the hydrogel out of the sample solution then exposing it to high temperature dry air to evaporate the bead's water content. This is a manual process and making it also solution based can facilitate simple automation of this process.

One way to dewater hydrogels has been to change pH of the solution that they are present in [26]. An experiment was conducted to determine if a pH change can dehydrate the hydrogel bead and lead to intensification of the color even in solution format. For this experiment, we used cored samples that were in the cylindrical format (6 mm diameter and 6 mm height) that were created using a biopsy punch from fully swollen beads (1.8 mm diameter). These pieces (n=4) were initially dehydrated before the experiment. During the experiment they were exposed to 25 ml Cu sample solution (with a concentration of 800 ppb) for 20 min. Following this, 125 µL of the colorimetric dye was added directly to the hydrogel without dehydration. Next, instead of thermal dehydration in an oven, the pH of the liquid sample was altered by addition of 2M KOH solution until the required pH is achieved. The beads were tested using addition of 0.4 mL of various pH solutions including pH 7, 10 and 13.

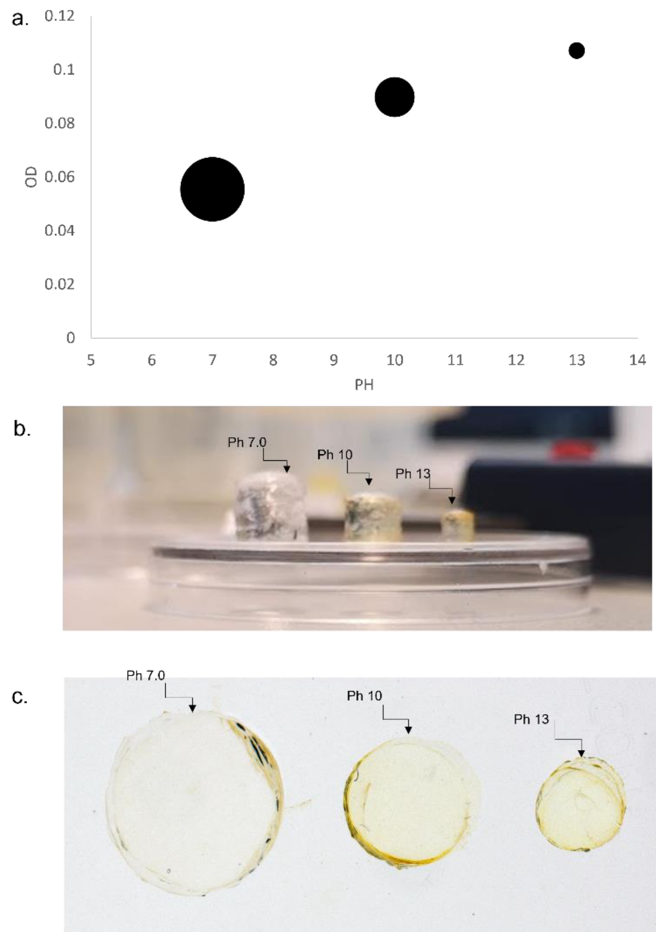

Figure 4-6 a) PH dependent OD measurements with circles area representing the measured colorimetric area. b) the hydrogel cores after exposure to different PH levels. And c) the top view of the cores with background illumination.

The results as shown in Figure 3-6a demonstrate that the pH of 10 and 13 induces a significant volumetric change in the hydrogel. The volumetric change was found to be 38% for pH 10 and 75% for pH 13. The measurement of OD in these shrunken beads also show increase as seen in Figure 3-6b. The change in OD was 62% for pH 10 and nearly 94% for pH 13 solution. Acrylate gels are pH sensitive due to protonation and deprotonation of the functional groups present in it that makes the polymer network more/less hydrophilic [26]. The gel is most hydrophilic at neutral and near neutral pH (6-8) as the polyelectrolytes present in it are stronger. At very low pH the Na^+ ions are replaced by H^+ making it a

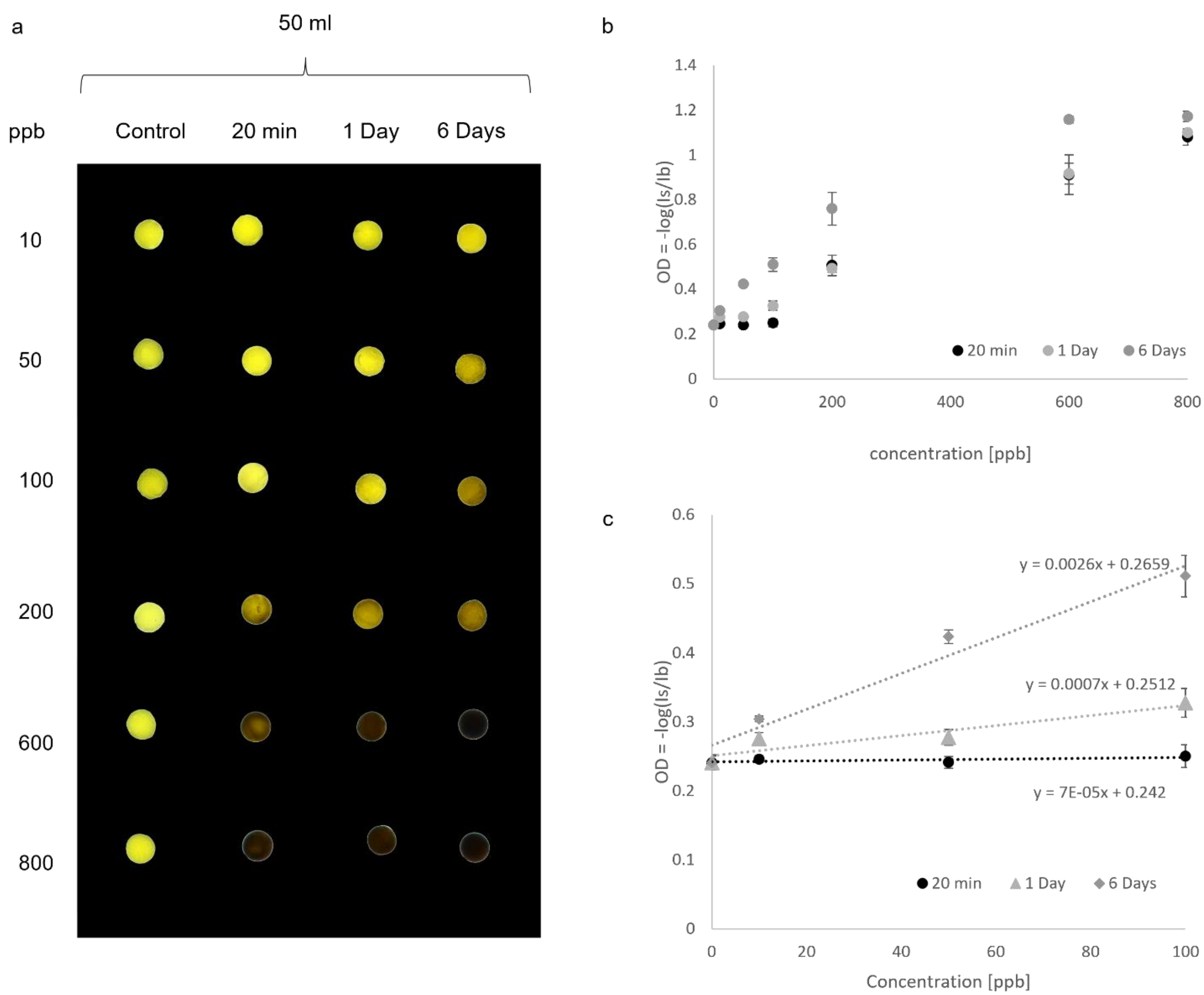

Figure 4-7 the beads (left) after adsorption for different time intervals of 20 min – 1 Day – 6 Days in sample volume of 50 ml with concentrations ranging 10 – 800 ppb. b) The OD measurements of those beads plotted with the linear region (c) with slopes shown on the bottom figure.

weaker polyelectrolyte and reducing the swelling. At high pH, condensation of counterions combined with charge screening due to excess Na^+ ions result in suppression of the polyelectrolyte swelling. Therefore, by change the pH from neutral to alkaline range we can dehydrate the gel leading to intensification of the color on the bead. This experiment shows that chemically induced pH change can result in dehydration that can be used to shrink the gel and replace thermal dehydration. This procedure can be used to automate and simplify the dehydration process. In the future, we can also consider generation of alkaline pH electrochemically to further automate the procedure.

4.4.5 Concentration and detection of Lead

This method of sensing is applicable to a wide range of ions that are capable of chelation to the gel and have a selective colorimetric reagent. To demonstrate this capability, we use detection of Lead around its regulatory limit in the few ppb (µg/L) range. Initial testing was carried out using various simulated sample solutions with concentration of 10 – 800 ppb as confirmed with ICP-MS measurement and with ions concentrations of Ca, Mg, and Na as described in the methods section. The beads were then placed in 50 ml samples for 20 min, 1 Day, and 6 Days. The results were then obtained by dehydrating the beads, adding 25 µl of 0.4 M Sodium Sulfide to each bead. The timed experiments shows that the mechanism of Pb ion accumulation on the beads is like Cu and increase with the time of exposure leading to higher accumulated amount on the bead. As shown in Figure 3-7a even at a low volume of 50 mL, concentrations of 10 ppb of Lead in solution can be differentiated by concentrating them on the bead. Figure 3-7b shows the change in both sensitivity and LOD for the different exposure intervals. The graph plots the different intervals in contrast to the control zero value. The OD was found to increase with the increase in duration of adsorption. At the low concentration range, less than 200 ppb, the OD obtained for a 20 minutes and 1 day exposure do not show any significant difference while adsorption over 6 days shows a significant increase. On the other hand, higher than 200 ppb concentration, there is a significant difference in OD even for 20 min exposure.

As shown in the graph of Figure 3-7c the 6 days exposure condition shows an increase of OD by around 100% at 100 ppb than that of the 20 minutes. At lower concentrations of 10 ppb and 50 ppb a 28% and 50% change in OD was also observed. This indicates that at lower concentrations, time of exposure to adsorption is indeed a crucial aspect that can significantly affect the OD and hence the sensitivity and

LOD. It's noticed that the sensitivity increases by 30% after 6 days compared to an increase by only 5% after one day. The LOD was measured to be at 40.8 ppb This method shows that it is possible to measure trace amounts of Lead in water samples with a simple, yet precise procedure. The design of experiments, precision of the beads in adsorption leading to repeatability and reproducibility allows for highly reliable quantitative measurements.

4.5 Conclusion

In this paper, we show that longer duration of exposure and large sample volumes allow collection of trace levels of heavy metal elements from water samples and enable sub ppb level detection of Copper (LoD of 0.14 ppb) using standard colorimetric dyes that are rated for >1 ppm level detection. We also use the same method for trace level of Pb detection at 40.8 ppb LoD using precipitation of PbS. The process relies on the preconcentration of ultra large samples of 2000 ml using Sodium Polyacrylate hydrogels. The Hydrogels are dehydrated after extended exposure to the sample volume in a flowing format for several days then ion-selective dye is used for each ion. The measurements are of colorimetric nature and the measurements were performed using a DSLR camera and the analysis using ImageJ Software. This process enables trace elements detection with regular colorimetric dyes that typically have high detection limits. The process expands the limit of detection by three orders of magnitude. Future work should focus on the expansion of this method utilizing different hydrogels and detection of more trace elements.

4.6 References

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Chapter 5 Lab in a Syringe: A Concept of solid-state reagent delivery and Colorimetric Sensing of Heavy Metals

Mohamed A. Fathalla[1], P. Ravi Selvaganapathy[1,2]

[1] Department of Mechanical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada

[2] School of Biomedical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada

Corresponding Author

P. Ravi Selvaganapathy – Department of Mechanical Engineering, McMaster University, Hamilton, ON, L8S 4K1, Canada; https://orcid.org/0000-0003-2041-7180

* selvaga@mcmaster.ca

Status:

Pending Submission

KEYWORDS: Super absorbent polymers; Hydrogel, Heavy metals; preconcentration; colorimetric detection; solid state

5.1 Abstract

Environmental pollution has been increasing over the past century mainly due to human activities such as mining and industrialization. Recognizing it, several regulations have been put in action to limit the exposure of the water resources to such contaminants. Nevertheless, measurement of the contaminants such as heavy metals which are toxic at very low concentrations by the average public is a challenge and requires rigorous sample collection, transfer to specialized facilities and require sophisticated analytical tests performed by experienced personnel. In this paper, we develop a system for simple, cost effective, reliable, and semi-automated measurement of heavy metals that can even be used by the public. We demonstrate that using hydrogels stacked inside standard dissolvable capsules and loaded with commercially available dyes, we were able to measure the presence of trace level heavy metals without the use of any sophisticated equipment. This allows for the concentration of a sample content on a piece of hydrogel that enables the detection of trace amounts of Copper (5 ppb). We also designed a colorimetric measurement platform built using an RGB sensor, an LED, a microcontroller, and constructed using LEGOs. We have built our device to be an off-shelf solution that can be universally implemented and replicated without the need for skilled personnel to perform the analysis and has the potential for use by the public.

5.2 Introduction:

Heavy metals are harmful even at trace levels, [1][2][3] however, over the past two centuries, industrialization and unsustainable waste management methods led to their increased prevalence in water at higher concentrations. In addition, Lead leaching from old water pipes into the drinking water is a growing concern and the infrastructure requires significant changes. The contamination of water resources due to industrial activities has resulted in severe poisoning of water supplies across the globe [4]. Many of the communities consuming the contaminated waters lack the tools to monitor if their drinking water is safe to drink [5][6]. Contaminated water has caused chronic diseases and deaths and continue to affect both humans and the ecosystem. Many rules were put in place to limit the exposure of water resources to the most dangerous toxins such as heavy metals released by unsustainable industrial activities[7][8][9]. Therefore, measuring heavy metals in water, soil, and industrial waste is essential to maintaining the wellbeing of humans and ensure properly functioning ecosystems.

However, current measurement techniques are not amenable for use by the public especially at the very low concentrations that the regulations demand for heavy metals. Sophisticated measurement techniques such as mass spectrometry are used currently in centralised labs where samples must be brought in from the field and subjected to time-consuming, expensive analysis [10]. Alternatively simpler electrochemical methods and colorimetric techniques can also be used for measurement of heavy metal ions albeit at higher concentrations. Some of these methods also require higher cost of materials (enzymes, DNA ligands, or nano coatings for electrodes), low temperatures for storage, or a sequence of unit operations to be performed in addition to high detection limits which limit their application in the field or for common use among the public. For instance, an interesting electrodeposition based approach has been taken to develop a low cost continuous monitoring impedance sensor that can detect 15 ppb of Lead in drinking water within 3 days [11]. Despite its interesting approach it required a minimum of 3 days of exposure to a relatively high concentration of the heavy metal ions for detection and must be replaced once a detection event has occurred. Previously, we have demonstrated a pre-concentration approach using hydrogel beads combined with colorimetric sensing to detect heavy metal ions such as Copper and Iron at a few ppb [12] range. Since, the low-cost chemical colorimetric dyes available commercially typically have a high detection limit, a pre-concentration step can be combined which will extend their range of sensing to much lower detection limits[13]. Although we have demonstrated the proof-of-concept demonstration that low detection limits are possible, the procedure is manual and the addition of the dye at the end requires a manual liquid reagent addition that may be difficult to automate. Moreover, the detection system used was a DSLR camera which was moderately expensive and could be simplified and made cheaper as well. Development of technology that is suitable for automation will be the first step in complete automation of this process. In addition, the automation process must also be simple and intuitive for the user so that errors in analysis are not made. One of the key challenges for such an automation is the ability to handle liquid reagents, aliquot them accurately and dispense them sequentially into the sample in a controlled fashion to perform the colorimetric reaction.

Here, we introduce a novel method of solid-state delivery of reagents to a sample. We have designed a dissolvable capsule-based delivery system that when exposed to the liquid sample can sequentially release different stages of reagents. The capsule is a custom designed multistage capsule that consists of two chambers. The first dissolvable chamber contains a hydrogel bead that is used to preconcentrate the sample solution. After the hydrogel and sample reach equilibrium, the second chamber is dissolved to release a dried solid-state hydrogel bead containing a dye that interacts with the liquid sample to release the dye which then reacts with the adsorbed species on the first bead. This method offers a simple and cost-effective way to preserve the reagents, aliquot them, sequentially inject them into the sample and perform the experiments in an automated fashion without the need for prior user knowledge. The capsules preserve the integrity of the dried hydrogel beads and prevent their moisture exposure while stored or during the sequential injection. In addition, we also demonstrate a simple LEGO based optical measurement system consisting of an LED and an RGB sensor connected to microcontroller (Arduino Uno) that can provide colorimetric measurements of the solid-state beads indicating the concentration of the heavy metal’s ions. These systems can be built at home using low-cost commercially available parts and can effectively be a sensing platform for this assay and other assays as well.

Our methods allow for the long-term preservation of species. Because it preserves the adsorbed species, preconcentrated for future examination, until the preserving environment parameters change. This allows

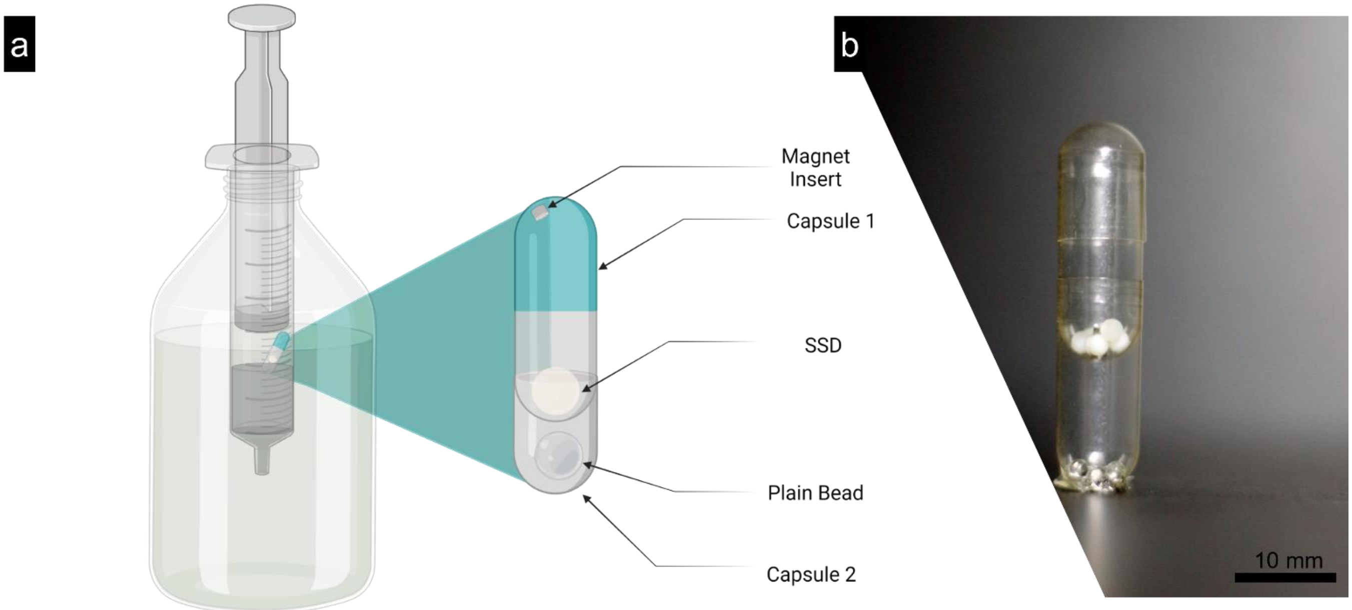

Figure 5-1 A capsules stack including two sets of beads and a magnet is inserted inside a 50 ml syringe. The first set is of plain beads for adsorption and the second set is solid state dye (SSD) in the form of dye loaded solid-state bead. The syringe plunger contains another magnet is assembled underneath the rubber section. This allows the capsule to attach to the plunger as it moves through the stroke.

for the transfer of samples in a far more cost-effective and user-friendly manner, removing the need for liquid handling. The solid-state based design enables the preconcentration of species within a hydrogel-based material and then allows the analysis to take place, utilising a solid state-based reagent released from another bead. This facilitates the identification of toxic, trace level, species using off shelf low-cost materials which can be useful in resource-constrained areas to test their own water supplies with a bench top solution.

5.3 Working Principle and Device Construction:

5.3.1.1 Syringe Assembly and Sequencing [Beads, Capsules]

We use solid state hydrogels as a key component for several processes in the analytical procedure. We utilize its capacity to absorb water samples and preconcentrate metal ions at trace level by adsorption on a hydrogel. We also utilize it as a solids state container to hold high concentration of reagents in small volume and release them into the sample solution in an appropriate manner. We use water dissolvable drug capsules that are commercially available to package the sodium polyacrylate hydrogel beads in different compartments, as shown in Figure 4-1, that can be utilized to sequence their release to automate the delivery process of the adsorption medium and the reagent. The capsules are stacked to form two compartments containing (i) a plain dried bead, (ii) a dye loaded dried bead, and (iii) two magnets (1/16" x 1/16") magnetized through thickness. We also use a 50 ml syringe that is commercially available, intuitively easy to use and graduated as a simple way for the user to perform sequential operation. The magnet holds the capsule to the plunger of the syringe under which another magnet (1/16" x 5/8") is fitted, and by moving the syringe plunger one can precisely move the capsule to different locations inside the syringe. The capsule is attached to the syringe plunger and the plunger inserted into a 50 mL syringe. The plunger is pushed till the 20 mL mark so that the capsule has some headspace. Six holes of 1 mm were drilled at 10 ml marker and one hole at the 50 ml marker. This enables the precise operation of the syringe allowing for venting excess air when required and disposal of the water sample prior final extraction. This configuration represents the initial configuration before the start of the assay.

The process sequence is presented in Figure 4-2. During operation, 30 mL of the sample solution was drawn inside the syringe by moving the plunger to the 50 mL mark. The initial draw also brings the tip of the capsule in contact with the sample which quickly dissolves that tip releasing a plain dried hydrogel bead into the sample solution. The dried hydrogel bead quickly absorbs the sample and swells. It is left to adsorb the trace metal ions from the sample for a period of three days. Next, the reagent loaded beads are delivered by pushing the syringe plunger to the 10 mL mark which brings the rest of the capsule in contact with water dissolving the capsule and releasing the reagent loaded bead into the solution. The reagent quickly diffuses out of the swollen bead and into the liquid sample where it can interact with the accumulated metal ions on the plain bead that has been in the liquid sample for three days. The diffusion of the reagent and its binding to the metal ions accumulated in the plain bead is complete in 90 minutes after which the bead is extracted out and dehydrated. Subsequently, the dried bead is imaged to measure the color intensity and relate it with the concentration of the ions present in the original sample.

5.4 Materials and Methods:

5.4.1 Materials

Sodium acrylate superabsorbent beads of diameter 2.7 mm (Water Beads Clear) and 1.8 mm (eboot 10000 Pieces Gel Soil Water Crystal Beads) were purchased from Water Beads Canada and Amazon eboot respectively. Lamotte 6446-E Copper reagent 1% Diethylcarbamodithioic acid) ordered from Cole-Parmer was used as an indication dye for Copper tested in samples of Copper Sulfate. The dye changes to a brown color upon binding with the Cu ion and the color develops instantly. A control experiment was performed where the standard colorimetric assay using the Copper sensing dye was used. Following the prescribed procedure, 125 µl of the indicator dye solution was added to 10 ml of the sample solution and measured using Lamotte Smart 3 colorimeter.

5.4.2 Dye loading onto a hydrogel bead

The solid-state dye loaded bead is prepared by adding ~125 µl (five drops) of the dye to dried hydrogel beads of 1.8 mm and 2.7 mm diameter. Under the pH of this dye the 1.8 mm bead only absorbs 75 µl while the 2.7 mm bed absorbs 110 µl. The beads are left to absorb the dye for an hour and then dehydrated in an oven at 75 ^{o}C for another hour. The result is solid state beads that retain the colorimetric dye. The dried bead when exposed to the sample starts absorbing it which then dissolves the loaded dye. Subsequently, the dissolved dye slowly diffuses out of the bead. The key factors influencing the release of reagents from beads are the swelling rate, and the diffusion constant of the dye within the swollen bead at different hydration states. The swelling rate affects the transition from glassy state to gel state, and the diffusion coefficient characterizes the dye release rate affecting the color development[14]. In this work we focus on the time required for the release of dye to induce colorimetric change.

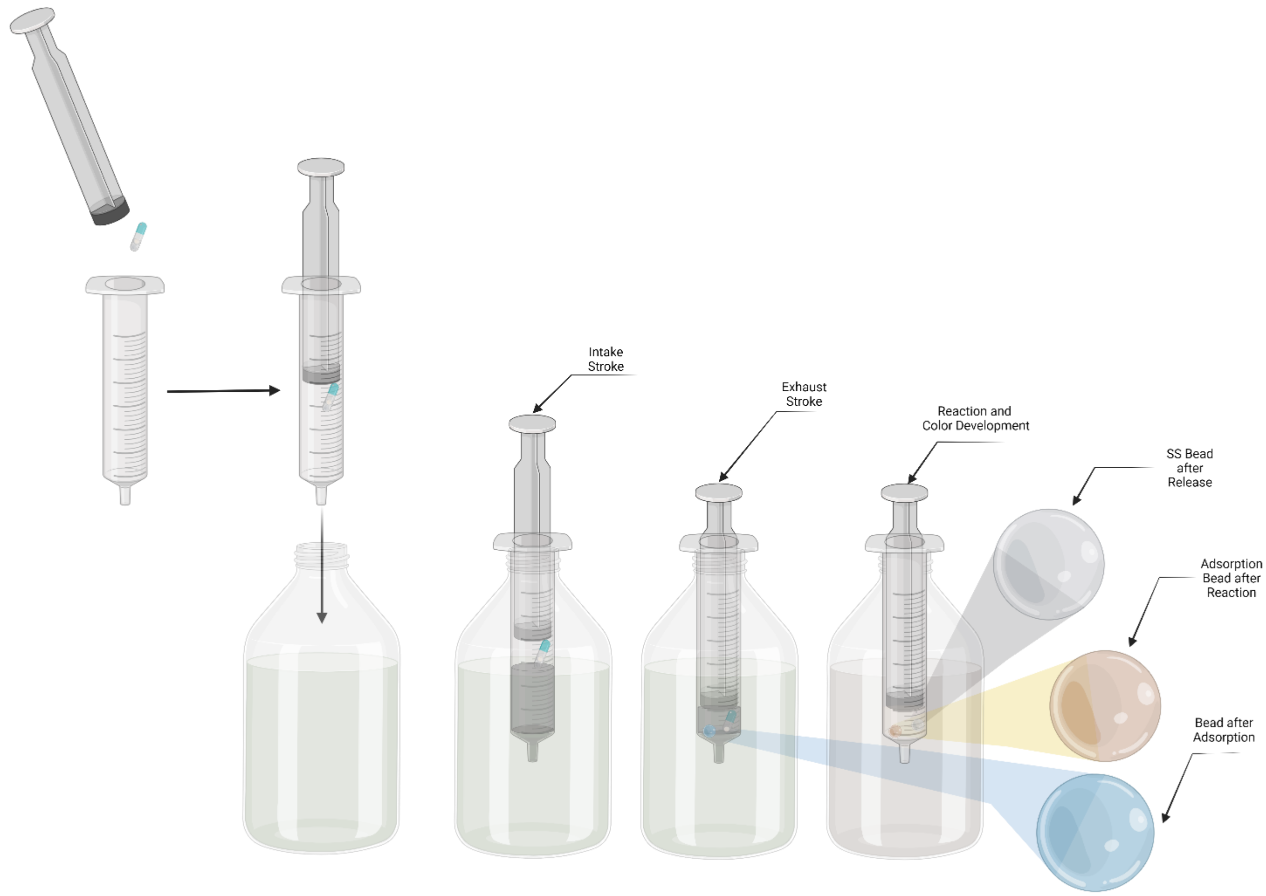

Figure 5-2 The process sequence is presented in this figure. The capsule assembly is inserted inside a 50 ml syringe and the plunger is moved to the 10 ml marker. The syringe is then inserted inside a one-liter bottle filled with the sample solution. the first capsule is exposed to the sample solution, dissolving, leading to the release of the plain-adsorption bead into the liquid sample inside the syringe. After three days the plunger is furtherly moved to the 10 ml marker exposing the second capsule to the sample solution. the capsule dissolves within 15 minutes and the dye loaded bead.

5.4.3 Colorimetric Sensor Platform

The hydrogel beads are perfectly spherical and have a smooth surface which makes them ideal ball lens. This format is quite suitable for sensing as the ball lens collects all the light transmitted through it into a focal point. Furthermore, the smooth surface of the hydrogel bead also minimizes scattering. Placing the detector at the focal plane of the hydrogel bead can then be an effective way to amplify the signal and make more sensitive detection.

The sensing platform (Rhino) was constructed based on the design as shown in Figure 4-3a where the light source is placed above the bead and in close contact. The four main components of the system are LED light source, the housing to position the bead, the Arduino Uno, and the RGB sensor as shown Figure 4-3b. The bead is placed directly above the RGB sensor where the light is focused. Using standard LEGOs a housing to position and align the 450 - 480 nm LED, and an RGB sensor (AS7262, Adafruit), was constructed. LEGO bricks are known for their standard sizes and ability to deliver precise geometric configurations, making them versatile for a wide range of use cases. The sensor and light source both were connected to a microcontroller (Arduino Uno) through external wiring as shown in Figure 4-3b. The assembly is topped with a grey cap, as shown in Figure 4-3d where it is also depicted in an assembled format with the control bead inserted. The other sub components of the system and its assembly are shown in Figure 4-3c,d and the fully assembled Rhino Platform is shown in Figure 4-3e.

The OD in the Rhino measurements was calculated based on the background measurements and the beads emitted color. The measurements were transferred through serial connection from Arduino Uno to Excel through data streamer add-on. The Adafruit library for the RGB sensor (AS7262, Adafruit) was used to

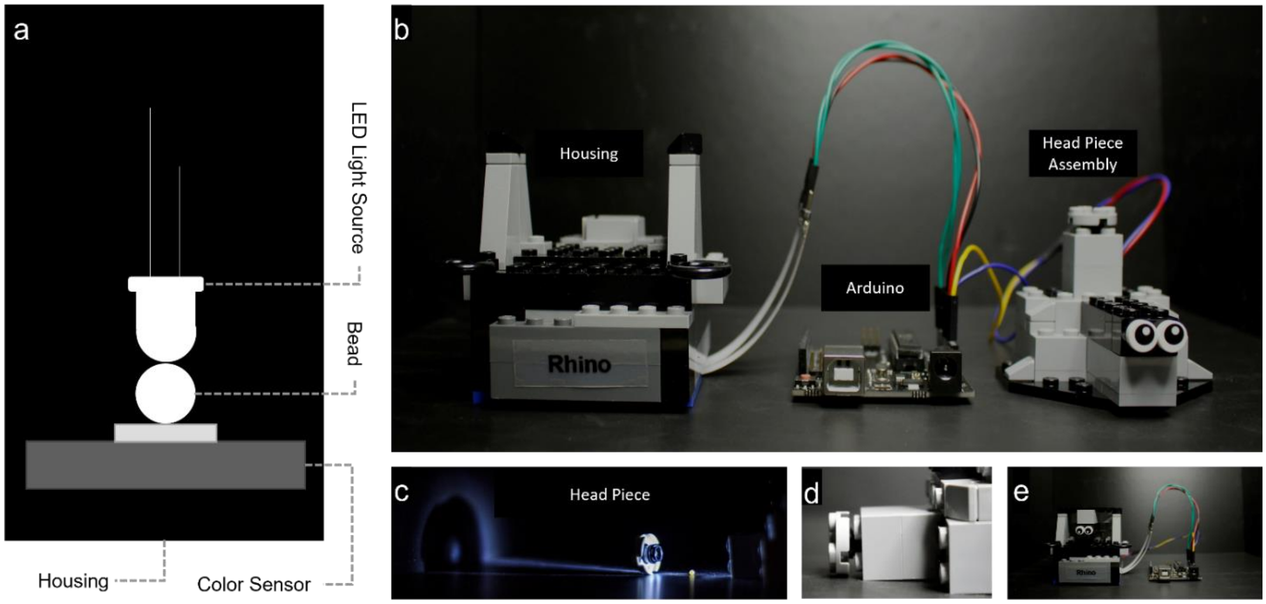

Figure 5-3 a) Shows the concept device with the control bead inserted inside the test gap. It has a stack of the RGB sensor at the bottom, topped by the solid-state bead, and the light source above the bead. This configuration is connected to a microcontroller (Arduino Uno) that reads the analog output of the RGB sensor converting it to a 255 RGB value that can be read on Excell using Serial Port connection. b) is the componens of the device where the housing on left houses the RGB sensor, and the compartment on the right houses the bead and the LED. c) shows the disassembled head piece where the bead is placed between the light source and the cap. d) shows the same components after assembly. e) The Top part on is the LED assembly housing the 450 µm LED through drilled holes in the 4x4 LEGO bricks.

do the data collection and the library installation can be found in their website. the optical density was calculated as -log(ls/lb) where (ls) represents the RGB component measured at a certain concentration and (lb) is the reference RGB value measured at the background intensity.

For calibration purposes and for comparison, another imaging setup was also used. It consisted of a NIKON DSLR FX with 105 mm lens that was placed inside a black box to isolate it from any light interference. An LED light source within the black box was used to reliably reproduce the illumination. Image capture settings of 1/125 seconds shutter speed, f - stop of f/45, and ISO Speed of ISO – 150 were used. RGB analysis of the obtained images was performed using open-source ImageJ software. The area of interest in these images were chosen to contain only the beads. The Red (R), Green (G) and Blue (B) channels showed an equivalent contribution to the increase in OD despite the R, and G values being the main components of the mean value with the B being of highest OD. The value optical density (OD) found in all the reported data is of the average of R, G, and B measurements which is typically used in obtaining the greyscale of an image and was found useful in representing the change of OD. The optical density is defined as (OD=-log (Is/Ib), where is the average of the pixel intensities in the region of interest, and Ib is the background intensity.

5.4.4 Simulated Water Sample

Tap water was obtained from drinking water taps at McMaster University and analysed using ICP-MS at Activation laboratories, Hamilton, Canada. The tap water samples contained around 39 ppm of Calcium, 14.9 ppm of Sodium, 9.4 ppm of Magnesium ions, respectively. It also contained 59.2 ppb of Copper. Simulated water samples were prepared by adding $CaCl_2$, $Mg(OH)_2$, and NaCl salts to DI water at the same concentration as in the tap water samples. Calibration samples were prepared from the simulated water samples by adding controlled amounts of Copper.

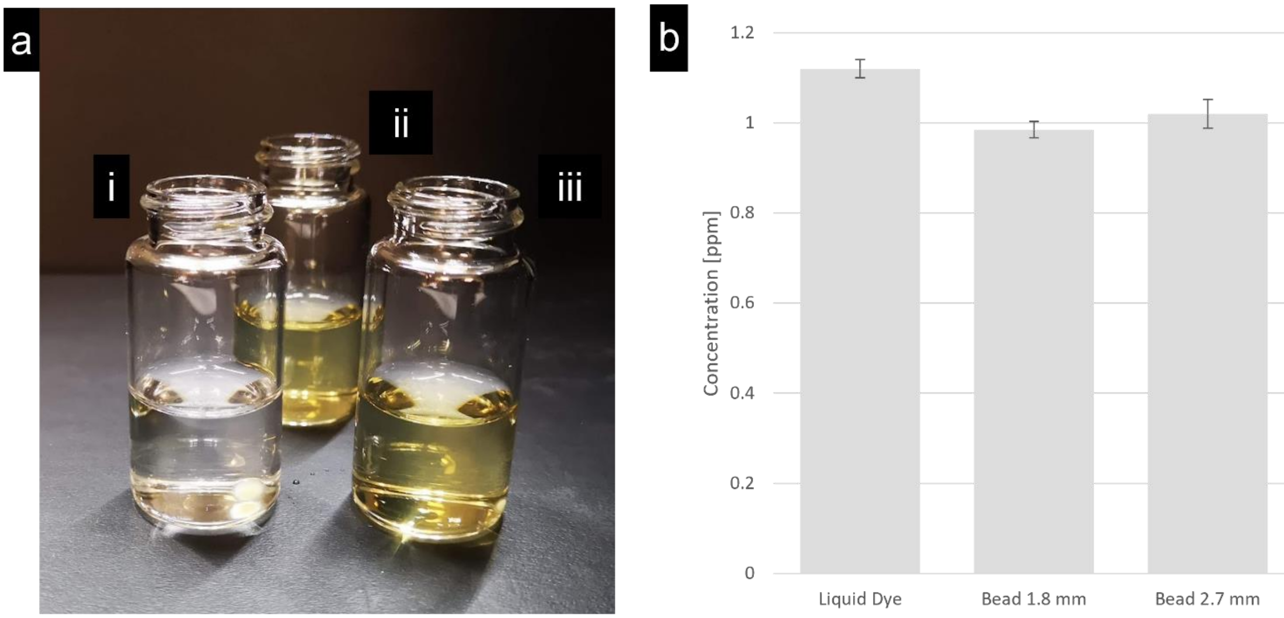

Figure 5-4 a) Shows the colorimetric intensity of liquid samples of 1 ppm before adding dye (i) and after adding liquid dye (ii) and after adding solid state dye bead (iii). The bar graph in b) presents the measurements of a sample showing a reduction in OD of 9% and 12% for the different sizes of beads used in the solid-state release. (n=4)

5.5 Results:

5.5.1 Solid State Dye delivery

To validate the effectiveness of solid-state dye loaded bead, the release of the dye from it was compared with the standard approach of adding a liquid dye directly to the sample and measured using a LaMotte 3 spectrometer. Beads of 1.8 mm and 2.7 mm diameter were loaded with five droplets of dye (~125 µL) and dried. Three vials of 10 ml, 1 ppm Copper solution were prepared, and 125 µl of liquid dye was directly added to one of them while the dye loaded 1.8 mm, and 2.7 mm beads were added to two other vials. The release of the dye from the beads started instantaneously, and the dried dye-loaded beads swelled rapidly within 5 minutes absorbing the sample solution. The released dye begins to change the color of the sample solution with Cu instantaneously and the color was fully developed within 5 min. The samples were then analysed against a control sample of the 1 ppm (without the dye) using the LaMotte Colorimeter 3.

The colorimetric response is shown in Figure 4-4a where (i) is the control, (ii) is the sample with direct addition of the liquid dye and (iii) is the sample with the solid state bead of 1.8 mm. the 2.7 mm were added. The addition of the dye changes the color of the sample, and the intensity represents the concentration. Quantitative measurement using a spectrometer of these differently loaded samples in Figure 4-4b demonstrate that the dye loaded bead was able to deliver sufficient amount of dye to the sample to induce color change similar to the direct addition and mixing of the liquid dye. The differences was only 9% and 12% with respect to the liquid dye indicating release efficiency of 88% and 91% for the 1.8 mm and 2.7 mm beads, respectively. This result confirms the usability of hydrogels as a solid-state medium for the colorimetric dye. It also improves the handling, and volumetric efficiency of the dye storage allowing for compact handling.

We designed another experiment to quantify the require time for the adsorped beads to reach colorimetric equilibrium. In order to achieve that, beads were swollen in DI water then placed in 10 ml of Copper solution of 100 ppm initial concentration. The beads were placed in the sample for two minutes then extracted, rinsed with DI water, then placed in 10 ml of DI water. The dye was then introduced into the liquid solution containing the bead in both liquid and solid state formats. The dye was added to three 10 ml vials, as shown in Figure 4-5, initially containing an adsorbed bead in 10 ml DI water each. Five droplets of liquid dye was added to the first vial. Five solid state dye (SSD) beads were added to the second vial, and one (SSD) bead was added to the last vial. The experiment was conducted until equilibrium is reached which was identified by the stability of the colorimetric response of the adsorbed beads.

Figure 5-5 The figure shows the release of dye in liquid sample containing beads after adsorption in 100 ppm copper solution for two minutes. The experiment studied the release time to equilibrium. We compared three beads of the same conditions exposing one to five droplets of liquid dye, another to five of the solid state dye beads (SSD) and lastly, only one SSD bead. results are shown over a timeline from left to right.

The time was recorded for all samples and the results are presented in Figure 4-5. At time zero it can be noticed that the beads instantaneously react colorimetrically leading to brown color in both the liquid and in the case where multiple SSD beads were present. Only the single SSD bead scenario where the dye release didn’t seem to Lead to colorimetric reaction instantaneously. The optical density increases over time and it was noticed that the liquid dye case reached equilibrium in about 48 mins which was sooner than the other two cases where the dye is released from the bead. The case where the dye was released from five SSD beads reached equilibrium at around 90 min than that of the liquid state. It is important to highlight that the five SSD beads contained five times the dye introduced in the liquid state. The last case where the dye was introduced in a single SSD bead reached equilibrium after 90 min, at the same time as the five SSD beads case, yet it didn’t reach the same optical density as that of either the first and second cases.

These results indicate that achieving equilibrium when releasing dye from SSD beads requires longer duration of time due to the kinetics of dye diffusion from the bead and also due to its uptake dynamics on to the ion adsorbed bead. Even though the start of the release of the dye is instantaneous, it takes a while for the dye to to released and for it to diffuse throughout the sample and equilibrate. Further, there is additional time required for the solvated dye to be taken up by the pre-concentrated bead and to change its color. These dynamics show that effective and stable color change can occur in around 90 mins which was chosen as the duration of the experiment.

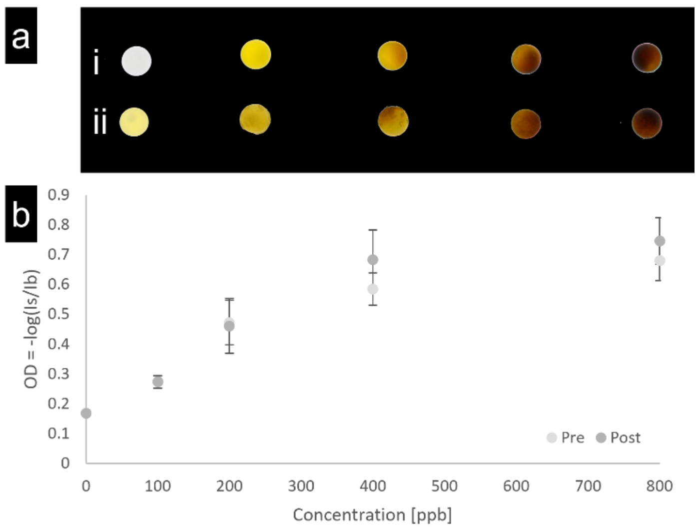

Figure 5-6 a) contrasts the colorimetric response of beads when the dye is added to them after adsorption in liquid solution (i) and after complete dehydration (ii). It can be noticed that there is no significant change in colorimetric response despite that of 200 ppb displaying a slight reduction in OD in the pre dehydration than that of the post dehydration dye addition. In b) the difference is characterized and the LOD in both cases is similar at 5.4 ppb.

5.5.2 Trace level Sensing

Trace level sensing of heavy metals require a series of unit operations to be performed which are amenable to automation that can prevent cross contamination and errors in aliquoting. To demonstrate that this design of the device makes it amenable for eventual full automation, we performed the sequence of steps as described in the methods section on simulated samples. We insert a capsule that contains both the sample adsorption bead and the dye loaded bead inside a 50 ml Syringe. We prepared four syringes and they were inserted into bottles containing one-liter samples with Copper ion concentration of 10 – 100 –

400 ppb as well as a control which did not have any Copper ions in it. The sample is then drawn into the syringe by moving the plunger to the 50 ml marker. The capsule is allowed to dissolve for 15 minutes, and the sample adsorption bead gets released. The bead is allowed to adsorb metal ions from the sample over three days. Subsequently, dye addition was performed by moving the plunger to the 10 ml marker, ejecting the excess sample from the syringe, and exposing the second capsule containing the dye bead to the sample solution. The second capsule gains exposure to the sample and dissolves within 15 minutes leading to the release of the dye loaded bead into the remaining sample solution. The colorimetric response in the sample starts instantaneously but requires 90 minutes for the bead's color to reach equilibrium. The beads are then extracted from the syringes and then dehydrated for colorimetric analysis to take place.

As reported in Figure 4-6b, concentrations as low as 10 ppb can be distinguished from control samples. The LOD was calculated to be 5.4 ppb. The increase in optical density is linear with a slope of 0.0014 [ppb^{-1}] As can be seen in Figure 4-6b The OD increases by 52% from 0 to 10 ppb and another 57% from 10 to 100 ppb. and the increase from 100 to 400 was 112% indicating a linear increase. The sensitivity in this experiment was 0.0014 [ppb^{-1}] which is higher by 350% than that of the sensitivity reported earlier (0.0004) using hydrogel beads but with a shorter exposure time to adsorb all the metal ions in the sample [12]. The higher sensitivity can be attributed to the longer exposure time, larger sample volume, and the uniform exposure to sample. This result indicates that not only that this process enabled simple operation, construction, and handling, but also enhanced the sensitivity significantly. This makes the process more reliable in the low spectrum of detection at the trace level.

5.5.3 Effect of Dye Diffusion Kinetics on Response

The procedure used in this device, especially in the dye addition step is different from our previous practice [12] of adding the dye to dehydrated bead containing the ions. Here, the dye is added to the sample solution that may contain some metal ions and has to diffuse to the sample loaded bead where the metal ions are concentrated and chelate with it to produce the color. In order to identify if the change in procedure has an effect on sensitivity and response, experiments were conducted by addition of dye to the fully swollen bead loaded with the metal ions and compared with the condition where dye was added when it was in the fully dehydrated state. We prepared 50 ml of Copper Sulfate samples of varying concentrations using simulated water background as described in the methods section. We added five beads to each 50 mL sample and exposed it for three days. We then extracted the beads from the sample solution then for a set of them (five at each concentration) added the dye directly to the fully swollen bead. To another set (5 beads), we added the dye after full dehydration. We then dehydrated the two sets before taking measurements using the DSLR camera based optical measurement system.

The results shown in Figure 4-6a are of the two sets of beads. The beads show very similar colorimetric response regardless of the state of the bead when the dye was added. It can be seen in Figure 4-6b that the response closely matches when the concentration of ions in solution is low and is a bit different at higher concentration. This may be due to presence of metal ions chelated at the interior of the bead that are exposed to the colorimetric dye much better when the dye is loaded onto a dehydrated bead as compared with a fully hydrated one. Our observation from previous experiments [12] is that chelation of the metal ions occurs initially at the surface sites and then upon filling proceeds to the bulk sites due to diffusion access of the ions in the sample to those sites. At low concentrations, there are sufficient surface sites that most of the ions are on the surface and have easy access to the dye as it diffuses from the solution. These experiments demonstrate that the new procedure where the solid-state dye loaded bead infuses the dye in the solution which then binds to the metal ions pre-concentrated on the plain bead should produce the similar results as the manual addition of the dye in the liquid form, thereby enabling automation.

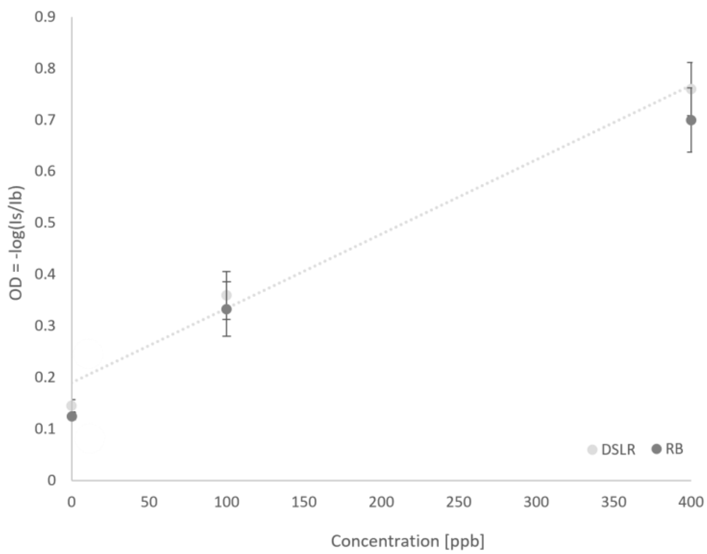

Figure 5-7 The measurements of the capsule-based sequencing using Rhino. This data set is of the beads reported earlier. The linear trend is the DSLR measurement, and the second order line is the measurements done by Rhino.

5.5.4 LEGO® Based Colorimetric Measurements Platform

The current optical measurement methods for colorimetric sensing are cuvette based and do not suit the solid-state bead form. Alternative methods such as microscopes or DSLR cameras are also expensive. We have designed a platform for low-cost benchtop sensing of solid-state colorimetric beads using the unique light focusing property of the beads to our advantage. Our novel design shown in Figure 4-3 can precisely and firmly hold the solid-state bead for the colorimetric measurement to be repeatable, precise, and avoid any external light noise or location error.

To assess the sensitivity and optical characteristics of the sensor, measurements were conducted on the samples that were previously reported using DSLR camera earlier in the previous section on trace level sensing. Those samples presented previously in Figure 4-6d using the capsule stacking technique were measured using a DSLR camera following the protocol described in the methods section. In this part of the study, we used the Rhino platform to measure the same beads. The beads were loaded in the device and measurements were conducted as described earlier in the methods section. The results are shown in Figure 4-7 where the measurements from the Rhino platform are plotted against the DSLR measurements.

The results indicate a shift in response where the OD from Rhino measurements are were lower by around 5% but with the same trend as those from DSLR camera. The values were close to each other at lower concentration and increase at higher concentrations. This is fundamentally due to the difference in the setup. The Rhino platform measures the transmittance of the light through the bead using the hydrogel bead as a ball lens while the DSLR setup measures the reflectance of the light from the bead. This result verifies both the sensitivity and limit of detection of the RGB platform (Rhino) to be similar to that of the more expensive DSLR setup.

5.5.5 Color sensor Sensitivity

Another experiment was designed to assess the sensitivity of the color sensor to slight variations in the solid bead's color. The solid-state samples were prepared using a protocol that intensifies the colorimetric response to provide higher variation in color intensities. The liquid water samples were prepared from a Copper stock solution of 10 ppm and the test samples were spiked with Ca, Mg, and Na as described in the methods section. Liquid samples were of 5 – 100 ppb at volumes 50 – 250 – 500 ml. The beads were placed in the liquid sample for three days to swell and adsorb the ions, then extracted and dehydrated. They were then exposed to 25 µl of liquid state colorimetric dye and dehydrated again after fully swelling the dye. The samples were chosen at these concentrations to characterize the ability of the RGB sensor to respond to slight variations in colors through solid state medium and semi-transparent objects.

The measurements using Rhino is presented in Figure 4-8 where combination of the (Blue – Red) components of the intensity was used to calculate the OD. The results shown in Figure 4-8 where the optical density was calculated as -log(ls/lb) with (ls) representing the component measured at a certain concentration and (lb) is the reference value measured at the background intensity. The OD increases with

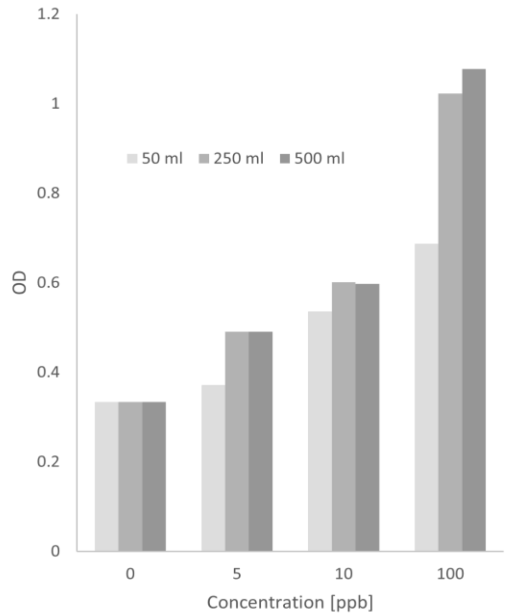

Figure 5-8 This graph presents an enhanced sensitivity of the colorimetric response. Obtained by combining the Red and Blue components as it increases the difference in OD leading to higher sensitivity.

the increase in concentration or volume. The samples at 250 and 50 ml with 5, 10 and 100 ppb concentration of Copper show similar colorimetric response suggesting similarity in response which aligns with the findings in our previous publication on sub ppb measurements. This indicates that the single cell RGB sensor has comparable sensitivity to that of the DSLR camera.

The data shown in Figure S2 shows the RGB channels of the samples indicating successful measurement of the beads transmitted color. The change in optical density (OD) was observed to decrease as the concentration of the heavy metal ions increased. This trend was observed for the red, and blue components of the colorimetric assay. The OD values were determined by measuring the colorimetric reading of the beads in the presence of the heavy metal ions and comparing it to the colorimetric value of the background measurement of the plain beads. The decrease in OD can be attributed to an increase in the red component, as the reported value is the negative logarithm of the ratio between the colorimetric reading of the red value of the bead and the colorimetric value of the background measurement of the plain bead. Additionally, the blue component also demonstrated a similar declining trend, except at a concentration of 800 ppb, where the 50 ml and 250 ml samples showed a positive increase. This deviation in trend is likely due to a shift in color at these concentrations, which resulted in a sharp decline in the green component. The green component on the other hand is of an inverted trend where the values decline below the background value. These findings validate the possibility of easy to construct, highly reliable, and cost-effective device that can detect trace level and sub ppb concentrations of heavy metals with no dependency on trained personnel or sophisticated experimental protocols.

5.6 Conclusion

In this paper we presented the design and implementation of a novel method to semi-automate the detection of heavy metals. We used solid state dye in the form of dye loaded hydrogel beads and utilized the hydrogel as a preconcentration medium. Stacking these beads in a multistage capsule enabled the passive sequencing of the process of preconcentration and release of reagent. A novel approach in deploying a syringe inside a one litre sample was developed enabling precise delivery of the adsorption material and the solid-state dye. The method proven effective detection of trace elements such as Copper. It was also reported to have a LOD of 1.2 ppb of Copper while maintaining linear response at the lower end of concentration span. This method is highly automatable since it utilizes solid state material enabling the prevention of cross contamination and the fast analysis in comparison to the time required for a traditional lab-based analysis using ICP-MS.

5.7 References

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Chapter 6 Conclusions and future direction

6.1 Contributions to knowledge

In this thesis, a solution inspired by the limited availability of in-field sensors for heavy metals was developed. The requirements for safe drinking water include a set of regulations for heavy metals concentrations. A solid-state reagent-based solution that utilizes an RGB sensing platform in the detection of trace quantities of Iron, Copper, and Lead was introduced as a cost effective approach to heavy metals monitoring. This thesis and the related research work was inspired by the need for an urgent intervention to limit and eventually stop water contamination. The challenge lies initially in identifying the sources of contamination and their effect on the human body and the ecosystem, and then, the challenge is to quantify and track the presence of those contaminants in order to keep them under control. There is a need for fast, selective, and widely deployable test methods that enable the public to take action and control over their water resources. Similar to the emergence of rapid testing solutions for COVID-19, which played a crucial role for the public to take control of their interactions, such a solution, if present, for the heavy metals will enable cost effective and accessible solution for ensuring safe drinking waters and food consumption. The background investigation for this research work offered a detailed review of the dangers associated with the exposure to heavy metal ions present in water, food, and soil in addition to a literature survey on the tools for the detection of heavy metals.

By initially designing a process for the detection of trace amounts of Copper and Iron, This method, presented in the second chapter, utilized off-shelf colorimetric dyes to determine the concentrations in complex water samples. The ions were preconcentrated prior to the colorimetric detection by exposing the samples to sodium polyacrylate (SPA) hydrogels. This novel approach in preconcentrating the ions on the SPA hydrogels enabled the extension of the detection limit of the colorimetric dye by two orders of magnitude. We were able to detect Copper a concentration with a limit of detection of 5.4 ppb. The method was used on tap water samples collected from McMaster University, Hamiton, Canada and provided a quantification similar to that of the gold standard (ICP-MS) with an error less than 3%. This solution is the first to utilize SPA hydrogels in the preconcentration of trace elements. Such hydrogels are typically used for the wastewater treatment processes at high concentrations. The literature was lacking studies of the trace level interaction between heavy metal ions and SPA hydrogels. This contribution forms a corner stone in the understanding of the exposure of hydrogels to trace amounts of heavy metals.

We then designed a technique that enabled the detection of nano grams per liter of heavy metals in complex water samples. Trace elements are hazardous at the low micro grams per liter level. To quantify the present heavy metal ions at concentrations below 10 ppb; very high sensitivity instruments are required. The conventional methods mentioned earlier in the introduction chapter rely on the high sensitivity of reagents. Instead, in this work an emphasis was put on the utilization of the high adsorption capacity of the SPA hydrogels to heavy metal ions and their ability to swell and retain liquid phase. Such feat has never been investigated before. This solution was designed to be reliable, easy to implement, and with very low limit of detection. The technology used the SPA hydrogels in the preconcentration of trace quantities of heavy metal ions in the sub ppb range then the dehydration of those hydrogels and finally adding the specific colorimetric dye. This method utilized ultra large liquid samples of 2000 ml with the hydrogels being exposed to a flowing sample for as long as seven days. This method was able to achieve LOD as low as 140 ng/l of lead and copper.

Our final contribution was the development of a system for the straightforward and semi-automated detection of trace quantities of heavy metals that is both reliable and cost-effective. This solution was developed as step towards the automation and subsequently the remote sensing of heavy metal sensors. The need for widely spread sensors in the communities prone to water contamination and at their water sources is a crucial step towards ensuring safe drinking waters. This solution was able to detect the presence of Copper at a concentration as low as 5 ppb without the need for expensive equipment. Using hydrogels loaded with readily available dyes and a colorimetric measuring platform constructed from readily available materials. The public may utilise our off-the-shelf solution without specific training because it can be widely deployed and duplicated. This work offers a quick and efficient technique for the identification of heavy metal pollution, which has the potential to dramatically improve environmental monitoring and public health.

6.2 Future directions

6.2.1 Multiple Heavy Metals preconcentration and colorimetric sensing using SPA Hydrogels

In this work we utilized SPA hydrogels in the reconcentration of heavy metal ions. We were able to preconcentrate and measure trace quantities of heavy metals using readily available selective colorimetric dyes. The main limitation was that we were only able to test one element at a time, and that the reagent is only providing response for a single element. This limitation is a common flaw of colorimetric reagents and markers. the next steps in the development of a cost-effective sensing solution would be the utilization of a wider selection of the selective colorimetric dyes that can provide colorimetric response to several ions. Such solution can rely on the segmentation of the liquid dye on solid state hydrogel beads and introducing them to the sample one at a time. This would allow for the testing of multiple analytes at a time and utilize the selective nature of those reagents.

6.2.2 pH Based Dehydration of the Hydrogels

In our work we relied on the dehydration of hydrogels as a method of extensive preconcentration of heavy metals. This enables the increase of the colorimetric response intensity allowing for the detection of trace quantities. The dehydration relied mainly on the hot air circulation over the hydrogels, it was demonstrated as well that it’s possible to use of PH alteration as a technique for dehydration, a future work can focus on the utilization of in liquid PH change where the dehydration can take place in liquid rather than the extraction of the hydrogels. The challenge in this work was that to introduce the PH varying medium, the hydrogels needed to be extracted the placed in a high PH solution, using a local-electrical method to alter the PH might be a viable solution that can implemented in-situ. It was discussed in this piece of work that changing the PH from 7 to 10 or 13, one can induce shrinkage of the hydrogel size which in turn intensifies the colorimetric response and reduce the limit of detection. A future solution can integrate this protocol into an in-line systems that extracts, aliquots, preconcentrate by then trigger shrinkage using a PH altering medium.

6.2.3 Solid-State Automated Process for the Detection of Heavy Metals

Our latest contribution is to make the detection of heavy metals easily accessible to the public which was achieved by the automation of the preconcentration and colorimetric detection. This method which we called (Lab in a Syringe) passively automate the preconcentration and solid-state dye delivery to the liquid sample. The process, despite being fairly passive, lacked the automation aspect. In order to operate the system an operator needed to interact with the device, do multiple steps, then extract the measurements. A better design would incorporate a mechanical handling system of the capsules, a liquid delivery system for the sample, and an extraction mechanism that can perform the dehydration autonomously. Automated delivery of solid-state dyes with the integration of capsule stacking and handling mechanisms can lead to a significant leap in the automation of colorimetric detection using solid-state reagents eliminating the need for several liquid reagents and preventing cross contamination.

Appendices

Appendix 1

Supporting information for:

Colorimetric Detection of Heavy Metal Ions Using Super Absorptive Hydrogels and Evaporative Concentration for Water Quality Monitoring

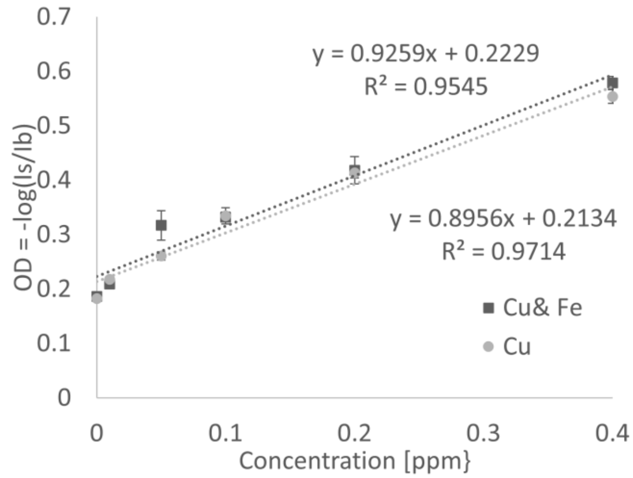

Figure S 2-1 – Copper and iron coexisting at similar concentrations. The measurements shown are of copper only, and copper + iron spiked DI water samples. The LOD is 7.9 ppb

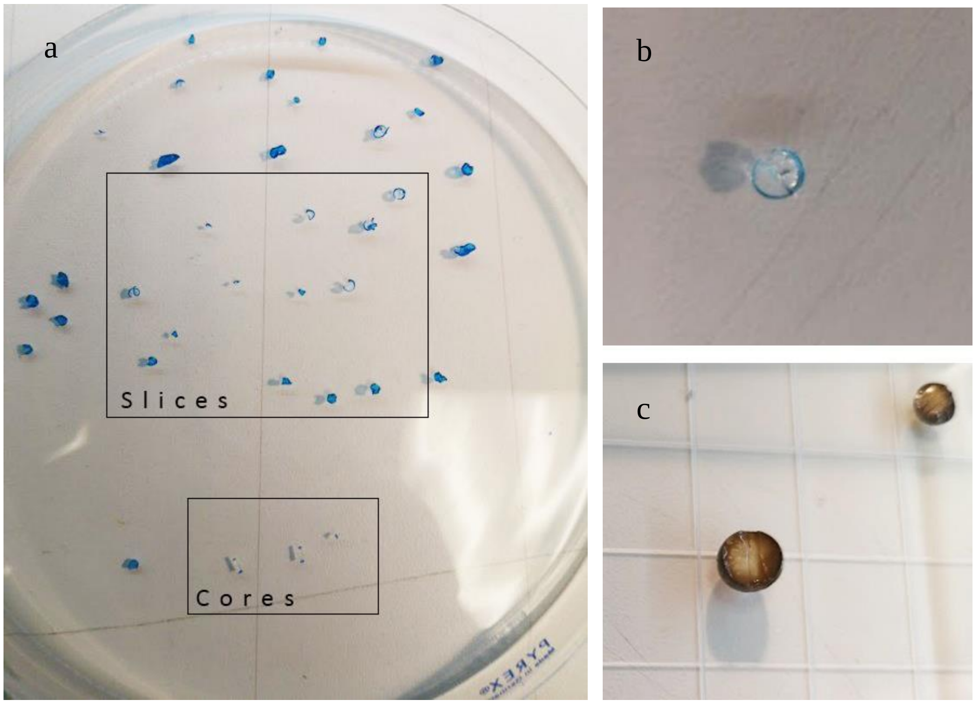

Figure S 2-32 - a) Slices and cores cut out of a hydrogel bead after swelling in Cu 100 ppm solution. Concentration zones can be noticed to be on the external surfaces of the bead. b) half a bead cut after swelling in Cu 10 ppm solution. c) dye added to the half bead in b.

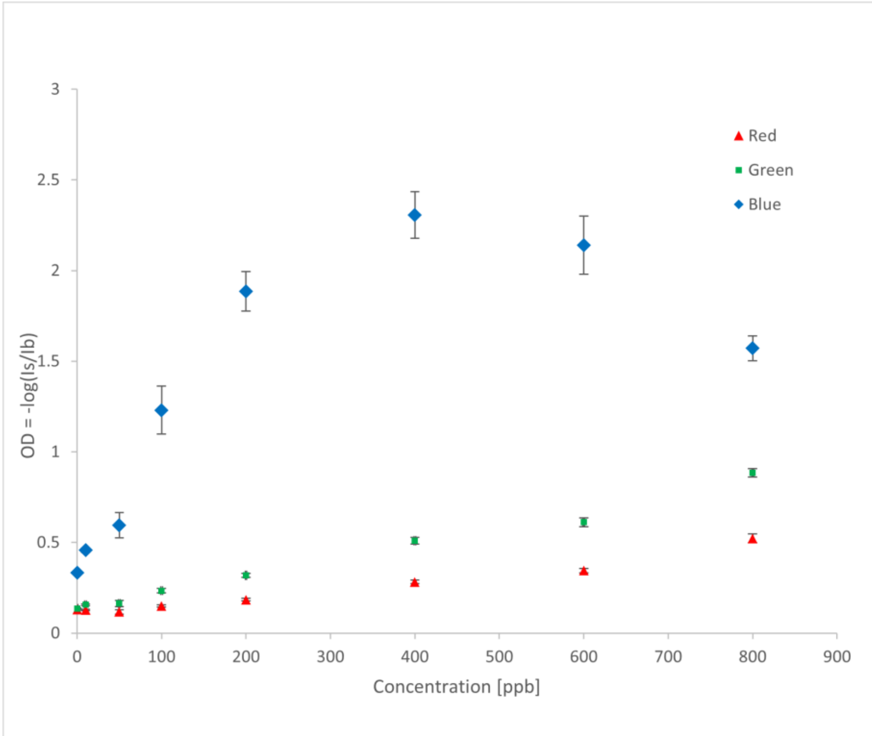

Figure S 2 - 3 – RGB color channels.

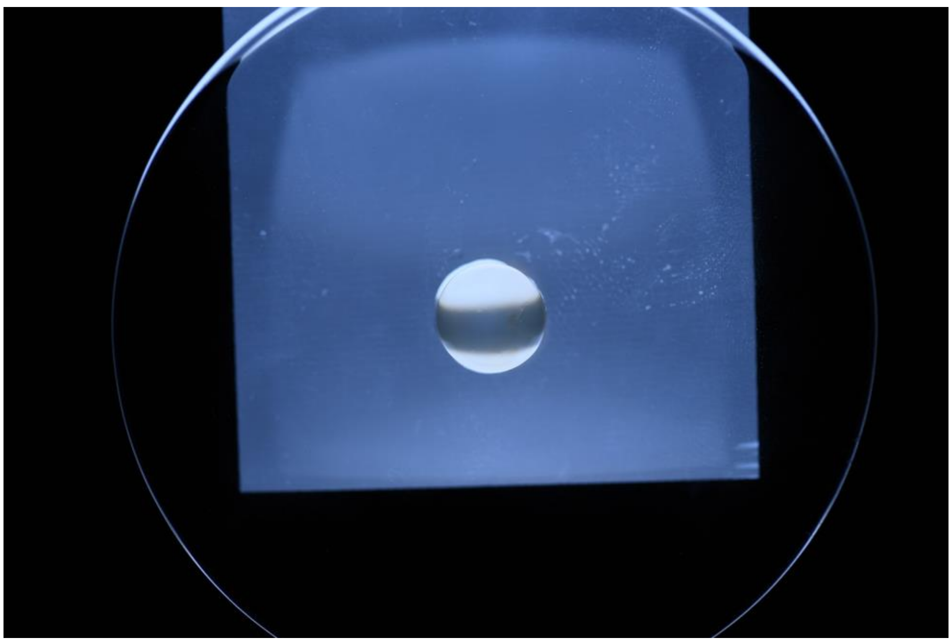

Figure S 2 - 4 – Fully swollen bead in 5 ppm solution showing an optical edge effect.

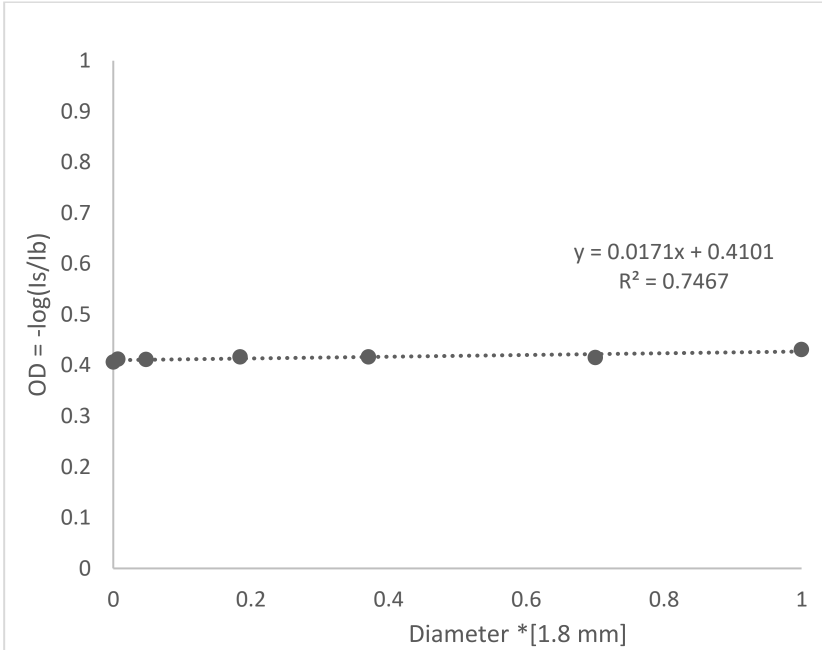

Figure S 2 - 5 - The Measurement at different distances from the center of the bead. Circles of different diameters ranging from 0 to 100% of the bead's diameter were used to quantify the optical density at these regions.

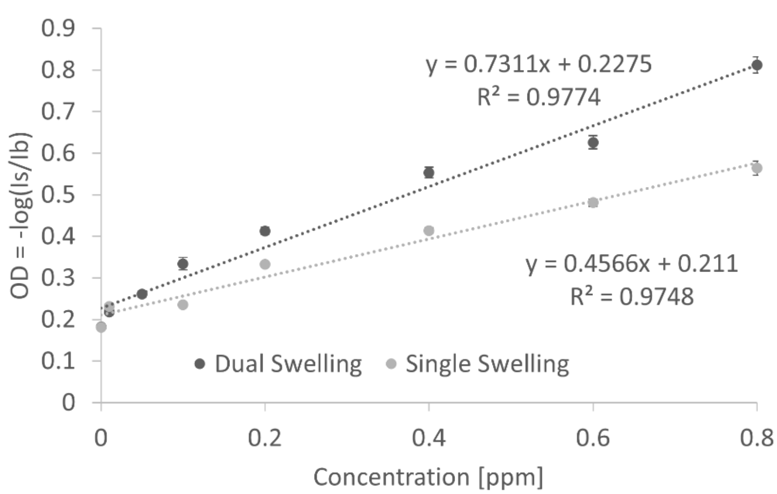

Figure S 2 - 6 – Multiple loading cycles.

Appendix 2
Supporting information for:

Sub ppb sensing of trace heavy metals using pre-concentration from large sample-volumes on solid state hydrogels

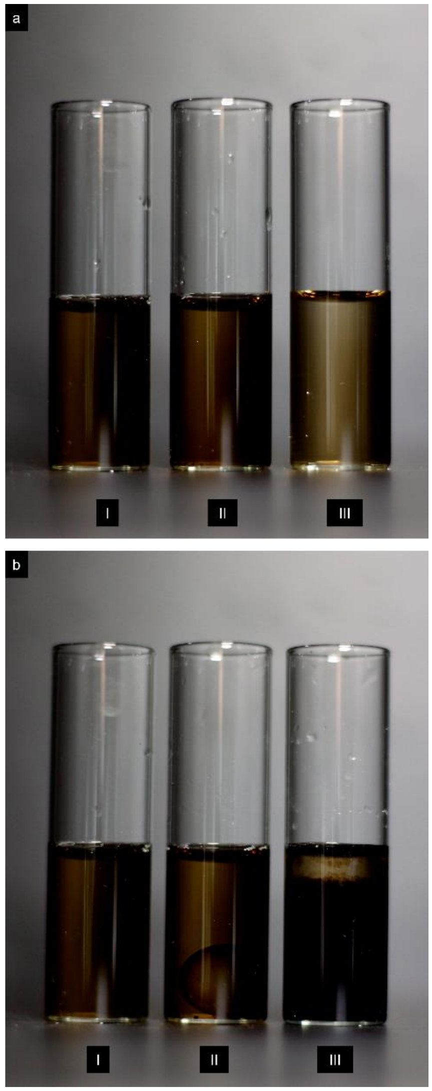

Figure S 3 - 1 a) the liquid samples of 40 ml at 10, 30, and 50 mg/l after the addition of 10 ml of sodium sulfide. The beads were then introduced with 2 ml of the dye (sodium sulfide 0.4 M) to then develop a black colour on the surface of the beads. b) shows the beads after adsorption for 1 day and the addition of 2 ml of dye to them. c) Shows the water samples after adsorption and removal of beads with the dye of 2 ml added to them.

a) the liquid samples of 40 ml at 10, 30, and 50 mg/l after the addition of 10 ml of sodium sulfide. The beads were then introduced with 2 ml of the dye (sodium sulfide 0.4 M) to then develop a black colour on the surface of the beads. b) shows the beads after adsorption for 1 day and the addition of 2 ml of dye to them. c) Shows the water samples after adsorption and removal of beads with the dye of 2 ml added to them.

Appendix 3
Supporting information for:

Lab in a Syringe: A Concept of solid-state reagent delivery and Colorimetric Sensing of Heavy Metals

White	R:	96	G:	84	B:	34	
Black	R:	0	G:	0	B:	0	
Brown	R:	255	G:	0	B:	0	
Off White	R:	130	G:	65	B:	17	
Grean	R:	5	G:	122	B:	20	
Magenta	R:	12	G:	87	B:	136	
Red	R:	227	G:	14	B:	23	
Purple	R:	234	G:	21	B:	33	
Pink	R:	184	G:	17	B:	53	

Figure S 4 - 1 Measurements of standard 2x2 LEGO blocks using the Rhino Platform.

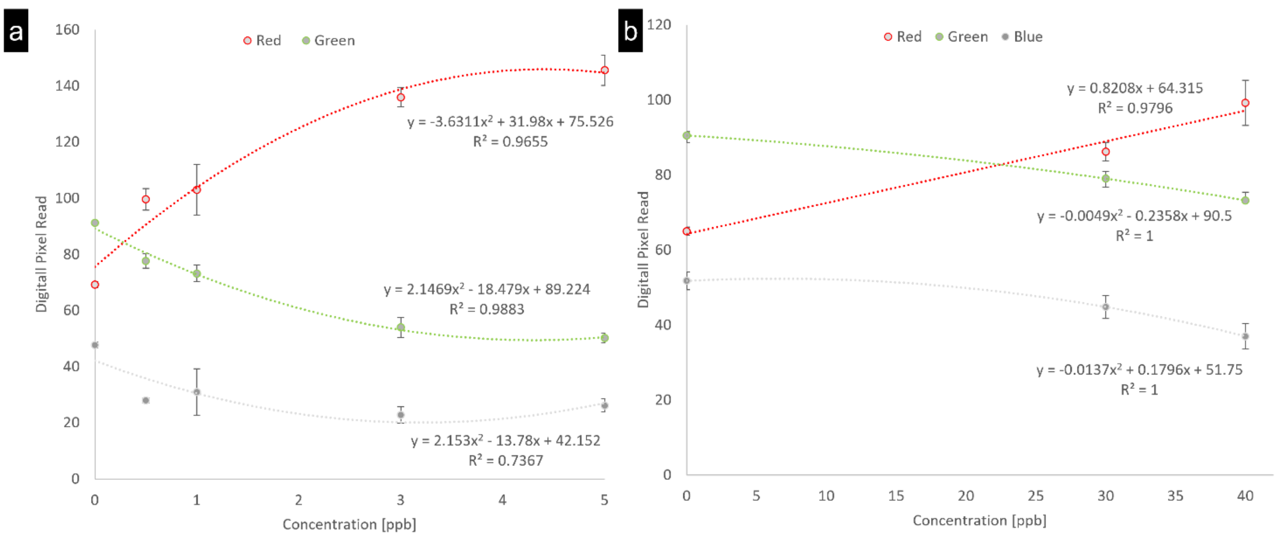

Figure S 4 - 2 a) Shows the copper measurements using the RGB sensor the readings indicate increase in the red component, and reduction in both green and blue. The plateau at the higher end of the spectrum indicates saturation where the colorimetric response isn't increasing in a linear proportion. As noticed in b) at the low concentrations of lead below 1 ppb the correlation can be assumed linear. Yet, the same trend in increasing red and reduction in both green and blue is similarly noticed.