THE SYNTHESIS AND INVESTIGATION OF ALIZARIN-BASED FUNCTIONAL CHROMOGENIC PROBES FOR HEAVY METAL SENSING IN AQUEOUS MEDIUM

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PENEXUPIFO LINEEKELOMWENE MARTIN

200612921

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SUPERVISOR: Prof. Veikko Uahengo (University of Namibia)

ABSTRACT

Heavy metal contamination of drinking water is a global concern. Conventional analytical techniques that are commonly used for metal ion detection in water, such as atomic absorption spectrometry and mass spectrometry, are not suitable for mass monitoring purposes. Suitable chromogenic probes could provide an alternative to conventional methods. Moreover, chromogenic probes such as off the shelf, commercially available organic reagents enable rapid and cost efficient *in situ* monitoring of water sources without the requirement of laborious sampling. Sensor AB and EF, an alizarin derivative was synthesised and characterised using UV-vis, ^{1}H-NMR and FT-IR spectroscopy. The aim of this study was to investigate a colorimetric method based on Schiff base synthesised sensors and other probes such as ready-to-use alizarin organic dye, as well as two organic reagents, dithizone and phenylfluorone for heavy metal detection in borehole water and commercially available water.

Sensor AB and EF showed strong colorimetric response upon the molar addition of various cations (including Fe^{2+}, Fe^{3+}, Hg^{2+}, Co^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}) and anions (including AcO^-, OH^-, CN^-) in DMSO, acetonitrile and DMF:water (1:9). Similarly, alizarin and phenylfluorone also showed strong colorimetric response in DMF:water (1:9) and ethanol solvent systems. Whereas vivid colour changes were observed when dithizone complexed with ions. Subsequently, in addition to sensing of cations and anions, all chromogenic probes were employed for signaling of commercial water and borehole water in different solvent systems. Sensor AB reported lack of sensitivity towards water samples, yielding in no colour change for water sensing. Furthermore, spectroscopy results of UV-vis and FT-IR as well as colorimetric results of sensor EF indicate close similarities to alizarin, demonstrating little to no effect of modification of alizarin.

Solvatochromism effect associated with dithizone, alizarin and phenylfluorone was observed. Varied and sensitive chromic behaviour was observed in dithizone, displaying colour variances in DMF, methanol, acetonitrile, chloroform, acetone and ethanol.

Keywords: *chemosensor; heavy metals; dithizone, sensing*

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±	Plus-minus
°C	degree Celsius
^{13}C - NMR	carbon-13 Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
AAS	Atomic Absorption Spectrometry
AcO^-	Acetate
AMCA	Aminomethylcoumarin Acetate
c	Concentration
CGM	Continuous Glucose Measurement
CH_3CN	Acetonitrile
cm^{-1}	Centimetre, per centimetre (wavenumber)
DFT	Density functional theory
DMF	N,N-dimethylformamide
DMSO	Dimethyl sulfoxide
EMA	European Medical Agency
EPA	US Environmental Protection Agency
ESIPT	Excited state intramolecular proton transfer
ESPT	Intramolecular Proton Transfer
Et_2O	Ethyl acetate
EtOH	Ethanol
ε	Molar extinction coefficient
Equiv.	Equivalence
FAAS	Flame Atomic Absorption Spectroscopy
FT-IR	Fourier Transform Infrared spectroscopy
h	Hour
h	Planck’s constant
H_2Dz	Dithizone
H_2O	Water

HOMO	highest occupied molecular orbital
HSDB	Hazardous Substances Data Bank
ICP-MS	Inductively Coupled Plasma–Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IPA	Isopropyl Alcohol/Isopropanol
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of Detection
LUMO	Lowest unoccupied molecular orbitals
MeOH	Methanol
MS	mass spectrometry
NIR	Near Infrared
nm	Nanometre
NMR	Nuclear Magnetic Resonance
PET	Photoinduced Electron Transfer
pH	Power of Hydrogen
ppm	parts per million
RBM	Round bottom flask
RDT	Rapid Diagnostic Test
RO	Reverse Osmosis
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Saline
TRITC	Tetramethylrhodamine
UV-Vis	Ultraviolet-Visible
VLSI	Very large-scale integration
WHO	World Health Organisation
XRF	X-ray fluorescence
β	beta
δ	Chemical shift
λ	Wavelength
$\lambda_{ex.}$	Excitation wavelength

$\lambda_{max.}$	Maximum absorption wavelength
π	Pi
π^*	pi-star

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DECLARATIONS

I, **Penexupifo L Martin**, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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1. INTRODUCTION

1.1 Background of the study

In the words of Jean-Marie Lehn, “The essence of chemistry is not only to discover, but to invent and, above all, to create.” A Chemist designs and creates molecules, materials and devices that are useful to everyday life. The dynamic complex systems of a human body and environmental systems has been the foundation upon which researchers in the field of science base their research analogies to come up with solutions and innovations that are essential for the betterment of our everyday life. Herein, we discuss the importance of chemical sensors (chemosensors) and their application in everyday life.

1.1.1 What is a Sensor?

The need to monitor our surroundings has evoked an increasing effort in the field of molecular recognition where researchers place their focus to develop a device that can receive information and turn that information into a form that is compatible with human perception, knowledge and understanding. In essence that is what a sensor is, a device that converts a physical measure into a signal that is read by an observer or by an instrument. In our everyday life, we see sensors performing important functions ranging from temperature regulation in ovens and kettles by thermocouples, to the monitoring of blood sugar levels by blood glucose sensors in the management of diabetes [1-5]. The definition of a sensor is often derived from its uses, applications and material used; there is therefore no uniform description of sensors or the process of sensing. Depending on the application of the sensors, a sensor is sometimes referred to as a transducer, a probe, a gauge, a detector among others. However, setting aside the various definitions, a sensor is generally a device that detects the changes and in response produces an output as an acknowledgement of change [1, 5-8]. According to the Merriam-Webster dictionary, the word *sensor* is derived from the Latin word, *sentire* which means to perceive [9].

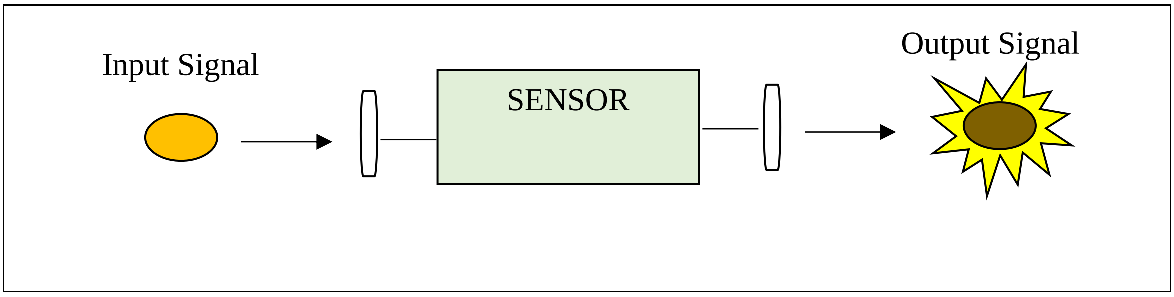


Figure 1: Simple generalised sensor setup

There are many types of sensors that can be used to measure or detect a vast variety of physical presence of flames, metals, leaks, levels, gas, light intensity, position, sound, chemicals, biological quantities, including proteins, bacteria, and many others. Some sensors are designed to sense physical properties such as temperature, pressure, or radiation, while others can detect motion or proximity [1, 3, 11].

The success of a sensor is limited to its performance characteristics, a senser should be able to understand the physical or chemical input signal and yield useful transduction and unambiguous output signal. The output signal measurements are therefore by default an assessment tool of the quality of the sensor [1, 3, 5]. Static characteristics of a sensor are those that can be measured after stabilisation of transient effects to their final or steady state values. This relates to issues such as how a sensor’s output changes in response to an input change, selectivity, robustness and stability. The most important static characteristics of sensors are as follows: accuracy, sensitivity, specificity, stability (ability to operate in different environmental conditions), repeatability, rapid response and ruggedness [1, 5, 12-14].

The field of sensor technology is extremely broad, and its development involves the interaction of nearly every scientific and technical discipline. Nowadays, our livelihoods and living standards have improved as we enjoy the results of continuous advancement due to research and development invested in the field of science and technology. We frequently rely on various types of sensor-based appliances or gadgets, such as computers, copy machines, mobile phones, microwave ovens, refrigerators, air conditioning and television remotes, smoke detectors, infrared (IR) thermometers, turning on and off lamps and fans, which help us interact with the physical environment. [1, 5, 6, 15].

With a vast field of sensors in nearly all disciplines, sensors are an enabling technology, applicable to a wide spectrum of uses, with the aim to improve our daily life dealings. **Figure 2** below shows a few examples of different types of sensors.

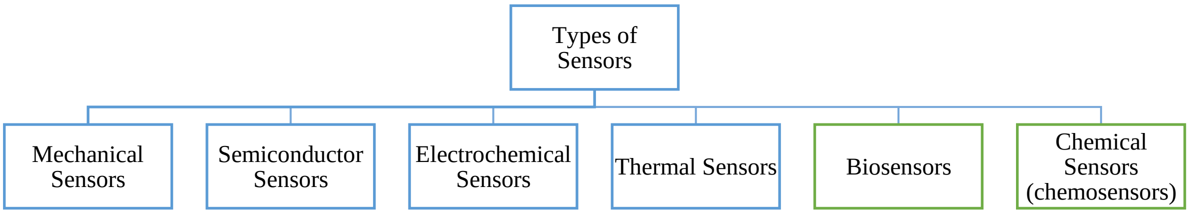


Figure 2: Different types of sensors [1, 5, 15].

Of the various sensor technologies, two key domains of sensors highlighted in section 1.1.1 are as follow: biosensors and chemosensors. These sensors are used in a wide array of diagnostics ranging from agriculture, mining to medical diagnosis, biomedical (bio processes), clinical applications to environmental applications and food quality monitoring [15-18].

1.1.2 Applications of biosensors and chemosensors

Chemosensors (chemical sensors) and biosensors have attracted the attention of many researchers because of their diverse applications. The definition of a chemosensor is discussed in section 1.1.1 of this study. Similarly, a biosensor is a device that measures both biological and chemical reactions by generating signals proportional to the concentration of an analyte in the reaction [19-21]. As a result of recent scientific and technological progress, biosensors have a very wide range of applications that aim to improve the quality of life. One of the main applications of biosensors is the detection of biomolecules that are either indicators of a disease or targets of a drug. For example, electrochemical biosensing techniques can be used as clinical tools to detect protein cancer biomarkers. Biosensors are now available over the counter for a large variety of consumer applications such as blood glucose monitoring, pregnancy test, cholesterol measurement, fertility monitoring, bacterial infection or exposure (such as Helicobacter pylori), allergies etc. [1, 18, 19, 21]. According to Kazemi-Darsanaki *et al.* [21] the history of biosensors began in the year 1962 with the establishment of enzyme electrodes by the scientist Leland C. Clark. Since then, experiment communities from different areas such as Physics, Chemistry, Material Science and VLSI, a branch of Mechatronics have come together to develop more sophisticated, reliable and mature biosensing devices for applications in the fields of medicine, drug discovery, agriculture, biotechnology, as well as the military for use of identification of chemical and biological warfare agents [18, 21-23].

The health and wellness market made major advances in moving from centralised laboratory testing to rapid diagnostic test (RDT) with current attention moving into the area of wearables. Recently, a novel approach for non-invasive or wearable biosensors is on the rise for Continuous Glucose Measurement (CGM). Unlike traditional invasive methods such as blood tests and finger pricks, wearable biosensors can provide essential body information conveniently and painlessly in real time. These methods do not require a blood sample but use the interstitial fluids such as sweat, saliva, tears and skin surface to detect glucose molecules [24 -27].

Figure 3: Illustration of miniaturisation of blood glucose monitoring technique [24].

Electronics for biosensors

Figure 4: Wearable biosensor [24].

Recent advancements in smartphone technology as portable sensors which uses a combination of chemical sensors and biosensors that enable multiple scientific disciplines to apply this technology in serving their needs cost effectively. In chemistry, smartphone technology is used in areas such as; detection of explosives, detection of illicit drugs, detection of heavy metals in aqueous media and pesticides. Moreover, easy-to-use smartphone colorimetric sensing android application can be employed to measure the molar concentration of the biochemical liquid analytes. The application enables users to perform rapid testing and measurement of material concentration based on the simple image capturing of carefully prepared samples [28, 29].

1.1.3 Supramolecular chemistry: Chemical Sensors

As stipulated in section 1.1.1, the definition of a sensor is usually based on its application. A chemical sensor or chemosensor is defined by Cattral as cited in Ming-Yan [30] as "a device that responds to a particular analyte of interest selectively through a physical or chemical interaction and can be used for determination of that analyte." That is, a molecule that can bind to a target analyte and transform this binding into useful information [30].

Lehn defined supramolecular chemistry as "chemistry beyond the molecule" [31]. This is a branch of chemistry that focuses on the development of functional complex entities through intermolecular bonding and not covalent bonding. In molecular chemistry, molecules making up a chemical sample are composed of a unique combination of elements held together by several types of bonds of different strengths. Supramolecular entities are formed by association of two or more chemical species that are held together by intermolecular bonding [31-36]. A large fraction of supramolecular chemistry research initially focused on the synthesis and use of artificial, abiotic receptors to mimic natural receptors, this is how the concept of molecular recognition and selective chemosensing which is derived from the "lock and key" concept for enzyme-substrate interactions was introduced to supramolecular chemistry [35-37]. While selective chemosensing techniques were the entry level to the success of molecular recognition when C. J. Pedersen discovered crown ethers, differential sensing is continuing to develop as an alternative to selective chemosensing techniques. This technique takes a cue from the process of human olfaction and gustation processes which are able to obtain qualitative and even quantitative data on single analytes and mixtures [37, 39-40].

Chemical sensor technologies have emerged as dynamic approaches for identifying and quantifying specific analytes for security, human threats and environmental monitoring and control to facilitate a better quality of life. The projected increase in global energy usage and unwanted release of pollutants has led to a serious focus on advanced monitoring technologies for environmental protection, remediation, and restoration [41]. Consequently, these global challenges induced growth in the chemical *sensor* market and increased the demand to develop rapid, molecular and chemical sensors is increasing within the analytical sciences. Hence, the research on chemosensors that combine supramolecular chemistry and analytical techniques that yield in rapid results at the point of testing.

1.1.4 Synthesis and Application of Fluorogenic and Chromogenic probes

Chemosensors are molecular or chemical sensing probes with indicators, chromophores, luminophores, or reporters. A good sensor fulfils a number of requirements such as: selective recognition, the interaction should be reversible and rapid to allow real-time readings [32, 33, 40].

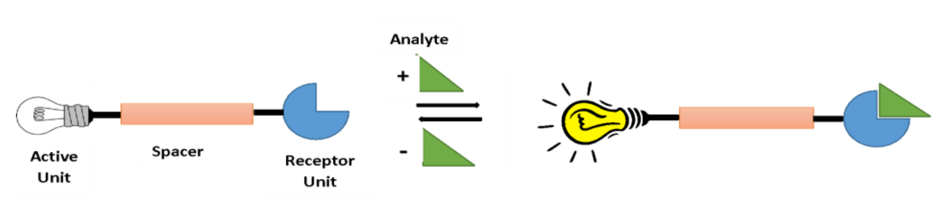


Figure 5: Schematic illustration of chemosensor [design concept adopted from 32, 33, 40].

In Figure 5 above, a chemical sensor consists of a receptor unit, which is a recognition element that is sensitive to stimuli produced by various chemical compounds (analyte) and a transduction element (spacer) that generates a signal whose magnitude is functionally related to the concentration of the analyte. Chemical sensors are broadly classified into gas, liquid, and solid particulate sensors based on the phases of the analyte. They are further categorized as optical, electrochemical, thermometric, and gravimetric (mass sensitive) sensors according to the operating principle of the transducer (spacer). A good sensor must fulfil a number of requirements such as: selective recognition, the interaction should be reversible and not slow to allow real-time readings [38, 41, 42]. This research focuses on alizarin and Schiff Base alizarin derivative probes for heavy metal sensing in aqueous medium. These probes are chromogenic (colorimetric) and fluorogenic and they fall under the purview of optical sensors. Fluorescence-based optical sensors represent a group of chemical sensors that can detect the change in the frequency of electromagnetic radiation emission. Optical sensors can be based on various optical principles including absorbance, reflectance, luminescence, and fluorescence covering different regions of the spectra, UV-visible region and IR, NIR regions allowing measurement whose intensity is proportional to the concentration of the analyte. However, for sensor applications, the most commonly applied methods in optical sensing are those based on light absorption or light emission that allow the detection of naked-eye colour change [43-46].

Fluorescent chemosensor is defined earlier as “compounds incorporating a binding site, a fluorophore, and a mechanism for communication between the two sites” (Figure 5). However, if the fluorescent sensor participates in an irreversible chemical reaction with a specific analyte, then it is generally called a fluorescent chemodosimeter or a fluorogenic reagent. Literature indicates that the terms fluorescent chemosensors and fluorescent chemodosimeters have been used interchangeably and fluorescent probes are now more commonly referred to as it is inclusive of both chemosensors and chemodosimeters [47-49].

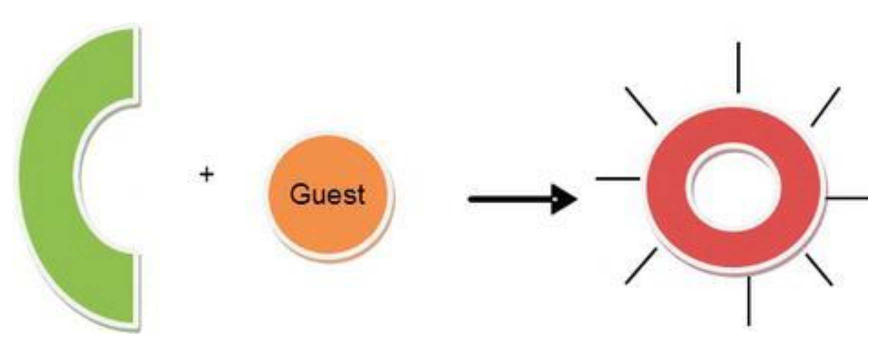


Figure 6: Schematic representation of the chemodosimeter [48].

Both chemosensors and chemodosimeters incorporate a binding site, a fluorophore, and a mechanism for communication between the two sites. Fluorophores are fluorescent molecules that respond distinctly to light compared to other molecules. They can absorb and emit light within a range of wavelengths, normally referred to as the absorbance (excitation) and emission spectra. They absorb light at a given wavelength and emit light at a higher wavelength, producing fluorescence in various colours that can be visually observed with a naked eye. Due to advancements in research, the vast selection of fluorophores today provides greater flexibility, variation and fluorophore performance than ever before. Fluorophores can be divided into three general groups: Organic dyes (synthetic organic dyes) e.g., fluorescein, alizarin, rhodamine, AMCA; Biological fluorophores e.g., green fluorescent protein, phycoerythrin, allophycocyanin and Quantum dots. Each fluorophore has distinct characteristics, which are considered when deciding which fluorophore to use based on its application [50, 51].

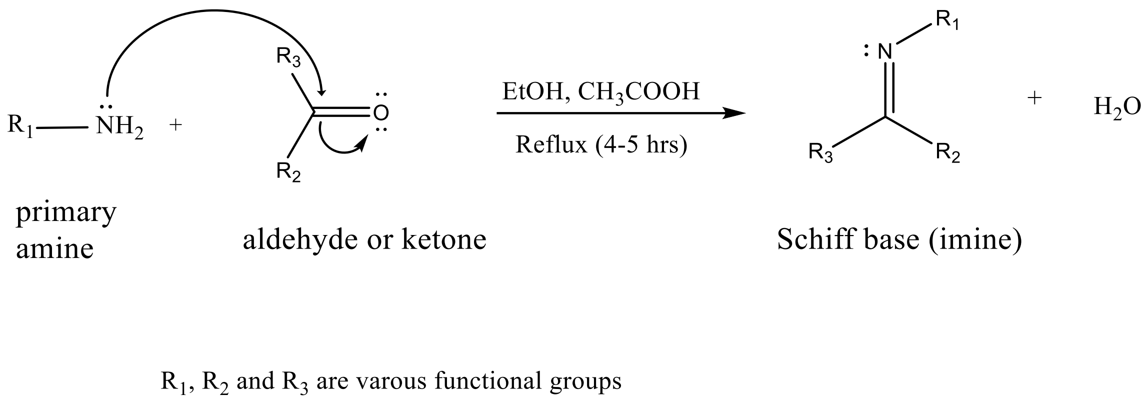
Amid a large variety of molecules utilised as chemosensors; dyes are more prominent owing to the diversity in their structural type as well as the physico-chemical characteristics [24]. The design of chromogenic and fluorogenic probes for cations and anions has received much attention over the years as a result of the significant roles they play in environmental and biological applications [52-53]. Fluorogenic sensors are capable of selective interaction with analyte, which leads to a change in the position or intensity of the fluorescence band, enabling the detection of ions or molecules *in situ* and *in vivo* with high sensitivity and efficiency [4, 54]. Moreover, in this field, the design of probes displaying changes in optical properties through a "turn-on" response and "turn-off" response is also an area of study that is prevailing.

1.1.5 Chemosensor synthesis and application

Ionic species are essential trace elements in the environment, in living organisms such as the human body, plants and animals. However, trace elements, especially heavy metals, are considered to be one of the main sources of environmental pollution since they have a significant effect on ecological quality. Consequently, there is more demand for highly selective chemosensors for metal ion and anion detection. Chemosensors represent an alternative to traditional analytical methods such as AAS, FAAS, ICP methods, atomic emission, and XRF for metal ion detection. These methods are often costly, confined to a laboratory, time consuming and require highly skilled operators. Alternatively, chemosensors utilise molecules that can bind selectively and reversibly with an anion or a cation. Moreover, they are cost effective, highly sensitive and able to detect very low ion concentration, simple to use yielding in real time response and colorimetric, which allows detection of colour change under visible light and in turn enabling on site detection [4, 46, 54-56].

One efficient way to detect cations and anions is through the use of optical sensors, both chromogenic and fluorogenic molecular probes, which show a shift in colour upon ion recognition. Anions used in agricultural fertilisers and pesticides can be harmful to the environment. Likewise, metal ion contaminants in the environment and in water can pose a health risk to both human and animal [55]. Some metal ions may cause corrosion, some are carcinogenic and toxic, affecting the central nervous system and other organs like kidneys, liver and skin. Therefore, the detection of metal ions in water and the environment is required to control and maintain permissible levels. The design and synthesis of fluorogenic probes or chemosensors for the naked eye detection of ionic species, particularly heavy metal ions and anions has received extensive attention over the years [57-62]. The detection of heavy metal in water with optical techniques can be achieved by various methods. In this study, we focused on Schiff Base inorganic chemosensors and an organic-natural dye, alizarin.

Schiff base ligands are organic compounds that contain the azomethine group, a carbon-nitrogen double bond (-C=N-). The nitrogen atom is connected to an aryl or alkyl group, not hydrogen. The Schiff bases are synthesised by the reaction of a primary amine and an active carbonyl compound (aldehyde or ketone). The reaction is a nucleophilic attack of amines with electrophilic carbon atoms that gives a consequential compound in which the C=N double bond substitutes the carbonyl (C=O) of an aldehyde or ketone as shown in scheme 1. The resulting compound is known as an imine, or Schiff base [63 -67].



Scheme 1: Schematic presentation of Schiff base reaction [66, 68 - 69].

Literature reveals there have been extensive studies over the past decades on Schiff bases due to their vast applications. Furthermore, the chemistry of metal complexes with Schiff base ligands containing oxygen (O) and nitrogen (N) as donor atoms has continued to receive considerable attention of researchers as these ligands are versatile and flexible capable of coordinating with various metal ions to give complexes with suitable properties for various applications. Schiff base ligands easily coordinate to transition metal ions via azomethine nitrogen to form stable complexes. This excellent chelating ability is owed to the easiness of preparation and the special property of C=N group, especially when a functional group like –OH or –SH is present close to the azomethine group. Moreover, the nature and the relative position of the donor atoms of Schiff base ligands allow a good control over stereochemistry of the metallic centres, as well as the number of the metal ions within homo- and hetero-polynuclear complexes. Additionally, Schiff bases are capable of forming complexes with certain metal ions which can exhibit unusual coordination that is thermodynamically favoured with good fluorescent properties and biological activities [70]. In conjunction with synthesis simplicity, all these advantages make Schiff bases a favourable choice of ligands to form metal complexes that are of interest in bioinorganic chemistry, catalysis, encapsulation, transport and separation process [66-71].

The synthesis and characterisation of transition metal complexes of Schiff bases is also one of the major areas of research focus because of their biological activities such as antibacterial, antifungal, anti-inflammatory [60-63]. Analgesic [65-66], anticonvulsant [67], antitubercular [67], antioxidant [72], anthelmintic [73], anti-tuberculosis and antitumour activity [74, 75]. The Schiff base transition metal complexes have also been used as biological models to understand the structure of biomolecules and the biological processes [67, 68, 72, 73]. Henceforth, the Schiff base reaction is undertaken as a means of design of chemosensors in this study.

1.1.6 Metal ion detection with alizarin dye

Besides their application in several fields of science, the extensive use of dyes in the field of chemosensing could be attributed to their simplicity, availability in sufficient quantities and the different classes of chromophores [24, 25]. Natural dyes and their identical synthetic analogues as well as synthetic dyes are used in a wide range of applications including textile industry, printing, food packaging industry, and cosmetic industry. Natural dyes can be obtained from plants, insect secretions, and rocks that are present in the environment. Natural dyes are preferred by environmentally conscious consumers as they are non-allergenic, non-toxic and degradable (i.e., does not cause environmental pollution). Plants are the main source of natural dyes. Dyes are organic substances with chromophore and auxochrome groups classified into several groups based on the presence of chemical group such as; anthraquinone (madder), alpha naphthoquinones (henna), flavones (weld), indigoids (indigo and tyrian purple) and carotenoids natural colorants of various structures for different applications.

One of the oldest known compound dyes is alizarin (1,2-dihydroxyanthraquinone), a natural red pigment which is extracted from the roots of *Rubia tinctorium L.* also known as "common madder" plant [76-78]. Alizarin is one of ten dihydroxyanthraquinone isomers. From a chemical point of view, alizarin is a condensed tricyclic anthraquinone dye that contains two carbonyls at positions 9 and 10 with two neighbouring hydrogen atoms (H) replaced by hydroxyl groups (–OH), forming a cis-diol [79-80].

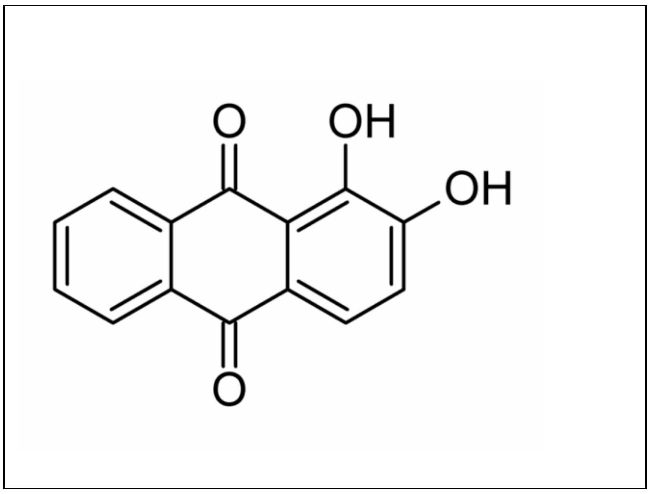
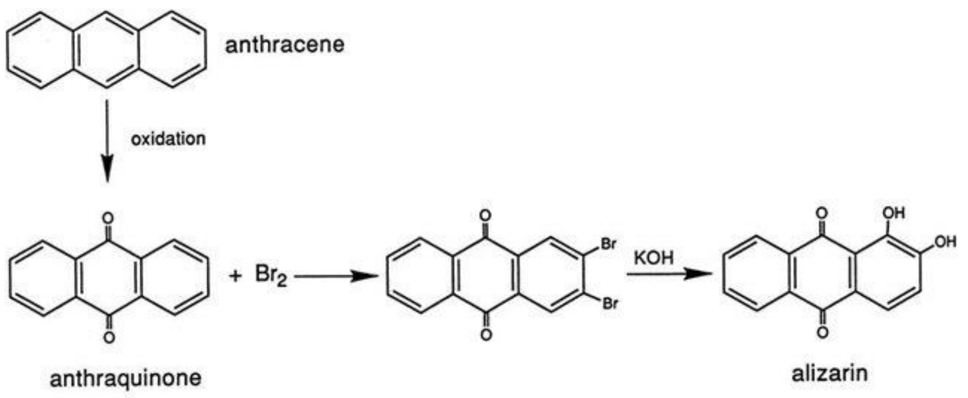


Figure 7: Chemical structure of 1, 2-Dihydroxyanthraquinone (alizarin), $C_{14}H_8O_4$ [81].

Alizarin can be used as a pH indicator; however, it is primarily used as a dye in the production of lake pigments [82]. The demand from the industrialisation of textile production has led to the support of synthetic chemistry to develop alternatives to popular natural dyes such as alizarin. In 1868 Charles Graebe and Charles Liebermann, German chemists in the Baeyer lab synthesised alizarin via oxidation of anthracene, bromination and hydroxylation as depicted in scheme 2. Subsequently, upon review of Graebe and Liebermann method, it was discovered that the method was not cost effective in view of the bromine price and a low yield [81].



Conversion of anthracene to alizarin, Graebe and Liebermann, 1868. [modern structural formulae]

Scheme 2: Graebe and Liebermann pathway for the synthesis of alizarin in 1868 [81].

The years that followed, research scientists discovered improved green methods of synthesising alizarin on a larger scale to meet the demands for natural dye. In recent years, alizarin has been synthesised from anthraquinone sulfonic acids [81, 83]. The most common method of obtaining alizarin lakes is by the complexation of 1, 2-dihydroxyanthraquinone with alumina. This reaction is based on the formation of a six-membered cyclic chelate, with a hydroxyl group (–OH) substituent at position 1 and the nearest oxygen atom from the carbonyl (C=O) [81].

Application of dyes in analytical chemistry are feasible because of the presence of chromophores and auxochromes. Most dyes form complexes with metal ions in aqueous media. Some dyes are used as titration indicators in analytical chemistry, and their complexes with metal ions in aqueous media are used in spectrophotometric analysis. The complexation between dyes and some essential metals including heavy metals allows their detection by the spectrophotometric method or chromatography. Furthermore, Tang *et al*. reports that alizarin has been widely used in anticancer agents and chemical reagents used in data recording and storage materials due to its unique activity [84]. Generally, hydroxyanthraquinones are known to be good photosensitiser with biological activities such as anti-tumour. In particular, dihydroxyanthraquinone has an important application as a prominent family of pharmaceutically active and biologically relevant chromophores. Additionally, alizarin also has strong antigenotoxic activity, mainly due to the transfer of ultrafast electrons in alizarin-metal oxide complexes and alizarin-TiO_2 complex materials, widely used as a photosensitiser in dye-sensitised solar cells. It is against this background that the alizarin chromophore has been favoured by many researchers [84-86]. Yoshida and Takabayashi as cited in Sasirekha *et al*. reported the effect of the substituent and intramolecular hydrogen bonding on the longest wavelength absorption band of substituted anthraquinone in various polar and non-polar solvents [86].

Alizarin and its isomers are known to form intramolecular hydrogen bonding between its conjugated π-system, the carbonyl and adjacent hydroxyl groups. When visible light is shone onto the molecule, an electron can be excited from the ground state orbital to an excited state orbital, the intramolecular proton transfer (ESIPT) reaction of the "locally excited (LE)" alizarin may occur to result in a "proton-transferred (PT)" tautomer of alizarin. The energy difference between the locally excited state (electronically excited molecule still in the ground state geometry) and the geometrically changed excited molecule provides the driving force for transformation. The absorption has a specific wavelength of energy. This process is responsible for colour change in the molecule that is detected by the human eye. Because the PT tautomer has a broken symmetry compared to the structure of alizarin, the emission from the PT tautomer appears at much longer wavelengths than that of alizarin [84-86].

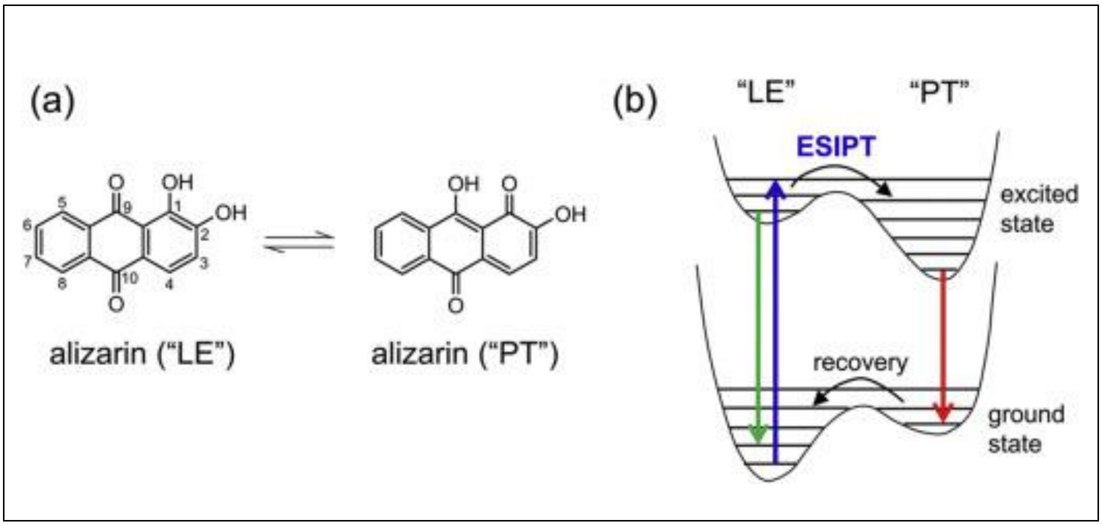


Figure 8: (a) Structures of alizarin (LE tautomer) and its internally proton transferred (PT) tautomer, (b) electronic structure of alizarin in LE and PT tautomer [85].

ESIPT characteristics of alizarin makes this compound an advantageous heavy metal probe because ESIPT characteristics makes alizarin solvatochromic. Solvatochromism is the phenomenon describing solvent effects on the absorption or emission spectra of the molecule following a change in the polarity of the solvent system or the concentration of the solvent. When absorption spectra are measured in solvents of different polarity, it is found that not only the position but also the intensity and shape of the absorption band can vary, depending on the nature of the solvent. Solvatochromism is a dynamic phenomenon that is not yet fully understood as it includes both the intermolecular forces of the molecular probe and that of the solvent. However, there have been intense studies to analyse the effect of solvent medium on chemical reactions and efforts have been made to correlate equilibrium constants, reaction rate constants, or positions of UV-vis absorption bands with the solvent polarity [86, 87 - 88].

1.2 Statement of the problem

Over the past decades, researchers established reliable techniques for the detection of analytes of metal ions and anions using analytical instruments such as AAS, ICP-OES, MS, and XRF among others. Although these methods are sensitive, highly accurate and feasible, there are several disadvantages associated with these analytical methods. They often require complicated sample preparation methods, trained instrument operators and costly instruments and do not offer the convenience to operate onsite or at the point of need. Contrary to conventional methods, chromogenic and fluorogenic probes offer a favourable approach to detect metal ions as they are low cost and have demonstrated simplicity, superiority in real-time response and to a greater extent, selectivity towards analyte detection even at low concentrations. Moreover, chromogenic and fluorogenic probes present the ability for in-field monitoring [4, 45, 54-56].

It is against this background that guest species by means of molecular frameworks (chemosensors) have become an alternative method for the determination of small molecules such as cations and anions due to their versatile nature and simple chemistry. Chemosensors has received much focus with intense research activities carried out in physiological, biological (medical diagnostics) and environmental processes. Special interest is devoted to developing highly selective and sensitive optical chemosensors for detecting heavy metals such as zinc, iron, copper, silver, cobalt, mercury, cadmium, nickel and aluminium [46, 55, 89-91].

There is limited literature on heavy metal detection in borehole water within the Khomas region and around Namibia at large. This leads to a lack of recommendations and solutions to avert heavy metal contamination detected in borehole water. There is a need to educate the public on the dangers of heavy metal water contamination and to put strict regulations in place to safeguard the quality of borehole water.

1.3 Objectives of the study

a) To synthesise and characterise an alizarin-based multifunctional chemosensor that is able to detect cations and anions in aqueous soluble medium.

b) To evaluate the chromogenic and fluorogenic behaviour of the synthesised chemosensors and alizarin probe with the selected heavy metals in aqueous medium.

c) To characterise the synthesised sensor using UV-vis, ^{1}H NMR and FT-IR.

d) To detect the presence of cations and anions in borehole water samples.

e) To evaluate the absorption properties of sensors using UV-vis titrations with some chosen analytes.

1.4 Significance of the study

The design and synthesis of chemosensors for metal ion detection using optical properties-based methods is effective and relatively economical, selective, rapid and sensitive enough to gain qualitative and semi quantitative information in real time with the convenience to perform outside the laboratory. Findings of the study towards aqueous based alizarin sensors can make a valuable contribution towards further research activities. Several studies on heavy metal sensing were conducted globally, however, such studies are limited in Namibia. The data obtained from this research will be used as a baseline statistic on the heavy metal detection in Khomas region borehole water, with fluorogenic and chromogenic probes.

1.5 Limitation of the study

The borehole water samples analysed in this research are limited to the Khomas and Otjozondjupa area only. Sample collection did not take into consideration the seasonal variations such as the effect of rainy and dry season on sampled borehole water. Moreover, the effect of geological structures on water was also not determined. Quantitative analysis of the water to determine whether the detected heavy metals are over the permissible limits was not conducted.

1.6 Delimitation of the study

Parameters such as temperature, pH and geological factors that would influence levels of heavy metals were not within the scope of this study.

2. LITERATURE REVIEW

2.1. Heavy metal toxicity and the environment

According to Michelle Bachelet, The United Nations High Commissioner for Human Rights: “Water is a basic human right and it is fundamental to human dignity” [92]. Unarguably, an adequate supply of safe drinking water is one of the major prerequisites for a healthy life. Water can be obtained from two major sources, groundwater and surface sources. The groundwater sources include boreholes and hand dug wells while surface water sources include rivers, streams, subsurface aquifers and lakes. Irrespective of the source of water, water is an essential tool for life, which is used for domestic, industrial and agricultural purposes.

Any activity which releases chemicals or waste into the environment, either intentionally or accidentally, has the potential to pollute the water. Contamination or pollution of water sources can render them unfit for consumption and can be costly and difficult to treat. Pollution of water sources can result from leaching of rocks, natural and chemical weathering resulting from natural disasters and industrial and agrochemical discharge, especially during the rainy season. These pollutants can affect the turbidity and the chemical constituents of the water source. Ultimately, water contaminants can distort the quality of the water and even add odour to the water thereby impacting negatively on economic activities. One major contaminant of water sources from the environment is heavy metals [92-95].

2.1.1 Toxicity of Heavy Metals

Although heavy metals are naturally occurring elements that are found throughout the earth's crust, their concentration in the earth has increased as a result of anthropogenic activities such as mining, transportation, and industrial activities. Examples are listed in Figure 8 below. Regardless of origin, increase of heavy metal concentration in water has become a very serious issue and needs special consideration because of their detrimental effect on living organisms.

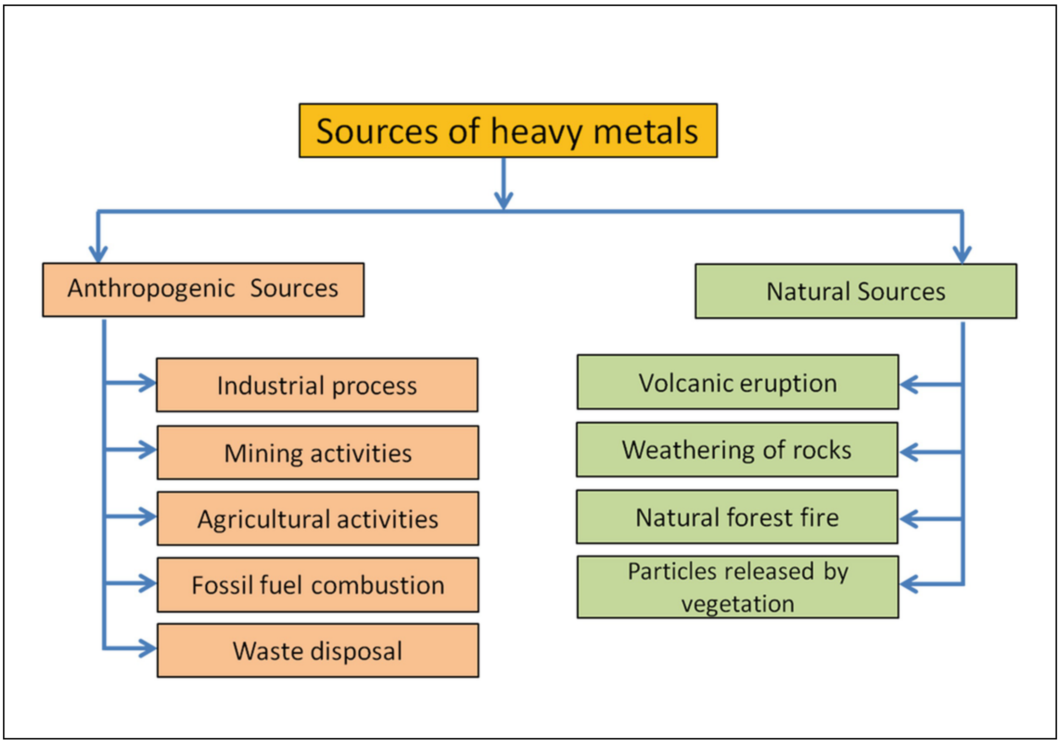


Figure 9: Different sources of contamination heavy metal in water & aquaculture [96].

The term 'heavy metals' is commonly used in the environmental literature to refer to metals and metalloids associated with environmental pollution, toxicity and adverse effects on the ecosystem. The term has been diversely defined in terms of density, relative atomic mass and atomic number [97, 98]. Some researchers define heavy metals as metallic elements that have a relatively high density compared to water and are toxic or poisonous even at low concentrations. The concept of a heavy metal is derived on the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure [99]. Due to a diversity of definitions, the use of the term “heavy metal” is regularly questioned by the scientific community. As the nomenclature of these elements is not clearly defined by any National Adhering Organisation such as IUPAC [94, 97-98].

2.1.2 Human exposure to heavy metals

Contrary to the traditional organic pollutant, heavy metals cannot be degraded or destroyed and can remain in the environment for a long time resulting in accumulation in various environmental compartments such as water and soil. Hence, there is a great chance of bioaccumulation in living organisms either directly or through the food chain. [99-104].

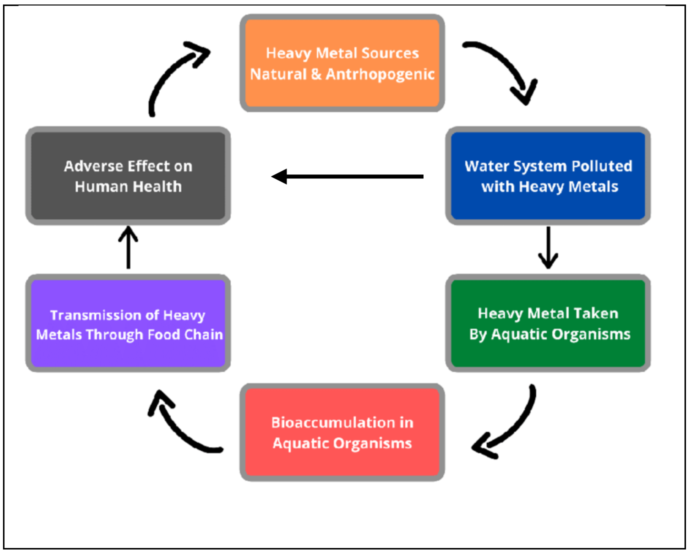


Figure 10: Conceptual framework of human exposure to heavy metal mixture from the environment [94].

Heavy metal ions in water include: lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), nickel (Ni), manganese (Mn) copper (Cu), cobalt (Co), selenium (Se), bismuth (Bi), iron (Fe), and the platinum group elements. Although most of the heavy metals are poisonous, some metal ions in minute amounts are generally regarded as nutritionally essential for animal and plant growth and are useful in chemical analysis under controlled conditions. However, when inside the body in undesirable quantities, metal ions can be converted to more toxic forms and can directly interfere with metabolic processes. This happens due to an additional benefit of their chemical coordination and oxidation-reduction properties that enable them to escape control mechanisms within the body cells [104-108]. As a result, metal toxicity has many adverse health effects that can put the body at risk of various diseases that can result in damage or reduced mental capacity, central nervous functions and damage to the body's vital organs. These metals bind with protein sites that are not made for them by displacing original metals from their natural binding sites resulting in the malfunctioning of cells and ultimately toxicity [108]. Repeated long-term contact with low concentration of some of these metals or their compounds may even cause cancer and neurological diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease and Wilson disease in humans [105, 107-108].

Moreover, what makes heavy metals toxic is their ability to form a complex with biological substances. The process can result in breakage of hydrogen bonds in proteins or inhibition of enzymes, and therefore, direct exposure can badly affect the nervous system, the liver, the kidneys, skin, teeth, and bones. Overall, human exposure to harmful heavy metals can occur in many different forms. In Figure 11 below, Sonone *et al*. illustrates the toxic effects of various heavy metal exposure on the human body.

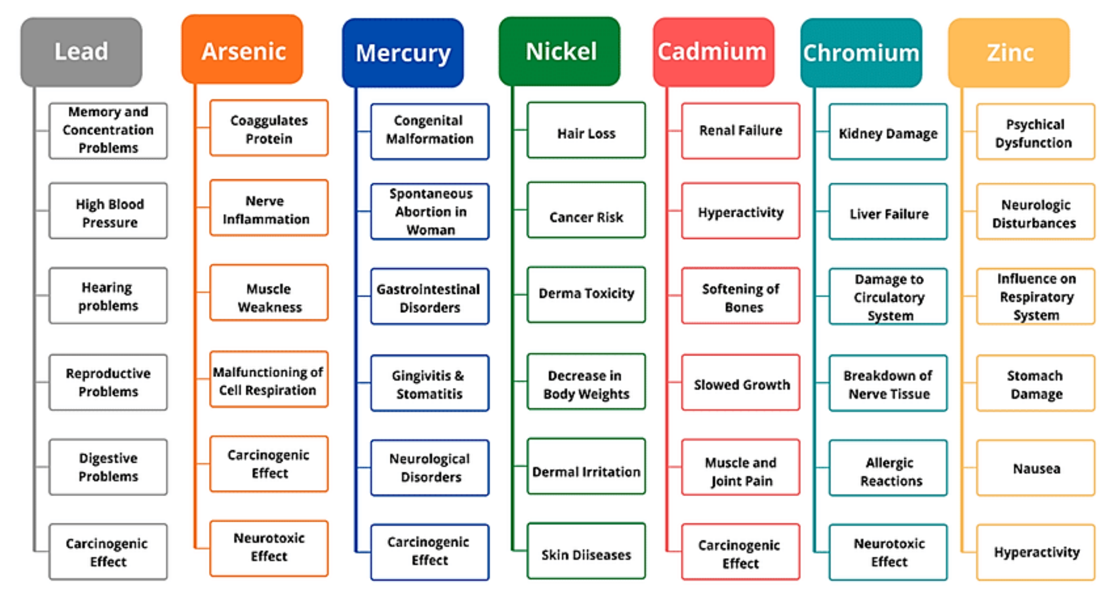


Figure 11: Toxic Effects of various heavy metals on human health [94].

The effects of heavy metals in humans are subject to their dosage, rate of emission and period of exposure. The concentration of heavy metal in water depends on pH, Total Dissolved Solids (TDS), seasonal temperature and rainfall variation in a region [100, 102-103, 109-110].

In addition to physical properties of water, it is equally important to assess the chemical parameters of water and accurately detect levels of heavy metals in water, in order to reduce the environmental and public health effects of heavy metal pollution. It remains a collective effort for governments, health agencies and controlling bodies to give additional attention to the environment and anthropogenic activities. Good water quality is an essential resource for domestic use to promote good human health and ensure a healthy nation. This is particularly true for an arid country such as Namibia, where water resources are limited and are regarded as a highly valued commodity. The methods employed to deliver clean water are often costly, placing a heavy burden on the government to provide water for all Namibian citizens. It has proven to be impractical to supply clean water throughout the country and the most convenient point setups. As a result, there has arisen private man-made water sources such as wells and boreholes, indiscriminately drilled by individuals, corporate organisations and even government agencies in different areas of Namibia with the aim to curb the inadequate water supply in the country. In its efforts to implement best practices to avoid water pollution, the Namibian government implemented policies to promote the maximum beneficial use of the country’s water supplies and to safeguard water supplies from avoidable pollution such as industrial process effluent discharge [111-113]. Toxic effects of metallic pollution coupled with the need of clean water supply for survival, various convenient strategies have been developed for the detection and the removal of metal ions from aqueous systems to uphold the quality of water. Against this background, it is undeniable that cost effective methods for detection of heavy metal in water is an area worth researching [104].

2.1.3 Molecular probes for monitoring heavy metals

There are three main categories to assess the water quality for the purposes of determining pollution indicators: physical, chemical and biological parameters [114-115]. There is a great demand for regular water quality monitoring and assessment in order to identify and prevent heavy metal pollution in water. Sensitive and selective methods are required for effective heavy metal monitoring in both groundwater and surface water. The widely used water monitoring method is largely based on manual water sampling followed by laboratory analysis [116]. Maximum permissible levels of heavy metal ions in the environment have been recommended by several environmental agencies including WHO, EPA and EMA. A wide range of chemical analysis techniques have been employed for heavy metal detection in water which can be divided into three main categories; spectroscopic detection techniques, electrochemical detection techniques and optical detection techniques. Spectroscopic methods for heavy metal ion detection include FAAS, AAS, ICP-OES, ICP-MS, and XRF among others [94, 104, 117]. Spectroscopy is defined as the interaction of light with matter [118]. Some of the drawbacks in operating these conventional methods is discussed in section 1.2. of this study. Laboratory based equipment requires regular maintenance and skilled operators. Moreover, sample collection and transportation can result in additional costs which vary depending on the frequency of sampling required and the distance between sampling point and the laboratory. Consequently, laboratory-based methods are not suitable for routine high frequency sample analysis. In light of these limitations, researchers have been attentive to cost effective, sensitive and selective methods that are easy to use and highly desirable for heavy metal monitoring [54-56, 119].

Researchers have made a significant breakthrough in the development of various electrochemical sensor modules and digital microfluidic modules for the detection of heavy metals. Electrochemical systems include techniques such as amperometry, voltammetry, potentiometry and coulometry, which make use of different sensors including the electrochemical enzyme sensor, electrochemical immunosensor, electrochemical nucleic acid sensor, the electrochemical whole cell biosensor, imprinted sensor and novel nanomaterial modified. Electrochemical sensors meet some of the requirements that are the driving force behind deviating from traditional analytical techniques. Electrochemical sensors are more cost effective, user friendly, reliable and allow fast analyses with experimental data obtained mostly in real time or in a few minutes making them suitable for in-field applications. Hence, on-line monitoring of water samples becomes possible, providing dynamic data of relevance for biogeochemical survey. Nevertheless, specific developments are still required for such applications, particularly to improve sensitivity, LOD and automation [104, 118, 120]. To date, electrochemical sensor devices have been successfully exploited for the detection of heavy metals in aqueous media.

 **Figure 12:** Portable hand-held device for heavy metal level detection [121].	 **Figure 13:** Differential pulse anodic stripping voltammetry (HM-5000P) [122].

Figure 12 and **Figure 13** above show self-contained portable electrochemical sensor instruments for qualitative and quantitative heavy metal analysis in aqueous media.

In optical methods of detection, metal ions can be detected by conventional analytical techniques of absorption, reflection or luminescence spectrometry. Fluorometric and chromogenic chemosensors, and indicator dyes and reagents (chemodosimeters) are often used in optical detection methods for selective recognition of metal ions, these methods are facile, rapid, and have good sensitivity and selectivity towards analytes. In earlier discussion of this report, a colorimetric sensor is an optical sensor that undergoes vivid colour change upon complexation with the analyte, which can be observed easily by the naked eye or electronic devices. Research focus on the technology of optical sensors is promising as they offer different features for detecting heavy metal ions [104, 123].

Sensor based probes for detection of metal ions in water have been designed to fit with the current needs for sample analysis. A simple example of a colorimetric sensing probe for metal ions is a diagnostic reagent strip. A reagent strip is a thin piece of paper impregnated with a reagent (a substance that causes a chemical reaction) to a specific substance, used in testing for that substance in a body of fluid [123-124]. Diagnostic reagent strips are commonly used in clinical analysis of biological samples, in particular for monitoring glucose concentration. Dry reagents such as dyes are applied in the construction of strips in a variety of ways. Test results are visually observed as thresholds providing semi-quantitative outputs. The reagents change colour, with the intensity of the colour proportional to the concentration of the analyte measured. Due to of their ease of use and convenience, researchers and manufacturers continue to expand the scope of application and technology for diagnostic reagent strips and improve sensitivity of current commercially available strips to adapt to market needs [123-124]. Similarly, the manufacturing methods of reagent strips are more advantageous contrary to commercially available heavy metal analysis techniques. The reagent test strips are manufactured in bulk through lean manufacturing principles and orders of several thousand can be available at single factories [126].

The Heavy Metal Test (HMT) test kit developed by NissenMedica Inc is an *in vitro* diagnostic tool used for the detection of free heavy metal ions in aqueous samples and bodily fluid samples such as urine and saliva. Metal ion testing with this kit is based on the dithizone reaction method by means of naked colour change. As a reagent, dithizone binds with metal ions to form a complex that depict an unambiguously colour change, indicating the presence of heavy metal ions in qualitative and in quantitative terms. Indicated in Figures 14 and 15 below are diagnostic reagent strips for heavy metal detection.

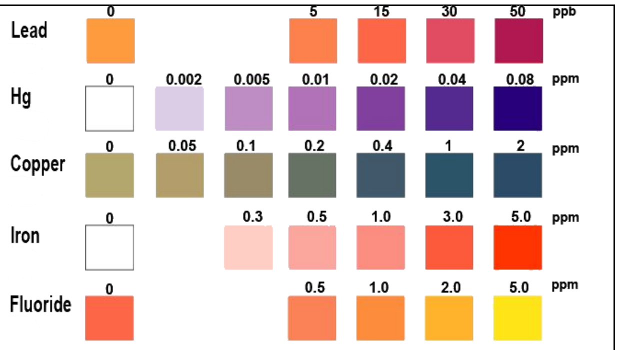
 Figure 14: NissenMedica Heavy metal screen test kit [127].	 Figure 15: ALLORA diagnostic reagent strip [128].

Figure 14 and **Figure 15**: Examples of chromogenic probe sensors for heavy metal analysis.

2.2 Organic reagent Colorimetric Methods

Colorimetric sensors are alternative analytical tools for heavy metal detection. However, research is now also directed to organic dyes for colorimetric probes for metal detection. In Figure 15 above, dithizone is used as a sensor for selectively detecting more than five kinds of heavy metal ions by the change of colour through naked eye observation [129]. This same approach is what we aim to achieve in this study with the organic dye, alizarin and alizarin derivatives. The principal goal of the present work is to study alizarin and alizarin derivative probes for the detection of metal ions in borehole water.

Organic reagents have played an important role in the chemical and physicochemical methods of analysis for a long time. Over the years, it has been noticeable that certain organic compounds that are generally used for analysis such as dithizone, alizarin, dimethylglyoxime and the pH indicators, are now part of a new class of analytical chemistry. Owing to the newly found applications of organic reagents, organic chemistry is merging into analytical procedures of qualitative and quantitative analysis of chemical elements and both organic and inorganic compounds, as well as for separation, concentration, masking and other auxiliary operations. Organic reagents may be used to simplify and to speed up methods and also to improve accuracy in analytical techniques. These organic analytical reagents are also applicable to trace metal analysis [130-132]. Theoretically, there are no limits to designing new organic compounds for analytical purposes; however, in practice only several dozens of classes of chemical compounds are effective as organic reagents [131]. The requirements for the organic reagent are dictated by the analytical method used and its intended application. Herein, the aspects of organic reagents such as dithizone and alizarin for the determination of metal ions in aqueous medium are investigated. The principal characteristics of organic reagent for the determination of metal ions depend largely on the presence of a certain functional group that enables its interaction with the determined element and subsequent observation of the respective analytical signal such as colour change. The remainder of the molecular structure to which they are joined is generally of secondary importance. Hence, there are in many instances, a series of organic compounds containing the same functional group, but in which the remainder of the molecule is different. Organic molecular structures can be bulky and complex which have a remarkable effect on the chemical reaction. Therefore, the design and synthesis of organic compounds or selection of organic reagents is carefully done with the intended application in mind, be it solubility in a particular solvent system or luminescence [131-132].

Not all organic compounds can form chelates with metal ions. However, organic compounds consisting of -OH, -SH, –NH groups or N, O and S atoms frequently form coordinate bonds with metal ions in forming chelating rings. Reagent colorimetric method refers to comparing or measuring the coloured substance solution, they are mainly applied for measurements of metal ions at trace levels. Generally, the analysis is dependent on a distinctive coloration which serves as a qualitative measurement. The intensity of the coloration is used to deduce quantitative estimation [130, 132].

2.2.1. The chemistry of Dithizone

1,5-diphenylthiocarbazone ($C_{13}H_{12}N_4S$) commonly known as “dithizone” is a sulfur-containing organic compound, usually abbreviated to either H_2-DPTC, or DTZ. Dithizone is one of the widely used organic chelating reagents that forms complexes with metals. Dithizone is a dark blue-green solid crystalline powder with a melting point of 168 °C and molecular weight, MW = 256 g/mol. It is a weak acid insoluble in water but soluble in alkaline media, alcohols, hydrocarbons, and chlorinated hydrocarbons such as chloroform and carbon tetrachloride. This bidentate ligand incorporates two potentially dissociable hydrogen atoms and has two sites for coordination with a metal, the sulfur and nitrogen donor atoms. When complexed to metal ions, dithizone gives a wide range of distinct colorimetric responses with numerous metal ions. Due to this, dithizone has become a useful tool to aid Chemists in isolating and determining traces of heavy metals at low concentration with an accuracy greater than that found in spectroscopic determinations [129, 130 – 135]. Furthermore, chelating reagents with thiol groups (such as dithizone) are used frequently in solid phase extraction for separation of heavy metals [130, 137-138].

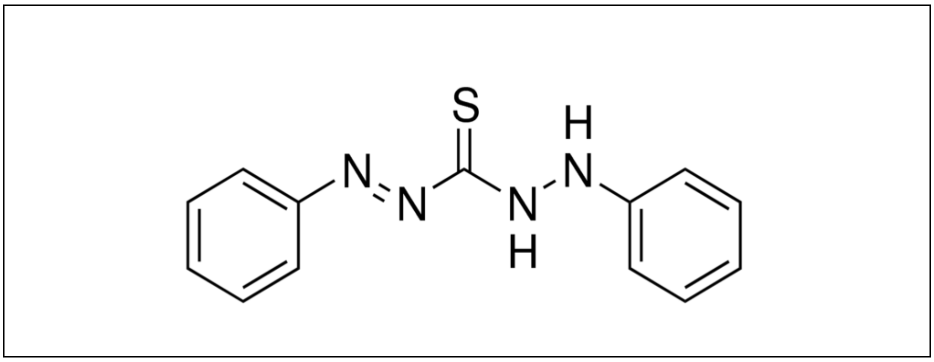


Figure 16: Molecular structure of Dithizone [135].

Dithizone was first synthesised and studied by Emil Fischer in 1878 during his investigation of the series of compounds resulting from the reactions of phenylhydrazine with carbon disulphide. However, it was used as an analytical reagent for the first time by Hellmut Fischer in 1925. His work showed that dithizone had great potential for the detection and determination of heavy metal ions. Fischer, studying metallic dithizonates and their possible applications in analytical chemistry, was the first to report that dithizone existed in two tautomeric forms he referred to as the “keto” and “enol” forms [130, 137, 138, 139, 140].

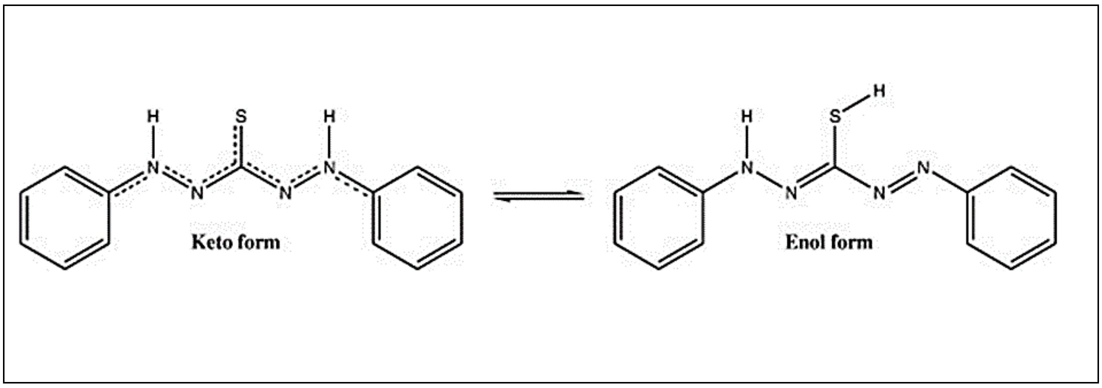


Figure 17: The two tautomeric forms of dithizone [137].

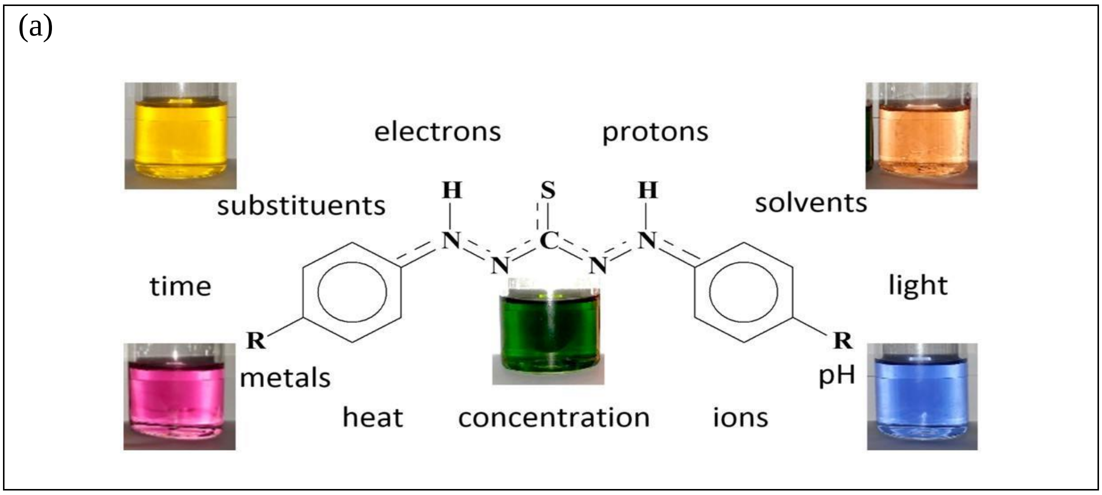
It is reported that the keto tautomer is formed predominantly in acid or neutral solution whereas the enol tautomer presents in alkaline solution or where there are insufficient quantities of dithizone [141]. Time dependent density functional theory (DFT) and X-ray crystallography studies conducted on dithizone and its oxidation products by von Eschwege *et al* in 2011 reveals the existence of at least six different tautomers [142].

Dithizone and its derivatives and complexes are among the most reported colourful compounds, being responsive to no less than seven different external stimuli that affect colour change. Dithizone originally earned its place in analytical chemistry because of its sensitivity towards numerous metal ions at low concentrations which leads to the formation of colourful complexes that are easily observed with the naked eye under visible light. The phenomenon by which a chemical substance changes colour when exposed to light and reverts back is known as photochromism. Remarkably, photochromism is a reversible transformation of a single chemical species being induced in one or both directions by electromagnetic radiation (UV, visible or IR radiation) between two states having distinguishable absorption spectra [143-144].

For better understanding of the chromism phenomena, there are two basic types of chromisms; direct and indirect. Direct chromism is when the stimulus itself causes an almost instantaneous change in colour, for example, photochromism by light, whereas with indirect chromisms, immediate colour change is not observed but occurs via an intermediate index, which can be either chemical or physical, for example, chronochromism which is observed after some time has elapsed. Another form of chromism is electrochromism, which is a reversible colour change upon oxidation or reduction brought about by an electrical current or electric potential. Thermochromism and solvatochromism are colour changes brought about by heat and by solvent system respectively.

Herein, solvatochromism of alizarin was briefly discussed in section 1.1.6. of this study. Likewise, dithizone is solvatochromic; literature reveals the effect on colour change of dithizone when dissolved in different organic solvents systems. Furthermore, other chromisms include: concentratochromism, halochromism and chronochromism. The list of chromisms is endless, and the chromic phenomena will continue to grow with new discoveries in research. Overall, these colour change phenomena, whether known as chromic or chromogenic, are classified and named after the stimulus that causes changes in the differential absorption, and reflection and/or scattering of white light [143, 145].

Fries *et al*. reports that photochromism was first reported by Fritsche in 1867, who observed that tetracene with air and light produced a colourless material which regenerated to tetracene by heating [144, 146]. On the other hand, Rawat *et al*., reports that photochromism was first recognized by Markwald in 1899. It is further reported that in 1950, Hirschberg described this phenomenon as “Photochromism ", derived from the Greek word, Phos (light) and Chroma (colour) [147]. Following the first discovery of photochromism or photochromic or photochromatism, there is considerable continued interest in photochromic materials and it remains an active field of basic and applied research [143, 144, 146]. According to Ntoi *et al.* it was not until 1977 when Irving published an extensive overview on dithizone photochromatism. The first signs of chromism were noticed by him and Webb independently discovering photochromism in the orange bis dithizonatomercury (II) complex. Fifteen years later Meriwether, building on the foundations of Irving, reported similar observations for several metals [143].



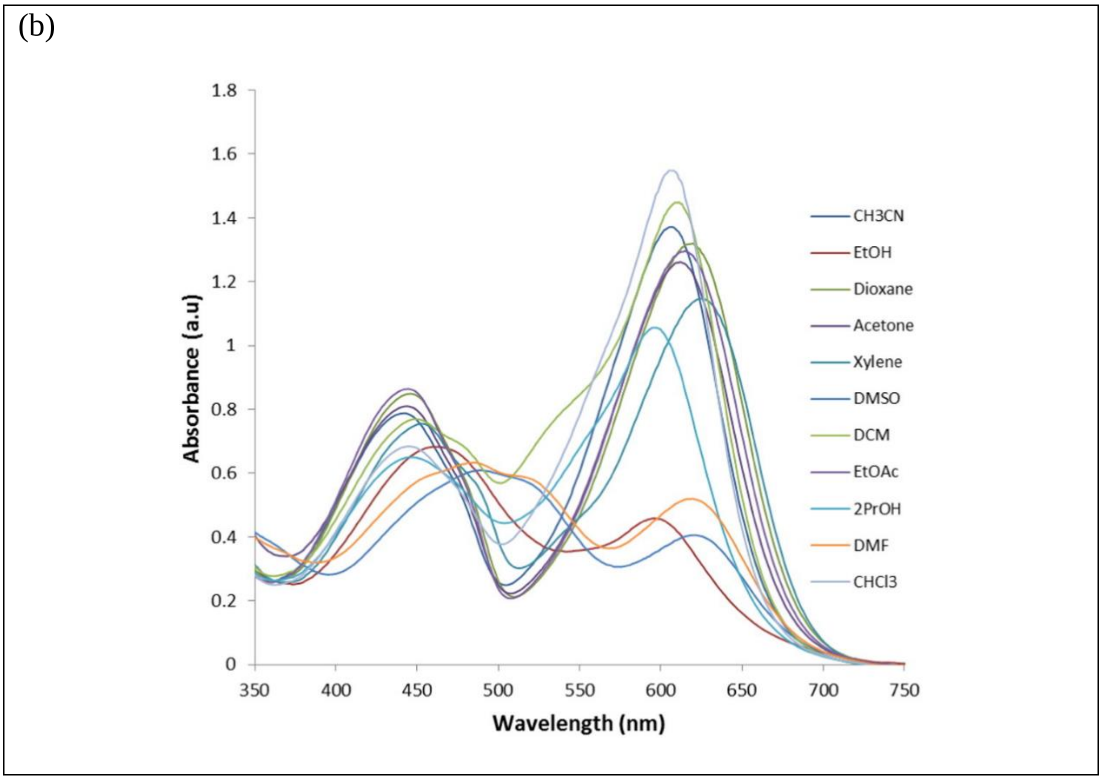


Figure 18: The chromisms associated with dithizone. (a) seven chromisms of dithizone reported by Ntoi *et al.* [143]. (b) the absorption spectra of dithizone in various solvents [137].

In 2017, Ntoi *et al.* reported seven chromisms associated with dithizone metal complexes, attributed to isomerisation around the carbon-nitrogen (C−N) bond. The seven chromisms reported in Figure 18 (a) are based on reversible or irreversible transformation of dithizone molecule, evident through difference in absorption of electromagnetic radiation. The chromisms are: (1) Photochromism - a light-induced transformation. (2) Solvatochromism - A phenomenon whereby a compound changes colour when dissolved in solvents with different polarities [143]. In this report, solvatochromism of dithizone is briefly studied. In Figure 18 (b), Rauf *et al.* [137] reported results obtained through investigation of dithizone spectra, where dithizone was employed as a probe in various solvents. Figure 17 indicate two forms of dithizone molecule, due to the isomers of dithizone, the change in peak position of the absorption band of the keto form of dithizone in different solvents is an indication of the interaction of the probe molecule with the solvent. It was revealed that the keto form of dithizone belongs to the longer wavelength band (620 nm) in the UV–visible spectroscopy, while the shorter wavelength band (450 nm) arises from the overlap of the keto and the enol forms. Consequently, the higher wavelength band arises due to the changes in the $n \rightarrow \pi^*$electronic transition, with the participation of lone electron pair of sulphur [137]. (3) Electrochromism-reversible colour change due to electron transfer or oxidation/reduction process. (4) Halochromism – colour change induced by change in pH. (5) Thermochromism – colour change caused by heat. (6) Concentrochromism- colour change that occurs as a result of a change in concentration. (7) Chronochromism - change of colour as a result of defined period of time.

Linear geometry of the π-electron delocalized backbone of dithizone enables colour-changing isomerization gives rise to a HOMO that stretches along the entire ligand, including the two phenyl end groups. The elongated HOMO persists in the ligand also when complexed to metals [143]. In section 1.1.6, it was highlighted that when visible light is induced on an alizarin molecule, its π-conjugated system induces ESIPT. Likewise, the chromism effect in dithizone is attributed to Intramolecular Proton Transfer is attributed to isomerisation around the Carbon−Nitrogen(C-N) bond. In this study, solvatochromism of dithizone was briefly researched by observing naked eye colour change of dithizone in various solvent systems.

2.3 A review on synthetic organic dyes as chromogenic probes (chemodosimeter)

The use of organic colorants extracted from a variety of natural sources for textile dyeing purposes have been used since ancient times. Dyes differ from pigments, which are finely ground solids dispersed in a liquid. Synthetic dyes are by far the most widely applied colourants in industry. From manufacturing of paint and coating products to fabric dying. Synthetic dyes are applied practically in all areas of our daily life. Literature reviews indicate that a significant percent of dyes from anthropogenic activities persist as pollutants in the environment through industrial effluents. Dye contaminated sources often require complex and costly, intensive treatment. As a result, environmental and sustainability considerations have led to an increasing effort to substitute inorganic dyes with safer and more sustainable equivalents. One promising class of alternatives is the naturally occurring anthraquinone dyes or simply anthraquinones that can be synthesised, i.e., synthetic organic dye. Anthraquinone is present in most common red natural colorants used extensively in textile dyeing. Anthraquinones constitute the largest group of natural quinoids, a class of cyclic organic compounds characterised by a saturated benzene ring that contains two oxygen atoms that are bonded to carbonyls and have sufficient conjugation to show colour under visible light [148-150]. Therefore, the elucidation of anthraquinone dyes in recent decades has gained momentum in the field of science and is becoming one of the most interpretative and reliable approaches for colorimetric analysis of heavy metal detection and recently as biomedical diagnostic tool [149,151-152].

The first synthetic organic dye, mauve (aniline) was discovered by Henry W Perkin in 1856. This was a major breakthrough which subsequently led to the commercialisation of synthetic organic dyes to replace natural dyes. The advantageous ripple effect of this has since then, reduced the consumption and application of natural dyes and inorganic dyes for textiles [153-154]. Dyes are aromatic compounds whose molecular structures consist of delocalised electrons and often with multiple functional groups. Unlike most organic compounds, dyes possess colour because they absorb light in the visible spectrum (400–700 nm). Dyes have certain features responsible for colour and when any one of these features are lacking from the molecular structure, the colour is lost. The features of the dyes are as follow:

1. They have at least one chromophore (colour-bearing group) such as nitro (NO_2,) azo (-N=N), methine (-CH=), Carbonyl (C=O), amine (-C=N), and -N=O [76, 155-156]. A chromophore is the part that is responsible for providing a particular colour [157].
2. They have a conjugated π-bonding system (for example, a structure with alternating double and single bonds) [155].
3. Dyes also contain an auxochrome, a functional group of atoms attached to the chromophore which modifies the ability of the chromophore to absorb light (colour helpers). Examples of chromophores include carbonyl group, sulfonate group, amino, and hydroxyl groups. While these groups are not responsible for colour, their presence can shift the colour of a colourant and they are most often used to influence dye solubility [76, 155-156].

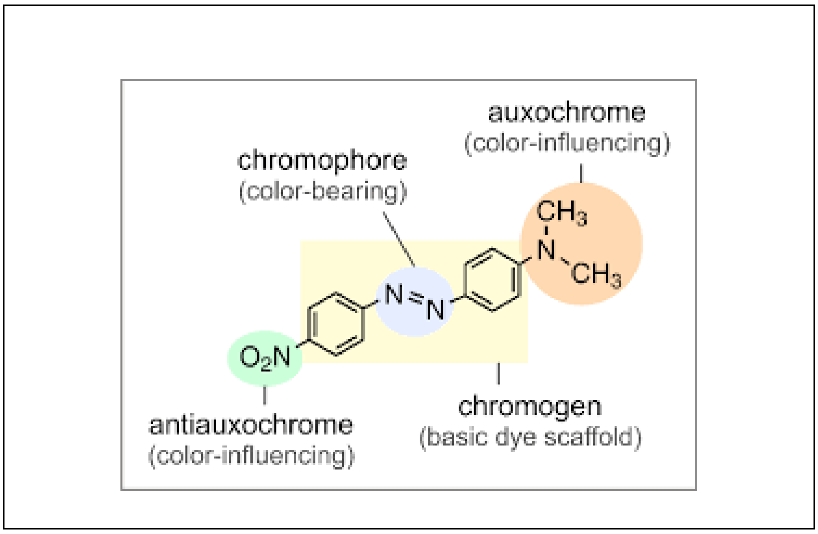


Figure 19: Chemical structure of an organic dye molecule consisting of the dye features [158].

The use of dyes in spectrophotometry, titration and chromatography is well known. The area of electrochemistry metal ion detection is also a fast-growing research area. Owing to its application in industrial process control, environmental monitoring, and different applications in medicine. Dye features discussed in this section clarify the need to use organic dyes as colorimetric probes. Dye applications in analytical chemistry are proven feasible because of the presence of chromophores and auxochromes [76]. Therefore, investigation and evaluation of dyes play a vital role in the process of introducing novel metal indicators.

2.3.1 Alizarin as chromogenic probes

This section provides a theoretical framework on alizarin dye as an analytical reagent, including an overview of alizarin dye and alizarin derivatives, with emphasis on heavy metal sensing in aqueous medium. The natural dye alizarin was introduced in chapter 1, section 1.1.6 of this research report. Alizarin (1,2-dihydroxyanthraquinone) is an organic dye of the anthraquinone compounds from *Rubia tinctorum L*. Alizarin is widely used in the textile industry. Generally, hydroxyanthraquinones have attracted the attention of many researchers owing to their possible applications related to the photoactivity based on their chromatic properties such as the presence of chromophores and auxochromes. It is reported that dihydroxyquinones are known as a prominent family of pharmaceutically active chromophores with diverse biological effects including antibacterial, antitumor and antioxidation [159-160]. Furthermore, alizarin is also used as a biological dye to stain pure calcium and calcium in bones and other body tissues. In geology, alizarin is used as a stain indicator for minerals such as calcium carbonate, calcite and aragonite [161].

Alizarin is one of the oldest dyes and it is widely studied, resulting in abundant availability of experimental data. Figure 19 above indicates different features of a dye that makes dyes good colorimetric probes. In Figure 20 below, the two carbonyls at positions 9 and 10 and the two hydroxyls bound to carbons 1 and 2 are the chromophore functional groups and they are responsible for alizarin's optical properties in the visible region. With an exception of the carbonyl in position 10, they can also act as binding groups between the dye and the support base through an intermediate metal atom called mordant [162].

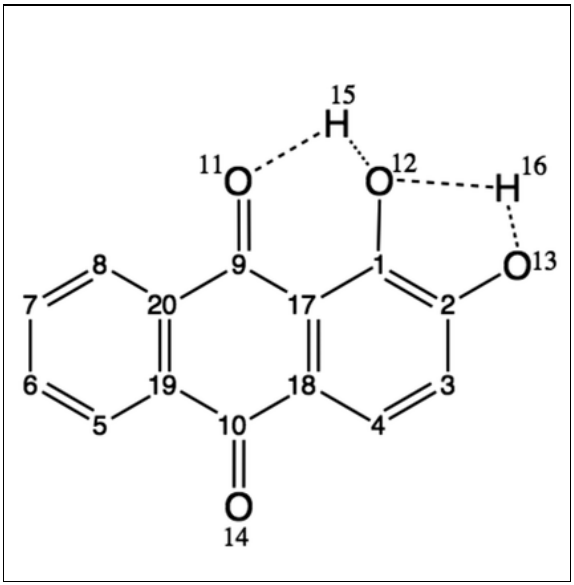


Figure 20: Molecular structure and atom labelling of alizarin [162].

According to Fain *et al.*, alizarin was the first of the hydroxyanthraquinones to be proposed as an analytical reagent for qualitative and quantitative determination of metals. Literature reviews indicate that its water-soluble derivative alizarin red S (1,2-dihydroxy-9,10-anthraquinone-3-sulfonic acid) became mostly employed as a colorimetric reagent in analytical chemistry [160, 162, 164]. Very little has been reported on unmodified alizarin and its derivatives for heavy metal sensing in water.

One of the well-studied alizarin-metal complexes is that of alizarin and aluminium ion (Al^{3+}) because it is a well-known dye named Turkey Red used to dye cotton and wool.

This research briefly discusses the dynamics of aluminium-alizarin complex, which will set the basis for understanding alizarin-metal ion complexation in aqueous medium. David [165] reports that Wunderlich and Bergerhoff conducted studies in 1994 on X-ray crystallography studies of an aluminium-alizarin complex crystallised from dimethylformamide. Their study concluded that the complex was polynuclear bonded with two aluminium cations and two calcium cations chelated to four alizarin molecules, with the added twist that the two central aluminium ions were linked by hydroxyl bridges [165].

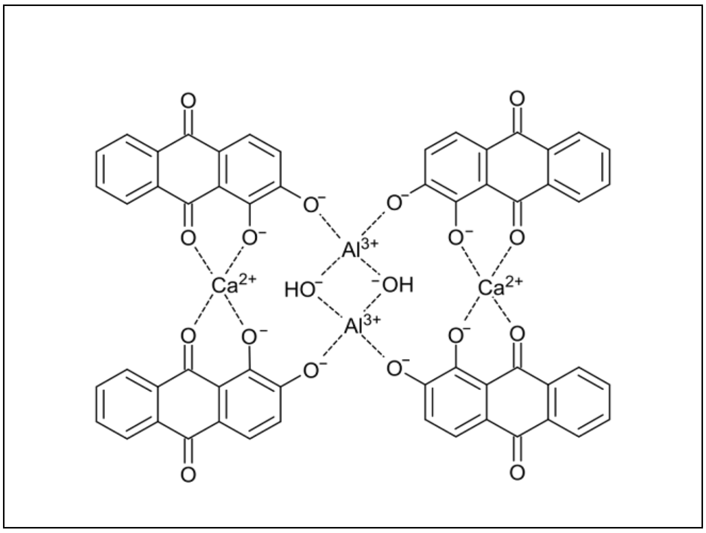


Figure 21: Aluminium-alizarin complex according to Wunderlich and Bergerhoff, 1994 [165-166].

Literature reveals that there may be a range of different aluminium-alizarin complexes depending on the conditions of the dye bath, especially the nature of the alkali used [165]. Vankar [166] states that alizarin molecules are capable of forming six-membered chelate rings with aluminium ions. Zhuang et al. [168] reports that there are six possible coordinated structures of aluminium-alizarin complexes. It is perceived that aluminium cations are coordinated with O atoms from H_2O or OH^-. i.e., $[AlX_6]$, $[AlX_5]$ and $[AlX_4]$ (X refers to H_2O or OH^-). [75]. It was discussed earlier in chapter one that alizarin complexes occur as a mixture of isomers with the ortho-diphenol and peri-hydroxycarbonyl 9,10-anthraquinoid structures [164]. Moreover, many computerised models of the binding and orientation of alizarin towards an aluminium metal ion are proposed. In literature there are two possible coordination sites that have been proposed and these are shown in Figure 22.

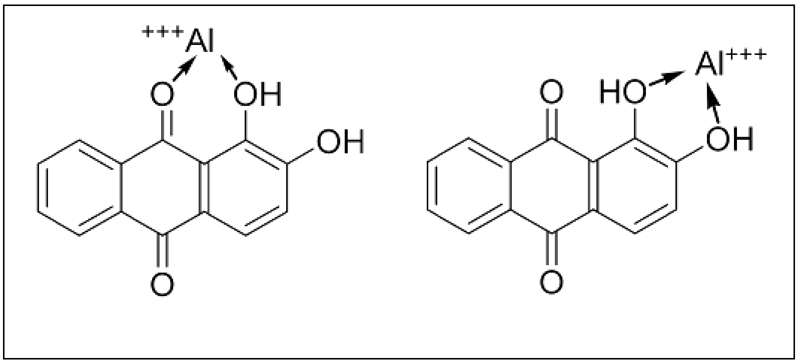


Figure 22: Two possible coordination sites formed between alizarin and Al^{3+}: Kiel and Heertjes model (left); and Wunderlich and Bergerhoff model (right) [164, 165, 167, 169].

Mohandoss *et al* report a highly selective and sensitive alizarin **(1)** colorimetric and fluorescent sensor probe (chemosensor) and alizarin: β-cyclodextrin (β-CD) **(2)** an alizarin derivative chemosensor for the detection of cobalt, Co^{2+} ion and nitrite NO^{3-} ion in aqueous medium. Chemosensor **2** showed good fluorescence behaviour upon interaction with various cations such as Ca^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Mg^{2+}, Hg^{2+}, Zn^{2+} and Fe^{3+} and it displayed strong fluorescence quenching with cobalt. Moreover, when the **2**-cobalt complex was tested with various anions, only nitrate (NO^{3-}) enabled cobalt binding (as $CoNO_3$) and led to fluorescence enhancement [170]. Tajmir-Riahi *et al.* describe Fluorescence quenching as the decrease in the quantum yield of fluorescence from a fluorophore, induced by a variety of molecular interactions with quencher molecule(s) [171]. Whereas fluorescence enhancement is the increase of fluorescence emissions/signal. One of the ways to enhance fluorescence signals is to increase the number of fluorophores available for detection [172]. For illustration purposes, below are UV absorption profiles adopted from Pandey *et al*. [173]. The downward arrow indicates fluorescence quenching and the upward arrow indicates fluorescence enhancement.

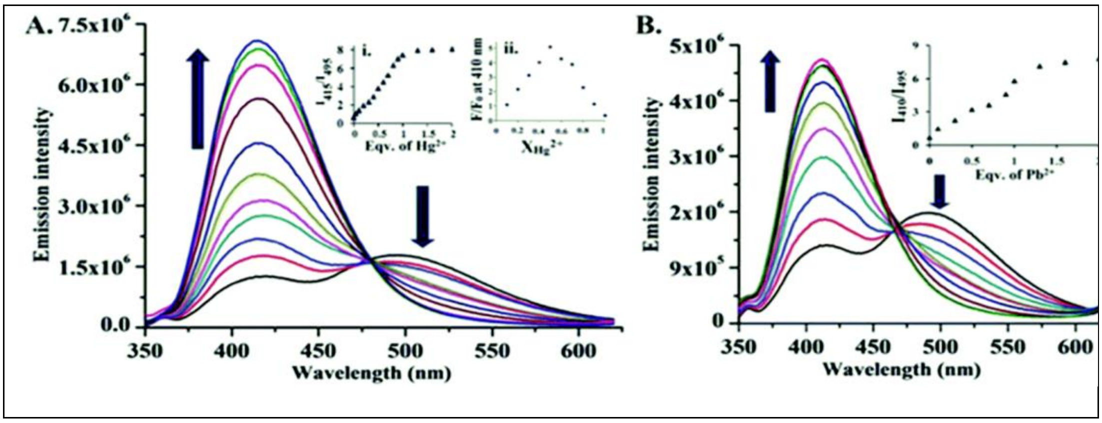


Figure 23: UV absorption profiles of Zn(II), Cu(II), and Cd(II) based complexes as fluorescent probes for cations [173].

Chemosensors were investigated by means of UV-visible and fluorescence spectroscopy complemented by computational studies and confocal laser scanning microscopy. It is reported that β-CD: alizarin derivative demonstrates colorimetric recognition behaviour toward Co^{2+} ion by colorimetric colour change. It is further revealed that the binding of alizarin with Co^{2+} ion resulted in large blue-shifts in the absorption spectra at wavelength maxima: 234, 266.5 and 530.5 nm. While other metal ions produced insignificant changes, excluding copper (II) at wavelength maxima: 236, 262.5 and 524.5 nm. Copper (II) showed slight interference. Moreover, in the presence of miscellaneous metal ions, **1** and **2** exhibited absorption peak shifts as well as colour changes from violet to brown and yellow, visible to the naked eye as indicated in Figure 24 below:

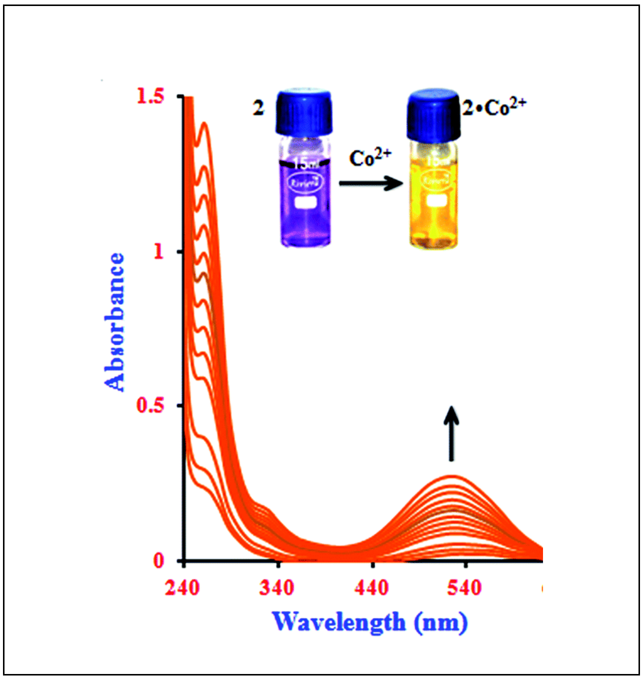


Figure 24: Enhancement absorption spectra of **2** with the addition of Co^{2+} ion concentrations from 0.3 to 0.85 μM [171].

A comparative study conducted by Naimhwaka *et al*. [174] reported the investigation of a naphthoquinone based colorimetric sensor (**N**) bearing hydrazone receptors in aqueous media and its recognition properties towards biologically important anions in DMSO–water mixture (9:1) using spectroscopic methods. It is further reported that hydrazone-based receptors showed selectivity towards several anions. Firstly, it was established whether there is any chemical interaction between sensor **N** and all respective anions, a series of prepared anionic solutions (1×10^{-3} M) in DMSO–H_2O were added drop wise, each separately, to 3 ml of N (1×10^{-5} M) in a similar solution (DMSO–H_2O), at room temperature. Naked eye colour change was observed from green to light blue for F^-, CN^- and AcO^- and colour change from green to violet for OH^- as indicated in Figure 25 below.

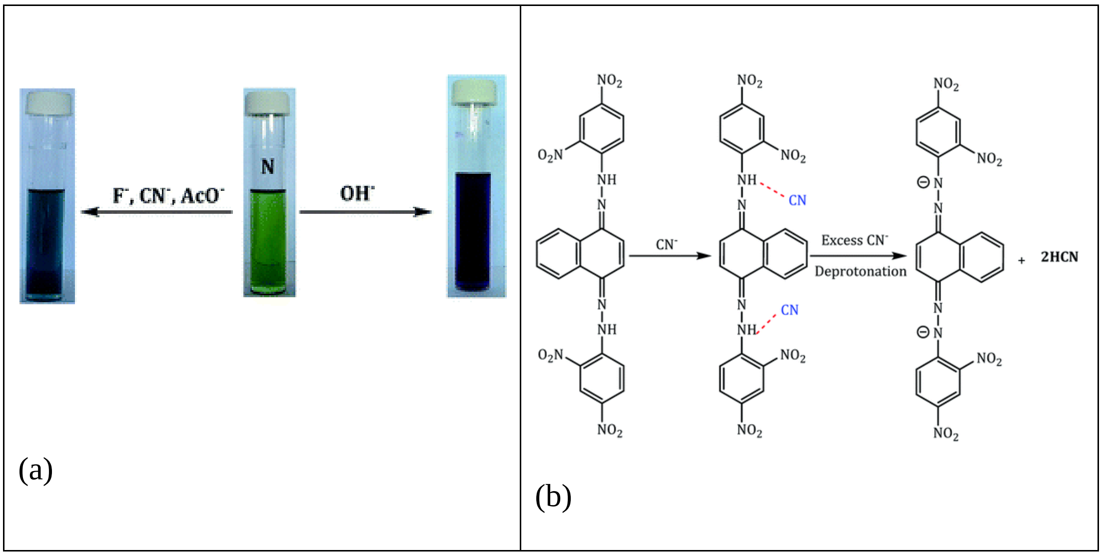


Figure 25: (a) Visual colour changes of N in DMSO–H_2O (1 × 10−5 M), upon addition of 3 equivalence of CN^-, F^-, AcO^- and OH^- respectively. (b) Proposed binding mechanism of N on interaction with anions (CN^- ion) in DMSO–H_2O as reported in Naimhwaka *et al.* [174].

Positive test results were obtained when sensor **N** was tested for fluoride detection in two different commercially available toothpaste brands. Moreover, test paper strips prepared from **N** were able to detect the presence of cyanide (KCN) and hydroxide (NaOH) in tap water [174]. Similarly, the concept of selective qualitative metal analysis in aqueous medium is discussed in section 2.1.3. in this study, we investigate alizarin and alizarin derivative probes for the detection of several cations and anions. The sensor approach is similar to the method employed by Naimhwaka *et al*. We also studied the UV-absorption spectra of sensor probe-ion complexes.

3. RESEARCH METHODOLOGY

3.1 Research design

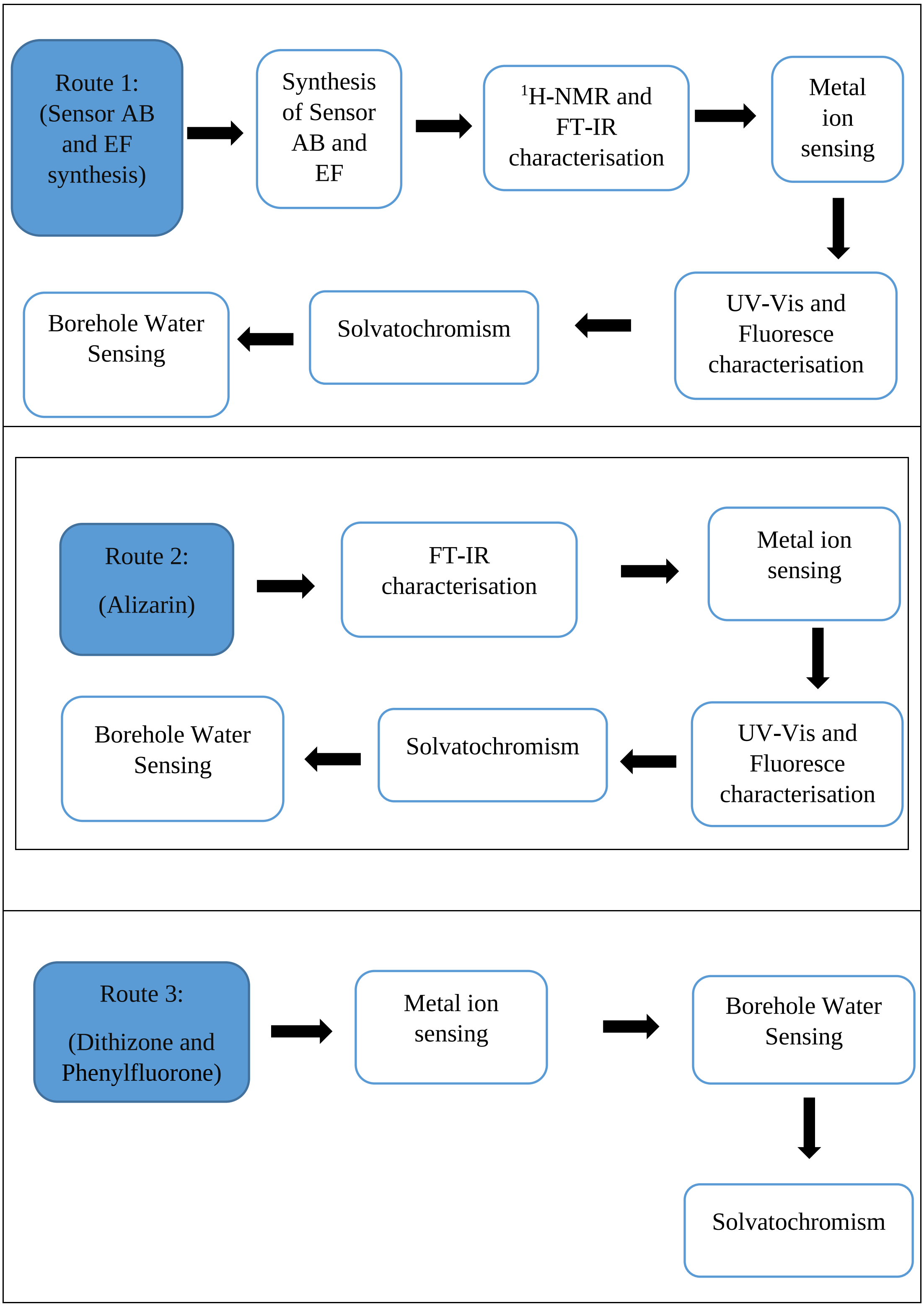
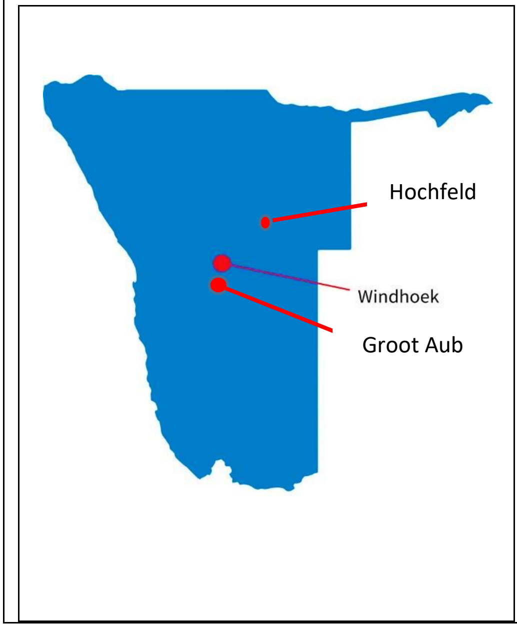


Figure 26: A flow diagram representing the research design

3.2 Sample collection



Site	*Borehole Water Sample*
1	Sample 1
2	Sample 2
3	Sample 3
4	Sample 4
5	Sample 5
6	Sample 9

Figure 27: Borehole water sampling site [Namibian Map, 175].

3.3 Research Instruments and Reagents

All reagents and solvents were obtained from commercial suppliers and they were of analytical grade. All reagents were used without further purification. Milli-Q (18.2 MΩ) water was employed as the preparatory medium. UV-vis spectroscopy was performed with the PerkinElmer Lambda 35 spectrophotometer in a standard 3.0 ml quartz cuvette with 1 cm path length. UV-fluorescence spectroscopy was performed with SpectraMax M2 spectrometer in a standard 3.0 ml quartz cuvette with 1 cm path length. FT-IR spectra were measured on a PerkinElmer Spectrum 100 FTIR spectrometer. ^{1}H NMR spectra were recorded using a Varian Mercury VX-300 MHz spectrometer in d6-DMSO solvent. The pH values were measured on a Thermo Scientific™ Eutech CON 450 Handheld pH Meter model. All the measurements were carried out at ambient temperature (25 °C).

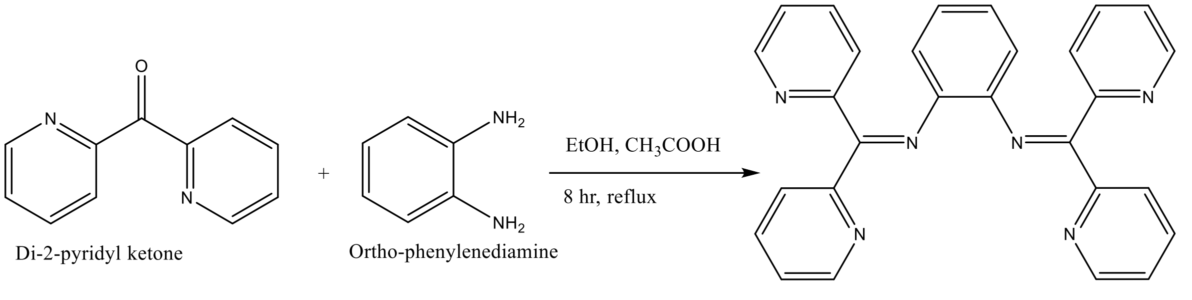
3.4 Methods and Procedures

Commercially available Alizarin, Dithizone and Phenylfluoron were applied as fluorogenic probes without further purification. For synthesis of sensor AB and EF (also referred to as fluorogenic probes), starting material organic molecules were selected based on features such as: aromatic rings and presence of soft donor atoms (O, N, and S). Solvent system was selected based on solvent selection guidelines outlined in Byrne *et al*. [176]. Syntheses of sensor AB and EF was based on Schiff's base, which is the condensation of aldehyde/ketone and primary amine derivatives in ethanol – at varying temperatures (room temperature and reflux at 60 ˚C) as illustrated, using the approach of green chemistry via a simple, low cost and one-step-reaction of Schiff base condensation reaction [109]. The procedure for sensor AB and EF synthesis was adopted from a report by Roy *et al*. [177].

In order to establish whether there is any chemical interaction between chromogenic probes, all respective anions and cations, a series of prepared ionic solutions (1×10^{-3} M) in selected solvent systems (a) EtOH, (b) DMF-Water (1:9), (c) Acetonitrile and (d) DMSO were added dropwise, each separately, to chromogenic probe (1×10^{-5} M) in respective solvent systems, at room temperature. The following ions were investigated for any interaction with chromogenic probes: Cu^{2+}, Sn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Co^{2+}, Cr^{3+}, Bi^{3+}, Al^{3+}, Cd^{2+}, Mn^{2+} and Ag^{2+}, Ca^{+}, Cs^{+}, Fe^{2+}, Fe^{3+}, CN^{-}, F^{-}, OH^{-} and acetate (AcO^{-}). Commercially available and literature fluorescent probes, with core structures based on traditional fluorophores (alizarin, dithizone and phenylfluorone) were employed as chromogenic probes.

3.4.1 The synthesis of sensors

3.4.1.1 The synthesis of sensor AB



A

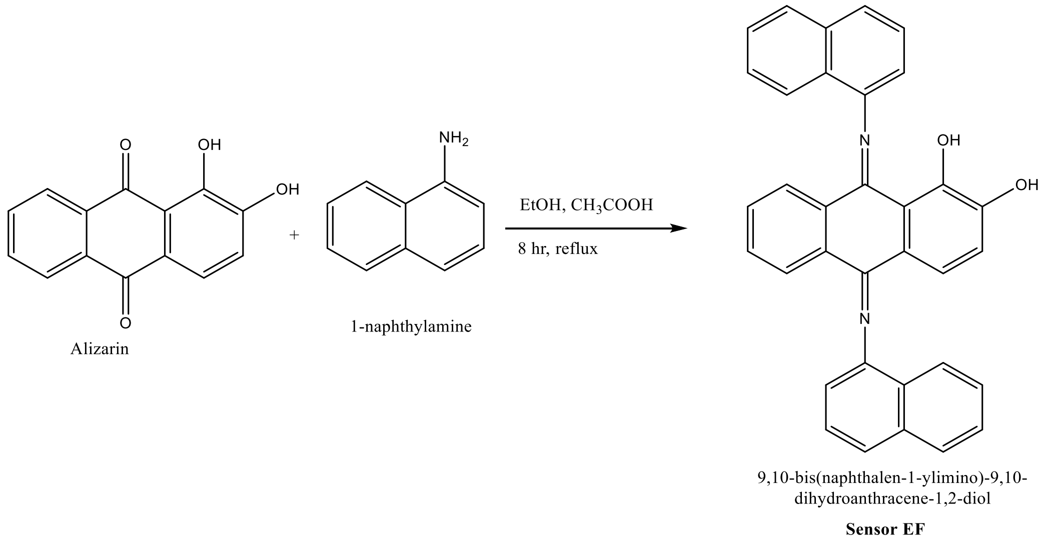
B

Sensor AB

Scheme 3: Schiff base synthesis of sensor AB

Di (2-Pyridyl) ketone (1.20 g, 6.515 mmol, 2 equivalence) was dissolved in 50 mL of EtOH. Ortho-Phenylenediamine (0.352 g, 3.257 mmol) was dissolved in 50 mL of EtOH. Both starting compounds were added together in 250 mL RBM and were magnetically stirred, the reaction was charged with 10 mL EtOH solution dropwise. The resulting solution mixture was catalysed with 3 ml of acetic acid and refluxed at 95°C for four hours under nitrogen gas for inert condition. The product solution was crystallised by chilling at 0 to -5° C and extracted through filtration on a pad of silica gel by washing three times with warm ethanol and two times with water. Yielding a brown crystalline product (AB). Yield: 48%. The synthesised compound was elucidated using ^{1}H NMR.

3.4.1.2 The synthesis of sensor EF



Scheme 4: Schiff base synthesis of sensor EF.

Alizarin (E) (1.00 g, 4.163 mmol) was dissolved in 50 mL of EtOH. 1-naphthylamine (F) (1.20 g, 8.326 mmol, 2 equivalence) was dissolved in 50 mL of EtOH. Both mixtures were added in 250 mL RBM and were magnetically stirred, the reaction was charged with 10 mL EtOH solution dropwise. The resulting mixture was catalysed with 3 ml of acetic acid and refluxed at 95°C for four hours under nitrogen gas for inert condition. The product solution was crystallised by chilling at 0 to -5° C and extracted through filtration on a pad of silica gel by washing three times with warm ethanol and two times with water. Yielding a deep orange powder (EF). Yield: 94.2%. The synthesised compound was elucidated using ^{1}H NMR.

3.4.2 Borehole water sample collection

Borehole water was sampled at six boreholes located in Khomas region and Otjozondjupa region, namely Groot Aub, Brakwater and Farm Hochfeld. Water was collected in pre-cleaned, dry 500 ml plastic containers. To ascertain that the samples were a true representative of the borehole water, the sample containers were rinsed with respective borehole water, twice. Sampling was done by opening the tap at each sampling site, draining out the water for 30 seconds. Water samples were collected between July and September 2021. The pH of the water was determined; however, other physico-chemical parameters of the borehole water were not determined.

3.4.3 Bottled water sample collection

Six branded bottles of still water were purchased at a local supermarket without employing any particular selection method.

3.4.4 Determination of water pH

The electrometric method has been used to measure pH values in water samples. Buffer solutions of pH 4.0, 7.0 and 10.0 were employed to calibrate the instrument. A volume of about 50 ml was taken from each sample and poured into a clean glass beaker (100 ml) and the electrode inserted into it (after being calibrated and rinsed with distilled water), the pH was directly read from the pH meter instrument and recorded as per instrument manual.

3.4.5 Characterisation of the sensors and their interaction with analyte

3.4.5.1 UV-vis spectrophotometry

A double beam PerkinElmer UV-Vis spectrophotometer Lambda 35 model was used to study the photocatalytic properties of the probes: AB, EF and Alizarin. The absorption spectra of the sensors were recorded using a 1 cm quartz cuvette at room temperature, over a wavelength range of 200–800 nm unless otherwise stated. The UV-Vis absorption spectra of AB, EF and alizarin were obtained by preparing a solution of (1.0 x 10-5 M) in 100 mL acetonitrile, DMF-Water (1:9) and DMSO. The sensor was titrated with 0.03 M analyte (cation or anion). Background correction was done for all the spectra by taking the blank solvent absorbance for each measurement.

3.4.5.2 UV- Fluorescence

UV-Fluorescence spectroscopy was used to investigate the properties of the synthesised sensors in terms of emissivity by using the SoftMax Pro 6.4 software. Steady-state fluorescence emission measurements of sensor EF and AB was obtained by preparing a solution of (1.0 x 10-5 M) in 100 mL acetonitrile, DMF-Water (1:9) and DMSO at different excitation wavelengths ranging from 260 nm to 480 nm at an interval of 5 nm. The sample was analysed by transferring 3.0 ml of the sensor solution into a fluorescence absorbance quartz cuvette with 1 cm path length and by scanning through a fixed monochromatic beam of light at different excitations within the emission wavelength range of 400-800 nm. The sensor was titrated with 0.03 M analyte (cation or anion). All spectra were recorded at room temperature and the blank fluorescence for the background correction was taken for each measurement beforehand.

3.4.5.3 Proton nuclear magnetic resonance spectroscopy

The Nuclear magnetic resonance spectroscopy (NMR) phenomenon is a technique which exploits the magnetic properties of nuclei of atoms. In principle, NMR is applicable to any nucleus possessing spin. It is based on the same principle as magnetic resonance imaging (MRI). The ^{1}H-NMR and ^{13}C-NMR spectroscopy is widely applied in organic chemistry. When placed in a magnetic field, NMR active nuclei (such as ^{1}H or ^{13}C) absorb at a frequency characteristic of the isotope. The resonant frequency, energy of the absorption and the intensity of the signal are directly proportional to the strength of the magnetic field. The shift is converted into a field-independent dimensionless value known as the chemical shift which in turn is a relative measure of the reference resonance frequency. For the nuclei ^{1}H, ^{13}C TMS (tetramethylsilane) is commonly used as a reference [178-180].

The synthesised Schiff base sensors AB and EF, were characterised using ^{1}H-NMR spectrometer installed with Bruker software package at Cardiff University, Cardiff, Wales. All samples were prepared in deuterated chloroform ($CDCl_3$) and the respective spectra were obtained at a frequency of 400 MHz. ^{1}H-NMR chemical shifts (δ) are reported in ppm and recorded using the residual solvent peak or external reference. Data was processed using MestReNova.

3.4.5.4 Fourier Transform Infrared spectrometry

FT-IR spectrometer was used to characterise the peaks of different functional groups in the sensors to evaluate the purity of the sensor. The FT-IR analysis was done using potassium bromide disks. FT-IR spectrum measures the infrared intensity versus wavelength (wavenumber) of light absorption; the wavenumber is directly related to the energy and frequency. The absorption corresponds to vibrational frequencies between the bonds of the atoms making up the synthesised sensors and analytical reagents with the frequency ranges measured as wavenumbers, typically over the range of 700 to 400 000 nm and wave numbers from 14 000 to 25 cm^{-1}. Based on the wavenumber, the infrared portion of the electromagnetic spectrum corresponds to the wavenumbers between 14,000 and 10 cm^{-1} (or to a wavelength between 0.8 and 1000 nm), and can be categorized into three portions: near infrared (14000–4000 cm^{-1}), mid infrared (4000–400 cm^{-1}) and far infrared (400–40 cm^{-1}), named for their relation to the visible spectrum [118, 120, 181-182].

3.5 UV-vis evaluation of sensor AB and EF

The binding capacity of sensor AB and EF toward various metal ions was measured by UV-vis absorption studies. When continuous radiation is passed through a quartz cuvette, a portion of the radiation may be absorbed yielding in an absorption band. The absorption bands show information obtained at different wavelengths resulting in appearance and disappearance of bands due to analyte binding. Quenching or enhancement bands are observed based on the affinity of a particular analyte. Electromagnetic spectrum of the UV and visible regions have wavelengths (λ) ranging from 190 nm to 800 nm. Transitions that result in the absorption of electromagnetic radiation in this region is from the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbitals (LUMO) [112-113]. The Beer Lambert's Law was used to describe the absorption of the sensor and its interaction with the analyte. The principles of absorption follow the Beer–Lambert law which states that, under ideal conditions, there is a linear relationship between absorbance and concentration (*c*) and between absorbance and the length of the light path (*b*), which is equal to the width of the cuvette. The absorptivity, ε, is a constant for each molecule for each wavelength.

$$A = \varepsilon \cdot b \cdot c$$

A (λ) is the intensity of light at a specified wavelength, λ (nm); ε (λ) is the molar extinction coefficient (mol^{-1}. L cm^{-1}) of the sensor, it depends on both the nature of the sensor and the wavelength; c (mol L^{-1}) is the concentration of the absorbing molecular sensor species and l (cm) is the path length of light through the absorbing species (this depends on the width of the sample holder, usually the cuvette being used for analysis) [90]. The principles of absorption follow Beer-Lambert Law. However, this law does not hold when fluorescent compounds or compounds changed by irradiation are present. Herein, characteristics of absorption bands are studied based on the solvent system. Moreover, the absorption spectrum of a sensor is obtained, and can be compared to detectable changes observed in metal complex (sensor-metal ion) spectra for accurate elucidation of results. Sensor complexation with the metal ions and anions is studied by the fluorescence method where the chemosensor intensity is quenched in the presence of increasing concentration of metal ion.

4. RESULTS AND DISCUSSION

The initial aim of this study was to investigate alizarin and its Schiff base derivative (EF) and their metal ion and anion complexes as well as analytical applications for heavy metal detection in borehole water. However, during these experiments and through literature review, it was discovered that dithizone and phenylfluorone molecules also feature other chromogenic phenomena which became of interest. This study consequently reports on synthesis, solvatochromism and colorimetric observation of EF, AB, alizarin, dithizone and phenylfluorone in water samples.

The water samples investigated in this research were obtained from different sources. The pH of the water was determined, this is the potentiality of concentration of hydrogen ions in water. Although pH alone is not the primary determinant of adverse effects of water, pH is one of the most important operational water quality parameters and for this reason careful attention to pH control is necessary. In water treatment plants, the pH of water entering the distribution system is controlled to minimise the corrosion of water mains and pipes in household water systems and to prevent aesthetic problems, such as an alkali taste to the water. pH usually has no direct impact on consumers. However, the toxicity of metals in water depends on the presence of anions cations and their degree of solubility in water. There is no health standard for pH; however, controlling organisations such as EPA and WHO recommends that public water systems maintain pH levels of between 6.5 and 8.5, this is a good guide for borehole and well owners as municipal water is usually regulated before entering the distribution systems [101-108].

4.1 The evaluation of Sensor AB

4.1.1 Characterisation of Sensor AB by ^{1}H-NMR

The synthesis of sensor AB followed a Schiff base route as shown in Scheme 3, where two molecules of the primary amine, ortho-phenylenediamine reacted with the carbonyl group (ketone) of Di-2-pyridyl ketone via a nucleophilic attack followed by proton transfer, removal of water and finally deprotonation to form an imine derivative, known as a Schiff base (sensor AB) consisting of the azomethine (C=N function). The yield was much lower than expected.

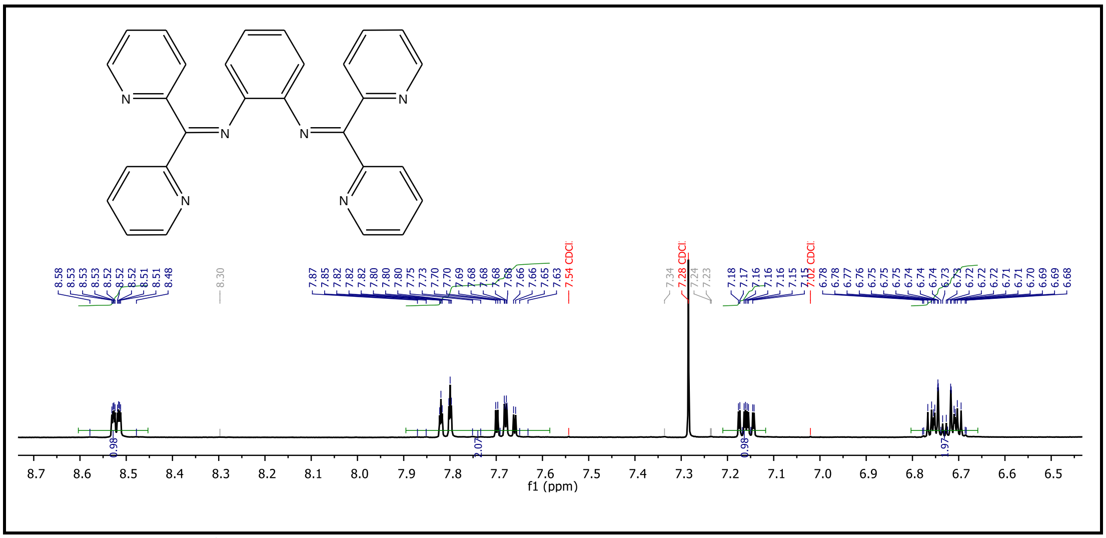
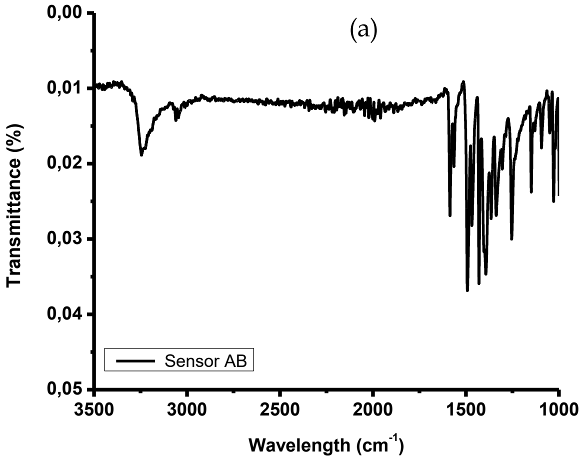


Figure 28: The ^{1}H-NMR structure for sensor AB

The ^{1}H-NMR structure of AB in Figure 28 above shows the presence of multiplet which extends from δ 6.68 to 6.78 ppm due to the aromatic ring protons of the two molecules of di-2-pyridyl ketone.

4.1.2 Characterisation of Sensor AB by FT-IR



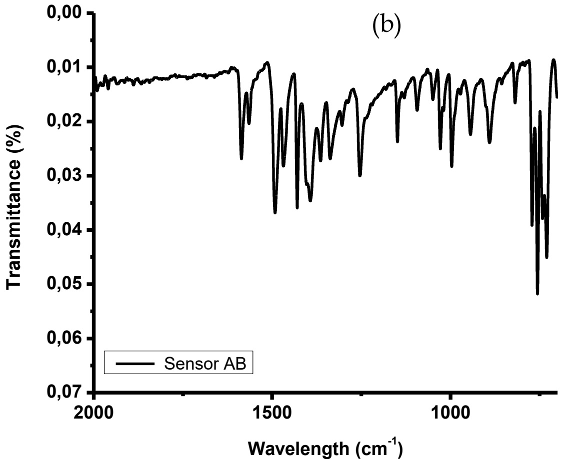


Figure 29: Experimental FT-IR spectrum of sensor AB; (a) Full range spectra and (b) IR spectra fingerprint region (2000-700 cm^{-1}) of sensor AB.

The IR spectrum of AB in Figure 29 is characterised by C—H, C═C, C═N and C—N vibrational bands. The presence of multiple weak bands appearing in the 3247-3047 cm^{-1} region of the spectrum is assigned to the C—H vibrational stretching of the aromatic rings. The intense bands in the fingerprint region, 1586-817 cm^{-1}, are due to aromatic C═C stretching observed and C═N and C═C stretching absorptions are also found at 1489 to 1390 cm^{-1} for aromatic amines. The vibrational stretching band of moderate intensity at 1494 cm^{-1} is the C═N band.

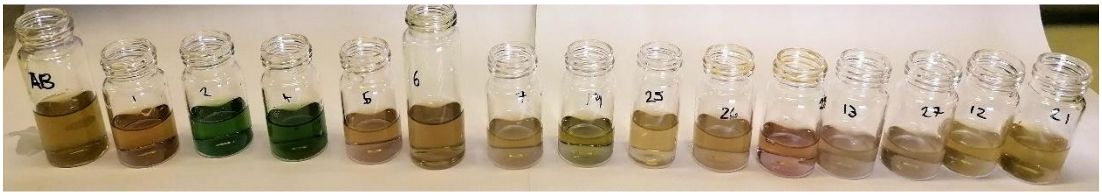
4.1.3 Sensitivity and selectivity of Sensor AB as a metal ion sensor

Following the synthesis and characterisation of sensor AB, was the evaluation of its sensing ability. Sensor AB was interacted with various metal ions the behaviour upon complexation with metal cations and anions was investigated through naked eye visual colour observation in three solvent system media, namely; acetonitrile, DMSO and DMF: Water (1:9). The results obtained are indicated in figure 30.

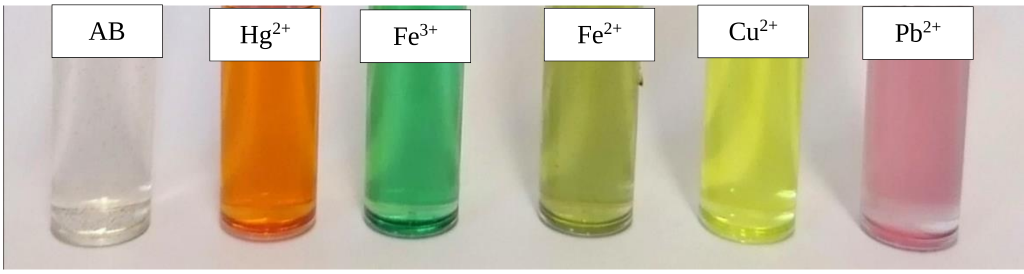
Table 1: The pH measurements of the water samples

No.	**Water Sample**	pH on water packaging label	Measured pH
1	Sample 1	-	7.77
2	Sample 2	-	7.85
3	Sample 3	-	7.85
4	Sample 4	-	7.56
5	Sample 5	-	7.86
6	Sample 6	-	8.20
7	Sample 7	-	7.71
8	Sample 8	-	7.60
9	Sample 9	-	7.71
10	Sample 10	8.90	7.38
11	Sample 11	6.5	7.86
12	Sample 12	7.20	7.50
13	Sample 13	-	7.85
14	Sample 14	7 - 8	7.09
15	Sample 15		6.85

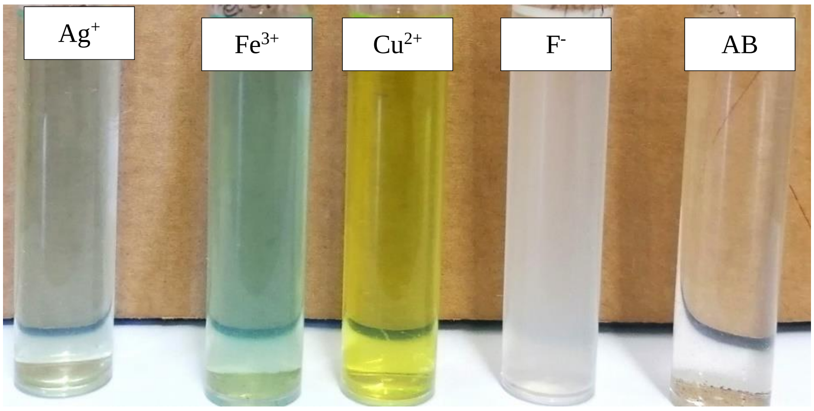
The pH values of all bottled water brands ranged from 6.85 to 7.86 and the pH values of borehole water ranged from 7.56 to 7.86 including the tap water at 7.71 and 7.85 as shown in Table. 4.1. The pH values of all water samples are within the WHO recommended limits. Moreover, all the pH value measurements determined in the laboratory are marginally different to those indicated on labels of bottled water.



(a) Sensor AB in DMF: Water (1:9)

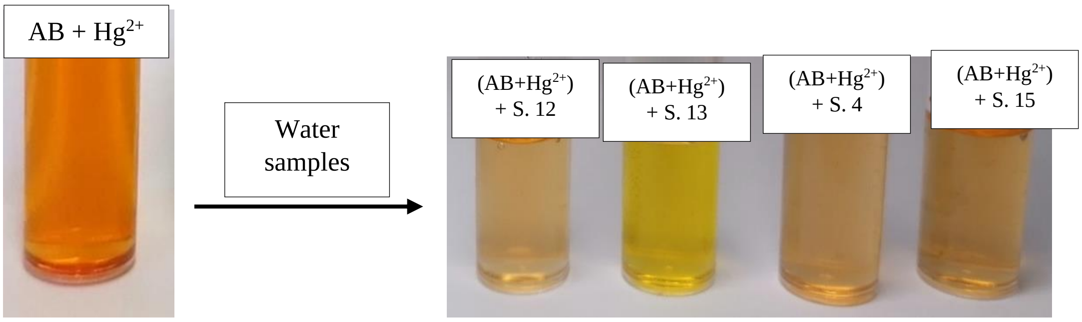


(b) Sensor AB in DMSO

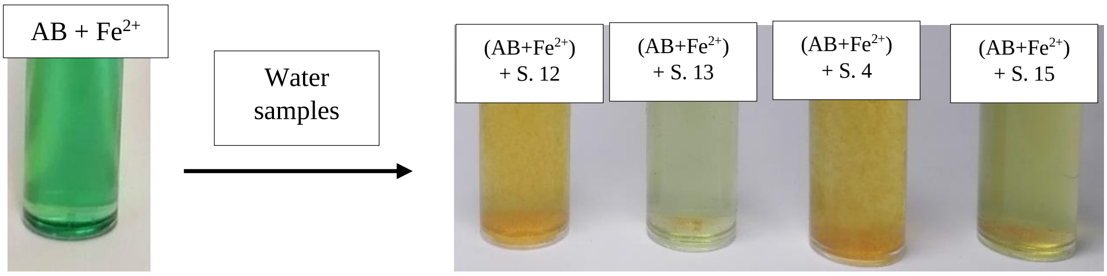


(c) Sensor AB in Acetonitrile

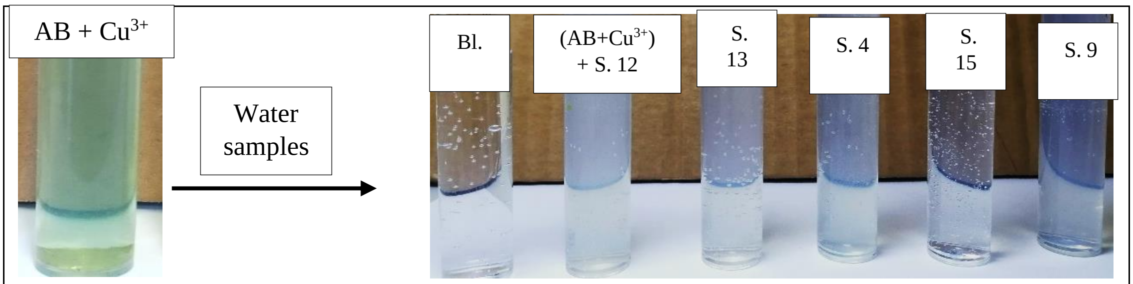
(d) Sensing water samples Sensor AB in DMF: Water (1:9)



(e) **T_1:** Reaction of water samples to sensor AB complexed mercury in DMSO. Key: **T_1** = Test One; **S.** = Water Sample. i.e., Water Sample 12; 13; 4 and 15



(f) **T_2:** Reaction of water samples with AB-iron (II) complexed in DMSO. Key: **T_2** = Test two; **S.** = Water Sample. i.e., Water Sample 12; 13; 4 and 15



(g) **T_3:** Reaction of water samples to sensor AB-cupper (III) complexed in CH_3CN. Key: **T_3** = Test 3; **S.** = Water Sample. i.e., Water Sample 12; 13; 4, 15and 9; Bl. = blank.

Figure 30: Visual colour changes of Sensor AB ion sensing in various solvent systems (a) DMF–H_2O (1:9) 1 x 10^{-5} M), (b) DMSO (c) Acetonitrile

Table 2: Colorimetric Test of Receptor AB in DMSO with anions and cations sensor AB.

No.	Cation/anion	Colorimetric Observation (DMF: Water)	Colorimetric Observation (DMSO)	Colorimetric Observation (Acetonitrile)
Bl	Blank	x	x	x
1	OH^-	x	x	x
2	Fe^{3+}	√	√	√
3	Hg^{2+}	x	√	x
4	Fe^{2+}	√	√	x
5	CN^-	√	x	x
6	Co^{2+}	x	x	x
7	Ni^{2+}	√	x	x
8	Co^{2+}	x	x	x
11	Cr^{3+}	x	x	x
12	F^-	x	x	√
13	Zn^{2+}	x	x	x
14	No_3^-	x	x	x
15	Cd^{2+}	x	x	x
16	Sn^{2+}	x	x	x
17	Ni^{2+}	x	x	x
18	Ag^+	√	x	√
19	Cu^{2+}	√	√	√
20	Pb^{2+}	x	√	x
21	Al^{3+}	x	x	x

Key: √ = *colour change*; x = *no colour change*.

The results summarised in Table 2 indicate a strong interaction of sensor AB in DMSO and 1:9 (DMF: water) solvent system compared to acetonitrile. Furthermore, the sensing of AB with water samples in DMF: water, 1:9 ratio did not yield any visible colour change as indicated in Figure 30 (d). Similarly, sensor AB in CH_3CN and DMSO did not yield colour change as well.

In order to understand the selectivity and sensitivity of sensor AB towards metal ions and anions in water samples was observed in Figure 30 (e) - (g) which interestingly shows displacement of metal ions initially complexed with sensor AB in the presence of water samples. In Figure 30 (e) AB-mercury complex DMSO solution changed colour from orange to yellow when five drops of (Water) sample 13 was added. Addition of sample 12, 4 and 15 did not yield any colour change. In addition, AB-iron (II) complexed in DMSO interaction with water samples 12 and 4 yielded colour change from green to orange, forming a precipitate. Samples 13 and 15 did not yield colour change. Similar investigations were carried out on sensor AB-cupper (III) complexed in CH_3CN which yielded in the dissociation of cupper (III) from sensor AB in the presence of water samples 12, 13, 4 and 9. Vivid colour change from blue to milky white was observed. Based on the colour change observation, it can be deduced that, upon addition of water to AB-cupper (III) complexed in CH_3CN, F^- ion are formed which have a higher affinity for AB in comparison to cupper (III).

All things considered, the presence of selective water samples in AB-ion complex destabilises and weakens the hydrogen bonding interaction, resulting into the dissociation of various metal ions from AB this creates an effective reversal system for water sensing.

4.1.4 UV-Vis characterisation of Sensor AB

Further evaluation of sensor AB interaction with metal ions was determined by performing UV-Visible titrations involving incremental addition of 1.0 x 10^{-5} M metal ion solution to a solution of sensor AB (0.03 M) as shown below.

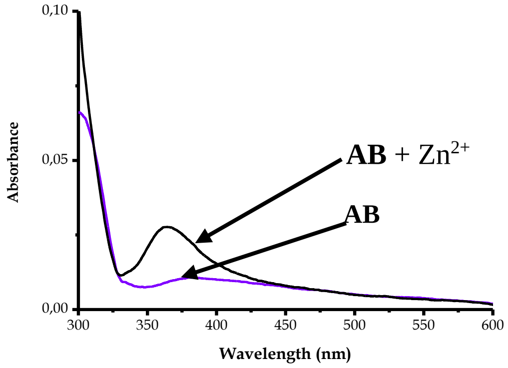


Figure 31: UV Spectra of sensor AB before and after titration with 2.4 equivalence of 0.03 M Zn^{2+} in acetonitrile. λ_{max} = 364 nm.

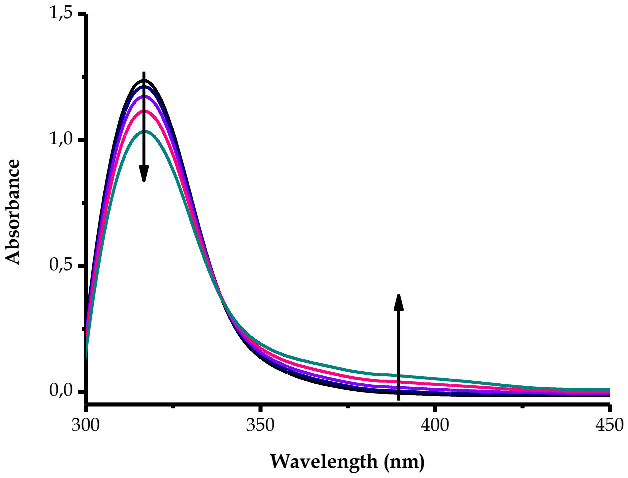


Figure 32: UV Spectra of sensor AB in the presence of increasing Ni^{2+} concentration in DMSO medium. λ_{max} = 316 nm and 389 nm.

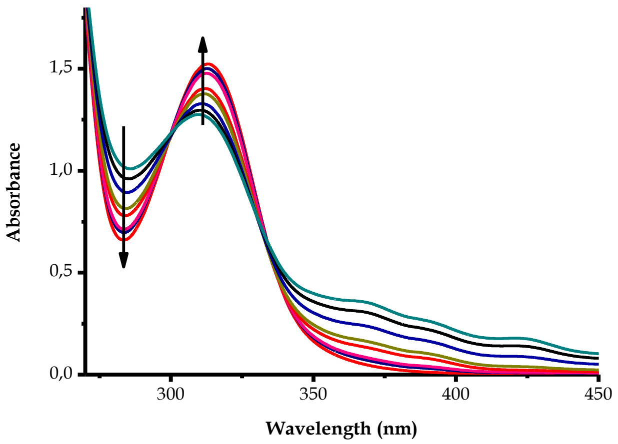


Figure 33: UV Spectra of sensor AB titration with varying concentration of Au^{3+} DMSO medium.

The interaction of AB with zinc (II) showed spectral changes in Figure 31, similar changes in UV-vis absorption behaviour is observed when sensor AB interacted with Ni^{2+} and gold (III) in Figure 32 and Figure 33 respectively. The solution of AB immediately turns orange on the addition of zinc (II). In Figure 33, titration with varying concentration of gold (III) shows progressive decrease in absorbance exhibited by a sharp band at 282 nm. But after some time, a progressive increase is observed at 313 nm with two isosbestic points of conversion at 300 nm and 334 nm. On the other hand, the interaction of AB with Ni^{2+} exhibited a main absorption band in the 300–340 nm range.

4.2 The evaluation of alizarin and its derivative (EF)

4.2.1 Characterisation of Sensor EF by ^{1}H-NMR

The synthesis of sensor EF followed a Schiff base route as shown in Scheme 4 where two molecules of the primary amine, 1-naphthylamine reacted with the carbonyl groups (ketone) of alizarin via a nucleophilic attack followed by proton transfer; removal of water and finally deprotonation to form an imine derivative, known as a Schiff base (sensor EF) having the C=N function.

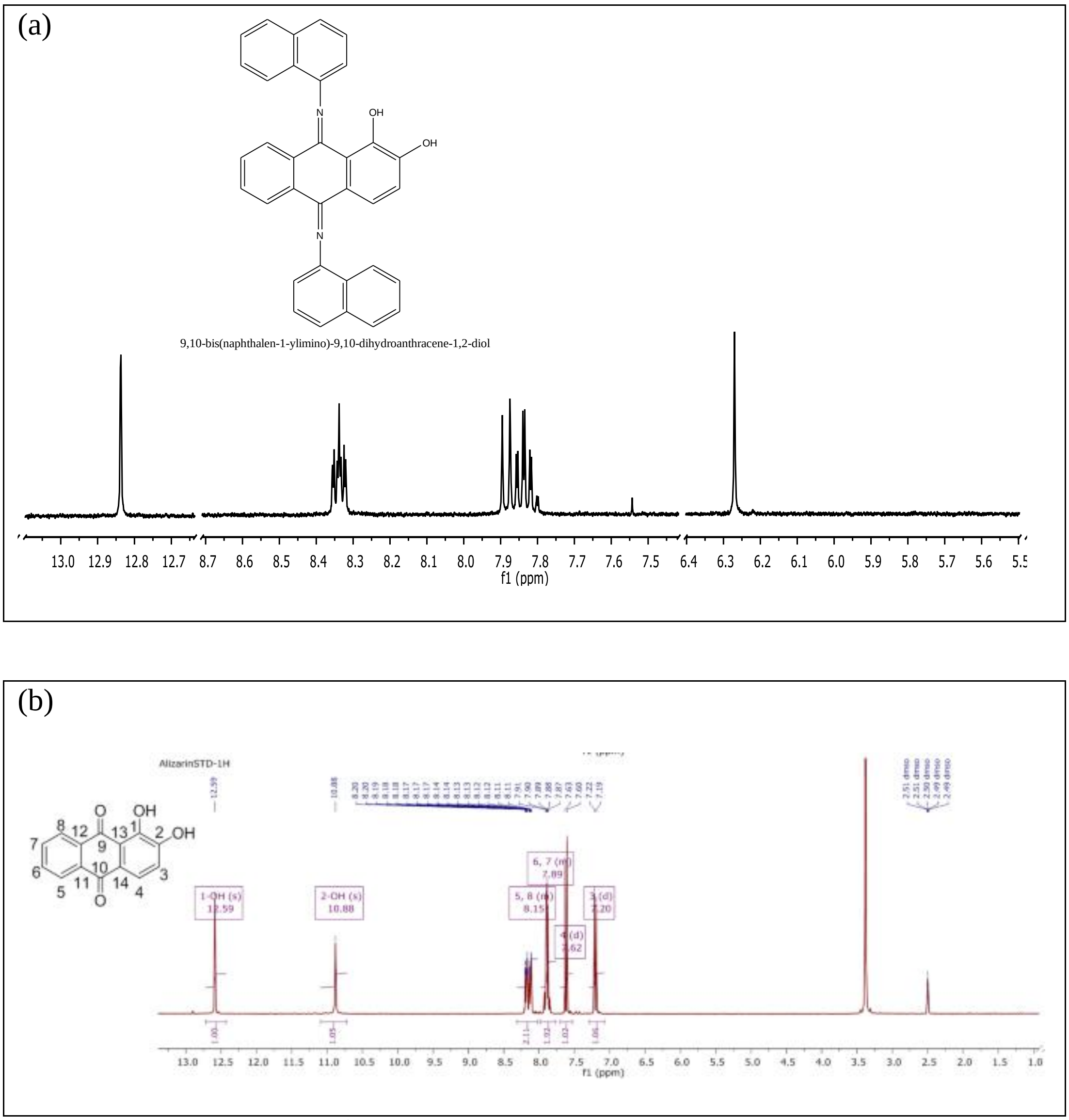
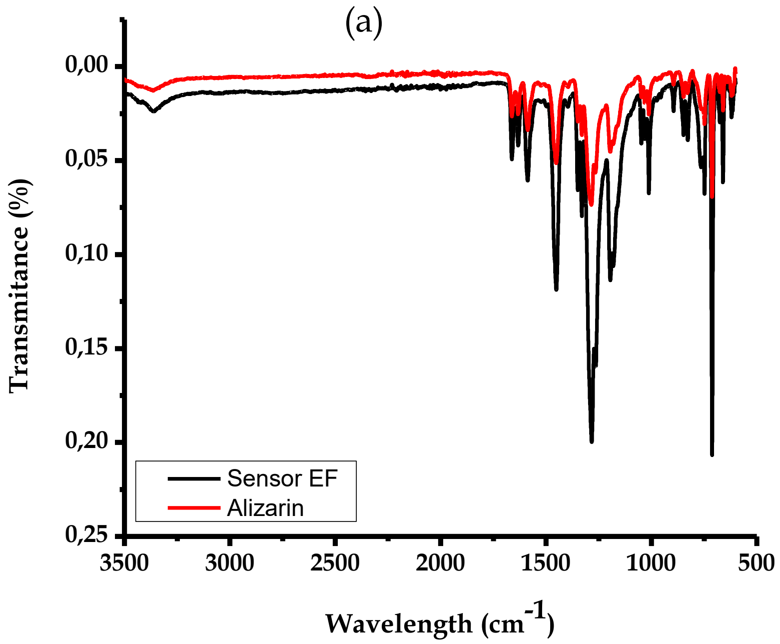


Figure 34: Comparison of ^{1}H-NMR analysis of alizarin derivative, sensor EF (a) and alizarin (b) reported in Nguyen *et al*. [91].

The ^{1}H NMR spectrum in Figure 34 (a) confirms the synthesis of the EF compound. Alizarin acts as a bidentate ligand on forming complexes with metal ions. However, the effect of complexation of alizarin with metal ions and anions has not been subjected to ^{1}H-NMR spectroscopy studies in this research. Likewise, the effect of anion and cation complexation of alizarin derivative (sensor EF) was not studied with ^{1}H-NMR technique. Consequently, the chemical shift difference between the free sensor and the metal ion complexed sensor was not determined. In Figure 34 the ^{1}H-NMR spectrum of sensor EF was compared to the ^{1}H-NMR spectrum of alizarin found in literature, there are similarities observed in the downfield chemical shift of the spectra.

4.2.2 Characterisation of Alizarin and Sensor EF by FT-IR



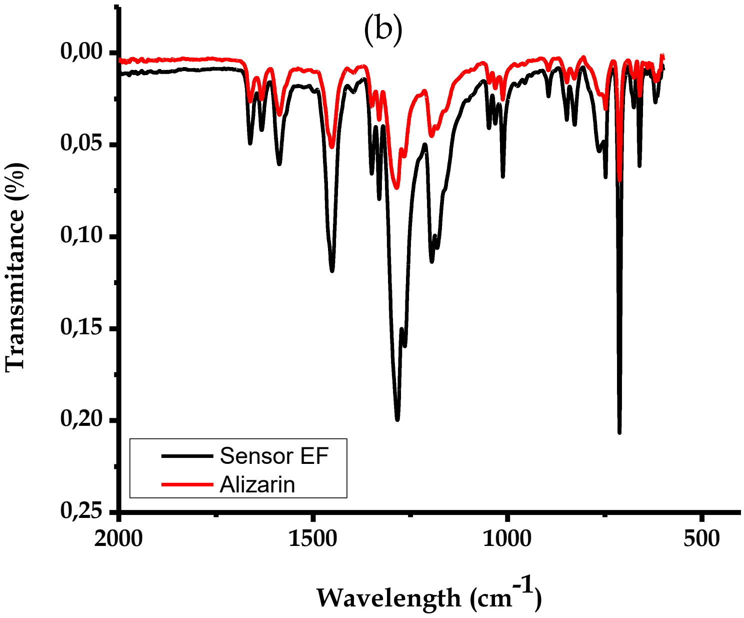


Figure 35: Experimental FT-IR spectrum of sensor EF and alizarin; (a) Full range spectra and (b) its fingerprint region (2000-400 cm^{-1}) of the IR spectra.

By contrast, UV-vis analysis and FT-IR analysis of alizarin and its derivative, sensor EF show close similarities. This indicates that for the modification of alizarin to form alizarin derivative, EF have little to no effect on the performance of alizarin. The FT-IR spectrum of alizarin and sensor EF in Figure 35 indicate the stretching vibrations O—H weak band at 3365 and 3374 cm^{-1} respectively. The bands at 1657 and 1582 cm^{-1} are assigned to ═C–H stretching vibrations, while the moderately strong bands at 1444 and 1175 cm^{-1} are assigned to C—C stretching vibrations. The strong bands at 1288 and 825 cm^{-1} are assigned to C═N stretching vibrations, while the bands between 1045 and 845 are assigned to C–H stretch. The bands at 752 cm^{-1}is assigned to C=C–C.

4.2.3 Sensitivity and Selectivity of Alizarin and Sensor EF as a metal ion sensor

Naked eye observation of chemical interaction of alizarin and sensor EF with anions and cations was carried out. A series of ionic solutions in selected solvent systems (a) EtOH, (b) DMF-Water (1:9), (c) Acetonitrile and (d) DMSO were added dropwise, each separately, to a vial containing 15 ml of chromogenic probe in respective solvent systems, at room temperature. The following ions were investigated for sensing sensitivity or interaction with chromogenic probes: Cu^{2+}, Sn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Co^{2+}, Cr^{3+}, Bi^{3+}, Al^{3+}, Cd^{2+}, Mn^{2+} and Ag^{2+}, Ca^{+}, Cs^{+}, Fe^{2+}, Fe^{3+}, CN^{-}, F^{-}, acetate (AcO^{-}), K^{-}, Cl^{-} and OH^{-}. The resulting observations were recorded as indicated in Figure 36 below.

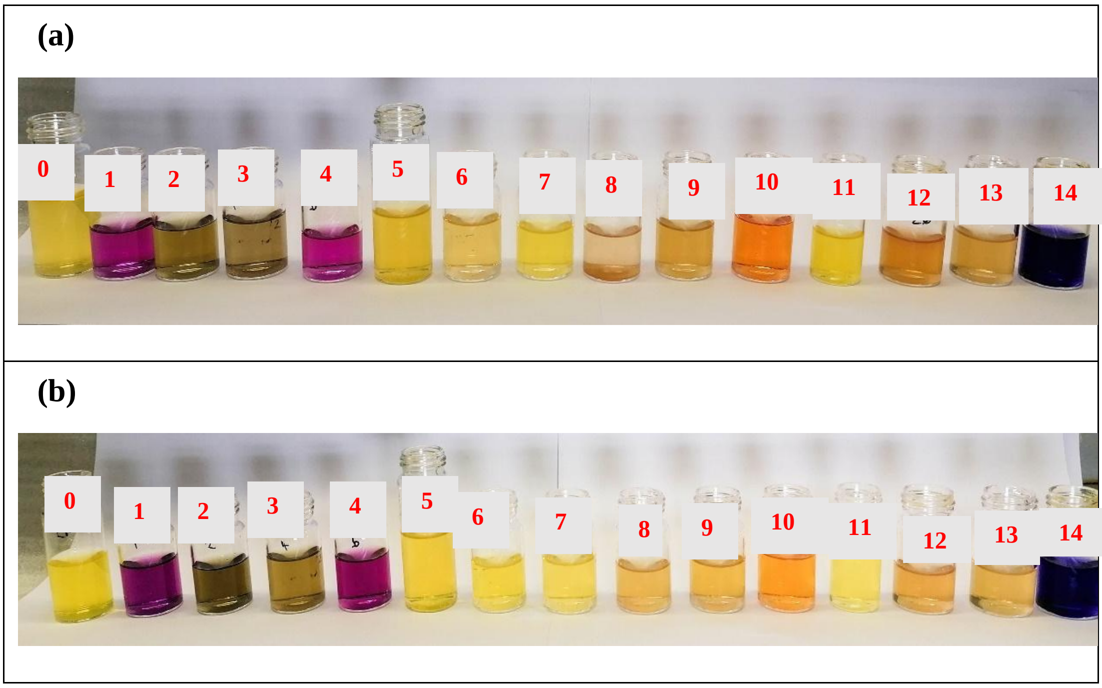


Figure 36: Visual colour changes of (a) alizarin, (b) sensor EF in DMF–H_2O (1:9) (1 x 10^{-5} M), upon addition of 3 equiv. of various cations and anions.

Alizarin and sensor EF dissolved in DMF–H_2O (1:9) and in EtOH yielded a bright yellow solution. Visible colour change observed in Figure 36 was due to interaction of alizarin and alizarin derivative (EF) with metal ions and anions. The reference sample,

Sample 0 in Figure 35 (a) and (b) is alizarin and sensor EF in the respective solvent medium without addition of cation or anion. The results are reported in Table 3 below. In addition to the results reported in Figure 36 above, interaction of EF and alizarin with the following metal ions was also investigated, however they were non responsive as there was no colour change observed: Sn^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Co^{2+}, Cr^{3+}, Cs^{+}, Bi^{3+}, Al^{3}, Mn^{2+} and Ag^{2}.

Table 3: Colour observation of various metal ions with sensor EF and alizarin in in DMF–H_2O (1:9).

No.	**Cation/anion added**	**Observation**
0	Blank	x
1	OH^- (NaOH)	√
2	Fe^{3+}	√
3	Fe^{2+}	√
4	CN^-	√
5	Co^{2+}	x
6	Ni^{2+}	x
7	F^-	x
8	Zn^{2+}	√
9	Cu^{2+}	√
10	Al^{3+}	√
11	Na^+	x
12	K^+	√
13	AcO^-	√
14	OH^- (KOH)	√

Key: √ = *colour change*; x = *no colour change*.

Based on the colorimetric results obtained, it is clear that there is no noteworthy difference between alizarin-ion interaction and EF-ion interaction. This is an indication that the modifications made to alizarin through the synthesis of sensor EF do not have an apparent effect on alizarin's interaction with the studied cations and anions in water: DMF medium and ethanol medium. This does not rule out the possibility of a different result if sensor EF and alizarin were subjected to different solvent systems. In section 1.16 the concept of solvatochromism was discussed. The ESIPT characteristics of alizarin makes it solvatochromic, hence it can be perceived that alizarin and its derivative, EF can display colour difference when dissolved in different solvent systems. Solvatochromism is not the focus of this study, however, this is an area worth researching as it complements the colorimetric chemosensing approach studied in this research.

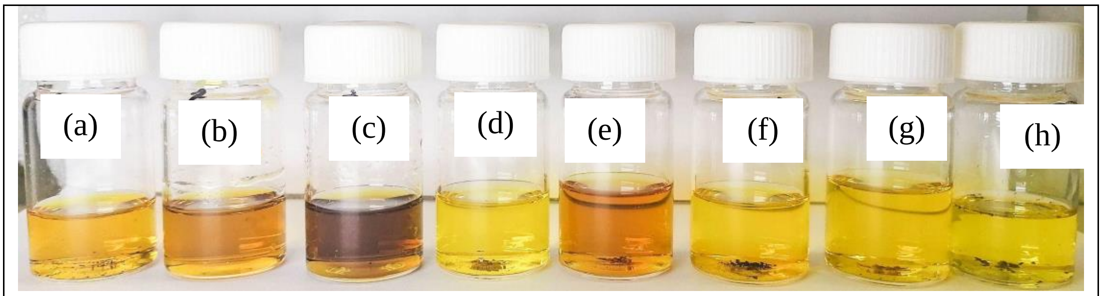
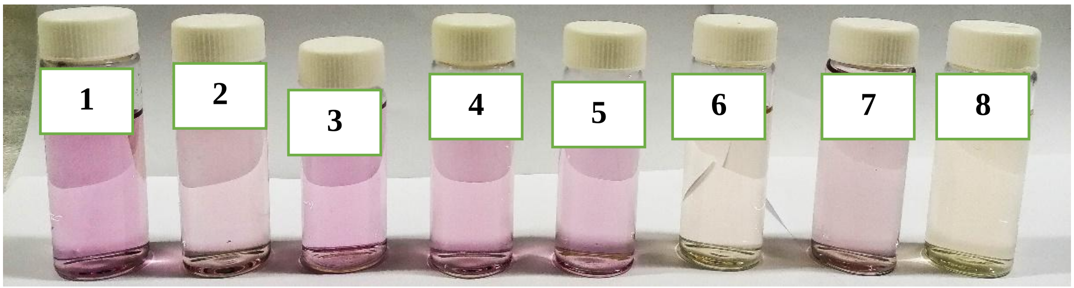


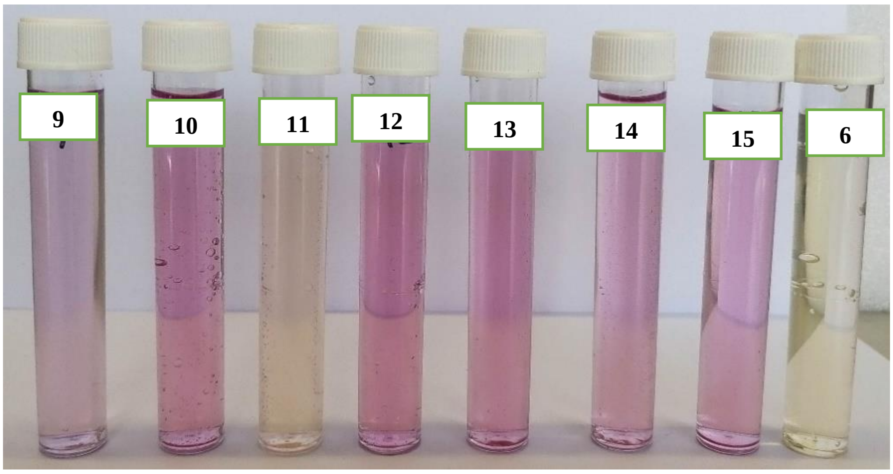
Figure 37: Alizarin solvatochromism in various solvent systems ((a) acetone; (b) DMSO; (c) DMF; (d) acetonitrile; (e) MeOH; (f) Et_2O; (g) EtOH; (h) chloroform.

Solvatochromism is a phenomenon whereby a compound changes colour when dissolved in solvents with different polarities, either by a change in the absorption or emission spectra of the molecule. In Figure 37 the solvatochromism of alizarin was observed through visual colour observation with colour change ranging from yellow to orange.

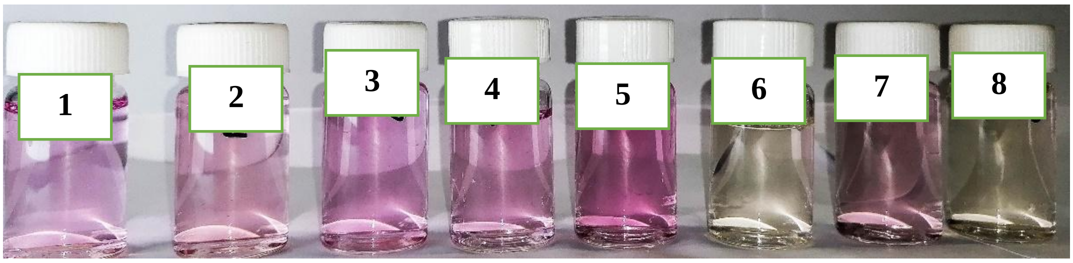
(a) Alizarin in DMF-Water (1:9) Sample 1- 8



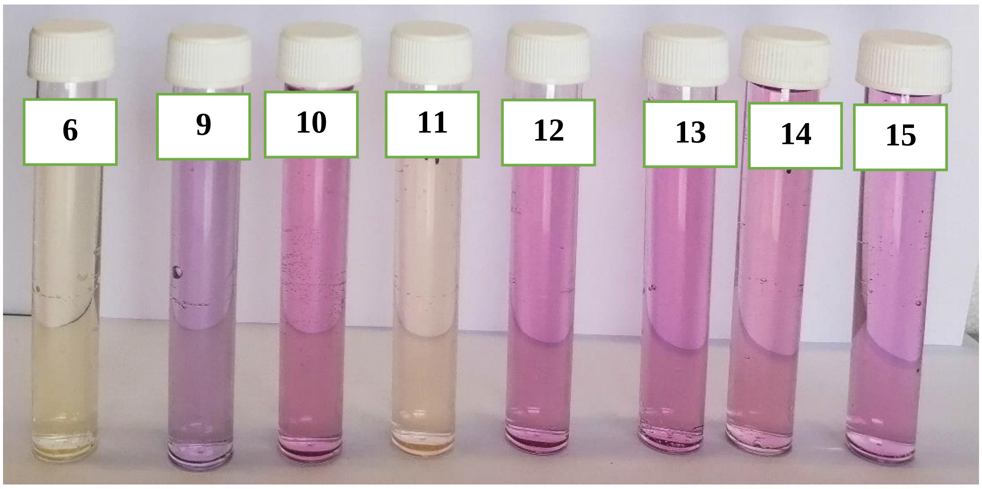
(b) Alizarin in DMF-Water (1:9) Samples 9- 15



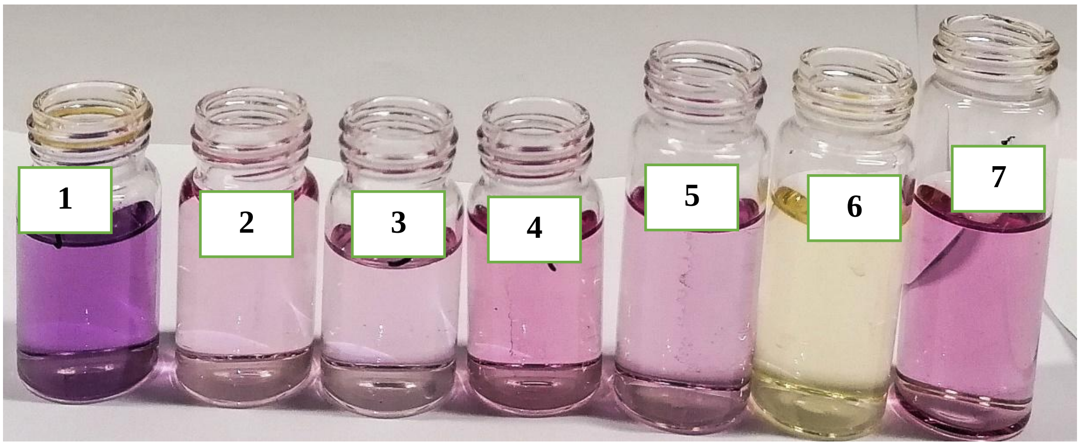
(c) EF in DMF-Water (1:9) Samples 1-8



(d) EF in DMF-Water (1:9) Samples 9-15



(e) Alizarin in EtOH



(f) Sensor EF in EtOH

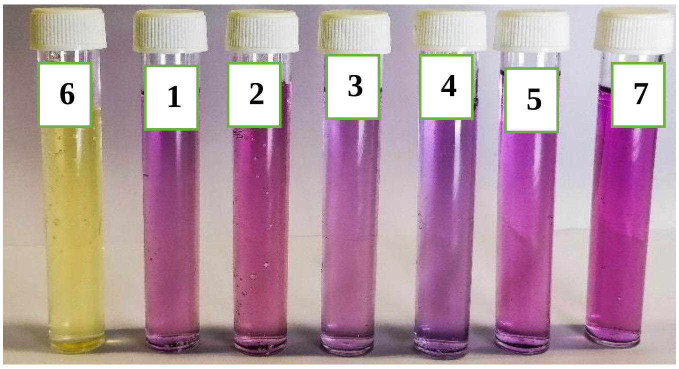


Figure 38: Colour change observation indicating cation and anion in water Samples with sensor EF and alizarin. Sample 6 (Milli-Q) water is a reference.

Table 4: Alizarin and EF colorimetric results for anion and cation detection in water samples, DMF-dH2O solvent system.

No.	**Water Sample**	**Observation**
1	Sample 1	√
2	Sample 2	√
3	Sample 3	√
4	Sample 4	√
5	Sample 5	x
6	Sample 6	x
7	Sample 7	x
8	Sample 8	√
9	Sample 9	√
10	Sample 10	√
11	Sample 11	√
12	Sample 12	√
13	Sample 13	√
14	Sample 14	√
15	Sample 15	√

Key: √ = *colour change*; x = *no colour change*.

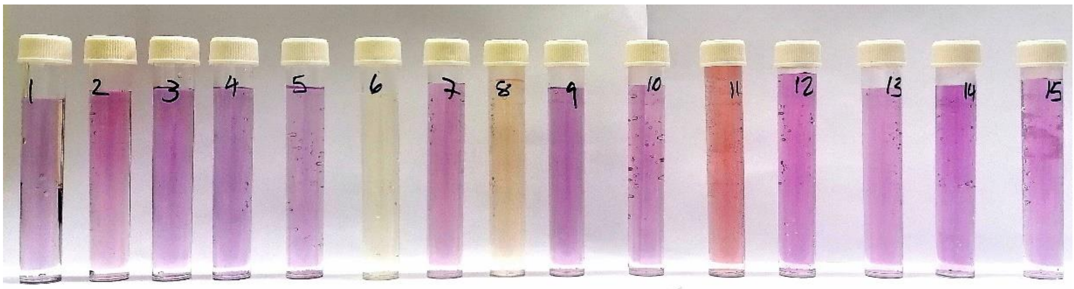


Figure 39: Alizarin (acetonitrile) sensing results for water samples.

Metal ion sensing in water samples yielded colour change ranging from pink to purple in tap water, bottled branded water and borehole water after dropwise addition of the molecular probes (EF and alizarin). There was no colour change observed in sample 8. Although colour change was observed within a few seconds of adding the sensor to the water samples, it is not apparent which metal ions present in the water is responsible for the colour change. The colour change from yellow to purple observed in Sample 1 can cautiously be attributed to the presence of CN^- or OH^-. This presumption is deduced from the results obtained in Figure 39. Moreover, there was no distinct difference observed between the two solvent systems; DMF-dH_2O and EtOH. However, the colour intensity appears to be more enhanced in EtOH solvent systems.

Cation and anion sensing with alizarin and sensor EF in some water samples resulted in an unexpected response of colour change from yellow to pink. A pink colour was not observed in the initial investigation (Figure 36(b)) of metal ion interaction with the molecular probes as indicated. Therefore, another metal ion which is beyond the scope of this study may have a higher affinity for alizarin resulting in the pink colour or the pink colour could be a result of the combination of ions. Naked eye observation was supported by UV-Vis and fluorescence spectroscopy characterisation. Sensing of water samples with alizarin in acetonitrile medium show a red colour change in sample 11. The vivid colour changes from yellow-to pink and purple were noticed for the probes in the presence of ions, whereas no such visual changes were observed for the ion free water (Milli-Q and distilled water). Hence, from this observation, sensor EF and alizarin can be used as a quick diagnostic test to determine if the water contains ions. The results obtained indicates good sensor sensitivity towards low quantities of cations and anions in aqueous medium. However, correct comparison with other analytical techniques is required by determining the limit of detection (LOD) of the sensor.

4.2.4 UV-Vis characterisation of Alizarin and Sensor EF

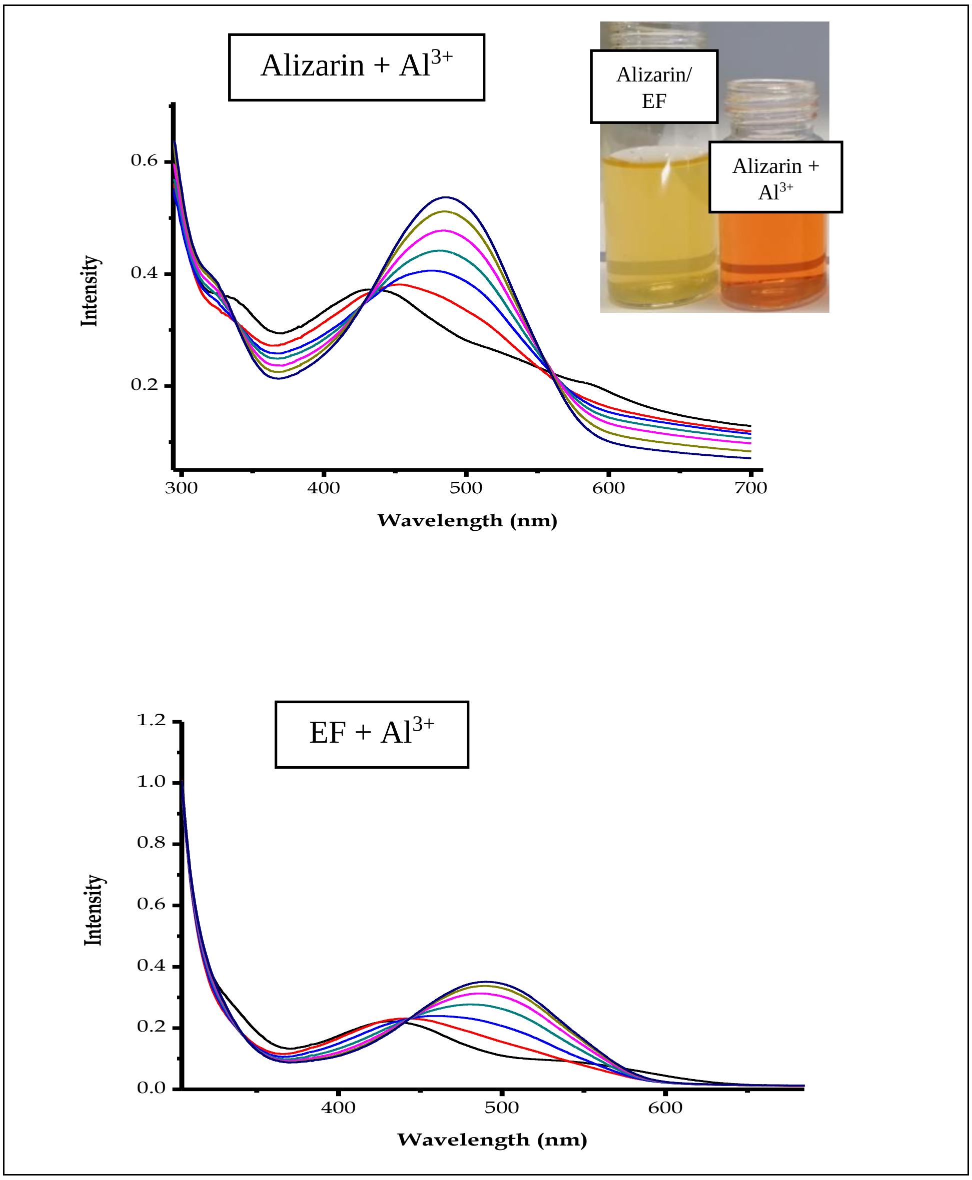


Figure 40: UV-vis absorption spectra of EF and alizarin in DMF–H_2O (1:9) 1 x 10^{-5} M upon titration with Al^{3+}.

The absorption spectra of EF and alizarin interaction with aluminium ion in DMF–H_2O was characterised by a prominent absorption band at λ_{max} = 490 nm. An orange-red colour change was observed when a colourless aluminium solution was added dropwise to the yellow alizarin solution. UV-vis absorption spectra of alizarin titrations titrated with aluminium ion in varying concentrations of shows progressive decrease in absorbance in the 300 nm region. Two isosbestic points of conversion at 450 nm and 560 nm was observed.

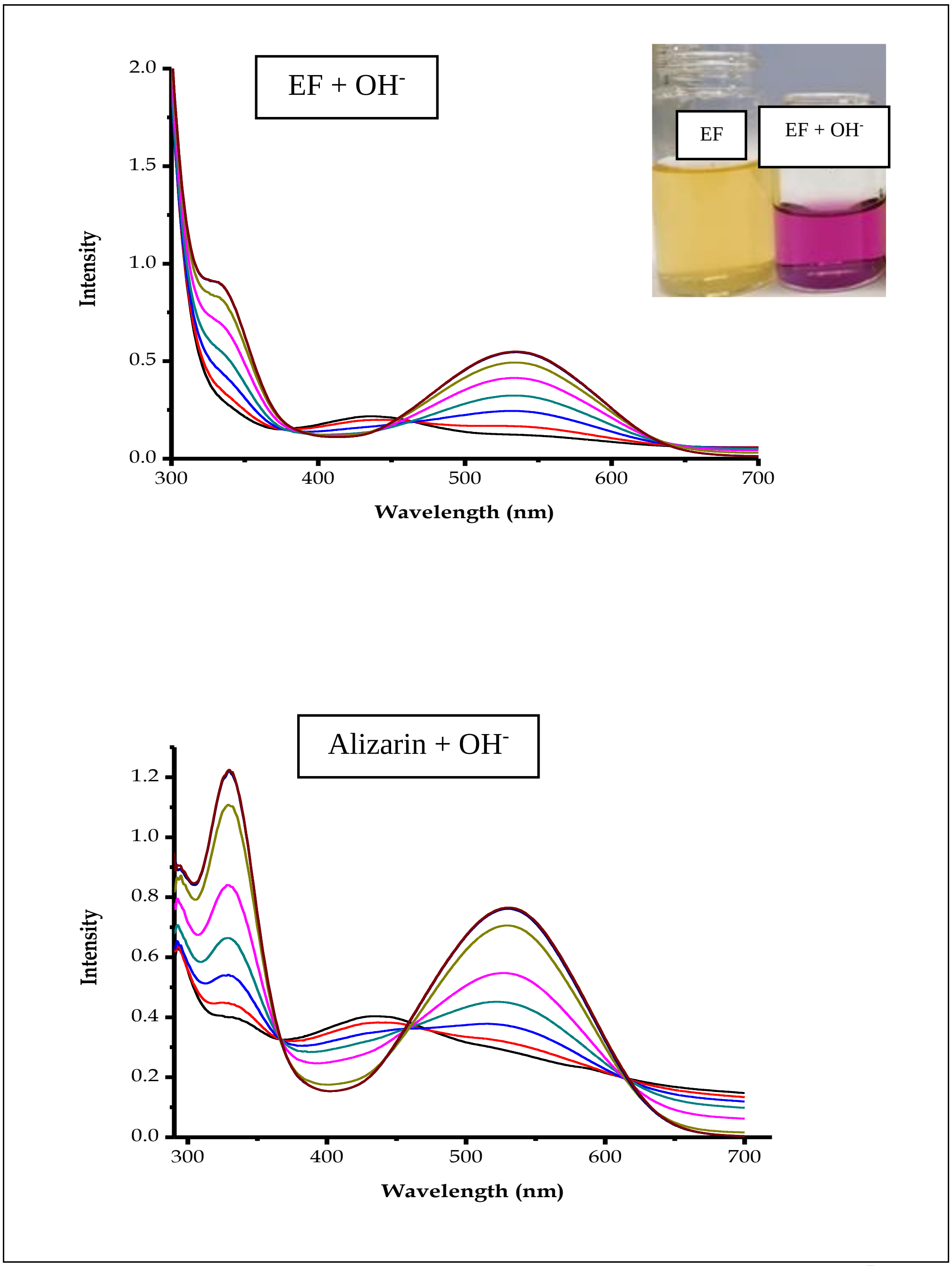


Figure 41: UV-vis absorption spectra of EF in DMF–H_2O (1:9) (1 x 10^{-5} M) upon titration with OH^-.

4.3 Analytical reagents for anion and cation sensing

4.3.1. Anion and cation sensing with dithizone

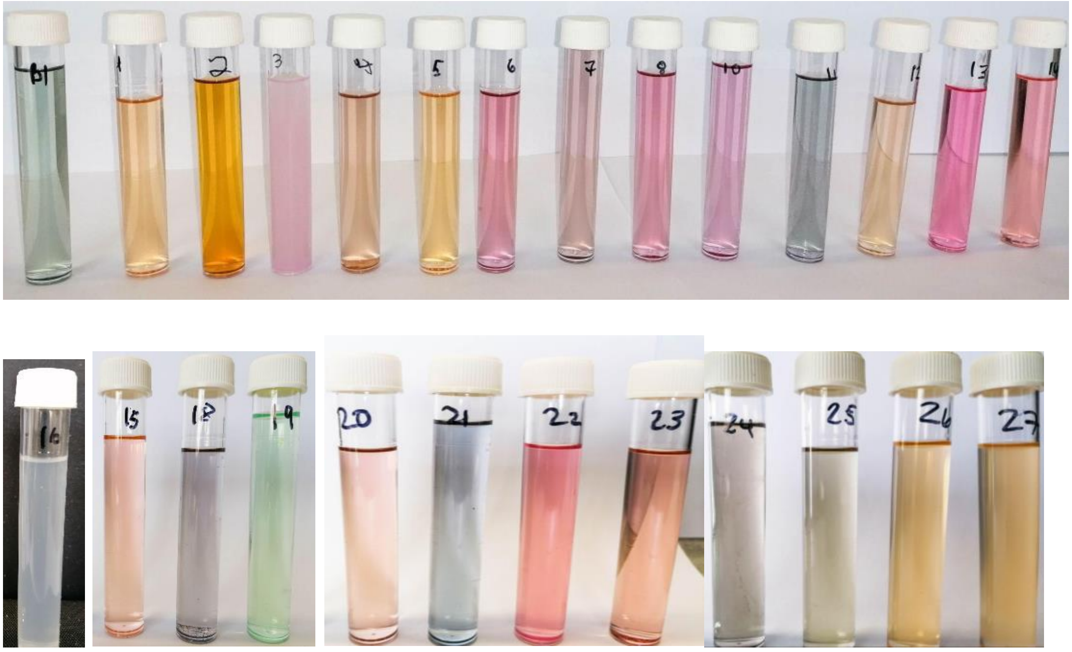


Figure 42: Visual colour changes of dithizone in EtOH (1 x 10^{-5} M), upon addition of 3 equivalence of various cations. *Bl* (blank) on the far left is used as a reference.

Visual colour changes observed indicate that dithizone is highly sensitive towards anions and cations. This is vindicated by colour change observed when dithizone is reacted with twenty different ions. When 1 x 10^{-5} M dithizone is dissolved in ethanol, a blue-green solution is formed. Upon addition of various cations and anions, visual colour change from blue-green to other colours was observed. Whilst all studied anions and cations are sensing, only Cr (III) did not depict naked eye colour change. Moreover, the solvatochromism properties of dithizone was observed through colour change observation.

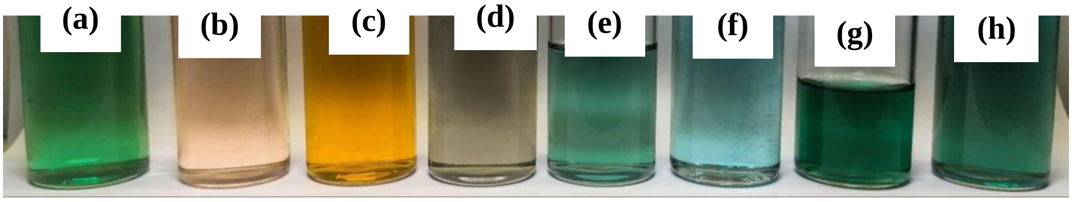


Figure 43: Dithizone solvatochromism in various solvent systems: (a) diethyl ether; (b) DMF; (c) MeOH; (d) DMSO; (e) acetonitrile; (f) chloroform; (g) acetone; (h) EtOH).

Solvatochromism of dithizone was investigated through visual colour observation. About 0.003g of dithizone was added to 15.0 mL of various solvents. Polar solvents, non-polar and polar aprotic solvents were used to study its effects on the colour of dithizone. Conditions like concentration and temperature were otherwise kept constant. In section 2.2.1 the discussion on dithizone indicate Density Functional Theory calculation studies conducted by Ntoi *et al*, who reported seven colour change (chromisms) associated with dithizone metal complexes, attributed to intramolecular proton transfer from the backbone of the carbon-nitrogen (C−N) bond. They further reported that the solvatochromic effect may be ascribed to different tautomers of dithizone.

Figure 44: Sensing of water samples with dithizone in EtOH.

Table 5: Sensing of water samples with dithizone in EtOH.

No.	Water Sample	Observation	Colour observation
1	Sample 1	√	Orange
2	Sample 2	√	Orange
3	Sample 3	√	Orange
4	Sample 4	√	Orange
5	Sample 5	√	Orange
6	Sample 6	x	N/A
7	Sample 7	√	Red
8	Sample 8	√	Orange
9	Sample 9	√	Orange
10	Sample 10	√	Orange
11	Sample 11	√	Orange
12	Sample 12	√	Orange
13	Sample 13	√	Red
14	Sample 14	√	Orange
15	Sample 15	√	Orange

Key: √ = *colour change*; x = *no colour change*.

4.3.2. Anion and cation sensing with phenylfluorone

There is relatively limited literature on phenylfluorone as a chromogenic probe. Ion sensing with various ions and yielded results as indicated in Figure 45 below.

Figure 45: Visual colour change of phenylfluorone in DMF: H_2O (1:9) with various cations and anions.

Table 6: Sensing various cations and anions with phenylfluorone in DMF: H_2O (1:9).

No.	Cation/anion	Colorimetric Observation (DMF: Water)	Colorimetric Observation (DMF: Water)
Bl	Blank – Milli-Q H_2O	x	No colour change
1	OH^- (NaOH)	√	Red
2	Fe^{3+}	√	Brown
3	Hg^{2+}	√	Red
4	Fe^{2+}	√	Brown
5	CN^-	√	Red
6	Co^{2+}	√	Light red
7	Ni^{2+}	x	No colour change
8	Co^{2+}	√	Red
9	N/A		
10	Ag^+	√	Light red
11	Cr^{3+}	√	Light red
12	F^-	x	No colour change
13	Zn^{2+}	√	Red
14	No_3^-	x	No colour change
15	Cd^{2+}	x	No colour change
16	Sn^{2+}	√	Red
17	Ni^{2+}	N/A	
18	Ag^+	x	No colour change
19	Cu^{2+}	√	Red
20	Pb^{2+}	√	Red
21	Al^{3+}	x	No colour change
22	Bi^{3+}	√	Red
23	Mn^{2+}	x	No colour change
24	Cs^+	x	No colour change
25	Na^+	x	No colour change
26	K^+	x	No colour change
27	$AcO^−$	√	Red
28	OH^-	√	Magenta

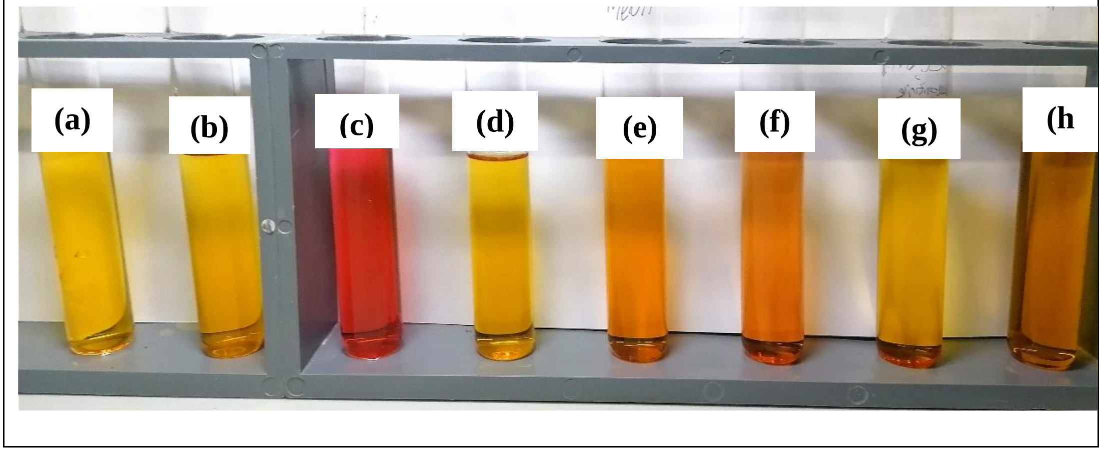


Figure 46: Phenylfluorone solvatochromism in various solvent systems: (a) DMSO (b) EtOH (c) DMF (d) ethyl acetate (e) MeOH (f) acetone (g) CH_3CN (h) chloroform.

The aim of this research was not to assess the quality of bottled water; however, it is evident that RO water (sample 8) behaved similar to the blank, Milli-Q water (sample 6) indicating the absence of ions in water sample 6. Based on visual colorimetric observations, sample 8 showed no colour change. Due to these similarities, it can be cautiously interpreted that sample 8 does not contain any minerals. Overall, results of the chromogenic probe sensing of water samples shows that anion and cation concentration in the different water samples differs, this is indicated by difference in colour and by the intensities of the colour depicted.

5. CONCLUSION

Two facile, low cost, and efficient chemosensors (sensors) were synthesised through Schiff base condensation reactions scaffold via supramolecular principles. Sensor AB being a Di-2-Pyridyl ketone-based organic sensor and sensor EF an anthraquinone-based sensor. The two organic sensors exhibit good characteristics of light absorption in the UV-visible region. Sensor AB and EF were tested for their chemosensing properties through the recognition of different anions and cations in water. Both sensors showed effective detection behaviours of UV–vis absorption by the addition of various cations and anions.

In addition, three commercially available organic reagents were employed as chromogenic probes for the detection of heavy metal ions and anions in water samples. In comparison, sensor EF was slightly polar compared to sensor AB, therefore sensor EF was able to recognise/sense the presence of ions in water samples through one step reaction, whereas, sensor AB achieved ion recognition in water samples via displacement reaction. Moreover, all three chromogenic probes were able to sense ions in water through one step reaction. For structure elucidation, FT-IR as well as the ^{1}H-NMR spectroscopy was carried out to confirm the structure of the sensors. The functional groups in the FT-IR spectra were identified and confirming the molecular structures of synthesised sensors and of commercial chromogenic probes. Furthermore, the ^{1}H-NMR signals were identified, to further confirm the speculated structures of sensor AB and EF.

The measured pH values of different water sources and colour change observed through ion sensing, can cautiously lead to the conclusion that there is no significant difference in the chemical quality of the different water samples.

Considering the need for simple chromogenic probes for cation and anion sensing, the commercially available reagent dyes such as alizarin, dithizone and phenylfluorone reported in this research has showed promising results that induce the need for further research. The colour changes upon interaction with ions is vivid, with dithizone showing 96% of sensing sensitivity towards the studied metals. Direct sensing performance of synthesised sensor AB was assessed and found to be ineffective in polar solvent systems. However, the reversible colorimetric activities were observed with sensor AB in water samples which is attributed to highly competitive nature of hydrogen bonding in water which is able to outcompete off the AB-ion association in CH_3CN and DMSO.

6. RECOMMENDATIONS

Despite the many advantages associated with chromogenic based methods such as sensitivity, simplicity and rapid response, the lack of selective sensitivity encountered in this study was a drawback. Although colour change was observed in water samples, the results obtained in this research do not provide enough information on the ion/s responsible for colour change in water. Furthermore, solvatochromic effects of the sensors studied in this research were solely observed through naked eye colour observation, UV-Vis characterisation is recommended to determine the pre-eminent solvent system based on photocatalytic enhancement. Further research in colorimetric probes for metal ion detection methods in water is needed to allow selective sensor development. Further recommendations for future work include studying the effect of solute concentration (concentratochromism) for UV-vis studies of the sensor interaction with ions this in turn will provide information regarding the binding affinity of the chromogenic probes with anions and cations and also determine the limit of detection of various ions. Devising a procedure to evaluate the limit of detection (LOD) in chromogenic sensors for correct comparison with other analytical techniques is recommended. Lastly, colorimetric competitive experiments to determine selectivity and sensitivity of a chromogenic sensor towards a particular metal ion or anion in the presence of other competing metal ions and anions is a recommended study.

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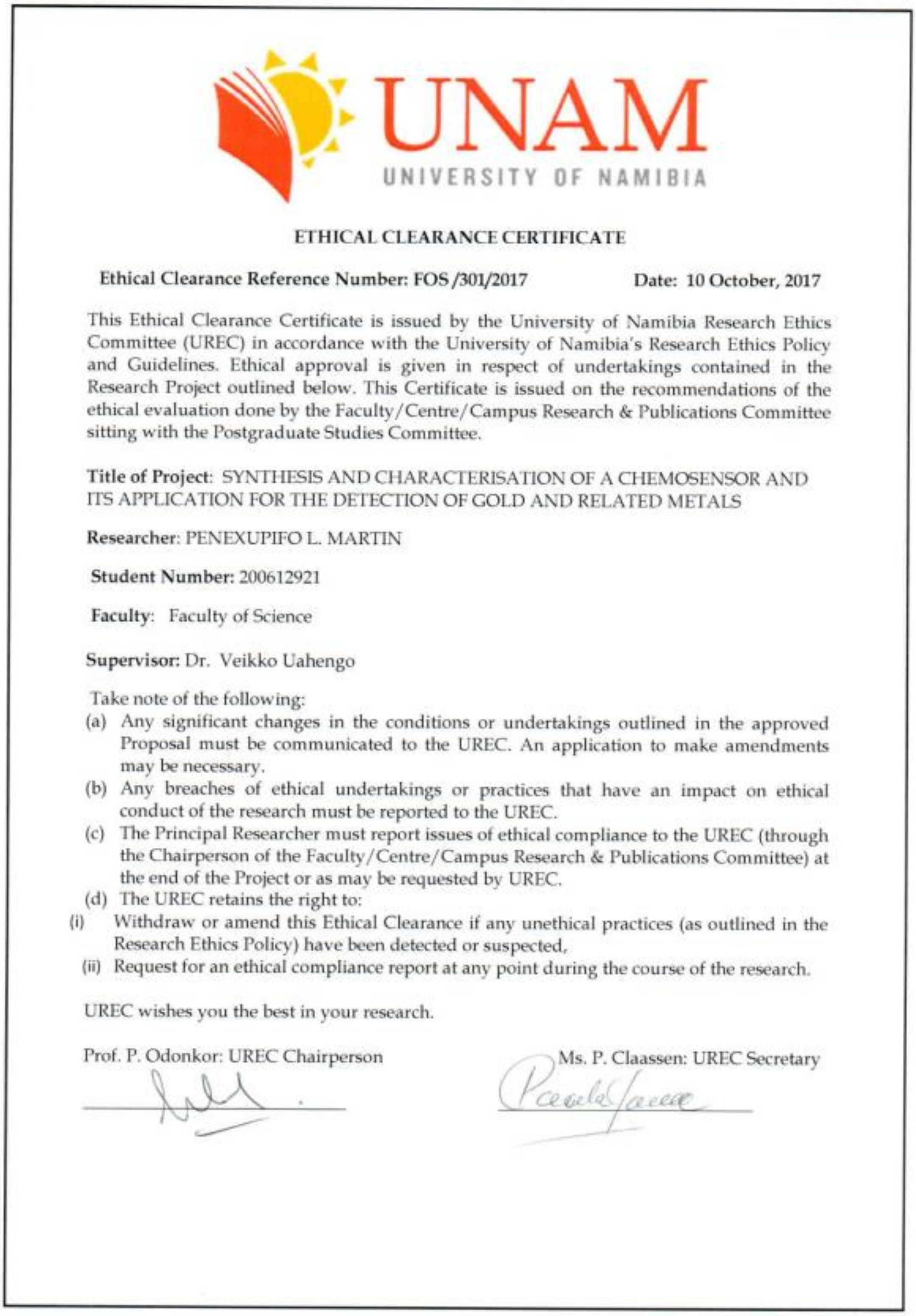
APPENDICES

Appendix A: University of Namibia Ethical Clearance Certificate



UNAM

UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: FOS /301/2017 **Date: 10 October, 2017**

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: SYNTHESIS AND CHARACTERISATION OF A CHEMOSENSOR AND ITS APPLICATION FOR THE DETECTION OF GOLD AND RELATED METALS

Researcher: PENEXUPIFO L. MARTIN

Student Number: 200612921

Faculty: Faculty of Science

Supervisor: Dr. Veikko Uahengo

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.

(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.

(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.

(d) The UREC retains the right to:

(i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

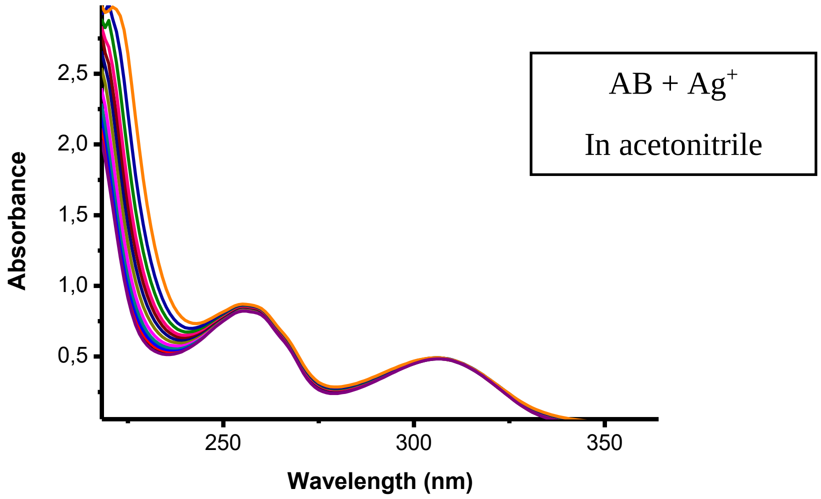
(ii) Request for an ethical compliance report at any point during the course of the research.

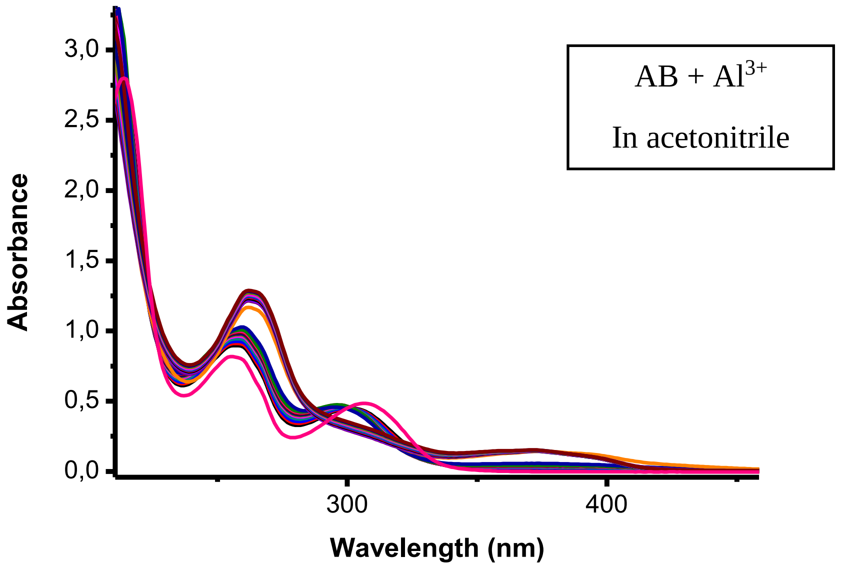
UREC wishes you the best in your research.

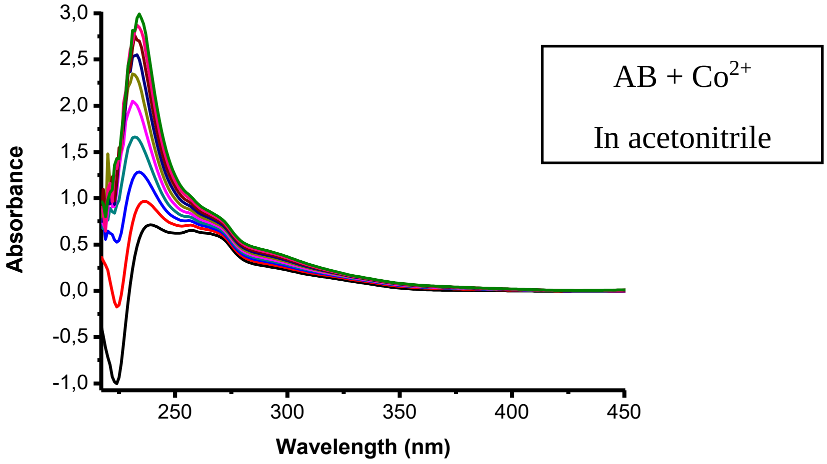
Prof. P. Odonkor: UREC Chairperson

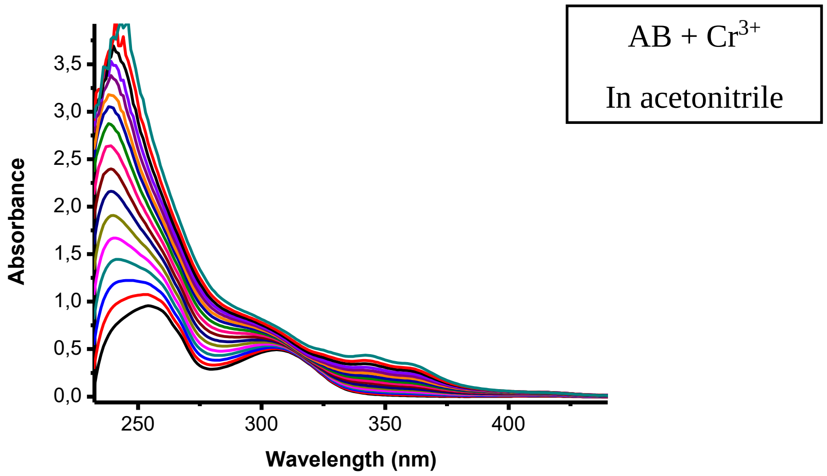
Ms. P. Claassen: UREC Secretary

Appendix B: Supplementary information






AB + Co^{2+}
In acetonitrile
Absorbance
3,0
2,5
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
250
300
350
400
450
Wavelength (nm)


AB + Cr^{3+}
In acetonitrile
Absorbance
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2,5
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1,5
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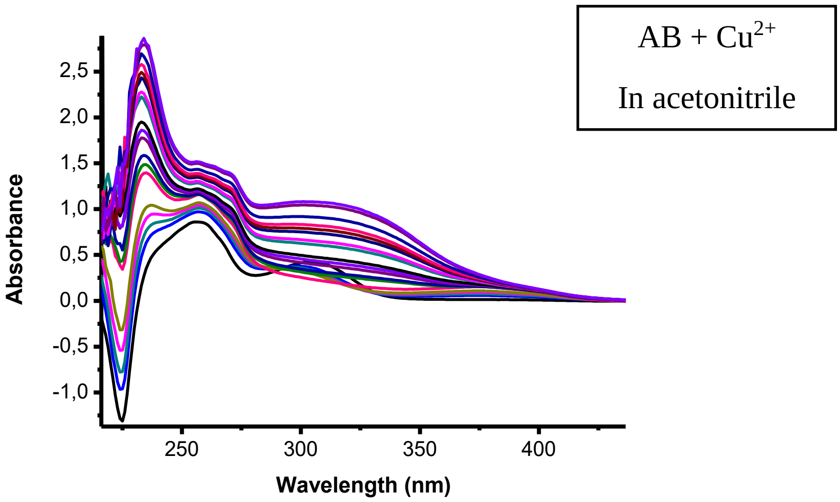
0,0
250
300
350
400
Wavelength (nm)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In acetonitrile
Absorbance
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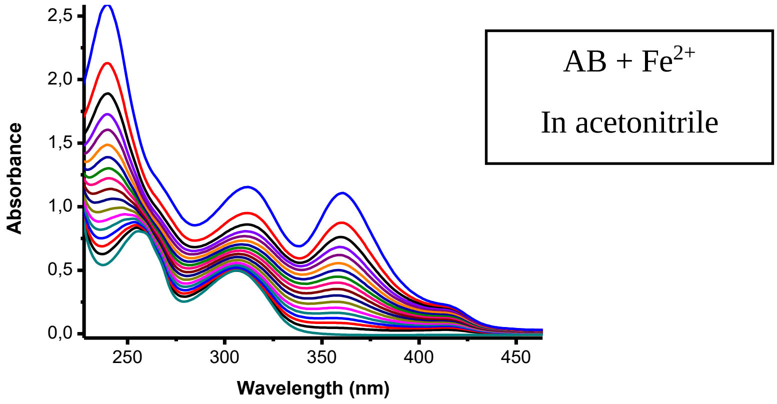
1,5
1,0
0,5
0,0
-0,5
-1,0
250
300
350
400
Wavelength (nm)


AB + Fe^{2+}
In acetonitrile
Absorbance
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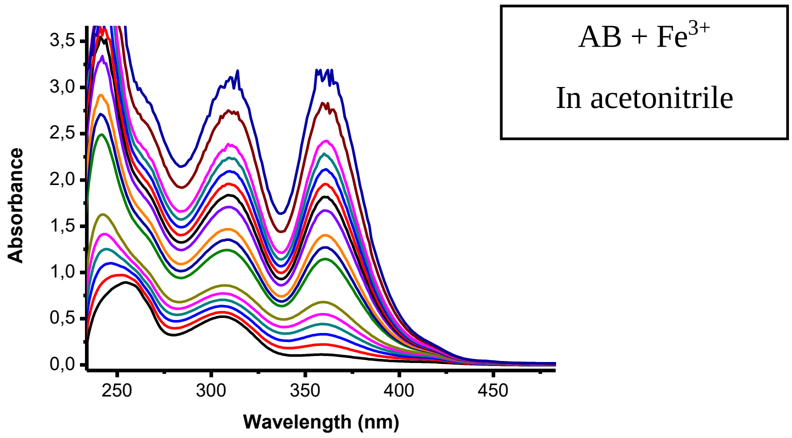
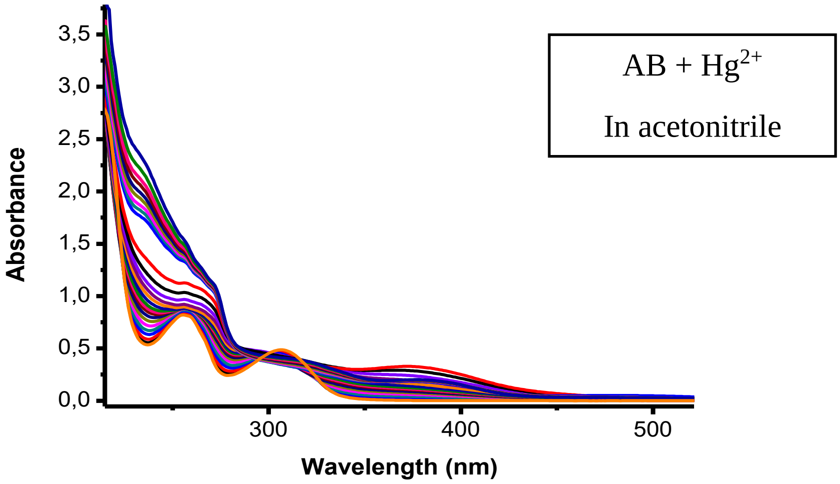
2,0
1,5
1,0
0,5
0,0
250
300
350
400
450
Wavelength (nm)


AB + Fe^{3+}
In acetonitrile
Absorbance
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
250
300
350
400
450
Wavelength (nm)


AB + Hg^{2+}
In acetonitrile
Absorbance
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
300
400
500
Wavelength (nm)

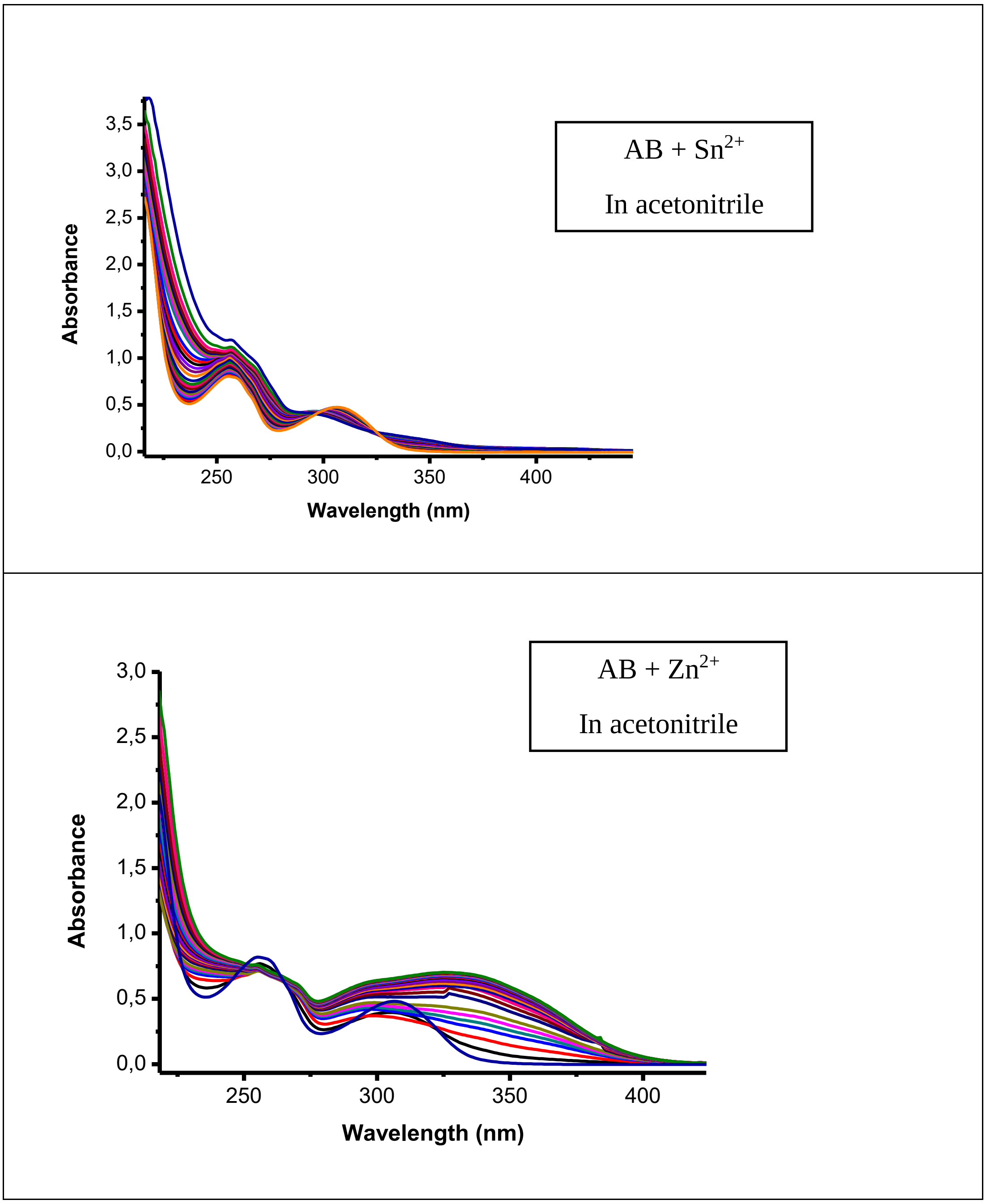
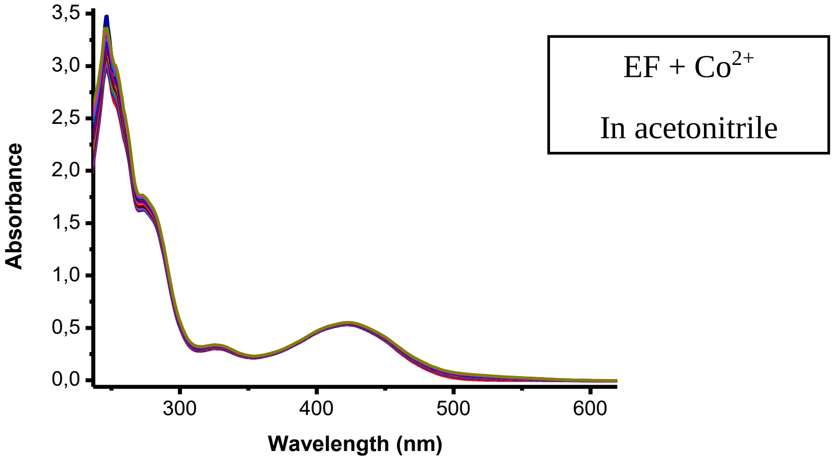
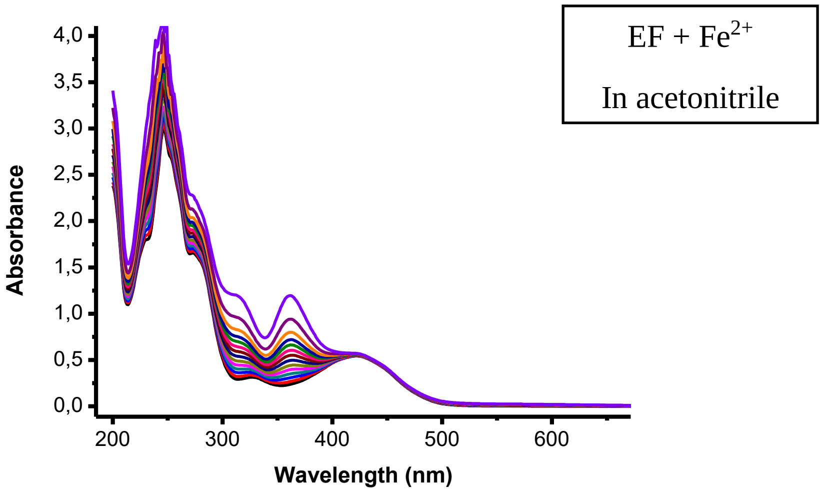
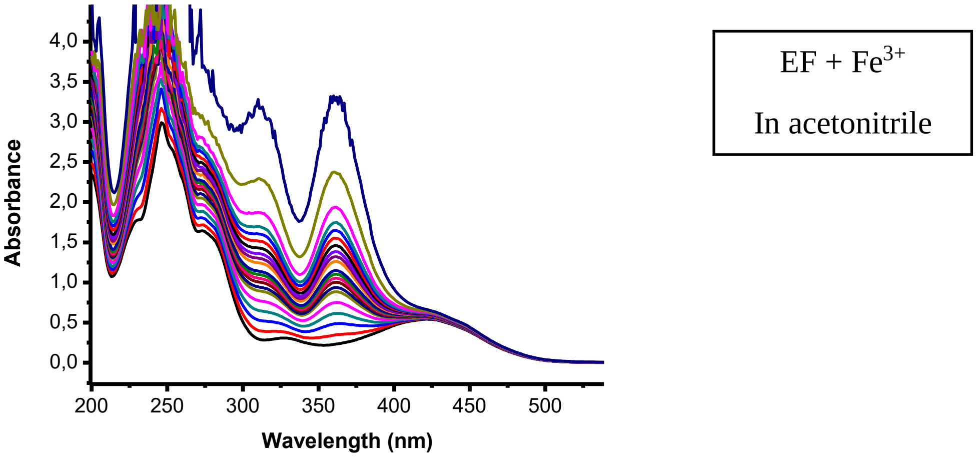
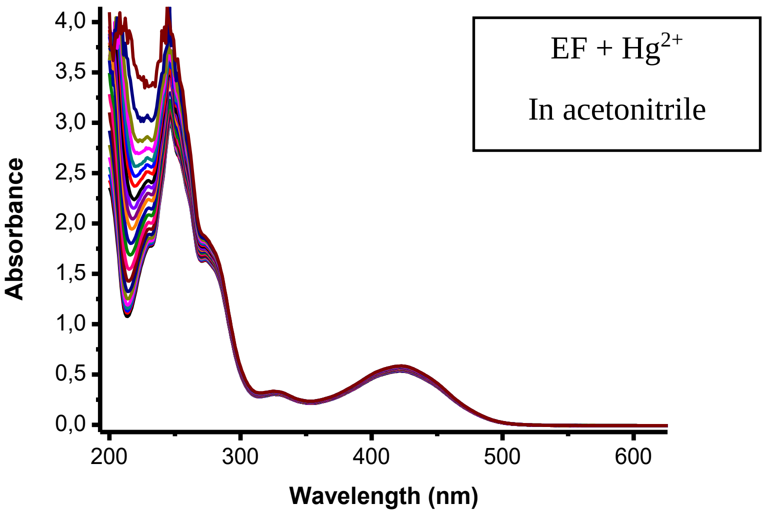


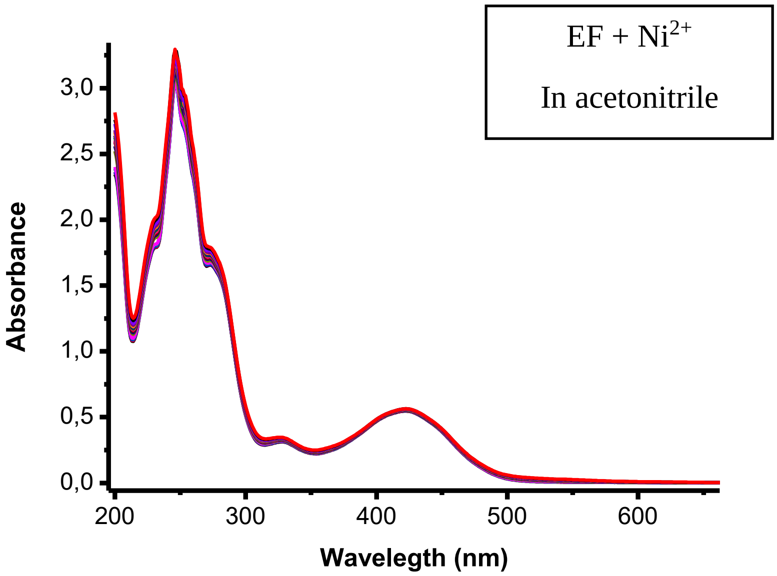
Figure S 1: UV-vis absorption spectra of AB in acetonitrile (1 x 10^{-5} M) upon the molar addition (0–20 µL equiv.) of various cations.


EF + Co^{2+}
In acetonitrile
Absorbance
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
300
400
500
600
Wavelength (nm)


EF + Fe^{2+}
In acetonitrile
Absorbance
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
200
300
400
500
600
Wavelength (nm)


EF + Fe^{3+}
In acetonitrile
Absorbance
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
200
250
300
350
400
450
500
Wavelength (nm)


EF + Hg^{2+}
In acetonitrile
Absorbance
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
200
300
400
500
600
Wavelength (nm)


EF + Ni^{2+}
In acetonitrile
Absorbance
3,0
2,5
2,0
1,5
1,0
0,5
0,0
200
300
400
500
600
Wavelegth (nm)

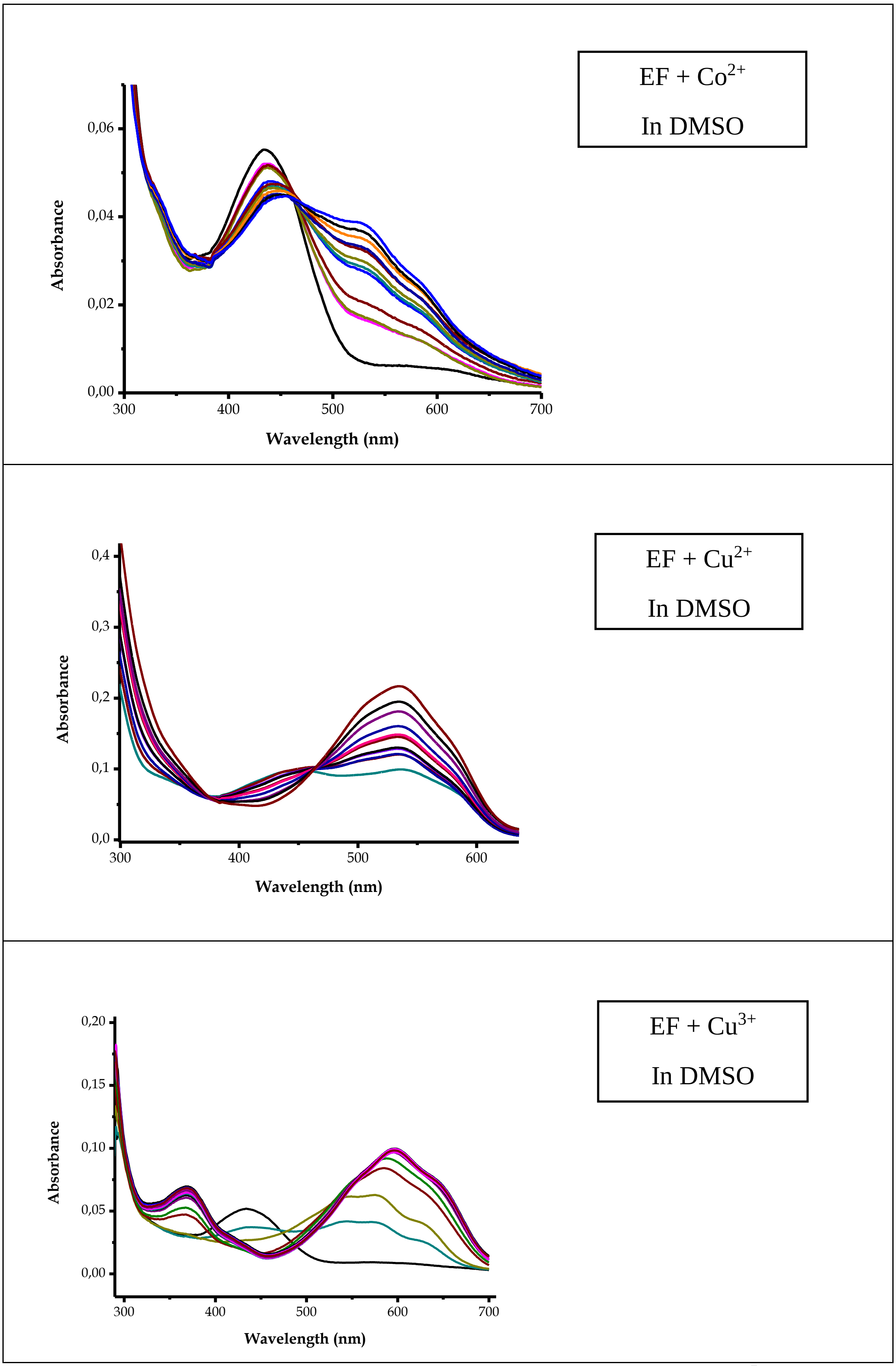
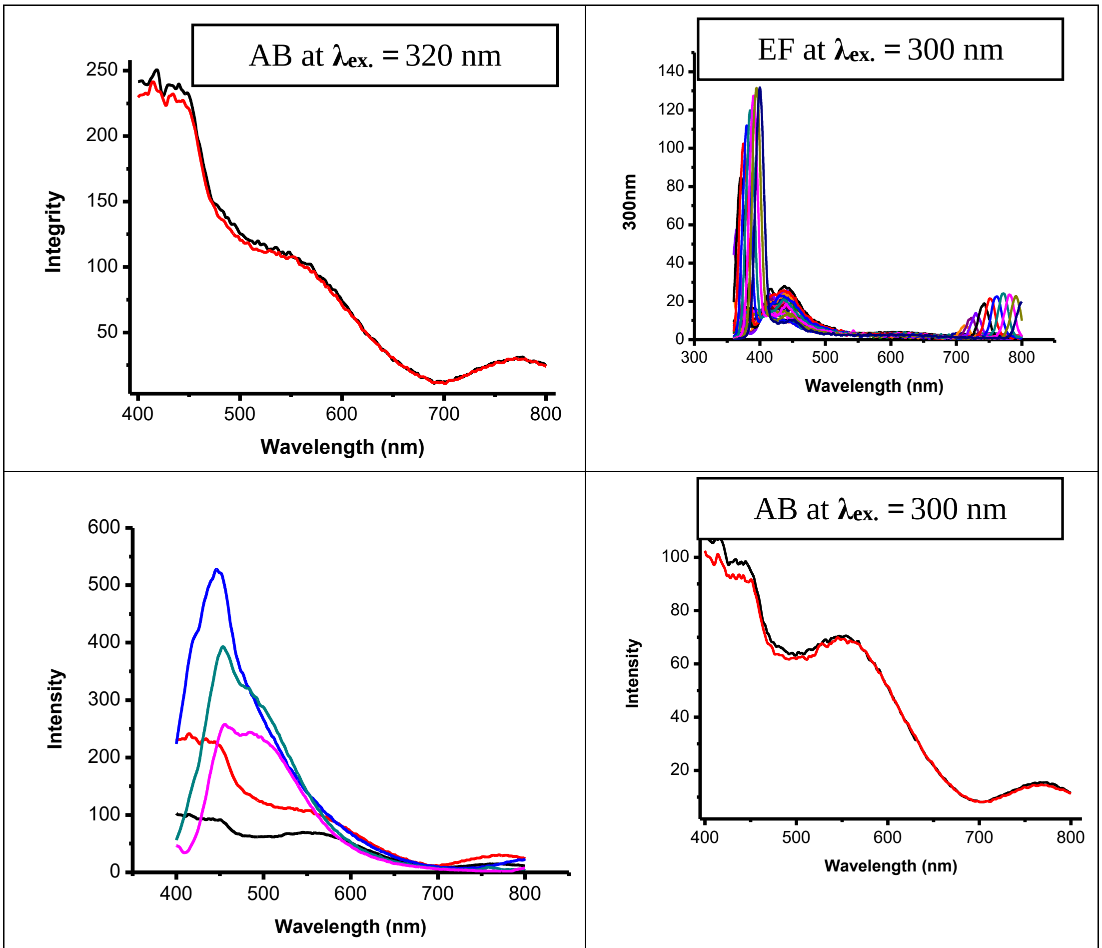
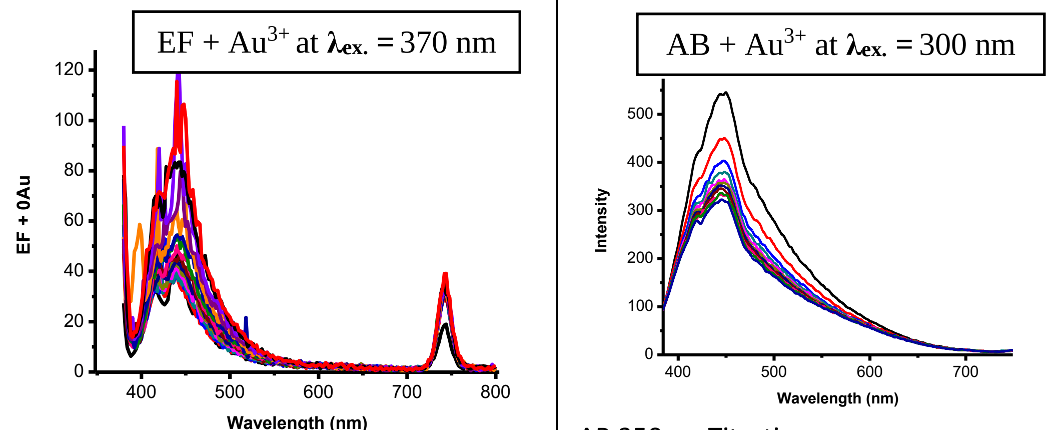


Figure S 2: UV-vis absorption spectra of AB and EF in DMSO (1 x 10^{-5} M) upon the molar addition (0–20 µL equiv.) of various cations.



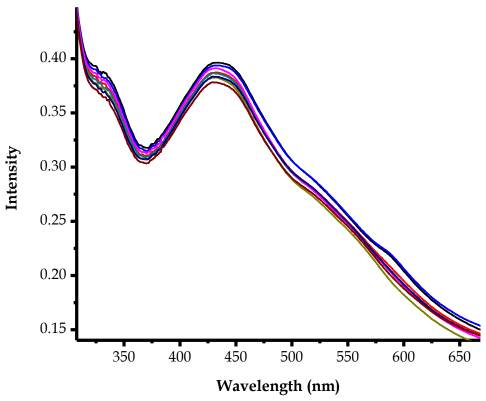
Sensor AB 300,320,350,380,400 + AU 5ul



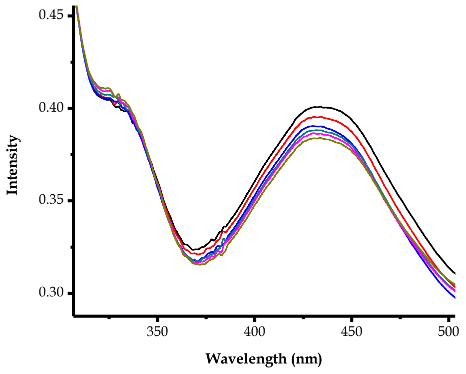
AB 350nm Titrations

Figure S 3: UV-vis fluorescence spectra of EF and AB in DMSO (1 x 10^{-5} M) upon the molar addition (0–20 µL equiv.) of various cations.

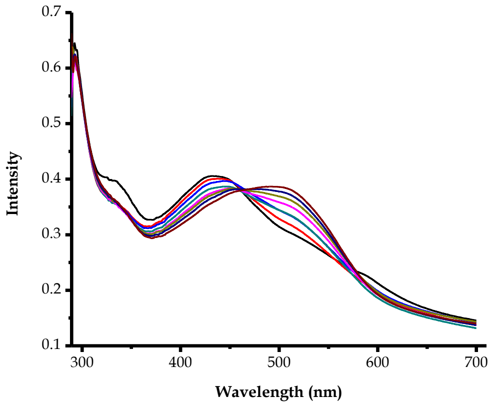
Alizarin + Ni^{2+}



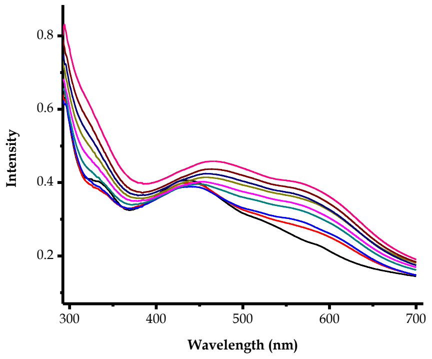
Alizarin + F^{-}



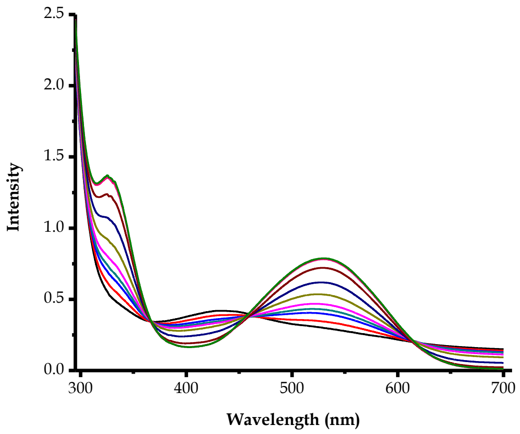
Alizarin + Cu^{2+}



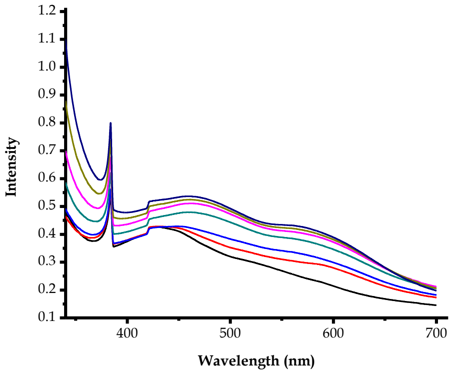
Alizarin + Fe^{2+}



Alizarin + CN^{-}



Alizarin + Cl^{3+}



Alizarin + Zn^{2+}

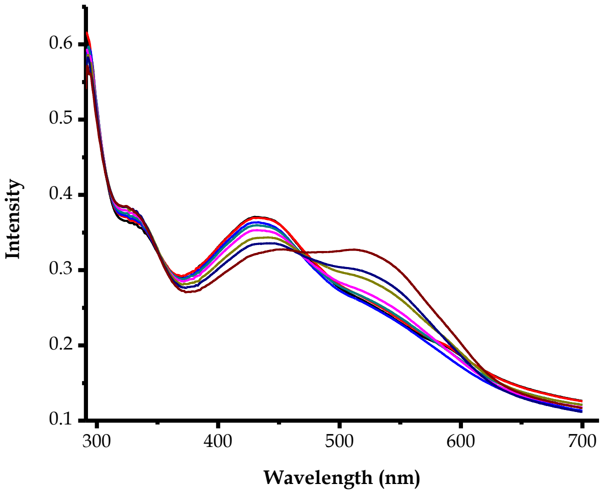
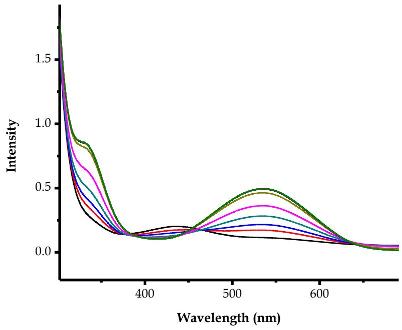
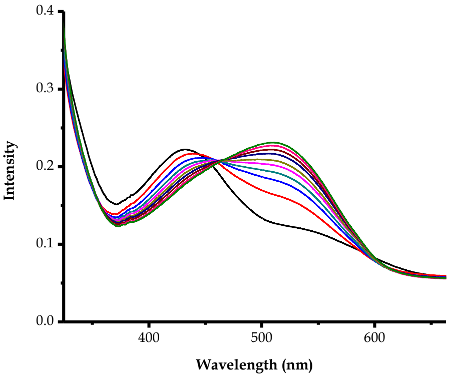


Figure S 4: UV-vis absorption spectra of alizarin in DMF–H_2O (1:9) (1×10^{-5} M) upon titration with cations and anions.

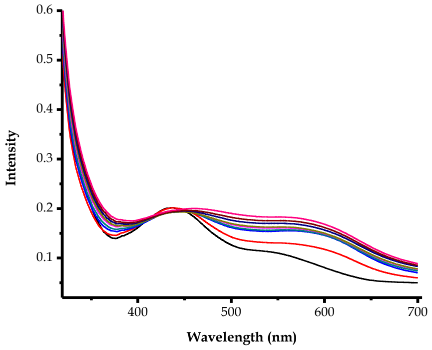
EF + CN^-



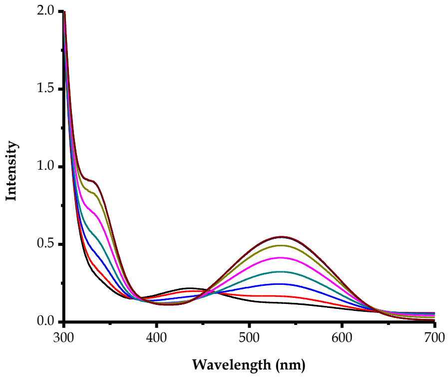
EF + Cu^{2+}

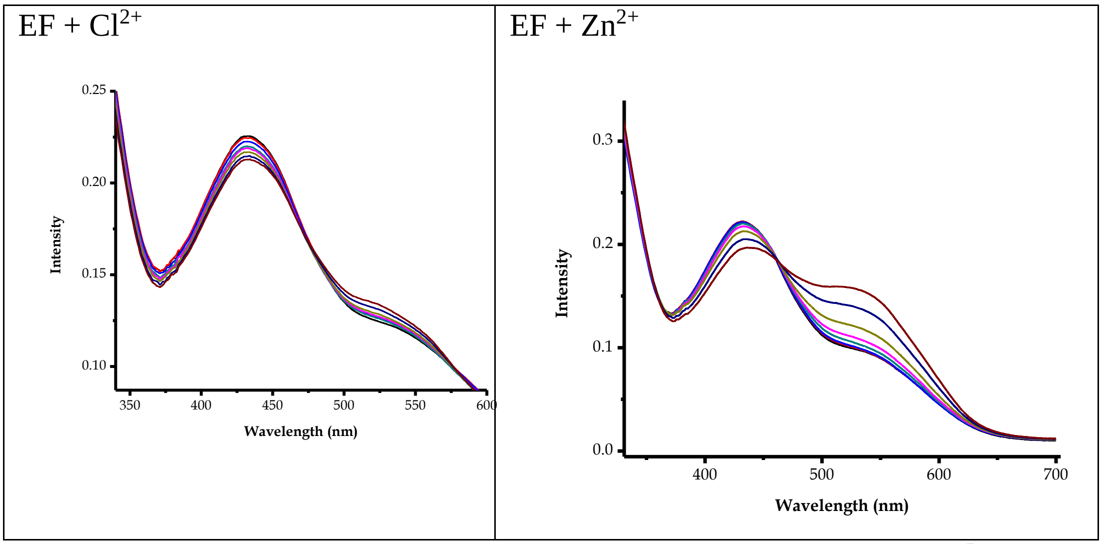


EF + Fe^{2+}



EF + Fe^{3+}

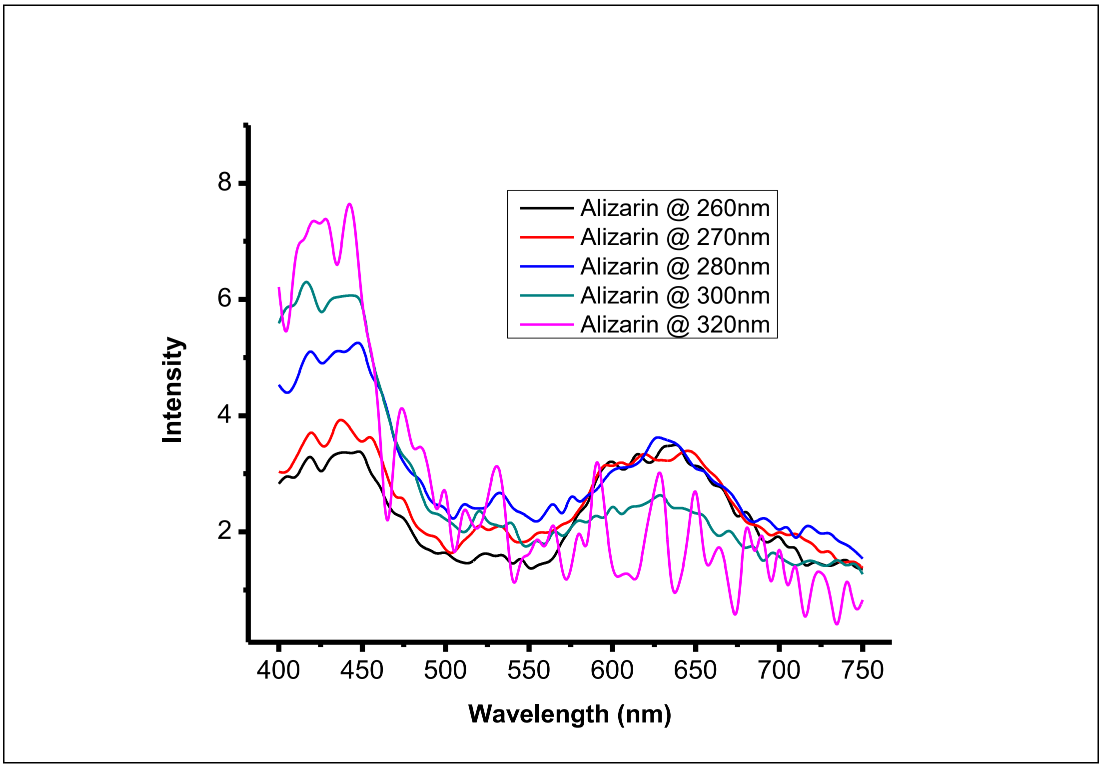




EF + Cl^{2+}

EF + Zn^{2+}

Figure S 5: UV-vis absorption spectra of EF in DMF–H_2O (1:9) (1×10^{-5} M) upon titration with cations and anions.



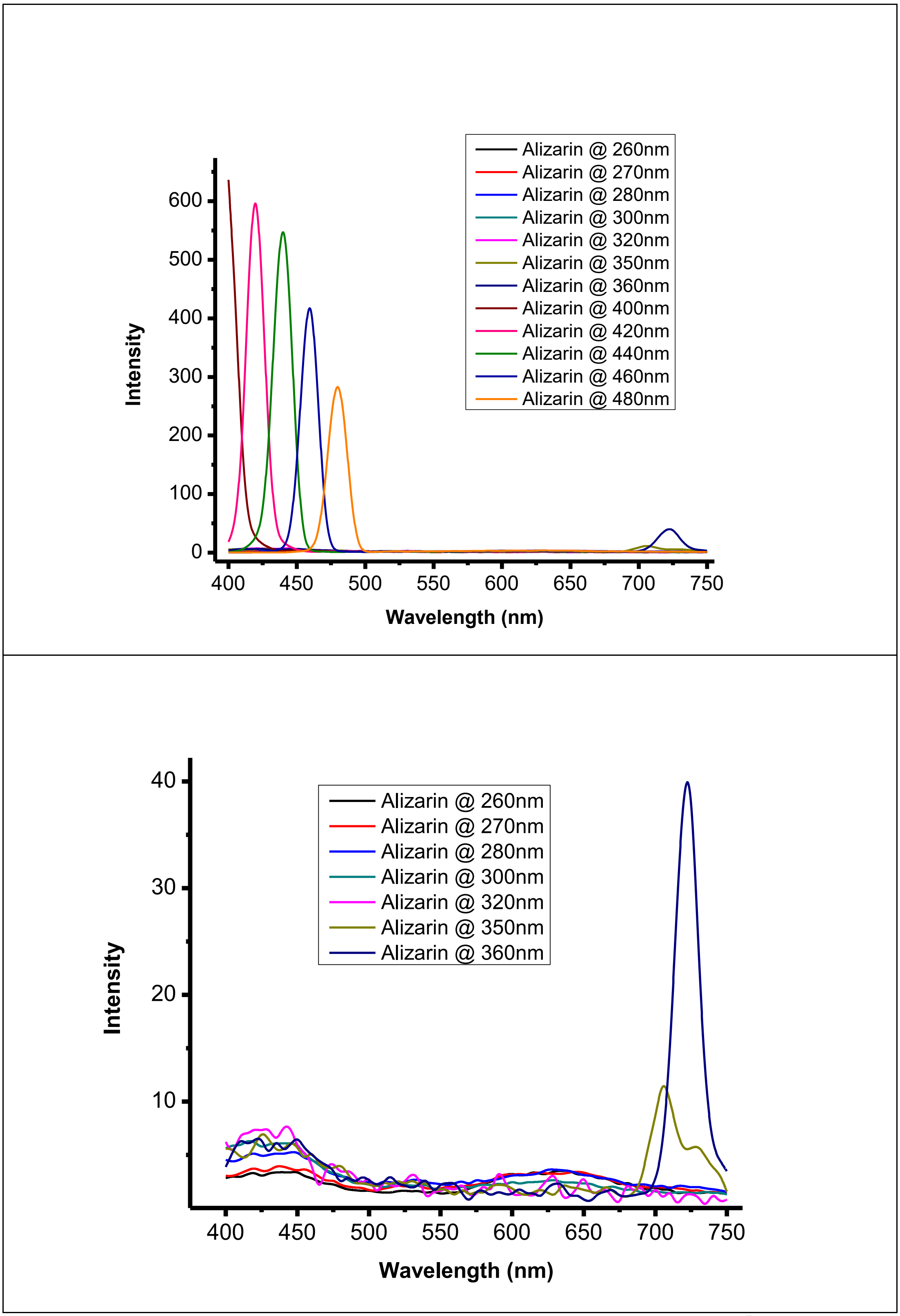
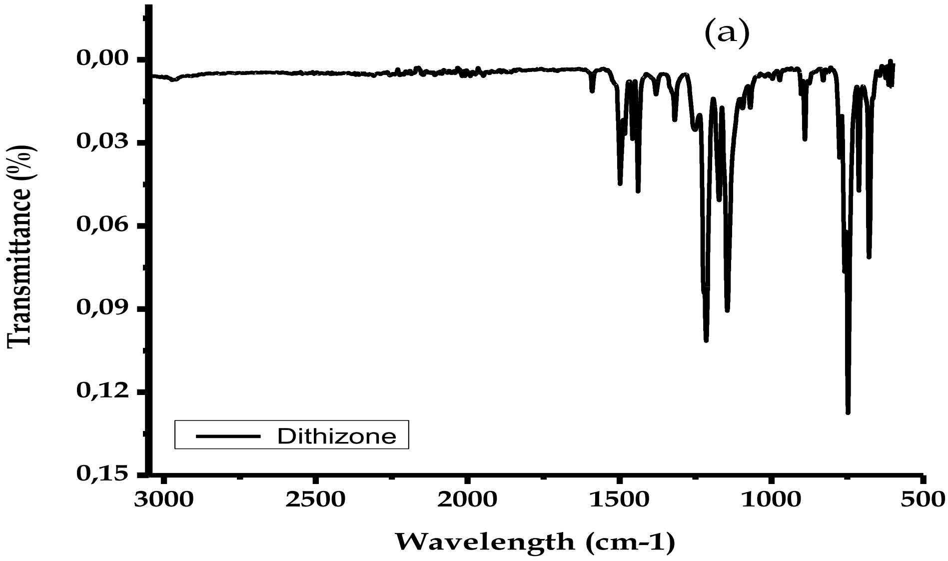


Figure S 6: Fluorescence spectra of alizarin in DMF–H_2O (1:9) (1×10^{-5} M) at various wavelengths (λ_{ex} = 260 to 480 nm).



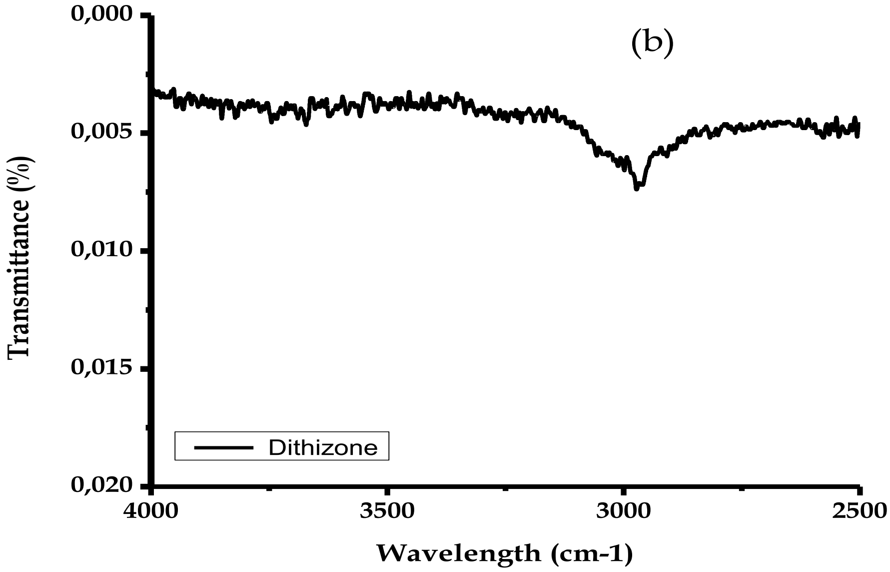


Figure S 7: FT-IR spectrum of dithizone; (a) Full range spectra and (b) its fingerprint region (4000-2500 cm-1) of the IR spectra.

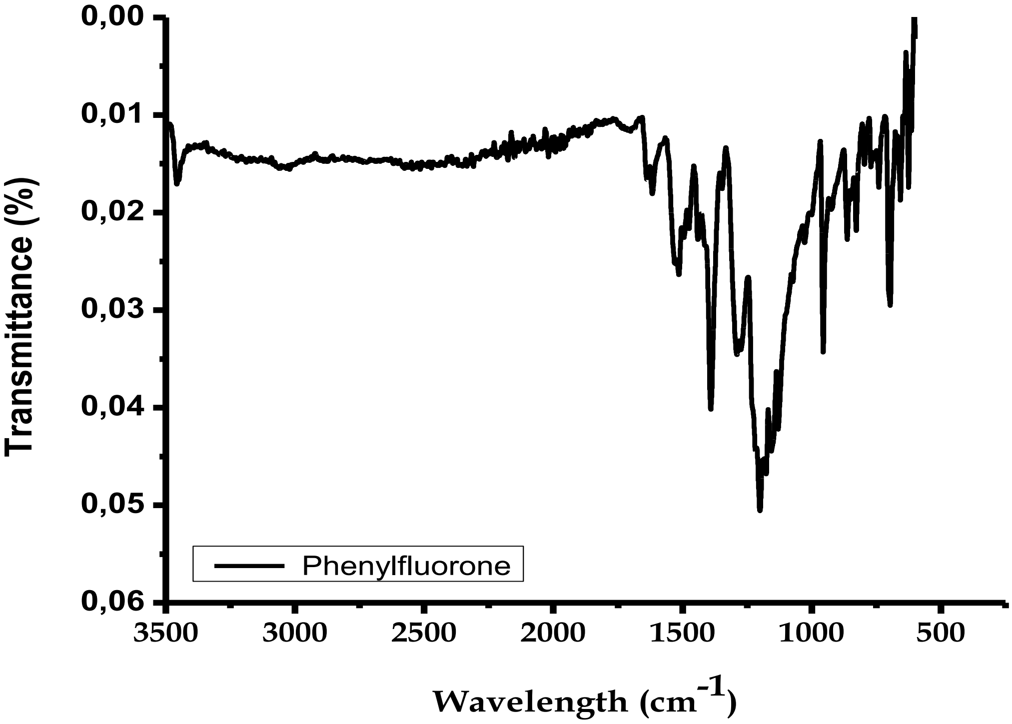


Figure S 8: FT-IR spectrum of phenylfluorone