Development of Nanosensor for Detection of Pollutants in Water

Thesis submitted

By

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List of Publications

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Conference Proceedings

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2. **Dei, J**., Bandyopadhyay, R. and Behera, B.K., 2024. Nano-biosensor Applications for Water Quality Monitoring. Current Trends in Fisheries Biotechnology (pp. 65-78). Singapore: Springer Nature Singapore.
3. Ghosh, D., Mal, D.D., Kumar, S., **Dei, J**., Sarkar, D.J., Parida, P.K., Behera, B.K., Pal, S., Das, B.K. and Pradhan, D., 2024. Electrochemical sensing platform using cerium tungstate for highly sensitive sensing of chromium in water. ***Electrochimica Acta,*** p.144553.
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Statement of Originality

*I, **Jyotsna Dei**, registered on **10th June, 2019**, do hereby declare that this thesis entitled "**Development of Nanosensor for Detection of Pollutants in Water**" contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.*

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 9%.

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This Thesis is dedicated

to

Lord Jagannath

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Abstract

Due to the increase in urbanization and industrialization, the load of toxicants in the environment is alarming. The most common toxicants, including heavy metals and metalloids such as hexavalent Chromium, have severe pathophysiological impacts on humans and other aquatic biotas. Hexavalent chromium or Cr(VI) is highly toxic for humans as it causes high oxidative reactions inside cells, leading to diseases like chronic ulcers and damage to the kidneys, mucous membranes, throat, skin, and respiratory tract. Due to various anthropogenic activities, Cr(VI) comes into our food chain through unmonitored and uncontrolled application in agriculture fields, refineries, mills, the tanning industry, automobiles, road works, etc. Presently, the standard Cr(VI) detection is done using conventional processes, which, though accurate, has severe drawbacks in on-the-spot rapid detection in the field. Therefore, developing a portable rapid detection device for such toxicants in the aquatic environment is necessary.

This work portrays the development of a field-portable image analysis device coupled with 3,3’,5,5’-tetramethylbenzidine (TMB) as a sensing probe for chromium(VI) detection in the aquatic ecosystem. Sensor parameters, such as reagent concentration, reaction time, etc., were optimized for the sensor development and validation using a commercial UV-Vis spectrophotometer. The chemoreceptor integrated with a uniform illumination imaging system (UIIS) revealed the system’s applicability toward Cr(VI) detection. The calibration curve using the R-value of image parameters allows Cr(VI) detection in the linear range of 25 to 600 ppb, which covers the prescribed permissible limit by various regulatory authorities. Furthermore, the adjusted R2 = 0.992 of the linear fit and correlation coefficients of 0.99018 against the spectrophotometric method signifies the suitability of the developed system. This TMB-coupled field-portable sensing system is the first-ever reported image analysis-based technology for detecting a wide range of Cr(VI) in aquatic ecosystems to our knowledge.

Further, a handheld potentiostat has been developed in the present study for the detection of trace-level Cr(VI) in aquatic environments. A Cr(VI) specific DNA aptamer immobilized screen printed electrode (SPE) has been used as the main biosensor. The device operates on an electronic peak current dumping event through mass deposition in the presence of an aptamer coupled with Cr(VI) onto the working electrode. The working range of the developed prototype is in the range of 0–1000 ppb Cr(VI), where the maximum linearity has been observed in the range of 0–500 ppb with a limit of Detection (LOD) as low as 10 ppb. The device has exhibited an excellent correlation with commercially available electrochemical workstations with a coefficient of 0.972. Moreover, the applicability of the developed device has been validated for seven different types of water samples. To our knowledge, this is the first-ever reported simplistic resource-limited on-spot aptasensing device for Cr(VI) detection in an aquatic environment.

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List of Abbreviations

EPA : Environmental Protection Agency
EDCs : endocrine disrupting chemicals
WHO : World Health Organization
CWC : Central Water Commission
Cr : Chromium
Fe : iron
Pb : lead
Ni : nickel
Cd : cadmium
Cu : copper
Hg : mercury
As : arsenic
Tl : thallium
AAS : atomic absorption spectroscopy
ICP-MS : inductively coupled plasma mass spectrometry
EDXRF : energy dispersive X-ray fluorescence
Zn : Zinc
Sb : Antimony
Mn : Manganese
Co : Cobalt
GC : Gas chromatography
HPLC : High-performance liquid chromatography
EEM : emission matrix
SFS : synchronized fluorescence spectroscopy
CDOM : chromophoric dissolved organic matter
FAAS : flame atomic absorption spectrometry
PUF : Polyurethane foam
ETAAS : electrothermal atomic absorption spectrometry
GFAAS : graphite furnace atomic absorption
DUSSS : direct ultrasonic slurry sampling
DPC : acidic diphenylcarbazide

CA	:	chromotropic acid
IC	:	ion chromatography
AMU	:	atomic mass units
LSPR	:	localized surface plasmon resonance
GCE	:	glassy carbon electrode
ITO	:	indium tin oxide electrode
ERGO	:	reduced graphene oxide electrode
SPE	:	screen-printed electrode
LEDs	:	light-emitting diodes
CMOS	:	complementary metal-oxide-semiconductor
CCD	:	charge-coupled device
UIIS	:	uniform illumination imaging system
TMB	:	3,3’,5,5’-tetramethylbenzidine
CNTs	:	Graphene and carbon nanotubes
GNR	:	graphene nanoribbons
ICT	:	intra/intermolecular charge transfer
PET	:	Photo-induced Electron Transfer
ESIPT	:	Excited State Intramolecular Proton Transfer
CHEF	:	Chelation Enhanced Fluorescent Resonance
FRET	:	Fluorescence Resonance Energy Transfer
TBET	:	Through Bond Energy Transfer
DNA	:	Deoxyribonucleic acid
RNA	:	Ribonucleic acid
SPR	:	surface plasmon resonance
ss	:	single-stranded
SELEX	:	Systematic Evolution of Ligands by Exponential Enrichment
ssDNA	:	single-stranded DNA
RE	:	reference electrode
WE	:	working electrode
RE	:	reference electrodes
CE	:	Counter Electrode
CA	:	control amplifier
LSV	:	Linear sweep voltammetry

PV : Pulse voltammetry

DPV : Differential pulse voltammetry

CIS : contact image sensor

ND : Not Detected

WVGA : Wide Video Graphics Array

LCD : touch screen Liquid Crystal Display

SFDA : Safe Water Drinking Act

POC : point-of-care

RAT : rapid antigen test

OPD : o-Phenyldiamine

ABTS : 2,2'-azino-bis (3- ethylbenzothiazoline-6-sulfonic acid)

H_2O_2 : hydrogen peroxide

$K_2Cr_2O_7$: potassium dichromate

$CrCl_3.6H_2O$: Chromium chloride hexahydrate

Z_nSO_4 : Zinc sulphate

$(Pb(C_2H_3O_2)_2.3(H_2O))$: Lead acetate trihydrate

Na_2SO_4 : Sodium sulfate

K_2CO_3 : Potassium carbonate

$NaHCO_3$: Sodium hydrogen carbonate

$(Ba(NO_3)_2)$: Barium nitrate

$(Cd(NO_3)_2 \cdot 4H_2O)$: Cadmium nitrate tetrahydrate

$HgCl_2$: Mercury chloride

DPC : 1,5-diphenylcarbazide

ELISA : enzyme-linked immunosorbent assay

LOD : Limit of Detection

LOQ : Limit of Quantification

ppb : parts per billion

ppm : Parts per million

RE : recovery error

FSSAI : Food Safety and Standards Authority of India

DCNNs : Deep Convolutional Neural Networks

FSL : Few-Shot Learning

GAN : Generative Adversarial networks

LOD	:	limit of Detection
WHO	:	World Health Organization
AAS	:	atomic absorption spectrometry
ICP-AES	:	inductively coupled plasma-atomic emission spectrometry
XRF	:	X-ray fluorescence
KCl	:	potassium chloride
$NaHCO_3$	:	Sodium hydrogen carbonate
$FeCl_3.6H_2O$	:	ferric chloride hexahydrate
$K_4[Fe(CN)_6]\cdot 3H_2O$	:	potassium ferrocyanide
$K_3[Fe(CN)_6]$	:	potassium ferricyanide
CV	:	cyclic voltammetry
SEM	:	Scanning Electron Microscopy

Chapter 1

Introduction

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Chapter 1

Introduction

1.1. Background of research

Water is essential for all living organisms on Earth. Water comprises approximately two-thirds of the Earth's surface. Land comprises the residual portion, which totals 71.4% of the Earth's surface. Precipitation that descends from the atmosphere and enters rivers, lakes, and other surface water bodies is referred to as the surface freshwater source [1]. The demand for freshwater is gradually increasing as a result of factors such as population growth, climate change, and decreasing water availability due to erratic weather. It is possible to prevent approximately 9% of all diseases and 6% of all fatalities worldwide by implementing global initiatives to enhance water safety, hygiene, and sanitation. Chemical contamination from agricultural operations, industry, urban areas, and mining regions is endangering the purity of water worldwide, in addition to the global waterborne disease epidemic (Fig.1.1). The long-term effects of numerous chemical pollutants are inadequately understood, while some have significant adverse health effects. As of now, the Environmental Protection Agency (EPA) has classified more than 40,000 water bodies in the United States as "impaired," indicating their inability to sustainable environment with non-polluted water quality criteria. Water pollution is the term used to describe the presence of an excessive quantity of a substance in water that has capability to cause harm or damage to both humans and the environment [1]. The quantity of pollutants (chemical, biological, or physical actions) in water that all contribute to the extent of water pollution. Although water contamination may occasionally result from natural events such as volcanic eruptions or evaporation. Nevertheless, human activity on land is the primary source of pollution. Water contaminants have the potential to migrate between various water reservoirs during the various phases of the water cycle. It is inevitable that river contamination has the potential to be transferred to another body of water, such as the ocean, where it may result in additional complications. Groundwater pollution can be a concern due to its sluggish flow and tendency to remain in one location for an extended period [2]. Distinct point sources, which are plainly distinguishable and relatively small, or non-point sources, are the means by which pollutants enter water bodies. Integrated sewer systems that treat wastewater by combining street sewage and storm water discharge may be significant sources of pollutants. Storm water runoff has the potential to exceed the sewer system's capacity during periods of heavy rain, resulting in the direct discharge of untreated effluent into surface waterways and the system becoming overburdened [3]. Nonpoint sources of pollution include urban areas, agricultural regions, and defunct mines. Pollutants from lawns and agricultural fields, like pesticides and herbicides fertilizers, are collected by precipitation as it travels across the terrain and penetrates the soil. It also accumulates acidic and poisonous components from abandoned mines, as well as substances such as animal excrement, urban road and salt oil. Subsequently, this contamination is transferred to both surface and groundwater [1]. Although not every water contaminant has an immediate harmful effect on health of lifeform, its prolonged consumption may lead to severe conditions [2]. The flora and fauna of the environment are at risk due to the prevalence of excessive contaminants in the water, which leads to the mortality of aquatic species. The consumption of these organisms poses substantial health risks to individuals, including the development of severe diseases such as cancer, disruptions in hormone levels, and damage to internal organs as kidneys, liver, heart, and brain. Two primary factors may be responsible for the origin of water pollution. Initially, it is the result of the natural processes that are involved in the production of a variety of products, which lead to the presence of pollutants in water. Secondly, it is a result of the use of synthetic products in industrial, agricultural, or domestic settings. Hazardous compounds, insecticides, pesticides, byproducts of petrochemicals, substances containing heavy metals, and radioactive elements are among the most notable examples of water contamination caused by these activities [3]. Numerous human health issues are precipitated by the presence of endocrine disrupting chemicals (EDCs) in water, such as heavy metal Cr(VI) in water. Precision-site identification of heavy metal concentrations in water is essential for its treatment to preserve aquatic ecosystems.

Fig.1.1. Tannery effluents containing heavy metal chromium discharging to river Ganga at Kanpur

1.2. Motivation of research

Water influences multiple facets of society, including healthcare for humans and animals, the food chain, and the tourism sector [4]. Consequently, enhancing water quality can stimulate a nation's economic growth and significantly aid in poverty alleviation. As per a survey by the WHO, 785 million individuals limited availability to essential drinking water services, while 144 million rely on surface water. Worldwide, a minimum of 2 billion individuals utilize drinking water sources that are polluted. In recent decades, the rise of industry and urbanization has resulted in a significant worry regarding the prevalence of heavy metals and its impact on human well-being. Detection of heavy metals attracted considerable interest due to the substantial leakage of these not break down naturally materials. Latest study from the CWC (Central Water Commission) reveals that two-thirds of consumable water samples were tainted with toxic heavy metals [5]. Chromium (Cr), lead (Pb), Iron (Fe), cadmium (Cd), nickel (Ni) and copper (Cu) are identified as the primary pollutants in the samples. Heavy metal poisoning is prevalent not just in water but also in food. This primarily results from inconsistent irrigation, inadequate sewage management, unsanitary food processing, and inappropriate food storage. Many food items contain measurable amounts of heavy metals. As a result, one major issue that academics worldwide are tackling is the identification of metallic substances in food items. By different traditional testing procedures, with modern novel sensors the toxic metals can be identified. The heavy metals possessing a substantial atomic mass and high relative density greater than 5 g/cm^3, which can induce significant toxicity even at very less amount in biological environments. The utmost hazardous heavy metals include chromium (Cr), mercury (Hg), thallium (Tl), lead (Pb), cadmium (Cd) and arsenic (As) as they may induce detrimental health consequences at minimal dosage levels. Furthermore, chromium, as a primary hazardous heavy metal in water, is particularly hazardous in its hexavalent form. Chromium present in Cr(III) and Cr(VI) form in environment, both requires evaluation for a precise assessment of health problems associated with drinking water. As of today, traditional methods continue to be the benchmark for identification and quantification of heavy metals. The principal diagnostic methods are atomic absorption spectroscopy (AAS) [6], flame or furnace spectroscopy [7], inductively coupled plasma mass spectrometry (ICP-MS) [8], and energy dispersive X-ray fluorescence (EDXRF) spectrometry [9], among others. Regrettably, these traditional analytical approaches are inadequate for rapid detection [10]. While traditional methods exhibit great selectivity and sensitivity for detecting heavy metals at the nanogram level, they generally lack field portability and necessitate a professional crew and considerable time for operation [11]. Furthermore, their operational expenses are elevated. Due to the industrial growth there is substantial rise in heavy metal ions contamination, along with the risk of bioaccumulation and toxicity in organisms if unregulated, has necessitated a swift, portable and automated on-site detection system to be developed. This study addresses health concerns and toxicity thresholds of the most perilous heavy metal Cr(VI), while evaluating several detection technologies. While most review articles concentrate on specific detection techniques, this research intends to develop field deployable nanosensor devices for detection of hazardous heavy metal, Cr(VI) in aquatic bodies.

1.3. Origin of water pollution

Contaminants originate from natural occurrences on Earth, the alteration of natural compounds, and their artificial production. The particles may inherently influence the overall exposure levels within the ecological framework (Fig.1.2). A considerable quantity of these chemicals is eliminated from the organisms or neutralized during detoxification. Naturally occurring pollutants encompass nitrogen oxides, heavy metals, hydrocarbons, and radioactive substances [12]. Pollutants may emerge from the modification and concentration of naturally occurring compounds during household, agricultural, or industrial use. Examples of toxins resulting from this include agrochemicals, pesticides, sewage and wastewater, heavy metals, radio nuclides and petrochemical hydrocarbons. Numerous chemicals are synthetic, and the pollution they produce is exclusively anthropogenic. The manufacture of various pesticides, plastics, petrochemicals and surfactants, has caused the discharge of several chemicals into the environment, resulting in considerable ecological problems [13].

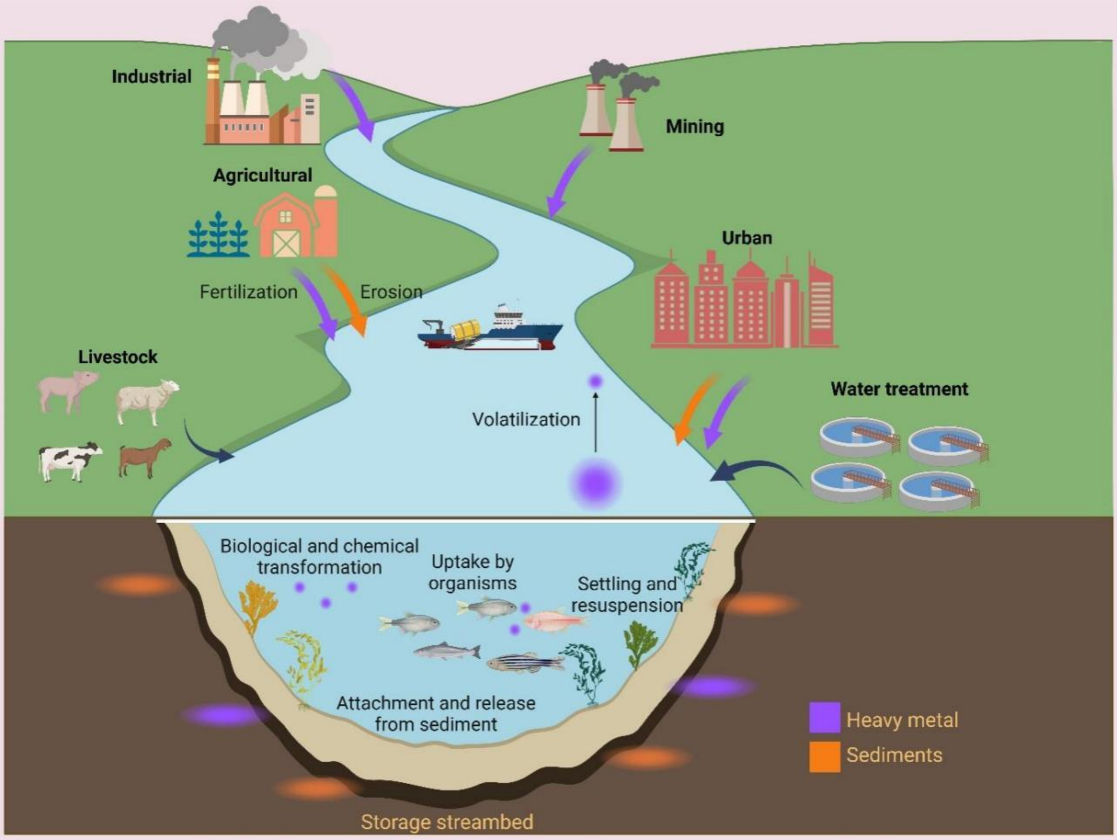


Fig. 1.2. The origin of water pollution

1.4. Water pollutants sources

The primary causes of water pollution leading to contamination include both direct and indirect sources (Table 1.1), as well as various other factors. Direct pollution transpires when pollutants are released directly into aquatic environments, illustrates by an industry discharging contaminated water or hazardous materials into the ocean or river. This results in water toxicity, occasionally leading to the mortality of fish and other amphibians. However, animals also ingest this water, perhaps resulting in adverse health effects or mortality. It may also affect individuals. Swimming or participating in activities such as canoeing in polluted water is a significant risk, as it may result in illness or even death [14].

Indirect water pollution occurs when pollutants are not immediately put into the water but instead accumulate within. An example is the progressive migration of fertilizer and pesticide chemicals through the soil, ultimately infiltrating groundwater and subsequently entering various watercourses. This incorporates the pollution of lakes, streams, and beaches, making the water hazardous for the organisms inhabiting these environments [14].

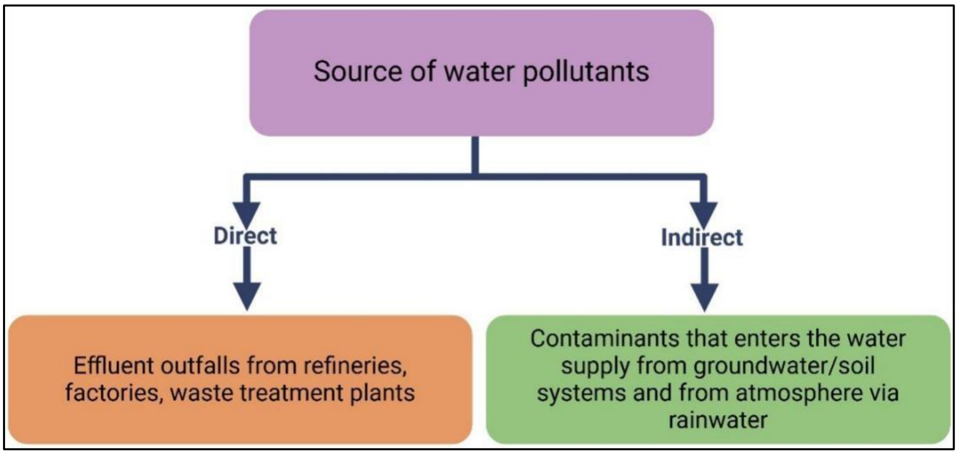


Fig.1.3. The schematic representation of different sources of water pollution

Irrespective of whether water pollution originates from direct or indirect sources (as seen in Fig. 1.3), the outcomes remain uniform: the risk of illness and mortality for any organisms that reside in or ingest the contaminated water. Therefore, it is essential for individuals and organizations to implement strategies to minimize pollution levels and lessen the impact on environment [15]. The different ways of water contamination can be entirely man-made or natural, including the discharge of domestic and industrial wastewater.

1.4.1. Runoff and natural sources

The water bodies can be contaminated through several actions as, surrounding vegetation, rainwater, volcanoes, underground rocks, natural run offs and atmosphere (dust, storms).

Precipitation is a significant natural contributor to water pollution since it absorbs contaminants from the atmosphere and carries them along with suspended particles. For example, acid rain occurs when acid gasses, such as sulfur and nitrogen oxides, dissolve in raindrops. Dry deposition refers to the direct settling of particles due to gravity, which may contribute to water contamination. The deposition of leaves, twigs, and other organic matter from the surrounding vegetation may help enhance the nutrient content of water bodies. The existence of sub-aquatic rock formations and volcanic activity may contribute to the formation of certain types of salts [16].

1.4.2. Domestic sewage

Domestic sewage consists of liquid waste from the general population and is composed of around 99% water and 1% particulates. Seventy percent of the substances found in sewage are organic, whereas the remaining thirty percent are inorganic. The organic ingredients consist of 10% fat, 25% carbs and 65% proteins. The inorganic component of sewage consists of salts, metals and grit in different amounts.

The primary issues related to sewage include the emission of foul smells, the transmission of enteric infections, as well as the contamination of water bodies with organic matter, resulting in a loss of oxygen levels and the death of fish. Sewage includes significant amounts of nutrients, such as N_2 and PO_4^{3-}even after undergoing secondary treatment. A prevalent method of disposal of sewage involves treating the land or irrigating crops. Nevertheless, disposing of waste in this manner, without taking scientific factors into account, might result in significant health risks and long-term degradation of the soil [17].

1.4.3. Agricultural wastes

Agricultural waste pertains to the refuse primarily generated from the runoff of agricultural lands and livestock. Currently, farmers use a variety of agrochemicals to enhance the development of fruits and vegetables, which unfortunately leads to several environmental issues. Additionally, it may induce toxicity in aquatic organisms [18].

1.4.4. Industrial wastes

Industrial waste can directly contaminate water reservoirs. The composition of industrial waste varies by industry and throughout time, influenced by factors such as materials employed, processes implemented, and operational considerations. Industrial wastes are said to include a significant number of organic materials [19].

These contaminants consist of several components, each with its own specific consequences on human health. Therefore, it is essential to regulate these pollutants in order to prevent them from reaching dangerous levels that might harm people.

Table 1.1. Sources and types of water pollution along with their effects

Source	**Type**	**Pollutants**	**Effects**
Industrial byproducts	Direct	Numerous hazardous substances, including heavy metals such as Chromium, lead and mercury, sulfur-containing compounds, nitrates, and asbestos	The alteration in water color is a consequence of the proliferation of compounds discharged into the environment. Additionally, abrupt temperature fluctuations occur, posing significant risks to aquatic life.
Sewage Wastewater	Direct	Pathogens like harmful fungus, bacteria, viruses, parasites etc.	The pathogen facilitates the proliferation of organisms that serve as carriers, ultimately resulting in fatal diseases.
Mining Activities	Indirect	Toxic elements, heavy metals, sulfides, fluorides	These result in severe health complications, including chronic cancer and respiratory diseases.
Marine Waste Dumping	Direct	The garbage produced by domestic activities and industries in the form of paper, plastic, food, rubber, glass, etc.	This is very harmful to animals in the sea. e.g., Corals present in the ocean are destroyed.
Accidental Oil Leakage	Direct	Petroleum products include gasoline, diesel fuel, paraffin wax., etc.	It damages different species in the ocean.
Burning of fossil Fuels	Indirect	Release of ash and poisonous gases into the atmosphere	The reaction between ash and water vapor produces acid rain. Additionally, the emission of carbon dioxide contributes to global warming.
Chemical fertilizers and pesticides	Indirect	Pesticides, fungicides, herbicides, insecticides, bactericides	When these chemicals combine with precipitation and enter rivers and canals, they cause severe harm to aquatic life. Additionally, it is capable of migrating off-site and leaching into groundwater and surface water, posing grave severe risks to humans.
Global Warming	Indirect	Greenhouse effect	It increases the temperature of water bodies, due to which there occurs death of aquatic animals and marine species.
Radioactive Waste	Direct/ Indirect	Elements e.g. radium, uranium; radioactive gases e.g. radon; artificial radionuclides from nuclear reactions	It causes severe environmental hazards if not disposed of properly. The diseases caused by exposure to radioactive waste include cancer, anemia, leukemia, hemorrhages, and cardiovascular diseases.

1.5. Different types of water pollutants

There are different kinds of pollution, which are listed below.

1.5.1. Organic pollutants

Organic pollutants are harmful chemical compounds made up of carbon, hydrogen, oxygen, sulphur and nitrogen. They could remain in the atmosphere for a long duration and accumulate in living organisms. These substances exhibit resistance to natural processes of biological, chemical, and physical deterioration. Some examples of these substances include dyes, polyaromatic hydrocarbons, pesticides, insecticides, phenols, biphenyls, and herbicides [20].

1.5.2. Inorganic pollutants

Inorganic pollutants include hazardous substances such as chromium, mercury, cadmium, arsenic, and lead. They also include metal compounds, inorganic salts, metal-organic complexes, sulfates, cyanides, and some nonmetals that possess toxicity even at low concentrations [21]. Developing nations express concern over the potential harmful substances, like heavy metals, ammonium nitrate and nitrite, in their potable water sources. The high amount of inorganic nitrogen pollutants (ammonium, nitrite and nitrate) as well as inorganic phosphates pollutant in water bodies, resulting the discharge of water from agricultural areas and the discharge of sewage from cities or industries, among other sources, contribute to a multitude of health issues. Nitrite is a carcinogen, meaning it has the potential to cause cancer. It specifically raises the chance of developing stomach, liver, and esophageal cancer. Additionally, it may result in elevated amounts of ammonium in the body [22]. Hexavalent Chromium is extremely harmful to humans, resulting in chronic ulcers and impairing the kidneys, skin, and respiratory system.

Heavy metal ions exist naturally in environment, and their recent rise in biological organisms necessitates that regulatory bodies develop critical guidelines for their safe intake [5]. Table 1.2 defines the array of international and national regulatory bodies that formulate and supervise guidelines to ensure the security of drinking water. This list emphasizes variations in threshold constraints resulting from regional differences and drinking restrictions across diverse communities about drinking water.

1.5.3. Radioactive pollutants

Radioactive material exists abundantly in the structure of the earth's crust and may dissolve in drinking water on the surface. Anthropogenic radioactive material is released by nuclear weapons testing, nuclear power plants and the production and use of radioactive substances also contaminate water. Typically, drinking water may include radionuclides such as uranium, thorium, and aluminium as well as naturally occurring substances including uranium, radioactive gas radon and radium. These pollutants have a detrimental impact on human health. Radium is a carcinogen that may lead to the development of bone cancer. Uranium is known to have carcinogenic effects in bone tissue and has harmful effects on the kidneys [12].

1.5.4. Suspended solid

Suspended solids refer to the contaminants found in municipal and industrial wastewater treatment facilities as well as sewage treatment plants. Three distinct forms of suspended solids are often seen, including (a) sand and other particulate matter that may be present during washing processes, (b) Unusable organic material, (c) Solid particles floating in the effluent [19].

1.5.5. Pathogens

These are microorganisms which are responsible for causing diseases. They include bacteria, viruses, fungi, and some parasites. The viruses often found in wastewater include Hepatitis, Norwalk virus and Candida. The Salmonella is responsible for causing food poisoning, whereas *Vibrio cholerae* is the specific bacteria that leads to the development of cholera. Pathogens including Cryptosporidium and Schistosoma are parasites that may result in diarrhoea, whereas all wastewater pathogens have the potential to cause severe gastrointestinal disease [19].

1.5.6. Agricultural pollutants

The widespread application of chemical based fertilizers in the field of agriculture results in numerous environmental issues stemming from the existence of heavy metal and high concentrations of radionuclides in certain fertilizers. The manufacturing of fertilizers involves the presence of excessive amounts of heavy metals, with cadmium, mercury, nickel, arsenic, lead, copper and chromium. The heavy metal fertilizers are administered to the soil and absorbed by vegetation. Plants assimilate nutrients from the soil, which may then infiltrate the food chain and pollute the water [18].

1.5.7. Thermal pollution

Thermal pollution arises from abrupt fluctuations in the temperature of aquatic ecosystems. The discharge from thermal power plants results in a temperature increase of 10 °C in the aquatic system. It arises because of activities such as the emission of coolants by power plants and industry, or the discharge of ice water from reservoir bases into rivers. The abrupt rise in water temperature results in a decrease in oxygen levels, leading to the mortality of aquatic organisms and causing changes in the structure of the food web, ultimately resulting in a decline in biodiversity [23].

Table. 1.2. Maximum permissible amount of various heavy metals according to other controlling agencies

Sl. No	**Heavy Metal**	**FSSAI** (mg L^{-1})	**WHO** (mg L^{-1})	**EU** (mg L^{-1})	**FDA**	**Health Risk**	**Ref.**
1	Cd	0.003	0.003	0.005	0.005	Diarrhea Renal failure Vomiting Muscles Cramps Liver injury Convulsions	[24–34]
2	Zn	15	3.00	5.00	5.000	Nausea Diarrhea Vomiting Body Cramps	
3	As	0.05	0.01	0.01	0.01	Encephalopathy Heart disease, Diarrhea Numbness, Cancer Abdominal pain, Darker skin	
4	Cu	1.5	2.00	2.00	1.000	Hemolytic anemia Jaundice, Gastrointestinal distress Vomiting Damage to the kidneys and liver Hypotension (low blood pressure)	
5	Ni	1.5	0.02	0.02	0.1	Allergy Kidney and cardiovascular diseases Chronic bronchitis	
6	Cr	0.05	0.05	0.05	0.100	Liver and kidney diseases Anxiety, Schizophrenia	
7	Sb	–	0.02	0.005	0.006	Vomiting Lungs disease Irritation of the skin, lungs and eyes	

8	Mn	–	0.4	0.3	0.05	Glucose intolerance Skin problems Birth defects Neurological symptoms Lowered cholesterol levels	
9	Fe	0.3	2.00	0.2	0.300	Bloody vomiting Stomach Pain Nausea and vomiting	
10	Hg	0.001	0.006	–	–	Effects on the immune, nervous, digestive systems Headaches Insomnia and memory loss	
11	Co	–	0.05	–	–	Vision problems Thyroid damage Pneumonia Stomach ulcers Heart problems Diarrhea	

1.6. Conventional water monitoring techniques

Water quality monitoring technologies aid in the identification of water pollution, a very intricate process. Various water monitoring approaches are used to identify water pollution, including conventional laboratory-based water analysis using sophisticated and bulky apparatus, spectroscopic devices, chemical and biological sensors, and microfluidic devices. When selecting a method, it is important to consider the intended aim of the study, such as whether it is qualitative, quantitative, or a combination of both. Below is a concise summary of the conventional methods used for monitoring water.

1.6.1. Chromatography

It is a method that separates different parts of a mixture by dividing the components of a sample between the moving and fixed phases. The stationary phase may consist of either solids or liquids, whereas the mobile phase can consist of either liquids or gases. The constituents of the mixture exhibit distinct interactions with the mobile and stationary phases, depending upon their solubility in each phase. If the component has a greater affinity for the stationary phase, it will need a longer duration to traverse the column. The varying affinity results in distinct migration rates of the components within the mixture, eventually leading to a divergent rate of pollutant separation. The intensity peaks in chromatograms indicate the occurrence of pollutants throughout time. The identification of a contamination is established by comparing its chromatogram with chromatograms that are like those of the suspected compounds. The ultimate concentration of pollutants was determined by calculating the area beneath the peak of each pollutant. Various forms of chromatography are used to analyze different categories of contaminants. Liquid chromatography is a method used to examine chemical molecules and metal ions that are present in water. Gas chromatography (GC) is used to separate volatile organic compounds, like benzene and ethylbenzene, from automobile exhaust and is used to monitor their presence in drinking water. Thin layer chromatography is a method used to identify and analyze substances such as pesticides, insecticides, and dyes. High-performance liquid chromatography (HPLC) can separate less volatile compounds, such as herbicides. On the other hand, ion exchange chromatography is suitable for isolating inorganic ions, including nitrates and sulphates, from water that has been polluted by excessive fertilizers or leaky septic systems [35].

1.6.2. Atomic absorption spectroscopy

The fundamental concept behind AAS is to analyze absorption of electromagnetic radiation by individual atoms to identify the specific elements present in the water. The absorption peak identifies the precise wavelength value associated with the element, while the intensity indicates the concentration of the element. The concentration is determined using the Beer-Lambert equation.

Atomic absorption spectroscopy has exceptional selectivity, allowing for the analysis of over sixty elements at concentrations equal to or below ppm. Nevertheless, this method requires thorough sample preparation, rendering it more intricate compared to electrochemical devices. Atomic absorption spectroscopy may be classified into two types: Furnace and flame AAS (atomic absorption spectroscopy). In flame AAS, metal ions are first dispersed as a fine mist using a high-temperature flame. Subsequently, these ions are converted into individual atoms that specifically absorb light emitted by hollow cathode lamps. This approach exhibits restricted sensitivity, capable of measuring just a single element at any one moment and demonstrates low linearity. Furnace atomic absorption spectroscopy can detect very low metal concentrations, even in tiny samples, with a sensitivity of more than 1 ppb. Instead of using a flame, a slender carbon tube is used to achieve optimal atomization of the sample, resulting in enhanced sensitivity and detection limits in comparison to flame spectroscopy [36].

1.6.3. Fluorescence spectroscopy

Fluorescence is the result of molecules being stimulated by high-energy electromagnetic radiation. This method is used to quantify organic chemicals found in polluted water. Two methods for recording the fluorescence spectra of polluted water are excitation and emission matrix (EEM) and synchronized fluorescence spectroscopy (SFS). Within the framework of EEM, a three-dimensional scan is captured and shown as a contour plot, which displays the relationship between excitation and emission wavelengths and the corresponding fluorescence intensity. EEM is becoming more often used in the field of water quality investigation for the examination of chromophoric dissolved organic matter (CDOM). CDOM encompasses many chemicals such as humic acids, amino acids, fulvic acids, other decomposing components, and disinfection by-products that arise from water treatment procedures. In SFS, the spectrometer's excitation and emission monochromators are systematically moved with a constant difference in wavelength in order to acquire spectra. Fluorescence spectroscopy is a fast method that offers benefits such as not requiring reagents, requiring little sample preparation, and providing high sensitivity and non-invasive measurements [37].

1.7. Heavy metal, Chromium(VI) on which this research work was conducted

A heavy metal is a metallic element that possesses a relatively high density and exhibits toxicity at low doses. Common heavy metals include copper, chromium, mercury, cadmium, lead and nickel. The Earth's crust contains heavy metals as a naturally occurring element that are indestructible and non-degradable. They enter human bodies in minute quantities through consumption, hydration, and respiration. Certain heavy metals, like copper and zinc are vital for human metabolic processes; nonetheless, elevated levels can result in toxicity. Heavy metals pose a risk due to their propensity for biological accumulation. Bio-accumulation refers to the elevation in quantity of a chemical molecule within a biological component throughout time, relative to its concentration in surrounding environment [38].

1.7.1. Heavy metal Chromium(VI) properties

Cr derives its name from the Greek term ‘chroma,’ which signifies color which is a hazardous metal, present in trace amounts in soils, fauna, volcanic emissions, rocks and similar sources. Chromium can produce numerous salts, with Na and K_2CrO_4 being very common [39]. Starting from Chromium 0 to Chromium 6, it can exist in several oxidation states. Chromium 3 and Chromium 6, among all the valence states, exhibit environmental stability. Chromium 3 is found naturally in vegetation, geological formations, volcanic emissions and soils [40]. Chromium 3 is non-harmful, beneficial to human health. Chromium 6 primarily occur as chromates due to human activities like chromium plating, wood preservatives, pigments, etc. [39]. The chromium in Cr(VI) form provide the most significant risk to environmental health [41]. With changes in pH of solution the species of Chromium 6 changes. At pH less than 1, Chromic acid (H_2CrO_4) is the major form of Chromium 6, while dichromate is the primary species from pH 2 to 4. The $Cr_2O_7^{2-}$ and $HCrO_4^{-}$ ions are in equilibrium when pH ranges from 4 to 6. In mildly acidic medium pH greater than 6.5, the main species is the Chromium(VI) ion [42].

1.7.2. Industrial use of Cr(VI)

Diverse array of Chromium 6 compounds is extensively utilized in the manufacturing of leather tanning, pigments, dyes, chrome plating and textiles serving as catalysts for halogenations, hydrocarbons catalytic cracking and alkylation [43]. In the steel industry, for the production of stainless steel, chromium is utilized as ferrochromium. China is the foremost producer of stainless steel, whereas South Africa dominates chromite ore production, accounting for about fifty percent of global output (Fig. 1.4). In the chrome plating business, dichromates and chromates are utilized to inhibit metal corrosion and enhance paint adhesion. Absorption of dichromate and chromate ions of heavy metals in water are limited, they are employed as dyes [44].

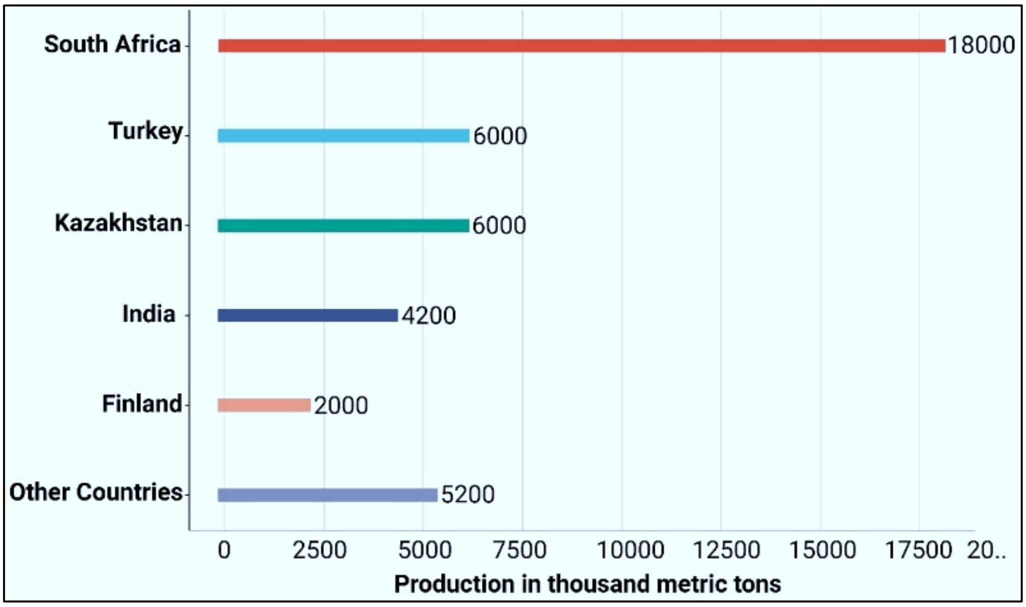


Fig.1.4. Chromium supply percentages in various countries for the year 2023

1.7.3 Impact of Chromium(VI) on ecosystems and human health

Chromium(VI) is considered a significant environmental pollutant. The harmfulness of Cr(VI) is correlated to its oxidation form which is highly dissolvable (e.g., polychromates, dichromates, chromates and chromic acid anhydride), whereas Chromium(III) is less harmful and less movable due to its tendency to form insoluble hydroxides. Nevertheless, prolonged exposure to elevated dosages of Cr(III) can result in adverse health effects [45]. Cr(VI) is usually 500 to 1000 times more poisonous than Cr(III) [46,47]. Typically, through industrial activities Chromium(VI) is released into the atmosphere. Chromium(VI) penetrate profound into the soil and contaminate subterranean water bodies. In various natural water bodies, Chromium(VI) remains stable and experiences modest difference because of low amount of reducing agents [48].

The concentration of chromium typically varies from 0.1 to 0.5 μgL^{-1} in seawater, 0.3 to 0.6 μgL^{-1} in land area and unpolluted river water, and 5 to 50 μgL^{-1} in contaminate river water; however, up to 200 μgL^{-1} , it may rise in severely contaminated water bodies [49]. US Environmental Protection Agency established the chromium limit in crops at 100 μgL^{-1}. In drinking water, Chromium(VI) must regulated to remain below the maximum contamination limit (MCL) of 50 μgL^{-1} [50]. Chromium(VI) can readily penetrate biological barriers and penetrate into the human system by direct skin contact, respiration and oral absorption. It will cause skin ulcers, nasal irritation, gastrointestinal disturbances, renal and hepatic dysfunction, and potentially death [39]. Furthermore, occupational exposure to Cr(VI) compounds results in several clinical issues, including nasal septum perforation, pneumonitis, asthma, dermatitis, bronchitis, and skin allergies, among others [51]. The WHO and the IARC has categorized Chromium(VI) as a major carcinogen [52] [53].

1.7.4. Conventional detecting techniques of Cr(VI)

Different conventional processes for identifying Chromium 6 include atomic absorption spectroscopy, ion chromatography and spectrophotometry [54–58].

1.7.4.1. Atomic absorption spectrometry

The most popular technique for detecting chromium is atomic absorption spectrometry (AAS) [54]. This technique requires breaking down a sample into its constituent atoms before measuring the concentrations of gas-phase atoms using light absorption. An atom in its ground state absorbs energy as light of a specific wavelength. As the quantity of atoms escalates, the energy of light correspondingly amplifies. The light content of an unidentified element can be ascertained by equating it to an average with a known value of the component [59]. When analyzing the concentration of total Cr, AAS is typically utilized. A sample needs to be preconcentrated or separated in order for AAS to distinguish between Cr(VI) and Cr(III) [60]. One of the most used methods for determining Cr(VI) is flame atomic absorption spectrometry (FAAS), which has a high degree of accuracy and precision [61]. However, this approach has certain drawbacks, such inadequate sensitivity or matrix interferences. An online packed column pre-concentration and/or separation stage can resolve these problems. Polyurethane foam (PUF) is among the best sorbent materials for packing columns [62]. It can persist in bases, organic solvents, and acids (except from intense sulfuric and nitric acids). Furthermore, when the temperature reaches 180°C, its structure won't alter [63]. It will therefore undoubtedly be a suitable material for online pre-concentration column packing. Cr(VI) traces, in the form of dithiocarbamate complexes with APDC, refers to a chemical complex formed when a metal ion binds to the ligand "ammonium pyrrolidine dithiocarbamate" can be detected in a mini-column containing unloaded PUF by an online time-based column pre-concentration method for FAASn [61]. The LOD was 2.0 μg L^{-1} at a sample frequency of 36 per hour and a pre-concentration period of 60 seconds. There was a 28-point enhancement factor and a 3.6% relative standard deviation (sr) for precision at a concentration of 50 mg L^{-1}. By enabling matrix removal prior to the atomization stage and extending the analyte's residence time in the atomizer, electrothermal atomic absorption spectrometry could increase the sensitivity, around two to three times when compared to FAAS [64]. Another advancement over AAS is graphite furnace atomic absorption (GFAAS). In GFAAS, an electrically heated graphite tube is utilized in place of the flame used in AAS. Once the sample is deposited inside the tube, the tube is heated to eliminate the main solvents and matrix components. Limitations of identification and selectivity are enhanced when the entire analyte, including the remainder, is atomized [59,65,66]. A novel analytical method for identifying the species of Chromium 6 and Chromium 3 in various aquatic specimens was created recently. Using SPE, DUSSS and ETAAS with nanometer-sized ZrO_2 as the sorbent material, under ideal conditions, the limit of detection for Chromium(III) was 0.48 μg L^{-1} and the limit of quantification was 1.61 μg L^{-1}. For Chromium(VI), corresponding values were 0.27 μg L^{-1} and 0.90 μg L^{-1} [67].

1.7.4.2. Spectrophotometry

Spectrophotometry quantifies the intensity of light as it traverses a sample solution to ascertain the extent of light absorption by a chemical molecule. The absorption or transmission of light of each molecule happens within a defined wavelength range, as per the fundamental principle. In Noroozifar's work, acidic diphenylcarbazide (DPC) was used as the reagent to back extract Cr(VI) in order to reach a LOD of 2.22ngL^{-1} [68]. As a reagent employing chromotropic acid (CA) in an acidic environment, Demetrius G. etc.,[69] reported a straight forward yet efficient spectrophotometric approach to measure Cr(VI) in aqueous solution. The entire detection process was made simpler by using a spectrophotometric detection without a sample has not been concentrated or purified before analysis. Zhao et al. [70] further examined this fundamental reaction among CA and Cr(VI) to detect Cr(VI) by means of an UV- spectrophotometric technique in liquid mixtures, attaining a LOD of 0.024μgmL^{-1}.

1.7.4.3. Ion chromatography

Ions in a sample are separated using ion chromatography (IC). Less than 500 AMU (atomic mass units) an ion exchange resin should be used to preserve the ionic species for ions. A conductivity detector measures the eluting mobile phase's electrical conductivity. This technique can be used to identify species with varying oxidation states in addition to differentiating anions and cations [59]. The concurrent separation of chromium is infrequently accomplished because Chromium(III) inherently present as a positively charged complex ion containing both water molecules (aqua) and hydroxide ions (hydroxo) coordinated to a central metal ion, while Chromium(VI) is predominantly found as chromate anions, chromium oxoanions, or divalent inorganic anions [71]. Two strategies have been used to address this issue. To maintain both species, one method is to employ a mixed-mode column or a series of cation-exchange guard and anion exchange columns [72–74]. In the latter, a derivitization process is involved, wherein Cr(III) forms an anionic complex with a ligand. Anion-exchange chromatography [75–77] can used to separate Cr(VI) and Cr(III).

1.8. Limitations of the above techniques and need for specific sensors

The above techniques discussed for quality gradation of water has certain limitations, which are discussed below.

The high-end analytical instruments are sophisticated and costly. Despite of producing accurate results, they are time consuming, complicated, requires skilled operator. Cost effectiveness and simplicity is a major factor in the research area. Due to these practical difficulties, these methods cannot be adopted for real time quality assessment of water bodies. Chromatography is a labor-intensive method for compound separation, necessitating substantial volumes of solvents, which renders it costly. The automated procedure gets intricate and consequently expensive due to its low separation efficiency.

Atomic absorption spectroscopy can only analyses elements that efficiently absorb light in the UV-Visible spectrum, hence restricting its use to a certain range of elements. The sample preparation process for AAS can be laborious and may include intricate digesting techniques. Fluorescence spectroscopy, while a powerful technique, also comes with certain disadvantages Auto florouscence/ biolumiinescence/ chemiluminescence:

Certain samples, especially biological ones, inherently emanate fluorescence from endogenous fluorophores, potentially complicating the identification of the target molecules.

1.9. Literature survey based on colorimetry and aptasensing sensors for heavy metal detection

1.9.1. UV-Visible colorimetric nanosensors for identification of heavy metal

UV-Visible Colorimetric nanosensors can be classified into two types based on their fabrication method: (1) Sustainable synthesis of nanomaterials and (2) synthetic nanoparticles using chemical or biological methods [78]. The core principle of UV-visible colorimetric nanosensors is the interaction and binding among nanosensors and metal ions, resulting in, change in absorbance. UV-visible optical nanosensors advancement is intricately linked with, selection of assembly materials. Colorimetric detection mechanism can be linked to two factors: the localized surface plasmon resonance (LSPR) phenomenon and the material's nanozyme or nanozyme-like features. Colorimetric sensors based on LSPR are typically constructed using metal nanoparticles, such as gold or silver, due to their distinct absorbance bands. This enables them to exhibit a targeted response to metal with substantial density (heavy metal). The fundamentals of LSPR-based UV-visible optical sensors involves a color change in the metal nanoparticle caused by either its aggregation or etching, resulting from unique absorption band. Nanoscale materials having catalytic properties are utilized in the manufacture of nanozyme-assisted colorimetric sensors to sense less quantities of heavy metals. This nanozyme's catalyst activity augmented or suppressed upon interaction with or adsorption of metal ions onto their surface [79,80]. A color change occurs during certain chemical processes (Table1.3).

Table 1.3. Summaries of the various types of colorimetric sensors

Sl. no	Target analyte	sample	Materials used	Detected color changes	LOD	Linear range	Ref
1	Cu^{2+}	Water	Thiazolylazopyrimidinefunctionalized TiO_2nanosensor (TiO_2-TAP)	Yellow to Red	2.51 nM	0.01–12.5 µM	[81]
2	Fe^{3+}, Cu^{2+}, and Cr^{6+}	Environmental water samples	AgCl NPs	Brown to Light Brown Brown to White Brown to Orange	1.69 ppb for Fe^{3+}; 3.18 ppb for Cu^{2+}; 5.05 ppb forCr^{6+}	0–100 ppb	[82]
3	Cr^{3+} andCu^{2+}	Environmental water samples	Silver Nanoplates	Yellow to Purple Yellow to Colorless	8.0 nM for Cr^{3+}; 0.27 µM for Cu^{2+}	25–400 nM for Cr^{3+}; 0.3–10 µM for Cu^{2+}	[83]
4	Cu^{2+}	Lake water	TMB	Colorless to Blue	0.1 nM	_	[84]
5	Cu^{2+}	Tap water	TMB	Colorless to Blue	0.11 nM	_	[85]
6	Cr^{+6}	Surface water	Alizarin cyanine green (ACG)	_	50 ngL^{-1}	0.2–6.0	[86]
7	Hg^{2+}	Tap, lake and waste water	TMB/COF-AuNPs	Colorless to Blue	0.75 nM	5–300 nM	[87]
8	Hg^{2+}	Tap, river and lake water	TMB/AuNPs@Tp-Bpy	Colorless to Bright Blue	0.33 nM	1–400 nM	[88]
9	Hg^{2+}	Water	L-Cysteine functionalized graphene oxide nanoarchitectonics CGO	Blue color (TMB oxidation)	7.6 μgL^{-1}	0–200 μgL^{-1}	[89]

10	Hg^{2+}	Environmental water samples	Graphene oxide stabilized AgNPs	Yellow to Colorless	0.64 nM	10–100 µM	[90]
11	Pb^{2+}	Environmental water samples	G-AuNPs	Red to Gray	1.07 µM	10–80 µM	[91]
12	Hg^{2+} And Pb^{2+}	Water samples	2-thiazoline-2-thiolfunctionalized AuNPs	Red to Purple (Hg^{2+}); Red to Blue (Pb^{2+})	~100 ppb	0.1–10 µM	[92]
13	Cu^{2+}	Tap water and green tea	TMB/Au@PtNHs	Blue to Deep Blue	4.0 nM	20–500 nM	[93]
14	Cd^{2+} and Ni^{2+}	Environmental water samples	Ag NPs	Yellow to Pale Yellow	0.2 nM	0.05–100 µM	[94]
15	Hg^{2+} and Cd^{2+}	Water samples	ssDNA (Hg) functionalized Mn_3O_4NPs	Green to Yellow	3.8 μgL^{-1} for Hg^{2+} and 2.4 μgL^{-1}forCd^{2+}	Not reported	[95]
16	Hg^{2+}	Blood and waste water	TMB/g-3N4/CeO_2	Blue to Colorless	0.085nM	0.20 nM -1.0 µM	[96]
17	Hg^{2+}	Yellow River and Tap water	TMB/MoS2-Au	Colorless to Blue	5 nM	0.02–20 µM	[97]
18	Hg^{2+}	Jialing River	TMB/2D Ni/Fe MOFs	Colorless to Blue	0.1–200 µM	100 nM	[98]
19	Hg^{2+}	Environmental water samples	Aptamer-modified cationic AuNPs	Blue to Red	4.9×10^{-11} M	$8.2\ \times10^{-10}$ M~6.2×10^{-8} M	[99]
20	Cr^{6+}	Tap water and lake water	TMB/Au@Hg	Colorless to Intense Blue	0.71 nM	1 nM-2 µM	[100]
21	Hg^{2+}	Aqueous solution	Ag NPs	Yellow to Pink	5×10^{-6} M	Not reported	[101]

1.9.2 Portable electrochemical nanosensors for the identification of heavy metal in the environment samples

The electrochemical aptamer sensor, composed of biomolecular elements and signal transducers, has attracted growing interest for the advancement of portable electrochemical nanosensors (Table 1.4). The signal sensor generally consists of a substrate for electrodes, an electrochemical signal detecting equipment and an enhanced layer. The main substrates for the electrodes include screen-printed electrodes, GCE, reduced GO, indium tin oxide and gold electrode. Diverse electrode materials demonstrate varying degrees of signal amplification influenced by a layer that has been correctly adjusted, with a different nanomaterial available for this modification. Aptamer is immobilized to the electrode's surface by interactions between molecules with the changed layer. This procedure will alter electrode's impedance and induce variation in current. Consequently, the alteration of nanomaterials in electrodes is a crucial aspect of electrochemical sensor fabrication. Electrochemical signal identification device consists a signal amplifier, a processing unit, and a display device. The electrochemical sensor has become broadly utilized in present time owing to its compactness and high effectiveness, great sensitivity and heightened specificity. Fig.1.5 illustrates a year-on-year increase in the publication of articles and patents containing the keywords “Electrochemical” and “Aptamer” from 2012, with data for 2022 available only up to March [102].

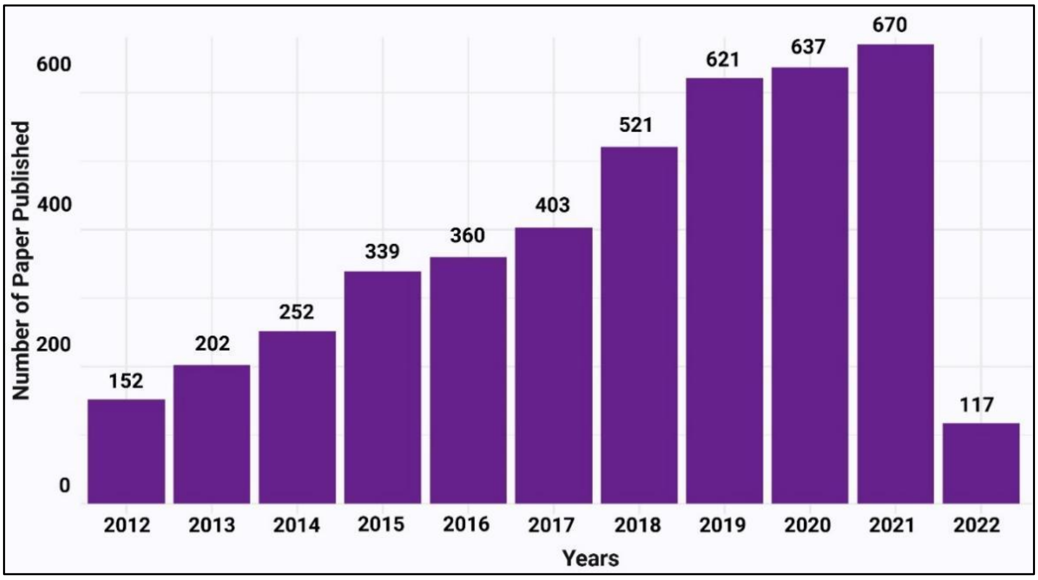


Fig. 1.5. Website knowledge statement detailing with quantity of publications and patents concerning the implementation of the E-apt sensor.

Table 1.4. Utilization of the electrochemical biosensor for detection of heavy metal ions

Serial no.	**Target**	**Sample**	**Electroc-hemical methods used**	**Aptamer sequence**	**LOD (nM)**	**Linear range (nM)**	**Ref.**
1.	Hg^{2+}	Lake water	DPV	5'-SH-$(CH_2)_6$-AAAAATTTCCTTTGCTTT-3'	0.03	0.01~100	[103]
2.	Hg^{2+}	Tap water	EIS	5'-CCCCCCCCCCCCTTCTTTCTTCCCCTTGTTTGTT-30'	0.071	0.1~50	[104]
3.	As^3	River water	DPV	5'-HS-GGTAATACGACTCATAAGGGAGATGCTTATTCAATTTTACAGAACACCAAGTCGCTTACTTCTTCATCGAGATAGTAAGTGCAATCT-3'	4×10^{-5}	0.13~130	[105]
4.	Hg^{2+}	Water	EIS	5'-TTTCTTCTTTCTTCCCCCCTTGTTTGTTT-3'	25	25~500	[106]
5.	Cd^{2+}	River water	EIS	5’-ACCGACCGTGCTGGACTCTGGACTGTTGTGGTATTATTTTT GGTTGTGCAGTATGAGCGAGCGTTGCG-3'	0.275	1~1. 10^6	[107]
6.	Hg^{2+}	Juice	SWV	5'-MB-CGCTTTAGATG-3'	1.79	10~100	[108]
7.	Cd^{2+}	Lake water	PEC	5'-GGACTGTTGTGGTATTATTTTTGGTTGTGCAGTATG-3'	1.8×10^{-3}	5×10^{-23}-29	[109]
8.	Cd^{2+}	Tap water	DPV	5'-ACCGACCGTGCTGGACTCTGACTGTTGTGGTATTATTTTT GGTTGTGCAGTATGAGCGAGCGTTGCG-3'	5×10^{-5}	1×10^{-3} ~ 10	[110]
9.	Hg^{2+}	Lake water	DPV	5'-(NH_2C_6)-CTT GCT TTC TGT-3'	5×10^{-3}	0.025~1 $\times10^{-6}$	[111]
10.	As^{3+}	Tap water	EIS	5'-TGATGTTTGTTTACGCATGTGTGAGGAGGCTGGGGTGATG AATCCCAATCCC-3'	0.26	1.3~6.5	[112]
11.	Pb^{2+}	Serum Water	EIS	5'-HS-TTTTTTCGATAACTCACTATrAGGAAGAGATG-3'	1.67×10^{-3}	5×10^{-3}~1	[113]
12.	Hg^{2+}	Lake water	DPV	5’-SH-$(CH_2)_6$-ACCGTGTTTGCCTTTGAC CTC-3’	0.03	0.1~100	[114]

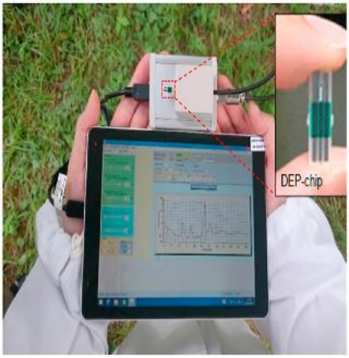
13.	Pb2+	Spring water	DPV	5'-GGGTGGGTGGGTGGGT-3'	4.3×10^{-9}	1.0×10^{-8}~ 5.010^{-5}	[115]
14.	Pb^{2+}	Tap water	DPV	5'-GGGTGGGTGGGTGGGTAT-3'	0.018	0.05~1 $\times10^{3}$	[116]
15.	Hg2+	Tap water	ECL	5'-NH2-TTGTTTGTCCCCTCTTTCTTA-(CH2)3-SH-3'	0.01	0.05~1 $\times10^{3}$	[117]
16.	Hg2+	Tap water	PEC	5'-NH2-(CH2)6-TTTTTTTTTTTTTTTTTTTT-3'	3.33×10^{-6}	1×10^{-5}~10^{-3}	[118]
17.	Pb2+	Tap water	PEC	5'-NH2-(CH2)6-TTGGGTGGGTGGGTGGGT-3'	0.05	0.1~50	[119]
18.	Hg^{2+}	Water	ECL	5'-amino-$(CH_2)_6$-O-TCTCCAGCGTCGTTTGTTTGCGGGAGCTTTCTTAAATCTCGAGCTAAA-3'	4×10^{-5}	1×10^{-4}~0.01	[121]
19.	Pb^{2+}	River water	ECL	5'-SH- $(CH_2)_6$-TTTTTACCCAGGGTGGGTGGG-TGGGT-(CH2)6-NH2-3'	3.82×10^{-6}	1.0×10^{-5} ~ 1.0×10^{-2}	[121]
20.	Hg^{2+}	River water	DPV	5'-COOH-CTTCTTCCCCCCCCTTCTTC-SH-3'	2.9×10^{-3}	0.01~1 $\times10^{5}$	[122]
21.	Cd2+	Tap water	SWV	5'-$HS(CH_2)_6$GGACTGTTGTGGTATTATTTTTGGTTGTGCAGTATG-3'	0.014	0.1~5	[123]
22.	Pb^{2+}	Reservoir water	PEC	50-NH2-(CH2)6-TTGGGTGGGTGGGTGGGT-P-30	1.67×10^{-5}	5×10^{5}~1×10^{3}	[124]
23.	As^{3+}	Tap water	EIS	5'-TGATGTTTGTTTACGCATGTGTGAGGAGGCTGGGGTGA TGAATCCCAATCCC-3'	0.26	1.3~6.5	[125]
24.	Hg^{2+}	Tap water	DPV	5'-SH-$(CH_2)_6$-TCATGTTTGTTTGTGGCCCCCCTTCTTTCTTA-Fc-3'	5×10^{-3}	0.01~500	[126]
25.	Pb^{2+}	Tap water	DPV	5'-MB-GGTGGTGGTGGTTGTGGTGGTGGTGG-3'	5.1×10^{-7}	1×10^{-6}~1	[127]
26.	Hg^{2+}	Tap water	SWV	5'-SH-AATTCTCTCTTCGACGTTGTGTGTT-3'	1×10^{-4}	2×10^{-3}~20	[128]
27.	Cd^{2+}	Lake water	PEC	5'-SH-GGACTGTTGTGGTATTATTTTTGGTTGTGCAGTATG-NH2-3'	0.011	0.03~40	[129]

28	Hg^{2+}	River Water	SWV	5’-SH-(CH2)6-CTGTTTTCTTTCGGACGA CCCCCCTCGTCCGT TTGTTTTCAG-MB+-3'	0.094	1~5 ×10^3	[130]
29	Pb^{2+}	River water	PEC	5'-NH_2-$(CH_2)_6$-TTGGGTGGGTGGGTGGGT-3'	0.34	1~1 ×10^4	[131]
30	Pb^{2+}	Tap water	DPV	50-GGGTGGGTGGGTGGGTAT-30	0.018	0.05~1 ×10^3	[132]
31	Hg^{2+}	Tap water	DPV	5'-Bio-TCTTTCTTCCCTTGTTTGT-3'	0.33	1~200	[133]
32	As^{3+}	Tap water	EIS	5'-TGATGTTTGTTTACGCATGTGTGAGGAGGCTGGGGTG ATGAATCCCAATCCC-3'	0.26	1.3~6.5	[125]
33	Cd^{2+}	Valley water	DPV	5'-GGGGGGGGACTGTTGTGGTATTATTTTTGGTTGTGCAGT-MB-3'	6.5 ×10^{-7}	1×10^{-6}~1	[134]
34	Hg^{2+}	River water	SWV	5’-SH-$(CH_2)_6$-CTGTTTTCTTTCGGACGA CCCCCCTCGTC CGTTTGTTTTCAG-MB+-3'	0.094	1~5 ×10^3	[135]
35	Pb^{2+}	Tap water	PEC	5'-TTGGGTGGGTGGGTGGGT-3'	3 ×10^{-4}	1 ×10^{-3}~5	[136]
36	Pb^{2+} As^{3+}	River water	EIS	5'-CAACGGTGGGTGTGGTTGG-3' 5'-GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTTTACAGA ACAACCAACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTGCAATCT-3'	2.27 ×10^{-3} 6.73 ×10^{-3}	0.01~10.0	[137]

1.9.3 Field-portable available commercial instruments for detection of heavy metals

The commercially accessible handheld devices that are extensively utilized and circulated for heavy metals detection is discussed here. The integration of several components for signal generation, transduction, and reception, target recognition and processing signal conclude in a commercially viable heavy metal ion recognition device, superseding conventional sensors for signals and workstations. A number of tools and materials have been examined for miniaturize the units [138]. Digitization have been employed to establish the quantifiable study of heavy metal. The objective of shrinking is attainable, thanks to sophisticated computer technologies, including neural networks, Big Data, and, cloud computing, enabled by intelligent processors [139]. The recent advancements in LEDs, charge-coupled device cameras, corresponding metal-oxide-semiconductor, smartphones, and miniaturized electronic components aid the creation of handheld sensing instruments used for the analysis of electrochemical or optical signals. This introduces an innovative method to replace conventional large and expensive components by including a smart phone, camera, for quantitative detection that is portable, quick, and affordable. Table 1.5 enumerates an array of commercially available equipment for identifications of heavy metals.

Table1.5. Commercially available equipment for the identification of heavy metals

S.No.	Handheld Device	Heavy Metals	Detection Principle	Image	Specifications	Ref.
1.	uMED	Pb, Cd, Zn	SWASV		Limit of Detection = 4 µg^{-L} - suitable for analysis in limited resource condition - Rapid Detection and screening	[140]
2.	DEP-Chip	Cu, Cd, As, Pb, Zn	DPV		Limit of Detection = 15.5 µg^{-L} (Cu), 2.6 µg^{-L} (Cd), 5.0 µg^{-L} (As), 4.0 µg^{-L} (Pb), 14.4 µg^{-L} (Zn). - DEP-On-Go	[141]

3.	Portable heavy metal ion detector INE-SJB-801	Zn, Hg, Cd, As in consuming water and Cd in rice analysis	ASV		Low cost than AAS ➢ The GCE system offers accuracy, a low detection limit, and suitability for precision measurements. ➢ Equipped with a SPE test device is ideal for speedy on-site detection.	[5]
4.	ANDalyze	Pb, Zn, Cd, Cu	Fluorescence-based sensing		Limit of Detection = 2 μg^{-L} (Pb), 1 μg^{-L} (Zn), 0.6 μg^{-L} (Cu), 0.1 μg^{-L} (Cd) ➢ Rapid identification (Less than 2 min) ➢ Digital outcome monitoring ➢ One-minute re-sampling time ➢ No toxic substances, eco-friendly ➢ Intuitive detection protocol requiring no user training ➢ Highly sensitive and selective	[5]

5.	AppliTrace	Cd, As, Cu, Pb, Fe, Zn	ASV		Limit of Detection = 1 μg^{-L} - Pre-concentration techniques for enhanced quantification - Hot acid Digestion	[5]

1.10. Objectives

This work focuses on progression of Nanosensors for identification of Chromium(VI) in water. The Electrochemical-based sensors enable direct, reliable and reproducible measurements. There is an urgent need for development of nanosensor with simple sample preparation procedure, resulting in lower cost, faster response and user-friendliness for determining existence of Chromium(VI) in water.

The following summarizes the goals of the thesis work:

- Determination of heavy metal pollutant, Cr(VI) in water using the conventional techniques.
- Development of electrochemical nano-biosensor, spectroscopic instrumentation for detection of heavy metal pollutant, Cr(VI) in water.
- Portable, onsite, real time, affordable electronic system anchored with advanced embedded system technology.
- Field testing of the developed prototype and validation.

1.11. Thesis layout

In view of the above-mentioned objectives, the entire work has been classified into five chapters. A brief chapter wise organization of the thesis is presented below.

Chapter 1 describes the purpose of this work and exemplifies the goals of this thesis work. Water pollution is a major environmental issue. The heavy metal, Cr(VI) pollution in water creates various human health hazards. The Cr(VI) pollution in aquatic environment has been discussed in details. The various conventional technologies used for detection of heavy metal, Cr(VI) are included. Nanosensors provide the most advanced detection technique for pollution analysis, encompassing water quality assessments. Various types of nanosensors and their features include portability and the flexibility for on-site deployment etc. are also included. The challenges in development of nanosensor technology for discovery of pollutions in water is discussed. Literature survey regarding the usage of nanosensor technique for ifentification of heavy metal, Cr(VI) in water has been done thoroughly.

Chapter 2 discusses the hardware device used and developed in the present study. Different types of nanosensors and chemosensing systems have been discussed. Design, characteristics and principle of sensors are included. The development and identification of suitable aptamers through SELEX technology have been discussed. Transducers based Biosensors and Poetentiometric biosensors used in present work are included.

Chapter 3 introduces the advancement of a portable image analysis apparatus integrated with TMB as a sensing agent for the detection of chromium 6 in aquatic environments. The optimization of sensor parameters, including reagent concentration and reaction time, was conducted for sensor development and validation utilizing a commercial UV-Vis spectrophotometer. The relevance of a chemoreceptor combined with UIIS for identification of Cr(VI) has been examined. This study reports that the TMB-coupled field-portable sensing system utilizes image processing technology to detect a broad spectrum of Chromium(VI) in water bodies.

Chapter 4 provided a portable potentiostat for measurement of trace concentrations of chromium 6 in aquatic bodies. This study utilized a DNA aptamer which is specific for Chromium(VI) mounted on a SPE as the primary biosensor. The instrument functions by utilizing electronic peak current, dumping event via mass deposition, involving an aptamer conjugated with Chromium(VI) on the WE. This chapter elucidates experimental and characterization information. The efficacy of the created gadget has been confirmed for seven distinct categories of water samples. This is a basic, resource-constrained, on-site aptasensing system for the recognition of chromium 6 in aquatic bodies.

Chapter 5 shows general outline of the work done and highlights the finishing up comments. The important points and weaknesses of the proposed framework have been examined here with certain suggestions and a few proposals are presented which may be taken up in future.

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Chapter 2

Hardware device used for the development of Nanosensor for detection of Cr(VI)

List of Sections

- **Introduction**
- **Nanosensors**
- **Chemosensing system**
- **Biosensor**
- **Types of working electrodes used for this work**
- **The device used in the present thesis for chemosensor development (uniform illumination imaging system)**
- **The device used in the present thesis for potentiostat development (electrochemical aptasensing technology)**

Chapter 2

Hardware device used for development of Nanosensor and detection of Cr(VI)

2.1. Introduction

A sensor is a device that identifies alterations in physical parameters, like humidity, temperature, motion, pressure, force, electrical metrics like current and transforms these variations into signals for detection and analysis [1,2]. A transducer is an instrument that converts energy from one form to another. A sensor serves as essential component of a measuring apparatus. Idyllic sensor must exhibit particular properties, including, range, calibration, sensitivity, drift, high resolution, linearity, reaction time repeatability, selectivity and reproducibility [3,4]. Advancement of sensor technology has gained significance due to its diverse uses, including food quality monitoring, healthcare, environmental and medical diagnostics, automotive and industrial production, along with space, security and defense [5].

Recent advancements in nanomaterial technology facilitate the construction of innovative miniature nanomachines. The nanomaterials can be classified in three different categories: quantum dots, carbon-based nanomaterials and metallic nanoparticles. Diverse metal nanoparticles, including magnetic iron, copper, gold, zinc, and silver, have been widely employed in numerous nanosensor applications [6]. The exceptional capability of these nanoparticles to interact well with targeted analytes (e.g., heavy metals, pathogens, antibodies, DNA) has been utilized in the development of meticulously targeted nanosensors [7].

Nanoscale substances like quantum dots are composed of semiconductor materials. Those are recognized for their nanoscale (1-10 nm) sizes and possess distinctive optical characteristics compared to conventional fluorescent dyes. They possess highly effective broad narrow and excitation ranges, adjustable emissions dependent upon size[8]. The elevated sensitivity of fluorescence emission offered by these Quantum Dots can be employed to develop effective selective nanosensors [9]. New nanosensor employing Graphene quantum dots for the identification of Metronidazole in biological samples has recently been developed [10]. Graphene and CNTs are frequently used in the construction of nanosensors due to their superior thermal conductivity, extensive surface area and exceptional electrical conductivity [11]. The accuracy of an electrochemical sensing unit has been improved by augmenting the sensitivity of glassy carbon electrodes and screen-printed carbon electrodes [12].

The miniaturized nanosensors design will be beneficial due to substantial advancements in nanomaterials and nanotechnology, facilitating the creation of highly selective nanosensors capable of probing earlier inaccessible domains, like pollutant detection and disease verdict. A general nanosensor node [13] comprises an energy supply module, a communication component, a processing remote node equipped with nano memory, and a sensing and actuation module [14].

The presence of Contaminants creates a problem in the air, soil, and water, among other environmental media. Heavy metals, nutrients, microorganisms, and precedence pollutants such as pesticides, industrial chemicals and petroleum hydrocarbons are examples of water contaminants. Moreover, like Pharmaceuticals, misuse medications, surfactants, personal hygiene products, hormones, plasticizers, steroids, insecticides, industrial additives, flame retardants, perfluorinated compounds, gasoline, and their byproducts are only a few of the many substances that are considered emerging pollutants [15]. There is a risk to human health since many environmental pollutants can endure in the environment for extended durations and often traverse extensive soil regions before contaminating water supplies [16]. To address the increasing demands of water requirements for both the quantity and reliability of information provide, in addition to sensitivity and rapid selectivity, innovative devices and methods, such as nanosensors, are under investigation [17,18].

2.2. Nanosensors

Nanosensors represent most recent advancements in identification technology for pollutant detection, encompassing water quality assessment, food analysis and quality control evaluation. Nanosensors offer benefits including portability and the ability for on-site deployment. Various advantageous attributes, such as elevated selectivity and sensitivity, position nanosensors as a prominent trend in forthcoming detecting technology [19]. Nanosensors can identify biological and chemicals substances in aquatic bodies. Nanosensors are regarded as a novel method for water quality monitoring because of its durability, sensitivity, and precision in measurement [20,21]. The behaviour and properties of nanoscale particles can be seen and communicated at the macroscopic level through the use of a nanosensor. For use in nanoscale sensors, nanoparticles have specific chemical, mechanical, and optical characteristics [22]. Nanomaterials or biomolecules [23,24] may be employed as recognition components, and they can be directly coupled to an optical, electrochemical, or mechanical physical transducer. The signal generated by the interaction of recognition elements with samples such as heavy metals, hydrocarbons, pesticides, toxins, and endocrine-disrupting substances can be processed and amplified [25]. An electrical signal is produced by transducer from a physicochemical change that is caused by a nanosensor's sensitive layer, or recognition element, reacting to chemical, concentration, mechanical, gravitational, thermal, magnetic, volume, electrical stimuli [22,26] (Fig. 2.1).

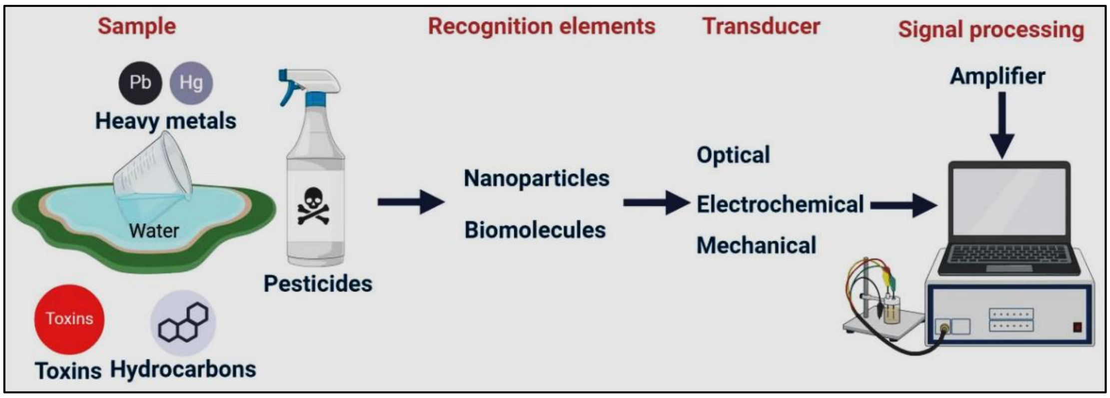

Fig. 2.1. Graphical representation of primary components and detection methodology of nanosensors

2.2.1. Different types of nanosensors

Nanosensors are engineered to react to real-world optical, chemical, or biological events by translating them into an electrical signal. Nanosensors can be divided into four main categories [27,28] (Fig. 2.2).

2.2.1.1. Mechanical nanosensors

Mechanical nanosensors detect changes in physical properties like pressure, force, and mass at the nanoscale. They operate based on the principle of mechanical resonance, where the sensor's mechanical structure resonates at a specific frequency when exposed to physical stimuli. Mechanical nanosensors are used in various fields, including environmental monitoring, structural health monitoring, and medical diagnostics. They can detect tiny changes in pressure or mass, making them ideal for high-precision applications [29].

2.2.1.2. Optical nanosensors

Using the optical properties of nanomaterials, optical nanosensors function. Applications for them include the healthcare, chemical industry, human protection and environmental security [30]. Utilizing nano-optical devices, a technique for early cancer detection has recently been presented [31]. The mechanism of action relies on modifications in optical characteristics (such as absorption, transmission, emission, etc.) brought about by the analyte's interaction with an immobilized indicator.

2.2.1.3. Chemical nanosensors

These are based on nanomaterials may exhibit altered optical characteristics due to factors such as target-induced aggregation or anti-aggregation and surface modifications [18].Chemical nanosensors are designed to measure numerous parameters, including gas concentrations, the presence of certain molecules, or particular chemical structures [14]. Operational mechanism of them relies on alterations in the electrical characteristics of carbon nanotubes (CNT) or graphene nanoribbons (GNR) due to the absorption of various types of molecules. The principle of response is based on a chemical reaction involving the emission, reception, or consumption of electrons by ions. A chemical reaction occurs among a ligand and a target analyte, leading to a quantifiable alteration in the transduced signal, like electric current or voltage [32]. The electrochemical signal is quantified and examined in the sample solution when the analyte of interest is present. There are various sorts of electrochemical detection methods that can be classified based on the signal they produce. These include amperometry, voltammetry, electrochemical impedance spectroscopy and potentiometry [14,33].

2.2.1.4. Biological nanosensors

These are analytical instruments that integrate a molecular recognition element with an electrical component to generate a measured signal and identify specific substances in a target. The recognition phenomena generate a biological signal, which is subsequently converted into a quantitative measure by the transducing unit. The resulted signal can be visualized through optical means such as luminescence, fluorescence, or surface plasmon resonance, or through electrical methods like capacitance, impedance, or voltammetry. It can also be detected using magnetic field, flux, permeability, or other formats. This signal indicates the existence of many biological molecules in the environment [34,35].

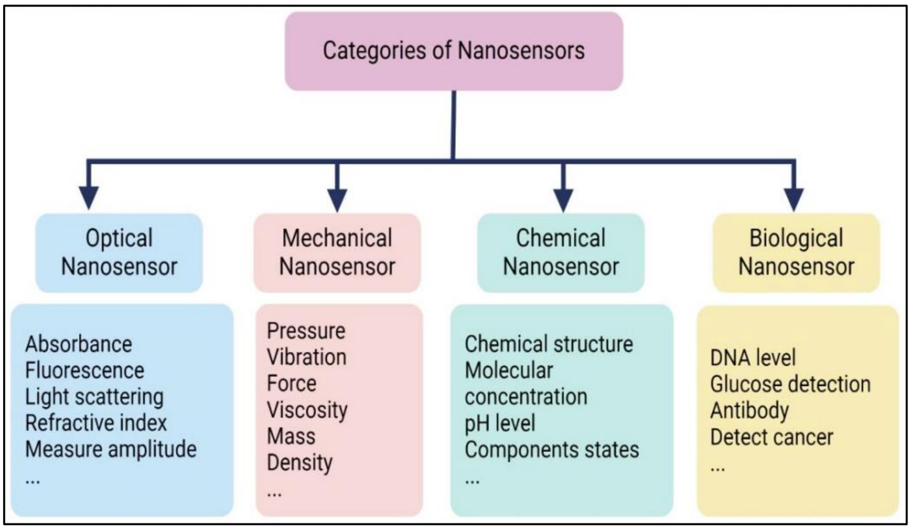

Fig. 2.2. Different categories of Nanosensors

2.3. Chemosensing system

A chemo-sensor is a different name for a chemical sensor. It is a instrument that transforms chemical data into a dependable and appropriate signal for examination. The "Cambridge definition" provides a suitable and accurate definition of a chemical sensor. Chemical sensors are small devices that can provide immediate and continuous information about existence of particular chemicals or ions in even complicated samples. Chemical sensors utilize specialized transduction mechanisms to obtain information about analytes.

The chemical information can originate from either a chemical reaction including the analyte or a physical feature of the system under investigation [36]. Chemical sensors generally comprise a receptor module and a transducer module. The receptor component of a sensor transforms chemical information into a distinct kind of energy that is quantifiable by the transducer. A transducer is a device that can translate the chemical information contained in a sample into a suitable analytical signal. The transducer exhibits a deficiency in selectivity [37]. Chemo-sensors can be categorized into two kinds based on the type of signal generated by the signaling sub unit.

2.3.1. Colorimetric chemosensor

A colorimetric chemosensor refers to the alteration in color that takes place following the interaction of a receptor with a particular analyte [38]. The chemosensor's signaling unit undergoes a color change after binding the receptor and the analyte [39]. Colorimetric chemosensors have garnered considerable attention because they offer the potential to gather qualitative and quantitative data using only visual observation, without the need for complicated methods. These phenomena are derived from the modification of electronic properties by intra/intermolecular charge transfer (ICT) [40–42].

A chemosensor utilized in the colorimetric approach must incorporate a chromophore group as signaling unit. Chromophore is accountable for hue of chemosensor in its unbound state and the distinct alteration in color following its contact with a specific analyte. Hence, the colorimetric approach operates by quantifying the alteration in absorbance subsequent to the inclusion of the analyte [43]. The colorimetric chemosensor has been employed in this investigation to detect Chromium(VI) in aquatic system.

2.3.2. Fluorescent chemosensor

Fluorescence chemosensors are chemical species that possess enhanced fluorescence properties, allowing them to interact with the analyte and produce easily quantifiable changes in various properties such as electrical, electronic, thermal, magnetic, and especially optical alterations [44–47]. Typically, optical signaling chemosensors are created by utilizing the changes in light-related properties when detecting several types of substances, including metal cations, anions, neutral molecules, and small biological structures, through the use of supramolecular host guest chemistry [48–52]. Fluorescent chemosensors are compounds that consist of a binding site, a fluoroscope, and a mechanism for communication among these two sites. Fluorogenic sensors that include, PET, CHEF, ESIPT, TBET, FRET and excimer–exciplex synthesis [53–59].

The colorimetric chemosensor agent includes a sensing mechanism that leads to a naked eye color change when interacting with the analyte. In the meantime, the fluorometric chemosensor agent works over two mechanisms, i.e., fluorescent-OFF à ON and fluorescent-ON à OFF (Fig. 2.3).

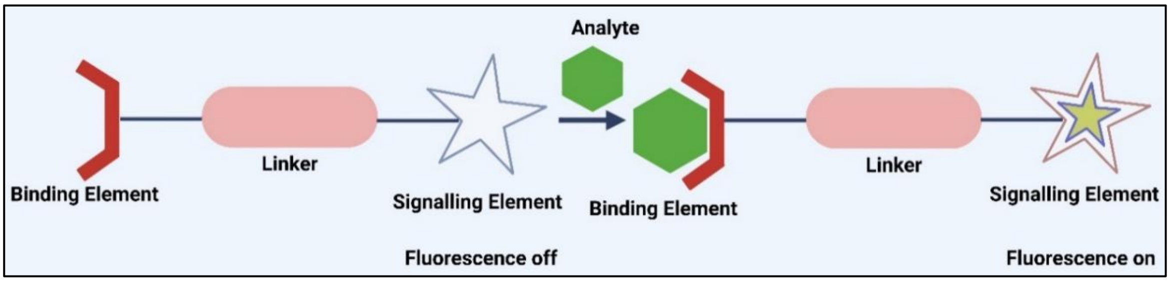

Fig. 2.3. The working principle of fluorescent chemosensor

2.3.3. Need for Chemosensor

There are numerous instrumental techniques available for monitoring ionic analytes, as well as inductively AAS, flow-injection analysis, ICP-MS, flame photometry, ion chromatography, ion-sensitive electrodes, capillary electrophoresis, electrochemical methods, neutron activation analysis, and spectrophotometric methods. Still, these methods are expensive and require a significant amount of time. In addition, they need a large quantity of samples [60]. Optical sensing technologies have gained significant attention in recent times owing to their superiority over other approaches. Among these methods, chemo-sensors are particularly renowned for their exceptional selectivity and sensitivity towards a wide range of harmful ionic analytes. These sensor technologies are simple to use.

2.4. Biosensor

2.4.1. Principle and design

A biosensor is an apparatus that includes a biological component, like enzyme, nucleic acids, antibody and lectins etc. with an electrical component to provide a measurable signal. The electronic component records, identifies and communicates data regarding physiological alterations or the presence of numerous biological or chemical substances in the atmosphere. Biosensors are of separate dimensions and forms and possess the capability to identify and quantify even minute amounts of particular diseases, harmful substances, and pH conditions. A biosensor typically involves of five key components: an analyte, a bioreceptor or molecular recognition element, electronic circuitry, a transducer and a display (Fig.2.4) [61].

(a) **Analyte:** A chemical that is being identified or detected that is of interest, such as heavy metal, ammonia, lactose, glucose and alcohol.

(b) **Bioreceptor:** A biomolecule capable of identifying the specific substrate, also known as an analyte, is referred to as a bioreceptor. Examples of bioreceptors contain cells, deoxyribonucleic acid (DNA or RNA), antibodies, aptamers and enzymes. Bio-recognition refers to the process of signal synthesis, which can take the form of light, pH, heat, tissue of plant or animal, microbial products, and, charge or mass change. This signal production occurs throughout the conversation between a bioreceptor and an analyte [5].

(c) **Transducer:** The apparatus that changes the form of energy. It changes the bio-recognition occurrence into a quantifiable electrical signal that reflects amount or existence of a biological or chemical objective. The transducer is a crucial component in a biosensor. The method of converting energy into signals is referred to as signalization. Transducers are classified into several categories based on their operating principle, including optical, electrochemical, gravimetric, and electronics transducers[62]. Transducers generate optical or electrical signals which are directly linked to the number of interactions between analytes and bioreceptors.

(d) **Electronics:** The signal that has been converted is analyzed and prepared for presentation. The transducer's electrical impulses are amplified and transformed to a digital format. The signals that have been processed are quantified by the display unit.

(e) **Display:** It is a unit comprises a user interface device, like a computer system or printer, which produces results in a format readily understandable by the user. The result may manifest as a numerical, tabular value or figure, graphical, dependent upon the end-user's specifications [5].

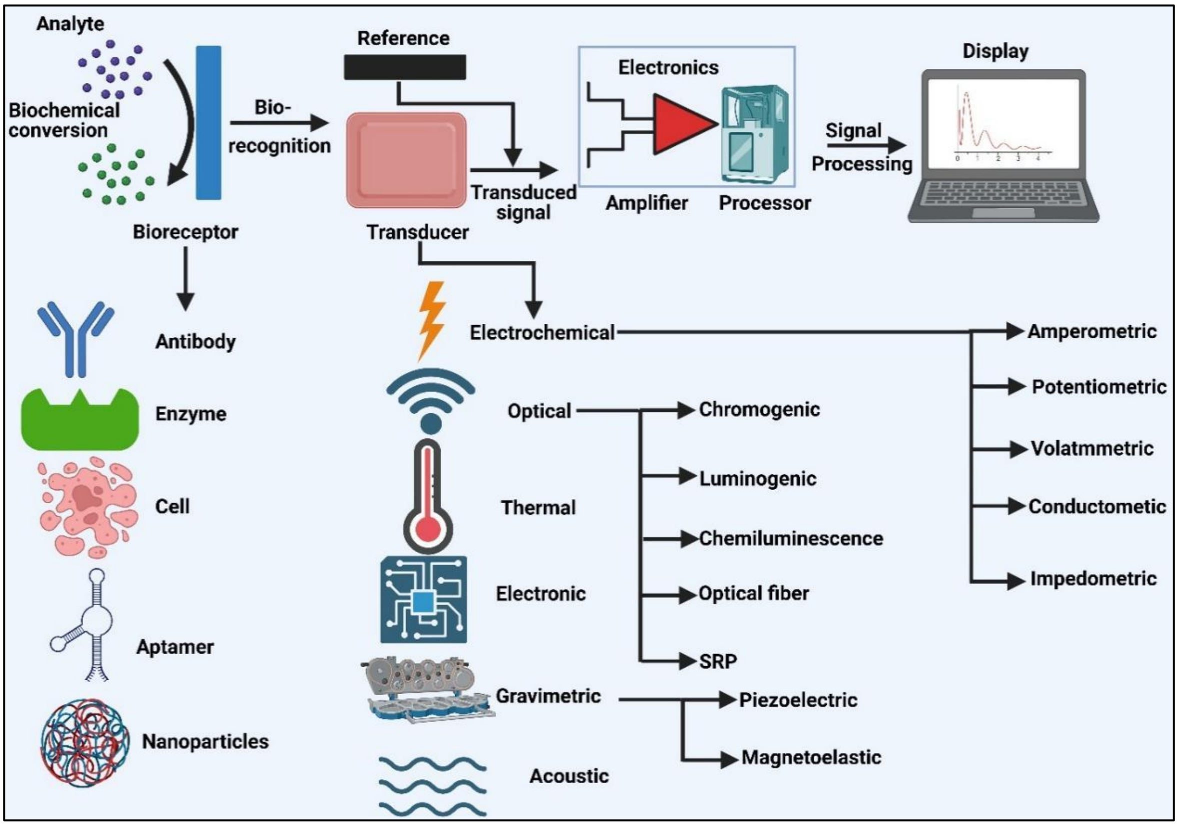

Fig. 2.4. Schematic diagram of a conventional biosensor comprising a bioreceptor, transducer, electrical system (including processor and amplifier), and display (such as a Personal Computer or printer), along with numerous categories of bioreceptors and transducers utilized in biosensors

2.4.2. Characteristics of biosensors

In order to create a biosensor system that is both efficient and capable, dynamic criteria and specific static must be met. By adhering to these specific requirements, the efficiency of the biosensors can be enhanced for commercial applications [63–65].

(a) **Selectivity:** Selectivity is a vital characteristic to contemplate while choosing a bioreceptor for a biosensor. A bioreceptor has the ability to identify a particular target analyte molecule in a sample that contains a mixture of spices and undesired impurities.

(b) **Sensitivity:** To validate the existence of traces amount of analyte in the sample, the lowest amount of analyte that can be reliably identified in the fewest procedures and at low concentrations (nanograms per milliliter).

(c) **Linearity:** Linearity enhances precision of measured outcomes. Greater linearity corresponds to increased sensitivity in detecting substrate concentration.

(d) **Response time:** The duration essential to obtain 95% of outcomes.

(e) **Reproducibility:** The capacity to produce accurate and consistent findings when measuring a sample more than once is known as reproducibility. It also includes a sensor's ability to produce a mean value with each measurement that is nearer the genuine value. The biosensor's reproducibility refers to its capability to consistently generate identical results while measuring the same sample multiple times [5].

(f) **Stability:** Stability is a crucial feature in biosensor applications that necessitate ongoing monitoring. Degree of vulnerability to environmental disturbances together inside and outside biosensing apparatus is referred to as stability. Two primary elements affect a system's stability: the bioreceptor's affinity, or the strength of bond between the bioreceptor and the analyte and the bioreceptor's gradual disintegration [5].

2.4.3. Biosensors classification

The arrangement of biosensors encompasses a wide range of disciplines and areas of study. The classification of biosensors involves multiple criteria, as depicted in Fig. 2.5. Bioreceptors are the main component employed in the development of biosensors, as previously mentioned. The Biosensors are categorized into different classes based on the type of bioreceptor they use. The immunosensors are highly specific and sensitive, which are particularly useful in diagnosis. For microbial strains and analytes that contain nucleic acid, aptamer-based biosensors exhibit a high specificity. Finally, there are whole-cell or microbial biosensors. Sensors are categorized as electrochemical (amperometric, conductometric, potentiometric and impedance), optical, thermal biosensor, mass-based or gravimetric, electronic biosensorin the second classification, which is based on the transducer. Bioreceptor-analyte combinations are included in another category, however they have a few limitations. Classifications are based on the type of detection method used, such as electrical, optical, electronic, mechanical, thermal and magnetic. They can also be categorized based on the technology employed, such as biosensors-on-chip (lab-on-chip), nano, deployable, surface plasmon resonance (SPR) and electrometers [5].

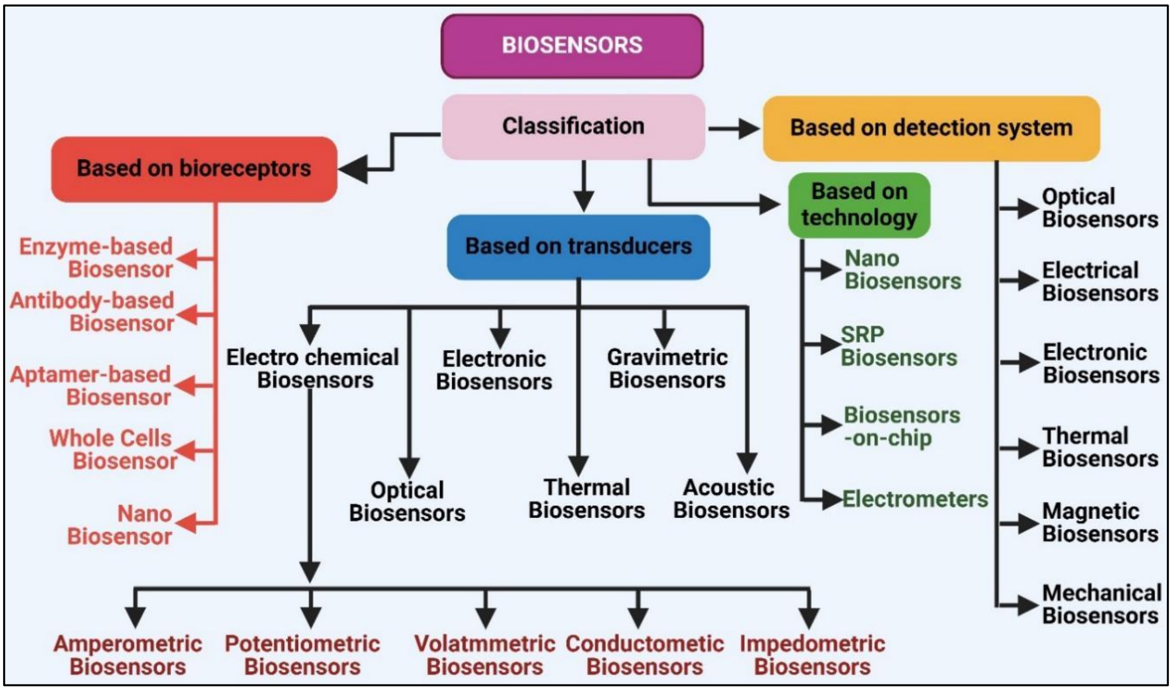

Fig. 2.5. Classification of biosensors according to the diverse bioreceptors and transducers employed

2.4.4. Bioreceptor (aptamer based biosensors) used in the present work

Aptamers have gained a lot of recognition in the realms of medicines and bio-analytical applications since their discovery in the early 1990s [66]. Aptamers are short synthetic sequences of single-stranded (ss) oligonucleotides (RNA/DNA/XNA) or peptides that range from 30 to 100 nucleotides. A technique known as SELEX is used in the lab to make them. An aptamer sequence is selected from an extensive, randomly generated pool of nucleic acid libraries via SELEX, followed by amplification [67,68]. As a recognition mechanism, the target-specific aptamer changes conformation to produce a particular G-quadruplex structure when it binds to the target. The presence of nucleases causes the ribonucleic acid (RNA) aptamers to become unstable and have a shorter lifespan in biological fluids or when analyzed in actual samples. On the other hand, the single-stranded DNA (ssDNA) aptamer is a great option for biosensing applications because to its intrinsic stability and resistance to degradation [67]. When compared to natural receptors like aptamers offer improved selectivity, antibodies or enzymes, ease of modification, simple labeling, and long-term stability because of their great affinity and specificity for their selected targets [68]. The aptamer's excellent target selectivity makes aptasensors well-suited for bio-analytical applications.

The advancement of the *in-vitro* SELEX procedure offers a method to extract oligonucleotide sequences that have the ability to specifically and strongly bind to different target molecules. The SELEX method consists of five fundamental steps: binding, partitioning, elution, amplification, and conditioning. These processes are illustrated in Fig. 2.6. Using a predetermined set of experimental conditions, the synthetic oligonucleotides (ssDNA/ssRNA) sequence library is first randomized, with numbers ranging from several hundred to several thousand[69,70]. Prior to binding, it is customary to carry out a pre-treatment procedure in which the oligonucleotides (single-stranded DNA or single-stranded RNA) library is exposed to a selection matrix that lacks the target molecule (known as negative SELEX). This phase serves to exclude any sequences that bind non-specifically. Binding occurs when random sequences of oligonucleotides are incubated with specific target analyte during initial phase of the SELEX cycle. Moreover, distinguishing target-bound sequences of loosely-bound or unbound oligonucleotides by various separation techniques, such as chromatography, flow cytometry, and electrophoresis, is a crucial step [71]. Next, the bound sequences are extracted and subsequently amplified using Polymerase Chain Reaction. The augmented collection of oligonucleotides is reintegrated into the subsequent SELEX cycle. Through amplification rounds in each cycle and iterative selection, the pool of nucleotides is progressively reduced to a smaller number. This smaller pool consists of sequences that have the specificity and highest affinity to analyte. Total SELEX cycles conducted varies expressively between experiments and is influenced by several aspects, including the structure of the target molecule and the efficiency of the selection conditions and the separation step. Process of aptamer-based sensor detection and attaching to their specific analyte is defined by the substantiation arrangement of molecular shapes, as well as the interactions involving Van der Waals forces, stacking of aromatic rings, electrostatic forces and hydrogen bonding. Aptamer-based sensors possess advantages of exceptional specificity, speed, selectivity, versatility and stability in terms of measurement methodologies, rendering them appropriate for a diverse array of analytes. Researchers are highly interested in the application of aptasensors for analyzing pollutants in water. They aim to develop dependable and economical methods that necessitate little or no need of sample preparation.

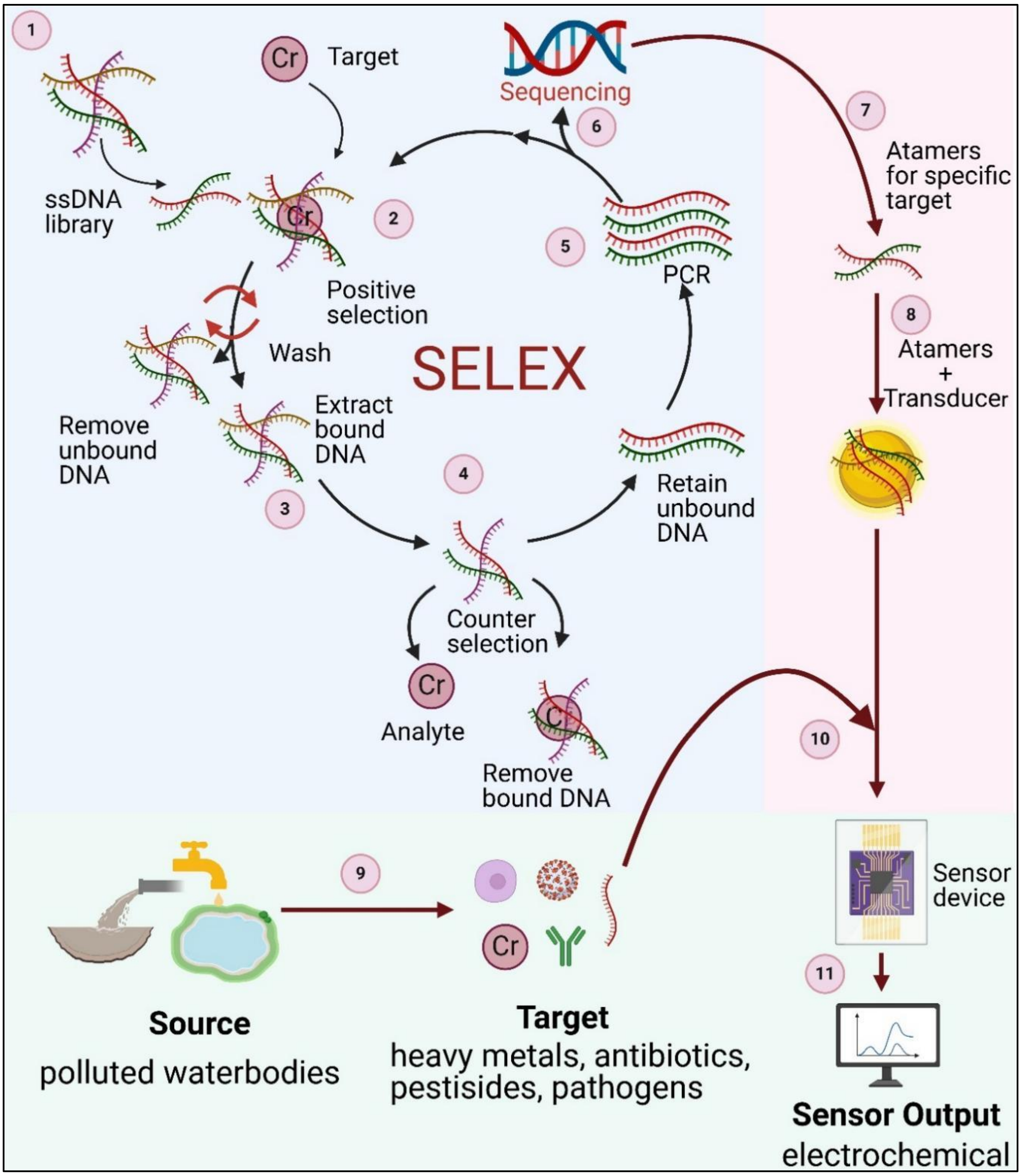

Fig. 2.6. Schematic depiction of the SELEX Process (illustrating all five key steps): The processes shown by solid arrows pertain to ssDNA aptamer selection, while those represented by dotted arrows relate to RNA aptamer selection

2.4.4.1. Biosensors with immobilization techniques

To enhance the Molecular Recognition Element component of biosensor, the binding of biomolecules on the transducer's surface is crucial (Fig.2.7). Choosing the right immobilization method is one of the most important parts of sensor preparation. This is due to the possibility of the bio-recognition element or molecule becoming inactivated or leaking away after immobilization. When using biosensors, biological molecules must maintain their biological function, composition, and activity. The two main immobilization techniques, chemical (irreversible) and physical (reversible)are widely recognized. Choosing an appropriate technique depends on the analyte's properties, the physical surroundings, chemical environment, biomolecular recognition element and transducer selected [5,72,73].

The method of physical or reversible immobilization involves affixing enzymes to the transducer's surface without forming chemical bonds. Physical entanglement and Physisorption are two examples of immobilizations [73–77]. The chemical bonds, are formed among the transducer surface and the functional groups of bio-recognition elements in the chemical or irreversible immobilization approach. Immobilization techniques of chemical are classified as (a) covalent cross-linking based on the chemical bonding and (b) direct covalent bonding [72,73,75,76,78].

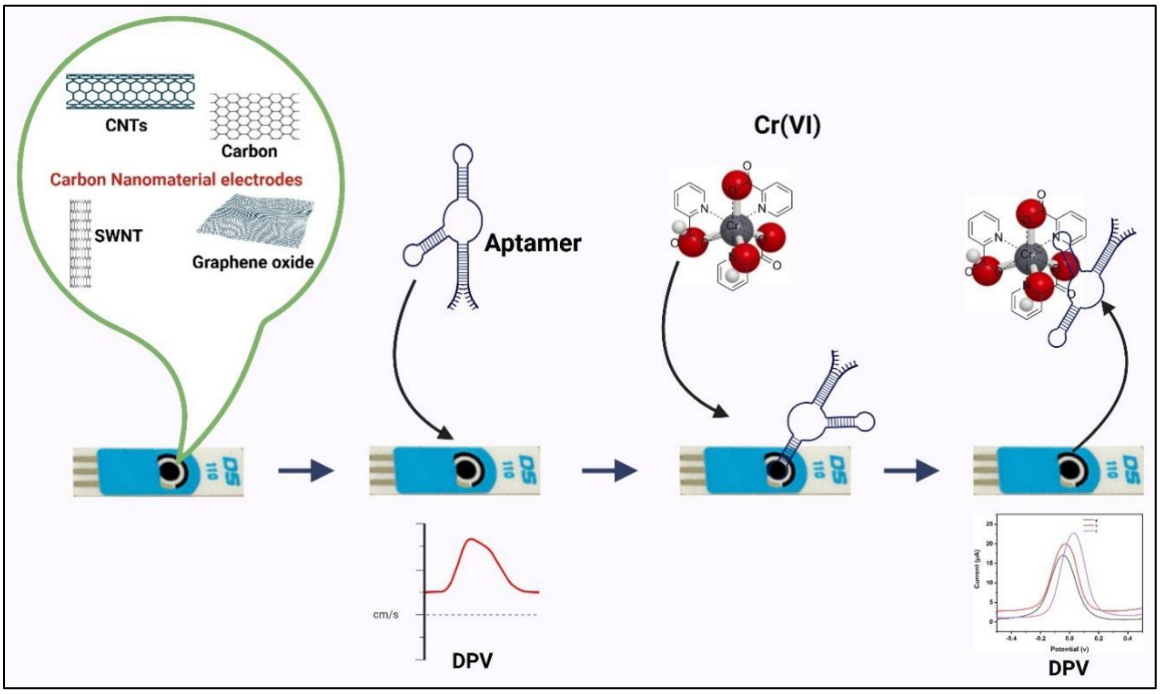

Fig.2.7. Cr(VI) Detection technique for four distinct screen-printed carbon materials electrodes

2.4.5. Transducers (electrochemical biosensors) used in present work

Transducers are classified into many categories based on their operating principle, namely electrochemical, optical, thermal, electronic, and gravimetric. These categories are illustrated in Fig. 2.5. Electrochemical biosensors are extensively studied and often used biosensors that operate based on electrochemical characteristics of cognate molecule and transducer. Electrochemical biosensors provide exceptional sensitivity, detection capabilities and selectivity. In biosensor, a transducer surface facilitates an electrochemical interaction between a bioreceptor and an analyte. This reaction generates detectable electrochemical signals, such as voltage, current, impedance, and capacitance [79,80]. Electrochemical biosensors are classified into five categories based on the transduction principle: voltammetric, amperometric, impedimetric, conductometric, and potentiometric [79–82].

2.4.5.1. Voltammetric biosensors

The predominant electrochemical techniques utilize voltammetry, wherein the current is quantified through an electrode within a varied potential range [83]. Voltammetric sensors sense the analyte by calculating current while continually adjusting applied voltage. Sensors offer the benefits of exceptionally sensitive readings and the ability to detect numerous analytes simultaneously [81].

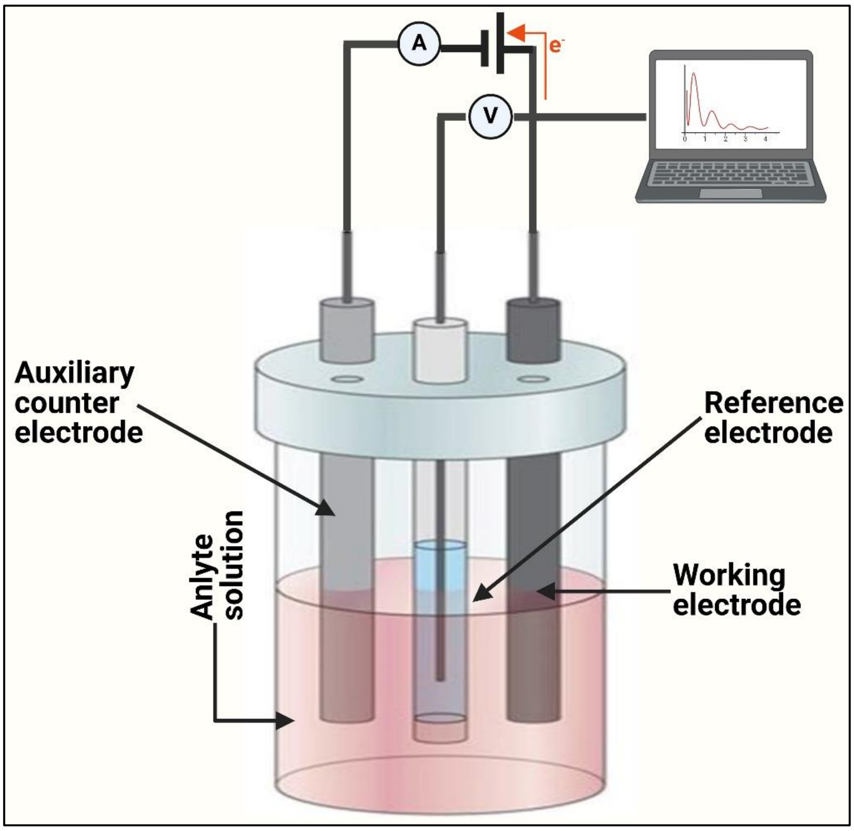

Fig.2.8. Diagram of voltammetric system

In voltammetric biosensors, the voltage is modulated within a defined range to maintain the electroactive chemical reaction at surface of WE. The quantitative data of target species can be acquired through the peak current produced by molecule absorption at the surface of WE. Fig. 2.8 illustrates the three-electrode electrochemical cell [84,85]. In contrast to potentiometry, reference electrodes (RE) are utilized to sustain a reference potential relative to the working electrode (WE) through the assistance of a power source or potentiostat. The potentiostat is analogous to a regulated voltage source in an electrical system by causing redox reactions at the electrode interface, it generates current and measures and regulates the voltage applied across WE. Induced current flowing via CE and WE convey significant information regarding the reaction kinetics of the system and its electrochemical characteristics. The connections and the three-electrode configuration show in Fig. 2.9.

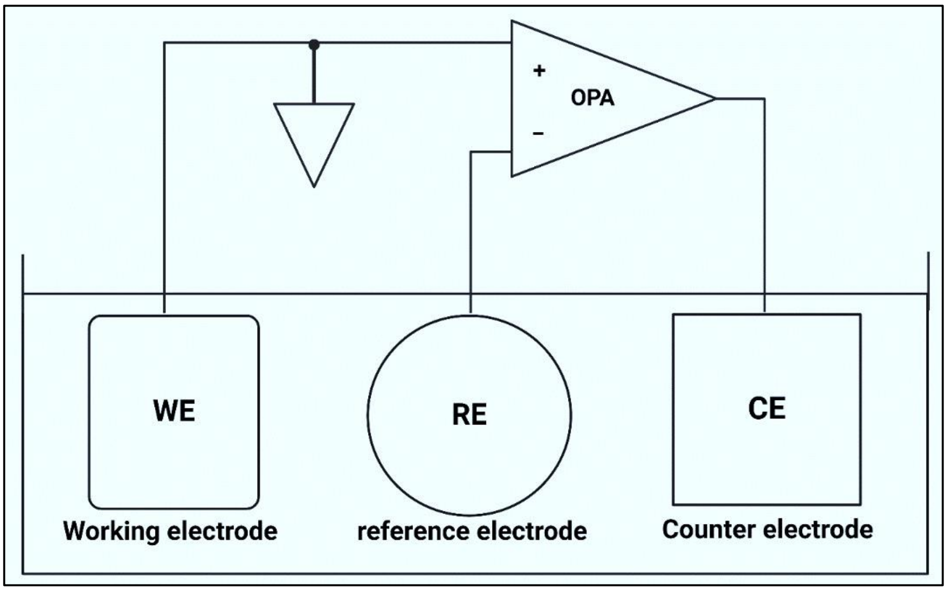

Fig. 2.9. Schematic illustration of electrochemical cell

Potentiostat

Fig. 2.10 illustrates a basic electronic device utilizing an operational amplifier, intended to regulate a three-electrode cell capable of conducting various electro-analytical experiments. The third electrode (CE) maintains a fixed potential differential among WE and RE. When current flows, the potential between the electrodes alters, indicating that the electrodes are polarized. The current at the CE is adjusted to maintain the working electrode's potential at constant level relative to reference electrode. Consequently, to attain a steady potential, no current should be permitted to flow through the reference electrode. A potentiostat must possess a significantly high input resistance to achieve the requisite null condition.

- Fig. 2.10 illustrates the fundamental electrical circuitry of a potentiostat. The control amplifier (CA) block is linked to the counter electrode (CE), which obstructs the current passage through the electrochemical cell. Conversely, WE are linked to the current follower circuit. A commercial potentiostat has several additional components:
- An input resistor serves as a protective component in the reference electrode input.
- Between RE and CE, a phase correction capacitor is connected.
- The input current signal is transmitted from the reference electrode input through a potential buffer for amplification, while the voltage remains constant. Consequently, the

reference electrode is subjected to minimal currents, allowing the output to support significant loads.

- Potentiostatic CA with high open-loop gain.
- To convert the current into equivalent voltage, a high precision operational amplifier is used as trans-impedance amplifier.

The present study developed a potentiostat for detection of Cr(VI).

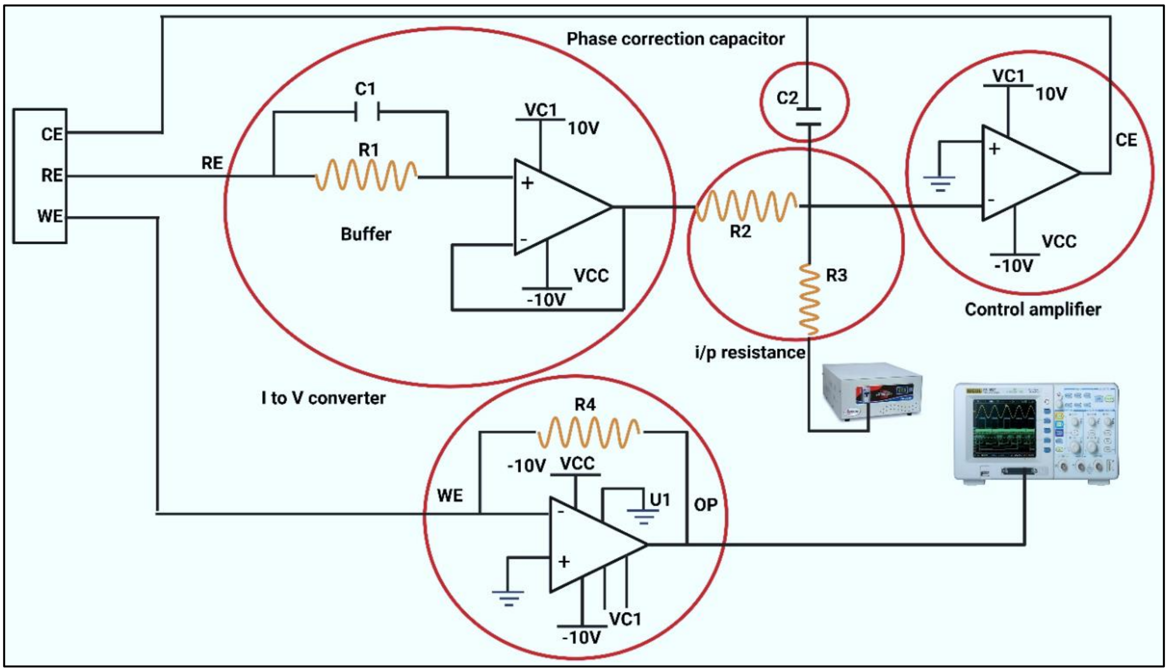

Fig 2.10. Basic circuitry of a potentiostat

2.4.5.1.1. Traditional types of voltammetric measurements

A. Linear sweep voltammetry (LSV)

The current generated in an electrochemical cell is measured with reference to the time-dependent linear variation of potential among WE and RE. The characteristics that dominate the LSV include the rate of electron transfer processes, reactivity of electroactive species, and scan rate [83]. In this context, an increase in the scan rate results in the generation of capacitive current, which cannot be electrochemically compensated.

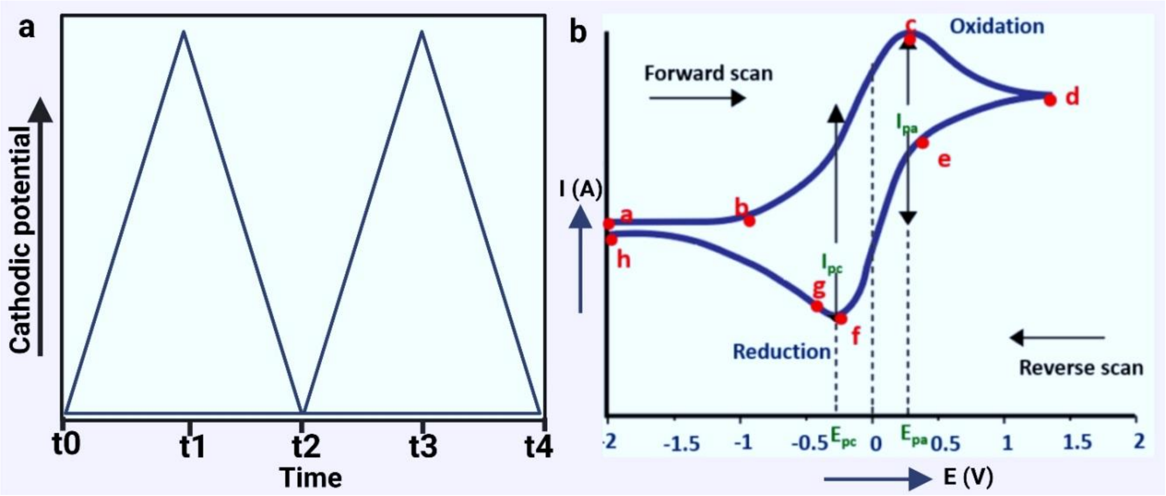

Fig. 2.11. (a) Cyclic voltammetry waveform; (b) Typical cyclic voltammogram showing the different parameters for a reversible reaction

B. Cyclic voltammetry

It is the most commonly utilized potentiodynamic electrochemical measurement, in which potential is varied linearly over time [83]. Upon attaining the saturation potential, the potential of working electrode decreases in reverse to revert to the initial potential value. The voltammogram is illustrated in Fig. 2.11(a) and 2.11 (b), depicting the cathodic current (*Ipc*) and the anodic current (*Ipa*) associated with oxidation and reduction processes, respectively, at varying time periods. The reaction is triggered by the triangle pulse, as seen in Fig. 2.11 (a). The current rises with input potential during the forward scan (t_0-t_1). The oxidation process occurs swiftly due to the significant quantity of reactants available. On further applying voltage, the reactants' supply become diffusion limited and the current value reduces to a lower or commonly, to a constant value. During the reverse sweep (t_1 to t_2), the cathodic peak is observed by the reduction of the oxidized species.

C. Pulse voltammetry (PV)

PV acts in relation to a potential pulse based on the variance in rate of charging and faradaic currents. Faradaic current diminishes at a slower pace than charging current, which decays exponentially according to a function of $1/(\text{time})^{1/2}$. This voltammetry techniques were created by Barker and Jenkin [86] and it detects analyte up to 10^{-8}Mconcentration level. Based on the applied waveform and the associated current profile, PV is categorized as follows:

- **Normal pulse voltammetry (NPV)**

  A sequence of monotonically increasing potential pulses trigger the output current at the end of each pulse thereby providing some time for charging current decrease. The pulse duration (t) in response to the potential (E_i) varies from 1 to 100 ms, with the corresponding interval ranging from 0.1 s to 5 s. The voltammogram exhibit sampled current on vertical axis in relation to a stepped pulse on the horizontal axis.

- **Differential pulse voltammetry (DPV)**

  Fig.2.12 illustrates a standard Differential Pulse Voltammetry (DPV) approach [53]. Besides the applied potential, which is incrementally increased in this instance, there are other resembles between the NPV and DPV approaches. The baseline potential corresponds to the alteration resulting from the application of successive pulses, maintained for a specified length until the subsequent pulse is applied.

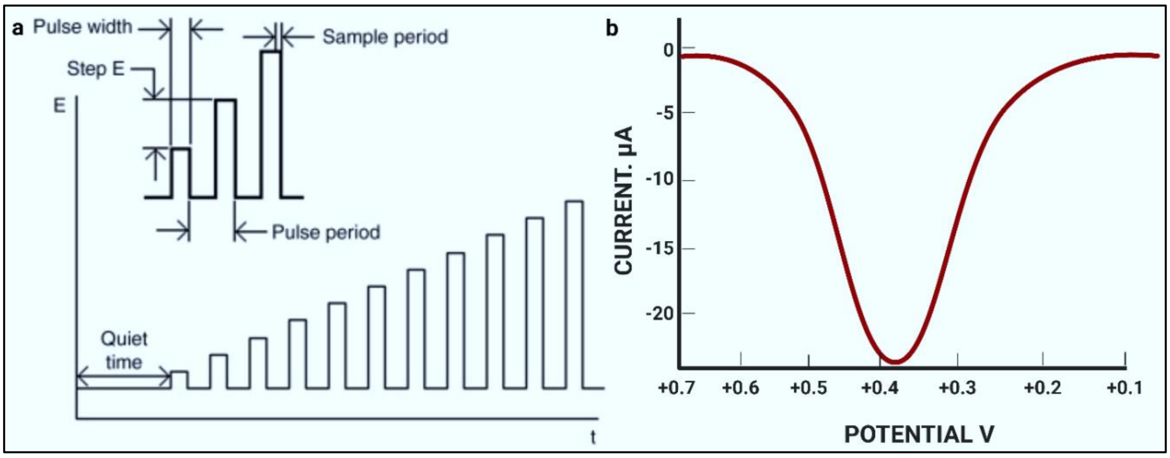

Fig. 2.12. (a) Excitation potential waveform for differential pulse voltammetry; (b) A typical differential pulse voltammograms

The sequential current is measured prior to and at the conclusion of each pulse, as illustrated in Fig. 2.12 (a). The resultant discrepancy between these two measured current values is subsequently documented for quantitative analysis of the target analyte. Therefore, DPV appears to be the most sensitive approach, exhibiting a lower limit of detection relative to other types of PVs.

- **Square wave voltammetry**

In this form of voltammetry, symmetrical square wave pulses of amplitude E_{sw} are assembled such that the forward pulse aligns with the phase of the staircase [87]. The resulting current is figured out based on the variation between the oxidation and reduction currents. The amplitude of the peak and the concentration of the electroactive species exhibit a linear dependence, with the lower detection limit reaching around 0.1 nM.

D. Stripping voltammetry

Sensitive stripping voltammetry technique is typically applied for detection of less amounts of metals in a solution [87]. Firstly, to activate the deposition of target metal ions on surface of electrode, certain potential is applied onto it. The solution is continuously agitated to optimize the quantity of metal ions available for deposition. The stirring procedure stopped once the deposited ions adhere to the electrode surface. In the final stripping state stage, the deposited metal ions are stripped off the electrode by scanning the potential and current which is produced proportional to amount of metal present in mixture or experimental solution. There are two types of voltammetry based on the positive or negative potential scan of the stripping process: anodic and cathodic stripping voltammetry.

The selection of a voltammetry technique is contingent upon the specific application, the characteristics of the analyte, and the objectives of the analysis. Every strategy possesses distinct advantages and limits, making the selection of the suitable method essential for achieving reliable and significant outcomes. CV and DPV have been employed throughout the thesis. CV has been employed to examine the kinetics of the electrochemical system, while differential pulse voltammetry, being significantly more sensitive, has been utilized to evaluate concentration variations.

2.4.5.2. Amperometric biosensors

This sensor function with either a two-electrode or three-electrode configuration (Fig. 2.8) and compute electric current produced by the electrochemical reduction or oxidation of electro-active compounds at working electrode. This transpires when a fixed voltage is given to WE in relation to RE. Electric current generated on WE's surface is directly proportional to analyte concentration in solution [79–82]. In contrast to potentiometric biosensors, this approach offers a sensitive, rapid, accurate, and linear response, rendering it more appropriate for large-scale manufacturing. Nevertheless, the drawbacks of these sensors include inadequate selectivity and interferences caused by other electroactive chemicals [88,89].

2.4.5.3. Conductometric biosensors

It measures alteration in conductance among two electrodes due to an electrochemical reaction, which causes a change in the conductivity properties of the analyte (Fig. 2.13). Conductometric and impedimetric biosensors are commonly employed for the purpose of monitoring metabolic processes in living biological systems [81,89].

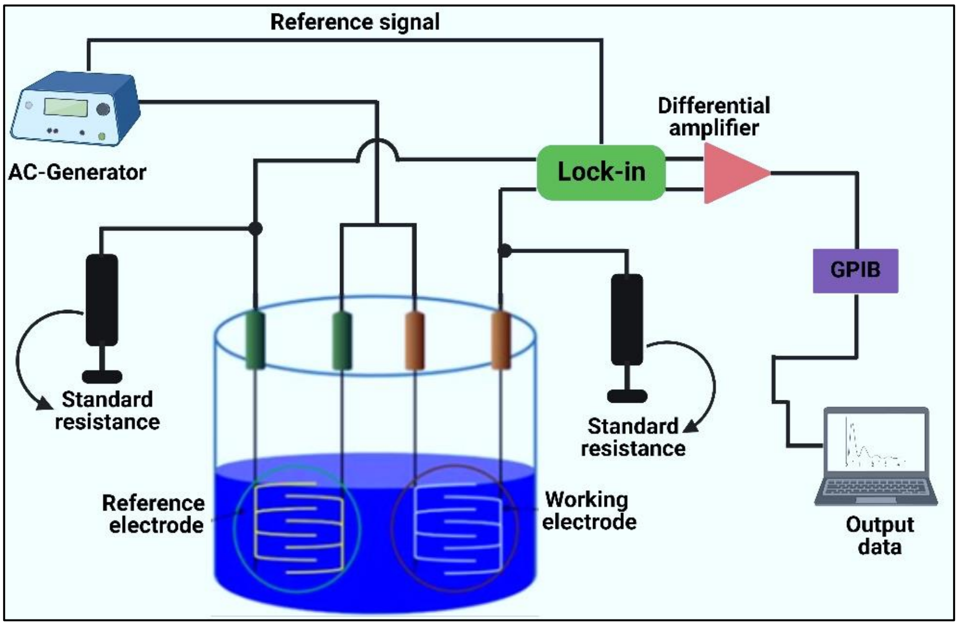

Fig. 2.13. Schematic diagram of conductometric biosensors

2.4.5.4. Impedimetric biosensors

This sensor quantitatively assesses electrical impedance generated at interface between the electrolyte and electrode by applying tiny sinusoidal stimulation pulse (Fig.2.14). The process is applying a low amplitude alternating current to a sensor electrode and then measuring current response, both in-phase and out-of-phase, as a function of frequency by an impedance analyzer [81,90].

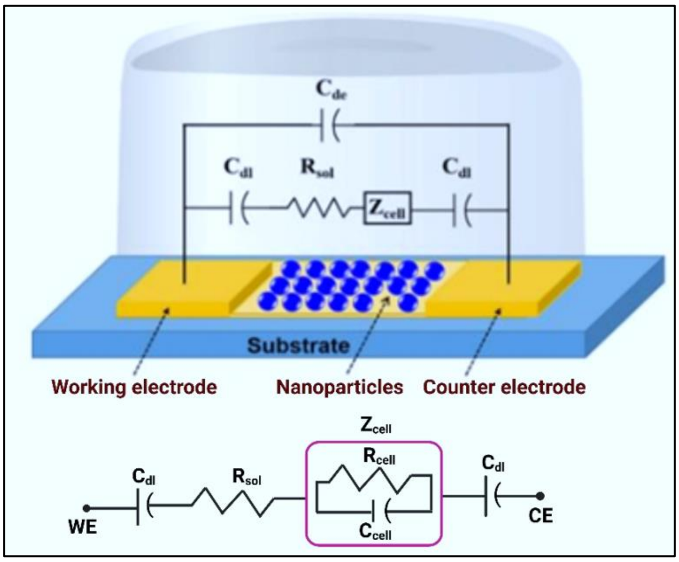

Fig.2.14. Schematic representation of circuit of impedimetric biosensor (C_{dl} = double-layer capacitance of the electrodes, Cde = electrode capacitance, R_{sol} = solution resistance, Z_{cell} = impedance contributed by bound nanoparticles, and R_{cell} and C_{cell} represent the parallel resistance and capacitance, respectively)

2.4.5.5. Poetentiometric biosensors

Potentiometric biosensors are highly adaptable instruments with emerging applications in several fields such as industrial, security, environmental safety, and human health (Fig.2.15)[5]. Potentiometric biosensors quantify the charge buildup resulting from the interaction among the analyte and bioreceptor [91] at the WE, in comparison to the RE, with no current flow. Ion-selective electrodes and ion-sensitive field-effect transistors are employed to convert a biological reaction into a potential signal [79–81,88].

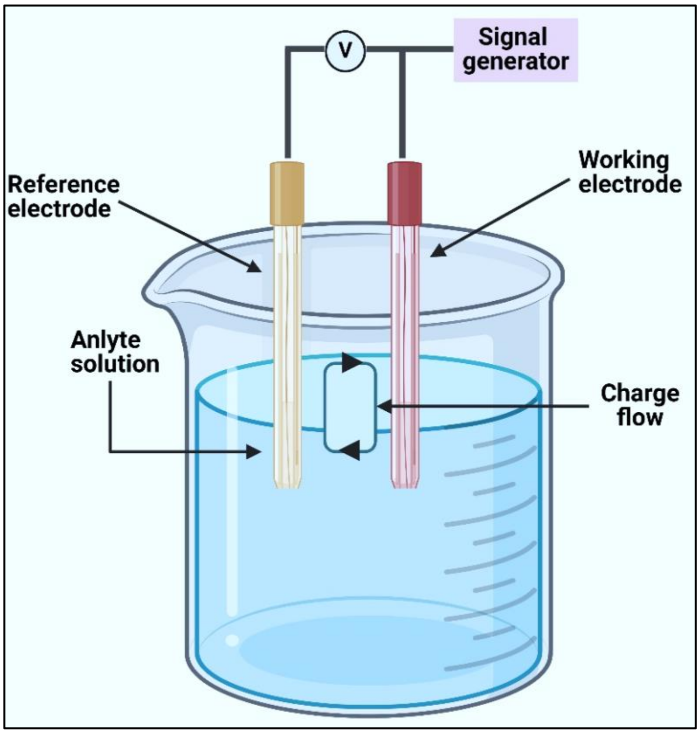

Fig.2.15. Schematic diagram of potentiometric

While measuring the potential difference between a WE and a RE via an interface, ensur that the current flowing is extremely small (about 10^15 A). This approach has several benefits since it is simple, effective, and consumes minimal power [92]. In addition, the minimal current flow indicates that the approach is more resistant to interference effects [93] and considerations of ohmic drop in comparison to voltammetric sensors. Ultimately, potentiometry shown a limited sensitivity to the size of the electrode [94], indicating that it is possible to miniaturize without losing sensitivity.

An alternative approach to developing a potentiometric biosensor entails immobilizing the molecular recognition element(s) onto the surface of a standard electrode, which comprises carbon rods or metal or disks enclosed in an insulating cover of glass or plastic, or onto a configuration of SPE. Process of immobilization could be achieved through several means, including the formation of self-assembled monolayers, polymeric membranes, hydrogels and other techniques. The discovery of new materials or the creative application of existing materials to enhance the sensitivity, stability and selectivity of biosensors.

Several research groups have examined several polymer materials for immobilizing biorecognition elements [95,96]. These materials include Nafion [95,97], Aquivion [96], chitosan, synthetic polymer [95,98,99], and 4-mercaptobenzoic acid, which can be used as substitute to conventional thiol-bonding procedures [100]. Additional researchers have incorporated metal oxides, specifically ZnO [97] and AZO (aluminum-doped zinc oxide) [97], as well as nanomaterials [100], into immobilization matrix to enhance the function of the sensor. Although a majority of these biosensors employ oxidase enzymes for the detection of tiny molecules such as glucose [95,97,100], certain research teams are investigating the utilization of direct electron transfer enzymes [101].

2.5. Types of working electrodes used for this work

The screen-printed carbon electrodes (SPCEs) are a unified device consisting of three different electrodes (Fig. 2.16)[102].

(a)The working electrode: Their response is dependent on the concentration of the analyte.

(b)Reference electrode: This technique enables the application of a predetermined electric potential that is unaffected by the concentration of other ions and analyte. The potential remains fixed and potential of the WE are calculated in relation to RE.

(c)The auxiliary electrode: This AE often known as the counter electrode, is a component utilized in electrochemical systems. The electrode functions as the final link in the potentiostatic circuit, enabling flow of electric current. This facilitates examination of methods involving transfer of electrons.

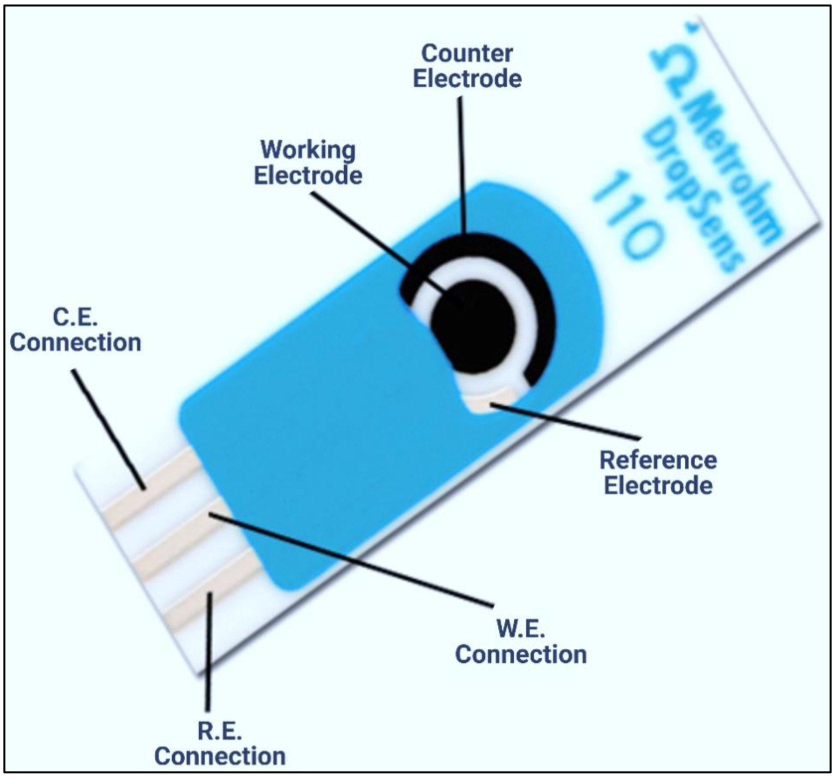

Fig. 2.16. SPE with three-electrode scheme, reference electrode, working electrode and auxiliary or counter electrode

Three electrodes have capability to be printed on various substrates such as plastic or ceramic and may be created using a wide range of inks [103,104]. The predominant inks consist of carbon and silver, although they could also be formulated with other metals viz. platinum, copper, palladium or gold. Furthermore, electrodes have the capability to undergo modifications using aptamers, carbon nanotubes, enzymes, complexing agents or polymers and metallic nanoparticles, [103,104]. The selection of the composition of electrode ink is based on specific use and desired level of sensitivity and selectivity, for the study [103,105,106].

2.6. The device used in the present thesis for chemosensor development (uniform illumination imaging system)

Recently it's a trend to develop field portable tools for assessing environmental pollution. Advance analysis technique and procedures facilitated for sample surveillance, assessment of geographical, pattern of adulterant, investigation of contaminants stretch and assessment of the remediation progress [107]. Advancements in electronic equipment production and procedural innovations now enable original data processing without sacrificing quality standards [108]. The sampling and capture of definitive data during on-site measurement are difficult. Consequently, on the spot evaluation necessitates reliable, precise and accurate outcomes within a brief time frame. Due to the notable characteristics such as light-weight design, compact dimensions, swift analytical capabilities, and a simple operational framework, including a portable energy source, as well as the comparability of data to that generated by lab system, information technology and electronics-based field-deployable instruments could be employed for the study of diverse agri-environmental samples [109,110]. Furthermore, field analytical analysis, permit accurate measurements of a significantly greater number of samples than those that might be evaluated in a laboratory at a higher expense [111]. The integration of analyzed statistic data and validating analysis is beneficial for producing justifiable conclusions regarding ecological health threat [112]. Moreover, origins of mistakes related to sampling process could be eradicated. Moreover, numerous laboratories, field-portable equipment is employed as stationary devices. Consequently, these divices, integrated with analyte-targated sensors could contribute towards modern technologies, enabling sophisticated field-portable devices to enhance quality and increase data.

The necessity for accurate, sensitive, specific, rapid, and user-friendly procedures for addressing growing need for legal measures for controlling environmental pollution and early warning, taking into consideration of increasing diversity of pollutants, necessitates the advancement of modern analytical tools [113]. Other techniques for analysis, such as GC, HPLC and ELISA are documented for identification of environmental pollutants. Despite the robustness of these approaches, the necessity for complex pre-treatment procedures necessitates a professional personnel and costly instrumentation, hence limiting their utility for on-site determination [114]. Currently, biosensing technology has become a crucial instrument for high-throughput analysis and detection, characterized by low cost and hand-held system [115].

Traditional assessments of environmental toxins are crucial information that is set to leverage advancements in biotechnology and equipment. The existing system results from a methodology that has responded to scientific advancements by progressively augmenting test protocols or including additional tests without assessing the testing framework concerning comprehensive risk assessment and risk management requirements. The increased expenses of testing, utilization of laboratory apparatus, sample preparation, and the duration required to produce and assess data made the system more complicated. Furthermore, the generated data could be insufficiently ideal for addressing inquiries related to human health risks.

Numerous traditional techniques for detecting environmental pollutants depend on laboratory microwell plate (96, 384, or 1536) readers for quantification and identification. The utilization of a microplate reader constrains the practical application of these tests because it necessitates highly skilled personnel. Traditional plate-reader machines are inadequate for the requirements of rapid, cost-effective, and accurate analysis in portable settings. Consequently, there is a considerable demand for development of a compact and economical high-efficiency color reader for point-of-care and mobile health applications, as well as use in resource-constrained environments. Recent reports indicate the development of cell-phone based microwell plate readers. Despite the easy process of developing these systems, challenges persist in scaling up and addressing inefficiencies related to the mismatches in field view among the light source, microwell plate and cell phone. The considerable distance between the microwell plate and mobile phone, along with the inconsistent camera resolution limits the applicability of smartphone-based analysis [116]. Despite potential benefits, high-efficiency imaging system for screening have been sluggish to emerge due to the unique challenges associated with imaging well plates. A significant issue is size of the plates which are big, yet each individual well is small in size; thus, photographing an extensive region composed of little, dark targets necessitates extremely sophisticated camera and lens systems[117], which makes it prohibitively exclusive.

Chromium (VI) presence in wastewater released from tanneries, electroplating, and chemical industries has resulted in environmental contamination, jeopardizing both the ecosystem and human health. Traditional Cr(VI) detection methods are ineffective, requiring costly equipment and supplementary chemical reagents. Sensors can be utilized for Cr(VI) detection to tackle these challenges, owing to their superior selectivity, sensitivity, and responsiveness. Consequently, rapid identification and accurate quantification of trace levels of Cr(VI) are becoming increasingly significant. Under response to the above concerns, a straightforward, economical, and portable device for color detection under field conditions has been created. This study utilized the prototype of the colorimetric system as a nanosensor, verified for upcoming applications, specifically the detection of the endocrine-disrupting chemical Cr(VI) in water.

2.6.1. Instrumentation and fabrication of UnIIScan

FUJITSU Image Scanner fi-65 F, one of the market-available scanner devices, has been purchased. Additionally, this scanner has been customized for Uniform Illumination Imaging System (UIIS) prototypes. Fig. 2.17 displays pictures of the created prototypes. The imaging array technology used by the UIIS device is a contact image sensor (CIS). CIS has therefore replaced the mirrors, filters, CCD array, lens and lamp with rows of diodes that emit green, blue light and red. The glass plate on which the object rests on is very close to the image sensor technique, which consists of three hundred to six hundred sensors spread across the assess area. White light produced during picture scanning by the combination of the light-emitting diodes. The lighted image is captured by the row of sensors. As a result, the instrument alone determines the uniform illumination.

Additionally, a white object was positioned differently on the object bed during the instrument development process. The numerical optimization technique has been employed to enhance uniform illumination on an intended trajectory. The efficiency of reflection of uniform illumination distribution was computed. To attain highly uniform illumination dispersion on the intended trajectory, the object function has been further minimized. A simulated annealing approach has been used to optimize the array's light emitting diode coordinates in order for the object function to achieve its lowest possible value. Details of algorithm and equations are available elsewhere [118]. This method was applied for creating software algorithms. If there is a divergence from the previously observed data, the calibration technique can be applied. A graphical user interface is included into the prototype. The prototype has a dpi of 600. A speech synthesis output has been integrated to the software. The prototype is capable of incorporating one 96-microwell plate. These UIIS were used to capture 300 dpi images, which were saved to a digital recorder via USB. The 96-well plate sample was positioned in the center of the acquisition bed on a slide in the UIIS. The images were in 24-bit BMP format. Additionally, original information in the form of fundamental Red, Green, and Blue values has been extracted from the BMP images. These Red, Green, and Blue values have been converted to Hue, Saturation, Intensity and CIE Lab color values for convenience of analysis. Using the global thresholding technique, segmentation of the background might be accomplished by establishing the pixel in the backdrop to zero without altering the values of the front-and center pixels.

Fig. 2.17. Developed UIIS prototype images

Visual C++, Version 6.0, Standard Edition from Microsoft has been utilized for original information or raw data acquisition, statistical evaluation, and GUI display. There are training and testing modules in the software development process. The training module has been created using established concentrations of Cr(VI) remnant. The program algorithm was initiated with the building of a database of machine knowledge. as seen in Fig. 2.18. The 96-microwell plate, displaying colors from established concentrations, has been inserted into the device. The technology originally captured 24-bit RGB pictures. The separation of the background procedure has been performed on the obtained images. Now the data analysis model has been developed using connected component labeling and obtaining color data that is particular to labels, employing Mahalanobis distance computation. A calibration curve has been established with a standard sample, Cr(VI), and Mahalanobis distance. additionally, the database of knowledge-based machines has been established through successive measurements utilizing known quantities of Cr(VI).

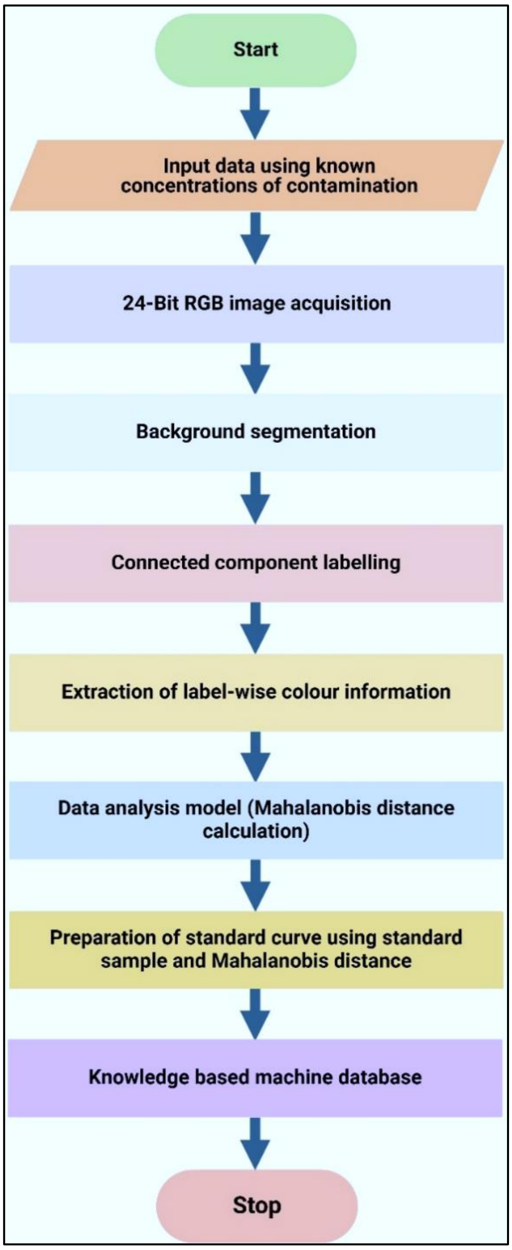

Fig. 2.18. Software algorithm training module development flow chart

During the development of software testing, the preceding stages have been executed up to the calculation of Mahalanobis distance utilizing unidentified concentrations of Cr(VI). Subsequent to the aforementioned stages, the computed Mahalanobis distance derived from the depictions of unidentified specimens has been verified against the standard data base. If the anticipated pollution is within the calibration curve range, the device computes and presents the outcome accompanied by a voice-guided index; if not, the result is shown as 'Not Detected: ND.'. The algorithm's architecture is illustrated in Fig. 2.19.

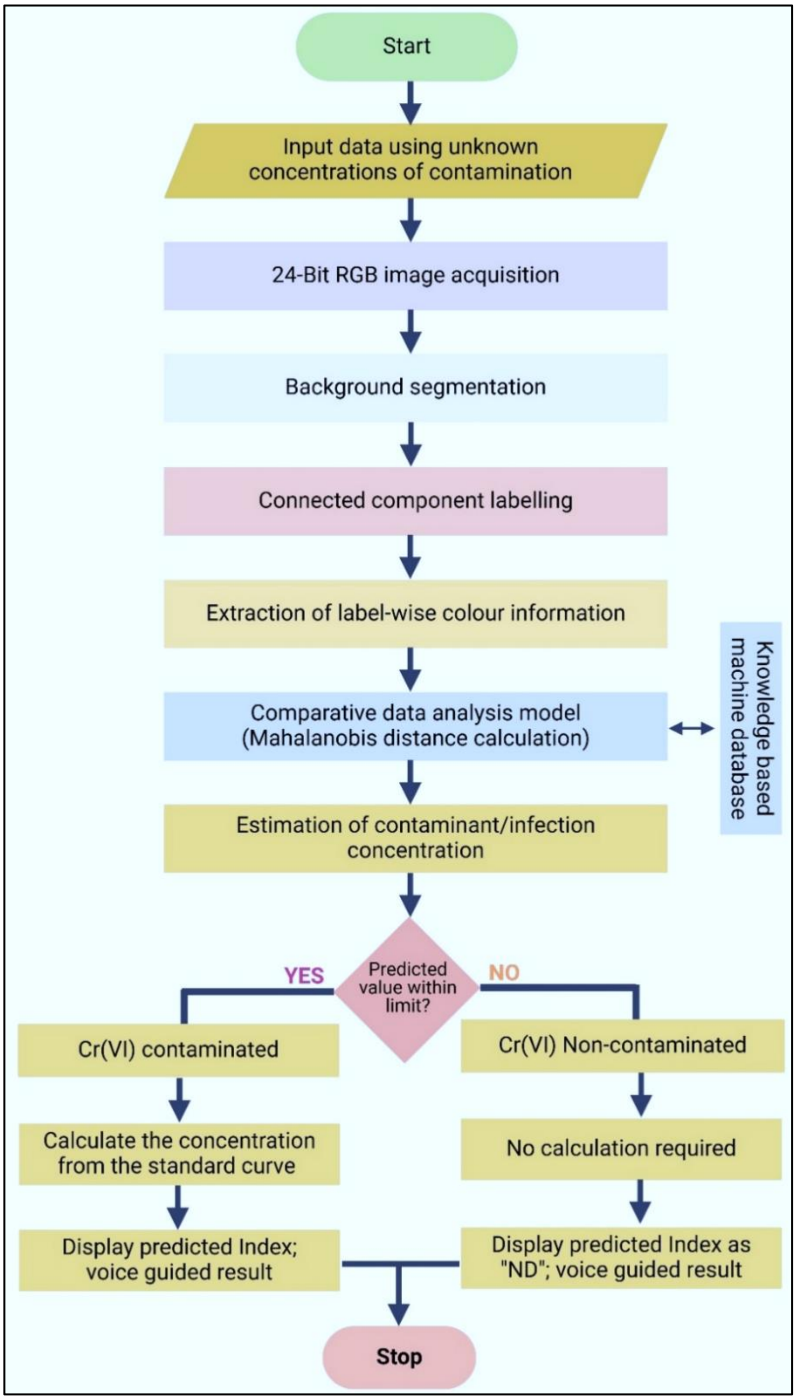

Fig 2.19 Flow diagram of the software algorithm testing module development

2.7. The device used in the present thesis for potentiostat development (electrochemical aptasensing technology)

The device consists of a digital end and an analog front end. The analog front end consists of various analogue circuitries to produce chronoamperometry, cyclic voltammetry and other potentiostatic experimentations. Configuration of three electrodes involves reference, working and counter. The potential between WE and RE should be maintained constant with the help of digital to analogue conversion and certain Op-Amps. The current from the working electrode and counter electrode can be measured with the help of a trans-impedance circuits. The current should be multiplied with an impedance to produce a specific voltage further analyzed by a high-definition analog-to-digital conversion. The overall work would be controlled and monitored with the help of an 8-bit microcontroller. The Op-Amps amplifier would be electrometer type, so that high precision could be achieved. The digital endpoint comprises a microprocessor that oversees the analog front end. The device features a real-time clock, microSD for data recording with a digital timestamp, and a Wide Video Graphics Array (WVGA) touchscreen Liquid Crystal Display (LCD) for clear visuals and user interaction (Fig. 2.20). The WE in an electrochemical device, where the reaction of interest transpires, is typically employed with a CE and a RE in a tri-electrode apparatus. The WE are designated as either anodic or cathodic, contingent upon whether the reaction occurring on it is a oxidation or reduction. Standard WE may comprise noble metals like silver, platinum or gold to noble carbon materials such as glassy carbon, as well as mercury drop and film electrodes. The auxiliary electrode, sometimes referred to as CE, is utilized in a three-electrode electrochemical cell for voltammetric analysis or other operations involving anticipated electrical current flow. The auxiliary electrode establishes the electrical potential against which different potentials may be measured. A reference electrode possesses a stable and precisely defined electrode potential. The elevated stability of the electrode potential is often achieved by utilizing a redox system with a consistent (buffered or saturated) concentration of each reactant in the redox reaction.

The cabinet has been divided into two segments. One part of the device is for electronic circuits, and the other is the sample-handling unit. These two segments have been designed to ensure that the liquid samples are not exposed to the electronic parts of the system. The sensing system consists of a data acquisition card and power supply.

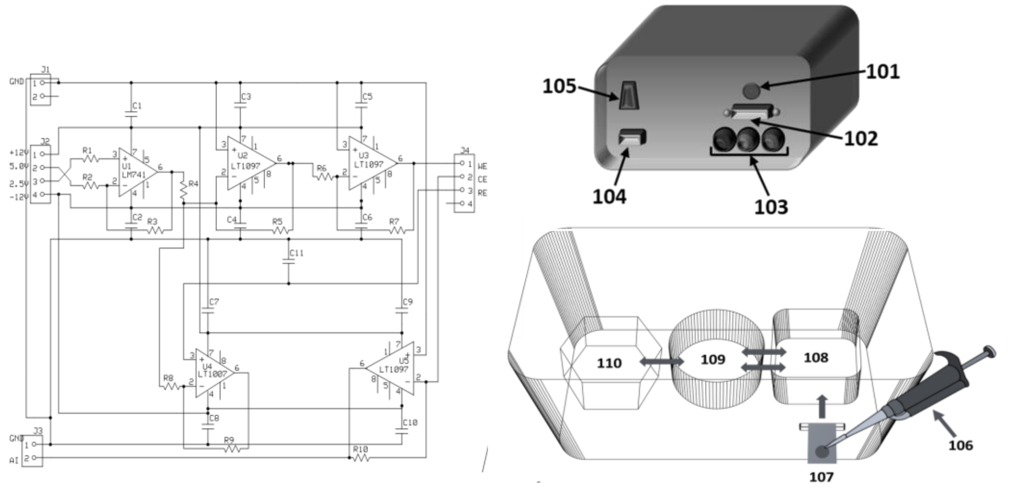

Fig.2.20. Block diagram of hardware design

A data acquisition card has been used for communicating the electronic system with the computer. During the testing period of the sample liquid, continuous information or data has been gathered with this really dependable data acquisition card. The data acquisition card can measure the potential difference between the membrane electrode and the RE. The software created for this system regulates all activities of the evaluation phase. After collecting testing signals, the system logs all the data in spreadsheet files and back up them with separate file names with date and time stamping for future references. Suitable software algorithms have been designed for data pre-processing and analysing with the multivariate data analysis and finally co-relating the data with the pre-defined tested sample. The created software's front end was designed for the options of “testing” or "training" the device. The system was initially trained using known pollution values of specimen to establish a database, in the system so it is possible to correlate unknown sample data with the trained database during testing (Fig. 2.21).

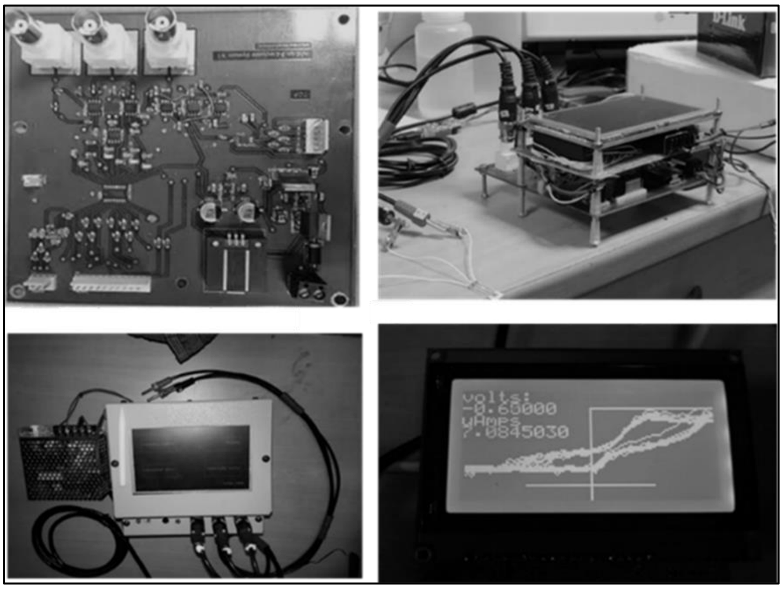

Fig. 2.21. Image of data acquisition and processing protocols by the system

The algorithm has been designed so that the system can build a database independently. The operation only takes one click to execute testing cycles. Upon concluding the testing cycle, the system has been configured to provide the results of the testing procedure and to specify the quality parameters of the sample (Fig.2.22).

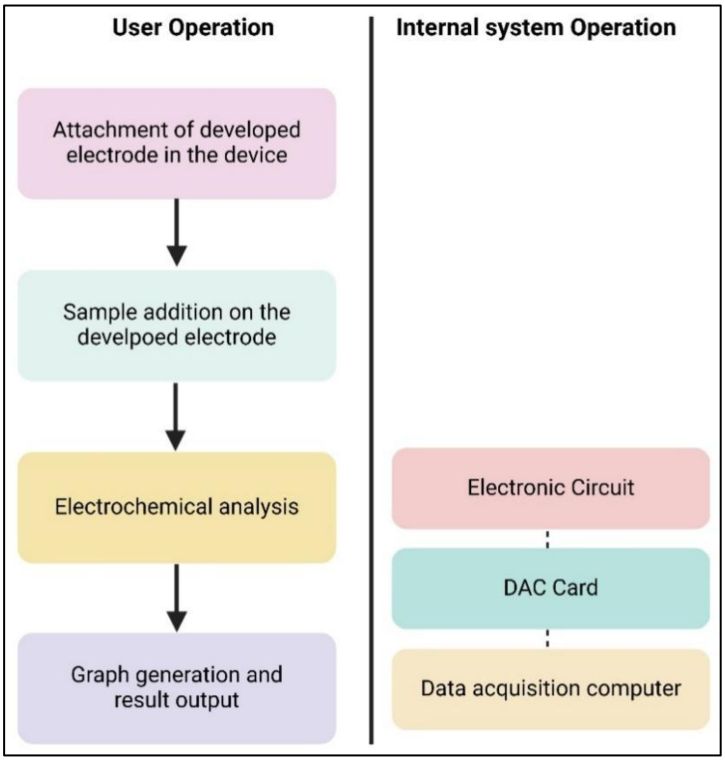

Fig. 2.22. Flow diagram of system operation

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Chapter 3

Cr-Detector: A simple chemosensing system for onsite Cr(VI) detection in water

List of Sections

- **Introduction**
- **Materials and Methods**
- **Results and Discussion**
- **Conclusion**

Content of this chapter is based on the following publication:

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Chapter 3

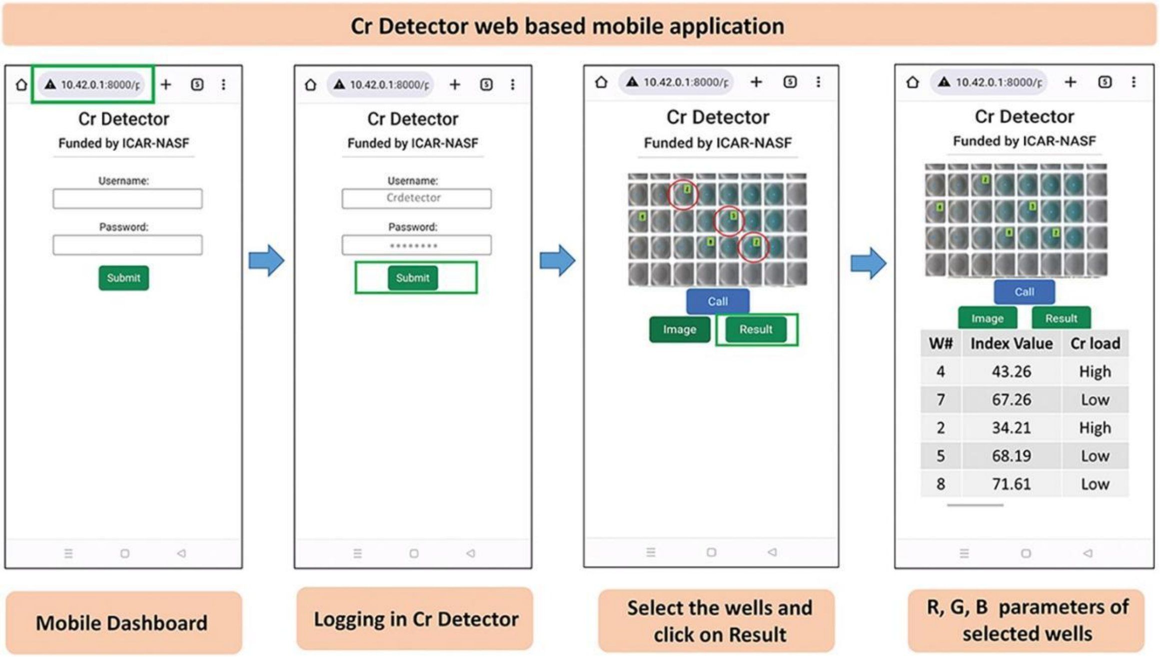
Cr-Detector: A simple chemosensing system for onsite Cr(VI) detection in water

3.1. Introduction

Industrial advancement has played a vital role in improving human life while presenting environmental challenges. One such challenge faced by our societies and the sustainability ofour planet is the ongoing release of toxic heavy metals and metalloids into the aquatic environment. The toxic metalloids have been infiltrating our environments through industrialdischarge, mining activities, and domestic discharge into surrounding water bodies, thus polluting the aquatic ecosystem [1]. Through bioaccumulation and bio-amplification across food chains, these heavy metal species have significantly influenced biodiversity and life on Earth [2]. Chromium(Cr), present in the effluents from several industrial activities, such as chemical synthesis, chrome plating, leather tanning, production of dyes, metallurgy, mining, and manufacturing of various alloys, has become one of the most toxic heavy metals in our environments. Because of their high toxicity, chromium and its compounds, commonly utilized in industrial chemical processes, have become environmental contaminants of genuine concern [3]. A massive influx of chromium pollution in soil, groundwater, and air due to industrial activities has been associated with an elevated risk of carcinogenicity, mutagenicity, chromosomal breakage, DNA damage, and genotoxicity, which are responsible for a range of debilitating diseases [4]. The Cr(VI) contamination was observed in higher concentrations in Changhua County compared to Taichung City, resulting in 60 times more gastric cancer incidence in the former compared to Taichung City. Overexposure to Cr(VI) was observed in China, where upper stretches of rivers such as the Luo and Sandaoexceeded Cr(VI) permissible levels, whereas lower and middle stretches did not [5]. Similar reports of chromium contamination of water supplies have been registered globally from USA [6], Australia, India [7], Bangladesh [8], Greece [9], Japan [10], and Iraq [11], and many resulting in successful lawsuits.

Chromium has two stable oxidation states viz. Cr(III) and Cr(VI), of which Cr(VI) is more soluble, mobile, and bioavailable in alkaline to slightly acidic conditions and, as a result, has a higher level of toxicity [12]. Cr(VI) is also regularly found in drinking and public water systems. In 1991, as a part of the Safe Water Drinking Act (SFDA), the United States EPA placed chromium under the maximum contaminant level goal to have a maximum contaminant level of 100 ppb total chromium. However, even though it is recognized that Cr(VI) is significantly toxic while Cr(III) is not, the US EPA has not yet been able to establish MCL specifically for Cr(VI). However, according to research from the US National Toxicology Program, California could establish a state-wide drinking water standard of 10 ppb MCL for Cr(VI), under further review [13]. In addition, Japan and China also regulate Cr(VI). The chromium is detected by traditional laboratory tests using AAS [14] and ICP-MS [15]. For instance, for regulatory compliance, the total chromium in water is currently measured by ICP-MS using the US EPA Method 200.8 [16]. These tests are sensitive and reliable but require expensive equipment with a large footprint and trained technicians, making on-site detection challenging and delaying decision-making. Importantly, these assays do not allow specific detection of toxic Cr(VI). A rather complex anion-exchange chromatography-based US EPA Method 218.7 can be followed [6]. These limitations have led to an emerging interest in developing new methods for specific Cr(VI) detection in a point-of-care (POC). Notably, very few reported methods demonstrated the ability to detect Cr(VI) selectively. These methods have utilized various transducer/detection platforms, including fluorescence spectrometry [17] electrochemistry [18], and others. Among different transducers, colorimetric detection offers the key advantage of more practical deployment due to the ease of analyte detection by an unaided eye, as is evident from the success of COVID rapid antigen test (RAT) kits, and other standard POC devices such as pregnancy strips. Thus, colorimetric methods offer the opportunity to develop low-cost portable devices for rapid onsite monitoring of important target analytes, both qualitatively and semi-quantitatively. Chromium monitoring using the colorimetric method based on 1, 5-diphenylcarbazide dye is a well-known process [19], but over time it has faced many interference issues, and the better chromogenic substrate is well sought.

The traditional method of Chromium(VI) in Water demands trained personnel, laborious data preliminary treatment, and complex equipment, including AAS, ICP-MS, UV-Vis spectrometry, surface-enhanced Raman scattering spectroscopy, fluorescence, electrochemistry, and X-ray Fluorescence, etc. which are more specific and sensitive towards minute concentrations of Cr(VI) [20,21]. But, detection through conventional systems is costly, requires skilled human resources for device and software handling, is time- consuming, and lacks field portability. These drawbacks of the conventional system have encouraged the development of inexpensive sensor-based rapid detection systems with high field portability and sensitivity. The precise detection of toxic metalloids using suitable sensing techniques may also flourish their separation and elimination techniques towards increasing the safety status of the usable water [22]. Moreover, the sensor development studymay also be integrated with novel nanomaterials and quantum dots [23] to increase the stability and sensitivity of the developed sensor. Not only that, but such sensors can also be used in conventional adsorption studies towards developing novel adsorption beds in water treatment plants, as mentioned by Ismail et al. [24]. In this study, a portable field device has been developed by exploiting TMB as a chromogenic substance. The first part of this endeavor depicts the choice of material through UV-Vis Spectroscopy. Furthermore, after establishing chemosensor selectivity and specificity towards Cr(VI), UIIS^{Scan}1.1, an advanced image array technology-based field-portable high-throughput sensory system, was [25] exploited towards a simple, rapid and on-site analysis of Cr(VI). The tailored system has been upgraded and trained with an image-processing algorithm. A software named 'Cr- Detector' was developed for this purpose (Fig. 3.1).



Username: Crdetector

W#	Index Value	Cr load
4	43.26	High
7	67.26	Low
2	34.21	High
5	68.19	Low
8	71.61	Low

Fig. 3.1. Software operation flow diagram of graphical user interface

The term "WELL" refers to the individual compartments of a 96-well plate used in this sensor study. Water samples were placed in these wells for analysis. In the chemosensing system, the R, G, and B parameters represent the red, green, and blue color components respectively. After capturing an image, the RGB color information is extracted from that image. This RGB data undergoes comparative analysis using Mahalanobis distance calculations, which converts the three separate RGB values into a single "Index Value." Each standard concentration produces a specific Index Value derived from its unique RGB characteristics, creating a reference knowledge base. When analysing unknown samples, their RGB values are compared against this established knowledge base. If the estimated contamination level falls within the range of the standard curve, the instrument calculates the corresponding index value and provides the analytical result.

3.2. Materials and methods

This section outlines experimental design details performed experiments and data analysistechniques. At first, suitable chromogenic compounds are selected based on the observable and measurable color change with different concentrations of Cr(VI). Next, different image analysis algorithms have been explored, and suitable color analysis techniques have been finalized for data collection. Furthermore, experimentations of different concentrations of Cr(VI) with suitable chromogen have been performed, and optimized reaction parameters. Next, repetitive experimentation with all optimized reaction parameters and selected concentrations of Cr(VI) have been executed towards cumulative data collection and standard curve generation. Different image parameters have been extracted from the images captured through the portable field device and evaluated towards selective output signal generation and finalization of the algorithmtowards accurate sample analysis and validation. Further selectivity study of the developed sensor with different positive and negative ions has also been performed. Furthermore, recovery analysis and accurate sample analysis have also been executed.

3.2.1. Reagents

3,3′,5,5′-tetramethylbenzidine (TMB), o-Phenyldiamine (OPD), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) hydrogen peroxide (H_2O_2, 30%), and DMSO, were obtained from Sigma-Aldrich. In addition, potassium dichromate ($K_2Cr_2O_7$), Chromium chloride hexahydrate ($CrCl_3.6H_2O$), Zinc sulphate (Z_nSO_4), Lead acetate trihydrate ($Pb(C_2H_3O_2)_2.3(H_2O)$), Sodium sulfate (Na_2SO_4), Potassium carbonate (K_2CO_3), Sodium hydrogen carbonate ($NaHCO_3$), Barium nitrate ($Ba(NO_3)_2$), Cadmium nitrate tetrahydrate ($Cd(NO_3)_2·4H_2O$), Mercury chloride ($HgCl_2$) were purchased from HiMedia. Na_2SO_4 and $ZnSO_4$ were used to obtain SO_4^{2-} ions which were further used to check the specificity/cross-reactivity of the developed sensor intended to detect Cr(VI). In this article, water and other aqueous solutions with ultrapure water were purified with a Millipore system (18.2 MΩ·cm).

3.2.2. Instruments and apparatus

Previously, a uniform illumination imaging system (UIIS) integrated with a portable scanner was reported that, allows the detection of an analyte through Mahanalobis Distance calculations [25]. The UIIS system can capture images of 96 well plates in 600 dpi resolution and operate at a wide temperature range, making it effective for spot experimentations in fields. The present study used a similar UIIS system with further modification. For capturing an image of the reaction colour, a different image analysis technique was explored to develop the algorithm for chromium detection. Here we have collected the ‘R’, ‘G’, and ‘B’ values of the reaction colour that signifies the ‘RED’, ‘GREEN’, and ‘BLUE’ parameters. Then we tried to find a predictive pattern with different colour parameters toward developing the standard curve. 96-well plates used in the study was obtained from Costar®, Corning Incorporated. For comparison with commercial spectrophotometer absorbance at a specific wavelength was measured by CLARIOstar^{plus}, BMG Labtech.

3.2.3. Reaction parameter optimisation

Different parameters regarding the Cr(VI) mediated oxidation of TMB were optimised initially. The optimisation of TMB concentration and H_2O_2 concentration was done by single variable method and using various concentrations of the reagents. The reaction time optimisation was performed in the Cr-Detector system.

3.2.4. TMB concentration optimization

The TMB amount ranging from 0.1 to 1.5 mM were used for the above experiment. A fixed concentration of H_2O_2 and Cr(VI) was then added to reaction mixture. Finally, volume was adjusted to 200 µL with ultrapure deionised water. The Abs. maxima values at 650 nm of the concentrations were documented.

3.2.5. H_2O_2 concentration optimization

The different concentrations of H_2O2 (10 to 300 mM) were used for the optimisation experiment. At first, a fixed amount of TMB was added to wells, followed by different concentrations of H_2O_2 and a fixed concentration of Cr(VI). Next, reaction volume was adjusted to 200 µL with ultrapure deionised water. Finally, the absorbance maxima values at 650nm were recorded.

3.2.6. Reaction time optimization

The reaction time was optimised with fixed TMB, H_2O_2 concentration, and different Chromium 6 concentration ranges from 0 ppb to 1000 ppb. In addition, a kinetics study of the reaction was performed for 25 minutes with a time interval of 5 minutes. The image parameter values were recorded in the Cr-Detector system every 5 minutes.

3.2.7. Colorimetric determination of Chromium(VI)

In the present study, various parameters have been optimized to get a proper signal for sensing chromium 6 up to allowable limit by EPA. Solutions of Cr(VI) with concentrations ranging from 1ppb to 1000ppb were formed by serial dilution to obtain the detectable linear range of the Cr(VI). Concentrations of 1, 25, 50, 75, 100, 200, 400, 600, 800 and 1000 ppb were used for the test. The different concentrations of Cr(VI) were added to the reaction mixture containing optimum concentrations of TMB and H_2O_2. The reaction volume was then adjusted to 200 µL with ultrapure deionised water, and absorbance was recorded at 650 nm after the optimized incubation. Further, the mentioned colorimetric experiment for Cr(VI) detection was repeated 500 times in the developed Cr-Detector towards the development of the calibration curve.

3.2.8. Specificity test

Solution of other metals and ions like Hg^{2+}, Pb^{2+}, Ba^{2+}, Cr^{3+}, As^{3+}, Cd, SO_4^{2-} $CO_3{}^{2-}$ and HCO^{3-} were prepared from their respective salts. A fixed concentration of these ions, along with Cr(VI), was added to optimum concentrations of TMB and H_2O_2. The reaction volume was adjusted to 200 µL with ultrapure deionised water followed by measuring absorbance at 650 nm.Further anti-interference tests were performed using a mixture of different metal ions in the same reaction mixture. All mentioned tests were carried out in triplicates and repeated more than once.

3.3. Results and discussions

3.3.1. Choice of materials

Cr(VI) and Cr(III) redox conversion is ubiquitous in the environment. In other words, Cr(III) can easily be oxidised to Cr(VI) by an oxidant and Cr(VI) can quickly be reduced to Cr(III) by a reductant [25]. Several attempts have been envisaged toward colorimetric Cr(VI) detection. One such testimony is 1,5-diphenylcarbazide (DPC), which can produce a strong colour change for quantitative or semi-quantitative analysis [26,27]. The DPC-based sensor is one the favourites among several other chromogens for environment monitoring. Despite its advantages, the use of DPC is limited due to its unstable nature, reduced sensitivity, and narrow linear range [28]. Research works have been conducted to increase the stability of DPC by coating variouspolymers, which, moreover, increases the sensor fabrication cost. Therefore, in this study, other different chromogenic substances such as 3,3’,5,5’-tetramethylbenzidine (TMB), o-Phenyldiamine (OPD), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) are envisaged towards cost effective colorimetric detection of Cr(VI). 3,3′,5,5′- tetramethylbenzidine (TMB) is one of the most used chromogenic substrates among peroxidase probes. In the presence of a catalyst, TMB can be oxidised by H_2O_2 to oxidised 3,3',5,5'-tetramethylbenzidine forming intense blue colour, which can be visualised with naked eye and spectroscopy. The absorbance peak can be recorded at 650 nm [29]. On the other hand, o-phenylenediamine (OPD)is a chromogenic substrate that generates yellow colour upon oxidation. The oxidation by H_2O_2 results in the formation of 2, 3-phenazinediamin, generating bright yellow colour and yellow fluorescence. The absorbance maxima of the colour change were measured at 420 nm [30]. Similarly, ABTS is a chemically stable and highly water-soluble peroxidase substrate. When oxidised by H_2O_2, it serves as a peroxidase substrate and creates a metastable cation by forming $ABTS^{4+}$. The oxidation produces blue-green colour, which can be quantified by recording absorbance maxima at 734 nm. This work chose TMB as the chromogenic substrate because of its superior sensitivity over OPD and ABTS comparatively. For example, a fixed concentration of the OPD and ABTS failed to produce a measurable colour change for different Cr(VI) concentrations in the presence of 50 mM H_2O_2, while the same concentration of TMB produced excellent measurable colour changes in the presence of 50mM H_2O_2 (Fig. 3.2). Also, TMB was found as chosen substrate in most of the literature regarding peroxidise, nanozyme and DNAzyme activities [31].

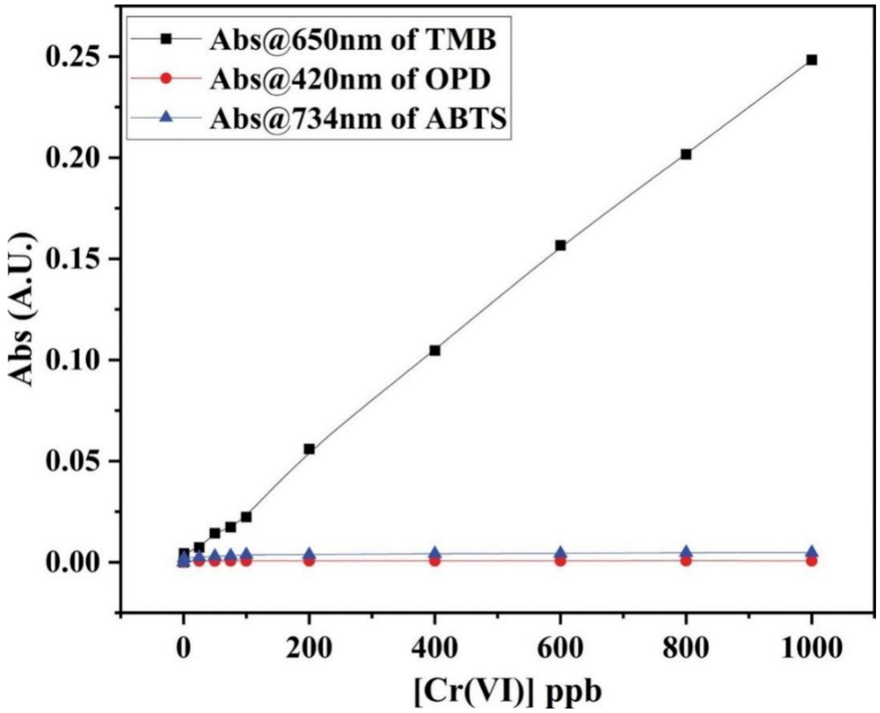


Fig. 3.2. Taken TMB, OPD and ABTS against Chromium 6 conc. under condition of H_2O_2 = 50 mM in UV-Vis Spectrophotometer

3.3.2. Reaction parameter optimisation

3.3.2.1. TMB concentration optimization

The absorbance maxima values at 650 nm of the TMB concentrations of 0.1 mM to 1.5 mM were found to be in a range of 0.0047 to 0.0685. It was observed that, from 0.9 mM concentration, the absorbance values got saturated. Therefore, as per this study, 0.9 mM concentration of TMB was found to be optimum for further experiments. The graph for TMB concentration optimization is represented in Fig. 3.3.

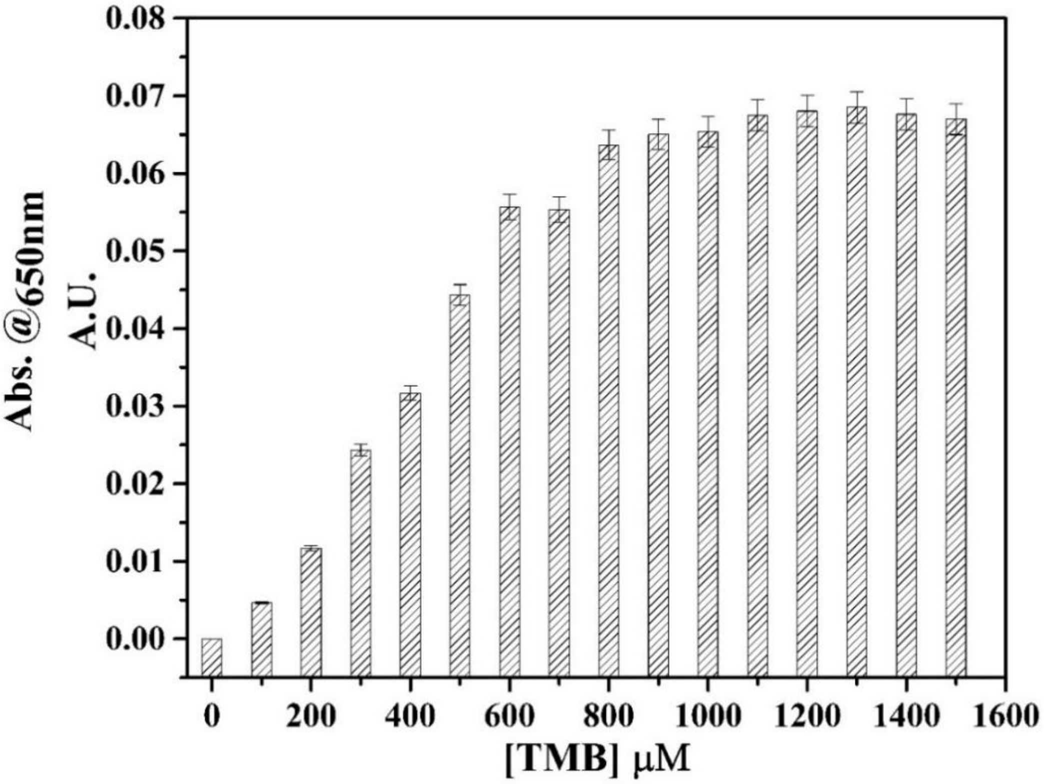


Fig. 3.3. TMB concentration optimization for Cr(VI) detection through UV-Vis spectrophotometer

3.3.2.2. H_2O_2 concentration Optimisation

The absorbance maxima values at 650 nm of the H_2O_2 concentrations of 10 mM to 300 mMwere found in a range of 0.0243 to 0.1523. From the absorbance maxima values, it was observedthat, the absorbance values get saturated from H_2O_2 concentration of 50 mM to 300 mM. Thus, 50 mM H_2O_2 was finalized as the optimum H_2O_2 concentration for further experimentation. Thegraph for H_2O_2 concentration optimization is represented in Fig. 3.4.

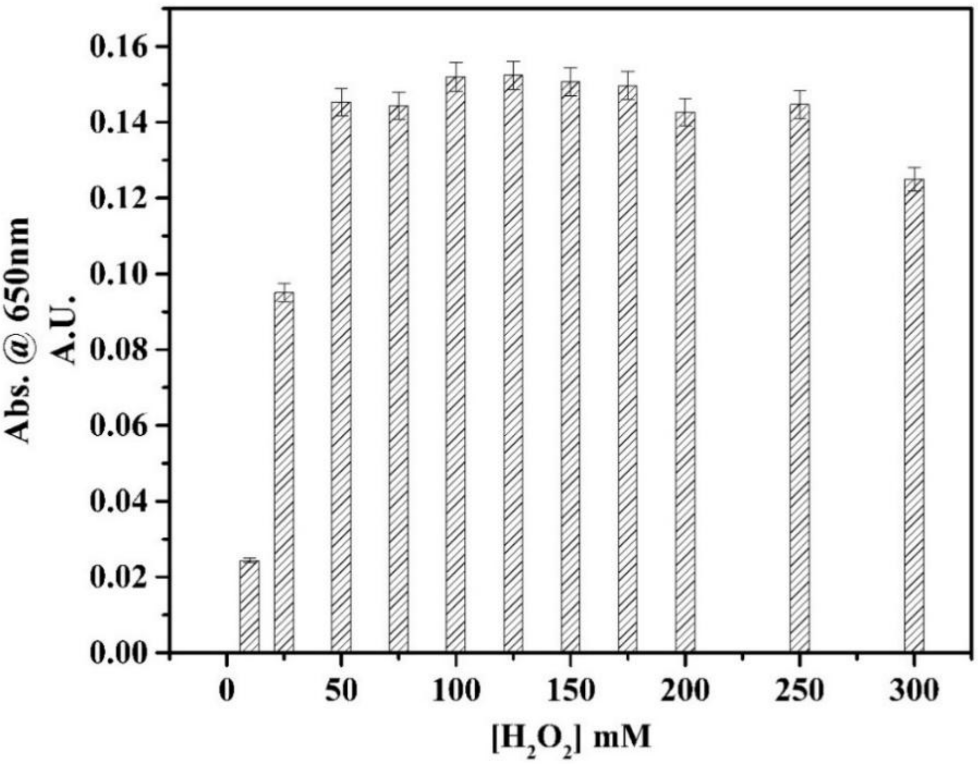


Fig. 3.4. H_2O_2 *concentration optimization for chromium 6 identification through UV-Vis spectrophotometer*

3.3.3. Colorimetric identification of Cr(VI) through spectrophotometer

In this work, ELISA substrate TMB is utilized as a instrument for colorimetric detection of chromium 6. Sensing mechanism of this colorimetry is based on oxidation of TMB by Cr(VI) in an acid media with the assistance of H^+ ions produced by H_2O_2. TMB is converted to oxidized TMB when chromium 6 is reduced to chromium 3 in an acidic environment. When TMB is oxidized from its colorless form, it becomes blue. Maximum absorption for this blue color occurs at 650 nm wavelength. As a result, by measuring absorbance at 650 nm, the intensity of the generated color is assessed spectroscopically. The absorbance maxima values at 650 nm of different Cr(VI) concentrations of 1 ppb to 1000 ppb were found to be in the range of 0 to 0.2435. Furthermore,it was found that, as the amount of Cr(VI) increased, Abs. at 650 nm increased steadily. The inset of Fig. 3. 5 provides images of TMB-H_2O_2 solutions mediated by various Cr(VI) concentrations for naked-eye examination. The linear fit analysis for the standard curve depicted excellent goodness of fit value of 0.994 (Fig. 3. 5). The equation for the standard curve is as follows:

$$Y_1 = m_1 \times X_1 + c_1$$

Where 'Y_1' is the absorbance value at 650 nm, 'm_1' is the slope, i.e., $2.55744 \times 10^{-4} \pm$

6.25491×10^{-6}, 'X_1' is chromium 6 concentration and 'c_1' is intercept value, i.e., -0.00178 ± 0.00281.

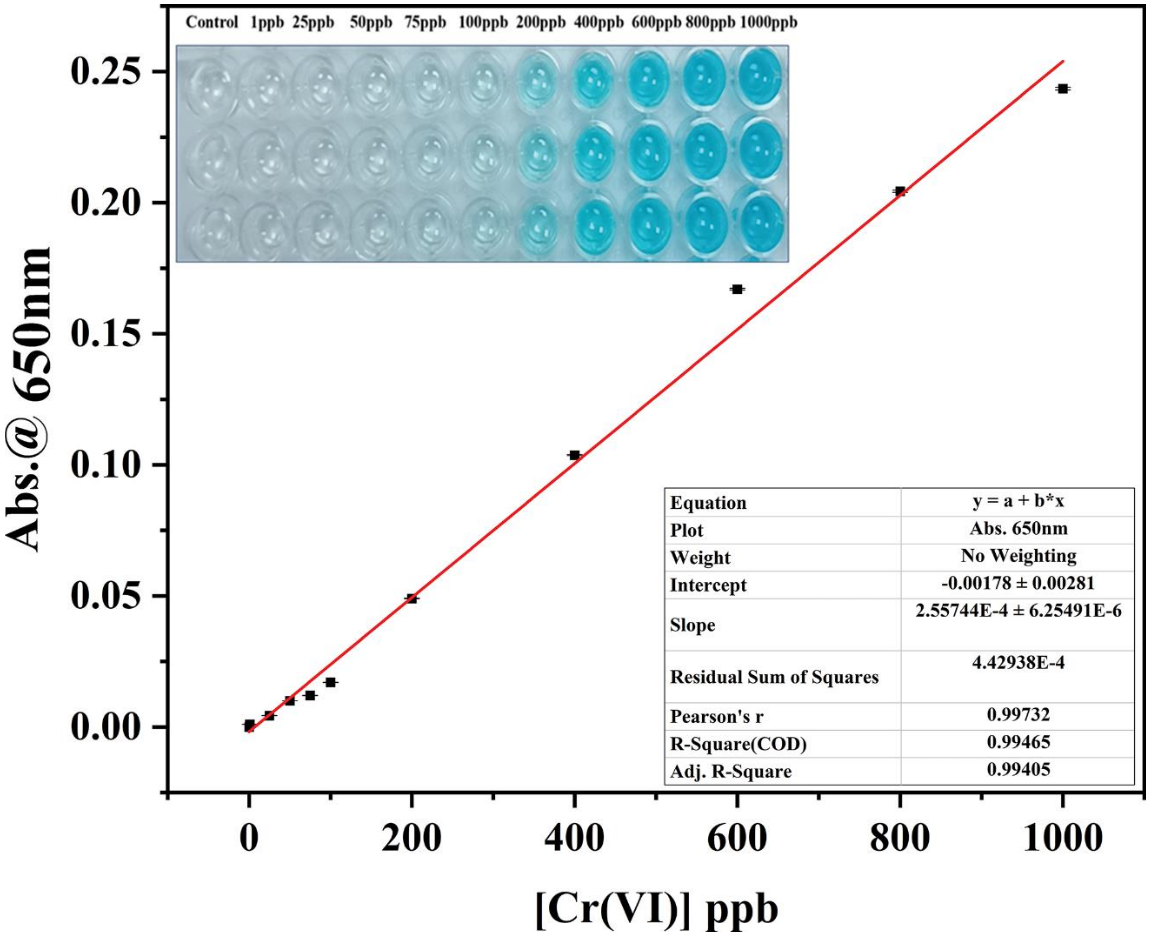


Fig. 3. 5. Standard curve of Chromium 6 under the optimized condition of [TMB] = 900 µM and [H_2O_2] = 50mM using UV-Vis Spectrophotometer; Captured image of visual colour change against different [Cr(VI)] (Inset)

3.3.4. Effects of oxidized ions on specificity and anti-interference

To confirm the selective/specific response for detecting Cr(VI), numerous oxidized ions, including cations and anions (Cr^{3+}, Pb^{2+}, As^{3+}, Hg^{2+}, Cd^{2+}, Ba^{2+}, SO_4^{2-}, HCO^{3-}, PO_4^{3-}) were supplemented in the reaction medium in presence and absence of Cr^{6+}. The Abs. maxima values at 650 nm were noted for several other ions in the same TMB-H_2O_2 reaction system. Fig. 3. 6. shows naked-eye color formation only in the wells where Cr(VI) was added. Also, there is a significantly high signal output in case of Cr(VI), unlike other ions when the absorbance values were studied.

To further investigate the viability of the new colorimetric chemosensor for Cr(VI), an anti- interference experiment was conducted. Under the optimized experimental conditions, equal amounts of other metals and other ions were added to the reaction system, and the absorbance maxima values were then measured at 650 nm. As shown in Fig. 3. 6., The existence of intruding ions has no obvious impact on the Abs. intensity at 650 nanometers of TMB solution after adding Cr(VI). These results demonstrated the novel visual chemosensor's excellent specificity and anti-interference for a qualitative Cr(VI) study.

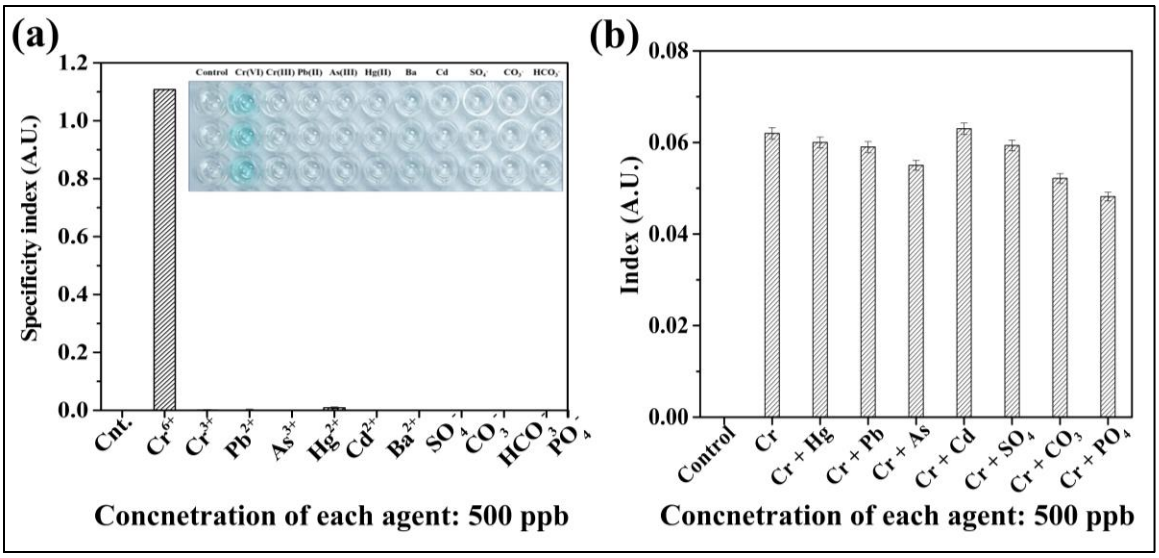
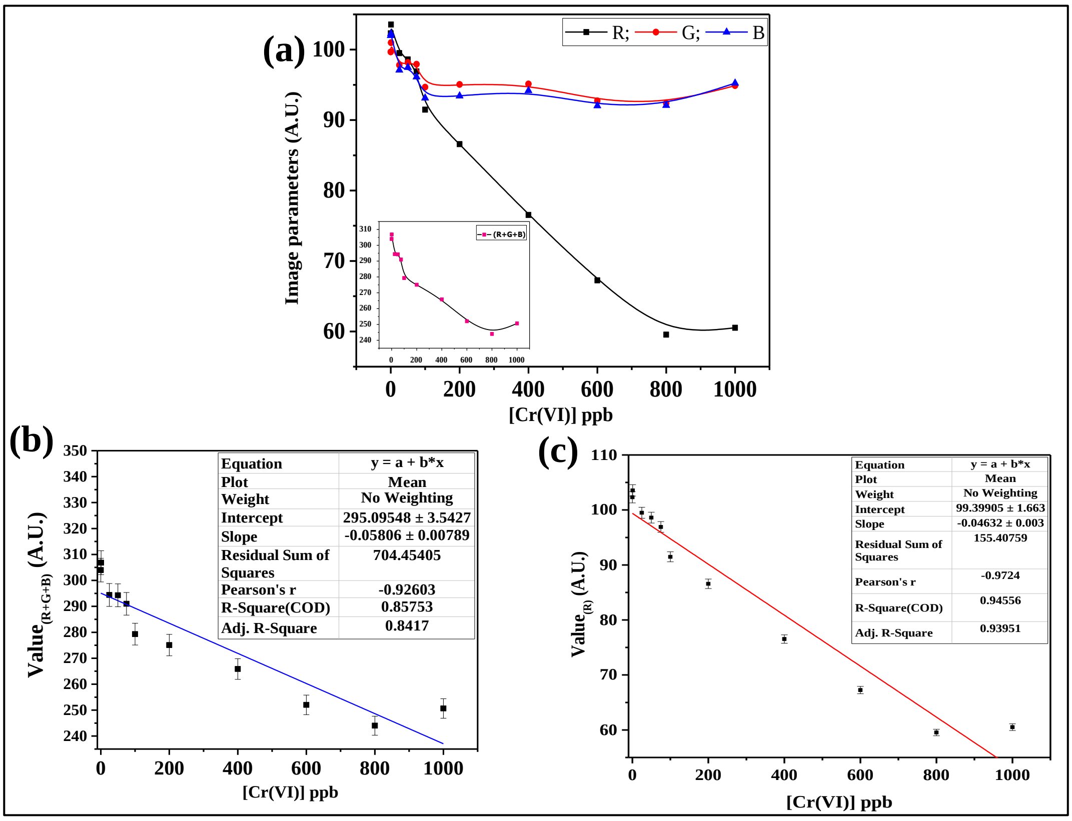


Fig. 3. 6. Specificity analysis of Cr(VI) through TMB oxidation in UV-Vis spectrophotometer; (a) Specificity index vs. different analytes; (b) Specificity index vs. mixture of individual analytes with Cr(VI)

3.4. Development of UIIS sensing system for Cr(VI) detection image parameter selection

In this work, different image parameter values, such as RED (R), GREEN (G), and BLUE (B), are extracted from the Cr-Detector system for further data analysis. When the G and B values are plotted against the chromium 6 concentrations, the different Cr(VI) amount cannot be clearly distinguished based on either G or B values, as both the G and B values for different concentrations appeared to be similar and thus no distinguishable pattern is observed. The linear fit equations for the plot of Cr(VI) concentration vs. G value and Cr(VI) concentration vs. B value provide poor goodness of fit values of 0.50 and 0.26. In contrast, the R-value and R+G+B value expressed a distinguishable pattern. The R-value for each concentration of Cr(VI) appeared to be much more distinguished; the linear regression analysis provided an excellent adjusted-R^2 value of 0.94. A similar pattern is observed for the R+G+B value vs. Cr(VI)concentration plot, where the linear fit with adjusted-R^2 value is 0.84. From this, R-value was targeted among other image parameters for all the experiments of colorimetric identification of chromium 6 due to its highest linearity and adjusted-R^2 value. The image parameter selection is represented in *Fig. 3. 7*.



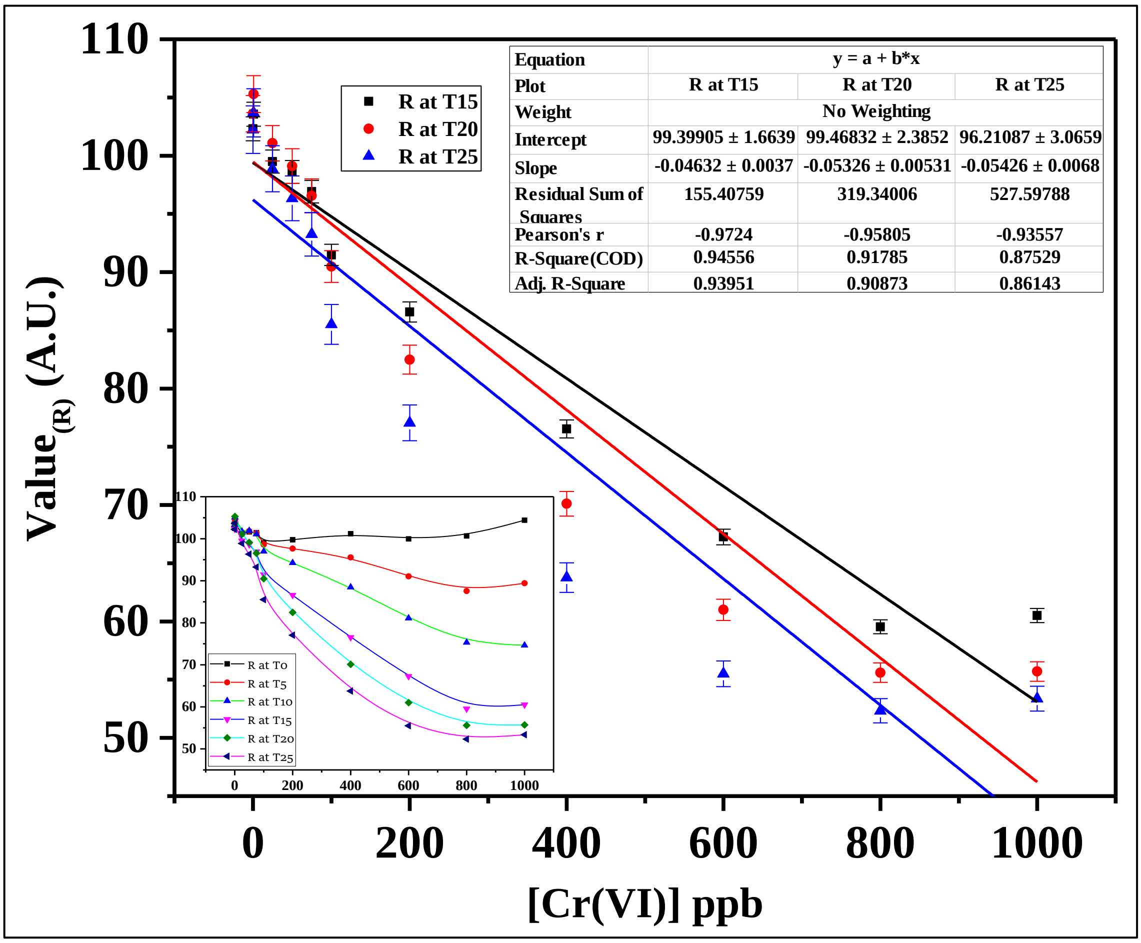
Equation	y = a + b*x
Plot	Mean
Weight	No Weighting
Intercept	295.09548 ± 3.5427
Slope	-0.05806 ± 0.00789
Residual Sum of Squares	704.45405
Pearson's r	-0.92603
R-Square(COD)	0.85753
Adj. R-Square	0.8417

Equation	y = a + b*x
Plot	Mean
Weight	No Weighting
Intercept	99.39905 ± 1.663
Slope	-0.04632 ± 0.003
Residual Sum of Squares	155.40759
Pearson's r	-0.9724
R-Square(COD)	0.94556
Adj. R-Square	0.93951

Fig. 3. 7. Image parameter selection for Cr(VI) detection through Cr-Detector: (a) plot of Cr(VI) concentration vs. different image parameter values such as R, G, B, and R+G+B (inset of a); (b) Linear fitting curve of Cr(VI) concentration vs. R+G+B value plot and goodness of fit calculation; (c) Linear fitting curve of Cr (VI) concentration vs. R-value plot and goodness of fit calculation

3.4.1. Reaction time optimization

The reaction time was optimized with fixed TMB, H_2O_2 concentration, and different Cr(VI) concentration ranges from 0 ppb to 1000 ppb. Then, a kinetics study of the reaction is performed for 25 minutes with a minimum interval of 5 minutes. We considered the ‘RED’ (R) value among the other image parameters for further analysis. The data generated every 5 minutes are plotted against the Cr(VI) concentration ranges from 0 ppb to 1000 ppb. Visual colour changes for the concentrations appeared from 15 minutes of the kinetics study. Before 15 minutes, the visual colour changes were negligible. The plot explained that the difference between the ‘R’ values of all the concentrations before 15 minutes of the kinetic study was significantly less and thus produced flat patterns with minimum slope. Whereas distinguishable differences appeared for the different concentrations from 15 to 25 minutes of the kinetic study, thus producing patterns with a higher slope. The linear regression analysis of the patterns observed at 15 minutes, 20 minutes and 25 minutes of the experiments showed adjusted goodness of fit values (R^2) as 0.93, 0.90 and 0.86, respectively. From this, it was concluded that the reading could be taken in theCr-Detector after 15 minutes of incubation of the reaction mixture to get the best possibleoutput. The reaction time optimization through Cr-Detector is represented in *Fig. 3. 8*.



Equation	y = a + b*x		
Plot	R at T15	R at T20	R at T25
Weight	No Weighting		
Intercept	99.39905 ± 1.6639	99.46832 ± 2.3852	96.21087 ± 3.0659
Slope	-0.04632 ± 0.0037	-0.05326 ± 0.00531	-0.05426 ± 0.0068
Residual Sum of Squares	155.40759	319.34006	527.59788
Pearson's r	-0.9724	-0.95805	-0.93557
R-Square(COD)	0.94556	0.91785	0.87529
Adj. R-Square	0.93951	0.90873	0.86143

Fig. 3. 8. Reaction time optimization for Cr(VI) detection through Cr-Detector: linear regression analysis of Cr(VI) concentration vs 'R' value plot at different incubation times (15 Min, 20 Min and 25 Min). The inset represents scatter plot of Cr(VI) concentration vs 'R' value at different incubation times (T=0, 5, 10, 15, 20, 25 Minutes)

3.4.2. Colorimetric identification of Cr(VI) through Cr-Detector

Further, the mentioned protocol for colorimetric detection of Cr(VI) was repeated 500 times in Cr-Detector to generate the R-values of the image acquired through scanning the

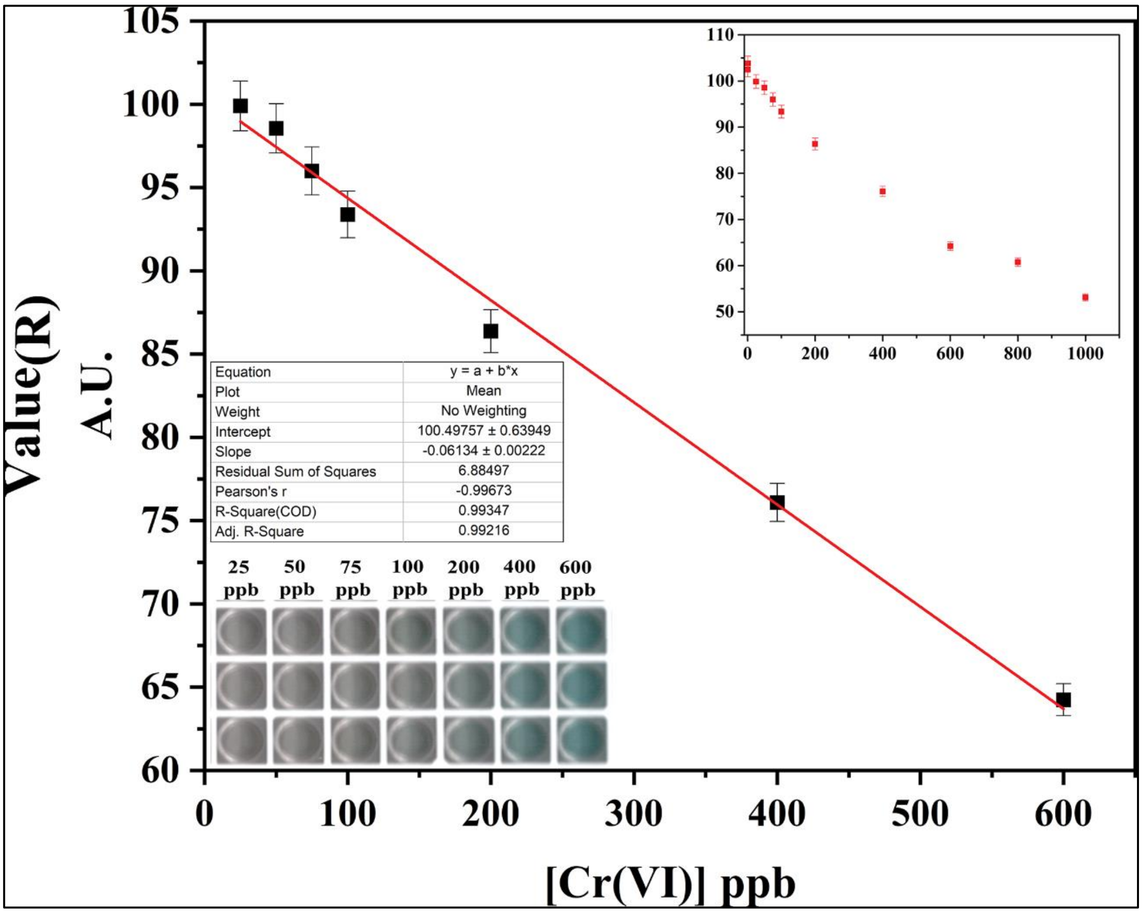
96 well plates towards developing the calibration curve (*Fig. 3. 9*).

The average R-values of the concentrations range from 0 ppb to 1000 ppb and are plotted against the concentrations towards the development of the calibration curve (Represented in the inset of *Fig. 3. 9*). The plot demonstrates the calibration curve with the linearity found from 25 ppb to 600 ppb of Cr(VI).

The equation of the calibration curve is found to be:

$$Y_2 = m_2 \times X_2 + c_2$$

Where 'Y_2' is R-value generated in Cr-Detector, 'm_2' is the slope, i.e., -0.06134 ± 0.00222, 'X_2' is the Cr(VI) concentration, and 'c_2' is the intercept value, i.e., 100.49757 ± 0.63949. The adjusted-R^2 value is found to be 0.99216. The calculated theoretical LOD and LOQ values from the calibration curve is 0.12 ppb and 0.36 ppb, respectively, with a linear detection range 25 to 600 ppb.



Equation	y = a + b*x
Plot	Mean
Weight	No Weighting
Intercept	100.49757 ± 0.63949
Slope	-0.06134 ± 0.00222
Residual Sum of Squares	6.88497
Pearson's r	-0.99673
R-Square(COD)	0.99347
Adj. R-Square	0.99216

Fig. 3. 9. Standard curve of different [Cr(VI)] with optimized parameters in Cr-Detector; Full experimental range of different [Cr(VI)] from 0 ppb to 1000 ppb under optimized parameters (Inset)

3.4.3. Recovery analysis

A recovery analysis has been carried out to check the device's applicability. 50, 100, 250 and 500 ppb of Cr(VI) were spiked in Milli-Q water, and recovery percentages of spiked concentrations are 101.056%, 100.026%, 100.0324% and 99.9716%, respectively. The recovery error (RE) percentages for 50, 100, 250 and 500 ppb are -1.056, -0.026, -0.3024 and 0.0284, respectively (Table 3.1).

Table 3.1. Recovery analysis of Cr (VI) in Cr-Detector

[Cr (VI)] Added (ppb)	**Developed device**		
	[Cr (VI)] Found (ppb)	**RE (%)**	**Recovery (%)**
	Mean ± SD		
50	50.528 ± 0.79	-1.056	101.056
100	100.026 ± 0.99	-0.026	100.026
250	250.756 ± 0.84	-0.3024	100.3024
500	499.858 ± 1.69	0.0284	99.9716

3.4.4. Real sample analysis

Further, 27 samples from the southern belt of river Hooghly of West Bengal, India, were collected where most industries are located. The specimens were evaluated according to the aforementioned reaction technique, and results were collected with the spectrophotometer and Cr-Detector. The data are represented in Table 3.2. Out of 27 samples, except 3, the rest of the samples' detection was comparable with the conventional spectrophotometric analysis. The correlation coefficient of the UIIS data with spectrophotometer data was 0.99018.

Table 3.2. Comparison of predictive data after analysis of the river water sample through commercial UV-Vis Spectrophotometer and Cr-Detector

Sample ID	**Spectrophotometer**		**Cr-Detector**		
	O.D. Value	**Predictive [Cr(VI)] (ppb)**	**UIIS Index (A.U.)**	**Predictive [Cr(VI)] (ppb)**	**Error percentage (%)**
1	0.0005	20.643	86.213	232.875	10.281
2	0.0430	187.412	89.779	174.740	0.068
3	0.0716	299.569	83.092	283.752	0.053
4	0.1420	575.647	65.795	565.741	0.017
5	0.0001	19.176	N.D.*	N.D.	N.A.#
6	0.0251	117.255	93.702	110.785	0.055
7	0.1101	450.549	73.373	442.200	0.019
8	0.0448	194.471	88.976	187.831	0.034

9	0.0770	320.745	81.041	317.192	0.011
10	0.1160	473.686	71.234	477.072	0.007
11	0.0100	58.000	97.994	40.815	0.296
12	0.0260	120.745	93.523	113.703	0.058
13	0.0002	19.569	N.D.	N.D.	N.A.
14	0.0200	97.216	94.593	96.260	0.010
15	0.1130	461.922	72.660	453.824	0.018
16	0.0160	81.529	N.D.	N.D.	N.A.
17	0.0603	255.255	84.607	259.057	0.015
18	0.1010	414.863	75.959	400.042	0.036
19	0.0003	19.961	N.D.	N.D.	N.A.
20	0.0011	23.098	N.D.	N.D.	N.A.
21	0.1060	434.471	73.908	433.478	0.002
22	0.1400	567.804	66.686	551.216	0.029
23	0.1470	595.255	64.623	584.848	0.017
24	0.0308	139.569	75.200	412.416	1.955
25	0.0520	222.706	86.218	232.794	0.045
26	0.0280	128.588	93.345	116.605	0.093
27	0.0148	76.824	96.109	71.545	0.069

*N.D.=Non-Detectable (when, 25 ppb<predictive concentration>600 ppb); #N.A.= Not Applicable

3.5. Comparison of ‘Cr-Detector’ with other sensors for detection of Cr(VI)

Lots of similar work on sensor development for identification of chromium 6 have been reported in earlier studies. The result obtained from ‘Cr-Detector’ was compared with other sensors and it correlates well with other reported literature in Table 3.3.

Table 3.3. Recent advancements in sensor-based detection of Cr(VI)

Developed sensor	**Sensor design**	**Transduction principle**	**Linear Range**	**Limit of Detection**	**Ref.**
Colorimetric paper-based analytical device	Diphenylcarbazide on µPAD	Optical	10-90 ppb	3 ppb	[32]
Wireless portable device	Using 1, 5 diphenyl carbazide aschromogen	Optical	-	2 ppb	[33]
POC instrument for field detection of Cr(VI) in wastewater	Using citrate-capped silver nanoparticles	Optical	10-700 ppb	26 ppb	[34]
Photoelectrochemical sensor for identification of trace Cr(VI)	By photon-excited electron-hole pair separation coupledby light-sensitive quercetin	Photo-electrochemical	1.4-2592.3 ppb	0.44 ppb	[35]
Portable sensing instrument for idetection of Cr(VI) in wastewater and living cells	Devicethin layer (~1 mm) of polyvinylidene fluoride-co-hexafluoropropylene membrane	Optical	-	3.4 ppb	[36]
Choromium 6 identification with disposable SPE modified with AuNPs	AuNPs electrodeposition-based voltammetric identification	electrochemical	10-5000 ppb	5 ppb	[37]
Au-Ag NPs modified electrochemical sensor for identification of Cr(VI) in wastewater specimen	Ag-Au bimetallic NPs by electrochemical deposition	electrochemical	50–5000 ppb	0.1 ppb	[38]
Electrochemical identification of	g-C3N4 decorated with Ag molybdate	electrochemical	5.199–36.39	0.0831	[39]

chromium 6 in specimen using modified GCE	immobilizedwith nafion modified GCE		ppb	ppb	
Flower-like self-assembly ofAuNPs on a GCE for detection of chromium(VI)	The first is sol–gel film derived fromMPTS. The second gold nanoparticle layer is self-assembled on surface ofsol–gel film	electrochemical	0.01–1.2 ppb	0.0029 ppb	[40]
Electrochemical identification of Cr(VI) using Nickel Oxide NPs	Sol–gel synthesized Nickel OxideNPs coated onto the fuorine doped tin oxide plate	Optical	5000-50000 ppb	5000 ppb	[41]
Electrochemical identification of chromium 6 based on AuNPs decorated titania nanotube arrays	Au-NPs-designed TiO_2NTs grown on a titanium substrate	electrochemical	5.199-5448 ppb	1.55 ppb	[42]
Electrochemical identification of ppb level Cr(VI)using AuNPs	AuNPs grown on a conducting substrate modified with sol–gel-derived thiol functionalized silicate network	electrochemical	0.2-3 ppb	0.1 ppb	[43]
A miniaturized portable electrochemical device for Chromium 6	SPCE with AuNPs modification	electrochemical	20-200 ppb	5.4 ppb	[44]
Electrodeposited Gold nanoparticles /Graphene oxide Nanocomposite as sensor for Cr(VI) determination in water	Electrodeposition of reduced graphene oxide on Glassy Carbon Electrode by electroreduction of Graphene Oxide using CV	electrochemical	5.19-1559 ppb	2.39 ppb	[45]
Ion-imprinted chitosan-graphene nanocomposites for identification of chromium 6	Ion-imprinted polymers electrodeposition of chitosan-graphene nanocomposites (IIP-S)	electrochemical	0.051-520 ppb	0.33 ppb	[46]
‘Chromium-Detector’ system for specific identification of Cr(VI) in water	Image parameter extraction and analysis for TMB oxidation by chromium 6 presence of H_2O_2	Optical	25-600 ppb	0.12 ppb	Present study

3.6. Conclusion

Herein we report development of a chemo-sensor, TMB, for identification chromium 6 in water using spectrophotometric process. The sensor was tested against various interfering parameters. In the presence of those interfering substances, the chemo-sensor exhibited excellent specificityand selectivity towards Cr(VI). Using the spectrophotometric method, the linear range was 1 to 1000 ppb with an adjusted-R^2 value of 0.994. Furthermore, the chemo-sensor was integrated with Cr-Detector to demonstrate a lab-to-field application that, extended the sensing system's applicability. Using a uniform illumination-based system, linear range was determined to be 25 to 600 ppb of Cr(VI) with LOD 0.12 ppb, which is well comparable with other reported literature concerning the linear range and detection limit, as presented in Table 3.2.

Furthermore, the exhibited wider linear range covers the prescribed permissible limit by USEPA and FSSAI. Real sample analysis revealed a good correlation with spectrophotometric data. Integrating TMB as a chemo-sensor and Cr- Detector, a selective, rapid, portable, user-friendly device for measuring Cr(VI) is first reported in this endeavor, to our knowledge. Image analysis-based field portable sensor and sensing system development for low-cost rapid onsite monitoring of environmental toxicants will encourage the future development of array-based sensor integrated devices that will enable cumulative detection and multi-parametric analysis of more than one analyte at a time. Moreover, the advancement in soft computing has revolutionized data analytics for sensor development. The rapid rise of machine learning was effectively utilized to enhance the empirical performance of colorimetric assessment (e.g., UV-Vis spectrophotometer and colorimetric test paper), involving blurring, acknowledgment, and summarization of every minor characteristic variation in each image [47–50]. One example is Deep Convolutional Neural Networks (DCNNs) [51]. DCNNs have excellent potential to increase the sensitivity and selectivity of any biosensor by rapid and effective decision generation. In comparison to human methods, the automated aspect of DCNNs enables quick and effective analysis. The rapid onsite one-click picture processing can be achieved through DCNNs. Moreover, Users with different degrees of image analysis or colorimetric sensing experience can utilize this one-click method since it is user-friendly and accessible. Again, Few-Shot Learning (FSL) and Generative Adversarial networks (GAN) can increase the accuracy of DCNNs. These soft computing technologies may be explored to develop effective decision support systems with superior sensitivity and selectivity in future.

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Chapter 4

Development of field portable potentiostat using electrochemical aptasensing technology for detection of Cr(VI) in aquatic environment

List of Sections

- **Introduction**
- **Materials and methods**
- **Result and discussion**
- **Conclusion**

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Chapter 4

Development of field portable potentiostat using electrochemical aptasensing technology for detection of Cr(VI) in aquatic environment

4.1. Introduction

Water is a premier resource of livelihood for various organisms, including humans. In addition, it has been a source of economy for agriculture, fisheries, healthcare, and the food chain due to its wide range of usages. Numerous industrial anthropogenic activities become discern to the water sources [1]. Growth in human activities and rapid industrialization quickly raise the risk of heavy metals in water, resulting in an escalation of metallic substances in aquatic and terrestrial environments [2]. Heavy metals increased in water due to their activities include, cultivation, factories and the tanning industries, livestock, chicken manure, automobiles, and road works to increase the heavy metal percentage. As a result, many heavy metals are present in the environment, like arsenic (As), cobalt (Co), chromium (Cr), lead (Pb), zinc (Zn), copper (Cu), etc., and become harmful to living beings [3,4]. Hence, the severity of illnesses rises and becomes a silent killer for human beings. Thus, the need of the hour is to facilitate safe water for the growth of the economy and better human livelihoods.

The presence of Cr is of more significant concern in the environment because Cr is present in two states in environment, chromium 3 and chromium 6. It is naturally present in plants, soils, rocks, and sediments and is a vital micronutrient essential in the daily diet [5]. Cr(VI) toxicity is a severe concern, being frequently used in wood preservation, metal plating, ink producing, pigment dyes, ceramics, tanning, and textile industries that percolate into the groundwater [6]. High levels of oxidation inside the cells can result from Cr(VI), which may harm the kidney, salivary glands, throat, skin, and lungs in addition to causing chronic ulcers [7,8]. WHO and FSSAI had prescribed the safety limit of Cr(VI) in drinking water to be 50 $\mu g\ L^{-1}$ [9].

Presently the standard detection of Cr(VI) is done using inductively coupled plasma-mass spectrometry (ICP-MS) [10], atomic absorption spectrometry (AAS) [11], chromatography, fluorometry [12], Raman scattering spectroscopy [13], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [14], X-ray fluorescence (XRF)

[15]. All these methods are sensitive and exhibit low detection limits, though they require trained workforces, sample pre-treatment, and a longer time to detect, with huge set-up costs. Moreover, due to size and sophistication, these instruments are non-portable. Currently, in the area of analytical research, most active regions is development of chemical and biological sensors. Nanomaterial-induced biochemical and chemical sensors in the field of environmental analyte monitoring have flourished in recent technological advancements [16–19]. There are several outcomes as reported recently, where fluorescence measurement based sensors are applied in the trace level detection of chromium in water [20,21]. Other than optical sensors, electrochemical sensor-based detection of Cr(VI) is also reported in current studies. The transduction through electrodes is being used as a working principle of electrochemical sensors, a subclass of chemical sensors. As far as the electrochemical sensor is concerned, the sensors usually convert chemical signals to electrical signals. Analytes, when they interact with the working electrode, a core component of the electrochemical sensor, generally activate chemical reactions and result in the ion movement at the electrode interface governed by an applied electrolyte. This ion movement can be measured as voltage, current, conductivity or impedance [22]. In this case, the reference electrode, another core component of electrochemical sensor, helps in sustaining consistent reference potential that ensures accurate measurements. The analyte concentration can easily be determined though the resultant electrical signal generated, thus ensures the vast applicability of such type of sensors in diverse domains. These devices have the capability and portability with a variety of significant uses in medical, industrial, ecological, and agricultural assessments [23]. Therefore, modification of electrodes with sensitive films and usage of pulsed electrochemical procedures have been extensively used to detect Cr(VI) in water in recent times. These techniques are preferable because of their fast detection, excellent stability, simple operation, high selectivity, and sensitivity [24]. Several important disclosures on metal-organic framework, heterometallic compound-based electrochemical, photoelectrochemical sensing and removal of trace level of Cr(VI) have paved the path for rapid development of electrochemical sensing systems[25–30]. Several colorimetric and electrochemical devices have been reported in the field of Cr(VI) detection, which also comments on the real-time monitoring of Cr(VI) [31–37]. Moreover, these works on development towards the detection of Cr(VI) claim the applicability of such sensors in environmental diagnostics and open vast opportunistic pathways for significant improvement in the simplicity of the sensor fabrication and development of field portable devices.

The main objective of the current study was to develop a handheld electrochemical aptasensor device for the qualitative and quantitative measurement of Cr(VI) in water under the WHO-prescribed maximum permissible limit of 50 ppb. This endeavour depicts a simplistic method for electrochemical detection of Cr(VI). The recent work sequentially describes the screen printed electrode designing and fabrication, its electrochemical characterization, modification with bio-receptor, i.e. DNA aptamer, handheld potentiostat designing and fabrication, experimentation with spiked samples of different Cr(VI) concentrations, real sample analysis, stability and interference studies of the developed sensor and the future scope of present work. In this study, Cr(VI) detection was represented using electrochemical aptasensing. The usage of single-stranded DNA aptamers towards the development of biosensors for environmental diagnostics is of prime interest in recent scenarios. Despite the disintegration issue of DNA aptamers under harsh acidic conditions, these biomolecules possess remarkable strengths to justify their applicability in diverse fields. The single-stranded DNA aptamers have the inherent property of stable three-dimensional folding, enabling selective affinity toward the target analyte. Moreover, compact size and cost-effective synthesis allow the DNA aptamers to be used in low-cost sensor fabrication and application [38]. Additionally, these biomolecules also allow their integration in different sensor platforms with minor modifications and to be linked with different signal transduction principles such as optical, electrochemical, piezoelectric, Surface Plasmon Resonance, etc., ensuring applications in diverse domains. The peak current measurement at a constant potential of 0.20 V has been utilized for the detection of limited concentrations of Cr(VI) in aquatic environments. Initially, a hand-held electrochemical potentiostat development with the incorporation of microelectronics and soft computing was elaborated on in this work. The spiking and recovery studies have been explored to benchmark the developed method that could be used for real water samples for the detection of Cr(VI). Moreover, the device has been utilized for real sample analysis.

4.2. Materials and methods

4.2.1. Materials and instrumentation

Potassium dichromate (99.0%), potassium chloride (KCl) (99.0-100.5%), Sodium Citrate dehydrate (99%), sodium hydroxide (97.0%), concentrated hydrochloric acid (36.5-38.0%), sodium acetate (99%), sulfuric acid (98%), Sodium hydrogen carbonate ($NaHCO_3$), Sodium sulfate (Na_2SO_4), Potassium carbonate (K_2CO_3), Zinc sulphate ($ZnSO_4$), Cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$), Barium nitrate ($Ba(NO_3)_2$), Mercury chloride ($HgCl_2$), were obtained from Sigma Aldrich. The citric acid (99.5%) was obtained from Hi-Media Laboratories Pvt. Ltd. (India). Nafion™ perfluorinated resin solution (20 wt%), ferric chloride hexahydrate ($FeCl_3.6H_2O$), potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$), potassium ferricyanide ($K_3[Fe(CN)_6]$) have been procured from Sigma-Aldrich India. Cr(VI) aptamer has been procured from SYNGENX INDIA. Laboratory-based electrochemical measurements have been performed on Autolab PGSTAT302N (Metrohm Autolab B.V., Netherlands). Custom-made screen-printed electrodes (SPE) have been fabricated from PalmSens B.V., Netherlands. Micropipettes (eppendorf®, Germany) have been used in all sample preparations. The pH meter has been procured from Mettler Toledo, 8606, Switzerland. The instrument materials, such as a Raspberry pi compatible DAQ (USB 6008 DAQ) card, were obtained from DIGILENT, and a Raspberry pi motherboard for internal software processing and data visualization was obtained from Raspberry pi. The wires and battery were obtained locally, and the units were fabricated using AutoCAD® and Flashforge adventure 3D printer from Hydrotech 3D.

4.2.2. SPE fabrication

In the current day scenario, screen-printed electrodes (SPE) are widely chosen as the main component of the electrochemical sensing system development by various scientists. The electrode surfaces of the SPEs can easily be modified with several biochemical agents, which allows further immobilization of various biomolecules towards target molecules for detection and quantitation. In this work, we have designed SPE by AutoCAD. The specifications are notified in Fig. 4.1. The SPEs have been fabricated using PalmSens B.V., Netherlands facilities. Here, in these SPEs, the working electrode is made of carbon, where platinum and silver/silver chloride were used as CE and RE.

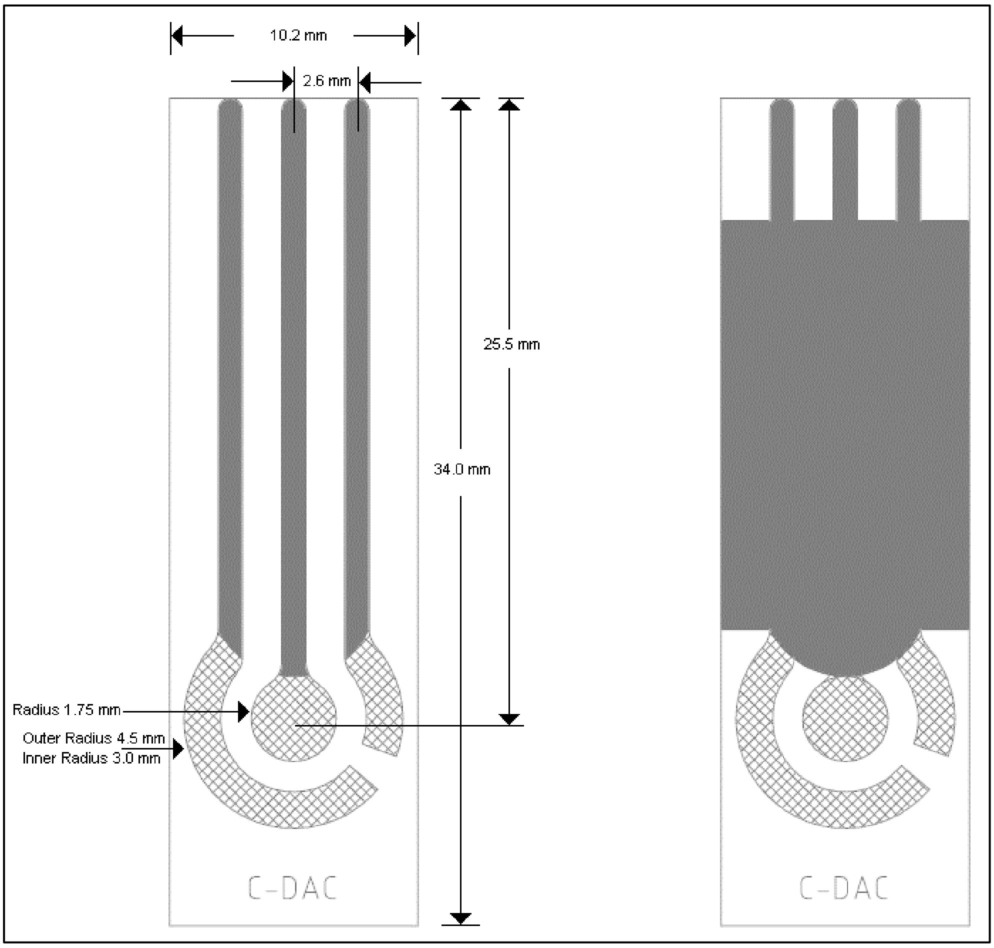

Fig. 4.1. Schematic drawing of SPE with the actual dimension

4.2.3. Aptamer preparation

The 1000 nM 62 base long Cr(VI) DNA aptamer with sequence 5’-CCACGCATAGGGCAAATCAAGCACACCCTCTAATGTTGCCTCTGATTCTGGCCTATGCGTGC-3’ [39] has been procured from SYNGENX INDIA. After procurement, the aptamer was suspended in DNase-free water. Then, the aptamer solution was heat shocked at 92 °C for 8 minutes, followed by rapid cold shock in the ice bath. [40]. Further, the heat and cold shocked aptamer solution was diluted to 250 nM working concentration using DNase-free water. This aptamer concentration was further used for modification of the electrode surface.

4.2.4. Modification of SPE

4.2.4.1. Electrode surface cleaning

The cleaning of the developed electrodes has been performed with 0.05 M phosphate buffer solution. Further, the chronoamperometric method at a fixed potential of +1.7 V for a time period of 180 seconds was applied to clean the developed electrodes in the presence of a mixture of 0.1 M KCl and the phosphate buffer solution. Following that, the SPEs were dried at room temperature.

4.2.4.2. Prussian Blue (PB) modification

The PB solution was prepared by mixing equal volumes of two solutions, solution A and solution B. Solution A was the mixture of 500 mL of 0.1 M KCl and 500 mL of 0.1 M HCl. At the same time, solution B was prepared by mixing 10 mL of 0.1 M $FeCl_3.6H_2O$ and 10 mL of 0.1 M Potassium ferrocyanide. Deionized water was used for all preparations. Finally, 60 µL of freshly prepared PB solution was taken onto the SPE surface for further electro-deposition of PB through the chronoamperometry method. The voltage was applied at +0.4 V for 50s. Excess PB was rinsed with deionized water and dried using an N_2 flush after deposition. Finally, using the deposited layer in solution A, CV (scan rate of 50 mV/s, fixed potential range from +0.4 V to +1.4 V, cycles 30) was used to modify the electrodes. The modified electrodes were further kept for baking in the environmental chamber at 100 °C for 90 min.

4.2.4.3. Nafion-aptamer modification of SPEs

For further modification of the PB-modified electrodes, Nafion and Cr(VI) aptamer were mixed in several volume ratios such as 1:0.05, 1:0.10, 1:0.25, 1:0.50, 1:1, 0.50: 1, 0.25:1, 0.10:1 and 0.05:1. The Nafion and aptamer mixtures were prepared by diluting the stock concentrations (Nafion 20-wt% and aptamer 250 nM) with deionized water. 2 µL of this has been uniformly placed on each of the working carbon electrodes and left at room temperature for drying overnight. CV of the modified electrodes was measured as per the above-mentioned specifications, and the Nafion to aptamer ratio was optimized by observing the peak current values (Fig. 4.2).

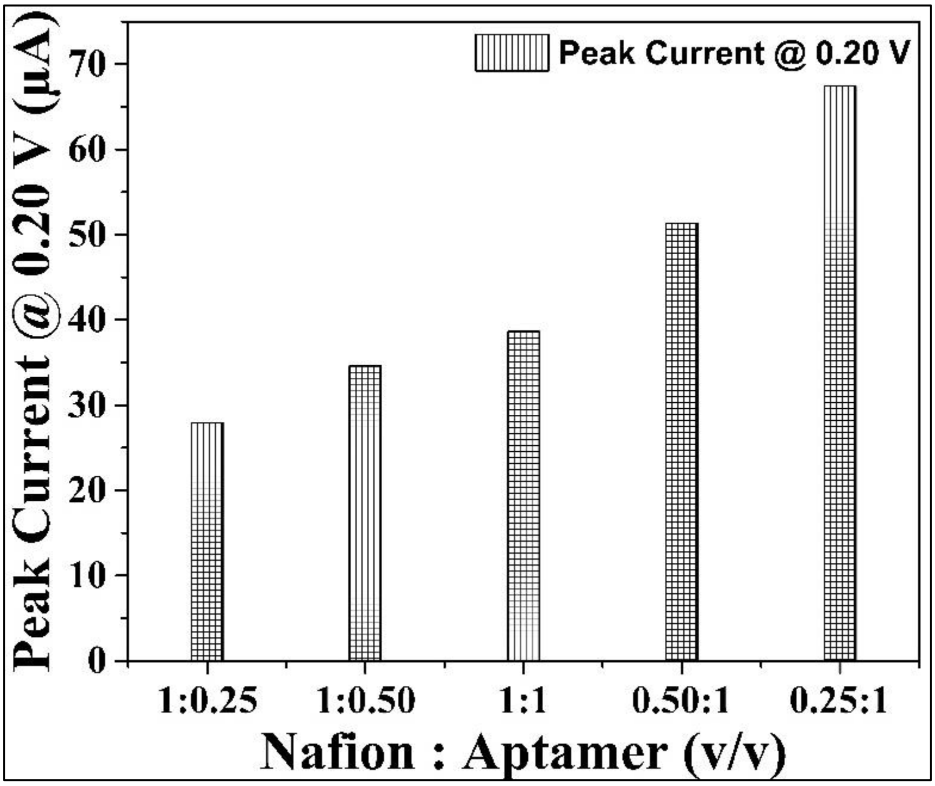

Fig. 4.2. Nafion to aptamer volume optimization

4.2.4.4. Characterization of modified SPE

The SPEs have been characterized at various stages of modification, such as a bare electrode, after PB modification, after Nafion modification, and finally after modification with a Nafion-aptamer mixture. Cyclic voltammetry (CV) has been carried over the potential range from -0.5 V to 0.6 V with a scan rate of 10 mV S^{-1}. Moreover, the modified surface of the electrodes is also scanned by SEM to acquire knowledge on the modification (Fig. 4.3).

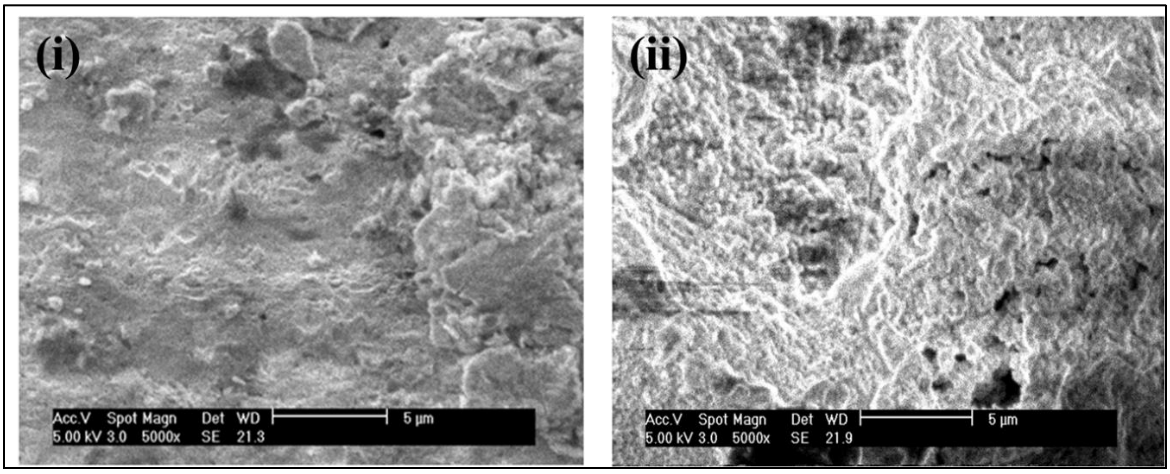

Fig. 4.3. SEM images of SPE modification; (i) working electrode surface of PB modified SPE; (ii) working electrode surface of Nafion-aptamer modified SPE

4.2.4.5. Sensory system designing and fabrication

The handheld potentiostat design has an analogue front end and digital end. A variety of analogue circuits are used at the analogue front end to generate CV, chronoamperometry, and other potentiostatic experiments. The three-electrode system in the form of SPEs was implemented in the device. Op-Amps were further used in the device to keep the constant potential among working and reference electrodes of SPEs. Trans-impedance circuit current to voltage conversation was used to measure the current from the working and counter. To create a specific voltage that might be further read by a high-resolution analogue to a digital converter, the current was multiplied by an impedance. An 8-bit microcontroller was used to monitor and control the entire project. The digital end consists of a microcontroller to control the analogue front end. The system has a real-time clock and microSD for data logging with a digital time stamp. The proposed system would be based on the voltammetry technique.

A cabinet having two segments, such as a segment to accommodate electronics and another segment that is to be used as a sample-handling unit that enables easy-to-use operations and testing procedures, has been created. The developed device also consisted of a data acquisition card (DAQ card) and battery. Continuous data was collected through this highly reliable DAQ card during the sample testing. The software developed for this system controls all the operations of the testing process. The developed device named ‘metalloSTAT’ is represented in Fig. 4.4.

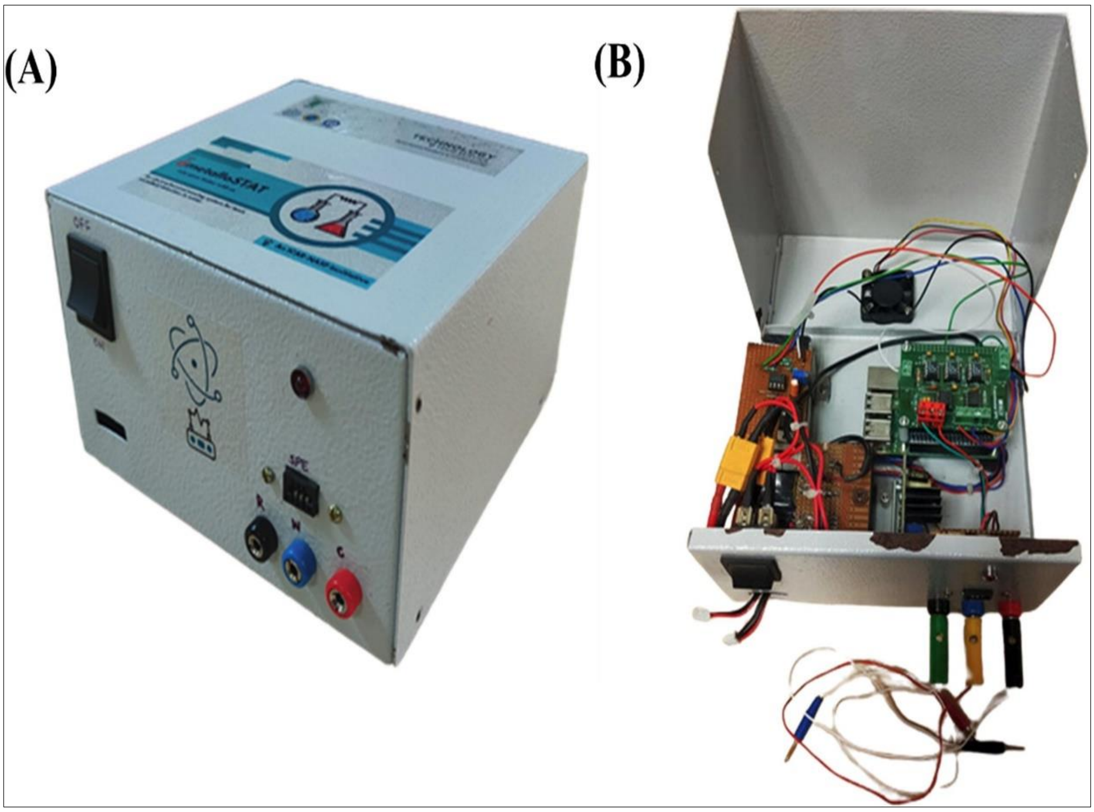

Fig. 4.4. (A) Image of the final version of battery-operated handheld prototype "metalloSTAT", (B) The internal image of the electronics of 'metalloSTAT'

The front panel of the developed software has been designed to offer the option of either "training" or "testing" the system. Initially, the system has been trained with known concentration values of samples to create a database in the system so that at the time of testing, the data of unknown samples can be correlated with the trained database. The algorithm has been designed in such a way that the system has the ability to create a trained database on its own. The testing cycles were operated with a single mouse click, and after completing the testing cycle, the system was to generate the outcome of the testing process and indicate the quality parameters of the sample.

4.2.5. Electrochemical sensing of Cr(VI)

Various amount of chromium 6 have been formulated by combining suitable amount of dichromate in Milli-Q water. For this study, concentrations ranging from 1000 ppb to 0 ppb were prepared through serial dilution. Initially, 100µL of Milli-Q water was drop casted onto the SPEs to record the blank reading of the modified SPEs. Then, the same volume of the different concentrations of the prepared standards was drop casted onto the SPEs for DPV analysis. Cyclic voltammetry (CV) has been carried over the potential range from -0.5 V to 0.6 V with a scan rate of 10 mV S^{-1}. The peak current values for different concentrations of Cr(VI) were plotted against concentrations towards development of the calibration curve. Similar experimentation has been repeated in commercially available electrochemical workstations to correlate the results obtained further through our developed metalloSTAT device.

4.3. Result and Discussion

4.3.1. Electrochemical characterization of modified SPEs

After cleaning the electrode surface, cyclic voltammetry (CV) exhibited no significant changes in the current, whereas the PB modification revealed a significant reversible oxidation peak at 0.15 V. The layer formed due to Nafion and aptamer modification showed enhanced sensitivity at a fixed potential with a decrease in peak current, as represented in Fig. 4.5. This has been due to the resistance offered in the layer during the transfer of electrons. Moreover, three different concentrations of Cr(VI), such as 50 ppb, 100 ppb and 500 ppb, were spiked and tested with the modified and unmodified electrodes. When the ability of the screen-printed electrodes modified with aptamer was compared with the unmodified electrodes in Cr(VI) detection, an interesting result was observed and represented in Table 4.1. According to the results, it was found that in the case of SPEs unmodified with aptamer, no mass deposition was observed, as there was no aptamer present on the surface of the working electrode to bind and deposit a substantial amount of Cr(VI). Hence, the electrode response was similar to the blank response and was also inconsistent. At the same time, the modified electrodes showed a significant decrease in the peak current signal through the serial increase in the mass deposition in the presence of the spiked sample. From that, the use of aptamer-modified SPEs was optimized for selective detection of Cr(VI) in water.

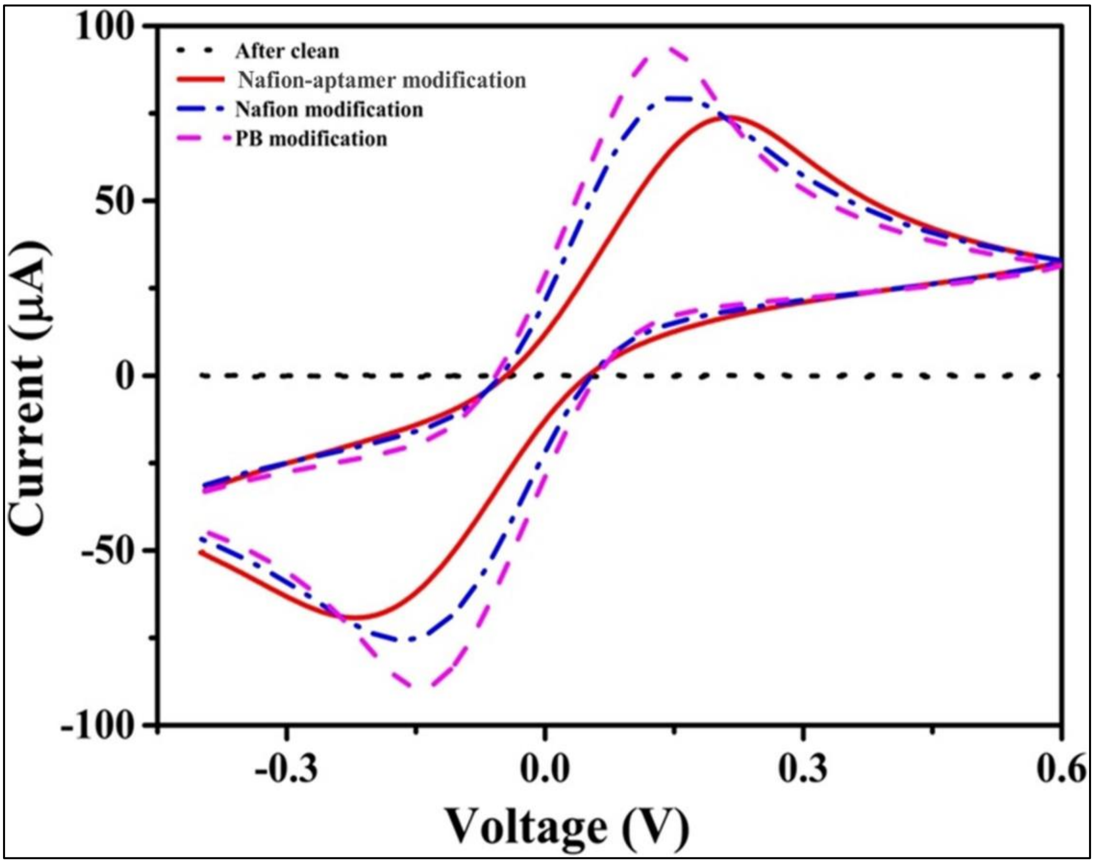

Fig. 4.5. Characterisation of stepwise modification of SPE using cyclic-voltammetry

Table 4.1. Recovery study using spiked samples for determination of device specificity

Cr (VI) Spiked Samples	**Peak current @ 0.20 V (µA)**	
	Unmodified SPE	**Aptamer modified SPE**
50 ppb	70.03	63.12
50 ppb	78.93	63.49
50 ppb	71.44	63.09
100 ppb	76.03	56.5
100 ppb	72.01	56.23
100 ppb	70.91	56.63
500 ppb	70.47	31.04
500 ppb	69.43	30.69
500 ppb	75.36	31.21

4.3.2. Temperature & pH optimization

The temperature of the system has been varied from 20 °C to 45 °C. The SPEs were preheated using a hot plate, and the temperature of the working electrode was monitored using an IR thermometer. The experiment has been conducted with 100 ppb Cr(VI) at pH 7. The maximum current has been obtained as a response at 30 °C. Also, the developed sensor exhibited stable response up to 35 °C. It has been observed that the peak current value for 100 ppb Cr(VI) decreased with a further increase in the temperature, which may be caused by partial evaporation of the minute volume liquid on the surface of the working electrode. Similarly, for low temperatures such as 20-25 ℃, the peak current was found to be less than that of 30 ℃ but not as low as a higher temperature of 45 ℃, which was due to the less mobility of the ions in the solution. Further experiments have been carried out at 30 ℃. The temperature stability at 35 ℃ revealed that the sensors could result in stable signals in Indian climate conditions.

Further, the pH optimization study for the aptamer immobilized SPE biosensor was performed. The pH of the sample, ranging from 3.5 to 10, was adjusted using acids or bases. It was observed that at pH 7.0, the peak current for 100 ppb of chromium 6 was maximum, with a decrease in the peak current for further increasing and decreasing the pH of the sample medium. This phenomenon was observed due to the presence of DNA aptamer-based receptor elements that are usually susceptible to instability at acidic and basic pH. The sensitivity, lowest response time, and dynamic range have been observed as the maximum/largest at pH 7.0.

4.3.3. Electrochemical sensing of Cr(VI)

Different Cr(VI) concentrations from 0 ppb to 1000 ppb were tested with the aptamer- modified SPE in our developed potentiostat as per the mentioned protocol. The peak current observed from the blank electrode with aptamer modification has been found to be 67.42 µA at 0.20 V. For increasing concentration of Cr(VI), the peak current has been observed to decrease. This phenomenon is observed due to serial damping of the current signal for an increase in the mass deposition in the working electrode. When Cr(VI) binds with the aptamer present in the working electrode, the mass deposition onto the working electrode surface increases due to the presence of Cr(VI) in the bound state, with the increasing concentration of Cr(VI) in solution, higher will be the formation of Cr(VI) aptamer complex and hence, the mass deposition of the working electrode increases. This provides resistance in the layers during electron transportation, which in turn affects a gradual increase in the damping of the current output value. The overall signal dumping has been represented in the inset of Fig. 4.6.

The device showed an excellent linear response in range of 0 ppb to 500 ppb, as represented in Fig. 4.6, with a LOD of 10 ppb. The same experiment has been reproduced in a standard electrochemical workstation device, and the result demonstrated an excellent correlation coefficient of 0.972 with the metalloSTAT data.

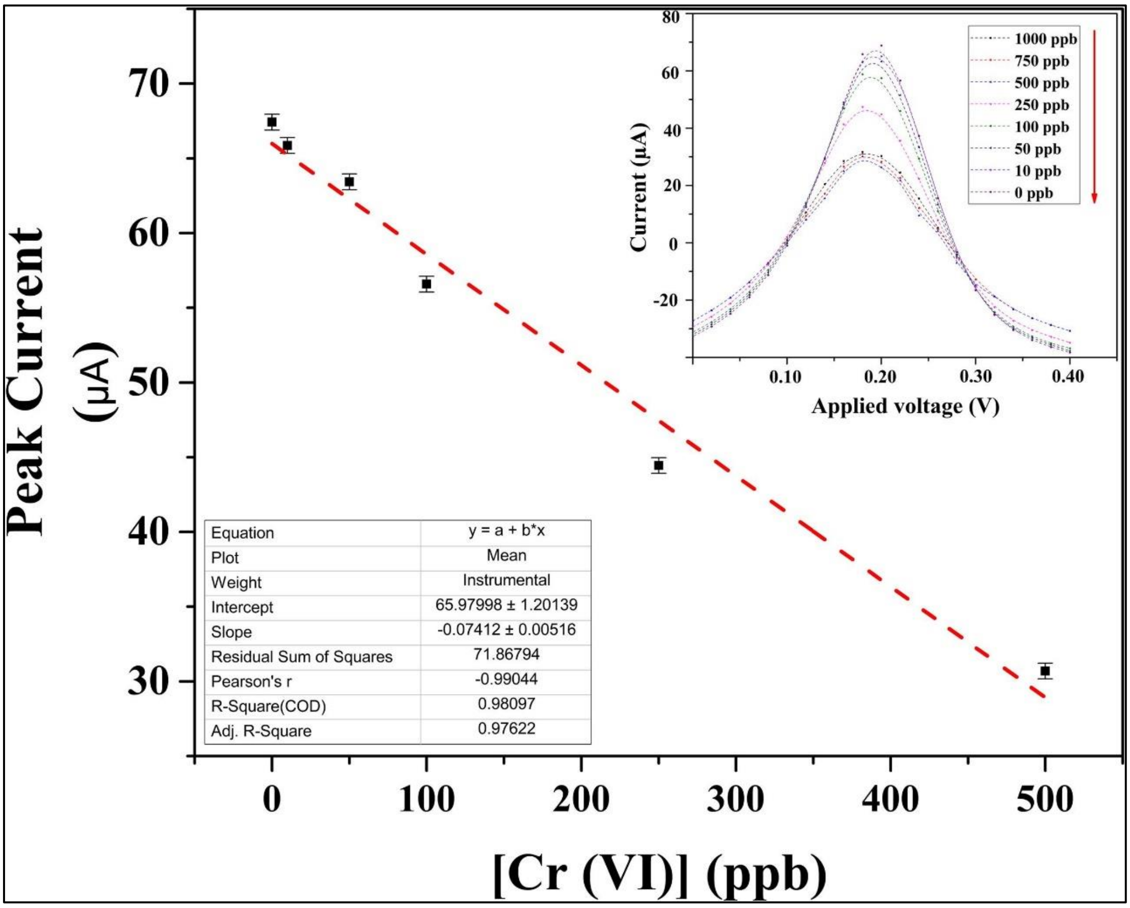

Fig. 4.6. Calibration curve of metalloSTAT device; Inset: Applied Voltage Vs. Peak current of Cr(VI) concentration from 0 ppb to 1000 ppb

4.3.4. Recovery analysis

A recovery analysis has been carried out to check the device's applicability. 10, 50, 100 and 250 ppb of Cr(VI) were spiked in Milli-Q water, and recovery percentages of spiked concentrations are 116.34%, 93.84%, 115.98% and 102.91%, respectively. The recovery error (RE) percentages for 0, 50, 100 and 250 ppb are -16.34, 6.16, -15.98 and -2.90, respectively (**Table 4.2**).

Table 4.2. Recovery analysis of Cr(VI) in Cr-Detector

Sl. No.	[Cr]+6 added in ppb	[Cr]+6 found in ppb (Mean ± SD)	RE (%)	Recovery (%)
1	10	09.06 ± 0.32	−16.34	116.34 ± 6.68
2	50	41.09 ± 0.89	6.164	93.84 ± 2.66
3	100	85.31 ± 0.79	−15.98	115.98 ± 4.30
4	250	239.02 ± 0.54	−2.90	102.91 ± 1.22

4.3.5. Specificity Test

Solution of other metals and ions like Cu^{2+}, Hg^{2+}, As^{3+}, Cr^{3+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, CO_3, $HCO3^-$, $SO4^{2-}$, $PO4^-$ were prepared from their respective salts. 100 ppb fixed concentration of these ions, along with chromium 6, was added to electrode, and mentioned protocol was run in developed potentiostat. The mentioned experiments were performed in triplicates and repeated more than once. Only for the Cr(VI), a high specificity index of 1.2 has been observed in the assay, which signifies the selective nature of the Cr(VI) aptamer towards its target analyte. The cross-reactivity result has been represented in Fig. 4.7.

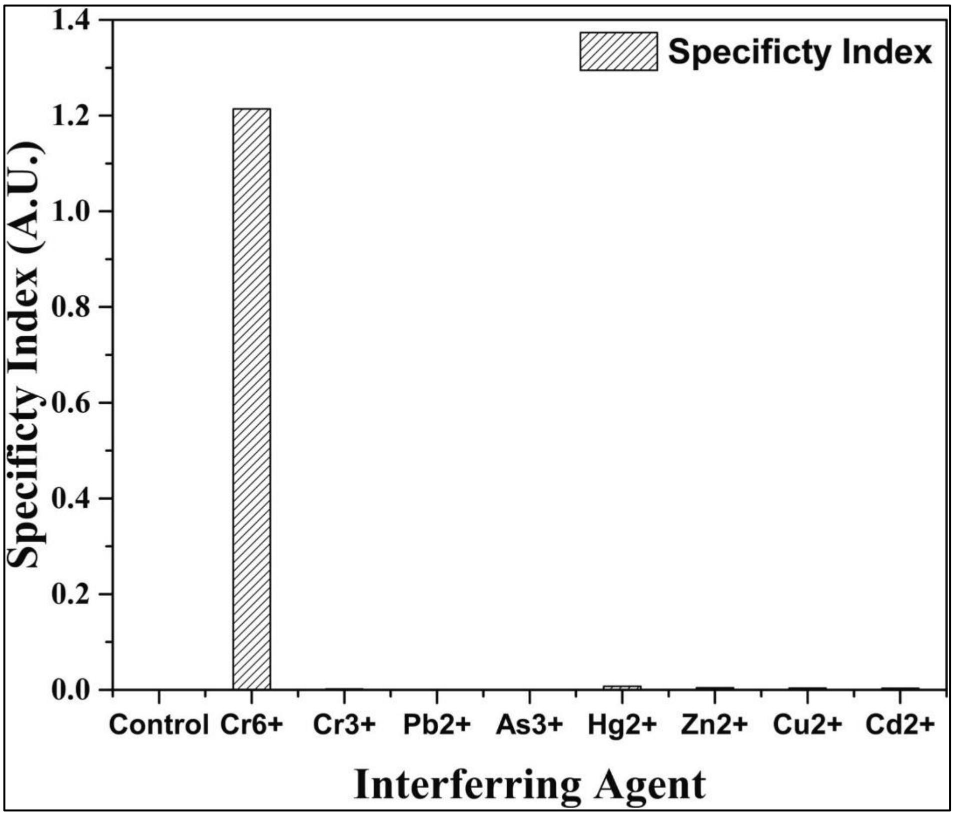

Fig. 4.7. Cross-reactivity studies of different metals with the developed device metalloSTAT

4.3.6. Sensor Performance

The storage stability of the developed aptamer immobilized SPE has been examined at 4 °C and measured thrice every day with washing steps over sixty days (Fig. 4.8). A loss of 4.20% of the initial response after 60 days has been observed when aptamer has been immobilized with the retention of more than 95% of the initial values. The intra-batch device performance (N=5) was evaluated for the developed sensor. The resulting data is presented in Table 4.3. An excellent reproducibility with almost no loss of nanocomposite activity has been obtained with R.S.D. of 0.465 % and 0.315 % against 10 and 50 ppb Cr(VI), respectively. These results suggested that the developed Cr sensor showed acceptable reproducibility.

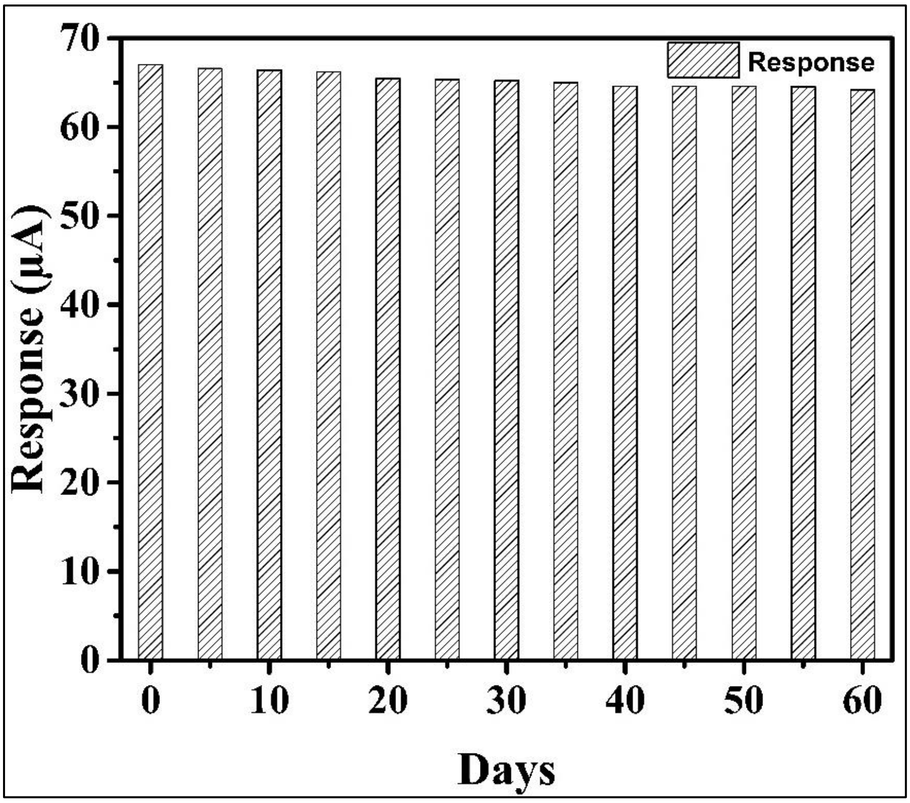

Fig. 4.8. Stability in days for the developed aptamer modified SPEs

Table 4.3. Intra-batch device performance (N = 5) for the developed sensor

[Cr (VI)] ppb	Current Response (µA)	Mean ± Standard deviation (µA)	R.S.D (%)
10	66.21	66.042 ± 0.307685	0.465
	66.42		
	65.65		
	66.11		
	65.82		
50	64.177	64.0368 ± 0.201746623	0.315
	64.075		
	63.72		

4.3.7. Real sample analysis & validation against ICP-MS

Real water sample testing through the developed sensor requires consideration of several essential indicators of the collected samples to get accurate results. These essential indicators that can affect the sensor's accuracy are the pH of the samples, the presence of interfering agents and organic compounds, turbidity of the samples, and temperature. By meticulously considering these essential indicators, the reliability and practicality of the developed electrochemical aptasensor for Cr(VI) detection in real-world water samples can be ensured. The pH of the collected water samples was adjusted to 7.0 to further reduce the acidic degradation of the aptamers during the pre-treatment of the collected waters. Moreover, after collecting the samples, filtration with Whatman Grade 42 filter paper to reduce the turbidity effect on the sensor performance. The developed aptasensor also showed excellent specificity towards Cr(VI), thus reducing the interfering effect of other ionic compounds. Finally, the sensor also represents the thermal stability up to 35 °C without affecting the peak current output. Most of the time, industrial effluent-containing water samples are found to be acidic in nature. However, as discussed above, the developed sensor was found to be working optimally at pH 7.0 due to the presence of DNA as a constructing material for the sensor element. Hence, the acidity of the collected real samples was first adjusted to pH 7.0 using 10 M NaOH prior to application onto the sensor element. Seven different water samples, like Distilled Water, Tap Water, Ground Water, Drainage Water, River Water, Sewage Water, and Industrial effluent Water, were collected from eastern Kolkata, which is the hub of several heavy industries. The samples were analyzed per the above reaction. The results represented in Table 4.4 showed the similarity of the interpretation of the developed Potentiostat with benchmarking conventional devices like ICP-MS/MS and Electrochemical Workstation. Though the results obtained in the developed Potentiostat were found to be less than that of ICP-MS/MS and Electrochemical Workstation, they were found to be correlative with the benchmarking standard devices with less than 5% of error for most of the real samples.

Table 4.4. Device validation study using ICP-MS/MS, Electrochemical Workstation, and developed potentiostat using the developed protocol

Sl. No.	Sample Type[a]	ICP-MS/MS	Electrochemical workstation	Developed potentiostat
1	Distilled Water	BLOQ[b]	0.00	0.00
2	Tap Water	0.11	0.00	0.00
3	Ground Water	90.12	88.31	65.52
4	Drainage Water	293.18	287.31	275.59
5	River Water	334.49	327.80	314.42
6	Sewage Water	642.89	630.03	604.32
7	Industrial effluent Water	851.22	834.20	800.15

[a] Site Collected Samples.
[b] BLOQ: Below Limit of Quantification.

4.4. Conclusion

In the mentioned study, we have successfully developed a handheld potentiostat named ‘metalloSTAT' for the detection of Cr(VI) in aquatic environments. The Cr(VI) aptamer- modified SPE expressed high selectivity towards Cr(VI) and was able to detect up to 10 ppb of the Cr(VI). The electrodes showed damping of the peak current output in the presence of Cr(VI), which was utilized for the calibration curve development of the developed device. The device incorporates electrochemical analysis of the water samples in presence of chromium 6 aptamer-modified screen-printed electrodes. The developed sensor has demonstrated excellent stability for up to 60 days. Moreover, the device has expressed a correlation coefficient of 0.97 with commercially available electrochemical workstations. The device has been utilized for real sample analysis with seven different samples, and the results have expressed a good correlation with the electrochemical workstation. To our knowledge, metalloSTAT is the first ever developed simplistic electrochemical handheld potentiostat for rapid identification of chromium 6 in water bodies. Furthermore, the developed potentiostat can also be envisaged in several heavy metals and toxic metalloids detection in further studies with minor modifications in the receptor element of the SPE. This will enable a complete solution of on-spot toxic metalloid detection for aquatic environmental safety studies.

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Chapter 5

Conclusion

List of Sections

- **Introduction**
- **Summary of the findings**
- **Recommendations**
- **Future scope of this work**
- **Conclusion**

Chapter 5

Conclusion and future scope

5.1Introduction

Life-threatening contaminants called heavy metals and metalloids are typically exist in industrial effluent have an impact on both the atmosphere and human well being. [1,2,3]. The most hazardous heavy metals that occur in nature and commonly used in manufacturing is chromium(Cr). Natural sources of Cr, particularly the earth's crust, can release it [4], in two forms that is chromium 3 and chromium 6 [5]. Chromium 6 is extremely poisonous, soluble, and mobile , it has more detrimental effects on both people and animals [1]. In contrast, Cr(III) is insoluble and mostly associated with organic matter, rendering it stationary in ambient conditions. It is less toxic than Cr(VI) [6,7]. The predominant environmental emissions Cr comes from manufacturing processes and products, such as those dealing with leather tanning, electroplating, minerals, metal plating, cement making, steel, textile dyeing, chemicals, and metallurgical operations [1,2]. These sectors generate substantial wastewater, including solid sludge and chromium-bearing waste, such as effluent from tanneries, resulting in significant chromium contamination globally [8]. The chrome discharge enduring from coalfields and penetrated water are recognized as significant contributors to soil and water pollution, raising substantial concerns [9]. In India, places such as Kanpur of Uttar Pradesh, Ranipet of Tamil Nadu, Sukinda Valley of Odisha and Ranaghat–Fulia of West Bengal are at elevated danger due to increased levels of Cr in both water bodies and soil [6,10].

This endeavor discusses several traditional developments alongside new advances, such as nanosensor advancements for field mobility in the identification of Chromium(VI) in water. This endeavor discusses several traditional developments alongside new advances, such as nanosensor advancements for field mobility in the identification of Chromium(VI) in water. Conventional detection technologies have been shown to be inadequate for rapid assessment and operational mobility. Sensors are combined with receptors and DSS, facilitating attributes such as speed and portability. These sensory systems include transducers, receptors and digital signal processing (DSP) components with a digital display. Novel sensor like as aptamers and ion-imprinted receptors have demonstrated superiority in evolution from traditional receptors like enzymes and antibodies to intelligent receptors. A substantial number of researches focused on the development of stable receptors and their immobilization, resulting in a robust foundation and expertise in this domain. Previously, receptors were primarily employed for identification of minimal levels of toxic metals in diverse aquatic environments, as essential need for generating a signal in presence of a molecular recognition element. Consequently, extracting toxic metals from soil, vegetation etc. proved challenging. Now a day's advancements in the efficient extraction of toxic metal from solid matrices facilitate applications including vegetables, fruits and soils. Nonetheless, the quantities of other toxic metals and orientation of receptors are aspects that require significant focus to mitigate interference and noise. Considering the advancements in the development of field-portable sensors for toxic metal identification, optical and electrochemical sensors are emerging as a more cost-effective and straightforward alternative to many other methods. Nonetheless, there is a necessity for enhanced repeatability and resistance to interference in these sensor devices. Consequently, this study focuses on optical and electrochemical sensors for identification of Chromium(VI) in aquatic environment.

5.2 Summary of the findings

5.2.1 Hardware device used for development of Nanosensor for detection of Cr(VI)

(i) Chemosensor development using Uniform Illumination Imaging System (UIIS)

As part of this effort, we have created a field-portable device that uses a uniform illumination imaging system (UIIS) to make colorimetric biochemical sensing easier. To identify and quantify chromium 6 in water, the created field-portable, wavelength-independent UIIS was put to use. This system demonstrated a strong correlation when compared to two other traditional methods, namely, LC-MS/MS ($r = 0.998877399$) and multi-plate reader ($r = 0.9991938$), and it only took 5 minutes to analyze 95 test samples. The sophisticated algorithm for data analysis has been studied and created by including the Mahalanobis distance computation. A good relation with a traditional plate reader ($r = 0.915389612$) was achieved by transforming the entire dataset into a matrix format. In contrast to the traditional, labor-intensive method of sending samples to labs for processing, this newly-developed UIIS will aid in the rapid detection of possible public health dangers [11].

(ii) Development Potentiostat using Electrochemical Aptasensing Technology

Two parts make up the gadget: the digital end and the analogue front end. Several analogue circuits make up the analog front end, which is used to produce potentiostatic experiments, amperometry, and CV. The reference, working, and counter electrodes form a trio. With the use of digital-to-analog conversion and specific Op-Amps, the potential among WE and RE should be kept constant. The working and counter currents can be measured using a trans-impedance circuit. For a precise voltage that can be study with a high-resolution ADC operation, the current must be increased by impedance. With the aid of an 8-bit microprocessor, the entire operation could be controlled and monitored. To ensure pinpoint accuracy, the Op-Amps amplifier would be of the electrometer kind. In order to manage the analog front end, the digital end is composed of a microcontroller. With its built-in digital time stamp, real-time clock, and Wide Video Graphics Array (WVGA) touch screen Liquid Crystal Display (LCD), this gadget is perfect for logging data and displaying it clearly. There are now two halves to the cabinet. The electronic circuitry and the sample-handling unit are two separate components of the device. The liquid samples will not come into contact with the system's electrical components thanks to these two sections. Data acquisition card and power source make up the sensor system.

5.2.2. Development of a basic chemosensing system for onsite Cr(VI) detection in aquatic system

Heavy metals, hexavalent chromium, are among the most prevalent toxicants, and they have devastating physiological effects on aquatic biotas and humans. In order to detect chromium(VI) in aquatic environments, this study details the creation of a field-portable image analysis apparatus that is connected with 3,3',5,5'-tetramethylbenzidine (TMB). With commercial spectrophotometer, the sensor's development and validation involved optimizing sensor parameters like reagent concentration and reaction time, among others. The usefulness of chemoreceptor coupled with a UIIS for Chromium(VI) identification was demonstrated to detect Cr(VI) within linear range of 25ppb - 600 ppb. The standard curve based on correlation coefficient value of the image quality parameters was developed. This range encompasses the allowed limit specified by several regulatory bodies. To the best of our knowledge, this 3,3′,5,5′-Tetramethylbenzidine with the developed device detects a extensive range of Chromium(VI) in water bodies, making it the first image analysis-based technology ever described. We present here the results of developing a chemo-sensor, TMB, for spectrophotometric detection of Cr(VI) in water. Several factors that could interfere with the sensor's performance were tested. While those interference compounds were present, the chemo-sensor nevertheless showed remarkable selectivity and specificity for Chromium(VI).

5.2.3. Invention of field portable potentiostat utilizing electrochemical aptasensing technique for detecting Cr(VI) in water

The development of a portable potentiostat for identification of trace levels of chromium 6 in water is achieved. The primary biosensor has been a screen-printed electrode (SPE) immobilized with a DNA aptamer that is specific to Cr(VI). The working electrode of the device is coated with an aptamer that is linked with Cr(VI), and the device runs by means of mass deposition during an electrical peak current dumping event. The established prototype has a LOD 10 ppb and a working range of 0ppb to1000 ppb Chromium(VI), with greatest linearity reported in a range of 0-500 ppb. Also, seven distinct kinds of water samples were used to confirm the device's usefulness. This on-the-spot aptasensing gadget is the first of its kind to detect Cr(VI) in water using just basic, modest resources. The created sensor has shown remarkable stability for a period of sixty days. Applying the device to seven distinct samples in a real-world setting yielded findings that showed a strong relationship with the electrochemical workstation.

5.3 Recommendations

The following applications in the Cr (VI) detection can be suggested for the proposed thesis study in its current form.

- The developed simple chemosensing system can be used for onsite identification of heavy metal chromium 6 contamination in polluted water.
- The developed field portable potentiostat using electrochemical aptasensing technology can be used for qualitative and quantitative identification of Chromium(VI) in water bodies.
- Addition, with small adjustments to the SPE's receptor element, the potentiostat that was produced might potentially be used to detect a variety of heavy metals and dangerous metalloids in future research.
- Studies on the safety of aquatic environments will be able to use these nanosensors as a whole to identify harmful metalloids in the water as they occur.

5.4 Future scope of this work

There are several areas in which the proposed methodology discussed in the previous sections can undergo improvement. These are listed as follows:

- The sensors should be made more commercially viable for implementation in the water pollution monitoring. It may be done using disposable sensors or sensors made using screen printed electrodes. By employing such types of methodologies, the problem of regenerating the surface every time the electrode is exposed to target analyte, may be dissolved. Moreover, those sensors will also be able to yield a greater level of precision.
- All the sensors described herein, were tested using a limited number of water samples collected from various polluted sites of India. To prepare an electrode that can be implemented in the industry level it must be trained with datasets corresponding to different types of water samples from the sensors when it is exposed to different genres of water sample corresponding to different polluted sites of India. Further, the electrodes should also be exposed to various concentrations of chromium 6 to procure a wide range of samples for the performance validation of the electrodes.
- To make this device portable, some more future work needs to be done. Firstly, a function generator, which should have the provision of applying various types of voltammetric techniques should be there. Secondly an on-board power supplier like batteries or power banks with proper power rating needs to be placed and a proper charging circuit also needs to be designed. Apart from this a proper calibration of the device is needed to generate accurate results.
- IoT empowered decision-making tool can be leveraged by the application of big data analytics in uniform illumination imaging system (UIIS) in future.