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POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

Development of a novel electrochemical bismuth sulfide-based biosensor for the detection of Zn ions in cancer

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Département de génie biomédical

Mémoire présenté en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

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Ce mémoire intitulé :

Development of a novel electrochemical bismuth sulfide-based biosensor for the detection of Zn ions in cancer

présenté par **Alexis MARION**

en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

a été dûment accepté par le jury d'examen constitué de :

Abdellah AJJI, président
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Edward HARVEY, membre externe

DEDICATION

This thesis is dedicated to my parents whose unwavering support has been the driving force towards the realization of my academic achievements.

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RESUMÉ

Les maladies liées au cancer sont l'une des principales causes de décès et représentent le plus grand obstacle à l'augmentation de l'espérance de vie dans le monde. Le fardeau du cancer augmentant chaque année, il est indispensable de mettre au point de nouveaux dispositifs médicaux performants et peu coûteux pour aider à établir un diagnostic et/ou un pronostic sur le lieu de soins. Pour être applicables sur le lieu de soins, les senseurs médicaux doivent être entre autres rentables, faciles à utiliser et miniaturisés. Les senseurs électrochimiques offrent tous ces avantages et d'excellentes performances en détection pour une multitude de biomarqueurs de maladies. Parmi les biomarqueurs du cancer, les métaux libres et les protéines contenant des ions métalliques tels que le Zn sont aujourd'hui des cibles populaires pour le développement de senseurs en raison de leur rôle dans de nombreux processus biologiques liés à la maladie. Récemment, des senseurs électrochimiques à base de bismuth se sont révélés très prometteurs pour la détection à haute sensibilité du Zn. Dans ce travail, nous proposons le développement d'un senseur micro-électrochimique à base de nanoparticules de sulfure de bismuth pour la détection du Zn. Nous avons mis au point une synthèse hydrothermale simple et novatrice à trois réactifs pour du sulfure de bismuth carboxylé, qui présente l'avantage unique d'avoir des groupements fonctionnels d'acides carboxyliques en sa surface, ce qui permet d'autres modifications chimiques de la surface du dispositif. La réticulation de l'acide aminé l-histidine à la surface du senseur a été étudiée comme moyen d'en améliorer les performances électrochimiques. Des senseurs basés sur les nanoparticules de sulfure de bismuth et le sulfure de bismuth modifié par l'histidine ont été préparés et testés électrochimiquement par des techniques voltamétriques afin d'en optimiser les paramètres de détection. Le senseur préparé à partir des nanoparticules non modifiées a montré une limite de détection du Zn dans une solution tampon de TBS en laboratoire de 1,98 µg/mL et une relation linéaire entre le courant et la concentration de Zn dans la plage 5µM - 50µM, qui est d'une pertinence biologique. Le biocapteur de sulfure de bismuth modifié par l'histidine a montré une performance électrochimique supérieure en voltampérométrie cyclique par rapport aux nanoparticules non modifiées. Cependant, la détection du Zn dans ce système n'a pas encore été optimisée pour réduire le bruit des mesures à un degré permettant la caractérisation et l'étalonnage précis du dispositif. D'autres expériences axées sur l'optimisation des paramètres de détection sont nécessaires sur le senseur à base de sulfure de bismuth modifié par l'histidine afin de développer

pleinement le prototype. En conclusion, ce travail a permis de mettre au point un nouveau système de sulfure de bismuth hautement personnalisable pour la détection électrochimique du Zn, avec des caractéristiques hautement souhaitables pour une application potentielle au niveau du point de services en santé.

ABSTRACT

Cancer-related diseases are a leading cause of death and represent the greatest barrier to increasing life expectancy globally. With the burden of cancer increasing annually, there is a great need to develop new low-cost and performant sensors to help establish a diagnosis and/or prognosis in the point-of-care setting. To be applicable to the point of care, medical sensors must be cost efficient, easy to operate and miniaturized among other characteristics. Electrochemical sensors offer all these advantages with excellent detection capabilities for a multitude of disease biomarkers. Among cancer biomarkers, free metals and metal ion-containing biomarkers such as Zn are popular targets for the development of electrochemical sensors today because of their key activity in many disease-related processes. Recently, bismuth-based electrochemical sensors have shown great promise in high sensitivity Zn detection. In this work, we proposed the development of a bismuth sulfide nanoparticle-based microelectrochemical sensor for the detection of Zn. A novel and simple 3 reagents one-pot hydrothermal synthesis of carboxylated bismuth sulfide was developed, with the unique prosperity of having surface carboxylic acid functional groups, allowing further chemical modifications of the sensor surface. Cross-linking of amino acid l-histidine was investigated as a mean to enhance sensor performance. Sensors based on the bismuth sulfide nanoparticles and histidine-modified bismuth sulfide were prepared and tested electrochemically through voltametric techniques to optimize detection parameters. The sensor prepared from the unmodified nanoparticles showed a limit of detection of Zn in laboratory TBS buffer solution of 1.98 µg/mL and a linear relationship between current and concentration in the 5µM - 50µM range, which is biologically relevant. The histidine modified bismuth sulfide biosensor showed superior electrochemical performance in cyclic voltammetry compared to unmodified nanoparticles. However, Zn sensing in this system has not yet been optimized to reduce noise to a degree allowing precise sensor characterization and calibration. More experiments focusing on optimization of the detection parameters are needed on histidine-modified bismuth sulfide in order to fully develop the system prototype. In conclusion, a novel highly customizable bismuth sulfide framework for the electrochemical sensing of Zn was developed in this work, with highly desirable features for a potential point-of-care application.

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LIST OF SYMBOLS AND ABBREVIATIONS

AUC Area Under the Curve

BiSNP Bismuth Sulfide Nanoparticles

BiSNP-H Histidine-modified Bismuth Sulfide Nanoparticles

CA Carbonic Anhydrase

cBiSNP Carboxylated Bismuth Sulfide Nanoparticles

CE Counter Electrode

CV Cyclic Voltammetry

DPV Differential Pulse Voltammetry

E Potential

ECM Extracellular Matrix

EIS Electrical Impedance Spectroscopy

ELISA Enzyme-Linked Immunosorbent Assay

GCE Glassy Carbon Electrode

I Current

LOD Limit of Detection

LOQ Limit of Quantitation

MMP Matrix Metallopeptidase

MS Mass Spectrometry

NP Nanoparticle

RADT Rapid Antigen Detection Test

REF Reference Electrode

SEM Scanning Electron Microscope

SPR Surface Plasmon Resonance

SWSV Square Wave Stripping Voltammetry

TBS Tris(hydroxymethyl)aminomethane-buffered Saline

WE Working Electrode

Z Impedance

ΔI Forward current pulse minus reverse current pulse

CHAPTER 1 INTRODUCTION

Cancer-related diseases are a leading cause of death and represent the greatest barrier to increasing life expectancy globally [1]. The search for easily detectable cancer markers is an ongoing challenge, decidedly complicated by the diseases' heterogeneity. With the worldwide burden of cancer increasing yearly [2], there is a great need to develop new medical devices suitable for early, point-of-care detection of occult cancers and to monitor disease progression and treatment efficacy, or lack thereof [3]. Moreover, most cancers still lack an effective and non-invasive early detection method [4].

Biomarkers, as defined by the World Health Organisation are measurable substances within the body or its products which are predictive of a disease (diagnosis) or an outcome (prognosis) [5]. Biomarkers can also give valuable information on exposition and help in disease screening or staging [6]. Given that most biomarkers are specific to cancer subtypes and not applicable in all situations, a marker cannot by itself serve as primary means of screening [7]. However, they can be of great utility in the clinical setting to support another diagnostic modality, to suggest undertaking further tests, differentiate between malignant or benign disease or evaluate the response to treatment [8]. Figure 1.1 presents a classification scheme for biomarkers according to their biological source, molecular nature and use in supporting medical care. The ideal biomarker would achieve all of the above aspects, while being accessible to detect in terms of simplicity and cost of the equipment required, in order to be implemented as part of a point-of-care solution [7]. It is important to note that while many powerful tools are available to researchers for identification and quantification of biomarkers, the widespread availability of low-cost and accessible point-of-care solutions remains a challenge to this day. Searching the proteome is advantageous in this area as many protein-based markers can be found circulating freely in the extracellular environment and in bodily fluids, rendering them easy to detect with the appropriate non-invasive or minimally invasive techniques such as blood draws and urine or saliva collections, which are considered ideal [3, 9, 10].

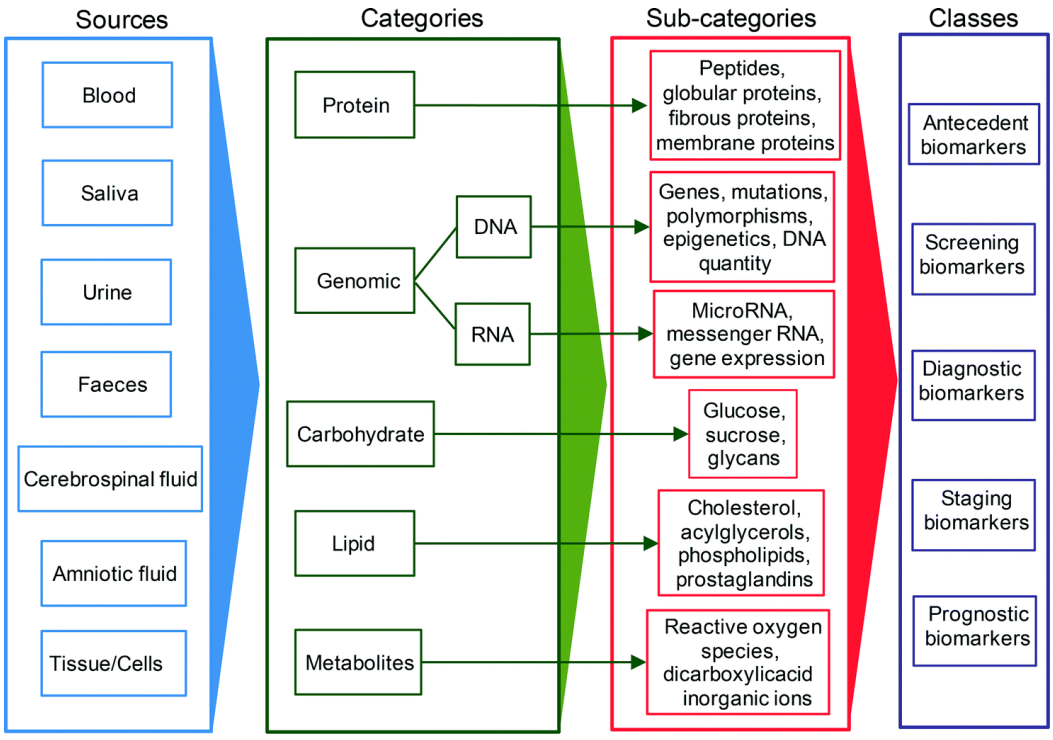

Figure 1.1 Overview of biomarker source, nature and clinical usage [6]

Technologies to detect biomarkers can be largely divided into antibody-dependant and antibody-free techniques. Historically, antibody-mediated approaches have been and are still seen as the mainstay for biomarker detection in clinical and laboratory settings, most notably the enzyme-linked immunosorbent assay (ELISA) and immunofluorescent assay. They are the most accurate, precise and specific, however they also require more complex tools and advanced technical expertise, and are slow to display results [11]. ELISA is currently the gold standard when it comes to biomarkers [12] and new emerging technologies are always compared to it to assess their performance and suitability. Other antibody-mediated techniques such as rapid antigen detection tests (RADT), while being generally specific, have limited sensitivity and do not allow for precise quantification of the target marker. Another major drawback of these techniques is the destruction of the tissue, which limits their use in further applications. While antibody-free techniques are generally more prone to non-specific interactions and interference, they tend to be significantly faster, cost-efficient and accessible. Of which, two major groups of technologies are the electrochemical and optical methods. They make ideal candidates for point-of-care use and for

population screening, whereas immunoassays are likely to remain uncontested for laboratory usage [11].

Electrochemical sensing is a well-established and heavily researched technology from which we have seen developed sensitive, rapid and cost-effective sensory devices for detecting a multitude of molecules and elements of interest in diverse environments. It relies on the principle of conversion of chemical energy into electrical energy, which can be measured as a signal through the transfer of electrons and ions between substances or materials. The technology is most known for its use in advanced batteries, fuel cells and wastewater treatment and analysis. However, there is an increasing amount of literature applying electrochemical sensors to cancer research [13]. Numerous advances in nanomaterials and surface functionalization have been made in the last decade of such sensors, with increasing biological or chemical complexity in order to achieve optimal detection performance as well as miniaturization [14].

Among cancer biomarkers, free metals and metal ion-containing biomarkers are popular targets for the development of electrochemical biosensors today because of their key activity in many biological processes. Among metals, Zn, the second most abundant in the human body after iron, is of particular interest in cancer research because of its role in cell differentiation, cell growth, transcription, and protein synthesis, which can be upregulated in cancer and therefore represent a valuable source of information for monitoring the disease [15]. In fact, numerous enzymes and transcription factors regulating such physiological functions depend on Zn^{2+} binding to exert their function, notably the redox-active metallopeptidases class of metalloproteins. Metallopeptidases (also known as collagenases) are a large family of divalent ion -mostly zinc- dependant enzymes which have the biological function of cleaving different proteins and peptide chains, found involved in all of the major processes of life [16]. A subclass of particular interest for monitoring cancer are the matrix metallopeptidases (MMP), which are involved in digesting extracellular matrices (ECM) by cleaving many of its constituents such as collagen [17, 18]. These enzymes play an especially important role in cancer pathogenesis as the degradation of the ECM, whose loss of integrity is considered a hallmark of the disease, allows proliferating neoplastic cells to invade nearby tissue and thus contribute to metastasis [19-21]. Many studies have demonstrated the correlation between expression of MMP and the staging of cancer and metastatic potential of a tumour. Out of the many subtypes, MMP2 and MMP9 (type IV collagenases/gelatinases) have

been found to be upregulated in almost all cancers, and are believed to be excellent candidates for early cancer detection and following treatment progression in the brain, breast, lung, colon, head & neck, renal, bladder, pancreatic, oesophageal, stomach and ovarian carcinomas [22-25]. MMPs can be found simultaneously in the tumour microenvironment and in biofluids, namely serum and urine [26].

Since these MMPs of clinical interest are comprised of a functional Zn^{2+} ion within their core, they are excellent candidates for electrochemical detection. Our hypothesis is that Zn can be detected electrochemically in laboratory solutions using an nanometal-enhanced glassy carbon electrode (GCE), and that these results would be applicable to MMP detection in further experiments not part of this project. To this end, post-transition metals such as bismuth (Bi) have shown a great affinity for Zn and other electronegative heavy metal ions in electrochemical measurement settings [27, 28]. The excellent properties of Bi-modified electrodes render them ideal for this project, where we aim to:

(1) Synthetize and characterize Bi nanostructures with a high surface-to-volume ratio and high Zn affinity.

(2) Chemically modify the nanostructures with specific amino acids such as histidine to enhance electrocatalytic activity.

(3) Modify the surface of a microelectrochemical probe with the synthetized Bi nanostructures and investigate Zn detection capabilities in *in vitro* buffer solutions.

Chapter two of this work will present a review of the recent literature on Zn and Zn-dependant enzyme sensing in medicine, with a particular focus on electrochemical technologies, their strengths, and their fields of application. Chapter three will present the detailed methodology applied for this project, whereas its results will be presented in chapter four. Finally, chapter five will outline limitations and future recommendations from our work to conclude this thesis.

CHAPTER 2 LITERATURE REVIEW

Precise and sensitive Zn detection in biological systems can therefore represent a crucial source of information on health and disease and could drastically improve performance in delivering proper and timely healthcare to certain patients through effective diagnostic strategies.

2.1 Zn and Zn-binding Proteins in Mammalian Physiology

Zn, an essential micronutrient and trace element, is the second most abundant metal in mammalian physiological systems after Fe and the second most abundant divalent ion after Ca^{2+}. It notably serves as a cofactor for numerous and enzymatic and transcription factor proteins, which contributes to the regulation of gene expression, cell signalling, and immune functions [15]. The field of metalloproteomics, which studies exclusively metal-dependent proteins and their role in biology is a newly established field of study, it is gathering significant attention since it is estimated that one third of all proteins in the human body require a metallic co-factor to exert their function [29]. More specifically, it is currently known that over 300 enzymes and 3000 transcription factors depend on Zn. It is also the only element known to be involved in all six classes of enzymes, being oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases [29-31]. Zn can be found in majority bound to these proteins or in its free, unbound form. While free Zn represents the lesser fraction of total Zn, it is an important signalling molecule and alteration in its basal levels have been found to be closely associated with certain cancers [31-33]. This is thought to be a consequence of DNA damage caused by increased oxidative stress. While the role of free Zn is poorly understood, it is known to be by itself an antioxidant and helps to protect cells against excessive production of reactive oxygen species. Moreover, a loss of DNA repair functions from Zn-dependent enzymes is a contributing factor to genomic instability and development of cancer. Free Zn in the intra and extracellular environments is tightly regulated by specific transporters and protein levels, to which most is bound [34]. Reported concentrations in human tissues are found in the $\mu g * mL^{-1}$ order of magnitude [35], whereas physiological Zn concentration in serum is known to be in the $0.1\ \mu g * mL^{-1}$ order of magnitude [36]. With these data from the literature, benchmarks for the LOD required in free Zn sensor design can be set.

2.2 Zn in Cancer

While there is a clear association between Zn deficiency and risk of cancer development, it is to note that high levels of Zn have also been associated with certain cancers. Although not fully understood, this contradiction can be explained in part by differential dysregulation of certain Zn transporters in the different cancers [33]. Furthermore, free Zn measurements are often imprecise, as the quantities being measured are small and could be affected by many variables such as the cellular microenvironment and the measurement techniques used. Significant controversies still exist, and more research would be needed to elucidate the roles and mechanisms of Zn in cancer pathogenesis and progression. Notably, regarding prostate cancer, literature describing both an increase and a decrease of free Zn in the cancerous tissue can be found, with each side being strongly critical of the other [37-39]. Table 2.1 presents a selection of some of the most incident cancers worldwide [40] and their corresponding Zn-associated measurements in cancerous tissues reported in the literature. Quantitative measurements of Zn have been performed on tumor samples and in the plasma or serum of cancer patients and healthy controls. Cancers of the breast, colon, head and neck, liver, lung, pancreas, prostate and stomach have been included in the table because of their high incidence and/or high mortality and the amount of literature investigating the connection between Zn contents and the cancer. However, many more cancers have been investigated in relation to Zinc and other divalent metal ions and a great review on the topic can be read from Wang and colleagues [33].

Table 2.1 Overview of frequent cancers with associated Zn related findings

Cancer	Main cell types	Age-standardized incidence rate (per 100,000 pers-years) for males and females	Zn findings in cancer vs healthy	Reference
Breast	Adenocarcinoma	F: 47.8 [40]	High Zn in breast Low Zn in serum	[33, 41]
Colon	Adenocarcinoma	M: 13.1 F: 10.0 [40]	Low Zn in colon Low Zn in serum	[42, 43]
Head and neck	Squamous cell carcinoma	M: 12.3 F: 4.3 [40]	High Zn in saliva Low Zn in serum	[44, 45]
Liver	Hepatocellular carcinoma	M: 14.1 F: 5.2 [40]	Low Zn in liver Low Zin in plasma	[46]
Lung	Adenocarcinoma Squamous cell carcinoma	M: 31.5 F: 14.6 [40]	High Zn in lung Low Zn in serum	[33]
Pancreatic	Adenocarcinoma	M: 5.7 F: 4.1 [40]	High Zn in pancreas Low Zn in plasma	[33, 47]
Prostate	Adenocarcinoma	M: 30.7 [40]	Low Zn in prostate*, Low Zn in serum	[33, 37, 48]
Stomach	Adenocarcinoma	M: 15.8 F: 7.0 [40]	Low Zn in stomach Low Zn in serum	[42, 43]

* Controversial

2.3 Zn-dependent metalloproteins

As previously stated in this work, many proteins and enzymes requiring Zn to function can be found involved in biological processes leading to cancer and can be detected electrochemically or by other means. This section will present a selection of important proteins with an established interest in electrochemical detection with a particular focus on MMPs, whose detection represents the rationale behind this work.

2.3.1 Matrix Metallopeptidases

MMPs are a family of Zn^{2+}-dependant endopeptidases involved in the crucial biological function of remodeling the ECM, which is a dynamic network of proteins providing structural integrity to cells and tissues. ECM remodeling by MMPs is done by enzymatic cleaving of its constituents, namely collagen, gelatin, laminin and fibronectin at specific sites. For this reason, MMPs are also commonly referred to as collagenases. This remodeling process allows for tissue growth and organ development, wound healing, neovascularization as well as regulation of inflammation [17, 18]. Moreover, MMPs target directly other factors involved in cellular behaviour such as other proteinases or protease inhibitors, cell adhesion and chemotaxis molecules, growth factors and surface receptors [49]. There are a total of 23 different MMPs found in humans which can be divided into secreted and membrane-bound, and are expressed by stromal cells (fibroblasts, endothelial cells, neutrophils, lymphocytes and macrophages among others) [50]. Overexpression of MMPs has been found in virtually all cancers [22]: at least three key steps in metastatic transformation are mediated by processes requiring MMP action. First, degradation of the ECM allows transformed cells to escape their anatomical niche. Second, degradation of surface receptors allows for immune system evasion and removal of cell-to-cell adhesion, which modifies cellular behaviour. Third, neovascularization promoted by MMP allows for sustained growth of tumors, which is otherwise limited by lack of available nutrients and oxygen [51, 52]. Overall, the microenvironment finds itself altered which may result in it being more suitable to support neoplastic transformations.

MMPs inhibitors are actively being investigated for cancer treatment, however, translational research has proven challenging, with little to no improvement in patient outcomes as of recently [53]. There is a high functional redundancy within MMP structures and cleaving sequences on

peptides, which in part explains the difficulty of designing specific inhibitors for cancer treatment [54]. This also explains the difficulty in designing MMP-specific biosensors based on cleaving sequences alone, as was experienced by our research group and will be detailed further in this chapter.

Among MMPs, MMP2 and MMP9 are the most investigated in the field, as higher levels of these proteins have successfully and repeatedly been associated with higher risk of metastases and are a marker of poor prognosis in numerous cancers [55-58]. A pan-cancer review of MMPs and their diagnostic and prognostic values by Gobin and colleagues [22] shows MMP9 has an area under the curve (AUC) of receiver operating characteristics (ROC) between 0.90 and 0.95 for oesophageal carcinoma and head and neck squamous cell carcinomas, which is considered of excellent diagnostic value [59]. Overall, for almost every The Cancer Genome Atlas cancer type, there was an MMP with an AUC of at least 0.9. Interestingly, MMP11 was found to have the highest number of significant AUC values, despite being less investigated in research. We can therefore establish the important diagnostic and prognostic values of MMPs sensing, including MMP9, which could become highly beneficial for cancer monitoring and a valuable diagnosis aid in the point-of-care setting.

2.3.2 Carbonic Anhydrase

Carbonic anhydrases (CA) are another family of Zn-dependent enzymes which have the important function of catalyzing the reversible conversion between carbon dioxide (CO_2) and water (H_2O) to bicarbonates (HCO_3^-) and protons (H^+). This reaction, which takes place extracellularly and intracellularly, serves to regulate acid-base balance, pH and carbon dioxide transport [60]. In cancer, CA function has been linked to tumor growth and metastasis. Cancer cells, which have an altered metabolism in order to survive and sustain their growth, require a slightly more alkaline intracellular pH and acidic extracellular pH in the microenvironment. This equilibrium can be mediated by CA action. Otherwise, acidosis due to high lactic acid and poor tumor perfusion impairs growth [61]. Certain isoforms of CA, notably CAIX and CAXII have been found to be overexpressed in cancers and these enzymes are actively being investigated as biomarkers for detection and monitoring of the disease [62, 63]. They are believed to be excellent candidates for

electrochemical detection as their catalytic activity allows for an strong electron signal to be picked up by appropriate electrodes.

2.3.3 Other Zn Metalloenzymes

Of the more than 3000 Zn-dependent metalloproteins found to this day, many have been found to have relevance in cancer pathological processes. Table 2.2 presents an overview of some of the most significant Zn-dependent metalloproteins not previously described in this work, which could also be of diagnostic or prognostic value with the appropriate sensors.

Table 2.2 Overview of Zn-dependent metalloproteins

Protein	Function	Significance in cancer	Sensors available	References
Metallothioneins	Metal ion binding and homeostasis	Antioxidant, DNA protection, inhibition of apoptosis, cell proliferation, angiogenesis	Yes	[64-66]
Zinc-finger proteins	Varied: DNA binding, RNA binding, Protein binding	Acts as tumor suppressors, transcription factors, genome stability and DNA repair Other functions	Used as aptamer, no direct sensor	[67-71]
S100	Intra and extracellular signalling, metal homeostasis	Regulation of proliferation, differentiation, apoptosis, energy metabolism, cell migration and invasion Overexpressed in cancer	No	[72-75]

2.4 Techniques and Methods for Zn and Zn-dependant Protein Sensing

Early detection is crucial in cancer and patient survival is improved when detected early. Currently, about half of cancers are detected at a late or advanced stage, where treatment options are reduced and sometimes limited to palliative care [76]. To this day, advances in medical imaging and pathology performed from tissue biopsy which is the gold standard in diagnostic, are considered inefficient in early stage detection since they rely on tumor phenotypic properties [77]. Early and specific biomarker detection, including Zn dependant makers, could greatly impact how quickly a diagnosis can be made, and therefore treatment initiated since biomarker expression changes begin in very early stages of the disease, in preneoplastic stages [10].

With the diagnostic and prognostic value of Zn-dependant proteins in cancer solidly established by the literature, the design of sensors capable of detection of these elements is an active area of ongoing research. Here, sensors must have a low enough LOD and limit of quantitation (LOQ), the latter being the lowest quantity of analyte which can be reliably not only detected but measured with acceptable accuracy and precision [78] . As mentioned in section 2.3.1, free Zn is found in a range of $\mu g * mL^{-1}$, and differences between healthy and cancerous tissue can be very low, ranging from few to over 80 $\mu g * mL^{-1}$ [79], although this is highly dependent on tissue type and cancer type, and more research would be needed in this area to set diagnostic standards for free Zn in tissues or serum [80]. In a point-of-care setting, markers circulating in the serum, saliva or urine make for a more appropriate approach, where minimally invasive collection procedures can be employed, and a tissue biopsy cannot be performed or isn't indicated yet. These "peripheral" sensors would complement pathology or suggest that further tests are needed, as it is unlikely a single marker can in itself be of diagnostic value [11, 77].

Many techniques are currently used in the laboratory setting for Zn and Zn-dependant proteins. Some are exclusive to free ions or proteins and others are capable of being used for both free ions and proteins. Versatile techniques are considered advantageous in the point-of-care setting, as reduced equipment can translate into reduced costs.

2.4.1 Features for a Point-of-Care-Ready Sensor

While many powerful instruments are commercially available for biomarker sensing in clinical medicine and in research, few possess the ensemble of qualities and features required for it to be applicable to the point of care setting. Ideally, on top of being of acceptable sensitivity and accuracy sensors developed for this purpose must be accessible, in terms of cost and ease of operation. Figure 2.1 highlights the proposed ideal specifications of a point-of-care ready sensor.

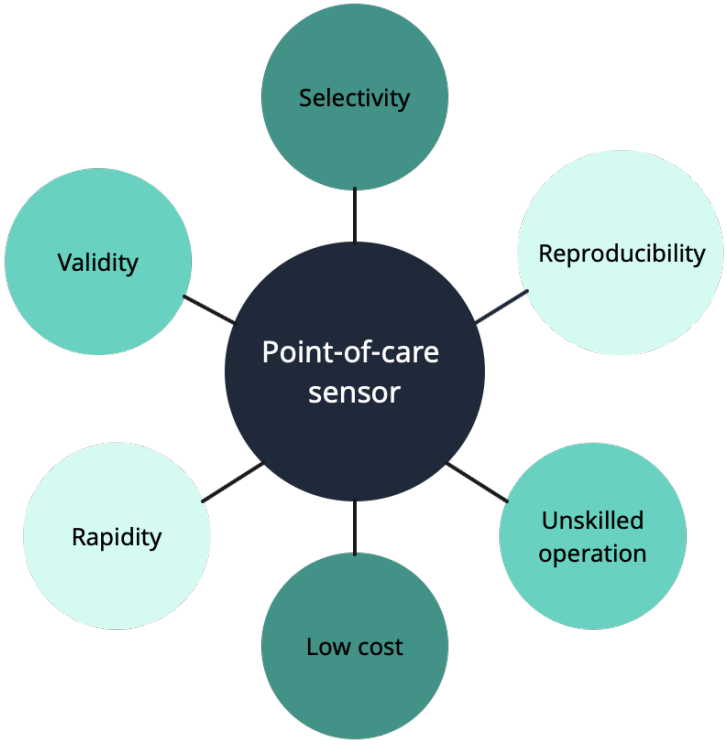

Figure 2.1 Desired characteristics for a point-of-care biomarker sensor

Firstly, validity is the prime measure for any diagnostic test, including sensors. They refer to the ability of a test to be accurate in acceptable performance for the context and when compared to the gold standard. Validity is measured by sensitivity and specificity [81]. Selectivity refers to the crucial ability of a sensor to respond to a given specific analyte or biomarker in an environment with contaminants or substances which may interfere with it [82]. An example of this would be an electrochemical sensor not differentiating the signal between Zn^{2+} and Cu^{2+}. Depending on the exact target of the sensor, such as a particular protein isoform, this can be a difficult task.

Repeatability and reproducibility are two aspects of precision: the former refers to the ability of a test to produce the same result to an acceptable variability and precision when conditions of the test are constant and it is performed by the same operator and instrument; the latter is the ability to generate the same result to an acceptable variability and precision when the test is performed by different operators using different instruments, in different conditions [83]. This characteristic is crucial for a sensor to reach the market. Lastly, the rapidity to deliver results, the cost-effectiveness and the ease of use are features which serve to differentiate a point-of-care system from other mainstream laboratory techniques. They must be easily implantable in smaller clinics and remote or underdeveloped areas, which may lack the expertise to run expansive analytical systems, and which may also lack the funds for expensive machinery [84]. Trade-offs with performance can sometimes be made in this category to better adapt the technology to the point-of-care.

2.4.2 Spectrometric Techniques

In biological fluids, free metals such as Zn and others are often detected through spectrometry, an analytical technique measuring the mass-to-charge ratio of ions. Large molecules can also be identified and precisely measured using these techniques, which involves ionization, then separation and identification of the ions [85]. Among these techniques, mass spectrometry (MS) is especially powerful and often considered a universal analytical tool as it can be applied to any possible situation, in known or unknown samples [86]. Techniques such as MS and atomic absorption spectrometry are routinely used in clinical laboratories for metal detection and are generally highly sensitive and fast, however require expensive investments, skilled operators and considerable laboratory space. Most techniques also require extensive sample preparation and the method is destructive [87, 88]. This renders them poorly applicable to the point-of-care setting and their use is confined to specialized laboratories or research. For large protein biomarkers, spectrometric methods are rarely used in clinical laboratories in favor of immunological assays or spectrophotometric techniques, although they have the potential to greatly outperform them in terms of detection capabilities [89, 90]. More recently, mass spectrometry coupled to liquid chromatography (LC-MS) has been gaining ground in clinical laboratories for proteomic studies and some of the limitations have being addressed, however the method is still too complex and too expensive for widespread and routine usage, despite greater sensitivity and specificity [90-92].

Ongoing research and development is being performed on spectrometric techniques, aiming towards simplification, miniaturization and cost reduction in order to render them better suited to the clinical laboratories and the point-of-care [86, 93].

2.4.3 Immunological Assays

Immunological assays are bio-analytical techniques widely used to measure circulating large analytes, protein biomarkers, RNA, DNA and cells in biofluids. They rely on antibody binding to an antigen (analyte) for signal transduction. These methods are the most commonly used in clinical laboratories today as well as research owing to their high-throughput, high sensitivity and specificity, and the wide range of possible applications [94]. Known techniques such as immunofluorescence, flow cytometry, radioimmunoassay and ELISA belong to this category. Clinically, ELISA represents the workhorse technique for protein biomarker detection today [95]. While many variants of the technique exist, it generally involves immobilization of the analyte of interest onto a surface and using targeted antibodies labeled with enzymes to bind and produce a measurable signal, typically through luminescence, indicative of the concentration of the analyte within the sample [96]. The direct ELISA approach is illustrated in figure 2.2. As previously mentioned, ELISA is the current gold standard when it comes to clinical diagnostic protein biomarker detection and quantification and is a benchmark to which new and emerging techniques are compared to assess validity [97]. Although ELISA shows many strengths as a diagnostic tool, the technique presents difficult to address shortcomings: it is time-consuming since multiple incubation steps are needed, requires expertise and specialized instruments. Cost, while not being as prohibitive as spectrometry, could be improved upon as large quantities of reagents and materials are needed [98-100]. Furthermore, immunoassays in general may not be sufficiently sensitive for the detection of very low quantities of biomarkers, such as what would be found in the very early stages of diseases, limiting its effectiveness in early diagnosis and screening [77]. Immunoassays have and will continue to have an important role in clinical laboratory diagnosis in the near future for simple and routine assays, however it is theorized that they may get replaced by more performant and cost-effective spectrometric techniques in the long run, as technology progresses over time [101].

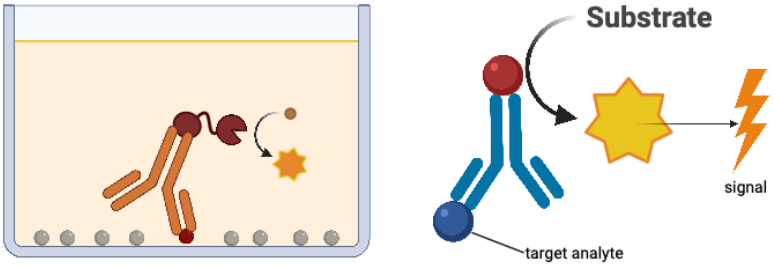

Figure 2.2 Working principle of direct ELISA

2.4.4 Optical Techniques

Optical sensing is a group of technologies using light or plasmonic fields and their interaction with recognition elements for detection analytes in a sample. They can be performed with or without labels, and are generally found to be highly sensitive and specific, rapid, easy to use and cost-effective [102]. Label-free techniques are highly advantageous as sample manipulation can be virtually eliminated from the detection steps. Surface plasmon resonance (SPR) and Raman spectroscopy (SERS) are among the main optical techniques used and researched today. While powerful, they show limitations in preventing non-specific interactions (SPR) [103] and may require expensive equipment not always available to resource-limited settings (Raman) [104]. However, multiple improvements have been achieved with optical technologies and platforms now exist addressing most shortcomings, such as integrated microfluidics which offer optical biosensing with portable and simple tools, and are promising candidates for point-of-care implementation [105]. The detection limit of an SPR-based sensor today is typically in the order of 10 pg/ mL [106].

2.5 Electrochemical Sensors

The fundamental principle of electrochemistry is the conversion of chemical energy into electrical energy, which can be measured as a signal through the transfer of electrons and ions between substances or materials. These processes occur in systems named electrochemical cells, which consist of electrodes immersed in an electrolyte solution where the reaction involving electron transfers takes place. Electrochemical sensors utilize this reaction between the electrodes and the

analyte in solution to detect and measure its concentration [107]. Electrochemistry-based sensors measure electrical potential or current changes in specific processes originating from a reduction-oxidation reaction (redox), where the electron transfer from the chemical species to an electrode can be precisely measured through a choice of techniques, the most popular of which include the powerful and flexible cyclic voltammetry (CV) and square-wave stripping voltammetry (SWSV) [108, 109]. Electrochemical techniques can be classified as voltametric, amperometric, potentiometric or impedimetric, which depends on the current injection and/or electrical measurement modalities [110].

Electrochemical sensing is an established technology with a history of use in many industries, such as environmental and food monitoring, especially in the detection of trace metals [111, 112]. More recently, there has been significant research focused on biomedical applications, most notably for glucose sensing in diabetes [113], which represents its major clinical use today, but also in detection of bacterial infections [104, 114] and traumatic brain injury [115]. Electrochemical sensors are currently receiving significant attention for their potential applications in point-of-care cancer monitoring, since they possess the advantage of being extremely low-cost, simple to design and operate, fast, miniaturized, and easily mass-manufactured. Of all their advantages, perhaps the most significant is the endless potential of chemical modifications that can be applied to the electrodes' surfaces, enabling a wide variety of applications and strategies to be put forth to augment sensitivity and selectivity of these sensors [116].

Electrochemical sensors are often designed around recognition elements, which can be antibodies, enzymes or aptamers to facilitate signal transduction and to ensure higher specificity in the presence of interfering substances. Furthermore, nanomaterials as an electrode structural modification are becoming increasingly popular as they allow for significant increase in surface area available to the electron transfer reaction to take place, therefore enhancing sensitivity [117]. Conductive nanomaterials Also allows direct electron transfer from the active site of the metalloprotein to electrode surface by tunneling of electrons when distance is about 10 Å or less [118]. Certain metals exhibit a particular surface catalytic activity for substrates, or chemical selection, and therefore contributing to increasing specificity in the presence of interfering substances [119]. This is notably the case for bismuth and Zn, where Bi-modified electrodes have shown the potential to interact with metallic ions at a more positive reduction potential than

otherwise expected from carbon electrodes, allowing their detection [120]. Figure 2.3 depicts these typical electrochemical sensor design strategies. Design elements depicted in can also be combined for even more performant sensors, where recognition elements can be attached to nanoparticles [121]. To be detected with an electrochemical apparatus in an approach not involving recognition elements, a biomarker must therefore be redox-active. In the case of a protein marker, this would translate into having an metal ion cofactor such as the metalloproteins, or aromatic compounds [122]. Zn-dependant proteins expressed in cancer are thus excellent candidates for electrochemical detection.

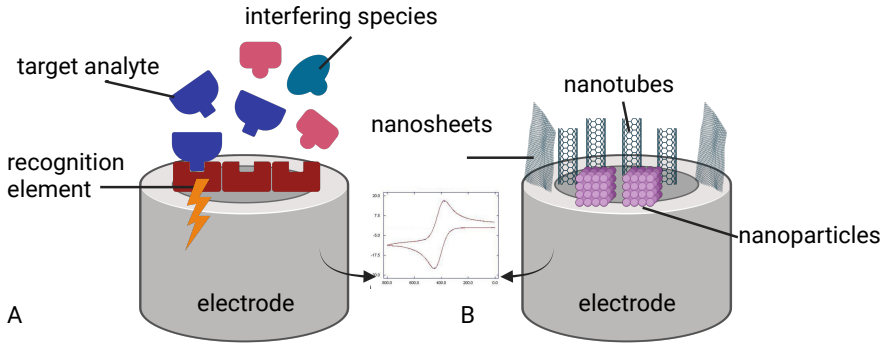

Figure 2.3 Common design strategies of electrochemical sensors. Recognition elements (A) including antibodies, enzymes and aptamers for specificity. Nanomaterials (B) used to augment surface-to-volume ratio and sensitivity.

2.5.1 The Three-electrode Electrochemical Cell

The electrochemical cell is the basic functional apparatus of electrochemical measurements and the three-electrode setup has been the most widely used in studies historically [123]. Connected to a potentiostat/galvanostat unit, it allows to perform a multitude of electrochemical techniques such as CV, electrical impedance spectroscopy (EIS), chronoamperometry, and SWSV without changing its setup. In the form used for this project, it consists of three electrodes: namely, the working electrode (WE), the counter electrode (CE) and the reference electrode (REF). Figure 2.4 describes an electrochemical cell as used for this project. The cell contains an electrolyte solution

required for the flow of current within the system and “close” the electrical circuit. In *in vitro* experiments, a supporting electroactive salt must be added to the liquid in order to enable electrochemical experiments to take place within the cell. They are often KCl combined with tetrabutylammonium tetrafluoroborate (NBu_4BF_4), potassium ferricyanide (K3[Fe(CN)6]) or sodium perchlorate ($NaClO_4$) [108]. Commonly used buffer solutions such as phosphate-buffered saline and tris-buffered saline (TBS) which mimic physiological conditions are often suitable on their own without any added electrolyte.

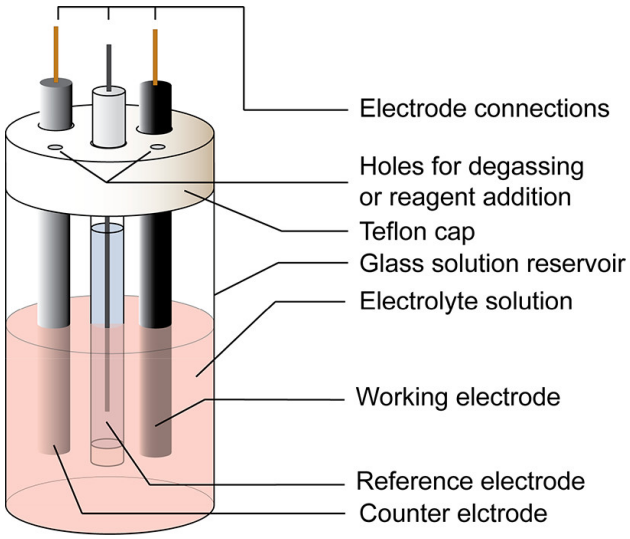

Figure 2.4 The three-electrode electrochemical cell setup [108]

The WE controls the electrochemical event taking place in the cell, on which an electrical potential is applied in reference to the REF through the potentiostat. The reaction of interest takes place at the surface of the WE, and therefore physico-chemical modifications are to be applied at this site to enhance affinity for the analyte and favour electron transfer. It is also the location at which peptides can be grafted to the system, where changes in impedance (the resistance to the flow of current) will be detected at the electrode-solution interface. Glassy carbon and gold are the two of the most used materials for WEs as they are excellent conductors, easy to modify chemically and to clean, and inexpensive [124, 125]. Moreover, there is now a significant amount of literature regarding surface modification of glassy carbon electrodes (GCE) which greatly facilitates the development of novel sensors [126].

The REF acts as a reference point to measure and apply currents within the cell. In a system, the redox potentials for a species will vary according to the make of the REF. The most used electrodes for such systems are silver chloride (Ag/AgCl) and saturated calomel (SCE). Lastly, the counter electrode completes the circuit since current is flowing between the WE and the CE in the cell. When oxidation occurs at the WE, reduction occurs at the CE and vice-versa. Since the rate of reaction is determined by the surface area of the electrode on which it takes place, it is imperative to have a much larger surface area on the CE than the WE to make sure the former is not the rate-determining step in the combined redox reaction: we are interested in studying the component of the reaction occurring on the WE only [127]. High surface area platinum coil wires or meshes are typically used as they are chemically inert in oxidation and good conductors [128].

2.5.2 Cyclic Voltammetry

In a solution, CV employs the three-electrode setup to perform an electrical potential (E) sweep across a given range, while recording the current response (I) of the system. In a given chemical environment, an electroactive species will be oxidized or reduced at a specific potential, which in turn produces a current response proportional to the species' concentration, which is indicative of the reaction rate of the electron transfer. The voltammogram is further influenced by several other factors to be taken into account, such as the scan rate, or the speed at which E is swept between the set limits, the diffusion coefficient of the electroactive species, or the speed at which it moves in a solution, the electrolyte solution composition and pH, and the physico-chemical properties of the electrode itself, namely the chemical selection and surface area available for the reaction [108]. Figure 2.5 depicts an example cyclic voltammogram of one cycle from a solution containing one electroactive analyte at a given scan rate. CV is a fast and simple technique to obtain measurements of analytes and to characterize an electrode surface, however it lacks sensitivity when compared to stripping voltammetry techniques and in such it is rarely used alone for precise detection of small quantities of analytes. It serves more as an exploratory technique. This low sensitivity is explained by the faradic current -the current generated by the flow of electrons involved in the electrochemical reaction, which is linear to the analyte's concentration- being interfered with by capacitive current, arising from the sweep charging/discharging of the electrical double layer at the electrode-electrolyte interface [109].

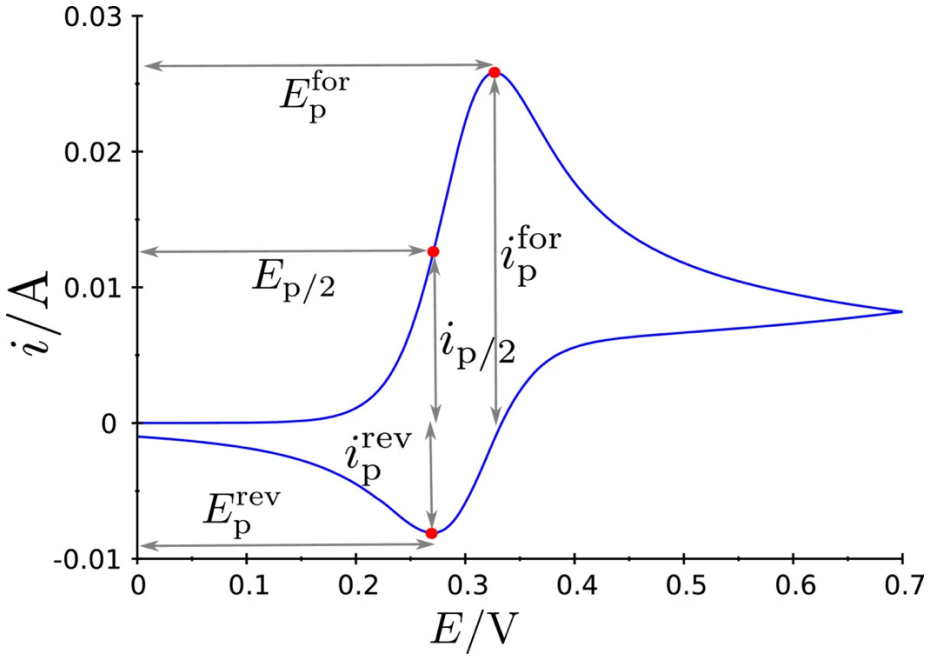

Figure 2.5 A standard cyclic voltammogram of current (I/Amperes) vs potential (E/Volt) showing reversible oxido-reduction of an electroactive species. Forward direction: oxidation, reverse direction: reduction. [129]

Trace metals such as Zn can easily be detected by CV as Zn undergoes the following reaction at the surface of the WE: $Zn \rightleftharpoons Zn(II) + 2e^{-}$ [130]. The oxido-reduction peak can be easily seen on the cyclic voltammogram which also allows with a high enough resolution to differentiate between the different ions should there be interfering species present (Cu2+, Pb2+ or other divalent metal cations), such as depicted in figure 2.6. Few sensors have been described in the literature using only this modality: CV is first employed to characterize the electrode's conductivity and chemical selection for the desired species, then typically a method of stripping voltammetry is used to determine the sensitivity of the system and the lowest possible LOD and LOQ.

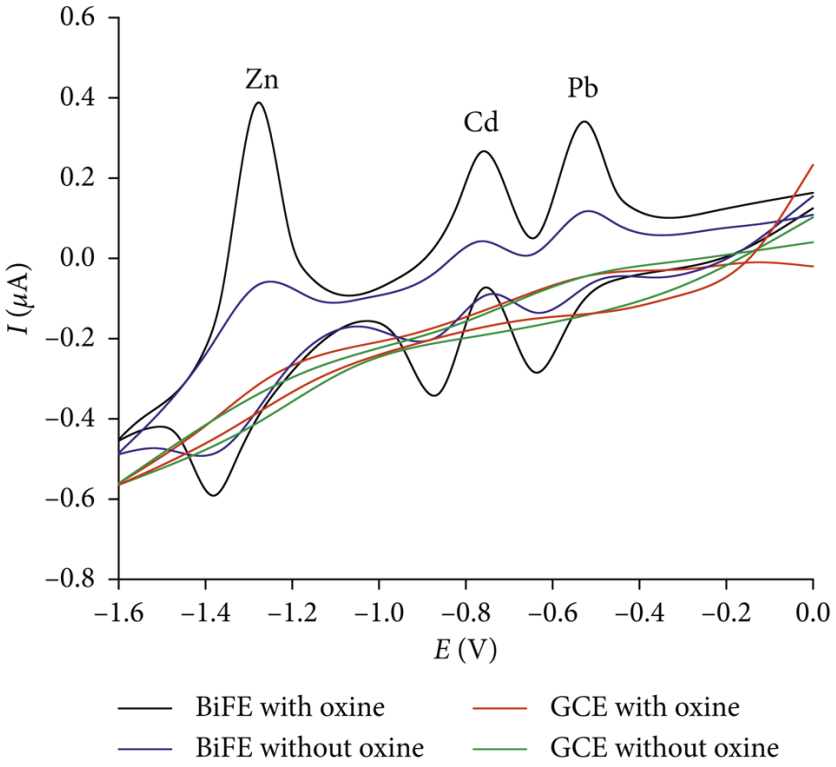

Figure 2.6 Cyclic voltammogram showing the discrete oxido-reduction peaks of Zn, Cd and Pb with modified Bismuth Film electrodes (BiFE) and Glassy carbon electrodes (GCE) [131].

2.5.3 Stripping Voltammetry

Stripping voltammetry is a popular and powerful technique to quantitatively measure ultra-low quantities of trace elements in electrochemistry. It involves two key steps: (1) electrodeposition of the electroactive species and (2) stripping.

(1) The electrodeposition step involves the accumulation of the electroactive species through either the application of a reduction (anodic) or oxidation (cathodic) potential between the REF and the WE. The oxidized or reduced species will be deposited on the WE surface. In the case of trace metal analysis, anodic stripping is mostly used as the application of a sufficiently negative potential will reduce the metal ion M^{n+} to M, where the zero-valence metal will easily deposit to the electrode surface [132]. In the case of Zn, the following reaction will occur:

$$Zn^{2+}(aq) + 2e^- \rightarrow Zn(s) \quad (1)$$

(2) A reversal potential will be applied to oxidize or reduce the species and revert the previous reaction which occurred in the electrodeposition step. In anodic stripping voltammetry, oxidation of the metal back to its ionic state is done via sweeping the potential in the anodic direction. Equation 2 illustrates this reaction with Zn following the reaction in equation 1. The potential at which a stripping peak occurs and its current/AUC are recorded, which are dependent on the nature and quantity of material previously deposited [132].

$$Zn(s) \rightarrow Zn^{2+} + 2e^- \quad (2)$$

Equations 1 and 2 in the context of anodic stripping voltammetry are illustrated in figure 2.7. In the stripping step, the potential sweep may be performed linearly, or using other waveforms such as SWSV or differential pulse voltammetry (DPV). Linear sweep voltammetry is the simplest technique and requires the least complex instruments [133].

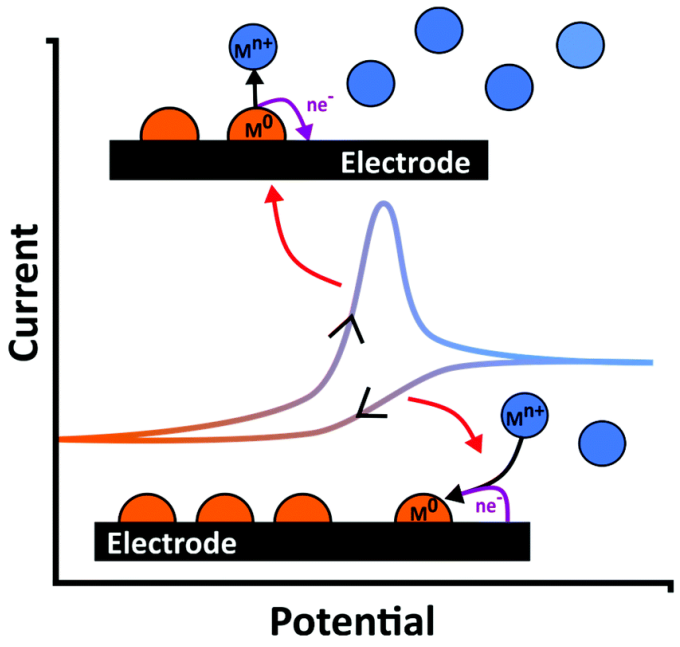

Figure 2.7 Electrodeposition (top) and stripping (bottom) steps in conventional stripping voltammetry techniques [134]

SWSV and DPV both offer greater sensitivity than linear sweeps, however they require instruments capable of generating and analyzing precise current pulses or current waveforms. Compared to

DPV, SWSV is generally believed to possess the best advantages and considered the most advanced technique since it is faster, more sensitive and offers the greatest reduction in capacitance current [135], which can interfere with measurements as discussed in the previous section. However, it is to note that some controversy exists on which stripping voltammetry technique is the most sensitive [136]. Detection limits of SWSV based electrochemical sensors are regularly in the 10^{-10} M, or 1 ng * mL for trace ions, while for DPV the limits are in the 10^{-8} M range [137]. To calibrate a stripping voltammetry sensor, the peak reduction/oxidation current is recorded at a given concentration of analyte, then a curve can be plotted consisting of peak current (I) vs concentration. An LOD and LOQ can then be calculated from the linear regression, which is shown in figure 2.8.

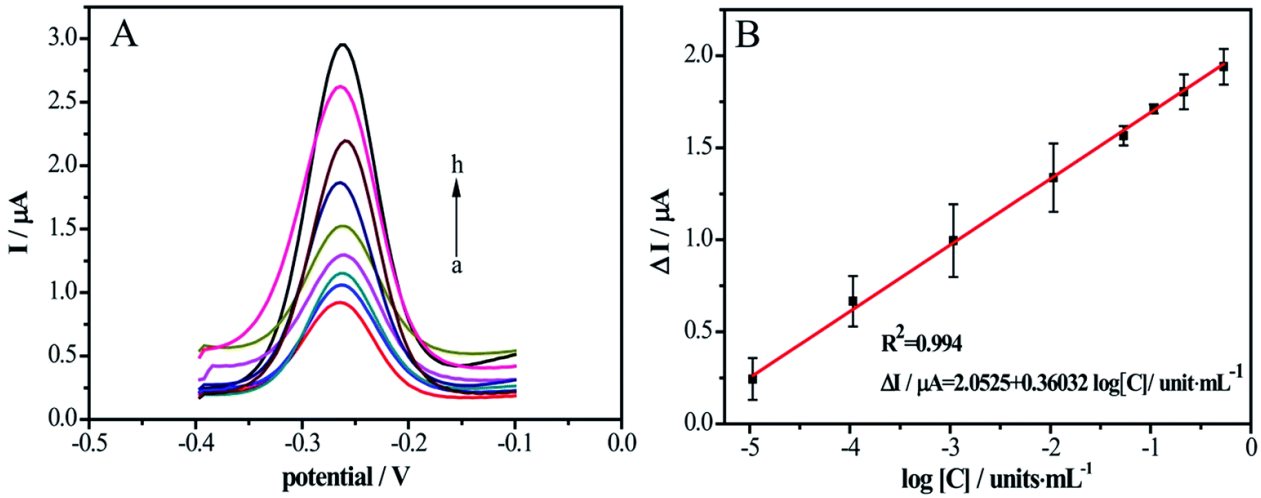

Figure 2.8 Example of square wave stripping voltametric curves and corresponding calibration curve for determination of analyte concentration [138]

While effective for free ions and small molecules, SWSV is rarely used for the detection of large molecules such as proteins where a labelled or recognition element approach is preferred. In the design of voltametric sensors, great care must be employed to ensure that the sensor is resistant to interference, as biological samples contain many interfering species [139].

2.5.4 Electrical Impedance Spectroscopy

Lastly, EIS is an important technique used in biomarker detection when recognition elements are employed. It relies on the principle of measuring the impendence (Z, the opposition to the flow of a current in an electrical circuit), at the electrode-electrolyte interface [140]. Recognition elements can serve to bind the target analyte, in which case resistance to the transfer of charge will increase as the electrode gets obstructed by the analyte. Another popular method for the detection of enzymes is to chemically link a degradable peptide chain to the electrode, which will get cleaved by the target analyte, therefore decreasing the obstruction and resistance at the electrode surface [141]. EIS is regarded as an ultra-sensitive, low cost and non-destructive analytical method with a resistance to interference as recognition elements are often present, however, the careful consideration in choice of said element must be made in order to avoid nonspecific binding in the case of biosimilar molecules and makers, a major limitation of the technique [142].

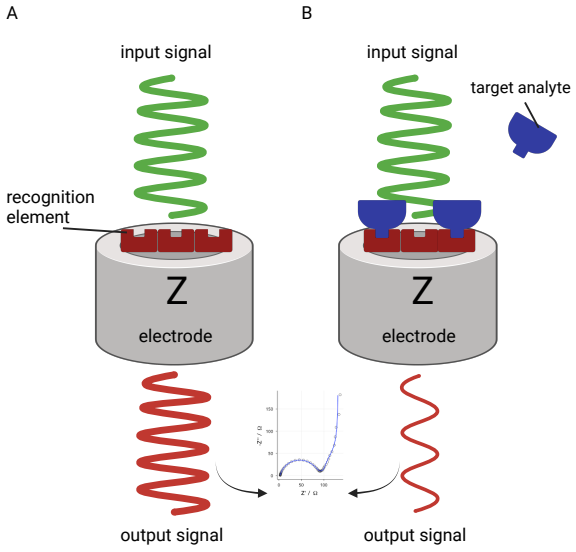

Figure 2.9 Working principle of an electrochemical EIS biosensor. Impedance (Z) increases with binding of the target analyte, decreasing the electrical output and signal of the apparatus.

To conclude this review, a selection of sensors based on technologies described in this chapter will be presented, along with their lowest reported LOD and catalyst or recognition element strategy used. Table 2.3 presents a selection of sensors for free Zn ions. Among chosen sensors, the lowest

LOD belongs to an optical SERS biosensor, which achieved a staggering and unprecedented 10^{-14} M, however the reproducibility is low and SERS devices are still poorly adaptable to the point-of-care [143]. Optical sensors generally outperform electrochemical sensors by three orders of magnitude, however SWSV sensors have shown to be on the same performance level with an LOD of 9 ng/L for free Zn ions [144]. For the detection of Zn-dependant proteins, a selection of sensors targeting cancer biomarker proteins discussed in this chapter are presented in table 2.4. For protein detection however, performance of electrochemical sensors is comparable to other technologies such as optical techniques and the gold-standard ELISA. A SWSV electrochemical sensors has even achieved an LOD of $3*10^{-15}$ M for MMP-7 using the strategy to link peptides with MMP-7 cleavage specific sites to the electrode for selectivity and signal amplification, which allowed such a high sensitivity [145]. Overall, electrochemical sensors show adequate performance compared to other available technologies and current gold standards in biomarker detection, combined with their highly desirable advantages for the point-of-care implementation. This renders them an important and promising research area for the field of medical sensors for cancer monitoring, more specifically free Zn and Zn-dependant metalloproteins in cancer.

Table 2.3 Selection of free Zn ion sensors with their characteristics and performance

Technology	Method	Recognition Element or Catalyst	LOD	Reference
Electrochemistry	SWSV	Bismuth	0.05 μg/ml	[146]
Electrochemistry	Cyclic voltammetry	Bismuth + carbon fibre	1.0 μg/L	[147]
Electrochemistry	SWSV	Bismuth oxide	1.0 μg/L	[148]
Electrochemistry	DPV stripping voltammetry	Carbon nanotubes	17 μg/L	[149]
Electrochemistry	SWSV	Sulfonated carbon+ Bismuth NP	4.8 ng/mL	[120]
Electrochemistry	SWSV	Carbon nanotube threads	9 ng/L	[144]
Optical	Fluorescence	1-((2-(2-(2-((2-hydroxynaphthalen-1-yl)methyleneamino)phenylamino)ethylamino) phenylimino)methyl)naphthalen-2-ol (H_2L)	3ng/L	[150]

Optical	SPR	Chitosan–TBTDS	10 μg/L	[151]
Optical	SPR	Gold-Nanocrystalline cellulose	10 μg/L	[152]
Optical	SERS	Zincon	0.1 μmol/L	[153]
Optical	SERS	N′N-bis(2-hydroxybenzylidene)-4-aminophenyl disulfide (HBA)	10 fmol/L	[154]
Spectrometric	Microwave plasma torch MS	None	10 μg/L	[155]

Table 2.4 Selection of Zn-dependant protein cancer biomarker sensors with their characteristics and performance

Technology	Method	Target	Recognition Element or Catalyst	LOD	Reference
Electrochemistry	EIS	MMP-9	Cleavage site specific peptide	15 ng/mL	[141]
Electrochemistry	DPV	MMP-2	Anti-MMP2 Antibodies on Au-NP	0.11 pg/mL	[156]
Electrochemistry	SWV	MMP-7	Cleavage site specific peptide	3.1 fg/mL	[145]
Electrochemistry	Chronoamperometry	CA	Sulfonated carbon+ Bismuth NP	11 ng/ml	[120]
Electrochemistry	Chronoamperometry	CA II	Methazolamide	6 ng/mL	[157]
Immunoassay	ELISA	MMP-9	Anti-MMP9 Antibody	4 pg/mL	[158]
Optical	SPR	MMP-9	Anti-MMP9 Antibody	8 pg/mL	[159]
Spectrometry	MALDI-TOF MS	MMP-2	Binding peptide probe	10 ng/mL	[160]

Spectrometry	LC-MS	MMP-3	None	0.5 ng/mL	[161]

CHAPTER 3 CHALLENGES, RESEARCH OBJECTIVES AND GENERAL METHODOLOGY

3.1 Challenges & Objectives

Upon reviewing the literature on Zn electrochemical sensors and their strategies, it was clear bismuth materials offered a promising path to developing performant yet accessible point-of-care sensors. New materials are always being investigated to develop sensors with the lowest possible LOD and recently bismuth-based electrochemical devices have reached limits in the ng/mL [120] which are biologically relevant in medical sensing. However, there is still the possibility to improve the sensitivity of those sensors as there is a gap between the most performant electrochemical sensors and devices from optical technologies such as SPR which can currently reach LODs in the fmol/mL [154] for free Zn detection. These devices however require complex fabrication and a high cost to acquire the technology, which is a major hurdle for implantation in the point-of-care setting when compared to electrochemistry, which tends to produce easy to fabricate and accessible sensors.

In conclusion, despite the recent progress made in medical sensing devices, there still remains a gap for accessible, point-of-care-ready sensors for the detection of free Zn in cancer with ultra-high sensing performance comparable to optical technologies. It is believed bismuth materials may help bridge this gap by providing electrochemical sensors with a high chemical selectivity and affinity for Zn ions.

The aim of this work is to develop the basis of a novel electrochemical sensor for the detection of free Zn ions with a simple fabrication scheme and accessible utilisation. The objectives are divided as follows:

O1. First, to synthetize and characterize Bi nanostructures with a high surface-to-volume ratio and high Zn affinity.

O2. Second, to chemically modify the nanostructures with specific amino acids such as histidine to enhance electrocatalytic activity.

O3. Third, modify the surface of a microelectrochemical probe with the synthetized Bi nanostructures and investigate Zn detection capabilities in *in vitro* buffer solutions.

3.2 General Methodology

The following chapter will present a detailed methodology for the development and *in vitro* testing of the electrode concepts. Based on a review of recently developed similar devices and following fundamental electrochemical principles, the work on sensors was mostly based on trial and error to optimize the many parameters and variables involved in order to maximize detection capabilities.

3.2.1 Synthesis of Bismuth Sulfide Nanoparticles

Bismuth sulfide nanoparticles (BiSNP) were chosen as basis for the Zn detection electrocatalytic ink. While the Bi shows excellent electrocatalytic activity for the reduction of Zn, S allows covalent modifications. The NP were designed to incorporate carboxylic acids as terminal functional groups (-COOH) on their surface linked to S to allow for further chemical modification of the framework through cross-linking through popular reaction such as NHS/EDC [162], used to attach a terminal carboxy group to a primary amine, such as those present in amino acids. Using this method, the BiSNP framework could theoretically support a variety of peptides, proteins and polymers grafted upon it to improve its performance as a biosensor. Amino acid histidine was chosen as the recognition element for its affinity with divalent metals, which should enhance selectivity and sensitivity of the carbon electrode. L-histidine has been shown to form complexes with Zn^{2+} and Cu^{2+} and has been found useful in the detection of these ions with voltammetry techniques [163].

3.2.2 Carboxylated Bismuth Sulfide Framework

A hydrothermal reaction was employed to synthetize carboxylated bismuth sulfide nanoparticles (cBiSNP) in a one-pot synthesis, which is considered an efficient approach in synthetic chemistry, minimizing chemical waste, saving time and simplifying steps [164]. In the presence of thioglycolic acid (TGA), bismuth nitrate, $Bi(NO_3)_3$, can react to form bismuth sulfide (Bi_2S_3) at relatively low temperatures if pressure is kept very high such as in a hydrothermal process. At reaction temperatures of 160-180^{o}C, a nanorod-like structure can be obtained [165, 166]. The

reaction was completed with thioacetamide (TAA), another source of sulfur ion. Combining TGA and TAA with a metal cation in a hydrothermal process results in a carboxylated sulfur ion, where TGA acts as the -COOH source [167]. The reaction scheme is illustrated in figure 3.1.

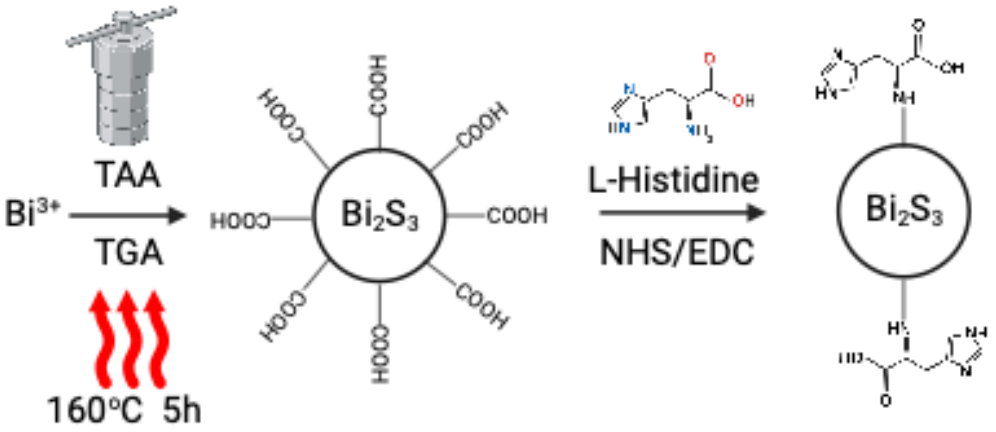

Figure 3.1 Reaction scheme of carboxylated bismuth sulfide nanoparticles with NHS/EDC cross-linking of amino acid L-histidine (not to scale).

The reaction scheme depicted in Figure 3.1 can be explained in the following proposed mechanism: firstly, bismuth nitrate dissolves in water to form free Bi^{3+} ions, which combined with sulfur from TAA form Bi_2S_3 nanoparticles when heated at 160°C for 5h. The carboxylic acid group of TGA can then undergo a reaction with the surface sulfide (S^{2-}) ions present in the nanoparticles: the thiol group of TGA will be displaced by the S^{-2}, resulting in surface-bound carboxylic acid groups covalently linked to the nanoparticles themselves.

3.2.3 Nanoparticle Physico-chemical Characterisation

The synthetized cBiSNP and bismuth sulfide nanoparticles coated with histidine (BiSNP-H) were characterized with Fourier-transform infrared spectroscopy (FTIR) to inspect surface functional groups and high-resolution images were taken with a scanning electron microscope (SEM). Average size of nanoparticles was measured manually through SEM images taken at 1.2k magnification using Fiji software [168] and data was presented as mean ± SD.

3.2.4 Preparation of Bismuth NP-modified Electrode

GCE with 5mm diameter were used as the basis electrode for this sensor design. An electrocatalytic ink was prepared consisting of 1 mg/mL of the chosen NP, 16 μL/mL of Nafion117 (5% w/v polymeric solution, acting as a molecular binder [169] to coat the electrode with our NP), in a 80% v/v ethyl alcohol in water. GCE were prepared by first careful polishing for 5 minutes in a 0.05 μm alumina polishing solution, then washed and sonicated in an ultrasonic bath for 10 minutes in water and 10 minutes in acetone. The electrodes were then dried under N_2. 20 μL of the electrocatalytic ink was then dropped casted on the surface of a GCE and set to dry at room temperature. The prepared Bi NP electrodes were then ready to be placed in a three-electrode electrochemical cell, acting as the WE.

3.2.5 Electrochemical Measurements and Zn Sensing

A three-electrode electrochemical cell was used as described in chapter 2. Platinum coil wire was used as CE and Ag/AgCl was used as REF. To assess electrical conductivity of the designed cBiSNP and BiSNP-H electrodes and compare them with a bare GCE for control, simple CV were conducted in a 10 mM aqueous potassium ferricyanide solution. Control CVs were performed in a range of -0.4 to 0.8 V at a scan rate of 50 mV/s.

SWSV was chosen for Zn sensing for its superior sensitivity compared to other electrochemical techniques and TBS was chosen as electrolytic solution to carry the measurements as it is an effective buffer solution, maintaining a stable pH between the 5.8-7.2 range, which is important as many electrochemical reactions are affected the pH value. Furthermore, TBS shows excellent biocompatibility as it is isotonic, non-toxic to cells and is often used as a nucleic acid stabilizing and storage solution. To determine the reduction potential of Zn in our system that will be applied during pre-concentration step of SWSV, a simple CV was performed in TBS pH 5.1 with 1 mM Zn nitrate in the range of -1.4 to -0.8 V at a scan rate of 20 mV/s. Through the resulting cyclic voltammograms, a cathodic reduction peak of Zn was identified. pH at which Zn is detected was optimized with SWSV measurement of Zn. 5mM Zn nitrate-TBS (deposition parameters: -1.4 V for 300 seconds; SVW parameters: pulse amplitude 25 mV, step height 0.2 mV, frequency 25 Hz, range -1.4 to -1 mV) was used and pH of the Zn solution was adjusted from 3.6 to 7.1 by adding NaOH or HCl as needed. Once the optimal detection pH was identified, SVSW Zn measurements

were undertaken with the same parameters in Zn nitrate-TBS solutions ranging from 5 to 50 μM. Peak current in the stripping step was recorded and used to plot a calibration curve of concentration vs I. In all SWSV experiments, current I was calculated from ΔI (F-R), which is the differential DC current obtained by subtracting the reverse pulse reading from the forward pulse reading, providing with the differential output as is common practice in pulse voltammetry techniques. LOD was determined from the formula $LOD = 3.3 * \frac{\sigma}{S}$ were σ is the standard deviation of the intercept (calculated as SE*sqrt(N)) and S the slope of the curve. LOQ was determined from the formula $LOQ = 10 * \frac{\sigma}{S}$.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Carboxylated Bismuth Sulfide Nanoparticles

cBiSNP were synthetized according to step one of the reaction scheme depicted in figure 3.1 using bismuth nitrate, TAA and TGA in a hydrothermal reaction for 5h at 160ºC. A second reaction cross-linking amino acid histidine was carried on the nanoneedle-shaped NP using NHS/EDC. To confirm successful synthesis of BiSNP and the presence of surface carboxyl groups on the NP, physicochemical characterization was performed on the powder resulting from the first reaction step. Macroscopically, the two end products were indiscernible from each other, and the thin black powder appearance form the first synthesis was preserved. SEM pictures of the NP were taken before (figure 4.1 A) and after (figure 4.1 B) histidine cross-linking. The nanoneedle morphology confers a high surface area to volume ratio, therefore enhancing greatly the available electrocatalytic surface of the electrodes.

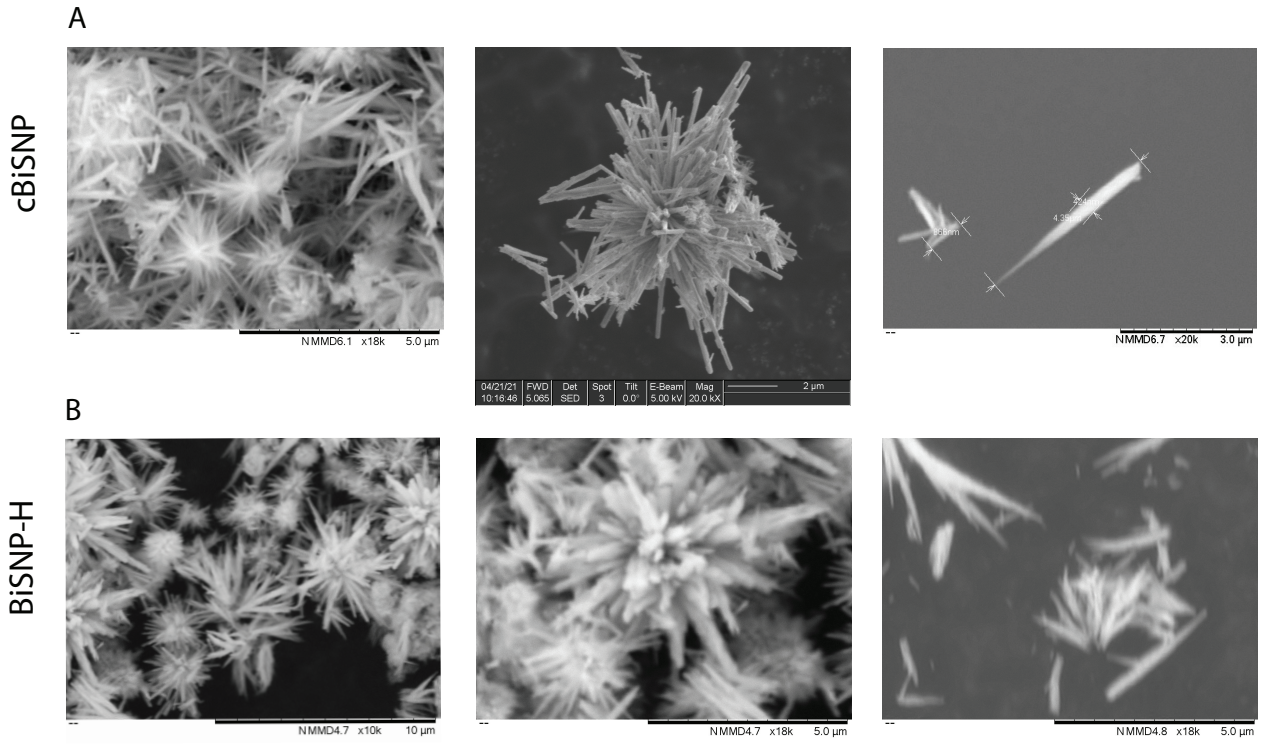

Figure 4.1 SEM images taken of cBiSNP powder (A) and cBiSNP-H (B) after histidine cross-linking. Images show nanoneedle morphology is preserved after chemical modification.

Figure 4.1 shows that the morphology of the cBiSNP nanoneedles was preserved after NHS/EDC cross-linking of histidine and the structural arrangement of the NP in a flower-like configuration remains unchanged. Sizes of cBiSNP were measured manually from SEM pictures and are presented in Figure 4.2. A total amount of 215 NP were individually measured, and average NP length was determined to be 1.782 ± 0.832 µm.

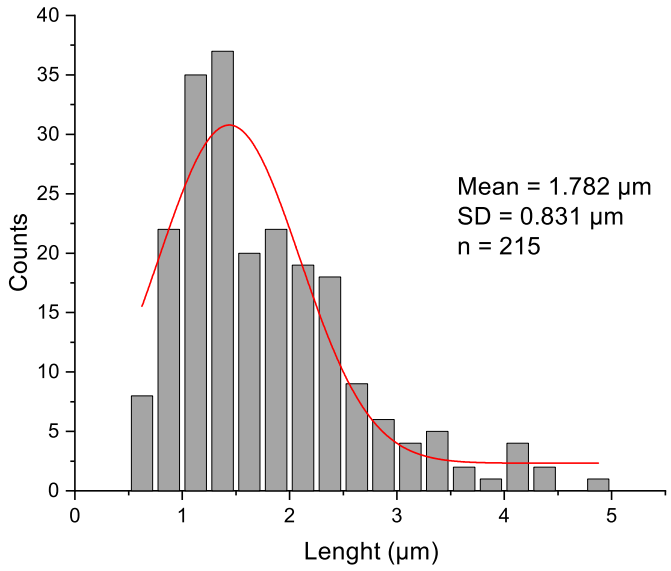

Figure 4.2 Size distribution of cBiSNP

To characterize the composition and surface functional groups of the synthetized NP, FTIR was performed on cBiSNP, shown in figure 4.3. Firstly, the IR spectrum shows the presence of bismuth and sulfur with two strong peaks at 770 & 692 cm^{-1} characteristic of Bi_2S_3 and attributed to Bi-S bending vibrations [170, 171] and to C-S stretching, respectively [172]. The peak located at 1200 cm^{-1} may be attributed to bending of C-O bonds. Furthermore, the strong peaks seen at 1515 cm^{-1} and 1350 cm^{-1} may be attributed to carboxylate salts themselves, which would indicate the presence of the surface carboxyl groups in their ionized form. The two bands are typical of the ionized groups in the sodium carboxylate form [173]. The weak broad band seen in the 3500-3000 cm^{-1} region is also characteristic of carboxylic acid O-H stretch. As the region peak is weaker than the carboxylate peaks, we can infer the dominant form of the functional group in the cBiSNP is the sodium carboxylate.

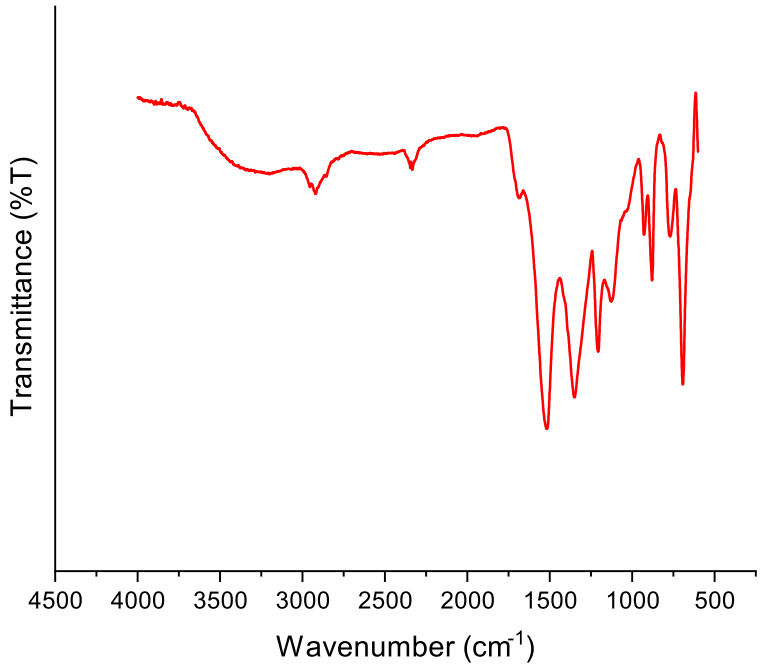

Figure 4.3 FTIR spectra of the carboxylated Bi_2S_3 nanoneedles in powder form

4.2 Electrochemical Characterization of cBiSNP and BiSNP-H Electrodes

Electrical conductivity of the newly designed electrodes was assessed using potassium ferricyanide solution $[K_3Fe(CN)_6]/[K_4Fe(CN)_6]$ as electron transfer agent. The obtained cyclic voltammograms are depicted in figure 4.4 for a bare GCE negative control electrode (black curve), GCE modified with the cBiSNP electrocatalytic ink (red curve) and GCE modified with the BiSNP-H (containing histidine) electrocatalytic ink (blue curve). As shown by the inset of figure 4.4, peak anodic current density at the surface of the electrode decreased from 194 $\mu A/cm^2$ to 132 $\mu A/cm^2$, which represents a 32% decrease in conductivity of the surface. However, this decrease in conductivity is expected to be greater as the electrode surface was coated with high surface area to volume ratio NP, and a higher surface area leads to higher currents. Despite a greater surface area, the new cBiSNP electrode showed a significant decrease in current density, indicating of the very low conductive nature of our material. This is to be expected as Bi is less of an electrical conductor than traditional carbon structures [174]. In its Bi_2S_3 form, bismuth has a reported electrical conductivity (σ) of 6 Siemens * m^{-1} [175] while glassy carbon has a conductivity of 6803 Siemens * m^{-1} [176], which is

over 1000 times greater. The hypothesis is that by using bismuth to coat a carbon electrode, specificity will be gained at the loss of electrical conductivity. Then, the bare GCE was coated with the BiSNP-H based electrocatalytic ink to investigate the effects of histidine linking on conductivity. As shown on figure 4.4, anodic peak current density of the BiSNP-H is 62 $\mu A/cm^2$. This represents a further 53% decrease from the observed conductivity of the cBiSNP electrode and a 68% decrease from the bare GCE. This decrease can be explained by the histidine assembly forming an insulating layer retarding the electron transfer from the analyte to the electrode Bi_2S_3 surface. Despite a lower electrical conductivity from the addition of histidine to cBiSNP, sensitivity to Zn ions is expected to be greater as histidine forms complexes with ions in the solution.

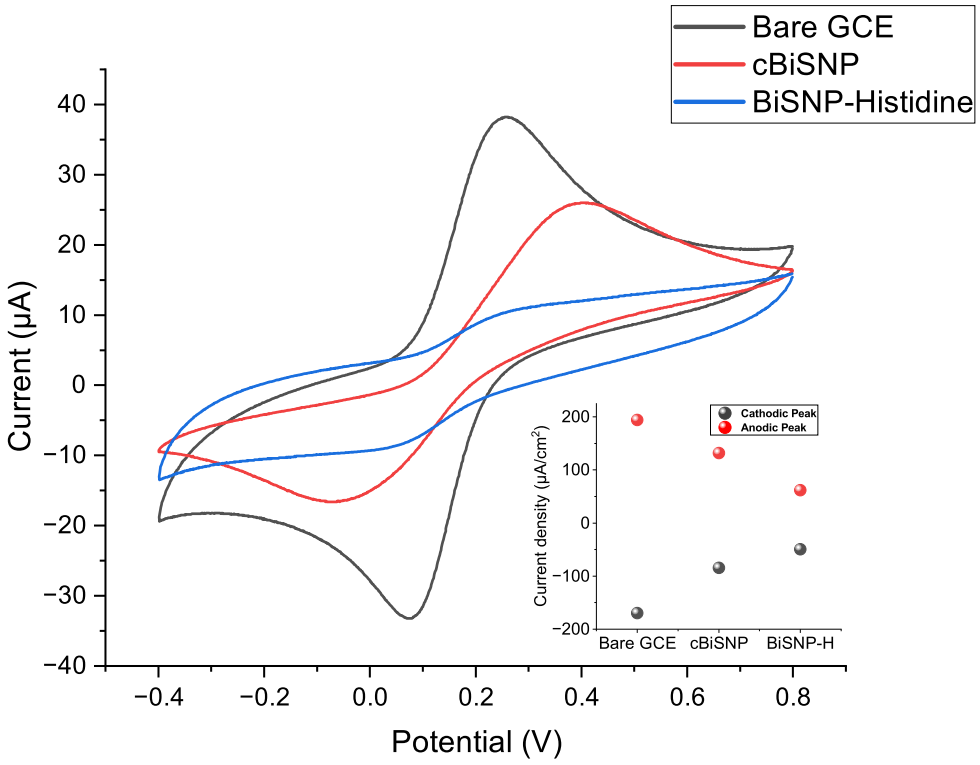

Figure 4.4 Cyclic voltammograms in 10 mM $[K_3Fe(CN)_6]$ of a bare 5mm GCE (black), cBiSNP modified GCE (red) and BiSNP-Histidine modified GCE. Inset shows oxidoreduction potentials as current densities for a 5mm electrode for each modified electrode.

4.3 Zn Electrodetection

Firstly, Zn reduction peak at the surface of our designed electrodes needed to be elucidated in order to later apply the correct potential in the deposition step of the SWSV for optimal detection performance. This is an intrinsic property of the system and is highly dependent on surface coating, and therefore cannot be inferred from similar electrode designs in the literature. To this end, CVs were performed in TBS at pH 5.1 with a high Zn concentration (1 mM) to observe the peak cathodic potential, at which Zn reduction and deposition on the electrode surface occurred and the resulting current generated by the system. cBiSNP modified and BiSNP-H modified GCEs were compared, and the results are shown in figure 4.5. Cathodic peak potential occurred more negatively for the BiSNP-H (-1.32 V) versus the cBiSNP (-1.25 V). This would theoretically enhance specificity as the Zn reduction reaction is pushed to more negative potentials, it can be more easily differentiated from interfering species reacting at the same surface. Furthermore, a greater cathodic current density was generated by the reaction on BiSNP-H (-510 $\mu A/cm^2$) versus cBiSNP (-415 $\mu A/cm^2$) which would suggest superior affinity for Zn reduction and superior electrochemical performance for BiSNP-H compared to cBiSNP (23% increase). However, in future works, more repeats of the measurements under the same conditions should be completed in order to establish statistical significance. The observed difference in current densities for Zn reduction could be explained by the surface histidine forming complexes with Zn ions in solution, therefore increasing the amount of Zn in close proximity of the electrocatalytic surface, which are then readily available to undergo redox.

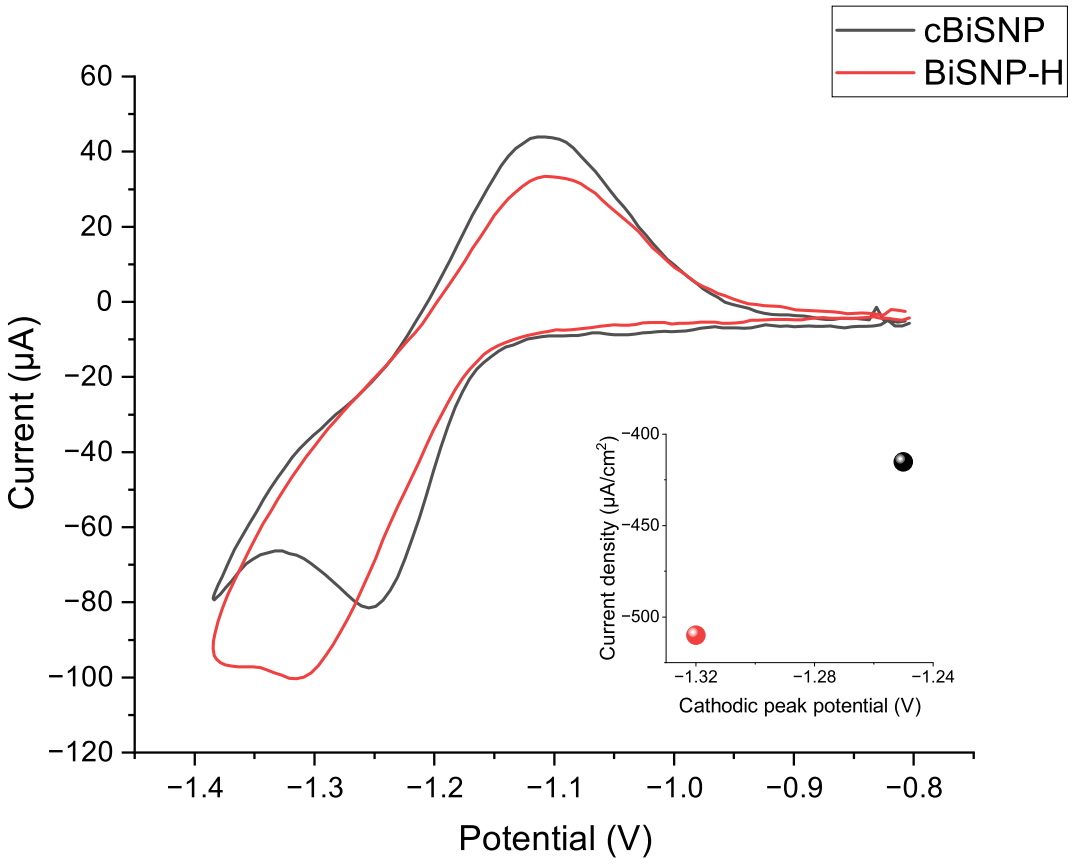

Figure 4.5 Cyclic voltammograms of cBiSNP and histidine-modified BiSNP (BiSNP-H) 5mm GCE in TBS pH 5.1 with 1mM Zn nitrate. Inset shows the cathodic peak potentials and current densities.

BiSNP-H modified GCE were chosen to investigate the effect of pH on Zn sensing. SWSV was performed in 5mM zinc nitrate TBS solution, varying the pH from 3.6 to 7.1. The voltammograms are shown in figure 4.6A. Overall, the BiSNP-H electrode showed measurement stability across a wide pH range, as illustrated in figure 4.6 B. At pH 3.5, measured current was 60µA, which decreased to 51 µA close to physiological pH at 7.1. This represents a change of -15% over this range. Peak sensor performance in TBS occurred in a pH range between 4.3 and 5.0. This is consistent with the literature, which reports Bi film electrodes to operate at highest electrochemical performance in stripping voltammetry in the 4 to 5 pH range [177]. Other works also report a greater potential windows in Bi hydrolysis in lower pH [27], which would make this range

theoretically ideal for a performant Bi-based Zn sensor. While the relationship between potential window and current is not straightforward, generally a higher potential window for an electrode can lead to greater currents being generated by the studied reaction. However, due to practical considerations, the use of a pH closer to physiological pH is more commonly used in Zn biosensors in the literature, since biologically relevant samples have a pH range between 6.5-7.4 [178]. Therefore, a pH of 6 was chosen to conduct further Zn sensing trials in TBS.

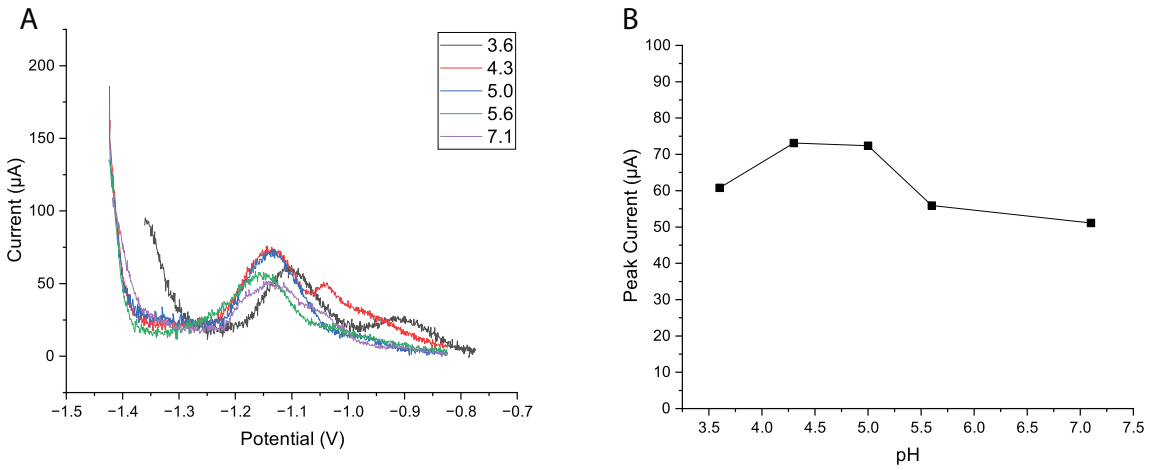

Figure 4.6 SWSV voltammograms (A) of BiSNP-H modified 5mm GCE in TBS buffer with 5 mM Zn nitrate, varying the pH of the buffer solution from 3.6 to 7.1. Peak SWSV current (B) as a function of TBS buffer pH.

cBiSNP were investigated and optimized first for their Zn detection performance in TBS. SWSV was performed on 5mm GCE with the previously designed electrocatalytic ink. The physiologically relevant range of 0 to 50 µM was investigated, in Zn increments from 0, 5, 10, 20, 30 and 50 µM. Figure 4.7 A shows the resulting voltammograms. The lowest concentration tested, 5 µM, results in a current density of 70.0 µA/cm^2, which is 3.3 times greater than the current measured when no Zn was present (21.1 µA/cm^2) suggests a signal of good quality would have been obtained if lower concentrations were to be tested on the system and that 5 µM does not represent the limit of the system. The peak SWSV current for each Zn concentration was plotted in a calibration curve

presented in figure 4.7 B. Within the 5 µM to 50µM range, our sensor shows a linear relationship between ΔI and [Zn], with an adjusted r-square of 0.93. The linear regression was Y = 2.93*[Zn] +71.16. Based on a linear regression including 0 µM Zn (regression: Y = 3.49*[Zn]+ 52.13, adjusted r-squared 0.88), LOD was calculated according to the IUPAC method of 3.3*SD/S to be 30.23 µM and the LOQ (10*SD/S) to be 100.80 µM, or 1.98 µg/mL and 6.60 µg/mL, respectively.

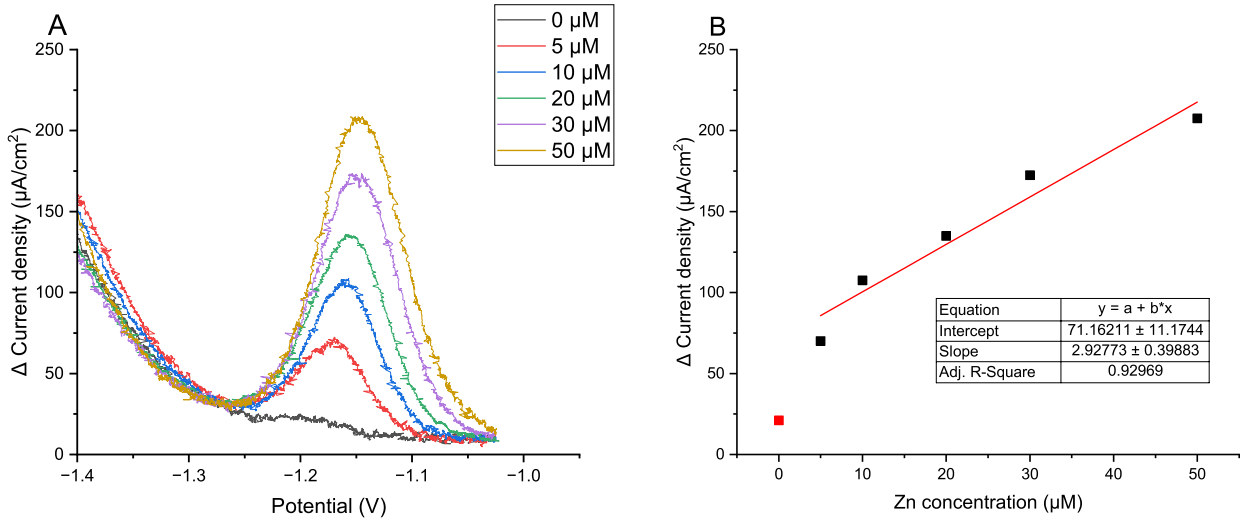

Figure 4.7 SWSV voltammograms (A) for Zn detection from cBiSNP-modified GCE in TBS pH 6.1. The electrodes were exposed to Zn solutions of 5µM, 10µM, 20µM, 30 µM and 50 µM. Calibration curves (B) of the sensor showing linear relationship between ΔI and [Zn] with linear regression in the 5-50 µM range.

4.4 Preliminary BiSNP-H Results

Zn detection from BiSNP-H are only available as preliminary results and need more experimentations to optimize the detection performance and stability of the electrocatalytic ink in SWSV. The current state of the biosensor is presented in figure 4.8. The signal obtained from the sensor currently has a very low signal-to-noise ratio, limiting its analysis. Furthermore, the current obtained for this sensor is greatly diminished at the same Zn concentration when compared to the cBiSNP-based sensor (53.5 µA/cm^2 vs 172.5 µA/cm^2) and shows a reduction of 69% at 30 µM Zn. A linear regression was performed in the 5 to 50 µM range, which resulted in an adjusted r-square of 0.56, which is too low to establish the linear relationship between ΔI and [Z]. For this reason,

an LOD and LOQ were not calculated. The system however responds to Zn and considering the results of the CV in high Zn concentrations in Figure 4.5 which show superior electrochemical performance of BiSNP-H compared to cBiSNP, it is hypothesized that given more experiments to optimise the new coating BiSNP-H will show superior performance in SWSV for Zn detection.

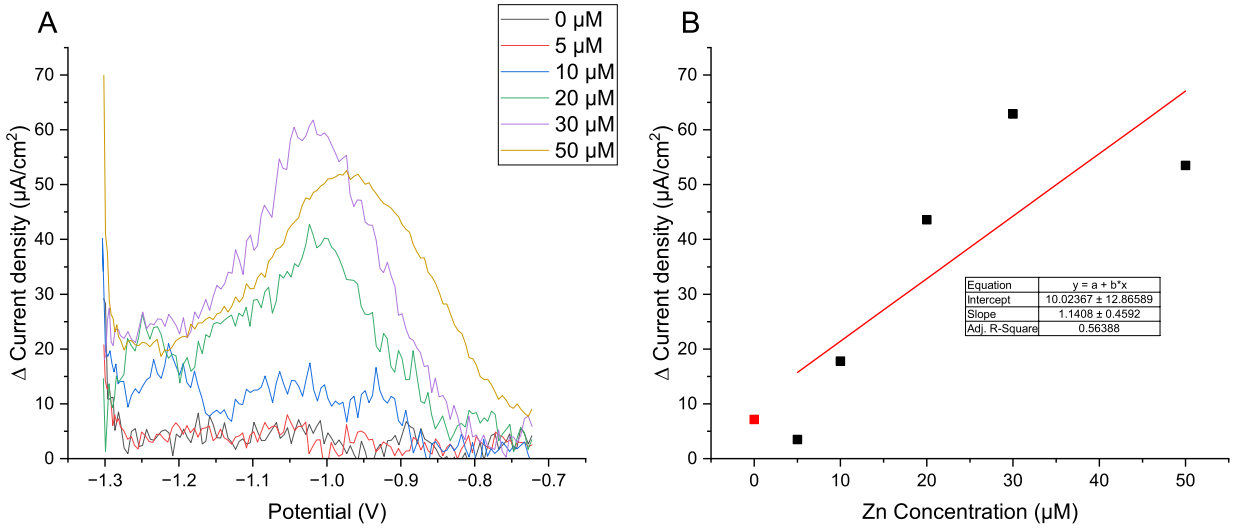

Figure 4.8 SWSV voltammograms (A) for Zn detection from BiSNP-H modified GCE in TBS pH 6.1. The electrodes were exposed to Zn solutions of 5µM, 10µM, 20µM, 30 µM and 50 µM. Calibration curves (B) of the sensor showing linear regression between ΔI and [Zn] in the 5-50 µM range.

4.5 General Discussion

The aim of this thesis was to develop an electrochemical sensor to detect a cancer biomarker, free Zn ions, in laboratory solutions. To this end, a modified bismuth sulfide-based carbon electrode was designed with high affinity for Zn, and the successful engineering of this sensor was demonstrated in this work. Further modifications and optimizations of the sensor have been undertaken and are in the preliminary stage at the time of writing this thesis.

A challenge in developing electrochemical Zn sensors is optimizing the chemical selectivity of the surface. While carbon is an excellent conductor, it does not allow to easily differentiate our target analyte from interfering species, namely other divalent ions. To address this challenge, bismuth in

its Bi_2S_3 form was chosen, which has been shown to possess great affinity for Zn in the electrochemical context, as the basis of our sensor which would then be modified to attempt to further enhance its sensing performance. First, the Bi_2S_3 was successfully prepared in the form of nanoneedles with an average size of 1.78 µm, whose morphology bears a high surface-to-volume ratio. This allows for a greater surface area in contact with the solution and enhance the electrochemical reaction taking place, which would translate into a higher sensitivity for the sensor. The novelty in this material is the one-pot hydrothermal synthesis resulting in NP with surface carboxylic acid functional groups (cBiSNP), which has not been previously described in the literature. We therefore designed an electrochemical Bi_2S_3 framework which has the potential to be further modified according to one's needs and objectives in sensing. The surface carboxylic acids can be involved in a multitude of chemical reactions to attach other molecules or recognition elements to the framework, and their presence was confirmed through FTIR (figure 4.3). The cBiSNP modified electrodes were tested through CV and showed a decrease in cathodic peak (I_{cp}, -32%) and anodic peak (I_{ap}) currents when compared to GCE. This was expected as bismuth is a lesser conductor than carbon, however this is at the advantage of higher selectivity for Zn ions. Furthermore, the cBiSNP were investigated as Zn sensor through SWSV and the following results were obtained: an LOD of 30.23 µM or 1.98 µg/mL and a linear relationship between ΔI and [Zn] in the physiologically relevant 5-50 µM range. Due to the low number of experimental repeats, the LOD is higher than would have been expected from to the calibration curves (figure 4.7), and this is due to the high standard error of the intercept. Performing more experiments would allow to reduce this error and increase the LOD. This LOD is comparable to other electrochemical Zn sensors published in the literature, and the same order of magnitude has been achieved as seen in table 2.3. A sensor based on a carbon fibre/bismuth electrode achieved an LOD of 1.0 µg/mL [147], which is similar to what was achieved in this work from the cBiSNP. However, the cBiSNP sensor's LOD is approximately 3 orders of magnitude of the most sensitive electrochemical sensor described in table 2.3, which achieved an LOD of 4.8 ng/mL using sulfonated carbon and bismuth [120]. Both of the published sensors mentioned here used a slightly different strategy where the bismuth is coupled to another more conductive material (in this case carbon) as basis for the electrocatalytic surface. Since bismuth is a poor conductor, coupling it with carbon proves an effective way to increase surface area while also retaining a high conductivity at the electrode

surface, which was not seen in the cBiSNP alone. Therefore, modifying this framework to couple it to carbon could be an interesting lead for further experiments aiming at increasing the sensitivity of this sensor.

The second aim of this thesis was to chemically modify the surface of our designed framework with amino acids to attempt to enhance its electrochemical sensing performance. L-histidine was chosen as it is reported to form complexes with Zn ions in solutions and therefore would increase sensitivity of the sensor through this affinity. From the cBiSNP framework, a cross linking reaction was performed following the well-established NHS/EDC protocol. The presence of histidine on the Bi_2S_3 NP could not be verified though physicochemical characterization techniques and further experiments should be undertaken to elucidate their composition. The newly formed BiSNP-H were investigated through CV and showed a further decrease in electrical conductivity from the I_{cp} (-68%) when compared to the GCE and 58% less than the cBiSNP. This suggests the presence of an additional layer over the cBiSNP which we hypothesize is histidine. When investigated through CV for the detection of Zn, BiSNP-H showed a slightly superior electrochemical performance than cBiSNP electrodes: peak Zn reduction I was at -510 $\mu A/cm^2$ for BiSNP-H and -415 $\mu A/cm^2$ for cBiSNP, which represents a 23% increase in I. Zn sensing through SWSV was only available as preliminary results in this work. In its current state, the BiSNP-H sensor shows a low signal-to-noise ratio, rendering performance analysis difficult at this stage (figure 4.8). Furthermore, Δcurrent density was significantly lower at every Zn concentration in SWSV for BiSNP vs cBiSNP. For these reasons, an LOD was not calculated with the data of this sensor. More experimentations would be needed to optimize the stability of the BiSNP-H coating and detection parameters in order to obtain higher quality sensing data. The hypothesis is that the histidine layer changes the electrochemical behavior of the system and the same detection parameters which were applicable to the cBiSNP sensor are not applicable to the BiSNP-H, and that given more experiments, would achieve a superior electrochemical performance in the detection of Zn, as suggested by the CV data.

The novel electrochemical sensor framework presented in this work offers an exciting potential avenue to the field of Zn sensor development with a high degree of flexibility as many parameters of the design can be configurable and optimized according to varying needs and hypotheses.

CHAPTER 5 CONCLUSION (AND RECOMMANDATIONS)

5.1 Conclusion

In conclusion, a novel sensitive Bi_2S_3 NP-based electrochemical Zn sensor was successfully developed and tested in laboratory solutions.

A highly customizable Bi_2S_3 framework with high surface-to-volume ratio was designed which allows easy chemical modifications to be applied. Electrochemical testing of the nonmodified framework through SWSV shows Zn sensing performance comparable to the current literature and an LOD of 1.98 µg/mL was achieved.

The proposed sensor contributes to research in the development of point-of-care medical devices in cancer care, which would greatly benefit patients as better and earlier monitoring of the disease has been shown to significantly improve outcomes. Clinicians would have access to an easy-to-use and rapid sensor providing information about the stage of the disease and helping in clinical decision making when further tests would be needed. The developed sensor framework is inexpensive and easier to operate than current gold standard medical laboratory technologies and offers possibilities for miniaturization.

The novel electrochemical sensor framework presented in this work offers a contribution to the field for future Zn sensor development with a high degree of flexibility as many parameters of the design can be configurable and optimized according to varying needs and hypotheses.

5.2 Limitations

- A high degree of variability exists on individual electrodes repeatedly used in laboratory settings. Furthermore, the fabrication process not being optimized represents an added source of variability in the measurements. Standardized manufacturing of single-use sensors would palliate this flaw when implemented.
- The presence of histidine on the surface of cBiSNP could not be elucidated and therefore the observed effects of histidine-modified NP could be due to another by product of the NHS/EDC cross-linking reaction.

- Due to practical issues, triplicates for electrochemical sensing measurements could not be performed. Therefore, statistical significance of the presented data cannot be established and the observed results could be due to chance alone.
- The developed sensor was only tested in controlled laboratory TBS buffer solution. The complex nature of biological fluids was not taken into account and could greatly affect the electrochemical behavior of the sensor.
- Measurements were obtained with a laboratory-grade benchtop potentiostat/galvanostat unit, which are expensive and offer greater sensitivity than portable units, which are more desirable for point-of-care implementation. Therefore, a miniaturized version of the sensor might be less applicable to the point-of-care than the work presented in this thesis.

5.3 Future Recommendations

- More physico-chemical characterizing experiments should be undertaken on both developed NP, namely cBiSNP and BiSNP-H to precisely elucidate their structure, composition and properties. Experiments such as MS, FTIR, Energy-dispersive X-ray spectroscopy and X-ray crystallography would completement the data acquired in this thesis.
- More experiments should be dedicated to optimizing the stability and deposition methods of the electrocatalytic ink used to apply the NP to the electrodes.
- More SWSV experiments should be performed after optimization of the technique and parameters to establish statistical significance in the results.
- Novel surface modifications of the developed cBiSNP framework could be investigated to developed new and more performant sensors, and possibly aiming at different analytes. Examples would be different amino acids than histidine, possibly smaller molecules which would cause less physical hinderance, or molecules which show high affinity to Zn or other ions such as peptides and biomimetic nanostructures [179].
- Future works should investigate whether the ability of the sensor to detect Zn translates into detection of Zn-dependant proteins such as MMPs and CA.

5.4 Closing Remark

I would like to close this thesis by reiterating my gratitude to my supervisor and mentor, professor Géraldine Merle, and the friends and colleagues of the Lab of Diagnostic & Therapeutic Technologies research group without whom this work would not have been possible. This study was supported by grants of the Fonds de Recherche du Québec – Santé (FRQS).

REFERENCES

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68(6) (2018) 394-424.

[2] F. Bray, A. Jemal, L.A. Torre, D. Forman, P. Vineis, Long-Term Realism and Cost-Effectiveness: Primary Prevention in Combatting Cancer and Associated Inequalities Worldwide, JNCI: Journal of the National Cancer Institute 107(12) (2015).

[3] M. Nagpal, S. Singh, P. Singh, P. Chauhan, M.A. Zaidi, Tumor markers: A diagnostic tool, Natl J Maxillofac Surg 7(1) (2016) 17-20.

[4] X. Chen, J. Gole, A. Gore, Q. He, M. Lu, J. Min, Z. Yuan, X. Yang, Y. Jiang, T. Zhang, C. Suo, X. Li, L. Cheng, Z. Zhang, H. Niu, Z. Li, Z. Xie, H. Shi, X. Zhang, M. Fan, X. Wang, Y. Yang, J. Dang, C. McConnell, J. Zhang, J. Wang, S. Yu, W. Ye, Y. Gao, K. Zhang, R. Liu, L. Jin, Non-invasive early detection of cancer four years before conventional diagnosis using a blood test, Nature Communications 11(1) (2020) 3475.

[5] O. World Health, S. International Programme on Chemical, Biomarkers in risk assessment : validity and validation, World Health Organization, Geneva, 2001.

[6] S. Nahavandi, S. Baratchi, R. Soffe, S.-Y. Tang, S. Nahavandi, A. Mitchell, K. Khoshmanesh, Microfluidic platforms for biomarker analysis, Lab on a Chip 14(9) (2014) 1496-1514.

[7] B. Hayes, C. Murphy, A. Crawley, R. O'Kennedy, Developments in Point-of-Care Diagnostic Technology for Cancer Detection, Diagnostics (Basel) 8(2) (2018) 39.

[8] S. Sharma, Tumor markers in clinical practice: General principles and guidelines, Indian J Med Paediatr Oncol 30(1) (2009) 1-8.

[9] X. Wang, K.E. Kaczor-Urbanowicz, D.T. Wong, Salivary biomarkers in cancer detection, Med Oncol 34(1) (2017) 7.

[10] S. Kumar, A. Mohan, R. Guleria, Biomarkers in cancer screening, research and detection: present and future: a review, Biomarkers 11(5) (2006) 385-405.

[11] R. Liu, X. Ye, T. Cui, Recent Progress of Biomarker Detection Sensors, Research 2020 (2020) 7949037.

[12] C. Li, Y. Yang, D. Wu, T. Li, Y. Yin, G. Li, Improvement of enzyme-linked immunosorbent assay for the multicolor detection of biomarkers, Chemical Science 7(5) (2016) 3011-3016.

[13] J. Baranwal, B. Barse, G. Gatto, G. Broncova, A. Kumar, Electrochemical Sensors and Their Applications: A Review, Chemosensors 10(9) (2022) 363.

[14] M.R. Hasan, M.S. Ahommed, M. Daizy, M.S. Bacchu, M.R. Ali, M.R. Al-Mamun, M.A. Saad Aly, M.Z.H. Khan, S.I. Hossain, Recent development in electrochemical biosensors for cancer biomarkers detection, Biosensors and Bioelectronics: X 8 (2021) 100075.

[15] C.T. Chasapis, P.-S.A. Ntoupa, C.A. Spiliopoulou, M.E. Stefanidou, Recent aspects of the effects of zinc on human health, Archives of Toxicology 94(5) (2020) 1443-1460.

[16] N. Cerdà-Costa, F.X. Gomis-Rüth, Architecture and function of metallopeptidase catalytic domains, Protein Sci 23(2) (2014) 123-144.

[17] A. Page-McCaw, A.J. Ewald, Z. Werb, Matrix metalloproteinases and the regulation of tissue remodelling, Nat Rev Mol Cell Biol 8(3) (2007) 221-33.

[18] W.C. Parks, C.L. Wilson, Y.S. López-Boado, Matrix metalloproteinases as modulators of inflammation and innate immunity, Nat Rev Immunol 4(8) (2004) 617-29.

[19] E.C. Filipe, J.L. Chitty, T.R. Cox, Charting the unexplored extracellular matrix in cancer, Int J Exp Pathol 99(2) (2018) 58-76.

[20] H. Nagase, R. Visse, G. Murphy, Structure and function of matrix metalloproteinases and TIMPs, Cardiovascular Research 69(3) (2006) 562-573.

[21] T. Klein, R. Bischoff, Physiology and pathophysiology of matrix metalloproteases, Amino Acids 41(2) (2011) 271-290.

[22] E. Gobin, K. Bagwell, J. Wagner, D. Mysona, S. Sandirasegarane, N. Smith, S. Bai, A. Sharma, R. Schleifer, J.-X. She, A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential, BMC Cancer 19(1) (2019) 581.

[23] Q. Xue, L. Cao, X.-Y. Chen, J. Zhao, L. Gao, S.-Z. Li, Z. Fei, High expression of MMP9 in glioma affects cell proliferation and is associated with patient survival rates, Oncol Lett 13(3) (2017) 1325-1330.

[24] E.J. Slapak, J. Duitman, C. Tekin, M.F. Bijlsma, C.A. Spek, Matrix Metalloproteases in Pancreatic Ductal Adenocarcinoma: Key Drivers of Disease Progression?, Biology (Basel) 9(4) (2020) 80.

[25] H. Huang, Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances, Sensors (Basel) 18(10) (2018) 3249.

[26] R. Roy, J. Yang, M.A. Moses, Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer, J Clin Oncol 27(31) (2009) 5287-5297.

[27] P. Jothimuthu, R.A. Wilson, J. Herren, X. Pei, W. Kang, R. Daniels, H. Wong, F. Beyette, W.R. Heineman, I. Papautsky, Zinc Detection in Serum by Anodic Stripping Voltammetry on Microfabricated Bismuth Electrodes, Electroanalysis 25(2) (2013) 10.1002/elan.201200530.

[28] G. March, T.D. Nguyen, B. Piro, Modified electrodes used for electrochemical detection of metal ions in environmental analysis, Biosensors (Basel) 5(2) (2015) 241-275.

[29] B. Roberts, A. Lothian, D. Hare, C. Masters, T. Ryan, R. Grimm, Metalloproteomics: principles, challenges and applications to neurodegeneration, Frontiers in Aging Neuroscience 5 (2013).

[30] B.L. Vallee, K.H. Falchuk, The biochemical basis of zinc physiology, Physiol Rev 73(1) (1993) 79-118.

[31] E. Ho, Zinc deficiency, DNA damage and cancer risk, J Nutr Biochem 15(10) (2004) 572-8.

[32] A. Kocyła, J. Adamczyk, A. Krężel, Interdependence of free zinc changes and protein complex assembly – insights into zinc signal regulation†, Metallomics 10(1) (2017) 120-131.

[33] J. Wang, H. Zhao, Z. Xu, X. Cheng, Zinc dysregulation in cancers and its potential as a therapeutic target, Cancer Biol Med 17(3) (2020) 612-625.

[34] B.J. Grattan, H.C. Freake, Zinc and cancer: implications for LIV-1 in breast cancer, Nutrients 4(7) (2012) 648-75.

[35] N.C. Lim, H.C. Freake, C. Brückner, Illuminating Zinc in Biological Systems, Chemistry – A European Journal 11(1) (2005) 38-49.

[36] H. Yokokawa, H. Fukuda, M. Saita, T. Miyagami, Y. Takahashi, T. Hisaoka, T. Naito, Serum zinc concentrations and characteristics of zinc deficiency/marginal deficiency among Japanese subjects, J Gen Fam Med 21(6) (2020) 248-255.

[37] L.C. Costello, R.B. Franklin, Zinc is decreased in prostate cancer: an established relationship of prostate cancer!, J Biol Inorg Chem 16(1) (2011) 3-8.

[38] A. Banas, W.M. Kwiatek, K. Banas, M. Gajda, B. Pawlicki, T. Cichocki, Correlation of concentrations of selected trace elements with Gleason grade of prostate tissues, JBIC Journal of Biological Inorganic Chemistry 15(7) (2010) 1147-1155.

[39] A.M. Banas, K. Banas, Response to commentary "Zinc is decreased in prostate cancer: an established relationship of prostate cancer!", JBIC Journal of Biological Inorganic Chemistry 16(1) (2011) 9-13.

[40] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA: A Cancer Journal for Clinicians 71(3) (2021) 209-249.

[41] L. Jouybari, F. Kiani, A. Akbari, A. Sanagoo, F. Sayehmiri, J. Aaseth, M.S. Chartrand, K. Sayehmiri, S. Chirumbolo, G. Bjørklund, A meta-analysis of zinc levels in breast cancer, J Trace Elem Med Biol 56 (2019) 90-99.

[42] P. Christudoss, R. Selvakumar, J.J. Fleming, G. Mathew, Zinc levels in paired normal and malignant human stomach and colon tissue, Biomedical Res 21 (2010) 445-50.

[43] A. Boz, O. Evliyaoğlu, M. Yildirim, N. Erkan, B. Karaca, The value of serum zinc, copper, ceruloplasmin levels in patients with gastrointestinal tract cancers, Turk J Gastroenterol 16(2) (2005) 81-4.

[44] B.K. Ayinampudi, M. Narsimhan, Salivary copper and zinc levels in oral pre-malignant and malignant lesions, J Oral Maxillofac Pathol 16(2) (2012) 178-82.

[45] A.K. Kudva, S.V. Raghu, P.K. Achar, S. Rao, S. Suresh, M. Shrinath Baliga, Study of Serum Zinc and Copper Levels and Tumor Pathology: A Pilot Study in People Affected with Head and Neck Cancers, Indian Journal of Otolaryngology and Head & Neck Surgery 74(3) (2022) 6007-6015.

[46] L.C. Costello, R.B. Franklin, The status of zinc in the development of hepatocellular cancer: an important, but neglected, clinically established relationship, Cancer Biol Ther 15(4) (2014) 353-60.

[47] J. Yang, Y. Zhang, X. Cui, W. Yao, X. Yu, P. Cen, S.E. Hodges, W.E. Fisher, F.C. Brunicardi, C. Chen, Q. Yao, M. Li, Gene profile identifies zinc transporters differentially expressed in normal human organs and human pancreatic cancer, Curr Mol Med 13(3) (2013) 401-9.

[48] N. Karunasinghe, Zinc in Prostate Health and Disease: A Mini Review, Biomedicines 10(12) (2022).

[49] M.D. Sternlicht, Z. Werb, How matrix metalloproteinases regulate cell behavior, Annu Rev Cell Dev Biol 17 (2001) 463-516.

[50] K. Kessenbrock, V. Plaks, Z. Werb, Matrix metalloproteinases: regulators of the tumor microenvironment, Cell 141(1) (2010) 52-67.

[51] S. Quintero-Fabián, R. Arreola, E. Becerril-Villanueva, J.C. Torres-Romero, V. Arana-Argáez, J. Lara-Riegos, M.A. Ramírez-Camacho, M.E. Alvarez-Sánchez, Role of Matrix Metalloproteinases in Angiogenesis and Cancer, Frontiers in Oncology 9 (2019).

[52] D.E. Kleiner, W.G. Stetler-Stevenson, Matrix metalloproteinases and metastasis, Cancer Chemother Pharmacol 43 Suppl (1999) S42-51.

[53] K. Augoff, A. Hryniewicz-Jankowska, R. Tabola, K. Stach, MMP9: A Tough Target for Targeted Therapy for Cancer, Cancers (Basel) 14(7) (2022).

[54] P. Cieplak, A.Y. Strongin, Matrix metalloproteinases - From the cleavage data to the prediction tools and beyond, Biochim Biophys Acta Mol Cell Res 1864(11 Pt A) (2017) 1952-1963.

[55] R. Patil, A. Mahajan, G.L. Pradeep, N. Prakash, S. Patil, S.M. Khan, Expression of matrix metalloproteinase-9 in histological grades of oral squamous cell carcinoma: An immunohistochemical study, J Oral Maxillofac Pathol 25(2) (2021) 239-246.

[56] J.F. Liu, P.C. Chen, T.M. Chang, C.H. Hou, Thrombospondin-2 stimulates MMP-9 production and promotes osteosarcoma metastasis via the PLC, PKC, c-Src and NF-κB activation, J Cell Mol Med 24(21) (2020) 12826-12839.

[57] Z. Yao, T. Yuan, H. Wang, S. Yao, Y. Zhao, Y. Liu, S. Jin, J. Chu, Y. Xu, W. Zhou, S. Yang, Y. Liu, MMP-2 together with MMP-9 overexpression correlated with lymph node metastasis and poor prognosis in early gastric carcinoma, Tumour Biol 39(6) (2017) 1010428317700411.

[58] H. Jiang, H. Li, Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis, BMC Cancer 21(1) (2021) 149.

[59] J.N. Mandrekar, Receiver Operating Characteristic Curve in Diagnostic Test Assessment, Journal of Thoracic Oncology 5(9) (2010) 1315-1316.

[60] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nature Reviews Drug Discovery 7(2) (2008) 168-181.

[61] A. Ibrahim-Hashim, V. Estrella, Acidosis and cancer: from mechanism to neutralization, Cancer Metastasis Rev 38(1-2) (2019) 149-155.

[62] S. Zamanova, A.M. Shabana, U.K. Mondal, M.A. Ilies, Carbonic anhydrases as disease markers, Expert Opin Ther Pat 29(7) (2019) 509-533.

[63] C.P. Potter, A.L. Harris, Diagnostic, prognostic and therapeutic implications of carbonic anhydrases in cancer, Br J Cancer 89(1) (2003) 2-7.

[64] M. Si, J. Lang, The roles of metallothioneins in carcinogenesis, Journal of Hematology & Oncology 11(1) (2018) 107.

[65] M. Ryvolova, S. Krizkova, V. Adam, M. Beklova, L. Trnkova, J. Hubalek, R. Kizek, Analytical methods for metallothionein detection, Current Analytical Chemistry 7(3) (2011) 243-261.

[66] N. Thirumoorthy, A. Shyam Sunder, K.T. Manisenthil Kumar, M. Senthil kumar, G.N.K. Ganesh, M. Chatterjee, A Review of Metallothionein Isoforms and their Role in Pathophysiology, World Journal of Surgical Oncology 9(1) (2011) 54.

[67] M. Cassandri, A. Smirnov, F. Novelli, C. Pitolli, M. Agostini, M. Malewicz, G. Melino, G. Raschellà, Zinc-finger proteins in health and disease, Cell Death Discovery 3(1) (2017) 17071.

[68] J.H. Laity, B.M. Lee, P.E. Wright, Zinc finger proteins: new insights into structural and functional diversity, Curr Opin Struct Biol 11(1) (2001) 39-46.

[69] J. Jen, Y.-C. Wang, Zinc finger proteins in cancer progression, Journal of Biomedical Science 23(1) (2016) 53.

[70] C.S. Fang, K.-S. Kim, B. Yu, S. Jon, M.-S. Kim, H. Yang, Ultrasensitive Electrochemical Detection of miRNA-21 Using a Zinc Finger Protein Specific to DNA-RNA Hybrids, Anal Chem 89(3) (2017) 2024-2031.

[71] J. Lee, A. Tatsumi, K. Tsukakoshi, E.D. Wilson, K. Abe, K. Sode, K. Ikebukuro, Application of a Glucose Dehydrogenase-Fused with Zinc Finger Protein to Label DNA Aptamers for the Electrochemical Detection of VEGF, Sensors 20(14) (2020) 3878.

[72] R. Donato, B.R. Cannon, G. Sorci, F. Riuzzi, K. Hsu, D.J. Weber, C.L. Geczy, Functions of S100 proteins, Curr Mol Med 13(1) (2013) 24-57.

[73] O.V. Moroz, K.S. Wilson, I.B. Bronstein, The role of zinc in the S100 proteins: insights from the X-ray structures, Amino Acids 41(4) (2011) 761-772.

[74] A.R. Bresnick, D.J. Weber, D.B. Zimmer, S100 proteins in cancer, Nat Rev Cancer 15(2) (2015) 96-109.

[75] S.S. Cross, F.C. Hamdy, J.C. Deloulme, I. Rehman, Expression of S100 proteins in normal human tissues and common cancers using tissue microarrays: S100A6, S100A8, S100A9 and S100A11 are all overexpressed in common cancers, Histopathology 46(3) (2005) 256-69.

[76] D. Crosby, S. Bhatia, K.M. Brindle, L.M. Coussens, C. Dive, M. Emberton, S. Esener, R.C. Fitzgerald, S.S. Gambhir, P. Kuhn, T.R. Rebbeck, S. Balasubramanian, Early detection of cancer, Science 375(6586) (2022) eaay9040.

[77] V.S.P.K.Sankara A. Jayanthi, A.B. Das, U. Saxena, Recent advances in biosensor development for the detection of cancer biomarkers, Biosensors and Bioelectronics 91 (2017) 15-23.

[78] D.A. Armbruster, T. Pry, Limit of blank, limit of detection and limit of quantitation, Clin Biochem Rev 29 Suppl 1(Suppl 1) (2008) S49-52.

[79] P. Rusch, A.V. Hirner, O. Schmitz, R. Kimmig, O. Hoffmann, M. Diel, Zinc distribution within breast cancer tissue of different intrinsic subtypes, Arch Gynecol Obstet 303(1) (2021) 195-205.

[80] J. Gumulec, M. Masarik, V. Adam, T. Eckschlager, I. Provaznik, R. Kizek, Serum and Tissue Zinc in Epithelial Malignancies: A Meta-Analysis, PLOS ONE 9(6) (2014) e99790.

[81] R. Parikh, A. Mathai, S. Parikh, G. Chandra Sekhar, R. Thomas, Understanding and using sensitivity, specificity and predictive values, Indian J Ophthalmol 56(1) (2008) 45-50.

[82] N. Bhalla, P. Jolly, N. Formisano, P. Estrela, Introduction to biosensors, Essays Biochem 60(1) (2016) 1-8.

[83] A. Han, M.P. Andre, L. Deiranieh, E. Housman, J.W. Erdman, Jr., R. Loomba, C.B. Sirlin, W.D. O'Brien, Jr., Repeatability and Reproducibility of the Ultrasonic Attenuation Coefficient and Backscatter Coefficient Measured in the Right Lobe of the Liver in Adults With Known or Suspected Nonalcoholic Fatty Liver Disease, J Ultrasound Med 37(8) (2018) 1913-1927.

[84] A. Kaushik, M.A. Mujawar, Point of Care Sensing Devices: Better Care for Everyone, Sensors (Basel) 18(12) (2018).

[85] P.L. Urban, Quantitative mass spectrometry: an overview, Philos Trans A Math Phys Eng Sci 374(2079) (2016).

[86] X. Zhou, W. Zhang, Z. Ouyang, Recent advances in on-site mass spectrometry analysis for clinical applications, Trends Analyt Chem 149 (2022) 116548.

[87] B.J. Bolann, R. Rahil-Khazen, H. Henriksen, R. Isrenn, R.J. Ulvik, Evaluation of methods for trace-element determination with emphasis on their usability in the clinical routine laboratory, Scandinavian Journal of Clinical and Laboratory Investigation 67(4) (2007) 353-366.

[88] H. Tavallali, D. Abdardideh, M. Aalaei, S. Zahmatkesh, New Application of Chemically Modified Multiwalled Carbon Nanotubes with Thiosemicarbazide as a Sorbent for Separation and Preconcentration of Trace Amounts of Co(II), Cd(II), Cu(II), and Zn(II) in Environmental and Biological Samples Prior to Determination by Flame Atomic Absorption Spectrometry, Journal of the Chinese Chemical Society 59(1) (2012) 114-121.

[89] C.A. Crutchfield, S.N. Thomas, L.J. Sokoll, D.W. Chan, Advances in mass spectrometry-based clinical biomarker discovery, Clinical Proteomics 13(1) (2016) 1.

[90] E.M. Mahabee-Gittens, M.J. Mazzella, J.T. Doucette, A.L. Merianos, L. Stone, C.A. Wullenweber, A.B. S, G.E. Matt, Comparison of Liquid Chromatography Mass Spectrometry and Enzyme-Linked Immunosorbent Assay Methods to Measure Salivary Cotinine Levels in Ill Children, Int J Environ Res Public Health 17(4) (2020).

[91] B.V. Chikkaveeraiah, A.A. Bhirde, N.Y. Morgan, H.S. Eden, X. Chen, Electrochemical immunosensors for detection of cancer protein biomarkers, ACS Nano 6(8) (2012) 6546-61.

[92] H.G. Gika, G.A. Theodoridis, R.S. Plumb, I.D. Wilson, Current practice of liquid chromatography–mass spectrometry in metabolomics and metabonomics, Journal of Pharmaceutical and Biomedical Analysis 87 (2014) 12-25.

[93] S. Banerjee, Empowering Clinical Diagnostics with Mass Spectrometry, ACS Omega 5(5) (2020) 2041-2048.

[94] I.A. Darwish, Immunoassay Methods and their Applications in Pharmaceutical Analysis: Basic Methodology and Recent Advances, Int J Biomed Sci 2(3) (2006) 217-35.

[95] S.K. Vashist, J.H.T. Luong, Chapter 5 - Enzyme-Linked Immunoassays, in: S.K. Vashist, J.H.T. Luong (Eds.), Handbook of Immunoassay Technologies, Academic Press2018, pp. 97-127.

[96] S. Aydin, A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA, Peptides 72 (2015) 4-15.

[97] A. Thiha, F. Ibrahim, A Colorimetric Enzyme-Linked Immunosorbent Assay (ELISA) Detection Platform for a Point-of-Care Dengue Detection System on a Lab-on-Compact-Disc, Sensors (Basel) 15(5) (2015) 11431-41.

[98] D.S. Kinnamon, J.T. Heggestad, J. Liu, A. Chilkoti, Technologies for Frugal and Sensitive Point-of-Care Immunoassays, Annu Rev Anal Chem (Palo Alto Calif) 15(1) (2022) 123-149.

[99] K.W. Kahl, J.Z. Seither, L.J. Reidy, LC-MS-MS vs ELISA: Validation of a Comprehensive Urine Toxicology Screen by LC-MS-MS and a Comparison of 100 Forensic Specimens, Journal of Analytical Toxicology 43(9) (2019) 734-745.

[100] J.F. Rusling, C.V. Kumar, J.S. Gutkind, V. Patel, Measurement of biomarker proteins for point-of-care early detection and monitoring of cancer, Analyst 135(10) (2010) 2496-2511.

[101] T.G. Cross, M.P. Hornshaw, Can LC and LC-MS ever replace immunoassays?, Journal of Applied Bioanalysis 2(4) (2016) 936.

[102] S. Akgönüllü, A. Denizli, Recent advances in optical biosensing approaches for biomarkers detection, Biosensors and Bioelectronics: X 12 (2022) 100269.

[103] F.E. AHMED, J.E. WILEY, D.A. WEIDNER, C. BONNERUP, H. MOTA, Surface Plasmon Resonance (SPR) Spectrometry as a Tool to Analyze Nucleic Acid–Protein Interactions in Crude Cellular Extracts, Cancer Genomics - Proteomics 7(6) (2010) 303-309.

[104] S. Yadav, A. Parihar, M.A. Sadique, P. Ranjan, N. Kumar, A. Singhal, R. Khan, Emerging Point-of-Care Optical Biosensing Technologies for Diagnostics of Microbial Infections, ACS Applied Optical Materials (2023).

[105] B. Kaur, S. Kumar, B.K. Kaushik, Recent advancements in optical biosensors for cancer detection, Biosensors and Bioelectronics 197 (2022) 113805.

[106] H.H. Nguyen, J. Park, S. Kang, M. Kim, Surface plasmon resonance: a versatile technique for biosensor applications, Sensors (Basel) 15(5) (2015) 10481-510.

[107] B.A. Patel, Chapter 1 - Introduction to electrochemistry for bioanalysis, in: B. Patel (Ed.), Electrochemistry for Bioanalysis, Elsevier2020, pp. 1-8.

[108] N. Elgrishi, K.J. Rountree, B.D. McCarthy, E.S. Rountree, T.T. Eisenhart, J.L. Dempsey, A Practical Beginner's Guide to Cyclic Voltammetry, Journal of Chemical Education 95(2) (2018) 197-206.

[109] F.R. Simões, M.G. Xavier, 6 - Electrochemical Sensors, in: A.L. Da Róz, M. Ferreira, F. de Lima Leite, O.N. Oliveira (Eds.), Nanoscience and its Applications, William Andrew Publishing2017, pp. 155-178.

[110] M. Choudhary, K. Arora, Chapter 8 - Electrochemical biosensors for early detection of cancer, in: R. Khan, A. Parihar, S.K. Sanghi (Eds.), Biosensor Based Advanced Cancer Diagnostics, Academic Press2022, pp. 123-151.

[111] G. Hanrahan, D.G. Patil, J. Wang, Electrochemical sensors for environmental monitoring: design, development and applications, Journal of Environmental Monitoring 6(8) (2004) 657-664.

[112] A. Curulli, Electrochemical Biosensors in Food Safety: Challenges and Perspectives, Molecules 26(10) (2021).

[113] A. Heller, B. Feldman, Electrochemical Glucose Sensors and Their Applications in Diabetes Management, Chemical Reviews 108(7) (2008) 2482-2505.

[114] A.A. Karbelkar, A.L. Furst, Electrochemical Diagnostics for Bacterial Infectious Diseases, ACS Infectious Diseases 6(7) (2020) 1567-1571.

[115] A. Ledo, E. Fernandes, J.E. Quintero, G.A. Gerhardt, R.M. Barbosa, Electrochemical Evaluation of a Multi-Site Clinical Depth Recording Electrode for Monitoring Cerebral Tissue Oxygen, Micromachines (Basel) 11(7) (2020).

[116] F. Cui, Z. Zhou, H.S. Zhou, Review—Measurement and Analysis of Cancer Biomarkers Based on Electrochemical Biosensors, Journal of The Electrochemical Society 167(3) (2020) 037525.

[117] N. Wongkaew, M. Simsek, C. Griesche, A.J. Baeumner, Functional Nanomaterials and Nanostructures Enhancing Electrochemical Biosensors and Lab-on-a-Chip Performances: Recent Progress, Applications, and Future Perspective, Chem Rev 119(1) (2019) 120-194.

[118] T. Zheng, J. Li, Y. Ji, W. Zhang, Y. Fang, F. Xin, W. Dong, P. Wei, J. Ma, M. Jiang, Progress and Prospects of Bioelectrochemical Systems: Electron Transfer and Its Applications in the Microbial Metabolism, Frontiers in Bioengineering and Biotechnology 8 (2020).

[119] K. Beaver, A. Dantanarayana, S.D. Minteer, Materials Approaches for Improving Electrochemical Sensor Performance, The Journal of Physical Chemistry B 125(43) (2021) 11820-11834.

[120] D. Vieira, E. Harvey, G. Merle, Stripping metalloprotein with bismuth nanomaterials tethered on carbon surface, Applied Surface Science 635 (2023) 157636.

[121] X. Dong, C. Zhang, X. Du, Z. Zhang, Recent Advances of Nanomaterials-Based Molecularly Imprinted Electrochemical Sensors, Nanomaterials 12(11) (2022) 1913.

[122] B.T.P. Quynh, J.Y. Byun, S.H. Kim, Electrochemical Behavior of Aromatic Compounds on Nanoporous Gold Electrode, Journal of The Electrochemical Society 165(10) (2018) B414-B421.

[123] C.G. Zoski, Handbook of electrochemistry Elsevier, Amsterdam, The Netherlands, 2006.

[124] A. Cirocka, D. Zarzeczańska, A. Wcisło, Good Choice of Electrode Material as the Key to Creating Electrochemical Sensors—Characteristics of Carbon Materials and Transparent Conductive Oxides (TCO), Materials 14(16) (2021).

[125] R. Porada, K. Jedlińska, J. Lipińska, B. Baś, Review—Voltammetric Sensors with Laterally Placed Working Electrodes: A Review, Journal of The Electrochemical Society 167(3) (2020) 037536.

[126] L.d.S. Vieira, A review on the use of glassy carbon in advanced technological applications, Carbon 186 (2022) 282-302.

[127] P. Westbroek, 1 - Fundamentals of electrochemistry, in: P. Westbroek, G. Priniotakis, P. Kiekens (Eds.), Analytical Electrochemistry in Textiles, Woodhead Publishing2005, pp. 3-36.

[128] R. Chen, C. Yang, W. Cai, H.-Y. Wang, J. Miao, L. Zhang, S. Chen, B. Liu, Use of Platinum as the Counter Electrode to Study the Activity of Nonprecious Metal Catalysts for the Hydrogen Evolution Reaction, ACS Energy Letters 2(5) (2017) 1070-1075.

[129] M. Bogdan, D. Brugger, W. Rosenstiel, B. Speiser, Estimation of diffusion coefficients from voltammetric signals by support vector and gaussian process regression, Journal of Cheminformatics 6(1) (2014) 30.

[130] X.-b. Wu, Z.-l. Zhu, H. Kong, Y.-q. Fan, S.-w. Cheng, Z.-s. Hua, Electrochemical reduction mechanism of Zn2+ in molten NaCl−KCl eutectic, Transactions of Nonferrous Metals Society of China 32(9) (2022) 3088-3098.

[131] N.M. Thanh, N.D. Luyen, T. Thanh Tam Toan, N. Hai Phong, N. Van Hop, Voltammetry Determination of Pb(II), Cd(II), and Zn(II) at Bismuth Film Electrode Combined with 8-Hydroxyquinoline as a Complexing Agent, Journal of Analytical Methods in Chemistry 2019 (2019) 4593135.

[132] T. Wang, W. Yue, Carbon Nanotubes Heavy Metal Detection with Stripping Voltammetry: A Review Paper, Electroanalysis 29(10) (2017) 2178-2189.

[133] N.W. Khun, E. Liu, Linear sweep anodic stripping voltammetry of heavy metals from nitrogen doped tetrahedral amorphous carbon thin films, Electrochimica Acta 54(10) (2009) 2890-2898.

[134] A.J. Borrill, N.E. Reily, J.V. Macpherson, Addressing the practicalities of anodic stripping voltammetry for heavy metal detection: a tutorial review, Analyst 144(23) (2019) 6834-6849.

[135] V. Mirceski, R. Gulaboski, M. Lovric, I. Bogeski, R. Kappl, M. Hoth, Square-Wave Voltammetry: A Review on the Recent Progress, Electroanalysis 25(11) (2013) 2411-2422.

[136] G. Hussain, D.S. Silvester, Comparison of Voltammetric Techniques for Ammonia Sensing in Ionic Liquids, Electroanalysis 30(1) (2018) 75-83.

[137] J.P. Metters, C.E. Banks, 3 - Nanoparticle modified electrodes for trace metal ion analysis, in: K.C. Honeychurch (Ed.), Nanosensors for Chemical and Biological Applications, Woodhead Publishing2014, pp. 54-79.

[138] C. Li, X. Chen, N. Wang, B. Zhang, An ultrasensitive and label-free electrochemical DNA biosensor for detection of DNase I activity, RSC Advances 7(35) (2017) 21666-21670.

[139] S.N. Topkaya, M. Azimzadeh, M. Ozsoz, Electrochemical Biosensors for Cancer Biomarkers Detection: Recent Advances and Challenges, Electroanalysis 28(7) (2016) 1402-1419.

[140] H.S. Magar, R.Y.A. Hassan, A. Mulchandani, Electrochemical Impedance Spectroscopy (EIS): Principles, Construction, and Biosensing Applications, Sensors (Basel) 21(19) (2021).

[141] A. Biela, M. Watkinson, U.C. Meier, D. Baker, G. Giovannoni, C.R. Becer, S. Krause, Disposable MMP-9 sensor based on the degradation of peptide cross-linked hydrogel films using electrochemical impedance spectroscopy, Biosensors and Bioelectronics 68 (2015) 660-667.

[142] A. Bogomolova, E. Komarova, K. Reber, T. Gerasimov, O. Yavuz, S. Bhatt, M. Aldissi, Challenges of electrochemical impedance spectroscopy in protein biosensing, Anal Chem 81(10) (2009) 3944-9.

[143] G. Xu, P. Song, L. Xia, Examples in the detection of heavy metal ions based on surface-enhanced Raman scattering spectroscopy, Nanophotonics 10(18) (2021) 4419-4445.

[144] D. Zhao, X. Guo, T. Wang, N. Alvarez, V.N. Shanov, W.R. Heineman, Simultaneous Detection of Heavy Metals by Anodic Stripping Voltammetry Using Carbon Nanotube Thread, Electroanalysis 26(3) (2014) 488-496.

[145] Y. Zheng, Z. Ma, Dual-reaction triggered sensitivity amplification for ultrasensitive peptide-cleavage based electrochemical detection of matrix metalloproteinase-7, Biosensors and Bioelectronics 108 (2018) 46-52.

[146] J. Kim, W.R. de Araujo, I.A. Samek, A.J. Bandodkar, W. Jia, B. Brunetti, T.R.L.C. Paixão, J. Wang, Wearable temporary tattoo sensor for real-time trace metal monitoring in human sweat, Electrochemistry Communications 51 (2015) 41-45.

[147] W.-J. Wang, Y.-L. Cai, B.-C. Li, J. Zeng, Z.-Y. Huang, X.-M. Chen, A voltammetric sensor for simultaneous determination of lead, cadmium and zinc on an activated carbon fiber rod, Chinese Chemical Letters 29(1) (2018) 111-114.

[148] M. Khairy, R.O. Kadara, D.K. Kampouris, C.E. Banks, Disposable Bismuth Oxide Screen Printed Electrodes for the Sensing of Zinc in Seawater, Electroanalysis 22(13) (2010) 1455-1459.

[149] G. Ringgit, S. Siddiquee, S. Saallah, M.T. Mohamad Lal, A sensitive and rapid determination of zinc ion (Zn2+) using electrochemical sensor based on f-MWCNTs/CS/PB/AuE in drinking water, Scientific Reports 12(1) (2022) 18582.

[150] R. Azadbakht, M. Koolivand, J. Khanabadi, A new fluorescence chemosensor for Zn2+ with a remarkable red shift in emission spectra, Analytical Methods 9(32) (2017) 4688-4694.

[151] Y.W. Fen, W.M. Yunus, Z.A. Talib, N.A. Yusof, Development of surface plasmon resonance sensor for determining zinc ion using novel active nanolayers as probe, Spectrochim Acta A Mol Biomol Spectrosc 134 (2015) 48-52.

[152] W.M.E.M.M. Daniyal, Y.W. Fen, N.A.A. Anas, N.A.S. Omar, N.S.M. Ramdzan, H. Nakajima, M.A. Mahdi, Enhancing the sensitivity of a surface plasmon resonance-based optical sensor for zinc ion detection by the modification of a gold thin film, RSC Advances 9(71) (2019) 41729-41736.

[153] Y. Teng, Z. Ren, Y. Zhang, Z. Wang, Z. Pan, K. Shao, Y. She, Determination of prostate cancer marker Zn2+ with a highly selective surface-enhanced Raman scattering probe on liquid–liquid self-assembled Au nanoarrays, Talanta 209 (2020) 120569.

[154] H. Zhuang, Z. Wang, X. Zhang, J.A. Hutchison, W. Zhu, Z. Yao, Y. Zhao, M. Li, A highly sensitive SERS-based platform for Zn(ii) detection in cellular media, Chemical Communications 53(11) (2017) 1797-1800.

[155] T. Jiang, X. Xiong, S. Wang, Y. Luo, Q. Fei, A. Yu, Z. Zhu, Direct Mass Spectrometric Analysis of Zinc and Cadmium in Water by Microwave Plasma Torch Coupled with a Linear Ion Trap Mass Spectrometer, International Journal of Mass Spectrometry 399-400 (2016) 33-39.

[156] G. Yang, L. Li, R.K. Rana, J.-J. Zhu, Assembled gold nanoparticles on nitrogen-doped graphene for ultrasensitive electrochemical detection of matrix metalloproteinase-2, Carbon 61 (2013) 357-366.

[157] A. Moradi, H. Adibi, V. Akbari, A.R. Jalalvand, Developing a novel amperometric method for biosensing of carbonic anhydrase II based on conventional and multi-way chemometric analyses of its inhibition by acetazolamide, dorzolamide and methazolamide, Sensing and Bio-Sensing Research 37 (2022) 100516.

[158] G. Ruiz-Vega, A. García-Robaina, M. Ben Ismail, H. Pasamar, T. García-Berrocoso, J. Montaner, M. Zourob, A. Othmane, F.J. del Campo, E. Baldrich, Detection of plasma MMP-9 within minutes. Unveiling some of the clues to develop fast and simple electrochemical magneto-immunosensors, Biosensors and Bioelectronics 115 (2018) 45-52.

[159] S. Mohseni, T.T. Moghadam, B. Dabirmanesh, S. Jabbari, K. Khajeh, Development of a label-free SPR sensor for detection of matrixmetalloproteinase-9 by antibody immobilization on carboxymethyldextran chip, Biosensors and Bioelectronics 81 (2016) 510-516.

[160] D. Yu, P. Lai, T. Yan, K. Fang, L. Chen, S. Zhang, Quantifying the Matrix Metalloproteinase 2 (MMP2) Spatially in Tissues by Probe via MALDI Imaging Mass Spectrometry, Frontiers in Chemistry 9 (2021).

[161] P. Kotnik, M.K. Krajnc, A. Pahor, M. Finšgar, Ž. Knez, HPLC–MS/MS method optimisation for matrix metalloproteinase 3 and matrix metalloproteinase 9 determination in human blood serum using target analysis, Journal of Pharmaceutical and Biomedical Analysis 150 (2018) 137-143.

[162] D.V. Shepherd, J.H. Shepherd, S. Ghose, S.J. Kew, R.E. Cameron, S.M. Best, The process of EDC-NHS Cross-linking of reconstituted collagen fibres increases collagen fibrillar order and alignment, APL Mater 3(1) (2015).

[163] D.C. Clark, G.T. Cheek, Electrochemical Studies of L-Histidine, ECS Transactions 85(13) (2018) 1123.

[164] Y. Hayashi, Pot economy and one-pot synthesis, Chemical Science 7(2) (2016) 866-880.

[165] M. Salavati-Niasari, Z. Behfard, O. Amiri, E. Khosravifard, S.M. Hosseinpour-Mashkani, Hydrothermal Synthesis of Bismuth Sulfide (Bi2S3) Nanorods: Bismuth(III) Monosalicylate Precursor in the Presence of Thioglycolic Acid, Journal of Cluster Science 24(1) (2013) 349-363.

[166] M. Salavati-Niasari, D. Ghanbari, F. Davar, Synthesis of different morphologies of bismuth sulfide nanostructures via hydrothermal process in the presence of thioglycolic acid, Journal of Alloys and Compounds 488(1) (2009) 442-447.

[167] H. Wang, Y. Sun, W. Yue, Q. Kang, H. Li, D. Shen, A smartphone-based double-channel fluorescence setup for immunoassay of a carcinoembryonic antigen using CuS nanoparticles for signal amplification, Analyst 143(7) (2018) 1670-1678.

[168] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D.J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, Fiji: an open-source platform for biological-image analysis, Nature Methods 9(7) (2012) 676-682.

[169] M.A. Gómez, A.J. Navarro, J.J. Giner-Casares, M. Cano, A.J. Fernández-Romero, J.J. López-Cascales, Electrodes based on nafion and epoxy-graphene composites for improving the performance and durability of open cathode fuel cells, prepared by electrospray deposition, International Journal of Hydrogen Energy 47(29) (2022) 13980-13989.

[170] M. Riahi, C. Martínez-Tomás, S. Agouram, A. Boukhachem, H. Maghraoui-Meherzi, The effects of thermal treatment on structural, morphological and optical properties of electrochemically deposited Bi2S3 thin films, Thin Solid Films 626 (2017) 9-16.

[171] S. Subramanian, P. Chithra lekha, D. Pathinettam Padiyan, Inclusion of polyaniline in electrodeposited bismuth sulphide thin films: Synthesis and characterization, Current Applied Physics 9(5) (2009) 1140-1145.

[172] J. Arumugam, A. Dhayal Raj, A. Albert Irudayaraj, Solvent effects on the properties of Bi2S3 nanoparticles: photocatalytic application, Journal of Materials Science: Materials in Electronics 28(4) (2017) 3487-3494.

[173] J.-J. Max, C. Chapados, Infrared Spectroscopy of Aqueous Carboxylic Acids: Comparison between Different Acids and Their Salts, The Journal of Physical Chemistry A 108(16) (2004) 3324-3337.

[174] Y. Astuti, S. Hartinah, A. Darmawan, H. Widiyandari, Synthesis and characterization of bismuth oxide/commercial activated carbon composite for battery anode, Open Chemistry 20(1) (2022) 1476-1484.

[175] J. Recatala-Gomez, H.K. Ng, P. Kumar, A. Suwardi, M. Zheng, M. Asbahi, S. Tripathy, I. Nandhakumar, M.S.M. Saifullah, K. Hippalgaonkar, Thermoelectric Properties of Substoichiometric Electron Beam Patterned Bismuth Sulfide, ACS Applied Materials & Interfaces 12(30) (2020) 33647-33655.

[176] S. Nimbalkar, E. Fuhrer, P. Silva, T. Nguyen, M. Sereno, S. Kassegne, J. Korvink, Glassy carbon microelectrodes minimize induced voltages, mechanical vibrations, and artifacts in magnetic resonance imaging, Microsystems & Nanoengineering 5(1) (2019) 61.

[177] S.B. Hočevar, B. Ogorevc, J. Wang, B. Pihlar, A Study on Operational Parameters for Advanced Use of Bismuth Film Electrode in Anodic Stripping Voltammetry, Electroanalysis 14(24) (2002) 1707-1712.

[178] T. Romih, S.B. Hočevar, V. Kononenko, D. Drobne, The application of bismuth film electrode for measuring Zn(II) under less acidic conditions in the presence of cell culture medium and ZnO nanoparticles, Sensors and Actuators B: Chemical 238 (2017) 1277-1282.

[179] B.-C. Lee, T.K. Chu, K.A. Dill, R.N. Zuckermann, Biomimetic Nanostructures: Creating a High-Affinity Zinc-Binding Site in a Folded Nonbiological Polymer, Journal of the American Chemical Society 130(27) (2008) 8847-8855.