Fabrication and characterization of barium strontium titanate film based biosensor

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FABRICATION AND CHARACTERIZATION OF BARIUM STRONTIUM TITANATE FILM BASED BIOSENSOR

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SUMMARY

In this project, biosensors based on a sol-gel derived Barium Strontium Titanate (BST) composite film are studied. Poly(TMS-*r*-NHS) [TMS: 3-(trimethoxysilyl)propyl methacrylate; NHS: N-hydroxysuccinimide] is used to modify the BST surface for protein immobilization. Two types of biosensors were developed and investigated. First, Bovine Serum Albumin (BSA) was immobilized on the poly(TMS-*r*-NHS) followed by anti-BSA. On the second type of biosensors, cloned Epstein-Barr virus (EBV) was immobilized on the poly(TMS-*r*-NHS) followed by anti-EBV. Significant impedance increments were observed on both biosensors. These impedance increments were attributed to three factors. Firstly, the BSA/anti-BSA molecules and the EBV/anti-EBV molecules that immobilized on the respective BST surfaces prevent the water molecules from contacting the BST grains and induce less water molecules to dissociate into mobile ions given that BST is a highly polar material and susceptible to humidity. When polar water molecules contact the BST grain surfaces, they dissociate into H^+ and OH^- which contribute to the charge transfer between the two electrodes when an electrical field is applied. Secondly, the immobilized BSA/anti-BSA molecules and the EBV/anti-EBV molecules act as obstacle barriers to the charge transfer between the solid/liquid interfaces. Thirdly, the immobilized BSA/anti-BSA molecules and the EBV/anti-EBV molecules block the sample's surface and thus preventing DI water from penetrating into BST film. Consequently the impedance increases due to the less penetrated DI water and hence higher bulk BST impedance. Good linear relationship between impedance change and logarithm of anti-BSA concentration in a wide range is obtained.

Summary

Two types of BST composite films were also studied to investigate the effect of film morphologies on the device sensitivity. The composite films based on the original commercially purchased BST powder have higher film porosities as compared to the composite films based on the high-energy ball-milled BST powder. Higher porosity composite films provide larger specific area that provides more functional groups for protein immobilizations and in turns leads to higher device sensitivity.

Comparing to the conventional electrochemical biosensors, our present sensor based on BST composite films is promising as it can offer higher device sensitivity without the need for the liquid electrolyte probe. The sensor surface modification by poly(TMS-r-NHS) also demonstrates the capability of immobilizing different proteins onto the device. This shows that the biosensors structure is suitable to be used in determining a wide range of antibody or antigen concentrations.

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CHAPTER ONE

INTRODUCTION

1.1 Motivation

Biosensors are analytical devices incorporating biological materials such as enzymes, tissues, micro-organisms, antibodies, cell receptors or biologically derived materials or a biomimic component in intimate contact with a physico-chemical transducer. Transducers are the components that convert a biochemical signal into a quantifiable electrical signal. Biosensors have found immense applications in medical diagnostics, environmental monitoring and genetics, food processing industries and defense. Due to their simplicity, high sensitivity and potential ability for real-time and onsite analysis, biosensors have widely been applied in various fields including industrial process, clinical detection, environmental control etc. [1-4].

Biosensors can be classified based on the transduction methods they employ. A great variety of transduction methods have been utilized for biosensors in the last two decades. Most forms of transduction can be categorized in the following four main classes: optical techniques [5-6], electrochemical techniques [7-8], piezoelectric techniques [9-10] and mass-sensitive techniques [11-12]. Each of these four main classes contains many different subclasses, creating a nearly infinite number of possible transduction methods or combination of methods. However, new types of transducers are constantly being developed for use in biosensors.

Chapter 1 Introduction

Barium strontium titanate (BST) has been intensively studied for developing sensors, such as bolometers [13-14], humidity sensors and gas sensors [15-17]. The bolometers, humidity sensors and gas sensors may employ BST films as the middle dielectric layers in a capacitor structure with the capacitance change or leakage current change when the BST films adsorb radiant heat, water vapor or gas. The humidity sensors may also have the BST film to form a resistor structure. When the water molecule adsorbed onto the grain surface, it dissociates into hydrogen ion and hydroxyl ion since BST is a highly polar material. The resistance of the film will decrease due to charge transport occurs through proton hopping, Grotthuss's chain reaction [17] or electrolytic conduction.

In this investigation we explore BST films as a new electrode material to compose a novel electrochemical impedimetric biosensor. Since BST is a highly polar material, it can dissociate water molecules into hydrogen ions and hydroxyl ions when DI water contacts BST surface. It is observed, the DI water impedance becomes lower when the water molecules contact BST grain surface. This process is used as a signal amplification method in our biosensor since formation of bio-recognition complex will prevent DI water from contacting BST surface and as a result makes the DI water impedance higher. The surface immobilized bio-complex will also act as obstacle barriers to the charge transfer between the solid/liquid interfaces and results in higher interface impedance. In addition, this effect has another amplification mechanism by blocking the sample's surface and thus preventing DI water from penetrating into BST film. Consequently the impedance increases due to the less penetrated DI water and hence higher bulk BST impedance. Another advantage of

our biosensor is that does not use the harmful redox probe $[\text{Fe}(\text{CN})_6]^{4-/3-}$ which is widely used in conventional electrochemical biosensor. The $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox probe is believed that it denatures protein and it is not good for health [18].

1.2 Objectives

The objectives of the research work in developing and characterizing a novel electrochemical impedimetric biosensor based on BST films are summarised as follow:

1. To synthesis and characterize BST composite film.
2. To construct an electrochemical impedimetric biosensor based on BST composite film. This includes device structural design, fabrication and surface modification for protein immobilization.
3. To characterize the BST based biosensor for anti-Bovine Serum Albumin (BSA) sensing.
4. To characterize the BST based biosensor for anti-Epstein-Barr Virus (EBV) sensing.

1.3 Major contributions of the thesis

1. An optimised sol-gel technique has been successfully developed to fabricate BST composite film, including using high-energy ball milling to derive the nano-sized powders, creating a uniform BST colloids from the powder and film deposition by inter-layering BST sol-gel solutions and BST colloids to form thick, uniform and porous composite films.
2. The BST composite film based biosensor has been well designed and fabricated, including substrates fabrications using in-house sputtering systems for bottom electrode platinum sputtering and BST film surface modification using functionalized polymer for bio-molecules immobilization.
3. Developed and characterized BSA/anti-BSA and EBV/anti-EBV types of biosensors. Linear relationship was obtained between the device impedance change and the logarithmic antibody (anti-BSA and anti-EBV) concentration in a wide range.
4. Studied the effect of porosity on sensor performance in terms of surface areas of the deposited film.
5. An equivalent circuit for modelling the constructed biosensor has been successfully setup. The sensing mechanism of the BST based biosensor has been well understood.

1.4 Organization of the thesis

This thesis emphasizes on the synthesis, fabrication and electrical characterization of the BST material and its composite films. Further, the sensing devices fabricated based on the sol-gel derived BST composite films were characterized and the sensor performances were investigated. At a glance, the thesis is organized in the following chapters:

In Chapter One, motivation of the project is emphasize. An introduction of biosensors and the performance factors of biosensors are described. The objectives as well as the contributions of the thesis and organization of the thesis are presented.

Chapter Two presents the literature survey on biosensors, Barium Strontium Titanate (BST) and the ceramic materials properties. Background study on colloids system stability and measurement methods for biosensors are presented. The targeted analytes, Eistein-Barr Virus (EBV) that leads to nasopharyngeal carcinoma (NPC) is described.

In Chapter Three, the experimental procedures used in this research work are presented. The preparation of substrates, BST solution using sol-gel techniques, BST nano-sized powder using high energy ball-milling process and the slurry consists of the BST solution and the BST powder are described in detail. In addition, device structure and the electrical characterization setup are illustrated.

Chapter Four presents the characterization results of BST composites as well as the immunosensors based on the BST composites. The detail results of the material

Chapter 1 Introduction

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characterizations and the sensing mechanisms are discussed. Two different immunosensors developed in this project were presented in detail.

Chapter Five concludes the project and recommendations for future work are suggested.

CHAPTER TWO

LITERATURE SURVEY

2.1 Biosensors

Biosensors have major applications in various fields, from medicine, food and processing industry to environmental diagnostics. A biosensor consists of a bio-recognition element and a physico-chemical transducer, as shown in Figure 2.1. Specific interaction between the bio-recognition element of the biosensor with the target analyte give rise to a chemical or physical change, which results in a measurable response from the transducer [19-21]. This signal can be a visible light, pressure, or a difference in voltage, etc. When specific biological receptors derived from nature, such as antibodies, binding proteins or specific oligo-nucleotides are used as the binding domain, a very specific sensor with a very high specificity for its target molecule is produced [22].

Professor Leland C. Clark Jnr proposed the concept of an enzyme based glucose biosensor in 1962 at a New York Academy of Science Symposium [23]. Since there are millions of bio-molecules with specific bonding in the nature, it is expected that the application of biosensor is unlimited.

Fig. 2. 1: General Configuration of a Biosensor

There is a wide variety of biosensors and they can be grouped according to the transduction technique used. The 6 main types of techniques are optical, electrochemical, electrical, piezoelectric thermal and magnetic.

2.1.1 Optical Biosensor

In 1975, to measure the concentration of CO_2 and O_2 , the first optical chemical sensor was developed based on the changes of absorption spectrum [24]. Besides this, there is a large variety of optical methods employed in chemical sensors and biosensors applications, such as absorption, ellipsometry, fluorescence, and surface Plasmon resonance. Optical biosensor gives the desired quantities by measuring reflective index, absorbance, fluorescence properties, and dispersion of analyte molecules or chemo-optical transducing mediums. Fluorescence and surface plasmon resonance (SPR) have the highest sensitivity, which explains their popularities.

2.1.2 Electrochemical Biosensor

In 1962, using the electrochemical technique as the transduction method, the first glucose biosensor was introduced [23]. Electrochemical biosensor offers simplicity, low cost, and fast detection. However, as compared to the previous optical detection, electrochemical biosensor has lower sensitivity. There are mainly four measurement modes for electrochemical biosensor [25].

- Potentiometric– measure potential difference between a biochemical sensitive electrode and a reference electrode.
- Amperometric– current produced during oxidation and reduction of electroactive species during analyte recognition process.
- Conductimetry– impedance measurement of electrode and circuit with an AC signal applied.
- FET– conductivity measurement of a surface modified FET, which is sensitive to solution pH changes.

2.1.3 Piezoelectric Biosensor

In 1959, a simple, cost-effective, and high-resolution piezoelectric biosensor was introduced [26]. As discussed before, piezoelectric crystals acquire charges due to induced polarization when subjected to mechanical strain. This is a good transducer effect that translates signal between electrical and mechanical oscillation. Piezoelectric biosensors are further sub-classified to three different sensing modes:

- Quartz Crystal Microbalance (QCM)
- Surface Acoustic Wave resonator (SAW)
- Shear-Horizontal Acoustic-Plate-Mode resonator (SH-APM)

Of all three types of biosensor, QCM is the most widely used. This is because QCM biosensors are more robust, readily available and affordable. However, they are less sensitive relative to SAW or SH-APM biosensors. The detection range of QCM covers monolayer surface with small molecules to larger mass molecules. It can detect monolayer surface coverage by small molecules or polymer films at the low mass end. It is also capable of detecting much larger masses bound to the surface.

2.1.4 Electrical Biosensor

Electrical biosensors work by detecting the changes of conductivity and have been explored since 1980s [27-28]. An electrical biosensor has a pre-immobilized bio-recognition element on its surface, which can bind with the analyte directly. The binding will be detected directly by measuring the electrical impedance or capacitance values.

In conclusion, sensing and transduction techniques of various types of biosensors have its strengths and weaknesses. The electrical biosensors are portable and able to provide direct interface to electrical analyzers.

2.2 Ferroelectric ceramics

From the standpoint of crystal structure, ferroelectric materials are a further subset of pyroelectric and piezoelectric materials. As with pyroelectrics, ferroelectrics also display a spontaneous polarization in the absence of an applied electric field. The distinguishing feature of ferroelectrics, however, is that the spontaneous polarization must be re-orientable under an applied electric field of magnitude lower than the material own dielectric breakdown strength.

The ferroelectric properties of materials are intimately related to their domain and crystal structures. In the most widely used class of ferroelectric ceramics, the ABO_3 perovskite materials as showed in Fig. 2. 2[29], the spontaneous polarization that occurs on cooling below the Curie transition temperature results from a crystal structure change from a centrosymmetric to a noncentrosymmetric structure. For example, on cooling from temperatures greater than approximately 125 °C, BaTiO_3 transforms from a cubic paraelectric structure to a tetragonal ferroelectric structure in which the *B*-site titanium ions are displaced slightly off-center. In BaTiO_3 , there are six equivalent directions in which the titanium may be displaced during this transformation. Since the displacement of the titanium species in one unit cell affects the displacement of the titanium species in neighbouring cells, ferroelectricity is considered to be a cooperative ordering effect. This results in the formation of regions of homogeneous polarization in the materials, referred to as domains, which typically encompass thousands of unit cells. The adjacent domains with different polarization directions are separated by domain walls that are typically a few unit cells thick. With cooling in the absence of an electric field, the domains are randomly

oriented, and the net polarization of the material as a whole is thus zero. The domains may become oriented with the application of an electric field.

Fig. 2. 2: The perovskite crystal structure [29].

Another distinguishing feature of ferroelectric behavior is the polarization versus electric field (P - E) hysteresis loop. The hysteresis loop results from the domain re-orientation which occurs as the electric field direction is varied. The size and shape of the loop is determined by the magnitude of the dipole moment of the unit cell and the domain-switching characteristics of the material. Hysteresis loop behavior is measured using either a Sawyer-Tower Circuit or a Diamant-Pepinsky bridge [30]. Thin film properties have also been measured with these two types of set up and in addition, a commercially available RT66A Ferroelectric test system has been widely used [31].

2.2.1 Ceramic capacitor materials

Ceramic materials suitable for capacitor applications such as charge storage applications are dependent on the dielectric properties of the material. For these

applications, materials with high dielectric constants are typically desired.

Capacitance is defined as

$$C = k\epsilon_0 \frac{A}{d} \quad (2.1)$$

where k is the dielectric constant, ϵ_0 is the permittivity of free space, A is the electrode area and d is the thickness of the dielectric. Although the dielectric constant is considered to be a fundamental material property, it is also dependent on factors such as measurement or use frequency, electric field strength, stress and temperature. Physical features of the ceramic, such as porosity and grain size, may also affect the observed dielectric constant.

Since the 1940s, ceramic capacitors have typically been fabricated from doped barium titanate compositions, since these materials display dielectric constants greater than 2000. To achieve the desired room temperature dielectric constant and dielectric constant versus temperature characteristics, researches have added various substituents BaTiO_3 . For instance, the additions of strontium titanate to barium titanate shift the Curie point to lower temperatures. Typical results are shown in Fig. 2. 3 for various BST compositions. BST materials have also been used in multilayer configurations to achieve even greater capacitance efficiencies [32].

Fig. 2. 3: Effect of the barium titanate to strontium titanate ratio on the dielectric properties of barium titanate-strontium titanate composites [32].

2.2.2 Barium Strontium Titanate (BST)

Ferroelectric barium strontium titanate (BST) ceramic thin films have been widely investigated as potential materials for the applications of microelectronic devices such as dynamic random access memories, infrared detectors, microwave devices, and hydrogen gas sensors owing to their high dielectric constant, relatively low dielectric loss tangent, large electric-field tunability, long lifetime, and good temperature stability [33-37].

Alternatively, porous perovskite BST ceramic and thick films can be used as humidity sensitive material [38-43]. The principle of humidity sensor is based on the change in electrical conductance and capacitance due to water vapor chemisorption and physisorption and/or capillary condensation in the pores of the sensor material.

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In perovskite BST, the atoms of Ba and Sr are susceptible to humidity. Since water is a polar molecule, the negatively charged oxygen is electrically attached to the positively charged $\text{Ba}^{2+}/\text{Sr}^{2+}$ ions of the sensor material. The initial monolayer is chemisorbed due to formation of a chemical bond under the influence of high electrostatic field between $\text{Ba}^{2+}/\text{Sr}^{2+}$ and oxygen of the water layer. This initial chemisorbed layer is tightly attached to the BST grains surface. Once this initial layer formed, it is not further affected by exposure to humidity. The irreversible reaction for the first layer can be given as:

Once the first layer is formed, subsequent layers of water molecules are physically adsorbed. These physisorbed water molecules dissociated because of the high electrical fields in the chemisorbed water layer, as given below:

The charge transport occurs when H_3O^+ ions release a proton to neighboring water molecules, which accepts it while releasing another proton and so on. This is known as Grotthuss's chain reaction [44-45]. Charge barrier height at the BST grain boundary will be reduced, leading to an exponential increase in conductivity of the film. Capacitance increase due to water molecule reorientation and mobile charges accumulated at the electrodes and pore necks.

Most of the research work has been done on bulk ceramics and thick films, which need very high sintering temperatures in excess of 1200 °C. Thus, it limits further

miniaturization and integration with silicon IC. On the other hand, BST thin films can be integrated with silicon IC, however, the fundamental limitation is that the BST thin films have inferior dielectric, piezoelectric, and pyroelectric properties compared to their bulk counterparts. In some cases, there may be an order of magnitude difference in the electrical properties of the bulk ferroelectric and its thin-film form [46]. This reduction is usually attributed to compositional and microstructural inhomogeneities, defects, and internal stresses. Internal stresses in thin films arise due to a variety of reasons including the lattice mismatch between film and the substrate in the case of epitaxial films, differences between thermal expansion coefficients of film and substrate, and phase transformations. Epitaxy-induced stresses were shown to have a profound effect on the physical properties of ferroelectric films especially in the vicinity of a phase transformation. It was shown that the choice of substrate and also the film thickness can be used to adjust the internal stress level and thus to tune physical properties. The latter is due to the thickness dependence of stress relaxation by misfit dislocation formation at the deposition temperature [47].

2.3 Colloidal System Stability and Zeta Potential

Colloidal systems include solid-solid (metal alloys); solid-liquid (a suspension such as muddy water); solid-gas (smoke, airborne dust, aerosol inhalers); liquid-solid (butter, creams, ointments, lotions, photographic emulsions, paints); liquid-liquid (an emulsion such as milk); liquid-gas (fog, mist, aerosol sprays); gas-solid (marshmallows); and gas-liquid (foams). The term “colloidally stable” means that the

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particles in the colloidal system do not aggregate at a significant rate. The precise connotation depends on the type of aggregation under consideration. For example, a concentrated paint is termed stable by some people because oil and pigment do not separate out at a measurable rate, and unstable by others because the pigment particles aggregate into a continuous network [48]. In general, aggregation denotes a group of particles held together and it is usually used to describe the structure formed by the cohesion of colloidal particles. When a sol is colloiddally unstable, the formation of aggregates is called coagulation implying the formation of compact aggregates. The rate of aggregation is in general determined by the frequency of collisions and the probability of cohesion during collision. When larger aggregates are formed, action of gravity towards the aggregates is more significant. This may cause sedimentations. Sedimentation is the settling of suspended particles under the action of gravity or a centrifugal field [48-49].

Zeta Potential is a very good index of the magnitude of the interaction between colloidal particles and Zeta Potential measurements are normally used to assess the stability of colloidal systems. Colloidal particles dispersed in a solution are electrically charged due to their ionic characteristics and dipolar attributes. Each particle dispersed in a solution is surrounded by oppositely charged ions called the fixed layer. Outside the fixed layer, there are varying compositions of ions of opposite polarities, forming a cloud-like area. This area is called the diffuse double layer, and the whole area is electrically neutral. As shown in Fig. 2.4, when a voltage is applied to the solution in which particles are dispersed, particles are attracted to the electrode of the opposite polarity, accompanied by the fixed layer and part of the

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diffuse double layer, or internal side of the "sliding surface". Zeta potential is considered to be the electric potential of this inner area including this conceptual "sliding surface". As this electric potential approaches zero, particles tend to aggregate [48].

The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. It is noticed that the most important factor that affects zeta potential is pH since it is an indication of acid or base level of a system. Acidic colloid contains large number of positive ions whereas base colloid contains large number of negative ions. The ions create the suspension effect of the particles within the system and thus determine the stability of the system.

Fig. 2.4: Colloidal systems and zeta potentials.

2.4 Impedance Spectroscopy Measurement

Impedance spectroscopy is an effective method for probing the features of surface-modified electrodes in electrochemical sensors [50][51][52]. A small-amplitude perturbing sinusoidal voltage signal is applied to the electrochemical cell and the resulting current response is measured. The impedance is calculated as the ratio between the system voltage phasor, $U(j\omega)$ and the current phasor, $I(j\omega)$ which are generated by a frequency response analyzer during the experiment. From Eq. (2.3), where $j = \sqrt{-1}$, ω and f (excitation frequency) have units of rad/s and Hz respectively, the complex impedance $Z(j\omega)$ can be presented as the sum of the real, $Z_{re}(\omega)$ and imaginary, $Z_{im}(\omega)$ components that originate mainly from the resistance and capacitance of the cell respectively.

$$Z(j\omega) = \frac{U(j\omega)}{I(j\omega)} = Z_{re}(\omega) + jZ_{im}(\omega) \quad (2.3)$$

where $\omega = 2\pi f$. A general electronic equivalent circuit (Randles and Ershler model [53]) which is very often used to model interfacial phenomena, includes the ohmic resistance of the electrolyte solution, R_s , the Warburg impedance, Z_W , resulting from the diffusion of ions from the bulk electrolyte to the electrode interface, the double-layer capacitance, C_{dl} , and the electron transfer resistance, R_{ct} , that exists if a redox probe is present in the electrolyte solution as shown in Fig. 2. 5. The parallel elements of C_{dl} and $Z_W + R_{ct}$ of the equivalent circuit are introduced since the total current through the working interface is the sum of distinct contributions from the Faradaic process, I_F and the double-layer charging, I_C . Since all of the current must

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pass through the uncompensated resistance of the electrolyte solution, R_S , it is inserted as a series element in the circuit. The two components of the electronic scheme R_S and Z_W represent bulk properties of the electrolyte solution and diffusion features of the redox probe in solution. Therefore, these parameters are not affected by chemical transformations occurring at the electrode surface. The other two components in the scheme, C_{dl} and R_{ct} depend on the dielectric and insulating features at the electrode/electrolyte interface. The double-layer capacitance depends on the dielectric permittivity introduced into the double-charged layer molecules, ϵ_{dl} and for the high polar molecules the capacitance should be higher as shown in Eq. 2.4, where $\epsilon_0 = 8.85 \times 10^{-12} F/m$ is the dielectric constant of the vacuum, ϵ_p is the effective dielectric constant of the layer separating the ionic charges and the electrode surface, A is the electrode area and δ is the thickness of the separating layer.

$$C_{dl} = \frac{\epsilon_{dl} A}{\delta} \text{ where } \epsilon_{dl} = \epsilon_0 \epsilon_p \quad (2.4)$$

In the equivalent electronic circuit the double-layer capacitance, C_{dl} can be represented as the sum of a constant capacitance of an unmodified electrode, for instance Pt electrode and a variable capacitance originating from the electrode surface modifier, C_{mod} connected as series elements. Any electrode modifier of insulating features decreases the double-layer capacitance as compared to the pure metal electrode. Thus, the double-layer capacitance could be expressed as:

$$\frac{1}{C_{dl}} = \frac{1}{C_{Pt}} + \frac{1}{C_{mod}} \quad (2.5)$$

The electron transfer resistance, R_{ct} controls the electron transfer kinetics of the redox probe at the electrode interface. Thus, the insulating modifier on the electrode is expected to retard the interfacial electron transfer kinetics and to increase the electron transfer resistance. The electron transfer resistance at the electrode is given in Eq 2.6 where R_{Pt} and R_{mod} are the constant electron transfer resistance of the unmodified electron and the variable electron transfer resistance introduced by the modifier in the presence of the solubilised redox probe respectively. These resistances are also connected as series elements in the equivalent electronic circuit.

$$R_{ct} = R_{Pt} + R_{mod} \quad (2.6)$$

Fig. 2. 5: General electronic equivalent circuit (Randles and Erschler model).

2.5 Epstein-Barr Virus (EBV)

Epstein-Barr Virus frequently referred to as EBV was discovered by Denis Burkitt in 1864. It is a member of the herpesvirus family and one of the most common human viruses. The virus occurs worldwide, and most people become infected with EBV sometime during their lives. Seroepidemiological and molecular studies also pinpointed an association between EBV infection and anaplastic nasopharyngeal carcinoma (NPC) [54-55], a geographically and genetically restricted tumor which is very common in southern China [46]. However, for this tumor, EBV does not act as

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the sole aetiological agent but is probably one of several necessary co-factors in the evolution of the tumor. Symptoms of infectious mononucleosis are fever, sore throat, and swollen lymph glands. Sometimes, a swollen spleen or liver involvement may develop. EBV also establishes a lifelong dormant infection in some cells of the body immune system.

In this project, cloned EBV is immobilized onto poly(TMS-*r*-NHS) modified BST surface which was spin-coated on the Pt coated silicon substrates. The device is then able to detect anti-EBV target analyte. Detail discussions on device measurements and characterizations are described in Chapter 4.

CHAPTER THREE

EXPERIMENTAL PROCEDURES

In this chapter, the BST composite film deposition process is discussed, including the substrate preparation, BST sol-gel solution preparation, BST nano-sized powder preparation, BST slurry preparation and selection of composite pH and its zeta potential. BST film deposition structure, BST film surface modification for bio-molecules immobilization and device electrical measurement setup are also described.

3.1 BST composite film deposition

3.1.1 Substrate preparation

Prior to the thin film deposition, wafers were prepared by using the Leybold Sputtering system. Titanium dioxide, TiO_2 was deposited at the substrate temperature of 400 °C for 10 minutes followed by the platinum, Pt for 20 minutes. The thickness of TiO_2 is estimated at around 80 nm while the thickness of Pt is estimated at around 150 nm. The Pt layer will serve as a bottom electrode for the device.

3.1.2 BST sol-gel solution preparation

0.2M $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ (BST) sol-gel solution was developed based on the flow chart shown in Fig. 3.1. Barium acetate ($\text{Ba}(\text{CH}_3\text{COO})_2$) and strontium acetate ($\text{Sr}(\text{CH}_3\text{COO})_2$) powder were dissolved in glacial acetic acid at 60 °C for 10 minutes. De-ionized (DI) water was added to promote the dissolving of acetate powder at the molar ratio of 1:4 ($M_{(\text{Ba}+\text{Sr})} : M_{\text{DI}}$).

The clear solution provides barium and strontium sources for the

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final BST product. On the other hand, titanium butoxide ($\text{Ti}[\text{O}(\text{CH}_2)_3\text{CH}_3]_4$) was mixed with acetylacetone ($\text{C}_5\text{H}_7\text{OOH}$) in molar ratio of 1:1 at 80 °C for 10 minutes. The acetylacetone helps to stabilize and prevent the titanium butoxide from hydrolyzing when exposed to the moisture. By replacing one butoxide group with acetylacetone, the hydrolysis of the modified titanium butoxide is greatly reduced.

The two solutions were then mixed at room temperature for 10 minutes and the viscosity of this yellowish solution was adjusted by adding ethylene glycol monomethyl ether ($\text{C}_3\text{H}_8\text{O}_2$) with continuous stirring for 30 minutes. The final solution was filtered by using 0.1 μm filter and stored in the amber bottles.

Fig. 3. 1: Flow chart for pure BST sol-gel solution, BST milled powder slurry and BST original powder slurry preparations.

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3.1.3 BST nano-sized powder preparation

High energy ball milling method was applied to reduce the commercially purchased $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ (BST) powder particle size to nanometer. The ball milling machine used is the Fritsch Pulversisette 5 planetary ball milling machine from Fritsch GmbH. A container with tungsten carbide (93 wt% WC + 6 wt% Co) vials and balls are used. The vial has a volume of 250 ml with an inner diameter of 75 mm. The balls have diameters of 10 mm and 20 mm. Commercial BST powder is used with a weight ratio of 1:20 to balls. The milling speed is set at 200 rpm. The milling is carried out in 30 minutes cycle with 20 minutes of milling followed by 10 minutes of pause to prevent overheating of the milling system. The powder was oven-baked dry at 110 °C for 12 hours prior to high-energy milling process. Sample powder was taken from the milling container every 10 hours for powder characterization.

3.1.4 BST slurry preparation

Upon reaching 35 hours net milling time, ESL #400 commercial available organic vehicle was added as dispersant to prevent conglomeration of BST powder. The mass ratio of dispersant to powder is around 1:20. Milling process was continued for another 6 hours of net milling time. Upon completion of the ball milling process, the nano-sized powder was ready to be added to the 0.2M BST sol-gel solutions at the weight ratio of 1:2 to form nano-composite colloids. In order to obtain homogenous slurry, the combination of sol-gel solution and the nano-composite powder were subjected to another 12 hours ball milling process at 160 rpm to form homogenous BST milled powder slurry. This process is illustrated in Fig. 3.1.

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To further examine the contributions of BST powder in the biosensor, the unprocessed original powder purchased commercially was added with 0.2M sol-gel solutions directly and undergone 12 hours ball milling process at 160 rpm. It was observed that the homogeneity of this BST original powder slurry is comparable to the BST milled powder slurry. The process flow is also illustrated in Fig. 3.1. Both BST milled powder slurry and BST original powder slurry were then spin coated on separate substrates for further evaluations and characterizations.

3.1.5 Selection of composite pH and its zeta potential

The pH value for the 0.2M BST solutions varies from 1.2 to 6.5 depending on the volume ratio of acetic acid and Ethylene Glycol Monomethyl Ether added. The pH can be further adjusted up to 11 by adding ammonium hydroxide.

A Brookhaven ZetaPALS zeta potential analyzer was used to perform zeta potential measurement for the particles in the solutions with different pH value. An isoelectric point (IEP) was found at pH 2.5 as shown in Fig. 3.3. The surface of the BST particles are positively charged at lower pH values and negatively at higher pH values. The highest absolute zeta potential value is presented in the basic pH region. Therefore, stabilization of the slurry can be achieved at these pH values.

Sedimentation curves for pH of 2, 3.75 and 8.25 are shown in Fig. 3.4. Some time after pouring the slurry into the test tube, an interface was evident between a perfectly clear region, at the top of the tube, and a cloudy region at the bottom of the

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tube. The height of such interface was measured as a function of the elapsed time.

The sedimentation test results are consistent with zeta potential measurement results. For pH of 2, the zeta potential of the suspension is near IEP, therefore sedimentation happened very fast. For pH of 8.25, the slurry is very stable. No significant sedimentation happened even after 66 hours of observation. The stability of the slurry with suspension zeta potential of 3.75 is between those of pH of 2 and 8.25.

Fig. 3. 2: Zeta potential of BST powder vs. pH in BST sol-gel solution.

Fig. 3. 3: Sedimentation curves for BST powder dispersed in BST solution at different pH values.

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3.2 Deposition of BST composite film

The BST composite was spun onto the self-prepared Pt-coated Si substrate wafer by using a spin-coating machine at 2000 rpm for 30 seconds. Figure 3.4 illustrates the flow of BST composite spin-coating steps. At each layer of the coated film, it was then put on the hotplate at 200 °C for 2 to 5 minutes and follow by 400 °C for another 2 to 5 minutes to pyrolyze the film. The main purpose is to remove organic solvent and hence promote the formation of the BST film. In this experiment, the final deposited films were annealed at different temperature range from 475 °C to 875 °C for 1 hour at the end of the entire process.

Fig. 3. 4: The flow-chart for BST composite film spin coating.

Figure 3.5 illustrates the structure of the BST composite film developed on a substrate. Inter-layers of sol-gel and slurry are chosen to develop high density BST thick film. The intermediate sol-gel layers helped to smoothen out the inter-composite slurry layers whereas the final top 3 layers of sol-gel thin films served as the capping layers to fill up the micro-cracks on the film surface. pH level of slurry plays an important role to ensure stability of slurry during sensor fabrication and provide good surface uniformity.

Fig. 3. 5: Basic structure of BST composite film.

3.3 Surface modification and bio-molecules immobilization

The development of biosensors concerns the interaction between inorganic material such as BST composite films and organic material such as the EBV bio-receptors. The demand for high sensitivity requires a covalent and tailored coupling of the bio-molecules to the inorganic transducer surface. Therefore, immobilisation of bio-molecules needs to be evaluated and optimised in all its different aspects and steps. Self-assembled monolayers (SAMs) of thiols and silanes are realised as a first step in

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the immobilisation process. In this project, phosphoric acid is chosen to form the SAMs on the surface of BST composite films. The covalent bonded phosphonate groups with the BST composite films to provide stable hydroxyl groups that are able to react with the silane group of 3-propyl methacrylate (TMS). TMS is a very sensitive and reactive reagent that is able to immobilize protein onto the film surface. TMS is functionalized with N-hydroxy succinimide methacrylate (NHS). The composition and the molecular weight can be easily designed and polymerized.

Firstly, the BST film was rinsed with acetone followed by DI water, then dried at 120 °C for 5 hours. Secondly, the clean BST film was soaked in 9.5% phosphoric acid aqueous solution for 20 min and dried at 60 °C for 8 hours to form a self-assemble monolayer (SAM) on the surface of BST film. Thirdly, the phosphoric acid modified BST film was then immersed in poly(TMS-r-NHS) [TMS: 3-(trimethoxysilyl)propyl methacrylate (99%, Fluka); NHS: N-hydroxysuccinimide (99%, Sigma)] methanol solution (5 mg/ml) for 3 min and dried at 200 °C for 24 h. Finally, the modified BST thin film was immersed in tetrahydrofuran (THF) solution with constant Ar bubbling for 30 min, and dried at 120 °C for 1 h.

It is known that N-hydroxysuccinimide reacts smoothly with primary or secondary amino groups giving the corresponding amides [58]. N-hydroxy succinimide methacrylate (NHS) can be synthesized and used to prepare a random co-polymer with 3-(trimethoxysilyl)propyl methacrylate (TMS) via Atom Transfer Radical Polymerization technique (ATRP). The procedure of synthesis of NHS is shown in Fig. 3.6. The precursor TMS is functionalized with NHS. The poly(TMS-r-NHS) is synthesized via ATRP technique (shown in Fig. 3.7). The procedure of rough BST

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surface modification with poly(TMS-*r*-NHS) is shown in Fig. 3.8. Fig. 3.9 shows the cross-sectional schematic view of our prototype device.

Fig. 3. 6: The procedure of synthesis of NHS

Fig. 3. 7: Synthetic route for the poly(TMS-*r*-NHS).

Fig. 3. 8: Surface modifications of BST composite film.

Fig. 3. 9: Cross-sectional schematic view of the prototype BST film based biosensor.

3.3.1 Synthesis steps

3.3.1.1 N-Acryloxysuccinimide (NHS)

To a stirred solution of g N-hydroxysuccinimide in 40 ml of dry dioxane, cooled at 5 °C with an ice-bath, 3.5 ml methacrylic acid and 8.66 g N, N'-dicyclohexylcarbodiimide (DCC) were added. The mixture was stirred at 0 °C for 2 h. The cooling bath was removed and the mixture was stirred for a further 4 h while it reached room temperature. The precipitated N,N'-dicyclohexylurea was then removed by filtration and the solvent was evaporated in vacuum. The residue was extracted exhaustively with hot n-heptane. The combined n-heptane extracts were cooled to 0 °C for crystallization of the product. The product was then recrystallized from n-heptane, m.p. 106 °C.

3.3.1.2 Poly(TMS-r-NHS)

To a dry 25 ml Schlenk flask with a magnetic stirring bar, CuCl (0.027 g, 0.268 mmol), and NHS (0.2 g, 1.08 mmol) were added and the flask was evacuated and flushed with argon. Degassed 1,4-dioxane (3 ml), hexamethyl-triethylenetetramine (HMTETA) (0.073 ml, 0.268 mmol) and TMS (2 ml, 4.02 mmol) and were introduced to the Schlenk flask using degassed syringes and the mixture was stirred for 10 min. The mixture was then degassed by three freeze-pump-thaw cycles. Finally, EBrIB (0.020 ml, 0.134 mmol) was added using a degassed syringe. The flask was then placed in a preheated oil bath maintaining at 50 °C. The reaction was allowed to proceed with constant stirring for 4.2 h. The polymer was precipitated in n-hexane and resolved in dry THF. The polymer had M_n , GPC = 11,100 and polydispersity index (PDI) = M_w/M_n = 1.26.

3.4 Electrical Characterization Setup

Figure 3.10 illustrates the test jig setup for electrical measurements. The device sample with exposed Pt bottom electrode at the side is placed on a poly(dimethylsiloxane) (PDMS) stage. 10 μ L DI water is dropped onto the device surface to act as a contact media between Au electrode on the glass substrate and the test device. The Au top electrode of 100 nm thickness is E-beam evaporated on the top glass substrate through a patterned shadow mask. The height and the diameter of the DI water are approximately 0.8 mm and 4 mm, respectively. The Pt bottom electrode is exposed by using diluted HF solution as an etchant.

Fig. 3. 10: Test jig setup for device measurements.

In summary, Chapter Three is describing the entire experimental procedures and material preparation. Substrate preparation and sensor fabrication process have been discussed in detail. Platinum substrates serve as bottom electrodes for the BST composite film based biosensors. Nano-sized BST powder was carefully prepared by using ball milling method for subsequent slurry preparation. In order to prevent conglomeration of the BST powder, pH level of sol-gel solution was carefully selected prior to slurry formation through ball milling process. Pure sol-gel solution and slurry were intermediately spun on the Pt substrate to form BST composite film with good uniformity for surface modification and protein immobilization.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Characterization of BST composites

4.1.1 DTA and TGA of BST composites

Thermogravimetric (TGA) and Differential Thermal Analysis (DTA) analyses were carried out at a heating rate of 2 °C/min from room temperature to 900 °C by using Perkin-Elmer 7. Fig. 4. 1 illustrates the thermal decomposition behavior of $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ sol-gel solution, composite slurry as well as the powder. Based on the Fig. 4. 1(a), the sol-gel solution experiences a significant weight loss at below 600 °C due to the evaporation of residual solvent in the solution as well as a solid reaction of $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$. Two exothermic peaks are observed at 300 °C and 400 °C in the DTA curve, corresponding to the decomposition of metal-organic precursors. It is known that the precursors of barium acetate and strontium acetate are decomposed at around 300 °C, and the titanium butoxide is decomposed at around 400 °C [37]. The resulting $(\text{Ba,Sr})\text{TiO}_x$ complex is stable up to 550 °C, and shows a flat region in the TGA curve. With further increasing temperature, a step is found in the TGA curve between 550 °C and 600 °C, accompanied by an exothermic peak in the DTA curve. The exothermic peak at these temperatures are due to the decomposition of the intermediate phase $(\text{Ba}_{0.67}\text{Sr}_{0.33})\text{TiO}_3 + \text{CO}_2$, and crystallization of BST perovskite phase which is accompanied by a weight loss.

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The weight loss is not significant for the composite slurry as shown in Fig. 4. 1(b). This is due to the composite slurry consisting of the mixture of sol-gel solution and the purchased polycrystalline BST powder at the ratio of 2:1. Therefore, the solvent content is relatively lower as compared to pure sol-gel solution.

To further analyze the thermal decomposition behavior of $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$, an analysis of commercially purchased powder were carried out as shown in Fig. 4. 1(c). It is clearly shown that the purchased powder is thermally up to stable 900 °C. Thus, we can conclude that the weight loss and heating changes to temperatures in composite slurry are due to the reaction of sol-gel solution.

Fig. 4. 1: TGA and DTA curves of: (a) 0.2M $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ sol-gel solution, (b) $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ composites with powder to solution ratio of 1:2, (c) Original $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ powder.

4.1.2 XRD of BST powders and BST composite films

Rigaku Dmax 2200 x-ray diffractometer was used to analyze crystal structure of BST powder and BST composite film. X-ray diffraction patterns of BST powder were shown in Fig. 4. 2 and Fig. 4. 3. From Fig. 4. 2, we noticed that the effect of the organic vehicle is not significant in changing the BST powder crystalline structure except promoting the dispersion of the powder. In Fig. 4. 3, XRD patterns of BST powder without organic vehicle in different milling hours were taken to observe the effect of milling hours versus the change of BST crystalline structure. It is observed that the powder is stable and the crystalline size changes at different stages. The crystalline size estimation is discussed in subsequent section.

Fig. 4. 2: X-ray diffraction patterns of BST powders with and without vehicle.

Fig. 4. 3: Corresponding X-ray diffraction patterns of $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ powder with different milling hours.

The XRD patterns of different BST composite film samples annealed in air for 1 hour at 600 °C is shown in Fig. 4. 4. Typical perovskite structure of BST was observed for the deposited BST composite film.

Fig. 4. 4: X-ray diffraction patterns of BST composite films annealed at 600 °C.

4.1.3 Grain size estimations

The determination of BST powder crystalline size is based on the concept of XRD peak contracting or broadening after the high-energy ball milling process. Peak contracting at half height intensity of the XRD patterns is induced by an increment in crystalline size. On the other hand, a reduction in crystalline size causes broadening of the XRD peak at its half height intensity.

The crystalline size can be calculated using the Scherrer equation [56]:

$$\beta = \frac{\zeta \lambda}{d_c \cos \theta} \quad (4.1)$$

where β in radians is the broadening of the diffraction peak due purely to crystalline size measured at half of its maximum intensity; d_c is the diameter of crystalline size and ζ is a constant. The constant ζ depends mainly on the crystalline shape and the indices. It has a value of about 0.9. The key in determining crystalline size of powder particles from peak broadening is to determine d_c purely from the pure breadth β of the diffraction peak. However, there are several factors that can contribute to peak broadening, namely, decrease in crystalline size, internal strain and broadening due to the x-ray machine itself.

In Scherrer's original investigation, the broadening parameter B is purely due to the change in crystalline size and strain was obtained from the measured broadening of the diffraction peak, B_M and the measured breadth B_I is due to the instrument at half maximum intensity of the peak. The broadening B_I is measured under similar geometrical conditions using a material with a crystallite size well in excess of

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100nm. Among the many methods that have been proposed to include the correction on broadening due to instrument, Warren's is the simplest and a more accurate method. That is:

$$B^2 = B_M^2 - B_I^2 \quad (4.2)$$

In order to obtain B_I , a well-annealed specimen with grain size above $1\mu\text{m}$ should be used for the XRD measurement. Since there is no residual strain in a well-annealed specimen, the broadening measured can be assumed to be purely due to the instrument.

Besides the broadening due to the instrument, non-uniform strain as a result of heavy deformation will also lead to a broadening of XRD peaks. The broadening due to non-uniform strain can be written as:

$$B_S = -2 \frac{\Delta d}{d} \tan \theta = C \tan \theta \quad (4.3)$$

where B_S is the broadening of the XRD peaks due to residual strain, d is the lattice spacing and Δd is the change in lattice spacing and $C = -2\Delta d/d$.

Therefore, the broadening parameter, B can be written as:

$$B = B_S + \beta \quad (4.4)$$

Substituting Eq. (4.1) and Eq. (4.3) into Eq. (4.4):

$$B = C \tan \theta + \frac{\zeta \lambda}{d_c \cos \theta} \quad (4.5)$$

Substituting Eq. (4.2) into Eq. (4.5):

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$$\sqrt{B_M^2 - B_I^2} \cos \theta = C \sin \theta + \frac{\zeta \lambda}{d_c} \quad (4.6)$$

To solve Eq. (4.6) graphically, $\sqrt{B_M^2 - B_I^2} \cos \theta$ may be plotted as a function of $\sin \theta$, namely:

$$Y = C \sin \theta + A \quad (4.7)$$

where $Y = \sqrt{B_M^2 - B_I^2} \cos \theta$, $A = \frac{\zeta \lambda}{d_c}$ and λ is the wavelength of radiation at 1.54056 Å.

Based on the data extracted from the XRD obtained for the $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ powder at different milling duration as shown in Fig. 4. 3, the crystalline size of the powder can be calculated and plotted as shown in Fig. 4. 5 by using Eq. (4.6) and Eq. (4.7).

Fig. 4. 5: Changes of $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ powder in terms of crystalline size in different milling hours.

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The average grain size of the commercial BST powder decreased rapidly to minimum at 10 hours; at the same time the lattice constant increased rapidly and peaked at 10 hours. It is possible that the material bonds were broken due to the great impact energy derived during high-energy ball milling, and the material is at the highest energy state. Between 10 and 40 hours, the average grain sizes of the powder increased slightly. At the same time, the lattice constants of the powder decreased gradually. This is because the powder is at the extremely deformed state at 10 hours of milling time, and as the surface stress of the powder was released, the powder changed from the unstable highest energy state at 10 hours of milling time to the meta-stable state at 40 hours of milling.

4.1.4 Particle size observations

A JEOL JSM-6340F Field Emission Microscope was used to observe the commercial and high-energy ball-milled powder. Fig. 4. 6 shows the FE-SEM image of the original BST powder. It is shown that the particle size of the original powder is around 500 nm to 1 μm . After high-energy ball milling for 40 hours the particle size was reduced to around 200 nm as shown in Fig. 4. 7. It is believed that the smaller particle size is beneficial to achieve denser and smoother film.

Fig. 4. 6: FESEM images for BST original powder ($\times 10,000$).

Fig. 4. 7: FESEM image for powder after high-energy ball milling (×40,000)

4.1.5 Laser Particle Size Analysis

Fig. 4. 8: Particle size analysis for original purchased BST powder and 40 hour ball-milling BST powder.

A Brookhaven ZetaPALS laser particle size analyzer was used to analyze particle size of the original powder and the ball-milled powder. Fig. 4. 8 shows that after 40 hours of high energy ball milling, the particle size shows two distributions. Most of the particles have been reduced significantly to the range of around 230 nm whereas

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a small amount of particles show bigger size at around 500 nm. These two distributions were analyzed to because of the highly humidity sensitive property of BST powder. Because of the adsorption of water molecules in atmosphere, portion of the powder stick firmly on the wall of ball milling vial. The powder stuck on the wall has bigger size when compared to the loose powder in the vial. The result from the laser particle size analyzer consist with the result observed by using SEM.

4.1.6 BET surface area analysis for BST powders

The Micromeritics' ASAP 2020 Accelerated Surface Area System has been used to analyze the surface area characteristics of BST powders. The BST powder was contained in an evacuated sample tube and cooled to cryogenic temperature at about 100 K. It was then exposed to nitrogen gas at a series of precisely controlled pressures. At the cryogenic temperatures, weak molecular attractive forces will cause the gas molecules to adsorb onto the powder. The pressure in the sample vessel was measured after each nitrogen dosing. There is a direct relationship between the pressure and the volume of gas in the sample vessel. By measuring the reduced pressure due to adsorption, the ideal gas law can then be used to determine the volume of gas adsorbed by the sample.

As adsorption proceeds, the thickness of the adsorbed film increases. Any micropores in the surface are quickly filled and the free surface becomes completely covered. Finally larger pores are filled. On the other hand, the desorption process begins when the pressure is systematically reduced resulting in the liberation of the adsorbed molecules. Similar to the adsorption process, the changing quantity of gas

on the solid surface is quantified. These two sets of data describe the adsorption and desorption isotherms. Analysis of the isotherms yields information about the surface characteristics of the material.

The analysis shows that the BET surface area for the original BST powder is 1.6 m²/g. For the 40 hours ball-milled powder with ESL #400 organic vehicle, the BET surface area is significantly larger at 29.2 m²/g. This can be understood that by going through the ball-milling process, the BST powder size is reduced and hence the effective surface area is increased.

The results of BET surface area analysis for the powders agree with the Laser particle size analysis as both showed that the powder size is reduced after the ball-milling process.

4.1.7 Surface morphology observations of the composite films

A JEOL JSM-6340F Field Emission Microscope was used to observe the surface morphology of the deposited film. The FESEM images shown in Fig. 4. 9 demonstrate the effect of tuning pH level of BST sol-gel solution. It can be clearly seen that when the pH level is at 3.75, the film structure is not uniform due to the agglomeration of nano-sized BST powder. When the pH level is increased, the zeta potential of the colloids is increased and hence the agglomeration of the BST powder is reduced. More uniform film surface and denser structure can therefore be formed.

Fig. 4. 10(a) and (b) are the images of the composite films formed by using high energy ball-milled BST powder. Fig. 4. 10(c) depicts the cross-section for the

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composite films at $\times 1000$ and $\times 5000$ magnification respectively. Comparing to the images shown in Fig. 4. 10(d) (e) and (f) for the composite films formed by using the original BST powder without going through the high energy ball-milling process, the films composed of ball-milled powder show smoother surface. That is because the gaps between ball-milled powders are smaller and easier to be filled with BST sol. The surface morphologies of the composite films for both milled and unmilled BST powder are uniform.

4.1.8 Porosimetry test for composite films

The Mercury porosimetry test using AutoPore Porisimeters from Micromeritics was performed to characterize the porosity of the composite films. To perform an analysis, the sample is loaded into a penetrometer, which consists of a sample cup connected to a metal-clad, precision-bore, glass capillary stem. The penetrometer is sealed and placed in a low pressure port, where the sample is evacuated to remove air and moisture. The penetrometer's cup and capillary stem are then automatically backfilled with mercury. As pressure on the filled penetrometer increases, mercury intrudes into the sample's pores. Mercury porosimetry characterizes a material's porosity by applying various levels of pressure to a sample immersed in mercury. The pressure required to intrude mercury into the sample's pores is inversely proportional to the size of the pores.

Two types of samples, ball-milled composite film and original composite film were tested. It is observed that the composite film based on the original BST powder has 24% higher total pore area compared to that of the composite film based on the ball-

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milled BST powder. The data is consistent with the observations made from the FESEM images.

Fig. 4. 9: FESEM images. (a) BST composite film surface at pH 3.75. (b) BST composite film surface at pH 5.5. (c) Cross-sectional view of BST composite film at pH 3.75. (d) Cross-sectional view of BST composite film at pH 5.5.

Fig. 4. 10: FESEM observations for BST composite films: (a) (b) (c) consist of ball-milled BST powder for 35 hours follow by 6 hours with 5wt% ESL #400 organic vehicle. (d) (e) (f) consist of original BST powder without going through high energy ball-milling process.

4.2 Characterization of Bio-sensing Devices

4.2.1 Immobilized BSA observations

Fluorescent isothiocyanate (FITC) is currently the most commonly-used fluorescent dye for Fluorescence activated cell sorter (FACS) analysis. FITC is a small organic molecule, and is typically conjugated to proteins via primary amines (i.e., lysines). Usually, between 3 and 6 FITC molecules are conjugated to each biomolecule. Fluorescence is typically excited by the 488 nm line of an argon laser, and emission is collected at 530 nm. In order to evaluate the properties of modified BST surface, the modified samples were characterized by fluorescent microscopy measurement after binding with Bovine Serum Albumin (BSA)-FITC. In the experiment, the modified samples were immersed into BSA-FITC (1mg/ml) PBS buffer solution (pH = 7.4) for 16 hours at 4 °C and then washed with PBS thoroughly.

The fluorescent microscopy images shown in Fig. 4. 11 can be clearly seen that the composite film developed from the original BST powder shows higher fluorescent intensity than that of the film developed from the ball-milled BST powder. From the Mercury porosimetry analysis, we already know the composite film based on the original BST powder has higher surface area compared to that of the composite film based on the ball-milled BST powder. Therefore, more functional groups provided by the polymer layer can be modified onto the surface of original powder composite film. In hence, there are more protein bindings on the surface of original powder composite film.

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Fig. 4. 11: The Fluorescent Microscopy of polymer layer on BST surfaces with and without BSA. (a) Ball-milled powder composite film without BSA, (b) Original powder composite film without BSA, (c) Ball-milled powder composite film with BSA, (d) Original powder composite film with BSA.

4.2.2 Impedance analysis of BSA Immunosensors

Fig. 4. 12: Impedance spectroscopy for bare BST surface, 100 mg/mL poly(TMS-*r*-NHS) modified BST surface and 10 mg/ml BSA immobilized surface, (a) Bode plot; (b) Nyquist plot.

The BSA immunosensor was developed based on the high energy ball-milled BST slurry that spun-coated on Pt layered Si substrate. The fabrication process and the device structure are as shown in Fig. 3.4 and Fig. 3.5 respectively. Fig. 4.12 shows the impedance measurement results of bare BST surface, poly(TMS-*r*-NHS) modified BST surface as well as BSA immobilized surface. When the BST film surface is being modified by poly(TMS-*r*-NHS), the impedance spectroscopy shows a significant higher interfacial resistance, indicating that the poly(TMS-*r*-NHS) hinders the electron transfer at the solid-liquid surface. After the surface is being immobilized with BSA, the measurement result shows more significant interfacial

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resistance change, indicating plenty of BSA molecules are immobilized onto the surface. Fig. 4. 13 shows impedance spectroscopy for anti-BSA reacted surface. The sample incubated in higher concentration of anti-BSA solution gives higher interfacial resistance, indicating more anti-BSA molecules are bonded with the immobilized BSA molecules.

Fig. 4. 13: Impedance spectroscopy for different concentration of anti-BSA reacted surface, (a) Bode plot; (b) Nyquist plot.

The impedance change before and after antigen-antibody interaction can be calculated as follows:

$$\Delta Z = Z_{Ab-Ag} - Z_{Ab} \tag{4.1}$$

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where Z_{Ab-Ag} is the value of impedance after anti-BSA binding to BSA, Z_{Ab} is the value of the BSA immobilized surface. From Fig. 4. 14, a linear relationship is observed between the impedance change and the logarithmic anti-BSA concentrations. Therefore, it shows that the sensor can be used as immunosensor to determine the concentration of BSA antibody. When other types bio-molecules are immobilized onto the device surface, our sensor can also be used to detect the counter part of the immobilized bio-molecules, such as immobilized human IgG can be used to detect anti-human IgG.

Fig. 4. 14: Impedance change after anti-BSA binding to BSA vs. anti-BSA concentration.

An equivalent circuit as shown in Fig. 4.15 has been developed to fit into the measurement data. As a result, the sensing mechanism can be explained. The circuit model includes the ohmic resistance of water, R_s , the Warburg impedance, Z_w , resulting from the diffusion of ions from bulk water to the electrode/water interface, the double layer capacitance, C_{dl} , charge transfer resistance, R_{ct} , the resistance and capacitance of the Pt electrode modifying layers that includes BST composite film,

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poly(TMS-r-NHS) polymer layer, immobilized BSA and anti-BSA layer represented by R_m and C_m , the R_s and Z_w represent the bulk properties of the water and diffusion of the dissociated ions, respectively. Therefore, they are not affected by chemical transformations occurring at the electrode interface. Both C_{dl} and R_{ct} depend on the dielectric and insulating features at the electrode/water interface. R_m and C_m represent insulating and the dielectric features of the electrode surface modifying layer. The total impedance is determined by all of the components. Fig. 4. 16 shows a very good agreement between the measured result and the numerical fitted result from the equivalent circuit.

Fig. 4. 15: Equivalent circuit used to fit the measured impedance of the sensor.

Fig. 4. 16: The fitting result of the impedance spectroscopy and the data measured at 10 µg/ml anti-BSA surface. Solid Square denotes experimental value; Solid Line denotes numerical fits from the equivalent circuit.

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The corresponding parameter values of the equivalent circuit elements with different anti-BSA concentration reacted surfaces are shown in Table 4.1. When the anti-BSA concentration increases from 100 ng/ml to 1 mg/ml, R_s , R_{ct} and R_m are increased whilst C_{dl} and C_m are decreased. The increment of R_s is because of the reduced quantity of dissociated H^+ and OH^- when more anti-BSA molecules bind onto the surface. R_{ct} increases due to the charge transfer rate at the electrode/water interface is further hindered when more anti-BSA molecules are bonded to the surface. The insulating characteristics of the anti-BSA increases the resistivity of the electrode modifying layer. C_{dl} decreases due to the formation of the antigen-antibody complex that increases the thickness of the double layer existing at the electrode /water interface. C_m decreases also because of the thickness increase of the electrode modifying layer. No significant change is observed for Z_w parameters. Among all the components, R_{ct} is the dominant one for the impedance at the central part of the measured frequency range. Comparing to typical electrochemical immunosensor, our sensor has the extra impedance contributing parts of R_s , R_m and C_m . Therefore, the sensitivity of the sensor is enhanced by considering the net effects of all those parameters, since when more bio-molecules are immobilized on the surface, all the parameters' change will result in higher impedance value of the device.

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Electrode surface condition	R _s (k ohm)	R _{ct} (k ohm)	C _{dl} (f)	Z _w			R _m (k ohm)	C _m (f)
				R (k ohm)	T	P		
1mg/ml anti-BSA	2720	105970	4.0E-11	71926	0.0074	0.42	561440	6.2E-12
100 µg/ml anti-BSA	2465	60865	6.8E-11	88726	0.0086	0.42	454710	6.8E-12
10 µg/ml anti-BSA	2174	28867	1.1E-10	85202	0.0073	0.41	372150	7.4E-12
1 µg/ml anti-BSA	1872	13596	2.3E-10	70704	0.0087	0.41	317480	8.5E-12
100 ng/ml	1604	8392	4.2E-10	76565	0.0073	0.40	263720	9.9E-12

Table 4. 1: The corresponding parameters of circuit elements at different surface condition for the BST biosensor.

4.2.3 Impedance analysis of EBV Immunosensors

Upon obtaining the linear relationship between the impedance change and the logarithm of anti-BSA concentration on the BSA immunosensor, two types of BST composite films were developed based on the original BST powder and the BST powder under high energy ball-milling process to examine the sensitivity of the devices. Four samples were chosen for the experiments. Sample 1 and sample 3 were developed based on the original BST powder whilst sample 2 and sample 4 were developed based on the ball-milled BST powder. All of the four samples were subjected to the same surface modification by poly(TMS-r-NHS) to capture protein molecules subsequently. The impedance spectroscopy is measured on poly(TMS-r-NHS) and it is noticed that the impedance of the four samples are in the same order. They are 234 kΩ, 212 kΩ, 181 kΩ and 247 kΩ at 1 kHz respectively.

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Subsequently, the samples were immersed into 0.85 mg/ml EBV solution for 16 hours for immobilization. Buffered solution in the mixture of 10 mM 2-amino-2-hydroxymethyl-1,3-propanediol (Tris) HCl, 200 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA) and 10 mM maltose, with a pH 7.4 was used. After 16 hours of incubation at 4 °C, the samples were taken out and rinse thoroughly with PBS and DI water to remove non-specific EBV bindings on the device surfaces. After the EBV immobilization, all of the samples were then immersed into 10 mg/mL BSA for another 16 hours to block and fill the function groups on the poly(TMS-r-NHS) that may not be filled by the EBV molecules. The basis is to eliminate non-specific binding of anti-EBV to the available function groups on the poly(TMS-r-NHS) subsequently. The impedance measured on EBV-BSA filled surfaces of sample 1 and sample 3 at 1 kHz are 954 k Ω and 995 k Ω whilst the impedance of sample 2 and sample 4 are 655 k Ω and 575 k Ω . This shows that the surfaces of sample 1 and sample 3 are able to immobilize more protein molecules compare to the sample 2 and sample 4. This is due to the surfaces of sample 1 and sample 3 are derived based on the original powder giving higher film porosity and thus providing more functional groups for protein immobilizations. This is consistent with the Fluorescence observation. Two human serum samples, one from a normal person as well as one from a nasopharyngeal carcinoma (NPC) patient, were obtained to examine the feasibility of the developed EBV immunosensor. The serum from the NPC patient was confirmed to contain anti-EBV using ELISA. 50 μ L of the normal person's serum was each dropped onto the surface of sample 1 and sample 2. 50 μ L of NPC patient's serum was each dropped onto the surface of sample 3 and sample 4. After 1 hour of incubation at room temperature, the samples

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were rinsed with PBS and DI water thoroughly, and impedance spectroscopies were measured on the devices. In sample 1, which consists of composite film developed based on original powder and tested with the normal person serum, the impedance obtained at 1 kHz was 1187 k Ω . The impedance change in percent due to the reaction of the serum with the EBV immunosensor was 24.4%. On the other hand, the sample 3 which also consists of the composite film developed based on the original powder was tested with the NPC patient's serum and the resultant impedance obtained at 1 kHz was 1329 k Ω yielding a the impedance change of 33.7%. In contrast, sample 2, which consists of the composite film developed based on ball-milled powder and tested with the normal person's serum, the impedance obtained at 1 kHz was 929 k Ω . The impedance changed due to the reaction of the serum with the EBV immunosensor was 41.8%. Sample 4, which also consists of the composite film developed based on the ball-milled powder was tested with the NPC patient's serum and the resultant impedance obtained at 1 kHz was 817 k Ω yielding a impedance change of 42.2%.

It is observed that the device impedances of the four samples have been increased with the binding to the normal and patient's serum. This is due to the normal person's serum also contains anti-EBV but the concentration is much lower than that in the patient's serum. Comparing both types of EBV immunosensor derived based on the original BST powder as well as the ball-milled BST powder, it can be clearly seen from Table 4. 2 that the immunosensor derived based on the original BST powder has higher ability to distinguish patient's and normal person's serum. The impedance changed due to the normal person's serum was 24.4% and the impedance

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changed due to the patient’s serum was 33.7%. Therefore, it is able to make a clear distinction of 9.3% shift between normal and patient’s serum yielding higher sensitivity as compare to the immunosensor derived based on the ball-milled BST powder which has merely 0.4% shift. Details of the impedance measurement data are tabulated in Table 4. 2 and the measurement results for the four samples are plotted in Fig. 4. 17.

At 1kHz	Impedance measured (kOhm)	Impedance changed (%)
Sample 1:		
Polymer on Original powder	233	-
EBV-BSA	954	309
Normal anti-EBV	1187	24.4
Sample 3:		
Polymer on Original powder	211	-
EBV-BSA	994	370
Patient anti-EBV	1329	33.7
Sample 2:		
Polymer on Milled powder	181	-
EBV-BSA	655	261
Normal anti-EBV	929	41.8
Sample 4:		
Polymer on Milled powder	247	-
EBV-BSA	574	133
Patient anti-EBV	817	42.2

Table 4. 2: Comparisons of Normal and Patient’s anti-EBV response on Original powder composite films and Milled powder composite films.

This chapter describes the BST composites characterization results through DTA, TGA and XRD analyses. BST powder is characterized by using particle size analyzer

to estimate the powder size, FESEM to observe the BST powder formation before and after the ball milling process. The BET surface area analysis technique has helped to re-affirm the results obtained from the Laser particle size analyzer that the powder size is reduced after the ball milling process. Immobilized BSA was observed through Fluorescent microscopy and it can be clearly seen from images obtained that the composite film developed from the original BST powder shows higher fluorescent intensity than that of the film developed from the ball-milled BST powder. The bio-sensing devices were characterized by using impedance analysis techniques. The impedance analyzer sweeps across a wide spectrum from 0.1Hz to 0.1MHz.

It is observed that impedance is stable at 1kHz for the bio-sensing devices and hence this reference frequency is chosen for subsequent analysis. BSA Immunosensors are characterized with comparison of bare BST surface and bare 100mg/mL poly(TMS-*r*-NHS) modified BST surface through impedance analyzer. The impedance of BSA immobilized surface is the highest compared to impedance measured on bare 100mg/mL poly(TMS-*r*-NHS). Impedance measured on BST surface is the lowest among these 3 samples at 1kHz. This is due to when the BST surface is modified by poly(TMS-*r*-NHS), the poly(TMS-*r*-NHS) hinders the electron transfer at the solid-liquid interface. When BSA is immobilized onto poly(TMS-*r*-NHS), more significant interfacial impedance is observed.

Fig. 4. 17: Impedance spectroscopy for four BST surface modified samples immobilized with EBV-BSA reacted with different anti-EBV concentrations.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- (1) A simple and sensitive immunosensor device with labelless analyte detection capability was developed. It has simplified the sensing process significantly.
- (2) The impedance spectroscopy has proved to be an effective measurement method for the immunosensor structure developed in this project. The measurement results provide information on the kinetic process of biomolecule interactions and the charge-transfer behaviours.
- (3) The surface modification by poly(TMS-r-NHS) on BST composite films demonstrates the capability of immobilizing different proteins of BSA and EBV onto the device. Thus the immunosensor structure is suitable to be used in determining a wide range of antibody or antigen concentrations.
- (4) A novel impedimetric immunosensor based on sol-gel derived BST composite films have been successfully developed. The BST composite film plays an important role in dissociating the water molecules into H^+ and OH^- which contributed to the charge transfer between the two electrodes when an electrical field is applied across the immunosensor. The phenomenon has increased the device sensitivity considerably.

Chapter 5 Conclusions and Recommendations

- (5) BSA immunosensors were developed and characterized. Linear relationship was observed between the device impedance change and the logarithmic of anti-BSA concentrations.
- (6) To further explore the device capabilities, two types of EBV immunosensors were developed and characterized based on the original BST powder and the ball-milled BST powder. The device based on the original BST powder revealed higher film porosity yielding higher sensitivity.

5.2 Recommendations

- (1) It has been found that BST composite films with higher porosity provide larger surface specific area and leads to higher device sensitivity. Therefore, a study of the relationship between the film porosity and device sensitivity would help to further improve the device performance.
- (2) Till now only one type of NPC patient serum that contains considerably high concentration of anti-EBV was obtained for sensor characterizations. In order to determine the device detection limit and sensitivity, a wide range of anti-EBV concentrations is needed. One of the possible processes to obtain various concentration of the anti-EBV serum is by diluting the existing NPC patient's serum.

Chapter 5 Conclusions and Recommendations

- (3) In principle, the immunosensor developed is able to immobilize any receptor, in this case, antibody or antigen on the modified BST surface to detect the analyte of interest. Therefore, other types on antibody-antigen interaction on the immunosensor should be studied.
- (4) Need to study the specificity by introducing interfering antigens and antibodies into the analyte of interest.
- (5) Since the immunosensor is small in size, and the measurement technique is effective and could produce rapid results, a sensor array could be developed to detect a wide range of antibodies or antigens simultaneously.
- (6) The immunosensor based on the BST material has made the sensor compatible with silicon integrated circuit. Therefore, an intelligent biosensor integrated circuit could be developed in near future.

AUTHOR PUBLICATIONS

- [1] LingLing Sun, Ooi Kiang Tan, **Lay Im Tan**, ZhiHong Wang and WeiGuang Zhu, “Structure and electrical properties of $\text{Ba}_{0.67}\text{Sr}_{0.33}\text{TiO}_3$ thick films fabricated using a modified sol-gel technique”, 9th International Conference on Advanced Materials (ICAM 2005), July 2005, Singapore, Accepted
- [2] Sun Lingling, Tan Ooi Kiang, Mao Baowei, **Tan Lay Im**, Gan Leong Huat, Gan Yik Yuen, “ A novel impedimetric immunosensor based on sol-gel derived Barium Strontium Titanate composite film”, the 5th IEEE conference on sensors, October 2006, Korea, Accepted

REFERENCES

- [1] Reshetilov AN, "Microbial, enzymatic, and immune biosensors for ecological monitoring and control of biotechnological processes", *Applied Biochemistry And Microbiology* 41 (5) (2005) 442-449.
- [2] Retama JR, Mecerreyes D, Lopez-Ruiz B, et al., "Synthesis and characterization of semiconducting polypyrrole/polyacrylamide microparticles with GOx for biosensor applications", *Colloids And Surfaces A-Physicochemical And Engineering Aspects* 270 (2005) 239-244.
- [3] Srivastava S, Singh P, Bhagat R, et al., "Application of bacterial biomass as a potential metal indicator", *Current Science* 89 (7) (2005) 1248-1251.
- [4] Tkac J, Svitel J, Sturdik E, "Biosensors in the determination of saccharides", *Chemicke Listy* 93 (9) (1999) 563-569.
- [5] S. L. R. Barker, R. Kopelman, T. E. Meyer and M. A. Cusanovich, "Fiber-optic nitric oxide-selective biosensors and nanosensors", *Anal. Chem.*, (1998) 70 971.
- [6] N. Isola, D. L. Stokes and T. Vo-Dinh, "Surface-enhanced Raman gene probe for HIV detection", *Anal. Chem.*, (1998) 70 1352.
- [7] F. Tobalina, F. Pariente, L. Hernandez and H. D. Abruna, "Carbon felt composite electrodes and their use in electrochemical sensing: a biosensor based on alcohol dehydrogenase", *Anal. Chim. Acta*, (1998) 358 15.

References

- [8] O. V. Fedosseeva, H. Uchida, T. Katsube, Y. Ishimaru and T. Iida, "Cholinesterase-based biosensor using surface photovoltage technique", *Electrochemistry*, (1999) 67 755.
- [9] C. Kosslinger, S. Drost, F. Aberl, H. Wolf, S. Koch and P. Woias, "Quartz crystal biosensor for measurement in liquids", *Biosens. Bioelectron.*, (1992) 7 397.
- [10] N. J. Geddes, E. M. Paschinger, D. N. Furlong, Y. Ebara, Y. Okahata, K. A. Than and J. A. Edgar, "Piezoelectric crystal for the detection of immunoreactions in buffer solutions", *Sens. Actuators B*, (1994) 17 125.
- [11] A. Hengerer, J. Decker, E. Prohaska, S. Hauck, C. Kosslinger and H. Wolf, "Quartz crystal microbalance (QCM) as a device for the screening of phage libraries", *Biosens. Bioelectron.*, (1999) 14 139.
- [12] T. Wessa, M. Rapp and H. J. Ache, "New immobilization method for SAW-biosensors: covalent attachment of antibodies via CNBr", *Biosens. Bioelectron.*, (1999) 14 93.
- [13] K. Hashimoto, H. Xu, T. Mukaigawa, R. Kubo, H. Zhu, M. Noda, M. Okuyama, "Si monolithic, microbolometers of ferroelectric BST thin film combined with readout FET for uncooled infrared image sensor", *Sens. Actuators A* 88 (2001) 10–19.
- [14] M. Noda, K. Hashimoto, R. Kubo, H. Tanaka, T. Mukaigawa, H. Xu, M. Okuyama, "A new type of dielectric bolometer mode of detector pixel

References

-
- using ferroelectric thin film capacitors for infrared image sensor”, *Sens. Actuators A* 77 (1999) 39–44.
- [15] W. Zhu, O. Tan, Q. Yan, J. Oh, “Microstructure and hydrogen gas sensitivity of amorphous (Ba, Sr)TiO₃ thin film sensors”, *Sens. Actuators B* 65 (2000) 366–370.
- [16] X. Chen, W. Zhu, O. Tan, “Microstructure, dielectric properties and hydrogen gas sensitivity of sputtered amorphous Ba_{0.67}Sr_{0.33}TiO₃ thin films”, *Mater. Sci. Eng. B* 77 (2000) 177–184.
- [17] G. Sharma, S. Agarwal, “Humidity sensing properties of (Ba, Sr)TiO₃ thin films grown by hydrothermal-electrochemical method”, *Sens. Actuators B* 85 (2002) 205–211.
- [18] Rickert J, Gopel W, Beck W, et al., “A 'mixed' self-assembled monolayer for an impedimetric immunosensor”, *BIOSENSORS & BIOELECTRONICS* 11 (8) (1996) 757-768.
- [19] W. Zhang, G. Li, "Third-Generation Biosensors Based on the Direct Electron Transfer of Proteins," *Analytical Science*, vol. 20, (2004) 603-609.
- [20] A. F. Collingsy, F. Carusozx, "Biosensors: recent advances," *Rep. Prog. Phys*, vol. 60, (1997) 1397-1445.
- [21] R. S. Freire, C. A. Pessoa, L. D. Mello, L. T. Kubota, "Direct Electron Transfer: An approach for Electrochemical Biosensors with Higher
-

References

- Selectivity and Sensitivity," *Journal of the Brazilian Chemical Society*, vol. 14, (2003) 230-243.
- [22] Jasper Akerboom, Wageningen University, 2005, "<http://www.ftns.wau.nl/micr/bacgen/Jasper/>", Retrieved 28 February 2006 from the World Wide Web.
- [23] L. C. Clark and C. Lyons, "Electrode systems for continuous monitoring in cardiovascular surgery," *Annals of the New York Academy of Sciences*, vol. 102, (1962) 29-35.
- [24] D. W. Lubbers and N. Opitz, "Eine neue pCO₂-bzw: pO₂-Messonde zur Messung des pCO₂ oder pO₂ von Gasen und Flüssigkeiten," *Zeitschrift Fur Naturforschung C*, vol. 30, (1975) 532-533.
- [25] D. R. Thevenot, K. Toth, R. A. Durst, and G. S. Wilson, "Electrochemical Biosensors: Recommended definitions and classification", *Biosensors and Bioelectronics*, vol. 16, (2001) 121-131.
- [26] G. Sauerbrey, "Verwendung von Schwingquarzen zur Wagung dünner Schichten und zur Mikrowagung", *Z. Phys*, vol. 155, (1959) 206-222.
- [27] D. C. Cullen, R. S. Sethi, and C. R. Lowe, "Multi-analyte miniature conductance biosensor", *Analytica Chimica Acta*, vol. 231, (1990) 33-40.
- [28] M. Stelzle and E. Sackmann, "Sensitive detection of protein adsorption to supported lipid bilayers by frequency-dependent capacitance measurements

References

-
- and microelectrophoresis", *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 981, (1989) 135-142.
- [29] P. Vincenzini, R.W. Whatmore, "Fundamentals of ceramic engineering", Elsevier Applied Science, Chap. 5, (1991).
- [30] Yimnirun R, Ananta S, Laoratanakul P, "Dielectric and ferroelectric properties of lead magnesium niobate-lead zirconate titanate ceramics prepared by mixed-oxide method", *JOURNAL OF THE EUROPEAN CERAMIC SOCIETY* 25 (13) (2005) 3235-3242.
- [31] Zhu C, Yong Z, Yang CT, et al., "PbO volatilization and annealing conditions investigation of $\text{Pb}(\text{Zr}_{0.52}\text{Ti}_{0.48})\text{O}_3$ thin films fabricated by sol-gel method", *MATERIALS SCIENCE AND ENGINEERING B-SOLID STATE MATERIALS FOR ADVANCED TECHNOLOGY* 123 (2) (2005) 143-148.
- [32] E.N. Bunting, G.R. Shelton and A.S. Creamer. "Ceramic properties and its application", *J. Res. Natl. Bur. Std.* 38, (1947) 337-349.
- [33] H.J. Cho, C.S. Kang, C.S. Hwang, et al., "Structural and electrical properties of $\text{Ba}_{0.5}\text{Sr}_{0.5}\text{TiO}_3$ thin films on Ir and IrO_2 electrodes", *Jpn., J. Appl. Phys.* 36 (7A) (1997) 874– 876.
- [34] T. Kawakubo, S. Komatsu, K. Abe, et al., "Ferroelectric properties of $\text{SrRuO}_3/(\text{Ba,Sr})\text{TiO}_3/\text{SrRuO}_3$ epitaxial capacitor", *Jpn. J. Appl. Phys.*, 37 (9B) (1998) 5108– 5111.
-

References

-
- [35] H. Zhu, M. Noda, T. Mukaigawa, et al., "Application of ferroelectric BST thin film prepared by MOD for uncooled infrared sensor of dielectric bolometer mode", T. IEE Jpn. 120-E (12) (2000) 554–558.
- [36] W. Zhu, O.K. Tan, J. Ding, et al., "Preparation, property, and mechanism studies of amorphous ferroelectric (Ba,Sr)TiO₃ thin films for novel metal–ferroelectric–metal type hydrogen gas sensors", J. Mater. Res. 15 (6) (2000) 1291–1302.
- [37] O.K. Tan, X.F. Chen, W.G. Zhu, "Ferroelectric (Ba,Sr)TiO₃ thin films for H₂ gas detection by sol–gel and RF sputtering", Ferroelectrics 225 (1999) 295–302.
- [38] H. Arai, S. Ezaki, Y. Shimizu, O. Shippo, T. Seiyama, "Semiconductive Humidity sensor of perovskite type oxide", Proc. Int. Meet. Chem. Sens. Fukuoka, Japan 19-22 (1983) 393-398.
- [39] Y. C. Yeh, T. Y. Tseng, "Trans. Components", Hybrids Manufact. Technol. 12 (1989) 259.
- [40] Y. C. Yeh, T. Y. Tseng, "Analysis of the d.c and a.c. properties of K₂O doped porous ceramics", J. Mater. Sci. 24 (1989) 2739-2745.
- [41] Y. C. Yeh, T. Y. Tseng, "Humidity sensitive electrical properties of (Ba_{0.5}Sr_{0.5})TiO₃", J. Mater. Sci. Lett. 7 (1988) 766-768.
- [42] H. Janez, S. Jaroslav, H. Marko, Sens. Actuators B 26 (1995).
-

References

- [43] S. Jaroslav, H. Janez, H. Marko, C. Miran, *Sens. Actuators B* 7 (1992) 439-442.
- [44] J. J. Fripiat, A. Jelli, G. Poncelet, J. Andre, *J. Phys. Chem.* 69 (1965) 2185-2197.
- [45] J. H. Anderson, G. A. Parks, *J. Phys. Chem.* 72 (1968) 362-3669.
- [46] Dorothy H. Crawford, "Principles and Practice of Clinical virology", John Wiley & Sons, Ltd., (2000) 117-139.
- [47] M. AlgueroÂ , M. L. Calzada and L. Pardo, "The Effect of Film Thickness on the Ferroelectric Properties of Sol-Gel Prepared Lanthanum Modified Lead Titanate Thin Films", *Journal of the European Ceramic Society* 19 (1999) 1481-1484.
- [48] Robert J. Hunter, "Foundations of colloid science", Oxford University Press, (2001) Chap. 1.
- [49] Drew Myers, "Surfaces, interfaces, and colloids: principles and applications", Wiley-VCH, (1999) Chap. 1.
- [50] Sarah Grant, Frank Davis, Karen A. Law, Andrew C. Barton, Stuart D. Collyer, Seamus P.J. Higson, Timothy D. Gibson, "Label-free and reversible immunosensor based upon an ac impedance interrogation protocol", *Analytica Chimica Acta*, 537 (2005) 163–168.

References

- [51] Eugenii Katz, Itamar Willner, “Probing Biomolecular Interactions at Conductive and Semiconductive Surfaces by Impedance Spectroscopy: Routes to Impedimetric Immunosensors, DNA-Sensors, and Enzyme Biosensors”, *Electroanalysis* 2003, 15, No. 11.
- [52] Zhaopeng Chen, Jianhui Jiang, Guoli Shen, Ruqin Yu, “Impedance immunosensor based on receptor protein adsorbed directly on porous gold film”, *Analytica Chimica Acta*, 553 (2005) 190–195.
- [53] Mikhail A. Vorotyntsev, Jean-Pierre Badialib, Gyorgy Inzeltc, “Electrochemical impedance spectroscopy of thin films with two mobile charge carriers: effects of the interfacial charging”, *Journal of Electroanalytical Chemistry*, 472(1), (1999) 7-19.
- [54] Old, L.J., Boyse, E.A., Oettgen, H.F. et al. “Precipitating antibody in human serum to an antigen present in cultured Burkitt’s lymphoma cells”, *Preceedings of the National Academy of Sciences of the USA* 56, (1966) 1699-1704.
- [55] Wolf, H., Zur Hansen, H. and Becker, V. “EBV viral genomes in epithelial nasopharyngeal carcinoma cells”, *Nature New Biology*, 244, (1973) 245-247.
- [56] Li Lu, Man On Lai, “Mechanical Alloying”, Kluwer Academic Publishers, (1998), Chap. 1.

References

- [57] F.M. Pontes, E. Longo, E.R. Leite, J.A. Varela, "Study of the dielectric and ferroelectric properties of chemically processed BST thin films", *Thin solid film*, 386, (2001) 91-98.
- [58] B.W. Mao, L.H. Gan, Y.Y. Gan, "Ultra high molar mass poly[2-(dimethylamino)ethyl methacrylate] via atom transfer radical polymerization", *Polymer*, 47 (2006) 3017–3020.
- [59] Bear, R., Bankier, A. T., Biggin, M.D. et al. "DNA sequence and expression of the B95-8 Epstein-Barr virus genome", *Nature*, 310, (1984) 207-211.
- [60] Roizman B. *Herpesviridae*. In fields BN, Knope DM, Howley PM (eds) "Field's Virology", Philadelphia: Lippincott-Raven, (1996) 2221-30.
- [61] Lindsey Hutt-Fletcher, "Persistent Viral Infections", John Wiley & Son Ltd, (1999) 243-268.
- [62] Xiaojing Yang, Xi Yao, Liangying Zhang, "The dielectrical properties of (B,Si) doped (Ba,Sr)TiO₃ thin films fabricated by sol-gel technique", *Ceramics International* 30 (2004) 1763–1765.
- [63] Y. Drezner, S. Berger, "Nanoferroelectric domains in ultrathin BaTiO₃ films", *J. Appl. Phys.* 94 (10) (2003) 6774– 6778.
- [64] Xu, J., Shaikh, A.S., Vest, R.W., "High K BaTiO₃ films from metalloorganic precursors, *Ultrasonics, Ferroelectrics and Frequency Control*", IEEE Transactions Vol. 36, (1989), 307-312.

References

- [65] Shaobo Liu, Meidong Liu, Yike Zeng, Churong Li, Shi Chen, Yanqiu Huang, Donglin Xia, "Preparation and characterization of $\text{Ba}_{1-x}\text{Sr}_x\text{TiO}_3$ thin films for uncooled infrared focal plane arrays", *Materials Science and Engineering C* 22 (2002) 73–77.
- [66] Brian R. Eggins, "Chemical sensors and biosensors", John Wiley & Sons, Ltd., (2002) 102-124.
- [67] Feng Yan, Peng Bao, Zhigang Zhang, Jinsong Zhu, Yening Wang, Helen L.W. Chan, Chung-Loong Choy, "Dielectric properties of $(\text{Ba}_{0.5}\text{Sr}_{0.5})\text{TiO}_3$ thin films", *Thin Solid Films*, 375 (2000), 184-187.
- [68] D. N. Kouvatsos, V. Ioannou-Sougleridis, A. G. Nassiopoulou, "Charging effects in silicon nanocrystals within SiO_2 layers, fabricated by chemical vapor deposition, oxidation, and annealing", *Applied Physics Letters*, Volume 82, (2003) 3.
- [69] Seema Agarwala, S.B. Samanta, G.L. Sharma, "Influence of pH on structural and electrical properties of sol-gel derived (Ba, Sr) TiO_3 thin films under humid conditions", *Thin Solid Films*, 447–448 (2004) 502–508.
- [70] Zheyao Wang, Jianshe Liu, Tianling Ren, Litian Liu, "Fabrication of organic PVP doping-based $\text{Ba}_{0.5}\text{Sr}_{0.5}\text{TiO}_3$ thick films on silicon substrates for MEMS applications", *Sensors and Actuators A*, 117 (2005) 293–300.
- [71] M. Alguero, M. L. Calzada and L. Pardo, "The Effect of Film Thickness on the Ferroelectric Properties of Sol-Gel Prepared Lanthanum Modified Lead

References

- Titanate Thin Films”, Journal of the European Ceramic Society, 19 (1999) 1481-1484.
- [72] H.Z. Jin, J. Zhu, P. Ehrhart, F. Fitsilis, C.L. Jia, S. Regnery, K. Urban, R. Waser, “An interfacial defect layer observed at (Ba,Sr)TiO₃/Pt interface”, Thin Solid Films, 429 (2003) 282–285.
- [73] A.L. Kholkin, R. Martins, H. Aguas, I. Ferreira, V. Silva, O.A. Smirnova, M.E.V. Costa, P.M. Vilarinho, E. Fortunato, J.L. Baptista, “Metal-ferroelectric thin film devices”, Journal of Non-Crystalline Solids, 299–302 (2002) 1311–1315.
- [74] Desheng Fu, Kazuyuki Suzuki, Kazumi Kato, “Polarization switching in CaBi₄Ti₄O₁₅ ferroelectric thin films”, Key Engineering Materials, Vol. 269 (2004), 41-44.
- [75] Jae Hyuk Jang, Ki Hyun Yoon, Ki Young Oh, “Electrical fatigue of ferroelectric PbZr_{0.5}Ti_{0.5}O₃ and antiferroelectric PbZrO₃ thin films”, Materials Research Bulletin, 35 (2000) 393–402.
- [76] Apurba Laha, S.B. Krupanidhi, “Effect of electric field on dielectric response of PMN–PT thin films”, Materials Science and Engineering B, 113 (2004) 190–197.
- [77] A.I. Burkhanov, A.V. Shil’nikov, S.A. Satarov, K. Bormanis, K.A. Sternberg, A. Kalvane, “Specifics of polarisation switching in PbNi_{1/3}Nb_{2/3}O₃–PbTiO₃–

References

- PbZrO₃ ferroelectric ceramics”, *Journal of the European Ceramic Society*, 24 (2004) 1541–1544.
- [78] B. Wang, K.W. Kwok, H.L.W. Chan, C.L. Choy, K.Y. Tong, E.Z. Luo, J.B. Xu, I.H. Wilson, “Study of polarization switching in PZT films with RuO₂ electrodes by conducting atomic force microscopy”, *Materials Characterization*, 48 (2002) 249–253.
- [79] Ronald Pethig, Douglas B Kell, “The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology”, *Phys. Med. Biol.*, Vol. 32, No 8, (1987) 933-970.
- [80] A. Dhahri, J. Dhahri, S. Zemni, M. Oumezzine, H. Vincent, “Structural, magnetic and magnetocaloric effect in double perovskite Ba₂CrMo_{1-x}W_xO₆”, *Journal Of Alloys And Compounds*, 420 (1-2), (2006) 15-19.
- [81] Cerecedo C, Valcarcel V, Guitian F, “Assisted sintering via sol-gel deposition in low-fired ceramic performs”, *Journal Of The American Ceramic Society*, 88 (8), (2005) 2300-2303.
- [82] Lupp PB, Sokoll LJ, Chan DW, “Immunosensors - principles and applications to clinical chemistry” *Clinica Chimica Acta*, 314 (1-2), (2001) 1-26.
- [83] Ghindilis AL, Atanasov P, Wilkins M, Wilkins E, “Immunosensors: Electrochemical sensing and other engineering approaches”, *Biosensors & Bioelectronics*, 13 (1), (1998) 113-131.
-

References

- [84] Lin JH, Ju HX, "Electrochemical and chemiluminescent immunosensors for tumor markers", *Biosensors & Bioelectronics*, 20 (8), (2005) 1461-1470.
- [85] Campanella L, Attioli R, Colapicchioni C, et al., "New amperometric and potentiometric immunosensors for anti-human immunoglobulin G determinations", *Sensors And Actuators B-Chemical*, 55 (1), (1999) 23-32.
- [86] M. Jain, S.B. Majumder, R.S. Katiyar, A.S. Bhalla, "Structural and dielectric properties of heterostructured BST thin films by sol-gel technique", *Thin Solid Films*, 447-448 (2004), 537-541.
- [87] S. Tosatti, R. Michel, M. Textor, N.D. Spencer, "Self-Assembled Monolayers of Dodecyl and Hydroxy-dodecyl Phosphates on Both Smooth and Rough Titanium and Titanium Oxide Surfaces", *Langmuir* 2002, 18, 3537-3548.
- [88] Ashok Kumar, Sriraj G. Manavalan, "Characterization of barium strontium titanate thin films for tunable microwave and DRAM applications", *Surface & Coatings Technology*, 2004.
- [89] Tomokazu Tanase, Yoshio Kobayashi, Toshihide Nabatame, Takao Miwa, Mikio Konno, "Dielectric properties of lead zirconate titanate thin films seeded with barium strontium titanate nanoparticles", *Thin Solid Films*, 471 (2005), 71-75.
- [90] Chun-Sheng Liang, Jenn-Ming Wu, "Electrical properties of W-doped $(\text{Ba}_{0.5}\text{Sr}_{0.5})\text{TiO}_3$ thin films", *Journal of Crystal Growth*, 274 (2005), 173-177.

References

-
- [91] X. Xhu, S.Lu, H.L.W.Chan, C.L. Choy, K.H. Wong, "Microstructures and dielectric properties of compositionally graded $(\text{Ba}_{1-x}\text{Sr}_x)\text{TiO}_3$ thin films prepared by pulsed laser deposition", *Applied Physics A*, 76, (2003) 225-229.
- [92] T.J. Zhang, H. Ni, W. Wang, "Preparation and Characterization of Epitaxial-Grown $\text{Ba}_{0.65}\text{Sr}_{0.35}\text{TiO}_3$ Thin Films by the Sol-Gel Process on Pt/MgO Substrates", *Journal of Materials Synthesis and Processing*, Vol. 10, 2002.
- [93] L.B. Kong, J. MA, T.S. Zhang, "Preparation of antiferroelectric lead zirconate titanate stannate ceramics by high-energy ball milling process", *Journal of Materials Science: Materials in electronics* 13, (2002) 89-94.
- [94] D. O'Neill, G. Catalan, F. Porras, R.M. Bowman, J.M. Gregg, "Thin film ferroelectrics for capacitor applications", *Journal of Materials Science: Materials in electronics* 9, (1998) 199-205.
- [95] Seema Agarwal, S.B. Samanta, G.L. Sharma, "Influence of pH on structural and electrical properties of sol-gel derived $(\text{Ba}, \text{Sr})\text{TiO}_3$ thin films under humid conditions", *Thin Solid Films*, 447-448, (2004) 502-508.
- [96] S.B. Krupanidhi, C.J. Peng, "Studies on structural and electrical properties of barium strontium titanate thin films developed by metallo-organic decomposition", *Thin Solid Films*, 305, (1997) 144-156.
- [97] N.V. Giridharan, R. Jayavel, P. Ramasamy, "Structural, Morphological and Electrical Studies on Barium Strontium Titanate Thin Films Prepared by Sol-Gel Tehncique", *Cryst. Res. Technol.*, 36, (2001) 65-72.
-

References

- [98] Z.G. Ban, S.P. Alpay, "Phase diagrams and dielectric response of epitaxial barium strontium titanate films: A theoretical analysis", *Journal of Applied Physics*, Vol. 91, (2002) 11.
- [99] Iwao Suzuki, Masahiro Ejima, Kenichi Watanabe, Yi-Ming Xiong, Tadashi Saitoh, "Spectroscopic ellipsometry characterization of $\text{Ba}_{0.7}\text{Sr}_{0.3}\text{TiO}_3$ thin films prepared by the sol-gel method", *Thin Solid Films*, 313-314, (1998) 214-217.
- [100] Hyun Jin Chung, Jae Hyoung Choi, Jeong Yong Lee, Seong Ihl Woo, "Preparation and electrical properties of $(\text{Ba}, \text{Sr})\text{TiO}_3$ thin films deposited by liquid source misted chemical deposition", *Thin Solid Films*, 382, (2001) 106-112.
- [101] M Yavuz, H Maeda, L Vance, H K Liu, S X Dou, "Effect of ball milling materials and methods on powder processing of Bi2223 superconductors", *Supercond. Sci. Technol.*, 11, (1998) 1153-1159.
- [102] I.C. Cosentino, E.N.S. Muccillo, R. Muccillo, R.M. Vichi, "Low-temperature sol-gel synthesis of single-phase ZrTiO_4 nanoparticles", *Journal of Sol-Gel Science Technology* 37, (2006) 31-37.
- [103] John. J. Steela, James P. Gospodyn, Jeremy C. Sit, Michael J. Brett, "Impact of morphology on high-speed humidity sensor performance", *IEEE Sensors Journal*, Vol. 6, (2006) 1.

References

- [104] Jerzy P. Lukaszewicz, Magdalena Skompska, "A novel carbon-based ionic conductor for humidity sensors", *Sensors and Actuators B*, 113, (2006) 970-977.
- [105] N. Kavasoglu, A.S. Kavasoglu, M. Bayhan, "Comparative study of ZnCr_2O_4 - K_2CrO_4 ceramic humidity sensor using computer controlled humidity measurement set-up", *Sensors and Actuators A*, 126, (2006) 355-361.
- [106] Thomas Carlsson, Janne Halme, Peter Lund, Petri Konttinen, "Moisture sensor at glass/polymer interface for monitoring of photovoltaic module encapsulants", *Sensors and Actuators A*, 125, (2006) 281-287.
- [107] J.D. Baniecki, R.B. Laibowitz, T.M. Shaw, P.R. Duncombe, D.A. Neumayer, D.E. Kotecki, H. Shen, Q.Y. Ma, "Dielectric relaxation of $\text{Ba}_{0.7}\text{Sr}_{0.3}\text{TiO}_3$ thin films from 1m Hz to 20 GHz", *Applied Physics Letter*, Vol. 72, (1998) 4.
- [108] J.M. Liu, B. Pan, K.F. Wang, H. Yu, "Frequency response and hysteresis dispersion scaling in ferroelectric $\text{SrBi}_2\text{Ta}_2\text{O}_9$ and $\text{Pb}(\text{Ti}_{0.48}\text{Zr}_{0.52})\text{O}_3$ thin films", *Ceramics International* 30, (2004) 1471-1475.
- [109] C. Schyberg, C. Plossu, D. Barbier, N. Jaffrezic-Renault, C. Martelet, H. Maupas, E. Souteyrand, M.H. Charles, T. Delair, B. Mandrand, "Impedance analysis of Si/SiO₂ structures grafted with biomolecules for the elaboration of an immunosensor", *Sensors and Actuators B*, 26-27, (1995) 457-460.

References

- [110] G. Di Francia, A. Castaldo, E. Massera, I. Nasti, L. Quercia, I. Rea, "A very sensitive porous silicon based humidity sensor", *Sensors and Actuators B*, 111-112, (2005) 135-139.