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An optical fiber sensor for the determination of hydrogen peroxide

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AN OPTICAL FIBER SENSOR FOR THE DETERMINATION OF HYDROGEN PEROXIDE

By

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Hydrogen peroxide is used in various fields, such as food preservative, bleaching, oxidizing, reducing, and chemical reaction reagents. Herein is described the effort to develop an optical fiber chemical sensor based on the evanescence wave absorbance that can detect the presence of, and measure the concentration of, hydrogen peroxide. For the H_2O_2 optical fiber sensor, a Nafion membrane was coated on the fiber optic. Titanium ions dispersed in the Nafion membrane can form a TiO-H_2O_2 complex with the H_2O_2 diffused into the membrane. The complex is shown to absorb light with a maximum absorption near 360 nm. The intensity of the absorbance peak is directly proportional to the concentration of H_2O_2. Additionally, coating polydimethylsiloxiane (PDMS) outside the fiber optic can detect H_2O_2 in high concentration 300ppm and high temperature 70^{o}C. Finally, the use of the developed optical fiber chemical sensor allows the direct determination of H_2O_2 in milk.

DEDICATION

I dedicate this work to my parents, Hu Haifeng and Zhang Cuirong; my husband, Zu Chengli; my daughter, Zu Xinyue (Caroline), and my son, Zu Xinqi (Kevin).

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CHAPTER I

INTRODUCTION

1.1 History

An "oxygenated acid" was synthesized through a simple experiment by Louis Jacques Thénard in 1818.[1] This so-called "oxygenated acid" later shown to be hydrogen peroxide. He dissolved barium peroxide in nitric acid in an ice bath, and observed gaseous bubbles were coming off from the solution the next morning he came back to the lab. The evolved gas was identified to be oxygen. At that time he concluded that the acid became oxygenated. Thenard noticed that the oxygenated acid (the liquid leftover from the reaction mentioned above) could be evaporated without forming solid residue. In fact, a number of acids and barium peroxide are able to form the oxygen rich liquid that is actually hydrogen peroxide solution. Thenard also prepared anhydrous hydrogen peroxide whose oxygen content was measured by gasometric analysis.

There are three approaches to prepare hydrogen peroxide. Firstly, barium peroxide reacts with acids (nitric acid, hydrochloric acid and sulfuric acid, etc.) by the discovery of Louis Jacques Thénard.[1] The disadvantages of this method in industrial production are: low concentration, low purity and high cost.[2] Secondly, water and oxygen can be directly converted to hydrogen peroxide by an electrochemical processes.[1] The third method is utilization of auto-oxidizable compounds (i.e.

alkylanthrahydroquinone) reacting with oxygen in air under suitable solution. The formed hydrogen peroxide can be separated from the reaction mixture, and the oxidized alkylanthrahydroquinone (alkylanthraquinone) is reduced back to alkylanthrahydroquinone by hydrogen under catalyst. This process can produce concentrated hydrogen peroxide with high purity. A typical reaction scheme is shown in Figure 1-1 (Adapted from reference 2)[2].

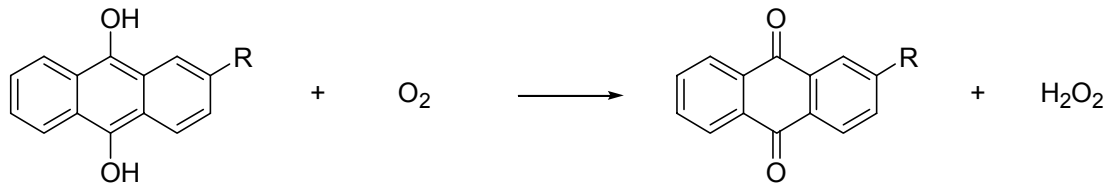

Figure 1- 1. Anthrahydroquinone autooxidation process for the manufacture of hydrogen peroxide.

1.2 Structure And Physical Properties

Hydrogen peroxide is a simple inorganic compound with the formula H_2O_2.[1] Hydrogen peroxide has a skew chain configuration (see Figure 1-2). This configuration minimizes repulsion between the oxygen lone pairs and the O-H bond pairs. It is relatively stable at ambient conditions. By a single-crystal neutron-diffraction study[3], the O-O-H angle is determined to be 102.7°. The dihedral angle is 90.2° in solid state.

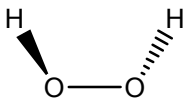

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Figure 1- 2. Structure of hydrogen peroxide, **1**.

Hydrogen peroxide is a clear, colorless liquid. Hydrogen bonds can form between hydrogen peroxide and water and these liquids are miscible in all proportions. There are different physical properties for different concentration of hydrogen peroxide. The physical properties of hydrogen peroxide are much different from water, even though they seem to be similar. Table 1-1 (adapted from reference 1 and 2) shows a comparison of the important properties between anhydrous hydrogen peroxide and water. High concentration hydrogen peroxide can be obtained by distillation.

Table 1- 1. Physical properties of hydrogen peroxide and water.[1, 2]

Property	Hydrogen peroxide (H_2O_2)	Water (H_2O)
Molecular weight	34.016	18.016
Boiling point (^{0}C)	150.2	100
Freezing point (^{0}C)	-0.43	0
Heat of vaporization ($Jg^{-1}K^{-1}$)		
25 ^{0}C	1519	2443
b.p	1387	2258
Specific heat ($Jg^{-1}K^{-1}$)		
Liquid (25 ^{0}C)	2.629	4.182
Gas (25 ^{0}C)	1.352	1.865
Relative density (g cm^{-1})		
0 ^{0}C	1.4700	0.9998
20 ^{0}C	1.4500	0.9980
25 ^{0}C	1.4425	0.9971
Dielectric constant, 20^{o}C	73.1	80.4

1.3 Chemical Properties And Applications

The applications of hydrogen peroxide can be classified based on its chemical properties. Because the oxidation state (–1) of oxygen in hydrogen peroxide is between

oxygen molecule (0) and water (-2), both oxidation and reduction reactions can occur readily. As an oxidizing reagent, H_2O_2 can be used in a number of fields[2], such as in industrial purification, bleaching, and as an oxidizing reagent in organic synthesis. Compared to oxygen, production of hydrogen peroxide is simpler in operation and higher in selectivity.

Hydrogen peroxide plays an important role in chemical purification in industry. Good color, odor and purity are desired in every industrial product. Contaminants, in crude oil and coal can be removed by the use of hydrogen peroxide. Also, hydrogen peroxide is a good bleaching reagent. Dilute solutions of hydrogen peroxide can be used to bleach wool, hair, fur, and feather, which are easily damaged by stronger bleaching solutions[1].

Hydrogen peroxide can also be used as a reducing reagent. In acidic solution, hydrogen peroxide can reduce manganese (IV) oxide to manganese (II) salts and form oxygen gas[2].

These oxidation and reduction reactions can occur at the same time. One example is the decomposition of hydrogen peroxide under heat, light irritation or high pressure. (see Figure 1-3)

$$2H_2O_2 \xrightarrow{\triangle} O_2\uparrow + 2H_2O\uparrow$$

Figure 1- 3. Decomposition of hydrogen peroxide under heat or light irritation.

Hydrogen peroxide can also be used in the production of building materials[1]. The gas released by hydrogen peroxide helps produce porous blocks. One of the advantages

of using hydrogen peroxide is that the gas (just oxygen gas) does no harm to the environment.

Hydrogen peroxide is a good reagent to dissolve some metal ions (vanadium, molybdenum and tungsten)[2], which are difficult to dissolve in water. When adding hydrogen peroxide, these metals form complexes that are easy to dissolve in water with hydrogen peroxide. Additionally, the H_2O_2-metal ion complexes usually present colors different from the free metal ions; therefore it can be used as an analytical reagent in the titration of metal ions. For example, titanium ion can be detected by observing the yellow color change when hydrogen peroxide is added[4].

1.4 Hazards

Hydrogen peroxide is useful in various fields, but workers should be aware of its hazards, especially when concentrated hydrogen peroxide is used. Improper usage may cause unpredicted incidents.

When hydrogen peroxide is used as a bleaching reagent, the solvent should be carefully selected. Ketone cannot be used as solvent since an explosion can occur[1]. Highly concentrated hydrogen peroxide can whiten skin upon contact due to its oxidation property. Therefore, it is necessary to use proper protection equipment, like gloves. Highly concentrated hydrogen peroxide also induces eye irritation. Dilute hydrogen peroxide is usually considered safe.

The decomposition of hydrogen peroxide is a disadvantage in its storing. The decomposition of hydrogen peroxide can cause high pressure. In sealed equipment, H_2O_2

may induce explosion[1]. It can pose a combustion hazard due to the formation of pure oxygen gas.

1.5 Analytical Methods

Since hydrogen peroxide is widely used in various fields, the demand to determine concentration levels in various applications has led to the development of many analytical methods. A wide variety of approaches are available for this goal, such as chemiluminescence,[5-10] electrochemistry,[11-19] spectrophotometry,[20-23] and fiber-optic sensors[24-27].

1.5.1 Chemiluminescence

When a chemical reaction only gives out light (luminescence) but not heat, this phenomenon is called chemiluminescence. This process is expressed in two equations below: (adapted from reference 5)

$$\mathbf{A} + \mathbf{B} \rightarrow \mathbf{C}^* + \mathbf{D}$$

$$\mathbf{C}^* \rightarrow \mathbf{C} + h\nu$$

where, **A** and **B** are reactants, and **C*** is the excited state of the product **C**.[5] When **C*** releases energy to go back to its ground state **C**, light can be emitted. The intensity of emission can be related back to the concentration of reactant.

Hydrogen peroxide can react with some chemiluminescent reagents to form compound in its exited state. A calibration line can be constructed for the determination of concentration of hydrogen peroxide in solution[6]. For example, luminol is a common

chemiluminescence reagent. It reacts with hydrogen peroxide in the presence of catalyst, such as cobalt (II)[6]. Recently, Yamada et al have reported a chemiluminescent flow sensor for hydrogen peroxide using luminol as chemiluminescent reagent[10]. The luminol solution was pumped through Dowex-50W resin containing immobilized Co (II)-monoethanolamine complex. Once hydrogen peroxide was injected, the rapid decomposition of hydrogen peroxide was observed as emitted luminescence.

Other compounds also can be used for this goal, such as pyrogallol[28] or lucigenin,[29] directly or indirectly, in weakly alkaline solutions. In each case, the intensity of chemiluminescence is proportional to the concentration of hydrogen peroxide.

1.5.2 Electrochemistry

Electrochemical methods have been found useful for determination of hydrogen peroxide due to their low detection limits and rapid response times. These methods are based on direct reduction or oxidation of hydrogen peroxide, where voltage (or current) change can be detected. There are a lot of publications to detect the concentration of hydrogen peroxide with the method. However, it is limited by harsh working conditions, such as high acid or the presence of electric field and poor reproducibility.[14, 30]

1.5.3 Spectrophotometry

Spectrophotometric methods require a reagent that can sense the presence of hydrogen peroxide. The signal can be detected with a spectrophotometer. The reagent usually forms complexes with hydrogen peroxide therefore leading to the color change, light absorption or fluorescence enhancement.

One of the most commonly used reagents is titanium (IV), which turns to a yellow color upon the addition of hydrogen peroxide[22]. The color change can be observed by the naked eye or a spectrometer. Takamura and co-workers[23] reported the use of titanium in the presence of a dye molecule for determination of hydrogen peroxide. A trimeric complex of Ti (IV), 2 - ((5 - bromopyridyl) azo) – 5 - (N – propyl – N - sulfopropylamino) phenol disodium (abbreviated as PAPS), and hydrogen peroxide in solution was thought to be formed at a ratio of 1:1:1 (see Figure 1-4 adapted from reference 23). The complex has a red-purple color (λ_{max} 539 nm), which is different from that (λ_{max} 450 nm) of Ti (IV)-PAPS. The absorbance of the complex at λ_{max} 539 nm is proportional to the concentration of hydrogen peroxide.

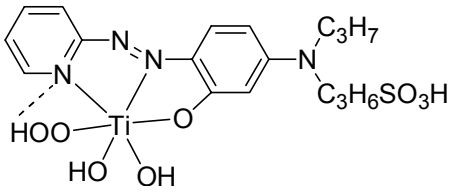


Figure 1- 4. The structure of 1:1:1 Ti-PAPS-H_2O_2 complex.

1.5.4 Optical Fiber Sensors for Hydrogen Peroxide

Fiber optic sensor is also an important method for determination of trace amounts of hydrogen peroxide. One example is the use of spectrophotometer as detector by immobilizing the indicator dye Meldola Blue (MB) into sol-gel material, which is coated outside the fiber optic. However, the disadvantage of this method is its irreversibility.[24] In 1988, Wolfbeis et al.[25] developed an optical fiber sensor for determination of hydrogen peroxide. It utilized the decomposition reaction of hydrogen peroxide to produce oxygen.

The amount of oxygen was quantitatively measured via fluorescence quenching of a dye molecule due to the production of oxygen gas. The dye molecule was embedded into the sol-gel material, allowing a continuous detection.[26] Later, they reported another type of optical fiber sensor using europium tetracycline (EuTc) embedded into a polyacrylonitrile-co-polyacrylamide polymer matrix as an indicator. The intensity of fluorescence is quantitatively enhanced with the amount of hydrogen peroxide in solution.[27]

1.6 Overview Of Optical Fiber Sensors

1.6.1 Structure of fiber optics and light guiding in the fiber optics

Fiber optics are made up of an optical fiber core, a cladding layer coated outside the optical fiber core and a jacket. The optical fiber core can be fused silica, metal oxide doped silica, or organic polymer (see Figure 1-5). The optical fiber core guides light wave by the total internal reflection. The cladding layer protects the light from leaking out of the optical fiber core. The cladding layer is usually an organic polymer, which has smaller refractive index n_2 compared with the refractive index n_1 of the core. Most of the jackets are also an organic polymer, which can prevent the breaking of the optical fiber core.

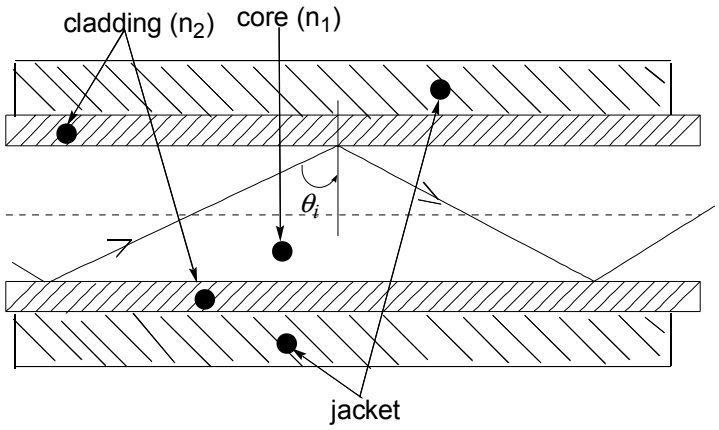


Figure 1- 5. Diagram of the structure of an optical fiber and a light beam propagating inside the optical fiber core by total internal reflections.

The light wave propagating inside the optical fiber core by the total internal reflection is governed by Snell's law:[31] (see Figure 1-6)

$$n_1 \bullet \sin(\theta_i) = n_2 \bullet \sin(\theta_r) \tag{1.1}$$

where θ_i is the angle of incident light in medium one, which is the optical fiber core; while θ_r is the angle of the refracted light in medium two, which is an optical fiber cladding. n_1 is the refractive index of medium one, while n_2 is the refractive index of medium two.

The refractive index (n) is defined as:

$$n = \frac{c}{v} \tag{1.2}$$

where c is the velocity of light in vacuum (3×10^8 m/sec), and v is the velocity of light in the media of interest. The refractive index is always greater than 1.

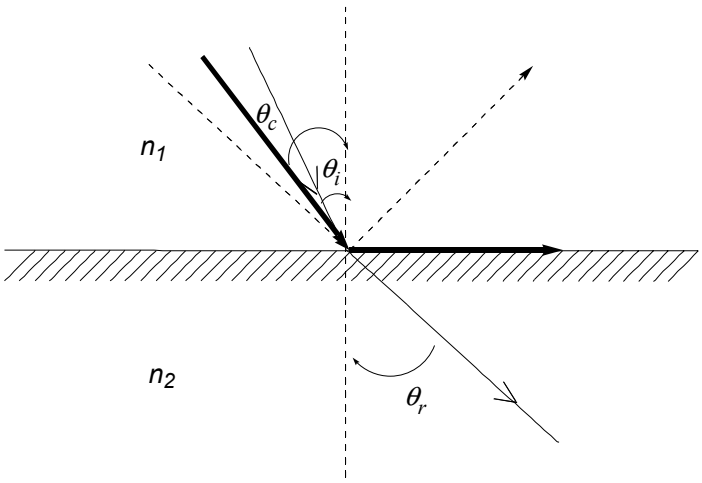


Figure 1- 6. Snell's law.

Since the refractive index of medium one is larger than that of medium two ($n_1 > n_2$), the angle of the incident light is smaller than that of the refracted light ($\theta_i < \theta_r$). When the angle of the refracted light (θ_r) reaches to 90 degrees, there is an equation as belows.

$$\theta_i = \theta_c = \sin^{-1}(\frac{n_2}{n_1}) \qquad \text{if } n_1 > n_2 \tag{1.5}$$

where θ_c is the critical angle of incidence. If the angle of incidence (θ_i) is larger than the critical angle (θ_c), the light wave is reflected completely back to medium one, therefore propagating inside the fiber with little loss in intensity. Also, transparent materials are one of the requirements for both the optical fiber core and the cladding in the long distance propagation.

1.6.2 Evanescence wave of optical fiber chemical sensors

When the light propagates within an optical fiber, it can be absorbed or scattered by the analyte. The change of intensity of the light is proportional to the concentration of the analyte. According to the location of the analyte, optical fiber chemical sensors can be divided into active core optical fiber chemical sensors and evanescence wave optical fiber chemical sensors. For active core optical fiber chemical sensors, the analyte is immobilized in the optical fiber core. The absorption or scattering of the light occurs in the optical fiber core. For evanescence wave optical fiber chemical sensors, the analyte is immobilized in the optical fiber cladding. The absorption or scattering of the light occurs in the optical fiber cladding.

Evanescence wave of optical fiber chemical sensors, in optical theory, can be formed when the sinusoidal incident light waves propagates in the optical fiber core by the total internal reflection. Evanescence, in literal theory, means "tending to vanish". Exactly, the intensity of evanescent wave decays exponentially with the distance from the optical fiber core and cladding interface. The intensity of evanescence wave with the distance can be expressed as belows[32].

$$I(x) = I_0 \exp\left(-\frac{x}{d_p}\right) \tag{1.6}$$

where I is the intensity at the distance x from the optical fiber core and cladding interface; I_0 is the intensity at the optical fiber core and cladding interface, and d_p is the penetrated depth into the optical fiber cladding, which can be given by

$$d_p = \frac{\lambda_0}{2\pi\sqrt{n_2^2 \sin^2\theta - n_1^2}} \tag{1.7}$$

where θ is the angle of incident light, and λ_0 is the detected wavelength of the analyte.

Figure 1-6 shows the light path of evanescent wave optical fiber chemical sensor with interacts of the analyte in the optical fiber cladding.

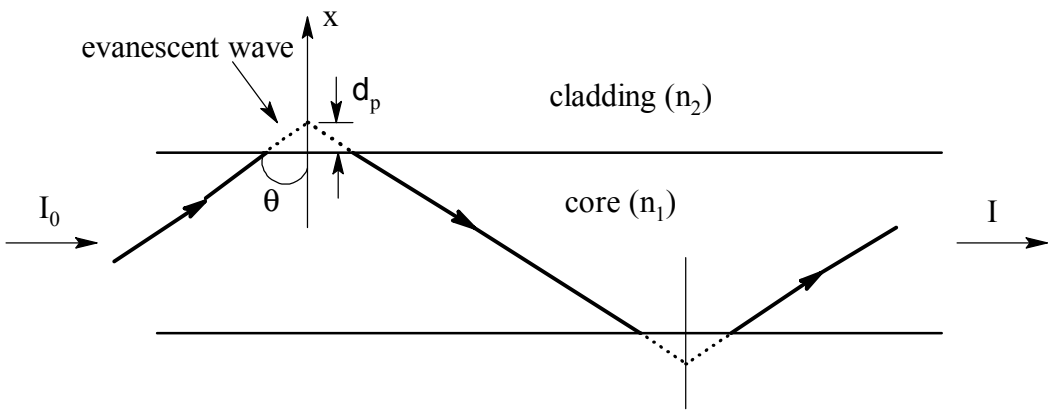


Figure 1- 7. Light path of an evanescent wave optical fiber chemical sensor.

It is important to achieve the highest intensity of evanescent wave for an optical fiber chemical sensor based on evanescent wave. There are a number of factors to affect the intensity of evanescent wave, such as the material of an optical fiber core, the material of an optical fiber cladding, or the conformation of an optical fiber. Khijwania et al.[33] reported that the sensitivity of evanescent wave in an optical fiber sensor was increased when the fiber optic was bent to U-shape. The less the bending radium of the probe in a certain range was, the stronger the sensitivity of evanescent wave was. T. Lee S. et al.[34] also reported that the microbent optical fiber chemical sensor has higher sensitivity of evanescent wave than conventional optical fiber chemical sensor. The

microbent optical fiber chemical sensor can detect the concentration of the analyte down to ppm level.

1.6.3 Principle of an optical fiber chemical sensor based on the absorbance

Wherever the location of the analyte is in an optical fiber core or in an optical fiber cladding, the absorbance of the spectrum in certain wavelength is the same as the conventional analytical spectroscopy. Lambert-beer's law[5] represents the relationship between the absorbance and the concentration of the absorbing analyte.

$$A = \log \frac{I_0}{I} = \varepsilon bc \tag{1.8}$$

where A is the absorbance of the absorbing analyte, ε is the absorption coefficient, , c is the concentration of the absorbing analyte in the optical fiber sensor, and b is the length of the interaction. b can be obtained as bellows.

$$b = \frac{l}{\sqrt{1 - \sin^2 \theta}} \tag{1.9}$$

where l is the length of an optical fiber sensor and θ is the angle of incident light into an optical fiber core.

In the experiment, every substance has its maximum absorbance peak at certain wavelength. Intensity varies accordingly with the concentration of analyte, such that calibration curve can be constructed in order to accurately measure the concentration of an analyte in unknown samples.

1.6.4 Applications of optical fiber sensors

Optical fibers have been found useful for environmental, industrial, biotechnological, food, medical, and related applications. Michel et al.[35] reported the use of chalcogenide glass optical fibers for in situ monitoring pollutants in wastewater by infrared spectroscopy along with evanescent wave spectroscopy. Tao et al.[36] developed an optical fiber sensor for detection of ammonia. A dye molecule, bromocresol purple, was absorbed into sol-gel, which was coated onto the surface of optical fiber. The sample gas was flowing across the coated area continuously. The absorption wavelength of dye molecule will change upon complex with ammonia. The intensity at this wavelength was used to construct a calibration line for quantitative determination of ammonia in unknown samples.

Fiber optic chemical sensors make it possible to record optical spectra on sites remote or inaccessible to conventional spectroscopy.[37] The working principles of fiber optic sensors can be divided into either direct or indirect sensing schemes. In the first case, the properties (i.e., refractive index, absorption, or emission) of the analyte itself can be detected directly. In the second case, an immobilized indicator, label or any other optically detectable bioprobe is needed for the detection of the analyte.[37]

Compared to conventional sensors, the optical fiber sensors have advantages as listed below:[38]

1. An optical fiber is small in size, light in weight, and it is easy to fabricate and operate. Also, it has low cost.

2. An optical fiber is not only strong but flexible. Protective coating called the jacket, outside the optical fiber, helps strengthen the glass inside. It also increases the tension of the optical fiber.
3. An optical fiber, whether made from glass or plastic, is an insulator; therefore no electric interference should be observed. In addition, the light wave propagates inside the optical fiber, so it cannot be interrupted by other signals.
4. An optical fiber can be fabricated in long lengths without the loss of light when proper connectors are used.

Also, fibers allow optical real time detection of analytes. These features make the optical fiber sensor ideal for environmental, biotechnological, food, pharmaceutical, medical and related applications.

1.7 Objective

The goal of this work is to develop an optical fiber sensor for the real time determination of hydrogen peroxide using a UV-vis detector. The responsivity, reversibility and reproducibility of the sensor were evaluated. The sensor is expected to have application in human health, in complex samples and in inaccessive environments. It is an important and easy method for food applications.

CHAPTER II

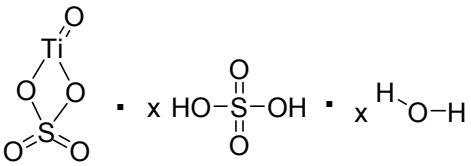
EXPERIMENTAL

In the experiment, the monitoring of the analyte is based on the evanescent wave absorbance. Nafion whose refractive index is 1.38 is the cladding. It was coated outside the optical fiber core whose refractive index is 1.45. It is obvious that the total internal reflection can occur under the condition. Hydrogen peroxide does not absorb at a useful wavelength, so a titanium reagent that can absorb light at wavelength 360 nm by forming the yellow complex with hydrogen peroxide was selected. The titanium reagent was immobilized into the Nafion, therefore, it is an evanescence wave optical fiber hydrogen peroxide sensor.

2.1 Reagents

All reagents were obtained from Sigma-Aldrich and used without further purification. Titanium (IV) oxysulfate-sulfuric acid complex hydrate **2** and titanium (IV) oxyacetyl-acetonate monohydrate **3** were used as indicators. Hydrogen peroxide (30%) was diluted with de-ionized water to the appropriate concentration before use. Polydimethylsiloxane (PDMS) **5** was used as a protection membrane for both high - temperature and high - concentration hydrogen peroxide tests. (See Figure 2 – 1 for structures).

Nafion **4** (5 wt. % in mixture of lower aliphatic alcohols and water, and contains 45% water) is a perfluorinated sulfonic acid ionomer.[39] As a copolymer of tetrafluoroethylene and sulfonyl fluoride vinyl ether, Nafion shows a reverse micelle morphology in the dry state. The ionic clusters are dispersed in the tetrafluoroethylene phase. The attached sulfonic acid group of the vinyl ether is responsible of providing hydrophilicity. The hydrophobic backbone contributes chemical, mechanical, and thermal stability. In addition, Nafion resists interferences from anions and biological macromolecules and has good biocompatibility.[40]



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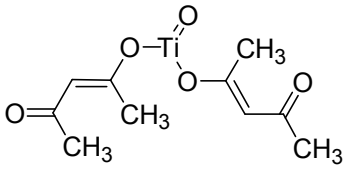

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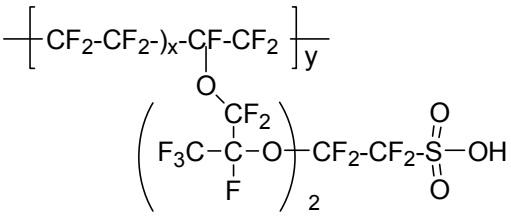

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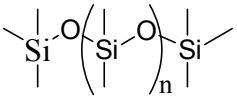

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Figure 2- 1. Structures of titanium (IV) oxysulfate-sulfuric acid complex hydrate **2**, titanium (IV) oxyacetyl-acetonate monohydrate **3**, Nafion **4** and PDMS **5**.

2.2 Preparing Hydrogen Peroxide Sensing Probe

2.2.1 Pretreatment of the fiber optic

The 300 μm core multimode fiber (FT-300-UMT) was purchased from Thorlab, Inc. (Newton, New Jersey). The ends of the fiber were polished with sandpaper (from rough, medium to fine grit sequentially). The central part of a conventional silica optical fiber was inserted into a small flame to burn off the jacket and the cladding of central part of the optical fiber. The bare silica fiber core was further bent into a "U" shape in the flame. The "U" shaped fiber core of the bent probe was cooled to room temperature before it was soaked in a $K_2Cr_2O_7/H_2SO_4$ solution for 1 day, and then rinsed with deionized water. Finally, it was soaked in a 5% NaOH solution for 2 hours to activate the hydroxyl groups on the surface of the silica fiber core.

2.2.2 Coating of the indicator onto the fiber optic

Initially, titanium (IV) oxysulfate-sulfuric acid complex hydrate **2** was used as an indicator for hydrogen peroxide. To 1 mL of deionized water, about 0.01 g of **2** and two drops of concentrated H_2SO_4 were added. Then 0.5 mL Nafion solution was added to the titanium solution. The solution was dip-coated into the fiber optics. They were allowed to dry at ambient conditions for several days before use. The coating process for titanium (IV) oxyacetyl-acetonate monohydrate **3** is the same as that **2**. The concentration of titanium (IV) oxyacetyl-acetonate monohydrate **3** was introduced in following chapter.

2.3 Instrument Set-Up

The ends of the coated bent fiber optic probe were connected to a fiber optic compatible visible light source (Model DT-100 CE, Analytical Instrument System Inc., Flemington, NJ) and an fiber optic compatible UV-Vis spectrometer (Model SD-2000, Ocean Optics Inc., Dunedin, FL). The coated bent part of the fiber optic probe was sealed inside a small flow cell as shown in Figure 2–2. The sample solution was pumped into the flow cell at 1 mL/min. The optical absorption signal of the bent optical fiber probe was recorded with the UV-Vis spectrometer and the data was stored and processed with a computer.

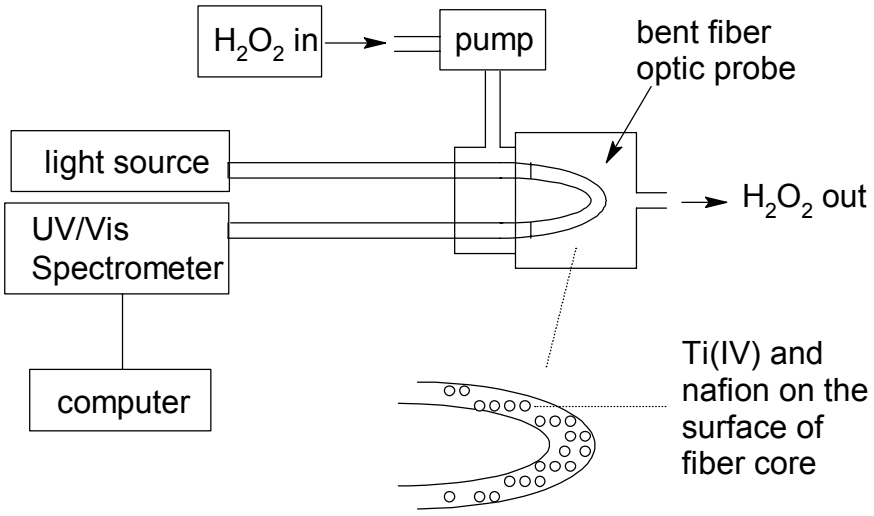

Figure 2- 2. A schematic diagram of a laboratory set-up for testing a fiber optic hydrogen peroxide sensor.

CHAPTER III

FABRICATION AND VALIDATION OF AN OPTICAL FIBER SENSOR FOR THE TRACE AMOUNT OF HYDROGEN PEROXIDE IN WATER

Many methods have been developed for the detection of a trace amount of hydrogen peroxide with spectrophotometry. Most of these methods use indicators to complex with the hydrogen peroxide, leading to a color change. The absorbance can be observed with a spectrometer, and the intensity of absorbance is proportional to the concentration of hydrogen peroxide in solution. Many titanium compounds have been found useful for this purpose. Sellers[41] used potassium titanium (IV) oxalate to detect hydrogen peroxide. A yellow titanium (IV) – peroxide complex was produced, which absorbs with a λ_{max} of about 400 nm. Takamura and co-workers[20] reported the use of a water-soluble titanium (IV)-porphyrin complex to enhance the spectrophotometric determination of a trace amount of hydrogen peroxide. The absorbance of this complex decreased at 432 nm as hydrogen peroxide was added, owing to the consumption of the complex following the formation of a new titanium (IV) – porphyrin – peroxide complex. The same research group also reported that a mixture of titanium (IV) and 2-((5-bromopyridyl)azo)-5-(*N*-propyl-*N*-sulfopropylamino) phenol (Ti-PAPS) useful for the spectrophotometric determination of a trace amount of hydrogen peroxide.[23] In a

reciprocal manner, hydrogen peroxide has been used as a reagent for the determination of titanium.[42] The similar principle applied in this case, where a yellow titanium (IV) – hydrogen peroxide complex was formed and its color was matched against the standard solution to determine the approximate concentration of TiO_2 in the solution.

Herein, the effort to develop an optical fiber sensor is demonstrated using titanium (IV) as an indicator for the determination of hydrogen peroxide. Nafion was used to form a thin film outside the fiber core in order to prevent titanium ion from leaching during the analysis. It is believed that titanium (IV) forms a yellow $TiO\text{-}H_2O_2$ complex with hydrogen peroxide and the complex absorbs light at around 360 nm.

3.1 Comparison of titanium (IV) oxysulfate – sulfuric acid complex 2 and titanium (IV) oxyacetyl - acetonate 3 as indicators

3.1.1 Transmission electron microscopy (TEM)

The structures of the indicators titanium (IV) oxysulfate - sulfuric acid complex **2** and titanium (IV) oxyacetyl – acetonate **3** within the Nafion matrix were performed with transmission electron microscopy (TEM)(Model JEM-100CX II, JEOL USA, Inc.). Briefly, the suspension containing either **2** or **3** and Nafion used for the fiber optic sensor coating was placed on copper grids to form a thin film, and then dried for the TEM experiment (accelerating voltage: 80 kV and magnification: 20,000X).

Figure 3–1 is a TEM image of titanium (IV) oxysulfate-sulfuric acid complex **2**. The black dots represent titanium randomly dispersed in the Nafion film. The crystals are relatively small as compared with the TEM image of titanium (IV) oxyacetyl-

acetonate monohydrate **3**, which is shown in Figure 3–2. It can be clearly seen that titanium (IV) oxyacetyl-acetonate monohydrate **3** disperses in the Nafion film with good shapes and larger sizes.

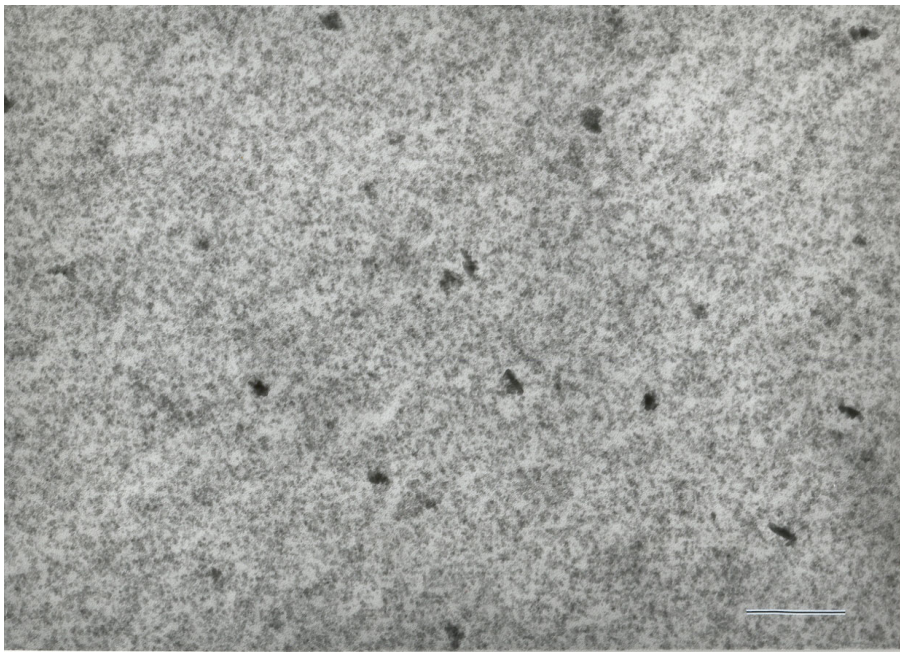

Figure 3- 1. The TEM image of titanium (IV) oxysulfate-sulfuric acid complex hydrate **2**. (microbar = 0.5 μm).

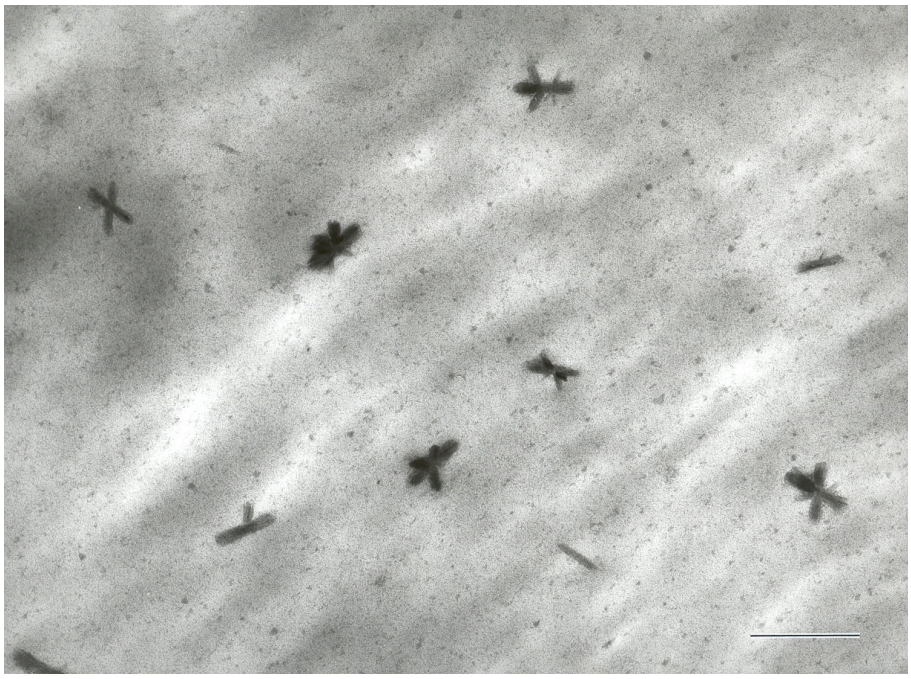

Figure 3- 2. The TEM image of titanium (IV) oxyacetyl-acetonate monohydrate **3**. (microbar = 0.5 µm).

3.1.2 Sensitivity and stability

Initially, titanium (IV) oxysulfate-sulfuric acid complex hydrate **2** was used as an indicator. The aqueous solution of about 0.01 g of **2** was mixed with 0.5 mL Nafion to form a suspension. Nafion was used to provide a more mechanically stable titanium film on the surface of the fiber optic. The "U"-shape fiber optic was dipped into the suspension to produce an indicator coating. The coated fiber optic sensor was installed in the same manner as shown in Figure 2–2. A hydrogen peroxide solution of 150 ppm was pumped through the sample cell at ~ 1 mL/min. The signal was recorded with a UV-vis detector.

Figure 3–3 shows the normalized absorbance of titanium (IV) oxysulfate-sulfuric acid complex hydrate **2** as an indicator. The fiber optic sensor was scanned every several

minutes. There is a maximum absorbance observed around 360 nm. It was also observed that the intensity of the absorbance for **2** decreased with time while the flow rate of hydrogen peroxide solution was constant. The absorbance at 360 nm dropped 22% (from 0.045 to 0.035) in 22 minutes.

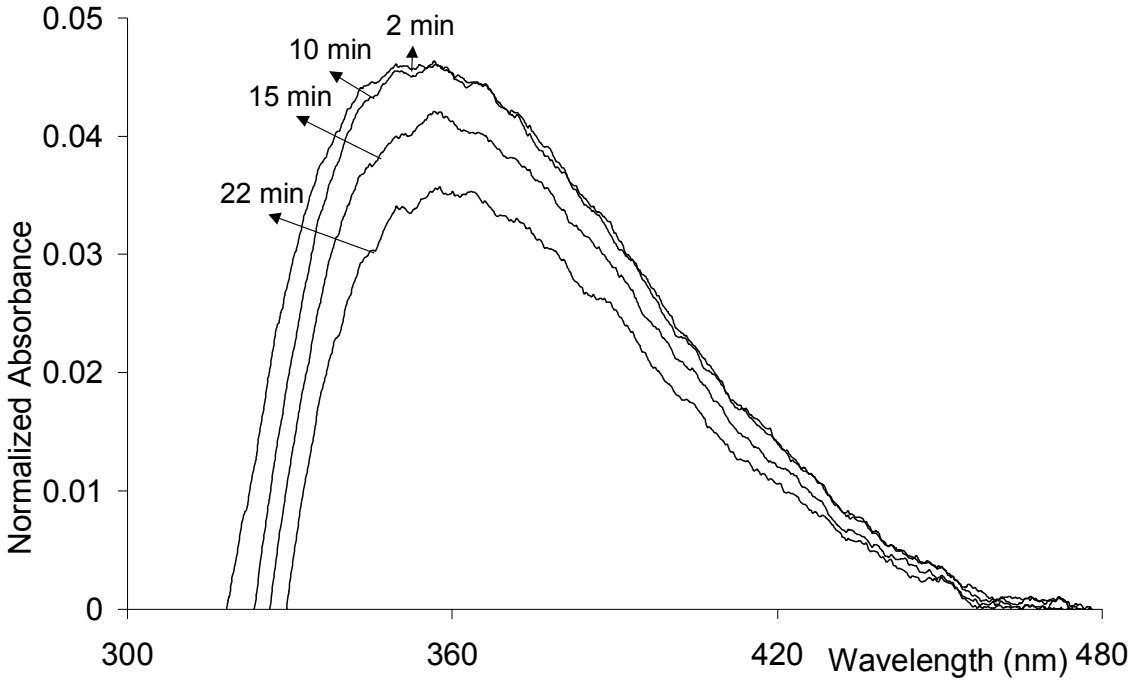

Figure 3- 3. Absorbance of titanium (IV) oxysulfate-sulfuric acid complex hydrate **2** at 2, 10, 15 and 22 min.

Similarly, about 0.01 g of titanium (IV) oxyacetyl - acetonate **3** (0.04 mmol) mixed with 5 mL ethanol and 0.5 mL Nafion. The solution was dip-coated on a fiber optic. Both the concentration and the flow rate of hydrogen peroxide solution were kept the same as before.

Figure 3–4 shows the normalized absorbance of titanium (IV) oxyacetyl - acetonate **3** as an indicator. It also was scanned every several minutes for over one hour.

It is similar to figure 3-3 that the maximum absorbance was observed around 360 nm, but the absorbance value was almost invariable with time.

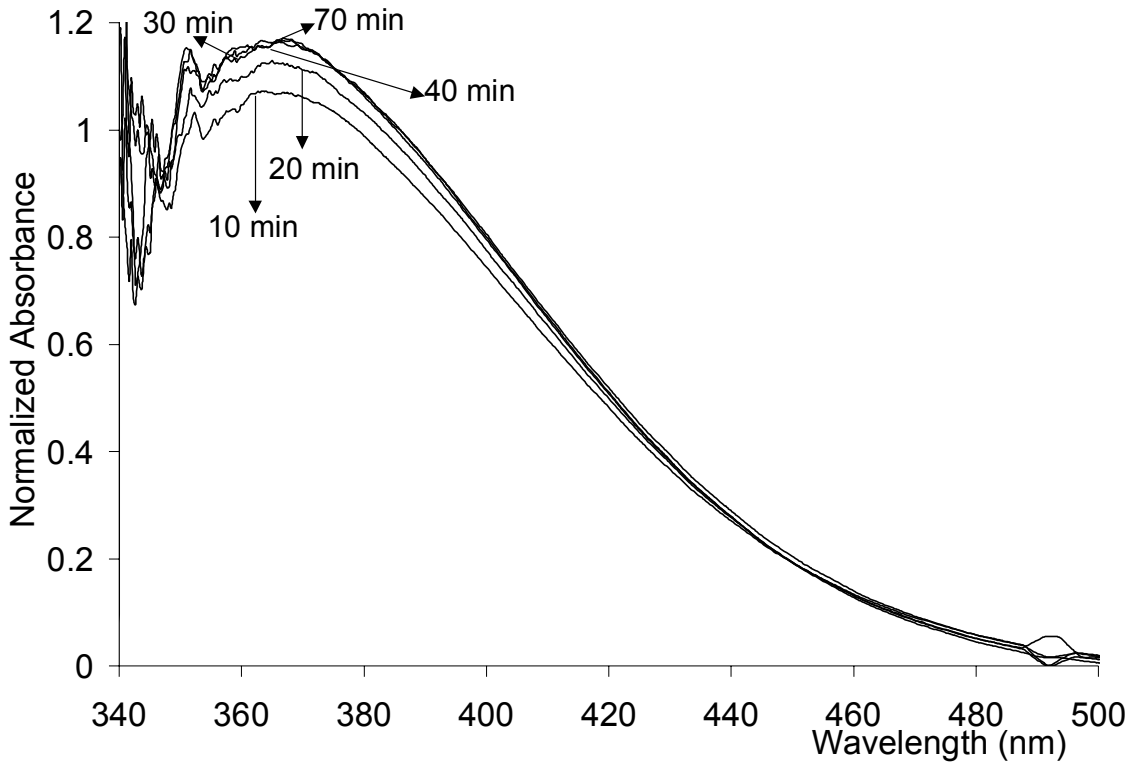

Figure 3- 4. Absorbance of titanium (IV) oxyacetyl-acetonate monohydrate **3** at increments spanning at one hour.

In order to compare the performance and stability of compounds **2** and **3** as indicators, the absorbance at λ_{max} as a function of time are shown in Figure 3–5. With compound **2** as an indicator, the intensity of absorbance decreased after 10 minutes. It was thought that the inorganic titanium compound diffused from the surface of fiber optic into the sample solution. Using compound **3** as an indicator, the intensity was relatively constant. This indicates that Nafion can hold the organic titanium compound 3 tightly with little leaking during the analysis. Finally, the sensor was flushed with pure water in

order to examine the reversibility of the complex between **3** and hydrogen peroxide. It was observed that the intensity dropped to baseline after about 1 hour. This indicates that the optical fiber sensor with **3** as an indicator can be used repeatedly.

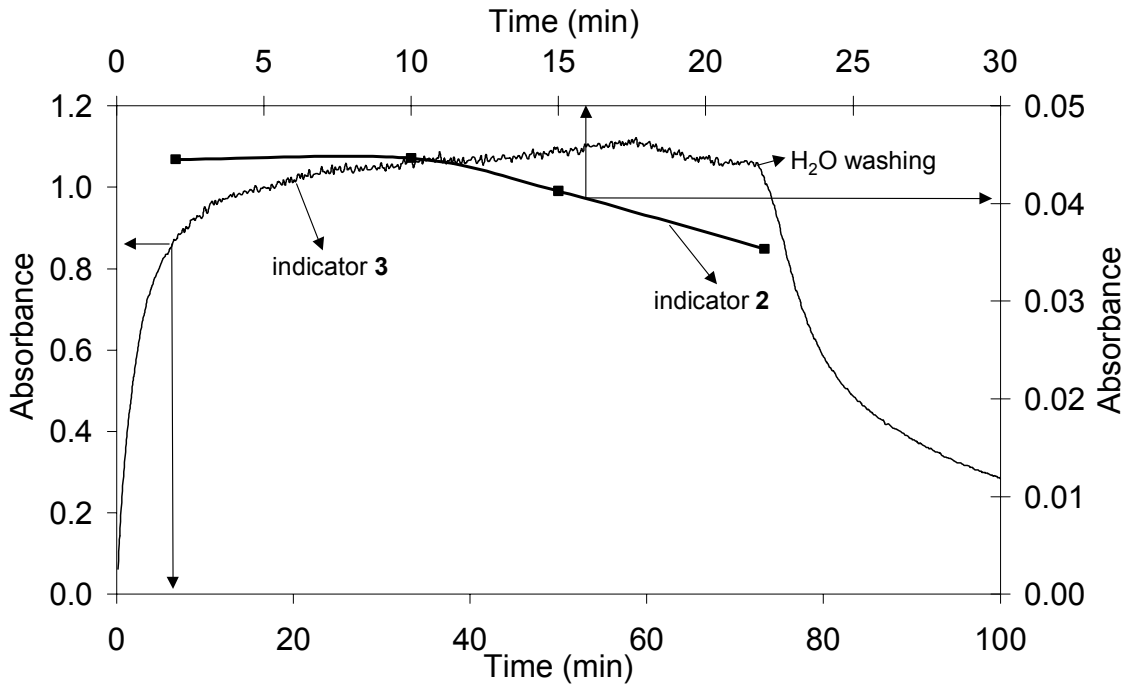

Figure 3- 5. The absorbance comparison of two titanium reagents in 150 ppm hydrogen peroxide solution at wavelength 360 nm.

3.2 Titanium (IV) oxyacetyl-acetonate monohydrate 3 as an indicator

3.2.1 Qualitative Analysis

Owing to the satisfactory stability and good sensitivity of **3** for detection of hydrogen peroxide, it was chosen as the indicator in the following experiments. The responsivity, repeatability and reversibility were analysized. Figure 3-6 shows the comparison of various absorbance curves of a Ti (IV)-H_2O_2 complex formed on the

optical fiber sensor while the solution is flowing. The fiber was dip-coated with about 0.01 g of titanium (IV) oxyacetyl-acetonate monohydrate ethanol solution in the presence of 0.5 mL Nafion. Rather than using the intensity at the endpoint of the response (which would take more than one hour), the 11 signals obtained after 10 minutes were used. It can be observed that the higher the concentration of hydrogen peroxide, the stronger the intensity of absorbance was at λ_{max} 360 nm.

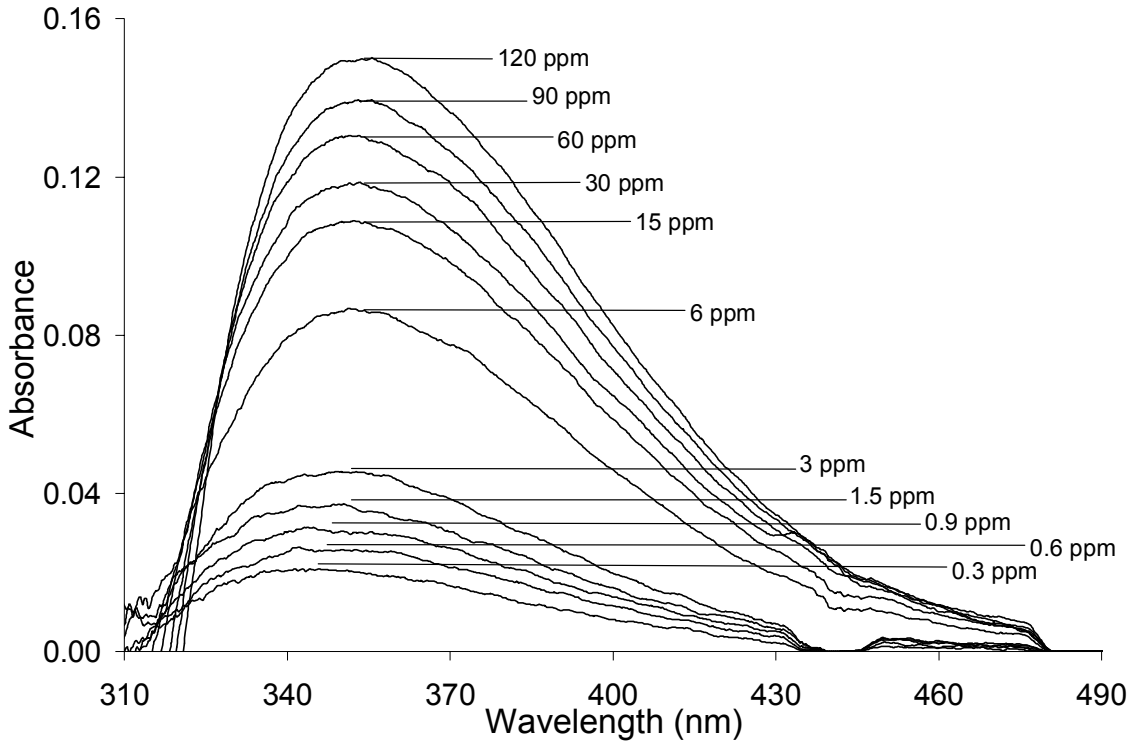

Figure 3- 6. Absorbance of different concentration hydrogen peroxide solutions at 10 min.

Figure 3-7 shows the reproducibility and reversibility of **3** for the detection of hydrogen peroxide. A 3 ppm-H_2O_2 solution was pumped into the sample cell at ~1 mL/min. The absorbance was monitored at 360 nm. In order to eliminate fluctuation of

the signal, which results from air bubbles in solution, instability of the instrument and other causes, the absorbance was normalized with respect to the absorbance at 470 nm. The response increased rapidly to 0.04AU in 15 minutes. At this point, the sample cell was flushed with water, and the intensity of absorbance decreased over 15 minutes to baseline. The reproducibility was evaluated by comparing four successive cycles and calculating the highest response signal change. The standard deviation was found to be 0.0003.

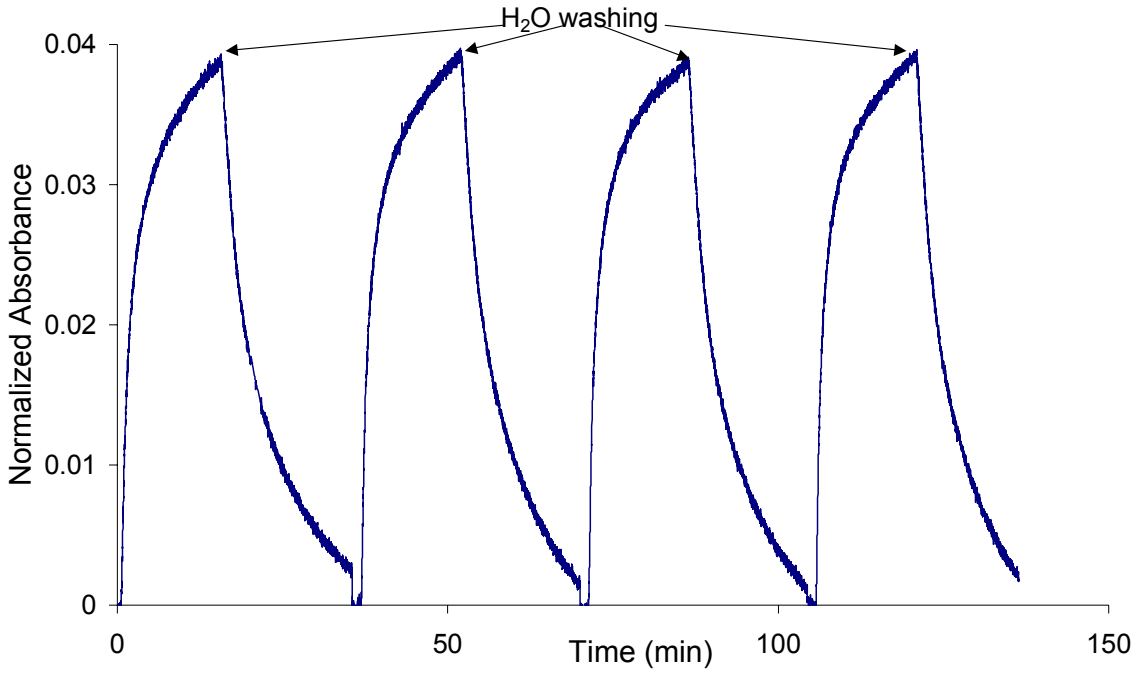

Figure 3- 7. Reproducibility and reversibility of the optical fiber H_2O_2 sensor in 3 ppm H_2O_2 solution (the absorbance was normalized at wavelength 360 nm and 470 nm).

3.2.2 Scanning electronic microscope (SEM)

The response time is concerned with the thickness of the Nafion film. The thickness of the Nafion film was measured with scanning electronic microscope (SEM) (Model JSM-6500F field emission, JEOL USA, Inc.). The fiber optic coated with titanium (IV) oxyacetyl-acetonate monohydrate Nafion solution was transected and the surface was scanned to measure the thickness of the coating film. The thickness of film was roughly 30 nm for the single dip-coating fiber (see Figure 3-8), and 157.5 nm for the five dip-coating fiber (see Figure 3-9).

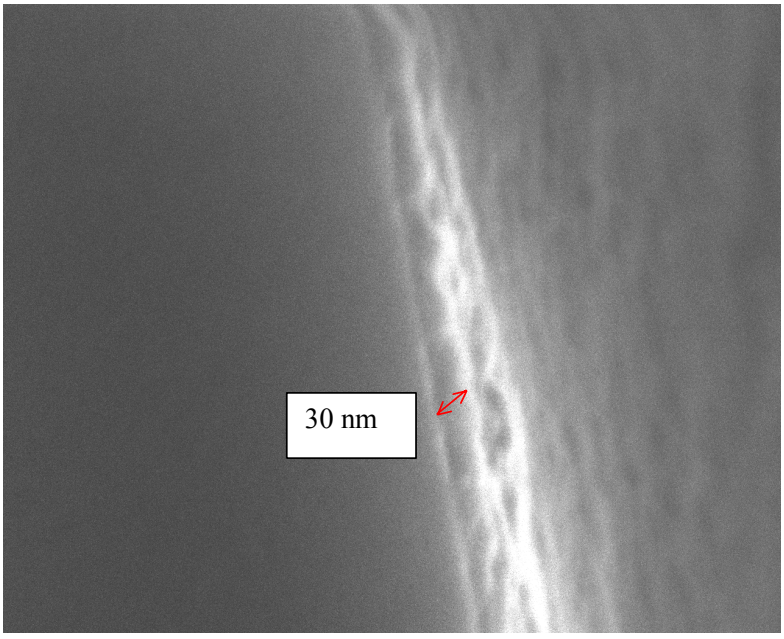

Figure 3- 8. The scanning electron microscope image of the section of an optical fiber Ti-1 dip-coated one time (Accelerating voltage: 5.0kV and magnification: 200,000X)

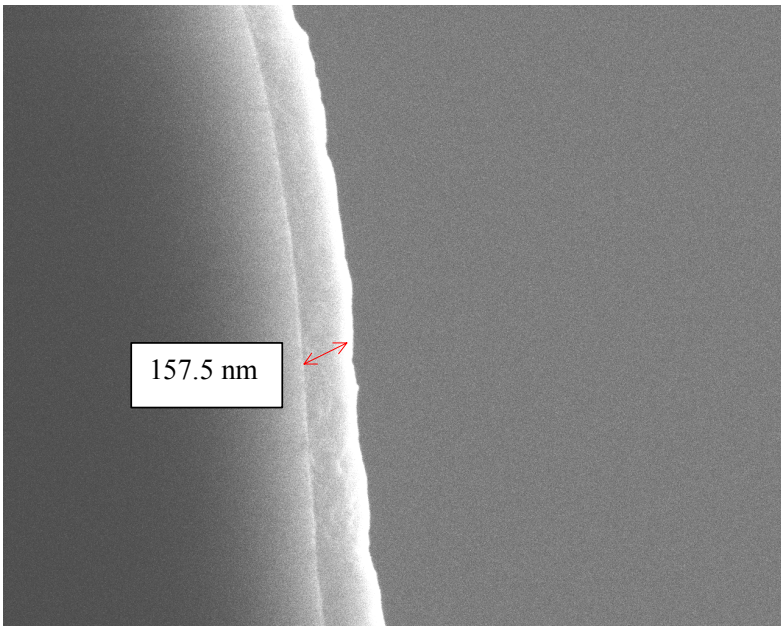

Figure 3- 9. The scanning electron microscope image of the section of an optical fiber Ti-1 dip-coated five times. (Accelerating voltage: 10.0kV and magnification: 50,000X)

Figure 3-10 shows the comparison of absorbance for hydrogen peroxide using sensors produced by dipcoating the fibers different numbers of times under the same conditions. They were dip-coated one, three, and five times, respectively. The results demonstrated that the thicker the coating, the higher the intensity. However, the sensor with a thicker coating usually exhibits a longer response time. The response time (time to reach 90% of the maximum response) for the single dip-coating is about 10 minutes, 20 minutes for the three dip-coating, and 50 minutes for the five dip-coating.

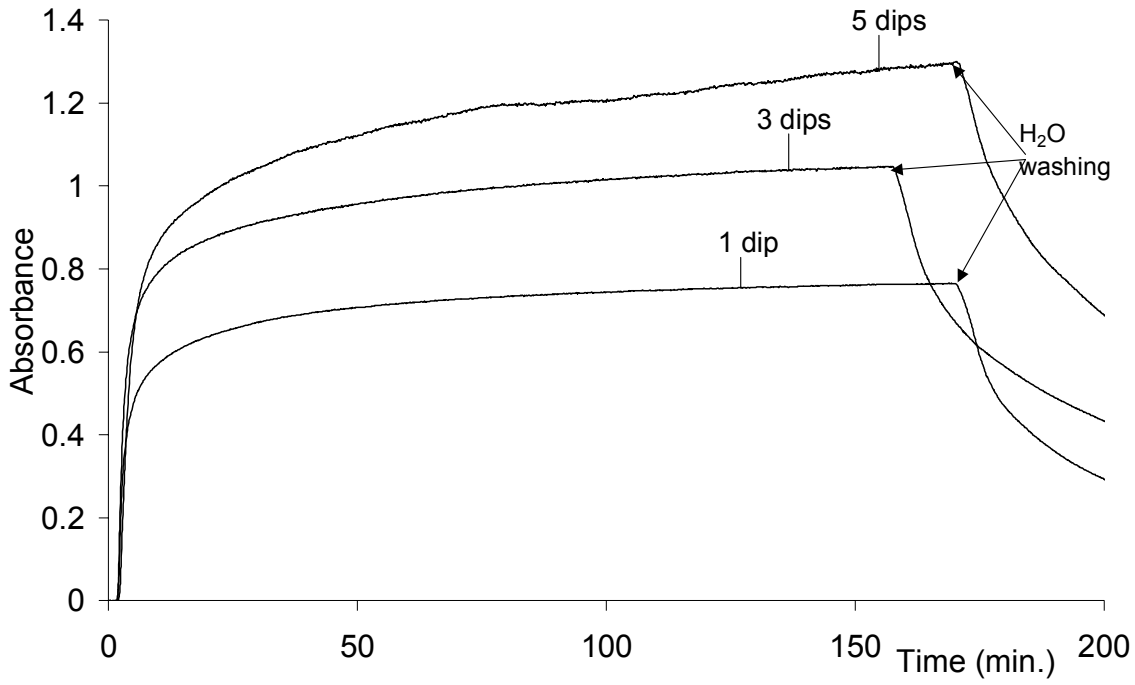

Figure 3- 10. Comparison of absorbance of 15-ppm H_2O_2 using optical fibers prepared 1, 3 and 5 dip-coatings at 360 nm.

Table 3-1 shows the comparison of the coating thickness on the effects of response time. The thickness of the coating was measured by SEM to be 30 nm for one-dip-coating sensor, and 157.5 nm for three-dip-coating sensor. It was observed that the response time changed with thickness: the thicker the coating, the longer the response time. The intensity of absorbance also increased with increasing thickness.

Table 3- 1. Comparison of coating thickness on effects of response time and intensity of absorbance of sensors.

Dip-coating	Response time (min)	Absorbance (AU)	Thickness (nm)
One	10	0.757	30
Three	20	1.0468	Not analyzed
Five	50	1.2982	157.5

3.2.3 Quantitative analysis

0.05 g (0.19 mmol) of titanium (IV) oxyacetyl-acetonate monohydrate **3**, 0.5 mL Nafion solution and 5 mL ethanol were mixed. Nominally, the concentration of titanium (IV) oxyacetyl-acetonate monohydrate **3** was 3.468×10^{-2}M. The mixture was sonicated for 20 min before use. The solution reached saturation and was not clear. Then the solution was dip-coated into a fiber optic for one time and dried for a few days before use.

Figure 3-11 shows the sequential response of the sensor to a series of hydrogen peroxide solutions at different concentrations. The concentration of hydrogen peroxide was varied from as low as 0.03 ppm to as high as 9 ppm. The 0.03-ppm solution was first tested at 360 nm. The next solution was not pumped to the sample cell until the response of the current solution reached a plateau. According to Figure 3-12, the absorbance increased proportionally to increasing hydrogen peroxide concentrations. And it can return to baseline with H_2O washing.

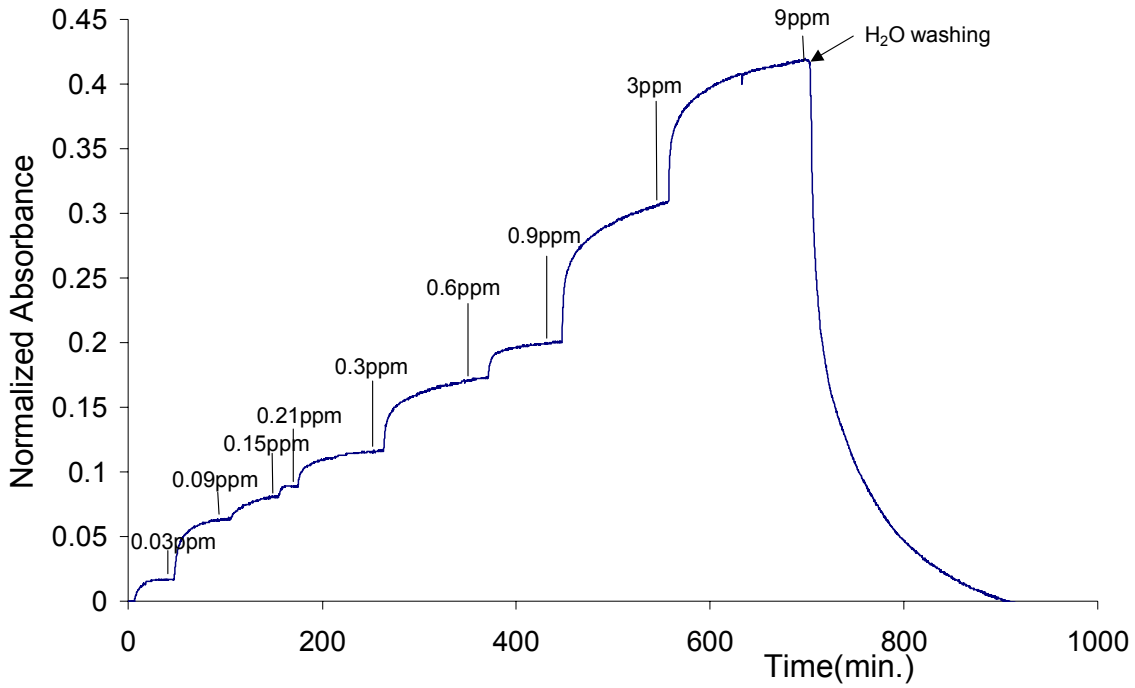

Figure 3- 11. The successive time response of the sensor to a series of hydrogen peroxide solutions (normalized absorbance at 360 nm and 470 nm).

Figure 3-12 and 3-13 show the calibration for the concentration range between 0.09-9 ppm of hydrogen peroxide by using the fiber optic coated with titanium (IV) oxyacetyl-acetonate monohydrate Nafion solution dip-coating one time at 360 nm. Hydrogen peroxide solutions were pumped through the sensor successively starting from the lowest (0.09 ppm) to the highest (9 ppm). The higher concentration hydrogen peroxide was not pumped until the previous one reached its equilibrium and kept for around one hour (see Figure 3-12, a flat or almost flat line was seen). 20 data points were taken from relative flat lines on every concentration curve. The average value was used to construct a calibration line. At a low concentration range (0.09-0.6 ppm), the intensity of absorbance is linearly proportional to the concentration of hydrogen peroxide (see

Figure 3-12). The correlation coefficient is 0.9944. According to the equation $\frac{3\sigma}{S}$ (σ is the ratio of signal and noise and S is the slope), the detection limit is 0.01 ppm.

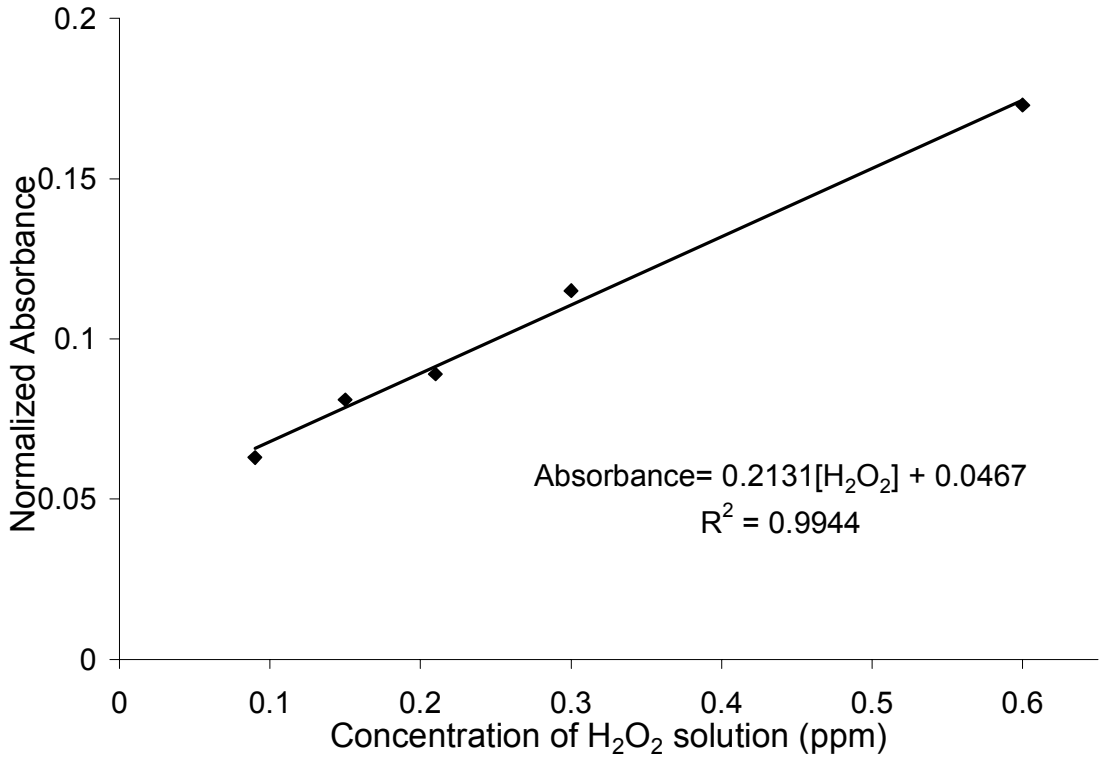

Figure 3- 12. A calibration for detecting the concentration of hydrogen peroxide from 0.09 ppm to 0.6 ppm (normalized absorbance at 360 nm and 470 nm).

At a higher concentration range (0.3-9 ppm), a logarithmic calibration curve was obtained with a correlation coefficient of 0.9967 (see Figure 3-13).

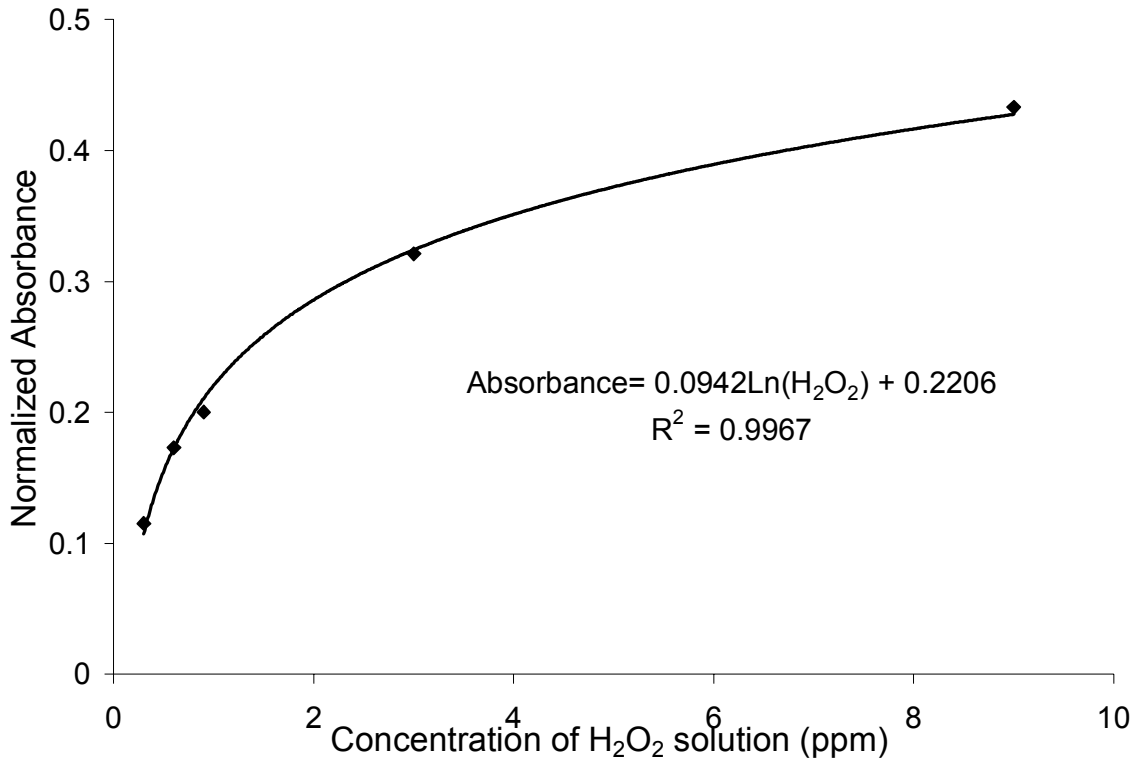

Figure 3- 13. A calibration for detecting the concentration of hydrogen peroxide from 0.3 ppm to 9 ppm (normalized absorbance at 360 nm and 470 nm).

3.2.4 Polydimethylsiloxane (PDMS) coating

In order to develop a reliable method for the determination of hydrogen peroxide at high concentration, the fiber optic coated with titanium (IV) oxyacetyl-acetonate monohydrate Nafion solution dip-coating one time was used for the analysis of 300 ppm solution of hydrogen peroxide. Figure 3-15 shows absorbance of the sensor with the time for the determination of 300 ppm hydrogen peroxide solution (black curve). The absorbance was monitored at 360 nm and the flow rate was 1 mL/min. The absorbance reached its maximum of ~0.8 AU within 15 minutes and then decayed rapidly with time. It seems that high concentration hydrogen peroxide deteriorated the Nafion film of the sensor. This is likely due to the titanium ion leaching out of Nafion film. Figure 3-14 demonstrates the process of titanium ion leaching out of Nafion film with a concentrated hydrogen peroxide solution flowing across it. As is mentioned in Chapter 2, inorganic titanium oxysulfate **2** is soluble in aqueous solution, therefore leaching quickly from nafion film. In contrast, organic titanium reagent **3** is less soluble in aqueous solution, which prevents it being dissolved into flowing solution. It is assumed that the formed H_2O_2-TiO complex is more soluble in aqueous solution than its precursor, **3**, accounting for the leaching of titanium reagent. At concentrated hydrogen peroxide solution, the reaction goes to the right, which results in the rapid leaching of titanium reagent out of nafion film.

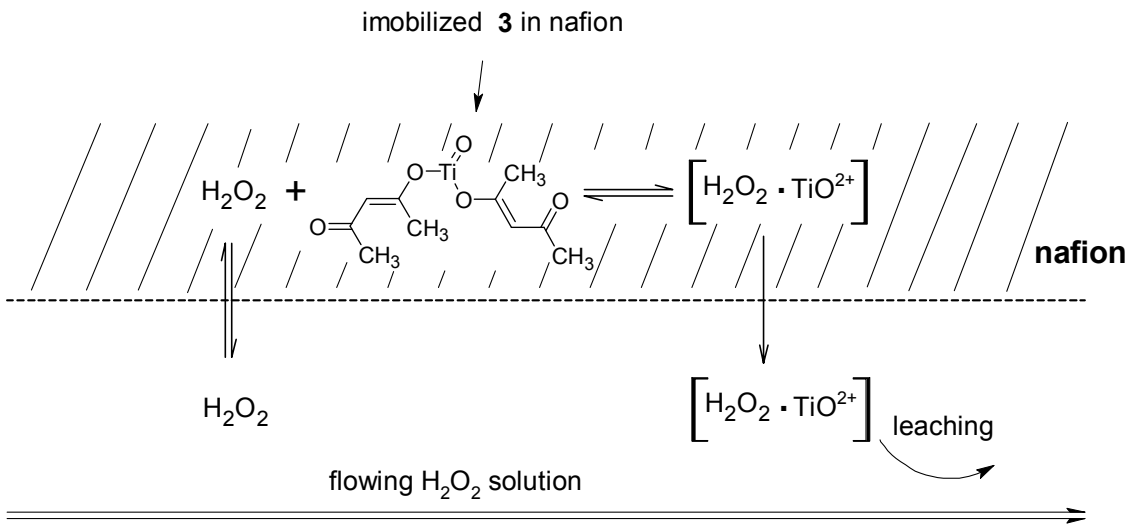

Figure 3- 14. Demonstration of titanium leaching out of Nafion film.

Polydimethylsiloxane (PDMS) was coated on the outside of the Ti (IV)-Nafion film in order to analyze concentrated hydrogen peroxide. PDMS is a commercially available polymer. It is made up of KE-108 with CAT-108 (5% KE-108). Compared to other polymers, PDMS has many important physical and chemical properties. They are low glass transition temperature ($T_g \sim -125\ ^0C$), high gas permeability and compressibility. And it is used in a wide temperature range from $-100\ ^0C$ up to $+100\ ^0C$. It is more important that PDMS is non-toxic. It has been used as a membrane to trap ammonia that can be detected by a fluorescent optical fiber sensor[36]. In this case, the gaseous small molecules can pass through the PDMS membrane while the big molecules or (aggregates of molecules) are blocked. It was assumed that Henry's law was followed with the PDMS acting as a protection membrane to the optical fiber sensor. Equilibrium is established between the pressure in the gas phase and the concentration in solution

phase of the interest analyte. The absorbance changes regularly with the gas pressure, and is therefore indirectly related to the concentration of the analyte.

An optical fiber sensor for concentrated hydrogen peroxide was prepared by dip-coating a fiber with Ti (IV)-Nafion (same mixture as before), and then dip-coating with PDMS (preparation 5 mL KE-108 mixed with 0.25 mL (5% KE-108) CAT-108 and a small amount toluene to reduce the thickness).

The gray curve in Figure 3-15 represents the time response of a PDMS-coated sensor to a 300 ppm hydrogen peroxide solution. Unlike the uncoated sensor, the response reached a steady plateau and remained constant for over 100 min. It was also found that the PDMS-coated sensor response was reversible. The response decreased quickly when the H_2O_2 solution was replaced with deionized water (data not shown). This preliminary experiment indicates that the sensor protected with PDMS may suffice in the determination of hydrogen peroxide at high concentrations.

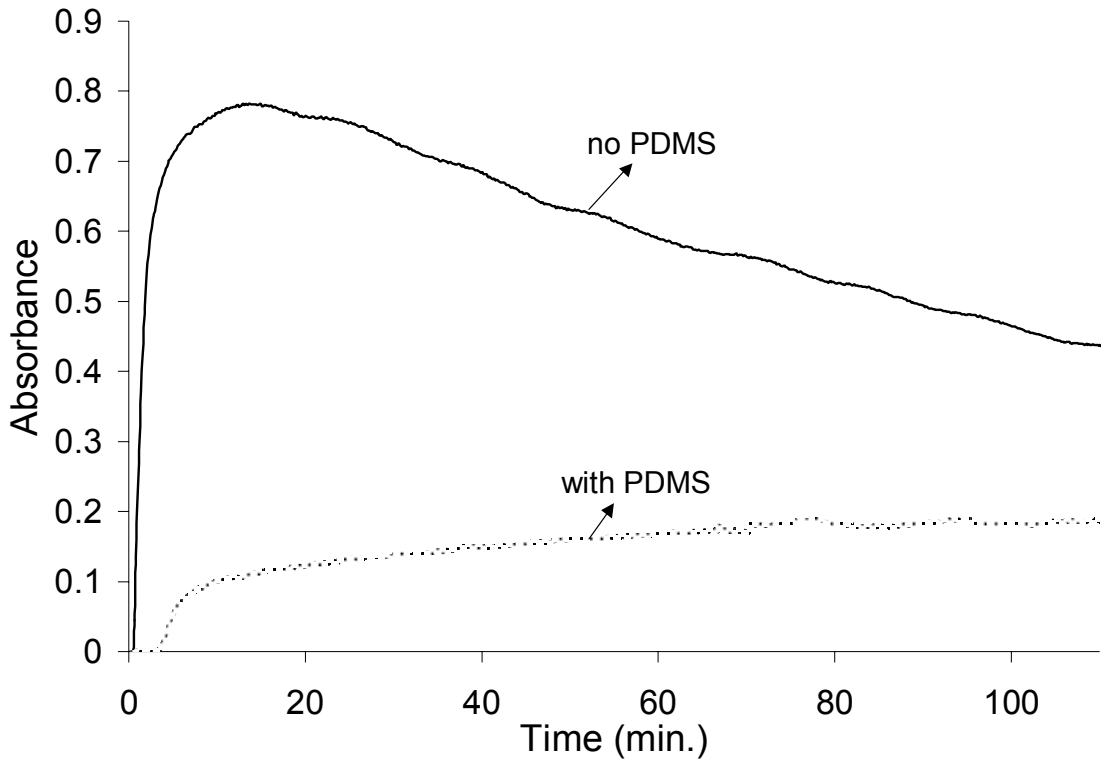

Figure 3- 15. Comparison of time responses of optical fiber sensors without PDMS and with PDMS for a 300 ppm H_2O_2 solution.

Some cases demand the determination of hydrogen peroxide at high temperature. For example, fuel cells produce hydrogen peroxide as a by-product, which is harmful to the lifetime of the fuel cell. The working temperature of fuel cells is much higher than room temperature. Fortunately, PDMS can withstand high temperature and also prevent leaking out of the titanium ion.

Figure 3-16 shows the comparison of fiber optic H_2O_2 with PDMS. It is obviously that the absorbance intensity (0.4AU) without PDMS is stronger than that (0.045 AU) with PDMS. And the response time (20 min.) without PDMS is shorter than that (50 min.) with PDMS. Though there are lower absorbance intensity and longer

response time with PDMS, it can make the optical fiber sensor work at high concentration (300 ppm) and high temperature (70^0C).

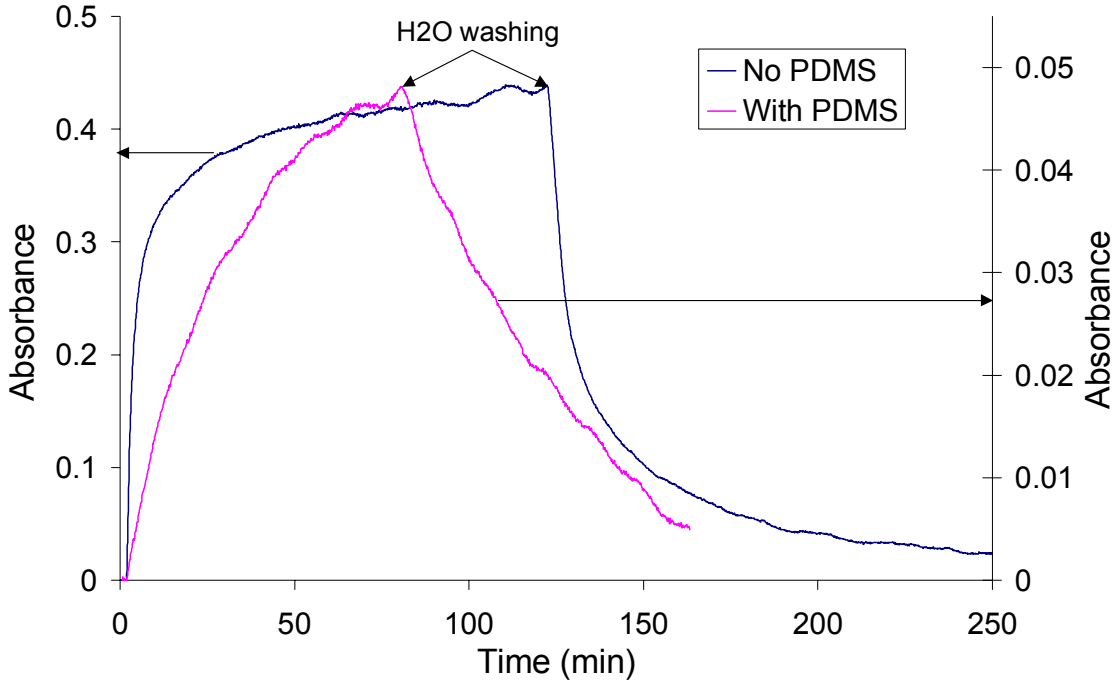

Figure 3- 16. Comparison of fiber optic sensor with PDMS.

Figure 3-17 shows the time response of the sensor for a series of hydrogen peroxide solutions from 48 ppm to 300 ppm at 70 ^{0}C (this is the highest temperature that the current device can tolerate due to the inability of degassing the air bubble). Solutions were successively pumped into the sample cell after the previous solution reached equilibrium. As shown in Figure 3-17, the absorbance increased directly with an increase in hydrogen peroxide concentration. The signal was relatively unstable due to the existence of bubbles in solution at high temperature. The absorbance unit shown in figure 3-17 is the absorbance at 360 nm normalized with respect to that at 470 nm.

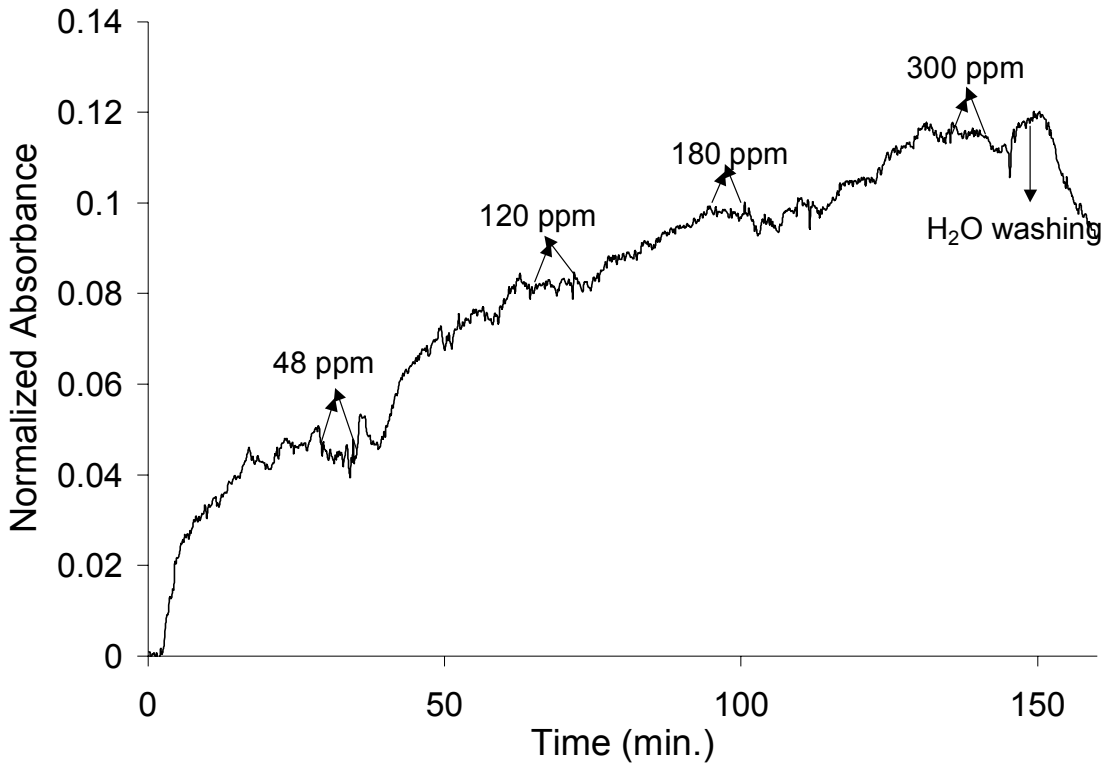

Figure 3- 17. Successive time response of the optical fiber sensor Ti-1 protected with PDMS to 48, 120, 180, and 300-ppm H_2O_2 solutions at 70 ^{0}C.

Figure 3-18 shows the calibration curve for the concentration range between 48-300 ppm of hydrogen peroxide using the optical fiber sensor protected with PDMS at 70^{0}C. The average value of 20 data points (taken from the relative flat line on every concentration curve) is used as the data point to get the calibration curve. A logarithmic relationship between normalized absorbance and hydrogen peroxide was obtained with a correlation coefficient of 0.9995.

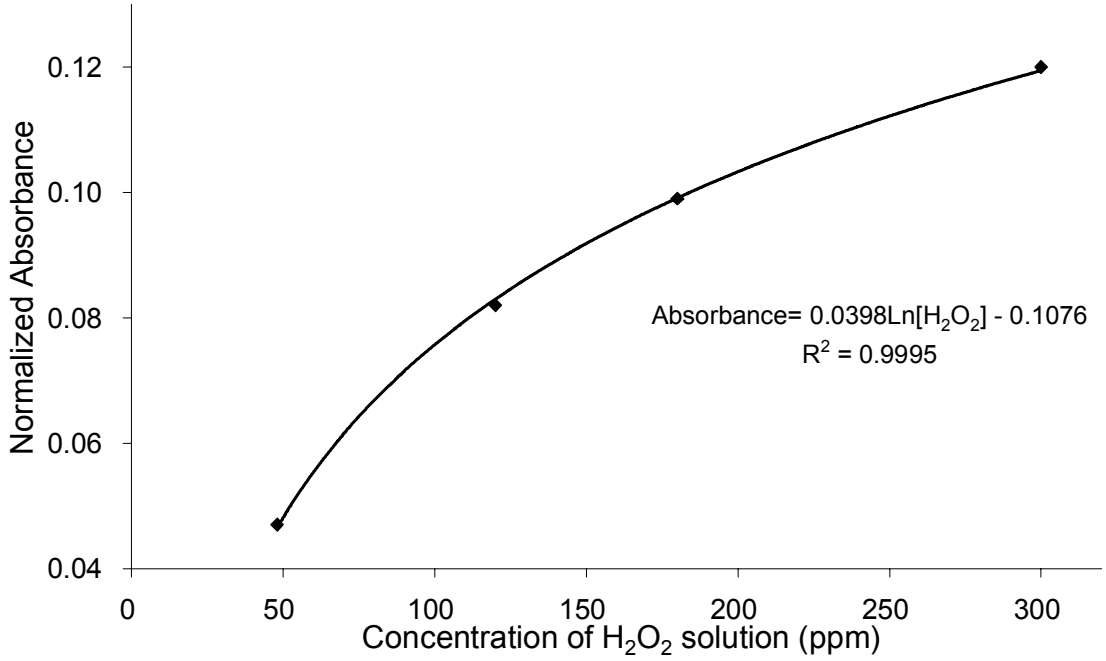

Figure 3- 18. A calibration for detecting hydrogen peroxide using the optical fiber sensor Ti-1 with PDMS at 70 ^{0}C.

3.3 Summary

Two titanium compounds were compared as indicators for sensing hydrogen peroxide in water with an optical fiber sensor. The indicator was doped in a Nafion film on the surface of a fiber core. TEM images confirmed the immobilization of titanium ions in the Nafion film. The organic titanium reagent, titanium oxyacetonate is superior to the inorganic reagent, titanium oxysulfonate sulfuric acid due to its stabilities and sensitivity as in response to concentration. The time response of the titanium indicator is proportional to the concentration of hydrogen peroxide. The reproducibility of the sensor also was demonstrated. However, the sensor did not function well at either high

temperature or high concentration of hydrogen peroxide. A PDMS-coating over the TiO-Nafion film allowed the sensor to work normally under these harsh conditions.

CHAPTER IV

DETECTION OF HYDROGEN PEROXIDE IN MILK WITH TITANIUM (IV) OXYACETYL-ACETONATE MONOHYDRATE AS THE INDICATOR

4.1 Introduction

Hydrogen peroxide is widely used as a chemical preservative for food[43] and is used in milk as activation to the enzyme that is naturally present in milk[44]. It can make raw milk store in air for 7-8 hours at 30^0C and even longer if the temperature is lower. It is thought to be one of the bactericidal factors.[45] The ability to rapidly determine hydrogen peroxide concentrations in milk is of importance for quantitative control. Many studies have shown that a small amount of hydrogen peroxide is not harmful to human health, However, a higher concentration of hydrogen peroxide is toxic. Hankin reported that a 0.45% hydrogen peroxide solution given to rats instead of pure water depressed fluid intake and food consumption and reduced body weight.[46]

As early as the early 1900's, Amberg[47] developed a method to determine the amount of hydrogen peroxide in milk. He used a sulfuric acid solution of titanic acid to qualitatively determine the presence of hydrogen peroxide in milk. Peinado and Toribio[48] proposed a kinetic nonenzymatic method for determination of hydrogen peroxide in coffee, tea, and milk samples. The method was based on the oxidizing ability

of hydrogen peroxide. It oxidizes 2-hydroxynaphthaldehyde thiosemicarbazone with Mn(II) as the catalyst. However, reports regarding optical fiber applications in determination of hydrogen peroxide in milk have not been reported. Herein, the detection of hydrogen peroxide in milk using an optical fiber chemical sensor is discussed, where Titanium (IV) doped in Nafion film is used as an indicator.

4.2 Detection Of Hydrogen Peroxide In Milk

The regular milk was diluted 10-fold with deionized water before use. Since the sensitivity of the optical fiber Ti-1 was highest and the response time of one dip-coating was shortest, the optical fiber sensor Ti-1 one dip-coating was used.

The diluted milk was pumped into the sample cell and monitored in the wavelength range of 300 – 800 nm. Figure 4-1 shows the absorbance from 330 – 510 nm recorded after pumping the sample for 1 hour (dotted curve). The maximum absorbance was still observed at ~ 360 nm though the peak was extremely wide due to the sample matrix of milk. In order to verify that the maximum absorbance corresponds to the complex formed between Ti (IV) and hydrogen peroxide, a small amount of hydrogen peroxide solution (nominally 3-ppm) was spiked into the sample. The intensity of absorbance at 360 nm increased significantly (see Figure 4-1).

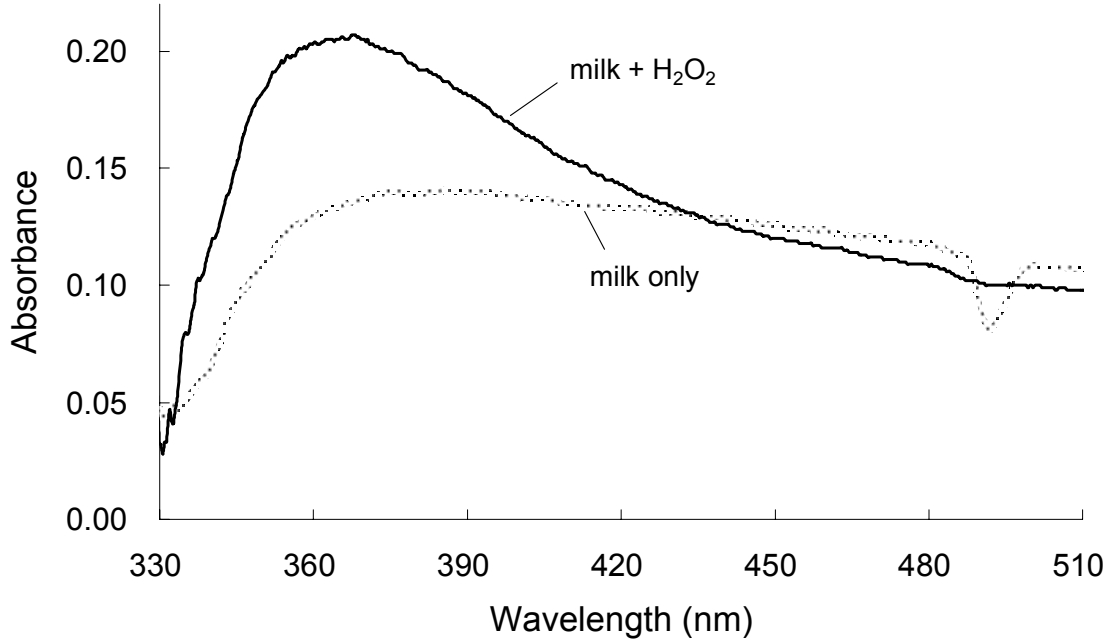

Figure 4- 1. Absorbance of milk sample (dotted line) and sample spiked with 3-ppm H_2O_2 (solid line) using Ti-1 one dip-coating sensor.

Figure 4-2 shows the response of the sensor for 3-ppm hydrogen peroxide spiked in a milk sample and the reversibility upon washing with water. The absorbance was monitored at 360 nm. As shown in Figure 4-1, the intensity of absorbance reached 0.2 AU within 100 minutes. Then the sensor was washed with water and the intensity decreased significantly, showing the reversibility of the senor for hydrogen peroxide in milk.

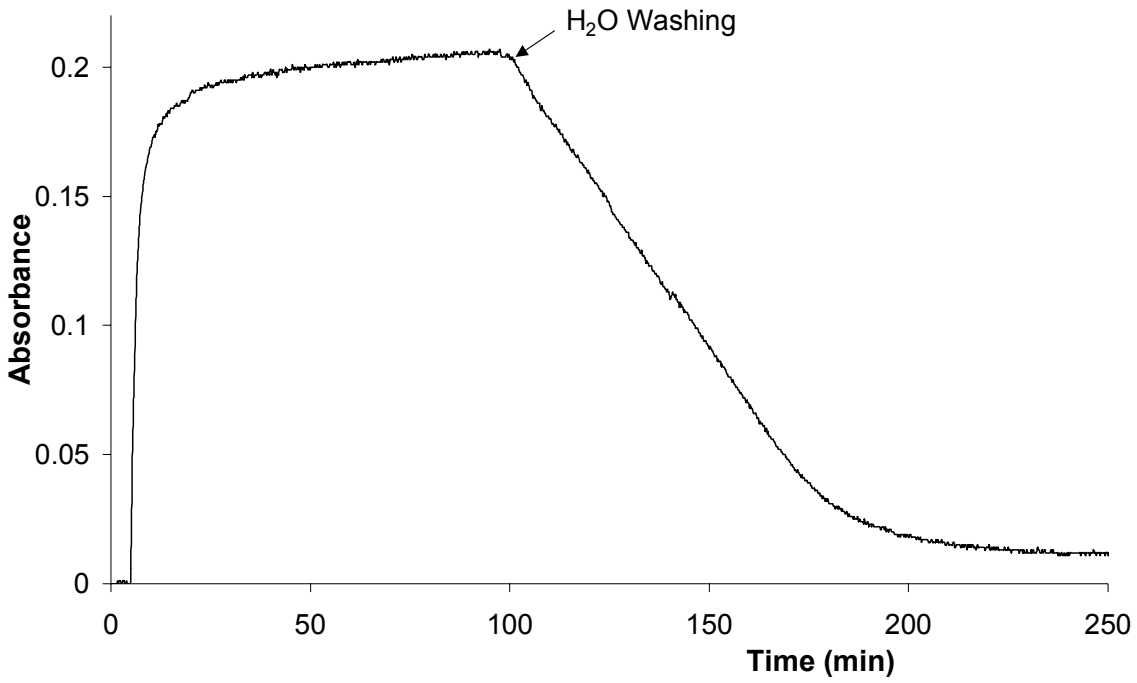

Figure 4- 2. Response with time and reversibility of the fiber optic sensor for hydrogen peroxide in milk.

4.3 Detection Of Hydrogen Peroxide In Milk Using Standard Addition

There are many other components present in a milk sample matrix such as proteins, fats, and calcium ions. These substances could potentially interfere with the detection of hydrogen peroxide. They may interact with hydrogen peroxide to change the response, or themselves interfere with the response of the sensor to the analyte, leading an error in the determination of hydrogen peroxide.

One way to overcome this difficulty is to use the "standard addition method". Briefly, a standard solution of a known concentration of the analyte is added (spiked) in a blank sample containing the same sample matrix as the unknown sample. A series of spiked solutions are prepared in different spiked concentrations of the analyte. These

solutions and a sample are then tested. The concentration of the analyte in the unknown solution can then be extrapolated and determined.

Figure 4-3 shows the absorbance spectra of the sensor exposed to a set of spiked milk solutions. 20 mL of raw milk was diluted 10 fold to 200 mL with deionized water. Spiked solutions were prepared by measuring 20 mL of raw milk, adding the calculated amount of concentrated hydrogen peroxide solution and then diluted to 200 mL with deionized water. The concentrations of hydrogen peroxide in the spiked sample were 0.03-ppm, 0.09-ppm, 0.9-ppm and 3-ppm. Solutions were pumped into the sample cell at 1 mL/min, and the absorbance was monitored at 360 nm. After analysis of each sample, the sensor was washed with deionized water exhaustively. As shown in Figure 4-3, it is observed that the absorbance at 360 nm did not change significantly when the concentration of added hydrogen peroxide was below 0.09 ppm. With the concentration above 0.9 ppm, the absorbance at 360 nm increased with concentrations of added hydrogen peroxide accordingly. It was considered that the original concentration of hydrogen peroxide in milk might be higher than at least 0.09 ppm so that the added small amount of hydrogen peroxide had no obvious effect to the response. It should be mentioned here that the lowest concentration producing significant response is not 0.9 ppm. It must be somewhere in between 0.09 ppm and 0.9 ppm.

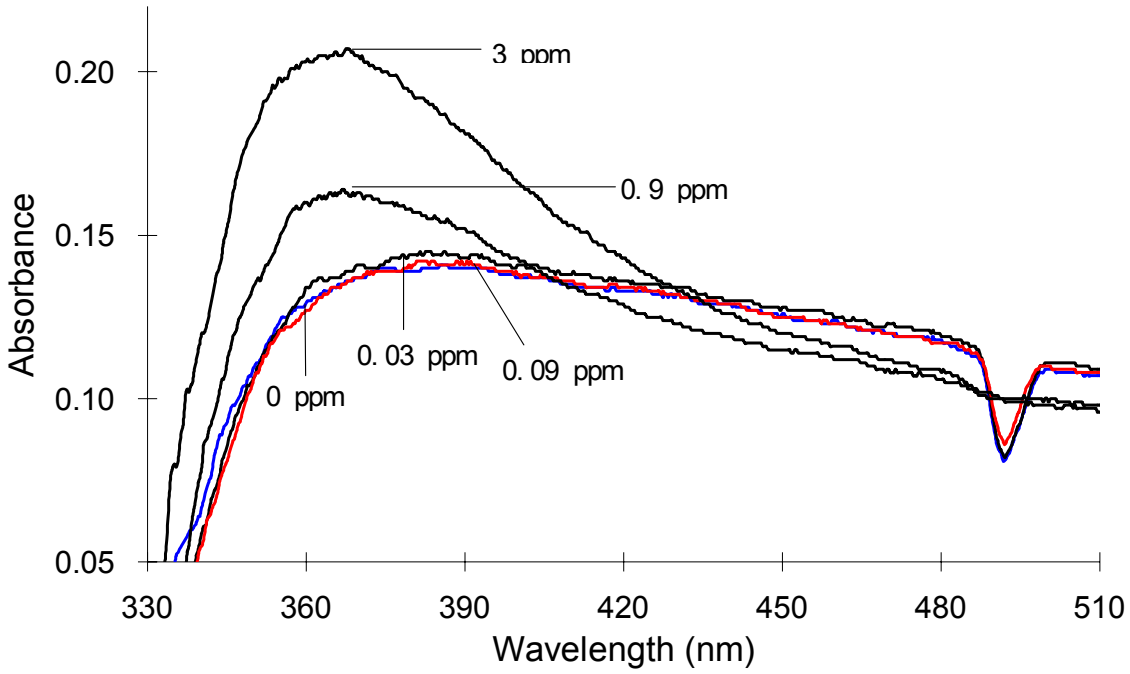

Figure 4- 3. Absorbances of spiked milk solutions.

Figure 4-4 shows the normalized absorbance as a function of the concentration of the spiked hydrogen peroxide. Again, the absorbance at 360 nm was normalized with respect to the absorbance at 470 nm. According to the principle of standard addition method, the plot is extrapolated to zero and the absolute value of the x-intercept is the concentration of hydrogen peroxide in raw milk. In this case, the concentration of the 10-fold dilution of milk is 1.65-ppm; therefore, the original concentration of peroxide in the milk sample is 16.5-ppm.

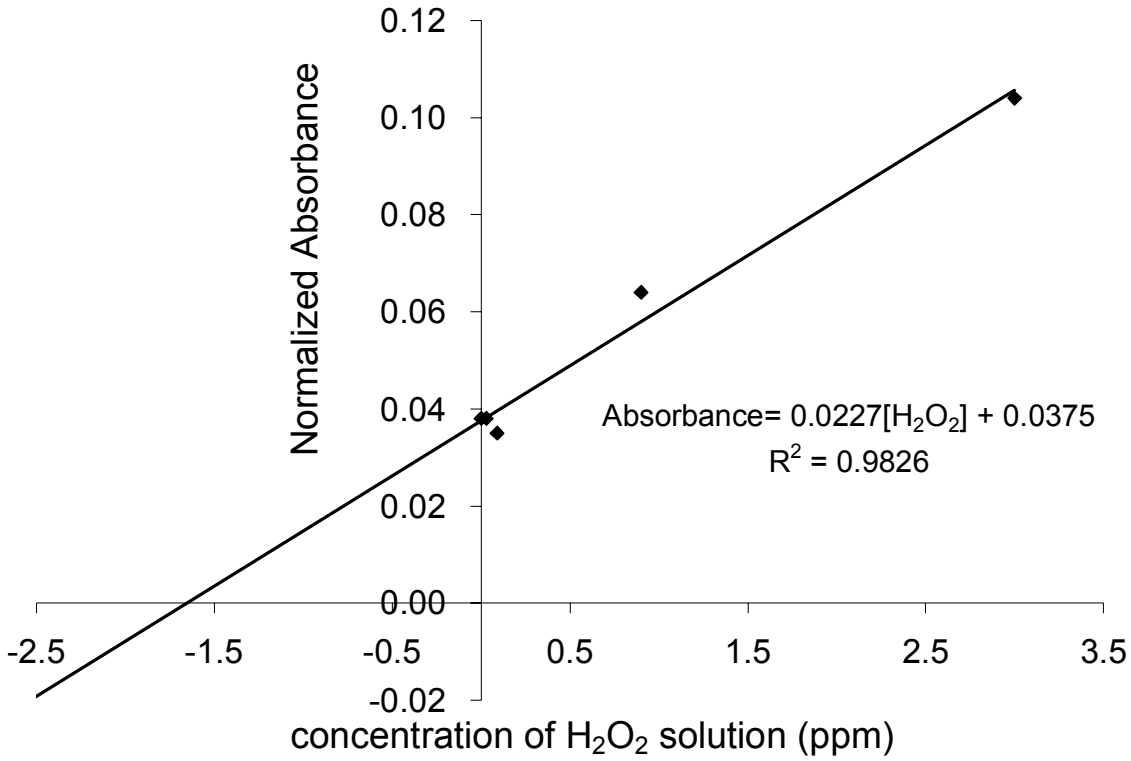

Figure 4- 4. Plot of absorbance versus the concentration of spiked H_2O_2. The absorbance is normalized with respect to the absorbance at 470 nm. The x-intercept is the concentration of the original H_2O_2 in the diluted milk.

4.4 Summary

The analysis of hydrogen peroxide in milk was performed with the optical fiber sensor developed in this study. Since milk is a complex sample matrix, the standard addition method was applied for the determination of trace amount of hydrogen peroxide in milk. The intensity of absorbance is increased with the addition of hydrogen peroxide proportionally. The plot of the absorbance is linear with the added concentration of hydrogen peroxide and the x-intercept of the extended line is taken as the original concentration of hydrogen peroxide in milk.

CHAPTER V

CONCLUSIONS

The aim of this work was to develop a method to quantify hydrogen peroxide using an optical fiber chemical sensor. The optical fiber chemical sensor is of great interest to scientists. This is because of its high sensitivity, easy fabrication, wide application and low cost of application.

The optical fiber chemical sensor in this work was prepared by dip-coating in a titanium(IV)-Nafion mixture. Nafion was used to form a membrane on the surface of fiber core in order to hold titanium ions. Titanium was used as an indicator to signal the presence of hydrogen peroxide by forming the Ti (IV)-H_2O_2 complex.

The wavelength of the maximum absorbance is 360 nm. Two different titanium compounds (titanium oxysulfate sulfuric acid and titanium oxyacetyl - oxyacetonate) were compared as indicators. It was proved that the inorganic titanium oxysulfate sulfonic acid couldn't be held tightly by Nafion therefore the response decreased rapidly with time. The titanium with organic ligands (titanium oxyacetonate) had no obvious leaking after a relative long time use. Transmission electron microscopy (TEM) was used to verify the existence of titanium in the Nafion film.

The response of the optical fiber titanium sensor increased proportionally with the increase in concentration of hydrogen peroxide in solution. At low concentrations, the

plot of the response versus the concentration is linear, while at higher concentrations (larger than 0.6 ppm), the response has a logarithmic relationship with the concentration. Additionally, the numbers of dip-coatings affect the response of the sensor to the same hydrogen peroxide solution. The more dip-coatings it has, the higher the intensity will be. After each analysis, the sample cell was flushed with deionized water and the response went back to the base line rapidly, showing the good reversibility of the sensor for hydrogen peroxide. In addition, the sensor also had satisfying reproducibility.

The use of PDMS as a protecting membrane was examined. It was observed that PDMS membrane could prevent the leaking of titanium from the film in the presence of either concentrated hydrogen peroxide or high working temperature. A calibration curve with a high correlation coefficient was obtained by analyzing a series of relative high concentration of solutions at 70 ^{0}C.

The determination of hydrogen peroxide in milk was performed using the optical fiber titanium sensor developed in this work. The milk was diluted 10 fold with water before use. Since the milk is a complex matrix, the standard addition method was used in the determination of the trace amount of hydrogen peroxide in milk. The concentration of hydrogen peroxide in the commercial milk sample studied here was determined to be 16 ppm.

Generally, hydrogen peroxide plays an important role in various fields. In this work, a reliable and simple method for the determination of hydrogen peroxide has been developed. Nafion proved to be useful in holding the titanium ions on the surface of the fiber. PDMS was determined to be beneficial in preventing the destruction of the Ti-Nafion film under harsh conditions. This method also provides a guide for the direct

detection of hydrogen peroxide in a complicated environment with an optical fiber sensor. More experiments are necessary to explore the application of this sensor to other fields.

REFERENCES

1.Schumb, W. C.; Satterfield, C. N.; Wentworth, R. L., *Hydrogen peroxide*. Reinhold publishing corporation: New York, 1955.

2.Jones, C. W., *Applications of hydrogen peroxide and derivatives*. The Royal Society of Chemistry: Widnes, UK, 1999.

3.Busing, W. R.; Levy, H. A., Crystal and molecular structure of hydrogen peroxide: A Neutron-Diffraction study. *The Journal of chemical physics* **1965,** 42, (9), 3054-3059.

4.Marczenko, Z., *spectrophotometric determination of elements*. 1976.

5.Skoog, D. A.; Holler, F. J.; Nieman, T. A., *Principles of instrumental analysis*. Fifth ed.; Thomson,brooks/cole: 1997; p 374-376.

6.Qin, W.; Zhang, Z.; Li, B.; Liu, S., Chemiluminescence flow-sensing system for hydrogen peroxide with immobilized reagents. *Analytica Chimica Acta* **1998,** 372, 357.

7.Hool, K.; Nieman, T. A., Immobilized luminol chemiluminescence reagent system for hydrogen peroxide determinations in flowing streams. *Anal. Chem.* **1988,** 60, 834-837.

8.Nakashima, K.; Maki, K.; Kawaguchi, S.; Akiyama, S.; Tsukamoto, Y.; Imai, K., Peroxyoxalate chemiluminescence assay of hydrogen peroxide and glucose using 2,4,6,8-tetrathiomorpholinopyrimido[5,4-d]-pyrimidine as a fluorescent component. *Anal. Sci.* **1991,** 7, 709-713.

9.Lin, J. M.; Arakawa, H.; Yamada, M., Flow injection chemiluminescent determination of trace amounts of hydrogen peroxide in snow-water using KIO4-K2CO3 system. *Analytica Chimica Acta* **1998,** 371, 171.

10.Hanaoka, S.; Lin, J. M.; Yamada, M., Chemiluminescent flow sensor for H2O2 based on the decomposition of H2O2 catalyzed by cobalt(II)-ethanolamine complex immobilized on resin. *Analytica Chimica Acta* **2001,** 426, 57.

11.Jönsson, G.; Gorton, L., An electrochemical sensor for hydrogen peroxide based on peroxidase adsorbed on a spectrographic graphite electrode. *Electroanalysis* **1989,** 1, (5), 465-468.

12.Morales, A.; Cespedes, F.; Munoz, J.; Martinez-Fabregas, E.; Alegret, S., Hydrogen peroxide amperometric biosensor based on a peroxidase-graphite-epoxy biocomposite. *Analytica Chimica Acta* **1996,** 332, (2-3), 131.

13.Wollenberger, U.; Wang, J.; Ozsoz, M.; Gonzalez-Romero, E.; Scheller, F., Bulk modified enzyme electrodes for reagentless detection of peroxides. *Bioelectrochemistry and Bioenergetics* **1991,** 26, (2), 287.

14.Li, J.; Tan, S. N.; Ge, H., Silica sol-gel immobilized amperometric biosensor for hydrogen peroxide. *Analytica Chimica Acta* **1996,** 335, (1-2), 137.

15.Sánchez, P. D.; Blanco, P. T.; Alvarez, J. M. F.; Smyth, M. R.; O'Kennedy, R., Flow-injection analysis of hydrogen peroxide using a horseradish peroxidase-modified electrode detection system. *Electroanalysis* **1990,** 2, (4), 303-308.

16.Frew, J. E.; Harmer, M. A.; O. Hill, H. A.; Libor, S. I., A method for estimation of hydrogen peroxide based on mediated electron transfer reactions of peroxidases at electrodes. *Journal of Electroanalytical Chemistry* **1986,** 201, (1), 1.

17.Qian, J.; Liu, Y.; Liu, H.; Yu, T.; Deng, J., Characterization of regenerated silk fibroin membrane for immobilizing peroxidase and construction of an amperometric hydrogen peroxide sensor employing phenazine methosulphate as electron shuttle. *Journal of Electroanalytical Chemistry* **1995,** 397, (1-2), 157.

18.Degani, Y.; Heller, A., Electrical communication between redox centers of glucose oxidase and electrodes via electrostatically and covalently bound redox polymers. *J. Am. Chem. Soc.* **1989,** 111, (6), 2357 - 2358.

19.Ye, J.; Baldwin, R. P., Catalytic reduction of myoglobin and hemoglobin at chemically modified electrodes containing methylene blue. *Anal. Chem.* **1988,** 60, (20), 2263-2268.

20.Matsubara, C.; Kawamoto, N.; Takamura, K., Oxo[5, 10, 15, 20-tetra(4-pyridyl)porphyrinato]titanium(IV): an ultra-high sensitivity spectrophotometric reagent for hydrogen peroxide. *Analyst* **1992,** 117, 1781 - 1784.

21.Harms, D.; Than, R.; Krebs, B.; Karst, U., Flow injection analysis of hydrogen peroxide in disinfectants. *Fresenius' Journal of Analytical Chemistry* **1999,** 364, (1-2), 184-188.

22.Yoe, J. H., Photometric chemical analysis. *Chapman & Hall, Limited* **1928,** Vol. I. Colorimetry.

23.Matsubara, C.; Kudo, K.; Kawashita, T.; Takamura, K., Spectrophotometric determination of hydrogen peroxide with titanium 2-((5-bromopyridyl)azo)-5-(N-propyl-N-sulfopropylamino)phenol reagent and its application to the determination of serum glucose using glucose oxidase. *Anal. Chem.* **1985,** 57, (6), 1107-1109.

24.Lobnik, A.; Cajlakovic, M., Sol-gel based optical sensor for continuous determination of dissolved hydrogen peroxide. *Sensors and Actuators B: Chemical* **2001,** 74, 194.

25.Wolfbeis, O. S., Fiber-optic chemical sensors and biosensors. *Anal. Chem.* **2004,** 76, (12), 3269-3284.

26.Posch, H. E.; Wolfbeis, O. S., Optical sensor for hydrogen peroxide. *Microchimica Acta* **1989,** 97, (1), 41.

27.Wolfbeis, O. S.; Schäferling, M.; Dürkop, A., Reversible optical sensor membrane for hydrogen peroxide using an immobilized fluorescent probe, and its application to a glucose biosensor. *Microchimica Acta* **2003,** 143, (4), 221.

28.Nakano, S.; Fukuda, M.; Kageyama, S.; Itabashi, H.; Kawashima, T., Flow injection determination of chromium(III) by pyrogallol chemiluminescence. *Talanta* **1993**.

29.Kamidate, T.; Ichihashi, H.; Segawa, T.; Watanabe, H., Enhanced chemiluminescence of lucigenin with epinephrine in cationic surfactant micelles containing periodate. *Journal of Bioluminescence and Chemiluminescence* **1995,** 10, (1), 55-61.

30.Schachla, K.; Alemub, H.; Kalcher, K.; Jeözkovac, J.; öSvancarac, I.; Vytörasc, K., Amperometric Determination of hydrogen peroxide with a manganese dioxide-modified carbon paste electrode using flow injection analysis. *The Analyst* **1997,** 122, 985-989.

31.Ungar, S., *Fibre Optic: Theory and Applications*. John Wiley & Sons Company: New York, 1990.

32.Stewart, G.; Johnstone, W., *Opt. fiber sens.* 1996; p 69-101.

33.Khijwania, S. K.; Gupta, B. D., Maximum achievable sensitivity of the fiber optic evanescent field absorption sensor based on the U-shaped probe. *Optics Communications* **2000,** 175, 135-137.

34.S, T. L.; George, N. A.; Sureshkumar, P.; Radhakrishnan, P.; Vallabhan, C. P. G.; Nampoori, V. P. N.

35.Michel, K.; Bureau, B.; Boussard-Pledel, C.; Jouan, T.; Adam, J. L.; Staubmann, K.; Baumann, T., Monitoring of pollutant in waste water by infrared spectroscopy using chalcogenide glass optical fibers. *Sensors and Actuators B: Chemical* **2004,** 101, (1-2), 252.

36.Tao, S.; Xu, L.; Fanguy, J. C., Optical fiber ammonia sensing probes using reagent immobilized porous silica coating as transducers. *Sensors and Actuators B: Chemical* **2006,** 115, (1), 158.

37.Wolfbeis, O. S., Fiber-optic chemical sensors and biosensors. *Anal. Chem.* **2006,** 78, (12), 3859-3874.

38.Palais, J. C., *Fiber optic communications*. Third ed.; 1992; p 21-25.

39.Patil, Y. P.; Seery, T. A. P.; Shaw, M. T.; Parnas, R. S., In situ water sensing in a nafion membrane by fluorescence spectroscopy. *Ind. Eng. Chem. Res.* **2005,** 44, (16), 6141-6147.

40.Yang, W.; Li, Y.; Bai, Y.; Sun, C., Hydrogen peroxide biosensor based on myoglobin/colloidal gold nanoparticles immobilized on glassy carbon electrode by a Nafion film. *Sensors and Actuators B* **2006,** 115, 42-48.

41.Sellers, R. M., Spectrophotometric determination of hydrogen peroxide using potassium titanum (IV) oxalate. *Analyst* **1980,** 105, 950-954.

42.Yoe, J. H., Photometric chemical analysis. Chapman & Hall, Limited: London, 1928; Vol. I. Colorimetry.

43.Luck, H., The use of hydrogen peroxide as a dairy preservation. *Dairy Science Abstracts* **1956,** 18, 364-368.

44.Benefits & potential risks of the lacto-peroxidase system of raw milk preservation. Bilingual edition ed.; Food & Agriculture Org: 2006.

45.Reiter, B.; Oram, J. D., Bacterial inhibitors in milk and other biological fluids. *Nature* **1967,** 216, (5113), 328.

46.Hankin, L., Hydrogen peroxide ingestion and the growth of rats. *Nature* **1958,** 182, (4647), 1453.

47.Amberg, S., A method for the determination of hydrogen peroxide in milk, together with some observations on the preservation of milk by this substance. *J. Biol. Chem.* **1905,** 1, (2), 219-228.

48.Peinado, J.; Toribio, F.; Perez-Bendito, B., Fluorometric reaction-rate method for determination of hydrogen peroxide at the nanomolar level. *Anal. Chem.* **1986,** 58, (8), 1725-1729.