Estimation of Chemical Oxygen Demand in WasteWater using UV-VIS Spectroscopy

by

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Abstract

The aim of this research is to build a portable system to perform real-time analysis of waste water samples. Thus, that can significantly improve existing waste water treatment technology. In waste water treatment plant, an important parameter, chemical oxygen demand is needed to be measured. The amount of chemical oxygen demand determines the degree of water pollution by organic material. The conventional method for measuring chemical oxygen demand requires sample preparation and pre-treatment using chemicals. These conventional techniques are time consuming and labour intensive. To overcome these problems, a system is developed in this work that offers significant advantages over conventional methods. The system employs ultraviolet and visible spectrometer in order to correlate the absorbance of light after it passes through a waste water sample with the conception of the target chemicals. The spectrometer limits the use and disposal of chemicals for the measurement of chemical oxygen demand. In addition, a software system is developed to control the spectrometer and to detect the most sensitive spectral region and wavelength for chemical oxygen demand content. Moreover, an algorithm is introduced by performing linear regression analysis on acquired concentration and light absorbance from the acquired spectra. The development and validation of this spectroscopic tool and software system are described in this thesis.

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List of Acronyms

APHA American Public Health Association

BOD Biochemical Oxygen Demand

CCD Charge Coupled Device

COD Chemical Oxygen Demand

DO Dissolved Oxygen

EMR Electromagnetic Radiation

FAS Ferrous Ammonium Sulfate

GUI Graphical User Interface

IR Infrared Ray

KHP Potassium Hydrogen Phthalate

MSE Mean Standard Error

SNR Signal to Noise Ratio

SMA Sub Miniature Version A

UI User Interface

UV Ultra-Violet

UV-VIS Ultra-violet and Visible

VIS Visible

WWTP Waste Water Treatment Plant

Chapter 1

Introduction

Water pollution and its impacts on the environment are serious issues for present world. To limit the water pollution and improve the water quality, advanced wastewater treatment technologies are invented. These technologies are implemented by removing physical, chemical and biological contaminants from wastewater and producing an environmentally safe fluid waste stream (treated effluent) and a solid waste (treated sludge)[1, 2]. It may then even be possible to reuse sewage effluent for drinking water with the help of more advanced technologies. If untreated wastewater containing contamination enters into the surface and ground water resources, it leads to a serious environmental and human health risk [3]. To minimize the potential risks from untreated wastewater entering freshwater resources, industrial wastewater plants go through a water quality assessment by monitoring some parameters. Water quality professionals assess water quality by measuring the concentrations of these parameters and comparing with their standards [2, 4]. Some of the unique analytical parameters of the water pollution control industry are biochemical oxygen demand, chemical oxygen demand, taste, odor, color, chlorine demand, hardness, alkalinity and biodegradability tests [2, 5]. Finding excessive levels of one or more of these parameters can serve as an early warning of potential pollution problems. One of these

parameters are COD and BOD that indicate the amount of organic pollution and water degradation.

COD is defined as the amount of oxygen equivalents consumed in oxidizing the organic compounds of samples by strong oxidizing agents such as dichromate or permanganate. It is expressed in milligrams per liter (mg/L) that indicates the mass of oxygen consumed per liter of solution [6]. The higher the chemical oxygen demand, the higher the amount of pollution in the water sample [7]. COD is considered one of the most important quality control parameters of an effluent in wastewater treatment facility [8]. COD values are used to monitor wastewaters before (influent) and after (effluent) treatment, and, therefore, their reliability is important to protect the environment and to guarantee the economical sustainability of the treatment facility [9].

COD measurements are commonly made on samples of wastewater treatment facility or of natural waters contaminated by domestic and industrial wastes. COD is measured as a standardized laboratory assay in which a closed water sample is incubated with a strong chemical oxidant under specific conditions of temperature and for a particular time. A commonly used oxidant in COD assays is potassium dichromate ($K_2Cr_2O_7$) which is used in combination with boiling sulfuric acid (H_2SO_4)[10, 11].

BOD is the amount of oxygen consumed by bacteria in the decomposition of organic material. It also includes the oxygen required for the oxidation of various chemical in the water, such as sulfides, ferrous iron and ammonia. While a dissolved oxygen test tells you how much oxygen is available and BOD test give information about how much oxygen is being consumed [10].

BOD is determined by measuring the dissolved oxygen level in a freshly collected sample and comparing it to the dissolved oxygen level in a sample that was collected at the same time but incubated under specific conditions for a certain number of days. The difference in the oxygen readings between the two samples in the BOD is recorded in units of mg/L. Unpolluted, natural waters should have a BOD of 5 mg/L or less. Raw sewage may have BOD levels ranging from 150 mg/L to 300 mg/L [12].

Chemical oxygen demand is related to BOD. However, BOD only measures the amount of oxygen consumed by microbial oxidation and COD and BOD do not necessarily measure the same types of oxygen consumption.

1.1 Objectives and Motivation

The objective of this study is to develop a rapid, inexpensive and accurate spectral analysis method for the determination of COD in industrial effluent. The developed system can omit the conventional time consuming sample preparation process and can avoid chemical reagents and chemical by products . Finally, simplified User Interface (UI) for the spectrometer is developed which will guide the user to estimate accurate COD content instantly.

Ultra-violet and Visible (UV-VIS) spectroscopy is the best solution for quantitative determination of organic compounds through absorbance analysis [13]. Thus, the COD value can be obtained by UV-VIS absorbance analysis, which is based on Beer-Lambert law [14]. According to Beer-Lambert law the light absorbance through a solution is directly proportional to the concentration of the absorbing species in the solution and the path length of sample holder. For a fixed path length, the absorbance of a solution changes with the concentration of that solution. In this project, spectral absorbance of the solutions were

analyzed to evaluate unknown COD concentration value from the water samples. Real wastewater samples were collected directly from Waste Water Treatment Plant (WWTP), which were then analyzed with the developed spectrometric system. Throughout this process, a linear relationship of absorbance and COD concentration was established. Moreover, this developed system is portable and does not require any chemicals to process. Real time measurements can be achieved by minimal sample preparation and less labour.

1.2 Thesis Structure

Chapter 2 provides a literature review which explains the various water quality assessment parameters and mostly emphasizes on the impact of chemical oxygen demand and biochemical oxygen in wastewater treatment plant. It also describes basic principle of UV-VIS spectrometer and its application for determining COD and BOD.

Chapter 3 provides the detailed explanation about the spectral system. Detailed description of each components of the UV-VIS spectrometer has been presented in this Chapter.

Chapter 4 summarizes the developed algorithm for detecting the maximum sensitive wavelength and region for measuring industrial COD by linear regression analysis of industrial samples and interfacing this algorithm with spectrometer. In addition,this Chapter also outlines the development processes of MATLAB based UI.

Chapter 5 demonstrates the results obtained from statistical analysis to get a perfect linear relationship of spectrometer parameter and COD concentration of industrial samples which will lead to achieve any unknown COD content from any WWTP. In addition, this

Chapter also shows the validation processes of the obtained results.

Finally, Chapter 6 gives an insight into the future work and presents the conclusion of this research.

Chapter 2

Literature Review

2.1 Water Quality Assessment Parameters

A rapid development in science and technology and a vast usage of industrial processes are generating and releasing more and more pollutants into the environment. The number of pollutants and their concentrations in water are continuously increasing. Therefore, the analysis of chemical pollutants in water has become a serious concern to the professional world and to the authorities in charge of water quality [8, 15].

Some of the unique analytical parameter characteristics of the water pollution control industry are biochemical oxygen demand, chemical oxygen demand, acidity, alkalinity, conductivity, hardness, hardness, metal, pH, turbidity tests. Water quality depends on these parameters and their characterization tests [5].

2.1.1 Acidity

Acidity of water is determined when reacts with a strong base to a designated pH. Acidity indicates of how corrosive water is and the most common source of acidity in unpolluted

water is carbon dioxide in the form of carbonic acid. Acidity is a measure of an aggregate property of water, and which can be interpreted in terms of specific substances only when the chemical composition of the sample is known [16, 5].

Acidity is classified by the pH value of a titration end point. Acidity caused by mineral acids contains a pH below 4.5 [17]. Industrial waste waters containing high mineral acidity must be neutralized before they are subjected to biological treatment or direct discharge to water sources. Standard methods for the examination of water and waste water recommends titration with sodium hydroxide to an end point pH of 3.7 to determine mineral acidity [18].

2.1.2 Alkalinity

Alkalinity is a measure of the capacity of water to neutralize acids. Alkalinity of water indicates the presence of bicarbonate, carbonate, and hydroxide ions. In waste water treatment, alkalinity is a quality parameter to determine the amenability of waste to the treatment process [5]. Waste water becomes alkaline when receiving alkalinity from the water supply, ground water, domestic use (detergents and soap-based products) and acid rain [16].

Alkalinity is significant in the treatment processes for water and waste water. Alkalinity is expressed as phenolphthalein alkalinity or total alkalinity [18]. Both types can be determined by titration with a standard sulfuric acid solution to an end point pH, evidenced by the color change of a standard indicator solution. The pH also can be determined with a pH meter. Phenolphthalein alkalinity is determined by titration to a pH of 8.3 (the phenolphthalein end point) [18].

2.1.3 Conductivity

Conductivity is a measure of how well water can pass an electrical current. It is an indirect measure of the presence of inorganic dissolved solids. The presence of chloride, nitrate, sulfate, phosphate, sodium, magnesium, calcium, iron and aluminium increases conductivity of water. In addition, organic substances like oil, alcohol, and sugar conduct less electricity which indicates low conductivity of water [19].

The measurement of conductivity is generally expressed in S/cm (or mS/cm). The scale for conductivity starts at 0.05 μS/cm (at 25 $^\circ$C) for ultra pure water [20]. The conductivity of natural waters, such as drinking water or surface water is typically in range of 100-1000 μS/cm.

2.1.4 Hardness

Hardness is frequently used as an assessment of the quality of water supplies. The hardness of a water is governed by the content of calcium and magnesium salts (temporary hardness), largely combined with bicarbonate and carbonate and with sulfates, chlorides, and other anions of mineral acids (permanent hardness).To distinguish the contributions of such anions from carbonates,hardness is sometimes termed as "carbonate hardnesss" or "noncarbonate hardness" and expressed as mg $CaCO_3$ [21]. Hardness can be measured by calculation from the concentration of calcium and magnesium ions in the sample [22].

2.1.5 Metals

The effects of metals in water and wastewater range from beneficial through troublesome to dangerously toxic. Some metals are essential, others may adversely affect water consumers, wastewater treatment systems, and receiving waters. Some metals may be either

beneficial or toxic, depending on concentration [16].

2.1.6 Nitrogen as Ammonia

Ammonia (NH_3) is one of the most important pollutants in the aquatic environment because of its relatively highly toxic nature and its presence in surface water systems. It is discharged in large quantities in industrial, municipal and agricultural wastewaters. In aqueous solutions, ammonia assumes two chemical forms: NH_4^+ - ionized (less toxic/nontoxic) and NH_3 - unionized (toxic). The relative concentration of ionized and unionized ammonia in an ammonia solution are principally a function of pH, temperature and ionic strength of the aqueous solution [23].

2.1.7 Nitrogen as Nitrate

Nitrate (NO_3^-) generally occurs in trace quantities in surface water. It is the essential nutrient for many photosynthetic autotrophs and has been identified as the growth limit nutrient. It is only found in small amounts in fresh domestic wastewater. In effluent of nitrifying biological treatment plants, nitrate may be found in concentrations up to 30 mg nitrate as nitrogen/L [16].

2.1.8 Nitrogen as Nitrite

Nitrite (NO_2^-)is extremely toxic to aquatic life, however, is usually present only in trace amounts in most natural freshwater systems because it is rapidly oxidized to nitrate. In sewage treatment plants, nitrification process is used to convert ammonia to nitrate. The process may be impeded though causing discharge of nitrite at elevated concentrations into receiving waters [24].

2.1.9 Phosphorus

Phosphorus is often the limiting nutrient for plant growth. Phosphorus usually occurs in nature as phosphate, which is a phosphorous atom combined with four oxygen atoms (PO_4^{-3}). Phosphate that is bound to plant or animal tissue is known as organic phosphate. Phosphate that is not associated with organic material is known as inorganic phosphate. Both forms are present in aquatic systems and may be either dissolved in water or suspended. Inorganic phosphate is often referred to as orthophosphate or reactive phosphorous. It is the form most readily available to plants, and thus may be the most useful indicator of immediate potential problems with excessive plant and algal growth. Testing for total phosphorous (both inorganic and organic phosphate) provides with a more complete measure of all the phosphorus that is actually in the water [19].

2.1.10 pH

pH is an important limiting chemical factor for aquatic life. If the water in a stream is too acidic or basic, the H^+ or OH^- ion activity may disrupt aquatic organisms biochemical reactions by either harming or killing the stream organisms. pH is expressed in a scale with ranges from 1 to 14. A solution with a pH less than 7 has more H^+ activity than OH^-, and is considered acidic. A solution with a pH value greater than 7 has more OH^- activity than H^+, and is considered basic. The pH scale is logarithmic and making up and down the scale, the values change in factors of ten. An one-point pH change indicates the strength of the acid or base has increased or decreased tenfold [22].

Streams generally have a pH values ranging between 6 and 9, depending upon the presence of dissolved substances that come from bedrock, soils and other materials in the watershed. Changes in pH can change the aspects of water chemistry. For example, as pH

increases, smaller amounts of ammonia are needed to reach a level that is toxic to fish. As pH decreases, the concentration of metal may increase because higher acidity increases their ability to be dissolved from sediments into the water [19].

2.1.11 Turbidity

Turbidity is a measure of the cloudiness of water [25]. Cloudiness is caused by suspended solids (mainly soil particles) and plankton (microscopic plants and animals) that are suspended in the water [26]. Moderately low levels of turbidity may indicate a healthy, well-functioning ecosystem, with moderate amounts of plankton present to balance the food chain. However, higher levels of turbidity pose several problems for water systems. Turbidity blocks out the light needed by submerged aquatic vegetation. It also can raise surface water temperatures above normal because suspended particles near the surface facilitate the absorption of heat from sunlight. Suspended soil particles may carry nutrients, pesticides, and other pollutants throughout a water system [27]. Turbid waters may also be low in dissolved oxygen. High turbidity may result from sediment bearing run-off, or nutrients inputs that cause plankton blooms [19].

2.1.12 Total Solids

Total Solids is a measure of the suspended and dissolved solids in a body of water. Thus, it is related to both conductivity and turbidity. To measure total suspended and dissolved solids, a sample of water is placed in a drying oven to evaporate the water, leaving the solids. To measure dissolved solids, the sample is filtered before it is dried and weighed. To calculate the suspended solids, the weight of the dissolved solids is subtracted from the total solids [19]. Important components of the total solid load from the treatment plants include phosphorus, nitrogen, and organic matter.The amount of solids in wastewater is

frequently used to describe the strength of the waste. The more solids present in a partic-
ular wastewater, the stronger that wastewater will be. If the solids in wastewater are mostly
organic, the impact on a treatment plant is greater than if the solids are mostly inorganic.
Gravimetric is the approved procedure total solids, total dissolved solids, total suspended
solids, fixed and volatile solids and Volumetric is the method for measuring settleable solids
[18].

2.2 Chemical Oxygen Demand

COD is defined as the amount of dissolved oxygen to oxidize and stabilize a sample when
organic or inorganic matter of sample solution is responsive by a strong chemical oxidant.
The COD value indicates the mass of oxygen consumed per liter of solution and expressed
in milligrams per liter (mg/L). The higher the chemical oxygen demand, the higher the
amount of pollution in the water sample. However, COD is considered one of the important
quality control parameter of an effluent in wastewater treatment facility [9].

The complete oxidation of organic compounds under such strong oxidizing conditions
produces carbon dioxide (CO_2) and water (H_2O). The COD for any organic compound
can be theoretically calculated from writing a balanced equation [11]. Using glucose as an
example:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O \tag{2.1}$$
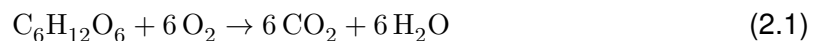
The theoretical COD of glucose can be calculated as :

$$\text{COD} = \frac{\text{grams of oxygen used}}{\text{grams of substrate used}} = \frac{(6\times32)}{(6\times12)+12+(6\times6)} = \frac{192}{180} = \frac{1.067 \text{ g COD}}{\text{grams of substrate utilized}}$$

Glucose is converted to carbon dioxide and water by a stoichiometric amount of oxygen
(1.067 mg of oxygen per mg of glucose). In wastewater, if the concentration of glucose and

Table 2.1: Theoretical COD values of 1 gm of variuos organic compounds.

Component	Formula	COD (mg/L)
Oxalic Acid	$C_2H_2O_4$	0.18
Formic acid	CH_2O_2	0.35
Citric acid	$C_6H_8O_7$	0.75
Acetic acid	$C_2H_4O_2$	1.07
Glycerol	$C_3H_8O_3$	1.22
Phenol	C_6H_6O	2.38
Acetone	C_3H_6O	2.21
Ethanol	C_2H_6O	2.09
Methanol	CH_4O	1.50
Ethane	C_2H_5	3.73
Methane	CH_4	4.00

concentration of biomass is calculated before and after biological treatment, we can know how much oxygen was consumed during the conversion of glucose to biomass and end products. When this procedure for the COD test is strictly followed, the experimental result will not differ more than a few percent from the theoretical value. A list of the theoretical oxygen demand of a variety of other substances [28] which can be contented in municipal and industrial wastewater is given in Table 2.1. It shows that 1 mg/L of oxalic acid has a theoretical COD of 0.18 mg/L where 1 mg/L of acetic acid has a theoretical COD of 1.07 mg/L.

2.2.1 Methods to Measure COD

The dichromate method is the American Public Health Association (APHA) standard method for determining COD with the use of potassium dichromate. The amount of dichromate is determined by direct titration using Ferrous Ammonium Sulfate (FAS) as the titrant and fer-roin (1, 10 phenanthroline ferrous sulfate) as the indicator [11]. During the course of the titration, the titrant (Fe_2^+) reacts instantly with hexavalent chromium (Cr_6^+) to form trivalent

chromium (Cr_3^+) and ferric ion (Fe_3^+) which is shown below:

$$3Fe^{2+} + Cr^{6+} \rightarrow 3Fe^{3+} + Cr^{3+} \tag{2.2}$$
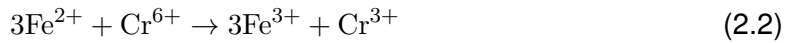
The final hexavalent chromium level is then subtracted from the initial level to determine the amount of hexavalent chromium reduced during the digestion. This difference is used to calculate the COD by following equation

$$COD, \text{ mg/L} = \frac{(A - B) \times N \times 8000}{\text{mL sample}} \tag{2.3}$$

where, A is the mL FAS required for the titration of the blank, B is the mL FAS required for the titration of the sample, N is the normality of FAS. In 1951 Medalaia presented the total pollution load of most wastewater discharges by this COD analysis. In 1990 the Association of Official Analytical Chemists described, the COD as a degree of pollution and one of the most important parameters to characterize pulping effluents [29]. The conventional method for COD determination was then performed by adding a known amount of oxidant (such as potassium dichromate, potassium permanganate) to the sample to oxidize the organic matter in a digestion process at high temperature in open containers and then titrating the excess oxidant. The COD value was then calculated by subtracting the excess amount of oxidant from the initial. However, the conventional method has some drawbacks, such as time-consuming and requiring troublesome manual operations. Back titration of dichromate after sample digestion is an insensitive method of detection, and the method also consumes some expensive and toxic chemicals such as Ag_2SO_4 and $HgSO_4$ [30]. Recently, manganese III sulfate contributes for COD tesing as an oxidant because of its non-hazardous properties. Beside these, $Ce(SO_4)_2$, K_2S_2O, KIO_3 are used as oxidant in COD determination. However, the only interference in determining COD is presence of Chloride [31]. It can be eliminated by mixing mercuric sulfate ($HgSO_4$) in COD vials

and diluting samples proper enough to reduce the chloride concentration. Also, titrimetric procedure requires a high degree of operator skill and take longer time to perform. Figure 2.1 shows the conventional method of measuring COD in wastewater.

Figure 2.1: Conventional method of measuring COD in wastewater.

Colorimetric measurement of COD is then considered faster and easier to perform than titrimetric analysis. The sample is digested in an ampule, culture tube or vial under closed reflux conditions [11]. A spectrophotometer is needed to make a standard calibration curve by measuring concentration of dichromate and their absorbance. Normally, COD vials are commercially available for COD measurement and Potassium Hydrogen Phthalate (KHP) is used as a reference standard for colorimetric analysis [32].

In the last decade, chemometrics has been developed rapidly in modern chemical measuring instrument and the development in computer technology. Chemometrics has

evolved to an important analytical method which was widely received as a rapid and efficient analytic technique. In addition, chemometrics has been widely used in the development of analytical methods through experimental design and systematic optimization. Specially, chemometricsassisted spectrophotometry is a very good combination of chemometrics with analytical chemistry [33]. It increases the Signal to Noise Ratio (SNR), improve selectivity of determination, optimize experimental conditions, increase analytical operation efficiency, and provide much more scientific information than conventional spectrophotometry [34]. Hence, chemometricsassisted spectrophotometry is rapidly attracting analysts attention and is used for simultaneous determination of multi-components in recent years. In analytical chemistry, chemometrics is usually used to resolve some complex problems of calibration [35]. Among the linear regression methods that have been proposed for multicomponent analysis [36]. Based on the results of spectroscopic measurement, mathematical models for the relationship between multi-wavelength spectral signals of samples and the corresponding concentration were established, and the concentration of unknown samples can be predicted by inputting their spectral signals into mathematical models.

It is difficult to determine COD in wastewater system using the absorbance at single or a few wavelengths because wastewater is a complex system which consists of multi-components. Therefore, we have developed a intelligent system, which can automatically determine most sensitive wavelength and determine COD content in effluent in this work.

2.3 Biochemcial Oxygen Demand

BOD is defined as the amount of oxygen required by microorganism to stabilize decomposable organic matter at a particular time and temperature. If a small amount of biomass

Table 2.2: Theoretical BOD values of organic compounds.

Condition	BOD (mg/L)
Pristine river	≤ 1mg/L
Moderately polluted river	2-8 mg/L
Well-treated municipal sewage	20 mg/L
Untreated sewage	≥ 200 mg/L
Waste water BOD_5	≈ 250 mg/L
Effluent BOD_5 from waste water	≤ 30 mg/L
Industrial waste water	$30,000$ mg/L

(primarily bacteria) seed is mixed in wastewater (containing of organic matter such as car-bohydrates, proteins and fats) and added nutrients for biomass growth, biodegration of organic matter will be increased and dissolved oxygen will be decreased. The general form of the equation for decomposition of organic matter during BOD test is [37]

$$\text{matter + nutrients} + O_2 \xrightarrow{biomass} CO_2 + H_2O + \text{biomass} \tag{2.4}$$
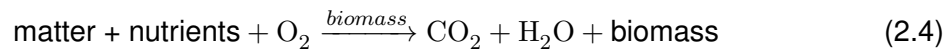
The organic matter is the growth substrate (carbon and energy source) for the generation of new biomass. Biomass is considered as the catalyst for the reaction where oxygen is consumed. Theoretically infinite time is required for complete biological oxidation of organic matter of domestic sewage. But for all practical purposes, the difference between the initial amount of oxygen and the remaining amount of oxygen after 5 days of water sample yields the "biochemical oxygen demand after 5 days" or the BOD5 value. All these water samples should be kept at $200°C$ for proper biodegrading which has impact on dissolved oxygen and thus BOD [28].

There is a co-relation between COD and BOD_5 and BOD_5/COD ratio is known as biodegradability index. Considering domestic wastewaters, this ratio value varies from 0.4 to 0.8 for domestic [38]. If BOD_5/COD is ≥ 0.6 then the waste is fairly biodegradable and an effective biological treatment can be possible. If BOD_5/COD ratio is between 0.3 and

0.6, then seeding is required to treat it biologically. If BOD_5/COD ratio is <0.3 then the probability of biological treatment is less [38].

2.3.1 Methods to Measure BOD

The most conventional method is 5 days BOD determination method. In this standard BOD test, a small sample of the wastewater to be tested is placed along with dilution water in a BOD bottle (300 ml). The Dissolved Oxygen (DO) concentration of the mixture in the bottle is measured. The bottle is incubated for 5 days at $20°$C and the DO concentration is measured again [38]. Oxygen consumed by the organics is determined from the difference and the BOD is calculated as follows:

$$\text{BOD (mg/L)} = \frac{(A_1 - A_2)V_2}{V_1} \tag{2.5}$$

where,

A_1 is the initial concentration DO, mg/L in the dilution water prepared

A_2 is concentration DO, mg/L in the diluted sample after 5 days incubation

V_1 is the volume of diluted sample

V_2 is the volume of the BOD bottle, mL

An important disadvantage of the BOD_5 test is that it requires a long time, which can be reduced only conditionally on the basis of special empirical values and on-line measurement is not possible for this method. In 2009 Hach proposed a graphical method to determine BOD value [39]. In this method, a series of diluted samples are prepared and incubated for 5 days where initial quantity of DO is not required to measure. Only remaining DO after incubation is measured. A graph of mL samples diluted vs mg/L DO after incubation is plotted over best-fit points. The slope resulted in line is equal to the quantity

of DO consumed (as mg/L) per mL of sample. And BOD is calculated from the following equation [39].

$$\text{BOD (mg/L)} = (slope = V_2) - y - intercept + \text{sample DO} \tag{2.6}$$

where V_2 is the volume of the BOD bottle, mL.

Yeast integrated on membrane has been used to determine BOD of domestic wastewater and pharmaceutical industries [40]. This measurements process require 30 minutes. The main disadvantage of this method is sample handling, necessity of filtration. In some wastewater treatment facility, isolation technique has been implemented on microorganisms from activated sludge which takes only 6 minutes to determine BOD value. Samples are needed to store at 4°C temperature which can result limited lifetime and stability of this method [41].

Biofilm with pre-ozonation of refractory organic compounds are also used to determine BOD of artificial wastewater which need sample pre-treatment by using many chemicals. These chemicals make those samples pH and temperature sensitive and takes 5 min-20 min to complete the whole procedure. Measurement of N-BOD can be done by disposable microbial sensor. This process is suitable for Ammonium standard solution and municipal wastewater. Samples remain stable in maximum 3 days and gives short lifetime [40, 42].

The most quick method to determine BOD is Ultra-Violet (UV) spectral measurements and multivariate calibration for mainly surface waters. If sample handling, acquisition of reference spectra and calibration are in a done properly, this method can result within few minutes [43].

Real time monitoring of BOD of surface wastewater is done by optical scattering method. Although this method is selective for plant site and whole method is limited to pH and temperature. UV absorption at 280 nm is considered to determine BOD of farm wastes which is similar to UV spectral measurement [44]. The only deference is its performance in only one wavelength which limits the result on influence of particles and toxic metals [44].

In recent years, some modelling and virtual sensors are developed to measure BOD. For paper mill effluent, prediction using artificial neural network is implemented. Another mathematical prediction can be done from COD values to measure BOD. Very recently a simplified method was proposed in which the BOD_5 value results as the difference of two COD values one of which is measured immediately and the other after standing for 2 hrs in the presence of excess air. It can give linear relationships all the time though not considered as real measurement [44].

2.4 Spectroscopy

The term "spectroscopy" defines a large number of techniques that use radiation to obtain information on the structure and properties of matter. All spectroscopic techniques focus on to response of sample when it stimulates by Electromagnetic Radiation (EMR). The response is recorded as a function of radiation wavelength which can refer as a spectrum. When this spectroscopic technique is used to measure the concentration or amount of a given species, the phenomenon is known as spectrometry [45]. Spectroscopy/spectrometry is used for the identification or quantification of substances through the spectrum emitted from or absorbed by them. And spectrometer or spectrograph is the instrument to operate such phenomenon.

Many atoms emit or absorb Visible (VIS) light and the spectrum is studied in absorption or emission. VIS absorption spectroscopy is often combined with UV absorption spectroscopy in UV-VIS spectroscopy. UV-VIS spectroscopy is the best solution for quantitative determination of organic compounds through absorbance analysis. The UV-VIS spectrometry system is increasingly employed in predicting COD and BOD recently for its significant advantages compared with traditional standard chemical method [46].

2.4.1 UV-VIS Spectroscopy

UV-VIS spectroscopic processes rely on the fact that EMR interacts with atoms and molecules in discrete ways to produce characteristics absorption or emission profiles. UV-VIS radiation comprise only a small part of the electromagnetic spectrum with other forms of radiation as radio, Infrared Ray (IR) radiation, cosmic, and x-rays [47].

EMR can be considered a combination of alternating electric and magnetic fields that travel through space with a wave motion. Because radiation acts as a wave, it can be classified in terms of either wavelength or frequency, which are related by the following equation [48]:

$$v = \frac{c}{\lambda} \tag{2.7}$$

where λ is frequency (in seconds), c is the speed of light (3×10^8 ms^{-1}), and λ is wavelength (in meters) [49].

In UV-VIS spectroscopy, wavelength usually is expressed in nanometers (1 nm = 10^{-9} m). It follows from the above equations that radiation with shorter wavelength has higher energy. In UV-VISspectroscopy, the low-wavelength UV light has the highest energy.
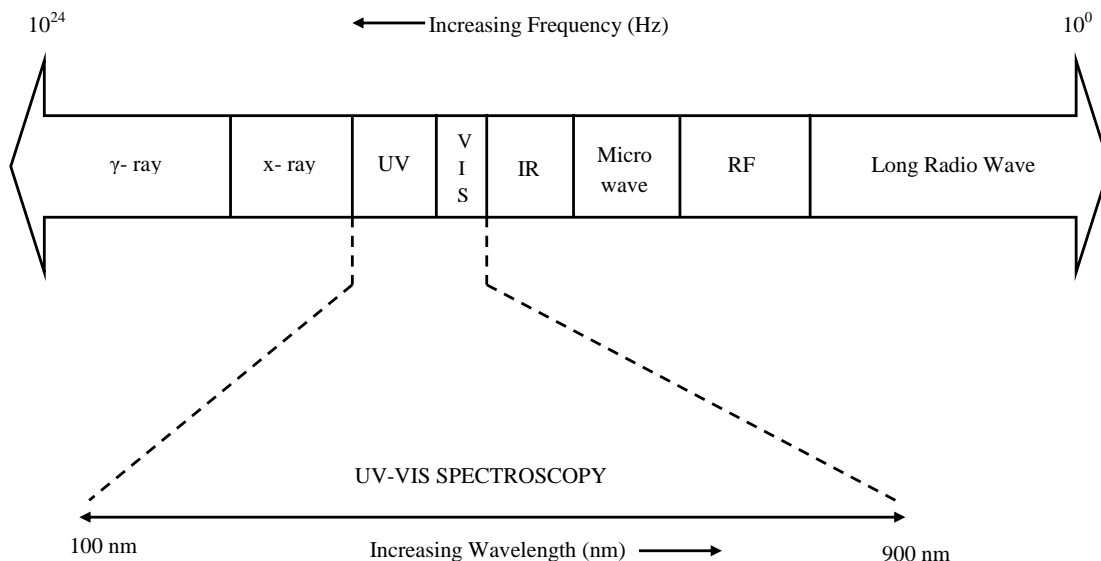
Figure 2.2: The spectrum of electromagnetic radiation from lowest energy to highest energy range

In some molecules and atoms, photons of UV-VIS light have enough energy to cause transitions between the different electronic energy levels. The wavelength of light absorbed is that having the energy required to move an electron from a lower energy level to a higher energy level.

When light passes through or is reflected from a sample, the amount of light absorbed is the difference between the incident radiation (I_o) and the transmitted radiation (I). The amount of light absorbed is expressed as either transmittance or absorbance [13]. Transmittance usually is given in terms of a fraction of 1 or as a percentage and is defined as follows:

$$T = \left(\frac{I}{I_0}\right) \times 100\%$$

(2.8)

This mathematical formulation was stated by Lambert in 1760 and since then this equation is known by Lambert's law. In the later time, the Lambert's law was combined with another law named Beer's law to produce a relationship between absorbance (A) and transmittance (T). Beer's law states that the absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path [14].

$$A = -\log_{10} T = \epsilon c b \qquad (2.9)$$

where A is the absorbance, ϵ is the molar absorptivity (dm^3 mol^{-1} cm^{-1}), c is molar concentration (mol dm^{-3}) and b is path length (cm)[50] [51].

2.4.2 Spectroscopic Methods for COD and BOD Measurements

UV-VIS spectroscopy was suggested as fast and versatile monitoring tools for BOD and COD in water samples. Continuous water quality data from COD or BOD value can be achieved by UV-VIS spectrometer by following Beer-Lambert's law [52]. This spectroscopy has also advantage of limiting measurement time for BOD from 5 days to few minutes and also limiting the usage of a large amount of expensive reagents.

According to Beer-Lambart's law, when three samples (standard solutions) having identical absorption are introduced into a beam of monochromatic light and each of the samples is chosen so that precisely one half of the intensity of the incident radiation is transmitted (T = 50%), the intensity after each sample will be as follows (the intensity of the incident radiation is 100%T)

after Sample 1 = 1 x 0.5 = 50%T

after Sample 2 = 50% x 0.5 = 25%T

$$\text{after Sample 3} = 25\% \text{ x } 0.5 = 12. \text{ } 5\%\text{T}$$

The expression relating A to T ($A = -\log_{10} T$) shows that the absorbance after each sample will be:

$$\text{after Sample 1} = 0.301$$

$$\text{after Sample 2} = 0.602$$

$$\text{after Sample 3} = 0.903$$

The three samples may be considered as known concentrations of an absorbing medium and it results an exponential graph of concentration against transmission and linear graph of concentration against absorbance. These two graphs are shown in Figure 2.2.
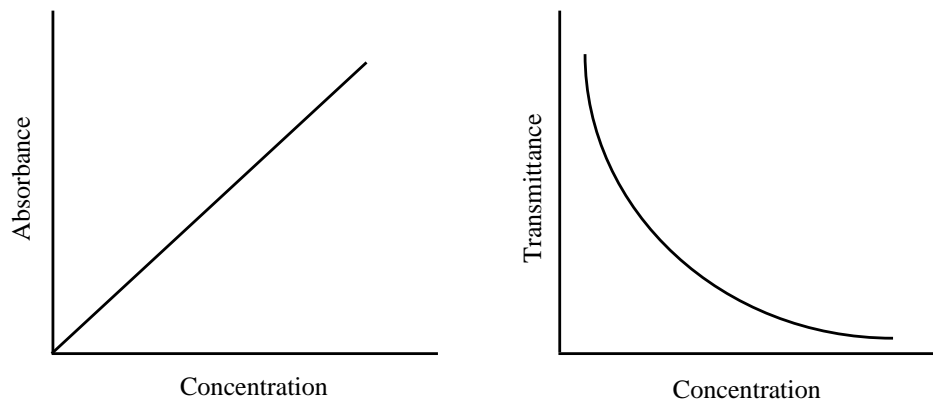
Figure 2.3: Absorbance and transmittance of photon plotted v-s concentration of solutions.

Same principle will be applied while measuring COD. If we take Acetic acid as our standard sample which has theoretical COD value of 1.07 mg/L and dilute it several times, absorbance will be differentiate in change of concentration. The more we dilute, concentration of the samples decrease and thus absorbance decreases.

Once the graph of absorbance values vs added concentration is achieved, the extrapolation to zero absorbance will give the desired unknown concentration of analyte. In 2011,

Guoqing established COD quantitative analysis model by linear calibration of KHP standard solution after irradiating with 254 nm of UV light and extent of water pollution were examined from the absorbance value. Figure 2.3 shows the absorption spectra of 25-600 mg/L of standard solution of KHP taken with Ocean Optics CHEM USB-4000 spectrometer [53].
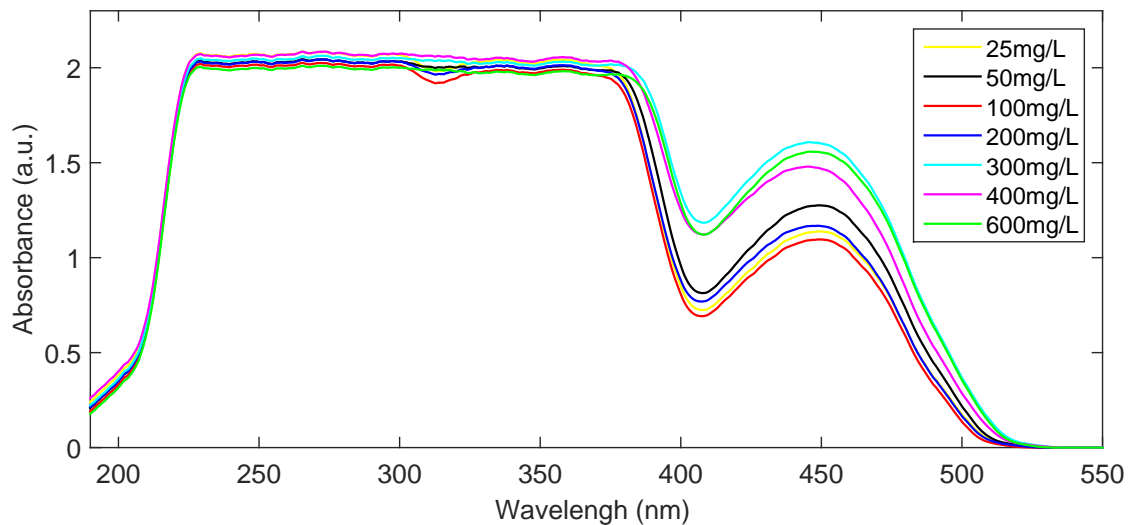
Figure 2.4: UV-VIS absorbances spectra of sample

2.4.3 Challenges of Using Spectrometers for BOD/COD Measurement

In spite of the progress in the optical spectroscopy system, there are still some limitations that need to be addressed. Spectroscopic instruments are affected by surrounding condition variations and temperature change. One of the important components of the spectroscopic system is optical fiber. Optical fibres are susceptible to physical perturbations, such as bending, kinking and crushing. Problems with optical fiber will generate uncertainty in the measurements. However, the problem of optical fiber can be easily solved by a compact design of spectrometer, which will eliminate the use of optical fiber in the spectrometer.

Optical sensor is the most essential part of the spectrometer. This component is also susceptible to vibration, temperature and radiation damage and that will cause degradation of resolution of detector [54]. In addition, the assembly of optical components for the sensor also requires tremendously high precision and accuracy, especially for placement of optical components, which normally has a tolerance of less than a micrometer. This tolerance is vital to guarantee the reliability of the spectroscopic system. In addition, spectroscopic system requires cuvettes to place the samples in side of the spectrometer. These cuvettes need to be very high standard, otherwise that will absorb photon in the UV region.

UV-VIS spectrometer gives the absorbance result of each wastewater sample in the range of 200 nm to 1100 nm. In wastewater samples, COD and BOD amounts are not same. Thus, spectrometer gives individual absorbance spectrum for COD of a sample and BOD of a sample. It will be challenged to measure COD and BOD of samples in a same time using UV-VIS spectrometer. To overcome this problem, linear relationship of absorbance and COD and BOD content will be made separately. Samples should be placed in a proper cuvettes for which material will be chosen in respect to BOD and COD characteristics.

Chapter 3

Integrated Spectroscopic System

As mentioned in previous chapters, the purpose of this project is to monitor the COD content in waste water using the UV-VIS spectroscopic technique. The general arrangement of the UV-VIS spectrometer and its components are presented in this chapter. In addition of spectrometer components, the operating procedure of spectrometer, selection process of sample holder materials, sample preparation process and collection methods are also presented in this chapter.

3.1   System Components

Spectrometric system for COD content analysis consists of Ocean Optic's CHEM USB-4000 spectrometer equipped with 1 cm quartz cells, 22 cm long optical fiber and Toshiba TCD-1304AP Linear Charge Coupled Device (CCD) Array detector with 3648 pixels. For UV-VIS analysis, a deuterium tungsten light source is used to provide a spectral range of 180 nm to 1100 nm. A schematic view of the experimental setup is shown in figure 1. To control and process the experimental setup, a MATLAB based Graphical User Interface (GUI) is developed. An intelligent algorithm is also included in this software to perform

statistical analysis on experimental data. The development process of GUI and software will be presented in chapter 4. Deionized water (DI) is used as a blank solution for the analysis. All spectra is recorded in the range of 180 nm to 900 nm which is limited by the characteristics of quartz cuvette. In literature [53], wavelength (λ) of 254 nm is reported as optimal wavelength for COD content analysis in waste water treatment facility as most of the organic materials absorb light and have response in that particular wavelength. However, this system is flexible enough to analyze full spectral range of 180 nm to 900 nm with any user defined spectral bandwidth (BW) to evaluate maximum sensitive wavelength λ_{max} for organic pollutant by performing statistical analysis. Besides, the user can overwrite this option to perform manual analysis based on user defined λ_{max} and BW. Followed by these processes, system will perform linear regression analysis on samples with known COD values to generate standard calibration curve which will finally be used to evaluate unknown concentration in the raw wastewater samples.
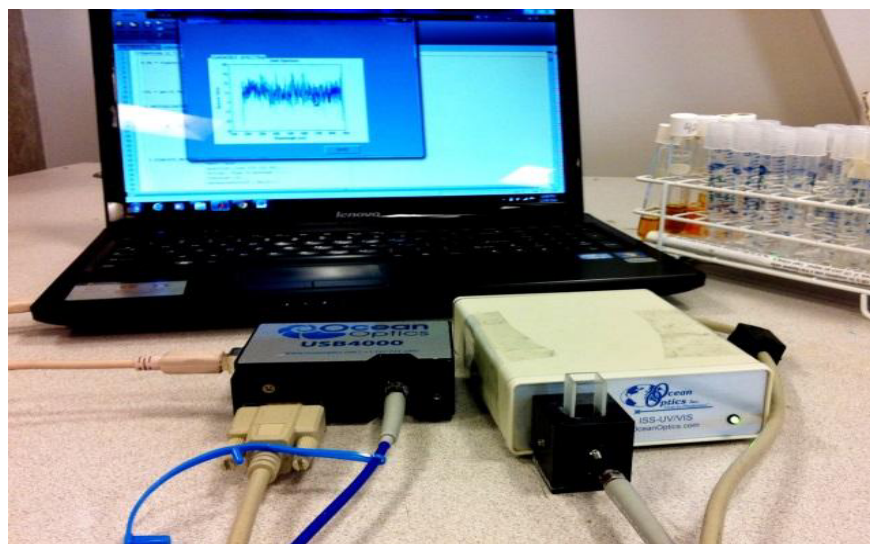
Figure 3.1: A photograph of complete experimental apparatus.

Radiation
Source

Monochromator

Sample

Detector
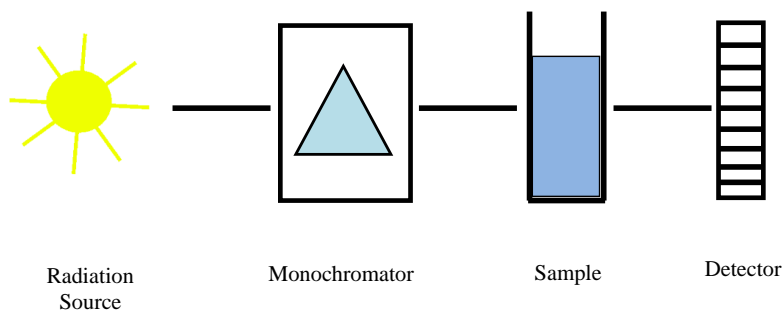
Figure 3.2: Schematic of single beam UV-VIS spectrometer.

3.2 UV-VIS Spectrometer

For this absorbance measurements, Ocean optic's CHEM USB-4000 UV-VIS spectrometer [55] is preferred as the standard instrument which is constructed by low-cost standardized optical components such as light source, solid-state wavelength separation filters and gratings, diode array detectors, fiber optics, and embedded computation system. CHEM USB-4000 UV-VIS spectrometer has the key components which are described below and schematic of this spectrometer is shown in Figure 3.2.

A dispersion device that selects from the broadband radiation of the source. Normally, prisms and holographic gratings are commonly used in UV-VIS spectrometers. Prisms are simple and inexpensive, but the resulting dispersion is angularly nonlinear and temperature sensitive. As a modern spectrometer, Ocean optic's USB-4000 contains holographic gratings instead of prisms. These devices are made from glass blanks, onto which very narrow grooves are ruled. The dimensions of the grooves are of the same order as the wavelength of light to be dispersed. Finally, an aluminum coating is applied to create a reflecting source. Light falling on the grating is reflected at different angles, depending on

Table 3.1: Different types of UV-VIS detectors and useful working ranges in nanometers.

Detector Type	Useful working range (nm)
Silicon photodiode	350-1100
Photomultiplier tube	160-1100
CCD (charge-coupled devices)	180-1100
Photodiode array	180-1100

the wavelength. Holographic gratings yield a linear angular dispersion with wavelength and are temperature insensitive.

Detector is one of the most important equipment of spectrometer. Detectors are used to measure the intensity of the incoming radiation through samples from the radiation sources. A detector converts this incoming light signal into an electrical signal. A variety of detectors are available for spectrometric analysis. Spectrometers normally contain either a photo-multiplier tube detector or a semiconductor detector. Table 3.1 is showing a list of different types of detectors and their working range [51]. In our experimental setup, Ocean Optic's USB-4000 spectrometer contains Toshiba TCD1304AP linear CCD array detector [55]. CCD array is an integrated circuit which is etched onto a silicon surface forming light sensitive elements called pixels. The photons incident on this surface to generate charge that can be read by electronics and turned into a digital signal which represent the light patterns falling on the device. This CCD array has 3648 pixels that enables the optical resolution. This CCD array has best response for the wavelength from 180-1100 nm. This detector also facilities an electronic shutter which allow to control the spectrometer remotely using the software interface.

Two sources are commonly used in UV-VIS spectrometers. One is deuterium arc lamp which results a good intensity in the UV and VIS region. Having low noise makes deuterium arc lamp more acceptable to user. However, the intensity of this lamp decreases

steadily over time. Typically, this lamp has a half-life of 10^3 hrs. The other UV-VIS source is tungsten-halogen lamp which also results good intensity over UV and VIS region with useful life of 10^4 hrs. For absorbance analysis for COD, both types of light sources are suitable. For this system, a tungsten-halogen lamp was chosen based on the half-life of the source. Figure 4.3 shows the full spectral output of the tungsten-halogen lamp. This spectrum was taken with Ocean Optics CHEM USB-4000 spectrometer. This light source was controlled and operated using the MATLAB based UI. The details about the UI based control system will be presented in Chapter 4.

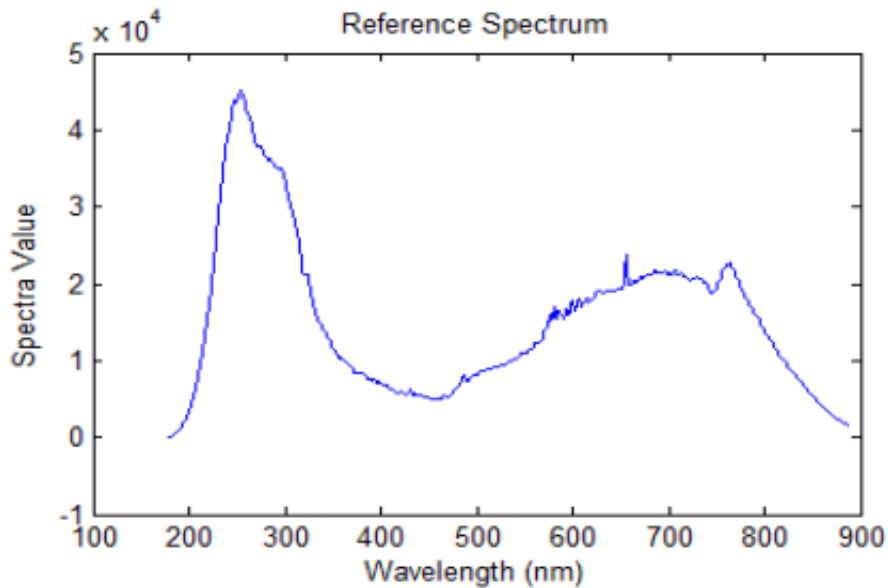Figure 3.3: Spectral output of Ocean Optics ISS UV-VIS tungsten-halogen lamp. The spectrum was taken with Ocean Optics CHEM USB-4000 spectrometer.

Ocean optic's 300 μm diameter solarisation resistant optical fiber consists of silica core, surrounded by a silica cladding material. The fiber is coated by aluminium. This solarisation-resistant fibers are used for regions below 250 nm for UV absorbance measurement.

Table 3.2: Photon transmission range through different cuvette window materials

Materials	Best transmission range (nm)
Glass	334-2500
Borosilicate	325-2500
UV Silica	220-2500
Quartz	190-3500

A cuvette is a small tube of circular or square cross section, sealed at one end, made of plastic, glass, or fused quartz and designed to hold samples for spectroscopic experiments. These cuvettes may have limited transmission through the samples for specific range of wavelength depending on the cuvette window materials. Normally plastic cuvettes are chosen for spectroscopic analysis over cuvettes made with other materials to limit the amount of cleaning. Table 3.2 shows the best photon transmission behaviour for different wavelength range through different cuvette window materials.

In addition, Table 3.2 shows glass materials are good for visible wavelength range but not in UV wavelength. This table also shows that silica has moderate behavior in UV wavelength range. According to this table, only quartz materials show the perfect standard transmission behavior in both UV and VIS wavelength ranging from 180 nm to 3000 nm [56].

For this work, our target is to analyze waste water to accurately determine COD in waste water samples. The COD contents show maximum response in the UV-VIS wavelength region and according to literature, at 254 nm, COD contents show maximum response. For this reason, a deuterium-halogen light was chosen for this work, which can provide continuous light spectrum 180 nm to 900 nm as output. Based on cuvette window materials characteristics in the wavelength range 180 nm to 900 nm, quartz was chosen as the perfect cuvette material for our experimental analysis. Figure 3.4 shows the light transmission

characteristics through glass and quartz cuvette. These spectra were taken with CHEM-4000 USB UV-VIS spectrometer. In the time of these measurements, data taking time and other conditions was remained identical. Figure 3.5 shows light transmission rate through glass cuvette is almost reduced by factor 2 compared to quartz cuvette for the wavelength below 300 nm. This result is also indicate that quartz cuvette is best for our experiment compared to other materials.
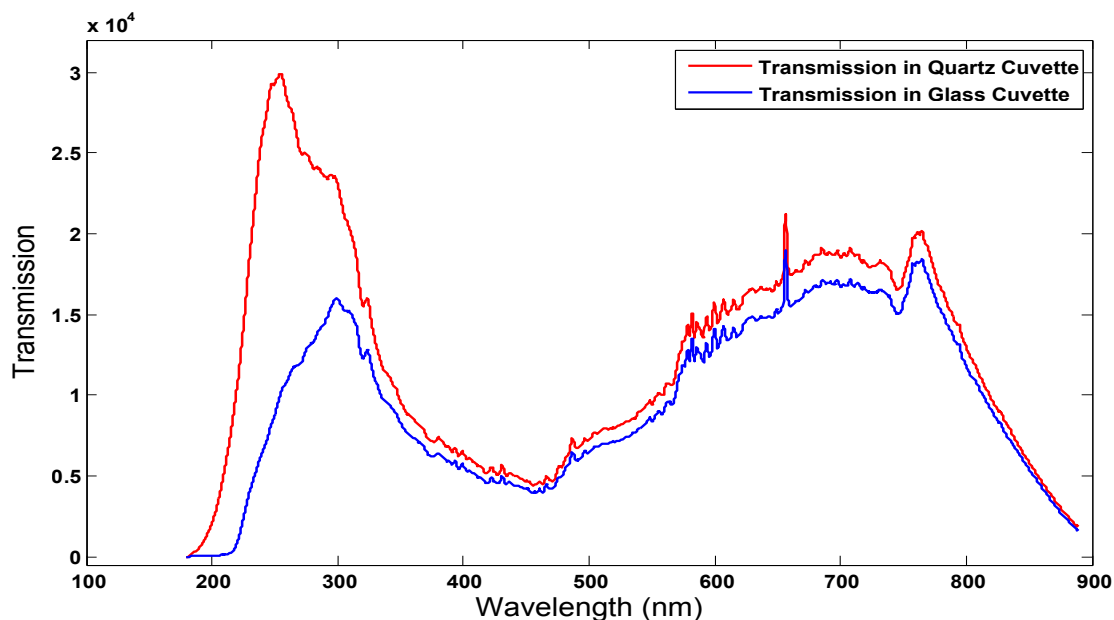
Figure 3.4: Transmission behaviour of quartz and glass cuvette. Transmission in glass cuvette is half compared to quartz cuvette for the wavelength below 300 nm.

3.3 Operating Procedure

Ocean optic's UV-VIS integrated sampling system is a combination of a RF deuterium source with a tungsten halogen bulb connected to a cuvette holder for 1cm cuvettes. The cuvette holder attaches directly to the light source and has a 5 mm diameter f/2 collimating lens to collect the light. UV-VIS can be operated manually or through the spectrometer

operating software. The spectrometer's power can be off by disconnecting it from the PC or detaching the USB cable. An optical fiber is connected on one end of spectrometer and other end of light source through Sub Miniature Version A (SMA) connector. For manual use of lamp, there are three positions: On, Off and Remote. For 0.3% peak-to-peak stability and lamp should be allowed 30 minutes warm-up time before taking measurements.

The Ocean Optics USB-4000 Spectrometer is a miniature fiber-optic spectrometer with an advanced detector and powerful high-speed electronics. The USB-4000 Spectrometer connects to a computer via the USB port or serial port. The USB-4000 can be controlled by Ocean Optic's SpectraSuite software that operates on Windows, Macintosh and Linux operating systems. Also user's custom made software can operate this spectrometer. At the beginning, a basic LabView based acGUI was developed to control and characterize the spectrometer. However, finally a MATLAB based GUI has been developed to operate the spectrometer and monitor wastewaters COD value in on-line system. This GUI first sends instruction to spectrometer to perform the measurements on waste water samples and then collect data in the hard-drive and in GUI function memory. After collecting data this GUI is also capable to execute automatic statistical model on recorded data for COD analysis. Details about this GUI development and operating processes are described in Chapter 4.

3.4   Sample Preparation

On-line monitoring is of great interest for fault prevention in the wastewater treatment facility. However, it is important to detect the accurate quantity of chemical oxygen demand (COD) in the wastewater treatment facility. For these purpose, the COD analysis processes and performance of the spectrometer need to be characterized. To characterize and optimize

the performance and operation of spectrometer for COD and BOD analysis, system was tested with laboratory prepared samples and with industrial samples which was directly collected from wastewater treatment facility. Details about these samples preparation and collection processes are described in the subsequent sections. Different solutions for laboratory samples and industrial samples were chosen to accomplish two main objectives: to compare the results obtained from different samples, relationship between results from different samples to obtain a uniform statistical model for wastewater analysis and to compare the ability to distinguish different concentrations of the same solution.

3.4.1 Laboratory Prepared Samples

To prepare laboratory samples four different chemicals acetic acid glacial ($C_2H_4O_2$), $\alpha-D$ glucose anhydrous ($C_6H_{12}O_6$), L$-$glutamic acid ($C_5H_9NO_4$) and Dextrin (($6\,H_{10}O_5$)n) were used. All these chemicals were purchased from Sigma-Aldrich, USA . These chemicals were used to prepare four different laboratory samples stock solution. As the concentrations of these chemicals are known, so the preparations of stock solutions are straightforward. Preparations of stock solutions only required, weighting out of solid/liquid chemicals of known purity and dissolving these chemicals in a required volume of suitable solvents. In our case, chemicals were dissolved in deionized water (DI). The stock solutions of these four chemicals were diluted with DI to prepare samples with COD concentrations between 1 mg/L to 200 mg/L. Dilution of stock solutions was performed using the Eq 3.1:

$$V_1 M_1 = V_2 M_2 \tag{3.1}$$

where,

V_1 = Volume of stock solution

M_1 = Concentration of stock solution

Table 3.3: COD concentrations of laboratory made standard glucose solutions

Solution Concentration (mg/L)	COD Concentration (mg/L)
186.92	200
93.46	100
46.73	50
23.37	25.01
11.68	12.50
5.84	6.25
2.9	3.10

V_2 = Desired volume of final solution

M_2 = Desired concentration of final sample

This method of dilution was used to prepare the samples from stock solution to reduce the chemical waste. Dilution processes were performed at SFU chemistry laboratory by using proper glassware equipments to reduce the uncertainty in the dilution processes. For each chemical, the COD concentrations from these solution concentrations were determined analytically as described in Chapter 2. Table 3.3 shows the laboratory made standard glucose solutions actual solution concentration and the analytically calculated COD concentrations for these sample solutions. Similar to glucose, samples for other three chemicals were also prepared from their stock solution. After completing the preparation processes, these samples were poured in test tubes and labeled properly. Finally, all laboratory samples were stored in refrigerator at constant 4°C for future use.

3.4.2 Collected Wastewater Samples

As mentioned earlier, UV-VIS spectrometer system and analysis algorithm need to be tested with both laboratory prepared samples and real wastewater samples for accurate detection mechanism of COD contents. Real wastewater samples were collected from

Figure 3.5: All industrial samples were stored in test tubes with proper labeling. These labeled samples were stored in refrigerator at constant 4°C for future uses.

Metro Vancouvers Annaces Island WWTP, British Columbia, Canada. The Annacis Island WWTP treats about 175 billion litres of wastewater every year which is Located at Delta, British Columbia, Canada. The plant provides secondary treatment to wastewater for over 1 million residents in 14 municipalities and wastewater is discharged into the Fraser River once it is treated. In this study, real wastewater samples were collected from two different wastewater treatment facilities of WWTP. Effluent 140.5 mg/L and Influent 251.33 mg/L samples were collected from facility 1 and facility 2, respectively. Samples collected from these facilities were combined in different ratios to prepare a series of samples with COD concentration from 140.5 mg/L to 251.33 mg/L. Before combining these samples, they are first filtered by 0.2μm syringe filter to get rid of all solid particles from samples. Figure 3.6 shows the preparation processes of the samples. After preparation, all samples were stored in test tubes with proper labeling. These labeled samples were stored in refrigerator at constant 4°C for future uses as shown in Figure 3.6. These series of COD samples were

Table 3.4: COD concentrations of industrial samples used in this analysis

Samples	COD Concentration (mg/L)
Sample 1	140.50
Sample 2	162.67
Sample 3	180.40
Sample 4	207.00
Sample 5	233.60
Sample 6	251.33

mixed using the Eq 3.2:

$$M_1 V_1 + M_2 V_2 = M_3 V_3 \tag{3.2}$$

where,

M_1 = Concentration of 1st sample

V_1 = Volume of 1st sample

M_2 = Concentration of 2nd sample

V_2 = Volume of 2nd sample

V_3 = Desired concentration of final solution

M_3 = Desired volume of final solution

Table 3.4 shows the series of industrial samples COD concentrations that will be used in this analysis to develop statistical model to evaluate the unknown COD concentration. Similar to laboratory prepared samples, these industrial samples were poured in test tubes, labeled properly and finally all samples were stored in refrigerator at constant 4°C for future use.

Chapter 4

System Interface and Signal Processing

4.1 Signal Processing

In Chapter 3, the details of UV-VIS spectrometer system components, operating procedure of spectrometer and sample preparation techniques were presented. In this section, the development of algorithm processes for unknown COD measurement will be presented. Initially the LabView based program was developed to control the CHEM USB-4000 spectrometer, to acquire signal from spectrometer and process the acquired signal for final result. Finally, a MATLAB based program and algorithm was developed to serve these purpose. The MATLAB program and data flow diagram is presented in Figure 4.1.

This MATLAB program is communicating with spectrometer through USB ports. This program allows the user to enter customize data for these initialization parameters, the data are stored in MATLAB workspace to be used in other operations. Once the user has specified all the initialization parameters, and then the program will automatically communicate
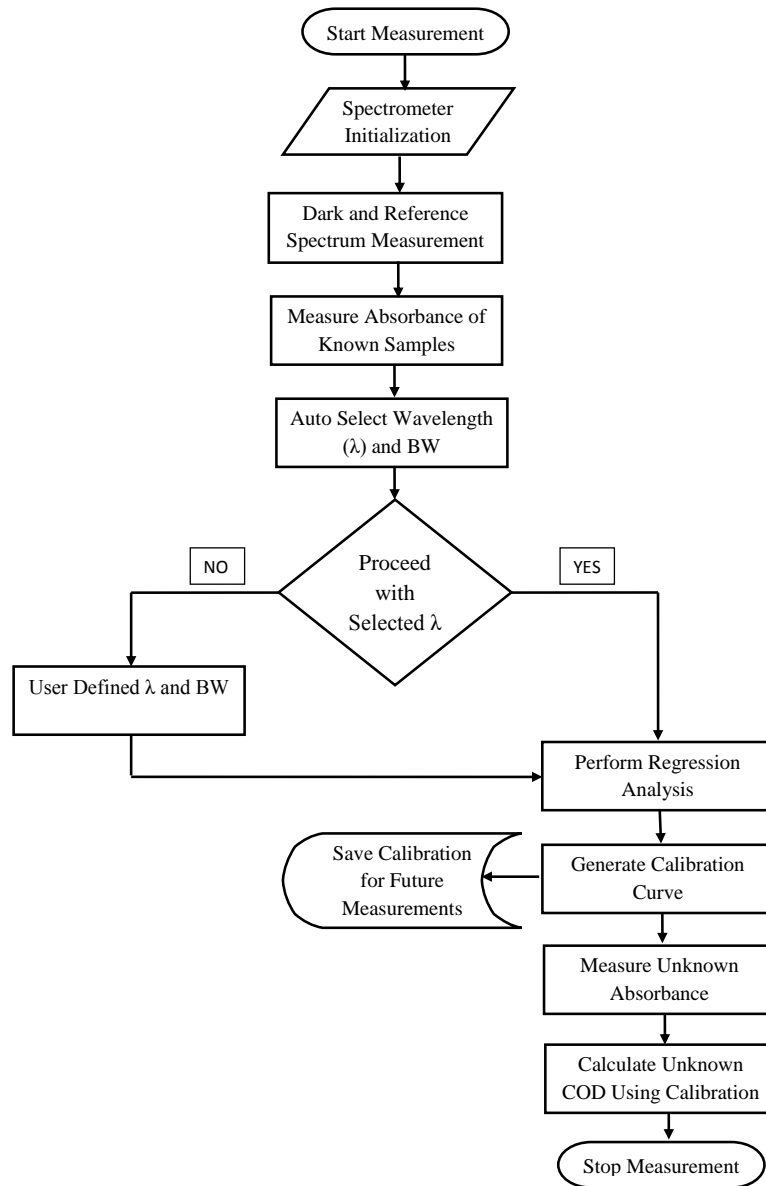
Figure 4.1: Flowchart of MATLAB Program sequence.

with Ocean Optics Spectrometer and performed the initialization.

First parameter the program sends to spectrometer is integration time, which specifies the active photon detection time of the spectrometer's detector. The higher the integration time, the longer the detector monitors the incoming photons. For this instrument, standard integration time is identified as 22 ms. Second parameter is scan to average value, which defines the number of discrete spectral acquisitions that the device driver accumulates before MATLAB receives a spectrum. Scan to average value provides better SNR. The last parameter of initialization is boxcar width, which averages a group of adjacent detector elements value to generate a smooth spectral data. A higher value of boxcar width improves the SNR, however a much higher value will reduce the spectral resolution.

Spectrometer must need to be calibrated before performing any measurements. This calibration is performed by taking dark and reference spectrum by the program. A dark spectrum is the spectrum recorded at spectrometer's CCD array in the absence of light source. This dark spectrum is important to reduce the background created by ambient light in the CCD array. To perform the dark spectrum measurement, light source needs to be disconnected. In this experimental setup, halogen-deuterium light source is used in remote mode. This mode allows to control the light source operation through serial port communication. This MATLAB program sends disable signal to light source, and then the light path from light source to sample is blocked by inserting an opaque shutter. After blocking the light path, program measures the dark spectrum from spectrometer. The measurement of dark spectrum by the program is presented in Figure 4.2. Measured dark spectrum is stored in certain folder of local hard drive memory in text format for future use and in MATLAB function memory for absorbance calculation of wastewater sample.
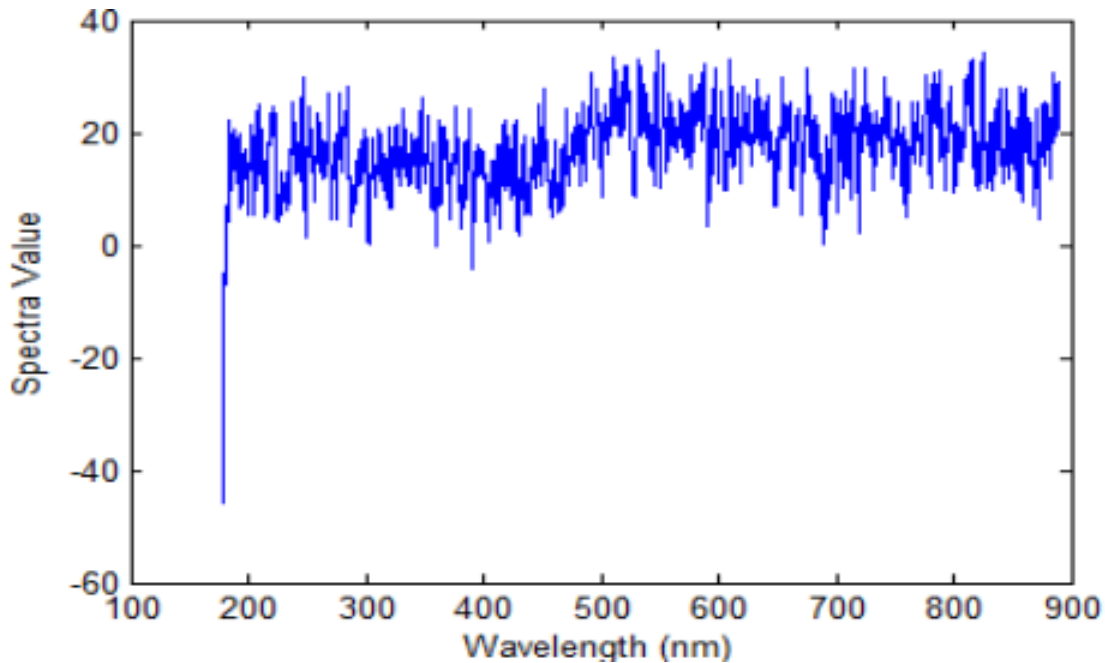
Figure 4.2: Dark spectrum recorded at MATLAB from spectrometer.

To measure the absorbance of a sample, a reference spectrum is also needed to be measured. A reference spectrum is the spectrum recorded at spectrometer's CCD array in the presence of light source without any sample present in the sample holder. Reference spectrum identifies the absorbance of sample holder materials. To measure the reference spectrum, the program automatically sends enable signal to light source, which remove the opaque shutter from the light path. Reference spectrum is recorded in hard drive memory and in MATLAB function memory like dark spectrum. Figure 4.3 shows the acquired reference spectrum from the spectrometer's CCD array.

This program performs absorbance measurements of known COD samples. To generate COD calibration curve from measured absorbance of known COD samples, this program asks for number of known COD samples and guides the user to insert the number of known COD samples that are used in absorbance calculation. These imported data are
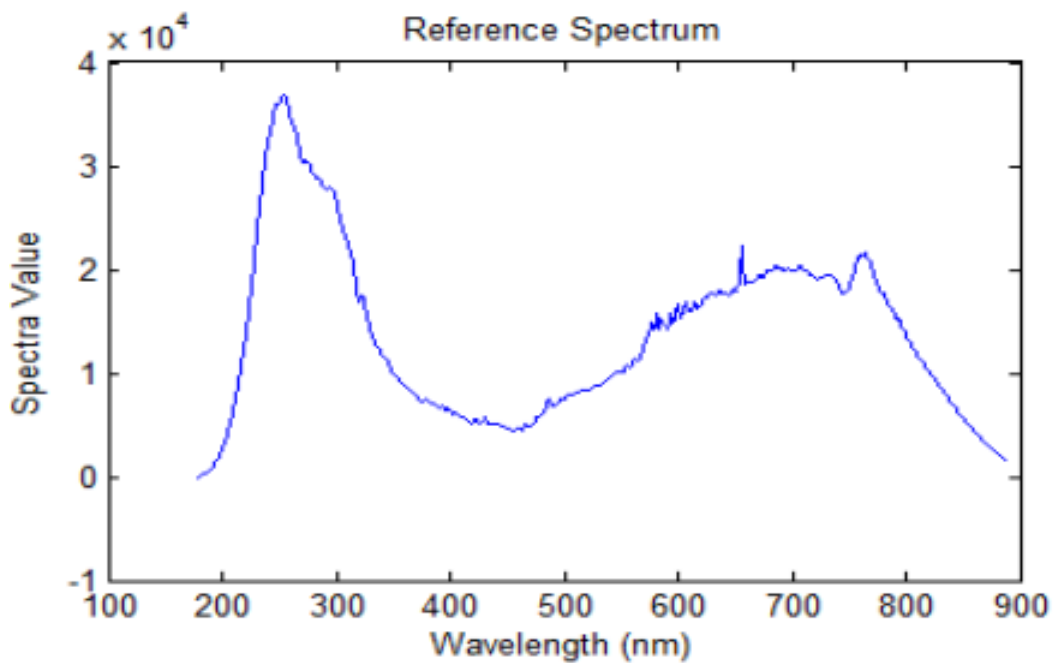
Figure 4.3: Reference spectrum recorded at MATLAB from spectrometer.

processed and saved in MATLAB work space to guide the user in sample spectra measurement and absorbance calculation processes.

In the process of absorbance calculation, the user inserts known COD concentration values in the program. The program records these value in the MATLAB workspace. Based on these values, program instructs user to input known COD concentration values and then place the cuvette with COD sample in the spectrometer's cuvette holder. This program records and stores the sample spectrum in hard drive memory and MATLAB function memory for absorbance calculation.

At the last stage of absorbance calculation, this program communicates with different MATLAB functions memory and obtains values of dark and reference spectra. The program uses every single pixel of the CCD array detector and calculates the absorbance using the

Eq 4.1:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right) \tag{4.1}$$

where, S_λ is sample intensity at wavelength (λ), D_λ is dark intensity at wavelength (λ) and R_λ is reference intensity at wavelength (λ) [51]. Figure 4.4 shows the calculated absorbance values using Eq. 4.1 and effect of applying MATLAB's smoothing filter with different span size. This absorbance values are stored in hard drive and MATLAB workspace after receiving user-defined input.
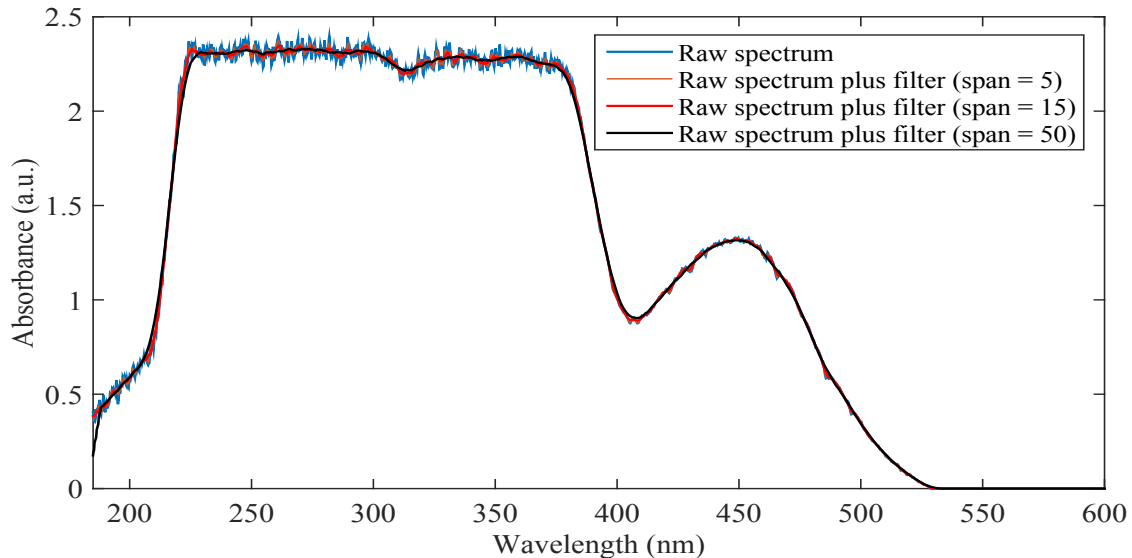
Figure 4.4: Absorbance spectrum recorded at MATLAB from spectrometer and spectra are processd by applying filter at span 0 to 50 to improve the SNR of the absorbance spectrum.

A key feature of this program is to evaluate the maximum sensitive region of absorbance vs. wavelength spectrum. As mentioned in Chapter 2, the absorbance of light in sample strongly depends on the wavelength of light. Absorbance analysis is a strong tool to probe in the sample as based on electronic structure certain sample will absorb light only on

specific wavelength. This absorbance of light also depends on concentration of the sample and follows the Beer's law. For this analysis, this program processes the absorbance and wavelength data from MATLAB workspace. This program scans the absorbance values for the wavelength values starting from 180 nm to 900 nm with 20 nm step size.

User can choose different BW for spectral analysis. Larger size BW will eliminate statistical fluctuation in recorded absorbance values and will improve SNR in the signal. However, very large BW will cause spectral resolution degradation, which is not acceptable for measurement. For this measurement, it is important to find out a optimized value for BW, which will improve SNR in signal without degrading much spectral resolution. After performing different analysis, we found BW = 10 nm is suitable for our analysis. For each wavelength this program utilizes spectral region BW = 10 nm and determine the average absorbance values for each samples.These average absorbance values are stored in MATLAB workspace in matrix format. Figure 4.5 shows different chosen spectral region (BW = 10 nm) at different λ values in the absorbace vs. wavelength spectrum.

After identifying different spectral region, this program will then perform linear regression analysis on the stored average absorbance and known COD concentration values using the MATLAB's statistical built-in toolbox regstat [57] function. According to Beer's law, absorbance caused by COD content will show linear relationship with the concentration of the sample. This program will evaluate the linear correlation between absorbance and sample concentration by using the result of performed linear regression analysis. The best relationship is identified by using the R^2 value and Mean Standard Error (MSE) values from this statistical analysis. The R^2 value represents how good the model is represented the data points of the spectral region. This program creates a map of wavelength and the R^2 value for the each selected spectral region based on the linear relationship between
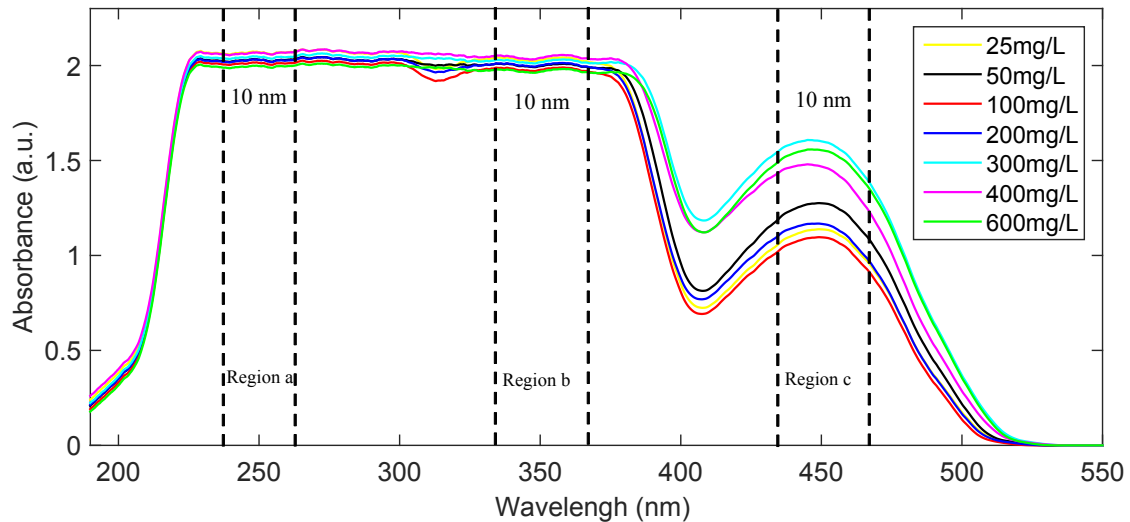
Figure 4.5: Sample spectral range for a specified BW (= 10 nm) at different wavelengths to develop the statistical analysis.

samples absorbance and concentration. As mentioned maximum R^2 shows the best linear relationship between absorbance and concentration. This program uses maximum R^2 value which eventually direct towards maximum sensitive wavelength . Additionally, user can define this λ value independently by editing the automatically chosen λ value in the program input interface. As mentioned earlier, BW = 10 nm was used for spectral region to choose maximum sensitive λ value. However, similar to maximum sensitive λ value, user can customize this BW input by editing predefined BW value in the program input interface. Finally, these maximum sensitive λ and BW values were stored in MATLAB workspace.

The program performs statistical analysis on stored data to generate the calibration curve. This calibration curve has a great significance on the evaluation of unknown COD sample concentration. To perform the statistical regression analysis, this program processes all the stored absorbance data from MATLAB's workspace.

Using the λ and BW values from the MATLAB workspace, this program will evaluate the average of absorbance value for the specified λ. The absorbance values and concentrations of all known samples are processed for statistical analysis. The program uses the MATLAB's statistical toolbox built-in regstat [57] function to perform this analysis. This regstats function is able to perform multi-linear regression of the responses on the predictors. The input Model controls the regression model types. Linear, quadratic and purequadratic regression analyses are possible types using this regstats function. In this analysis, known COD samples concentration used as predictors input and the absorbance values are used as observed responses input for the function. As mentioned in chapter 2, the absorbance of light in sample and the concentration of sample show linear relationship according to Beers law.

The result of this analysis will be used to calculate the unknown COD concentration. Additionally, coefficients values from the linear regression fitted model were also saved at hard drive memory for future use. This data automatically saved in designated location as ASCII format. All saved files are arranged according to date-time stamp of the experiment.

The main purpose of the program is to measure the concentration of unknown COD samples. The concentration is calculated using the measured absorbance of the unknown sample. This program follows similar processes like absorbance calculation for the evaluation of the absorbance of unknown COD samples. For these calculations, the program uses Eq. 4.1. According to Eq. 4.1, the absorbance calculation requires dark spectrum, reference spectrum and sample spectrum. To obtain the sample spectrum of unknown COD sample, this program will instruct user to place the cuvette with unknown COD sample in the spectrometer's cuvette holder. Then the program will record and store the sample spectrum from the CCD array in the hard drive memory and MATLAB function memory for
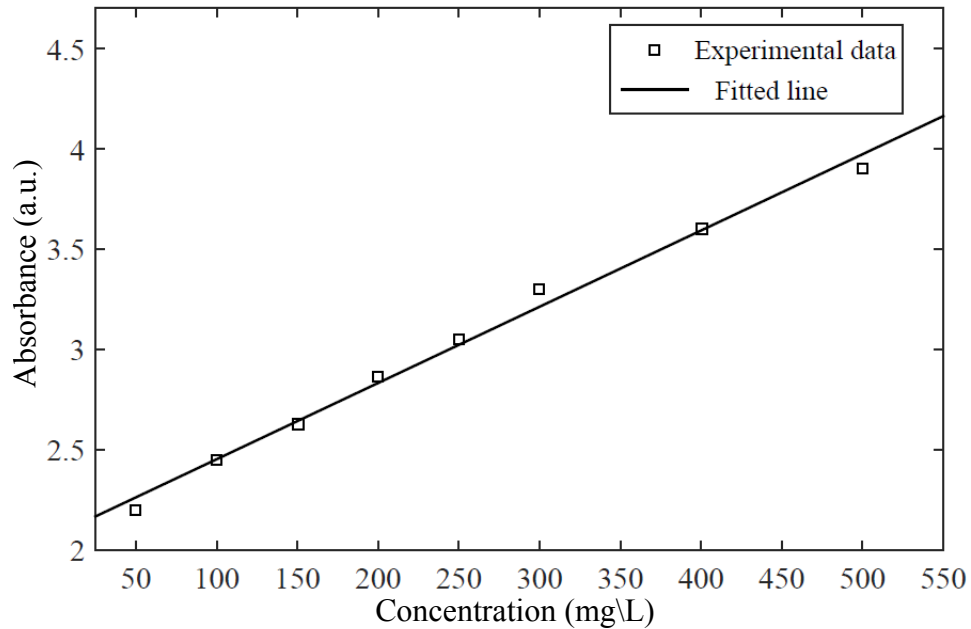
Figure 4.6: Using selected wavelength and BW, this program performs linear regression analysis on all known wastewater COD sample absorbance spectral data and generate calibration curve. These calibration values are automatically recorded in text files for future use. Besides, these calibration values will be used to evaluate unknown wastewater COD value.

the absorbance calculation of unknown sample.

After sample spectrum measurement, the program will calculate the absorbance spectrum using the every single pixel of the CCD array detector and store these values for future use. Then, this program will obtain the coefficient values of the linear regression fitted line. The concentration of unknown COD sample is determined using the Eq 4.3:

$$\text{Unknown Sample Concentration}, C_{un} = (A_{un} - \beta_1)/\beta_0 \tag{4.2}$$

where A_{un} is calculated unknown sample absorbance, β_0 and β_1 are the coefficients of fitted line.

4.2 MATLAB Interface

A graphical user interface (GUI) has been developed to control the spectrometer and monitor COD value in on-line system. The creation of a GUI within MATLAB [57] allows user to access the hardware providing a dynamic interface instead of writing code which makes it accessible to a wider audience of engineers. The purpose of the GUI is to allow the user to operate/control the spectrometer and light source interactively while the developed algorithms in GUI modules automatically performs calculations and generates relevant analysis graphs. This GUI makes the operating process of the spectrometer simple and allows the user to evaluate unknown COD value instantly. This section of the thesis will provide an overview of the interface, how the user interacts with it and the underlying implementation details.

Graphical User Interfaces for spectrometer control and wastewater analysis consists of six GUI's:

- Initialization GUI

- Spectrometer Calibration GUI

- Absorbance Calculation GUI

- Automatic Maximum sensitivity Selection GUI

- Regression Analysis GUI

- Target COD evaluation GUI

Each of the GUIs is designed to implement a particular task. Details outlook of developed GUI is presented in Appendix A.

Chapter 5

Experimental Results and Verification

The main objective of this work is to develop an automatic portable device, which can probe into wastewater to detect different kind of variation of pollutants COD by performing UV-VIS spectral analysis. As mentioned earlier in Chapter 2, these COD measurements are performed using conventional techniques. The conventional technique requires sample collection and pre-treatment at COD reactor, which involves heating of the samples in laboratory to estimate these COD contents. These processes are time consuming and manual labour oriented. However, this system is developed to overcome these problems. The developed system can estimate the pollutant degradation (change in COD concentration) in wastewater instantly without performing any pre-treatment on the samples. Therefore, this system can act as an important fault prevention device in wastewater treatment facility. Hence, it is important to characterize and investigate the reliability of the developed system to find out if this system can detect the variation of the chemical content in the influent of the treatment system. To perform these investigations, this system was tested with laboratory prepared samples and collected samples from wastewater treatment facility. Sample

preparation and collection processes were described in details in section 3.5. COD measurement process using laboratory samples and industrial samples are described in details in the following sections.

5.1 COD measurements

5.1.1 Using Laboratory Prepared Samples

To test the feasibility of experimental setup, glucose standard solution samples were used. These samples were utilized to obtain the correlation between UV-VIS spectral absorbance values acquired from spectral system with known COD standard concentration. This correlation will be used as a standard calibration source for the system to evaluate unknown sample's COD concentration.
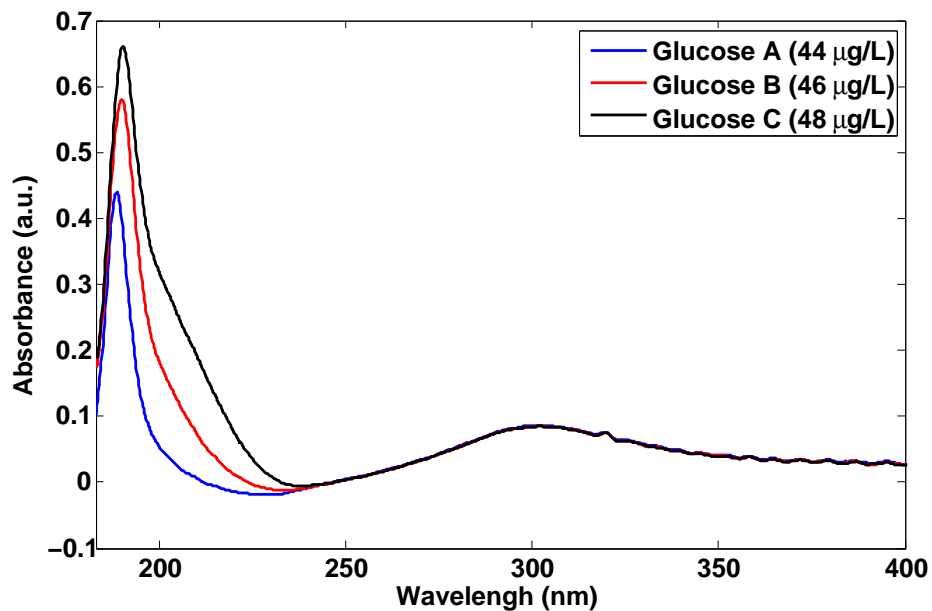

Figure 5.1: UV-VIS spectra acquired using ocean optics CHEM-4000 spectrometer for some laboratory prepared glucose samples.

To perform systematic tests, 50 mg/L glucose stock solution was prepared and from there using the dilution process 41.3 mg/L, 42.2 mg/L, 43 mg/L, 45 mg/L and 44.08 mg/L concentrated six glucose solutions were prepared in the laboratory. As mentioned earlier in Chapter 2, the theoretical COD value of a specific compound can be calculated from stoichiometric considerations. For all the prepared solutions, theoretical COD content concentrations were calculated and values are 44.20 mg/L, 45.20 mg/L, 46.02 mg/L, 48.15 mg/L, 49.13 mg/L and 47.17 mg/L, respectively. Spectrum was acquired for each glucose samples using CHEM-4000 spectrometer and our developed software. Details about these data taking processes were explained in Chapter 4. Figure 5.1 shows the acquired UV-VIS spectra of some laboratory prepared glucose samples using ocean optics CHEM-4000 spectrometer. This figure shows absorbance of photon increases in the samples as the concentration of the samples increases by following the Beer's law. In the UV-VIS spectra, a variation can be easily detected by a change on spectra's shape. For this analysis, spectra was taken for UV-VIS spectral range from 180 nm to 900 nm. Several spectral ranges were selected and studied. The results between different regions were achieved to find out the amount of relevance information for each region. This collected information was utilized to identify the most sensitive wavelength and spectral region for COD measurement. For the spectral range selection purpose, we used 10 nm bandwidth at different wavelength.

Figure 5.2 shows two 10 nm spectral region was selected at 225 nm and 250 nm to extract the information about absorbance for building the statistical model. As mentioned in Chapter 4, this system's algorithm is capable to automatically identify the most sensitive wavelength at which all solutions have maximum response activity. Here, 225 nm was chosen automatically by the system and 250 nm was chosen manually as this wavelength was reported at literature for COD analysis.
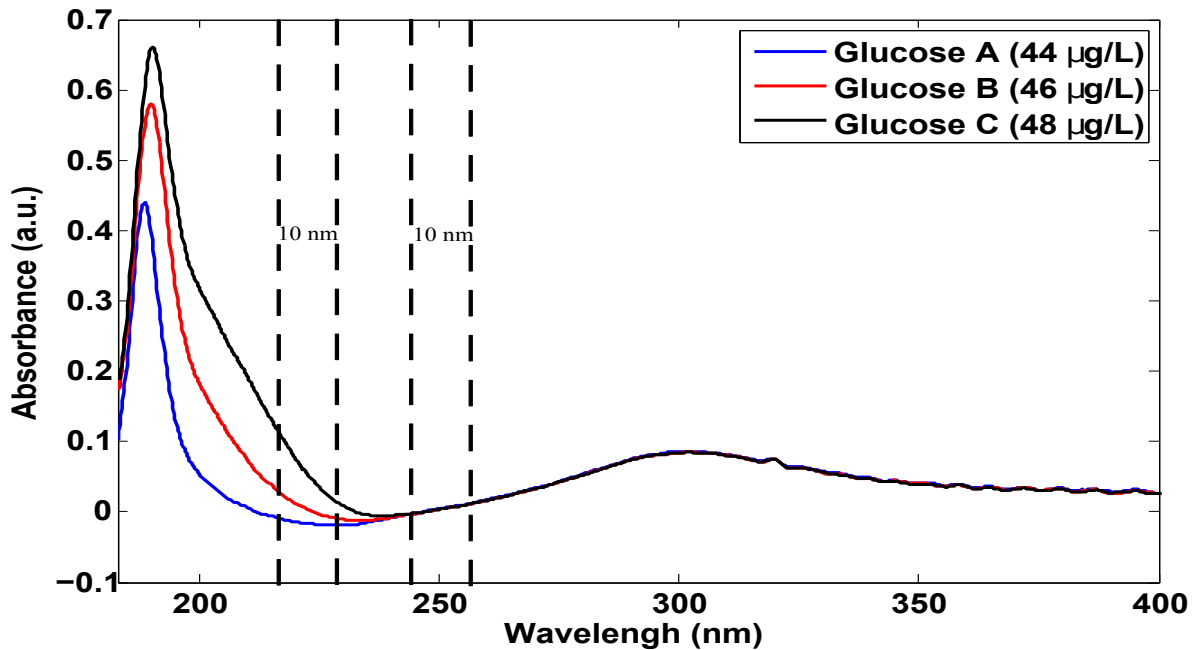
Figure 5.2: Absorbance values were chosen from different spectral region at different wavelength for statistical model. For this analysis BW= 10 nm was used at 225 nm and 250 nm for spectral region.

Average absorbance values from the selected spectral region for all samples and their known COD concentration values were used in developing linear regression model. Details about the development of regression model from absorbance and COD concentration using MATLAB's statistical toolbox built-in function $regstat$ was mentioned in section 4.1.5. Figure 5.3 shows the correlation between laboratory prepared standard glucose COD concentration and spectral absorbance at 225 nm. Similarly, Figure 5.4 shows the correlation between laboratory prepared standard glucose COD concentrations and literature reported optimal absorbance at 250 nm, respectively.

Table 5.1 shows the R^2, adjusted R^2 and MSE results from the linear regression model for the selected region at 225 nm and 250 nm, respectively. The obtained R^2 value is more than 96% and MSE value is small at selected spectral regions for the both wavelengths. At
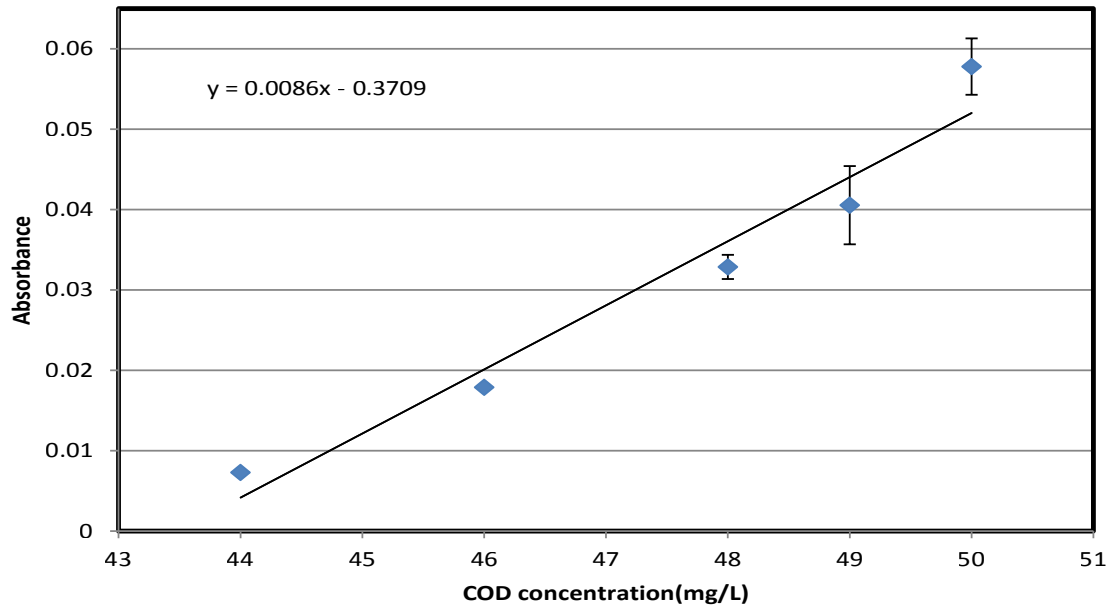
Figure 5.3: Corelation between laboratory prepared standard Glucose samples with COD concentartion from 44 mg/L to 50 mg/L with UV-VIS absorbance derived from spectral system at λ_{max} of 225 nm using 10 nm BW for spectral region.

selected region at 225 nm, statistical test results an intercept with co-efficient of - 0.3709, standard error of 0.0459, t-stat of - 8.0737, p-value of 0.004. In same region, X variable presents co-efficient of 0.0086, standard error of 9.4×10^{-4}, t-stat of 8.8967, p-value of 0.0031. At selected region at 250 nm, statistical test results an intercept with co-efficient of - 0.0202, standard error of 0.0022, t-stat of - 9.2805, p-value of 0.0026. In same region, X variable presents co-efficient of 5.2×10^{-5}, standard error of 4.59×10^{-5}, t-stat of 11.9314, p-value of 0.0015. The obtained regression coefficients, standard error on coefficients and statistical test results indicate that this model is representing data properly. In addition, Table 5.2 shows predicted absorbance values and their deviation from the original values. The regression model shows good results in terms of mean standard error (MSE) and regression coefficients. Here, the R^2 values from the linear regression model for the selected region at 225 nm and 250 nm are greater than 96% and 97%, respectively.
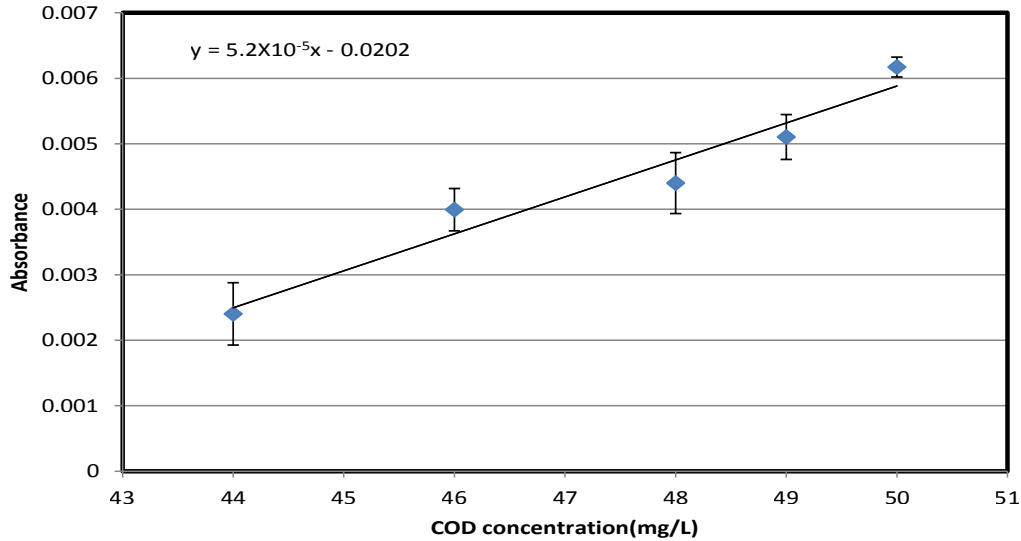
Figure 5.4: Corelation between laboratory prepared standard Glucose samples with COD concentartion from 44 mg/L to 50 mg/L with UV-VIS absorbance derived from spectral system at λ_{max} of 250 nm using 10 nm BW for spectral region.

Table 5.1: Results obtained for COD calibration with UV-VIS immiscible probe by performing linear regression analysis.

Wavelength	225 nm	250 nm
R^2	0.9627	0.9774
Adjusted R^2	0.9502	0.9699
Standard Error	2.15×10^{-5}	4.81×10^{-4}

The R^2 value obtained from the regression analysis indicates that UV-VIS spectral analysis can be a suitable tool for COD concentration analysis. After developing the correlation between spectral absorbance and standard COD concentration (standard calibration curve), a predetermined 44.08 mg/L glucose solution was utilized to determine the accuracy of the system. Spectral analysis was performed on this sample using the spectral system and standard calibration curve. Resulted obtained from the analysis gave COD concentration (44.38 mg/L $\pm$ 8.02 % mg/L) considering systematic and vibration errors.

Table 5.2: Regression analysis predicted values and residual or deviation from the original values is presented for COD calibration with UV-VIS immersible probe at different wavelength.

Wavelength (nm)	Original Absorbance	Predicted Absorbance ($\hat{y}$)	Residual
225 nm	0.0075	0.0054	0.0021
	0.0180	0.0225	0.0045
	0.0344	0.0311	0.0033
	0.0440	0.0482	-0.0042
	0.0611	0.0567	0.0034
250 nm	0.0029	0.0029	6.10×10^{-6}
	0.0039	0.0039	4.86×10^{-5}
	0.0045	0.0045	2.39×10^{-5}
	0.0052	0.0055	$- 3.02 \times 10^{-5}$
	0.00625	0.0060	2.44×10^{-4}

5.1.2   Using Real Waste water Samples

In this study, real samples were collected from two different wastewater treatment facilities at Annaces Island wastewater treatment plant located in Metro-Vancouver. Effluent 140.5 mg/L and Influent 251.33 mg/L samples were collected from facility 1 and facility 2 respectively. Samples from two different facilities was combined in different ratios to prepare a series of samples from 140.5 mg/L to 251.33 mg/L which will be used to generate standard calibration curve.

As described in test with laboratory section, prepared series of solutions were used in spectral analysis to obtain correlation between spectral absorbance with COD concentrations of industrial samples. For this analysis process λ_{max} chosen by system algorithm was 225 nm which is not really close to literature reported value. Figure 5 and 6 are showing correlation between Industrial COD concentration from reactor 1 and spectral absorbance at λ_{max} at 225 nm and 254nm respectively. After preparing the calibration curve, 233.6 mg/L concentrated industrial sample was taken as unknown sample to check the accuracy of the spectral system with industrial samples and system measured value was 238.36
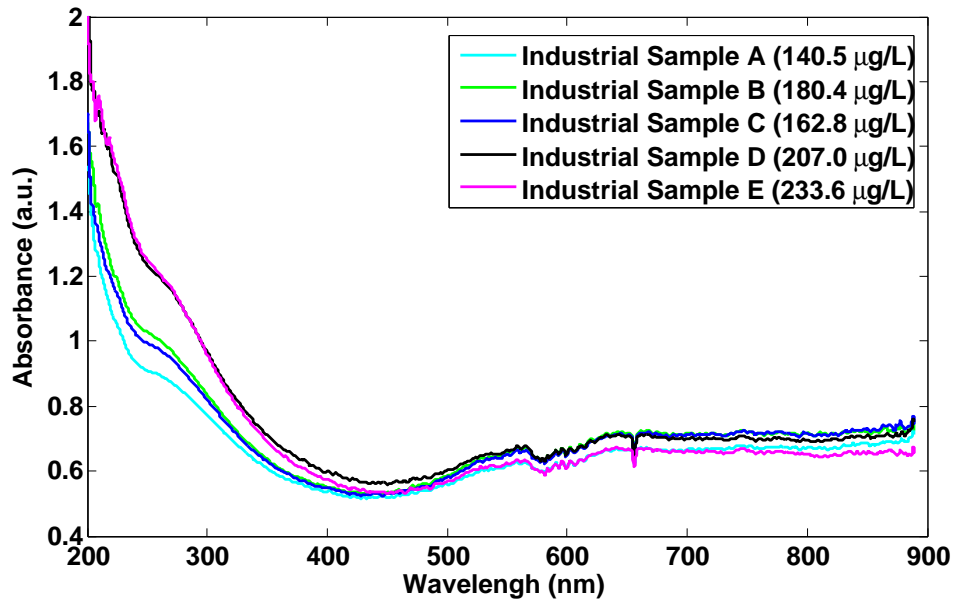
Figure 5.5: UV-VIS spectra acquired using ocean optics CHEM-4000 spectrometer for industrial samples collected from metro Vancouver's Annacis Island Wastewater Treatment Plant. These absorbance spectra were obtained for COD samples with concentration ranging from 140 mg/L to 250 mg/L.

mg/L (± 8.02 % mg/L).

Similarly to the measurement with laboratory samples section, experimental setup was similarly investigated using industrial samples. In this study, real samples were collected from two different wastewater treatment facilities at Annaces Island wastewater treatment plant located in Metro-Vancouver. Effluent with COD concentration of 140. 5 mg/L and Influent with COD concentration of 251.33 mg/L samples were collected from facility 1 and facility 2 respectively. Samples from two different facilities were combined in different ratios to prepare a series of samples with different concentration ranging from 140. 5 mg/L to 251. 33 mg/L. Industrial samples collection and dilution processes were described in details at section 3.5.2.
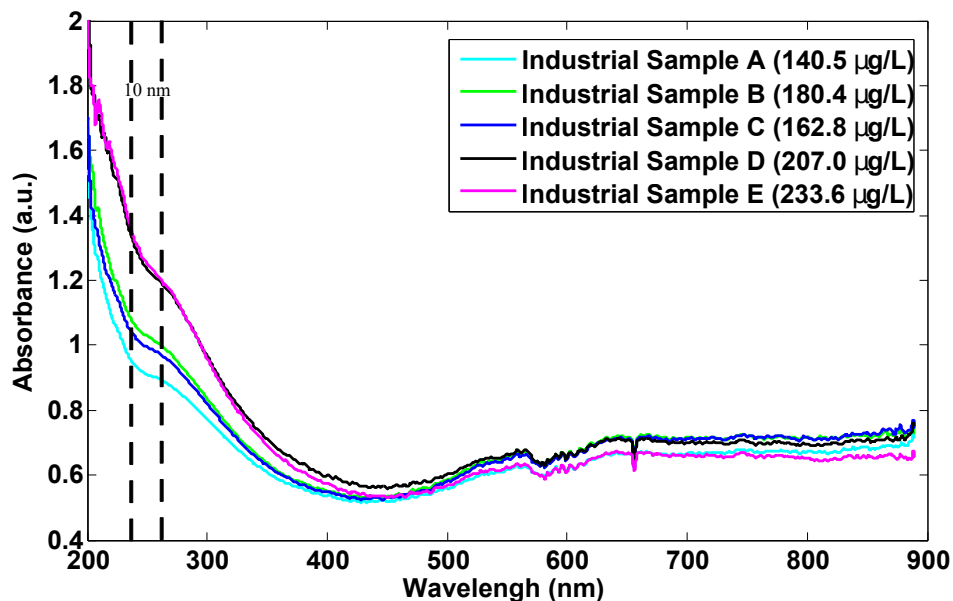
Figure 5.6: Absorbance values were chosen from different spectral region at different wavelength for statistical model. For this analysis BW = 10 nm was used at 225 nm for spectral region.

As described in previous section, these industrial samples were utilized to obtain the correlation between UV-VIS spectral absorbance values acquired from spectral system with known COD concentration of these samples. Similar to measurement with laboratory standard samples, spectrum was acquired for each industrial samples using CHEM-4000 spectrometer and our developed GUI system. Figure 5.3 shows UV-VIS acquired spectra using ocean optics CHEM-4000 spectrometer for all industrial samples. This figure shows similar photon absorbance pattern in samples like the glucose samples. Here, photon absorbance also increases in the industrial samples as the concentration of the samples increases following the Beer's law. Similar to glucose spectra, in the UV-VIS spectrum of industrial samples a variation in concentration can be easily detected by only looking at the change of spectra's shape. Again, these samples, spectra were taken for UV-VIS spectral range from 180 nm to 900 nm and several spectral ranges were selected and studied to
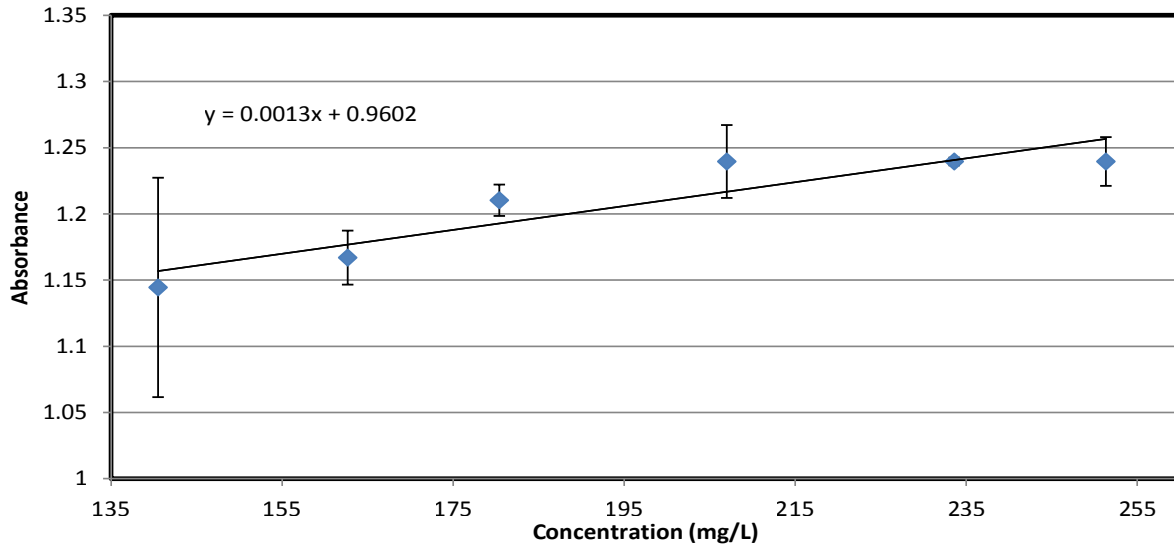
Figure 5.7: Corelation between COD samples colleced from wastewater treatment plant with COD concentartion from 140 mg/L to 250 mg/L with UV-VIS absorbance derived from spectral system at λ_{max} of 225 nm using 10 nm BW for spectral region.

investigate the results between different regions. Similar to previous process, developed system identified the most sensitive wavelength and spectral region for COD measurement using the same 10 nm bandwidth at different wavelengths. Figure 5.6 shows a 10 nm spectral region at 225 nm, which was selected automatically as most sensitive region for building the statistical model. In addition, literature reported 10 nm spectral region at 25 nm was chosen manually to compare the results with our system selected wavelength.

As described in previous section, average absorbance values from the selected spectral region for all samples and their known COD concentration values were used in developing linear regression model. Figure 5.7 shows the correlation between COD concentration of industrial samples and spectral absorbance at 225 nm. Similarly, Figure 5.8 shows the correlation between COD concentrations of industrial samples and literature reported optimal absorbance at 250 nm respectively.
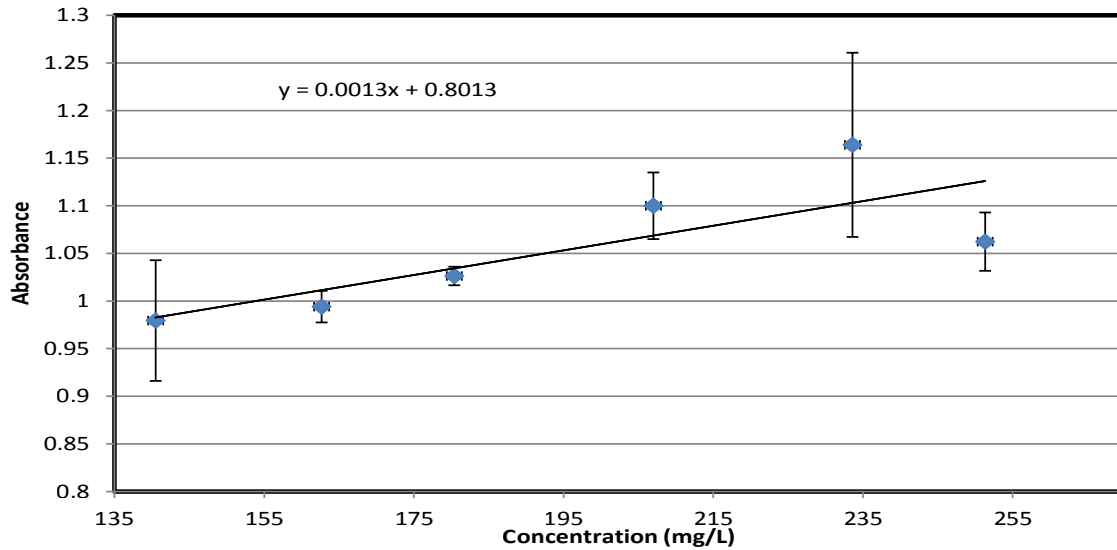
Figure 5.8: Corelation between COD samples colleced from wastewater treatment plant with COD concentartion from 140 mg/L to 250 mg/L with UV-VIS absorbance derived from spectral system at λ_{max} of 250 nm using 10 nm BW for spectral region.

Table 5.4 shows the R^2, adjusted R^2 and MSE results from the linear regression model for the selected region at 225 nm and 250 nm, respectively. Here, the obtained R^2 value at 225 nm is more than 93% and MSE value is small at that spectral region. Whereas, the R^2 value is around 62% at 250 nm and MSE value is factor 8 larger compared to MSE value at 225 nm. At selected region at 225 nm, statistical test results an intercept with co-efficient of 0.9602, standard error of 0.0345, t-stat of - 27.8127, p-value of 9.94×10^{-6}. In same region, X variable presents co-efficient of 0.0013, standard error of 1.72×10^{-4}, t-stat of 7.6822, p-value of 0.0015. At selected region at 250 nm, statistical test results an intercept with co-efficient of 0.8016, standard error of 0.1004, t-stat of 7.9838, p-value of 0.0013. In same region, X variable presents co-efficient of 0.0013, standard error of 5.02 $\times 10^{-4}$, t-stat of 2.5704 , p-value of 0.0620. The obtained regression coefficients, standard error on coefficients and statistical test results indicate that this model is representing data

Table 5.3: Results obtained for COD calibration with UV-VIS immiscible probe by performing linear regression analysis.

Wavelength	225 nm	250 nm
R^2	0.9365	0.6229
Adjusted R^2	0.9207	0.5286
Standard Error	2.69×10^{-5}	0.0023

properly. The obtained regression coefficients, standard error on coefficients and statistical test results indicate that our developed system can identify most sensitive region accurately and developed statistical model can represent data properly. In addition, Table 5.5 shows model predicted absorbance values and their deviation from the original values at 225 nm and literature reported 250 nm. The R^2 value, regression coefficients and mean standard error were achieved for the system at chosen wavelength and spectral region from this statistical model. The achieved values are satisfactory and promising, which can be used as accurate COD concentration measurement tool.

Table 5.4: Regression analysis predicted values and residual or deviations from the original values are presented for COD calibration with UV-VIS immiscible probe at different wavelength.

Wavelength (nm)	Original Absorbance	Predicted Absorbance ($\hat{y}$)	Residual
225 nm	1.14	1.147	-0.0067
	1.17	1.176	-0.0062
	1.21	1.199	0.0103
	1.24	1.245	0.0049
	1.29	1.27	0.0196
	1.272	1.294	-0.0219
250 nm	0.9795	0.9827	-0.0032
	0.9941	1.0114	-0.0173
	1.0263	1.0343	-0.0080
	1.1	1.0686	0.0313
	1.164	1.1030	0.0609
	1.0623	1.1259	-0.0636

5.2   Verification

As mentioned in previous section, a correlation (standard calibration curve) between average absorbance of all the industrial samples and their known COD concentration has developed. To develop this correlation all the experiments were performed in three replicates. A relative error of the UV-VIS spectral model is around 8.00% by considering the forced manual vibration error $\approx 5\%$. Excluding the forced vibration error this relative error is less than 3%. Considering relative error, COD concentration in waste water treatment facility results 83.5 ± 6.68 mg/L of its content by our UV-VIS spectral method.Simultaneously, conventional method gives 90 ± 9 of COD value for waste water sample.

From this result, it is possible to conclude that the relative error of UV-VIS system is lower than the conventional method. The relative error of these models are indicating that the UV-VIS spectral method provide better predictability of COD concentration in waste water treatment facility over the conventional method.

Chapter 6

Conclusion and Future Work

This chapter summarizes the conclusion and future work of this research.

6.1 Conclusion

A rapid, inexpensive and accurate UV-VIS spectrometric method has been developed for determination of COD in the waste water treatment facility. The results from this work has permitted to conclude that the developed system could monitor and estimate the accurate COD concentration in wastewater treatment plant. A self-guided user friendly MATLAB based UI has developed to control the UV-VIS spectrometer, halogen-tungsten light source and electronic shutter of the detector. The developed system comes with a smart algorithm, which is capable to automatically identify maximum sensitive spectral region and wavelength for COD content in the waste water system. It was possible to conclude that UV-VIS spectrometer system can probe in raw waste water sample and distinguish between solutions with different composition. These changes could be visually identified by only looking at UV-VIS spectrum acquired with developed system. However, the system limits the response of sample which has COD content with other suspended solid. To

characterize the spectrometric system, different tests were performed with different system imbalances in order to obtain the maximum information about the system response and test the ability of the probes to detect them. Laboratory made standard samples and collected samples from different industrial facility were used in these tests. After acquiring UV-VIS spectra from these samples using the developed system, a linear relationship of the spectral absorbance and COD concentration were established. This linear relationship was achieved without using any kind of chemical reagent. The result obtained from statistical model for industrial samples showed good result for system chosen wavelength and spectral region and for literature reported spectral region. Only one issue of this system is achievement linear relationship should be done by more than one samples. The obtained regression coefficients, mean standard error, standard error on coefficients and residual indicate that this model is representing data properly. The COD concentration obtained from the system was validated using traditional method. The developed system showed accurate result with very small error range. In summary, we developed a system for rapid measurement of COD in filtered wastewater samples that can provide accurate measurements after initial calibration. The system does not require any chemical treatment of the sample and is therefore safer and less expensive to use compared to the conventional COD measurement techniques. Finally, it is possible to integrate the system components into a portable system for real-time measurement of COD on-site.

6.2   Future Work

Regarding the importance of this type of studies for the development of more suitable techniques for monitoring and control of waste water parameters in WWTP, the following ideas are suggested as future research:

- The developed program can easily be converted to executable file format to utilize in

portable electronic devices with multi touch screen so that user can bring the portable UV-VIS spectrometry system to remote location and analyse sample directly from there.

- Developed system and experiments were only focused on accurate determination of COD concentration instantly from waste water facility. In future, the developed system can be extended for determining BOD, TOS, NO_2 or NO_3 from WWTP.

- The developed system can be improvised by adding a submersible online UV-VIS spectrometer probe. This online probe can monitor waste water parameter continuously directly from the WWTP or any other remote location and trigger an alarm when the system reads outer range data and prevent the fault in WWTP system.

Bibliography

[1] Naima Bradley, Henrietta Harrison, Greg Hodgson, Robie Kamanyire, Andrew Kibble, and Virginia Murray. *Essentials of Environmental Public Health Science: A Handbook for Field Professionals*. Oxford University Press, 2014.

[2] KH Mancy. Instrumental analysis for water pollution control. *Instrumental analysis for water pollution control*, 1971.

[3] George Tchobanoglous and Franklin L Burton. Wastewater engineering. *Management*, 7:1–4, 1991.

[4] Suman Mor, Khaiwal Ravindra, RP Dahiya, and A Chandra. Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site. *Environmental monitoring and assessment*, 118(1-3):435–456, 2006.

[5] Mogens Henze. *Wastewater treatment: biological and chemical processes*. Springer Science & Business Media, 2002.

[6] Ashok Kumar, BS Bisht, VD Joshi, AK Singh, and Amitabh Talwar. Physical, chemical and bacteriological study of water from rivers of uttarakhand. *Journal of Human Ecology*, 32(3):169–173, 2010.

[7] RK Trivedy and PK Goel. *Chemical and biological methods for water pollution studies*. Environmental publications, 1984.

[8] Baisheng Chen, Huanan Wu, and Sam Fong Yau Li. Development of variable pathlength UV-vis spectroscopy combined with partial-least-squares regression for wastewater chemical oxygen demand (COD) monitoring. *Talanta*, 120:325–330, 2014.

[9] Guoqing Wu, Weihong Bi, Jiaming Lv, and Guangwei Fu. Determination of chemical oxygen demand in water using near-infrared transmission and UV absorbance method. *Chiese Optics Letters*, 09(s1):310705, June 2011.

[10] BP Rajbhandari and S Shrestha. An assay of rupa lake water from the perspectives of livelihoods. *NEPALESE JOURNAL OF AGRICULTURAL SCIENCES*, page 126.

[11] Wayne Boyles. The science of chemical oxygen demand. *Technical information series, Booklet*, (9):24, 1997.

[12] Eugene R Weiner. *Applications of environmental chemistry: a practical guide for environmental professionals*. CRC press, 2010.

[13] Heinz-Helmut Perkampus, Heide-Charlotte Grinter, and TL Threlfall. *UV-VIS Spectroscopy and its Applications*. Springer, 1992.

[14] I Botev. A new conception of bouguer-lambert-beer's law. *Fresenius' Zeitschrift für analytische Chemie*, 297(5):419–419, 1979.

[15] World Health Organization. *Guidelines for drinking-water quality: recommendations*, volume 1. World Health Organization, 1993.

[16] American Public Health Association. Water environment federation. *Standard methods for the examination of water and wastewater*, 19, 1995.

[17] Nicholas P Cheremisinoff and Anton Davletshin. *Hydraulic Fracturing Operations: Handbook of Environmental Management Practices*. John Wiley & Sons, 2015.

[18] American Public Health Association, American Water Works Association, Water Pollution Control Federation, and Water Environment Federation. *Standard methods for the examination of water and wastewater*, volume 2. American Public Health Association., 1915.

[19] Tom Murdoch, Martha Cheo, and Kate O'Laughlin. The streamkeeper's field guide: watershed inventory and stream monitoring methods. 1996.

[20] Mike Cable. *Calibration: A Technician's Guide*. ISA, 2005.

[21] Robert G Wetzel. *Limnology: lake and river ecosystems*. Gulf Professional Publishing, 2001.

[22] P Patnaik. *Handbook of environmental analysis: chemical pollutants in air, water, soil, and solid wastes*. 2010.

[23] Gary M Rand and Sam R Petrocelli. Fundamentals of aquatic toxicology: methods and applications. Technical report, FMC Corp., Princeton, NJ, 1985.

[24] Kenneth Bendschneider and Rex J Robinson. A new spectrophotometric method for the determination of nitrite in sea water. 1952.

[25] Joel Schwartz, Ronnie Levin, and Rebecca Goldstein. Drinking water turbidity and gastrointestinal illness in the elderly of philadelphia. *Journal of epidemiology and community health*, 54(1):45–51, 2000.

[26] Sushil Tuladhar and Zachary Lenth. Water quality monitoring of prairie lake. 2011.

[27] David J Welsch. *Riparian forest buffers: function and design for protection and enhancement of water resources*, volume 7. US Department of Agriculture, Forest Service, Northeastern Area, State & Private Forestry, Forest Resources Management, 1991.

[28] Pavel Pitter and Jan Chudoba. Biodegradability of organic substance in the aquatic environment. 1990.

[29] W Horwitz AOAC. Official methods of analysis of the association of official analytical chemists. *Association of Official Analytical Chemists, Arlington, VA, USA*, 1990.

[30] Honglei Chen, Yuancai Chen, Huaiyu Zhan, and Shiyu Fu. Chemometrics-assisted spectrophotometry method for the determination of chemical oxygen demand in pulping effluent. *Environmental Monitoring and Assessment*, 175:321–329, 2011.

[31] Richard A Dobbs and Robert T Williams. Elimination of chloride interference in the chemical oxygen demand test. *Analytical chemistry*, 35(8):1064–1067, 1963.

[32] Timothy M LaPara, James E Alleman, and P Greg Pope. Miniaturized closed reflux, colorimetric method for the determination of chemical oxygen demand. *Waste Management*, 20(4):295–298, 2000.

[33] Mike J Adams. *Chemometrics in analytical spectroscopy*. Royal Society of Chemistry, 2004.

[34] Kunwar P Singh, Amrita Malik, Dinesh Mohan, Sarita Sinha, and Vinod K Singh. Chemometric data analysis of pollutants in wastewatera case study. *Analytica Chimica Acta*, 532(1):15–25, 2005.

[35] James N Miller and Jane Charlotte Miller. *Statistics and chemometrics for analytical chemistry*. Pearson Education, 2005.

[36] Richard G Brereton. *Chemometrics: data analysis for the laboratory and chemical plant*. John Wiley & Sons, 2003.

[37] American Public Health Association. Water environment federation (1998) standard methods for the examination of water and wastewater, 1994.

[38] T Srinivas. *Environmental biotechnology*. New Age International, 2008.

[39] Clifford C Hach, Robert L Klein Jr, and Charles R Gibbs. Biochemical oxygen demand. *Tech. Monogr*, (7), 1997.

[40] Jing Liu and Bo Mattiasson. Microbial bod sensors for wastewater analysis. *Water Research*, 36(15):3786–3802, 2002.

[41] A Vaid, C Kopp, W Johnson, and AG Fane. Integrated waste water treatment by coupled bioreactor and membrane system. *Desalination*, 83(1):137–143, 1991.

[42] K Riedel, K-P Lange, H-J Stein, M Kühn, P Ott, and F Scheller. A microbial sensor for bod. *Water Research*, 24(7):883–887, 1990.

[43] Harald Martens. *Multivariate calibration*. John Wiley & Sons, 1992.

[44] Wilfrid Bourgeois, Joanna E Burgess, and Richard M Stuetz. On-line monitoring of wastewater quality: a review. *Journal of chemical technology and biotechnology*, 76(4):337–348, 2001.

[45] Lawrence Berliner and Chris Bender. *EPR: Instrumental Methods: Instrumental Methods*. Number 21. Springer Science & Business Media, 2004.

[46] M Nataraja, Y Qin, and E a Seagren. Ultraviolet spectrophotometry as an index parameter for estimating the biochemical oxygen demand of domestic wastewater. *Environmental technology*, 27(March 2013):789–800, 2006.

[47] Mujeeb Khan and Basis Seminar. Ultraviolet / Visible. (April), 2009.

[48] H Förster. Uv/vis spectroscopy. In *Characterization I*, pages 337–426. Springer, 2004.

[49] Sl Upstone. Ultraviolet/visible light absorption spectrophotometry in clinical chemistry. *Encyclopedia of Analytical Chemistry*, pages 1699–1714, 2000.

[50] JA Van Loon. *Analytical atomic absorption spectroscopy: selected methods*. 2012.

[51] Jerry Workman Jr and Art Springsteen. *Applied spectroscopy: a compact reference for practitioners*. Academic Press, 1998.

[52] Dr Hinna Hamid. Pharmaceutical analysis. pages 24 – 25, 2007.

[53] Guoqing Wu Guoqing Wu, Weihong Bi Weihong Bi, Jiaming Lv Jiaming Lv, and Guangwei Fu Guangwei Fu. Determination of chemical oxygen demand in water using near-infrared transmission and UV absorbance method. *Chinese Optics Letters*, 9:s10705–310709, 2011.

[54] S Rudoler, Ofer Hadar, Moshe Fisher, and Norman S Kopeika. Image resolution limits resulting from mechanical vibrations. *Optical engineering*, 30(5):577–589, 1991.

[55] Ocean Optics Documentation. http://oceanoptics.com/.

[56] Starna Scientific Documentation. http://www.starna.co.uk/.

[57] MATLAB - The Language of Technical Computing. http://www.mathworks.com/. Accessed: 2013.

[58] Md Mizanur Rahman, Mohammad Mizanur Rahman Khan, and Mohammad Mazedul Hosain. Analysis of Vitamin C (ascorbic acid) Contents in Various Fruits and Vegetables by UV-spectrophotometry. *Bangladesh Journal of Scientific and Industrial Research*, 42(4):417–424, 2008.

[59] J. Randall Etheridge, François Birgand, Jason a. Osborne, Christopher L. Osburn, Michael R. Burchell Ii, and Justin Irving. Using in situ ultraviolet-visual spectroscopy to measure nitrogen, carbon, phosphorus, and suspended solids concentrations at a high frequency in a brackish tidal marsh. *Limnology and Oceanography: Methods*, 12:10–22, 2014.

[60] Alexandra M E Viana da Silva, Ricardo J N Bettencourt da Silva, and M. Filomena G F C Camões. Optimization of the determination of chemical oxygen demand in wastewaters. *Analytica Chimica Acta*, 699(2):161–169, 2011.

[61] Am Pisarevsky, Ip Polozova, and Pm Hockridge. Chemical oxygen demand. *Russian journal of applied Chemistry, Moscow*, 78(1):101–107, 2005.

[62] WC Navidi. *Statistics for engineers and scientists*. 2008.

[63] Graciela Ruderman, Ernesto R Caffarena, Ines G Mogilner, and Eduardo J Tolosa. Hydrogen Bonding of Carboxylic Acids in Aqueous SolutionsUV Spectroscopy, Viscosity, and Molecular Simulation of Acetic Acid. 27(10), 1998.

[64] GF Kirkbright. The application of non-flame atom cells in atomic-absorption and atomic-fluorescence spectroscopy. A review. *Analyst*, 1971.

[65] Jus Irving and Greg Land. PNCWA 2012 - Wastewater Influent Monitoring to Minimize Process Upsets. 2012.

[66] Shoshana Fogelman, Huijun Zhao, and Michael Blumenstein. A rapid analytical method for predicting the oxygen demand of wastewater. *Analytical and Bioanalytical Chemistry*, 386:1773–1779, 2006.

[67] DM Haaland and EV Thomas. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Analytical Chemistry*, 1988.

[68] a W Mohammad, N Hilal, and L Y Pei. Treatment of landfill leachate wastewater by nanofiltration membrane. *International Journal of Green Energy*, 1(1):251–263, 2004.

[69] DL Massart, BG Vandeginste, and LMC Buydens. Handbook of chemometrics and qualimetrics: Part A. 1997.

[70] DC Montgomery and GC Runger. *Applied statistics and probability for engineers*. 2010.

[71] J Kopáček, L Prochazkova, J Hejzlar, and P Blažka. Trends and seasonal patterns of bulk deposition of nutrients in the Czech Republic. *Atmospheric Environment*, 1997.

[72] GM Rand and SR Petrocelli. Fundamentals of aquatic toxicology: methods and applications. 1985.

[73] RD Down and JH Lehr. *Environmental instrumentation and analysis handbook*. 2005.

[74] Boehringer Mannheim, R-biopharm Enzymatic Bioanalysis, and Food Analysis. Acetic acid.

[75] John Makemson. Lab Week 2 - Spectrophotometry. 2012.

[76] Andreja Drolc, Magda Cotman, and Milenko Roš. Uncertainty of chemical oxygen demand determination in wastewater samples. *Accreditation and Quality Assurance*, 8:138–145, 2003.

[77] RG Wetzel. *Limnology: lake and river ecosystems*. 2001.

[78] Richard a. Dobbs, Robert H. Wise, and Robert B. Dean. The use of ultra-violet absorbance for monitoring the total organic carbon content of water and wastewater. *Water Research*, 6(1966):1173–1180, 1972.

[79] North Wyke. Technical Note Estimation of Biochemical Oxygen Demand in Slurry a N D Effluents Using Ultra-Violet. 31(2):372–374, 1997.

[80] Abdul Bari and Shaukat Farooq. Measurement of wastewater treatment efficiency by fluorescence and UV absorbance. *Environmental Monitoring and Assessment*, 5:423–434, 1985.

[81] Joep Van Den Broeke, Günter Langergraber, and Andreas Weingartner. On-line and in-situ UV / vis spectroscopy for measurements : a brief review. *Spectroscopy Europe 1*, 18(4):4–7, 2006.

Appendix A

Operating Manual

Some steps of MATLAB based GUI are presented in this Appendix.

As discussed in Chapter 4, Graphical User Interfaces for spectrometer control and wastewater analysis consists of six GUI's. Each of the GUIs is designed to implement a particular task. Figure A.1 shows a road map for creating MATLAB GUI.

Defining the task then drawing and testing the GUI by hand are the components of the design phase. Writing and testing the code are the components of the implementation phase. It is an important fact that the design phase should be completed before starting the implementation phase. The road map also shows that arrows flow in both directions; this reflects that the project is likely to go through the design and implementation phases multiple times.

Spectrometer Initialization: As COD is measured by a UV/VIS spectrometer, an initialization will be adjusted with 2.2×10^4 μs integration time, 5 scan to average and 3 boxcar width.Figure A.2 shows the initialization step for GUI.
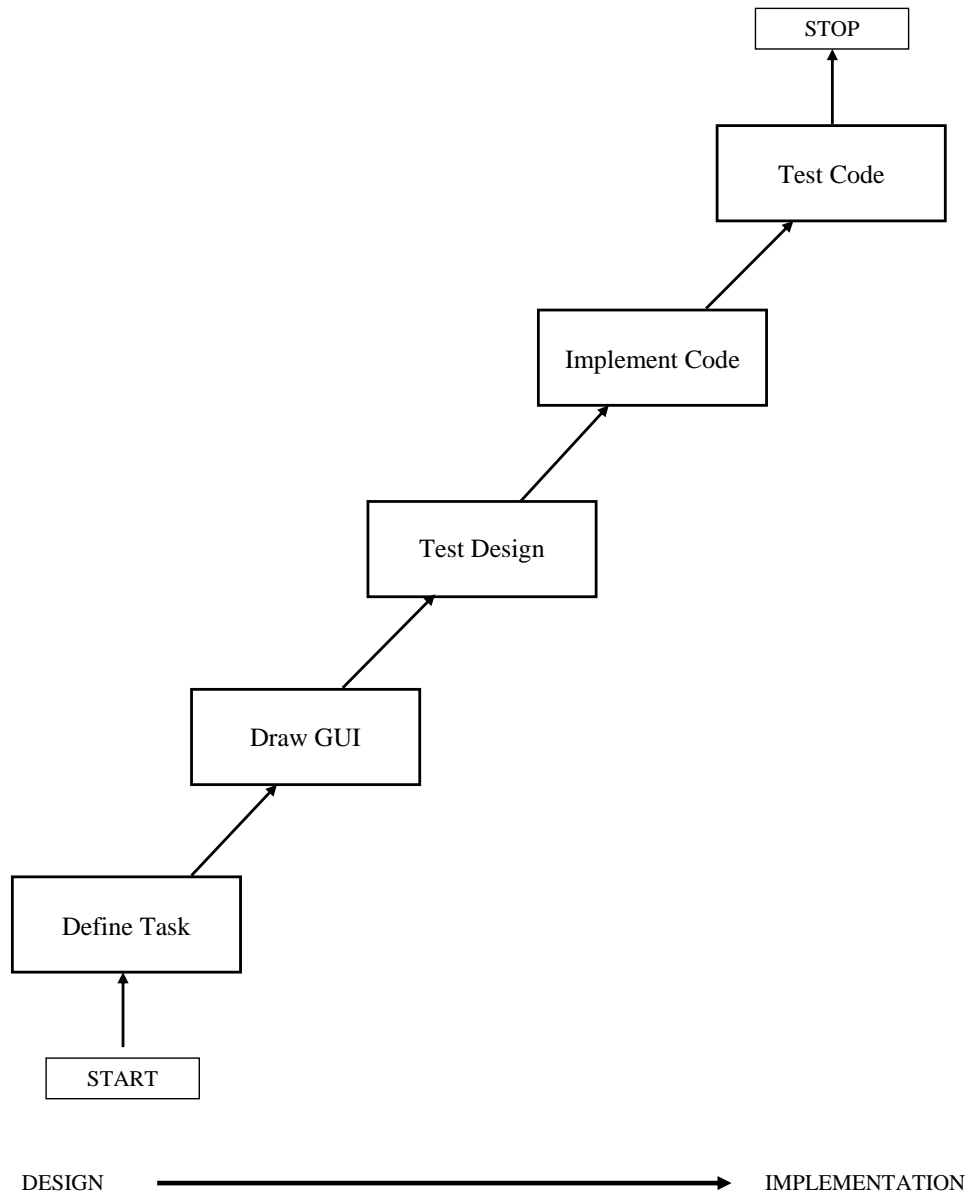
Figure A.1: GUI development process road map.
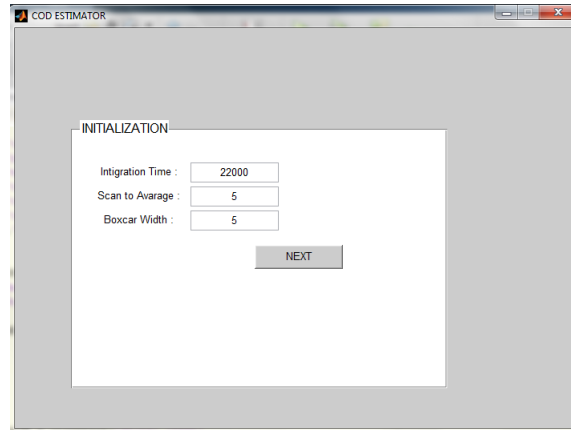
Figure A.2: Initialization GUI, accepts user defined parameters to initialize spectrometer.

Save Dark and Reference: For every COD sample, absorbance is saved by measuring dark and reference spectrum.Recorded Dark and Reference spectrum from spectrometer are shown in Figure A.3 and Figure A.4
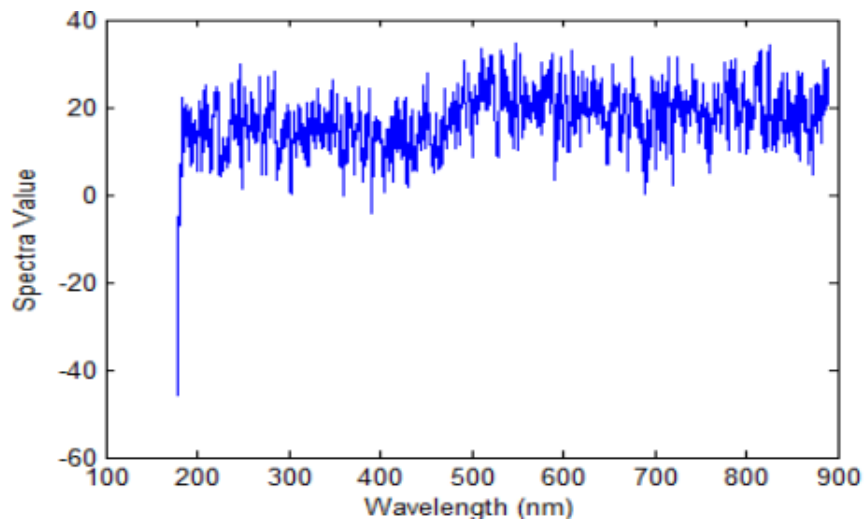

Figure A.3: Dark spectrum recorded at MATLAB from spectrometer.

Amount of samples: After initializing the spectrometer and absorbance value, amount of known COD will be inserted. Figure A.5 shows that GUI receives number of known
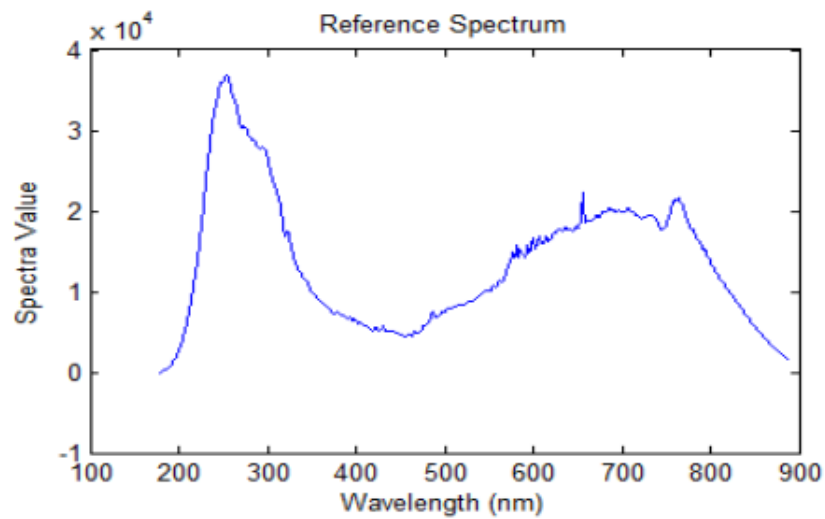
Figure A.4: Reference spectrum recorded at MATLAB from spectrometer.

samples in advance to guide the user dynamically and guides for absorbance calculation GUI to perform absorbance analysis.
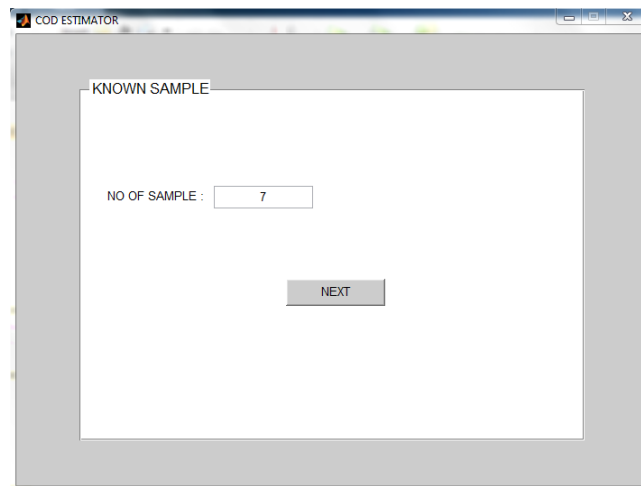
Figure A.5: Amount of samples inserted as user input.

Insert known sample: The concentration of each known sample will be inserted. All sample spectrum are recorded at absorbance calculation GUI from spectrometer at the
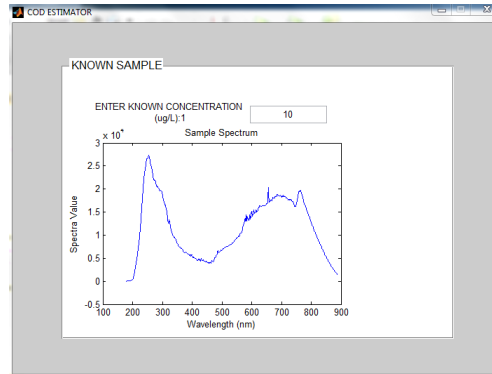
Figure A.6: Sample spectrum of known concentration.

time of testing with known concentration of waste water sample. Sample spectrum of known concentration is shown as Figure A.6

Achieve absorbance for known sample: After inserting known sample, the absorbance for each known sample will be achieved by following calculation.

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right) \tag{A.1}$$

where, S = sample intensity at wavelength (λ), D = dark intensity at wavelength (λ) and R = reference intensity at wavelength (λ).

Figure A.7 shows recorded known waste water samples absorbance at absorbance calculation GUI from spectrometer at the time of testing with known concentration of waste water sample.

Insert Wavelength and BW(If known): Automatic Maximum sensitivity Selection GUI, automatically perform analysis on recorded known absorbance spectral data to evaluate maximum sensitive region and maximum sensitive wavelength. This maximum sensitive wavelength is suggested to the user by pre-defined wavelength and bandwidth (BW). User
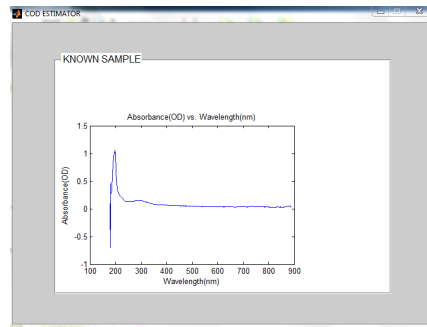
Figure A.7: Absorbance for each known sample.

Figure A.8: User specified wavelength and BW.

can proceed with this selected value or customize these parameter, which will be used in regression analysis to generate calibration curve.
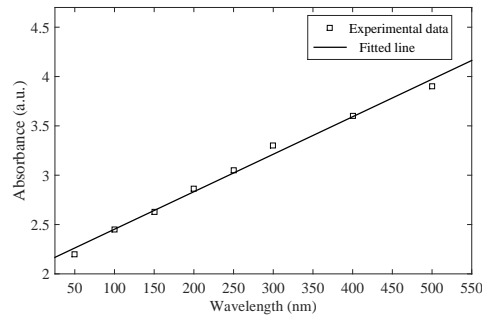

Figure A.9: Linear relationship for absorbance vs known sample concentration of waste water sample.

Regression Analysis: Using selected wavelength and BW, this program performs linear regression analysis on all known waste water COD sample absorbance spectral data and generate calibration curve. These calibration values are automatically recorded in text files for future use. Besides, these calibration values will be used to evaluate unknown waste water COD value.Figure A.9 shows the resulted linear regression relationship of absorbance and known sample concentration.
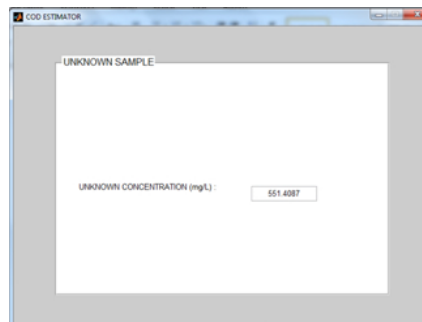

Figure A.10: Evaluated COD concentration of unknown waste water sample.

Find unknown concentration from linear calibration curve: The Unknown sample will be given into calibrated model. Thus, we will get concentration for that unknown sample.COD evaluation GUI is presented unknown waste water sample's evaluated COD concentration in Figure A.10