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Investigation of a Fiber-Optic Chemical Sensor for *in-situ* Measurement of pH in Soils.

Shalveen Sharika

Master of Science

Investigation of a Fiber-Optic Chemical Sensor for *in-situ* Measurement of pH in Soils.

By

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(BSc, PGD) *U.S.P*

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

Department of Chemistry

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AUTHOR'S DECLARATION

I earnestly declare that this thesis is a report of my own research work and has not been submitted for a higher degree at any other University to the best of my knowledge. Acknowledgements have been duly made where information has been incorporated from other published work.

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List of Abbreviations

FOCS	Fiber optic chemical sensor
UV/Vis	Ultra violet/ Visible
M	moles/litre
nm	nanometer
μm	micrometer
ppb	parts per billion
m	meter
km	kilometer
g	gram
ssm mode	scope sample mode
LED	Light-emitting diode
TBPSP	3,4,5,6 tetrabromophenolsulfonephthalein
BCG	Bromocresol green
BCP	Bromocresol purple
BTB	Bromothymol Blue
MR	Methyl red
PR	Phenol red

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Abstract

An investigation was carried out on developing a pH sensor for soils based on fiber optic technology. The design is based on immobilizing a pH sensitive indicator dye on an inert substrate and coupling these with optical fibers that allow pH to be monitored optically. In the first part of the study, common acid-base indicator dyes were screened to identify those which would give appropriate optical signals for the pH range commonly encountered in soils. Soils generally have a pH value in the range of about 5 to 8. The optical signals of pH indicators when present in solution form were obtained to select the appropriate indicators to be utilized for sensor development. The five selected indicators were bromocresol green (BCG), bromocresol purple (BCP), bromothymol blue (BTB), methyl red (MR) and phenol red (PR).

The next phase of the project dealt with immobilization of these indicators on selected inert substrates or supports. The supports chosen were three different Amberlite resins: XAD-2, XAD-4 and XAD-7. The five indicators were immobilized on the three resins to give a set of fifteen indicator/resin combinations. For sensor development, the best indicator/resin combination is one that is most stable and most sensitive. One of the requirements for stability is that the indicator does not readily leach from the resin and to establish this, leaching studies were carried out for all indicator/resin combinations. Resins with immobilized indicators were packed in mini-columns and eluted with a cycle of acidic and basic buffers, and distilled water. Leaching of the relevant indicator was monitored optically by measuring absorbance of the leachate at the characteristic absorption wavelength of the indicator.

Leaching tests showed XAD-2 to be the best binding substrate as lowest amounts of indicators leached from this resin. XAD-7 was the most unsuccessful membrane in binding with all the indicators. Amongst the indicators, methyl red and phenol red

leached out the most from all the resins. Certain indicators such as methyl red from XAD-4, methyl red, bromocresol purple and phenol red from XAD-7 leached out completely. Tests to establish optical signal characteristics after immobilization showed certain indicators giving very poor or no response at all with change in pH. These were almost all indicators adsorbed on XAD-2, except bromocresol green. The pKa values of the indicator in solution form appear to change from the pKa after immobilization. This gives a variation in the response range of the indicator after immobilization. The indicators which had a shift in the pH range of response after immobilization were bromothymol blue on XAD-4 and XAD-7 and phenol red on XAD-4 and XAD-7. Based on tests on stability, reproducibility, response time and pH range; bromocresol green on XAD-2, XAD-4 and XAD-7 were selected as the most appropriate combinations for pH sensor development.

The next stage focused on testing performance in soils of pH 5 and 8. The pH of a sub sample of a soil with an existing pH value of 5 was adjusted to 8 by addition of the required amount of calcium carbonate and the original and treated soils were used in the study. One of the most critical factors in sensor performance in soils is the soil moisture content so a series of samples at both pH 5 and pH 8 were prepared containing different amounts of moisture. The soil: water (w/v) ratios used were 1:2.5, 1:1.5, 1:1 and 1:0.5. The performance of the sensor in detecting changes in soil pH was determined by allowing the sensor to first equilibrate in a pH 5 buffer solution and then placing it in a soil sample of pH 8 of given moisture content to determine its response characteristics. For the reverse cycle, it was allowed to equilibrate in pH 8 buffer solution first and then subjected to a pH 5 soil. The time required for the sensor to reach an equilibrium signal in the soil was recorded as the response time and this showed a strong dependence on moisture content.

One of the major issues of sensor performance in soils was soil particles interfering with the optical signal of the sensor. Fine soil particles penetrated through the nylon membrane separating the sensing phase from the soil. To overcome this problem, less porous membranes can be used but time did not permit this in this study. It might also be appropriate to let the sensing probe rest gently on the soil surface rather than being forcefully pushed into it but whilst this may inhibit soil particle penetration, it could increase response time. Based on the results obtained in this study, good potential has been demonstrated for real-time monitoring of pH in soils but further development work is necessary to inhibit the penetration of soil particle.

1.0 Introduction

Fiber Optic Chemical Sensors (FOCS) as an Analytical Method

Optical methods are some of the oldest and best established techniques for sensing chemical analytes. Initially as early as 1970s, optical fibers were used for telecommunications with their ability to allow light and signal transmission over long distances. There was a major accomplishment in the chemical sensing world when optical fibers were coupled with the optical sensing method (Narayanaswamy, 1993). Advances in the design and manufacturing technology of optical fibers, optoelectronics and semiconductors components had enabled fiber-optic sensors to have vast application in almost all areas of sensing and measurement (Taib and Narayanaswamy, 1995; Dyke and Cheng, 1988; Brewster and Anderson, 1982).

The term "optrode" is used to describe this type of device. It dictates the similar principles of optical sensors with electrodes (Janata, 1987). Since most chemical analytes of interest do not have distinctive inherent optical characteristics, an indirect, transducer based method is often used to extract useful optical signals from them. Analyte sensitive chemical recognition reagent phases are immobilized on various supports, and attached to the tip of fiber optic sensing probes. The immobilized chemical reagent interacts with the analyte and gives a colorimetric or fluorimetric indication of the chemical change and activity of the analytical species, which is measured as the change in magnitude of the optical signals in the form of absorbance, reflectance or luminescence, etc (Arnold, 1992; Oscroft, 1992). The whole concept behind these sensors is to take a physical quantity and transform it into an optical and then electrical signal which becomes more convenient means of recording and analyzing (Tucker, 1991).

1.1 Advantages and Disadvantages of FOCS

Many authors have highlighted various advantages and disadvantages of the designed optical sensors over other conventional devices in their field of applications (Seitz, 1984; Kirkbright, 1984; Jackson, 1992; Narayanaswamy, 1993; Norris, 1989).

1.1.1 Advantages:

Some of the essential advantages are:

- **Small size and flexibility -**The sensor consists of a light guiding core of fiber typically ranging from 3-1000μm in diameter and fibers of core diameter 50-200μm is normally used in chemical analysis. Also the plastic cladding material (silica fibers) of diameter 200μm can be bent round 1 cm radius mandrel. This flexibility enables optical fiber to be used in very small and otherwise inaccessible sensing areas (Norris, 1989).

- **Chemically and thermally stable -**Amorphous silica is the basic material composition of optical fibers. Amorphous silica is chemically inert which means it can be used in strongly acidic or moderately alkaline environments. However, it is still sensitive to strong acid HF and strong alkalines (pH >13). Heat resistance capability is high for both silica and glass material whereby pure silica softens above 500 $^{\circ}$C. But the thermal stability is limited for the surrounding polymer or the optical fibers that can only tolerate increase in temperature up to 125°C. However small developing polymers (polyamide) and metallic coatings would be able to perform at temperatures ≥ 400°C (Norris, 1989).

- **Electrically isolated and suitable for real-time monitoring, and for remote (hostile environment) *in-situ* measurement-** optical fiber sensors are non-electrical, hence are intrinsically safe and capable of operation in hostile (remote) environments (Norris, 1989; Narayanaswamy, 1993; Jackson, 1992).

- **Freedom from electromagnetic interference-** The glass fibers are insulators, hence are unaffected by the electromagnetic interference (EMI). Thus it can be used in electronically noisy environments (Norris, 1989; Narayanaswamy, 1993; Seitz, 1984; Jackson, 1992).

- **No reference sensor-** Unlike potentiometric sensors, they do not require a separate reference sensor. Referencing can be carried out optically and internally. This is performed most frequently by comparing the optical intensity at two different wavelengths, only one of which is affected by the analyte whilst both are dependent on the optical characteristics of the remainder of the optical system (Norris, 1989; Seitz, 1984)

- **Potential of distributed sensing-** The fiber optical chemical sensors can be designed to particular analyte sensitivity where the presence of that analyte modifies the optical properties, enabling a large area to be monitored. Likewise the sensitized fiber can also be interrogated by the optical equivalent of radar to give a measurement of the analyte concentration as a function of position over the extended area (Norris, 1989).

- **Multiwavelength Measurement-** Optical sensors provide multiwavelength and temporal information. For instance, the sensor can respond simultaneously to two or more analytes distinguished by measurements at two or more probe-detection wavelength concentrations. If the optical sensor involves luminescence, then time

- **Low loss of signals-** The cladding material of optical fiber behaves like a glass whereby the light passing through the cladding material of optical fiber undergoes total internal reflection without any distortion of the signals. These characteristics enable fiber optic to carry light long distance measurements with minimal transmission loss. This ensures good sensitivity to be maintained over long distances (Norris, 1989).

- **Reagent phase flexibility-** Since reagent phase is not physically in contact with the fiber optic, it can be changed easily for disposal. Also reagent phase can be in different environment from the fiber optic (Seitz, 1984).

- **Low Cost -** Fiber-optic sensors are also corrosion resistant and less expensive, has low mass of instrumentation and can be easily miniaturized (Narayanaswamy, 1993; Seitz, 1984).

1.1.2 Disadvantages:

- **Ambient light-** In fiber optic sensing, ambient (surrounding) light can interfere with the measurement of the optical signals. This can be alleviated by pulsing the interrogated light and using phase sensitive detection to remove background or basically using the sensor in a dark environment (Norris, 1989; Narayanaswamy, 1993; Seitz, 1984).

- **Response time-** As for all multi-phase chemical sensing techniques, the response time can be long. This is because the chemical indicator (reagent) and the analyte are in different phases. The delay would be due to mass transfer between the different phases that require chemical equilibrium to be established between the phases. This can be minimized by designing smaller probes that incorporates only thin fiber of the reagent phase (Norris, 1989; Seitz, 1984).

- **Long term stability-** Long-term stability could be a problem with optical sensors based on the use of indicator phases, as a result of photodegradation or leaching effects of the indicator reagents which often are organic in nature. Changes in the optical characteristics or source of detection, sensing transducers can also result due to fouling which limits the long-term system stability. This can be compensated by the use of ratio of optical signals of two-measurement wavelengths (Norris, 1989; Narayanaswamy, 1993; Seitz, 1984).

- **Limited dynamic range-** Sensors usually have a limited dynamic range of measurements. This range can be extended by the use of multiple sensors that can measure at different levels of the analyte. This however is not a big disadvantage in environmental work as most environmental parameters, such as pH, are well buffered within a given range (Norris, 1989; Narayanaswamy, 1993; Seitz, 1984).

- **Reagent phase-** For many types of optical measurements, the intensities observed are proportional to the amount of reagent phase. So if amount of reagent phase is small, intensities can still be increased by using intense probe radiation, but this will result in reagent photodegradation. Hence the amount of reagent phase and probe radiation affects long term stability of sensors (Narayanaswamy, 1993; Seitz, 1984).

1.2 Applications of Fiber Optic Chemical Sensors

Fiber-optic sensors offer the capability of continuous determination of chemical analytes in remote, inaccessible, hazardous, or in vivo environments. (Taib and Narayanaswamy, 1995; Norris, 1989). These sensors represent an emerging technology likely to have important applications in clinical diagnosis, pollution monitoring, and other fields (Cordek *et al*. 1999, Cheng and Chau, 2003 and Epstein *et al*. 2002). Many different analytes have been determined including ions, small molecules and macromolecules. FOCS's have also been successfully established for measuring pH in solutions, oxygen, carbon dioxide, nitrogen oxide, glucose, temperature, vapor of polar organic solvents, narcotics, organochlorine compounds, water contaminants, ammonia, moisture, copper and potassium ions. Many authors have outlined detailed reviews on their design features and the outcome of their applications for chemical analysis (Lee, 2003; Lee *et al*.2002; Lin, 2000; Wolfbeis, 2000; Wolfbeis, 2002; Posch *et al*.1988).

1.2.1 Ion Sensing

FOCS for detection of ions (other than H^+) have been the subject of interest for many researchers. The design of such a sensor involves immobilizing an indicator molecule that selectively binds the ion of interest at the tip of a fiber optic device. The sensors have been developed for determination of mainly cations with single as well as multiple charges.

The single-charged cations that were detected were Li^+, Na^+ K^+, Ag^+ and NH_4^+. Alder *et al*. (1987) constructed a potassium sensor utilizing a chromogenic crown ether, which responded reversibly to aqueous potassium ions in the concentration range 10^{-3}-10^{-1}M with a K^+/Na^+ selectivity ratio of 6.4. A new type of absorbance-based optical sensor,

with integrated waveguide absorbance optrode was designed by Puyol *et al*. (1999) which was combined with a potassium-selective bulk optrode.

Qin *et al*. (2002) developed a fiber-optic fluorescence sensor based on a controlled-release reagent for the determination of lithium ion in organic solvents. Narayanaswamy *et al*. (1988) have also reported a highly sensitive and reasonably selective, Alizarin Fluoride blue ternary complex method of determining single-charged anion, the fluoride ions.

The double-charged cations that were detected were Co^{2+}, Fe^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Mg^{2+} and Ca^{2+}. Zhang *et al*. (2002) synthesized a novel fluoroionophore for preparation of Hg^{2+}-sensitive fiber-optic chemical sensor for determination of Hg^{2+} in water samples. Chau and Porter, (1990) presented a calcium-selective optical sensor based on electrostatically immobilizing the calcichrome on a porous ion-exchange polymer film. The change in optical properties of the bound indicator as a function of Ca^{2+}concentration was monitored in a single-beam diffuse reflection mode. Suzuki *et al*. (1989) showed the preparation and response characterization of an optical sensor for detection of Mg^{2+} and Ca^{2+} using the natural carboxylic polyether antibiotic. It forms a complex with these two ions due to which a decrease in the fluorescence intensity was observed. For determination of copper in aqueous solution, Mahendra *et al*. (2003) developed a fiber-optic chemical sensor based on immobilizing copper (Cu^{2+}) specific reagent α-benzoinoxime on an Amberlite XAD-2 polymer series. The reagent reacted with copper to form a colored complex. Ueberfeld *et al*. (2002) chose the metallofluorochromic dye calcein as an indicator for detection of Cu^{2+} via fluorescence quenching system.

The triple-charged cations that were detected were Al^{3+}, Ga^{3+}, In^{3+} and Cr^{3+}. Oliveira and Narayanaswamy, (1992) immobilized dithizone on resins to develop a flow-cell optical sensor for lead. Saari and Seitz, (1983) prepared a sensor based on fluorescence for sensing of Al^{3+} by immobilizing morin on cellulose powder. They further used a similar system to design a sensor for detection of beryllium ions (Saari and Seitz, 1984). Szunerits and Walt, (2002) developed a fiber-optic imaging sensor with fluorescent dye morin which formed a chelate with Al^{3+} and helped in identifying means of corrosion at the aluminum surface. Carroll *et al*. (1989) constructed a time-resolved fluorescence based sensor for determination of Al^{3+} and Ga^{3+} or In^{3+}.

Certain researches have made successful attempts on developing optical ion sensors for depiction of multiple ions. Andres and Narayanaswamy, (1995) had immobilized urease on aminopropyl glass through different bifunctional coupling reagents (cyanuvic chloride, glutanaldehyde, hexamethylence diisocyanate, and phenylene diisothiocyanate) to compare the metal inhibition characteristic of these reagents. The inhibition studies were performed using a fiber optic biosensor configuration, wherein the pH change resulting from the biocatalytic hydrolysis of urea was compared before and after the exposure to the metal samples. The metal immobilization behavior differed for every different immobilized urease. Urease bound on cyanuvic support and urease bound on glutanaldehyde-activated support were shown to be more sensitive to the inhibitions of metal solutions tested (Hg^{2+}, Ag^{+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, and Co^{2+}). Urease bonded through hexamethylene diisocyanate coupling reagent had slightly lesser inhibition while urease bonded through phenylene diisocyanate showed excellent metal inhibition.

More recently, Kuswandi *et al*. (2001) described cation-selective optical sensors relying on the specific recognition of ions by synthetic or natural ion receptors. These sensors mainly consist of cation-selective receptors and hydrogen-selective chromophores co-

immobilized in plasticized poly (vinyl chloride) PVC membrane. Such sensors are based on ion exchange mechanisms, where transport of ions by the carrier into the PVC membrane is also coupled by a proton release by the protonated dye in order to maintain electroneutrality in the membrane. As the dye deprotonates, it undergoes a change in color or fluorescence property that is detected using fiber optics. By varying the ion phase and chromophore composition, optical sensing of different cations or organic cationic compounds can be achieved.

Bright *et al*. (1988) had constructed a fluorimetric ion sensor based on measurement of ion quenching or enhancement of the reagent Rhodamine 6G fluorescence entrapped on a Nafion film. It was found that ion such as Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, $Ni^{2+}$and NH_4^+ rapidly quench the reagent at an initial rate which also depends and determines the concentration of the ions. The quenching of the reagent is then rapidly reversed by added ions, H^+, Li^+, Na^+ K^+, Ca^{2+}, Mn^{2+}, Zn^{2+} and Mg^{2+} which gives the concentration of non-quenching ions. Egorov and Ruzicka, (1995) have utilized Cr^{4+} sensitive reagents that showed good response with increase in ion concentration.

1.2.2 Gas Sensing

Fiber optic chemical sensors have also been reported for gas-sensing, particularly for oxygen, carbon-dioxide and ammonia.

Oxygen

FOCS for measurement of oxygen are based on fluorescence quenching of an immobilized fluorophore that is either trapped within or positioned behind a gas-permeable barrier (Rharbi *et al*, 1999). Arnold, (1992) has discussed a series of

quenching agents and their limits of detection of oxygen. Hauser and Tan (1993) employed oxygen determination membrane on a fluorescence-based fiber-optic chemical sensor.

Carbon-dioxide

The principle of operation for carbon-dioxide sensors is analogous to that of a CO_2 electrode. Jennifer *et al*. (1993) discussed the means of determining the concentration of CO_2 in relation to H^+ concentration governed by the Henderson-Hasselbalch model. Munkholm *et al*. (1988) further converted a fibre-optic pH sensor into a carbon dioxide sensor by introducing a semipermeable membrane that excludes protons and only allows CO_2 to enter the pH sensing region, where it reacts with water in hydrophilic microenvironment of the dye containing polymer matrix, changing the pH and thereby altering the fluorescence intensity.

Ammonia

FOCS for this can be constructed based on the entrapment of an appropriate indicator solution (Rhines and Arnold, 1988). It simply consists of a thin-layer of a pH indicator and an ammonium chloride solution, entrapped between a microporous teflon membrane and an optical fiber probe. Ammonia gas formed from enzyme-catalyzed reactions can diffuse across the membrane, enter the internal solution and change the pH of the ammonium chloride buffer (Kuswandi *et al*. 2001; Kar and Arnold, 1993). The resulting change in pH is reflected as a change in the absorbance of fluorescence of the dye, which is monitored using optical fibers. Baxter *et al*. (1994) have utilized an integrated fiber-optic detection system for analysis of ammonia. This system combines the advantages of gas diffusion and stopped flow, making the overall assay very sensitive.

1.2.3 Water Analysis

In addition to the possibility of measuring various inorganic analytes such as cations and anions which has already been described in an earlier section, various workers have reported the potential for monitoring organic contaminants in water samples. Chudyk *et al*. (1985) carried out remote UV-laser-induced fluorescence measurements on model and actual contaminated groundwater samples at instrument/analyte distances from 1-25 m. The analyzed contaminates were mainly phenol, *o*-cresol, toluene, xylenes at a detection level of 1 ppb. These analyzed contaminants are important components of gasoline and humic acid.

1.2.4 Clinical and Biomedical field

The determination of metabolites in blood, urine, and other biological fluids is essential for assessment of metabolic conditions and treatment of disease. Fiber-optic biosensors are perceived as a good means of analysis for this with rapid response times, miniature size that allows penetration into bodily tissues, and their non-electric nature which renders its safe when in contact with the body.

A fluorescence fiber-optic urea sensor based on the use of trisodium 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) as the indicator was tested for the determination of urea concentration in human serum (Xie *et al*. 1991). A fiber-optic biosensor for uric acid is constructed by co-immobilizing uricase and horseradish perioxidase on to borine albumim via glutaraldehyde.

Together with sensors for determination of penicillin, creatinine and glucose, biosensor for analyzing *L*-lactate is important in the diagnosis of respiratory insufficiencies, heart diseases and also in sport medicine (Kulp *et al*. 1987; Dempsey and Wang, 1993).

Peterson *et al*. (1984) described a fiber-optic probe for in vivo measurement of oxygen partial pressure of blood.

1.2.5 Food Analysis

To maintain good quality food, it is important to analyze the different substances present in food samples. Several enzyme-based biosensors for this have been reported. Sulfite in different food sample (potato flakes, red wine, and beer) was determined using sulfite oxidase and perioxidase membranes. Biosensors based on oxygen transducers have also been used for analyzing sulfite and phenols in tea. Xie *et al*. (1994) constructed a sulfite biosensor for determination of sulfite contents of various food samples e.g. dried fruits, potato flakes and lemon juice which was based on the enzymatic oxidation reaction of sulfite catalyzed by sulfite oxidase (SOD).

Flow-injection systems based on chemiluminescence measurements and enzyme reactors have also been applied for determination of ethanol in wine and glucose in fruit juice. The biosensor integrated with a flow-injection assembly has been used for analysis of milk products, ascorbic acid in fruit juices, lactic acid in milk products, protein (Flora and Brennan, 1999) and glutamate in flavor enhancer products (Kuswandi *et al*. 2001).

1.2.6 pH Sensing

The measurement and control of pH is required in practically all fields including chemical, biomedical and environmental science. The Fiber-optic pH sensor was mainly utilized for monitoring blood pH and study brain tissues (Peterson *et al*. 1980; Grant and Glass, 1997; Grant *et al* 2001). Noui *et al*. (1998) described optical pH sensor that can be applied for study of fermentation processes of biochemical monitoring system. The work done on the development of optical pH sensor and its applications are discussed in detail in section on literature review.

1.3 Research Needs and Potential for pH Sensing in Soils

The soil pH is arguably one of the most important properties controlling a soil's behavior including those related to plant growth. The availability of plant nutrients, activities and nature of microbial populations, solubility and fate of toxic additives, soil corrositivity and activities of certain pesticides are all influenced by soil pH (Donahue *et al*. 1983; Tan, 1982; Townsend, 1973; Batjes, 1995 and Adams *et al*. 2000). Methods currently employed for measuring soil pH include either pH meters or more broad range measurements based on colorimetric reagents (Simpon, 1983; Oliver *et al*., 1998; Helyar *et al*., 1990; Clarke, 1971; Faniran and Areola, 1978; Hausenbuller, 1975; and Strong, 1997). The first involves preparing a soil solution of ratio close to 1:1 (soil: water or $CaCl_2$) and the second involves making a paste of the soil with the colorimetric reagent but both require removal of the soil from the field for measurement. In addition, the second method is very sensitive to extraneous dust, finger perspiring and other hindrance during sampling that makes it less appropriate for real-time analysis. Also, measuring the pH of the soil when in contact with a substantial amount of solution determines only the amount of H^+ ion around the colloidal that dissociates in water. This measured value doesn't truly correspond to the actual pH, as it is dependent on the equilibrium established between the phases and the dissociation constant of the sorbed hydrogen ion.

Soil pH is also an intensive factor that can vary over relatively short time and spatial scales and this can have important implications for the mobility of substances such as plant nutrients and soil contaminants. Existing methods of pH measurement do not allow assessment of pH variations on these scales as they require substantial amounts of soils to be removed from the field and result in major alterations to the soil configuration. Significant inroads can be made in understanding the dynamics of nutrients and other substances' mobility in soils if continuous real-time monitoring of soil pH were to be possible. Fiber optic sensors offer great potential in this regard.

Fiber-optic sensors are flexibile and can be miniaturized which would allow interrogation of various areas of the soil in the field, for example the root zone of a plant, on a very small scale (Motellier *et al*., 1995). The small size of the probe could also mean that the moisture already present in the soil might be sufficient for the sensor to be able to signal pH and so no further alterations to the soil configuration would be necessary. In spite of the great advantages that can be obtained by applying the fiber optic approach to soil pH sensing, very little research on this is evident in the literature. In this study, the potential for this application is investigated.

2.0 Literature Review

Using Fiber Optical Sensors for pH Sensing

The measurement of pH is essential basically for all scientific study including environmental and clinical studies. As already stated in a previous section, the most widely used method is based on electrochemical principles but optical based methods are being reported that offer significant advantages over electrochemical methods.

2.1 The aspects of a Fiber Optic Chemical Sensor in pH sensing

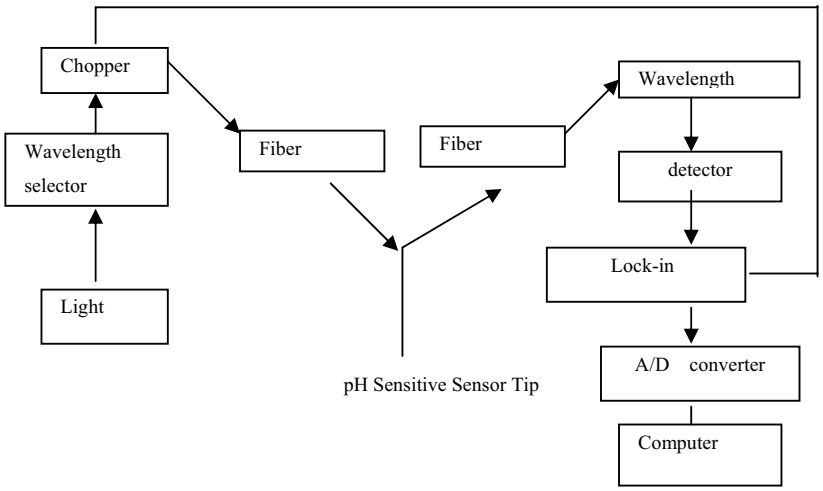
A chemical sensor is a device that can be used to determine the detectable presence, the concentration or activity of a chemical species in a sample of interest. In the chemical sensors designed for pH sensing, light from a suitable source is instigated into the optical fiber and directed to the sensing region where it interacts with the pH sensitive chemical transducer. The most commonly used interactions are based on selective binding or complexation reaction. The extent to which the H^+ ions interact with the chemical recognition phase determines the magnitude of the signal. The modulations in the reflected optical signal in correspondence to the optical changes of the transducer are characterized by a photodetector and the output defines the response (Narayanaswamy, 1993).

2.2 Instrumentation

The equipment associated with fiber optical pH sensors is one or more optical fibers as the light guides, a light source, a transducer to translate the concentration of the H^+ ion species into measurable modulated light component, modulator, signal couplers, photodetector and a detector output system (Norris, 1989; Seitz, 1984; Kuswandi *et al*., 2001; Taib and Narayanaswamy, 1995; Narayanaswamy, 1993).

The following diagram outlines the basic components of the fiber-optic pH sensor;

Fig 2a: **Instrumental Components required for a FOCS**



2.2.1 Optical fiber

Structure of Fibers

An optical fiber consists of an inner core of light-conducting material and a surrounding cladding with a refractive index which must be less than that of the core. This arrangement enables total internal reflection of light signals at the core-cladding interface, allowing the light to propagate along the length of the fiber (Oscroft, 1992; Seitz, 1984).

Types of optical fiber

- ***Bifurcated optical fiber:***

  In such optical fibers, separate fibers are engaged to transmit incident and detected optical radiation. A bifurcated bundle of optical fibers can be used for immobilizing the indicator chemistry at the tip of the common end. Large bundles are used to bring light to this sensing region and to carry the light to the detector optics.

- ***Single optical fiber***: For such fibers it is necessary to distinguish between the incident and the detected radiation. When the indicator chemistry is immobilized at the distal tip of the single fiber, the incident radiation enters the fiber at one end and then travels the length to the distal end to the indicator. Together with exciting the fiber, incident radiation also excites the indicator. The resulting portion of luminescence is collected by the same optical fiber which then travels the length of the fiber to the detector optics.

2.2.2 Chemical Transducer

Chemical analysis using fiber-optic chemical sensing is mainly dependent on the ease and the manner in which the chemical transduction can be designed and interfaced with fiber optics. Most commonly used chemical transducer consists of immobilized chemical reagents or pH indicators, which are mostly analyte specific and are usually in solid phase for convenient handling. The reagent phase is localized in the sensing region of the optical fiber by either of the two ways; direct deposition on the fiber or encapsulation with a polymeric membrane (Badini *et al*., 1996; Narayanaswamy, 1993 and Hauptmann, 1991).

2.2.3 Source of light

The source of illumination should be able to provide an intense and stable incident radiation. The types of light sources that can be employed in optical sensors are as follows;

- *Incandescent Lamps*- These emit a broad spectrum of optical radiation and are mostly used as sources of ultraviolet and visible light in short-range optical fiber sensors. Some of these lamps include tungsten lamps and quartz-halogen lamps.

- *Gas Lasers*- These are useful general-purpose sources of highly intense and coherent radiation and are mostly used for long-distance (remote) sensing.

- *LEDs*- The light-emitting diodes (LEDs) are miniature sources of high-intensity monochromatic radiation. It produces incoherent light with a spectral bandwidth of 40-50nm. These are mostly useful in short range fiber optic sensing (1km).

- *Semiconductor Injection Lasers*- These radiate a coherent beam of light with narrower spectral bandwidth (5-10nm) which enables it to be an excellent source for transmission of light in fibers of greater length.

2.2.4 Monochromators

Before the light is led to the photodetector, wavelengths other than that relating to the species have to be excluded. Monochromators are used for this purpose. It offers high efficiency and can be adapted for different wavelengths. Monochromators consist of entrance and exit slits, a prism or diffraction grating and lenses or mirrors to collimate or focus radiation. The essential feature of monochromators is that it converts polychromatic radiation from the light source into monochromatic radiation.

2.2.5 Optical couplers

Optical couplers are basically needed to focus the light beam to the optical fiber and to direct radiation from the return fiber to the photodetector.

2.2.6 Modulator

Ambient light can interfere with the measurement by gaining entrance into the sensing region of the optical fiber. This interference can be eliminated by modulating the light

source so that optical signals derived from it can be differentiated from the extraneous radiation.

2.2.7 Photodetector

A photodetector system is a photo-counting device that converts optical signals into electrical signals, which can easily be amplified by electronic means. There are various types of photodetectors that have been incorporated for optical chemical sensing. These include photomultiplier tubes, positive intrinsic negative (PIN) photodiodes, photodiode arrays and avalanche photodiodes.

2.2.8 Detector

Light detection is performed with either a solid-state diode or a photomultiplier tube (PMT) in combination with a computer.

2.3 Indicator dyes

Organic indicator dyes which have routinely been used previously in acid-base titrations and pH indicator papers can now be utilized to provide pH-dependent spectral properties in fiber-optic sensing techniques. The optical property measured (reflectance, absorbance or fluorescence) depends on the configuration of the device and nature of the indicator. The pKa of the immobilized indicator is the primary factor to consider when identifying a suitable indicator for a particular application.

The indicator dye molecules that are used for optical measurement of pH are mostly weak acids or bases. The physical and chemical properties of the surface on which the indicator is immobilized can strongly influence the apparent acid-base properties of the indicator. The loss or gain of a proton changes the electronic structure of the molecule which modifies the manner in which molecules interact with light. On gain or loss of protons,

indicators change their acidic and basic forms, which changes the color for each form whether as free in solution or chemically bound to a supporting membrane. The equilibrium achieved on proton change leading to the acid and base forms of the dye can be described as:

$$Dye^{Z} + H^{+} \overset{K_a}{\leftrightarrow} Hdye$$

$$K_a = \frac{a_{H^+} \cdot a_{Dye^z}}{a_{Hdye}}$$ where Z = valence of molecule, a= activity

The optical measurement is based on the color change of the indicator dye with pH. The sensitivity of measurement is greatest at the equilibrium where both acid and base forms of indicator are present in equal concentrations and where the pH of the solution is equal to the pKa of the indicator dye (Sheppard and Guiseppi-Elie, 1999).

The pKa of the immobilized indicator can be quite different than that of the indicator in solution. The apparent pKa is defined as the pH where response is half way between the minimum and maximum values (Jin *et al*., 2000). In addition, the effective pKa is also dependent on ionic strength and temperature. Accurate measurements demand equivalent ionic strength and temperature for the standards and the sample solutions.

Baker and Narayanaswamy (1995) investigated the influences of the support matrix [Dowex-4, IR-45, Amberlite IR4-400] on 3,4,5,6 tetrabromophenolsulfonephthalein (TBPSP) which was immobilized by electrostatic attraction and XAD-2, XAD-4, XAD-7 (immobilizing by adsorption). They also discussed the impact of the immobilized indicator concentration and the ionic strength on the pH response.

The electronic properties of the support matrix increases the pKa of the immobilized indicator for electron donating matrices such as XAD-2, XAD-4 and XAD-7 with

indicator immobilized via adsorption, while pKa decreases for electron withdrawing matrices such as anion exchange resins Dowex-4, Amberlite IR-45 and Amberlite IRA-400 on which indicator was electrostatically immobilised. The increase in pKa was explained in terms of the formation of π - π electron donor-acceptor complexes between the XAD (hydrophobic styrene/divynl benzene copolymers) matrix as the donor and bromine substituted phenyl ring of the indicator as the acceptor. Donation of π electrons from resins causes a destabilization effect on the phenolate anion of the indicator.

The decrease in pKa for electron withdrawing resins were explained in terms of the attraction of sulphonate negative charge in the ion-exchange group present on the styrene/divinylbenzene of resin that stabilizes the phenolate anion of 3,4,5,6 tetrabromophenolsulfonephthalein (TBPSP) by allowing better distribution of phenolate negative charge.

Increase in the concentration of indicator loading on resins has shown a decrease in pKa value of indicator because the electronic microenvironment of the resin remains largely unchanged but the electron withdrawing influence of the matrix increases with more of anion exchange sites occupied by indicator molecular which leads to increase in pH sensitivity of the immobilized reagent phase. For 3,4,5,6 tetrabromophenolsulfonephthalein (TBPSP) immobilized on Amberlite IRA-400 resin matrix, an increase in ionic strength increased the pKa of the indicator.

Motellier *et al.* (1995) also explained that an increase in pressure causes the resins to shrink, which, in turn, increases the charge density of the indicator-resin system hence consequently increasing the apparent pKa. John Peterson (1980) and Edmonds *et al* (1988) also elaborated on the principles of the pKa of the bound dyes.

2.4 Immobilization

Immobilization of chemical reagents can be carried out either chemically or physically. Physical methods are based on gel entrapment, adsorption and electrostatic attraction in use with simple economical procedures. Chemical methods are based on formation of covalent bond between the reagent molecule and an activated or functionalized form of the polymeric solid support (Narayanaswamy, 1993; Dybko *et al.,* 1997; Kuswandi *et al*., 2001).

2.5 Optical Signals

Optical sensor tactics are based on changes in optical properties through absorption, emission, fluorescence and reflection or scattering of light. The changes in the optical properties will be reflected in the modulation of the following properties; wavelength (colour), amplitude, phase, or polarization (Taib and Narayanaswamy, 1995; Narayanaswamy, 1993; Norris, 1989; Seitz, 1984; and Kuswandi *et al*., 2001).

2.6 Sensor response chemistry

The optical chemical response function depends on the manner in which the analyte interacts with the reagent phase (indicator) with the chemical transducer (Seitz, 1984; Narayanaswamy, 1993 and Norris, 1989). If a reagent (R) is reacting with the analyte species (A) forming a produce (AR), the equilibrium established between the analyte and the immobilized reagent at the 1:1 stoichiometery reaction can be represented as:

$$A + R \leftrightarrow AR$$

The component R or AR is usually absorbing or luminescent and can be therefore measured optically. The chemical transduction is mostly based on the equilibrium established in between which describes the equilibrium constant K_e as:

$$K_e = \frac{[AR]}{[A][R]}$$

Note: the square brackets indicate equilibrium concentration of the species involved.

As the chemical reaction proceeds, R is consumed resulting in the production of AR. Hence as more R is consumed, more AR is produced. This signifies that as the reaction proceeds, decrease in R will result in decrease in absorption or luminescence due to R. Moreover as more R will be consumed, more AR is produced which results in increase in absorption or luminescence due to AR. Either way these changes in optical properties can be related to the concentration of R or AR which in turn can be related to concentration of analyte A causing changes in the measured optical property.

So if R is consumed, its total initial concentration (C_R) is given by

$$C_R = [AR] + [R]$$

Now if the optical property of R is measured, the analyte concentration related to the concentration of R can be expressed as;

$$[A] = \frac{1}{k_e}\left[\frac{C_R}{[R]} - 1\right]$$

A more linear relationship of [R] with respect to [A] can also be derived from equation.

$$\frac{C_R}{[R]} = 1 + K_e[A]$$

A plot of C_R / [R] vs. [A] give a linear graph with y intercept as 1.

Ratio relationship

Another approach of relative [AR], [R], to [A] is to take the ratio

$$\frac{[AR]}{[R]} = K_e[A]$$

The ratio is directly proportional to [A] and is also not dependent on, thus insensitive to slow loss of reagent. This approach has an additional advantage since it is less sensitive to instrumental fluctuations. However the limitations of the approach are that AR, R should be present in sufficient amounts to be measured with adequate precision, hence the dynamic range is limited.

2.7 Response Time

The response time is defined as the time required for 95% of the total signal change (Safavi and Bagheri, 2003; Kostov et al, 1993). The limiting factor for the response time is the rate of diffusion of the protons into the core of the resin. The rate of diffusion of protons also depends on certain factors such as; particle size (size of the resins), quantity of the matrix at the sensing tip, indicator concentration, direction and magnitude of the pH changes, shape of the sensor tip, solution temperature, ionic strength of the analyte solution, indicator dynamic range and the pressure applied to shrink the resins at the sensing tip (Rayss and Sudolski, 2002; Baker and Narayanaswamy, 1995; Alabbas *et al*., 1996; Fuh *et al*., 1987).

2.7.1 Particle size

Sensors with larger particles have shorter response times. The difference may be connected with the fact that the pore size between larger particles is greater than between the small particles and the external surface /volume ratio is on the other hand larger for smaller particles.

2.7.2 Quantity of the matrix effect

As the thickness of the sensing layer increases, the response time also increases (Dybko *et al*., 1997; Zhujun and Seitz, 1986 and Xie *et al*., 1991).

2.7.3 Indicator Concentration

The sensor response time increases with increases in the indicator concentration on the membrane via immobilization (Xie *et al*., 1991). Dybko *et al*. (1993) illustrated that higher concentration of Bromothymol blue shifts the pH range to lower pH values and the response time of going from alkali to acidic medium is less than going in reverse.

2.7.4 Direction and Magnitude of pH changes

When transferring the probe from one pH solution to another, the sensor response time may depend on the pH difference between the two solutions. When going from lower pH to higher values, the response time increases. The bigger the difference, greater is the response time. Hence, the response time of the sensor is a function of both the magnitude and the direction of pH change (Lee *et al*., 2001).

2.7.5 Shape of the Sensor tip

Alabbas *et al*. (1996) derived an equation based on thickness of membrane with respect to proton transfer to explain that a cylindrical tip-shaped sensor takes roughly five times longer to reach steady state than a sensor with a hemispherical tip.

Cylindrical tip

Hemispherical tip

2.7.6 Solution Temperature

The response time of the sensor decreases with increase in temperature probably due to the increase rate of diffusion of the analyte with increase in temperature (Kirkbright *et al*, 1984; Zhujun and Seitz, 1986).

2.7.7 Ionic Strength

With the increase in the ionic strength of the analyte solution, the response time decreases (Suah *et al*., 2003; Kirkbright *et al*, 1984; Jaanta, 1987, Christian and Seitz, 1988).

2.7.8 Indicator Dynamic Range

The response time is shorter at the extremes of the dynamic range i.e. if for analysis for pH (2-7), between pH (2-3) and pH (6-7), it takes the sensor shorter time for equilibration while in the mid-pH range of pH 3-4, pH 4-5, pH 5-6 (more sensitive), it takes it longer response time. A variation in the relative concentrations of the components of the indicator system varies the effective equilibration constant and thus can change the range of analyte concentration sensed by the indicator (Dybko *et al*. 1993).

2.8 Sensor Optimization

Much of the work reported in the literature on fiber optic pH sensing is on optimization of the designs. Many authors have showed means of improving the response times and maintaining the life-time of the probes.

Peterson *et al.* (1980) developed the first fiber optic absorbance-based sensor for monitoring pH of blood over the physiological pH range of 7.0-7.4. The earliest pH sensor based on fluorescence was reported by Saari and Seitz, (1982). Although fluorescence-based sensors have employed increase preference to absorption-based sensors because of increased sensitivity, greater latitude in geometric design, wide dynamic range, wavelength sensitivity, and linearity at low concentration; it still possesses some difficulties in practical application. One of these difficult is the "inner filter effects" resulting from absorption of the excitation radiation by various species in solution or matrix analyzes (primary inner filter effect), or absorption of the emitted fluorescence radiation by these same competitive species (secondary inner filter effect). Correction for the effect is restricted. Jordon and Walt (1987) explored the possibility of linking an absorber's specificity for a particular analyte to a fluorescent signal.

Fuh *et al*. (1987) observed that the bifurcated fiber optic system had greater response times, owing to the requirement that the analyte diffuse across the membrane and into a layer of immobilized reagent. In addition, bifurcated optics is less efficient than single optical fiber as large volume being excited and the emission observation regions do not coincide.

For an optical fiber to serve as a sensor, the reagent phase must be in the vicinity of the distal end of the fiber. Mere attachment of a fluorescent compound to the surface of optical fiber results in no detectable signal due to limited surface area available. Munkholm and David (1986) suggested the use of polymers which can serve to increase the surface area and result in multiple sites of attachment for the fluorescence species. Jin *et al.* (2000) developed a stable polyaniline for use in optical pH sensors that was suitable for pH (2-12).

Lobnik *et al.* (1998) compared two techniques of immobilization: covalent linkage of the dye to a suitable matrix and the chemical doping method. Covalent binding is said to be firmly bound to the matrix without any leach, and longer-term stability is unsurpassed. For relatively small and robust probe molecules, covalent linkage is often used because of its simplicity; however the immobilization event often pacifies complex probe molecules. Entrapment within, or to, a semi-permeable membrane, or in a sol-gel matrix has been used to immobilize complex probe molecules. But without careful storage, the sol-gel matrix shrinks, leading to cracks and eventually leakage of probe matrix into measured solution. With concept Bobbitt and Fry (2001) tried to show that the hydrophobic surface is immune to hydrolysis and the immobilization of hydrophobic derivatives of fluorescein, a pH-sensitive dye as mentioned above, potentially allows pH sensing at the extremes of pH scale.

The following tables give an indication of the work done so far on pH sensing. It summaries the type of indicators used, the type of membrane the indicators were immobilized on, the method of immobilization, the optical signal used, the pH range and the response time, the concepts discussed on optimizing the techniques or any field of application and the respective references for all the work done.

Table 2a) Fiber-optic pH Sensors with pH-sensitive indicator dye-based transducers.

	Immobilization Membrane	**Immobilization Method**	**Optical signal and response time and pH range**	**Concept and application**	**Reference**
Bromocresol green	Sol-gel encapsulation	Bromocresol green was impregenated in sol-gel films	Absorbance	The pKa of BCG in solution was estimated as 4.96 and the absorbance of protonated/deprotonated form of indicator was around 444nm and 614nm respectively	Ismail *et al*, 2002
Bromothymol Blue	Amberlite XAD-2 resin (polystyrene-divinyl benzene copolymer	Bromothymol blue was adsorbed on the XAD-2 resin and the coplymer was retained on the sensing tip using membrane polytetrafluoroethylene (PTFE)	pH 7-12 Reflectance	Probe lifetime and probes response time dependence on temperature solution ionic strength and indicator concentration was discussed	Kirkbright *et al*, 1984
Bromothymol Blue	Amberlite XAD-2 resin (polystyrene-divinyl benzene copolymer	Bromothymol blue was adsorbed on the XAD-2 resin and the coplymer was retained on the sensing tip using membrane polytetrafluoroethylene (PTFE)	pH 6-10 reflectance based with LED	The pH range of common indicators has been specified. Bromocresol purple has range 5-7. Bromothymol blue has range 7-9.	Kirkbright *et al*, 1984
Bromothymol Blue	Amberlite XAD-2 resin (polystyrene-divinyl benzene copolymer	BTB was physically adsorbed on the Amberlite XAD-2 resin	Reflectance	Showed application of Kubelka -Munk Theory to a pH sensor	Guthrie *et al*,1988
Bromothymol Blue	Tetramethoxysilane (TMOS) doped with Eu+3complex	Sol-gel route	pH 5-9 fluorescence	Thick layers of TMOS were prone to cracking when coated shortly before gelation	Lobnik *et al*, 2001
Bromothymol Blue	Ion-exchange XAD-1180 resin	The surface of optical fiber was treated with polyvinyl chloride (PVC) in rectified tetrahydrofuran (THF). The PVC coat was inserted into Amberlite XAD-1180 powder and then dipped in BTB	pH 7.7-9.4 Reflectance	The higher concentration of BTB shifts the pH range to lower pH values. The response time of going from alkali to acidic medium is less than going in reverse.	Dybko *et al*, 1993
Methyl red	Cladding material of silica optical fiber	Methyl red doped in poly(methyl methacrylate) (PMMA) was coated on the naked core of the fiber	pH 5.0-7.0 Absorbance	High concentrations of dye does not permit to satisfy internal total reflection index hence thickness of film and dye concentration is important for complete absorption	Egami *et al*, 1996

Cont'd Table 2a)Fiber-optic pH Sensors with pH-sensitive indicator dye-based transducer

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Phenol Red	Polyacrylamide microspheres	The polyacrylamide microspheres provided an open hydrophilic gel structure for ionic diffusion of covalently bound phenol red dye	pH 7-7.4 Absorption	Concept: Phenol red was chosen because its pKa was appropriate and it's not toxicologically hazardous. Principles of pH measurement and pKa of bound dye was well elaborated. Performance of probe with respect to response time, temperature coefficient, solution ionic strength coefficient, effective path length and stability of probe was studied. Application: The very first designed Fiber-optic pH sensing probe was utilized to test blood pH	Peterson *et al*, 1980
Phenol Red	Amberlite XAD-4 resin	0.2% Phenol red prepared in ethanol was physically absorbed on the XAD-4 resin	pH 7-10 Absorption Retention time 3-5min	Concept: Use of small beads may limit the diffusion layer thickness when soaking the bead of resin in the indicator solution, thus reducing the equilibration time of the probe. The influence of organic matter, ionic strength and pressure on signal response time was discussed.	Motellier *et al*,1995
Phenol Red	Agarose polysaccharide material	Phenol red was entrapped in the agarose gel matrix	pH 6-8 Absorbance	Severe leaching of phenol red was controlled by further coating with cellulose acetate membrane and storing the probe in 0.01M NaOH with similar concentration of phenol red as in matrix	Hao *et al*, 1993
Phenol Red	The silanized fiber	The polymerized medium of acrylamide solution, N,N'-methylenebis-(acrylamide)(BIS) solution and phenol red was covalently attached on the silanized fiber tip.	pH 4.49-8.60 Fluorescence Response time 0.3secs	The new approach of designing a sensor based on interaction between two moieties species where one pH-insensitive species acts as the donor transferring energy to pH-sensitive absorber.	Jordon and Walt David, 1987
Phenol Red	The sensor was developed to determine pH of solution containing phenol red.	The indicator wasn't immobilized but used in solution form in pH buffers.	pH 6.8-9.7. Absorbance	Phenol red existed in two tautomeric forms absorbed in pH range 6.8-9.7	Benaim *et al*,1986
Bromophenol Blue (BPB)	Sol-gel matrix with tetraetylortho-silicate	BPB was coated on the sol-gel film	pH 2-12 Absorbance	The artificial neural network approach shows ability to predict the response of sensor with minimum error	Suah *et al*, 2003
Bromophenol Blue (BPB)	Sol-gel film of tetramethylorthosilicate (TMOS)	BPB was entrapped in the sol-gel film	pH 3-8	The developed probe Can be applied for study of fermentation processes of biochemical monitoring system	Noui *et al*, 1998

Cont'd Table 2a)Fiber-optic pH Sensors with pH-sensitive indicator dye-based transducer

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Bromophenol Blue (BPB)	Amberlite XAD-7	BPB was physically adsorbed on hydrophobic organic polymer amberlite XAD-7	pH 2-12 reflectance	The limitation of artificial neural network approach is that such study is on long network training time.	Suah *et al*, 2003
Bromocresol purple, bromocresol green bromophenol blue, bromothymol blue cresol red, methyl red and phenol red	The indicators prepared in dilute sodium hydroxide were added in pH buffers	The indicator wasn't immobilized but used in solution form in pH buffers.	Absorbance	The pK_a of all the indicators in solution was determined. The pKa of BCG is 4.7, BCP is 6.2, BTB is 6.8, methyl red is 4.9 and phenol red is 7.7	Edmonds *et al*, 1988
Phenol red, cresol red and bromophenol blue	Unclad portion of fiber	A thin porous film of glass with dye was entrapped on unclad portion of fiber using sol-gel technology	Evanescent wave absorption pH 4-11	Phenol red showed response from pH 7-12.Cresol red showed response from 6.5-11.0.Bromophenol blue had pH range 4-7.5	Gupta and Sharma, 1997
Thymol blue Methyl orange bromophenol blue Bromocresol green, Methyl red, Chlorophenol red Bromocresol purple Bromothymol blue Phenol red, cresol red phenolphthalein Thymolphthalein	The absorption of a solution of dye at number of different pH was absorbed	The indicator wasn't immobilized but used in solution form in pH buffers.	Absorbance	The pKa and wavelengths in acidic/basic form of bromocresol green, bromocresol purple, bromothymol blue, methyl red and phenol red in solution are as 4.7(444nm, 617nm),6.3(433nm,591nm),7.1(433nm,617nm),5.0(530nm, 427nm) and 7.9 (433nm, 558nm) respectively.	Sheppard and Guiseppi-Elie, 1999
Cresol red, bromophenol blue and chlorophenol red	Unclad portion of fiber	A thin porous film of TEOS with dye was entrapped on side-polished unclad portion of fiber using sol-gel technology	pH3-14 Evanescent wave absorption	To make the probe less fragile the single mode fiber was fixed in a groove fabricated on the surface of the quartz block.	Sharma and Gupta, 2003
Bromothymol Blue and azo dye	Hyrogel matrix	The pH indicator dyes were co-immobilized with the ruthenium(II) metal complex on a hydrogel matrix	pH7-9 Luminescent	The very first luminescent based pH was designed by use of two indicators. The response time of going from basic-acidic condition was greater than in reverse	Kosch *et al*, 1998

Cont'd Table 2a)Fiber-optic pH Sensors with pH-sensitive indicator dye-based transducer

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Congo red and azo dye	Cellulose acetate film	The dyes were adsorbed on the cellulose-based polymers	pH 2.0-10.9 absorbance	The acidic strength of the dye increased after immobilization	Jones and Porter, 1988
cresol red, chlorophenol red and bromophenol blue	Unclad portion of fiber	A thin porous film of glass with dye was entrapped on unclad portion of fiber using sol-gel technology	Evanescent wave absorption pH 4.5-13.0	Sol-gel films have more resistant than polymer films in aggressive environments	Gupta and Sharma, 1998
cresol red, chlorophenol red and bromophenol blue	Unclad U-shaped fiber optic probe	A thin porous film of glass with dye was entrapped on unclad portion of U-shaped fiber using sol-gel technology	Evanescent wave absorption pH 4.0-13.0 response time =15sec	It was observed also that for a given pH, as the bending radius of the U-shaped probe decrease, the sensitive increases.	Gupta and Sharma, 2002
Thymol Blue and Bromophenol Blue	Amberlite XAD microspheres based on methods described Peterson(1980) and Kirkbright *et al*, (1984)	BlueOptrode built with Thymol Blue and Bromophenol that was dissolved in 0.1% alcohol solution and immobilized on Amberlite XAD microspheres.	Absorbance pH 0.8-3.2, pH 10-13 and pH 3.2-7.0 for respective dye. Response time was 30secs.	The aspects of utilizing optical fibers for remote on-line process controls are discussed.	Boisde *et al*, 1988
Bromothymol blue and bromophenol blue	Amberlite XAD-2 resin (polystyrene-divinyl benzene copolymer	Bromophenol red, Bromothymol blue was adsorbed on the XAD-2 resin and the coplymer was retained on the sensing tip using membrane polytetrafluoroethylene (PTFE)	Diffuse Reflectance spectrometry	Factors such as matrix quantity, indicator solution concentration, direction and magnitude of pH change, shape of sensor tip, temperature of analyte solution and transition intervals of indicator relative to pH of the solution that affects the response time of optical fiber pH sensor was discussed.	Alabbas *et al*,1996
Bromocresol purple (BCP) and bromocresol green (BCG)	Unclad portion of multi-mode optical fiber	BCP and BCG were directly coated on the cladding core of the optical fiber by sol-gel route.	Evanescent Wave Absorption spectrometry Response time from low to high pH was 5secs, in reverse was 30secs	pKa of BCP shifts after immobilization from 6 in solution to 9 in sol-gel matrix, pKa of BCG in solution is 4.4 and in sol gel its 8. Likewise the pH range of BCP in solution is 5.2-5.8 but in sol-gel, its 7.5-10.5, the range of BCG in solution is 3.6-5.2 but in gel, its 6.5-9.5. Insufficient film thickness gives poor sensitivity, but thick layer cracks .The response time has an adverse effect by increase the number of film layer. Response time of going from less pH(acidic) to higher pH(alkaline) is less than as when going in reverse.	S.Thomas Lee *et al*, 2001

Table 2b) Fiber-optic pH Sensors with pH-sensitive reagent- based transducers

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Fluoresceinamine	Controlled pore glass and cellulose	Fluoresceinamine in borate buffer of pH 8.5 was bound to oxidized glass and with cellulose	pH 3-8 fluorescence Response Time (15-30secs)	In solution, fluorescein behaves similarly to the glass-bound fluoresceinamine as signal increases significantly from pH3 to 6 and then slightly increases above pH8. Above pH8 cellulose-bound fluoresceinamine response decreases which was caused by hydrolysis of immobilized fluoresceinamine	Linda Saari, 1982
Fluoresceinamine	Acrylamide methylenebis (acrylamide) copolymer	Fluroesceinamine was incorporated into an Acrylamide-methylenebis (acrylamide) copolymer that was covalently attached to a glass fiber via thermal or photopolymerization.	pH 4-8 Fluorescence Response time 9secs	The disadvantage of polymer-modified fiber is lack of reproducibility due to difficulty of removing fiber from bulk polymer	Munkholm and Walt, 1986
Fluoresceinamine	Acrylamide methylenebis (acrylamide) copolymer	Fluroesceinamine was incorporated into an Acrylamide-methylenebis (acrylamide) copolymer that was covalently attached to a glass fiber via thermal or photopolymerization.	pH 4-9 Fluorescence Response Time in millisecs	Benefits of Submicron Optic fiber pH Sensor which gives a very quick and highly stable response is acknowledged. Application: Sensor was tested using a "Nucleopore" porous polycarbonate membrane	Tan *et al*, 1992
Sodium Fluorescein	Porous sol-gel films	Sol-gel claddings was prepared with immobilized Sodium Fluorescein	pH 7-11 Fluorescence	Intense dye leaching from the sol-gel matrix was observed	Wallace *et al*, 1997
Fluoresceinamine	Acrylamide methylenebis (acrylamide) copolymer octadecylsilane	The C-18 derivatized fibber was incubated in solution derivatives of fluorescein	pH 7.5-10 Fluorescence	The fluorescein derivatives used are shown to form non-fluorescent and non-pH-sensitive lactone after immobilization but by decreasing the densities of the hydrophobic layer, pH sensing can be achieved.	Fry and Bobbitt, 2001
Derivatives of fluorescein	Uncharged proton-permeable hydrogel cocktails	Ethanolic dye solutions were stirred with polymer solutions resulting in a thin, transparent and inert film.	pH 4.5-8.0. Absorbance based at 470nm and 530nm	The synthesis of fluorescein derivative have been discussed. The effect of ionic strength, proteins and photosensitivity on the response on the membranes.	Weidgans *et al*, 2004.
2',7'-dihexyl-5(6)-*N*-octadecyl-carboxamidofluorescein (DHFA) and 2',7'-dihexyl-5(6)-*N*-octadecyl-carboxamidofluorescein ethyl ester (DHFAE)	Uncharged proton-permeable hydrogel matrix	DHFA and DHFAE were physically entrapped in a polyurethane hydrogel	pH 7.2-9.2	Designed for pH measurements in marine environment.	Schroder *et al*, 2005

Cont'd Table 2b)Fiber-optic pH Sensor with pH-sensitive reagent -based transducers

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Fluorescein isothiocyanate (FITC)	Porous Glass Beads	FITC was covalently bonded to a porous glass bead	pH 3-7 Fluorescence Response time (20-35sec)	The range of pH 3-7 can be expended by decreasing the dye load.	Ming-ren *et al*, 1987
Fluorescein isothiocyanate (FITC)	Base-catalyzed organo-silica sol-gel film	FITC was covalently immobilized on the hydrohillic sol-gel	pH 2.4-6.4 Fluorescence	Response time of sensor going from acid-basic medium was less than in reverse.	Nivens *et al*, 1998
Fluorescein isothiocyanate (FITC)	Gels	FITC gels were impregnated with silylating agent	Fluorescence pH 6-8 response time was 20-30secs	The use of silylating agent was found to improve immobilization and decrease leaching. But the gels get harder and cracks easily	Badini *et al*, 1995
8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS)	Ethylene-vinyl acetate (EVA) polymer matrix	Reagent 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) was entrapped into the ethylene-vinyl acetate (EVA) polymer matrix	pH 5.5-8.0 Fluorescence	Effect of path length of signal, and indicator load on the response time was discussed together with implementing a modified reservoir-like set-up was suggested	Luo and Walt, 1989
Prussian blue and N-substituted polypyrroles	Non conductive polystyrene cuvettes	Prussian blue and N-substituted polypyrroles were deposited on a conductive polystyrene cuvettes	Absorbance pH 5-9	The insoluble crystalline membranes developed by this method could overcome problems of swelling, hysteresis and leaching	Koncki and Wolfbeis, 1998
Hydroxypyrenetri-sulfonic acid (HPTS)	The bare fiber tip was inserted in the dye solutions mixed with 100µL of phosphate buffer and 100µL of each stock dye solutions	The indicator wasn't immobilized but used in solution form in pH buffers.	Fluorescence pH 6.5- 8.0	Described the enhancement of response by primary and secondary inner filter effects of dyes	Gabor and Walt, 1991
Pyrenebutyric acid(PBA) and 7-hydroxy-4-methylcoumarine-3-acetic acid (HMCA)	Porous glass tips of single optical fibers	(PBA) and (HMCA) were bound on the porous glass tips of single optical fibers	Fluorescence pH 6-8	There was a decrease in pKa value of HMCA after immobilization	Bromberg *et al*, 1996

Cont'd Table 2b) Fiber-optic pH Sensors with pH-sensitive reagent- based transducers

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Carboxy-SNAFL-2-dextran	Distal cuvette chamber of the probe	Carboxy-SNAFL-2 encapsulated with dextran was immobilized in the distal cuvette chamber of the probe	pH 5-9 Fluorescence	Carboxy-SNAFL-2 encapsulated with dextran exhibited lifetime changes in presence of the analyte	Thompson and Lakowicz, 1993
Congo red	Triacetylcellulose	Congo red was bound to alkaline hydrolyzed triacetylcellulose membrane.	Absorbance pH 2-5	The influence of different parameters such as pk_{ind} e, diffusion coefficient and thickness of membrane on the dynamic characteristic of the sensor was explained.	Kostov *et al*, 1993
3,4,5,6 tetrabromophenol-sulfonephthalein (TBPSP)	Amberlite IRA-400 Amberlite IRA-45. XAD-2, XAD-4, XAD-7	TBPSP was electrostatically attracted on anion exchange resins; Amberlite IRA-400 and Amberlite IRA-45.TBPSP was also physically adsorbed on XAD-2, XAD-4, XAD-7	pH 3.0-10.5 Reflectance	Influence of resin support matrix on pK_a of TBPSP and the influence of indicator concentration and ionic strength on pH response of the probe was investigated.	Baker and Narayanaswamy *et al*, 1995
Seminaphthorhodamine (SNARF)	In-conjugated Poly(hydroxyethyl methacrylate	SNARF was conjugated with PHEMA forming a polymer which was attached to the Fiber tip	pH 6.8-7.8 Fluorescence emission	The pKa of the dye was sensitive to the structure of the polymer.	Parker *et al*, 1993
Seminaphthorhodamine (SNARF)	Unclad fiber optic	SNARF- 1C was coated on the fiber by sol-gel encapsulation method	pH 6.8-8.0 fluorescence	The sensor was tested in human blood and brain tissues Application: It was proposed to be utilized to monitor stroke patients	Grant and Glass, 1997
Seminaphthorhodamine (SNARF)	Unclad fiber optic	SNARF- 1C was coated on the fiber by sol-gel encapsulation method	pH 6.8-8.0 fluorescence	The sensor was tested in human blood Application : It was useful in tracking brain tissue pH and to monitor stroke patient	Grant *et al*, 2001
Victoria Blue (VB) and dipicrylamine (DPA)	triacetylcellulose	Victoria Blue (VB) and dipicrylamine (DPA) was optically transparent triacetylcellulose	pH 0-14 Absorbance	The back-propagation artificial neural network(ANN) model extends the measuring pH range	Safavi and Bagheri, 2003
α-naphtholphthalein	Fiber cladding material	α-naphtholphthalein sol gel was coated on SiO_2 fiber optic	Absorption pH 4-11 response time= 40secs	The dynamic pH range by absorption method was higher than fluorescence based sol-gel fiber sensors	Ben-David *et al*, 1997
Porphyrin	Indium (tin) oxide glass slides	porphyrin was electropolymerized on the surface of indium (tin) oxide glass slides	pH 8-12. Absorbance	Since the film consisted covalently linked ionophore monomers with no additional polymer matrix, leaching of membrane components into sample solution was minimized.	Blair *et al*, 1993

Cont'd Table 2b) Fiber-optic pH Sensors with pH-sensitive reagent- based transducers

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Kraton G1652	The polymer was coated on the tip of a single optical fiber	Free-radical polymerized Kraton G1652, vinylbenzyl chloride, and divinylbenzene was coated on the tip of the fiber.	pH 6.5-8.0 Reflectance	Emphasis on factors affecting response time and scattering effect.	Shakhser and Seitz, 1994
Ruthenium complex	Nafion Film	Ruthenium complex immobilized in Nafion	pH 1-8 Luminescent	The quenching effect of oxygen on luminophores has been studied.	Chan *et al*, 1998
Aminofluorescein (AF)	Glass support	Sol-gel purcursors with covalently immobilized AF	pH 4-9 Fluorescence	Effects of storage conditions affect the pKa of the indicator where under basic conditions the pKa of the indicator values increases.	Lobnik *et al*, 1998

Table 2c) Fiber-optic pH Sensors based on transducers developed by other means
other than the Colorimetric method

Indicator	Immobilization Membrane	Immobilization Method	Optical signal and response time and pH range	Concept and application	Reference
Silica Films	Optical Fiber core	Porous silica film made by means of sol-gel method was cladded onto optical fiber core.	pH 7.0-10.5	The surface (negative) charge of silica changes with pH of solution by adsorption of cations which modifies the refractive index of the film, modifying the light propagation with within optical fiber. Influence of film preparation conditions and ionic strength on response time was investigated	Rayss and Sudolski, 2002
Polyaniline	Core of Silica Optical Fiber	A section of cladding from a single strand silica optical fiber was replaced by the conductive polymer polyaniline film.	pH 3-12 An evanescent wave absorption spectroscopy Response time 4min	The optical response time to pH change was a function of the polyaniline film thickness on the optical fiber core as response time decreases for thinner films	Ge *et al*, 1993
Polyaniline	The prepared film was directly attached to the sensing tip of the fiber	The aniline went under electrochemical oxidative polymerization to form polyaniline films	Absorbance pH 5-8	During protonation and deprotonation, the pore size and permeability of films varies. This hysteresis effect decreases porosity for diffusion of hydroxide ions hence less absorbance drift is observed in basic solutions	Jin *et al*, 2000

The pH sensitive dyes which were utilized in this study had previously been applied in some other studies. However the method of development of the sensor and its application in pH monitoring varied from the work done in the literature. The indicators dyes have been mostly used in solution buffers to show change in optical response with variation in pH (Sheppard and Guiseppi-Elie, 1999; Edmonds *et al*., 1988; Benaim *et al*., 1986). The indicators have also been directly attached to the cladding material of silica optical fiber (Lee *et al*., 2001; Gupta and Sharma, 1997; Jordon and Walt David, 1987; Egami *et al*., 1996). Bromocresol green has also been impregenated in sol-gel films in some studies (Ismail *et al*., 2002). The bromothymol blue has been immobilized on Amberlite resins such as XAD-2 resin (Kirkbright *et al*., 1984; Guthrie *et al*., 1988). Phenol red was immobilized on XAD-4 resin (Motellier *et al*., 1995) and Ion-exchange XAD-1180 resin (Dybko *et al*., 1993).

Amongst the Amberlite resins used in this study, only Bromothymol blue have been immobilized on XAD-4 and Phenol red was immobilized on XAD-4 in literature as mentioned in previous paragraph. In this investigation, extensive study was performed by immobilizing Bromocresol green, Bromocresol purple, Bromothymol blue, Methyl red and Phenol red on three different Amberlite resins, XAD-2, XAD-4 and XAD-7 resins and its response and stability was demonstrated in pH buffers and also in soil solutions.

3.0 Materials and Methods

This study was carried out in the Chemistry Department laboratories at the University of the South Pacific in Fiji.

The chemicals used for preparation of aqueous solutions of pH buffers were; NaOH, KH_2PO_4 ,H_3BO_3, KCl, citric acid, and Na_2HPO_4 . The common pH indicators; bromocresol green, bromocresol purple, bromothymol blue, methyl red and phenol red were used for sensor development. All the reagents and chemicals were obtained from the Chemistry Department, University of the South Pacific, Fiji.

The substrates on which the indicators were immobilized were the resins, Amberlite non-ionic absorbent polymer beads (XAD-2, XAD-4, XAD-7) of dimension 100-250 microns. The beads were purchased from Sigma-Aldrich Pty Ltd.

The resins were first washed thoroughly with distilled water, followed by acetone and then methanol. After washing, the resins were left overnight in the oven at 105^{o}C for drying. The dried resins were then ground and sieved to obtain the required size of 100-250μm, and then packed in vials and stored for later use.

All the reagents and chemicals were used without further purification. Distilled water was used for all solution preparation throughout the study.

3.1 Indicator Selection

All pH sensitive indicator dyes were considered to select the most suitable ones for pH sensing in soils using the fiber optic approach. Selected ones were; bromocresol green,

bromocresol purple, Bromothymol blue, methyl red and phenol red as they have color changes in the desirable range for soils. Buffer solutions in pH range 5.0 - 8.0 at incremental of 0.2 pH units were prepared using NaOH and KH_2PO_4 at various compositions. Equal amounts of each 0.1% indicator solutions prepared in methanol (Kirkbright *et al*., 1984) were then added to the buffers. The pH of these solutions was taken before and after adding the indicators, as well as their absorbance (400nm-800nm) and reflectance signals (200nm-800nm). Most appropriate indicators were selected based on relatively large changes in absorbance or reflectance signals in the pH range 5 to 8.

3.2 Immobilization

To minimize the response time and to ease the light path going from one phase to another, choice of the membrane to immobilize the indicator was important. The first attempt was to immobilize the indicators on very thin polymer films. Phenol red indicator solution at concentrations 2.5%, 5%, 10%, 25%, and 50% were prepared in ethanol and were deposited on polyacetate films and 10% Polystyrene films prepared in Dichloromethane. The films were left to dry overnight. All indicators completely leached out from both the polymer films on washing. The next option was to physically adsorb the indicators on the amberlite resins and the chosen beads were XAD-2, XAD-4, and XAD-7.

The resins were left in the bath of the 0.1% indicator solutions prepared in methanol for 4 hours. The submerged resins were then washed with distilled water until the washings showed no visible indicator color. The set of 15 resin/indicator combinations were prepared having 3 different types of beads (XAD-2, XAD-4, XAD-7) immobilized with the 5 different indicators.

3.3 pH Range

After immobilization, small amount of resin was spread on a paper and was treated with little amount of different pH buffers ranging from pH 2-12 to select the pH range at which the indicator showed best response after being immobilized. Lower pH buffers were prepared with composites of Na_2HPO_4 and Citric Acid and higher pH buffers were prepared with composites of KCl and H_3BO_3 in NaOH. This difference in pH response of indicator before and after immobilization is due to change in its pKa values as in aqueous form and then when loaded on electron withdrawing membrane such as resins (Baker and Narayanaswamy, 1995; Motellier *et al*., 1995).

3.4 Leaching Test

The leaching test is important to obtain a stable response from the sensor. The resin was put into a mini column prepared in a Pasteur pipette with glass-wool packing (refer to fig 3a). Cycles of acidic and the basic buffers together with distilled water were poured through the column respectively and the washings were collected and scanned using UV-absorbance to depict the amount of leachate from each resin.

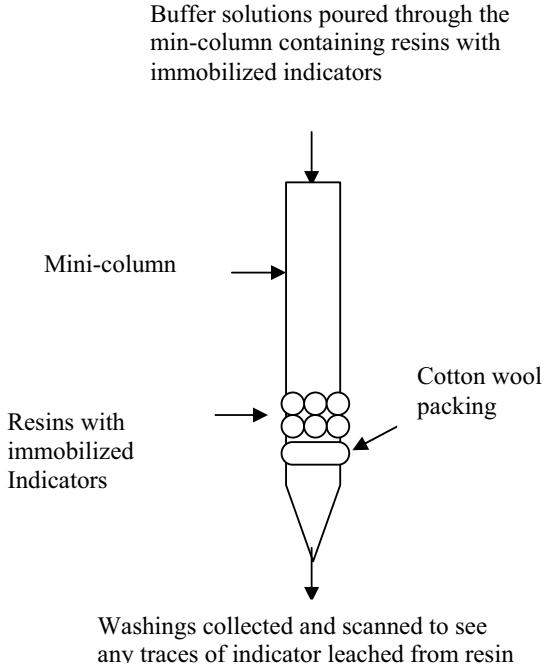


Fig 3a: Showing the set-up of the leaching test process

There was a series of leaching test organized for all resins. The resins were rinsed with the buffers after one night stay of resins in buffers, one week, after two weeks and then weekly basis till there wasn't any evidence of indicator leachate in the washings collected after each rinsing. For some cases indicators completely leached out on washing.

3.5 Probe Design:

Preparation of fiber optic pH probe:

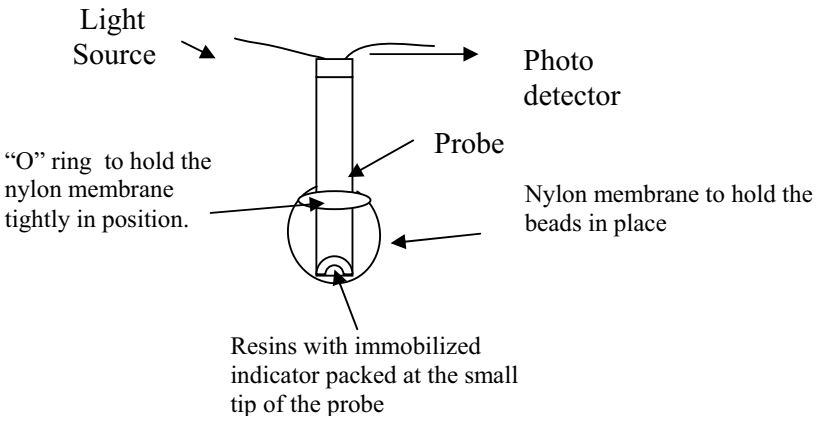


Fig 3b: Showing the design of the fiber optic pH probe.

A bifurcated fiber-optic material was utilized to carry light from the tungsten halogen lamp (LS-I) to the transducer material and collect and carry the reflected signals from the sensing tip to the photodetector (Ocean Optics, Inc S1000 Fiber-optic spectrometer) which modulates and displays the signals as characterized by SpectraScope software (v2.5(c) 1993/1995 Ocean Optics Inc).

The transducer was prepared with immobilized pH-sensitive indicator dyes on amberlite resins which were held to the sensing tip with a porous nylon membrane. A pair of "O" rings was used to hold everything intact to the probe (refer to fig 3b).

3.5.1 Stirring Effect:

When the probe is immersed in the buffers and soil solution, efficient diffusion of the hydrogen ions from the analyte species to the membrane of the reagent phase on the resins is essential to increase the response time with respect to change in color of the indicator at different pH's. Continuous stirring of the solutions increases the diffusion rate of the hydrogen ions across the membrane (Tsafack *et al*. 2000).

3.6 Wavelength Selectivity

The resins that had the immobilized indicators still bound to the membrane after leaching test were packed at sensing tip. The resins were immersed in different pH buffers and scanned using FOCS to determine the wavelength at which the sensor shows best response with pH. The FOCS was calibrated at the most suitable wavelength of 650 nm.

3.7 Response Time

The time required for the probe to equilibrate between pH 5 and pH 8 was determined under history channel at optimum wavelength 650 nm. Each scan under the history channel mode lasted for 25 minutes. All resins were mainly equilibrated between the pH range of interest pH 5-8 and the pH range depicted at stage 3.3 for respective indicator combination after immobilization. Those indicators which showed weak response in narrow pH range were also equilibrated for wide pH range as pH 2 and pH 12 or between any pH values where they showed any signal change. The equilibrations were mostly between 5 minutes or ten minutes (600secs) time intervals.

After determining the response time and the pH range in which the immobilized indicator shows best response, the resins were again scanned for real-time monitoring specifically for respective pH's only and within the depicted response time only.

Table 3a: The sequencing of the trials for this scanning was as follows;

Resins	**Trial: respective pH scanned for 1min**
XAD-2 with bromocresol green	pH 5, pH 6, pH 7, pH 8
XAD-2 with bromocresol purple	pH 5, pH 6, pH 7, pH 8
XAD-2 with Bromothymol blue	pH 5, pH 6, pH 7, pH 8
XAD-2 with Methyl red	pH 5, pH 6, pH 7, pH 8
XAD-2 Phenol red	pH 5, pH 6, pH 7, pH 8
XAD-4 with bromocresol green	pH 4, pH 5, pH 6, pH 7
XAD-4 with bromocresol purple	pH 5, pH 6, pH 7, pH 8
XAD-4 with Bromothymol blue	pH 5, pH 6, pH 7, pH 8
XAD-4 with Methyl red	Leached out
XAD-4 Phenol red	pH 8, pH 9, pH10, pH11, pH12
XAD-7 with bromocresol green	pH6, pH 8, pH 10, pH 12
XAD-7 with bromocresol purple	Leached out
XAD-7 with Bromothymol blue	pH 8, pH 9, pH 10, pH 11, pH 12 and in reverse order.
XAD-7 with Methyl red	Leached out
XAD-7 Phenol red	Leached out

The response of the sensor on the ssm mode wasn't so reliable when compared to the scanning efficiency showed by the history channel mode.

3.8 Probe Testing

After all previous optimizations, only three out of the 15 resins/indicator combinations were appropriate for good response in close range of pH 5-8. These were bromocresol green on XAD-2, XAD-4 and XAD-7. These resins were further tested in soil samples. Since optimum pH range for soil is pH 5-8, it was important to obtain soil samples of pH 5 and pH 8 for probe testing. A soil sample of pH 5 was utilized for the work which was also further treated with base to produce soil subsamples of pH close to 8.

3.8.1 Derivation of a Buffer curve to adjusting the soil pH within the required range of pH5-8

Soil solutions were prepared in distilled water incorporated within the ratio of 1g soil: 2.5ml distilled water. 0.1M KOH was added in significant amounts to increase the soil pH from pH 5 to pH 8. The buffer curve was calibrated based on the amount of KOH needed to increase the soil pH from pH 5 to pH 8.

Table 3b: The ratio of the amount of water and KOH that was added to 4g of soil to increase the pH of soil from 5-8.

Soil Sample	**Ratio of soil(g):volume of water: volume of 0.1M KOH**	**pH**
1	4g:10ml:0ml	5.12
2	4g:9ml:1ml	5.70
3	4g:8ml:2ml	6.20
4	4g:7ml:3ml	6.60
5	4g:6ml:4ml	6.84
6	4g:5ml:5ml	6.99
7	4g:4ml:6ml	7.30
8	4g:3ml:7ml	7.52
9	4g:2ml:8ml	7.67
10	4g:1ml:9ml	7.86
11	4g:0ml:10ml	7.99
12	4g:0ml:12ml	8.14
13	4g:0ml:14ml	8.37

3.8.2 Determination of the amount of $CaCO_3$ required to increase the soil pH 5 to pH 8.

Correlating with the amount of KOH base required from the buffer curve, the amount of $CaCO_3$ to be added to the soil to adjust the soil pH 5-8 was determined. For 4 g of soil, approximately 13 ml of 0.1 M KOH were required to increase the soil pH from 5 to 8. So for 60 g of soil close to 0.975 g of $CaCO_3$ was essential to adjust the pH to 8.

Series of 3 lots of soil were prepared with addition of 0.95 g, 1.1 g and 1.3 g of $CaCO_3$ to 60 g of soil. Calcium Carbonate was well mixed with the soil. About 30 ml of distilled water was added to each soil to assist complete reaction of calcium carbonate with the soil. The soil mixture was left overnight for completion of reaction and stored for usage.

3.8.3 Monitoring the amount of moisture requisite for pH sensing

To obtain consistent results, it is important to maintain the moisture condition of suspension close to the moisture condition of the field. A series of subsamples of soils at pH 5 and pH 8 were prepared containing different amounts of moisture by soil: water (w/v) ratio of 1:2.5, 1:1.5, 1:1 and 1:0.5 respectively. The least amount of moisture content at which soil pH was depictable was determined.

3.8.4 The performance of the prepared probe

The performance of the sensor in detecting changes in soil pH was determined by allowing the sensor to equilibrate between pH 5 and pH 8. Bromocresol green adsorbed on XAD-2, XAD-4 and XAD-7 was equilibrated in pH 5 buffers and in soil of pH 8 at respective moisture content. The probes were also equilibrated in pH 8 buffer and in soil of pH 5 to observe the reproducibility of the results.

The time required for the sensor to reach an equilibrium signal was recorded as the response time. Based on the response of the three resin/indicator combination, the most preferable indicator/resin for soil pH sensing was selected.

Results and Discussion

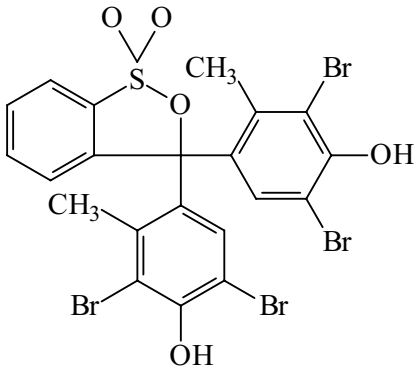
The fiber-optic pH-sensing probe was constructed based on physical absorption of pH-sensitive indicators on polymer resins. The selected indicators were; bromocresol green, bromocresol purple, bromothymol blue, methyl red and phenol red. These indicators were prompt to show good response with respect to change in pH particularly in the pH range of interest pH 5-8, essential for monitoring pH of soil. The performance of these indicators were first observed in solution form and then also after immobilization on the resins. The suitable membranes on which these indicators were immobilized on were Amberlite resins, XAD-2, XAD-4 and XAD-7. A total of 15 sensing tips were prepared, and the response of each prepared probe was studied in aqueous buffers of pH 2-12 and in soil of pH 5-8.

4.1 Indicators:

4.1.1) Bromocresol green

4.1.1.1.) Structure of Bromocresol Green and pKa of bromocresol green in solution:

Ismail *et al*. (2002) estimated pKa of bromocresol green in solution as equal to 4.96. Edmonds *et al*. (1988) and Sheppard and Guiseppi-Elie (1999) gave pKa of bromocresol green in solution as 4.7. Lee *et al*. (2001) reported the pKa of bromocresol green in solution as 4.4.



Bromocresol Green

4.1.1.2) Absorbance of Bromocresol Green in buffer solutions of pH 5-8.

Bromocresol green in solution absorbed mainly at 420.9nm and 617.9nm. The molar absorptivity of the base form of the indicator is generally greater than the absorptivity of acidic form hence response will be stronger for the basic form of the indicators. The protonated form of bromocresol green absorbs at 444nm and the deprotonated form of bromocresol green absorbs around 614nm-617nm (Ismail *et al*., 2002; Sheppard and Guiseppi-Elie, 1999).

4.1.1.3) pH range of Bromocresol Green in solution

The indicator absorbed strongly in acidic and basic buffers. There was an increase in absorbance shown for the indicator in buffers 5.0-5.6. From pH> 5.8, there was fluctuation in the trend of amount of absorbance versus the pH of the solution (refer to fig 4.1.1a). Hence the predicted range of bromocresol green in solution can only be confirmed between pH 5.0-5.6. Lee *et al*. (2001) reported the range of bromocresol green in solution as 3.6-5.2. There is a decrease in reflectance response with increase in pH from 5.0-5.6 at then equilibrates to constant value for pH > 5.6 (refer to fig 4.1.1b). This indicates that this indicator in solution form responds better for lower pHs.

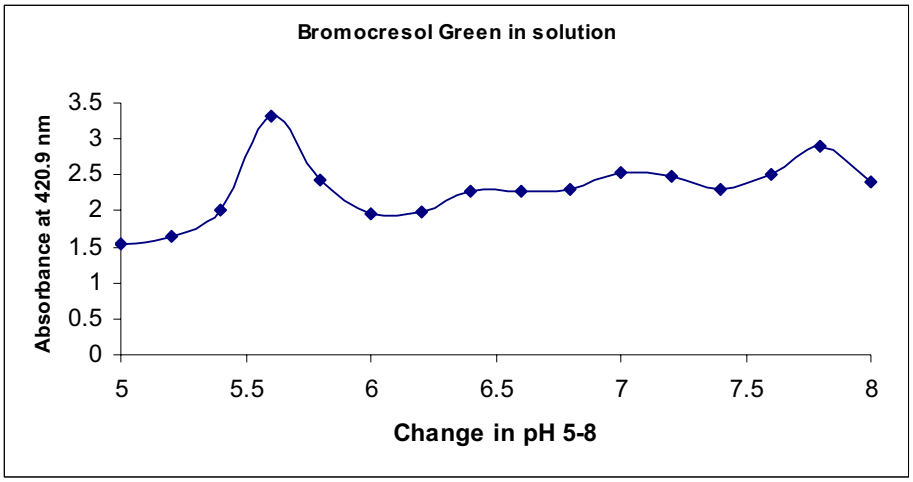


Fig 4.1.1a) Shows the absorbance of indicator bromocresol green in buffers of pH 5.0-8.0. (±0.2).

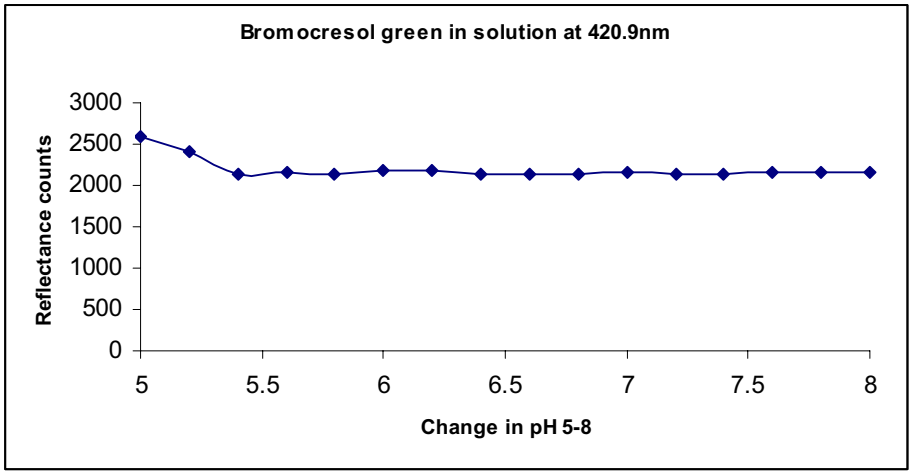


Fig 4.1.1b) Shows the reflectance of indicator bromocresol green in buffers of pH 5.0-8.0. (±0.2).

4.1.1.4) Immobilization of Bromocresol green on resins

Bromocresol green was physically absorbed on amberlite resins; XAD-2, XAD-4 and XAD-7. The resins were soaked in the bath of the 0.1% indicator solutions prepared in methanol as mentioned in the method. The indicator on XAD-2 appeared yellowish green in colour after immobilization. The indicator on XAD-4 appeared dirty green in colour after immobilization. The indicator on XAD-7 appeared yellowish green in colour after immobilization.

4.1.1.5) pH range of Bromocresol green after immobilization

The resins with immobilized indicator were spread on a paper and drops of buffers of pH 2-12 were added onto it to observe the colour change of the immobilized indicators at different pH's.

4.1.1a) The change in colour of the immobilized indicators when exposed to different pH buffers.

Indicator(initial color)	**Color change with pH**	**pH range**
Bromocresol green on XAD-2 (light yellowish green)	pH 2-5: light yellow pH 5: light green pH 6-8: turned to darker green pH 8-12: dark green	pH 5-8
Bromocresol green on XAD-4 (dirty green)	pH 2-3: light yellow pH 4-6: greenish yellow pH 7-12: dirty green	pH 4-7
Bromocresol green on XAD-7 (light yellowish green)	pH 2-5: light green pH 6: dark green pH 7: bluish green pH 8: blue pH 9: dark blue pH10-12: turns darker blue	pH 6-12

4.1.1.6) Leaching Test

The three resins, XAD-2, XAD-4 and XAD-7 with bromocresol green were rinsed with buffers of low pH (pH ≤ 2) and high pH (pH ≥ 11) and distilled water to remove any excess and/or weakly adsorbed indicator molecules in the sensing medium before its application (refer to fig 4.1.1c)..

Bromocresol green leached heavily from the resins on washing. It was also observed that the indicator leached more in the basic medium and distilled water when compared to the amount leached in the acidic medium. This is mainly because bromocresol green is acidic

in nature with pKa ≤ 5. At high pH, the indicator molecules are converted to its ionic form which is more soluble in aqueous medium, hence more leaching is observed in high pH buffers. After certain period of washing, the leaching of indicator was minimized.

Bromocresol green absorbed on XAD-7 leached more when compared with other two resins (refer to fig 4.1.1c). XAD-2 resins formed the strongest bound with bromocresol green. After 7 weeks of leaching test, there weren't much signs of further leaching of the indicator and little amount of the indicator was still left bound to the resins to be utilized for further testing.

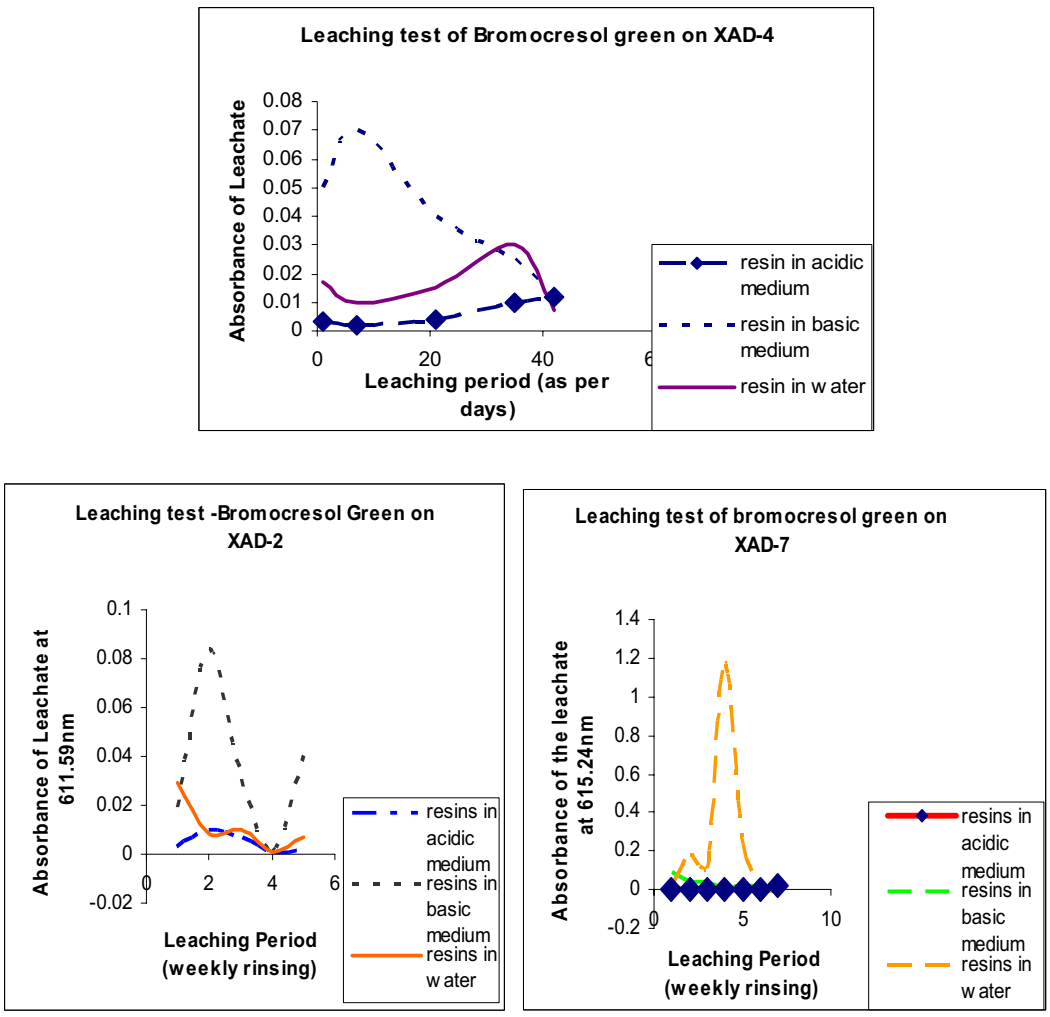


Fig: 4.1.1c) Shows the absorbance of leached indicators from XAD-2, XAD-4 and XAD-7 resins placed in different pH medium. The amount of absorbance indicates the amount of the indicators leached; hence more indicators have leached in the basic medium and water.

4.1.1.7) Response of immobilized indicator in buffer solutions of pH 2-12 after immobilization at 650 nm.

It was important to observe the response of these resins in the pH range of interest pH 5-8. Also if these resins weren't so effective in pH 5-8, further investigation was carried out to confirm the pH range of bromocresol green after immobilization on amberlite resins.

From the previous study at stage 4.1.1.5, it was shown that bromocresol green after immobilization on XAD-2 shows good colour change within pH range 5-8. Based on these findings, series of trials were performed on this XAD-2 bromocresol green indicator/resin combination. In the first trial, the resins with the indicator were immersed in buffers pH 5 and pH 8 respectively at every 5 minutes intervals. The response was little slow. However, very good trend in response was observed on equilibration of the probe in these two pH values. During the first five minutes (300secs), the probe was immersed in buffer of pH 5. A decrease in signal occurred. In the next 5-ten minutes (600secs), it was swapped to a buffer of pH 8. A further decrease in signal was observed. From 10-15 (600-900secs), the probe was immersed back in buffer of pH 5, which gave an increase in signal. From fifteen- twenty minutes, the probe was again immersed in pH 8 buffer, which showed a decrease in signal again. The reflectance signal increases again after twenty five minutes (300secs) when the probe was placed back in buffer of pH 5.

Similar trend was observed when the probe was equilibrated in pH 5 and pH 8 at ten minutes (600secs) intervals (refer to fig 4.1.1d). Two more trials were performed on the probe to observe its response in broader range in equilibration between pH 2 and pH 12 and also in successive pH buffers of pH 5, pH 8, pH 12 and pH 2 swapped at five minutes (300secs) interval. There was a decrease in reflectance signal when the probe was placed

in higher pH buffers and an increase in signal when it was placed in low pH buffers. The results indicate that the probe with bromocresol green immobilized on XAD-2 is suitable for pH sensing in the range of pH 5-8.

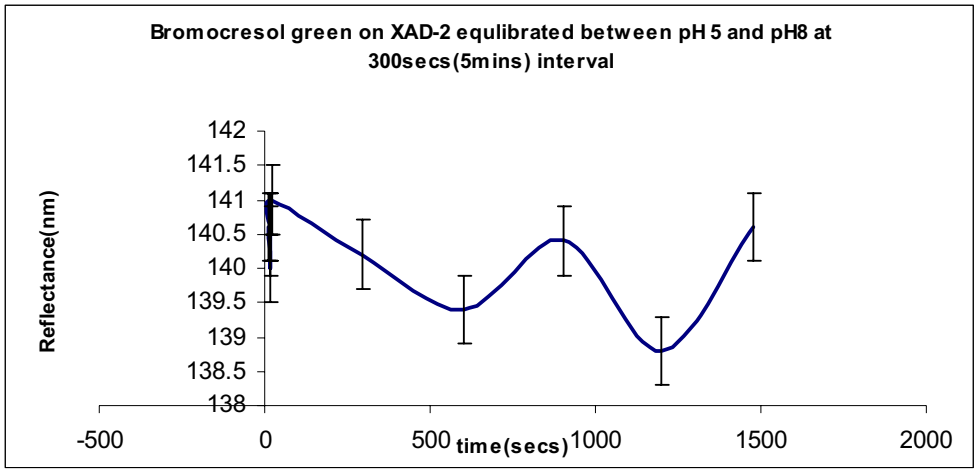


Fig: 4.1.1.d) Shows the change in response of the XAD-2 with bromocresol green probe when immersed in pH 5 and pH 8 respectively at every 5 minutes intervals.

From the previous study at stage 4.1.1.5, it was depicted that bromocresol green after immobilization on XAD-4 shows good colour change within the pH range 4-7. Based on these findings, series of trials were performed on this XAD-4 bromocresol green indicator/resin combination. In the first trial, the resins with the indicator were immersed in buffers pH 4 and pH 7 respectively at every 5 minutes interval. Avery good response was observed on equilibration of the probe in this two pH values. During the first five minutes (300secs), the probe was immersed in buffer of pH 4. A decrease in signal occurred. In the next 5 minutes, it was swapped to a buffer of pH 7. A further decrease in signal was observed. After fifteen minutes, the probe was immersed back in buffer of pH 4, which gave an increase in signal. From twenty- twenty five minutes (300secs), the probe was again immersed in pH 7 buffer, which showed a decrease in signal again. The reflectance signal increases again after twenty five minutes (300secs) when the probe was placed back in buffer of pH 4 (refer to fig 4.1.1e).

Similar trend was observed when the probe was equilibrated in pH 4 and pH 7 at ten minutes (600secs) intervals. Another trial was performed on the probe to observe its response in broader range in equilibration between pH 2 and pH 12. Two more trials were performed in sets of successive pH buffers of pH 4, pH 5, pH 6 and pH 7swapped at five minutes (300secs) intervals from lower pH to higher and in reverse. There was a decrease in signal when the probe was placed in higher pH buffers and an increase in signal when it was placed in low pH buffers. These trials have shown that Bromocresol green on XAD-4 responses best between pH 4-7.

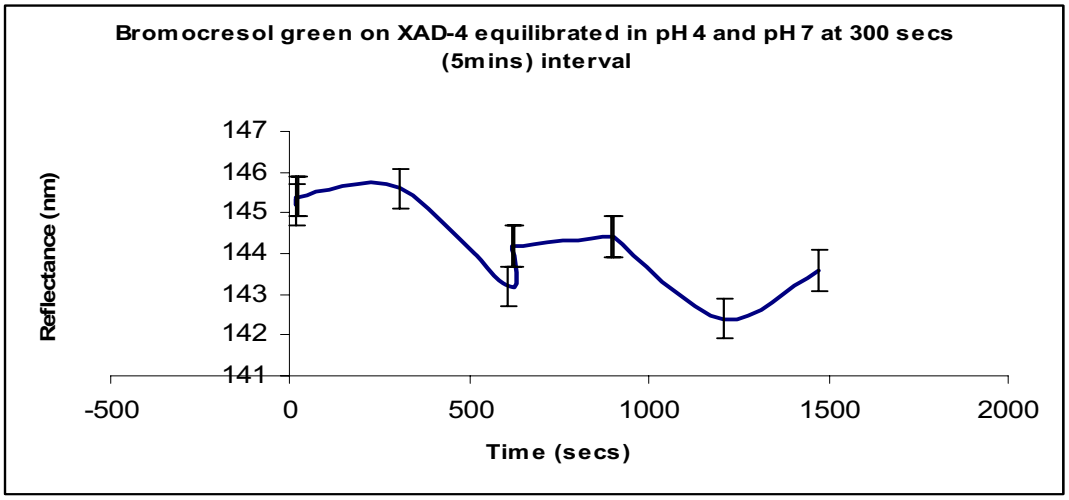


Fig: 4.1.1.e) Shows the change in response of the XAD-4 with bromocresol green probe when immersed in pH 4 and pH 7 respectively at every 5 minutes intervals.

From the previous study at stage 4.1.1.5, it was depicted that bromocresol green after immobilization on XAD-7 shows good colour change within pH range 6-12. Based on these findings, series of trials were performed on this XAD-7 bromocresol green indicator/resin combination. In the first trial, the resin with the indicator was immersed in buffers pH 6 and pH 12 respectively at every 5 minutes interval. A very good response was observed on equilibration of the probe in these two pH values.

Similar trend was observed when the probe was equilibrated in buffers on pH in reverse order of pH 12 and pH 6 at ten minutes (600secs) intervals (refer to fig 4.1.1f). During the first ten minutes the probe was immersed in a buffer of pH 12. A decrease in signal

was observed. In the next ten minutes (600secs) the probe was kept in the pH 6 buffer which gave an increase in signal. At 20-25 (1200-1500secs) it was placed into pH 12 buffer again which showed a decrease in signal again. The response of indicator decreases with increase in pH.

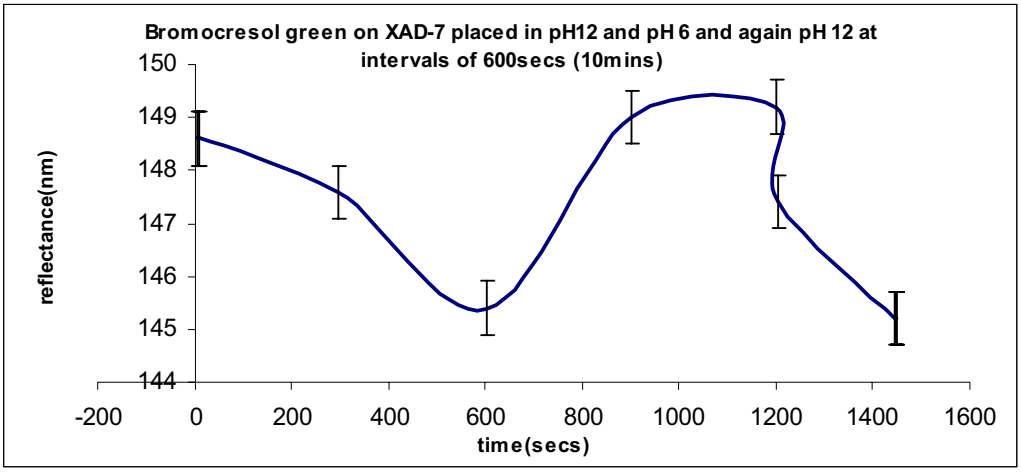


Fig: 4.1.1.f) Shows the change in response of the XAD-7 with the bromocresol green probe when immersed in pH12 and pH 6 respectively at every ten minutes (600secs) intervals.

Two more trials were performed on this probe to observe the equilibration of the probe in successive pH buffers of pH 6, pH 8, pH 10 and pH 12 (refer to fig 4.1.1g) and in reverse order (refer to fig 4.1.1h) swapped at five minutes (300secs) intervals. There was a decrease in signal when the probe was placed in higher pH buffers and an increase in signal when it was placed in low pH buffers. The results indicate that Bromocresol green immobilized on XAD-7 is suitable for pH sensing at the range of pH 6-12.

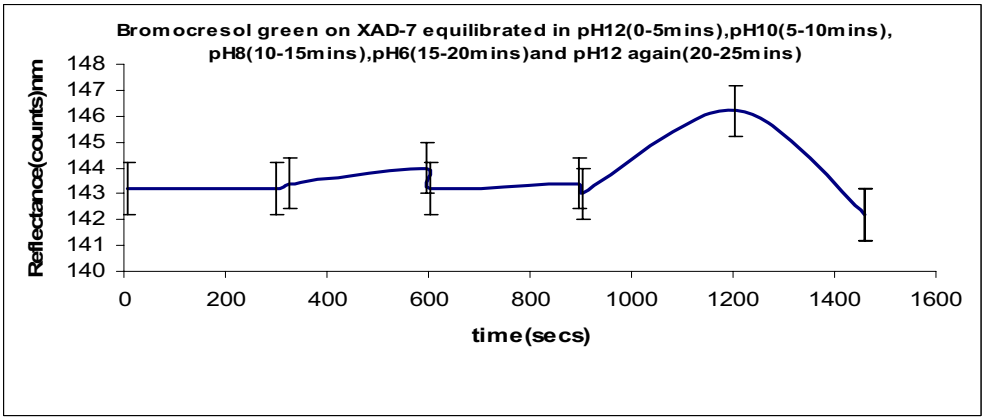


Fig:4.1.1.g) Shows the change in response of the XAD-7 with bromocresol green probe when immersed in pH 12, pH 10, pH 8, pH 6 and again in pH 12 at 5 minutes intervals.

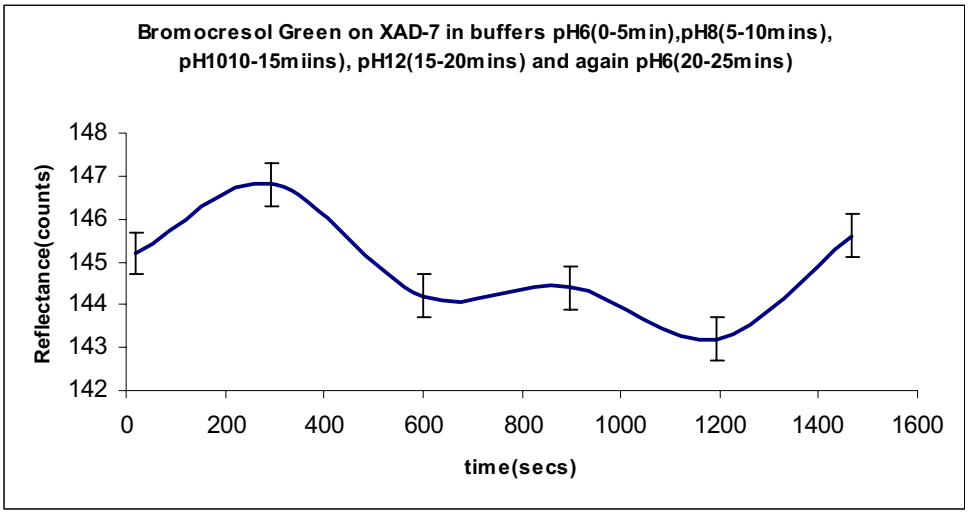
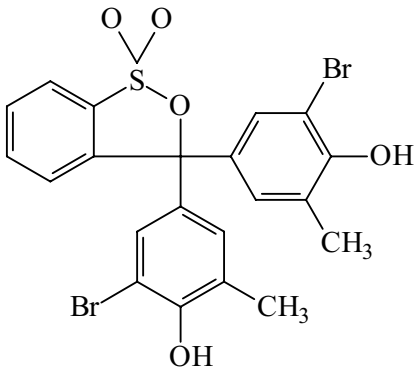


Fig: 4.1.1.h) Shows the change in response of the XAD-7 with bromocresol green probe when immersed in pH 6, pH 8, pH 10, pH 12 and again in pH 6 at 5 minutes intervals.

4.1.2 Bromocresol Purple

4.1.2.1). Structure of bromocresol purple and pKa of bromocresol purple in solution:

Edmonds *et al*. (1988) estimated the pKa value of bromocresol purple in solution form as 6.2. Sheppard and Guiseppi-Elie (1999) gave pKa of bromocresol purple in solution as 6.3. Lee *et al.* (2001) reported the pKa of BCP in solution as close to 6.



Bromocresol Purple

4.1.2.2) Absorbance of Bromocresol purple in buffer solutions of pH 5-8.

Bromocresol purple in solution absorbs mainly at 419.0nm and 588.9nm. The molar absorptivity of the base form of the indicator is generally greater than the absorptivity of acidic form hence response will be stronger for the basic form of the indicators. The protonated form of bromocresol purple absorbs at 433nm and the deprotonated form of bromocresol purple absorbs around 519nm (Sheppard and Guiseppi-Elie,1999).

4.1.2.3) pH range of Bromocresol purple in solution

The indicator absorbed strongly in acidic and basic buffers. There is a continuous increase in absorbance of the indicator with increased pH in the range pH 5.0-6.0. Except for the slight fluctuation in absorbance (refer to fig 4.1.2a) and reflectance (refer to fig 4.1.2b) response from pH 6.2 -7.4, the indicator showed good response with increase in buffer pH. Overall there was an increase in absorbance shown for the indicator in buffers 5.0-8.0. Hence the predicted range of bromocresol purple in solution can be confirmed between pH 5.0-8.0.

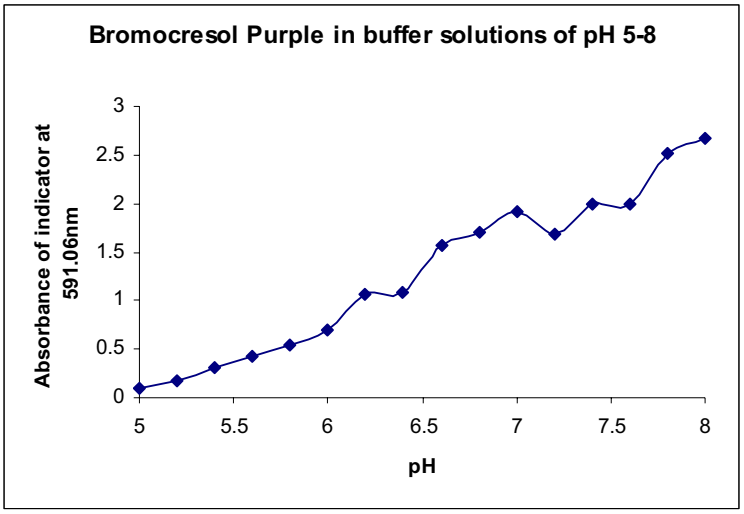


Fig 4.1.2a) Shows the absorbance of indicator bromocresol purple in buffers of pH 5.0-8.0.(±0.2)

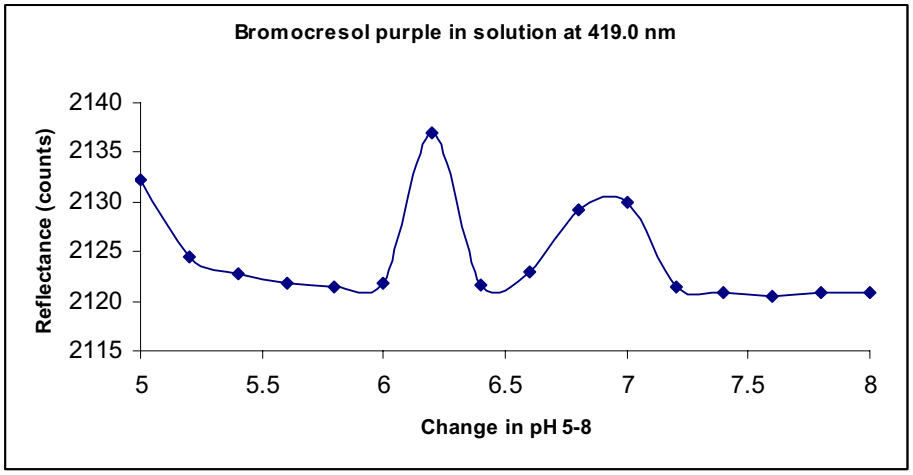


Fig 4.1.2b) Shows the reflectance of indicator bromocresol purple in buffers of pH 5.0-8.0.(±0.2)

4.1.2.4) Immobilization of Bromocresol purple on resins

Bromocresol purple was physically absorbed on amberlite resins; XAD-2, XAD-4 and XAD-7 as per described in the method. The indicator on XAD-2 appeared light yellow in colour after immobilization. The indicator on XAD-4 appeared bright yellow in colour after immobilization. The indicator on XAD-7 appeared sparkling yellow in colour after immobilization.

4.1.2.5) pH range of Bromocresol purple after immobilization

The resins with immobilized indicator were spread on a paper and drops of buffers of pH 2-12 were added onto it to observe the colour change of the immobilized indicators at different pH's.

Table 4.1.2.a) The change in colour of the immobilized indicators when exposed to different pH buffers.

Indicator(initial color)	**Color change with pH**	**pH range**
Bromocresol purple on XAD-2 (light yellow)	pH 2-12 no color change	pH 2-12
Bromocresol purple on XAD-4 (bright yellow)	pH 2-12 no color change	pH 2-12
Bromocresol purple on XAD-7 (sparkling yellow)	pH 2-5: light yellow pH 6: dull yellow pH 7: light green pH 8: dark green pH 9: dark blue pH 10-12 dark purplish blue	pH 5-12

4.1.2.6) Leaching Test

The three resins, XAD-2, XAD-4 and XAD-7 with bromocresol purple were rinsed with buffers of low pH (pH ≤ 2) and high (pH ≥ 11) and distilled water to remove any excess and/or weakly adsorbed indicator molecules in the sensing medium before its application.

Bromocresol purple leached heavily from the resins on washing. It was also observed that the indicator leached more in the basic medium and distilled water when compared to the amount leached in the acidic medium. This is mainly because bromocresol purple is acidic in nature. At high pH, the indicator molecules are converted to its ionic form which is more soluble in aqueous medium, hence more leaching is observed in high pH buffers. After washing, the leaching of indicator was minimized.

Bromocresol purple adsorbed on XAD-7 leached more when compared with other two resins. After certain trials of washing, the indicator completely leached off the XAD-7 resin. XAD-2 and XAD-4 resins formed the stronger bonds with bromocresol purple (refer to fig 4.1.2c). After 7 weeks of leaching test, there weren't much signs of further leaching of the indicator on XAD-2 and XAD-4 and little amount of the indicator was still left bound to the resins to be utilized for further testing.

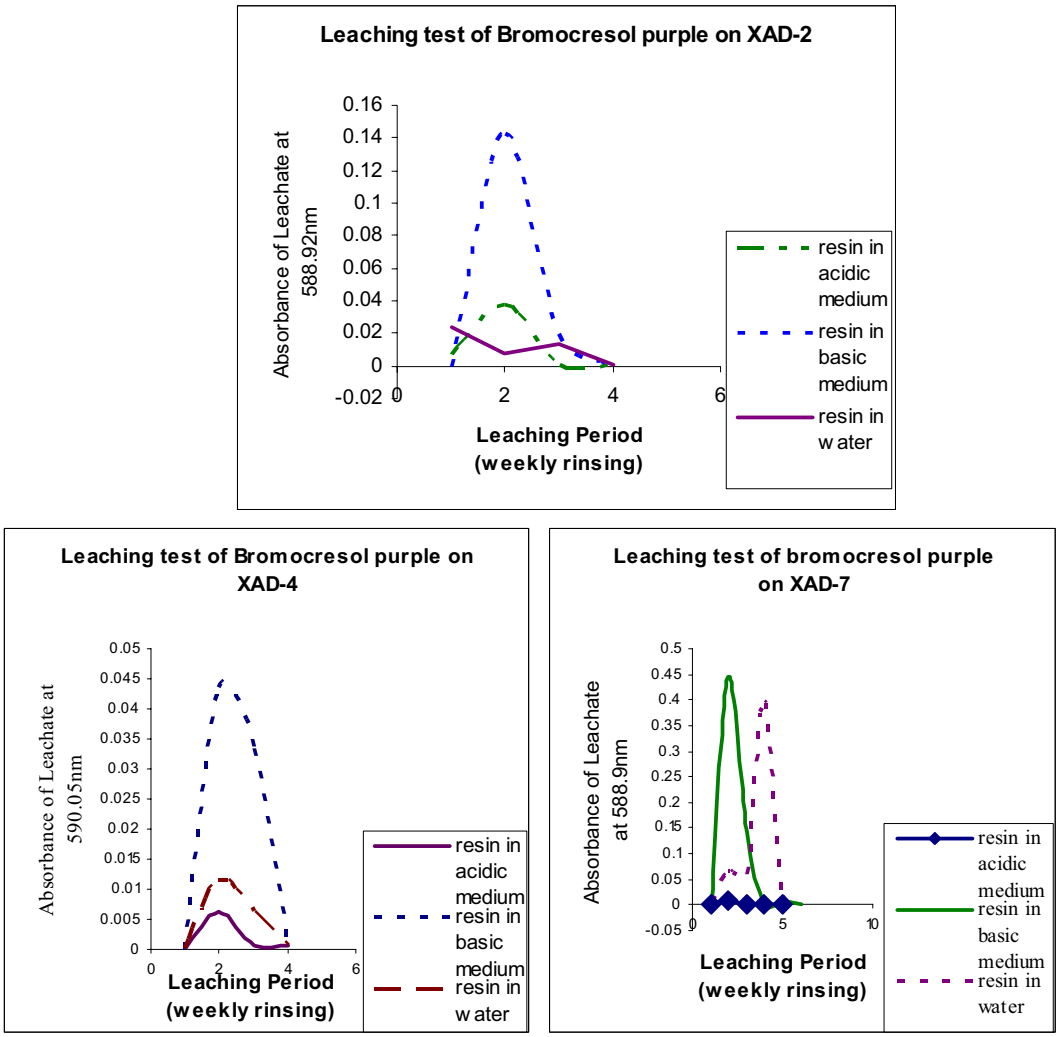


Fig 4.1.2c) Shows the absorbance of leached indicators from XAD-2, XAD-4 and XAD-7 resins placed in different pH medium. The amount of absorbance indicates the amount of the indicators leached, hence more indicators have leached in the basic medium and water.

4.1.2.7) Response of immobilized indicator in buffer solutions of pH 2-12 after immobilization at 650 nm.

Further investigation was carried out to confirm the pH range of bromocresol purple after immobilization on amberlite resins. From the previous study at stage 4.1.2.5, it was depicted that bromocresol purple after immobilization on XAD-2 only showed good response for wider change in pH between pH range 2 and pH 12. Based on these findings, series of trials were performed on this XAD-2 bromocresol purple indicator/resin combination. In the first trial, the resins with the indicator were immersed in buffers pH 2 and pH 12 respectively at every ten minutes (600secs) intervals (refer to fig 4.1.2d). A very good response was observed on equilibration of the probe in this two pH values. At the first ten minutes, the probe was immersed in buffer of pH 2. In the next 10-20 minutes (600-1200secs), it was swapped to a buffer of pH 12. A decrease in signal was observed. From 20-25 (1200-1500secs), the probe was placed again in pH 2 buffer which showed an increase in signal again.

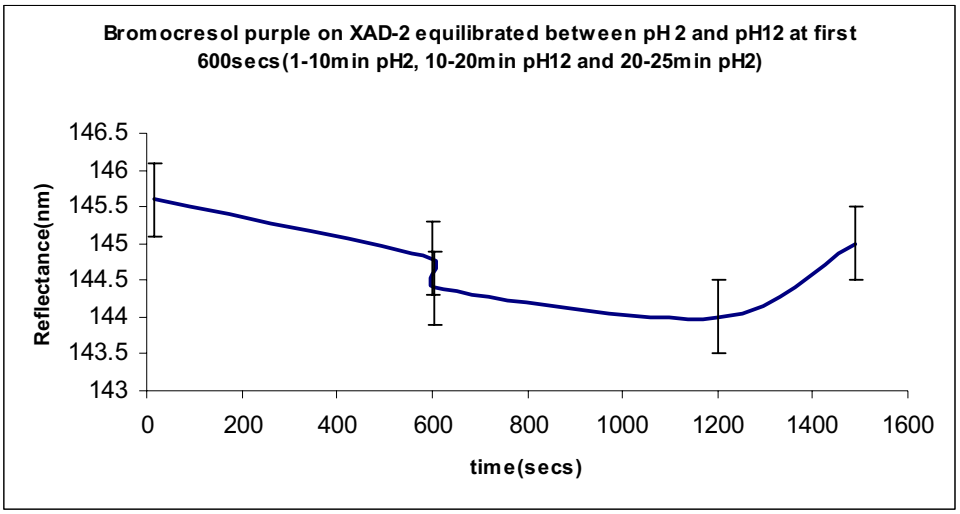


Fig 4.1.2d) shows the change in response of the XAD-2 with bromocresol purple probe when immersed in pH 2 for first ten minutes (600secs) and in pH 12 for 10-20 minutes (600-1200secs) then again in pH 2 from 20-25 (1200-1500secs).

Two more trials were performed on the probe to observe its response in equilibration between pH 5 and pH 8 (refer to fig 4.1.2e) at five and ten minutes intervals and also in successive pH buffers of pH 5, pH 8, pH 12 and pH 2 swapped at five minutes (300secs) intervals (refer to fig 4.1.2f). Kirkbright *et al*. (1984) reported a good response of bromocresol purple within small change in pH between pH 5-7. However in this study there wasn't much change in signal shown on equilibration of probe in pH 5 and pH 8 but the response was only significant with broad change in pH.

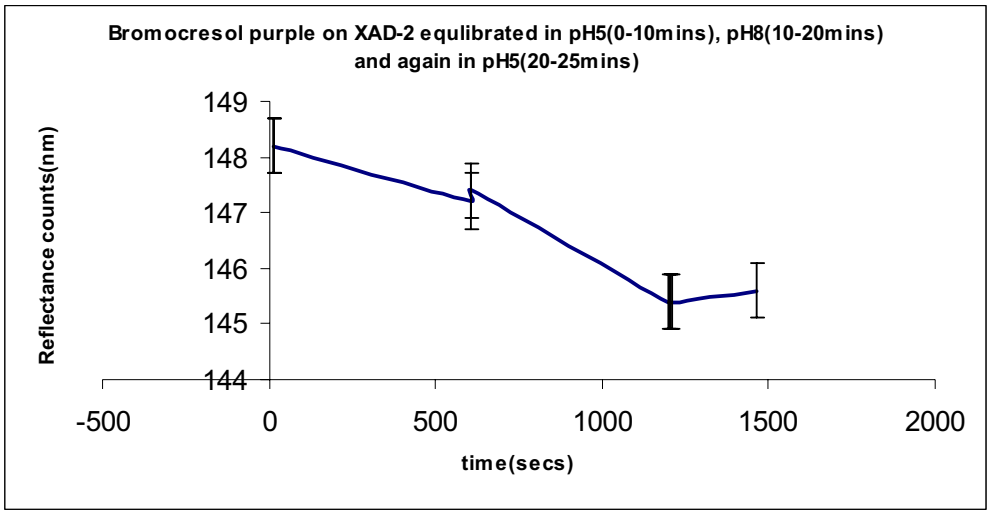


Fig 4.1.2e) the equilibration of XAD-2 bromocresol purple probe in pH 5 and pH 8 showed gradual decrease in signal with no good response with change in buffer pH.

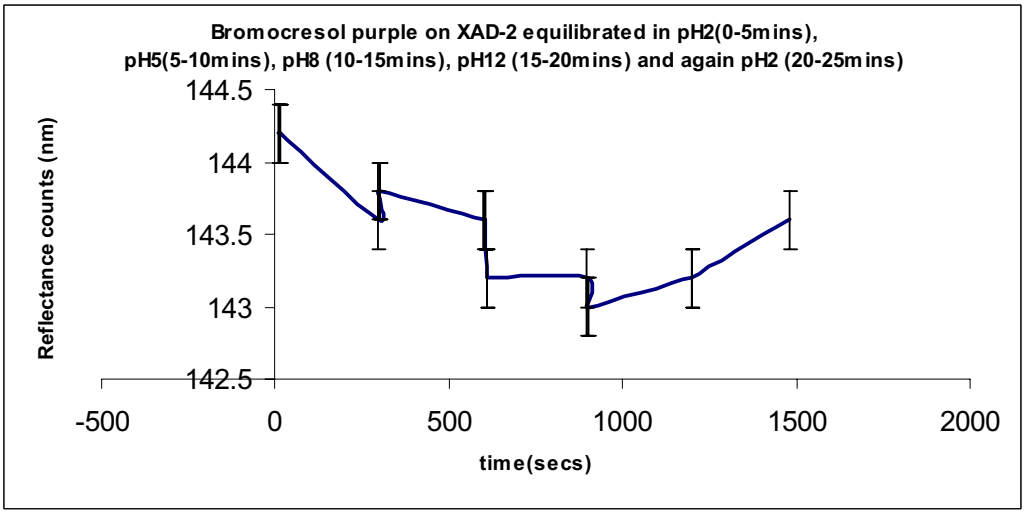


Fig 4.1.2f) shows response of XAD-2 bromocresol purple probe immersed in pH 2, pH 5, pH 8 and pH 12. There wasn't much difference in signal from pH 5-8.

From the previous study at stage 4.1.2.5, it was depicted that bromocresol purple after immobilization on XAD-4 also shows good color change within pH range 2-12. Based on these findings, series of trials were performed on this XAD-4 bromocresol purple indicator/resin combination. In the first trial, the resins with the indicator were immersed in buffers pH 2 and pH 12 respectively at every ten minutes (600secs) intervals. A very good response was observed on equilibration of the probe in this two pH values. At the first ten minutes, the probe was immersed in buffer of pH 2. A decrease in signal occurred. In the next 10-20 minutes (600-1200secs), it was swapped to a buffer of pH 12. A further decrease in signal was observed. From 20-25 (1200-1500secs), the probe was placed again in pH 2 buffer which showed an increase in signal again (refer to fig 4.1.2g). Hence this study indicates that Bromocresol purple with XAD-4 is suitable for only sensing wide difference in pH.

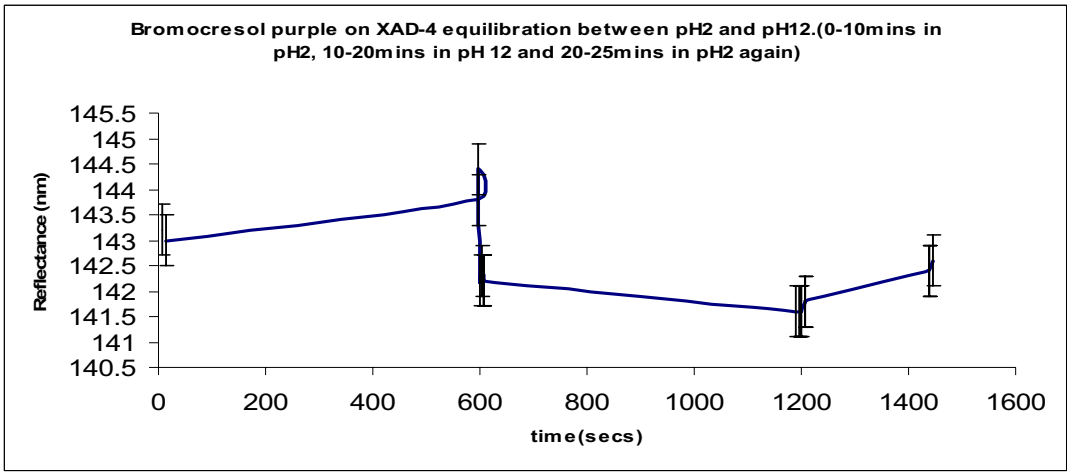


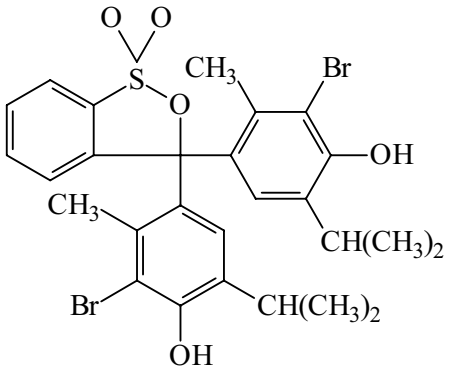
Fig 4.1.2g) shows the change in response of the XAD-4 with bromocresol purple probe when immersed in pH 2 for first ten minutes (600secs) and in pH 12 for 10-20 minutes (600-1200secs) then again in pH 2 from 20-25 (1200-1500secs)

Bromocresol purple completely leached off the XAD-7 resin after series on washing in the leaching test hence it was not used for further studies.

4.1.3 Bromothymol Blue

4.1.3.1). Structure of Bromothymol blue and pKa of bromothymol blue in solution:

Edmonds *et al.* (1988) estimated the pKa of bromothymol blue in solution as 6.8 and Sheppard and Guiseppi-Elie (1999) gave pKa of bromothymol blue in solution as 7.1.



Bromthymol Blue

4.1.3.2) Absorbance of Bromothymol blue in buffer solutions of pH 5-8.

Bromothymol blue in solution absorbed at 420.8nm and 616.9nm. The molar absorptivity of the base form of the indicator is generally greater than the absorptivity of acidic form hence response will be stronger for the basic form of the indicators. The protonated form of Bromothymol blue absorbs at 433nm and the deprotonated form of Bromothymol blue absorbs around 617nm (Sheppard and Guiseppi-Elie, 1999).

4.1.3.3) pH range of Bromothymol blue in solution

The indicator absorbed strongly in acidic and basic buffers. There was an increase in absorbance shown for the indicator in buffers of pH 5.0-8.0 (refer to fig 4.1.3a) and a decrease in reflectance for the indicator in buffers of pH 5.0-8.0 (refer to fig 4.1.3b). Hence the predicted range of Bromothymol blue in solution can be confirmed as pH 5.0-8.0.

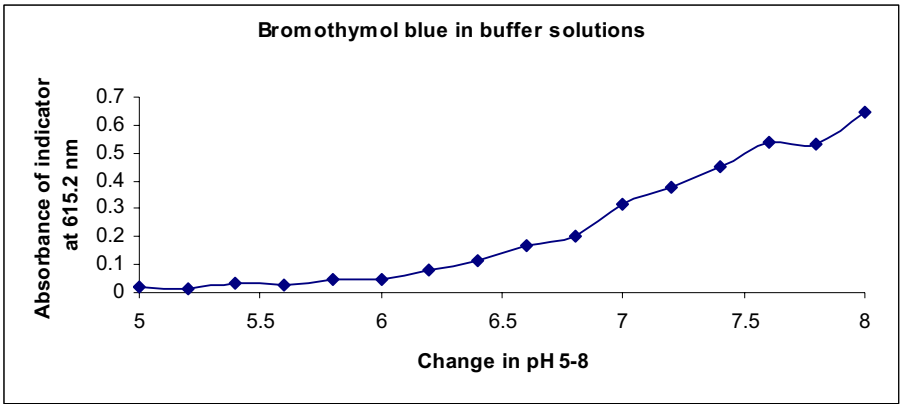


Fig 4.1.3a) shows the absorbance of indicator Bromothymol blue in buffers of pH 5.0-8.0 (±0.2).

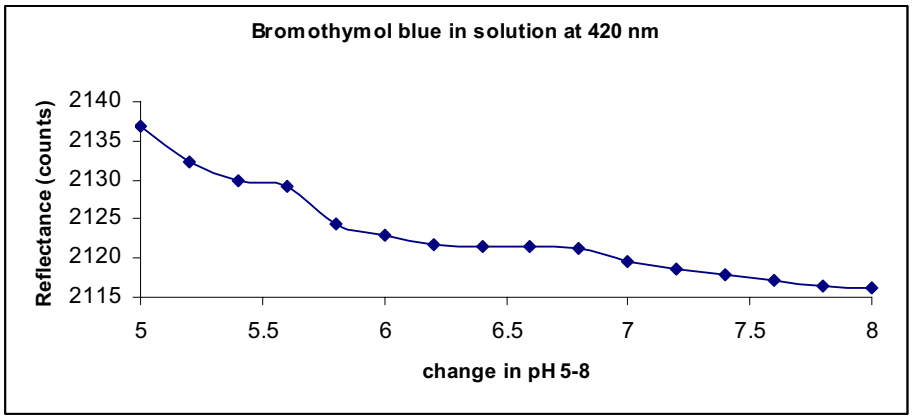


Fig 4.1.3b) shows the reflectance of indicator Bromothymol blue in buffers of pH 5.0-8.0.(±0.2)

4.1.3.4) Immobilization of Bromothymol blue on resins

Bromothymol blue was also physically absorbed on amberlite resins; XAD-2, XAD-4 and XAD-7 by soaking the resins in the bath of the 0.1% indicator solutions prepared in methanol as mentioned in the method. Guthrie *et al.* (1988) and Kirkbright *et al*. (1984) immobilized bromothymol blue on XAD-2 for developing pH sensing probes.

The indicator on XAD-2 appeared creamy orange in colour after immobilization. The indicator on XAD-4 appeared light orange in colour after immobilization. The indicator on XAD-7 appeared bright yellow in colour after immobilization.

4.1.3.5) pH range of Bromothymol blue after immobilization

The resins with immobilized indicator were spread on a paper and drops of buffers of pH 2-12 were added onto it to observe the colour change of the immobilized indicators at different pH's.

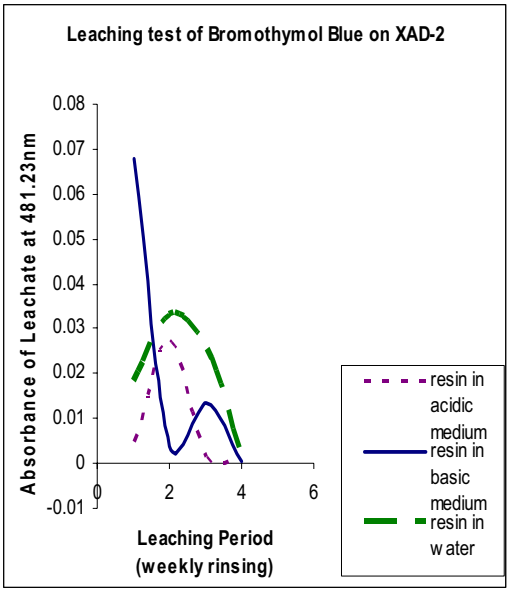
Table 4.1.3a) The change in colour of the immobilized indicators when exposed to different pH buffers.

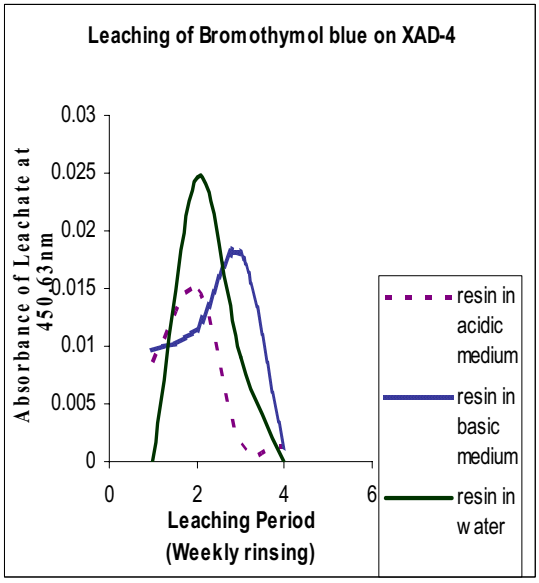
Indicator(initial color)	**Color change with pH**	**pH range**
Bromothymol blue on XAD-2 (creamy orange)	pH 2-12 no color change	pH 2-12
Bromothymol blue on XAD-4 (light orange)	pH 2-12 no color change	pH 2-12
Bromothymol blue on XAD-7 (bright yellow)	pH 2-8: light yellow pH 9: light green pH 10-12: dark green	pH 8-12

4.1.3.6) Leaching Test

The three resins, XAD-2, XAD-4 and XAD-7 with Bromothymol blue were rinsed with buffers of low pH (pH $\leq$ 2) and high (pH $\geq$ 11) and distilled water to remove any excess and/or weakly adsorbed indicator molecules in the sensing medium before its application.

Bromothymol blue has pKa value close to neutral value of 7. Unlike other indicators Bromothymol blue didn't leach much in the basic medium (refer to fig 4.1.3c). Bromothymol blue was one of the best bound indicators to all the resins. After 7 weeks of leaching test, there weren't much signs of further leaching of the indicator and sufficient amount of the indicator was still left bound to the resins to be utilized for further testing.





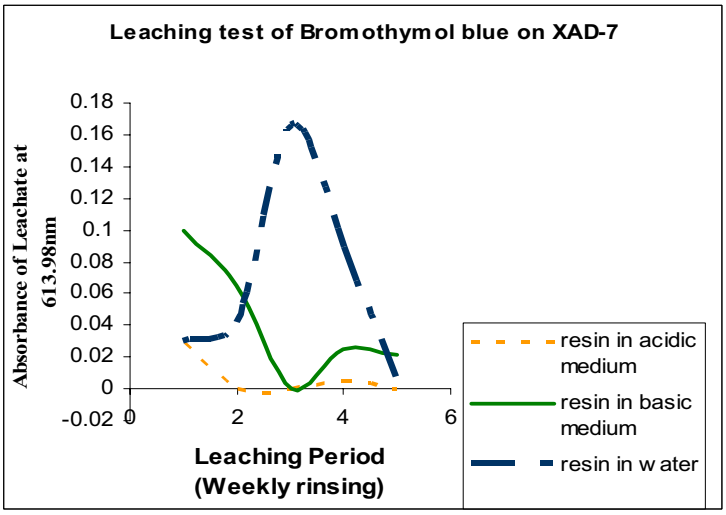


Fig 4.1.3c) shows the absorbance of leached indicators from XAD-2, XAD-4 and XAD-7 resins placed in different pH medium. The amount of absorbance indicates the amount of the indicators leached; hence more indicators have leached in the basic medium and water.

4.1.3.7) Response of immobilized indicator in buffer solutions of pH 2-12 after immobilization at 650 nm.

It was important to observe the response of this resin in the pH range of interest pH 5-8. Also if in-case these resins weren't so effective in pH 5-8, further investigation was carried out to confirm the pH range of Bromothymol blue after immobilization on amberlite resins.

From the previous study at stage 4.1.3.5, it was depicted that Bromothymol blue after immobilization on XAD-2 only responded with wide change in pH. Based on these findings, series of trials were performed on this XAD-2 Bromothymol blue indicator/resin combination. In the first trial, the resins with the indicator were immersed in buffers pH 5 and pH 8 respectively at every 5 minutes intervals and ten minutes (600secs) intervals. There wasn't much change in signal on equilibration of probe between pH 5 and pH 8 (refer to fig 4.1.3d). Two more trials were performed on the probe to observe its response in broader range in equilibration between pH 2 and pH 12 and also in successive pH buffers of pH 5, pH 8, pH 12 and pH 2 swapped at five and ten minutes interval. There was a decrease in signal when the probe was placed in higher pH buffers and an increase in signal when it was placed in low pH buffers (refer to fig 4.1.3e). The response of indicator decreases with increase in pH. This indicates that bromothymol blue after immobilization on XAD-2 is only suitable for sensing of wide change in pH.

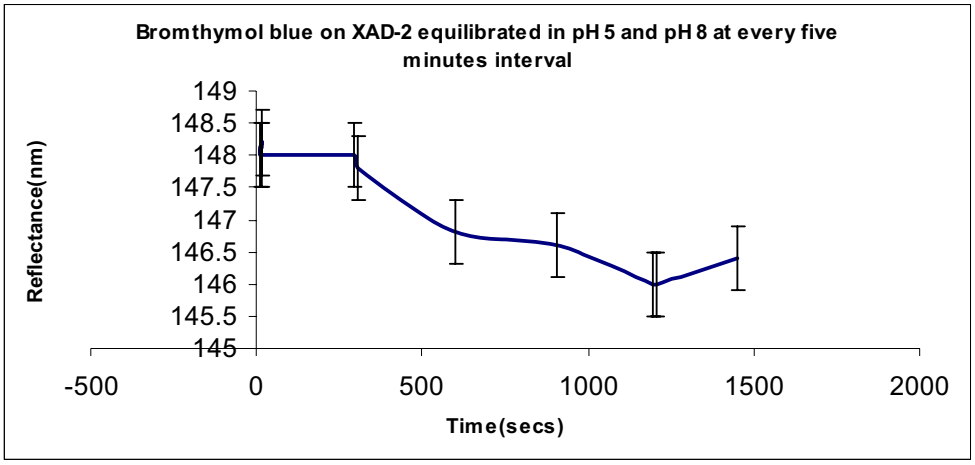


Fig 4.1.3d) shows the response of BTB-XAD-2 probe equilibrated in pH 8(0-300secs, 600-900secs, 1200-1500secs) and pH 5(300-600secs, 900-1200secs). There wasn't much difference in signal with change in pH of buffers.

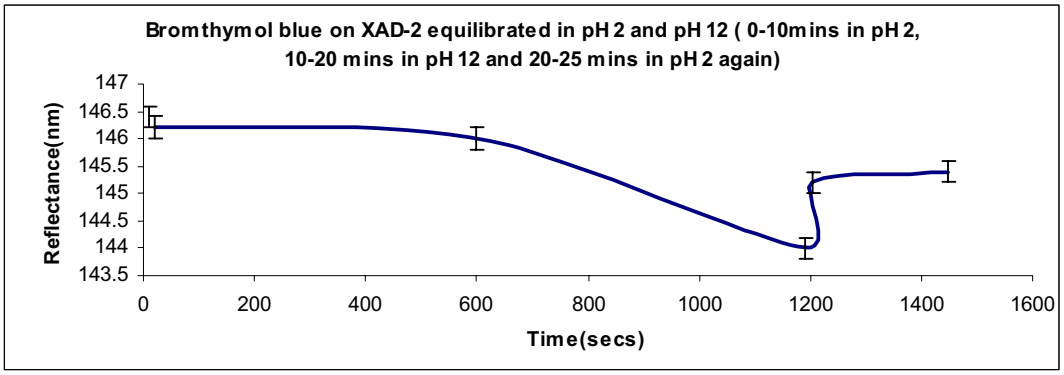


Fig 4.1.3e) shows the change in response of the XAD-2 with Bromothymol blue probe when immersed in pH 2 for first ten minutes (600secs) and in pH 12 for 10-20 minutes (600-1200secs) then again in pH 2 from 20-25 minutes (1200-1500secs).

From the previous study at stage 4.1.3.5, it was depicted that Bromothymol blue after immobilization on XAD-4 also only responds for wide change in pH. Based on these findings, series of trials were performed on this XAD-4 Bromothymol blue indicator/resin combination.

In the first trial of equilibration between pH 5 and pH 8 buffers, Bromothymol blue on XAD-4 did not show good response within pH 5-8. There was a continuous decrease in signal in both low and high pH buffers (refer to fig 4.1.3f).

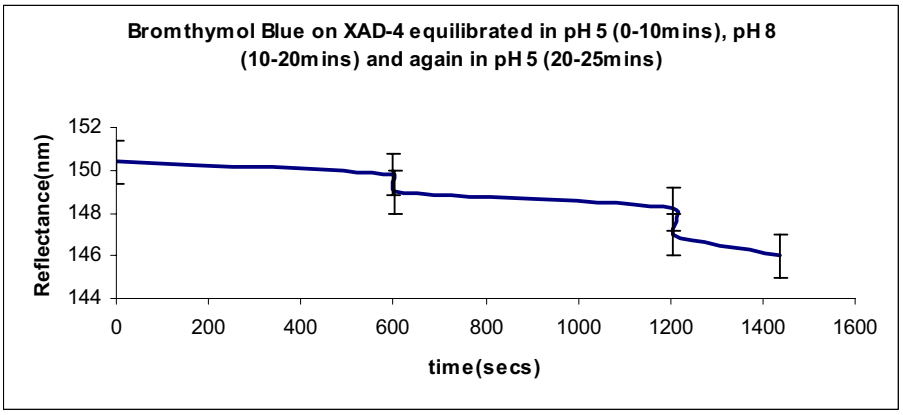


Fig 4.1.3f) shows the change in response of the XAD-4 with bromothymol blue probe when immersed in pH 8 (0-600secs), pH 5 (600-1200secs), pH 8 (1200-1500secs). It shows inverse response in acidic and basic condition.

The resin with the indicator was also immersed in buffers pH 2 and pH 12 respectively at every 5 minutes intervals. A very good response was observed on equilibration of the probe in this two pH values.

Two more trials were performed in sets of successive pH buffers of pH 5, pH 8, pH 12 and pH 2 swapped at five minutes (300secs) intervals. There wasn't much difference in signal on equilibration between pH 2-8 and then between pH 8-12 however the signal increased as the probe was equilibrated between pH 12 and pH 2 (refer to fig 4.1.3g,h). Hence this indicates that bromothymol blue on XAD-4 is suitable for sensing of only wide difference in pH.

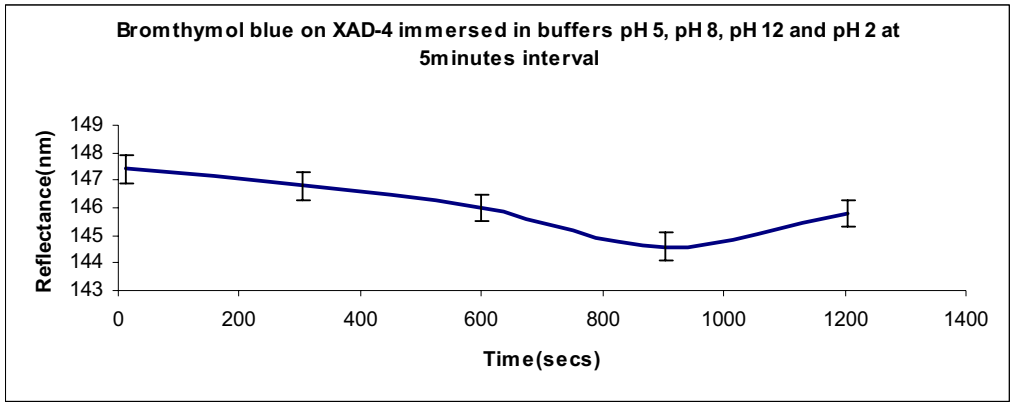


Fig 4.1.3g) shows the change in response of the XAD-4 with Bromothymol blue probe when immersed in pH 5 (0-300secs), pH 8 (300-600secs), pH 12 (600-900secs) and in pH 2 (900-1200secs).

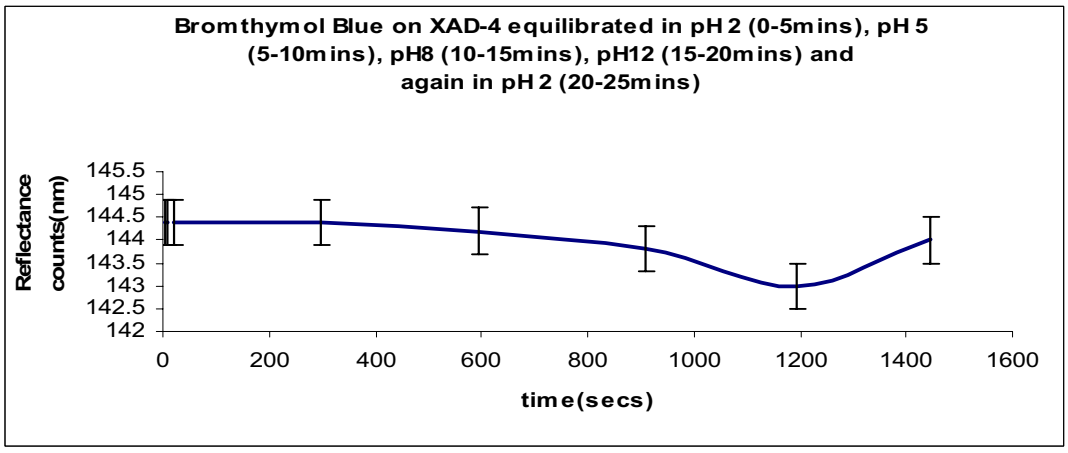


Fig 4.1.3h) shows the change in response of the XAD-4 with Bromothymol blue probe when immersed in pH 2 (0-300secs), pH 5 (300-600secs), pH 8 (600-900secs), pH 12 (900-1200secs) and again in pH 2 (1200-1500secs).

From the previous study at stage 4.1.3.5, it was depicted that Bromothymol blue after immobilization on XAD-7 shows good colour change within pH range 8-12. Based on these findings, series of trials were performed on this XAD-7 Bromothymol blue indicator/resin combination. In the first trial, the resin with the indicator was immersed in buffers pH 5 and pH 8 respectively at every 5 minutes and ten minutes (600secs) interval. There wasn't much change in signal on equilibration of probe in pH 5 and pH 8.The probe was also equilibrated between broad pH range pH 2 and pH 12. The response was very significant with decrease in signal in stronger pH and increase in signal at low pHs (refer to fig 4.1.3i).

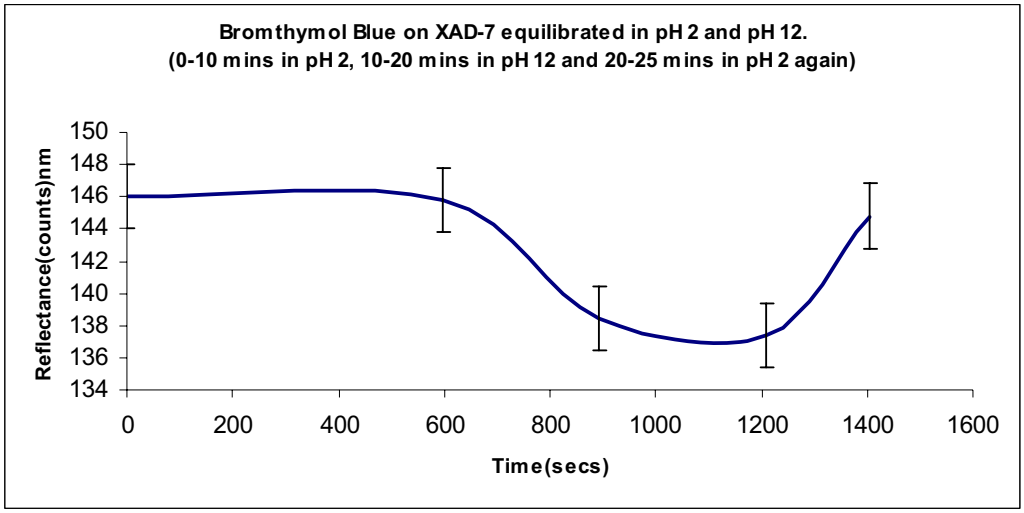


Fig 4.1.3i) XAD-7 BTB probe equilibrated in pH 2 and pH 12. Signal increases in acidic condition and decreases in basic conditions

Two trials of equilibration were performed in successive pH buffers of pH 8, pH 9, pH 10 pH 11 and pH 12, (refer to fig 4.1.3j) and between pH 2, pH 5, pH 8 and pH 12 (refer to fig 4.1.3k) at five minutes (300secs) intervals. There was a decrease in signal when the probe was placed in higher pH buffers and an increase in signal when it was placed in low pH buffers. This indicates that bromothymol blue on XAD-4 is suitable for detection of pH in the range pH 8-12 as well for wide variation of pH of acidic solutions of pH 2 to very basic solutions of pH> 8.

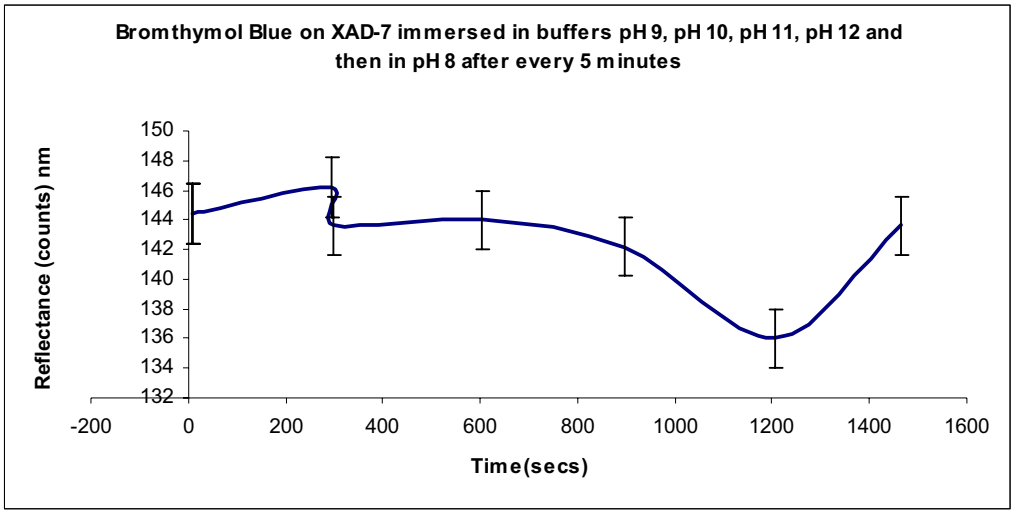


Fig 4.1.3j) XAD-7 BTB probe equilibrated in pH 9 (0-300secs), pH 10 (300-600secs), pH 11 (600-900secs), pH 12 (900-1200secs) and pH 8 (1200-1500secs). Signal increases in acidic condition and decreases in basic conditions

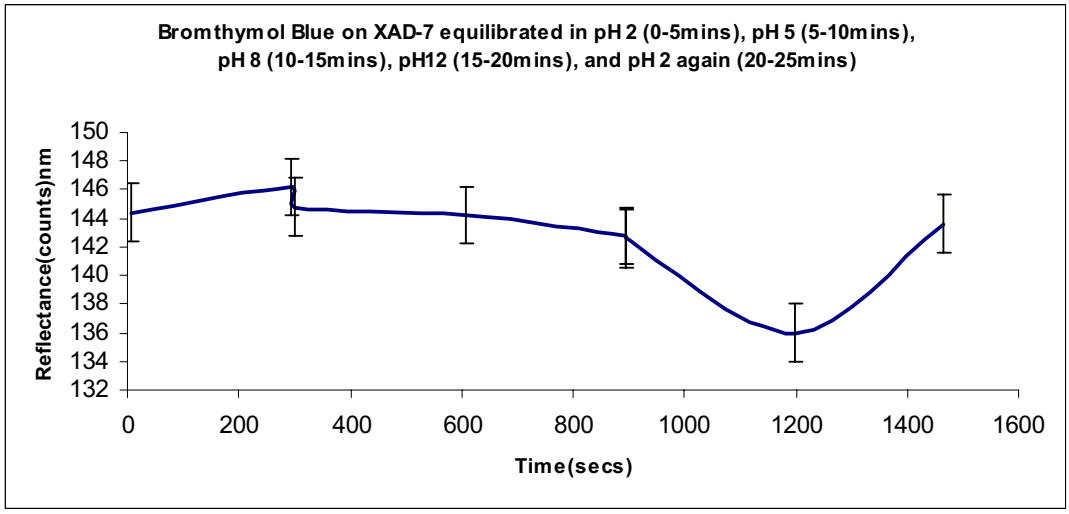
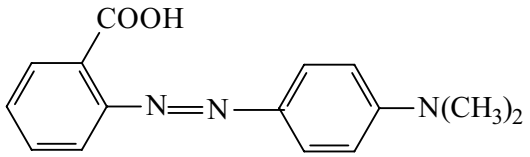


Fig 4.1.3k) XAD-7 BTB probe equilibrated in pH 2 (0-300secs), pH 5 (300-600secs), pH 8 (600-900secs), pH 12 (900-1200secs) and pH 2 (1200-1500secs). Signal increases in acidic condition and decreases in basic conditions. There was much difference in signal between pH 5-8.

4.1.4 Methyl red

4.1.4.1). Structure of Methyl red and pKa of Methyl red in solution:

Edmonds *et al.* (1988) gave the pKa value of methyl red in solution as 4.9 while Sheppard and Guiseppi-Elie (1999) gave pKa of methyl red in solution as 5.0.



Methyl Red

4.1.4.2) Absorbance of Methyl red in buffer solutions of pH 5-8.

Methyl red in solution absorbed mainly at 419nm and 520.6nm. The molar absorptivity of the base form of the indicator is generally greater than the absorptivity of acidic form hence response will be stronger for the basic form of the indicators. The protonated form of methyl red absorbs at 427nm and the deprotonated form of methyl red absorbs around 530nm (Sheppard and Guiseppi-Elie, 1999).

4.1.4.3) pH range of Methyl red in solution

The indicator absorbed strongly in acidic and basic buffers. There was an increase in absorbance shown for the indicator in buffers of pH 5.0-6.8 at 419 nm. Between pH 6.8-7.2, there was fluctuation in the trend of amount of absorbance versus the pH of the solution. However at 520 nm, with little fluctuation between pH 5.0-5.4, the signal decrease continually between pH 5.4-6.4 and then equilibrates to constant level for pH> 6.4 (refer to fig 4.1.4a). The reflectance signal only showed good response at pH 5.0-5.6 (refer to fig 4.1.4b).There were fluctuations in reflectance response with respect to pH change at all other pH's.

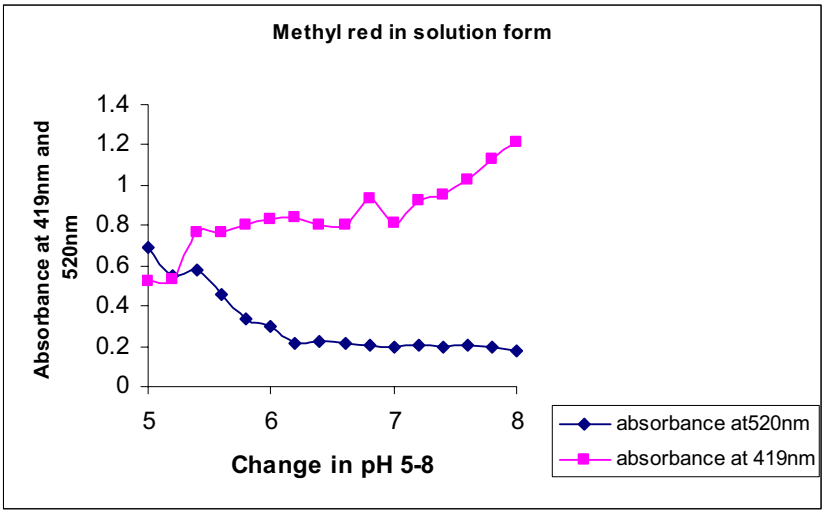


Fig 4.1.4a) shows the absorbance of indicator methyl red in buffers of pH 5.0-8.0. (±0.2)

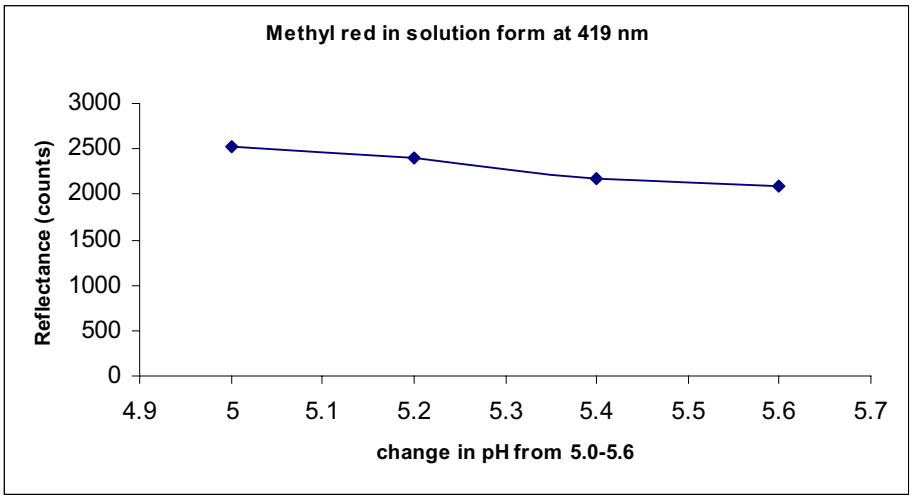


Fig 4.1.4b) shows the reflectance of indicator methyl red in buffers of pH 5.0-5.6 (±0.2).

4.1.4.4) Immobilization of Methyl red on resins

Methyl red was also physically absorbed on amberlite resins; XAD-2, XAD-4 and XAD-7 by soaking the resins in the bath of the 0.1% indicator solutions prepared in methanol as mentioned in the method. The indicator on XAD-2 appeared maroonish red in colour after immobilization. The indicator on XAD-4 appeared bright red in colour after immobilization. The indicator on XAD-7 appeared dark red in colour after immobilization.

4.1.4.5) pH range of Methyl red after immobilization

The resins with immobilized indicator were spread on a paper and drops of buffers of pH 2-12 were added onto it to observe the colour change of the immobilized indicators at different pH's.

4.1.4a) The change in colour of the immobilized indicators when exposed to different pH buffers.

Indicator(initial color)	**Color change with pH**	**pH range**
Methyl red on XAD-2 (maroonish red)	pH 2-7 no color change pH8-12 turns dull red in color	pH 7-8
Methyl red on XAD-4 (bright red)	pH 2-12 no color change	pH 2-12
Methyl red on XAD-7 (dark red)	pH 2-6 red pH 7-8 yellowish red pH 9-12 yellow	pH 6-12

4.1.4.6) Leaching Test

The three resins, XAD-2, XAD-4 and XAD-7 with methyl red were rinsed with buffers of low pH (pH $\leq$ 2) and high (pH $\geq$ 11) and distilled water to remove any excess and/or weakly adsorbed indicator molecules in the sensing medium before its application.

Methyl red leached heavily from the resins on washing. It was also observed that the indicator leached more in the basic medium and distilled water when compared to the amount leached in the acidic medium (refer to fig 4.1.4c). This is mainly because methyl red is acidic in nature with pKa $\leq$ 5. At high pH, the indicator molecules are converted to

its ionic form which is more soluble in aqueous medium, hence more leaching is observed in high pH buffers.

Methyl red absorbed on XAD-4 and XAD-7 leached off completely after washing with cycle of buffers and distilled water. XAD-2 resins formed the strongest bound with methyl red.

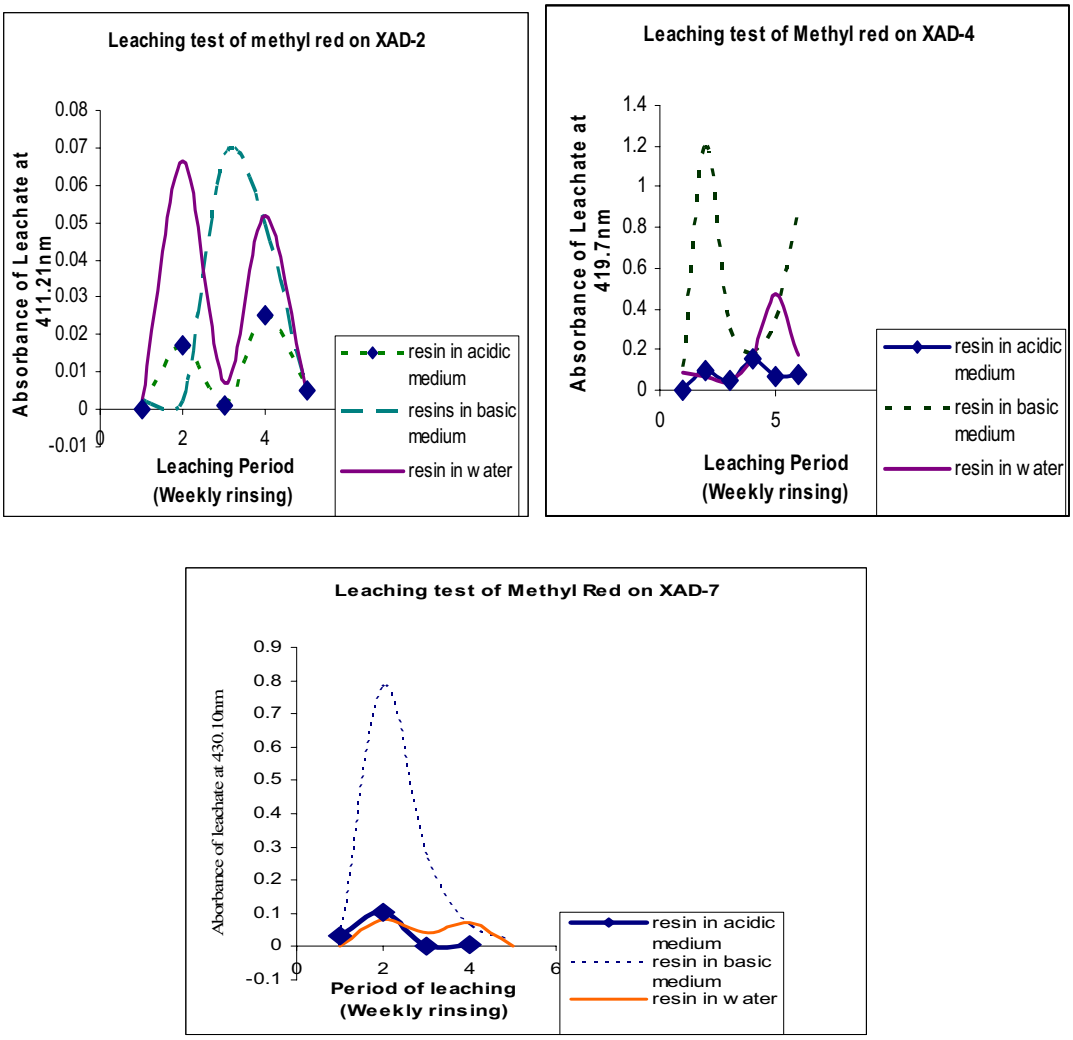


Fig 4.1.4c) shows the absorbance of leached indicators from XAD-2, XAD-4 and XAD-7 resins placed in different pH medium. The amount of absorbance indicates the amount of the indicators leached; hence more indicators have leached in the basic medium and water.

4.1.4.7) Response of immobilized indicator in buffer solutions of pH 2-12 after immobilization at 650 nm.

It was important to observe the response of these resins in the pH range of interest pH 5-8. Also if in-case these resins weren't so effective in pH 5-8, further investigation was carried out to confirm the pH range methyl red after immobilization on amberlite resins.

From the previous study at stage 4.1.4.5, it was depicted that methyl red after immobilization on XAD-2 shows good colour change within pH range 7-8. Based on these findings, series of trials were performed on this XAD-2 methyl indicator/resin combination.

The probe was immersed in successive pH buffers of pH 5, pH 6, pH 7 and pH 8 swapped at five minutes (300secs) intervals. There was a continuous decrease in signal when the probe was placed in higher pH buffers and low pH buffers (fig 4.1.4d). However Egami *et al.* (1996) reported a good response of methyl red in the range pH 5.0-7.0. The sensing probe was prepared by coating the naked core of the fiber with methyl red doped in poly (methyl methacrylate) (PMMA).

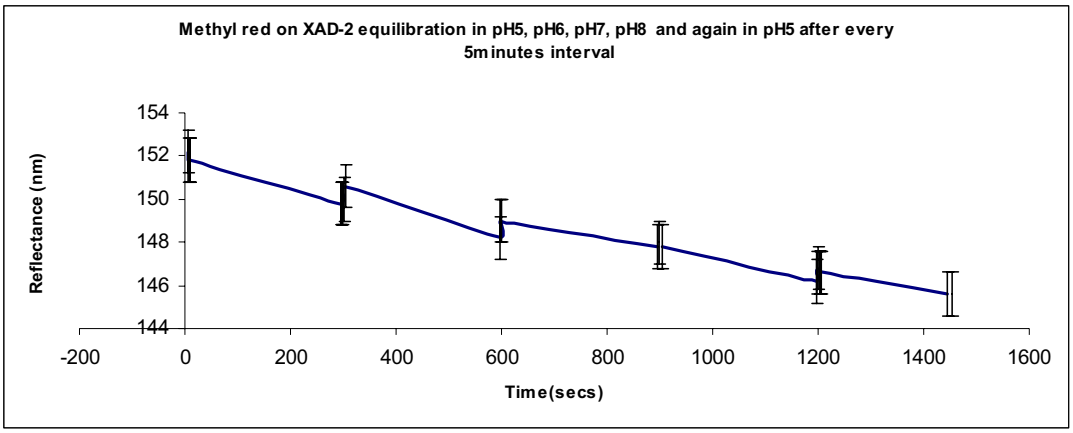


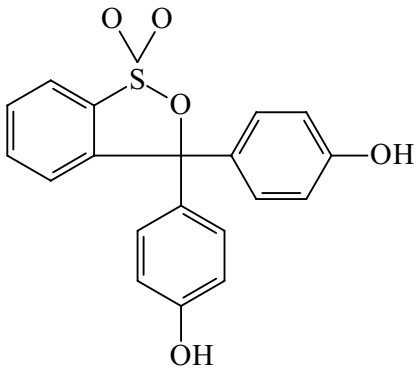
Fig 4.1.4d) The XAD-2 with methyl red equilibrated between pH 5-8. There is continuous decrease in signal both in acidic and basic conditions.

Methyl red immobilized on XAD-4 and XAD-7 completely leached out on washing, hence wasn't utilized for further testing.

4.1.5 Phenol Red

4.1.5.1). Structure of Phenol red and pKa of phenol red in solution:

Edmonds *et al.* (1988) gave the value of phenol red in solution as 7.7 while Sheppard and Guiseppi-Elie (1999) gave pKa of phenol red in solution as 7.9.



Phenol Red (phenolsulfonphthalein)

4.1.5.2) Absorbance of phenol red in buffer solutions of pH 5-8.

Phenol red in solution absorbed at 420.9nm and 558.6nm. The molar absorptivity of the base form of the indicator is generally greater than the absorptivity of acidic form hence response will be stronger for the basic form of the indicators. The protonated form of phenol red absorbs at 433nm and the deprotonated form of phenol red absorbs around 558nm (Sheppard and Guiseppi-Elie, 1999).

4.1.5.3) pH range of phenol red in solution

The indicator absorbed strongly in acidic and basic buffers. There was an increase in absorbance shown for the indicator in buffers 5.0-6.8. There is a continuous increase in absorbance with pH for pH 7.0-8.0 but the difference in response of indicator in this range is very limited (refer to fig 4.1.5a) .Hence the predicted range of phenol red in

solution was confirmed as between pH 5.0-8.0. The reflectance signal decrease continuously with increase in pH (refer to fig 4.1.5b). Benaim *et al*. (1986) also reported a good response of two tautomeric forms of phenol red in solution within pH 6.8-9.7.

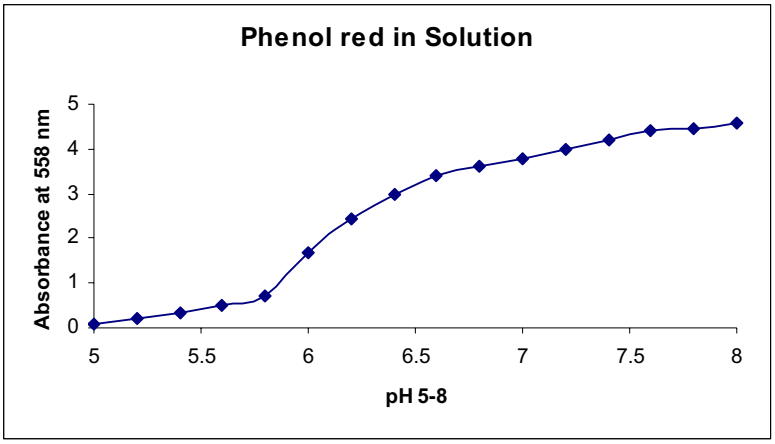


Fig 4.1.5a) shows the absorbance of indicator phenol red in buffers of pH 5.0-8.0. (±0.2) at 558nm

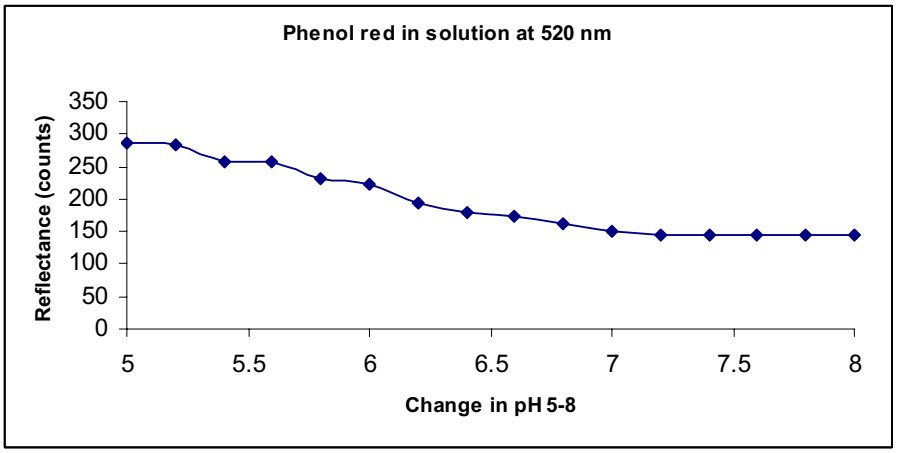


Fig 4.1.5b) shows the reflectance of indicator phenol red in buffers of pH 5.0-8.0. (±0.2).

4.1.5.4) Immobilization of phenol red on resins

Phenol red was also physically absorbed on amberlite resins; XAD-2, XAD-4 and XAD-7 by soaking the resins in the bath of the 0.1% indicator solutions prepared in methanol as mentioned in the method. The indicator on XAD-2 appeared creamy yellow in colour after immobilization. The indicator on XAD-4 appeared creamy light yellow in colour after immobilization. The indicator on XAD-7 appeared bright yellow in colour after immobilization.

4.1.5.5) pH range phenol red after immobilization

The resins with immobilized indicator were spread on a paper and drops of buffers of pH 2-12 were added onto it to observe the colour change of the immobilized indicators at different pH's.

Table 4.1.5a) The change in colour of the immobilized indicators when exposed to different pH buffers.

Indicator(initial color)	**Color change with pH**	**pH range**
Phenol red on XAD-2 (creamy yellow)	pH 2-5: no color change pH 5-6: light yellow pH 6-8: yellow pH 8-12: yellow	pH 5-8
Phenol red on XAD-4 (creamy light yellow)	pH 2-8: no color change pH 9: dark yellow pH10-12:dark reddish yellow	pH 8-12
Phenol red on XAD-7 (bright yellow)	pH 2-5: light yellow pH 6-7: creamy yellow pH 8: greenish yellow pH 9-12:maroonish orange	pH 5-9

4.1.5.6) Leaching Test

The three resins, XAD-2, XAD-4 and XAD-7 with phenol red were rinsed with buffers of low pH (pH $\leq$ 2) and high (pH $\geq$ 11) and distilled water to remove any excess and/or weakly adsorbed indicator molecules in the sensing medium before its application.

Phenol red leached heavily from the resins on washing. It was also observed that the indicator leached more in the basic medium and distilled water when compared to the

amount leached in the acidic medium. At high pH, the indicator molecules are converted to its ionic form which is more soluble in aqueous medium, hence more leaching is observed in high pH buffers.

Phenol red absorbed on XAD-7 leached more when compared with other two resins (refer to fig 4.1.5c). XAD-2 resins formed the strongest bound with phenol red. After 7 weeks of leaching test, phenol red leached out completely from amberlite XAD-7 but there little amount of the indicator was still left bound to the XAD-2 and XAD-4 to be utilized for further testing.

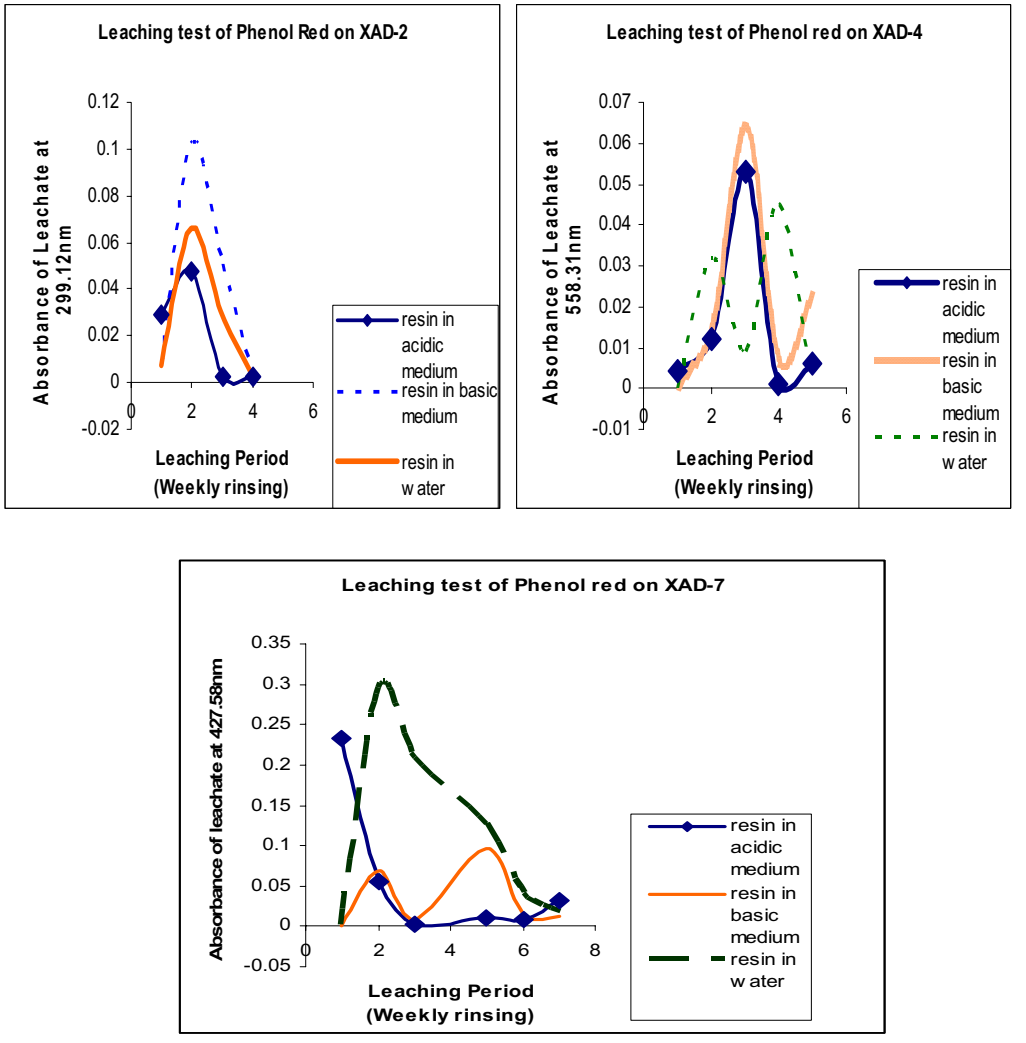


Fig 4.1.5c) Shows the absorbance of leached indicators from XAD-2, XAD-4 and XAD-7 resins placed in different pH medium. The amount of absorbance indicates the amount of the indicators leached; hence more indicators have leached in the basic medium and water.

4.1.5.7) Response of immobilized indicator in buffer solutions of pH 2-12 after immobilization at 650 nm.

It was important to observe the response of this resin in the pH range of interest pH 5-8. Also if in-case these resins weren't so effective in pH 5-8, further investigation was carried out to confirm the pH range of phenol red after immobilization on amberlite resins.

From the previous study at stage 4.1.5.5, it was depicted that phenol red after immobilization on XAD-2 shows good colour change within broad difference in pH of range 5-8. Based on these findings, series of trials were performed on this XAD-2 phenol red indicator/resin combination. In the first trial, the resin with the indicator was immersed in pH 5 and pH 8 buffers (refer to fig 4.1.5d). There wasn't any change in signal with variation in pH from pH 5-8.

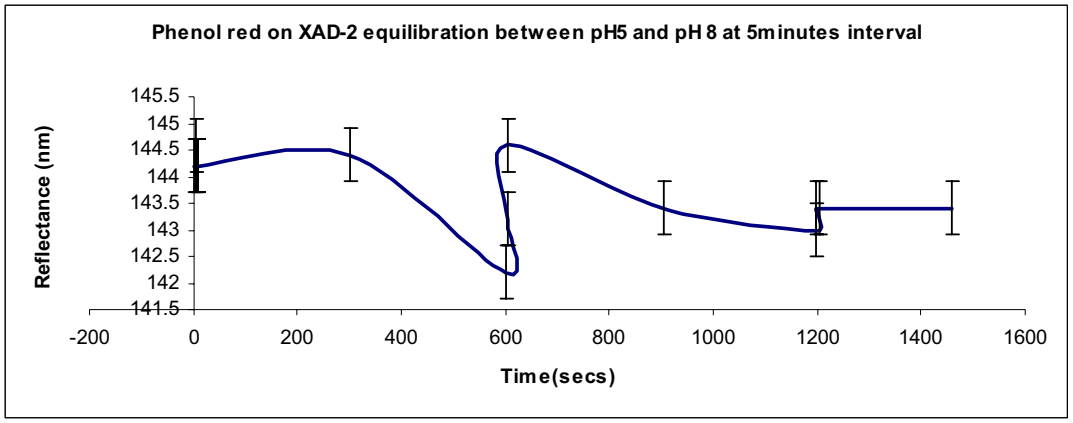


Fig 4.1.5d) shows XAD-2-phenol red probe equilibrated in pH 5 buffer (0-300secs, 600-900secs, 1200-1500secs) and pH 8 buffer (300-600secs, 900-1200secs).

More trials were performed on the probe to observe its response in pH buffers pH 12 and pH 2 swapped at five minutes (300secs) intervals. A good response was observed with increase in signal in the acidic medium and decrease in signal in the basic medium (refer to fig 4.1.5e).

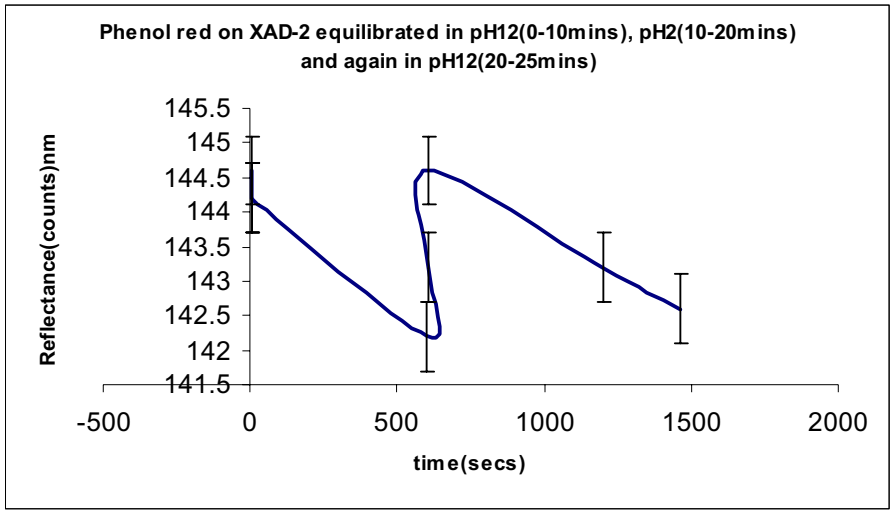


Fig 4.1.5e) shows XAD-2-phenol red probe equilibrated in pH 2 buffer (600-1200secs) and pH 12 buffer (0-10 mins, 1200-1500secs).

From the previous study at stage 4.1.5.5, it was depicted that phenol red after immobilization on XAD-4 shows good colour change within pH range 8-12. Based on these findings, series of trials were performed on this XAD-4 phenol red indicator/resin combination. In the first trial, the resin with the indicator was immersed in pH 8 and pH 12 buffers (refer to fig 4.1.5f). A very good response was observed on equilibration of the probe in this two pH values.

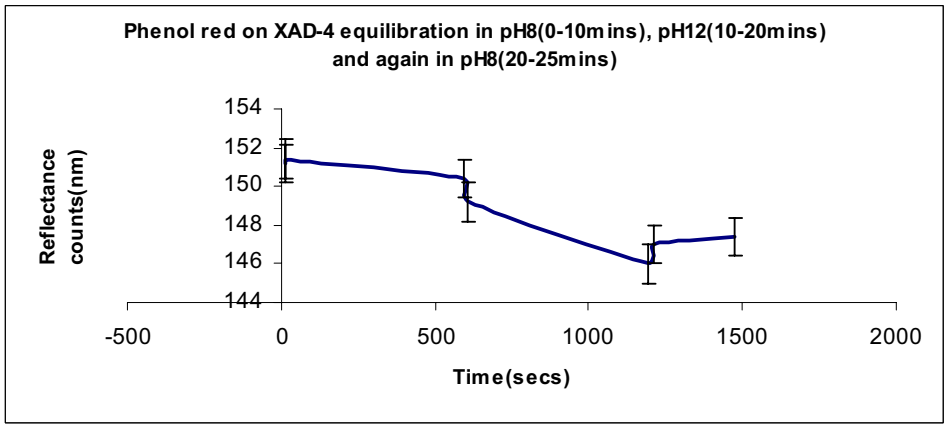


Fig 4.1.5f) XAD-4 with phenol red equilibrated in pH 8 and pH 12.

Two more trials were performed in sets of successive pH buffers of pH 8, pH 9, pH 10, pH 11 and pH 12 swapped at five minutes (300secs) interval from lower pH to higher and in reverse. There was a decrease in signal when the probe was placed in higher pH buffers and an increase in signal when it was placed in low pH buffers (refer to fig

4.1.5g,h). Hence phenol red immobilized on XAD-4 showed good response between pH 8-10. Motellier *et al*. (1995) have demonstrated the response of the probe with phenol red immobilized on XAD-4 at a range of pH 7-10.

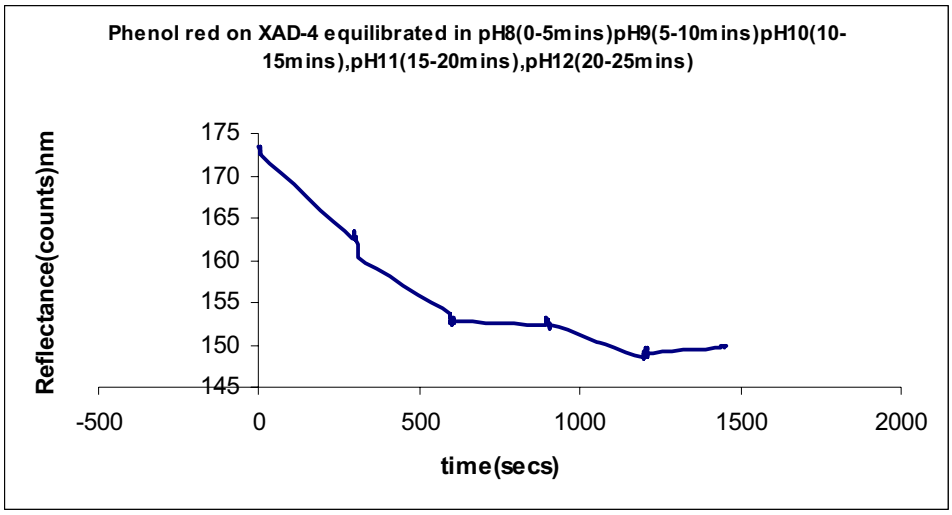


Fig 4.1.5g) XAD-4 with phenol red equilibrated in pH 8, pH 9, pH 10, pH 11 and pH 12 at 5 minutes intervals. The signal decreased with increase in buffer pH

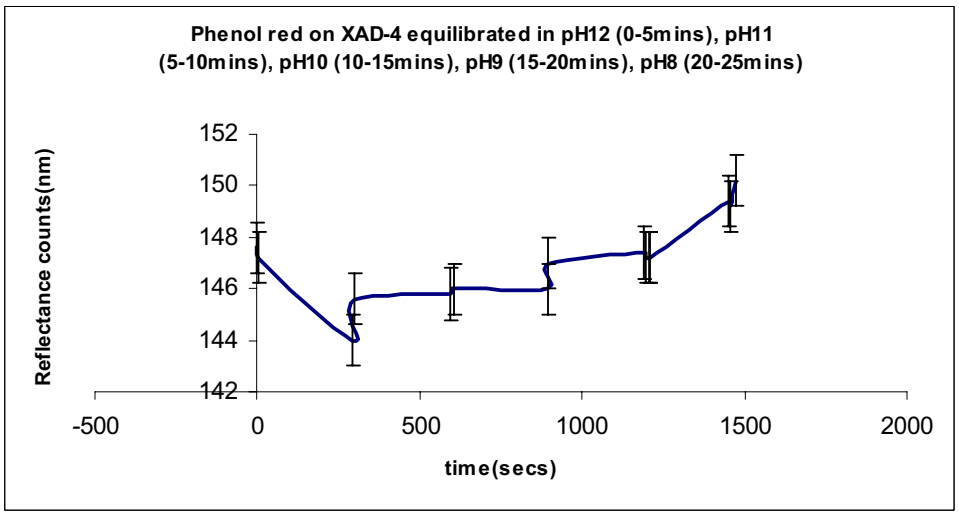


Fig 4.1.5h) XAD-4 with phenol red equilibrated in pH 12, pH 11, pH 10, pH 9 and pH 8 at 5 minutes intervals. The signal increased with decrease in buffer pH.

Phenol red absorbed on XAD-7 completely leached out after the cycle of washings in the leaching test hence it wasn't utilized for further testing.

4.2 FOCS-pH sensor in soil

4.2.1 Preparation of soil samples

A soil of pH 5 was taken to prepare the subsamples of soils of different pH. 0.1 M KOH was added to the soil solution of pH 5(prepared in water by ratio 1:2.5w/v; soil: water) to increase the pH of the soil.

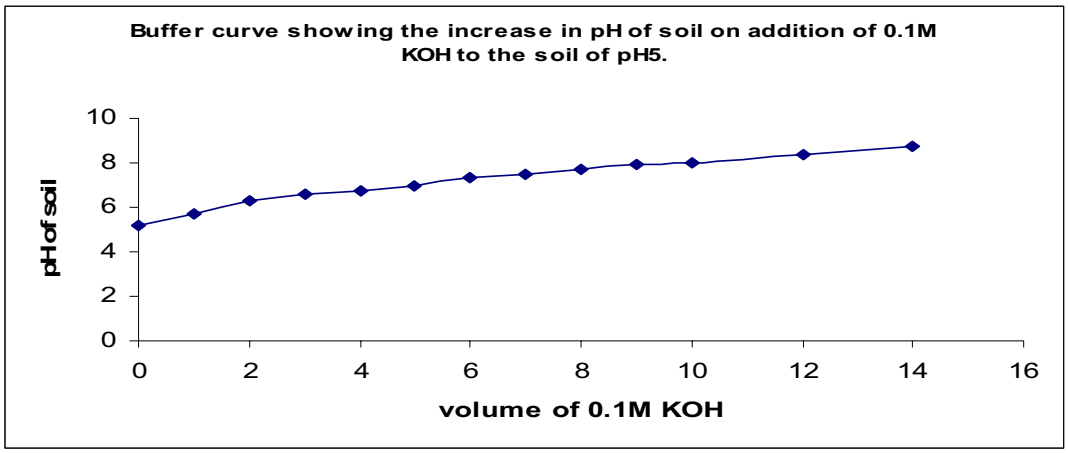


Fig 4.2.1a) shows the amount of 0.1 M KOH was added to increase the pH of soil solution (4 g in 10 ml water) from pH 5 to pH close to 8.

Determination of amount of $CaCO_3$ to be added to increase soil pH

To increase the pH of 4 g of soil of pH 5 to pH 8, approximately 13 ml of 0.1 M KOH was required.

4 g soil required 13 cm^3 of 0.1 M KOH

$$\frac{13 \times 0.1}{1000} = 1.0 \times 10^{-3} \text{ moles}$$

1 mol of $CaCO_3$ neutralizes 2mol of H^+

1 mol of $CaCO_3$ = 100g

100g of $CaCO_3$ will neutralize 2mol of H^+

So for neutralization of 1mol of H^+ = 50g of $CaCO_3$ is required

For 1.3×10^{-3} mol = $1.3 \times 10^{-3} \times 50$

$= 6.5 \times 10^{-2}$ g

6.5×10^{-2} g of $CaCO_3$ is required to increase pH of 4 g of soil sample from pH 5 to close to pH 8.

For 60 g of soil sample;

6.5×10^{-2} g of $CaCO_3 \rightarrow$ 4 g of soil

$\times \rightarrow$ 60g of soil

$\times \rightarrow$ 0.975g of $CaCO_3$ is needed to increase pH of 60g of soil sample from pH 5 to pH 8.

The subsamples of soil were prepared by adding 0.95 g, 1.1 g and 1.3 g of $CaCO_3$ to 60g of soil. The pHs of the prepared subsamples was between pH 7.8-8.2.

To determine the least amount of moisture required that would be sufficient for sensing the pH of soil, a series of soil subsamples were prepared with different moisture content at ratios 1:2.5, 1:1.5, 1:1 and 1:0.5 (w/v ; soil: water).

4.2.2 Response of FOCS in soil

From the results of stage 4.1, it was determined that bromocresol green adsorbed on all three resins, XAD-2, XAD-4 and XAD-7 were the most appropriate resin/indicator combinations to be utilized for pH sensing of soil in the range pH 5-8. The probes were prepared with each resin attached to the sensing tip of the fiber optic chemical sensor. A porous nylon material was clipped around the probe with a “o” ring to hold the resins in position. The probes were equilibrated between pH 5 buffer and pH 8 soil and vice versa in pH 5 soil and pH 8 buffer.

XAD-2 with bromocresol green was equilibrated in pH 5 buffer and all pH 8 sub samples of soils prepared with moisture contents (1:2.5, 1:1.5, 1:1, 1:0.5 w/v soil:water).

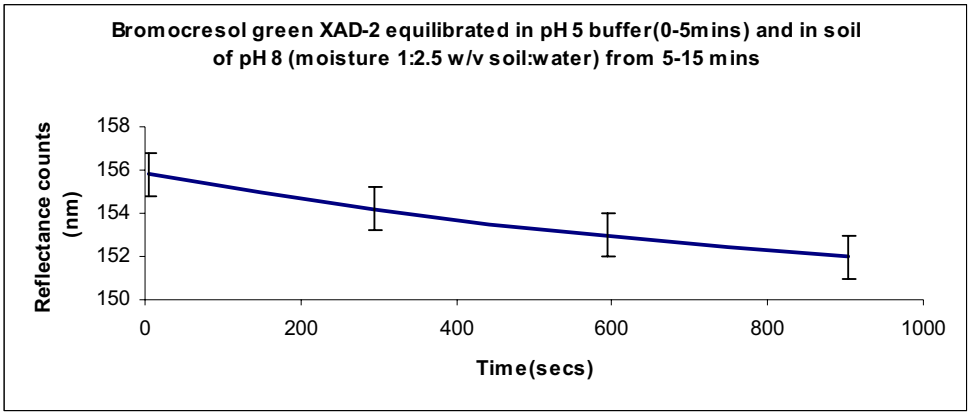


Fig 4.2.2 a) Response of bromocresol green on XAD-2 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:2.5 w/v; soil : water

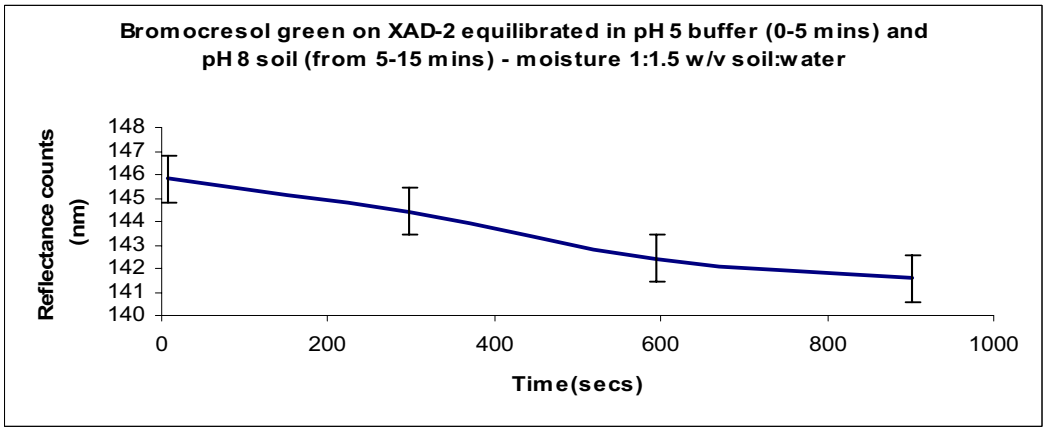


Fig 4.2.2 b) Response of bromocresol green on XAD-2 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:1.5 w/v; soil : water

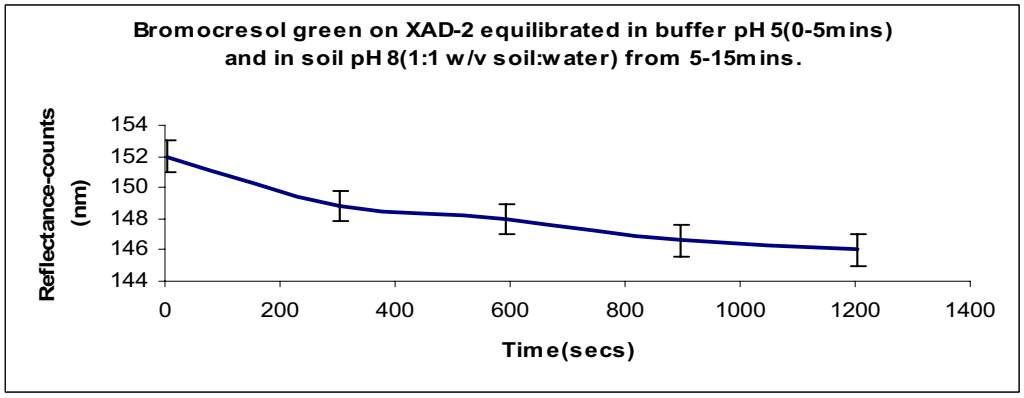


Fig 4.2.2 c) Response of bromocresol green on XAD-2 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:1 w/v; soil : water

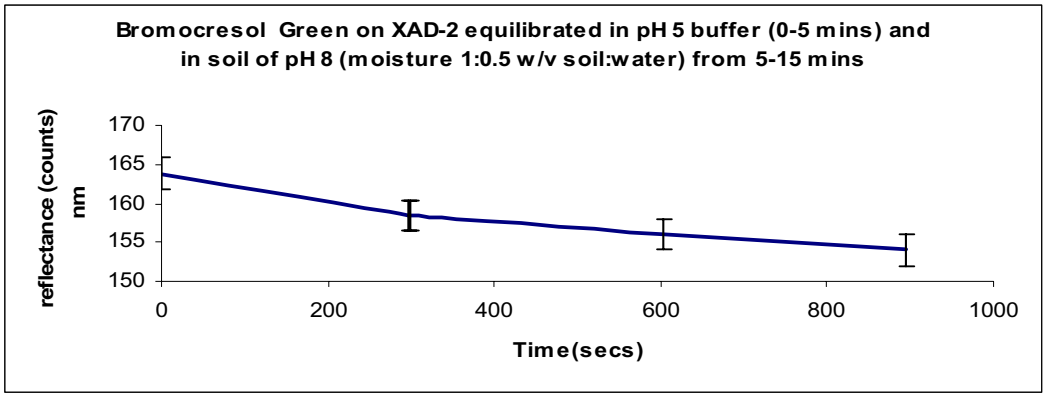


Fig 4.2.2 d) Response of bromocresol green on XAD-2 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:0.5 w/v; soil : water

XAD-2 with bromocresol green was also equilibrated reversely from more basic pH in pH 8 buffer to acidic soil solution of pH 5 with moisture contents (1:2.5, 1:1.5, 1:1, 1:0.5 w/v soil: water) to observed the response in reverse conditions.

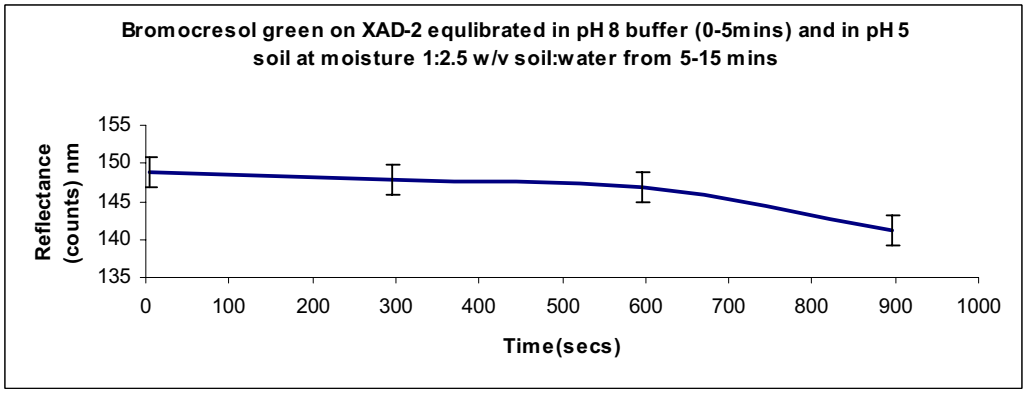


Fig 4.2.2 e) Response of bromocresol green on XAD-2 equilibrated in pH 8 buffer and pH 5 soil at moisture 1:2.5 w/v; soil : water.

XAD-4 with bromocresol green was equilibrated in pH 5 buffer and all pH 8 sub samples of soils prepared with moisture contents (1:2.5, 1:1.5, 1:1, 1:0.5 w/v soil:water).

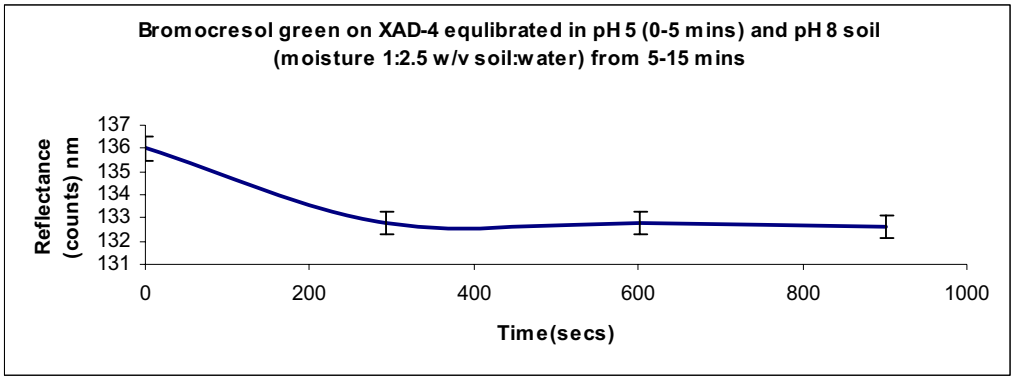


Fig 4.2.2 f) Response of bromocresol green on XAD-4 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:2.5 w/v; soil : water

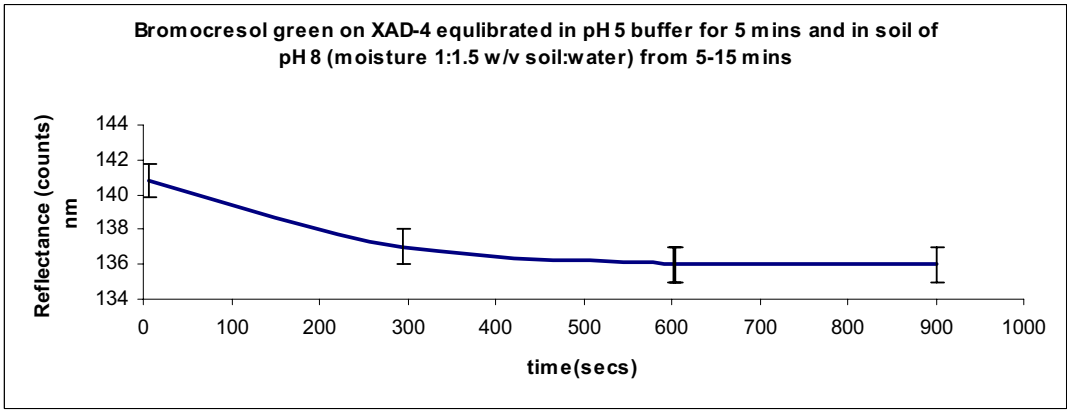


Fig 4.2.2 g) Response of bromocresol green on XAD-4 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:1.5 w/v; soil : water

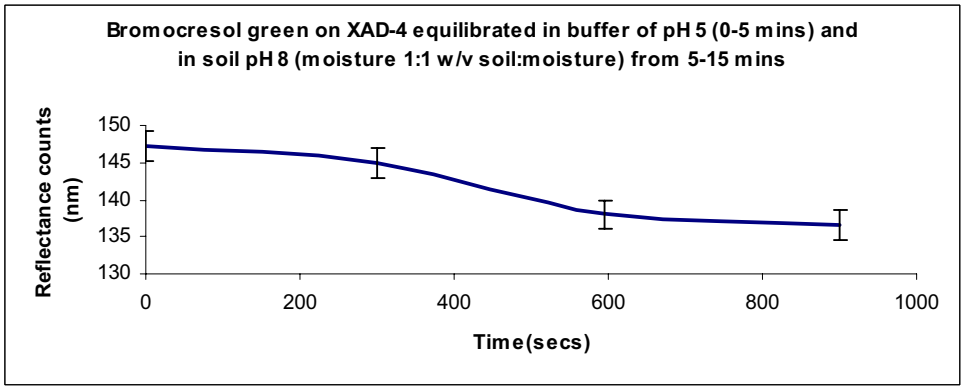


Fig 4.2.2 h) Response of bromocresol green on XAD-4 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:1 w/v; soil : water

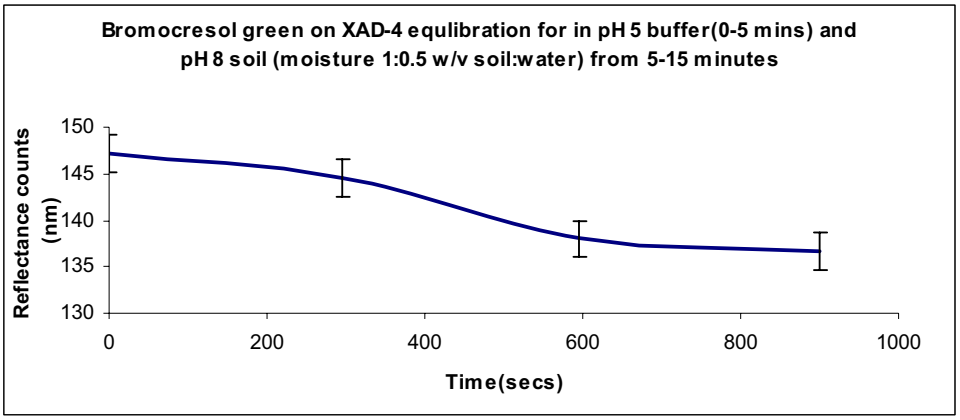


Fig 4.2.2 i) Response of bromocresol green on XAD-4 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:0.5 w/v; soil : water

XAD-4 with bromocresol green was also equilibrated reversely in pH 8 buffer and all pH 5 subsamples of soils prepared with moisture contents (1:2.5, 1:1.5, 1:1, 1:0.5 w/v soil: water) to observed the response in reverse conditions.

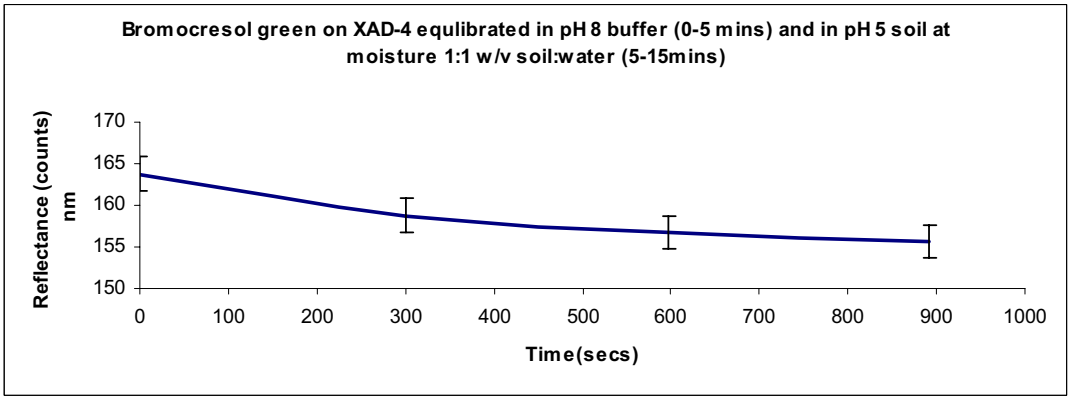


Fig 4.2.2 j) Response of bromocresol green on XAD-4 equilibrated in pH 8 buffer and pH 5 soil at moisture 1:1 w/v; soil : water

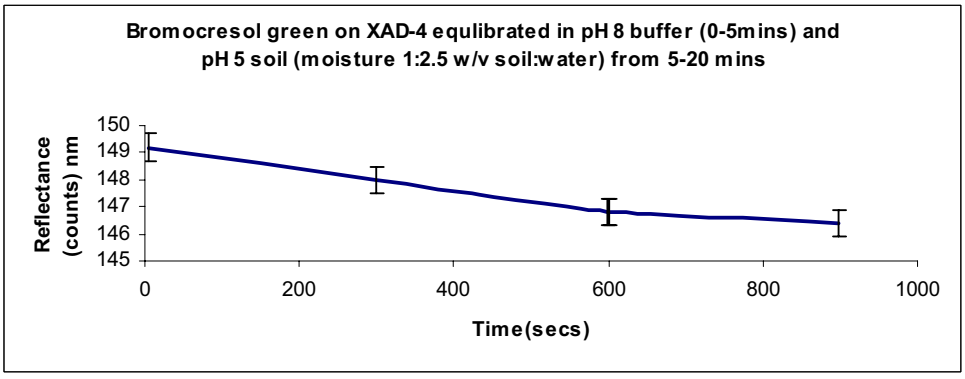


Fig 4.2.2 k) Response of bromocresol green on XAD-4 equilibrated in pH 8 buffer and pH 5 soil at moisture 1:2.5 w/v; soil : water.

XAD-7 with bromocresol green was equilibrated in pH 5 buffer and all pH 8 sub samples of soils prepared with moisture contents (1:2.5, 1:1.5, 1:1, 1:0.5 w/v soil:water).

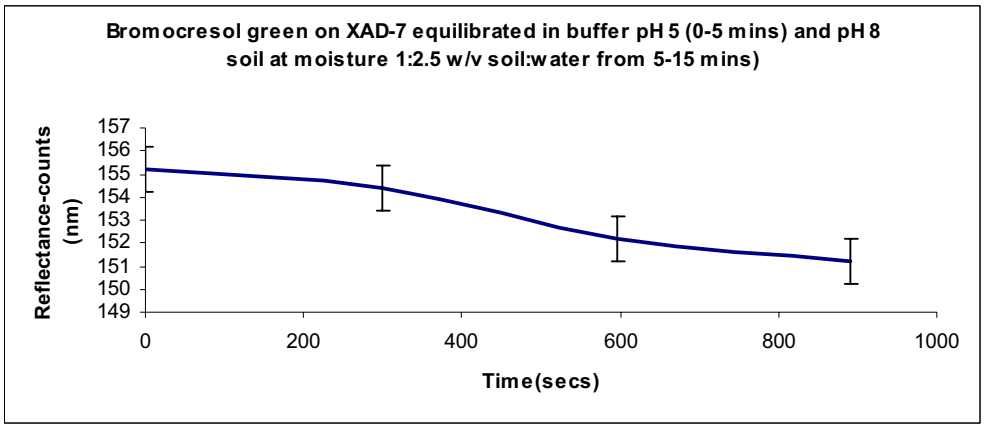


Fig 4.2.2 l) Response of bromocresol green on XAD-7 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:2.5 w/v; soil : water

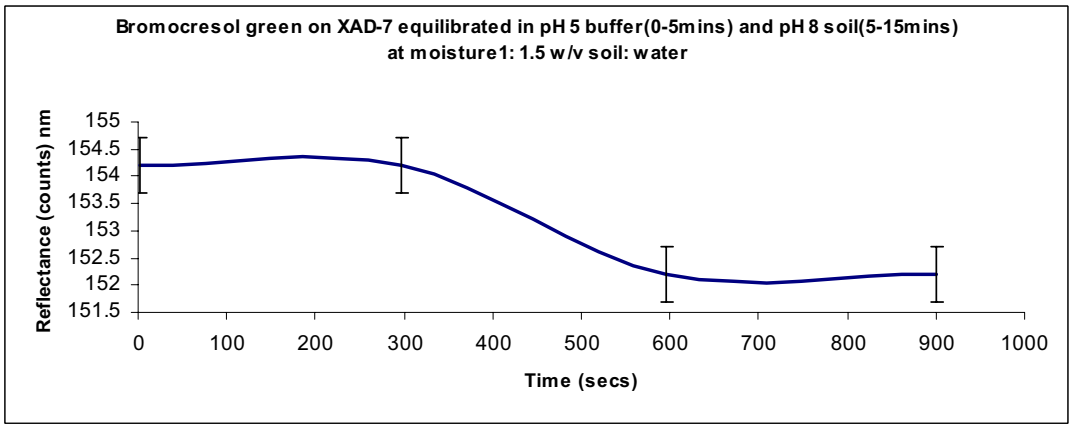


Fig 4.2.2 m) Response of bromocresol green on XAD-7 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:1.5 w/v; soil : water

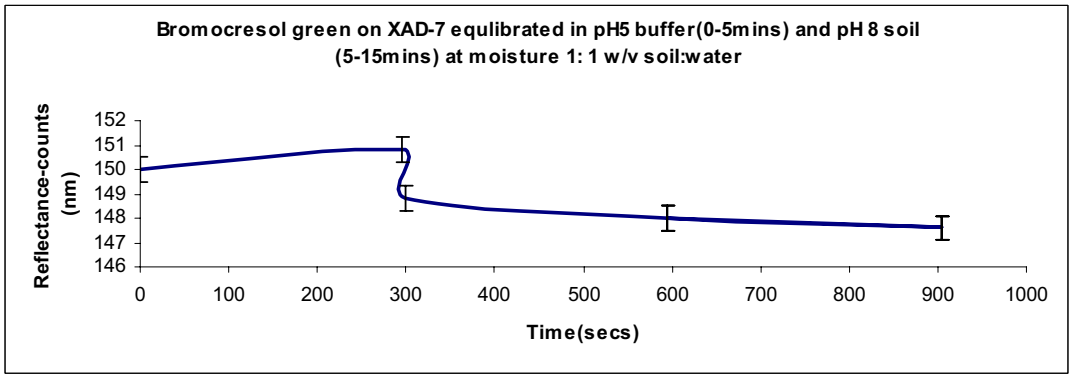


Fig 4.2.2 n) Response of bromocresol green on XAD-7 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:1 w/v; soil : water

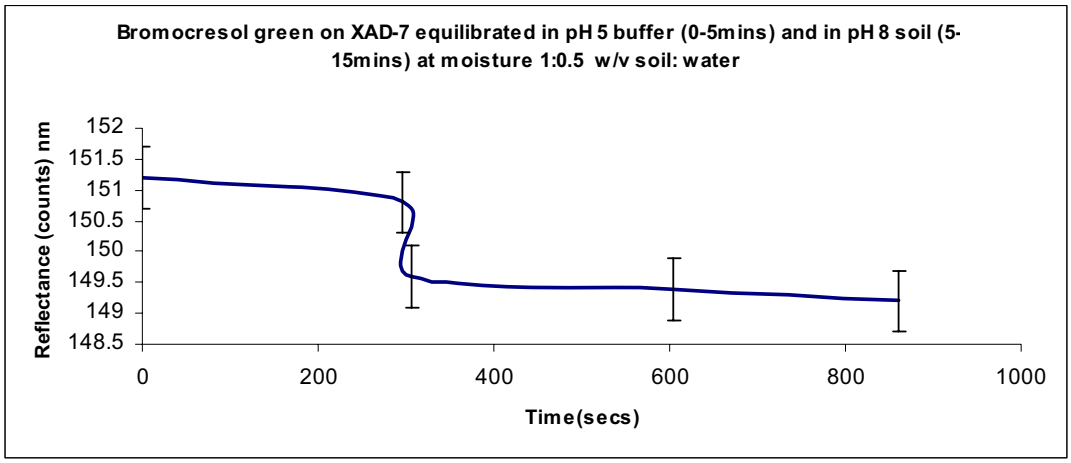


Fig 4.2.2 o) Response of bromocresol green on XAD-7 equilibrated in pH 5 buffer and pH 8 soil at moisture 1:0.5 w/v; soil : water

XAD-7 with bromocresol green was also equilibrated reversely in pH 8 buffer and all pH 5 subsamples of soils prepared with moisture contents (1:2.5, 1:1.5, 1:1, 1:0.5 w/v soil: water) to observed the response in reverse conditions.

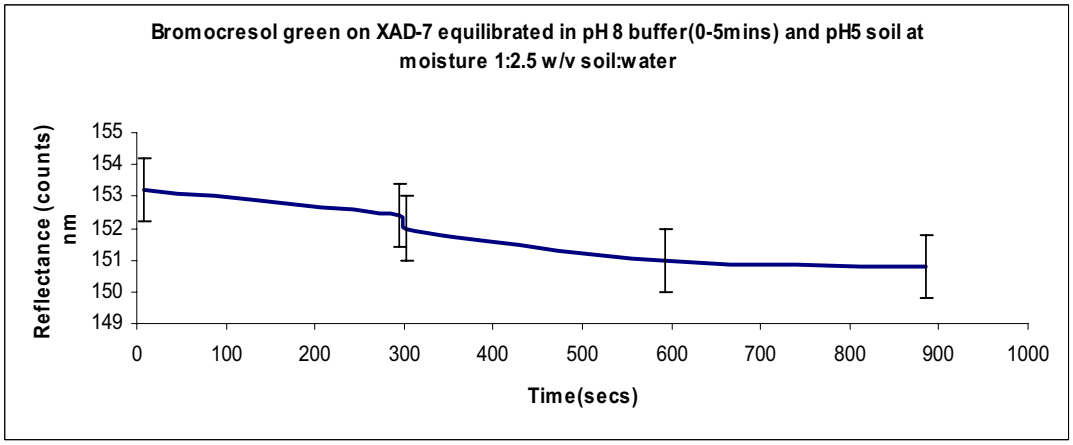


Fig 4.2.2 p) Response of bromocresol green on XAD-7 equilibrated in pH 8 buffer and pH 5 soil at moisture 1:2.5 w/v; soil : water

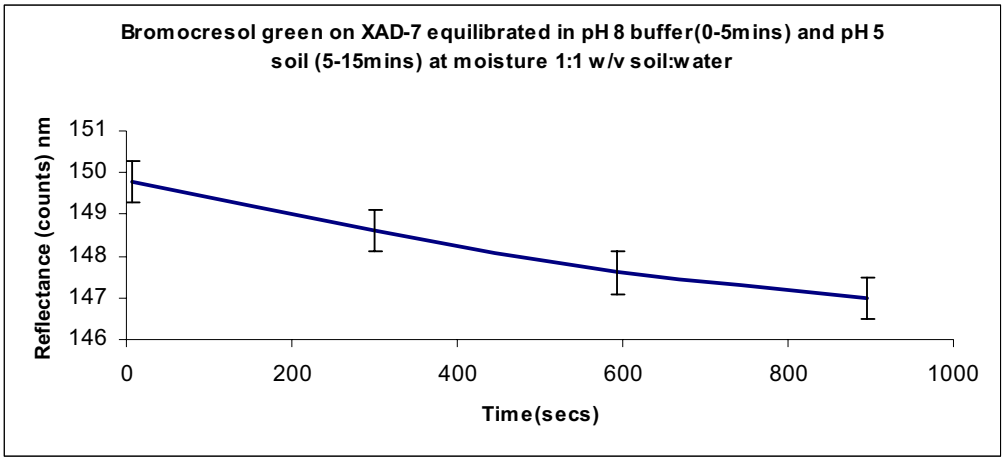


Fig 4.2.2 q) Response of bromocresol green on XAD-7 equilibrated in pH 8 buffer and pH 5 soil at moisture 1:1 w/v; soil : water

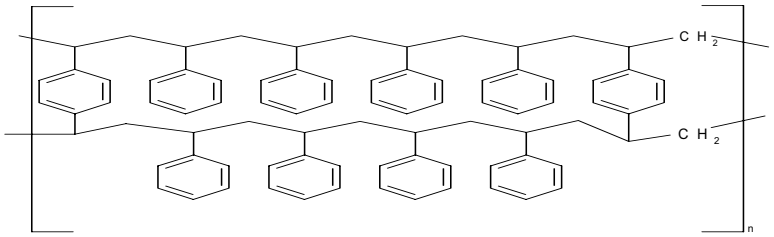
4.3 Discussion

4.3.1 Response in Buffer solution

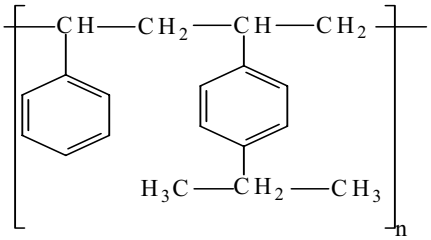
The pH range of indicator solutions for each indicator was as follows; bromocresol green responded best between pH 5.0-5.6, bromocresol purple responded best in the range pH 5.0-8.0, Bromothymol blue responded best the range pH 5.0-8.0, methyl red responds best in pH 5.0-6.2 and pH 7.0-8.0, and phenol red responded best in the range pH 5.0-8.0. The pH of the solution was taken before and after addition of indicators. There wasn't any shift in pH values of buffers after addition of any indicators.

4.3.2 The characteristics of the membrane Amberlite resins and the shift in pH range of indicators after immobilization.

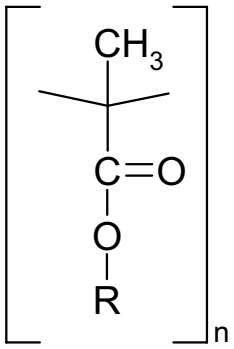
All the five indicators were immobilized on the three Amberlite resins, XAD-2, XAD-4, and XAD-7.



Amberlite XAD – 2: Cross linked styrenedivinylbenzene



Amberlite XAD – 4



Amberlite XAD – 7: Cross linked methylmethacrylate

According to the suppliers of Amberlite resins Sigma-Aldrich Company and Rohm and Hass Company, XAD-2 polymeric adsorbent is a hydrophobic crosslinked polystyrene copolymer resin, supplied as 20-60mesh size white insoluble beads. Amberlite XAD-2 resin is characterized by its unique macroreticular porosity, broad pore size distribution and large surface area, and a chemically homogeneous nonionic structure. Each bead consists of an agglomeration of many very small microspheres, giving a continuous gel phase and a continuous pore phase. The open-cell porous structure allows aqueous solutions to penetrate the pores easily. Amberlite XAD-4 is a polymeric adsorbent, supplied as white insoluble beads. It is a non ionic crosslinked polymer. It has high surface area and the aromatic nature of its surface gives the physical adsorbent an excellent physical, chemical and thermal stability which makes it an excellent choice for the adsorption of organic substances of relatively low molecular weight.

Baker and Narayanaswamy, (1995) have explained how the electronic properties of the support matrix increases the pKa of the immobilized indicator for electron donating matrices such as XAD-2, XAD-4 and XAD-7 with indicator immobilized via adsorption. The increase in pKa was explained in terms of the formation of π - π electron donor-

acceptor complex between the XAD (hydrophobic styrene/divynl benzene copolymers) matrix as the donor and the bromine substituted phenyl ring of the indicator as the acceptor. Donation of π e^- from resins causes a destabilization effect on the phenolate anion of the indicator.

4.3.3 Leaching Effect

The indicators were very weakly bound to the resin XAD-7 as compared with the other two types of resins. Much of the indicators bound on XAD-7 leached out on washing. XAD-2 formed the strongest bound with almost all the indicators with very little amount of the indicator being leached. Methyl red and phenol red had weak binding with all the resins whereby very little amount of or no indicator remained on the resin after continuous rinsing of the resin with the buffers during the leaching test. Bromocresol green and bromothymol blue had the most effective bonding with all the resins.

On washing with buffers and water, methyl red, phenol red and bromocresol purple completely leached out from XAD-7. Bromocresol green gradually leached out from XAD-7. Methyl red also leached out completely from the XAD-4 resins.

4.3.4 Response time and Reproducibility in reverse trials.

The response time of all the indicators after immobilization was < 20secs. However, indicators absorbed on XAD-2 took longer time to equilibrate as compared to indicators on other resins. Whereas the indicators attached on XAD-7 were the fastest in response.

The reproducibility of the probe was very poor mainly because of the intense leaching of the indicators. All the probes were first equilibrated between acidic medium to basic medium and vice-versa. It was observed that there was a quicker response of the

indicators when it was equilibrated from acidic medium to a basic medium. When the probe was placed back into acidic medium, though the response was fast but there was very little change in signal intensity (refer to figures; 4.1.2g, 4.1.3e and 4.1.5f).

4.3.4 The most suitable indicator/ resin combination for monitoring soil pH

The optimum pH range of soils for proper plant growth is between pH 5-8. Out of the 15 indicator/resin combination, four of them weren't utilized for further testing because all indicators leached out on washing. These were; methyl red on XAD-4 and XAD-7 (fig 4.1.4c), phenol red on XAD-7 (fig 4.1.5c) and bromocresol purple on XAD-7 (fig 4.1.2c). Methyl red and phenol red leached heavily from all the resins; hence it has shown weak reproducibility of results.

There was a shift in the pH range of all indicators after immobilization on respective resins (table 4.1.1a, 4.1.2a, 4.1.3a, 4.1.4a, 4.1.5a). Bromocresol green absorbed on XAD-2 had pH range 5-8; bromocresol green absorbed on XAD-4 had pH range 4-7 and bromocresol green on XAD-7 had pH range 6-12. Bromocresol purple absorbed on XAD-2 had very little response even in wide difference in pH from pH 2 and pH 12 and bromocresol purple absorbed on XAD-4 also had very little response even in wide difference in pH from pH 2 and pH 12. Bromothymol blue absorbed on XAD-2 had responded only with wide difference in pH for pH 2 and pH 12; bromothymol blue absorbed on XAD-4 also only responded for wide difference in pH of 2 and 12 and bromothymol blue on XAD-7 had pH range 8-12. Methyl red absorbed on XAD-2 showed no response in the pH range 5-8 hence is only suitable for wide difference in pH such as pH 2 and pH 12. Phenol red absorbed on XAD-4 responded well in the pH range 8-12 while phenol red absorbed on XAD-2 only response in wider difference in pH.

Bromocresol green on the three resins XAD-2, XAD-4 and XAD-7 (fig 4.1.1d-h) showed good response in pH 5-8 (the optimum soil pH range). However, bromocresol green leached heavily from XAD-7 (fig 4.1.1c). Bromocresol green was also slower in response after immobilization on XAD-2 (fig 4.1.1d).

Hence in terms of stability (fig 4.1.1c), reproducibility and response time (as discussed above), bromocresol green adsorbed on XAD-4 proves out to be the most appropriate indicator/resin combination to be utilized for monitoring of pH of soil.

4.3.5 Summary of response in soil

The response of the probes with bromocresol green absorbed on amberlite XAD-2, XAD-4 and XAD-7 was very similar. The reproducibility of the results wasn't reliable since the signals decreased at both basic and acidic conditions (refer to figures 4.2.2a-4.2.2q). This limitation was due to the interference of soil particles. The fine soil particles penetrated through the nylon membrane and got settled around the indicator vicinity at the sensing tip. These particles darken the whole zone at the sensing tip, which shows a quick drop in the signal. The soil particle interference was observed during equilibration of soils at all moisture content but it was more intensely common for soils at lower moisture contents of 1:1w/v and 1:0.5w/v; soil: water. This interference by the soil particles prohibits observing the true activity of the soils liquid layer with H^+ with the immobilized indicator.

4.4 Optimization

4.4.1 Standardization of peaks of FOCS for indicator in solution form

Initially the Fiber-optic instrument was giving problems in response for analysis of indicators in solution form, as there were fluctuating signals for one sample itself. This was due to the path length of light going through the sample to the next reflecting surface. To standardize the signals, the path length was monitored. The probe was modified by designing a probe case (refer to fig 4.4.1a) which would allow the probe to have a consistent amount of sample entering in to the probe (the probe case had two holes each side to allow sample in) and have consistent path length for all running samples (on top of the cover, was a lid that holds the probe to one distance from the reflecting surface which in this case was a mirror attached at the bottom of the case for total reflection of light).

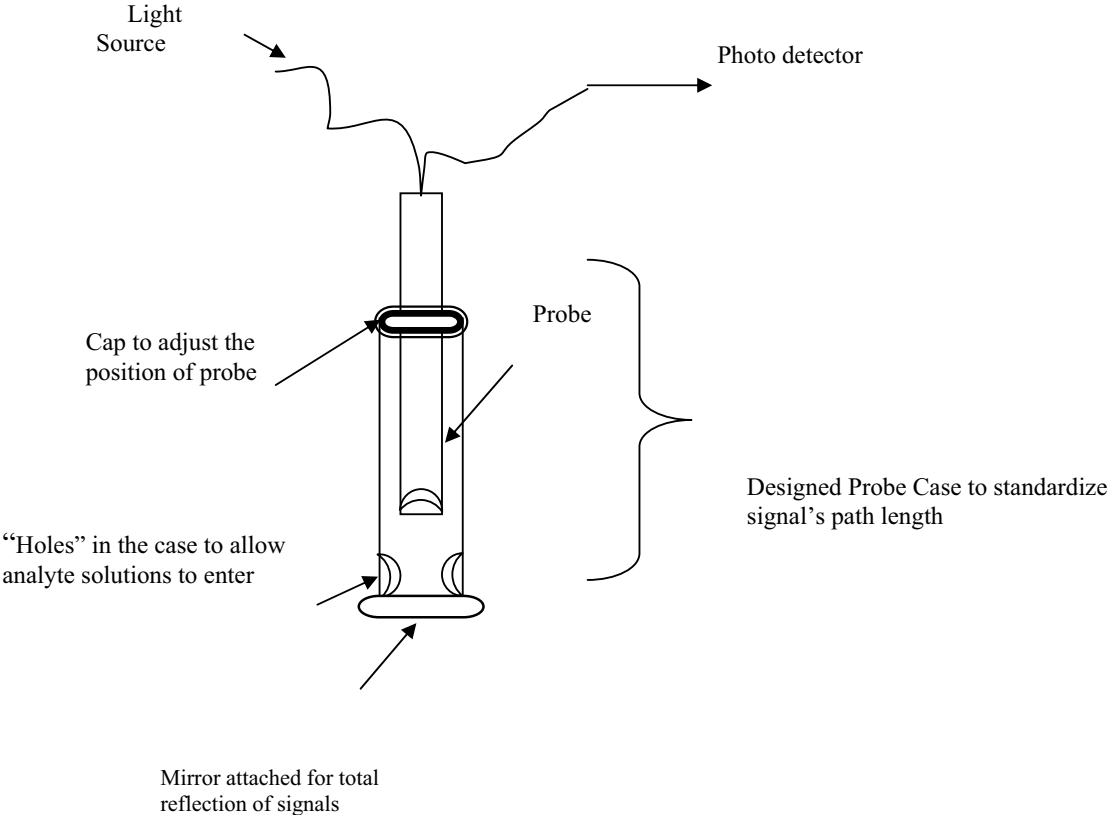


Fig 4.4.1 a) Design of probe set-up for standardization of signal at equal path length.

4.4.2 Immobilization Technique

Little amount of the indicator at various concentrations was applied on the polyacetate and polystyrene films and was left stand for certain period of time. The films with coated indicators were then rinsed with distilled water to see any leaching and it was observed that the indicator completely leached out after frequent washing.

This signifies that polymer films are not appropriate binding membranes for the indicator to be immobilized on. However the surface of the polymer can be activated with reagents giving functional groups that would help to bind with the indicators depending on the structure of the indicators. But it is a tough task to decide on those activating reagents at this stage with consideration that the activated polymer doesn't affect optical properties of the indicator.

4.4.3 Soil particle interference

The penetration of soil particles to the vicinity of the sensing area can be minimized if less porous membranes or filters are used that would stop penetration of soil particles and should only permit the aqueous layer to pass through. Attempts have been made to overcome this limitation. Less porous filters were fixed at the tip. It minimized the penetration of soil particles but micro filters cracks very easily and is non-durable to fit the shape of the probe. Hence the filters should be pasted on a flat surface which should be attached to the tip. It will also be appropriate to have less pressured intact of the sensing probe to the soil sample. Allowing the probe to stay in very slight contact with the soil has minimized the problem of soil particle penetrations but it also increases the response time. The delay would be due to mass transfer between the different phases that requires more time to attain chemical equilibrium between the phases.

The following diagram is the proposed design of the sensing system which can enable proper measurement of soil pH using the fiber-optic pH sensor.

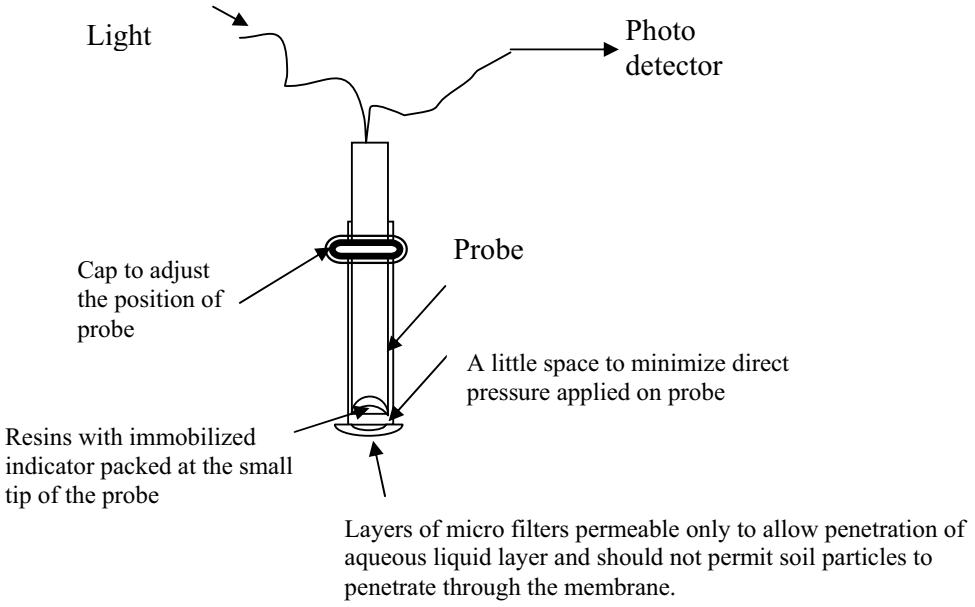


Fig 4.4.3 a) Design of probe for limiting soil particle interference.

CONCLUSION AND RECOMMENDATIONS

The soil pH varies over relatively short time scales which highly affects the mobility of plant nutrients. The optimum range of pH 5-8 is required for proper plant growth and to maintain the soil pH within this range, continuous monitoring of soil pH is very essential. Previously soil pH was analyzed using pH meters, taking photometric or electrometric measurements and the colorimetric method. In these techniques mostly soil was sampled and analyzed via preparation of soil solutions or by applying indicators. The plants uptake essential nutrients from its roots hence in order to have proper control of pH for proper plant growth, continuous real-time monitoring of pH of the soil around the roots of the plant is necessary rather than monitoring the pH of the bulk soil. The small size flexible fiber-optic sensor can be employed for monitoring of soil pH at the inaccessible vicinity of the plant roots.

In this study, pH indicators sensitive at the range of pH 5-8 were physically adsorbed on the amberlite resins XAD-2, XAD-4 and XAD-7. The selected indicators were; bromocresol green, bromocresol purple, Bromothymol blue, methyl red and phenol red. Some of the pH sensitive dyes mainly bromothymol blue and phenol had previously been applied in some other studies. However the method of development of the sensor using all three resins and its application in monitoring soil pH wasn't practiced in literature before. The leaching tests have indicated that this method of immobilization was most suitable for resin XAD-2 and indicators Bromothymol blue and bromocresol green. The XAD-7 resin was the weakest binder with all the indicators. Certain indicators such as methyl red, phenol red and bromocresol purple leached out mostly from all the membranes. The pH range of the respective indicators before and after immobilization was determined. It was observed that there was a shift in pH range of the indicators after immobilization due to the shift of the pKa values of the indicators on the electron

donating resins. The immobilized phenol red, Bromothymol blue and bromocresol green showed very effective and reproducible response in aqueous buffers within pH 2-12.

Based on the response of the indicators after immobilization and the stability of the probe, the most suitable resin/indicator for sensing pH of soil was chosen out of the prepared fifteen combinations. Bromocresol green adsorbed on all the three resins proved out to be the best responding indicator at the range pH 5-8. The three probes were equilibrated between acidic buffers and basic soil and vice versa for all soils prepared at moisture contents of ratios (1:2.5w/v, 1:1.5w/v, 1:1w/v and 1:0.5w/v) soil: water.

The reproducible equilibration of the immobilized indicator in the soil samples was difficult to attain due to the interference of the soil particles at the vicinity of the sensing tip. The soil particle penetrated through the nylon membrane settles around the sensing tip and darkens the atmosphere which eventually decreases the signal during equilibration at both low and high pH. This limitation was common for the indicator on all the three resin and was more obvious in muddy soil samples will lower moisture content.

Several attempts have been made to overcome this limitation. A less porous membrane should be utilized that would only be permeable for liquid layer of soil with H^+ ions and will not permit penetration of soil particles. A design with attached micro-filters near sensing tip of the probe is described in Fig 4.4.3 a) that would avoid soil particle penetration and can be used for measurement of soil pH.

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