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Title Investigation of the colorimetric measurement of pH and metal ions by using reagents doped in sol-gel glasses for potential on-line monitoring

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INVESTIGATION OF THE COLORIMETRIC MEASUREMENT OF pH AND METAL IONS BY USING REAGENTS DOPED IN SOL-GEL GLASSES FOR POTENTIAL ON-LINE MONITORING

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A thesis submitted to the University of Luton for the degree of Doctor of Philosophy

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DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of Luton. It has not been submitted before for any degree or examination in any other University.

Muhammad Azhar Javaid
13th day of July, 1998.

INVESTIGATION OF THE COLORIMETRIC MEASUREMENT OF pH AND METAL IONS BY USING REAGENTS DOPED IN SOL-GEL GLASSES FOR POTENTIAL ON-LINE MONITORING

M A JAVAID

ABSTRACT

Sol-gel porous glasses have been doped with indicator molecules for colorimetric measurements of pH and metal ions in solution. pH measurements were made in real time (20 seconds) to a wide pH range 3-8 by using bromophenol blue doped in sol-gel thin films. New methods of sol-gel coating on the inside of test tubes and tubing have been introduced for simple, non invasive, on the spot chemical sensing. pH indicator doped films were also successfully autoclaved for biological applications without affecting their chemical and physical properties. The pore structure of thin films has been controlled for minimising the effect of ageing on response time by introducing dimethyl formamide (DMF). The effect of light, temperature and salt on thin films have been studied. The results show that they are relatively stable between 20-37^0 C and less affected (< 0.03 absorbance unit decrease in 3 weeks) by light. However their response to pH is changed by adding salt in solution with concentration higher than 0.01M. Fourier transform infra red (FTIR) study of films has been conducted to elucidate the effect of ageing, DMF and autoclaving on their chemical structures. It was found that ageing continues after four weeks of fabrication and addition of DMF helps to reduce ageing and increase porosity. The long term stability of these pH indicator doped films in various solvents has been established. Thin films on microscope slides were deposited by using a newly designed spin coater and have been demonstrated as reusable pH slides. Sol-gel films were also doped by different metal reagents. Eriochrome cyanine doped thin films were found to be sensitive to copper ions in solution. Copper (Cu^{++}) was measured to a low concentration of 0.6 ppm. The effect of light and temperature on Eriochrome copper complex was studied. Interferences of other metal ions were examined.

A fibre-optic pH sensor has been demonstrated by coating an optical fibre with a sol-gel film (0.8 μm thick) doped with bromophenol blue. The sensor has shown fast response (5 seconds) to pH changes from pH 3 to 8 and no leaching or cracking during repeated use. It is simple to fabricate and easy to use as an interchangeable pH fibre probe. It has potential application in biological processes as an integral part of an on-line monitoring system.

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CHAPTER 1. GENERAL INTRODUCTION

1.1 THE IMPORTANCE OF pH

The term pH is used to describe the hydrogen ion activity of a system. It is defined by the expression pH = -log aH^+. Here aH^+ is the activity of the hydrogen ion. In dilute solutions the activity is essentially equal to the concentration and the pH may be approximately defined as pH = -$\log_{10}$ [H^+], where [H^+] is the concentration of the hydrogen ion in moles per litre. The use of pH makes negative exponents unnecessary in describing the hydrogen ion activity. In a system in which the hydrogen ion activity is 10^{-3} moles per litre, the pH is 3. The term is seldom used to describe solutions in which the hydrogen ion activity is 1 or greater. The expression defining the pH is closely related to the free energy of the hydrogen ion with respect to a standard reference state (Bates, 1964).

Many of nature's processes are highly dependent on pH. This is also the case for many chemical reactions which take place in industry or in the laboratory. pH is one of the variables most often controlled in biotechnology because enzymatic activities and, therefore, metabolism is very sensitive to its change. In 1909, the founder of the modern pH concept, S. P. Sorensen, proved that control of H^+ concentration is essential for many enzymatic processes. One example is the cleavage of cane sugar using invertase.

pH can also have an influence on the colour of certain dyestuffs. For example, although cyanidin chloride gives the cornflower its blue hue, it is the same dyestuff which gives a rose its red colour. The explanation for this is that cyanidine chloride is blue at a high pH while it is red at a low pH. It is essential as regards living organisms that the pH of the biological fluids is maintained within a narrow range.

Swimming pool water is disinfected using a chlorine. The chlorine's optimal effectiveness and the avoidance of eye irritation can only be assured at a specific pH level. In galvanic baths, quality and current efficiency is critically dependent on the correct pH. When the residual metals in the rinse water from such baths are precipitated, pH also plays a very important role.

These few though wide ranging examples illustrate the importance of pH. It is appropriate to mention at this point that it is the pH value which is of significance and therefore not the total concentration of acid or alkaline species.

1.2 BACKGROUND HISTORY OF pH

1.2.1 Acid-base theories

Acids and bases are two interrelated classes of chemical compounds. Their precise definitions have varied considerably with the development of chemistry. These changing definitions have led to frequent controversies, some of which are still to be resolved (Bates, 1981). Acids initially were defined only by their common properties. They were substances which had a sour taste which dissolved many metals and which reacted with bases to form salts. It was believed following the work of Lavosier that a common constituent of all acids was the element oxygen but gradually it became clear that if there were an essential element it was hydrogen not oxygen. The definition given by Liebig in 1840 as a hydrogen-containing substance which will generate hydrogen gas on reaction with metals was proved to be satisfactory for about 50 years.

Bases initially were defined as substances which reacted with acids to form salts. The alkalies, sodium and potassium were the best known bases. The acidity and basicity of a solution is frequently an important factor in chemical reactions. Several theories have been put forward to describe the acid-base properties of substances in water as well as other solvents. Some of the important theories on which the foundation of pH was built are described here.

Arrhenius-Ostwald theory

When the concept of ionisation of chemical compounds in aqueous solution became established, some considerably different definitions of acids and bases became popular. Arrhenius and Ostwald in 1894 presented an acid-base theory which defined acid as a substance that ionises partially or completely in water to give hydrogen ions.

$$HA + H_2O \longrightarrow H_3O^+ + A^-$$

A base was defined as a substance which ionises in water to give hydroxyl ions.

$$B + H_2O \longrightarrow BH^+ + OH^-$$

The use of this theory makes it possible to discuss acid and base equilibrium and also the strength of individual acids and bases. The concentration of the species H^+ and OH^- in water are not independently variable. This is because water itself is both a weak acid and a weak base ionising very slightly according to the following equation

$$H_2O \rightleftharpoons H^+ + OH^-$$

For pure water the concentration of H^+ and OH^- are equal. At room temperature roughly $2x10^{-7}$ % of the water is present as ions. As a result of this ionisation of water the ionic concentration is related through equation given below.

$$\frac{[H^+][OH^-]}{[H_2O]} = K$$

At 25 ^{0} C and with concentrations in moles per liter, the product $[H^+]$ $[OH^-]$ is equal to $1x10^{-14}$. A major result of this interdependence is that measurement of the concentration of either H^+ or OH^- in an aqueous solution permits immediate calculation of the other. This fact led S. P. Sorenson in 1909 to propose the use of a logarithmic pH scale for the concentration of hydrogen ions in water. He defined the pH of a solution as $pH = -\log [H^+]$, where $[H^+]$ represents the concentration of hydrogen ions in solution.

The Arrhenius-Ostwald theory of acids and bases has many attractive features but it has also presented some difficulties. A major problem was that solvents other than water can be used for acids and bases and thus need consideration. For many of the solvents of interest the necessary extension of the water theory are both obvious and plausible. For example, with liquid ammonia as the solvent one can define NH_4^+ as the acid ion and NH_2^- as the base ion and the former can be thought of as an hydrogen ion combined with a molecule of solvent. However for aprotic solvents such as liquid sulphur dioxide the extensions are less obvious. Considerations of such systems has led to some solvent oriented theories of acids and bases (Coetzee and Ritchie, 1969).

Theory of the solvent system

Franklin presented the solvent system concept of acids and bases in 1905. This theory recognises the ionisation of solvent to give cations and anions. An acid is defined as a solute that gives the cations of the solvent while a base is a solute that yields the anion of the solvent.

$$2H_2O \longrightarrow H_3O^+ + OH^-$$

$$2NH_3 \longrightarrow NH_4^+ + NH_2^-$$

The theory has been useful in emphasizing the need to consider non-aqueous systems. It is similar to the Arrhenius theory but applicable to other ionisable solvents. However for non-ionisable solvent such as benzene it is not suitable.

The Bronsted-Lowry theory

In 1923 Bronsted and Lowry described a theory known as the Bronsted-Lowry theory of acids and bases. This theory gives a unique role to the hydrogen ion and appears to have the justification for this. One justification of course is the historically important role already given to hydrogen in defining acids. A rather different justification involved the unique structure of the hydrogen ion. It is the only common ion containing a proton. The small size of the hydrogen ion makes it reasonable to postulate that protons are never found in a free state but rather always exist in combination with some base. The Bronsted theory emphasises this by proposing that all acid-base reactions consist simply of the transfer of a proton from one base to another.

The Bronsted and Lowry theory states that an acid is any substance that can donate a proton and a base is a substance that can accept a proton. Compared to the Arrhenius theory, this represents only a slight change in definition of an acid but considerable extension of the term base. In addition to hydroxide ions, the bases now include a wide variety of uncharged species, such as ammonia and the amines as well as anions of weak acids. In fact every acid can generate a base by loss of a proton. Acids and bases which are related in this way are called conjugate acid-base pairs.

The Bronsted concepts can be applied without difficulty to other solvents which are amphoteric in the same sense as water and data are available for many non aqueous solvents e.g. methyl alcohol, formic acid. Bronsted acid-base reactions can also be studied in aprotic solvents such as hexane but in this case both acid and base must be added to the solvent (Logowski, 1976).

A fact which merits consideration in any theory of acids and bases is that the speeds of large numbers of chemical reactions are greatly accelerated by acids and bases. This phenomenon is called acid-base catalysis and the major reason for its wide prevalence is that most proton transfers are themselves exceedingly fast. The existence of this general acid base catalysis constituted an important item in the wide acceptance of the Bronsted definitions (Christian, 1994).

Lewis theory

Studies of catalysis played a major role in the acceptance of a set of different definitions of acids and bases which are due to G. N. Lewis. These definitions were originally presented at the same time as those of Bronsted but they gained wide consideration only after Lewis restated them in 1938.

The Lewis theory defined an acid as a substance that can accept an electron pair whereas a base is a substance which can donate an electron pair. These definitions are very similar to the terms electron donors (Lewis bases) and electron acceptors (Lewis acids) introduced by N. V. Sidgwick in 1927. Bases under the Lewis definitions are very similar to those defined by Bronsted, but the Lewis definition for an acid is very much broader. For example every cation is an acid such as $AlCl_3$, BF_3. An acid base reaction now typically becomes a combination of an acid with a base, rather than a proton transfer. Many types of reaction which are characteristic of proton acids occur between Lewis acids and bases such as neutralization and the colour change of indicators.

For several reasons the Lewis theory has not been universally accepted. One reason is that the term electron donor and electron acceptor had been widely accepted and appear to serve similar predicting and classifying purposes. It is the drawback of the Lewis theory of acids and bases that the relative strengths of acids vary widely with the choice of base and vice versa. For example with the Bronsted definitions hydroxide ion is always a stronger base than ammonia, whilst with the Lewis definition hydroxide ion is a weaker base than ammonia when reacting with a silver ion but is stronger than ammonia when reacting with a hydrogen ion. Another discrepancy of this theory is that some common acids such as HCl and $H_2 SO_4$ do not naturally fit the Lewis definition since they cannot accept electron pairs (Small, 1954).

Inspite of these difficulties the use of the Lewis definitions is increasing. Since there does not appear to be any simultaneous tendency to give up the Bronsted definitions, chemistry seems to be stepping into a period when the term acid needs a qualifying adjective for clarity, for example Lewis acid or proton acid (Pearson, 1966).

Hard and soft acids and bases

As pointed out earlier one of the drawbacks of the Lewis theory is that it is hard to systematise the behaviour of acids and bases towards each other. Attempts have been made to classify Lewis acids and bases into categories with respect to their mutual behaviour. Pearson in 1963 proposed a simple classification based in part on earlier methods that appears to be promising in its application to a wide variety of Lewis acid base behaviour.

Lewis bases are classified as soft if they have high polarisability low electronegativity, are easily oxidised or possess low lying empty orbitals. They are classified as hard if they have opposite tendencies e.g. H_2O is hard and NH_3 is soft. Acids are divided more or less distinctly into two categories hard and soft with few intermediate cases. Hard acids are of low polarisability, small size and high positive oxidation state and do not easily excited outer electrons. Soft acids have several properties of high polarisability, low or zero positive charge, large size and easily excited outer electrons. For example H^+ is a hard acid and Cu^+ is a soft acid.

Hard acids prefer to associate with hard bases and soft acids with soft bases. This rule is successful in correlating acid-base behaviour of a wide variety of chemical systems including covalent bond formation, metal-ligand interaction and hydrogen bond formation. It is to be emphasised that the hard and soft acid and base concept is a means of classification and correlation and is not a theoretical explanation of acid-base behaviour. The reason why hard bases prefer hard acids and soft prefer soft are complex and varied. However the already established concepts of ionic and covalent bonds seem to be helpful. Hard acids and hard bases with small sizes and high charge would be held together with stable ionic bonds.

Usanovich theory

Another comprehensive theory also known as positive-negative theory was presented by Usanovich in 1939. According to this theory acids are defined as substances which form salts with bases, give up cations and add themselves to anions and to free electrons. Bases are similarly defined as substances which give up anions or electrons and add themselves to cations. Two examples of acid-base reaction under this theory are stated as follow.

$$Na_2O + SO_3 = 2\,Na^+ + SO_4^{--}$$
$$2\,Na + Cl_2 = 2\,Na^+ + 2\,Cl^-$$

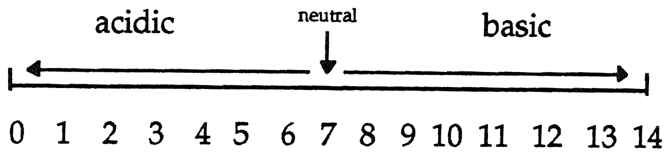
The Usanovich scheme also includes oxidation-reduction in the definitions. So far this theory has had little acceptance because the definitions are too broad to be very useful.

1.3 pH MEASUREMENTS

pH is defined as

$$pH = -\log [H^+]$$

The pH is a measurement of the activity of dissolved hydrogen ions, $[H^+]$ and describes in numerical values as to whether an aqueous solution is acidic, neutral or basic. The pH scale usually ranges from pH 0 to pH 14. pH 7 is neutral, below pH 7 is acidic and above 7 is regarded as basic.



pH of an aqueous solution can be measured by electrochemical methods and colorimetric methods.

1.3.1 Electrochemical methods

The hydrogen electrode

The basis of the invention of the hydrogen electrode was the Nernst's theory of electrolytic solution pressure introduced in 1889. Nernst stated that when metals are immersed in liquids their atoms tend to pass into the ionic state. He defined the normal hydrogen electrode electromotive force (EMF) as that given by a solution containing 1 gram equivalent of hydrogen per liter.

Early measurements of $[H^+]$ and hence pH were made using electrochemical cells fitted with a hydrogen electrode.

$$\begin{array}{lllll} \mathrm{Pt\,H_2} & \left|\,\mathrm{H^+}\,\right| & \mathrm{KCl}\,\big| & \mathrm{Hg_2Cl_2}\,\big| & \mathrm{Hg} \\ \text{1atm.} & & \text{satd.} & \text{satd} & \text{reference electrode} \end{array}$$

The emf of this cell at 25^0 C is given by

$$\begin{aligned} E &= \text{constant} - 0.0591 \log [H^+] \\ &= \text{constant} + 0.0591\mathrm{pH} \end{aligned}$$

i.e. the potential of the cell changes by 59.1 mV per pH unit.

The hydrogen electrode can be used to measure the pH of solutions which do not contain substances which interfere with the attainment of equilibrium in the electrochemical reaction as follow:

$$H_2 = 2H^+ + 2e^-$$

Solutions which contain strong oxidising or reducing substances may react with hydrogen.

Unfortunately, a hydrogen electrode is not easy to set up, a source of hydrogen is required and the platinum electrode is easily poisoned by small amounts of impurities in the solution. Hence the electrode is not suitable for routine measurements of pH (MacInnes, 1961).

The quinhydrone electrode

The quinhydrone electrode was first studied by Haber and Russ in 1904 but its value as an electrode for measurement of pH was recognised and developed by Biilman in 1927. The principle of the quinhydrone method depends upon electrical effects of the change from quinone into the anion of hydroquinone by the addition of two electrons which can be neutralised by 2 H^+.

$$C_6H_4O_2H_2 = C_6H_4O_2 + 2H^+ + 2e^-$$

The EMF can be measured and is greater than the 0.1 N calomel half-cell to the 1N hydrogen half cell, 0.7042 volt instead of 0.3380 volt at 18^0 C. Therefore quinhydrone half cell is positive to the calomel half cell and equation can be written as follow.

$$\text{pH} = \frac{0.3668 - \text{EMF obs.}}{0.0577}$$

The EMF is for a short duration and therefore readings must be taken quickly. For pH measurements with the quinhydrone electrode the solution should be saturated with quinhydrone. Errors may arise if an unsaturated solution is used (Biilman, 1927).

The antimony-antimony trioxide electrode

This electrode consist of metallic antimony in contact with solid antimony trioxide.

$Sb; Sb_2O_3$, solution x : Saturated KCl : reference electrode

The electrode reaction at such an electrode is given below;

$$2Sb + 3H_2O = Sb_2O_3 + 6H^+ + 6e^-$$

The potential will therefore depend not only upon the activity of the hydrogen ion constituent but also on that of water. This is important only if concentrated solutions are measured because the activity of water in dilute aqueous solution changes very little. The electrode has the advantage of simplicity and of not requiring a current of hydrogen. The antimony-antimony trioxide electrode can not be used with strong oxidising or reducing substances. It also cannot be used with very strong acid or basic solutions because of the solubility of the oxide in such solutions. Moreover it is not very accurate (Bates, 1964).

The glass electrode

A glass electrode which responded specifically to the concentration of H^+ was introduced in the early 1950's. Development of this electrode coupled with the tremendous advances in electronics have led to pH meters fitted with glass electrodes being available in every laboratory and the measurement of pH is now a routine operation. Electrodes covering the entire pH range are available and when meters have been carefully calibrated pH measurements can be made to an accuracy of 0.001 units.

The Nernst equation for the potential of a glass pH electrode is given by

$$E = E^0 + (2.3\,RT/F)\log[H^+]$$

The factor 2.3 RT/F is called the slope and corresponds to the change of potential per pH unit. The slope at 25°C is 59.1 mV. Therefore the equation at this temperature can be written as

$$E = E^\circ - 0.0591\,\text{pH}$$

The typical constructions of glass and reference electrodes for a combination pH electrode are shown in figure 1.1.

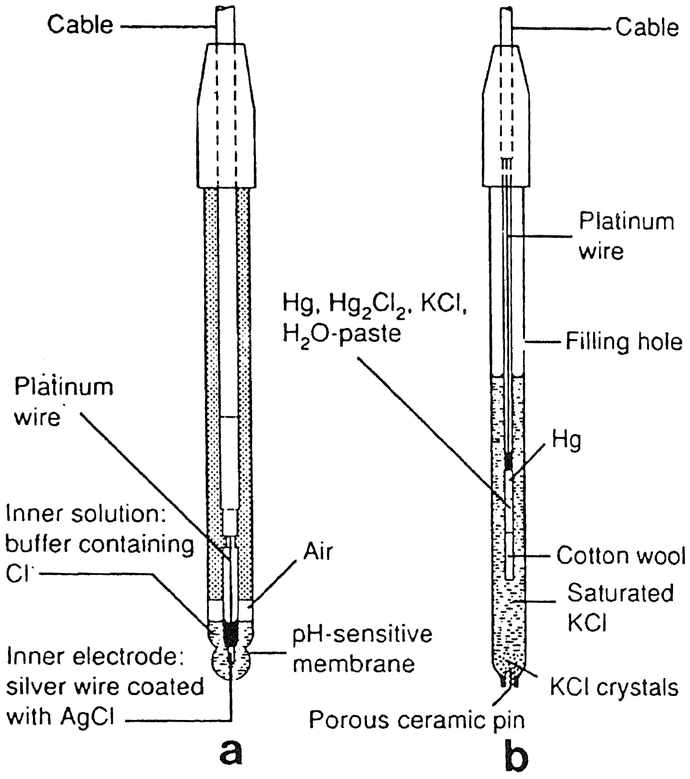


Fig. 1.1 Typical construction of (a) glass electrode and (b) reference electrode.

Glass electrodes linked to modern pH meters would seem to be ideal for on line monitoring of pH. However the electrodes do have limitations (Brezinski, 1983 ; Covington, et al., 1985; Davison, 1987; Herczeg & Hesslein, 1984; Illingworth, 1981; Holobar, et al., 1993).

1 They require a reference electrode.
2 The liquid-liquid junction is easily perturbed by external factors such as sulphide solutions, particulates, bacteria and yeast cells.
3 They are relatively fragile.
4 They can not be readily miniaturised.
5 They are easily contaminated by impurities in solutions, particularly surface active compounds.

6 They cannot be autoclaved at high temperatures.

7 They are not very accurate in measurements on solutions with low ionic strength.

8 Their semi permeable membranes may be clogged by biomass in bioreactors.

9 They exhibit alkaline errors (where electronic readings are affected by high concentration of Na^+, which is mistaken, partially, for H^+ unless special glass is used).

1.3.2 Colorimetric method

Measurement of colour by visual matching of the test colour with a series of predetermined standard colours made from mixing of three primary colours red, yellow and blue was introduced and devised by J.W. Lovibond in 1880. Lovibond units of colour have received international recognition, and standardising bodies in over 20 different countries quote colours in terms of units of Lovibond scale. The colour changes of indicators are most easily identified by visual comparison with Lovibond permanant glass colour standards.

Gillespie (1920) used dilutions of standard acid or standard alkali on indicator drops to match drops of the test fluids plus indicator. Wagnar and others in 1916 used buffer solutions and indicators in drops to match the test fluid plus indicator.

Small (1926) successfully measured the pH of plant juices by using a series of indicators. He introduced the method called Small's range indicator method of pH measurement. By this method several indicators were used to obtain the confirmation of upper and lower limits of observed pH range and an overlapping which narrows the range within which lies the actual pH of the material being examined.

The most useful addition to the methods of determining pH values of solutions and soils has been the introduction of indicator test papers by Johnson and B. D. H in the early 1940's. pH sensitive dyes adsorbed on cellulose have found extensive use as indicator strips which are still indispensable in a chemical laboratory.

The measurement of pH by pH papers suffers certain drawbacks. They fail in diluted, poorly buffered or coloured solutions and also in cases where high accuracy is required (Offenbacher, 1986). However, if the indicator is added to the solution and the absorbance of the indicator measured using a spectrophotometer the pH can be obtained with an accuracy equivalent to that of the glass electrode (Thomas and Chamberlin, 1980).

A pH indicator is a substance which changes colour over a certain limited range of hydrogen ion concentration. Most indicators are either weak acids or weak bases, the acid form exhibiting one colour and the basic form a different colour.

Consider a monobasic acid indicator:

$$\underset{\text{acid}}{HA} \rightleftharpoons H^+ + \underset{\text{base}}{A^-}$$

The equilibrium constant is given by

$$K_{In} = \frac{[H^+][A^-]}{[HA]}$$

$$[H^+] = K_{In} \frac{[HA]}{[A^-]}$$

$$\log H^{+} = \log K_{In} + \log \frac{[HA]}{[A^{-}]}$$

$$pH = -\log_{10} [H^{+}]$$

$$pK_{In} = -\log_{10} [K_{In}]$$

$$pH = pK_{In} + \log_{10} \frac{[A^{-}]}{[HA]}$$

$$pH = pK_{In} + \log_{10} \frac{[\text{ basic form}]}{[\text{ acid form}]} \qquad 1.1$$

The equation 1.1 is known as the Henderson-Hasselbalch equation.

A distinct colour change for an indicator is normally observed when the concentration of one of its forms changes from 10% to 90%. This means that the indicator changes colour over a pH range of approximately 2 units.

Indicators have not been used for routine determination of pH because the indicator had to be added to the solution and the absorbance of the solution then measured using a spectrophotometer. Once added the indicator could not be removed from the solution, making this a destructive analytical technique, whereas the glass electrode does not affect the solution in which it is immersed. In order to make the colorimetric method useful for continuous pH measurements, the indicators have to be immobilised on some substrate like glass, fibres, polymers etc. (Leiner & Wolfbeis, 1991). Advances in immobilisation techniques and their application as optical sensors have stressed the following advantages over the electrochemical methods (Avnir, et al., 1994; Weigl, et al., 1994).

1 No need for a reference electrode
2 The signal is not influenced by electrical "noise".
3 The sensor can be manufactured as a miniature disposable element mechanically connected to the optical fibre.
4 Simple factory or one point calibration.
5 Simultaneous measurement of several analytes by co-immobilised sensor reagents at different wavelengths.
6 Optical control of the baseline stability.

Electrochemical devices are inherently limited to reactions involving ion movements or electron transfer, while a modification of reactant optical properties is almost inherent in every chemical reaction. The current practices are richer in optical detection techniques rather than electrochemical (Shtelzer and Braun, 1994). Optical methods for rapid and continuous pH monitoring provide a promising alternative to the glass pH electrode (Sipior, 1994). However optical sensors also have certain limitations (Avnir, et al., 1994).

1 Limited dynamic range.
2 Signal amplitude depends upon the amount of reagent in the optical pathway.
3 Photobleaching or photodegradation of the sensor reagent.

1.4 IMMOBILISATION OF pH INDICATORS

Over the years a number of methods have been developed to immobilise reagents. These methods can be divided into three main categories (Taylor, 1991; Ingersoll and Bright, 1997); 1- physisorption, 2- covalent attachment and 3- entrapment. Physisorption is the simplest of all the methods but it suffers several disadvantages when used to immobilise sensing chemistries. Random orientation of the recognition element on the substrate can lead to its inaccessibility to the analyte in question. Moreover the immobilised agent often leaches or desorbs from the surface (Mohr and Wolfbeis, 1994).

Covalent attachment methods generally form stable interfaces and the recognition element rarely leaches from the substrate. However such schemes involve more complicated chemistry and can be expensive and time consuming (Hall, 1990). Glass and polymers have been extensively used as substrates for covalent attachment of colorimetric indicators (Reetz, et al., 1995). Polymeric substrates interact not only with dopants but also with external and internal impurities and quenchers. They have serious deformation problems after swelling and also fluoresce in the blue or violet under UV excitation (Allen and McKeller, 1980; Saavedra and Reichert, 1990).

Compared to polymers, glass provides the properties suitable as sensor substrates such as high surface area, ruggedness, high chemical and thermal stability and low optical absorption in the visible and near infra red range (Harper, 1975; Lev, et al., 1995).

The covalent binding of indicator to glass surface requires chemical silanization. This process is deterrent to functional activities of most indicators. Therefore the native properties of covalently immobilised agent do not remain intact due to structural modification by silanization (Bacci, et al., 1991). Many studies in the past have been conducted to immobilise the indicators (colorimetric and fluorescent) covalently to substrates such as glass, polymers, cellulose etc. to measure pH (Kirkbright et al., 1984; Peterson and Goldstein, 1982; Saari and Seitz, 1982; Parker et al.,1993; Wolthuis et al., 1992; Wolfbeis et al., 1992 & 1994; Janata et al., 1994).

Entrapment of the recognition chemistry within a porous three dimensional network has become an attractive means to immobilise the recognition species for chemical and biochemical sensing. However the leaching of dopants can be an obstacle (Shtelzer, 1992).

Recent developments in the field of sol-gel processing (Aharonson, et al., 1994; Avnir, et al., 1994a) has led to the possibility of trapping pH indicators in sol-gel glasses to avoid many of these problems. These glasses formed by hydrolysing alkoxysilanes have a porous structure in which the indicator molecules can be trapped. The pores are small enough to retain the indicator molecules but small ions such as H^+ and other cations can diffuse in and create a colour change. This process of colour change is reversible.

Glass obtained by sol-gel processing provides an excellent host matrix and the molecules immobilised by this method retain their functional characteristics to a large extent (Avnir, et al., 1992; Avnir, 1997). Moreover these functionalised glasses can be prepared so that they are optically transparent, permitting monitoring of the spectroscopic properties of the encapsulated reagent. These sol-gel glasses make it possible to retain the specificity and reactivity of dopants in the solid state and provide morphological and structural control that are not available when they are simply dissolved in aqueous media. The amorphous nature of the glassy material does not impart a geometric order to the entrapped molecules; many of the characteristics of the liquid state are retained despite the fact that the molecule is trapped in solid material (Barreau and Miller, 1996).

1.5 FORMATION OF SOL-GEL GLASSES

Glass prepared by traditional melting of silica is not feasible as the host matrix for most of the organic dopants, because of the high temperatures required. By contrast sol-gel processing involves low temperature hydrolysis of suitable alkoxysilane precursors and is highly suitable for microencapsulation of a variety of molecules which cannot withstand extreme temperatures (Hench and West, 1992).

The sol-gel process can be broadly defined as one in which a useful solid product is prepared from a solution or suspension of precursor materials by removal of solvent. A sol is defined as the suspension of small particles of one phase in another major (usually liquid) phase. When particles in a sol aggregate and become heavy they precipitate. But if they form a continuous (usually three dimensional) network, the sol loses its mobility and becomes able to maintain its shape without the mold. This means the sol has formed a gel. A gel is a solid, usually soft and with low elastic modulus which resembles the parent sol in composition and solid to liquid ratio.

Sol-gel glasses are usually produced from alkoxysilanes such as:

TETRAETHOXYSILANE (TEOS)	$Si(OC_2H_5)_4$
TETRAMETHOXYSILANE (TMOS)	$Si(OCH_3)_4$

There are five steps involved in the formation of sol-gel glasses; hydrolysis, condensation, polymerisation, gelation and drying. Each step will be discussed briefly.

1.5.1 Hydrolysis

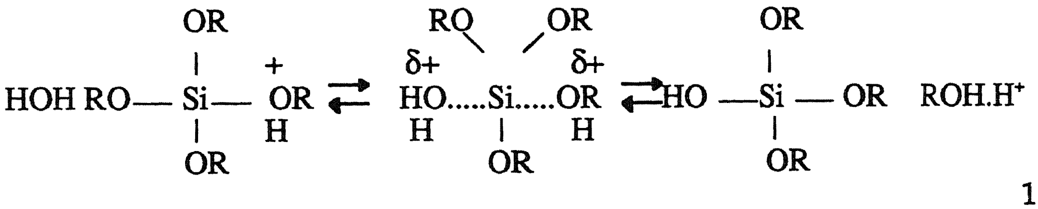
Alkoxysilanes are usually hydrolysed in the presence of water and alcohol at acidic or basic pH to form silanol.

$$Si(OR)_4 + H_2O \longrightarrow HO\text{-}Si(OR)_3 + ROH \qquad 1.2$$

The reaction rate is very much dependent on the pH of the reaction medium. Acidic catalysis increases the rate of hydrolysis and delays the condensation whereas base hydrolysis produces rapid condensation and slows the rate of hydrolysis (Rabinovich, 1994).

Hydrolysis is most rapid and complete when catalysts are added (Brinker, 1990). The catalysts may be acidic or basic in nature. The most commonly used acid catalyst are HCl and HNO_3. The commonly used basic catalyst is NH_4 OH. The acidic catalysts favour the hydrolysis reaction by way of electrophilic substitution and produces weakly branched structures. The basic catalyst on the other hand yields a particulate structure and the reaction mechanism is nucleophilic. There is a linear relationship between the hydrolysis rate and the hydrogen ion concentration in an acid catalysed reaction medium. The base hydrolysis rate is also proportional to the base concentration (Keefer, 1984).

Under acidic conditions, it is likely that the alkoxide group is protonated in a rapid first step. Electron density is withdrawn from silicon, making it more electrophilic and thus more suceptible to the attack of water. The positive charge of the protonated alkoxide is correspondingly reduced and allows the alcoholic group to leave.

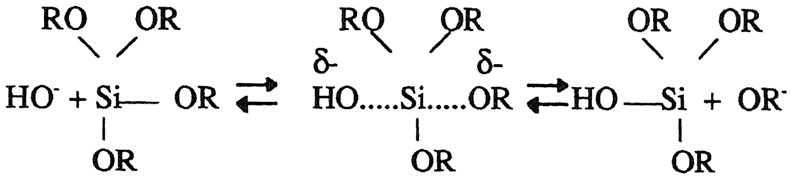


$$HOH\ RO-\overset{\overset{OR}{|}}{\underset{\underset{OR}{|}}{Si}}-\underset{H}{\overset{+}{OR}} \rightleftarrows \underset{H}{\overset{\delta+}{HO}}\ldots\ldots\overset{RO\diagdown\ \diagup OR}{\underset{\underset{OR}{|}}{Si}}\ldots\ldots\underset{H}{\overset{\delta+}{OR}} \rightleftarrows HO-\overset{\overset{OR}{|}}{\underset{\underset{OR}{|}}{Si}}-OR\quad ROH.H^+$$

1.3

The hydrolysis rate is increased by substituents that reduce the steric crowding around silicon. Electron providing substituents (alkyl groups) that help to stabilise the developing positive charges should also increase the hydrolysis rate.

Under basic conditions the water dissociates to produce nucleophilic hydroxyl anions in a rapid first step. The hydroxyl ions then attack the silicon atom. The mechanism of base catalysed hydrolysis in which OH^- displaces OR^- with inversion of the silicon tetrahedron is stated as follow.



$$HO^- + Si(OR)_3{-}OR \rightleftarrows \overset{\delta-}{HO}\cdots Si(OR)_3\cdots \overset{\delta-}{OR} \rightleftarrows HO{-}Si(OR)_3 + OR^- \qquad 1.4$$

This mechanism is affected by steric and inductive factors. However steric factors are more important because silicon requires little charge in the transition state (Pohl and Osterholtz, 1985). The isoelectric point of silica at which the electron mobility and the surface charge is zero, occurs at approximately pH 2. This pH value forms the boundry between acid catalysed hydrolysis ($pH < 2$) and base catalysed hydrolysis ($pH > 2$).

Acid catalysis is associated with fast hydrolysis rates and relatively long gel time whereas, under basic conditions hydrolysis is slow and condensation rates are faster giving rise to shorter gel times (Brinker, 1990). Another important parameter, along with the pH of starting solution, is the amount of water used for hydrolysis. Theoretically a water : TEOS ratio, R, of 2 is sufficient for complete hydrolysis and condensation as water is formed in the condensation process but generally the reactions do not go to completion under these conditions because of the formation of the intermediate species (Brinker, 1990). Below pH (<2) and at a low R (R<2) the gel evolves as a weakly branched microporous structure with pore sizes <2 nm. Under conditions of pH>2 and R>2 a particulate gel is formed with larger pores. Increasing the R value increases the hydrolysis and decreases the condensation rate for a particular pH value (McDonagh et al, 1996). Moreover an increase in R value also decreases porosity, thickness and pore sizes in thin films (Fardad et al, 1995).

1.5.2 Condensation

Two types of condensation reactions occur:

A) alcohol condensation

$$R_3O\text{-}Si\text{-}OR + HO\text{-}Si(OR)_3 \longrightarrow R_3O\text{-}Si\text{-}O\text{-}Si(OR)_3 + ROH \qquad 1.5$$

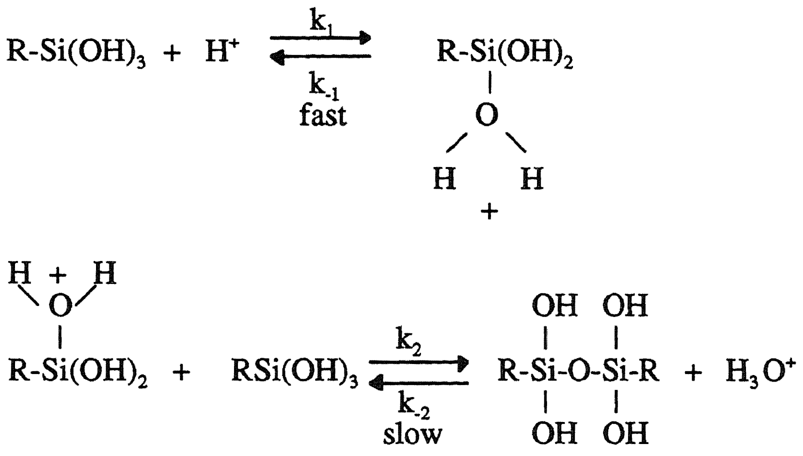
B) water condensation

$$R_3O\text{-}Si\text{-}OH + HO\text{-}Si(OR)_3 \longrightarrow R_3O\text{-}Si\text{-}O\text{-}Si(OR)_3 + H_2O \qquad 1.6$$

Condensation reactions involving the silanol groups produce siloxane bonds and the byproducts alcohol or water. Under most conditions condensation starts before hydrolysis is complete. As water and alkoxysilanes are immiscible a mutual solvent such as alcohol is normally used as an homogenising agent (Avnir, 1995; Lev, 1995). Alcohol produced as the byproduct of the condensation reaction is sufficient to homogenise the initially phase separated system. It should be noted that alcohol is not simply used as solvent but it can participate in esterification or alcoholysis reactions.

The rate of condensation reaction also depends on the pH of the initial reaction mixture. There are different mechanisms of condensation reactions which operate in acidic and basic media. The acid catalysed condensation mechanism involves a protonated silanol species.

Protonation of the silanol makes the silicon more electrophilic and thus more susceptible to nucleophilic attack.



$$R\text{-}Si(OH)_3 + H^+ \underset{\substack{k_{-1}\\ \text{fast}}}{\overset{k_1}{\rightleftharpoons}} R\text{-}Si(OH)_2\text{-}\overset{+}{O}H_2$$

$$\overset{+}{O}H_2\text{-}R\text{-}Si(OH)_2 + RSi(OH)_3 \underset{\substack{k_{-2}\\ \text{slow}}}{\overset{k_2}{\rightleftharpoons}} R\text{-}\overset{OH}{\underset{OH}{Si}}\text{-}O\text{-}\overset{OH}{\underset{OH}{Si}}\text{-}R + H_3O^+$$

1.8

$-d[\text{silanetriol}]/dt = k_1\, k_2 / k_{-1}\, [RSi(OH)_3]^2\, [H^+]$

The most basic silanol species are the most likely to be protonated. Therefore condensation reactions more likely occur between neutral species and protonated silanols situated on monomer end groups of chains (Pohl and Osterholtz, 1985).

The base catalysed condensation mechanism stated below involves the attack of a nucleophilic deprotonated silanol on a neutral silicate species.

$$R\text{-}Si(OH)_3 + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R\text{-}Si(OH)_2O^- + H_2O \qquad 1.9$$

fast

$$R\text{-}Si(OH)_2O^- + RSi(OH)_3 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R\text{-}Si(OH)_2\text{-}O\text{-}Si(OH)_2R + OH^-$$

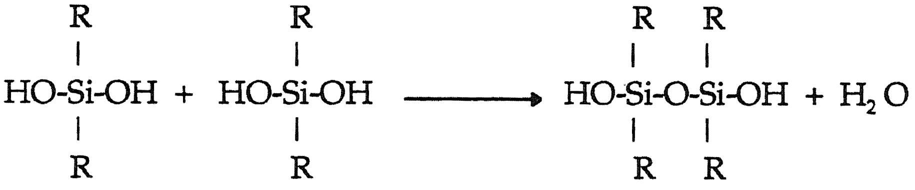
slow

$-d[\text{silanetriol}]/dt = k_1\, k_2 / k_{-1}\, [RSi(OH)_3]^2\, [OH^-]$

In the mechanism hydroxyl ions react reversibly with silanetriol in a rapid first step leading to the equilibrium concentration of the silanolate anion.

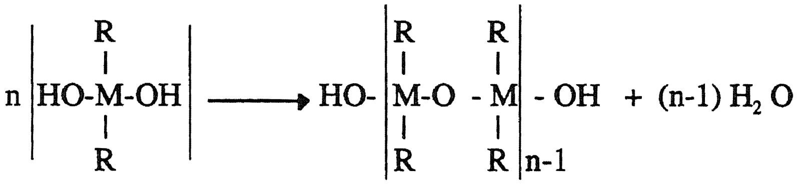
1.5.3 Polymerisation

Organic polymers evolve through the formation of dimers, trimers, linear and branched chains containing siloxane bonds.



$$\mathrm{HO{-}\overset{R}{\underset{R}{Si}}{-}OH + HO{-}\overset{R}{\underset{R}{Si}}{-}OH \longrightarrow HO{-}\overset{R}{\underset{R}{Si}}{-}O{-}\overset{R}{\underset{R}{Si}}{-}OH + H_2O} \qquad 1.10$$

dimer



$$n\left|\mathrm{HO{-}\overset{R}{\underset{R}{M}}{-}OH}\right| \longrightarrow \mathrm{HO{-}}\left|\mathrm{\overset{R}{\underset{R}{M}}{-}O{-}\overset{R}{\underset{R}{M}}}\right|_{n-1}\mathrm{{-}OH} + (n-1)\,\mathrm{H_2O} \qquad 1.11$$

Chain

Polymerisation occurs in three stages (Iller, 1979):

(I) Polymerisation of monomer to form particles.

(II) Growth of particles.

(III) Linking of particles into chains then networks that extend throughout the liquid medium, thickening it to a gel.

At pH above 2 the formation of dimers is low, but once dimers are formed they react preferentially with monomers to form trimer and tetramers. At this point cyclisation is rapid due to the proximity of the chain ends and the substantial depletion of the monomer population. The solubility of silica is low between pH 2-7, particle growth stops when the particles reach 2-4 nm where the solubility and size dependence of solubility is greatly reduced. The base catalysed polymerisation mechanism is given below:

$$\equiv\text{Si-OH} + \text{OH}^- \longrightarrow \equiv\text{Si-O}^- + \text{H}_2\text{O} \qquad 1.12$$

$$\equiv\text{Si-O}^- + \text{HO-Si} \longrightarrow \equiv\text{Si-O-Si}\equiv + \text{OH}^-$$

The rate of polymerisation below pH 2 is proportional to hydrogen ion concentration. The acid catalysed polymerisation mechanism involves a siliconium ion intermediate ($\equiv \text{Si}^+$)

$$\equiv\text{Si-OH} + \text{H}_3\text{O}^+ \longrightarrow \equiv\text{Si}^+ + 2\text{H}_2\text{O} \qquad 1.13$$

$$\equiv\text{Si}^+ + \text{HO-Si} \longrightarrow \equiv\text{Si-O-Si}\equiv + \text{H}^+$$

1.5.4 Gelation

The process in which the three dimensional network is formed through cross linking of polymers produced after polymerisation is called gelation. Because of the bond-bridging between the sol particles, the viscosity increases rapidly and finally leads to the formation of a solid mass. The control over processing parameters at this stage allows one to shape the sol into a desired form, e.g. monoliths, fibres or films. The mechanical strength of the gel depends on the material porosity. It can be increased by allowing the polycondensation reaction to continue. The rate of gelation can be increased by increasing the temperature at which the reaction is being carried out (Burzynski and Prasad 1994).

1.5.5 Drying

At this final stage of sol-gel glass formation alcohol and water are evaporated from the sol-gel matrix by heat treatment at low temperature usually (40- 80^0 C) and the resultant gel solidifies. As liquid evaporates, the solid network is drawn into the remaining liquid to prevent exposure of the energetic solid-vapour interface. The network is sucked under the surface of the liquid by the capillary tension and as long as the network remains sufficiently compliant, the liquid-vapour meniscus remains at the exterior surface and the evaporation rate is constant (Smith, et al., 1995). Once the network becomes hard enough to withstand pressure, the evaporation drives the meniscus into the body and the evaporation rates begin to drop. For a time liquid is able to flow along contiguous channels of liquid and evaporates at the exterior; later evaporation occurs within the body and transport to the surface requires diffusion of vapours. If the pressure in the liquid is uniform the network will be hydrostatically compressed and there will be no risk of cracking. However because of low permeability of the gel network the tension is greater in the liquid near the drying surface and this produces differential strain leading to fracturing of the glass. The greatest stress and the highest probability of fracture occurs when the shrinkage stops and the meniscus begins to recede into the body (Brinker, 1990).

Different approaches have been employed to avoid fracturing of sol-gel glasses. These include prolonged ageing before heating, strengthening the gel by reinforcement, enlargement of pores, reducing the surface tension of pore fluid, drying the gel under supercritical conditions of the pore fluid and freeze drying (Chaudhuri and Sarkar, 1994).

Hench and coworkers (1986) have added a drying control chemical additives (DCCA) such as formamide to the sol to avoid fracture problems. They suggested that the DCCA's control the rate of hydrolysis and condensation, pore size distribution, pore liquid vapour pressure and drying stress. To the extent these additives are effective, their success can be attributed to coarsening of the microstructure and strengthening of the network. They may also provide a medium through which more volatile components (water and alcohol) can diffuse, thereby allowing diffusion to reduce the pressure differential within the matrix.

1.6 PHYSICAL CONFIGURATIONS OF SOL-GEL GLASSES

1.6.1 Monoliths

Monoliths are defined as bulk gels (smallest dimension >1mm) cast to shape and processed without cracking. They have a pore size in the order of 10 nm and a surface area of 600-700 m^2/g.

The simplest kind of sol-gel platform to build for chemical and biochemical sensing is a monolith. Monoliths are simple to fabricate and have a relatively long path length for optical measurements. Many characterisation studies and initial sensing measurements have been made on monolithic sol-gels.

The method of doping sol-gel glass monoliths with pH indicators has been applied to the preparation of pH sensors. (Zusman et al., 1990; Rottman et al., 1992; Lev, O.,1992; Badini et al.,1995). Ellerbee et al., (1992) entrapped metalloproteins in sol-gel monoliths. Visible absorbance spectrophotometry was used to show that in both wet and dried gels, entrapped proteins could reversibly bind soluble ligands. The characteristics of immobilised bovine serum albumin and myoglobin in monoliths were studied by Edmiston et al (1993). They showed reduced native activities of these doped proteins compared to that of aqueous solution.

Weetall, (1996) entrapped bacteriorhodopsin in monoliths. He showed maximum enzyme activity at 4 ^{0} C which reduces at room temperature and is completely lost at 100^0 C. Lipases were reported to have a maximum activity and storage stability in porous monolithic silicate glasses (Reetz et al., (1996).

Avnir et al., (1994) reviewed the growing field of entrapment of purified enzymes, whole cells, antibodies and other proteins in sol-gel matrices. Shtelzer et al., (1994) described a glucose sensor based upon glucose oxidase immobilized in a sol-gel silicate matrix. Dave and co-workers (1994) immobilised biological molecules in sol-gel monoliths to determine whether these proteins retain their native function in such composites. A series of proteins and enzymes (myoglobin, hemoglobin, glucose oxidase, and oxalate oxidase) encapsulated in sol-gel monoliths retained their native ability to detect biologically relevant analytes (e.g., oxygen, carbon monoxide, glucose and oxalate).

The application of doped sol-gel monolith glasses in detection of heavy metals has been reported. (Kuyavskaya et al., 1992; Zusman et al., 1990; Kuseman et al., 1993). The advantages of sol-gel glasses mentioned by Zusman, et al., (1990) and Dave, et al., (1994) are

1 The glass matrix is transparent down to 250 nm, making it highly suitable for quantitative spectrophotometric and spectrofluorometric tests.

2 The glasses are chemically inert, photostable and thermally stable making them highly suitable for application in harsh environments or in food technologies.

3 The preparation of the doped glasses is technically simple. Thus the trapping procedure is straightforward and non-specific as compared with covalent binding of a reagent to a solid support.

4 Glass blocks of various shapes as well as thin films are easily prepared.

1.6.2 Films

Thin, transparent films (<1 µm in thickness) can also be produced via sol-gel technology. (Brinker et al., 1990; Brinker et al., 1992). Entrapment of pH indicators in thin films coated onto the surface of glass slides to make optical waveguide pH sensors have been described. (Yang et al., 1995; Lee et al., 1994). Bambot et al., (1994) reported the optical pH sensing by using flourescence resonance energy transfer in sol gel films. They gave the following advantages of this pH sensor:

1 Fast response times (seconds) and short optical paths (microns) result from thin coating.

2 The sol-gel film possesses robust physical properties, such as the ability to withstand repeated wiping with tissue.

3 Entrapment in a sol-gel matrix permits facile substitution of dyes, allowing for changes in the pH range of the sensor or wavelenghth of the excitation light.

4 Significantly, the sensor has undergone repeated autoclaving, an important attribute for utilization in sensitive environments such as bioreactors or the bloodstream.

Shahriari et al., (1994) used a fiber optic coated by a thin film of sol-gel silica doped with colorimetric pH indicators to measure pH changes in the range 3.5-9.5 by a single probe. pH indicators entrapped in sol-gel glasses change pH at the same values as they do in solutions (Zusman et al., 1990).

Prasad et al, (1995) used steady state and time-resolved fluorescence spectroscopy to determine how the micro environment around a dopant entrapped within sol-gel matrix evolves with time. On the basis of this information they managed to develop sol-gel thin film chemical sensors based on artificial recognition elements, enzymes and intact antibodies. More recently the use of sol-gel technology for trapping a series of biomolecules in sol-gel matrices were extensively reviewed (Livage, 1996).

To date there are quite a few sol-gel pH sensors which have been reported by making use of fluoresent dyes incorporated in sol-gel glasses (MacCraith et al., 1991; Badini et al., 1989; Badini et al., 1995a). These sensors have the advantage of high sensitivity due to fluorescence but at the same time the doped fluorescent dyes have inherent problems of quenching in atmospheric oxygen and hence this imparts short life to them. On the other hand colorimetric indicators attached with absorption detection techniques have been less explored in sol-gel chemical sensor fabrication (Avnir et al., 1994a).

Inspite of the increasing application of sol-gel technology in the chemical sensing field there are still improvements to be made in long term stability and chemical durability of doped sol-gel glasses.

1.7 AIMS OF PROJECT

The major objective of the present study is to investigate the use of indicator doped sol-gel glasses for the measurement of pH and possible cations in an on-line system. It is envisaged that the sol-gel glass could be used as a window in a spectrophotometric cell and the technique could be developed for the continuous monitoring of the pH of a solution in on line systems by measuring the absorbance. This would provide an alternative method in situations where pH electrodes are unusable.

It can be shown that

$$pH = pK_{In} + \log \frac{A - A_B}{A_A - A}$$

where A = absorbance of the solution

A_A = absorbance of indicator when completely unionised

A_B = absorbance of indicator when completely ionised

Other workers in this field have primarily used reflectance and fluorescent techniques. This work aims to use conventional absorbance methods to measure pH and metal ions. It is further intended to optimise the conditions required for the stable preparation of sol-gel glasses and their use as chemical sensors. The project is also directed to build an understanding of the structural evolution of these glasses with passage of time. It is also planned to use the newly designed, high resolution spectrophotometer, at CAMAS, for detection of light signals from sol-gel coated optical fibres in conjunction with continuous pH measurements. The new spectrophotometer would be specifically adapted for fibre-optic sensors to enhance its sensitivity by efficient use of the light signal.

CHAPTER 2. MONOLITHS DOPED WITH pH INDICATORS

2.1 INTRODUCTION

Sol-gel processing provides an excellent method for doping chemical reagents into porous glasses without affecting their chemical properties. Rottman et al (1992) doped a series of pH indicators in sol-gel porous glasses by polymerisation of tetramethyl orthosilicate (TMOS) in the presence of cetyltrimethylammonium bromide (CTAB). They described the properties of doped monoliths including spectral shifts, shifts in pH, sensing range, cycle repeatability, leachability, rates of response and isosbestic points. They attributed the spectral shifts of doped monoliths mainly to the interactions of CTAB with indicators. The kinetics of water consumption during the early stage of the TMOS sol-gel polymerisation process was studied in detail by Kaufman et al, (1988). They reported that changes in water/ silane ratio and in pH affect the kinetic behaviour of water consumption.

Badini et al (1995 a) reported the preparation of monoliths in which a pH sensitive fluorescent agent, fluorescein isothiocyanate (FITC) was incorporated at the liquid sol stage. Impregnation of the sol-gel monolith with pH-sensitive fluorescent dye was carried out by Badini et al (1995 b). They explained problems of fracturing, aqueous and dry storage stabilities of these gels. They used silylating agents to improve the immobilisation of dye to the substrate and to minimise leaching.

Aharonson et al (1994) reported solving leaching problems in monoliths doped with methyl-red by silylating the dopant before entrapment. They also showed no leaching in monoliths doped with polyclonal antibodies when polymerisation was carried out at high acidity and low water content. Avnir and coworkers (1994) pointed out the problems of cracking in sol-gel glasses. They reported that monoliths tend to fracture when immersed and reimmersed in aqueous solution. They also recommended the use of quaternary ammonium compounds for reducing this tendency of monolithic sol-gel glasses. The addition of drying control chemical additives (DCCA) to sol-gel glasses for resolving the cracking problems during drying were suggested by Hench, (1986) and Brinker, (1990).

The present work is intended to measure a pH of aqueous solution by using pH colorimetric indicator doped monolith as a window in a spectrophotometric cell. It is also aimed to enhance the aqueous stability of these glasses and to reduce their pathlength by making thinner monoliths.

2.2 MATERIALS

Tetramethylorthosilicate (TMOS) and tetraethylorthosilicate (TEOS) were obtained from the Aldrich chemical company. pH indicators, bromophenol blue, bromocresol purple and bromothymol blue were from the BDH chemical company. The reagents for buffers were obtained from BDH. All the chemicals used were of analytical grade.

2.3 APPARATUS

The pH of buffers was measured by a C14 WPA pH meter using a BDH combination electrode No 309/1050/06. Absorption spectra of pH indicators in solution were measured using a Perkin-Elmer lambda 3B spectrophotometer and absorption spectra of indicators entrapped in sol-gel glasses were measured using a Philips PU 8740 spectrophotometer.

2.4 PREPARATION OF INDICATOR DOPED MONOLITHS

The gels were prepared by hydrolysis of TMOS in a water/ethanol mixture at pH 2-3. The molar ratio (R) of water : TMOS was 5, which was selected to achieve crackfree monoliths with optimum ageing time at 50°C. Five ml TMOS, 3.2 ml distilled water, 2.0 ml ethanol, 4 ml of mixture of dyes containing 0.1 mM solution of each bromophenol blue, bromocresol purple and bromothymol blue in ethanol were mixed and a catalytic amount of 0.1 M HCl (two drops) was added. Gelation was carried out at room temperature in a 100 ml (50 mm diameter) glass beaker covered with aluminium foil. After 24 hours the beaker (still covered) was placed in an oven at 50°C for one week. The solvent was then allowed to evaporate slowly by piercing the foil and kept in the oven for further three days. The xerogels were cooled to room temperature and uncovered.

2.5 RESULTS

Sol-gel glasses doped with mixture of dyes were washed several times with distilled water to remove loosely bound dyes. Washing was continued till no further leaching was observed in the water. Then doped sol-gel glasses were studied for colour change by immersing in the appropriate pH buffer (0.1M citrate and 0.1M di sodium hydrogen ortho pyrophosphate) covering the range of pH 3 to 8.5 (Wilson and Walker, 1994). The absorption spectrum of the doped sol-gel glass monolith was recorded. The change in the absorbance at the wavelength of maximum absorbance was shown to be related to the pH of the solution. Consistent results were obtained provided that measurements of the absorbance were made after a fixed time (5 minutes). However if the monoliths were immersed for longer periods further changes in absorbance occured showing that the diffusion of H^+ into the matrix had not reached equilibrium.

2.5.1 Comparative stability of different monoliths

The comparative stability of sol-gel glasses made from TEOS and TMOS was investigated. Parameters like response time, storage stability, aqueous stability and % leaching in each case were brought into consideration (Table 2.1). Various methods were used to try to improve the stability of the glasses such as heat treatment at varied temperature, addition of drying control chemical additives (DCCA) like DMF, triton X 100, polyethylene glycol 4000 and glycerol to the gelation phase. Both TEOS and TMOS were mixed together in equal volume ratio (2.5: 2.5) for gelation and tested the stability of the resulting glasses. The quantification of leached dyes was carried out by difference of absorption spectra before and after immersing the glasses in distilled water for a specific period (three days). The dried gel (TMOS) with the additive of 2 ml of DMF showed no cracking during the experiment but it cracked when left immersed in distilled water for one day. However it was stable when stored in a damp or wet environment. The glasses obtained by mixing TEOS and TMOS in equal proportions and TMOS added with 20%, 1 ml glycerol cracked within a few minutes after immersing in distilled water. The comparative stability of DMF added glasses was exploited by using TEOS and a mixture of TMOS & TEOS with the same additive. TMOS and TEOS with 1ml DMF as DCCA showed no cracking in dry and wet conditions. The immersing and reimmersing of these glasses in liquid did not cause them to fracture. However DMF affected the pH of the resultant doped glasses and hence gave them green colour instead of the normal yellow. Therefore before subjecting them to spectrophotometric study the glasses were turned back to yellow by 0.1 M HCl. The response times noted in each case were in the order of 2 to 3 hours. The summary of these results have been shown in table 2.1.

Table 2.1. Stability and aqueous durability of various types of monoliths immobilized with a mixture of pH indicators aged for fifteen days.

gel type	dry storage stability	stability in liquid	response time	extent of leaching	effect of immers- -ing & reimmers- ing in liquid
TEOS	months	2-3 days	2-3 hours	25% in 3 days	cracked
TMOS	months	2-3 days	2-3 hours	25% in 3 days	cracked
TEOS_ TMOS	days	cracked into powder	2 hours	not recorded	cracked
TMOS glycerol	days	cracked into powder	2 hours	not recorded	cracked
TEOS_ DMF	months	weeks	2-3 hours	40% in 9 days	remained stable
TMOS_ DMF	months	weeks	2-3 hours	34% in 9 days	remained stable
TEOS-TMOS-DMF	months	1-2 days	2-3 hours	not recorded	cracked
TMOS-TRITON X 100	months	1-2 days	2-3 hours	not recorded	cracked
TMOS-PEG 4000	months	1-2 days	2-3 hours	not recorded	cracked

2.5.2 Physical appearance of dried gel

The dried gel (Xerogel) was transparent yellow in colour (fig. 2.1). It was 23 mm in diameter and 3.2 mm in thickness. It had shrunk 49% in diameter and 32% in thickness of the initial gelled form. Its weight was 2.47 gm which constituted 24% of the weight of the initial gel phase. It was slightly concave due to uneven drying. Any attempt to cut or grind the glass caused it to crack.

Fig. 2.1. Photograph of a dried monolith enlarged by three times the original size.

Cracking during drying can be overcome by using glass containers for gelation instead of plastics, a proper cover with aluminium foil and suitable heat treatment. Crack free monoliths have been obtained by using drying temperature of 50° C for six days.

2.5.3 Leaching measurements

2.5.3.1 *cracking and leaching in distilled water*

Xerogels (TMOS) with 4-5 months ageing were stable in distilled water for many days and resisted cracking when immersed and reimmersed in buffers of different pHs. They also showed no or negligible leaching when kept in distilled water for 15 days. The sol-gel glasses with fifteen days ageing cracked in aqueous solution in 24 hours. However those containing PEG 4000 as a surface active agent remained stable in distilled water for 48 hours.

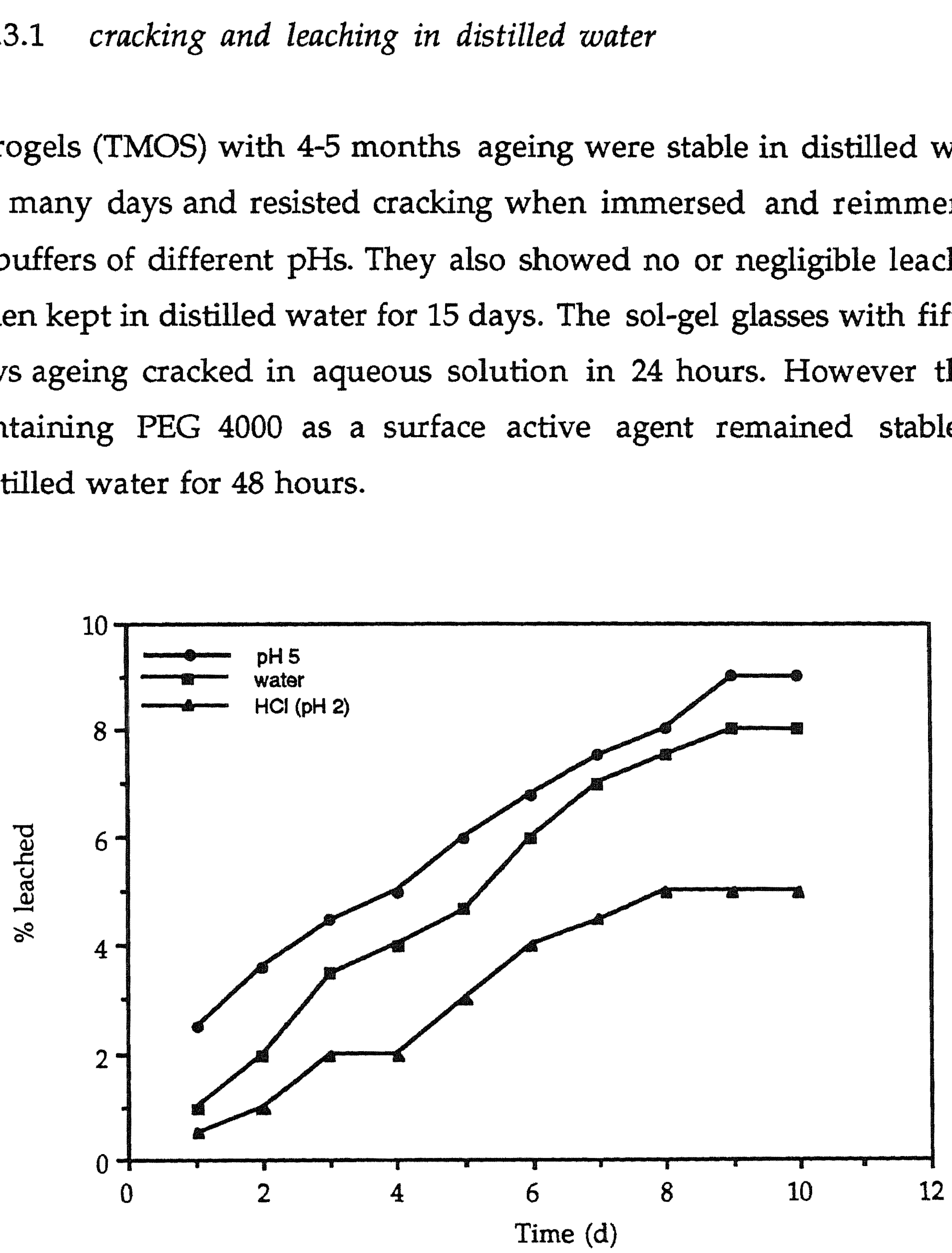


Fig. 2.2. Leaching measurements of four months old monoliths at 22^{0}C in different solvents.

2.5.3.2 *cracking and leaching in various pH buffers*

In the higher pH buffer range 8-9 the sol-gel glass cracked and the dye leached out over a period of few hours. Four month old samples stayed stable in freshly prepared 0.1M citric acid-phosphate buffer pH 5, for one month. Some slight leaching of indicator was observed during this period. The least leaching (5%) occured in HCl pH 2 when monoliths were left immersed for 12 days. The comparative stabilities of 4 month aged samples at different pH are shown in figure 2.2.

2.5.4 Spectral behaviour of entrapped indicators

The mixture of three indicators i.e. bromophenol blue, bromocresol purple and bromothymol blue entrapped in sol-gel showed peaks (high absorbance) at 438nm and peaks (lower absorbance) at 590nm by treating with different pH buffers ranging from 3 to 8.5 (figure 2.4). The absorption spectra of this mixture in solution (figure 2.5) at same pH values showed difference in peak heights and maximum absorbance at 430nm and 590nm.

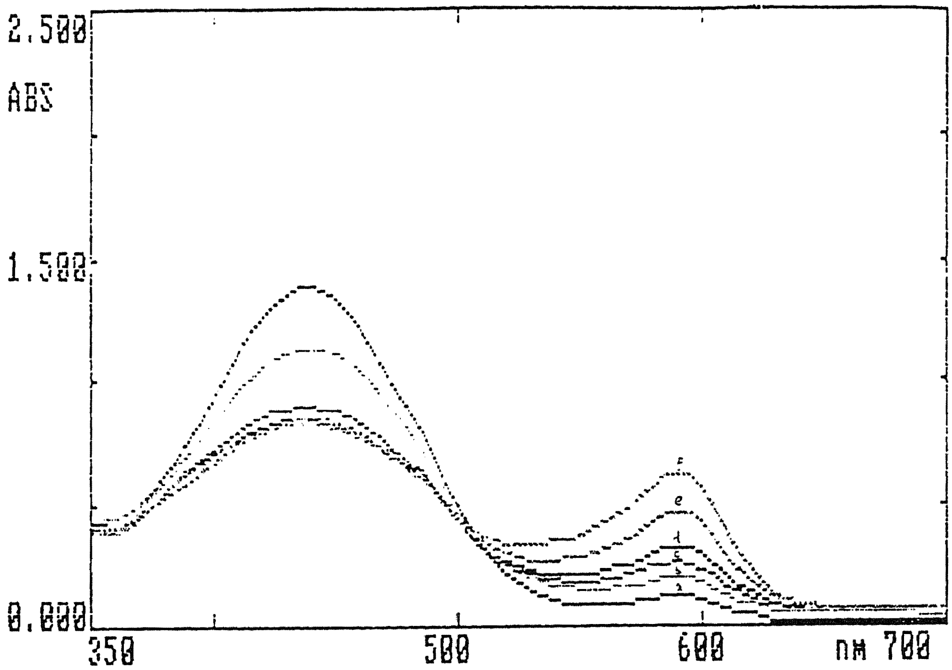


Fig. 2.3 Absorption spectra of bromophenol blue (0.1mM) entrapped in a sol-gel monolith glass at various pH values; (a) pH 3.0 (b) pH 4.0 (c) pH 5.6 (d) pH 6.4 (e) pH 7.4 (f) pH 8.5

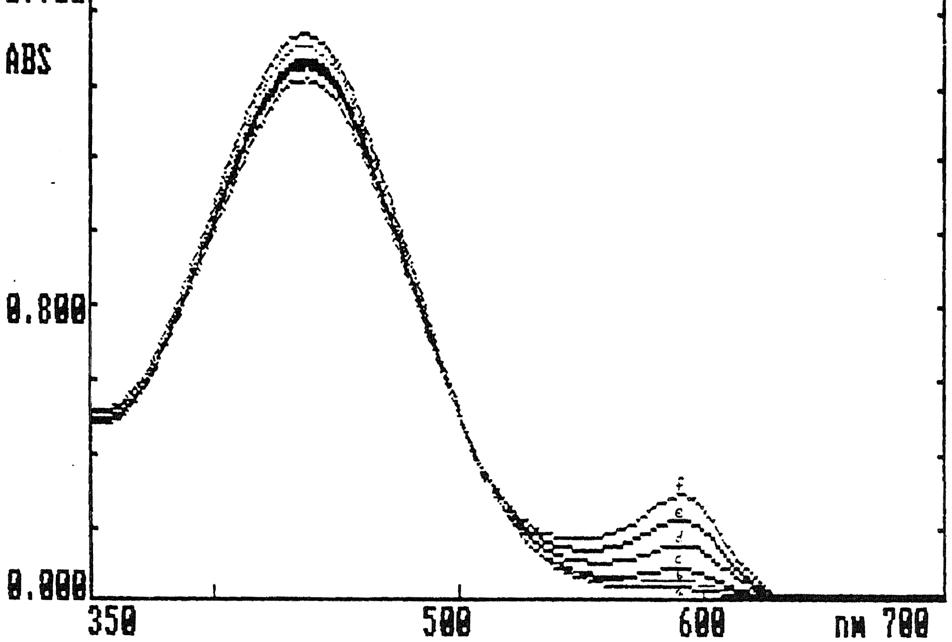


Fig. 2.4 Absorption spectra of a mixture of dyes (bromophenol blue, bromocresol purple and bromothymol blue, each in 0.1 mM concentration) entrapped in a sol-gel monolith at different pH levels; (a) pH 3.0 (b) pH 4.5 (c) pH 5.6 (d) pH 6.6 (e) pH7.3 (f) pH 8.5.

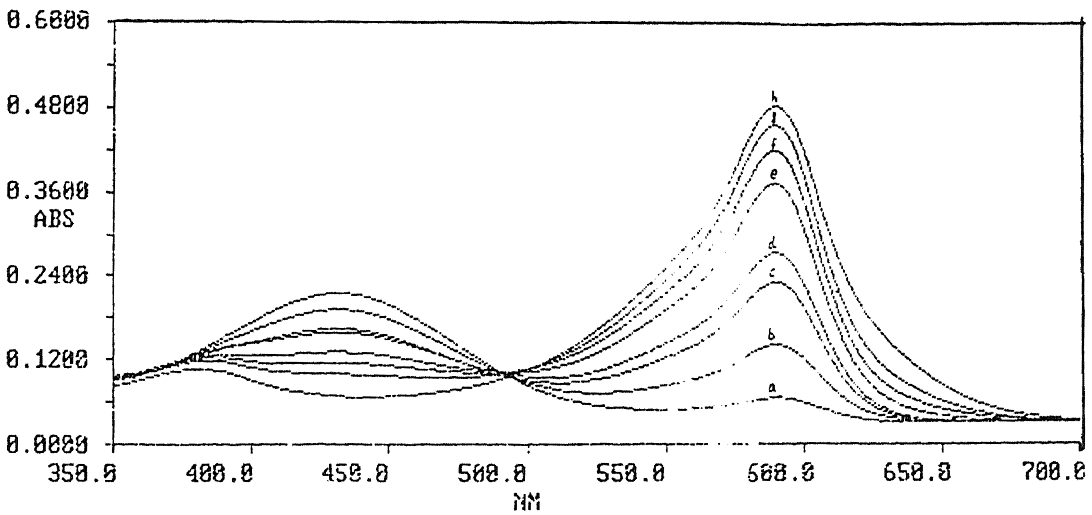


Fig. 2.5 Absorption spectra of a mixture of dyes (bromophenol blue, bromocresol purple and bromothymol blue, each in 0.1 mM concentration) in 0.1M citrate phosphate buffer at different pH levels; (a) pH 3.3 (b) pH 4.0 (c) pH 4.7 (d) pH 5.3 (e) pH 6.2 (f) pH 6.7 (g) pH 7.0 (h) pH 7.6.

Bromophenol blue in sol-gel glass showed maximum absorbance at 440nm and 590nm with pH ranging from 3to 8.5 (figure 2.3). It changed the colour from yellow to light brown in acidic pH and from brownish yellow to dark purple in basic pH. The lapse time set was 5 min. The sol-gel glasses took hours to completely change their colour in different pH.

Bromocresol purple and bromothymol blue showed no maximum absorbance (peaks) at 590 nm. They did not change their colour in different pH solutions even after immersing them in each solution for 50 minutes. They do not act as indicators in the sol-gel matrix probably because they are chemically bound to the glass through their pH active groups. The chemistry involved requires further investigation.

The most active indicator in the mixture was bromophenol blue, the other two were either very slow to react or completely inactive. The maximum absorbance at 590 nm shown by the mixture was mainly due to the presence of bromophenol blue (figure 2.4). The low peak heights at this wavelength in response to different pH as compared to the individual spectra of bromophenol blue can be attributed to the interference of other indicators.

Figure 2.3 shows that over a wide range of pH the indicator has not completely changed from one form to the other. This suggests that only indicator in the surface layers is affected and H^+ is unable to diffuse into the bulk region.

In order to decrease the response time monoliths of thinner samples (1.40 mm and 0.40 mm) were made but the response time could not be largely improved (it remained in order of 50 minutes to 2 hours).

2.6 DISCUSSION

Doped sol-gel monolith glasses made from TMOS and TEOS showed similar properties in term of physical stability (resistance to cracking chemical stability, rate of leaching and dry storage stability). They had similar response times. TMOS was used for further research because of its methanol by product which has a polarity closer to water and hence was suitable for organic dopants (Jorden, et al., 1995). Moreover it has high rate of hydrolysis and reaches gelation point faster than other alkoxides (Allain, 1997).

During the drying phase, some of the larger pores are emptied while smaller pores remain wetted by the solvent, creating large internal pressure gradients. This stress causes cracks in large monoliths and is also responsible for fractures in dry monolithic sensors upon immersion in water (Lev, et al., 1995). Drying control chemical additives are used to increase the rate of drying. They help to prevent cracking by keeping porosity uniform through even drying (Brinker, et al., 1990). Among chemical additives DMF was found to be most effective in stopping fracture of dried monoliths after repeated immersion in water. However, its higher concentration in the matrix may affect the transparency of the sol-gel glass.

Long aged (4-5 months) samples showed comparatively good chemical stability and low leaching of doped dyes. This is attributed to the shrinking of the internal structure as ageing continues after drying for several months. The shrinkage reduces the pore size and surface area of monoliths and make it stronger internally (Lev, et al., 1995). The long aged (well-dried) samples withstood repeated immersion in aqueous solution.

Three pH indicators bromophenol blue, bromocresol purple and bromothymol blue were selected for covering the desired pH range 3-8. The absorption spectra of mixture of these indicators in solution (figure 2.5) showed one isosbestic point instead of three. This can easily be exploited for measuring pH. Bromophenol blue in a mixture of indicators entrapped in sol-gel monolith glass was found to be dominating in the colour change between pH 3 to 8. The other two indictors were either very slow to react with the analyte or do not work in this pH range. Entrapped indicators gave maximum absorbances at the same wavelength as they give in solution. This agrees with Rottman, et al., (1992). The difference in absorption spectra of a mixture of indicators in solution (fig. 2.5) and in monolith (fig. 2.4) suggests that doped indicators molecules deep inside monolith did not change their colour during exposure (5 min) to pH solution due to slow diffusion of H^+.

Monolith-based pH sensors are not practical for real-world detection because of the inherently long response times associated with the long, tortuous diffusion path length of the analyte and the limited accessibility of an entrapped indicators to analyte. They require a long processing time to stabilise and have a tendency to crack if repeatedly used in aqueous solution. Thus this area of research was abandoned in favour of doped thin films.

CHAPTER 3. THIN FILMS DOPED WITH pH INDICATORS

3.1 INTRODUCTION

Thin films can be produced by sol-gel processing through the method of dip coating or a spin coating (Brinker, 1990). The deposition of sol-gel films with regard to time scales, drying theory, tendency towards cracking and development of microstructure were discussed by Brinker et al (1992). They reported that the final film microstructure depends on the structure of entrained inorganic species in the original sol (e.g. size and fractal dimension), the reactivity of these species (e.g. condensation or aggregation rates), the time scale of the deposition process (i.e. related to evaporation and film thickness) and magnitude of shear forces and capillary forces that accompany film deposition i.e. surface tension of solvents and surface tension gradients. Scriven (1988) studied in detail the physics and applications of dip and spin coating. The merits and demerits of these two methods were investigated by Thomas (1994). He also gave the following advantages of the spin coating method over that of dip coating;

1 It requires a smaller quantity of coating solution.
2 It is very rapid.
3 It is good for multilayer applications.

Despite these advantages, the conventional spin coating technique still needs to be improved. For example, it requires a more controlled environment to reduce the risk of air contamination and to coat more than one microscope slide at the same time. It is further required to achieve striation free film with improved smoothness for optical measurements (Du, et al., 1995).

Recent developments in sol-gel thin film technology include the production of films for use as protective coatings and for optical sensor applications (McDonagh, 1996; MacCraith, 1993; Ingersoll et al., 1997).

Lee and Saavedra (1994) reported the entrapment of pH indicator, bromophenol blue, in a sol-gel thin film prepared from a starting mixture of 1 ml TEOS, 164 μl of deionised water, 112 μl of 0.04 M HCl and 50 μl of 60 mg/ ml bromophenol blue in water, coated on a microscope slide. They showed fast response of indicator to pH change. Using attenuated total reflectance spectrometry the indicator performance was assessed. It was proved that indicator is non-leachable and responds to pH changes in a reversible manner.

Yang and Saavedra (1995) developed a new optical sensing platform based on a combination of planar waveguide and sol-gel processing technologies. The sensor element consisted of a porous glass layer composed of a 12: 6: 16: 1 (v/v) mixture of methyltriethoxysilane (MTES), titanium tetrabutoxide, absolute ethanol, silicon chloride containing an immobilised pH indicator (bromocresol purple) coated over a single mode planar integrated optical waveguide. Dissolved analytes that diffuse to and react with indicator were detected via attenuated total reflection (ATR). The response to pH was both sensitive and rapid. They measured pH over wide pH range 3.5 to 9.5 by using a single indicator. However the sensor suffered serious leaching problems, about 50% dye leached out in one hour in pH 5 buffer.

Bambot et al (1994) doped a pH sensitive fluorescent indicator (Texas red hydrazide) in a sol-gel film. The sensor was based on the phenomenon of fluorescence energy transfer. They showed fast response of the sensor to changes in pH 6 to 8. They also mentioned the long term stability of doped films in pH 8 buffer and their sensitivity to salt and ionic strength. They failed to reproduce the response of sensor after steam autoclaving. Shahriari et al (1994) reported the preparation of thin film from starting mixture of TEOS 52%, deionised water 9%, anhydrous ethyl alcohol 38.9%, HCl (12M) 0.1% on porous fibres doped with two pH indicators (bromocresol purple, bromocresol green). They reported that optrode intensity changes by 2% per ^{0} C with rise in temperature from 20 ^{0} C to 45 ^{0} C and also stated that increase in NaCl concentration in buffer shifts the optrode pH calibration curve to lower pH end. They used a laser diode as light source which may cause photobleaching of entrapped indicators.

The purpose of the present work is to develop a stable pH indicator doped sol-gel thin film for on-line pH measurement in real time by using absorption spectroscopy. The aim is to coat thin films on different glass substrates for achieving a robust sensor. It is also planned to study the different factors affecting the pH sensing and long term stability of these doped films.

3.2 THIN FILM ON A MICROSCOPE SLIDE

3.2.1 Materials

Microscope slides (75x25 mm) were obtained from Coulter electronics, GMBH, Germany. Sodium dichromate, ethanol (98%) and reagents for phosphate buffers were supplied by BDH.

3.2.2 Preparation of the thin film

Thin films were prepared by hydrolysis of TMOS with water at pH 1-3, with a water : alkoxide molar ratio (R) of 2. The films fabricated with R values 4 and 5 were found to either crack or were unhomogeneous after drying.

A stock solution of sol-gel was prepared by mixing 7.3 ml TMOS, 1.7 ml deionized water and 0.1 ml 0.04 M HCl. The mixture was sonicated at room temperature for 10-20 minutes to create a single phase, clear solution. This solution was then placed (covered) in the dark at room temperature for 24 hours.

3.2.2.1 *Coating solution*

2.0 ml of partially hydrolysed stock sol solution was added to 1 ml, 10 mM phosphate buffer pH 6 and 1 ml, 40 mM bromophenol blue (bromocresol purple or bromothymol blue or mixture of three) solution in ethanol. The coating solution was aged for 3 days to get film of optimum thickness without cracking. Then 2.0 ml of this solution was diluted with 1ml of ethanol for coating glass substrates.

3.2.2.2 *Preparation of glass substrates*

The glass substrates (test tubes, microscope slides) to be coated were cleaned with chromic acid solution prepared according to the method described by Vogel, (1953). 10 g of sodium dichromate in 10 ml distilled water was added to 200 ml concentrated sulphuric acid. The glass substrates were left dipped in the chromic acid solution for half an hour. Afterwards they were washed several times with distilled water. The surface activation (make hydrophilic by activating OH groups) of the glass substrates was carried out by immersing them in 2 M NaOH, 2 M HCl and distilled water respectively for 10 minutes each. In between they were washed several times with distilled water. All glass substrates were dried in vacuum at room temperature before coating.

3.2.2.3 *Method of coating*

Microscope slides were coated by a dip and a spin coating method. In the dip coating method the microscope slide was immersed in 20 ml coating solution placed in small beaker. It was then withdrawn manually at the rate of 10 cm/min. One side of the slide was blocked by cellotape. The final film was solid, thin but non-uniform.

For spin coating 2 ml coating solution was placed on the surface of the slide and was spun lying horizontally in the spin coater at 600 rpm for 20 seconds. The sol-gel solution was evenly spread on the slides, the excessive solution was dragged out leaving a very thin film by centrifugal force acting outward. The final film was thin, transparent, uniform and solidified. There was no visible cracking observed on the surface. The slides were then stored covered in the dark at room temperature.

3.2.3 Results

Thin films obtained by dip coating were non uniform and hence were inadequate for the study of absorption spectra for trapped indicators but they however changed colour in changing pH and did not crack. There are some other disadvantages attached to this method. For example it requires a large quantity of coating solution which is not desirable, it coats on both sides of slides (sealing one side with tape caused contamination in solution and more complications in keeping that side clean) (Thomas, 1994).

Spin coating is a more sophisticated and reliable method for making uniform sol-gel thin films. It has tremendous applications in the microelectronics industry (Vorotilov, et al., 1995).

3.2.3.1 *New spin coater*

The spin coater was a new design to enable eight microscope slides to be coated at the same time. It has variable speed to control the thickness of films. The whole process is carried out in a closed system to avoid any contamination. The different views of the spin coater can be seen in Figures 3.2, 3.3. The spin coater consisted of a circular metal disc which is cut on the top into eight equal size compartments to hold standard microscope slides. The slide compartments are encircled by a circular drain to collect excess coating solution. The metal disc is mounted on the spindle of a bench centifuge to get control of the speed. The spin coater has two safety lids to seal the whole system.

3.2.3.2 *Uniformity of the thin film on a microscope slide*

The film deposited on the microscope slide by the new spin coater was subjected to uniformity testing. The newly fabricated film (1 hour aged) was measured for absorbances at various points by a spectrophotometer. The number of measurements were plotted against

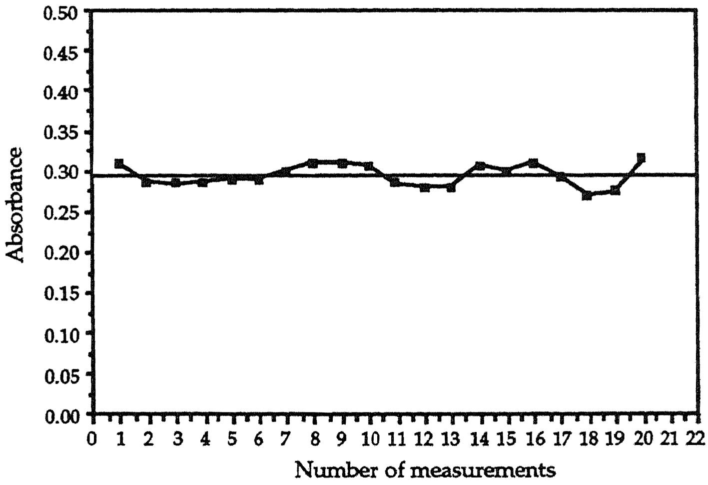
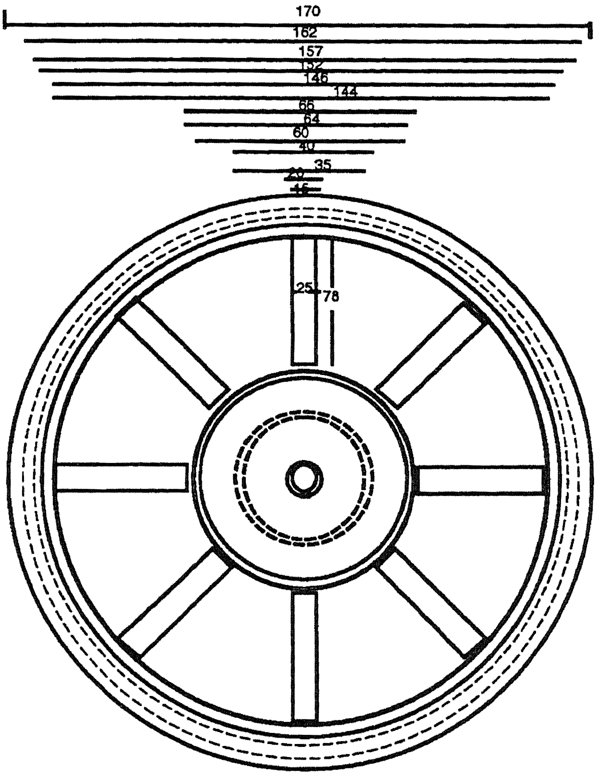
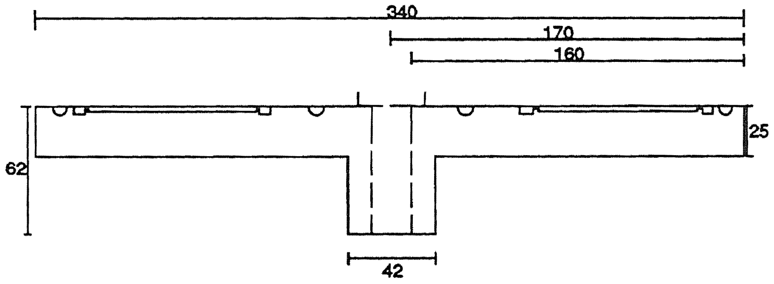


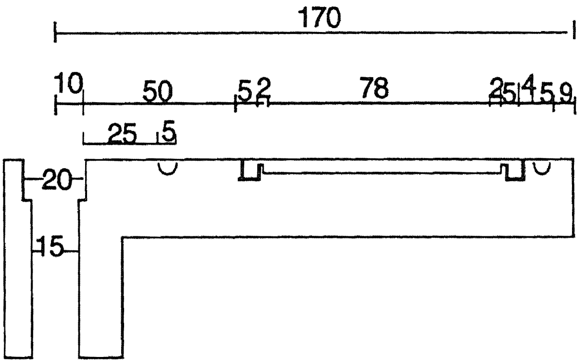
Fig. 3.1 Measurements of uniformity of a thin film on a glass microscope slide deposited by a spin coating method.



Top view



Side view



Section view

Fig. 3.2 Engineering diagram of a new spin coater (dimensions in mm).

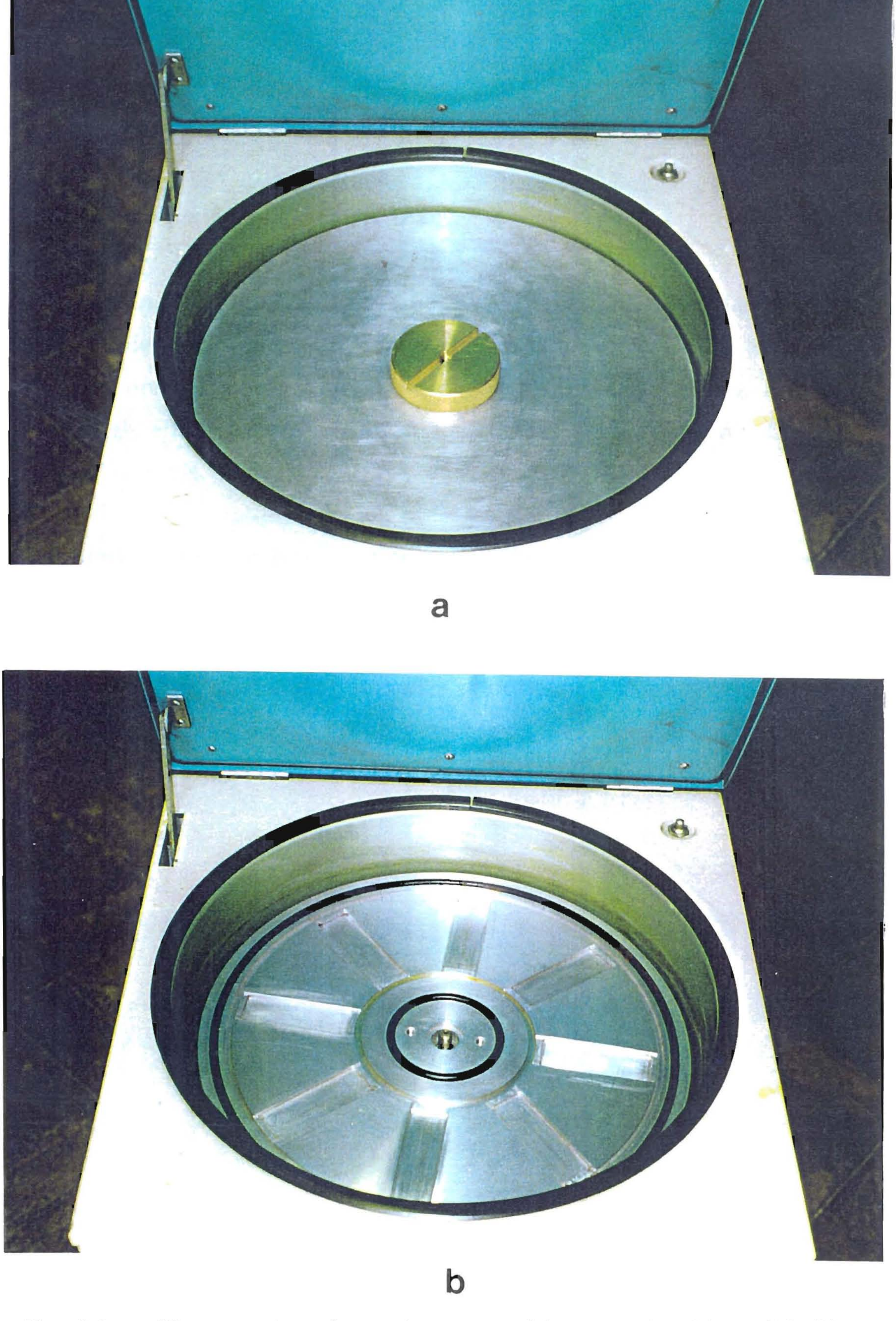


Fig. 3.3 Photographs of a spin coater (a) covered with a lid (b) uncovered showing microscope slide compartments on a circular metal disc mounted on a motor spindle of a bench centrifuge.

absorbances to find the uniformity of the film (fig. 3.1).

3.2.3.3 *Response of film to pH change*

Sol-gel thin film doped with bromophenol blue coated on glass slides were studied for colour change in pH buffer ranging from pH 3 to pH 8. The response time i.e. time required by film to change the colour completely and to become stable in a particular pH solution was found to be 20 seconds.

The films as obtained from spin coater after fabrication exhibited a dark yellow colour which showed a series of colour change, from light yellow to dark purple on increasing pH 3 to 8. The colours developed by doped film after immersing in series of buffers are displayed in figure 3.4.

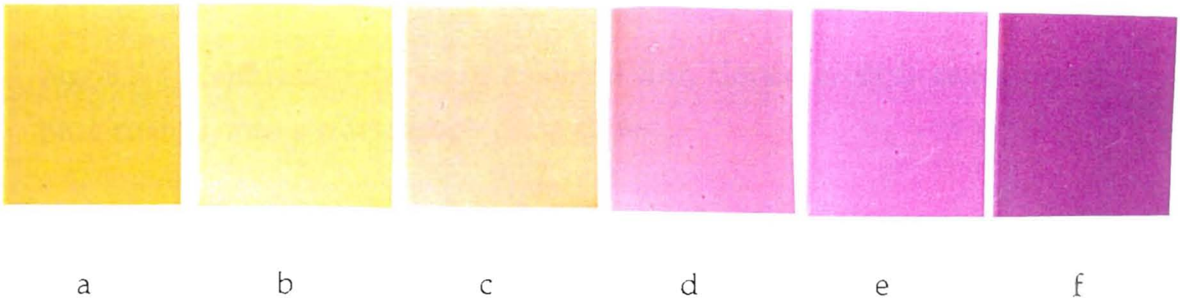


Fig. 3.4 Series of colours developed by bromophenol blue doped in a sol-gel film on a microscope slide in various pH solutions. (a) pH 3 (b) pH 4 (c) pH 5 (d) pH 6 (e) pH 7 (f) pH 8.

The coated glass slides were subjected to spectral analysis for each colour change in pH 3 to 8 to draw a calibration curve. The coated slides were read at a single marked point with back ground correction with an uncoated slide. The absorbances were recorded at each pH for an average of three samples.

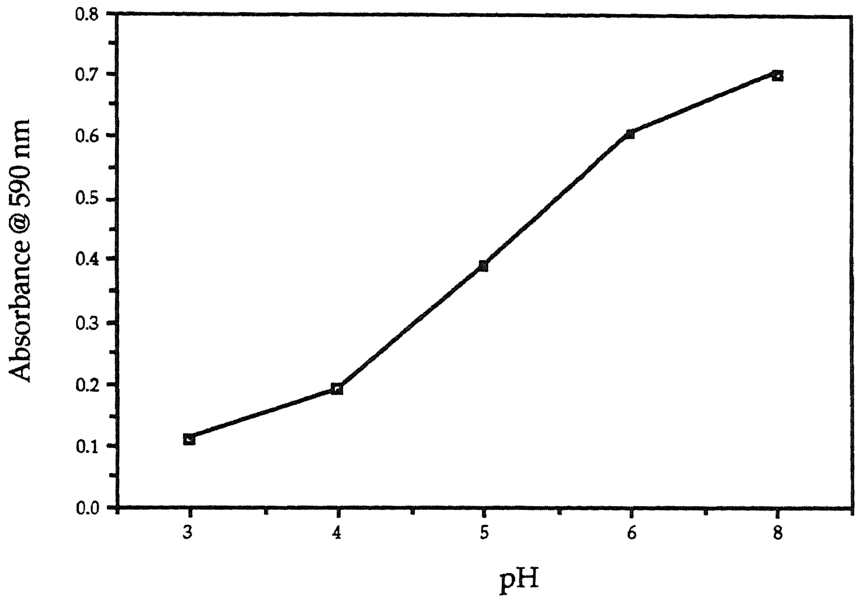


Fig. 3.5 Calibration curve of a sol-gel film doped with bromophenol blue coated onto a microscope glass slide.

3.2.4 Discussion and conclusion

Spin coating differs from dip coating in that the deposition film thins by centrifugal draining and evaporation (Brinker et al 1992). This process can be divided into four stages; deposition, spin-up, spin off and evaporation, although for sol-gel coating evaporation normally overlaps the other stages (Scriven, 1988). An excess of liquid is dispensed on the surface during the deposition stage. In the spin-up stage the liquid flows radially outward driven by centrifugal force. In the spin-off stage excess liquid flows to the perimeter and leaves as droplets. As the film thins the rate of removal of excess liquid by spin-off slows down, because the thinner the film, the greater the resistance to flow and the concentration of non-volatile components increases, raising the viscosity (Brinker, et al., 1992).

The films obtained as a result of spin coating were found to be uniform under light as shown in the photographs given in fig 3.4. The uniformity of film was also tested by spectroscopy. The film after deposition was measured for absorbances at various points by slowly moving the slide up and down and across (whilst standing vertically) the light beam in the spectrophotometeric cell. The results in figure 3.1 suggest that film is evenly deposited with small differences in absorbances at different points. These differences can be attributed to uneven glass or fine solid particles in the film formed as a result of the condensation of the sol. According to Scriven (1988), the advantage of spin coating is that the film of liquid tends to become uniform in thickness. The optimum speed for producing uniform, thin (< 1μ thickness) and crack free films was achieved at 600 rpm for 20 seconds. Higher than this caused cracking of the film after drying and lower speed gave inhomogeneity. Thicker films (> 1μ) usually crack either during drying or immersing in aqueous solution (see also section 6.4).

Cleanliness can be a problem because, during spinning, air borne particles land on the film due to the fly paper effect (Thomas, 1994). In the newly designed spin coater this problem was kept to a minimum by using two safety lids to make the whole system air tight.

The films were brought into operation 1 hour after deposition. The colour change was reversible and time taken for this reverse change was also found to be 20 seconds. No visible leaching or cracking of doped films was observed when subjected to various pH solutions for recording their pH responses. The colour chart in figure 3.4 can be used as reference for measuring pH in the range from 3-8. The calibration curve of coated slides (fig. 3.5) showed the dynamic pH range of trapped bromophenol blue in between pH 3 to 8.

The microscope slides deposited with doped sol-gel film have the following advantages over pH paper;

1. They are reusable.
2. A single slide can cover a wide pH range 3 to 8.
3. They are relatively resistant to chemical attacks like strong acids, organic solvents (Lin and Brown, 1997).
4. They can be plunged into soil for measuring soil pH.
5. They can be measured in a spectrophotometer to give accurate results.

However for continuous pH measurements while immersed in sample liquids, these slides have to be cut down to fit in standard cuvettes or used as waveguides measuring via the attenuated total reflection (ATR) technique (Yang and Saavedra, 1995). These methods are complicated and time consuming. For more simple and rapid continuous pH measurements, it was decided to coat test tubes and tubing.

3.3 THIN FILMS ON THE INSIDE OF TEST TUBES AND GLASS TUBING

3.3.1 Materials

Soda glass test tubes (75x12 mm) were supplied by Samco company. Soda glass tubing (5 mm diameter) were obtained from BDH limited.

3.3.2 Preparation of film

2 ml coating solution was placed in a pre activated (see sec. 3.2.2.2) glass test tube and was rotated by hand in a horizontal position to make sure it was coated evenly inside. The tube was then allowed to stand in inverted position for a few seconds just to drain off the excess solution. The tube standing inverted was then dried under vacuum in a dessicator for 5 minutes. The drying of the film was carried out immediately (within one minute) after coating. The final film obtained was solid, robust to gentle rubbing, thin, visibly uniform, transparent and crackfree. The coated tubes were then stored covered in the dark at room temperature for 24 hours for ageing. The same procedure was adopted to coat sol-gel thin films inside of glass tubing.

3.3.3 Results

3.3.3.1 *Spectral analysis of pH indicators entrapped in sol-gel film*

Thin coatings (aged for one week) entrapped with Bromophenol blue, Bromocresol purple, Bromothymol blue and a mixture of three indicators were subjected to pH solution ranging from 3 to 10 separately (figures 3.6-3.9). The buffer used to cover the pH range 3-8.5 was 0.1M citrate-phosphate and for pH 9-10, 0.1 M sodium borate was used. The overlapping spectra in each case were obtained by using a Philips spectrophotometer (PU 8740). The lapse time in each buffer was set to five minutes to compare the results with monoliths. However the diffusion of H^+ in thin film had normally reached equilibrium in less than 1 minute (fig. 3.13, 3.14).

Each indicator in solution was also scanned for absorption spectra (figures 3.10 to 3.12) in order to compare with the doped one. The concentration of indicator in solution was $1x10^{-4}$ M and to get similar sensitivity in thin film each indicator was added at a concentration of $2x10^{-2}$ M in the sol-gel solution. Each doped pH indicator showed wider pH response than when free in solution (fig. 3.6-3.8). The mixture of three indicators trapped in sol-gel gave a response to pH changes from 3 to 9 (fig. 3.9) but these films cracked after a few days of ageing. However individually doped films stayed stable for weeks. The results on ageing effect will be presented in the next section.

Bromophenol blue doped films were selected for further experiments for their good sensitivity and response to pH changes from 3 to 8, the useful range for most industrial and environmetal applications. The response times of these films in changing from pH 3 to pH 8 and vice versa were recorded to be 20 seconds (fig. 3.13, 3.14).

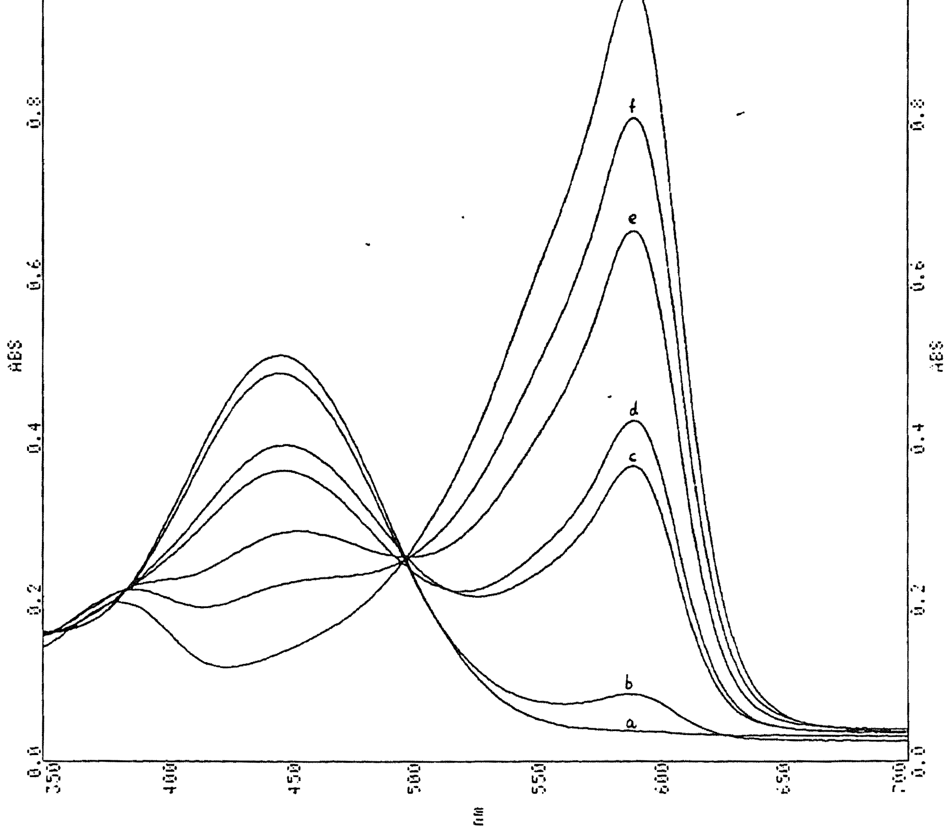


Fig. 3.6 Absorption spectra of bromophenol blue (40 mM) in a sol-gel thin film at various pH levels; (a) pH 3.0 (b) pH 4.0 (c) pH 4.5 (d) pH 5.0 (e) pH 5.5 (f) pH 6.0 (g) pH 8.0.

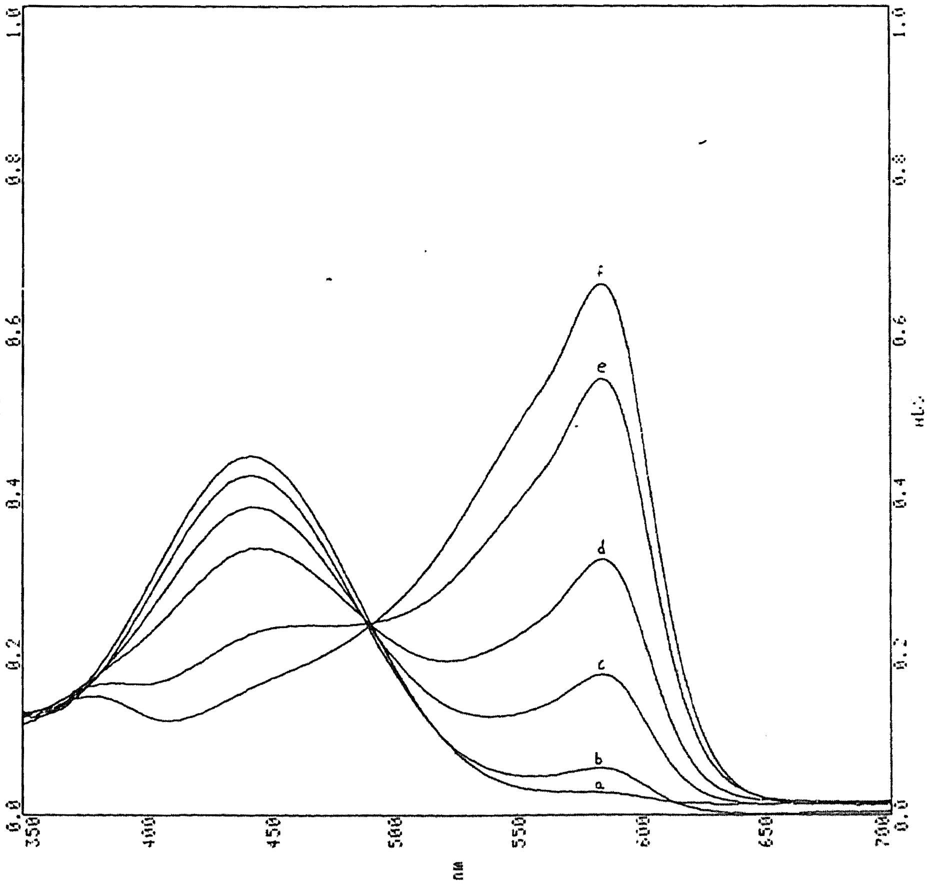


Fig. 3.7 Absorption spectra of bromocresol purple (40 mM) in a sol-gel thin film at various pH levels; (a) pH 5.0 (b) pH 6.0 (c) pH 7.0 (d) pH 8.0 (e) pH 9.0 (f) pH 10.

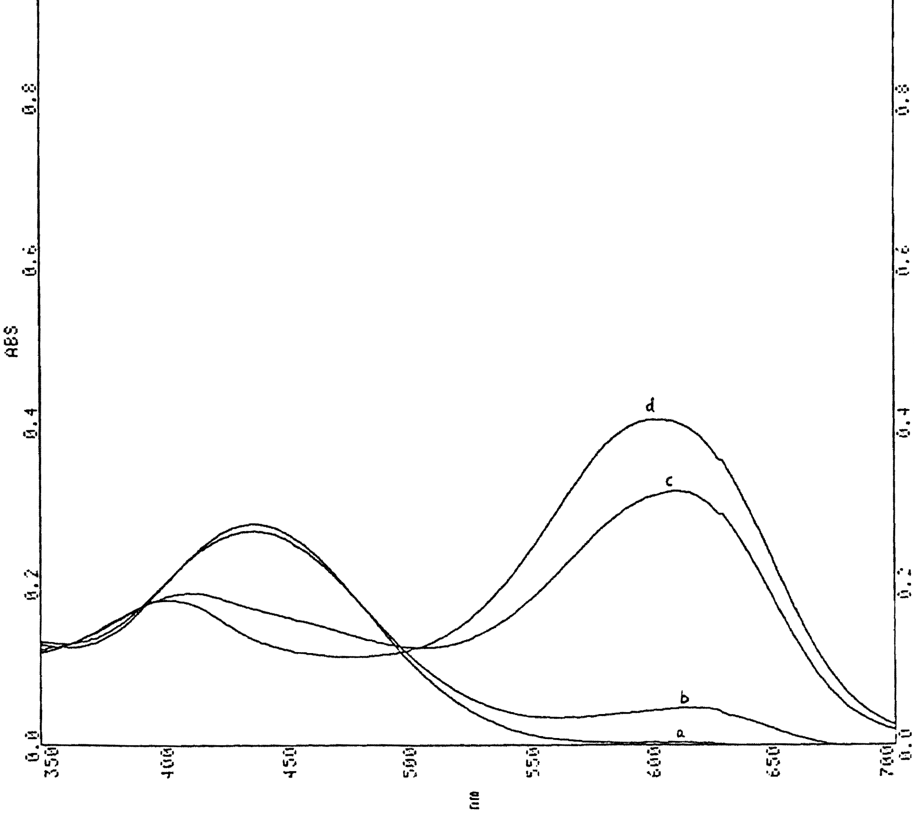


Fig. 3.8 Absorption spectra of bromothymol blue (40 mM) in a sol-gel thin film at various pH levels; (a) pH 7.0 (b) pH 8.0 (c) pH 9.0 (d) pH 10.

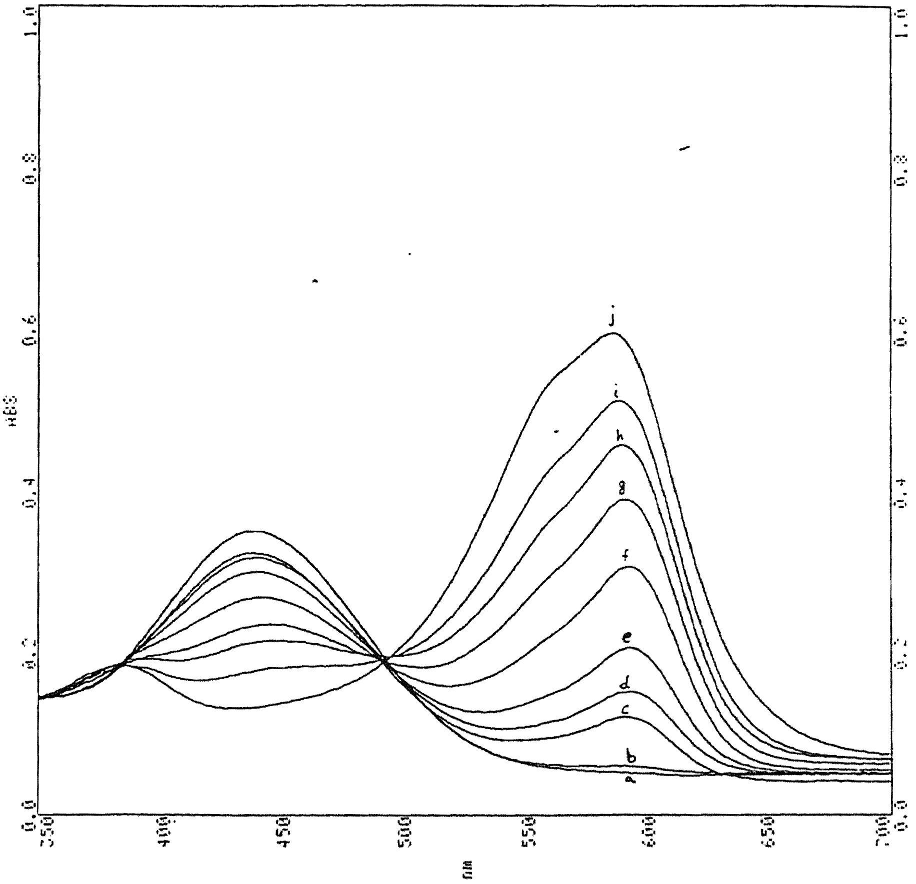


Fig. 3.9 Absorption spectra of a mixture of indicators (bromophenol blue, bromocresol purple and bromothymol blue, each in 40 mM concentration) in a sol-gel thin film at various pH levels; (a) pH 3.0 (b) pH 4.0 (c) pH 4.5 (d) pH 5.0 (e) pH 5.5 (f) pH 6.0 (g) pH 7.0 (h) pH 8.0 (i) pH 8.5 (j) pH 9.0.

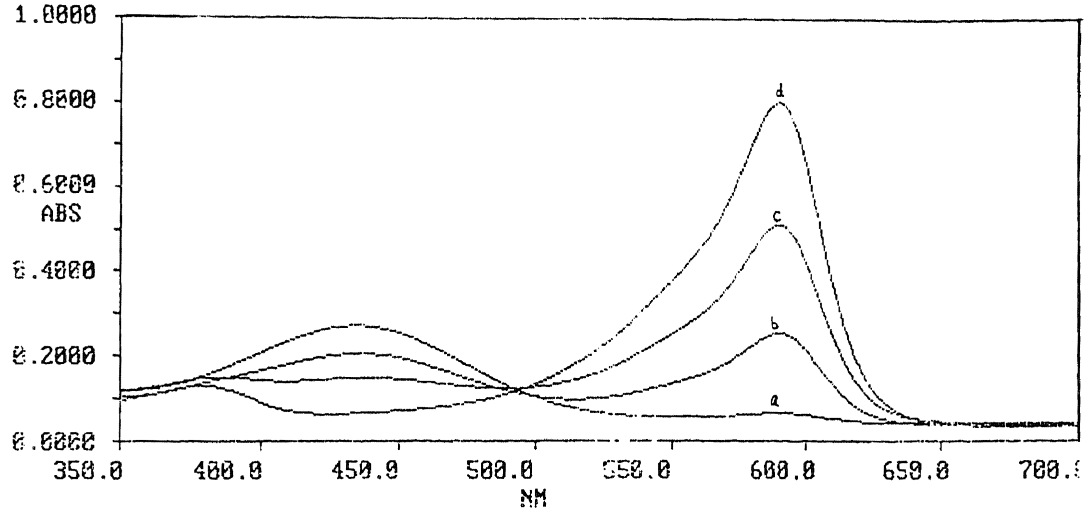


Fig. 3.10 Absorption spectra of bromophenol blue (0.1 mM) in 0.1 M citrate phosphate buffer at various pH levels; (a) pH 2.3 (b) pH 3.3 (c) pH 4.0 (d) pH 5.1.

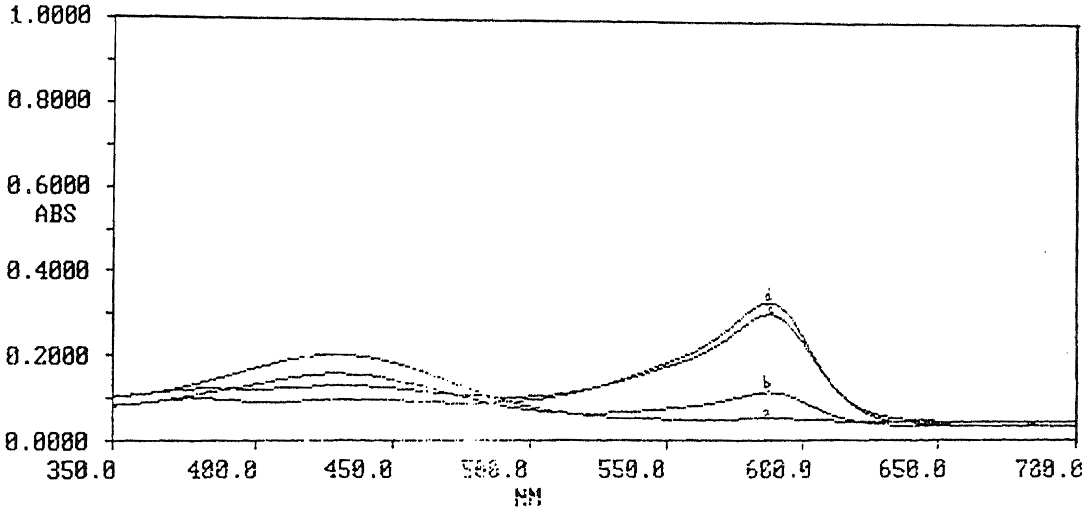


Fig. 3.11 Absorption spectra of bromocresol purple (0.1 mM) in 0.1 M citrate phosphate buffer at various pH levels; (a) pH 4.2 (b) pH 5.5 (c) pH 6.3 (d) pH 6.5.

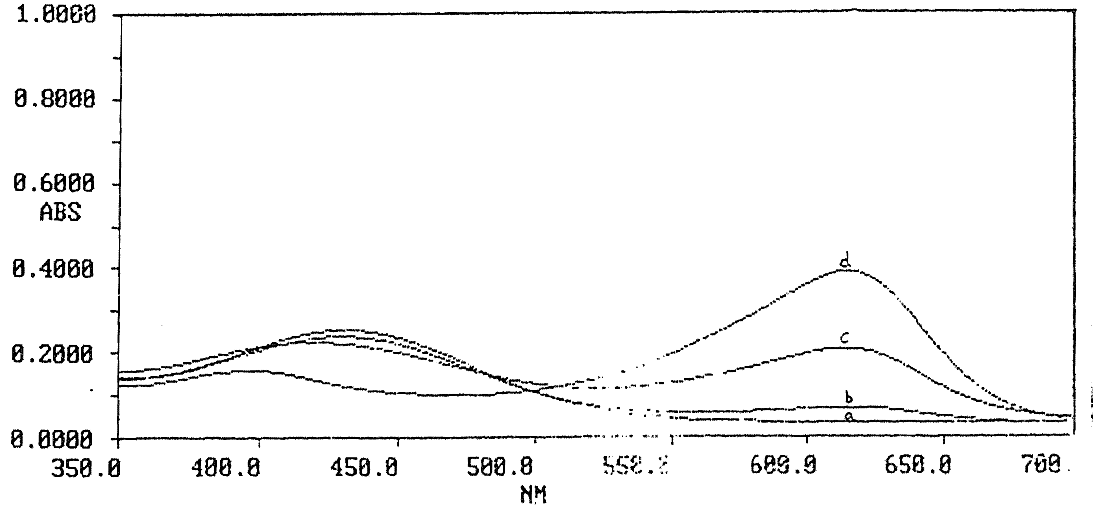


Fig. 3.12 Absorption spectra of bromothymol blue (0.1 mM) in 0.1 M citrate phosphate buffer at various pH levels; (a) pH 4.7 (b) pH 5.9 (c) pH 6.8 (d) pH 8.4

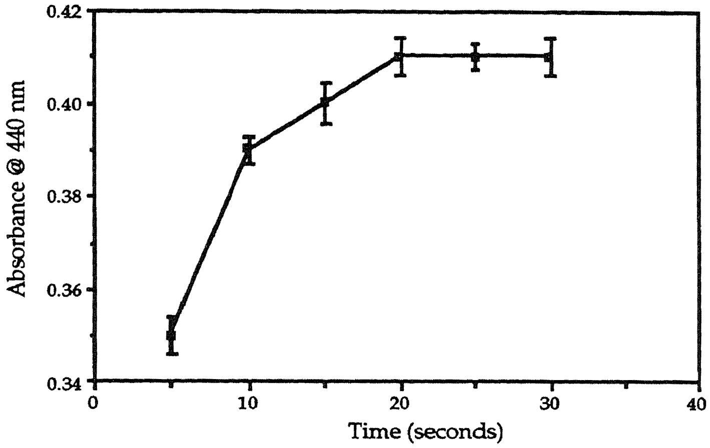


Fig. 3.13 Response of sol-gel film doped with bromophenol blue to pH 3 buffer at room temperature (22 ^{0}C).

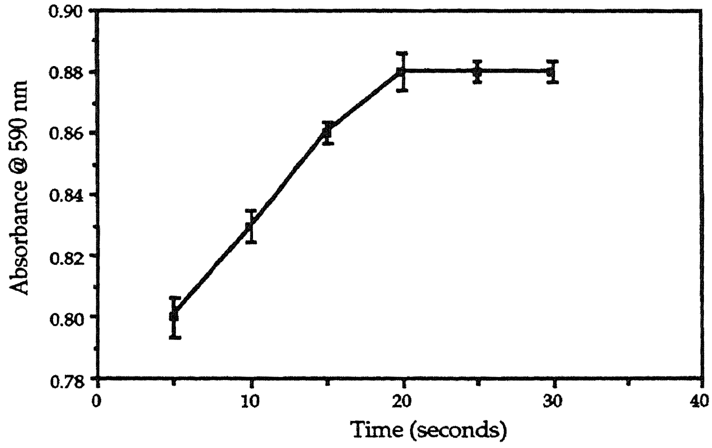


Fig. 3.14 Response of doped film to pH 8 buffer at room temperature .

3.3.3.2 *Comparative studies of sol-gel films aged for different periods*

Two types of ageing effect were noticed on doped thin films. One on the pattern of absorption spectra and the other on the response time.

The absorption spectra of entrapped bromophenol blue and bromocresol purple in a thin sol-gel film aged for one week and four weeks were comparatively studied (figures 3.15, to 3.18). In case of four weeks old samples at higher pH i.e. 7 to 10 there was some absorbance at the wavelength of 560 nm. This can be attributed to the changing distribution of pore sizes with ageing that restricts the movement of OH^-, H_2O (but not H^+) in relatively narrowed pores at basic pH. Notably the ageing affected more the shape of the absorption spectra of bromocresol purple with pH range 5 to 10 (fig. 3.18) than bromophenol blue with pH range 3 to 8 (fig. 3.13). This also favours the choice of bromophenol blue for further studies.

The response time of four weeks old samples in pH 8 buffer increased to 10 minutes (fig. 3.19). However ageing did not affect the response time of thin films in pH 3 buffer because the diffusion of H^+ through narrow pores remained uneffected due to their small size.

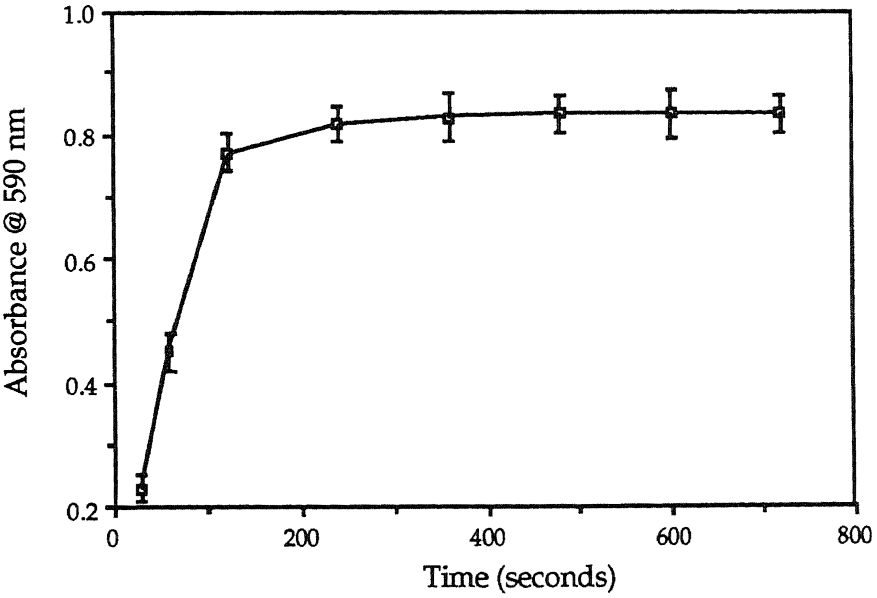


Fig. 3.19 Response of four weeks old bromophenol blue doped film to buffer pH 8.

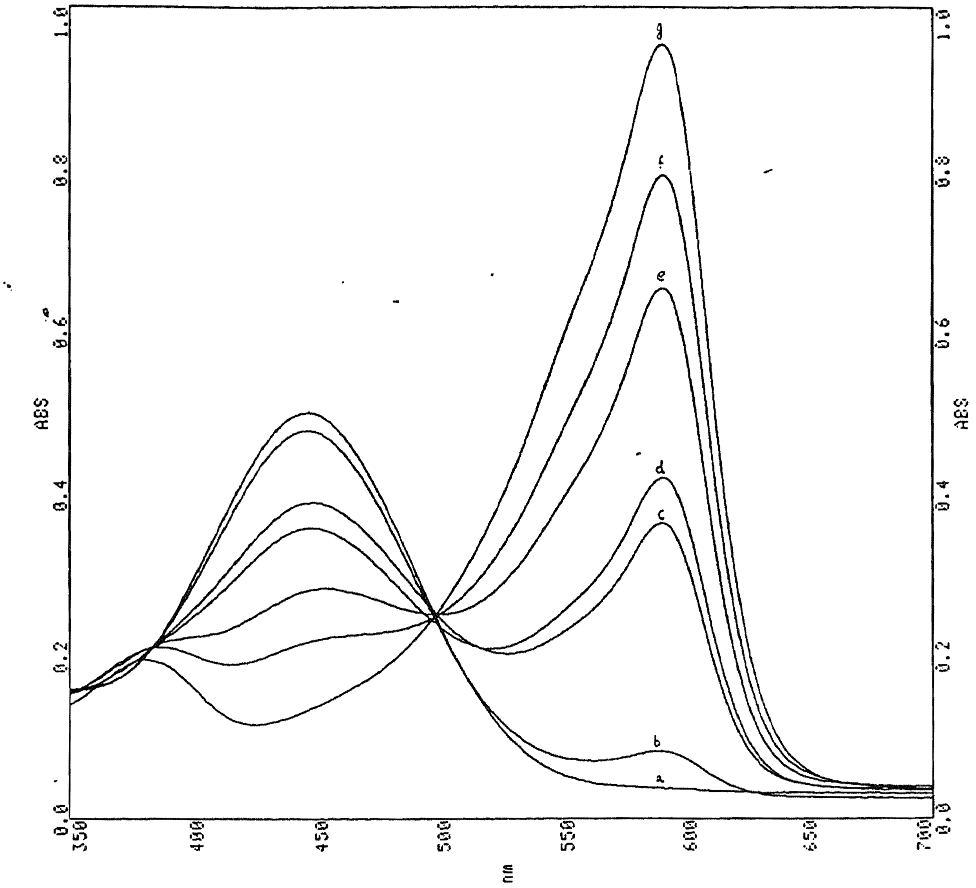


Fig. 3.15 Absorption spectra of bromophenol blue (40 mM) in a sol-gel thin film aged for one week (a) pH 3.0 (b) pH 4.0 (c) pH 4.5 (d) pH 5.0 (e) pH 5.5 (f) pH 6.0 (g) pH 8.0.

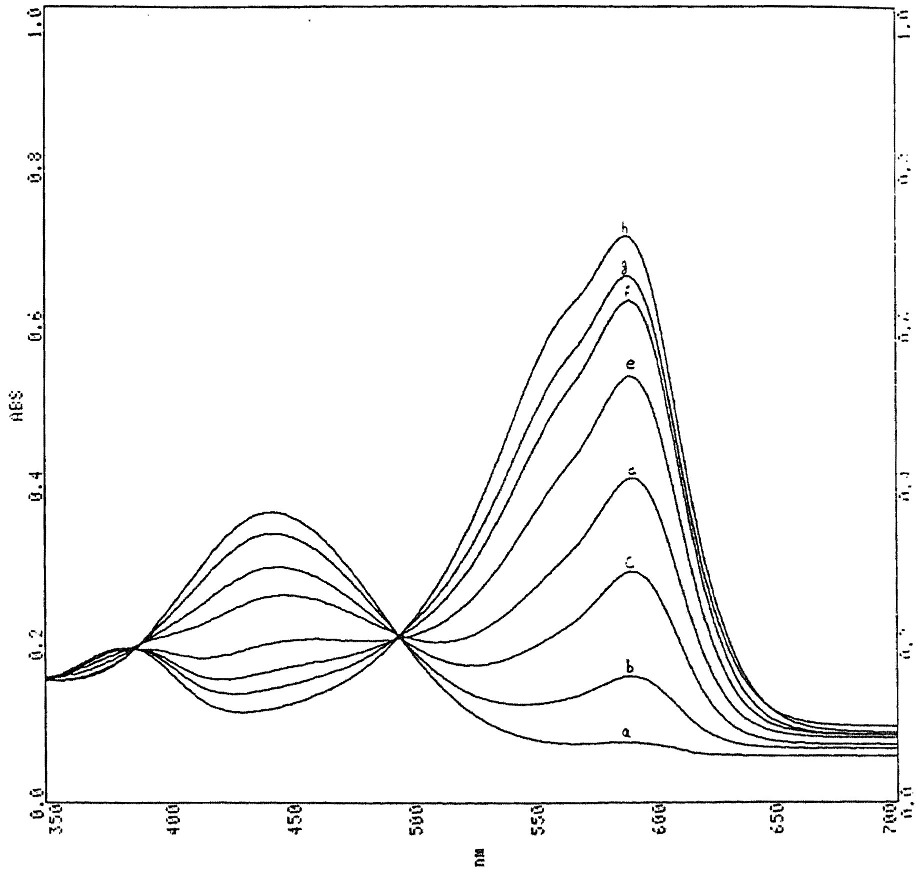


Fig. 3.16 Absorption spectra of bromophenol blue (40 mM) in a sol-gel thin film aged for four weeks (a) pH 3.0 (b) pH 4.0 (c) pH 4.5 (d) pH 5.0 (e) pH 5.5 (f) pH 6.0 (g) pH 8.0.

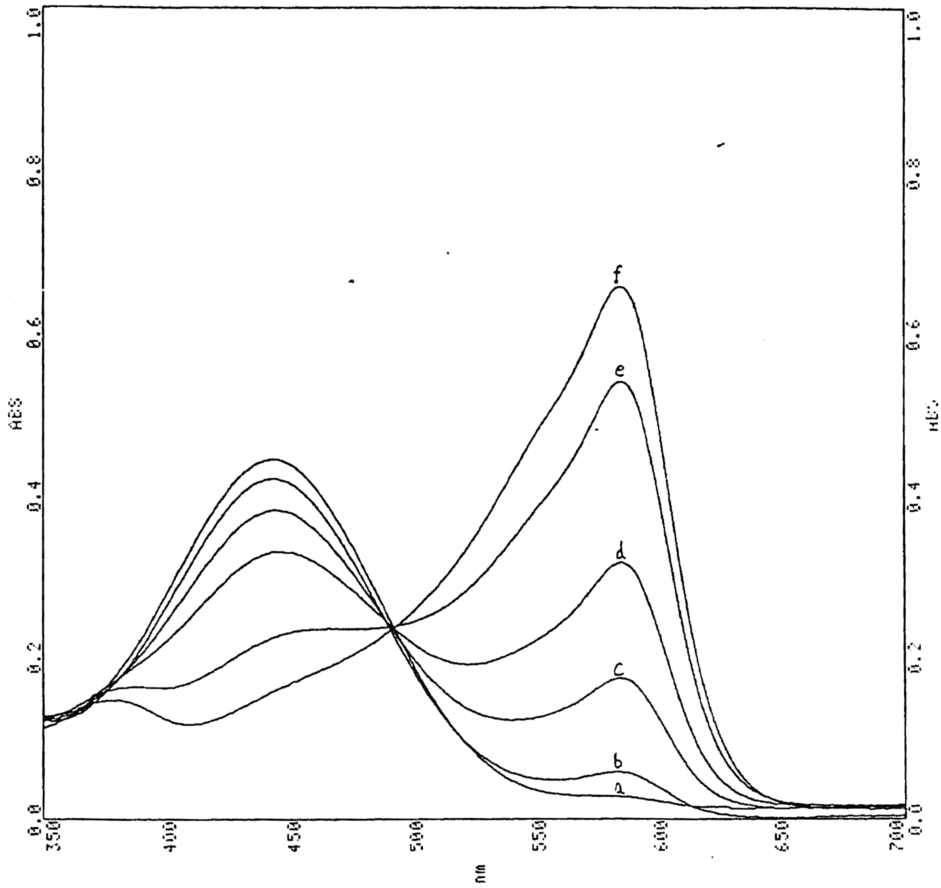


Fig. 3.17 Absorption spectra of bromocresol purple (40 mM) in a sol-gel thin film aged for one week (a) pH 5.0 (b) pH 6.0 (c) pH 7.0 (d) pH 8.0 (e) pH 9.0 (f) pH 10.0.

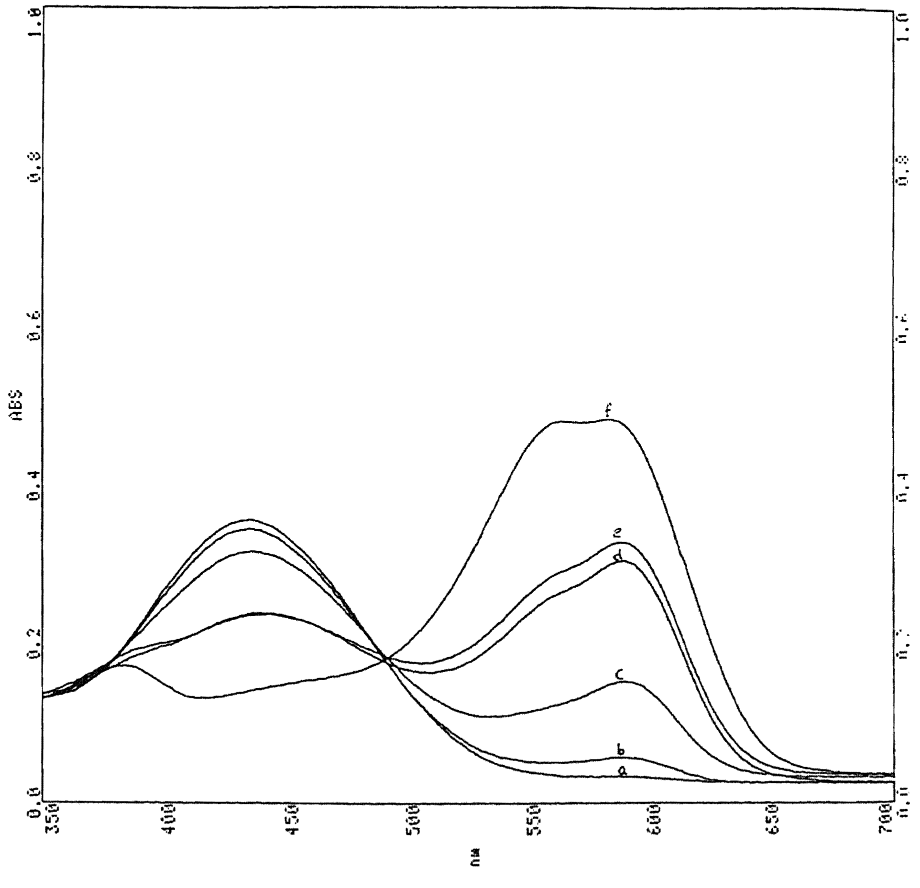


Fig. 3.18 Absorption spectra of bromocresol purple (40 mM) in a sol-gel thin film aged for four weeks (a) pH 5.0 (b) pH 6.0 (c) pH 7.0 (d) pH 8.0 (e) pH 9.0 (f) pH 10.0.

3.3.3.3 *Effect of DMF*

DMF (dimethyl formamide) is a drying control chemical agent (DCCA) which helps to speed drying of the sol-gel matrix deep inside and to avoid cracking by reducing surface tension. DMF was added to TMOS in a final volume ratio of 0.10 : 2.00 before hydrolysis. Indicator doped films containing DMF were steam heated at 120^0 C after fabrication. Four weeks old samples were tested for response time in pH3 and pH 8 buffers. They were found to exhibit 20 seconds response time in both buffers contrary to films without DMF.

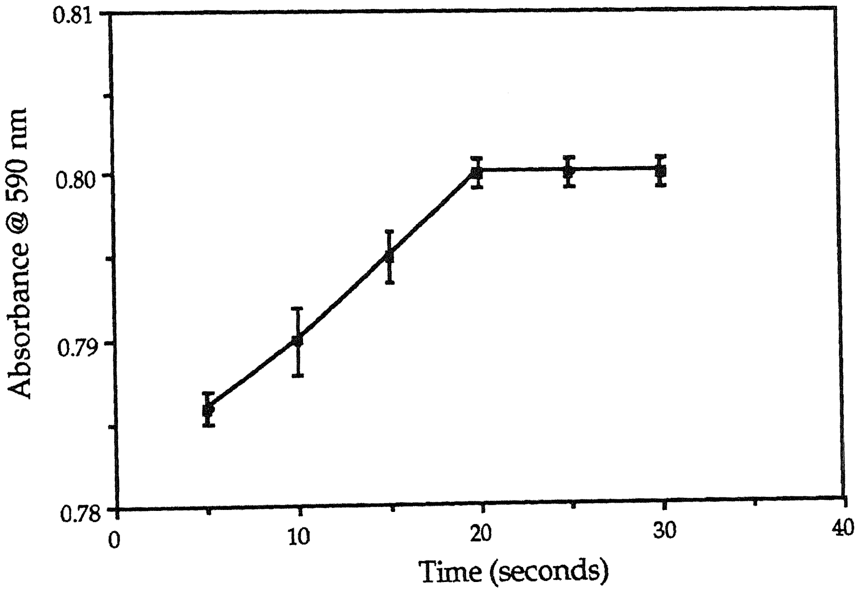


Fig. 3.20 Response of a four week aged thin film with added DMF to buffer pH 8.

3.3.3.4 *Autoclaving a thin film*

A thin film doped with indicator coated inside a test tube was steam autoclaved under standard autoclaving conditions i.e. heated at 120 ^{0} C, under 15 psi pressure for 15 minutes. The thin film for autoclaving was prepared by the addition of DMF. The autoclaved thin film showed no cracking and had the similar response time to that of unautoclaved samples. Indicator doped thin films without DMF when steam autoclaved, failed to give any response to pH change even after immersion in various pH buffers for many hours.

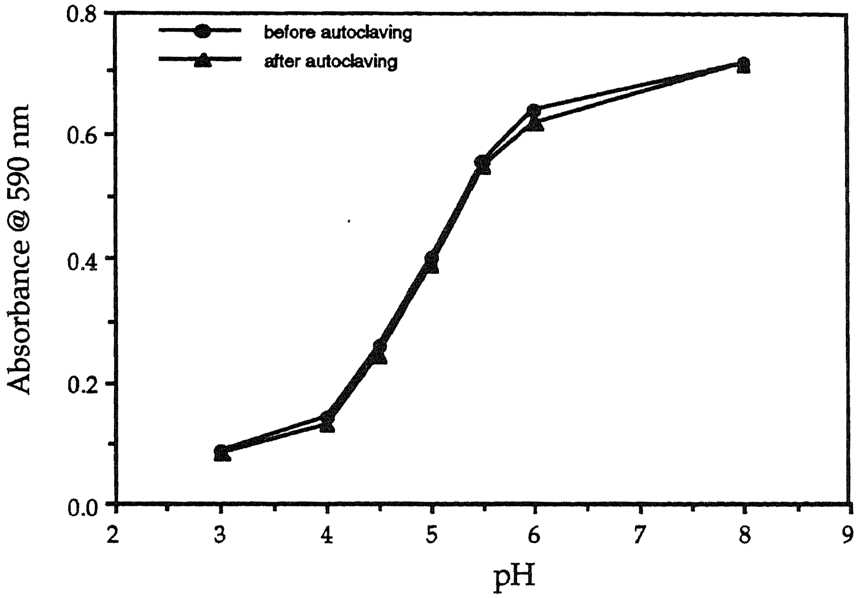


Fig. 3.21 Effect of autoclaving on pH calibration curve of sol-gel thin film doped with bromophenol blue.

3.3.3.5 *Effect of temperature on thin film sensor*

Sol-gel films aged for four weeks were used to study the effect of temperature on pH sensing. Temperature was varied from 20^0 C to 60 ^{0} C. The buffer was citrate phosphate of pHs 3, 5, and 8. The buffer was found unaffected by temperature. The tubes coated with thin films containing pH buffer were heated in a water bath at each temperature for one hour. The temperature effect was determined by extent of leaching and increase in absorption of indicator.

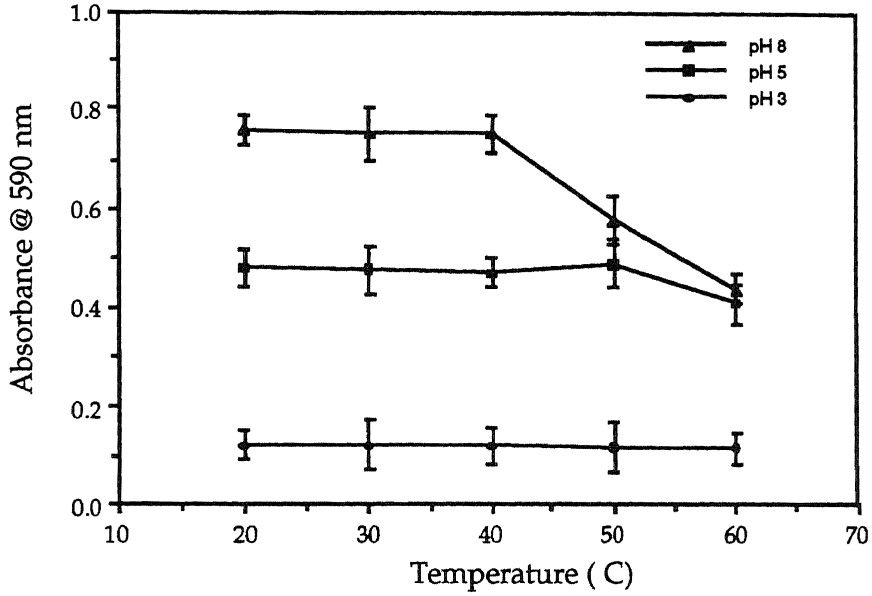


Fig. 3.22 Effect of temperature on a sol-gel film doped with bromophenol blue.

Leaching was higher at temperatures above 40 ^{0} C. The colour of indicator found to be intensified slightly at 50 ^{0} C in pH 5 therefore an increased absorbance of indicator was recorded. The temperature effect was found to be at a minimum ($\leq$ 2% absorbance unit change) between 20^0 C to 40^0 C. This is the desired temperature range for most of the biological and environmental applications.

3.3.3.6 *Effect of light*

The effect of light on the sensor at various pHs was studied by keeping the doped films under a tungsten light for five weeks. Films were first stabilised in buffer pHs 3, 5 and 8. After that they were sealed and exposed to tungston light (100 W) at room temperature (22° C). The tubes were left under moist condition for avoidance of any possible dessication of films due to light and heat. The light effect was minimum between 0 to 3 weeks at pHs 5 and 8 but some decrease in absorbance was found after this period. However the effect of light was almost negligible at pH 3.

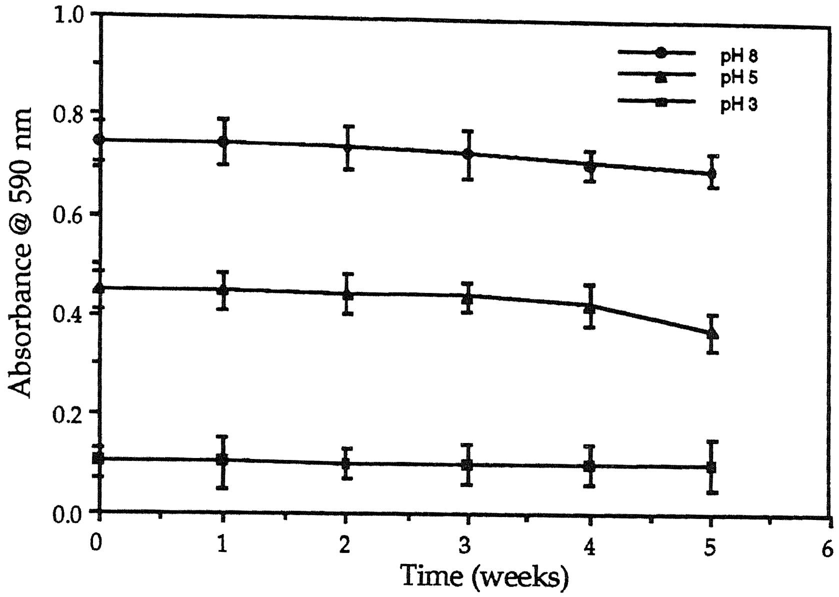


Fig. 3.23 Effect of light on entrapped bromophenol blue in a sol-gel thin film.

3.3.3.7 *Effect of salts*

It is important to find the effect of ionic strength on pH sensing properties of the sol-gel film. In this study a series of buffers containing various amount of NaCl was used to vary the ionic strength. The concentrations of NaCl used were 0.001 M, 0.01 M and 0.1 M. The calibration curve was not affected by NaCl in concentrations 0.001 M and 0.01 M (fig. 3.24). However, it was changed by a 0.1 M concentration of NaCl. The results shown in figure 3.25 suggested that 0.1 M KCl had a more severe impact on the calibration curve than 0.1 M NaCl. To determine the effect of K^+ on the curve, the sensor was tested using another salt of potassium as the nitrate. The results indicated that 0.1 M KNO_3 has a similar effect on the calibration curve as does 0.1 M KCl.

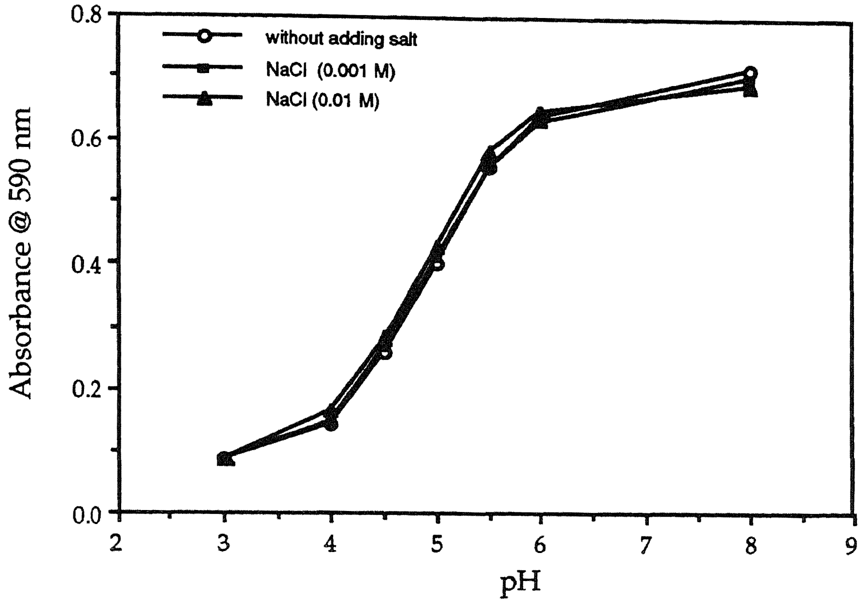


Fig. 3.24 Salt effect of sodium chloride on the calibration curve of the indicator doped sol-gel film.

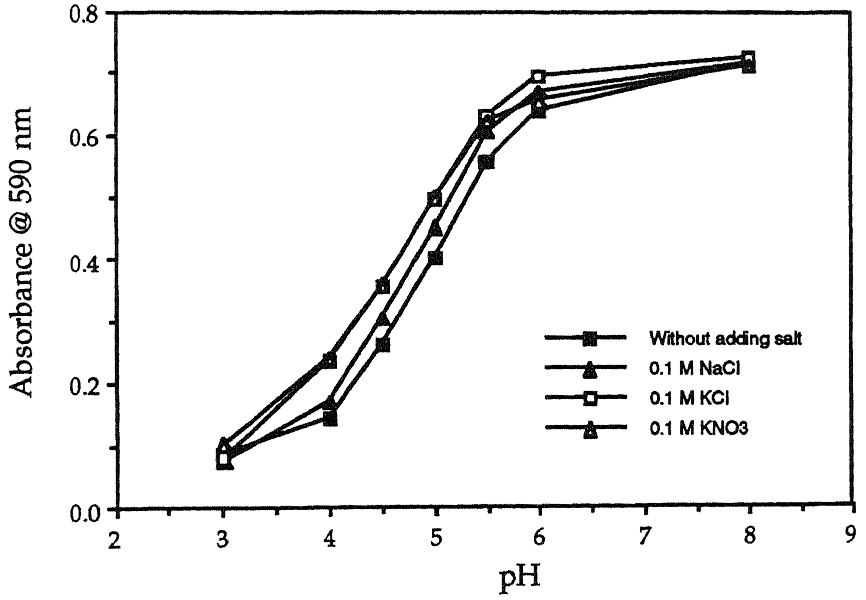


Fig. 3.25 Salt effect of sodium chloride, potassium chloride and potassium nitrate on the calibration curve of doped thin film.

3.3.3.8 *Long term stability of the sensor*

In the development of on-line sensors, one of the vital issues is the chemical stability of immobilised indicators. Though sol-gel processing provides a generic method of trapping both organic and inorganic indicators without harming their molecular structures, it is also important for the sensor to have a prolonged life time after repeated usage. Therefore a study of long term stability of thin film sensors was conducted.

In order to test the stability of doped thin film sensors, the films were placed in buffer pHs 3, 5 and 8 and distilled water for four weeks. All films were aged for four weeks under ambient conditions and immersed in water for 3 days prior to the experiment. Films at pH 8 dissolved after two weeks. Some inital leaching (11%) was observed in pH 5 but films were found stable after 3 weeks. However the sensor was found to be most stable in pH 3 and to less extent in distilled water. Almost negligible leaching was noticed in these solvents.

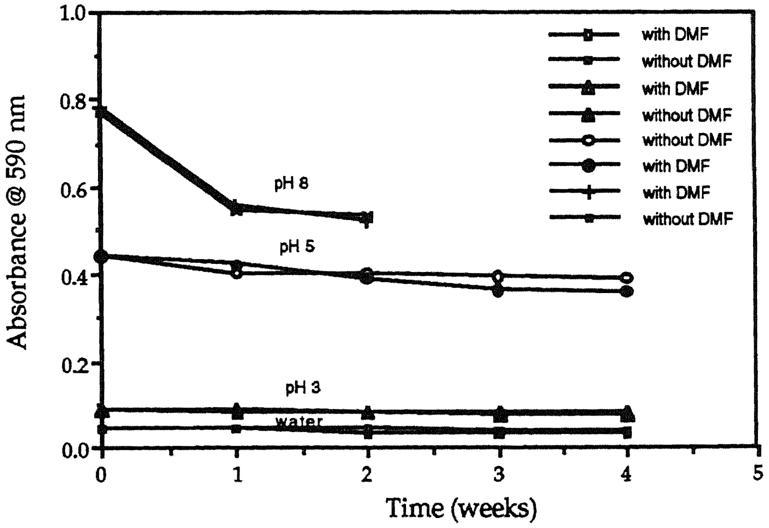


Fig. 3.26 Stability measurements of sol-gel films doped with bromophenol blue.

The comparison of long term stabilities of such films with those of DMF added films revealed no significant differences. However it was observed that DMF added heated films have a longer storage life (8-10 months) as compared to other films (having storage life 3-4 months).

3.3.4 Discussion and conclusion

Sol-gel thin films doped with pH indicator coated inside tubes were studied for spectral analysis. Each tube was carefully selected for approximate uniform absorbance on all sides before coating. The coated tube for spectrophotometric studies was marked for its position in the spectrophotometer and then all readings for the same set of experiments were taken by keeping the tube standing in the same position. The background was corrected by scanning the uncoated tube containing distilled water. Soda glasses for coating sol-gel thin film were also selected by Yang and Saavedra, (1995).

The comparison of absorption spectra of individual indicators and mixture of indicators in sol-gel thin films (fig. 3.6-3.9) and in solutions (fig. 3.10-3.12) suggested resemblences in maxima and isosbestic points at the same wavelengths. Thin film doped indicators however showed differences in term of pK values and pH ranges. The pH range of bromophenol blue in solution is 2.3 to 5.1 but in sol-gel thin film it shows response to pH changes from 3 to 8 (fig. 3.6). The linear pH range of bromophenol blue in sol-gel film is 4 to 6 with a midpoint about 4.9. The pK of bromophenol blue in solution is 4.1. This change in pK shows that the chemical environment of the dye may be altered by entrapment in sol-gel. The negative surface charge of the glass probably affects the protonation and deprotonation of indicator at some sites. (Lee and Saavedra, 1994). The broad range over which the thin film pH sensor responds may indicate that immobilization of bromophenol blue in sol-gel layer changes slightly its spectroscopic properties due to modification of degree of freedom in pores resulting from the interactions of functional sites with the pore walls.

The response time in one month old samples was slightly higher than one week old samples in pH 8 buffer. This suggests that with the ageing the sol-gel thin film shrinks and its pore size reduces. Therefore the analytes take more time to diffuse through the thin film (Narang, et al,. 1994). However notably aging did not affect the response time in pH 3 buffer. This can be explained that the relatively narrowed pores in aged film still allow the H^+ to diffuse in and out and hence rate of protonation is uneffected but they hinder the movement of bigger analytes such as H_2O, OH^- (proton acceptors) which are required to deprotonate indicator at basic pH.

Leaching studies of one month old thin films at 25°C and 40°C in various pH buffers showed promising results. After inital leaching all samples gave a stable response. However the continuous immersion of thin film for a week in pH 8 buffer caused partial dissolution of the film. No difference in results of leaching experiments and chemical stabilities at these two temperatures was recorded.

Autoclaving did not appear to affect the pH sensing properties of thin films incorporated with DMF. But thin films prepared without DMF when autoclaved, showed no response to pH change. The heating of a thin film at $120 \pm 3\ ^0C$ for 15 minutes under pressure causes the pore structure of the sol-gel to shrink and makes the pores even smaller for H^+ to diffuse in. However DMF in the thin film helps to avoid shrinkage and hence keeps the pore size intact. It is also responsible for bigger and uniformly distributed pores in the sol-gel matrix.

The effect of temperature on the colour of an indicator in a given solution is determined (a) by the effect of temperature on the pH of the solution and (b) by its effect on the response of the indicator. (Shahriari and Ding, 1994). The buffer used in the experiment affects the pH very little upon heating and cooling. Therefore the change in absorbance of the doped thin film may not be due to this. To understand the effect of temperature on response of indicator, the dissociation constant (K_{in}) must be taken into account.

The temperature coefficient of an indicator dissociation reaction can be expressed in the following thermodynamic relationship;

$$d \ln K/dT = \Delta H/RT^2$$

Standard enthalpy change ΔH varies with temperature according to the equation given below;

$$d\,\Delta H/d\,T = \Delta\,C_p$$

where $\Delta\,C_p$ is the difference in molar heat capacities of products and reactants. According to Shahriari and Ding (1994), ΔC_p is independent of temperature for many pH indicators dissociation reactions. Therefore it can be written as, $\Delta H = \Delta H^0 + \Delta C_p\,T$ where ΔH^0 is hypothetical enthalpy change at absolute zero. For many indicators ΔH^0 is positive and ΔC_p is negative quantity. At certain temperature, T_m derived from $T_m = -\Delta H^0/\Delta C_p$, ΔH and $d \ln K/d\,T$ are zero and ionisation constant goes to maximum. The maximum ionisation occurs when increased proton transfer tendency decreases the tendency of charge seperation. In indicators which are cationic acids, there is no net separation of charge hence ionisation constant changes regularly with rise in temperature.

However indicators which are molecular or anionic acids show smaller changes of K_{in} with temperature than for cationic acids. Bromophenol blue is an anionic acid. The absorbance changes were minimum with rise in temperature between 20^0 C to 40 ^{0}C. The indicator starts leaching after 40^0 C in pH 8 because of the dissolution of the film. However surprisingly higher temperatures did not affect the film and hence there is negligible leaching in pH 3. Acidic pH found friendly to the sol-gel matrix. It appeared to decrease slightly the pore sizes by protonating the silanol groups on the surface of pore walls and hence reduced leaching. The slight increase in absorbance at 50^0 C in pH 5 can be attributed to the shift of indicator equilibrium towards base formation.

Light affected the doped indicator in two ways either by photobleaching or by photoleaching. Photobleaching is the process by which the light reduces the colour intensity of indicator at a particular pH and hence causes a decrease in absorbance. Photoleaching is the phenomenon in which light enhances the leaching of entrapped indicator from the matrix of sol-gel glasses. Photoleaching is not the serious problem in thin films as compared to bulk gels (Wolfbeis, et al., 1992). Some leaching was however observed after 3 weeks of exposure to light in pH 8 film. Photobleaching appeared to be the main cause of decrease of absorbance at pH 5 after 3 weeks. Little decrease in absorbance was noted at pH 3 through the whole exposure period.

The doped pH indicator showed photostability over a period of 3 weeks. Nemoto and Hirokawa, (1996) also reported minimum effect of light on doped pH indicators (methylene blue, eosin, uranine) in sol-gel films. They demonstrated a negligible photobleaching effect in short term exposure. However the doped indicators are relatively more photostable in sol-gel matrix than in water (Avnir et al., 1984).

Neutral electrolytes such as sodium chloride can contribute to the colour change of pH indicators in two ways; (i) change in the colour intensity (absorbance) of indicators and (ii) effect of the electrolytes on the indicator equilibrium. Of these two effects, the first one is more likely to be the serious at relatively high ionic strength (Shahriari and Ding, 1994). Sodium chloride in concentrations 0.01 M and 0.001 M does not affect the calibration curve (fig. 3.24). The change in calibration curve of doped bromophenol blue caused by KCl is more than that of

NaCl with same concentrations. Shahriari and Ding, (1994) also reported that KCl shifts the calibration curve of sol-gel immobilised indicators, bromocresol purple and bromocresol green to higher pH end as compared to that of NaCl. The similar change brought about by 0.1 M KNO_3 to the curve (fig. 3.25) led to the conclusion that the effect of K^+ on the indicator is not dependent on chloride.

The stability measurements of doped films in different solvents suggested that films can be used in pH sensing below pH 8 for 3-4 weeks without any significant leaching. In pH 8 buffer after one week of leaching the films were found to be stable for the next 7 days. The doped films however can be used for many repeated applications providing they are stored in an acidic pH solution (pH 2-3) when not in use.

The comparative studies of thin films and monoliths doped with indicators revealed the following additional advantages in favour of thin films.

1 All pH indicators, bromophenol blue, bromocresol purple and bromothymol blue, were found to be active in changing colour both individually as well as in a mixture when immobilised in the thin film.

2 Chemical stability of the thin films in different pH buffers is more than that of monoliths. Neither leaching of dopants nor cracking on repeated immersion in aqueous solution was observed in the case of the thin film.

3 Thin films do not require heat drying. They can be brought into operation after 48 hours of aging.

4 Thin films doped with indicators showed absorption spectra similar to that of solution.

5 Thin films can be coated on the inside of the flow cell for continuous monitoring of pH.

CHAPTER 4. FTIR SPECTROSCOPY OF THIN FILMS

4.1 INTRODUCTION

There have been extensive studies for the past ten years concerning the relationship between IR absorption spectra of silica based monoliths and their structure at different temperatures (Brinker, 1990).

Nevertheless, due to the differences between monolithic and thin film gels, studies of the monolith are generally inappropriate for interpreting structural information on films obtained by dipping or spinning: the structures of the films are often considerably more compact than those of the corresponding bulk xerogels prepared from identical precursors. (Brinker, 1990; Almeida and Pantano, 1990).

Almeida, et al (1990) studied the structure of thin films by drying them either at room temperature or densified at 400-450^0 C. The densified films showed the presence of residual OH groups mostly hydrogen bonded, whereas very little molecular water or organic species was left. Parril, (1993) investigated structural modification in thin film formed by spin coating, acid-catalysed TEOS-based sol-gel, on silicone substrates after heat treatment at 300-1000^0 C. He stated that porosity decreases with increase in temperature. Structural changes of TEOS derived thin films on silicon wafers as function of ageing were discussed by Parril, (1992). He reported the increase in peak heights at 580 and 1130 cm^{-1} with solution age time.

Farded et al, (1995) studied the effect of H_2 O on the structure of thin films deposited on silicon wafers by IR spectroscopy. They proved reduction in porosity and pore sizes with increase in molar ratios R, of water: TEOS. The peak height at 1070 cm^{-1} was also reported to increase with increasing R. Almeida, et al, (1994a & 1994b) studied the structure of silica and silica-titania sol-gel films in term of porosity and refractive index after heat densification. They reported maximum porosity and minimum refractive indices in these films.

The effect of the drying control chemical additives (DCCA) such as formamide, DMF on the sol-gel processing has been established through different studies (Brinker, 1990 ; Klein, 1994). The FTIR spectroscopy of the sol-gel containing formamide was carried out recently by Viart and Rehspringer (1996). They reported rapid condensation and fast cross linking in these sol-gels.

The purpose of this work is to understand the structural behaviour of TMOS derived silica gel film, deposited on commercially available IR cards, as a function of ageing. It is further intended to study the effect of DMF on the chemical structure of the film in relation to its stability and porosity.

4.2 MATERIALS AND METHODS

IR cards (type 61) made by 3M, USA were used for sample analysis. TMOS, DMF, and other chemicals were bought from Sigma and were of analytical grade. The Perkin-Elmer FTIR spectrophotometer (model 1710) was used for obtaining transmission spectra.

The preparation of sol-gel solution was carried out by the method described earlier. Films containing DMF were prepared from solution having DMF mixed with TMOS in a volume ratio of 0.10 : 2.00. The sol-gel film on IR card was deposited by placing two drops of solution in the centre whilst the card was standing in a slanting position in order to drain off the excess solution quickly and to minimise the thickness. The samples used for investigating ageing effect were prepared in triplets (appendix A table 1,2) and an average of 20 scans were used for each measurement made by the FTIR spectrophotometer. Films were stored in vacuum for ageing experiments.

4.3 RESULTS

4.3.1 The ageing effect on chemical structure of thin films

The FTIR study was conducted to explain the effect of ageing on structure of sol-gel film aged for different periods. Films were aged from 1 minute to 4 weeks after fabrication. The results shown in fig. 4.3 indicated that there are significant changes in the peak at 1463 cm^{-1}. This band reduced intensity in the first hour than started moving upward. Its height increases continuously after two weeks but stabilises between 3 to 4 weeks of ageing. Figure 4.1 showed a rapid change in the band in the first day of ageing but later it becomes relatively slow in the upward increase until almost stable after 3 weeks of ageing.

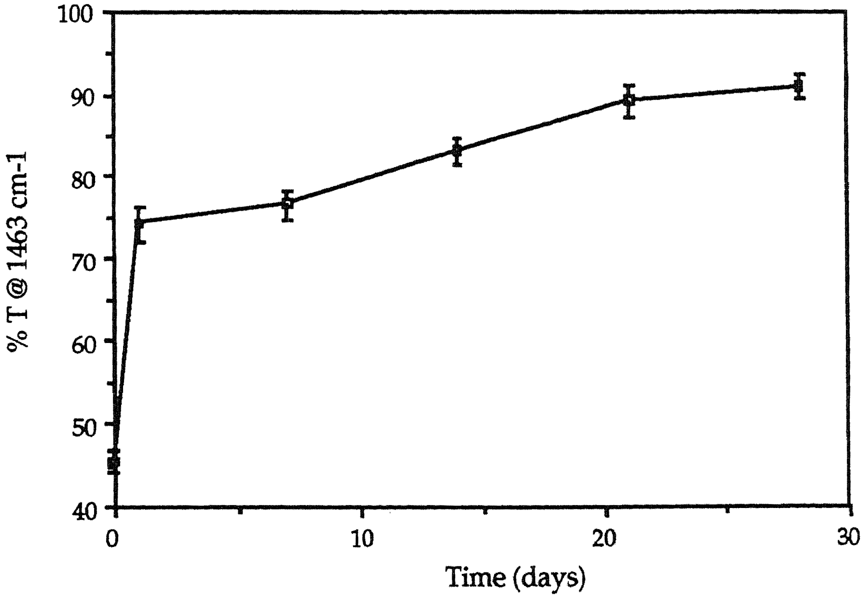


Fig. 4.1 Effect of ageing on C-H bending at 1463 cm^{-1} in sol-gel film.

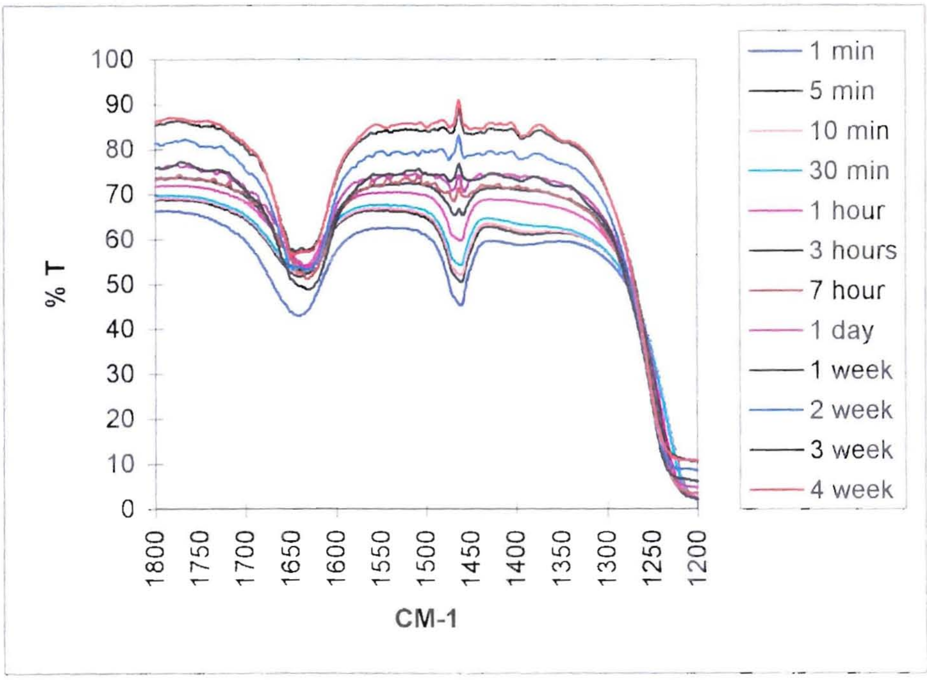


Fig. 4.3 FTIR spectra of sol-gel film aged for different periods after fabrication.

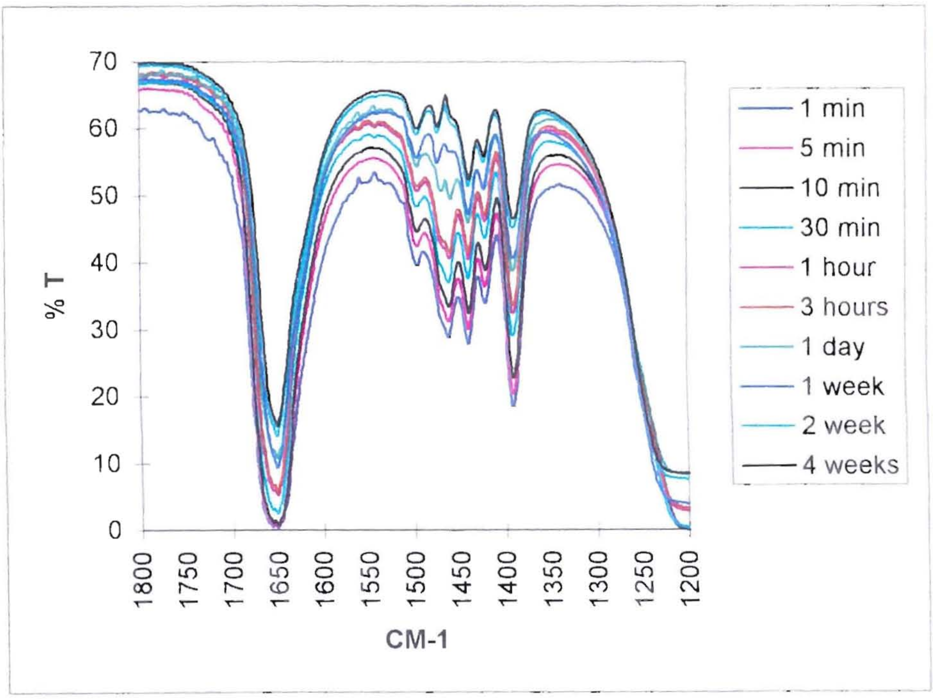


Fig. 4.4 FTIR spectra of sol-gel film added with DMF aged for different periods after fabrication.

4.3.2 The ageing effect on structure of thin films containing DMF

DMF is the drying control chemical additive (DCCA) which increases the condensation of alkoxide and produces gels with larger pores. In order to understand how ageing affects the films containing DMF, films aged for different periods were subjected to FTIR study. The band at 1463 cm^{-1} shown in figure 4.4 was used as an ageing indicator. This band showed reduction in the first hour, then increases gradually upward for a couple of weeks. Its height increases until, at the end of the second week of ageing, becomes stable after that (fig. 4.2). The bands at 1390cm^{-1}, 1408 cm^{-1}, 1440 cm^{-1} and 1500 cm^{-1} are related to DMF which by the passage of time reduced due to evaporation from the film as substrate facilitates the evaporation of solvent.

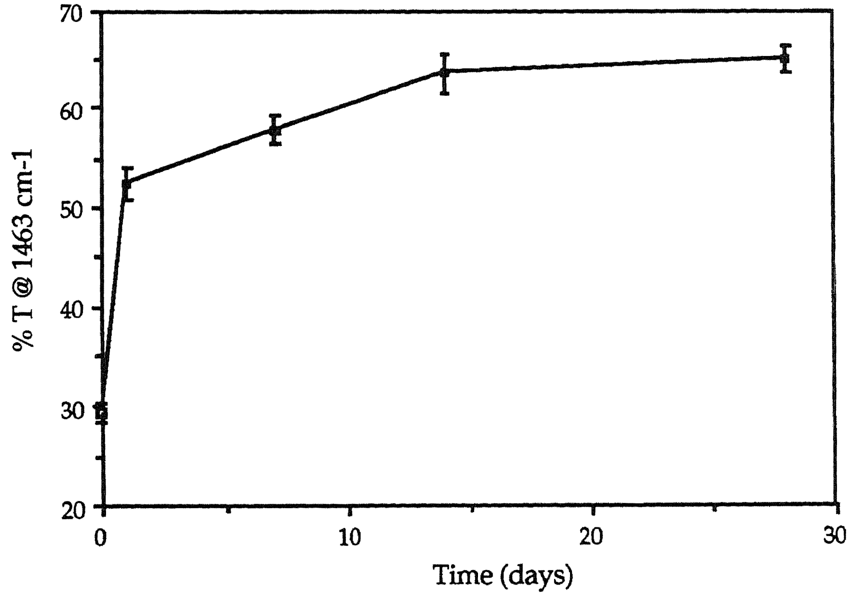


Fig. 4.2 Effect of ageing on C-H bending at 1463^{-1} in sol-gel film added with DMF.

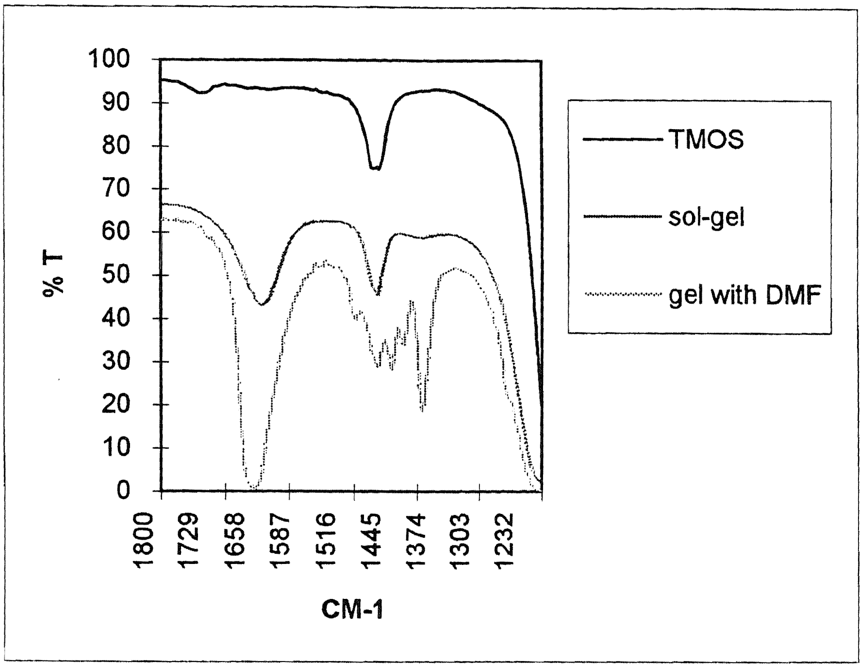


Fig. 4.5 Comparative FTIR spectra of films (with and without DMF) and starting material TMOS showing bands at 1463 cm-1.

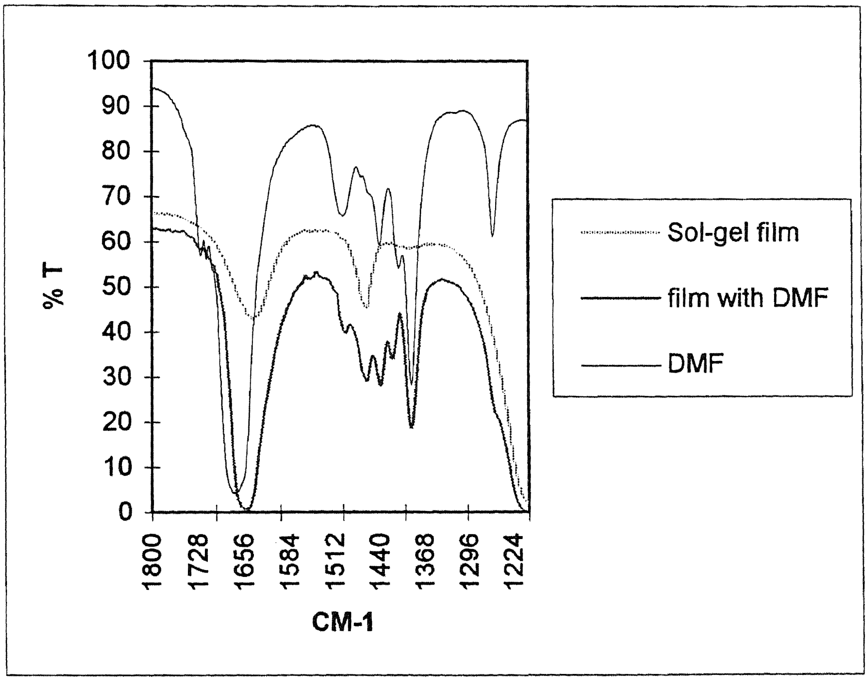


Fig. 4.6 FTIR spectra of sol-gel films with and without DMF and neat DMF for comparison of band at 1463 cm-1.

4.3.3 Comparative study of FTIR spectra of films with and without DMF, TMOS and neat DMF

The band at 1463 cm^{-1} was found to be useful in understanding the ageing process in both type of films i.e. with and without DMF. In order to co-relate it to the starting material, TMOS, it was important to run the spectra of pure TMOS. The transmission spectrum of TMOS when compared with those of the two film types shown in fig. 4.5 confirmed the presence of a band at 1463 cm^{-1}.

This particular band in thin films containing DMF was examined comparatively with the neat DMF for marking out the other closely associated DMF bands (fig. 4.6). The C-N stretching band appearing at 1390 cm^{-1} and symmetric (δ_s CH_3) and asymmetric (δ_{as} CH_3) bending vibrations due to methyl groups attached to amide {(-CON-$(CH_3)_n$} occuring at 1408 cm^{-1} and 1440 cm^{-1}, 1500 cm^{-1} respectively (Silverstein, et al., 1974) indicate DMF in the film. The C-H bending at 1463 cm^{-1} is mainly due to the alkyl groups in TMOS.

4.3.4 The effect of autoclaving on the structural evolution of films

To determine the effect of steam autoclaving on the structure of the film with added DMF, the films deposited on IR cards were subjected to an FTIR study before and after autoclaving at 121^0C for 15 minutes. The substrate did not appear to be affected by steam autoclaving. The results shown in fig. 4.7 indicated that autoclaving reduced the DMF bands. The band at 1463 cm-1 is narrowed and sharp as compared to that before autoclaving. Moreover a side band appeared at 1468 cm^{-1} after autoclaving. This band which can also be seen in fig. 4.4 corresponds to more than 1 week aged film. This suggests that autoclaving enhances condensation.

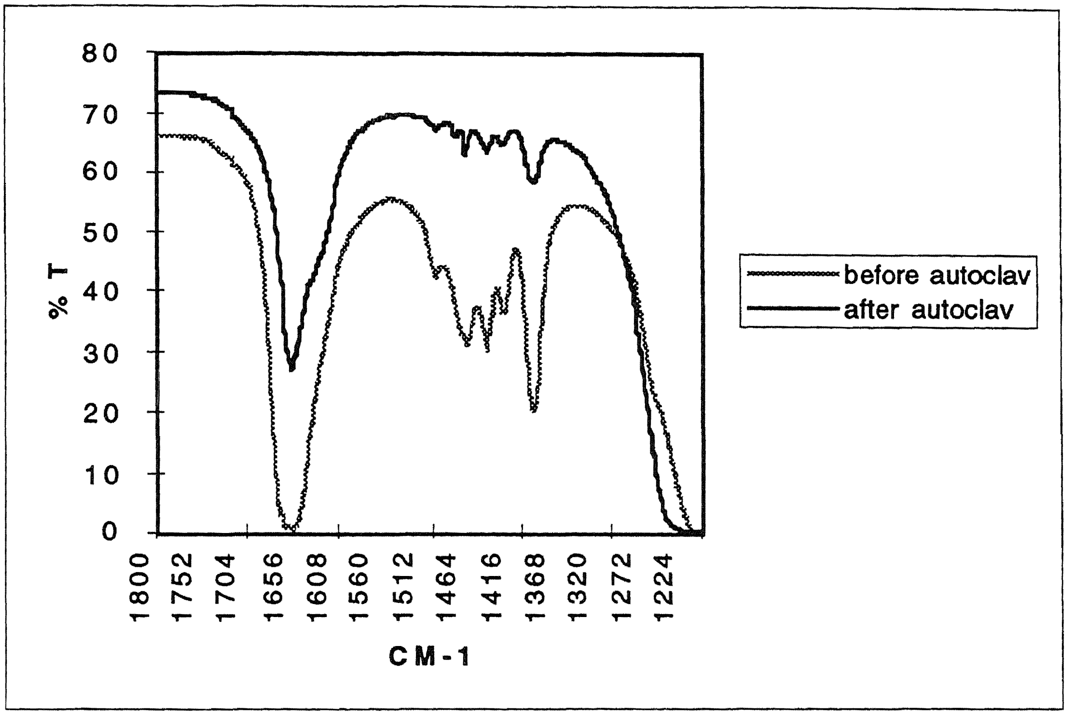


Fig. 4.7 FTIR spectra of sol-gel film before and after steam outoclaving at 121 ^{0}C for 15 minutes.

4.4 DISCUSSION AND CONCLUSION

FTIR is a technique that has been applied to a variety of silicon oxide films, and is used to identify changes in molecular structure of the films as a function of ageing, solution composition and temperature treatment. (Parrill, 1991).

It was also found to be a good method of following the hydrolysis and condensation reactions in sol-gel processes (Viart, et al, 1996).

IR cards are easy to use and they do not absorb between 1200 cm^{-1} to 2000 cm^{-1} the range of interest. The fabrication of the film on the card is a straight forward process and does not require any sophisticated handling.

FTIR transmission band at 1463 cm^{-1} is the C-H bending of CH_3 groups present in the starting material (Parril, 1992; Kamalasanan et al., 1996). This band appeared in neat TMOS found in films after the hydrolysis and the condensation stages. The changes in the band reflect the structural evolution of gel with ageing. When hydrolysis is incomplete there is a high content of residual organic groups (Parril, 1992) which are continuously used up as reaction approaches condensation and then polymerisation. Some of alkoxyls (OR) are retained in the matrix and can only be removed by curing at temperature 400-500 ^{0} C (Farded, et al., 1995). The increase in the intensity of C-H bending at 1463 cm^{-1} with ageing at room temperature (22^0 C) suggests that some unreacted (OCH_3) groups accumulated in matrix (Rabinovich, 1994) as the film evolves to the final condensed form in four weeks. Most of the structural changes occured in the sol-gel film within four weeks after fabrication. Similar structural behaviour was reported in pyrene-doped thin film by Dunbar et al., (1996).

DMF like formamide slows down the hydrolysis but enhances the condensation and polymerisation (cross linking) and hence contribute larger pores to the sol-gel matrix (Viart, et al., 1996; Wallington, 1995). The increase in the intensity of the band at 1463 cm^{-1} started in the first hour in case of non-DMF sample, but in DMF films it started after 2 hours of fabrication. This suggested that hydrolysis is slowed in the DMF added film. However this band stabilised in DMF films in 2 weeks as compared to non-DMF films where it stabilised after 3 weeks. This suggests that DMF speeds up the condensation reaction and cross linking and hence reduces shrinkage by decreasing surface area (Wallington, 1995). The presence of DMF bands in the sol-gel film at a similar position as they appear in neat DMF indicate that this chemical is not making any chemical bonds with the silica of the sol-gel film. The DMF is an aprotic solvent which is not involved in the sol-gel processing reactions due to lack of sufficient electrophilic protons and hence is unable to be deprotonated to form strong nucleophiles (Brinker, 1990). However its presence in the sol-gel matrix helps to minimise the shrinkage by activating condensation which results in stronger pore walls and hence larger pores, resistant to collapse.

Autoclaved films showed reduction in DMF bands at 1390 cm^{-1}, 1408 cm^{-1} and 1440 cm^{-1}. This suggests that by heating some of the DMF is removed but it is not necessarily completely eliminated. The reduced peaks show that DMF is still present in the matrix of film even after steam autoclaving at 121 ^{0} C for 15 minutes. The C-H bending at 1463 cm^{-1} is reduced by autoclaving. Heating decreases the intensity of C-H stretching bands in thin films (Kamalasanan, et al., 1996) and also increases consumption of OCH_3 and OH groups in sol-gel matrix by enhancing condensation (Farded, et al., 1995). The partial removal of DMF by heating from sol-gel matrix contributes even larger pores to the film. Similar results were reported using bulk gels (Brinker, 1990).

The FTIR study of films revealed that ageing is longer in non-DMF films as compared to DMF added films because of the relatively low rate of condensation. It also suggested that changes at 1463 cm^{-1} correspond to shrinkage caused by condensation reactions going on in the films. Four week old films are structurally stable for pH sensing experiments. DMF promotes bigger pores in the film by controlling the condensation and cross-linking. Autoclaving enhances the pore sizes by partially eliminating the DMF from the pores and reducing alkoxy groups by increasing hydrolysis and condensation.

CHAPTER 5. INCORPORATION OF REAGENTS FOR METAL IONS

5.1 INTRODUCTION

The determination of metal ions in solution is of growing interest to industries and in the environment. In contrast to well-established laboratory methods, such as atomic absorption spectrometry and voltammetry, chemical sensors enable the on-line monitoring of pollutants. Analysis by using sensor based systems would be easier, faster and inexpensive.

For determination of metal ions different indicators immobilised in various matrices were discussed by Lerchi et al., (1992). The doping of metal reagents in sol-gel glasses offers several advantages over other organic matrices such as photochemical stability, variability of preparation conditions, hydrophilicity and porosity of the resulting layers (Plaschke, et al., 1995).

Most of the work has been done by using doped sol-gel monolith glasses as metal ion detectors. The problem with such sensors is the long response time which is not desirable in real time analysis. Both fluorimetric and colorimetric indicators have been reported to be immobilised in sol-gel glasses.

Kuyavskaya, et al (1992) described the spectrophotometric method of detection of cobalt and divalent iron in solution by trapping 1-nitroso-2-naphthol and o-phenanthroline in monoliths. They observed cracking of monoliths and leaching of doped dyes in most of samples. Lev, (1992) described a list of metal reagents doped in monoliths for determining various metal ions in solution. He succeeded only in 50% of immobilised indicators to get reasonable chromophoric response.

Kuselman and Lev, (1993) explained the method of iron(II) detection by using a sol-gel based tube detector. The sensor was consisted of capillary glass tubes filled with porous sol-gel silica powder doped with o-phenanthroline. Its response time was reported over 15 minutes.

Wallington, et al (1997) described the determination of Al^{3+} and Cu^{2+} in aqueous solution by monolithic sol-gel doped with fluorescent reagent (morin) and colorimetric reagent (eriochrome cyanine red) respectively. They prepared bulk gels by hydrolysis of TMOS with a water : TMOS molar ratio of 8 and showed the reponse time of eriochrome doped gels over 2 days.

Plaschke, et al (1995) determined mercury fluorimetrically by immobilising porphyrin in sol-gel film. The detection limit of Hg (II) was reported to be 1.4 μg/l. Ahmad, et al (1995) entrapped fluorimetric indicators (morin, porpurin) in sol-gel films to demonstrate detection of aluminium ions. They failed to reproduce the response of the sensor.

Most of the research has been done in trapping fluorimetric indicators in thin films. In this work different colorimetric indicators were immobilised in a thin film but many of them were found to be less sensitive. The indicators doped in the sol-gel films are listed in table 5.1. Among these reagents o-phenanthroline and dithio-oxamide were found to give some spectral change but leaching and cracking were maximum in these films. The most promising results were obtained by doping Eriochrome cyanine RC for Cu^{2+}. Therefore further research was carried out by using these doped films.

Table 5.1. Reagents doped in sol-gel film for detection of metal ions.

No	Reagents	metal ions
1	o-phananthroline	Fe^{2+}
2	dimethyl glyoxime	Ni^{2+}
3	dithio-oxamide	Cu^{2+}
4	alizarin red s	Al^{3+}
5	cuproin	Cu^{2+}
6	titan yellow	Mg^{2+}
7	pyrocatechol violet	Al^{3+}
8	pyrogallol red	Al^{3+}
9	malachite green	Cr^{6+}
10	eriochrome cyanine RC	Al^{3+} , Cu^{2+}

5.2 PREPARATION OF FILMS

The films were prepared by the method described earlier with some modification. TMOS, distilled water, ethanol and HCl (0.1M) in the volume ratio of 2: 0.46: 0.50: 0.1 were mixed and aged for one day hydrolysis. The coating solution was prepared just before coating by adding to it 1 ml, 20 mM eriochrome cyanine red RC. The test tubes were coated by the same method described earlier. Films were brought into operation after one hour of ageing.

5.3 RESULTS

5.3.1 Measurement of copper

Copper ions formed a reversible complex with doped eriochrome cyanine RC. Films were placed in 0.01, 0.02, 0.03, 0.04, and 0.05 mM copper solutions buffered to pH 6.5. The maximum absorbance was recorded at 565 nm. The detection limit of copper ions in solution by using eriochrome doped sol-gel was found to be 0.6 ppm (fig. 5.1).

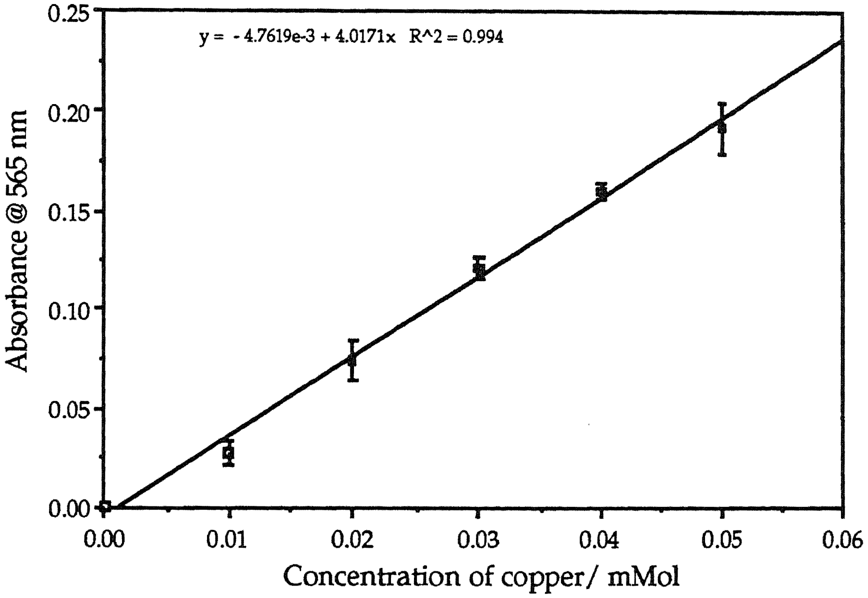


Fig. 5.1 Calibration curve of eriochrome doped sol-gel thin film.

Copper was displaced from a thin film by placing films in 2 M HCl for 5 minutes. The films were re-equilibrated in pH 6.5 buffer (10 mM sodium acetate) without the copper ions for one hour and no copper-eriochrome complex colour (purple) could be found. This suggested that all copper ions are displaced from the films. Films were reused for copper detection by placing them in different concentrations of copper and were found to give good reproducibility (appendix A table 3).

5.3.2 Interference of other metal ions

Interference study was carried out at pH 6.5 with concentrations 0.25 and 0.50 mM of each Fe^{+2}, Mg^{+2}, Al^{+3}, Ni^{+2}, Cr^{+2}, Zn^{+2} ions. Only Fe^{+2} showed some absorbance at the wavelength 565 nm with concentration 0.50 mM (fig. 5.2). There was no interference in term of reduction or increase in peak height of Cu^{+2} with concentration 0.05 mM in mixture of metal ions each in 0.25 mM concentration (fig. 5.3).

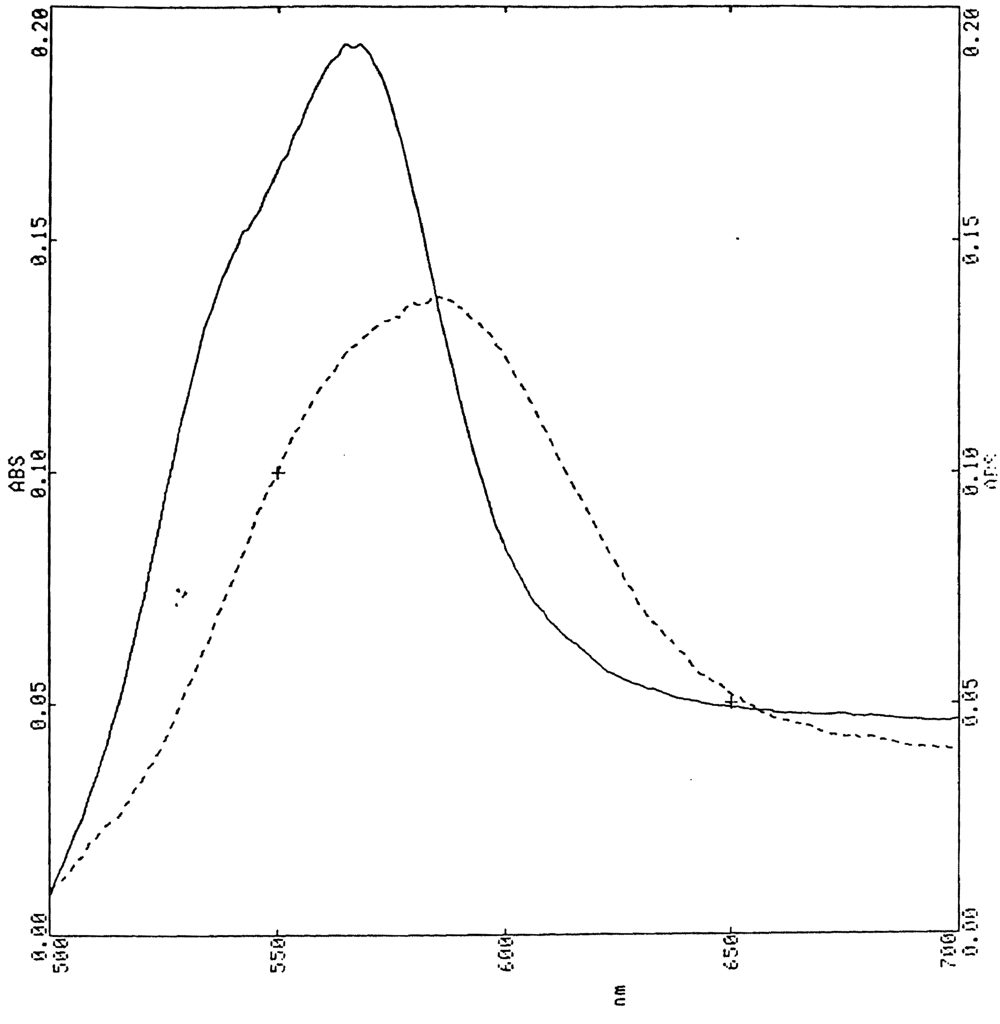


Fig. 5.2 Absorption spectra of an eriochrome doped film complexed with (a) Cu^{++} (____) and (b) Fe^{++} (------) in a mixture each in 0.05 mM and 0.50 mM respectively.

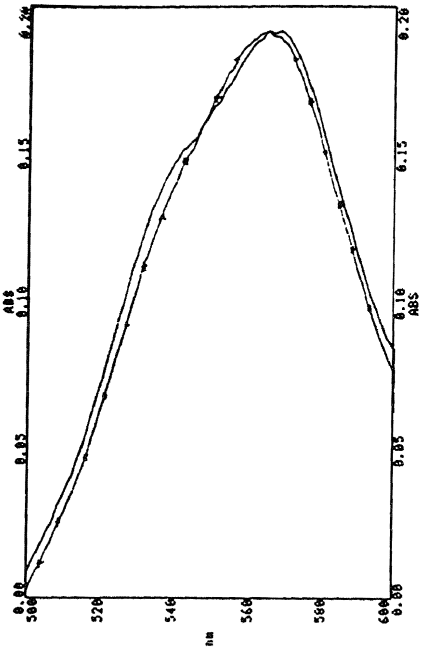


Fig. 5.3 Absorption spectra of eriochrome doped film in copper solution (a) with (xxxx) and (b) without (____) possible interfering metal ions (Fe^{+2}, Mg^{+2}, Al^{+3}, Ni^{+2}, Cr^{+2}, Zn^{+2}).

5.3.3 Effect of light on eriochrome-copper complex

It is important to examine the effect of light on the reagent copper complex. Thin films coated inside test tubes doped with eriochrome cyanine RC were first stabilised in copper solution (0.25 mM) subjected

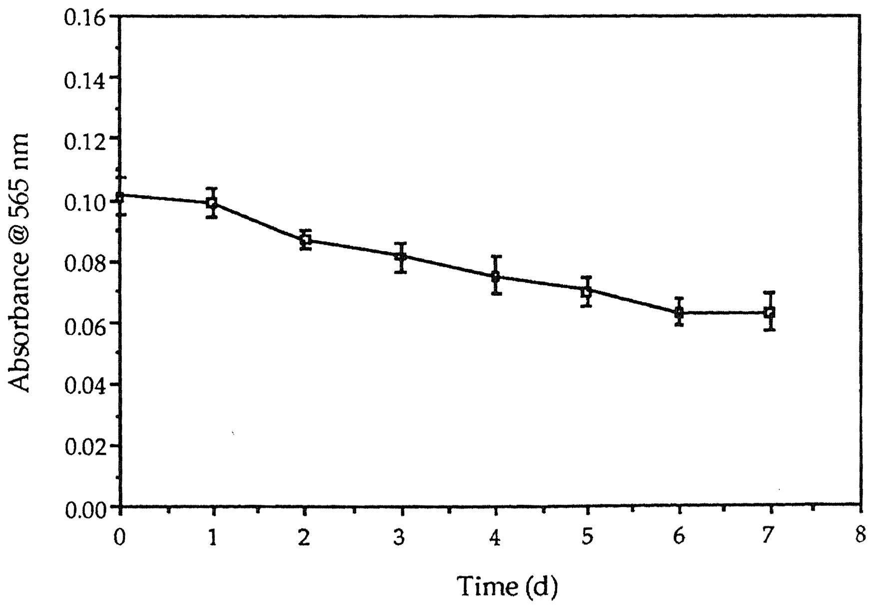


Fig. 5.4. Effect of light on eriochrome-copper complex in a sol-gel film.

to tungston light for one week. Longer exposure to light was not desirable because of low stability of these films. The result showed that light affected the complex. The intense purple colour faded until stable after six days. Photobleaching could be the main cause. In order to avoid any leaching of indicator, sample tubes were exposed to light emptied, covered with foil under moist conditions.

5.3.4 Effect of temperature on Eriochrome copper complex

Temperature is an important parameter to be considered in monitoring downstream processes. The thin film sensors are likely to be used in on-line systems. Therefore it was necessary to subject them to changes in temperature. To examine whether the complex formation is stable or not, the tubes containing copper solution (0.04 mM) were placed in a water bath at various temperatures ranging from 20 ^{0} C to 50 ^{0} C for 1 hour each.

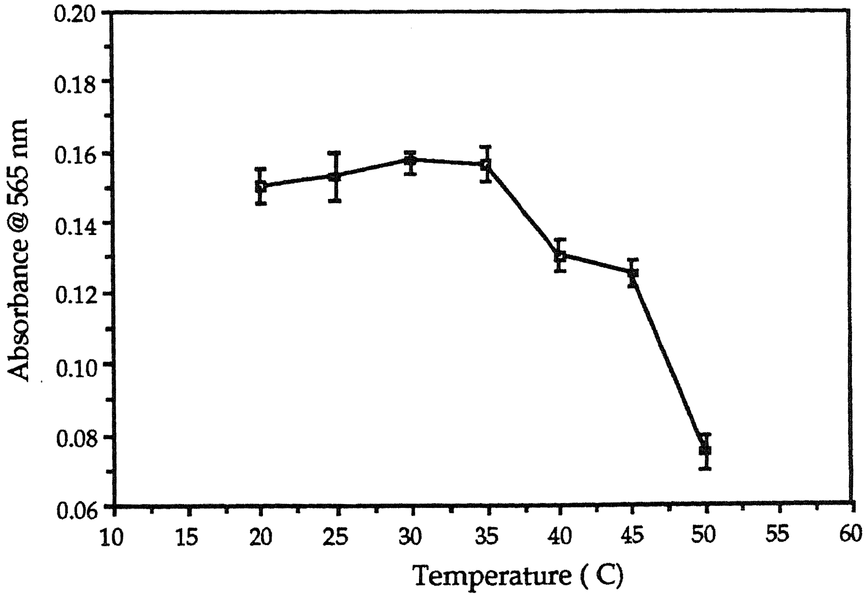


Fig. 5.5. Effect of temperature on eriochrome-copper complex in sol-gel thin film.

The results shown in fig. 5.5 indicated that rise in temperature between 20^0 C to 35^0 C slightly increased the absorbance. The indicator started leaching after 35 ^{0} C and almost leached out at higher temperature i.e. 50^0C.

5.3.5 Stability measurement of doped film

The stability of thin films doped with eriochrome cyanine was tested in solvents like distilled water, buffer pH 6.5 and HCl (2 M). Samples were aged for two days in wet condition before performing the experiment. Stability is expressed in terms of the extent of leaching with passage of time. In buffer pH 6.5 the indicator leached out overnight because of delamination of film. In distilled water films were found to be relatively stable after six days of initial leaching (37%). Stability of films was found to be good in HCl (2 M) for first three days. No or negligible leaching (<3%) was observed during this period. However significant leaching (40%) was noticed in the next four days (fig. 5.6).

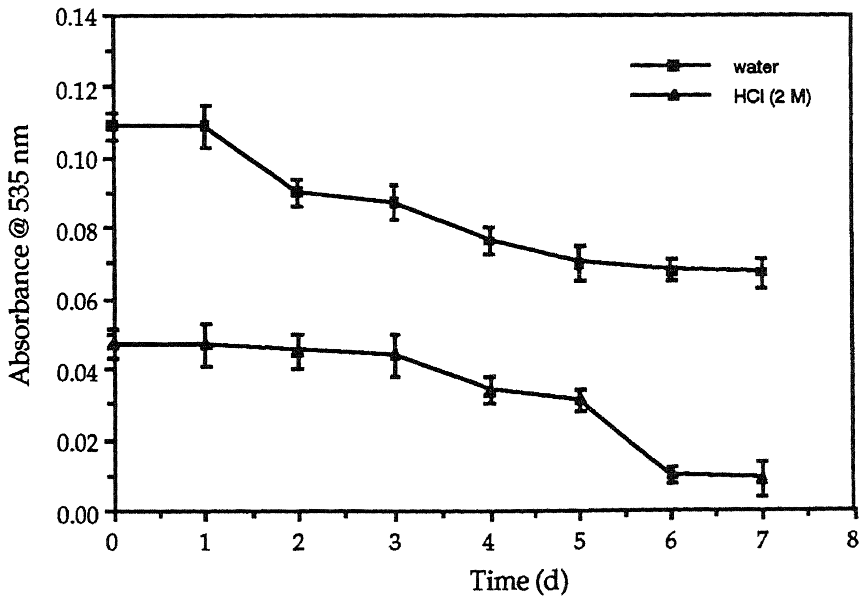


Fig. 5.6. Stability measurements of eriochrome doped sol-gel film.

5.4 DISCUSSION AND CONCLUSION

Doping of metal reagents listed in table 5.1 manifested some problems. The most important was the sensitivity of reagents in thin films even at high concentration such as 10-20 mM. Some reagents caused cracking of films while drying and some others have leaching problems. The most effective among the reagents was found to be the eriochrome cyanine RC.

Eriochrome cyanine doped in sol-gel film gave a stable complex with Cu^{++} at pH 6.5. It showed spectral changes between pH 5.5-6.5 but absorbance was found to be maximum and stable at pH 6.5 agreeing with Wallington, et al., 1997. The absorbance maximum was obtained at 565 nm which is different from that reported by Wallington et al., (1997). They showed a maximum absorbance peak of eriochrome copper complex at 580 nm. This difference could be due to variations in pathlengths of thin film and monolith and the concentrations of eriochrome doped in them. Thin films gave response times of 5-10 minutes to become stable in copper solution. More time was taken, about 10 minutes, when a higher concentration was used. This is likely to be due to low availability of active sites of reagent molecules because of the increased amount of complexes.

The concentration of eriochrome in film (20 mM) was used to get sensitivity and to optimise the doping conditions. This concentration of indicator did not cause any cracking or leaching of dye from the matrix when in operation.

A higher concentration of Cu^{++} can be measured by using another calibration curve (Kuselman, et al., 1993). But in the present application lower concentrations of copper (< 1 ppm) were required to be monitored.

The reversibilty of the copper reagent complex formation was negated in the studies by Wallington et al, (1997). They reported that eriochrome doped monoliths stabilised in copper solution for 2 days when placed in H_2SO_4 did not displace copper ions from the complex. However doped sol-gel films having a thickness of less that 1μm makes a stable complex with copper in a short time (5-10 min). Such complex formations are reversible. When these films were placed in HCl (2 M) pH 0.5 for 5 minutes they showed complete displacement of Cu^{++}. These contradictory results are attributed to the micro structural differences of bulk gels (monoliths) and thin films (Almeida & Vasconcelos, 1994) which lead to variations in their porosity, size selectivity and optimise entrapment and binding of metal ions to doped reagent. Moreover HCl appears to be an effective solvent to displace Cu^{++} from the eriochrome-copper complex. Barrero et al (1995) also reported the cleavage of metal-ligand complex in sol-gel glass by using HCl in order to demonstrate continuous measurements of metal ions.

The complex formation of eriochrome with Fe^{++} may not interfere with Cu^{++} detection because both complexes give maximum absorbances at slightly different wavelengths (fig. 5.2). The other metal ions mentioned earlier do not interfere with copper measurements even at a presence of five times higher concentration than copper in solution. This suggests that eriochrome doped in sol-gel thin film is selective to Cu^{++} and to lesser extent to Fe^{++}. However in solution this dye makes strong complex with Al^{+++} (Marczenko, 1976). This change in the selectivity of eriochrome in sol-gel matrix is due to thermodynamic effects of complexation within small pores. The restricted translational freedom of water molecules in small pores prevent the development of the full entropy change when the ion sphere is displaced by multidentate ligand (Sommerdijk and Wright, 1997; Sommerdijk, et al., 1998).

The continuous exposure of the eriochrome copper complex to a tungsten lamp for one week showed a (appx. 33%) decrease in absorbance. Photobleaching was not found a problem when intermittent light was used. As films were not stable over more than one week the light effect was recorded only for a similar period.

Temperature affected the eriochrome-copper complex in two ways;

1 It slightly increased the absorbance by taking up more copper and hence intensified the colour of complex.

2 It increased leaching of the reagent from the matrix when the temperature was raised above 35 ^{0} C. The second effect was noticed due to the cracking and dissolving of the film.

Eriochrome doped films aged two days lasted a week in distilled water and HCl. A longer dry ageing found a problem of cracking with these films. They were preferably aged under moist condition without contact with water. These films were used repeatedly for 2 days while being immersed in HCl (2 M) without any leaching. It was found that once the film stabilised in the copper solution it exhibited more resistance to cracking and leaching. Films can be brought into operation after one hour of fabrication.

The eriochrome doped films constitute a useful reusable sensor for the measurement of Cu^{++} in real time. The detection limit of Cu^{++} is 0.6 ppm which can be lowered to 0.1 ppm at the expense of low sensitivity. However sensitivity can be enhanced by making relatively thick films.

CHAPTER 6 FIBRE-OPTIC pH SENSORS

6.1 INTRODUCTION

The problem of detecting chemical species in real time and in situ is one which is receiving growing attention because of its importance in industrial process control, industrial safety and in the protection of the environment. Such problems initiate the necessity of development of robust, inexpensive chemical sensors which are capable of accurate remote sensing. Fibre optic technology gives promising solutions to many of these problems. (Culshaw, 1984 ; Yang, et al.,1993). They provide an efficient and inexpensive method for selective, in situ, real-time chemical sensing. (Browne, et al., 1996).

The active sensor region of a fibre can be either immobilised at the distal end of an optical fibre (extrinsic) or distributed along the length of the fiber-optic waveguide (intrinsic). (Yang, et al., 1993).

Peterson and Goldstein (1982) developed a miniature fibre-optic pH sensor for physiological use. The sensor was made by connecting two plastic optical fibres with their distal ends to cuprophan dialysis hollow fibre packed with phenol red. The proximal end of one fibre was used for a light source and the other was attached to a light sensor. It was able to measure pH between 7.0 to 7.4.

Wolthuis et al (1992) developed a fibre-optic pH sensor based on the covalent binding of an indicator to cellophane which was linked to a light source and detector by fibre-optics. Similarly a fibre-optic pH probe made by covalent binding of a fluorescent dye to porous glass beads attached to single, multimode optical fibre was reported by Fuh et al (1987). The dynamic range of this probe was shown to be between pH 3 to 7. Ge et al (1993) reported the construction of an optical pH sensor by immobilising a conductive polymer onto the core of silica fibre. The absorption of evanescent wave in the near-infrared region was detected by Fourier transform near infra red spectrometry.

The application of the sol-gel process to fibre optic chemical sensors was first proposed by Badini et al, (1989). In their work, silica gels incorporating fluorescein isothiocyanate were prepared and coated onto a glass slide. Cracks developed for most of the coatings and difficulties in the detection of spectral changes were also reported in their work due to very low sensitivity.

MacCraith, et al., (1991) developed a pH sensor that uses evanescent wave excitation of fluorescein-doped silica cladding at the distal end of a fiber-optic waveguide, and an intrinsic sol-gel based fibre-optic oxygen sensor based on oxygen quenching of the luminescence of sol-gel incorporated ruthenium complexes.

Browne, et al., (1996) described fluorescein-doped silica xerogel clad pH sensors and an undoped aluminosilica xerogel clad quinone sensor as an intrinsic sol-gel clad fiber-optic sensor. Similarly fibre-optic chemical sensors based on fluorescent indicators trapped in a sol-gel coating to monitor O_2 , H_2 S and CO were reported by Krihak and Shahriari, (1995).

Shahriari and Ding, (1994) demonstrated a pH sensor prepared by co-immobilising absorption type pH indicators (bromocresol green and bromocresol purple) into a silica gel which was coated as a thin film into a porous core silica glass fiber. They chemically etched the fibres to make them porous and used laser as light source.

Yang, et al., (1993) developed a distributed optical fiber chemical sensor based on immobilisation of a fluorescent indicator into a sol-gel film coated onto a fibre. The dye laser, pumped by a short pulse N_2 laser was used to produce blue light pulses at 440 nm to launch in fibres.

Tabacco and DiGiuseppe, (1996) developed fibre-optic chemical sensors for environmental control and system management. They tested several optical sensors which utilize novel glass and polymeric materials as the sensor substrates. Analytes are detected using an immobilised colorimetric indication system or molecular recognition elements.

Kurauchi, et al., (1992) reported fluorimetric determinations of Zinc, Cadmium and Gallium ions with a fibre-optic sensor having a pyridoxal isomer-modified chitosan agarose gel as a sensing probe.

Ingle, et al., (1996) developed fibre-optic chemical sensors for volatile organics and metal ions. They described a renewable reagent sensor based on vacuum aspiration of samples and its application in fluorometric determination of aluminium and chemiluminescence determination of chromium.

The purpose of this work is to test the potential of a sol-gel thin film immobilised with bromophenol blue coated onto fibres as on-line fibre-optic pH sensors. The fibre-optic sensor carries an evanescent light wave signal which is not affected by the turbidity of solution. Recently Gupta and Sharma (1997) and Noire et al (1997) reported fibre-optic sensors based on evanescent wave absorption using silica-gel immobilised indicators. These sensors were fabricated by creating a sensing area in the middle of a fibre and launched the light at one end and detecting at the other end of same fibre. This configuration for sensors has complications in the preparation of fibres, which require stripping and coating in the middle. Moreover they are not easy to use as a fibre-probe in on-line measurement and are difficult to replaced or recoat. The fibre-optic pH sensor reported here is more practical in use and simple to fabricate. It can be easily replaced and recoated without disturbing the optical set-up, which is critical. The novel spectrometer (CCD based) controlled by computer was used to detect the evanescent wave signal reflected by the mirror at the end of the probe.

6.1.1 Principle

Fibre-optic sensors can be divided into intrinsic or extrinsic depending upon whether fibre itself is part of sensing the medium or acting as a mere waveguide. The schematic diagram of an intrinsic fibre-optic sensor is presented in fig. 6.1. The basic principle of a fibre-optic sensor is using the evanescent mode of light that interacts with indicator trapped in a porous sol-gel film coated onto a fibre-optic core. The light wave penetrates into the film less than 1 µm and undergoes total internal reflection because of the low refractive index of sol-gel film compared to the fibre. The colour changes produced in the indicator by analyte (H^+) can be measured by the absorption spectrophotometer by analysing the reflected evanescent light signal. The coated end of the fibre is mirrored to get a better reflection of light signal.

Two optical fibres are used at the proximal end which are coupled together by a three port fibre-optic coupler. One fibre is connected to a light source for sending the light in and the other is attached to the spectrophotometer for detecting the reflected light signal.

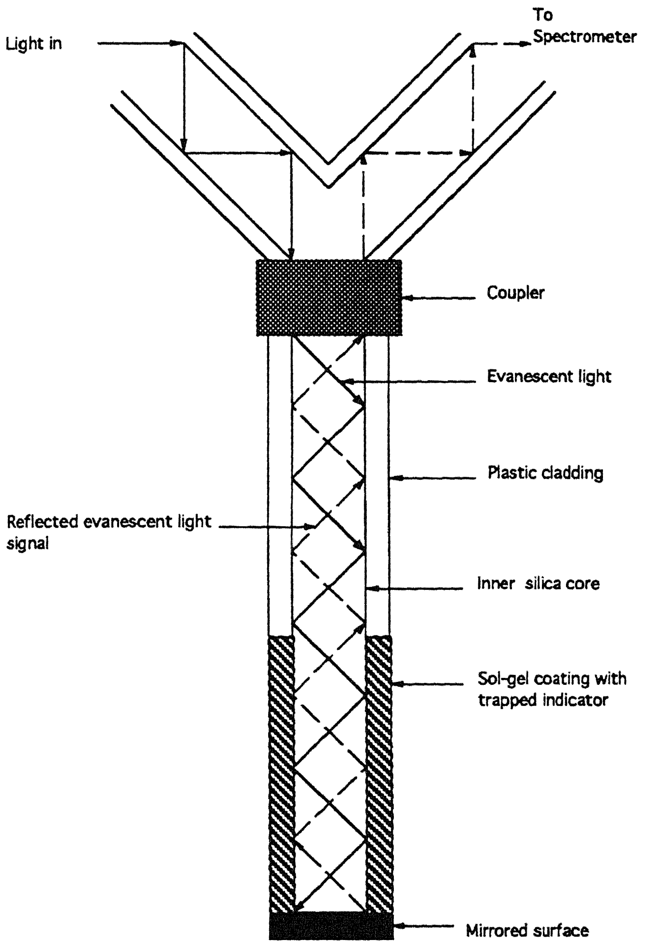


Fig. 6.1 Principle of the fibre-optic sensor.

6.2 SENSOR PREPARATION

6.2.1 Materials

Multimode optical fibres (25 cm long) with internal core diameter of 600 μm and refractive index 1.453 were obtained from SpecTran. Panasolve 100 was purchased from Eurobond Adhesives Ltd.

6.2.2 Uncladding of fibres

Optical fibres were uncladded (7 cm from the end) by burning and chemical methods. In the burning method the outer plastic coating was first burnt out carefully by a hot needle. The underneath cladding was then removed by immersing fibres in panasolve 100 for 48 hours. The chemical dissolved the cladding and hence exposed the core. To ensure the cladding had been properly removed, the fibres were examined under a microscope and tested by passing light through them. Light coming out from the fibres indicated that the cladding had dissolved. Uncladded fibres were cleaned by immersing in 5% chromic acid solution for half an hour and then washed thoroughly with distilled water. They were then air dried and mirrored at the tips by aluminium coating (exposing to aluminium vapours under vacuum) which were then protected by epoxy resin.

6.2.3 Coating of fibres

Sol-gel coating solution was prepared by mixing 7.3 ml TMOS, 1.7 ml deionised water and 0.1 ml 0.1 M HCl. The mixture was sonicated at room temperature for 10-20 minutes to create a single phase, clear solution. This solution was placed, covered, in the dark at room temperature (22^0 C) for 24 hours of hydrolysis. The partially hydroylsed solution was then added with 4.5 ml 40 mM bromophenol blue solution in ethanol. The coating solution was aged for three days at room temperature before coating the fibres. Uncladded fibre cores were made hydrophilic (surface activated) by immersing in 2 M NaOH, 2 M HCl, and distilled water respectively for 15 minutes each. The fibres were then air dried and coated by a mechanical, computer controlled device (fig. 6.2) for withdrawal from solution at a uniform speed of 1 cm/second. The device consisted of a stepping motor attached to a computer through an interface card (National Instrument NB-MIO-16).

The fibre was connected to the motor via a piece of cord. The programming language used to control the device was LabVIEW, which enabled to create a control panel on the screen with programming options such as variable speeds, time. The different withdrawal speeds with corresponding film thicknesses are shown in table 6.1. The optimum thickness was achieved at 1 cm/ sec.

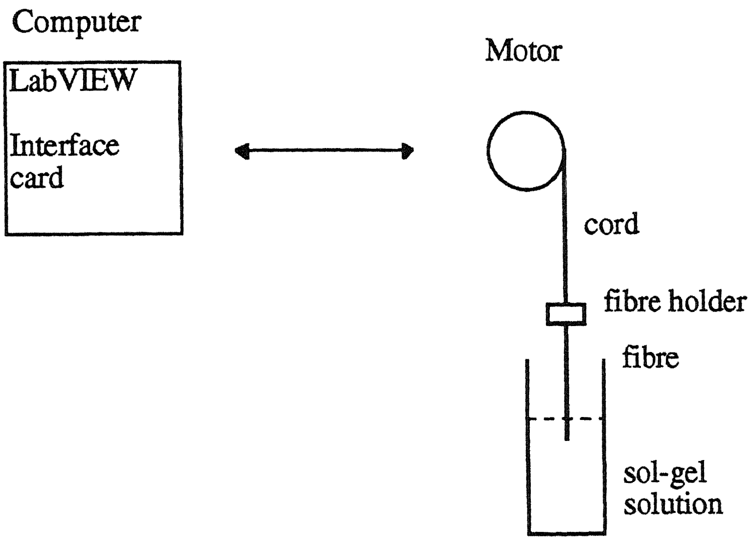


Fig. 6.2 A computer controlled device for fibre-optic coating.

6.2.4 Experimental set-up for sensor measurements

A schematic diagram of the optical arrangement of the sensor is shown in fig. 6.3. Two fibres (600 μm diameter) were spliced together by a three port coupler (ATI 1 x2, 50/50 ratio). The light from a tungsten halogen lamp (10 W) was focused on one fibre held in front of it. The other fibre was plugged into the detector based in a spectrophotometer specifically adapted for it. The outgoing fibre from the coupler was fitted with a connector at the distal end. The coated fibre with reflection coating at the end was joined to the connector which was removable. The coated fibre was immersed in different pH buffer solutions ranging from pH 3-8. The absorption spectrum at each pH was obtained through the spectrometer controlled by a computer.

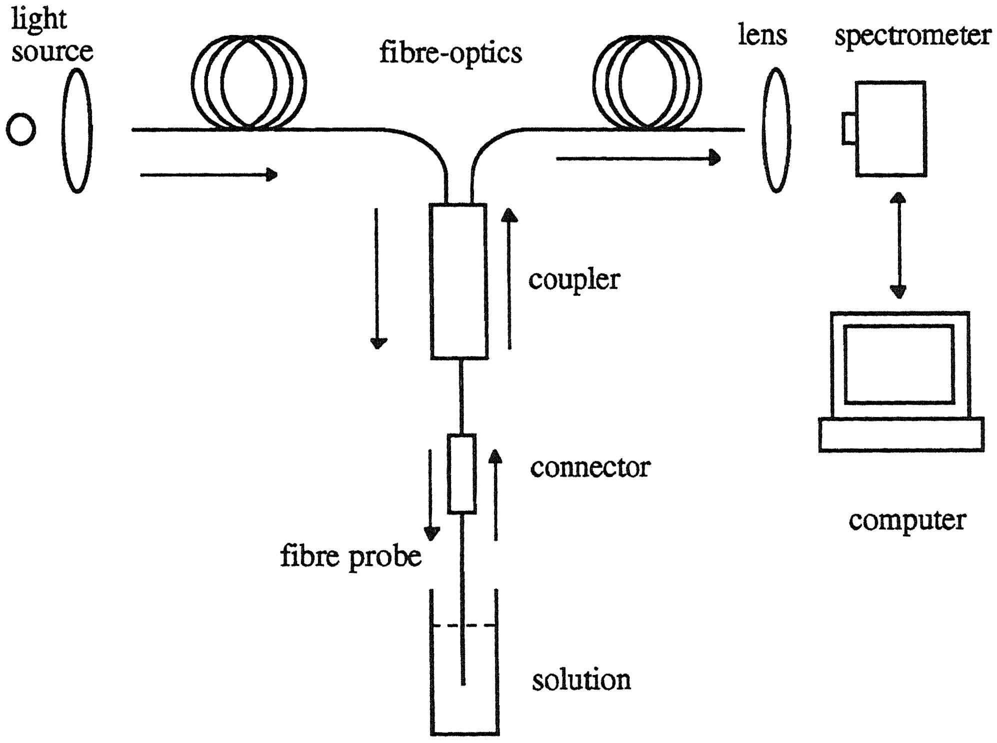


Fig. 6.3 Experimental arrangement for a fibre-optic pH sensor.

6.2.4.1 The novel spectrophotometer

A novel spectrophotometer was used to detect the reflected light beam from the fibre-optic probe. The spectrophotometer utilises a concave holographic flat field diffraction grating and a linear semiconductor Charge Coupled Device (CCD) sensor. Such a device offers a continuous spectrum with optimum noise reduction, allowing several analytes to be monitored in real time. A spectrophotometer was designed, constructed, and evaluated. Figure 6.4 shows the optical layout of the spectrophotometer.

The instrument consists of a set of fused silica lenses to collimate the light from the fibre-optics, a mechanical shutter, a spectrometer unit with no moving parts, and the driving electronics.

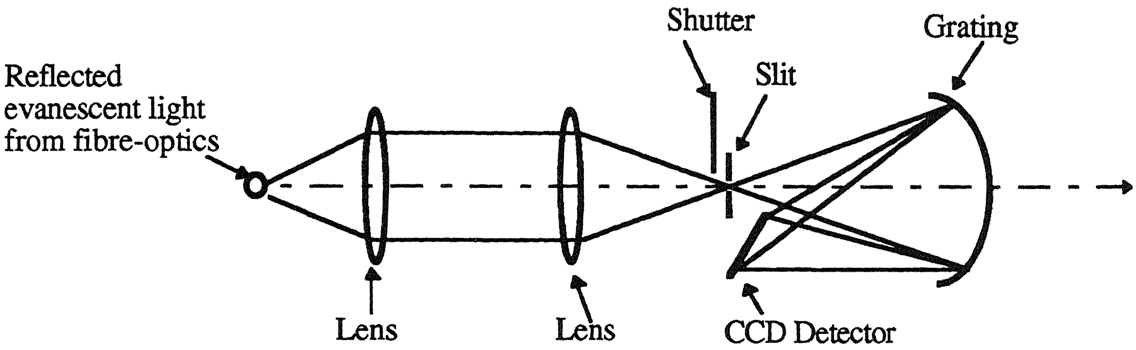


Fig. 6.4 Optical layout of the spectrophotometer.

The spectrophotometer is CCD-based, computer controlled, and can hold fibre-optics. The interface board is a multifunction board known as Lab PC+ and was supplied by National Instruments Ltd. It has a total of 8 analogue input channels, which can be configured either as 8 single-ended, or 4 differential analogue inputs. The latter configuration was selected for this application. One differential analogue input channel was used to acquire the video signal of the CCD. The Lab PC+ board also has 24 programmable digital I/O lines. Two digital lines were used to set the exposure of the CCD. A third digital line was used to activate the shutter in order to take account of the dark current of the CCD. User-friendly software for control of the instrument, data acquisition, data processing and data display was developed using the

LabVIEW programming language, supplied by National Instruments. All controls of the spectrometer are programmable from the computer on the control panel as shown in figure 6.5. A dial control was assigned to control the exposure time of the CCD. A numeric control was used to set the number of averages of the spectrum to be captured. Captured spectra can also be smoothed by averaging a window of pixels, and moving the window across the spectrum. A binary control is used to switch between the capturing of a reference and sample spectra.

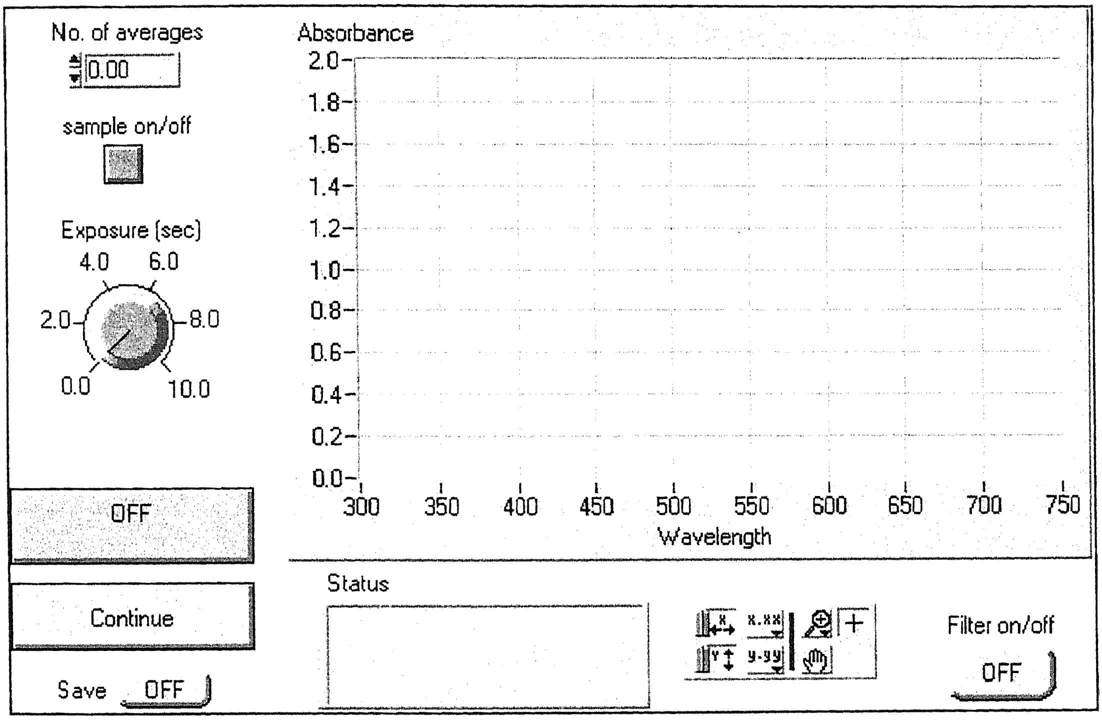


Fig. 6.5 Control panel of the spectrophotometer created with LabVIEW programming language.

The software calculates the absorption of a sample using the transmission spectra of a reference and a sample and the dark current spectrum as follows:

$$A = \log(Ir - Id)/(Is - Id)$$

where *A* represents the absorbance spectrum, *Ir, Is* and *Id* are the reference, signal and dark current spectra, respectively. Two logical controls allow the operator to save the spectrum or continue with more measurements. A window indicator shows the status of the spectrophotometer as the measurement progresses. Four exposure times of the CCD are possible, i.e. 1/2, 1/4, 1/8, or 1/16 sec..

The spectrophotometer has the following characteristics which give advantage over conventional spectrophotometers; 1- instant output, 2- high spectral resolution (0.3 nm) 3- miniaturisation, 4- robust instrumentation, 5- precision (0.001Au) 6- good reproducibility (absorbance repeatability) and 7- dynamic range. All these features helped to improve the sensitivity of fibre-optic pH sensor by a factor of two.

6.3 RESULTS

6.3.1 Spectral analysis

Fibre-optics coated with doped sol-gel thin films were aged one day before investigating their spectral characteristics. Sensors were subjected to spectral analysis by placing them in a series of pH buffer solutions (0.1 M citrate phosphate) ranging from pH 3 to 8. The spectra were recorded in the visible wavelengths (317 nm to 750 nm) with an average of 20 at each pH. A shift of 40 nm in the maximum absorption peak was noticed from pH 3 to 8 (fig. 6.6). The optimum sensing wavelength of the sensor was found to be 625 nm. The response time of the fibre-optic sensor at pH 3 to pH 8 and reverse was noted as 5 seconds. The figure 6.7 shows the response of the sensor to changing pH from pH 8 to 3. The spectrum was recorded after every half second.

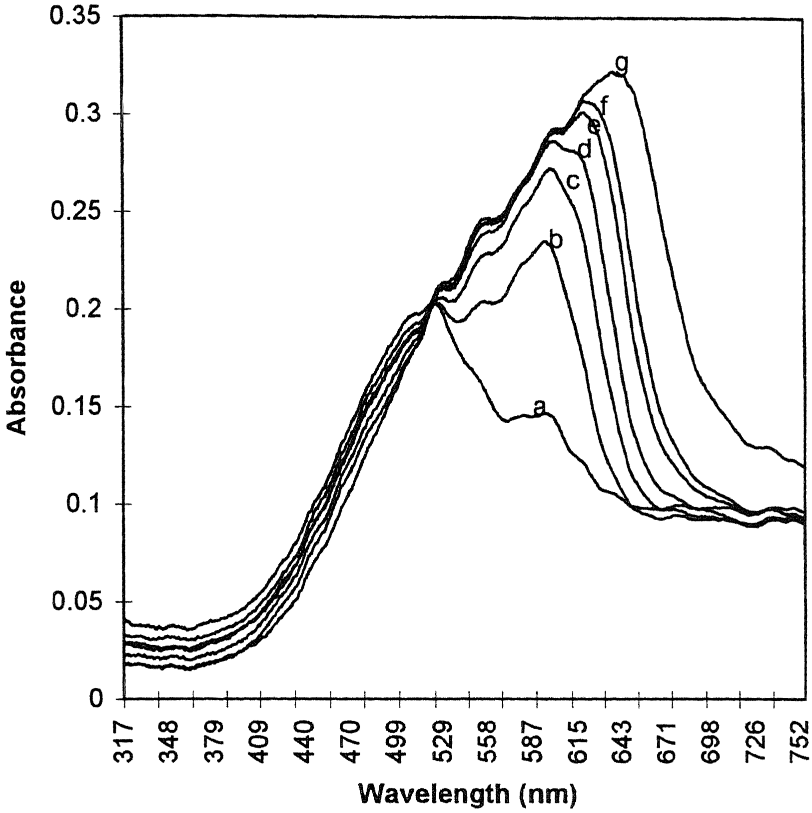


Fig 6.6 Absorption spectra of sol-gel immobilised bromophenol blue coated onto fibre-optics at various pH values: (a) pH 3.0 (b) pH 4.0 (c) pH 4.5 (d) pH 5.0 (e) pH 5.5 (f) pH 6.0 (g) pH 8.0.

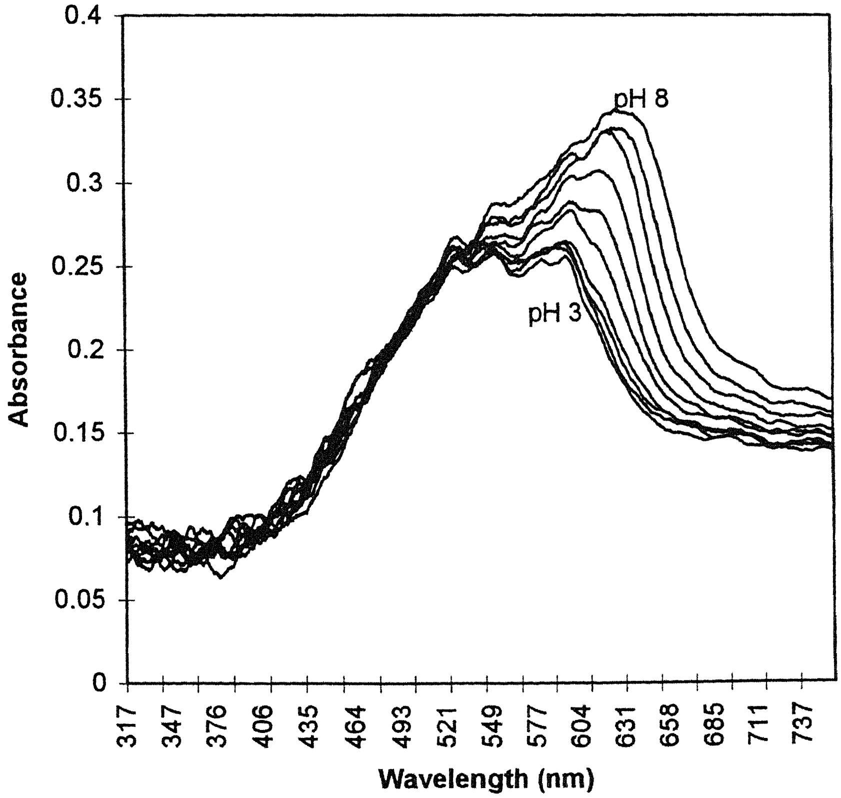


Fig 6.7 Response of fibre-optic sensor to changing pH from pH 8 to pH 3 and vice versa. Spectra were recorded at 0.5 second interval with total time of 5.0 seconds.

6.3.2 Film thickness measurements

A scanning electron microscope (SEM) was used to measure the thickness of film coated onto fibres. Films of different thicknesses were obtained by withdrawing fibres from solution at different speed. They were measured and comparatively studied. The measured thicknesses of different films against withdrawal speeds are reported in table 6.1. Each measurement was done in triplicate and result was subjected to standard deviation. All films were fabricated at room temperature ($22^0 \pm 1^0$ C) with the same coating solution. Scanning electron micrographs showing films with different thicknesses are presented in figure 6.8.

Table 6.1 Measurements of film thicknesses coated onto fibre-optics at various withdrawal speeds from sol-gel solution.

withdrawal speed	film thickness
1 cm/ 1.5 second	0.74 ± 0.02 µm
1 cm/ second	0.80 ± 0.05 µm
1 cm/ 0.3 second	1.4 ± 0.14 µm
1 cm/ 0.03 second	1.9 ± 0.10 µm

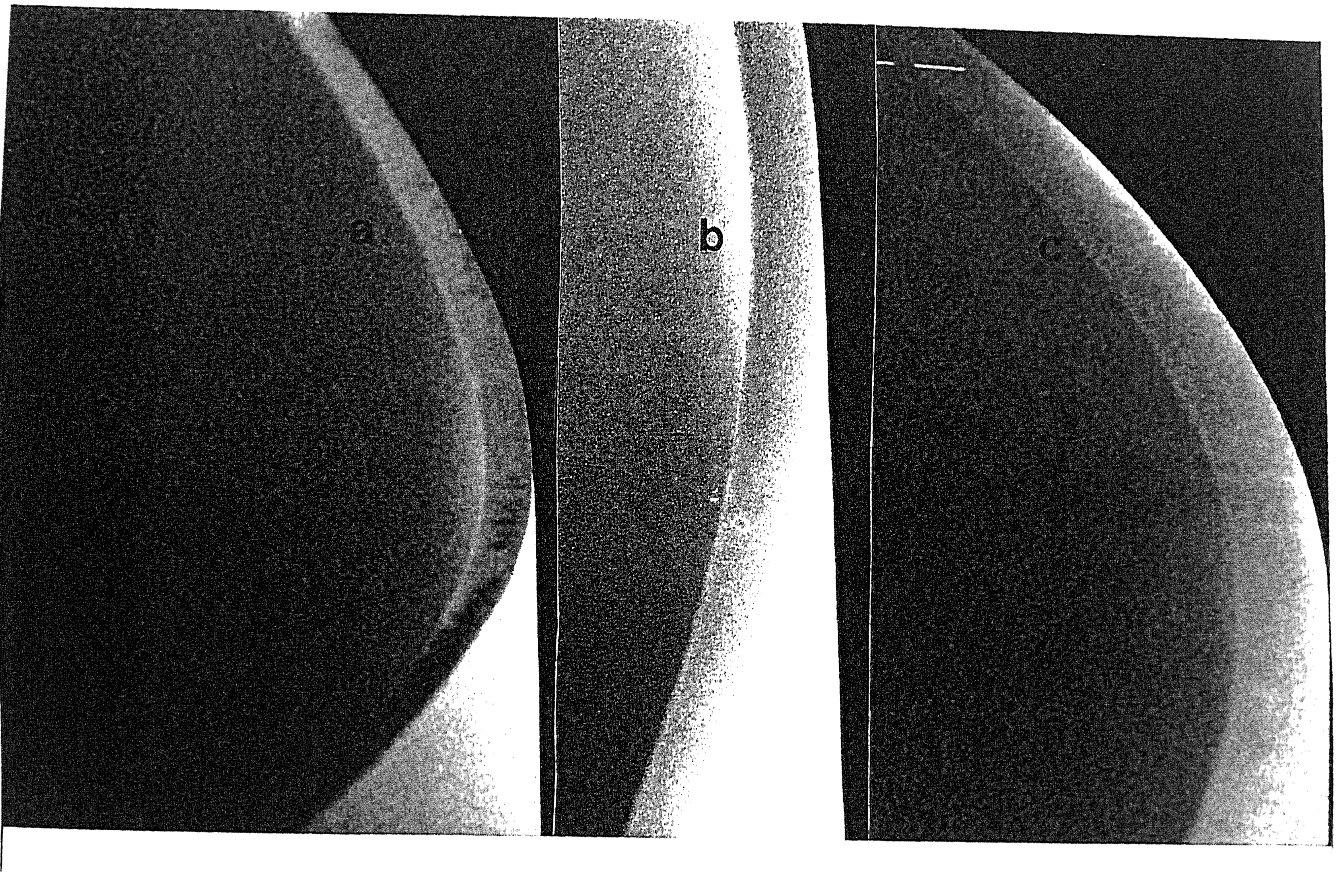


Fig. 6.8 Scanning electron micrographs of sol-gel films coated onto fibre-optics with different thicknesses: (a) 0.8 μm (b) 1.4 μm (c) 1.9 μm.

The sample in the SEM is prepared under high vacuum which causes the film to crack. The vacuum pump sucked out the water contents from the film and forced it to dry immediately. This resulted in the collapse of the porous sol-gel matrix and cracks appeared on its surface. The films are thin enough to resist external pressure and therefore cracks and dries quickly inside, which affects its binding with the fibre. At many places films come off from the surface of the fibres as individual cracked pieces. These pieces were focused at their width (raised edges) under high magnification (6700 x) and micrographs were prepared. This magnification was set to achieve best images of defined edges of various films and their differences in thicknesses. This method gave precise measurements of film thicknesses.

6.3.3 Stability measurements

A fibre-optic sensor was tested for the long term stability at an optimum pH 5. The coated fibre (probe) aged one week was placed in pH 5 buffer solution at room temperature (22^0 C) for three weeks. The absorption spectrum of the sensor was recorded every day. Buffer solution was replaced regularly after every 2 days. The maximum absorption peak was noticed at 620 nm. The graph was plotted between maximum absorbances verses time (days) to evaluate the leaching of indicator from sol-gel film coated onto fibres (fig. 6.9).

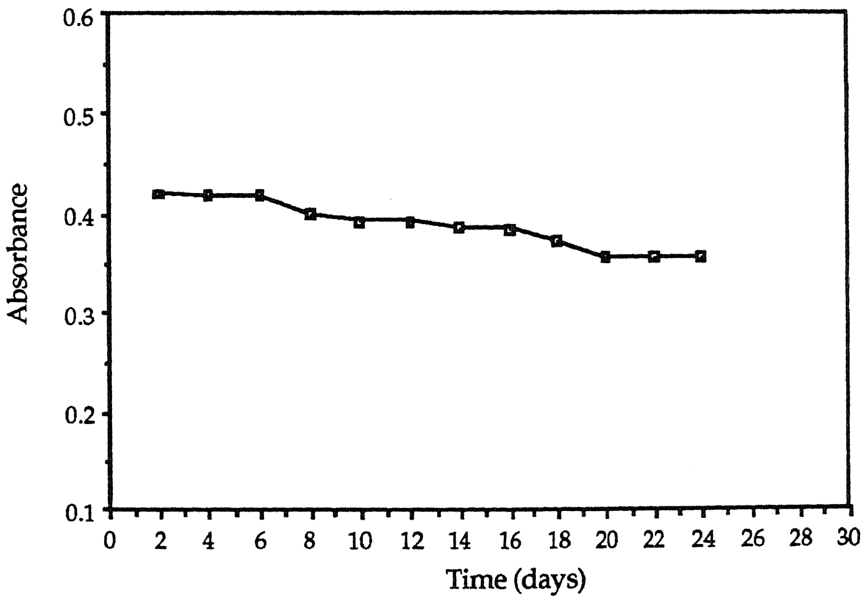


Fig 6.9 Stability measurements of a fibre-optic sensor in pH 5 buffer solution.

6.4 DISCUSSION AND CONCLUSION

The sensor was found to be stable during its spectral analysis. Leaching and cracking of the sol-gel film was not observed during the repeated use of the sensor for absorption spectra. The shift in the maximum absorption peak and difference in the shape of absorption spectra from the previous one (fig. 3.6) can be attributed to the evanescent mode of the light signal through the fibres which is different from simple transmission or propagation of light through doped film. Here light undergoes the total internal reflection after hitting dye molecules entrapped in coated film and hence changes its characteristics. Further the evanescent light signal after sensing was not transmitted but reflected to the spectrophotometer which also gave the different absorption spectra. The shift in maximum absorbances of fibre-optic sensors was also reported by Gupta and Sharma (1997) and Noire et al (1997).

Other researchers (Butler et al, 1995) used a transmission mode of evanescent wave light signal through a fibre, reported no shift in wavelength with change in pH and also showed the isosbestic point of entrapped indicators (bromophenol blue, bromocresol purple) in sol-gel film coated onto fibre-optics. The disappearance of the isosbestic point and shift in maximum absorbances with changing pH in figures 6.6, 6.7 with respect to figure 3.6 can not be attributed to the chemical differences of the two films because the films were prepared by similar composition of hydrolysing sol mixture (R=2). Moreover these differences may not be due to poor calibration or alignment of CCD spectrophotometer because the same spectrophotometer when used to scan a bromophenol blue solution in a cuvette gave the maximum absorbances at 590 nm with a clear isosbestic point (fig. 6.10). The reflective aluminium coating might interfere with the light signal and also light losses from the coupler and connectors could have an impact on the final outcome of the absorption spectra of doped indicator. However the exact answer to this deviation in the absorption spectra by a fibre-optic sensor requires further investigations.

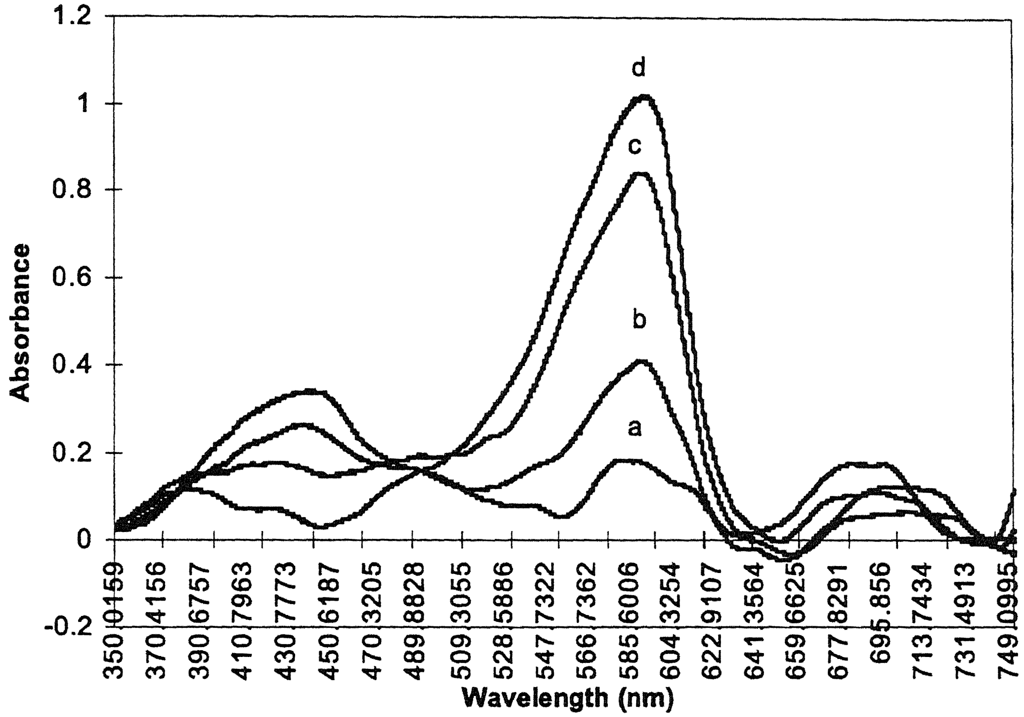


Fig. 6.10 Absorption spectra of bromophenol blue (0.1 M) in solution as scanned by CCD spectrophotometer at pH values; (a) pH 2.4 (b) pH 3.4 (c) pH 4.0 (d) pH 5.1.

Scanning electron microscopy (SEM) was found a simple and reliable method of comparative measurements of sol-gel film thicknesses. The use of the SEM was previously reported by Dunbar et al., (1996) and Plaschke et al., (1995) for the study of morphology and thickness of sol-gel films coated on a fibre and a glass disc respectively. The former did not give detail method of measurements and the later used long scan profiler in conjunction with SEM. Moreover these workers only measured the thickness of a single speed coating. In the present research the thicknesses of the films coated at different withdrawal rates were comparatively measured (table 6.1). Films with optimum thickness of 0.8 µm were used for sensing because of their sensitivity to pH changes and stability during continuous use. Other films with thicknesses (0.7 µm, 1.4 µm, 1.9 µm) were found either less sensitive or poorly stable in solutions.

Thickness of the film was found to be related to the withdrawal speed. Faster withdrawal gave thicker films and the slower gave thinner (table 6.1). According to Scriven (1988), the viscous drag force by which substrate (fibre) lifts the coating solution is higher at faster withdrawal and hence thicker film is deposited by dragging along excess solution. On the other hand at slower withdrawal the viscous drag force is smaller which results in coating thinner film.

The sensor stability was tested for short term and long term use in pH solutions. In short term the repeated uses of fibre-optic probes did not show leaching and maximum peak absorbances remained the same. The sensor was however kept dry when not in operation during this period. For determination of long term stability a fibre-optic sensor was kept in pH 5 buffer solution for three weeks. Sensor showed 14% decrease in absorbance after this prolonged immersion in pH 5 buffer solution. This is attributed to the leaching of the indicator from the sol-gel matrix. Long term stability was carried out with one week aged sensing film. The leaching can be further reduced if the sensor is aged for a couple of weeks before subjected to stability testing. The DMF was not added to the doped films coated onto fibres because in its absence the films were found to be stable over a long period without any effect on their response times. The long dry ageing (12 months) of one of the coated fibres did not affect the response time of sensing film and its binding to the surface of silica core.

The fibre-optic pH sensor described in this chapter has the advantage of simplicity in use. It can easily be removed from the main fibre assembly without affecting the optical alignment which is very critical. Sensing fibres can be recoated and reused without any harm to aluminium coating at the tip. The procedure of uncladding is simple and environmentally friendly. It has good sensitivity to changing pH between pH 3 to 8. The sensor has potential applications in on-line fermentation and downstream processes as part of a biochemical monitoring system.

CHAPTER 7 CONCLUSIONS

Sol-gel glasses derived from TMOS were used to dope the colorimetric indicators for optical measurements of pH and metal ions in solution. The glasses are usually categorised as monoliths and thin films according to their physical configurations. The monoliths are simplest in form and can easily be employed as a window inside a spectrophotometric cell to monitor changes in pH. Some initial studies were conducted to use monoliths as pH sensors (Zusman, et al., 1990; Rottman, et al., 1992; Lev, 1992; Badini, et al., 1995). They pointed out the problems of cracking and the long response time of these gels. However these studies did not give data of improvement of aqueous stability of doped gels by using DMF or long ageing. They also failed to give results on spectral analysis and their differences from that of the solution in order to explain their slow response times. Also there is no report of any study to reduce the thickness (< 1 mm) of monolithic sensors for decreasing response time. In this study (chapter 2) the sol-gel monoliths doped with pH indicators were prepared crack free. They were improved in their stability during fabrication and immersion in solution through the use of drying control chemical additives especially DMF and ageing for longer periods. The absorption spectra of doped monoliths were compared with that of the indicators in solution to evaluate the behaviour of the trapped indicators. Response times of these glasses were also decreased from few hours to 50 minutes in pH 8 buffer by producing thinner monoliths (0.40 mm thickness). However with all these improvements the monoliths were still found to be inefficient for pH measurements in real time.

Sol-gel thin films doped with indicators on different substrates have a bright future as chemical sensors because of their rapid response times

(20 seconds) and relatively good stability in various solvents. They were found resistant to cracking and exhibited very low leaching in an extended period of time. They can be easily fabricated and brought into operation after a few hours of drying. The sol-gel thin films were coated on glasss slides by using a newly designed spin coater. The coater enabled an even coating under a controlled environment with minimum risk of contamination. It also made possible coating more than one slide at a time. A novel method was introduced to coat test tubes and tubing. The sol-gel coated test tubes were used for simple and fast measurements. They can easily fit into a spectrophotometer and can be read out directly for absorbance changes. In the present study absorption spectroscopy was used to assess the performance of the sensor irrespective of the other workers who predominantly used reflectance techniques (Lee and Saavedra, 1994; Yang and Saavedra, 1995). Moreover other workers did not give a comparison of spectra of indicators trapped in thin films and free in solution which was carried out in the present research for understanding their responses in the two different environments. The thin films were initially doped with three different indicators, bromphenol blue, bromocresol purple and bromothymol blue but after comparative study of their absorption spectra and effect of ageing on their performances, bromophenol doped films were selected for further characterisation. These bromophenol doped films were found to be less effected by ageing and sensitive to pH changes from pH 3 to 8. The effect of temperature, light and salts were recorded on these films which are not reported.

pH measurement using a doped sol-gel film showed stable response to a rise in temperature between 20 ^{0} C to 37^0 C which is a required range in most of industrial applications. The light effect was not significant on immobilised indicator. Salt was found to affect the pH calibration curve of doped indicator at high concentration such as 1 M. However it can be eliminated by reducing the concentration to 0.01 M in the sample. Sol-gel films showed good stability (no leaching) after repeated uses in short term period of 1-2 weeks. These films can be used as disposable pH sensors because of the very low cost and simple and quick preparation.

DMF proved to be a wonderful DCCA which helps to control the pore sizes and responsible for quick drying and hence add stability to films. The DMF in other studies (Brinker, 1990; Wallington, 1995) was mainly used to stop the fracture of bulk gels during preparation and to measure their surface area and porosity after drying. However they were no intention to study the effect of DMF on the pH indicator doped monoliths or thin films. This chemical is believed to be introduced for the first time in this research to control the ageing and porosity in the bromophenol blue doped thin films. It was concluded from the results that the response time of DMF containing films are not affected by ageing due to bigger pore sizes. The toxicity of DMF was minimised by using small volume (0.1 ml DMF to 2 ml TMOS) and finally partial elimination from the matrix of the sol-gel film by steam autoclaving.

FTIR spectroscopy was used to understand the effect of ageing on the chemical structure of thin films with and without DMF. The changes in C-H bending at 1463 cm^{-1} were found to be a useful indicator to follow the structural evolution of the films. The results suggested that most of the drastic structural changes resulting from condensation and polymerisation reactions occur in the sol-gel film within the first four weeks of fabrication. After that the film become relatively stable and can be used for stability experiments. The results further favoured the DMF films in term of short ageing (polymerisation) and more porosity after autoclaving.

Sol-gel films doped with eriochrome cyanine gave accurate measurements of Cu^{++} in solution to a concentration of 0.6 ppm in 5-10 minutes. They have low long term stability but can be used repeatedly for 2 days without any leaching. They are affected by light if exposed constantly. However intermittant use of these films avoid photobleaching. Films can be aged without affecting their chemical sensing capacities in HCl for many hours. The literature does not report the use of eriochrome cyanine in sol-gel thin films. However Wallington et al., (1997) immobilised monoliths with this reagent but they reported long response time (2 days) and irreversible complex formation.

Fiber-optics coated with indicator doped sol-gel film can be used efficiently to measure pH in on-line systems due to the evanescent mode of light signal carried through the fibres which is not affected by turbidity of the sample solution. Fibre-optic sensors developed in the project have exhibited fast response (5 seconds) to changing pH from pH3 to 8 and chemical stability in repeated uses. Its sensitivity has been enhanced by a factor of two by using a novel spectrophotometer based on a CCD sensor. It is more practical to use as a pH probe for on-line/ at-line measurements. It can be recoated and reused many times without affecting the reflective aluminium tip coating and hence reduce the cost of sensor. It is simple to fabricate and can be easily plugged in and out of a main optical set up. The sol-gel film coated onto the fibre was of medium thickness (0.8 μ) which showed reasonable sensitivity of entrapped indicator with changing pH and did not crack during prolonged use. However thicker films fractured after one day use in solution.

7.1 FUTURE WORK

Future work can be split into two areas; (a) further improvement in the long term stability of the doped films by controlling their pore sizes with addition of DMF at different stages of formation of the films and (b) improvement in applications as chemical sensors in on-line systems by increasing sensitivity.

The pores of sol-gel films could be controlled so that they become the right size for required analytes such as H^+ and can restrict the entry of other bigger ions such as Na^+ , K^+ . This would help to solve the problem of leaching and eliminate the effect of salt on doped indicator. It is further required to exploit the possibility of making relatively thicker films for improving the sensitivity of doped metal reagents.

A future plan for using thin film coated test tubes or tubings for on-line chemical sensor should be based on making use of them as flow cells. The figure 7.1 represents the schematic diagram of such a flow cell.

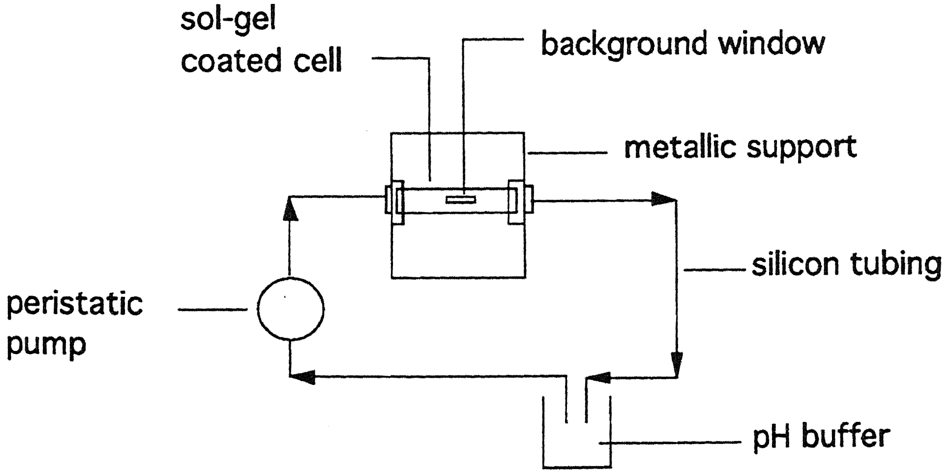


Fig. 7.1 Schematic diagram of a flow through sol-gel coated cell.

The doped thin films coated glass slides can be plunged into soil to measure soil pH. They can be a good alternative to pH paper for many routine pH measurements because of their rigidness, less prone to attack by most organic solvents and acids and also they are reusable. The test tube coated thin films constitute a generic method for chemical sensing, which can be adopted in future to other cations, anions and metabolites as potential applications in water and environment industries.

The fibre-optic sensor mentioned in the thesis require further investigation to improve sensitivity by reducing light losses through the optical set-up. This can be achieved by using more efficient fibre-optic couplers and connectors. Further sensitivity can be enhanced by coating more thick films onto fibres which could last longer in repeated uses. This increase in sensitivity would enable the sensor to be used for other applications as well such as detection of metal ions, organics and biological species in on-line processes.

REFERENCES

Aharonson, N., Altstein, M., Avidan, G., Avnir,D., Bronshtein, A., Lewis, A. and Liberman, K. (1994) Recent developments in organically doped sol-gel sensors: A microns-scale probe; successful trapping of purified polyclonal antibodies; solutions to the dopant-leaching problem. *Mat. Res. Soc. Symp. Proc.*, **346**, 519-530.

Ahmad, M. and Narayanaswamy, R. (1995) Entrapment of some aluminium (III)-sensitive reagents in sol-gel films. *Bull. Singapore Nat. Inst. Chem.*, **23**, 55-60.

Allain, L. R., Sorasaenee, K., and Xue, Z. (1997) Doped thin film sensors via a sol-gel process for high-acidity determination. *Analytical chemistry*, **69**, 3076-3080.

Allen, N. S. and McKeller, J. F. (1980) *Photochemistry of dyed and pigmented polymer.* Applied science publication, London.

Almeida, R. M., Pantano, C. G. (1990) Structural investigation of silica gel films by infrared spectroscopy. *J. Appl. Phys.*, **68**, 8, 4225-4232.

Almeida, R. M. and Orignac, X. (1994a) Silica-based sol-gel films doped with active elements. *Journal of sol-gel Science and Technology*, **2**, 465-467.

Almeida, R. M., and Vasconcelos, H.C. (1994b) Relationship between infrared absorption and porosity in silica-based sol-gel films. *SPIE*, **2288**, 678-687.

Avnir, D., Levy, D. and Reisfeld, R. (1984) The nature of the silica cage as reflected by spectral changes and enhanced photostability of trapped rhodamine 6 G.*The journal of physical chemistry*, **88**, 5956-5959.

Avnir, D., Kaufman, V. R., Pines-Rojanski, D. and Huppert, D. (1988) Water consumption during the early stages of the sol-gel tetramethylorthosilicate polymerization as probed excited state proton transfer. *Journal of Non-Crystalline solids,* **99**, 379-386.

Avnir, D., Braun, S., Lev, O. and Ottolenghi, M. (1992) Chemically active organically doped sol-gel materials: Enzymatic sensors,chemical sensors and photo active materials. *SPIE* , Sol-gel Optics II, **1758**, 456-463.

Avnir, D., Braun, S., Lev, O., Levy, D., and Ottolenghi, M. (1994a) Organically doped sol-gel porous glasses: chemical sensors, enzymatic sensors, electrooptical materials, luminescent materials and photochromic materials. In *Sol-gel optics: processing and applications.* Kluwer academic press, Boston, 540-582.

Avnir, D., Braun, S., Lev, O. and Ottolenghi, M. (1994b) Enzymes and other proteins entrapped in sol-gel materials.*Chemical materials,* **6**, 1605-1614.

Avnir, D. (1995) Organic chemistry within ceramic matrices: doped sol-gel materials. *Acc. Chem. Res.,* **28**, 328-334.

Avnir, D. (1997) Sol-gel materials doped with organic molecules-applications across chemistry and recent advances. *Sol-gel conference '97 abstracts,* **A1** (I), 5.

Bacci, M., Baldini, F. and Bracci, S. (1991) Spectroscopic behavior of acid-base indicators after immobilization on glass supports. *Applied spectroscopy,* **45**, 9, 1508-1515.

Badini, G. E., Grattan, K. T. V., Palmer, A. W. and Tseung, A. C. C. (1989) Development of pH-sensitive substrates for optical sensor applications. *Springer proceedings in physics,* **44**, 436-442.

Badini, G. E., and Grattan, T. V. (1995 a) Sol-gels with fiber-optic chemical sensor potential: Effects of preparation, aging, and long-term storage. *Rev. Sci. Instrum.*, **66,** 8, 4034-4040.

Badini, G. E., Grattan, K. T. V., and Tseung, A. C. C. (1995 b) Impregnation of a pH-sensitive dye into sol-gels for optic chemical sensors. *Analyst,* **120,** 1025-1028.

Bambot, S. B, Sipior, J., Lakowicz, J. R., and Rao, G. (1994) Lifetime-based optical sensing of pH using resonance energy transfer in sol-gel films. *Sensors and Actuators,* **B 22,** 181-188.

Barreau, S., and Miller, J. N. (1996) Sol-gel systems for biosensors. *Analytical Communications,* **33,** 5H-6H.

Barrero, J. M., Camara, C., Perez-Conde, M. C., San Jose, C. and Fernandez, L. (1995) Pyroverdin-doped sol-gel glass for the spectrofluorimetric determination of iron (III). *Analyst,* **120,** 431-435.

Bates, R. G. (1964) *Determination of pH: theory and practice.* John Willey, New York.

Bates, R.G. (1981) The modern meaning of pH. *CRC Crit. Rev. anal. Chem.,* **10,** 247-278.

Biilmann, E. (1927) *Bull. Soc. Chim.,* **41,** 213.

Brezinski, D. P. (1983) Kinetic, static and stirring errors of liquid junction reference electrodes. *Analyst,* **108,** 425-442.

Brinker, C. J., Hurd, A. J., Frye, G. C., Ward, K. J., and Ashley, C. S. (1990 a) Sol-gel thin film formation. *Journal of Non-Crystalline Solids,* **121,** 294-302.

Brinker, C. J. and Sherer, G. W. (1990 b) *Sol-gel science: The physics and chemistry of sol-gel processing.* Academic press, Boston.

Brinker, C. J., Hurd, A. J., Schunk, P. R., Frye, G. C. and Ashley, C. S. (1992) Review of sol-gel thin film formation. *Journal of Non-Crystalline Solids,* **147 &148,** 424-436.

Browne, C .A., Tarrant, D. H., Olteanu, M. S., Mullens, J. W., and Chronister, E. L. (1996) Intrinsic sol-gel clad fiber-optic sensors with time-resolved detection. *Analytical Chemistry,* **68,** 2289-2295.

Burzynski, R., and Prasad, P. N. (1994) Photonics and non-linear optics with sol-gel processed inorganic glass: Organic polymer composites. In: *Sol-gel optics: Processing and applications,* Klein, L. C. Ed., Kluwer academic publishers, Boston, 417-449.

Butler, T., MacCraith, B. D., and McDonagh, C. (1995) Development of an extended-range pH sensor using evanescent wave absorption of sol-gel entrapped pH indicators. *Proc. SPIE,* **2508,** 190-198.

Chaudhuri, S. R. and Sarkar, A. (1994) Large silica monoliths. In: *Sol-gel optics: processing and applications,* Klein, L. C. Ed., Kluwer academic publishers, Boston, 83-107.

Christian, G. D. (1994) *Analytical chemistry,* John Willey and sons, New York.

Clark, W. M. (1928) *The determination of hydrogen ions.* 3rd edition, Williams and Wilkins co., Baltimore.

Coetzee, J. F. and Ritchie, C. D. (1969) *Solute-solvent interactions.* Dekker, USA.

Covington, A. K., Whalley, P. D. and Davison, W. (1985) Improvements in the precision of pH measurements; A laboratory reference electrode with renewable free-diffusion liquid junction. Analytica Chim. Acta., 169, 221-229.

Culshaw, B. (1984) *Optical fibre sensing and signal processing.* Peter perengrinus.

Dahmouche, K., Bovier, C., Dumas, J., Serughetti, J., and Mai, C. (1995) Densification of acid-catalysed silica xerogels. *Journal of Materials Science,* **30**, 4149-4154.

Dave, B. C., Dunn, B., Valentine, J. S., and Zink, J. I. (1994) Sol-gel encapsulation methods for biosensors.*Analytical chemistry* **66**, 22, 1120A-1127A.

Davison, W. and Woof, C. (1985) Performance tests for the measurement of pH with glass electrodes in low ionic strength solutions including naturl waters. *Analytical Chemistry,* **57**, 2567-2570.

Du, X. M., Orignac, X., and Almeida, R. M. (1995) Striation-free, Spin coated sol-gel optical films. *J. Am. Ceram. Soc.,* **78**, 8, 2254-2256.

Dunbar, R. A., Jordan, J. D., and Bright, F. V. (1996) Development of chemical sensing platforms based on sol-gel derived thin films: Origin of film age vs performance trade-offs. *Analytical Chemistry* , **68**, 604-610.

Edmiston, P. L., Wambolt, C. L., Smith, M. K. and Saavedra, S. S. (1994) Spectroscopic characterization of albumin and myoglbin entrapped in bulk sol-gel glasses. *Journal of colloid and interface science,* **163**, 395-406.

Ellerby, L. M., Nishida, C. R., Nishida, F., Yamanaka, S. A., Dunn, B., Valentine, J. S., Zink, J. I. (1992) Encapsulation of proteins in transparent porous silicate glasses prepared by the sol gel method. *Science* , **255**, 1113-1115.

Farded, M.A., Yeatman, E.M., Dawnay, E.J.C., Green, M., and Horowitz, F. (1995) Effects of H_2 O on structure of acid-catalysed SiO_2 sol-gel films. *Journal of Non-Crystalline Solids,* **183**, 260-267.

Fuh, M. S., Burgess, L. W., Hirschfeld, T., Christian, G. D. and Wang, F. (1987) Single fibre optic fluorescence pH probe. *Analyst,* **112,** 1159-1163.

Ge, Z., Brown, C.W., Sun, L. and Yang, S.C. (1993) Fibre-optic pH sensor based on evanescent wave absorption spectroscopy. *Analytical chemistry* , **65** , 2335-2338.

Gillespie, C. J. (1920) Colorimetric determination of hydrogen ion concentrion without buffer mixtures, with special reference to soils. *Soil Science,* **IX,** 115.

Goldstein, S. R., Peterson, J. I. and Fitzgerald, R. V. (1980) A miniature fibre optic pH sensor for physiological use. *J. Biomech. Eng* . **102,** 141-146.

Gomes, M. C., Fernandes, A. C., Almeida, B. S., and Almeida, R. M. (1995) Influence of the wettability of silicon substrates on the thickness of sol-gel silica films. *Journal of Materials Science* , **30,** 3893-3896.

Gupta, B. D., Sharma, D. K. (1997) Evanescent wave absorption based fibre optic pH sensor prepared by dye doped sol-gel immobilisation technique. *Optics Communications,* **140,** 32-35.

Haber, F. and Russ, R. (1904) *Physik. Chem.,* **47,** 257.

Hall, E. A. H. (1990) *Biosensors.* Open university press, Milton Keynes, 1-29.

Harper, G. B. (1975) Reusable glass-bound pH indicators. *Analytical chemistry,* **47** , 2, 348-351.

Hench, L. L. (1986) *Science of ceramic chemical processing.* Wiley, New York.

Hench, L. L. and West, J. K. (1992) *Chemical processing of advanced materials,* Wiley, New York.

Herczeg, A. C., Hesslein, R. H. (1984) Determination of hydrogen ion concentration in softwater lakes using carbon dioxide equilibria. *Geochim. Cosmochim. Acta.*, **48**, 837-845.

Holobar, A., Weigl, B. H., Trettnak, W., Benes, R., Lehman, H., Rodringuez, N. V., Wollschlager, A., Wolfbeis, O. S. (1993) *Sensors and Actuators*, **B 11**, 425-430.

Iller, R. K. (1979) *The chemistry of silica.* Wiley, New York.

Illingworth, J. A. (1981) A common source of error in pH measurement. *Biochem. J.*, **195**, 259-262.

Ingersoll, C. M. and Bright, F. V. (1997) Using sol-gel-based platforms for chemical sensors. *CHEMTECH*, **27**, 26-31.

Ingle, J. D., Louch, J., Waddle, R. D., and Siemion, E. V. (1996) Fiber optic chemical sensors for volatile organics and metal ions. *Proc. SPIE-Int. Soc. Opt. Eng.*, **2872**, 112-113.

Janata, J., Josowicz, M. and Devaney, D. M. (1994) Chemical Sensors. *Analytical Chemistry*, **66**, 207R-28R.

Jones, T.P. and Porter, M.D. (1988) Optical pH sensor based on the chemical modification of a porous polymer film.*Analytical Chemistry*, **60**, 404-406.

Jordan, J. D., Dunbar, R. A., and Bright, F. V. (1995) Dynamics of acrylodan-labeled bovine and human serum albumin entrapped in a sol- gel derived biogel. *Analytical chemistry*, **67**, 2436-2443.

Kamalasanan, M.N., Kumar, N.D., and Chandra, S. (1996) Fourier transform-infrared and optical studies on sol-gel synthesized $SrTiO_3$ precursor films. *Journal of Materials Science*, **31**, 2741-2745.

Keefer, K. D. (1984) In: *Better ceramics through chemistry*, Brinker, C. J., Clark, D. E., and Ulrich, D. R. ed., North-Holland, New York, 15-24.

Kirkbright, G. F., Narayanaswamy, R., and Welti, N. A. (1984 a) Fibre-optic pH probe based on the use of an immobilised colorimetric indicator. *The Analyst,* **109,** 1025-1028.

Kirkbright, G. F., Narayanaswamy, R. and Welti, N. A. (1984b) Studies with immobilized chemical reagents using a flow cell for the development of chemically sensitive fibre optic devices. *Analyst,* **109,** 15-17.

Klein, L. (1994) *Sol-gel optics: Processing and applications.* Kluwer academic press, Boston, MA, USA.

Krihak, M. K. (1996) The optical, chemical and photochemical properties of organic molecules trapped in sol-gel materials for fiber optic chemical sensors. *Dissertation Abstracts International,* **57,** 2, 1368-B.

Krihak, M., and Shahriari, M.R. (1995) Fiber optic chemical/ environmental sensors based on active coatings. *SPIE,* **2508,** 358-367.

Kurauchi, Y., Hayashi, R., Egashira, N., and Ohga, K. (1992) Fluorometric determination of zinc, cadmium and gallium ions with a fiber-optic sensor having a pyridoxal isomer-modified chitosan/agarose gel as a sensing probe. *Analytical sciences,* **8,** 837-840.

Kuselman, I. and Lev, O. (1993) Organically doped sol-gel based tube detectors: determination of Iron (II) in aqueous solutions. *Talanta,* **40,** 5, 749-756.

Kuyavskaya, B. I., Gigozin, I, Ottolenghi, M., and Avnir, D. (1992) Spectrophotometric detection of heavy metals by doped sol-gel glass detectors. *Journal of Non-Crystalline solids,* **147&148,** 808-812.

Lee, J. E. and Saavedra, S. S. (1994) Evanescent sensing in doped sol-gel glass films.*Analytica Chimica Acta* , **285,** 265-269.

Leiner, M. J. P., and Wolfbeis, O. S. (1991) In: *Fiber optic chemical sensors and biosensors*. CRC press, Boca Raton, FL, 359f.

Logowski, J. J. (1976) *The chemistry of non-aqueous solvents*. Volume 4, Academy press, USA.

Lerchi, M., Bakker, E., Rusterholz, B., and Simon, W. (1992) Lead-selective bulk optodes based on neutral ionophores with subnanomolar detection limits. *Analytical Chemistry*, **64**, 1534-1540.

Lev, O. (1992) Diagnostic applications of organically doped sol-gel porous glass. *Analusis*, **20**, 543-553.

Lev, O., Tsionsky, M., Rabinovich, L., Glezer, V., Sampath, S., Pankratov, I. and Gun, J. (1995) Organically modified sol-gel sensors. *Analytical Chemistry*, **67**, 1, 22A-30A.

Lin, J. and Brown, C. W. (1997) Sol-gel glass as a matrix for chemical and biochemical sensing. *Trends in analytical chemistry*, **16**, 4, 200-211.

Livage, J. (1996) Bioactivity in sol-gel glasses. *C. R. Acad. Sci. Paris*, **t. 322**, Serie II b, 417-427.

MacCraith, B. D., Ruddy, V., Potter, C., (1991) Optical wave guide sensor using evanescent dye in sol-gel glass. *Electronics letters*, **27**, 14, 1247.

MacCraith, B. D., McDonagh, C. M., O'Keefe, G., Keyes, E. M., O'Kelly, B., and McGilp, J. F. (1993) Fibre optic oxygen sensor based on fluorescence quenching of evanescent- wave excited ruthenium complexes in sol-gel derived porous coatings.*Analyst*, **118**, 385-388.

MacInnes, D. A. (1961) *The principles of electrochemistry*. Dover publications, New York.

Maher, M. H., and Shahriari, M. R. (1993) A fiber optic chemical sensor for measurement of ground water pH. *Journal of Testing and Evaluation*, **21**, 448-52.

Marczenko, Z. (1976) *Spectrophotometric determination of elements*, Halsted press, John Wiley, pp. 51-115.

McDonagh, C., Sheridan, F., Butler, T., MacCraith, B. D. (1996) Characterisation of sol-gel-derived silica films. *Journal of Non-Crystalline Solids*, **194**, 72-77.

Mohr, G. J., and Wolfbeis, O. S. (1994) Optical sensors for a wide pH range based on azo dyes immobilized on a novel support. *Analytica Chimica Acta* **292**, 41-48.

Narang, U., Jordan, J. D., Bright, F. V., and Prasad, P. N., (1994a) Probing the cybotactic region of PRODAN in tetramethyl orthosilicate-derived sol-gels. *J. Phys. Chem.*, **98**, 8101-8107.

Narang, U., Prasad, P. N., Bright, F. V., Ramanathan, K., Kumar, N. D., Malhotra, B. D., Kamalasanan, M. N. and Chandra, S. (1994b) Glucose biosensor based on a sol-gel derived platform. *Analytical chemistry* , **66**, 3139-3144.

Narang, U., Wang, R., Prasad, P. N., and Bright, F. V. (1994c) Effects of ageing on the dynamics of rhodamine 6G in tetramethyl orthosilicate-derived sol-gels. *J. Phys. Chem.*, **98**, 17-22.

Narang, U., Prasad, P. N., and Bright, F. V. (1994d) A novel protocol to entrap active urease in a tetraethoxysilane-derived sol-gel thin film architecture. *Chemical Materials*, **6**, 1596-1598.

Nemoto, S., and Hirokawa, N., (1996) Spectral transmittance of organic dye-doped glass films obtained by the sol-gel method. *Applied Optics*, **35**, 16, 3052-3058.

Noir, M. H., Gojon, C., Marty, P., Gonter, J. and Bouzon, C. (1997) A new sol-gel derived optical fibre sensor for high acidity measurements: application in nuclear fuel reprocessing. *Sol-gel conference '97 Abstracts,* **F13**, 48.

Offenbacher, H., Wolfbeis, O. S. and Furlinger, E. (1986) Fluorescence optical sensors for continuous determination of near-neutral pH values. *Sensors and Actuators,* **9**, 73-84.

Parker, J. W., Lacksin, O., and Yu, C. (1993 a) Fiber optic sensors for pH and CO2 using a self reference dye. *Analytical chemistry* , **65** , 2329-2334.

Parker, J. W., Laksin, O., Yu, C., Lau, M. L., Klima, S., Fisher, R., Scott, I. and Atwater, B. W. (1993 b) Fiber-Optic sensors for pH and carbon dioxide using a self-referencing dye.*Analytical Chemistry,* **65**, 2329-2334.

Parrill, T. M., (1992) Transmission infrared study of acid-catalyzed sol-gel silica coatings during room ambient drying. *J. Mater. Res.,* **7**, 2230-2239.

Parrill, T. M. (1994) Heat treatment of spun-on acid catalysed sol-gel silica films. *J. Mater. Res.,* **9**, 3, 723-730.

Pearson, R. G. (1966) Acids and bases. *Science,* **151**, 172.

Peterson, J. I., Goldstein, S. R., Fitzgerald, R. V. and Buckhold, D. K. (1980) Fiber optic pH probe for physiological use. *Analytical chemistry,* **52**, 6, 864-869.

Peterson, J. I. and Goldstein, S. R. (1982) A miniature fiberoptic pH sensor potentially suitable for glucose measurements.*Diabetes Care* **5**, 3, 272-274.

Pfeifer, K. B. (1995) Heat of adsorption and thin film surface area studies of a silica sol-gel film exposed to HCl and H_2O. *Langmuir,* **11,** 4793-4796.

Plaschke, M., Czolk, R., and Ache, H. J. (1995) Fluorimetric determination of mercury with a water-soluble porphyrin and porphyrin-doped sol-gel films. *Analytica Chimica Acta,* **304,** 107-113.

Pohl, E. R. and Osterholtz, F. Z. (1985) In: *Molecular characterisation of composite interfaces,* Plenum, New York, 157.

Prasad, P. N., Bright, F. V., Narang, U., Wang, R., Dunbar, R. A., Jordan, J. F. and Gvishi, R. (1995) Novel organic-inorganic composite materials for photonics. *ACS Symposium Series,* **585,** 317-330.

Rabinovich, M. E. (1994) Sol-gel processing - general principles. In: *Sol-gel optics: processing and applications.* Klein, L. C., Kluwer academic publishers, MA, USA, 1-37.

Reetz, M. T., Zonta, A., and Simpelkamp, J. (1996) Efficient immobilisation of lipases by entrapment in hydrophobic sol-gel materials. *Biotechnology and bioengineering,* **49,** 527-534.

Rottman, C., Ottolenghi, M., Zusman, R., Lev, O., Smith, M., Gong, G., Kagan, M. L. and Avnir, D. (1992) Doped sol-gel glasses as pH sensors. *Materials Letters ,* **13,** 293-298.

Saari, L. A. and Seitz, W. R. (1982) pH sensor based on immobilized Fluoresceinamine. *Anal. Chem.,* **54,** 821-823.

Saavedra, S. S., and Reichert, W. M. (1990) Prism coupling into polymer integrated optical waveguides with liquid superstrates. *Applied Spectroscopy* **44,** *7,* 1210-1217.

Scriven, L. E. (1988) Physics and applications of dip coating and spin coating. *Mat. Res. Soc. Symp. Proc.* **121,** 717-729.

Severin-Vantilt, M. M. E., and Oomen, E. W. J. (1993) The incorporation of Rhodamine B in silica sol-gel layers. *Journal of Non-Crystalline Solids,* **159** , 38-48.

Shahriari, M. R., and Ding, J. Y. (1994) Doped sol-gel films for fiber optic chemical sensors. In: *Sol-gel optics processing and applications,* Klein, L. C. Ed., Kluwer Acadamic Publishers, Boston, 279-301.

Shtelzer, S., Rappoport, S., Avnir, D., Ottolenghi, M. and Braun, S. (1992) Properties of trypsin and of acid phophatase immobilized in sol-gel glass matrices.*Biotechnology and applied biochemistry,* **15,** 227-235.

Shtelzer, S., and Braun, S. (1994) An optical biosensor based upon glucose oxidase immobilized in sol-gel silicate matrix.*Biotechnology and Applied Biochemistry,* **19,** 293-305.

Silverstein, R. M., Bassler, C. G. and Morrill, T. C. (1974) *Spectrometric identification of organic compounds,* pp. 73-140. John Wiley, New York.

Sipior, J., Bambot, S. B., Lakowicz, J. R. and Rao, G. (1994) Lifetime-based optical sensing of pH using resonance energy transfer in sol-gel sensors. *Proc. SPIE-Int Soc. Opt. Eng,* **2136,** 315-324.

Small, J. (1926) Hydrogen ion concentration of plant tissues. I. The method. *protoplasma,* **I,** 324.

Small, J. (1954) *Modern aspects of pH with special reference to plants and soils.* Baillieve, Tindall and Cox publishers, London, 1-21.

Smith, D. M., Scherer, G. W. and Anderson, J. M. (1995) Shrinkage during drying of silica gel. *Journal of Non-Crystalline Solids,* **188,** 191-206.

Sommerdijk, N. A. J. M. and Wright, J. D. (1997) Matrix effects on selective chemical sensing by sol-gel entrapped complexing agents. *Sol-gel '97 abstracts,* **F 14,** 49.

Sommerdijk, N. A. J. M., Poppe, A., Gibson, C. A., and Wright, J. D. (1998) Unexpected complexion behaviour of sol-gel immobilised dye: the development of an optical copper (II) sensor. *J. Mater. Chem.*, **8**, 565-567.

Sorensen, S. P. L. (1909) Comptes-Rendus des travaux du laboratoire de Carlsberg 8me volume 1re livraison, Copenhague.

Tabacco, M. B. and DiGiuseppe, T. G. (1996) Optical chemical sensors for environmental control and system management. *Adv. Space Res.*, **18**, 4/5, 125-134.

Taylor, R. F. (1991) *Protein immobilisation: Fundamentals and applications*. Marcel Dekker, New York, 263-303.

Thomas, I. M. (1994) Optical coating fabrication. In: *Sol-gel optics processing and applications*, Klein, L. C. Ed., Kluwer academic publishers, Boston, 141-158.

Thomas, L. C. and Chamberlin, G. J. (1980) *Colorimetric chemical analytical methods*. The Tintometer Ltd, Salisbury.

Viart, N. and Rehspringer, J. L. (1996) Study of the action of formamide on the evolution of a sol by pH measurements and Fourier transformed infra-red spectroscopy. *Journal of Non-Crystalline Solids*, **195**, 223-231.

Vogel, A. I., (1953) *Quantitative inorganic analysis: Theory and practice*. Longmans, Green and company, London.

Vorotilov, K., Petrovsky, V., and Vasiljev, V. (1995) Spin coating process of sol-gel silicate films deposition: Effect of spin speed and processing temperature. *Journal of Sol-gel Science and Technology* , **5**, 173-183.

Wallington, S. A. (1995) *Sol-gel materials for optical chemical sensing*. Published PhD thesis, University of Kent.

Wallington, S. A., Labayan, T., Poppe, A., Sommerdijk, A. J. M. and Wright, J. D. (1997) Sol-gel materials for optical sensing of solvents and metal ions. *Sensors and actuators,* **B 38-39**, 48-52.

Wang, R., Narang, U., Prasad, P. N., and Bright, F. V. (1993) Affinity of antifluorescein antibodies encapsulated within a transparant sol-gel glass. *Analytical chemistry,* **65**, 2671-2675.

Weetall, H. H. (1996) Retention of bacteriorhodopsin activity in dried sol-gel glass. *Biosensors and bioelectronics,* **11**, 3, 327-333.

Weigl, B. H., Holobar, A., Trettnak, W., Klimant, I., Kraus, H., O'Leary, P., Wolfbeis, O. S. (1994) Optical triple sensor for measuring pH, oxygen and carbon dioxide. *Journal of biotechnology,* **32**, 127-138.

Wilson, K., and Walker, J. M. (1994) *Principles and techniques of practical biochemistry.* Cambridge university press, Cambridge.

Wolfbeis, O. S., Rodriguez, N. V., and Werner, T. (1992) Led-compatible fluorosensor for measurement of near-neutral pH values. *Mikrochimica Acta,* **108**, 3-6, 133-141.

Wolfbeis, O. S. and Mohr, G. J. (1994) Optical sensors for a wide pH range based on azo dyes immobilized on a novel support.*Analytica Chimica Acta* , **292**, 41-48.

Wolthuis, R., Mccrae, D. and Saaski, E. (1992) Development of a medical fibre optic pH sensor based on optical absorption.*IEEE Transactions on biomedical engineering* (ISSN 0018-9294), **39** , 531-537.

Wu, S., Ellerby, L. M., Cohan, J. S., Dunn, B., El-sayed, M. A., Valentine, J. S. and Zink, J. I. (1993) Bacteriorhodopsin encapsulated in transparent sol-gel glass: a new biomaterial. *Chemical materials,* **5**, 115-120.

Yang, L., and Saavedra, S. S. (1995) Chemical sensing using sol-gel derived planar waveguides and indicator phases.*Analytical Chemistry,* **67**, 1307-1314.

Yang, Y., Wallace, P. A., and Campbell, M. (1993) Distributed optical fiber chemical sensor. *Proc. SPIE-Int. Soc. Opt. Eng.,* **2594,** 233-242.

Zusman, R., Rottman, C., Ottolenghi, M. and Avnir, D. (1990) Doped sol-gel glasses as chemical sensors. *Journal of Non-Crystalline solids,* **122,** 107-109.

APPENDIX A. TABLES

Table A-1 Data used to measure the effect of ageing on sol-gel films without DMF.

Days	% Transmission @ 1463^{-1}			
	1	2	3	SD
0	45.52	44.86	43.43	1.06
1	74.19	73.45	70.36	2.03
7	76.64	73.43	74.25	1.66
14	83.11	84.07	80.17	2.03
21	89.17	90.14	85.39	2.50
28	90.95	88.04	91.95	2.03

Number of samples = 3, SD = Standard deviation

Table A-2 Data used to record the effect of ageing on sol-gel films added with DMF

Days	% Transmission @ 1463^{-1}			
	1	2	3	SD
0	29.36	28.20	30.30	1.05
1	52.37	50.06	54.31	2.12
7	57.76	55.90	53.70	2.03
14	63.60	60.05	66.09	3.03
28	65.06	61.38	64.63	2.01

Number of samples = 3, SD = Standard deviation

Table A-3 Maximum absorbances used for caliberation curve of sol-gel film doped with eriochrome cyanine at different concentrations of copper.

Copper concentration (mMol)	Absorbances @ 565 nm 1	2	3	4	5	6	SD
0.01	0.03	0.023	0.025	0.028	0.03	0.027	0.006
0.02	0.075	0.080	0.065	0.075	0.075	0.074	0.010
0.03	0.120	0.125	0.120	0.117	0.123	0.121	0.006
0.04	0.158	0.152	0.150	0.171	0.173	0.160	0.004
0.05	0.182	0.189	0.192	0.198	0.199	0.192	0.012

No of samples = 6, SD = Standard deviation

Table A-4 Effect of temperature on films doped with bromophenol blue.

Temperature °C	Absorbances* @ 590 nm					
	pH 3	SD	pH 5	SD	pH 8	SD
20	0.121	0.04	0.480	0.04	0.757	0.03
30	0.120	0.03	0.475	0.05	0.750	0.05
40	0.118	0.05	0.470	0.03	0.749	0.04
50	0.115	0.02	0.490	0.05	0.577	0.05
60	0.115	0.03	0.410	0.04	0.437	0.03

SD = Standard deviation, * average of 3 samples

Table A-5 Data for response times of bromophenol blue doped films coated inside test tubes in pH 3 and pH 8.

Time seconds	Absorbances @ 590 nm pH 3	Absorbances @ 440 nm pH 8
5	0.350±0.004	0.800±0.006
10	0.390± 0.003	0.830±0.005
15	0.400± 0.004	0.860± 0.004
20	0.410± 0.004	0.880±0.006
25	0.410±0.003	0.880±0.004
30	0.410 ± 0.004	0.880±0.004

Table A-6 Data used for measuring effect of light on pH sensor.

Time Weeks	Absorbances @ 590 nm pH 3	 pH 5	 pH 8
0	0.102± 0.030	0.447±0.04	0.748± 0.04
1	0.102± 0.05	0.447 ± 0.04	0.747± 0.046
2	0.101± 0.03	0.445 ± 0.04	0.738± 0.043
3	0.100± 0.04	0.442 ± 0.03	0.729±0.05
4	0.100± 0.04	0.426 ± 0.045	0.710± 0.03
5	0.099± 0.05	0.373 ± 0.035	0.700±0.033

Table A-7 Data used for stability measurements of eriochrome doped films.

Time days	Absorbances @ 535 nm	
	water	HCl
0	0.109 ± 0.004	0.047±0.004
1	0.109 ±0.006	0.047±0.006
2	0.090 ± 0.005	0.045±0.004
3	0.087 ± 0.006	0.044±0.005
4	0.076 ±0.004	0.034± 0.004
5	0.070 ± 0.003	0.031±0.005
6	0.068 ± 0.002	0.010± 0.003
7	0.067 ± 0.005	0.009± 0.004

Table A-8 Maximum absorbances used for measuring effect of light on erichrome copper complex.

Time days	Absorbances
0	0.101±0.006
1	0.099±0.005
2	0.087±0.003
3	0.081±0.005
4	0.075±0.006
5	0.070±0.005
6	0.063±0.004
7	0.063±0.006

Table A-9 Data used for measuring effect of temperature on eriochrome copper complex.

Temperature °C	Absorbances
20	0.150±0.005
25	0.153±0.007
30	0.157±0.003
35	0.156±0.005
40	0.130±0.005
45	0.125±0.004
50	0.075±0.005

Table A-10 Maximum absorbances used for a calibration curve of doped sol-gel films coated on glass microscope slides.

	Absorbances @ 590 nm		
pH	slide1	slide 2	slide 3
3	0.112	0.110	0.115
4	0.192	0.210	0.173
5	0.401	0.369	0.432
6	0.602	0.623	0.581
8	0.723	0.754	0.692

APPENDIX B. PUBLICATIONS DURING THE COURSE OF THIS RESEARCH

L. Noui, M. A. Javaid and P. J. Keay (1998) Development of a fibre-optic pH sensor for on-line measurements. *Applied Optics and Optoelectronics 1998, Proceedings of the Applied Optics Divisional Conference of the Institute of Physics (16-19 March),* Brighton, UK, 175-180.

M. A. Javaid and P. J. Keay (1998) A non-invasive, reusable optical chemical sensing in solution by doped sol-gel thin films on inside test tubes. *Journal of sol-gel science and technology* (submitted).

Development of a fibre-optic pH sensor for on-line measurements

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Abstract. The development of an optical pH probe is described. The fibre-optic sensor is based on evanescent wave absorption using a pH indicator immobilised in a sol-gel film coated onto an optical fibre. The pH probe was set up to work in reflection mode by coating the tip of the fibre with aluminium, thereby improving sensitivity by a factor of two. The sensor is sensitive to changes in pH between 3 and 8, and has a response time of 5 seconds. Moreover, it can be re-coated and used for prolonged periods. The pH sensor is connected to a compact spectrophotometer for direct spectral read-out. The sensor has the advantage of simplicity for making at-line or on-line measurements. It is potentially useful as an integral part of a monitoring system for biological processes.

1. Introduction

Fibre-optics have been recently exploited for sensing various chemicals in solution [1-2]. They have been used in either the extrinsic mode or intrinsic mode of sensors [3-5]. The continuous measurement of pH in real time is a growing requirement of many industrial and environmental processes. Fibre-optic sensors provide an accurate, fast, robust and inexpensive method of pH measurement [6-8]. The first fibre-optic pH sensor based on an immobilised colorimetric indicator was reported by Peterson *et al* [9]. Since then, many fibre-optic sensors employing the techniques of fluorescence, optical reflectance bulk absorption and energy transfer have been reported [10-13]. Sol-gel technology was first used for fibre-optic chemical sensors by MacCraith *et al* [11,14] and Ding *et al* [15].

Sol-gel technology offers a simple, quick and low temperature method of immobilisation of pH indicators in porous silicate thin films. These films are transparent, inert, resistant to harsh environments and intrinsically bound to the fibre core.

Sol-gel derived optical fibre sensors based on evanescent wave absorption have recently been developed by Gupta and Sharma [16]. They launched a light beam into one end of a single fibre and detected the evanescent light wave signal at the other end. The arrangement required stripping the optical fibre in the middle, which is not easy to do. This sensor configuration is also not practical to use as the sensing area is located in the middle of the fibre which is difficult to use as a probe.

We report in this paper the development of a fibre-optic pH sensor based on evanescent wave absorption using a fibre optic coupler to direct the light beam into the fibre-optic probe and to send the reflected beam from the probe to the detector (CCD-based spectrometer) [17]. This arrangement is more practical as it allows the use of the coated fibre as an interchangeable dip probe. The indicator used was bromophenol blue which was entrapped in a thin film (derived from tetramethylorthosilicate (TMOS) by the sol-gel method) and coated onto the fibre.

2. Experimental

2.1. Preparation of fibres

Optical fibres with a core diameter of 600μm and a refractive index of 1.453 (SpecTran Corporation) were used. The optical fibre cladding was removed by burning and chemical methods. In the burning method the outer plastic sheath was melted using a hot needle and stripped from the fibre. The cladding was then removed by immersing the fibres in Panasolve 100 (Eurobond adhesives Ltd) for 48 hours. The chemical dissolved the cladding and hence exposed the core. The fibre cores were cleaned by placing them in a 5% chromic acid solution for half an hour and then thoroughly washed with distilled water. The fibres were then left to dry, then the tip was coated with aluminium and protected with epoxy resin.

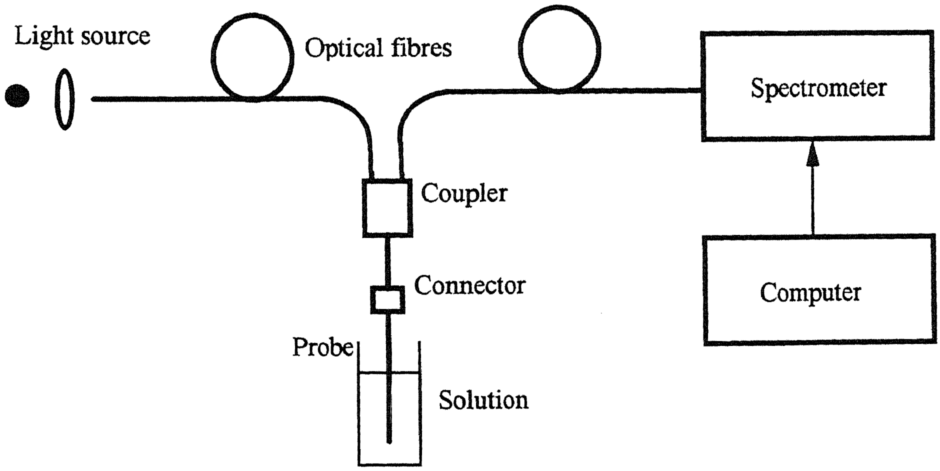
2.2. Sol-gel preparation and coating of fibres

Sol-gel solution was prepared by mixing 7.3 ml TMOS, 1.7 ml deionized water and 0.1 ml 0.1 M HCl. The mixture was sonicated at room temperature for 10-20 minutes to create a single phase, clear solution. This solution was then covered and placed in the dark at room temperature for 24 hours. 4.5 ml 40 mM bromophenol blue/ethanol solution was then added and the solution was left at room temperature for three days before being used to coat the fibres. The exposed fibre cores were made hydrophilic by immersion in 2M NaOH, 2M HCl, and distilled water respectively for 15 minutes each. The fibres were then left to dry and coated by mechanical withdrawal from the sol-gel solution at 1cm/sec.

2.3. Optical arrangement

The optical arrangement is shown in figure 1. It consists of a light source, a 1x2 optical fibre coupler, an optical fibre probe, a connector, and a CCD-based spectrometer. A white light beam is launched into one arm of the coupler and then coupled to the fibre probe through a connector. The light beam interacts with the entrapped indicator in the sol gel coating and is reflected back off the tip of the fibre. The reflected beam is then directed to the spectrometer through the coupler. The spectrum was then captured and analysed by a Personal Computer.

Figure 1: Optical layout



3. Results and discussion

Figure 2 shows the absorption spectra recorded using the pH probe in a series of buffers (0.1 M citrate phosphate) ranging in pH from 3 to 8. The measurement of changes in absorbance due to changes in pH of solutions is obtained by using a spectrum of the uncoated fibre as a reference and measuring the spectrum of the reflected light beam when the probe is immersed in the solution under test. The results shown in figure 2 were obtained by averaging 20 spectra at each pH. The probe is clear sensitive to variations in pH, particularly in the spectral region above 510 nm. There is also a shift of about 40 nm of the peak absorbance as the pH changes from 3 to 8. The shift in absorption peaks could be attributed to the characteristics of the evanescent wave mode of operation. Gupta and Sharma [16] also reported a similar shift in maximum absorbances of fibre-optic pH sensors. Figure 3 shows an overlay of spectra acquired in real time as the probe measured changes in pH from 3 to 8. The response time of the fibre-optic pH sensor was found to be 5 seconds. The sensor was also found to be chemically stable during spectral analysis. Leaching and cracking of the film were not observed in repeated uses.

4. Conclusion

The pH sensor described here has many advantages. It is simple to use. It can easily and quickly be removed or interchanged without affecting the optical alignment. The sensing fibres can be re-coated and reused. The probe is sensitive to changes in pH in the range of pH 3 to 8, and has a response time of 5 seconds. The developed pH probe has potential applications in fermentation processes as part of a biochemical monitoring system. Further work is envisaged to study the long term stability and leaching effects of the probe, as well as extending the technique to other chemical parameters.

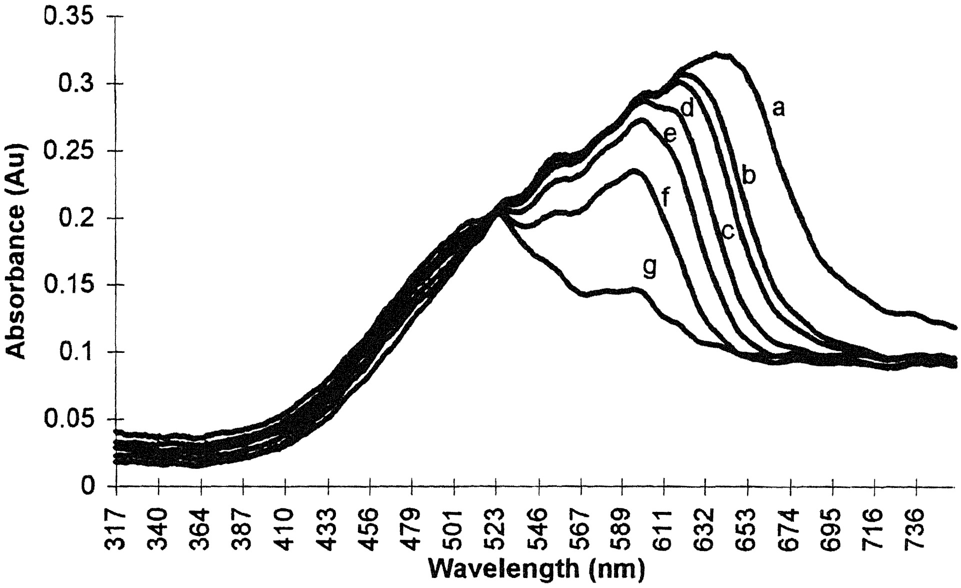


Figure 2: Response of the probe to changes in pH. (a) pH 8, (b) pH 6, (c) pH 5.5, (d) pH 5, (e) pH 4.5, (f) pH 4, (g) pH 3

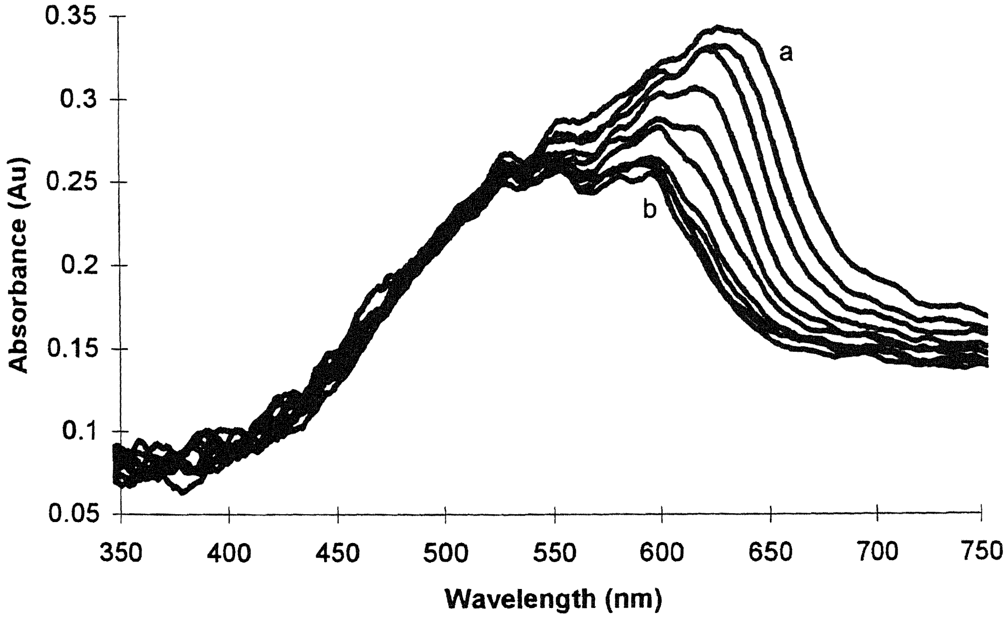


Figure 3: Response time of the probe to changes in pH from pH 8 (curve (a)) to 3 (curve (b)). Spectra were recorded at 0.5 sec interval, the total time being 5 seconds.

References

[1] Boisde G, Harmer A, 1996 *Chemical and biochemical sensors with optic fibers and waveguides* (Boston: Artech house)

[2] Wolfbeis O S 1991 *Fiber-optic chemical sensors and biosensors* **I,II** (London: CRC press)

[3] Hobbs J R 1992 *Laser focus world* 83-86

[4] Ge Z, Brown C W, Sun L, Yang S C 1993 *Anal. Chem.* **65** 2335-2338

[5] Browne C A, Tarrant D H, Olteanu M S, Mullens J W and Chronister E L 1996 *Anal. Chem.* **68** 2289-2295

[6] Barnard S and Walt D 1991 *Environ. Sci. Technol.* **25** 1301-1304

[7] Moslehi B, Shahriari M, Schmidlin E, Anderson M and Lukasiewicz M 1992 *Laser Focus World* 161-168

[8] Arnold M A 1992 *Anal. Chem.* **64** 1015A-1025A

[9] Peterson J I, Goldstein S R, Fitzgerald R V and Buckhold D K 1980 *Anal. Chem.* **52** 6 864-869

[10] Baldini F 1990 *Proc. SPIE* **1368** 184

[11] MacCraith B D, Ruddy V and Potter C 1991 *Electron Letters* **27** 1247

[12] Thompson R B, Lakowicz J R 1993 *Anal. Chem.* **65** 853

[13] Zhang S, Tanaka S, Wickramasinghe Y A B D and Rolfe P 1995 *Med. Biol. Eng. Comput.* **33** 152

[14] MacCraith B D, McDonald C M, O'Keefe G, Keyes E M, O'Kelly B and McGilp J F 1993 *Analyst* **118** 385-388

[15] Ding J Y, Shahriari M R and Siegel G H 1991 *Electron. Lett.* **27** 1560

[16] Gupta B D and Sharma D K 1997 *Optics Communications* **140** 32-35

[17] L. Noui, J. Hill, P. J. Keay, T. Smith , K. Young, G. Habib and M. Hoare *To be published in the J. of Biotechnology*