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Porous Glaxss Substrates with Potential Applications to Fiber Optic Chemical Sensors

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Porous glass (PG), used in fiber optical chemical sensors as a support for the sensitive reagent, was investigated. Microanalysis was employed to assess the effect of certain reaction parameters on the efficiency of each of the two steps involved in immobilizing an appropriate fluorescent dye onto the glass surface. It was found that the low organic content of the derivatized samples limits the overall usefulness of this analytical approach. The effect of various treatments was investigated and the fluorescence response of the derivative porous glass, both dry and in solution, was recorded. The fiber optic configuration used in the investigation resulted in strong fluorescence signals but a less-than-optimal response time. Generally, it was found that the fluorescence intensity of the dry PG was greater when lower concentrations of the fluorescent agent, fluorescent isothiocyanate (FITC), was employed in the immoblization step.

1. Introduction

Various techniques have been used in order to enable chemically sensitive species employed in fiber optic chemical sensors (FOCS) to be accessible to the environment in which the measurement is being taken (typically in a liquid or gas), and yet to remain attached to a probe to enable optical measurement of the induced change. A successful

formula for this has been one of the most difficult aspects of current research in the field, and there have been many attempts to produce the most satisfactory solution over the past ten years or so. The problem rarely lies in the optical nature of the sensitive species—changes easily measured in the liquid phase, such as those of fluorescence or absorption intensity, wavelength shift of notable features, or variations in decay times (especially of fluorescence) are clear indications of a change in the chemical nature of the environment. Glass and polymers have been used as substrate materials for the immobilization of indicators in optical sensors.[1–5] Organic carriers are photochemically and thermally unstable, which are limitations since excited states may react with the surroundings. Polymeric networks, being less rigid than glass, may cause greater problems of this type and reactions with oxygen or humidity in the air, as well as with internal impurities such as indicators, solvents and monomers are possible, especially at high temperatures. Polymers may swell and deform, while in contrast, glass is more stable, has a higher surface area, is rugged and has high chemical stability. However, the ranges of indictors which can be immobilized and of structural changes during the process are limited. These factors have stimulated the search for new and better host materials for immobilized indictors.

In addition to, for example, the sensing of specific gases, which is important for a number of industrial applications, the measurement of pH change is very important in the monitoring of groundwater quality, human physiological stability or the extent of a chemical reaction, to illustrate but a few current applications. For some years there has been a need to apply the most appropriate and simple optical methods. Optical techniques require transparent matrices for the active dye and are sensitive to any micro-heterogeneities that may be present.[1] Whether such methods are absorption or fluorescence based, and especially in the latter case, the use of appropriate substrates onto which the optical active medium can be attached is paramount, and remains a subject of continuing research. The authors reported one such approach using sol-gels in an earlier publication,[1] and discussed the context of such work extensively in that paper.

Following early and highly limited work (in 1974) on extrinsic chemical sensors, where the indicator was separated from the analyte by a semipermeable membrane, for example, in the use of oxygen-sensitive trypaflavin adsorbed on silica gel attached to a fiber optic,[2] the first glass-bound pH indicators were accidentally discovered by workers at Corning Glass. This occurred during the process of activating high-surface area glass in the binding of enzymes. In 1980, a major step forward came with the development of the pH probe for physiological use which was reported by Peterson *et al.*[3] It was based on the use of a dye bound to polyacrylamide microspheres and was able to operate above the pH region of 7.0 to 7.4, utilizing a reflectance-based absorption radiometric principle. The first fluorescence-based sensor of this type demonstrating the principle of the method was reported in 1982[4] and involved fluoresceinamine covalently coupled to cellulose and glass. Whilst the choice of an appropriate indicator is relatively straightforward, with the aim of achieving appropriate sensitivity, photochemical stability, excitation and emission within a convenient band and compatibility with the substrate, the choice of the substrate is more difficult. The essential criteria are that the substrate be optically transparent, rugged, chemically inert in the sampling environment and easily configured, have a high surface area to maximize the amount of indicator that can be attached, yet be able to adsorb the

indicator without adversely affecting its properties and possess an open network of pores to minimize the response time.

It is hardly surprising that, given the above, most approaches so far have represented compromises, some of which are more satisfactory than others. The work of Avnir *et al.*[5] directed effort at what has subsequently been a very worthwhile approach in the use of sol-gels as matrices. However, there are protracted difficulties in ensuring the optimum conditions for the use of such matrices, and porous glass offers a solution that may potentially be easier for the less experienced experimenter to implement. Polymers used as supports may offer a high degree of processability, which will allow the sensing material to be readily attached. [However, they have other limitations such as hydrophobicity, a tendency to swell and, occasionally, an inherent intrinsic fluorescence (*e.g.*, both polystyrene and polythene fluoresce in the blue-wavelength region) and the cellulose is not very rigid.] By contrast, glass has the advantage of being rugged, transparent and resistant to bacterial attack, which is important if long-term storage is necessary. Porous glass (PG) can provide the desired high specific surface area and is available in various forms ready to be reacted with the indicator. However, it may be difficult to attach it to a fiber optic probe and, thus, a compromise whereby polyorganic systems which synergetically combine the properties of polymers and glasslike substrates could be developed may be best.

2. Immobilization Methods

Much of the published knowledge base accumulated on immobilization techniques, vital for effective fiber optic sensors, has arisen from protein-related studies which can be effectively applied to the problems of chemically sensitive dye immobilization. Four major approaches may be considered, as illustrated schematically in Fig. 1.[6] It is important to assess these briefly in order to indicate the most appropriate approach for fiber optic chemical sensor development. Physical methods including gel entrapment, adsorption and electrostatic attraction (Figs. 1(a) to (c)) were used in earlier work (*e.g.*, Kirkbright *et al.*, 1984[7]). Here, bromothymol blue was immobilized on beads of styrene-divinylbenzene copolymer by soaking the beads in the dye solution, but this method suffered from, as may be expected, dye leaching effects. Chemical immobilization (Fig. 1(d)) requires a more complex synthesis process to modify the substrate to provide it with a sufficiently reactive function and thus can provide, in principle, a more stable substrate. Harper,[8] for example, commented that, in many cases, the colors of the bound pH indicators differed from those of the free indicators, suggesting a change in their characteristics. Similarly, in the absorption of a series of indictors on the polymer XAD-2, the dynamic response was shifted to slightly higher pH values. This microenvironmental effect[9] can be a result of charges or physiochemical properties of the supporting matrix, or it may be caused by diffusion limitations. Simple chemical immobilization to the tip of the fiber has resulted in weak signals from such sensors (see *e.g.*, ref. 10) and subsequent advances have, for example, placed the sensor dye in the fiber core (which may be porous [11]) and thus improved the signal-to-noise ratio, but at the expense of a longer response time. The coupling of evanescent fields to a reagent immobilized onto the surface of the fiber can

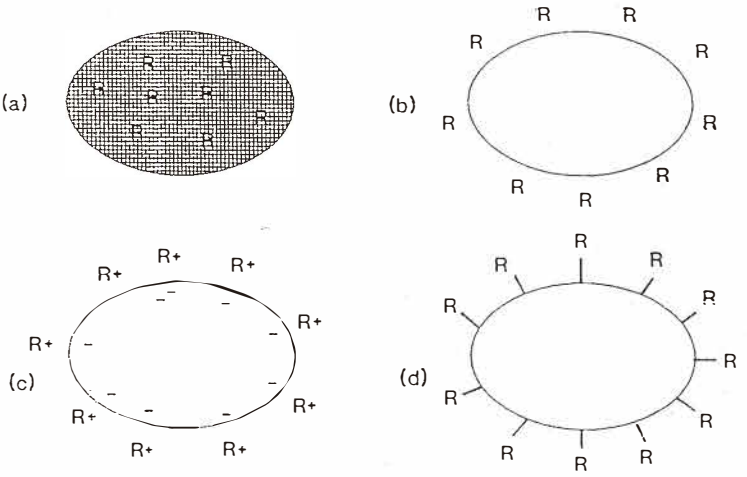


Fig. 1. Schematic of attachment of species, R, the reagent, to a substrate in fiber optic chemical sensors: (a) gel entrapment, (b) absorption, (c) electrostratic attraction, (d) chemical immobilization.

improve the situation. Several variations on this have been reported since the pioneering work of Lieberman and Brown.[12]

Thus, faced with the complexity of the physiochemical situation, in this work, a decision was made to carry out a study of the immobilization of a simple and effective fluorescent pH-sensitive dye, fluorescein isothiocyanate (FITC) onto PG. The synergy of the preservation of the optical and chemical properties of the resultant product is emphasized, and its potential application in fiber optic chemical sensor systems is discussed.

3. Choice of Fluorescent Reagent – Fluorescein Isothiocyanate (FITC)

FITC is widely used in fluorescent immunoassay techniques due to its high quantum yield, good photostability and relatively low temperature coefficient. This isothiocyanate group provides a convenient site for immobilization of the molecule onto suitable substrates. Furthermore, it has convenient absorption and emission spectra, absorbing in the blue-wavelength region and emitting strongly at a band centered at 530 nm.[1] FITC is attractive as a reagent as it can undergo rearrangement, deprotonation and protonation reactions which affect its resonance structure and thus it fluorescence. Its fluorescence response increases with pH as a result of the changes in the molecule, especially at pH around 6.7, which is an important region for a number of applications.

4. Porous Glass and Its Application in FOCS

Essentially, PG consists of pure silica with a microscopic system of interconnecting pores and channels. The preparation is well known, and dates from the 1930's,[13] and requires heat treatment and acid washing to obtain the required material. The pore volume can be accurately controlled through the preparation process, and the surface of the PG can be thought of as being covered by silanol (SiOH) groups, where the number of these groups per unit area governs the amount of material that can be conveniently immobilized onto the glass surface. In analyzing a PG, it is potentially difficult to differentiate the hydroxyl groups due to the absorbed water involved in the preparation from those due to the surface, and in reality the temperature at which physisorbed water is completely removed is not well known over the 100°–350°C range.

Conventionally, bifunctional silanes of the $(YR')_nSiR_{4-n}$ form are used to modify the SiO_2 structure (where Y and R are specific organic groups).[14] In a FOCS, the main function of the silane coupling agent is to provide a bond between the indicator reagent (organic) and the substrate carrier (inorganic). In this work, 3-aminopropyltriethoxysilane (3APTS) was used as the reagent, which shows excellent affinity for glass silanols. The immoblization scheme is shown schematically in Fig. 2[15] and may be summarized in terms of two stages where Stage 1 involves the condensation of three ethoxy groups with the glass hydroxyl groups to form stable siloxane bonds. The glass surface now carries an active amine functionality which, in Stage 2, can undergo further reaction with the isothiocyanate group of the FITC. The coupling of the ethoxy group to the surface silanols, however, is incomplete, and this accounts for some of the observations discussed later.

5. Experimental Procedure

The PG used throughout this investigation was of a specific surface area of 32.4 m^2g^{-1} (Micrometrics Surface Area Analyzer). The particle size distribution (Malvern Instruments) was confirmed to be 160–100 mesh (152–251 μm). All other chemicals used in this work were obtained from recognized international suppliers, and the subsequent thermal analyses were performed using a Mettler Thermal Analyzer 2.

PG will turn brown when left in an ordinary laboratory atmosphere, due to the incorporation of organic materials from the air. Various methods have been suggested in the literature to remove the contamination; however, in the present investigation, the PG was gently stirred in a 1:1 v/v HNO_3:H_2SO_4 mixture for several hours, washed repeatedly with quantities of distilled water until the washings indicated a pH close to neutral on universal indicator paper, filtered, dried in an oven at 100°C and then stored in a vacuum desiccator over silica gel until used, or stored under distilled water until thermally treated.

A number of thermal treatments were investigated to determine if they had any significant effect on the extent of the derivatization of the PG. Table 1 summarizes the methods used where a multistage reaction process was undertaken, as discussed below.

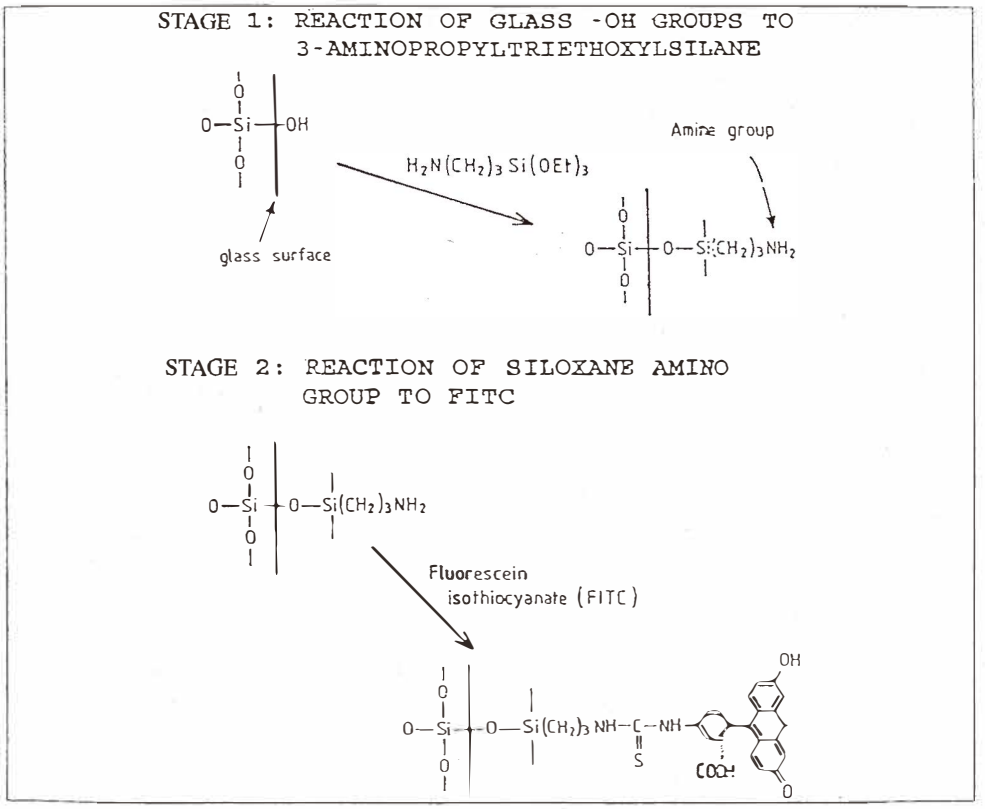


Fig. 2. Schematic of reactions involved in immobilization of FITC on glass.

Stage 1 Reaction: Silylation of PG Surface

Toluene (220 ml) was dried by refluxing over NaPb alloy for several hours followed by distillation. Subsequent to the thermal pretreatment, each batch of PG (2–3 g) was treated by one of the methods shown in Table 2. The PG was then filtered off and washed in sequence with methanol (MeOH), H_2O, MeOH, H_2O, MeOH and ethanol (EtOH). The samples were dried in an oven at 80°C.

Stage 2 Reaction: Immobilization of FITC onto Silylated PG Surface

The aminopropylated PG samples were allowed to stand in solutions of FITC overnight, filtered, washed with copious quantities of distilled water and then dried in an oven at 80–100°C. Four different concentrations of FITC were used, as indicated in Table 3. After solvent treatment, washing and drying, the PG was left to stand in a 2.0 mg $(100\ ml)^{-1}$ FITC solution in dry O_2. Each sample was analyzed for carbon, hydrogen and nitrogen[16]

Table 1
Summary data for thermal pretreatment of the porous glass. (Figures in brackets indicate temperature of thermal treatment in°C.)

Notation	Thermal Pretreatment					Comments
	Air (550)	O_2 (450)	O_2 (550)	N_2 (150)	N_2 (550)	
1						No heating
2				X		
3					X	
4		X				
5			X			
6					X	As for 3
7	X					
8		X				
9	X					As for 7
10	X					As for 7

Table 2
Treatment methods used in Stage 1.

Notation	Method
1	Added immediately to a 1% v/v 3APTS solution in dry toluene (100 ml) and then left to stand for several hours.
2	Added immediately to a 1% v/v 3APTS solution in dry toluene (100 ml) and then refluxed at 80–90°C with gentle stirring for several hours.

Table 3
Concentrations of FITC used in the work.

Notation	[FITC] in mg $(100\ ml)^{-1}$
1	4.0
2	2.0
3	1.0
4	0.5

contents, and the results expressed as percentages of the total sample weight, using the standard procedure with the Mettler Thermal Analyzer.

5.1 *Fluorescence analysis*

The excitation spectrum of a sample of the dry derivatized PG was measured using a simple fiber optic link to the spectrophotometer used. This enabled the optical properties to be remotely analyzed relatively easily, by providing this coupling to the standard instrument, as discussed in earlier work.[17]

The fluorescence response of the sensor to pH was also measured using the same simple coupler. The probe was dipped into an aqueous solution of known pH and the spectrum was recorded once the emission intensity, at a wavelength of 530 nm, reached a steady value. The probe was rinsed thoroughly with distilled water between each measurement.

The response time of the probe configuration to a step change in pH was determined using a simple fiber optic link to the excitation argon ion laser used. Distilled water was acidified by addition of a small amount of HCl, then the FITC probe was immersed in the solution (which was magnetically stirred). After 5 min, pH recordings of the solution and fluorescence intensity were made versus time. Once the output reaching was stable, a small amount of concentrated NaOH was added. The recordings were continued until a total of 5 min had elapsed.

5.2 *Appearance of porous glass samples*

Samples which had undergone the two-step derivatization procedure were yellow to dark orange in color (depending mainly on the concentration of FITC used) and this color was retained even after the samples were immersed in water for a number of days (although some leaching of the dye into solution did occur). This contrasted with samples that had only been immersed in the FITC solution without undergoing the silylation reaction, or that had been immersed in 3APTS solution without any refluxing or thermal pretreatment — these samples adopted a pale coloration which was readily washed out.

Carbon and hydrogen data obtained via the thermal treatment analysis discussed were found to be in good agreement with values predicted both from the results of a simple model and from reported values for the concentrations of reactive groups on the silica surface, which influence the uptake of FITC by the PG. The result of this analysis, the details of which are beyond the scope of this paper, led to the conclusion that the derivatization of the FITC was quite low, based on the recorded carbon values as a weight percentage. By measuring this recorded weight percentage of carbon to nitrogen, and assuming values of the uptake of FITC, agreement between theoretical and experimental values was found to yield approximately one 3APTS group in 100 undergoing reaction with the indicator, FITC. Subsequent analysis showed that samples which were refluxed in 3APTS showed a greater uptake of FITC than those which were simply immersed in the indicator, and that using different concentrations of FITC solution does not lead to a significant difference in the measured quantities of the indicator dye present in the sample. Figure 3, obtained from the results of the thermal analysis, illustrates that there is a correlation between the Stage 2 and Stage 1 carbon and nitrogen contents. The relationships deviate from linearity at the higher Stage 1 concentrations. The use of different concentrations of FITC solution does not lead to a significant difference in the measured quantities, even though the appearance of the sample was different in some cases.

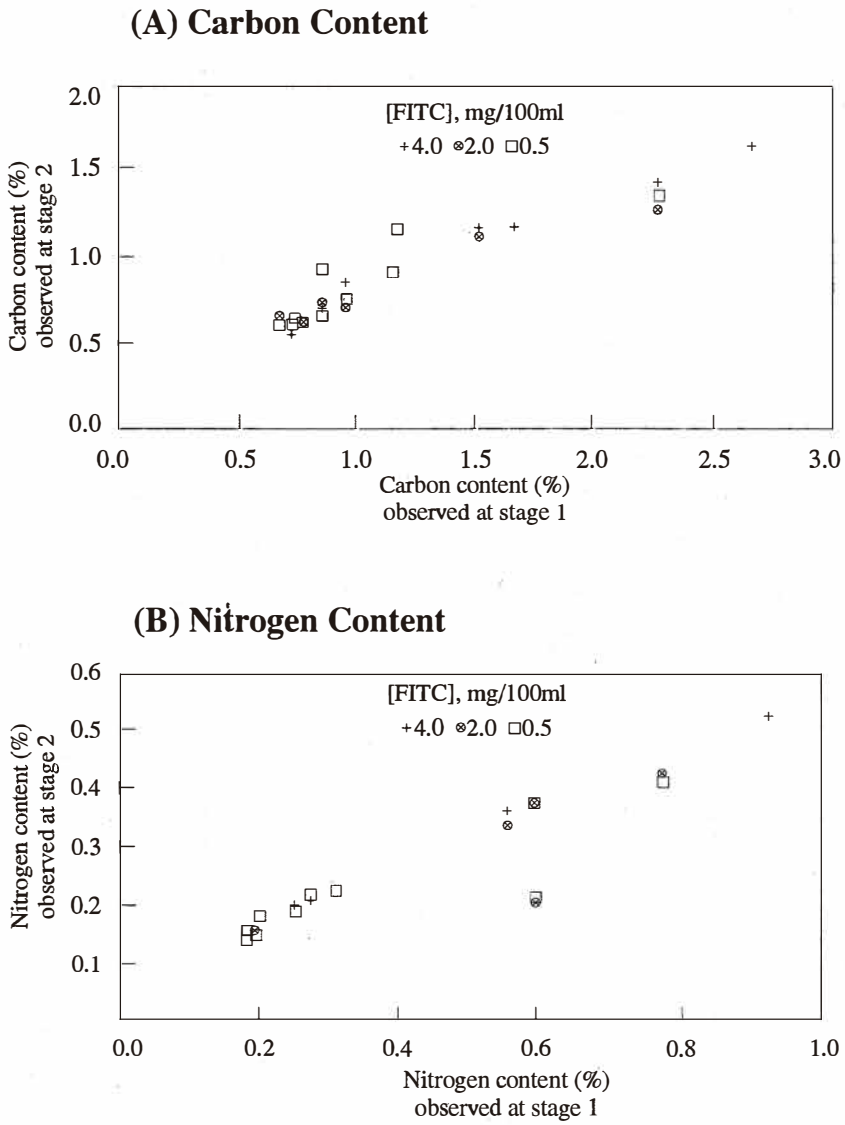


Fig. 3. Effect of concentration of FITC solution on observed carbon and nitrogen contents.

Thus an effective mechanism for the preparation of adequate samples for further analysis, and agreement between a simple theoretical and experimental investigation on the attachment of the FITC dye to the PG, were obtained. This provided the background for the spectral analysis of the PG, which is important in light of its use in FOCS systems.

6. Fluorescence Analysis of Derivatized PG

6.1 *Excitation spectra*

The excitation spectra for the derivatized PG samples and FITC in aqueous solution were measured using a Perkin Elmer (PE) spectrophotometer with a fiber optic link constructed for the purpose[17] of such tests. The sample glass was viewed by means of an optical fiber attached to the spectrophotometer. The intention in this work was not to optimize the fiber optic probe, but to investigate the use of the PG in the probe itself. To that end, the probe arrangement is simply used as a convenient method to carry light from the PG to the detection system (the spectrophotometer) without any attempt to produce a probe design that could be employed more widely or represent a prototype for commercial development. Figure 4 shows comparative excitation spectra, with a peak at wavelength λ ~ 495 nm, of aqueous FITC (4 mg/100 ml water) with FITC-PG, the scales of which were normalized to allow for concentration differences. The cut-off at $\lambda \leq 320$ nm reflects the attenuation of the probe fiber used. A slight shift in the absorption band of the immobilized dye, as compared to the aqueous dye, is to be expected.[18]

6.2 *Emission spectra*

The main purpose of the work carried out at this stage of the study was to investigate certain aspects of specific substrates used to support a fluorescing reagent, with a fiber optic link attached to a Perkin Elmer (PE) fluorescence spectrophotometer, type MPF-4. This enabled the accumulation of reliable and reproducible measurements of optical fluorescence of the systems under investigation, as shown in Fig. 5.

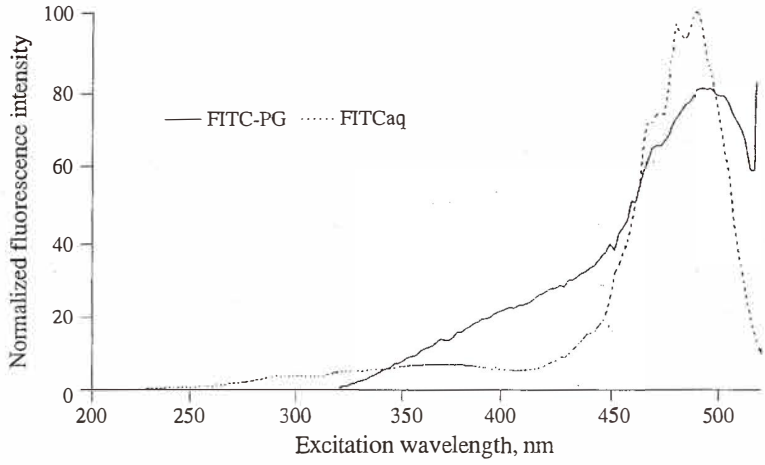


Fig. 4. Comparison of excitation spectra of FITC-PG and FITCaq (FITC attached to porous glass and in aqueous solution, respectively).

Light from the spectrophotometer lamp was focused onto the central fiber of a fiber bundle fabricated for this work from a series of eight 1-m-long fibers of 600 μm diameter and propagated along its length to the distal end. The reflected or fluorescence radiation emerging from the sample under study was gathered by the seven surrounding fibers and carried back to the fluorometer. Although the arrangement used was probably not optimal in terms of the optical alignment, it was effective in that it ensured minimal pick-up of background light and readily measurable light signals from the sample.

The instrument shown in Fig. 6 was designed in order to further assess some features of the fluorophore and FOCS. The argon ion laser is a convenient source of 488 nm radiation used in this work, and its collimated output was easily coupled to an optical fiber. This

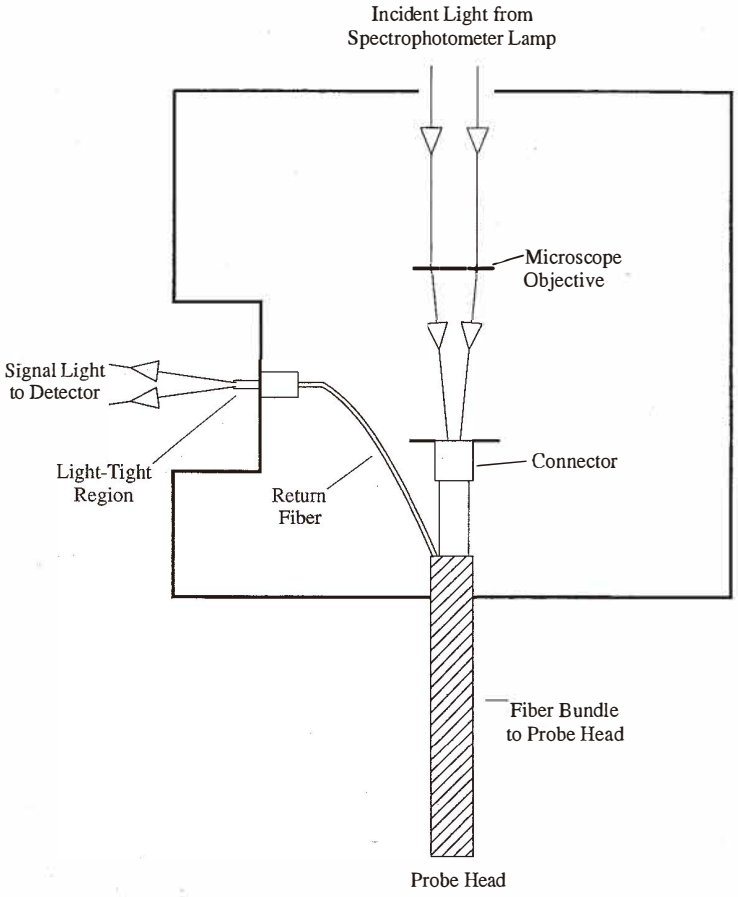
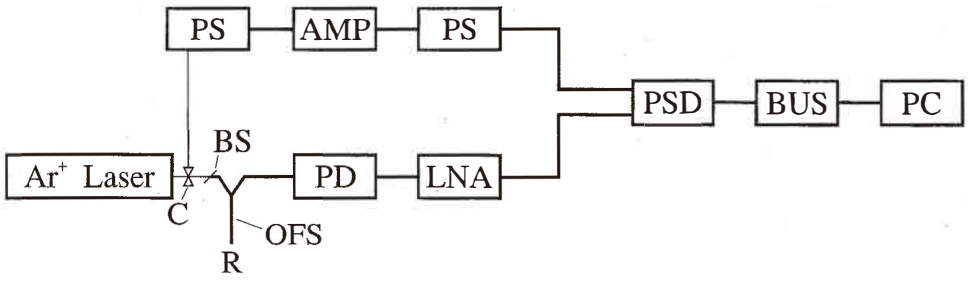


Fig. 5. Fiber optic link for the spectrophotometer used in this work.



C - Chopper to modulate light
BS - Beam splitter
PD - Photodiodes
OFS - Optical fiber sensor with
R - reagent at end of fiber
PS - Phase shifter
LNA - Low noise amplifier
PSD - Phase sensitive detector
BUS - IEEE 488 interface
PC - Portable computer

Fig. 6. Optoelectronic arrangement for the emission spectra obtained.

device is less practical for compact FOCS systems, but is valuable for laboratory investigations. The new generation of frequency-doubled fiber lasers and ultrabright blue LEDs offers the possibility of replacing the argon ion laser with more convenient, powerful compact sources, especially when coupled with sensitive (*e.g.*, APD-based) solid-state detectors.

In the arrangement discussed, two main sources of error are unwanted radiation from objects surrounding the measurement apparatus and noise from the photodetector and its amplifier. In the configuration illustrated in Fig. 6, the light emerging from the argon ion laser is modulated by a chopper and then split to give excitation and reference signals. Since the optical signal is modulated, resulting in an output which is alternating current (a.c.), the direct current (d.c.) component due to background light is eliminated by using a.c.-coupled amplification in the detection circuit. By using a phase-sensitive detector, maximum readings are obtained when the reference and signal voltages are in phase, which reduces the error mentioned above. The detection circuit was optimized by the manufacturer and used as is; details of the components employed can be found in the literature associated with the device.

6.3 *Dry derivatized porous glass emission spectra*

The emission spectra of dry derivatized PG, as shown in Fig. 7, exhibits a slight shift toward the long wavelength region compared to the dye in solution. The overall effect on

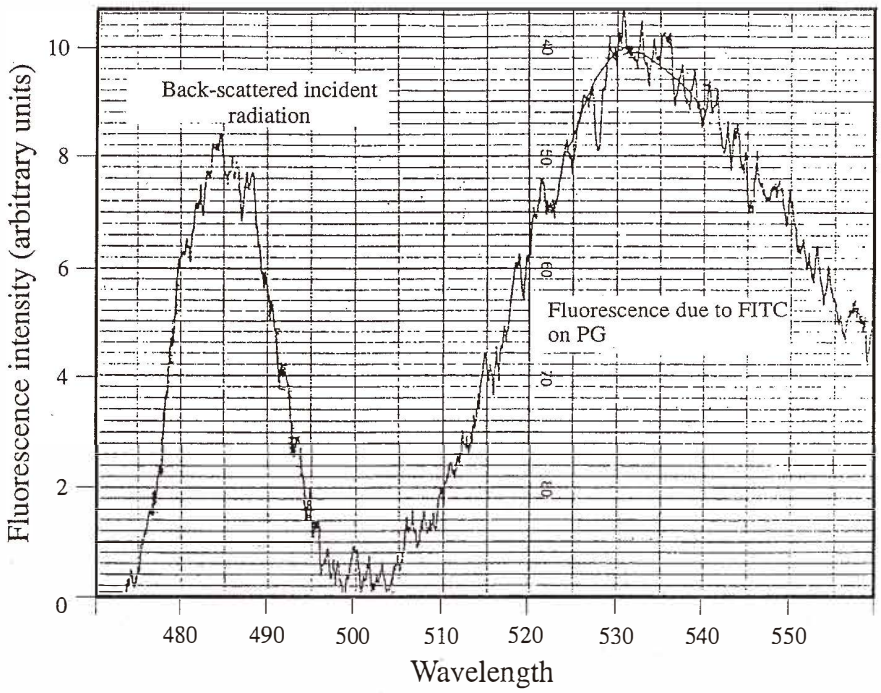


Fig. 7. Emission spectrum for dry derivatized PG-FITC.

the Stokes shift, however, is minimal, and occurs for both the immobilized and solution FITC where the difference in excitation and emission maxima is about 50 nm. A shift of this order can result in the effective separation of short and long wavelengths using a proprietary optical filter, which is important in fiber optical chemical sensing. This means that more sophisticated wavelength separation techniques may not be required, which can effect a saving of cost.

6.4 *Fluorescence response as a function of pH*

The spectra for immobilized FITC in different pH solutions obtained using the apparatus discussed are shown in Fig. 8. The total emission intensities were calculated from the areas under the curves and compared with the measured fluorescence intensity of the FITC in solution. For pH values below 8, as shown in Fig. 9, the total (normalized) emissions are similar for both the immobilized FITC and the dye in solution. The sensitivities in the linear range using the two approaches are similar in terms of percentage change (i.e. the slopes across the points of inflexion are nearly the same). In absolute terms, however, the sensitivity of the FITC in solution is much greater, although the actual values will depend on the concentration of the solution.

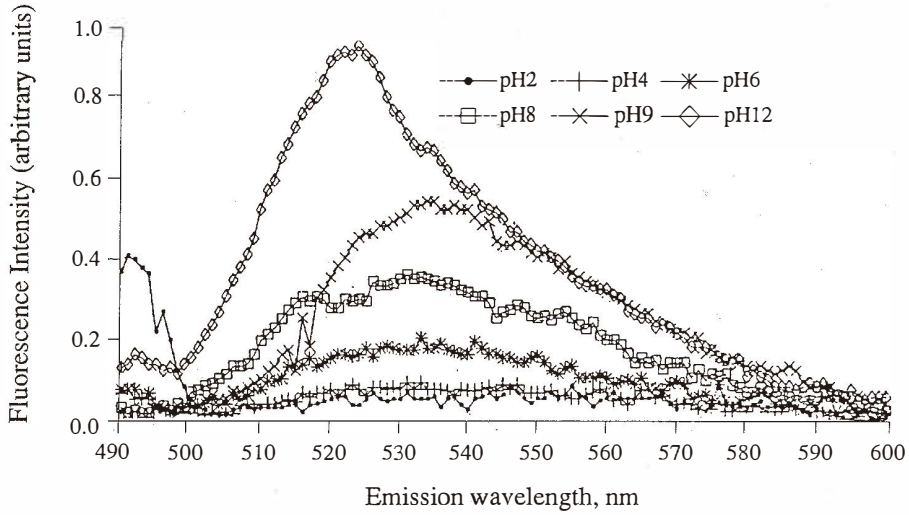


Fig. 8. Emission spectrum of PG-FITC at different pH values.

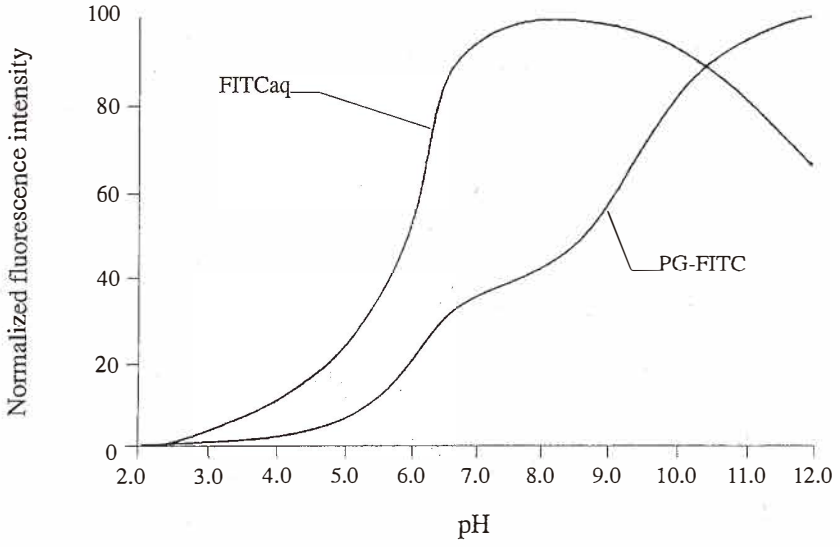


Fig. 9. Fluorescence response of PG-FITC and FITCaq between pH 2 and pH 12.

When the fluorescence response (Fig. 9) of the probe and solution FITC are compared at high pH values, some marked differences are observed. At pH values above 8, the intensity of the probe fluorescence increased in contrast to that of the solution FITC. This observation can be explained by noting that in alkaline environments the siloxane bond undergoes cleavage which results in the immobilized dye going into solution. Dye in solution is known to possess higher quantum efficiencies than immobilized dye.[4] The construction of the optical probe was such that any dye which became unbound was retained in the macroporous membrane and would diffuse slowly into the surrounding environment. Separate leaching studies, in which the absorbance of solutions surrounding known quantities of derivatized PG was measured, confirmed that FITC is much more readily leached at high pH values.

The reproducibility error seen in the use of the fiber optic link was calculated on the basis of the variation of the areas under three separately recorded emission curves measured at pH 7 and found to be within 1%. The optical probe was not moved for the duration of the three measurements. On repeated use, however, the intensity of the response diminishes and the extent of the reduction depends mainly on the intensity of radiation and on the pH of the test solutions. This is probably due to saturation effects, and implies that the intensity of the excitation light should be kept as low as possible with an acceptable signal. This will reduce any errors due, for example, to photobleaching of the dye. Furthermore, there may be an effect due to the time taken to return to a neutral pH of the dye within the matrix. As a result of this variation in the reading with time, such a setup would require calibration prior to use, reflecting the need for regular recalibration of any reusable probe.

6.5 *Fluorescence response of the fiber optic probe to a step change in pH*

Since the solution surrounding the probe was stirred during this measurement, it could be expected that the measured fluorescence intensity was mostly due to the immobilized FITC and not that leached from the PG. An exponential fluorescence response was observed, as shown in Fig. 10, which requires to ~ 1.5 min for a halfway response (pH 7) and 4.4 min for the response to achieve 95% of the maximum value. This is significantly longer than the response of the pH electrode, and represents a potential problem for the use of such glasses when a faster response is needed.

The reproducibility of this approach was not determined quantitatively, but it would be expected to depend mainly on the way the fiber optic probe material was prepared. The response time was not evaluated for other than the most stable of the glasses prepared—those glasses which showed greater degrees of leaching were less suitable overall and rejected before this stage of evaluation. In commercial development, standardization of the probe design and construction would be an essential feature of the fabrication process, but was beyond the scope of this investigation. A long response time for PGs over, for example, sol-gels is one of the demerits of the use of this material, which is advantageous more in terms of stability.

In some applications, for example, some groundwater monitoring uses, a response time of the order found here would be too long to be acceptable. In many instances, there is a need for a probe which will react much more rapidly to changes in the analyte concentra-

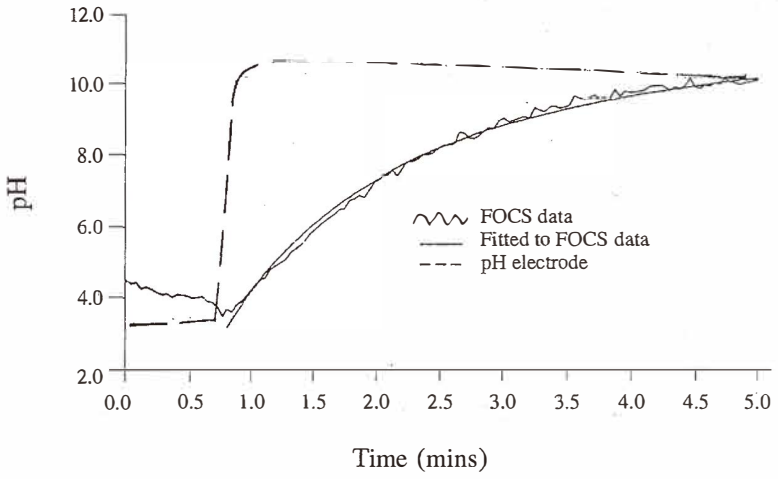


Fig. 10. Fluorescence response of PG-FITC to a step change in pH, over time.

tion. Some workers have managed to achieve this, but their approaches have drawbacks. Fuh *et al.*[19] attached a single sphere of FITC derivatized PG to the end of a single fiber optic fluorescence pH probe. Response times of 20 to 35 s were reported, but apart from the limitations owing to the increased susceptibility to dye loss, the experimental setup also required the presence of a more sensitive detection instrumentation. Given the differences in the preparation and analysis of the PGs used in their work with those reported herein, a comparison is difficult, other than to quote the figures given. In the work reported herein, due to the larger fiber bundle used and the relatively strong degree of doping of the glass achieved, in a stable manner, with FITC, detection problems were not experienced. However, there would appear to be a trade-off between stable response time and leaching due to the nature of the interaction in the doped glass. In another approach (Kawabata *et al.*[20]), the FITC was immobilized directly to the distal end of the single fiber optic probe, which gave an intrinsic response time of less than 1 s. The overall response time of the sensor, however, was determined by the time constant of the lock-in amplifier used. In order to achieve an adequate signal-to-noise ratio, this had to be set at 30 s. It is apparent that there must be a compromise between signal intensity, speed of measurement and response time, and that the optimum balance will depend on the specific construction, and thus the desired application, of the probe.

6.6 *Effect of FITC immobilization on the fluorescence of dry derivatized PG*

Here, from the recording of the fluorescence spectra of the dry derivatized PG materials, the height of the fiber bundle probe from the surface of the sample was adjusted, using a mounting stage, until the maximum signal was achieved at 530 nm. It was noted that for

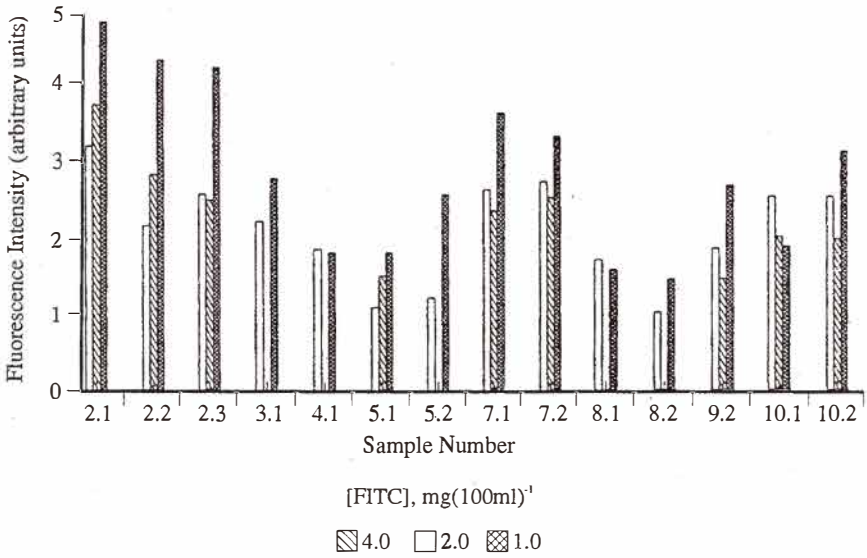


Fig. 11. Fluorescence response of PG-FITC samples for various values of FITC concentration (different shading).

the more strongly colored samples, the probe tip need not contact the material, whereas for samples which were lighter in color, the tip of the probe must be immersed into the bulk of the material to achieve the desired signal levels.

No correlation could be found between the carbon or nitrogen content of the sample and the intensity of the fluorescence response. However, when the fluorescence intensities are plotted against the range of preparation concentrations used, as shown in Fig. 11, it can be seen that, in most cases, the fluorescence intensity increases when lower concentrations of FITC solutions are used.

The PG sample which was neither preheated nor refluxed was not brightly colored like the other samples and did not exhibit any significant fluorescence, showing the inadequacy of this method of preparation.

7. Conclusions

It has been shown that PG is a useful potential substrate for consideration with alternatives such as sol-gels in the development of fiber optic chemical sensors. An analysis of different methods of derivatization has suggested that refluxing has the greatest effect on the loading of the dye onto the PG and that thermal pretreatment does not produce

significant advantages. The effect of the concentration of the FITC solution on tbe final dye loading can be observed by inspection and in the fluorescence responses, but these differences could not be detected by the microanalytical method used here. The preparation of derivatized PG for fiber optic chemical sensing seems best achieved with relatively low loadings of the dye. In practice, this means that it should not be necessary to reflux preheated porous glass to achieve adequate silylation.

In other work by the authors,[1] an important alternative approach is investigated with an emphasis on sol-gel technology, which can be used to prepare pH-sensitive substrates that can overcome some of the short-comings of PG. At the expense of a more complex preparation procedure, however, the sol-gel approach is not without complexity and difficulty in meeting the criteria for a substrate given earlier.

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