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Fiber Optic pH Sensor Based on Phase Fluorescence Lifetimes

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A fiber optic pH sensor based on single fiber phase fluorescence lifetime measurements of commercially available fluorescence indicators is described. The apparatus is a straightforward modification of an existing phase fluorometer and exhibits accuracy and precision of approximately 0.02 pH unit. The approach is applicable to other analytes and indicators, as well as evanescent wave sensing schemes.

INTRODUCTION

Fiber optic chemical sensors represent an emerging technology likely to have important applications in clinical diagnosis, pollution monitoring, oceanography, chemical process monitoring, and other fields.[1-4] Fiber optic sensors offer the capability of continuous determination of chemical analytes in remote, inaccessible, hazardous, or in vivo environments. Many different analytes have been determined or quantitated using fiber optic sensors, including ions, small molecules, and macromolecules. While some chemical sensors based on absorbance and other phenomena have been described,[5,6] most fiber optic sensors are based on photoluminescence effects, especially fluorescence.[7] The sine qua non of fluorescence-based sensors is transducing the presence or concentration of the analyte as a change in fluorescence observable through a length of optical fiber. While performing fluorometry through optical fibers is not trivial,[8,9] much of the effort in the field is directed at achieving desirable levels of sensitivity, selectivity, dynamic range, and accuracy through development of the transducing mechanism.

Among the fluorescence-based sensors, most have relied upon monitoring a change in fluorescence intensity at a single wavelength which is correlated with the presence and amount of the analyte. While the virtues of fluorescence in analytical chemistry are well-known, the drawbacks of intensity measurements are also well-known, including the susceptibility to photobleaching and quenching, variation in probe concentration, inner filter effects, scattering, source level variations, susceptibility to temperature variations, and the presence of interfering fluorescent materials and Raman scatter. Some of these problems are straightforward to address, but others cannot be alleviated for many applications. Recognizing this, many workers have employed fluorescent techniques wherein the presence of the analyte causes a wavelength shift in the excitation or emission, permitting the amount of analyte to be correlated with the *ratio* of fluorescence intensities at two wavelengths. These ratiometric techniques are relatively insensitive to photobleaching or the fluorophore amount, source intensity fluctuations, quenching, and influences such as temperature; they are also easier to calibrate. Among the analytes determined in this way are pH,[10] Ca^{2+},[11] Mg^{2+}, and inhalation anesthetics.[12] Unfortunately, this approach is not immune to inner filter effects or fluorescent interferents, and it is evidently difficult to design fluorescent indicators that have the requisite selectivity for and sensitivity to a particular analyte and also exhibit a suitable shift in fluorescence excitation or emission. It is particularly difficult to envision such indicators for analytes other than atoms or small molecules.

Recently, Lakowicz,[13] Wolfbeis,[14] and others[7] have shown that the presence or level of an analyte can be correlated with a change in fluorescence *lifetime* of a suitable indicator and that this approach has some important advantages over analytical methods based on fluorescence intensity changes. Among these advantages are insensitivity to excitation source fluctuations, scattering or absorption of excitation or emission, variation in fluorophore levels due to washout or photobleaching, facile calibration, and reduced susceptibility to fluorescent interferents and influences such as temperature. Lakowicz and Szmacinski[15] also demonstrated an important advantage of the lifetime approach, namely a broad dynamic range. Of course, an ordinary pH indicator operating under the law of mass action will exhibit 10–90% of its total response (absorbance or fluorescence intensity) over a range of 100-fold in concentration, and the total dynamic range cannot be more than about 3 orders of magnitude or so because greater absorbance or fluorescence measurement precision is difficult to achieve in practice. By judicious selection of excitation and emission wavelengths to favor particular forms of the indicator, Lakowicz and Szmacinski showed that a single indicator (not exhibiting multiple equilibria), such as carboxy-SNARF-6, could measure pH over a range from pH 5.5 to 10. This property is of particular interest when an indicator cannot be synthesized with precisely the desired equilibrium constant. Moreover, it seems likely that the design of indicators exhibiting lifetime changes in the presence of the analyte may be easier than for those exhibiting wavelength shifts. Finally, instrumentation for the measurement of fluorescence lifetimes has become simpler and cheaper, due to advances in laser, electronics, and computer technology.[13,16-19] For all these reasons, fluorescence lifetime-based chemical analysis is likely to grow.

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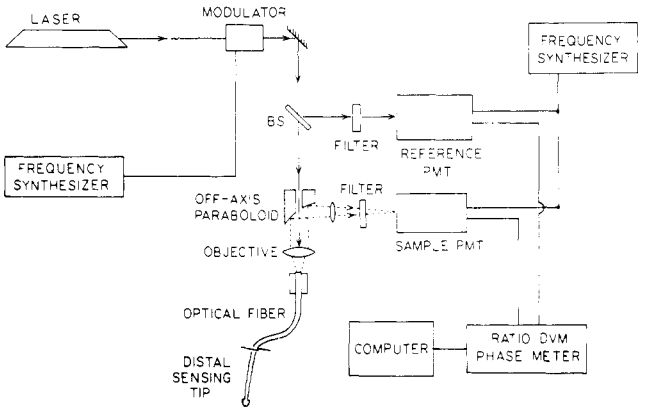

Figure 1. Apparatus for measuring phase fluorescence lifetimes through a single optical fiber. The cuvette holder in a commercial phase fluorometer is replaced by the off-axis paraboloid, objective, and fiber holder as described in the text.

From the point of view of optical fiber sensors, lifetime-based sensing offers additional advantages. First, due to microbending, mode transformation, and passage through slip rings, the apparent fluorescence intensities measured through multimode optical fibers can fluctuate, even if other factors such as source intensity are held constant. These problems are reduced or absent for single mode fibers, but such fibers are difficult to use for routine fluorescence sensing. For remote sensing applications such as in oceanography, ease of calibration of the lifetime approach is a decisive advantage; we note that wavelength ratiometric approaches require distance-dependent calibration in most fibers due to the steep wavelength dependence of fiber transmission with length. Hieftje and Bright[9] and later Bright[24] described fiber optic phase fluorometers which differ somewhat from the apparatus described in Figure 1. In particular, they used much more powerful laser sources in a two-fiber configuration, and measured fluorescence lifetimes of several compounds. The two-fiber configuration, where excitation and emission are conducted through different fibers, has some drawbacks[7] including unsuitability for evanescent wave excitation, the difficulty of constructing the sensing tip, occasional problems with getting good registration between the portion of sample illuminated and the fluorescence collected, and the additional complexity and cost of the second fiber. While they did not use their devices for sensing per se, the data in Bright[24] are comparable to ours. Apart from the drawbacks of the two-fiber approach, the apparatus of Bright should be satisfactory for lifetime-based sensing.

For these reasons we chose to adapt our existing fiber optic sensor design[8,20] to the measurement of fluorescence lifetimes using the frequency domain technique and apply this apparatus to the measurement of pH as an example. We note that this approach is not only feasible with pH but other chemical analytes as well.

EXPERIMENTAL SECTION

Apparatus. The fiber optic phase fluorometer is a modification of our existing design,[8,20] adapted for use with a commercial phase fluorometer (ISS K2, Champaign, IL); the apparatus is depicted schematically in Figure 1. The turret is removed from the sample chamber and replaced with a 6- × 6-in. breadboard, on which are mounted an off-axis parabolic mirror (Janos A8037-105), with a 0.062-in. hole along its mechanical axis, and a precision fiber positioner (Newport-Klinger FP-1015), with its microscope objective replaced with a 25-mm $f/1$ synthetic fused-silica lens (Newport-Klinger SBX-019); both positioner and mirror are conveniently mounted on small three-axis translators. The optical fiber (General Fiber Optics Catalog No. 16-200S 200-μm core plastic clad silica, 2–5 m long) is held in the positioner with a fiber holder, a glass capillary tube, or an SMA 905 connector (Amphenol). In addition, the emission polarizer holder was removed and replaced with a 75-mm focal length fused-silica lens (Newport-Klinger SBX 025) to focus the fluorescence emission on the PMT photocathode. Excitation was provided by a Liconix 4214NB helium cadmium laser (442 nm, 10 mW cw) or an Ion Laser Technology 5000 Series air-cooled argon ion laser (50 mW all lines). For alignment purposes it was convenient to launch a HeNe laser beam back down the fiber into the fluorescence collection optics. Excitation intensity was enhanced by insertion and adjustment of a $^1/_4$ wave plate (Karl Lambrecht, Chicago, IL) in the laser beam prior to its passage through the beam-splitting polarizer.

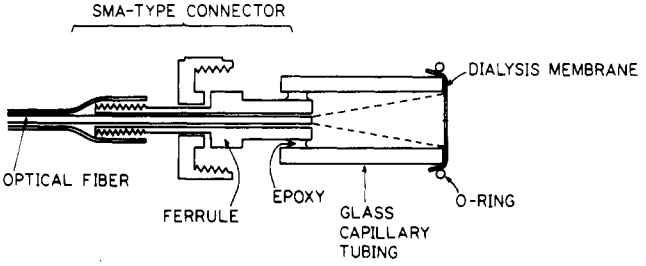

Figure 2. Distal sensing tip schematic. Glass capillary tubing is epoxied to an SMA connector attached to the distal end of the fiber. The dialysis membrane is secured by the O-ring, forming a small chamber that contains the c-SNAFL-2 dextran conjugate.

For some experiments the fluorescent indicators were dissolved in solution at low micromolar concentrations and the distal end of the fiber was dipped therein. For sensing applications the indicator was immobilized at the distal end by covalent attachment to 70 000 molecular weight dextran and confined in a chamber adapted from an SMA 905 connector (Figure 2) or a Zeppezauer tube. Dialysis tubing stretched over the end permits passage of ions while retaining the indicator.

Reagents. Resorufin (sodium salt), carboxy-SNAFL-2, and SNAFL-2 dextran were products of Molecular Probes, and Rose Bengal was from Aldrich; all were used without further purification. Water was distilled and passed through a Milli-Q water purification system; buffer salts were analytical reagent grade and were used without further purification.

RESULTS AND DISCUSSION

We decided to compare the precision and relative accuracy of the fiber optic phase fluorometer depicted in Figure 1 to those routinely achieved with non fiber optic instrumentation in order to determine if the approach was feasible and to form some estimate of the accuracy and precision by which analytes such as pH might be determined by this method. We took the results in ref 15 as being perfectly accurate with regard to what phase angle, demodulation ratio, or complete frequency response of a given indicator corresponds to a particular pH. Our goal is to assess any *additional* error introduced by the use of an optical fiber in the measurements. Our assessment of accuracy is therefore based on the closeness of our results to those in ref 15 and on the χ_R^2 for fits to complete frequency responses. The χ_R^2 is an indicator of accuracy because in this case, where we have a good understanding of the form of the data (the model) and an independent measure of the precision of the data from the standard deviations of the measurements at each frequency,

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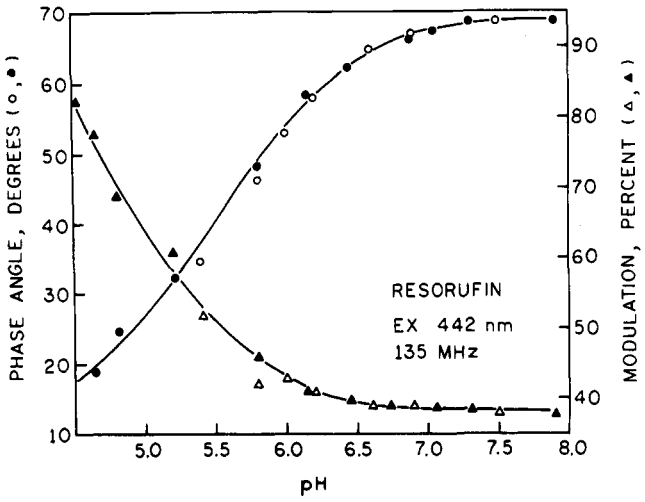

Figure 3. pH-dependent phase shifts (circles) and demodulation factors (triangles) measured for resorufin in a cuvette (filled symbols) and through an optical fiber (open symbols). The cuvette data are from ref 15.

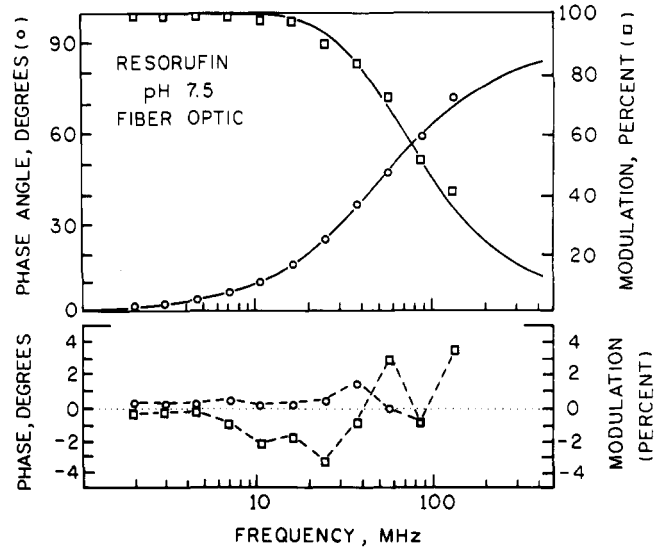

Figure 4. Frequency-dependent phase shifts (circles) and demodulation ratios (squares) measured through 2 m of optical fiber for resorufin in 0.1 M potassium phosphate buffer pH 7.5 versus Rose Bengal as a standard.

the χ_R^2 cannot be low for an inaccurate result. Thus we measured the phase shift and demodulation of resorufin in 0.1 M citrate–phosphate buffer solutions at various pH's using resorufin at pH 7.5 as a standard and compared them with the results of Lakowicz and Szmaczinski,[15] as depicted in Figure 3. The conditions of excitation (442 nm), modulation frequency (135 MHz), and emission filtration (Corning 3-76) match those of Lakowicz and Szmaczinski, except that an additional 2-mm path length fused-silica liquid filter containing 1% potassium dichromate in water was inserted in the detection optical train between the 3-76 filter and the off-axis paraboloid to prevent photoluminescence being excited in the 3-76 by stray laser light;[8] we do not anticipate that this affected the results much, as the liquid filter does not absorb appreciably at the wavelengths of resorufin emission. For the samples at lower pH the detector gain and/or the amount of the resorufin indicator in the sample were increased to obtain adequate intensity; a virtue of this method is that these factors do not affect the results much,[13] as can also be seen in Figure 3.

It is evident from Figure 3 that the apparent phase angles and demodulation factors are very similar to those measured previously by Lakowicz and Szmacinski,[15] and thus it is quite feasible to measure pH through an optical fiber by this method. The low fluorescence intensity and quantum yield of resorufin at low pH prevented acquisition of data in this case at pH's below 5.4. We note that the modulated laser power launched in the fiber was less than 0.5 mW and that more powerful excitation should increase the fluorescence intensity and permit measurements at lower pH using resorufin. Note that using much higher laser power or high peak powers with a small fiber core can result in damage to the core or nonlinear effects. Apparently, the data obtained through the optical fiber are not much less accurate than those observed in a cuvette, and their precision as indicated by the standard deviations of the individual measurements is less good, but comparable. The average standard deviations of the phase and modulation data in Figure 3 measured through an optical fiber (three repetitions each of sample and reference) are 0.351° and 0.007, respectively, which are about 2-fold larger than the values typically seen in this laboratory, when samples are measured in cuvettes, and used in calculating χ_R^2. Much of this difference can be attributed to the low fluorescence intensity due to modest excitation power and to the inefficiency of optical fibers for collecting emission.

As an independent measure of the accuracy of the method, and its utility at frequencies other than 135 MHz, we measured the frequency-dependent phase shifts and demodulation ratios of resorufin in 0.1 M sodium, potassium phosphate pH 7.5 buffer using Rose Bengal as a reference[21] and used these data to determine its fluorescence lifetime in the usual manner. The best fit value for the results shown in Figure 4 was a single component of 3.36 ± 0.11 ns, in good agreement with the results of Lakowicz and Szmacinski[15] (2.96 ns), which were obtained in 80 mM Tris–HCl buffer at pH 7.3. The accuracy of the individual phase and modulation measurements can be adduced from the resulting value of χ_R^2 when the average standard deviations of the phase and modulation measurements (0.351° and 0.7%, respectively) are employed in the calculation. The resulting χ_R^2 is 9.9, indicating that the measurements are reasonably accurate.

Our results indicate that rather accurate and precise pH measurements can be made with this apparatus. In the case of resorufin, in the pH range roughly from 5 to 6, where the phase angle varies approximately linearly with pH, a change of 1.0 pH units corresponds to a phase of 27° (Figure 3) or 0.037 pH unit/deg. Thus if we ordinarily obtain accuracy and precision in our phase measurements of 0.35°, we can expect pH measurements accurate to 0.015 pH unit. For some applications, such as blood pH monitoring[22] or microbial cell growth monitoring, this accuracy is adequate, but for other applications, such as monitoring oceanic CO_2, it is not.[23] Resorufin represents a favorable case in that it exhibits a very large lifetime (and thus phase) difference between the protonated and unprotonated forms, whereas other probes are not so good; of course, the phase difference cannot be greater than 90°. However, the precision of these measurements might be very much improved in a phase fluorometer optimized for operation at a single frequency, rather than the more flexible, broad-band commercial phase fluorometer used in these experiments. Note also that given the ability to vary the wavelengths of excitation and emission for a particular probe, the optimum response can be achieved.[15]

Comparable results were obtained for the pH indicator carboxy-SNAFL-2 in solution using an argon ion laser for excitation, using a different excitation wavelength (514 nm) than those of Lakowicz and Szmacinski (543 and 563 nm). In particular, Figure 5 depicts phase angles and demodulations measured through the optical fiber using the apparatus depicted in Figure 1 and in a cuvette. The slight systematic

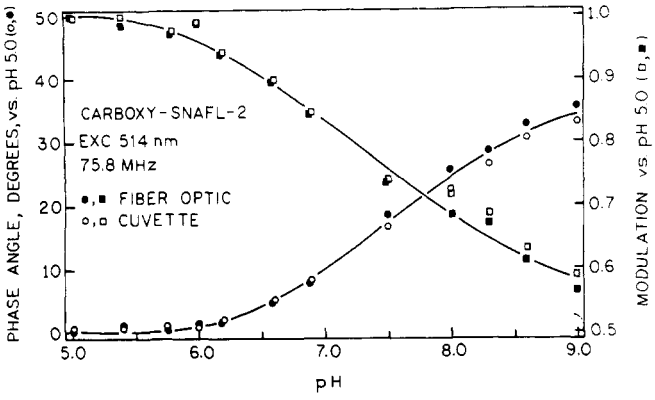

Figure 5. pH-dependent phase shifts (circles) and demodulation ratios (squares) measured for carboxy-SNAFL-2 in a cuvette (open symbols) and for carboxy-SNAFL-2 conjugated to dextran through an optical fiber (filled symbols).

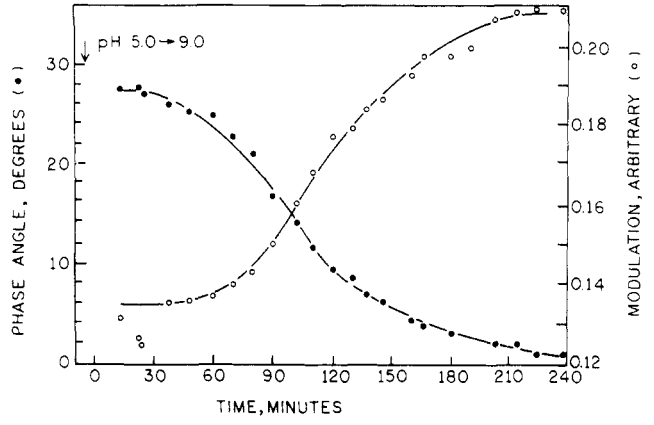

Figure 6. Time response of fiber optic sensor. The sensing tip depicted in Figure 2 is switched from a pH 5.0 buffer solution to a pH 9.0 buffer solution at time $T = 0$, and the phase angle (filled circles) and modulation (open circles) was monitored at intervals.

error apparent at high pH suggests that some slight aging of the samples may have occurred over a 1-month period, but otherwise they appear to be quite robust. In many cases it is impractical to add a fluorescent indicator such as resorufin or carboxy-SNAFL-2 to a sample whose pH is to be determined. In these cases it is preferable to immobilize an indicator at the distal end of the fiber. In this instance we chose to encapsulate carboxy-SNAFL-2 as a conjugate to 70 000 molecular weight dextran in the distal cuvette chamber depicted in Figure 2. Dialysis tubing secured over the end served to prevent leakage of the SNAFL–dextran, while permitting the passage of protons. In addition to being convenient, the isolation of the indicator from some fluorescent interferents which may be present in solution is also an advantage.[2] The rapidity of response of pH probes is of importance in many applications, and therefore the kinetics of the phase shift of carboxy-SNAFL-2 immobilized in the distal cuvette-type probe in Figure 2 were measured as the pH was abruptly varied. The results of such an experiment are depicted in Figure 6. Under these conditions of rapid mixing, the estimated time for mixing is 10–15 s, which is somewhat more than the instrumental time constant of the ISS phase fluorometer. The drift in phase angle was about 1 deg in a period of 2 h. In the experiment depicted in Figure 6, a time constant of over 2 h is observed. This is overtly slow for nearly all applications, and clearly the slow response can be attributed to slow diffusion through the dialysis tubing and mixing within the distal cuvette chamber. Since some miniature electrochemical pH probes exhibit time constants of less than 30 s, it is evident that the fiber optic sensor time constant could also be improved by miniaturization of the sensing tip and substitution of a different membrane or by covalently attaching the fluorescent indicator to the distal end of the fiber.

In these experiments, the results are unaffected by demodulation of the excitation due to modal or material dispersion due to the short length (2–5 m) of fiber employed.[7] The plastic clad silica fiber employed here has a bandwidth of approximately 15–20 MHz km and an estimated material dispersion of several hundred picoseconds per kilometer per nanometer. For more remote experiments, it might be necessary to employ gradient index multimode fibers with bandwidths due to modal dispersion of 400 MHz km, which is a higher frequency response than the detector used in these experiments, the Hamamatsu R928 PMT. For multikilometer sensing, it might be necessary to employ single mode fibers with single frequency near-IR lasers, but fluorescent indicators useful with such excitation sources are not yet available.

CONCLUSIONS

It is difficult to predict the ultimate performance achievable with the fiber optic lifetime-based sensor, but there seems to be no overt barrier to performance comparable to typical pH electrodes. Obviously, the fiber approach is unsuited to routine applications which require low cost, but for other applications it is likely to be useful or even indispensible. Among the important issues remaining are improved accuracy and precision, together with faster response time. A really useful development would be the synthesis of fluorescent lifetime indicators excitable by diode lasers. Phase fluorometry using diode lasers as a source is straightforward,[17,25] and this approach has many advantages from the standpoint of cost, durability, detection limits, low interference, and simplicity.[16,26] In addition, light of red and near-IR wavelengths is much less attenuated in optical fibers than shorter wavelengths, which is important for remote sensing applications.

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