Sol-Gel Based Fiber-Optic Sensor for Blood pH Measurement

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Abstract

This paper describes a fiber-optic pH sensor based upon sol-gel encapsulation of a self-referencing dye, seminaphthorhodamine-1 carboxylate (SNARF-1C). The simple sol-gel fabrication procedure and low coating leachability are ideal for encapsulation and immobilization of dye molecules onto the end of an optical fiber. A miniature bench-top fluorimeter system was developed for use with the optical fiber to obtain pH measurements. Linear and reproducible responses were obtained in human blood in the pH range 6.8 to 8.0, which encompasses the clinically-relevant range. Therefore, this sensor can be considered for *in vivo* use.

Introduction

Continuous monitoring of blood pH is an important concern for critically ill patients; therefore, numerous efforts have been directed toward the development of both electrochemical and fiber-optic pH sensors for this purpose [1-7]. In some medical applications, fiber-optic pH sensors may offer certain advantages over other types of sensors. In particular, they are perceived as being safer for *in vivo* use since no electrical currents are passed. Electromagnetic interference is not a concern for fiber-optic sensors. Since optical fibers avoid crosstalk, a small, compact, multisensing fiber is possible. Remote sensing can be achieved since the optical signal can be carried over lengthy distances. Fiber optic sensors are compatible with magnetic resonance imaging (MRI). Finally, depending on their design, fiber-optic pH sensors can be made at very low cost without compromising accuracy.

Most fiber-optic pH sensor designs are based on the immobilization of pH-sensitive dye molecules onto the tip or sides of an optical fiber. The encapsulation method must not invalidate the recognition chemistry (i.e., pH dependence) leading to spectroscopic changes for the dye. Immobilized dye molecules must not leach out of the network, which may cause an adverse reaction if used for *in vivo* sensing. Most researchers have either used covalent chemical linking or simple physical encapsulation techniques to immobilize dye molecules. Surface modification of the optical fibers is required for covalent attachment of dye molecules. For example, HPTS (8-hydroxy-1,3,6-pyrinetrisulfonic acid tetrasodium salt) and fluorescein have been covalently linked to thin films of cellulose or cellophane which are then applied to sensing tips in schemes for pH sensing [8, 9]. Fluorescence-based pH sensors have been developed which use a dye covalently bonded to a cross-linked hydrogel which is then dip coated or micropipeted onto a fiber [10]. Although covalent attachment results in excellent immobilization of the pH-sensitive dye, these methods are often more difficult to implement and may lead to loss of dye sensitivity or result in poor fluorescence properties [11].

Noncovalent immobilization techniques for pH sensors have involved methods of entrapment and adsorption. For example, sensing schemes using pH-sensitive dyes adsorbed on polymeric

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supports and entrapment behind semipermeable membranes have been discussed [12]. Dialysis membranes, which permit the passage of ions while retaining the pH indicator dye, have been used for this purpose [13, 14]. As a drawback, coatings based upon noncovalent methods of dye immobilization suffer from leachability, making long-term *in vivo* use impractical.

Recently, sol-gel methods for reactant/indicator immobilization have been developed [15-18]. The basic inorganic sol-gel technique consists of hydrolysis of metal alkoxides in an alcoholic solution followed by condensation to form oxides. The sol-gel method produces glasses with higher purity and homogeneity, is a simple fabrication process, and can be done at low temperature. Also, the ability to tailor sol-gel derived glasses to obtain optimized cell/pore sizes (<50 nm) and very high surface areas (400–1000 m^2/g) also make them attractive materials for sensor applications.

Sol-gel immobilization involving entrapment of indicators within monolithic gels has been used to fabricate bulk pH indicators [19]. Rottman and coworkers [20] prepared a series of pH-sensitive dyes immobilized in sol-gel glasses and determined that leachability is dependent on the trapped dye and the pH of the solution in which the sol-gel monolith is placed. They observed various rates of leaching ranging from essentially undetectable to very rapid leaching. Because sol-gel monoliths provide a large optical path, sensitivity was reported to be high in this study, but response time was slow.

Planar pH sensors can also be produced by coating thin layers of sol-gel immobilized indicators onto appropriate surfaces [19]. Wolfbeis and coworkers entrapped 5–(and 6–) carboxynaphthofluorescein within a thin layer sol-gel matrix on glass plates and examined the spectroscopic response to changes in pH [8]. It was suggested that physical entrapment of the dye within a sol-gel matrix would be a suitable coating for evanescent wave-type fiber-optic sensors. Similar schemes have been used by other groups to develop evanescent wave sensors for other analytes, for instance, an oxygen sensor based upon immobilization of ruthenium complexes in sol-gel coatings [21].

Ding and coworkers fabricated sensors by dipcoating porous optical fibers into a sol-gel solution containing a pH-sensitive dye [22]. This absorption-type sensor used bromocresol purple and bromocresol green as indicators within the sol-gel matrix. Dipcoating the sol-gel solution onto the surface of a porous fiber generated a higher surface area sensor coating, resulting in increased sensitivity. However, the dynamic pH range was 3.8–5.4 for the bromocresol purple and 5.2–6.8 for the bromocresol green, which are not appropriate for blood pH sensing.

MacCraith and coworkers also developed a pH sensor based on fluorescence of evanescent-wave excited fluorescein dyes encapsulated within sol-gel coatings on optical fiber tips [23]. They determined a response time of approximately 5 s with no leaching of fluorescein after repeated washing. However, photobleaching of fluorescein occurred at higher incident laser power.

To develop a sol-gel based fiber-optic pH sensor for *in vivo* measurements, the constituents and pH of the sol-gel solution must be optimized to achieve the desired porosity, and a pH indicator dye suitable for blood pH sensing must be used. Fiber-optic pH sensors using the ratiometric measurement method (i.e., ratio emission intensity at two different wavelengths) are impervious to stray light, source fluctuations, and bending losses, and generally yield improved signal-to-noise ratios [10]. A convenient pH indicator that can be used in ratiometric sensing is seminaphthorhodamine-1 carboxylate (SNARF-1C). It absorbs with a maximum at 533 nm (green LED), and its emission spectrum is pH sensitive, thereby imparting two spectrally distinguishable forms, e.g., an acid form with an emission peak at approximately 580 nm, and a base form with an emission peak at approximately 640 nm. The pH can be determined by evaluating the peak emission intensity ratio, R, of the acid and base forms, or the ratio of the peak emission intensity for either the acid or base form and the intensity at the isobestic point.

This paper reports on the development of a sol-gel based fiber-optic pH sensor for *in vivo* measurements. The fiber-optic sensor encapsulates SNARF-1C within a sol-gel matrix. The sensor has a linear and reproducible pH response in the physiologically relevant range for human blood pH measurement. The sol-gel method offers the advantage of tailor-made porosities with low leachability for the indicator molecule. Parameters for sol-gel encapsulation of the pH

sensitive dye were determined by spectroscopically measuring the fluorescence properties of bulk sol-gel matrices. Then, the procedure for the fabrication of a fiber-optic pH sensor was developed and the sensor was tested in human blood. The results indicate that the sensor is suitable for blood pH measurement.

Experimental Procedures

Reagents. All reagents were reagent grade unless otherwise indicated. Tetramethyl orthosilicate (TMOS) was used as received from Aldrich (Milwaukee, WI). Phosphate buffered saline (PBS) was obtained from Sigma (St. Louis, MO). Seminaphthorhodamine-1 carboxylate (SNARF-1C) was obtained from Molecular Probes (Eugene, OR). The dye was mixed with 50:50 v/v of ethanol (EtOH) and water or mixed with PBS to make solutions which were 0.441 M. All sol-gel solutions were prepared using deionized water: stock solution A was prepared by mixing 30 ml of TMOS with 20 ml of distilled water. Stock solution B was prepared by mixing 10 ml distilled water with 10 ml methanol and 30 μl of ammonium hydroxide. Proportional amounts of each stock solution were added to a beaker and then stirred for 5 minutes. SNARF-1C solution was added while stirring to obtain the desired dye concentration. The approximate pH of the sol-gel solution was 6.0. The SNARF-1C doped sol solutions were then placed in cuvettes. Gelation occurred in approximately 20 minutes. In addition to the sol-gels, a series of EtOH-H_2O solutions were prepared containing various concentrations of "free" SNARF-1C (pH 6) as well as solutions of varying pH containing a constant concentration of SNARF-1C.

To prepare the sol-gel solutions used in dipcoating the optical fibers, 0.5 ml TMOS, 1.0 ml deionized water and 10 μl of 0.04 M HCl were sonicated in an ice bath for approximately 30 minutes. To 0.5 ml of this solution was added 1.0 ml PBS**.** To increase porosity, additional PBS was added to the sol solution which increased the pH to about 7. SNARF-1C was added to this solution to achieve various concentrations.

Probe Construction. Silica optical fibers were obtained from General Fiber Optics (Fairfield, NJ). The 125 µm o.d. fibers were multimode, step index fibers with a numerical aperature of 0.41. The fiber tips were polished using a series of graded alumina slurries ending with a 0.05 µm particle size slurry. Approximately 1 cm of the cladding was stripped and the ends were tapered by etching with concentrated HF down to a 50 µm tip diameter. Tapering increases the capture of fluorescence back into the fiber core for more efficient evanescent wave sensing [24]. After tapering, the tips were rinsed, dried, and then dip coated into the sol-gel solution and immediately placed in an oven at 75^OC for approximately 1 min. This procedure formed smooth homogeneous coatings approximately 1 µm or less in thickness on the fiber tips.

Apparatus. A Perkin–Elmer luminescence spectrometer was used to perform the fluorescence measurements for the bulk SNARF-doped sol-gel samples. The excitation wavelength was maintained at 533 nm for all measurements. For the optical fiber sensors, a miniature bench top fluorimeter system was designed and is shown in Fig. 1. A ratiometric-based measurement system was utilized. Excitation was provided by a 50 W tungsten halogen lamp. This light passed through an excitation filter and a set of biconvex lenses, and was then modulated by a chopper wheel at 1 kHz. The light was reflected by a dichroic lens and coupled to the optical fiber whose SNARF-1C coated tip was immersed in solution. The emitted fluorescence traveled back through the fiber and was transmitted through the dichroic lens**.** It was directed to a second dichroic lens which split the fluorescence into two bands corresponding to the two spectrally distinguishable forms, i.e., the acid and the base forms. The two bands were then filtered and directed into photomultiplier tubes (Model No. H5784-01, Hamamatsu, Japan). The PMTs and chopper

control were connected to lock-in amplifiers which were interfaced to a computer where the ratio was calculated and displayed.

Procedure. PBS and human whole blood were used as the test media. The pH of the solution was changed by adding small amounts of 0.1 M HCl or 0.1 M NH4OH (1.0 to 5.0 µl increments). The pH was measured using a conventional glass pH electrode. In all cases, approximately 2 ml of the test medium was placed in a vial and stirred with a magnetic spinning bar and maintained at a temperature of 37^oC using a hotplate stirrer. The fiber-optic pH sensors were placed in these solutions while the pH was varied.

Results and Discussion

Bulk samples. The emission spectra for SNARF-1C in ethanol-water solution at varying pH was first determined. The pH ranged from 6.0 to 8.0. The acid tautomer emission peak is in the 585 to 590 nm range, while the base tautomer emission peak occurs in the 635 to 640 nm range. The isobestic point is observed at approximately 610 nm. The ratio between the base and acid peak emission intensities or between the base peak and isobestic point emission intensities, determined as a function of pH, can then be used to establish calibration plots for pH measurement.

It is desirable that the encapsulation method not alter the fundamental acid-base equilibrium of the encapsulated dye such that pH sensitivity is lost. Alternatively, if an alteration of properties does occur as a result of encapsulation, it is important that this happens in a predicatable fashion so that the sensor can still function as a reliable pH sensor. Fig. 2 compares the spectra for free SNARF-1C in EtOH-H_2O solution with sol-gel encapsulated SNARF-1C obtained within 10 minutes after initiation of gelation. The two spectra have similar characteristics; however, the sol-gel form has diminished emission intensity, likely due to light scattering from the clusters froming in the cuvette, with the acid peak shifted slightly to shorter wavelength and the base peak shifted slightly to longer wavelength.

Upon mixing the SNARF-1C concentration was 1.0 mM for both the free and sol-gel solutions. However, as gelation occurs and the gel dries, the intensity increases. After 1 week of aging under ambient conditions, the encapsulated dye demonstrates an almost 1000% increase in emission intensity. The reason for this is not entirely understood; however, in large part this intensity increase is due to an effective increase in concentration of the dye. This occurs because water and alcohol evaporate from the gel as it dries, which, due to capillary forces, can cause the structure to collapse to less than 10% of its original volume [25].

Once the dye is encapsulated, it must not leach out into its surroundings. If this occurs, adverse inflammatory reactions can occur if used as an *in vivo* type sensor. After drying for 2 days under ambient conditions, aged samples were placed in either distilled water or PBS solutions (pH = 7.4) for 30 days to test for leaching of SNARF-1C into solution. After 30 days, the solutions were poured into cuvettes and their fluorescence was determined. Fig. 3 shows a comparison of the spectra for a PBS solution used to leach SNARF-1C along with that for the bulk aged sample. Relative to Fig. 2 it is noted that the base emission in Fig. 3 is more pronounced since the sample had been immersed in the more basic PBS solution. Very little fluorescence is observed from the PBS solution. A rough comparison, based upon peak intensities from the base form, reveals that only approximately 7 % of the dye has leached out of the bulk material after 1 month in an aqueous environment. This small amount of leachant could be accounted for in large part by the fraction of dye molecules which were more loosely bound to the surface rather than completely embedded in the gel.

Fiber-optic sensor measurements. Fig. 4 shows the pH response (base to acid from emission intensity ratio) of the sensor in 2 ml PBS. The pH was changed by additions of 5 μl increments of 0.1 M HCl or NH_4OH. The τ_{90} response time is less than 15 s. As expected, as the pH is increased, a step-wise increase in the base to acid intensity ratio occurs. This effect is

mirrored by a corresponding intensity ratio decrease as the pH is decreased. Over the timescale for the measurements shown, a small amount of drift is observed at some pH values.

Fig. 5 (acid to base intensity ratio) shows the sensor response in 2 ml of whole blood upon pH change. The response is linear in the pH range 6.7 to 8.2, again with a response time less than 15 s. The equation that describes the data shown in Fig. 5 is given as $y = -0.1035x(\pm 0.0022) + 3.814 (\pm 0.0167)$, which confirms linearity. Regression analysis performed on the data shown in Fig. 5 yield a standard error of 0.00295.

Conclusions

It has been shown that sol-gel encapsulation provides a convenient method for fabricating fiber- optic pH sensors using the self-referencing dye SNARF-1C. While there are not immediate significant changes in the emission intensity of acid and base forms of SNARF-1C upon sol-gel encapsulation, as the gel dries the fluorescence intensity increases approximately 1000%. This increase can in part be attributed to the collapse of the gel structure due to capillary forces, leading to an effective increase in concentration of the dye. Once encapsulated, leaching of SNARF-1C out of the sol-gel matrix is very low, which is advantageous for any type of sensor but especially for those proposed for use *in vivo* . The fiber-optic sensor was tested in phosphate buffered saline and in whole blood. Linear pH response with low drift was obtained in both cases. In particular, in blood, a linear response in the pH range from 6.7 to 8.2 was obtained with response times less than 15 s. Improvements in the coating process and the optoelectronics is expected to eliminate any drift problems.

Acknowledgments

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Figure Captions

Figure 1. Miniature bench-top fluorimeter setup.

Figure 2. Spectra for free SNARF-1C in ethanol-water solution (——) and the bulk
encapsulated forms (------). Intensity for these are shown on the left axis.
Spectrum for a sample aged one week (— - —) is also shown (right axis).

Figure 3. Comparison of the emission spectrum from a PBS solution used to
leach SNARF -1C (----) to that for a bulk aged sample (—).

Figure 4. Fiber optic sensor response in PBS solution at varying pH.

Figure 5. Fiber optic sensor response in whole human blood. Increasing pH (■) and
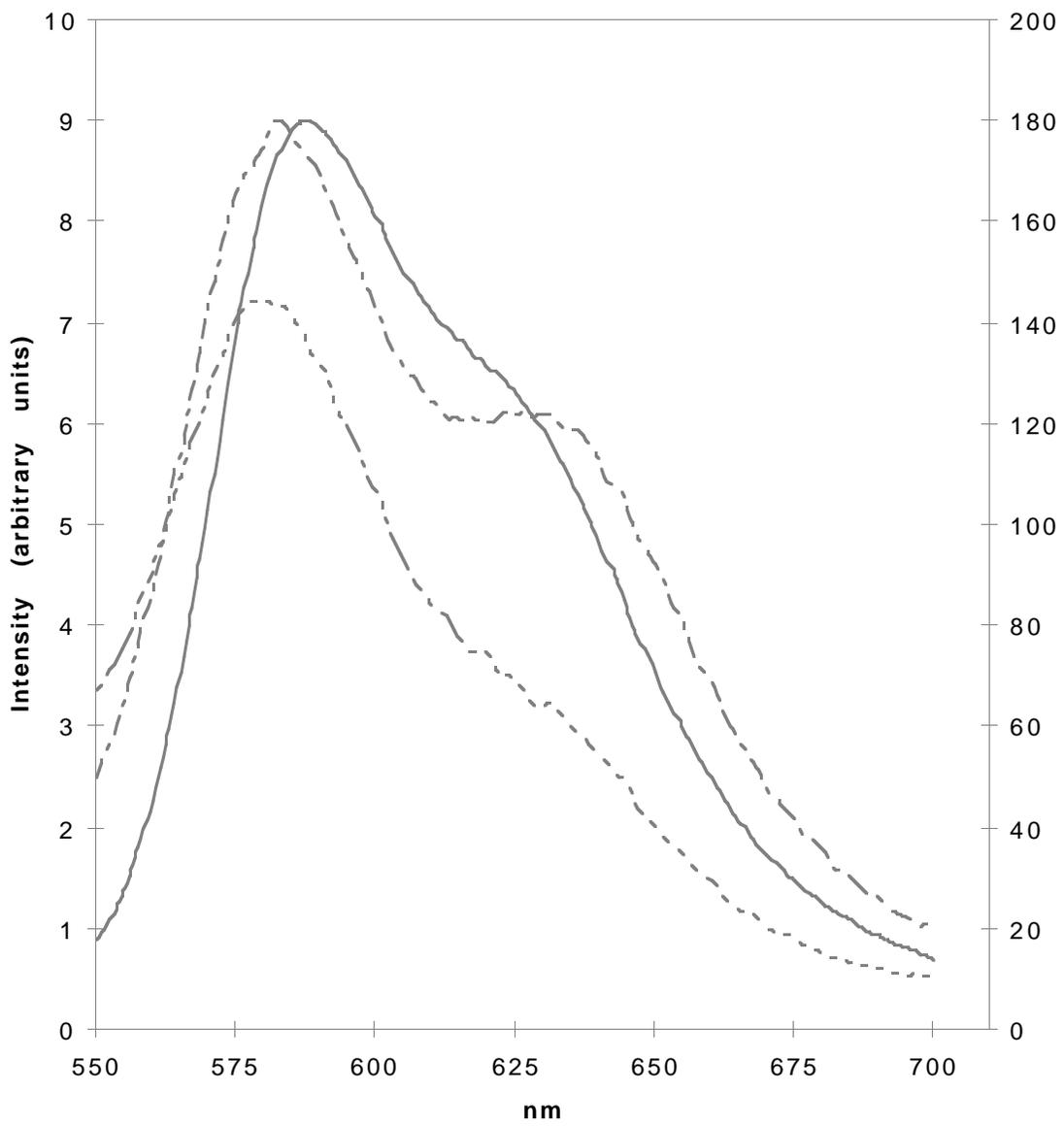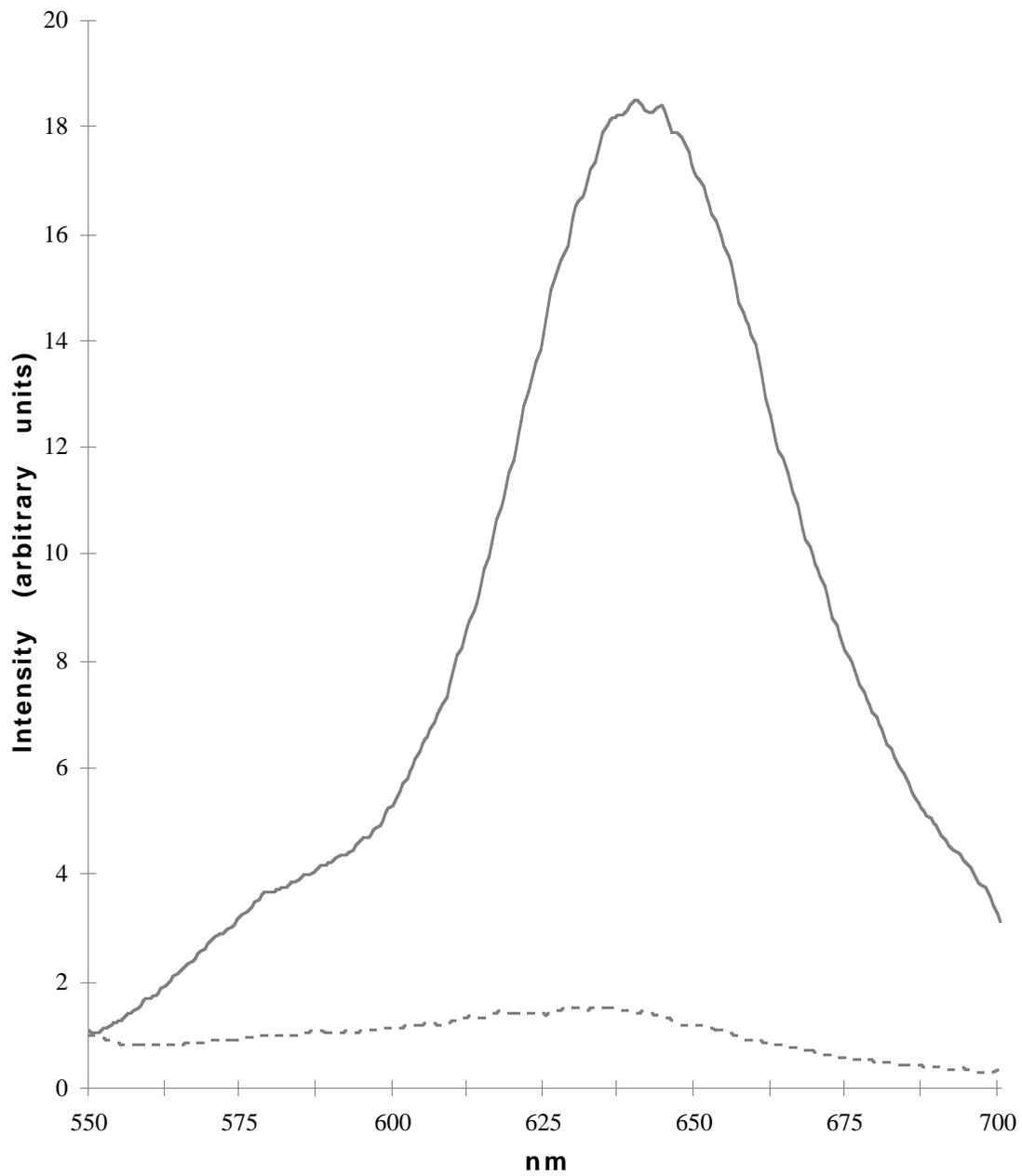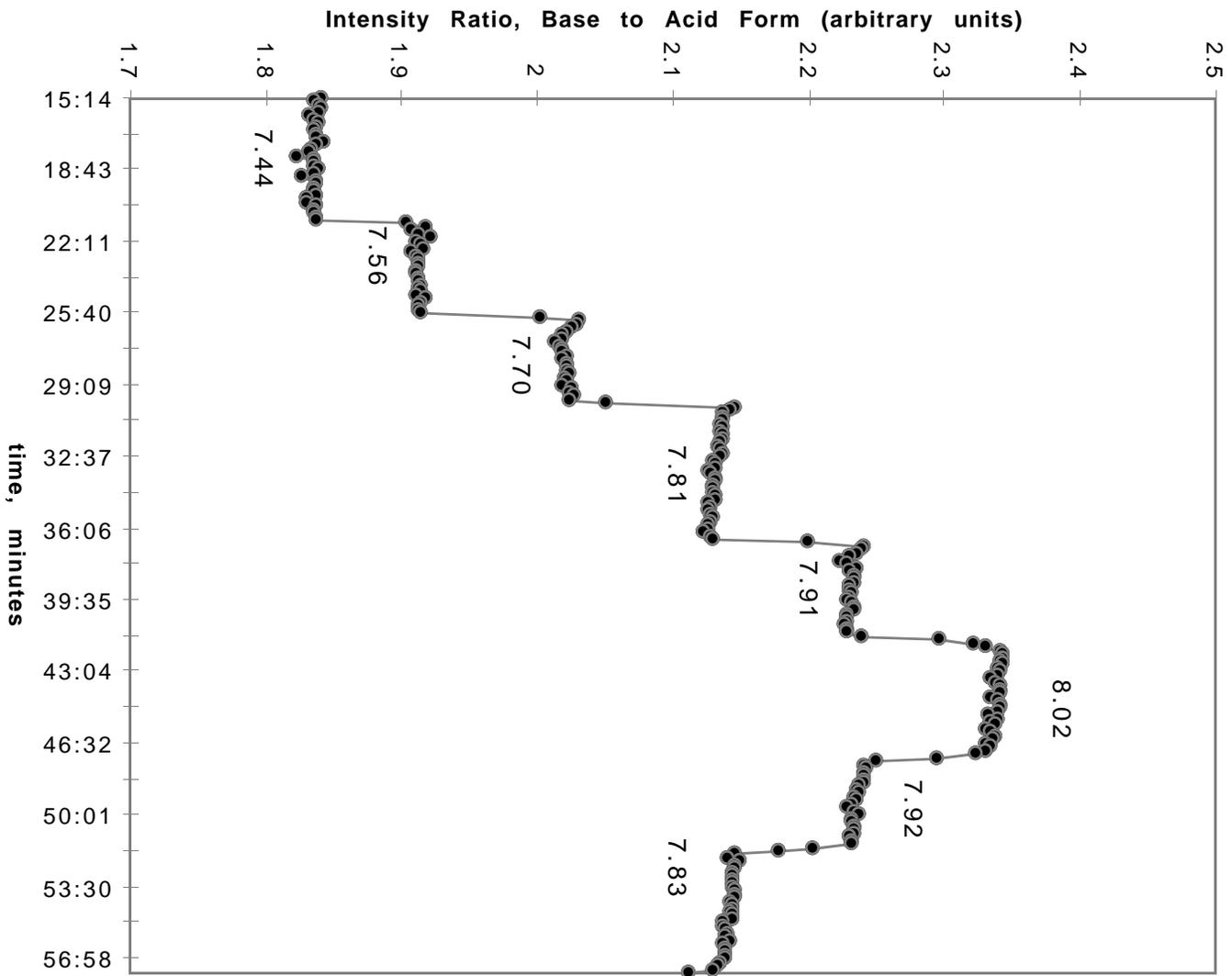decreasing pH (O) are shown.
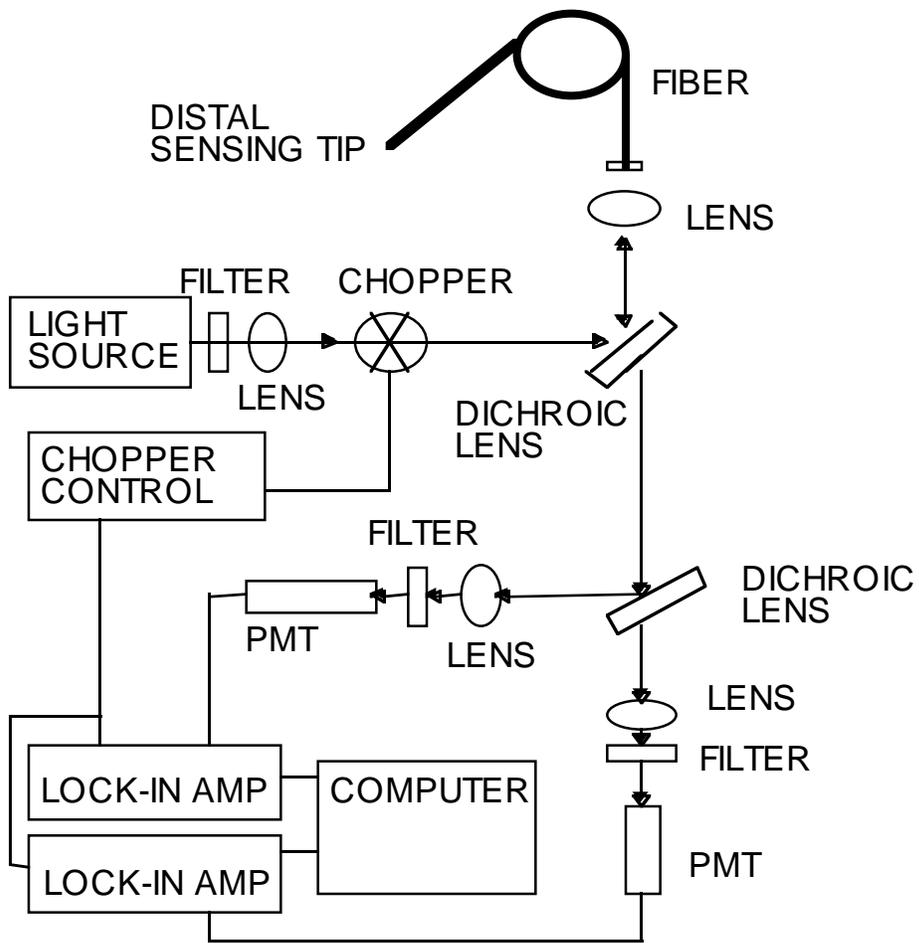
Figure 1

Figure 2

Figure 3

Figure 4

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Figure 5