Characterization of a Fast Response Fiber-Optic pH Sensor and Measurements in a Biological Application

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ABSTRACT

Optical, and especially fiber-optic techniques for the sensing of pH have become very attractive and considerable research progress in this field has been made over a number of years. The determination of the pH level across a broad range of applications today, e.g. in life sciences, environmental monitoring, industry and widely in biologically research is now accessible from such optical sensors. This arises because familiar sensors are often limited in terms of their response time and drift, which reduces the use of the current group of such fiber-optic sensors in wider applications. A new compact sensor design has been developed in this work, based on a specially-formed fiber-optic tip that was coated with a pH-sensitive dye, covalently linked to a hydrogel matrix to provide high stability. The sensor developed has a very fast response time (to 90% of saturation, Δt_{90}) of < 5 seconds, a sensing uncertainty of about ± 0.04 pH units and given the covalently bonded nature of the dye, leeching is reduced and the probe is very stable over many days of use. During extended continuous use over ~12h in pH 7, this stability was confirmed, with drift of < 0.05 pH/h. Preliminary experiments in an important biological application, monitoring over pH levels from pH 5 to pH 8.5, are shown and discussed.

Keywords: pH measurement, fast response time, fiber-optic, optical fiber sensors, fluorescence, luminescence, biomedical measurements

1. INTRODUCTION

The determination of the pH level is very important across a broad range of applications today: in life sciences, environmental monitoring, biomedical research and widely in industry globally. For example, there is particular interest in detecting pH values using optical techniques for areas as diverse as biological studies of tissues[1] and cells[2, 3], process control in bioreactors[4–8], seawater analysis[9, 10] or even corrosion monitoring[11]. Classical pH glass electrodes have been well known and used for the measurement of pH values over many decades. However, these types of sensors have the disadvantages of often being bulky and their glass surface makes them fragile and subject to breakage, unless they are handled carefully – not necessarily the case in many industrial situations. Further, the signal obtained can be subject to interference from stray electromagnetic fields. Optical, and especially fiber-optic sensors have become very attractive for these sorts of measurements in recent times since these sensors are usually reversible and easy to miniaturize (< 50µm). Due to the use of small diameter fiber, they are inexpensive and can be employed where electromagnetic interference would mean that conventional sensors would often fail. Therefore, there is increasing demand for fiber-optic pH sensors of this type, for a wide range research fields as well as commercial and industrial uses[12].

In general, an optical sensor system requires an indicator dye that changes its optical properties, when interacting with solutions of different pH values. Examples of widely used dyes are SNARF, SNAFL, HPTS[13–15] and several novel NIR indicators[16–18]. Typically, the indicator dye is held or immobilized in a supporting matrix (e.g. polymers or hydrogels[19]) that need to be permeable to hydrogen ions. There are three widely used methods for the immobilization of a dye in a solid matrix: adsorption, physically entrapment and covalent binding[14]. Compared to the other techniques, covalent binding is usually complex and time-consuming and needs an appropriate material suitable for the immobilization. However, where dyes are used as pH-sensitive materials, covalent binding is often preferred, as it can eliminate leaching effects[14, 12] which can prevent the use of the sensor in many situations e.g. biological or clinical applications. In a typical fiber-optic sensor design, the pH sensitive material is then applied to the fiber tip using a suitable coating procedure amongst which the most popular are dip coating, spin coating or photo-polymerization. Depending on the sensing materials chosen, the signal change measured could be as a result of different types of interactions, such as absorbance/reflectance or luminescence (intensity or lifetime), which are the most widely used optical techniques to detect pH values[14–16].

The sensitivity and response time to pH as well as the detection range and long-term stability of a sensing system depends primarily on both the sensing element (dye and supporting matrix) and the optical platform (in this case, the fiber tip) to which the coating is attached[14, 20]. One approach which can offer a significant improvement in the sensor performance is to optimize the design of the fiber tip, with the potential to reduce the response time as a result. Further, the physical parameters and characteristics of the optical fiber chosen need to be taken fully into account, since these significantly influence the light guidance in the optical fiber chosen[21, 22].

Currently available luminescence-based pH sensors typically have reported response times (to 90% of saturation, Δt_{90}) from ~20 seconds to an extreme of ~50 minutes[1, 3, 9, 11, 23–26]. As in many applications such shorter response times are important, in this work the focus has been on the *reduction of this parameter* while still maintaining the sensitivity and stability of the device. To achieve this, the fiber-optic pH sensor thus developed and reported here has been based on a specially-formed and treated tip with an optimized light emission to allow for the use of thinner coatings, and thus making this ideal to generate luminescence-based sensors (here for pH monitoring) with faster response times than has been reported. The tip has been coated with an indicator dye, selected to be suitable for the physiologically important pH range of pH 5 to pH 8.5, and for stability and to minimize leaching covalently bound in a hydrogel. The sensing method used detects pH changes through measuring the pH-induced changes in the luminescence intensity, using a newly developed instrument that also reduces the effect of photobleaching and stray light, which are problems in many conventional sensors and which limit the performance[15, 16].

The sensing system developed in this way has been shown to be very stable over a long period of operational time, to exhibit a short response time and importantly for biomedical applications in particular, demonstrate no dye leaching. Finally, the system comprising the sensor probe and the instrumentation for analysis has been briefly tested in a biological application to monitor the pH level of an AMES' medium, which is important to maintain the metabolism of retinal tissues. However, the new optical platform created for the sensor has the potential to be used in the development of faster fiber-optic sensors, with sensitivities to other important analytes, such as oxygen[27], carbon dioxide or ammonia.

2. PRINCIPLE OF pH MEASUREMENT BASED ON FLUORESCENCE INTENSITY

The pH scale was introduced by Søren P. L. Sørensen in 1909 where pH firstly was described as the negative logarithm of hydrogen ions concentration[28] and thus the principle of pH measurement is well known. Nowadays pH is defined in terms of hydrogen ion activity a_{H^+}:

$$\mathrm{pH} = -\log a_{H^+} \tag{1}$$

Classical electrochemical sensors directly measure the activity of hydrogen ions in aqueous solutions and optical pH sensors the concentrations of the protonated and deprotonated form of the indicator dye. By contrast, the fiber-optic sensor scheme designed and reported in this paper is based on a fluorometric determination of pH, where this effect results in a change of the luminescence intensity observed. The well-known Henderson-Hasselbalch equation is commonly used to determine the value of pH from the changes of the deprotonated [A^-] and protonated [HA] form, using an optical signal mehtod[14]:

$$\mathrm{pH} = \mathrm{pK_a} - \log\frac{[\mathrm{HA}]}{[\mathrm{A^-}]} \tag{2}$$

where pK_a is the acid-base constant of the indicator dye. [A^-] and [HA] are then related to fluorescence intensities observed from the sensor through [A^-] = I_m - I_{min} and [HA] = I_{max} - I_m. The signal I_m is the measured luminescence intensity from the indicator in the sensor system. Defining I_{max} as the maximum luminescence intensity signal of the deprotonated form and I_{min} as the minimum luminescence intensity signal of the protonated form, the value of pH in solution which causes that particular change can be calculated by substituting these expressions into Eq. (2):

$$\mathrm{pH} = \mathrm{pK_a} - \mathrm{b} \cdot \log\left(\frac{\mathrm{I_{max}} - \mathrm{I_m}}{\mathrm{I_m} - \mathrm{I_{min}}}\right) \tag{3}$$

where b is the numerical coefficient to determine the slope of the function. Rewriting Eq. (3) in terms of I_m results in the well-known sigmoidal function centered on the pKa value [29]:

$$I_m = I_{min} + \frac{I_{max} - I_{min}}{1+10^{-\left(\frac{pH - pKa}{b}\right)}} \quad (4)$$

3. pH SENSOR DESIGN AND EXPERIMENTAL SETUP

Figure 1 shows an illustration of the fiber-optic sensor design developed in this research. A commercially available fiber is combined with the new tip design, to which the pH sensitive material selected was attached. The fiber itself is protected in a retractable housing. The housing and the small-sized tip (Ø < 50 µm) used specifically in this work makes it ideal for biomedical and physiological applications, given that often only small sample volumes are available for the measurement.

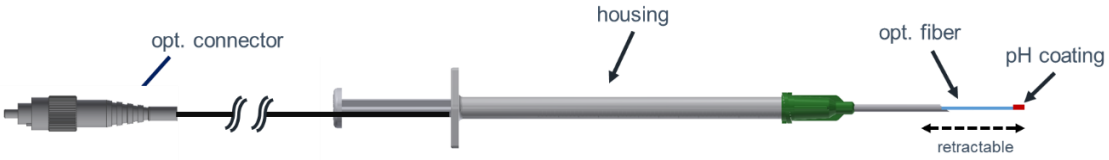

Figure 1. Illustration of the fiber-optic sensor design with the new sensor tip and the pH sensitive layer attached (red). The optical fiber is protected in a retractable housing.

3.1 Fabrication of the fiber-optic pH sensor probe

For the fiber-optic pH sensor probe design a commercially available 400/430 µm (core/cladding) optical fiber was selected. The tip was specially formed during a thermal process with the goal being to decrease the fiber diameter to < 50 µm. In a next step, the prepared tip was chemically treated to remove any remaining parts of the fiber cladding and thus to optimize the light emission and increases the effective area of the fiber over which the coating could be applied. Preparing the coating mixture, the fluorescent monomer fluorescein O-methacrylate was chosen (being appropriate for a detection range from pH 5.0 to pH 8.5) that exhibits a strong luminescence signal and is polymerizable. The use of such polymerizable fluorescent monomers is preferred and allow a covalent binding to overcome dye leaching problems[30]. Further, it has fluorescein that acts as an indicator with the least negative charges compared to other indicator dyes such as HPTS and thus has a lower dependence on the ionic strength[31]. Details of spectral properties of the material can be found in Section 4.1.

The fluorescent monomer itself, an additional monomer to create the hydrogel, a photo-initiator and a cross-linker to adjust the mechanical resistance in aqueous solutions were dissolved and later used to form a thin pH-sensitive layer on the shaped fiber tip in a photo-polymerization process. The mixture prepared, while in essence comparable to that shown in the literature[32] was modified to improve the coating process developed here where photo-polymerization, a simple process which allows for easy and inexpensive fabrication, was used.

3.2 Experimental setup for pH measurement including calibration procedure

Figure 2 shows a schematic of the experimental setup used for evaluating the pH sensors developed. This newly designed instrument was designed specifically to monitor the very weak luminescence emission signals from the pH-sensitive coating attached to the fiber tip and thus to give an accurate determination of the pH value. This new instrument is important in that it minimizes the effect of stray light and can work well with very low excitation intensities, critical to reduce any photo-bleaching of the dye, which is the active element of the sensor. In order to characterize the pH sensors produced in this way, each fiber-optic sensor was connected to the optical port and the output monitored when the probe was dipped into 5 different pH buffers (pH 5, pH 6, pH 7, pH 8 and pH 8.5). Each measurement was performed under constant environmental conditions (stable temperature and pressure) and the pH values of the buffer solutions used were checked, both before and after each measurement (using a reference pH electrode).

In the course of the experiment carried out to evaluate the system, first the relative intensity (monitored in arbitrary units (a.u.)) was measured in each buffer solution and the system calibrated using the Henderson-Hasselbalch equation

(Eq. 3). To analyze the response time, Δt_{90}, the pH sensors were first dipped into the pH 7 buffer solution and then quickly (< 1s) transferred to the pH 8 buffer solution. Further, a measurement of any drift in the sensor response when the probe was maintained in the pH 7 buffer solution was performed, over a 12h period, with a continuous irradiation of the sensor tip to determine if any evidence of photo-bleaching could be seen (given these extreme irradiation conditions for 12 h).

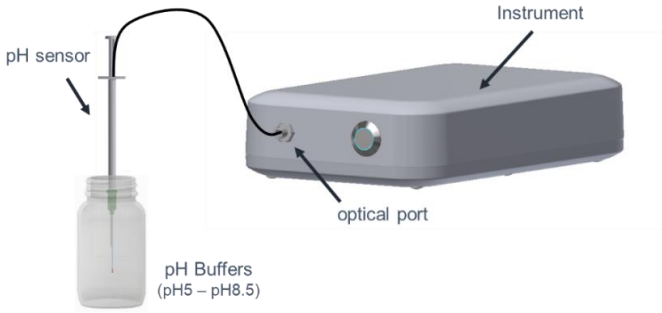

Figure 2. Illustration of the experimental setup for calibrating the pH sensors, used with the newly developed detection instrument, allowing luminescence intensity measurements to undertake the calibration. Five different pH buffer solutions (from pH 5 to pH 8.5) in glass bottles were used for sensor system characterization.

4. EXPERIMENTAL RESULTS AND DISCUSSION

This section shows the characterization of the fiber optic pH sensor probe developed in this work, with respect to key parameters which include spectral properties, calibration, accuracy, long-term stability and response time. Finally, preliminary results of a measurement in a biological application (AMES' medium) are shown, which compare favorably with the performance of a commercially available system.

4.1 Optical and spectral properties

Since a fluorescein-based monomer is used as pH sensitive indicator, the sensor has maximum absorbance at 490 nm and a peak emission wavelength at 520 nm[33]. Figure 3 shows the emission spectrum of the sensor tip when excited with light from a 470 nm LED (saturated region). For an efficient separation of excitation and emission light, a 470 nm LED with an optical filter was used to excite the sensor tip and the emission light was detected through a bandpass filter. Further, this optical set-up, with the electronics specifically designed for it, reduces the effect of stray light to a minimum and thus is suitable for use in the bright environments where most measurements are needed.

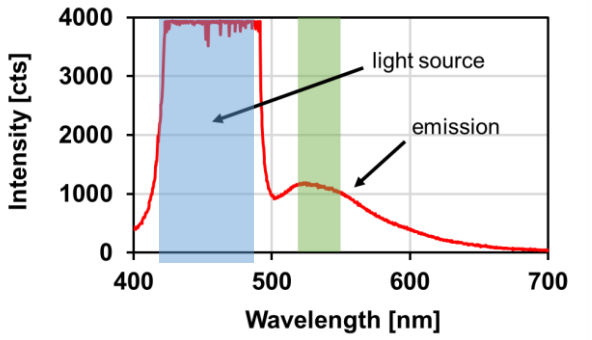

Figure 3. Emission spectrum (in the spectral range ~500 to ~700 nm) of the sensor tip when excited with light from a ~470 nm LED (seen from the saturated signal over the spectral range between ~400 and ~500 nm). The graph shows signal intensity (in arbitrary units, counts (cts) as a function of wavelength in nm). The blue area represents the approximate range of the excitation filter and the green area that for the emission filter.

4.2 Calibration and sensor response

The sensor developed exhibited an increase in fluorescence intensity with increasing pH values over the range studied. Figure 4 shows the measured steady state signal intensities obtained from the fiber-optic pH sensor when immersed in the different buffer solutions used. For each pH value, the relative intensity was determined by averaging the signals over a time interval of 10 seconds. The dashed line in the figure represents the fitted titration curve, applying Equation (3) with a $pK_a = 8.22 \pm 0.07$. The determined pK_a value is higher than that often reported for fluorescein in literature[33] ($pK_a = 6.4$). However, the pK_a value of fluorescein varies depending on its environment[34] and can shift to a higher value when immobilizing an indicator into a polymer, due to a change in the polarity of the microenvironment[29]. The equation used describes the experimental results very precisely (with a high correlation coefficient, $R_2 = 0.999$) and further tests carried out show that the calibrated pH sensors have a good repeatability. The evaluation undertaken shows a sensor accuracy of about ± 0.04 pH units (at pH 7), using a conventional glass pH electrode as reference standard. Figure 5 shows the measured changes in pH over the range from pH 5 to pH 8.5, for the sensor after calibration and showing a maximum signal variation which is equivalent to < 0.01 pH units, obtained for pH values greater than pH 6. The new sensor system design using as it does a very thin coating, in combination with the developed instrument, shows an excellent signal-to-noise ratio.

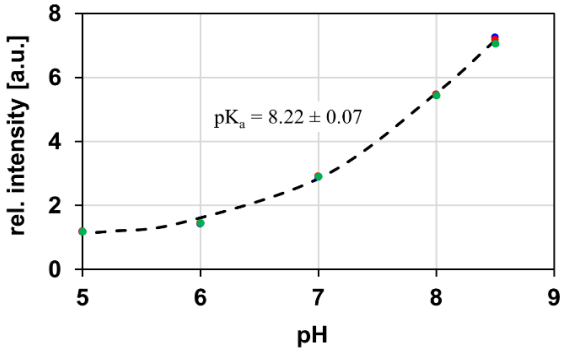

Figure 4. pH dependence of the luminescence intensity recorded over the range from pH 5 - pH 8.5 (and reproducible when measured three times). The dashed curve represents the fit using Equation (3) with $R^2 = 0.999$ and $pK_a = 8.22 \pm 0.07$.

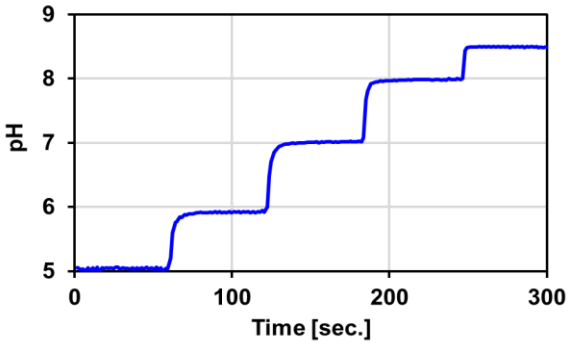

Figure 5. Measured pH changes in aqueous solutions after calibration, stepping over the range pH 5 to pH 8.5 and illustrating the rapid response of the probe.

The rapid response time of the newly developed pH sensor is an important feature, which was studied in some detail, as shown in Figure 6. The sensor has a very fast response to pH changes, of $\Delta t_{90} < 5$s (with the period of ~1s taken to move the sensor physically from one buffer solution to another also included in that measurement), and studied over the range from pH 7 to pH 8. In this latter case, it was observed that the sensor needed an additional 10s to reach the steady state, which was surprising as this phenomenon was not observed for all the sensor designs studied. It could therefore be assumed that this rogue result was related to an inhomogeneous layer distribution and irregular thickness on the fiber tip that influences the pH changes locally. However, this very low, and importantly very fast response time is unusual, when compared to the data published on a wide range of pH sensors described in the literature. Moreover, the response time depends on the initial and final pH value: for pH values below pH 7 the response time was slightly slower; however, this was faster for pH values greater pH 8.

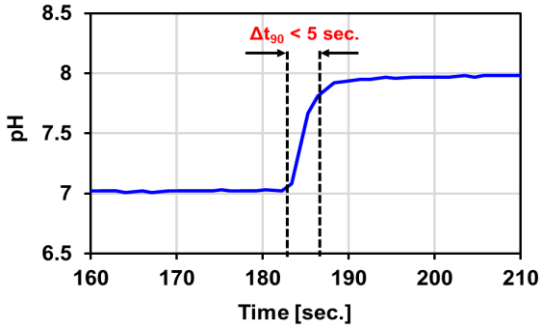

Figure 6. Response time of pH sensor probe due to a sudden (~1s) transfer from pH 7 to pH 8, showing the response time of the probe, $\Delta t_{90} < 5$s.

4.3 Long-term stability (photostability)

Figure 7 shows a measurement made in a stable pH 7 buffer solution for an extended period of ~12h, subjecting the probe to continuous irradiation to examine if any signal drift occurred or there was evidence of photo-bleaching. It was pleasing to note that the pH sensor probe was very stable (with the drift seen being < 0.05 pH/h), even though the determination of pH is based on a luminescence intensity measurement. This represents and extreme situation – and one which would not be seen in practice – as the probe would rarely be used with continuous irradiation. In a typical measurement situation, the duty cycle would be set with long 'off-phases' of the excitation light source between actual measurements as the probe has Δt_{90} < 5s, and thus stable value of pH can be reached in considerably under 1 minute (< 60s).

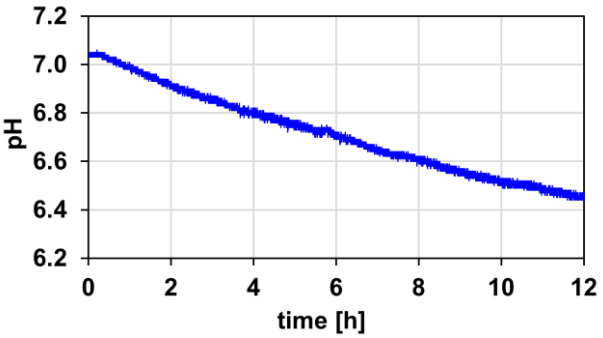

Figure 7. Signal decrease seen under continuous irradiation for ~12 h of the probe placed in a buffer solution of pH 7. The initial measurement (at t = 0) is 7.0 ± 0.05 with a measured drift in the probe response over that period shown to be < 0.05 pH/h

4.4 Experiment in a test-chamber for retinal studies

To provide an initial evaluation of the usability, accuracy and stability of the pH probe developed in this work for long-term measurements in a biological application, a simple experiment was carried out where the sensor was integrated in a test-chamber designed for retinal studies. The aim was to monitor the pH value in an AMES' medium close to the tissue – a substance which is important to maintain the metabolism of retinal cells and thus keeping these active. The AMES' medium used in this research is specially designed for these specific cell types and very well established, as well as commercially available. A typical formulation as well as the preparation is described in the manufacturer's literature[35]. Before the experiment, the pH value of the AMES' was adjusted to the optimal pH range for this study (between pH 7.4 and pH 7.5) using 1N HCL or 1N NaOH[35]. During the measurement process carried out, the AMES' medium passed through the test-chamber at a constant volume flow of 3 ml/min while its pH was measured for 40 minutes with the pH monitoring instrumentation developed in this work. To provide a comparison, a commercially available fiber-optic pH sensor was used as a reference.

The preliminary results in Figure 8 show that the pH monitor developed in this work has a better precision to the adjusted pH value of the AMES' medium (initial pH value between pH 7.4 and 7.5) while with the reference system a greater pH offset was measured. Unexpectedly, only the reference system shows a significant signal drop between 26 and 40 minutes, that might result from locally induced pH changes due to the volume flow of the AMES' medium or the pH sensor itself. This phenomenon is not fully explained as yet and will be studied in further experiments. However, it could be a first indication that the new fiber-optic pH sensor developed has a better stability in a flow system than the conventional device and thus would be much more suitable for experiments of this type. In addition, the new pH-sensor shows a lower signal drift during the entire measurement process, which confirms the excellent photostability discussed earlier.

As it is recognized that this evaluation is simply a 'proof of principle' of the sensor system, in further studies a more detailed and extensive investigation will be carried out. In such work, a pH regulation system would be implemented by automatically fumigating the AMES' medium with Carbogen (CO_2-bicarbonate) to allow better control of the pH level, between pH 7.4 and pH 7.5. Thus it could be expected that the much faster response time of the newly developed pH sensor reported here will show its major advantage when rapid pH changes occur. This will be the subject of further evaluation work.

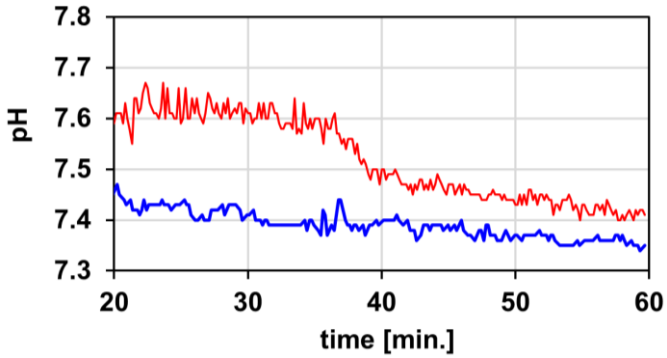

Figure 8. Results of a preliminary study to evaluate the performance of the sensor system for pH measurement in an AMES' medium for retinal studies – with the main experiment started at ~20 minutes after the system was inserted into the medium. The line in blue shows the response of the pH system developed in this work and red the data from a reference fiber-optic pH system used for comparison.

5. SUMMARY AND OUTLOOK

A new fiber optic-based pH sensor probe system has been developed, allowing the investigation of the new, fast response tip design created. The system was evaluated and preliminary results reported are highly promising and show a significant improvement in the sensor performance on current devices, with an extremely fast response time to pH changes (Δt_{90} < 5s). With this new sensor design, highly accurate measurements of pH are possible using very small volumes, because of the design involving small diameter optical fibers and small tip diameters, important for monitoring of the small volume samples often only available from biological or clinical work. In combination with the newly developed signal processing instrumentation used, the pH sensors developed have been shown to be very stable over a long period of time – during a measurement for 12 hours at pH 7 with continuous irradiation, a low signal drift (of less than 0.05 pH/h) and a negligible influence of stray light were observed. The sensor tip itself has a significant effective area but includes a very thin coating that shows an almost homogeneous coating distribution, making it ideal for fast response sensor designs since small differences in thickness can result in large changes in the dynamic response of the sensor.

Figure 7 shows the change in the reading with long, continuous irradiation and this signal drift could be reduced or eliminated by changing the duty cycle used, with longer 'off phases' of the excitation light source. Further R&D work is planned to optimize the sensor further, including to analyze the long-term mechanical stability and to optimize the thickness of the sensor layers (but maintaining the positive trade-off between the layer thickness and the response time), as well as evaluate in more detail and thus reduce or eliminate any cross sensitivities to other parameters (in particular temperature and ionic strength changes).

A very positive conclusion of the study carried out is that it has clearly shown that this new sensor design has the potential to extend the breadth of applications for fiber-optic pH sensors, taking full advantage of the fast response times seen from this design. As has been shown in the preliminary biological experiment reported, the pH value monitored in an AMES' medium for retinal tissue can readily be measured during a long-term measurement. It is recognized that further experiments of this type are needed and further, the detection range of the pH sensors is ideal for measurements in physiological applications, where a pH range from pH 5 to pH 8.5 is necessary and small sensors are needed. This is the subject of on-going research work. A further benefit of this design for biological or clinical applications is that the manufacturing process for these sensors is relatively simple and thus volume production, at low cost, is feasible, which will also suit a range of applications including those where disposal of the sensor probe is required after each use.

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