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Tapered Optical Fiber Sensor for Detection of pH in Microscopic Volumes

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Abstract: A compact and robust tapered optical fiber microsensor is presented for detection of pH in a range from 5.8 to 7.5 in sub-microliter volumes. The sensor is based on a pH transducer 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt immobilized in a xerogel matrix onto the tip of a optical fiber taper with a tip diameter below 20 μm. The sol-gel method and two silicon alkoxides is used for preparing the matrix. A ratio of the fluorescence emission intensities measured at 518 nm after the excitation at 400 and 450 nm is used for evaluating the sensor response to pH. This ratiometric approach enables to reduce effects of ambient light, bleaching of the sensitive layer and geometry of the probe to the fluorescence signal and achieve the resolution of about 0.07 pH units. *Copyright © 2014 IFSA Publishing, S. L*

Keywords: Optical sensor, Ph, Microfiber tip, Fluorescence, HPTS, Ratiometrc method.

1. Introduction

Recent progress at investigation and development of luminescence micro- and nanosensors has created new possibilities for chemical detection in samples with microliter volumes or for local chemical detection in biological objects such as cells, tissues, viruses etc. Micro- and nanosensors extend the performance of fluorescence-microscopic techniques broadly used in medicine or biology [1-4]. Such microscopic techniques can detect the fluorescence emitted from different places in biological samples as images or photometric data from which intensities and emission spectra can be deduced. They enable us to precisely locate intracellular biomolecules, determine their diffusion coefficients, transport characteristics, or study interactions with other biomolecules. Moreover, the response of the fluorescence to localized environmental variables makes possible to determine pH, viscosity, refractive index, concentrations of ions, solvent polarity etc. in living cells and tissues [1, 2]. Thus, these techniques have a great potential for measurements in biology and medicine. However, they need either molecules with fluorescence (phosphorescence) labels or luminescence transducers loaded into samples.

In a luminescence micro- or nanosensor a suitable luminescence transducers is immobilized in its sensitive part (nanoparticle surface or volume, fiber tip) that has dimensions on a level of micrometers or nanometers. This sensing part is brought into contact with a sample and optical changes in the sensitive part caused by interactions of the transducer with its local environment are detected.

Luminescence micro- and nanosensors have been employed for detecting different ionic and molecular

species such H^+, Ca^{2+}, Zn^{2+}, NO, O_2, proteins, etc [3-16]. Especially the detection of protons (hydroxonium ions), i.e. pH detection, plays very important role in cell investigations [7-9]. Intracellular pH have very important role in cellular processes and is highly regulated in every organelle. The structural stability and function of proteins are tightly associated with pH. For example, cell cycle progression and programmed cell death have both been linked to changes in intracellular pH [10]. This example shows that the quantification of pH fluctuations in cells cal help us to understand cellular processes.

Nanosensors can be divided into nanoparticle-based nanosensors and fiber-optic nanosensors. Three main classes of nanoparticle-based nanosensors can be distinguished, namely quantum dot-based nanosensors, probes encapsulated by biologically localized environment - PEBBLEs and phospholipid-based nanosensors [3]. Such nanosensor can employ changes of the luminescence of quantum dots or transducers immoblized on PEBBLEs or phospholipides that is caused by interaction of these nanoparticles with analytes.

Usually large quantities of luminescent nanoparticles inserted in a cell, either by a gene gun or a similar device. Then, the entire cell is illuminated and the fluorescence signal from the indicator is measured over the autofluorescent background of the cell. A strong autofluorescence of the cell can be overcome by using magnetically modulated version of PEBBLEs [3].

Fluorescence images of by nanoparticle-based nanosensors are usually detected by an inverted fluorescence microscope, by a suitable fluorescence immaging system or by a confocal microscope equipped with a proper excitation source [7-9, 11, 12].

Nanoparticle-based nanosensors have been employed for measuring pH in animal cells [3, 8, 9, 11]. For example, intracelluar pH in HepG2 cancer cells was determined by confocal microscopy using two pH-sensitive fluorescence dyes (fluorescein and oregon green) and one reference dye (rhodamine) [8, 9]. Such nanosensors have been shown to measure pH in a range from 4.1 to 7.5. Novel techniques, such as surface-enhanced Raman spectroscopy (SERS), with silver or gold nanoparticles modified with pH transducers (e.g. indocyanine green) whose SERS spectra are sensitive to pH have also been tested for very sensitive intracellular pH measurements [11].

In plant cells nanoparticle-based nanosensors have been investigated mainly for oxygen sensing [6, 12]. An optical multifrequency phase-modulation technique has been used for separating the signal from the transducer and the strong autofluorescence of the plant tissue [16].

Fiber-optic micro- and nanosensors are based on micro- and nanofiber tips. These tips can be prepared by two methods. The first one, used more often, is based on by a controlled elongation of a silica optical fiber to the breaking point [3, 4, 13, 14]. The second method is based on controlled etching of silica optical fibers in a solution of HF [15]. Nanofiber tips with diameters from 20 -100 nm have been prepared [3, 4, 13-17]. The tapered side walls of such fiber tip are usually coated with a metallic layer (a thickness of about 100-200 nm) that enables propagation of the excitation light through the tapered part of the micro- or nanofiber. Such fiber tips are further modified by the application of a suitable luminescence transducer. For such a purpose a photo/nanofabrication technique can be used [13] that is based on photopolymerization of a polymer solution containing the transducer directly on the fiber tip by using light transmitted through the fiber.

For dimensions of fiber-optic tips below the wavelength of light a special technique, near-field microscopy is used for measuring signals from a localized light source, i.e. the fiber tip, with dimensions on nanometer scales [3, 13-17]. This technique enables the surface inspection with high spatial, spectral and temporal resolving power. The resolution of the image is limited by the size of the detector aperture. A sensor system usually consists of an excitation source (typically a laser), an inverted fluorescence microscope, imaging detector intensified charge-coupled device (ICCD), a photon-counting detector (PMT), and computer [14]. Light from the source is guided through the tapered nanofiber to a transducer immobilized on the fiber tip and excites there fluorescence that is collected via the inverted fluorescence microscope.

In luminescence sensors employing fiber-optic tips with tip diameters higher than one micrometer the excited luminescence can be launched in the fiber used for the excitation and transmitted to a photomultiplayer or photodiode [18, 19]. Instead of the direct immobilization of luminescent transducers on the fiber tip polymeric microbeads immobilizing fluorescence transducers have also been attached to a bifurcated fiber tip with a diameter of 140 μm and used for dual sensing of oxygen a pH [19]. Chemical sensors employing such microsensing elements with a minimum tip diameter of about 50 μm are also available commercially [20, 21].

Similarly, as in the case of sensing nanoparticles measured luminescence intensities provide us with information about the chemical environment of the fiber tip. However, the placement of fiber-optic micro-and nanosensors can be controlled much easily than that of nanoparticle-based micro- and nanosensors. Fiber-optic nanosensors also provide the spatial resolution necessary to obtain simultaneous measurements of multiple analytes by using arrays of fiber tips [3, 22].

Fiber-optic micro-and nanosensors have been employed for measuring pH [13, 23, 24]. Fluorescent transducers such as different carboxyfluoresceins [13] or 8-hydroxy-pyrene-1,3,6-trisulfonate (HPTS) [23, 24] have been tested. The transducers were immobilized in polymeric or ormosil matrices.

Luminescence micro- and nanosensors measure usually the fluorescence intensity. As the luminescence intensity can be influenced by random fluctuations, ratiometric methods or phase modulation techniques have been adapted for measurements [8, 9, 13, 23, 24]. Phase modulation techniques are based on the phase shift of the luminescence excited by a sinusoidally modulated excitation light. These techniques provide us with information on pH-dependent luminescence decay time that practically does not suffer from random fluctuations. Such techniques have been used for pH detection both with nanoparticle-based nanosensors [13] and with fiber-optic pH microsensors [23].

Ratiometric techniques employing the ratio of the luminescence of sensing transducer and that of reference transducer have also been used with nanoparticle-based nanosensors [8, 9]. In one of our articles we have presented a fiber-optic microsensor for pH detection in plant exudates that was based on HPTS as the detection pH transducer and Ru complex as the reference transducer. Both transducers were immobilized in a porous matrix prepared by the sol-gel method from 3-Glycidyloxypropyl)trimethoxysilane and phenyltrethoxysilane.

This paper presents results on preparation and characterization of a microsensing fiber-optic element based on a microfiber tip with a diameter up to 20 μm. The tip is modified with a luminescence pH transducer HPTS in a porous matrix prepared by the sol-gel method. A ratiometric method is described in the paper which is based on measuring fluorescence intensities at 518 nm both for the excitation of the transducer at 405 and 450 nm, respectively.

2. Experimental

2.1. Preparation of Sensing Elements

Fiber tips were prepared from a graded-index optical fiber produced at the Institute. The fiber with cladding and core diameters of 125 and 50 μm, respectively, numerical aperture of 0.2 and attenuation of 1-2 dB/km at 1300 nm was used. Nearly adiabatic biconical fiber tapers with a waist diameter of about 5-10 μm were prepared by indirect flame heating of the fiber while pulling its ends apart at a controlled speed. These tapers were cut at a desired diameter with a thin and sharp steel blade under a microscope. The resulting tips were grinded at an angle of about 30° to the fiber axis on a laboratory micro-beveller using a lapping foil with roughness of 2 μm. The tips were used in experiments without coating with any metallic layer.

The sol-gel method was used for preparing sensitive layers on a fiber tip. An input sol was mixed of two different sols [10]. The first one was prepared from 3-Glycidyloxypropyl)trimethoxysilane (GLYMO, 98 %, Aldrich, CR), absolute ethanol (≥ 99.5 % absolute, Sigma-Aldrich, CR), 1-methylimidazole (MI, > 99 %, Sigma-Aldrich, CR) and deionized water. In this preparation 22 ml of GLYMO was dissolved in 36.7 ml of absolute ethanol at first. Into this solution a solution of 5.5 ml of MI and 7.2 ml of deionized water was added dropwise while the whole mixture was stirred on a magnetic stirrer. The sol with a water/alkoxide ratio of 4.0 and alkoxide concentration of 1.4 mol/l was prepared. The second sol was prepared by mixing 21.5 ml of ethyltriethoxysilane (ETES, 97 %, Fluka, CR) and 37 ml of EtOH with 7 ml of 0.1 M hydrochloric acid (Fluka, CR). Second sol was prepared with a concentration of 1.7 mol/l and water/alkoxide ratio of 3.7. Finally, both these sols were mixed together in 1:1 ETES-to-GLYMO molar ratio.

The fluorescent ion-paired opto-chemical transducer (c-HPTS) was obtained by reaction of 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS, > 97 %, Aldrich, CR) with hexadecyl-trimethylammonium bromide (CTAB, > 99 %, Sigma, CR) using a procedure described elsewhere [10]. An amount of 5 mg of c-HPTS was added into 1 ml of the input sol and the resulting sol was aged for at least 72 hrs at laboratory temperature.

Prepared beveled fiber-optic tips were shortly dipped into a small drop of the aged sol using an inverted microscope and micro-manipulators (Narishige). In the experiments of about 10 μL of the sol with the dye was dropped onto a glass-slide and after approximately 1 min. when the sol viscosity increased the tip was shortly immersed in the drop and withdrawn. The coated tips were dried at a hot-air drier at 140 °C for 4 hrs.

2.2. Optical Set-up

The experimental set-up consisted of a fiber taper tip, two high-power light emitting-diodes with heat sinks and nominal wavelengths of 400 nm and 450 nm (OEM, maximum power of 1.3 W), two multi-mode fiber couplers (Opneti, China) with coupling ratios of 1:99 and 1:1, and UV-VIS pocket-size USB spectrometer (SD2000, Ocean Optics, USA) with a laptop. These components were interconnected according to the scheme at Fig. 1. All fiber components were spliced by arc fusion-splicing.

2.3. Tests of Sensing Elements

For testing the response of prepared sensing elements to pH a set of eight Britton-Robinson pH buffer solutions with an ionic strength of 0.15 mol/l was used. This set covered a pH range approximately from 4 to 8. In calibration experiments a sensing element was immersed in each buffer solution for several tens of seconds and emission spectra were measured for the excitations at 450 nm and 400 nm.

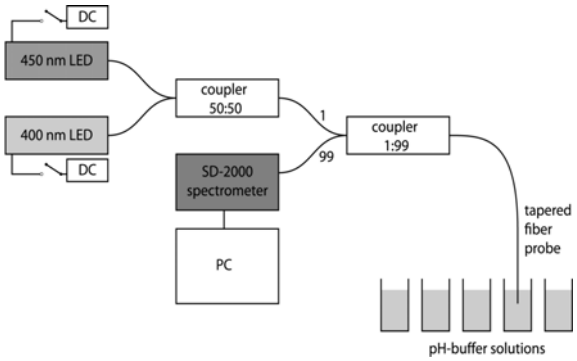

Fig. 1. Scheme of the optical set-up used for characterization of pH response of the prepared microfiber tip.

The measurement with the set of pH solutions was repeated for three times. The spectra were taken with an integration time of 1 s and "boxcar" smoothed 50 adjacent values.

In order to determine the time response of prepared elements to pH changes, a sensing element was immersed several times in buffer solutions of pH 4.18 and 7.95 and the fluorescence intensity at 518 nm was measured for the excitation at 450 nm.

3. Results and Discussion

An example of the tapered fiber tip with a diameter of about 15 μm prepared by cutting a bicconical taper is shown in Fig. 2. All data presented in the paper were obtained with this taper. In order to improve the penetration of fiber optic tapered elements into cell samples, decrease the intensity of back-reflected excitation light and to increase the fluorescence intensity, the fiber tip was beveled at an angle of about 30 ° to the axis (see Fig. 3).

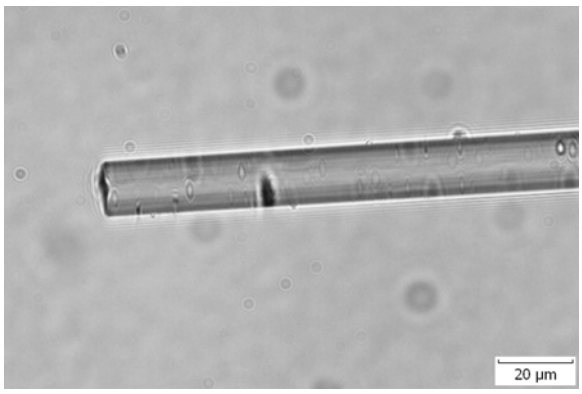

Fig. 2. The tip of the tapered fiber as cut.

Two alkoxides, namely ETES and GLYMO were used for the preparation of the input sols in order to decrease effects of xerogel matrix relaxations and bleaching of the transducer from the matrix. Special attention has been focused on the preparation of the sensing layer on the microfiber tip. Since the sol containing the sensitive dye tends to rise along the taper surface during immersing the fiber tip in the sol, the duration of the sol application was checked by using an inverted microscope equipped with micro-manipulators. The contact of the tip and sol drop and effects of capillary phenomena are shown in Fig. 4. The resulting sensing element after the hot-air drying can be seen at Fig. 5.

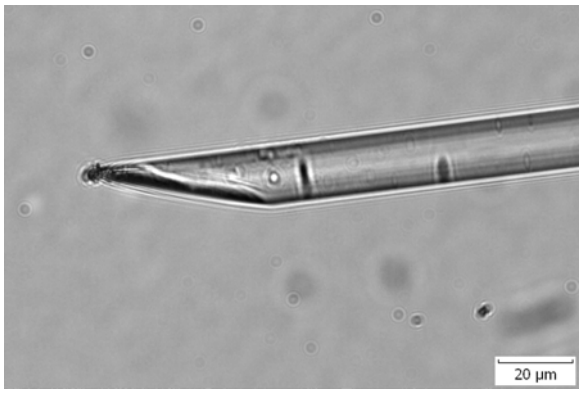

Fig. 3. The beveled fiber-optic tip.

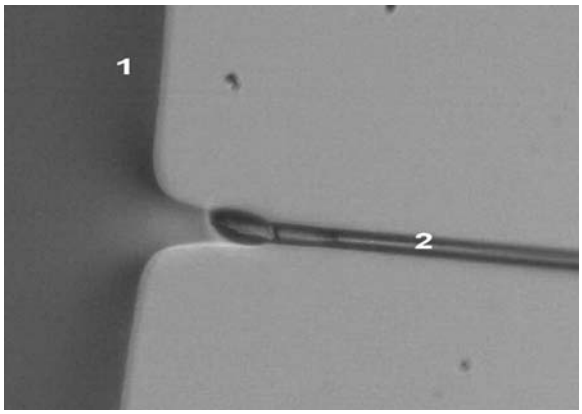

Fig. 4. Application of the sol on the fiber tip: 1-a drop of the sol; 2-the tapered and beveled optical fiber tip.

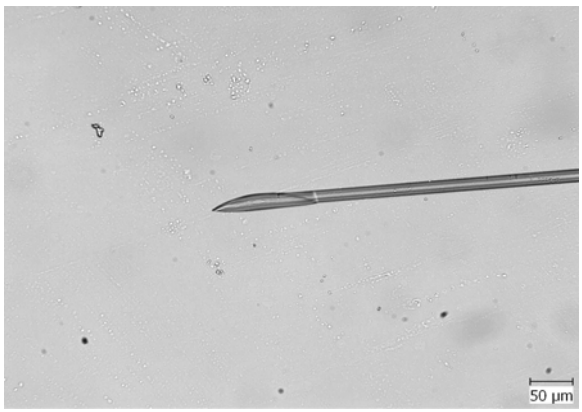

Fig. 5. The prepared sensing mikrfiber tip.

Differences between emission spectra taken at pH 4 and 7.5 (see Fig. 5) were used to identify a suitable emission wavelength for obtaining the calibration curve. From the emission curves in Fig. 6 it is evident that the maximum of the curve measured for the excitation at 450 nm shifts to shorter wavelengths when pH increases (compare the solid lines). A very small shift of the curve maximum can be observed for the excitation at 400 nm (compare the dashed lines). These shifts can be explained by the wavelengths of the excitation LEDs used. In a wavelength region of 400-500 nm HPTS has two absorption bands [25]. The height of the first one that is centered at 405 nm practically does not depend on pH and thus this band can be used for the internal reference. On the contrary the height of the second band centered at 460 nm depends strongly on pH. In this research, the excitation at 450 nm instead of 460 nm was used, which can explain the observed spectral effects. By analyzing emission spectra measured for the excitation at 450 nm highest changes of the emission intensity with pH were found for a wavelength of 518 nm. Therefore, emission intensities at this wavelength were used for constructing the calibration curve.

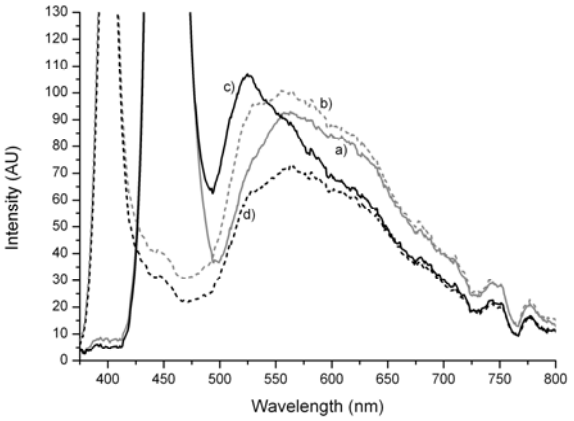

Fig. 6. Spectra taken at pH 4 (grey – a,b) and at pH 7.5 (black – c,d) after excitation at 450 nm (solid lines – a,c) and 400 nm LED (dashed lines – b,d).

A ratio of fluorescence intensities I_{450} and I_{400} measured at 518 nm for the excitation at 450 nm and 400 nm, respectively was used for the evaluation of the sensor response to pH and construction of the calibration curve. This ratio R is defined by Eq. (1)

$$R(pH) = \frac{I_{450}}{I_{400}} \tag{1}$$

The calibration curve of the sensing microfiber showing its pH response is depicted in Fig. 7

The calibration curve in Fig. 7 was obtained by fitting the experimental data with the Boltzmann sigmoidal function (see Eq. (2) [23]) using the least-square method.

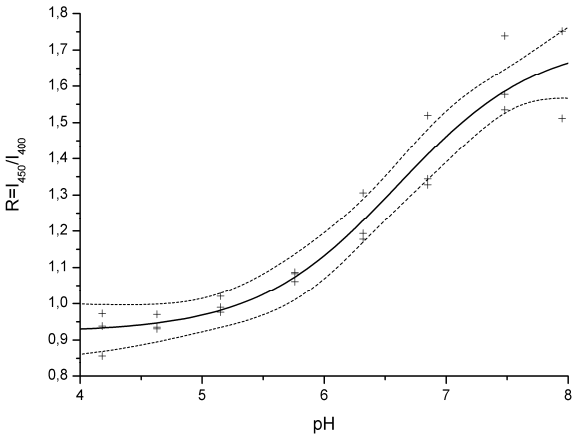

Fig. 7. The measured values (crosses), the calibration curve of the sensor (solid line) and 95% confidence levels (dashed lines) of the calibration curve.

$$R = \frac{A_1 - A_2}{1 + e^{\frac{pH - pH_0}{d}}} + A_2, \tag{2}$$

where A_1 and A_2 are the horizontal asymptotes, pH_0 is the center of the inflection, and d is the "width" or "rate" (the change of pH corresponding to the most significant change of R). The parameters of this fitting with their variances are summarized in Table 1.

Table 1. Parameters of the Boltzmann function with standard errors.

A_1	A_2	pH_0	d
0.92±0.04	1.73±0.11	6.60±0.19	0.58±0.17

The value of pH_0 in Table 1 corresponds to the value of pK_a of c-HPTS. This value is higher than a value of 6.20 published elsewhere [25].

From Fig. 7 one can see that the sensing element exhibits a linear response in a pH range from about 5.8 to 7.5. By fitting the experimental data in this range with a linear function Eq. (3) was obtained.

$$R=-0.75\ 1\ 0.32\ pH \tag{3}$$

Standard errors of the constant and slope in Eq. (3) are 0.32 and 0.03 respectively. The detection sensitivity of 0.32 pH^{-1} follows from Eq. (3). Taking into account the precision of the emission intensity measurements, a limit of detection of about 0.07 pH units can be estimated from the sensitivity value. The comparison of the fitting the experimental data with the Boltzmann sigmoidal function and linear function can be seen from Fig. 8.

Since the experimental setup did not allow an automatic switching of the excitation LEDs the intensity ratio could not be recorded as a function of time. In order to obtain the time response of the

sensing element to a pH change intensity changes at the excitation at 450 nm were measured as a function of pH and time. Results of these measurements are shown in Fig. 9.

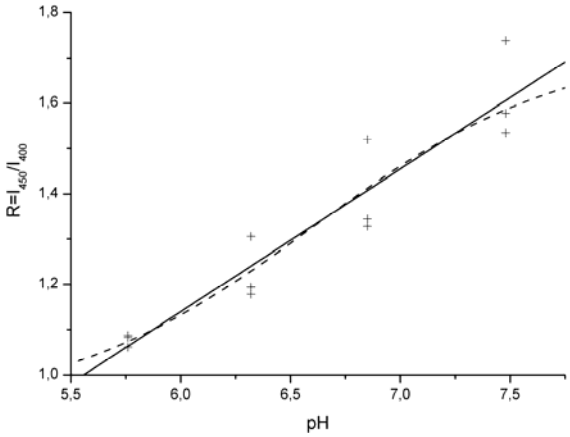

Fig. 8. Linear fit (solid line) of the sensor compared to Boltzmann sigmoidal fit (dashed line) .

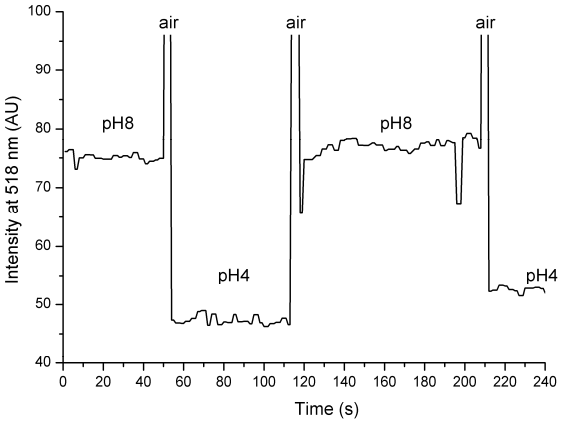

Fig. 9. The time response of the sensor to pH changes.

4. Conclusions

We have prepared a microfiber tip for microscopic-scale measurements of pH. The element has a linear response to a pH range from 5.8 to 7.5 with a time response better than 20 s. The dimensions of the sensor and its operation range make it useful e.g. for *in vivo* biological and micro-biological experiments.

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References

[1]. H. C Ishikawa-Ankerhold, R. Ankerhold, G. P. C. Drummen, Advanced fluorescence microscopy techniques—FRAP, FLIP,FLAP, FRET and FLIM, *Molecules,* Vol. 17, 2012, pp. 4047-4132.

[2]. B. O. Leung, K. C. Chou, Review of super-resolution fluorescence microscopy for biology, *Appl. Spectrosc.*, Vol. 65, 2011, pp. 967-980.

[3]. B. M. Cullum, Optical nanosensors and nanobiosensors, Dekker Encyclopedia of Nanoscience and Nanotechnology, 2nd Edition-Six Volume Set, Edited by C.I. Contescu and Karol Putyera, *CRC Press,* 2008, pp. 3258-3268.

[4]. Y. Zhang, A. Dhawan, T. Vo-Dinh, Design and fabrication of fiber-optic nanoprobes for optical sensing, *Nanoscale Res Lett*, Vol. 6, 2011, Article 18, 6 pages.

[5]. K. J. Cash, H. A. Clark, *In Vivo* histamine optical nanosensors, *Sensors*, Vol. 12, 2012, pp. 11922-11932.

[6]. C. Ast, E. Schmälzlin, H.-G. Löhmannsröben, J. T. van Dongen, Optical Oxygen Micro- and Nanosensors for Plant Applications, *Sensors*, Vol. 12, 2012, pp. 7015-7032.

[7]. K. P. McNamara, N. Rosenzweig, Z. Rosenzweig, Liposome-Based Optochemical Nanosensors, *Mikrochim. Acta,* Vol. 131, 1999, 57/64.

[8]. R. V. Benjaminsen, H. Sun, J. R. Henriksen, N. M. Christensen, K. Almdal, T. L. Andresen, Evaluating nanoparticle sensor for intracellular pH measurements, *ACS Nano,* Vol. 5, 2011, pp. 5864–5873.

[9]. H. Sun, T. L. Andresen, R. V. Benjaminsen, K. Almdal, Polymeric nanosensors for measuring the full dynamic pH Range of endosomes and lysosomes in mammalian cells, *J. Biomed. Nanotechnol.*, Vol. 5, 2009, pp. 1–7.

[10]. M. Thangaraju, K. Sharma, D. Liu, S. H. Shen, C. B. Srikant, Interdependent Regulation of Intracellular Acidification and SHP-1 in Apoptosis, *Cancer Res.*, Vol. 59, 1999, 59, pp. 1649–1654.

[11]. J. Kneipp, H. Kneipp, B. Wittig, K. Kneipp, Novel optical nanosensors for probing and imaging live cells, *Nanomedicine: Nanotechnology, Biology, and Medicine,* Vol. 6, 2010, pp. 214–226.

[12]. E. Schmalzlin, J. T. van Dongen, I. Klimant, B. Marmodee, M. Steup, J. Fisahn, P. Geigenberger, H.-G. Lohmannsroben, An optical multifrequency phase-modulation method using microbeads for measuring intracellular oxygen concentrations in plants, *Biophysical Journal,* Vol. 89, 2005, pp. 1339–1345.

[13]. W. H. Tan, Z-Y. Shi, R. Kopelman, Miniaturized fiber-optic chemical sensor with fluorescent dye-doped polymers, *Sens. Act. B-Chemical,* Vol. 28, 1995, pp. 157-163.

[14]. T. Vo-Dinh, C. P. Kasili, Fiber-optic nanosensors for single-cell monitoring, *Anal. Bioanal. Chem.,* Vol. 382, 2005, pp. 918–925.

[15]. T. Vo-Dinh, P. Kasili, M. Wabuyele, Nanoprobes and nanobiosensors for monitoring and imaging individual living cells, *Nanomedicine: Nanotechnology, Biology, and Medicine*, Vol. 2, 2006, pp. 22–30.

[16]. Z. Rosenzweig, R. Kopelman, Analytical properties and sensor size effects of a micrometer-sized optical fiber glucose biosensor, *Anal. Chem.,* Vol. 68, 1996, pp. 1408-1413.

[17]. T. Vo-Dingh, Optical Nanosensors for Detecting Proteins and Biomarkers in Individual Living Cells, *Methods in Molecular Biology*, Vol. 300, 2005, pp. 383-401.

[18]. U. Kosch, I. Klimant, O. S. Wolfbeis, Long-lifetime based pH micro-optodes without oxygen interference, *Fresenius J. Anal. Chem.,* Vol. 364, 1999, pp. 48-53.
[19]. A. S. Kocincova, S. M. Borisov, C. Krause, O. S. Wolfbeis, Fiber-optic microsensors for simultaneous sensing of oxygen and pH, and of oxygen and temperature, *Anal. Chem.*, Vol.79, 2007, pp. 8486-8493.
[20]. http://www.oceanoptics.com/, December 2012.
[21]. http://www.presens.de/, December 2012.
[22]. E. J. Park, K. R. Reid, W. Tang, R. T. Kennedy, R. Kopelman, Ratiometric fiber optic sensors for the detection of inter- and intra-cellular dissolved oxygen, *J. Mater. Chem*., Vol. 15, 2005, pp. 2913–2919.
[23]. A. Song, S. Parus, R. Kopelman,, High-performance fiber optic pH microsensors for practical physiological measurements using a dual-emission sensitive dye, *Anal. Chem*., Vol. 69, 1997, pp. 863–867.
[24]. I. Kasik, J. Mrazek, T. Martan, M. Pospisilova, O. Podrazky, V. Matejec, K. Hoyerova, M. Kaminek, Fiber-optic pH detection in small volumes of bio-samples, *Anal. Bional. Chem.,* Vol. 398, 2010, pp. 1883-1889.
[25]. D. Wencel, B. D. MacCraith, C. McDonagh, High performance optical ratiometric sol–gel-based pH sensor, *Sens.& Act.,* Vol. 139, 2009, pp. 208-213.