Development and comparision of pH microoptodes for use in marine systems

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ABSTRACT

Traditionally microscale measurements of pH are based on potentiometric measurements with a pH glass microelectrode. The preparation of these electrodes is, however, very time consuming. We developed pH microoptodes for use in seawater in the range of pH 7-9. The optodes are based on immobilized acid-base indicators, which change their color and/or fluorescence properties as a function of the pH. Various dyes were immobilized directly on the tip of a tapered optical fiber by different techniques. We then investigated these pH optodes with respect to response time, mechanical stability and calibration characteristics. Dependent on the optical properties of the indicator material we used different Light Emitting Diodes (LED´s) as the light sources and either a photodiode or a photomultiplier as detector.

Keywords: Optode, optical microsensor, pH, fiber-optic, environmental sensing, seawater

1. INTRODUCTION

The pH value is an important parameter in aquatic systems. The equilibria of the carbon dioxide- and hydrogen sulfide systems are pH dependent and to understand the biogeochemistry of the carbon and sulfur cycles it is thus necessary to do direct fine scale measurements of pH, H_2S and CO_2 in sediments and biofilms. Here steep gradients form over distances ranging from <0.1 to 1 mm and only the use of microsensors with a tip diameter <50 µm can provide a sufficiently high spatial resolution for measuring the distribution of e.g. pH in these systems. For optical pH sensing, a sensitive dye is immobilized at the tip of a fiber. The matrix material has to prevent the leaching of the dye and has to be permeable for the analyte. In order to reach a high proton concentration in the matrix and a fast sensor, a hydrophilic material has to be used. Here problems with the long term stability due to leaching may occur. Thus the interaction between the dye and the matrix material has to be stronger than between the dye and water.
In the literature many pH sensitive dyes are described. Two classes of dyes can be distinguished: 1) absorption dyes, and 2) luminescence dyes. Both types of indicators are useful for the optical pH measurements with microsensors. In case of absorptive dyes some amount of the non-absorbed light is reflected and measured at the same wavelength as the illumination light, and in case of luminescent dyes the emitted luminescence is measured. After exchange of the optical filters and depending on the signal size, the same optoelectronic measuring systems are applicable to both principles.

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The mathematical description of both kinds of sensors is given by the Henderson-Hasselbach equation. This equation shows the influence of the temperature, given by the thermodynamic constant, and an influence of the ionic strength, dependent on the activity coefficient of the acid and base form of the indicator dye. The calibration curve (pH versus light signal) of an optical pH sensor therefore exhibits a sigmoidal form. pH measurements with chemical indicators can thus be performed at pH values up to ± 2 units from the pK of the indicator dye. The operational pK of the sensor material can be changed by interaction of the dye with the matrix material, swelling processes of the matrix at higher pH´s, an addition of a second indicator, and the addition of phase transfer catalysts, plastizers etc.
We developed fiber optic pH sensors for application in marine systems over a pH interval between pH 6.5 and 9.5. The ionic strength depends on the salinity of the seawater and is constant during one measurement. But it may change in every application. Therefore, the calibration curve of the pH sensor has to be determined for different salinities before an accurate measurement can be performed. Investigations in this field show a pK-shift of the dye to lower values at high salinities [1]. As mentioned above, the temperature also influences the calibration curve [2]. Usually the pK shifts to higher values at higher temperatures [3].
Most applications of the pH microsensors involve measurements of pH profiles in marine sediments and microbial mats. Thus the mechanical stability of the sensor, especially the adhesion of the dye matrix to the fiber tip, has to be sufficiently high for profile measurements in abrasive material. For example if the sensor hits a sand grain and cannot proceed during the profiling, the dye matrix will be compressed and the light signal is changed without a change of the analyte concentration (activity). Furthermore reflections of the illumination light or additional luminescence of chlorophyll and carotenoids induced by the excitation light can occur during the measurement within the biological system. Therefore, the dye matrix has to be hard and should prevent an optical interaction of the measuring light and the sample.
Exsisting pH-microelectrodes do not exhibit such interactions of light with the sensor chemistry and the sample. However, electrochemical measurements can suffer from other measuring problems: interactions with electromagnetic fields or - in case of the pH-electrode - interference by the high sodium content in seawater and insufficient spatial resolution. A pH microelectrode has a proton-permeable glass at the sensor tip which has dimensions in the range of 30 µm in diameter and up to 200 µm in length [4]. We present here a fiber-optic microoptode that overcomes most of the measurement problems mentioned above.

2. EXPERIMENTAL

2.1 Reagents and materials

The fluorescent dye 4´-aminofluorescein (A-fl), the matrix materials cellulose acetate and tetraethyl orthosilicate and the scattering material titanium dioxide were obtained from Aldrich-Chemie (Germany). Tridodecylmethylammonium chloride (TDMA), ethanol, sulfuric acid, sodium hydroxide, sodium chloride and aceton were bought from Fluka (Switzerland). The absorption dye N9 was obtained from Merck (Germany), polymethyl methacrylate from Goodfellow (Germany).
Buffer solutions for the calibration measurements were prepared from 0.025 M Tris-buffer and sodium chloride. The pH value of the buffers was checked with a commercially available electrode (Type InLab 412, Mettler Toledo, Switzerland).

2.2 Preparation of the sensors

Microoptodes were constructed from multimode silica/silica step index fibers with a 100 µm core and 140 µm cladding diameter (Radiall, Germany). The fiber was tapered by heating the bare fiber in a small flame of a gas burner [5] or in the light arc of a fusion splicer (RXS, Siemens, Germany). Fiber tips from 20 to 40 µm diameter were achieved by cutting the taper at this diameter. Three different kinds of sensor chemistry were tested. The optodes were fabricated by dipping the fiber into the indicator/polymer solution (cocktail).

Type I: The sensor cocktail which includes a luminescence dye and the matrix material in a solvent was prepared from 2 ml tetraethyl orthosilicate, 3 ml ethanol, 1.2 ml water, 0.5 ml 0.1 M hydrochloric acid and 1.4 mg aminofluorescein as the pH sensitive luminescence dye. The procedure of the sol-gel process based on the work of Strawbridge et al. [6]. This mixture was stored for 72 hours at room temperature. At the end of the curing time the sol-gel should be well prepared and the tapered fibers were dip coated. In order to evaporate the solvent and finish the sol-gel reaction sensors were tempered at 100 °C for 40 hours. The drying step of sol-gel at higher temperatures decreased the leaching of the dye [7].

Type II: Additionally to sensor type I the cocktail of type II included 2.29 mg tridodecylmethylammonium chloride. The sol-gel process was done under the same conditions.

Tpy III: This sensor based on a covalently bound absorption dye N9. By this procedure we achived a carrier layer for the immobilization matrix which contained cellulose acetate as the basic material [8]. For spectroscopical investigations of the absorption sensor a planar optode with 5 cm in diameter was prepared under the same conditions using a polystyrene disk as the transparent support.

2.3 Measuring system

Spectral light measurements of the sensor material were made with a Photonic Multichannel Analyzer (PMA-11, Hamamatsu) and a fiber-optic halogen lamp (KL 1500, Schott, Germany). The spectra were corrected by substraction of the lamp spectra that illuminated an indicator free matrix by use of the PMA Application Software (Version 1.0, Hamamatsu). The measuring system for the pH microoptodes is shown in Figure 1. We used a blue and a yellow LED with emission maxima at 450 or 590 nm as illumination/excitation light. The light was filtered (short-pass) and coupled via a spherical lens into one branch of a 2x2 optical fiber coupler (Gould Inc., USA). The other branch was not used. The light from the sensor was filtered (520 nm long-pass for the luminescence measurement) and measured by a detector (photomultiplier (PMT), photodiode). In order to allow measurements under ambient light conditions, the intensity of the LED emission was modulated (f_{mod}= 1 kHz) and a lock-in-amplifier was used for the signal conditioning. For all fiber connections standard ST-connectors were used. All measurement were performed at room temperature

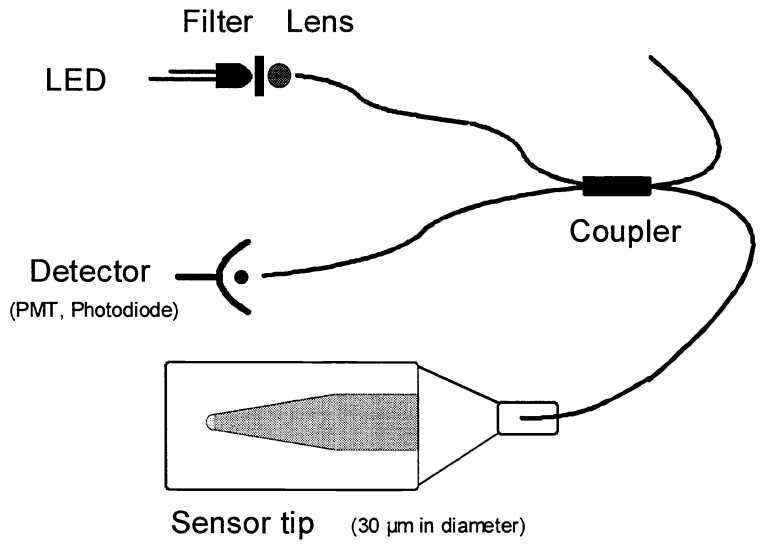


Figure 1: Setup for fiber-optical pH-sensing

3. RESULTS AND DISCUSSION

3.1 Spectral characteristic of the indicators

The spectral characteristic of aminofluorescein is well known and was therefore not investigated in this work. In Figure 2 the absorption spectrum of covalently bound N9 at different pH values and a salinity of 35 ‰ is shown.

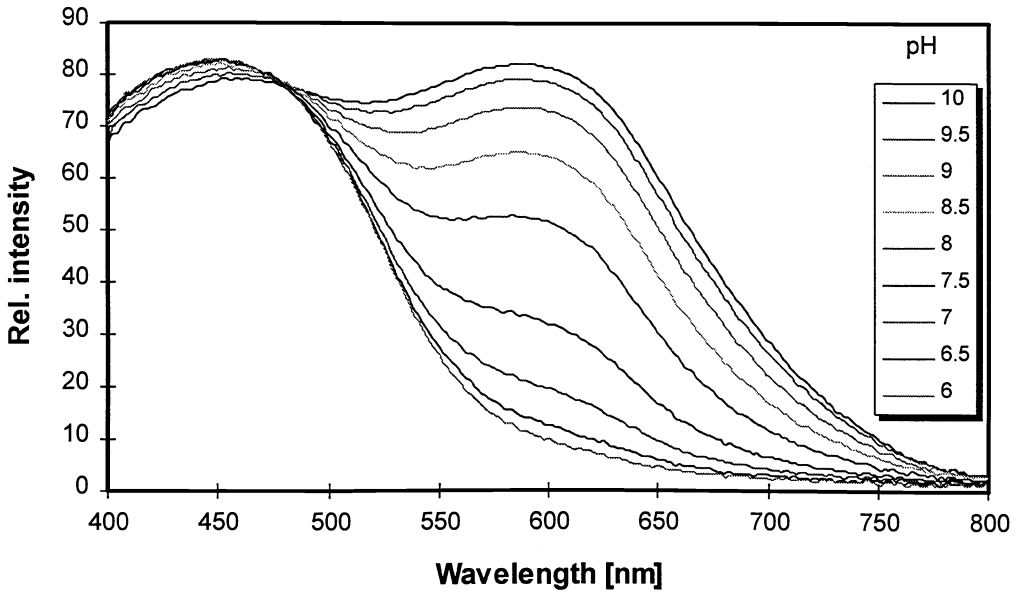


Figure 2: Absorption spectrum of N9

In the visible part of the spectrum there were two pH sensitive maxima at 450 and 590 nm. Both maxima fit to commercially available LED's. pH independent wavelengths were found at 480 nm and wavelengths > 800 nm. Such wavelengths are useful for a referencing system in order to determinate artefacts based on reflection in the sample. In Figure 3 the signal at the pH sensitve wavelength of 450 and 590 nm is shown as a function of pH.

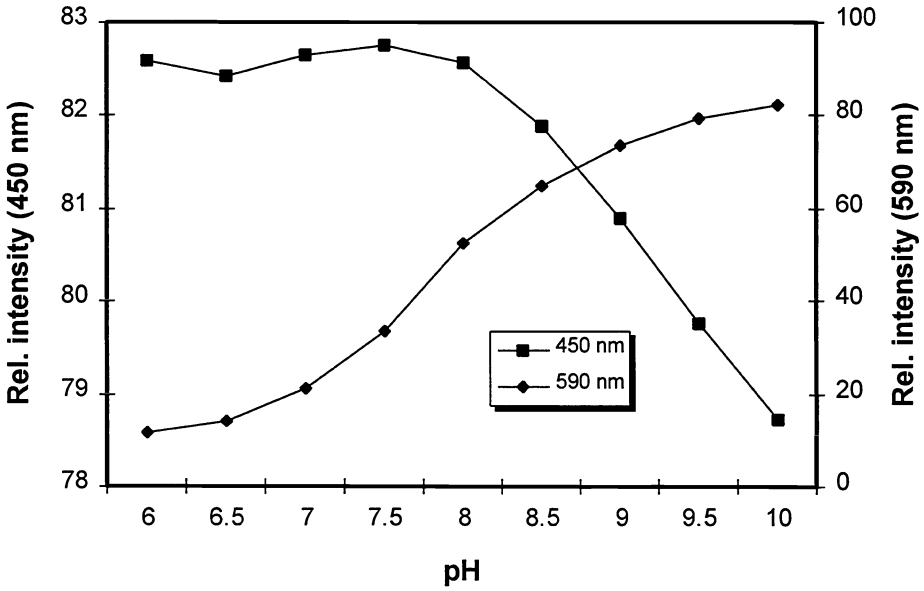
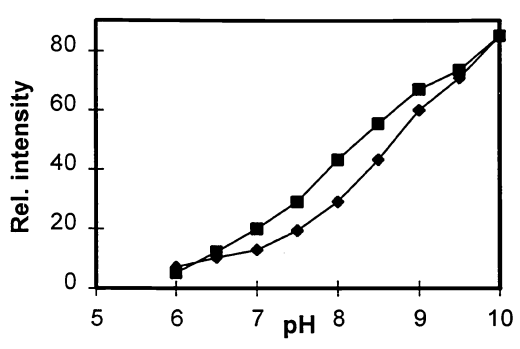


Figure 3: Signal of the blue (450 nm) and yellow (590 nm) absorption of N9 at different pH values.

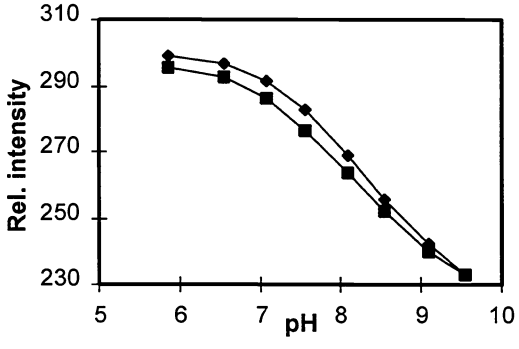
Measurements with yellow light showed a clearly detectable correlation between the pH and the intensity signal. The expected sigmoidal signal dependence was obtained with a pK value in the range of the seawater pH at 8.2. The non-pH sensitive amount of the signal was 10 % of to the total signal. In the blue part the conditions were reciprocal. In comparision to the yellow signal a high intensity was obtained at low pH values, the signal/noise ratio did not allow accurate measurements, but a comparision between both signals may allow an additional referencing system.

3.2 Sensor characterisation

The most important parameters of the pH sensor are the signal stability, the response time and the pK value of the used dye. In Figure 4 a) - c) typical calibration measurements in buffer solutions at a salinity of 35 ‰ are shown. The measurements were done from pH 6 up to pH 10 and down again to pH 6.



a)



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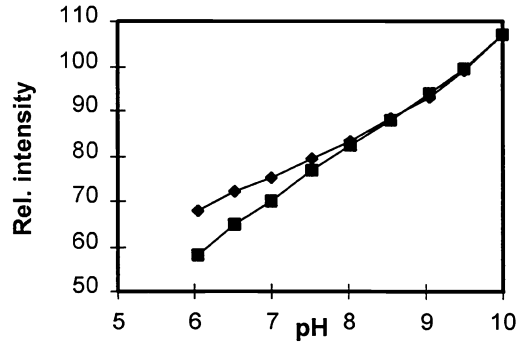


c)

Figure 4: Calibration measurements in buffer solutions of a) sensor type I (A-fl), b) sensor type II (A-fl, TDMA) and c) sensor type III (N9); [◆ up, ■ down]

In every measurement we obtained a decrease of the signal over time due leaching of the dye and/or a drift of the electronical part of the system. The sensor types I and III showed the expected sigmoidal curve. The sensor type II showed a different correlation. At low pH values a strong difference between the intensity values was measured. This could be caused by leaching of the TDMA and therefore a change of the matrix conditions. The hysteresis of sensor type I could be due to a long response time, where the matrix system didn't reach its equlibrium. The response times, drift behaviour and sensitivity oft the different pH optodes are summarized in Table 1.

Table 1: Characteristics of the investigated pH microsensors

Parameter	Type I (A-fl)	Type II (A-fl, TDMA)	Type III (N9-yellow)
range of max. sensitivity	pH 7.5-9	pH 7-9	pH 7-9
response time from pH 8 to pH 9 in 0.025 M buffer solution [min]	≈ 30	< 1	< 1
drift at pH 8 [pH units per hour]	≈ 0.2	≈ 0.2	< 0.05

The pH sensitve range of every investigated sensor was useful for application in marine systems. The response time of sensor type I would however be too slow for measurements in dynamic systems. Sensor type II would be applicable in the upper pH range. The response time and drift behaviour of sensor type III allows pH measurements in seawater systems under on-line and in-situ conditions. Therefore this sensor was choosen for more detailed investigations.
In order to investigate the influence of the salinity we made measurements at 3 ‰ and 38 ‰ salinity in a buffer solution, i.e. salinities ranging form brackish water to seawater levels. The measurements were done with a planar optode and the PMA-11. No significant difference in the spectral characteristic was detected in the different buffer salinities. In Figure 5 the absorption at 590 nm (corresponding to the yellow LED) is shown.

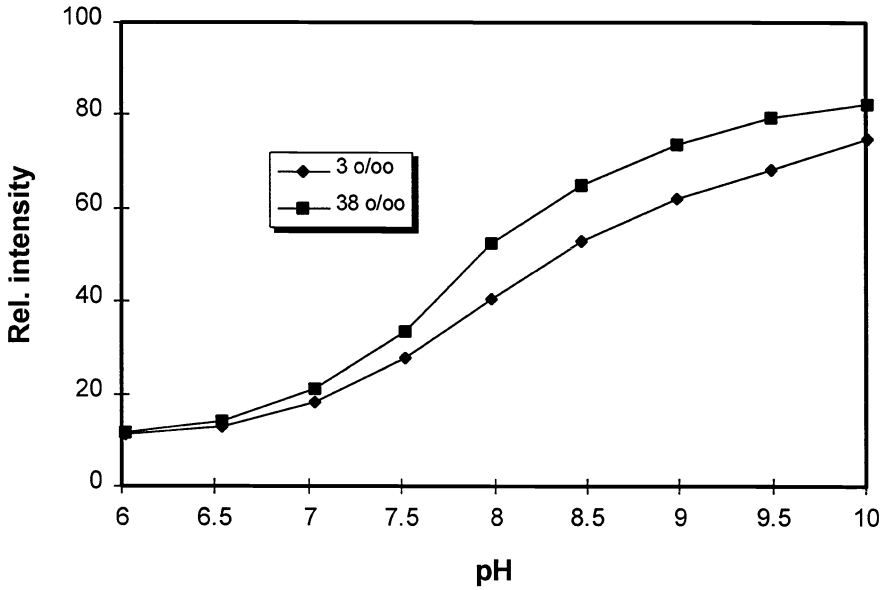


Figure 5: The yellow light absorption of immobilized N9 at different salinities

A pK shift between the measurements was observed. At lower salinities the pK was shifted to higher pH values. The difference was approximately 0.5 pH units between pH 8 and 9. Therefore, the calibration of the sensors must be done at the same salinity as is present in the environmental sample.

3.3 Microsensor application

Based on the absorption dye (sensor type I) we developped a pH microoptode and tested it in various marine sediments. In Figure 6 a pH profile in a sandy coastal North Sea sediment (Sylt, Germany) is shown.

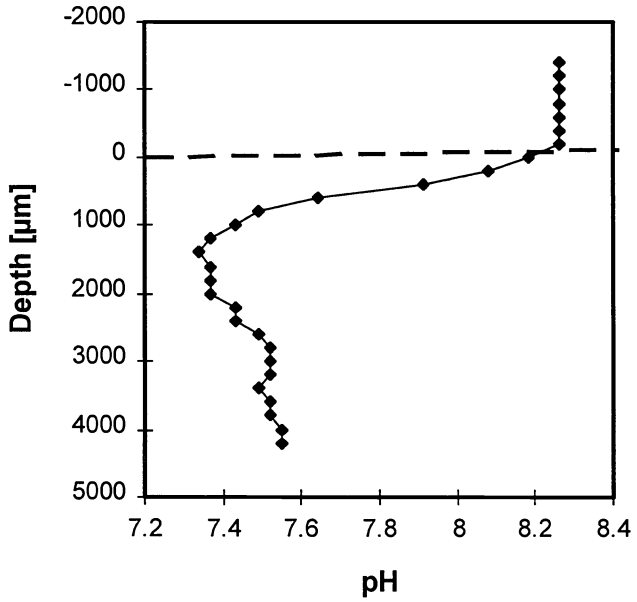
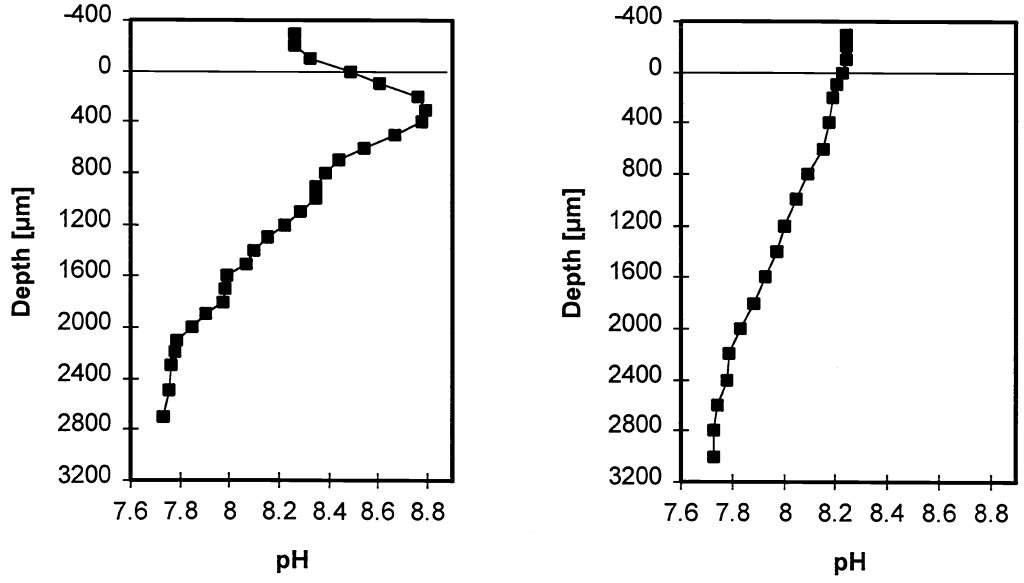


Figure 6: pH profile in a North Sea sediment.

In the water column we measured a constant pH value of 8.25. Inside the sediment the pH value decreased and reached a minimum of approximately pH 7.35 at 1.5 mm depth. In deeper layers, the pH value increased up to 7.55. The measured pH profile is typical for such sediments [9].
Another application was done in a microbial mat which was obtained from Solar Lake (Sinai, Egypt). The mat was immobilized in a layer of agar and placed in a laminar flow chamber. The measurements were performed in the presence of light from a halogen lamp (Figure 7 a) and under dark conditions (Figure 7 b).



a) b)

Figure 7: pH profile in a microbial mat a) in light and b) dark conditions

In the presence of light the pH increased and reached pH 8.75 0.4 mm below the mat surface. Below this depth pH decreased to pH 7.8 at 2.5 mm. In the dark a different profile was measured. The pH value decreased down to pH 7.8 at 2.5 mm depth similar to the measurement in the sediment. Illumination allowed photosynthesis in the upper layers [10], where cyanobacteria took up the carbon dioxide and thus the pH shifted to higher values.

4. Summary and Conclusions

We investigated 3 types of optical microsensors for the measurement of pH in seawater systems. The sensors showed a high sensitivity over the pH range found in marine systems. The response time of the pure sol-gel sensor (type I) was too high compared to drift phenomena. The sol-gel sensor with additional TDMA and the absorption sensor had faster response times. In this case smaller drift phenomena were neglectable because profile measurement can be performed in a short periode of time. The sensor type II (A-fl, TDMA) was useful for measurements of pH 8 and higher. At lower values the drift was too high. A better long term stability was reached by the absorption sensor (type III). The accuracy was better than ± 0.05 pH units (between pH 7 and 9). The application of this type of sensor in marine systems showed results which agree well with previous investigations in these systems. More detailed investigations of the temperature and salinity influence of the sensor have to be done in the future. A further demand will be the investigation of the mechanical and optical interaction of the sensor with coarse sediment sand very cohesive materials. For this a referencing system is necessary.

5. Acknowledgement

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