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A high-precision ratiometric fluorosensor for pH: Implementing time-dependent non-linear calibration protocols for drift compensation

Aron Hakonen, Stefan Hulth*

Department of Chemistry, GöteborgUniversity, SE-412 96 Göteborg, Sweden

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ABSTRACT

We present a versatile time-dependent non-linear calibration protocol for optical sensors, implemented on the pH sensitive ratiometric fluorophore 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) immobilized in ethyl-cellulose. The calibration protocol individually compensated for the progressive drift of calibration parameters, whereby sensor precision and accuracy, as well as applicable lifetime were improved. A severely reduced photoacidity was observed for the immobilized fluorophore, for which excited state dynamics was characterized and benefited from during measurements. Due to the significantly reduced photoacidity of HPTS immobilized in the ethyl-cellulose sensing membrane, a dual excitation/dual emission (F_1, ex/em: 405/440 nm and F_2, ex/em: 465/510 nm) ratiometric ($R_{F_1,F_2} = F_1/F_2$) sensing scheme could be used to amplify sensor response. The signal to noise (S/N) ratio was enhanced by ~400% utilizing the dual excitation/dual emission ratiometric sensing scheme, rather than the more commonly used protocol of dual excitation/single emission for HPTS fluorescence. Apparent pK_a of the fluorophore ranged from 6.74 to 8.50, mainly determined by the immobilization procedure. The repeatability (IUPAC, pooled standard deviation) over three pH values (6.986, 7.702 and 7.828) was 0.0044 pH units for the optical sensor, compared to 0.0046 for the electrode used for standardization. Sensor analytical characteristics were thereby in principle limited by the performance of the standardization procedure.

1. Introduction

The activity of hydrogen ions (pH) is a key analytical parameter reflecting the thermodynamic state of acid-base processes and overall balances between multiple reactions. In 1980, Peterson et al. [1] developed the first optical sensor (optode) for pH measurements utilizing absorbance of the indicator dye phenol red. Shortly after, the first fluorescence-based pH-optode was described [2]. Up to date, numerous optodes have been developed for pH-measurements, realizing a variety of immobilization and spectroscopic techniques [3–15]. Overall, optodes have many appealing features, of which no need for a reference signal is perhaps the most advantageous. Further, optodes are normally not sensitive to electrical interferences and are comparably easy to miniaturize, e.g. for in-vivo measurements [16–18]. Optodes are also suitable for high-resolution imaging of solute distributions in complex environments such as aquatic sediments [6], and there are exciting possibilities for multi-parameter and multi-analyte sensing [19–21]. Important drawbacks of most optical sensors include an inherent sensitivity to changes in ionic strength of the sample matrix [22]. They are also influenced by variations

* *Corresponding author*. Tel.: +46 31 772 2878; fax: +46 31 772 2785.
E-mail address: hakonen@chem.gu.se (A. Hakonen).
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in excitation light intensity, and often have a limited long-term stability due to leaching and photobleaching of the indicator dye [23–25]. In fact, optical sensors are commonly susceptible to a drift in sensor response, a phenomenon only rarely fully compensated for by appropriate analytical protocols.

There are, however, several spectroscopic techniques and analytical calibration protocols designed to normalize for signal drift and a sensor response not associated with changes in analyte activity. Such procedures include time and frequency-domain fluorescence lifetime measurements. Lifetime-based sensing schemes often demonstrate a significantly reduced susceptibility for intensity and wavelength dependent interferences [11,13]. Though lifetime-based sensing of fluorescence provides an analytical tool versatile for many applications, direct investigations on the quantitative importance and analytical character of drift during lifetime measurements are to the best of our knowledge missing. In addition, fluorescence lifetime measurements are usually associated with complex and costly instrumentation.

Ratiometric normalization schemes provide an alternative technique to time and frequency-domain fluorescence lifetime measurements. During the last few decades, ratiometric probes and ratiometric normalization schemes, more recently including time-domain dual lifetime referencing (t-DLR) [13], have frequently been used to reduce artifacts during reversible fluorescence detection [3,23]. Ratiometric procedures are feasible when the solute specific fluorescent probe induces a spectral shift in response to changes in analyte concentrations [3]. Preferably, changes in analyte concentrations are associated with signal amplification during the ratiometric protocol, i.e., the individual fluorescence signals are anti-correlated [25]. Reversible ratiometric probes have up to recently been in diminutive supply, but lately ratiometric probes have been developed for several ions e.g., Zn^{2+}, Mg^{2+}, F^-, PO_4^{3-} and molecular oxygen [26–30]. Until the study by Kurihara et al. [31], ratiometric analytical schemes were only applied on solute specific probes with intrinsic ratiometric properties. However, implementing ionophores as analyte carriers in conjunction with solvent (or polarity) sensitive fluorescent dyes that exhibit a spectral shift upon change of environment, has realized ratiometric sensing schemes for a progressively increasing number of solutes. Examples for which ionophores and principles of coextraction have been utilized for ratiometric solute detection include Na^+ and NH_4^+ [31,32]. The ratiometric normalization procedure is normally assumed to remove artifacts that induce a sensor response not related to changes in analyte concentrations, e.g., variations in ambient and excitation light intensities, uneven dye concentration, photobleaching and wash-out of the indicator dye [23]. However, there are at present only few investigations that have qualitatively and quantitatively evaluated the efficiency of the ratiometric approach. For example, Stromberg and Hulth [25] demonstrated that variations in excitation light intensity (10–100% incident light) were found more or less completely eliminated by a ratiometric sensing scheme. It was, however, not possible to compensate for artifacts associated with fluctuations in temperature, effective probe concentration, and sample ionic strength.

Analytical improvements to normalize and control signal drift over time have encompassed a ratiometric sensing scheme coupled to a time-correlated calibration procedure [33,34]. The main objective of this study was to further evolve the time-correlated ratiometric calibration procedure to also include non-linear parameterization for quantification. To apply this we also developed a fluorescence-based ratiometric pH sensor that is straightforward and quick to manufacture for high-precision detection of pH.

2. Experimental

2.1. Materials

To prepare the sensor layer the ratiometric fluorophore 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS; 2.0 mg of the tri-sodium salt; n_{HPTS} = 3.70 μmol, >97%, Sigma–Aldrich) was dissolved in tetraoctylammonium (TOA^+) hydroxide solution (200 μL of 10% TOA^+ in methanol, n_{TOA+} = 33.3 μmol, Purum, Fluka) and diluted with ethanol (800 μL, 99.5%, Kemetyl AB). Ethyl-cellulose solution was prepared by dissolving 200 mg ethyl-cellulose (48% ethoxyl content, Sigma–Aldrich) in an ethanol (1400 μL, 99.5%, Kemetyl AB)/toluene (2000 μL, HPLC grade, Fisons Scientific Equipment) mixture. The sensor cocktail was finalized by mixing 320 μL HPTS solution with 680 μL cellulose solution. The cocktail was spin-coated on a ~100 μm thick transparent plastic film (Brilliant transparency film, Art. no. 901121). Deionized water utilized for buffers was further purified by a Milli-Q system (Millipore Corp.) to a resistivity of >18 MΩ cm^{-1}. Phosphate buffers were prepared from Milli-Q and H_3PO_4 (85%, J.T. Baker), where pH and ionic strength were adjusted by appropriate additions of NaOH (p.a., Merck) and NaCl (p.a., Merck).

2.2. Instrumentation

Spin-coating of the sensor cocktail was performed by a spin-coating device made in-house, resulting in a sensing layer thickness of ~10 μm. The final thickness was verified by fluorescence microscopy (Leica Microsystems DMI 6000B). The sensor was mounted onto a plexiglass support and secured in a specially designed flow-through plastic cuvette (3 mL), placed in the spectroflourometer (Fluoromax-3, SPEX Instruments Int. Inc.). To minimize background noise and reflection from incident light, the angle of incidence for the excitation light was set to 60°. Matrix scans (excitation 300–480 nm, emission 430–550 nm) were performed at pH 6.25 and 9.20 to determine the optimal excitation/emission wavelength pairs for this pH interval. The ratiometric pH response (R_{F_1,F_2}) of the sensor was determined as the ratio between F_1 and F_2 (i.e., $R_{F_1,F_2} = F_1/F_2$). Matrix scans were performed at 1.00 nm resolution, 0.5 s integration time and 2.00 nm slit widths.

Working solutions for calibrations were prepared by mixing different ratios of two phosphate buffer solutions of pH 6.00 and 8.50 (ionic strength; I_{Tot} = 3.0 mM and $[PO_4^{3-}]_{Tot}$ = 1.95 mM). Mixing of the two buffers was performed on-line with an HPLC pump with multiple input solutions and mixing chambers. The mixed solution was pumped into the flow-through cuvette secured in the spectrofluorometer. The pH of the final solution was measured on-line with a pH-electrode (model 6.0232.100, 780 pH meter; Metrohm Ltd.),