Direct UV-LED lifetime pH sensor based on a semi-permeable sol-gel membrane
immobilized luminescent Eu\textsuperscript{3+} chelate complex

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Abstract

A new optical luminescent-lifetime pH sensor based on the sol-gel doped europium chelate has been developed. Near-UV to visible (350-400) nm excitation of the europium (III) chelate of a 4-trifluoromethylenecarbostyrl derivative of diethylenetriaminepentaacetic acid (DTPA) exhibits a typical lanthanide line-like emission, the highest peak being at 615 nm. Due to antenna-mediated effects, the heterocyclic chromophore (parent emission at 450 nm) transfers its energy to the close europium having isolated very weak quantum efficiency. The complex was co-immobilized with the non-fluorescent pH indicator bromothymol blue (BTB) in a semi-permeable sol-gel layer. It can be shown, that not only the line emission spectra but also decay lifetimes depend strongly on pH changes. Since the sensitive dyestuff is highly stable and not quenched by oxygen, a sensor in the most interesting pH range 4 to 9.5 could be produced, having its highest sensitivity at around physiological pH.

Keywords: quinolin-2-one, europium-complex, bromothymol blue, sol-gel, pH sensor, luminescence decay time
**Introduction**

Sol-gel doped indicator species can provide an active layer for pH optical lifetime-based sensors, where the analyte affects the indicator decay time, a parameter that is conveniently independent of the signal intensity [1-3]. Especially the long-lived species, such as lanthanide and transition metal complexes, which display decay times between some hundreds of nanoseconds up to several milliseconds, can be useful in applications for decay time sensing. Lifetime methods using such complexes offer excellent suppression of stray light and other undesirable optical background signals, firstly because of their convenient temporal variation, which distinguishes them from constant background light levels, and secondly because the luminescence of most useful chelates lasts much longer than elastic scattering and short-lived fluorescence from many other contaminants [3-6].

Our recent state-of-the-art survey has revealed several lifetime-based pH sensors. An illustration, which uses the pH-dependence of photo-induced electron transfer (PET) from an intermediate compound, was presented [7]. It is a UV-excitable pyrene derivative having a lifetime about 100 ns. Indicator Resorufin, doped in a sol-gel copolymer was proposed as lifetime-based sensor [2]. The Resorufin pH dependence was also exploited in a fibre optic sensor [8]. Recently, a decay time-based sensor incorporating a fluorescent indicator Acridine into a Nafion matrix was presented, being most sensitive between pH 8 and 10 [9]. Complexes based on ruthenium (Ru) and rhenium (Re) were successfully applied in different optodes [10,11]. Another strategy to realize lifetime-based pH optodes is the use of so-called "Förster-type" fluorescence energy transfer (FET), which occurs from a pH-insensitive luminescent donor to a pH-sensitive coloured acceptor. Recently, FET-based optodes have been realized, where the decay time is measured as a useful analyte-dependent parameter. These are sol-gel and hydrogel (polyurethane type) doped optodes, incorporating Texas Red
Hydrazine (TRH) and Ru-complexes as donors and pH indicator dyes, such as Bromothymol blue and N-9 (Merck type), as receptors [1,12-14].

The encapsulation of a number of lanthanide ions, mainly Eu(III) and Tb(III), in the ligand-type shielding cages offer the possibility of meeting the optical requirements for many new potential applications [15,16]. Long-lived luminescent lanthanide antenna-chelates are excellent alternatives to classic fluorescent sensor dyes especially when probes display significant autofluorescence [17]. Such dyestuffs can be also effective tools to investigate (static- or time-varying) inter- and intramolecular distances using energy-transfer experiments [18]. The advantage of lanthanide chelates over other transition metal complexes, such as Ru- and Re-complexes, is that lanthanides display much longer decay times [19] (from several micro-seconds up to several milli-seconds) than the latter [20,21] (usually several hundreds of nano-seconds) thus enabling easier temporal variation of measurements [22,23]. Lanthanides also display specific line-like emission peaks, which is not the case of Ru- and Re-complexes.

In a recent review on (fiber) optical chemical sensors and biosensors, indicator dyes immobilized on sol-gels can be found for different sensor schemes and for various analytes [24]. Among them are optical sensing of pH, ammonia, ionic species and oxygen [24,25]. The attraction of the sol-gel method, as a basis for the immobilization of a new family of europium-based hybrid materials has been demonstrated [26-28]. These xerogels are obtained as transparent elastomeric monoliths, which are essentially amorphous and thermally stable (up to 200 °C). They form novel multi-wavelength emitters, in which the narrow reddish Eu⁴⁺ luminescence merges with broad blue-green emission of the hybrid backbone. Eu⁴⁺ and Tb⁴⁺ complexes, both incorporated in a sol-gel matrix, have been reported by Parker in intensity-based pH sensors [29] or as an oxygen sensor [30]. Gunnlaugsson et al presented the incorporation of europium complex using a quinaldine chromophore into a water-permeable methylmethacrylate type hydrogel based on luminescence intensity measurements [31]. Here,
complex diffusion out of the membrane was observed after a prolonged soaking period. In contrast, the sol-gel process has the advantage of non-leachable entrapment of water-soluble indicators, without the need to add reactive groups to provide covalent bonding [32-34].

The present work is developed from our previous approach for pH sensing [26], where varying Eu\textsuperscript{3+} emissions based on the absorption of antenna generated luminescence by the pH indicator Bromothymol blue (BTB) in a sol-gel membrane were used. Here, we demonstrate that the same optode scheme is useful for decay time sensing. A setup using the same dyestuff combination to measure pH changes by carbon dioxide has been published by a Japanese group [35,36]. A BTB indicator has previously been utilized by Kessler [37] to study the dynamic quenching of time-resolved luminescence in an aqueous solution using a europium terpyridine chelate as the antenna chromophore. In our work, a solid phase version using a long wavelength antenna dye is advanced. This leads to the use of sensors working with a simple LED emitting close the visible region [38,39]. Eu\textsuperscript{3+}-complex and BTB were co-immobilized, both trapped within a sol-gel layer under acidic conditions on a glass support, providing a pH-sensitive optode layer with decay times in the microsecond range. Our heterocyclic antenna is a modified carbostyril [40,41], which shows a broad excitation maximum at 370 nm, useful in the range between 350 and 400 nm. A lifetime-based pH sensor incorporating a lanthanide complex in a sol-gel polymer support has not been introduced yet. Moreover, compared to Ru- and Re-complexes, it displays narrower emission bands and by about 500 times longer decay time. Eu\textsuperscript{3+}-chelate complex was non-leachably entrapped in a sol-gel and the sensor showed reproducible results.

**Experimental**

*Reagents and solutions*
A combination of Eu\(^{3+}\)-complex and bromothymol blue (BTB, from Aldrich, prod. No. 11,441-3) was entrapped in a sol-gel matrix using a commercial precursor tetramethoxysilane (TMOS, from Fluka, prod. No. 87,682). For pH testing, buffer solutions of defined pH were prepared using standard analytical grade citrate, phosphate and carbonate buffers (from Fluka). All buffer compositions were adjusted to constant ionic strength by adding analytical reagent grade potassium chloride.

**Instrumentation**

Continuous fluorescence measurements were performed with a Shimadzu (Kyoto, Japan) RF-5001PC spectrofluorometer. Sensing layers were mounted in a self-constructed flow-through cell to cover the inner wall. Light from 150 W xenon source was directed to the sensing position at an angle of typically 35\(^{\circ}\), and fluorescence was measured after passing the emission monochromator at a typical slit width of 5 nm.

Unless otherwise described, separate check of pH value was taken with a commercial, thermally-compensated pH-meter (from WTW, Germany and type pMX 2000).

**Lifetime measurements**

The decay time characterization of the fibre optic sol-gel sensor was performed as an arrangement suitable for interrogation of short fluorescence lifetime (Figure 1). Compared to the possible arrangement via an optical fibre probe for both excitation and fluorescence collection, the present arrangement improves the signal-to-noise ratio, because the luminescent coating is directly illuminated by a UV LED via optical filters and the light is collected with a convenient optical fibre collector probe. The illumination arrangement (a) illuminates a large coating area, (b) reduces a significant optical power loss that would
otherwise occur when coupled from the LED into an optical fibre, (c) it also reduces the background auto-fluorescence signal that might be generated from various optical components, if the light were passed to and from the sample using common optical and fibre-optical paths for the launch and return signals. The fibre probe is then fed back into the interrogation system. From here it falls, via suitable long-pass optical filtering for removing residual excitation light, onto a PMT (Hamamatsu H6180) which operates in a photon counting mode. Custom electronics and computer software allow either real time measurement of fluorescence lifetime via a rapid lifetime detection (RLD) system [42,43] or a measurement of the fluorescence decay response as a function of time, as used in our experiments.

Figure 1

For the reproducibility evaluation, the sensor layer was tested for its time dependent luminescence measurements using a commercially accessible Varian Cary Eclipse Spectrometer at 20 °C. The set up parameters were used as follows: PMT voltage: high, 1 flash, delay time (gate time): 0.05 ms, Ex/Em slit: 5/5, acquisition cycles: 50, total decay time: 5 ms. Lifetime calculation was performed using the built in software over the span of at least 7 lifetimes.

Preparation of sol-gel sensor layer

The indicator layer was prepared as follows: 2 mg of Eu$^{3+}$-complex and 1.3 mg of BTB were diluted in 2.5 mL of Ethanol. After a minute of sonication, 2 ml of TMOS precursor was added. After an additional minute of sonication, the sol-gel process proceeded under acidic conditions by adding 1 mL of 1×10^{-3} mol/L HCl. 5 minutes sonication was finally carried out. After 24 hours, the glass slides (10 × 15 × 0.19 mm$^3$) were dipped into this sol-gel cocktail
and allowed to dry for 2-4 h in ambient atmosphere and finally at 70 °C for 24 h. For glass slides activation procedure, see details in [26].

Results and discussion

Selection of materials

A sol-gel glass based on TMOS was employed as our preferred matrix for use in pH sensing rather than hydrogel or PVC [12]. Sol-gel technology uses water and ethanol as starting components, which is very suitable for the actually hydrophilic Eu$^{3+}$-complex dye. Its molecule and spectral characteristics are shown in Figure 2. Although Eu$^{3+}$-complex luminescence in water is diminished above pH 6.5, its entrapment into the sol-gel net keeps its emission intensity stable in aqueous medium of pH between 2 and 10. In contrast, our preliminary experiments in hydrogel polymer showed similar properties of Eu$^{3+}$-complex as in water, but significant leaching of the complex was observed. Sol-gel material was also chosen because of its excellent adhesion to glass substrates, as required to provide good mechanical stability of sensing layers. Furthermore, sol-gels allow high indicator loading, display adequate ion permeability and have excellent optical transparency.

It was initially found that a Eu$^{3+}$-complex, which showed useful pH sensitivity in aqueous solution, lost this sensitivity when immobilized in sol-gel matrices. In order to recover this property, the pH indicator BTB was added to the starting sol-gel components and it was found that the useful pH response that was present in water was not only restored, but was actually improved on. The BTB was selected to design an optical sensor for the pH range between 4 and 9, covering thus the physiological range. Note that Eu$^{3+}$-complex in the absence of BTB in sol-gel matrix showed no decay time dependence on pH.

Figure 2
Lifetime measurements

Fluorescence lifetime interrogator performance

A common problem with fluorescence interrogators is auto-fluorescence that is fluorescence induced in the optical coupling components or on contaminated optical surfaces by excitation light. The auto-fluorescence can cause a constant undesirable background that can also exhibit its own fluorescence decay. For checking for the presence of such signal, a fluorescent decay function was recorded with a LED and source filters were aligned to point directly into the fluorescence-detecting probe. By coupling a much larger portion of excitation light into the capturing probe, diffused scattering reflection from the slide or the presence of lower levels of any possible auto-fluorescence could be observed. This is certainly also a good test that the source filters and detector filters do their required job of (a) removing the source light in the spectral region where fluorescence is expected to be measured and (b) removing any elastically scattered source light from the layer from the desired fluorescent light detection. The received detector light showed that the filters effectively blocked the undesired light, and the optical decay function of the luminescent light was plotted to be compared with the normal decay function taken with the experimental arrangement, as shown in Figure 3. The received auto-fluorescence signal intensity was found to be almost unnoticeable, less than 0.5%, and was, therefore, assumed to be negligible.

Figure 3

Luminescence decay profiles of Eu\(^{3+}\)-complex-impregnated coating were then examined, while the coatings were immersed in three different pH buffers (pH 4.0, 7.0 and 9.2). The pH of buffer solutions was verified using a commercial electrode-type pH meter (Jenway 3030) before each experiment. After the mathematical retrieval using the non-linear regression function in Sigma Plot 2000, and fitting the data to the equation (1), the luminescence lifetime
of the coating was investigated. Decay times vary strongly with pH and are plotted in Figure 4.

\[ I = y_0 + a \cdot e^{-b \cdot t} \]  

Figure 4

At pH 4, the Eu\(^{3+}\)-complex lifetime is about 640 \(\mu\)s. Decay time is decreased by about 1.6-fold at pH 7 and by about 2.4-fold at pH 9.2. Ionic strength used in this experiment was 0.1 (KCl); decay time was determined after 15 minutes soaking time between measurements. By linear fitting an equation \( y = -68.54x + 891.6 \ (r^2 = 0.996) \) was obtained, where \( y \) represents the sensor lifetime and \( x \) is the pH. The two values recorded at pH 7 were taken at the beginning and at the end of the experiments demonstrating the excellent reversibility. Our linear working response is between pH 4.8 and 9.5 and is shown in the inset of Figure 5.

Figure 5

Effect of ionic strength

In addition to the previous measurement (Figure 4), a second experiment (Figure 5) was performed on a Varian spectrofluorometer in order to check the reproducibility of the sol-gel doped Eu\(^{3+}\)-complex coating and the possible effect of ionic strength on the sensor performance.

Variations in the ionic strength are known to cause very high changes in the reading of optical sensors [26,44], although a study also has been proposed where pH fluorescence sensors were developed with negligible sensitivity towards ionic strength interference [45]. However, in our case, ionic strength affected the performance of the lifetime-based pH sensor. The effect of ionic strength on the sol-gel layer was tested with buffer solutions adjusted with KCl (I = 0.1, 0.5 and 1.0). It was found that with increasing ionic strength, the apparent pK\(_a\) values shifted to lower values: from pK\(_a\) 7.0 (I = 0.1) to 6.1 and 5.1, respectively. Not only the
pKₐ values of the sensor layer shifted, but also the sensor sensitivity is significantly reduced from 50% of the total signal to 25% at ionic strength 1. Figure 5 shows decay time values obtained at the most common ionic strength 0.1 over the pH range between 3 and 10. At pH 3, the decay time is about 400 μs and rises up to 710 μs at pH 4.3. Then, the signal decreases linearly with rising pH and shows at pH 10 again the lifetime of 400 μs. The lifetime decreases by 45% from pH 4.3 to pH 10. At the pH of the apparent pKₐ, 7, the decay time was 570 μs. Interestingly, europium decay times around 600 μs are found most often in the literature for aqueous solutions [46,47]. Hence, the environment for the complex entrapped inside the sol-gel should mimic aqueous conditions.

Finally, the data obtained from the fluorescence lifetime interrogator (A; Figure 4) and from the Varian fluorometer (B; Figure 5) were compared, namely, by the slopes of the obtained linear fits. The slope values are -68.54 and -68.10 for A and B, respectively. The estimated deviation between the two values is minimal, about 0.6%. We can, therefore, conclude that the two instruments gave comparable data and the pH lifetime sensor performance was reproduced.

Comparison with known developed optical lifetime-based pH sensors

The established Eu³⁺-complex/BTB sol-gel doped lifetime-based sensor represents a promising new device that displays specific advantages over other reported sensors based on the specific luminophore pH dependent decay emission. Table 1 gives a comparison between the developed lifetime-based pH sensors and ours with respect to various parameters, such as indicator used, polymer matrix, operating pH range, pKₐ, and decay time.

Hydrophilic D4TMI-PEG-J and hydrogel polymers were used to incorporate Ru- and Re-complexes [7,10-14], while sol-gel and its PVA copolymer, hydrogel and nafion matrixes
were used to incorporate various other fluorophore systems, such as TRH/BTB [1], Resorufin [2], DaPy [7] and Acridine [9]. While hydrogel [1,14] and D47MI-PEG-J [10] are noted to cause indicator leaching, the sol-gel matrix for Eu$^{3+}$-complex/BTB sensor provides a stable membrane, where no component leaching was observed at all.

The approximate lifetime values at pK$_a$ range for short-lived fluorophores [1,2,7,9] from 0.003 up to 0.160 µs, whereas for Ru- and Re-based indicators [10-14] the decay time is about 1 µs. The latter is by about 500-fold shorter than the Eu$^{3+}$-complex/BTB system displays it. Such long decay times of lanthanide-complexes are especially useful in time-resolved luminescence technique, where the discrimination of interfering fluorescence is of key importance.

Looking at the operating ranges in Table 1, it can be seen that sensors mostly operate in the range over 2 to 3 pH values, except the one based on hydrogel doped diethylaminomethyl pyrene sensor [7], spanning 4 pH values and our sensor, spanning 4.5 pH values. The wide working range of Eu$^{3+}$-complex/BTB is corroborated by the advantage of having the pK$_a$ value at a neutral pH 7. This is a useful sensor property, which enables the determination of pH in some certain biological samples (human blood for instance), where little deviations around pH 7 might cause irreparable consequences. Neutral and near-neutral pK$_a$ values are also exhibited by the TRH/BTB system [1], Resorufin [2], Re-complex [11] and Ru(didipy)/N9 [14], whereas the diethylaminomethyl pyrene derivative sensors [7], have the pK$_a$ moved to pH 7.7, the acridine pK$_a$ is at 9 [9] and Re- and Ru-complexes may have pK$_a$ values at around 8 [11,12]. The Ru(Ph$_2$-phen)$_2$DCbpy D4TMI-PEG-J doped sensor was described for working in acidic conditions [10].

Table 1

<table>
<thead>
<tr>
<th>Sensor</th>
<th>pH Range</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH/BTB</td>
<td>2-3</td>
<td>Mostly operates</td>
</tr>
<tr>
<td>Resorufin</td>
<td>2-3</td>
<td>Neutral and near-neutral pK$_a$ values</td>
</tr>
<tr>
<td>Diethylaminomethyl pyrene</td>
<td>4</td>
<td>Widely working range</td>
</tr>
<tr>
<td>Acridine</td>
<td>9</td>
<td>pK$_a$ at pH 7.7</td>
</tr>
<tr>
<td>Ru(didipy)/N9</td>
<td>8</td>
<td>Neutral and near-neutral pK$_a$ values</td>
</tr>
<tr>
<td>Ru(Ph$_2$-phen)$_2$DCbpy D4TMI-PEG-J</td>
<td>4.5</td>
<td>pK$_a$ at pH 7.7</td>
</tr>
</tbody>
</table>

12
Conclusions

We have described a new lifetime-based sensor using a long-lived Eu$^{3+}$-complex. Together with a non-fluorescent pH indicator bromothymol blue, it was co-immobilized in a sol-gel matrix. The sensor layer's resistance against leaching and photostability were excellent. The sol-gel polymer showed good mechanical stability of sensor layers, adequate ion permeability and excellent optical transparency. The long decay time of the europium complex (several hundreds of microseconds) is advantageous, because it enables us to discriminate all the background, short-lived fluorescence, by simple temporal gating, so that only changes the europium lifetime are observed. The complex has the strongest peak emission at 615 nm. The technique is insensitive to inconveniences such as optical misalignment or sample turbidity, which are usual obstacles in intensity-based schemes [41]. Since many published luminescent sensors require high UV excitation frequencies and, consequently, more expensive light sources [10], the utilization of the blue LED in our case, places the pH sensor among cheaper devices. The presented illumination arrangement for interrogation of short fluorescence lifetime improves the signal-to-noise ratio because the sol-gel coating is illuminated directly with a filtered UV LED source, rather than via an optical fibre probe. Ru- or Re-complex based lifetime-based pH sensors display shorter decay times [20] than our Eu$^{3+}$-complex, may have pK$_a$ values moved to acidic pHs [21] or narrow sensor operating range [11]. In contrast, our sol-gel doped Eu$^{3+}$-complex luminescent pH sensor is privileged because it displays much larger “aqueous type” lifetimes (570 µs at pH 7), wider working range (linearity spans between pH 4 and 9.5) and a neutral pK$_a$ value (7). Such physiological pH range is the most interesting to be observed, therefore, the sensor seems to be suitable for applications where near-neutral conditions have to be controlled. Nevertheless, the main negative aspect of the presented sensor is that it shows high dependence on the ionic strength, which might possibly limit its utilization.
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References


Biographies

Matejka Turel received her Ph.D. from the University of Maribor, Faculty of Mechanical Engineering (Slovenia) in 2006 after her research work on the field of luminescent determination of inorganic water parameters using the lanthanide complexes. She is currently a member of the Centre of Sensor Technology of the Faculty of Mechanical Engineering in Maribor. Her research interests are in the area of analytical chemistry, mostly in optical chemical sensing using the lanthanide chelates.

Merima Čajlaković received her M.Sc. from the University of Ljubljana, Faculty of Chemistry (Slovenia) in 2002. She worked on a field of analytical chemistry and sol-gel based optical sensors for determination of hydrogen peroxide and pH. Since spring 2004 she has been employed as a research scientist at the Institute of Chemical Process Development and Control at Joanneum Institute (Austria). Within the field of Opto-chemical gas Sensors (O₂ and CO₂) she is currently working on her dissertation.

Ed Austin completed his Ph.D. in optics from the University of Southampton (Great Britain) in 2002, after researching novel methods for gas sensing using fluorescence from immobilized chemical indicators. This involved theoretical studies to optimize systems, and construction of prototype instruments. Ed’s following areas of study have included: optical gas sensing using correlation spectroscopy, microstructured fibre gas sensors, remote gas sensing via multi-km fibre links, sensors for oceanographic measurements, optical fibre ASE source enhancement and optimization, satellite-mounted remote sensing instrumentation and gas sensing at very low pressures.
John P Dakin completed his Ph.D. and is nowadays a Research Professor at Southampton University, supervising research and development in optical instrumentation, having particular interests in optical fibre sensors and other optical measurement instruments. He has since spent two years in Germany at AEG Telefunken, twelve years at Plessey UK and two years with York Limited/York Biodynamics before returning to the University. He is the author of over 200 technical and scientific papers, and over 120 patent applications, and has edited or co-edited six books on optical fibre sensors. He has recently co-edited a major reference book "Handbook of Optoelectronics" for CRC press. He was previously a visiting professor at Strathclyde University (UK) and was the technical programme committee chairman of the major OFS '89 Conference in Paris. He is frequently an invited speaker and chair at major international conferences, and has served on the editorial board of a number of optoelectronics journals. Professor Dakin has won a number of awards, including "Inventor of the Year" for Plessey Electronic Systems Limited, the Electronics Divisional Board Premium of the IEE and open scholarships to both Southampton and Manchester Universities. He has also been responsible for a number of key electro-optic developments.

Georg Uray is a Professor of Organic Chemistry at the Department of Chemistry at Karl-Franzens University Graz, Austria. His fields of interest are preparation of chiral stationary phases ("ULMO") and HPLC analysis of enantiomers and the optimization of luminescent dyes, especially studies in the series of carbostyrils and their function as antenna molecules for lanthanide ions.

Aleksandra Lobnik is a Professor at the University of Maribor, Faculty of Mechanical Engineering, Slovenia, where she is leading Centre of Sensor Technology. She has got her Ph.D. degree in chemistry from Karl Franzens University in 1998 in Graz, Austria. Her research experiences are in development and application of various polymers, especially sol-
gel materials in the area of optical chemical sensors. She has been working in the field of optical chemical sensors for several years and she has published over 100 scientific and conference papers on this topic. She is frequently an invited speaker and chair at major international conferences.
Figures

Figure 1: Optical arrangement for the interrogation of the weak fluorescence signals

Figure 2: Europium complex and its electron spectra in buffer of pH 6.5 (continuous mode)

Figure 3: Comparison between the auto-fluorescence signal and the Eu$^{3+}$-impregnated coating signal (sol-gel layer)

Figure 4: Sol-gel doped Eu$^{3+}$-complex decay times, measured as a fluorescence lifetime interrogation performance at pH 4, 7 and 9.2; soaking time = 15 min, I$_{KCl}$ = 0.1

Figure 5: Sol-gel doped Eu$^{3+}$-complex decay times, versus pH range from 3 to 10, inset - linear sensor operating range; soaking time = 15 min, I$_{KCl}$ = 0.1 measured on a Varian spectrofluorometer
Table

Table 1: Figures of merit for optical lifetime-based pH sensors
Luminescence from Eu-indicator coated slide (in pH 9 buffer)

Auto-fluorescence baseline
Figure 4
Click here to download high resolution image

\[ y = -68.54x + 891.6 \]
\[ r^2 = 0.996 \]

\[ \tau \text{ (\mu s)} \]

\[ \text{pH} \]
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Indicator</th>
<th>Polymer</th>
<th>Operating pH range</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
<th>τ&lt;sup&gt;a&lt;/sup&gt; (in µs)</th>
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<tr>
<td>[1]</td>
<td>Texas Red Hydrazide + BTB&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sol-gel</td>
<td>6 – 8</td>
<td>7</td>
<td>n.d.&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>[2]</td>
<td>Resorufin</td>
<td>Sol-gel/PVA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.8 – 8</td>
<td>~ 6.5</td>
<td>0.003</td>
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<td>[7]</td>
<td>DaPy&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Hydrogel (PU&lt;sup&gt;f&lt;/sup&gt;)</td>
<td>6 – 10</td>
<td>7.7</td>
<td>0.160</td>
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<tr>
<td>[9]</td>
<td>Acridine</td>
<td>Nafion</td>
<td>8 – 10</td>
<td>~ 9</td>
<td>0.019</td>
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<tr>
<td>[10]</td>
<td>Ru(Ph&lt;sub&gt;2&lt;/sub&gt;-phen)&lt;sub&gt;2&lt;/sub&gt;DClpy&lt;sup&gt;x&lt;/sup&gt;</td>
<td>D4TMI-PEG-J&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 – 5</td>
<td>~ 3.5</td>
<td>0.850</td>
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<td>[11]</td>
<td>Re-complex&lt;sup&gt;y&lt;/sup&gt; (probe) + Re-complex&lt;sup&lt;y&lt;/sup&gt; (reference)</td>
<td>D4TMI-PEG-J&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 – 9</td>
<td>~ 7.4</td>
<td>1.3</td>
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<td>[11]</td>
<td>Re-complex&lt;sup&lt;y&lt;/sup&gt; (probe) + Re-complex&lt;sup&lt;y&lt;/sup&gt; (reference)</td>
<td>D4TMI-PEG-J&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 – 9</td>
<td>~ 7.9</td>
<td>1.2</td>
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<td>[12]</td>
<td>Ru(dph-bpy)&lt;sup&gt;mm&lt;/sup&gt; + BTB&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hydrogel (PU&lt;sup&gt;f&lt;/sup&gt;)</td>
<td>6.5 – 9</td>
<td>~ 8</td>
<td>1.2</td>
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<td>[14]</td>
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<td>7.4</td>
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<td>[r]</td>
<td>Eu-complex&lt;sup&gt;x&lt;/sup&gt; + BTB&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sol-gel</td>
<td>4 – 9.5</td>
<td>7.0</td>
<td>570</td>
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Approximate lifetime at pKₐ

Bromthymolblue

not defined data. Tetra Red Hydrazide belongs to a Rhodamine class of fluorophores, the sensor lifetime is, therefore, supposed to be in nanosecond range [20]

Polyvinylalcohol

Diethylaminomethyl pyrene

Polurethane

[Ru(Ph₂-phen)₂DCbpy]²⁺; (Ph₂-phen) = 4,7-diphenyl-1,10-phenanthroline,

(DCbpy) = 4,4’-dicarboxy-2,2’-bipyridine]²⁺

D4TMI-PEG-Jeffamine polymer – see [10] for synthesis

Re(CO)₃(t-but₂bpy)(py-3-OH)ClO₄; (t-but₂bpy) = 4,4’-bis(tert-butyl)-2,2’-bipyridine,

(py-3-OH) = 3-hydroxy pyridine

Re(CO)₃(phen)(ppp)ClO₄; (phen) = 4,7-diphenyl-1,10-phenanthroline,

(ppp) = 4-(3-phenylpropyl)pyridine

Re(CO)₃(phen)(py-3-OH)ClO₄; (phen) = see ³, (py-3-OH) = see ¹

Ru-tris-4,4’-diphenyl-2,2’-bipyridyl

Ru-tris(diphenyl-diprydyl) perchlorate

N9 – Merck pH indicator

Eu-4-trifluoromethyldicyostyl derivative of diethylenetriaminepentaacetic acid

[τ] Our sensor