Absorbance-based pH Sensor Utilizing a Sol-Gel Matrix Suffers From Leaching Based Upon Form of Dye

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Statement of Purpose: The purpose of this research is to develop a stable, intravascular optical pH sensor capable of real-time, continuous monitoring. The measurement of arterial pH, along with the partial pressures of O_2 and CO_2, are used to determine base-excess and –deficient disorders that can be caused by a number of problems including cardiac failure, renal failure, and asthma (Kaplan LA. Clinical Chemistry: Theory, Analysis, Correlation. 2003:462-476). Real-time measurement of these parameters may improve treatment through trend monitoring and reducing the time before a therapy is administered. A major problem for intravascular sensors is thrombosis on the sensor surface. Thrombosis interferes with the sensor's ability to measure the actual analyte levels in the bulk blood (Mahutte CK. Clin Biochem. 1998:31:119-130). A well characterized and stable sensor is needed to show that a reduction in thrombosis will improve sensor performance in vivo.

Blood pH may be measured using an optical or electrochemical sensor. Optical sensors have many benefits over electrochemical sensors such as ease of miniaturization, no reference electrode, and not being susceptible to electrical interference (Mahutte CK. Clin Biochem. 1998:31:119-130). Absorbance-based optical sensors utilize a dye that changes color based upon what form it is in (i.e. protonated or deprotonated). When the form of the dye changes, the affinity of the dye for the sol-gel matrix also changes and the dye may leach out of the matrix at pH-dependent rates, thus rendering the sensor unstable for continuous monitoring.

To reduce the leaching of the dye from the matrix, Wang (Wang E. Anal Chim Acta. 2003:495:45-50) suggests adding phenyltriethoxysilane (Ph-triEOS). Adding a covalently linked aromatic ring to the backbone of the sol-gel increases the affinity of the dye, such as cresol red, for the matrix due to π-stacking between the multiple aromatic rings present in the dye's structure and the pendant phenyl group on the additive.

Methods: The sol-gels were prepared using tetraethyl orthosilicate Ph-triEOS, HCl, cresol red or phenol red, ethanol (Sigma-Aldrich, St. Louis, MO), and distilled-deionized water. The sol-gel was applied to glass microscope slides using spin coating (1500 rpm for 30 seconds). An additional layer of cresol red sol-gel was applied over the top of the initial cresol red sol-gel layer on one of the slides three hours later using the same spin coating technique. The slides were allowed to air dry for 12 days , were cut up, and soaked on a shaker table in 5 mL of a phosphate buffered saline (PBS) adjusted with HCl to the desired pH. Dye present in the PBS soaking solution was measured using a Lambda 35 UV/Vis Spectrometer (PerkinElmer, Waltham, MA). The slides were placed in a fresh aliquot of PBS on the shaker table and this was repeated until all of the soaking time points had been collected.

Results: An initial leaching test of cresol red and phenol red sol-gels suggests that the dyes leach out of the sol-gel at a pH of 7.8 faster than at a pH of 6.8 or 7.4 (unpublished data). Solutions with a pH of 6.8, 7.4, and 7.8 are chosen to look for leaching within the physiologically relevant range of blood. Since the leaching is most pronounced at pH 7.8, PBS at pH 7.8 was used to study dye leaching out of the matrix with respect to time. Figure 1 shows a running total of the absorbance of the soaking PBS for a single layer and a double layer of cresol red sol-gel at $\lambda_{571\ nm}$. The wavelength is chosen because it is the visible range spectral peak for the dye in the deprotonated form. Twice as much dye leaches out of the double layered slide compared to single layered slide over a given time interval. After 10 h of soaking, the slides do not contain a detectable amount of dye, suggesting that all the dye has leached out of the matrix.

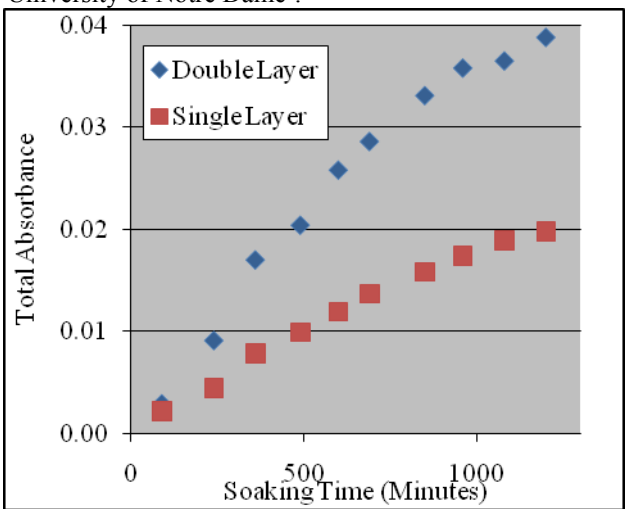

Figure 1. Total Leaching of Cresol Red from a Single Layer and Double Layer Sol-Gel in PBS (pH 7.8)

Conclusions: Adding Ph-triEOS to increase the affinity of the dye to the sol-gel is not sufficient to counteract the affect of changing the charge of cresol red due to deprotonation. The cresol red readily leaches out of the sol-gel when it is immersed in an aqueous solution with a pH high enough to change the dye to its deprotonated form. Unfortunately, the protonation and deprotonation of the dye results in the color change that makes the dye useful as a pH indicator which makes this dye entrapment method undesirable for a pH sensor used under physiological conditions. Other methods of immobilizing the dye in sol-gels, such as covalently linking the dye to the sol-gel or a filler particle are currently under investigation in the laboratory. Ultimately, this work is leading toward the development of an optical intravascular pH sensor that will incorporate nitric oxide release, a strategy that has been proven to greatly enhance in vivo performance of blood-contacting devices and allow for the development of a continuous monitoring system.