

Dual-Fluorophore Silica Microspheres for Ratiometric Acidic pH Sensing

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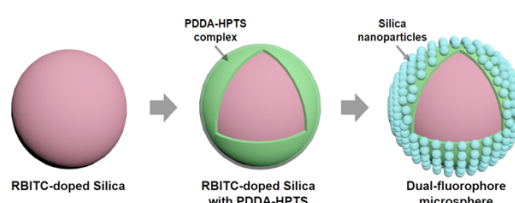
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Abstract: Encapsulation of fluorophores in silica matrix offers many advantages such inhibition of photobleaching and possibilities for ratiometric pH sensing. Dual-fluorophore pH-responsive silica microspheres, incorporating pyranine (HPTS) and rhodamine B isothiocyanate (RBITC), were synthesized by Stöber method, followed layer-by-layer depositions. The resulting dual-fluorophore silica microspheres were then characterized by SEM, TEM, fluorescence spectroscopy and imaging. The incorporation of two dyes in the microspheres allowed ratiometric quantification of pH.

The ratiometric approach has been proven to reduce the influences of external perturbations and unequal dye concentration in silica matrix during measurements. The dynamic range for pH was from 1.5 to 4. The sensing microspheres could be applied to determine acidic pH. Additionally, the sensing microspheres exhibited a high colloidal and long-term stability and also allow a fast detection of pH due the porosity of the microspheres. Such structured microspheres could be optimized, using multiple dyes for multianalyte detection.

Keywords: ratiometric pH measurement, silica, dual-fluorophore, fluorescence spectroscopy.



1. Introduction

pH remains an important parameter in agricultural, chemical, biological and medical fields.¹ Over the years, pH have been monitored using electrochemical and optical transduction methods. Among these methods, fluorescence measurement techniques offer many benefits such as reduced electromagnetic interferences, high sensitivity, easy miniaturization, relatively low cost² and noninvasiveness. Fluorescence sensors may be implemented by simply measuring the absolute value of the fluorescence intensity or by a ratiometric measurement involving the ratio of the emissions at two or more wavelengths to correlate the concentration of the analyte of interest. However, unequal dye distribution in the sensing membrane, as well excitation source fluctuations and variations in environmental conditions can undesirably affect the accuracy of fluorescence intensity based measurements.³⁻⁶ This unreliability in intensity-based measurements can be overcome with a ratiometric measurement scheme by providing an internal referencing standard.⁷

Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS

or pyranine) remains one of the gold standard fluorescent pH-indicator dye because of its excellent photostability, high quantum yield and biocompatibility.^{1,8-10} To increase the operational life and stability of pH dyes for sensing, it is required to incorporate dye molecules in polymeric supports. The polymeric support guards the dye molecules from photobleaching and solvatochromic effects,¹¹ as well as reducing the contamination of free dye molecules in the sensing medium. Silica presents itself as an inert and chemically stable matrix for dyes.¹² Other benefits of using silica as a polymeric support include the availability of established protocols to attach dye molecules, low toxicity¹³ and colloidal stability of silica particles due to the abundant negative charges on their surfaces. Though the incorporation of dyes in silica NP offers advantages, embedding anionic dyes such as HPTS into silica has been problematic owing to the electrostatically repulsive interactions between silica matrix and the negatively charged dye molecules. An approach introduced by Mills *et al.*,¹⁴ uses quaternary ammonium salts as phase transfer agents to transfer anionic pH dyes into an organic phase and then reacted with silica precursors to form lipophilic organically modified silica (ORMOSIL). The ORMOSIL could then be coated on substrates or grinded into powder to produce pH-sensitive fluorescent particles. However, this approach churns out non-uniform particles of low hydrophilicity. Moreover, it is particularly daunting to control the geometry of the particles produced. Conversely, microemulsion and Stöber methods can

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