

Optical nanosensors for intracellular pH measurements

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Introduction: We present the development of fluorescent nanoscale optical sensor for intracellular pH measurements. For this purpose core-shell type silica nanospheres were synthesized using a modified Stöber method in the 50-100 nm size range. The sensing was based on the dual lifetime referencing (DLR) method [1]. Two fluorescent indicators with overlapping emission bands, one pH-sensitive, short-lived indicator and a pH-insensitive reference with a decay time in the μ s range have been chosen. N-allyl-4-piperazinyl-1,8-naphthalimide (APN) have been synthesized according to the literature [2] and have been used as fluorescent pH indicator while ruthenium(II) tris(diphenylphenanthroline) (Ru(dpp)) complex has been used as reference fluorophore.



Fig. 1. Fluorescence enhancement mechanism



Fig. 2. APN fluorescence at different pH

Experimental: The pH sensing principle of APN is shown in Fig. 1, and its fluorescence properties on Fig. 2. The core of the silica sphere was prepared by dissolving Ru(dpp) in the ethanol tetraethoxysilane (TEOS) and water were added. The mixture was stirred and sonicated for 30 minutes. The mixture was thermostated at 25°C. Ammonia catalyst was rapidly added and the mixture was stirred for further 24 h, then centrifuged (5000 RPM, 15 minutes), dried and annealed at 200 °C. The sensing shell of the sensor was prepared in two steps. First APN and vinyltriethoxysilane (VTES) were dissolved in ethanol, and irradiated with UV (250nm) lamp for 1 hour. In the second step the appropriate amount of APN/VTES solution, 0.01% of APTES was added to suspension of the reference silica spheres and sonicated for 30 minutes. The ammonia catalyst was added and the mixtures were stirred for 24 hours. The resulting nanosensors was centrifuged and washed with ethanol and DI water. Finally it was dispersed and stored in DI water until further use. This way the reference indicator was encapsulated in the core of the silica beads that was covered by pH sensitive indicator containing shell. The calibrating curves were obtained both in intensity and in life-time domain. The fluorescence measurements were made with AVANTES AvaSpec 2048 spectrophotometer, by exciting the samples with LED (430 nm) through a 400 broad range IF filter, while a long pass filter with cut off wavelength of 510 nm was placed in front of detector. Phase shift measurements were performed with dual-phase lock-in amplifier (DS2P-650, Stanford Research Inc.) in a home made flow through cell. Optical system consisted of a blue led (430 nm) a 430 broad range band pass filter, bifurcated fiber bundle, and Hamamatsu (R15760-01) PMT equipped with 510 nm cut off wavelength long pass filter.

Results: SEM images of the synthesized nanospheres are shown in the Fig. 3. The main size of the particles was 210 nm. The particles formed a stable suspension in water. Hydrogen ions strongly enhance the fluorescence intensity of APN, which shows no fluorescence above pH 12. The fluorescence properties of pH sensing nanospheres are presented on Fig. 4. The phase shift of the modulation frequency as a function of the modulation frequency are shown in the Fig. 5. It has been found that the pH sensing spheres have the largest phase shift around 10 kHz modulation frequency. Fig. 6. shows the obtained calibration curve of the sensing nanospheres in B-R buffer at 300mM ionic strength.



Fig. 3. SEM image of the pH sensing nanospheres

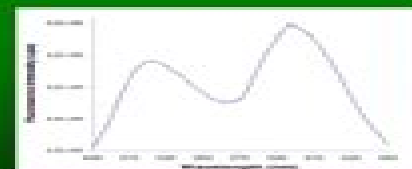


Fig. 4. Fluorescence of the pH sensing nanospheres



Fig. 5. Phase shift as a function of modulation frequency



Fig. 6. Calibration of sensing layer

Conclusion: A pH sensing nanospheres has been prepared with covalently bonded pH sensitive dye and co-immobilized reference dye. The spectral properties of the nanosensor are suitable for imaging measurements with most CMOS and CCD cameras [3].

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