# Three-Dimensional pH Microprobing with an Optically-Manipulated Fluorescent Particle

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A novel pH microprobe has been developed, which makes it possible to measure inhomogeneous pH distributions with submicrometer three-dimensional resolution. A polyacrylamide particle incorporated with pH-sensitive fluorescein-acrylamide is manipulated with a laser trapping technique. It is demonstrated that the local pH in a water/glass interface is different from that of bulk water.

Concentration gradient of ionic molecules is generally induced in liquid/solid interface layers by surface electric charges due to adhesion of ions on solid surfaces and ionic dissociation of surface molecules.[1] Furthermore, if the surface forms two-dimensional microstructures, consisting of some different materials such as microelectrodes, microfabricated patterns, biological membranes, and catalytic particles, three-dimensionally inhomogeneous distributions of ionic molecules are created in the liquid layers. The precise observation and analysis of the ion concentration distributions will make it possible to understand various interesting phenomena characteristic of surfaces and interfaces and, furthermore, to control their processes. A pH-sensing microscope was developed by Nakao et al.,[2] however, it is applicable only to two-dimensionally inhomogeneous systems and its resolution cannot be improved beyond the optical diffraction limit.

Kopelman et al. proposed a fiber-optical pH sensor fabricated by a near-field photopolymerization technique.[3,4] A nanometer-sized polymer probe containing a pH-sensitive dye was prepared with the evanescent field that was formed on the tip of a tapered and aluminum-coated optical fiber. This pH microsensor has excellent detection limit and fast response time, as well as high spatial resolution.[3] In case of surface and interface analyses, however, the fiber tip introduces the aluminum surface into the sample solution, and limits the volume between the liquid/solid interface and the tip surface, which may affect the physical and chemical conditions in the observation field. In addition, the mechanical positioning sometimes causes a crash of the tip and also damages easily soft samples.

In this paper, we propose a new pH microprobing method with a single fluorescent particle manipulated by radiation pressure of a focused laser beam, which has the advantage of being a noncontact, nondestructive, and nonperturbative measurement, as well as having the high spatial resolution.

pH microprobes were prepared in the following procedures. All chemicals used were purchased from Aldrich Chemical Co., and they were used without further purification. A phosphate buffer solution (pH=6.5, 10 mL) of acrylamide (3 g) and N,N-methylenebis(acrylamide) (150 mg) was prepared according to the procedures of Hicks and Updike.[5] Then the solution was mixed with an ethanol solution (0.4 mL) of N-fluorescein-acrylamide (2 mg) that was prepared by the method described previously.[4] The mixture was put in a Teflon vial and bubbled with nitrogen for 15 min at room temperature. A catalyst solution (0.4 mL) containing potassium persulfate (16 mg) was added to the mixture solution, and then the polymerization reaction was allowed to stand in an oil bath heater at 65 °C for 25 min, with maintaining the nitrogen atmosphere during the reaction. After washing with water, the resulting polymer solid was ground by a mortar and a pestle to form submicrometer-sized particles, and then washed again with pure water by repeated decantations.

To control the position of the probe particle against the thermal motion, gravity, and convection, we used a laser manipulation technique based on radiation pressure of a focused laser beam.[6] A cw Nd:YAG laser (Spectron, SL902T, 440 mW) was used as a trapping laser source. The laser beam was introduced into an optical microscope (Nikon, Optiphot 2) and focused into an ~1 $\mu$m spot by an objective lens (x100, NA=1.30) so that the probe particle was trapped three-dimensionally. The positioning control of the particle was performed by moving the external mirrors and lens, which was controlled by a computer. A mercury lamp (491.6 nm) was used for excitation of the pH probe. Fluorescence from the particle was selected by a pinhole with a diameter of 200 $\mu$m that corresponded to 1.3 $\mu$m in the sample, and the spectrum was observed by a polychromator (McPherson 2045, 100 G/mm) and a double-intensified multichannel photodiode array detector (Princeton Instruments, D/SIDA-700G(B)). The exposure time for measuring each spectrum was 1.0 s.

The accuracy of the present positioning control was theoretically estimated on the following assumptions; radiation pressure provides a harmonic potential well, and the probe particle is so small that the scattered field can be calculated under the Rayleigh approximation.[7] The result indicates that the displacement of the Brownian motion in the trapping potential well at room temperature of 20 °C is 11 nm in the x and y directions for a 300-nm particle irradiated with a 440-mW laser beam, while that in the z direction is 29 nm.

In order to test the pH response of the prepared copolymer, fluorescence spectra of a large particle (diameter ~300 $\mu$m) were observed in eight different buffer solutions from pH 4 to 8, as shown in Figure 1A. Since the buffer solutions were repeatedly washed away and replaced, the particle was held between a glass needle and a microscope glass plate. The spectral observation was performed several min after the change of each buffer solution so that the solution homogeneously penetrated into the probe particle composed of hydrophilic polymer.[4] As shown in Figure 1A, the fluorescence intensity increases with increasing pH, and spectral shape also varies depending on pH. After prolonged irradiation of intense excitation light, the absolute fluorescence intensity for each pH buffer solution was always slightly lower than the previous one; this might be ascribed to photobleaching of the dye inside the polymer particle. On the other hand, the change in the spectral shape was negligibly small in the repeated measurements, hence we used the fluorescence intensity ratio at 540 nm and 610 nm for estimating pH.[3] Figure 1B shows the pH dependence of the intensity ratio calculated from Figure 1A. This result demonstrates that the prepared polymer particle is useful as a pH probe. In the present work we used the curve of Figure 1B for each probe particle in the following experiments, since all the probe particles used in this work were fragments of a single polymer solid so that their spectral responses would be assumed to be the same. It was also confirmed that the fluorescence spectrum did not change with irradiation of a trapping laser.

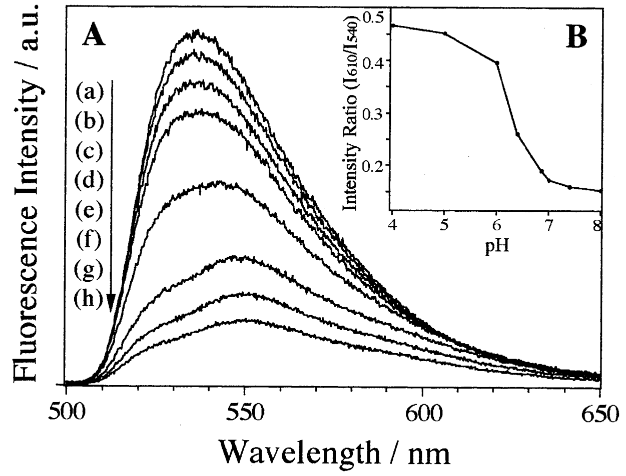

**Figure 1.** **A**: Fluorescence spectra of a fluorescein-incorporated acrylamide-copolymer particle (diameter ~300 $\mu$m) observed in eight buffer solutions with (a) pH=8.0, (b) 7.4 (c) 7.0, (d) 6.8, (e) 6.4, (f) 6.0, (g) 5.0, and (h) 4.0, respectively. **B**: pH dependence of the fluorescence intensity ratio at 540 nm and 610 nm, calculated from Figure 1A.

We have applied the present system to a pH analysis of water in a surface double layer formed on a glass plate. Water (in air atmosphere, pH~6.0) containing probe particles was sandwiched between two glass plates (Matsunami Glass), and placed on the microscope sample stage. Since the probe particle is sensitive in the pH range from 6 to 7, as shown in Figure 1B, it was better to use deionized water. Unfortunately, contamination of carbonate ions could not be avoided under the present measurement system. Under the microscope, a single probe particle with a size of <1 $\mu$m, that was smaller than the spatial resolution of the optical microscope, was selected and optically trapped in bulk water (Position A; ~20 $\mu$m distance from the bottom glass plate) to observe the fluorescence spectrum. Then the particle was manipulated to the interface between the water and the bottom glass plate (Position B), and the pH at this position was analyzed. After that, the particle was returned to the original position in bulk water (Position C) to confirm the reproducibility of the fluorescence intensity ratio.

As shown in Table 1, the fluorescence intensity ratios at 610/540 nm in bulk water (positions A and C) were obtained with good reproducibility to be ~0.39. This ratio corresponds to pH of ~6, which well agrees with pH of water used in this experiment. On the other hand, the intensity ratio in the water/glass interface layer (position B) was 0.49 (pH<4.0). The statistical errors were enough small to distinguish the observed pH between the bulk water and the interface layer. It should be noted that the pH measured in the interface layer was the value averaged in the volume of the particle (=sub$\mu$m$^3$), which would be slightly higher than pH at the interface. Fortunately, if the spatial response function as well as the size of a probe particle can be determined in separate experiments, pH distributions can be estimated from observed pH profiles by a mathematical deconvolution technique.

**Table 1.** Difference of the fluorescence intensity ratio between bulk water and a water/glass interface. The probe particles (<1 $\mu$m) were optically trapped in bulk water (A), manipulated to a glass surface (B), and returned to the bulk (C) with monitoring the fluorescence spectra (see text).

| Position | Fluorescence Intensity Ratio (I610/I540) |
|---|---|
| A | 0.38±0.03 |
| B | 0.49±0.05 |
| C | 0.40±0.02 |

In conclusion, a new pH microprobing method with an optically-manipulated fluorescent particle has been proposed for analyses of inhomogeneous pH distributions. The spatial resolution of the pH measurement is not determined by the diffraction limit of the optical system but by the size of the probe particle and the accuracy of the optical positioning control. The noncontact manipulation of the probe particle has the advantages of reducing a perturbation on the physical/chemical conditions of the sample solution and of avoiding damages to the probe and the sample. This method can be extended to analyses of various ionic molecules or other analytes in small domains.

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