pH Microscopy Applications

Nomura Satoshi, Horiba R&D Center

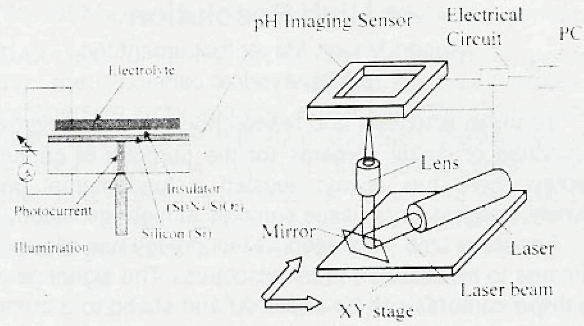
A scanning chemical microscope utilizing a flat pH sensor has proven effective for analyzing a number of biological specimens including human teeth surfaces, plant leafs, and rice.

The key to this microscope, the "pH imaging sensor", is an electrochemical semiconductor that can be made to function as an array of point sensors when scanned by backside laser illumination. Measured pH values at each measurement point can be processed into a pH image. So far a resolution of 5 µm with a 0.01 pH sensitivity has been attained by this imaging technology, although most images are made using 100 µm resolution. The time to acquire an image is, of course, dependent on the size of the measured area. For example, a 6.4 mm x 6.4 mm image with a resolution of 100 µm can be acquired in 25 s. Linear pH profiles can also be obtained at a speed of 1 cm/s.

Producing a pH image

The pH sensor is flat and consists of an electrolyte insulated from Si by a Si_3N_4/SiO_2 layer (see following figure) Because the surface of this structure is proton sensitive, placing the sample on the surface will result in a photocurrent output at an illuminated point. By using a tightly-focused semiconductor laser beam as the source and a thinned sensor structure, microscopic pH values can be recorded. Because sensor output current is proportional to the pH level of the sample at the illuminated point, by placing the laser and the related optical system on a scanning XY stage, 2-D images of the sample's pH values can be attained.

Measurement Principle & Instrument Setup



The flat pH imaging sensor enables the pH distribution in a semi-solid electrolyte solution, such as agar film, to be detected and observed. Placing solid samples in contact with the agar film sometimes generates small pH distributions that indirectly describes the surface condition or even the inner condition of the solid sample.

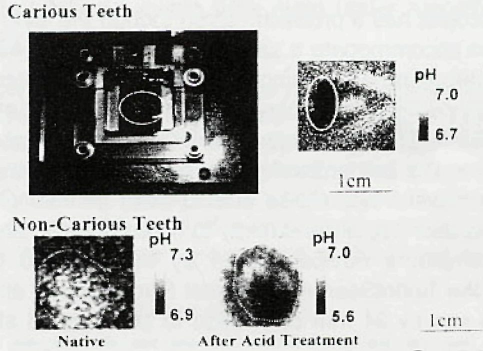
The cause of the pH distribution may be only just proton or hydroxide adhering to the sample surface. Or, it may be those species contained inside the sample and released from it. And, of course, a surface reaction may cause the pH distribution. By studying how fast the pH distribution expands or how large the change of pH values at a specific point may give valuable information relating to the sample characteristics such as proton release capacity, amount of surface-adhered acid, impurities on the surface, sample reactivity and so on.

In order to conduct pH-based experiments, a thin agar film is formed on the sensor and a solid sample placed on this film. Because the diffusion of the proton is reduced in the agar film, pH distribution of the sample can be imaged.

Applications

The surface analysis of freshly extracted human teeth has been accomplished using the pH imaging technique. The analysis was conducted by placing sliced discs of freshly extracted teeth on the agar film. Teeth samples that were parallel to the long axes of teeth were used to compare the pH distribution between the carious and the noncarious teeth. Horizontally sliced non-carious teeth were used to compare the results of a phosphoric acid treatment. At the caries lesion, a lower pH was detected than with the intact lesion. The decrease in pH may be attributed to the decalcification caused by the acid from which the bacteria causing the carious teeth are made. On the other hand, some lower pH values could be attributed to the hydroxyappatitie, which is the main component of teeth. The result shows that the carious lesion could be identified in the pH image thus showing that this technique can be applied to caries assessment.

Surface Analysis of Human Teeth

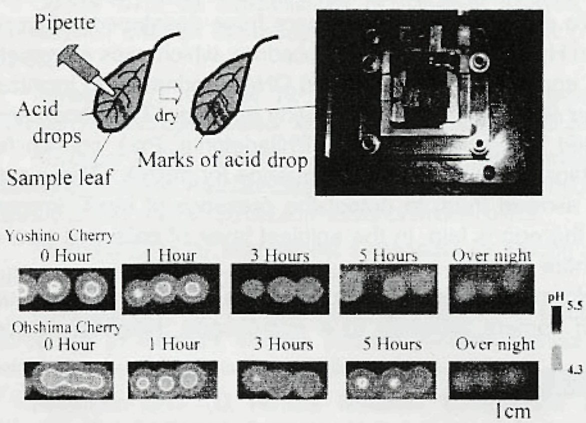


Non-carious samples were treated with 37% phosphoric acid and then thoroughly rinsed. At the enamel lesion, remarkable decreases in pH were confirmed even though the testing surface was rinsed thoroughly. The magnitude of the changes might be due to the remaining acid, or because it is easier for enamel to be decalcified.

Leaf testing

The pH imaging microscope was use to estimate plant leaf surface response to the acid. Small acid droplets, 0.1 μm of 10 mN sulfuric acid, were placed on plant leaf surfaces and allowed to dry naturally. Small leaf discs, containing the marks made by the droplets, were cut at various times after the acid drips had dried. The pH of the agar film was first adjusted to be 5.5, which was the same as the surface on the leaf samples. In turn each disk was placed on the agar film on the pH imaging sensor so that the acid-contaminated surface of the leaf was contacting the top layer of the agar film. The pH distribution formed in the agar film by each leaf was then imaged.

Plant Leaf Response to Acid

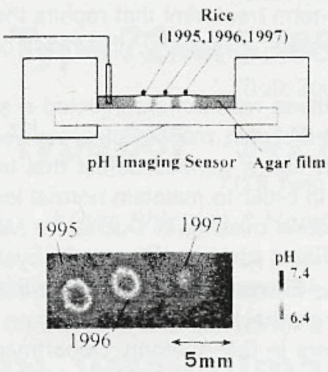


These pH images show pH distributions obtained with Yoshino cherry and Ohshima Cherry. Here the initial pH region is shown in blue and the initial acid affected region is in red. As shown, generated pH distribution regions became smaller in time after the acid droplets were placed and dried up. This result indicates that the sample leaves neutralized the surface acid by ion exchange with basic cations from their surface tissues and that they recover from strong temporary acid stress, such as acid rain fog, in a relatively short time.

Rice Freshness

The degree of rice freshness was also evaluated with the pH imaging sensor. Three grains of rice that were harvested at different times were placed on the agar film and tested at 100 μm intervals after 5 minutes. The resulting pH values are depicted so that the neutral pH region is blue and the acidic region is red. It was confirmed that the pH of the lower pH region corresponded to the harvest time of the rice thus verifying that the surface of the rice becomes more acidified as it grows older.

Rice Evaluation



Summary

A new type of pH imaging microscope has been developed and a new analytical method for evaluating biological solid sample has been illustrated. The placing of samples on the thin agar film formed on the sensor is a new idea in chemical analysis. Future testing will explore other applications of this microscope.