Feature Article

New Challenge to pH Measurement

What will come next to the glass electrode?

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Abstract

Since HORIBA developed the first glass-electrode pH meter in 1950 in Japan, HORIBA has greatly contributed to the development of science and technology through its sophisticated pH measuring technologies. On the other hand, requirements for pH measurement have greatly diversified in this half century, leading to the development of pH measuring technologies, which HORIBA has not been involved in so far, other than the glass-electrode method. In this article, the pH measuring methods other than by the glass-electrode pH meter will be reviewed. In particular, those applied to biological research will be explained as well as pH measuring technologies related to the most up-to-data nanotechnologies.

1 Introduction

The pH is one of the most important parameters that indicate the physical properties of solutions, and it also can control many phenomena occurring around us. The idea of pH was proposed nearly one century ago[1], and the original form of glass electrode-type pH measurement, which is most popular at the present, was put forward a few years later than the idea of pH had been proposed[2]. Since its foundation, HORIBA has continued to improve and spread the glass-electrode pH measurement technology, enabling to measure easily and accurately pH and contributing to the development of science and technology.

The glass electrode-type pH measurement has not necessarily been a universal technique in pH measurement. Since the idea of pH was proposed, many people have coped with this important parameter of pH using various means. HORIBA has also made every effort to widen the possibility of pH measurement by devising various shapes and sizes of the glass electrode. However, in the rapidly developing technical fields such as semiconductor, biotechnology, and nanotechnology, understanding of chemical reactions at atomic and molecular levels is required and therefore, the application of glass electrode-type pH measurement is reaching to its limitations.

In order to contribute to the further development of science and technology through HORIBA's pH and proton measurement technology, we need to review all the pH measurement techniques proposed in the past and to devise pH measurement techniques that can contribute to the future science and technology.

In this report, setting aside the conventional pH measurement method, I would like to focus on reviewing both various pH measurement techniques other than the glass electrode method proposed and worked by our predecessors and their future prospects. As a member of HORIBA that has led Japan's and world's pH measurement fields with the glass electrode method for this half century, I would like to continue to be involved in pH and proton measurement.

2 Electrochemical pH Measurement Technology without Using Glass Electrodes

In this section, I will explain electrochemical pH measurement techniques without using glass electrodes on how they are proposed and are applied up to now.

2.1 Metal Oxide Electrodes and Liquid Membrane-type pH Selective Ion Electrodes

Attempts to use non-glass electrodes in the potentiometry based on electrochemical principles have been made since old times[3].

One example is metal oxide electrodes applying the principle that a certain metal oxide will generate an oxidation-reduction potential proportional to the concentration of hydrogen ions or hydroxide ions when the electrodes are immersed in a test solution. In the case of the typical antimony oxide electrodes, they generate an electromotive force in accordance with oxidation-reduction reaction when they are immersed in a test solution. Since this electromotive force is proportional to the pH of the solution, the value of pH can be obtained when the force is measured.

Since metal oxide electrodes have advantages that their sensing parts are durable and easy to handle, various electrodes have been developed up to now. For example, micro pH electrodes using iridium oxide have already been put to practical use[4]. However, because of the disadvantages that the pH indication can vary depending on the polishing conditions of the electrodes and that the reproducibility is not good, the electrodes are used only in limited applications now.

Another example is liquid membrane-type ion selective sensors. They are fabricated by dissolving chemical compounds called an ionophore, which selectively reacts with certain ions, in an organic solvent before being held by a polymer matrix material like PVC[5]. If we use a substance that reacts with protons or hydroxides as an ionophore, we can fabricate a pH sensor. Since this type of electrode does not have a glass-membrane sensing part, it is suitable for the miniaturization or the microfication and therefore, is now being widely used in the field of biology.

2.2 pH Sensor Using Semiconductor Technology

In the 1970s, new types of pH sensors were invented by the combination of semiconductor technology and pH measurement technology[6], leading to opening the way to realize a compact and high-function pH meter. The first-invented new sensor is an ISFET (ion sensitive field effect transistor), a so-called one-chip sensor formed on one silicon substrate that substitutes for both a conventional glass electrode and pH meter's first stage amplifier. In the ISFET, a MOSFET gate itself is a pH sensing part and therefore, the ISFET does not need a high-impedance amplifier as glass-electrode pH meters do. As a pH sensing membrane, thin films such as chemical-vapor-deposited (CVD) Si_3N_4, Al_2O_3, and Ta_2O_5 are frequently used. The ISFET can be formed in a very small size and a free shape because it is produced using the semiconductor process.

Further, another silicon substrate-based sensor, the LAPS (Light Addressable Potentiometric Sensor), was invented in the 1980s. The LAPS consists of an insulator (Si_3N_4/SiO_2) and a semiconductor (Si)[7]. When voltage is applied between the silicon and the electrolyte and modulated light is radiated to the silicon, alternating photocurrent passes through the inside of the silicon. As this current varies according to the pH of the electrolyte, the pH can be measured by using the current.

Since the LAPS has an advantage that it can have a flat, small pH sensing part, it can measure independently each pH value of minute areas. As the name of “light addressable” indicates, the LAPS can “address” measurement points.

As examples of LAPS application in practical use, there are an instrument evaluating a cell metabolism from changes in pH generated around the cell and an instrument monitoring enzyme reactions occurring in two or more places on the sensor surface, which take advantages of flatness of the sensor[8].

In addition to these, there is a pH sensor having a liquid membrane-type ion selective electrode fabricated on a silicon substrate by screen printing technology[9]. Since the electrode and the pH sensing part are formed by screen printing, it is possible to make a compact and flat sensor easily.

More than ten years ago, HORIBA developed sheet-type pH composite electrodes using screen printing technology (Fig.1). Handy pH meters with the sheet-type electrodes such as the card-type Cardy series and the stick-type Twin series (Fig.2) are being used in various industry fields.

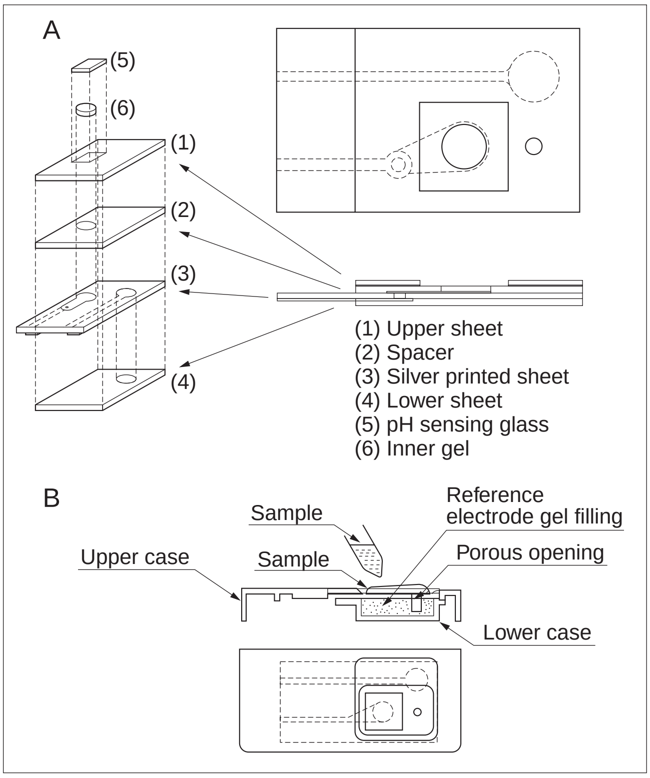

Fig. 1 Sheet-type pH Composite Electrode

Fig. 2 Compact pH Meter (Twin pH)

3 pH Measurement under Special Environment

Challenges to pH measurement in biology

In this section, I will explain the studies in the field of biology as an example of pH measurement under a special environment. Since old times, biologists have made efforts to observe living things with dividing them into the smallest units by using a variety of means. One of the results of these efforts is the microscope technology. Measurement of pH in cells, which are the smallest units of living things, was also one of their targets. This was because a majority of bioreactions is regulated by pH in the cells, and the measurement of pH in the cells was a significant challenge for the development of biology[10]-[11].

3.1 Microelectrode Method

This method is to measure pH in cells by sticking a microfied glass or a liquid membrane-type ion selective electrode directly into cells. To minimize the impact on the cells to be stuck, the electrode must be as small as possible. Conventionally, a pH sensing glass is attached to the tip of a glass micropipet[12] or a hydrogen ion-selective liquid membrane was stuffed in a pipet[13]. The latter has been mainly used because the electrode can be made minute and is easy to fabricate. However, the microfication is still limited, and it is mainly applied to the measurement of comparatively large cells.

3.2 pH Measurement Using Weak Acid and Weak Base as a Marker

A liphophilic weak acid and a weak base accumulate inside cells through a cell membrane. The amount of both the weak acid and the weak base passing through the membrane depends on the difference in pH between the inside and the outside of the cells. Therefore, it is possible to determine pH by measuring the concentration of both the liphophilic weak acid and the weak base. To measure the concentration, a marker isotope and the weak acid and the weak base with fluorescent and light absorbing properties are used, while studies on marker reagents have been reported. On the other hand, though this pH measurement method is applicable to very small cells, it is difficult to continuously trace rapidly changing pH values since the added weak acid and weak base require a long time until reaching to the equilibrium state. This method has been recently used to measure pH in more minute parts like vesicles in cells[11].

3.3 pH Measurement Using Fluorescent Reagent

This method is to measure pH in cells by injecting a fluorescent pH indicator into cytoplasm to measure the fluorescence spectrum excited by a certain wavelength of light. Fluorescein was used at first[14] as a fluorescent dye and then its derivative, BCECF*1, has become the mainstream[15].

This method has become popular because it is less invasive to the cells and can continuously measure rapidly changing pH values. However, it has still some disadvantages such as the limited measuring time (10-12 minutes) because of the extinction time of fluorescence and the influence of protein on fluorescence intensity.

However, this method has led to developing new pH measurement technologies such as optodes and fluorescent microscopes described below and greatly contributed to biology.

*1: 2’, 7’-bis (carboxyethyl)-5, 6-carboxyfluorescein

3.4 Optode

This is a simple sensor utilizing the extinction phenomenon of fluorescence and is used for measuring various ions, same as ion selective electrodes[16]. Since this sensor has a structure of holding an ion-sensing fluorescent material at the tip of optical fiber, it is called “optode (or optrode)” in contrast with “electrode”. Optodes for pH measurement use proton-selective ionophore as an ion-sensing material.

Since the optode was invented, it has undergone various improvements such as microfication of the measuring part and stable-holding of the ion-sensing fluorescent material. Optodes far smaller than the conventional microelectrodes have been developed[17] and are put in practical use such as the measurement of the inside of a rat’s uterus[18].

3.5 pH Measurement by NMR Method

The nuclear magnetic resonance (NMR) spectrum of a solution containing the ^{31}P, isotope of phosphorus will shift in accordance with the pH of the solution. Although the pH measurement by this method has problems such as being poor in accuracy and requiring large-scale equipment, it is an epoch-making that this method can measure pH inside cells without attacking them[19]-[20].

4 Observation of Localized Proton Distribution

All the pH measurement methods mentioned above are to make the quantitative evaluation of protons evenly distributed in solution. However, it is inevitable that the localization of protons occurs during reaction processes in which pH change is involved. Understanding of the localization of protons is very important in deepening our grasp of chemical phenomena around us. From this point of view, I would like to introduce here the pH imaging technology that is currently receiving a great attention as a method to grasp or “observe” the distribution of localized protons.

The pH imaging technology studied in early times is a method using a fluorescence microscope or a confocal laser microscope. The method was developed further by biologists who have pursued the smallest unit of life. Thanks to the recent remarkable progress in optical technology, we have achieved to obtain a μm-order of spatial resolution and a millisecond-order of time resolution. As a result, we can observe now in real time the movement of protons in and between cells associated with life phenomena[21].

On the other hand, in the area of the electrochemical method, researchers specialized in metallic corrosion proposed the scanning reference electrode method that measures two-dimensional distribution of pH by mechanically moving a reference electrode[22]. This method was further developed into the scanning vibrating electrode method.

In addition to these methods, there is an attempt to attach a fine-pointed metal oxide pH sensor to a scanning tunneling microscope (STM) in place of a tunneling probe to obtain pH images in a μm-level of spatial resolution taking advantage of the high-precision mechanism of STM[23].

HORIBA has developed a pH imaging microscope based on the above-described LAPS technique (Fig.3). This microscope is used in research and development in various fields[24]-[28]. In Fig.4, the measurement principle of the pH imaging microscope is shown.

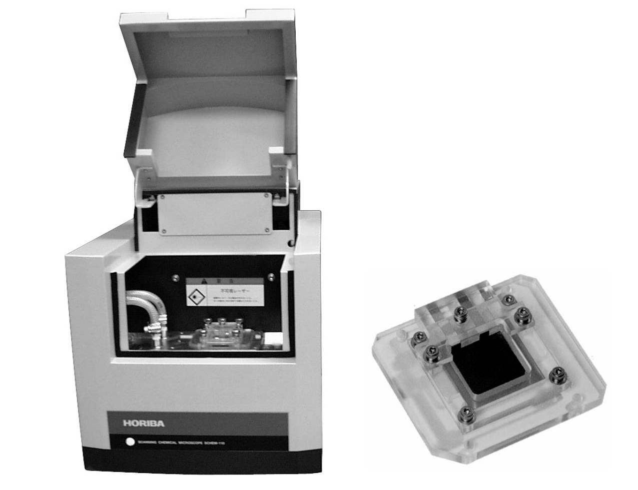

Fig. 3 pH Imaging Microscope and Sensor

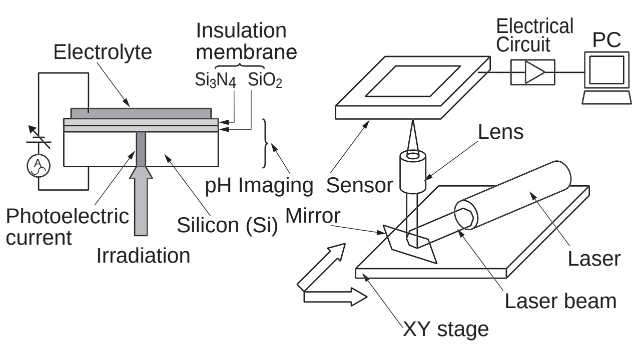

Fig. 4 Measurement Principle of pH Imaging Microscope

5 Directions in the Future pH Measurement

Nanotechnology and pH/proton

In recent years, there have been remarkable advances in nanotechnology and biotechnology. In parallel with the trends, the pH measurement technology is also required to develop. In concluding this report, I would like to explain the state-of-the-art pH measurement technology together with the trends in the forefront of science and technology.

Achievements in development of nanometer-level materials such as nano-wires and nano-particles are closely linked to the development of new pH sensors. For example, by using a nanowire-doped semiconductor, the ISFET having a nanometer-sized sensing part has been realized[29]. Since the gate size can be microfied to a nanometer-level, it will become feasible to detect the proton movement in the ion channel of a biomembrane on a channel-by-channel basis.

On the other hand, the nearfield optics, which is one of the prime impetus of the advance in nanotechnology, is contributing to the microfication and the sophistication of the above-described optodes. In addition, a new pH measurement technique using nano-particles, PEBBLE*2, has been proposed, providing a clue to obtain information on the more inner parts of cells without contacting them[30].

These new pH measurement techniques using nanotechnology are expected to make a great contribution in understanding the origin of life phenomena. Of course, they will be also expected to be used for controlling pH in minute areas in the domains of both the development and the manufacture of nanomaterials.

According to the concept of our conventional pH measurement techniques, the range of pH that can be measured with glass electrodes is generally 1 to 14, and the resolution is 0.001 pH. If we assume that hydrogen ion concentration at pH=14 is

10^{-14} M, the existing number of protons in the solution becomes 10^9. Since the smallest pH that can be resolved by glass electrodes is 0.001 pH, the glass electrodes are capable of detecting only the difference of 10^{20} protons. If the discussion of the molecular and atomic levels that nanotechnology is now aiming is applied to protons, we must challenge the quantity of protons smaller than in 9-digit to 20-digit order.

So, we would like to continue the challenges for enhancing pH and proton measurement with aiming at achieving our corporate slogan, “HORIBA, Always A Step Forward in High-tech”.

*2: Probes Encapsulated By Biologycally Localized Embedding

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