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NOTAS DE APLICACIONES

GENERAL

Determination of pH in Non-Aqueous Solutions

A simple extraction procedure using water is recommended for measuring the pH of non-aqueous solutions containing water-immiscible solvents. Water is added and mixed thoroughly with the sample. After reaching equilibrium, the solvent phase is separated and the pH of the water phase is then measured.

Introduction

Liquids can serve as solvents to dissolve solutes (i.e., solid, liquid or gaseous) to form solutions. The most common solvent is water. Solvents other than water are called non-aqueous solvents. Some examples of non-aqueous solvents are hexane, alcohol, oil, etc. These are often mixed with water or some other non-aqueous solvents to form mixed solvents appropriate for certain applications in chemical research or industrial processes. Non-aqueous solvents that tend to mix with water to form homogeneous mixture are called water-miscible (e.g., methanol, acetone) while those which separate or form a layer when mixed with water are water-immiscible (e.g., oil, hexane, toluene).

pH measurement in non-aqueous and mixed solutions poses a number of issues such as dissociation of the solvent, different pH scale, and liquid junction potential to name a few. The typical problems encountered during measurement with pH electrodes are slow response time, unstable readings, and erroneous results. According to Frant[2], the electrode should have an adequate outward flow from the junction and the junction design should permit easy cleaning for optimum performance. These two key features prevent memory effects at the junction and minimize liquid junction potential.

The Sleeve ToupH 9481-10C electrode (PN 3200611631) is our recommended product for pH measurement in non-aqueous and mixed solutions. It is a refillable, double-junction, glass-body, combination pH electrode. The cable length is 1m and the connector is BNC, compatible with any pH meter that has BNC input. The movable glass sleeve allows easy cleaning of the liquid junction and prevents clogging. The applications include testing of non-aqueous solvents, viscous solutions, and samples containing non-aqueous solvent (e.g., cosmetics, paints, etc). If a combination pH electrode with built-in temperature sensor is desired, the Sleeve ToupH 9681S-10D electrode (PN 3200585463) meets this requirement. This electrode is compatible with HORIBA pH meters only.

Solvent Miscibility and Solubility

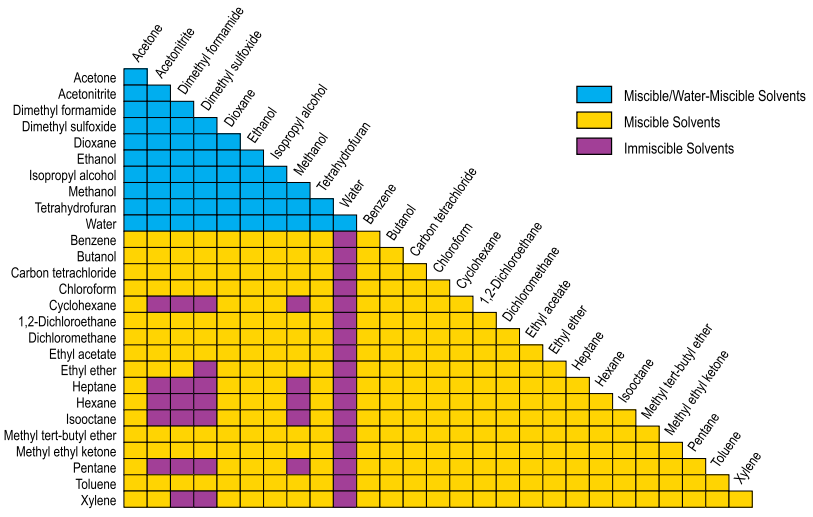

Figure 1: **Solvent Miscibility and Solubility**
(Source: Restek http://www.restek.com/techtips/Solvent-Miscibility-and-Solubility)

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Method

Calibrate the meter and electrode system according to manufacturer's instructions with at least two pH buffers that bracket the expected sample pH.

Sample Preparation And Measurement

The method described below is based on US EPA Method 9045D.

1. Add 20ml of water to 20g sample in a beaker or container. Cover and stir for 5 minutes.
2. Let the solution stand for 15 minutes or centrifuge it to allow the sample and water to separate.
3. Measure the pH of water phase. Record the pH value and the temperature.

To obtain accurate results, standard buffer solutions and samples should be measured at the same temperature. If the electrode is coated with oily material from a sample, clean it with detergent and warm water.

Results And Benefits

As non-aqueous solvents have very low conductivity and can dehydrate the glass membrane, it is difficult to use glass electrodes in measuring pH directly. There must be some electrical conductivity through the solution and glass membrane must be hydrated to function well.

For water-immiscible non-aqueous solvents and non-aqueous solutions with water-immiscible solvents, this measurement can be accomplished by adding water as described in the method above. Pure water with very low buffering capacity and no dissolved salts should be mixed thoroughly with the solvent. Once the two phases are in equilibrium with each other, the activity of any dissolved species should be the same in both phases. After separating the solvent phase, the pH of water phase is then measured.

For water-miscible non-aqueous solvents and mixed aqueous/water-miscible non-aqueous solutions (e.g., water and methanol), a reproducible measurement process can be achieved if the solvent background is known and constant. To do this, it is important to describe the choice of pH electrode, calibration standards, sample preparation, and electrode conditioning.

1. pH electrode

A glass-body electrode resists chemical attack. A flowing reference should be used to eliminate or minimize liquid junction problem. An aqueous filling solution may be used, but most often develops a large or unstable junction potential. This can be reduced by changing the filling solution so that it is compatible with the sample (e.g., methanol saturated with KCl, 90% glacial acetic acid plus 10% saturated aqueous LiCl).

2. Calibration Standards

Ideally, the calibration standards should have the same background as the sample. A constant solvent background may be used in testing the measuring system. A "check" standard made with dry buffer and same solvent background can be measured after calibrating the electrode/meter system in aqueous pH buffers. Its reproducibility is a good test although the reading will be different from the aqueous values. In pH measurement of non-aqueous and mixed solutions, only relative readings can be obtained.

3. Sample Preparation

The ionic strength of non-aqueous solvents can be increased by adding a neutral electrolyte such as a quarternary ammonium salt.

4. Electrode Conditioning

When measuring pure solvents or samples with less than 20% water, the contact time of the electrode to the sample should be kept to a minimum as solvents may dehydrate the glass membrane. Between measurements and after use, it should be soaked in buffer or KCl solution to hydrate the glass membrane. Dried electrodes can be drifty and sluggish.

References And Suggested Readings

1. US Environmental Protection Agency Method 9045D Soil and Waste pH, Revision 4 November 2004
2. Frant, Martin. How to Measure pH in Mixed and Non-Aqueous Solutions. American Chemical Society. 1995
3. C. Westcott. pH Measurements. Academic Press Inc. New York, USA. 1978

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LAQUA pH Combination Electrodes Lineup

9415-10C	9425-10C	9481-10C	9418-10C	9480-10C	6069-10C	6061-10C
ToupH Standard	Field plastic	ToupH Sleeve	ToupH Micro	ToupH Long	Long	Flat

Horiba Instruments (Singapore) Pte Ltd
83 Science Park Drive, #02-02A,
The Curie, Singapore 118258
Tel. +65 6908 9660
E-mail: laqua@horiba.com
http://www.horiba-laqua.com

IMS
HORIBA Group is operating Integrated Management System (IMS)
ISO9001 JOA-0298 / ISO14001 JOA-E-90039 / ISO13485 JOA-MD0010 / OHSAS18001 JOA-OH0068

Application Note

Determination of pH as Quality Control Test in Culture Media

pH affects the physical appearance of culture media and their ability to grow microorganisms. Most bacteria grow in pH 6.5 - 7.0 while animal cells thrive in pH 7.2 – 7.4. Measure the pH value of culture media sample in final form at room temperature with a 6261-10C flat glass pH electrode or 0040-10D ISFET pH electrode and pH meter to ensure the quality of each batch.

Introduction

In laboratories, microorganisms such as bacteria, yeast, fungi, animal cells, and plant cells are cultivated in favourable growth environments known as culture media or growth media for various microbiological tests. Culture media contain nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and sometimes solidifying or gelling agents (agar) to support the growth and survival of microorganisms. They can be prepared in-house by mixing individual components or purchased commercially as complete dehydrated media and may form as liquid (broth), semi-solid, or solid (agar plate, slant, or deep tube) depending on the solidifying or gelling agent content. Different types of culture media are designed for different types of microorganisms and applications.

Apart from complete nutritional composition, right and stable pH is another important requirement for optimum microbial growth in culture media. The pH of a culture medium should be suitable to the microorganisms that will be grown. Most bacteria grow in pH 6.5 - 7.0 while most animal cells thrive in pH 7.2 – 7.4. As certain microorganisms like bacteria tend to release acidic products that can interfere with their growth, buffers are added in culture media to stabilize the pH. Media manufacturers adjust the pH values of dehydrated media so that the final pH values of the finished culture media conform to the specifications on the product labels when cooled to 25°C.

Prior to use, each batch of culture media must undergo quality control tests. The most important chemical test is pH measurement as pH influences the performance of culture media. If the pH of the finished culture medium is outside the recommended range, not only the growth of the microorganisms that the culture medium is intended to grow is inhibited, but also physical changes such as precipitation of components or soft gelling of agar may occur. Routine pH checks in liquid, semi-solid, or solid culture media can be simply carried out with pH meter and pH electrode after proper calibration with pH buffers.

Flat pH electrodes, also called flat bottom pH electrodes, flat tip pH electrodes, and flat surface pH electrodes, are commonly used in measuring pH of culture media, especially for agar plates. Both the sensing membrane and reference junction of the flat pH electrode are constructed on the flat surface tip of the electrode body. This tip configuration is perfect for measuring pH of single drop or small volume of liquid samples as well as moist surfaces of soft solid or semi-solid samples such as meat, paper, skin, cloth, cheese, leaves, leather, bread dough, and culture media.

HORIBA offers two types of flat pH electrodes which are based on two different electrode technologies, the 6261-10C combination flat glass pH electrode and 0040-10D ion sensitive field effect transistor (ISFET) pH electrode. The pH sensitive part of the former is a glass membrane based on pH glass electrode technology while that of the latter is a miniature semiconductor-based sensor using pH transistor technology. The 6261-10C is a refillable combination pH electrode with glass body that is resistant to chemical attack and sleeve junction that prevents clogging because of its relatively high flowrate compared to conventional ceramic junction. The 0040-10D is designed with ISFET chip and non-glass body, which make it rugged, unbreakable, low maintenance, and waterproof. The advantages of 0040-10D over 6261-10C are as follows:

Features of 0040-10D ISFET pH electrode:

- The sensor is replaceable, easy to clean with soft toothbrush, and can be stored dry.
- The robust epoxy body is ideal for applications and environments where glass material is unacceptable.

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- It is integrated with temperature sensor for automatic temperature compensation (ATC) and accurate pH reading.
- It has improved electrostatic protection circuit for reduction of static electricity effect.
- It gives fast response and reduces acidic or alkaline errors in extreme pH conditions.
- It shuts off automatically when not in use.

Model	 6261-10C	 0040-10D
Description	Combination Flat glass pH Electrode	Ion Sensitive Field Effect Transistor (ISFET) pH Electrode
pH Range	0 – 12	0 – 14
Temperature Range (°C)	0 – 50	0 – 60
Reference Junction	Sleeve	Porous sintered polyethylene
Reference Electrode	Ag/AgCl	Ag/AgCl
Temperature Sensor	—	Built-in
Replacement Sensor	—	0141
Material	Glass	Epoxy
Dimensions (mm)	150 x 12	190 x 10
Cable length	1m	1m
Connector(s)	BNC	BNC & phono jack
Fill Solution	3.33M KCl	—
Power	—	CR2032 x 2
Part No.	3014081807	3200367925
 Scan the QR code for more information		

Method

Meter Set-Up And Calibration

Since pH is temperature-dependent, use a pH meter with ATC capability. If 6261-10C pH electrode is used, measure the temperature of pH buffers using a calibrated thermometer and enter the value into the pH meter. This will allow the pH meter to compensate the temperature effect in the calibration.

1. Prepare the pH electrode according to the instruction manual.
2. Set the pH buffer group (e.g. USA or NIST) and resolution (e.g. 0.01 pH) in the pH meter and connect the pH electrode.
3. Select at least two pH buffers (usually, pH 4.01, 7.00, and 10.01 USA buffers are used) that bracket the expected pH value of the culture medium. Pour small amount of fresh pH buffers in beakers for calibration.
4. Rinse the tip of pH electrode with distilled or deionized water and blot dry with soft tissue.
5. Calibrate the pH electrode / meter system with pH buffers according to manufacturer's instructions.

After calibration, each slope should be within 95 – 105%. If slope is not within this range, change the pH buffers and clean, drain / refill (only applicable for 6261-10C), and condition the pH electrode according to instruction manual.

Sample Preparation And Measurement

Measure the pH of culture media at room temperature (20 – 25°C) unless otherwise specified by the media manufacturer. To obtain accurate results, pH buffers and samples should be at the same temperature.

Laboratory-prepared media should be checked for pH and adjusted with 1M NaOH or 1M HCl (if necessary) before dispensing for sterilization. Verify pH after sterilization as changes in pH may occur. In contrast, commercially available dehydrated media usually require no pH adjustment if properly prepared, so a single sample of sterilized finished culture media can be checked for pH. For agar-based culture media, take the pH of the solidified sample.

1. Place the tip of the flat pH electrode on the surface of the culture medium sample to measure the pH. Make sure that the tip touches the culture medium and no gap between them.
2. Record the pH and temperature displayed on the meter once stable.
3. Refer to literature for the desired pH range of the culture medium. For commercially purchased dehydrated media, refer to the product label to check whether the pH is within the stated range. If not, follow the media manufacturer's recommendations.
4. Before measuring another sample, rinse the tip of pH electrode with distilled or deionized water and blot dry with soft tissue.
5. Discard samples after testing.

Always store the pH electrode clean. To remove protein residues from pH electrode, use HORIBA 250 cleaning solution and warm water. For more information on cleaning and maintenance, refer to the electrode instruction manual.

References And Suggested Readings

1. Baird, R., Denyer, S. & Hodges, N. Handbook of Microbiological Quality Control in Pharmaceuticals and Medical Devices, p. 27.
2. Control of Microbiological Culture Media by Nordic Committee on Food Analysis. Retrieved from http://www.nmkl.org/dokumenter/prosedyrer/sk/PROC10_no.pdf on 25 September 2016.

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HORIBA Instruments (Singapore) Pte. Ltd.
83 Science Park Drive, #02-02A, The Curie, Singapore 118258
Phone: 65 6908-9660
Fax: 65 6745-8155
www.horiba-laqua.com
e-mail: laqua@horiba.com

HORIBA UK Limited
Kyoto Close, Moulton Park, Northampton NN3 6FL
Phone: 44 (0) 1604 542567
Fax: 44 (0) 1604 542699
www.horiba.com/uk
e-mail: waterquality@horiba.com

www.horiba-laqua.com