TRIS Buffers - What electrodes can I use?

TRIS buffers are used by biochemists to control pH in the physiological range (about 7 to 8 pH) because phosphates cause undesirable side reactions with the biological substances in their test samples.

However, when pH measurements are to be made on these solutions, another type of "undesirable side reaction", involving the pH electrode system, must be recognized. The common Silver-Silver Chloride reference electrode used with most combination pH electrodes has a Potassium Chloride salt-bridge which is saturated with Silver Chloride. This salt-bridge system works well in most samples, but not in biological samples containing proteins or related materials. The quite low concentration of Silver ion (about 0.0001 M) is sufficient to react with proteins and produce an insoluble precipitate in the porous liquid junction structure of the electrode and thus cause errors in pH measurement due to the development of substantial "liquid junction" potentials across this plug of precipitate.

This problem can be avoided quite simply by using an electrode with a calomel reference internal element, or a "double-junction" design. Either reference internal cell is contained in its own glass tube structure within the salt-bridge, and the Potassium Chloride solution does not contain heavy metals. Thus, none of the undesirable precipitates form, the reference junction remains unplugged, and the liquid junction potential remains negligible.

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Accurate pH Measurements in Tris Buffer Solutions

Introduction

Tris, or tris (hydroxymethyl) aminomethane, has been widely used as a pH buffer in biological media for approximately thirty-five years. The almost ideal characteristics of this physiological buffer account for its popularity. Tris is not hydroscopic is easily dissolved in water, and is available in high purity. It does not precipitate calcium salts, is stable in solution at room temperature for months, and does not appear to inhibit many enzyme systems. During the late '60s, it was reported that incorrect pH readings were obtained when the reference electrode used to measure the pH of tris had a linen fiber liquid junction. This problem, though easily eliminated, has raised a number of questions concerning the proper reference for use with tris buffers. Additionally, because of the chemical and physical properties of tris buffers, improper use may lead to erroneous pH measurements. The purpose of this bulletin is to point out the possible sources of error and to recommend the most appropriate electrode system for pH measurement when tris buffers are used.

Effect of Chemical & Physical Properties of Tris Buffers on pH

Table I contains compositions, buffer values, dilution values, and an approximate temperature coefficient of tris buffer over its practical buffer range (pH 7 to 9). The data in Table I indicates that tris in the pH range frequently used for physiological measurements (pH 7 to 7.5) does not have a large buffering capacity. Therefore, caution should be exercised when using tris buffers to control sample pH. The rather large temperature coefficient of tris (- 0.028 pH/°C) also warrants consideration. For example, if a tris buffer is used to standardize a meter, and the tris temperature is 20°C, using the pH value at 25°C will result in an error of

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+0.14 pH units ($\Delta T = -5^\circ, = \frac{\Delta pH}{\Delta T}$ - 0.028 pH/°, Δ pH = (- 5) (- 0.028) = +0.14)

Clearly, for best results, the temperature pH dependency must be used when standardizing with tris. It directly follows that when tris is used to control the pH of a sample, the degree of control will be dependent on sample temperature. If the sample temperature varies by 2°C, the pH control varies by approximately 0.06 pH units. The desired pH control will dictate the maximum allowable temperature fluctuations.

Tris is a primary aliphatic amine and can react with some samples. When tris is used to control sample pH, the possibility of a reaction with the sample should be considered. For example, tris forms a stable complex with Silver ion. The presence of Silver ion in the sample may rule out using tris to control the pH of the sample.

PH Measurements Using Tris Buffers

1. Use an electrode pair or combination with a ceramic or sleeve-type junction.

2. Calomel or Silver/Silver Chloride type references will be satisfactory for most measurements. However, the possibility of introducing heavy metal ions (Ag+, Hg22+) should be considered. This problem is less likely to occur when a calomel reference is used due to the lower solubility of Mercurous Chloride versus Silver Chloride. Introducing heavy metals into the sample can be eliminated by using a double junction system with either reference.

3. Tris buffers should be prepared from high purity tris and HCl using C02-free distilled or deionized water.

4. The pH of tris buffers is temperature dependent. For most accurate work, all solutions should be thermostated.

Table 1

COMPOSITIONS, BUFFER VALUES ß, AND DILUTION VALUES ApH I/2,
OF SOME TRIS BUFFER SOLUTIONS AT 25°C

50mL 0.1 M tris (hydroxymethyl)-aminomethane, x mL 0.1 M HCI,
Diluted to I00mL dpH/dt = – 0.028 unit deg.-1; I = 0.001X

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pH	x	β	ApH [1/2]
7.00	46.6	-	-0.02
7.10	45.7	0.010	
7.20	44.7	0.012	
7.30	43.4	0.013	
7.40	42.0	0.015	
7.50	40.3	0.017	-0.02
7.60	38.5	0.018	
7.70	36.6	0.020	
7.80	34.5	0.023	
7.90	32.0	0.027	
8.00	29.2	0.029	-0.02
8.10	26.2	0.031	
8.20	22.9	0.031	
8.30	19.9	0.029	
8.40	17.2	0.026	
8.50	14.7	0.024	
8.60	12.4	0.022	
8.70	10.3	0.020	-0.01
8.80	8.5	0.016	
8.90	7.0	0.014	
9.00	5.7	-	-0.01

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