



Prepare Mobile Phase with Accurate pH for Drug Analysis using Agilent pH Meters

Technical Overview

Introduction

High performance liquid chromatography (HPLC) is an important tool for the analysis of pharmaceutical drugs for drug monitoring and quality assurance. The pH value of the mobile phase is an important factor in controlling the sample solution during HPLC.

This technical overview describes the use of an Agilent pH meter/electrode combination for a simple and accurate method to make 0.025 mol/L KH_2PO_4 at pH 3, a mobile phase common in drug analysis [1]. Agilent electrochemistry meters and electrodes are designed for easy operation, even by non-technical users, while still producing the most reliable readings in the shortest possible time.



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Materials

Meters: Agilent 3200P pH meter (p/n G4383A), 3200I ion meter (p/n G4386A), or 3200M Multi-Parameter Analyzer (p/n G4387A)

Holder: Agilent 3200EA electrode holder (p/n G4389A)

Stirrer: Agilent 3200SA (p/n G4388A)

Electrodes: Agilent P3211 combination electrode (p/n 5190-3988), T7111 ATC probe (p/n 5190-3998)

Buffer: pH buffer package (p/n 5190-0533)

Sample: 0.025 mol/L KH_2PO_4

pH adjustment solution: Phosphoric acid, analytical grade

To prepare the phosphate stock solution, accurately weigh 3.40 g KH_2PO_4 and dissolve in DI water. Make up to a constant volume of 1,000 mL in a volumetric flask.

Calibration

The calibration process is as follows:

1. Rinse electrode with DI water to clean it.
2. Press **Calibrate** and **Enter**.
3. Dry the electrode and put in pH 4.01 buffer. The reading should be 177 ± 30 mV.
4. When the display is stable, press **Enter**.
5. Remove the electrode, rinse with DI water and put in pH 10.01 buffer. The reading should be -178 ± 30 mV, and 330 to 360 mV different from the potential at pH 4.01. The slope should exceed 90%.
6. Remove electrode, rinse with DI water and place in pH 7.00 buffer. A value of $\text{pH } 7.00 \pm 0.1$ indicates that the electrode can be used.

Typical results

To adjust the pH of the solution to the desired pH, pour 500 mL of the stock solution into a beaker and insert the P3211 electrode. The pH reading will be around pH 4.77. While stirring continuously, add phosphoric acid until the reading is as close as possible to pH 3; this requires three to four drops of the acid, or about 0.1 mL

Table 1. pH and stabilization time of three buffers measured using an Agilent pH meter/electrode combination.

Temperature (°C)	Buffer pH 4.01		Buffer pH 10.01		Slope	Buffer pH 7.00		Measured pH value
	mV	Stabilization time (s)	mV	Stabilization time (s)		mV	Stabilization time (s)	
24.5	184.3	29	-160.8	33	97.23	6.8	30	7.010

Clean the electrode and repeat the exercise with the other half of the stock solution to obtain a second reading, again as close as possible to pH 3. Table 1 shows example buffer readings and Table 2 is a summary of the pH values that can be expected.

Table 2. pH of phosphate solutions.

Temperature (°C)	Sample	Phosphate solution	Final pH
24.5	1	0.025 mol/L KH_2PO_4	4.766
	2	0.025 mol/L KH_2PO_4 + phosphoric acid	3.007
	3	0.025 mol/L KH_2PO_4 + phosphoric acid	3.004

Conclusions

By adjusting pH twice, the end point value obtained meets the requirement for a phosphate solution with an accurate value of pH 3. This shows that the buffer system is very stable when used with an Agilent pH meter/electrode combination.

Reference

1. Anon. Pharmaceutical Applications with HPLC. Solution guide, Agilent Technologies, Inc. Publication number 5968-2635E (2000).

Appendix

pH values of National Institute of Standards and Technology (NIST) buffers at different temperatures.

Temperature (°C)	pH of NIST buffer		
	4.01	7.00	10.01
10	4.001	7.058	10.179
15	4.001	7.035	10.118
20	4.003	7.015	10.063
25	4.01	7.00	10.01
30	4.015	6.980	9.968
35	4.023	6.979	9.926
40	4.024	6.973	—

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