The Preparation of Buffers with **ZirChrom**'s Buffer Wizard

http://www.zirchrom.com

What is pH ?

- A scale for measuring solution acidity/basicity

$$pH = -\log a_{H^+} \cong -\log [H^+]$$

- In dilute solution, H^+ activity is essentially equal to the H^+ ion concentration.
- pH in water is commonly between 0 and 14

What is a pH buffer?

- A solution that resists changes in pH when small amounts of acids or bases are added to the solution or when the solution is diluted.
- The buffer solution is commonly prepared from a weak acid and its conjugate base or from a weak base and its conjugate acid.

How does a pH buffer resist changes in pH?

- A pH buffer contains species that can neutralize small amounts of acids & bases.

pH and Liquid Chromatography

- pH of the eluent may be changed by the sample being analyzed.
- The pH changes affect acid-base equilibria in the system.
- Shifts in the acid-base equilibria may affect reproducibility, selectivity, and peak shape for ionizable compound.
- High & low pH can also destabilize silica-based columns.

Why Control/Adjust pH?

- To improve (increase/decrease) retention.
- To adjust band spacing.
- To improve peak shape (tailing).
- To make results reproducible.
- To improve detection (UV, MS).

Buffer Capacity: A Key Concept

- Ability of a buffer to resist changes in pH upon addition of an acid or a base.
- Defined as the number of moles of strong acid or base per liter required to add to the solution to produce a unit change in pH.

$$\beta = dC_b / dpH = - dC_a / dpH$$

Acetic Acid

A Simple Monoprotic Acid-Base Equilibrium

$$HA \Leftrightarrow H^+ + Ac^-$$

$$K_a = \frac{[H^+][Ac^-]}{[HAc]}$$

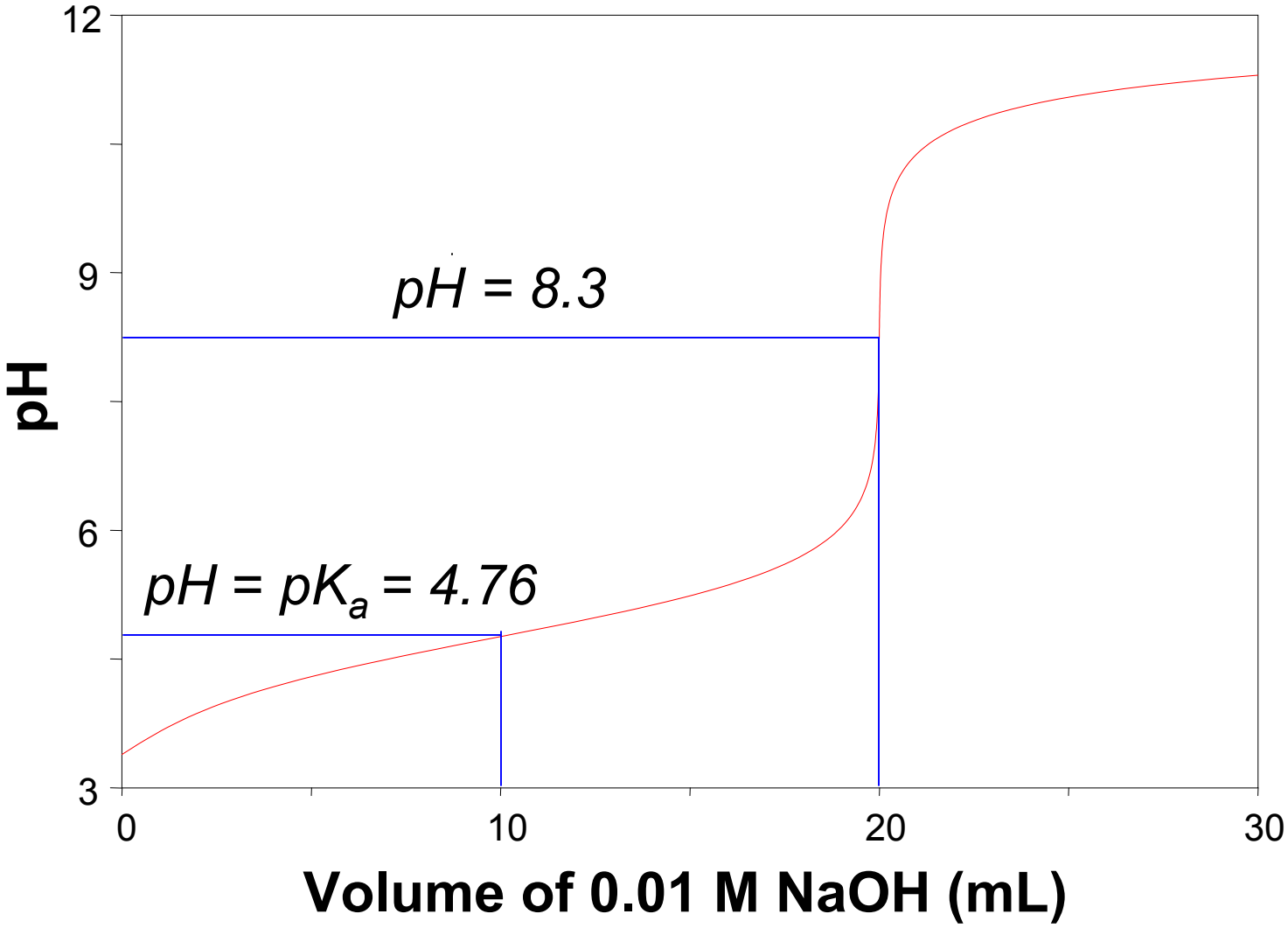
$$[H^+] = K_a \cdot \frac{[HAc]}{[Ac^-]}$$

$$pH = pK_a + \log \frac{[Ac^-]}{[HAc]}$$

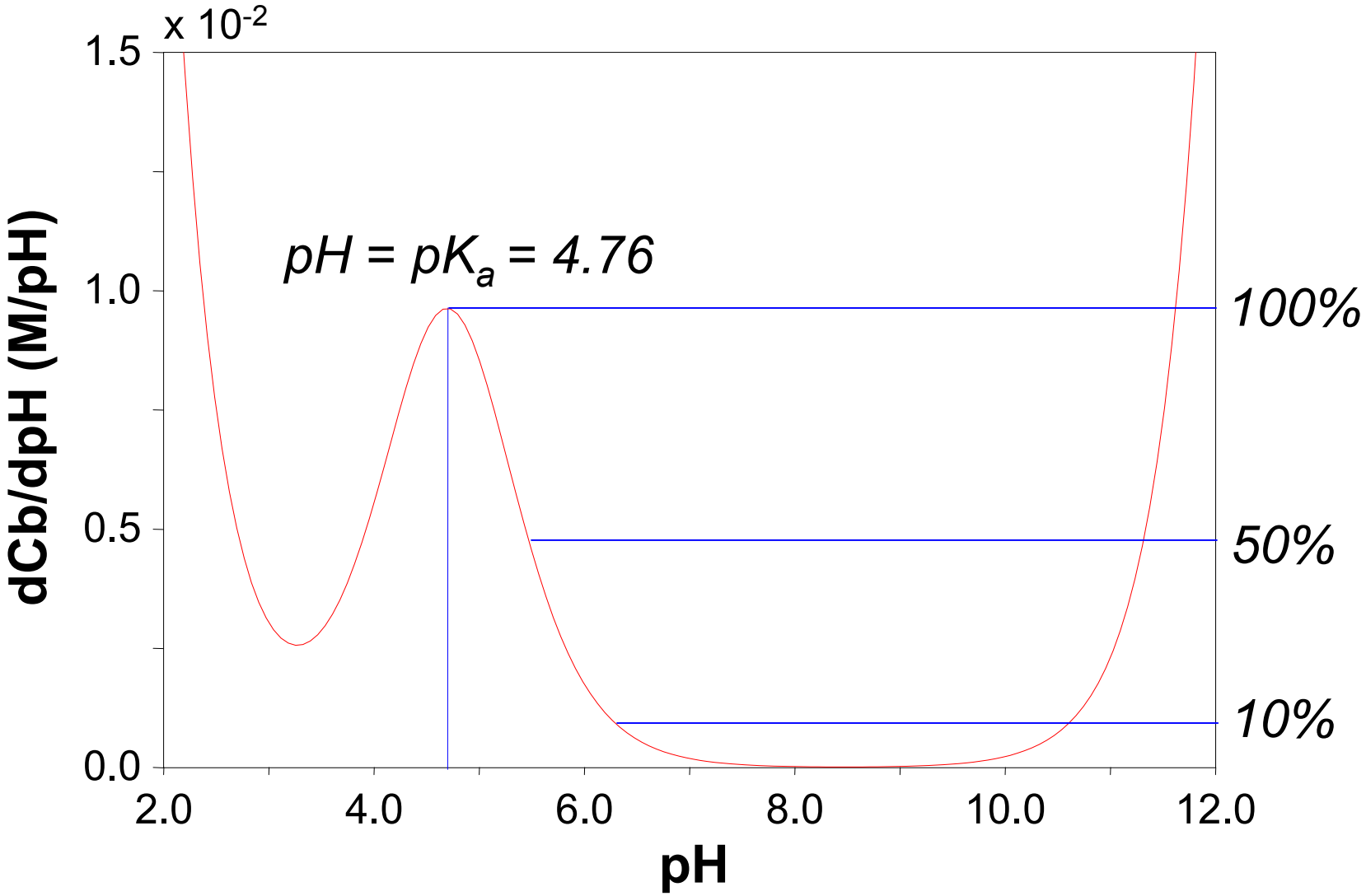
Henderson - Hasselbalch Eq.

$$pK_a = 4.76$$

Titration of 20 mL 0.01 M HAc with 0.01 M NaOH



Buffer Capacity of 0.01 M HAc



Phosphoric Acid

A Triprotic Acid-Base System

$$H_3PO_4 \Leftrightarrow H^+ + H_2PO_4^- \qquad K_{a,1} = \frac{[H^+][H_2PO_4^-]}{[H_3PO_4]}$$

$$H_2PO_4^- \Leftrightarrow H^+ + HPO_4^{2-} \qquad K_{a,2} = \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]}$$

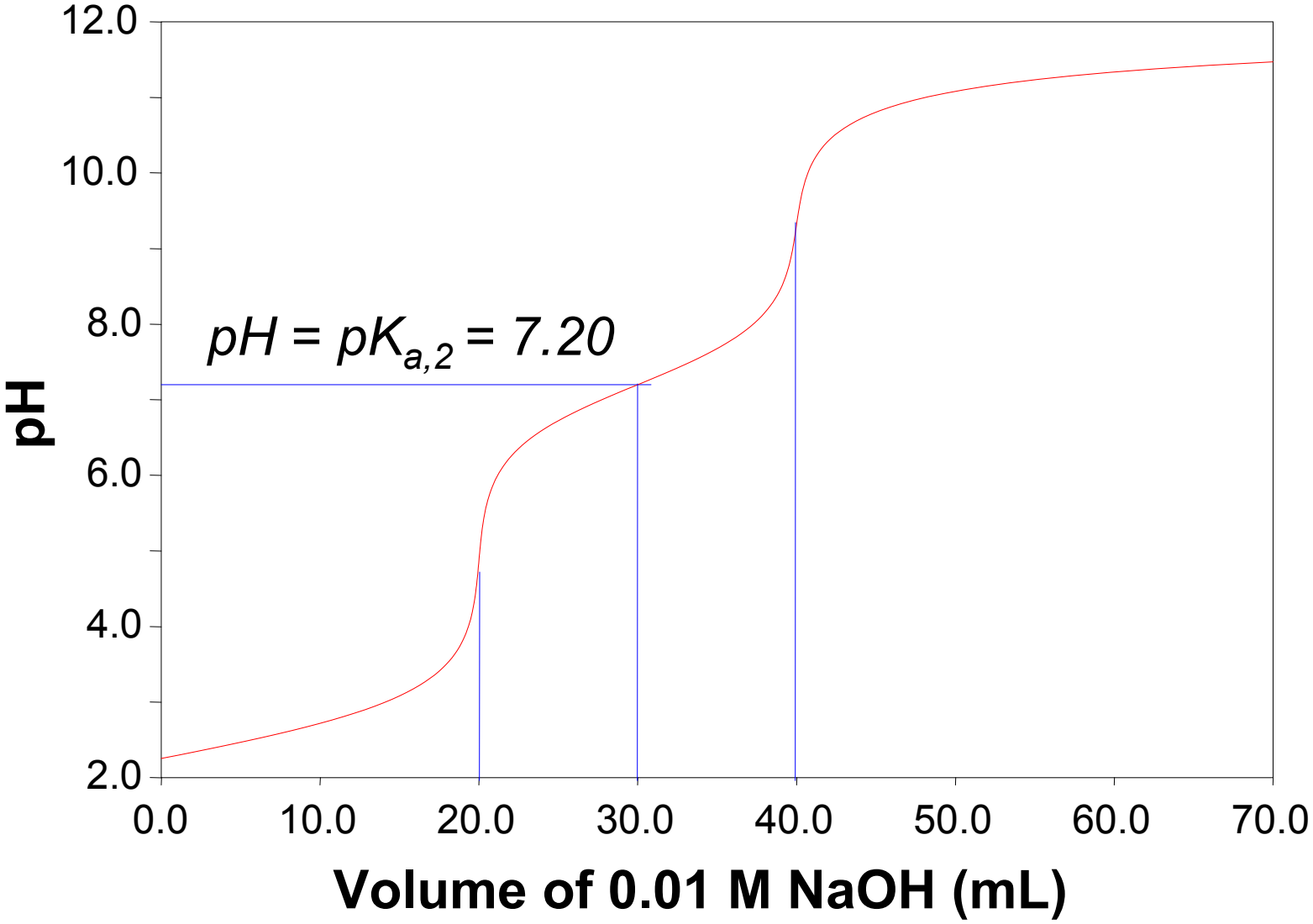
$$HPO_4^{2-} \Leftrightarrow H^+ + PO_4^{3-} \qquad K_{a,3} = \frac{[H^+][PO_4^{3-}]}{[HPO_4^{2-}]}$$

$$pK_{a,1} = 2.15$$

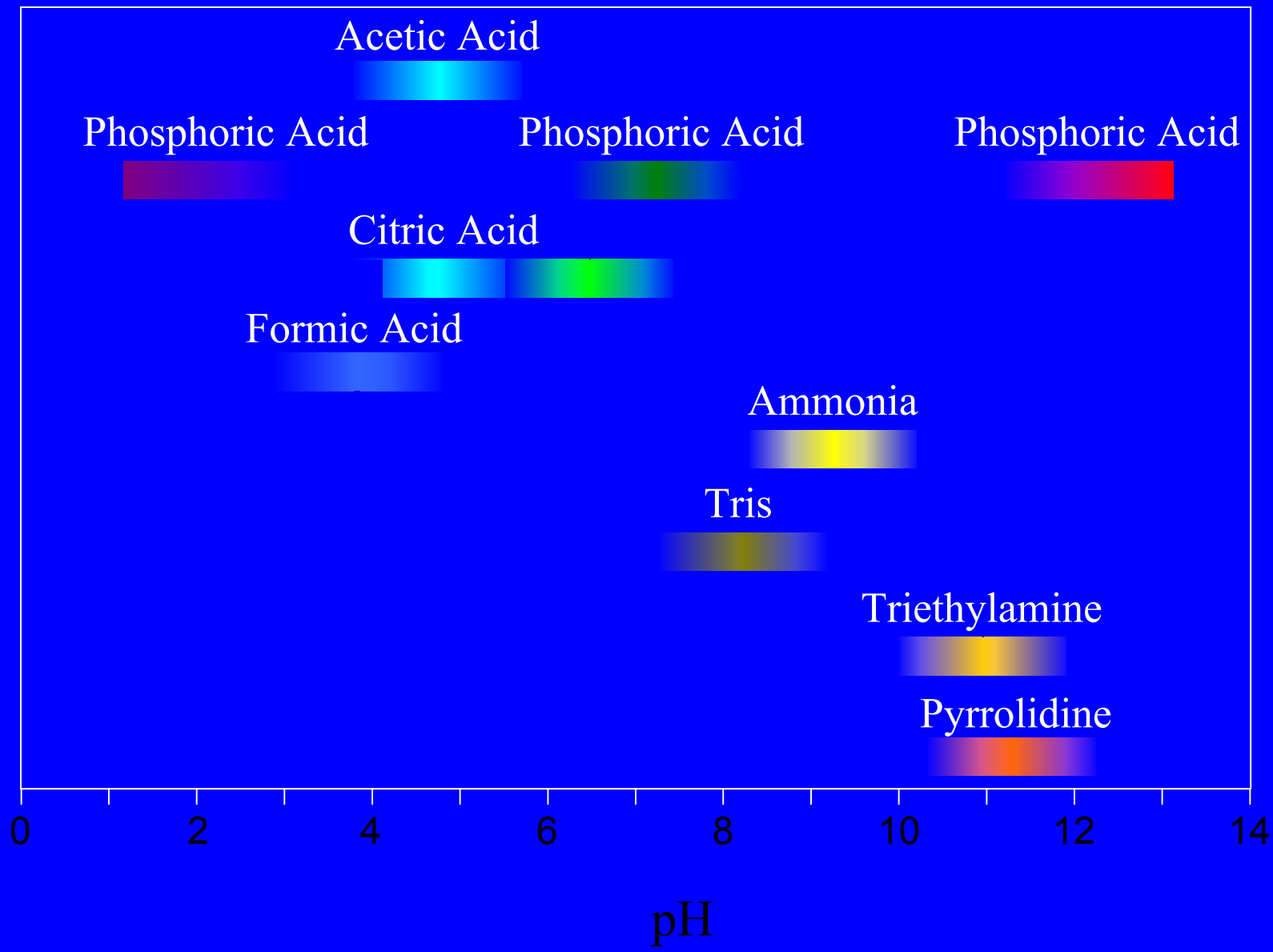
$$pK_{a,2} = 7.20$$

$$pK_{a,3} = 12.15$$

Titrating 20 mL 0.01 M H_3PO_4 with 0.01 M NaOH



Common pH Buffers



Acetic Acid + Ammonium Hydroxide

A Mixture of Two Monoprotic Acid-Base Systems

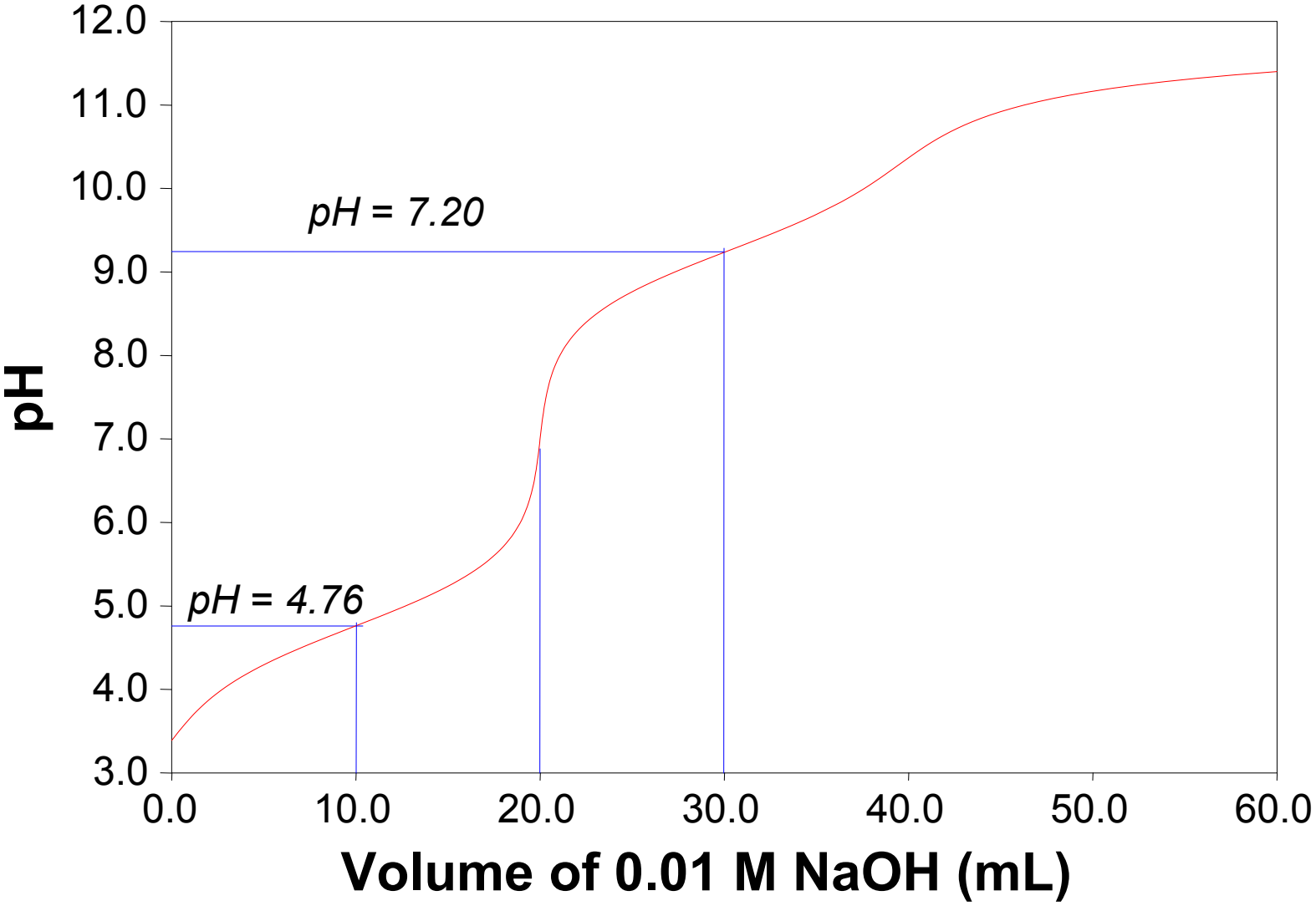
$$HAc \Leftrightarrow H^+ + Ac^- \qquad K_{a,HAc} = \frac{[H^+][Ac^-]}{[HAc]}$$

$$NH_4OH \Leftrightarrow H^+ + NH_3 + H_2O \qquad K_{a,NH_4OH} = \frac{[H^+][NH_3]}{[NH_4OH]}$$

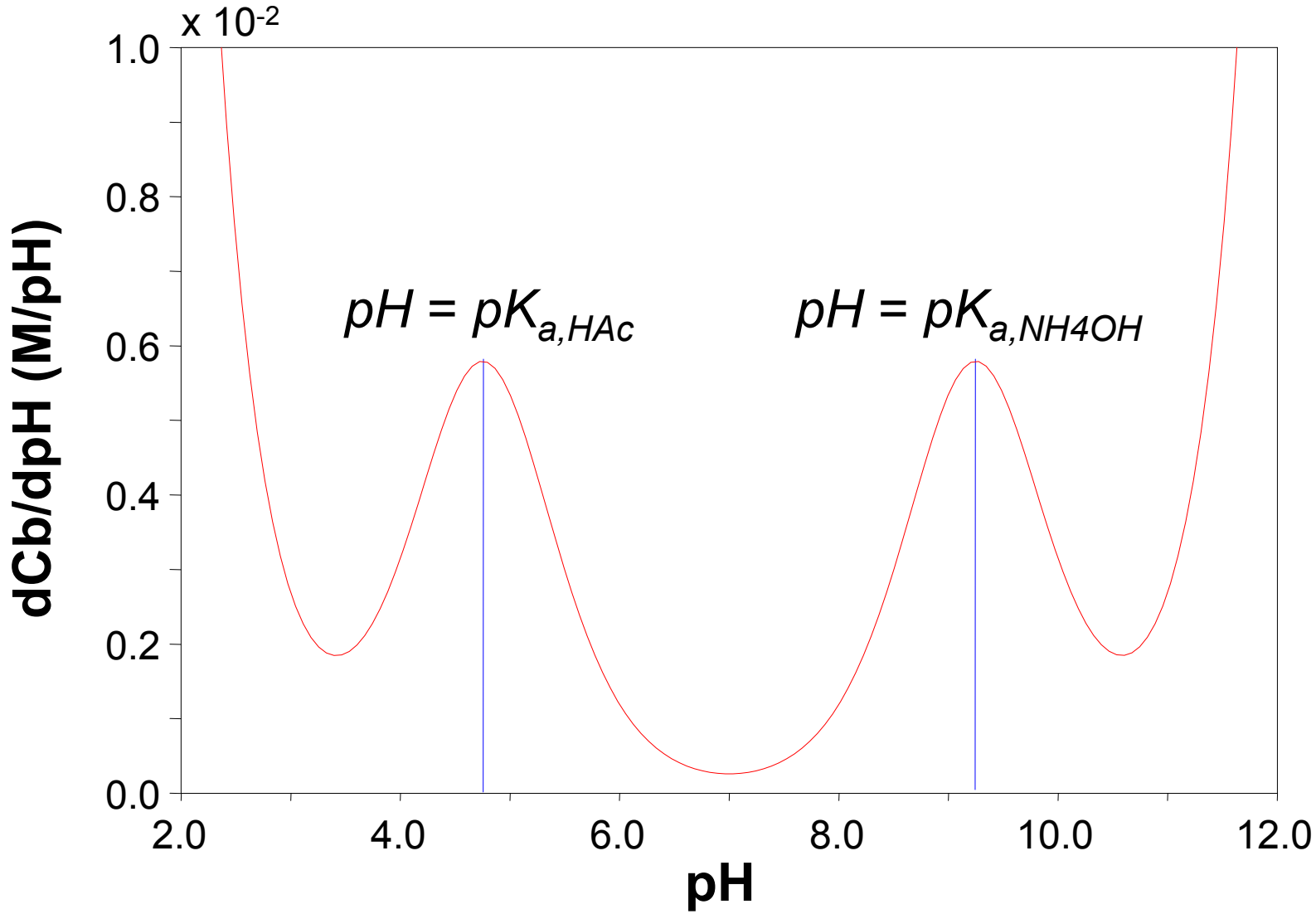
$$pK_{a,HAc} = 4.76$$

$$pK_{a,NH_4OH} = 9.24$$

Titrating 20 mL 0.01 M HAc + 0.01 M NH_4OH with 0.01 M NaOH



Buffer Capacity of 0.01 M HAc + 0.01 M NH_4OH



Buffer Action upon Dilution

- Weak acid + Weak base

  0.085 M HAc + 0.015 NaAc

  Initial pH: 4.0

  pH after 10 times dilution: 4.0

- Strong acid

  0.0001 M HCl

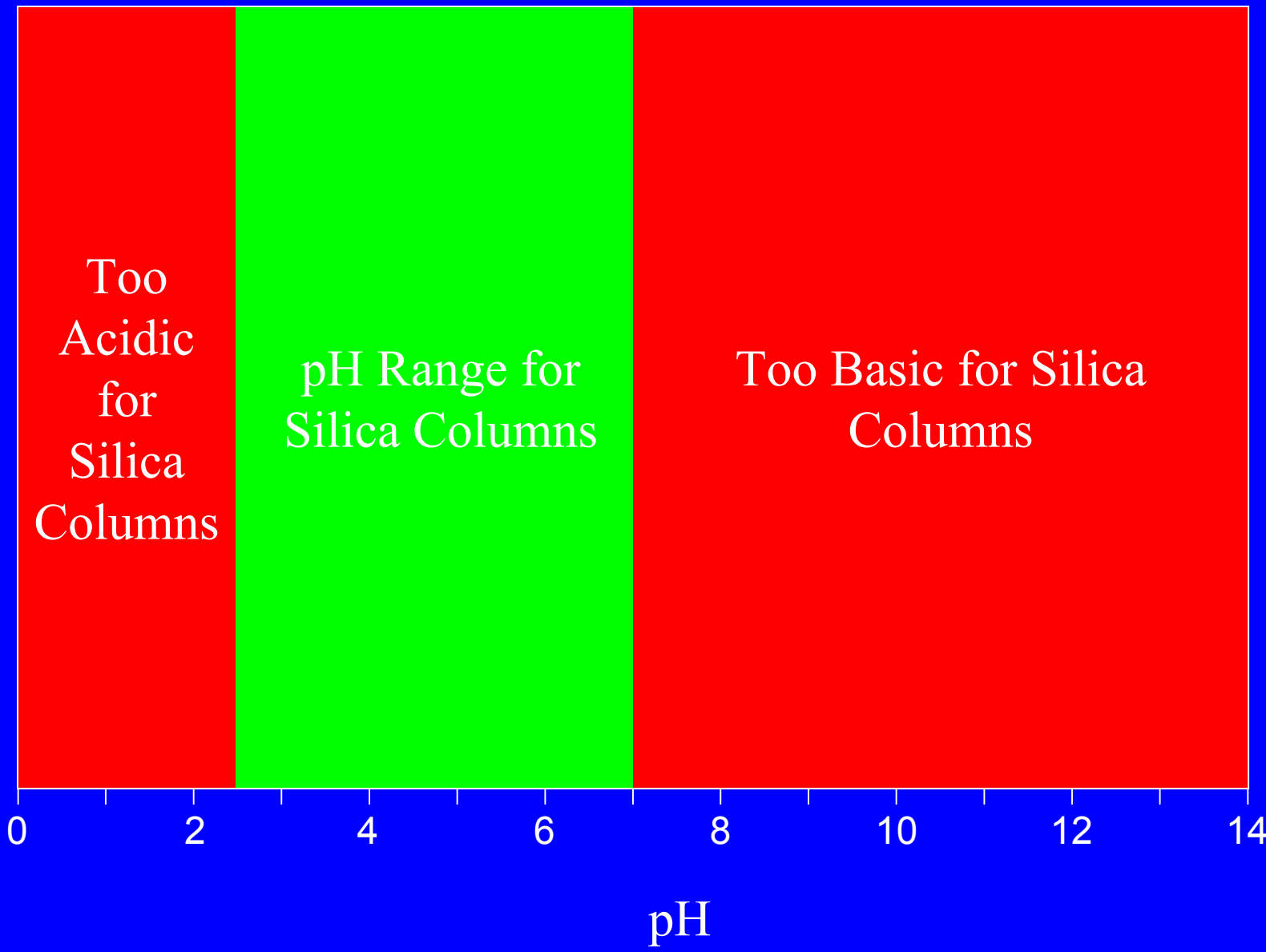
  Initial pH: 4.0

  pH after 10 times dilution: 5.0

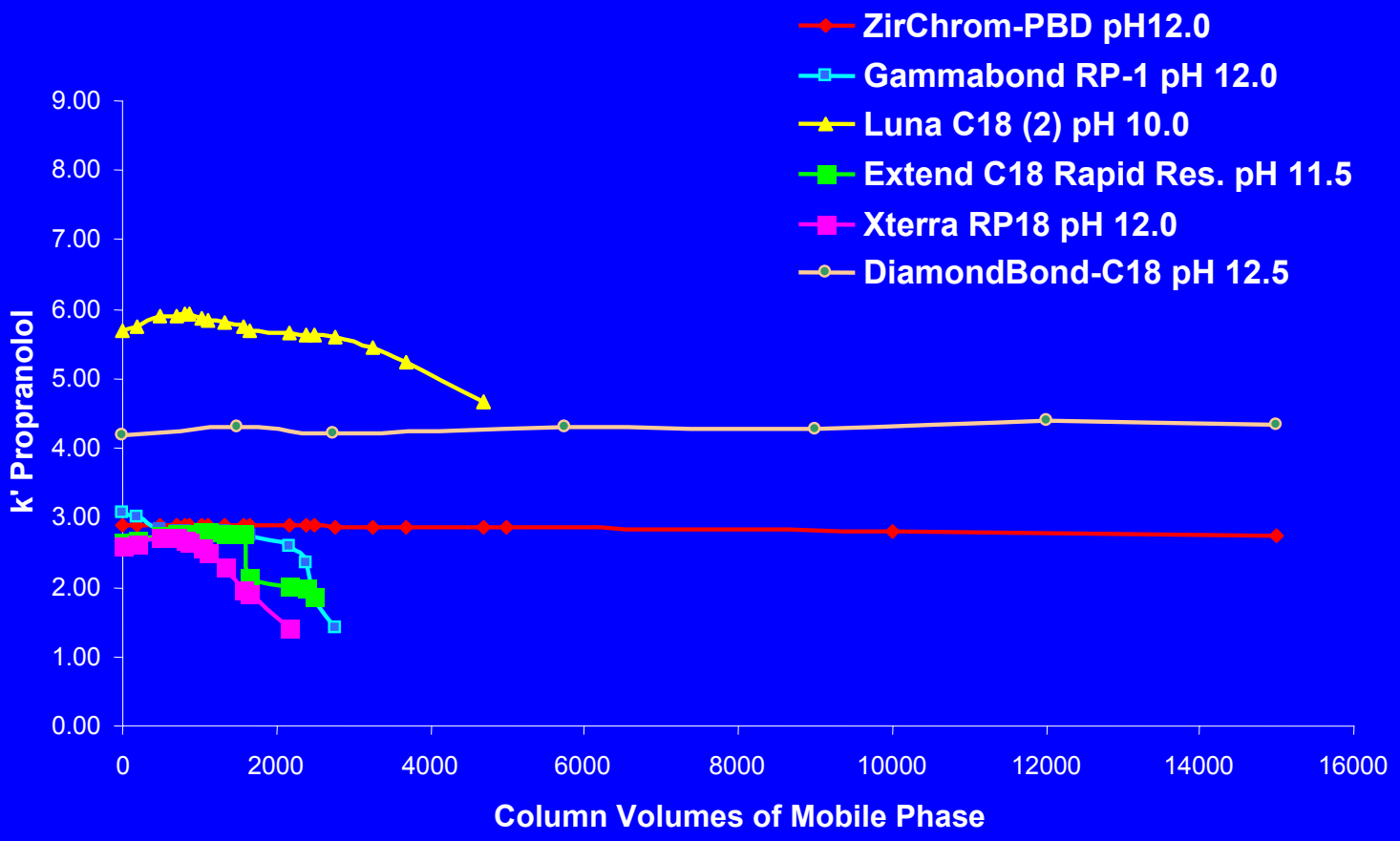
Key Issues in Buffer Preparation for HPLC

- Adequate buffer capacity—pick buffer with pK_a closest to the desired pH.
- Buffer solubility & compatibility with organic modifier.
- UV cut-off.
- Volatility for LC-MS.
- COLUMN STABILITY!

pH Range for Silica Columns



High pH Stability Comparison*

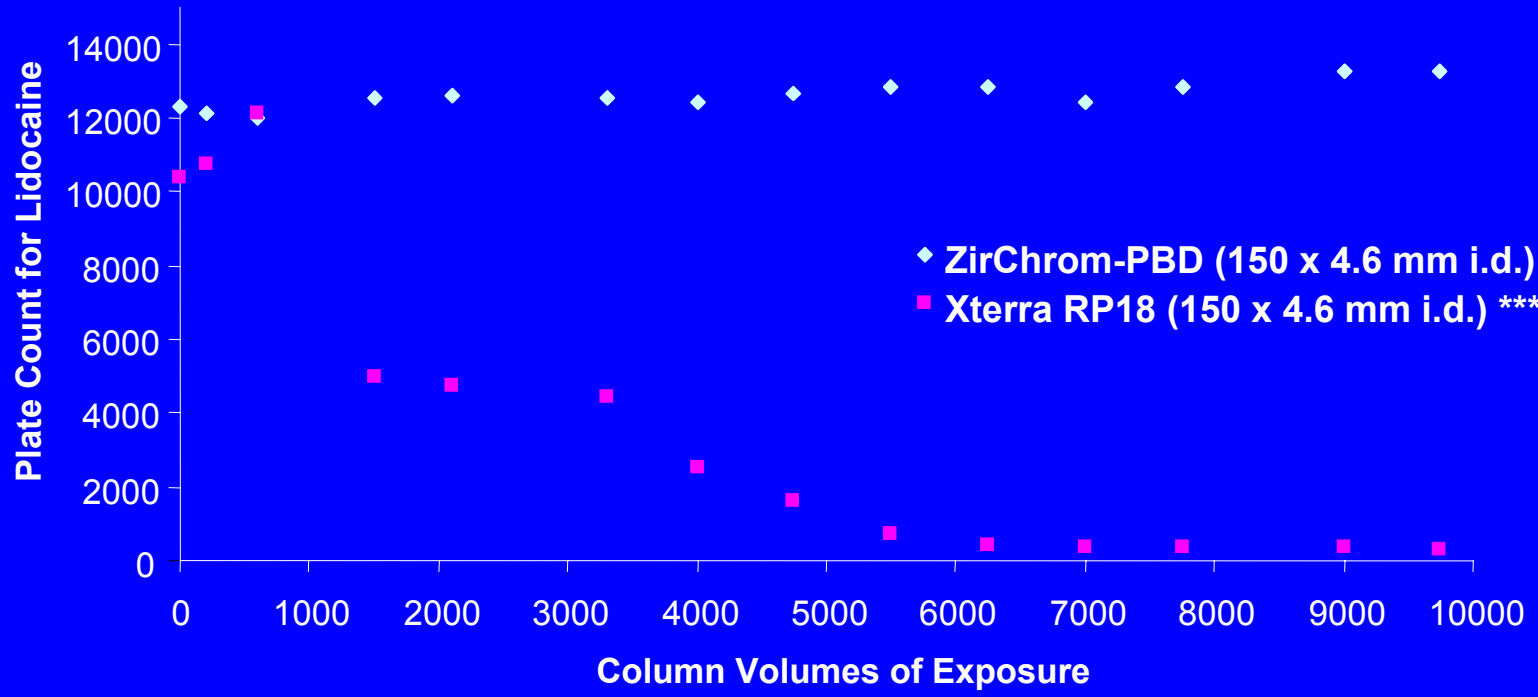


Exposure Conditions: Mobile phase, ACN/50mM Potassium phosphate buffer at indicated pH; Temperature, 30 °C.

LC Conditions: Mobile phase, ACN (or THF)/50mM Potassium phosphate buffer at indicated pH; Flow Rate, 1.0 mL/min.; Temperature, 30 °C; Injection Volume, 5 uL; Detection, 254nm.

* Column names are the trademarks of their respective manufacturers.

80 °C Aging Study
pH 7 ZirChrom-PBD vs. Xterra RP18*

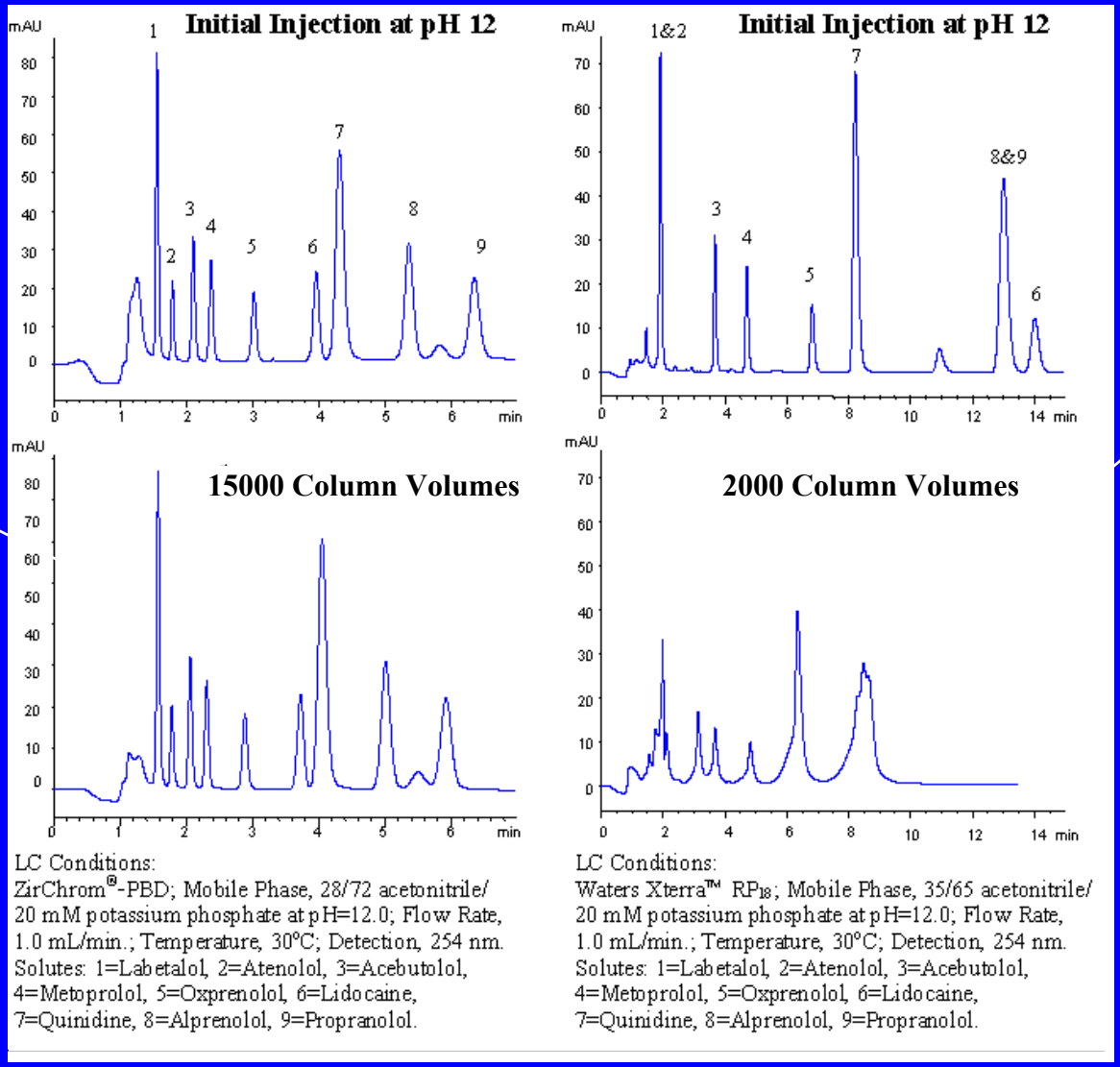


LC Conditions: Mobile phase, ACN/50mM Potassium phosphate, pH 7.0; Flow rate, 1.0 ml/min.; Temperature, 80 °C, Injection Volume, 5 uL; Detection, 254nm. **25/75 ACN/Buffer ***30/70 ACN/Buffer

* Column names are the trademarks of their respective manufacturers.

ZirChrom®-PBD vs. Xterra® RP18

ZirChrom®-PBD

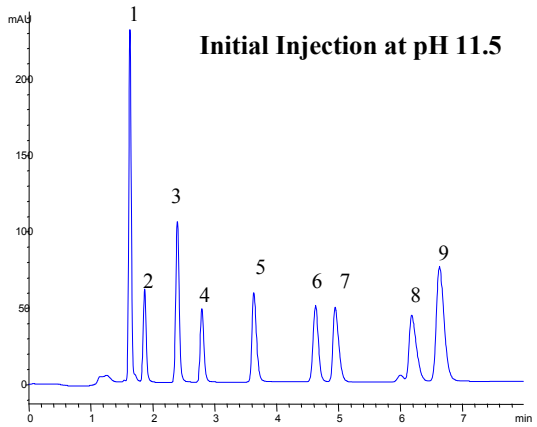


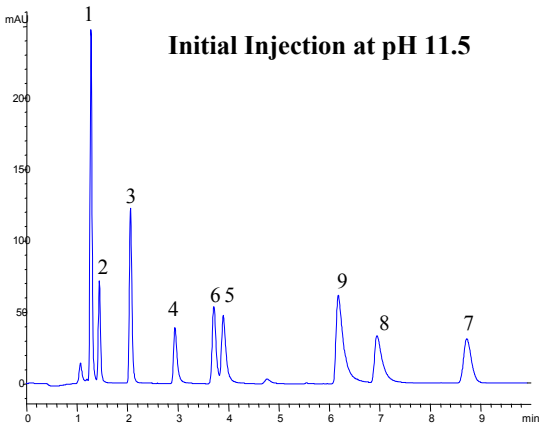
LC Conditions:
ZirChrom®-PBD; Mobile Phase, 28/72 acetonitrile/ 20 mM potassium phosphate at pH=12.0; Flow Rate, 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

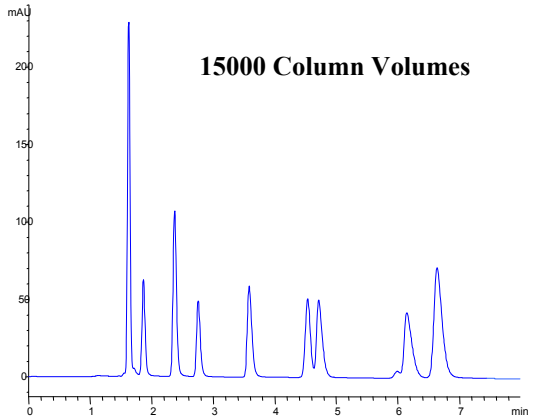
LC Conditions:
Waters Xterra™ RP_{18}; Mobile Phase, 35/65 acetonitrile/ 20 mM potassium phosphate at pH=12.0; Flow Rate, 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

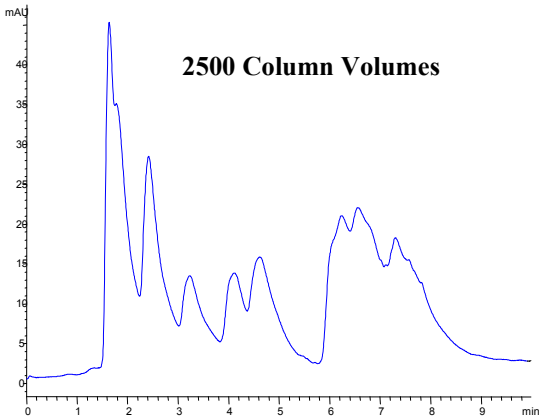
Xterra® RP18

ZirChrom®-PBD vs. Extend C18 Rapid Resolution









LC Conditions:
ZirChrom®-PBD; Mobile Phase, 28/72 acetonitrile/ 20 mM potassium phosphate at pH=11.5; Flow Rate, 1.0 mL/min.;Temperature, 40°C; Detection, 254 nm. Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

LC Conditions:
Extend C18 Rapid; Mobile Phase, 45/55 acetonitrile/ 20 mM potassium phosphate at pH=11.5; Flow Rate, 1.0 mL/min.;Temperature, 40°C; Detection, 254 nm. Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

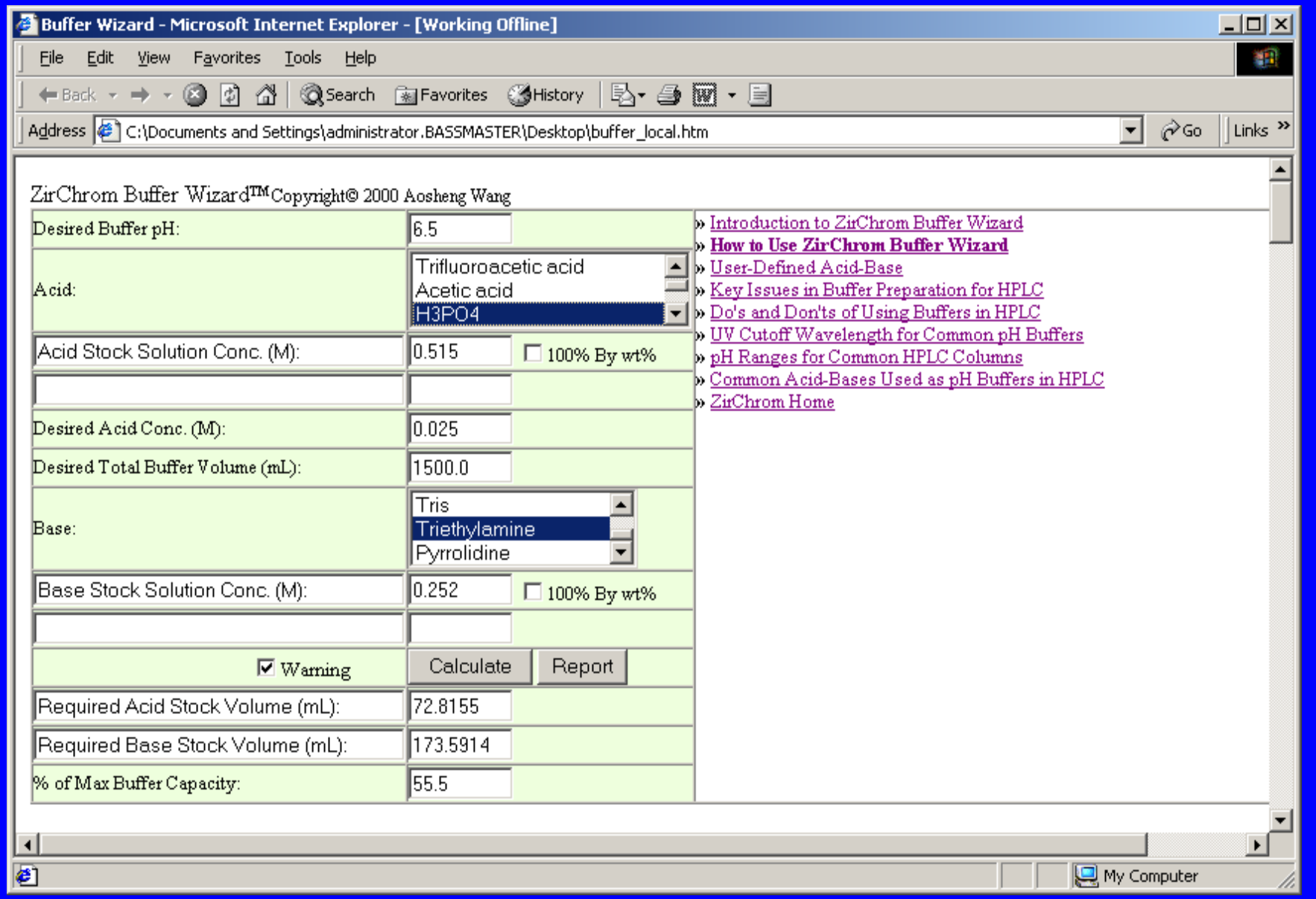
The Preparation of Buffers with ZirChrom's Buffer Wizard

http://www.zirchrom.com

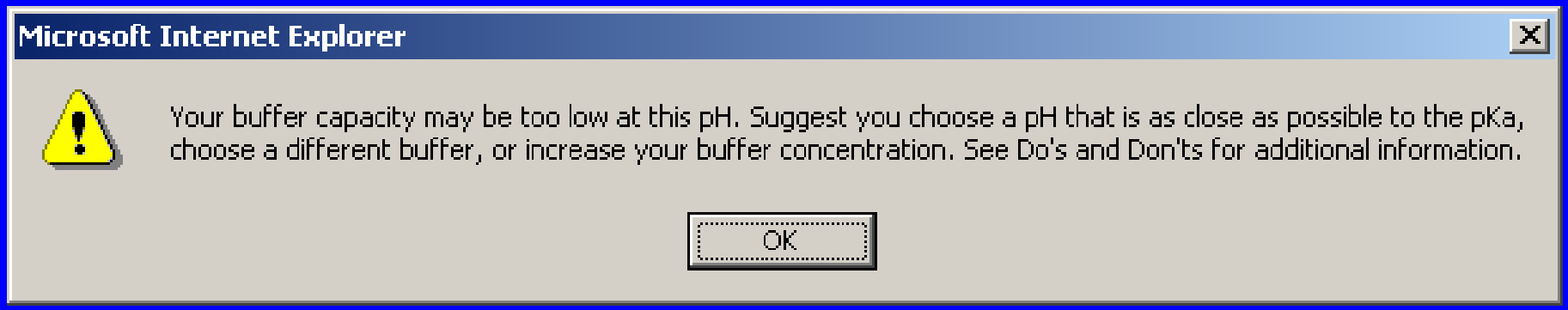
How to Prepare a Buffer

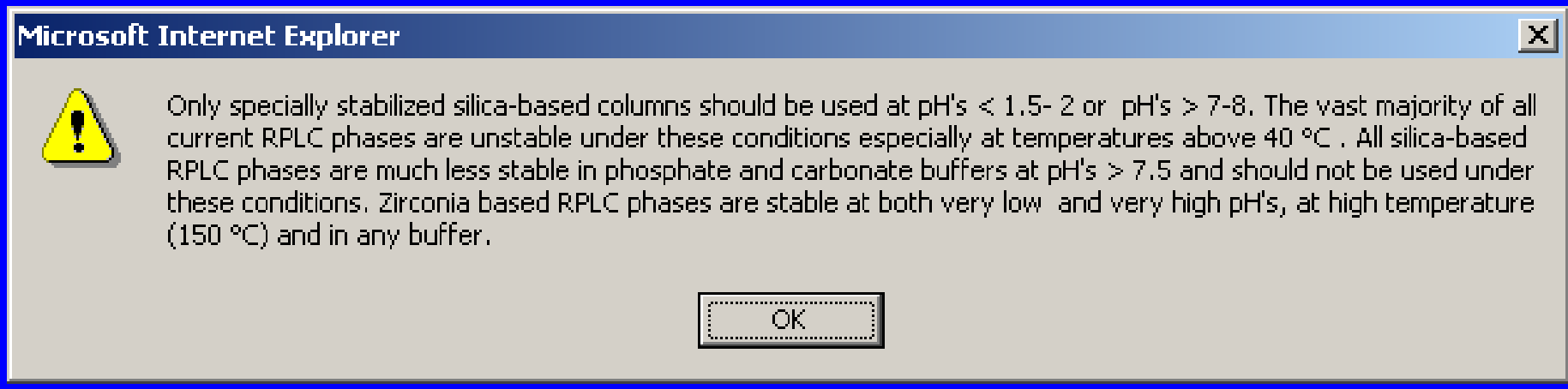
- Use Henderson-Hasselbalch equation to compute amount of acid and base needed for simple monoprotic systems.
- Get out your book on quantitative analysis and calculate for an hour after studying for a day.
- Use ZirChrom Buffer Wizard for all pH buffers.

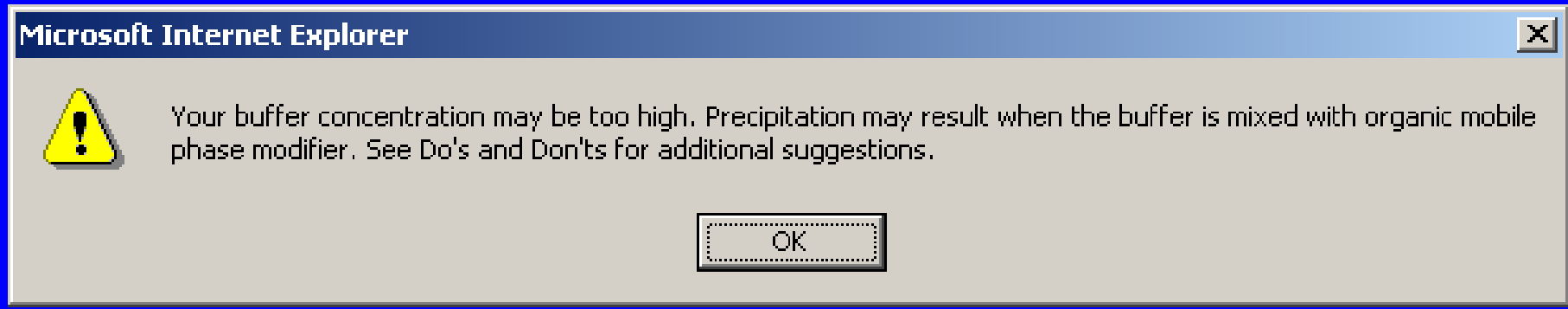
http://www.zirchrom.com


Buffer Wizard - Microsoft Internet Explorer - [Working Offline]
File Edit View Favorites Tools Help
Back Search Favorites History
Address C:\Documents and Settings\administrator.BASSMASTER\Desktop\buffer_local.htm
Go Links
ZirChrom Buffer Wizard™ Copyright© 2000 Aosheng Wang
Desired Buffer pH: 6.5
Acid: Trifluoroacetic acid Acetic acid H3PO4
Acid Stock Solution Conc. (M): 0.515 100% By wt%
Desired Acid Conc. (M): 0.025
Desired Total Buffer Volume (mL): 1500.0
Base: Tris Triethylamine Pyrrolidine
Base Stock Solution Conc. (M): 0.252 100% By wt%
Warning Calculate Report
Required Acid Stock Volume (mL): 72.8155
Required Base Stock Volume (mL): 173.5914
% of Max Buffer Capacity: 55.5
» Introduction to ZirChrom Buffer Wizard
» How to Use ZirChrom Buffer Wizard
» User-Defined Acid-Base
» Key Issues in Buffer Preparation for HPLC
» Do's and Don'ts of Using Buffers in HPLC
» UV Cutoff Wavelength for Common pH Buffers
» pH Ranges for Common HPLC Columns
» Common Acid-Bases Used as pH Buffers in HPLC
» ZirChrom Home
My Computer

Buffer Wizard Warning Panels







Key Issues in Buffer Preparation for HPLC

- Adequate buffer capacity.
- Buffer solubility & compatibility with organic modifier.
- UV cut-off.
- Volatility for LC-MS.

The Big 14 Do's & Don'ts in Using Buffers in HPLC

1. Check pH of buffers ***before*** adding organic modifier not after.
2. Phosphate at pH > 7 destabilizes silica based columns. Should not be used > 40 ^{o}C. Silica columns are seriously destabilized at pH < 2.5 and pH > 7-8. ***Use zirconia based columns for highest stability at any pH or temperature***.

Do's & Don'ts of Using Buffers in HPLC

3. Always ***pre-check buffer solubility*** by mixing the buffer with highest amount of organic modifier. Let sit for a minimum of 30 minutes to see if buffer precipitates. The buffer cation seriously effects its solubility. Sodium salts tend to be least soluble.

Do's & Don'ts of Using Buffers in HPLC

4. The buffer cation ($Na^+ < K^+ < NH_4^+ < TEA^+$) can help suppress silanophilic interactions in the order listed.
5. It is important to flush silica based columns with 15-20 volumes of buffer free eluent and then with pure methanol or pure acetonitrile (preferred) prior to overnight storage.
6. 200 ppm of sodium azide or alternatively a minimum of 20% (by volume) organic modifier should be added to the buffer to suppress bacterial growth.

Do's & Don'ts of Using Buffers in HPLC

7. 10-50 mM buffer is usually adequate. ***25 mM is good to start*** . Most inorganic buffers are more soluble in methanol than acetonitrile or THF.

Do's & Don'ts of Using Buffers in HPLC

8. If peak shape of acidic or basic analytes is poor increase the buffer capacity, or decrease the amount of sample injected. ***Use more than 5 mM buffer and make sure that the buffer capacity is at least 20% of the maximum possible for the concentration used.***

Do's & Don'ts of Using Buffers in HPLC

9. Buffers containing ammonium or triethylammonium are more volatile than those containing Na^+, K^+, etc.
10. Buffers containing acetate, formate, fluoride, are more volatile than phosphate, citrate, etc.
11. Carbon dioxide can be lost by excessive sparging of the buffer. pH increases.

Do's & Don'ts of Using Buffers in HPLC

12. TFA and TEA degrade with time and the UV cutoff deteriorates.
13. TFA in glass ampoules gives better UV cutoff than bottled TFA.
14. Phosphate, has better UV cutoff than TFA, acetic acid or citric acid.

Effect of Organic Solvent on pH Buffers

- I. Canals, J. A. Portal, E. Bosch, M. Roses, "Retention of Ionizable Compounds on HPLC. 4. Mobile Phase pH Measurement in Methanol/Water", *Anal. Chem.* 2000, 72, 1802-1809.

Thanks ***very much*** for listening!

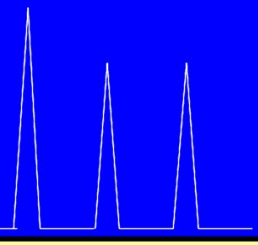
ZirChrom Separations & Cabot Corporation
Partners in Chromatography

PLEASE VISIT BOOTH 220-222

For more information and web access to the
free **Buffer Wizard: www.zirchrom.com**

PRP SiO_2

ZirChrom Separations



SOLVENT	UV Cutoff (nm) A=0.02	A=1.0
Methanol	250	205
Acetontitrile	210	190
THF	300	212
Isopropanol	250	205
Water	<190	<190

Column Dead Volumes (cc)[a]

L(cm)	Diameter (mm) 1	2.1	4.6
5	0.0271	0.119	0.573
10	0.0542	0.239	1.146
15	0.0812	0.358	1.719

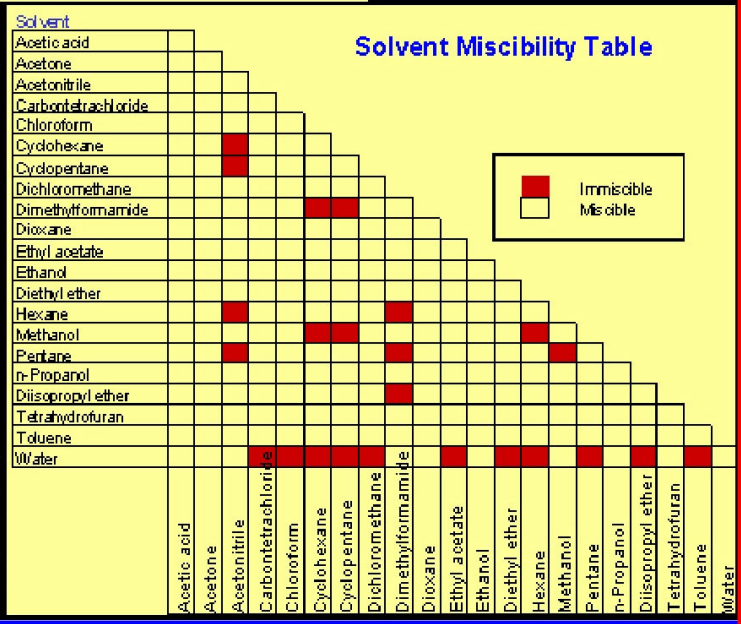
a. Total porosity = 0.69.

Acid/Base	pKa
COOH	4.5-5.5
Aliph. N	9.0-11.0
Pyridine	5.17
Aniline	4.6

BUFFERS	pKa	Buffer Range	UV Cutoff (10mM) in nm
Trifluoroacetic*	0.5	1.5-2.5	210
H_3PO_4	2.1	< 3.1	<200
	7.2	6.2-8.2	
	12.3	11.3-13.3	
Citric acid	3.1	2.1-6.4	230
	4.7		
	5.4		
Formic acid*	3.8	2.8-4.8	210
Acetic acid*	4.8	3.8-5.8	210
Carbonate	6.4	5.4-7.4	<210
	10.3	9.3-10.3	
Bis-tris propane	6.8	5.8-7.8	215
	9.0	8.0-10.0	
Tris	8.3	7.3-9.3	205
Ammonia*	9.2	8.2-10.2	200
1-methylpiperidine	10.1	9.1-11.1	215
Triethylamine*	11.0	10.0-12.0	<200

* denotes volatile buffer

Solvent Miscibility Table



To access our FREE automated HPLC Buffer Wizard calculator and a great deal of other HPLC data visit our web site at http://www.zirchrom.com

World's Most Robust HPLC Columns. Telephone: 763 -421-5264, Fax: 763-421-2319

Product List

Part #	Product Name	Chromatographic Mode and Uses
ZR01	ZirChrom®-CARB	Reversed-Phase
ZR02	ZirChrom®-PHASE	Normal Phase and SEC
ZR03	ZirChrom®-PBD	Reversed-Phase
ZR04	ZirChrom®-WCX	Weak Cation-Exchanger
ZR05	ZirChrom®-WAX	Weak Anion-Exchanger and Sugar Analysis
ZR06	ZirChrom®-SAX	Strong Anion-Exchanger
ZR07	ZirChrom®-SHAX	Strong Hydrophilic Anion-Exchanger
ZR08	ZirChrom®-PEZ	Cation-Exchanger for Proteins