Guidelines for Using UNOsphere™ S Cation Exchange Support

UNOsphere S support exhibits a pH shift during conductivity fluctuations. These pH fluctuations can be modulated through careful selection of buffering and sample conditions. This phenomenon is common in strong cation exchangers containing carboxyl groups in their support structures, as well as in weak carboxymethyl (CM)-containing cation exchange resins. The following recommendations (Table 1) will help you choose the appropriate buffers and experimental conditions when using UNOsphere S support.

Table 1. Recommendations for experimental conditions.

	Guideline	Rationale
Buffer Selection	Use an anionic or zwitterionic buffer.	Standard cation exchange chromatography buffers (anionic (–) and zwitterionic (+/–)) will not associate with the negatively charged groups on the cation exchanger and will be more available to provide pH control in the mobile phase.
	Use a buffer that has a pK_a within 0.5 pH units of the desired pH of the experiment (Table 2).	Buffering capacity and pH control are greatest near the pK_a of the buffer. Any typical cation exchange buffer may be used; however, Table 2 lists the best buffers, organized by target pH.
Buffer Preparation	Do not back-titrate buffer pH.	Use particular care when using buffer salts to ensure that back-titration does not occur. If the pH is overshot during preparation, discard the buffer and begin again. Back-titration will produce inconsistent buffers due to the formation of differing amounts of salt depending on the severity of the back-titration.
Rapid Column Equilibration	Preequilibrate the column (Figure 1).	A 1 column volume (CV) preequilibration with the appropriate buffer at a concentration of at least 0.5 M will bring the column into operating conditions more efficiently than using equilibration buffer alone. Experiments may be conducted to further optimize equilibration, possibly by lengthening the preequilibration step (Figure 1).
	Use an equilibration buffer with a concentration of at least 0.05 M (Figure 2).	Equilibration with 0.05 M buffers reduces the equilibration time and provides better pH modulation than 0.02 M buffers (Figure 2).
Sample Preparation	The salt concentration and conductivity of the sample should be as close as possible to that of the equilibration buffer.	pH fluctuation can occur if the sample conductivity and salt concentration differ from that of the equilibration buffer. This can create changes in protein and contaminant binding. If the pH is reequilibrated during sample loading, these effects should be minimized.
Elution Buffer Selection and Conditions	Avoid the use of NaCl* to produce conductivity gradients; use higher buffer concentrations to formulate elution buffer.	Maximum pH control upon elution occurs when elution is with a concentrated buffer instead of a salt gradient. Using buffers to increase conductivity increases buffering capacity and dramatically reduces the amplitude and duration of pH transients in comparison to NaCl.*
	If NaCl* must be used to create conductivity gradients, make the steps as shallow as possible.	The amplitude and duration of the pH transient is proportional to the change in NaCl* concentration. Minimizing the interval of the step will minimize the severity of the pH fluctuation.
	When making multiple gradient steps, allow the pH to return to its target value during one step before starting the next step.	Allow the operating pH to be restored before the next gradient step. If the pH is not allowed to return to its target value prior to the next gradient step, the pH fluctuation may result in significant changes in selectivity that may or may not be reproducible. This can be avoided by extending each gradient step until reequilibration to the target pH is achieved.
	Conduct method scouting and development with step gradients rather than linear gradients.	The pattern of pH transients with linear gradients is different from the pattern with step gradients. Steps produce a transient pH value before reachieving pH equilibrium. Linear gradients suffer from a lag in reequilibration. The combination of pH fluctuation and changing salt concentration could make it difficult to predict the correct step based on a linear gradient.

* NaCl is used as an example in this paper, but other salts will have similar effects.

Table 2. Buffers that provide the lowest total equilibration volume (<10 CV) and most effective pH control for UNOsphere S support.

pH	Recommended Buffer	Alternative Buffers
4.0	Succinate	Citrate
4.5	Acetate	Succinate, citrate
5.0	Citrate	Succinate
5.5	Citrate	MES
6.0	MES	Citrate
6.5	MES	—
7.0	Phosphate	—

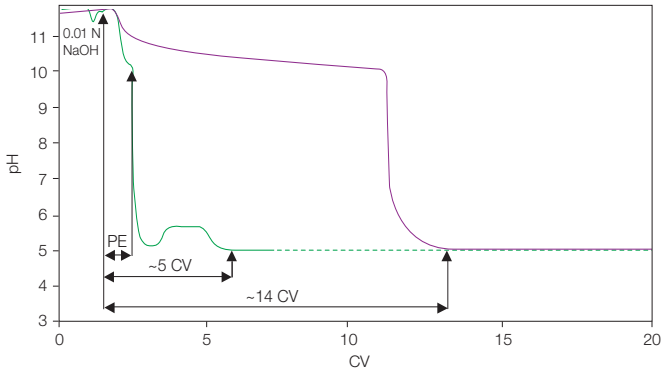

Fig. 1. Importance of preequilibration (PE) with concentrated buffer. A 10 ml UNOsphere S column (10.4 cm x 1.1 cm) was run at 250 cm/hr. Equilibration with 0.05 M acetate, pH 5.0 was preceded by a 1 CV preequilibration with the same buffer (—) or with 0.5 M acetate, pH 5.0 (—). The total number of CV required for the column to equilibrate was determined by calculating the volume from the beginning of preequilibration until the pH stabilized at pH 5.0. The trend is consistent for other buffer systems, but the degree of improvement varies from buffer to buffer.

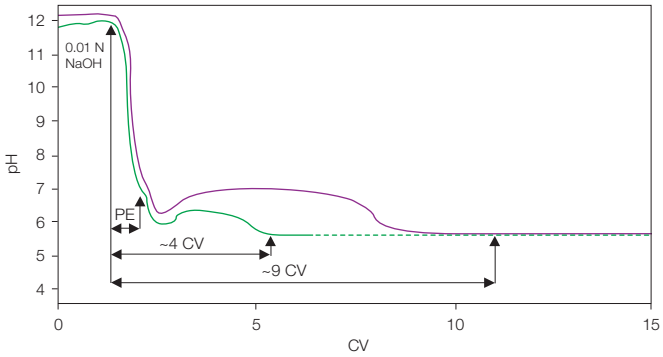

Fig. 2. Effect of buffer concentration on the number of CV required to achieve equilibration following a 1 CV preequilibration (PE) with 0.5 M buffer. A 10 ml UNOsphere S column (10.4 cm x 1.1 cm) was run at 250 cm/hr. A 1 CV preequilibration with 0.5 M citrate, pH 5.5 was followed by equilibration with either 0.02 M citrate, pH 5.5 (—) or 0.05 M citrate, pH 5.5 (—). The total number of CV for the column to equilibrate was determined by calculating the volume from the beginning of preequilibration until the pH stabilized at pH 5.5. The trend is consistent for other buffer systems, but the degree of improvement varies from buffer to buffer.

Ordering Information

Catalog #	Description
156-0111	**UNOsphere S Support,** 25 ml
156-0113	**UNOsphere S Support,** 100 ml
156-0115	**UNOsphere S Support,** 500 ml
156-0117	**UNOsphere S Support,** 10 L

For more information about UNOsphere S support, refer to the UNOsphere™ Q and S Ion Exchange Media instruction manual, contact your local Bio-Rad sales representative, or visit Bio-Rad Technical Support on the Web at **consult.bio-rad.com**

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