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Choosing HPLC Mobile Phase Buffers

Buffers are used in HPLC mobile phase preparations in order to achieve reproducible chromatography. They are needed when an analyst is dealing with an ionizable sample species. In reversed phase chromatography samples are separated based on their hydrophobicity. The less polar a sample is the longer it is retained on the column. When an analyte is ionized it becomes more polar and subsequently less retained on the column. Acids become ionized as the pH increases, conversely bases become ionized as the pH decreases.

To develop a rugged method buffers should be employed that are at least 2 pH units away from the analyte pKa . This is drawn from the Henderson-Hasselback Equation:

$$\text{pH} = \text{pKa} + \log\left(\frac{[A^-]}{[HA]}\right)$$

Essentially, operating at a pH near to the pKa of the sample analyte means that it will be in a partially dissociated state, the analyte will partially in its weak acid or base form and partially in its conjugate form. This will cause peak distortion in the chromatography are poor peak reproducibility. Operating with a mobile phase at least 2 pH units away from the analyte pKa ensures that in excess of 99% of the sample will be in a single state.

Once the proper pH range for the mobile phase is determined choosing the correct buffer can begin. Buffer capacity is optimized at or near a pH equal to the pKa of the buffer. As a rule of thumb, most buffers work suitably well within ± 1 pH unit of their pKa.

Buffers for Reversed Phase HPLC

Buffer	pKa	Buffer Range	UV Cutoff (nm)	
Phosphate	pK1	2.1	1.1-3.1	210
	pK2	7.2	6.2-8.2	
	pK3	12.3	11.3-13.3	
Citrate	pK1	3.1	2.1-4.1	230
	pK2	4.7	3.7-5.7	
	pK3	5.4	4.4-6.4	
Formate		3.8	2.8-4.8	210
Acetate		4.8	3.8-5.8	230
Tris(hydroxymethyl) aminomethane		8.3	7.3-9.3	220
Borate		9.2	8.2-10.2	210
Triethylamine		10.8	9.8-11.8	200

Another consideration when choosing a buffer is the type of detector being used. Citrate may not be suitable for some UV/Vis applications due to its high UV cutoff limit. Likewise, if mass spec detection is used, a volatile buffer such as TEA or acetate should be employed while non-volatile buffers such as phosphate or citrate should be avoided.

Once a buffer range and type are identified the proper concentration must be used. Ideally, unless using the buffer as ion-pair reagent, the buffer should have negligible effect on the overall separation and retention of your sample analytes. The concentration should be set just high enough to control the mobile phase pH. But still low enough to avoid possible precipitation of the buffer salts in the presence of organic solvents. Typically a buffer concentration in the range of 20-50mM is suitable.

It is advisable that you check the miscibility of your aqueous buffer solution in the highest concentration of organic mobile phase that will be present during the course of a gradient HPLC run before putting it on your system. This can easily be checked by mixing the aqueous portion with the correct amount of organic in a beaker and observe the presence of any salting out. Also, recall that pH is only defined in an aqueous system. So, when adjusting the pH of the mobile phase, it must be done prior to the addition of the organic solvent.

Proper selection and preparation of your mobile phase will help ensure good peak shape in your chromatography. An understanding of your analyte pKa and applying the recommendations outlined above will help in choosing and preparing the right buffer for your application.