

Using Volatile Buffers to Adjust Drop pH and Induce Crystallization

Crystal Growth 101

Volatile buffers, when added only to the reagent reservoir of a vapor diffusion experiment, can alter the pH of the crystallization drop by vapor diffusion of the volatile acid or base component from reservoir into the drop. This may be particularly useful when the sample is known to have pH dependent solubility and may be used to induce crystallization.¹⁻⁷ Acetic acid can be added to the reservoir to lower the pH of the drop. Ammonium hydroxide can be added to the reservoir to raise the pH of the drop. Final pH, the actual final volatile buffer concentration in the drop, rate and overall time of equilibration will vary with drop and reservoir volume, geometry and temperature.

The following table offers a general guideline when using a volatile buffer to manipulate drop pH to induce crystallization.

Reservoir Volume	5.2 M Volatile buffer	Final Drop [Volatile buffer]	Drop pH (CH ₃ CO ₂ H / NH ₄ OH)
1,000 µl	20 µl	0.1 M	3/9
500 µl	10 µl	0.1 M	3/9
100 µl	2 µl	0.1 M	3/9
75 µl	1.5 µl	0.1 M	3/9
50 µl	1 µl	0.1 M	3/9

Using 5.2 M Acetic acid, the approximate final drop concentration will be 0.1 M Acetic acid. The pH of 0.1 M Acetic acid is approximately 3 but the actual final drop pH after addition of Acetic acid will depend upon the sample buffer and crystallization reagents in the drop.

Using 5.2 M Ammonium hydroxide, the approximate final drop concentration will be 0.1 M Ammonium hydroxide. The pH of 0.1 M Ammonium hydroxide is approximately 9 but the actual final drop pH after addition of Ammonium hydroxide will depend upon the sample buffer and crystallization reagents in the drop.

The volatile buffer may be added at the time of initial drop/reservoir set up. In this method, the initial drop pH will be that of the sample and crystallization reagent but change over time as the volatile buffer vapor diffused from the reservoir to the drop.⁷ Alternatively, as a salvage method, to induce crystallization or improve the crystal, the volatile buffer can be added after the drop has fully equilibrated with the reagent reservoir.¹⁻⁶

References

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0.1 M Acetic acid (1 ml), 0.1 M Ammonium hydroxide (1 ml)
- HR2-853** 5.2 M Acetic acid, 10 ml
- HR2-855** 5.2 M Ammonium hydroxide, 10 ml

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