

Using Volatile Buffers to Adjust Drop pH and Induce Crystallization

Crystal Growth 101

Adding Volatile Buffers to the Reagent Well to Manipulate Drop pH

The volatile buffers Acetic acid ($\text{CH}_3\text{CO}_2\text{H}$) and Ammonium hydroxide (NH_4OH) 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.

Table 1. Adding Volatile Buffer to the Reagent Well

Reservoir Volume	Volume of 5.2 M ($\text{CH}_3\text{CO}_2\text{H}$ or NH_4OH)	Final Drop [($\text{CH}_3\text{CO}_2\text{H}$ or NH_4OH)]	Drop pH When Using $\text{CH}_3\text{CO}_2\text{H}$ or NH_4OH
1,000 μl	20 μl	0.1 M	3 or 9
500 μl	10 μl	0.1 M	3 or 9
100 μl	2 μl	0.1 M	3 or 9
75 μl	1.5 μl	0.1 M	3 or 9
50 μl	1 μl	0.1 M	3 or 9

As shown in Table 1, adding 20 μl of 5.2 M Acetic acid to a reagent well of 1,000 μl 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.

As shown in Table 1, adding 20 μl of 5.2 M Ammonium hydroxide to a reagent well of 1,000 μl 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

The Last Gasp

Alternatively, as a salvage method, to induce crystallization, the volatile buffer can be added after the drop has fully equilibrated with the reagent reservoir.¹⁻⁶ This pH driven re-solubilization and re-equilibration of the sample is a last gasp method to try and produce crystals before abandoning the experiment.

When a vapor diffusion crystallization experiment is on the verge of disposal, the drops may be clear, but more often contain amorphous precipitate, phase separation or some equivalently hopeless result. The drop has by now fully

equilibrated with the reservoir and is likely way beyond the sample's solubility limit. The addition of a volatile base or volatile acid will often dissolve most or all of the precipitate protein in the drop. Since the volume of added volatile is so small compared to the reagent well and vapor space, with the passing of time, the added volatile base or acid will leave the drop through the vapor phase and the pH will return to or close to the original drop value, again producing a supersaturated solution. It is during or shortly after this second equilibration that an opportunity exists for crystals to appear.

To try the Last Gasp, open the experiment, and add to the drop only, an amount of 0.1 M Ammonium hydroxide equal to that of the final equilibrated drop volume. For example, if the initial vapor diffusion experiment consisted of a drop composed of 0.5 μl of sample plus 0.5 μl of reagent, the final equilibrated drop volume is approximately 0.5 ml; therefore 0.5 μl of 0.1 M Ammonium hydroxide should be added to the drop. Note, If the sample is not partially or fully solubilized with Ammonium hydroxide, repeat the process with 0.1 M Acetic acid. Next, reseal the experiment. Allow up to one week for the drop to equilibrate and produce a small miracle (crystal). "If no crystal forms, dump the samples in the sink and curse the darkness", Alexander McPherson.¹

References

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