MAKING GOOD'S BUFFERS GOOD FOR FREEZING: THE ACIDITY CHANGES AND THEIR ELIMINATION VIA MIXING WITH SODIUM PHOSPHATE [1]

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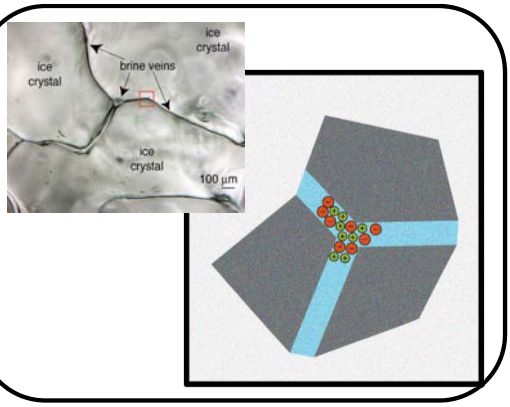
Introduction: Freezing of (bio)chemicals often leads to deviations from the optimum pH which cause compound's degradation, e.g., protein aggregation[2] .So, we would like to present a straightforward and efficient approaches to reduce the freezing-induced acidity changes by blending two different buffers which are commonly used for biological samples . This approach can be highly helpful in both the pharmaceutical domain and those branches of science where freezing is the preferred method for long-term preservation.

A. Freeze concentrate solution

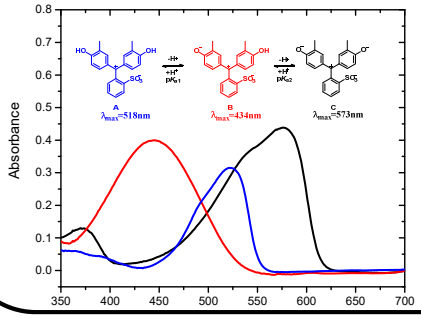


How we measure the pH Change?

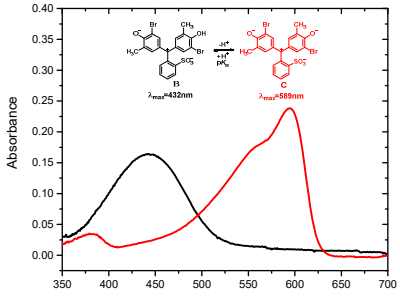
Molecular Probes for H0

$$H_{2-} = pK_{a,Ind} + log\frac{c_{Ind^x}}{c_{HInd^{x+1}}}$$

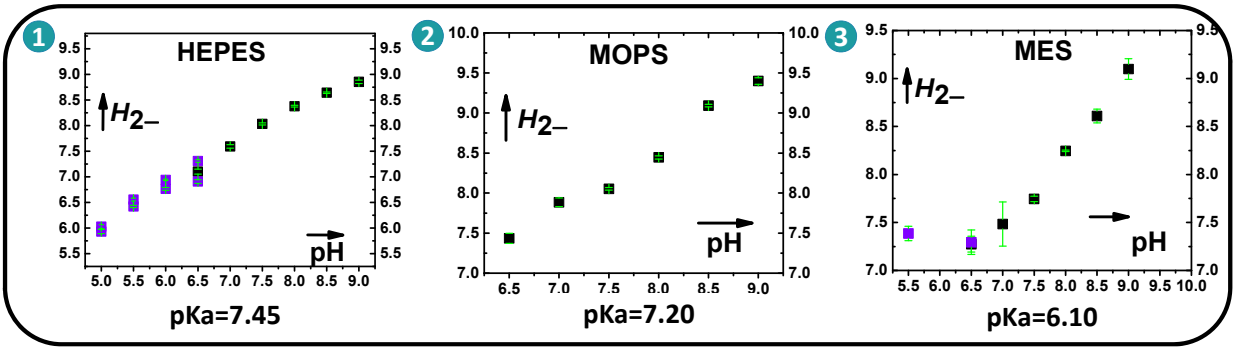
1 **Cresol Red (CR)**



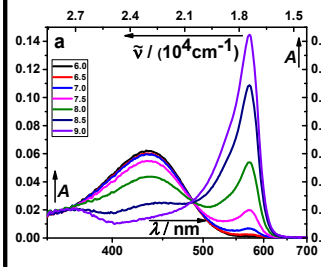
2 **Bromocresol Purple (BCP)**



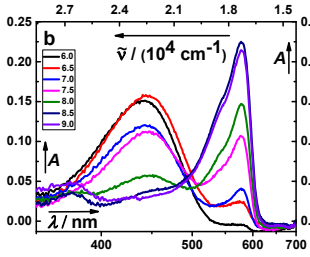
B. Freezing–induced acidity changes in Good's Buffers[1]



UV-Vis Spectra of HEPES Buffer

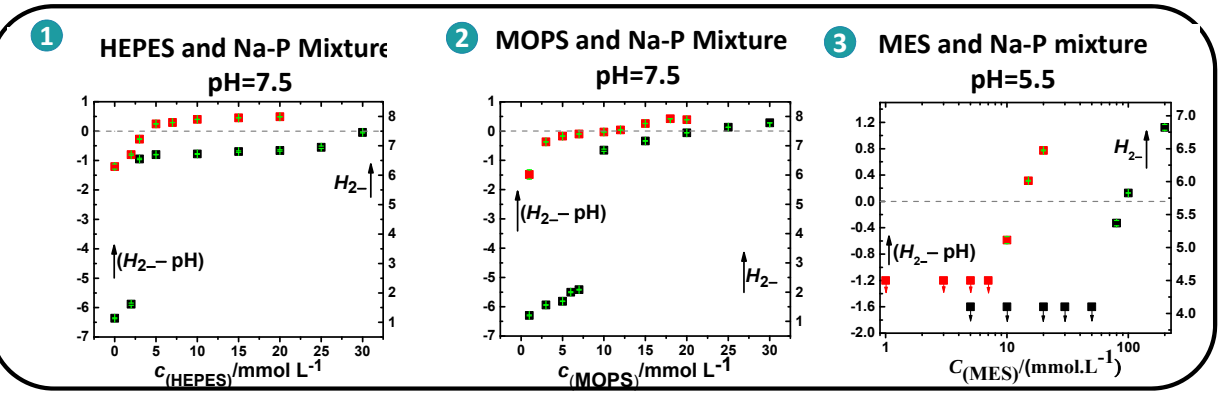


Room Temperature(RT)

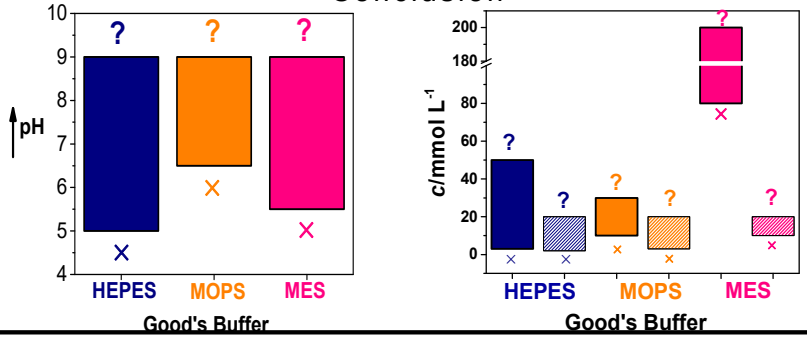


Fast Frozen (FF)

C. What happens when we Mix Good's Buffers with Sodium Phosphate Buffer ?



Conclusion



References

[1] Lukáš Veselý, (2021)" Making good's buffers good for freezing: The acidity changes and their elimination via mixing with sodium phosphate" Int J Pharm 593, 120128

[2] Zbacnik, T. J., R. E. Holcomb, D. S. Katayama, B. M. Murphy, R. W. Payne, R. C. Coccaro, G. J. Evans, J. E. Matsuura, C. S. Henry and M. C. Manning (2017). "Role of Buffers in Protein Formulations." Journal of Pharmaceutical Sciences 106(3): 713-733.

[3] Thorat, A. A. and R. Suryanarayanan (2019). "Characterization of Phosphate Buffered Saline (PBS) in Frozen State and after Freeze-Drying." Pharm Res 36(7): 98.