JBScreen pH-2D

Extended-range buffer systems

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Introduction

Choosing the right buffer/pH is vital for the successful performance of a crystallization experiment, where the buffering chemical is usually employed in rather high concentrations, e.g. at 100 mM. The buffer is of particular importance since it adjusts the pH of the protein solution during crystallization and it can also form specific interactions with protein molecules.

For a successful optimization of crystallization conditions it is of advantage to alter independent factors of the crystallization cocktail. For conventional buffers this is certainly difficult, since the buffering chemical and the appropriate pH range are strongly correlated. Thus, screening of a broad pH range would require several chemically distinct buffer substances.

One way to sample a broad pH range without changing the chemical composition of the buffering component is the application of extended range buffer systems [1].

The JBScreen pH-2D

JBScreen pH-2D contains 6 extended-range buffer systems; each composed of a mixture of 3 individual buffers with a distinct chemical nature and well separated pK_a-values.

Table 1. *Composition of the extended range buffer systems*

No	Buffer composition	Ratio	pH low	pH high
1	Succinic Acid Sodium dihydrogen Phosphate Glycine	2 7 7	4.0	10.0
2	Citric acid HEPES CHES	2 3 4	4.0	10.0
3	Malonic Acid Imidazole Boric Acid	2 3 3	4.0	10.0
4	Sodium Acetate ADA Bicine	1 1 1	4.0	9.0
5	L-Malic Acid MES Tris	1 2 2	4.0	9.0
6	Sodium Tartrate dihydrate Bis-Tris Glycylglycine	3 2 2	4.0	9.0

Each buffer system is composed of a low-pH and a high-pH stock solution. The low-pH stock solution is preset at pH 4.0 and the high-pH solution either at pH 9.0 or 10.0 (see Table 1) using HCl or NaOH as appropriate. Thus, mixtures of the low-pH and high-pH stock solutions in different proportions allow to cover the entire pH range from 4.0 – 9.0 or 4.0 – 10.0, respectively.

As already stated, each buffer system is composed of three single components. To ensure a linear pH curve upon mixing the low-pH and high-pH stock solution, the best ratio between the single components had to be determined [1]. An example of the pH curve for the buffer system Citric Acid, HEPES, CHES is depicted in Fig.1. The two stock solutions were mixed in different ratios ranging from 10:0 to 0:10 and the resulting pH values were measured. The best results were obtained, when the stock solutions were composed of Citric Acid, HEPES, CHES in a ratio of 2:3:4 [1].

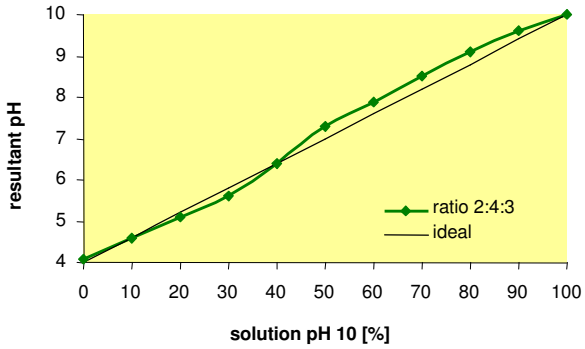

Figure 1. *Plot of the pH-curve for buffer system Citric Acid, HEPES, CHES, which comprises two stock solutions preset at pH 4.0 and 10.0, respectively. The two stock solutions were mixed in different ratios ranging from 10:0 to 0:10 and the resulting pH values were measured.*

Usage

The low-pH and the high pH-stock solution of a buffer system are designed to be mixed in different ratios to sample a broad pH range. The stock solutions are 1M in buffer and have to be diluted in the crystallization experiment, i.e. upon adding precipitant and salt solutions. **JBScreen pH-2D** can also be incorporated into existing screens, replacing the conventional buffering component.

Furthermore, **JBScreen pH-2D** is particularly suitable for application with liquid handling robots where number of stock solutions is limited.

References

[1] Newman (2004) Novel buffer systems for macromolecular crystallization. *Acta Cryst. D***60**:610.