First Order Corrections of pKa Values for Crystallization Optimization Trials

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Outline

- Crystallization background
- Introduction of problem
  - Preliminary results
- Purpose / Goal
- Methods
- Results and discussion
- Conclusion
Crystallization Background

- Given biological macromolecule
  - Proteins, polysaccharides, nucleic acids, etc.

- Convert into a crystalline state with a high degree of internal order

- Achieved by careful manipulation of environmental parameters to slowly reduce solubility
  - pH, concentration, temperature, etc.
Problems Associated with Macromolecular Crystallization

- As many as 25 controllable parameters
- Givens with limited predictive value
- Strong interdependence amongst variables
- Difficulty in applying theory of thermodynamics at the bench
- Limited time, material and human resources
Crystallization Optimization Trials

- Extensive scanning of existing crystallization results to identify promising conditions
- Trials completed via grid screen.
- 1-2 parameters systematically varied amongst the original combination of conditions surrounding the successful crystallization
  - pH and concentration
- Implementation of grid screen:
  - By hand or by automated crystallization robots
  - Often poses errors in accuracy and precision
  - Avoid errors by use of the 4 corners method
Problem

Recent experiments using the buffer solution, BTP (1,3 bis(tris(hydroxymethyl) methylamino)propane) has not achieved the level of accuracy required for crystallization optimization trials.

- High accuracy, in this context, is defined as measured values falling within ±0.05 units of target values.
Preliminary Results

Ideal Behavior vs. Nonideal Behavior Displayed Across a Gridscreen

HEPES is acting more ideally than BTP
Neither HEPES nor BTP can be considered ideal solutions

- Salt additive

However, BTP is subject to a greater level of nonideal behavior which has shifted its pKa value – or acid dissociation value.

- There is a different volume of acid in the wells than we expected.
Goal

- Calculate the error in the acid dissociation value
  - Determine actual volume of acid present in the wells.
- Readjust the spreadsheet which controls the crystallization robot
- Run a second trial and hope for improvement!

If results fail to improve trial accuracy:
- Error in the published pKa value
- Effect of the second titrateable group
Methods – Calculate error in the pKa

The Henderson Hasselbalch equation:

\[
pH = pK_a + \log \frac{[A^-]}{[HA]}
\]

- Which can be rewritten as:

\[
pH = pK_a - \log \frac{x}{(1 - x)}
\]

Where \( x = \) volume of acid
**Methods** – Calculate error in the pKa

**Taylor Series** is used to develop an approximation of a function where $\delta pK$ is very small.

$$pK_a = pK_{a,0} + \delta pK$$

- Subsequent terms in the series allow for a greater approximation

$$f(x_0 + \delta x) \approx f(x_0) + \left(\frac{df(x_0)}{dx}\right)(\delta x) + \left(\frac{1}{2}\right)\left(\frac{d^2f(x_0)}{dx^2}\right)(\delta x^2) + \ldots \left(\frac{1}{n}\right)\left(\frac{d^nf(x_0)}{dx^n}\right)(\delta x^n)$$

- We performed a first order correction
Methods – Calculate error in the pKa

$$\delta p_k = \left(\frac{1}{\alpha_i}\right) \times (x_i - x_{c,i})$$

Where $1/\alpha_i$ is the scaling factor
Where $x_i$ is the measured volume of acid present in the wells
Where $x_{c,i}$ is the calculated volume of acid present in the wells
Using the algorithm under false circumstances

**HEPES**

$$
(4-(2\text{-hydroxyethyl})\text{-}1\text{ Piperazineethanesulfonic acid})
$$

- Buffer solution acting under ideal behavior
- pKa = 7.55
- Use algorithm with false pKa values 7.10 and 8.00

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Using the algorithm under false circumstances

- **Iteration** – a succession of approximations, each building on the one preceding till a certain degree of accuracy is achieved.

- After 10 iterations, we obtained the correct pKa value of 7.55.
- The $\delta$pK reduced by roughly half with each correction.
- We expected similar results for BTP.
Using the algorithm under false circumstances

It Works!
Using the algorithm on the problem buffer:

Using our experimental pH values for BTP, we calculated the error in the pKa.

The number of iterations was twice that required of HEPES.

- Effect of second, non-adjusted, titratable group

We obtained a pKa of approximately 7.27
Using the algorithm on the problem buffer:

**DELTA PKA PROGRESSION**
from pKa = 6.8

**ESTIMATED PKA PROGRESSION**
from pKa = 6.8
Results and Evaluation

Success! The margin of error has reduced to values within the permitted ±0.05 pH units.

Possible sources of withstanding error:
- Experimental error; pH meter
References

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BBSI Members

Thank You!
Questions?