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A correction to the calculation of the Gibbs free energy of adsorption for biomolecules in ion-exchange systems

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Abstract

We wish to propose a correction to the methodology introduced by Gerstner et al. [J.A. Gerstner, J.A. Bell, S.M. Cramer, Biophys. Chem. 52 (1994) 97–106] for the calculation of Gibbs free energies of adsorption of biomolecules to ion-exchange systems. Our approach is based on the requirement that the mobile phase and stationary phase concentrations be expressed in exactly the same units and the equilibrium constant be strictly dimensionless. The Gibbs free energies of ion-exchange calculated based on this correction appear to be more negative than those originally calculated by Gerstner et al. © 1997 Elsevier Science B.V.

Keywords: Gibbs free energy of adsorption; Ion-exchange systems; Stationary phase; Mobile phase

1. Introduction

The steric mass-action model of Brooks and Cramer [1] offers an elegant theoretical framework for the facile chromatographic determination of the binding characteristics of ionic biomolecules to oppositely charged surfaces. The theory of Brooks and Cramer is more successful than Langmuir's for the description of ion-exchange because it explicitly takes into account the electroneutrality at all times of the adsorbing ionic surface and allows for adsorbate bulkiness which is necessary for macromolecular species such as proteins. A set of chromatographic ion-exchange data on amino acids, polypeptides and proteins was recently analysed [2] using this theory [1] and the characteristic charges, ν_α, and ion-exchange free energies, ΔG_{exc}, calculated. The purpose of this paper is to recalculate the ΔG_{exc} of these compounds based on a correction to the theory as detailed below.

2. The correction

The correction that we wish to propose arises from the fact that Gerstner et al. [2] have ignored the subtle difference between the units of the mobile phase concentrations C_i, which are mol/volume of *solution*, and the stationary phase concentrations Q_i, which are mol/volume of *stationary phase*. To secure the strict non-dimensionality of the equilibrium constant K, in the definition Eq. (2), the Q_is must be

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Table 1
Originally calculated and corrected free energies of ion-exchange

Biomolecule	Characteristic charge (ν)	Original ion-exchange free energy (kcal/mol)	Correction (kcal/mol)	Corrected Ion Exchange free energy (kcal/mol)
α-chymotrypsinogen	4.8	2.8	−1.4	1.4
Cytochrome C	5.9	2.8	−1.8	1.0
Lysozyme	5.3	1.0	−1.6	−0.6
Ribonuclease A	4.9	3.4	−1.4	2.0
Lactoferrin	8.1	1.1	−2.6	−1.5
α-chymotrypsin	4.8	3.1	−1.4	1.7
Trypsin	5.3	3.9	−1.6	2.3
Arginine	1.1	0.34	−0.04	0.30
Lysine	1.1	0.80	−0.04	0.76
bz-ArgPheTrpLysThrPhe	0.9	−0.71	+0.04	−0.67
bz-ArgArgArgTyr	1.9	−1.3	−0.3	−1.6
Protamine	10.2	−8.2	−3.4	−11.6

multiplied by the column phase ratio β, which has units of volume of stationary phase per volume of mobile phase (contrary to the statement of Gerstner et al. in the List of Symbols section that β is dimensionless; notice that the authors have correctly multiplied Q_{protein} by β in Eq. (14) to ensure the non-dimensionality of the chromatographic capacity factor, k'). Therefore, the corrected Eq. (2)) should read:

$$K = \frac{1}{\beta^{\nu-1}}\left(\frac{G_{\text{protein}}}{C_{\text{protein}}}\right)\left(\frac{C_{\text{salt}}}{\bar{Q}_{\text{salt}}}\right)^{\nu} \tag{1}$$

and Eq. (9) should become:

$$K^{*} = \beta\frac{Q_{\text{protein}}}{C_{\text{protein}}} \tag{2}$$

which would imply:

$$K^{*} = k \tag{3}$$

Eq. (15) should read:

$$k' = \beta^{\nu}K\left(\frac{\Lambda}{C_{\text{salt}}}\right)^{\nu} \tag{4}$$

resulting in a corrected Eq. (16):

$$\log k' = \log(\beta^{\nu}K\Lambda^{\nu}) - \nu\log C_{\text{salt}} \tag{5}$$

Eq. (17a) remains unchanged, but Eq. (17b) becomes:

$$\text{intercept} = \log(K\Lambda^{\nu}\beta^{\nu}) \tag{6}$$

and Eq. (18) is corrected to:

$$K = \frac{10^{\text{intercept}}}{\beta^{\nu}\Lambda^{\nu}} \tag{7}$$

This implies that the corrected free energy of ion-exchange, $\Delta G''_{\text{exc}}$, can be obtained from the originally calculated value, $\Delta G'_{\text{exc}}$, as follows:

$$\Delta G''_{\text{exc}} = \Delta G'_{\text{exc}} - (\nu - 1)RT\ln\beta \tag{8}$$

At room temperature and for a typical value of $\beta = 0.5385$ mL stationary phase/mL mobile phase [3], one obtains:

$$\Delta G''_{\text{exc}} = \Delta G'_{\text{exc}} + 0.3665(\nu - 1)\text{kcal/mol} \tag{9}$$

Applying this correction to the values of Table 1 of [2] leads to the corrected values of Table 1 shown here which also presents the original values calculated by Gerstner et al. Most of the new ion-exchange free energies acquire a more negative value because β is lower than 1 and most νs are greater than 1.

A similar correction to Table 2 of [2] suggests that all free energies of adsorption should increase by +0.3665 kcal/mol.

References

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