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Note

Are skew concentration distributions of ampholytes in isoelectric focusing due to specific conductivity changes with pH?

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In the theory of isoelectric focusing[1], the specific conductivity is generally assumed to be constant throughout the zone of a focused ampholyte. This assumption leads to the well known Gaussian concentration distribution of a focused ampholyte in a linear pH gradient. In practice, however, the specific conductivity is not constant[2]: for some carrier ampholytes and at $5 > \text{pH} > 8$ its change with pH can amount to 100%.

In 1961, Svensson[3] pointed out that in a linear conductivity gradient a skew concentration profile of a focused ampholyte would occur. In recent years, this statement has sometimes been used to explain experimentally found non-Gaussian distributions[4,5]. Therefore, we thought it worthwhile to investigate how important the deviation from Gaussian distributions due to this conductivity effect really is in practical cases.

THEORETICAL

Starting from the differential equation

$$\frac{Cui}{q\kappa_0} = D \cdot \frac{dC}{dx} \tag{1}$$

representing the balance between electrophoretic and diffusional mass flow in the steady state, Svensson[3] derived for the concentration, C, of a focused ampholyte in a linear pH gradient at constant specific conductivity, κ_0, the equation

$$\left(\frac{C}{C_0}\right)_{\text{Gaussian}} = \exp\left(-\frac{pix^2}{2q\kappa_0 D}\right) \tag{2}$$

which is the symmetrical Gaussian function. In these equations the symbols have the following meanings:

u = electric mobility of the ampholyte constituent;
D = diffusion coefficient of the ampholyte;
i = electric current;

q = cross-sectional area of the focusing medium;
x = distance along the direction of the current ($x = 0$ at the concentration maximum C_0 of the ampholyte, and $x > 0$ towards the cathode);

$$p = -\frac{du}{dx} = -\frac{du}{dpH} \cdot \frac{dpH}{dx}.$$

Svensson[3] also showed that eqn. 1 can be solved in the case when the specific conductivity is a linear function of x:

$$\kappa = \kappa_0 + rx \tag{3}$$

The solution is then

$$\left(\frac{C}{C_0}\right)_{skew} = \exp\left[\frac{pi\kappa}{Dqr^2} \cdot \ln\left(1 + \frac{rx}{\kappa_0}\right) - \frac{pix}{Dqr}\right] \tag{4}$$

which is a skew distribution function. For $r \to 0$ this equation reduces to eqn. 2, as can easily be seen by using the approximation

$$\ln\left(1 + \frac{rx}{\kappa_0}\right) \approx \frac{rx}{\kappa_0} - \frac{1}{2}\left(\frac{rx}{\kappa_0}\right)^2$$

For a convenient comparison between the Gaussian and the skew distribution we introduce the quantities $A = pi\kappa_0/Dqr^2$ (dimensionless) and $B = r/\kappa_0$ (reciprocal length). Eqns. 2 and 4 can then be written as

$$\left(\frac{C}{C_0}\right)_{Gaussian} = \exp\left(-\frac{AB^2}{2} \cdot x^2\right) \tag{5}$$

and

$$\left(\frac{C}{C_0}\right)_{skew} = \exp\{-A\,[Bx - \ln(1 + Bx)]\} \tag{6}$$

In Fig. 1 $(C/C_0)_{skew}$ is plotted against x for two values of A (1 and 100) and for two ampholytes, *i.e.*, one focusing at pH_1 in a region with negative r (or B) and one focusing at pH_2 in a region with positive r. For comparison the Gaussian distribution $(C/C_0)_{Gaussian}$ is also shown.

In Fig. 1 x is expressed in $A^{-\frac{1}{2}}B^{-1}$ units. This unit corresponds to the x value of the inflection point in the Gaussian distribution curve. For values of $|Bx| \ll 1$ the ratio $C_{Gaussian}/C_{skew}$ can be written as:

$$\frac{C_{Gaussian}}{C_{skew}} \approx \exp\left(-\frac{1}{3}AB^3x^3\right) \tag{7}$$

as can be seen by dividing eqn. 5 by eqn. 6, expanding $\ln(1 - Bx)$ as a series in Bx and

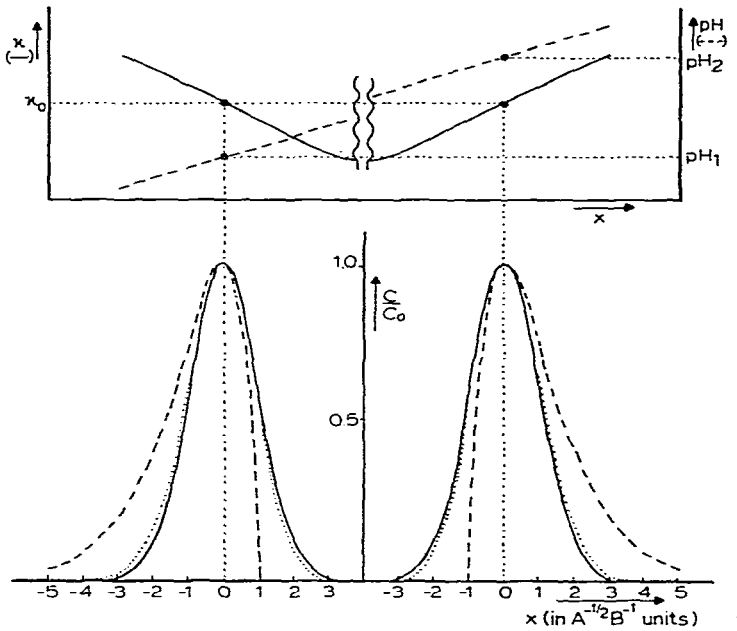

Fig. 1. Distribution curves of ampholyte concentration as a function of the distance along the pH gradient. Gaussian curve (———) and skew curves for $A = 1$ (— — —) and $A = 100$ (· · · · · ·).

neglecting the terms higher than the third power. Hence, the ratio $C_{\text{Gaussian}}/C_{\text{skew}}$ at the inflection points in the Gaussian curve is approximately

$$\left(\frac{C_{\text{Gaussian}}}{C_{\text{skew}}}\right)_{x = \pm A^{-\frac{1}{2}}B^{-1}} \approx \exp\left(\pm\frac{1}{3}A^{-\frac{1}{2}}\right) \tag{8}$$

This ratio is 1 ± 0.10, 1 ± 0.032, 1 ± 0.015 and 1 ± 0.003 for $A = 10$, 10^2, 10^3 and 10^4, respectively.

DISCUSSION

The deviation from the Gaussian distribution due to the conductivity effect thus depends on the value of A. Therefore, this value, which depends on the experimental conditions (dpH/dx, i, κ_0, q and r) and on the properties of the ampholyte (du/dpH and D) will be evaluated for one set of experimental conditions and three types of ampholytes.

The experimental conditions chosen are those of the focusing experiments of Gelsema *et al.*[2], as these provide values of the specific conductivity (κ_0 and r) pertaining to almost undisturbed, focused carrier ampholyte gradients in water. A is calculated for r values equal to $\pm$(dpH/dx · κ_0). These are the cases referred to in the *introduction* (dκ/dpH · $1/\kappa_0 = \pm 1$); they are representative of Ampholine gradients at pH ≈ 4.5 and Servalyte gradients at pH ≈ 8.5. For the other variables the following values are used: dpH/dx = 0.04 cm^{-1}, $i = 1 \cdot 10^{-4}$ A, $\kappa_0 = 1 \cdot 10^{-4}\Omega^{-1}$ cm^{-1} and $q = 0.125$ cm^2. For both cases the value of A is then

$$A = 200\left(\frac{-\text{d}u/\text{dpH}}{D}\right)$$

The factor $-du/dpH/D$ is evaluated below for a protein, a carrier ampholyte and a "poor" amino acid.

A protein. The classical values for myoglobin of Vesterberg and Svensson[6] are used*. With $(du/dpH)_{25} = -4.41 \cdot 10^{-5}$ cm^{2}V^{-1} sec^{-1} and $D_4 = 6.85 \cdot 10^{-7}$ cm^{2}sec^{-1}, the value of A is $1.3 \cdot 10^4$.

A carrier ampholyte. No experimental values of du/dpH and D are available. We can approximate, however, the value of $(-du/dpH)/D$ using the equation[7] $u = QD/kT$, where Q represents the charge of the ampholyte molecule, k is Boltzmann's constant and T the absolute temperature. From this equation we can derive

$$\frac{-du/dpH}{D} = \frac{1}{kT} \cdot \frac{-dQ}{dpH} = \frac{B}{kT} \cdot \frac{F}{N}$$

where B represents the molar buffer capacity of the ampholyte, F is the Faraday constant and N is Avogadro's number. With buffer capacity data taken from ref. 2 [using $B^* \approx 16$ μequiv. ml^{-1}, holding for 1% (w/v) Ampholines at pH $\approx$ 3.5 and a mean molecular weight for Ampholines $\bar{M} \approx 700$], $B \approx 1.1$ equiv. mole^{-1}. This gives** $(-du/dpH)_{25}/D_4 \approx 89$ V^{-1} and $A \approx 1.8 \cdot 10^4$.

A "poor" amino acid. In the literature[5,8-10] on isoelectric focusing an ampholyte that is isoelectric over a wide pH range is called a "poor" carrier ampholyte. Most amino acids belong to this class, as their isoelectric points are determined by the dissociation constants of the α-carboxyl and α-amino groups, which differ considerably. For the evaluation of du/dpH for such an amino acid we follow the approach of Rilbe[1]:

$$-\frac{du}{dpH} \approx \frac{U \cdot \ln 10}{1 + \left(\frac{K_1}{4K_2}\right)^{\frac{1}{2}}}$$

where U is the mobility of the univalent positively charged amino acid and K_1 and K_2 are the dissociation constants of the α-carboxyl and α-amino group, respectively. Taking for pK_1 and pK_2 typical values[11] of 2.3 and 9.6, respectively, and for U/D the values pertaining to infinite dilution of Edward and Waldron-Edward[12] ($U_{25}/D_4 = 74$ V^{-1})**, we calculate $(-du/dpH)_{25}/D = 0.076$ V^{-1} and $A \approx 15$.

It follows from Fig. 1 and from eqn. 8 that for much smaller A values (*ca.* 100) than calculated above for the protein and carrier ampholyte, the deviation from the Gaussian concentration distribution has already become very small. Hence, the effect of considerably varying specific conductivity (100% change) cannot give rise to a measurable skewness of the distribution curve in these cases. Further, the calculated A values hold for hypothetical ampholytes having the properties, in terms of du/dpH and D, of myoglobin, Ampholine and a "poor" amino acid, but focusing in a region with a steep conductivity gradient. However, the so-called "poor" amino acids focus

* The ratio $p\kappa_0/r^2$ in A can be assumed to be independent of temperature. Therefore, as the specific conductivity was measured[2] at 25°, du/dpH at 25° was taken. As the focusing experiments[2] were performed at 4°, D values at 4° were used.

** Multiplication by a factor $(277/298) \cdot (\eta_{25}/\eta_4)$ was performed to correct D values to 4° (where η is the viscosity of water).

at pH $\approx$ 6, where κ_0 is about three times smaller, but where r becomes exceedingly small, resulting in a far greater A value than calculated above for a "poor" amino acid. Thus, in all practical cases, the conductivity effect is much too small to account for asymmetry of the concentration distribution. Therefore, the origin of experimentally obtained skew distributions should not be sought in this effect, but rather in ampholyte inhomogeneity or in interaction of the ampholyte with other components in the focusing system, *i.e.*, with other (carrier) ampholytes or with the anticonvective medium.

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