

Abschliessend sei bemerkt, dass die beschriebene Methode auch zur zerstörungsfreien Revelation von Peptiden angewandt werden kann. In Verbindung mit der "Dünnschicht-Fingerprint"-Technik nach WIELAND UND GEORGOPOULOS<sup>7</sup> erleichtert sie die Sequenzanalyse von Peptiden.

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## Notes

### Non-ideality of the gas phase in frontal analysis

The activity coefficient at infinite dilution  $f_2^\infty$  of a volatile solute in an involatile solvent determined by gas-liquid chromatography is:

$$f_2^\infty = \frac{n_1^L RT}{p_2^0 V^G} \quad (1)$$

where  $n_1^L$  is the number of moles of solvent in the column,

$p_2^0$  is the vapour pressure of the solute at the column temperature  $T$ ,

$V^G$  is the true retention volume.

Equation (1) is derived by assuming, amongst other things, that the gas phase is ideal and that the activity coefficient is pressure independent. However, corrections must be made for the invalidity of these assumptions if activity coefficients measured by gas-liquid chromatography are to agree with static results<sup>1-3</sup>.

These corrections will be more important if the carrier gas is a mixture of an inert gas (by this is meant a gas that is virtually insoluble in the involatile solvent) and the solute, e.g. an elution peak is superimposed on the plateau obtained in frontal analysis. These "plateau" experiments would give the activity coefficient as a function of solute concentration thus allowing one to test solution theories in a concentration region which is inaccessible to static techniques.

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In this note an equation is derived which allows for the non-ideality of the gas phase and the pressure dependence of the activity coefficient. As a model for the thermodynamic effects in a column it is assumed that this is equivalent to a static system under the same conditions as the column, with the volume of the gas phase equal to the true retention volume and with equal amounts of solute  $n_2$  in both phases. This model readily gives eqn. (1) and the derivation of the full equation is a simple problem in thermodynamics<sup>4</sup> which will now be outlined.

The activity coefficient is defined unambiguously in terms of the absolute activities,  $\lambda$ , at zero pressure by:

$$\lambda_2^L(o) = \lambda_2^0(o) \alpha f_2(o) \quad (2)$$

where  $\alpha$  is the mole fraction of the solute in the solvent.

The absolute activities at zero pressure may be expressed in terms of their values at a particular low pressure by using the fact that the volumes of liquids are pressure independent. Thus eqn. (2) becomes:

$$\ln \lambda_2^L(\bar{P}) = \ln \lambda_2^0(p_2^0) \alpha f_2(o) + \frac{\bar{P} \bar{v}_2}{RT} - \frac{p_2^0 v_2^0}{RT} \quad (3)$$

where  $\bar{P}$  is the pressure of the static system,

$v_2^0$  is the molar volume of the solute,

$\bar{v}_2$  is the partial molar volume of the solute in the solvent.

The absolute activities of the solute in the gas phase for slightly imperfect gases at low pressure are:

$$\ln \lambda_2^G(\bar{P}) = \ln \frac{\gamma \bar{P}}{\varphi} + \frac{\bar{P}}{RT} \{ B_{22} - (1 - \gamma)^2 (B_{11} - 2 B_{12} + B_{22}) \} \quad (4)$$

$$\ln \lambda_2^G(p_2^0) = \ln \frac{p_2^0}{\varphi} + \frac{p_2^0 B_{22}}{RT} \quad (5)$$

where  $B_{ij}$  is the appropriate second virial coefficient,

$\gamma$  is the mole fraction of the solute in the gas phase,

$\varphi$  is the internal partition function of the solute and is independent of its environment.

Equations (3) and (4) are related by the condition for equilibrium, *i.e.* equality of absolute activities in both phases. This together with eqn. (5), the equation of state for the binary gas mixture:

$$\bar{P} V^G = (n_1^G + n_2) [RT + \bar{P} \{ (1 - \gamma)^2 B_{11} + 2 \gamma (1 - \gamma) B_{12} + \gamma^2 B_{22} \}] \quad (6)$$

and some manipulation leads to the full equation:

$$\begin{aligned} \ln f_2(o) = \ln \frac{n_1^L + n_2}{p_2^0} \frac{RT}{V^G} \left[ 1 + \frac{\bar{P}}{RT} \{ (1 - \gamma)^2 B_{11} + 2 \gamma (1 - \gamma) B_{12} + \gamma^2 B_{22} \} \right] \\ + \frac{\bar{P}}{RT} \{ B_{22} - \bar{v}_2 - (1 - \gamma)^2 (B_{11} - 2 B_{12} + B_{22}) \} - \frac{p_2^0}{RT} \{ B_{22} - v_2^0 \} \quad (7) \end{aligned}$$

In elution gas-liquid chromatography the solute concentration is vanishingly small so that eqn. (7) reduces to:

$$\ln f_2^\infty(o) = \ln \frac{n_1^L RT}{p_2^0 V_G} + \frac{\bar{P}}{RT} \left\{ 2 B_{12} - \bar{v}_2 \right\} - \frac{p_2^0}{RT} \left\{ B_{22} - v_2^0 \right\} \quad (8)$$

if  $\ln[1 + \bar{P}B_{11}/RT]$  is expanded and only first order terms retained. This equation has been derived previously and has been tested by measuring the pressure dependence of the  $k'$  factor<sup>3,5</sup>.

In frontal analysis, however, the solute concentration cannot be neglected and eqn. (7) should be used to correct for the non-ideality of the gas phase. Although this equation is complicated it may be simplified by expanding the logarithm of the term in square brackets and neglecting all but first order terms. This assumption will normally be valid so that eqn. (7) becomes:

$$\ln f_2(o) = \ln \frac{n_1^L + n_2}{p_2^0} \frac{RT}{V_G} + \frac{\bar{P}}{RT} \left\{ 2 \gamma B_{22} + 2(1 - \gamma) B_{12} - \bar{v}_2 \right\} - \frac{p_2^0}{RT} \left\{ B_{22} - v_2^0 \right\} \quad (9)$$

In many cases the volume of mixing is small so that the partial molar volume may be replaced by the molar volume. Thus for 0.1 mole fraction of *n*-octane in nitrogen at 30° the coefficient of  $\bar{P}$  in eqn. (9) is  $-0.0591 \text{ atm}^{-1}$  and the constant term is 0.0058 but at infinite dilution the coefficient is only  $-0.0254 \text{ atm}^{-1}$ . These calculations show that, as expected, the correction terms for the non-ideality of the gas phase may be large and if accurate values of activity coefficients are to be obtained these corrections should be made.

Finally the measurement of the pressure dependence of the  $k'$  factor may be used to measure the second virial coefficient of the solute in the same way that elution gas-liquid chromatography gives the mixed second virial coefficient.

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