The Use of RP-HPLC Capacity Factor as Lipophilicity Index for Aroma Compounds

G. Piraprez, M-F. Hérent & S. Collin*

Unité de Brasserie et des Industries Alimentaires, Faculté des Sciences Agronomiques, Université Catholique de Louvain, 2/7 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium

INTRODUCTION

Lipophilicity which represents the affinity of a molecule or a moiety for a lipophilic environment is commonly measured by its distribution behavior in a biphasic system either liquid-liquid (e.g., partition coefficient in *n*-octanol/water) or solid-liquid (e.g. retention time in RP-HPLC or TLC) systems.

Thermodynamically, the partition coefficient P is defined as a constant relating the activity of a solute in two immiscible phases at equilibrium. By convention, the ratio of concentration in the two phases is given with the organic phase as numerator.

$$P = \gamma_o C_o / \gamma_w C_w$$

In dilute solutions, it can be approximated by

$$P = C_o/C_w = \text{capacity factor}\left(\frac{\text{weight in the n-octanol phase}}{\text{weight in the water phase}}\right) \times \text{phase ratio}\left(\frac{\text{water volume}}{\text{n-octanol volume}}\right) \quad \textbf{Equation (1)}$$

Therefore, a positive log P value reflects a preference for the lipid phase while a negative value indicates a relative affinity for water (van de Waterbeemd & Testa, 1987).

In aroma research, lipophilicity of the flavouring compounds is an indicator of their behaviour in the food matrix, generally composed of water and less hydrophilic components such as proteins, lipids, polysaccharides, etc (Landy *et al*, 1995; Guyot *et al*, 1996; Piraprez & Collin, 1995; Roberts & Acree, 1995). The aroma lipophilicity has also a determinant role in the olfaction process (aroma transport across the aqueous mucus and binding to the olfactive receptors). Hérent *et al* (1995) have shown that among thirty-two green- and/or nutty smelling compounds, most of them pyrazine and thiazole derivatives, only very lipophilic compounds were green odorants and good ligands to the bovine and porcine olfactive binding proteins (OBP).

DETERMINATION OF LIPOPHILICITY

The 1-octanol/water partition coefficient (logP), measured by the shake-flask technique, was usually used to describe the lipophilicity. Unfortunately, this method has a number of practical disadvantages such as, (i) slowness; (ii) limitation to log P values above -2 and below 4,

owing to the required precision and sensitivity of the analytical technique; (iii) large errors caused by small impurities which, if they are e.g., strongly UV-absorbing, may seriously interfere with the quantitative determination of the solute; (iv) the formation of micelles and microemulsions in the aqueous phase; (v) instability of the solute in aqueous media; (vi) dissociation/association of polar solutes and (v) volatility of the solute (Braumann, 1986; El Tayar *et al*, 1985b). Therefore, other methods, including theoretical and experimental trials, have been explored in order to measure lipophilicity.

Theoretical calculations (Fujita *et al*, 1964; Rekker & de Kort, 1979) of partition coefficients still suffer from inaccuracy. So, to avoid the traditional laborious and standard shake-flask technique, some chromatographic techniques esp. Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC) and Centrifugal Partition Chromatography (CPC), were developed in order to determine the hydrophobic nature of drugs or other kind of compounds.

Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC)

The availability of alkyl-bonded phases provides a simple, accurate and reproducible method to determine the lipophilic character of a wide variety of compounds (Braumann, 1986). As eluent, mixtures have been used consisting of water and organic modifiers such as methanol, acetone, tetrahydrofuran or acetonitrile. Among them, methanol/water is the best « *n*-octanol/water-like » eluent in providing both strong hydrogen-bond donor and hydrogen-bond acceptor abilities at the interface.

In RP-HPLC, the lipophilicity is not issued from a concentration ratio (log P) but is given by a weight ratio (capacity factor « k ») (Equation (1)). Therefore, log P is correlated to log k by **Equation (2)** : $\log P = \log k + \text{constant}$ with $k = (t_R - t_0)/t_0$ where t_R and t_0 are the retention times of the analyte and of a non-retained compound, respectively.

Capacity factors determined at a given percentage of organic modifier « ϕ » (isocratic capacity factor) are sometimes used as lipophilicity index. The capacity factor « $\log k_w$ » obtained by extrapolation of retention data from binary eluents to 100 % water is however more interesting.

In order to avoid the adsorption of solutes on the residual silanol sites of the stationary phase (silanophilic interactions), lipophilic amines like *n*-decylamine (0.2 %) must be added (Braumann, 1986; El Tayar *et al*, 1985 a, b). At very low water concentrations, the mobile phase change from a water-like structure to an organic modifier-determined structure that exerts its own solvophobic effect. In that case, the addition of methanol will change the dielectric constant of the medium and decrease the hydrophilicity. According to these observations, El Tayar *et al* (1985 b) have suggested that extrapolation to $\log k_w$ should best be done by linear extrapolation of log k values measured :

(i) in the range $10<\phi<80$ for neutral and/or non-polar compounds;

(ii) in water-rich ranges of eluent composition for ionogenic polar compounds.

The silica gel matrix is unstable outside the 1.5-7.5 pH range. Therefore, many basic solutes are not eluted in their non-ionized state (Braumann, 1986). In that case, a correction which involves the pK_a value should be applied as shown by Equations (3) and (4) (Unger & Feuerman, 1979; El Tayar *et al*, 1985b)

$$\log k_w = \log k_w^{app} + \log\left(1 + 10^{pH - pK_a}\right) \text{ for acids;} \qquad \textbf{Equation (3)}$$

$$\log k_w = \log k_w^{app} + \log\left(1 + 10^{pK_a - pH}\right) \text{ for bases.} \qquad \textbf{Equation (4)}$$

For methanol-water eluents, the statistical significance of the log k_w - log P correlations is remarkably high (El Tayar *et al*, 1985 c; Braumann, 1986; Tsantili-Kakoulidou *et al*, 1987). In comparison with the *n*-octanol/water log P index, log k_w can, however, be expected to include more steric information due to the fact that the stationary phase is able to discriminate between solutes of different shapes.

RP-HPLC makes several important solute groups accessible to the experimental determination of their hydrophobicity. These include (i) complex structures of unknown partition behaviour for which the additivity of hydrophobic substituent constants may not be applied, (ii) permanently charged solutes and (iii) hydrophobic compounds with log P > 4 whose partition coefficients cannot be determined with sufficient accuracy by the shake-flask technique (Braumann, 1986).

The expression of hydrophobicity in terms of log k is relative in nature, and an established log k - log P correlation for a given class of compounds cannot be extrapolated either to different solutes or to other separation systems, even if the latter consists of an identical mobile phase and a stationary phase of nominally the same composition. The observed variations are induced by the stationary phase, by the volume fraction of organic modifier in the eluent and by the structure of the solute, which all together being regarded as a major drawback, in comparison with the classical *n*-octanol-water system, that provides a single, continuous hydrophobicity scale. However, should methanol-water eluents of comparable ionic strength at near neutral pH be used to measure retention, the stationary phase-induced variance of the resulting log k_w values is small (Braumann, 1986).

As a conclusion, RP-HPLC has a great number of advantages over the shake-flask technique, among others : (i) better accuracy and precision; (ii) applicability over a broader range of lipophilicity; (iii) decreased perturbations caused by impurities in the analytes; (iv) rapidity and minute material consumption (van de Waterbeemd & Testa, 1987).

The most recent chromatographic method used to determine partition coefficient is the « Centrifugal Partition Chromatography » (CPC). However, the limitation of this method is its cost and its restricted lipophilicity range as shake-flask measurements (van de Waterbeemd & Mannhold, 1996).

According to those statements, RP-HPLC has been chosen to determine the lipophilicity of hundred aroma compounds (pyrazines, thiazoles, other heterocyclic compounds, alcohols and phenols, ketones, esters, aldehydes, sulphur compounds and terpenes). The detailed method as well as the capacity factors, log k_w, obtained for pyrazine and thiazole derivatives have already been published by Hérent *et al* (1995).

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