Comparison of Experimental Methods for Measuring Infinite Dilution Volatilities of Aroma Compounds in Water/Ethanol Mixtures

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Several experimental methodologies exist for measuring volatilities; however, results show great dispersion and sometimes lack of agreement between different methods. The aim of our study was to compare the performance of three static headspace methods (vapor phase calibration, VPC; phase ratio variation, PRV; and liquid calibration static headspace, LC-SH) for determining gas/liquid partition coefficients of two aroma compounds in hydroalcoholic multicomponent solutions at infinite dilution. Comparison with literature data based on static and dynamic methods showed that PRV is simpler than VPC and LC-SH and that VPC and PRV are more accurate than LC-SH, which presented a significant bias (50% lower values).

INTRODUCTION

Flavor perception and distillation of alcoholic beverages are governed by the way aroma molecules distribute between gas and liquid phases. This is defined by the vapor−liquid equilibrium (VLE), which is characterized by the partition coefficient of a volatile compound between a vapor phase and a liquid phase. This coefficient is denoted k_i and is expressed by the ratio between the compound concentration in gas phase [C_i^{gas} in (mol or g)/m^3] and compound concentration in the sample [C_i^{liq} in (mol or g)/m^3] at equilibrium. VLE can also be quantified by the partition coefficient expressed in molar fractions, also called absolute volatility (K_i), or by the activity coefficient (γ_i), which represents the deviation from ideality. Both parameters are related by:

$$K_i = \frac{y_i}{x_i} = \frac{\gamma_i \cdot P_{i(T)}^{\circ}}{P_T} \tag{1}$$

with x_i and y_i as molar fractions in liquid and gas phases, respectively, $P_{i(T)}^{\circ}$ as the vapor pressure of pure component i at a given temperature T (Pa), and P_T as the total pressure (Pa).

In the particular case of infinite dilution [concentration values lower than 10^{-4} mole fraction, in food research (*1*)], the activity coefficient can be considered as constant and is noted γ_i^{∞}. The product $\gamma_i^{\infty} \cdot P_{i(T)}^{\circ}$ (eq 1) is also called the Henry's constant, He.

Aroma components are present in alcoholic beverages at very low concentrations, and they often have extremely low solubility in water; thus, aromas in these kinds of mixtures are normally found in the infinite dilution range. Unfortunately, measuring infinite dilution activity coefficients in these hydroalcoholic solutions is difficult, since they contain numerous aroma compounds and ethanol interferes with headspace measurements as it is also volatile. Furthermore, ethanol, even at a relatively low concentration (10% v/v), significantly reduces the volatility of the aromas in the mixture (*2*), given that most of them are hydrophobic. In addition, physicochemical interactions may exist between volatile compounds (*2, 3*). As a consequence, suitable methods for studying alcoholic beverages have to be sensitive enough in the infinite dilution region in hydroalcoholic solutions and should be able to measure volatilities in multicomponent systems.

A number of experimental methods are available for the direct measurement of gas−liquid partition coefficients leading to the determination of activity coefficients at infinite dilution γ^{∞}. The methods are often complementary since they cover different ranges of relative volatilities. The most frequently used methods for measuring volatility can be classed into three groups: differential methods, dynamic headspace, and static headspace, which we briefly review next.

Differential methods are based on differences between pure solvent and dilute solutions either on change of phase temperatures, such as boiling point [differential ebulliometry (*4*) or

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dew point (*5*) or pressure difference (*6*). These techniques are quite precise but not suitable for γ^{∞} measurements in multicomponent systems. One drawback of these methods is the sensitivity to errors in the liquid composition measurement (*7*).

Dynamic headspace methods allow one to obtain the γ^{∞} by bubbling an inert gas carrier through a binary dilute solution, such as the exponential dilution method (*8*), which has been frequently used to study aroma vapor−liquid equilibria (*9*, *10*), or the headspace stripping at equilibrium method (*11*−*13*), which, contrary to the exponential dilution method, needs an external calibration by injection of liquid standard solutions into the gas chromatograph. On the other hand, gas/liquid elution chromatography is used to find infinite dilution activity coefficients by measuring the elution time of the dilute species (solute) in an inert carrier stream through a column composed of the abundant species (solvent) (*14*).

Static headspace methods are based on measurements performed at thermodynamic equilibrium between liquid and gas phases. Two methods without use of an external calibration are the phase ratio variation (PRV) method used in environmental studies, which establishes the partition coefficient based on the fact that the headspace concentration changes as a function of the phase volume ratio (gas and liquid phases), while the partition coefficient remains constant (*15*, *16*), and the equilibrium partitioning in closed system (EPICS) method, which allows one to determine the Henry constant by measuring gas headspace concentration ratios from pairs of sealed bottles having different liquid volumes but the same quantity of volatile compound (*17*). The EPICS method limiting factor is that the precision of the method is associated with the difficulty of delivering equal quantities in the two bottles specifically for volatile compounds with very low solubility in water.

Static headspace methods that use external calibration determine the partition coefficient by analyzing the headspace using gas chromatography and establishing the volatile concentration by calibration with an external standard. The vapor phase calibration (VPC) method uses an external vapor standard for calibration (*18*, *19*). For this purpose, the pure component is completely vaporized in the vial prior to injection. Whitehead and Sandler (*20*) have modified the calibration by using a pure component at different temperatures and determining the relationship between solute vapor pressure and peak area. The partial pressure in equilibrium with the dilute solution is determined from the saturated pressure calibration curve. Last, the liquid calibration static headspace (LC-SH) method calibrates with an external liquid standard. It has been utilized for years (*21*) and is still frequently used (*22*).

Methods most frequently used for aroma research are static and dynamic headspace techniques (*23*), which often show lack of agreement (*23*, *24*). In addition, it is very difficult to assess the experimental discrepancies since aroma studies do not provide sufficient details of the methodology used. However, the use of dynamic headspace methods becomes impractical with volatile solvents and with solutes with low values of partition coefficients (*25*). Moreover, in the case of multicomponent systems, they may be unsuitable due to the delay introduced by the chromatographic separation step.

Although static headspace methods are flexible enough to be used to measure volatilities in multicomponent mixtures, they are less sensitive than dynamic methods (*26*) and some of them require a calibration (VPC, LC-SH, etc.). The vapor calibration method is particularly expensive since pure aroma compound vials are required.

The aim of this work is to compare the results of three static headspace methods in terms of accuracy and simplicity, within them and with literature results, for ethyl hexanoate and isoamyl alcohol in hydroalcoholic solutions. We have chosen these molecules because they have been extensively studied and because of their perception relevance in alcoholic beverages (*27*, *28*). Moreover, these two compounds have very different physicochemical properties (water solubility, volatility), which will allow us to define a range of validity of the three methods. The results are discussed in terms of experimental errors associated with operating conditions and with the determination of the range of applicability of each method for calculating infinite dilution partition coefficients in hydroalcoholic solutions in multicomponent mixtures.
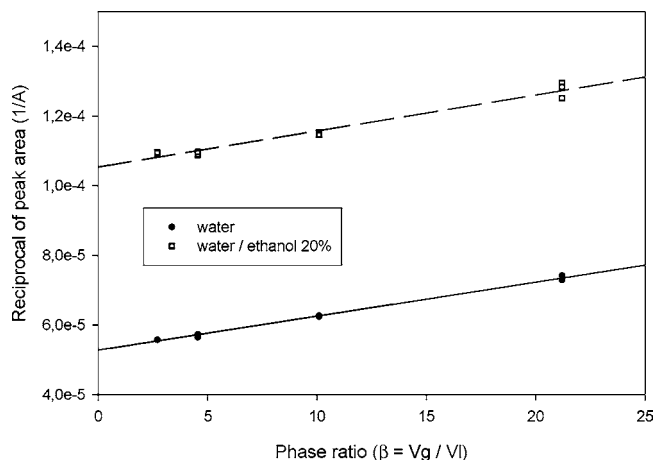
EXPERIMENTAL PROCEDURES

Hydroalcoholic Aroma Solution Preparation. Each sample was prepared by weighting an aroma quantity (ethyl hexanoate [CAS no. 123-66-0] 99% pure, Aldrich; or isoamyl alcohol [3-methyl-1-butanol CAS no. 123-51-3] 99% pure, Aldrich) and the solvent (water or ethanol or hydroalcoholic solution) on a high precision balance (PRECISA 404 A, 0.001 g precision). The hydroalcoholic solutions were prepared by weighting deionized water (milli-Q system, Millipore Waters, France) and pure ethanol (ethanol 99% RPE, Carlo Erba) and mixing them to obtain the adequate v/v concentration according to literature data (*29*).

In the case of multicomponent samples, solutions were prepared from a concentrated solution (20 000 ppm v/v) in ethanol of the 15 aroma compounds: dimethyl disulfide (99% pure, Aldrich), dimethyl trisulfide (95% pure, Acros), allyl isothiocyanate (99% pure, Aldrich), benzaldehyde (99% pure, Aldrich), hexanal (98% pure, Aldrich), 2-phenylethanol (98% pure, Sigma), isoamyl alcohol (99% pure, Aldrich), hexanol (99% pure, Lancaster), *cis*-3-hexenol (98% pure, Sigma), linalool (97% pure, Aldrich), geraniol (98% pure, Aldrich), α-terpineol (95% pure, Sigma), ethyl butyrate (95% pure, IFF), ethyl hexanoate (99% pure, Aldrich), and isoamyl acetate (99% pure, Aldrich). This concentrated mixture of 15 aroma compounds was stored at −80 °C and diluted for the preparation of the multicomponent solutions with concentrations of either 20 or 50 ppm for each compound.

Gas Chromatography Headspace Analysis. Glass vials (20 mL, Chromacol, France) were filled with aliquots of the volatile compound solution. The liquid volume introduced in the vial ranged from 50 μL to 10 mL depending on the headspace method. Vials were sealed using Teflon/Silicone septa in metallic caps (Varian, France). After equilibration at 25 °C for at least 4 h (time necessary to reach equilibrium in static conditions), a 2 mL sample of headspace was automatically withdrawn using a 2.5 mL gastight syringe, preheated to 35 °C in an automatic headspace sampler CombiPal (CTC Analytics, Switzerland), and analyzed on a Hewlett-Packard 6890 series gas chromatograph using a flame ionization detector (FID). The injection was direct, and the injector temperature was settled at 250 °C and injection rate at 100 μL s^{-1}. Only one headspace injection was made per vial, and three vials were analyzed for each solution. A 530 μm × 30 m capillary BP20 column (df = 1 μm) was used with a carrier gas (helium) flow rate of 6.7 mL min^{-1}. The oven temperature program was isothermal (80 °C) for binary solution (T_r = 3.8 and 4.4 min, respectively, for ethyl hexanoate and isoamyl alcohol). For the multicomponent mixture, the oven temperature program was set from 40 (2 min) to 80 °C at 4 °C/min and from 80 to 200 °C at 8 °C/min (T_r = 10.5 and 11.2 min, respectively, for isoamyl alcohol and ethyl hexanoate). Peak areas were measured using the Hewlett-Packard Chemstation integration software.

Static Headspace Method Sensitivity to Operation Parameters. A systematic study of the impact of operating conditions on the headspace analysis was performed. The four parameters tested were as follows: (i) the filling rate of the gas syringe (10, 100, or 500 μL s^{-1}); (ii) the gas injection rate (100, 250, or 600 μL s^{-1}); (iii) the volume ratio between the gas phase and the liquid phase in 20 mL headspace glass vials was varied from 220 to 2.2 corresponding to a liquid volume from 0.050 to 10 mL; and (iv) the time to reach equilibrium after the solution preparation: once the hydroalcoholic solution containing ethyl

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Figure 1. Reciprocal of peak area (1/*A*) vs phase ratio (*β*) for ethyl hexanoate in water/ethanol systems at 25 °C.

Table 1. Semiempirical Coefficients of Antoine's Law (A, B, C) for the Determination of Saturated Vapor Pressure of the Pure Volatile Compound at Different Temperatures

aroma compound (temperature validity range [°C])	A	B	C	ref
ethyl hexanoate (−9 to 130)	7.277	1651.270	209.213	*12*
isoamyl alcohol (10 to 130)	7.921	1666.375	200.158	*41*

Figure 2. Calibration curve of integrated peak area vs vapor pressure for pure ethyl hexanoate. Linear correlation coefficient = 0.996.

hexanoate was prepared at room temperature, the vials were filled and 2 mL of their headspace was injected every 30 min for more than 15 h. Each of the above parameters was varied one at a time.

Infinite Dilution Volatility Measurements. *PRV Method.* Glass vials (20 mL) were filled with variable volumes (from 0.05 to 2 mL) of solutions containing the aroma compound infinitely diluted (10, 20, and 50 ppm) in water or in water−ethanol mixtures. At equilibrium, a 2 mL sample of headspace was withdrawn from each vial and injected into the gas chromatograph. Ettre et al. (*16*) have established the following equation, based on mass balance equations, which allows us to obtain the partition coefficient k_i:

$$\frac{1}{A} = \frac{1}{f_i \cdot C_i^{\text{liq}}} \cdot \frac{1}{k_i} + \frac{1}{f_i \cdot C_i^{\text{liq}}} \cdot \beta \qquad (2)$$

where *A* is the peak area obtained at equilibrium, f_i is the proportional factor, *β* is the V_{g}/V_1 ratio with V_{g} the headspace volume and V_1 the liquid volume of the sample.

Using the linear equation $1/A = a + b \cdot \beta$, with $a = [1/(f_i \cdot C_i^{\text{liq}})] \cdot (1/k_i)$ and $b = 1/(f_i \cdot C_i^{\text{liq}})$, the *b/a* ratio corresponds to the partition coefficient expressed as a concentration ratio (k_i). For example, the reciprocal of the chromatographic areas for ethyl hexanoate was plotted against the phase ratio *β* (V_{g}/V_1) (**Figure 1**). A high linear correlation was obtained in water and in 20% (v/v) hydroalcoholic solutions for *β* smaller than 25. The k_i value can be converted into the ratio of molar fractions (K_i) following the conversion procedure proposed by Sander (*30*).
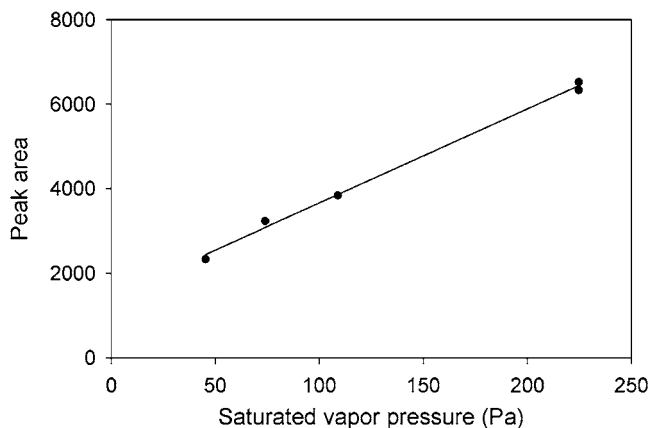
VPC Method. Glass vials (20 mL) were filled with 5 mL of the liquid solution containing aroma compounds. After 12 h, the headspace in equilibrium with the solution was analyzed in the same conditions as with the PRV method. To determine the aroma compound partial pressure corresponding to the FID signal, a gas calibration was used. This calibration was performed using the pure aroma compound. Glass vials (20 mL) were filled with 200 µL of pure ethyl hexanoate or isoamyl alcohol and equilibrated at different temperatures ranging from 9 to 25 °C for 12 h. After equilibration, 100 µL of headspace was analyzed in the gas chromatograph. Each FID response at each temperature was associated with a vapor pressure calculated from Antoine's law (*31*) (eq 3):

$$\log P^{\text{S}} = A - \frac{B}{C + (T - 273)} \qquad (3)$$

where P^{S} is the vapor pressure (mmHg), *T* is the temperature (in °C), and *A*−*C* are the three Antoine constants defined in **Table 1** for both compounds, ethyl hexanoate and isoamyl alcohol. It was found that for the range of conditions considered here the detector response (pure component peak areas) and the vapor pressure were linearly proportional (**Figure 2**). These calibration curves were used to determine the aroma compound molar fraction in the headspace [$y_i = p_i/P_{\text{T}}$ with p_i, the partial

pressure, and P_{T}, the total pressure, in the vial (1 atm)]. The molar partition coefficient, K_i, was obtained by a linear regression on the data of the gas molar fraction vs the liquid molar fraction, taking into account all of the different mixtures for which measurements took place. The variance of K_i then corresponds to the variance of the slope of the linear regression.

LC-SH Method. Conditions were similar to those used for the VPC method: glass vials (20 mL) were filled with 5 mL of solutions containing aroma compounds infinitely diluted in water−ethanol mixtures. A 2 mL sample of headspace was withdrawn from each vial at equilibrium and injected into the GC-FID. To obtain the headspace molar fraction (y_i), a liquid calibration was performed. For this purpose, glass vials (1 mL) were filled with solutions containing aroma compounds infinitely diluted in water−ethanol mixtures. Two microliters of liquid sample was withdrawn and injected into the GC-FID. A linear regression was fitted to data of the mass of aroma injected vs the FID response. The slope was used as a calibration parameter, accounting for the sensitivity of the FID detector. The response of the GC-FID was assumed to be the same when injecting a liquid or a gas sample.

Similar to the VPC method, the molar partition coefficient, K_i, was obtained by a linear regression on the data of the gas molar fraction vs the liquid molar fraction. The typical headspace concentrations vs liquid sample concentrations obtained with this method can be seen in **Figure 3**. This figure shows the results obtained when plotting C_i^{gas} vs C_i^{liq} in the case of ethyl hexanoate in water or in a 20% (v/v) hydroalcoholic solution. It also shows a linear dependence between C_i^{gas} and C_i^{liq} in order to access k_i through the slopes.

Data Analysis. We assumed that the mean *K* value obtained for each method had a *t*-student distribution (few samples from a normal distribution). The standard deviation (SD) and the 95% confidence interval were obtained for LC-SH and VPC methods by using the regression function in the data analysis package of Microsoft Excel 97. For PRV, the *K* value was obtained by averaging the results of different series, each one with a regression as stated in eq 2. The SD and the 95% confidence interval of the mean were obtained by using the descriptive statistics function from the data software previously mentioned.

The comparison between the *K* values obtained by two different methodologies, for example, K_{a} and K_{b}, was done by constructing a new variable $K_{\text{new}} = K_{\text{a}} - K_{\text{b}}$. This new variable also had a *t*-student

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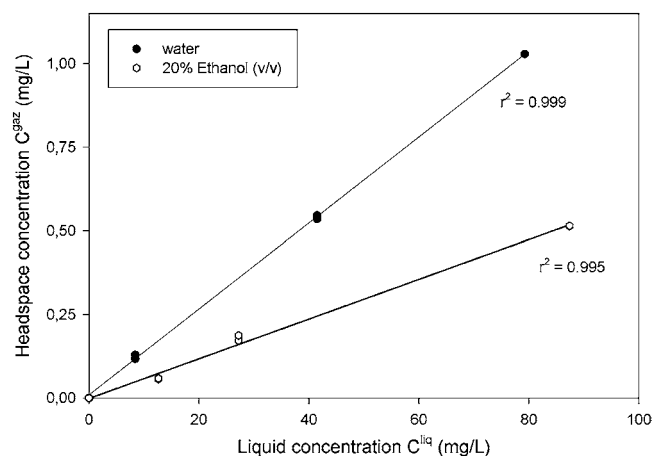
Figure 3. k_i calculation using LC-SH. Relationship between C^{gaz} and C^{liq} for ethyl hexanoate in water and 20% ethanol (v/v).

distribution with a SD and degrees of freedom (df) given by ref *32*:

$$SD_{new} = \sqrt{SD_a^2 + SD_b^2} \qquad (4)$$

$$df_{new} = min(df_a, df_b) \qquad (5)$$

Next, we tested the Ho probability (probability that the mean is zero) of the distribution of the subtraction of means (function T.DIST in Microsoft Excel 97).

RESULTS AND DISCUSSION

Effect of Operating Conditions on Headspace Concentration. We first studied the influence of some parameters of the headspace injection that could affect headspace analysis. The gastight syringe filling rate was tested between 10 and 500 μL s^{-1}, and the rate of injection into the gas chromatograph was tested between 100 and 600 μL s^{-1}. No effect of filling rate was observed in our headspace samples in the range studied. In contrast, increasing rates of injection resulted in a loss of volatile compound, with maximum headspace concentration detected at 100 μL s^{-1} and a minimum at 600 μL s^{-1}. For these reasons, we have chosen in our experiments to fill the syringe at 100 μL s^{-1} and to perform injection into the chromatograph at 100 μL s^{-1}.

The kinetics of volatile liberation, i.e., the time required to reach equilibrium inside the vial, was also studied. We noticed that headspace concentration increased logarithmically, reaching a maximum and constant value between 240 and 300 min approximately (between 4 and 5 h). The experiments were done with a 50 ppm solution of ethanol/water 20%, equilibrated at T = 20 °C (not shown). Hence, after the vials were filled, they were equilibrated at 25 °C for at least 4 h before injection into the GC-FID.

We further studied the influence of the liquid sample volume introduced into the 20 mL vial on aroma compound headspace concentration as described by Ettre and Kolb (*15*). Results presented in **Figure 4** showed that the headspace concentration above the hydroalcoholic solution of ethyl hexanoate was maximum for a liquid volume equal to or higher than 5 mL. Increasing the volume of the liquid sample in the vial did not result in higher headspace concentrations. Therefore, for our VPC and LC-SH experiments, we have chosen a sample liquid volume of 5 mL, since in this case the headspace concentration is independent of the liquid sample volume. On the contrary, for PRV experiments, we have chosen volumes inferior to 5
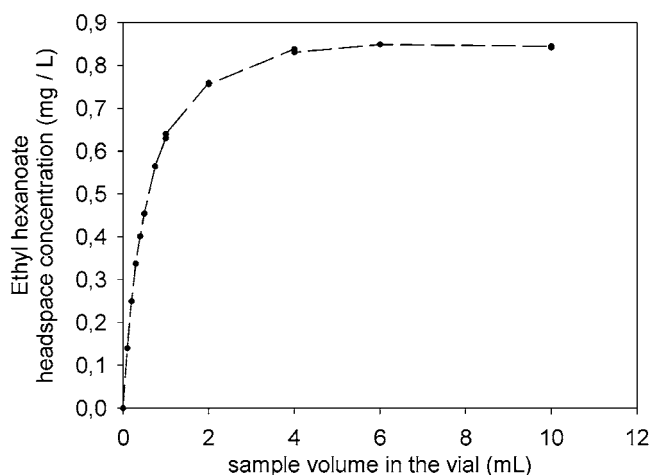
Figure 4. Influence of sample volume on ethyl hexanoate headspace concentration (a 100 ppm solution in water for a total vial volume of 20 mL).

mL, since in this case we needed to measure the headspace concentration in the zone sensitive to changes in the liquid volume.

Comparison of the Three Static Headspace Methods for Infinite Dilution Volatility Measurements. *Infinite Dilution Volatility Measurements in Water.* The measurements of partition coefficients at infinite dilution were first performed in water at 25 °C with the three methods, namely, PRV, VPC, and LC-SH. **Table 2** shows results for single and multicomponent mixtures, in the case of ethyl hexanoate alone in water or in the presence of the 14 other aroma compounds, called multicomponent mixtures. Statistical analysis for the mean distribution is included. PRV and VPC methods show very similar results. Moreover, the 95% confidence intervals of ethyl hexanoate overlap and the Ho test shows that the probability of the K mean value obtained with the PRV method is identical to VPC's one, which is 0.92. On the other hand, the LC-SH method always gives partition coefficients lower than half of those obtained by the two previous methods. The 95% confidence intervals help illustrate this, and the Ho test between PRV and LC-SH shows the very low probability of their K mean values being equal [p(Ho) = 0.007].

Moreover, the values obtained for multicomponent mixtures are similar to those obtained for single component mixtures, for all of the methods tested. It can be seen for ethyl hexanoate that the 95% confidence intervals overlap for the three methods when comparing single vs multicomponent results. However, the Ho test for each method is not very conclusive, since the p(Ho) is 0.36, 0.22, and 0.61 for PRV, VPC, and LC-SH, respectively.

As seen in **Table 2**, our results for volatilities of ethyl hexanoate in water at 25 °C with three static headspace methods show agreement between PRV and VPC but not with LC-SH. Further comparison against literature values shows even higher dispersion (**Table 3**). The comparison can be done considering the activity coefficients (γ^∞), the molar partition coefficient (K), or the concentration partition coefficient (k), since literature results are often expressed using only one of these variables. The parameters used for conversions between these three ways to express volatility are either well-known standard constants (R, T, P_T, MW, and ρ) or the saturation pressure of the pure compound at the corresponding temperature (P_{sat}). The observed dispersion within K and k values was similar, showing that the conversion procedure does not introduce additional errors. This

Table 2. Experimental Values for Ethyl Hexanoate Partition Coefficients K (mol/mol/mol/mol) in Water at 25 °C, with Three Static Headspace Methods, PRV, VPC, and LC-SH, for a Single or a Multicomponent Mixture[a]

ethyl hexanoate	PRV		VPC		LC-SH	
	K	SD	K	SD	K	SD
single component	36.21 (26.64–45.78)	3.01	35.88 (33.53–38.24)	0.99	16.20 (15.32–17.08)	0.39
multicomponent	40.05 (34.16–45.94)	1.85	43.84 (29.91–57.77)	5.89	15.17 (10.85–19.49)	1.91

[a] SD, standard deviation. Numbers in parentheses, 95% confidence interval for mean K value.

Table 3. Literature Values for Ethyl Hexanoate Volatility in Water at 25 °C[a]

γ^∞	standard P_{sat} at 25 °C	γ^∞ recalculated with P_{sat} at 25 °C from ref 12	K (mol/mol/ mol/mol)	k (mg/L/ mg/L)	liquid concn range (ppm or mg/kg)	method	ref
			dynamic HS methods				
(–)	(–)	26 323	**58.20**	*4.33E–02*	20–1000	exponential dilution	*33*
14 634	**346.13**	22 610	*49.99*	*3.72E–02*	30	exponential dilution	*34*
26 428	(–)	20 682	45.73	**3.40E–02**	20–1000	exponential dilution	*11*
13 300	(–)	13 300	29.41	2.19E–02	30	exponential dilution	*10*
15 481	**346.73**	23 960	*52.98*	*3.94E–02*	30–1000	exponential dilution	*9*
(–)	(–)	20 682	45.73	**3.40E–02**	20–1000	gas stripping at equilibrium*	*11*
16 195	**223.98**	16 192	**35.80**	*2.66E–02*	100	gas stripping at equilibrium*	*12*
18 954	(–)	18 954	41.91	3.12E–02	40	gas stripping at equilibrium*	*13*
			static HS methods				
6300	(–)	6300	13.93	1.04E–02	2–10	modified VPC	*27*
11 336	**223.93**	11 336	25.06	*1.86E–02*	10	liquid calibration	*35*

[a] Experimental values appear in bold font, calculated values using all parameters from the same work are in bold italic font, calculated values using R, T, P_L, MW, and ρ are in italic font, and calculated values using P_{sat} at 25 °C from ref 12 are in normal font. For ref 35, γ^∞ is given at 27 °C and recalculated with P_{sat} obtained with experimentally determined Antoine's equation parameters and with experimentally determined k. (*) The gas stripping at equilibrium method required a liquid calibration. (–) Value not given.

is expected, since only standard constants are used here. On the other hand, P_{sat} measurements show high dispersion in the literature; therefore, conversion between K and γ^∞ is likely to introduce errors. It can be seen in **Table 3** that there is a 50% difference between the minimum and the maximum P_{sat} values used in γ^∞ calculations. Moreover, the table shows γ^∞ differences higher than 300%. We have then recalculated γ^∞ for all of the works using a given P_{sat} value (ref *12*, in the third column of **Table 3**). Here, the differences in the calculated values of γ^∞ dropped from 300 to 200%, showing the significant influence of the dispersion of the P_{sat} values.

In addition, **Table 3** shows that volatility values obtained with dynamic headspace methods are systematically higher than those obtained with static headspace methods (LC-SH and modified VPC). This is so, since in dynamic methods the headspace sample is injected using calibrated automatic gas valves, which avoid gas losses. In contrast, in static headspace methods, headspace samples are injected using gastight syringes, which may introduce measurement errors due to leaks and adsorption of aroma compounds on its walls (*25, 36*), which may become important when operating in the ppm range (*37*).

However, our PRV and VPC results (**Table 2**) are as good as those obtained with dynamic HS methods (**Table 3**). If it is assumed that headspace injection measurements are subjected to the same relative error, it can be easily shown that the VPC method eliminates the bias introduced by gas leak and adsorption, since the K calculations consider the ratio between those measurements. In addition, as shown in the Supporting Information, the bias due to the use of syringes is also eliminated with the PRV method. Our LC-SH results (**Table 2**) matched those low K values found in the literature for the same method (**Table 3**). These values are much lower than those obtained with dynamic headspace methods, highlighting the detrimental effect of using liquid calibrations for quantifying gas injections. Even

though Conner et al. (*27*) did not use liquid calibrations, their K values (**Table 3**) are much lower than ours (**Table 2**). We have shown that the time required to reach equilibrium is approximately 4 h, while in the latter work they have only waited around 30 min; this may explain the differences.

Figure 5B shows that the above discussion regarding static methods is also applicable to isoamyl alcohol, which is much less volatile than ethyl hexanoate with a K around 1 in water at 25 °C, determined with PRV and VPC methods. Few literature data are available regarding this compound. With a static headspace method, van Ruth et al. (*38*) have obtained a value of gas−liquid partition coefficient at 37 °C of $k = 1.2$, that is to say $K = 1.62$ in molar fractions ratio. Considering the higher value of temperature in their work, we may conclude that our experimental determinations at 25 °C for this compound are quite consistent with their work. Sancho et al. (*24*) have obtained for isoamyl alcohol a γ^∞ of 206 with an exponential dilution method. Using this γ^∞ and a value of P_{sat} of 442 Pa at 25 °C (**Table 1**), we obtained a K value of 0.89, which is similar to our K values obtained with PRV and VPC; hence, these methods seem to work well in this case also.

Normally, physicochemical interactions between aroma compounds at the infinite dilution zone are neglected. Nevertheless, some studies have shown the existence of such interactions. Bohnenstengel et al. (*3*) have shown that when adding large amounts of a component in a solution containing between 100 and 200 mg L^{-1} of other components, interactions between aroma compounds seem to influence the headspace concentrations of each aroma compound. Besides, it has been shown that on hydroalcoholic solutions, when ethyl esters are present in concentrations above their solubility, they cause agglomerations that affect other aroma volatilities (*2, 39*). Although, it is worthwhile noting that the above findings do not consider the infinite dilution zone for all of the species.
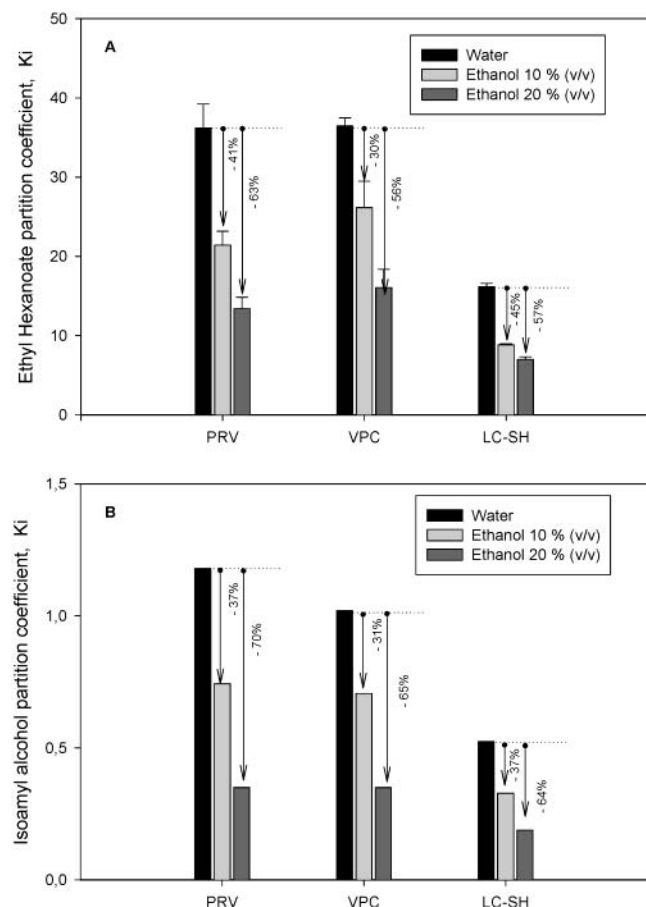
Figure 5. Ethyl hexanoate (**A**) and isoamyl alcohol (**B**) partition coefficients [K (mol/mol)/(mol/mol)] at 25 °C in water, in 10% ethanol (v/v), and in 20% ethanol (v/v), with three static headspace methods: PRV, VPC, and LC-SH.

Our results showed that a 15 component mixture at infinite dilution, either in water (**Table 2**) or in 20% v/v ethanol (not shown), presented no significant differences for ethyl hexanoate partition coefficients, when compared to single component mixtures. We assumed that if interactions exist between aroma compounds, these should be reflected on the volatility of ethyl hexanoate or isoamyl alcohol. Hence, on the basis of these preliminary results, we can conclude that little or no interactions exist between aroma compounds in spirits at the infinite dilution region. However, care must be taken since the number of aroma components quantified in real spirits is at least five times larger than 15 (*40*); hence, a specific study of interactions would require an even higher number of components in the mixture.

Infinite Dilution Volatility Measurements in Water−Ethanol Mixtures. The effect of ethanol [up to 20% (v/v)] on aroma partition coefficients was studied using the three static headspace methods previously discussed for water. **Figure 5** gives the results obtained for ethyl hexanoate (**A**) and isoamyl alcohol (**B**) in the presence of 10 (v/v) and 20% (v/v) ethanol. Whatever the method, an increasing quantity of ethanol in the mixture leads to lower partition coefficient values, a result that was also observed by Conner et al. (*27*).

There is no major difference between the three methods used, since each method gives similar reduction in volatility with increasing presence of ethanol (**Figure 5**). Indeed, for ethyl hexanoate, this reduction is on the average of $38 \pm 7\%$ in 10% (v/v) ethanol and $58 \pm 3.5\%$ in 20% (v/v) ethanol with the three methods. In the case of isoamyl alcohol, the presence of

10 (v/v) and 20% (v/v) ethanol led to partition coefficient reductions of 35 ± 3 and $66 \pm 3\%$, respectively. Considering the deviations to the averages values, one may conclude that the retention effect of ethanol is similar for both aroma molecules, even if their absolute volatilities in water are initially very different.

Measuring absolute volatilities in multicomponent mixtures is difficult with methods that require concentration values of the liquid sample, like VPC and LC-SH. This is so since volatilization of aroma compounds occurs during liquid sample preparation and weighing errors are almost unavoidable even with high precision balances. On the other hand, PRV seems the most suitable method to measure absolute volatilities in multicomponent systems. It does not require an external calibration and does not use liquid concentration measurements in the calculation of absolute volatilities. PRV only requires that the compounds are at infinite dilution and that the exact amount of liquid sample is delivered into the vial, since the measurement of the phase ratio (β) may become an important source of error.

In summary, we have shown that absolute volatilities in hydroalcoholic mixtures at infinite dilution can be measured accurately with PRV and VPC methods, since in both procedures the errors due to gas leaks and adsorption in gastight syringes are eliminated. On the other hand, the LC-SH method presents bias in K determination, since headspace injections are calibrated with liquid injections, which have different leak and adsorption patterns. In addition, the three tested methods are suitable to observe the ethanol retention effect up to 20% v/v for two different volatile aroma molecules. The use of the PRV method in the presence of more than 20% (v/v) ethanol is not advisable due to the reduction of absolute volatility. In this case, changes in headspace concentration are not sensitive enough to variations in the volume of the liquid sample in the vial (*16*).

Existing LC-SH data can be better appraised thanks to the experimental comparison presented in this work. Despite its widespread use in aroma research, since it is a low cost and simple method, LC-SH always underestimate absolute volatility values.

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