Experimental Validation of Perfumery Ternary Diagram® Methodology

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The new methodology based on Perfumery Ternary Diagram® (PTD®) used to predict the odor value (OV) of a perfume mixture was updated and validated through experimental measurements of odor threshold values and headspace concentrations, using a perfume test mixture of limonene, geraniol, vanillin, and ethanol. The influence of the odor threshold values on the PTD® was considered. The experimental OV values were compared with OV values predicted by PTD® model, for the ideal case (considering no molecular interactions) and nonideal case (using UNIFAC model to account for molecular interactions). It was found that PTD® results fit reasonably well the experimental data, except for vanillin. Nevertheless, the PTD® methodology has proven to be useful in predicting the odor of perfume mixtures and to study the influence of the liquid composition on the headspace compositions, odor values, and its smell. © 2007 American Institute of Chemical Engineers *AIChE J,* 54: 310–320, 2008

Keywords: perfume, fragrance, perfumery ternary diagram, odor value, odor threshold, headspace analysis

Introduction

The sense of smell can be quantified based on the measurement of headspace concentrations (C_{hs}) and odor threshold values (Thr). Odor threshold values are defined as the lowest concentration at which the odor of a chemical compound can be distinguished with certainty from a blank under standard conditions.[1] In spite of perfumes being produced and applied in the liquid phase, the nose is able to detect the smell only when the components have evaporated, and if the concentrations of the odorant molecules in the gas phase (headspace) are above their odor threshold values.

Odor threshold values are very valuable data for perfumery, since they determine the minimum amount of an ingredient to use in a perfume formulation in order that it will be perceived. They are also important indicators of their performance: comparing substances with similar volatilities and at the same concentration level, the lower threshold compounds show a stronger odor (high impact). Often it is found that the key components of a flavor or fragrance are only present in trace amounts but they strongly impact the overall sensory impression because of their extremely low odor threshold value.

Combining the information of headspace concentration (C_{hs}) and odor threshold value (Thr), a parameter named Odor Value (OV) is defined as the ratio of those two values.[2] It is assumed that the component with the highest OV will be the one perceived as having the strongest odor. In a perfume mixture, the dominant smell can be assumed as corresponding to the component with the highest OV—the strongest component model. Therefore, OV is used to infer how a fragrant component can contribute to the overall character of a perfume or flavor and evaluate its impact during the evaporation of the perfume. The OV values are also important to screen fragrant components that can "mask" some unpleasant odors, such as the smell of smoke on a room or on cloths, or the unpleasant off-notes of some cosmetic matrix.

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The use of the concept of OV to relate to odor intensity is a common practice in perfumery,[2–6] as an approximate relative measure of odor intensity and substantivity.[2] Although being an approximate approach, it is calculated based on measurable headspace concentrations and odor threshold values. Baydar et al.[3,4] use OV concept to obtain odor perception profile, compare fragrance performance and quantify fragrance diffusivity and substantivity on skin. They consider that "OV defines the probability of perceiving a fragrance and relates to fragrance intensity".

Using OV values to quantify odorant's intensity presents some limitations, namely that odor intensity does not vary linearly with concentration (follows a psychophysical power law described by Stevens[7]] and does not vary consistently between different materials[2,5,8,9] (which implicates that two odorants can be perceived with different intensities although they have the same OV). Moreover, it does not does not take into account interaction between components in a mixture.

However, it can be used for assessing the relative importance of a fragrance ingredient to the total odor and as a relative measure for odor intensity.[2,5] The application of OV value is a fast and easy-to-handle method, that reduces time-consuming and less precise sensory measurements, relying on the measurement of headspace concentration that can be done with high precision (by headspace gas chromatography) and on odor threshold measurement (by dynamic olfactometry), which delivers fairly reproducible data.[5]

There are several models of odor interaction[10,11] for predicting odor intensity of mixtures, namely Strongest Component Model, Vector Model, U model, among others. More recently, Cain et al. have compared five models of odor interaction.[11] They found that similar correlation fits for all models. The psychological model "Strongest Component Model", stating that the perceived intensity of mixtures tend to be closer to the one of the strongest component, was found the second best in terms of Pearson's correlation coefficient.

The problem of using other models for odor intensity is that measuring odor intensity is very difficult since perceived intensity varies from individual to individual, there is no objective scale[12] (since when comparing two odors, the scale will adjust to the difference between them) and has a very strong physiological subjectivity, since perceived intensity depends also on experience, cognitive factors,[13] familiarity[14] and hedonic tone (liking/disliking),[8,14] where people tend to react more strongly to odors they dislike, perceiving it more intensely.[8] Even the existing standards for measuring suprathreshold odor intensities, such as ASTM E 544, are not direct measures of odor intensity, since the odor intensity of the reference component itself (1-butanol) is not a linear function of its concentration.[15]

In most cases, odor intensity slopes can not be found or they exist only for a limited group of aroma chemicals. Devos et al. have made recently a compilation of standardized olfactory power law exponents.[9] However, these values are still controversial,[16] involving the reliability of data sets, the validity of the power law itself,[16] the fact that the slope may not be constant as assumed by Stevens but a function of the pair stimulus-subject,[2] the dependence upon the method used so that data can only be compared meaningfully when obtained at the same conditions.

Calkin and Jellinek suggest using a slope value of 0.35 for all the materials, which is a reasonable value considering that about 60% of the power law exponents of components applied in perfumery, that can be found in the compilation of Devos et al., lies between 0.3 and 0.4. However, for comparative purposes it is indifferent to use a general slope equal to 0.35 or 1.

The quantifiable aspects of odor perception are[17]: odorants' concentration, the odor threshold values, OVs, the determination of chemical structure fragrant molecules and the measuring of a few physiological signals (transduction on cell membranes of olfactive neurons, the frequency modulated nerve pulses and mapping brain neurons activated during smelling). In this work, we will focus on the first three aspects.

A new methodology named Perfumery Ternary Diagram® (PTD®) has been developed in our previous works,[18–20] for predicting the odor of a perfume mixture based on the concept of OV. In this work, PTD® was validated experimentally by measuring odor threshold values and headspace concentrations and comparing the OV values obtained experimentally and by simulation. The UNIFAC parameters used were also updated using more recent literature[21] than that used in our previous works.[22]

PTD® methodology

In our previous works, the innovative concept of PTD® was introduced, combining the Perfume Pyramid Structure with Engineering Ternary Diagrams.[18–20,23]

The PTD® model predicts the smell of perfume liquid mixtures, for all possible compositions. This way, the amount of trial and error experiments necessary to reach the right fragrance composition is considerably reduced.

PTD® methodology is based on the concept of OV as a measure of odor intensity. OV values are expressed as follows[2]:

$$\mathrm{OV}_i = \frac{C_{\mathrm{hs}_i}}{C\mathrm{thr}_i} \tag{1}$$

where OV is odor value, C_{hs_i} is headspace concentration (kg/m^3) and C_{thr_i} is the odor threshold value in air (kg/m^3).

As odor threshold values are the minimum concentration in the gas phase to be detected by the olfactory sense, the OV value of a fragrant component must be higher than one so that it can be perceived by the nose. The odor intensity will be stronger as the OV is higher.

It is important to notice that odor intensity is not an additive parameter.[24] So, in this work, it is considered that the perfume mixture will smell stronger to the component having the highest OV. Moreover, OV values are not absolute measurements for intensity but enable to compare odor intensities and predict the dominant smell, in the same way that the vapor pressure is used to characterize volatility.

The perfume system is considered as consisting in a complex fragrant liquid mixture and a corresponding air phase above it, the headspace, which we smell. Assuming that this system is in thermodynamic equilibrium, being the headspace an ideal gas mixture and the liquid phase a nonideal solution, the OV value of each component is calculated by the equation:

$$\mathrm{OV}_i = \gamma_i x_i \left(\frac{P_i^{\mathrm{sat}} M_i}{C_{\mathrm{thr}_i}}\right)\left(\frac{1}{RT}\right) \tag{2}$$

where OV_i is the OV of component i, γ_i is the activity coefficient of i in the liquid, x_i is the liquid phase molar fraction, P_i^{sat} is the saturated vapor pressure (Pa), M_i is the molar mass (kg/mol), C_{thr_i} is the odor threshold value in air (kg/m^3), R is the ideal gas constant (J mol^{-1} K^{-1}), and T is the absolute temperature (K). In this work, the values of γ_i were calculated using the UNIFAC method, which is based on molecular group contribution.[21]

The activity coefficient γ_i of a component i in a mixture provides a useful way of accessing the affinity of this odorant i to the surrounding medium.[25] In the ideal case, there are no molecular interactions and $\gamma_i = 1$. For $\gamma_i > 1$, the partial pressure of component i is higher than that predicted for an ideal solution, indicating that this volatile has no affinity to the medium and so it will be "pushed-out" of the liquid system into the gas phase. On the contrary, when $\gamma_i < 1$, implies that the component i has a high affinity to the medium, so its partial pressure will be lower, meaning that there is less material in the gas phase than expected if we consider the liquid an ideal solution and so there occurs an increased retention in the liquid.

The OV value of a perfume mixture of N components, $\mathrm{OV}_{\mathrm{perf}}$, is expressed as:

$$\mathrm{OV}_{\mathrm{perf}} = \mathrm{OV}_{\mathrm{MAX}} = \mathrm{MAX}\left(\mathrm{OV}_{\mathrm{A}}, \mathrm{OV}_{\mathrm{B},}\ldots, \mathrm{OV}_N\right) \tag{3}$$

For a perfume test mixture constituted by three fragrant components, it is possible to represent all the possible compositions in a ternary diagram and to show for each composition, which is the component having the maximum OV value, as is shown in Figure 1. For example, the points marked as triangles correspond to the compositions with maximum OV value equal to OV_{B}, and so these perfume compositions will smell strongly to component B.

Validating PTD® methodology

PTD® model can be validated by determining the OV value experimentally, based on measured odor threshold values and headspace concentrations. Some considerations on these two parameters will be presented next.

Odor threshold value

The perfumery materials have a wide range of odor threshold values, which vary several orders of magnitude between them, as for example, 440 ppb for limonene and 0.03 ppb for vanillin,[2] showing that the nose responds quite differently to the fragrances. To date, it is not possible to predict the odor of a molecule, though there are some theories that try to explain how the sense of smell works.[26–32] Therefore, odor thresholds must be measured.

The odor threshold values found in the literature for any fragrance or flavor component vary greatly from author to author, existing differences of several orders of magnitude. For example, for geraniol there are values between 1.20 × 10^{-5} mg/m^3 and 1.29 mg/m^3,[33] with a variation that covers five orders of magnitude. These big differences occur because these values depend on the sensory response (which vary from person to person) and on the experimental conditions (experimental methodology, purity of fragrant materials, ambient temperature, fragrance base in which is evaluated).[34] Therefore, it is necessary that perfumers determine their own odor threshold values and build their own database based on their individual sensory response at perfectly controlled conditions, since their work will depend on their sensitivity. It is like making the "calibration curve" of their nose. Taking this into account, in this work, there were measured our own odor threshold values, using an olfactometer, at our working conditions.

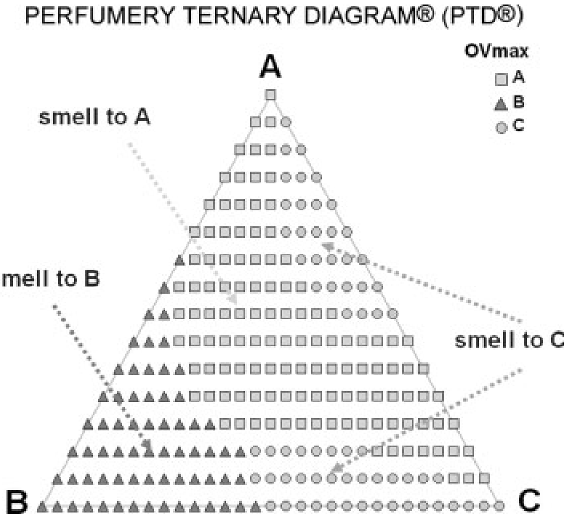

Figure 1. Perfumery Ternary Diagram®, a representation of all the perfume compositions and their smell, for ternary mixtures (components A, B, and C).

The composition in molar or mass fraction (in a basis without the solvent) is indicated by the position in the triangular diagram, and the smell by the shape of the point, corresponding to the component that has the maximum odor value, $\mathrm{OV}_{\mathrm{max}}$.

Headspace concentration

Any fragrant component to be perceived must be in the gas phase (headspace) so that it can reach the olfaction sense and be recognized as a smell. However, the components are mixed in the liquid phase to compose a perfume and they have different volatilities, so that the composition in the gas phase is significantly different from that of the liquid phase. Moreover, there are molecular interactions between the components, which affect the volatility of the components in the mixture and consequently, the headspace composition. The headspace concentrations can be measured by headspace analysis using gas chromatography (HS-GC).

There are two main techniques of HS-GC[35]: static direct injection of a gas sample, using a gastight syringe (S-HS); and using an adsorptive material to concentrate the sample and then release it for analysis, as solid phase microextraction and purge-and-trap (P&T).

Direct injection is the simplest technique and the one that measures the "true" headspace concentration, with no distortion in the concentration because of concentration effect and competition phenomena, as happens with solid phase micro-extraction.[36,37] In this work the headspace concentrations are high enough to be measured by GC and it is possible to quantify the high concentration of the solvent ethanol, without the risk of occurring any saturation. Therefore, direct injection was the technique employed for headspace analysis.

Experimental

Materials

The following pure fragrances or aroma chemicals were used: limonene (Fluka, 98%), geraniol (Sigma, 98%), vanillin (Fluka, 98%), and ethanol (Merck, 99.9%).

Olfactometer equipment and odor sample preparation

The equipment used to perform the odor threshold measurements was an Olfactometer Ecoma model T07 (Germany), built according to standards VDI3881 and EN13725. The recommended methods are described in several international standards.[38–41] The principle of measurement is based on a panel of people used to detect the smell and by making precise dilutions with neutral air of the odorant sample of known concentration, in a defined ratio, in a sequential way and at a fixed dilution step, and recording the results. The measurement of odor threshold concentration consists of a sequence of dilutions of a gas sample of known concentration. The concentration is presented in ascending order (decreasing dilution factor), starting at a point where no odor is detected, and using yes/no questioning to the panelists (method of limits). The threshold odor concentration is calculated by determining the dilution factor at which the odor starts to be sensed. Figure 2 shows a scheme of the measurement procedure for the determination of odor threshold values. Figure 2 shows a scheme of the measurement procedure for the determination of odor threshold values.

The measurement is run automatically and controlled by the software, using neutral air to make the dilution of the sample concentration. In the end, the results are saved in a file. There are four sniffing ports. There is a test leader who sets the valves manually to the dilution indicated by the software and gives indications to the panelists. The panelist should press the button when detects an odor. If no odor is detected, the next dilution step is set. The measurement sequence stops after two consecutive positive answers by at least 50% of the panelists, or when it reaches the end of the sequence at the lowest dilution factor (highest concentration). Each measuring test comprises three measuring sequences, with a rest period of 60 s between them. The sample is presented in ascending concentration series, to avoid adaptation phenomena of the sense of smell. There are 20% of random blanks that correspond to neutral air instead of odor sample, to check if there is any tendency for guessing. The olfactometer was used in the conditions shown in Table 1.

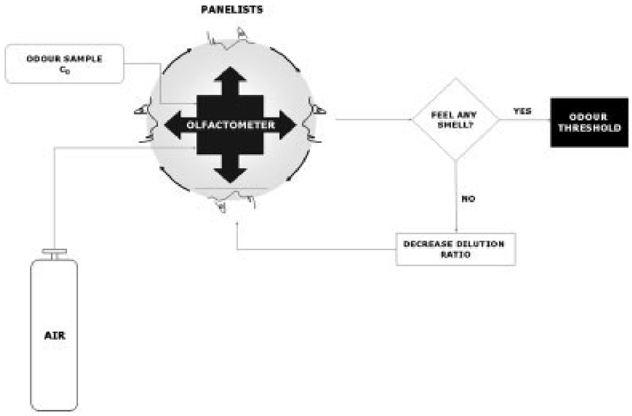

Figure 2. Scheme of the experimental measurement of odor threshold values.

Table 1. Conditions Used for the Olfactory Measurements

Parameter	Value Set
Dilution step*	2
Dilution range	2.5–64,000
Measuring sequences	3
Interval between sequences	60 s
Number of panelists	1–4
Breathing time	2.2 s
Air flow rate (inhaling)	1.2 m^3/h (1.5 bar)
Operating air	medicinal air
Inlet gas pressure	3 bar
Operating pressure	1.5 bar
Room temperature	25°C

*Dilution step = 2 means that the actual dilution ratio is set to half of the previous one, in a measuring sequence.

The odor sample is supplied to the olfactometer using a sample bag, made of PTFE material and with a stainless steel tube, used to insert the sample and to connect it to the olfactometer. The volume of the sample bag is 8.6 L. The fragrant sample was prepared using medicinal air and adding a known amount of the aroma chemical into the bag, using a small volume syringe (GC or HPLC syringe). The fragrant gas solution was left to equilibrate for at least 1 h, at controlled room temperature of 25°C, before measuring odor threshold values. All the measurements were made within a period of 15 days.

Measurement of odor threshold values

Three sets of data were obtained: two sets of odor threshold values obtained by two panelists, individually (P1 and P2), and one set with a panel constituted by four people (4 PANEL, coworkers in the laboratory), as can be seen in Figure 3. The measurements were carried out at an ambient controlled temperature of 25°C (by air conditioning equipment), at the same time of the day and the procedure of preparing the samples was the same for all the experiments. The panel members were constituted by three women and one man, non-trained, with ages comprised between 26- and 31-years old.

The dilution ratio at threshold, Z_{thr}, was calculated after obtaining two consecutive positive answers (Z_N and Z_{N+1}), as the geometric mean of the last step when no odor was smelled (Z_{N-1}) and the first step when the odor was first detected (Z_N), according to European standard EN13725.[39]

Figure 3. Measurement of odor threshold value using a panel of four people and an olfactomer.

The odor threshold value (g/m^3), C_{thr}, was finally calculated by:

$$C_{\mathrm{thr}} = \frac{C_0}{Z_{\mathrm{thr}}} \tag{4}$$

where C_0 is the gas concentration of the aroma chemical in the sample bag (g/m^3).

The resolution value (λ) corresponds to the standard deviation of the odor sensations, being a statistical parameter indicative of the dispersion of the odor sensations, defined as:

$$\lambda = \frac{1}{2}\log_{10}\left(\frac{Z_{16}}{Z_{84}}\right) \tag{5}$$

where Z_{16} is the reaction limit (odor impression in 16% of the panelists) and Z_{84} is the determination limit (odor impression in 84% of the panelists).

Headspace analysis

The headspace analyses were used to determine the concentration in the gas phase above the perfume test mixture and to determine the vapor pressure of the pure components (limonene, geraniol, vanillin, ethanol) at 25°C.

The mixture of limonene (A), geraniol (B), and vanillin (C) in ethanol (S) was chosen as a test perfume mixture, having one citric top note (A), one rose floral middle note (B) and one sweet vanillin base note (C). Three solutions were prepared with the compositions shown in Table 4. The molar fraction of ethanol was chosen as the minimum amount of solvent necessary to dissolve the vanillin (solid). Therefore, for point P1, with higher quantity of vanillin, the molar fraction of ethanol had also to be higher.

A 4 ml aliquot of the perfume mixture was placed in a 20 ml glass vial capped with aluminium crimp caps with Pharma-Fix septum. The samples were equilibrated overnight before the first headspace analysis and 1 h between analyses, at room temperature of 25°C. For the headspace analysis, it was used a gastight syringe (SGE, 1 ml). The sample was withdrawn and injected manually into GC/FID at a rate of 0.1 ml/s. The sampling volume was 0.5 ml of the headspace above the perfume test mixture. The syringe was flushed with air after the sampling, to prevent carry over of the sample between analyses. Each sample was analyzed in duplicate.

Gas chromatography GC/FID was carried out using a Varian CP-3800 instrument equipped with a split/splitless injector and a capillary polar column from Chrompack (CP-Wax 52 CB bonded fused silica, 50 m × 0.25 mm i.d., 0.2 μm film thickness). The oven temperature was programmed isothermal at 50 °C for 5 min, to 70°C at a heating rate of 2°C/min, to 200°C at 10°C/min and then held isothermal for 20 min. The injector was set at 250°C, splitless for the initial 5 min and afterwards with a split ratio of 1/50. The FID detector temperature was 250°C. The carrier gas was helium He N60, at a constant flow rate of 1 ml/min.

For the quantification of the volatile components (limonene, geraniol, vanillin, and ethanol) using gas chromatography (GC), the linear calibration curves for each component (GC peak areas vs. injected mass) were determined by the external liquid calibration method. Each calibration point corresponded to the average of three injections. The calibration curves had correlation coefficients higher than 0.999 for all the components.

The headspace concentration was finally calculated by:

$$C_{\mathrm{hs}} = \frac{m}{V_{\mathrm{inj}}} \tag{6}$$

where C_{hs} is the headspace concentration (kg/m^3), m is the injected amount withdrawn from the perfume headspace (kg) and V_{inj} is the injected volume (m^3). The amount m was determined through the GC peak and the respective calibration curve.

Determination of vapor pressure values

For the determination of vapor pressure values of the pure components, an amount of 4 ml of each pure component was placed in a 20 ml headspace vial. The samples were left to equilibrate overnight, at room temperature of 25°C. The analysis procedure was the same used for determining headspace concentrations of the perfume test mixtures. Each component was analyzed in triplicate (3 vials). The vapor pressure P_{v} of the fragrant component was then calculated by the following equation, based on the ideal gas law:

$$P^{\mathrm{sat}} = \frac{C_{\mathrm{hs}}}{M}RT \tag{7}$$

where C_{hs} is the headspace concentration (kg/m^3), M is the molar mass (kg/mol), R is the gas constant (8.314 J mol^{-1} K^{-1}) and T is the temperature (298 K).

Results

The PTD$^{®}$ methodology for predicting OVs was applied to a perfume test mixture, consisting of a ternary system of limonene (top note, A), geraniol (middle note, B) and vanillin (base note, C), in solution with ethanol (solvent, S). The validation of PTD$^{®}$ model consisted in four main steps: (i) measuring odor threshold values and vapor pressures for each component of the perfume system; (ii) getting "experimental" OV values by measuring the headspace concentra-

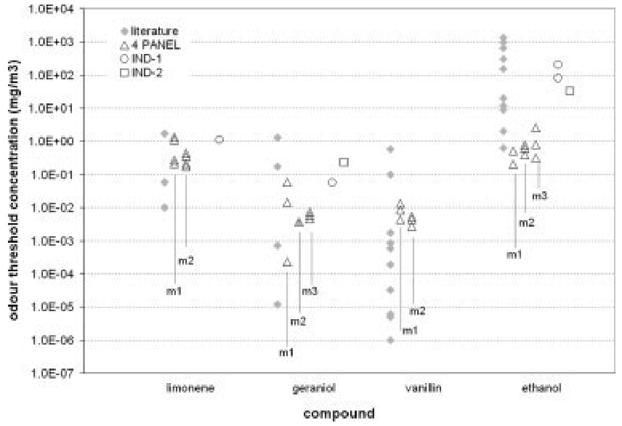

Figure 4. Odor threshold values measured experimentally, using individual panelists (IND-1and IND-2) and a panel of four individuals (4 PANEL).

Each column of data points (Δ) corresponds to one measurement and comprises 1–4 sensorial responses. Our experimental data is compared with values found in the literature[33] (data points with background color).

tions; (iii) obtaining the OV values simulated by PTD® model, using the experimental data of odor threshold values and vapor pressures as parameters; and finally (iv) comparing OV values obtained experimentally and by simulation.

Experimental Data

The odor threshold values measured experimentally at 25°C are shown in Figure 4 and Table 2. The values in the graphic correspond to the odor thresholds detected by all the panelists, for each component. For each measurement, corresponding to one column of data points in Figure 4, there are four points corresponding to the four panelists. In the case when the panelist failed to give a consistent answer, his/her result was excluded and therefore in some cases the total number of data points is inferior to four. Note that for the second measurement of odor threshold of geraniol, there are three points that overlap since three panelists gave exactly the same response. The odor threshold values obtained for each measurement (corresponding to the geometric mean of all the valid answers of the panelists, for each measurement) and the respective resolutions or standard deviations, using the four people panel, are shown in Table 2.

Table 2. Odor Threshold Values Measured* (C_{thr_i}) and Respective Resolution (λ) Obtained, Using a Panel of Four People (4 PANEL)

	Component	C_{thr_i} (mg/m^3)	λ
A	Limonene	5.94×10^{-1}; 2.73×10^{-1}	0.389, 0.196
B	Geraniol	5.76×10^{-3}; 3.63×10^{-3}; 5.65×10^{-3}	1.121, 0.000, 0.152
C	Vanillin	6.64×10^{-3}; 3.99×10^{-3}	0.244, 0.171
S	Ethanol	3.54×10^{-1}; 5.70×10^{-1}; 8.67×10^{-1}	0.252, 0.201, 0.368

The odor threshold values (C_{thr_i}) correspond to average values (geometric mean) of the four panelists' answers, obtained for each measurement (m1, m2, and m3, in Figure 4).

Table 3. Vapor Pressure and Odor Threshold Values (of Panelist IND-1) Measured Experimentally, for the Components Used in the Perfume Test Mixture

	Component	P_i^{sat} (Pa)	C_{thr_i} (P1) (mg/m^3)
A	Limonene	5.68×10^{1}	1.09×10^{-0}
B	Geraniol	4.13×10^{-1}	5.75×10^{-2}
C	Vanillin	2.70×10^{-2}	5.15×10^{-3}
S	Ethanol	3.76×10^{3}	$2.04 \times 10^{+2}$

We have made the option of using a nontrained panel since current perfume users and consumers do not have

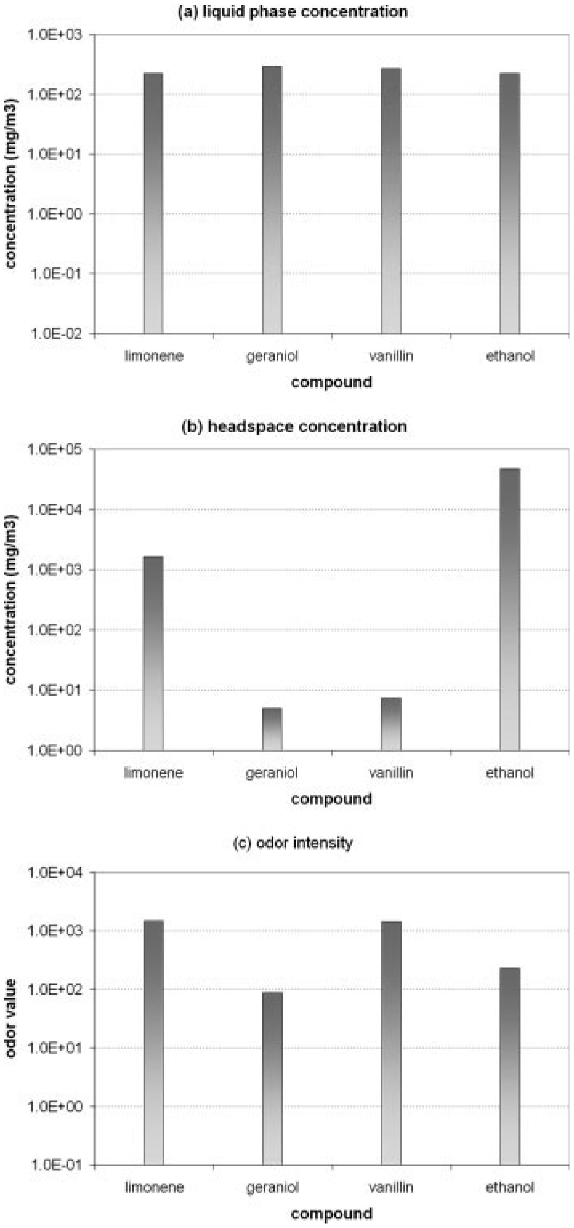

Figure 5. Composition profiles of the perfume test mixture at equilibrium and odor values, at 25°C.

(a) liquid phase; (b) headspace; (c) odor values.

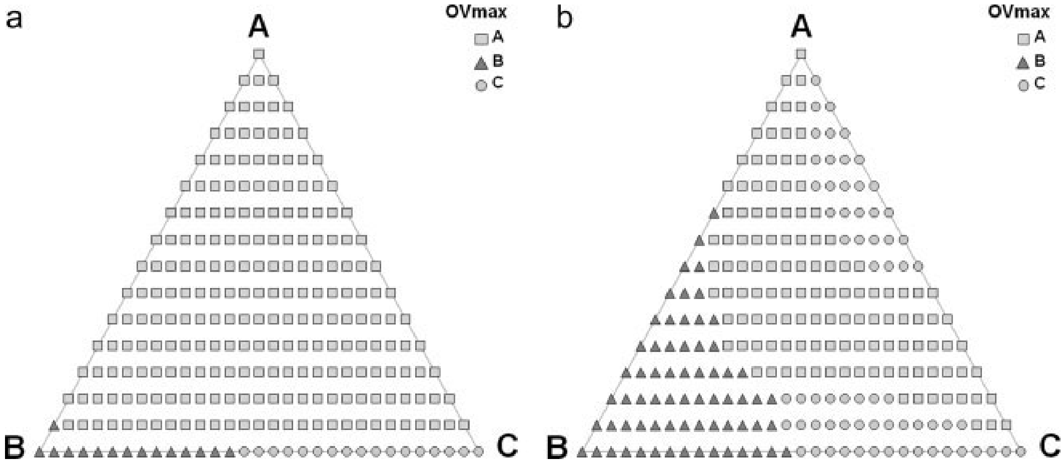

Figure 6. Perfumery Ternary Diagram® of the perfume test mixture composed of limonene (A), geraniol (B) and vanillin (C), without solvent.

The simulation was performed considering the non-ideal case, using odor threshold values and vapor pressure: (a) measured experimentally; (b) obtained from the literature.[2]

trained noses, being this way the results obtained more representative of reality.

The data obtained show a great variability, especially geraniol, although they were obtained at the same conditions, in a short period of time (15 days) and keeping the same procedure. These results confirm the difficulty in obtaining consistent sensory data since the olfactory sensitivity changes considerably from person to person. For example, there will be some people that will smell strongly to ethanol in the perfume if they have a lower odor threshold and others will not even feel it because their odor threshold is higher.

In the same plot, the experimental results are compared with the values found in the literature,[33] which also vary significantly over a wide range of values. It can be seen that the measured odor threshold values fall within the range of the literature values.

Therefore, the work of a perfumer should be based on his own odor threshold, since this is the only meaningful reference for him or her to evaluate the smell of his or her own perfume compositions. In this work, the odor thresholds used were the ones determined by the panelist IND-1, shown in Table 3.

The vapor pressures that were measured experimentally are also shown in Table 3.

Comparison of perfume liquid composition and its headspace

The composition profiles in liquid and gas phases and odor intensity are compared in Figure 5, for a perfume test mixture composed of approximately equal amounts of each component A, B, C, and S. Although all the fragrant components had approximately the same concentration in the liquid phase, it can be seen that the headspace concentrations were quite different because they have evaporated at different rates, because of their different volatilities. As expected, the most volatile components limonene and the solvent ethanol were the ones with the highest amount in the gas phase, having a concentration of three to four orders of magnitude higher than the other two.

Considering the odor intensity of the perfume test mixture, the higher concentration of ethanol in the headspace might lead to the conclusion that the component that would be smelled with more intensity would be that of ethanol. However, as can be seen in Figure 5c, limonene showed the maximum OV, followed very closely by the OV of vanillin, because although its headspace concentration is low, its odor threshold is also very low (see Table 3). This means that limonene was the component perceived with highest intensity, but vanillin was also felt relatively strong. In fact, the perfume test mixture smelled to lemon candies, with very sweet (vanilla) lemon odor, and the ethanol smell was not detected.

PTD® as a predictive tool

The PTD® simulations should be based on the perfumer olfactory sensitivity and therefore the OV values were calculated using the values of odor thresholds obtained by panelist IND-1 (see Table 3). Also the equilibrium vapor pressures used were measured experimentally at the working room temperature of 25°C (see Table 3) and in the same conditions as the headspace concentrations were determined.

The new PTD® profile, using our measured data and considering the nonideal case, is shown in Figure 6a. It can be seen that this test perfume is mostly dominated by the limonene (A) top note, where geraniol (B) and vanillin (C) are only perceived after A has completely evaporated (for the compositions on the line BC, which means binary mixtures of B and C), or for very high concentration of B (points on line AB, close to B edge). Comparing with the PTD® obtained in our previous works[19] using literature data[2] (Figure 6b), the profiles are very different owing to the fact that panelist IND-1 is very sensitive to limonene and less sensitive to geraniol and vanillin, thereby the region with $\mathrm{OV}_{\max} = OV_{\mathrm{A}}$ have become dominant and the lemon scent (A) overlays rose (B) and vanilla (C) smells.

The effect of the solvent on the PTD® based on experimental data is shown in Figure 7. It can be seen that the solvent concentration has little influence in the OV value, according to panelist IND-1 sensitivity, where ethanol at an amount as high as $x_{\mathrm{S}}=0.7$ becomes noticeable only after limonene has

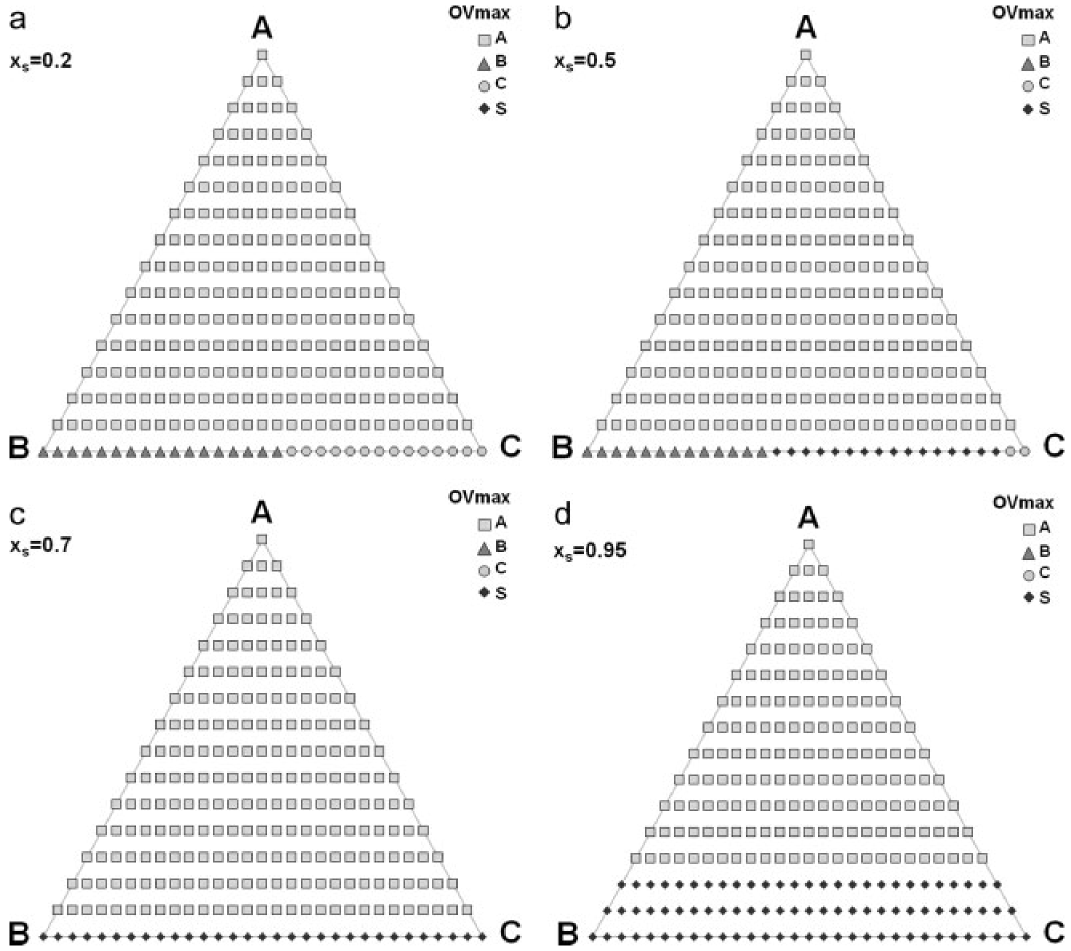

Figure 7. Effect of the solvent (ethanol) on PTD®, for a perfume test mixture composed of limonene (A), geraniol (B), vanillin (C), and ethanol (S), with different molar fractions of ethanol.

(a) $x_S = 0.2$; (b) $x_S = 0.5$; (c) $x_S = 0.7$; (d) $x_S = 0.95$.

completely evaporated (the values of $OV_{max} = OV_S$ are located at the binary line B-C, corresponding to $x_A = 0$).

Validation of PTD® methodology

The OVs determined based on experimentally measured data of headspace concentrations and odor threshold values were compared with the OVs predicted using the PTD® methodology, for the perfume test mixture, as presented next.

Three perfume test mixtures of limonene (A), geraniol (B), vanillin (C), and ethanol (S), A + B + C + S, were prepared with the compositions shown in Table 4. The composition points P1, P2, and P3 are also shown in the PTD® in Figure 8. All the compositions correspond to perfume test mixtures with limonene having the highest OV ($OV_{max} = OV_A$), although the amount of each component is considerably different. This was confirmed by smelling the headspace of the perfume test mixtures, which actually had the strongest odor of lemon (limonene, A).

The comparison of the experimental OV values and the simulated OV values, using PTD® model and considering the ideal case and the non-ideal case, is shown in Figure 9. The simulated ideal OV values constitute a reference for inferring about the affinity of the components towards the liquid media: if $OV > OV_{ideal}$, then the component is "pushed-out" from the liquid phase, while if $OV < OV_{ideal}$ then the component is retained in the solution—it occurs fixation.

It was concluded that the OV values of most of the components, with the exception of vanillin, were reasonably well predicted, being the experimental OV values closer to the OV values simulated for the nonideal case.

The experimental OV value of limonene for composition P2, with high concentration of limonene, was intermediary between the values predicted for ideal and nonideal case, being closer to the nonideal case. This means that limonene

Table 4. Composition of Perfume Test Mixtures Used in the Experiments, Expressed as Molar Fractions

	Component	P1	P2	P3
A	Limonene	0.129	0.289	0.096
B	Geraniol	0.129	0.145	0.339
C	Vanillin	0.129	0.048	0.048
S	Ethanol	0.614	0.517	0.517

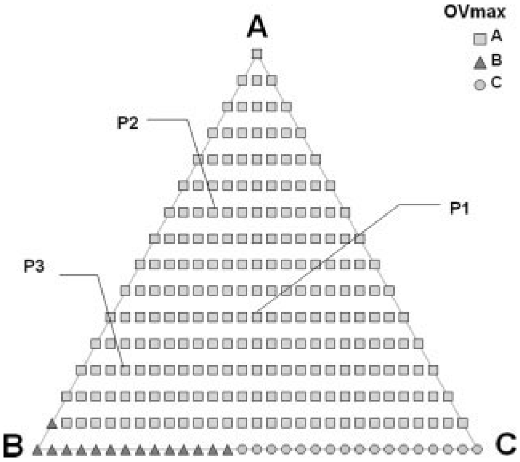

Figure 8. **Perfumery Ternary Diagram® and the composition points P1, P2, and P3 of a perfume test mixture with limonene (A), geraniol (B) and vanillin (C), in a basis without solvent ethanol (S), considering the nonideal case.**

is more retained in the solution than predicted and so the activity coefficient was overestimated. On the other hand, for more polar media, with compositions P2 and P3, the experimental OV value was higher than the simulated values, showing that limonene is being "pushed-out" from the liquid more than predicted. In these two cases, the activity coefficients were underestimated, especially at low concentrations of limonene (P3).

Considering geraniol, a medium polar component, the experimental OV values were slightly lower than the simulated OV values in all cases. The PTD® model overestimates the activity coefficients of geraniol, showing that in reality geraniol has a higher affinity towards the media than expected and so it is more retained in the liquid phase. Here, vanillin works as a fixative by decreasing geraniol headspace concentration (or OV value).

The opposite occurs for ethanol, the polar solvent used, which experimental OV value is higher than predicted by PTD® model, for all the compositions considered, being though closer to the simulated nonideal values. So the simulation underestimates the activity coefficients of ethanol, being the affinity of ethanol towards the media lower than predicted.

In the case of vanillin, a very polar component, the OV values obtained experimentally were quite different from the ones predicted by PTD® model. While the experimental OV values of the previous components differed from the OV simulated values in a range of 20–30%, in the case of vanillin the difference was 70% (P3) to 300% (P1). The experimental OV value was higher than simulated OV for high

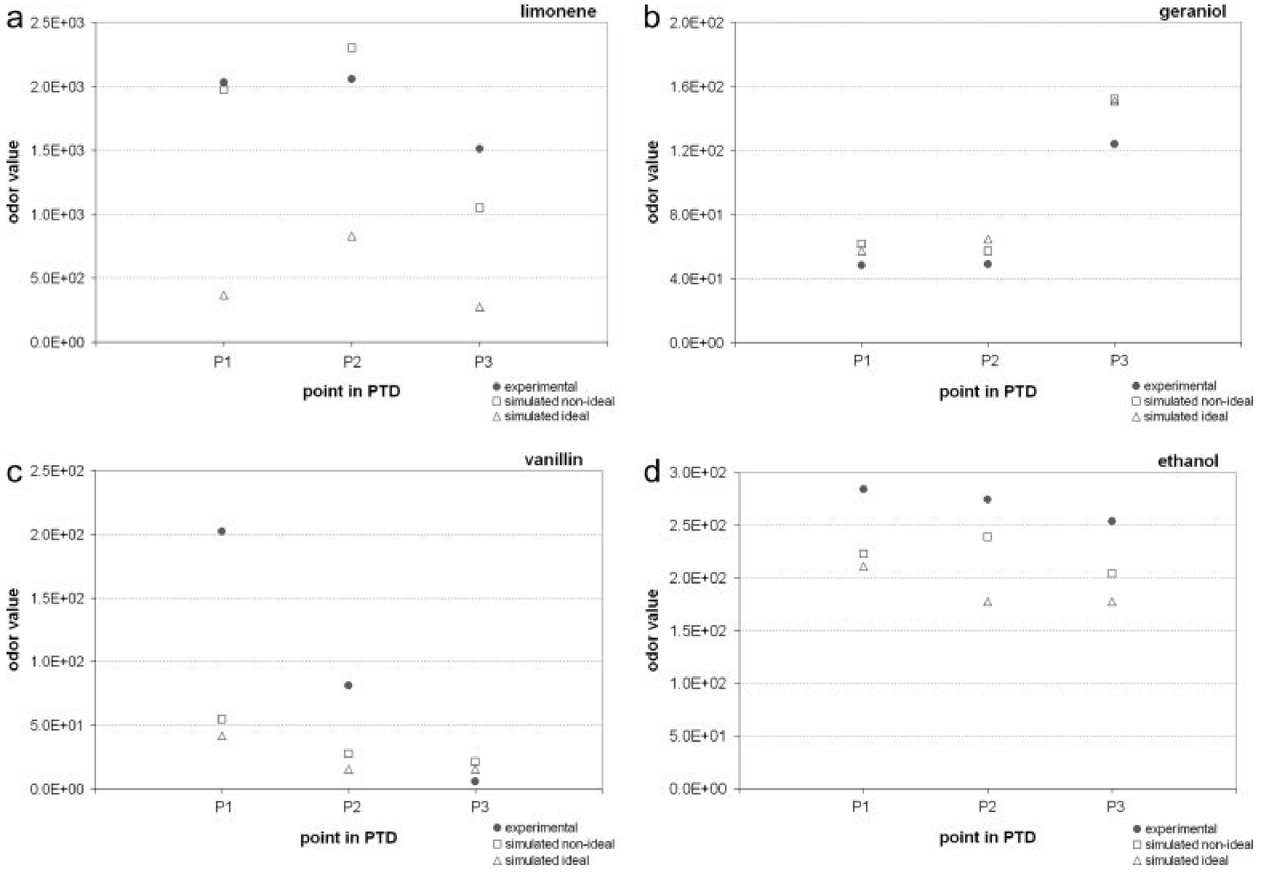

Figure 9. **Comparison of experimental and simulated odor values, at 25°C, obtained for the perfume test mixtures with limonene, geraniol, vanillin, and ethanol, for composition points P1, P2, and P3.**

The simulation was performed for ideal and nonideal case. The results are shown for each component: (a) limonene; (b) geraniol; (c) vanillin; (d) ethanol.

amounts of vanillin and limonene (P1 and P2), and lower for the smaller amount of limonene (P3). This means that the activity coefficients for vanillin were clearly underestimated in the case of P1 and P2 and overestimated in the case of P3. Considering compositions P1 and P2, the fact that the predicted values for non-ideal are lower than ideal case and in reality the experimental are higher than ideal case means that vanillin is actually being "pushed out" from the solution, instead of being retained as would be expected due to the affinity with geraniol. On the other hand, for composition P3, with low amount of limonene, the experimental OV values are smaller than simulated values, showing that in this case vanillin is more retained than predicted in the liquid phase.

It is apparent from these results that exists a special interaction limonene-vanillin, where the evaporation of vanillin is somehow enhanced in the presence of limonene, at relatively high concentrations (P1, P2). In fact, vanillin is one of the most difficult perfumery materials to work with, although it is one of the most important, being part of nearly every perfume.[2] In a perfume composition, at concentrations as low as 0.5% vanillin can overlay the odor of other components. Therefore, it is usually the last ingredient to add and the most effective concentration level is established by trials.

The reason for the differences found between simulated values and experimental results can be due to the special nature of the vanillin component. Another reason can be related to the UNIFAC model used to predict the molecular interactions and activity coefficients. In some cases, poor results are obtained using UNIFAC and so it is necessary to introduce new structural groups to improve the prediction of phase equilibrium, namely acetals, amides, lactones, aromatic esters and aldehydes, and many others.[42–44] Thereby, the PTD® model may be improved by introducing new structural groups, especially differentiating the cyclic from the linear structure, in order to better represent the complex nature of the fragrance materials.

Conclusions

Although the perfume is formulated in the liquid phase, it is the headspace concentration that will determine the smell of the composition. Our PTD® methodology predicts the smell of a perfume composition, based on the parameter OV, which in turn is determined based on measurable data, namely odor threshold values and headspace concentrations. In this work, PTD® model was validated by obtaining experimental data and then comparing the experimental OV values with OV values obtained through simulation.

It was concluded that the OV values were reasonably well predicted by PTD® model, except for vanillin. The UNIFAC model could be improved by introducing new structural groups that take into account the complexity of the fragrance molecules, which include cyclic components with many functional groups, or eventually use other thermodynamic models. Nevertheless, the methodology has proven to be useful in predicting OV values of perfume mixtures, giving some insight on the behavior of the components in solution, and how that affects the headspace composition and its smell.

Acknowledgments

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