

# Solvent screening for the purification of monoterpenoids by countercurrent and centrifugal partition chromatography

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## Abstract

**BACKGROUND:** Countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC) are efficient techniques to purify terpenoids from essential oils. These methods require suitable solvent systems for the partition between the two immiscible liquid phases. In this study, using the analytical shake-flask method, we measured the partition coefficients of three model monoterpenoids, namely carvone, eucalyptol and thymol, in 11 biphasic solvent systems, at 298.2 K. Moreover, the predictive COSMO-RS model was applied to represent the partition coefficients of data measured in this work and retrieved from the literature, the liquid–liquid equilibrium (LLE) data of three solvent families suitable for CCC/CPC separations (i.e., Arizona, Modified Arizona and Green Arizona) and the solute's partition coefficients in the three solvent families.

**RESULTS:** The partition coefficients obtained for thymol and eucalyptol are the first partition data available in the literature. The obtained root mean square deviations (RMSDs) between the experimental and predicted partition coefficients (log basis) varied between 0.28 and 0.49. For LLE, RMSDs of 0.040 and 0.048 were achieved for the Arizona and Green Arizona families, respectively.

**CONCLUSION:** The predictive COSMO-RS model describes the monoterpenoid partition coefficients and the LLE data of the solvent families well. The most favorable solvent systems to perform the separations were identified, showing that systems with low and intermediate-to-low polarities are the most promising options for separating the selected monoterpenoids from their natural matrices by CCC/CPC techniques.

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Supporting information may be found in the online version of this article.

**Keywords:** monoterpenoids; partition coefficient; COSMO-RS; solvent screening; countercurrent chromatography; partition chromatography

## INTRODUCTION

Terpenes and their oxygenated derivatives encompass a family of natural compounds.<sup>1</sup> These compounds have several biological functions and are widely present in plants, animals and microorganisms.<sup>2</sup> Current estimates are that about 100 000 terpenoids have already been identified.<sup>3</sup> Available in many types of biomass, terpenes are promising alternatives along with other feedstocks (e.g., carbohydrates, natural oils, lignin) to replace petrochemicals in many commercial products.

Monoterpenes and monoterpenoids represent one of the most important classes of the terpene family, composed of ten carbon atoms. Monoterpenes are hydrocarbons, while monoterpenoids consist of a wide range of oxygenated derivatives, including alcohols, ketones, ethers and phenolics.<sup>4</sup> With more than 2500 different structures already identified,<sup>4</sup> these compounds are the largest secondary plant metabolites.<sup>5</sup> Among their diverse potential applications, monoterpenes and monoterpenoids are widely used as fragrance and flavor additives in food and cosmetic products,<sup>6</sup> and as precursors

to developing biofuels,<sup>7</sup> fine chemicals,<sup>8</sup> pharmaceuticals<sup>5,9</sup> and pest management products.<sup>10</sup>

The major natural sources of monoterpenes and monoterpenoids are essential oils (EOs),<sup>5</sup> which are complex mixtures that can be synthesized by several plant organs, such as flowers, leaves, stems, seeds and fruits.<sup>11,12</sup> The fractionation and

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purification of EOs is a key step in obtaining high-added-value monoterpenes, since EO profiles may comprise more than 80% monoterpenes.<sup>13,14</sup> Among the available separation methods, scalable chromatographic techniques, such as countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC), have been applied to isolate monoterpenes from different EOs, including those obtained from anis,<sup>15</sup> clove,<sup>16</sup> ginger,<sup>17</sup> oregano,<sup>18</sup> perilla<sup>19</sup> and pepper<sup>20</sup> species. Unlike conventional chromatographic techniques with a solid stationary phase, CCC and CPC principles rely on the sample partition between two immiscible liquid phases: one that remains stationary, stabilized by centrifugal forces, and the other that is the mobile phase.<sup>21,22</sup> The benefits of using a liquid stationary phase instead of the conventional solid-liquid chromatographic techniques include higher loading capacity, total recovery of the injected sample, low solvent consumption and absence of sample adsorption.<sup>23</sup>

A key step when elaborating a protocol to isolate target compounds using CCC/CPC techniques is selecting the immiscible solvent system,<sup>24,25</sup> which might represent around 90% of the necessary work.<sup>26</sup> For a specific application, the selection of an appropriate biphasic solvent system in CCC/CPC would be equivalent to the simultaneous selection of the chromatographic media and liquid mobile phase in conventional liquid chromatography.<sup>27</sup> To achieve an efficient separation in countercurrent methods, the selected solvent system should be able to solubilize the target compounds<sup>28</sup> and offer good resolution and performance.<sup>24,27</sup> The solvent system polarity should match the target compounds to ensure good affinity, while solvent viscosity, interfacial tension, settling time and density difference between the two phases are relevant parameters for the performance of CCC/CPC separations.<sup>27,29</sup> However, one of the most important parameters to evaluate the suitability of a biphasic solvent system for a particular application is the target compound's partition coefficient ( $K$ ) between the immiscible phases. Ideally,  $K$  should range between 0.4 and 2.5 to avoid problems of resolution loss and excessive band broadening when operating CCC/CPC in the regular elution mode.<sup>30,31</sup>

Aqueous-organic biphasic systems composed of up to five components are often found in the literature as suitable immiscible phases for CCC/CPC separations, resulting in an almost limitless number of combinations.<sup>32,33</sup> Examples of multicomponent biphasic solvent systems commonly applied in CCC/CPC are mixtures of ethyl acetate-butanol-water (EBuWat),<sup>34</sup> hexane-ethyl acetate-methanol-water (HEMWat)<sup>31</sup> and heptane-ethyl acetate-methanol-water (Arizona).<sup>35</sup> In such systems, the composition of the constituents is varied to reach specific polarity and physicochemical properties. The EBuWat system, designed by Foucault,<sup>32</sup> is split into 11 different proportions of ethyl acetate-butanol-water, being indicated to isolate polar organic compounds, such as glycosylated flavonoids and phenolic acids.<sup>33,36</sup> On the other hand, the HEMWat family, also proposed by Foucault<sup>32</sup> and modified by Friesen and Pauli,<sup>31</sup> contains 17 different proportions, covering a polarity range from mid-range to lipophilic solvent systems.<sup>27</sup> This is probably the most popular solvent family used in countercurrent separation protocols to isolate natural compounds,<sup>27,33,37,38</sup> being the basis for formulating other multicomponent solvent families.<sup>33</sup> Nonetheless, using hexane and methanol in HEMWat raises some concern due to these compounds' high toxicity, which is partially overcome in the Arizona solvent family, where the less toxic heptane replaces hexane.<sup>33</sup> A more sustainable version of the Arizona system was proposed

by Faure *et al.*,<sup>39,40</sup> named Green Arizona, where methanol and heptane were replaced by ethanol and limonene, respectively. Compared to Original Arizona, the Green Arizona family presents more polar organic phases, and solvent systems with lower density differences between the phases.<sup>39,40</sup> The last might be an advantage in hydrostatic countercurrent columns or a severe drawback of hydrodynamic columns, which require a minimum phase-density difference to retain feasible amounts of the stationary liquid phase.<sup>39,40</sup>

Different strategies have been reported in the literature for solvent selection purposes in countercurrent separation methods.<sup>41</sup> Partitioning or shake-flask experiments are often employed for empirical solvent selection. In this approach, partition coefficients of the target compounds are measured in various solvent systems, aimed at identifying those presenting optimum values within the preferable polarity range (e.g.,  $0.4 \leq K \leq 2.5$ ).<sup>33,41</sup> The almost infinite number of possible solvent systems, however, might turn the empirical solvent selection into a labor-intensive and time-consuming task.<sup>33,42</sup> An alternative is applying rational procedures based on modeling strategies that have been applied to reduce the experimental efforts required in solvent selection.<sup>43,44</sup> Semi-predictive or predictive thermodynamic models such as the UNIFAC model,<sup>45,46</sup> NRTL-SAC model,<sup>47-49</sup> the Abraham solvation model<sup>42</sup> and the COSMO-RS model<sup>25,50-52</sup> have been used to correlate or estimate partition coefficients of selected solutes in different solvent systems, which are particularly useful in a solvent screening stage when no experimental data are available.

The main goal of this work was to evaluate the capabilities of the predictive COSMO-RS model<sup>53-55</sup> as a tool for screening biphasic solvent systems to be applied in CCC/CPC technologies to fractionate monoterpenoids from their natural matrices. Three monoterpenoids – carvone (ketone), eucalyptol (ether) and thymol (phenol) – that are commonly found in many EO profiles,<sup>56-61</sup> were selected as the model solutes. These compounds exhibit appealing pharmacological and therapeutical properties,<sup>62-67</sup> being promising substances to be explored by the pharmaceutical industry.

Due to the lack of experimental  $K$  values in the literature, the partition coefficients of the three solutes in five binary solvent systems (1-butanol-water, ethyl acetate-water, acetonitrile-heptane, methanol-heptane and methanol-limonene) and six quaternary solvent systems (Arizona N, Arizona T, Modified Arizona N, Modified Arizona T, Green Arizona N and Green Arizona T) were measured, at 298.2 K. The Modified Arizona N and T are less toxic versions of the Original Arizona N and T systems, in which the amounts of methanol are replaced by the equivalent amounts (volume basis) of ethanol. The Green Arizona N and T proposed by Faure *et al.*<sup>39,40</sup> have an additional modification, as both methanol and heptane are replaced by equal volumetric proportions of ethanol and limonene, respectively.

In this study, COSMO-RS was used to describe partition coefficient data measured in this work and collected from the literature, covering a wide solvent polarity range. The goal was to determine the best solvent systems for conventional CCC/CPC separations. The Original Arizona<sup>35</sup> and the Green Arizona<sup>39,40</sup> solvent system families were considered along with the proposed Modified Arizona family. For the Original Arizona and the Green Arizona families, the predicted compositions were compared with data available in the literature,<sup>39,68</sup> while no experimental values were found for the Modified Arizona systems.

## MATERIAL AND METHODS

### Chemicals

The structures, sources and mass purities of solvents and solutes are listed in Table 1. All the organic compounds were stored at room temperature and used as received from the supplier. Water of ultrapure quality (resistivity of 18.2 MΩ cm, free particles <0.22 μm and total organic carbon <5 μg dm<sup>-3</sup>) was used. Some physicochemical properties of the selected monoterpenoids are presented in Supporting Information, Table S1.

### Partition coefficient experiments

Partition coefficients were measured using the isothermal shake-flask method, following the methodology detailed in our previous work.<sup>51</sup> To ensure that the solute is at infinite dilution, it is recommended that solute concentration is lower than 0.01 mol L<sup>-1</sup> in both phases.<sup>69</sup> To do so, a sample of around 10 mg (±0.01 mg, ABT 100-5 M analytical balance, Kern, Frankfurt, Germany) of

the solute was dissolved in one of the solvents of the biphasic system before being transferred to an all-glass, graduated tube containing predefined volumes of the other solvents. Each tube contained a total volume of 14 mL.

The tubes were then manually inverted for 5 min (at least 100 times) to promote the distribution of the solutes in both phases, as recommended by Leo *et al.*<sup>70</sup> To reach equilibrium conditions, the pre-agitated tubes were placed in a thermostatic apparatus (ThermoMixer C, Eppendorf, Hamburg, Germany) operating at 298.2 ± 0.5 K, under continuous agitation (300 rpm), for at least 6 h, followed by a settling period of at least 15 h.

To determine the partition coefficients, samples were collected from each phase using all-glass syringes and diluted in ethanol-water, 50:50 wt% (thymol samples), or propanol (carvone and eucalyptol). The solute concentration in each sample was determined by UV-visible spectroscopy or gas chromatography. Further details on the quantification procedure are given in the

**Table 1.** Structures, CAS number, source and mass purity of the organic compounds used in this work

Compound	Structure	CAS number	Source	Mass purity (%) <sup>a</sup>
(S)-(+)-Carvone		2244-16-8	Acros Organics	≥98.0
Eucalyptol		470-82-6	Alfa Aesar	≥99.0
Thymol		89-83-8	Alfa Aesar	≥98.0
Methanol		67-56-1	JT Baker	≥99.9
Ethanol		64-17-5	Fisher	≥99.8
1-Butanol		71-36-3	Sigma-Aldrich	≥99.5
Acetonitrile		75-05-8	Fisher	≥99.9
Ethyl acetate		141-78-6	Carlo Erba	≥99.9
Heptane		142-82-5	Honeywell	≥99.0
(R)-(+)-Limonene		5989-27-5	Sigma-Aldrich	≥97.0

<sup>a</sup> The purity was obtained in the certificate of analysis issued by the manufacturer.

Supporting Information. At least two independent samples from each phase were analyzed for each tube.

## MODELING

### Framework

For a non-electrolyte organic solute  $i$ , the partition coefficient ( $K_i$ ), directly obtained from the ratio of the solute concentration in the upper phase ( $c_i^{u,\infty}$ ) and lower phase ( $c_i^{l,\infty}$ ), is also related to the activity coefficient at infinite dilution ( $\gamma_i^\infty$ ) by the following expressing obtained from the equilibrium thermodynamics condition<sup>47</sup>:

$$K_i = \frac{c_i^{u,\infty}}{c_i^{l,\infty}} = \frac{\gamma_i^{l,\infty} v_i^l}{\gamma_i^{u,\infty} v_i^u} \quad (1)$$

where  $v_i$  is the molar volume of species  $i$ , and the superscripts  $u$  and  $l$  stand for the immiscible upper and lower phases, respectively.

### COSMO-RS model

COSMO-RS<sup>44-46</sup> is an efficient model for predicting the thermodynamic properties of liquid mixtures,<sup>71</sup> and it has successfully been applied to describe partition coefficients of organic solutes in different biphasic solvent systems.<sup>72-78</sup> By combining a quantum chemistry-based treatment of the components with statistical thermodynamic calculations, the model can predict the activity coefficients of the solute in both phases of the solvent system and then calculate the partition coefficient through Eqn (1). Unlike group contribution and semi-predictive approaches that generally need fitting experimental data to estimate their parameters, COSMO-RS requires only information that is stored in so-called .cosmo files (i.e., optimized geometry, energy, electron density and polarization charge density) for each component to compute the partition coefficient values.<sup>79,80</sup>

COSMO-RS calculations were carried out using COSMOtherm software (version 21.0)<sup>55,81</sup> with the BP\_TZVPD\_FINE\_21.ctd level of parametrization. Further details on the calculation procedure are available in the COSMOtherm reference manual.<sup>82</sup> The required .cosmo files for water and most organic solvents were obtained from the COSMOtherm TZVPD-FINE database. For the monoterpenoids and solvents missing in the software database, COSMOconf 2021 software<sup>83</sup> coupled with TURBOMOLE V7.4,<sup>84</sup> using the BP-TZVPD-FINE-COSMO+GAS\_18 template, were applied to generate the .cosmo files. In these cases, all the obtained conformers generated in COSMOconf 2021 were considered to perform the calculations in COSMOtherm.

### Assessment of the deviations

The root mean square deviation (RMSD) was applied to quantify the deviations, for each solute  $K$ , between the experimental and predicted partition coefficients:

$$\text{RMSD}_K = \sqrt{\frac{\sum_i (\log K_i^{\text{exp}} - \log K_i^{\text{calc}})^2}{N}} \quad (2)$$

where  $N$  is the total number of data points considered in the dataset, the subscript  $i$  covers all the solvent systems, and the superscripts 'exp' and 'calc' mean experimental and calculated, respectively.

The deviations between the experimental and predicted liquid-liquid equilibrium (LLE) compositions for the Original, Modified, and Green Arizona solvent families were also assessed with the RMSD, described as follows:

$$\text{RMSD}_{\text{LLE}} = \sqrt{\frac{\sum_j \sum_i (x_{ij}^{\text{exp}} - x_{ij}^{\text{calc}})^2}{N}} \quad (3)$$

where  $x_{ij}$  is the mole fraction composition of component  $i$  in the solvent system  $j$ .

## RESULTS AND DISCUSSION

### Experimental partition coefficients

The experimental partition coefficients of carvone, eucalyptol and thymol in the biphasic solvent systems (1-butanol-water, ethyl acetate-water, acetonitrile-heptane, methanol-heptane, methanol-limonene, Arizona N, Arizona T, Modified Arizona N, Modified Arizona T, Green Arizona N, Green Arizona T) are given in Table 2, along with the main components present in the upper phases. The composition of the quaternary solvent systems (volume basis) is presented in Supporting Information, Table S2.

Low coefficients of variation inferior to 2.2% for carvone, 2.5% for eucalyptol and 4.7% for thymol were obtained. These values were calculated from at least four collected samples (two from each duplicate) for the upper and lower phases. Moreover, a comparison between the amount of solute placed in the tubes and obtained from the measured concentrations in each phase (i.e., material balance) confirms the consistency of the method, resulting in an average relative deviation of 2.5% and a maximum relative deviation of 4.9%.

The partition coefficients obtained in the binaries 1-butanol-water and ethyl acetate-water systems are much higher than unity, as expected from the mostly hydrophobic surface area of monoterpenoids.<sup>85</sup> On the other hand, the partition coefficients of carvone and thymol in the binary solvent systems with heptane were lower than unity, revealing that these solutes have a stronger affinity with the acetonitrile- or methanol-rich (lower) phase. In these solvent systems, the  $K$  values of thymol were lower than those obtained for carvone; this behavior was expected since the phenolic monoterpene is more polar (able to self and cross-associate), while the ketone has only one hydrogen-bond acceptor group. The partition coefficients measured for the more nonpolar monoterpene eucalyptol (an ether) in heptane-acetonitrile ( $K = 7.11$ ) and heptane-methanol ( $K = 0.961$ ) were higher than the values obtained for the other solutes.

In the case of the methanol/limonene system, the  $K$  values rank according to the polarity of the solutes: thymol > carvone > eucalyptol, which is the opposite order of that observed in the other organic binary solvent systems. In contrast to the heptane-methanol system, where the methanol-rich phase was at the bottom, the upper phase was richer in alcohol in methanol-limonene since methanol<sup>86</sup> has a lower density than limonene.<sup>87</sup> Thus, the trends observed in methanol-limonene are aligned with the other two organic binary solvents.

Regarding the quaternary solvent systems, the partition coefficients in all Arizona N systems (Original, Modified and Green) are higher than in the reciprocal Arizona T systems, probably due to the lower water content of the Arizona T systems compared to the Arizona N systems (i.e., the lower phase is less

**Table 2.** Experimental partition coefficients measured in this work, at 298.2 K<sup>a,b</sup>

Solvent system	Carvone	Eucalyptol	Thymol	Upper-phase main components
1-Butanol/water	97.5 ± 2.1	233 ± 2	256 ± 12	1-Butanol
Ethyl acetate/water	894 ± 8	320 ± 4	462 ± 1	Ethyl acetate
Heptane/acetonitrile	0.348 ± 0.006	7.11 ± 0.02	0.084 ± 0.001	Heptane
Heptane/methanol	0.427 ± 0.006	0.961 ± 0.007	0.127 ± 0.002	Heptane
Methanol/limonene	1.75 ± 0.01	0.626 ± 0.015	2.10 ± 0.02	Methanol
Arizona N	7.49 ± 0.15	9.67 ± 0.03	9.98 ± 0.01	Heptane and ethyl acetate
Arizona T	1.20 ± 0.01	3.54 ± 0.09	0.840 ± 0.026	Heptane
Modified Arizona N	3.36 ± 0.06	6.03 ± 0.10	6.66 ± 0.01	Heptane and ethyl acetate
Modified Arizona T	0.880 ± 0.012	2.68 ± 0.02	0.659 ± 0.005	Heptane
Green Arizona N	10.29 ± 0.05	12.48 ± 0.03	8.01 ± 0.18	Limonene and ethyl acetate
Green Arizona T	1.10 ± 0.01	7.24 ± 0.01	1.15 ± 0.02	Limonene

<sup>a</sup> Standard uncertainties for the data measured in this work:  $u(T) = 1.0$  K;  $u_r(p) = 0.05$ .

<sup>b</sup> Standard deviations are given after the '±' sign.

polar).<sup>39,68</sup> Moreover, replacing methanol (Original Arizona) with ethanol (Modified Arizona) leads to a decrease in the partition coefficients of the three solutes as, in general, all phases will be less polar. On the other hand, substituting heptane with limonene has the opposite effect in the systems with ethanol: the observed  $K$  values in the Green Arizona systems are higher than those obtained in the reciprocal Modified Arizona systems. Nevertheless, the results show that the less polar Arizona T systems (Original, Modified and Green) are more suitable to promote the separation of the studied terpenoids by CCC/CPC than the Arizona N systems, since most of the observed  $K$  values fall in the optimal separation polarity region ( $0.4 < K < 2.5$ ),<sup>31,34</sup> except for eucalyptol, where none of the studied quaternary solvent systems is within this range. For this solute, the less polar binary solvent systems methanol–limonene and heptane–methanol are more suitable to promote its purification by CCC/CPC methods.

The partition coefficients obtained in this work for carvone in the binary solvent systems and the Arizona N system are compared with literature data,<sup>88,89</sup> at  $298.2 \pm 1$  K, in Fig. 1. Data available at room temperature for the Arizona N system<sup>90,91</sup> were also included in Fig. 1 for comparison purposes. To the best of our knowledge, the partition coefficients of carvone in all Arizona systems (except original Arizona N) and the partition coefficients of eucalyptol and thymol in all solvent systems listed in Table 2 are reported for the first time in this work. For comparison purposes, the COSMO-RS predictions are included in Fig. 1.

The  $\log(K)$  values obtained in this work for carvone are in good agreement with the data reported by Marlot,<sup>89</sup> presenting absolute deviations inferior to 0.3 (a recommended value by OECD for data obtained by the shake-flask method)<sup>69</sup> for most solvent systems, except ethyl acetate–water. Besides, the data obtained for carvone in the original Arizona N system ( $\log(K) = 0.87 \pm 0.01$ ) is consistent with the average literature data ( $\log(K) = 0.68 \pm 0.19$ ).<sup>89–91</sup> Larger deviations are observed with the values reported by Janoschek *et al.*,<sup>88</sup> particularly for the 1-butanol–water solvent system, which is surprisingly low, suggesting that carvone has a stronger affinity with water than with alcohol. For most biphasic systems, the COSMO-RS predictions are consistent with the data obtained in this work and reported in the literature, presenting deviations within  $\pm 0.33$  log units. The only exception is observed for ethyl acetate–water, where, in the best scenario,

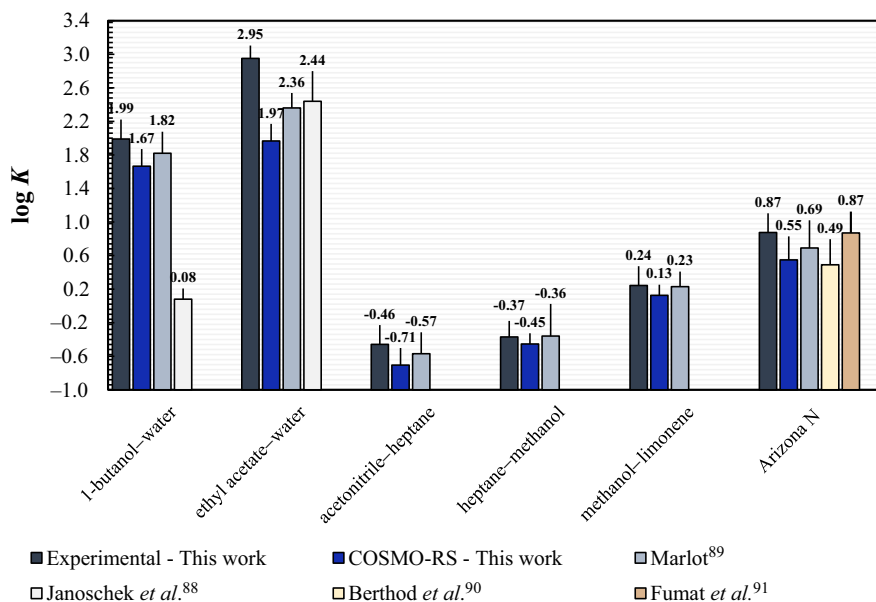
COSMO-RS underestimates the  $\log(K)$  value in 0.4 log units. The overall model predictions are discussed in the next section.

### Prediction of the partition coefficients with COSMO-RS

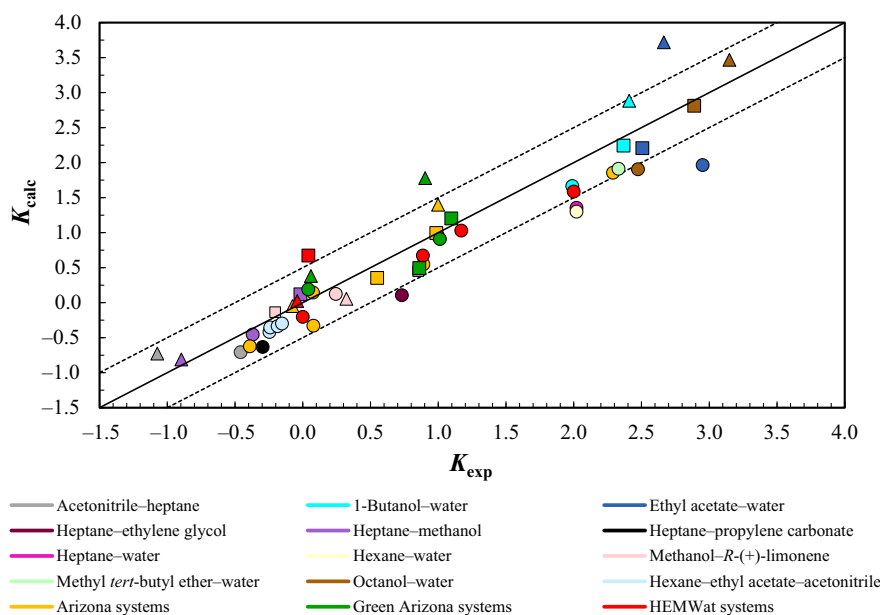
The partition coefficients predicted with COSMO-RS are compared with the data measured in this work and retrieved from the literature<sup>75,88–90,92–98</sup> (for other binary solvent systems) in Fig. 2. Since RMSDs of 0.5 for predicted  $\log(K)$  values have been considered acceptable,<sup>42,99</sup> dashed lines corresponding to  $\log(K) \pm 0.5$  are added in Fig. 2. The composition of the phases, required to perform the partition coefficient calculations in COSMO-RS, were also collected from the open literature.<sup>25,39,68,98,100–110</sup> The experimental LLE data for the HEMWat,<sup>25</sup> Arizona<sup>68</sup> and Green Arizona families<sup>39</sup> were measured at 295.2 K, and were used in the calculations as an approximation.

The results show that COSMO-RS adequately represents the partition coefficients of the monoterpenoids in the biphasic solvent systems, achieving RMSDs of 0.39 for carvone (27 data points), 0.28 for eucalyptol (11 data points) and 0.49 for thymol (11 data points). For 84% of the cases, the predicted values are distributed within the demarcated region in Fig. 2 ( $\log(K) \pm 0.5$ ), while the highest deviations are observed in ethyl acetate–water (RMSD = 0.78). A few other outliers are observed for carvone (octanol–water,<sup>75</sup> hexane–water,<sup>88</sup> heptane–water<sup>88</sup> and heptane–ethylene glycol<sup>97</sup>). For other solutes, only the predicted data for eucalyptol–HEMWat 0 (hexane–ethyl acetate–methanol–water, in 1/1/1/1 volumetric proportion)<sup>92</sup> and thymol–Green Arizona N are outside of the  $\log(K) \pm 0.5$  region.

It is worth mentioning that the RMSDs obtained in this work are comparable to the values reported in other studies that applied COSMO-RS to describe partition coefficients of organic solutes in biphasic systems.<sup>25,50,75,111</sup> Besides, the performance of COSMO-RS is similar to those provided by group contribution or semi-empirical models, such as UNIFAC,<sup>111</sup> QSAR,<sup>103</sup> NRTL-SAC<sup>51,112</sup> and the Abraham solvation model.<sup>42</sup> In contrast to these models, COSMO-RS presents a fully predictive character, a powerful tool to explore in preliminary solvent screening, particularly when no experimental data are available.



**Figure 1.** Comparison between the  $\log(K)$  data obtained in this work and found in the literature for carvone.



**Figure 2.** Comparison between the experimental and the predicted partition coefficients using the COSMO-RS model for carvone ( $\circ$ ), eucalyptol ( $\square$ ) and thymol ( $\triangle$ ). The dashed lines correspond to  $\log(K) \pm 0.5$ .

### Prediction of the LLE of solvent systems for CCC/CPC

Another potential contribution of COSMO-RS to solvent selection for CCC/CPC separations is the model's ability to estimate LLE data for multicomponent biphasic solvent systems.<sup>111,113-115</sup> Although experimental LLE data for some traditional solvent families (e.g., Original Arizona,<sup>68</sup> Green Arizona,<sup>39</sup> HEMWat<sup>25</sup>) have already been reported, searching for new solvent systems requires LLE data that might be unavailable, such as for the Modified Arizona family.

To assess the reliability of COSMO-RS in representing the LLE of quaternary solvent systems, the model was applied to predict the phase composition of the Original Arizona and Green Arizona

families. The predicted data were compared with experimental values reported by Garrard *et al.*<sup>68</sup> and Faure *et al.*<sup>39</sup> (both datasets at 295.2 K) in Supporting Information, Figs S1 and S2, respectively. In general, the model offers a good description of the LLE of the Original Arizona system, achieving a global RMSD of 0.040, which is slightly better than the results found by Frey *et al.*<sup>111</sup> (RMSD = 0.047). In their work, the authors also compared experimental LLE data with values predicted using COSMO-RS for other biphasic solvent systems (HEMWat, heptane-acetone-water, heptane-ethanol-water, cyclohexane-2-propanol-water), finding that the model's accuracy was sufficient for screening potential biphasic solvent systems for CCC/CPC separations.<sup>111</sup> For the

**Table 3.** COSMO-RS predicted solvent systems obeying the preferred operation region ( $-0.4 < \log(K) < 0.4$ )<sup>31,34</sup> for the studied monoterpenoids

Biphasic solvent system	Carvone	Eucalyptol	Thymol
Original Arizona	P–T	T–Z	S–U
Modified Arizona	P–U	S–W	R–U
Green Arizona N	R–W	U–W	T–W

Green Arizona family, an RMSD of 0.048 was obtained, which is slightly higher than the value achieved for the Original Arizona family. The global RMSD obtained for the Green Arizona family was comparable to the deviations reported by Frey *et al.*<sup>111</sup> for multicomponent biphasic solvent systems.

Considering the good performance of COSMO-RS in describing LLE data, the model was applied to predict the LLE data for the proposed Modified Arizona family, for which no data are available. In this solvent system, the global volumetric compositions of heptane–ethyl acetate–alcohol–water are the same as in the original Arizona family.<sup>68</sup> The global composition (volume and mole fraction basis) of the Original, Green and Modified Arizona solvent systems are presented in Supporting Information, Tables S3, S4 and S5, respectively. The estimated LLE data for the Modified Arizona solvent systems are compared to the experimental LLE for the Original Arizona system and the Green Arizona system in Supporting Information, Figs S3 and S4, respectively.

As shown in Supporting Information, Fig. S3, replacing methanol (from Original Arizona) with equal volumetric proportions of ethanol (Modified Arizona) leads to higher alcohol and water contents in the upper phase. In contrast, lower amounts of ethyl acetate and heptane are observed in the upper phase. In the lower phase, the opposite trends are observed for ethyl acetate, heptane and alcohol. The only exception is water, whose mole fraction compositions are higher in most Modified Arizona systems than in the reciprocal Original Arizona system. This behavior probably results from the higher global mole fraction composition of the water in Modified Arizona (Supporting Information, Table S5) than in the Original Arizona family (Supporting Information, Table S3).

When comparing the Modified Arizona and Green Arizona solvent families (Supporting Information, Fig. S4), it is evident that the limonene content in the upper phases of the Green Arizona systems is lower than that of heptane in reciprocal Modified Arizona's upper phases, while the opposite behavior is observed in the lower phases. Unlike heptane, due to its two double bonds, limonene might present  $\pi$ -polar interactions with polar solvents. Likewise, lower water contents are expected in the upper phases of the Modified Arizona solvent systems compared to those from the Green Arizona family with equivalent global hydrocarbon volumetric proportions, which is observed in most cases. Moreover, higher contents of ethyl acetate and ethanol are observed in the aqueous-rich phases of the Modified Arizona systems. In contrast, the opposite behavior is registered for ethanol in the upper phases.

### Prediction of monoterpenoid partition in the Arizona solvent families

To further investigate the predictive capability of COSMO-RS, the model was applied to describe the  $\log(K)$  of the studied monoterpenoids in the three versions of the Arizona solvent systems (Original, Modified and Green). The experimental LLE data

(measured at 295.2 K) was used for Original<sup>68</sup> and Green Arizona<sup>39</sup> solvent systems, whereas the predicted data with COSMO-RS, at 298.2 K, was considered for the Modified Arizona family. The results are depicted in Supporting Information, Fig. S5, while an overview of the solvent systems predicted with COSMO-RS that are distributed in the preferred operation range ( $-0.4 < \log(K) < 0.4$ )<sup>31,34</sup> is presented in Table 3. For visualization purposes, the preferred operation range is identified in a demarcated area in Supporting Information, Fig. S5.

In general, the  $\log(K)$  values decrease as the polarity of the system decreases (i.e., the content of the hydrocarbon increases) in the three analyzed solvent families. Although COSMO-RS captures this trend for all solutes, high deviations are observed for the most polar system (Arizona A or ethyl acetate–water), where the thymol partition coefficient is overestimated and the  $K$  values of carvone and eucalyptol are underestimated. In most cases, the available experimental  $\log(K)$  values confirm the COSMO-RS results presented in Table 3 (73% of cases), with some exceptions (eucalyptol–Arizona T, carvone–Arizona Y, and carvone–Arizona Z).

In the case of eucalyptol, the Original Arizona family presents the highest number of solvent systems distributed in the preferred CCC/CPC operating region (seven systems), followed by Modified Arizona (five systems) and Green Arizona (three systems), respectively. For carvone, the Modified Arizona presents six solvent systems in favorable polarity region – one more than the other two families. In contrast, the Modified and Green Arizona families exhibit the highest number of potential options (four each) for thymol – one more than the Original Arizona solvent system. The results suggest that the last solvent systems from the Green Arizona series (i.e., with the lowest polarity) are the most appropriate options from this family to separate the studied monoterpenoids by CCC/CPC. The same behavior is observed for eucalyptol in the other two solvent families. On the other hand, systems with intermediate-to-low polarity from these two families of solvents are the most suitable alternatives to purifying carvone and thymol using partition-based chromatographic techniques.

## CONCLUSIONS

This work reports experimental partition coefficients of carvone, eucalyptol and thymol in 11 biphasic solvent systems. To the extent of our knowledge, the data measured for carvone in the Modified and Green Arizona N and T systems and all data obtained for eucalyptol and thymol are reported here for the first time. Besides, the new partition coefficient values obtained for carvone in the biphasic solvent systems and Arizona N are in good agreement with most data available in the literature, with a few exceptions for the 1-butanol–water and Arizona N systems.

COSMO-RS delivered a very good description of the partition coefficients, achieving a global RMSD (log basis) of 0.37. For 84% of the analyzed cases, the predicted  $\log(K)$  values presented absolute deviations lower than 0.5 log units compared to the

experimental data, which is a very satisfactory performance for a fully predictive model. Moreover, COSMO-RS delivered a good representation of LLE data for the Original Arizona and Green Arizona solvent families, with a global RMSD of 0.040 and 0.048, respectively. Thus, the model was also employed to estimate the phase compositions of the proposed Modified Arizona solvent systems.

Lastly, COSMO-RS predicted the  $\log(K)$  profiles of the solutes in the Arizona, Modified Arizona and Green Arizona families, showing that solvent systems with low and intermediate-to-low polarities are the most promising options for separating the selected monoterpenoids from their natural matrices by CCC/CPC techniques. The experimental results confirm that the COSMO-RS predictions are more reliable in the more apolar biphasic systems.

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## DATA AVAILABILITY STATEMENT

All data are either provided in the paper or supplementary file.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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