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Assessing the hydrotropic effect in the presence of electrolytes: competition between solute salting-out and salt-induced hydrotrope aggregation

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ABSTRACT

Water solubility enhancement is a long-standing challenge in a multitude of chemistry-related fields. Hydrotropy is a simple and efficient method to improve the solubility of hydrophobic molecules in aqueous media. However, the mechanism behind this phenomenon remains controversial. Herein the impact of salt doping on the hydrotropy phenomenon is determined experimentally using the ionic liquid (IL) 1-butyl-3-methylimidazolium chloride ([C₄mim]Cl) as hydrotrope and vanillin as solute. Hydrophobic interactions were found to be central to the aggregation of the hydrotrope around the solute, which seem to drive hydrotropy. Furthermore, ¹H-NMR analysis indicates that hydrotrope-solute interactions present a degree of site-specificity. The addition of chloride salts in the presence of higher IL concentrations promotes a greater relative decrease of the vanillin solubility than in the corresponding system without IL. This was assigned to the negative impact of increased hydrotrope pre-aggregation in the presence of inorganic salts. The results were rationalised using statistical thermodynamic through which hydrotrope aggregation prior to solute addition is shown to be detrimental to the hydrotropic effect, seemingly confirming solute-induced clustering of the hydrotrope to be the predominant mechanism of hydrotropy.
INTRODUCTION

Improving the water solubility of sparingly water-soluble compounds has been a challenge in several chemistry-related fields. This physicochemical parameter greatly determines the bioavailability of a drug, and thus its therapeutic effectiveness.\textsuperscript{1,2} It is also crucial in several industrial processes, with emphasis on solvent chemistry.\textsuperscript{3} Hydrotropy has established itself as a promising method for water-solubility improvement due to its simplicity, compatibility with a wide pH range and ease of solute recovery through aqueous dilution.\textsuperscript{4} Hydrotropes are currently applied in a broad range of chemical industrial processes and pharmaceutical drug development.\textsuperscript{5–8} Hydrotropes can be ionic or neutral molecules and typically present an amphiphilic nature and high water solubility.\textsuperscript{4} However, the hydrophobic moiety of hydrotropes are shorter than those of surfactants, preventing their spontaneous self-assembly in aqueous solutions into organised structures such as micelles, and stressing the difference between hydrotropic and micellar solubilisation. In recent years, ionic liquids (ILs) have been proposed as a novel class of ionic hydrotropes, where both anion and cation can contribute to the hydrotropic solubilization if properly selected, and show promise in the drug solubilisation field.\textsuperscript{9–11} Combining the advantages of hydrotropy with the tuneable properties intrinsic to ILs, research on this class of compounds presents itself as a very promising approach towards water-solubility improvement.

The driving mechanism of hydrotropy remains a subject of extensive debate from which three main explanations arose: (a) the self-aggregation of hydrotrope molecules; (b) the disruptive effect of hydrotropes on the water structure; and (c) the formation of complexes between hydrotrope and solute molecules. The latter was initially proposed with on the basis of $\pi$–$\pi$ stacking interactions that lead to aggregate formation, but the later identification of hydrotropic effects being induced by non-aromatic and non-charged molecules showed it to be inappropriate.\textsuperscript{12,13} More recently, an alternative co-aggregative model has been proposed and complemented with statistical thermodynamics descriptions of hydrotropy.\textsuperscript{14–18} These describe the hydrotrope aggregation around the solute as pivotal to the phenomenon, driven by strong hydrophobic interactions between the hydrotrope and the solute. As the interactions between the hydrophobic moieties and water are weaker than the hydrogen bonding involved in water self-association, these moieties are led to associate between themselves, originating aggregation of the hydrotrope around the solute. The strength of this model, arising from its basis on statistical thermodynamics, is only challenged by the limited experimental evidence to support it.\textsuperscript{19}

The introduction of salt ions can alter the physical properties of molecules, hydrotropes included, in aqueous solutions by increasing the amount of overall possible interactions between the system
components provoking changes in solubility.\textsuperscript{20–23} The addition of electrolytes to an aqueous solution containing a solute is macroscopically characterised by the salting-in (increased solubility) or salting-out (decreased solubility) of the salt introduction on the solute and can be arranged according to the Hofmeister series.\textsuperscript{24} This series was initially explained based on the ability of ions to increase or decrease the water structuring in a solution. A given salt is said to provide a salting-out effect in relation to a certain solute if the solute-salt ion interaction is less favourable than both solute-water and salt ion-water interactions, and a salting-in effect in the case of stronger solute-salt ion interaction.\textsuperscript{25,26} Several works have studied the effect of salts in IL solubility in aqueous solutions, and found consistency with the Hofmeister series and with the entropy of hydration of the ions.\textsuperscript{27–30} However, more recently, the Hofmeister series is portrayed as not so universal, effectively showing the existence of a spectrum between direct, reverse, and altered versions of it.\textsuperscript{31} Furthermore, IL aggregation in aqueous media is dependent on its interaction with water.\textsuperscript{32} Therefore, the addition of electrolytes in the form of inorganic salts can influence this interaction and affect the self-aggregation rate and aggregate size, ultimately influencing the solubilization power of IL hydrotropes. Despite this, there are few works systematically addressing the influence of a salt series on the hydrotropic effect.

There is yet insufficient knowledge in both the hydrotropic properties of ILs as well as how the salting of hydrotropic solutions of ILs influences the solubility of hydrophobic compounds. The study of this subject could translate into a better understanding of the hydrotropic solubilization mechanism, of the IL behaviour in heavily electrostatic charged environments, and in the design of novel applications for ILs as solvent media and hydrotropes. In this work different techniques are employed to further the understanding of the characteristics of hydrotrope-solute interactions and to establish the effect of inorganic cations on the behaviour of hydrotropic solutions doped with their chloride salts. Vanillin, a phenolic compound with natural antioxidant and anti-inflammatory properties was chosen as the solute.\textsuperscript{33–36} The IL 1-butyl-3-methylimidazolium chloride (\([\text{C}_4\text{mim}]\text{Cl}\)) was selected as the hydrotrope as its properties are extensively characterised and is a known hydrotrope for vanillin.\textsuperscript{9} Matching the IL anion with the anion of the chosen salts to dope IL solutions, allowed to focus on the study of the effect of the salt cations on the hydrotropic effect. The solubility of vanillin in aqueous solutions of \([\text{C}_4\text{mim}]\text{Cl}\) containing different chloride salts is determined and the local environment of the solute and hydrotrope, both with and without the presence of additional salts, is characterised.
EXPERIMENTAL

Materials

The IL 1-butyl-3-methylimidazolium ([C₄mim]Cl, 99.0 wt.% purity) was purchased from IoLiTec, LiCl (99.0 wt.%) from Merck, NaCl (99.5 wt.%) from Fisher Scientific, KCl (99.5 wt.%) from Chem-Lab NV, anhydrous CaCl₂ (≥ 95.0 wt.%) from Panreac and all other chemicals including YCl₃·6H₂O (99.9 wt.%) and vanillin (99.0 wt.%) as well as deuterium oxide (99.96% D atoms) and 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt (TSP, 98 mol % D atoms) used in Nuclear Magnetic Resonance (NMR) analyses were acquired from Sigma-Aldrich. All chemicals were used as received without further purification. Ultrapure, double distilled water, passed through a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus, (18.2 MΩ.cm at 25 °C) was used for all experiments.

Hydrotropic solubility curves

The solubility curves of the solutes in the hydrotropic systems studied were experimentally determined using the isothermal shake-flask method. First, water/hydrotrope/inorganic salt solutions were prepared for two fixed [C₄mim]Cl concentrations of 0.5 mol.kg⁻¹ and 1.5 mol.kg⁻¹ to which were added various concentrations of individual inorganic chloride salts up their respective solubility limit in water. To these solutions, excess vanillin solute was added and the samples were left to equilibrate under constant agitation (1050 rpm) at 303.2 ± 0.5 K using an Eppendorf Thermomixer Comfort apparatus. Vanillin was added whenever necessary as to keep all solutions saturated over time. The procedure was stopped after no further vanillin dissolution was observed for 48 h. All solubility tests were performed in triplicate. After equilibration the samples were centrifuged at (303.2± 0.5) K at (4500 rpm) for 20 minutes, using a Hettich Mikro 120 centrifuge. Finally, samples of the liquid phase were carefully collected and appropriately diluted by a minimum factor of 2000 prior to vanillin quantification by UV spectrophotometry with a SHIMADZU UV-1700 Pharma-Spec spectrometer at a wavelength of 280 nm. The absorbance of an aqueous solution containing an equivalent [C₄mim]Cl concentration was subtracted from the sample spectra to control for the IL during vanillin quantification. Prior knowledge of the hydrotropic behaviour of [C₄mim]Cl in the tested conditions guaranteed that the limiting factor for the maximum experimental concentration of each salt was its own solubility in water, and that no phase separation was to be observed, allowing saturation of all tested solutions with vanillin to occur without impediment. An identical protocol was followed to determine the solubility of vanillin in inorganic salt and water solutions.
Probing hydro trope-solute interactions

$^1$H-NMR spectroscopy analysis was performed to provide insight on the changes of the chemical environment of the IL and vanillin promoted by the addition of salt, and on the interactions between the IL and vanillin, that is between the hydrotrope and the solute. Measurements were performed on a Bruker Avance 300 NMR spectrometer operating at 300 MHz, at 298 K. Samples were prepared as previously described for the hydrotropy solubility tests. They were analysed in a glass NMR tube with an added sealed capillary in a coaxial insert containing D$_2$O and trimethylsilylpropanoic acid as an internal reference. The formation of IL-vanillin aggregates was followed by dynamic light scattering (DLS) using a Zetasizer Nano-ZS photometer from Malvern Instruments. Prior to the analyses, all samples were filtered through a nylon 0.45 μm syringe, sonicated in an ultrasonic bath for 5 minutes to promote particle dispersion and degassing and left to rest in a sealed quartz cuvette overnight. DLS analyses were carried at 293.15 K, with an equilibration time of 120 seconds. Irradiation was performed at a wavelength of 565 nm by a helium-neon laser and scattered light was detected at a backscattering angle of 173°. The automatic mode was used for both measurements and data processing and was corrected for the viscosity (1.315 and 1.730 mPa.s) and refractive index (1.348 and 1.366) of both 0.5 M and 1.5 M aqueous IL solutions respectively. Analyses were performed in triplicate and extracted results are averages.

RESULTS AND DISCUSSION

Hydrotropic solubility measurements

In the following discussion the vanillin solubility in g.L$^{-1}$ is denoted by $S$ and the associated subscript indicates the aqueous system composition such that $S_{IL}$, $S_{Salt}$ and $S_{IL+Salt}$ corresponds to the solubility of vanillin in water containing the IL, an inorganic salt or a mixture of IL and inorganic salt respectively. Two [C$_4$mim]Cl concentrations were selected from the dilute region of the vanillin solubility curve previously reported to assess the influence of inorganic salts at these conditions.$^{11}$ The tested IL concentrations correspond to 0.5 M (9.0 wt. %) and 1.5 M (26.0 wt. %). The solubility curves of vanillin for 0.5 M and 1.5 M IL in the presence of mono-salt solutions are presented in Figures 1A and 1B respectively. The vanillin solubility data for each respective salt system is presented in Table S1-S4 of the ESI. The vanillin solubility values obtained in this work for both [C$_4$mim]Cl concentrations in the absence of salt, corresponding to the initial point in Figure 1A and 1B, are 43.2 and 279.8 g.L$^{-1}$ respectively. Chloride salts of varying valency from LiCl to YCl$_3$ were investigated, thereby eliminating the possibility of anion exchange. Furthermore, the investigated salts present limited coordination...
with chloride in aqueous media, avoiding the extensive formation of multiple chloro-complexes. The aqueous properties of the investigated salt cations relevant to the establishment of a salting-out trend and correlation with the Hofmeister series are summarised in Table 1. Table 1 includes bulk solution properties (ionic radius \(r\), molar entropy of hydration \(\Delta_{\text{hyd}}S\)) as well as more specific ones such as the ionic surface tension increment \(k\).\(^{21,30,38}\) Each system was investigated until the appearance of an observable precipitate in the absence of solute; the more dilute IL solution of 0.5 M allowed for a larger range of tested salt concentrations prior to precipitation. For comparative purposes, the solubility curves for vanillin in aqueous solutions of LiCl, NaCl, CaCl\(_2\) and YCl\(_3\) of varying molality were determined and shown in Figure 1C.

**Figure 1.** Aqueous vanillin solubility for starting [C\(_4\)mim]Cl concentrations of A) 0.5 M and B) 1.5 M in the presence of individual salts. Dashed lined correspond to the aqueous vanillin solubility of 13.1 g.L\(^{-1}\) at 303.2 K.\(^{11}\) C) Aqueous vanillin solubility as a function of the concentration (solute-free basis) of inorganic chloride salts in the absence of [C\(_4\)mim]Cl. Error bars represent the standard deviation from three independent measures. All measurements were performed at \(T = 303.2\) K.
Table 1. Thermodynamic parameters of the tested salt cations at 298.15 K. \( r \) denotes the ionic radius, \( \Delta_{\text{hyd}} \dot{S}^o \) the standard molar entropy of hydration, and \( k_i \) the ionic surface tension increment of aqueous ions. All values taken from Marcus et al.\textsuperscript{39,40} \( K_{\text{CH}_4} \) are the Setschenow coefficients for methane in aqueous solutions of alkali chloride at 298.15 K. (\( a \) - values for the La\textsuperscript{3+} ion; \( b \) – values for the chloride salt calculated from ref \textsuperscript{41}).

<table>
<thead>
<tr>
<th>Cation</th>
<th>( r ) (nm)</th>
<th>( \Delta_{\text{hyd}} \dot{S}^o ) (J.K(^{-1}).mol(^{-1}))</th>
<th>( k_i ) (mN.m(^{-1}).mol(^{-1}).L)</th>
<th>( K_{\text{CH}_4} ) (kg.mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li\textsuperscript{+}</td>
<td>0.069</td>
<td>-142</td>
<td>0.95</td>
<td>-0.112</td>
</tr>
<tr>
<td>Na\textsuperscript{+}</td>
<td>0.102</td>
<td>-111</td>
<td>1.20</td>
<td>-0.151</td>
</tr>
<tr>
<td>K\textsuperscript{+}</td>
<td>0.138</td>
<td>-74</td>
<td>1.10</td>
<td>-0.128</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}</td>
<td>0.100</td>
<td>-252</td>
<td>2.10</td>
<td>-0.246</td>
</tr>
<tr>
<td>Y\textsuperscript{3+}</td>
<td>0.090</td>
<td>-483</td>
<td>3.20\textsuperscript{a}</td>
<td>-0.334\textsuperscript{a}</td>
</tr>
</tbody>
</table>

The solubility of vanillin in aqueous \([\text{C}_4\text{mim}]\text{Cl}\) solutions consistently decreased in the presence of all inorganic salts, with the solubility curves at both \([\text{C}_4\text{mim}]\text{Cl}\) concentrations (Figure 1A-B) for each respective salt presenting a generally similar qualitative behaviour with small differences addressed further on. The addition of inorganic chloride salts to IL solutions decreased \( S_{\text{IL+Salt}} \) in line with the lower reported aqueous solubilities of phenolic compounds – including vanillin – in the presence of electrolytes.\textsuperscript{21–23,41} Even the addition of dilute salt concentration yields a non-negligible decrease in vanillin solubility. This could be of relevance as human body fluids contain approximately \([\text{NaCl}] \approx 0.15\) mol.L\(^{-1}\) and its impact must be considered when designing hydrotropic formulations. The extent of this decrease is salt-dependent and approximately related to the charge density of the salt cation. At a salt concentration of 1.0 M, the apparent trend is Y\textsuperscript{3+} > Ca\textsuperscript{2+} > Na\textsuperscript{+} > Li\textsuperscript{+} > K\textsuperscript{+} for 0.5 M IL, and (Y\textsuperscript{3+}) = Ca\textsuperscript{2+} > Li\textsuperscript{+} > Na\textsuperscript{+} \approx K\textsuperscript{+} for 1.5 M IL ranging from the salt cation most detrimental to vanillin solubility to the one the least detrimental. These trends hold for low salt concentrations; the greater solubility of LiCl allowed the investigation of concentrated salt systems and shows the presence of a vanillin solubility plateau for \([\text{LiCl}] \geq 4.0\) M. In fact, the \( S_{\text{IL+Salt}} \) values at the saturation concentration of LiCl, CaCl\(_2\) and YCl\(_3\) tend to be similar (Table S1), casting doubt on the predictability of solute solubility decrease based on the salt \( \Delta_{\text{hyd}} \dot{S} \) in concentrated electrolyte solutions. Similar behaviour was observed in the binary system of salt and vanillin at higher salt concentrations, Figure 1C.

To facilitate the comparison between salts, the Setschenow constant (\( K_{\text{IL+Salt}} \)) was adapted to treat the hydrotrope+solute+salt system for a fixed IL concentration as a pseudo-binary one by considering the solubility of vanillin in aqueous IL solution without salt as the reference concentration (\( S_\text{IL} \)):...
\[
\log \left( \frac{S_{\text{IL+Salt}}}{S_{\text{IL}}} \right) = K_{\text{IL+Salt}} C_{\text{Salt}}
\]

where \( S \) is the saturated vanillin concentration in the system (g.L\(^{-1}\)), \( C_{\text{Salt}} \) is the inorganic salt concentration (mol.kg\(^{-1}\)) and the subscript IL+Salt and IL correspond to the systems in the presence and absence of additional inorganic salts. The adapted Setschenow constant for each ternary IL+vanillin+salt was obtained from least-squares linear regressions of the plot \( \log(S_{\text{IL+salt}}/S_{\text{IL}}) \) against \( C_{\text{Salt}} \) for both IL concentrations, with the obtained \( K_{\text{IL+salt}} \) summarised in Table 2.

**Table 2.** Setschenow coefficients for the binary vanillin + salt systems (\( K_{\text{Salt}} \)) and ternary IL + vanillin + salt systems (\( K_{\text{IL+Salt}} \)) as well as the salt concentration range used to determine these ([\( C_{\text{Salt}} \)]).

<table>
<thead>
<tr>
<th>Salt</th>
<th>[C(_4)mim]Cl = 0.0 M</th>
<th>[C(_4)mim]Cl = 0.5 M</th>
<th>[C(_4)mim]Cl = 1.5 M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_{\text{Salt}} )</td>
<td>( K_{\text{IL+Salt}} )</td>
<td>( K_{\text{IL+Salt}} )</td>
</tr>
<tr>
<td>Li(^+)</td>
<td>-0.148</td>
<td>0.0-4.0</td>
<td>-0.101</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>-0.187</td>
<td>0.0-3.0</td>
<td>-0.120</td>
</tr>
<tr>
<td>K(^+)</td>
<td>-</td>
<td>-</td>
<td>-0.067</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>-0.228</td>
<td>0.0-2.0</td>
<td>-0.198</td>
</tr>
<tr>
<td>Y(^{3+})</td>
<td>-0.316</td>
<td>0.0-1.0</td>
<td>-0.270</td>
</tr>
</tbody>
</table>

The magnitude to which each salt decreases vanillin solubility should be dictated by the salting-out potential of each of the added salts and would be expected to follow the traditional Hofmeister series according to the sequence Y\(^{3+}\) > Ca\(^{2+}\) > Li\(^+\) > Na\(^+\) > K\(^+\). Attempts to establish a correlation between the obtained \( K_{\text{IL+salt}} \) values the various cation parameters previously related to the salting-out effect\(^{38,42}\) held best at lower ionic strengths as shown in Figure 2. The best correlation was obtained using the ionic surface tension increment (\( k_i \)) compared to standard hydration properties, as the formers reflect the lower-than-expected salting-out of Li\(^+\) relative to Na\(^+\).\(^{38,42}\) This is consistent with the work of Freire et al.\(^{43}\) in which the change in aqueous solubility of the bistriflimide IL [C\(_4\)mim][Tf\(_2\)N] in the presence of inorganic salts was also related to their surface tension as salting-out can be best understood as interfacial phenomena between water and the hydrophobic surface of an organic solute.
Figure 2. A) Setschenow coefficients and B) solubility decrease of the ternary systems relative to the binary solute-hydrotrope system as a function of the aqueous surface tension increment ($k_i$) of the inorganic salt cation for two IL concentrations. The $k_i$ value of La$^{3+}$ was used as a proxy for Y$^{3+}$, see Table 1.

Experimental Setschenow coefficients for the salt+vanillin ($K_{Salt}$) systems listed in Table 2 compare favorably to those predicted using the linear free energy of model of Burant et al.$^{21}$ ($K_{LiCl} = -0.116$, $K_{NaCl} = -0.164$ and $K_{CaCl_2} = -0.241$ kg.mol$^{-1}$) based on the octanol-water partition of vanillin as input, albeit slightly greater for the monovalent cations. Comparison of the $K_{Salt}$ to those of the ternary $K_{IL+Salt}$ in Table 2 for the higher valency cations Ca$^{2+}$ and Y$^{3+}$ presents a contrasting trend of the salt effect with the IL concentration. For 0.5 M IL, a decrease in the salting-out effect of Ca$^{2+}$ or Y$^{3+}$ towards vanillin is observed as $K_{Salt} > K_{IL+Salt}$ whilst the opposite is observed when the IL concentration is increased to 1.5 M. This trend is unexpected as in mixed salt solutions, the Setschenow coefficient is regarded to be additive based on the mole fraction of the salt in the system.$^{21}$ Considering the value of $K_{IL}$ of 1.017 kg.mol$^{-1}$ obtained from Figure 1A (concentration range of 0.0 to 0.3 mol.kg$^{-1}$), one should expect a higher $K_{IL+Salt}$ at 1.5M IL relative to 0.5 M in contradiction with the experimentally obtained trend in Table 2 for Ca$^{2+}$ and Y$^{3+}$ where $K_{IL+Salt}$ at 0.5 M IL > $K_{IL+Salt}$ at 1.5 M IL. Interestingly, for the weaker salting-out cations Li$^+$, Na$^+$ and K$^+$, $K_{Salt}$ is more negative than $K_{IL+Salt}$ regardless of the IL concentration. The
latter does not appear to impact the Setschenow coefficient as it, for a given monovalent cation, varies negligibly with IL concentration contrary to that observed for Ca$^{2+}$ and Y$^{3+}$. This suggests that [C$_4$ mim]Cl remains an efficient hydrotrope for vanillin in the presence of weak salting-out salts. More importantly, the system behaviour in the presence of stronger salting-out compounds is indicative of antagonistic effects driven by the increased hydrotrope-hydrotrope interaction and contradicts the theory proposing that hydrotropy is driven by the self-aggregation of hydrotrope molecules (as will be discussed below).

**Hydrotrope-solute interactions**

Towards a further understanding of the intermolecular interactions at play in the hydrotropic solubilization of vanillin, NMR and DLS analyses were performed on several combinations of IL/salt/vanillin solutions. The aim of the present NMR analyses is to take a closer look at which interactions effectively are at play, namely which moieties of the hydrotrope and solute interact and how they interact. A “nesting doll” approach is taken for the analysis in which the $^1$H-NMR shift ($\Delta \delta$) for a system is presented relative to the same system minus one component. For example, the shifts in a ternary system of IL + vanillin + salt are presented relative to those in the binary IL + vanillin system at same IL concentration. All systems were saturated in vanillin, implying that the final solute concentration will differ between systems and must be considered. Only the systems containing 1.5 M [C$_4$ mim]Cl were studied as no significant concentration effect was observed by $^1$H-NMR for dilute IL solution below 1.9 M (35.0 wt.%), Figure S1, suggesting that the presented conclusions in the following sections are transferable to the 0.5 M IL systems. The $^1$H-NMR shifts for the IL and vanillin are available in Table S5 and S6 respectively of the ESI. The binary system consisting of an aqueous IL solution saturated with vanillin, without added salt, was first considered, with the $\Delta \delta$ for the hydrotrope and solute presented in Figures 3A and 3B respectively.
Figure 3. A) Proton chemical shifts ($\Delta \delta_\text{H}$) of the IL cation in a 1.5 M solution of [C$_4$ mim]Cl in water, saturated with vanillin, relative to an aqueous 1.5 M IL solution with no solute. B) Vanillin molecule in saturated aqueous 1.5 M IL solution relative to vanillin dissolved in water to saturation.

In the case of both hydrotrope and solute, a clear site-specificity in the locus of interaction is observed manifested by the different $\Delta \delta_\text{H}$ values for each proton. A negative $\Delta \delta_\text{H}$ indicates that the immediate proton environment is more shielded as water is replaced from its coordination sphere by a more apolar group. There is a clear distinction between the change in environment of the IL ring with a delocalised charge and the more hydrophobic butyl chain in the presence of a solute. Whilst the smaller $\Delta \delta_\text{H}$ of the ring protons suggests these remain primarily hydrated, the addition of vanillin provided a significant shielding for all hydrogens of the hydrocarbon tail and suggests a preferred tail-solute interaction over a ring-solute one. When comparing vanillin dissolved to saturation in 1.5 M IL aqueous solution to vanillin in water (Figure 3B), all solute hydrogens are more shielded in the presence of hydrotrope. This effect is especially pronounced for the aromatic hydrogens H2 and H3 of the ring, see Figure 3B for hydrogen labelling. In contrast, the hydrogens closest to the more hydrophilic hydroxyl (H4), aldehyde (H1) and methoxy (H5) groups present the smallest $\Delta \delta_\text{H}$ as these groups are less prone to interact with the hydrotrope. The $\Delta \delta_\text{H}$ in Figure 3 suggests that the significant increased vanillin solubility in 1.5 M IL solutions occurs through the displacement of water molecules
around the aromatic ring of the solute by the alkyl chain segment of the hydrotrope, its most hydrophobic sites, thus revealing favoured hydrophobic hydrotrope-solute interactions. This is consistent with the emergence of aggregates with an average hydrodynamic diameter of 2.01 nm, as measured by DLS (Figure S2), whilst no aggregates were observed in aqueous [C₄mim]Cl solution (detection limit of 0.70 nm). This evidenced hydrophobic interaction is in support of the hydrotropy model proposed by Shimizu et al.¹⁴ The hydrotropic solubilisation mechanism is induced by the accumulation of the hydrotrope around the solute and the formation of hydrotrope-solute aggregates where the apolar moieties are associated while the polar moieties are kept mostly in contact with water. The ¹H-NMR results in Figure 3 confirm that the interaction between an hydrotrope and solute is site-specific, as significant interaction site selectivity is observed. This provides a partial explanation as to the higher solubilisation selectivity of hydrotropes when compared with surfactants and reinforces the difference between the two categories.

**Hydrotrope interaction in presence of a salt**

Aqueous solutions of 1.5 M IL doped with a fixed concentration of 1.0 M of individual salts without the vanillin solute were investigated by NMR and DLS with the results presented in Figure 4. Due to the similar qualitative behaviour of the ¹H-NMR shifts for the IL cation hydrogens the discussion will focus on the Δδᵢ of the alkyl tail terminal CH₃ group although, as observed in Figure 3A, the cationic butyl chain always tends to have a less electronegative environment than the imidazolium ring. The addition of an inorganic salt resulted in a shielding of all hydrogens relative to the aqueous IL solution. The magnitude of the induced Δδᵢ follows the sequence Y³⁺ > Ca²⁺ > Na⁺ > Li⁺ and correlates well with the salting-out extent of each salt represented by their respective surface tension increment, Figure 4A. More importantly, a comparison of the induced Δδᵢ and the average IL aggregate diameter in each salt solution in Figure 4B confirms that Δδᵢ is indicative of enhanced IL-IL interaction. The DLS profiles for the 1.5 M IL + 1.0 M salt solutions are presented in Figure S3 with the average hydrodynamic diameter increasing from Li⁺ (0.82 nm) < Na⁺ (0.93 nm) < Ca²⁺ (1.09 nm) < Y³⁺ (1.48 nm). It must be emphasised that these aggregates were polydisperse and do not correspond to any organised structures such as micelles.⁴⁴ Ultimately, the increased IL-IL interaction and dehydration in the presence of chloride salts in the absence of solute negatively affects its hydrotropic capacity as reflected by the decreasing KᵢL-Salt at 1.5 M IL with Δδᵢ shown in Figure 4C. This further accentuates that the decrease in vanillin solubility in the ternary system is partially determined by the aggregation (salting-out) of the IL component and not solely of the solute as could be expected.
Figure 4. Correlation between the average $\Delta \delta_H$ of an aqueous 1.5 M IL after and before the addition of 1.0 M of chloride salt (no solute) to A) the surface tension increment of each salt, B) diameter of the IL aggregates in solution and C) the $K_{IL+Salt}$ of the respective salt at 1.5 M IL.

Hydrotrope-solute interactions in the presence of a salt

Finally, 1.5 M [C$_4$ mim]Cl solutions containing 1.0 M of NaCl, CaCl$_2$, or YCl$_3$ and saturated with vanillin were investigated, with the $\Delta \delta_H$ relative to the salt solutions without solute presented in Figure 5A. No clear trends emerge in relation to the salt valency, with the magnitude of $\Delta \delta_H$ in Figure 5A indicating an inverse cation organizational series of Ca$^{2+}$ > Na$^+$ > Y$^{3+}$ for the IL imidazolium head hydrogens and Ca$^{2+}$ > Y$^{3+}$ > Na$^+$ for the methyl and butyl groups hydrogens in the ternary system with solute relative to the IL + salt system in Figure 4. This inversion in tendency between the binary and ternary system suggests that the formation of more apolar aggregates is primarily determined by the salting-out influence in the case of the higher charge density cation Y$^{3+}$. In contrast, the increased hydrogen shielding of the IL in the 1.5M IL + vanillin + 1.0 M CaCl$_2$, and to a lesser extent the NaCl system, can be attributed to the introduction of vanillin and not to the salt. This observation reinforces that hydrotropic solubilisation occurs preferentially through a tail-solute hydrophobic interaction over a ring-solute one. The site-specificity in the immediate environment of the IL cation is preserved in the ternary systems containing Ca$^{2+}$ and Y$^{3+}$, with the butyl chain hydrogens presenting more negative $\Delta \delta_H$ values when compared to the imidazolium head. In contrast, the ternary system containing Na$^+$ presents an inverse tendency with $\Delta \delta_H$ values with the exception of the H(4) hydrogens which appear as outliers. The NaCl salt induces shielding in every hydrogen of the IL cation. This is in sharp contrast to the specificity observed in the binary system of IL and solute in the absence of inorganic salts shown in Figure 3A although the justification as to this is unclear.
Figure 5. A) $\Delta \delta_H$ of the $[C_4\text{mim}]^+$ cationic hydrogen in the ternary system of IL + vanillin + salt relative to the binary IL + salt system for a fixed IL and salt concentration of 1.5 and 1.0 M respectively. Peak labelling corresponds to those in Figure 3A. B) Aggregate size distribution by intensity obtained by DLS in 1.5 M $[C_4\text{mim}]\text{Cl}$ aqueous solutions saturated with vanillin and doped with 1.0 M of inorganic chloride salts. The same solutions in Figure 5A were analysed by DLS to detect eventual aggregate formation with the diameters presented in Figure 5B. The results support the link between decreased solubility via salt addition and the formation of aggregates of increasing size. The smallest aggregate diameters were obtained for the binary IL+vanillin system (distribution centred at 2.01 nm) whilst the ternary IL+vanillin+$Y\text{Cl}_3$ system yielded a large aggregate distribution with a maximum at approximately 10.16 nm. Aggregates in the LiCl, NaCl and CaCl$_2$ systems present a partially overlapping distribution with the distribution maximum shifting from 2.48 nm for LiCl, 3.05 nm for NaCl and 3.91 nm for CaCl$_2$. The formation of consistently bigger aggregates along the Li$^+$ < Na$^+$ < Ca$^{2+}$ < $Y^{3+}$ series results from increases in the hydrotrope-hydrotrope interactions. With this promoted IL aggregation there are fewer IL cations available to take part in the hydrotropic solubilization of vanillin, which justifies the decreased solubility observed in the hydrotropic solubility measurements presented earlier. The smaller aggregate size in aqueous IL solutions doped with salt in contrast with the aggregates observed in the present analysis reinforces the understanding that the presence of solute is essential as a nucleation point for significant aggregation to happen around it, and thus to the hydrotropic effect.

Statistical thermodynamic rationalisation

The Setschenow constant is linked to the Kirkwood-Buff integrals (KBI) through a series of pair-wise interaction parameters.$^{45,46}$ In this section and the associated Appendix, the notation was modified
from that used in equation (1) to be consistent with previous derivations and generalise the discussion to any hydrotrope-salt-solute system. For a solute (S) in equilibrium with its pure phase and at any concentration, rigorous statistical thermodynamic theory of hydrotropy by Shimizu and colleagues can be extended to express the hydrotropic solubilization in the presence of salts and account for the hydrotrope (H), salt (A), and water (W) present in the system according to the equation below:

\[
kT \left( \frac{\partial \ln c_S}{\partial c_H} \right)_{T,P,c,A,W} = c_H \left( G_{S,H} - G_{S,W} \right) - \frac{c_H c_A \left( G_{S,A} - G_{S,W} \right) \left( G_{H,A} - G_{A,W} \right)}{1 + c_A \left( G_{H,A} - G_{A,W} \right)} \frac{\partial \mu_S}{\partial c_H} \bigg|_{T,P,c,A,W} = \mu_S^\circ - \mu_S^0 - \frac{c_A}{c_A + \frac{\partial \mu_A}{\partial c_A}} \left( G_{A,A} - G_{A,W} \right)
\]  

(2)

where \( G_{i,j} \) represents the KBI between the component \( i \) and \( j \), \( \mu_i \) is the chemical potential of the species \( i \) and \( c_i = \langle N_i \rangle / V \) is the bulk number density of the species \( i \), respectively. A detailed derivation of equation (2) is available in the Appendix.

A qualitative rationalisation of the experimental results is proposed below based on the change in the individual term contribution of equation (2) upon salt addition. Statistical thermodynamics shows that hydrotropic solubilization is mainly driven by solute-hydrotrope preferential interactions relative to solute-water, making \( (G_{S,H} - G_{S,W}) > 0 \) the primary contribution for solubilisation. Any loss in site-specificity, i.e., the loss of the prevalent hydrophobic hydrotrope-solute interactions in Figure 3, driven by changes in the hydrotrope or salt concentrations can decrease this term. In the present work, a third component is introduced in a hydrotropic solution/solute system. Let us look at the impact of each term on Eq. (2):

(a) The \( (G_{S,A} - G_{S,W}) \) factor: The unfavourable solute-salt interaction implies that \( (G_{S,A} - G_{S,W}) < 0 \) and reduces solubilisation as shown in Figure 1C and is dependent on the salt concentration. Note that only the chloride anion was assessed, and the above statement should be revisited in the presence of salts with salting-in anions.

(b) The \( (G_{H,A} - G_{A,W}) \) factor: Salt-water interaction is preferential to hydrotrope-salt interaction as a consequence of hydrotrope-hydrotrope interaction as clearly shown by the results in Figure 4, making \( (G_{H,A} - G_{A,W}) < 0 \).

(c) The \( 1 + c_A (G_{A,A} - G_{A,W}) \) factor: The self-association of salt preferential to salt-water association causes the deviation from 1 of the denominator but does not affect the sign of the second term in the bracket.
(d) The factor: This factor is positive as expected when considering the binary IL+vanillin system solubility curve\(^{11}\) but reduced by the hydrotrope self-association enhanced by the addition of salt.

From this analysis, two aspects contribute to the reduction of hydrotropic solubilization due to hydrotrope self-association enhanced by the addition of salt: (i) the negative overall second term in the bracket in equation (2) as can be concluded from (a)-(d) and (ii) the decrease in the term.

**CONCLUSIONS**

In this work, the solubility of vanillin in the aqueous solutions of the hydrotrope [C\(_4\)mim]Cl in the presence of inorganic chloride salts of varying valency was investigated. It was found that the addition of salt resulted in a reduced vanillin solubility, this reduction correlating with the surface tension increment induced by the salt. The results presented support the mechanism of hydrotropy proposed by Shimizu and colleagues based on statistical thermodynamics, with hydrotropic solubilization found to be linked with hydrotrope and solute aggregation and driven mainly by hydrophobic interactions.

Site specificity of hydrotrope-solute interactions was evidenced and seen to be dampened by the addition of salt. The addition of inorganic salts promotes IL-IL interaction even in the absence of vanillin and ultimately the formation of larger solute-hydrotrope aggregates. The formation of IL-IL aggregates hindered vanillin solubility, suggesting that pre-clustering of the hydrotrope is not beneficial towards hydrotropy. The particularities of solubilization behaviour in salted environments may have interesting and creative biological applications, since different in vivo locations possess different electrolyte environments. Only the cationic component was evaluated in this work that must be extended in a future work to the anionic contribution as the latter are known to exert a greater influence on the salting-in/out of the system.

**APPENDIX**

Under constant temperature and pressure, the chemical potential of a solute, \(\mu_s^*\), whose centre-of-mass is fixed at origin, can be expressed as
\[-d\mu^*_S = \sum_{i = W,H,A,S} \left( \langle N_i \rangle_S - \langle N_i \rangle \right) d\mu_i \]

where $\mu_i$ is the chemical potential of the species $i$. $\langle N_i \rangle_S$ and $\langle N_i \rangle$ express the ensemble average of the numbers of the species $i$ in the presence and absence of a fixed solute, respectively. The notation adopted here is consistent with “Statistical thermodynamic rationalisation” section of this paper. Using the definition of the Kirkwood-Buff integral,

\[c_i G_{S,i} = \langle N_i \rangle_S - \langle N_i \rangle \tag{A.2}\]

Using Eq. (A.2), Eq. (A.1) is rewritten as

\[-d\mu^*_S = \sum_{i = W,H,A,S} c_i G_{S,i} d\mu_i \tag{A.3}\]

where $c_i = \langle N_i \rangle / V$ is the bulk number density of the species $i$.

To express how the solvation free energy of a solute, $\mu^*_S$, is affected by the addition of a hydrotrope, the Gibbs-Duhem equation under constant $T$ and $P$ is used:

\[0 = \sum_{i = W,H,A,S} c_i d\mu_i \tag{A.4}\]

Eliminating $d\mu_W$ from Eq. (A.3) using Eq. (A.4) yields

\[-d\mu^*_S = \sum_{i = H,A,S} c_i (G_{S,i} - G_{S,W}) d\mu_i \tag{A.5}\]

We then consider an equilibrium between a solute in its pure phase $\mu^*_0$ and in solution $\mu_S$. Since $\mu^*_0$ only depends on $T$ and $P$, $\mu_S = \mu^*_0$ is a constant. Therefore,

\[d\mu_S = 0 \tag{A.6}\]

Because of Eq. (A.6), the solute-solute KBI (i.e., the last term of Eq. (A.5)) does not affect solubilization. Note that the solute-solute correlations affect $d\mu^*_S$ indirectly by modifying $G_{S,W}$, $G_{S,H}$ and $G_{S,A}$. We also use the well-known relationship between $\mu^*_S$ and $\mu_S$:

\[d\mu_S = d\mu^*_S + kT d\ln c_S \tag{A.7}\]

Combining Eqs. (A.5)–(A.7), the following relationship is obtained:

\[kT d\ln c_S = c_H (G_{S,H} - G_{S,W}) d\mu_H + c_A (G_{S,A} - G_{S,W}) d\mu_A \tag{A.8}\]

Here Eq. (2) of the main article is derived, i.e., solubilization by hydrotrope under a constant salt concentration. Differentiating Eq. (A.8) with respect to $c_H$ under constant $c_A$ yields

\[kT \left( \frac{\partial \ln c_S}{\partial c_H} \right)_{T,c_A P, \mu_S = \mu^*_0} = c_H (G_{S,H} - G_{S,W}) \left( \frac{\partial \mu_H}{\partial c_H} \right)_{T,c_A P, \mu_S = \mu^*_0} + c_A (G_{S,A} - G_{S,W}) \left( \frac{\partial \mu_A}{\partial c_H} \right)_{T,c_A P, \mu_S = \mu^*_0} \tag{A.9}\]
We again use Eqs. (A.1) and (A.3), this time with a fixed $A$ instead of a fixed $S$, to eliminate $\frac{\partial \mu_A}{\partial c_H}$. Using Eq. (A.7) for a fixed $A$, eliminating $\mu_A$ using Eq. (A.4), and under phase equilibrium (Eq. (A.6)), we obtain

$$d_{CA} \frac{c_A}{kT} = \left[ 1 + c_A(G_{AA} - G_{AW}) \right] d\mu_A + c_H(G_{HA} - G_{HW}) d\mu_H$$

(A.10)

Under constant $c_A$, Eq. (A.10) leads to

$$0 = \left[ 1 + c_A(G_{AA} - G_{AW}) \right] \left( \frac{\partial \mu_A}{\partial c_H} \right)_{T,P,c,H,S} + c_H(G_{HA} - G_{HW}) \left( \frac{\partial \mu_H}{\partial c_H} \right)_{T,P,c,H,S}$$

(A.11)

Combining Eqs. (A.9) and (A.11) leads to Eq. (2) of the main text.

**Conflicts of interest**

There are no conflicts to declare.

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