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Cold-Spray Ionization Mass Spectrometry of the Choline Chloride-Urea Deep Eutectic Solvent (Reline)

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Abstract

Cold-Spray Ionization mass spectrometry (CSI-MS) that can be compared to an electrospray ionization (ESI) source acting with a nebulizing gas cooled by liquid nitrogen, is used for analysing reline as Deep Eutectic Solvent (DES). The association of cholinium chloride salts with urea molecules is evidenced in negative CSI-MS through the chloride adduct formation. The structure of the supramolecular assemblies forming the reline ions that are observed on CSI mass spectra, is rationalized by chemical quantum calculation. The theoretical studies indicate that the ionic network organisation is only supported by a maximization of hydrogen bonds of the chlorides with the hydroxyl and methyl moieties of the cholinium cations and the amino groups of urea. The studies of gas-phase fragmentation of the supra-molecular ionic assemblies detected in CSI-MS are performed using the in-source collision induced dissociation experiments. The experimental measurements in CSI-MS, interpreted at the light

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of the molecular modelisation results suggest that the insertion of urea in adducts of chlorides with cholinium cations do not lead to the most stable ions.

Keywords: Cold-Spray Ionization, Deep Eutectic Solvent, Reline, Chemical Quantum Calculation, In-Source CID.

1 Introduction

Deep eutectic solvents (DESs) can be defined either as low-melting-temperature mixture with at least one salt as component or as a mixture of Lewis or Brønsted acids and bases that can contain a variety of species yielding an eutectic with a melting point much lower than each of components. This new generation of green solvents, described for the first time by Abbott *et al.* in 2003,¹ are known for their low toxicity, non-volatility and low production cost. Their organization is based on associations between a hydrogen bond acceptor (HBA) such as an ammonium halide and an hydrogen-bond donor (HBD).^{2,3} The possibility to substitute organic solvents and ionic liquids by DESs has been described in various application fields of chemistry such as extraction, synthesis, catalysis, carbon dioxide capture or electrochemistry.³⁻¹³ In order to better rationalize the DES molecular organization, theoretical and experimental studies have been performed by focussing on reline so-known as archetypical DES. Reline is a blend of choline chloride salt (ChCl) and urea molecule (U) (see Scheme 1) in molar ratio 1:2. The use of spectroscopic methods (RMN and IR),¹⁴⁻¹⁷ the analysis of Inelastic Neutron Scattering (INS) spectra together with the results of *ab initio* quantum chemical calculations and molecular dynamic simulations, have allowed to propose two main organization modes.¹⁸⁻²³ The associations of the reline constituents are then described through the optimization of the hydrogen bonds as the urea molecules get into the H-bond network initially formed by the interactions between the cholinium cations and the chloride anions. The HBD properties of the cholinium are due either to its hydroxyl moieties and the C-H bonds of their methyl groups while chloride is the HBA compound. In this model, the urea contributes to the DES organization first by disrupting this pre-existent network, then by maximizing the hydrogen bonds through the HBD character of their amino groups.^{17,19,21,22} Another organization mode proposes that ionic interactions could participate to the non-covalent stable complex assembly.¹⁸ In this case, the DES organization is ensured by the HBD character of urea molecule interacting with a chloride anion whereas the free rotor hydroxyl group of a choline maintains a strong hydrogen bond with this chloride whilst maximizing its interaction with urea

through Coulomb attractions between its ammonium and the carbonyl oxygen of urea. All these spectroscopic measurements and the associated theoretical studies, suggest that the H-bonds or the charge delocalization could be at the origin of a sandwich structure where each chloride would be in interaction with one choline cation and two urea molecules. This behavior described through analyses realized on DES models in condensed phase, should be completed by studies on the intrinsic stability of the basic structure assumed as being at the origin of the DES organization.

In this context, for attempting to better understand DES molecular organization, we have applied the Cold-Spray Ionization (CSI) Mass Spectrometry (MS) to reline analysis, together with quantum mechanical calculations to investigate in detail the interactions involved in the non-covalent associations disclosed by the interpretation of CSI mass spectra. Cold-spray ionization also called as cryo-spray is a low-temperature variant of electrospray ionization (ESI) that was developed for characterizing labile non-covalent complexes that cannot be observed by conventional mass spectrometry techniques.^{24,25} The CSI method can be described as an ESI source where the sprayer is a probe submitted to a coaxial nebulizing gas cooled by a liquid nitrogen device maintaining the temperature of the capillary below -30 °C.²⁶ In CSI, the shrinkage and the fissions of charged droplets are not thermally assisted in the desolvation chamber as in ESI. The ionization mechanism and the desolvation process are rather ensured by an increasing of the medium dielectric polarization (P) at low temperature, where: $P = \epsilon_r(\epsilon_0 - 1)E$, with ϵ_r is the relative permittivity and the strong electric field (E) at the surface of the charged micro-droplets and ϵ_0 is the vacuum permittivity.²⁵ This increased polarizability of the solvent molecules induced by the high values of ϵ_r at low temperature favours charge separations in the solution emerging from the capillary tip and leads to observe free-solvent gaseous “brittle” ionic species.²⁷ The CSI method has been namely used for characterizing the structures of labile inorganic and organometallic compounds in solution or investigating labile solution structures of biomolecules such as DNA.²⁸⁻³¹ The use of CSI-MS analysis in coordination chemistry allows for discriminating between no-covalent complexes in solution whether they are discrete or oligomeric coordination species.³²⁻³⁴ It has also been shown that CSI mass spectrometry as NMR spectroscopy and X-ray crystallography, allows to study the formation of large-scale aggregated chain structures or clusters in solution through intermolecular hydrogen bonds.³⁵

To our knowledge, the cold-spray ionization mass spectrometry has never been used to disclose no-covalent structures that can characterize a DES such as reline. The new experimental data

that are obtained from this work have been interpreted to the light of theoretical studies in order to complete the data already compiled from other spectroscopic methods and to bring additional criterions of rationalization of the DES organization.

2 Experimental

2.1 Chemical

The chemicals used for the preparation of the DES, choline chloride ($\geq 99\%$) and urea ($\geq 99.5\%$) were supplied by Acros Organics and used without further purification. UPLC/MS grade acetonitrile was purchased from Biosolve Chimie (Dieuze, France).

2.2 Preparation

Deep eutectic solvent reline was prepared by mixing choline chloride and urea in a molar ration 1:2 at 80°C under stirring for 4h until an homogeneous and transparent liquid was obtained. The DES was allowed to cool at room temperature and stored. The DES solution was diluted in pure acetonitrile at 0.5 % (v:v) and stored at room temperature for the ESI and CSI analyses.

2.3 Mass Spectrometry

Cold-Spray Ionization (CSI) and Electrospray Ionization (ESI) mass spectra were acquired using an AccuTOF CS mass spectrometer (JEOL, Tokyo, Japan). The scheme depicted in Figure 1, illustrates the different items of this dual ESI/CSI source. The needle voltage was set at -1.5 kV. Nitrogen was used as nebulizer and dry gas at flow rates of 1.0 and 2.0 L/min, respectively. The nebulizer gas temperature was set between - 40 and 0°C depending of the performed CSI-MS experiments with a desolvation chamber that does not exceed 30°C . For conventional ESI-MS, the desolvation chamber temperature was set at 230°C with a nebulizer gas that was introduced at room temperature. Unless indication mentioned in the text or in the figure captions, the voltages of the Orifices 1 and 2 lenses were generally set at 30 and -5 V, respectively. The Ring Lens voltage was set at -15V. The bias voltage of the Ion Guide has been set at -29 V. The Ion Guide peak voltage was fixed at 1100 V. The detector voltage was set at 2300 V. It can be noted that the value read for the Orifice 2 voltage, corresponds in fact to the potential difference, so-called OR2 in Figure 1, between this lens and the bias voltage applied on the Ion Guide. Then, the value read for the Orifice 1 voltage, represents the potential difference, so-called OR1 in Figure 1, between this item and the voltage applied on Orifice 2. Endly, the voltage value of the Ring Lens, so-called RL in Figure 1, is the potential difference

between this one and the Orifice 1. The mass spectra were recorded every second in the m/z range 50 to 1200 and with a resolution of 6000 (fwhm definition). The mass drift compensation procedure available on the main program that controls the AccuTOF CS, was used for compensating for the m/z drift in the range of m/z 100 - 1100 and performing the accurate mass measurements. The ion at m/z 174.0452 corresponding to the chloride adduct of the choline chloride dimer was selected as internal standard in negative electrospray and the ion at m/z 155.0336 corresponding to the chloride adduct of the urea dimer was selected as internal standard in negative cold-spray.

2.4 Chemical quantum calculations

Stationary points were optimized with the Gaussian 09 program³⁶ using the B3LYP³⁷ density functional and a 6-311++G(d,p) basis set. Dispersion corrections have been included via the D3-Grimme correction.³⁸ This procedure is consistent with the previous work of Hunt *et al.*²¹ on similar clusters but of smaller size. All reported structures were confirmed as energy minima, with no negative eigenvalue in the Hessian matrix. Initial structures were either hand constructed or taken from molecular dynamics simulations. We simulated a box containing 100 choline chloride:urea (1:2) pairs performed with AMBER18³⁹ and the GAFF v2.11 FF⁴⁰ simulated during 100 ns. Typical arrangements with the various selected m/z ratio were extracted from this simulation. Interaction energies (ΔE) within the different clusters were calculated as the difference between the total energy of the considered aggregate and the isolated components. Relative interaction energy for a specific conformation X (ΔE_{rel}) is the difference, for a given m/z , between the energy of X and the most stable one.

3 Results and discussion

3.1 Cold-Spray Ionization Mass Spectrometry of reline

The negative ion ESI and CSI mass spectra of a reline solution in acetonitrile, are reported in Figure 2a and 2b, respectively. The accurate mass measurement results and their comparison with theoretical masses calculated from elemental compositions (see Table 1) indicate that all the signals of Figure 2a correspond to chloride anion adducts with cholinium chloride units, *i.e.* $[(\text{ChCl})_n + \text{Cl}]^-$ where ChCl is the cholinium chloride depicted in Scheme 1 and $n = 2$ to 7. The only ion population observed on the ESI(-) mass spectrum of Figure 2a, is formed by the association of a chloride with an increasing number of cholinium chloride salt.

These results show that the ESI(-)-MS analysis do not lead for characterizing reline as a choline chloride and urea mixture, even though, in a first approximation, the species detected on the ESI mass spectrum are often characterized as the most stable assemblies emerging from charged micro-droplets.^{41,42} By contrast, the CSI mass spectrum of Figure 2b depicts supplementary signals that disclose the presence of urea molecules in interaction with cholinium chloride salts.

This assignment is confirmed by the attributions established by accurate mass measurements and reported in Table 2. The more labile species observed in CSI-MS (see Figure 2b and Table 2), are thus rather characteristic of reline since they are formed of two cholinium chloride (ChCl) as salt and urea (U) as molecule. The low temperature of the nebulizing gas of the CSI source (-30°C for the mass spectrum of Figure 2b) promotes the observation of such labile non-covalent complexes that cannot be detected in conventional ESI-MS.

More precisely, among the most abundant ions observed in CSI (see Figure 2b), the three ions m/z 155, m/z 234 and m/z 373, are not detected in ESI (see Figure 2a). The m/z 155 ion is the base peak in CSI-MS and corresponds to a chloride adduct with two urea molecules (see Table 2). This ion $[(U)_2+Cl]^-$ is even more abundant than the cholinium chloride ions $[(ChCl)_n+Cl]^-$ where $n = 1$ to 7, these ones are the only ionic species emerging from the electrosprayed solution (see Figure 2a and Table 1). From their elemental composition reported in Table 2, the m/z 234 and 373 ions are associations of one urea molecule (U) with one and two cholinium chloride units (ChCl) into a chloride adduct, respectively. Note that the signals of these ions noted $[(ChCl)_n+U+Cl]^-$ where $n = 1$ or 2, are many more intense than the m/z 294 ion $[(ChCl)_n+(U)_m+Cl]^-$ where $n = 1$ and $m = 2$, whereas this association of two urea molecules with one cholinium chloride has been previously pointed out as the putative basic structure in a DES organization (see Introduction). One will also remark that the relative intensity of the peaks decreases as the number of urea molecules increases in the ionic non-covalent complex observed in CSI-MS (see Table 2 and Figure 2b).

Considering that the primary difference in the step of charged micro-droplet formation between the ESI and CSI processes, is related to the spray temperature emerging from the inlet capillary tip, CSI mass spectra were performed by at -40 and 0°C of the nebulizing gas temperature (see Figure 3a and 3b).

From the mass spectra of Figure 3, the detected ions have been regrouped in four ion populations. These ones differ by the urea molecule number into the non-covalent ionic

assemblies (see caption in Figure 3). At low temperature of the spray, *i.e.* $-40\text{ }^{\circ}\text{C}$ (see Figure 3a), the more intense ions are those of the population represented by the chloride adducts $[(\text{ChCl})_n+\text{U}+\text{Cl}]^-$ (where $n = 1$ to 4). One must reach a 0°C spray temperature, for equalizing the intensities of these reline ions $[(\text{ChCl})_n+\text{U}+\text{Cl}]^-$ and the choline chloride adducts $[(\text{ChCl})_n+\text{Cl}]^-$ (see the marks \diamond and \bullet on Figure 3). The mass spectra of Figure 3 and their evolution with temperature show that the increasing of the number of urea molecule in reline ions, represented by the $[(\text{ChCl})_n+(\text{U})_2+\text{Cl}]^-$ and $[(\text{ChCl})_n+(\text{U})_3+\text{Cl}]^-$ adducts (see the marks * and \square on Figure 3), are more intense at lower temperature, *i.e.* -40°C . They are associated to lower signal intensities and this, all the more as the spray temperature is kept at 0°C (see Figure 3b). These observations allow to rule out an attachment process of neutral urea that would first emitted in the gas-phase from charged droplets during the desorption step and then added to the gaseous ions in the CSI interface due to a condensation phenomenon at low temperature. This is in agreement with the ion formation mechanism described by K. Yamaguchi *et al* that propose in their studies in coordination chemistry that the solvent adducts observed on CSI mass spectra, represent structures established in solution rather than gas-phase additions of neutral molecules due to the low temperatures of the CSI source.^{24,25,32,33}

In-source Collision Induced Dissociation (in-source CID) experiments have been performed by rising the voltage of the so-called Orifice 2 lens (OR2), *i.e.* from 2 to 15 V, while keeping constant the so-called Orifice 1 (OR1) voltage at 30 V. The aim of such experiments is to evaluate the relative stabilities of these ion populations by focusing on their gas-phase behavior under collision activation conditions due to the presence of residual nitrogen in this region of the mass spectrometer interface. One will note that the in-source CID experiments are usually performed by rising the Orifice 1 (OR1) voltage value. However, for the present studies it was appeared that the increasing of the OR1 voltage (with a 5 V fixed value for OR2) had no influence on the ion dissociation ratios such as depicted in Figure S1. By contrast whereas the increasing of the OR2 voltage value is not commonly used for such experiments, it has been observed for the present work that the ion intensities measured on the negative CSI mass spectra of reline, were related to this potential difference between the so-called Orifice 2 lens and the Ion Guide (see Figure 1). As a result, all the in-source CID experiments have been performed by rising the OR2 voltage value while keeping constant the Orifice 1 voltage value at 30 V. The later is the value for that the sum of ion intensities has been measured at its maximum as the OR 2 voltage was of 5 V (see Figure S2). By referring to the manufacturer data that indicates that the acting pressure of Ion Guide is close to 1.10^{-3} mbar, the collision processes that the reline ions undergo during the crossing of a part of this interface, can be

compared to the activation regimes occurring in a collision cell such as typically used in CID-MS-MS experiments. It should be noted that the AccuTOF CS mass spectrometer do not allow to performed these ion dissociation experiments after selection of precursor ion. So, from the recorded CSI mass spectra, the intensity of the ions of interest, have been summed at each Orifice 2 voltage value and the contribution of each peak has been reported as a fraction of the total ion intensity. The values of the fractions of the most abundant ions have been reported as a function of the OR2 voltage values and reported in Figure 4.

The graphs reported in Figure 4 indicate that the DES ions integrating two urea molecules are the less stable of the studied ion series under collision activation. Indeed, even though the m/z 155 ion $[(U)_2+Cl]^-$ stays as the major species detected on the mass spectra, its intensity dramatically decreases as the value of the OR2 voltage is rising. The same trends are observed for the m/z 453 and 294 ions but at a lower extend. The m/z 294 ion ($[ChCl+(U)_2+Cl]^-$) is also the minor ionic species. This reinforces the assumption that this is probably the non-covalent complex formed with the weakest intermolecular interactions of the series plotted in Figure 4. These mass spectrometry results show then that the association of two urea molecules with a cholinium chloride salt, in reline ions do not lead to obtain the most stable ionic non-covalent complex in the gas phase. The reline ions integrating only one urea molecule such as the m/z 234 and 373 ions appear slightly more stable than the m/z 453 and 294 ions. The proportion of the m/z 234 and 373 ions on the CSI mass spectra noticeably increases until a 5 V value of OR2. This could indicated that these ions would be produced by dissociations of adducts of higher m/z ratios. For instance, the loss of urea molecule from the m/z 453 and 294 ions under collision activation could produce the m/z 373 and 234 ions, respectively. The same interpretation can be proposed for the cholinium chloride anions (m/z 174, 313 and 452). Their respective fractions in the mass spectra increase whereas that of all the others is lowered under collision activation as rising the voltage of the Orifice 2. To summarize, the most stable ions in the gas phase appear to be the $[(ChCl)_n+Cl]^-$ (where $n = 1$ to 3) adducts and the addition of urea molecules in the non-covalent complexes is not a favourable parameter to the intrinsic stability of reline gaseous ions.

3.2 Theoretical study of reline ions

The conformation, electronic structure and energies of selected m/z aggregates have been examined by quantum mechanical calculations. We focused on the lighter ionic clusters detected by CSI-MS (*i.e.* m/z 155, 234, 294 and 373). In Figure 5, the most stable geometries

are represented while the various optimized structures as well as their corresponding energies are given in Tables S1 to S5. Note that the energies of the isolated species that are reported in Table S1, show that interactions between the cholinium cation and the chloride anion, either *via* the hydroxyl group or the ammonium group, lead to more stable species than the association of Cl⁻ with amino groups of urea.

Looking at the different structures, three general trends on typical interactions between choline, urea and chloride are observed: (i) in agreement with the work of Hunt *et al.*,²¹ the association of urea with chloride occurs *via* a single chelating urea[⋯]Cl⁻ interaction with the formation of two H-bonds except for the dimer [(U)₂+Cl]⁻ (*m/z* 155) where the two urea molecules are connected to the chloride *via* a bidentate interaction, (ii) the cholinium interact preferentially with the chloride anion *via* the hydrogen of its hydroxy group. A secondary interaction also occurs involving the methyl groups of the choline and thus the formation of three CH[⋯]Cl⁻ electrostatic interactions. The first interaction is 31.7 kJ.mol⁻¹ more favourable than the second one. (iii) No clear interaction between urea and cholinium is observed, the most stable conformation always involves structures with direct [Ch]⁺⋯Cl⁻ and urea[⋯]Cl⁻ coordinations.

All the structures given in Table S2 to S5 that follow these three tendencies are nearly isoenergetic. A good illustration is, for example, the *m/z* 234 anionic cluster, for which we found seven different minima, six of them have less than 9 kJ.mol⁻¹ difference and follow the three points cited above, while the last one is 60 kJ.mol⁻¹ higher in energy and shows no bidentate Urea[⋯]Cl⁻ interaction but only a single NH[⋯]Cl⁻ H-bond. Same conclusions can be made for the other *m/z* anionic clusters. Interestingly, we can notice that the molecular organization of the *m/z* 294 ion involves the formation of a stabilizing H-bond between the hydroxyl group and the chloride at the expense of the H-bond that an amino group of urea could establish with this HBA. This agrees with the intrinsic reactivity of the *m/z* 294 [ChCl+(U)₂+Cl]⁻ ion evidenced by CSI-MS under collision activation. The loss of bidentate interaction of the urea with chloride could participate to the dissociation of this [ChCl+(U)₂+Cl]⁻ ion by loss of an urea molecule, confirming then that this one is a non-covalent ionic assembly among the less stables into the gas-phase. The theoretical study discloses also that the presence of a supplementary urea molecule in the reline ions do not results to a simple attachment of a HBD on an chloride anion initially positioned into the structure, as could be suggest if this was compared to an electrostatic interaction as in the case a cation attachment, *e.g.* with Na⁺ or K⁺. If we compare in one hand, the structures of the ions *m/z* 234 and 294 and in other hand, that of the *m/z* 313 and 373 ions, it appears that the presence

of a supplementary urea molecule leads to modify the overall organization of the non-covalent complex. We can also observe from the structures of Figure 5, that the hydrogen bonds maintaining the supramolecular assemblies are no longer the same in the case of the m/z 294 and m/z 373 depending on whether we consider two or one urea molecule into the reline ion.

4 Conclusions

Cold-spray ionization mass spectrometry can be considered as an analytical method suitable for the determination of the composition in non-covalent complex of a deep eutectic solvent (DES) such as reline. The ions detected on the CSI mass spectra, are characteristic of an association of cholinium chloride salts and urea molecules into a chloride adduct. The interpretation of mass spectrometry observations by the way of the quantum chemical calculation results, describes an organization of the detected gaseous ions, through a maximization of the hydrogen bonds of the DES constituents with the chloride anions. The gas-phase dissociation studies indicate that it is not the increasing number of urea molecules that participate the most to the DES constituent stabilization. Finally, the assembly of two urea molecules with one cholinium chloride salt into a chloride adduct, appears to be the less stable gaseous species under collision activation whereas this supra-molecular organization is considered as the basic structure of the reline formation in condensed phase.

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TABLE 1. Results of Accurate Mass Measurements performed from the ESI mass spectrum of reline.

<i>m/z</i>	Proposed formula	Calculated mass (error in ppm)	Choline chloride
174.0452	C ₅ H ₁₄ NOCl ₂	174.0452 (0)	1
313.1202	C ₁₀ H ₂₈ N ₂ O ₂ Cl ₃	313.1216 (-4.5)	2
452.2000	C ₁₅ H ₄₂ N ₃ O ₃ Cl ₄	452.1980 (4.1)	3
591.2733	C ₂₀ H ₅₆ N ₄ O ₄ Cl ₅	591.2744 (-1.1)	4
730.3493	C ₂₅ H ₇₀ N ₅ O ₅ Cl ₆	730.3468 (2.5)	5
869.4313	C ₃₀ H ₈₄ N ₆ O ₆ Cl ₇	869.4272 (4.1)	6
1008.4970	C ₃₅ H ₉₈ N ₇ O ₇ Cl ₈	1008.5036 (6.0)	7

TABLE 2. Results of Accurate Mass Measurements performed from the CSI mass spectrum of relin.

<i>m/z</i>	Proposed formula	Calculated mass (error in ppm)	Choline chloride : Urea
95.0015	CH ₄ N ₂ OCl	95.00122 (2.8)	0:1
155.0336	C ₂ H ₈ N ₄ O ₂ Cl	155.0336 (0)	0:2
215.0664	C ₃ H ₁₂ N ₆ O ₃ Cl	215.0659 (2.0)	0:3
234.0867	C ₆ H ₁₈ N ₃ O ₂ Cl ₂	234.0776 (-1.4)	1:1
294.1113	C ₇ H ₂₂ N ₅ O ₃ Cl ₂	294.1010 (4.5)	1:2
354.1458	C ₈ H ₂₆ N ₇ O ₄ Cl ₂	354.1423 (8.9)	1:3
373.1531	C ₁₁ H ₃₂ N ₄ O ₃ Cl ₃	373.1540 (-2.4)	2:1
433.1865	C ₁₂ H ₃₆ N ₆ O ₄ Cl ₃	433.1864 (0.4)	2:2
493.2222	C ₁₃ H ₄₀ N ₈ O ₅ Cl ₃	493.2190 (7.0)	2:3
512.2305	C ₁₆ H ₄₆ N ₅ O ₄ Cl ₄	512.2304 (0.3)	3:1
572.2626	C ₁₇ H ₅₀ N ₇ O ₅ Cl ₄	572.2627 (0.2)	3:2
632.2925	C ₁₈ H ₅₄ N ₉ O ₆ Cl ₄	632.2951 (-4.1)	3:3
651.3036	C ₂₁ H ₆₀ N ₆ O ₅ Cl ₅	651.3068 (-4.9)	4:1
692.3256	C ₁₉ H ₅₈ N ₁₁ O ₇ Cl ₄	692.3275 (-2.7)	3:4

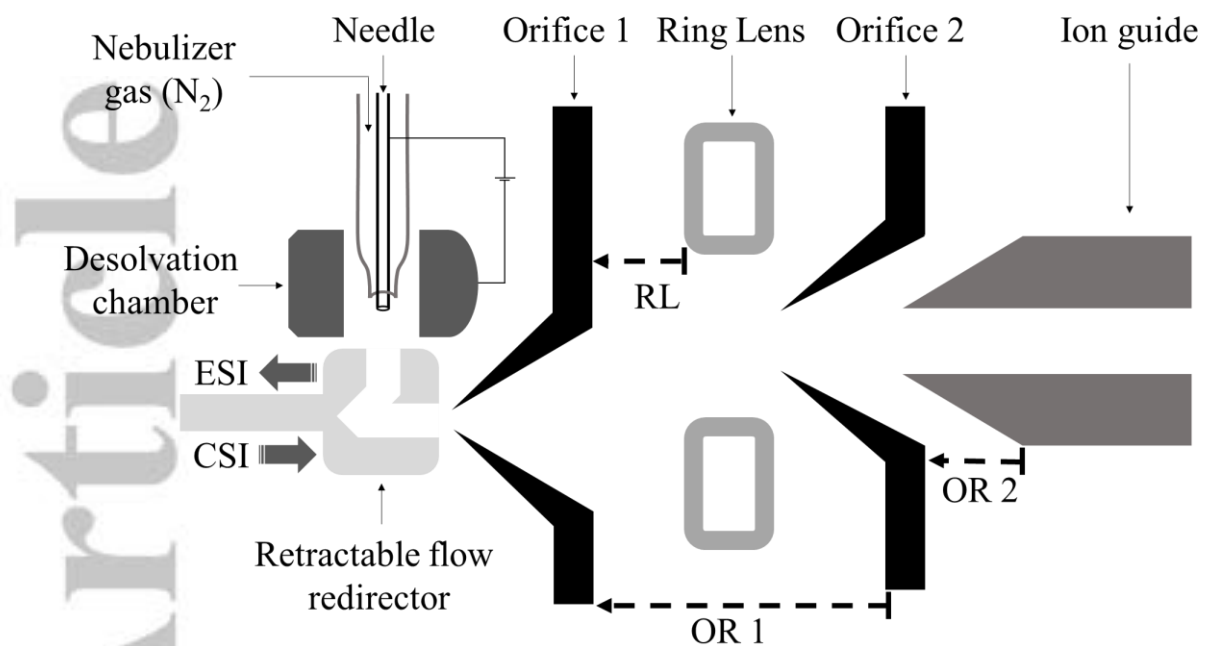


FIGURE 1: Dual source ESI/CSI of the AccuTOF CS mass spectrometer. Note that the change of electrospray ionization (ESI) for the cold-spray ionization (CSI) is ensured by the used of liquid nitrogen cooling device (not shown in the figure) for the desolvation gas and the positioning of the retractable flow redirector between the desolvation chamber and the Orifice 1 lens entrance.

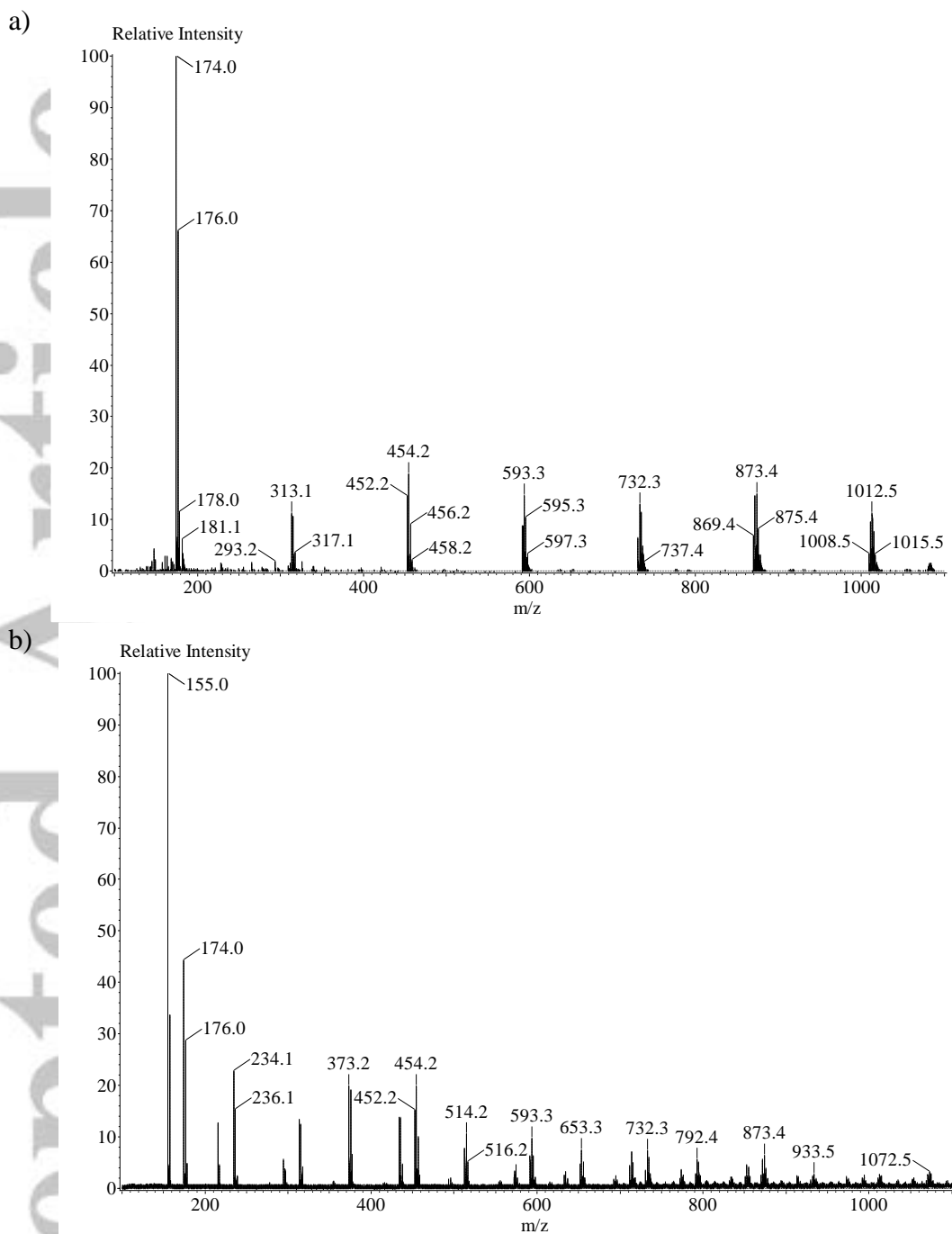


FIGURE 2. Comparison of **a)** ESI(-) and **b)** CSI (Spray and capillary temperatures fixed at -30 °C) mass spectra of relin.

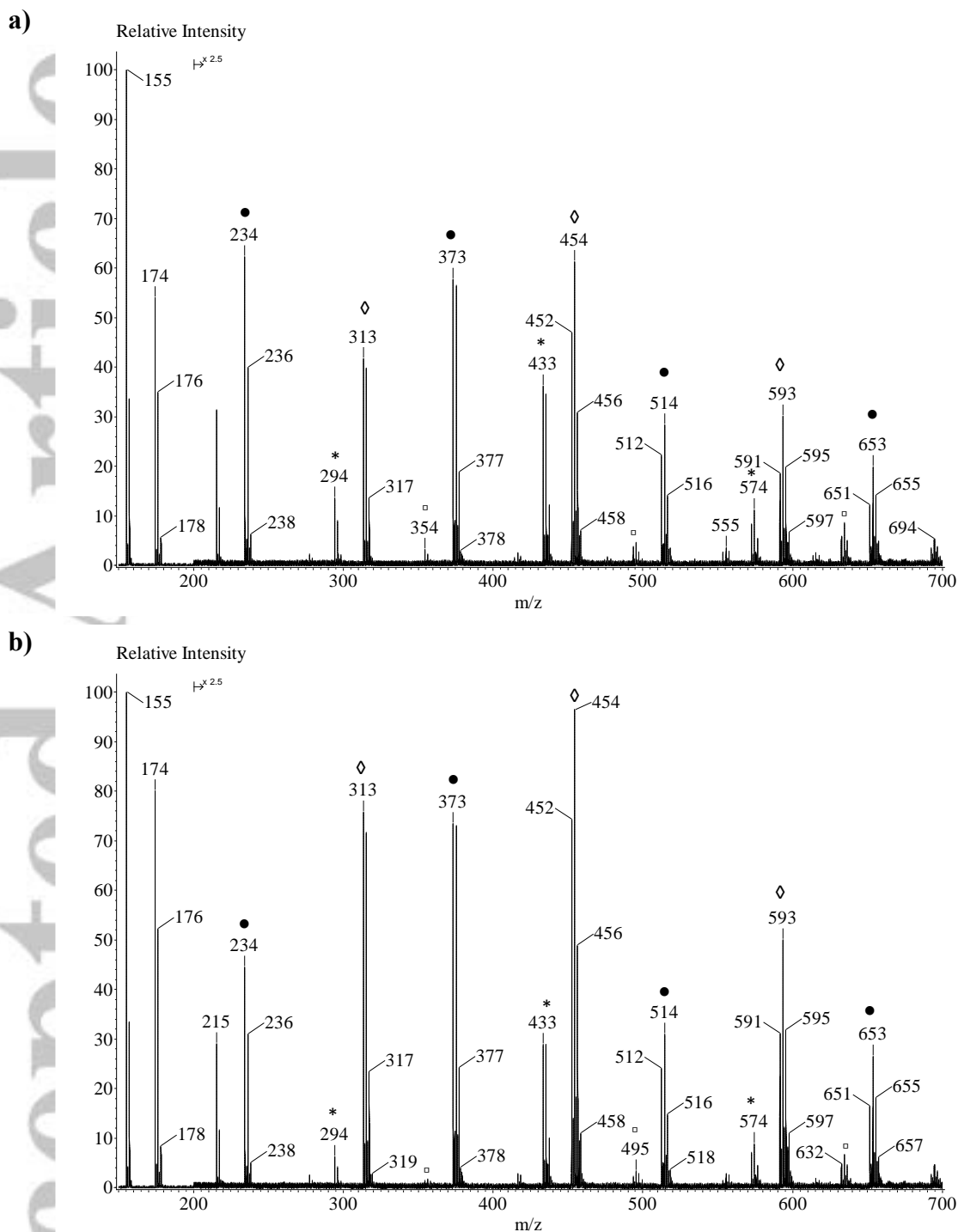


FIGURE 3. CSI(-) mass spectra of relin sample in acetonitrile at a nebulizing gas temperature of **a)** -40°C and **b)** 0°C , where the symbols (\diamond), (\bullet), (*), and (\square) indicate the ion populations that can be written such as: $[(\text{ChCl})_n+\text{Cl}]^-$, $[(\text{ChCl})_n+\text{U}+\text{Cl}]^-$, $[(\text{ChCl})_n+(\text{U})_2+\text{Cl}]^-$, $[(\text{ChCl})_n+(\text{U})_3+\text{Cl}]^-$, respectively, and where n ranges from 1 to 4.

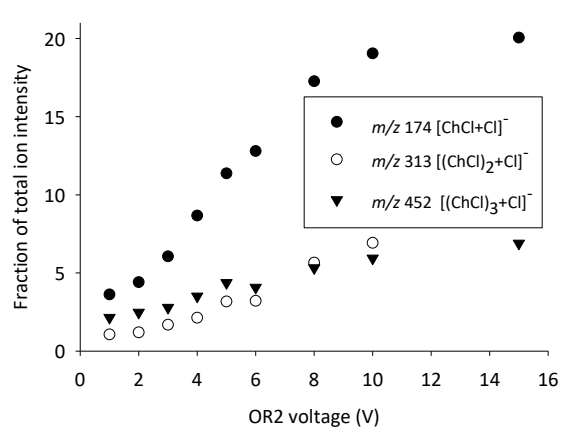
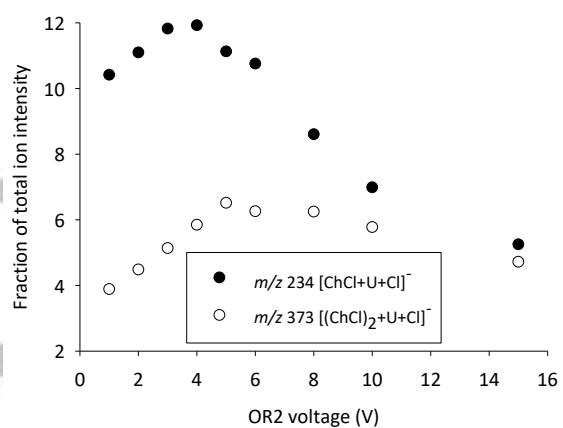
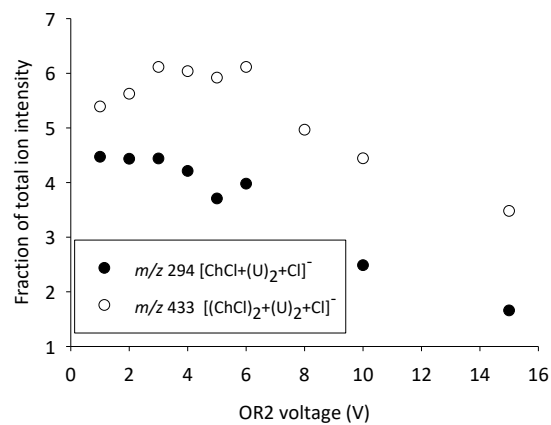
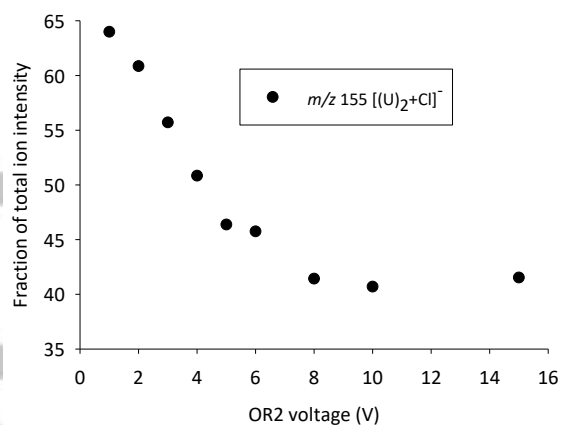


FIGURE 4. Fractions of intensities of the ions $[(U)_2+Cl]^-$, $[(ChCl)_n+(U)_2+Cl]^-$, $[(ChCl)_n+U+Cl]^-$ and $[(ChCl)_n+Cl]^-$, and where $n = 1$ to 3 reported as a function of the orifice 2 voltage values.

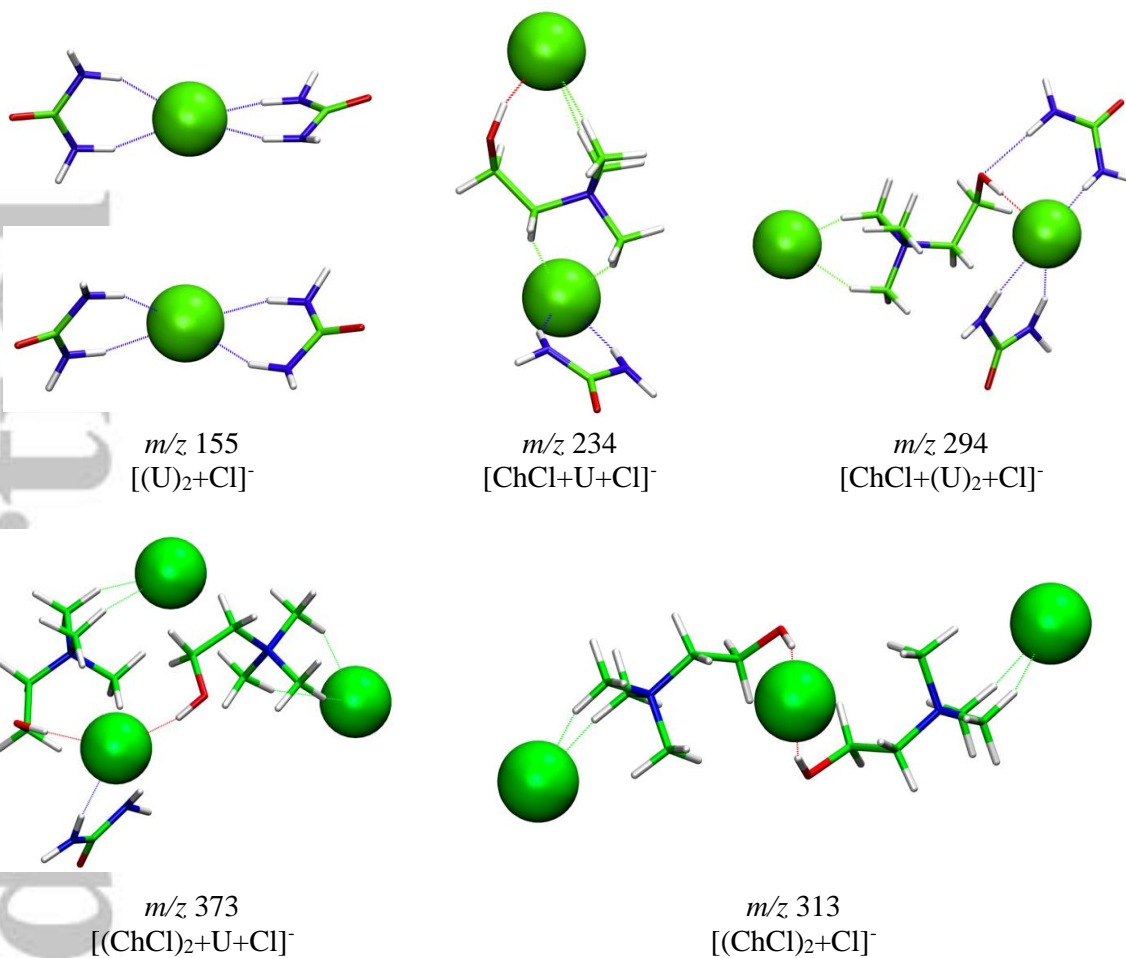
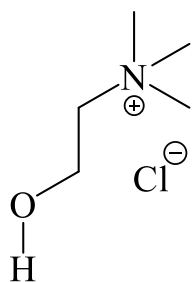
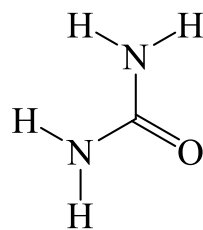


FIGURE 5. Snapshots of the most stable structures of the m/z ions (m/z from 155 to 373) obtained after QME(B3LYP/6-311++G(d,p) geometry optimization. For the energies see Table S1 to S5.



Cholinium chloride salt (ChCl)



Urea molecule (U)

Scheme 1: Chemical structures of compounds forming the Reline as Deep Eutectic Solvent