Ion mobility mass spectrometry with surface activated chemical ionisation as a method for studying the domain of water clusters

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Introduction

Water holds great relevance in various biological and biochemical systems pertaining to human life [1]. Lack of water would jeopardise several chemical reactions paralysing biological functions, and the importance of water for the cells, organs and life forms can hardly be overemphasised [2]. It is observed that water presents itself as the matrix and medium for the genesis and sustenance of various life forms [3].

Inherently simple, water behaves as an excellent solvent, a reactant, a product of the reaction and also a catalyst. And though exhibiting reasonably normal behaviour, it is complex and anomalous in many instances [1]. Water exhibits quirky behaviour during experiments and scientific explorations, necessitating the deployment of more than one theory or model to understand the unusual aspects of water. A tighter association between the water molecules, as compared to other material molecules of the same size and shape, results in much higher cohesion between the molecules [4]. Consequently, this leads to higher surface tension, melting point and boiling point. Water also exhibits evident volumetric anomaly when its solid form (ice) floats on the liquid (water), which is opposite to the general observation of a solid sinking in its liquid form. Again this is attributed to the minimum volume of water at 4°C or a temperature of maximum density (TMD) at 4°C, which is in defiance of the increasing volume of simpler liquid monotonically [4]. Further, the negative slope of the water’s pT equilibrium phase boundary between the solid and liquid results in liquification of ice due to pressure, against the customary solidification of liquid into solid when under pressure. In contrast to a maximum of one or two solid phases of other materials, water is known to have at least 17 distinct phases of solids. The quintessential hydrophobic effect of water represents its thermodynamic uniqueness while interacting with nonpolar molecules is another noteworthy feature of water.

The structure of water is more relevant and vital for scientific analysis rather than just focussing on the chemical composition of water. The organisation of the molecules, synergised by hydrogen bonds, builds up the structure of the water and, in the simplest form, is represented by (H₂O)₂, a dimer. These small clusters may associate with each other to form larger groups spread all over the water [5]. These water clusters significantly influence biological functions and can be studied experimentally or computationally [6].
Though water appears homogenous at the macroscopic level at the nanoscopic level, it is not. Two competing processes, namely the enthalpic and the entropic processes, are responsible for the structuring of liquid water [7].

The objective of this paper it to study the domains of water clusters in pure water samples and diluted arginine solutions using Ion mobility mass spectrometry with surface activated chemical ionisation and to determine the water behaviour in terms of cluster formation before and after the application of low-frequency irradiation. And further, in the discussion section, we also intend to discuss the nature of such clusters formation in view of theories and knowledge accumulated in recent years.

**Materials and Methods**

SACI ionisation technology is considered to be a very soft ionisation technique that does not modify the analyte-solvent coordination significantly [8]. This is substantiated by the fact that it has been employed to monitor the peptide-cation adduct in solution [9]. The SACI-CIMS is an evolved version of SACI technology that enables solvent cloud ion species by deploying mass spectrometry.

Ion-mobility mass spectrometry (IM-MS) synergises ion mobility with mass spectrometry enabling compounds of a predetermined (m/z) range in an analyte solution to be characterised together. In an IM-MS, an IM chamber is inserted after the ionisation source of the mass spectrometer (an electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI) source, for example) but before the mass spectrometer optics and analyser. Such placement of the IM chamber creates an additional analyte separation step which helps reduce the spectral noise [10].

During the IM-MS procedure, the analyte ions, generated in the ionisation chamber, enter the ion-mobility section and move through the chamber according to their charge, which gets created as a result of an electrostatic or electrodynamic force generated by an electric field. The Ion-mobility chamber contains the gas at almost the atmospheric pressure, and the moving analyte collides with it. Within the chamber, the collision cross-section and the charge help separate them. While characterising proteins using Mass spectroscopy, the inclusion of an IM step has been shown to be useful. The characterisation of different spatial isoforms is enabled by including an IM step due to the difference in their collisional cross-sections with the gas molecules generating different time-dependent isoform drifts.

Further, during the ionisation process, as a consequence of substantial solvent-ion clustering, the ESI and APCI sources generate noise. Dynamic IM-MS, e.g., high-field asymmetric waveform ion mobility spectrometry (FAIMS), has been employed to filter analyte ions by their m/z values before they enter the MS analyser and to retain ion-solvent clusters within the ionisation source, thereby simplifying the spectrum of an analyte sample by producing multiple spectra which individually report a subset of the analyte components. However, notwithstanding the ability of FAIMS to optimise the separation of analyte components, the choice of appropriate gas is vital for optimal performance.

The low-voltage ionisation source employed by a Surface-activated chemical ionisation (SACI) source increases the spectral sensitivity by ionising mainly analyte solute molecules [10]. SACI is a polarisation effect induced at a surface inserted into an APCI chamber that is subjected to a smaller potential (~50-300 V) than is employed by a classic atmospheric pressure ionisation source (~3000-6000 V). It has been observed that under SACI conditions, there is a considerable reduction in the number of solvent-ion clusters while the analyte ion population is noticeably enhanced. Under low-voltage SACI conditions, a highly interesting and uniquely unreported phenomenon was observed, which we call the “in-source Cloud Ion-mobility Mass Spectrometry” (CIMS) effect. This effect is observed singularly in the SACI mode when the surface potential is fixed and the entrance capillary voltage of the MS is varied. However, it is essential to note that ESI and APCI operate at high voltage ionisation conditions, unlike the SACI, which operates at low voltage. Consequently, the observations confirm that with increased internal capillary voltage ions with larger m/z values gain focus, while those with smaller ratios of m/z remain defocussed. It is further observed that the ion-cloud spatial distribution in the low-voltage SACI source depends on the rate of the in-source gas circulation. The scope and utilisation of the SACI-type MS are greatly enhanced due to the newly discovered CIMS effect as the additional ion filter located before the analyser substantially improves the spectral quality and definition of the target molecule [10].

The experiment was conducted using the following equipment and chemicals (Tab. I).

**Sample preparation**

SACI-CIMS was utilised to analyse two different types of water solution and a dilute solution of Arginine:

a. 100 µL Pure water without treatment, by direct infusion;
b. 900 µL of water electromagnetically irradiated at 7 Hz to 80 Hz for 5 minutes, using an electromagnetic frequency fields generator;
c. A dilute solution of Arginine was made with a concentration of 100 ng/mL. And this solution was exposed to radiated and non-radiated water, respectively.

**Mass spectrometry**

The data was acquired by means of the SANIST platform provided with a Surface Activated Chemical Ionization- Cloud Ion Mobility Mass Spectrometry (SA-CI-CIMS) [10]. The surface potential was regulated at 47 V for the experiment. The nebulising gas pressure was set at 75 Psi, and the flow rate of the dry gas parameter was regulated at 1.0 L/min. The experiment was performed with the nebuliser temperature equal to...
300°C while the dry gas was at a temperature of 320°C. The voltage at the entrance ion lens was set at 550 V. The charged water clouds between 100 and 800 intensity were focused for analysis by the mass analyser. An electromagnetic frequency fields generator was used to emit EM radiations between 7 Hz to 80 Hz. The low-frequency radio waves at 7 Hz to 80 Hz are able to deposit their energy more profoundly into the material because of better penetration but at the same time are non-ionising. These waves are able to agitate polar molecules leading to their vibration, and hence increase in temperature is achieved.

Results

Figure 1 depicts the MS result of the charged water cloud m/z ion species. As prominently highlighted in the chart, the mass difference of 18 Da between the peaks unambiguously corresponds to the H2O molecular weight. The peak at 220 corresponds to the water cluster (H2O)12. While the peak at 238 represents (H2O)13. And further, when one more molecule of water gets added, it results in m/z of 256, which is (H2O)14. Similarly, adding one molecule of water to the peak of 222.9 takes it 240.9 and finally to 258.9 with more additional H2O.

To further verify and confirm the presence of water clusters, the ion at m/z 256.8 [corresponding to (H2O)14] was isolated and fragmented further. The corresponding result was as depicted in Figure 1b. And in this particular case, a neutral loss of 18 Da emphatically vouches for the presence of neutral water species.

The experiment had two samples of water, out of which one was pure and not exposed to any radiations or physical effect, and the other was subjected to electromagnetic radiations between 7 Hz and 80 Hz for 5 minutes. The results of the MS analysis of both the samples are represented in the Figure 2a and 2b respectively.

Interestingly in both the samples, two water ion clusters are observed at average m/z 250 and 650, respectively. And these are (H2O)14 and (H2O)36, respectively. Further, it can be observed that the water ion cluster at m/z 250 of the untreated water registers an intensity of 1250 counts/s while the water ion cluster at m/z 250 in the case of irradiated water has an intensity of 400 Counts/s. Thus, intensity in the first case is more than double that of the second case.

Moreover, the abundance of the lower m/z cluster as compared to the higher ones in electromagnetically treated water is lower against the same parameter of the untreated water. However, the intensity of about m/z 650 in the case of treated water seems to be lower in absolute values compared to untreated water. And this indicates the lesser formation of larger clusters in treated water than in untreated water for the same value of m/z.

As the experiment progressed further, some water cluster species seemed to be localised near the analyte species, as observed in the full scan spectrum. A solution of Arginine at a high diluted concentration of 100 ng/mL was infused and exposed to treated and untreated water.

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**Tab. I. Equipment and chemicals using in the experiment.**

| Equipment   | Model                              | Manufacturer   | Revision date | Revision |
|-------------|------------------------------------|----------------|---------------|----------|
| Mass Spectrometer | SANIST platform                | ISB srl         | 13/09/2021    | 06/2022  |
| SACI        | SACI version for protein analysis | ISB srl         | 02/02/2021    | 06/2022  |
| Deionized water | VWR                        |                | 07/09/2023    |          |
| Arginine    | Sigma-Aldrich                   | 08/09/2022     | 137455541408312 | 2008111 |

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**Fig. 1. a) Water cloud ion species, b) Fragmentation spectrum of the water cluster species at m/z 256.8.**
respectively. These two samples were further analysed, and Figure 3a shows the result of untreated arginine water solution while Figure 3b shows the result of treated arginine water solution.

The first mass spectrum (Fig. 1b) does not show the presence of water clusters. However, in the second one, the treated Arginine solution (Fig. 3b), a clear ten-molecules water cluster peak at m/z 181 was observed (red arrow in Fig. 3b). It is interesting to note that water clusters exist in the presence of Arginine in water.

Figure 4a below shows the Total ion current mass chromatogram of arginine solution 100 ng/ml non-radiated (Red Line) and irradiated (Green Line), which is an aggregation of hundreds or even more of the mass to charge units revealed in the mass spectrums. It can be observed that the intensity of the radiated dilute arginine solution is lower than that of the untreated arginine solution. The radiated solution (green line) has comparatively lesser peaks and is relatively smoother as compared to the non-radiated sample of Arginine (red line).

The mass spectra of the two arginine solutions also indicate lower intensity values for the radiated sample compared to the non-radiated ones. Also, the number of visible peaks in the radiated sample is lower than that available for the non-radiated sample.

As indicated in Figure 4b, Arginine is prominently present at m/z 175.4; however, its intensity in the case of untreated Arginine solution seems higher than in the treated solution. Around m/z values of 193, the Arginine with water molecule can be detected. In general, the number of peaks in the irradiated samples is lesser than in the non-irradiated samples. The continuance of the study could perhaps lead to a better understanding of the relation between the duration of irradiation and the formation of water clusters. Further research and studies can be carried out to determine if the effect of irradiation is long-lasting or even permanent. In fact, the literature shares some hypotheses, wherein it is believed that the effects of the electromagnetic field treatment can be long lasting [11].

Discussion

The results of the current study deploying the SA-CI-CIMS method also evidenced a presence of water cluster...
clusters similarly to other experimental methods such as the ones conducted by Simone Konig and Henry Fales of National Institutes of Health, Bethesda, Maryland, USA [12] and also in line with the results of another experiment conducted by Frank N. Keutsch and Richard J. Saykally, Department of Chemistry, University of California [13]. Cluster formation is one of the peculiar properties of a water molecule. And this is due to its molecular structure, which is responsible for the critical role of water in chemistry, biology and life.

**Molecular Structure and Spectral Analysis of Water**

Based on the recent theories, analysis of the behaviour of water molecules resultant from its biphasic structure describes it as a dynamic dissipating system. A significant molecule on earth, water, is composed of one atom of oxygen and two of hydrogen. A covalent bond between the oxygen and the two hydrogen atoms builds the water molecule. The two hydrogen atoms share a pair of electrons with the oxygen atom. However, this arrangement renders the oxygen with an electronegative charge while the hydrogen atoms are electropositive. Consequently, the water molecule is a dipolar molecule. And the presence of this electric dipole enables water to orient itself in the presence of external electric fields.

The spectral analysis of the water molecule highlights the asymmetric nature of its structure, which explains many unusual properties of water. The two O-H covalent bonds are symmetrical with a length of 0.97 Angstroms) and an angle of 104.5° between them [14]. Due to the dipole property, a water molecule can build hydrogen bonds with four neighboring water molecules forming pentamers, as well as to form bigger aggregates (e.g. octa-, icosa- or higher eders) in various configurations, which are, however, highly dynamic [11]. The excellent solvent capabilities and properties of water can be attributed to its unique structure.

**The Water Structure**

It has been shown that at the molecular level, water does not have a homogeneous structure but, instead, is in dynamic equilibrium between the changing percentages of assemblies of different kinds of oligomers and polymers. The structure of these ‘clusters’ or the units themselves is dependent on temperature, pressure and composition [15]. Recent studies indicate the ability of the water molecules to rearrange themselves into different clusters depending on the electromagnetic fields that it is subjected to, rather than being just a homogenous fluid.

Frank and Wen introduced the concept of the flickering cluster model, resulting from water-water interaction wherein the hydrogen bond resonance between electrostatic and covalent bonding lead to the formation and destruction of aggregates of water molecules tetrahedrally linked by dipolar bonds [16]. Essentially the surrounding water is responsible for the cluster of water molecules created.

The model proposed by Emilio Del Giudice and Giuliano Preparata (1988) is characterised by a larger scale of clusters [16]. Based on quantum field theory, they de-
scribe sub-micron-sized coherence domains of water, each of which can contain many millions of molecules. The bonds between water molecules within these domains can be thought of as antennae receiving electromagnetic energy from outside. And with this received energy, the water molecules can release electrons, making them available for chemical reactions. The size of the coherence domains corresponds to the wavelength of the coherent field and, in the case of water, corresponds to about 1/10 micron.

Though it has been established in the last decade that magnetic and electromagnetic stimulations alter the physicochemical properties of water, the exact mechanism of the phenomena eludes the scientific community [17, 18].

**Quantum Field Theory**

This approach encouraged Emilio Del Giudice, Giuliano Preparata and many other researchers to study water and its characteristics from different perspectives. The generally accepted model assumes that water molecules are connected to each other through ‘hydrogen bridges’ that arise from their hydrogen atoms, which are supposed to be abandoned by the electrons migrated into the molecule’s electron cloud. Though the term hydrogen bridge is used in chemistry and biology as well, their conceptual meaning is different in either case. According to the classical model, the abandoned hydrogen atoms become a source of attraction for the electronic clouds of the adjacent molecules, which, swelling outwards, form a bridge with the hydrogen nucleus. This is why quantum physics interprets hydrogen bridges as the consequence of the dynamics of electrodynamic quantum coherence that leads the gas to collapse into a liquid and not as the cause of the cohesion of the liquid. However, in biological processes, molecules follow chemical codes (e.g. DNA, Krebs cycle, Respiration) which lead a given molecule to interact only with a pre-defined one. Del Giudice and Preparata, therefore, wondered what the physical dynamic is that gives rise to this ordering, i.e. that causes the molecule to interact only with a given one and not with the others. The answer to these questions comes from applying quantum field physics to describe the molecular model of water and its behaviour. In the light of quantum physics, water takes the form of a complex autopoietic system characterised by a structure consisting of two phases: coherent and incoherent [19].

At this point, it is worth introducing the theorem postulated by Giuliano Preparata, which encapsulates years of experimentation and the work of many scientists [20]. “Given an extended electromagnetic field with frequency $F_0$ and two molecules with frequencies $F_1$ and $F_2$ respectively, if the frequencies of these molecules are different, nothing ‘happens’, i.e. short-range interactions can take place. If the frequencies of these molecules and the field are equal, then the molecules attract each other with a tremendous force. This can happen even at a distance, depending on the size of the field. There is, therefore, a principle of selection that can take place even at great distances, thus providing an explanation for the rapidity of biological reactions”.

On the basis of this theorem, the group led by Del Giudice hypothesised the existence of a background electromagnetic field with a given frequency that is able to attract molecules vibrating at the same frequency. According to Giuliano Preparata, there is a threshold of matter density beyond which a ‘super-radiative phase transition’ occurs: the system reorganises itself and reaches a new stable configuration, in which the matter field and the electromagnetic field coherently oscillate at a common frequency [20].

This leads to a significant energy gain proportional to the density. Hence, the density increases enormously up to the limit value determined by the repulsive forces linked to the Pauli principle, as well as those linked to the Coulomb repulsion [21]. The cohesive part, highly structured in tetrahedral form, which simulates the so-called hydrogen bond, generates magnetic patterns capable of interacting in principle with weak electromagnetic signals and storing the information they carry [22].

The coherent network of Water Coherence Domains then becomes a candidate for being a dissipative system, as described by Prigogine. The interaction between water-protein has been accepted as a significant influence of chain folding, internal dynamics, conformational stability, binding specificity and catalysis for a substantial period of time [23].

The standard quantum theory fails to propose quantum coherence for liquid water because of its inability to recognise quantum fluctuations and interaction between light and water. Despite the fact that conventional quantum electrodynamics field (QED) theory applies only to gases, Giuliano Preparata, Emilio Del Giudice, and others broadened its scope to include the condensed phase of liquids. It was subsequently observed that large and coherent domains (CDs) form when the vacuum electromagnetic field interacts with water. In such CDs, the water molecule constantly oscillates between the stable ground state and the excited state close to the ionising potential of water. Present in water at ambient temperature and pressure, these CDs offer possible explanations for the bizarre behaviour of water and are responsible for the sustenance of life on the planet [24, 25].

The primary focus is observing the interaction between the Quantum Elementary Particles (atoms/molecules) with its radiative electromagnetic field. Several observations and research studies support the fact that under predetermined conditions, all the particles transit to a coherent self-consistent physical state oscillating in unison with the same phase between the ground state and an excited state just short of the first ionisation comprising of about a million of almost unethered electrons. Consequently, the inner periphery of the CD sphere is most likely negatively charged with complementary positively charged protons just external to the coherent domain. Thus, the surface of the Coherent Domain develops into a “redox pile”. This phenomenon of the stimulated CD lays the foundation for all the oxidation-reduction
energy metabolism that drives and sustains all the life processes [26]. The CD becomes a cavity for the electromagnetic field because the dynamics give the photon an imaginary mass, according to the Anderson-Higgs-Kibble mechanism. All the molecules within a CD show a larger volume than their ground state since they oscillate in unison between the ground state of the individual molecule and an excited state. The electrodynamic attraction is neutralised by thermal collisions that push the molecules out of phase. Therefore, at a non-zero temperature T, as in the case of Landau liquid helium, each liquid becomes a two-phase system, where a fraction Fc (T) of the constituent particles behaves consistently, while a fraction Fnc (T) = 1 - Fc (T) forms a dense gas trapped between the CDs. Since coherent molecules are larger than incoherent ones, the density of the coherent fraction is lower than that of the incoherent one; the density of the coherent fraction of liquid water was estimated to be 0.92 – the same value as the density of ice. According to thermal dynamics, inside the liquid, there is a continuous crossing of molecules between the two fractions (coherent and incoherent) so that, while the total number of coherent molecules remains constant at a given temperature T, their spatial distribution keeps changing. The same phenomenon, which has its roots in Coherent QED, maybe at the origin of the formation of water supramolecular structures, which can be obtained through various kinds of purely physical low-energy triggers, and at the same time, explain their permanence in time [27]. This characteristic explains why experiments such as neutron scattering, which have a greater resolution time than the typical time of change of coherent structures in space, find the liquid as homogeneous. Only experiments with a reasonably short resolution time (of the order of collision time, 10⁻¹⁰ s) could detect the actual uneven structure of liquid water. However, there are other kinds of experimental considerations that may indicate the presence of larger aggregates in aqueous solutions that could be traced to QED predictions on coherent domains [28].

Conclusion

In line with the successfully conducted experiment, the authors could further study the water clusters using Ion-mobility mass spectrometry and detect the presence of water clusters in the tested samples. The spectrometry results of the diluted arginine solution infused with treated water also confirm the presence of the water cluster near the analyte molecule ion in agreement with the observation of Elia et al. (2014) as mentioned above [27]. The analyte ion is able to surround itself with rearranged water clusters when the water is treated electromagnetically. This helps confirm the ionic/polar concept explaining the excellent solvent properties of water. It can also be concluded that electromagnetic radiations have the ability to reorient the structure of the water clusters. Water treated with low-frequency electromagnetic radiations water tends to form less of smaller clusters as compared to untreated water. It can also be concluded that exposure to electromagnetic radiations strengthens the hydrogen bonds of the water cluster resulting in a smaller number of low-intensity water clusters. However, this opinion is highly contested. Moreover, the presented experimental results are supported by the detailed survey analysis of current theories based on the Quantum Field Theory of Coherent Domains presented in the discussion part.

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Conflicts of interest statement

Authors declare no conflict of interest.

Author’s contributions

MB: study conception, editing and critical revision of the manuscript; SC, ML, SM, IZ, KD, SP: literature search, editing and critical revision of the manuscript. All authors have read and approved the final manuscript.

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