

Interactions of Glycerol with Water in the Gaseous State under Field Ionization Conditions

Marina V. Kosevich* and Vadim S. Shelkovsky

B. Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of Ukraine, 47, Lenin Avenue, 310164, Kharkov, Ukraine

The possibility of the formation of water-glycerol clusters in the gas phase is studied in connection with an earlier observation of the absence of sputtering of such clusters under fast-atom bombardment (FAB) of frozen water-glycerol solutions. It is shown that, during interactions in the gas phase under field ionization mass spectrometric conditions, the formation of mono- and dihydrates of glycerol (G) is possible, which ultimately result in GH^+ and $\text{G} \cdot \text{H}_2\text{O} \cdot \text{H}^+$ ions in the mass spectra. The use of heavy water allows the observation of a comprehensive process of exchange of mobile hydrogens of glycerol with the deuterium of heavy water in the course of the interactions of the neutral molecules in the gas phase. The possibility of clustering of glycerol with water in admixture in the gas-phase under field ionization and the absence of this phenomenon in the case of condensed samples under FAB conditions confirms a suggestion about the mechanism of ion formation from frozen mixtures under low-temperature FAB. This phenomenon consists in the separate sputtering of neat water and glycerol clusters from separate surface domains of different chemical composition, the domains being formed due to phase separation during the freezing of solutions.

The peculiarities of the intermolecular interaction of glycerol with water via hydrogen bonds form the basis of the valuable hydration properties of glycerol, that are widely applied in the pharmaceutical, cosmetics and food industries. One of the applications of glycerol lies in its role as a cryoprotector and depends upon the changes of the parameters of the phase transitions of water in the presence of glycerol. In our previous study of frozen water-glycerol solutions by means of low-temperature (LT) fast-atom bombardment (FAB) mass spectrometry^{1,2} it was found that no mixed water-glycerol clusters are formed under conditions in which large clusters of water $(\text{H}_2\text{O})_n\text{H}^+$ ($n=1-20$) and of glycerol G_mH^+ ($m=1-3$) are sputtered simultaneously. On the basis of the analysis of a number of LT FAB mass spectra of frozen mixtures, it was suggested that the process of cluster sputtering in LT FAB is sensitive to the morphology of the sample surface, and originates from component separation in the mixtures during freezing. Clusters of individual substances are sputtered from separate regions of concentration, while a necessary prerequisite for the formation of mixed clusters is the possibility of the mixing of the components in the solid state, either during co-crystallization or homogeneous amorphization. The morphology of the frozen water-glycerol solid^{3,4} allows independent sputtering of water clusters from the pure ice crystallites and of glycerol from eutectic channels.^{1,2} The concentration of glycerol at the eutectic point is 66.7% by weight,³ corresponding to a ratio of 2.5 water molecules to 1 glycerol molecule, which would seem to provide the possibility of mixed water-glycerol sputtering. But a rather complicated temperature behavior of the water-glycerol eutectic mixture may hinder this process. Upon heating of the sample, pre-cooled to liquid nitrogen temperature, crystallization of water in the glassy eutectic takes place at a temperature value of -75 to -83 °C,³ which is a little lower than the temperature threshold for sputtering of glycerol ions.¹ There is a possibility of the existence of so-called strongly bound water, which remains bound to solute molecules at low temperatures and thus is not involved in formation of water crystals. Nevertheless water-glycerol

clusters are not recorded even over a wide temperature range of measurements. In this connection, the question arose as to whether stable mixed clusters of water and glycerol can, in principle, exist in the gas phase, as it had been shown earlier that some compounds with donor-acceptor functional groups do not form clusters with water due to some peculiarities of their electron and geometrical structures.⁵

One of the methods for the study of weak van-der-Waals and hydrogen-bonded associates is field ionization (FI) mass spectrometry. Its potential has been demonstrated with model studies of hydration of biomolecules.^{6,7} The technique was applied in the present work to elucidate the possibility of formation of stable water-glycerol associates in the gas phase and to study the peculiarities of water-glycerol intermolecular interactions.

EXPERIMENTAL

Water-glycerol interactions in the gas phase were studied by means of a variation of the FI mass spectrometric technique, that had been developed earlier for the study of the hydration of biomolecules.⁶ A dedicated FI ion source, described elsewhere,^{6,7} was used. A tungsten needle-typer emitter was employed; the emitter potential was kept constant at +5 keV and the counterelectrode potential was varied from -1 to -5 keV. Provision was made for resistive heating of the emitter up to 200 °C.

A special sample-introduction system was used to ensure stable monomolecular flows of the substances under high vacuum conditions (Fig. 1), which included two separate glass evaporators. A crystalline hydrate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was utilized as a source of a water vapour monomolecular flow, stable due to the constant water-vapour pressure over the solid surface of the crystalline hydrate at any given temperature. For the analogous desorption of D_2O , the crystalline hydrate $\text{CuSO}_4 \cdot 5\text{D}_2\text{O}$ was produced.⁸ When D_2O was being investigated, the measurements were started only after at least 2–3 hours of high vacuum pumping of the ion source with samples inserted, to ensure the removal of much of the background undeuterated water.

To obtain a directed vapour flow of glycerol, a 0.5 μL

* Author for correspondence.

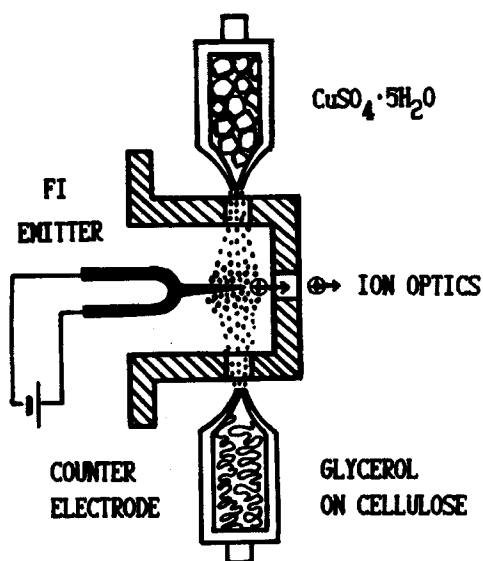


Figure 1. Samples introduction system of the field ionization ion source, dedicated to the study of intermolecular interactions in the gas phase.

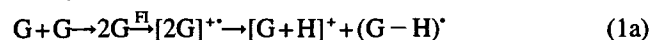
droplet was deposited into the second glass evaporator, where it was adsorbed on cellulose fibers to avoid hydrodynamic effects of sputtering of microdroplets of viscous liquid during pumping. The described sample inlet system can maintain the pressure in the ion source at a value $\leq 1 \cdot 10^{-6}$ Pa.

A sector magnetic mass spectrometer, MI-1201 ('Electron' Works, Sumy, Ukraine) was used.

RESULTS AND DISCUSSION

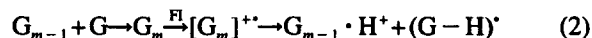
The measurements of FI mass spectra of the water-glycerol mixture were accompanied by some effects characteristic of polar substances. In the presence of an excess of glycerol, signals from water could not be detected and the total ion emission stopped a short time interval after applying the high field. To restore the ion emission, heating of the FI emitter up to nearly 100°C was necessary. The phenomenon described is connected with the known effect of the directed movement of polar molecules in the high electric field and field attraction.^{5,9} Glycerol molecules, having a dipole moment ($\mu=2.68\text{ D}^{10}$) higher than that of water (1.84 D),⁴ are adsorbed at the emitter surface first, thus shielding it from water molecules. At a certain thickness of the adsorbed layer field ionization is terminated. Optimization of the glycerol concentration and the emitter temperature allows one to obtain a stable ion current, but the intensity of water signals usually does not exceed the GH^+ ion intensity.

In the FI mass spectrum of neat glycerol the most abundant ion corresponds to GH^+ , m/z 93. There are also weak (<5%) ions G^+ , m/z 92, and $[\text{G-H}]^+$, m/z 91. The latter ion species is a witness to intermolecular interactions via hydrogen bonding and is formed by a mechanism:⁹



The abundance of protonated dimer ions G_2H^+ , m/z 185, is somewhat lower than that of monomer ions, GH^+ , but of the same order of magnitude, and is sensitive to variations in emitter temperature and field strength. Higher clusters, in contrast to what is observed in FAB, are absent. One should

keep in mind, however, that in the gas-phase FI conditions, neutral clusters are initially formed, and one of the components of this association serves as a source of a proton in the formation of the protonated species. Thus, the abundant G_2H^+ ion originates from virtually unstable trimer ($m=3$):



Fragmentation of glycerol under FI conditions is different from that in FAB: the main complementary fragments are m/z 31 $[\text{CH}_2\text{OH}]^+$ and m/z 61 $[\text{C}_2\text{H}_3(\text{OH})_2]^+$.¹¹

Water under FI at room temperature produces clusters $(\text{H}_2\text{O})_n\text{H}^+$, $n=1-5$.¹² Heavy water, under our experimental conditions, can undergo minor hydrogen-deuterium exchange with background (non-deuterated) water, and in this connection not just single peaks but groups of peaks with a number of masses equal to all those possible for every combination of light and heavy isotopes are present. The most abundant peaks are due to the ions D_3O^+ , m/z 22, and D_2HO^+ , m/z 21.

In the FI mass spectrum of a mixture of normal water and glycerol vapours, only one new peak different from those described for the monomers appears, corresponding to the protonated association of glycerol with one water molecule $\text{G} \cdot \text{H}_2\text{O} \cdot \text{H}^+$, m/z 111. Larger heteroclusters were not observed.

The following general mechanism of formation for hydrates of organic molecules has been proposed:⁹



According to this scheme, the GH^+ ion is formed from the monohydrate and the $[\text{G} \cdot \text{H}_2\text{O} \cdot \text{H}]^+$ ion from the dihydrate. Thus, it may be concluded, that glycerol can associate with one and with two water molecules in the gas phase. The addition of these two water molecules changes the electronic parameters of the glycerol molecule in such a way, that the interaction at its third hydrogen-bonding site (OH group) becomes unfavourable.

As to the possibilities of other mechanisms of formation of $\text{G}(\text{H}_2\text{O})_n\text{H}^+$ ions, it was proven earlier, that ion-molecule reactions characteristic of chemical ionization, e.g. clustering of neutral glycerol with H_3O^+ or proton transfer, are impossible under FI conditions due to the rapid extraction of the primary positive ions by the accelerating field. The time taken by an ion to cross the reaction zone near the emitter surface is of the order of $10^{-12}-10^{-13}$ s, which is insufficient for ion-molecule collisions which occur approximately once every 10^{-11} s, under the experimental conditions described.⁹

It should be mentioned that a certain fraction of the GH^+ ions may originate from glycerol dimers by Reaction (1a), but the ratio of the yields from Reactions (3) and (1a) is hard to estimate. The same is true of the formation of the protonated dimer of glycerol G_2H^+ , in which the proton may come either from the third glycerol molecule or from a water molecule. In any case, the absence of $[\text{2G} \cdot \text{H}_2\text{O} \cdot \text{H}]^+$ and higher clusters points to the possibility of the interaction of glycerol dimer with only one water molecule (resulting in G_2H^+ ions) and, essentially, a competition between hydration and dimerization of glycerol.

To obtain additional information about the source of protons in clusters, heavy water was used. But this does not give an unambiguous answer, as under these conditions the FI mass spectra undergo substantial changes connected with the hydrogen-deuterium exchange process, which are dependent on the relative quantity of heavy water and the

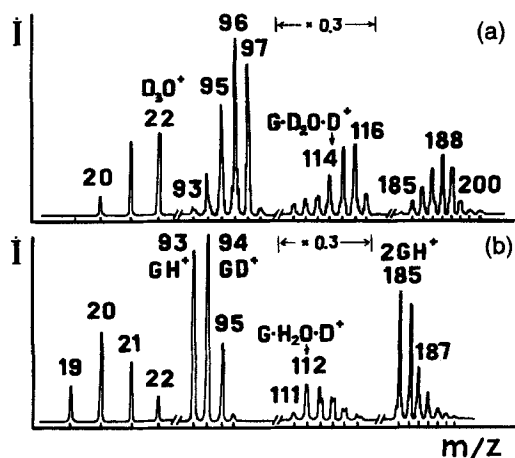


Figure 2. Fragments of FI mass spectra of the mixture of vapors of heavy water and glycerol in different concentrations (concentration of D_2O in (a) is higher than in (b)).

field strength. In Fig. 2(a) and (b), fragments of the FI mass spectra of water-glycerol mixtures with different ratios of normal to heavy water are presented. In these cases the masses corresponding to deuterated (XD^+ ions) of monomer, dimer, and hydrate are followed by groups of peaks, which point to the occurrence of a hydrogen-deuterium exchange process. The analogous groups of peaks at the masses expected for polyhydrates of glycerol monomer and dimer are absent.

The mass values in the groups of peaks show that all mobile hydrogens of the glycerol molecule may be substituted by deuteriums. At higher concentrations of D_2O the extent of exchange, naturally, increases (Fig. 2(a), (b)). The process of hydrogen-deuterium exchange may be described as follows. It is known, that the distribution of ions in FI mass spectra reflects the distribution of neutral particles, formed in the reaction zone near the surface of the FI emitter.⁹ In this zone, multiple collisions of the molecules take place, leading to formation and decay of hydrogen-bonded clusters. There is a certain possibility that, during the lifetime of a cluster based on the water-glycerol pair, an exchange event may occur. Several subsequent steps of formation and breaking of clusters may end in the substitution of the three mobile hydrogens on the OH groups of glycerol. The time-scale involved allows these processes. The mean lifetime of particles (molecules, clusters) in the neutral state near the FI emitter surface before they are ionized is of the order 10^{-6} s^{6,9} and, as mentioned earlier, the time interval between particle collisions is *ca.* 10^{-11} s. The time necessary for deuterium exchange when collision partners are in contact is roughly equal to the time for a proton transition between two positions in the hydrogen bond, which is *ca.* 10^{-14} s.

It is obvious, that the reverse exchange of deuteriums in the substituted glycerol molecule back to hydrogens can occur in the complex with any hydrogen-donating agent such as some other glycerol molecule or HOD species. It is hard to make a quantitative estimate of the contribution of all these processes to the final distribution of the exchange products, present in the spectra in Fig. 2. Nevertheless, one interesting peculiarity concerning the dimer of glycerol should be mentioned. The number of mobile hydrogens in such a dimer, plus the charge-donating proton/deuteron is equal to seven, but the peak corresponding to the presence of seven deuteriums in the dimer is somewhat lower than may be expected from isotopic distribution estimates. This

may be regarded as a sign of the larger residence time of one of the protons which is engaged in the stabilization of the dimer, by H-bond¹³ and thus temporarily excluded from the further steps leading to exchange.

The use of heavy water enabled us to register hydrogen-deuterium exchange between water and glycerol in the gas phase, but the exchange of protons, naturally, occurs in the case of ordinary water. In contrast to the liquid and gas phase, the proton transfer between water and glycerol molecules is absent in the case of solid frozen solutions. It is impossible to use heavy water to confirm this, as the hydrogen-deuterium exchange would be completed in the liquid before it freezes. But a characteristic feature of LT FAB, i.e. the abundant formation of $(H_2O)_nH^+$ water clusters and absence of glycerol characteristic ions at the lowest temperature range, provides evidence that the protons of water (ice) are not involved in the formation of GH^+ ions. The production of GH^+ ions only starts at a temperature value at which the molecular mobility in glycerol itself becomes sufficient for occurrence of ion-molecule reactions. It was mentioned earlier that the eutectic phase, containing both water and glycerol, may be solidified amorphously on rapid freezing down to liquid nitrogen temperature. The increase of molecular mobility on warming affects not only glycerol, but water molecules too. This allows water molecules, evenly spread in the amorphous eutectic, to assemble as ice crystals. The temperature value for the threshold of GH^+ ion sputtering from glycerol roughly coincides with the crystallization of water.^{1,3} Thus, this fraction of water is also excluded from direct intermolecular interactions with glycerol molecules in the solid state. The low intensity of $G \cdot H_2OH \cdot H^+$ hydrate obtained under FI conditions with a large excess of water demonstrates that the small quantities of the so-called strongly bound water, which may essentially remain bound to glycerol, are certainly insufficient for the formation of a detectable amount of heteroclusters, and may only make a very small contribution to the abundance of GH^+ .

As the possibility of clustering of water with glycerol in the gas phase has been demonstrated in the present work, it is evident that such gas-phase interactions are absent under LT FAB conditions. Obviously, the pressure of the spontaneously sublimed and sputtered particles over the surface of the frozen sample is insufficient for the mixing and association of water and glycerol in the gas phase.

The results of the above discussion lend support to the validity of the model based on sputtering of clusters of different substances from separate phases of the frozen solution under LT FAB conditions.

CONCLUSIONS

Analysis of the FI mass spectra of a gas-phase mixture of water and glycerol vapours provides information about the possibility of cluster formation between glycerol and one or two water molecules in the gas phase. Evidence is provided by the presence in the spectra of GH^+ and $G \cdot H_2O \cdot H^+$, formed due to decay of hydrate associates via characteristic mechanism.³ Hydrates of the glycerol monomer with a large number of water molecules as well as hydrates of the dimer (except for an uncertain preparation of G_2H^+) are absent in the spectra, which may be connected with the unfavourability of the existence of such structures in vacuum and the competition between hydration and dimerization processes for glycerol.

The use of heavy water demonstrated the high rates of

exchange for all mobile hydrogens in glycerol with deuterium in heavy water in neutral gas-phase associations.

The data obtained show that, under conditions which provide a sufficient concentration of water and glycerol in the gas phase mixture, mixed water-glycerol clusters are formed. The absence of this type of cluster under LT FAB conditions indicates that the above conditions are not fulfilled: simultaneous sputtering of water and glycerol to the gas phase is impossible due to phase separation in the frozen sample and spontaneous sublimation does not provide the necessary concentration of vapours over the sample surface at low temperatures. This provides support to the earlier proposed model of ion formation under LT FAB conditions¹ whereby the sputtering of water and glycerol (as well as any other component of the frozen water solutions) occurs independently from spatially separated surface domains of the frozen water-glycerol solution.

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REFERENCES

1. O. A. Boryak, M. V. Kosevich, V. S. Shelkovsky and Yu. P. Blagoy, *Rapid Commun. Mass Spectrom.* **9**, 978 (1995).
2. M. V. Kosevich, O. A. Boryak and V. S. Shelkovsky, *Abstr. 13th Informal Meeting on Mass Spectrom., 8-10 May 1995, Budapest, Hungary*, p. 29.
3. T. N. Yurchenko, V. F. Kozlova, B. A. Skornyakov, V. I. Strona and N. V. Repin, *The influence of cryoprotectors on biological systems*. Naukova Dumka, Kiev 1989 (in Russian).
4. F. Franks, *Water: a comprehensive treatise*, Vol. 7. *Water and aqueous solutions at subzero temperatures*. Plenum Press, New York, 1982.
5. M. V. Kosevich, V. S. Shelkovsky and S. G. Stepanian, *Biophysical Chemistry* **57**, 123 (1996).
6. L. F. Sukhodub, *Chem. Rev.* **87**, 598 (1987).
7. L. F. Sukhodub, V. S. Shelkovsky and K. L. Wierzhowski, *Biophys. Chem.* **19**, 191 (1984).
8. V. S. Shelkovsky and M. V. Kosevich, *Studia Biophys.* **136**, 193 (1990).
9. H.-D. Beckey, *Principles of field ionization and field desorption mass spectrometry*, Pergamon, London 1977.
10. K. D. Cook, P. J. Todd and D. H. Friar, *Biomed. Environ. Mass Spectrom.* **18**, 492 (1989).
11. K. A. Caldwell and M. L. Gross, *J. Am. Soc. Mass Spectrom.* **5**, 72 (1994).
12. L. F. Sukhodub, B. I. Verkin, V. S. Shelkovsky and I. K. Yanson, *Doklady Akademii Nauk USSR* **258**, 1414 (1981).
13. J. A. Sunner, R. Kulatunga and P. Kebarle, *Anal. Chem.* **58**, 1312 (1986).