

Water Clusters in Amorphous Pharmaceuticals

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ABSTRACT: Amorphous materials, although lacking the long-range translational and rotational order of crystalline and liquid crystalline materials, possess certain local (short-range) structure. This paper reviews the distribution of one particular component present in all amorphous pharmaceuticals, that is, water. Based on the current understanding of the structure of water, water molecules can exist in either unclustered form or as aggregates (clusters) of different sizes and geometries. Water clusters are reported in a range of amorphous systems including carbohydrates and their aqueous solutions, synthetic polymers, and proteins. Evidence of water clustering is obtained by various methods that include neutron and X-ray scattering, molecular dynamics simulation, water sorption isotherm, concentration dependence of the calorimetric T_g , dielectric relaxation, and nuclear magnetic resonance. A review of the published data suggests that clustering depends on water concentration, with unclustered water molecules existing at low water contents, whereas clusters form at intermediate water contents. The transition from water clusters to unclustered water molecules can be expected to change water dependence of pharmaceutical properties, such as rates of degradation. We conclude that a mechanistic understanding of the impact of water on the stability of amorphous pharmaceuticals would require systematic studies of water distribution and clustering, while such investigations are lacking. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association *J Pharm Sci* 103:2663–2672, 2014

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INTRODUCTION

Amorphous solid (glassy) states are ubiquitous in both natural (e.g., dehydrated drought- and frost-resistant organisms) and man-made or processed (e.g., freeze-dried pharmaceutical and biopharmaceutical) systems.^{1–9} In practically all the cases, such amorphous systems contain a certain amount of water, which can range from less than 1% (w/w) (as in freeze-dried materials) to 30% (w/w) or more, as with maximally freeze-concentrated solutions of sugars and proteins. Because of the ubiquity of amorphous states in pharmaceutical materials, and the fact that the presence of water has a major impact on their stability, there are numerous studies of various aspects of water relationships in amorphous solids. Major efforts have been devoted to the investigation of the impact of water on the molecular mobility, including the relationship between the glass transition temperature and water content^{10–12} and the interaction between water vapor and amorphous solids.^{13–16} More recently, the subject of the structure of amorphous solids has also attracted the attention of the pharmaceutical science community.^{17–20} In particular, the heterogeneous nature of amorphous solids, which are proposed to consist of domains with local (short-range) order resembling local arrangements in the crystal lattice, has been emphasized.¹⁸ The lack of translational and rotational coordination of molecules belonging to different domains results in the loss of the long-range order. The origin of this domain-structure concept is probably related to the Adam–

Gibbs theory²¹ of cooperatively rearranging clusters, with heterogeneity length scale corresponding to the cluster size.

In the reviews on the structure of amorphous pharmaceutical solids, however, water distribution has not been considered in any detail, and the intention of the present paper is to highlight this gap. In particular, one could raise several relevant questions, such as: (1) Are water molecules distributed homogeneously, or do they form clusters in certain conditions?; (2) What are conditions for cluster formation, that is, type of system, water content, and temperature?; (iii) What are the properties of these clusters, for example, size; Are they similar to three-dimensional droplets of a minor “phase” or two-dimensional chains of water molecules?; (iv) What is the impact of water clusters on the properties and performance of amorphous pharmaceutical materials? For example, it would be natural to expect that water impact on stability (both physical and chemical) could depend on the clustering patterns.

The paper starts with a brief overview of water distribution in solutions. This appears to be a necessary starting point as we consider the wealth of studies on water clustering and distribution in amorphous liquid systems. In addition, solution chemistry approaches were extended to describe particular features of amorphous pharmaceutical solids.^{22,23} After a consideration of the distribution of water in mixed solvent systems in section *Water Clusters in Solutions*, two important types of pharmaceutical glass-forming constituent materials, that is, polyhydroxycompounds (PHCs) and polymers, will be discussed in some detail in sections *Water Distribution in Concentrated Solutions of Sugars and Sugar Alcohols and Their Glasses* and *Water in Polymers*. The potential pharmaceutical relevance of water clustering will be highlighted in section *Significance for Pharmaceutical Science*, which will also emphasize the scarcity

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of direct studies of water clustering in pharmaceutical systems. We will conclude that water molecules can exist in unclustered form or as aggregates (clusters) of different sizes and geometry, both in solutions and amorphous solids. Clustering depends on water concentration, with unclustered water molecules existing at lower water contents, whereas clusters form at intermediate water contents. It is proposed that studies on water clustering would greatly facilitate the development of a quantitative understanding in such practically important areas as the relationships between water content and chemical degradation in freeze-dried and other amorphous pharmaceuticals, the stabilization of amorphous materials by annealing, and the cryopreservation of proteins and biopharmaceuticals.

WATER CLUSTERS IN SOLUTIONS

In contrast to other solid materials such as crystals and crystalline mesophases, amorphous solids do not possess long-range translational and rotational order. However, all condensed phases, including both amorphous solids (glasses) and amorphous liquids, have short-range structure (local ordering). Because of the structural similarity, and also considering the fact that many pharmaceutical glasses, such as freeze-dried powders, are produced from aqueous solutions, a brief consideration of the distribution of water molecules in the liquid state would be a logical starting point. In addition, there is emerging evidence that important features of chemical processes in glasses resemble those in solutions.²⁴

Various liquid systems exhibit clustering of solvent and/or solute molecules that results in inhomogeneity on the nm-length-scale.^{25–29} In particular, the concept of water clusters is central to the subject of the structure of water and aqueous solutions. In a seminal paper, Frank and Wen proposed that water consisted of flickering clusters of various size and shapes, with half-lives of 10^{-11} – 10^{-12} s.³⁰ The Frank–Wen model, with the addition of unclustered water proposed by Nemethy and Sheraga,³¹ is represented in Figure 1. More recently, a two-

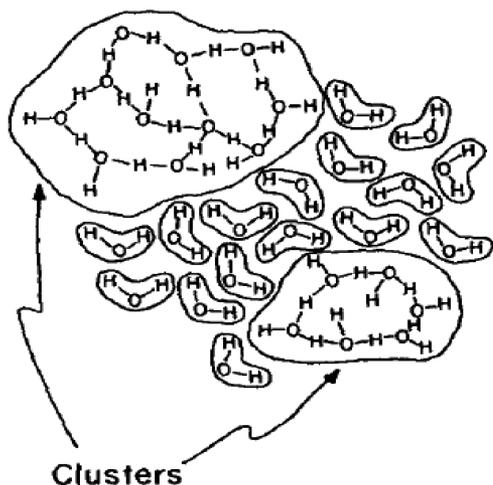


Figure 1. Schematic representation of the model of liquid water, showing hydrogen-bonded clusters and unclustered water molecules. The molecules in the interior of the clusters are tetracoordinated, but not drawn as such in this two-dimensional diagram. The representation is based on the Frank–Wen model of liquid water. The Figure is reproduced from Ref. 31 with permission of the AIP Publishing LLC.

fluid model of water and aqueous solutions has been suggested on the basis of quantum electrodynamic considerations,²⁵ in which coherent domains (or a coherent phase, CD) coexist with a noncoherent phase (NCP). In this model, CD molecules can be considered as equivalent to water clusters, whereas the NCP would represent unclustered water molecules.

It is commonly accepted that an understanding of local structure is essential in studies of various chemical and physical processes. In such fundamental processes, as proton transfer in water, for example, three-coordinated water molecules provide the most favorable configuration for proton transfer.³² Furthermore, a study of microscopic structure and clustering in water–alcohol mixtures was used to understand solvent effects in various physicochemical processes, such as fluorescence quantum yield for 10-hexylacridineorange, the fluorescence lifetime of acridine, the fluorescence pattern of pyrene, and the rate constant for the hydrolysis of t-BuCl.³³ In another example, the structure of water clusters, in particular, the formation of six-member rings with long-lasting hydrogen bonds (lifetime >2 ns), was shown to be essential to the nucleation of ice in supercooled water.³⁴ In addition, the size of the clusters associated with crystallization was observed to be 150–300 molecules, although the relatively small size of the system studied consisting of 512 water molecules might not be sufficient to make a solid conclusion about the size effects.

Even in highly miscible organic solvent–water mixtures, water and cosolvent molecules are not necessarily distributed homogeneously, as taken into account by various thermodynamics models of binary liquid solutions such as Wilson’s model, NRTL model, and UNIQUAC model. These models account for microheterogeneity; they assume that the local composition around a molecule of compound “1” is different from the local composition around a molecule of compound “2,” the local compositions being driven by the 1–1, 2–2, and 1–2 interaction energies.³⁵ In a systematic investigation of thermodynamic properties of water–alcohol systems,^{36,37} three composition regions have been identified in each of which the mixing behavior is qualitatively different from the other regions.³⁸ Region III, the low-water (water-poor) content region, is the most relevant from the perspectives of this paper. In this region, water behavior has been shown to depend on the molecular weight and chain length of the alcohol present. For smaller alcohol molecules, water molecules interact with the OH-groups of clustered alcohol molecules predominantly as single molecules. The water–water interaction is enthalpically unfavorable and entropically favorable for lower molecular weight alcohols. However, water–water interactions become more enthalpically favorable as water content increases. For larger alcohol molecules, water molecules form clusters around the alcoholic OH-groups even in the low-water region, water being more structured in these clusters than in bulk water.

A comprehensive investigation of water clusters in binary alcohol–water mixtures (methanol, ethanol, 1-propanol, and glycerol) at different temperatures has been performed by wide-angle neutron scattering.^{39–41} For example, water clustering was observed at a methanol concentration of 0.27–0.7 mol fraction, with the percolation threshold between 0.54 and 0.7 mol fraction of methanol.⁴⁰ It should be stressed again that formation of water clusters depends on water concentration, as shown in a neutron scattering study of water–glycerol solutions.⁴¹ At a lower water concentration of approximately 5 wt %, water exists as monomers with water molecules distributed

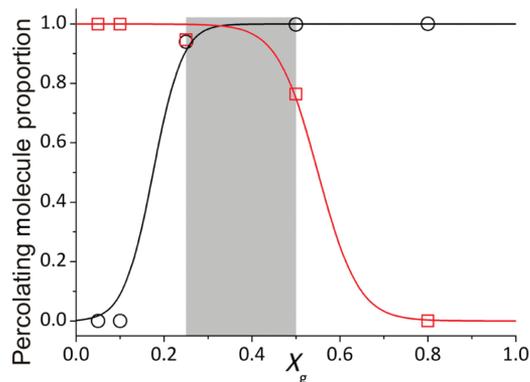


Figure 2. Proportion of molecules found in percolated clusters for glycerol (black circles) and water (red squares), obtained from wide-angle neutron scattering study of water–glycerol solutions. The gray region shows the concentration range for bipercolating clusters. Lines are shown as a guide for the eye. Reproduced from Ref. 41 with permission of the American Chemical Society.

homogeneously throughout the material, whereas water clusters with up to 50 molecules are observed at a molar glycerol fraction of 0.5 (16.4 wt % water). Note also that, in the clustering concentration region, water clusters coexist with non-clustered water molecules; for example, approximately 80% of water molecules exist as percolating clusters in glycerol–water systems at 16.4% water (Figure 2).⁴¹

This investigation of the glycerol–water system⁴¹ demonstrated that water clustering can be expected to depend on water concentration in any particular system, with a potential for a transition from clustering to single-molecule distribution when concentration of water is reduced below a threshold value.

Microheterogeneity has been observed by neutron scattering in water–acetonitrile solutions.⁴² Although this is a single-phase system above the upper critical solution temperature (272 K at molar fraction of acetonitrile of 0.38), a significant small-angle neutron scattering was observed at acetonitrile molar fraction of 0.3 and 0.4, with the acetonitrile–water correlation length of approximately 1 nm at 298 K. This finding has been confirmed by nuclear magnetic resonance (NMR)⁴³ and mass-spectrometry.⁴⁴ In particular, water clusters have been reported for water–acetonitrile mixtures at water mole fractions $X_w > 0.2$.⁴⁴ It was shown also that the local water concentration around solute molecules was different from the bulk concentration.⁴⁴ For example, for acetonitrile–water mixtures with bulk water mole fractions 0.5, 0.8, and 0.9, the local mole fractions of water around a hydrophobic solute, phenol, were decreased to 0.4, 0.5, and 0.7, respectively. One can speculate that such a reduction in the local water concentration at the solute molecule, if true, could have a significant impact on the rate of any hydrolytic process, the reaction rate being lowered by the reduced effective concentration of the water in accordance with the law of mass action.

Microscopic heterogeneity has also been suggested for aqueous solutions of the highly soluble solute dimethyl sulfoxide (DMSO). Liquid–liquid phase separation has been proposed in this system at molar water/DMSO ratios 15, 12, and 10 at higher pressures and lower temperatures $< -90^\circ\text{C}$ based on observation of two ice nucleation events.⁴⁵ The two phases were proposed to have different water structures, low-density liquid water like and high-density liquid water like. However, it

was not clear from the publication if these two phases were different in chemical composition (i.e., concentration of water). Also, there is an alternative explanation, which does not require liquid–liquid phase separation; as the study was performed at high pressure, the two ice nucleation events may be due to the sequential formation of high-pressure ice and hexagonal ice. In the same system, water clusters were reported at atmospheric pressure by mass spectrometry⁴⁴ in a similar concentration range, at water mole fractions $X_w > 0.93$ (water/DMSO molar ratio 13.3). In addition, microscopic liquid–liquid demixing in binary aqueous solutions of electrolytes has been suggested on the basis of molecular modeling, when the concentration of ions in the system was 5%–20%.⁴⁶ It has been proposed that the immiscibility is driven by the transformation of water to form a four-coordinated low-density amorphous (LDA) liquid. The dimension of the phase segregation was determined to be approximately 5 nm, and water fraction in the LDA phase of approximately 0.45, for the system with 10% of ions.

There are other examples of inhomogeneities and water clustering in aqueous mixtures of both low-molecular-weight materials and polymers. Aqueous solutions of such highly soluble solutes as glucose also may exhibit clustering and inhomogeneity.⁴⁷ Longer range inhomogeneity, on the micron-length scale, was reported for some polymer solutions.⁴⁸

To summarize the brief discussion of water clustering in solution: (1) water clusters have been observed in various mixed solvent systems and studied both experimentally by various methods and by molecular modeling; similar approaches can (and occasionally have been) applied to amorphous solids, as described in the following sections. (2) A transition from clustering to single-molecule distribution takes place when concentration of water is reduced below a certain (threshold) value; (3) water concentration corresponding to the transition from water clusters to nonclustered water depends on the cosolvent, as was demonstrated, for example, for water–acetonitrile (water clustering at $X_w > 0.2$) and water–DMSO (water clustering at $X_w > 0.93$).

WATER DISTRIBUTION IN CONCENTRATED SOLUTIONS OF SUGARS AND SUGAR ALCOHOLS AND THEIR GLASSES

Carbohydrates and other PHCs are widely represented in various pharmaceutical and biotech systems. They are used as cryoprotectors and lyoprotectors for proteins and other biological systems for frozen storage and freeze-drying, as well as excipients for solid dosage forms. For example, sucrose and trehalose are common lyoprotectors, sorbitol is used as a cryoprotector and as a stabilizer for freeze-dried formulations, and mannitol is used as a bulking agent for freeze-drying and as a diluent in tablets. Because of their practical importance, PHC warrant separate consideration.

An important insight into the structure of amorphous carbohydrate–water systems at nm and sub-nm levels has been obtained from molecular dynamics simulations. In computer simulation studies of concentrated water–monosaccharide⁴⁹ and water–sucrose solutions,⁵⁰ spatially isolated water clusters were found at lower water content, whereas the clusters became interconnected when the water concentration exceeded a percolation threshold. For the glucose–water system at 340 K, for example, the percolation threshold was estimated to be 18 wt %

water.⁴⁹ In another molecular dynamics simulation study,⁵¹ water clusters (defined as a set of water molecules connected to each other by at least one hydrogen bond with a defined geometric criterion) were studied in three disaccharides (trehalose, sucrose, and maltose) at concentrations up to 66 wt % sugar at relatively high temperatures (above 273 K). The weight-averaged size of water clusters was found to decrease from approximately 400 to approximately 20 water molecules, as the sugar concentration increased from 4 to 66 wt %.⁵¹ Molecular dynamics simulation studies of sugar–water systems revealed preferred water–water contacts at the expense of water–sugar contacts, with a loss of a percolating network of water–water hydrogen bonds near threshold sugar concentrations of approximately 60% (w/w).⁵⁰ Such results could be taken to support a model that predicts spatially isolated (but potentially temporarily connected) water “pockets” distributed in a sugar matrix.

Experimental, although indirect, support for the existence of water clusters in the water–sucrose system can be found in an NMR study where the temperature dependence of spin–lattice relaxation times (T_1) for water (D_2O) and sucrose was studied as a function of sucrose concentration.⁵² At high (>60 wt %) sucrose concentrations, the T_1 for water molecules became uncoupled from that for sucrose molecules when the temperature was near or slightly higher than the T_g , whereas at lower sucrose concentrations (<50 wt %), those of sucrose and water were coupled. Moreover, the T_1 values for water in the concentrated solutions were similar to that for pure D_2O . Based on those observations, it was suggested that sucrose molecules form a gel-like network, whereas water molecules retain at least their rotational mobility.⁵² Note that in the same study, it was reported that the “ideal glass transition temperature,” T_0 , for water in sugar glasses is approximately 115 K. Figure 3 represents the concentration dependence of T_0 for both water and sucrose (broken lines); it clearly shows a decoupling between the respective values for water and sucrose above approximately 60 wt % sucrose. Overall, the results indicate the decoupling of the mobilities of water molecules and the sucrose matrix. The findings, taken together, can be considered as indirect evidence of water clusters in which water molecules behave independently of the sugar matrix.

A thermally stimulated current (TSC) study of concentrated sorbitol–water and sucrose–water systems corroborated the NMR-derived conclusion on the low-temperature mobility of water in concentrated sugar solutions.⁵⁵ The TSC results reveal that there is a characteristic temperature (“ T_{gw} ”) associated with the onset of the mobility of water. The temperature range of the T_{gw} event was -125°C to -155°C at water concentrations 30–1 wt % (Fig. 3, rectangle symbols). The T_{gw} temperatures in sucrose–water and sorbitol–water systems are similar to the T_g values reported for the LDA, that is, -135°C to -150°C , as shown in Figure 3 by arrows. This observation of the similarity of the T_g in LDA and in sorbitol–water glasses is consistent with a recent neutron diffraction study, according to which the structure of water clusters in 70 wt % sorbitol solution was similar to that of LDA but different from the bulk water.⁶² The “ T_{gw} ” is apparently independent of the sugar type, as both sucrose and sorbitol samples furnished similar T_{gw} values at comparable water contents. From the concentration dependence of the T_{gw} , two regions could be identified, an intermediate water region (35%–10% water), and a low-water region (10%–0% water)

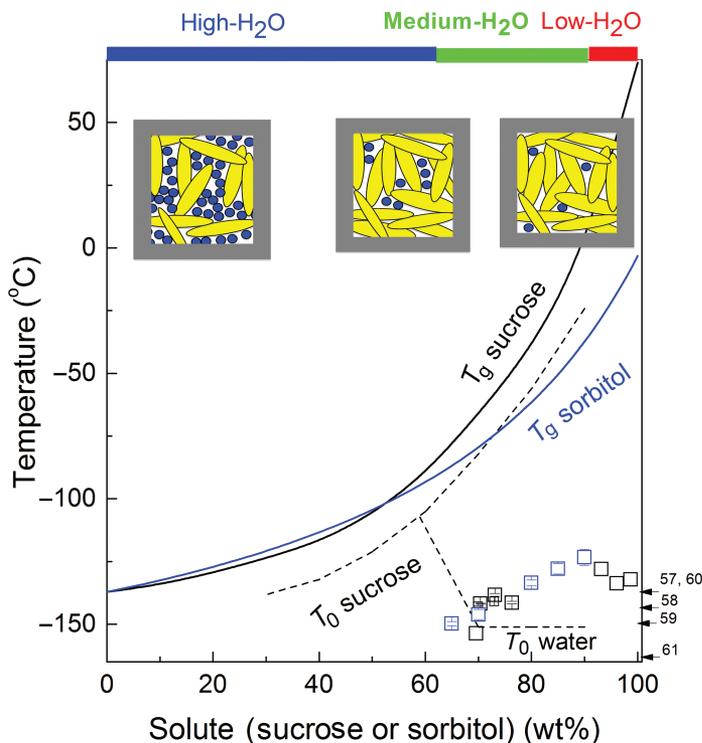


Figure 3. Calorimetric T_g and TSC events in sucrose–water (solid black line⁵³ and black symbols, respectively) and sorbitol–water (blue line and blue symbols, respectively) systems, and T_0 values for water–sucrose system (dotted lines). The T_g line for sorbitol was constructed by fitting the DSC T_g (onset) data from Refs. 54 and 55 to the Gordon–Taylor equation⁵⁶ with K as a fitting parameter $T_g = (w_1 T_{g1} + Kw_2 T_{g2}) / (w_1 + Kw_2)$ where T_{g1} and $T_{g2} = 136$ K are the glass transition temperatures and w_1 and w_2 are the weight fractions of sorbitol and water, respectively, and $K = 3.1 \pm 0.2$. The arrows indicate temperature of the T_g for pure water (-137°C for hyperquenched glassy water,⁵⁷ -144°C for the LDA form of pressure-amorphized hexagonal ice,⁵⁸ -149°C for LDA,⁵⁹ -138°C for vapor-condensate⁶⁰) and for the rotational mobility of water in hexagonal ice (Ih)⁶¹. Schematic representation of the microscopic structure of sorbitol–water system is presented at the top, with three regions of different water concentration. Water and sorbitol molecules are shown as blue and yellow objects, respectively. The figure is from a manuscript submitted to BOOK-ISOPW11 in 2011.⁵⁵

as represented schematically in Figure 3. In the intermediate water region, water mobility decreased (the T_{gw} increased) with a decrease in the water content, that is, the T_{gw} changes were parallel with the T_g changes in a semi-quantitative manner. In the low-water content region, however, water mobility did not change or even increased (i.e., T_{gw} decreased) with decrease in water content. One may therefore speculate that, based on the TSC results, water molecules form clusters (distribute inhomogeneously) in the “medium-water” region (more than 10 wt % water for sorbitol–water and sucrose–water systems), and that residual water is distributed monomerically (as unclustered water) in the “low-water” region.

In a recent study, concentrated sorbitol–water mixtures containing 70 wt % sorbitol were studied by wide-angle and small-angle neutron scattering (WANS and SANS) at temperatures between 298 and 100 K, that is, both above and below the calorimetric T_g ($T_g \sim 200$ K).⁶² WANS data were used to obtain the

site–site radial distribution functions (RDFs) and coordination numbers. This analysis revealed the presence of water clusters surrounded by (and interacting with) sorbitol molecules. The water clusters appear more structured compared with the bulk water and, especially at the lowest temperatures, resemble the structure of LDA ice. On cooling to 100 K, the peaks in the water RDFs become markedly sharper, with increased coordination number, indicating enhanced local (nm-scale) ordering while preserving the amorphous structure, with changes taking place both above and well below the T_g . On the mesoscopic (submicrometer) scale, although there are no changes between 298 and 213 K, cooling the sample to 100 K results in a significant increase in the SANS signal, which is indicative of pronounced inhomogeneities. This increase in the scattering is partly reversed during heating, although some hysteresis is observed. Furthermore, a power law analysis of the SANS data indicates the existence of domains with well-defined interfaces on the submicrometer length scale, probably as a result of the appearance and growth of microscopic voids in the glassy matrix. The SANS results provided another example of long-range inhomogeneity in aqueous solutions, and also suggested an intriguing possibility of a thermal memory retained by the glasses even after heating above the glass transition temperature.

WATER IN POLYMERS

Interaction of water with synthetic and biological polymers is a well-covered subject in scientific literature. A significant portion of publications is devoted to different “states” of water, with three “states” most commonly distinguished.⁶³ Any discussion of the merits of such classification is beyond the scope of this paper; instead, we consider evidence of water clustering (or the lack thereof) in several polymeric systems.

Clustering of water was studied in several hydrophilic polymeric systems with Zimm’s cluster function, G_{11}/v_1 .⁶⁴ The cluster function is defined via molecular pair distribution and represents the mean number of type 1 molecules in the neighborhood of a given type 1 molecule; it can be calculated from the concentration dependence of the activity coefficient of the solvent in question. The cluster size is determined as $c_1 G_{11} + 1$, where c_1 is the volume fraction of component 1 (solvent, e.g., water).⁶⁴ Figure 4 illustrates a major difference in solvent clustering behavior between different polymer–solvent systems. While solvent clusters are present in a benzene–rubber system at low solvent content of less than 0.01 volume fraction, the strongly negative value of the clustering integral for water–collagen is indicative of the lack of water clustering at water volume fraction values of 0.3 and below. The clustering function reaches a value of 1 (which corresponds to the onset of cluster formation) at water volume fraction of approximately 0.35,⁶⁵ as shown in Figure 4.

An approach for the determination of clustering in two-component systems was described by Zimm⁶⁴ and Zimm and Lundberg,⁶⁵ and applied to several polymer–water systems by Starkweather.⁵³ In that study, small water clusters of average size up to 3.5 water molecules [cluster size = $c_1 G_{11} + 1$, where c_1 and G_{11} are the volume fraction of component 1 (water) and the cluster integral, respectively] were observed in cellulose and several proteins (collagen, keratin, egg albumin, and serum albumin), albeit at higher relative humidity (RH) values. In

egg albumin, for example, clustering was observed at RH > 80%. Globular proteins (egg albumin and serum albumin) and cellulose formed water clusters at lower RH and lower water contents than fibrillar proteins (collagen and keratin). A similar approach was used to analyze the water sorption isotherm for maltodextrin–glycerol mixtures.⁶⁶ The Zimm–Lundberg clustering function was determined to be negative at the majority of water contents studied and was indicative of a highly dispersed state of water molecules (i.e., no water clusters) at water volume fraction below 0.06–0.08. It was further suggested that water likely forms small clusters at higher water contents and when the matrix enters the rubbery state.⁶⁶ One potential complication with the studies of water uptake by polymers should be noted. Relaxation of polymers is a slow process, which may take many months. Therefore, water sorption data, which are typically obtained within a few days, may not represent the equilibrium state. Indeed, one example of developing water clusters as a result of annealing was reported for nylon 66,⁵³ which is also considered in the next section.

Molecular dynamics simulation studies provide an additional insight into water distribution in polymers, although such investigations are somewhat limited by relatively small system sizes and short timescales. A polyvinylpyrrolidone (PVP)–water system was studied by molecular dynamics simulation at two different water contents, 0.5 and 10 wt %.⁶⁷ Water clusters were observed at 10 wt % water, whereas at the low water content of 0.5 wt %, clusters were not detected. At 10 wt % water, each water molecule had on average two neighboring water molecules, although a significant fraction of water molecules (approximately 0.1) remained unclustered. Consequently, one can expect a gradual transition from unclustered state of water to water clusters, with an increase in probability of clustering as the water content increases. Such gradual changes in the water structure were observed in the Raman study of PVP with various levels of hydration.⁶⁸ As the amount of water increases, the shape of the water peak starts more closely to resemble that of pure water. At the highest water content, the OH stretching region of water closely resembles that obtained for pure water.

In another polar and hydrogen-bonding polymer, poly(vinyl alcohol), no cluster formation was observed at water contents up to 2.6 and 5.2 wt % by MD simulations, with water molecules distributed homogeneously.⁶⁹

More hydrophobic polymers can be expected to have a higher tendency to form water clusters. In one study of the relatively hydrophobic polymer, poly(lactic-co-glycolic acid) (PLGA), differential scanning calorimetry (DSC) measurements of the concentration-dependent T_g were used to investigate polymer–water mixing behavior. In PLGA polymer, plasticization (decrease in the T_g) by water was observed in the water content range 0–2.6 wt %.⁷⁰ The plasticizing effect of water follows the Gordon–Taylor equation, which can be taken as a sign of an ideal mixing behavior, i.e., no water clustering. At a higher water content of 20%, the T_g was the same as at 2.6%, that is, water above approximately 3% does not have any impact on the T_g . This observation provides a strong indication of an aqueous phase separated from a hydrated polymer phase, thus suggesting an extreme case of water clustering.

The higher tendency of hydrophobic polymers to water clustering is consistent with the thermodynamic analysis of solvent

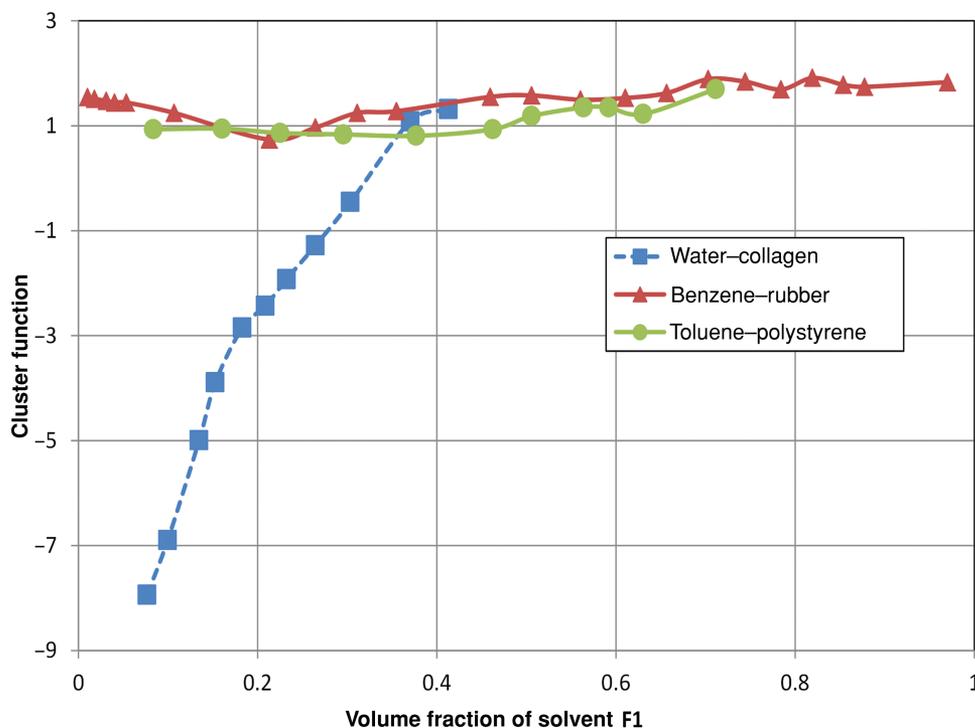


Figure 4. Clustering function of solvents versus volume fraction of solvents and polymers for benzene–rubber, toluene–polystyrene, and water–collagen at 25°C. Replotted from Ref. 65 with permission of the American Chemical Society.

clustering in polymers based on Flory–Huggins theory.⁷¹ It was shown that the amount of solvent clustering increases with an increase in the χ (interaction parameter), so that the average size of solvent cluster is greater for a poor solvent (such as water in hydrophobic polymers) than for a good solvent (such as water in hydrophilic polymers).

It should be noted that although there is substantial evidence of water clustering in both amorphous sugars and polymers, analysis of the water sorption data for several relevant systems results in a different conclusion (data not shown). In particular, Authelin⁷² has shown that the Type III water sorption isotherms, which are typical for amorphous solids including amorphous sorbitol up to 40 wt % water (Ref. 73, water sorption data from Ref. 74) and PVP9000 at up to 40 wt % water (Ref. 73, water sorption data from Ref. 75), would correspond to unclustered water molecules. This is different from the conclusions achieved in the neutron scattering study for sorbitol and the MD simulations for PVP, as discussed above. The apparent contradictions observed for two simple binary systems remains unexplained, and warrants further investigations.

SIGNIFICANCE FOR PHARMACEUTICAL SCIENCE

We should stress that there are very few direct studies of water clusters in pharmaceuticals, as outlined in this paper. Nevertheless, there are strong reasons to expect relationships between water clustering and stability of amorphous materials. In this section, we point out several potential mechanisms for such relationships, including heterogeneity and the impact of annealing on amorphous solids.

One probable consequence of the existence of water clusters is a heterogeneity in the interactions between water and the molecules of the active ingredient. The interactions can be expected to be different for water in the clusters versus unclustered molecules, and also could depend on the size of water clusters. Such heterogeneity would result in different populations of molecules of the active ingredient with different individual rate constants, especially for hydrolysis or water-catalyzed reactions. As the common experimental methods (e.g., HPLC) would measure bulk-averaged concentration of the reaction products or the extent of the conversion of a reactant, the apparent rate constant would represent a weight-averaged sum of the individual rates. In this case, even if the kinetics of an elementary reaction corresponds to a simple reaction order, for example, first order, the average kinetic curve would represent distribution of the individual rate constants, and therefore more complex kinetic curves can be expected. One such case was reported in Ref. 76 in which the impact of water on amide hydrolysis of a model drug, zoniporide, in the amorphous solid state was investigated and difference in the shape of the degradation curves between dry samples and samples with elevated water contents was noted. For a nominally dry sample (water content 0.1–0.3 wt %), the hydrolysis kinetics followed zero-order process, whereas the kinetic curves for samples exposed to RH 6% and 11% RH were best described using $C = f(t^n)$ dependence, where C is concentration of the degradant, t is time, and the exponent n was determined to be between 0.5 and 1. We hypothesize that this is the heterogeneity in water distribution, and therefore existence of species with different reactivity, which contributed to more complex kinetic curves observed for zoniporide samples with higher water content.

Properties of amorphous solids, because of their nonequilibrium nature, depend on their thermal and processing history, and it is reasonable to expect that the local structure may also be sensitive to the history of a system. In particular, annealing of glasses was demonstrated to reduce rates of chemical degradative processes,^{77–79} presumably by the creation of amorphous materials with lower free volume and lower molecular mobility. However, details of the structural changes leading to improved chemical stability are not fully understood. One may suggest that annealing can result in a change in water distribution, for example, an increase in water clustering that decreases water–matrix contacts and therefore rates of hydrolytic processes. In addition, water in clusters may have different structural arrangements (e.g., different extents of unsatisfied H-bonding ability) from unclustered water molecules, which may result in differences in the ability of water molecules to participate in hydrolytic chemical processes. An example of developing water clusters as a result of annealing was reported for nylon 66.⁵³ Water clusters were not initially observed in nylon exposed to 20% RH, whereas small water clusters were detected, based on the analysis of the water sorption isotherms, in an annealed sample. To expand the discussion on potential relationships between history and properties of amorphous materials, we should mention a relevant, and practically important, subject of preparing amorphous materials with variable water contents. A preparation of materials with different water levels is a common task in pharmaceutical R&D, which is used to study the impact of water on stability and setup water content specification for freeze-dried formulations. Two ways to achieve variable water contents are commonly used, that is, interruption of secondary drying during freeze-drying process and rehydration of a dried material through the gas phase. One can hypothesize that the “water history” could play a role in stability of amorphous materials, similar to the thermal history (annealing) mentioned above, and therefore difference in stability of amorphous materials having the same water content but prepared by two different methods (i.e., secondary drying interruption vs. rehydration) could be expected. However, a lack of studies of the impact of hydration history on amorphous structure (including water clustering and distribution), dynamics, and stability should be noted, as well as a few anecdotal evidences that chemical stability might be essentially independent of the hydration history in some cases.⁸⁰

As a special case of water redistribution in amorphous materials, partial crystallization of initially amorphous materials, which can take place during either manufacture or storage, should be mentioned. In such cases, water molecules are either expelled by growing crystals (e.g., crystallization of glycine, which forms anhydrous crystalline forms) or captured in the crystal lattice in cases when crystalline hydrates are formed (e.g., raffinose hydrate^{81,82}). Although crystallization of lyoprotectors is usually associated with destabilization of proteins^{83,84}, in part because of the increase in the local water content, there are cases when partially crystalline materials provide good stability both during processing and shelf-life. In such partially crystalline materials, timing of the crystallization, that is, if the crystallization takes place during initial cooling step of a freeze-drying process or during annealing, was shown to have a significant impact on storage stability of a protein,⁸⁵ possibly because of the difference in the local water distribution.

Comparisons of the sensitivity of various chemical processes in amorphous pharmaceuticals to water were performed in Ref. 24, and a wide range of water sensitivity was noted between different systems. For example, analysis of the data reported in Ref. 86 showed a weak impact of water on one of the beta-lactam compounds considered (cefalothin), which was in stark contrast to that for cefamandole, which demonstrated a much stronger water dependency. It was further suggested²⁴ that the cases with low water sensitivity (small changes in the reaction rate with change in water content) corresponded to water clustering, where increase in water content would result in growth of water cluster size without a significant increase in water–drug contacts, and therefore a weak sensitivity of the reaction rate to an increase in water content. One example of such leveling-off in the degradation rate with an increase in water content was reported in Ref. 87, where deamidation of a model tetrapeptide in amorphous polymeric matrixes was studied. The degradation rate increased with an increase in the water content from 6% to 8% (w/w), and then remained essentially constant while water content increased from 8% to 14% (w/w). Moreover, water clustering was suggested as a possible explanation of the “leveling” effect.⁸⁷ In another study, rate of chemical degradation of IgG was shown to be similar in a relatively wide water content range from 0.2% to 5.2%.⁸⁸ This result would be qualitatively consistent with water cluster formation, although we should note that a different interpretation was proposed in the original paper.

An important stability-related property is water mobility, and clustering could be expected to decrease mobility of water (as a result of increase in the cluster size). For poly-vinyl chloride, for example, it was suggested that clustered water lowered the diffusion coefficient, based on a comparison of the dynamic and static water sorption data.⁸⁹ Furthermore, diffusivity for the water cluster was reported to be one order of magnitude lower than that of a single water molecule in another hydrophobic polymer, polyethylene.⁹⁰ Interestingly, a different conclusion was achieved from the molecular dynamics simulations of hydrophilic glasses, that is, glucose and PVP, where water coordination was observed to increase water mobility.^{67,91} The opposite conclusions achieved for different systems indicate that the impact of clustering on water mobility may depend on the type of the system as well as on the diffusion length scale.

Water clustering and local water structure might also be relevant to cryoprotection of proteins. Protein molecules are commonly preserved in the frozen state with sugars and sugar alcohols. Such systems represent two-phase systems, consisting of ice and the freeze-concentrated solution, with water content ranging from typically 20 to 50 wt %, depending on the solutes present and the temperature. In a recent study of a representative system, that is, sorbitol–water glass containing 30 wt % water, water clusters with the structure resembling the LDA ice were observed. Since LDA was reported to have different properties from ordinary water, with salts being suggested not to be soluble in LDA,^{92,93} this could have significant implications for protein stability. If salts solubility is indeed inhibited, LDA would favor ion pairing (minimizing dissociation), thus disrupting electrostatic interactions known to be essential contributors to protein stability.

It should also be noted that water clusters may exist in crystalline pharmaceutical materials. For example, water clusters were reported for crystalline channel hydrates and other molecular crystals.^{72,94} Protein crystals represent similar type of

systems, with water molecules occupying well-defined “pockets” in the crystal structure. Although water ordering and cluster formation in such systems is important to our understanding of the physical stability of the crystal structure and chemical stability of active ingredients, a consideration of water clusters in crystalline materials is beyond the scope of the present paper.

CONCLUSIONS

Water clusters are reported in materials of various nature, including aqueous solutions of different solutes, amorphous sugars, and other PHCs, synthetic polymers, and proteins. Clustering depends on water concentration, with unclustered water molecules being found at lower water contents, whereas clusters are seen at higher, generally intermediate water levels. In amorphous sorbitol, for example, the transition from unclustered to clustered water is suggested to occur at approximately 10 wt % water,⁵² in PVP it appears to take place between 0.5 and 10 wt % water,⁶⁷ and in glycerol between water contents of 4.7 and 16.4 wt %.⁴⁰ In addition, analysis of the water sorption isotherms for some polymeric systems suggests that the transition from unclustered to clustered water may occur even at higher water content, for example, at approximately 35% water volume fraction for collagen.⁶⁵ It should be noted that these examples represent nonionizable solutes; ionic systems might have a different, and possibly lower, threshold concentration for water clustering.

The transition from water clusters to unclustered water molecules can be expected to result in a change in the water content dependence of various properties, such as degradation rates. In particular, change in the degradation rate with water content would probably be stronger in the “low-water” range (i.e., with unclustered water), and weaker when water clusters are present. We can conclude that although there is little doubt about the existence of clustered water in amorphous systems, systematic studies of water clustering in pharmaceutical materials are lacking. Furthermore, there are very limited (if any) discussions of merits of different approaches and methodologies to study water clusters, in particular in pharmaceutically relevant systems. These two gaps, that is, lack of studies on water clusters in pharmaceutical amorphous materials (such as lyophilized protein/sugar mixtures) and the deficiency with a clarity on which method(s) would be most appropriate to detect water cluster formation in pharmaceuticals, make it very difficult (if not impossible) to reach any definite conclusions on the extent of the water clustering in amorphous pharmaceuticals, water content threshold for cluster formation, and stability implications. A main goal of the present paper is to highlight these gaps in our understanding of structure of amorphous pharmaceuticals and to stress that without studies of relationships between water clustering and stability, the mechanistic understanding of the impact of water on the chemical and physical stability of amorphous pharmaceuticals would be incomplete.

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