

## The Effect of Geometrical Criteria on Hydrogen Bonds Analysis in Aqueous Glycerol Solutions

Cong Chen\*, Wei Zhong Li, Yong Chen Song, Lin Dong Weng and Ning Zhang

Key Laboratory of Ocean Energy Utilization and Energy Conservation of Ministry of Education, Dalian University of Technology, Dalian, Liaoning Province, P. R. China

### Abstract

Using a molecular dynamics simulation method, hydrogen bonds in aqueous glycerol solutions with different concentrations has been studied. The H...O distance was chosen as the basic definition of hydrogen bonds and the necessity of O...O distance, O-H...O angle and H-O...O angle was then analyzed. Results showed that O...O distance was not necessary in the hydrogen bonding definition when H...O distance was involved and O-H...O angle was a complementary geometrical constraint more strict than H-O...O angle. The mean numbers of hydrogen bonds per molecule (water and glycerol) and per atom (oxygen and hydrogen) and percentages of molecules and atoms with n hydrogen bonds have been reported and compared for different hydrogen bonding definitions. Definitions had a significant effect on hydrogen bonding analysis and the results for glycerol molecules were more sensitive to O-H...O angle and H-O...O angle than that for water molecules.

**Keywords:** Hydrogen bonds; Molecular dynamics simulation; Glycerol; Cryoprotectant agent

### Introduction

Glycerol has been chosen as a cryoprotectant agent (CPA) in this study for its capabilities in the long term preservation of cells and proteins and it is considered as one of the best cryoprotectant solvents [1]. Glycerol has been the subject of numerous studies both experimentally and numerically [1,5-12]. And there is an enormous amount of literature related to the investigation of hydrogen bonding in glycerol [5-12]. Although many studies of glycerol-water [13-21], glycerol-urea [22] and glycerol-water-sodium chloride [23-25] systems have been made, the studies of hydrogen bonding in glycerol solutions are rare [15].

In a classical molecular dynamics simulation, the definitions of hydrogen bond are in some way arbitrary due to the lack of information on the electron density [7]. Two criteria are often used for determining the hydrogen bonds, i.e., by energy and by geometry. With energetic criteria, a pair of molecules is defined as hydrogen bonded when the interaction energy is less than the threshold value [26]. With geometric criteria, the hydrogen bond is determined by the relative configuration of the two molecules [5,7,8,10,11]. The geometry of a hydrogen bond O-H...O involves an O...O distance, an H...O hydrogen bond length, an O-H covalent bond length, an O-H...O hydrogen bond angle, an H-O...O angle and an O...O...H angle, five of which are related with hydrogen bonds except the O-H covalent bond length which is determined by the chosen water model.

Atoms H and O are defined as hydrogen bonded when the O...O distance or H...O distance is less than the threshold value and limitation to the angle O-H...O or H-O...O are often used concomitantly. During the molecular dynamics study of glycerol and its solutions, the geometric criteria have been mainly used, but the criteria are quite different as summarized in Table 1 [7-9,11,27-29]. So, the purpose of the study is to evaluate the effect of geometrical criteria on hydrogen bonds analysis in aqueous glycerol solutions including the mean numbers of hydrogen bonds per water (glycerol, oxygen atoms and hydrogen atoms) and the percentages of water (glycerol, oxygen atoms and hydrogen atoms) with n hydrogen bonds  $f_n$  ( $n=0,1,2,\dots$ ).

### Computation details

Molecular dynamics simulations have been carried out with the

molecular dynamics package NAMD [30] using CHARMM22 format force field for glycerol employed by Reiling [31] and the SPC/E water model. A hexagonal ice lattice with 768 water molecules was generated using the procedure proposed by Hayward [32] and then melted for 600 ps at constant temperature 300 K and constant pressure 1.0 bar. The water box with a density of nearly 1 g/cm<sup>3</sup> was then replicated for two times in x and y dimensions to construct a larger box with 3072 water molecules. The water box with 3072 molecules is used to prepare the glycerol/water boxes by adding glycerol molecules at random positions. The glycerol molecules are randomly selected from an equilibrated glycerol box. The number of glycerol molecules added depends on the desired concentration of glycerol for each simulation box. Water molecules overlapping glycerol within 1.0 Å and additional randomly selected water molecules are removed to approximate the desired glycerol concentration.

Standard techniques for periodic boundary conditions and neighborhood lists have been applied. The neighborhood lists distance is 13.5 Å and lists are updated every 10 time steps with a time step of 2 fs. The nonbonded interactions are truncated using a switching function between 10.0 and 12.0 Å. The SHAKE algorithm [33] is used to fix the water molecule geometry and covalent bonds between hydrogen and the heavy atoms. Coulombic interactions are computed by the particle mesh Ewald method [34] every two time steps. Initial velocities are generated randomly from a Gaussian distribution. Multiple time step integration technique r-RESPA [35] is adopted to integrate the equations of motion. All simulations are performed in the NPT ensemble where the number of molecules N, pressure P and temperature T are fixed. The pressure is set to 1.0 bar using the

\*Corresponding author: Cong Chen, Key Laboratory of Ocean Energy Utilization and Energy Conservation of Ministry of Education, Dalian University of Technology, Dalian, Liaoning Province, P. R. China, 16024, E-mail: [congchen@dlut.edu.cn](mailto:congchen@dlut.edu.cn)

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| Literature | Solutions studied                           | O...O                  | H...O                  | O-H...O           | H-O...O              |
|------------|---|------------------------|------------------------|-------------------|----------------------|
| [27]       | Galanin in aqueous and nonaqueous solution  |                        | <2.4                   |                   |                      |
| [7]        | Glycerol condensed phases                   |                        | <2.45                  | >145 <sup>a</sup> |                      |
| [8, 28]    | Water and alcohols, aqueous ionic solutions | <3.4                   | <2.425                 |                   | <30                  |
| [11]       | Glycerol                                    |                        | <2.55                  |                   | <35(65) <sup>b</sup> |
| [29]       | Aqueous NaCl solutions                      | <3.5                   | <2.45                  |                   | <45                  |
| [9]        | Liquid alcohols                             | <3.4(3.5) <sup>c</sup> | <2.4(2.6) <sup>d</sup> |                   | <30                  |

a For intermolecular hydrogen bonds only;  
 b 35 for intermolecular hydrogen bonds and 65 for intramolecular hydrogen bonds;  
 c 3.4 for water molecules and 3.5 for alcohol molecules;  
 d 2.4 for water molecules and 2.6 for alcohol molecules

**Table 1:** Definitions of hydrogen bonds in literature (distance unit: Angstrom, angle: degree).

Langevin piston Nose-Hoover method which is a combination of the Nose-Hoover constant pressure method [36] with piston fluctuation control implemented using Langevin dynamics [37]. The temperature is maintained to 300 K using Langevin dynamics [38] with a damping coefficient of 5/ps. A first 1000 steps run is performed to minimize the system energy and then a 1 ns cycle is repeated until thermal equilibrium was reached at the desired starting conditions. An additional 1 ns cycle is run to analyze hydrogen bonding. Six boxes with different glycerol concentrations (1.75, 2.9, 4.6, 5.59, 7.49 and 8.54 M) are generated and simulated.

## Results and Discussions

### The distributions of hydrogen bonds related lengths and angles

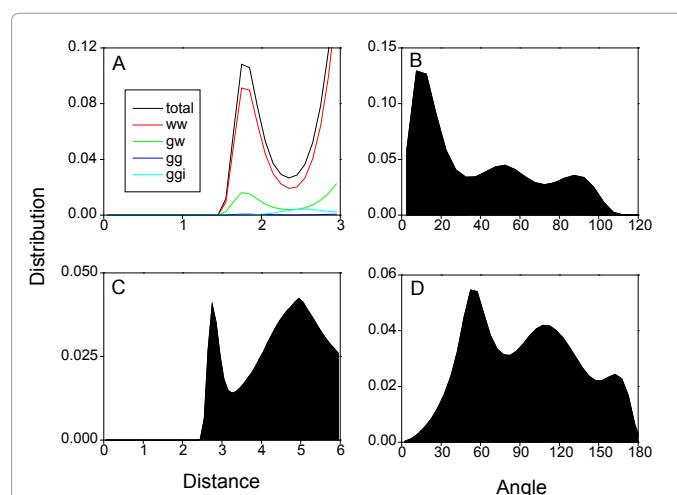
The average distributions of hydrogen bonds related lengths and angles have been calculated. The results for H...O distance (intermolecular), H-O...O angle, O...O distance and O-H...O angle have been illustrated in Figure 1. The distributions vary slightly with glycerol concentration and so only the results when glycerol concentration is 2.9M have been given. As can be seen, there is a peak at about 1.8 Å in H...O distance distribution function and the peak is a sufficient proof of hydrogen bonds. The position for the first minima is 2.4 Å which is usually chosen as the cutoff of hydrogen bonds in geometry criteria. The contributions from water-water (ww), glycerol-water (gw), different glycerol molecules (gg) and the same glycerol molecules (ggi) have also been given in Figure 1(A). It is clear that the positions for the first minima for total, ww and gw curves are nearly the same. It's reasonable to choose the same cutoff for water-water and glycerol-water hydrogen bonds. The distribution function of O...O distance is quite similar to that of H...O distance. The position for the first minima is 3.4 Å. The distributions of H-O...O and O-H...O angle are quite different. H-O...O angle ranges from 0 to 100° and never exceeds 110°. But the distribution of O-H...O angle is quite broad ranging from 0 to 180°. The distribution of H...O...O angle is illustrated in Figure 2. The H...O...O angle is quite small. Most of them are smaller than 20 and never exceed 30°. This is the reason that H...O...O angle is usually not involved in the definition of hydrogen bonds. So, in the following analysis, H...O...O angle will not be included.

### The necessity of O...O distance, H-O...O angle and O-H...O angle

As can be seen in Table 1, in all definitions, the H...O distance has been included. So, in the present study, the H...O distance has been chosen as the basic definition of hydrogen bonds. Is it enough to define hydrogen bonds using the single criteria? To answer this question, the necessity of other potential criteria should be analyzed. The distributions of O...O distance, H-O...O angle and O-H...O angle when the H...O distance is smaller than its cutoff have been calculated. Seven cutoff values of H...O distance have been used ranging from 1.8

to 3.0 Å with 0.2 Å larger each. The results for O-O distance, H-O...O angle and O-H...O angle when glycerol concentration is 2.9 M and H...O distance is smaller than 2.4 Å have been illustrated in Figures 3-5 respectively. The contributions from water-water (ww), glycerol-water (gw), different glycerol molecules (gg) and the same glycerol molecules (ggi) have also been given.

As can be seen in Figure 3, O...O distance is always smaller than 3.4 Å, which is exactly 1.0 Å larger than H...O distance cutoff. In fact, the same results have been gained in all cutoff values and glycerol concentrations. That's to say, O...O distance is NOT necessary in the hydrogen bonding definition when H...O distance has been involved. H-O...O angle ranges from 0° to 60° (see Figure 4) and O-H...O angle ranges from 100° to 180° when H...O distance is smaller than 2.4 Å (see Figure 5). The similar results have been gained in other H...O distance cutoff values and glycerol concentrations. The cutoff values of H-O...O angle are usually 30° [8, 28], 35° [11] and 45° [29] and the cutoff value of O-H...O angle is usually 145° [7]. Thus, H-O...O angle and O-H...O angle are necessary in the hydrogen bonding definition of glycerol aqueous solutions studied here. The distribution of H-O...O angle when O-H...O angle is larger than 145° and H...O distance is smaller than 2.4 Å has been calculated and illustrated in Figure 6. As can be seen, H-O...O angle is always smaller than 30°. The distribution of O-H...O angle when H-O...O angle is smaller than 30° and H...O distance is smaller than 2.4 Å has been drawn in Figure 7. As can be



**Figure 1:** The distributions of hydrogen bonds related lengths and angles when glycerol concentration is 2.9M: H...O distance (A), H-O...O angle (B), O...O distance (C) and O-H...O angle (D). Note that the contributions from water-water (ww), glycerol-water (gw), different glycerol molecules (gg) and the same glycerol molecules (ggi) have also been given for H...O distance distribution. The distributions for other glycerol concentrations are similar and have not been illustrated here.

seen, O-H...O angle is not always larger than 145°, which indicates that O-H...O angle is a complementary geometrical constraint more strict than H-O...O angle.

### The effect of geometrical criteria on hydrogen bonding analysis

The mean numbers of hydrogen bonds per molecule (atom)  $n_{HB}$  and the percentages of molecules (atoms) with  $n$  hydrogen bonds  $f_n$  play an important role in understanding hydrogen bonding network in solutions. To study the effect of geometrical criteria on hydrogen bonding analysis,  $n_{HB}$  and  $f_n$  with different hydrogen bond criteria have been calculated and compared. The results are similar for the glycerol concentrations studied and only the results for one glycerol concentration have been illustrated here. H...O distance  $R_{OH}^c$  is

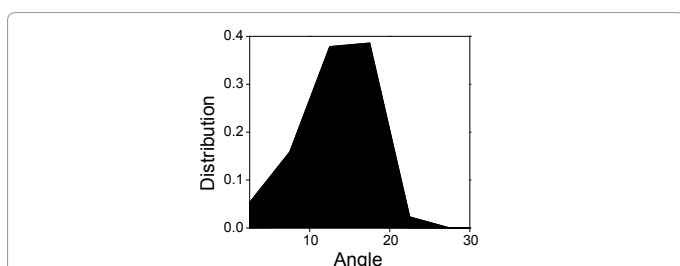


Figure 2: The distribution of H...O...O angle when glycerol concentration is 2.9 M.

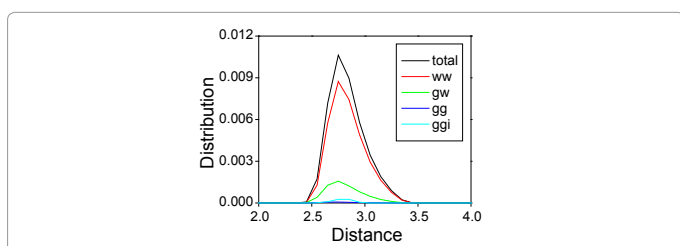


Figure 3: The distribution of O...O distance when glycerol concentration is 2.9M and H...O distance is smaller than 2.4 Å.

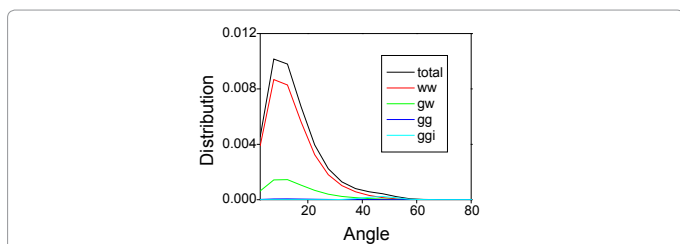


Figure 4: The distribution of H-O...O angle when glycerol concentration is 2.9M and H...O distance is smaller than 2.4 Å.

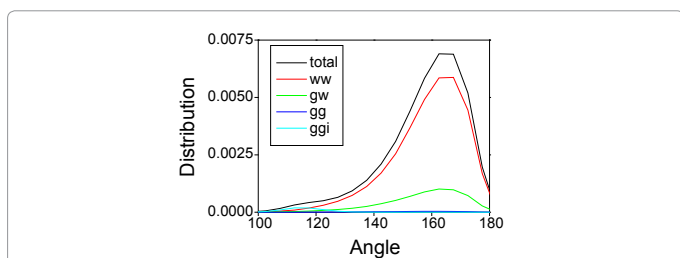


Figure 5: The distribution of O-H...O angle when glycerol concentration is 2.9M and H...O distance is smaller than 2.4 Å.

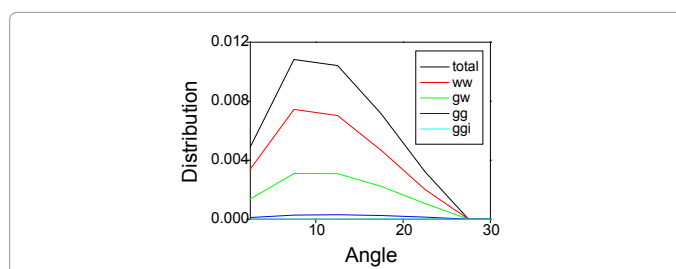


Figure 6: The distribution of H-O...O angle when O-H...O angle is larger than 145° and H...O distance is smaller than 2.4 Å (glycerol concentration is 7.49 M).

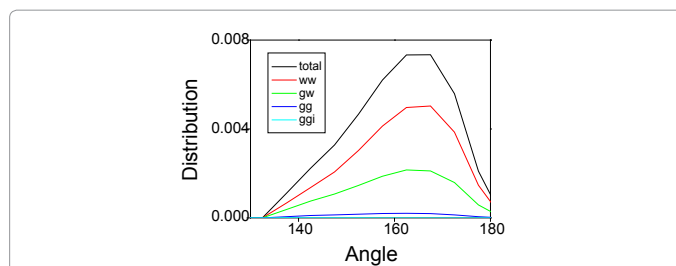


Figure 7: The distribution of O-H...O angle when H-O...O angle is smaller than 30° and H...O distance is smaller than 2.4 Å (glycerol concentration is 7.49 M).

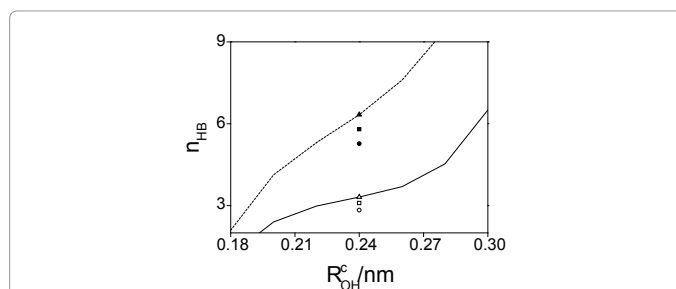


Figure 8: The mean numbers of hydrogen bonds per water (line) and per glycerol (dashed line) as a function of H...O distance cutoff  $R_{OH}^c$  (Å) as well as results for three additional definitions (glycerol: closed, water: open; AD1: square, AD2: circle, AD3: triangle). The glycerol concentration is 1.75 M.

recognized as a reference definition and three additional definitions have also been given:

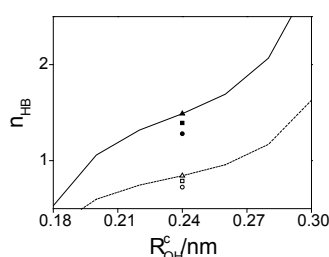
- (1) AD1: H...O distance is smaller than 2.4 Å and H-O...O angle (intermolecular) is smaller than 30° (without intramolecular constraints);
- (2) AD2: H...O distance is smaller than 2.4 Å and O-H...O angle is larger than 145°;
- (3) AD3: H...O distance is smaller than 2.4 Å and H-O...O angle (intramolecular) is smaller than 65° (without intermolecular constraints).

The mean numbers of hydrogen bonds per water/glycerol/oxygen/hydrogen under different hydrogen bonding definitions have been shown in Figures 8 and 9. As can be seen, the mean number of hydrogen bonds increases with the H...O distance cutoff value. The increasing speeds for glycerol molecule and oxygen atom are faster than that for water molecule and hydrogen atom respectively. The effect of AD2 is most significant, and AD3 nearly has no effect.

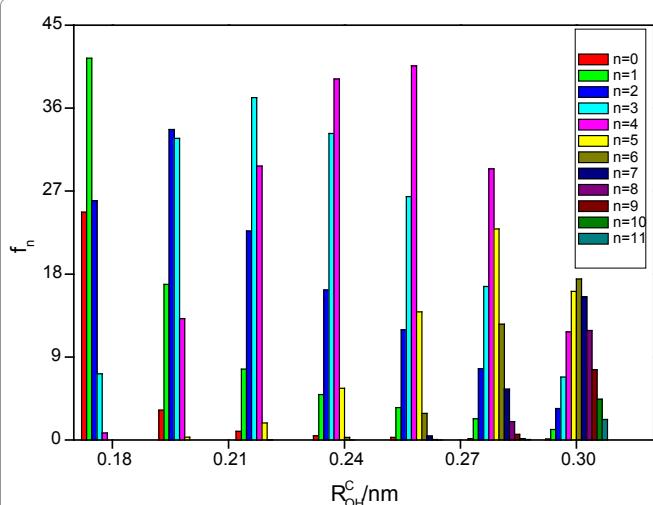
The percentages of water/glycerol molecules and oxygen/hydrogen atoms with  $n$  hydrogen bonds as a function of H...O distance cutoff

values have been illustrated in Figure 10 to 13 respectively. Two characteristic values  $i$  and  $j$  ( $i < j$ ) exist: as H...O distance cutoff value increases, when  $n \leq i$ , the percentages  $f_n$  decreases; when  $n \geq j$ ,  $f_n$  increases; when  $i < n < j$ ,  $f_n$  first increases and then decreases. This implies that as H...O distance cutoff value increases, the number of hydrogen bonds increases, which is consistent with the trends of mean number of hydrogen bonds as shown in Figures 8 and 9. The characteristic values  $i$  for water, glycerol, oxygen and hydrogen are 1, 3, 0 and 0 respectively. The values  $j$  for water, glycerol, oxygen and hydrogen are 6, 11, 3 and 2 respectively.

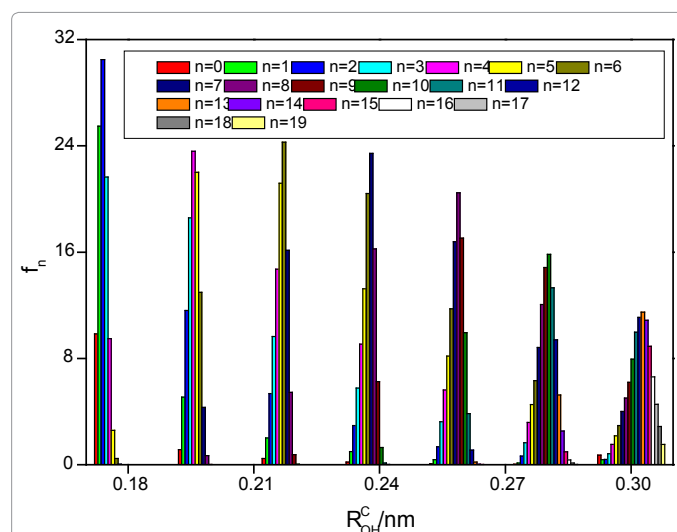
The percentages of water/glycerol molecules and oxygen/hydrogen atoms with  $n$  hydrogen bonds for AD1, AD2 and AD3 have been illustrated as well as the results for  $R_{OH}^c = 2.4 \text{ \AA}$ , as shown in Figure 14. The effects of AD1, AD2 and AD3 are similar as that in Figures 8 and 9. A conclusion can be made that hydrogen bonds are more sensitive to O-H...O angle than to H-O...O angle and this conclusion is consistent with that of necessity analysis: O-H...O angle is a complementary geometrical constraint more strict than H-O...O angle. The effect of AD1 and AD2 on glycerol is more significant than that on water. In fact, according to Masakazu's topological analysis [26], a simple decision by intermolecular O-H distance is most affordable. However, the results for glycerol molecules are more sensitive to O-H...O angle and H-O...O angle because glycerol interact with water molecules in



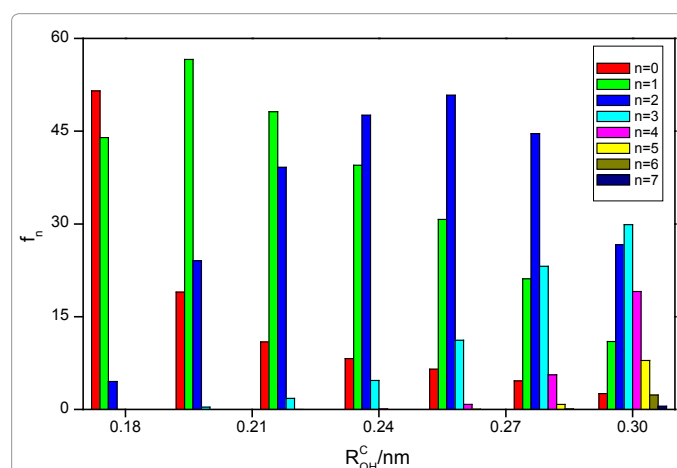
**Figure 9:** The mean numbers of hydrogen bonds per oxygen atom (line) and per hydrogen atom (dashed line) as a function of H...O distance cutoff  $R_{OH}^c$  (Å) as well as results for three additional definitions (oxygen: closed, hydrogen: open; AD1: square, AD2: circle, AD3: triangle). The glycerol concentration is 5.59 M.



**Figure 10:** The percentages of water molecules with  $n$  hydrogen bonds ( $n=0,1,2,\dots,11$ ) as a function of H...O distance cutoff  $R_{OH}^c$  (Å). The glycerol concentration is 4.6 M.



**Figure 11:** The percentages of glycerol molecules with  $n$  hydrogen bonds ( $n=0,1,2,\dots,19$ ) as a function of H...O distance cutoff  $R_{OH}^c$  (Å). The glycerol concentration is 4.6 M.



**Figure 12:** The percentages of oxygen atoms with  $n$  hydrogen bonds ( $n=0,1,2,\dots,7$ ) as a function of H...O distance cutoff  $R_{OH}^c$  (Å). The glycerol concentration is 5.59 M.

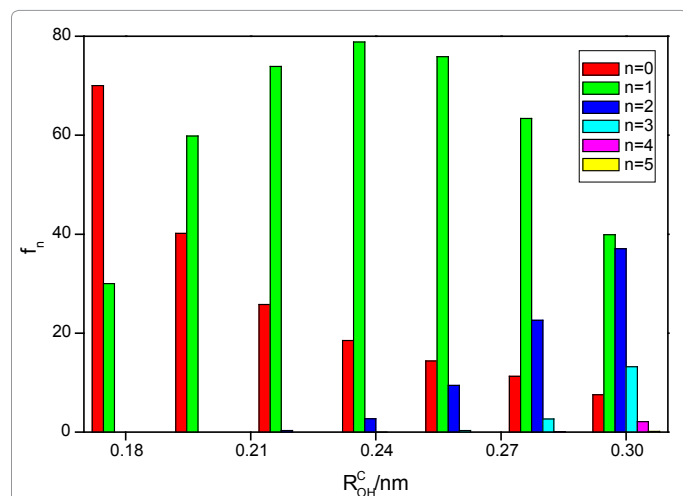
more linear hydrogen bonds to mimics the hydrogen bonding that would form in ice [15].

## Conclusions

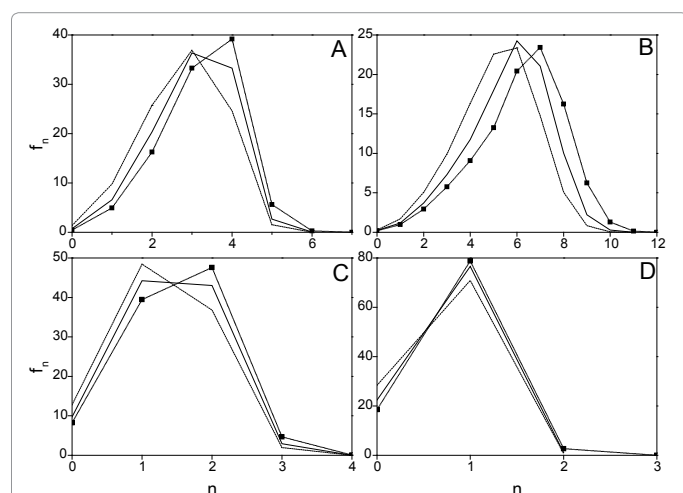
Although the geometric criteria have been mainly used during hydrogen bonding studies in aqueous solutions, the criteria are different. To evaluate the effect of geometrical criteria, hydrogen bonds analysis in aqueous glycerol solutions including the mean numbers of hydrogen bonds per water/glycerol/oxygen/hydrogen  $n_{HB}$  and the percentages of water/glycerol/oxygen/hydrogen with  $n$  hydrogen bonds  $f_n$  ( $n=0,1,2,\dots$ ) have been made compared with different hydrogen bonds definitions.

According to necessity analysis, O...O distance is not necessary in the hydrogen bonds definition when H...O distance has been involved and O-H...O angle is a complementary geometrical constraint more strict than H-O...O angle.

Definitions have a significant effect on hydrogen bonding analysis. Hydrogen bonds are more sensitive to O-H...O angle than to H-O...O



**Figure 13:** The percentages of hydrogen atoms with  $n$  hydrogen bonds ( $n=0,1,2,\dots,5$ ) as a function of  $H\dots O$  distance cutoff  $R_{OH}^c$  (Å). The glycerol concentration is 5.59M.



**Figure 14:** The percentages of molecules (A: water; B: glycerol) and atoms (C: oxygen; D: hydrogen) with  $n$  hydrogen bonds  $f_n$  as a function of  $n$  for hydrogen definition AD1 (lines), AD2 (dashed lines) and AD3 (dotted lines) as well as the results for  $R_{OH}^c = 2.4$  Å (squares). The glycerol concentrations are 4.6M for A and B and 5.59M for C and D.

angle and this conclusion is consistent with that of necessity analysis. Hydrogen bonds related with glycerol molecules are more sensitive to O-H...O angle and H-O...O angle than that for water molecules because glycerol interact with water molecules in more linear hydrogen bonds to mimics the hydrogen bonding that would form in ice. To make an accurate hydrogen bonding analysis, a suitable hydrogen bonding criteria must be found. In aqueous glycerol solutions, a complementary geometrical constraint with O-H...O angle or H-O...O angle must be given along with H...O distance.

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