CHAPTER 6

RESULTS AND DISCUSSION

In this Chapter, characteristics of the selected basis sets and the newly developed glucosamine-water potential have been reported. The structural properties of the solution have been discussed on the basis of the radial distribution functions, the corresponding integration numbers, distribution of coordination numbers, probability densities, etc.

6.1 Selection of the Suitable Basis Set for the SCF Calculations

In table 6.1, characteristics of the *ab initio* calculations for various basis sets, STO-3G, 3-21G, 6-31G, DZ, DZP and cc-pVTZ, performed with and without correction due to the basis set superposition error (BSSE) are summarized.

Taking into consideration the influences of the small basis set and of the BSSE, it is clear that, in term of stabilization energy and distance to the minimum (from oxygen atom of water molecule to center of glucosamine molecule) as well as their changes due to the BSSE, the STO-3G, 3-21G and 6-31G basis sets are too small for the investigated system while the DZ, DZP and cc-pVTZ basis sets give comparable results. The DZ basis set yield too high dipole moment of water as compare to the experimental measurement [47] and the cc-pVTZ basis set leads to the computational time of about 28 times longer than the DZP basis set. Therefore, the DZP basis set seems to be the most suitable one for our system.

TABLE 6.1 Total energy (E in atomic units), molecular dipole moment (μ in Debye), optimal stabilization energy with and without BSSE corrections (ΔE and ΔE_{BSSE} in kcal.mol^{-1}), corresponding distance from the O atom (of water molecule) to the center of glucosamine molecule (r and r_{BSSE} in Å), and CPU time for glucosamine-water complex on a PC LINUX PIII 550 (in hour:min:second), calculate from various basis sets.

Basis set	Water		Glucosamine-Water					
	μ	E	E	r	ΔE	r_{BSSE}	ΔE_{BSSE}	CPU time
STO-3G	1.73	-74.96	-729.64	3.88	-6.49	4.14	-2.25	00:00:23
3-21G	2.44	-75.59	-735.26	3.94	-15.23	4.03	-8.02	00:02:22
6-31G	2.63	-75.98	-739.06	4.01	-10.70	4.06	-8.41	00:04:07
DZ	2.68	-76.01	-739.22	4.02	-9.77	4.06	-8.17	00:13:37
DZP	2.23	-76.05	-739.59	4.04	-7.36	4.07	-6.34	00:41:10
cc-pVTZ	2.03	-76.06	-739.69	4.07	-6.76	4.10	-5.39	17:46:28

* Experimental dipole moment of water molecule is 1.85 Debye [47].

Consider an error due to the unbalance of the basis set, the *ab initio* results with and without including the Boys-Bernardi counterpoise correction using the DZP basis set in the configuration shown in Figure 5.2 are exhibited in Table 6.2 and plotted in Figure 6.1. As expected, the calculations using the DZP basis set without the BSSE correction cause too low stabilization energy of the complex. The energy minimum and the corresponding distance are shifted from –7.36 kcal.mol^{-1} and 4.04 Å to –6.34 kcal.mol^{-1} and 4.07 Å.

Table 6.2 Stabilization energies calculated using DZP basis set with and without BSSE corrections (ΔE_{BSSE} and ΔE, respectively, in kcal.mol^{-1}) at different distances (r, in Å) for the glucosamine-water configuration given in Figure 5.2.

r	ΔE	ΔE_{BSSE}
3.21	11.29	12.82
3.44	1.51	2.90
3.54	-1.38	-0.05
3.64	-3.81	-2.55
3.70	-4.81	-3.59
3.76	-5.65	-4.46
3.82	-6.32	-5.16
3.88	-6.82	-5.69
3.94	-7.15	-6.06
4.01	-7.34	-6.27
4.04	-7.36	-6.32
4.07	-7.35	-6.34
4.10	-7.33	-6.32
4.14	-7.27	-6.27
4.21	-7.06	-6.10
4.28	-6.77	-5.84
4.35	-6.42	-5.51
4.42	-6.01	-5.12
4.64	-4.65	-3.83
5.03	-2.55	-1.86
5.85	-0.35	-0.06
7.63	-0.01	0.00
9.50	0.00	0.00
10.45	0.00	0.00

It is cleared that the difference in the position of the minimum is almost negligible. Although the DZP yields an error of about 15% in terms of stabilization energy, however, it is known that the simulation results, especially structural data, are not sensitive to the energy of the system. Therefore, the DZP basis set without applying the BSSE correction is selected and used to calculate all data points reported in this study.

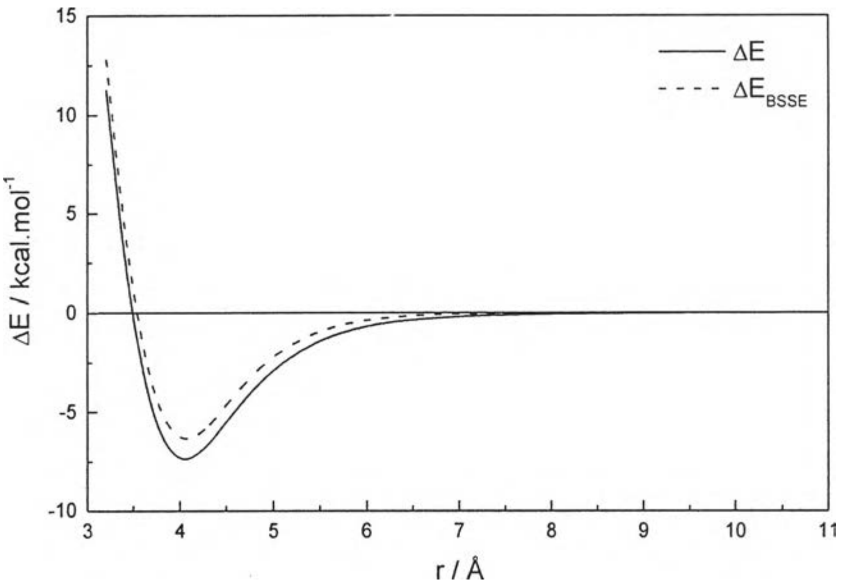


Figure 6.1 Glucosamine-water stabilization energies at the optimal configuration (see Figure 5.2) as a function of the distance between center of glucosamine molecule and oxygen atom of water molecule, calculated using DZP *ab initio* calculations with (dashed line) and without (solid line) BSSE corrections.

Table 6.3 Final optimized parameters for the *i*th atoms of glucosamine molecule interacting with the *j*th atoms of water molecule.

i—*j*	**A (Å^6 kcal.mol^{-1})**	**B (Å^{12} kcal.mol^{-1})**
C^1—O	0.000×10^0	8.089×10^5
C^2—O	0.000×10^0	1.026×10^6
N—O	0.000×10^0	1.005×10^5
O^1—O	4.234×10^2	5.207×10^5
O^2—O	8.261×10^2	5.914×10^5
O^3—O	2.798×10^2	5.323×10^5
O^4—O	1.880×10^3	9.648×10^5
H^1—O	0.000×10^0	4.247×10^3
H^2—O	0.000×10^0	2.884×10^3
H^3—O	1.048×10^2	3.361×10^2
H^4—O	3.196×10^1	3.162×10^{-2}
H^5—O	3.304×10^2	4.386×10^3
C^1—H	1.278×10^{-4}	2.575×10^4
C^2—H	4.641×10^{-4}	1.034×10^1
N—H	0.000×10^0	1.142×10^2
O^1—H	2.990×10^0	3.307×10^2
O^2—H	5.013×10^1	8.430×10^{-9}
O^3—H	0.000×10^0	3.697×10^2
O^4—H	1.874×10^1	5.786×10^1
H^1—H	0.000×10^0	2.789×10^1
H^2—H	0.000×10^0	8.388×10^0
H^3—H	0.000×10^0	1.378×10^2
H^4—H	9.313×10^{-5}	1.020×10^2
H^5—H	0.000×10^0	8.305×10^2

6.2 Glucosamine-Water Potential Function

The reliable functional form for glucosamine-water potential is the Lennard-Jones 12-6 one plus Coulombic term as shown in eq. (5.3). The optimized parameters, A and B, are summarized in Table 6.3. The physical meaningful values of the coefficients are achieved, attractive and repulsive interactions for A and B, respectively.

As mentioned before, structural data obtained from the Monte Carlo simulations depends on the shape of the analytical function rather than on the absolute interaction energies. Therefore, the most important factor indicating the quality of the function is the correlation between the positions of the energy minima, ΔE_{SCF} and ΔE_{FIT}. To examine this character, the stabilization energies obtained from the quantum chemical calculations were plotted versus those obtained from the function with parameters summarized in Table 6.3 and in the configuration shown in Figure 5.2 where α, β and γ are 90, 0 and 45 degree, respectively. The energies extracted from both sources are monitored in Figure 6.2. The differences in the position to the minima as well as the interaction energies are almost negligible. The small value of this function at the distance of about 7.5 Å justifies that correction of the Coulombic interactions beyond the cut-off distance in the Monte Carlo simulations is not necessary [27]. This as well as those observed in the literature [60] certify that the Ewald summation and reaction field methods which take care of long-range interaction is not necessary for this system.

Further confirmation of the quality of the fit is given by comparing ΔE_{SCF} and ΔE_{FIT} (Figure 6.3). Perfect agreement would have implies a straight line of unit slope, and the scatter about this line gives a graphical measure of the quality of the fit. The plot indicates a good agreement between both data, especially for the attractive ranges, which is important for prediction of the simulation results.

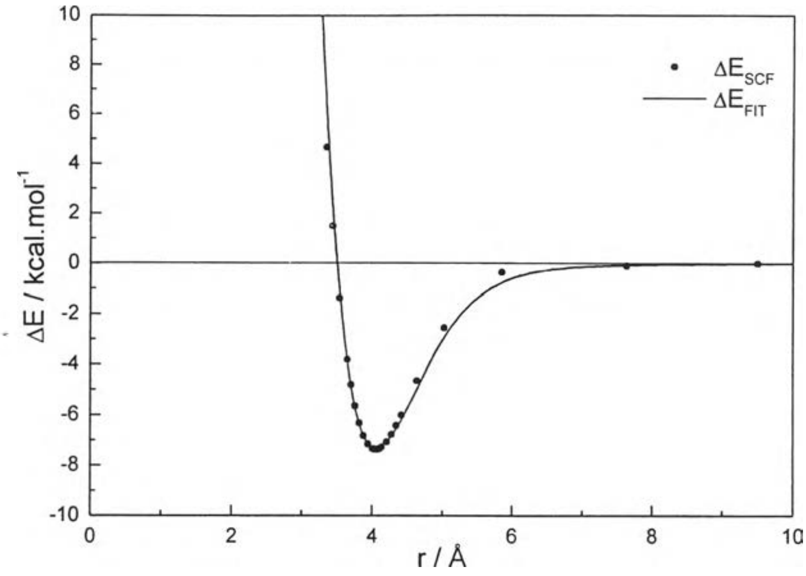


Figure 6.2 Glucosamine-water stabilization energies obtained from the DZP *ab initio* calculations (circle) and from the potential function (line) with the fitting parameters summarized in Table 6.3 for the complex configuration shown in Figure 5.2.

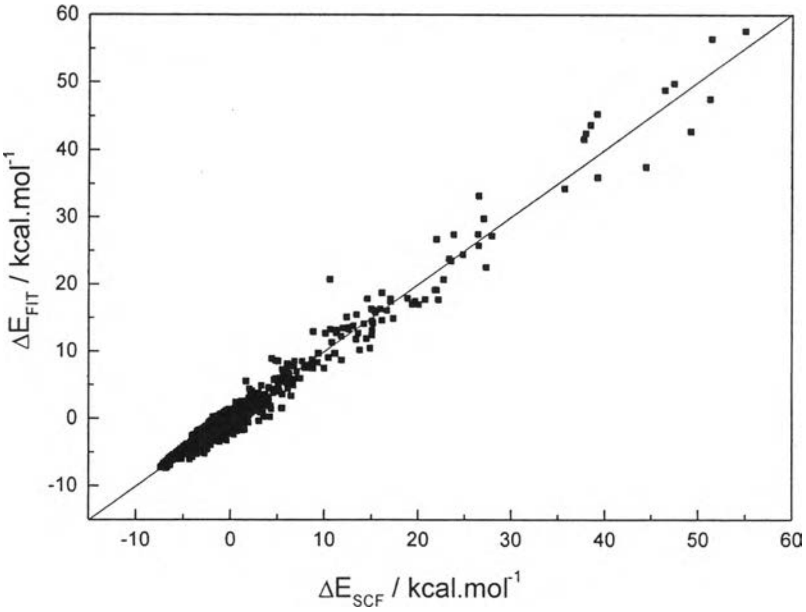


Figure 6.3 Comparison between the fitted energies (ΔE_{FIT}) and the SCF energies (ΔE_{SCF}) obtained from the DZP *ab initio* calculations and from the potential function with the fitting parameters summarized in Table 6.3.

Moreover, the quality of the fit is confirmed by the testing procedure. The new 370 data points of ΔE_{SCF} and ΔE_{FIT} are compared, as displayed in Figure 6.4. It shows that the fitted energies are in good agreement with the SCF energies.

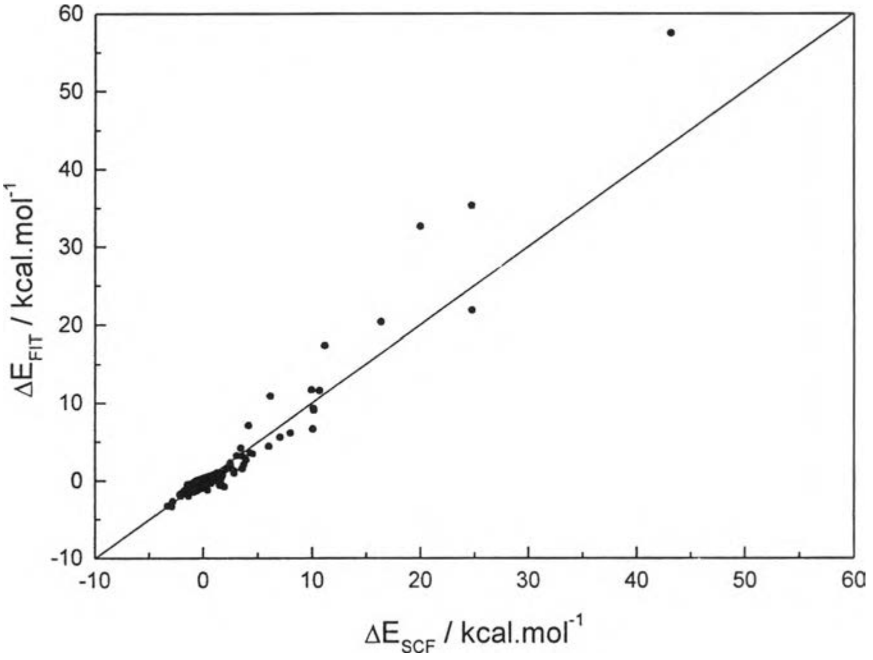


Figure 6.4 Comparison between the fitted energies (ΔE_{FIT}) and the SCF energies (ΔE_{SCF}) for the testing procedure.

6.3 Solvent Structure

In order to investigate structure changes of solvent molecules in dilute solution of glucosamine, atom-atom radial distribution functions (RDFs) for H_2O-H_2O—$g_{xy}(r)$: probability of finding atom of types y around x as a function of r—have been evaluated and compared with those of pure water [61]. In Figure 6.5, no significant difference between two sources of the RDFs is found. There are 6 water molecules in the first solvation shell and the average O-O distance is about 3.8 Å.

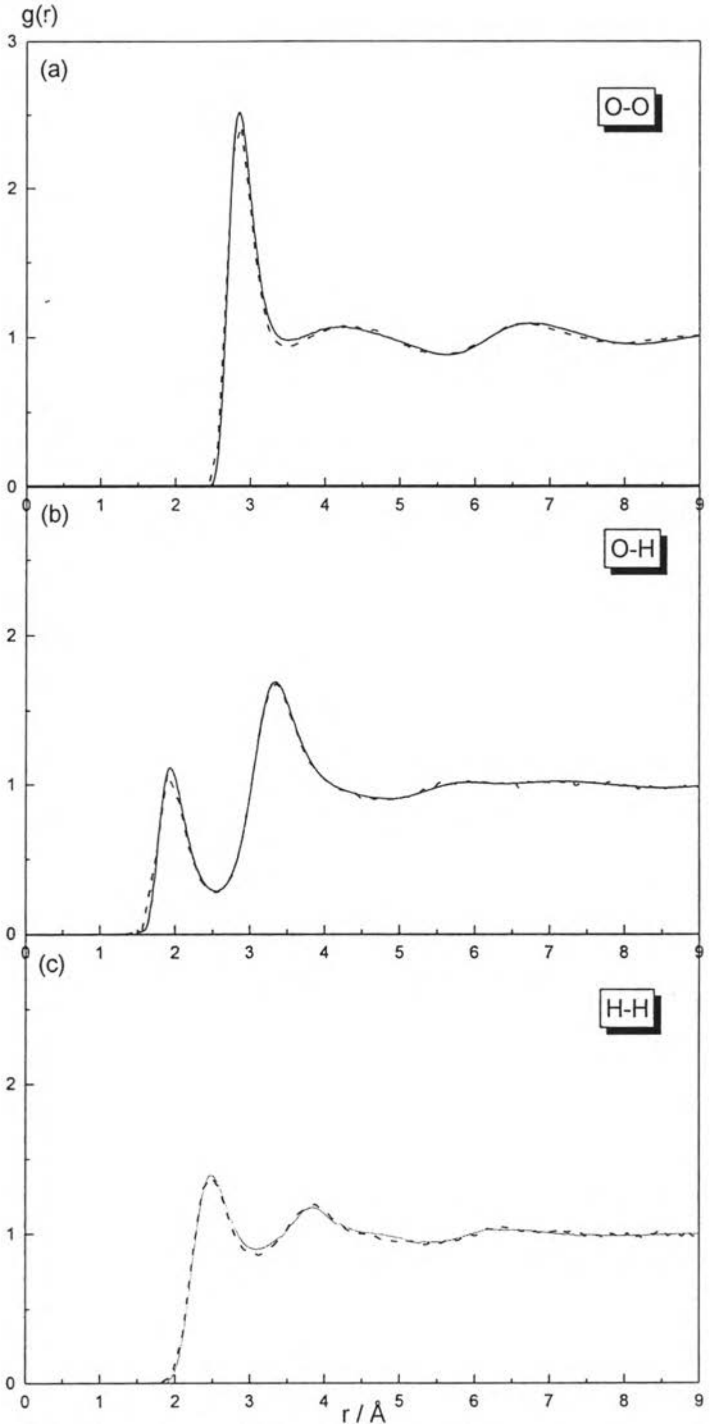


Figure 6.5 Water-water radial distribution functions obtained from the Monte Carlo simulations of water with one glucosamine molecule (solid line) and pure water (dash line) [61], (a) $g_{OO}(r)$, (b) $g_{OH}(r)$ and (c) $g_{HH}(r)$.

6.4 Water Structure Around the Glucosamine Molecule

In Figure 6.6, total RDFs referring to the center of the glucosamine for the entire volume (E), $g_{EO}(r)$ and $g_{EH}(r)$, are depicted. Due to complexity of the glucosamine molecule, the RDFs between atoms of glucosamine and those of water molecules overlap each other so this plot can not give more information. However, E-O RDF displays 2 peaks, centered at about 4.3 and 5.1 Å. Their running integration numbers, integrated up to the corresponding minima of 4.6 Å, and 6.0 Å, are 7 and 26 water molecules, respectively.

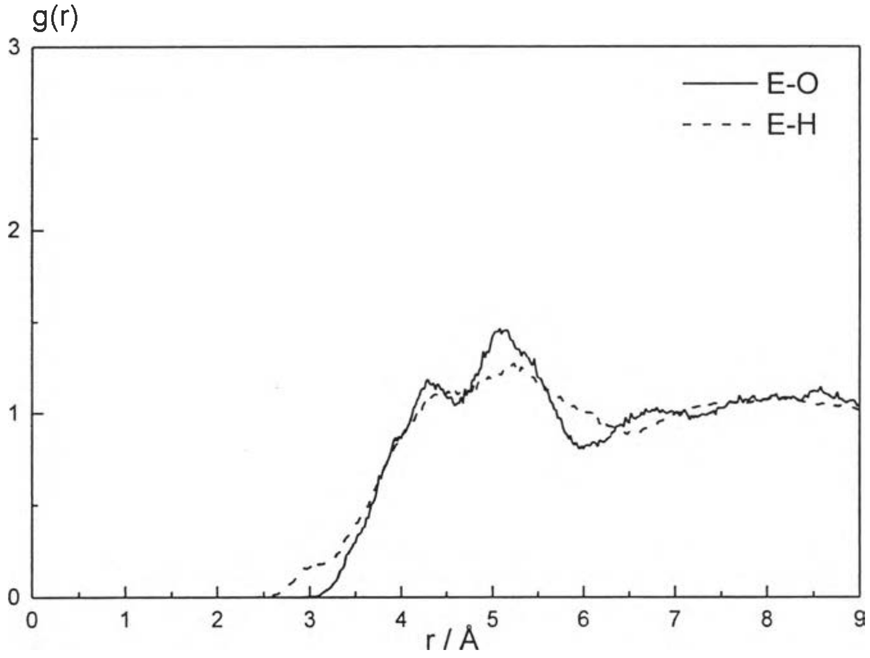


Figure 6.6 Radial distribution functions from O (solid line) and H (dash line) atoms of water molecules to the center of glucosamine molecule.

To overcome the difficult evaluation of the position and orientation of solvent molecules due to complexity of the ligand, space around glucosamine molecule was divided into four quadrants in which x-axis points from center of C4-C8 to that of C14-O18 bonds, and y-axis from C3 to C11 atoms (see Figure 6.7).

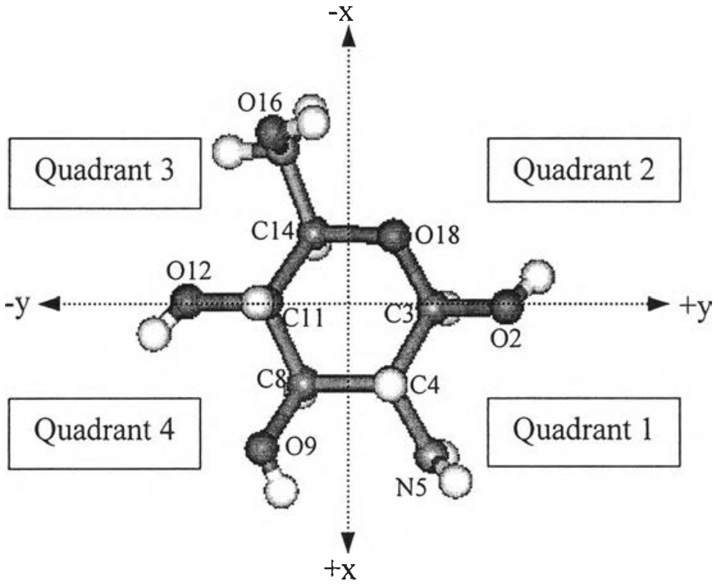
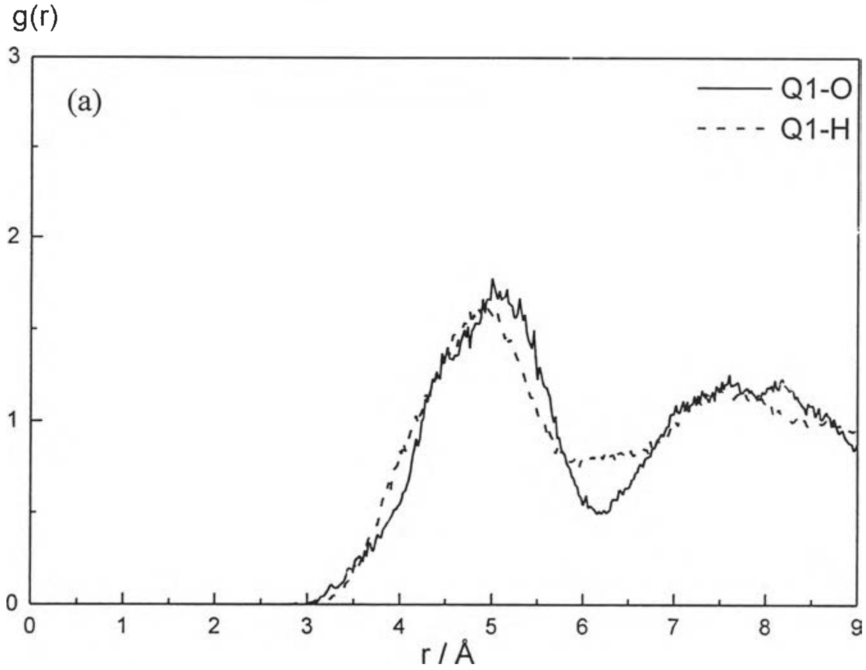
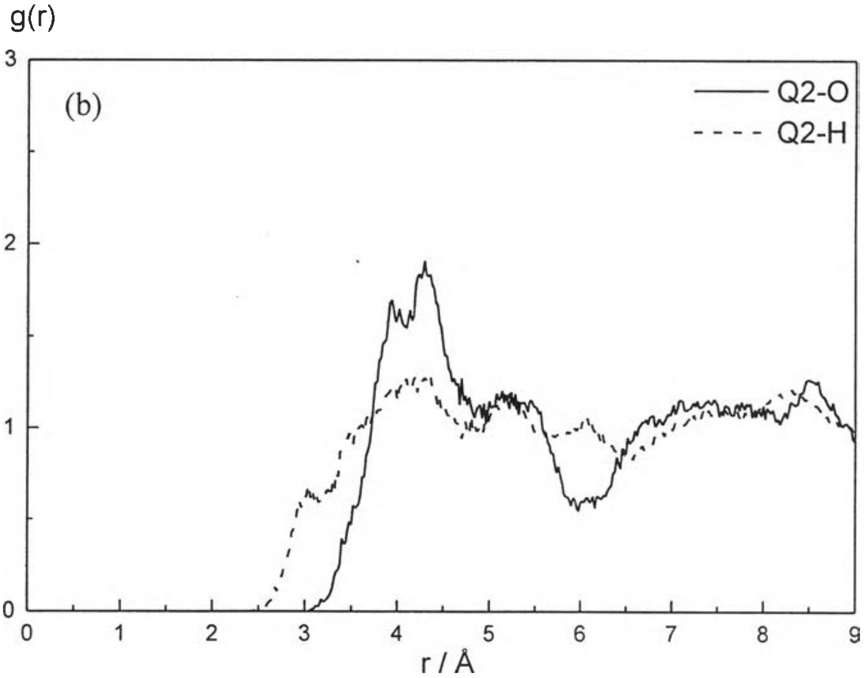


Figure 6.7 Space around glucosamine molecule was divided by x- and y-axis into 4 quadrants.

Figures 6.8a – 6.8d show the RDFs of water molecules to center of glucosamine molecule in each region. They exhibit a weak glucosamine-water interaction. The Q2-H appears at shorter distance than the corresponding Q2-O RDFs. This indicates preferential orientation of water molecules by pointing their hydrogen atoms to the glucosamine molecule, was detected only in quadrant 2, but not for the others. The running integration numbers up to 4.6 Å, which was taken from the distance to the minimum of the E-O RDF (Figure 6.6) and assumed to be the first solvation shell of glucosamine, of the Q1-O, Q2-O, Q3-O and Q4-O RDFs are 1.5, 3.0, 1.0 and 1.5 water molecules, respectively.


g(r)
(a)
Q1-O
Q1-H
0
1
2
3
0
1
2
3
4
5
6
7
8
9
r / Å


g(r)
(b)
Q2-O
Q2-H
0
1
2
3
0
1
2
3
4
5
6
7
8
9
r / Å

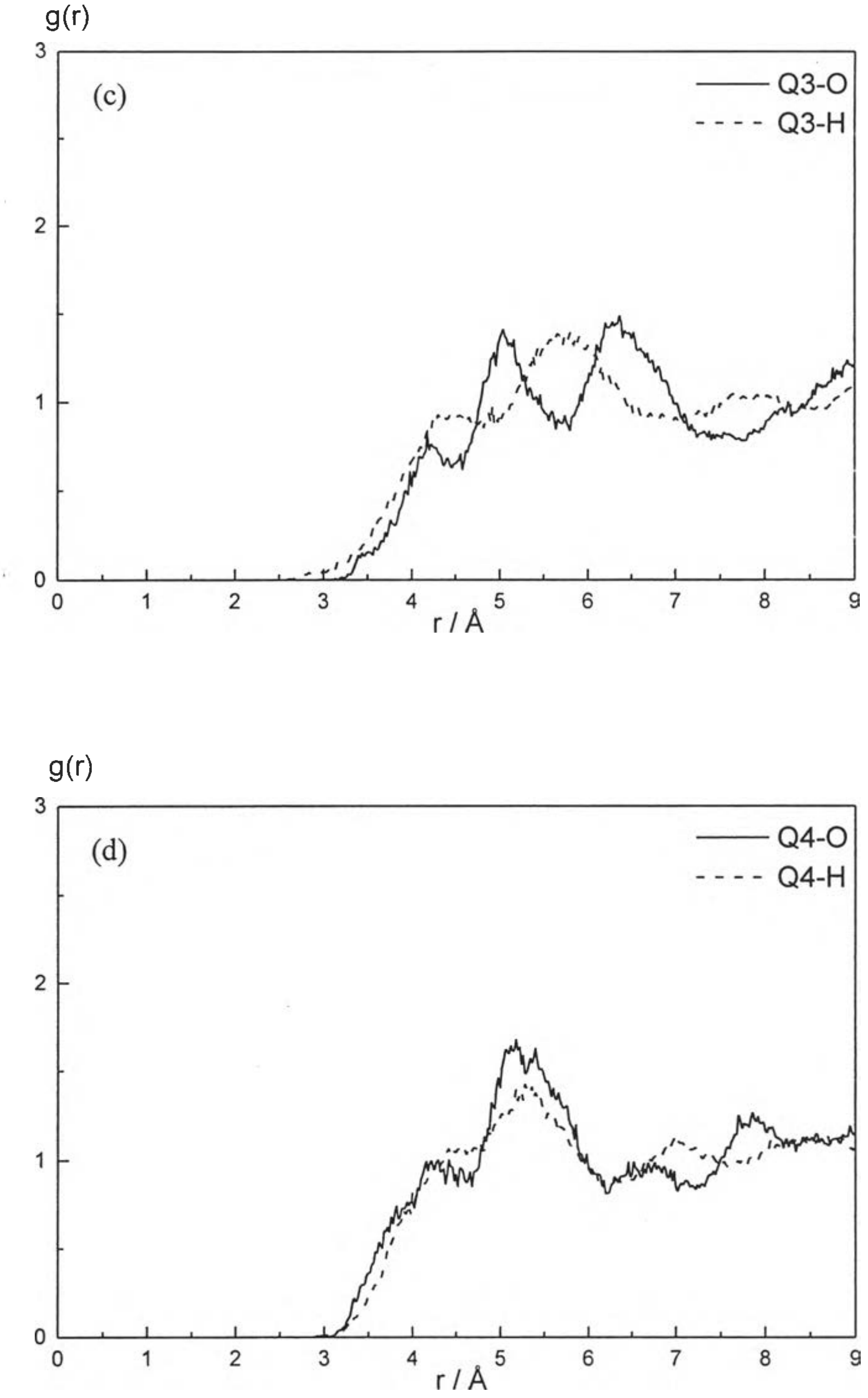
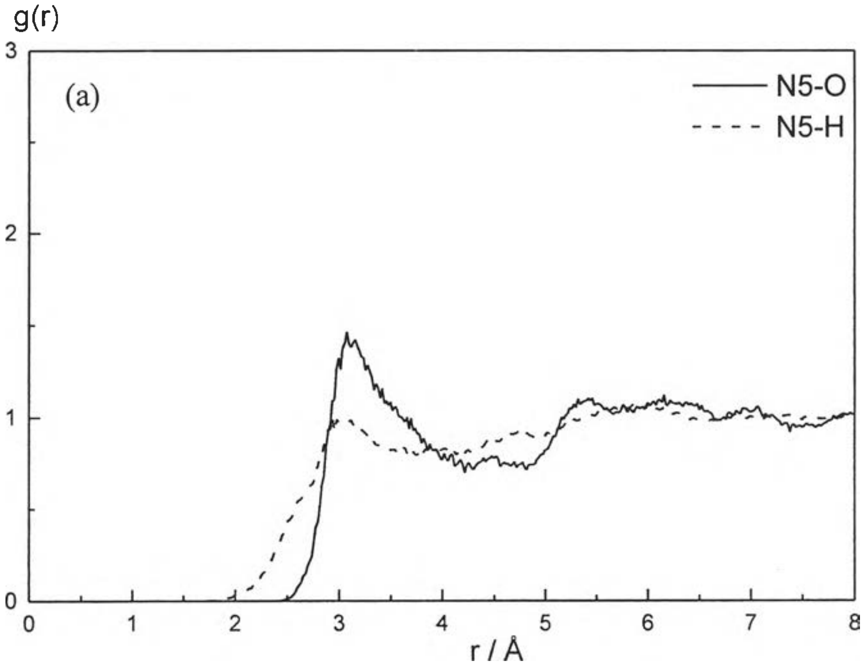


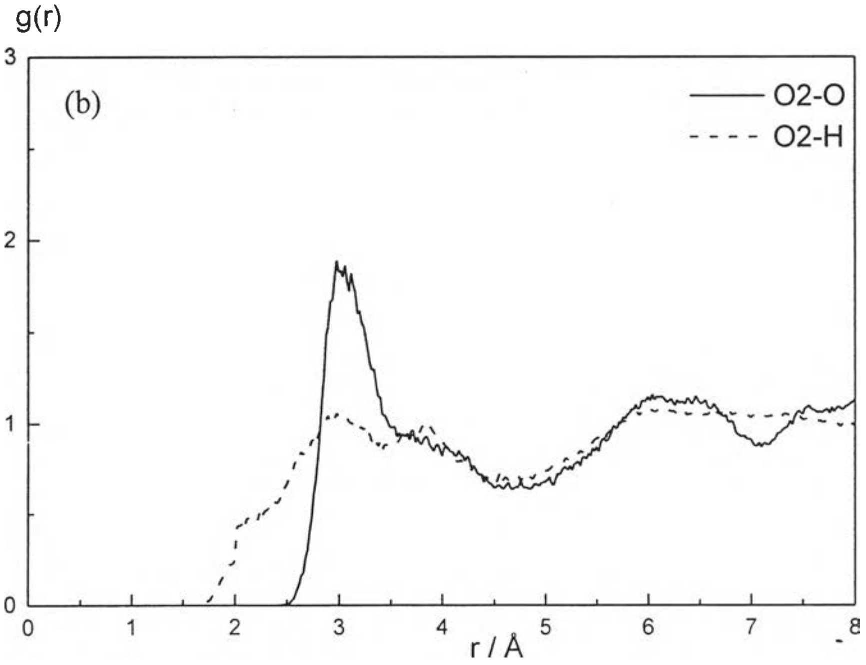
Figure 6.8 Radial distribution functions from center of glucosamine to O (solid line) and H (dash line) atoms of water molecules lying in (a) quadrant 1, (b) quadrant 2, (c) quadrant 3 and (d) quadrant 4.

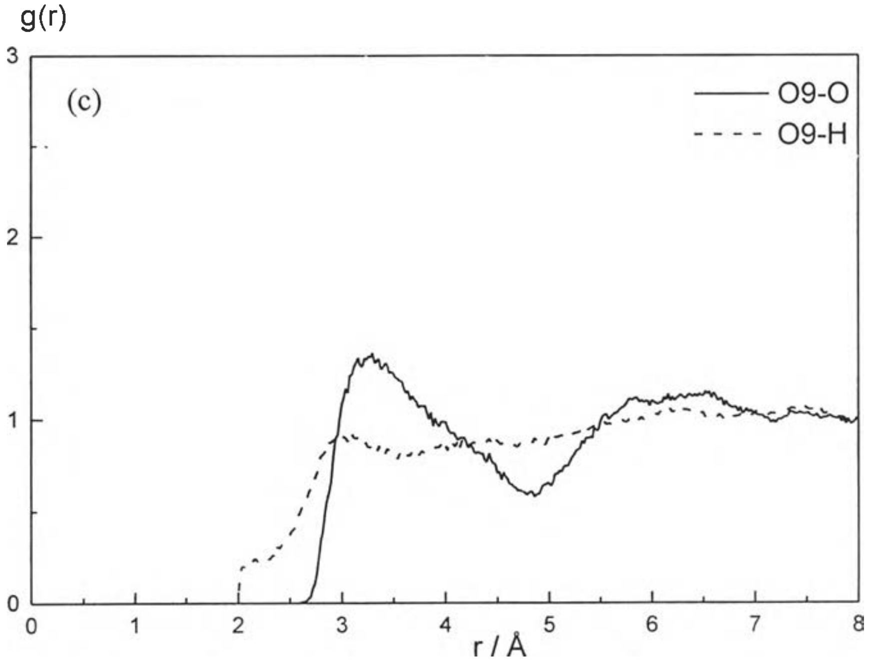
The atom-atom RDFs from nitrogen and oxygen atoms of glucosamine molecule to water molecules are displayed in Figures 6.9a – 6.9f. For all plots, the RDFs to the H of water are detected before those to the O atom. This indicates preferential orientation of water molecule in which one of its H atom is oriented to point toward the O or N atoms of glucosamine. The plots show well defined peaks, except that of the O16, centered at about 3 Å (Fig. 6.9e). The running integration numbers up to the first minima of the N5-O, O2-O, O9-O, O12-O and O18-O RDFs are 7.4, 4.7, 11.2, 7.7 and 2.2 water molecules, respectively. Since an H-bond is established when the distance between donor hydrogen and acceptor oxygen is less than 2.4 Å [62-63], the coordination numbers of hydrogen atoms of water molecules are integrated up to 2.4 Å and the values of the N5-H, O2-H, O9-H, O12- H, O16-H and O18-H RDFs are 0.2, 0.8, 0.3, 0.6, 0.5 and 1.0, respectively. This data as well as an appear of the first peak or the hump of the RDFs from the N or O atoms of the ligand to the H atom of water indicates clearly that water molecules lying under the peaks to O atoms at 3 Å form hydrogen bond with glucosamine molecule. The obtained coordinate numbers, which are quite high, do not indicate numbers of hydrogen bonding because of the overlapping of the RDFs as mentioned before. This fact is confirmed by the broaden or splitting of these peaks. More details investigation of number of water molecules as well as their orientation are given in the next paragraph. Note that: (i) the O18-H RDF shows very sharp first peak which indicates strong H-bond between water and O18, and (ii) the difference between the distance to the first maxima of the O18-O and O18-H RDFs which almost equivalent to the O-H bond length of water indicates a formation of linear H-bond.

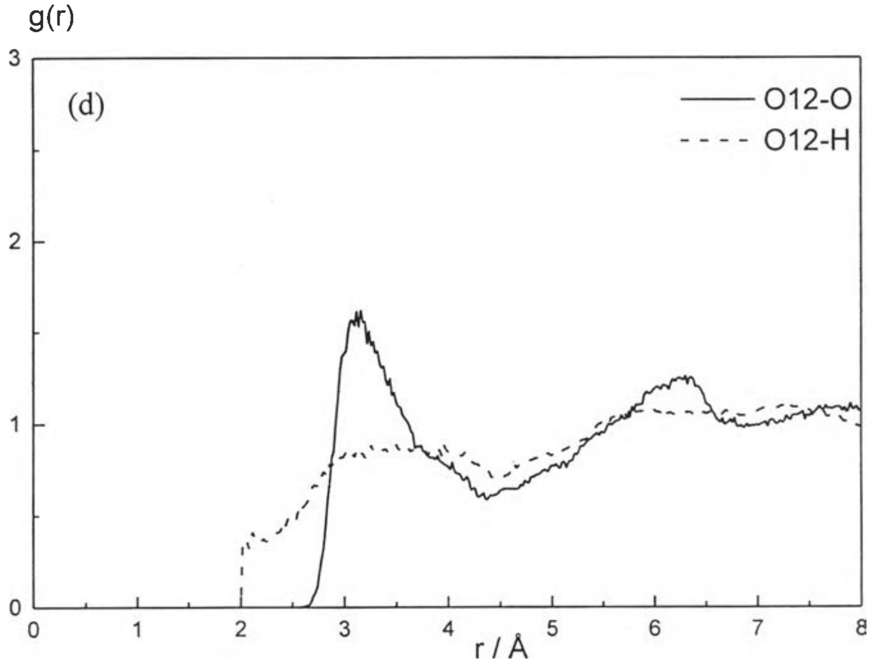
In Figures 6.10a – 6.10f, the atom-atom RDFs for the selected hydrogen atoms of glucosamine molecule to water molecules are delineated. The corresponding running integration numbers up to the H-bond distance, 2.4 Å, of the H1-O, H6-O, H7-O, H10-O, H13-O and H17-O RDFs are 0.8, 0.6, 0.5, 0.3, 0.3 and

0.9 oxygen atoms, respectively. The plots show rather strong H-bond formation at the H1 and H17 of glucosamine while the other are rather weak.






g(r)
(c)
O9-O
O9-H
0
1
2
3
0
1
2
3
4
5
6
7
8
r / Å


g(r)
(d)
O12-O
O12-H
0
1
2
3
0
1
2
3
4
5
6
7
8
r / Å

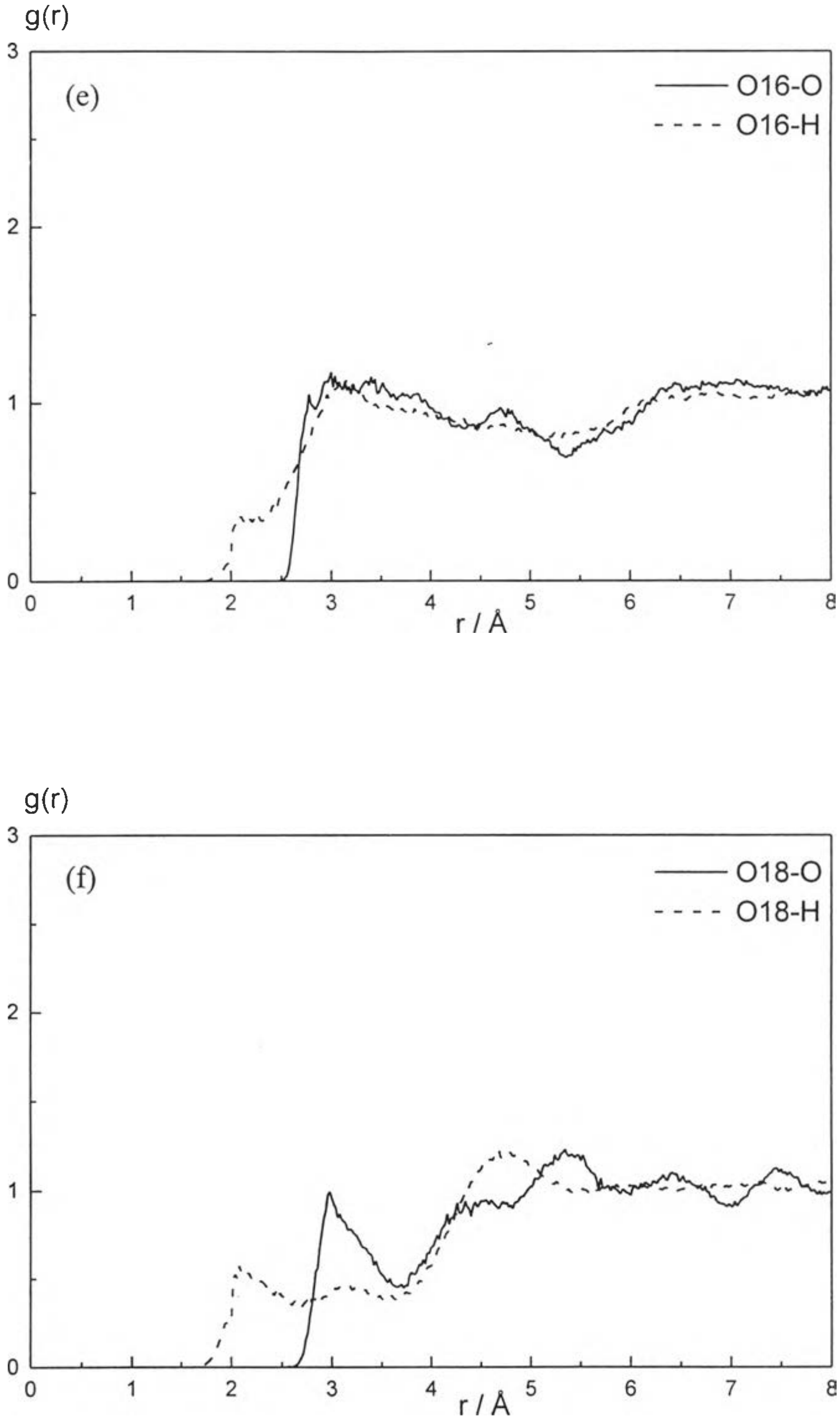
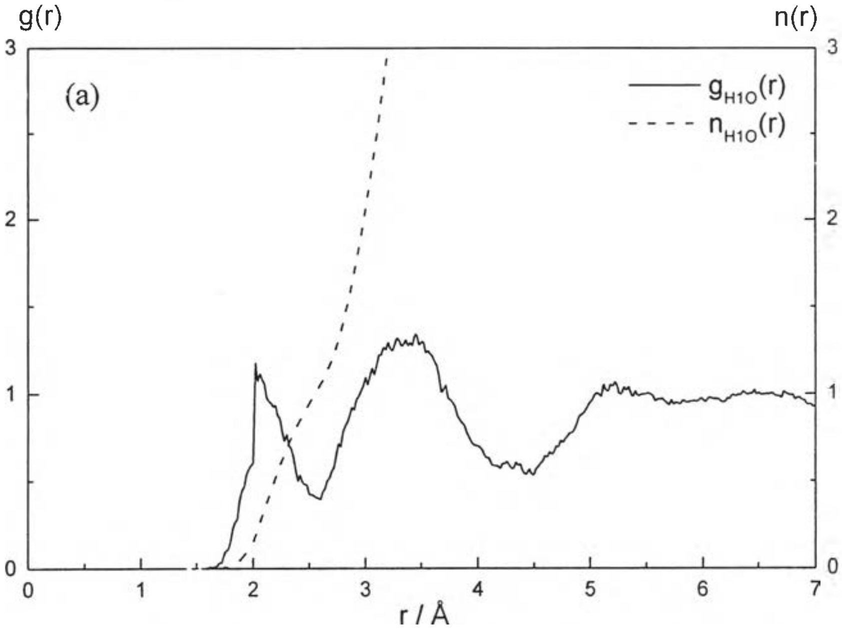
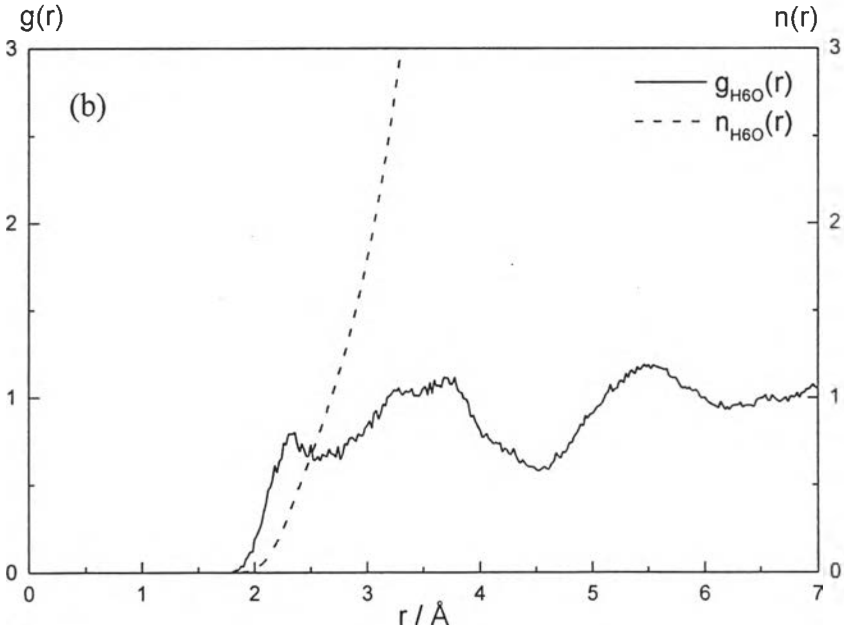
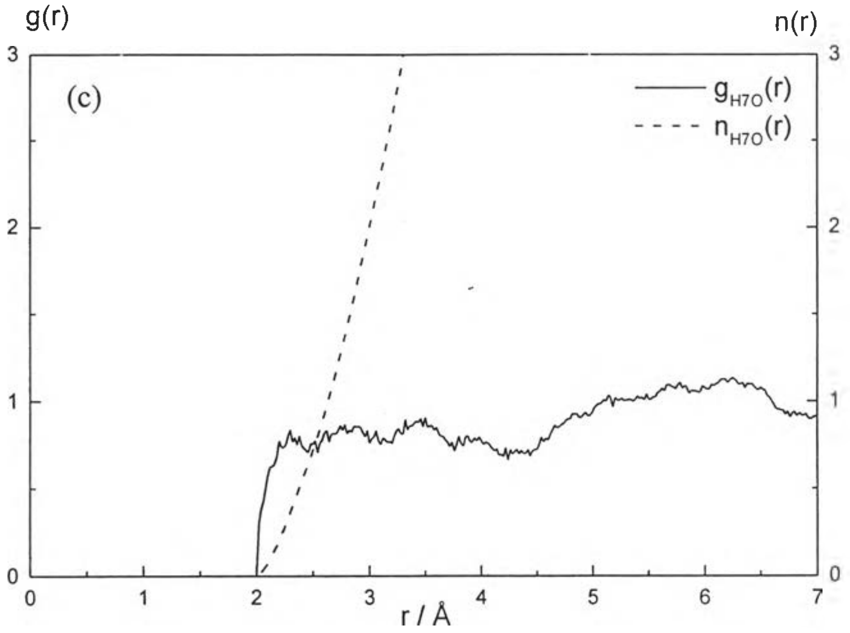
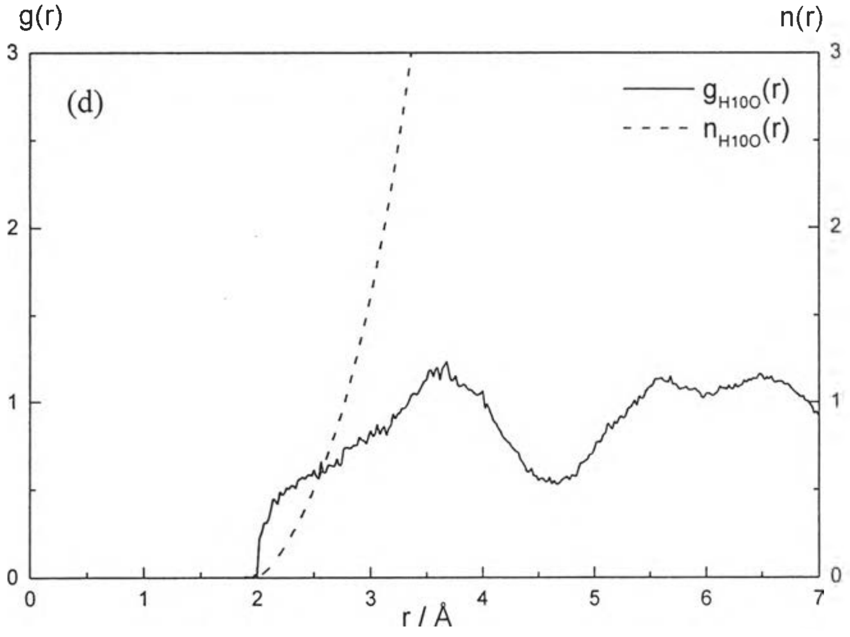


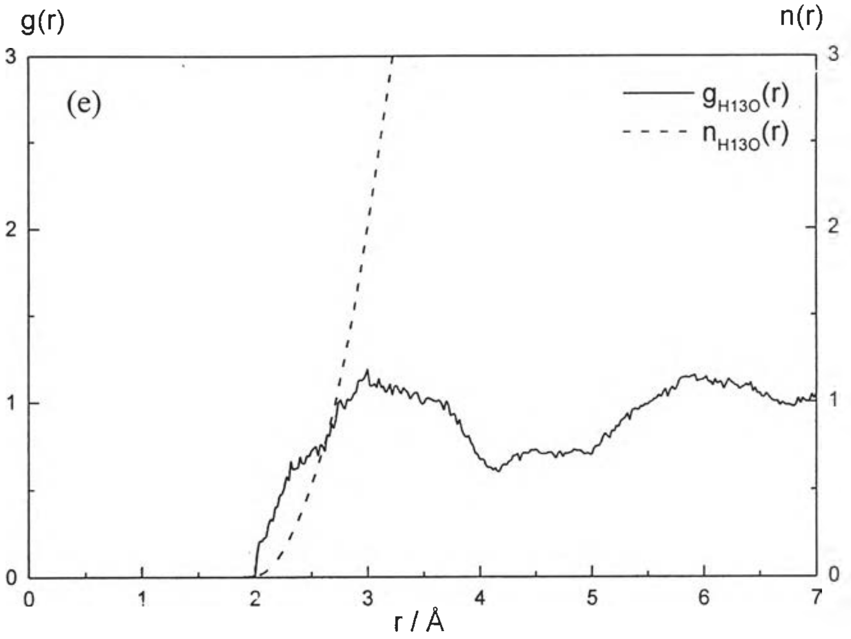
Figure 6.9 Radial distribution functions from O (solid line) and H (dash line) atoms of water molecules to (a) N5, (b) O2, (c) O9, (d) O12, (e) O16 and (f) O18 atoms (see Figure 5.1) of glucosamine molecule .


g(r)
n(r)
(a)
g_H1O(r)
n_H1O(r)
0
1
2
3
0
1
2
3
4
5
6
7
r / Å


g(r)
n(r)
(b)
g_H6O(r)
n_H6O(r)
0
1
2
3
0
1
2
3
4
5
6
7
r / Å


g(r)
n(r)
(c)
$g_{H7O}(r)$
$n_{H7O}(r)$
0
1
2
3
0
1
2
3
4
5
6
7
r / Å


g(r)
n(r)
(d)
$g_{H10O}(r)$
$n_{H10O}(r)$
0
1
2
3
0
1
2
3
4
5
6
7
r / Å



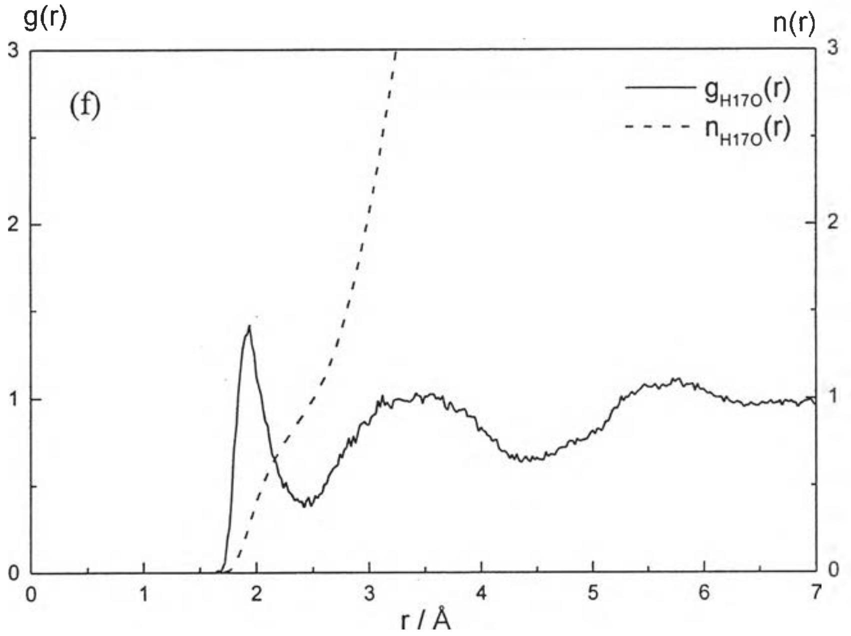


Figure 6.10 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules to (a) H1, (b) H6, (c) H7, (d) H10, (e) H13 and (f) H17 atoms (see Figure 5.1) of glucosamine molecule.

To investigate precise positions of the 7 water molecules in the first solvation shell of glucosamine, the periodic cube was sliced into several layers by the plane parallel to xy-plane. Parts A, B, C, D, -A, -B, -C and –D are the volumes where 0 Å ≤ z ≤ 2 Å, 2 Å < z ≤ 3 Å, 3 Å < z ≤ 4 Å, 4 Å < z ≤ 5 Å, 0 Å > z ≥ -2 Å, -2 Å > z ≥ -3 Å, -3 Å > z ≥ -4 Å and -4 Å > z ≥ -5 Å, respectively (Figure 6.11).

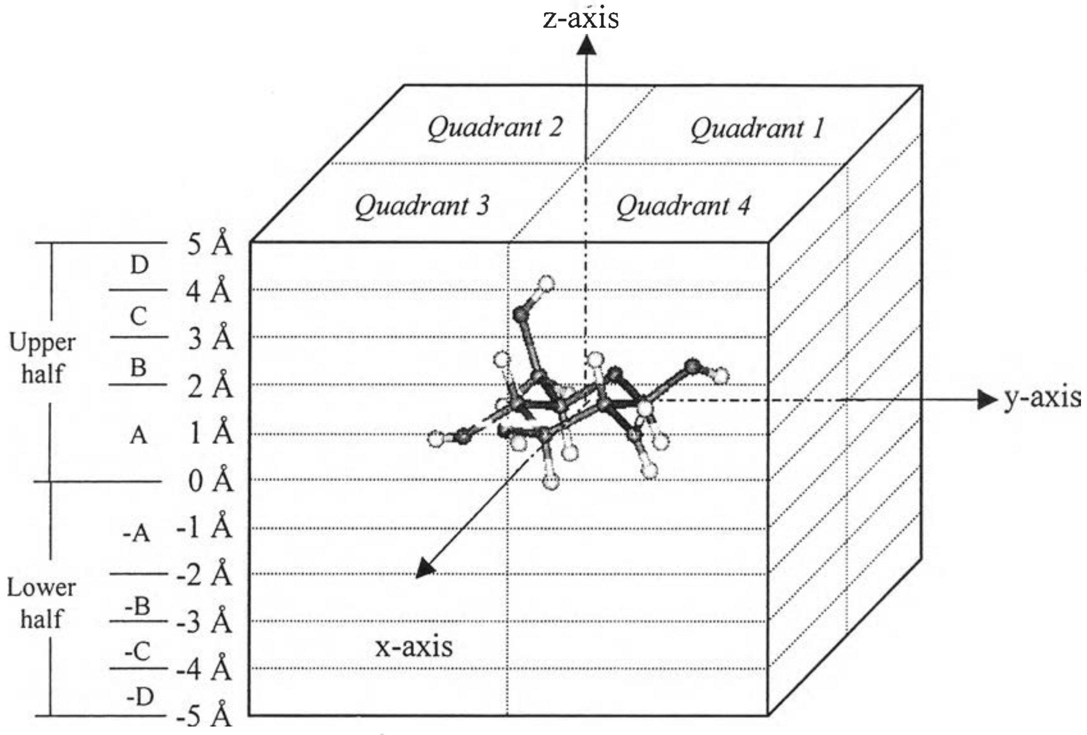


Figure 6.11 Periodic cube was sliced into several layers by the plane parallel to the xy-plane.

In Figures 6.12 – 6.19, the RDFs from oxygen atoms of water molecules lying in each quadrant and in each sliced as shown in Figure 6.11 to center of glucosamine molecule are plotted. The running integration numbers of water molecules up to the first solvation shell, 4.6 Å, of those plots are summarized in Table 6.4. To ascertain for more information for 7 water molecules in the first shell of glucosamine, Cartesian coordinate of these water molecules were projected into xy-plane. The resulted density contour plots are displayed in Figures 6.20 – 6.21.

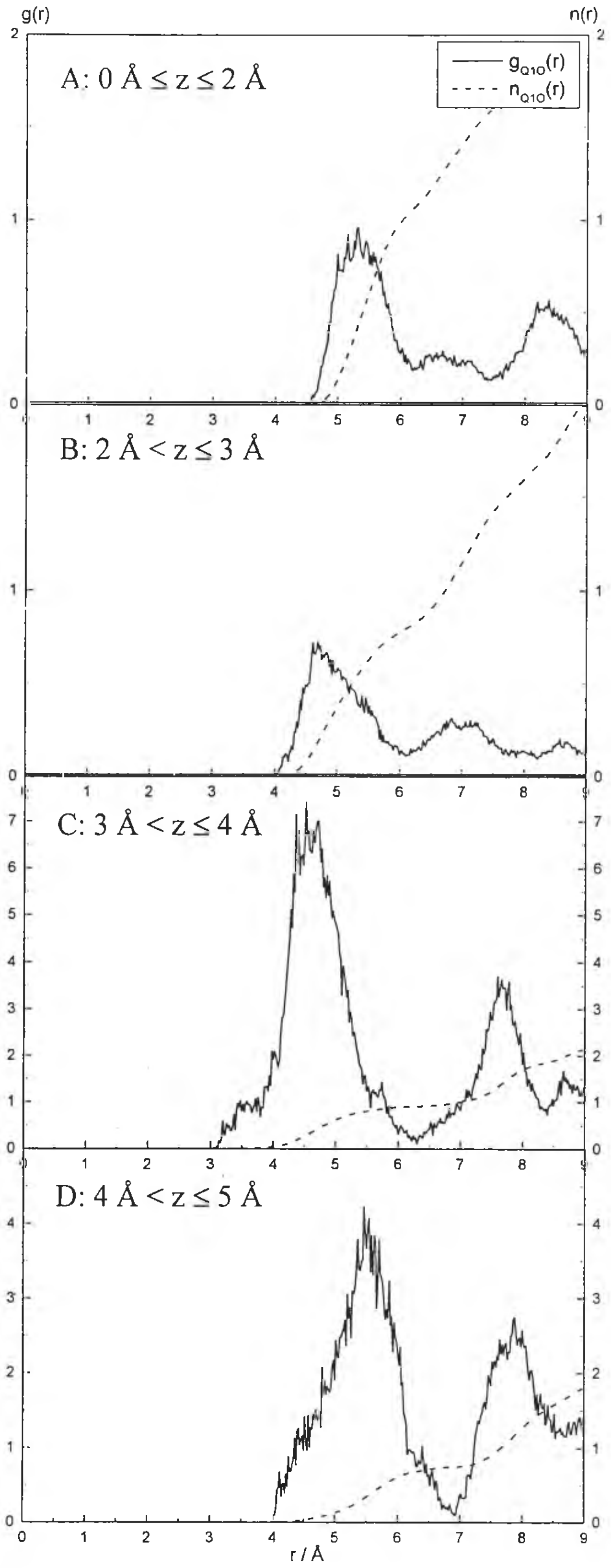


Figure 6.12 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in upper half (see Figure 6.11) of quadrant 1 to center of glucosamine molecule.

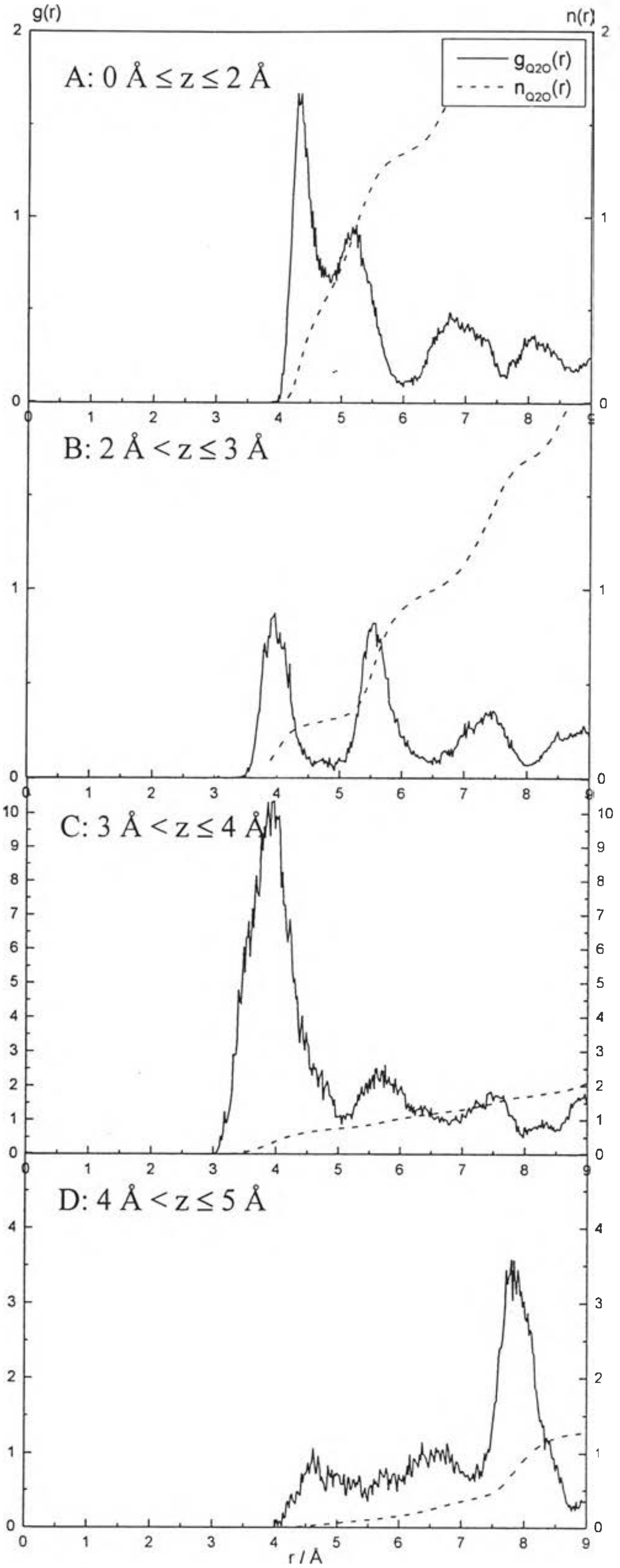


Figure 6.13 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in upper half (see Figure 6.11) of quadrant 2 to center of glucosamine molecule.

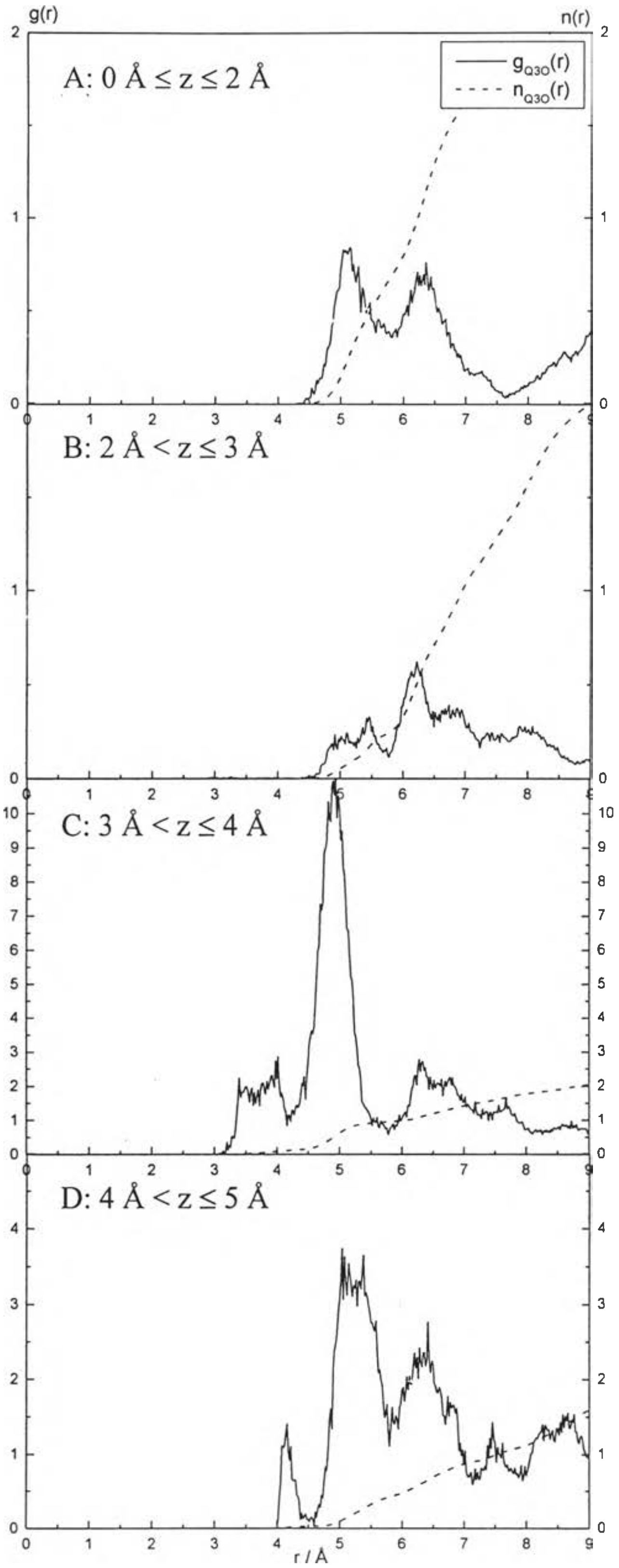


Figure 6.14 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in upper half (see Figure 6.11) of quadrant 3 to center of glucosamine molecule.

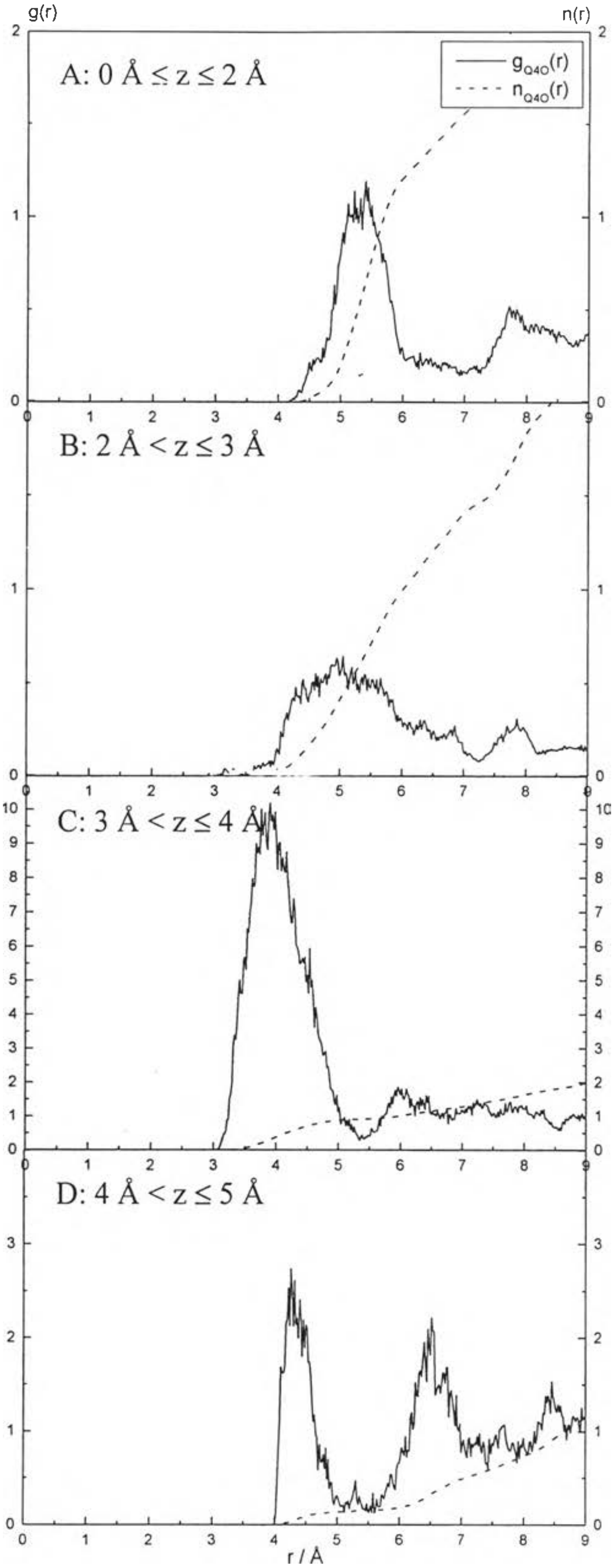


Figure 6.15 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in upper half (see Figure 6.11) of quadrant 4 to center of glucosamine molecule.

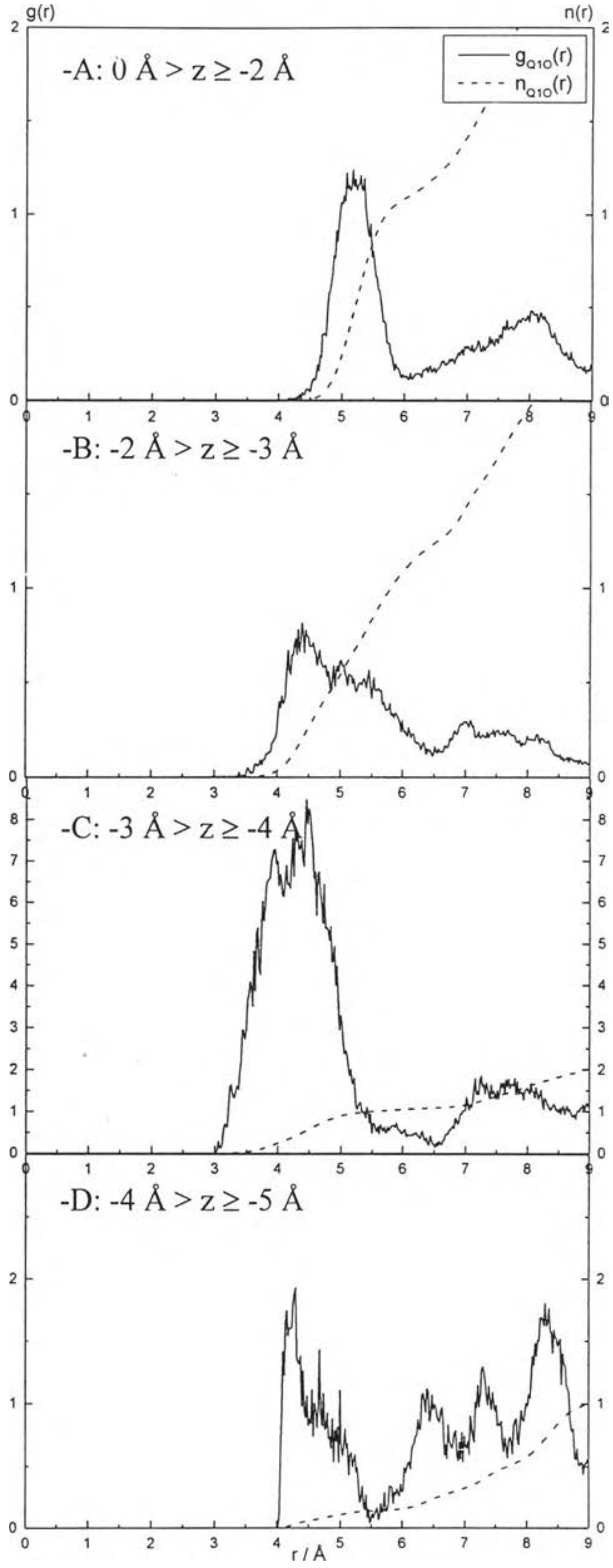


Figure 6.16 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in lower half (see Figure 6.11) of quadrant 1 to center of glucosamine molecule.

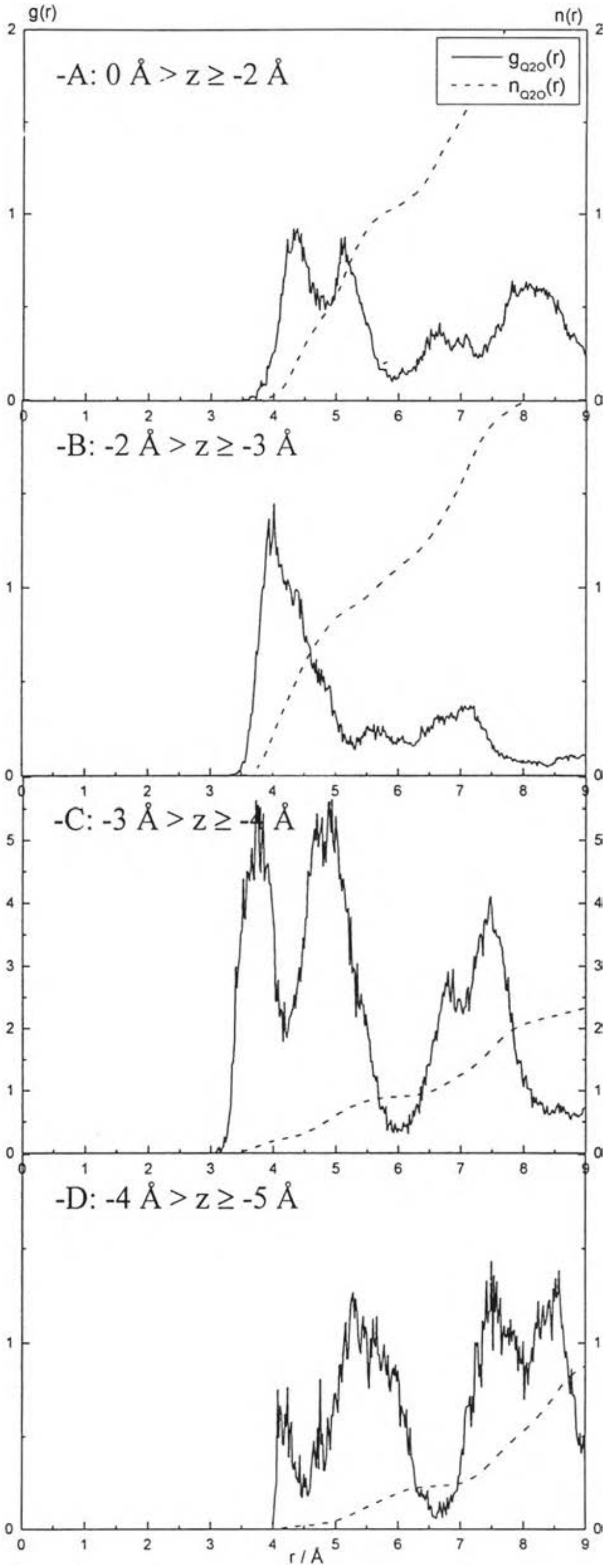


Figure 6.17 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in lower half (see Figure 6.11) of quadrant 2 to center of glucosamine molecule.

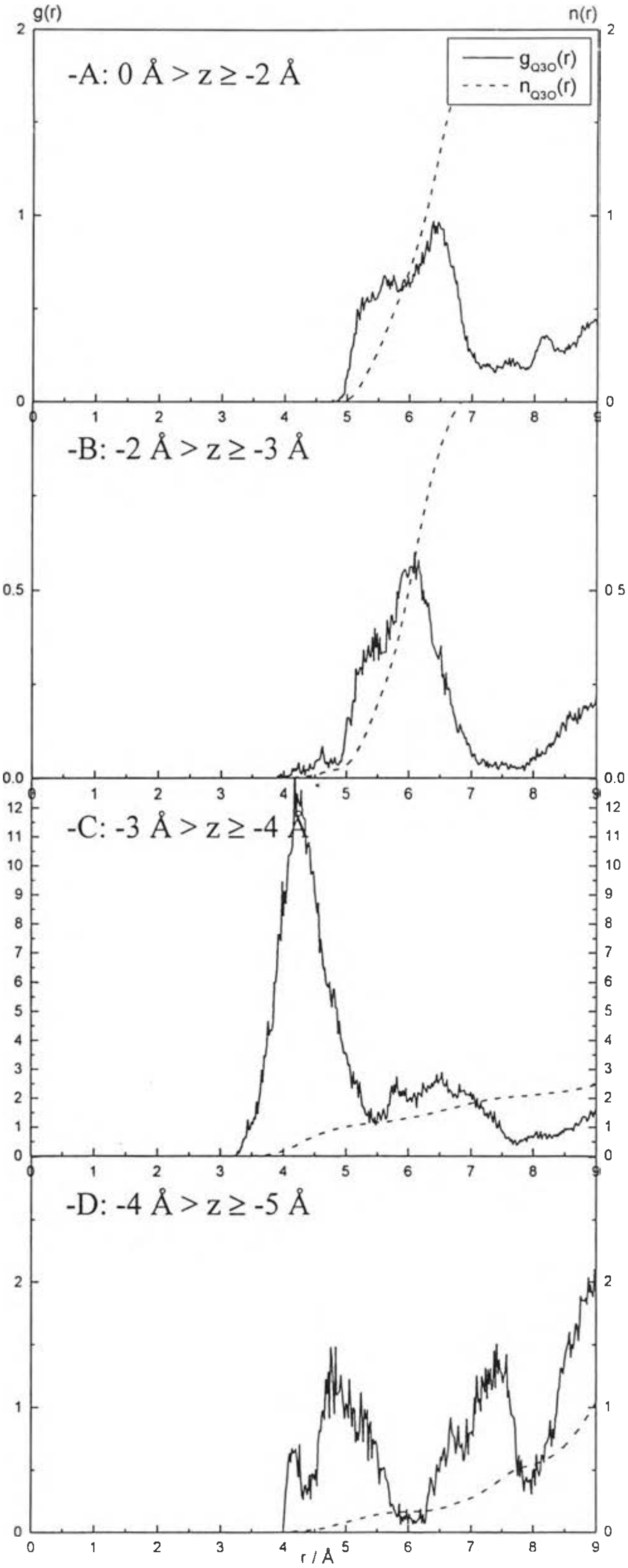


Figure 6.18 Radial distribution functions (solid line) and running integration numbers (dash line) from O atoms of water molecules lying in lower half (see Figure 6.11) of quadrant 3 to center of glucosamine molecule.

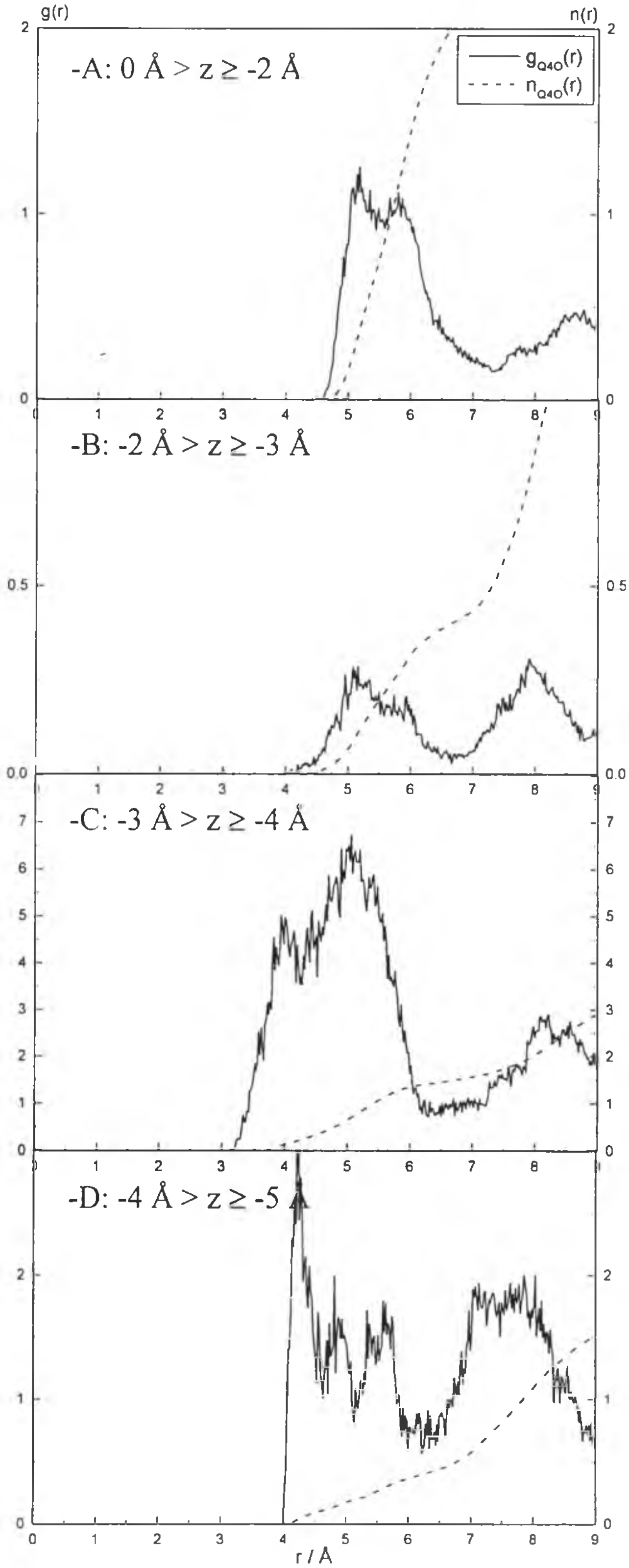


Figure 6.19 Radial distribution functions (solid line) and corresponding running integration numbers (dash line) from O atoms of water molecules lying in lower half (see Figure 6.11) of quadrant 4 to center of glucosamine molecule.

Table 6.4 Running integration numbers of water molecules integrated up to 4.6 Å of the RDFs in Figures 6.12 – 6.19 (a, b,... and m are the points in Figures 6.20 and 6.21).

Part	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
A: 0 Å $\leq$ z $\leq$ 2 Å	-	0.5 (a)	-	-
B: 2 Å < z $\leq$ 3 Å	0.1 (c)	0.3 (b)	-	0.2 (d)
C: 3 Å < z $\leq$ 4 Å	0.4 (c')	0.7 (b')	0.1 (f)	0.7 (d',e)
D: 4 Å < z $\leq$ 5 Å	-	-	-	0.1 (e')
-A: 0 Å > z $\geq$ -2 Å	-	0.5 (a')	-	-
-B: -2 Å > z $\geq$ -3 Å	0.3 (h,i)	0.8 (g)	-	-
-C: -3 Å > z $\geq$ -4 Å	0.7 (i',k)	0.2 (j,k)	0.9 (k,l)	0.4 (k,m)
-D: -4 Å > z $\geq$ -5 Å	-	-	-	0.1 (k')
Total	1.5	3.0	1.0	1.5

Consider upper half of quadrant 1 (Figure 6.20), water molecules have been found at points *c* in layer B and *c'* in layer C with the corresponding coordination numbers of 0.1 and 0.4, respectively (see Table 6.4). Because *c* and *c'* appear at the same xy-coordinate, it is reasonable to conclude that these points are contributed from the same water molecule. That is 0.5 water molecule coordinates to upper half of quadrant 1 at about 3 Å above the ligand's plane. Considering upper half of quadrant 2, 0.5, 0.3 and 0.7 water molecules have been detected at the layers A, B and C, which are labeled as *a*, *b* and *b*', respectively. Here, points *b* and *b*' are the same water molecule. Therefore, this quadrant contains 0.5 and 1.0 waters which centered at 0 – 2 Å and about 3 Å, respectively, above the xy-plane.

Only 0.1 water was found in quadrant 3 (point *f*) at the distance between 3 - 4 Å above xy-plane while four of them, *d*, *d'*, *e* and *e'*, were found in quadrant 4. Again, water molecule which contributions to points *d* and *d'* as well as *e* and *e'* are considered to be the same molecule as they appear at the same xy-coordinate. It is possible that these four points are the contribution from 1 water molecule because space in quadrant 4 and 2 Å < z ≤ 5 Å, which they are detected, is surely not enough to accommodate 2 water molecules. This assumption leeds to the conclusion that water coordination number of 1.0 in this region is highly mobile. In addition, higher density of the *e* plus *e'* than the *d* plus *d'* points means that this water prefers to lie above C8-C11 bond than above space between O9 and O12 atoms (see Figure 5.1).

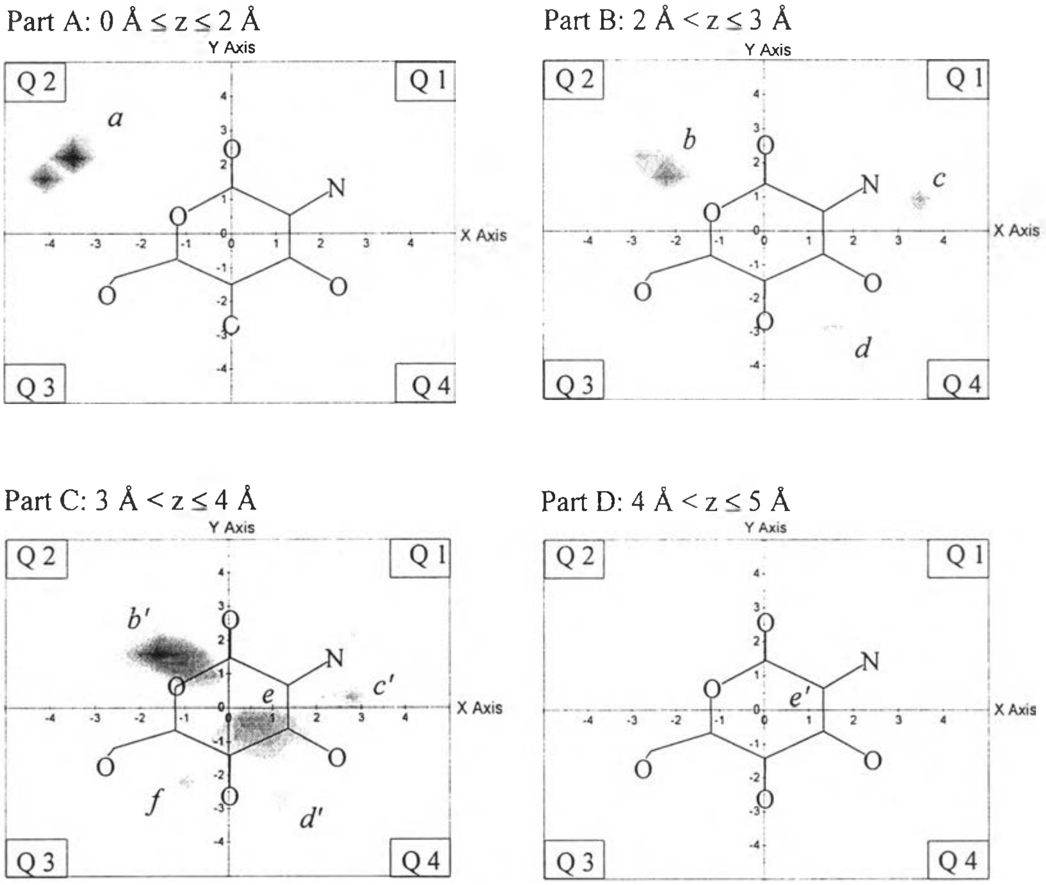
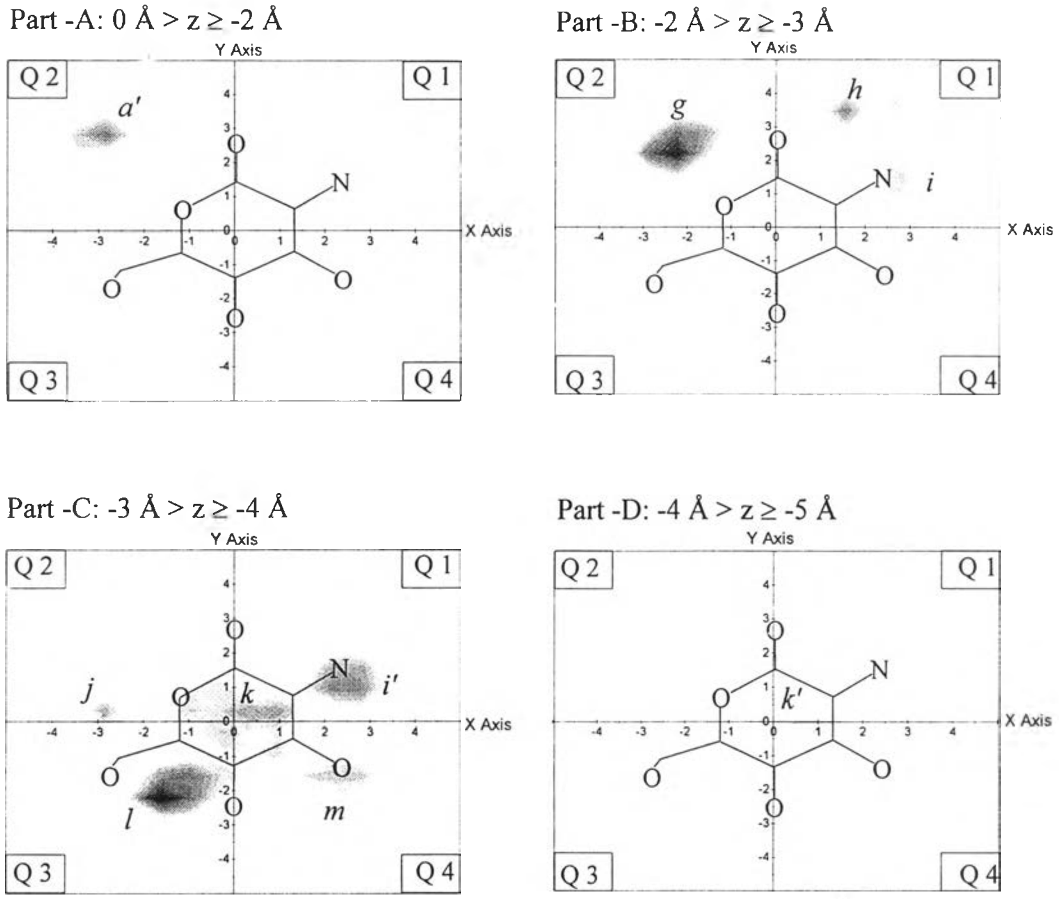


Figure 6.20 Contour plots of the xy-coordinate of water molecules in the upper half of each quadrant (Q) in the first coordination shell of glucosamine.

Figure 6.21 shows contour plots of in the lower half of each quadrant for each layer. It can be seen from this plot and Table 6.4 that coordination number of almost one water in quadrant 1 are from layers –B, -C and –D (points *i*, *i'*, *h* and *k*). For quadrant 2, total coordination numbers of 1.5 can be splitted into two parts, 1.0


Part -A: 0 Å > z ≥ -2 Å

Part -B: -2 Å > z ≥ -3 Å

Part -C: -3 Å > z ≥ -4 Å

Part -D: -4 Å > z ≥ -5 Å

Figure 6.21 Contour plots of the xy-coordinate of water molecules in the lower half of each quadrant (Q) in the first coordination shell of glucosamine.

and 0.5 molecules, by considering the xy-coordinate of the contour plots. Here, one water molecule lies between 2 Å and 3 Å below the xy-plane. The other 0.5 molecule, which contributes to point *a* in layer A in the upper half of this quadrant in the contour plot (see Figure 6.33) is considered to be the same molecule as that contributes to point *a'* in layer –A (lower half).

In quadrant 3, about one water molecule has been found at point *l*, between O12 and C15 atoms (see Figure 5.1) and 3-4 Å below the xy-plane. Quadrant 4 contains three points, point *m* and part of point *k* in layer C, and part of point *k'* in layer D. The point *k* and *k'* yield from the same water, which interact with the ring of the glucosamine molecule at more than 3 Å below the molecule plane. At this layer, point *m* indicates a partial solvation (total coordination number of 0.4 in layer –C shown in Table 6.4) O9 atom of glucosamine molecule. that sometime water molecule was interacted by O9 atom of glucosamine molecule.

It is interesting to note that the low density points on the contour plot, such as points *f*, *h* and *j*, relates to mobility of water molecules due to a weak glucosamine-water interaction. This event is supported by the undefine first-minimum of almost all RDFs, as well as the distribution of the coordination numbers depicted in Figure 6.22. Percentage of finding 5, 6, 7, 8 and 9 water molecules around the ligand up to the distance of 4.6 Å are 8.6, 22.6, 37.1 23.2 and 8.5%, respectively. These yield the average of the coordination number of 7.0 water molecules.

Taking into account all data mentioned above, precise positions of the 7 water molecules in the first solvation shell have been drawn and displayed in Figure 6.23 and the snapshot (one out of 16 million equilibrium configurations) is displayed in Figure 6.24.

As mentioned in Chapter 1, the data on the simulation of the glucosamine is not yet available. However, investigations have been done for similar ligand, glucose, using force-field molecular dynamics method [62] and first principles molecular dynamics [63]. The first one deals with the rubbery and glassy states of an 85% (w/w) aqueous solution of glucose. The results show the average hydration number of each glucose unit of 5. The first principles simulation performs for one glucose in 58 water molecules. It has been found that the average numbers of glucose-water hydrogen bonds for the β-, α_1- and α_2-glucoses are 10.2, 11.1 and 10.5, respectively. Although such data is not able to compare directly to our results but it indicates reliability of the first shell coordinate numbers of glucosamine of 7 which found in this study.

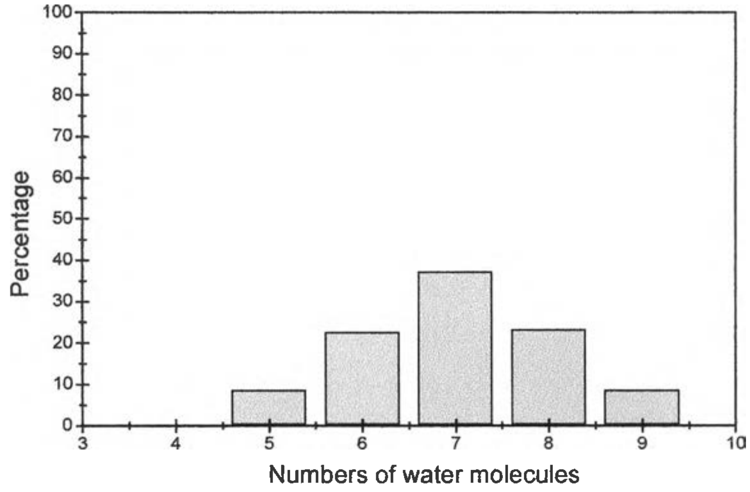


Figure 6.22 Distribution of running integration number in the first solvation shell of glucosamine (up to 4.6 Å).

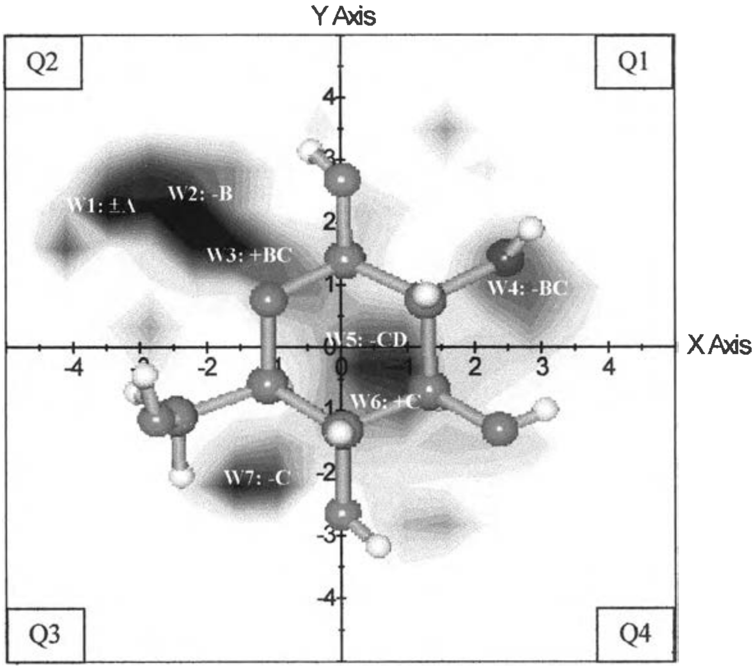


Figure 6.23 Summarized positions of 7 water molecules (W) in the first solvation shell of glucosamine (the letters A, B, C, and D and the signs plus and minus show the z-axis value; see Figure 6.11).

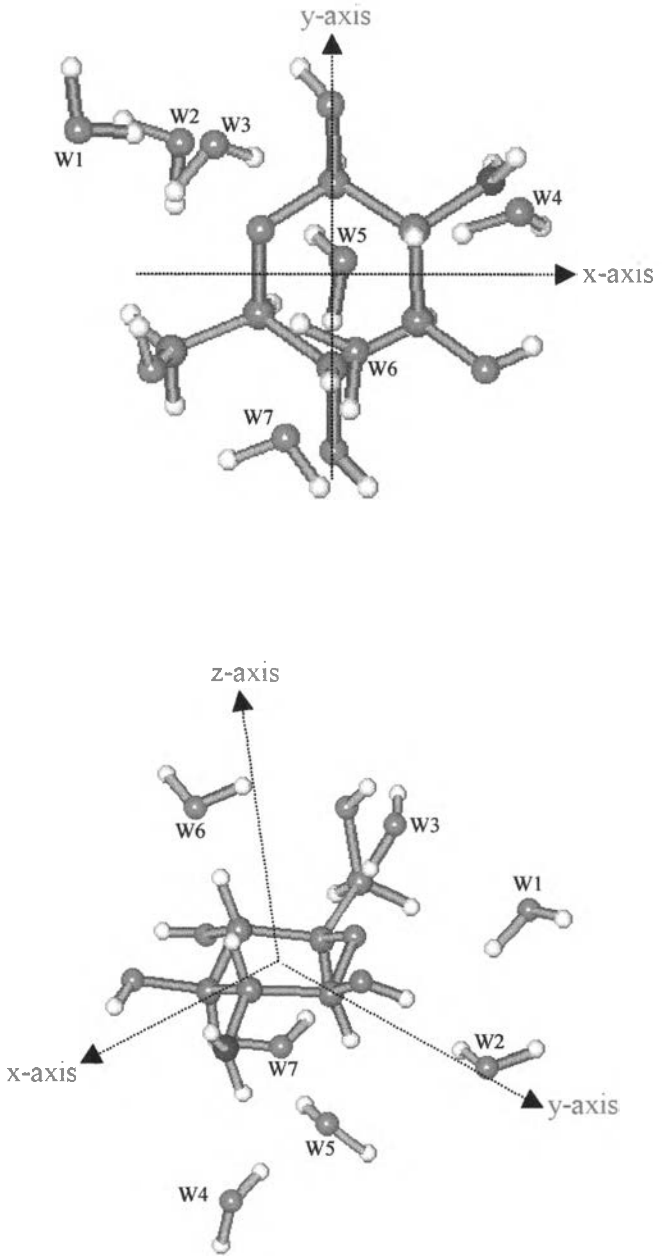


Figure 6.24 Snapshot (one out of 16 million equilibrium configurations) of the first solvation shell of glucosamine consisting of 7 water molecules.