1 \(^1\)H NMR Elucidation of Observed Stable Sugar-\(\text{NaCl}\)-water Complexes in Aqueous Solution

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6 KEYWORDS. \(^1\)H NMR elucidation; mono/disaccharides; stable sugar-\(\text{NaCl}\)-water complex; NaCl effect; aqueous solution.

7 Abstract:
8 The solvation of sugars in aqueous media matters in the understanding of biological systems
9 and carbohydrate transformations. Generally, 2 – 4 water units were proposed to interact with
10 each hydroxyl group in monosaccharides via different types of hydrogen bondings at room
11 temperature in previous studies. Presence of NaCl is known to perturb hydrogen bondings of
12 sugar hydrates. However, direct evidence to elucidate mechanism at atom level is very rare
13 even though the "NaCl Effect" was well known in biomass chemical transformations. Here we
14 report \(^1\)H NMR elucidation evidences of mono/disaccharides hydrates in different
15 concentrations of NaCl aqueous solutions. We here conclude two new findings: 1) under ideal
16 usage of NaCl, different mono/disaccharides hydrates are likely to be converted into a stable
17 sugar-\(\text{NaCl}\)-water form via a stepwise mechanism; 2) reactivity order (acidity difference) of
18 different hydroxyls in mono/disaccharides has intangible influence on hydrate form change
19 induced by NaCl. An ideal NaCl usage based on maximum of \(^1\)H NMR shift was proposed.

20 1.0 Introduction

21 Monosaccharides as basic units of glycogen, starch and cellulose, play very important roles in
22 biological systems and water is the only media\(^1\); hence solvation of monosaccharides by water
23 was studied for decades for better understanding of molecule mechanism. However, due to
24 lack of direct experimental evidence, exact interaction between monosaccharides like D-
25 glucose and water in solution is not yet fully understood, particularly regarding on how each
26 hydroxyl group is locally hydrated. In 1976, J. M. Harvey et al reported that each hydroxyl
27 group of D-glucose forms at least two hydrogen bonds with two water molecules, and four
28 solvated forms with different hydrogen bindings were proposed (Figure 1)\(^1,3\), via direct
29 measuring hydroxyl proton at low temperature. T. Suzuki’s simulation work also resulted into
30 a similar conclusion in 2008.\(^4\)

31 Figure 1. D-Glucose and its four different solvated forms in water.

32
The presence of sodium chloride was proved to perturb the hydrogen bonding network of water, resulting into sodium-saccharide interaction in water. Early studies via paper electrophoresis and optical rotation by S. J. Angyal showed that as a univalent cation sodium is highly hydrated in solution and a single hydroxyl group on monosaccharides cannot compete with water molecular in coordination; only two or three hydroxyl groups in a suitable arranged combination may result in sodium-monosaccharide complex in a very weak manner. A Raman-spectra study by F. Franks et al also indicated that sodium cation-glucose perturbation rather than sodium-glucose complex formation caused observed spectrum changes; and cations effect study also confirmed that the interaction induced by sodium cation is much weaker than other multivalent cations like Ca. Recently, NaCl usage in aqueous solution as an additive or a promoter for biomass transformations into valuable chemicals or biofuels is increasingly popular because they are abundant in nature and are very cheap; and “NaCl Effect” (or “salt effect”) became well known in biomass conversion and carbohydrate chemistry in the recent decade. Further system study of the role of metal halide in enhancing the dehydration of xylose to furfural by K. R. Enslow et al and insights from quantum mechanics/molecule dynamics on sodium ion interaction by H. B. Mayes et al both confirmed the previous hypothesis, which states that the cation has a stronger effect than the anion on glucose with the anions acting to stabilize critical intermediates. However, very recent higher yield productions of chemicals from monosaccharides in aqueous solution promoted by NaCl challenged the minor role hypothesis of chloride anion in a NaCl promoted system. The nature of complex-formation between sodium and monosaccharides is not yet well understood, particularly at atom level. No direct experimental evidence mapping bonding interaction between monosaccharides like glucose and NaCl in water has been reported to our best of knowledge, probably because of the instability nature of complex in solution and NMR spectrum was different depending on many factors. There are two important concerns yet to be answered: 1) would the presence of NaCl change reactivity order (acidity difference) of all hydroxyls in mono/disaccharides via perturbing hydrogen bonding networks in aqueous solution? 2) could maximum promotion (or activation) usage of NaCl be predictable? To answer these two concerns, here we reported 1H NMR
evidences of a relatively stable sugar-NaCl-water complexes observed on different mono/disaccharides in concentrated NaCl solution, which is not yet reported before. Combining with observed $^1$H NMR shifts correlated with a concentration of NaCl solution, a more detailed mechanism on role of chloride anion is proposed here for a better elucidation of “NaCl Effect”.21

It was known that solid state NMR and IR spectra both showed no obvious change, especially in spectral regions characteristic of the ordered and disordered regions of cellulose in the presence of NaCl.22,23, probably because of cellulose’s poor solubility in water. Inspired by a sharp difference of $^1$H NMR shifts of glucosamine in saturated NaCl solution, we envisaged an NMR angle by measuring $^1$H NMR shifts of monosaccharides and disaccharides might deliver more experimental evidences to understand the mechanism of “NaCl Effect”. Hence, water soluble monosaccharides including D-glucose, D-glucosamine hydrogen chloride, N-acetyl-D-glucosamine and D-fructose were initially chosen in order to gain some insights on the “NaCl effect” on the intramolecular/intermolecular hydrogen bonding in water via regular $^1$H NMR measurement. Variant concentration and saturated NaCl solutions were prepared for comparison study.

2.0 Experimental Section

2.1 Preparation for different concentrations of NaCl solutions M (wt%)

1. 0.08 M (0.5 %) NaCl solution: 29.2 mg NaCl was dissolved in 6 mL deionized water.
2. 0.33 M (1.9%) NaCl solution: 116.8 mg NaCl was dissolved in 6 mL deionized water.
3. 0.42 M (2.4%) NaCl solution: 146.1 mg NaCl was dissolved in 6 mL deionized water.
4. 0.85 M (4.8%) NaCl solution: 1 g NaCl was dissolved in 20 mL deionized water.
5. 1.71 M (9.1%) NaCl solution: 2 g NaCl was dissolved in 20 mL deionized water.
6. 2.56 M (13.0%) NaCl solution: 3 g NaCl was dissolved in 20 mL deionized water.
7. 3.42 M (16.7%) NaCl solution: 4 g NaCl was dissolved in 20 mL deionized water.

2.2 General procedure for $^1$H NMR investigation on monosaccharides

Monosaccharide (0.5 mmol) was added into a different concentration of NaCl solution (6 mL) and the mixture was stirred for 6 h at room temperature. After then, 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37°C. Removal of residual solvent in vacuum gave a crude product (dissolved in 0.4 mL D$_2$O) for $^1$H NMR to determine chemical shift.

2.3 General procedure for $^1$H NMR investigation volume impact (1.71 M NaCl solution) of “NaCl effect” on N-acetyl-D-glucosamine

N-Acetyl-D-glucosamine (0.5 mmol) was added into 1.71 M (9.1 wt%) NaCl solution (1.5 mL, or 3 mL or 6 mL or 12 mL) and the mixture was stirred for 6 h at room temperature. After that, 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37°C. Removal of residual solvent in vacuum gave a crude product (dissolved in 0.4 mL D$_2$O) for $^1$H NMR to determine chemical shift.
Note: sample concentration means the concentration of NaCl in NMR tube. In procedures 2.2 - 2.3, 1 mL of solution was taken out and the water was evaporated along with ethanol added to give a crude solid; the solid was redissolved into 0.4 mL D$_2$O and it led to a concentration increase in NMR tube (2.5 times of prepared solution).

2.5 Procedure for $^1$H NMR comparison study (glucose)

2.5.1 Preparation of 1.71 M (9.1 wt%) NaCl solution

2 g NaCl was dissolved in 20 mL deionized water.

2.5.2 Procedure

D-glucose (0.5 mmol) was added into a NaCl solution (1.71 M, 9.1 wt%, 6 mL) and the procedure is similar to procedure 2.2. Removal of residual solvent in vacuum gave a crude product (dissolved in 0.4 mL D$_2$O) for $^1$H NMR to determine chemical shift. Meanwhile another 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37 °C. Removal of residual solvent in vacuum gave a crude product (dissolved in 1 mL D$_2$O), part of them for $^1$H NMR to determine chemical shift for comparison.

2.5.3 Procedure for $^1$H NMR study of NaCl solution directed preparation in D$_2$O.

Preparation of 1.71 M (9.1 wt%) NaCl solution: 0.2 g NaCl was dissolved in 2 mL D$_2$O. D-glucose (0.1 mmol) was added into above NaCl solution (1.2 mL) and the mixture was stirred for 6 h at room temperature. After then, 0.5 mL of the reaction mixture was taken out (2 times of concentration in preparation) for $^1$H NMR to determine chemical shift.

2.5.4 Procedure for preparation a control (D-glucose in D$_2$O).

D-glucose (0.05 mmol) was added into D$_2$O (0.6 mL); and the reaction mixture was taken out for $^1$H NMR to determine chemical shift.

3.0 Results and Discussion

3.1 $^1$H NMR Study of monosaccharides in NaCl solution (0.20 M (1.25 wt%) to saturated solution (5.40 M))

Initially, 0.5 mmol of N-acetyl-D-glucosamine was dissolved into 6 mL of NaCl solution in a different concentration and the mixture continued to stir for 6 hours at room temperature before one portion was taken out for $^1$H NMR measurement. Five easily identified peaks, including hydrogens on 1-position carbon of both α-anomer and β-anomer$^{24}$, were marked (Figure 2) in order to track their changes in different concentrations of NaCl solutions.

Figure 2. $^1$H NMR Spectra of N-Acetyl-D-glucosamine and Five Peaks Marked for Tracking.
For N-acetyl-D-glucosamine, protons on 1-position carbon of α-anomer and β-anomer displayed at δ 5.18 and 4.67 respectively on 1H NMR spectrum in D2O, the former was at left side of D2O peak while the latter was at right side. In 0.20 M (1.25 wt%, in NMR tube) NaCl solution, influence of NaCl to 1H NMR shifts of all marked five peaks (△δ <= -0.02 ppm) were almost intangible; meanwhile the shifts became obvious (△δ = 0.03 - 0.13 ppm) in 0.83 M (4.75 wt%, in NMR tube) NaCl solution (Figures 3 and supporting information). An interesting observance was that the shift (△δ = 0.13 ppm) of the second marked peak (H1 of β-anomer) was the bigger than that (△δ = 0.05 ppm) of the first one (H1 of α-anomer) in 0.83 M (4.75 wt%) NaCl solution, indicating the presence of NaCl had a stronger influence on β-anomer. A significant deshielding effect was observed for all protons of N-acetyl-D-glucosamine (△δ = 0.26 ppm) in the presence of 2.13 M (12 wt%) NaCl solution (in NMR tube), which was induced by hydrated NaCl (Figures 3). A remarkable downfield shifting (△δ = 0.18 ppm) on 1H NMR shift for all five peaks was also observed by increasing of 2.13 M (12 wt%) NaCl solution to 4.28 M (22.8 wt%) NaCl solution (in NMR tube). Such an obvious shift is comparable to what K. R. Enslow et al. had observed with D-xylose in NaCl solution. Change (△δ <= 0.02 ppm) became intangible when further increase of concentration to saturated NaCl solution (in NMR tube). Based on these data (Figures 2), it could be concluded that maximized perturbation of intramolecular/intermolecular hydrogen bonding within N-acetyl-D-glucosamine molecule was reached in 4.28 M (22.8 wt%) NaCl solution and in which relatively stable sugar-NaCl-water complex might form. Meanwhile, such a stable complex was never observed before to our best knowledge.

Figure 3. 1H NMR Changes of N-Acetyl-D-glucosamine and D-Glucosamine Hydrogen Chloride Correlating with Concentration of NaCl solution and Molar Ratio by Tracking Marked Five/Six Peaks
In order to gain more clues, similar \(^1\)H NMR tracking investigation in different concentrations of NaCl solution were also performed with other three abundant monosaccharides (D-glucose, D-fructose and D-glucosamine hydrogen chloride) in nature (Figure 3 and 4). Above three monosaccharides were known to interact with sodium cation in aqueous solution differently. Similarly, in 0.20 M (1.25 wt%) NaCl solution, influence of NaCl to \(^1\)H NMR shifts of all marked six peaks (\(\Delta \delta \leq -0.05\) ppm) of D-glucosamine hydrogen chloride was almost intangible; meanwhile the shifts became obvious (\(\Delta \delta = 0.04 - 0.09\) ppm) in 0.83 M (4.75 wt%) NaCl solution (Figures 3 and supporting information). The shift change (\(\Delta \delta = 0.09\) ppm) of the second marked peak (H\(_1\) of \(\beta\)-anomer) and that (\(\Delta \delta = 0.07\) ppm) of the first one (H\(_1\) of \(\alpha\)-anomer) was narrow in 0.83 M (4.75 wt%) NaCl solution, indicating the presence of acetyl group had an obvious influence and the anomeric effect became not obvious. Interestingly, the possible stable complex could only be obtained when NaCl concentration is increased to saturated solution (in NMR tube). The significant delay to a stable sugar-NaCl-water complex
was likely due to free amine moiety of D-glucosamine hydrogen chloride (stronger inter/intramolecular bonding).

Figure 4. $^1$H NMR Changes of D-Glucose and D-Fructose Correlating with Concentration of NaCl solution and Molar Ratio by Tracking Marked Six Peaks.

In 0.20 M (1.25 wt%) NaCl solution, a downfield effect ($\Delta \delta = 0.02 - 0.03$ ppm) to all marked six peaks of D-glucose was observed (Figures 4 and supporting information), unlike the existence of both upfield effect and downfield effect to the marked five/six peaks of N-acetyl-D-glucosamine/D-glucosamine hydrogen chloride. Significant downfield changes ($\Delta \delta = 0.15 - 0.18$ ppm) were observed upon the increase of concentration of NaCl solution from 0.20 M (1.25 wt%) to 1.05 M (6 wt%) (in NMR tube). No change at all or little change ($\Delta \delta = 0 - 0.03$ ppm) for all marked peaks was found upon further increase of the concentration of NaCl solution from 1.05 M (6 wt%) to 1.71 M (9.1 wt%) or from 1.71 M (9.1 wt%) to 2.13 M (12 wt%).
wt%) or from 4.28 M (22.8 wt%) to saturated solution; meanwhile significant changes ($\Delta \delta = 0.26 - 0.32$ ppm) were observed upon the increase of concentration of NaCl solution from 2.13 M (12 wt%) to 4.28 M (22.8 wt%). A possible stable sugar-water-NaCl complex was formed in 4.28 M (22.8 wt%) NaCl solution. Trends of $^1$H NMR shifts of D-glucose in marked six peaks were pretty like a staircase, not a line, indicating a stepwise formation of hydrogen (H$_2$O) - chloride bonding.

For D-fructose, an upfield effect ($\Delta \delta = -0.02$ ppm) to all marked six peaks was observed in 0.20 M (1.25 wt%) NaCl solution, probably because of its rigid furanose structure. Similar to the observance for D-glucose, obvious $^1$H NMR shifts ($\Delta \delta = 0.10 - 0.11$ ppm) could still be observed for all six marked peaks when further increase of concentration to 1.05 M (6.0 wt%) from 0.20 M (1.25 wt%); meanwhile little change ($\Delta \delta = -0.01$ ppm) was shown upon further increase of concentration from 1.05 M (6.0 wt%) to 2.13 M (12 wt%). Surprisingly, anomeric effect was intangible for D-fructose. Continued increase to saturated NaCl solution led to small changes ($\Delta \delta = 0.08$ ppm) for all six marked peaks on $^1$H NMR spectrum. Such observed staircase-like trend indicated that correlation of $^1$H NMR shift of other monosaccharides (without amine moiety) to concentration of NaCl solution might also be similar, which is an important insight for understanding of “NaCl effect” at atom level.

Theoretically the change of $J$ coupling constants upon NaCl addition could be possible, however, it was concluded that the $J$ coupling constants rarely changed upon NaCl addition in our system based on tracking $^1$H NMR data on another random selected five peaks between 3.0 to 4.0 ppm (data and zoom-up spectrum with peaks picked see supporting information).

Hence, in our system the method to track marked peaks in our manuscript is trustable. It is likely because of the excellent stability of stereoechemistry structure and the lack of foldable structure in monosaccharides; and intramolecular interaction is also negligible under our conditions (relatively low concentration).

It is worth being noted that shifting trends of all marked peaks were similar in all four monosaccharides which indicated that acidity of proton has no observable effect on perturbation of hydrogen bonding, and generation of new hydrogen bonding networks was non-selective at relatively high concentration of NaCl solution. However, anomeric difference did exist for some monosaccharides in relatively low concentration of NaCl solution, in which such selective bonding might be present. Another important insight is that chloride anion likely plays major role on induction of $^1$H NMR shift because of two reasons: 1) sodium cation is known to have less specific locations of binding due to its univalent and readily hydrated property in water.\textsuperscript{25} Typically, two or three hydroxyl groups of monosaccharides are necessary to bind with each sodium cation and it is not so surprising that only one or two hydrated sodium cations can interact with each monosaccharide weakly. 2) Sodium cation is known to be buried inside water shells in its hydrated form meanwhile chloride anion is on the top of water cluster in its hydrated form\textsuperscript{26}. In addition, strong perturbation of anions by exerting on adjacent H atoms was reported\textsuperscript{27} through experimental Raman spectral measurements with classical MC simulations.

3.2 $^1$H NMR Study of disaccharides/oligosaccharide in NaCl solution (2.13 M (12 wt%) to saturated solution) at room temperature and 60°C.
Disaccharides and oligosaccharides have stronger hydrogen bonds with water because of more chelating bonds involved, and self-associate to form cluster is also involved in aqueous solution. Next, two disaccharides (sucrose and trehalose) and one tetrasaccharide (stachyose) were selected as targets for $^1$H NMR comparison study in higher concentrations of NaCl solutions at room temperature and $60^\circ$C (supporting information) in order to provide more viable references for NaCl promoted perturbation of hydrogen bonding of polysaccharides such as cellulose. As a disaccharide formed by a 1,1-glycosidic bond between two $\alpha$-glucose units, trehalose has a stronger hydrogen bonding network than D-glucose. Disaccharide containing fructose moiety and fructose need a higher concentration of NaCl solution to generate the possible sugar-NaCl-water complexes since the ring of fructose hydrate is more rigid than that of glucose hydrate, leading to less mobility of H-bonded water.

At room temperature in 2.13 M (12 wt%) NaCl solution (in NMR tube), $^1$H NMR shifts ($\Delta\delta = 0.27 - 0.30$ ppm) for all four marked peaks of trehalose were much bigger than those observed ($\Delta\delta = 0.15 - 0.16$ ppm) with sucrose (supporting information). It was easy to be understand because one molecule sucrose composed of two monosaccharides (glucose and fructose) and the moiety (fructose) in slow rate determined the rate of sucrose in perturbation of hydrogen bonding. Such difference between shifts of all four peaks of trehalose ($\Delta\delta = 0.34 - 0.49$ ppm) and those of sucrose ($\Delta\delta = 0.39 - 0.43$ ppm) became very little in case that concentration of NaCl solution was over 4.28 M (22.8 wt%) (in NMR tube).

Replacement of sucrose to tetrasaccharide (stachyose) led to little change on the correlation between $^1$H NMR shift and concentrations of NaCl solution (supporting information). Such similar change indicates that NaCl promoted stable sugar (moiety)-NaCl-water complexes are likely present as well for oligosaccharides in NaCl solution.

An increase of mixing temperature to $60^\circ$C had positive effect ($\Delta\delta = 0.11$ ppm) on perturbation of hydrogen bonding of sucrose (Table 1 and supporting information) in relatively low concentration of NaCl solution (2.13 M (12 wt%) in NMR tube); smaller shifts ($\Delta\delta = 0.02$ ppm) for all four marked peaks of stachyose were also observed upon the increase to $60^\circ$C (see supporting information). However, influence became intangible in relatively high concentration of NaCl solution (4.28 M, 22.8 wt%) for both disaccharides and stachyose (Table 1 and supporting information). Such observance indicated the formed sugar-NaCl-water complexes are relatively stable and could be prepared in relatively low concentration of NaCl solution under elevated temperature or in higher concentration at room temperature. For trehalose, a remarkable upfield effect ($\Delta\delta = -0.13 - 0.14$ ppm) was observed in 2.13 M (12 wt%) NaCl solution, probably because self-associate of trehalose was strongly accelerated at $60^\circ$C.

Table 1. $^1$H NMR shifts (Four marked peaks of sucrose) under different concentrations resulted from increase of temperature ($60^\circ$C).

<table>
<thead>
<tr>
<th>Concentration (NMR sample)</th>
<th>1 ($\Delta\delta$)</th>
<th>2 ($\Delta\delta$)</th>
<th>3 ($\Delta\delta$)</th>
<th>4 ($\Delta\delta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-0.011</td>
<td>-0.010</td>
<td>-0.010</td>
<td>-0.011</td>
</tr>
<tr>
<td>2.13 M (12.0%)</td>
<td>0.109</td>
<td>0.107</td>
<td>0.106</td>
<td>0.111</td>
</tr>
<tr>
<td>4.28 M (22.8%)</td>
<td>-0.032</td>
<td>-0.030</td>
<td>-0.029</td>
<td>-0.032</td>
</tr>
</tbody>
</table>
The significant shifts for all marked peaks of sucrose in the presence of 2.13 M (12 wt%) NaCl solution at room temperature and 60°C indicated the importance of temperature; particularly the temperature might play a more important role for polysaccharides that have poor solubility in water due to large molecular weight. NaCl concentration was well known to have strong effect on hydrogen bonding in water due to solvation\textsuperscript{29-31}. The higher concentration of NaCl solution, the better effect on perturbation of hydrogen bonding network based on \textsuperscript{1}H NMR shifts.

3.3 NMR instrument impact on \textsuperscript{1}H NMR shifts of D-glucose in D\textsubscript{2}O.

Table 2. \textsuperscript{1}H NMR shifts (six marked peaks of D-glucose) on different NMR instruments at different date.

<table>
<thead>
<tr>
<th>Entry (E)</th>
<th>Date (brand)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>(\Delta \delta (E_2 - E_1))</td>
<td>0.032</td>
<td>0.031</td>
<td>0.026</td>
<td>0.027</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>4</td>
<td>2018 (Bruker)</td>
<td>5.143</td>
<td>4.551</td>
<td>3.841</td>
<td>3.665</td>
<td>3.408</td>
<td>3.161</td>
</tr>
<tr>
<td>5</td>
<td>(\Delta \delta (E_4 - E_1))</td>
<td>-0.045</td>
<td>-0.056</td>
<td>-0.042</td>
<td>-0.048</td>
<td>-0.052</td>
<td>-0.051</td>
</tr>
</tbody>
</table>

As we discussed in the introduction part, many factors might have influence on absolute value of shifts of \textsuperscript{1}H NMR, here we investigated the impact of NMR instruments on six marked peaks of D-glucose by measuring the sample on different NMR instruments at different date. Both NMR instruments were in default working conditions at room temperature for users from chemistry laboratories. The error (\(\Delta \delta=0.03\) ppm) between two \textsuperscript{1}H NMR spectrum (in D\textsubscript{2}O) recorded on JEOL NMR instrument at Shanghai in Sept.2021 and Dec. 2021 was in acceptable error range (Entries 1 – 3, Table 2). The difference (\(\Delta \delta=0.04 – 0.06\) ppm) for \textsuperscript{1}H NMR spectrum of D-glucose in D\textsubscript{2}O (Entries 1, 4 and 5, Table 2) was slightly bigger when samples were recorded on different NMR instruments (JEOL at Shanghai, Dec. 2021 and Bruker at Guangzhou, 2018). Based on these comparison data, a general guideline is that a control sample is strongly suggested to be done at the same time for comparison in case samples in very low concentration of NaCl solution (< 0.20 M (1.25 wt%)) or a change to a new NMR instrument.

3.4 Volume effect

In procedures 2.1 - 2.4 in experimental section, 1 mL of solution was taken out and the water was evaporated along with ethanol added to give a crude solid; the solid was dissolved into 0.4 mL D\textsubscript{2}O and it led to a concentration increase in NMR tube (2.5 times of prepared solution). Sample concentration in Table 3 means the concentration of NaCl in NMR tube. A recent comparison study (Entries 1 – 3, Table 3) on another NMR instrument (JEOL) indicated the
difference ($\triangle \delta = 0.06 - 0.08$ ppm) of six marked peaks of D-glucose was very small between the two samples mixed in 4.28 M (22.8 wt%) solution (in NMR tube), considering the obvious shifts ($\triangle \delta = 0.44 - 0.52$ ppm) in comparison with D-glucose in the absence of NaCl (supporting information). Not surprisingly, an increase on concentration in NMR tube (2.5 times) led to a sharp change on shifts of all six peaks (Entries 2 and 4, Table 3). A control study in NaCl/D$_2$O solvent (1.71 M, 9.1 wt%) was also carried out and part of solvent mixture was directly taken out for $^1$H NMR measurement (Entries 5, Table 3); the shifts of all six marked peaks had little difference ($\triangle \delta <= 0.02$ ppm) with those via evaporation/dissolving process (Entries 4, Table 3).

**Table 3.** $^1$H NMR shifts (six marked peaks of D-glucose) on different NMR instruments at different date.

<table>
<thead>
<tr>
<th>Entry (E)</th>
<th>Concentration (sample)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.71 M (4.28 M, 0.4 mL D$_2$O) (2018, on Bruker)</td>
<td>5.633</td>
<td>5.061</td>
<td>4.276</td>
<td>4.129</td>
<td>3.926</td>
<td>3.664</td>
</tr>
<tr>
<td>2</td>
<td>1.71 M (4.28 M, 0.4 mL D$_2$O) (Dec. 2021, on JEOL)</td>
<td>5.568</td>
<td>4.992</td>
<td>4.217</td>
<td>4.051</td>
<td>3.850</td>
<td>3.588</td>
</tr>
<tr>
<td>3</td>
<td>$\triangle \delta$ (E2-E1)</td>
<td>-0.07</td>
<td>-0.07</td>
<td>-0.06</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.07</td>
</tr>
<tr>
<td>4</td>
<td>1.71 M (1.71 M, 1.0 mL D$_2$O) (Dec. 2021, on JEOL)</td>
<td>5.365</td>
<td>4.790</td>
<td>4.032</td>
<td>3.868</td>
<td>3.635</td>
<td>3.380</td>
</tr>
<tr>
<td>5</td>
<td>1.71 M (D$_2$O) (Dec. 2021, on JEOL)</td>
<td>5.383</td>
<td>4.799</td>
<td>4.053</td>
<td>3.885</td>
<td>3.654</td>
<td>3.397</td>
</tr>
</tbody>
</table>

Effects of volume of 1.71 M (9.1 wt%) NaCl solution (4.28 M (22.8 wt%) in NMR tube) was also investigated with N-acetyl-D-glucosamine (0.5 mmol) (Table 4). Downfield shift was observed along with increase of volume till 6 mL, indicated that after the bonding network became steady no change was observed on $^1$H NMR spectrum. The total usage of NaCl was 10 mmol under the turning point condition (6 mL) and molar ratio of NaCl to total hydroxyls (2.5 mmol) of monosaccharide was 4:1.

**Table 4.** $^1$H NMR Spectra of N-acetyl-D-glucosamine after mixing with different volume of 4.28 M (22.8 wt%) NaCl solution (in NMR tube) at room temperature.

<table>
<thead>
<tr>
<th>Volume (Molar ratio of $c_{NaCl}/c_{NAG}$)</th>
<th>1 ($\triangle \delta$)</th>
<th>2 ($\triangle \delta$)</th>
<th>3 ($\triangle \delta$)</th>
<th>4 ($\triangle \delta$)</th>
<th>5 ($\triangle \delta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 mL (5.13)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 mL (10.26)</td>
<td>0.050</td>
<td>0.054</td>
<td>0.048</td>
<td>0.048</td>
<td>0.058</td>
</tr>
<tr>
<td>6 mL (20.52)</td>
<td>0.236</td>
<td>0.229</td>
<td>0.206</td>
<td>0.227</td>
<td>0.231</td>
</tr>
<tr>
<td>12 mL (41.04)</td>
<td>-0.017</td>
<td>-0.003</td>
<td>-0.003</td>
<td>0</td>
<td>-0.004</td>
</tr>
</tbody>
</table>
3.5 $^1$H NMR chemical shifts referencing.

Peak of HDO was a reference (4.79 ppm) in processing all spectrum. The HDO peak position is known to be sensitive to salt concentration\textsuperscript{28a}, such shift was mainly due to the same mechanism of "salting-out effect" which was quite well understand; salts is dissolved in water and water clusters collapse around the ions, leading to a volume contraction, electrostriction.\textsuperscript{28b,c} Such salt induced cavitation change of water structure (global changes of the water H-bonding network and hydration upon salt addition) likely has similar effect on monosaccharides soluble in NaCl solution, resulting to some changes on $^1$H NMR shift of monosaccharides. Hence, by using HDO as a reference, the chloride-water-sugar bonding effect resulted $^1$H NMR shift could be decoupled from those that resulted from salt induced cavitation change of water structure, and this is crucial to this work to half-quantify the bonding ratio of the stable sugar-NaCl-water complex.

Key evidence to prove existence of both global salt solution effect and chloride bonding effect on $^1$H NMR shifts is the data from Table 4. Because all experiments were carried out under exactly the same concentration in reaction vials (1.71 M) and their samples were measured under exactly the same concentration in NMR tubes (4.28 M), $^1$H NMR shift change that resulted from salt induced cavitation change of water structure was supposed to be the same for all solute in the salt solution; however, significant $^1$H NMR shift changes were still observed when the same amount of sugar was dissolved in different volumes of salt solution leading to the change on the ratio of sugar to salt in NMR tubes. The change became intangible when the mole ratio of NaCl to hydroxyls (of saccharides) increased to 4:1, indicating all possible stable (OH)H–Cl bondings at adjacent position were all formed (it is easy to be understand that some Cl– would still remain in the solution as free hydrate anion in bulky water). Based on above reasons and data, HOD is the very suitable reference in our current investigation.


**Figure 5. Stable Complex of D-Glucose-NaCl-water in NaCl Solution.**

The staircase trend in correlation of $^1$H NMR shifts (D-glucose/fructose) showed the formation of hydrogen (H$_2$O)–chloride bond was likely stepwise (Figure 4). Both sodium cations and chloride anions play an important role in perturbing hydrogen bonds with bulk water in proposed hydrated forms and intramolecular hydrogen bonding of mono/disaccharides; however, chloride anions may contribute much more to stabilize the sugar-NaCl-water
complexes and to the observed $^1$H NMR shifts due to the very weak coordination between sugar and sodium cations.

Both J. M. Harvey$^{23}$ and T. Suzuki$^{s}$' findings indicated that each hydroxyl group of D-glucose forms two hydrogen bonds with two water molecules (Figure 1), and another discovery by J. D. Smith et al.\textendash found that$^{27}$ changes of hydroxyl (from monosaccharides) vibrational spectrum is induced by the action of halide anion’s electric fields on adjacent water molecule near to hydroxyl. Based on these findings, chloride anions likely bind with two water molecules adjacent to each hydroxyl (or acetyl amide) respectively forming sugar-NaCl-water complexes; only one or two hydrated sodium cations will bind with sugars and most are very mobile. Since theoretically only three protons on the two adjacent water molecules of each hydroxyl are available with chloride anions, two possible sugar-NaCl-water complexes (I and II) with single hydrogen (H$_2$O)-chloride bond, one possible sugar-NaCl-water complexes (III) with two hydrogen (H$_2$O)-chloride bonds as shown in Figure 5, and a theoretically possible sugar-NaCl-water complexes (IV) with three hydrogen (H$_2$O)-chloride bonds would be very bulky. Relatively stable sugar-NaCl-water complex I or II or both for monosaccharides likely form under 1 $\sim$ 2 M NaCl solution with a molar ratio of NaCl to monosaccharide over 5 (the first staircase in Figure 4 and data with 1.5/3 mL in Table 4), meanwhile a much higher concentration of NaCl solution with a molar ratio of NaCl to monosaccharide over 20 is quite necessary to enable formation of the second hydrogen (H$_2$O)-chloride bond generating stable complexes III (the second staircase in Figure 4 and data with 6/12 mL in Table 4); the complexes (IV) with three chloride anions per hydroxyl might be unstable due to negative charge repulsion of two chloride anions binding with the same adjacent water molecule. It is consistence with our observance that molar ratio of NaCl to total hydroxyls (2.5 mmol) of monosaccharide was 4 : 1 when a stable sugar-water complex II formed since the presence of half of chloride anion in hydrated in bulk water might be quite necessary in stabilization of the formed complex (data with 6/12 mL in Table 4). The structure of complexes in NaCl solution is likely the same when they reach a stable status regardless of initial parameters of concentration, volume and temperature, this is of much importance for understanding of the well-known “NaCl effect”.

3.7 $^1$H NMR Study of D-glucose in biological relevant NaCl solution (< 0.20 M (1.25 wt%).

$^1$H NMR shifts of D-glucose in 0.20 M (1.25 wt%) showed clear downfield change on the spectrum for all six marked peaks ($\Delta \delta = 0.03$ ppm); meanwhile an upfield change ($\Delta \delta = -0.01$ - -0.02 ppm) for all six peaks of fructose was observed under the same condition (Figure 4 and supporting information). Such observance might be of much value to understanding of sugars in the human body via NMR analysis techniques$^{22}$, as well as for understanding of biological mechanism of Na-dependent cotransport of sugars like glucose and fructose. It is known that Na dependent glucose transport has an approximate half maximal Na concentration of around 50$\sim$70 mM for the Na dependent glucose cotransporter, whereas fructose is not transported in a Na dependent manner.$^{33,34}$ A further exploration on $^1$H NMR shifts of D-glucose in biological relevant NaCl solution (100 mM and 140 mM) at room temperature was also performed; similar downfield changes were still observable (Table 5). This small but clear downfield change indicated glucose-NaCl-water complex (more like
complexes I or II with only some (not all) hydroxyls bonding with chloride anions might generate in biological system, meanwhile similar fructose-NaCl-water could not form in low concentration of NaCl solution (< 0.20 M (1.25 wt%)); such difference may explain some of the stereospecificity of this process. Further work with more sensitive $^1$C NMR to verify such difference would be performed in the future.

**Table 5.** $^1$H NMR shifts of D-glucose in biological relevant NaCl solution at room temperature.

<table>
<thead>
<tr>
<th>Concentration (In NMR tube)</th>
<th>1 ($\Delta \delta$)</th>
<th>2 ($\Delta \delta$)</th>
<th>3 ($\Delta \delta$)</th>
<th>4 ($\Delta \delta$)</th>
<th>5 ($\Delta \delta$)</th>
<th>6 ($\Delta \delta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM (100 mM, 0.58%)</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.008</td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td>70 mM (140 mM, 0.82%)</td>
<td>0.010</td>
<td>0.011</td>
<td>0.019</td>
<td>0.016</td>
<td>0.011</td>
<td>0.011</td>
</tr>
</tbody>
</table>

3.8 A proposed ideal NaCl usage.

Concerning frequent optimization on NaCl usage in literatures, we tentatively propose a calculation model recommending the best usage of NaCl for maximum perturbation of hydrogen bonding network of mono/disaccharides. It is also very relevant for reference in depolymerization of polysaccharides including starch, chitin and cellulose. Recommended absolute amount of NaCl in solution is 3.5 – 4.0 equivalent to total hydroxyl of saccharides (including protons on amine moiety if glucosamine-based saccharides were used) based on observed maximum $^1$H NMR shift forming stable sugar-NaCl-water complexes. This recommended ideal usage is consistent with optimized condition in recent reported literature. For temperature, room temperature is usually sufficient for monosaccharides and oligosaccharides, which are soluble in water; however, typical increase of temperature is pretty necessary for cellulose depolymerization according to a recent report, stating that “NaCl effect” is only obvious when the temperature is above 210°C.

**4.0 Conclusion**

In summary, $^1$H NMR evidences of stable sugar-NaCl-water complexes with monosaccharides and oligosaccharides at room temperature were obtained, and the staircase trend in correlation of $^1$H NMR shifts (D-glucose/fructose) showed that the formation of hydrogen (H$_2$O)-chloride bond was likely stepwise; this observance is of much importance to the further understanding of prevailing “NaCl effect” at atom level. The shifting trends of all marked peaks correlation to concentration of NaCl solutions were very similar in all four monosaccharides; that indicated that the reactivity order (acidity difference) has no observable effect on $^1$H NMR shifts and generation of new hydrogen bonding network (H-Cl bond) is non-selective. However, anomeric effect did exist for some monosaccharides at relatively low concentration of NaCl solution. Although both sodium cations and chloride anions promote perturbation of hydrogen bonding network (global salt solution effect), chloride anions may play a key role in the stabilization of sugar-NaCl-water complexes and the induction of $^1$H NMR shifts via bonding as proposed in **Figure 5**. Based on the maximum of the induced $^1$H NMR shifts reaching stable sugar-NaCl-water complexes, a general recommended NaCl usage of 3.5 –
4.0 equivalent mole of hydroxyls (including amines or amides) on saccharides was proposed. We envisage more insights may be achieved when a full characterization of the stable sugar-
NaCl-water complexes in aqueous media is available in the future.

**Supporting Information.**

Completed general information, all reaction procedures, $^1$H NMR shifts summarized in tables, figures for shift trends of disaccharides/stachyose, reproducing note and all $^1$H NMR spectrum with marked peaks as well as J coupling constants data (and zoom-up spectrum with peaks picked) were all included in supporting information.

**Author Contributions**

Concept was designed by L. Gu; G. Zhu and H. Li carried out experiments and collected related data; L. Gu supervised this project and analyzed the data; Y. Li co-supervised this project; the manuscript was written by L. Gu and all authors participated in revision. All authors have given approval to the final version of the manuscript.

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**References:**


[21] Part of this work was presented in ACS Spring 2021 National Meeting (Organic Chemistry Division) (live). A recorded presentation video see: https://doi.org/10.1021/scimettings.1c00812.

[24] Usually a mixture of α-anomer and β-anomer both exist due to dissociated equivalent.


