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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Molecular understanding of ion specificity at the peptide bond

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

5 The Hofmeister series has remained a mystery for more than a century. A detailed understanding of interactions in ions-dissolved systems is still in need since the classical theories have failed to accommodate the specific ion effects. In this study, the interactions between ions, solvent and model compound for protein were explored using a direct nuclear magnetic resonance (NMR) approach along with density functional theory (DFT) calculations. It was found that the chaotropic anions caused
10 increasing chemical shifts of model compound while kosmotropic anions resulted in decreasing ones, which might suggest that the latter were prevented from interacting with the model compound. The experimental results can be explained by a combination of local electrostatic interactions and hydrogen bonding. Although more efforts should be given to justify the NMR method applied in this study, the results could give a quantitative standard for defining kosmotropes/chaotropes and might provide a new
15 way of predicting the effects of unfamiliar ions in the future.

Introduction

The relative ability of inorganic salts to precipitate proteins from aqueous solution was first systematically studied by Hofmeister and his co-workers more than 120 years ago.^{1, 2} Their works
20 resulted in a typical order of the anion series: $\text{SO}_4^{2-} > \text{F}^- > \text{HPO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{I}^- > \text{ClO}_4^- > \text{SCN}^-$. Although cation has weaker effects, an analogous order for cations was later collaborated to be typically: $\text{N}(\text{CH}_3)_4^+ > \text{NH}_4^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Ca}^{2+} > \text{Mg}^{2+}$.³ These two orders were later
25 named after its founder or originator as Hofmeister series. Generally, the anions to the left side of Cl^- are more likely to cause salting-out of proteins while the ones to the right end tend to salt-in proteins into solutions. As a rule, a term called “kosmotropes”, which means structure makers were assigned to
30 the formers while the latter ones were referred to as “chaotropes” that means structure breakers.⁴ Since its first appearance, more and more covert chemical and biological phenomena caused by specific ions were ascribed to Hofmeister effects.⁵⁻⁸ However, the mechanism of Hofmeister effects has still remained largely
35 vague, which makes it one of most challenging phenomena.^{9, 10} An answer for it would bear so many merits due to profound impacts of specific ion effects within chemical and biological systems that extensive studies with the help of most advanced methods and tools strived to resolve this recurring puzzle.¹¹⁻¹⁴
40 In the past century, so many classical theoretical ideas failed to accommodate the series that some would compare its importance to that of Mendel’s study of genetics.¹⁰ In general, in these classical theories seemed incompetent in rationalizing the ion effects. For example, the salting-in phenomenon cannot be
45 explained by the plausible hydration theories. Other similar theories based on dipolar, electrostatic, and *van der Waals* forces,

unfortunately suffer from shortages alike. One reason for the stagnant development of theories is that previous studies tried hard to focus on separate interactions between ions, water and
50 solutes. In reality, it’s widely accepted that the simple solvation of ions is vital for understanding these ions effects.¹⁵ However, it has never been proved to be helpful or easy to do so since the Hofmeister series itself might be a result of interplays of these interactions.

55 Recently, significant progresses have been made and details of interactions between ions, water and proteins or its model compounds are emerging. Even better, two insightful mechanisms or theories were thereby proposed,^{16, 17} both of which stressed on specific binding sites in the aqueous solution,
60 about which we would later discuss along with our results. One of them showed that anions indeed preferred some certain sites of the protein molecule.¹⁶ The other theory was outlined in a scheme, where preferred binding sites for both cation and anion were depicted. Although these theories still need further
65 evaluation, they have demonstrated that getting specific about specific ion effects seem to be the right direction for unlocking the Hofmeister secret.¹⁸

However, those theories are still unsatisfactory for a few reasons. On one hand, there is no exact explanation for why and
70 how the distributions of various anions were different. In other words, lack of quantitative reasoning makes them merely conceptual theories. On the other hand, only a few frequently encountered anions and cations were tested, while there are more than thousands of salts existing in chemical world. Previous
75 studies seemed to try to justify their theories with limited data while a fact that the inquiry into the mysterious Hofmeister series has gone beyond the original description,¹⁹ was often neglected.

Here, we report our unexpected findings of the surprisingly matching correlation between surface electrostatic potentials of

ions and changes in chemical shift for protons of a model amide compound. Additionally, our NMR results could potentially give an explicit definition to terms “kosmotropes” and “chaotropes” in a quantitative way, which have never been explicitly defined where they were quoted. A mechanism based on a combination of local electrostatic interactions (EI) and hydrogen bonding (HB) was hereby proposed. The proposed mechanism explains the Hofmeister series very well and most importantly, could be possibly used to predict effects of unfamiliar ions in the future studies.

Experimental section

All sodium and chloride salts with purities at least higher than 98% and N-methylacetamide (NMA, > 99%) were purchased from J&K Scientific Ltd. and used in experiments as received. The salts purity has already been proved to be adequate enough in NMR experiments.¹⁶ The deuterium solvent (D₂O of > 99.9%D) was purchased from the Analytical Instrumentation Centre of Peking University, Beijing, P. R. China.

The ¹H-NMR experiments were carried out on Bruker Advance III 400 HD machine controlled by Icon NMR program, and at constantly 298.15 K. Particularly, the fid files were collected without further reference to internal or external standards. It is known that the spectra can still be obtained because the deuterium signal is used to lock the magnetic field in each NMR run. In Icon NMR: Automation program, the D₂O solvent was selected and other default settings were left unchanged. Then, the machine automatically preceded the field locking, shimming and scanning. The data of chemical shifts were later picked in MestReNova Program. The reason for why internal reference was not made was that water-soluble internal standard compounds such as sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) would bring in extra ions, which was unfavoured in the study of specific ion effects. Although external standard was applied in previous study,¹⁶ the idea of excluding external reference originated from that identical samples results in identical NMR spectra under identical experimental conditions since NMR method is sensitive enough to reveal any tiny differences caused by other factors such as experimental temperature, solution viscosity and ionic strength, the last two of which is the most likely ones as a result of adding different salts. Seeking a general relationship between these spectral differences and intrinsic properties of ions (in this study, electrostatic potential surfaces, EPS) is the main purpose of this study. And by analysing the original NMR data, we might find out what really happens in the first place after the addition of salts, which might be possibly obscured by referring to external reference. Nevertheless, the NMR experiments of a few samples with DSS as external reference (DSS/D₂O solution was loaded in inserted capillary tube) were also carried out. However, it seemed unlikely that there was a universal relationship between changing chemical shifts and properties (kosmotropic or chaotropic) of ions because the difference of changes in chemical shifts was very small.

The samples were prepared according to an identical procedure: predetermined amount of salts was dissolved in 0.6 ml of NMA/D₂O solutions (0.25 M). In a recent study,¹⁶ the NMR

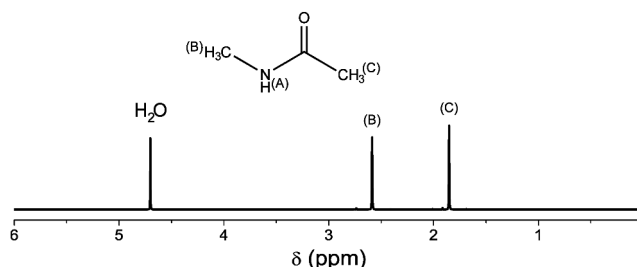


Fig. 1 Structural features of NMA: ¹H-NMR spectrum of NMA at 298.15 K. The solvent is D₂O and the concentration is 0.25 M. And this bulk solution of NMA in D₂O was used in all ¹H-NMR experiments later to measure the effects of specific ions on peptide bond. In all spectra, the proton A of NMA was not detected due to its activity.

spectra of solution containing monomer equivalents of ~42 mM seemed to be too dumpty to easily analysis the changing chemical shifts so that the concentration of NMA was increased by about five-folds in order to gain clean NMR spectra with sharp peaks that would be convenient and accurate in calculating changes in chemical shifts.

The calculations for electrostatic potential surfaces of ions and molecules were performed by using DFT at the B3LYP/6-311+G** level and 298.15 K under vacuum environment in Spartan software. Electrostatic potential surfaces were proved to be a useful qualitative guide in the study of non-covalent interactions such as halogen bonding,²⁰⁻²² cation-π²³ and hydrogen bonding.²⁰ Specially, electrostatic potential surfaces are proved to be useful and insightful in understanding the nature of halogen bond,²⁴⁻²⁶ which is recently being recognized as an equivalent of hydrogen bond in hydrophobic environment. In these works, Spartan software, DFT/B3LYP/6-311+G** basis set and vacuum environment were commonly applied.^{25, 26} Taking in the effects of hydration (calculating under water environment) would be more realistic since the hydration would reshape, to a more or less extent, the potential surfaces. And in order to be fair in comparison, additional calculations were performed as well. However, these additional calculating results were not complete because certain technical limitations prevented this from happening. The most pronounced one was that it is inappropriate to calculate the EPS of metal ions in water environment at 6-311+G** level or 6-31G** level. Also, to the best of our knowledge, there are rare reports about DFT-calculated EPS of metal ions in literature, without mentioning calculations in water environment. Nevertheless, hydration effects did not seem to cause significant changes in EPS of studied items.

Results and discussion

A model compound “N-methylacetamide (NMA)” was chosen to represent the peptide bond of proteins, which was an adequate model compound in previous studies.²⁷⁻²⁹ The ¹H-NMR spectrum of NMA was shown in Fig. 1. As mentioned above, the ¹H-NMR spectrum of NMA in D₂O was acquired without further external or internal references, which indicates that any slight difference in NMR spectra must be a result of changed chemical environment within the solution given that the changes in chemical shift cannot be affected by the instability of equipments because the ¹H-NMR test for bulk solutions of NMA was

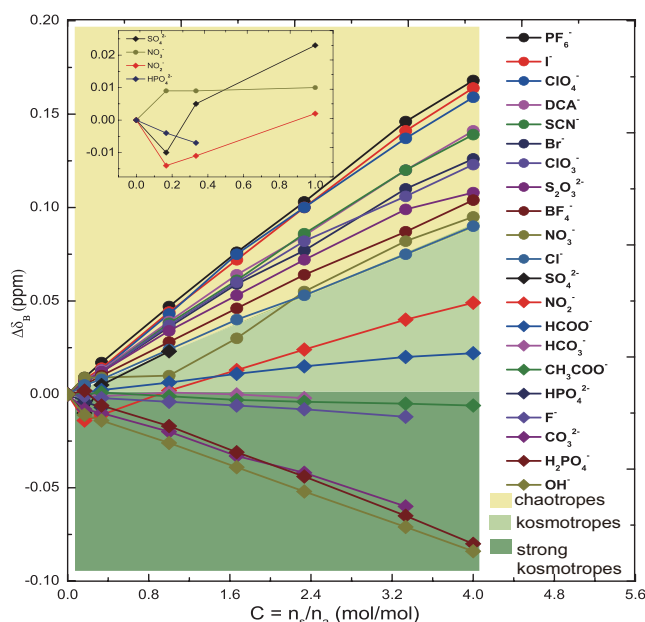


Fig. 2 Anionic effects on NMA when the cations are Na^+ . Linear relationships exist between the changes in the chemical shift for B proton of NMA and concentration of salts. The concentration was expressed in molar ratios, in which n_s stands for the mole of salts while n_a stands for mole of NMA. In this work, n_a was constantly 1.5×10^{-4} mole in 6 ml bulk solution. The inserted figure is the enlarged display of the whole figure at the concentration from 0 to 1.0 mol/mol. And DCA⁻ stands for the anion of sodium dicyanamide. The anions in yellowish region (inserted figure not included) were cataloged as chaotropes while light green and deep green colored regions represent the regimes of kosmotropes and strong kosmotropes, respectively.

repeated several times among more than 197 tests (Fig. S1).

In reality, Hofmeister series consists of two aspects, one of which is anionic effects while the other is cationic effects. The Hofmeister series were mostly referred to anionic effects, and most of the previous works focused on the anionic effects. Hence, our work started with anionic series. Upon addition of salts with different anions, there were significant changes in the chemical shifts for proton B and C of NMA (Fig. S2-S22, Fig. 2 and Fig. S23). For a given anion, a nearly linear relationship existed between the changes in the chemical shifts for proton B and C of NMA, and the concentration of ions, except for very low concentration (< 0.4 mol/mol or ~ 100 mM¹⁰) or a few anions such as NO_3^- . The salt concentration was specially expressed in molar ratios term instead of mol/L. Although the differences between molar ratio and volumetric concentrations might be negligible, this expression was made due to two considerations. For the one thing, it would be more accurate since high salt content leads to reductions in the volume of electrolyte solutions, which is also a much more complex issue itself. For the other, it would be closer to the origin since the ability of salts to precipitate the egg white and serum was measured by the required mass ratio in Hofmeister's works. In fact, when the protein content is extremely low, it would not be precipitated regardless of the salt content and thus the ability could not be distinct.

For various anions at a given concentration, the changes in the chemical shifts for B (and C) protons were remarkably different. Sodium salts with chaotropic anions such as I^- and

SCN^- caused increasing changes in the chemical shifts, whereas kosmotropic anions such as OH^- and CO_3^{2-} led to significantly decreasing ones. It is worth noticing that the directions for changes induced by $\text{SCN}^-/\text{Cl}^-/\text{SO}_4^{2-}$ in chemical shifts were opposite to, while the relative order remained consistent with, that of one of Cremer's works,¹⁶ which might be because that the sample spectra were externally referenced in his work. To be fair in comparison, additional NMR tests were carried out (Fig. S24-S28). It was found that the chemical shifts of NMA were decreasing with increasing concentrations of either chaotropic or kosmotropic salts (Fig. S29). This was consistent with previous results¹⁶ despite that the degree of downshifting was smaller (SCN^- and Cl^- anions), which might be due to the lower molar ratio (4 mol/mol in this work) of salts-to-NMA, which was up to 24 mol/mol in Cremer's work. It seemed there was a general correlation between NMR changes and anionic types (chaotropes or kosmotropes) if one only looks at a selected group of anions. However, it is astonishing that there was no general relationship between changes in chemical shifts and ion species (chaotropes or kosmotropes). For example, the extent of changes in chemical shifts caused by NaSCN that was a chaotropes was found to be located between two strong kosmotropes (Ac^- and H_2PO_4^- in Fig. S29). Within a limited selection of anions, there might be a general relationship between anionic species and changes in chemical shifts (as showed in previous study), whereas the relationship disappeared within a larger group of samples (Fig. S29). This indicated that the method of using external reference was somehow not accurate in predicting anionic effects on a larger scale although it has already been proved to be a reliable method,³⁰⁻³² since it could not differentiate chaotropes from kosmotropes. That might be the reason for that there is still no comprehensive NMR study of Hofmeister series, which should include at least the full anionic and cationic series.

However, the order in this study measured by changes in chemical shift at same concentration was consistent with typical orders for anions of complete Hofmeister series that includes ten anions (Fig. 2). Moreover, other unfamiliar anions such as PF_6^- , BF_4^- and $\text{N}(\text{CN})_2^-$ (DCA) were also put in tests because those anions were very common in study of ionic liquids (ILs). The anionic species greatly contribute to the phase behaviours (such as Lower Critical Solution Temperature, LCST) of ILs/water systems.³³⁻³⁵ And the contributions of anions to LCST seemed to follow Hofmeister series.³⁶ The results of 21 anions (actually 22, but the solubility of Na_3PO_4 is very poor in water and heavy water) were tested in order to seek a universal mechanism of Hofmeister series.

Overall, chaotropes caused increasing changes in the chemical shifts while kosmotropes caused decreasing ones. It is worth noticing that $\text{S}_2\text{O}_3^{2-}$ anion, traditionally viewed as a kosmotrope, shares the behaviour of chaotropes. An explanation for this phenomenon might be the contributions from extra Na^+ and the weak HBs between $\text{S}_2\text{O}_3^{2-}$ and solvent (would be later confirmed by anionic effects on water residue). It has to be pointed out that the concept of chaotropes or kosmotropes has never been quantitatively defined. Thus, there are actually some small deviations to anionic order based on different methods.^{37, 38}

Here, the anions studied in this work could be divided into three groups: chaotropes, kosmotropes and strong kosmotropes,

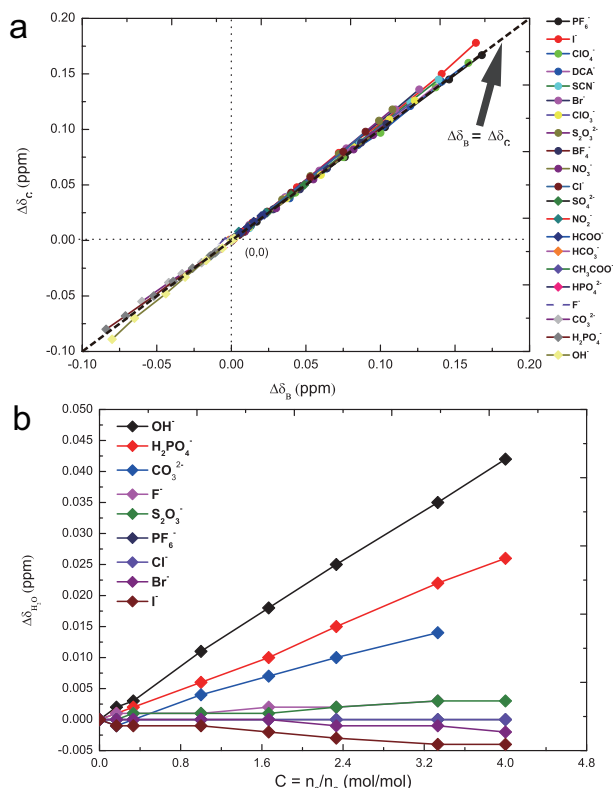


Fig. 3 Anionic effects on NMA and water residue. (a) Correlation between changes in the chemical shift for B proton and that for C proton. (b) Changes in the chemical shift for proton of water residue at different concentrations by specific anions.

based on the conventional concept that anions to the right side of Cl⁻ are considered as chaotropes and vice versa. At a given concentration ranging from 0.4 to 4 mol/mol, if a certain anion induces larger positive NMR changes than that by Cl⁻ it would be assigned to chaotropes. For example, S₂O₃²⁻ is chaotropic while SO₄²⁻, a traditional kosmotrope, was still in kosmotropic range since the changes in chemical shifts caused by it were smaller than that of Cl⁻. The Hofmeister series only consists of about ten anions, and this work has expanded the series into a comprehensive one consisted of more than twenty anions. Fig. 2 showed that some of kosmotropes (such as OH⁻) were so strong that they caused significant negative changes in chemical shifts. Thus, to be precise, they should be catalogued as strong kosmotropes (deep green-coloured regions in Fig. 2).

Either chaotropes or kosmotropes induced proportional changes in chemical shifts for B and C protons of NMA ($\Delta\delta_B \approx \Delta\delta_C$, Fig. 3a). Nevertheless, chemical shift for C proton changes a little faster than that for B proton when the anion is [X⁻] (X = I, Br, Cl), S₂O₃²⁻ or OH⁻. The changes in chemical shift for water residue supported the cataloguing of anionic types in the coloured regions of Fig. 2. Strong kosmotropes such as OH⁻ and H₂PO₄⁻ formed stronger HB with water proton in comparison with HB between water molecules, which induced up-shifting of chemical shift for water protons (Fig. 3b). Other kosmotropes caused slightly positive NMR changes for water protons while chaotropes induced slightly negative ones (Fig. 3b and Fig. S30). It is also needed to be pointed out that S₂O₃²⁻ still shared the

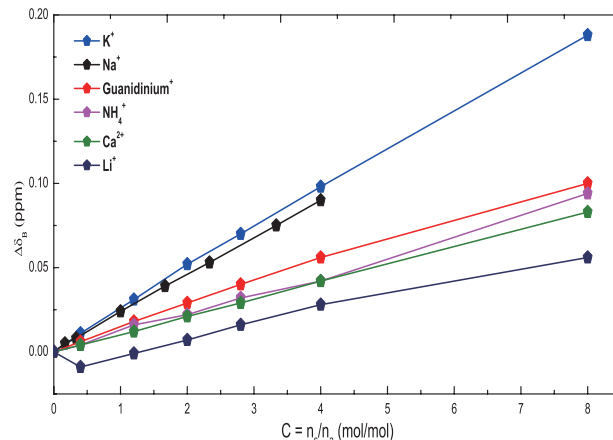


Fig. 4 Cationic effects on NMA when the anions are Cl⁻. A linear relationship exists between changes in the chemical shift for B proton and concentration of salts. The concentration range was doubled since cation has weaker effects than anions do.

behaviours of SO₄²⁻ in NMR changes for water protons, which might be due to that its S→O bonds can form as strong HB with water as SO₄²⁻, although it was catalogued as chaotropes in Fig. 2. So far, although the above mentioned characteristic changes in chemical shifts for model compound were slightly different from a recent important work¹⁶, these results were surprisingly compatible with Hofmeister effects on solubility of macromolecules such as polypeptides, PNIPAM and Poly(2-ethyl-2-oxazoline).^{16, 38-40} Originally, the term called chaotropes was assigned to these anions that cause salting-in of proteins while salting-out was induced by kosmotropes. In recent decades, thermally responsive polymers that contain amide bonds were also found to susceptible to Hofmeister series. For aqueous solutions of these substances, the addition of chaotropic anions caused significant increasing in LCST (or solubility at a given temperature) while kosmotropic anions posed opposite effects. In this study, we are now unable and have no intent to theoretically explain the differences with previous results,^{16, 41} but one should bear in mind that the Hofmeister series originated from the observations of solubility of protein in salt solutions¹, and thus results from mechanism study must be compatible with Hofmeister effects on solubility of macromolecules.

Another important aspect of Hofmeister series is cationic effects. Although cations usually maintain weaker effects on solutes, any attempt to uncover the Hofmeister secret without accommodating cationic series would be considered incomplete. Hence, a few of frequently encountered cations were also put into testing following the same procedures (Fig. S31-S35). It was found that cations indeed maintained weaker effects (about half strength of anions) on solute than anions did at a given concentration (Fig. 4 and Fig. S36). It was also found that all cations caused positive NMR changes, and they followed the order: K⁺ > Na⁺ > Guanidinium⁺ > NH₄⁺ > Ca²⁺ > Li⁺. The chemical shift for C proton changed slightly faster than that for B proton (Fig. 5a). If one reorders the cationic order according to the differences between changes in chemical shift for B and that for C proton, the order would look like this: Ca²⁺ > Li⁺ > NH₄⁺ > Guanidinium⁺ Na⁺ > K⁺. This was consistent with a recent study.⁴²

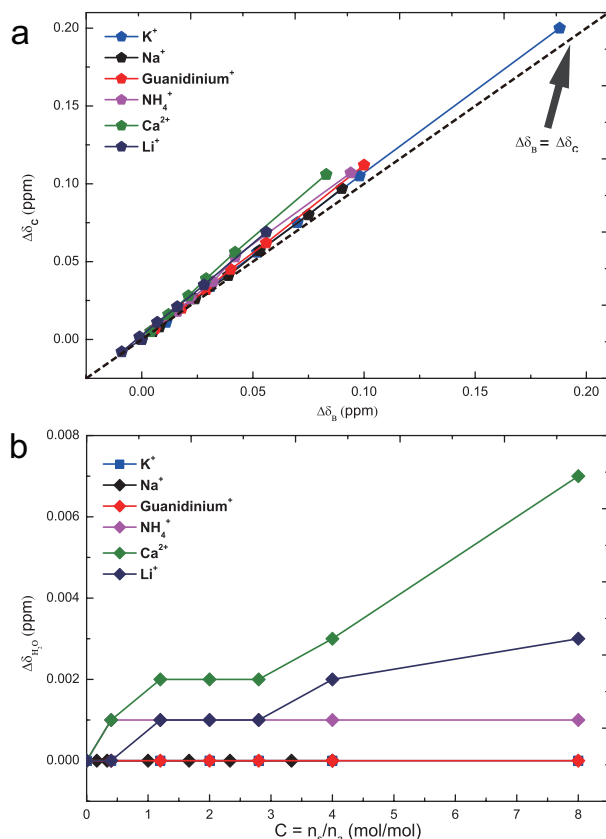


Fig. 5 Cationic effects on NMA and water residue. (a) Correlation between changes in the chemical shift for B proton and that for C proton. (b) Changes in the chemical shift for proton of water residue at different concentrations by specific cations.

Moreover, the chemical shift for water proton also changed according to the above cationic order except that the relative positions of Ca^{2+} and Li^{+} were overturned (Fig. 5b). However, it seemed that cations maintained very weak effects (almost negligible) on water protons, which might be due to that the electrostatic interactions existing between cations and water molecules were weaker than HB and thus they maintained little influences on chemical shifts of water proton.

It was worth noting that the above discussions were based on an assumption that the concentrations of ions remained unaffected after dissolving in the water. However, a question has been raised that to what extent the hydrolysis of weak acid anions such as CO_3^{2-} would affect the majority of ionic species. In other words, to what extent the ionic species would affect the pH of salts solutions and thus affect the effects of ions on the NMA molecules. In order to demonstrate the extent of salts effects on the pH of solutions, the pHs of all electrolyte solutions were measured. It was found that the extent of anions effects on pH was much greater than that of cations. The pH of sodium salts was ranged from 3 to 13 depending on the anionic species (Fig. 6a), whereas the extent of cation effects was within $\text{pH} \pm 1$ (Fig. 6b). And unexceptionally, the concentration at 100mM marks the beginning of Hofmeister ions effects.¹⁰ Thus, the specific ions effect should be discussed at concentrations above 100mM because the Hofmeister phenomena can be explained by classical

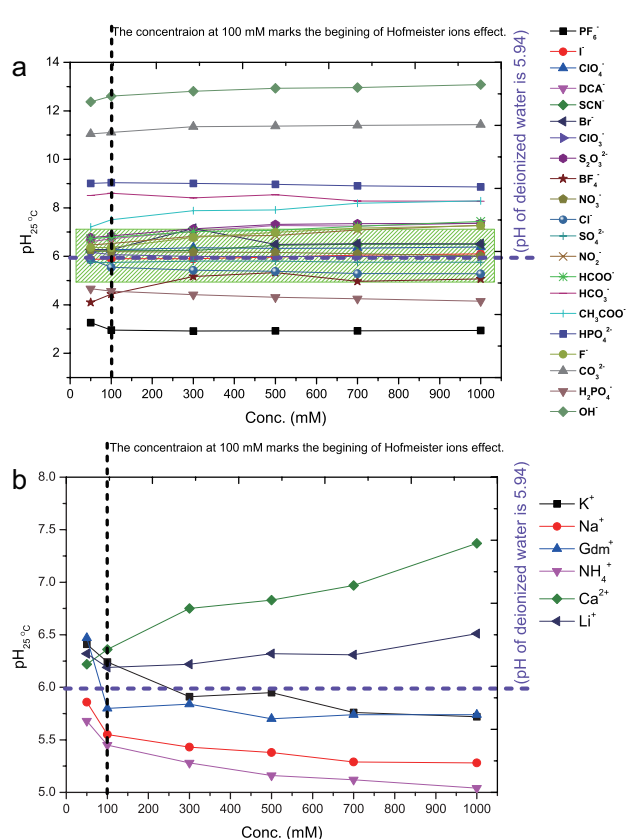


Fig. 6 The pH of salts solutions. (a) Anion effects on the pH of electrolyte solutions: the cation is sodium; the solvent is de-ionized water. All measurements were carried out with pH 11 Economy Meter (OAKTON Instruments). It is worth noticing that the pH of pure de-ionized water isn't ideally equal to 7, which might be due to the inevitable adsorption of small amount of gases such as CO_2 from air, even after the water was boiled and cooled down. (b) Cation effects on the pH of electrolyte solutions: the anion is chloride; the solvent is de-ionized water.

theories at concentration below that value.

The Fig. 6b showed that the amount of H^{+} or OH^{-} in chloride salts solutions ($\sim 10^{-5}$ - 10^{-7}M) was negligible compared to the amount of ions (10^{-1} - 1M). The situation for anions effects on pH was complicated. However, the hydrolysis of anions seemed not to change the ionic concentrations. For example, even for the strongest acid solution of PF_6^{-} whose acidity might come from the hydrolysis of PF_6^{-} , the amount of H^{+} in sodium salts solutions was only $\sim 10^{-3}\text{M}$, which is still much less than the anion concentration (~ 0.099 - 1M). It's a little bit strange that the pH of 1M NaOH is 13.08 rather than the ideal value 14. Although the already existed CO_2 might contribute partly for this eccentricity, the non-Nernstian behavior associated with high ionic strength might also contribute greatly.⁴³ Nevertheless, the total hydrolysis of weak acid anions can be considered as negligible for most of anions in our study (especially for those are within or close to the green marked region, Fig. 6a). Thus, the NaSCN system is mainly the mixtures of Na^{+} and SCN^{-} (>99%). Actually, HSCN is a strong acid rather than a weak one that usually tends to be subject to hydrolysis.

Until now, the NMR results have been perfectly compatible with experimental observations of Hofmeister effects on aqueous

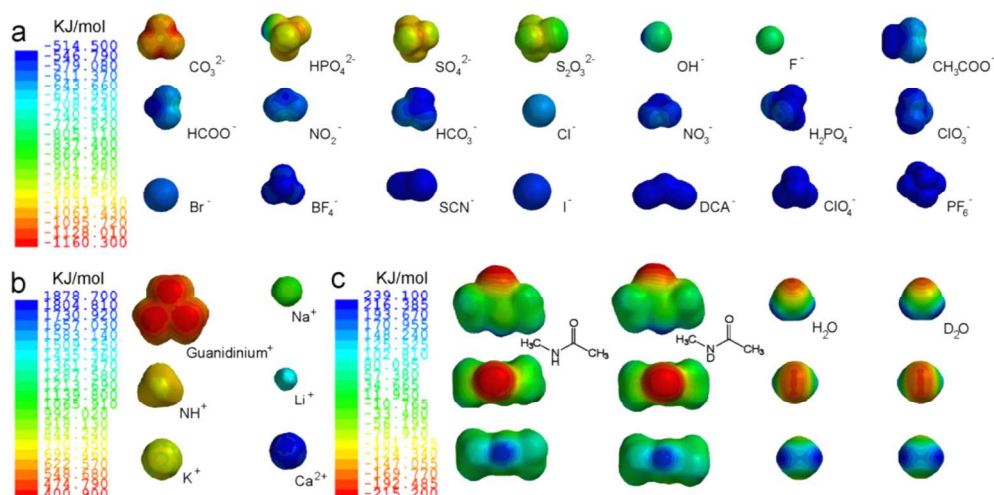


Fig. 7 The electrostatic potential surfaces of ions, solvents and solutes: (a) anions; (b) cations; (c) NMA and water and their deuterated counterparts.

solution. However, the question remains as usual as what's behind the apparent Hofmeister series.

As mentioned earlier, none of the existing classical theoretical ideas can provide a comprehensive understanding and prediction of ion specificity.¹⁰ Simple theories based on hydration force, water dipolar force, electrostatic force and etc. are too simplistic. However, an idea came across that the combination of these forces might provide a clear picture because the forces in reality are never separable or linearly additive. Here, a theory based on a combination of multi-factors was proposed to better understand the Hofmeister series. Two key factors including electrostatic interactions (EI) and hydrogen bonding ability (HB) of ions were taken into account in this study. The first one is the local electrostatic interactions, which exists between positively and negatively charged sites. The electrostatic interaction might be the most important one in solutions since both of hydrophobic anions such as SCN^- and hydrophilic anions such as OH^- can interact with cations or other positively charged particles by strong EIs. And it is fair to think that EI is a considerable part of the nature of HB. And by local it means anions and cations prefer differently charged sites of protein molecule if the barricade of hydration shell was not considered (in fact, this barricade should be taken into account). And according to the laws of physics, the strength of local electrostatic interaction between two particles depends on their quantity of electric charges and distance, which can be replaced by the term of electrostatic potential.

The second factor is the HBs between donors and acceptors. In effects, the existing HB network in aqueous solutions is a result of HBs between solvent molecules. Upon addition of ions with different HB ability, the network could be disrupted or enhanced. Take $\text{H}_2\text{PO}_4^{2-}$ as an example, it can form multiple HBs with water and thus enhance the HB network, which results in a thick hydration shell around it and the characteristic behaviours of kosmotropes (Fig. 2). However, SCN^- could not form HB with water and thus could disrupt the integrity of the network around it, which would define it as chaotropes.

To investigate the EIs, theoretical calculations of electrostatic potential surfaces (EPS) for anions, cations, solutes

Table 1 The calculating results for electrostatic potential surface of ions, solvents and solutes.

Entry	Item	EPS ^a (KJ/mol)	HB terminus ^{b/c}
1	PF_6^-	(-514.5, -462.5)	-
2	I^-	(-564.9, -556.6)	I
3	ClO_4^-	(-534.1, -460.6)	-
4	DCA ⁻	(-538.2, -390.0)	-
5	SCN^-	(-569.6, -412.8)	-
6	Br^-	(-599.9, -590.7)	Br
7	ClO_3^-	(-601.2, -364.8)	O
8	$\text{S}_2\text{O}_3^{2-}$	(-995.6, -778.2)	O
9	BF_4^-	(-570.7, -515.8)	-
10	NO_3^-	(-625.4, -451.3)	O
11	Cl^-	(-635.7, -625.7)	Cl
12	SO_4^{2-}	(-1050.5, -943.5)	O
13	NO_2^-	(-672.0, -536.0)	O
14	HCOO^-	(-686.3, -397.9)	O
15	HCO_3^-	(-653.9, -232.0)	H, O
16	CH_3COO^-	(-691.8, -300.3)	O
17	HPO_4^{2-}	(-1067.1, -588.8)	H, O
18	F^-	(-784.9, -764.5)	F
19	CO_3^{2-}	(-1160.3, -1014.4)	O
20	H_2PO_4^-	(-609.0, -154.5)	H, O
21	OH^-	(-790.3, -443.6)	H, O
22	K^+	(+807.3, +865.8)	-
23	Na^+	(+1033.6, +1139.2)	-
24	Gdm^+	(+400.9, +706.2)	H
25	NH_4^+	(+731.9, +821.7)	H
26	Ca^{2+}	(+1778.6, +1878.7)	-
27	Li^+	(+1336.4, +1546.3)	-
28	NMA	(-215.2, +215.9)	H, O
29	d-NMA	(-214.7, +213.3)	D, O
30	H_2O	(-173.7, +239.1)	H, O
31	D_2O	(-174.0, +238.8)	D, O

a: EPS stands for electrostatic potential surface; b: HB terminus means hydrogen bonding acceptors (such as halide anions and carbonyl group) and hydrogen bonding donors (such as hydroxyl group); c: There is no hydrogen bonding terminus or very weak one.

and solvents were first performed at DFT/B3LYP/6-311+G** level in vacuum environment (Fig. S37-S38, Fig. 7 and Table 1), which method was also employed in previous researches.^{25, 26} Naturally, hydration effects would more or less reshape the electrostatic potential surfaces in aqueous solutions. In order to find out the extent, to which hydration would reshape potential surfaces, additional calculations in water environment were also performed at 6-31G** level (Fig. S39). However, the calculations

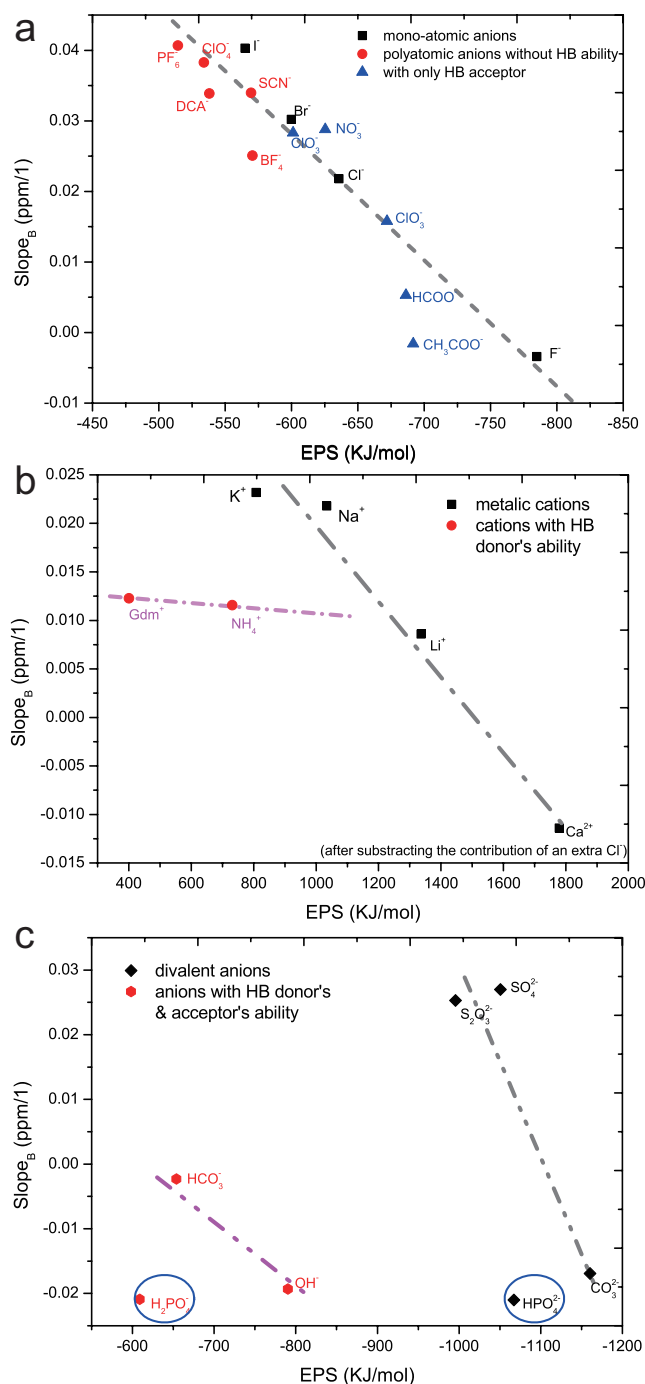


Fig. 8 Plots of slope_B as a function of EPS of ions. The values of slope_B were derived from Fig. 2&4 (see, Fig. S42 & Table S2).

in vacuum environments at 6-31G** level were also performed for comparison (Fig. S40), since the basis set of 6-311+G** was inappropriate for calculations in water environment. The calculating results showed that there was an insignificant difference between calculations in vacuum and in water environments (Table S1). For chaotropic anions such as PF₆⁻, the difference was negligible (upper, Fig. S41). Although there was a tangible difference for strong kosmotropic anions such as H₂PO₄²⁻ between in water and in vacuum (lower, Fig. S41), their maxima and minima remain close (Table S1). To make comparisons fair,

the differences between 6-31G** and 6-311+G** were also given (right column, Table S1). Although the average difference for anions was ~7%, the anionic series remained the same under a given calculating method. Thus, the results in Table 1 would still be used in the following discussions.

To make it easier to correlate the EPS with the effects of ions, the slopes of the fit lines in Fig. 2&4 were derived as slope_B (Fig. S42 & Table S2). After a semi-quantitative correlation between the EPS and slope_B was made, the anionic series ordered according to the values of most negative sites of anions (Table 1, the most negative number in the EPS brackets) roughly comply with NMR results. For example, the ordering for mono-atomic anions followed as F⁻ < Cl⁻ < Br⁻ < I⁻ (Fig. 8a) and that for polyatomic anions without HB ability followed as PF₆⁻ > ClO₄⁻ > DCA⁻ > SCN⁻ > BF₄⁻. It can be seen that the slope_B is a linear function of EPS. And there were some deviations from the linear fit line for the bigger and more complex multi-atomic ions, but there was still a nearly linear correlation between slope_B and EPS for most of single-charge anions. Similarly, the ordering for mono-valent cations followed as K⁺ > Na⁺ > Li⁺ > Ca²⁺ (Fig. 8b, after subtracting the contributions of extra Cl⁻ of CaCl₂). The data of Gdm⁺ and NH₄⁺ was deviated from the fit line, which might be due to the contributions of their HB donor's ability.

There was no linear correlations between the surface potentials of anions with HB ability and the values of slope_B, but it is still suitable to qualitatively predict the ordering for anions with similar HB terminus (with only HB acceptor-oxygen atoms). For example, the ordering for anions with "O" terminus followed as ClO₃⁻ > NO₃⁻ > NO₂⁻ > HCOO⁻ > CH₃COO⁻ (Fig. 8a). Also, given the same contributions from Na⁺, the ordering for divalent anions followed as S₂O₃²⁻ ~ SO₄²⁻ > HPO₄²⁻ ~ CO₃²⁻ (Fig. 8c). Ideally, the ordering for cations with only HB donor (hydrogen atom) should be as NH₄⁺ > Gdm⁺ because the surface potential of H-terminus of NH₄⁺ is higher than that of Gdm⁺ (Table 1, the most positive number in the EPS brackets) and thus the HB between NH₄⁺ and solvent is stronger (Fig. 5b). However, Gdm⁺ outnumbers NH₄⁺ in hydrogen atoms by two and thus the values of Δδ_B in Fig. 4 caused by Gdm⁺ are higher at the same concentration (Fig. 8b). It is easier to accommodate the ordering for anions with both of HB donor and HB acceptor such as H₂PO₄²⁻ and OH⁻ (Fig. 8c). But, the correlations between EPS and slope_B of anions with multiple HB abilities were far from being linear due to their multiple roles in affecting the HB networks enhanced by these anions (Fig. 8c, H₂PO₄²⁻ and HPO₄²⁻). It is also worth noting that the changes in chemical shifts of methyl groups of NMA (or peptide/protein in other works) are an overall result, with simultaneous contributions from both of anions and cations. Hence, the contributions from extra anions or cations should be taken into account when it comes to relating EPS values to NMR data in the future study (Fig. 8b).

Although Plots of slope as determined by fits to the data as a function of ΔS_{hydration} of anions^{38, 40} and the surface tension increment of the anions Δσ⁴⁰ were also successfully employed in the previous studies, it was laboring job to obtain these two data. In sharp contrast, it is much easier to obtain the EPS data of ions by simply performing DFT calculations in commercial programs and thereby more suitable to predict the effects of unfamiliar ions. All in all, a possible mechanism for specific ions effect has

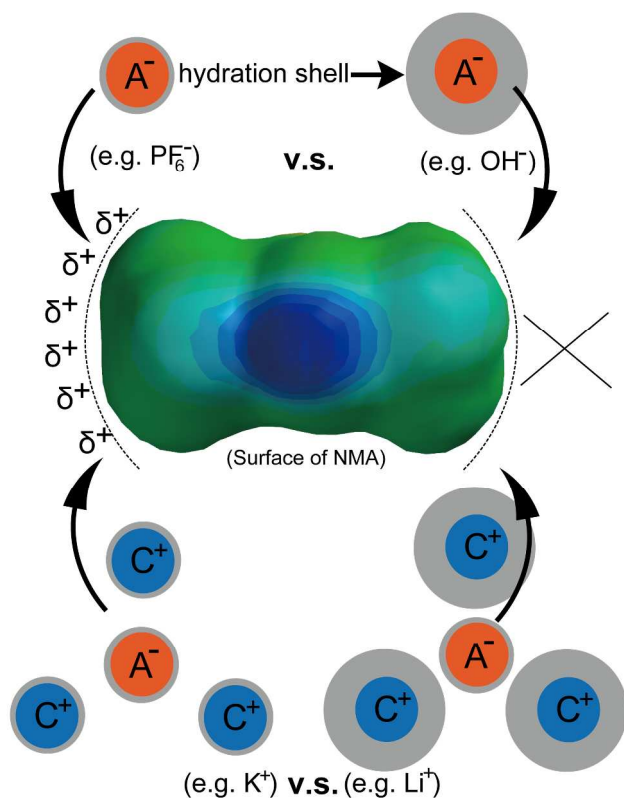


Fig. 9 A proposed mechanism for specific anions and cations effect: (upper section) Anions with low surface electrostatic potentials or without hydrogen bonding (HB) ability such as PF_6^- are negligibly hydrated and therefore could bind to the slightly charged methyl groups of NMA-in return, more positive charges are induced and emerge onto the surface of methyl groups; However, the thick hydration shell of anions with high surface electrostatic potentials or with strong HB ability such as OH^- completely shield their electric field-in return, there is less inductive positive charges or even negative charges on the surface of methyl groups; (middle section) electrostatic potential surface of NMA; (lower section) The hydration shell of cations tend to minimize the electric field of anions, but cations with low surface electrostatic potentials or without HB ability such as K^+ are less hydrated-as a result, the counterbalance is feeble; In sharp contrast, cations with high surface electrostatic potentials or with strong HB ability such as Li^+ and NH_4^+ are heavily hydrated-as a result, the effects of anions are offset. Therefore, the change in chemical shifts for Li^+ was close to 0.00 ppm or even below this value when the concentration was very low (Fig. 4).

emerged as shown in Fig. 9. Anions with low surface electrostatic potentials or without HB ability such as PF_6^- are negligibly hydrated and therefore could bind to the slightly charged methyl groups of NMA. In return, more positive charges are induced and emerge onto the surface of methyl groups. However, the thick hydration shells of anions with high surface electrostatic potentials or with strong HB ability such as OH^- completely shield their electric field. Therefore, there are less inductive positive charges or even more negative charges on the surface of methyl groups due to the net inductive effects of Na^+ . The hydration shell of cations tend to offset the hydration shells of cations owe to minimize the electric field of, but cations with low surface electrostatic potentials or without HB ability such as K^+ are less hydrated, as a result of which, the counterbalance is weak. In sharp contrast, cations with high surface electrostatic potentials or with strong HB ability such as Li^+ and NH_4^+ are hydrated (Fig. 5b) so that the effects of anions are offset. Therefore, the change

in chemical shifts for Li^+ was close to 0.00 ppm or even below this value when the concentration was very low (Fig. 4).

Conclusions

In summary, we have presented the specific ion effects on N-methylacetamide (NMA) that is a suitable model compound of protein. An unconventional ^1H -NMR experimental approach was applied, which clearly distinguished the specific ion effects on the methyl group adjunct to the peptide bond. Although more efforts are needed to give solid ground for this experimental approach, the changes in chemical shift could be a quantitative standard to define the two traditional terms for ions. And the surface electrostatic potentials could be used to accommodate the changes in chemical shift after grouping the ions into several classes such as with/without HB ability.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21473252, 21173267).

Notes and references

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- † Electronic Supplementary Information (ESI) available: [The plots of changes in chemical shift against concentration for proton B of NMA and for water proton and additional colorful displays of electrostatic potential mapping of molecules are provided in supplementary information]. See DOI: 10.1039/b000000x/
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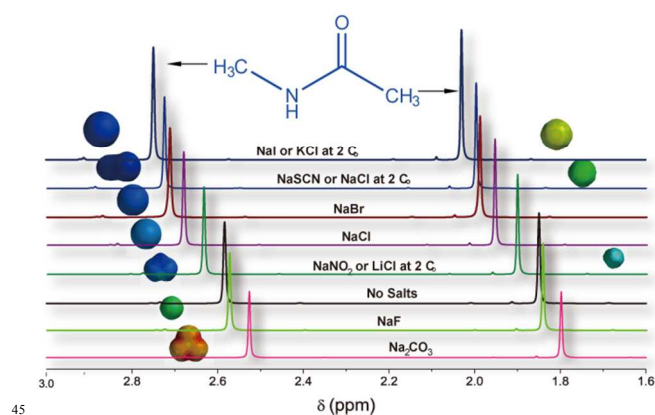


Table of Content (Graphic Abstract)

This work reveals the relationship between the Hofmeister ions effect and the electrostatic potential surfaces of the ions.