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## ARTICLE

Salt-induced LCST-type thermal gelation of methylcellulose:  
Quantifying non-specific interactions via fluctuation theoryReceived 00th January 20xx,  
Accepted 00th January 20xxNoriyuki Isobe<sup>\*,a</sup> and Seishi Shimizu<sup>\*,b</sup>

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What drives the phase separation of water-soluble polymers in the presence of electrolytes was quantified on a molecular scale via statistical thermodynamic fluctuation theory. Quantifying polymer-water and polymer-salt interactions enabled us to identify the dominant interaction for phase separation. As a model system, the lower critical solution temperature (LCST) type thermal gelation of methylcellulose (MC) in aqueous salt solutions was chosen. The Kirkwood-Buff integrals for intermolecular interactions, calculated from the published calorimetric and volumetric data, showed that 1) the accumulation of salts around MC molecules (favourable interaction between salts and MC) inhibits thermal gelation and the depletion of salts from MC (unfavourable interaction between salts and MC) promotes the gelation, and 2) this salt-MC interaction is the dominant factor (50-100 times stronger than the water-MC interaction). This insight from the fluctuation theory is at odds with the age-old consensus regarding the driving force of thermal gelation: water structure change in the presence of salts induces the promotion or inhibition of thermal gelation. However, our conclusion is founded upon the ability of the fluctuation theory to quantify water-MC and salt-MC interaction independently via the Kirkwood-Buff integrals. Flory-Huggins (FH) theory, on the contrary, could not separate these two interactions owing to the lack of a thermodynamic degree of freedom because the lattice solution is assumed to be fully packed. In addition, the dominant contribution from salt depletion cannot be captured by the  $\chi$  parameter, which is essentially the difference of contact energies. Our approach, requiring calorimetric and volumetric data alone as input, provides a simple and versatile method towards elucidating the effect of cosolvents on biopolymer phase separation of physiological importance.

## 1. Introduction

Biopolymer hydration, or how biopolymers interact with water, plays a crucial role in biological cells.<sup>1,2</sup> Indeed, the change of biopolymer hydration is one of the driving forces of liquid-liquid phase separation (LLPS),<sup>3</sup> which plays a key role to the health and diseases of living organisms.<sup>2,4-7</sup> Generally, LLPS is considered to be initiated when biopolymer concentration exceeds a certain threshold (namely, saturation)<sup>6-8</sup> and to exhibit an upper critical solution temperature (UCST) behaviour: dissolution upon heating and coagulation by cooling.<sup>4</sup>

Certain biopolymers, on the contrary, are reported to show a lower critical solution temperature (LCST): formation of gel-like clusters upon heating.<sup>9-11</sup> It is usually hypothesized as being driven by the exclusion of water molecules (namely, dehydration) from around the hydrophobic domain of the

biopolymers, leading to the formation of physical junction points. Despite the pharmaceutical importance of *in vivo* applications such as drug delivery system,<sup>12</sup> understanding biopolymers' LCST behaviour on a molecular scale is still far from complete.<sup>13</sup> This is because LCST is sensitive to the solution environment, such as the presence of electrolytes, as has been widely observed in physiological phenomena.<sup>13</sup>

In the presence of electrolytes, the molecular interactions underpinning biopolymer's LCST behaviour pose a serious challenge to the experimental approaches available at present:

- For small angle neutron/X-ray scattering (SAXS/SANS), a three-component mixture is too complex to deal with.<sup>14</sup>
- For dielectric relaxation spectroscopy,<sup>15</sup> the measurement is impossible in the presence of electrolyte.
- For calorimetric measurements analysed via the Flory-Huggins (FH) theory of polymer solutions, the  $\chi$  parameter, by definition, can deal only with the contact energy difference. (For example, polymer-salt  $\chi$  contains a difference in contact energies between self-contact (polymer-polymer and salt-salt) versus mutual-contact (polymer-salt)). The contact between adjacent sites as the inherent assumption means that the effect of solvent species without specific binding with polymer, considered to be crucial for the

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thermal collapse of hydration state, is hard to capture by the FH theory<sup>16</sup>

These difficulties can be overcome by analysing calorimetric data in the language of light/x-ray/neutron scattering.<sup>17</sup> Such a unified approach has been made possible by the statistical thermodynamic fluctuation theory, which can quantify both attractive and exclusion interactions in the water/electrolyte/polymer system by Kirkwood-Buff integral (KBI).<sup>18</sup>

The aim of this study is to clarify the mechanism of LCST type thermal gelation on a molecular scale in the presence of electrolytes by quantifying the changes of molecular interaction that accompany thermal gelation. Previously, KBIs for LCST polymer solutions were calculated from simulation.<sup>19,20</sup> Also KBI was determined using experimental data for sol-gel transitions exhibiting a UCST behaviour.<sup>21,22</sup> The significance of the present study lies not only in being the first experimental determination of KBI in LCST behaviour but also in demonstrating the advantage of the fluctuation theory and KBIs over the Flory-Huggins theory, which, despite being a common language of polymer science, is incapable of capturing the depletion of solvents from the vicinity of polymer which is crucial for understanding the LCST behaviour. Such an advantage can be gained simply from differential scanning calorimetry, which is rather ubiquitous compared with the SAXS/SANS facility. In addition, the calorimetric data can still be analysed in the same theoretical language as scattering via KBIs.<sup>17</sup> Hence, this versatile methodology combining fluctuation theory and calorimetry is valuable in the precise quantification of co-solvent-related biopolymer phase separation.

## 2. Results

### 2.1. Target data

We focus on the LCST type thermal gelation of an aqueous polymer in the presence of electrolytes. As a model system, we chose methyl cellulose (MC) in the presence of salts and water.<sup>23,24</sup> MC is a polymer based on cellulose, where some hydrogen atoms on the hydroxyl groups of cellulose are replaced with methyl groups. When more than 40% of the hydroxyl groups are replaced by methyl group, aqueous MC solution exhibits a thermally-reversible gelation with LCST, namely gelation upon heating and dissolution upon cooling.<sup>25,26</sup>

The thermal gelation of MC has been an object of intense research on its own, motivated by its wide-ranging applications in food and medicine.<sup>25,27</sup> MC gelation has been shown to be caused by dehydration, i.e., the exclusion of water from MC's hydrophobic domain.<sup>28,29</sup> In addition, the presence of salts, depending on their types,<sup>23,24,30,31</sup> can promote or inhibit gelation sensitively, just as in the case of UCST-type phenomena of biopolymers.<sup>13</sup> It should be emphasised that salts do not act as an ionic bridge in the gelation junction zone of MC, which does not contain any ionic groups. The lack of ionic groups in the MC junction zone means that the ion-specific effect, which has recently been

emphasised as crucially important to proteins,<sup>32</sup> may not play an important role in MC gelation. This sets MC gelation apart from other biopolymers that exhibits salt-induced gelation through salt bridges, to which our theoretical analysis based on KBI has already been applied.<sup>33</sup> Indeed, the lack of specific salt-polymer interactions makes MC gelation especially challenging for conventional approaches that are based on specific, short-range interactions (modelled as contact energies) founded on the  $\chi$  parameter in the FH theory.<sup>34–36</sup>

### 2.2. Theoretical framework

**2.2.1. The Kirkwood-Buff integral as a descriptor of molecular interactions in the fluctuation theory.** Here we consider a system comprised of water, salts, and polymer (MC), which are denoted as species 1, 2, and u, respectively, engaging in accumulative or depletion interactions among one another, beyond the reach of the  $\chi$  parameter. This can be captured by the Kirkwood Buff integral (KBI) defined as:<sup>37</sup>

$$G_{ui}^{(\alpha)} = \int [g_{ui}^{(\alpha)}(\vec{r}) - 1] d\vec{r} \quad (1)$$

where  $g_{ui}^{(\alpha)}(\vec{r})$  refers to the radial distribution function (RDF) of a species  $i$  around species  $u$ , and  $\alpha$  signifies the state: solution ("sol") or gel ("gel"). Unlike the  $\chi$  parameter based on contact energies, KBI can capture both accumulation and depletion of water and salts. This is achieved by the deviation of RDF (of  $i$  around species  $u$  in the state  $\alpha$ ) from its limiting (bulk) value (i.e., 1) far away from the solute. Hence a negative  $G_{ui}^{(\alpha)}$  takes place when  $i$  is depleted around  $u$ . The larger the region from which the salt is depleted the larger negative  $G_{ui}^{(\alpha)}$ , however, from a FH perspective, salt exclusion can be captured only indirectly through an effective enhancement of attraction between other components such as monomers or parts of polymers. Indeed, the degree of exclusion, quantifiable via negative KBIs, has a direct contribution to sol-gel equilibria, as will be shown in Section 2.2.2. In addition, the involvement of  $g_{ui}^{(\alpha)}(\vec{r})$  in Eq. (1) shows that calorimetry and scattering can be interpreted in the common theoretical framework of the fluctuation theory.<sup>38,39</sup> Furthermore, since  $g_{ui}^{(\alpha)}(\vec{r})$  is linked directly to the potential of mean force instead of contact energies, KBIs can capture the solution structure in the language common to the fluctuation solution theory. The use of KBIs is thus advantageous in capturing the solution phase behaviour responsible for gelation.

**2.2.2. Relating sol-gel transition to the Kirkwood-Buff integrals.** The key to gelation transition is the difference of KBIs between sol ( $s$ ) and gel ( $g$ ) states:

$$\Delta G_{ui} = G_{ui}^{(g)} - G_{ui}^{(s)} \quad (2)$$

How sol-gel transition depends on salt concentration and pressure can be exploited to evaluate KBIs. To do so, let us start from how the chemical potential of the state  $\alpha$  ( $= s$  or  $g$ ) is affected by the change of the chemical potential  $\mu_1$ , temperature  $T$  and pressure  $P$

$$d\mu_u^{(\alpha)} = \left( \frac{\partial \mu_u^{(\alpha)}}{\partial \mu_1} \right)_{T,P} d\mu_1 + \left( \frac{\partial \mu_u^{(\alpha)}}{\partial T} \right)_{\mu_1,P} dT + \left( \frac{\partial \mu_u^{(\alpha)}}{\partial P} \right)_{T,\mu_1} dP \quad (3)$$

Using  $\left(\frac{\partial \mu_u^{(\alpha)}}{\partial T}\right)_{\mu_1, P} = -S_u^{(\alpha)}$  (partial molar entropy) and  $\left(\frac{\partial \mu_u^{(\alpha)}}{\partial T}\right)_{\mu_1, P} = V_u^{(\alpha)}$  (partial molar volume), the chemical potential

difference between the sol ( $\alpha = s$ ) and gel ( $\alpha = g$ ) states can be expressed as

$$d\mu_u^{(g)} - d\mu_u^{(s)} = \left(\frac{\partial \Delta \mu_u}{\partial \mu_1}\right)_{T, P} d\mu_1 - \Delta S_{s \rightarrow g} dT + \Delta V_{s \rightarrow g} dP \quad (4)$$

in which  $\Delta \mu_u = \mu_u^{(g)} - \mu_u^{(s)}$ ,  $\Delta S_{s \rightarrow g} = S_u^{(g)} - S_u^{(s)}$  and  $\Delta V_{s \rightarrow g} = V_u^{(g)} - V_u^{(s)}$ .

Now we consider how the transition temperature ( $T = T_{s \rightarrow g}$ ), namely the temperature at which  $\mu_u^{(g)} - \mu_u^{(s)} = 0$ , is affected by  $\mu_1$  under isobaric condition. Putting  $dP = 0$ , Eq. (4) leads to<sup>17,18</sup>

$$\left(\frac{\partial \Delta \mu_u}{\partial \mu_1}\right)_{T = T_{s \rightarrow g}, P} = \frac{\Delta S_{s \rightarrow g} \delta T_{s \rightarrow g}}{RT \delta \mu_1} \quad (5)$$

Note that we have used  $\delta T_{s \rightarrow g}$  and  $\delta \mu_1$  instead of  $dT_{s \rightarrow g}$  and  $d\mu_1$ , in order to emphasise that experimental values have been used to calculate  $\frac{\delta T_{s \rightarrow g}}{\delta \mu_1}$ . Under dilute salt concentration,  $c_2 \rightarrow 0$ , using Raoult's Law (via the mole fraction of water,  $x_1$ ),

$$d\mu_1 \approx d(RT \ln x_1) = -\frac{1}{c_1^0} dc_2 \quad (6)$$

we can show that<sup>17,18</sup>

$$\left(\frac{\partial \Delta \mu_u}{\partial c_2}\right)_{T = T_{s \rightarrow g}, P, c_2 \rightarrow 0} = \frac{\Delta S_{s \rightarrow g} \delta T_{s \rightarrow g}}{RT \delta c_2} \quad (7)$$

(Note that  $c_2$  refers the concentration of ions; e.g. twice of salt concentration in the case of NaCl). The transition temperature is dependent also on pressure. Using Eq. (4) under constant  $\mu_1$ , this leads to

$$\Delta V_{s \rightarrow g} = \Delta S_{s \rightarrow g} \frac{\delta T_{s \rightarrow g}}{\delta P} \quad (8)$$

Our remaining task is to link Eqs.(5), (7), and (8) to KBIs. This can be done most straightforwardly through the inhomogeneous solution theory.  $\mu_u^{(\alpha)}$  can be expressed in terms of the grand partition functions in the presence ( $\Xi_u$ ) and absence ( $\Xi$ ) of the polymer as

$$\mu_u^* = -kT \ln \frac{\Xi_u(T, V_u, \mu_1, \mu_2)}{\Xi(T, V, \mu_1, \mu_2)} \quad (9)$$

where the superscript \* signifies that the centre of mass of the polymer is fixed. Since  $V_u = V_0$  at thermodynamic limit,<sup>40</sup> Eq. (9), under constant  $T$ , leads to the following key relationship between  $\mu_u^*$  and the change in number of species  $i$  around a solute,  $\langle N_i \rangle_u^{(\alpha)} - \langle N_i \rangle$ , as

$$-d\mu_u^* = [\langle N_1 \rangle_u^{(\alpha)} - \langle N_1 \rangle] d\mu_1 + [\langle N_2 \rangle_u^{(\alpha)} - \langle N_2 \rangle] d\mu_2 \quad (10)$$

Using the definition of the KBI,  $c_i G_{ui}^{(\alpha)} = \langle N_i \rangle_u^{(\alpha)} - \langle N_i \rangle$ , and taking the difference between the gel and sol states, Eq. (10) becomes

$$-d\Delta \mu_u^* = c_1 \Delta G_{u1} d\mu_1 + c_2 \Delta G_{u2} d\mu_2 \quad (11)$$

Now we can complete the connection between sol-gel equilibrium and KBIs. Combining Eqs. (8) and (11), we obtain

$$\Delta G_{u1} = -\Delta V_u = -\Delta S_{s \rightarrow g} \frac{\delta T_{s \rightarrow g}}{\delta P} \quad (12)$$

Combining Eqs. (7) and (11) leads to

$$\Delta G_{u1} - \Delta G_{u2} = \frac{\Delta S_{s \rightarrow g} \delta T_{s \rightarrow g}}{RT \delta c_2} \quad (13)$$

where  $\Delta V_u$ ,  $c_2$ ,  $\Delta S_{s \rightarrow g}$ , and  $T_{s \rightarrow g}$  represent the change in the molar volume of MC after gelation, molar concentration of salts, entropy change upon gelation, and gelation temperature. The superscript \* can be shown to disappear under the constancy of polymer concentration.<sup>18</sup> The  $\Delta G_{u1}$  and  $\Delta G_{u2}$  can be obtained by solving the simultaneous equations,

Eq. (12) and (13), based on experimental data as will be described in the following section. DOI: 10.1039/DOCP01687J

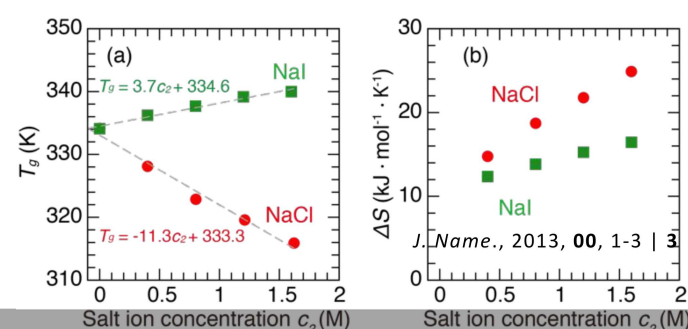
It should be noted that the determination of the KBI pertaining to each state, namely  $G_{ui}^{(s)}$  and  $G_{ui}^{(g)}$ , requires additional measurements such as solubility and small angle neutron/x-ray scattering.<sup>17,18,21,22</sup> However, the precise measurement of polymer solubility is technically difficult because of the high viscosity that increases exponentially upon polymer concentration. Also, the gelation can proceed only above certain polymer concentration, where polymer chains overlap with each other.<sup>41</sup> Under such a concentrated condition, the measurement of  $I(0)$  (i.e., scattering intensity at scattering vector  $q = 0$ ), which is indispensable for KBI determination,<sup>42</sup> is not possible due to the continuing increase of the scattering intensity toward  $q = 0$ , which arises from the large cluster domain caused by overlapping of polymer chains.<sup>43</sup> However, the  $\Delta G_{u1}$  and  $\Delta G_{u2}$  conveys the essential molecular-based information regarding sol-gel transition as will be shown in Section 2.4 and 2.5.

### 2.3. Analysing experimental data

How gelation temperature changes with salt concentration is the key information in KBI calculation, as shown by Eq. (13). The systematic data from Xu and co-workers<sup>23,24</sup> (Fig. 1) shows that gelation is promoted by NaCl and inhibited by NaI. Calculation of  $\Delta G_{u1}$  and  $\Delta G_{u2}$  from their data via Eqs. (12) and (13) is described here. Note that the experimentally-determined KBIs,  $\Delta G_{ij}$ , have the unit of L/mol, converted from the statistical thermodynamic formulae through Avogadro's constant, and thus signifies how many litres of species  $j$  accumulate (when the value is positive) or are depleted (when the value is negative) in the vicinity of 1 mole of species  $i$ .

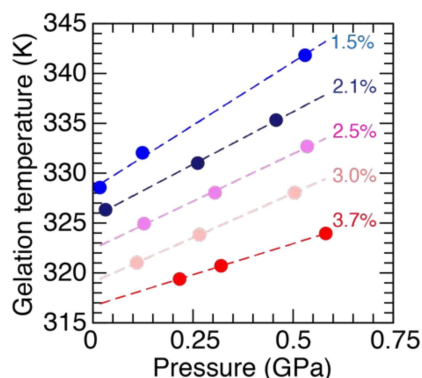
The target parameters are  $\Delta G_{u1}$  and  $\Delta G_{u2}$  that indicate respectively how the accumulation/depletion of water around MC and salts around MC change upon gelation. To obtain these parameters, Eq. 12 and 13 were solved.<sup>17</sup> For this, the values of  $\Delta G_{u1}$ ,  $\Delta S_{s \rightarrow g}$ , and  $\frac{\delta T_{s \rightarrow g}}{\delta P}$  were calculated as follows. Firstly,  $\Delta S_{s \rightarrow g}$  (Fig. 1b) was obtained from the DSC curves.<sup>23,24</sup> Then,  $\frac{\delta T_{s \rightarrow g}}{\delta P}$  was calculated from the literature data<sup>44</sup> shown in Fig. 2.

The slopes of dotted lines correspond to  $\frac{\delta T_{s \rightarrow g}}{\delta P}$ . The slopes got less steep with the increase in the concentration of MC. Therefore, we needed to extrapolate these  $\frac{\delta T_{s \rightarrow g}}{\delta P}$  values to the MC concentration (0.93 wt%) employed in the DSC data. However, the samples of MC employed in these studies were different: the one from Japan Synthetic Chemicals (Mw: ~550000) for the pressure study<sup>44</sup> in Fig. 2 and SM4000 from Shinetsu Chemical (Mw: ~310000) for DSC study<sup>23,24</sup> in Fig. 1. On this point, Nasatto and co-workers pointed out that not the molecular weight but the concentration of MC governs the thermal gelation of MC.<sup>45</sup> This suggests that the MC samples showing the same gelation temperature can be considered to be identical in terms of thermal gelation. The gelation





**Fig. 1.** Change in (a) gelation temperature ( $T_g$ ) and (b) entropy change ( $\Delta S$ ) upon the concentration of NaCl or NaI, retrieved from the systematic data by Xu and co-workers.<sup>23,24</sup> The equations in Fig. 1a are the results of linear fitting of data plots.



**Fig. 2.** Gelation temperature of MC plotted upon applied pressure retrieved from the literature data by Suzuki and co-workers.<sup>44</sup>

temperature of MC used in DSC study (Mw: ~310000) was 61.1°C.<sup>23,24</sup> The MC used in the pressure study (Mw: ~550000) should show the same gelation temperature at the MC concentration of 0.5 wt% as shown in Fig. S1. Based on this, we deemed that 0.5 wt% in the pressure study (Mw: ~550000) should be the experimental set-up identical to the one for the DSC study (Mw: ~310000). Thus, we extrapolated the  $\frac{\delta T_{s \rightarrow g}}{\delta P}$  value to 0.5 wt% as shown in Fig S2, and obtained the  $\frac{\delta T_{s \rightarrow g}}{\delta P}$  value of  $3.065 \times 10^{-8} \text{ (K} \cdot \text{m}^2/\text{N)}$ . This  $\frac{\delta T_{s \rightarrow g}}{\delta P}$  value was multiplied with  $\Delta S_{s \rightarrow g}$ , and then  $\Delta G_{u1}$  was obtained. As will be shown in Section 2.4,  $\frac{\delta T_{s \rightarrow g}}{\delta P}$ , and consequently  $\Delta G_{u1}$ , makes a minor contribution to sol-gel equilibria compared to  $\Delta G_{u2}$ , hence the potential error arising from our estimation makes little difference after all.

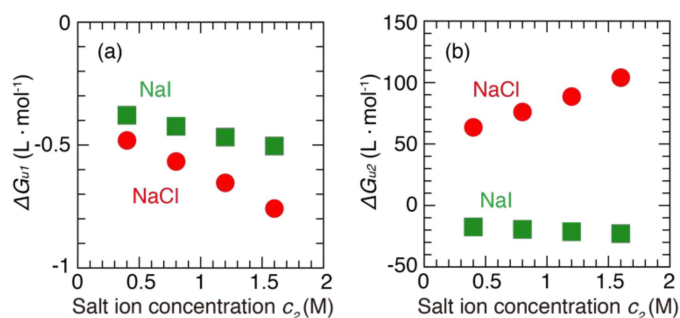
In order to obtain  $\Delta G_{u2}$ , Eq. (13),  $\Delta G_{u1} - \Delta G_{u2} = \frac{\Delta S_{s \rightarrow g} \delta T_{s \rightarrow g}}{RT \delta c_2}$ , must be solved, together with the  $\Delta G_{u1}$  evaluated above. For this, the  $\frac{\delta T_{s \rightarrow g}}{\delta c_2}$  was calculated by the linear fit of literature DSC data as shown in Fig. S3. By putting the obtained  $\Delta G_{u1}$  and  $\frac{\delta T_{s \rightarrow g}}{\delta c_2}$  into Eq. (13),  $\Delta G_{u2}$  was obtained.

#### 2.4. Quantified water- and salt-polymer interaction

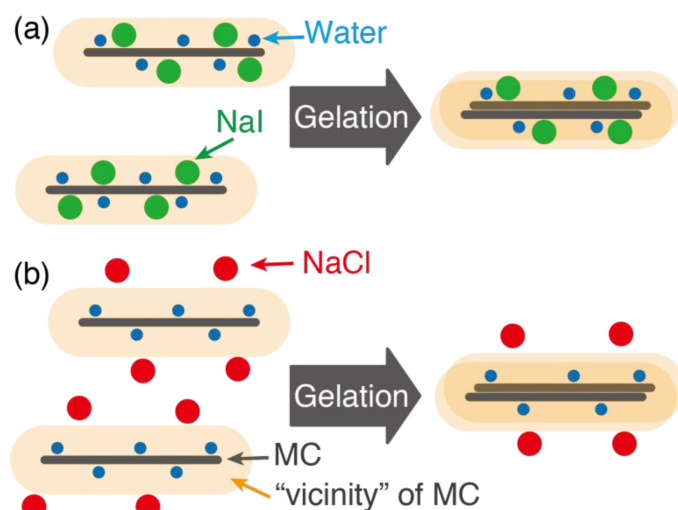
The water-MC interaction,  $\Delta G_{u1}$ , is shown in Fig. 3a.  $\Delta G_{u1}$  was slightly negative both in the presence of gelation-promoting (NaCl) and gelation-inhibiting (NaI) salts. These negative values show that water exclusion, namely dehydration, took place upon gelation, consistent with the previous suggestions.<sup>28,29</sup> In addition, the increase of dehydration with increasing salt concentration indicates the presence of salt-induced dehydration.

The salt-MC interaction,  $\Delta G_{u2}$ , is shown in Fig. 3b. Firstly, the absolute values of  $\Delta G_{u2}$  in both salts (25~50 L/mol at  $c_2 = 0.5 \text{ M}$ ) are 50~100 times larger than  $\Delta G_{u1}$  (0.3~0.5 L/mol at  $c_2 = 0.5 \text{ M}$ ). This shows that salt-MC interaction plays a dominant

role on gelation whereas the contribution of water-MC interaction is minor, contrary to the classical water structuring hypothesis, according to which gelation temperature change is induced by the enhancement or breaking of water structure in the presence of salts.<sup>23</sup>



**Fig. 3.** KBIs,  $\Delta G_{u1}$  (a) and  $\Delta G_{u2}$  (b), against salt ion concentration  $c_2$ .



**Fig. 4.** The role of salts on MC gelation in the presence of (a) NaI and (b) NaCl as revealed from the sign of KBIs.

To understand the role of salts upon gelation, the sign of  $\Delta G_{u2}$  is important.  $\Delta G_{u2}$  is positive in the presence of gelation-promoting (NaCl) and negative in gelation-inhibiting (NaI) salts. The negative  $\Delta G_{u2}$  for NaI signifies the decrease of KBI from the sol state of MC to its gel state, signifying the accumulation of NaI in the vicinity of MC sol state and its reduction upon gelation, as has been shown schematically in Fig. 4a.

In contrast, two possible explanations can be made for the positive change in KBI in the case of NaCl:

1. Upon gelation, NaCl molecules are incorporated in the junction zone, where the MC molecules overlap with each other, or
2. In the initial solution state, NaCl molecules are depleted from the vicinity of MC molecules due to the unfavourable interaction between NaCl and MC.

The possibility 1 is less likely because, unlike carrageenan and gellan gum, MC does not have any ionic groups that contribute to gelation via salt bridge formation.<sup>33</sup> Hence, the incorporation of NaCl molecules is unlikely. In addition, the

incorporation of such large volume ( $\sim 50$  L/mol at  $c_2 = 0.5$  M) in the junction zone, almost one-fourth of the volume of MC molecule (about 220 L/mol), would result in the drastic change in the mechanical property of the gel. In stark contrast to this expectation, the viscoelastic properties of MC gels with and without salts are known to be identical.<sup>24,46</sup>

Therefore, the possibility 2 is more likely as the gelation mechanism in the presence of NaCl (Fig. 4b). In the solution state (before gelation), NaCl molecules are excluded from the vicinity of MC owing to the unfavourable interaction between NaCl and MC ( $-8$  NaCl per 2 MC =  $-4$  NaCl per MC in Fig. 4b) being less favourable than between NaCl and water. The physical entanglement of MC molecules in the junction zone of MC molecules<sup>47,48</sup> leads to the reduction of exposed solvent-accessible surface area, leading to the decrease in the number of depleted NaCl molecules per MC molecule ( $-4$  per 2 MC =  $-2$  NaCl per MC in Fig. 4b). After all, the change in the number of NaCl molecules before and after gelation became net positive ( $-2 - (-4) = +2$  NaCl per MC upon gelation in Fig. 4b). Such an emergence of net-positive from the reduction of negative (i.e., salt exclusion) cannot be captured easily by the  $\chi$  parameter. Indeed, Fig. 4 shows that the sign of  $\Delta G_{u1}$  is the same regardless of the sign of  $\Delta G_{u2}$  and that the change of  $\Delta G_{u2}$  hardly affects  $\Delta G_{u1}$ . These observations necessitate a consideration of longer-range solution structure beyond the contact with the molecules in the first solvation shell (i.e., contact coordination).

To summarize, salt-MC interaction plays a dominant role in the thermal gelation of MC: NaI with a favourable (potential of mean force) interaction with MC inhibits gelation and NaCl having unfavourable interaction with MC promotes gelation. That solute-cosolvent interaction is the most important factor in the LCST-type phase separation of polymer solutions is at odds with the age-old consensus advocating the water structure as the dominant factor (e.g. Cl destroys the cage-like structures between MC chains and water molecules, increasing the hydrophobicity of MC in water<sup>23,24</sup>).

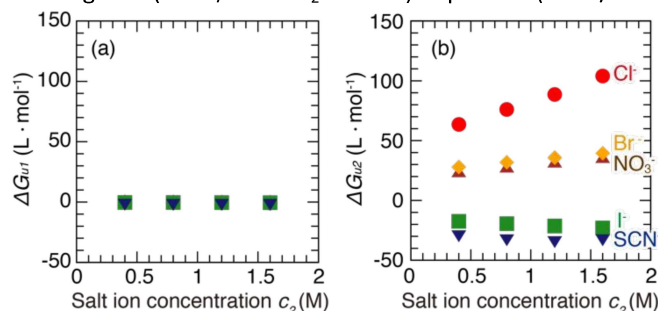
## 2.5. Effect of anions in Hofmeister series

Here we examine the effect of the Hofmeister series, focusing solely on the anions, because the effect of cation is known to be negligible in MC thermal gelation.<sup>23</sup> Firstly, the gelation temperature ( $T_g$ ) shows a linear relationship irrespective of the types of salts, unlike the case for starch and poly(N-isopropylacrylamide) (PNIPAM).<sup>22,49</sup> This linearity arises simply from the dilute salt concentration range in which the calorimetric measurements were performed. In addition, Eq. 4,  $\Delta G_{u1} - \Delta G_{u2} = \frac{\Delta S_{s \rightarrow g} \delta T_{s \rightarrow g}}{RT \delta c_2}$ , also showed linear relationship irrespective of the types of salts, as shown in Fig. S4. Here, one can find a similarity to protein denaturation, where the Gibbs free energy of denaturation is dependent linearly on denaturant concentration, and the  $m$ -value, the slope of the linear relationship, is considered to be related to the surface area of the hydrophobic domain.<sup>13,50</sup>

The salt-dependence of  $\Delta G_{u1}$ s exhibited similar values (Fig. 5a) and was negligible compared to  $\Delta G_{u2}$  (Fig. 5b), even though dehydration, which gives rise to negative  $\Delta G_{u1}$ , took place upon gelation in the presence of any salts (Fig. S5). This

underscores our finding that salt-MC interaction plays a dominant role on MC's LCST-type gelation while the contribution of water-MC interaction is minor.

However,  $\Delta G_{u2}$  is dependent on the types of salts, ranging from negative ( $-30$  L/mol at  $c_2 = 0.5$  M) to positive ( $+60$  L/mol



**Fig. 5.** (a)  $\Delta G_{u1}$  and (b)  $\Delta G_{u2}$  upon ion concentration  $c_2$  of NaCl (Cl<sup>-</sup>), NaBr (Br<sup>-</sup>), NaNO<sub>3</sub> (NO<sub>3</sub><sup>-</sup>), NaI (I<sup>-</sup>), and NaSCN (SCN<sup>-</sup>). For the convenience of comparison, the range of the vertical axis was set to be the same between Fig. 5a and 5b. Fig. 5a in higher magnification is included in Electronic Supplementary Information (Fig. S5).

at  $c_2 = 0.5$  M). Depending on the signs and magnitude,  $\Delta G_{u2}$  can be divided into three groups: 1) NaI and NaSCN, 2) NaBr and NaNO<sub>3</sub>, and 3) NaCl. The first group, NaI and NaSCN, showed a negative  $\Delta G_{u2}$ , indicating a favourable interaction between NaI/NaSCN and MC. The second group, NaBr and NaNO<sub>3</sub>, showed a positive  $\Delta G_{u2}$ , indicating unfavourable interaction between NaBr/NaNO<sub>3</sub> and MC. The third group, NaCl, showed a twice higher positive  $\Delta G_{u2}$  than the second group, indicating a strongly unfavourable interaction between NaCl and MC. This grouping clearly follows the order of Hofmeister series from so-called “kosmotropic” to “chaotropic” salts: Cl<sup>-</sup> > Br<sup>-</sup> > NO<sub>3</sub><sup>-</sup> > I<sup>-</sup> > SCN<sup>-</sup>. This implies that the “chaotropic” salts, NaI and NaSCN, have a favourable interaction with MC, accumulating presumably in the vicinity of the hydrophobic domain of MC and stabilizing the MC molecules in water even at the gelation temperature. The insights obtained here can be extended to other uncharged LCST-type polymers such as PNIPAM and elastin-like polymer (ELP).<sup>13,32,49</sup>

## 3. Discussion

### 3.1. Comparison between the fluctuation and Flory-Huggins theories

**3.1.1. KBIs versus the  $\chi$  parameter.** Here we aim to compare our approach based on the fluctuation theory with the classical Flory-Huggins approach to gelation. In preparation for this aim, let us first summarise the Flory-Huggins expression for the cosolvent-induced change of sol-gel transition temperature, interpreted by adapting the theory of polymer melting. In the presence of the cosolvent, the transition temperature in the pure polymer  $T_{s \rightarrow g}^*$  changes to  $T_{s \rightarrow g}$  as<sup>16,35,51</sup>

$$\frac{1}{T_{s \rightarrow g}} - \frac{1}{T_{s \rightarrow g}^*} = -\frac{RV_u}{\Delta H_{s \rightarrow g} V_1} \Gamma \quad (14)$$

where  $\Delta H_{s \rightarrow g}$  is the enthalpy of gelation and  $V_u$  and  $V_1$  are the partial molar volumes of the polymer and water, respectively.

When the polymer is dilute,  $\Gamma$  is expressed in terms of the  $\chi$  parameter between the species  $i$  and  $j$ , as

$$\Gamma = \left( \chi_{2u} \frac{\phi_2}{r} + \chi_{1u} \phi_1 \right) (\phi_1 + \phi_2) - \phi_1 - \frac{\phi_2}{r} - \chi_{12} \phi_1 \phi_2 \quad (15)$$

where  $\phi_i$  is the volume fraction of the species  $i$  and  $r_2$  is the size ratio between solvent and co-solvent.

Our goal is to obtain an expression analogous to Eqs. (14) and (15) from the fluctuation theory, through the use of KBIs. To do so, let us start from the following formulae that can be derived from Eqs. (5) and (13):<sup>17,18,21,22</sup>

$$\left( \frac{\partial \Delta \mu_u}{\partial \mu_1} \right)_{T,P,c_u \rightarrow 0} = \frac{\Delta H_{s \rightarrow g} \delta T_{s \rightarrow g}}{RT_{s \rightarrow g}^2 \delta \mu_1} \quad (16)$$

$$\left( \frac{\partial \Delta \mu_u}{\partial \mu_1} \right)_{T,P,c_u \rightarrow 0} = c_1 (\Delta G_{u2} - \Delta G_{u1}) \quad (17)$$

where  $T_{s \rightarrow g} \Delta S_{s \rightarrow g} = \Delta H_{s \rightarrow g}$  has been used to obtain Eq. (16). Combining Eqs. (16) and (17) yields

$$\delta \left( \frac{1}{T_{s \rightarrow g}} \right) = - \frac{R c_1}{\Delta H_{s \rightarrow g}} (\Delta G_{u2} - \Delta G_{u1}) \quad (18)$$

Integrating Eq. (18), assuming  $\Delta H_{s \rightarrow g}$  is a constant over the concentration range of interest, we obtain

$$\frac{1}{T_{s \rightarrow g}} - \frac{1}{T_{s \rightarrow g}^0} = - \frac{R c_1 \ln a_1}{\Delta H_{s \rightarrow g}} (\Delta G_{u2} - \Delta G_{u1}) \quad (19)$$

where  $T_{s \rightarrow g}^0$  is the transition temperature at  $c_2 = 0$ . This is the fluctuation theory alternative to the FH-based Eq. (14), expressed in terms of the KBIs.

**3.1.2. Comparison with the FH theory.** What drives the change of LCST ( $T_{g \rightarrow s}$ ) in the presence of cosolvents has been clarified, via Eq. (19), as the competition between  $\Delta G_{u2}$  and  $\Delta G_{u1}$ . These two interaction changes,  $\Delta G_{u2}$  and  $\Delta G_{u1}$ , have been determined from two independent relationships:<sup>52</sup>  $T_g$  change with respect to the water activity and the volume of gelation, as shown in the Results section.

In FH, on the contrary, it is not possible to quantify all the key interactions that drive gelation. Indeed, according to Eqs. (14) and (15), the three  $\chi$  parameters,  $\chi_{12}$ ,  $\chi_{1u}$  and  $\chi_{2u}$ , contribute to the cosolvent-induced change of LCST. Using the lattice model of solution,  $\chi_{12}$  may be determined from the activity data and by choosing an appropriate value for the solvent-cosolvent size-ratio  $r_2$ . Now, the remaining number of  $\chi$  parameters to be determined is two. However, evaluating both  $\chi_{1u}$  and  $\chi_{2u}$  is not straightforward, because there is no free volume in the classical FH theory;<sup>16</sup> since the system is fully packed, the pressure of the system is not an independent thermodynamic variable. Two remaining independent variables, pressure and activity, are utilised by the fluctuation theory for the independent evaluation of  $\Delta G_{u1}$  and  $\Delta G_{u2}$ , but only one variable (activity) is available for determining the two parameters  $\chi_{u1}$  and  $\chi_{u2}$  in FH theory.

Nevertheless, the determination of the three  $\chi$  parameters,  $\chi_{12}$ ,  $\chi_{1u}$  and  $\chi_{2u}$ , has been reported by Habeych and co-workers<sup>35</sup> in a ternary system consisting of starch, water, and co-solvents, as well as the prediction of melting temperature of starch in the presence of water and co-solvents. However, the  $\chi$  parameters were determined through statistical regression of the melting

temperature under a number of model assumptions (including the fully-packed lattice model) but without appearing to a set of independent measurements identical in number to the thermodynamic degrees of freedom governed by the Gibbs phase rule. In addition, the  $\chi$  parameter, by definition, is enthalpic in nature, due to its difference in terms of the difference in contact energies, as

$$\chi_{ij} = \frac{z}{RT} \left( w_{ij} - \frac{w_{ii} + w_{jj}}{2} \right) \quad (20)$$

where  $w_{ij}$  denotes the contact energy between the monomers of the species  $i$  and  $j$ , and  $z$  is the number of neighbouring sites.  $w_{ii}$ ,  $w_{jj}$  and  $w_{ij}$ , by definition, comes only from contacts with adjacent sites, which, by definition, neglect the long-ranged nature of interactions. (The entropic effect in the FH theory is taken care of by the size-dependent entropy of mixing.) Indeed, due to the contact energy nature of the  $\chi$  parameters and the inherent lack of free volume in the fully-packed lattice solution, there is no other way for the FH theory to model salt exclusion from polymer surfaces in terms of the difference of contact energies (Eq. (20)), which is different from the result of the fluctuation theory. However, the  $\chi$  parameters determined by regression may *implicitly* and *effectively* contain the effects beyond the lattice model and the contact assumptions of the FH theory, which may be the basis for its success as a prediction model. In a similar vein, the application of the FH theory to osmotic and scattering experiments may treat the contact-energy based  $\chi$  parameters beyond its original premise.<sup>53</sup>

One way of overcoming this difficulty is incorporating the free volume into the lattice model, as has been done before.<sup>54,55</sup> However, despite the increased complexity of free volume FH theory, the limitations of the lattice model persist still. Firstly, the  $\chi$  parameter only considers the contact energies between adjacent sites, and any contributions arising from longer range cannot be incorporated.<sup>54,55</sup> Indeed, the calculated KBIs, as shown in Results section, show that NaCl was excluded from the vicinity of MC molecules upon the thermal gelation. However, the FH theory cannot deal with such exclusion: it can only deal with the contact energies; exclusion is not merely the lack of contact, as has been shown in our formalism as well as by the crowding theories.<sup>56</sup> Indeed, the apparent lack of correlation between the signs of  $\Delta G_{u1}$  and  $\Delta G_{u2}$  (Figs. 3 and 5) shows salt binding (or exclusion) does not correlate with water exclusion (binding), indicating that solvation is more complex than an competitive over  $z$  contact (coordination) sites. Secondly, it is hard to imagine many organic co-solvents as flexible polymers with  $r_2$  water sites, or some ions being  $r_2$ -mer (where  $r_2 < 1$ ). Dealing with solvent-cosolvent size ratio via lattice model is therefore problematic.

Our approach, being free of these problems inherent in the basic assumptions of the lattice model, is powerful in the quantitative analysis of phenomena induced or suppressed by co-solvents. However, the principal drawbacks of our approach should be mentioned here. Firstly, it is impossible to dissect the KBIs of cation and anion. This is because one cannot independently change the number of cations or anions, formed by the dissociation of



salts.<sup>22</sup> Secondly, it is impossible to know the distribution of molecules (e.g. positive KBIs can arise either (i) from strong accumulation of salts in the polymer vicinity, or (ii) from weak but broad accumulation). To address this, small angle X-ray or neutron scattering experiments will be useful, because the structure factor at  $q = 0$ , or  $S(0)$ , corresponds to KBIs<sup>42</sup>, and the radial distribution function of each species can be obtained through the empirical potential structure refinement method.<sup>14</sup> In addition, a comparison between FH and the fluctuation theory (Eqs. (14) and (15) versus Eq. (19)) may provide a key to extracting a fully molecular interpretation out of a successful modelling of polymer gelation via FH theory, namely, to clarify solution structure incorporated implicitly into the  $\chi$  parameters.

#### 4. Conclusions

The LCST-type thermal gelation of MC is sensitive to salts present in the solution. Such an observation was given an explanation on a molecular scale via the statistical thermodynamics of fluctuation. The change of Kirkwood-Buff integrals (KBIs) upon gelation, calculated from calorimetric data, indicates that the dominant contributor is co-solvent-solute interaction, whereas the contribution from hydration changes due to water structuring and breaking are negligibly small. Drawing such conclusions on a microscopic and quantitative basis was beyond the reach of the Flory-Huggins (FH) theory, due to its twofold limitation, namely (i) the inability to capture interactions beyond contact energies and (ii) the restricted thermodynamic degrees of freedom making it impossible to quantify all the interspecies interactions. Our approach, on the contrary, is free of these limitations, and is effective in rationalizing the role of co-solvents on liquid-liquid phase separation of aqueous polymer mixtures in a quantitative manner, while being capable of incorporating the long-rangedness of solution structure.

#### Conflicts of interest

There are no conflicts to declare.

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Kirkwood-Buff integrals show the accumulation of salts inhibits and the depletion of salts promotes the LCST-type thermal gelation of methylcellulose.

