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Seishi Shimizu
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Seishi Shimizu

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Sugars and salts strongly affect the dimerization of caffeine in water. Such a change of dimerization, considered to be crucial for bitter taste suppression, has long been rationalized by the change of "water structure" induced by the additives; "kosmotropic" (water structure enhancing) salts and sugars promote dimerization, whereas "chaotropic" (water structure breaking) salts suppress dimerization. Based on statistical thermodynamics, here we challenge this consensus; we combine the rigorous Kirkwood–Buff theory of solution with the classical isodesmic model of caffeine association. Instead of the change of water structure, we show that the enhancement of caffeine dimerization is due to the exclusion of additives from caffeine, and that the weakening of dimerization is due to the binding of additives on caffeine.

1. Introduction

Tea and coffee have for a long time occupied an important place in many societies and cultures.[1,2] Their complexity in taste and flavour arises from the interplay between the molecules of different taste modalities, in which hydrophobicity is considered to play a crucial role.[3–11] In particular, sugars and salts affect the taste of tea and coffee at a molecular level: ion–caffeine interaction influences coffee extraction;[12–14] sugar's enhancement of caffeine dimerization suppresses the bitter taste.[3–6,15]

Here we investigate the molecular-based mechanism by which caffeine dimerization is affected by additives such as sugars and salts. This is a long-standing scientific question to which the decisive answer is yet to be found.[15–25] However, the following hypothesis seems to be the consensus, found most commonly in the literature:

Hypothesis: The change of water structure around caffeine, induced by the presence of additives, is the cause of the change of dimerization.[9–11,15,18,20,21] "Kosmotropic" additives such as sucrose enhance the hydrogen bond network of water around themselves, as well as around caffeine.[26,27] This strengthens the hydrophobicity of caffeine, thereby promoting its self-association.[11,15] "Chaotropic" additives such as $NaClO_4$, on the other hand, break the hydrogen bond network of water,[26,27] which weakens the hydrophobicity of caffeine, thereby suppressing its self-association.[18,23]

This hypothesis originally came from the study of hydrophobic hydration,[26,27] which has long been considered to be one of the major driving forces of protein folding and binding.[27–31] However, understanding how additives work had for a long time been hampered by a lack of a rigorous theoretical foundation.[31,32] Only recently, a rigorous statistical thermodynamics theory was established, which successfully clarified how additives work in a wide variety of phenomena, including biomolecular folding and binding,[32–37] drug solubilization,[38–41] and food gelation.[42] Based on this track record, here we examine whether water structure making and breaking caused by the additives is really the driving force of caffeine dimerization.

To this end, we will take the following strategy: to supplement a classical thermodynamic model of caffeine self-association, commonly referred to as the isodesmic binding model,[19–23] by a rigorous statistical thermodynamic theory, the Kirkwood–Buff (KB) theory of solutions.[32–45] The isodesmic model has for a long time been applied to caffeine dimerization in the presence of additives,[19–23] whereas the KB theory is rigorous, and has a track record from biophysics to chemical engineering.[32–45] By the combination of the two, more information can be drawn at a molecular basis than was previously possible from the isodesmic model, without any further approximations or assumptions.

2. Theory

2.1 Local *versus* bulk concentrations of water and additive molecules

Consider a pair of caffeine molecules in a mixture of water and additive molecules. A pair of caffeine molecules can take

York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK. E-mail: seishi.shimizu@york.ac.uk; Fax: +44 (0) 1904 328266; Tel: +44 (0) 1904 328281

monomeric and dimeric states. We adopt the following indexing scheme: water (i = w), additive (i = a) and caffeine (i = c), and the monomer (α = m) and dimer (α = d) of caffeine. The system is at temperature T and pressure P. The molar concentration of the species i in the solution is denoted by n_i.

How water and additive molecules interact with caffeine monomers and dimers can now be quantified, as schematically represented in Fig. 1, by the difference between the *local* concentrations of water and additive molecules around caffeine molecules, as compared to the concentrations in the *bulk* solution, which is the conclusion of the KB theory.[32–45] Such a difference between the local and bulk concentrations can be defined formally through the radial distribution function between caffeine and species i, $g_{ci}(r)$, where r is the centre-of-mass distance between c and i; the radial distribution function signifies the density of the species i at the position r relative to the bulk, which is a standard textbook quantity for the description of solution structure.[46] Far away from caffeine, when the solution structure is that of a bulk solution, radial distribution functions take the baseline value 1. The net deviation of the concentration of species i from the baseline, shown schematically in Fig. 2, can be expressed by the following KB integral defined as[32–45]

$$G_{ci}^{(\alpha)} = N_A \int dr 4\pi r^2 [g_{ci}^{(\alpha)}(r) - 1] \quad (1)$$

where N_A is Avogadro's number.

Thus the KB integral is the overall measure of attraction or repulsion.[32–45] If a species is overall attracted to caffeine, the radial distribution function exhibits peaks (higher than the bulk baseline, *i.e.*, 1) that contribute positively to the KB integral. If a species is overall repelled or excluded from caffeine, the radial distribution function exhibits troughs lower than the baseline 1 that contribute negatively to the KB integral. There is always a negative contribution from shorter distances due to steric repulsion between caffeine and water, as well as caffeine and additive.

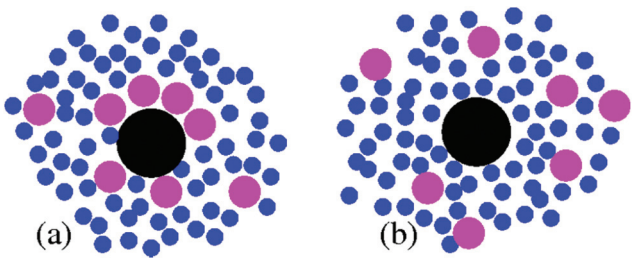

Fig. 1 Schematic representation of the local–bulk concentration difference. (a) Additive molecules (magenta) are more concentrated around the solute (black) compared to the bulk (far away from the solute). (b) Additives are less concentrated around the solute than in the bulk. Note that the local–bulk concentration difference of water is also considered in our theory.

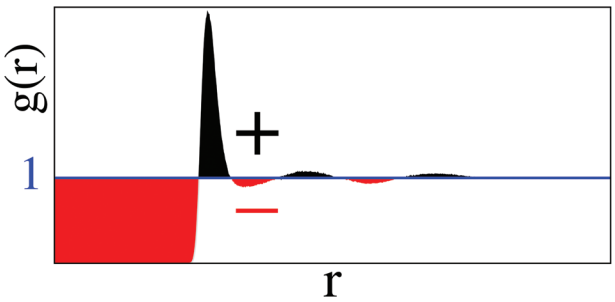

Fig. 2 Quantifying the local–bulk concentration difference by the use of the radial distribution function (introduced in the text) as a function of distance from the solute, r. The concentration of each species in bulk is normalized to 1 (blue line). The density deficit compared to bulk, coloured in red, contributes negatively, whereas the density excess, coloured in black, contributes positively. A quantitative measure of the local–bulk concentration difference can be obtained by adding up all these contributions, which is called the Kirkwood–Buff integral.

2.2 The changes of additive–caffeine and water–caffeine interactions upon dimerization

KB integrals can reveal the mechanism of the additive-induced changes in the self-association of caffeine. The key is the KB integral change that accompanies caffeine dimerization

$$\Delta G_{ci} \equiv G_{ci}^{(d)} - 2G_{ci}^{(m)} \quad (2)$$

This is because of a rigorous (approximation-free) relationship[32–34,39,40] between the dimerization constant K and the KB integrals ΔG_{c1} and ΔG_{c2}

$$-\left(\frac{\partial \ln K}{\partial n_a}\right)_{T,P,n_c;n_a\to 0} = \Delta G_{cw} - \Delta G_{ca} \quad (3)$$

This equation can be derived straightforwardly from basic chemical thermodynamics using the Gibbs–Duhem equations.[32–34,39,40] (Note that eqn (3) is the special case of KB theory at $n_a \to 0$,[32–34,39,40] which is particularly useful in connecting Setschenow constants[23,35] to the KB integrals, as will be discussed in section 2.4.) We emphasize here that ΔG_{ci}, as defined by eqn (1) and (2), is the change of KB integral which accompanies dimerization, rather than the change of Gibbs free energy. Note that KB integrals are usually denoted by G in the literature, because of the connection to the radial distribution function (Eq. (1)), which is expressed by $g(r)$.

Let us now clarify the meanings of eqn (2) and (3).

Eqn (2) can be interpreted in the following manner:

– When ΔG_{ci} is positive, species i is either more attracted to a caffeine dimer or less repelled (excluded) from a caffeine dimer than to a pair of caffeine monomers.

– When ΔG_{ci} is negative, species i is either more repelled (excluded) with a caffeine dimer or less attracted to a caffeine dimer than to a pair of caffeine monomers.

Eqn (3) signifies that the additive-induced dimerization change is due to the competition between the following two factors: the change of caffeine–water interaction ΔG_{cw} *versus* the change of caffeine–additive interaction ΔG_{ca}.[32–34,39,40]

– Caffeine dimerization is suppressed by the additive when $\Delta G_{cw} > \Delta G_{ca}$.[32–34,39,40]

– Caffeine dimerization is promoted by the additive when $\Delta G_{cw} < \Delta G_{ca}$.[32–34,39,40]

These two scenarios can be interpreted on a molecular basis, in conjunction with the interpretation of eqn (2). This will be done in section 3.2.

The key quantities ΔG_{cw} and ΔG_{ca} can be calculated solely from experimental data. This can be done by solving a pair of simultaneous equations, which consists of eqn (3) and the following:[32–34,39,40]

$$\Delta V_c = -\Delta G_{cw} \quad (4)$$

where ΔV_c is the change of partial molar volume that accompanies caffeine dimerization. Justification of eqn (4) will be given in section 2.4.

2.3 Hydration free energy of caffeine monomer

In addition to ΔG_{cw} and ΔG_{ca}, all the other KB integrals ($G_{cw}^{(m)}$, $G_{ca}^{(m)}$, $G_{cw}^{(d)}$, and $G_{ca}^{(d)}$) can be calculated from experimental data and the isodesmic model. This can be achieved by the well-established KB relationships analogous to eqn (3) and (4).[32–34,39,40] Firstly, the additive concentration (n_a) dependence of caffeine monomer solubility, $n_c^{(m)}$, is expressed in the following form analogous to eqn (3):[32–34,39,40]

$$\frac{1}{RT}\left(\frac{\partial \mu_c^{*(m)}}{\partial n_a}\right)_{T,P,n_c;n_a\to 0} = G_{cw}^{(m)} - G_{ca}^{(m)} \quad (5)$$

where $\mu_c^{*(m)}$ is the transfer free energy of a caffeine molecule from pure water to a water–additive mixture, which can be obtained from the solubility of caffeine monomer in the additive–water mixture $n_c^{(m)}$ and in pure water $n_c^{0(m)}$:

$$\frac{\mu_c^{*(m)}}{RT} = -\ln\frac{n_c^{(m)}}{n_c^{0(m)}} \quad (6)$$

The partial molar volume of the caffeine monomer, $V_c^{(m)}$, obeys the following relationship analogous to eqn (4):[32–34,39,40]

$$V_c^{(m)} = -G_{cw}^{(m)} \quad (7)$$

$G_{cw}^{(m)}$ and $G_{ca}^{(m)}$ can be calculated directly from $\frac{1}{RT}\left(\frac{\partial \mu_c^{*}}{\partial n_a}\right)_{T,P,n_c;n_a\to 0}$ and $V_c^{(m)}$, determined from experimental data through the isodesmic model,[22,23] by solving the simultaneous equations (eqn (5) and (7)).[32–34,39,40] Once ΔG_{cw}, ΔG_{ca}, $G_{cw}^{(m)}$, and $G_{ca}^{(m)}$ have been obtained, calculating $G_{cw}^{(d)}$ and $G_{ca}^{(d)}$ can be done straightforwardly by the use of eqn (2).

Eq. (7) can be justified in an intuitive manner:

– When r is very small, water molecules cannot penetrate and overlap with caffeine, hence $g_{cw}^{(m)}(r) = 0$. The contribution to KB integral from such a region, namely the integration $g_{cw}^{(m)}(r) - 1 = -1$ over this range of r (Eq. (1)), gives $-V_{ex}$ (V_{ex} is the excluded volume).

– At the peaks of $g_{cw}^{(m)}(r)$ higher than 1, the positive $g_{cw}^{(m)}(r) - 1$ would make a positive contribution to $G_{cw}^{(m)}$, thereby contributing negatively to the partial molar volume $V_c^{(m)}$.

Based on the above, eqn (4) can also be justified straightforwardly by taking the difference between the partial molar volumes of a dimer and a pair of monomers.

2.4 Connection to the isodesmic model and the Setschenow constant

Determination of the KB integrals requires the calculation of $-\left(\frac{\partial \ln K}{\partial n_a}\right)_{T,P,n_c;n_a\to 0}$ and $\frac{1}{RT}\left(\frac{\partial \mu_c^{*}}{\partial n_a}\right)_{T,P,n_c;n_a\to 0}$ from experimental data. This involves (1) the isodesmic model for caffeine self-association and (2) the calculation of Setschenow constants.[23,35] Both (1) and (2) will be explained below.

The isodesmic model has been used widely to model non-specific and non-cooperative self-association.[19–23,47–49] Non-specificity of self-association is modelled by infinite steps of monomer binding reactions (*i.e.*, monomer + monomer ⇄ dimer, dimer + monomer ⇄ trimer, and, in general, n-mer + monomer ⇄ n + 1-mer).[19–23,47–49] Non-cooperativity of self-association is modelled by assuming that the binding constant of a monomer to an n-mer does not depend on the aggregate size n.[19–23,47–49] The term isodesmic derives from the Greek words *isos* (equal) and *desma* or *desmos* (bond),[50] which, in this context, signifies "equal K".[47]

Based on the isodesmic model and experimental data, the determination of the following has been reported in the literature: (i) the additive concentration (n_a) dependence of caffeine dimerization constant K, (ii) the volume change upon caffeine dimerization, ΔV_c, (iii) n_a dependence of caffeine monomer solubility $n_c^{(m)}$, and (iv) partial molar volume of caffeine monomer, $V_c^{(m)}$.[21–23] The KB integrals in this paper will be calculated from (i)–(iv).

The solubility of caffeine monomer determined from the isodesmic model at various additive concentrations can be fitted by the following equation:

$$\frac{\mu_c^{*(m)}}{RT} = k_1^{(m)} n_a + k_2^{(m)} {n_a}^2 + \ldots \quad (8)$$

where $k_1^{(m)}$ and $k_2^{(m)}$ are fitting parameters. $k_1^{(m)}$ is known as the Setschenow constant, which is the standard quantitative measure for the effectiveness of additives on salting-in or -out.[23,35] Likewise, the dimerization constant K from the isodesmic model can also be fitted by

$$-\ln K = \Delta k_1 n_a + \Delta k_2 {n_a}^2 + \ldots \quad (9)$$

where Δk_1 and Δk_2 are fitting parameters. Here Δk_1 is the Setschenow constant for caffeine dimerization, *i.e.*, the effectiveness of additives on caffeine dimerization, which, parallel to eqn (2), is related to the monomer and dimer Setschenow constants as $\Delta k_1 = k_1^{(d)} - 2k_1^{(m)}$. Combining eqn (3), (5), (6), (8),

and (9), the Setschenow constants are shown to have the following direct links with the KB integrals:[35]

$$\Delta k_1 = \Delta G_{cw} - \Delta G_{ca} \quad (10)$$

$$k_1^{(m)} = G_{cw}^{(m)} - G_{ca}^{(m)} \quad (11)$$

Thus the determination of $G_{cw}^{(m)}$ and $G_{ca}^{(m)}$ (eqn (7) and (11)), as well as of ΔG_{cw} and ΔG_{ca} (eqn (4) and (10)), has been simplified significantly.

Thus the combination with the KB theory overcomes the limitation of the isodesmic model, which is incapable on its own of clarifying the true driving force of caffeine self-association.[22] Is it the additive-induced change of "water structure" which drives caffeine dimerization? Or is it the distribution of additives around caffeine molecules that drives caffeine self-association? These questions can now be answered with the help of the KB theory, which does not introduce any additional assumptions other than the isodesmic model.

3. Mechanism of additive-induced caffeine dimerization

Here we examine the validity of the water structure hypothesis, based on the combination of the KB theory and the isodesmic model of caffeine self-association.

3.1 Analysing caffeine dimerization data from the isodesmic model

According to eqn (10), $\Delta G_{ca} - \Delta G_{cw}$ can be calculated from the Setschenow constant for dimerization, Δk_1, which can be determined from the additive concentration (n_a) dependence of $-\ln K$, which has been shown in Fig. 3 for sucrose and a number of salts taken from the literature.[22,23] The data in Fig. 3 were fitted against eqn (9), as summarised in Table 1. According to eqn (4), ΔG_{cw} can be calculated from ΔV_c, which has again been taken from the literature.[21] Combining eqn (4) and (10), ΔG_{ca} can also be calculated. ΔG_{cw} and ΔG_{ca} calculated thus are summarised in Table 2.

Eqn (11) shows that $G_{ca}^{(m)} - G_{cw}^{(m)}$ can be calculated from the Setschenow constant for caffeine monomer, $k_1^{(m)}$, which can be determined from the additive concentration (n_a) dependence of the monomer hydration free energy $\left(\frac{\mu_c^{*(m)}}{RT}\right)$, which has been taken from the literature,[22,23] and shown in Fig. 4 for all the additives considered in this paper. The data in Fig. 4 were fitted against eqn (8), as summarised in Table 1. $G_{cw}^{(m)}$ can be calculated straightaway from $V_c^{(m)}$ from the literature,[21] through eqn (7). KB integrals, $G_{cw}^{(m)}$ and $G_{ca}^{(m)}$, can thus be calculated by solving the simultaneous equations (eqn (7) and (11)), which are summarised in Table 2. $G_{cw}^{(d)}$ and $G_{ca}^{(d)}$ can thenceforth be calculated straightforwardly from eqn (2), using ΔG_{cw}, ΔG_{ca}, $G_{cw}^{(m)}$, and $G_{ca}^{(m)}$. All the KB integrals thus determined are summarised in Table 2.

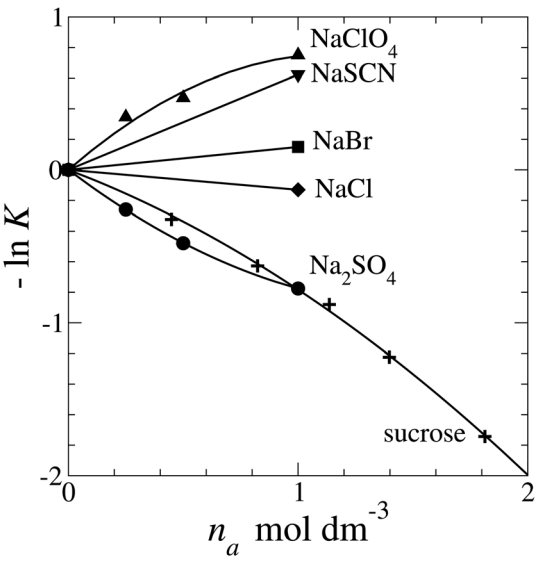

Fig. 3 $-\ln K$ (where K is the dimerization constant) against the molarity of additives, n_a, for various additives. Data are taken from ref. 22 and 23. The equations and parameters for the fitting curves (solid lines) are given in Table 1.

3.2 The dominance of the caffeine–additive interaction

First we examine whether the additive-induced changes in caffeine aggregation can really be rationalised by the additive-induced modification of the water structure.

If the water structure hypothesis, summarized in Introduction, were true, additives would modulate the water structure around the caffeine pair. Such a change of water structure inevitably leads to the change of caffeine hydration, namely ΔG_{cw}, and this ΔG_{cw} would be the dominant contribution to eqn (3).[32–34,39,40]

Thus the water structure hypothesis is equivalent to $|\Delta G_{cw}| \gg |\Delta G_{ca}|$.[31,34,35,38] In stark contrast, we observe that ΔG_{cw} is negligibly small compared to ΔG_{ca} for all the additives considered in this paper (Table 2). Thus the additive-induced water structure change does not account for the change of caffeine dimerization. Instead, it is ΔG_{ca} that is the true driving force of the additive-induced change in caffeine dimerization (Table 2). This leads us to the following new view:

New view: Caffeine–additive interaction is the cause of additive-induced change in caffeine dimerization.

1. Caffeine "dimerizers" (commonly called kosmotropes) tend to be excluded from the vicinity of caffeine, leading caffeine molecules to self-associate.

2. Caffeine "monomerizers" (commonly called chaotropes) tend to accumulate around caffeine, which makes caffeine molecules tend to be dissociated.

The new view rationalizes the sign and magnitude of the KB integrals summarized in Table 2. Let us take the strongest caffeine dimerizer (Na_2SO_4) and the strongest monomerizer ($NaClO_4$) as examples. Na_2SO_4 is excluded from caffeine monomers, since $G_{ca}^{(m)}$ is large and negative, whereas $NaClO_4$

Table 1 Fitting parameters for the dimerization and monomer hydration processes. For dimerization, the fitting equation is eqn (9), and for caffeine monomers, eqn (8). n_a has the unit of mol dm^{-3}. Experimental data are from ref. 22 and 23

		Na_2SO_4	NaBr	NaCl	$NaClO_4$	NaSCN	Sucrose
Dimerization	Δk_1	−1.13	0.150	−0.130	1.308	0.623	−0.568
Dimerization	Δk_2	0.357	0	0	−0.564	0	−0.215
Monomer	$k_1^{(m)}$	1.482	−0.108	0.248	−1.545	−0.796	0.455
Monomer	$k_2^{(m)}$	−0.219	0	0	0.608	0	0.103

Table 2 Kirkwood–Buff integrals (in cm^3 mol^{-1}) for the additive-induced dimerization of caffeine as well as the hydration of a pair of caffeine monomers and a dimer

		Na_2SO_4	NaBr	NaCl	$NaClO_4$	NaSCN	Sucrose
Dimerization	ΔG_{cw}	15[a]	15[a]	15[a]	15[a]	15[a]	15[a]
	ΔG_{ca}	1148	−135	146	−1293	−608	583
Monomer	$G_{cw}^{(m)}$	−145[a]	−145[a]	−145[a]	−145[a]	−145[a]	−145[a]
	$G_{ca}^{(m)}$	−1628	−36.9	−393	1400	651	−600
Dimer	$G_{cw}^{(d)}$	−275	−275	−275	−275	−275	−275
	$G_{ca}^{(d)}$	−2108	−209	−642	1508	694	−616

[a] Calculated from ref. 21, which reports −7.5 cm^3 mol^{-1} as the volume change upon dimerization per caffeine molecule, and $V_c^{(m)}$ = 145 cm^3 mol^{-1}.

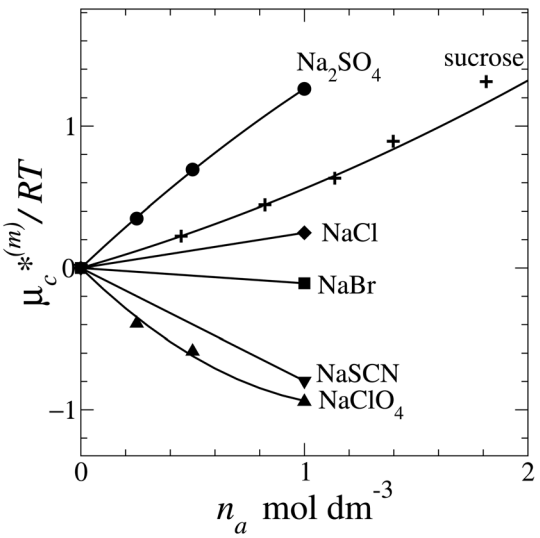

Fig. 4 Transfer free energy $\left(\frac{\mu_c^{*(m)}}{RT}\right)$ of caffeine monomers from pure water to a water–additive mixture, against the molarity of additives, n_a. Data are taken from ref. 22 and 23. The equations and parameters for the fitting curves (solid lines) are given in Table 1, which have been used to calculate the Setschenow constant, $k_1^{(m)}$.

accumulates around caffeine monomers, as shown clearly by a large and positive $G_{ca}^{(m)}$. Upon dimerization, ΔG_{ca} is large and positive for Na_2SO_4, which means that Na_2SO_4 is excluded less from a caffeine dimer compared to a pair of monomers. This large positive ΔG_{ca}, according to eqn (3), increases the association constant K. On the other hand, dimerization strongly reduces the accumulation of $NaClO_4$ around caffeine, as seen from a large negative ΔG_{ca}. This makes the association constant K decrease, according to eqn (3). The new view therefore successfully rationalizes the sign and magnitude of the KB integrals.

This new view on the role of additives in caffeine dimerization is consistent with the conclusions from other areas of research, such as protein denaturation and stabilization, drug solubilization by hydrotropes, as well as additive-induced change of food gelation, where additive accumulation and exclusion have been shown to be the dominant driving force.[32–44]

Following the classical insights from preferential solvation, how additives affect the dimerization process according to the new view can be understood in an intuitive manner.[32–42,51–53] Accumulation of chaotropes around the caffeine monomer ($G_{ca} > G_{cw} > 0$) makes its solvation more favourable $\left(\left(\frac{\partial \mu_c^{*(m)}}{\partial n_a}\right)_{T,P,n_c;n_a\to 0} < 0\right)$, according to eqn (5). Since the accumulation of chaotropes is favourable, caffeine dimerization is suppressed, because, intuitively speaking, where two caffeine molecules make contact, chaotropes cannot accumulate.[32–42,51–53] On the other hand, kosmotrope exclusion drives caffeine dimerization, which can be understood as the reverse of the chaotrope accumulation discussed above; exclusion of kosmotropes from caffeine monomer (for which G_{ca} has a *negative* sign) makes caffeine solvation more unfavourable $\left(\left(\frac{\partial \mu_c^{*(m)}}{\partial n_a}\right)_{T,P,n_c;n_a\to 0} > 0\right)$, according to eqn (5). This drives caffeine dimerization, because kosmotropes are excluded less from caffeine dimers than from caffeine monomers ($G_{ca}^{(d)}$ is *less negative* than $2G_{ca}^{(m)}$, hence $\Delta G_{ca} > 0$ in Table 2). Thus caffeine molecules, intuitively speaking, dimer-

ize in the presence of kosmotropes ($\left(\frac{\partial \ln K}{\partial n_a}\right)_{T,P,n_c;n_a\to 0} > 0$ in eqn (3) and Fig. 3) to minimize kosmotrope exclusion.[32–42,51–53] This is often referred to as minimizing the work or the "free energy of exclusion" of kosmotropes in the classical preferential solvation theory.[51–53]

This explanation based on the classical preferential solvation theory is consistent with the insight from the study of ion hydration, which has indicated the existence of a strong kosmotrope–water binding.[54,55] This is because, intuitively speaking, the stronger the additive–water binding, the more likely the additive molecules are attracted to the bulk phase of water, and hence are excluded from the vicinity of caffeine, provided that additive–caffeine interaction is weaker than additive–water.[42] The validity of this intuitive picture should further be confirmed through simulation, through which the affinity of the additive to bulk water should be compared to that to the vicinity of caffeine.

How does the new view compare with experiments? Spectroscopic studies have shown that the binding of kosmotropic ions to caffeine is weak, which is consistent with the new view.[24,25] A simulation study, on the other hand, reported evidence for sucrose–caffeine binding,[17] which may seem to be in apparent contradiction to the new view. However, it is important to realize that G_{ca} is made up of an integration of $g_{ca}(r) - 1$ over a long range of r.[32–44] Hence it is impossible to rationalize the sign of G_{ca} based on the behavior of $g_{ca}(r) - 1$ only at sucrose–caffeine contact; one must consider sucrose–caffeine configurations from a wide range of caffeine–sucrose distances r.[32–44] For this purpose, small-angle X-ray or neutron scattering measurements should be performed.

3.3. Advantages and limitations of our approach

The KB theory has shown that the validity of the water structure hypothesis can be examined simply by the comparison between ΔG_{cw} and ΔG_{ca}. This may be surprising, considering that the "water structure" is commonly linked to the entropy of hydration.[26,27] Why can the KB theory evaluate the water structure contribution without any reference to entropy? Chemical thermodynamics can dispel this doubt.[56–59] It is well-established that information on water structure can be probed by differentiating hydration free energy;[55,56] not only the entropy of hydration (*i.e.*, temperature derivative) but also ΔV_c (*i.e.*, pressure derivative; see eqn (4)) can give information on water structure.[56–59] Indeed, volumetric properties have been shown to be powerful in revealing the hydration mechanism of small molecules, as well as of macromolecules.[58,59]

We have used the KB theory at the $n_a \to 0$ limit, which has the following advantages: (i) much simpler in form than the KB theory without this limit;[31–35] (ii) a direct connection to the Setschenow constant can be made at this limit;[35] (iii) partial molar volume data of caffeine monomer and dimerization at finite additive concentrations, which have not been reported in the literature, are unnecessary.[31–35] The reason for (ii) can be understood directly from eqn (8) and (9): Setschenow constants can be calculated by taking n_a differentiation at $n_a \to 0$, which is indeed what eqn (3) and (5) do. Moreover, the magnitude of the dimerization constant and monomer solubility at finite n_a is determined by their n_a derivative at low n_a; this is why Setschenow constants have been used as a measure of effectiveness of salting-in and -out for over a century,[23,35,54,55] and it is this derivative that is the focus of the KB theory. However, understanding the behavior of caffeine dimer in the presence of concentrated additives requires further experiments.

The KB theory of solutions is rigorous and without any approximations.[32–45] On the other hand, the dimerization coefficient and the volume change upon dimerization both depend on the assumptions of the isodesmic model.[19–23,47–49] In this sense, the reliability of the present analysis depends solely on the accuracy of the isodesmic model. Even though there have been questions raised about the accuracy of one of its assumptions, *i.e.*, the independence of the association constant on the size of caffeine aggregates,[19–23,47–49] we consider that the conclusion of our paper, that caffeine–additive interaction plays the dominant role, is robust. This is because caffeine hydration data, from which the isodesmic model is derived, depend on the additive concentration far more sensitively than hydrostatic pressure,[20–23] which underscores our conclusion that caffeine–water interaction should be much weaker than caffeine–additive interaction.

In principle, it is possible to employ the KB theory of three component solutions, in which the solute (caffeine) is not dilute. Such a KB theory has already been published, yet the complexity of the equations involved therein is a hindrance towards a direct application.[60] Hence we had to adopt the isodesmic model, in order to focus on the initial dimerization process of a caffeine pair in dilution. However, a more complete treatment of caffeine aggregation in the future should directly employ the caffeine–caffeine KB integral G_{cc}.[60]

4. Conclusion

Caffeine dimerization is influenced strongly by the presence of additives. This molecular process, considered to be crucial for the enhancement and suppression of the bitter taste, has for a long time been rationalised through the effect of additives on the hydrogen bonding structure of water ("water structure"). Kosmotropes ("water structure makers" such as sucrose and Na_2SO_4) enhance dimerization, whereas chaotropes ("water structure breakers" such as $NaClO_4$ and NaSCN) weaken dimerization.[11,15,18,23]

This paper has challenged this classical hypothesis. Combining the KB theory of solution (which is a rigorous theory)[32–45] with the isodesmic model of caffeine dimerization,[19–23] we have shown that the "water structure", as well as the consequent additive-induced change in caffeine hydration, contributes negligibly to the change of dimerization. Instead of this classical view, the KB theory has established a new view: the true driving force is the caffeine–

additive interaction. Caffeine dimerization is enhanced when additives are excluded from caffeine molecules, and is suppressed when additives accumulate around caffeine.

What has emerged from this study seems to be a part of the general picture, which is valid for a variety of systems from biological macromolecules to drug solubilization: solute–additive interaction is the dominant cause of the equilibrium shift, rather than the indirect effect of the additive-induced hydration change.[31–35,38–41,44] The quest for understanding the role of the water structure in caffeine dimerization should therefore be directed towards a new line of enquiry: how caffeine–additive interaction is mediated by water.[31–35,38–41,44]

Abbreviation

KB Kirkwood–Buff

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