Unifying hydrotropy under Gibbs phase rule

Seishi Shimizu1,\* and Nobuyuki Matubayasi2,3

1York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom

2Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan

3Elements Strategy Initiative for Catalysts and Batteries, Kyoto University, Katsura, Kyoto 615-8520, Japan

**KEYWORDS:** Hydrotropy; Water; Cosolvent; Cooperativity; Minimum Hydrotrope Concentration

**AUTHOR INFORMATION**

**Corresponding Author:**

Seishi Shimizu

York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom

Tel: +44 1904 328281, Fax: +44 1904 328281, Email: seishi.shimizu@york.ac.uk

**ABSTRACT**

The task of elucidating the mechanism of solubility enhancement using hydrotropes has been hampered by the wide variety of phase behaviour that hydrotropes can exhibit, encompassing near-ideal aqueous solution, self-association, micelle formation, and micro-emulsions. Instead of taking a field guide or encyclopedic approach to classify hydrotropes into different molecular classes, we take a rational approach aiming at constructing a unified theory of hydrotropy based upon the first principles of statistical thermodynamics. Achieving this aim can be facilitated by the two key concepts: (1) the Gibbs phase rule as the basis of classifying the hydrotropes in terms of the degrees of freedom and the number of variables to modulate the solvation free energy; (2) the Kirkwood-Buff integrals to quantify the interactions between the species and their relative contributions to the process of solubilization. We demonstrate that the application of the two key concepts can in principle be used to distinguish the different molecular scenarios at work under apparently similar solubility curves observed from experiments. In addition, a generalization of our previous approach to solutes beyond dilution reveals the unified mechanism of hydrotropy, driven by a strong solute-hydrotrope interaction which overcomes the apparent per-hydrotrope inefficiency due to hydrotrope self-clustering.

**1. Introduction**

The difficulty in elucidating the origin and mechanism of hydrotropy has been a major stumbling block for its rational exploitation.1–7 This difficulty, as we believe, owes in large part to the following definition of hydrotropy (which is broad, inclusive and ambiguous) as small, organic and amphiphilic molecules that, when added to water, can drastically increase the aqueous solubility of hydrophobic solutes. 1–7 This definition admits urea (which forms near-ideal mixture with water) and surfactants (that self-associate in water to form micelles) under the same umbrella, leading to a classical misattribution of the mechanism of small molecule hydrotropy to hydrotrope self-association in the bulk phase, which is against the observed near-ideal mixing of urea with water.8–13 Although micelles are typically excluded from the category of hydrotropes in the modern literature, an analogy with micelles have often been invoked in the attempts to elucidate how hydrotropes work.1–7

Indeed, hypotheses on the mechanism of hydrotropy have been borrowed extensively from wide-ranging sub-disciplines, such as colloid and surfactant theories, the clathrate hypothesis of the hydrophobic effect,14,15 and chaotropy and kosmotropy.16–20 All these hypotheses, however, were qualitative, without any recourse to quantitative measures or “numerical reckoning”, indispensable for “true understanding”.21

Our recent approach to hydrotropy, based upon rigorous statistical thermodynamic principles, have brought a long-awaited clarity to the mechanistic understanding of hydrotropy.8–13 The Kirkwood-Buff (KB) integrals,22–24 the net excess of the radial distribution function from the bulk, quantifies the solvent-mediated interactions (potential of mean force) between the molecular species.25–28 For small molecule hydrotropes that do not form micelles, we have identified the non-specific solute-hydrotrope interaction as the major driving force of solubilization, modulated by the bulk-phase self-association of hydrotropes as the minor contribution.8–13 The sudden, cooperative onset of solubilization above the minimum hydrotrope concentration (MHC) has been attributed to the solute-induced enhancement of hydrotrope self-association.10,12,29 In addition, the origin of micellar hydrotropy has been attributed to be a large solute-micelle KB integrals as a major driving force which far more than compensates the minor contribution due to bulk-phase hydrotrope self-association.10,30

Establishing a unified theory of hydrotropy is still a challenging task, due to the diversity of hydrotropes admitted under the same definition, which spans over weakly-amphiphilic molecules, micelles and micro-emulsions.7 Such diversity in hydrotropic behaviour, however, can be classified rationally using the Gibbs phase rule, which can identify the degrees of freedom of the system31,32 and can clarify how many KB integrals, that represent the potential of mean force as integrals over the whole space, can be determinable in principle.27,33 Thus, the aims of the present Perspective article are:

1. To present a unified, coherent description of the new understanding of hydrotropy based upon rigorous statistical thermodynamic principles.
2. To clarify the degrees of freedom involved in hydrotropy based on Gibbs phase rule.
3. To extend the theory of hydrotropy beyond sparingly soluble solutes.

Aim (1) is necessitated by the fact that the basic theories have so far been presented incrementally in several papers8–11,13,29,34 and a unified perspective is now in order. Aim (2) is based upon a principle introduced in one of our biophysical papers27 as the way to establish a fundamental distinction between a solute and a surface, and will be exploited throughout this paper to distinguish apparently similar yet fundamentally different mechanisms at work in solubilization. Aim (3) comes from the fact that our previous papers, aiming at establishing a clear molecular picture, focused exclusively on solutes at infinite dilution.8–11,13,29,34 Yet our theory can be extendable to solutes with high solubility, even though the determination of the KBIs will require more experiments. A general picture of hydrotropy will emerge from this extension.

**2. Gibbs phase rules and the number of independent Kirkwood-Buff integrals involving the solute**

A simple consideration based upon the Gibbs phase rule is crucial in clarifying the number of independent KB integrals determinable in principle from experiments. Simple as it may sound, it is often difficult to exploit this relationship consciously for the interpretation of experimental data.27

Before moving onto the application to hydrotropy itself, let us briefly reiterate why applying such a simple approach has been difficult, which can be best illustrated by a controversy25,35–37 over the validity of the osmotic stress technique (OST) for the evaluation of hydration changes that accompany biochemical processes, i.e., to modulate the equilibrium constant (of binding, allosteric process, or channel opening) through the addition of osmolytes to evaluate the accompanying change of hydration. Whether the excess solvation numbers of water and osmolyte molecules around the biomolecule can both be determinable in principle was the central focus of this debate.25,35–37 A full resolution of this debate (which has led to a successful application of the KB theory to a number of systems34,38–41) required a simple realization that, for the determination of two independent quantities (excess numbers of water and osmolyte) the solvation free energy of a solute should be modulated via two independent variables (osmolyte concentration and pressure).25 This is indeed the basic consideration behind the solution of simultaneous equations, yet it was only much later that Gibbs phase rule was identified as the basis for the existence of independent variables that can be used to modulate the free energy of solvation, thereby governing the number of independently-determinable KB integrals.27,33

An important corollary of the above realization is the fundamental difference between a surface and a solute, even though the effect of cosolvents on solvation free energy can be written in a formally identical manner as the Gibbs adsorption isotherm.27 We will show that this fundamental difference comes from the phase rule itself, and is central to our understanding of hydrotropy.

**3. Hydrotropy at extremes**

**3.1. Cooperative solubilization in a single-phase aqueous hydrotrope solutions**

Here we consider the solubilization of hydrophobic solutes using small organic hydrotropes. Although the solubility increases can be 100-fold, we consider the cases in which the solutes are still dilute in the aqueous solution.8,9,11 And we consider the case in which aqueous hydrotrope solution can still be in a single phase and that the presence of a small quantity of solutes does not affect its phase behaviour.27 Whether the aqueous hydrotrope solution is in a single phase can be established by measuring how activities of water and hydrotrope depends on composition.

According to the Gibbs phase rule, since there are two components () (the solute is at the limit of infinite dilution so does not count as a component in this context) and the solution is in a single phase , the degrees of freedom of this system are . Thus , , and composition are independently changeable. Consider now a (locally) inhomogeneous solution which contains a solute molecule whose centre-of-mass is fixed in position.10 The presence of this external field does not change the phase rule.10 Under a constant temperature, both the composition and pressure are independently variable. Since the composition and pressure dependency of the solute’s chemical potential leads to two KB integrals, this system leads to an independent determination of solute-water and solute-hydrotrope KB integrals.27

Thus the solute-water and solute-hydrotrope KB integrals, denoted respectively as and , can be determined independently.8–11 This, together with the KB integrals for hydrotrope-water and hydrotrope-hydrotrope interactions, and , determinable from the bulk solution thermodynamics, gives the following hydrotrope concentration () dependence of the solvation free energy of the solute molecule (): 8–11

 (1)

Eq. (1) has revealed the following driving forces at work in hydrotropy:

1. A strong preferential solute-hydrotrope interaction compared to solute-water, i.e., a large positive is the major driving force for solubilization.8–11
2. A minor reduction in solubilization comes from the a large positive , which represents a bulk-phase self-association of the hydrotropes. 8–11

In addition, a detailed study of the second order derivative has shown that

1. A sudden onset of solubilization at MHC comes from the enhancement of hydrotrope-hydrotrope self-association induced by the solute molecule.10,12

We have thus clarified the driving forces for hydrotropic solubilization as well as its cooperativity based on the KB theory. This picture will be generalised to be applicable to concentrated solutes in Section 5.1.

**3.2. Apparent cooperativity in aqueous biphasic systems**

Here we consider another extreme case at the opposite end, i.e., solubilization in the aqueous biphasic systems, whose existence can be inferred by the phase diagram of the aqueous hydrotrope mixture.

Let us consider a 2-component system (), which separates into 2 phases (). According to the Gibbs phase rule, the degrees of freedom are . This means, under a constant temperature, either pressure or composition can be changed, but not both. This leaves us with only one thermodynamic parameter along which the solvation free energy can be changed. Therefore, in this system, two KB integrals (solute-water and solute-hydrotrope) cannot be determined independently. This is in contrast with the case of single-phase aqueous hydrotrope solution in which the solvation free energy could be modulated by two independent thermodynamic parameters, by which two KB integrals (solute-water and solute-hydrotrope) could be determined independently.

Suppose that the aqueous “hydrotrope” solution has relatively low saturation solubility of the hydrotrope. The addition of more hydrotrope after this composition will see the phase separation of the system into two phases. Using the mole fraction scale, let us denote the two hydrotrope concentrations on the coexistence curve as (water rich) and (hydrotrope rich). Let the molecular weight of water and hydrotrope respectively be and . According to the lever rule, the volume ratio between the amount of hydrotrope-rich phase (whose composition and mass density are and , respectively) *versus* the water-rich phases (whose composition and mass density are and , respectively) can be calculated between the two critical compositions as

 (2)

where and have been introduced for later convenience.

Let us solubilize a hydrophobic solute into this aqueous biphasic system. Due to the Gibbs phase rule, independent determination of solute-hydrotrope and solute-water interactions is impossible. The *relative* strength of the two interaction, however, can be determined. Let be the partition coefficient between the water-rich and the hydrotrope-rich phases as

 (3)

where and respectively express the solute molar concentration in the water-rich and hydrotrope-rich phases (now being viewed from the homogeneous solutions perspective). What is important from now onwards is that remains a constant regardless of .

Suppose that , which is the case for biphasic solubilization. Above the hydrotrope concentration , the relative amount of the hydrotrope-rich phase increases according to Eq. (2). Since is a constant, the more the hydrotrope-rich phase there is the more soluble the solute becomes. Hence solubilization, or the apparent partition coefficient can be expressed as

 (4)

and the total solubility can be expressed as

 (5)

This aqueous biphasic system can also exhibit what looks like cooperative solubilization, as can be seen from Eq. (5); a sudden increase of solubility takes place when approaches ( is actually not a constant with respect to the change in but varies only smoothly). However, this mechanism is different from the hydrotropic cooperativity clarified for the small molecule hydrotropy (Section 3.1) and from micellar hydrotropes (Section 4.1), both of which indeed have cooperative hydrotrope interactions as the basis of cooperative solubilization.

**4. Hydrotropy in-between**

**4.1. Cooperativity in micellar hydrotropy / solubilization**

Since the analogy between hydrotropy and micellar solubilization has often been invoked, here we consider the solubilization of a solute by mono-disperse micelles, which means that we consider a solute molecule (as a source of an external field of an inhomogeneous solution) in a system comprised of water, micelle, and monomer.10 This means an effective three-component solution () with one less degree of freedom due to micelle-monomer equilibrium; the solution is in a single phase. The degree of freedom therefore is , which is the same as in the case of small molecule hydrotropes. This means that, under a constant temperature, pressure and concentration can be changed independently to modulate the solvation free energy of a solute. Two independent variables for the solvation free energy means that two KB integrals involving the solute can be determined:27 solute-water and solute-micelle KB integrals. In turn, this means, due to the monomer-micelle equilibrium, that solute-micelle and solute-monomer KB integrals cannot be determined independently.

Consistent with the above argument based on the phase rule,27 we have derived the following relationship for micellar hydrotropy in our previous paper10

 (6)

where species 3 refer to the micelle. Eq. (6), derived under the condition that the surfactants are predominantly in the micellar form, shows that micellar solubilization is due to the stronger solute-micelle interaction compared to solute-water.10 But, in the current analysis of experimental data, surfactant concentration is commonly used instead of the micellar concentration. To conform to this common practice, we introduce here the total surfactant concentration , where is the aggregation number. In this framework, Eq. (6) can be rewritten as10

 (7)

Here, solubilization viewed in terms of the surfactant concentration contains , which refers to the effective reduction in the number of hydrotropes, hence is an apparent inefficiency due to micellization.10 However, the large more than compensates this factor, making many micellar hydrotropes effective solubilizers.10

An important task unfulfilled in our previous work is the link between Eq. (7) and the classical framework of micellar solubilization, namely the molar micelle-water partition coefficient and molar solubilization coefficient .42–44 Our aim here is threefold: (1) to show how the classical framework can be interpreted in terms of the KB theory; (2) to clarify the true molecular picture behind and ; (3) to clarify the difference between micelle-water partition coefficient and the biphasic partition coefficient.

The molar solubilization coefficient at a total surfactant concentration , in the experimental literature, has been defined in the following context

 (8)

where is the solubility of the solute and is that at CMC ().42–44 Molar micelle-water partition coefficient has been introduced in the context of rewriting Eq. (8) as30

 (9)

where is usually the concentration region used for solubilization.42–44 Note here that the “partition coefficient” introduced here refers to the partitioning of solute molecules between water and micellar interior, modelled as a “pseudo phase”, which even inspired classical studies on the correlation between and octanol-water partition coefficients.43,45,46 The approach based on is a further simplification of the stoichiometric modelling of solute-surfactant complexation.47 This is contradictory to accumulating evidence for the multiple possible locations (from interior to the surface) in the micellar system that a solute can be located.30,44,48,49

Hence, considering the water-micelle partition coefficient merely as a fitting parameter, now we combine the classical framework (Eq. (9)) and the rigorous KB theory (Eq. (7)). Since , one of us has shown30

 (10)

Eq. (10) is significant in the following manner (which addresses our three-fold aim).

(1) All the molar water-micelle partition coefficients determined in the literature42–44 can automatically be interpreted in terms of the KB integrals.30

(2) The KB integral interpretation of what is now the fitting parameter is consistent with accumulating evidence that a solute can be not only within the micelles but also embedded in the micellar layer or bound to the micellar surface.30,44,48,49 Such diversity in the solute location can be probed by simulation and can naturally be incorporated into the KB integrals as in the form of Eq. (10), but breaks the simple interpretation of as the partition coefficient of a solute between the aqueous phase and the micellar interior modelled as pseudo-phase.43,45,46

(3) This partition coefficient has an entirely different significance from the partition coefficient in biphasic systems, where the latter could not be decomposed further into solute-water and solute-hydrotrope interactions (corresponding to the potentials of mean force), due to the limited number of independent variables. In contrast, for micellar solutions, micelle-water partition coefficient comprises of a competition between solute-water and solute-micelle interactions both of which are independently-determinable, because the micelle-water mixture, being in a single phase, has one more degree of freedom that aqueous biphasic systems.

**4.2. Hydrotropy with ambiguous phase behaviour**

We have thus derived the two partition coefficients that are similar according to the classical theories but have fundamentally different origins.

1. Micelle-water solutions form a single phase, which means that, at a constant temperature, both pressure and micellar concentrations can be changed independently, thereby guaranteeing the existence of solute-water and solute-micelle KB integrals.
2. The amount of hydrotrope-rich phase in the biphasic system is determined by the lever rule along the tie line; the pressure and composition cannot be changed independently, thereby the solute-water (rich phase) and solute hydrotrope (rich phase) interactions cannot be separated.

These two partition coefficients, albeit their completely different molecular origins, can both give rise to cooperative-like behaviour. This poses a serious difficulty in the systems with ambiguous or unclear phase rules, such as micro-emulsions. This problem will be discussed more in depth in Section 5.4.

**5. Generalization to concentrated solutes**

Here we briefly present how our rigorous statistical thermodynamic approach to hydrotropy, based on the KB theory and the phase rule, can be generalized to solutes beyond infinite dilution and to the *n*-component systems in general.

**5.1. Small molecule hydrotropes**

For the sake of simplicity, let us focus on 3-component systems consisting of solute, water and hydrotrope, in which the solute, being no longer dilute, now counts as a component. (Generalization to -component systems including non-dilute solutes is straightforward). According to the Gibbs phase rule, if there are three components () and the solution is in a single phase , the degrees of freedom are . Thus, in addition to and , two composition ratios can be changed independently. This means, under constant temperature, that there are three variables that can be changed, guaranteeing the independent determination of solute-solute, solute-water and solute-hydrotrope KB integrals.

 In our previous papers, we have intentionally restricted our discussion to the case of dilute solutes, so as to clarify the mechanism of hydrotropy.8–13 However, we emphasise that the analysis of hydrotropy for concentrated solutes via the KB integrals can be performed using the inversion of the KB theory, applicable to in general to *n*-component systems.24 The matrix , whose elements are defined in terms of the KB integrals as

 (11)

can be determined from the following matrix inversion

(12)

in which the elements of defined as

 (13)

can be accessible from thermodynamic measurements.24

This matrix inversion approach (Eqs. (11)-(13)) can be used to generalize our earlier hydrotropy theory, derived for dilute solutes, into solutes with arbitrary concentrations. To do so, let us use the following well-known results:50–52

 (14)

where the cofactor is defined, and rewritten via KB integrals via Eq. (11), as

 (15)

and the determinant , which is positive due to the stability condition,52 can be expressed as

 (16)

Note that is the generalization of the solubilization inefficiency concept into the case of concentrated solutes.

Now our earlier proposal on hydrotropy mechanism (Eq. (1)) can be generalized to concentrated solutes. Firstly, a large positive drives solubilization, through making negative (a) by making its numerator large and negative (Eqs. (14) and (15)), and (b) by reducing the magnitude of its denominator and increase the solubilization efficiency when the solute-hydrotrope interaction is strong enough with attractive potential of mean force, which can be seen clearly by the contribution of to ,

 (17)

which is a negative contribution when is large enough. Secondly, hydrotrope self-aggregation contributes to increase the denominator, thereby making per-hydrotrope solubilization inefficient. This is seen in the following contribution of to

 (18)

in which can be proven to be positive based upon Schwartz inequality. This can also be proven through the thermodynamic stability condition using .52 We have shown that the two driving forces of solubilization identified in Section 2.1 still holds true even for concentrated solutes.

**5.2. Biphasic systems**

To illustrate the principle, let us consider the system of three components () with two phases () yields as the degree of freedom. Under isothermal condition (=constant), there are two other variables that can be changed independently. One cannot control the concentrations of the solute, water, and hydrotropes independently. Under this condition, there are only two independent KB integrals determinable in principle out of solute-solute, solute-water and solute-hydrotrope. This means that two relative KB integrals are determinable. Any one of the three KBI can be a reference, and Gibbs dividing surface is an example with the solute-water KBI as the reference. But suppose that we choose solute-solute as a reference. In this case the two relative KBIs, i.e., (solute-water)-(solute-solute) and (solute-hydrotrope)-(solute-solute), are determinable in principle. This is not the ideal of full information, yet it is informative enough to yields the partitioning of the solute into two phases. The KBIs can be determinable also by the inversion of the KB theory.

**5.3. Micellar solubilization**

For illustration purposes, we consider a four component () system comprised of water, monomer, micelle and solute. The system is in a single phase (). Hence . A further consideration of an additional stoichiometric constraint in terms of the monomer-micelle equilibrium reduces . This, under constant temperature, guarantees three more independent variables, leading to the independent determination of three KB integrals out of four that involve the solute. Note that the number of independent KB integrals involving solute is unchanged by the presence of micellar equilibrium from Section 5.1. The following two options are especially useful.

**KBIs relative to solute-solute.** Three KB integrals are fundamentally determinable relative to the one chosen as the reference. When the solute-solute KBI is taken as the reference, the solute-water, solute-monomer, and solute-micelle KBIs can be determined as the differences from the solute-solute KBI. This is in strong contrast to the arguments in the first paragraph of section 4.1. The contrast is only apparent, however. At finite concentration, one can define the solute-solute KBI and can use it as a reference, whereas with a single solute fixed at the origin of the system, the “solute concentration” does not exist as a controllable variable and *F* decreases by one. The monomer-micelle equilibrium condition thus reduces the rank of the A and B matrices by one, but the inversion procedure (Eqs. (11)-(13)) is still applicable to this system.

**Implicit treatment of micelle formation.** Instead of considering micelle as an additional species in the solution, it is possible to treat the solution as a three-component system (water, surfactant and solute) by considering micelle formation in terms of instead of adopting stoichiometric equilibrium and the aggregation number in the ad-hoc manner, as has been done earlier in this section. Hence the KB-based approach presented in Sections 3.1 and 5.1 are still applicable to micellar solutions. This approach is especially useful when the self-association or pre-clustering in the solution cannot be dealt with under the assumption of mono-dispersity. Indeed, the presence of solutes in solution can drastically affect the critical micelle concentration and the aggregation number.53,54 We shall demonstrate the explanatory power of this approach in the following.

**5.4. Effect of mesoscale pre-clustering on solubilization**

Let us again consider a three-component mixture consisting of water, hydrotrope and solute. Some hydrotropes, such as tert-butyl alcohol,55 are known to form mesoscale aggregates and droplets when mixed with water, that can be detected by scattering measurements using light, X-ray and neutron. Scattering intensity increase is observed in a region in the phase diagram close to de-mixing56–59 but the general consensus is that this fluctuation, corresponding to mesoscale aggregation or “bicontinuous phase”, is distinct from critical density fluctuations.59,60 The difference from Section 5.3 is that hydrotropes can form mesoscale structuring much larger than micelles and hence applying stoichiometry to model aggregation is not a good idea. Nevertheless, the system is still in a single macroscopic phase,59 and KB theory for ternary mixture (solute, water and hydrotrope) can be applied to clarify the driving forces for solubilization. This is why the same basic mechanism (Eq. (14)) still applies here, which are

1. Large positive drives solubilization (Eq. (15)).
2. Pre-clustering which reduces solubilization efficiency (Eq. (16)).

Here,

**Solute-hydrotrope interaction, .** In the case of micellar solubilization, a favourable solute-hydrotrope interaction, signified by a large positive , can be caused in a variety of manner, as can be seen from the location of the solute such as (a) incorporated inside the hydrophobic interior of the micelle, (b) embedded in the micellar layer, and (c) bound to the micellar surface.30,44,48,49 The multiplicity of solute locations depends on the chemistry of solute and hydrotrope molecules, and reflect the dominant interaction that is responsible for solubilization. Thus, in the present case, observation of different solute locations depending on hydrotrope-solute combination, ranging from the interior of the hydrotrope aggregates to within interfacial film, shows a variety of mechanisms by which is made large and positive.

**Pre-clustering, .** The relationship between solubilization and hydrotrope pre-clustering is an important question.55 In the language of the KB theory, pre-clustering refers to , which decreases solubilization efficiency (Eq. (16)), namely the apparent reduction of per-hydrotrope solubilization. But is not the sole contribution to solubilization. Section 5.1 has clarified that the major driving force of hydrotropic solubilization is , and that and are independent KB integrals.10 Hence the lack of a simple correlation between solubilization and hydrotrope pre-clustering55 (i.e., ) is not surprising nor does it constitute a refutation of or a counter-example to the rigorous statistical thermodynamic approach to hydrotropy.

Thus, the general principle of hydrotropic solubilization still holds true in the presence of mesoscale structuring: solubilization is driven by and attenuated by . A correlation between pre-clustering and solubilization55 alone cannot disentangle the cause and the effect. We hope that the wealth of scattering data55 will lead to an extensive evaluation of KB integrals to quantify each of the driving forces.

**6. Pre-ouzo effect and surfactant-free micro-emulsion**

So far we have focused on three component systems consisting of water, hydrotrope and solute. Here we extend our theory further to 4-component systems comprising of water, “solvent” or “cosolvent” (species 2, partly-miscible with water), hydrotrope (species 3, that mixes with water and solvent), and solute.7,55,60–65 A wealth of data has been published on the mixing properties of water-solvent-hydrotrope that exhibit mesoscale pseudophase domains,64 commonly referred to as the “pre-ouzo” or “surfactant-free microemulsions.”7,55,60–65 Here we show statistical thermodynamic principles can bring insights into the apparent complexity.

Extending our theory to the 4-component system, we obtain the following hydrotrope () concentration dependence of the solute chemical potential:50–52

 (17)

where the cofactor is defined as

 (18)

Note that, due to Schwartz inequality, we can show that

 (19)

**(i) Solute-hydrotrope interaction, .** Thanks to Schwartz inequality (Eq. (19)), a favourable solute-hydrotrope interaction, namely a large positive , leads to solubilization (negative ).

**(ii) Further solubilization through solute-solvent interaction.** Since two solvents are immicible, a large negative is expected from the first two terms of Eq. (18). The hydrotrope (species 3) interacts favourably with both solvents, yet this favourable interaction does not easily overcome the excluded volume effect to make and positive. Hence contribution to the first two terms of Eq. (18) is written as

 (20)

where the r.h.s. has been rewritten clearly to mark the positive sign within . This shows that favourable solute-solvent interactions ( and ) contribute towards solubilization (i.e., to make negative) in addition to the contribution from .

**(iii) Hydrotrope self-association for solubilization inefficiency.** The contribution of hydrotrope self-association, , to can be seen in its 4-component expression of the denominator of Eq. (17)

 (21)

The term is again positive, due to stability condition. Hydrotrope self-association thus increases , the denominator of Eq. (17), thereby reducing the solubilization efficiency per hydrotrope.

**(iv) Immiscibility contribution to solubilization efficiency.** We need to consider how a large negative contributes to (Eq. (22)). To extract the relevant terms

 (22)

It is difficult to draw a definitive conclusion without evaluating each of the KBIs in Eq. (22). Yet we can at least say that a strong solute-solvent (species 2) interaction contribute negatively to , thereby increasing the solubilization efficiency. For the sake of argument, we consider the case when hydrotrope makes very strong interaction, sufficient to make KBI positive, only with the solute, , and . In this case, when not only but also is positive, the first two terms can become negative (considering that is negative). The third term can become negative only when is sufficiently stronger than . Hence there is possibility that immicibility of two solvents may contribute to increase the solubilization efficiency.

The strong solubilization power of surfactant-free micro-emulsions should come from the combination of solute-hydrotrope affinity in addition to solute-solvent, which overrides the solubilization inefficiency. What we have carried out here is merely to identify some possible scenarios. A full analysis requires the determination of all the KBIs.

**7. Conclusion**

Against the long-standing confusion on the origin of hydrotropy, rigorous statistical thermodynamics, based on the KB theory of solutions, has brought clarity for the first time in elucidating how hydrotropes increase the solubility of solutes in aqueous solutions for some classes of hydrotropes.8–13 However, a great variety in the types of molecules encompassed under the aegis of “hydrotropes” has posed a major hindrance towards the establishment of the general theory.8

Such a hindrance, however, has been overcome. The aim of this *Perspective* article was to show that a truly general theory of hydrotropy requires the Gibbs phase rule as the foundation.27,33 The *apparent* variety of the hydrotropic mechanisms has been shown to arise from the difference in the number of degrees of freedom, classifiable by the Gibbs phase rule. This has clarified the difference between single phase, micellar and biphasic solubilization mechanisms, and have paved a way towards rationalizing how hydrotropes and their mixtures with mesoscale structuring work to solubilize solutes. The classical notion of the micelle-water partition constant has been generalized to incorporate the variety of locations within and around the micelle that the solute can be found.30 The difference between the biphasic partition function and the solute-micelle partition function have been clarified from the Gibbs phase rule.

The generalization of our previous theory8–13 to concentrated solutes in multiple component mixtures has revealed that the universal mechanism of hydrotropy is at work; solubilization is driven by a strong solute-hydrotrope interaction with attractive potential of mean force, which more than overcomes the attenuation by hydrotrope self-aggregation. This general theory is applicable even to hydrotropes that exhibit mesoscale pre-structuring.

To summarise, so far as the structuring within the solution phase does not reach the macroscopic scale, the basic driving forces of hydrotropic solubilization can be quantified via KBIs regardless of solute concentration or number of components. The Gibbs phase rule has always been the foundation which determines the number of independent KBIs determinable in principle.

**Acknowledgements**

We are deeply grateful to Steven Abbott for his generous help in careful and critical reading of the manuscript. We also thank Jonny Booth and Josh Reid for useful comments. This work was supported by the Gen Foundation, by the Grants-in-Aid for Scientific Research (Nos. 15K13550 and JP26240045) from the Japan Society for the Promotion of Science, by the Elements Strategy Initiative for Catalysts and Batteries and the Post-K Supercomputing Project from the Ministry of Education, Culture, Sports, Science, and Technology, and by the HPCI System Research Project (Project IDs: hp170097 and hp170221).

**References**

1 C. Neuberg, *Biochem. Z.*, 1912, **76**, 107–176.

2 D. Balasubramanian, V. Srinivas, V. G. Gaikar and M. M. Sharma, *J. Phys. Chem.*, 1989, **93**, 3865–3870.

3 S. E. Friberg, *Curr. Opin. Colloid Interface Sci.*, 1997, **2**, 490–494.

4 P. Bauduin, A. Renoncourt, A. Kopf, D. Touraud and W. Kunz, *Langmuir*, 2005, **21**, 6769–6775.

5 T. K. Hodgdon and E. W. Kaler, *Curr. Opin. Colloid Interface Sci.*, 2007, **12**, 121–128.

6 C. V. Subbarao, I. P. K. Chakravarthy, A. V. S. L. Sai Bharadwaj and K. M. M. Prasad, *Chem. Eng. Technol.*, 2012, **35**, 225–237.

7 W. Kunz, K. Holmberg and T. Zemb, *Curr. Opin. Colloid Interface Sci.*, 2016, **22**, 99–107.

8 J. J. Booth, S. Abbott and S. Shimizu, *J. Phys. Chem. B*, 2012, **116**, 14915–14921.

9 S. Shimizu, J. J. Booth and S. Abbott, *Phys. Chem. Chem. Phys.*, 2013, **15**, 20625–20632.

10 S. Shimizu and N. Matubayasi, *J. Phys. Chem. B*, 2014, **118**, 10515–10524.

11 J. J. Booth, M. Omar, S. Abbott and S. Shimizu, *Phys Chem Chem Phys*, 2015, **17**, 8028–8037.

12 S. Shimizu and N. Matubayasi, *Phys. Chem. Chem. Phys.*, 2016, **18**, 25621–25628.

13 S. Abbott, J. J. Booth and S. Shimizu, *Green Chem.*, 2017, **19**, 68–75.

14 H. S. Frank and M. W. Evans, *J. Chem. Phys.*, 1945, **13**, 507–532.

15 W. Kauzmann, *Adv. Protein Chem.*, 1959, **14**, 1–63.

16 R. W. Gurney, *Ionic processes in solution*, New York, 1953.

17 R. A. Robinson and R. H. Stokes, *Electrolyte solutions*, Butterworths, London, 1955.

18 H. S. Frank and W.-Y. Wen, *Discuss. Faraday Soc.*, 1957, **24**, 133.

19 O. Y. Samoilov, *Discuss. Faraday Soc.*, 1957, **24**, 141.

20 H. S. Frank and F. Franks, *J. Chem. Phys.*, 1968, **48**, 4746–4757.

21 W. Thomson (Lord Kelvin), *Popular lectures and addresses*, Macmillan, London, 1889.

22 J. G. Kirkwood and F. P. Buff, *J. Chem. Phys.*, 1951, **19**, 774–777.

23 D. G. Hall, *Trans. Faraday Soc.*, 1971, **67**, 2516–2524.

24 A. Ben-Naim, *J. Chem. Phys.*, 1977, **67**, 4884–4890.

25 S. Shimizu, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1195–1199.

26 S. Shimizu and C. L. Boon, *J. Chem. Phys.*, 2004, **121**, 9147–9155.

27 S. Shimizu and N. Matubayasi, *J. Phys. Chem. B*, 2014, **118**, 3922–3930.

28 S. Shimizu, R. Stenner and N. Matubayasi, *Food Hydrocoll.*, 2017, **62**, 128–139.

29 T. W. J. Nicol, N. Matubayasi and S. Shimizu, *Phys. Chem. Chem. Phys.*, 2016, **18**, 15205–15217.

30 N. Matubayasi, K. K. Liang and M. Nakahara, *J. Chem. Phys.*, 2006, **124**, 154908.

31 J. W. Gibbs, *Trans Conn Acad Arts Sci*, 1875, **3**, 108–248.

32 J. W. Gibbs, *Trans Conn Acad Arts Sci*, 1877, **3**, 343–524.

33 S. Shimizu and N. Matubayasi, *Biophys. Chem.*, 2017, DOI: 10.1016/j.bpc.2017.02.003.

34 S. Shimizu and S. Abbott, *J. Phys. Chem. B*, 2016, **120**, 3713–3723.

35 S. N. Timasheff, *Proc. Natl. Acad. Sci.*, 1998, **95**, 7363–7367.

36 V. A. Parsegian, R. P. Rand and D. C. Rau, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 3987–3992.

37 S. N. Timasheff, *Proc. Natl. Acad. Sci.*, 2002, **99**, 9721–9726.

38 S. Shimizu, W. M. McLaren and N. Matubayasi, *J. Chem. Phys.*, 2006, **124**, 234905/1-234905/4.

39 S. Shimizu, *Chem. Phys. Lett.*, 2011, **514**, 156–158.

40 S. Shimizu and N. Matubayasi, *J. Phys. Chem. B*, 2014, **118**, 13210–13216.

41 R. Stenner, N. Matubayasi and S. Shimizu, *Food Hydrocoll.*, 2016, **54**, 284–292.

42 D. Attwood and A. T. Florence, in *Surfactant Systems*, Springer Netherlands, Dordrecht, 1983, pp. 229–292.

43 F. A. Alvarez-Núñez and S. H. Yalkowsky, *Int. J. Pharm.*, 2000, **200**, 217–222.

44 C. De Oliveira, R. Yagui, A. Lineu Prestes, C. O. Rangel-Yagui, A. Pessoa-Jr and L. C. Tavares, *J Pharm Pharm. Sci*, 2005, **8**, 147–163.

45 J. H. Collett and L. Koo, *J. Pharm. Sci.*, 1975, **64**, 1253–1255.

46 D. E. Kile and C. T. Chiou, *Environ. Sci. Technol.*, 1989, **23**, 832–838.

47 E. H. K. Shinoda, *J. Phys. Chem.*, 1961, **66**, 577–582.

48 A. A. Dar, G. M. Rather and A. R. Das, *J. Phys. Chem. Bhysical Chem. B*, 2007, **111**, 3122–3132.

49 P. A. Bhat, G. M. Rather and A. A. Dar, *J. Phys. Chem. B*, 2009, **113**, 997–1006.

50 A. Ben-Naim, *Molecular Theory of Solutions*, Oxford University Press, Oxford, 2006.

51 P. E. Smith, *J. Chem. Phys.*, 2008, **129**, 124509.

52 D. Gazzillo, *Mol. Phys.*, 1994, **83**, 1171–1190.

53 T. J. Ward and K. D. Ward, in *Solubilization in surfactant aggregates*, eds. S. D. Christian and J. F. Scamehorn, Marcel Dekker, New York, 1995, pp. 518–540.

54 P. F. Garrido, P. Brocos, A. Amigo, L. García-Río, J. Gracia-Fadrique and Á. Piñeiro, *Langmuir*, 2016, **32**, 3917–3925.

55 T. Buchecker, S. Krickl, R. Winkler, I. Grillo, P. Bauduin, D. Touraud, A. Pfitzner, W. Kunz, R. Cubitt, S. Marelja and W. Kunz, *Phys. Chem. Chem. Phys.*, 2017, **19**, 1806–1816.

56 D. Subramanian and M. A. Anisimov, *J. Phys. Chem. B*, 2011, **115**, 9179–9183.

57 D. Subramanian, C. T. Boughter, J. B. Klauda, B. Hammouda and M. a Anisimov, *Faraday Discuss.*, 2013, **167**, 217–238.

58 D. Subramanian, J. B. Klauda, P. J. Collings and M. A. Anisimov, *J. Phys. Chem. B*, 2014, **118**, 5994–6006.

59 S. Schöttl and D. Horinek, *Curr. Opin. Colloid Interface Sci.*, 2016, **22**, 8–13.

60 S. Schöttl, J. Marcus, O. Diat, D. Touraud, W. Kunz, T. Zemb and D. Horinek, *Chem. Sci.*, 2014, **5**, 2909–3340.

61 O. Diat, M. L. Klossek, D. Touraud, B. Deme, I. Grillo, W. Kunz and T. Zemb, *J. Appl. Crystallogr.*, 2013, **46**, 1665–1669.

62 S. Schöttl, D. Touraud, W. Kunz, T. Zemb and D. Horinek, *Colloids Surfaces A Physicochem. Eng. Asp.*, 2015, **480**, 222–227.

63 J. Marcus, D. Touraud, S. Prévost, O. Diat, T. Zemb and W. Kunz, *Phys. Chem. Chem. Phys.*, 2015, **17**, 32528–38.

64 T. N. Zemb, M. Klossek, T. Lopian, J. Marcus, S. Schöettl, D. Horinek, S. F. Prevost, D. Touraud, O. Diat, S. Marčelja and W. Kunz, *Proc. Natl. Acad. Sci.*, 2016, **113**, 4260–4265.

65 T. Zemb and W. Kunz, *Curr. Opin. Colloid Interface Sci.*, 2016, **22**, 113–119.

**Graphical Abstract**

****