­­­­Origin of nonlinearity in phase solubility: Solubilisation by cyclodextrin beyond stoichiometric complexation

Thomas W. J. Nicol,1 Nobuyuki Matubayasi2,3, and Seishi Shimizu1\*

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

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**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](mailto:seishi.shimizu@york.ac.uk)

**ABSTRACT**

Low solubility of drugs, which poses a serious problem in drug development, can in part be overcome by the use of cyclodextrins (CD) and their derivatives. Here, the key to solubilisation is identified as the formation of inclusion complexes with the drug molecule. If inclusion complexation were the only contribution to drug solubility, it would increase linearly with CD concentration (as per the Higuchi-Connors model); this is because inclusion complexation is a 1:1 stoichiometric process. However, solubility curves often deviate from this linearity, whose mechanism is yet to be understood. Here we aim to clarify the origin of such non-linearity, based upon the Kirkwood-Buff and the McMillan-Mayer theories of solutions. The rigorous statistical thermodynamic theory shows that solubilisation non-linearity can be rationalised by two contributions: CD-drug interaction and the drug-induced change of CD-CD interaction.

**1. Introduction**

Cyclodextrin (CD) complexes have been used in pharmacy to improve the aqueous solubility of drug molecules, as well as targeted drug delivery in terms of controlled release.1–5 The ability of CDs to enhance solubility has been identified, within the literature, to stem from its formation of inclusion complexes with the drug molecule.6–8 However, accumulating evidence indicates that CD-drug complexation may not be the only driving force of CD-induced solubilisation.9–14 Despite this, elucidating quantitatively the origin and relative importance of non-complexation contributions has not yet been successful due to the lack of a rigorous theoretical framework. The aim of this paper is to clarify the origin and the magnitudes of such contributions, based upon a rigorous statistical thermodynamic theory.

The classical model for drug solubilisation by CDs was established by Higuchi and Connors (HC), which attributed the cause of solubility increase to stoichiometric 1:1 inclusion complexation.9 The HC model in its predominant form predicts a linear increase of solubility with CD concentration, in agreement with many experimental data.10,11 However, deviations from this linear solubility increase have also been reported in the literature,11–14 and standard nomenclature has been established to classify the different ways in which the so called phase solubility curves deviates from linearity (Figure 1).9,15 In addition, to address such deviation from linearity, the HC model was extended to incorporate different stoichiometries;12,16–18 yet these treatments cannot accurately model systems with large numbers of weak interactions.19,20 Hence, prominent questions still remain: what is the origin of non-linearity in phase solubility diagrams? What is the origin of the non-stoichiometric driving forces?

To give a clear answer to this historical question, a link between solubility behaviour and the molecular interactions causing it is indispensable. Such a link, provided by the rigorous Kirkwood-Buff (KB) theory of solutions,21–27 has recently made significant progress in clarifying the mechanism of drug solubilisation by the addition of hydrotropes.28–33 Drug solubility commonly exhibits non-linear dependence on hydrotrope concentration, often with a sudden offset of solubility increase at a threshold hydrotrope concentration referred to as the minimum hydrotrope concentration (MHC).28,29 The origin of MHC has been attributed by our rigorous KB-based theory to the solute-induced enhancement of the interactions among hydrotropes, which gives rise to a cooperative increase in solubility, showing that many-body interactions indeed play a crucial role in solubilisation.29–36 Our strategy, drawing from the theory of hydrotropy, is to construct a rigorous statistical thermodynamic theory of drug solubilisation by CDs to replace the purely phenomenological HC model.

In this vein, how drug solubility depends on CD concentration has previously been modelled by the McMillan-Mayer (MM) theory of solutions,37–40 another rigorous statistical thermodynamic theory;41–45 and though the MM theory has successfully produced regression equations for CD-drug systems,37–40 the molecular basis of non-linearity has remained unresolved.46–49 Here a clear understanding of the relationship between non-linear solubility and many-body interactions is indispensable.

The goal of this paper is to characterise drug solubility in ternary CD-drug aqueous systems, utilising statistical thermodynamics, through a rigorous theory superior to stoichiometric models. The relationship between non-linear solubility and many-body interactions will be clarified from both KB and MM theoretical bases. Such a theory will help extract molecular scenarios from the wealth of phase solubility data in the presence of CDs and provide a long-awaited understanding of the origin of the non-linear phase solubility curve.

**2. Theory of solubilisation in the presence of cyclodextrin**

**2.1. The goal and the formalism**

The goal of this paper is to rationalise how the solubility enhancement of drugs depends on the CD concentration and deviate from linearity.

To this end, consider a three component solution consisting of a drug (solute) (), water (), and CD () molecules. Let and respectively be the chemical potential and the number of species , and be the temperature and the pressure of the system. Let be the number density or concentration of species ; in the grand canonical ensemble, the ensemble average of is used instead to define , such that . The convention (where is the Boltzmann constant) is used throughout. Since is kept constant throughout this paper, it is often omitted in the subsequent discussions.

Solubility enhancement is quantified by the change of solute’s hydration free energy in the presence of CD from its value in pure water .24–26,28–32 Let us express this hydration free energy change, in terms of CD concentration . To achieve this goal, let us first expand in terms of CD fugacity, for convenience, and thereafter express in terms of . To this end, we need to calculate the following partial derivatives

(1)

We emphasise here that the insertion of the solute, as well as the addition of CD, are carried out under constant , and . This is different from the insertion condition of the MM theory, which is normally carried out under constant , and T.41,50,51

The understanding of CD-induced solubility enhancement has thus been reduced to the calculation of partial derivatives in Eq. (1).

**2.2. Basic concepts of the inhomogeneous Kirkwood-Buff theory**

The calculation of partial derivatives in Eq. (1) will be facilitated greatly by our recent development in the inhomogeneous KB theory.30 In this section we review the basic formulae necessary to achieve this goal.

The chemical potential of the solute fixed at an arbitrarily-chosen origin can be expressed in terms of the grand partition functions in the following manner:30

(2)

where the subscripts *u* and 0 denote the systems with and without the solute, respectively. As shown in our previous paper,30 the solution volume with the solute is treated as equal to that without solute *V* to assure that is an intensive property. In the following, is thus written as and the volume is the same whether the solute is present or absent.

Successive differentiation of Eq. (2) with respect to yields the following key relationships:30

(3)

(4)

where denotes ensemble averaging. The number averages and fluctuations in Eqs. (3) and (4) can be transformed to KB integrals through the following relationships:30

(5)

(6)

where is Kronecker’s delta.

**2.3. Cyclodextrin fugacity expansion of the hydration free energy**

**2.3.1. First order in**

Here we evaluate in Eq. (1). To do so, we need to bridge the VT ensemble in which the KB theory is constructed and the NPT ensemble in which solubilisation experiments are carried out.30 To this end, we employ the following thermodynamic relationship:

(7)

The evaluation of each term in Eq. (7) straightforwardly follows our previous paper.30 The first term of Eq. (7), using Eqs. (3) and (4) becomes

(8)

The second term of Eq. (7) can be evaluated by a combination of Eqs. (3) and (4), as well as the Gibbs-Duhem equation23,24,28–31,52 as

(9)

Combining Eqs. (7)-(9), we obtain

(10)

**2.3.2. Second order in**

**Overall strategy.** Here we evaluate in Eq. (1). To do so, as in 2.3.1., we need a relationship between the VT ensemble (the KB theory) and the NPT ensemble (solubilisation measurements).30 To this end, we express in terms of by virtue of the following thermodynamic relationship:

(11)

**Derivation of the thermodynamic relationship, Eq. (11).** We start from the following thermodynamic relationship:

(12)

Using Eqs. (7) and (9) for the first r.h.s. term, and Eq. (10) for the second r.h.s. term, Eq. (12) can be rewritten as

(13)

from which Eq. (11) can be obtained easily.

**Evaluation of in Eq. (11).** Differentiating Eq. (8) with respect to we obtain

(14)

The second term on the r.h.s. of Eq. (14) can be rewritten with the help of Eq. (4) as

(15)

Eqs. (14) and (15) can be rewritten in terms of KB integrals, with the help of Eq. (5) and (6), as

(16)

**Evaluation of** **in Eq. (11)**. Using Eqs. (3) and (4), we can show that

(17)

Eq. (17) can be rewritten in terms of KB integrals by the help of Eqs. (5) and (6) as

(18)

**Evaluation of in Eq. (11).** Again, using Eqs. (3) and (4), we can show that

(19)

Eq. (19) can be rewritten in terms of the KB integrals through the help of Eqs. (5) and (6) as

(20)

**Summary.** Combining Eqs. (11), (16), (18) and (20) we obtain

(21)

where denotes the contribution from solute-induced change of self-association, defined as

(22)

The equivalence between Eq. (21) and our previous expression for 30 can be shown straightforwardly in Appendix B.

**2.3.3. The CD fugacity expansion of hydration free energy to describe solubility enhancement**

Now we can conclude our quest for the -expansion of . Combining Eqs. (1), (10) and (21), we obtain

(23)

where the subscript 0 denotes the limit, and corresponds to with finite .

Solubility increase, namely ,28–31 can be expressed in the following manner using Eq. (23):

(24)

**2.4. CD concentration expansion of solubilisation**

The solubility of drugs in the presence of cyclodextrin is commonly plotted against the CD concentration, in phase solubility diagrams.9,48,49 Hence we need to change the variable of Eq. (24) from to . Note that the procedure of to conversion is different from that of the MM theory (VT ensemble),44,45,51,53 since the experiments are conducted entirely in the NPT ensemble.

Thus our goal in the forthcoming section is to determine a relationship between and in the NPT ensemble. To do so, let us start from a well-known relationship from KB theory, derived in the NPT ensemble:28–31,44,45

(25)

Eq. (25) indicates that can be expanded in the following manner

(26)

where is a constant. Note that originates from translational degrees of freedom. Substituting Eq. (26) into Eq. (25) at the limit yields

(27)

Note that is equivalent to . Eq. (27) leads to the following expansion for

(28)

where the existence of is justified clearly from the limiting behaviour.

Using Eq. (28), the expansion given by Eq. (24) can now be rewritten as

(29)

This is the CD concentration dependence of solubilisation that we sought after.

**2.5. Comparison to the McMillan-Mayer theory of solutions**

Part of the expansion presented above can be derived also from the MM theory. To demonstrate this, let us first appreciate the difference between the NPT process (Eq. (1)) and the osmotic equilibrium treated by the MM theory. The latter corresponds to

(30)

Note that , instead of , is kept constant in the partial derivatives; such partial derivatives have already been evaluated in Eqs. (8) and (16). Combining them with Eq. (30) yields

(31)

Eq. (31), which has been derived via the KB theory, can also be derived via the MM theory, which is shown in Appendices C and D. The insight from this comparison is that the solute-induced CD-CD interaction change term comes from MM theory, whereas those in CD-water and water-water interactions account for the difference between NPT and VT ensembles.

**3. Molecular basis of non-linearity in the phase solubility diagram**

Here we apply our theory to analyse the experimental solubility of drugs in the presence of CDs. The aim is to elucidate how non-linearity in phase solubility diagrams change as we modify the molecular structures of the drug and CD.

**3.1. Origin of non-linearity in the phase solubility diagram**

According to the classical 1:1 stoichiometric CD-solute complexation model of Higuchi and Connors,9 solubility is expected to increase linearly with the CD concentration. In reality, deviations from such linearity have been documented, suggesting that factors other than 1:1 CD-solute complexation are at work.11–14,54 Commonly observed modes for deviations from linearity in phase solubility data have been classified (Figure 1), 9,15 yet the molecular-based mechanism behind these different modes has remained a mystery.46,47 This was due to the lack of a true microscopic theory, which has been overcome by Eq. (29).

Interpreting phase solubility diagrams using the MM theory requires us first of all to have a clear physical meaning of each term in Eq. (29).

**The term** of Eq. (29), , clearly shows its equivalence to the expression of preferential solvation within KB theory.27 Thus the linear term of phase diagram, interpreted in the HC model as representing solute-CD complexation9 has now been generalised to drug-CD preferential interaction .

**The term** of Eq. (29) accounts for deviations from linear phase solubility. Positive and negative deviations from linearity, commonly referred to as and (Figure 1) can be attributed to its sign, yet it has a very complex expression with many terms. We aim, first of all, to simplify the term of Eq. (29) based upon experimental data. As will be demonstrated in Section 3.2, the following order of magnitude relationships hold true: , and . Note also that, at limit, , where is the isothermal compressibility, which makes a negligibly small contribution.45 Considering the above contributions, can be ignored. Hence

(32)

Using Eq. (32), Eq. (29) can be simplified as

(33)

The following two factors contribute to the non-linear term in Eq. (33):

1. , defined by Eq. (22), represents solute-induced change of the self-association of CD and water, as has been introduced previously.30,31 Because CD-solute interactions are much stronger than those between CD and water, we expect this term to be dominated by , the solute-induced CD-CD interaction change.31 Justification of this approximation is given in Appendix E. Furthermore, at the thermodynamic limit (i.e., ), we can show

(34)

1. can be interpreted as the CD-solute interaction.24,28–33 Hence the origin of non-linearity can be attributed to the interplay between CD-solute interaction and solute-induced increment of CD-CD interaction. The significance of each of the above factors’ contributions will be further discussed below through the use of experimental data.

On the other hand, phase solubility exhibits an apparently linear behavior when the third term of Eq. (33) is negligibly small compared to the second, namely , where here should represent the maximum CD concentration for experiment. To clarify the possible scenarios by which this condition is satisfied or broken would require an extensive analysis of experimental data, however, solute-induced weakening of CD-CD interaction sufficiently strong (but not too strong) to compensate would be required to make the nonlinear term vanish.

**3.2. Non-linearity of phase solubility diagram: effect of solute and CD structural changes**

When the structures of the solute and CD are modified, what is the consequent change in the non-linearity of phase solubility? Here we answer this question through the analysis of experimental data, in order to address the long standing need for a molecular-based interpretation of phase solubility diagrams in the presence of CDs.15,46 Non-linearity in phase solubility diagrams has now been attributed to the sign of , composed of the two factors as clarified in Section 3.1. Here we calculate their contributions from experimental data.

In order to understand the behaviour more clearly we have chosen data, from two drug molecules of comparable structure and two CD derivatives, to analyse using our method. Two specific solution comparisons stand out in terms of the insights that they can provide within our literature data survey (see Figure 2 for molecular structures):

1. Aqueous solubility of naringenin with β-CD *versus* with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD).2,55
2. Aqueous solubility of naringin versus naringenin with HP-β-CD.55

This set of examples was chosen for the following reasons, which are beneficial for investigating the structural cause of the nonlinearity:

1. Phase solubility of naringenin in β-CD exhibits close to linear AL (or a very weak positive deviation, AP) behaviour in contrast to a weak AP in HP-β-CD, which can be attributed to the derivatisation of β-CD to HP- β-CD.6
2. Entirely different deviations from phase solubility linearity between naringin (AN) and naringenin (AP) can be attributed to the additional neohesperidose (disaccharide) present in naringin.

The phase solubility diagrams of these drugs are shown in Figure 3. Naringin’s solubility behaviour does not change majorly between different CD derivatives, however, for completeness it will also be analysed.

Phase solubility data in the presence (S) and absence () of CD, taken from the literature,55 has been converted to through a well-established relationship: . Such data have been fit to the following polynomial (i.e., the second order truncation of Eq. (33)), whose fitting constants and are tabulated in Table 1.

(35)

The corresponding KB integrals can be determined through a comparison between Eqs. (33) and (35) as

(36)

And therefore

(37)

The approximations made to derive Eq. (37) (and Eq. (33)) can be justified using Table 2.

1. can be shown simply in from Table 2. Note that we have used a well-known relationship between and the osmotic second virial coefficient , ,38,45 to obtain . Combining this with (b) leads to .
2. can be justified through an order-of-magnitude analysis. According to the KB theory, holds true, ignoring the negligibly small contribution from the isothermal compressibility; hence can be obtained from the partial molar volume of solutes in pure water . Yet the experimental values for do not exist for naringin and naringenin to the best of our knowledge, likely due to their low aqueous solubilities. However, it is well-established observation that is in a similar order-of-magnitude to its crystal volume.56 Table 2 indeed shows that the crystal volumes are much smaller in magnitude than .
3. can be justified by the help of (a) and , which can be justified through a molecular crowding argument that CD-CD co-volume, which dominates , is much larger in magnitude than CD-water covolume, which dominates .23,24,30 Indeed, the available data supports this argument: dm3 mol-1, for -CD, which is much smaller than 12.6 dm3 mol-1.57

Based upon the above analysis, now we are in a position to examine changes in phase-solubility non-linearity due to solute or CD structural alterations. The key is the competition between and in Eq. (33), in which the latter is expected to be dominated by the solute-induced CD-CD interaction change (Appendix E);31 the former originates from the solute-CD interaction, the latter, when negative in value, signifies a case where the CD-CD interaction () is more attractive than the solute-induced CD-CD interaction (); such a case with will be referred to as the solute-induced weakening of CD-CD interaction.

**Naringin in β-CD and in HP-β-CD.**2,55Both CD derivatives’ solubility enhancement profiles exhibit negative deviation from linearity (), as shown in Figure 3 and Table 3; the deviation is larger in HP-β-CD compared to β-CD, which can be attributed to the stronger naringin-induced weakening of the interaction between HP-β-CDs, i.e., larger negative ) compared to that of β-CD, even though the interaction of naringin is stronger with HP-β-CD. The addition of hydroxypropyl groups, while strengthening the solute-CD interaction, enhances the solute-induced weakening of CD-CD interaction, leading to similar behaviour but enhanced solubility due to HP-β-CD’s higher solubility in water.

**Naringenin in β-CD (AL/P) *versus* in HP-β-CD (AP).** Naringenin-HP-β-CD interaction is stronger than that of naringenin-β-CD, as indicated by the magnitude of in Tables 1 and 3. (Understanding the detailed molecular basis of this requires simulation; yet the stronger interaction of HP-β-CD suggests that complexation is not driven by the hydrophobic effect alone; polar groups contributes significantly to CD-solute interaction). The positive deviation from linearity, albeit small, is still larger for HP- β-CD than for β-CD. This can again be understood in terms of the balance between solute-CD interaction and the solute-induced weakening of CD-CD interaction. Consistent with the previous case, the addition of hydroxypropyl groups increase the solute-induced weakening of CD-CD interaction, yet this increase is not great enough compared to the increase of solute-CD interaction contributions, thereby leading to a positive deviation for HP- β-CD.

**Naringenin *versus* Naringin.** An inspection of Tables 1 and 3 reveals a relatively simple picture. Naringenin interacts more strongly with both β-CD and HP-β-CD (as indicated by a larger positive or , suggesting that the presence of the disaccharide group in naringin weakens solute-CD interaction probably through steric hindrance. (This would also explain why β-CD to HP-β-CD increase of is less pronounced for naringin).

Consistent with the binding strength, naringenin induces the weakening of both β-CD to β-CD and HP-β-CD to HP-β-CD interactions more significantly than naringin, as indicated by larger negative . However, this naringenin-induced weakening of CD-CD interaction cannot overcome the CD-drug interaction contribution , whereas in naringin it can. It is this distinction which leads to the differences in phase-solubility behaviour. This may be attributed to the much smaller molecular size of naringenin compared to naringin (Figure 2), which is less effective for perturbing the CD-CD interaction.

We have shown that the molecular mechanism behind non-linear solubility enhancement can be attributed to the interplay between solute-CD interaction and solute-induced weakening of CD-CD interaction. The phase solubility classification (Fig. 1), employed widely in the literature,of an increase of solubility with CD concentration, in agreement with many experimental data,8–17 has now been given a molecular interpretation for the first time. The challenge now would be to quantitatively rationalise the competing factors, and , at a molecular structural basis. The values of , calculated from experimental data,2,55 are indeed helpful and lead to the description above, yet any interpretation beyond this requires extensive computer simulation studies.

**4. Conclusion**

Cyclodextrin (CD), through inclusion complexation, can improve the aqueous solubility of drugs.1–5 Phase solubility diagrams, determined extensively for drug-CD systems, indicate that formation of drug-CD inclusion complexes is not the only driving force of solubilisation.15,46 This has been evidenced by the non-linear dependence of drug solubility on CD concentration, which necessitates a rigorous statistical thermodynamic theory beyond the phenomenological Higuchi-Connors model.11–14

The molecular-based origin of such non-complexation contributions has been clarified in this paper through a rigorous statistical thermodynamic framework, based upon the rigorous Kirkwood-Buff and McMillan-Mayer theories of solutions.41,44,45,53 The leading contribution to the deviation from linear phase solubility (i.e., the term proportional to ) has been given a microscopic interpretation for the first time.

With the help of published thermodynamic and phase solubility data, we have identified the origin of the difference between the positive and negative deviation from linearity (AP and AN in Fig 1): the competition between drug-CD interaction and the drug-induced weakening of CD-CD interaction. AP behaviour is due to the overriding drug-CD interaction, whereas AN results from the overriding drug-induced weakening of CD-CD interaction. Our rigorous statistical thermodynamic approach thus led to a clarification of the factors determining the non-linearity of phase solubility, and the first guideline for further studies, which should concentrate on drug-CD, drug-CD-CD, and CD-CD interactions in water.

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**Appendix A: Kirkwood-Buff integrals, radial distribution functions, and the potentials of mean force interactions**

The origin of nonlinearity in phase solubility has been attributed, by our theory, to the KB integrals. Here we emphasise (using well-established statistical thermodynamic results44,45,53) that such KB integrals, namely and defined by Eqs. (5) and (6), can be linked directly to the two-body correlation functions (CF) and the orientationally-averaged radial distribution functions (RDF) as well as to the potentials of mean force (PMF) interactions between the species. Therefore, unlike activity coefficient models which indeed are useful in modelling and predicting thermodynamic quantities,58 our theory does not employ any model parameters to characterise interactions between species; we employ CFs and PMFs instead, that can be accessible directly by molecular dynamics simulations27,34,35. X-ray and neutron scattering can also yield information on RDFs.27 Hence our theory does not claim to be a predictable model but has a direct, rigorous link to the structure of solutions.

Let be the two-body distribution function between the species and located respectively at and . The corresponding two body CF is defined as

(A1)

which is linked directly to the potential of mean force between the species and as

(A2)

is the free-energy change required to bring molecule 1 of species *i* and molecule 2 of species *j* from their infinite separation to the positions and . It is not the direct interaction between the two molecules, but is determined under the influence of all the other molecules in the system. In this sense, it is the effective interaction mediated by the molecules in the solution.

Now we link CF to KB integrals. Firstly, it is easy to show that44,45,53

(A3)

Combining Eqs. (E1), (E3) and (5), we obtain

(A4)

Thus we have demonstrated that the KB integral has a direct link to the solution structure characterised by , as well as to the PMF interaction. In the inhomogeneous solution, in which a solute (drug) molecule is fixed at the origin, Eq. (E4) can straightforwardly be generalised to yield

(A5)

which again links the KB integral to the solution structure in the presence of the solute (drug) characterised by , and to the PMF.

Note the RDF, which is used frequently in molecular simulations, is the orientationally-averaged CF, which depends only on . RDFs are generally oscillatory with the periodicity of the molecular diameter of water, which possess narrow repulsive regions at short . For such RDFs, Eqs. (A2) and (A4) can be simplified as

(A5)

(A6)

which also link the KB integrals to the solution structure and PMF interactions.

**Appendix B: Equivalence between our theory and our previous paper.**

Here we show that our present result (Eq. (21)) is equivalent to a result in our previous paper (Eq. (39) of Ref 30). To do so, using the symmetry of indexes 1 and 2, Eq. (39) of Ref 30 is cast into the following form through the use of , defined in Eq. (22):

(B1)

To derive Eq. (B1) from Eq. (21), we exploit the following thermodynamic relationships which can easily be derived from the definition of :

(B2)

(B3)

Substituting Eqs. (10) and (21) into Eq. (B3) yields Eq. (B1).

**Appendix C: Rederivation of Eq. (31) via the McMillan-Mayer theory**

Here we derive Eq. (31) via the MM theory. The goal is to expand , defined below, in terms of the fugacity and concentration of CD.

(C1)

where the quantities with a subscript refer to those with solute fixed at the origin and the quantities with the subscript denote those without solute; note that when the solute is present, it is treated as a source of external field. To this end, let us start from the definition of grand canonical partition function of the three two component system42–45,53

(C2)

where *Q* is the canonical partition function; is the fugacity of the species *i* defined as:44,45,53

(C3)

Using Eq. (C2), the denominator and numerator of Eq. (C1) can be rewritten into the following manner: 41,43,45,53

(C4)

(C5)

where the cluster integrals and are defined respectively as

(C6)

(C7)

Eq. (C1), with the help of Eqs. (C4) and (C5), can be expanded into the series of as

(C8)

Using the definition of the KB integrals, Eqs. (D14)-(D16), Eq. (C8) can now be expressed as

(C9)

which can be shown through Eq. (D8) to be equivalent to Eq. (31) derived via the KB theory, since reduces to at the limit.

The variable can also be converted to via the MM theory (Eq. (D8)) to yield:

(C10)

Note that the -expansion from the MM theory (Eq. (C10)) is different from the KB theory (Eq. (29)) in two ways: (1) the lack of CD-water and water-water terms; and (2) the relationship between and are different between the VT ensemble (MM; Eq. (D8)) and the NPT ensemble (KB; Eq. (28)).

**Appendix D: Useful formulae of the McMillan-Mayer theory**

**Virial expansion.** The many-body interactions amongst the CD molecules show up most naturally in terms of the virial expansion of the osmotic pressures.41,44,45,53 Using grand canonical ensembles, osmotic pressures of the CDs in the bulk phase () and in inhomogeneous solution of aqueous CDs () can be expressed as:41,44,45,53

(D1)

(D2)

Combining Eqs. (C1), (D1) and (D2), we obtain the following fundamental expression for solubilisation:

(D3)

**Inversion of fugacity expansion.** Let us first note the following, which can be derived straightforwardly from Eq. (D2):

(D4)

where . Combining Eqs. (C4) and (D4), we obtain:41,44,45,53

(D5)

Using Eq. (D5), we now express in terms of . To do so, let us first assume the following:

(D6)

Substituting Eq. (D6) into Eq, (D5) yields

(D7)

Comparing r.h.s and l.h.s., , . Hence we obtain the following: 41,44,45,53

(D8)

**Connection to Kirkwood-Buff integrals.** Let us first differentiate Eq. (D5) with respect to :41,44,45,53

(D9)

Subtracting Eq. (D5) from Eq. (D9) yields:

(D10)

Combining Eq. (D10) with the first term of Eq. (D8), we obtain

(D11)

We can derive the following in a similar manner

(D12)

(D13)

Unless otherwise noted, all the ensemble averages are to be evaluated at .

Combining Eqs. (D11)-(D13) with the definitions of the KB integrals, we obtain the following relationship between s and the KB integrals:

(D14)

(D15)

(D16)

Note here that all the KB integrals are defined at infinite CD concentration, i.e., .

**Appendix E:** **On the solute-induced change of CD-CD interaction**

**Justification that .** Using experimental data, we can show that defined in Eq. (22) is indeed dominated by the contribution from for AP and AN phase solubility types. To do so, let us remember that in our theory, solubilisation was divided into two sub-steps (MM and the rest); each ensemble is expressed in two different, but equivalent (denoted by ) representations using the two sets of variables: and . Under this setup, the solubilisation process can be expressed as a change from (I) to (III) via (II):

1. Pure water: .
2. “Intermediate” aqueous CD solution: , where , where is the osmotic pressure.
3. Aqueous CD solution of experimental condition: Constant ensemble .

where the unprimed quantities correspond explicitly to experimental conditions, and temperature is kept constant throughout. The first step, (I) (II), corresponds to . The second step, (II) (III), corresponds to . To ensure this correspondence, in (II) is determined under the condition that

Using Eqs. (29) and (C10),

(E1)

in which we have ignored the negligibly small contribution coming from the isothermal compressibility , . Our aim here is to show that , i.e., the difference between the two is negligible in comparison to .

Let us first note that is intensive; this means that cannot be the function of extensive thermodynamic quantities. Hence and , where and are the mole fractions of the species 2. Consequently,

(E2)

Eq. (E2) can be decomposed into the following steps:

(E3)

**The first term of Eq. (E3)** can be approximated as

(E4)

where the final step involves a well-known KB relationship, , by ignoring a negligible term involving isothermal compressibility. Using Table 2, the magnitude is estimated to be 4-7 J mol-1 since atm and dm3 mol-1, which is negligible compared to . The term should be smaller than the above, and is expected to be negligible.

**The second term of Eq. (E3)**, let us start from

(E5)

In evaluating , we have to bear in mind that , since and are fixed in the partial differentiation in Eq. (E5); here, is really . Eq. (E5) can therefore be rewritten using the chain rule into the following form:

(E6)

Since is small, we use throughout in the following discussion. The first two factors of Eq. (E6) can be evaluated by the well-known KB relationships44,45

(E7)

(E8)

To evaluate the third factor of Eq. (E6), let and be the density of water respectively in systems (III) and (II);

(E9)

Using system (I) as the reference state, whose density is , Eq. (E9) can be rewritten as

(E10)

Since the first and the second terms correspond respectively to constant and constant processes

(E11)

The first term of Eq. (E11) can be evaluated by noting that is the function of

(E12)

in which we have used from the Gibbs-Duhem equation, the KB integral . The second can be simplified through ; 44,45 taking all together, we obtain the following leading term

(E13)

In combination with Eqs. (E7) and (E8), Eq. (E5) makes a negligible contribution because of the negligibility of .

We have thus shown that , namely the process IIIII makes a negligible contribution; this also means that . In Eq. (E1), can be estimated for naringin in -CD (exhibiting AN non-linearity) to be dm6 mol-2, much smaller than dm6 mol-2 (Table 3); considering that for HP--CD is larger yet in the same order of magnitude to that of -CD, still holds true also for AN and Ap types in HP--CD. Consequently, is much smaller than , thereby leading to for AN and Ap.

**An intuitive interpretation on the role of on phase solubility nonlinearity.** We have shown above that makes a dominant contribution to . According to Eq. (D3), the sign of is determined by a competition between and : solute-induced lowering of the osmotic pressure () increases and decreases solubility, whereas solute-induced increase of the osmotic pressure () decreases and increases solubility. Note that the second virial coefficients in the presence and absence of the solute are and , respectively. This simple setup rationalises how solute-induced changes in CD-CD interaction occur in non-linear behaviour. For example, AN is caused when , which contributes to the increase of and the decrease of solubility. From the relationship between the virial coefficients and the KB integrals, this takes place when , i.e., when the solute-induces weakening of CD-CD interaction. (Note that comes into term when considering solubility, i.e., .)

**Table 1:** CD concentration dependence of solubilisation; fitting parameters for Eq. (35). See Figure 3 for a comparison between experimental data and the fitting curves. Data used was obtained from the literature.55

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Solute | CD | *a* / dm3 mol-1 | *b* / dm6 mol-2 | R2 |
| Naringenin | β-CD | 1143 | 202.2 | 0.9965 |
| Naringenin | HP-β-CD | 2938 | 47230 | 0.9969 |
| Naringin | β-CD | 518.2 | -20240 | 0.9996 |
| Naringin | HP-β-CD | 632.4 | -23510 | 0.9978 |

**Table 2:** Comparative properties and KB integrals calculated from the published experimental data.55 B2 values for β-CD and HP-β-CD were taken from the literature.2 The values of in Table 1 are shown here again for comparison.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Solute | CD | / dm3 mol-1 | Crystal volume/dm3 mol-1 | / dm3 mol- 1 |
| Naringenin | β-CD | 1143 | 0.1832 | 12.6 |
| Naringenin | HP-β-CD | 2938 | 0.1832 | 2.83 |
| Naringin | β-CD | 518.2 | 0.3497 | 12.6 |
| Naringin | HP-β-CD | 632.4 | 0.3497 | 2.83 |

**Table 3:** Results for second order deviations calculated from published experimental data.55 See Eqs. (35)-(37). The values of in Table 1 are shown here again for comparison.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Solute | CD | /dm6 mol-2 | / dm6 mol-2 | a  / dm6 mol-2 | Type |
| Naringenin | β-CD | 2.0 | 1.3106 | -1.3 | AL/P |
| Naringenin | HP-β-CD | 4.7 | 8.6106 | -8.5 | AP |
| Naringin | β-CD | -2.0 | 2.7105 | -3.1 | AN |
| Naringin | HP-β-CD | -2.3 | 4.0105 | -4.5 | AN |