

Drug/cyclodextrin: beyond inclusion complexation

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Received: 28 October 2009 / Accepted: 11 February 2010 / Published online: 27 February 2010
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Abstract Development of cyclodextrins as enabling excipients began decades ago and during this time a conventional view of the substrate–cyclodextrin interaction was formed that has persisted in spite of numerous contradicting observations. Here the shortcomings of the phase-solubility method are elucidated. The limited permeability of drug/cyclodextrin complexes through semi-permeable membrane, as well as osmometric data and images from transition electronic microscopy (TEM) are used as convincing evidences of aggregation of cyclodextrins and their complexes. The necessity of updating the existing notions of cyclodextrin complexation is discussed.

Keywords Cyclodextrins · Nanoparticles · Aggregates

Introduction

Compounds like cyclodextrins, crown ethers and calixarenes can, due to their ability to form inclusion complexes with biologically active agents, serve as enabling excipients for pharmaceutical, food, cosmetic and other applications. Of these three groups only cyclodextrins have been approved for usage in both pharmaceutical formulations and food products. Consequently cyclodextrins have received continuous and increasing interest from both academic and industrial scientists. Development of cyclodextrins as enabling excipients began over 60 years ago

and during this time a conventional view of the substrate–cyclodextrin interaction was formed that has persisted in spite of numerous contradicting observations. According to this conventional complexation theory:

- (1) dissolved cyclodextrin molecules only interact with drugs through formation of inclusion complexes whose substrate–cyclodextrin (i.e. drug–cyclodextrin; D_mCD_n) stoichiometry ($m:n$) is most frequently 1:1, 1:2 and 2:1;
- (2) the complex formation is an equilibrium process characterized by a stability constant—complexes are constantly being formed and dismantled at average rates that range between nanoseconds and seconds [1];
- (3) the value of the stability constant is concentration independent but sensitive to temperature changes;
- (4) the aqueous substrate–cyclodextrin complex solutions are ideal solutions, i.e. the complexes do not interact with each other or any other dissolved excipients;

This view of substrate–cyclodextrin complexes is based on the pioneering work by Higuchi and Connors which however developed their phase-solubility classification systems on investigations of non-inclusion complexes [2]. Based on their studies of how complexing agents can enhance aqueous solubility of poorly soluble drugs, they created a simple and convenient method allowing determination of stability constants from so called substrate–ligand phase-solubility diagrams. This technique is presently one of the most common methods of stability constant determination. According to Higuchi and Connors there can be five types of phase-solubility relationship (Fig. 1): A_L -type where there is a linear relationship between substrate (drug) solubility and ligand (cyclodextrin) concentration and which is interpreted as formation of

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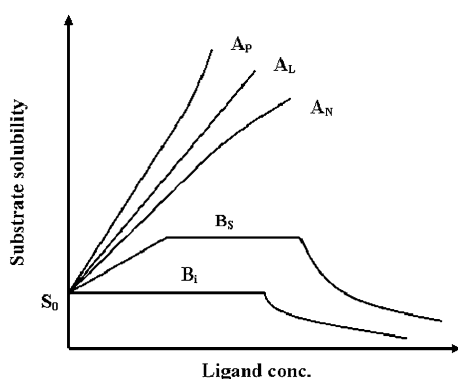


Fig. 1 Types of phase-solubility relationship according to Higuchi and Connors concept [2]

a complex with $m:1$ stoichiometry; A_P -type, which represents positive deviation from linearity which is described by a second-order polynomial function and which is usually interpreted as $m:n$ complex; A_N -type, which represents negative deviation from linearity with no reasonable explanation; and B_S - and B_I -types correspond to complexes possessing limited solubility, which is common in the case of the parent cyclodextrins.

It is worth mentioning that with few exceptions m and n do not simultaneously exceed 2, e.g. 2:2 drug–cyclodextrin complexes are rarely formed, and most often the stability constant value can be determined from A_L and A_P diagrams. For this purpose the following parameters are needed: slope of the plot, drug solubility values and the corresponding cyclodextrin concentrations. For 1:1 complexes the following equation applies:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (1)$$

Application of Eq. 1 is limited with important condition: $0 < \text{slope} < 1$ since $K_{m:n} > 0$. If this condition is not fulfilled and $1 < \text{slope} < 2$ then 2:1 stoichiometry is assumed and Eq. 2 is applied:

$$K_{2:1} = \frac{\text{Slope}}{S_0^2(2 - \text{Slope})} \quad (2)$$

In addition to the phase-solubility method, a series of methods have been assumed to give reliable values of stability constant. These include fluorometry [3], NMR [4], HPLC [5], calorimetry [6], drug degradation kinetics [7], phase distribution [8] and equilibrium dialysis [9]. Though with the methods mentioned different properties of solutions are monitored at diverse concentration ranges, they are expected to yield consistent data according to previously mentioned statements 3 and 4. The present communication is based on series of experimental facts that have lead to criticism of this conventional theoretical view of substrate–cyclodextrin complexes.

Phase-solubility discrepancies

Phase-solubility studies of NSAIDs with HP β CD often yield A_L -type phase-solubility diagrams with slope greater than unity, which according to the model of Higuchi and Connors indicates 2:1 complex formation. In particular, such results have been obtained by our team for ibuprofen and diflunisal—2-hydroxypropyl- β -cyclodextrin (HP β CD) complexes [10], and later for diclofenac and paracetamol—HP β CD complexes [11]. However, additional studies of complex stoichiometry by Job's plots from UV and NMR experiments showed that only 1:1 drug/HP β CD complexes were being formed. Besides, space filling docking studies of ibuprofen and diflunisal with HP β CD clearly demonstrated that there was no space left in the cyclodextrin cavity for another drug molecule (Fig. 2) [12]. Thus, the interpretation of complex stoichiometry based on phase-solubility diagrams is not as unambiguous as it is usually considered.

Another interesting observation is that frequently the experimental value of intrinsic drug solubility (S_0) demonstrates positive deviation from the value obtained by extrapolation of phase-solubility line (i.e. the intercept with the Y -axis). Moreover, the extrapolation value of S_0 can be negative which is obviously nonsense. It has been shown that such anomalous behavior is common among poorly soluble drugs with $S_0 < 0.1$ mM [13]. Such intrinsic solubility uncertainty can produce large fluctuations in the values of stability constant obtained from phase-solubility diagrams (see Eqs. 1 and 2).

As was previously mentioned, complex formation is thought to be both concentration and method independent, but comparison of stability constants available in the scientific literature gives numerous examples of that this assumption does not hold. Sometimes stability constant values obtained by different methods can differ by up to

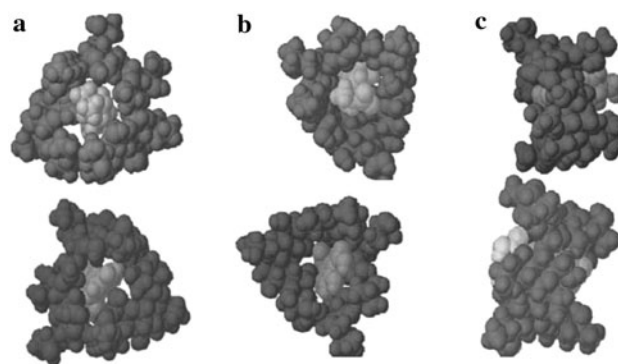


Fig. 2 Molecular modeling shows no possibility of 2:1 stoichiometry at ibuprofen/HP β CD (above) and diflunisal/HP β CD (below) complexation in spite of phase-solubility diagram slope exceeding unity (reprinted with permission from [12])

40 times as in case of ibuprofen/HP β CD with $K_{1:1}$ value ranging from near 100 M^{-1} obtained from phase-solubility diagrams [14] to 400 M^{-1} from fluorescence measurements [15] and up to 4000 M^{-1} from calorimetric determinations [6].

The listed examples clearly demonstrate deficiencies of the conventional view of drug/cyclodextrin relationships in aqueous solutions. The question is what can cause this anomalous behavior? What kind of interactions between solvent, complexing agent and substrate can motivate such discrepancies and which are not accounted for by the existing model. Recently it has been shown that parent cyclodextrins form aggregates in aqueous solutions and their existence can be detected by different analytical methods, such as viscosimetry, tensiometry, osmometry, light scattering, electronic microscopy [16–18]. In addition, hydrophobically modified cyclodextrins often behave as surfactants forming micelles and micelle-like structures [19, 20]. In this view, it is fair enough to suggest that inclusion of sufficiently hydrophobic guest to cyclodextrin cavity would impart surfactant properties to the cyclodextrin, which did not possess them alone. Such mechanism can be proposed for behavior of cyclosporine A in aqueous HP β CD solutions saturated with cholesterol [21]. It has been found that cholesterol not only does not compete with cyclosporine A for cyclodextrin cavity, but rather enhances HP β CD solubilization of the drug by 25%. A scheme of likely solubilizing mechanism is proposed in Fig. 3. Cyclosporine A has, in contrast to cholesterol, low affinity for the HP β CD cavity but can be solubilized through intrusion into lipophilic micelle core formed by cholesterol residues protruding from cyclodextrin cavity.

Permeation anomaly

Another strong argument supporting the aggregation hypothesis of drug/cyclodextrin complexes is their anomalous behavior during permeation through semi-permeable cellophane membrane. For example such behavior was observed when permeation of hydrocortisone was monitored from aqueous hydrocortisone/HP β CD system [22]. Since phase-solubility diagram of hydrocortisone in aqueous HP β CD solutions is of A_L -type, a linear relationship

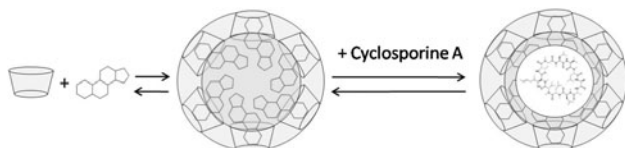


Fig. 3 A possible mechanism HP β CD solubilization of cyclosporine A in the presence of cholesterol

between drug flux and cyclodextrin concentration would be expected. Nevertheless, it has been found that at between 5 and 10% (w/v) cyclodextrin concentration (35–70 mM) the flux profile showed negative deviation from Fick's first law (Fig. 4), even when the membrane pore size was many times larger than the hydrocortisone/HP β CD complex diameter.

It was speculated that the observed negative deviation could possibly be due to increase in medium viscosity upon increasing HP β CD concentration but further investigations excluded such effect. Thus, the only reasonable explanation of limited permeability of hydrocortisone/HP β CD complexes is their aggregation to the sizes exceeding membrane pore diameter. Experiments with semi-permeable membranes with MWCO of 100,000 disclosed that aggregates can be composed over 50 hydrocortisone/HP β CD complexes [23]. Similar observations were made when permeation of hydrocortisone/HP γ CD and dexamethasone/HP γ CD complexes were studied.

Osmometry support

The osmotic phenomena are based directly on the number of completely solubilized particles rather than non-electrolyte molecules. Thus, analysis of osmolality concentration dependence may yield valuable qualitative information concerning aggregation processes in solution. Recently an attempt has been undertaken to evaluate aggregation of hydrocortisone/HP γ CD and hydrocortisone/ γ CD complexes as well as mixtures thereof [24]. Based on the results obtained it has been concluded that osmotic coefficient of solute tends to give negative deviation from its expected ideal trend, which could be interpreted as one more evidence of aggregate formation.

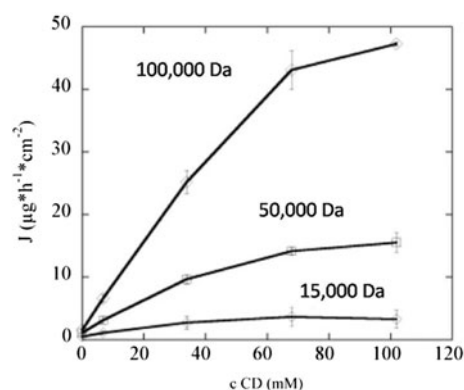


Fig. 4 Negative deviation from first Fick's law during hydrocortisone/HP β CD permeation through semi-permeable cellophane membrane with MWCO considerably exceeding conventional complex size

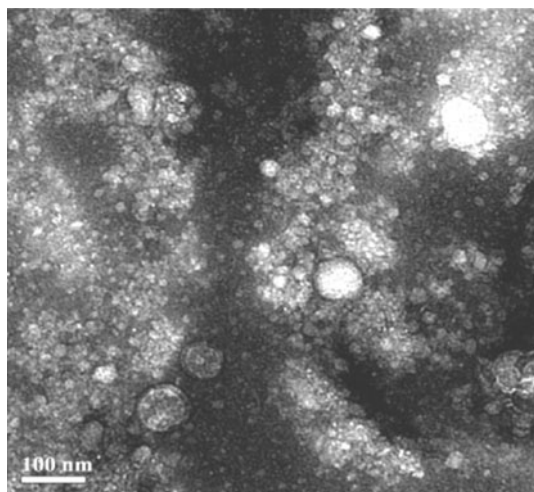


Fig. 5 TEM convincingly testifies the presence of nanoparticles in hydrocortisone/HP β CD aqueous solution [24]

TEM evidences

TEM is a convenient method for visual detection and analysis of nano- and micro-scale structures in aqueous solution. Although the samples are studied in solid (dried or frozen) state it should not impair the samples since the sample preparation is so fast that it preserves the sample from any structural deformations. As to cyclodextrin application TEM has been used to support the light scattering experimental results in the frame of patent cyclodextrins self-assembly studies [17, 25]. To further prove the hypothesis of drug/cyclodextrin complex aggregation Cryo-TEM has been used for characterization of 10% HP β CD and γ CD solutions, which have been saturated with hydrocortisone [24]. The Cryo-TEM images (Fig. 5) have supported formation of both cyclodextrin and drug/cyclodextrin aggregates. The observed particles represent diverse mixture of spheres with 10–80 nm diameter, which could correspond to aggregates containing up to several thousands complex units per particle.

Conclusion

Studies performed over the past few decades have resulted in a conventional model where cyclodextrins interact with substrates via formation of simple inclusion complexes. It is still assumed that inclusion complex formation is the main mechanism of cyclodextrin solubilization of poorly soluble substrates. In dilute aqueous solutions the substrate–cyclodextrin stoichiometry can be described by relatively simple mathematical models. However, in most recent years numerous experimental and computational evidences have shown that interactions

between cyclodextrin molecules and substrate molecules are not restricted to “host–guest” interactions and that more complex interactions like non-inclusion complexation or nanostructures formation do exist in aqueous cyclodextrin solutions. These non-conventional events have been supported by numerous experimental results obtained by a wide variety of different experimental techniques, such as permeation studies, osmometry and transmission electronic microscopy. Thus, the necessity to update the existing notions in cyclodextrin field is vital as it opens new horizons in understanding their essence and further application.

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