A Practical Integrated Approach to Supramolecular Chemistry. I. Equilibria in Inclusion Phenomena

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> Today chemistry is the science of molecules and their transformations. Over a period of a few hundred years art changed into science (with a convenient mythology to obscure how much art there still is in it), and instead of substances, chemists think of molecules.
>
> *Roald Hoffmann*

Supramolecular chemistry has been defined as "chemistry beyond the molecule", bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces (*1*). Molecular associations have been recognized and studied for many years, and the term *Übermoleküle* (i.e., supermolecules) was coined to describe entities of higher organization that arise from the association of coordinatively saturated species. The partners involved in a supramolecular species have received the names molecular receptor and substrate, the latter usually being the smaller component whose binding is being sought. While endoreceptors bind substrates in molecular cavities, exoreceptors rely on interactions between the surfaces of the receptor and the substrate. If the guest is situated within the cavity, the adducts are termed *inclusion compounds* (*2*, *3*). Conversely, if the guest molecule lies outside the cavity, the compounds are referred to as *association compounds.*

Despite the present importance of these phenomena, which have born fruit in the appearance of specialized journals such as *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry*, they receive very little attention in current texts on physical chemistry (*4*–*7*). An experiment for studying inclusion complexes by fluorescence has been proposed recently (*8*).

Cyclodextrins occupy an important place in the field of inclusion phenomena (*9*). They are cyclic oligosaccharides obtained from starch by enzymatic degradation. First isolated in 1891 as degradation products of starch (*10*), they were characterized as cyclic oligosaccharides in 1904 (*11*).

The most common α-, β-, and γ-cyclodextrins, or in more significant nomenclature, cyclohexa-, -hepta-, and -octaamyloses, are formed by six, seven, and eight units of D-glucose, respectively, bound by α-1,4 bonds. A cyclodextrin molecule resembles a hollow truncated cone (torus) and has approximate C_n symmetry. Figure 1 shows the structure of α-cyclodextrin and its functional structural scheme. The primary hydroxyl groups are located on the narrower side of the torus and the secondary hydroxyl groups on the broader side. The number of glucose units determines the dimension and size of the cavity, which is lined by hydrogen atoms and glycosyl oxygen bridges.

The peculiar arrangement of the functional groups of cyclodextrins means that their inside is lipophilic while their outside is hydrophilic. This allows them to harbor nonpolar organic molecules and their hydrophilic exterior affords them water solubility. Accordingly, one of their main applications is the formation of inclusion complexes. Among such applications, the possibility of using cyclodextrin dimers to strongly bind cholesterol (*12*) in order to remove cholesterol from dairy products is outstanding. They could also be used as the basis for a cholesterol detection system or in a pharmaceutical approach to lowering cholesterol levels in blood (*13*). Cyclodextrins have also proved useful in flavor technology (*14*), as mucosal absorption promoters (*15*), etc.

Within the framework of a project designed to develop new practical work in physical chemistry (*16*, *17*), in this article we describe an experiment aimed at familiarizing undergraduate students with the study of inclusion phenomena.

Equilibria in Inclusion Phenomena

In this experiment we propose a spectrophotometric study of the inclusion phenomenon of the azo-dye mordant yellow 7 (MY7), 3-methyl-5-(4-sulfophenylazo)salicylic acid, disodium salt, in α-cyclodextrin. MY7 was chosen as the inclusion species for two reasons: (i) its visible spectrum is altered to a considerable extent when it binds cyclodextrin; (ii) the methylsalicylate group of this molecule is too large to penetrate the cavity of α-cyclodextrin (*18*). As a result, only the phenyl ring of the sulfonate group can enter the cavity, simplifying the interpretation of the results considerably.

If S is used to designate the guest molecule and C is used to refer to α-cyclodextrin, the formation equilibrium of the

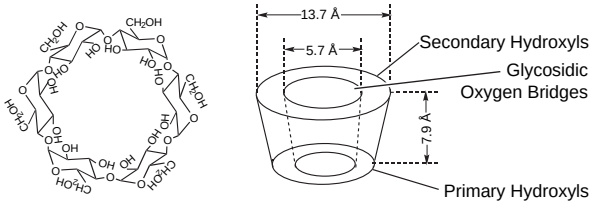

Figure 1. Structure and functional structural scheme of α-cyclodextrin.

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inclusion complex, CS, can be written thus:

$$S + C \rightleftharpoons CS$$

The stability of the complex can be described in terms of its formation (K_F) or dissociation ($K_D = 1/K_F$) constants:

$$K_F = [CS]/[S][C]$$

Figure 2 shows the absorption spectrum illustrating the evolution of the substrate when the concentration of α-cyclodextrin increases. Two isosbestic points can be observed (isosbestic: from the Greek *iso-*, "equal", and *sbestos*, "quenched"; i.e., equal extinction; think of "asbestos" = unquenchable, inextinguishable) at λ = 369 and 493 nm, indicating the formation of a complex with 1:1 stoichiometry (*18*). As expected, a bathochromic effect (bathochromic: from the Greek *bathos-*, "depth", and *chroma*, "color"; color-deepening; i.e., moving an absorption band to a region of longer wavelength) is also seen.

Data Analysis

If $[C]_o$ and $[S]_o$ are the initial cyclodextrin and substrate concentrations, respectively, x is the fraction converted into complex, and we work in such conditions that $[C]_o[S]_o >> x^2$ and $[C]_o >> [S]_o$, the dissociation constant of the complex can be calculated according to the Benesi–Hildebrand method (*19*, see also *8*):

$$\frac{[C]_o[S]_o}{\Delta A} = \frac{K_D}{\Delta\varepsilon} + \frac{[C]_o}{\Delta\varepsilon}$$

where the measured absorbance, A, is $A = A_{CS} + A_C + A_S$, such that $\Delta A = A - \varepsilon_C[C]_o - \varepsilon_S[S]_o = (\varepsilon_{CS} - \varepsilon_C - \varepsilon_S)x = \Delta\varepsilon x$.

Plotting the values of $[C]_o[S]_o/\Delta A$ against those of $[C]_o$ should afford a straight line. The quotient between the values of the intercept and the slope is the value of K_D. Table 1 shows the results obtained at five different wavelengths.

HINT: We propose working at five wavelengths to call students' attention to the variation in the molar absorption coefficient ε with the working wavelength. In particular, it seems appropriate to induce them to reflect on the suitability of measuring absorbance in the $0.20 \leq A \leq 0.70$ range for the following reason. If one starts out with the Beer–Lambert law

$$A = \ln(1/T) = \varepsilon l c$$

A being absorbance, T transmission, ε the molar absorption coefficient, l the optical path length, and c the concentration of the sample, one has that

$$dc/c = dT/(T \ln T)$$

If one accepts that the absolute error dT committed in the measurement of T is practically independent of the value of T, it is easy to show that the relative error affecting the measurement of concentration, dc/c (or, what amounts to the same, of $dT/[T \ln T]$) corresponds to $T = e^{-1}$; i.e., $A = 0.43$. Fortunately, since the relative error dc/c varies slowly with T in the proximity of the minimum, it makes sense to perform the absorbance measurements in the $0.20 \leq A \leq 0.70$ range (see Table 1).

Figure 3 shows the excellent fit to the Benesi–Hildebrand equation. Thus, it is seen that $K_D = (3.7 \pm 0.4) \times 10^{-4}$ M ($K_F = 2700 \pm 300$ M^{-1}), the mean of the values depicted in Table 1, in good agreement with the value reported in the literature ($K_F = 2800 \pm 300$ M^{-1} [*20*]).

Table 1. Dissociation Constants for Complexation of α-Cyclodextrin with the Azo Guest (Mordant Yellow 7)

λ / nm	Absorbance Range	$10^4 \times K_D$ / M
393	0.2813–0.3738	3.3 ± 0.3
383	0.3595–0.4322	3.8 ± 0.4
373	0.4206–0.4461	5.0 ± 1.7
363	0.4047–0.4457	3.8 ± 0.6
353	0.3396–0.4193	2.6 ± 0.2

NOTE: Values obtained from the Benesi–Hildebrand plot. Solvent, phosphate buffer; pH = 11.0; T = 298 K; $[S]_o = 3.338 \times 10^{-5}$ M; $[C]_o = 0$, 2.918×10^{-4}, 4.176×10^{-4}, 5.836×10^{-4}, 1.167×10^{-3}, 1.670×10^{-3}, 2.334×10^{-3}, and 3.341×10^{-3} M.

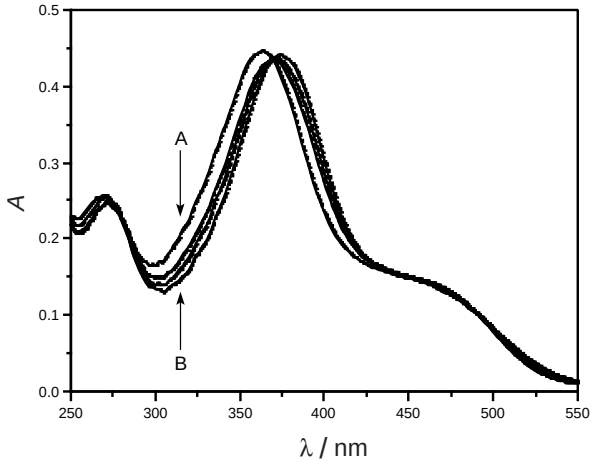

Figure 2. Absorption spectra for the guest mordant yellow 7 binding to α-cyclodextrin. Solvent, phosphate buffer; pH = 11.0; T = 298 K; $[S]_o = 3.338 \times 10^{-5}$ M; $[C]_o = 0$, 2.918×10^{-4}, 8.352×10^{-4}, 3.341×10^{-3}, and 6.682×10^{-3} M, read from A to B.

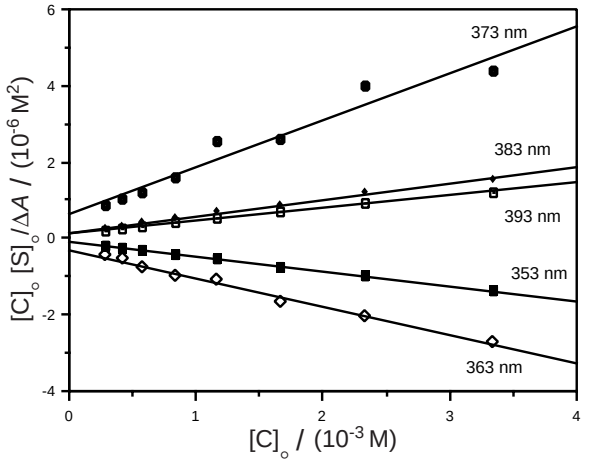

Figure 3. Determination of the equilibrium constant of the mordant yellow 7-α-cyclodextrin complex according to the Benesi–Hildebrand equation at different wavelengths.

Safety

Cyclodextrins are chemically stable and all toxicity tests have shown that orally administered cyclodextrin is harmless. According to reports of the FAO, enzymatically modified starch (this includes cyclodextrins) is also toxicologically harmless (*21*). Nor has any toxicity been reported for MY7 (*22*).

Conclusion

The experiment described here shows that simple absorption measurements provide a reliable way to detect the inclusion of an azo-dye with α-cyclodextrin and to measure the equilibrium constant of the formation of the corresponding inclusion complex.

Hardware and Chemicals List

1. A scanning double beam UV-Vis spectrophotometer with spectral subtraction capabilities and a temperature controlled cell holder. In the experiment described here, a Shimadzu UV-Vis 2101PC double beam spectrophotometer was used.
2. Thermostatted water bath for pre-equilibration of sample solution.
3. α-Cyclodextrin and MY7 were obtained from Fluka and Aldrich, respectively.

Acknowledgments

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