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ORIGINAL ARTICLE

Development of a competitive continuous variation plot for the determination of inclusion compounds stoichiometry

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Abstract The spectral displacement method in presence of methyl orange has been coupled to the continuous variation plot to allow the determination of the stoichiometries for inclusion compounds. Such a ''competitive continuous variation plot'' (CCV plot) is especially useful for the study of substrates which are not directly observable by spectroscopy or which are too poorly soluble to give observable signals. Moreover, when a mixture of complexes is observed, the position of the maximum in the competitive continuous variation plot gives information on the relative affinities of the complexes, which is not the case in the classical Job plot which also depends of the intrinsic spectral characteristics of each complex. The method is not restricted to β-cyclodextrin inclusion compounds, and it may be applied to any complexes if an appropriate competitive system is available.

Keywords β-Cyclodextrin · Continuous variation plot · Inclusion compounds · Methyl orange · Spectral displacement method

Abbreviations

CCV	Competitive Continuous Variation
βCD	β-Cyclodextrin
CD	Cyclodextrin
MO	Methyl orange
S	Substrate
SBE1	Sulfobutylated β-cyclodextrin, mean degree equal to 1
SBE7	Sulfobutylated β-cyclodextrin, mean degree equal to 7

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Introduction

The continuous variation plot is a well-known and useful tool for the determination of stoichiometries for any kind of complexes, and especially for cyclodextrin (CD) inclusion compounds [1–4]. This method is often applied with various analytical techniques, such as ^{1}H NMR, UV–vis spectroscopies or circular dichroism. In each case, the Job plot is based on the spectral change observed either for the host or the guest.

As an alternative to such ''direct'' observation, we developed the concept of a continuous variation plot based this time on the observation of a competitive guest. This ''competitive continuous variation plot'' (CCV plot) may be considered as the association of the classical Job plot with a spectral displacement method. Since the concentrations used may be lower than for classical Job plots, this competitive continuous variation plot could be a method of choice for substrates which could not be studied by usual methods. In our study, methyl orange (MO), which is probably one of the most used UV–vis probe for cyclodextrin complexes, has been employed as competitive guest. Thus, we present in this work the development of this competitive method and its application to β-cyclodextrin complexes. The advantages and limitations of such a method are also discussed.

Materials and methods

Chemicals

β-Cyclodextrin, adamantan-1-ol, methyl orange, sodium hydroxide and potassium dihydrogenophosphate (Aldrich) were all of analytical reagent grade and were used as received. Deionised water was used throughout this work. Sulfobutylated β-cyclodextrins with a mean degree of 1 and 7 (SBE1 and SBE7) were synthesised as described in literature [5].

Visible spectra

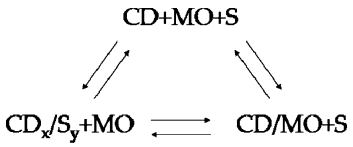
Spectra were recorded using a Perkin Elmer Lambda 2S double beam spectrometer and a quartz cell with optical path length of 1.00 cm at 293 K. All compounds were dissolved in phosphate buffer at pH 5.8. The control of temperature is realised by the use of a thermostated bath linked to the cell holder (accuracy: ±0.1 °C). Spectra were recorded between 350 and 650 nm, and the treatments were applied to the first derivatives of UV spectra in order to avoid any spectral influence of diffraction phenomena [6, 7]. In all experiments, the methyl orange concentration was fixed at 0.02 mM. In the followings, MO stands for methyl orange, CD for cyclodextrin and S for the studied substrate.

Titration experiments

The stability of the complexes formed between MO and the macrocyclic host is first obtained by the use of the well-known titration method [6]. CD concentrations were varied from 1 to 0.05 mM, MO concentration remaining constant. A dedicated algorithmic treatment has been applied for the determination of the formation constants for CD/MO complexes. Titrations were realised for the three studied cyclodextrins (genuine β-cyclodextrin βCD, SBE1, SBE7).

Spectral displacement experiments

Once the stability constants is known, the competition method may be applied [6, 8]. Such a method is based on the perturbation of the CD + MO equilibrium by the addition of a substrate S:



During the classical competition experiments, three spectrophotometric spectra need to be recorded, i.e. for MO, MO + CD and MO + CD + S solutions. The difference in absorbance between the MO + CD and MO + CD + S solutions is directly depending of the CD_x/S_y stabilities, which thus may be quantified. During the CCV plot experiments, more spectra are needed, and the absorbance of the aqueous MO solution is recorded in presence of varying amount of CD and S. As in classical Job plots, the total concentration of CD and S ($[CD]_T + [S]_T$) is remaining constant, while the concentration of each of these two species vary from 0 to a defined maximal concentration (1 mM in our experiments). Thus, the R ratio may be defined, by dividing the cyclodextrin total concentration by $[CD]_T + [S]_T$:

$$R = \frac{[CD]_T}{[CD]_T + [S]_T}$$

R is varying from 0 to 1. The treatment of each MO + CD + S spectra in order to obtain the CCV plot is developed in the following results and discussion part.

Results and discussion

Description of the method

When the spectra of a MO + CD + S solution is recorded, the CD concentration which is complexed with S may be calculated ($[CD]_{complexed}$). Indeed, the difference between the MO + CD and MO + CD + S solutions is caused by the fact that the CD concentration which is complexed with S is not available anymore to MO. Since the stability of the CD/MO inclusion compound is known, it is possible to estimate the CD concentration available to MO in presence of S, and thus the concentration of CD which is complexed with S. In the followings, k represents the formation constant of the CD/MO complex. ε_{MO} and $\varepsilon_{CD/MO}$ are the molecular absorptivity of MO and CD/MO, respectively. 1 is the length of the cell used for the spectroscopic experiments. A_{MO}, $A_{CD/MO}$ and $A_{CD+MO+S}$ are respectively the absorbance of the MO solution, of the pure CD/MO complex, and of the CD + MO + S solution:

$$A_{MO} = \varepsilon_{MO} * [MO]_T * 1 \quad (1)$$

$$A_{CD/MO} = \varepsilon_{CD/MO} * [MO]_T * 1 \quad (2)$$

$$A_{CD+MO+S} = \varepsilon_{MO} * [MO] * 1 + \varepsilon_{CD-MO} * [CD/MO] * 1 \quad (3)$$

With l being equal to 1, we may write the difference between the MO + CD + S and MO solutions as:

$$\begin{aligned} A_{CD+MO+S} - A_{MO} &= \varepsilon_{MO}*([MO] - [MO]_T) \\ &\quad + \varepsilon_{CD/MO}*[CD/MO] \\ &= -\varepsilon_{MO}*[CD/MO] + \varepsilon_{CD/MO}*[CD/MO] \\ &= [CD/MO]*(\varepsilon_{CD/MO} - \varepsilon_{MO}) \end{aligned} \quad (4)$$

Thus, the concentration of the complex formed between MO and CD may be expressed as:

$$[CD/MO] = \frac{A_{CD+MO+S} - A_{MO}}{\varepsilon_{CD/MO} - \varepsilon_{MO}} \quad (5)$$

In addition, the difference between the MO + CD and MO + CD + S is estimated by the following relation:

$$\begin{aligned} A_{CD/MO} - A_{CD+MO+S} &= -\varepsilon_{MO}*[MO] \\ &\quad + \varepsilon_{CD/MO}*([MO]_T - [CD/MO]) \\ &= -\varepsilon_{MO}*[MO] + \varepsilon_{CD/MO}*[MO] \\ &= [MO]*(\varepsilon_{CD/MO} - \varepsilon_{MO}) \end{aligned} \quad (6)$$

Thus, it is possible to calculate the free MO concentration:

$$[MO] = \frac{A_{CD/MO} - A_{CD+MO+S}}{\varepsilon_{CD/MO} - \varepsilon_{MO}} \quad (7)$$

As a consequence, the concentration of free CD may be obtained from the definition of the CD/MO stability constant:

$$\begin{aligned} [CD] &= \frac{[CD/MO]}{k^*[MO]} \\ &= \frac{((A_{CD+MO+S} - A_{MO})/(\varepsilon_{CD/MO} - \varepsilon_{MO}))}{k*((A_{CD/MO} - A_{CD+MO+S})/(\varepsilon_{CD/MO} - \varepsilon_{MO}))} \end{aligned} \quad (8)$$

And finally, it is possible to calculate the concentration of CD which is complexed with S:

$$[CD]_{COMPLEXED} = [CD] - \frac{A_{CD+MO+S} - A_{MO}}{k^*(A_{CD/MO} - A_{CD+MO+S})} \quad (9)$$

$[CD]_{COMPLEXED}$ is then estimated for various R values, with a constant value for $[CD]_T + [S]_T$. The plot of such concentration in function of the R ratio (from 0 to 1) may then be considered as a Job plot, the position of the maximum indicating the stoichiometry of the observed inclusion compound(s).

Application to cyclodextrins/adamantan-1-ol complexes

The competitive continuous variation plot has been applied to the complexes formed between adamantanol and three different β-cyclodextrins (βCD, SBE1 and SBE7). The solubility of such substrate hinders its study by ^{1}H NMR, and the absence of chromophoric unit prohibits a direct Job plot by UV–vis spectroscopy. Adamantanol is thus an appropriate candidate for the application of the competitive continuous variation plot.

The adamantane derivatives/βCD complexes have been claimed to be 1:1 inclusion compounds [9], since their characterization by titration methods has been fitted with such stoichiometry. Nevertheless, a Job plot would be of interest to ensure that only a 1:1 complex is formed in the case of adamantanol. A CCV plot has thus been realized, and some of the corresponding variations of absorbance are presented in Fig. 1.

For each experiment, the solution MO + CD + I results in an increase of the absorbance, as a result of the expulsion of the MO from the CD cavity. The highest variations are of course obtained for the weak values of R, since an excess of substrate as compared to the host leads to a greater displacement of MO. It is however necessary to mention that very weak values of R means weak concentrations of CD, so that the differences between the absorbance of MO and MO + CD solutions decreases as well, thus limiting the variation observed upon the addition of the substrate.

Each difference between MO and MO + CD solutions may then be used to calculate the concentration of complexed CD, and the corresponding CCV plot is illustrated in Fig. 2. The curve is centered on the 0.5 abscissa, thus confirming the unique formation of the 1:1 inclusion compound. In addition, the application of the spectral displacement method allows the determination of the formation constant, which is equal to 34,500 M^{-1}. Such value is in agreement with published data on adamantane derivatives [9].

The CCV plot has also been obtained for the complexation of adamantanol with the SBE1 and SBE7 cyclodextrins (Fig. 3). The curves are not centered on the 0.5 value anymore, indicating the existence of an higher stoichiometry. The deviation is weaker for SBE1 (maximum close to $R = 0.54$) than for SBE7 (maximum close to $R = 0.61$). This finding is in agreement with the obtained binding constants, since

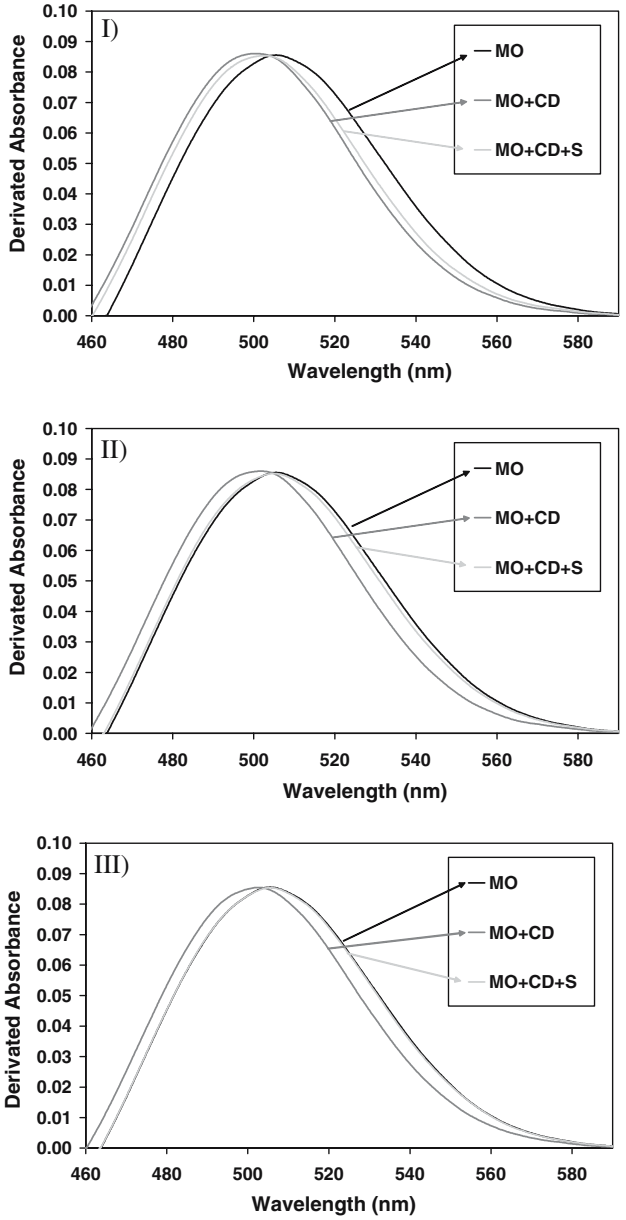


Fig. 1 First derivatives of the absorption spectra obtained for MO, MO + CD and MO + CD + S solutions, with a *R* ratio of 0.3 (I), 0.5 (II) and 0.7 (III)

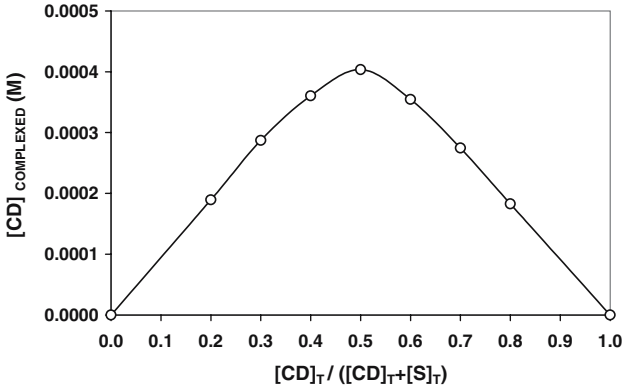


Fig. 2 CCV plot obtained for the βCD/adamantanol complex

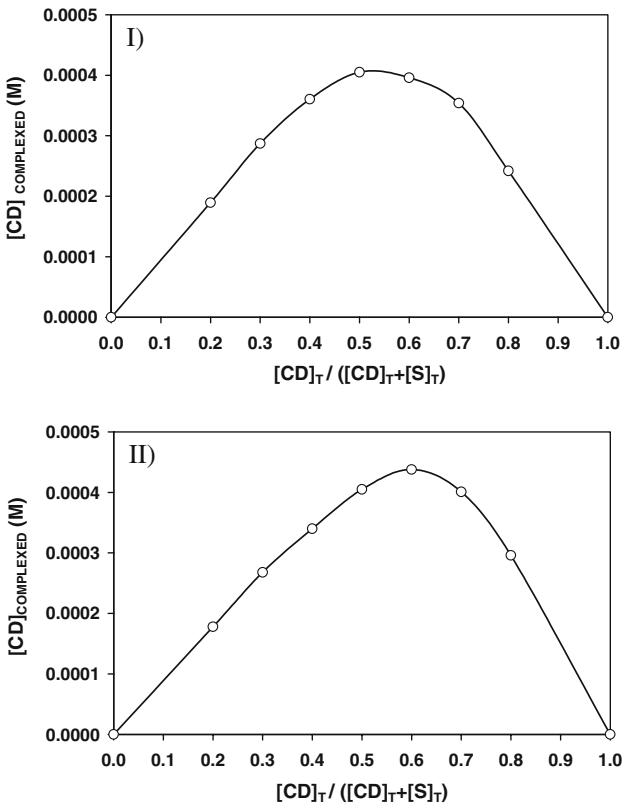


Fig. 3 CCV plots obtained for the SBE1 /adamantanol (I), and SBE7/ adamantanol (II) complexes

the 1:1 formation constant is weaker for SBE7 ($K_{1:1}$ = 12,500 M^{-1}) than for SBE1 ($K_{1:1}$ = 33,500 M^{-1}), while the 2:1 formation constant is greater for SBE7 ($K_{2:1}$ = 4000 M^{-1}) than for SBE1 ($K_{2:1}$ = 1000 M^{-1}).

Since the sulfobutyl arms extends the cavity of the genuine βCD, the need of a second modified cyclodextrin to fully encapsulate the adamantanol could seem unexpected, but these results lead to think that the association of two SBE molecules may afford a pseudo cavity by means of the sulfobutyl arms. The adamantanol thus could find a new complexation mode, explaining the formation of 2:1 complexes, stability of which is enhanced by the numbers of sulfobutyl arms.

Advantages and limitations of the competitive continuous variation plot

As already mentioned, the use of the CCV plot may be indicated for substrates which could not be studied by

other techniques, for spectral or solubility reasons. Moreover, since the plot is not based on the spectral observation of neither the studied host or guest, no unknown variable (as the intrinsic spectral characteristics of the complexes) interfere, so that the quantity used for the ordinate of the CCV plot represents the concentration of complexed cyclodextrin, and not only a quantity which is proportional to it. This implies that the formation constants may be easily obtained, in contrary to the classical Job plot. Moreover, when a mixture of inclusion compounds is observed, the position of the maximum does not correspond to typical value (i.e. 0.5 for 1:1 complexes, 0.67 for 2:1 complexes...); such a position is then depending on the relative stabilities of the various complexes but also, for classical Job plot, on the intrinsic spectral characteristics of the species. In the CCV plot, only the relative stabilities determine the shape of the curve, in such a way that the *R* value for the maximum is directly informative on the predominance of one stoichiometry as compared to the other.

If the CCV plot presents various advantages, two main limitations also arise. First of all, the difference between the MO + CD and MO + CD + I solutions decreases as the *R* value tends to 1, and it implies that a greater uncertainty is observed on the right side of the CCV plot. For weak complexes or substrates with too weak solubilities, this could result in a poor resolution of the plot, prohibiting any interpretation. Secondly, the influence of methyl orange has to be taken into account, since a Job plot requires the $[CD]_T + [S]_T$ total concentration to be constant. This is not exactly the case in the CCV plot, because of the host molecules which are complexed with methyl orange, and which are thus unavailable to the guest. The weaker the *R* value, the weaker the cyclodextrin concentrations, so that the CCV plot may be biased on its left side, for which a greater part of CD is complexed with MO. It implies that the weakest cyclodextrin concentration has to be largely superior to the MO concentration ($[CD]_T > 10 * [MO]_T$ in our experiments).

Conclusion

A new method for the determination of stoichiometry in the host–guest chemistry field has been developed, by associating the classical Job plot with the spectral displacement method. The resulting competitive continuous variation plot may be applied even with substrates which are not directly observable by spectroscopy. In association to a dedicated treatment, such a method could also allow an easy determination of the stability constants, the shape of the curve being controlled only by the stabilities of the various inclusion compounds.

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