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Complexation of (L)-Alanine-β-naphthylamide Hydrobromide with Methyl-β-cyclodextrin: An Ultrafiltration Study

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Abstract. The complexation constant for the system (L)-alanine-β-naphthylamide hydrobromide (S) and methyl-β-cyclodextrin (Me-β-CD) has been determined using an ultrafiltration method. Me-β-CD and its inclusion complex are retained by ultrafiltration (UF) membranes of 1 kDalton molecular weight cut-off with a rejection rate of 97%. As the substrate S passes freely throughout the UF membrane, the concentration of free and bounded S is easily determined. The value thus obtained for the complexation constant is in good agreement with those previously reported for similar inclusion complexes. In addition, the specific optical rotation of the complex has been determined. As expected, the specific rotations of S and CD are not additive.

Key words: Methyl-β-cyclodextrin, complexation constant, ultrafiltration, optical rotation, inclusion complex.

1. Introduction

Cyclodextrins (CDs) are cyclic compounds containing 6, 7 or 8 D-glucose units which are linked by an α-(1,4) bond. These supramolecular compounds are water soluble and possess a definite geometry and cavity size [1]. Inclusion of a wide range of molecules or ions such as iodine [2], dyes [3] or surfactants [4] in this hydrophobic cavity is possible and gives rise to the formation of inclusion complexes of various stoichiometries. Studies of inclusion complexes with cyclodextrins are of great interest as a model for molecular and enantioselective recognition or enzyme substrate interaction [5], and for analytical purposes in HPLC [6] and GC [7]. Many studies dealing with inclusion complexes with cyclodextrins have already been reported in the literature and have contributed to the development of the cyclodextrins as an important field of supramolecular chemistry [8].

Most of the methods used to study inclusion complexes with CDs are based on the variation of a spectrometric parameter during the formation of the complex. For example: UV spectroscopy, fluorescence and circular dichroism have been used to determine the complexation constants between CDs and substrates derived from

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amino-acids [9]. X-ray diffraction, NMR spectroscopy [10] and electrochemical methods [11] have also been used, and more recently MM2 calculations reproducing bimodal inclusion have been described [12].

We report here our investigation in this field and describe an alternative method for the determination of the equilibrium constant of inclusion complexes. As previously shown by Tawara *et al.* [3a] and more recently by Kokugan *et al.* [3b], this new method is based on the separation of high molecular weight compounds from small molecules and ions by means of an ultrafiltration technique.

2. Methods

Ultrafiltration (UF) is a technique that permits the highly efficient separation of dissolved macromolecules from aqueous solutions by means of a porous membrane. The separation can then be achieved by UF experiments using a batch equipment or preferably a tangential flow cell.

Tangential flow cells can be used in the stationary state if the filtrate (the permeate) is continuously recirculated in the feed solution (the retentate) as shown on the schematic diagram reported in Figure 1. After a short transitory period the concentration of all species in the permeate becomes independent of time. In this stationary state, the UF technique can be used as an analytical tool. In addition, the use of a high rate tangential flow (1–2 m/s or more) and a low differential pressure across the membrane, make it is possible to avoid concentration polarisation at the membrane interface. At the same time, adsorption and aggregation phenomena occurring onto the membrane surface are minimised. For these two reasons tangential flow ultrafiltration has been chosen for this study.

The permeation properties of UF membranes depend on the pore size and its associated characteristics, pore morphology and surface density. UF membranes are usually characterised by a molecular weight cut-off (MWCO) expressed in Dalton. The MWCO is the molecular weight of the largest solute that can permeate freely through the membrane. The rejection rate τ, used to evaluate the retention of any solute by a membrane, is defined as:

$$\tau = 1 - C_p/C_r \tag{1}$$

where C_p and C_r represent the concentration of the solute in the permeate and in the retentate, respectively.

Any substrate that could form a 1 : 1 inclusion complex with methyl-β-cyclodextrin gives rise to the thermodynamic equilibrium:

$$\text{Me-}\beta\text{-CD} + \text{S} \rightleftharpoons \text{Me-}\beta\text{-CDS}$$

where S, Me-β-CD, Me-β-CDS represent the substrate, the methyl-β-cyclodextrin and the inclusion complex, respectively.

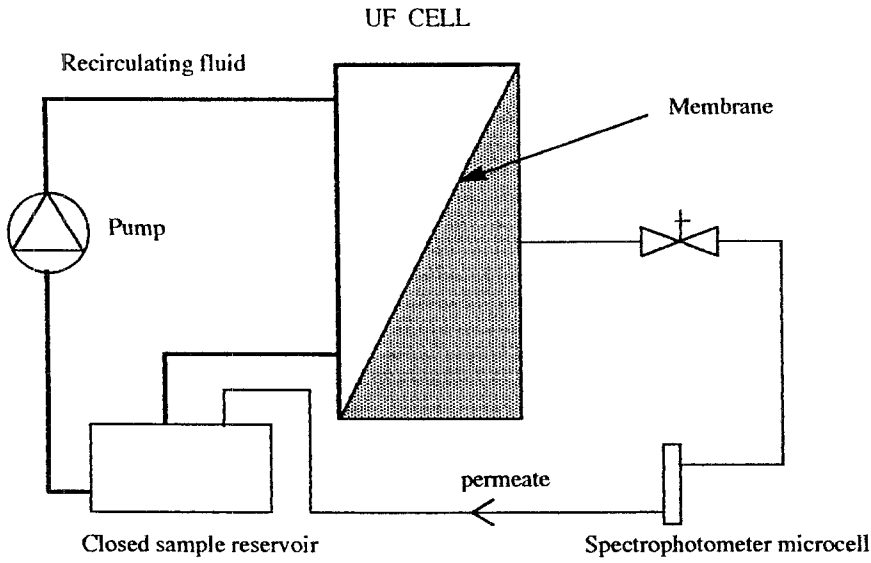


Figure 1. Schematic diagram of an ultrafiltration cross flow cell.

When a stationary state is established on both sides of the membrane, the cyclodextrin and its inclusion complex, whose size is equal to or greater than the size of the cyclodextrin itself, are retained by the membrane. In contrast, the substrate is equally distributed between the permeate and the retentate if its molecular weight is less than the MWCO of the membrane. Thus, the determination of the complexation constant K defined as:

$$K = \frac{[\text{Me-}\beta\text{-CDS}]}{[\text{Me-}\beta\text{-CD}][\text{S}]} \tag{2}$$

can be achieved by measuring the concentration of the substrate in both phases by an adequate method (spectrophotometry for example) and by using a mass balance equation.

3. Experimental

3.1. Materials

Methyl-β-cyclodextrin (average degree of substitution: 1.8) and (D, L) alanine-β-naphthylamide hydrochloride (98%) were from Aldrich: (L)-alanine-β-naphthylamide hydrobromide (Sigma grade) was from Sigma. All reagents were used as received.

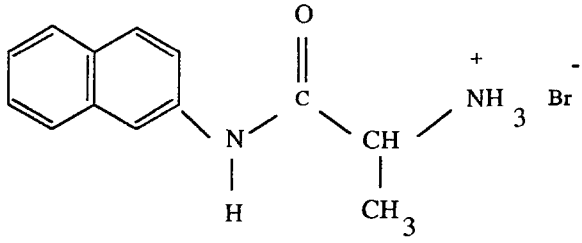
3.2. Ultrafiltration

Cellulose membranes with 1 kDalton MWCO were purchased from Millipore and used in a Minitan-S ultrafiltration module. This module is a plate and frame system

for cross-flow filtration. Circulation of process fluid (aqueous solution) parallel to the membrane is operated in a thin channel laminar flow at room temperature (20 ± 1 °C). Pressure and flow rate (250 mL/mn) are obtained by a peristaltic pump. The pressure drop, measured at the cell inlet, is adjusted to 0.8 bar by means of a clamp placed on the circulating fluid. The absorbance of the permeate was monitored on line by an Hitachi 100-80 spectrophotometer at a wavelength of 278 nm or at 241 nm (λ_{max} of the substrate). In addition and when needed, samples of 10 mL of solution (the retentate volume was approximately 200 mL) were taken during the ultrafiltration run to determine the optical rotation of the permeate and retentate. Polarimetric measurement were performed at 436 nm and at 20 °C on a Perkin-Elmer 141 polarimeter equipped with a Hg lamp. Substrate absorbance measurements were determined after dilution if required at 278 nm ($\epsilon = 6987$) and at 241 nm ($\epsilon = 34640$) when the concentration was less than 10^{-5}.

4. Results and Discussion

A great number of molecules and ions give rise to the formation of inclusion complexes with cyclodextrins. Those containing an aromatic ring capable of being included in the hydrophobic cavity of β-cyclodextrin have been extensively studied. (L)-Alanine-β-naphthylamide hydrobromide **1** has been taken as a model molecule for this study because of its ability to form 1 : 1 inclusion complexes with β-cyclodextrin derivatives [10]. In addition, the presence of the naphthyl group in this molecule facilitates the determination of its concentration in an aqueous medium by means of UV spectrophotometry.



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As the molecular weight of **1** is much lower than the MWCO of the membrane, it is not expected to be retained by the UF membrane. In order to verify this assumption and to determine if no adsorption phenomena at the membrane surface occur during the ultrafiltration experiments, the rejection rate of **1** has been measured as a function of its concentration. The results are given in Table I.

The apparent rejection rate of **1** strongly increases as the concentration in the feed is decreased below 8×10^{-5} mol/L thus indicating adsorption onto the membrane surface. As expected, the retention rate drops to nearly zero as the concentration in the feed solution exceeds the above value. Under these conditions, the amount

Table I. Permeate concentration (C_p), retentate concentration (C_r) and rejection rate τ, of **1** as a function of its concentration in the feed solution (C_1).

$10^4\ C_1$ (*)	0.4	0.6	0.8	1	10
$10^5\ C_p$ (*)	2.59	2.80	7.06	6.75	72.5
$10^5\ C_r$ (*)	4.62	4.17	6.82	6.59	72.9
τ	0.44	0.33	0	0	0

(*) Concentrations in mol/L.

Table II. Permeate concentration (C_p), retentate concentration (C_r) and rejection rate τ for Me-β-cyclodextrin as a function of its concentration in the feed solution (C_0).

$10^4\ C_0$ (*)	0.5	1	10	20	40	50
$10^5\ C_p$ (*)	2.76	5.0	7.07	10.6	17.2	22.2
$10^4\ C_r$ (*)	5.79	7.95	11.0	21.6	50.3	68.0
τ	0.95	0.94	0.94	0.95	0.97	0.97

(*) Concentration in mol/L.

of adsorbed solute becomes negligible and no steric hindrance for the permeation of the solute is observed. In order to avoid having to take into account amounts of adsorbed solute in mass balance equations, all UF experiments have been performed using concentrations higher than 8×10^{-5} mol/L.

Three kinds of cyclodextrins are commonly used for complexation studies i.e. α, β and γ cyclodextrins. More recently, partially alkylated and acetylated cyclodextrin derivatives have been prepared and commercialised. Among these derivatives, methylated β-cyclodextrin (Me-β-CD) exhibits an enhanced solubility as compared to β-cyclodextrin. The main advantage of using Me-β-CD is that it enables one to use more concentrated feed solutions. In addition, better retention by the UF membrane is expected for this compound as its molecular weight is higher.

The rejection rate of Me-β-CD on the 1 kDalton UF membrane was determined by measuring the optical rotation of the retentate and permeate solutions at 20 °C and 436 nm ($[\alpha]_{\text{Me-}\beta\text{-CD}} = +394.9$). Results of UF experiments are reported in Table II.

From the results reported in Table II, it can be seen that the rejection rate of Me-β-CD depends weakly on its concentration in the feed solution. In the range of concentration under study, the largest rejection rate is obtained for concentrations higher than 2×10^{-3} mol/L. However, even at these concentrations, Me-β-CD is not perfectly retained by the membrane. This is probably due to the presence of some larger pores than that corresponding to the nominal MWCO of the UF membrane. Nevertheless the permeation of a small amount of Me-β-CD will not

perturb the analytical results and particularly, the determination of the value of the complexation constant K. Results are given within an error limit not greater than 3–5%.

4.1. Determination of the complexation constant K

The equilibrium constant for the formation of the 1 : 1 inclusion complex of **1** with Me-β-CD was determined by UF experiments in which the Me-β-CD concentration was held constant at 5×10^{-3} mol/L. The concentration of **1** was varied from 8×10^{-4} to 3.5×10^{-4} mol/L. After the establishment of the stationary state, the concentration of the free substrate in the permeate was determined by measuring its absorbance $A_{1,p}$:

$$[S] = \frac{A_{1,p}}{\varepsilon_S}$$

where ε_S is the molar absorptivity of **1** at the wavelength considered. The concentration of the inclusion complex can be deduced from the absorbance of the retentate since in the stationary state the substrate is evenly distributed between the two sides of the UF membrane. The mass balance equation for the substrate gives:

$$[\text{Me-}\beta\text{-CDS}] = \frac{A_{1,r} - A_{1,p}}{\varepsilon_S}$$

where $A_{1,r}$ is the absorbance of the retentate at the same wavelength. Using a mass balance equation for the cyclodextrin, the concentration of the free Me-β-cyclodextrin is given by:

$$[\text{Me-}\beta\text{-CD}] = C_0 - \frac{A_{1,r} - A_{1,p}}{\varepsilon_S}$$

where C_0 is the formal concentration of the cyclodextrin in the retentate. The value of the complexation constant is given by the following expression:

$$K = \frac{\varepsilon_S(A_{1,r} - A_{1,p})}{\varepsilon_S A_{1,p} C_0 - A_{1,p}(A_{1,r} - A_{1,p})}.$$

The mean value for K (calculated from five UF experiments) is 255 on the molar scale. This value is in good agreement with those previously published for the complexation of **1** with trimethyl-β-cyclodextrin ($K = 234$) [10a] or diacethyl-β-cyclodextrin ($K = 228$) [10a]. All these values indicate that the interaction between **1** and these cyclodextrins is not very strong and gives rise, under usual conditions, to an equilibrium between the inclusion complex and the free species.

4.2. DETERMINATION OF THE SPECIFIC OPTICAL ROTATION OF THE INCLUSION COMPLEX

From the determined complexation constant, and the measurement of the optical rotation of solutions containing both Me-β-CD and **1**, the specific rotation of the complex can be inferred.

The presence of optically active substances in the feed or permeate solution rotates the plane of polarised light by an angle α:

$$\alpha = [\alpha]_S[S] + [\alpha]_{Me\text{-}\beta\text{-}CD}[Me\text{-}\beta\text{-}CD] + [\alpha]_{Me\text{-}\beta\text{-}CDS}[Me\text{-}\beta\text{-}CDS]$$

where $[\alpha]_S$, $[\alpha]_{Me\text{-}\beta\text{-}CD}$ and $[\alpha]_{Me\text{-}\beta\text{-}CD}$ are respectively the specific rotation of the substrate, the methyl-β-cyclodextrin and the inclusion complex.

According to the following equilibrium equation:

$$\text{Me-}\beta\text{-CD} + S \rightleftharpoons \text{Me-}\beta\text{-CDS}$$

t_o	C_0	C_1	
t_{eq}	$C_0(1-X)$	$C_1 - C_0X$	C_0X

the expression for α can be rearranged to:

$$\alpha = [\alpha]_S(C_1 - C_0X) + [\alpha]_{Me\text{-}\beta\text{-}CD}(C_0 - C_0X) + [\alpha]_{Me\text{-}\beta\text{-}CDS}C_0X \qquad (3)$$

where C_1 and C_0 are the initial concentration of (L)-alanine-β-naphthylamide hydrobromide and Me-β-cyclodextrin, respectively. X is the solution of the equation:

$$K = \frac{C_0(1-X)(C_1 - C_0X)}{C_0X}$$

and is given by:

$$X = \frac{1 + KC_0 + KC_1 - [(1 + KC_0 + KC_1)^2 - 4K^2C_0C_1]^{1/2}}{2KC_0}$$

$[\alpha]_S$ and $[\alpha]_{Me\text{-}\beta\text{-}CD}$ can be obtained from polarimetric measurement using solutions containing S or Me-β-CD at various concentrations. They are determined from the slope of the linear relation obtained by plotting the rotation angle versus the concentration of S or Me-β-CD: $[\alpha]_S = +22.5$ and $[\alpha]_{Me\text{-}\beta\text{-}CD} = +394.87$ at 20 °C and 436 nm with C expressed in mol/L.

Despite the complexity of the expression for α for a solution containing both S and Me-β-CD the specific rotation of Me-β-CDS can easily be obtained by a simulation technique.

For given C_1 and C_0 values, X can be calculated. As X is known α can be calculated for any starting value of $[\alpha]_{Me\text{-}\beta\text{-}CDS}$. On Figure 2 α (calculated) is

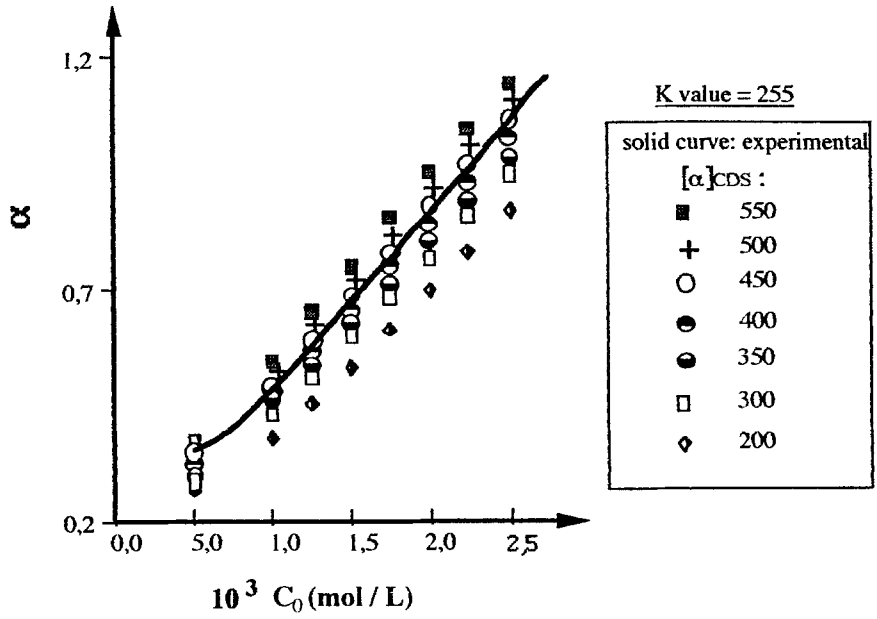

Figure 2. Light angle deviation versus initial cyclodextrin concentration for Me-β-cyclodextrin solution containing (L)-alanine-β-naphthylamide **1**.

plotted as a function of C_0 for different values of $[\alpha]_{\text{Me-}\beta\text{-CDS}}$ ranging from 200 to 550. For the same values of C_1 and C_0, α can be determined experimentally by polarimetric measurement. The experimental values thus obtained are also reported on Figure 2 as a function of C_0 (solid curve). As shown on the graph, the best fit with experimental values is obtained for $[\alpha]_{\text{Me-}\beta\text{-CDS}} = +450 \pm 50$.

In order to determine $[\alpha]_{\text{Me-}\beta\text{-CDS}}$ more precisely, the contribution of the complex to the experimental rotation α (i.e. $[\alpha]_{\text{Me-}\beta\text{-CDS}}\ C_0\ X$ in Equation 3) has been recalculated from experimental data according to:

$$[\alpha]_{\text{Me-}\beta\text{-CDS}}(C_0X) = \alpha - [\alpha]_S(C_1 - C_0X) - [\alpha]_{\text{Me-}\beta\text{-CD}}(C_0 - C_0X).$$

As shown in Figure 3 the plot of $[\alpha]_{\text{Me-}\beta\text{-CDS}}$ $(C_0\ X)$ as a function of [Me-β-CDS] $(= C_0\ X)$ is linear, as expected. The slope of this linear plot gives $[\alpha]_{\text{Me-}\beta\text{-CDS}} = 450 \pm 20$ at 436 nm. This result means that the specific rotation of the complex is by no means given by the sum of the specific rotation of the substrate and the Me-β-cyclodextrin.

4.3. ENANTIOMERIC SEPARATION

In order to test if it was possible to realize an enantiomeric separation with this method a UF experiment has been carried out on a solution containing Me-β-CD ($C_0 = 10^{-2}$) and DL-alanine-β-naphthylamide ($C_1 = 10^{-2}$). 40 mL of permeate

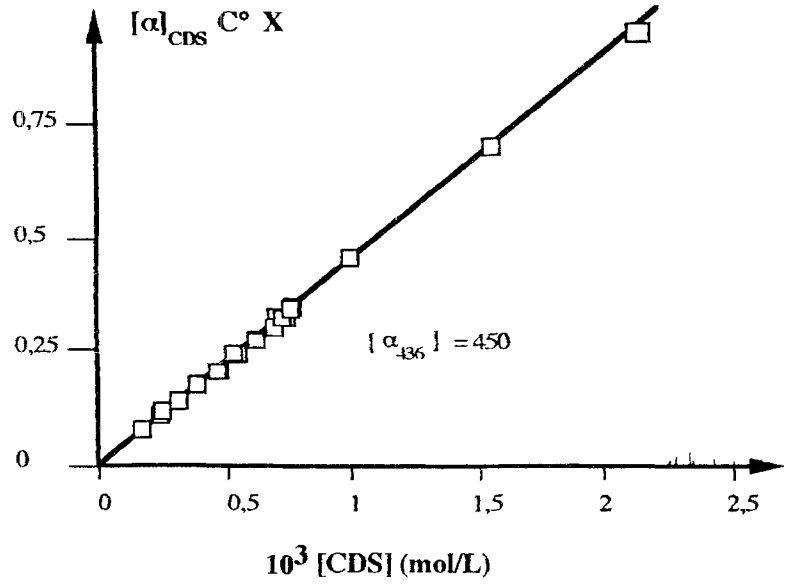

Figure 3. Contribution of the Me-β-cyclodextrin complex to the light deviation versus its concentration.

was obtained from an initial volume of 100 mL of this solution. The optical rotation of the permeate solution is very low ($\alpha_p = +0.091$) compared to that of the retentate ($\alpha_r = +6.141$).

Under the conditions used, the leakage of the membrane for Me-β-CD cannot be neglected since Me-β-CD contributes to the rotation of the plane of polarised light. Thus the optical rotation measured on the permeate is the sum of a different contribution, as expressed in the following equation:

$$\alpha_p = [\alpha]_S([L] - [D]) + [\alpha]_{Me\text{-}\beta\text{-}CD}[Me\text{-}\beta\text{-}CD]$$

In this condition, the term $[\alpha]_{Me\text{-}\beta\text{-}CD}$ [Me-β-CD] appeared to be predominant mainly as $[\alpha]_{Me\text{-}\beta\text{-}CD}$ has a high value compared to $[\alpha]_S$. Then the enantiomeric term (i.e. $[\alpha]_S([L]\text{-}[D])$) is very low and cannot be determined.

It can be concluded that no enantiomeric separation of the racemic **1** can be achieved under the conditions used with this method. This is mainly due to the fact that the specific rotation of the substrate is too low compared to that of the Me-β-CD.

5. Conclusion

In this work, we show that ultrafiltration coupled with spectroscopic and polarimetric measurements can be a convenient method to determine host-guest complexation constants with cyclodextrin derivatives. In addition, polarimetric measurements allow the determination of the specific rotation of the inclusion complex

formed in solution, as soon as the value of the complexation constant is known. This method is well adapted to evaluate the complexation constant in mixtures as an efficient separation between large host or host-guest solutes and small molecules and ions is obtained. Nevertheless, no enantiomeric separation has been achieved by ultrafiltration of a solution containing Me-β-CD and racemic substrate. This is mainly due to the fact that (i) the specific optical rotation of the substrate is too low as compared to that of the Me-β-CD and (ii) that the membrane leakage for Me-β-CD is not negligible. It seems that only multiple equilibria such as those occurring when cyclodextrins are used as chiral phases in a chromatographic column are able to overcome this handicap for the separation of enantiomers.

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