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Molecular Interactions and Thermodynamic Aspects of the Complexation Reaction between Gentian Violet and Several Cyclodextrins

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Abstract. The complexation process between gentian violet (CV^+) and four different cyclodextrins (α-, β-, γ-, and HP-β-CDs) has been investigated under different reaction conditions (pH, solvent, and temperature) by electronic absorption and ^{1}H NMR (NOE and NOESY) spectroscopies. All the binding constants were determined by the direct spectroscopic method. The ΔH and ΔS complexation values have been evaluated and discussed according to the diverse factors that affect the chemical interactions in these systems. A simple association takes place between the secondary hydroxyl or the hydroxypropyl groups of α and HP-β-cycloamyloses, respectively, with the amine group of the gentian violet, while the binding between CV^+ and β- or γ-CDs corresponds to a real inclusion. Also, a CV_2^{2+} dimeric species within the γ-CD cavity was detected in aqueous solution, while two molecules of α-CD react with one molecule of gentian violet in DMSO at 294 K. In all the reaction media the β-CD forms 1 : 1 complexes, but in the buffered aqueous solution at pH 7.5 the inclusion is deeper than in the other solvents. It is important to point out that the solvophobic effect is the most important binding factor in the complexation of the CV^+ with the α- and HP-β-CDs, while the complexes with β-, and γ-cyclodextrins are mainly stabilized by van der Waals interactions between the guest and the host cavity. In all cases, the inclusion orientation is probably determined by the ion-dipole interactions between gentian violet and the solvent.

Key words: gentian violet, cyclodextrin complexes, organic media, molecular interactions, thermodynamic parameters.

1. Introduction

The main purpose of this paper is to investigate the complexation equilibria of gentian violet with α, β, γ, and HP-β-cyclodextrins under different reaction conditions, and to relate the resulting thermodynamic parameters with the driving forces for complexation.

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Cyclodextrins are cyclic molecules produced from starch by enzymatic transglucosydation and cyclization. They represent a research subject of host–guest chemistry. Multiple applications have been widely investigated in food science, cosmetics, pharmacy and analytical chemistry [1, 2].

Due to the existent parallelism between the inclusion phenomenon and enzyme-substrate and drug–receptor interactions [3], it is very important to establish what driving forces lead to the selective binding of a substrate to a receptor.

Gentian violet or crystal violet ([4-[bis[*p*-(dimethyl-amino)phenyl]methylene]-2,5-cyclohexadiene-1-yliden] ammonium chloride) is a triphenylnaphthylmethane acid-base indicator. It is a monocationic species in aqueous solution at pH higher than 1.8 and lower than 9. CV^+ can form aggregates due to its planar molecular shape [4, 5]. It has been reported that the association constant of the dimer (CV_2^{2+}) is K_d = 600 mol^{-1} dm^3 at 298 K, in aqueous solution [6]. Changes of color intensity of this dye are related to its capacity to be adsorbed on surfaces. This effect has been named metachromasy by Ehrlich [7, 8].

The CV^+ electronic absorption spectrum in the visible region usually presents two characteristic bands, α at 588 nm and β at 542 nm, corresponding to monomeric and dimeric forms of this dye. Also, a third band, μ at 510 nm, can appear when the concentration increases. This latter is associated with the presence of higher aggregates in solution, either trimers or tetramers [9].

Some published works concerning triarylmethyl dye aggregation agree with the lack of aggregates in organic solvents due to the solvophobic effect [8, 10]. Therefore, to some extent, the association of CV^+ depends on the nature of the solvent [11].

In the literature there are some reports about the formation of the [$CV_2 \subset \gamma$-CD]$^{2+}$ complex in aqueous solution. Using a temperature-jumped method, rate constants and inclusion mechanism were reported [6, 12]. Also, Hirai et al. [13] studied the stability of several dyes with different cyclodextrins (α, β and γ). In this case, the complexation was shown on the basis of the electronic absorption and induced circular dichroism spectral changes due to changes of the dye concentration. Gentian violet was further used as a cationic species to study the charged cyclodextrin binding properties [14].

In spite of the above reports concerning the formation of CV^+-cyclodextrin inclusion compounds, the influence of temperature and molecular interactions between the CV^+ cation and different solvents had not been taken into account. Additionally, studies of guest-cycloamylose interactions in organic media are scarce.

2. Experimental

2.1. MATERIALS

Gentian violet (96% purity), hydrochloric acid, volumetric standard, 1.029 N solution in water, D_2O (99.9 atom% D), DMSO-d_6 (99.9 atom% D) and NaH_2PO_4 were purchased from Aldrich, and were used without further purification. NaOH (AR) and NaCl (AR) were acquired from Mallinkrodt. α-, γ-, and HP-β-cyclodextrins were kindly donated by Cerestar USA Inc. and were used as received. β-cyclodextrin provided by ARANCIA (México) was purified by successive solvent washings.

2.2. METHODS

Five different sets of solutions were prepared, an aqueous phosphate buffer solution 0.1 M at pH 7.5 and ionic strength 0.1 M NaCl, DMSO and DMSO/water mixtures (25%, 50%, and 75%). The electronic absorption spectroscopic studies were carried out at five different temperatures (294, 298, 302, 306 and 310 K) only for the aqueous buffer at pH 7.5 and for DMSO. Therefore, ΔH and ΔS values were obtained for the complex formation processes. The remaining water/DMSO solutions were studied only at 298 K. In all cases, the direct spectroscopy method was used for obtaining the binding constants. Stock solutions of gentian violet 5×10^{-6} M ($[CV^+]_t$) were prepared in all the solvents, and subsequent cyclodextrin dilutions were prepared from this guest solution (from 1×10^{-2} to 1×10^{-4} M). Sample measurements were made within 24 hours of being prepared.

Dye adsorption, especially on glass and silica surfaces, is a source of experimental error because of the metachromatic effect exhibited by the dye adsorbed on the cuvette walls [4]. To avoid this problem a special glassware cleaning care was followed in each spectroscopic measurement. Schubert and Levine have argued that the gentian violet staking is due to its π electron interactions [9]. The metachromic effect is intensively observed in the visible region, hence the spectral data were collected in the ultraviolet region where this effect is negligible. Only the γ-cyclodextrin system in aqueous buffer solution at pH 7.5 was studied considering the visible bands. This is a special case due to the guest dimerization process into the γ-CD cavity.

Absorption electronic spectra were recorded on a diode array HP8452A spectrophotometer equipped with a HP 8909OA temperature regulator Peltier system, and the cell sample solution was continuously stirred.

^{1}H NMR studies have been carried out in D_2O and in some cases in DMSO-d_6. In a 300 MHz Varian Unity Plus System 1D ^{1}H NMR spectra were collected by using a frequency of 299.95 MHz, with a 45° pulse (6.7 μs), 3.002 s of acquisition time at 298 K. The 2D NOESY experiments were collected in the same spectrometer, equipped with a broad band switchable probe. 90° pulse of 13.5 μs, 32 scans

were made for each increment and 128 increments were made. Delay time between each scan was 2 s.

Molecular mechanics studies were accomplished to support the experimental data on the inclusion compound formation. Structures of host and guest molecules either isolated or in the form of inclusion complexes were optimized with Hyperchem (1995 Hypercube, Inc.) module by using the molecular mechanism option with the MM2 forcefield and the conjugate gradient optimizer. The convergence criterion was 0.1 Kcal/mol Å in energy gradient.

The pKa values of CV^+ and its inclusion complex with HP-β-CD were obtained from spectrophotometric titrations, and the pH values were measured using an Orion 710A pH meter with a glass body electrode ROSS, Model 8102BN, 0-14 pH.

3. Results and Discussion

3.1. ELECTRONIC ABSORPTION SPECTRA OF GENTIAN VIOLET

Taking into account that CV^+ does not always follow the Lambert Beer law, we have studied what happens under the studied conditions. In aqueous solution (buffer pH = 7.5, 298 K and $[CV^+]_t = 5 \times 10^{-6}$ M), the dimerization process hardly takes place; the concentration of CV_2^{2+} has been calculated and is 0.6% of the total CV^+ concentration in solution, so CV_2^{2+} has no significant effect on the equilibrium, disregarding the interaction with γ-CD.

In DMSO and in the water/DMSO mixtures, the spectrum shape remains invariable, but a bathochromic shift is observed. No dimerization takes place.

3.2. BINDING CONSTANTS AND GEOMETRIC CHARACTERISTICS OF THE INCLUSION COMPLEXES BETWEEN CV^+ AND DIFFERENT CYCLODEXTRINS

The complex formation reaction was rationalized in terms of spectral shifts and absorption enhancement of the gentian violet.

3.2.1. *UV-visible spectroscopy*

The CV^+ absorption spectra in aqueous solution, pH = 7.5, at 298 K, with and without α-CD show that the absorption at 250 and 302 nm increases with the α-CD concentration, while the absorption at 542 and at 588 nm decreases. It is accompanied by the appearance of an isosbestic point at 476 nm. Very similar changes are observed with the β- and the HP-β-CDs. With the β-CD, an isosbestic point at 490 nm and a red shift of 8 nm on the visible absorption maximum are observed (Figure 1a). Also with the HP-β-CD, visible peaks are shifted 14 nm to longer wavelengths. However, the behavior with γ-CD is quite different (Figure 1b). UV region changes are similar to those shown by the other cycloamyloses, but a systematic shift of

the maximum absorbance in the visible region takes place at shorter wavelengths when the γ-CD concentration increases. Also, two isosbestic points appear at 502 and 618 nm. These spectral changes suggest interaction between the two species.

In general, it seems that the complexes are also present in the different DMSO/water solutions, since spectral changes are similar to those observed in aqueous solutions. No significant changes among the UV-visible spectra obtained at different temperatures in aqueous and DMSO solutions have been found.

CV^+ could be considered as a tridentate molecule and there is the possibility of cyclodextrin binding through any of its three aromatic rings. However, taking into account the size relationship between them, the formation of a 1 : 1 complex is more probable.

Scheme I shows the general complexation reaction equilibrium:

$$\mathrm{mCD} + \mathrm{nCV}^+ \overset{K_b}{\rightleftharpoons} [\mathrm{CV}_n \subset \mathrm{CD}_m]^{n+}$$

Scheme I.

Here, $[\mathrm{CV}_n \subset \mathrm{CD}_m]^{n+}$ represents a $n : m$ complex, and K_b is the equilibrium constant for this reaction. When a 1 : 1 complex is formed, the binding constants are obtained from Equation 1, where K_b and $\Delta\varepsilon$ are obtained by a non-linear iterative least squares method based on the Levenberg-Marquard mathematical method [15].

$$\Delta A = \frac{K_b[S_t][\alpha\text{-CD}]\Delta\varepsilon}{1 + K_b[\alpha\text{-CD}]}. \tag{1}$$

Here, ΔA is the difference in absorbance between CV^+ with and without α-CD. S_t is the total gentian violet concentration. $\Delta\varepsilon$ is the difference between the extinction coefficient of the inclusion compound and of the guest.

Only gentian violet with α-CD in DMSO at 294 K and this guest with γ-CD in aqueous solution at the five studied temperatures do not present the former behavior.

The simple 1 : 1 stoichiometry does not adequately describe the complexation equilibrium with α-CD in DMSO at 294 K. From the ΔA vs [α-CD] plot, we deduce the presence of a 1 : 2 complex, $K_{1:2} = 22000 \pm 2000$; $\chi^2 = 5 \times 10^{-7}$.

Equation (2) was used in this case:

$$\Delta A = \frac{K_b[S_t][\alpha\text{-CD}]^2\Delta\varepsilon}{1 + K_b[\alpha\text{-CD}]^2} \tag{2}$$

In the other case, gentian violet with γ-CD in aqueous solution, spectral changes suggest the presence of the 1 : 1 inclusion complex and 2 : 1 inclusion complex (Scheme II):

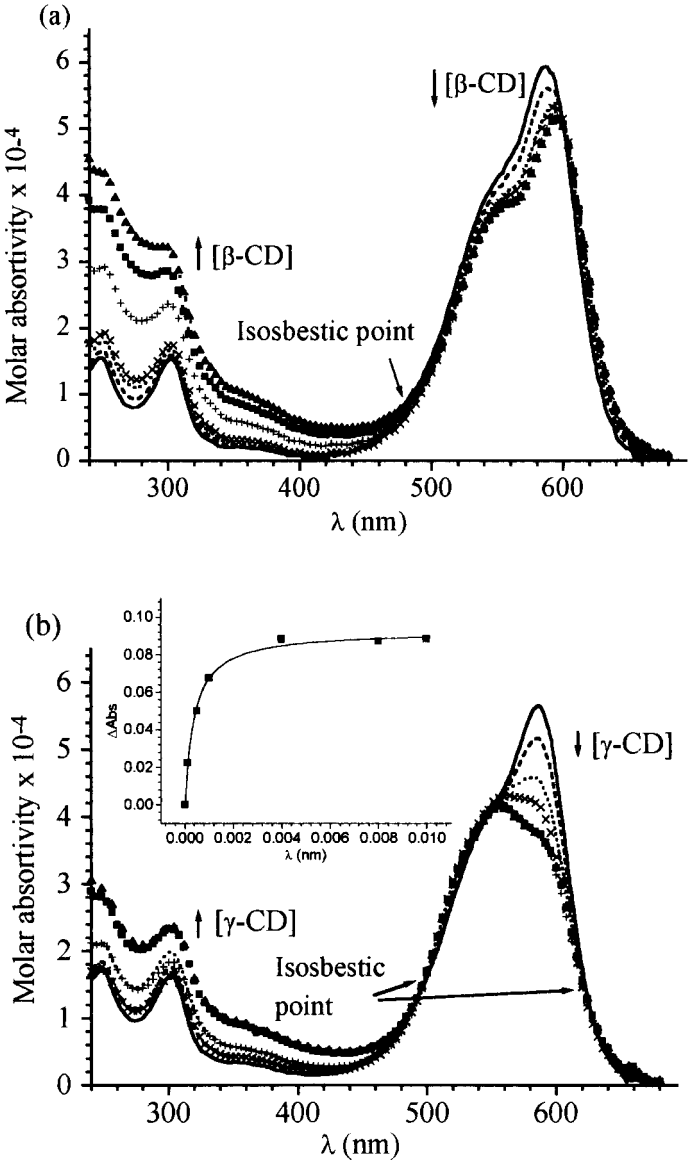


Figure 1. Electronic absorption spectra of CV^+ and CV^+ with several β- and γ-CDs concentration (a) and (b), respectively: (—) 0 M; (- - -)10^{-4} M; ($\cdots$) 5×10^{-4} M; (xxx) 10^{-3} M; (+++) 4×10^{-3} M; (-■-■-) 8×10^{-3} M and (-▲-▲-) 10^{-2} M, in aqueous solution at 298 K. The insert in (b) shows the corresponding binding isotherm for γ-CD, ■ experimental data, ___ 2 : 1 model fitting.

$$CV^+ \overset{+\gamma\text{-CD} \quad K_{1:1}}{\rightleftharpoons} [CV \subset \gamma\text{-CD}]^+ \overset{+CV^+ \quad K_{2:1}}{\rightleftharpoons} [CV_2 \subset \gamma\text{-CD}]^{2+}$$

Scheme II.

where $K_{1:1}$ and $K_{2:1}$ are the binding constants for the 1 : 1 and the 2 : 1 complexes, respectively.

We have simplified the problem considering that only one reaction takes place, one guest molecule enters into the cyclodextrin by the narrow edge and the other one through its wide part, resulting in a dimer within the cavity. In this case, Equation (3) was used, deduced from the simplified Scheme II, for obtaining the binding constants (Table I):

$$\Delta A = \frac{(\varepsilon_{CI} - 2\varepsilon_H)(1 + 4K_{2:1}[\gamma\text{-CD}][CV^+] - \sqrt{1 + 8K_{2:1}[\gamma\text{-CD}][CV^+]})}{8K_{2:1}[\gamma\text{-CD}]} \tag{3}$$

where ε_{CI} and ε_H are the extinction coefficients for the inclusion complex and free guest respectively. Table I shows equilibrium constants for different cyclodextrins in different reaction media at 298 K.

We consider that the method used for obtaining the constants is acceptable since the magnitude of the errors is small.

3.2.2. ^1H *NMR spectroscopy*

The NOESY spectrum of CV^+ with α-CD in aqueous solution at 298 K (Figure 2a) shows interaction between the methyl groups of the guest and the H(5) and H(3) corresponding to the α-CD. From this NMR spectrum and from optimization by molecular mechanics, we can establish that the CV^+ is associated through the α-CD wide part (Figure 2b), and a complex 1 : 1 can be observed.

1D ^{1}H NMR spectra (NOE) of CV^+ with β-CD in D_2O at 298 K have been recorded. A great downfield shift of all the CV^+ protons is noticed (0.7 ppm), and a H(5) shift is observed (0.2 ppm).

Analyzing this spectrum, we deduce the interaction among H_b of the guest with H(5) and H(3) of the β-CD. Also, there are interactions among H_a of the guest and H(6) and H(5) of the host. From here, we infer that one guest molecule is introduced within one cyclodextrin through its wide part and one complete phenyl group is accommodated in its interior.

A NOESY study was performed with CV^+ and β-CD in DMSO-d_6 at 298 K. The cross-peaks indicate that the hydroxyl groups on the C(2), β-CD wide part, and the anomeric proton of β-CD give the NOE effect with H_a and H_b of the CV^+. Also, we observed the interaction between the methyl group and the interior protons of the cycloamylose. This suggests that in this solvent the structure of the complex is different than in aqueous solution, as the guest is less included into the CD cavity.

Table I. Binding constant ($1:1$: mol^{-1} dm^3 or $2:1$: mol^{-2} dm^6 in the bold case) of gentian violet with several CD in different reaction media at 298 K, obtained from electronic absorption data

	Reaction media									
CDs	χ^2	Aq. solution	χ^2	25% DMSO	χ^2	50% DMSO	χ^2	75% DMSO	χ^2	DMSO
α-CD	3.8×10^{-6}	195 ± 7	1.3×10^{-6}	112 ± 5	5.6×10^{-8}	9.13 ± 0.08	2.7×10^{-6}	22.4 ± 0.6	8.9×10^{-8}	24.0 ± 0.5
β-CD	1.7×10^{-4}	330 ± 70	5×10^{-7}	45 ± 1	4.3×10^{-9}	110.2 ± 0.4	7.7×10^{-7}	150 ± 7	9×10^{-7}	250 ± 50
γ-CD	2×10^{-5}	$\mathbf{8 \times 10^7 \pm 2 \times 10^7}$	2.8×10^{-6}	10.6 ± 0.4	4×10^{-6}	34 ± 1	1.1×10^{-6}	120 ± 10	7.1×10^{-7}	126 ± 7
HP-β-CD	4.5×10^{-4}	180 ± 30	1.2×10^{-6}	110 ± 10	6×10^{-7}	33.1 ± 0.7	2×10^{-5}	29 ± 4	4.1×10^{-6}	23.5 ± 0.6

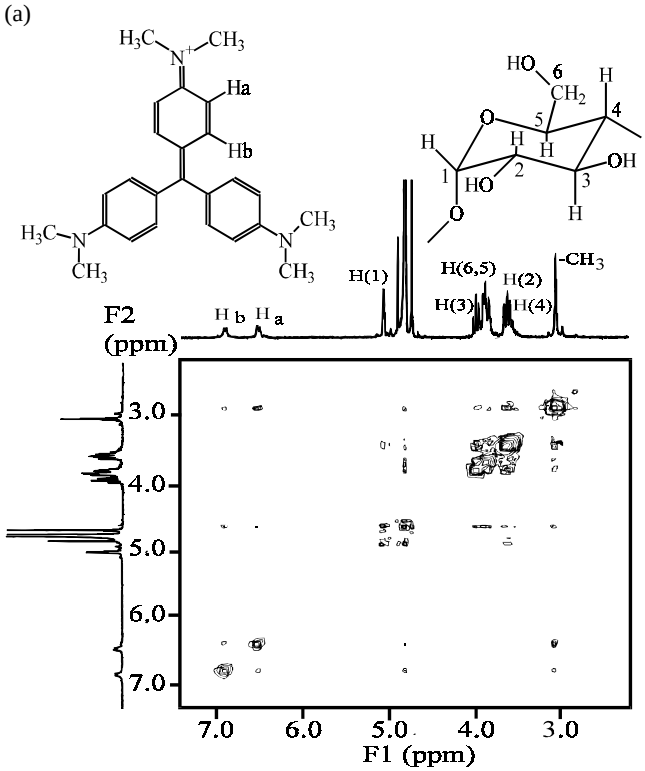


Figure 2. (a) ^{1}H NMR spectrum (NOESY) of CV^{+} with α-CD in D_2O. (b) Minimized structure for the association complex of gentian violet with cyclohexamylose.

Figure 3a shows the NOESY spectrum of gentian violet with γ-CD in D_2O at 298 K. Here we can observe interactions between the methyl groups of the CV^+ and H(5), H(6) and H(3) of the γ-CD. These signals support the assumption of formation of the dimer within the γ-CD cavity. Figure 3b shows the proposed structure of the $[CV_2 \subset \gamma\text{-CD}]^{2+}$ complex, which is in accordance with the minimized structure obtained from molecular mechanics. These results are in agreement with those obtained by UV-visible spectroscopy (Figure 1b), and with the dimeric structure proposed by Lueck et al. [16].

We assume that the orientation of the guest within the cycloamyloses is probably determined by the ion-dipole interactions between the gentian violet and the solvent, therefore the charged group of the molecule remains outside the cavity.

3.3. EFFECT OF DIFFERENT CYCLODEXTRINS ON THE COMPLEXATION

In general, the size of the cyclodextrins is a very important factor in the inclusion phenomena. In particular, HP-β-CD is a special case, the hydroxypropylation means an extension of the host cavity and it accentuates its hydrophobic character [17, 18]. In spite of this, there is no enhancement of the interactions with CV^+. Probably, the HP-β-CD interferes with the guest inclusion by steric hindrance.

In aqueous solution, the UV-visible spectra changes are consistent with the binding constants in the following order: γ-CD $>$ β-CD $>$ α-CD $>$ HP-β-CD, (Table I), and this is directly related with the size ratio CD/CV^+, which indicates that the annular radii of the cyclodextrin exert a substantial degree of selectivity for a particular guest. It seems that the cavities of α-, β- and HP-β-CDs cannot include a CV_2^{2+} as does the large cavity of γ-CD (Figure 3b).

On the other hand, for the other reaction media the latter behavior is not observed, because of this we propose that the binding constants also depend on other factors besides the size of the cyclodextrins.

3.4. THE SOLVENT EFFECT

For a long time it was believed that cyclodextrin inclusion complex formation occurred exclusively in aqueous solution. After some research, it was concluded that the complexation in nonaqueous and water-organic solvent mixtures is weaker than in pure aqueous solutions [19]; however, Nelson et al. [20] reported the opposite behavior for pyrene complexes in water/*t*-butanol mixtures. On this basis, one can conclude that the solvent plays a key role in the complexation process [14, 20, 21].

We consider the organic solvents influence on the inclusion complexes stability due to the great importance of the solvophobic effect on the inclusion phenomena. We have chosen DMSO due to its poor inclusion into the cyclodextrin cavities. It shows small binding constants, 0.41 M^{-1} with α-CD [22], and 1.8 M^{-1} with β-

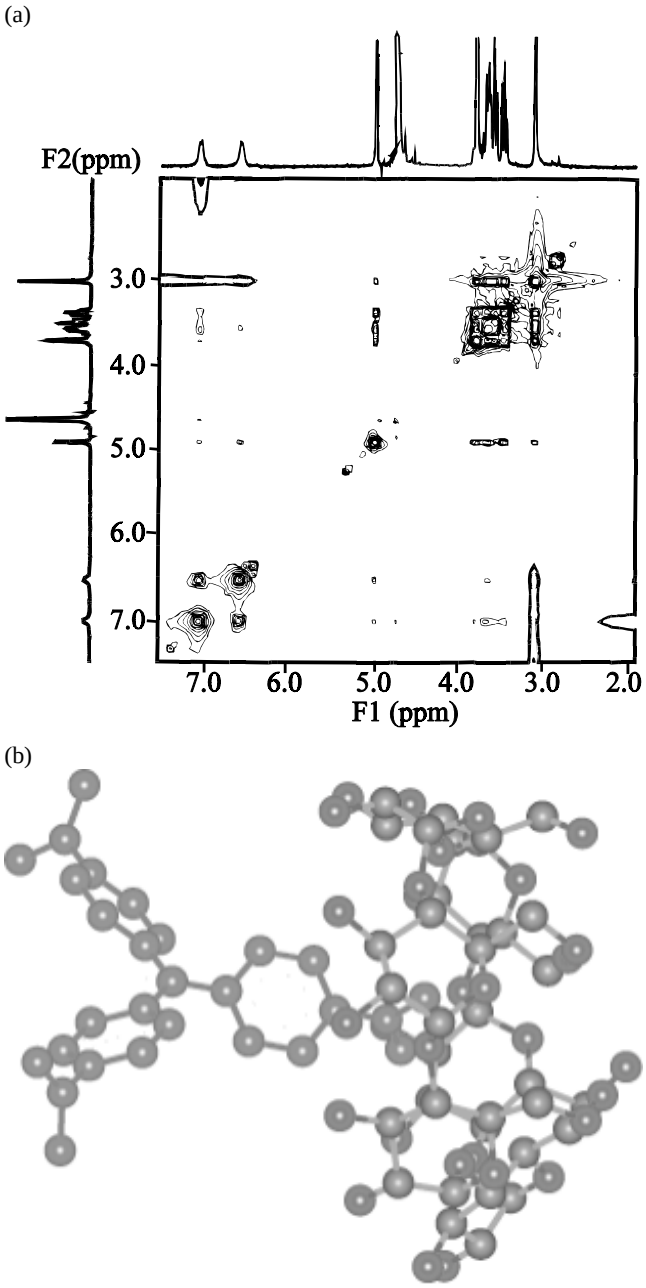


Figure 3. (a) ^{1}H NMR (NOESY) of CV^{+} with γ-CD in D_2O at 298 K. (b) Minimized structure of the $[CV_2 \subset \gamma\text{-CD}]^{2+}$ complex in aqueous solution. Signal assignment is displayed in Figure 2.

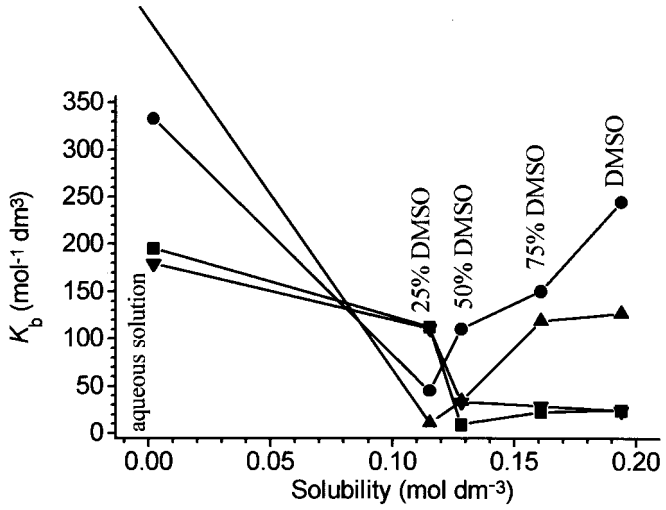


Figure 4. Binding constants vs CV^+ solubility in the different used media: (—■—) α-CD; (—●—) β-CD; (—▲—) γ-CD and (—▼—) HP-β-CD.

CD [14]. Besides, it allows us to maintain the cyclodextrin concentration used in aqueous solutions.

The guest solubility increases with the amount of DMSO in the reaction medium, that is the CV^+ solvation is higher when the ratio of DMSO/water is increased.

Figure 4 shows the binding constants as a function of CV^+ solubility in different media. When the guest solubility increases (solvophobic effect decreases), the stability constant decreases for α- and HP-β-CDs. For these, we suggest that a simple association takes place (Figure 2).

In view of the results, and assuming that these complexes have the same geometry in all solvents for each cyclodextrin, we consider that the reaction of CV^+ with α- or HP-β-CDs is mainly driven by the solvophobic effect.

When the interactions between CV^+ and γ-CD are studied, the aqueous solution results can hardly be compared with those from other reaction media, because the inclusion complex stoichiometry is different. Considering that the CV^+ solubility and the binding constants increase in parallel, we suggest that van der Waals forces are more important in these cases than the solvent effect.

We consider that the complex geometry in all the solvents is very similar for the above cases with γ-CD, excluding those in aqueous solution.

According to the above mentioned NMR studies, the geometry of the inclusion compounds between CV^+ and β-CD in water and DMSO is different. Probably due to the lower solvophobic effect and the higher CV^+ solvation in DMSO. In the mixtures and in pure DMSO, we assume that the inclusion complex geometry is very similar and the complex stability mainly depends on van der Waals interactions more so than the solvent effect.

Table II. Thermodynamic parameters studied for gentian violet with different cyclodextrins in aqueous solution and DMSO

CDs	Reaction media	Thermodynamic parameters	
		ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
α-CD	Aqueous solution	44.37 ± 0.05	184.8 ± 0.2
	DMSO	-4.20 ± 0.03	12.21 ± 0.01
β-CD	Aqueous solution	14.99 ± 0.05	97.5 ± 0.2
	DMSO	-32 ± 2	-63 ± 8
γ-CD	Aqueous solution	-103 ± 5	-195 ± 17
	DMSO	-31 ± 3	-60 ± 10
HP-β-CD	Aqueous solution	13.45 ± 0.05	88.8 ± 0.2
	DMSO	40.0 ± 0.2	162.7 ± 0.6

3.5. THE TEMPERATURE EFFECT

Tables I and II show the thermodynamic parameters (K_b, ΔH, and ΔS) for the studied complexation reactions. The ΔH and ΔS values have been obtained from a van't Hoff analysis [23, 24] (Figures 5a and 5b).

The enthalpy and entropy values are the sum of several contrasting effects: release of the water molecules from the interior of cyclodextrins [25–28], relief of cyclodextrin strain energy [29, 30], the inclusion depth and guest solvation (solvent effect), and finally the contacting surface magnitude between host and guest (van der Waals interactions).

Jenks [31] pointed out that a favorable ΔH may result from solute-solute interactions or from increased solvent-solvent interactions, which are made possible by the greater solvent order [32]. An unfavorable ΔS suggests that the formation entropy is largely associated with a loss of the cyclodextrin and guest mobility. The contacting surface between host and guest varies depending on the depth of inclusion and on the guest size. Consequently, van der Waals interactions as well as the complex stabilizing force will be affected.

Taking into account all the factors that participate in the complexation phenomena, it is possible to discuss thermodynamic parameters in relation to each other. From our experimental data, we may deduce that the difference in the number of water molecules excluded from the cyclodextrin cavity, and relief of ring strain are not the most important driving forces in the gentian violet complexation. The entropy of complexation would be expected to be less negative as the cyclodextrin cavity became larger, since the movement of its glycosidic linkages was less restricted by substrate penetration. But, in general, in our systems, ΔS follows the order: in aqueous solution (γ-CD < β-CD < α-CD) and in DMSO (β-CD < γ-CD < α-CD). Probably this behavior results because the depth of complexation is of the opposite order, as demonstrated by the spectroscopic studies. However, the HP-

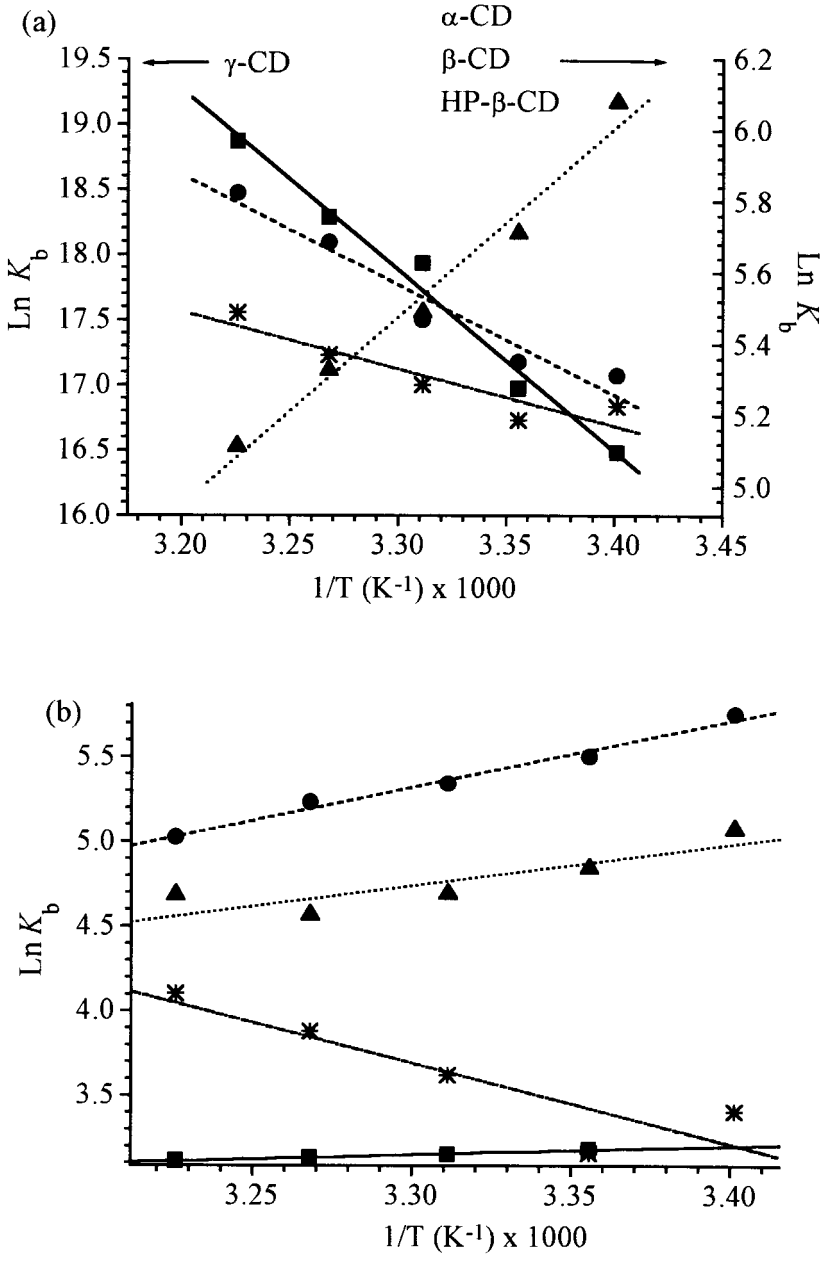


Figure 5. Van't Hoff plots of the CV^+ with α, β, γ and HP-β-CDs in aqueous solution and DMSO, (a) and (b) respectively. (—■—) α-CD; (—●—) β-CD; (—▲—) γ-CD and (—∗—) HP-β-CD.

β-CD thermodynamic behavior, in DMSO, is different from that of the other three hosts. We assume that it is related with its cavity depth, hydrophobic characteristics of the hydroxypropylated chains and the freedom of movement of these groups.

Considering that the complex geometry in both solvents, for α and HP-β-CDs, is very similar, their different behavior in each medium cannot be explained only by the differences in host-guest interactions, but is also due to specific contributions by the solvent. In general, their ΔS and ΔH values are typical of classical solvophobic interactions [33–36].

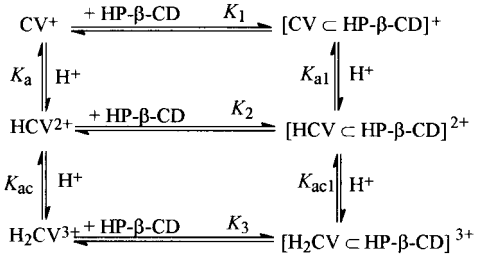
It is known by bidimensional ^{1}H NMR spectroscopy (Figure 3a) that the interior of cyclooctamylose, in aqueous solution, is completely occupied by two guest molecules. However, in DMSO, the depth of complexation is different and consequently also the host-guest and solvent-solute interactions. In both solvents, negative ΔH and ΔS values are characteristic of van der Waals interactions, therefore, we suggest that they are the most important driving forces in these systems.

^{1}H NMR studies for [CV $\subset$ β-CD]$^+$ complexes in aqueous solution and in DMSO indicate different inclusion depth. Thermodynamic parameters obtained in buffer, the favorable ΔS and unfavorable ΔH, indicate the great importance of solvophobic interactions. A very large similarity exists between the behavior of β- and γ-CDs in DMSO. van der Waals interactions are the most important component in the complex stability for these systems.

3.6. INFLUENCE OF pH

Spectroscopic titration of CV^+ and CV^+ with HP-β-CD were carried out with hydrochloric acid (Figure 6), and the influence of pH upon complexation can be observed.

CV^+ can accept two protons in its two uncharged amine groups. Scheme III shows the different equilibria that we have taken into account.



Scheme III.

The binding constants change with pH as the hydrophobic character of the charged CV^+ molecules decreases. At pH values in which uncharged molecules exist, better inclusion binding is obtained [24].

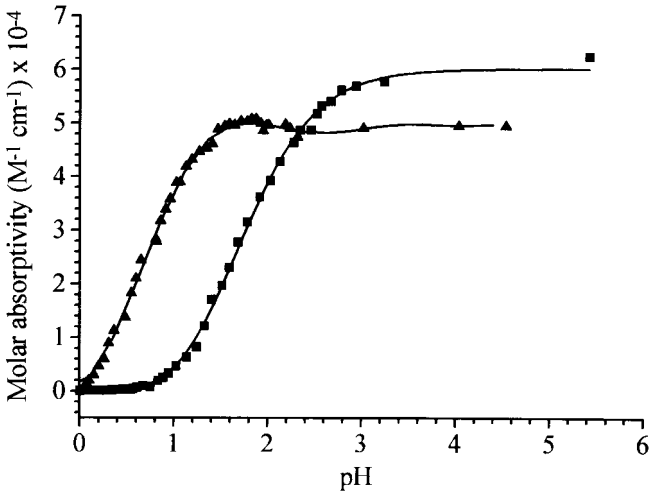


Figure 6. Molar absorptivity vs pH of CV^+ with (—▲—) and without HP-β-CD (–■—).

We have obtained pK_a for complexed gentian violet with HP-β-CD (pK_{a1} = 0.75 ± 0.02, pK_{ac1} = 1.14 ± 0.11) and compared them with pK_a for the uncomplexed form (pK_c = 1.07 ± 0.09, pK_{ac} = 1.88 ± 0.14). Complexing between CV^+ and HP-β-CD leads to a decrease in the basic strength of the substrate, which is equivalent to saying that the monoionized CV^+ reacts more strongly than does the trivalent cationic form. The study at various pH values shows that both the tricationic and the monocationic forms of CV^+ interact with hydroxypropylated beta cyclodextrin, the latter more strongly (Figure 6).

Electrostatic forces were not found to be important interactions for the inclusion compound formation in this study.

4. Conclusions

Our studies indicate that cyclodextrin size influences the complexation, but it is not the most important factor. On the other hand, in our systems the inclusion phenomenon is usually mostly entropically driven with a minor or major enthalpy contribution. Solvation plays an essential role in the binding of these systems and the differences in solvation are very important to elucidate some of the main features characterizing host-guest interactions in solution. In all cases, the inclusion orientation is probably determined by the ion-dipole interactions between the gentian violet and the solvent. Finally, we can say that two different behaviors are observed in this research; one was present with both α and HP-β-CDs, while a different behavior was shown by β and γ-CDs. Solvophobic effect is the most important binding factor in the former systems, but van der Waals interactions lead the complexation reaction in the latter ones.

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