J Incl Phenom Macrocycl Chem (2008) 60:329–338
DOI 10.1007/s10847-007-9382-4

ORIGINAL ARTICLE

Spectral properties and supramolecular inclusion complex formation between 2-styrylbenzothiazolium dye and cyclodextrins

Ivan Petkov · Asya Petinova · Stanimir S. Stoyanov · Stefan Metsov · Stefan I. Stoyanov

Received: 15 May 2007 / Accepted: 13 October 2007 / Published online: 31 October 2007

Abstract The effect of native and randomly methylated β-CDs on the absorption and steady-state fluorescence spectra of 2-(4-dimethylaminostyryl)-3-(2-hydroxyethyl)-benzothiazolium chloride (DHB) in aqueous buffer solutions with various pH values was studied. The inclusion with both CDs at pH 7.2 barely changed the UV spectra, whereas significant variations were produced in the emission spectra in all buffer solutions. In all cases the CDs increase guest fluorescence. The 1:1 stoichiometry of the inclusion complexes of the dye with both CDs was established according to the modified Benesi-Hildebrand method. Binding constant values were calculated using the iterative nonlinear least-squares regression approach. The pH of the solution and the type of the CD affected complex stability. The results indicate that native β-CD possesses better complexing ability towards DHB than randomly substituted β-CD and that the most stable inclusion complexes are formed in basic medium because of the structural changes in the guest molecule. In basic medium an attempt is made to interpret the proposed mechanism in terms of molecular rearrangement which take place as the dye penetrates the CD cavity.

I. Petkov · A. Petinova (✉) · S. S. Stoyanov
Department of Organic Chemistry, Sofia University, James Bourchier Blvd. 1, 1164 Sofia, Bulgaria
e-mail: asya_petinova@chem.uni-sofia.bg

I. Petkov
e-mail: ipetkov@chem.uni-sofia.bg

S. Metsov · S. I. Stoyanov
Department of Applied Organic Chemistry, Faculty of Chemistry, Sofia University, James Bourchier Blvd. 1, 1164 Sofia, Bulgaria

Keywords Cyclodextrins · Inclusion complexes · Optical spectroscopy · Stability constant · Styrylbenzothiazolium dyes

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six or more α-D-glucopyranoside units with hydrophilic exterior and hydrophobic internal cavity. This feature provides CDs the possibility to form inclusion complexes with a variety of organic molecules in aqueous solutions [1]. The formation of inclusion complexes is based on interactions of a noncovalent nature and on steric effects. The stability of inclusion complexes in aqueous solutions is mainly due to van der Waals forces and hydrophobic interactions [2] in which the solvent water pushes the hydrophobic side of the guest molecule into the hydrophobic CD cavity. In this way the organic guest can enter the cavity in whole or in part depending on geometrical factors. Therefore, methylation of the hydroxyl groups of CDs would lead to a change in the stability, solubility, and structure of the inclusion complexes. The complexation by CDs can alter some of the physical and chemical properties of guest molecules, such as solubility, chemical reactivity and the spectroscopic and electrochemical properties. The reduced polarity and the protection provided by the cyclodextrin cavity have a strong and often favorable influence on the properties of the included solute. The key observation is that the formation of supramolecular complexes of analytes with CDs provokes an increase of their fluorescence quantum yield [3, 4] or even the appearance of room-temperature phosphorescence [5, 6].

The styryl dyes with electron donor–acceptor moieties on either side of the styryl fragment are particularly attractive for their spectral sensitivity towards local environment and for

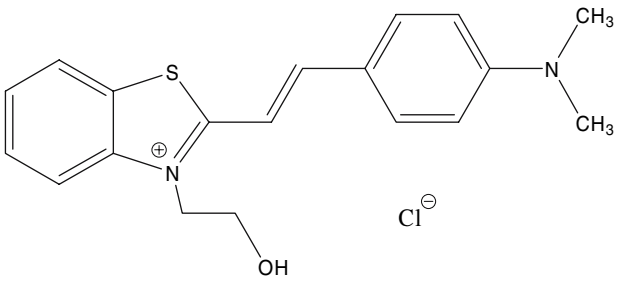
their optical and electronic properties. They are widely used in medicine for the fluorescent labeling of biomolecules [7, 8], such as dyes for optical records for image and information storage, as active materials in dye lasers, as chromoionophores in trace metal detection, etc. Relatively few works have been devoted to the spectral study of CD complexes with hemicyanine dyes, and these focus mainly on their photostability. Kasatani [9, 10] and Matsuzawa [11] observed enhancement of dimerization and an increase of the light stability of cyanine dyes with long polymethine chain in the presence of β- and γ-CD. Styrylindolium, styrylpyridinium and styrylbenzothiazolium cationic dyes are unsymmetrical polymethine dyes which were used as chromophores by modification of crown ethers [12–14] and calixarenes [15] for spectrophotometric detection of metal ions and surfactants. For the first time a 2-hydroxyethyl group was used as quaternary function in benzothiazolium dyes by synthesis of symmetrical trimethinecyanines with higher photographic sensitization [16]. Petkov et al. investigated the spectral and photochemical properties of a trimethinecyanine dye, derivative of 3H-indole, containing a 2-hydroxyethyl group on the nitrogen atom depending on the polarity and proticity of the solvent [17] and in polymer films [18]. However there is no evidence on the kinetics of cyclization of this 2-hydoxyethyl substituent neither in solution nor in restricted medium.

Our interest was focused on the inclusion of dye substrate into the CD cavity since these species could be candidates for optical sensors of organic molecules. In this contribution, we would like to extend our work which is related to the investigation of supramolecular systems between styrylheterocy -clic dyes as guests molecules and cyclodextrins as hosts, and to the efficiency of complex formation according to some special features of the medium [19]. Here we report on the study of the environmental effect on the spectral properties of a representative styrylbenzothiazolium dye containing quaternary N-2-hidroxyethyl substituent in the heterocycle, namely 2-(4-dimethylaminostyryl)-3-(2-hydroxyethyl)-benzothiazolium chloride (DHB) (Scheme 1). We chose native β-CD and its derivative methyl-β-CD (randomly substituted) as hosts in order to evaluate the influence that substitution may exert on complex stability and the stoichiometric ratio. These factors could also be influenced by the pH value of the medium because of the structural changes in the guest molecule. The differences in the optical properties of free and complexed dye have been discussed.

Experimental

Reagents

All experiments were performed with analytical grade chemicals. β-CD (obtained from Fluka, purum $\geq$99%, HPLC) and Me-O-β-CD (average M = 1,310 g/mol) purchased from Aldrich were used as received. The dye 2-(4-Dimethylaminostyryl)-3-(2-hydroxyethyl)-benzothiazolium chloride (DHB) was synthesized as described earlier and its structure was proved by means of IR and ^{1}H NMR spectroscopies [20]. The purity of the dye in these samples had been established by thin layer chromatography and optical spectroscopy. Distilled water and phosphate buffer solution (pH 7.20), citrate-hydrochloric acidic buffer (pH 2) and boric acid/potassium chloride–NaOH buffer (pH 9) obtained from Merck were used to control the pH-value. All other solvents used were of the spectroscopic grade commercially available.



Scheme 1 Structure of 2-(4-dimethylaminostyryl)-3-(2-hydroxyethyl)-benzothiazolium chloride (DHB)

Apparatus

The absorption spectra were recorded on a Thermo Spectronic instrument Unicam UV 500 UV–Visible double-beam spectrophotometer using 1 cm quartz cells. Data processing was performed on a Hewlett Packard computer with Vision-Pro software. Samples were scanned from 190 to 900 nm. Steady-state fluorescence measurements were performed with Cary Eclipse Fluorescence spectrophotometer from Varian. The excitation source was a xenon flash lamp. The fluorescence spectra of each set of solutions were measured using identical experimental conditions (excitation and emission bandwidths, excitation wavelength). All experiments were carried out at room temperature.

Solution preparation

The dye stock solutions were prepared by dissolving an appropriate amount of DHB in aqueous buffer solutions. The standard solutions of β-CD and Me-O-β-CD in the corresponding buffer solutions were prepared with concentration 3.5×10^{-3} M. In order to study the influence of the matrix on the dye intensity in each medium, several cyclodextrin solutions were prepared, by maintaining

constant dye concentration and varying the CD concentration. The concentration of the DHB solutions was of the order 1×10^{-5} to 4×10^{-5} mol/L for the separate measurements. An aliquot of the guest stock solution was placed in a 10-mL volumetric flask, an appropriate amount of cyclodextrin solution was added to reach the final CD concentration and it was diluted with the solvent to the final volume of 10.0 mL. All the measurements were carried out after at least 8-h dark storage.

Results and discussion

Absorption spectra

Influence of the solvent

We have studied the effect of solvent polarity on the UV–Vis spectra of DHB. In line with the literature data for other substituted 2-styrylbenzothiazolium salts [21], the position of the absorption band is very sensitive to the change of the solvent and the maximum shifts hypsochromically as solvent polarity increases. The longest absorption maximum of DHB in various solvents is presented in Table 1. With increasing solvent polarity [22], there is a large blue shift of the π–π^* band which suggests that the ground electronic state has a larger solvation than the first excited singlet state. It can be safely concluded that the dipole moment of the ground state is larger then that of the excited state.

It can be seen from Table 1 that, in the presence of β-CD, there is an additional blue shift of the absorption maximum compared to the aqueous solution. It is well known that the polarity of the CD cavity is much lower than water polarity. So this shift is not a result of a change of the environment polarity; it is rather the result of a change in the structure of the dye. When DHB penetrates into the CD nanocapsule, host–guest hydrogen bonding between the nitrogen atom from the dimethylamino-substituent and hydroxyls from the primary rim of CDs takes place. As a result of hydrogen bonds, the donor ability of the N-atom decreases, the conjugation, which covered the whole molecule, is partially hindered and the dye absorbs light with higher energy. Hence we assumed that DHB and CDs form host–guest inclusion association in water solution.

Influence of pH of the medium

The absorption spectrum of DHB in aqueous buffer solution with pH 7.2 and the changes of electronic spectra which occur when the acidity and the basicity of the water solution are increased are shown on Fig. 1. The presence of both donor and acceptor ends in the parent chromophore system (dialkylamino- and benzothiazolium quaternary nitrogen atoms, respectively) accounts for the appearance of a broad long-wave absorption charge-transfer band (CT) at about 517 nm (solid line in both spectra). Electronic charge is transferred from the amino-substituent of the phenyl ring to the nitrogen atom of the heterocycle upon excitation to the lowest excited state. By serial dilutions of the dye stock solution, we have determined that DHB obeys the Beer-Lambert law with the concentration used and the molar absorptivity at 517 nm and pH 7.2 is 49,200 L mol^{-1} cm^{-1}.

The increase of the acidity of the medium (Fig. 1a) is accompanied by a considerable colour change in the DHB solution (purple to colourless) due to the protonation of the dye molecules on the atom with higher basicity: $-N(CH_3)_2$. In this case, the intramolecular CT does not occur, the free electron pair cannot take part in delocalization with another unsaturated part of the molecule and the long-wavelength band disappears. A new absorption band appears in the UV region due to localized π–π^* at the cost of the CT band (Scheme 2).

A clear isosbestic point (408 nm) is observed in the absorption spectrum suggesting the existence of only two absorbing species under these conditions, which turn into each other.

In the other case, the increase of the basicity of the medium (Fig. 1b) resulted in a substantial change of the electronic spectrum of DHB and in bleaching of the solution. The intensity of the long-wavelength band at 517 nm decreased, whereas a new band at around 355 nm appeared.

This medium exerts an influence on both host and guest molecules. In the host molecule, the basic medium destroys the intramolecular hydrogen-bond belt on secondary rim of the CD, which facilitates the association. The increased basicity of the DHB solution provokes a deprotonation of the N-2-hydroxyethyl group, formation of an open form ($-CH_2CH_2O^-$) and a subsequent cyclization of the substituent to an oxazolidine cycle (Scheme 3). The

Table 1 Effect of solvent polarity and β-cyclodextrin on visible spectra of DHB. Solvent static dielectric constants ε_{stat} (all quoted from [22])

Solvent	Chloroform	Pyridine	Acetone	Ethanol	Methanol	Acetonitrile	Water	Water + CD
ε_{stat}	4.7	12.3	20.7	24.3	32.6	36.2	78.5	–
λ_{max} (nm)	548	539	523	528	524	523	517	508

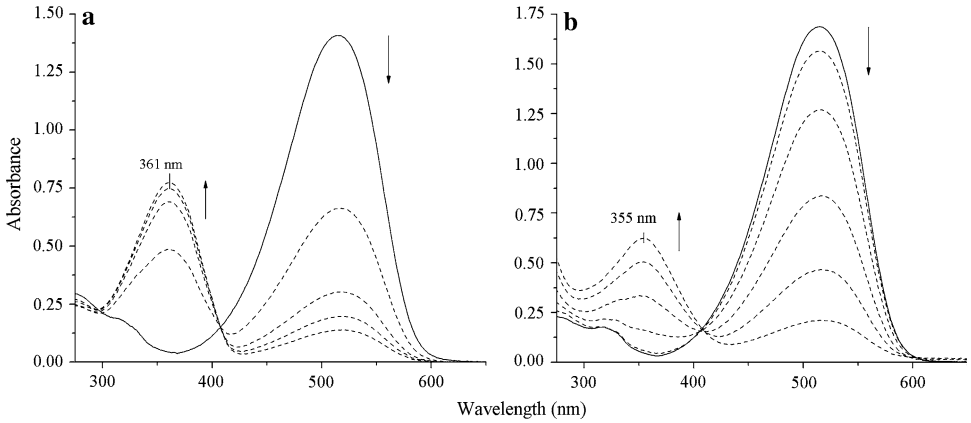
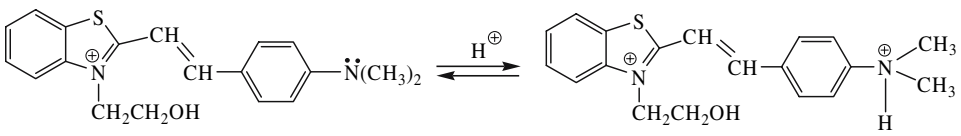


Fig. 1 Absorption spectra of DHB at pH 7.2 (solid line) at various concentrations of HCl ($2.2 \times 10^{-2} \div 8.6 \times 10^{-2}$ M) (**a**) and at various concentrations of NaOH ($0.8 \times 10^{-2} \div 2.3 \times 10^{-2}$ M) (**b**) (dashed lines)

Scheme 2 Conversion of DHB by increasing the acidity of the medium



517 nm 361 nm

cyclization disturbs the conjugation and eliminates the acceptor end in the dye molecule. As a result, in basic medium, DHB absorbs in a shorter wavelength region—colourless (closed) form. The isosbestic point at 407 nm provides strong evidence for the existence of only two forms and the presence solely of the process open-closed form.

Effect of CDs on the absorption spectra of DHB

We have traced the influence of native and modified CDs on the absorption spectra of the dye at various pH values by increasing their concentrations. When CD concentration was increased, the intensity of the maximum slightly decreased and the maximum shifted to shorter wavelengths relative to the spectrum of DHB alone (from 517 to 507 nm), which suggests host–guest interaction (Fig. 2). Furthermore, the absorption spectra exhibit multiple isosbestic points due to the existence of various absorbing species in the solutions.

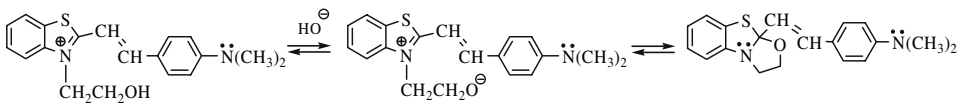
The increase of Me-β-CD concentration leaded to analogous changes in the absorption spectra of DHB, but to a lesser extent. Perhaps the substitution by O-methyl groups at both the narrow and the wide rim of β-CD reduces the inner diameter of the cavity and prevents the inclusion of the dye.

As mentioned above, there are two absorbing species in the solution in acidic medium—nonprotonated DHB and protonated $DHBH^+$ which absorb at 517 nm and 361 nm, respectively. The change in the absorption spectra of DHB when β-CD is added at pH 2 is shown on Fig. 3.

The increase of host concentration leads to a decrease of short-wavelength intensity and to an increase of the long-wavelength absorbance (Fig. 3). There is a well defined isosbestic point, which indicates the conversion of the protonated form into non-protonated. The changes of the absorption intensity of the guest upon addition of CDs at pH 2 are good pronounced. Moreover evidence for the formation of the inclusion complexes gives up not only the intensity changes but also the shift of the absorption maximum relative to the spectrum of DHB alone. This finding indicates that the dye molecule changes its environment and forms an inclusion complex with CDs. In our case the spectral increase which corresponds to the non-protonated DHB (517 nm) is accompanied by a hypsochromic shift of the maximum (511 nm) upon addition of CD.

Such behaviour in acidic medium shows that CDs interact with the DHB molecule and, furthermore, this interaction takes place only with the non-protonated form. By adding host molecules, an inclusion complex between this form and the macrocycles is formed and the equilibrium moves to the left (Fig. 4).

Scheme 3 Conversion of DHB by increasing the basicity of the medium



cationic form (517 nm) zwitter-ionic (open) form (517 nm) spiro (closed) form (355 nm)

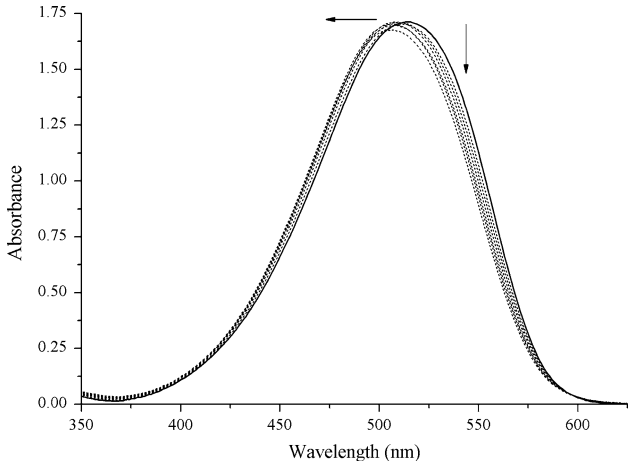


Fig. 2 Absorption spectra of DHB (3.4×10^{-5} M) in aqueous buffer solution (pH 7.2) (solid line) at various β-CD concentrations (dashed lines): 0; 3.5×10^{-4}; 7×10^{-4}; 1.1×10^{-3}; 1.4×10^{-3}; 1.8×10^{-3}; 2.1×10^{-3} M

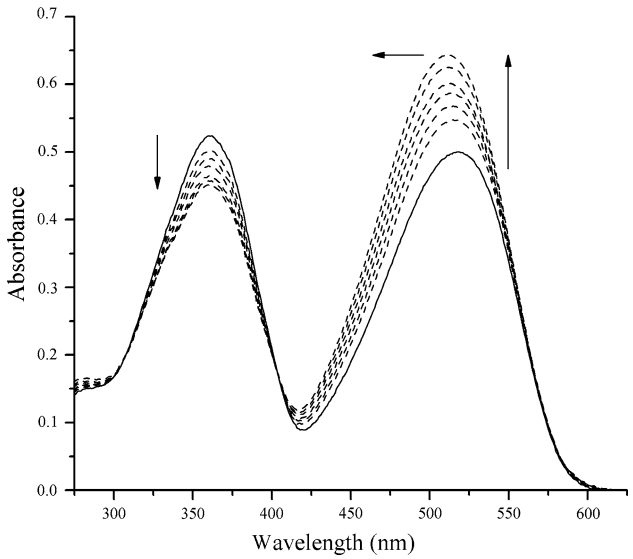


Fig. 3 Absorption spectra of DHB (2.7×10^{-5} M) in aqueous buffer solution (pH 2) (solid line) at various β-CD concentrations (dashed lines): 7×10^{-4}; 1.1×10^{-3}; 1.4×10^{-3}; 1.8×10^{-3}; 2.1×10^{-3}; 2.5×10^{-3} M

Based on these spectral observations on the complex formation of DHB, we assumed that the dye penetrates the cyclodextrin cavity by means of its phenyl ring together with the *N*,*N*-dimethylamino group and not with the heterocycle. The former group remains hidden for the hydrogen ions of the bulk solution. Besides, this type of complexes could be stabilized through the formation of hydrogen bonds between the amino-N-atom and hydroxyls from the primary rim of CDs.

In basic medium, DHB exists in two forms—ionic and neutral molecules, which are in equilibrium. As reported by Petkov et al. [17], this equilibrium between open and closed forms depends on the peculiarity of the environment. We used absorption spectroscopy to determine if the cyclodextrin macromolecule influences the process of cyclization and its rate. We monitored this response at equal intervals of time for the solutions of DHB alone and in the presence of β-CD after addition of 0.1 mL 0.1 M NaOH to both solutions. The rate of the cyclization process obeys a first order rate law (Fig. 5). For the first order process, the plot of ln [DHB] versus the reaction time is linear, with a slope k, which is the rate constant of the open-closed process. It was found that the cyclization rate constant in the presence of β-CD (2.5×10^{-3} M) is greater ($k = 1.43 \times 10^{-3}$ s^{-1}) than that of the free dye ($k = 8.46 \times 10^{-4}$ s^{-1}). Thus the rate of cyclization of the 2-hydroxyethyl substituent in the presence of β-CD is 1.7 times greater than the said rate without the CD.

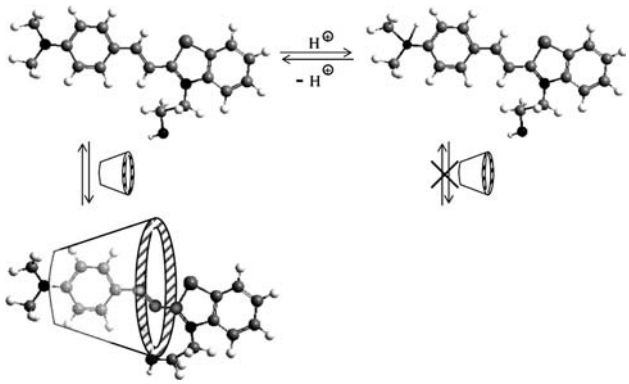


Fig. 4 Disturbance of the equilibrium after addition of CD and the proposed structure of the 1:1 inclusion complex

Similar behaviour could be readily predicted when we know that inclusion complexes between dye and CD are

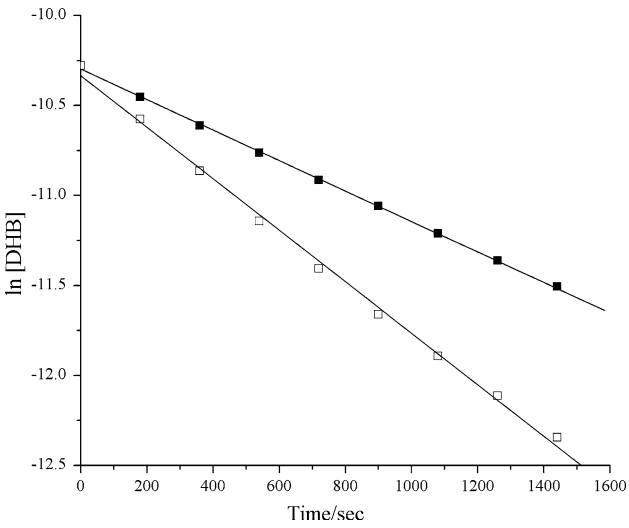


Fig. 5 Difference in the slope of the first-order plots for open-closed process of DHB alone (■) and in the presence (□) of β-CD (2.5×10^{-3} M). Reaction followed spectrophotometrically at 517 nm

formed. There is a reduction of the donor ability of the N-atom from the dimethylamino-substituent in the cavity of the CD, as a result of hydrogen bonding between $-N(CH_3)_2$ and β-CD-OH groups. The disrupted conjugation leads to enhancement of the partial positive charge of the C-atom from the heterocycle (spiro C-atom) and it makes the cyclization more favored. Thus the two processes—intramolecular charge transfer and formation of C–O bond, are competitive.

It should be pointed out that the kinetic experiments are made with solutions of DHB and DHB with β-CD (inclusion complexes) prepared in advance in neutral aqueous solution and with a subsequent alkalization of these solutions. Whereas the UV–Vis and fluorescence measurements were made by diluting the dye in the buffer solution (both open and closed forms) and subsequently adding of the CDs solution, diluted in the same buffer solution.

An interesting behavior was observed when CD concentration in DHB aqueous buffer solutions with pH 9 was varied (Fig. 6). The intensity of the longest wavelength absorption band (517 nm) decreased gradually and this was accompanied by a hypsochromic shift of the maximum upon inclusion into the CD cavity. We also observed that, in the presence of CD, an equilibrium is established with a greater quantity of cyclic product, which absorbs at 355 nm, than in the solution of pure dye in this medium. This is due to the fact that the cyclization reaction in the cavity of CD becomes easier. The position and intensity of the cyclic form do not change by further increase of CD concentration.

Fluorescence spectra

Effect of CDs on the fluorescence spectra of DHB

The most common feature of a fluorescent molecule resulting from the complexation with CDs or CD derivatives in aqueous media is the intensity enhancement and the spectral blue shift of its fluorescence spectrum. This is mainly due to the hydrophobic nature of the CD cavity—this feature is similar to the one observed when the solvent medium is changed from water to a less polar solvent. In this respect, DHB shows relatively low fluorescence in aqueous solution which increases regularly as host molecules are added (Fig. 7). The emission spectrum of DHB alone (pH 7.2) shows a maximum at about 597 nm which shifts to 587 nm as host concentration increases. Upon excitation in the maximum absorption (517 nm), the emission maximum of the free dye remains unaffected (in the region 594−597 nm) in the various aqueous buffer solutions and there are changes only in the intensity because of the presence of some other absorbing species in the solution with pH 2 and pH 9. The excitation and emission bandwidths were both set at 5 nm, and the excitation wavelength was 517 nm.

As Fig. 7 shows, when CDs are added, the fluorescence intensity of DHB is enhanced, which is accompanied by a shift of the fluorescence maximum to shorter wavelengths from 5 to 10 nm, depending on the type of CD. These findings indicate that the dye molecule changes its environment and interacts with the corresponding CD. The factors accounting for the variations in fluorescence

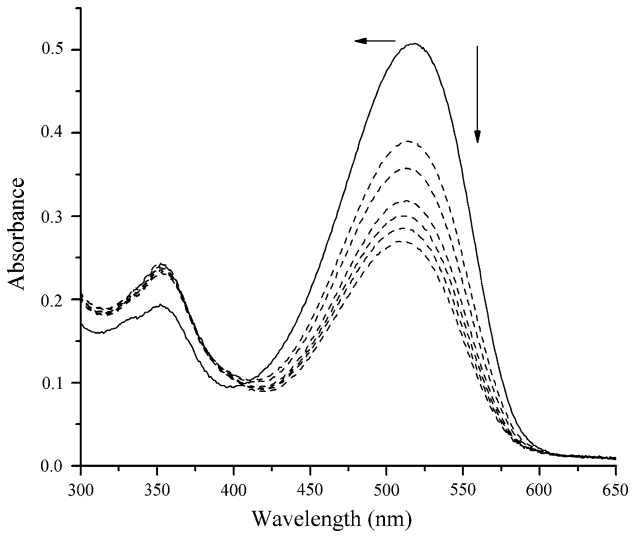


Fig. 6 Absorption spectra of DHB (2.2×10^{-5} M) in aqueous buffer solution (pH 9) (solid line) at various β-CD concentrations (dashed lines): 7×10^{-4}; 1.1×10^{-3}; 1.4×10^{-3}; 1.8×10^{-3}; 2.1×10^{-3}; 2.5×10^{-3} M

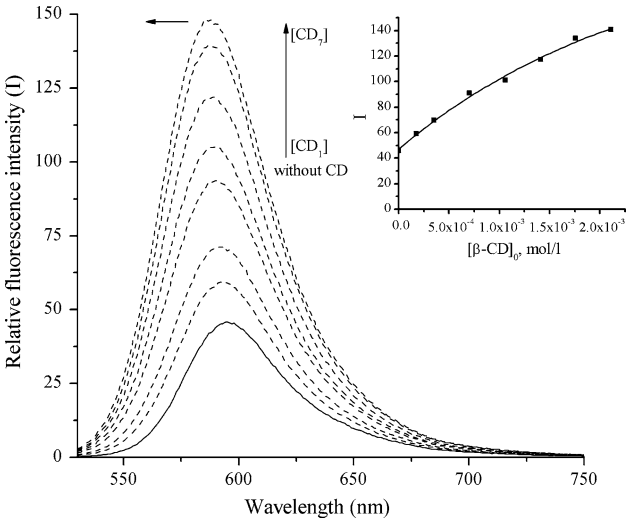


Fig. 7 Relative fluorescence spectra of DHB (2.2×10^{-5} mol/L) as a function of various β-CD concentrations (pH 7.2), excitation wavelength 517 nm: (—) without β-CD, (- - -) with β-CD: $[CD_1]$–$[CD_7] = 1.8 \times 10^{-4}$–$2.1 \times 10^{-3}$ mol/L; Inset: the plot of fluorescence intensity of DHB vs. β-CD concentration

intensity are the protection of the analyte molecule in the complex from quenching of the water molecules and, moreover, the increased viscosity in the CD cavity with a corresponding reduced quenching caused by oxygen. As a consequence of the inclusion, the host cavity can protect the excited state of DHB from the nonradiative processes and from the quenching that normally occur in an aqueous solution. Thus the fluorescence intensity of DHB increases about three times compared to the intensity of DHB alone.

The results of the fluorescence titration experiments described above are quantified by determining the integrated area under the measured fluorescence spectrum (total fluorescence) of the guest in the presence of CD (F) and by dividing it by the integrated area of the guest in the absence of CD (F_0). This ratio gives a direct measurement of the effect of the CD on the guest fluorescence intensity [23]. The ratios of the total fluorescence of complexed and free dye molecules (F/F_0) in aqueous buffer solutions with various pH values are shown in Table 2.

The values in Table 2 show higher fluorescence enhancement obtained in native β-CD relative to randomly substituted Me-O-β-CD probably because of a change in the size and shape of the macrocycle. Apparently, the methylation of hydroxyl groups increases the water solubility of host molecules by distorting the hydrogen bonds on the CD rim, but on the other hand, it narrows the inner diameter of the cavity and prevents the penetration of the guest [24]. Besides, the substitution of the OH-groups in Me-O-β-CD reduces the possibility of hydrogen bonding with the amino-N-atom of the dye, which further lowers the complex formation.

Molecular binding ability

The inclusion complexation with CDs is a result of the simultaneous contribution of a wide variety of weak interactions, among which van der Waals and hydrophobic interactions are the most important ones [25]. Both of these depend on how the size and/or shape of a guest molecule fit into the host cavity. The enhancement of the fluorescence intensity measured as a function of host concentration can be used to obtain the association constants and stoichiometry of the inclusion complexes by means of the modified Benesi-Hildebrand approach [26]. The total concentration of DHB is maintained constant, whereas the concentration of CDs is varied, provided that $[DHB]_0 << [CD]_0$ (Fig. 7) for both β-CD and Me-O-β-CD. The 1:1 modified Benesi-Hildebrand equation (1) was used to calculate the values of the equilibrium constant K_{11} for the DHB-CDs complexes.

$$\frac{1}{I - I_0} = \frac{1}{I_1 - I_0} + \frac{1}{K_{11}[CD]_0(I_1 - I_0)} \quad (1)$$

where I and I_0 are the fluorescence intensities of DHB in the presence and absence of CD, respectively, and I_1 is the expected fluorescence intensity when all guest molecules are included in a complex.

The assumption for 1:1 association between both β-CD and Me-O-β-CD and DHB reveals a linear relationship with good correlation coefficients ($r > 0.99$) for all pH values (Fig. 8). A plot assuming 1:2 association of DHB:CD reveals a downward curvature as CD concentration is increased. This deviation suggests that the stoichiometry of the complexes is not 1:2.

However, the relative errors in measuring the binding constants of CD complexation according to the Benesi-Hildebrand method are usually essential at high CD concentrations. Therefore, a better estimation can be made using nonlinear regression analysis. The nonlinear method provides estimates for K_{11} by fitting the data through iteration, i.e., by varying the values of the initial parameters I_0, I_1 and K_{11}, using the following equation (assuming a 1:1 association):

$$I = \frac{I_0 + I_1 K_{11}[CD]_0}{1 + K_{11}[CD]_0} \quad (2)$$

The nonlinear regression method is based on the Levenberg-Marquardt algorithm and the goodness-of-fit between the calculated and experimentally measured values of I was judged by means of the chi-square value (χ^2) and the correlation coefficient (r). The K_{11} values which give the best fit (smallest χ^2 and r close to unity) between the calculated and experimental values of I have been chosen as experimentally-determined constants and are shown in Table 3.

Table 2 Ratios of the total fluorescence of included and free dye molecules at various pH values for the same concentration of CDs (2.1×10^{-3} mol/L)[a]

	F/F_0					
	β-CD	Me-O-β-CD	β-CD	Me-O-β-CD	β-CD	Me-O-β-CD
DHB (2.2×10^{-5} M)	2.64	2.50	3.45	3.36	2.69	2.32
pH values	2		7.2		9	

[a] Aqueous buffer solutions with pH 2, 7.2 and pH 9

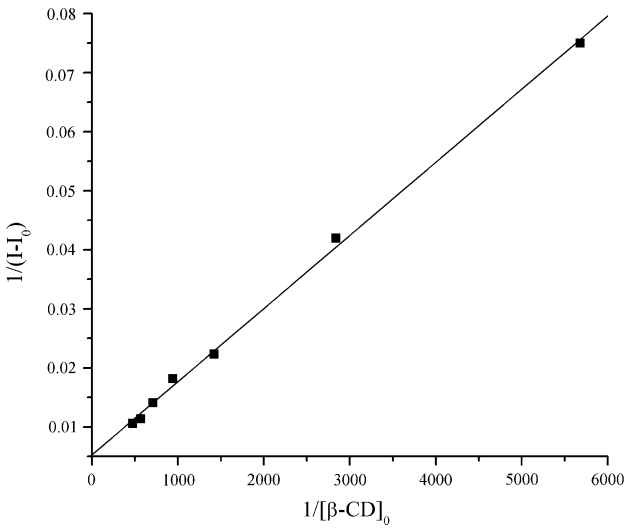


Fig. 8 Benesi-Hildebrand plot for the assumption of 1:1 stoichiometry of the complex between β-CD and DHB in aqueous buffer solution (pH 7.2)

As Table 3 shows, comparatively higher stability constants were obtained with native β-CD than with methyl-β-CD for all pH values, which suggests that β-CD possesses better complexing ability than randomly substituted β-CD towards DHB. The possible reasons could be the steric factor or size–shape incompatibility. The methylation of hydroxyl groups leads to distortion of the intramolecular hydrogen bonds of the β-CD rim and to an increase in solubility. On the other hand, the random substitution of β-CD narrows cavity diameter and hinders association [24, 27]. Besides, the substitution of the OH-groups in Me-O-β-CD reduces the possibility of hydrogen bonding with the amino-N-atom of the dye, which additionally lowers complex stability.

As mentioned above, the hydrophobic interaction is a major factor for the stability of host–guest complexes. As a rule, inclusion of charged molecules in the cavity is less favored than that of uncharged molecules. This leads to lower K_{11} values at pH 7.2 and pH 3 compared to those at pH 9 (950, 867 M^{-1}). DHB exists in ionic form in neutral and acidic media. In alkaline solution, the formation of oxazolidine cycle leads to receiving uncharged (neutral) guest molecules and consequently to the formation of much more stable complexes. Besides, the elimination of the acceptor group (quaternary nitrogen atom) as a result of cyclization increases the basicity of the amino-nitrogen atom and facilitates the formation of hydrogen bonds with primary OH-groups of both CDs.

Table 3 Stability constants K_{11} (mol^{-1} L) for 1:1 complexes of DHB and CDs at various pH values

Macrocycle	pH value	K_{11}	Log K_{11}
β-CD	2	131 ± 55	2.12
	7.2	256 ± 78	2.41
	9	950 ± 134	2.98
Me-O-β-CD	2	97 ± 30	1.99
	7.2	136 ± 89	2.13
	9	867 ± 159	2.94

The smallest binding constants correspond to the inclusion complexes between DHB and the two CDs in acidic medium. At pH 2, the dye exists in two forms—non-protonated, which absorbs at 517 nm, and protonated—$DHBH^+$, which absorbs at 361 nm. However, as the absorption spectra show (Fig. 3), only the non-protonated form penetrates the cavity but its content in this medium is reduced as a result of hydrogen ion association.

Fluorescence spectra in acidic and alkaline media

In acidic and in basic medium, there are other absorbing species in buffer solutions as the electronic spectra have shown, and consequently both the ionic and the neutral emission bands appear. The fluorescent spectra under such conditions are obtained by excitation at the maximum of short-wavelength absorption ($\lambda_{ex} = 361$ nm and $\lambda_{ex} = 355$ nm at pH 2 and pH 9, respectively). By increasing CD concentration at pH 2, the emission intensity at 452 nm decreases at the cost of the intensity at 595 nm, which corresponds to the main structure of DHB. There is an isoemissive point in the emission spectrum (Fig. 9a) which confirms the transformation of $DHBH^+$ into DHB when hosts are added.

In basic medium, the situation is different. There is an increase of fluorescence intensity for both closed and open forms and the neutral band is more intense than the cationic band. So the excitation slit was lowered to 2.5 nm. The addition of CDs provokes just a single, not a regular change in the emission spectra at 483 nm, as the absorption spectra have shown (short-wavelength band) (Fig. 6). The ratio of the total fluorescence of the complexed and the free closed form (F/F_0) is 4.03, whereas the ratio of the complexed and the free open form is 2.69 (Table 2) for the same concentration of β-CD (2.1×10^{-3} M). Obviously, this is due to the higher rigidity of the spiro form compared to the open form. The former structure becomes even more rigid in the cavity of the macromolecules and this prevents the energy of the excited state from being lost by torsional vibrations of the molecule.

Such a single change in the absorption and emission spectra of closed DHB in basic medium as the concentration of CDs is increase provoked our interest. At the same time, the absorption and fluorescence intensities of the zwitter-ionic form changed regularly as the host

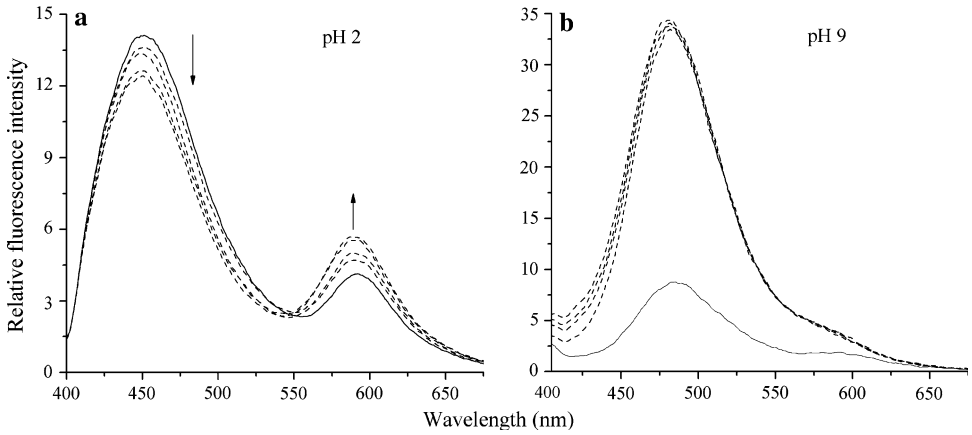


Fig. 9 Relative fluorescence spectra of DHB (2.2×10^{-5} mol/L) at: (**a**) pH 2, and (**b**) pH 9 aqueous buffer solutions (solid line) as a function of various β-CD concentrations (dashed line)

concentration increased. It is most likely that the complexation of the closed form with β-CD is faster than that with the open form since the former is not only more hydrophobic but also has more compact molecular topology compared to the latter. Besides the steric factors, electronic factors are also quite probably at work in complex formation, i.e. the lone electron pair of N-atom ($-N(CH_3)_2$) in the closed form participates, but to a lesser extent, in the conjugation, and the formation of hydrogen bonds with the primary OH-groups of β-CD is easier. Therefore, we suppose that the addition of CD moves the equilibrium open-closed form (Scheme 3) first to the right. At the same time, we assume that CD incorporates in its cavity the open form too, but with lower rate, because the decrease in absorption intensity is accompanied by a blue shift of the maximum (Fig. 6). At a certain moment, the ionic molecules arrange spatially so that a new, strong H-bond is formed between the secondary OH-groups of β-CD and the ethoxy anion ($-CH_2CH_2O^{-}\cdots H-O-\beta$-CD). This complexation requires time (suitable host–guest orientation) for the existent intramolecular H-bonds between the secondary hydroxyls of CD to be destroyed, and for the new, more stable intermolecular H-bonds to be formed.

This complex (open form—β-CD) is highly stabilized (highest values of K_{11}) as a result of the two types of H-bonds, and the conversion into the closed form is actually hindered. Therefore, as the β-CD concentration is further increased, only the intensity of the open form changes.

This behavior was confirmed also by the UV–Vis and the fluorescence spectra of DHB in the presence of Me-O-β-CD.

Conclusion

We used the absorption and fluorescence spectroscopies to study the host–guest complex formation between native and modified CDs and a representative styrylbenzothiazolium dye in aqueous buffer solutions with various pH values. The behavior of the dye in neutral, acidic and basic media varies substantially as host molecules are added, mainly due to changes in its structure. Nevertheless, for all absorption spectra, a shift of the long-wavelength absorption maximum occurs as the CDs concentration is increased, which is an evidence of inclusion association. Besides, supporting evidence for the formation of CD/dye complexes is provided by the total fluorescence enhancement (about three times) of the long-wavelength emission relative to the free dye. The change in the fluorescence intensity is most likely caused by the suppression of the competitive radiationless processes of DHB in the complex as a result of reduction of the degrees of freedom and space for relaxation. When the molecule penetrates the CD cavity, its structure becomes more rigid, the free rotations are restricted and the dye loses the absorbed energy through emission of light. We defined which part of the dye molecule interacts with the two hosts and determined that β-CD accelerate the process of cyclization by alkalization of the medium. The stoichiometry of all complexes for each pH value was 1:1 and the binding constants were determined using the changes in fluorescence intensity as a function of CD concentration using nonlinear regression analysis. K_{11} values for Me-O-β-CD are lower than those for β-CD, which is related mainly to steric hindrance due to the reduction of cavity diameter by methylation and to the lowering of the ability for hydrogen bonding between primary hydroxyls and the amino-N-atom. The most stable complexes are formed in basic medium due to the strongest intermolecular hydrogen bonding.

Acknowledgement Financial support from the National Science Foundation—Bulgaria, is gratefully acknowledged.

References

1. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
2. Connors, K.A.: The stability of cyclodextrin complexes in solution. Chem. Rev. **97**, 1325–1357 (1997)

3. Baglole, K.N., Boland, P.G., Wagner, B.D.: Fluorescence enhancement of curcumin upon inclusion into parent and modified cyclodextrins. J. Photochem. Photobiol. A **173**, 230–237 (2005)
4. Maafi, M., Laassis, B., Aaron, J.J., Mahedero, M.C., Munoz de la Pena, A., Salinas, F.: Photochemically induced fluorescence investigation of a β-cyclodextrin: Azure A inclusion complex and determination of analytical parameters. J. Incl. Phenom. Mol. **22**, 235–247 (1995)
5. Gonzalez, M.A., Lopez, M.H.: Determination of fluorene in seawater by room temperature phosphorescence in organised media. Analyst **123**, 2217–2221 (1998)
6. Scypinski, S., Cline Love, L.J.: Cyclodextrin-induced room-temperature phosphorescence of nitrogen heterocycles and bridged biphenyls. Anal. Chem. **56**, 331–336 (1984)
7. Patonay G., Salon, J., Sowell, J., Strekowski, L.: Noncovalent labeling of biomolecules with red and near-infrared dyes. Molecules **9**, 40–49 (2004)
8. Gadjev, N.I., Deligeorgiev, T.G., Kim, S.H.: Preparation of monomethine cyanine dyes as noncovalent labels for nucleic acids. Dyes Pigments **40**, 181–186 (1999)
9. Ohashi, M., Kasatani, K., Shinohara, H., Sato, H.: Molecular mechanics studies on inclusion compounds of cyanine dye monomers and dimers in cyclodextrin cavities. J. Am. Chem. Soc. **112**, 5824–5830 (1990)
10. Kasatani, K., Ohashi, M., Kawazaki, M., Sato, H.: Cyanine dye-cyclodextrin systems. Enhanced dimerization of the dye. Chem. Lett. 1633–1636 (1987)
11. Matsuzawa, Y., Tamura, S., Matsuzawa, N., Ata, M.: Light stability of a β-cyclodextrin inclusion complex of a cyanine dye. J. Chem. Soc., Faraday Trans. **90**, 3517–3520 (1994)
12. Mitewa, M., Mateeva, N., Antonov, L.: Spectrophotometric investigation on the complexation between chromo- and flouroionophores containing aza-15crown-5 moiety and alkaline and alkaline-earth metal ions. Quim. Anal. **16**, 153–162 (1997)
13. Gromov, S.P., Fedorova O.A., Alfimov, M.V., Druzhinin, S.I., Rusalov, M.V., Uzhinov, B.M.: Crown-containing styryl dyes. 14. Synthesis, luminescence, and complexation of the trans-isomers of chromogenic 15-crown-5-ethers. Izv. Akad. Nauk., Ser. Khim. **10**, 2003–2008 (1995)
14. Lednev, I.K., Hester, R.E., Moore, J.N.: Benzothiazolium styryl dyes containing a monoazacrown ether: protonation and complexation with metal and ammonium cations in solution. J. Chem. Soc., Faraday Trans. **93**(8), 1551–1558 (1997)
15. Nishida, M., Ishii, D., Yoshida, I., Shinkai S.: Molecular association of water-soluble calixarenes with several stilbene dyes and its application to the facile determination of cationic surfactant concentrations. Bull. Chem. Soc. Jpn. **70**, 2131–2140 (1997)
16. E.v Lare, Brooker, L.G.S.: Acyloxy polymethime dyes, US Patent 2,691,652, 12 Oct 1954.
17. Petkov, I., Charra, F., Nunzi, J.M., Deligeorgiev, T.: Photochemistry of 2-[(1,3,3-trimethylindoline-2(1H)-ylidene)propen-1-yl]-3,3-dimethylindolino[1,2-b]-oxazolidine in solution. J. Photochem. Photobiol. A **128**, 93–96 (1999)
18. Petkov , I., Charra, F., Nunzi, J.M., Deligeorgiev, T.: Photo- and thermoinduced ring opening reaction of 2-[(1,3,3-trimethylindoline-2(1H)-ylidene)propen-1-yl]-3,3-dimethylindolino[1,2-b]-oxazolidine in polymer films. Cent. Eur. J. Chem. **2**(2), 1–12 (2004)
19. Petinova, A., Metsov, S., Petkov, I., Stoyanov, S.: Photophysical behaviours of some 2-styrylindolium dyes in aqueous solutions and in the presence of cyclodextrins. J. Incl. Phenom. Macrocycl. Chem. doi:10.1007/s10847-007-9313-4 (2007)
20. Metsov S., Dudev T., Koleva V.: Infrared and NMR study of some 2-styrylindolium dyes. J. Mol. Struct. **350**, 241–246 (1995)
21. Zheng, J., Wu, D., Zhai, J., Huang, Ch., Pei, W., Gao, X.: Highly efficient photoinduced electron transfer on a benzothiazolium styryl dye Langmuir-Blodgett monolayer modified-ITO electrode. Phys. Chem. Chem. Phys. **1**, 2345–2349 (1999)
22. Gordon, A.J., Ford, R.A.: The Chemist's Companion. Wiley Interscience Pubs. (1972)
23. Wagner, B.D.: The effect of cyclodextrins on guest fluorescence. In: Douhal, A. (ed.) Chemical, Physical and Biological Aspects of Confined Systems, vol. 1, pp. 27–59. Elsevier, Amsterdam (2006)
24. Sueishi, Y., Hishikawa, H.: Complexation of 4-dimethylamino-azobenzene with various kinds of cyclodextrins: effects of cyclodextrins on the thermal cis-to-trans isomerization. Int. J. Chem. Kinet. **34**, 481–487 (2002)
25. Liu, L., Guo, Q.X.: The driving forces in the inclusion complexation of cyclodextrins. J. Incl. Phenom. Macrocycl. Chem. **42**, 1–14 (2002)
26. Benesi, H.A., Hildebrand, H.J.: A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. J. Am. Chem. Soc. **71**, 2703–2707 (1949)
27. Yoshida, N., Fujita, Y.: Dynamic aspects in host-guest interactions. 3. Kinetics and mechanism for molecular recognition by hexakis(2,6-di-o-methyl)-α-cyclodextrin of some azo guest molecules. J. Phys. Chem. **99**, 3671–3677 (1995)