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PHOTOCHEMISTRY

Vibronic Structure of Electronic Absorption and Fluorescence Spectra of Pyrene in the Complex with β-Cyclodextrin in the Presence of Nonpolar and Polar Solvents

V. G. Avakyan[a], V. B. Nazarov[b], A. V. Koshkin[a], and M. V. Alfimov[a]

[a] Photochemistry Center, Russian Academy of Sciences, ul. Novatorov 7a, Moscow, 119421 Russia
[b] Institute of Problems of Chemical Physics,, Russian Academy of Sciences,
pr. Akademika Semenova 1, Chernogolovka, Moscow oblast, 119421 Russia
e-mail: avak@photonics.ru

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Abstract—The feasibility of using pyrene as a fluorescent probe in complexes cyclodextrin–pyrene–analyte in aqueous solutions was studied for several analytes (A) of different polarity. Bands that prove the formation of the complex $Py_2\beta CD$ were found for the first time in the UV–VIS spectrum of an aqueous solution of pyrene in the presence of β-cyclodextrin. A characteristic of a fluorescent probe is the ratio I_3/I_1 in the fluorescence spectrum, where I_3 is the intensity of the third vibronic line at ~800 cm^{-1}, and I_1 is the intensity of the 0–0 transition. The value of I_3/I_1 for $Py_2\beta CD$ is highly sensitive to the addition of an analyte with a concentration of several μM/L to the aqueous solution. Dependences of $(I_3/I_1)/C$ on the function of the dielectric constant ε of the analyte $f\varepsilon = (\varepsilon - 1)/(2\varepsilon + 1)$ are divided into two straight lines with different slopes for nonpolar and polar solvents. Energies of insertion of analytes into $Py_2\beta CD$ were calculated by the quantum-chemical PM3 method. An increase in $(I_3/I_1)/C$ corresponds to the more thermodynamically stable complex $2APy_2\beta CD$.

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Because of the dependence of relative intensities of vibronic bands in the fluorescence spectrum on the nature of the solvent, the pyrene molecule is a highly sensitive fluorescent probe [1]. The ratio of the intensity of the fluorescence line of the 0–0 transition to the intensity of the transition to the vibronic level at ~800 cm^{-1} of the ground state I_1/I_3 is used as the main characteristic of this fluorescence probe. According to the data for 94 solvents [2], the value of I_1/I_3 is characteristic of the solvent and, generally, increases with the dielectric constant of the solvent [3]. However, no universal relationship with a reasonable correlation coefficient has been found. The analysis of the data demonstrates that the plot of I_1/I_3 as a function of the dielectric constant ε is divided into three branches, which involve nonpolar and low-polarity solvents (hexane, cyclohexane, isopentane, CCl_4, benzene, toluene, and dioxane), solvents of medium polarity (diethyl ether, tetrahydrofuran, chlorobenzene, chloroform, and dichloromethane), and highly polar solvents (acetone, acetonitrile, ethanol, methanol, and dimethylformamide). However, the property of pyrene as a fluorescent probe, whose fluorescence spectrum is sensitive to the polarity of the solvent, remains a prerequisite for the development of a sensitive chemosensor intended for the detection of small amounts of compounds that were mentioned as solvents in the works referred above. In this work, these compounds are called analytes (A) for convenience.

Using the previous experience, which demonstrated that the luminescence spectrum of the naphthalene molecule in the complex with β-cyclodextrin (βCD) is sensitive to the insertion of an analyte as the third component of the complex [4], we studied whether the Py@βCD complex can be used as a chemosensor. β-Cyclodextrin is a molecule which resembles a basketball basket in shape, consists of seven α-D-glucopyranoside units linked by (1–4) glucoside bonds, and has a hydrophobic inner cavity.

It was demonstrated [5, 7] that in the presence of excess βCD in an aqueous solution, pyrene forms the complex with a ratio of 1 : 2 (Py@2βCD), where pyrene is located inside the βCD dimer. From the geometric size of the dimer [6], it followed that the Py@2βCD cavity can contain, in addition to pyrene, at least two analyte molecules, which can affect the fluorescence spectrum of pyrene. Indeed, the addition of alcohols to the Py@2βCD complex efficiently affects the value of I_1/I_3 because of the formation of the complex of composition 2 : 1 : 2 · 2A · Py@2βCD [5b, 7]. The ability of *tert*-butylamine and propylamine to insert into Py@2βCD as the third component [5c] was confirmed by fluorescence spectroscopy with the use of the ratio I_1/I_3 and PMR.

Specific quantities $(I_3/I_1)/C$, dielectric constants ε, functions of dielectric constant f_ε, and energies of insertion of analyte molecules into the Py@2βCD complex

Analyte	$(I_3/I_1)/C$	ε	f_ε	Δ*E**
Benzene	0.0873	2.28	0.230	–34.5
Toluene	0.0803	2.37	0.239	–35.1
Cyclohexane	0.0603	2.02	0.202	–28.4
Methylcyclohexane	0.0775	2.071	0.208	–33.6
Hexane	0.0489	1.89	0.186	–26.5
Acetone	0.0781	20.7	0.465	–19.7
Acetonitrile	0.0285	37.5	0.480	–14.1
Ethanol	0.144	24.55	0.470	–22.3

* Δ*E*, kcal/mol.

The aim of this work was to find regularities in the effect of analytes in A–Py–βCD complexes on the vibronic structure of the fluorescence spectrum of pyrene. For this purpose, A–Py–βCD complexes in aqueous solutions were obtained with analytes of different polarity, their electronic absorption and fluorescence spectra were studied, and correlations between I_1/I_3 on the one hand and dielectric properties of analytes and quantum-chemical formation energies of three-component complexes on the other hand were revealed.

EXPERIMENTAL

Reagent-grade pyrene (Reakhim) was used after double recrystallization in ethanol. For the preparation of complexes, β-cyclodextrin (Cyclolab, Hungary) without additional purification and doubly distilled water were used. As analytes, we studied benzene, toluene, ethanol, acetone, acetonitrile, hexane, and cyclohexane, which substantially differ in the value of *D* and exhibit minimum solubility in water. Benzene 98.8% (Komponent-reaktiv), toluene of HPLC grade (Fisher Scientific), and methylcyclohexane (Aldrich) were used without further purification. Cyclohexane and hexane were purified by chromatography on a silica gel column. The other solvents were purified by distillation. Purity was controlled by absorption and luminescence spectra.

An aqueous solution of βCD with a concentration of 5×10^{-3} mol/L, at which pyrene is completely involved in the Py@2βCD complex [3], was prepared in a 10-mL test tube. A portion of a pyrene solution in hexane was evaporated in another test tube, so that the calculated pyrene concentration of about several micromoles per liter was obtained by adding 10 mL of an aqueous βCD solution. The test tube with pyrene and βCD was held for several hours at 50°C under ultrasonication in a UVZ-3/100-MP-RELTEK thermostatically controlled bath. The resulting solution was used after storing for a day at room temperature.

For measuring spectral characteristics of the complex, 2 mL of the solution was sampled into a fused-silica cell from the test tube after intensive shaking. To obtain three-component complexes, an aqueous solution of an analyte was prepared by the addition of 30 μL of the solvent to 500 mL of water, and several tens of microliters of this solution was introduced into a cell with a solution of the binary complex.

Electronic absorption spectra were measured on a Specord-M40 spectrophotometer with a resolution of 20 cm^{-1}. Fluorescence spectra of pyrene were measured on an Elyumin-2M spectrofluorometer at a spectral slit width of 2 nm (both instruments with digital recording). Fluorescence was excited with a UVTOP300HS light emitting diode with the emission peak at 302 nm. For the convenience of processing, the fluorescence spectra were normalized by the 0–0 transition band, and the variation of vibronic band intensities was characterized by the ratio I_3/I_1 (Table).

The size of particles in solutions was measured by the dynamic light scattering method on a Photocor Complex photometer using a He–Ne laser with λ 632.8 nm and power 10 mW (Coherent, model 31-2082) at a temperature of 24°C. The signal integration time was 3 min; the results were averaged over 10 measurements. The experimental data were approximated using the model of multimodal particle size distribution taking into account the intensity of light scattering by particles of different sizes.

Quantum-chemical calculations with full geometry optimization for all compounds and complexes

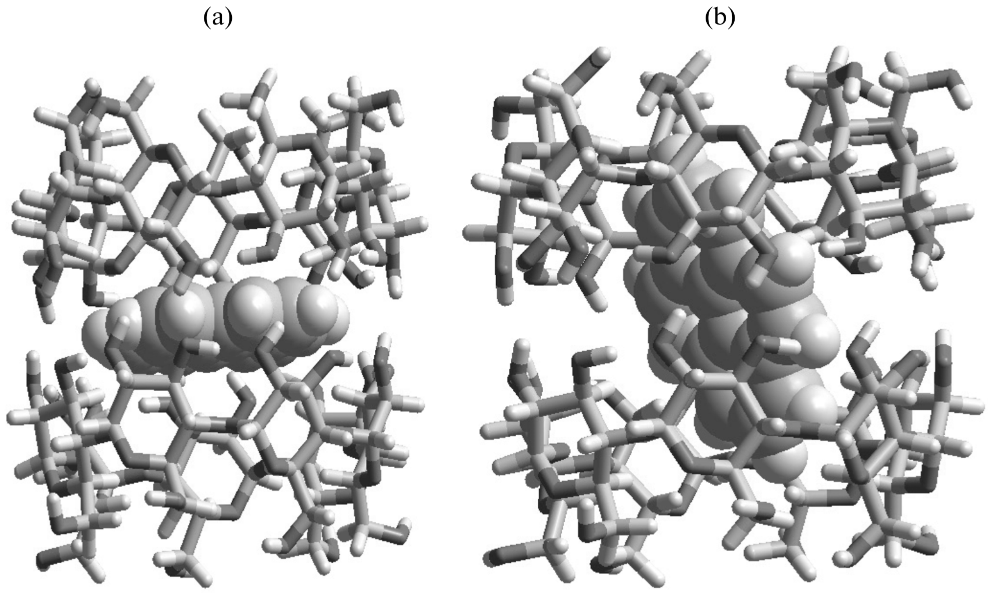

Fig. 1. Two structures of the Py@2βCD complex calculated by the PM3 method.

were performed by the semiempirical PM3 method with the standard set of parameters [8] (program Firefly by A.A. Granovsky, Moscow State University [9]). The formation energies of complexes Py@2βCD, $\Delta E_{\text{complex}}$ and the energies of analyte insertion producing complexes 2A · Py@2βCD (ΔE) were calculated (table). The values of $\Delta E_{\text{complex}}$ and ΔE were calculated as differences between ΔH_f of the fully optimized structure of the complex and the sun of ΔH_f of its components. Complexes Py@2βCD can exit in two configurations 1a and 1b (Fig. 1), of which 1b is thermodynamically more favorable.

In Fig. 1, it is seen that cavities in dimers have enough free space for the insertion of analyte molecules, which can form complexes of composition nA · Py@2βCD. The structures and energies of analyte insertion into Py@2βCD were calculated for complexes of both types. The table presents values of ΔE for 2A like 1a.

RESULTS AND DISCUSSION

Absorption and Fluorescence Spectra of an Aqueous Pyrene Solution in the Presence of β*CD: Evidence for Complexation*

Although organic molecule–cyclodextrin insertion complexes are formed in aqueous solutions [11], it was found impossible to obtain direct evidence for the formation of the Py@2βCD complex, e.g., by the PMR method [5c] because the micromole concentration of complexes controlled by the concentration of pyrene, which is poorly soluble in water, precludes the use of this method. Therefore, the main evidence for the insertion of the substrate into the cyclodextrin cavity was an increase in intensity and a change in the vibronic structure of fluorescence.

However, studying the electronic absorption spectra of an aqueous solution of pyrene before and after the addition of βCD, we found two new bands that provided evidence for the formation of the insertion complex with βCD by pyrene. Figure 2 shows the absorption spectra of pyrene solutions in water, ethanol, and hexane and the spectra of the aqueous solution of pyrene after the addition of βCD.

It is seen that the spectra of the pyrene solutions in water, ethanol, and hexane in the range of 29000–32000 cm^{-1} exhibit two intense bands at ~29800 and

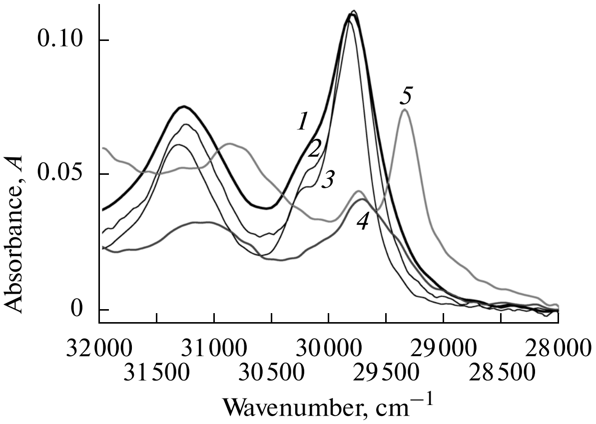

Fig. 2. Electronic absorption spectra of pyrene in (*1*) water, (*2*) ethanol, and (*3*) hexane. Spectrum (*4*), immediately after the addition of βCD to and aqueous solution of pyrene; (*5*), within a day after holding and stirring the cell with Py@2βCD.

~31 300 cm^{-1} and shoulders at ~30 250 cm^{-1}. According to calculations, the first band corresponds to the 0–0 $S_0 \rightarrow S_2$ transition in the electronic absorption spectrum of pyrene, and the shoulder and the second band correspond to vibronic satellites with $\Delta\nu$ ~ 420 and 1500 cm^{-1} [11]. Previously [3] bands at 29 673.6 and 31 055.9 cm^{-1} were observed in the absorption spectrum of pyrene in water; the positions of these bands are in agreement with our data. The comparison of spectra *1–3* recorded in different solvents demonstrates that the observed maxima are shifted with respect to each other by no more than 40 cm^{-1}.

On the addition of βCD to an aqueous solution of pyrene, the band of the 0–0 transition exhibits a weak bathochromic shift (to 29 710 cm^{-1}) and a weak shoulder appears on the low-frequency edge at 29 336 cm^{-1} (spectrum *4*). After holding and stirring the cell with the solution, new bands peaked at 29 336 and 30 834 cm^{-1} appear (spectrum *5*), which correspond to the formation of the complex Py@2βCD. Since the difference between these bands $\Delta\nu$ is 1500 cm^{-1}, it is evident that the new bands still correspond to the 0–0 $S_0 \rightarrow S_2$ band and its most intense vibronic transition shifted to the red region as a result of complexation. The appearance of new bands after stirring the cell with the solution allows for the conclusion that the Py@2βCD complex enters into the composition of a sedimented aggregate. Another piece of spectral evidence for the aggregation of Py@2βCD complexes in an aqueous solution is a decrease in the intensities of the 29 710- and 31 112-cm^{-1} bands with increasing concentration of βCD, which occurs strictly in parallel with the change in the ratio I_1/I_3 in the fluorescence spectrum (see below). Hence, it follows that new vibronic bands appear in the spectrum of the aggregated complex, which are absent in the spectra of solutions of pyrene in water and solvents of different polarity.

To confirm the assumption of aggregate formation, sizes of particles corresponding to spectra *4* and *5* were measured by the dynamic light scattering method. The hydrodynamic diameter of aggregates measured after stirring the cell with the solution was ~30 nm; taking into account the height of the βCD basket of 0.8 nm, it corresponds to a particle consisting of four βCD molecules with the pyrene molecule inside. Thus, it can be assumed that the aggregate consists of either one Py@2βCD complex (type 1a or 1b) surrounded by two "empty" βCD molecules, or a dimer of Py@2βCD.

In the fluorescence spectrum of the pyrene solution in water, the I_3/I_1 ratio is 0.53 [2] or 0.63 [1], whereas this ratio is greater than 1 in the majority of solvents, especially hydrophobic. After the addition of βCD to the aqueous solution of pyrene, the I_3/I_1 ratio also increases for a day and more and reaches the value 1.55 ± 0.12 (averaged over 14 values). This is indicative of a rather slow reaction of the formation of Py@2βCD complexes and their subsequent aggregation.

Change in the Vibronic Structure of Pyrene Fluorescence Spectra on the Addition of Analytes

The cavity of "empty" βCD can admit —five to six water molecules [12]; therefore, the value $I_3/I_1 = 1.55$ corresponds to the vibronic structure of the fluorescence spectrum of pyrene involved in the aggregate $(H_2O)_n \cdot$ (Py@4βCD), where $n = 10–12$, and, starting from this value, I_3/I_1 further increases after the addition of other analytes. We obtained values of I_3/I_1 for analytes of different polarity (benzene, toluene, hexane, cyclohexane, and methylcyclohexane) and polar liquids (ethanol, acetone, and acetonitrile). The selection of analytes was based on the requirement that these compounds must be soluble in water at least in micromole amounts. Figure 3 shows the fluorescence spectra of the pyrene–βCD complex after the addition of benzene or cyclohexane.

It is seen that in both cases the band contour only slightly changes on the addition of the analyte; however, as the concentration of the analyte increases, the intensity of all vibronic bands grows and band *3* becomes the most intense of them.

In Fig. 3a, it is also seen that the addition of benzene is accompanied by an increase in the intensity of the vibronic component at ~26 000 cm^{-1}, which reaches a maximum (I_3/I_1 = 3.13) at a concentration of 27.7 μmol/L, whereas I_3/I_1 is 0.95 for a solution of pyrene in benzene [2]. Consequently, the addition of benzene to the Py@2βCD complex even is small amounts leads to a substantial increase in the I_3/I_1 ratio. A similar situation is observed in the case of addition of cyclohexane (Fig. 3b): the I_3/I_1 ratio increases, although to a lesser degree, as well as on the addition of other analytes irrespective of their polarity.

Thus, the main specific feature of these spectra is an increase in I_3/I_1 within a characteristic period of time about ten minutes after the addition of an analyte with the general similarity of vibronic contours. This is the feature that basically differs the spectra of three-component complexes from the spectra of pyrene in liquid solutions of the same analytes, for which the fluorescence spectrum exhibits an individual ratio of intensities of vibronic components [1, 3]. Hence, it follows that the mechanism of the analyte effect on I_3/I_1 in nA · Py@2βCD complexes differs from the effect of the same analyte taken as the solvent for pyrene.

Since different concentrations of introduced analytes cause different increments in I_3/I_1, we characterized the individual properties of analytes in complexes by the specific quantity $(I_3/I_1)/C$ obtained by dividing the maximum value of I_3/I_1 by the corresponding concentration C of the analyte introduced. The values of $(I_3/I_1)/C$ are presented in the table.

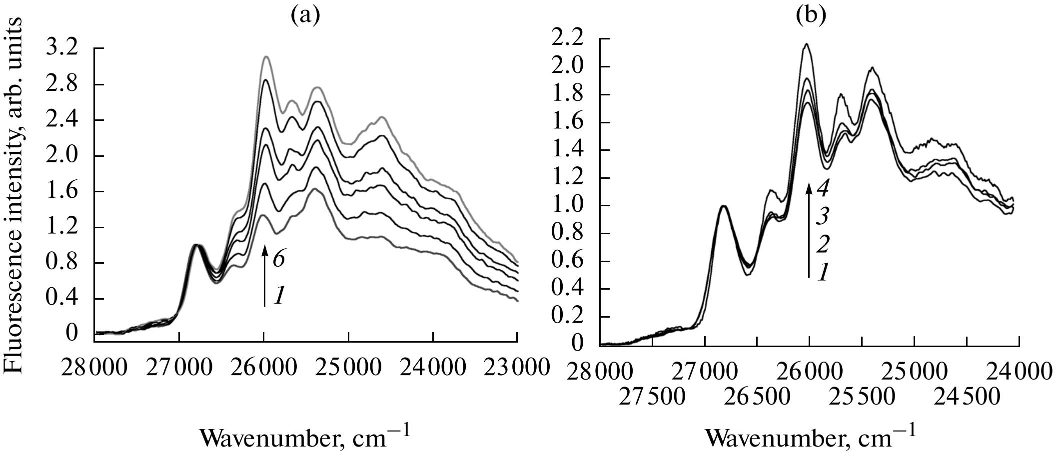

Fig. 3. Normalized (to the 0-0 band at 26 750 cm^{-1}) fluorescence spectra of the Py@2βCD complex (*1*) in water, concentrations of pyrene and βCD 1.7×10^{-6} and 5×10^{-3} mol/L, respectively, (a) after the addition of benzene with (*2–6*) *C* = 4.3, 8.5, 12.7, 16,7, and 27.7 μmol/L, respectively, and (b) after the addition of cyclohexane with *C* = (*2*) 6.5 μmol/L, (*3*) 32 μmol/L, and (*4*) 40 μmol/L.

As the universal characteristic of polarity, we used a function of the macroscopic dielectric constant of the analyte ε, $f_\varepsilon = (\varepsilon - 1)/(2\varepsilon + 1)$ [3]. Functions of this kind are used in the continuum Onsager theory for calculating the solvent effect on spectral characteristics of dissolved compounds [13]. The values of ε and f_ε are presented in the table.

Three-Component Complexes n(A) · (Py@2βCD)

Using the quantum-chemical PM3 method, the structures of 2A · Py@2βCD complexes were fully optimized and energies of the insertion of analytes into Py@2βCD complexes of both types Δ*E* were calculated (Fig. 1). The values of Δ*E* n(A) · (Py@2βCD) for complexes of the first type were larger than for the second type. These values are presented in the table. However, the equilibrium between the structures of both types in an aqueous solution is not improbable.

Changes in the Vibronic Structure of Fluorescence Spectra of Pyrene in n(A) · (Py@2βCD) Complexes by the Effect of Analytes

Figure 4a presents the dependence of the specific quantity $(I_3/I_1)/C$ of analytes on f_ε. The plot is divided into two parts: the left-hand part is the dependence for nonpolar compounds and the right-hand part is that for polar compounds. For nonpolar analytes, $(I_3/I_1)/C$ increases with f_ε. For polar analytes, the situation is seemingly opposite, although with a very poor correlation coefficient, evidently because of a small number

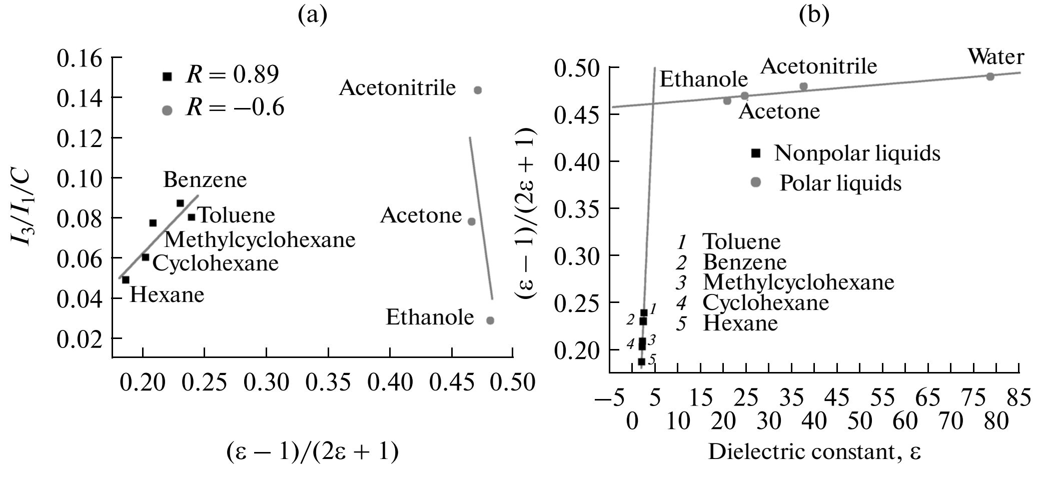

Fig. 4. (a) Dependence of I_3/I_1/C on the function of the dielectric constant of the analyte (ε – 1)/(2ε + 1). (b) Dependence of the function (ε – 1)/(2ε + 1) on the dielectric constant ε of the analyte.

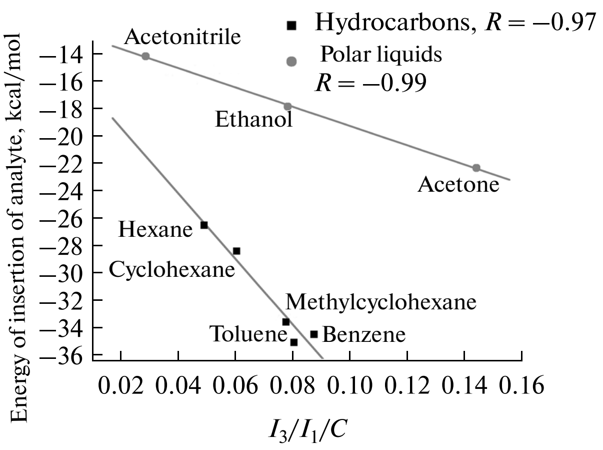

Fig. 5. Dependence of the energy of insertion of analyte molecules into the Py@2βCD complex on (I_3/I_1)/C.

of data points. Nevertheless, the dependence in Fig. 4a resembles the plot reported by Nakajima [3], who attempted to find a universal correlation that relates solvents of any polarity. It was found that the reason for the separation of the relationship into two straight lines with slopes of 0.109 for polar analytes and 4×10^{-4} for nonpolar analytes consists in the behavior of f_ε as a function of dielectric constant ε (Fig. 4b), which is also divided into two straight lines. Tests demonstrated that there is a relationship between the energy of insertion of an analyte into the Py@2βCD complex and the specific ratio of intensities $(I_3/I_1)/C$ (see Fig. 5).

It is seen that the dependence of ΔE on $(I_3/I_1)/C$, in accordance with the character of f_ε, is divided into two straight lines. The plot demonstrates that the energy (enthalpy) of insertion of an analyte into Py@2βCD increases with $(I_3/I_1)/C$. In other words, a larger value of $(I_3/I_1)/C$ is characteristic of the more thermodynamically stable complex must be 2A · Py@2βCD for both nonpolar and polar analytes. A general conclusion follows from the division of the dependence of ΔE on $(I_3/I_1)/C$ into two separate branches: the value of the specific ratio of intensities can be used as a characteristic of an analyte separately for nonpolar hydrocarbons and polar liquids.

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