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Guest—host complexes of spin-labeled indoles with cyclodextrins in the solid phase: an ESR study

V. A. Livshits, B. G. Dzikovskii, E. A. Samardak, and M. V. Alfimov*

Photochemistry Center, Russian Academy of Sciences,
7a ul. Novatorov, 114719 Moscow, Russian Federation.
Fax: +7 (495) 936 1255. E-mail: livsh@photonics.ru

Inclusion complexes of spin-labeled pyrrolidine- (**1**) and piperidine-containing (**2**) indole derivatives with β-cyclodextrin and γ-cyclodextrin (CD) were prepared in the solid phase and studied by ESR in a wide temperature interval. For all complexes and free spin probes in solvents of different polarity, local environment polarities of the NO group of the guest molecules were determined from the outer extrema separations in the ESR spectra measured at 77 K. From analysis of the Saturation Transfer (ST) ESR spectra and temperature dependences of linear ESR spectra of the complexes it follows that both guest molecules in γ-CD undergo rapid librations. The libration amplitude of the p-orbit axis of the NO group in molecule **2** increases with temperature and reaches about 16° at 333 K. The ESR lineshape of the β-CD complexes depends on the spin probe/β-CD molar ratio (ρ) even at $\rho < 0.01$. Lineshape analysis of the spectra recorded at different ρ showed that they consist of two components, one of them corresponding to strong spin-spin interaction between guest molecules and the other corresponding to almost absence of this interaction. The spectral components can be attributed to microphases of the complexes and to isolated complexes in the β-CD matrix, respectively. Simulation of the ST ESR and linear ESR spectra of the magnetically diluted complexes showed that the guest motion inside the CD cavity is better described by rotational jumps rather than Brownian diffusion in an orientation potential. In the temperature range 238—333 K, the rotational frequencies of **1** and **2** are in intervals $1.8 \cdot 10^7$—$6 \cdot 10^7$ s^{-1} and $4 \cdot 10^7$—$1.3 \cdot 10^8$ s^{-1}, respectively. The rotation occurs over the whole solid angle. Significant differences in the character of molecular dynamics in the γ-CD and β-CD complexes can be explained by different stoichiometry, namely, 1 : 1 for the former and 2 : 1 for the latter and by different orientation of guest molecules in the complexes. In both cyclodextrins the rotational mobility of molecules **2** is higher than that of **1** owing to intramolecular conformational transitions in the piperidine ring of **2** and steric hindrances produced by the methyl group in **1**.

Key words: γ-cyclodextrin, β-cyclodextrin, inclusion complexes, molecular dynamics, ESR spectra, saturation-transfer ESR spectra, spin-labeled indoles, environment hydrophobicity, spin-spin interaction.

Inclusion complexes with cyclodextrins (CDs) have been a subject of extensive studies carried out by various methods (see, *e.g.*, Refs 1—7). Nevertheless, a number of important aspects concerning the structure and especially the molecular dynamics of these compounds are still unclear. In particular, data on the dynamics of guest molecules inside the CD cavity are scarce. This is due to the fact that in solution studies by conventional methods (NMR spectroscopy and fluorescence polarization) the intramolecular dynamics in complexes is masked by Brownian rotation of a complex as a whole, while solid-phase studies are accompanied by dramatic increase in light scattering and, as a rule, loss of high resolution of NMR spectra, which complicates the use of these methods. Some results on the solid-phase dynamics of complexes were obtained from ^{13}C and ^{2}H spin relaxation measurements.[8—11]

A promising approach to the study of the structure of inclusion complexes and the dynamics of molecules located inside the CD cavity involves the use of spin-labeled guest molecules.[12] First, when applied to complexes in the solid phase, the method is free from the drawbacks listed above. Second, the ESR spectra of spin probes are highly sensitive to rotational dynamics of the probes in a wide range of correlation times, to the environment polarity, and to intermolecular interactions. The guest molecules used in this work were spin-labeled derivarives of indole (fluorophore), containing pyrrolidine and piperidine radical fragments (**1** and **2**, respectively). Earlier,[13,14] we determined the stoichiometry and the binding con-

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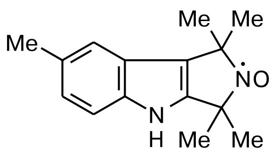
stants of these complexes in aqueous solution, the environment polarity, and the availability of the NO group of the guest molecules to water-soluble paramagnetic ions. The aim of this work was to study the structure of complexes and the molecular dynamics of spin-labeled guest molecules in complexes with β-CD and γ-CD in the solid phase.



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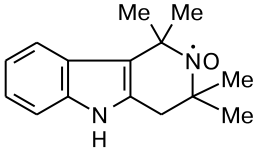

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Experimental

Reactants used in this work were β-CD and γ-CD (both purchased from Aldrich), ethanol, toluene, glycerol, ethyl acetate ("chemically pure" and "chemically pure for analysis" grade). The syntheses of spin-labeled indoles **1** and **2** were reported earlier.[15,16] These compounds were kindly granted by Dr. A. B. Shapiro (N. M. Emanuel´ Institute of Biochemical Physics, Russian Academy of Sciences).

Complexes of compounds **1** and **2** with β-Cd and γ-CD in aqueous solutions were prepared as follows. A solution of a spin probe in ethanol was evaporated, an aqueous CD solution of necessary concentration was added to the dry film, and the mixture was vigorously stirred for ~0.5 h. This resulted in extraction of spin probes to the aqueous phase in the form of complexes with CD. Solutions of the complexes were evaporated with a rotary evaporator at 50 °C until formation of a crystalline residue. An important problem in solid-phase studies of complexes is to minimize spin-spin interactions between the spin-labeled guest molecules, which can significantly reduce the spectral resolution. In this connection the inclusion of spin probes into CD was carried out in a large excess of CD; the concentrations of the spin probes included into CDs in solutions were $2 \cdot 10^{-5}$—$2 \cdot 10^{-4}$ mol L^{-1} (*cf.* 10 mmol L^{-1} for β-CD and 50 mmol L^{-1} for γ-CD).

ESR spectra were recorded with a Bruker ER-200D instrument in cylindrical tubes thermostatted with an accuracy of ±0.5 °C. Linear ESR spectra were recorded at low microwave power ($P = 32$ dB, $H_1 \approx 0.0125$ G) and small modulation amplitude, which excluded spectral distortion. Second-harmonic out-of-phase absorption ESR spectra detected at 90 degrees with respect to the modulating field under conditions of partial microwave saturation (Saturation Transfer ESR spectra, V'_2) were recorded as decribed earlier.[17] In this case the Zeeman modulation frequency in the cavity was 50 kHz, the microwave field amplitude, H_1, was 0.25 G, and the modulation amplitude was 5 G. The modulation phase was set by the "selfnull" method, as reported earlier.[17]

For most temperatures, linear ESR spectra of the complexes in the solid phase formally correspond to the so-called region of "slow" rotations (Fig. 1).

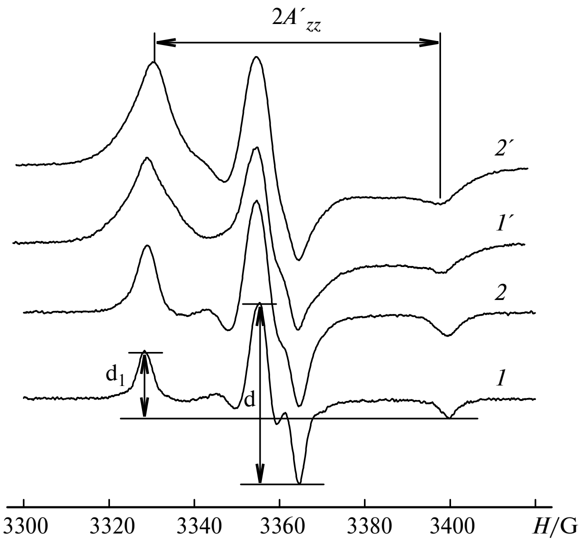

Fig. 1. ESR spectra of complexes of spin-labeled indoles **1** (*1*, *1´*) and **2** (*2*, *2´*) with γ-CD (*1*, *2*) and β-CD (*1´*, *2´*) at 293 K obtained for a probe/CD molar ratio of 0.005.

As a spectral parameter characterizing the molecular rotational mobility, we used the distance between the outer extrema, $2A'_{zz}$. Methods for determining the frequency and amplitude characteristics of rotational motion of guest molecules are considered below.

In order to characterize the environment polarity of the guest molecules, we used the parameter $2A'_{zz}$ measured at 77 K, which corresponds to almost frozen molecular motion. In the limiting case (frozen molecular rotation) this parameter nearly equals the doubled z-component of the HFC tensor ($2A_{zz}$). By analogy with solutions it is possible to introduce a dimensionless hydrophobicity parameter for the solid phase,[18]

$$h_c = \frac{2A^{w}_{zz} - 2A_{zz}}{2A^{w}_{zz} - 2A^{t}_{zz}}, \qquad (1)$$

where $2A_{zz}$, $2A^{t}_{zz}$, $2A^{w}_{zz}$ are the $2A_{zz}$ values for the complex under study, nonpolar solvent (toluene), and water, respectively. In polar environment (water), $h_c = 0$ and in hydrophobic environment, $h_c = 1$.

We measured the $2A_{zz}$ parameters at 77 K not only in toluene and water but also in solvents of intermediate polarity (ethyl acetate, ethanol, 50% ethanol, and 50% glycerol). For both spin probes the $2A_{zz}$ values are linearly related to the isotropic HFC constant (a) in these solvents:

$$2A_{zz} - 2A^{t}_{zz} = p(a - a^{t}),$$

where $p = 2.94 \pm 0.09$ and 3.59 ± 0.16 for the complexes of compounds **1** and **2**, respectively. First, these correlations make it possible to determine the A_{zz} values in frozen aqueous solutions, which can not be done for the experimental spectra in this system because of strong exchange and dipolar broadening. Second, it follows from linearuty of the $2A_{zz}$-*vs.*-a correlations that the hydrophobicity parameters h (in solution) and h_c (in the solid phase), measured in the same environment, should be the same.

Table 1. Hydrophobicity parameters (h_c) of the environment of spin-labeled indoles **1** and **2** in complexes with β-CD and γ-CD in the solid phase

Spin probe	Cyclodextrin	$2A_{zz}^{77\ K}$	h_c
1	β	72.2	0.41±0.02
1	γ	72.32	0.38±0.02
2	β	71.8	0.45±0.02
2	γ	72.64	0.315±0.02

Results and Discussion

Complexes with γ-CD. *Environment polarity of NO group of guest molecules. The* $2A_{zz}$ values and the hydrophobicity parameters (h_c) derived from ESR spectra at 77 K for the complexes of **1** and **2** with γ-CD and β-CD, are given in Table 1. As can be seen, the environment of the NO group for both spin probes in the complexes with γ-CD is characterized by rather high polarity. Measurements in solvents of different polarity (see Experimental) showed that the environment polarity is higher than that of ethanol but lower than those of aqueous solutions of these complexes (*h* values are equal 0.17 and 0.21, respectively[13,14]). This means that both spin-labeled indoles in the complexes with γ-CD form weaker H-bonds in the solid phase compared to solutions, probably, owing to a decrease in the water content inside the γ-CD cavity.

Molecular dynamics of guest molecules in complexes with γ-CD. We studied the temperature dependences of the ESR spectra of the complexes of both spin probes with γ-CD in the temperature range 130—333 K. Changes in the spectral shape for the complex **2**—γ-CD at different temperatures are shown in Fig. 2, *a*. Qualitatively, the ESR spectral pattern remains unchanged in the whole temperature interval; raising the temperature mainly results in line narrowing and a decrease in the parameter $2A'_{zz}$. The temperature dependences of the parameters $2A'_{zz}$ for both complexes are shown in Fig. 3.

Note that these changes are most likely due to rotational mobility of the guest molecules located in the CD cavities rather than rotation of the complex as a whole. Indeed, a ^{2}H NMR spectroscopy and spin relaxation study[9] revealed no rotation of perdeuterated β-CD in the crystal lattice of nondeuterated β-CD. To determine the amplitude-frequency parameters of mobility of the guest molecules, which leads to partial averaging of the $2A_{zz}$ component of the HFC tensor with an increase in temperature, one should know the character of molecular motion. Indeed, the same change, $2A_{zz} - 2A'_{zz}$, can be due to either slow rotational diffusion in the whole solid angle (or in the azimuth angle in the case of uniaxial rotation) or fast librations in a cone or sector.

The choice between these limiting models can be done by performing spectral simulations. In the range

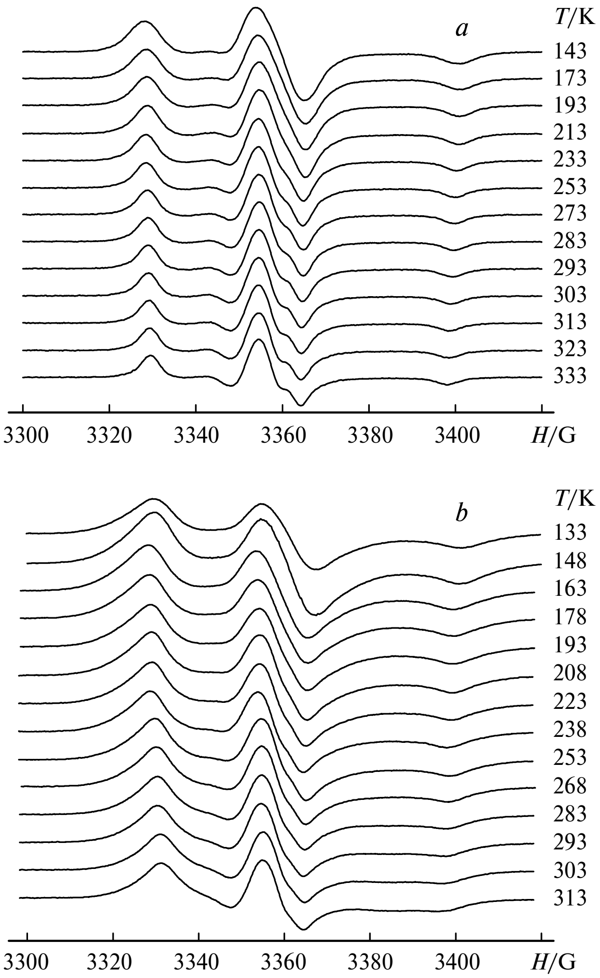

Fig. 2. ESR spectra of spin-labeled indole **2** in the solid phase in the complexes with γ-CD in the temperature range 133—333 K (*a*) and with β-CD at 133—313 K (*b*) obtained for a probe/CD molar ratio of 0.005.

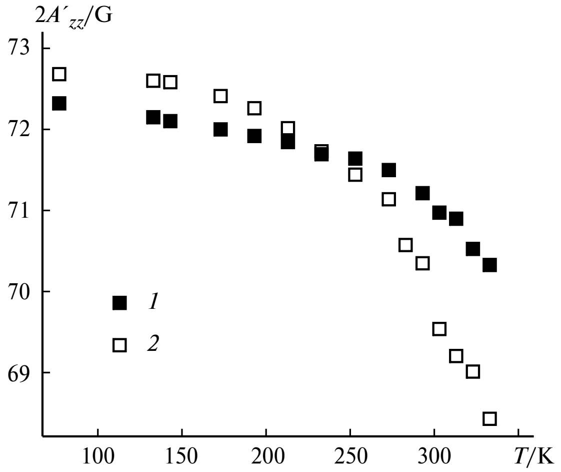

Fig. 3. Temperature dependences of the parameter $2A'_{zz}$ in the ESR spectra of complexes of spin-labeled indoles **1** (*1*) and **2** (*2*) with γ-CD.

140—300 K, the $2A'_{zz}$ parameters for compounds **1** and **2** in complexes with γ-CD vary by 0.9 and 2.6 G, respectively. Within the framework of the model of unrestricted slow rotational diffusion,[19] these shifts respectively correspond to correlation times $\tau_c = 2 \cdot 10^{-7}$ and $5 \cdot 10^{-8}$ s at 300 K. Would this model realize, one should observe broadening of the outer components of the HFC (0.1 and 0.2 of the initial linewidth in the absence of motions at these τ values, respectively).[19] However, such changes in the linewidths cannot be detected, because the narrowing rather than broadening of these components is experimentally observed on raising the temperature. For instance, an increase in temperature from 140 to 300 K is accompanied by a decrease in the halfwidth of the $m = +1$ component from 3.1 to 2.04 G in the spectrum of complex **1**—γ-CD and from 4 to 2.6 G for the complex **2**—γ-CD. This narrowing is probably due to the fact that inhomogeneous broadening observed at low temperatures is averaged owing to molecular motions. Note that librations should not be accompanied by pronounced relaxation-induced line broadening if the libration frequency is high compared to the change in the resonance field under librations, expressed in frequency units.

Earlier,[20,21] we proposed another approach to discriminate between the above two limiting models of motion. It involves the use of the V'_2 spectra, which are widely used in studies of "superslow" rotations (detailed analysis of this field of application of ST ESR spectra was reported in Ref. 17). It was shown[20] that for the fast small-amplitude librations with correlation times $\tau_c \leq 10^{-9}$ s the V'_2 spectral shape similar to that obtained in the case of no rotation, whereas it is strongly different for the rotation in a wide cone angle with τ_c lying in the range 10^{-8}—10^{-5} s. The strongest differences between these two types of spectral pattern are observed in the region of maximum change in the resonance field as function of the angle θ between the z axis of the probe and the magnetic field, at $\theta \approx 45°$ (Fig. 4, *a*, this region is denoted as L''). If no rotation or fast angular restricted librations occur, the spectral amplitude in this region is large, while in the case of slow rotation in a wide cone angle it decreases down almost to zero. The V'_2 spectra of probes **1** and **2** in the complexes with γ-CD are shown in Fig. 4, *a*. Would the model of slow diffusion in the whole solid angle be realized, at the τ_c values mentioned above ($2 \cdot 10^{-7}$ and $5 \cdot 10^{-8}$ s) the V'_2 shape should be strongly different from the spectral shape obtained in the absence of rotation.[17,20,21] However, the relative amplitude in the L'' region (L''/L ratio) is large for both spin probes (see Fig. 4, *a*), which indicates the absence of slow rotation with correlation times in the range $10^{-8} \leq \tau_c \leq 10^{-5}$ s.

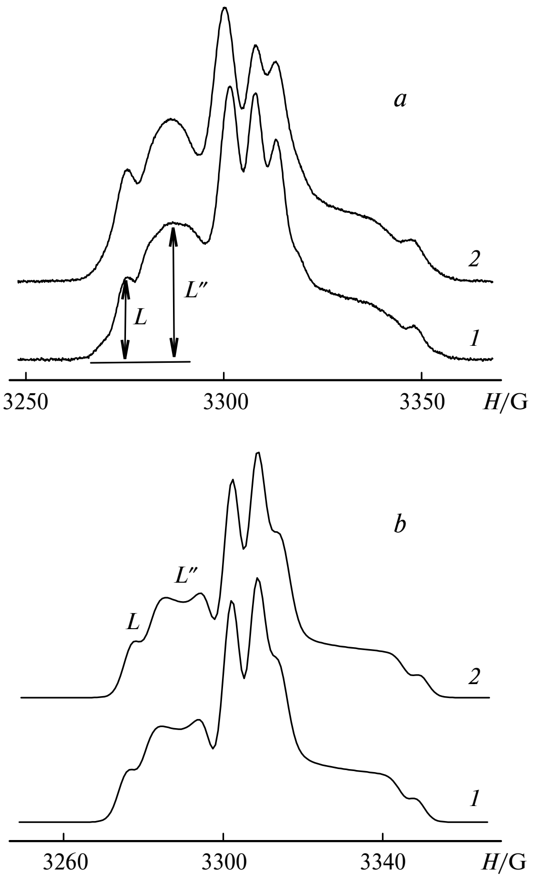

Fig. 4. *a*. The V'_2 spectra of the complexes of spin-labeled indoles **1** (*1*) and **2** (*2*) with γ-CD in the solid phase for a spin probe/γ-CD molar ratio, ρ, of 0.005 at 298 K; *b*. The V'_2 spectra simulated using the model of librations in a cone with cone angles of 8.5 and 12.5° corresponding to the averaging of the $2A'_{zz}$ parameters of the ESR spectra of complexes **1** (*1*) and **2** (*2*) in γ-CD at 298 K; correlation times are 10^{-10} s.

We also simiulated the ST ESR spectra in terms of a model of rotation in a cone angle using a program similar to that developed earlier.[20] Fig. 4, *b* presents the simulated V'_2 spectra obtained for the case of fast rotational jumps in the orientation cones with angles 8.5 and 12.5°, corresponding to experimentally observed averaging of the A_{zz} component in the complexes of compounds **1** and **2**; the τ_c times were set to $\tau_c = 10^{-10}$ s. Further reduction of τ_c has almost no effect on the V'_2 spectral shape. Detailed simulation of experimental V'_2 spectra was not performed because calculations were carried out using a number of approximations (adiabatic approximation for the resonance field, axial symmetry of the A and g tensors, and small modulation amplitude compared to the linewidth). Nevertheless, the simulated spectra are similar to the experimental V'_2 spectra of the γ-CD complexes in key parameter, the L''/L ratio, which is only weakly sensitive to non-axiality of the A and g tensors. Thus, it follows from simulations of the ST ESR spectra that at least at $T \leq 300$ K both probes in the complexes with γ-CD undergo fast angular restricted librations that lead to partial averaging of the A_{zz} components.

Using the A'_{zz} values, in the framework of the libration model it is possible to estimate the average libration amplitudes of the anisotropy axis (z), which coincides with the direction of the p-orbital axis of the unpaired electron of the NO group. The A'_{zz} values depend on the average angular libration amplitude[22] as follows:

$$A'_{zz} \cong A_{zz}\left\langle\cos^2(\Delta\theta)\right\rangle + A_{\perp}\left\langle\sin^2(\Delta\theta)\right\rangle. \tag{2}$$

Therefore,

$$\left\langle\sin^2(\Delta\theta)\right\rangle = \frac{A_{zz} - A'_{zz}}{A_{zz} - A_{\perp}}. \tag{3}$$

Here $A_{\perp}$ are the A_{xx} and A_{yy} components of the HFC tensor ($A_{xx} = A_{yy}$) and $\Delta\theta$ is the angle between the instantaneous and average orientation of the z axis for each complex. As A_{zz} values, the values measured at 77 K were used. The $A_{\perp}$ components of compounds **1** and **2** differ only slightly and therefore they were set equal for both radicals ($A_{\perp} \approx 6$ G).

The $\langle\Delta\theta\rangle$ values determined from Eqn. (3) are shown in Fig. 5. From these data it follows that, first, the amplitudes of spin probe librations inside the γ-CD cavity are relatively small, being at most 16° at 333 K. The libration amplitudes become large at temperatures above 250 K. Next, the libration amplitudes for compound **2** are much larger than for **1**. Both spin probes have the same molecular weights but different spatial structures. The most significant difference is that molecule **1** is planar, whereas in molecule **2** the NO radical fragment is included in the piperidine ring that can undergo chair—boat conformational transitions with a change in the orientation of the z axis of the unpaired electron. Besides, in molecule **1** there is a Me group, which can produce steric hindrances to the motion of this molecule inside the cavity. Both these distinctions allow one to explain a lower mobility of molecule **1** compared to **2** in the γ-CD cavity.

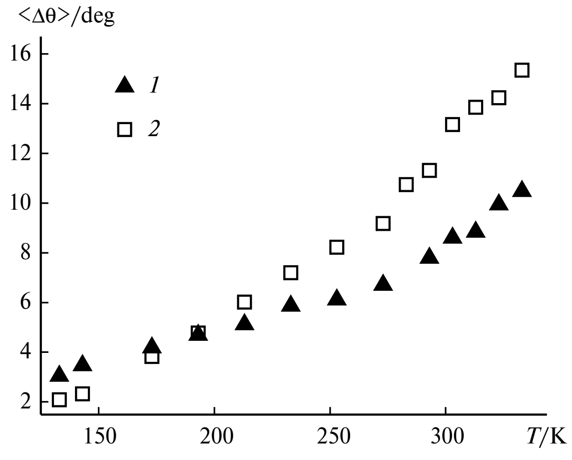

Fig. 5. Temperature dependences of average libration amplitudes of the HFC axial anisotropy axis (z axis) for the complexes of **1** (*1*) and **2** (*2*) with γ-CD.

Complexes with β-CD. *Concentration dependence of the ESR spectral pattern.* The spectra of the **2**—β-CD and **1**—β-CD complexes prepared at a spin probe/β-CD molar ratio, ρ, of 0.005 are additionally broadened compared to the corresponding γ-CD complexes (see Fig. 1). This broadening is indicative of spin-spin interaction between the guest molecules (see Fig. 1). In order to evaluate this interaction and obtain the spectra of magnetically diluted complexes, we studied the dependences of the ESR spectral pattern on ρ. To evaluate the spin-spin interaction, we used the parameter[23] d_1/d (see Fig. 1) and the half-width, $\Delta H^{+1}_{0.5}$, of the $m = +1$ component. The ESR spectra of the **2**—β-CD complexes obtained at different ρ values are shown in Fig. 6, *a*, while the dependences on 1/ρ of d_1/d and $\Delta H^{+1}_{0.5}$ are plotted in Fig. 6, *b*. As can be seen, the d_1/d parameter monotonically increases as ρ increases while with a decrease in ρ it approaches a plateau at a minimum ρ value of 0.001 (Fig. 6, *b*). At this ρ value, the d_1/d parameter equals 0.46 (*cf.* $d_1/d \approx 0.44$ for the complex **2**—γ-CD). Besides, an additional maximum appears witn an increase in ρ at intermediate fields (arrowed in Fig. 6, *c*), whereas the amplitude of the outer high-field extremum decreases and almost disappears at ρ = 0.025 (see Fig. 6, *c*). The appearance of a maximum at intermediate field and disappearance of the high-field extremum are likely due to the fact that not only the dipole-dipole but also an exchange interaction occurs between the spin-labeled guest molecules, which leads to partial averaging of the $2A'_{zz}$ parameter.

The spectral line shapes corresponding to different ρ suggest that the spectra can be represented by the sum of two components, one with almost no dipolar broadening and the other characterized by strong dipolar and exchange interactions. In order to obtain the shape of the second component, we subtracted spectrum *1* from spectrum *4* until disappearance of the high-field extremum in the difference spectrum (denoted as *dip* in Fig. 6, *c*). Then, the shape of the magnetically diluted component was determined by subtracting the *dip* component from spectrum *1* until a minimum amplitude in the region between the high-field and central maxima. The shape of the resultant difference spectrum (*dil*) is similar to that of the initial spectrum *1* (see Fig. 6, *c*), the contribution of the *dip* component to spectrum *1* being less than 20%. In order to validate the correctness of the hypothesis about two-component character of the spectra of the complex **2**—β-CD, we reconstructed an intermediate spectrum corresponding to ρ = 0.005 (curve *3*) using the two components mentioned above, *dil* and *dip*. The simulated spectra, *sum1* and *sum2*, are in reasonable agreement with the experimental spectrum *3* (see Figs 6, *a*, *c*).

Earlier,[23] calibration curves relating the d_1/d ratio to the average concentration of radicals in a glassy matrix at 77 K in the region $d_1/d \leq 1.1$ were obtained. These curves show that the d_1/d value of 1.1 corresponds to $\langle r\rangle \approx 17$ Å.

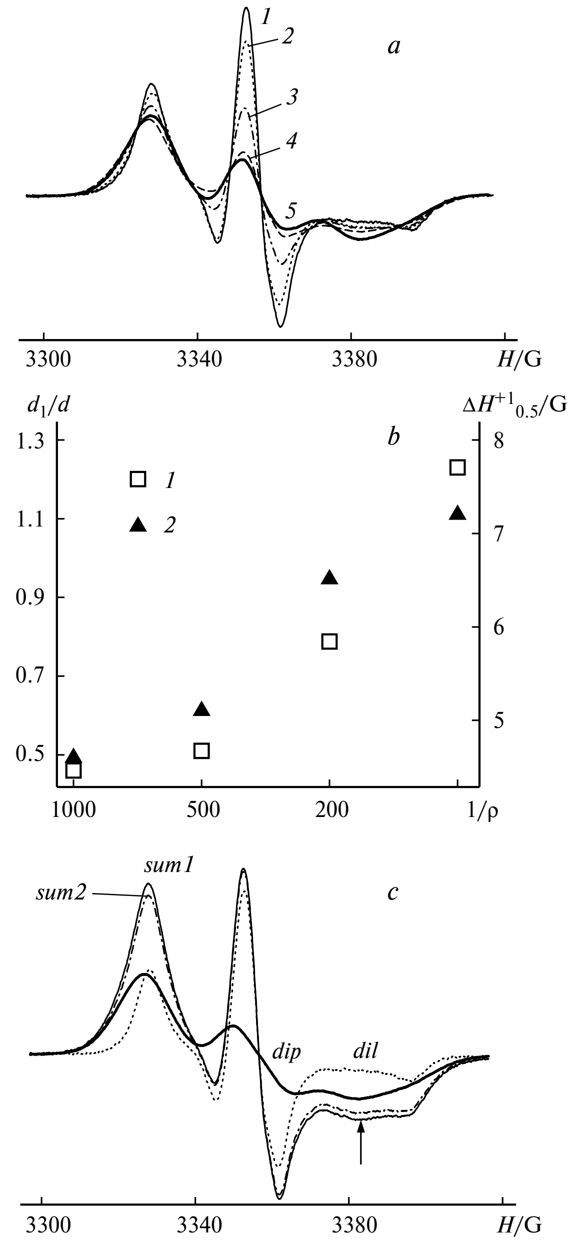

Fig. 6. *a*. ESR spectra of complex **2** with β-CD at 295 K obtained at probe/β-CD molar ratios of 0.001 (*1*), 0.002 (*2*), 0.005 (*3*), 0.01 (*4*), and 0.025 (*5*). Spectra *1—4* were normalized to the double integrals; *b*. Dependences of the d_1/d parameter (*1*) and the halfwidth of the $m = +1$ component ($\Delta H^{+1}{}_{0.5}$) (*2*) on the spin probe/β-CD molar ratio (ρ) in the range 0.001—0.01; *c*. Spectral subtraction and addition for the separation of the dipolar broadened (*dip*) and magnetically diluted (*dil*) components. To obtain the *dip* component, spectrum *1* should be subtracted from spectrum *4* with a weight factor of 0.2 (*dip* = *4* — 0.2 • *1*), to obtain the *dil* component, the *dip* component should be subtracted from spectrum *1* using the same weight factor, 0.2 (*dil* = *1* — 0.2 • *dip*). To validate the two-component spectral decomposition, the experimental spectrum *3* was reconstructed using the *dip* and *dil* components, see spectra *sum1*, *sum2* (*sum1* = *1* + 0.9 • *dip*, *sum2* = *dil* + 0.95 • *dip*). Obtained at a temperature of 295 K.

A similar estimate of ⟨*r*⟩ was obtained from a d_1/d-*vs.*-⟨*r*⟩ calibration curve for a random quasi-one-dimensional distribution of radicals in a glassy matrix.[24] In our case (ρ = 0.01) the d_1/d parameter is formally equal to 1.23, which corresponds to a smaller ⟨*r*⟩ value. However, it should be noted that the appearance of a maximum at an intermediate field (see Fig. 6, *c*) makes this parameter senseless. The spectrum obtained at ρ = 0.025 exhibits almost no high-field extremum and the d_1/d parameter loses its meaning. Apparently, the spectrum for ρ = 0.025 corresponds to a much smaller ⟨*r*⟩ value than 17 Å. Nevertheless, it is interesting to compare this strongly overestimated upper bound of the average distance between spin-labeled indole molecules in the solid complexes with the crystal lattice parameters of β-CD and its complexes.[25—27] X-ray analysis data obtained for a large number of complexes with different guests show that the lattice constants *a*, *b*, and *c*, which characterize the distances between the centers of gravity of the neighbor molecules are in the range 10—25 Å. Thus, the estimates of the average distance between the guest molecules made using the dipolar broadened component of the ESR spectrum of the complex fall in the range of distances between the nearest neighbors in the lattice for the β-CD complexes. Therefore, we can conclude that two types of microphases (microcrystals) are formed on crystallization of the β-CD complexes. These are (i) magnetically diluted microphases, in which the complex molecules are surrounded by β-CD molecules, and (ii) microphases of the complexes in which a strong spin-spin interaction between guest molecules occurs. The ratio of both microphases depends on the probe/β-CD molar ratio and on crystallization conditions; however, even at small ρ values (ρ ≥ 0.01) the predominant fraction of complexes crystallizes in a separate phase. Unlike β-CD, γ-CD and its complexes with compounds **1** and **2** seem to crystallize identically and therefore no separate complex phase is formed.

Environment polarity of the NO group of guest molecules. The complexes of both guest molecules with β-CD are characterized by higher h_c values compared to the γ-CD complexes (see Table 1). However, they are somewhat smaller than those of the corresponding complexes in aqueous solutions.

Molecular dynamics of guest molecules in the complexes with β-CD. Changes in the ESR line shape at high temperatures are more pronounced for the β-CD complexes (especially, **2**—β-CD) than for the γ-CD complexes (see Fig. 2, *b*). For instance, at ρ = 0.005 the $2A'_{zz}$ parameters of the complexes of compounds **1** and **2** with β-CD decrease by 2.8 and 4.5 G, respectively, in the temperature range 133—300 K. In the model of isotropic Brownian rotation,[17] this corresponds to correlation times of $3.0 \cdot 10^{-8}$ and $5.0 \cdot 10^{-8}$ s, respectively, at 300 K. The corresponding changes in the linewidth should be of the

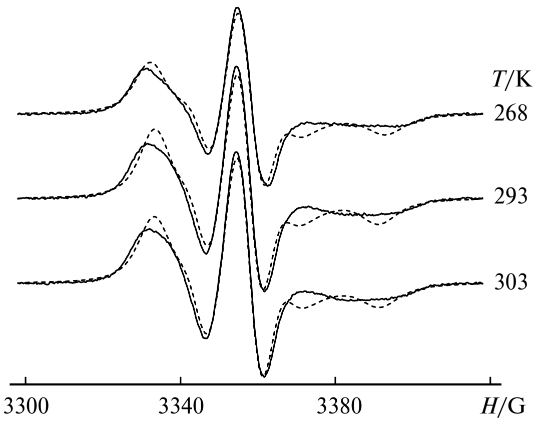

Fig. 7. Linear ESR spectra of magnetically diluted fraction of complexes **2**—β-CD with a spin probe/β-CD molar ratio of 0.001 (solid lines) and the best fit ESR spectra (dashed lines) obtained using the model of Brownian rotation in the orientation potential.[29]

order of 1.5 G;[17] however, as for the complexes with γ-CD, the experimental linewidths decrease rather than increase with temperature.

Examples of ESR spectra of magnetically diluted complexes (ρ = 0.001) recorded at several temperatures are given in Fig. 7. At all temperatures, these spectra correspond to a higher rotational mobility than the "two-component" spectra corresponding to ρ = 0.005. In order to determine the dynamic parameters, we simulated the linear ESR spectra of magnetically diluted complexes using a model of Brownian rotational diffusion in an orientation potential.[29] The experimental and best fit spectra at several temperatures are shown in Fig. 7. The high-field region of the experimental spectrum exhibits an appreciable rotation-induced broadening, which is absent in the simulated spectra. At the same time the simulated spectra exhibit two well-resolved extrema in this region. These discrepancies can not be eliminated by varying the magnetic tensors within the reasonable limits or by representing the spectrum as a superposition of two components with different rotational mobilities of guest molecules.

The V'_2 spectra of the complexes of compounds **1** and **2** with β-CD at 298 K are shown in Fig. 8, *a*. They are significantly different from the V'_2 spectra of the complexes with γ-CD (see Fig. 4); namely, they exhibit a "hole" in the L'' region, corresponding to the maximum angular dependence of the resonance field. As for the γ-CD complexes, no detailed simulation of the V'_2 spectra was performed because of approximations in the calculations mentioned above. Comparison with the V'_2 spectra simulated using the rotational jumps model shows that the experimental V'_2 spectra of radicals **1** and **2** in the complexes with β-CD are in qualitative agreement with rotation in the whole solid angle at frequencies ~$7 \cdot 10^7$ and $1.5 \cdot 10^7$ s^{-1}, respectively (Fig. 8, *b*).

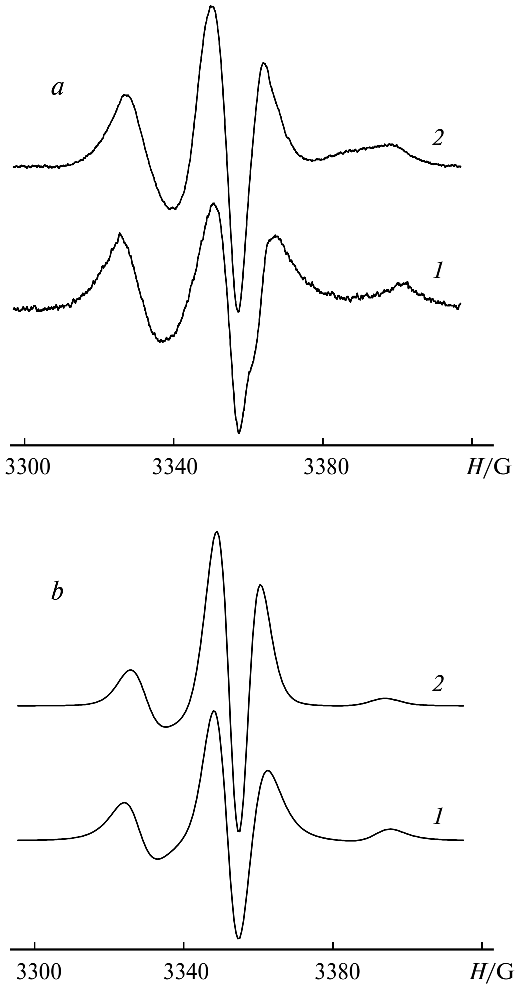

Fig. 8. *a*. ST ESR (V'_2) spectra of complexes **1**—β-CD and **2**—β-CD at a spin probe/β-CD molar ratio of 0.001 and a temperature of 293 K; *b*. Simulated V'_2 spectra calculated using the model of rotational jumps in a cone with the π/2 cone angle and frequencies $7 \cdot 10^7$ (*1*) and $1.5 \cdot 10^7$ s^{-1} (*2*).

We also simulated linear ESR spectra (Fig. 9) using the rotational jumps model. Figure 9 shows that in spite of the differences from the experimental spectra, this model better represents appreciable broadening in the high-field spectral region and, hence, describes the experiment more correctly than the model of Brownian rotation in the orientation potential. Differences from the experimental spectra are probably due to the approximations of the model, that is, isotropic rotation and adiabatic approximation for the resonance field. The temperature dependences of the rotational frequencies of molecules **1** and **2**, determined by simulating linear experimental ESR spectra, are shown in Fig. 10. First, it should be noted that the rotational frequencies derived from the linear spectra are similar to the values mentioned above that were found by simulating the quadrature spectra (V'_2). Second, in the range 238—333 K the rotational frequencies depend only

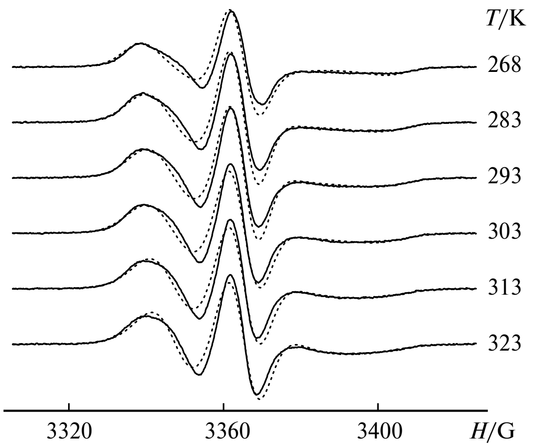

Fig. 9. Linear experimental (solid lines) and the best-fit (dashed lines) ESR spectra of complex **2**—β-CD for a spin probe/β-CD molar ratio of 0.001 at different temperatures. The spectra were calculated using the model of rotational jumps in a cone with the π/2 cone angle. The rotational frequencies are given in Fig. 10.

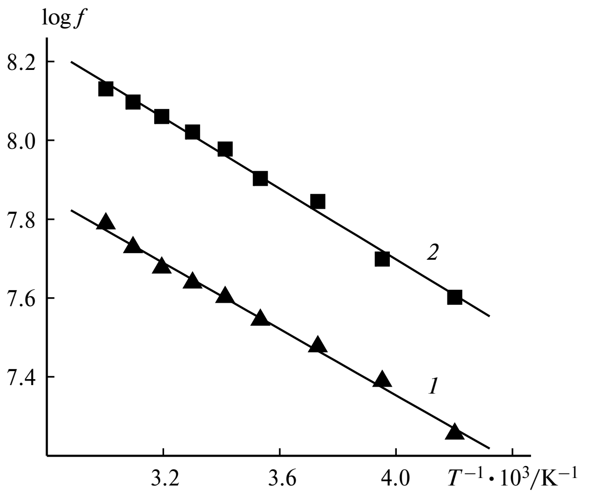

Fig. 10. Arrhenius plots of temperature dependences of rotational frequencies (f) of spin-labeled indoles **1** (*1*) and **2** (*2*) in the complexes with β-CD. Rotational frequencies were determined by simulating linear ESR spectra using the model of rotational jumps in a cone with the π/2 cone angle.

slightly on temperature, namely, the rotational frequencies of **1** and **2** vary in the range $1.8 \cdot 10^7$—$6 \cdot 10^7$ and $4 \cdot 10^7$—$1.3 \cdot 10^8$ s^{-1}, respectively. The temperature dependences of rotational frequencies for both guest molecules are linear in the Arrhenius coordinates with similar activation energies for **1** and **2** ($\varepsilon_A \approx 1.93 \pm 0.05$ and 2.1 ± 0.1 kcal mol^{-1}, respectively). Higher rotational frequencies of **2** compared to **1** are likely due to the contribution of intramolecular transitions in the piperidine ring in molecule **2** and to steric hindrances produced by the methyl group in molecule **1**.

As mentioned above, the character of rotation of the same probe (**1** or **2**) is different in the β-CD and γ-CD complexes. Indeed, this is rotation in the whole solid angle for the former and angle-limited librations for the latter complexes. These differences seem to be due to different stoichiometry of the complexes, namely, one host molecule per guest molecule for the γ-CD complexes and two host molecules per guest molecule for the β-CD complexes.[14] In the cavity of the β-CD dimer the free volume necessary for reorientation of a guest molecule is larger than in the γ-CD monomer cavity.

* * *

As mentioned above, there are a few studies on the molecular dynamics of guest molecules in the cyclodextrin cavities. In complexes of α-CD with *p*-nitrophenol, the activation energy for reorientation of the guest molecules about the C(4)—C(1) axis is 50 kJ mol^{-1} (obtained from the temperature dependence of the NMR linewidth).[8] ^{13}C NMR spectroscopy was also employed[9—11] for studying cyclodextrin conformations and dehydration of β-CD hydrate. ^2H NMR spectra and spin relaxation data of perdeuterated hydrate β-CD suggest that macrocycles do not move as a whole.[9] The ^{13}C and ^1H spin-lattice relaxation times and cross-polarization in the rotating frame were measured for benzaldehyde complexes with α-Cd, β-Cd, and γ-CD. It was assumed[11] that large differences between the cross-polarization of the guest molecule in three complexes with these CDs are due to molecular motions with correlation times of 0.1—0.5 ms. Molecular motions were also detected for ferrocene and ruthenocene in their complexes with cyclodextrins in the solid phase.[8—11]

In this work the spin-labeled indoles were used as guest molecules because indole is a constituent of an important aromatic acid, tryptophan, being also a fluorophore. Therefore, ESR data on the structure and molecular dynamics can be used for interpreting the fluorescence parameters of indoles in the complexes with CD. Radicals **1** and **2** have the same molecular weight but different spatial structures. Because of this, it was also interesting to assess the influence of the spatial structure of the guest species on its mobility inside the CD cavity.

We carried out ESR studies of solid complexes of spin-labeled indoles **1** and **2** with β-CD and γ-CD over a wide temperature range. This made it possible to determine a number of structural and molecular dynamics parameters and reveal some specific features of crystallization of these complexes. In particular, in the presence of excess γ-CD solid solutions are formed, where the complex molecules are surrounded by the host molecules, which manifests itself in the absence of spin-spin interaction between guest molecules. In the case of β-CD, both pure complex microphases (with strong dipolar and exchange interaction between neighboring guest molecules) and solid solution microphases (where complexes are uniformly distributed in the β-CD matrix so that the spin-spin interaction is almost absent) are formed. Pure complex microphases predominate already at a spin probe/β-CD molar ratio of 0.005.

For all the four complexes, we determined the environment polarity of the NO group of guest molecules. In γ-CD, the environment hydrophobicity of the NO group in the solid phase is higher than in solution, whereas the reverse occurs for the β-CD complexes. Differences in polarity are likely due to different number of water molecules in the complexes in solution and in the solid phase.

Guest molecules inside the γ-CD cavity undergo fast librations whose amplitude increases with temperature. The absolute value of the libration amplitude of the magnetic anisotropy axis (z), which coincides with the orientation of the p-orbit of the unpaired electron of the probe NO group, is at most 16° at 333 K. Simulation of linear and ST ESR spectra of the β-CD complexes shows that the libration frequencies are lower and the libration amplitudes are much larger than for γ-CD. These differences are most probably due to different stoichiometries of the complexes and different orientation of the guest molecules inside the β-CD and γ-CD cavities. According to quantum chemical calculations,[14] molecules **1** and **2** are incorporated into 1 : 1 complexes with γ-CD in such a fashion that the free volume accessible to reorientation is minimum. In the β-CD complexes, guest molecules are incorporated into the β-CD dimer in such a way that their long axes are nearly parallel to the dimer axis; in this case, the free volume accessible to guest reorientation in the dimer cavity increases compared to γ-CD.

In both CD molecules the rotational mobility of molecule **2** is higher than that of **1** due to the contribution of intramolecular conformational transitions of the piperidine ring, and, probably, to steric hindrances produced by the methyl group in molecule **1**.

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