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Complexation of Nitroxide Radicals with Cyclodextrins: Kinetics of Crystal Complex Formation

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Abstract

Using the ESR method in combination with the stop-flow technique the kinetics of crystal complex formation of hydrophobic nitroxide radicals with cyclodextrins has been studied upon mixing cyclodextrin aqueous solutions with nitroxides emulsified in aqueous milieu. The α-, β- and γ-cyclodextrins and the piperidine nitroxide radicals with a $OC(O)C_mH_{2m+1}$ (m = 7, 10, and 17) substituent in a para-position were used. It was established that the complexation process in the system emulsion of probe–cyclodextrin solution consists of two main stages. The first stage is the transfer of probes from drops into aqueous solution and the formation of complexes RC_n, where R is the probe, C is cyclodextrin, n = 1, 2 and 3. The second stage is crystal complex formation and growth from solution of RC_n complexes. The results obtained indicate that mainly RC_3 complexes take part in crystallization. It was observed that the characteristic time of crystallization is approximately inversely proportional to the concentration of RC_3 complexes. Equilibrium constants of the processes R + C $\rightleftarrows$ RC, RC + C $\rightleftarrows$ RC_2 have been determined. It was found that complexation and further crystallization lead to the formation of monodispersed microcrystals.

Introduction

Cyclodextrins are cyclic polysaccharide molecules in which six (α-cyclodextrin), seven (β-cyclodextrin), or eight (γ-cyclodextrin) glucose monomers are linked to form a truncated conical structure. Cyclodextrins are of great use because of their unique ability to form inclusion complexes with a wide variety of different molecules. In such complexes the molecule or a part of it is placed in the cavity of cyclodextrin.

The main studies of the complexation with cyclodextrin deals either with a dynamic equilibrium complexation in solution or with crystalline inclusion complexes. A great number of papers are devoted to the study of a dynamic equilibrium complexation in solution described by the equation

$$C + R \rightleftarrows CR,$$

where C is cyclodextrin, R is the guest molecule, and CR is the inclusion complex. Equilibrium constants have been obtained for the complexation with various molecules [1–13].

Crystal complexes have also been studied by different methods. There are data on their structure, composition and properties [1, 14].

However, there is still no information on the rates and stages of the crystal complex formation in the literature. It is obvious that knowledge of the kinetic patterns for the whole sequence of the complex formation is closely correlated with the possibility of controlling the crystal complex properties.

The aim of the present study was to determine the sequence of processes leading to the formation of crystal complexes and to obtain their characteristic rates.

An experimental approach was based on a spin probe technique. A set of spin probes which form inclusion complexes with cyclodextrins has been chosen. Complexation of these probes, initially in the dispersed phase of aqueous emulsions, was examined.

Materials and methods

Cyclodextrins from Cyclolab company were used without additional purification. The nitroxyl piperidine probes with a $OC(O)C_nH_{2n+1}$ substituent in the para-position (see Figure 1) were chosen as guest molecules. Probes with n = 7, 10 and 17 were synthesized by A.B. Shapiro in the Institute of Chemical Physics of Russian Academy of Sciences according to [15]. Systems used in the study were emulsions of these probes in aqueous milieu. The aqueous milieu was a 1 wt% aqueous solution of the non-ionic surfactant proksanol-268 (pluronik F-68 type), which was used to stabilize the emulsions. Emulsions were prepared by adding the aqueous surfactant solution to a weighed probe sample, after which the system was dispersed with the aid of an ultrasonic generator (22 kHz) for 5 min. Probes R_7 and R_{10} are liquids at room temperature but probe R_{17} is a solid substance (with a melting temperature close to 40 °C). Therefore probes R_7 and R_{10} were dispersed at room temperature and probe R_{17}

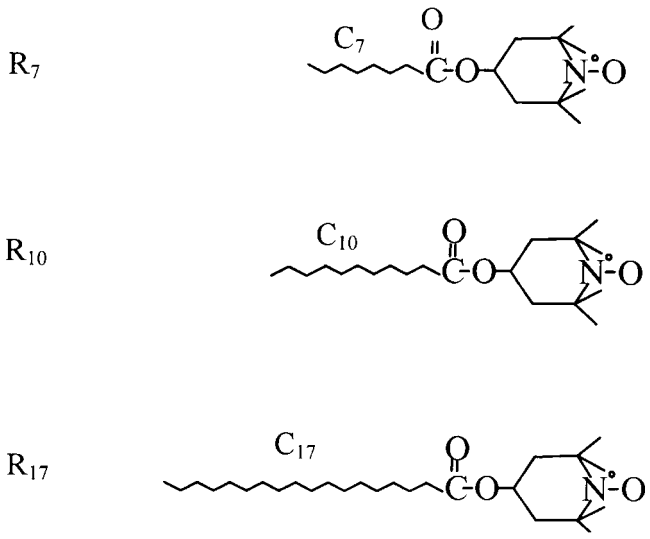

Figure 1. The structures of the paramagnetic probes used.

was dispersed at 50–60 °C. The emulsions obtained in such a way are stable for 1 to 3 days without considerable change of droplet size within the time of the experimental series (3–5 hours).

To determine a probe : cyclodextrin ratio for the crystal complexes, the emulsions of probes were mixed with cyclodextrin solutions in equal volumes. The obtained systems of volume 0.5 mL were placed in thin-walled glass ampoules with internal diameter 2 mm. After the complete precipitation (5–10 days) the precipitates obtained were desiccated and then weighed. The amount of probes in the precipitates was determined by double integration of the ESR spectra of the precipitates.

The analysis of the dispersity of the emulsions and systems after crystallization was carried out using an electron microscope. The average diameter of droplets of the dispersed nitroxide measured with the electron microscope was 0.03–0.06 μm.

To investigate the kinetics of complexation emulsions of the probes were mixed with aqueous cyclodextrin solutions in equal volumes with use of a stop-flow block, combined with an ESR spectrometer. After that the change of probe localization was monitored by recording the change in ESR spectrum. The dead time of the system was about 0.03 sec. The common X-band spectrometer ESR-V (Institute of Chemical Physics of Russian Academy of Sciences) was used.

The probe concentration in the emulsions studied was in the range $10^{-4} - 2 \times 10^{-3}$ M. Solutions of probe R_7 with concentration 5×10^{-5} M were also investigated. The concentration of the cyclodextrin solutions was varied from 2×10^{-4} M to quantities close to the solubility limits (solubility of α-, β-, and γ-cyclodextrins are 0.15 M, 0.016 M, and 0.18 M respectively). The experiments were carried out at room temperature.

Results and discussion

In general, the process of crystal complex formation in an emulsion can be described by the following a priori scheme:

$$R_d \leftrightarrows R_s \qquad (0)$$

$$R_s + C \leftrightarrows RC \qquad (1.1)$$

$$RC + C \leftrightarrows RC_2 \qquad (1.1)$$

$$\cdots\cdots$$

$$RC_{n+1} + C \leftrightarrows RC_n \qquad (1.n)$$

$$RC_n + RC_m \leftrightarrows R_2C_{n+m} \qquad (2.n+m)$$

$$\cdots\cdots$$

$$R_mC_n + R_kC_s \leftrightarrows R_{m+k}C_{n+s} \qquad (m+k.n+s)$$

where C is cyclodextrin, R_d is the probe in the emulsion drop, R_s is the probe dissolved in aqueous milieu, R_mC_n is the probe-cyclodextrin complex; $n \geq 1$, $m, k, s \geq 0$.

The first process is the transition of a probe from a drop into solution and back. Equilibrium (0) is easily observed before mixing the emulsion with cyclodextrin solution: the ESR spectrum is a superposition of a singlet line (probe in dispersed particles) and a triplet signal (probe in solution) (Figure 2(2)). Treatment of spectrum 2 (Figure 2), for example, shows that 96% of probes are located within emulsion droplets and 4% of probes are in the water milieu.

Processes $(1.n) - (m + k.n + s)$ correspond to the formation of low molecular complexes dissolved in aqueous milieu and the further formation of crystal complexes.

It was found experimentally that complexation leads to the two types of spectra. The first one is a triplet. Its line width is 10% more and the isotropic constant is 0.13 G less than the corresponding parameters for the spectrum of an isolated probe in solution (Figure 2 (spectrum 3)). The second spectrum observed (Figure 2 (spectrum 4)) coincides with the spectrum of precipitates from this systems. We attribute the triplet signal to the dissolved RC_n complex in which the only probe binds n cyclodextrins ($n \geq 1$). According to the estimates, the distance between neighbouring probes in the R_mC_n complex ($m \geq 2$) should be less than 40 Å (at $m/n \leq 4$, according to the data obtained on the composition of precipitates, see below). Considerable line broadening occurs at such separation between probes [16].

Thus, in all systems studied a triplet signal was considered to be a sum of signals from probes and complexes RC_n ($n \geq 1$) dissolved in aqueous milieu. Changing the concentration of probes and complexes RC_n in solution did

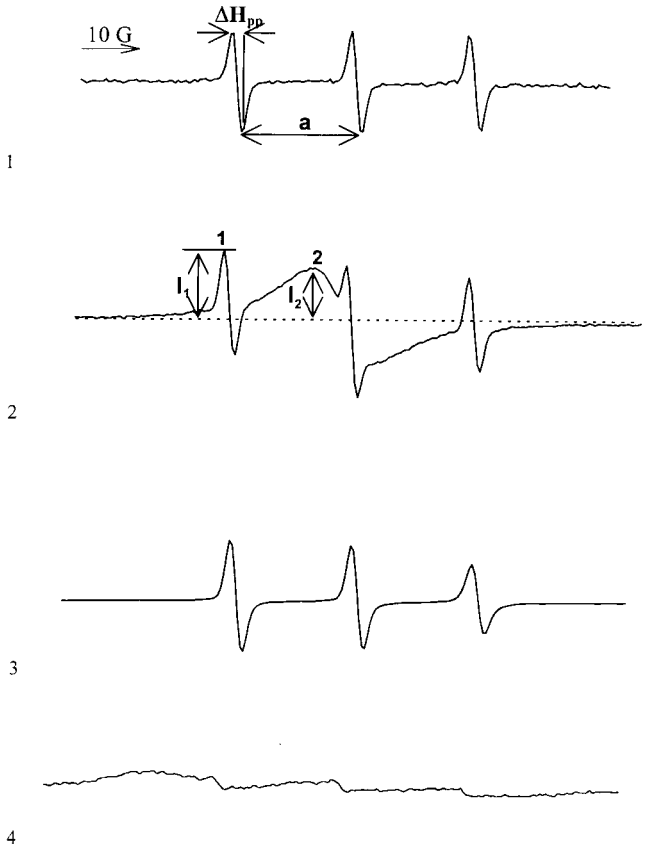

Figure 2. ESR spectra of probe R_7 in various systems at room temperature. (1) Saturated solution of probe R_7 in aqueous milieu ([R] = 8×10^{-5} M). (2) Emulsion of probe R_7 ([R] = 2×10^{-3} M). ΔH_{pp} = 1.64 G, a = 16.47 G. (3) Mixture of 2×10^{-3} M emulsion of probe R_7 with 10^{-2} M solution of α-cyclodextrin. (4) Mixture of 2×10^{-3} M emulsion of probe R_7 with 10^{-2} M β-cyclodextrin solution 2 min after mixing. The magnification for system 3 is 10% of the magnifications for other systems involved.

not lead to any observable change in line-width of the corresponding components. If the shape of the ESR spectrum does not change its intensity is proportional to the concentration of a probe in the corresponding state. Thus, the intensity of the triplet is linearly related to the concentration of the probes dissolved and with the total concentration of RC_n complexes in solution.

Generally speaking, the direct attack of emulsion drops by cyclodextrin molecules (i.e., $R_d + C \rightarrow RC$) is possible. But as shown below, the direct attack can be excluded from the consideration because of their negligible velocity.

The goal of the present study was to obtain experimental data that would give a possibility to detail the processes in a priori scheme and to obtain their characteristic rates.

The complexation of probes R_7, R_{10} and R_{17} with all types of cyclodextrins (α, β, and γ) has been examined. Probes R_7, R_{10} and R_{17} differ in the length of their hydrophobic tails and consequently in their solubility in aqueous milieu. Probe solubility, obtained by treatment of emulsion spectra, are $(8 \pm 3) \times 10^{-5}$ M for probe R_7, $(2 \pm 1) \times 10^{-5}$ M for probe R_{10}, and $\sim 10^{-7}$ M for probe R_{17}.

It was found that crystal complex formation take place in all systems with the exception of the probe R_7 – α-cyclodextrin and probe R_{10} – α-cyclodextrin systems. The absence of crystallization in the latter systems enables determination of the equilibrium constants of the processes (1.1) and (1.2). The results of this determination are presented below. The results of studying the kinetics of crystal complex formation are reported and discussed together with the data on crystal complex morphology obtained with electron microscopy.

Determination of equilibrium constants

It was found that the spectrum of the system upon mixing an emulsion of probe R_7 or R_{10} with α-cyclodextrin solution differs from the spectrum of the emulsion of the probe only by a more intensive triplet component and less intensive singlet signal. Such a spectrum settles in 0.1–0.4 sec after mixing and remains unchanged for more than 10^5 sec. The triplet signal corresponds to the dissolved probes and complexes RC_n ($n \geq 1$) in solution. Thus, the system reaches the equilibrium, which is determined by the processes (0), (1.1) – (1.n) in the general complexation scheme.

It is clear that the dissolution velocity at the initial stage (i.e., far from equilibrium) is determined by the following processes: a) transition of the probe from drop to solution ($R_d \rightarrow R_s$) with subsequent complexation in solution ($R_s + C \rightarrow RC$) and b) the direct attack ($R_d + C \rightarrow RC$). In order to understand what processes are really responsible for dissolution the dependence of the dissolution velocity on the cyclodextrin concentration was examined. It was observed that the dissolution velocity is independent of the cyclodextrin concentration. This means that the dissolution velocity is determined by process a) and that the direct attack can be excluded from the consideration.

Let us consider now the system after equilibrium has been reached. It is easy to obtain for the equilibrium concentrations the following expressions:

$$[RC]=K_1[R_s][C]$$

$$[RC_2] = K_1K_2[R_s][C]^2$$

$$\cdots$$

$$[RC_n]=K_1 \cdot \ldots \cdot K_n[R_s][C]^n$$

were K_i, $i = 1, \ldots, n$ are equilibrium constants of the processes (1.1) – (1.n) respectively. $[R_s]$ is equal to the probe solubility as long as emulsion drops are present in the system. The value of $I \equiv [R_s] + [RC] + \cdots + [RC_n]$ can easily be determined experimentally by measuring the triplet intensity and taking into account that $[R_s]$ is the probe solubility. The experimental dependencies of the I value on total cyclodextrin concentration $[C]_t$ was measured in order to calculate the equilibrium constants (Figure 2). The equilibrium constants were estimated assuming that only the first three processes determine equilibrium. The formation of RC_n-complexes with $n \geq 4$ is improbable for probes R_7

Table 1. Decimal logarithm of equilibrium constants K_i ($[K_i] = M^{-1}$) for the processes $R_s + C \leftrightarrows RC$ (first value), $RC + C \leftrightarrows RC_2$ (second value) and $RC_2 + C \leftrightarrows RC_3$ (third value) and characteristic crystallization times τ, sec (in square brackets) for various probe-cyclodextrin systems. The characteristic time was measured at $[R]_t = 10^{-3}$ M, $[C]_t = 5 \times 10^{-3}$ M. [∞] – no crystallization observed for 20 days

Probe	Cyclodextrin					
	α		β		γ	
R_7	3.0	[∞]	3.1	[3.5]	2.8	[$\sim 10^5$]
	2.6		2.8		2.1	
	≤ 1.4		≤ 1.7		≤ 1.0	
R_{10}	3.6	[∞]	–	[28]	–	[$\sim 10^6$]
	3.0					
	≤ 1.4					
R_{17}	4.5	[$\sim 10^5$]	–	[$\sim 10^5$]	–	–
	–					

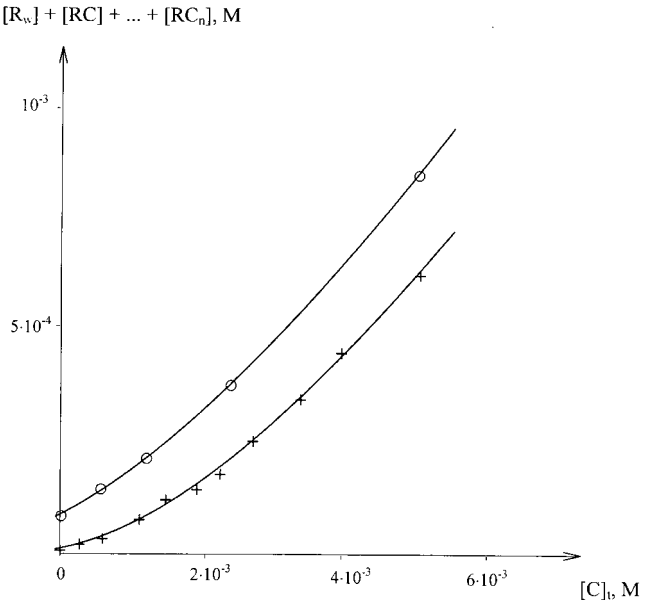

Figure 3. Experimentally observed dependencies of 1 vs $[C_t]$ ($I \equiv [R_s + [RC] + \cdots + [RC_n]$, $[C_t]$ is the total α-cyclodextrin concentration) for the probe R_7 – α-cyclodextrin system ('○') and the probe R_{10} – α-cyclodextrin system ('+'). Total probe concentration is 10^{-3} M. Solid lines – the appropriate dependencies obtained by fitting.

and R_{10} because of the geometric inconsistency of the R-tail length to the total width of the four cyclodextrin molecules. The supposition mentioned leads to two simple equations:

$$3K_1K_2K_3[R][C]^3 + 2K_1K_2[R][C]^2 + K_1[R][C] + [C] = [C]_t$$

$$K_1K_2K_3[R][C]^3 + K_1K_2[R][C]^2 + K_1[R][C] + [R] = I$$

These equations relate I with $[C]_t$. This relationship was used to determine the equilibrium constants by fitting (Figure 2). The constants obtained for the processes (1.1) and (1.2) for the systems probe R_7 – α-cyclodextrin and probe R_{10} – α-cyclodextrin are given in Table I. Unfortunately, the determination accuracy allowed us only to estimate the upper limit of the K_3 value. The estimated value is $K_3 \leq 50 \ll K_1$, K_2 (Table 1). This fact confirms the above assumption of negligible concentration of RC_n-complexes with $n \geq 4$.

The dependencies of I vs $[C]_t$ have also been obtained for some systems in which crystallization take place, namely probe R_7–β-cyclodextrin, probe R_7–α-cyclodextrin and probe R_{17}–α-cyclodextrin. This was possible because at sufficiently low cyclodextrin concentration the characteristic crystallization time in these systems is rather long (see Section 3.2). Only the K_1 value was obtained for the probe R_{17}–α-cyclodextrin system from the initial slope of I vs $[C]_t$ curve. The peculiarities of complexation in the probe R_{17}–α-cyclodextrin system (where $n = 4$ is probable) will be published elsewhere.

Kinetics of complexation

To study the kinetics of complexation emulsions of probes were mixed with cyclodextrin solutions after which the change of probe localization was monitored by recording the change in ESR spectrum. Due to the impossibility of rapid recording of a whole spectrum the change of spectrum intensity was registered only at points 1 and 2 of the field (Figure 2 (spectrum 2)). These points correspond to the maximum of a low-field triplet component (point 1) and to the maximum of a singlet line (point 2). The intensity of the spectrum of a system at point 1 (I_1) with acceptable accuracy is linearly related to the concentration of the probes dissolved and with the total concentration of RC_n ($n \geq 1$) complexes in solution. The intensity at point 2 (I_2) is approximately proportional to the amount of probes in the emulsion droplets.

As an example, shown in Figure 4, are these dependencies for the system upon mixing 2×10^{-3} M emulsion of probe R_{10} with 10^{-2} M β-cyclodextrin solution. The curve $I_1(t)$ consists of two main parts: the initial growth of intensity to maximum value, and the subsequent decrease to a value close to zero. The increase of I_1 is accompanied by the abrupt decrease of I_2. Because of the linear dependencies between I_1 and the concentration of complexes RC_n, $n = 1$ in solution and between I_2 and the amount of probes in emulsion drops, it is clear, that the initial parts of these curves correspond to transition of probes from drops into solution and to formation of RC_n complexes. According to the results reported above n = 1, 2 and 3. At the end of the initial part (approximately 0.3 sec after mixing) about 15% of probes transfers from drops into solution of RC_n complexes. The subsequent decrease of I_1 corresponds to the decrease of concentration of RC_n complexes, because of the formation of crystal complexes. Simultaneously the decrease of the amount of probes in drops take place. Thus, the two main stages of complexation can be pointed out from kinetic curves $I_1(t)$ and $I_2(t)$: the first stage are the processes (0)–(1.n) in the general complexation scheme leading to dissolution of emulsion drops and formation of a supersaturated solution of RC_n complexes, and the second stage is the formation and growth of crystals from RC_n complexes in solution.

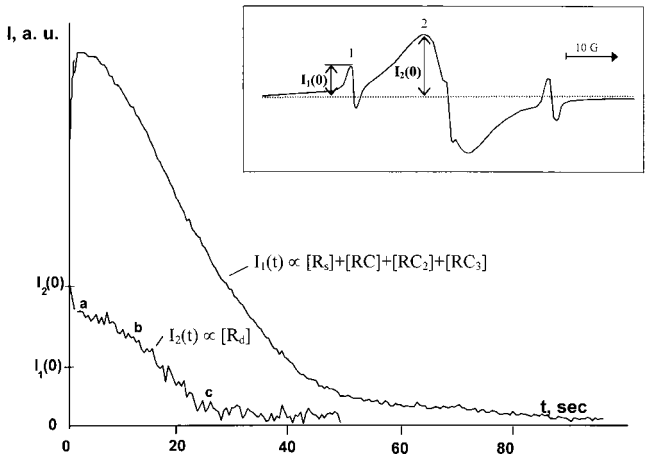

Figure 4. Kinetics of complexation in a system upon mixing 2×10^{-3} M emulsion of probe R_7 with 10^{-2} M β-cyclodextrin solution. Insert – initial spectrum of the system. $I_1(t)$, $I_2(t)$ – the dependencies of the spectrum intensity at points 1 and 2 of field on the time after mixing.

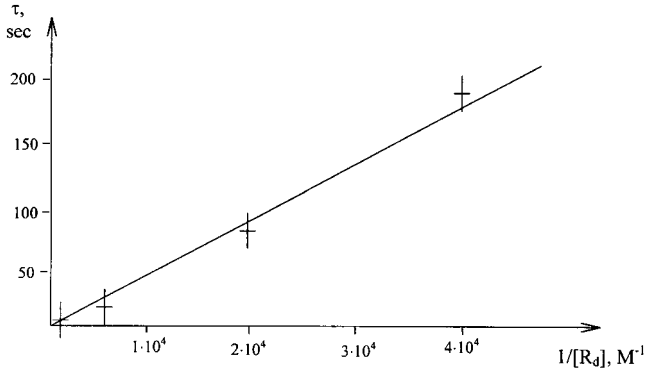

Figure 5. The dependence of the characteristic crystallization time in the probe R_7–β-cyclodextrin system on the $1/[R]_t$ value, where $[R]_t$ is the total probe concentration. The total cyclodextrin concentration is 5×10^{-3} M.

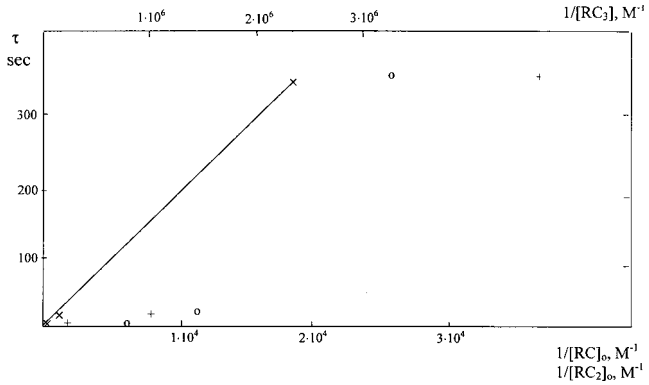

Figure 6. The dependence of the characteristic crystallization time (τ) of the probe R_7–β-cyclodextrin system on the initial concentrations of RC ('○', lower axis), RC_2 ('+', lower axis) and RC_3 ('×', upper axis) complexes. K_1, K_2 from Table 1 and K_3 = 20 M^{-1} were used in calculations.

The above two stages were experimentally observed in all probe–cyclodextrin systems where crystallization takes place.

The basic parameter that was determined from the kinetic curves is the characteristic time of crystallization τ, that is the time for which the intensity $I_1(t)$ decreases 2.7 times in relation to the maximum value.

Table 1 gives the characteristic crystallization times obtained for various probe–cyclodextrin systems. Characteristic times of the first stage (the initial dissolution of droplets) are about 0.1–0.3 sec and the time of the second stage greatly exceeds that of the first stage for all systems studied. That is, as long as emulsion droplets are present in the system the concentration of RC_n complexes in solution is mainly determined by processes (0)–(1.n) and the current cyclodextrin concentration. At 10^{-3} M probe and 5×10^{-3} M cyclodextrin concentrations the total dissolution of drops takes place only for the probe R_7 – β-cyclodextrin system. For other systems, there is a gradual decrease of the amount of probes in drops (after initial rapid partial dissolution) with simultaneous crystal growth.

As measurements of paramagnetic centers concentration in solution after precipitating have shown, the solubility of the crystals are less than 5×10^{-6} M.

All kinetic curves have slow and fast parts of concentration decrease (ab and be in Figure 4). This fact is in agreement with the generally accepted concept of the kinetics of crystallization from solution. The slow part corresponds to the formation of the crystal nuclei and their growth to such an extent that the formation rate of the new phase becomes more appreciable (particularly due to the increase of crystal surface).

The system probe R_7–β-cyclodextrin that has the highest crystallization rate was chosen to study the concentration dependencies of the crystallization time. The probe concentration was varied from 2.5×10^{-5} M to 5×10^{-4} M at constant cyclodextrin concentration (5×10^{-3} M) in the first series of experiments. So the cyclodextrin was in excess and there was a total dissolution of droplets in all systems.

Experiments showed that within the range of experimental errors the crystallization time is approximately inversely proportional to the initial probe concentration (Figure 5). It is easy to show that in the case of total dissolution each of the initial RC_n concentrations is proportional to $[R_t]$. For example one can conclude by using the equilibrium constants obtained that $[RC] = 0.2\ [R]_t$, $[RC_2] = 0.7\ [R]_t$, $[RC_3] = f \cdot [R]_t$ where $f \leq 0.1$. So, the dependencies of τ vs $[RC]^{-1}$, $[RC_2]^{-1}$ or $[RC_3]^{-1}$ are all straight lines.

In order to find out which of the RC, RC_2, RC_3 complexes give the most contribution to the crystal growth another series of experiments was performed. The initial cyclodextrin concentration was varied within the range $5 \times 10^{-3} - 5 \times 10^{-4}$ M at constant probe concentration (10^{-3} M). Variation of the cyclodextrin concentration leads to unproportional change of RC, RC_2 and RC_3 complexes concentrations in solution. Figure 6 shows the dependencies of the crystallization characteristic time on the maximum values of RC, RC_2 and RC_3 complexes concentrations, which have been calculated using the equilibrium constants obtained. K_3 = 20 M^{-1} was used for calculations. The uncertainty in the K_3 value (see Table 1) does not affect the shape of the corresponding curve. One can see that τ is approximately inversely proportional to the RC_3 complex concentration but not to RC and RC_2 complexes concentrations. This fact indicates that crystallization takes place mainly from RC_3 complexes.

This supposition is in agreement with the data obtained on the composition of precipitates from the probe R_7–β-cyclodextrin system, according to which the probe : cyclodextrin ratio in the crystal complex is 1 : 3.

Electron microscope photographs of crystals forming in the system probe R_{10}–β-cyclodextrin were obtained. The samples for study were taken at various times after mixing 2×10^{-3} M probe emulsion with 10^{-2} M cyclodextrin solution. It was observed that the resulting suspension consists of crystals that have a parallelepiped shape and approximately equal size of about 0.5 μm. The shape and the crystal size does not depend on the time after mixing at least within a period of 10 sec–10 days. The above fact is probably due to high supersaturation arising after droplet dissolution due to complexation with cyclodextrin molecules.

Conclusions

For the first time, the kinetics of crystal complex formation in an emulsion drop-cyclodextrin system has been studied by the spin probe method. As a model system the emulsions of nitroxide radicals with a $OC(O)C_nH_{2n+1}$ substituent in the para-position were used. The principal stages of this complexation has been established and their kinetic characteristics have been examined. The main results obtained are the following.

1. The complexation process in the emulsion of probe–cyclodextrin solution system consists of two main stages: the first stage is the transition (fully or partially) of probes from drops into solution of complexes RC_n, were R is the probe, C is cyclodextrin, n = 1, 2 and 3 and the second stage is crystal complex formation and growth from RC_n complexes solution.
2. RC_3 complexes mainly take part in crystallization.
3. It was observed that the characteristic time of crystallization is approximately inversely proportional to the concentration of RC_3 complexes.
4. Equilibrium constants of the processes $R + C \leftrightarrows RC$, $RC + C \leftrightarrows RC_2$ have been determined.
5. It was found that complexation and further crystallization lead to the formation of monodispersed microcrystals.

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