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Abstract

Microscopic size particles of the cholesteric double-stranded DNA (RNA) liquid–crystalline dispersions, containing the ions of the rare earth elements in their content, have been obtained for the first time. The properties of these particles differ from those of classical DNA cholesterics noticeably. The local concentration of the rare earth elements in a particle reaches 200 mg/ml. The particles of the liquid–crystalline dispersion of the (DNA–gadolinium) complex maintain the properties for a long time. The combination of the microscopic size of particles, high concentration of gadolinium in particles and their stability opens a way to practical application of this new biomaterial.

Keywords: Nucleic acids; Liquid–crystalline dispersions; Neutron capture therapy; Rare earth elements; Magnetometric and neutron activation analysis

1. Introduction

Neutron capture therapy (NCT) is a cancer treatment that utilizes nuclear neutron capture reaction (NCR) of radiation-producing elements administrated in vivo by thermal neutron irradiation [1].

In the “classical” version of NCT, 10B-compounds are typically used as radiation-producing elements. These compounds were delivered to tumor intracellularly in order to obtain an antitumor effect, because 10B emits α-particles whose ranges are equal to (about 10 μm) a cell diameter or shorter. 10B-NCT was successfully used for patients suffering from malignant melanoma and other diseases. However, developers of NCT are still interested in other radiation-producing elements to make NCT more effective.

The current attention to radiation-producing elements is essentially focused on gadolinium for its favorable properties, even though extensive studies have been reported on the other elements.

Gadolinium neutron capture therapy (Gd-NCT) utilizes the following NCR of 157Gd by thermal neutron irradiation [2]:

\[ \text{157Gd} + \text{n}_{\text{th}} \rightarrow \text{158Gd} + \gamma\text{-rays} + \text{internal conversion electrons} \]

→ 158Gd + γ-rays + Auger electrons + characteristic X-rays

NCT with 157Gd has a few advantages over 10B typically used as radiation producing element in NCT.

Abbreviations: CD, circular dichroism; dsNA, double-stranded nucleic acid; LCD, liquid–crystalline dispersion; NA, nucleic acid

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Materials and methods

The formation of the cholesteric LCD based on dsDNA or synthetic polyribonucleotides as a result of the phase separation from PEG-containing water–salt solutions was described in detail earlier [20,21]. In physicochemical sense, the initial system from PEG-containing water–salt solutions was described in synthetic polyribonucleotides as a result of the phase separation of rare earth elements, were obtained. The goal of this study was to develop Gd–enriched dsDNA (RNA) liquid–crystalline constructions as systems capable of retaining gadolinium in the tumor tissue [5].

Besides, Auger electrons increase the amount of DNA double-strand breaks [6]. Due to large total kinetic energy of these electrons, they increase the possibility of hitting the tumor cells by intensive destruction of DNA in neoplastic cells [7,8].

The γ-rays induce DNA single- and double-strand breaks [9]. Due to large total kinetic energy of these electrons, they increase the possibility of hitting the tumor cells by intensive destruction of DNA in neoplastic cells [7,8].

For this reason, it is necessary to use ligands that form stable complexes with Gd3+ before it is administrated to patients. This means that the developers of NCT are interested in particular molecular constructions with very high affinity to gadolinium, which are able to retain a sufficient gadolinium concentration. The chelate complexes of gadolinium with polymeric molecules [14–16] nanoparticles of various origin [10,17,18] have been produced as potent constructions for targeting a site and controlled release of a drug. Biodegradable, biocompatible chitosan nanoparticles [19] have recently received considerable attention as systems capable of retaining gadolinium in the tumor tissue during a Gd–NCT trial.

Thus, a key factor in the success of the current Gd–NCT trial is the use of molecular constructions by means of which Gd can be delivered efficiently and retained inside tumor tissues and/or cells during thermal neutron irradiation.

In the present paper, the particles of the cholesteric dsDNA (or ds synthetic polyribonucleotides used as a sterical model of dsDNA) liquid–crystalline dispersions, highly loaded by the rare earth elements, were obtained. The goal of this study was to develop Gd–enriched dsDNA (RNA) liquid–crystalline construction as a potential biomaterial for Gd–NCT.

2. Materials and methods

The formation of the cholesteric LCD based on dsDNA or synthetic polyribonucleotides as a result of the phase separation from PEG-containing water–salt solutions was described in detail earlier [20,21]. In physicochemical sense, the initial system was composed of isotropically distributed in poly(ethylene glycol)–water–salt (NaCl) solution, liquid–crystalline particles formed by dsDNAs.

The rare earth salts (99.99% of purity) were purchased from the Institute “Giredmet” (Moscow, Russia). The absorption spectra of all solutions were taken on a spectrophotometer (“Specord M40”, Germany) and the CD spectra were recorded by a portable dichrometer SKD-2 (manufactured by the Institute of Spectroscopy of the RAS, Troitsk, Moscow Region). In all cases, the quartz cells with 1 cm optical path have been utilized.

The morphology of the dsDNA LCD particles treated by GdCl3 was examined using a commercial Atomic Force Microscope P47-SPM-MDT (produced by NT-MDT, Russia). To isolate these particles, the solution in which they were formed was filtered through a poly(ethylene) nitrilate (PETP) nuclear membrane filter with size of pores of 0.15 μm (produced by the Institute of Crystallography of the RAS), that allowed us to immobilize particles; filters were dried in air for no less than 1 h.

To estimate the number of Gd3+ ions in the content of the LCD particles, the magnetic properties of the dsDNA LCD treated by GdCl3 have been measured and total magnetic moment of Gd3+ ions was determined. The dsDNA LCD after GdCl3 treatment was centrifuged (5000 rpm; 40 min; 15 °C), the obtained pellet was flushed a few times by a distilled water to erase the extra amount of nonspecifically absorbed GdCl3 and utilized as a sample for the further analysis. (The treatment of the dsDNA LCD by nonmagnetic LaCl3 and formation of the corresponding pellet was used as a control for the magnetic measurements.)

The magnetic properties of the samples were measured at magnetic field of 71.29 mT (712.9 Oe) at sample position by the superconducting interferometer device (SQUID-magnetometer) produced by D. Mendeleev University (Moscow, Russia) [22]. The sample was characterized magnetically before and after magnetic field introduction within temperature range from 4.2 K to room temperatures. The cooling to helium temperatures was carried out at zero magnetic field (ZFC), then a magnetic field was introduced, and temperature was increased to 100 K.

An amount of quanta (M) of magnetic flux through the pellet of dsDNA LCD treated by GdCl3 was measured continuously during this procedure. The M value is proportional to the magnetic moment (Pm) of the sample used. In our case, Gd3+ ions bounded to the dsDNA molecules in the content of the LCD play the role of the magnetic centers [2].

The determination of magnetic moment, Pm, characteristic of the pellet of dsDNA LCD treated by LaCl3, was measured for comparison.

The temperature dependence of magnetic moment, Pm, characteristic of the pellet of dsDNA LCD treated by LaCl3, was measured for comparison.

The concentration of the gadolinium in the dsDNA LCD particles was additionally checked by the neutron activation analysis [23]. The solution in which they were formed was filtered through a nitrocellulose filter (Sigma, USA; the size of pores is 0.20 μm), that allowed us to immobilize particles in the structure of this filter. The total mass of the DNA complexed with gadolinium was 300 μg. The determination of the DNA concentration in the obtained filtrate showed that here the concentration of the DNA molecules is practically zero. The filter containing all the dsDNA LCD particles was flushed a
few times by a distilled water to erase the extra amount of nonspecifically absorbed GdCl₃, dried in air for no less than 1 h and utilized as a biosample (BS) for the neutron activation analysis.

BS was irradiated by 14.8 MeV neutrons using the neutron generator NG-400 [24]. The fast neutrons beam with a flux of \(2 \times 10^9\) neutron cm\(^{-2}\)s\(^{-1}\) was produced using stationary titanium-tritium targets [25]. For the purposes of signal calibration and flux monitoring, two pure standard samples, Gd-3 and Gd-4, were irradiated as well. In the neutron beam, the BS was placed between two standard samples. The fast-neutron irradiation of the samples (during the time \(T_I\)) leads to neutron-induced reaction \(^{160}\text{Gd}(n,2n)^{159}\text{Gd}\), the reaction cross-section is 1.75 barns. The \(/\gamma\)-decay of radioactive \(^{159}\text{Gd}\) nuclide (its half-life is 18.6 h) generates the \(/\gamma\)-rays with a typical line at 363.6 keV.

After a cooling down of samples (during the time \(T_C\)) the \(/\gamma\)-activity of all samples \(A\) was measured; the measurement time was \(T_M\). For this aim we have used the gamma-spectrometers based on the 200 cm\(^3\) coaxial HPGe detector IGC-45 (ORTEC) with the energy resolution of 2.2 keV and relative efficiency of 45.5% at 1.33 MeV (\(^{60}\text{Co}\)).

3. Results and discussion

3.1. The CD spectra of the linear nucleic acids treated by the rare earth ions

In Fig. 1, as an example, the CD spectra of the linear dsDNA, treated by GdCl₃, are shown.

As a result of interaction of Gd\(^{3+}\) ions with DNA, the amplitude of the positive band (\(\lambda \approx 280\) nm) in the CD spectrum decreases and at certain concentration of GdCl₃ (\(\approx 25\) mM) the band changes its sign. The similar alterations of the shape of the CD spectrum are observed earlier at interaction of multivalent ions with dsDNA [26,27].

The changes in the CD spectra of dsDNA are explained within the framework of idea about a high flexibility of the secondary structure of these molecules, i.e. the secondary structure can be altered as a result of complex formation of the rare earth elements with nitrogen bases and/or phosphate groups [28,29].

The minor changes in the amplitude of the positive band in the CD spectrum detected in the case of the ds poly(I) × poly(C) LCD, treated by the rare earth salts (Fig. 2), reflect, probably, an existence of more rigid secondary structure of polyribonucleotides of A-family.

3.2. The CD spectra of the cholesteric LCD of nucleic acids treated by the rare earth ions

In Fig. 3, as an example, the CD spectrum of the initial dsDNA LCD is compared with the CD spectra of this dispersion treated
by GdCl$_3$. The amplitude of the intense (abnormal) negative band ($\lambda \approx 270$ nm) in the CD spectrum, characteristic of the initial cholesteric dsDNA LCD, is increased, and its maximum is displaced to the red area of the spectrum. The change in the shape of the CD spectrum testifies that the Gd$^{3+}$ ions (as well as La$^{3+}$, Nd$^{3+}$ or Sm$^{3+}$ ions) interact with dsDNA in the content of particles of the LCD.

Similar pronounced changes in the shape of the CD spectrum are observed for ds poly(I) × poly(C) LCD with the abnormal positive band in the CD spectrum (Fig. 4). It should be noted that the increase in the amplitudes of abnormal bands in the CD spectra occurs within a very short time, more exactly, simultaneously with the LCD treatment by the rare earth salts.

Hence, despite the different signs of abnormal bands in the CD spectra, the treatment of the LCDs of dsNA by rare earth ions is accompanied by similar changes in the amplitudes of these bands. However, the concentration of GdCl$_3$ or Sm(NO$_3$)$_3$ necessary to induce the changes in the CD spectrum of the LCD formed by poly(I) × poly(C) is approximately three times lower in comparison to that of the DNA LCD.

It should be noted that complexes between the rare earth ions and the phosphate groups have a low solubility [28,29], and the particles of the dsDNA LCD treated by these ions begin slowly to precipitate. This process is accompanied by decrease in the amplitude of the abnormal band. However, the intensive shaking of the solution containing the obtained pellet results in a full restoration of the initial abnormal band in the CD spectra of the LCDs, i.e. the coalescence of the particles of these LCDs does not happen.

The above-obtained results speak in favor of the assumption that gadolinium ions can easily induce the alteration of the secondary structure of dsDNA, induced by their complex formation with several metals [26,27].

In the framework of the theory [30] that describes the abnormal optical properties of the particles of the initial cholesteric LCD formed by dsNA molecules, one can expect that there are two main reasons responsible for amplification of the abnormal band in the CD spectra:

The first reason is related to the modification of the mode of the dsNA molecules packing in the content of particles of their LCD. To prove this assumption, the dsDNA were treated first by various concentrations of GdCl$_3$ and from these molecules the LCDs were formed. Fig. 5 shows that this treatment is accompanied by a full disappearance of the intense band in the CD spectrum of the LCD formed by these molecules, but the treatment does not influence the capability of these molecules to form the LCD, which scatters the UV irradiation.

Comparison of Figs. 1 and 5 shows unequivocally that gadolinium ions can easily induce the alteration of the dsDNA secondary structure in the case of the linear DNA molecules. Such an alteration prevents the formation of the cholesteric LCD from so-modified DNA molecules. This is typical of violation of regular character of the secondary structure of nucleic acids, induced by their complex formation with several metals [26,27].

However, in the case of the preformed cholesteric LCD from DNA molecules, one can observe a quite different effect. Because separation of chains of the dsDNA molecules in the content of particles of the LCD is impossible due to the sterical reasons [31,32], the alteration of the dsDNA structure, induced by gadolinium treatment, is “transformed” into the change in the mode of spatial packing of the neighboring DNA molecules in these particles. Hence, the first reason is the change of the pitch of the cholesteric structure of the dsDNA LCD as a result of interaction of gadolinium ions with these molecules.

The second assumption is the increase in the diameter of the initial cholesteric LCD [30] particles induced by the rare earth ion’s interaction.

To check the probability of these reasons, it was necessary, first, to estimate the concentration of the rare earth ions in the
content of the LCD formed by dsNA molecules and second, to evaluate the diameter of these particles.

3.3. Magnetic properties of Gd$^{3+}$ ions fixed on the dsDNA molecules ordered in the spatial structure of the LCD

Taking into account similarities in the “optical behavior” of all rare earth ions, and considering the specific magnetic properties of gadolinium ions, the concentration of these ions in the content of the dsDNA LCD was estimated as an example.

The temperature dependence of the magnetic susceptibilities (χ = P/mH) for the pellets formed by cholesterol dsDNA LCDs treated by GdCl$_3$ (and LaCl$_3$) was measured.

Fig. 6 shows that in a curve of this dependence the magnetic contributions both from paramagnetic and diamagnetic centers. In our case, the paramagnetic centers are only Gd$^{3+}$ bound to the dsDNA in the content of LCD. The diamagnetic contribution is expected to be caused by the “rest water” molecules in the case of Gd$^{3+}$-containing sample or La$^{3+}$ ions, because these ions are nonmagnetic and important for the diamagnetic part of susceptibility. The diamagnetism in the first case was taken into account at calculation of a “pure” paramagnetic Gd$^{3+}$ ions issue.

The number of paramagnetic centers (N$^\pi$; N$^\pi$ = 3 × k × C$_{ds}$/μ$\text{Gd}^3+$) is the number of Gd$^{3+}$ in 41$^\pi$-state, calculated from the temperature dependence of magnetic susceptibility [33], which was measured in the temperature range from 50 K to 300 K, i.e. in the temperature range with a linear dependence of χ upon 1/T. It should be noted that C$_{ds}$ value was obtained according to an equation: χ = χ$_0$ + C$_{ds}$/μ$\text{Gd}^{3+}$ and application of the fitting procedure to the theoretical curve and experimental data (see Fig. 6).

Taking into account that an effective magnetic moment of one Gd$^{3+}$ ion in a sample (μ$\text{Gd}^3+$) was estimated to be 7.94 μ$_B$, and the Curie-Weiss constant (C$_{ds}$) obtained from the temperature dependence of the magnetic moment was equal to 99.6 × 10$^{-6}$ erg K/μ$_B^2$, the number (N$^\pi$) of Gd$^{3+}$ ions was evaluated as 7.64 × 10$^{14}$. (The magnetic measurements showed that the concentration of Gd$^{3+}$ ions in a solution obtained after the washing of the pellet of the DNA LCD treated by GdCl$_3$ was insignificant. This confirms that Gd$^{3+}$ ions are tightly bounded to the DNA molecules.)

The above-obtained N$^\pi$ value was used to perform a few practically important estimations. Let us denote the molecular mass of one dsDNA molecule as $m_{\text{dsDNA}}$. This value was about 8 × 10$^6$ Da for the sample used or $m_{\text{dsDNA}}$ is equal to 1.33898 × 10$^{-18}$ g. Taking into account all the experimental conditions, as well as 100% precipitation of the DNA LCD treated by GdCl$_3$ in the course of centrifugation, one can say that the number of DNA molecules in the pellet, $N_{\text{dsDNA}}$, is equal to 2.98735 × 10$^{12}$.

Consequently, there are (N$^\pi$Gd$^{3+}$)N$_{\text{dsDNA}}$ = 2557 Gd$^{3+}$ ions per each dsDNA molecule. As the number of helical turns in dsDNA molecule is equal to (8 × 10$^6$)/(6.6 × 10$^3$) ≈ 120, so 2557/120 ≈ 21.3 Gd$^{3+}$ ions are located on each turn of DNA helix. Because each turn of the DNA helix carries 20 negatively charged phosphate groups, one can suppose that under conditions used one Gd$^{3+}$ ion is bounded approximately to one DNA phosphate group, i.e. each phosphate group of the DNA molecules, carrying one "effective" negative charge, is neutralized by Gd$^{3+}$ ion, that carries three positive charges.

3.3.1. Neutron activation analysis of the dsDNA LCD particles added with GdCl$_3$ (BS)

The concentration of gadolinium was additionally checked by neutron activation analysis of the dsDNA LCD particles. The results of analysis performed on the neutron generator NG-400 of the Institute for Nuclear Research of the RAS (Moscow) are presented in Table 1.

The amount of gadolinium in the BS, $m_{\text{Gd}}$, was obtained from the equation

$$m_{\text{Gd}} = m_{34} \frac{A_{\text{BS}}}{A_{34}} \frac{T_{\text{BS}}^{15} - T_{\text{BS}}^{16}}{T_{\text{BS}}^{15} - T_{\text{BS}}^{10}} \cdot \eta,$$

where $m_{34}$ is the total mass of samples Gd-3 and Gd-4, $A_{\text{BS}}$ and $A_{34}$ is the measured activity of the BS and the total activity of standard samples Gd-3 and Gd-4, respectively. $T_{\text{BS}}^{15}$ and $T_{\text{BS}}^{16}$ are the measurement times for the standard samples and the BS, respectively. $\eta$ = 1.086 is the correction coefficient, compensating the difference of values of times $T_{\text{BS}}$ and $T_{\text{BS}}$ for different samples, provided the decay of $^{155}$Gd is taken into account.

Using Eq. (1), the amount of gadolinium in the BS was estimated as $m_{\text{Gd}}$ = 128 μg. (Note that this concentration is higher

Table 1: Neutron activity of irradiated samples

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than the gadolinium density in all samples, used for NCT earlier [17,34].

Taking into account the experimental conditions and the amount of the DNA in the sample (300 μg; see Section 2), a very simple calculation shows that the obtained amount of gadolinium (128 μg) corresponds approximately to one atom of gadolinium per one phosphate group of the DNA molecule.

Hence, the results of determination of Gd\(^{3+}\) ions concentration in the content of DNA LCD particles treated by GdCl\(_3\) obtained by magnetometric and neutron activation analysis are very similar. Under used experimental conditions (see Section 2), this result is evident, because it reflects the saturation binding of Gd\(^{3+}\) ions to phosphate groups of the DNA molecules.

The obtained data correspond to the data on the saturation binding of gadolinium to nucleotide pairs of pBR 322 plasmid DNA published earlier [4].

However, multivalent Gd\(^{3+}\) ions are not simply agents to screen the long-range electrostatic interaction. These ions play a complex role in DNA–DNA interaction. Indeed, an appearance of noncompensated positive surface charge on the dsDNA molecules will induce an additional attraction between the neighboring DNA molecules. (The physical origin of this attraction mediated by strong correlation interaction is related to either electrostatic or entropic contributions.) Besides, an appearance of the surface charge on the LCD particles can result in the stabilization of the spatial structure of these particles after their treatment with GdCl\(_3\). This means that, as a result of interaction of gadolinium ions with the dsDNA in the content of the LCD particles, these particles can lose their ability to coalescence. This opens a gate for their visualization by AFM.

3.4. Visualization of particles of the dsDNA LCD treated by GdCl\(_3\)

It should be noted that the particles of initial cholesteric dsDNA LCD do not exist at all in the absence of the osmotic pressure of a solvent and their immobilization on a membrane filter and, hence, the visualization is just impossible.

Fig. 7A shows the AFM image of dsDNA LCD particles after their treatment with GdCl\(_3\) and immobilization on nuclear membrane filter, and Fig. 7B demonstrates the size distribution of these particles as well as the pores in the filter.

First of all, one can see that these particles exist as independent, individual objects. Second, the visualization of single particles (Fig. 7A) testifies that at treatment of particles of dsDNA LCD by GdCl\(_3\), the "liquid" character of the DNA packaging in these particles is disappeared and the particles have a rigid spatial structure. Third, the mean size of particles makes 4500–5000 Å, i.e. the mean diameter of the dsDNA LCD particles after gadolinium treatment coincides with the mean diameter of initial cholesteric dsDNA LCD particles [20,21].

Hence, the results of AFM measurements allow one to suppose that the neutralization of phosphate groups of dsDNA molecules by the rare earth ions is not accompanied by alteration in the mean size of the LCD particles and the second reason to explain the amplification of the abnormal band in the CD spectra must be rejected.

One can summarize the results shown above as the following. The treatment of the dsDNA LCD by the rare earth salts, in particular by GdCl\(_3\), is accompanied not only by neutralization of phosphate groups of the DNA molecules by Gd\(^{3+}\) ions, but by a significant attraction between the neighboring DNA molecules. Disappearance of the fluidity of the dsDNA LCD particles proves a short-range attractive interaction between the charged DNA molecules arising from interlocking Gd\(^{3+}\) ions, sometimes called as "counterion cross-links". An existence of independent particles speaks in favor of an appearance of non-compensated positive surface charge on the LCD particles. This, in turn, prohibits the coalescence of these particles and the creation of a uniform phase, i.e. is accompanied by the stabilization of spatial structure of individual particles of the LCD. Indeed, it was shown that despite a slow precipitation of these particles with accompanying disappearance of abnormal optical activity, the simple shaking results in a full restoration of their optical activity. In addition, the thermal "training" of the pellet of the dsDNA LCD particles, treated by GdCl\(_3\), is not accompanied by obtaining a homogeneous phase with a reference cholesteric texture. Hence, surface properties of the obtained LCD particles differ from the initial particles of cholesteric DNA LCD, which...
generate at thermal training a cholesteric phase characterized by the “finger-print” texture, easily recorded by the polarizing microscope [21]. Although the pellet of the LCD DNA particles treated by GdCl$_3$ scatters the X-rays intensively, a specific maximum on X-ray scattering curve, conforming to the mean distance between dsDNA in the LCD particles, does not exist (in contrast to the pellet of the initial cholesteric dsDNA LCD characterized by 31–32 Å Bragg reflection [20,21]). This, again, can be explained in the framework of supposition about the “isotropic” behavior of the obtained LCD particles, i.e. their incapability to form the homogeneous phase. It is necessary to add that taking into account that the mean distance between the initial dsDNA molecules in the particles of the LCD is characterized by the Bragg reflection of 31–32 Å, one can estimate the local concentration of the dsDNA molecules in the content of the LCD particles as 400 mg/ml. If we accept that, the content of the LCD particles as 400 mg/ml. If we accept that, can estimate the local concentration of the dsDNA molecules in the particles of dsDNA LCD can reach 200 mg/ml.

3.5. The possible reasons for amplification of the amplitude of the band in the CD spectra of the dsDNA LCD treated by the rare earth ions

The results of the CD studies, AFM and magnetometric measurements allow one to suppose that the neutralization of phosphate groups of dsNA molecules by the rare earth ions can lead to the alteration of charge distribution on the surface of the dsNA molecules. This provides an additional contribution to the chiral interaction between the neighboring dsNA molecules, ordered in the structure of the particles of the LCD. An arising contribution results in change of the helical twist of the cholesteric structure formed by the dsNA molecules complexed with the gadolinium, which can considerably influence the dsDNA secondary structure (see Fig. 1), the correlation between the theoretical and experimental means for the amplification of abnormal band in the CD spectra of the dsNA LCD, treated by GdCl$_3$, in particular, the influence of $P$ value on abnormal optical activity of the dsNA LCD. Assuming that in the case of the dsRNA LCD, $D$ value of particles is about 0.4 μm, the initial $P$ value is about 3 μm and the initial $ΔA$ value is 2000 × 10$^{-6}$ optical units, the theoretical curve (Fig. 8) for $(ΔA–P)$ relationship was calculated. This curve shows that the decrease in $P$ value is accompanied by the increase in $ΔA$ value, i.e. the smaller the pitch of a cholesteric structure, i.e. the greater the twist, the more intense is the amplitude of an abnormal band in the CD spectrum.

The experimental means of $ΔA$, i.e. the observed amplitudes of the abnormal band in the CD spectra of the LCD formed by poly(I) × poly(C) LCD treated by GdCl$_3$, correspond fairly well to the theoretical curve (Fig. 8).

Besides, taking into account the known concentration of gadolinium, one can find a correlation between the $P$ values and gadolinium concentration in solution (Fig. 9).

Hence, in the case, when the secondary structure of initial molecule is not altered, the “$P$-effect”, i.e. an amplification of the band as a result of the alteration of the helical twist of the cholesteric structure of the dsDNA LCD, is evident.

However, in the case of dsDNA LCD, i.e. in the case, where the gadolinium can considerably influence the dsDNA secondary structure (see Fig. 1), the correlation between the theoretical curve and the experimental means of $ΔA$ is not so good. This discrepancy can be caused by two reasons. First, one can

\[
\begin{align*}
L & = \frac{x \Delta y + \pi}{2} + \frac{1 + 3}{2} \left( \frac{1 + \frac{1}{2} - \sin x}{\cos x - \sin x} \right) + 2 \frac{\sin x - \sin x}{\sin x - \sin x} \\
& + 2 \frac{\sin x}{\sin x}
\end{align*}
\]
suppose that the dielectric properties of the dsDNA molecules (or the LCD particles) are strongly changed, and the expression (2) does not hold. Second, the dsDNA secondary structure is altered as a result of gadolinium interaction. This alteration is probably related to a high flexibility of the DNA secondary structure and, more exactly, with a change in the inclination angle of the DNA nitrogen bases induced by gadolinium interaction. This means that besides the \( P \) value decrease, there is an additional contribution to the amplitude of the band in the CD spectra in the case of the dsDNA LCD treated by the rare earth salts. It should be noted that the second supposition above seems to be more realistic, but such an effect is not considered in the framework of the used theory. Hence, the additional theoretical analysis is necessary, and is now in progress.

Comparison of the experimental results and the theoretical calculations confirms, in general, the supposition about correlation between the decrease in \( P \) value and the increase of an abnormal optical activity of the LCD particles, treated by the rare earth salts. However, there are some peculiarities of this process, which depend on the concrete parameters of the dsDNA secondary structure, changed by these salts. This means that the physicochemical properties of particles of dsDNA LCD, added with the rare earth salts, differ from the properties of initial cholesteric dsNA LCD noticeably.

In conclusion, one can say that the dsDNA molecules in the content of the LCD represent complexing agents for the rare earth ions, in particular, gadolinium. This allows one to create the particles of dsDNA LCD, which are strongly enriched by gadolinium, i.e. the formed biomaterial represents a depot for gadolinium. High abnormal optical activity of particles of dsDNA LCD, added with GdCl\(_3\), high and controlled concentration of Gd\(^{157}\) ions in the content of these particles, open a gate for application of the obtained biomaterial not only in medicine (as a platform for neutron capture therapy), but also in technique for application of the obtained biomaterial not only in medicine but also in technique for neutron capture reaction in the case of 157 Gd atoms results in an average 1.55 DNA single-strand breaks and 0.21 double-strand breaks in the immediate vicinity (\( \sim 40 \) nm) of the neutron capture. Of course, the direct application of these results to our condensed, liquid–crystalline system is not possible and additional calculations are necessary. But, one can expect that despite the possible effect of “self-shielding”, the outer DNA molecules (as a minimum) in the particles of LCD saturated with gadolinium are capable of emitting the irradiation that can kill closely located tumor cells.

To check this possibility, we have attempted to estimate a possible therapeutic effect of the BS irradiation by thermal neutrons. The question is how much surrounding tumor tissue will be affected by secondary radiation. In the paper [17], it is reported that the cancer tumor growth has been suppressed due to bio-samples with gadolinium, irradiated by thermal neutron flux of \( 2 \times 10^9 \) neutrons cm\(^{-2}\) s\(^{-1}\) during 1 h. In another paper [34], authors have estimated a dose rate and DNA double-strand breaks (DSB) probability for local tumor irradiation by secondary Auger electrons, conversion electrons and photons emitted due to sample irradiation by thermal neutron flux of \( 10^9 \) neutron cm\(^{-2}\) s\(^{-1}\). The major contribution to the dose rate (95%) and DSB (84%) has been produced by conversion electrons and photons. Our dsDNA LCD particles added with GdCl\(_3\) have the mean size of about 0.5 \( \mu \)m (Fig. 7). Auger electrons, emitted inside the particle, have a shorter path in the matter and with a high probability will be absorbed in the same particle. However, conversion electrons (average energy 46.3 keV) and photons (above 70 keV) have a much longer path in matter and will not be absorbed inside the particles [2]. Taking into account the fact that the conversion electrons and photons are responsible for the major contribution to the DSB in the NCT, we may expect that the therapeutic effect of NCT with our particles will not be affected by the material of these particles and the dose will be delivered to the surrounding tumor cells. The neutron generator NG-400 produces thermal neutron beam with a flux of \( 8.5 \times 10^7 \) neutron cm\(^{-2}\) s\(^{-1}\). Taking into account that the gadolinium concentration in the new biomaterial is relatively high, this flux is becoming to be close to the necessary therapeutic values. This means that the new biomaterial opens an opportunity to carry out the effective irradiation by thermal neutrons using smaller and safer machines with a smaller flux and to reduce harmful effects of the irradiation.

References