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MICROEMULSIONS BASED TEMPLATES FOR SYNTHESIS OF DNA MATERIALS MODIFIED WITH LANTHANIDE NANOPARTICLES

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In the last decade, many studies on deoxyribonucleic acid (DNA) based materials for photonics applications have been developed. Lanthanide doped materials showed improved fluorescence properties and thus good applicability in different fields. This paper aims at obtaining lanthanide nanoparticles incorporated in DNA functionalized with hexadecyltrimethylammonium chloride (CTMA) by microemulsion technique. Firstly, the ternary phase diagrams in water/CTMA/1-butanol and water/CTMA/solutions of DNA-CTMA in 1-butanol systems were constructed at different temperatures. By using the phase diagrams, specific working conditions have been selected in order to choose the optimum template for preparation of the new materials based on praseodymium hydroxide nanoparticles incorporated in biopolymer matrix with applications in electronics.

Keywords: ternary phase diagrams, DNA-CTMA, lanthanide nanoparticles, praseodymium hydroxide nanoparticles

1. Introduction

In our days, nanomaterials often display new properties, including chemical, physical, mechanical and also optical behaviors. Numerous methods have been developed for obtaining nano-structured materials of different chemical natures, among them an important role being played by *the deoxyribonucleic acid* (DNA) [1-4].

DNA is a very interesting biopolymer, which is used as a material for application in molecular electronics and in photonics, due to its special and unique properties associated with the construction of DNA double helix.

Nanoscience can use the DNA sequence to plan and implement efficient and complex self-assembly and self-directing processes. Due to the fact that the DNA has a phosphate group in the side chain, the DNA molecule can be

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functionalized by different species, such as metal nanoparticles, proteins, carbon nanotubes or organic dyes. It retains its self-assembly abilities, providing a straightforward method for organizing an ample collection of these nano-sized objects into well-defined structures [5].

The rare earth complexes exhibit high fluorescence intensities and long fluorescence lifetimes, which determine the synthesis of a large number of functional materials containing lanthanides [6–8], including organic low-molecular rare earth complexes and inorganic rare earth complexes [9, 10].

Nanoparticles represent the core of intensive research because of their scientific and technological importance. Due to their special properties, synthesis of nanoparticles with specific compositions, sizes, shapes and controlled dispersion becomes very important [11]. As a result of their unique optical, electronic, catalytic and magnetic properties, compared to the corresponding bulk materials, these nanomaterials are employed in many fields such as optics, biotechnology, industry, life sciences, pharmacy, medicine, catalysis, magnetism, mechanics, and energy science [12–15].

Incorporation of different particles (organic/inorganic) into biopolymer matrix is a difficult task in the field of nanomaterials, and the study of these composites represents an important step in the utilization of these biomaterials for potential applications.

One way to obtain this kind of biomaterials is the use of microemulsion within ternary system water/surfactant/oil. The advantage of using this method consists in obtaining of nanostructures with controlled shape and size.

In general, there are two main applications of microemulsions: templates for reactions taking place in nanodrops as nanoreactors, and obtaining adequate compositions for thin film preparation by spin coating, drawing, etc. [16-18].

Microemulsions are thermodynamically stable, clear and isotropic dispersions of two immiscible liquids, like oil and water, stabilized by an interfacial membrane formed by the surfactant molecules. When a low surfactant concentration is used, there is a sequence of equilibrium between phases, named Winsor phases. Winsor I (WI) has two phases (O/W, O), where the microemulsion (O/W) is in equilibrium with the oil phase (O) in excess. Winsor II (WII), has also two phases (W/O, W), the microemulsion (W/O) in equilibrium with the water phase (W) in excess. Winsor III (WIII) is a bicontinuous microemulsion, with three phases, where the middle microemulsion phase (O/W plus W/O) is in equilibrium with the oil and water excess phases, while Winsor IV (WIV) is a macroscopically single-phase microemulsion [19, 20]. For obtaining and utilization in optimal conditions of some nanodomains well defined in size and shape, it is necessary to limit the regions of interest for water/ surfactant/oil system by drawing the phase diagrams.

This paper presents the synthesis procedure of lanthanide hydroxide nanoparticles incorporated in DNA-CTMA matrix by microemulsion technique in ternary system. Firstly, it was necessary to construct the ternary phase diagrams in water/CTMA/solution of DNA-CTMA in 1-butanol system at different temperatures. Secondly, the working conditions, like composition and temperature, have been selected in order to find the specific domains, large enough to facilitate nanostructured templates useful in preparation of nanomaterials, especially to obtain the lanthanide nanoparticles incorporated in DNA-CTMA matrix.

The overall aim of the study was to obtain new materials based on praseodymium hydroxide nanoparticles incorporated in biopolymer matrices for applications in photonics, knowing that several studies proved that lanthanides based materials could be used in many fields of science, e.g. colored lighting [21], bio-imaging [21], luminescence tracing [21], detecting systems [21], forensic applications [21], biological labeling [22] or in catalytical applications [23].

2. Materials and methods

2.1. Materials

The deoxyribonucleic acid (DNA), extracted from salmon waste, purity 96 %, was supplied from Chitose Institute of Science & Tehnology, CIST, Japan. In order to solubilize DNA (Fig. 1 – a.) in organic solvents, it was functionalized with a cationic surfactant, hexadecyltrimethylammonium chloride (CTMA) which has a purity of 99 %. The CTMA surfactant (Fig. 1 – b.) was purchased from Acros, Organics.

1-butanol provided by Sigma–Aldrich was used as solvent. The ammonia solution 25 % (NH_4OH) was from Merck. Ultra-pure water (Millipore Corporation) was used for the sample preparation.

Praseodymium (III) chloride hydrate, purity 99.9 %, was purchased from Sigma–Aldrich. Solutions of DNA-CTMA and also of praseodymium (III) chloride hydrate were freshly prepared prior to use.

All the chemicals, solvents and reagents were of analytical grade and were used as received without further purifications.

2.2. Determination of phase diagram

In order to find out the region where microemulsion (μE) can be formed, ternary phase diagram was built using surfactant titration method at different temperatures (298 K and 333 K), following a procedure already known [16]. This involves successive additions of surfactant in small quantities in water/oil mixtures, over the entire composition domain corresponding to the mass fraction

w_{H_2O} = 0.1 ÷ 0.9. The samples were taken in sealed test tubes and vigorously shaken to ensure a proper mixing and then kept in a thermostatic device at the desired temperature. The phase transition was observed by using bromothymol blue (BTB) as color indicator.

The microemulsions used in this study were composed by water, CTMA and 1-butanol, or solutions of DNA-CTMA in 1-butanol with different concentrations 5 g/L, 15 g/L, respectively 30 g/L.

Taking into account that the temperature plays an essential role on surfactants solubilisation and on the microemulsion type, the ternary phase diagrams were constructed at two temperatures 298 K and 333 K.

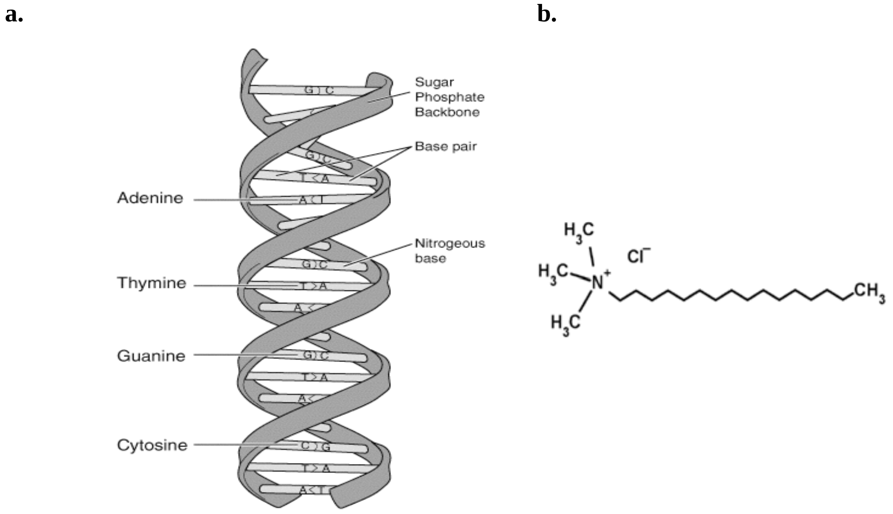


Fig. 1. Chemical structure of DNA (a) and CTMA (b)

2.3. Particle size measurements

The lanthanide hydroxide nanoparticles (LnNPs) size distribution and polydispersity index were determined by dynamic light scattering (DLS) method using a Zetasizer Nano ZS, (Malvern Instruments Ltd.). DLS measurements were performed at 298 K and 333 K in triplicate.

3. Results and discussion

For limiting and using the microemulsion domains in optimum and adequate conditions for a specific purpose, a detailed study was realized on the phase transitions in various water/surfactant/oil systems. The concentration of surfactant and DNA-CTMA complex is useful for synthesis of simple and embedded lanthanide nanoparticles in biopolymer matrix.

3.1. Design of microemulsion based templates

3.1.1. Phase diagram in ternary system water/surfactant/1-butanol

The ternary phase diagram was visually determined by mixing the surfactant with solvent/water mixtures until a clear, homogenous and transparent solution appeared. This method is advantageous because all types of microemulsions can be obtained by using a minimum surfactant quantity. The cationic surfactant has been used as self-assembling agent. The phase diagram drawn in ternary system water/CTMA/1-butanol, realized at 298 K, is shown in Fig. 2. From this figure one can observe that the solubilisation of water and 1-butanol increases proportionally with the surfactant concentration for a given water/oil volumetric ratio, R = V_W/V_O. Due to the fact that the 1-butanol and water is partially soluble, in this case, the WII microemulsion area is narrow and begins at lower surfactant concentrations (~2 %). WIV single-phase microemulsion appears for a CTMA concentration ranging between 3 and 7 %.

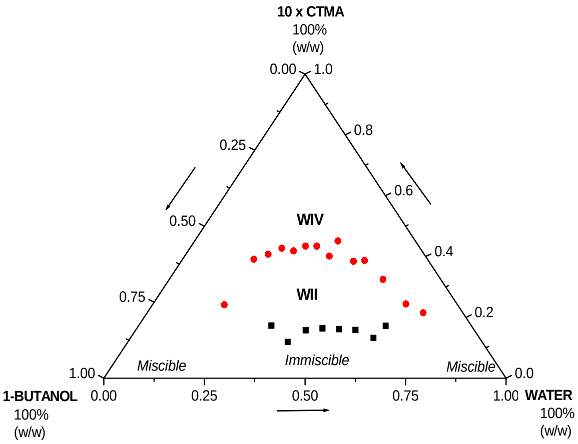


Fig. 2. Phase diagram in water/CTMA/1-butanol ternary system at 298 K

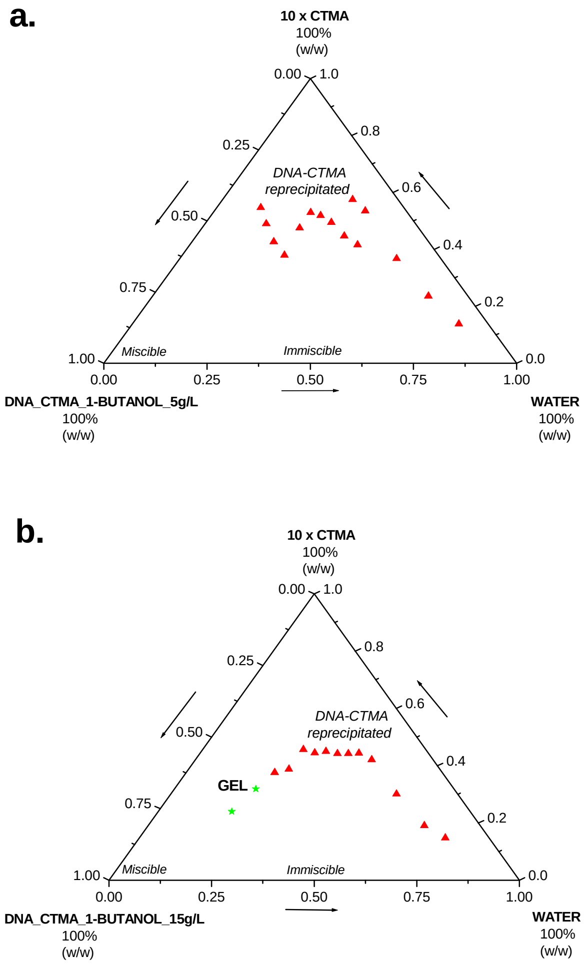
3.1.2. Room temperature phase diagrams in ternary system water/surfactant/solution of DNA-CTMA in 1-butanol

Fig. 3 presents the phase diagrams of the ternary system of water/CTMA/DNA-CTMA-1-butanol at T = 298 K. From this figure it can be seen that DNA-CTMA reprecipitated when the aqueous phase was added in all three cases. Increasing the DNA-CTMA concentration in the organic phase, this reprecipitation takes place when smaller quantities of water were added.

At the same time a gel phase was observed for higher DNA-CTMA concentration in organic phase. This behavior is due to the high molecular mass of DNA (8000 kDa), which leads to the increasing of the solution viscosity.

In these conditions the phase diagrams obtained revealed that it was not possible to obtain a WIV domain which is the most desirable for obtaining proper microemulsion.

Thus it can be assumed that, in this case, on one side the CTMA surfactant does not facilitate the obtaining of a microemulsion and on the other side the organic phase is not properly chosen.



c.

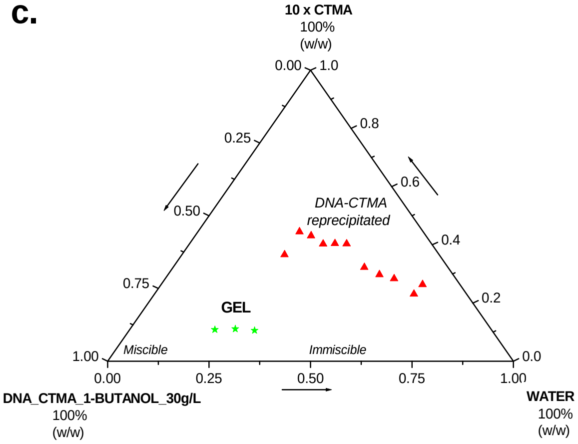


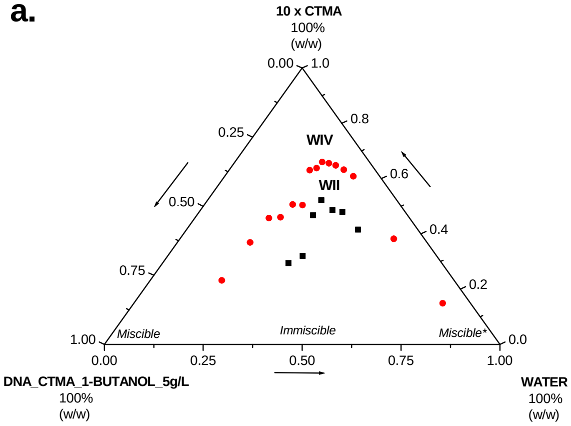
Fig.3. Phase diagrams at 298 K for different concentrations of DNA-CTMA in 1-butanol: 5 g/L (a), 15 g/L (b) and 30 g/L (c)

Taking into account all these aspects, it seems that the system water/CTMA/DNA-CTMA-1-butanol do not allow to get a template for the synthesis of nanoparticles at room temperature. In this context, experiments have been carried out at higher temperature.

3.1.3. High temperature phase diagrams in ternary system water/surfactant/solution of DNA-CTMA in 1-butanol

The phase diagrams of the ternary system water, CTMA and DNA-CTMA-1-butanol at 333 K are presented in Fig. 4.

a.



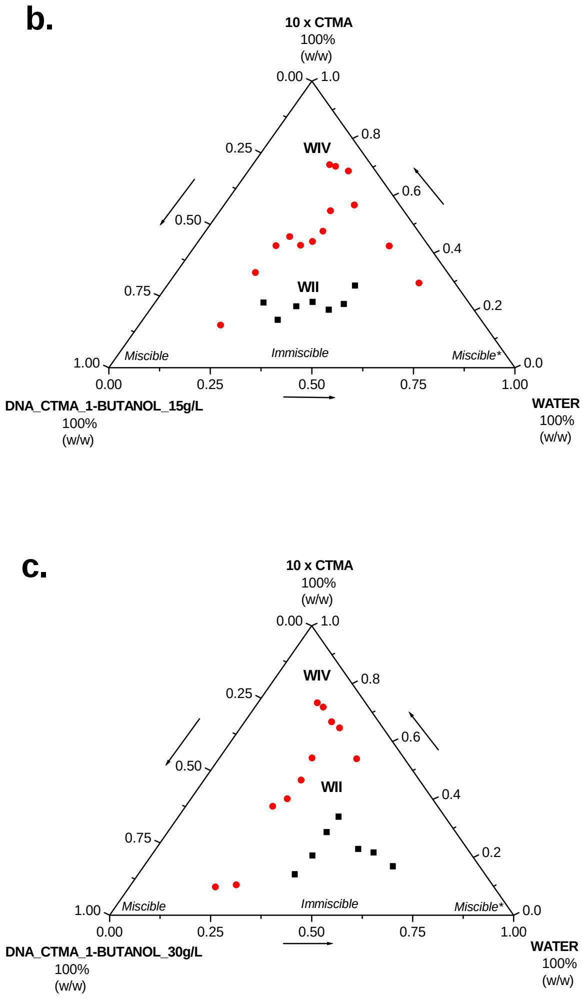


Fig. 4. Phase diagrams at 333 K for different concentrations of DNA-CTMA in 1-butanol: 5 g/L (a), 15 g/L (b) and 30 g/L (c)

One can observe that, in the case of R<1, the formation of WII microemulsion requires a low concentration of surfactant. For the system with solution of DNA-CTMA in 1-butanol with concentration 5 g/L, the surfactant concentration is between 6 and 9 % (w/w), while for 15 g/L, and 30 g/L the CTMA concentration is between 2 – 3 %, and 2 %, respectively. The area of WIV microemulsion corresponds to a surfactant concentration of around 8 % (w/w).

This behaviour enables choosing a proper composition from these ternary systems water/CTMA/DNA-CTMA-1-butanol, to obtain a stable microemulsion, which can be used for synthesis of nanoparticles incorporated in DNA-CTMA matrix. It can be observed in Fig. 4, that the left side of the diagram corresponds to an organic solution of surfactant, the water being present only in small amounts, corresponding to a system of reverse micelles. The right side of the diagram corresponds to an aqueous gel containing small amounts of oil phase. The solubilisation of water and solutions of DNA-CTMA in 1-butanol proportionally increases with the surfactant concentration for a given water/oil volumetric ratio, $R = V_W/V_O$.

3.2. Synthesis of DNA materials modified with praseodymium hydroxide nanoparticles

Lanthanide hydroxide nanoparticles - $Pr(OH)_3$NPs - incorporated in DNA-CTMA matrix were synthesized *via* W/O microemulsion, using a ternary composition formed by aqueous solution of $PrCl_3 \cdot xH_2O$ (0.001M), CTMA and solution of DNA-CTMA in 1-butanol (5 g/L).

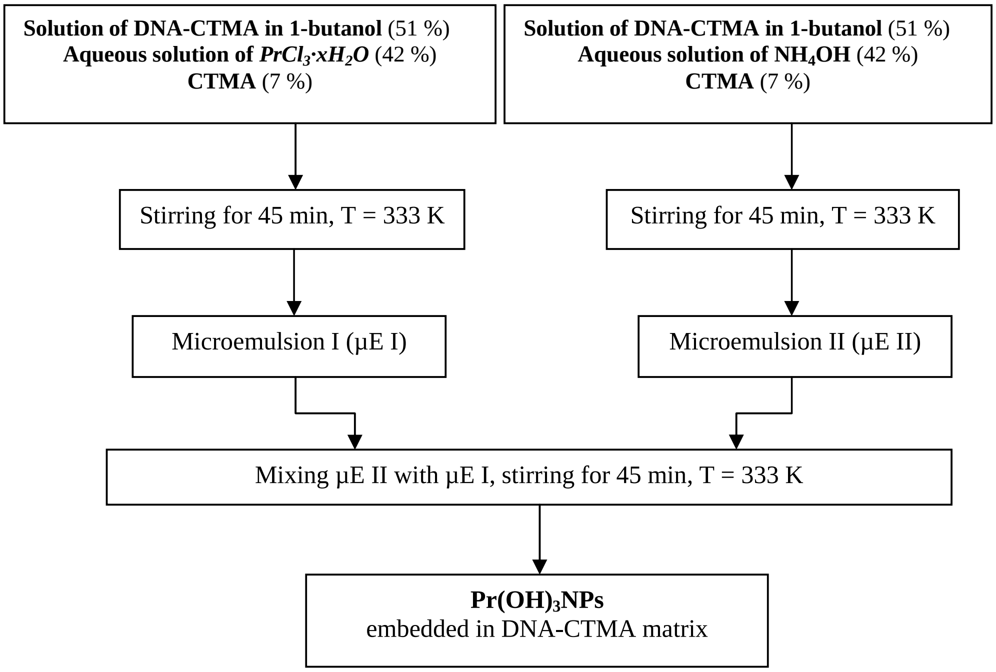


Fig. 5. Synthesis procedure of hydroxide lanthanide nanoparticles at 333 K

The $Pr(OH)_3$NPs incorporated in DNA-CTMA matrix were prepared by mixing two ***water-in-oil microemulsions***: the first one (μE I) containing Pr^{3+}

water solution, CTMA and solution of DNA-CTMA in 1-butanol, and the second one (µE II) formed from CTMA, an organic phase (solution of DNA-CTMA in 1-1-butanol – 5 g/L) and an aqueous solution of reduction agent - NH_4OH. Before mixing the µE II with µE I, every microemulsion was stirred for 45 min. The two mixed microemulsions were stirred for other 45 min. at 333 K.

The aqueous core from µE I was formed by the electrolyte of lanthanide salt, Pr^{3+}, while the aqueous core from µE II by the reduction agent - NH_4OH. The main steps of the synthesis of lanthanide hydroxide nanoparticles are shown in Fig. 5.

The diameters of $Pr(OH)_3$NPs incorporated in DNA-CTMA matrix are around 380 nm (Fig. 6). In this case, the sample has a polydispersity around 0.5, the curve is multimodal, indicating that the $Pr(OH)_3$NPs are not all incorporated in DNA-CTMA matrix and there are also other aggregates, which are most likely due to further aggregation of some nanoparticles.

As it can be observed in Fig. 6, when the $Pr(OH)_3$NPs were embedded into DNA – CTMA chains a strong contraction of DNA-CTMA coils from 662 nm to 380 nm is produced.

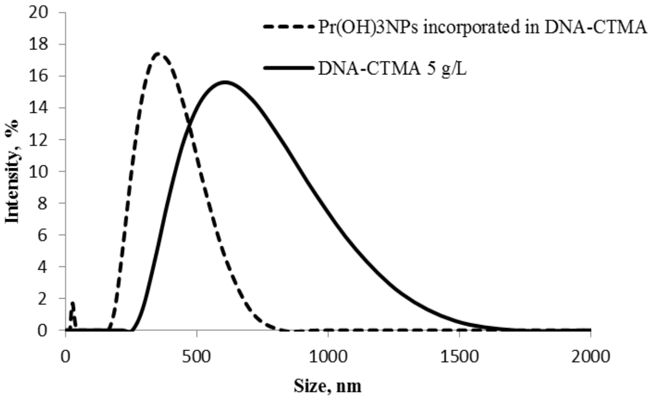


Fig. 6. Size estimation of the investigated systems by DLS measurements

4. Conclusion

The phase diagram in ternary system composed by aqueous solution of $PrCl_3 \cdot xH_2O$, CTMA and solution of DNA-CTMA in 1-butanol has enabled choosing the optimum composition domain for synthesis of praseodymium hydroxide nanoparticles embedded in DNA-CTMA matrix at 333 K.

Indeed, at 298 K, the system water/CTMA/DNA-CTMA-1-butanol has not enabled choosing a template for the synthesis of nanoparticles, because DNA-CTMA was reprecipitated when the aqueous phase was added. At 333 K, a proper composition of a stable microemulsion was selected for the synthesis of nanoparticles incorporated in DNA-CTMA matrix. Consequently, the temperature has an important role in finding a good template for the synthesis of lanthanide nanoparticles embedded in DNA-CTMA matrix.

Praseodymium hydroxide nanoparticles incorporated in DNA-CTMA matrix have been synthesized, *via* W/O microemulsion at 333 K, by mixing two water-in-oil microemulsions. The size of these DNA materials modified with $Pr(OH)_3$NPs are around 380 nm. The $Pr(OH)_3$NPs embeddeded into DNA – CTMA chains caused a strong contraction of DNA-CTMA coils.

In conclusion, the microemulsion technique represents a very promising procedure for obtaining materials based on DNA - rare-earth complexes for applications in electronics.

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