MORPHO – STRUCTURAL CONTROL OF FUNCTIONALIZED DNA BASED SYSTEMS

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There are described the results obtained on functionalized DNA thin film processing from solution by the spinning method and their thickness dependence on different parameters: rotation speed at film deposition, molar mass and biopolymer complex concentration and substrate used for deposition. Method of dynamic light scattering has been applied to study size dependence of DNA coils (aggregates) on DNA and chromophore concentration.

The obtained results are important since they help to choose the optimum parameters of thin film fabrication: chromophore, biopolymer matrix, their concentration, support etc. function of the purpose followed in the applications related to the design of electro optics devices envisaged.

Keywords: DNA – CTMA, thin films, chromophores, thin film thickness

1. Introduction

For the sake of assuring humanity a sustainable development the scientists turned recently their interest towards biomaterials, originating from renewable resources. One of them, and certainly the most important one, is the deoxyribonucleic acid (DNA). Its fascinating helical, double strand structure, discovered by Watson and Crick [1, 2] in 1953 attracted a lot of interest of biologists, chemists, and later, of physicists. This macromolecule plays an essential role in the growth, development and heritage transmission of living species of not only humans and animals but also of the vegetal ones.

DNA consists of base pairs of molecules: adenine with thymine and guanine with cytosine, and of helix backbones made of sugar and phosphate groups, joined internally by the esther bonds. The base pairs are linked together by the strong hydrogen bonds. The outside groups, which are phosphates provide

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a net negative charge to DNA macromolecule. This negative charge is compensated by the non-localized sodium counter ions Na^+. These ions can move freely along the macromolecular chain surface [3].

The encoding of genetic information of a given species is contained in the sequence of base pairs. The size of DNA depends on the level of development of the given species. Usually it is expressed in the number of base pairs (bp) and spans from several tens of bp, as for *Escherichia coli* (76bp), to 3 000 Mbp for Human DNA [4].

DNA is an abundant biomaterial and it can be obtained from the waste produced by the food processing industry. Therefore it is cheap and eco-friendly. Not protected DNA decays rapidly being "consumed" by bacteria. It can be functionalized by the ionic exchange reaction with certain surfactants, particularly cetyltrimethylammonium chloride (CTMA) giving stable, water insoluble (the only solvent of DNA) complexes [5] with improved physico-chemical properties [6]. The solubility of DNA-surfactant complexes in a large number of solvents allow their functionalization with a large number of photosensitive or conductivity increasing molecules for application in photonics and in electronics. DNA may replace or bring more than the presently used synthetic polymers with a long decay time. Also its particular double strand helical structure provides a kind of protection for active molecules against molecule aggregation and their photo-thermal degradation. It exhibits also a higher optical damage threshold [7, 8] what is important for applications in photonics.

The thin film processability, their optical quality and fabrication reproducibility are important aspects concerning the applicability of these materials in electronics and in photonics, and more particularly in integrated optics. It is also an important aspect concerning characterization of their physico-chemical properties. This paper describes our recent studies devoted to the DNA-CTMA thin film processing from solution by the spinning methods and the studies of their thickness dependence on different parameters, such as rotation speed, molar mass and biopolymer complex concentration and kind of substrate used. We report also results of our studies by the dynamic light scattering method of the DNA aggregates (coils) size dependence on DNA and chromophore concentration, functionalization and molar mass of DNA. A comparison with theoretical values will be also presented.

2. Materials and Methods

A series of DNA – CTMA based thin films were prepared by spin coating (Laurell – Model WS – 400B – 6NPP/LITE). The varied parameters were: spinning rate, type and concentration of dyes, molar mass of biopolymer, matrix and solutions concentration as well as the substrate on which the films were

deposed. The thin films thickness was measured using a profilometer model Dektak 120 from KLA Tencor.

DNA and DNA – CTMA were used as matrices. Deoxyribonucleic acid, abbreviated DNA, purity of 96%, was purchased from Chitose Institute of Science & Tehnology, CIST, Japan. Its molecular mass was reduced in several steps using the Sonic, model VC-250. In order to solubilize DNA in organic solvents DNA was functionalized with *hexadecyltrimethylammonium chloride* (CTMA) of purity 99%, from Acros, Organics.

The chromophores tested were: Disperse red 1 (DR1) (Aldrich), Rhodamine 610 (Rh 610)(Excitation), Rhodamine 590 (Rh 590) (Excitation), Disperse Orange 3 (DO3)(Sigma), Nile Blue (NB) (Sigma). The chemical structures of these dyes are presented in Fig. 1.

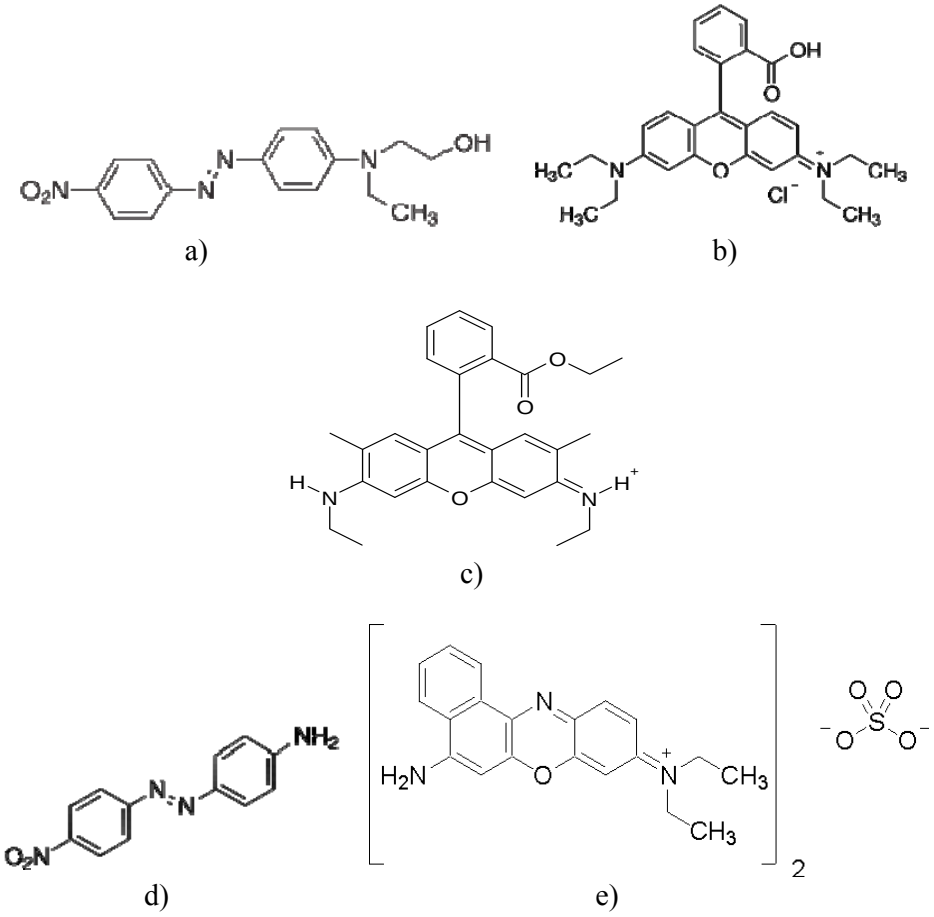
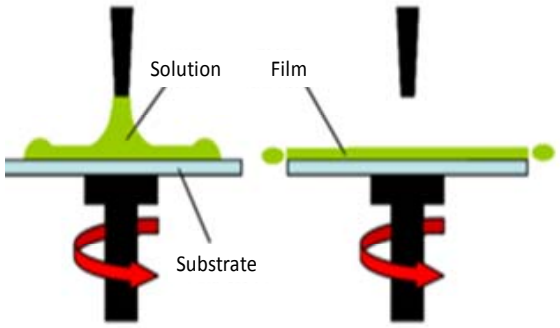


Fig. 1. Chemical structure of choromphores used a) DR1, b) Rh 610, c) Rh 590, d) DO3, e) NB

The thin films were deposed on very clean glass slides or ITO coated glass slides by spin coating technique. This technique consists in deposing a small quantity of polymer solution on the surface of a substrate which is then rotated. Usually the acceleration as well as rotation speed can be programmable. Two distinct rotations are used: first one serving for a good spreading out of the solution (fig. 2A) whereas the second one serves to evacuate the solvent (fig. 2B). The rotation speeds used are of about a few thousands rpm (rotations per minute) and depend on the desired thickness, solution adherence to the substrate and its viscosity. Fig. 2 shows schematically the set up for this technique. The initial stage involves delivering a quantity of solution to the surface of the substrate. The film thickness depends on the solution viscosity and on the rotation speed. On the other hand the solution viscosity depends on temperature, solution concentration, on polymer used, its molar mass and its dispersity as well as on the solvents used. The spinning technique is very frequently used in thin film fabrication for optical applications and often it leads to a partial orientation of polymer chains (cf. e. g. Koynov, K., [9]), with usually the polymer chains preferably oriented parallel to the substrate plane and randomly distributed within.



A B

Fig. 2. Schematic representation of the spin coating technique

In order to determine the size of DNA based aggregates Dynamic Light Scattering technique (DLS) was used.

DLS is a technique that offers information related to the size distribution profile of small particles in suspension or polymers in solution. This technique measures the diffusion of particles moving under Brownian motion. By this technique it is possible to estimate the size of DNA coils, DNA functionalized or not, and doped or not with dye.

3. Results and discussion

3.1. Thin films thickness variation

3.1.1. Thickness of thin films variation function of spinning rate

Thin films were deposed using the following spinning programmes:

- Program 1:
  - speed 1: 500 rpm for 40 sec
  - speed 2: 1000 rpm for 60 sec;
- Program 2:
  - speed 1: 1000 rpm for 40 sec
  - speed 2: 1500 rpm for 60 sec;
- Program 3:
  - speed 1: 1500 rpm for 40 sec
  - speed 2: 2000 rpm for 60 sec;
- Program 4:
  - speed 1: 2000 rpm for 40 sec
  - speed 2: 2500 rpm for 60 sec;

Fig. 3 presents the thin films thickness variation versus spinning rate. It can be seen that, as expected, the thickness of thin films is decreasing with the increase of spinning rate.

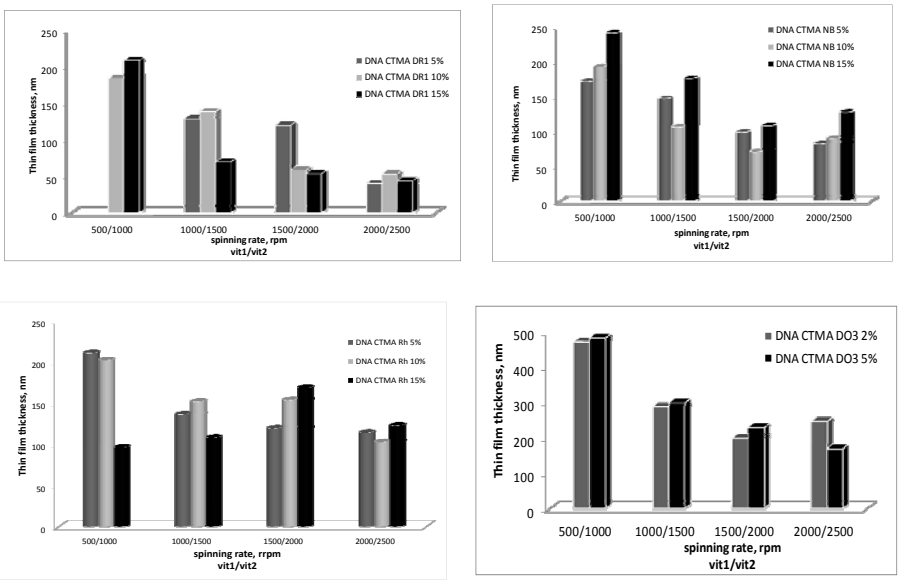


Fig. 3. Variation of thin films thickness for different spinning programs

3.1.2. Variation of thin films thickness function of type and chromophore concentration

Fig. 4 presents the dependence of thin film thickness with the type and cromophore concentration. It can be observed that low speeds of rotation and small concentrations of chromophore have no influence on the thickness of the thin films, whereas at high speeds of rotation and big concentrations of chromophores the thinnest thin films are obtained when the biopolymer is doped with DR1, a chromophore with small dimensions which can intercalate in polymer matrix.

In Fig. 5 results obtained for thin films of DNA doped with two kinds of rhodamine are comparatively presented. It can be noticed that films with Rh 610 are systematically thinner. This fact could be explained based on chemical structure which is different even the chromophores have the same molar mass. Rhodamine 610 possesses tertiary amino groups while rhodamine 590 presents secondary amino groups. On the other side substitution at the phenol ring is with a carboxylic group for rhodamine 610 and with an ethylic ester for rhodamine 590 leading to a relatively larger molecular volume and in this way to thicker films in case of doping with Rh 590.

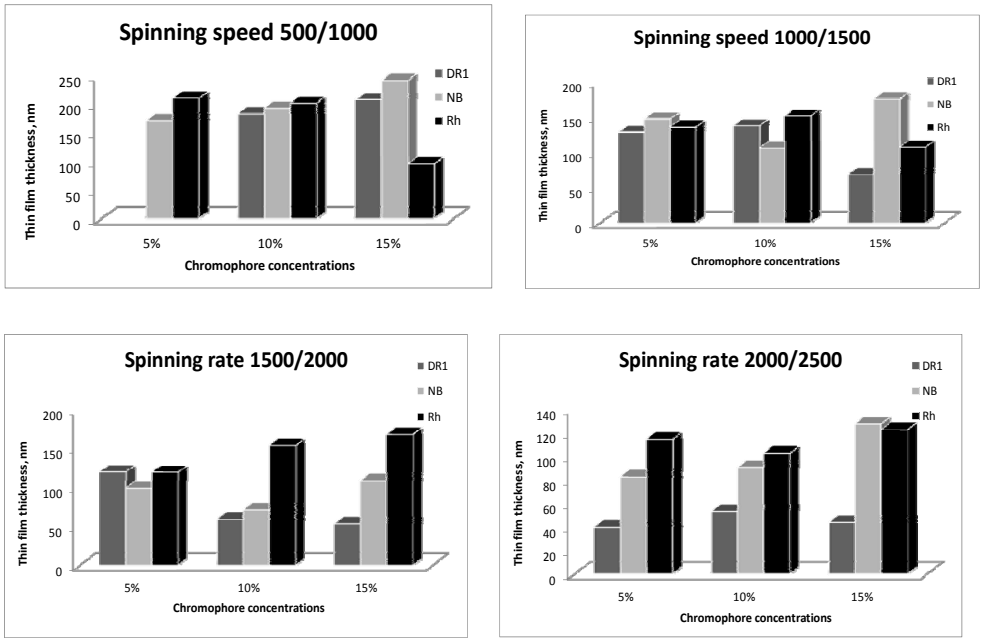


Fig. 4. Influence of chromofore type and concentration on thin film thickness for the four spinning programs used

3.1.3. Biopolymer molar mass influence on the thickness of thin films

In Fig. 6 are presented results obtained for DNA-CTMA based thin films prepared with DNA with different molar masses. Rotation speed used was 1500 and 2000 rpm. It can be seen that the films thickness decreases with the increase of molar mass. Deposition of thin films by centrifugation can determine an ordering of DNA chains. Longer the chain are, easier the ordering is explaining in this way the thin film thickness decrease.

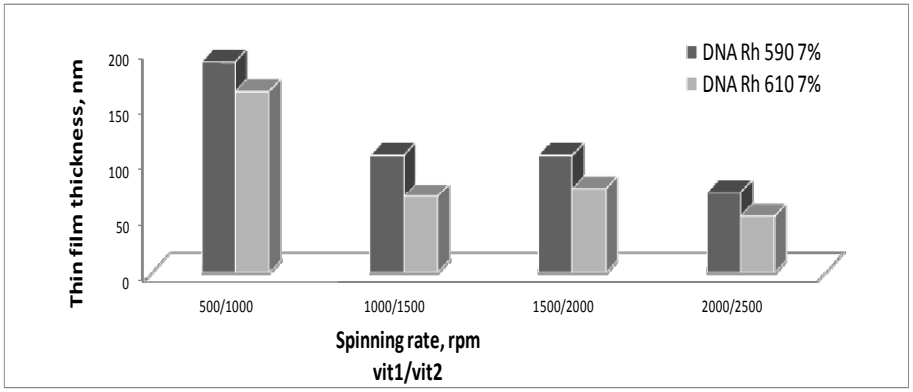


Fig. 5. Dependence of thin film thickness on rhodamine type for the four spinning programs

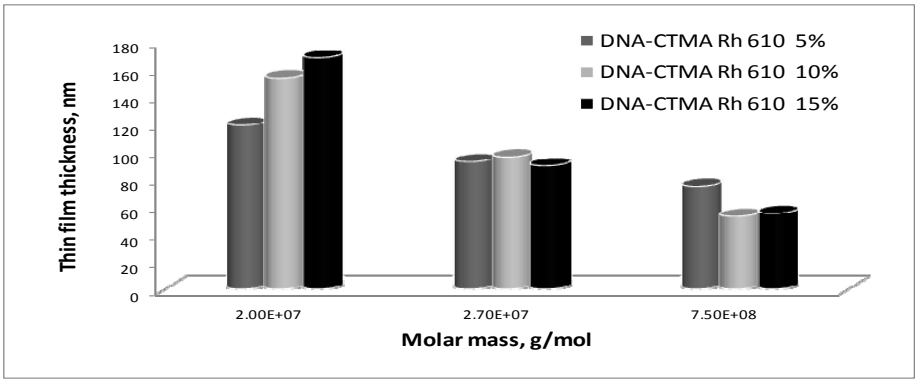


Fig. 6. Dependence of thin film thickness on molar mass of biopolymer

3.1.4. Influence of matrix and solutions concentration on the thin films thikness

Figs. 7 and 8 present the results obtained for chromophore Rhodamine 610 embedded in two different matrices: DNA and DNA – CTMA. It can be observed that films based of DNA – CTMA are thicker that those with DNA. This fact could be assessed to the solvent used. The films with DNA are obtained from 6g/L DNA aqueous solutions while films with DNA-CTMA are obtained from 30

g/L DNA – CTMA solutions in butanol. At the same time it could be seen that when the dye concentration increases, film thickness increases no matter the matrix used (fig. 8). Increasing the DNA – CTMA concentration in solutions leads to thicker films also (fig. 9).

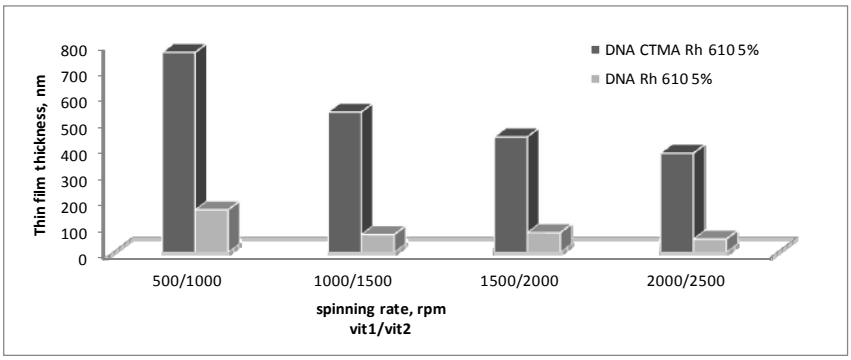


Fig. 7. Variation of thin film thickness with type of biopolymer used as matrix and spinning rate program

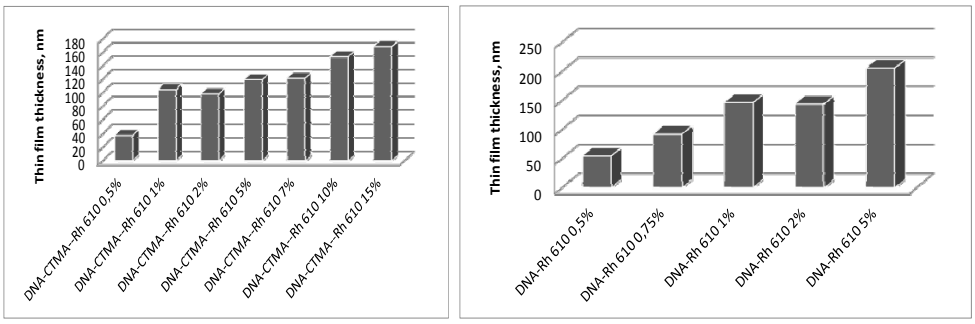


Fig. 8. Variation of thin film thickness with Rh 610 concentration for the two matrices used

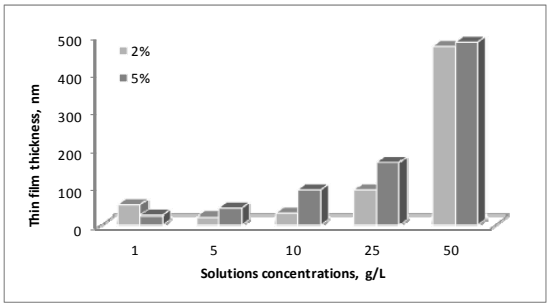


Fig. 9. Variation of thin film thickness with solutions of DNA – CTMA concentration for two DO3 concentrations

3.1.5. Substrate influence on the thin films thikness

Experimental results obtained showed that the thickness of films deposed on ITO (substrate with high electrical properties) or on glass is not significantly influenced by the substrate type on which the thin film is deposed (fig. 10).

Nevertheless the thin film thickness depends on the adherence degree of the material on the support.

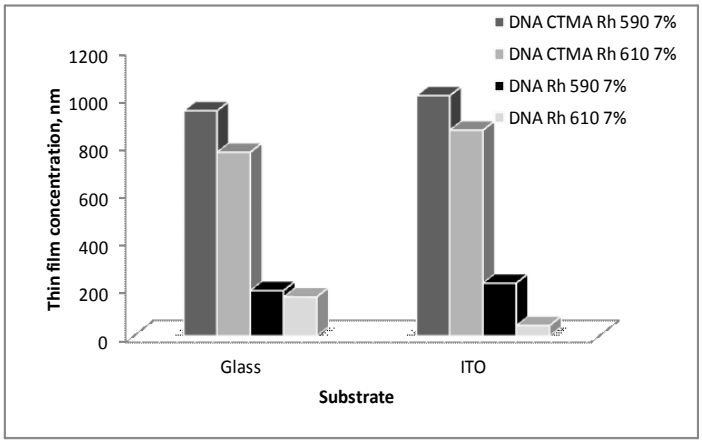


Fig. 10. Substrate influence on thin film thickness

3.2. Self assembling of DNA chains as function of DNA concentration and molar mass

Two systems: DNA rhodamine 610 and DNA – CTMA doped with rhodamine 610 were studied and the results obtained were compared with those calculated using equation 1 [10] taking into account that DNA aggregates are of sphere shape.

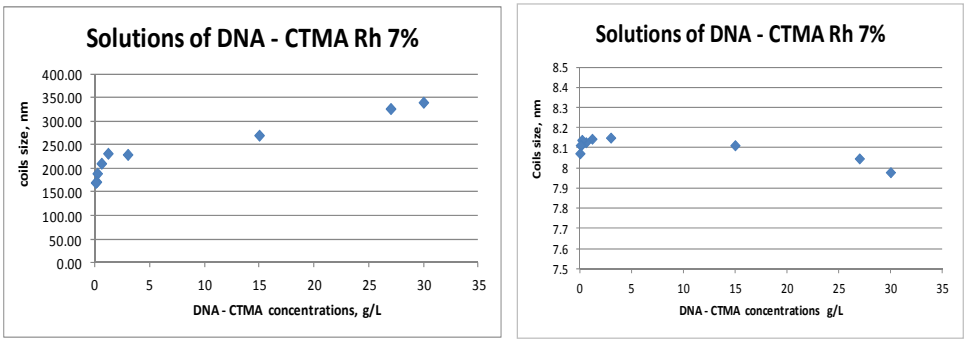
$$R_g = 0.0569^3 \sqrt{v_S \left(\frac{cm^3}{g}\right) M \left(\frac{g}{mol}\right)} \tag{1}$$

where N_A is Avogadro number and by replacing its value eq. 1 becomes:

$$R_g = 0.0569^3 \sqrt{v_S \left(\frac{cm^3}{g}\right) M \left(\frac{g}{mol}\right)} \tag{2}$$

v_S is the specific volume and M is the molar mass determined experimentally.

Fig. 11 shows, as example, the variation of aggregate size of DNA – CTMA doped with 7% rhodamine 610 (with respect to DNA – CTMA) *versus* DNA – CTMA concentration in solution. It can be observed that experimental measured aggregate size is increasing more rapidly for small concentrations of solutions of DNA – CTMA than for solutions with higher concentrations. Generally it can be said that the aggregates size is increasing with DNA – CTMA concentration. This behavior is completely different as compared to prediction of theoretical calculations, the experimental coils size being by two magnitude orders higher than the calculated values. On the other hand, the evolution of theoretical coils size with concentration seems to reach a maximum at 5% DNA – CTMA. However, these values show minor deviations around 8 nm size.

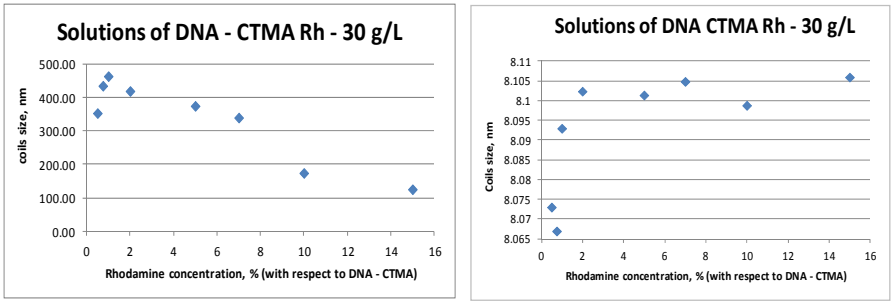


Experimental (A) Theoretical (B)

Fig. 11. Experimental (A) and theoretical calculations (B) of the aggregates size function of DNA – CTMA concentration

Variation of DNA – CTMA – Rh dimensions with concentration of rhodamine is illustrated in Fig. 12. Size of aggregates increases until 1% concentration of rhodamine followed by a decrease in two steps while according to theoretical predictions the coils size should be constant. This dependence of aggregates size with the choromophore concentration could be explained by rhodamine embedding in DNA – CTMA for small concentrations of Rh 610. When the Rh 610 concentration increased a contraction of DNA – CTMA coils takes place due to probably the excess of Rh molecules which surround the DNA – CTMA coils. Other possible explanation is that the aggregates are not sphere, but they are rods. Similar behavior is observed when DNA is not functionalized with CTMA. It is worth to notice that the coils of DNA are ten times larger than DNA – CTMA aggregates (see also fig. 14). This could be again explained by the solution concentrations (6g/L DNA and 30 g/L DNA – CTMA) and by functionalization. When the concentration is increasing the aggregates density is increasing leading to a contraction of coils. CTMA chains, which are bounded to

DNA, will also lead to the aggregate contraction. In this way one could be explained why experimental coils size are larger for DNA, while from theoretical prediction the larger size aggregates are for DNA – CTMA. As expected the aggregate size is increasing with molecular mass of DNA (fig. 15). Again increasing the Rh 610 concentration leads to a decreasing of coils size and experimental values obtained are several times larger than the predicted values.

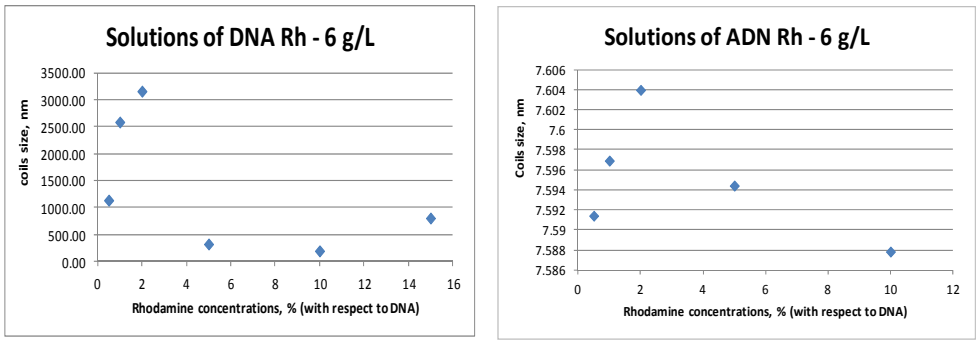


Experimental (A) Theoretical (B)

Fig. 12. Experimental (A) and theoretical calculations (B) of the aggregates DNA – CTMA size function of concentration of rhodamine

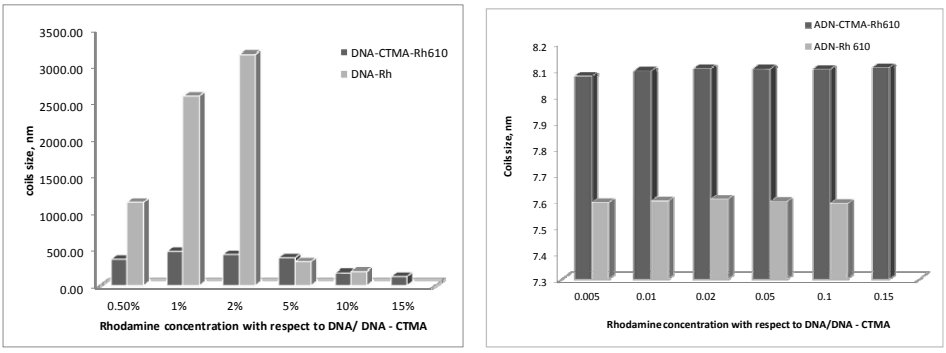
This disagreement between predicted data and experimental values obtained could be due to the fact that the theoretical calculus of the radius of gyration does not take into account the interactions between DNA chains and between DNA and chromophores.

When comparing the results obtained with literature data [10], it can be concluded that our results are in agreement with these data. The fact that for DNA the size measured and predicted are different could suggest that the DNA aggregates have the shape of rods or the aggregates are swollen sphere.



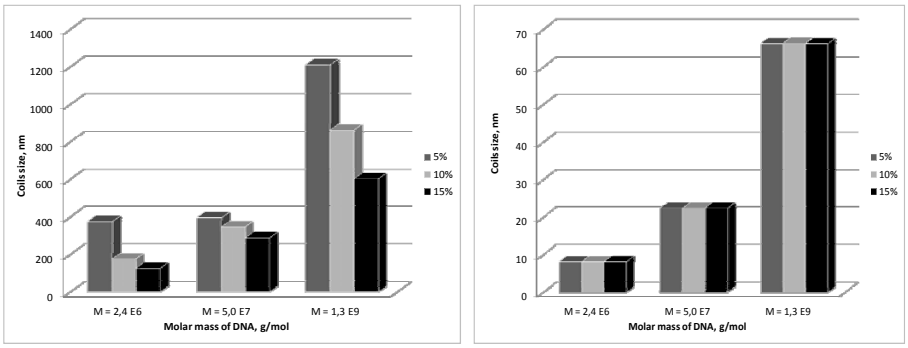
Experimental (A) Theoretical (B)

Fig. 13. Experimental (A) and theoretical calculations (B) of the aggregates DNA – Rh size function of concentration of rhodamine



Experimental (A) Theoretical (B)

Fig. 14. Experimental (A) and theoretical calculations (B) of the size of aggregates function of the matrix



Experimental (A) Theoretical (B)

Fig. 15. Experimental (A) and theoretical calculations (B) of the aggregates function of molar mass for different concentrations of rhodamine

3. Conclusions

This study showed how the thin films thickness is influenced by different processing parameters like spinning rate, solution concentration, molar mass of biopolymer, and chromophore type. The chromophore concentration (with respect to DNA or DNA – CTMA) and the substrate on which the thin film is deposed seem to have less influence on film thickness.

The aggregates size in solutions of DNA and DNA – CTMA could be controlled by DNA or DNA – CTMA concentration, chromophore concentration and molar mass of DNA. The results obtained showed that the DNA and DNA CTMA aggregates exhibit rod or swollen sphere shape.

This study is important since the results obtained allow choosing the optimum parameters of thin film fabrication like chromophore type, biopolymer matrix, and its concentration, etc. function of the purpose and applications of electro optics devices envisaged.

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