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Na-DNA FILMS WITH IONS OF METALS

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The structures of films received by evaporation from solutions of Na-DNA of calf thymus with concentration of 0.2 mg/ml, containing salts Li, K, Na, Cs, Mg, Zn, Ca are analyzed at various levels of relative humidity and temperatures. The solution of salt of Na-DNA in volume of 0.5 ml was poured into a glass quartz cell placed in a hermetic closed vessel with inputs for air pumping. The cell has an area of 420 mm^2 and height of lateral walls of 1 mm. The error of stabilization of temperature in the thermostat came to 0.5ºC, the error of humidity is 2%. The research was carried out at temperatures of 30 and 40ºC**.** The received films of Na-DNA were photographed in white polarized light. The volume of the formed liquid crystal phase depends on the type of ions in Na-DNA solutions and defines correlation between the area of structure on the film and the area of all the film and depends on the correlation of concentrations of salt of metal and DNA. The area occupied by liquid crystal structures was calculated as the area limited to a curve, connecting boundary elements of structures. The relative areas of structures received from solutions containing salt of NaCl with concentrations of 20 and 1.25 mM have values of 0.73 and 0.09 accordingly. The films containing salt of $MgCl_2$ has spherulite structures collapsing under the conditions of room temperature and humidity. The films received from the solutions containing salt LiCl, CsCl, $CaCl_2$ or $MgCl_2$ with the concentration of 10 mM do not form structures with the allocated direction of growth of clusters. The films received from the solutions containing salt NaCl with the concentration of 10 mM and salts $ZnCl_2$, or $CuCl_2$ with the concentration of 0.4 mM and 0.2 mM respectively do not form structures with the allocated direction of growth of clusters also. It is assumed, film can be characterised by a set of values of fractal dimensions; one of the signs of existence of DNA in native form is the presence of such fractal structures as percolation anisotropic cluster in the received film.

KEY WORDS: solution, ion, DNA, liquid crystal, film, humidity, fractal.

Such polyelectrolytes as DNA, fabric proteins and polysaccharides are components of numerous high-molecular associates *in vivo*, therefore the understanding of phase behaviour of these polymers has fundamental importance. On the other hand the methods promoting transition of molecules of DNA into compact condition are of great interest for gene therapy, however, the basic mechanisms of dehydration self-organising of solutions of DNA and other biological liquids remain not clear in many respects [1, 2]. One of compaction ways is creation of conditions for formation of liquid crystal (LC) phase by the molecules of DNA. Formation of LC phases occurs at concentration comparable with concentration *in vivo*. So, in the work [3] the conditions of formation of liquid crystals of holesteric and smectic types by the molecules of DNA are analyzed; in the work [4] the mechanisms of formation and the structural organisation of liquid crystals of holesteric spherulite type from DNA solutions are investigated; in the work [5] possibility of formation of liquid crystals of holesteric and nematic type by molecules of DNA with low molecular weights in the range of temperatures 60-85°C was investigated with the change of properties of solvent.

Streamlining of semifixed polymers at high concentration occurs spontaneously and with the purpose of minimisation of the excluded volume. The phase behaviour of polymer depends on effective volume of polymer; the critical concentration for streamlining of DNA molecules is the function of ionic force and the type of counterions [6].

The following reasons conducing to diminishing of effective volume of DNA are known:

1) change to a more ordered phase; 2) consolidation counterion layer; 3) change of DNA conformation; 4) changes in hydration cover.

The last two factors mentioned depend on humidity and temperature of the environment.

As it is known, ions of metals can be divided into 2 basic groups depending on their way of linkage with various centres on DNA [7,8]. The ions of alkaline (Na^+, K^+) and alkaline earth metals (Mg^{+2}, Ca^{+2}) co-operating basically with phosphatic groups of DNA both at direct contact and through the hydration cover of DNA can be referred to the first group; thus, it is necessary to note that the influence of ions Na^+, K^+ on the accuracy of synthesis of DNA is minimum in relation to ions of other metals. Such ions of transition metals as Zn^{+2} and Cu^{+2} which bind with the DNA bases and which make essential impact on the accuracy of synthesis of DNA, being, in fact mutagenic substances, [9,10] can be referred to the second group. During evaporation of water the concentration of DNA in the formed disperse environment comes closed to the value sufficient for the formation of LC phase; on the other hand, the process of evaporation of moisture leads to the dehydration of DNA molecules which is one of the interfering factors for the formation of LC phases. Besides, the presence of salts of metals in the dispersion influences the value of volume of the formed LC phase and, therefore, on the value of the area of the formed structure. Thus, the specified factors directly influence the character of ordering of DNA molecules during the drying of solution and hence, on the formation of the structure of the film received from solutions of DNA.

The offered work analyzes the structures of films with ions of alkaline and alkaline earth metals received by evaporation from solutions at various values of relative humidity and temperatures are presented.

EXPERIMENTAL DETAILS

Films have been received from solution of Na-DNA of calf thymus with concentration of 0.2 mg/ml. Salt of Na-DNA was dissolved in 0.01 M solution of NaCl. The solution of salt of Na-DNA in volume of 0.5 ml was poured into a glass quartz cell 1 placed in a tightly closed vessel 2 with inputs for air pumping (Fig.1). That vessel also contains the thermometer and hygrometer for the control of parameters of environment. The solution is filled in a cell having area of $21*21\ mm^2$ and height of lateral walls of 1 mm. The solution filled in a cell moistens lateral walls completely therefore after drying the film forms in the center of cell. If walls of cell have height more than 1 mm, the film is formed along walls of a cell because of action of forces of a superficial tension. Air pumping was carried out with the help of the vacuum pump 3 with the velocity of 0.5 l/min. The vessel with the cell was located in the thermostat 4. The error of stabilization of temperature in the vessel came to 0.5ºC, the error of humidity is 2%. The received films of Na-DNA were photographed in white polarized light. The value S_r of the relative area of structures

$$S_r = \frac{\sum S_i}{S} \tag{1}$$

defined as the relation of the total area of structures $\sum S_i$ to the whole area of film S characterises the ability of the substance existing in the solution to influence on forming of LC phase [11,12]. The research was carried out at temperatures of 30 and 40ºC.

RESULTS AND DISCUSSION

The structures of the films received from solutions containing salt of NaCl with concentrations of 20, 5 and 1.25 mM, accordingly, are given in Figs. 2 - 4.

The relative area of structures S_r decreases with the reduction of concentration of salt from S_r=0.73 at the concentration of salt NaCl equal to 20 mM to S_r = 0.09 at the

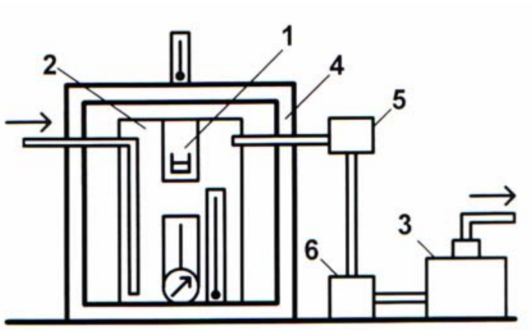

Figure1. The principal scheme of the equipment.
1-the glass quartz cell; 2-vessel with the inputs for air pumping; 3- vacuum pump; 4- thermostat; 5- manometer; 6- vacuum valve.

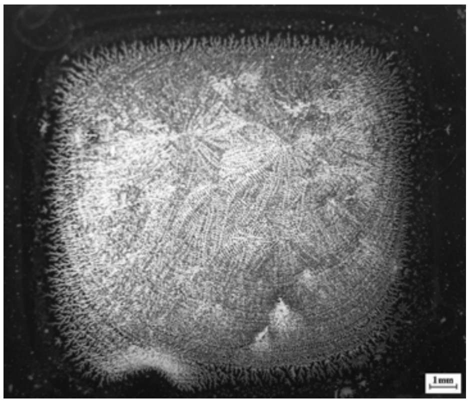

Figure 2. The film from the solution containing 20 mM NaCl. T=40ºC ; H=38%.

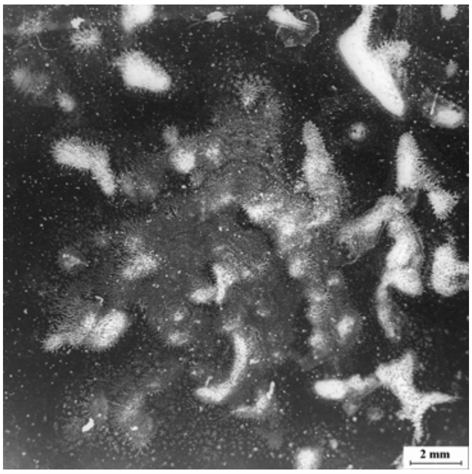

Figure 3. The film from the solution containing 5 mM NaCl. T=40ºC, H=38%.

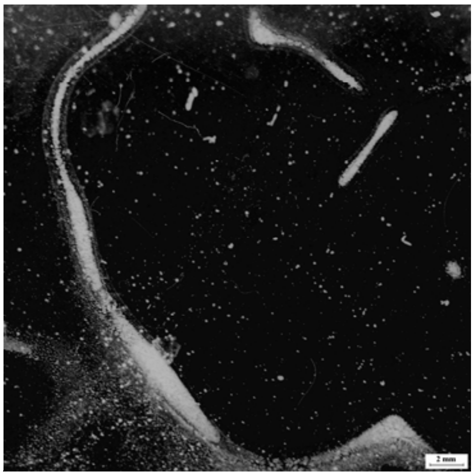

Figure 4. The film from the solution containing 1.25 mM NaCl. T=40ºC, H=38%.

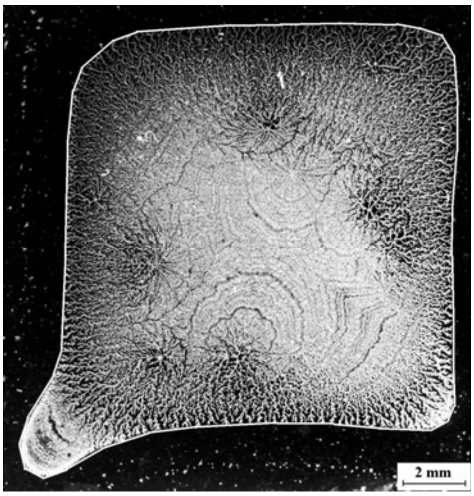

Figure 5. The film from the solution containing 10 mM NaCl. T=30, H= 43%. The white line connects boundary elements of structure to allocate its area.

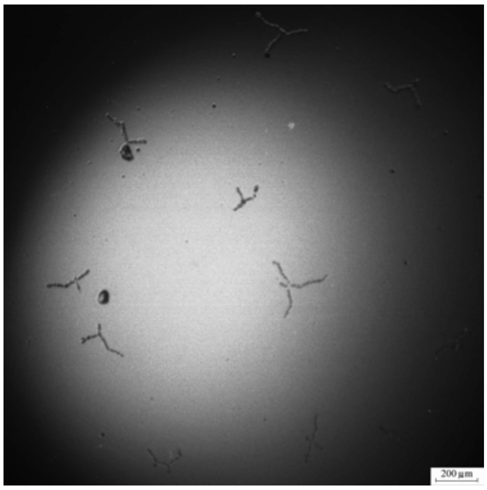

Figure 6. The film from the solution containing 10 mM LiCl. T= 40ºC, H=30%.

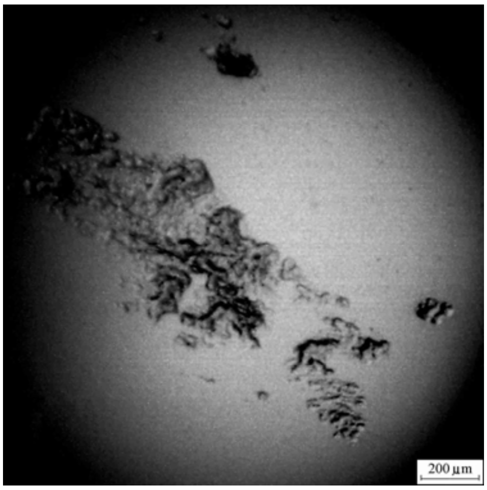

Figure 7. The film from the solution containing 10 mM CsCl. T=40ºC, H=38%.

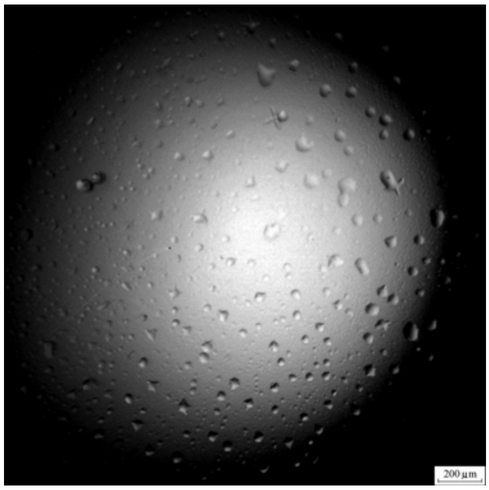

Figure 8. The film from the solution containing 10 mM $CaCl_2$. T= 40ºC, H=24%.

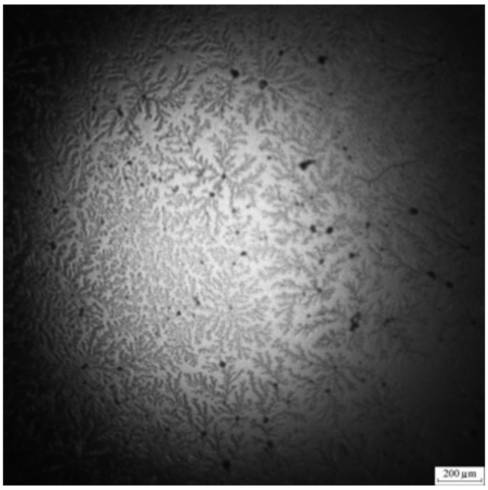

Figure 9. The film from the solution containing 10 mM $MgCl_2$. T=40ºC, H=16%.

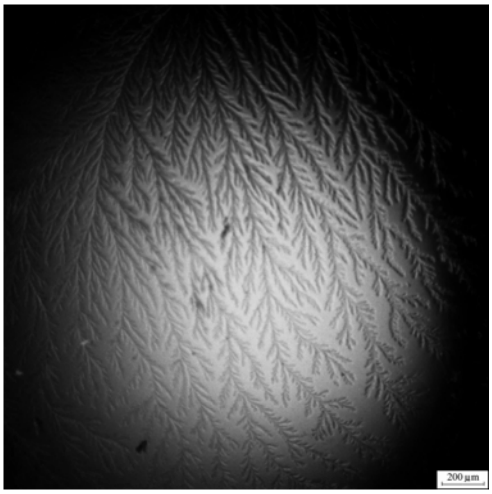

Figure 10. The film from the solution containing 10 mM $MgSO_4$. T= 40ºC, H=22%.

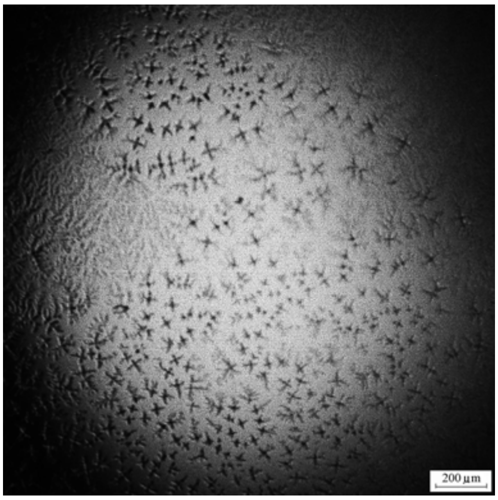

Figure 11. The film from the solution containing 0.4 mM $ZnCl_2$. and 10 mM NaCl. T=40ºC, H=18%.

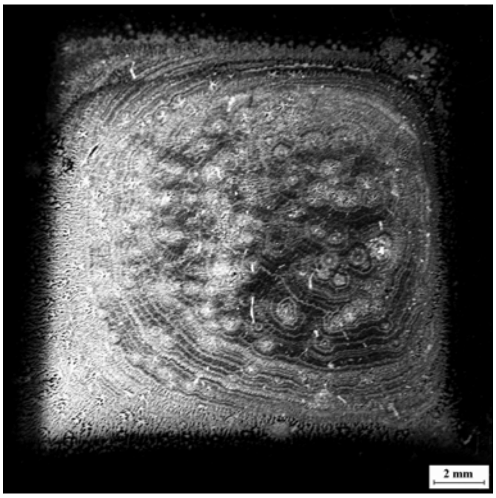

Figure 12. The film from the solution containing 0.2 mM $CuCl_2$ and 10 mM NaCl. T=40ºC, H=38%.

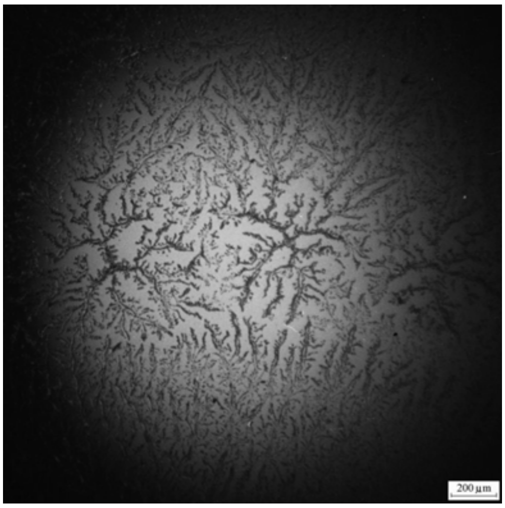

Figure 13. The film from the solution containing 0.2 mM $CuCl_2$ and 10 mM NaCl. T=40ºC, H=38%.

Figure 14. The film from the solution containing 10 mM KCl. T= 40ºC, H=38%.

Figure 15. The film from the solution containing 10 mM NaCl. T=30ºC; H= 30%.

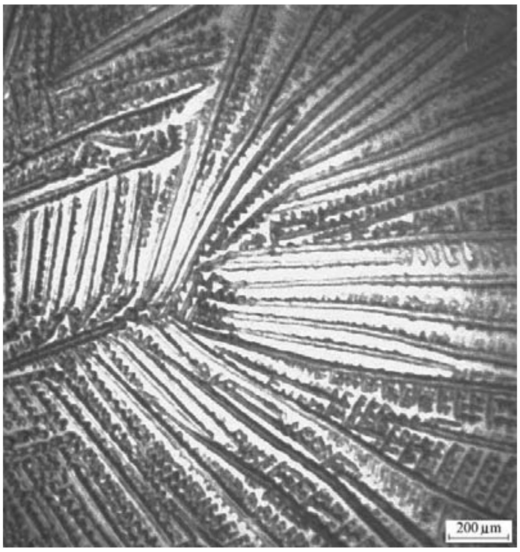

Figure 16. The film from the solution containing 100 mM NaCl. T=40ºC; H= 23%.

concentration of 1.25 mM. The area occupied by LC structures was calculated as the area limited to a curve, connecting boundary elements of structures as presented in Fig. 5. As it has been found out during the carried experiments, the films received from the solutions with salts of LiCl, CsCl and CaCl practically do not contain LC structures as can be seen in Figs. 6-8.

The distinction between the properties of films of DNA with salts of $MgCl_2$ and $MgSO_4$ (Figs. 9 and 10) is of interest. After the extraction of the cell with the film containing salt of $MgCl_2$ from the thermostat, spherulite structures can be seen on the film surface. However, after 2 minutes spherulite structures collapse under the conditions of room temperature and humidity (Fig. 9). The value of the clusters formed in this case are approximately order of magnitude less than the values of the clusters formed on the film from the solution containing salt $MgSO_4$.

The solution containing the salt of NaCl with the concentration of 10 mM keeps DNA in the native form, at the same time, it is known, that ions of Zn^{+2} when interacting with DNA in solution at the concentration of 0.4 mM show mutagen character [9], i.e. convert the DNA from native to denatured form. Accordingly, the films received from the solutions containing salt NaCl with the concentration of 10 mM and salt $ZnCl_2$ with the concentration of 0.4 mM do not form structures with the allocated direction of growth of clusters as shown in Fig. 11. The similar influence on the structures of films is made by ions of Cu^{+2}. In Figs. 12-13 the structure of the film received from the solution containing salt of $CuCl_2$ with concentration of 0.2 mM is presented. In Figs. 14-16 the structures of the films received from the solutions, containing salts of NaCl and KCl with the concentrations of 10 mM are presented. From the illustrations (Figs. 14-16) it is possible to see that there are some allocated directions of primary growth of fractal clusters, therefore, the specified structures can be defined as percolation anisotropic clusters [13]. The quantitative characteristics of fractal structures are their dimension. As it is possible to see from the presented illustrations, the forms of fractal structures in the centre of a cell and on its periphery differ (Fig. 2) which can be explained as a result of redistribution of components of the solution by capillary stream during drying [14]. Thus, the film can be characterised by a set of values of fractal dimensions depending on the choice of a site on which calculation is made.

CONCLUSIONS

The areas of structures on films depend on properties of the ions present in the solutions and on the correlation of concentrations of salt of metal and Na-DNA in the solution, thus, the majority of structures are the structures of fractal type. It is shown that one of the signs of the existence of DNA in native form is the presence in the film of such fractal structures which can be characterized as anisotropic clusters.

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