Influence of Glass and Air on Our Perception of DNA

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Abstract: The chemical and physical properties of glass do not allow it to be considered as an inert material. Therefore, it is necessary to discuss the ability of laboratory glassware to influence the state and properties of aqueous solutions, including DNA solutions. As a demonstration of this ability, it is shown here how contact with glass affects the state of salts in aqueous solutions. In terms of the topic under discussion, it is especially important that sodium salts are very sensitive to these contacts. Thus, it is shown here that contact with glass can affect the formation of fibers from sodium salts of DNA, which were mainly used in X-ray studies aimed at determining the molecular structure and parameters of DNA molecules. Special attention is paid to the fact that laboratory glassware is also sensitive to contact with aqueous solutions, including DNA solutions. That such sensitivity may be important to biologists is also shown here. In particular, it was shown how ignoring the sensitivity of silica glass, from which the photometric cells are made, to electrization gave rise to misconceptions about the spectral properties of aqueous solutions, including DNA solutions. The effect of air dissolved in aqueous DNA solutions on the spectral properties of both these solutions and the photometric cells containing them is also shown. In particular, it was shown that UV absorption of aqueous DNA solutions, which does not contain air, is completely insensitive to heating, and UV absorption of aqueous DNA solutions, which are saturated with air, is supersensitive to heating. In other words, it is shown here that our understanding of the thermal denaturation of DNA, which is reflected in the UV absorption of its aqueous solutions, was formed due to these two types of sensitivity. In the end, a conclusion is proposed here about the significant contribution of glass and air to the creation of traditional ideas about the structure and properties of DNA.

Keywords: DNA, Structure, Fibers, UV Absorption, Spectra, Melting, Phenazines

1. Introduction

It has long been known that glass is capable of absorbing electrons, thereby suppressing chemical reactions between free radicals, in particular-chain reactions occurring by a free radical mechanism. So, the walls of laboratory glassware are able to absorb electrons that are part of chemically active molecules and atoms of hydrogen and oxygen (H* + glass wall → inactive H, and O_2* + glass wall → inactive O_2) and stop their interaction, preventing the combustion of hydrogen gas in an oxygen atmosphere. For this reason, glassware is traditionally considered to be an inhibitor of free radical chemical reactions [1, 2].

Considering the above, it is not surprising that glass also absorbs negative charges from aqueous media [3], forming an electric field near its surface. It is also not surprising that

there are phenomena that confirm the existence of this field. Thus, the spiral (!) filaments appearing in aqueous solutions of salts near the walls of glassware (Figure 1) [4] clearly show both the existence of this field and its shape-forming action.

All this suggests that the DNA salt, the aqueous solution of which is placed in a glass capillary, is also subject to the fiber-forming effect of glass. Considering that the X-ray studies predominantly used fibers formed in drying solutions of sodium salts of DNA enclosed in glass capillaries [5], this may be important. For this reason, the validity of this assumption is discussed in detail here.

In addition, taking into account the selective sorption capacity of glass in relation to negative charges [1-3], it can be assumed that a number of its physicochemical properties also change during electrization. That these changes may be of interest to biologists is demonstrated here by the example

of quartz glass from which photometric cuvettes are made. In particular, this aspect analyzes the fact that the electrization of the inner surfaces of photometric quartz cells is accompanied by an increase in their absorption in the wavelength range: 180 – 240 nm (Figure 2) [6].

Figure 1. The fibrous spiral structures that form at the wall of the glass Petri dish, which is filled with an aqueous solution of $CuSO_4$, are clearly visible here [4].
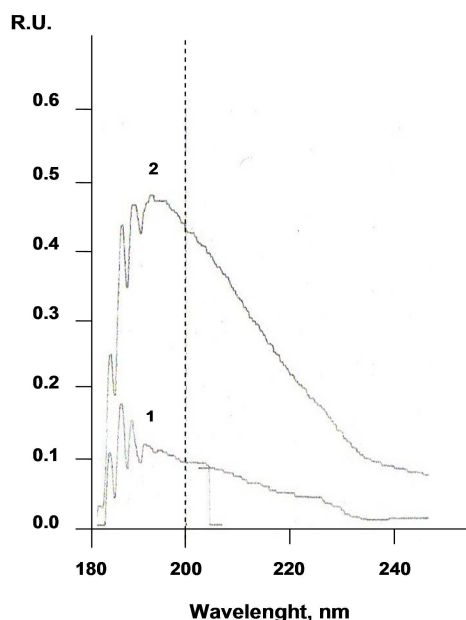
The spectra were not processed [6].

Figure 2. UV absorption spectra of an empty quartz photometric cuvette: 1 – before electrization of the cuvette; 2 – after negative electrization of the inner surfaces of the cuvette.

Taking into account the revealed dependence (Figure 2), here we analyzed the influence of some factors capable of electrifying both quartz photometric cuvettes and their contents. As an example of such factors, the electrifying ability of air oxygen is considered here. This choice is based on the fact that air is constantly in contact with both quartz photometric cuvettes and their contents.

2. Materials and Methods

Oppositely charged waters were obtained as in [6].

To UV spectra recording Specord UV VIS (Carl Zeiss Jena, Germany) was used.

Sodium salt of DNA was purchased from Fluka (Switzerland); other reactive were purchased from Ukrreachim (Ukraine).

The potassium salt of DNA was obtained from the sodium salt of DNA by dissolving it in 0.1 M KCl, followed by precipitation. The deposition was carried out in open bottles in the dark; the solubility of DNA decreases under such conditions, as was shown earlier [4].

3. Results

3.1. Formation of Fibers on Glass Surfaces

At first, it is useful to make sure once again that highly visible fibers are formed in aqueous solutions of salts in contact with the glass surface (Figure 3).

Figure 3. These are the fibers formed after the $CuCl_2$ solution dries on the glass surface.

Probably, the ability to form fibers is inherent in the surfaces of all silicates. Numerous filaments that form on the surface of silica gel from an aqueous solution of NaCl (Figure 4), suggest that this assumption corresponds to reality.
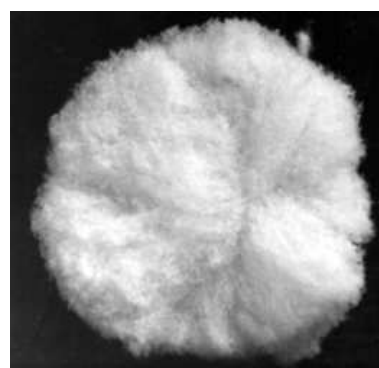

Figure 4. This is how the accumulation of filaments formed on the surface of drying silica gel, which was originally moistened with an aqueous solution of NaCl, looks like; up close it looks like real cotton wool.

It should be noted here that all types of glass, including quartz glass (whose properties are discussed below), are silicates. In other words, they are all derivatives of silica gel, which is a sorbent for aqueous hydroxyl ions, OH^-[3].

Thus, the ability of a glass surface to form filamentary structures from small salt molecules is beyond doubt (Figure 1, 3). Most likely, this ability of the glass surface is due to its

negative electrization. This assumption is based on the fact that many salts form filaments in negatively charged aqueous solutions, but do not form filaments in aqueous solutions that are positively charged [4, 6]. In aspect of the topic discussed, it is especially important that the sodium saltsform filaments in the negatively charged aqueous solutions (Figure 5).
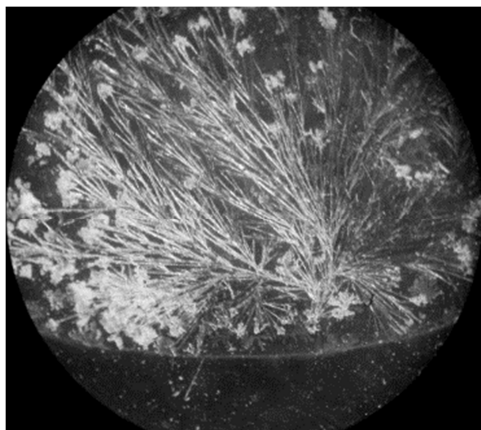
Figure 5. *Real Bouquets of flowers"are formed in drying NaCl solutions prepared in negatively charged water [4].*

Proceeding from this, the fact that the glass surface exhibits the same structure-forming properties in relation to sodium salts of DNA seems quite probable. Thus, the fact that X-ray studies predominantly use filaments that are formed in drying solutions of sodium salts of DNA enclosed in glass capillaries [7] may be due to the special nature of the interaction of Na-DNA with the negatively charged surface of the capillaries. In particular, this choice explains why potassium salts of DNA are not used in such studies, despite the fact that potassium cations are the most common cations in cells of living organisms [7, 8]: unlike sodium salts of DNAs, their potassium salts do not form fibers even in highly negatively charged aqueous solutions (Figure 6).


Figure 6. *This is the appearance of precipitation of fibers of sodium (left) and potassium powder (right) DNA salts formed in water with a potential of -300 mV; for contrast, the water was tinted with a phenazine dye [4, 6].*

3.2. The Influence of Quartz Glass on the Formation of Ideas Aboutthe Spectral Properties of Aqueous DNA

Previously, it was shown that the UV absorption spectra of

negatively charged water have a peak with a maximum in the range $190 \div 200$ nm (Figure 7, spectrum 2), and the UV absorption spectra of positively charged water have a peak with a maximum in the range $200 \div 220$ nm (Figure 7, spectrum 3) [5, 6].
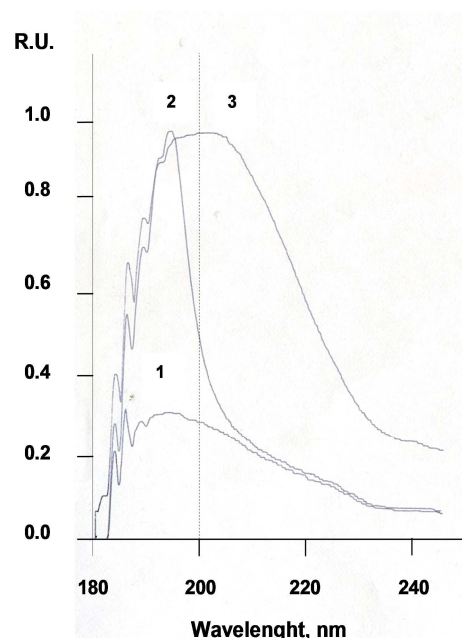
The spectra were not processed [6, 7].

Figure 7. *UV absorbance spectra of the water: 1 – fresh distilled water; 2 – water, filtered through activated carbon; 3 – water, filtered through silica gel.*

Since these peaks are usually present in the UV absorption spectra of aqueous DNA [7, 9], their origin has been thoroughly investigated. These studies made it possible to finally make sure that both of these peaks (Figure 7) reflect the electrization of silica glass, from which photometric cells are made for studying UV absorption spectra of liquids (Figure 2). Accordingly, it was also concluded that both of these peaks (Figure 7) are not associated with the presence of aqueous DNA in quartz photometric cuvettes, contrary to generally accepted views [7, 9]. All this allows us to conclude that these views have appeared due to inattention to the fact that UV absorption of silica glass is sensitive to its electrization.

3.3. Air Participation in the Formation of Concepts of Thermal Melting of Aqueous DNA

Naturally, all this stimulated the study of factors capable of causing electrization of laboratory equipment, in general, and, in particular, photometric cuvettes, as well as aqueous solutions of DNA. Since it is omnipresent, the air got our attention in the first place. Thanks to this attention, we found that heating of aqueous DNA solutions that do not contain dissolved air is not accompanied by an increase in their ultraviolet absorption in the range of $200 \div 300$ nm. It was also found that the heating of aqueous solutions of DNA, which are

specially saturated with air, is accompanied by their ultraviolet hyperchromism, the value of which is 100÷150%. Additional studies have led to the conclusion that thermal UV hyperchromism of aqueous DNA solutions is observed only for those solutions that contain dissolved air [10].

Thus, the air actually determined our ideas about the thermal denaturation of DNA, the degree of which is estimated by the thermal UV hyperchromism of their aqueous solutions, which occurs during their heating [7, 9].

3.4. Participation of Air and Glass in Interactions of Phenazine-N-oxides With Aqueous DNA

As a result, all this made it possible to assume that glass and atmospheric oxygen are capable of influencing the reactions of DNA with OH-radicals, which are generated by phenazine-N-oxides (Figure 8) [11].
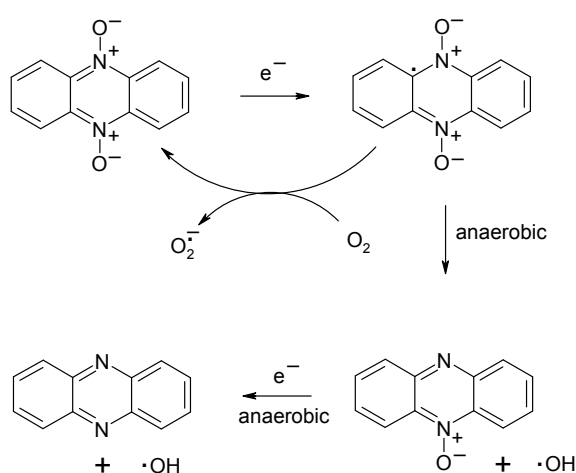
Figure 8. This is a suggested reaction scheme involving phenazine-N-oxides [11].

As you can see (Figure 8), free electrons are needed for such reactions. Thus, the ability of glass to absorb electrons [1, 2] suggested its inhibitory effect on the interaction of phenazine N-oxides with DNA in aqueous solutions. To test this assumption, the water used was preliminarily filtered through a finely porous glass filter or silica gel, which adsorbsaqueous negative charges [3]. Further studies showed that the investigated reactions do not occur in the water obtained after this filtration [12].

At the same time, we were convinced that these reactions successfully proceed in aqueous solutions prepared with water filtered through activated carbon, which adsorbs hydrogen ions H^+ [3] that is, in water enriched with uncompensated negative charges [12].

Also, taking into account the proposed reaction scheme (Figure 8), the ability of oxygen to absorb electrons was investigated. In the course of such studies, it was found that the studied reactions do not proceed in water previously saturated with air or gaseous oxygen. It was also found that these reactions successfully occur in aqueous solutions previously saturated with gaseous hydrogen, which is an electron donor in relation to water and aqueous solutions [3, 12].

Thus, glass and air additionally demonstrated their influence on the experimentally recorded (!) chemical properties of DNA.

4. Discussion

It is a fact that in most X-ray studies, mainly those filaments that form drying solutions of sodium salts of DNA in glass capillaries were used [5]. As shown above, this selection could be due to the special nature of the interactions that occur between Na-DNA and the glass surface. This suggests that our views on the molecular structure of DNA molecules and their parameters were mainly determined by these interactions.

Something should be added here. As is known, cellular sodium cations are concentrated mainly in the cell nuclei, where their concentration can be 10 times higher than in the cytoplasm [5, 8, 13, 14]. At the same time, it is known that cationic potassium is the most abundant cation in the cytoplasm [5, 8]. The existence of both of these distributions allows us to conclude that interphase DNA exists exclusively in the form of sodium salts, which are able to form filaments (Figure 6, left), while mitotic DNA exists mainly in the form of potassium salts, which do not form filaments (Figure 6, right). Taking into account this difference (Figure 6), it is quite natural to assume that the molecular parameters and molecular structures of sodium and potassium salts of DNA can differ significantly. At least, it is quite natural to assume that the molecular parameters and structure of DNA sodium salts cannot be equated with the molecular parameters and structure of DNA potassium salts.

Moreover, the parameters of DNA molecules whose salts form filaments, i.e. participate in intermolecular interactions, cannot be identified with the parameters of individual DNA molecules that do not interact with other DNA molecules.

In any case, the parameters of DNA molecules in the form of salts cannot be equated with the parameters of DNA molecules in acidic form, as it was suggested [15]. It is surprising that such identification was accepted unconditionally. This is analogous to the assumption that the macro-and micro-structural parameters of HCl and NaCl are absolutely identical, which is rather strange considering that HCl is a gas and NaCl is a solid under normal conditions. Thus, it is not surprising that such identification raises doubts about the correctness of traditional molecular models of DNA [4, 16, 17]. At the same time, it is surprising that such identification does not raise doubts among the majority of researchers.

Thus, it seems indisputable that the generally accepted concepts of the structure and parameters of DNA molecules were determined by the structure-forming properties of the glass surface in relation to sodium salts of DNA. No less indisputable is the fact that these concepts should not be extrapolated to native DNA, which a priori does not come into contact with glass, especially with glass capillaries.

It is no less plausible that the emergence of traditional concepts of UV absorption of aqueous solutions of DNA is due to the spectral properties of electrified silica glass and the

presence of air in such solutions. Something should be said here about the contribution of air to the thermal hyperchromism of aquatic DNA. In fact, this phenomenon is very similar to the result of the authors, who showed that oxygenation of aqueous DNA solutions that maintain room temperature is accompanied by an increase in their A_{260} [18, 19]. This similarity suggests that only reactive oxygen causes UV hyperchromism in aqueous DNA solutions. Thus, the content of reactive oxygen in aqueous solutions can be increased both by means of their long-term oxygenation [18, 19] and by means of their heating [10].

Obviously, all this does not agree with the traditional concepts, according to which the thermal UV hyperchromism of aqueous DNA does not exceed 40% [5, 9]. It is also obvious that all this is a consequence of the tradition of ignoring the presence of air in working DNA solutions and, therefore, not taking into account its influence on the spectral characteristics of aqueous DNA, including A_{260}. At the same time, all this shows that A_{260} is not able to adequately reflect the DNA concentration, as noted earlier [20].

It should be additionally noted that the results obtained (Figures 2, 7) require a revision of views on the nature of UV absorption of aqueous DNA near 200 nm, which is traditionally explained by the transfer of electrons in DNA bases [9]. Obviously, traditional views on the nature of UV hyperchromism of aqueous DNA, including thermal, which do not involve oxygen or air, also need to be revised.

Equally important is the established effect of glass and air on the reactions of DNA with OH-radicals generated by phenazine-N-oxides (Figure 8) [11, 12]. This influence should undoubtedly be taken into account when determining the kinetic parameters of DNA reactions with OH-radicals, which are one of the most active forms of oxygen [21]. Since damage to mitochondrial and nuclear DNA by reactive oxygen species causes many human diseases, including senile and cancer [21-29], it can be important to take this effect into account.

5. Conclusion

In vitro studies, contact of biological objects with glass and air, including dissolved air, is inevitable. For this reason, the consequences of such contacts must be considered. Inattention to these contacts can be the reason for the appearance of distorted ideas about the structure and properties of the biological objects under study. In particular, such inattention has become the reason for the emergence of peculiar ideas about both the structure of DNA molecules and the physicochemical properties of DNA in aqueous solutions. This, in turn, caused insoluble contradictions in explaining the manifestations of the functional activity of DNA.

In any case, it is incorrect to identify the parameters and properties of DNA inherent in them in vitro (upon contact with glass and air) with the parameters and properties of native DNA (which do not contact with glass and air). It is also incorrect to extrapolate the structural parameters of DNA salts to DNA acids.

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