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STUDY OF INTERACTION OF HOECHST 33258 AND DNA IN WATER–DMSO MIXED SOLVENTS

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Interaction of well-known DNA minor groove binder Hoechst 33258 and Calf Thymus DNA without and in the presence of increasing amount of dimethyl sulfoxide (DMSO) was studied using UV-vis spectroscopy methods. Thermal melting results show that the melting temperature of Hoechst–DNA complex decreases while increasing of DMSO content. It was shown that DNA is more stable at low DMSO content (5% v/v) rather, than at high concentrations (10–20%).

***Keywords*:** Hoechst 33258, Calf Thymus DNA, UV/vis spectroscopy, thermal melting, dimethyl sulfoxide, molecular interactions.

Introduction. The binding of small molecules targeting different parts of DNA has studies drawn considerable attention. These studies show that many of small compounds can affect on gene expression by inhibiting the binding of regulatory proteins to DNA [1]. Particularly, ligands targeting preferentially AT bases of DNA were studied widely, as the parts of DNA, which are rich with AT bases playing a key role in the formation of genome in eukaryotic organisms. DNA minor groove binders such as Hoechst 33258 (H33258), tripyrrole peptide, distimycin A, bind to DNA in the parts containing at least four AT base pairs [2–6]. Among these minor groove binders H33258 is widely studied as a fluorescence stain and potential anti-cancer agent. Biophysical studies with H33258 performed on poly[d(A-T)$_2$], poly[d(A)·d(T)] and poly[d(G-C)$_2$] revealed the existence of multiple binding modes between the drug and synthetic DNA [7, 8].

On the other hand, dimethyl sulfoxide (DMSO) is widely used in biochemistry and in biomedicine. Particularly, DMSO is a supplement for polymerase chain reactions (PCR). DMSO reduces the melting point of DNA, thus, allowing to perform PCR reactions at low temperatures and avoid from structural irreversible changes caused by high temperatures [9, 10]. The effect of DMSO and its homologues (diethyl sulfoxide and dipropyl sulfoxide) on the thermal stability of DNA was investigated by fluorescence spectroscopy, and was shown that the increasing of hydrophobic chain length causes increase of their denaturating ability. Authors explain this phenomenon by the increase of hydrophobic interactions between DNA and dialkyl sulfoxides [11].

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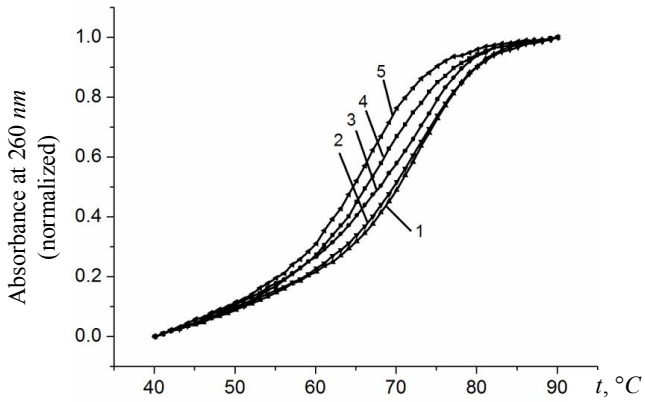
Above stated confirms the importance of studying DNA–H33258 system in the presence of DMSO to further reveal of their interaction characteristics.

Materials and Methods. DMSO, H33258 and Calf Thymus DNA were purchased from "Sigma" (USA). Bidistilled water was used for the preparation of solutions. All measurements were conducted in SSC buffer containing 20 *mM* sodium citrate, 50 *mM* NaCl, pH 6.8. The DNA and H33258 concentrations were determined by spectrophotometry. The extinction coefficients of ε_{260}=6600 $M^{-1}cm^{-1}$ and ε_{343}=42000 $M^{-1}cm^{-1}$ were used for the Calf Thymus DNA and H33258 respectively. DNA concentration was equal to $6.8 \cdot 10^{-5}$ *M* and the ratio concentration of H33258 to that of DNA was 0.1. DMSO content was varied from 0 to 20% (v/v). UV-vis measurements were carried out on Specord 50 spectrophotometer recording the absorbance of the solution at 260 *nm*. Spectral measurements were carried out in hermetically sealed quartz cuvettes with optical path lengths of 1 *cm*, placed into the thermostated cell holders of the spectrophotometer, and heated at a rate of 0.25°*C*/*min*. The investigated solutions were heated continuously with a Lauda Alpha A24 thermostat. All data were analyzed with Origin 8.0 software.

Results and Discussion. The study of ligand–DNA interaction is on the focus of many research groups. Particularly, H33258–DNA system was investigated widely. Meanwhile, the effect of different co-solvents was not studied widely. The study of DNA–H33258 system in the presence of DMSO can serve as a model for other ligands, which are not soluble in water or have limited solubility, as the adding of small amounts of DMSO will promote their solubility. In this work we have studied the thermal stability of H33258–Calf Thymus DNA complex by means of UV-vis spectroscopy. Our investigation was conducted at a constant ionic strength of the solution in order to study the effect of DMSO on the melting point of H33258 complex with DNA.



Melting curves of H33258–Calf Thymus DNA complexes in solutions with and without DMSO: 1 – 0% DMSO (v/v); 2 – 5%; 3 – 10%; 4 – 15%; 5 – 20%.

To obtain melting curves in the Figure, the dependence of solution absorbance at 260 *nm* from temperature was plotted. Our results show that the increasing of DMSO content reduces the melting temperature of H33258–DNA complex, as the melting curves moves to the low temperature region (see Figure). This can be explained by the structural modifications of Calf Thymus DNA caused by DMSO.

It is well known that H33258 is characterized with high affinity towards double stranded DNA, meanwhile it has very low affinity towards single stranded DNA [7]. It was shown earlier that the addition of DMSO causes lowering in the melting temperature of DNA and distortions in DNA double helix structure [12–14]. Thus, the lowering of melting temperature of H33258–DNA complex can be explained by the distortion of DNA double helix. The destabilizing effect of DMSO on DNA in the mentioned works was explained by strong interactions of DMSO with DNA bases of the minor and major grooves, which cause distortion of hydrogen bonds between DNA bases and thus destabilizing DNA double helix.

Melting temperatures in absence and presence of different content of DMSO

Content of DMSO, % v/v	T_m, °C
0	71.2
5	69.5
10	68.2
15	66.3
20	64.6

Based on melting temperatures of the complex, it can be concluded that the initial addition of DMSO (5% v/v) causes significant decrease in the thermal stability of DNA, but not as strong as in the cases of higher concentrations (see Table). This can be explained by hydrogen bonds distortion between DNA base pairs, which causes DMSO. Further addition of DMSO reduces the melting temperatures of the complex till 64.6°*C*. Thus, our results reveal the quantitative description of the effect of DMSO on the thermal stability of Hoechst 33258–Calf Thymus DNA complex.

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