

Effect antimicrobial agents on structure of nucleic acids

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Sodium hypochlorite (NaClO) is in the “top-100” list of chemical compounds, which are most relevant to practice. It is widely used as a bactericidal and sterilizing agent. It is known that the antimicrobial activity of the sodium hypochlorite is due to the presence of hypochlorite ion and its ability to oxidize and hydrolyse proteins. High concentrations of the compound can also destroy the cell membrane of bacteria. However, the effect of NaClO on DNA remains underinvestigated. In this work, we analyse the interaction of sodium hypochlorite with different mono- and polynucleotides using FTIR and UV spectroscopy.

1. Materials and methods

Concentration of mono- and polynucleotide samples was determined spectrophotometrically. The concentration of sodium hypochlorite in aqueous solution was determined spectrophotometrically using the extinction coefficient $\mathcal{E}_{292} = 350 \text{ M}^{-1}\text{cm}^{-1}$.

Samples for IR spectroscopy were freeze dried and KBr pellets were formed. IR spectra of the pellets were recorded using Tensor 27 FTIR spectrometer (Bruker), purged with dry nitrogen and equipped with MCT detector. Each spectrum was recorded with 4 cm^{-1} resolution and averaged by 128 accumulations.

2. UV Spectroscopy

To analyse the effect of NaClO on mono- and polynucleotide samples we obtained UV and IR absorption spectra of their complexes with NaClO at different [Cl]/[P] ratios R. The typical absorption spectra of the ctDNA ($C=0.040 \text{ mM}_p$) in presence of sodium hypochlorite are presented in Figure 1. From the obtained spectra, it can be seen that, the absorption of DNA gradually decreases and shifts towards longer wavelengths with increasing NaClO concentrations revealing considerable modification of the chromophore (i.e. nitrogenous bases) structure.

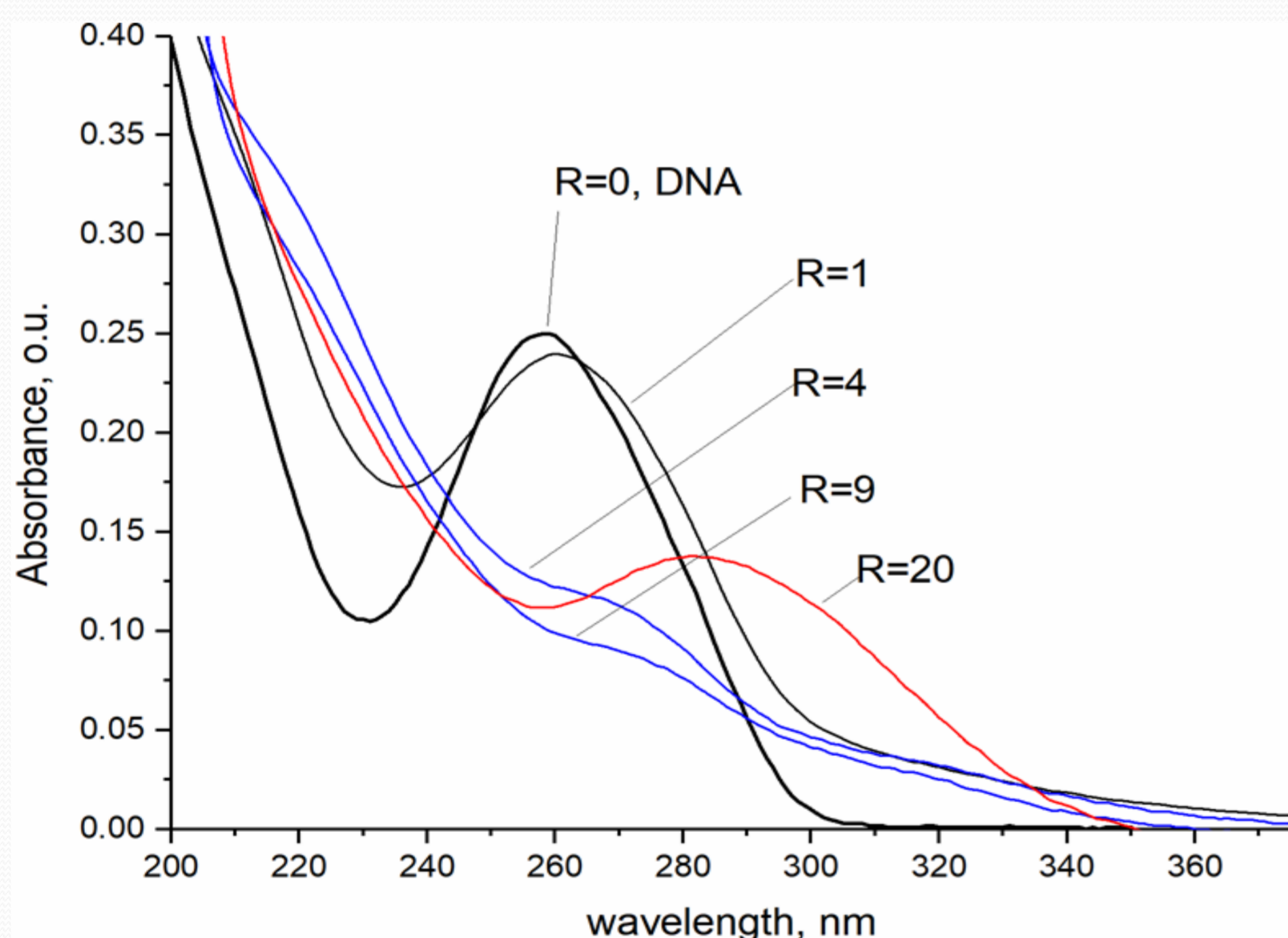
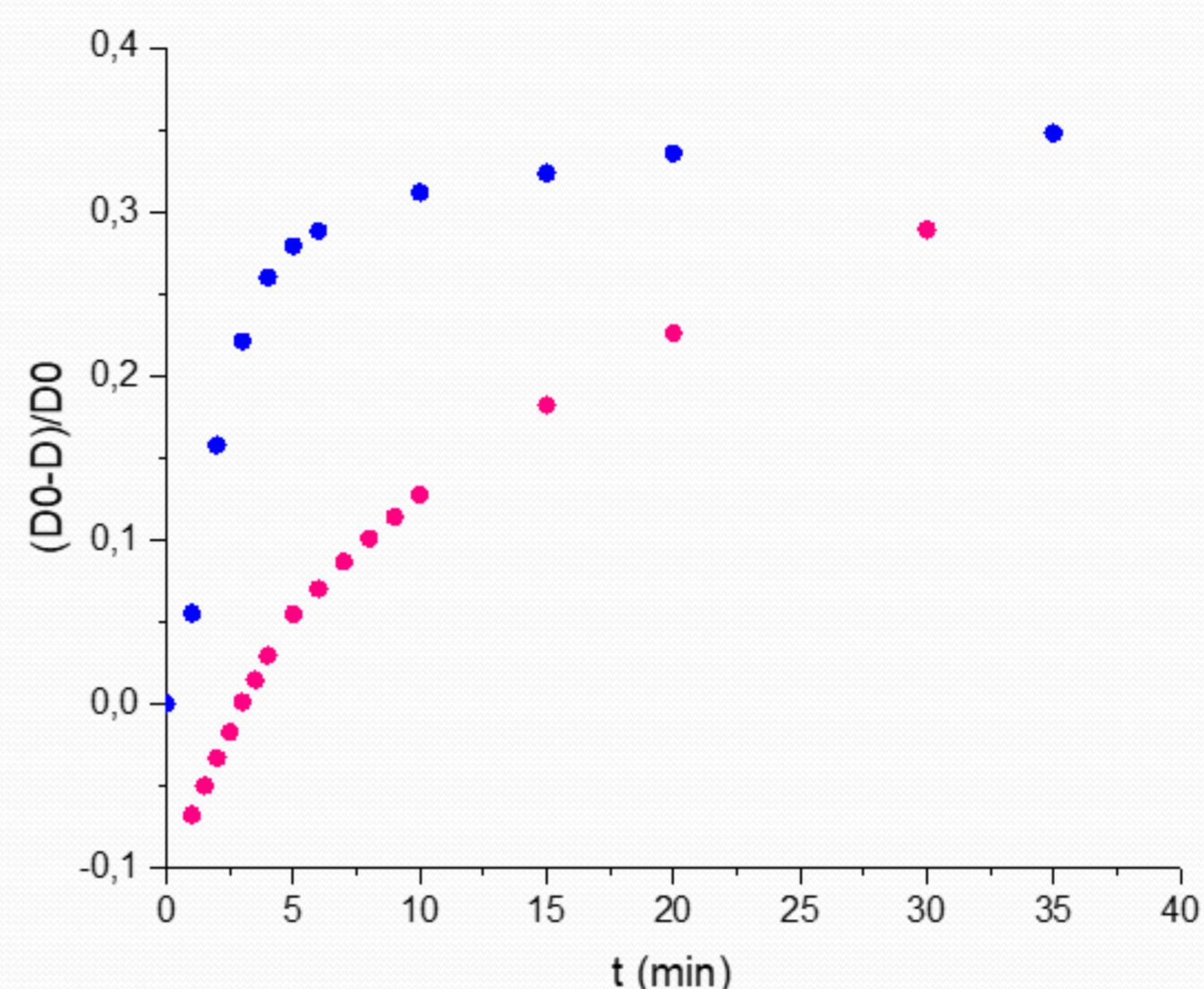


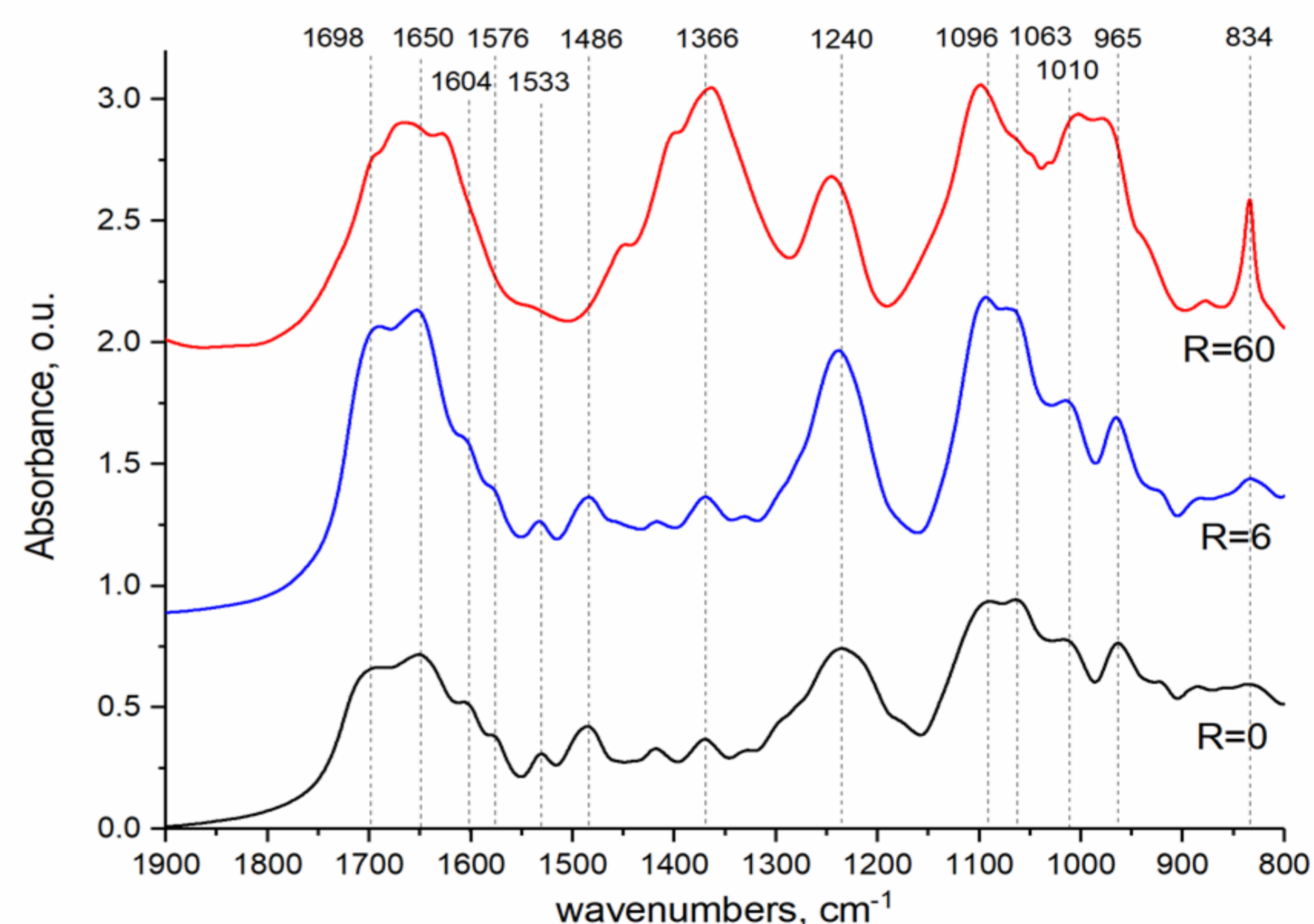
Figure 2 shows the dependence of the relative change in the absorption maximum of the complexes polyA-NaOCl (blue) and ATP-NaOCl (pink) on time. One may conclude that the destruction of cycles of nitrogenous bases at the same concentration of hypochlorite occurs faster and more efficiently in a polynucleotide.



3. IR Spectroscopy

Comparison of the absorption spectra of pure DNA and the spectra of the complexes reveals the appreciable changes in vibrations at 1698 and 1576 cm^{-1} . The former might be due to the guanine (G) C=O stretching and NH_2 scissoring vibrations and interaction of the bases in the pairs, while the latter is typical for the interactions with N7 atom of guanine. Also the changes in vibrational modes of other bases, mostly those of cytosine, can be clearly seen at 1650 , 1604 , 1533 and 1486 cm^{-1} . Vibration at 1650 is attributed to the hydrogen bonded cytosine C4=O4, while the others at 1604 , 1530 and 1490 cm^{-1} are involved in in-plane ring vibrations, NH and CH in-plane deformation modes of hydrogen bonded cytosine, adenine and thymine.

Figure 3 represents the mid-IR spectra of DNA contain two major regions corresponding to the vibrations of the nitrogenous bases ($1800 - \text{ca. } 1300 \text{ cm}^{-1}$) and to the vibrations of the sugar phosphate backbone ($\text{ca. } 1300 - 800 \text{ cm}^{-1}$).



Conclusion

The analysis of the data obtained confirmed that the chemical reaction between NaClO and NAs involves two stages. At low concentrations of hypochlorite the loss of the DNA secondary structure and formation of nucleotide chloramines occur. At higher concentrations of hypochlorite the destruction of the cyclic structure of nitrogenous bases is observed. Based on the analysis of FTIR spectra we confirmed that the reaction of hypochlorite with DNA causes its denaturation and destruction of cycles of nitrogenous bases.

Also, during the study, it was found that, although sites for chlorination are more accessible in free nucleotide complexes than in polynucleotides, in all cases, a more effective effect of hypochlorite was observed in polynucleotide complexes. This circumstance allows us to conclude that the destruction of nitrogenous bases at the selected concentrations is achieved due to the action of radicals on interacting nucleotides.