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Raman spectroscopic study on the subpicosecond dynamics in calf-thymus DNA, upon lowering the pH and in the presence of Mn^{2+} ions

Cristina M. Muntean * and Ioan Bratu
National Institute of Research & Development for Isotopic and Molecular Technologies, Cluj-Napoca, Romania

Abstract. In this paper the Raman total half bandwidths of calf-thymus DNA vibrations have been measured as a function of pH (3.45–6.4), in the presence of Mn^{2+} ions, respectively. The dependencies of the half bandwidths and of the global relaxation times, on DNA molecular subgroup structure and on pH, are reported. It is shown that changes in the subpicosecond dynamics of molecular subgroups in calf-thymus DNA can be monitored with Raman spectroscopy.

Particularly, the Raman band parameters for the vibrations at 728 cm^{-1} (dA), 787 cm^{-1} (dC), 1093 cm^{-1} (PO_2^-), 1376 cm^{-1} (dA, dG, dT, dC), 1489 cm^{-1} (dG, dA) and 1578 cm^{-1} (dG, dA) of MnDNA complexes, at reduced and low pH values, are presented. In our study, the full widths at half-maximum (FWHM) of the bands in calf-thymus DNA are typically in the wavenumber range from 11 to 27 cm^{-1}. It can be observed that the molecular relaxation processes studied in this work, have a global relaxation time smaller than 0.965 ps and larger than 0.393 ps. The limit values are characteristic for dA and dC residues, respectively (vibrations at 728 and 787 cm^{-1}).

Low pH-induced melting of double helical structure in calf-thymus DNA, in the presence of Mn^{2+} ions, results for some bands in smaller global relaxation times, and larger bandwidths, respectively, as a consequence of the increased interaction of the base moieties with the solvent molecules. This behaviour is most evident for the bands at 787 cm^{-1} up to pH 3.8, at 1578 cm^{-1} up to pH 3.45 and is partially confirmed for the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1}.

The fastest molecular dynamics was obtained for the adenine band at 728 cm^{-1} in the pH interval 3.45–3.8 (global relaxation time 0.885 ps), for the cytosine ring breathing mode near 787 cm^{-1} around the pH 3.8 (global relaxation time 0.393 ps), for the band at 1093 cm^{-1} in the pH interval 3.8–4.4 (global relaxation time 0.518 ps) and for the vibration near 1578 cm^{-1} at pH 3.45 (global relaxation time 0.544 ps).

A comparison between different time scales of the vibrational energy transfer processes, characterizing the protonated MnDNA structural subgroups has been given.

We have found that metal ion's type and concentration are modulators for the (sub)picosecond dynamics of protonated DNA molecular subgroups.

Keywords: Subpicosecond dynamics, calf-thymus DNA, pH, Mn^{2+} ions, Raman spectroscopy

1. Introduction

Vibrational relaxation plays a crucial role in many aspects of chemistry, physics and biology, e.g., reaction dynamics, electron transfer, photochemistry, thermal chemistry, excimer formation and photo-

*Corresponding author: Dr. Cristina M. Muntean, National Institute of Research & Development for Isotopic and Molecular Technologies, P.O. 5, Box 700, R-400293 Cluj-Napoca, Romania. E-mail: cmuntean@itim-cj.ro.

biological processes such as vision and photosynthesis ([1] and references therein).

Studies of molecular relaxations in liquids are valuable in providing information about the intermolecular interaction processes in condensed matter [2]. Among the techniques available for the study of molecular motions in liquids, Raman scattering has the distinct advantage, that it enables simultaneous analyses of both reorientational and vibrational processes ([2,3] and references therein).

Raman spectra of complex molecules do not consist of a set of sharp lines, as time-dependent forces broaden the vibrational bands [4]. Vibrational frequencies depend on static molecular parameters as force constants, bond distances and angles, atomic masses and electric charges. Dynamic parameters of atomic and molecular motions determine the vibrational band shapes [4,5].

The macromolecular motion in fluids is generally too slow to be observed in the Raman time window that is accessible in the frequency domain. On contrary, the motion of molecular subgroups can be fast enough [6].

A number of dynamical processes have been considered to broaden the vibrational bands of nucleic acids in the frequency domain, e.g. vibrational energy exchange, vibrational resonance coupling, vibrational dephasing and rotational broadening ([4] and references therein) [5]. Different relaxation mechanisms may characterize different vibrations, and this kind of information is available from spontaneous Raman measurements ([4] and references therein) [5].

The dynamics and vibrational relaxation processes of biomolecules in aqueous solutions have motivated several researches [1].

Upon changing the structure of the nucleic acid by the presence of proteins, ionic salts, pH, metal ions and intercalators, different vibrational modes of the molecule can behave quite different [5,7,8].

Raman bandwidths in polynucleotides range from 8 wavenumbers to 35 wavenumbers, and the corresponding time scale of the perturbing forces ranges from fractions of a picosecond to several picoseconds [4].

Isotropic and anisotropic spontaneous Raman spectra were obtained from solutions of poly(rA) and rAMP in buffer [9]. The temperature dependence of these spectra was measured to elucidate the influence of macromolecular dynamics and solvent dynamics on the bandwidths of base vibrations in the single stranded polynucleotide poly(rA) [9]. It has been found that the full widths at half-maximum (FWHM) of the bands in poly(rA) are typically [9] of the order of 12–20 cm^{-1}.

Besides, a confocal Raman microspectroscopic study into the vibrational half bandwidths of molecular subgroups in calf-thymus DNA, upon lowering the pH, and in the presence of Na^+, Ca^{2+} and Mg^{2+} ions, respectively, was previously reported by us [8]. In this study the half bandwidths of the vibrations in calf-thymus DNA were typically in the wavenumber range from 7.4 to 31 cm^{-1}. The bandwidths in the Raman spectra were sensitive to a dynamics active on a time scale from 0.34 to 1.44 ps [8].

Also, recently, the dependencies of the full widths at half-maximum (FWHM) and of the global relaxation times on DNA molecular subgroup structure, and on Mn^{2+} and Na^+ ions concentrations, respectively, have been reported [5].

In the study of nucleic acids, phosphate groups are particularly very important in the structure, dynamics and interactions of mono- and polynucleotides [5,10,11]. A study of the dynamics of the $PO_3{}^{2-}$ group in aqueous solution can give information on the mononucleotide mobility and interactions in its natural solvent [11].

A band-shape analysis of the IR $\nu_s(PO_3{}^{2-})$ band of disodium deoxycytidine 5′-monophosphate, 5′-dCMP, in ^2H_2O and H_2O has been performed in relation to the relaxation processes of this vibrational mode [1]. The second derivative spectra reveal the presence of 5′-dCMP aggregates when concen-

tration reaches ~0.28 mol dm^{-3}. A similar self-association process was detected for the mononucleotide 5′-CMP [1].

The IR $\nu_s(PO_3{}^{2-})$ band shape of cytidine 5′-monophosphate (5′-CMP) in H_2O solution at different concentrations, 0.009–0.3 mol dm^{-3}, has been analyzed in relation to the dynamics of the phosphate group of the nucleotide. The relaxation of this mode in aqueous solution seems to be predominantly vibrational [11]. Besides, FTIR measurements on the $\nu_s(PO_3{}^{2-})$ band shape of 5′-CMP in ^2H_2O solution at different concentrations, 0.002–0.58 mol dm^{-3}, and temperatures, 10–55°C have been interpreted in terms of the dynamics of the $PO_3{}^{2-}$ group and the self-association processes of this mononucleotide [12]. Second derivative spectra and integrated intensities reveal a possible aggregation of 5′-CMP in ^2H_2O at concentrations higher than 0.3 mol dm^{-3} [5,12].

In this paper, the complex system of calf-thymus DNA, in an aqueous buffer solution, is studied by Raman spectroscopy, at reduced and low pH values, in the presence of Mn^{2+} ions, respectively. The molecular relaxation processes of DNA subgroups are presented. Monitoring the changes in the Raman vibrational full widths at half-maximum (FWHM) and, correspondingly, in the global relaxation time of the molecular subgroups in DNA, upon lowering the pH and in the presence of a constant concentration of Mn^{2+} ions, is of interest.

2. Experimental procedure

The experimental details were given in Muntean et al. [13] for MnDNA complexes, at reduced and low pH values. Calf-thymus DNA samples were obtained in the presence of 150 mM NaCl and 10 mM $MnCl_2$. Raman spectra were measured at pHs 6.4, 5.8, 5.1, 4.4, 3.8 and 3.45. The pH values were controlled in the dialysis buffers and in the DNA samples, respectively. The Raman spectra were recorded with a Raman spectrometer T64000 (Jobin Yvon, France) equipped with a liquid nitrogen-cooled charge-coupled-device (CCD) detector, at the Max-Delbrück-Centrum für Molekulare Medizin, Berlin–Buch, Germany and are presented elsewhere [13].

Raman data were analyzed with the software packages LabSpec (Jobin Yvon, France) and GRAMS (Thermo Galactic, USA). Solution spectra were corrected by subtraction of the averaged buffer spectrum and fluorescence background that was approximated by a polynomial curve [13–15].

Peak positions and FWHM of the bands were determined using SpectraCalc software. The FWHM was evaluated from the half-maximum Raman bands.

3. Results and discussions

One of the well-known procedures of obtaining the relaxation times and the activation energy, in the study of the molecular relaxation processes, was developed by Rakov [5,8,16].

The total half bandwidth of the depolarized Raman lines contains in this approximation, two contributions [5,8]:

- an intrinsic bandwidth, δ_0, considered temperature independent in that time;
- another contribution $\Delta(T)$ which is temperature dependent.

The total half bandwidth can be written as:

$$\Delta\nu_{1/2} = \delta_0 + \Delta(T) = \delta_0 + \frac{1}{\pi c \tau_r}. \tag{1}$$

The potential barrier against reorientation can be obtained as:

$$\tau_r = \tau_0 \exp\left(\frac{U_{\text{or}}}{kT}\right), \tag{2}$$

where τ_0 is the period of the molecule oscillation around the equilibrium position, and U_{or} is the energy barrier or the activation energy [5,8].

The Rakov relationship can be written as:

$$\Delta\nu_{1/2} = \delta_0 + \frac{1}{\pi c \tau_0} \exp\left(\frac{-U_{\text{or}}}{kT}\right). \tag{3}$$

From the $(\Delta\nu_{1/2} - \delta_0)$ *vs* $10^3/T$ dependencies one can obtain U_{or} as the slope of this linear dependence [5,8].

The temperature "independent" part, due to the vibrational relaxation, δ_{v}, presents small temperature dependence, opposite as the one due to the reorientational relaxation.

The vibrational contribution becomes important for large molecules, in aqueous solutions. Using polarized light, it is possible to do the selection of these two contributions from Raman measurements [5,8]. One can assume, into a first approximation, the existence of a global relaxation time, τ, obtained from the total Raman half bandwidth. This band parameter can be related with the intrinsic parameters of the analyzed system through the relationship:

$$\tau_{v,1R,2R} = \frac{1}{\pi c \Delta\nu_{1/2}^{v,1R,2R}}, \tag{4}$$

where the half bandwidth includes the vibrational ($\Delta\nu_{1/2}^{v}$) and rotational ($\Delta\nu_{1/2}^{1R,2R}$) contributions and c is the velocity of light. $\Delta\nu_{1/2}^{1R,2R}$ is obtained from IR and Raman bands, respectively [5,8].

One can control the dominant contribution of one or another molecular relaxation process through:

(a) the selection of the molecular system; in the case of large molecules, solved in polar media (e.g. water), one can assume that the vibrational relaxation dominates (is the most efficient relaxation mechanism); the reorientational contribution can be neglected, being a very slow molecular motion [5,8];
(b) temperature dependence; the half bandwidth increases, with the temperature increase, see Eq. (1); if one observe a weak temperature dependence or even a decrease of the half bandwidth with the temperature increase, the dominant contribution is the vibrational relaxation [5,8];
(c) a proper selection of the solvents, e.g. in strong polar media, the vibrational contribution is dominant; in the case of inert, non-polar solvent media, on the contrary, the rotational relaxation must be taken into account.

Molecular dynamics studies for mononucleotides [12] or deoxymononucleotides [11] in aqueous solutions were done by using these approximations [5,8].

The development of fast and accurate curve fitting programs allows the analysis of the vibrational spectra of complicated biological molecules containing often more than 40 vibrational bands ([4] and references therein).

In this paper we will concentrate on the vibrational bandwidths. Only the relatively isolated nucleic acids vibrations are considered [5,8]. A study into the Raman vibrational bandwidths of molecular subgroups in calf-thymus DNA, upon changing the pH, and in the presence of Mn^{2+} ions, is of interest. It is shown that changes in the subpicosecond dynamics of molecular subgroups in calf-thymus DNA can be monitored with Raman spectroscopy.

For the case of aqueous solutions of DNA molecules we can suppose that the dominant relaxation mechanism is the vibrational one. The values of the global relaxation time suggest also the existence of a vibrational relaxation time, because the reorientational movement is much more slower for the DNA macromolecule in aqueous solution [5]. Particularly, the absence of reorientational broadening in polynucleotides indicates that the bases in polynucleotides reorient through an angle of 41° in times slower than 21 ps ([4] and references therein).

The Raman band parameters obtained for the adenine vibration at 728 cm^{-1} [15,17–19] the cytosine ring breathing mode near 787 cm^{-1} [8,13,18,20,21], the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1} [14,18,20], the purines (dA, dG) and pyrimidines (dT, dC) residues band near 1376 cm^{-1} [13,19,20], the guanine (N-7) and adenine rings vibration at 1489 cm^{-1} [18,19] and the purines (dG, dA) 1578 cm^{-1} vibration ([22] and references therein) of calf-thymus DNA, at different pH values, in the presence of Mn^{2+} ions, are summarized in Tables 1–2, respectively.

The full widths at half-maximum (FWHM) of the Raman bands in calf-thymus DNA, in the presence of 10 mM $MnCl_2$, are presented at 6 different pH values, and are typically in the wavenumber range from 11 to 27 cm^{-1} (see Table 1) [23]. Besides, the global relaxation times were evaluated on the basis of Eq. (4). From the vibrations at 728, 787, 1093, 1376, 1489 and 1578 cm^{-1} it can be observed that the global relaxation times, for molecular subgroups in dissolved calf-thymus MnDNA complexes, at reduced and low pH values, are slower than 0.393 and faster than 0.965 ps (see Table 2). The limit values are characterizing the dC and dA residues, respectively (vibrations at 787 and 728 cm^{-1}). As a general rule, the bandwidths in the Raman spectra are sensitive to a dynamics active on a time scale from 0.1 to 10 ps [4,5].

Table 3 presents the pH dependent wavenumber (cm^{-1}) of the band maximum for different vibrations in calf-thymus DNA, in the presence of Mn^{2+} ions.

Figures 1–4 present the total half bandwidths (FWHM) characteristic to molecular subgroups vibrations in calf-thymus MnDNA complexes, as a function of pH, for the modes at 787 cm^{-1} (dC), 1093 cm^{-1} (PO_2^-), 1376 cm^{-1} (dA, dG, dT, dC) and 1578 cm^{-1} (dG, dA), respectively [23].

Table 1
pH dependent Raman total half bandwidths (cm^{-1}) of different vibrations in calf-thymus DNA, in the presence of Mn^{2+} ions

pH	Vibrational modes, ν (cm^{-1})					
	728 (dA)	787 (dC)	1093 (PO_2^-)	1376 (dA, dG, dT, dC)	1489 (dG, dA)	1578 (dG, dA)
	FWHM, $\Delta\nu_{1/2}$ (cm^{-1})					
3.45	12.0	24.5	19.5	24.0	20.5	19.5
3.80	12.0	27.0	20.5	23.5	19.5	18.0
4.40	11.0	26.0	20.5	23.5	20.5	17.5
5.10	11.5	25.0	20.1	24.0	20.1	18.0
5.80	11.0	25.0	20.0	25.0	20.5	18.0
6.40	11.5	24.8	20.5	22.5	19.5	17.0

Table 2
pH dependent global relaxation times, for different molecular subgroups in calf-thymus DNA, in the presence of Mn^{2+} ions

pH	Vibrational modes, ν (cm^{-1})					
	728 (dA)	787 (dC)	1093 (PO_2^-)	1376 (dA, dG, dT, dC)	1489 (dG, dA)	1578 (dG, dA)
	Relaxation time, τ ($s \times 10^{-12}$)					
3.45	0.885	0.433	0.544	0.442	0.518	0.544
3.80	0.885	0.393	0.518	0.451	0.544	0.590
4.40	0.965	0.408	0.518	0.451	0.518	0.607
5.10	0.923	0.425	0.528	0.442	0.528	0.590
5.80	0.965	0.425	0.531	0.425	0.518	0.590
6.40	0.923	0.428	0.518	0.471	0.544	0.624

Table 3
pH dependent wavenumber (cm^{-1}) of the band maximum for different vibrations in calf-thymus DNA, in the presence of Mn^{2+} ions

pH	Vibrational modes, ν (cm^{-1})					
	728 (dA)	787 (dC)	1093 (PO_2^-)	1376 (dA, dG, dT, dC)	1489 (dG, dA)	1578 (dG, dA)
	Wavenumber, ν (cm^{-1})					
3.45	728.5	786.5	1093.0	1376.0	1488.0	1577.5
3.80	728.3	786.8	1093.3	1375.3	1488.8	1578.3
4.40	728.0	787.0	1093.0	1376.0	1489.5	1577.5
5.10	728.1	787.1	1093.6	1376.1	1489.1	1577.8
5.80	728.4	786.9	1092.9	1375.4	1488.4	1577.4
6.40	728.9	786.9	1093.9	1375.8	1489.8	1578.3

Figures 5–8 present the global relaxation times of molecular subgroups in calf-thymus MnDNA complexes, as a function of pH, for the modes at 787 cm^{-1} (dC), 1093 cm^{-1} (PO_2^-), 1376 cm^{-1} (dA, dG, dT, dC) and 1578 cm^{-1} (dG, dA), respectively.

Figures 9–11 present the pH dependent wavenumber (cm^{-1}) of the band maximum for different vibrations in protonated MnDNA complexes. Modes at 728 cm^{-1} (dA), 787 cm^{-1} (dC) and 1489 cm^{-1} (dG, dA), respectively, are taking into account.

pH dependent MnDNA structural changes are responsible for the dynamical behaviour of DNA molecular subgroups.

Low pH-induced melting of double helical structure in calf-thymus DNA results for some vibrations in smaller global relaxation times and larger bandwidths, respectively, as a consequence of the increased interaction of the base moieties with the solvent molecules [8,23]. This behaviour is most evident for the bands at 787 cm^{-1} up to pH 3.8, at 1578 cm^{-1} up to pH 3.45 and is partially confirmed for the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1}. For this last band, the global relaxation time increases at 0.531 ps for the pH value 5.8, before starting to decrease up to pH 3.8 (global relaxation time 0.518 ps). The best vibrational energy transfer processes were obtained for the adenine band at 728 cm^{-1} in the pH interval 3.45–3.8 (global relaxation time 0.885 ps), for the cytosine ring breathing mode near 787 cm^{-1} around the pH 3.8 (global relaxation time 0.393 ps), for the band at 1093 cm^{-1} in the pH interval 3.8–4.4 (global relaxation time 0.518 ps) and for the vibration near 1578 cm^{-1} at pH 3.45 (global relaxation time 0.544 ps) [23].

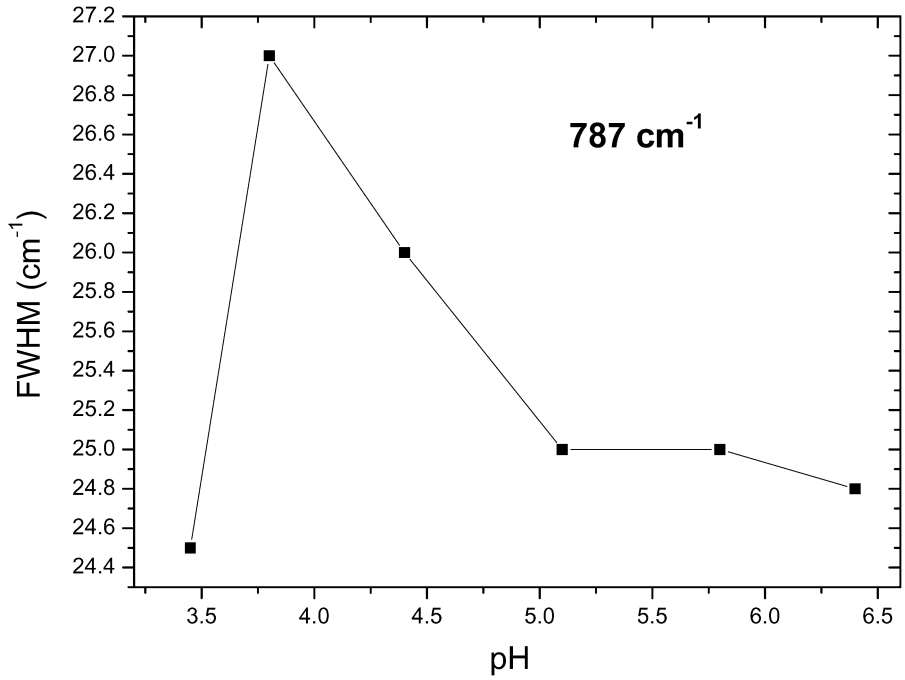

Fig. 1. Full width at half-maximum (FWHM) of the cytosine vibration at 787 cm^{-1} in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

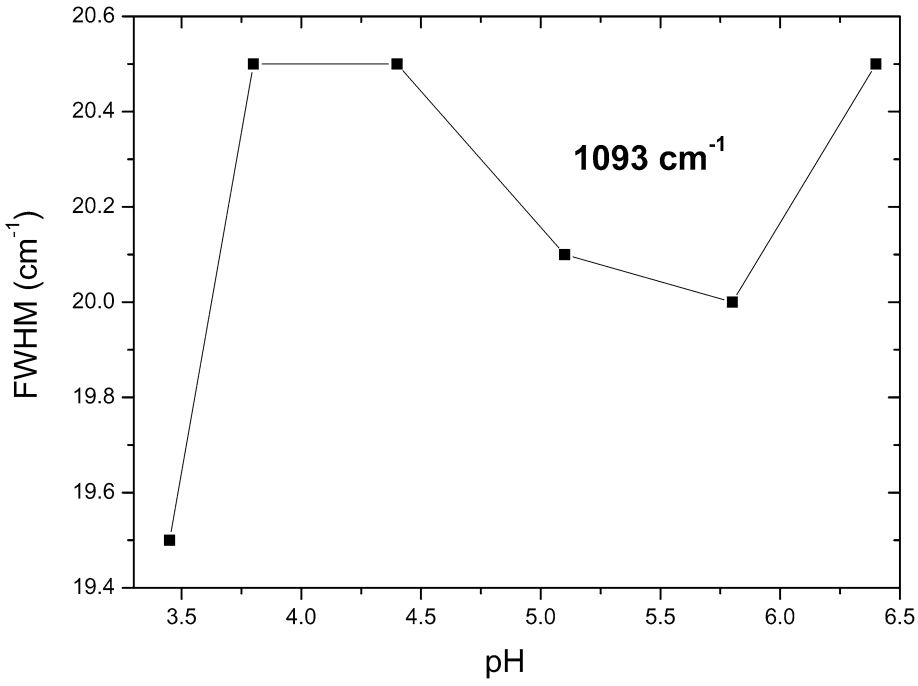

Fig. 2. Full width at half-maximum (FWHM) of the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1} in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

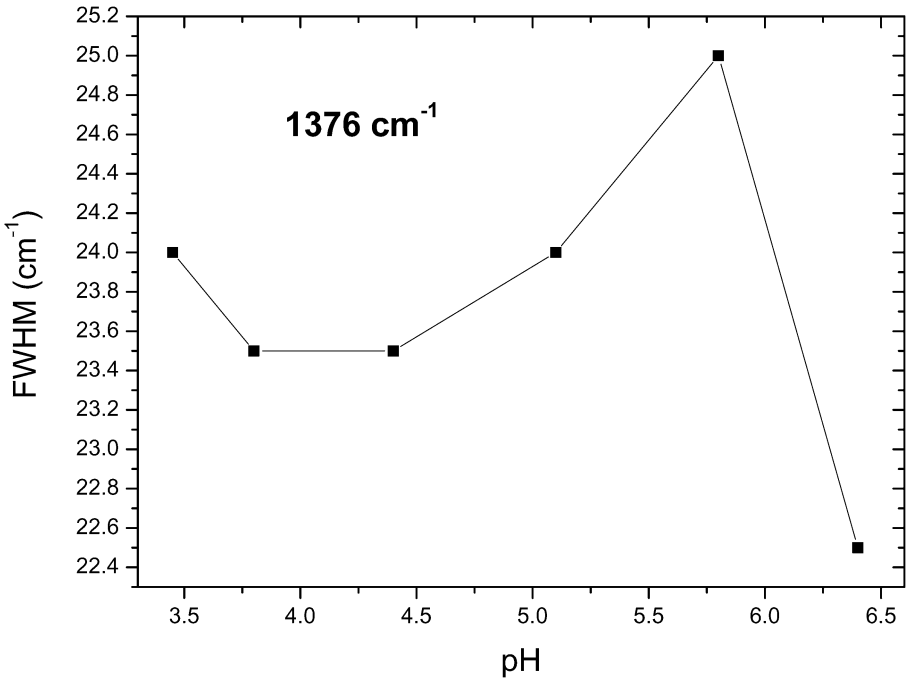

Fig. 3. Full width at half-maximum (FWHM) characteristic to the purines (dA, dG) and pyrimidines (dT, dC) residues vibration at 1376 cm^{-1} in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

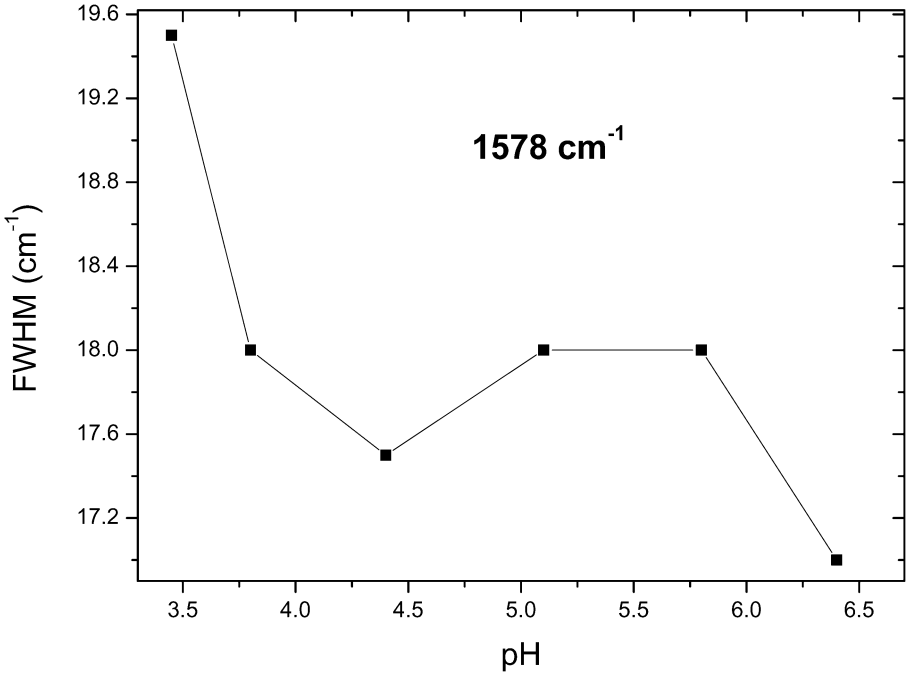

Fig. 4. Full width at half-maximum (FWHM) of the purines (dG, dA) 1578 cm^{-1} vibration in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

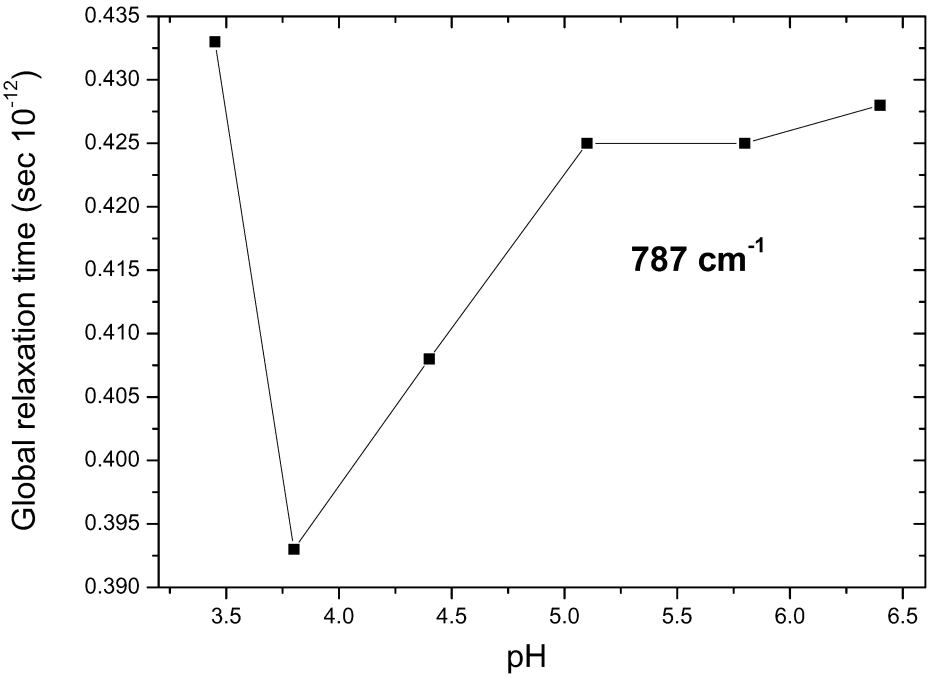

Fig. 5. Global relaxation time of the cytosine vibration at 787 cm^{-1} in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

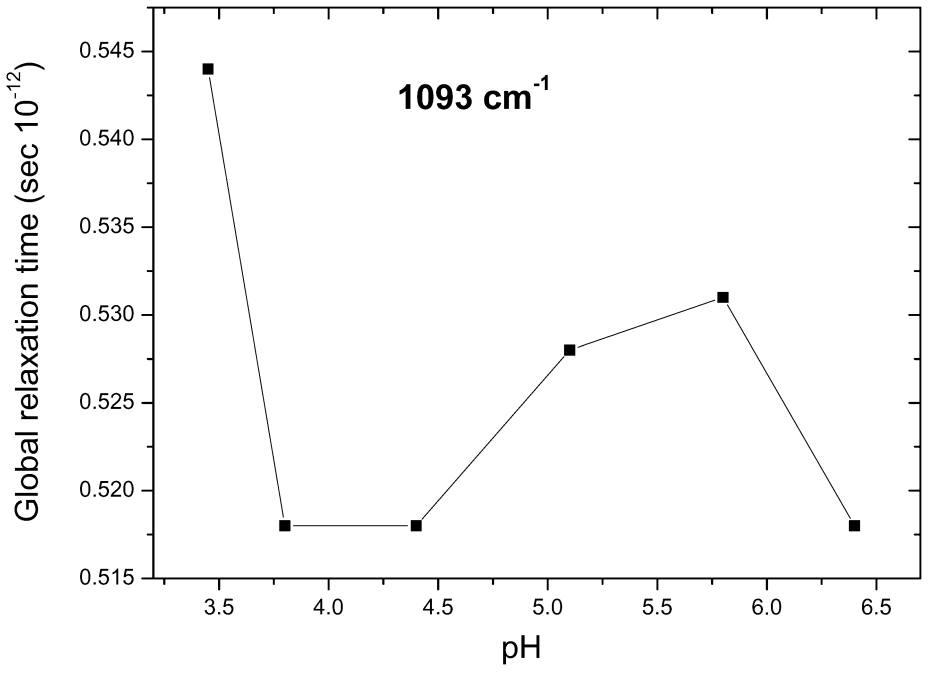

Fig. 6. Global relaxation time of the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1} in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

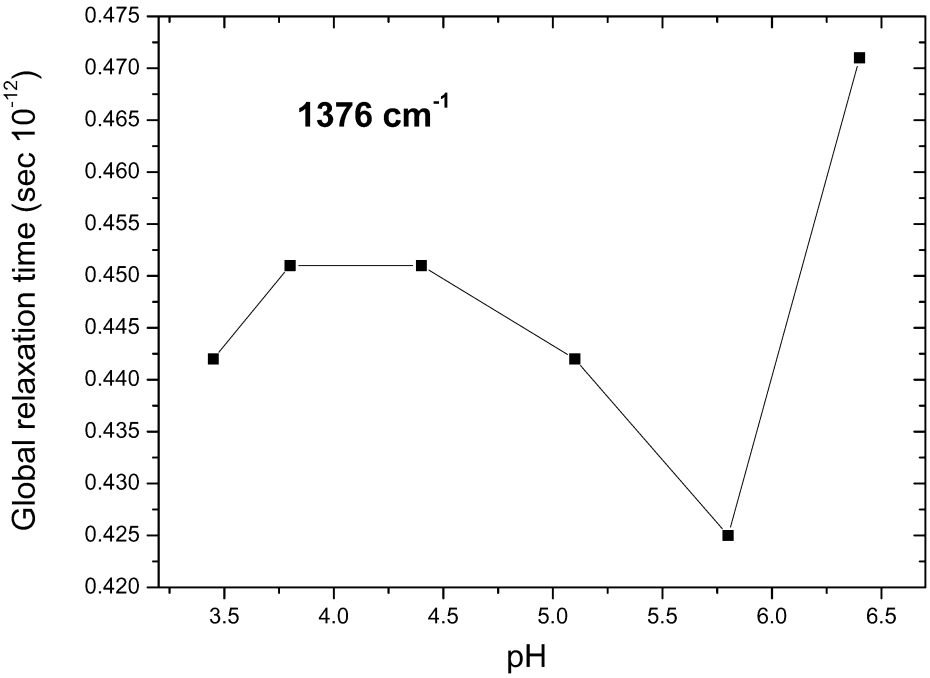

Fig. 7. Global relaxation time characteristic to the purines (dA, dG) and pyrimidines (dT, dC) residues band at 1376 cm^{-1} in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

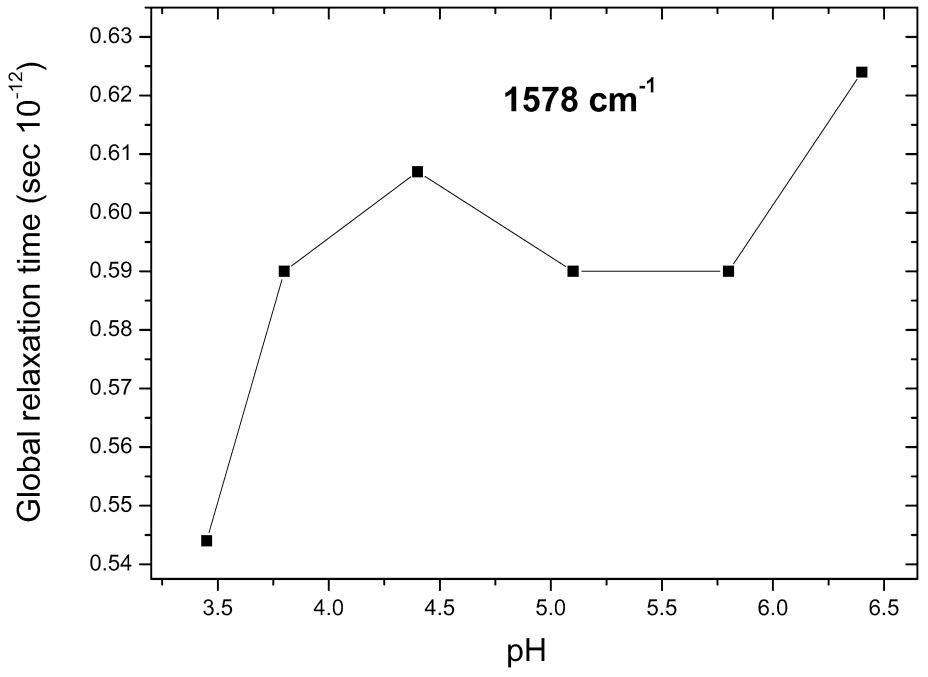

Fig. 8. Global relaxation time of the purines (dG, dA) 1578 cm^{-1} vibration in calf-thymus DNA, as a function of pH, in the presence of Mn^{2+} ions, respectively.

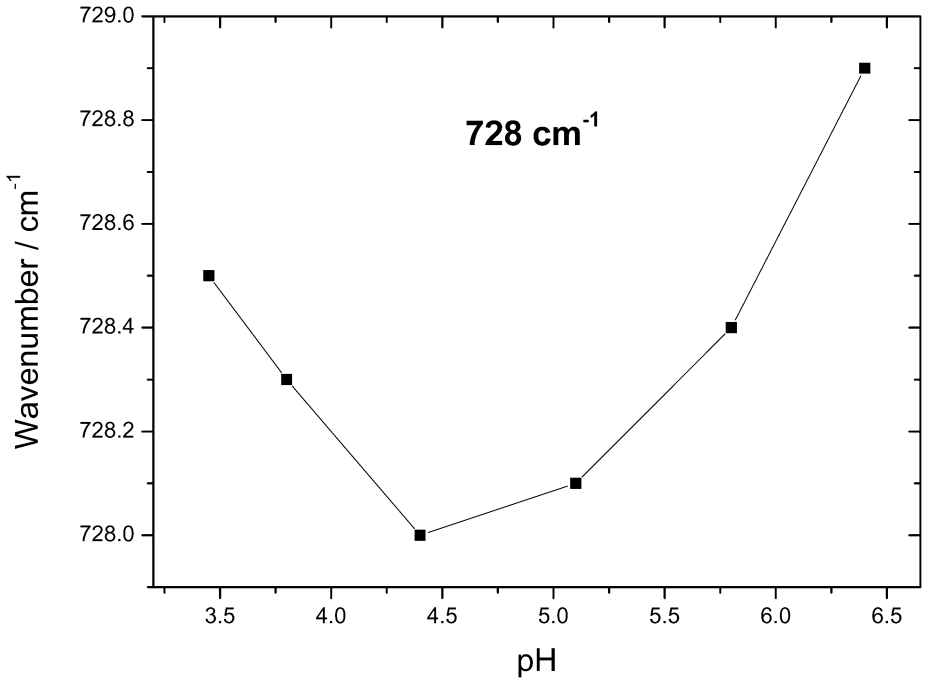

Fig. 9. pH dependent wavenumber of the band maximum for the adenine vibration at 728 cm^{-1} in calf-thymus DNA, in the presence of Mn^{2+} ions, respectively.

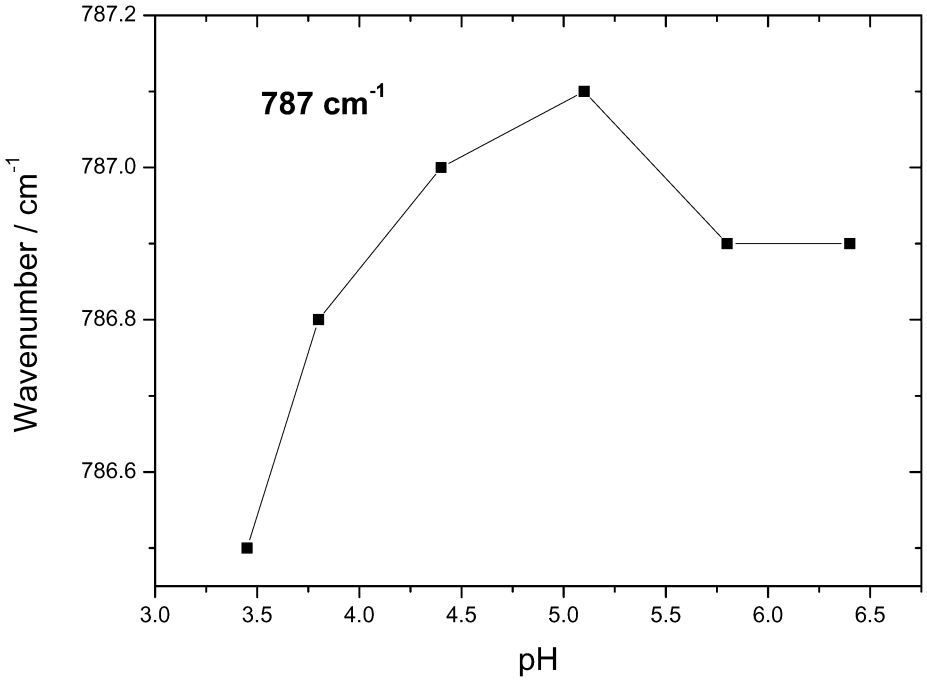

Fig. 10. pH dependent wavenumber of the band maximum for the cytosine vibration at 787 cm^{-1} in calf-thymus DNA, in the presence of Mn^{2+} ions, respectively.

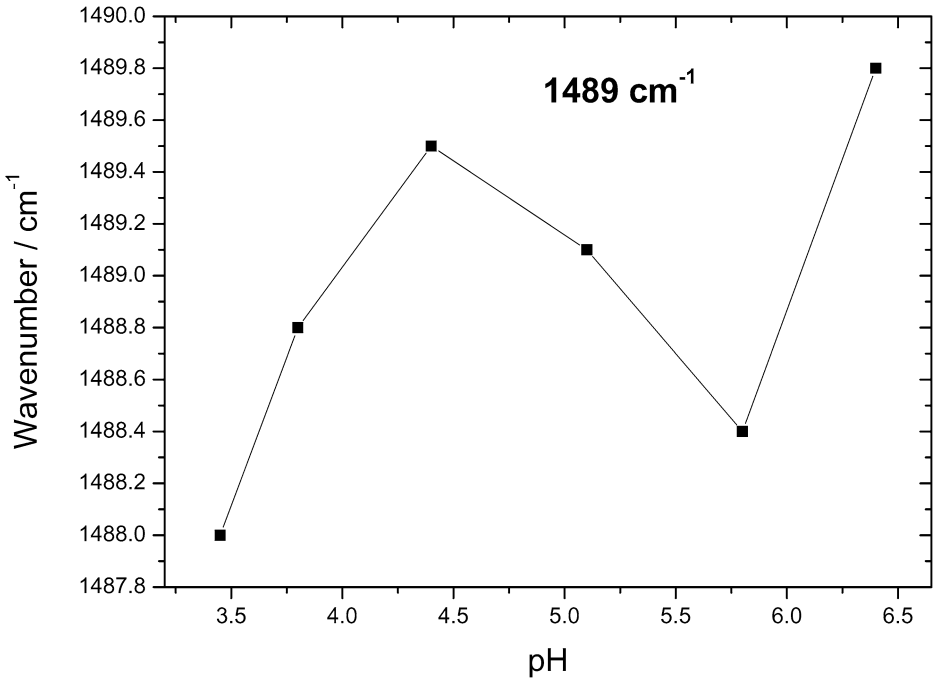

Fig. 11. pH dependent wavenumber of the band maximum for the guanine (N-7) and adenine rings vibration at 1489 cm^{-1} in calf-thymus DNA, in the presence of Mn^{2+} ions, respectively.

Upon lowering the pH under these values, the global relaxation times increase at the highest values for the bands at 787 and 1093 cm^{-1}, indicating a slower dynamics for the corresponding calf-thymus DNA structural subgroups.

Besides, upon lowering the pH from 4.4 to 3.8, a sudden increase of the global relaxation time to 0.544 ps is to be observed for the guanine (N-7) and adenine rings vibration near 1489 cm^{-1}, but upon further DNA protonation the global relaxation time decreased at 0.518 ps.

The fastest molecular dynamics observed for the DNA subgroups corresponding to vibrations at 728, 787, 1093 and 1578 cm^{-1} is probably due to the denaturation process of the double helical DNA [23].

Apart from the data above, the global relaxation time of the band near 1376 cm^{-1} of the purines (dA, dG) and pyrimidines (dT, dC) residues [24], decreases from 0.471 ps at pH 6.4 to 0.425 ps at pH 5.8. Upon further decreasing the pH up to 3.8, the global relaxation time of this band has a tendency to increase. Similar features of the molecular relaxation processes at reduced pH values, were observed in this study for the guanine and adenine rings band at 1489 cm^{-1}.

Previously, it was established [4,8] that the bandwidths of the 1336, 1480 and 1575 cm^{-1} adenine vibrations increase toward the value for the mononucleotides upon thermal melting of poly(rA). This temperature behavior of the bandwidths of poly(rA) were assigned to an increasing exposure of the bases to the solvent upon melting of the secondary structure. Moreover all the studied adenine, thymine, and uracil vibrations have a smaller bandwidth in stacked structures than in unstacked structures and mononucleotides [4,8].

On the basis of our experimental results, the molecular relaxation processes are slower for the dA residues (characteristic band around 728 cm^{-1}), as compared to those of the dC residues (characteristic vibration at 787 cm^{-1}). This result is similar with an observation made previously by us [5]. Besides,

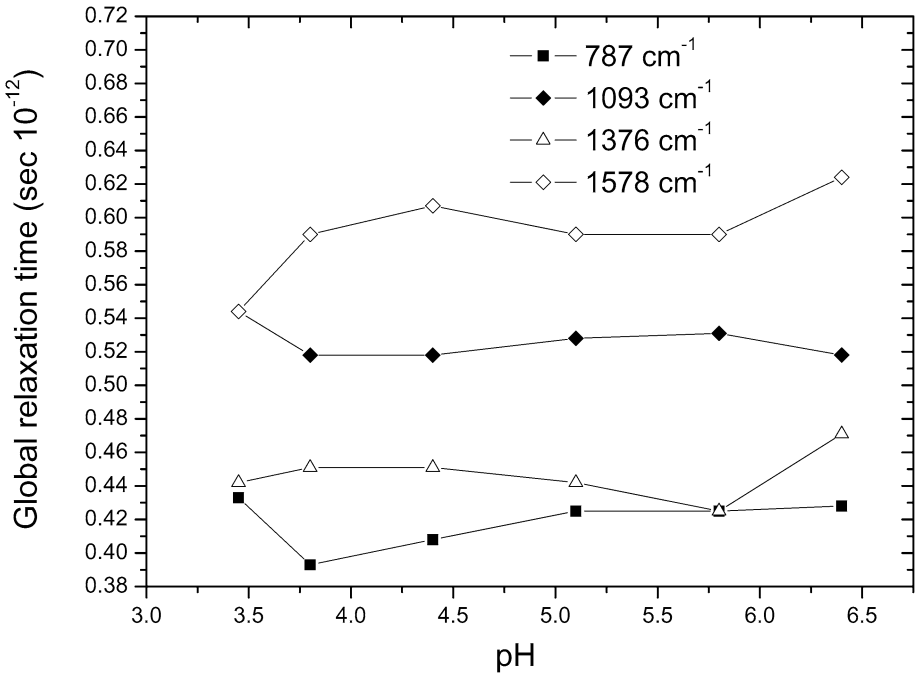

Fig. 12. A comparison between different time scales of the molecular relaxation processes in calf-thymus MnDNA complexes, as a function of pH.

the dynamics characterizing the vibration at 1376 cm^{-1} is faster than the molecular relaxation processes characterizing the band near 1489 cm^{-1} and both are faster than the vibrational energy transfer processes of the band at 1578 cm^{-1}. We have found that upon increasing the pH value, the global relaxation times characterizing the bands at 1093 and 1489 cm^{-1}, have the same variation interval.

A comparison between different time scales of the molecular dynamics, characterizing several protonated DNA subgroups is given in Fig. 12. It is shown that the Raman band parameters of the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1} and of the purines (dG, dA) band at 1578 cm^{-1} ([22] and references therein) of calf-thymus DNA have an opposite behaviour in the pH interval 3.45–6.4. Besides, the molecular dynamics characterizing the band at 1093 cm^{-1} is faster than that characterizing the vibration at 1578 cm^{-1}, with the exception of the vibrational energy transfer processes at pH 3.45.

Differences in the molecular relaxation processes of DNA subgroups are to be observed upon lowering the pH, in the presence of Mn^{2+} ions, as compared to the corresponding dynamics, found previously by us, for protonated DNA subgroups, in the presence of Mg^{2+} ions [8]. Hence, metal cation's type and concentration are modulators for the (sub)picosecond dynamics in protonated calf-thymus DNA complexes [5,8].

4. Conclusions

The most significant information concerning the behaviour of a condensed system in the picosecond time scale is offered by vibrational spectroscopy (infrared and Raman) ([3] and references therein).

Among the techniques available for the study of molecular dynamics, Raman scattering has the distinct advantage of simultaneously analyzing both reorientational and vibrational relaxation processes [2,3].

This work is a Raman spectroscopic study into the vibrational half bandwidths of molecular subgroups in calf-thymus DNA, upon lowering the pH from 6.4 to 3.45, and in the presence of Mn^{2+} ions. In addition, the corresponding global relaxation times have been derived. The Raman band parameters were obtained for the modes at 728 cm^{-1} (dA), 787 cm^{-1} (dC), 1093 cm^{-1} (PO_2^-), 1376 cm^{-1} (dA, dG, dT, dC), 1489 cm^{-1} (dG, dA) and 1578 cm^{-1} (dG, dA) of calf-thymus DNA [23,25].

A sensitivity of the vibrational half bandwidths of calf-thymus MnDNA complexes to the pH value has been found. Moreover, this proved to be dependent on the vibration under study [8,23].

The Raman half bandwidths of protonated calf-thymus MnDNA vibrations reveal a dynamic picture on a subpicosecond time scale. The full widths at half-maximum (FWHM) of the bands in calf-thymus DNA, analyzed in this study, are typically in the wavenumber range from 11 to 27 cm^{-1}. The bandwidths in the Raman spectra are sensitive to a dynamics active on a time scale from 0.393 to 0.965 ps.

The vibrational energy transfer processes, characterizing different Raman bands in MnDNA complexes, at reduced and low pH values, have been discussed.

Low pH-induced melting of double helical structure in calf-thymus DNA results for some bands in smaller global relaxation times, and larger bandwidths, respectively, as a consequence of the increased exposure of the bases to the solvent molecules upon melting of the secondary structure [8,23]. This behaviour is most evident for the bands at 787 cm^{-1} up to pH 3.8, at 1578 cm^{-1} up to pH 3.45 and is partially confirmed for the DNA backbone PO_2^- symmetric stretching vibration at 1093 cm^{-1}. The fastest dynamics was obtained for the adenine band at 728 cm^{-1} in the pH interval 3.45–3.8 (global relaxation time 0.885 ps), for the cytosine ring breathing mode near 787 cm^{-1} around the pH 3.8 (global relaxation time 0.393 ps), for the band at 1093 cm^{-1} in the pH interval 3.8–4.4 (global relaxation time 0.518 ps) and for the vibration near 1578 cm^{-1} at pH 3.45 (global relaxation time 0.544 ps) [23].

A comparison between different time scales of the molecular relaxation processes, characterizing the protonated MnDNA subgroups structures has been given.

Differences in the molecular dynamics of MnDNA subgroups are to be observed upon lowering the pH, as compared to the corresponding dynamics, found previously by us, for protonated DNA subgroups, in the presence of Mg^{2+} ions [8]. Hence, metal cation's type and concentration are modulators for the (sub)picosecond dynamics of protonated DNA structural subgroups [5,8].

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