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Infrared Spectroscopy of Nucleic Acids

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The review deals with infrared and Raman spectroscopic data for DNA, RNA, and their components and with the assignment of the infrared bands to the vibrations of individual bonds. An approach to a theoretical treatment of the normal vibrations of the nucleic acid skeleton is described. The applications of infrared spectroscopy to the study of the specificity of the interaction of the heterocyclic bases, the secondary structure of nucleic acids, and their interactions with proteins, metal ions, and certain biologically active substances is considered.
The bibliography includes 218 references.

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I. INTRODUCTION

The vigorous development of molecular biology has been accompanied by a large-scale application of physical and physicochemical methods in the investigation of the composition and structure of biologically important compounds. Infrared spectroscopy is acquiring increasing importance among these methods. The infrared spectroscopic method has been successfully employed to solve various structural and physicochemical problems in molecular biology, including the recognition of compounds with weak structural characteristics, the determination of the structural formulae of biologically important compounds, and the study of the steric configuration of biopolymers.

The infrared spectra of biologically important compounds such as aminoacids, peptides, heterocyclic bases of nucleic acids, lipids, steroids, and vitamins and also the spectra of biopolymers—proteins, nucleic acids, and polysaccharides—are being investigated at the present time. Major advances have been achieved in the study of the structure of polypeptides and proteins with theoretical analysis of the normal vibrations. However, for nucleic acids a complete description of the normal vibrations is still lacking, most probably due to the complexity of their molecular structure. Therefore the fundamental bands in the infrared spectra of the bases, polynucleotides, and nucleic acids have been interpreted by a semiempirical method based on the study of the absorption by individual atomic groups in the low-molecular-weight components. This reduces the reliability of the conclusions arrived at and, what is most important, limits the information which may be derived from the infrared spectra.

Nucleic acids consist of highly polymerised unbranched molecules the main valence chain of which comprises alternating phosphoric acid and sugar units; purine and pyrimidine bases are attached to the sugar residues (Fig. 1)†. The molecular structure of nucleic acids and also of synthetic polynucleotides is now fairly well known, as can be seen from the numerous papers, reviews, and monographs [1-9].

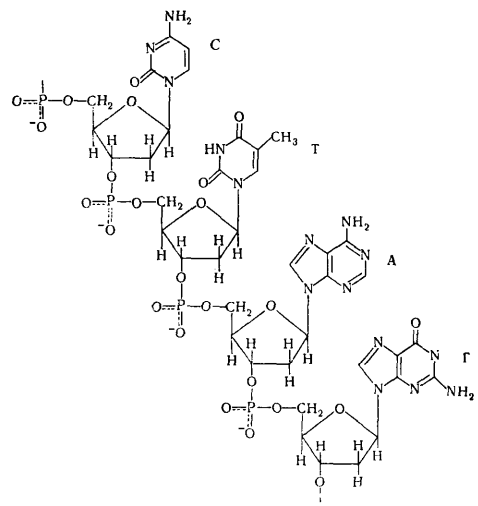


Figure 1. Schematic representation of the primary structure of the DNA chain.

There are no reviews on the infrared spectroscopy of nucleic acids in the Soviet literature. Abroad a review was published in 1965 by Shimanouchi et al. [10], in which the authors considered mainly the results of their own infrared spectroscopic studies on nucleotides, sugars, phosphates, synthetic polynucleotides, DNA, and RNA, while during the preparation of the present review Tsuboi [11] published a review on the infrared spectroscopic studies on the structure of nucleic acids. During recent years very many studies on the infrared spectra of nucleic acids, not included in the above reviews, have been published.

†The following abbreviations have been adopted: A = adenine, G = guanine, T = thymine, C = cytosine, U = uracil, I = inosine, GMP = guanosine monophosphate, poly-A = polyriboadenylic acid, poly-(G/C) = double helix of polyguanylic and polycytidylic acids.

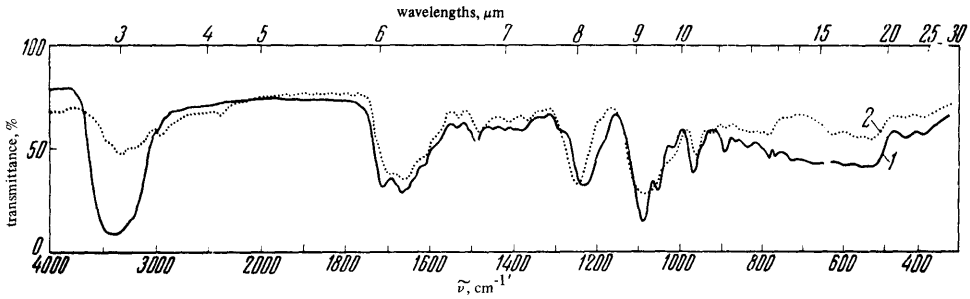


Figure 2. Infrared spectrum of the sodium salt of DNA for 75% (curve *1*) and 0% (curve *2*) relative moisture constants. Calf thymus DNA; $\tilde{\nu}$—vibration frequency (wave-number).

II. VIBRATIONAL SPECTRA OF NUCLEIC ACIDS

The infrared spectrum of DNA was described for the first time in 1948–1953 by Blout and coworkers for solid specimens in the range 4000–700 cm^{-1} [12,13] and for solutions in D_2O in the range 1750–1500 cm^{-1}. [14,15] Non-oriented DNA films and non-polarised radiation were employed in these investigations. The spectra of non-oriented DNA films were first published by Fraser [16] in 1951, but he did not take into account the effect of the moisture content of the specimens on the spectra. Subsequently a number of workers [10,17-23] studied the vibrational spectra of nucleic acids in the range 4000–400 cm^{-1} using all the possibilities of the method at present available.

The infrared spectrum of DNA [10] shown in Fig. 2 is comparatively simple: it consists of a fairly "diffuse" pattern, the majority of the bands of which are diffuse and only a few are fairly distinct. Numerous bands have become appreciably broadened and overlap with those adjoining them.

Three regions may be arbitrarily distinguished among the groups of absorption bands in the spectrum: 4000 to 2000 and 1700–1500 cm^{-1}, usually attributed to the vibrations of the bases and the CH groups of deoxyribose in DNA, and 1300–1000 cm^{-1} attributed to the vibrations of the sugar and the phosphate groups in the DNA molecular skeleton. The assignment of the bands in the infrared spectrum of DNA to the individual bonds of the skeleton and the DNA bases is given in Tables 1 and 2; for the majority of the bands, with the exception of those in the region 3500–2000 cm^{-1} and at 1705, 1220, and 1084 cm^{-1}, the assignment must be regarded as only approximate.

One should also bear in mind that the vibrations of the double bonds in the rings of the bases are strongly coupled. In the spectra of deuterated DNA, a shift of the majority of the bands towards lower frequencies is observed owing to the H–D isotope exchange in the NH_2 and NH groups of the bases [10,18].

The form of the infrared spectrum of DNA changes somewhat as the moisture content of the specimen is varied (Fig. 3)—a number of bands are displaced and the spectrum becomes systematically improved with increase of the relative moisture content to 92% [17,19] (a particular moisture content is achieved usually by placing the DNA film in a hermetically sealed chamber over a saturated solution of a particular salt or by blowing through the chamber a gas with a specified moisture content). Many bands become narrower, the degree of overlapping diminishes, and the overall diffuse background of the spectrum becomes less pronounced. A similar pattern

Table 1. Skeletal vibration bands in the infrared spectra of nucleic acids.

Group	No. of groups	Intensity	Expected vibration wave-number, cm^{-1}	Wave-number in spectrum, cm^{-1} of DNA	of RNA	of poly-adenylic acid
References			24–26	10, 17, 18	27	28
	1	s. s.	ν_{as} 1220 ν_s 1085	1230 ⊥ 1224 ∥ 1084–89 ⊥	1225 ⊥ 1084 ∥	1240 ⊥ 1090 ∥
—C—O—P	2	s. s.	ν 1100 $\nu \sim 900$	1053 ⊥ 965 ⊥	1070 ∥ 937 ⊥	— 920 ∥
—O—P—O—	1	med. w.	ν_{as} 820 ν_s 760–750	820 ∥ 784 ∥	810 ⊥	820
—C—O—C— rings	1	s. s. w. s. s.	ν_{as} 1100–1070 ν_s 940–910 γ 650–600 ν_{as} 2970–2920 ν_s 2860–2850	1050 ⊥ 1024 ∥ 970 ⊥ 2900 2800	990 ⊥, 960 ∥	990 ⊥, 965 ∥ 645 ∥, 630 ∥
	2	w. w. w.	δ 1465–1445 $\omega \sim 1380$ r 825–800	1450 ⊥ 1301 ⊥ 825 ∥	890 ∥ 860 ⊥	880 ∥ 860 ∥
—OH	1	s. s. w.	ν 3630 δ 1250 β 750–650	— —	1250 ⊥ 780, 730	780 ∥
	1	s. s.	$\nu_{degener}$ 1125–1085 $\nu_s \sim 800$	— —	1130–1120 ⊥ 810 ⊥	1116 ⊥ 1130 ∥ 820 ⊥
	3	m. w. w.	ν 2980–2890 δ 1440–1400 β 900–650	2850 1410 ⊥	1400 ⊥	1420 ⊥
—C—C ring	2	w.	ν 955	915	910	

Note. ∥, ⊥ -polarisation; s = strong (intense); m = medium; w = weak; ν—stretching vibration; δ—in-plane vibration; ρ—out-of-plane vibration; r—rocking vibration; ω—fanwise deformation vibration; γ—ring deformation vibrations.

is observed in the study of the vibrational spectra of amorphous synthetic polymers [36,37] and this suggests that the above spectroscopic behaviour is due to an appreciable structural imperfection of the test material. This conclusion is partly confirmed by an examination of the infrared spectrum of the double helix of polyriboadenylic acid (poly-A) [28] shown in Fig. 4. Simple elimination of the irregularity in the alternation of bases appreciably improves the spectroscopic pattern.

The polarisation of the infrared absorption bands of DNA also depends on the moisture content, the dichroism of the majority of the bands being shown fairly distinctly,

although it does not lead to an appreciable improvement of the quality of the spectrum (Fig. 3). The dichroic ratio of many bands usually increases with the relative moisture content of crystalline DNA specimens.

Table 2. Vibration bands of the bases in the infrared spectra of nucleic acids.

Group	Type of vibration	Expected wavenumber, cm^{-1}	Intensity	Wave-number in spectrum, cm^{-1}						
				C	U	T	A	G	DNA "light"	DNA "deuter"
References		24–26		10, 29–32	10, 29, 33, 34	14, 33, 35	10, 29, 31, 32	10, 31 32	10, 17–19, 23	
$-NH_2$ bound	ν_{as}	3350	s.	3367	—	—	3280	3310	3350	3103
	ν_s	3180		3155			3105	3077	3210	
—NH—bound	ν	3180—3000	m.	2778	3110 2924	3175	3000	2840	2950	
$-CH_3$	ν_{as}	2960	s.	—	—	3035	—	—	2950	
	ν_s	2880—2820				2900				
=CH	ν	3000—2750	m.	3081 2947	3000	2780	2780	2680	3090	
—C=O	ν	1690—1650	s.	1655	1724 1680	1735 1670	—	1700	1705	1685
$-NH_2$	δ	1680—1600	s-m	1667	—	—	1672	1681	1609	1557
—NH—	δ	1680—1560	s-m	1538	1666 1418	1570		1555	1528	
—C=C—	ν	1680—1620	m	1621	1623	1640	1578	1569	1575	1575
—C=N—	ν	1680—1630	m-w	1630	—	—	1605	1639	1655	1607
$-NH_2$	β	1450	s-m	1458	—	—	1451	1418	1444	
—NH—	β	1300—1200	m.		1388		1250	1263 1172	1296	
$-CH_3$	δ_{as}	1460±20	s.	—	—	1500	—	—	1450	1458
	δ_s	1380—1370	m.			1380			1375	1375
Ring	ν	1480—1200	s.	1527 1362 1236	1389 1238	1200	1418 1333	1477 1375	1503 1477	1516 1476
C—CH_3	ν	1250—1200	w.	—	—	1245	—	—	1292	1300
C—NH_2	ν	1300—1250	s.	1275	—	—	1206	1215	1331	1331
=C—H	δ	1460	w.		1451	1380		1464	1456	1453
=C—N—	ν	1200—1150	w.	1156			1124			
—C—N—	ν	1250—1100	w.	1011	1099	1200			1296	1300
$-NH_2$	β	900—600	m.		—	—	1022, 797	949	893	778
—NH	β	820—720	m.	823	822, 760	835	870	877, 776, 689	778	
—C—C	ν	1000—950	w.	995	994	1000	938		860	851
=C—H	β	860—700	w.	784	851	705		789, 703	795	794
Ring	γ	850—700	w.	794	780	740	722		727	715

In the range 1750–1550 cm^{-1}, where the in-plane vibrations of the rings of the bases are active, all the bands of Na- and Li-DNA are polarised at right angles to the direction of the orientation of the film. An analogous polarisation is observed also for the stretching and in-plane deformation vibrations of the NH_2, NH, and CH groups in the range 1600–1300 cm^{-1}.[18,19] It is interesting that the band at 1240 cm^{-1}, corresponding to the antisymmetric vibrations of the PO_2^- groups of Na-DNA,[10] does not exhibit dichroism at a relatively low moisture content, but, on increasing the latter, it is displaced from 1240 to 1220 cm^{-1} and is split into two components. Tsuboi believes[11] that this finding implies that the antisymmetric vibrations of each PO_2^- groups are not independent and that adjacent PO_2^- groups appreciably interact with one another, generating a series of vibrations which are propagated throughout the chain. The component with a lower wave-number (1215 cm^{-1}) and parallel dichroism may be assigned to the in-phase vibrations of the PO_2^- groups and the component with a higher wave-number (1225 cm^{-1}) and perpendicular dichroism may be assigned to the vibrations of the PO_2^- groups with a phase difference of $2\pi/n$, where n is the number of nucleotides corresponding to one turn of the DNA spiral. The wave-number difference between these components (~10 cm^{-1}) may be regarded as reflecting the strength of the interaction between the PO_2^- groups in the given crystalline Na-DNA specimen.

The band due to the symmetrical vibrations of the PO_2^- groups of DNA (1089 cm^{-1}) exhibits perpendicular dichroism. An analogous polarisation is observed also for the absorption bands in the range 1100–900 cm^{-1}, which are mainly due to the vibrations of the sugar and phosphate groups. The bands in the range 900 to 700 cm^{-1}, where the out-of-plane deformation vibrations at the N–H bond are active, also show parallel dichroism[17–19].

The effect of an increase of temperature from 22° to 90°C on the infrared spectra of DNA has been discussed by a number of investigators[10,20,38,39] and has been attributed to the breakdown of the secondary DNA structure (see Section V).

The infrared spectra of RNA have now been investigated in fair detail. All the general characteristics of the spectrum indicated for DNA hold also for RNA (Fig. 5). The vibrational spectrum of RNA has a set of intense absorption bands in the regions 3500–2000, 1700–1500, and 1250–1050 cm^{-1}. However, in the range 1100 to 800 cm^{-1}, where the sugar components of nucleic acids absorb, there is an appreciable difference between the DNA and RNA spectra[10,39,40]. The RNA spectra have bands at 1120 and 810 cm^{-1}, which are absent from the DNA spectra, and conversely the 1050 cm^{-1} band is characteristic only of polydeoxyribonucleotides. The spectroscopic characteristics of various types of RNA—ribosomal, transport, and viral—are in many ways similar, but the details of the spectra in the ranges 1750–1400 and 840 to 760 cm^{-1} are somewhat different, which is probably due to the different quantitative compositions of the bases and different percentage contents of the base pairs[27,41–43]. The infrared spectra of moist films of viral RNA show a weaker polarisation than DNA, which, however, may be due to the lower degree of orientation of the films. It is important to note that the symmetrical and antisymmetrical vibrations of the double helical RNA of the dwarf rice virus shows a dichroism which differs from that of DNA (Table 8). Quantitative data on the orientation of DNA and RNA films are normally quoted with the spectra, which greatly complicates their interpretation and comparison of spectra in polarised radiation. Oriented films of transfer RNA cannot be obtained owing to their low molecular weight.

The effects of changes in moisture content and temperature on the infrared spectra of RNA have been investigated to a lesser extent than for DNA. In contrast to DNA, the infrared spectrum of a solution of double stranded viral RNA is still lacking, but the spectra of transfer RNA have been recorded both for films and for D_2O solutions[11].

Synthetic polynucleotides exist either in a single-chain state or form double or triple helices, depending on a number of conditions (concentration, ionic strength, pH of the medium, etc.[6,44,45]). The infrared spectra of films comprising the double helices of polyadenylic acid (poly-A) and poly-(G/C) and the triple helix of poly-(A/2U) have been described[11,28] and the spectra of D_2O solutions of double-helical poly-(A/U), poly-(G/C), and poly-(I/C) have been measured by Miles and coworkers[46–52].

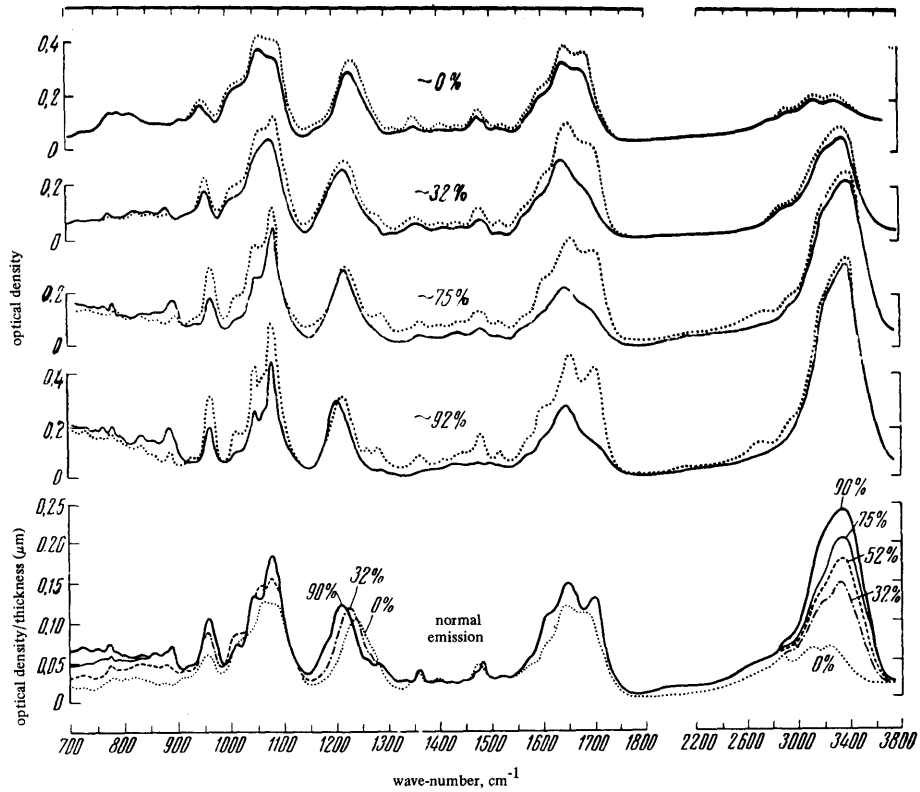


Figure 3. Polarised infrared spectra of oriented films of the sodium salt of DNA with different relative moisture contents[17]; dashed curves—emission vector perpendicular (⊥) to direction of specimen orientation; continuous curves—emission vector parallel (∥) to direction of specimen orientation.

The attempts to ob in the Raman spectra of aqueous DNA and RNA solutions remained unsuccessful for a long time. Only the Raman spectrum of polyadenylic acid in the single-chain state was known[53]. The laser Raman spectra of solutions of calf thymus DNA and the ribosomal RNA of *E. coli* were obtained by Tobin in 1969 [54] and Thomas in 1970 [55]. The positions of the bands in the Raman and infrared spectra of DNA largely coincide except for a discrepancy in the range 1700–1600 cm^{-1}, while the bands in the Raman spectrum of RNA from *E. coli* coincide satisfactorily with the bands in the corresponding region of the infrared spectrum[42].

III. VIBRATIONS OF THE MAIN VALENCE CHAIN OF NUCLEIC ACIDS

The theory of the normal vibrations of high-polymer molecules has recently developed considerably and, since nucleic acids are polymeric, it is quite reasonable to attempt a description of their vibrational spectra within the framework of the concepts formulated for polymeric systems[37,56].

A complete analysis of the vibrational spectrum of a polymer—a determination of the total number of its normal vibrations, their polarisation, and the selection rules for the infrared and Raman spectra and also the calculation of the frequencies and forms of the normal vibrations—is possible only for systems with a high degree of three-dimensional order. Despite their capacity for being packed in crystal lattices[57], neither DNA nor RNA possess such an order owing to the irregular sequence of bases. Only the molecules of synthetic polynucleotides such as poly-A and poly-C exhibit a rigorous chemical and steric (in the crystalline phase) periodicity and can be regarded as regular. Unfortunately the data for the vibrational spectra of these systems available in the literature are extremely limited[10,28], and yet they may prove to be indispensable in the interpretation of the vibrational spectra of nucleic acids. At the same time the main chains (skeleton) of the DNA and RNA molecules have an irregular chemical structure and under certain conditions also an irregular conformational structure[5,58–60], which allows an interpretation of the normal vibrations of the molecular skeleton using the periodic properties. Strictly speaking, the vibrational spectra of such helix complexes should be due to the vibrations of the atoms in both chains and to their interactions. However, one may suppose that the interaction of the chain vibrations in the double helix should be extremely insignificant, since the chains are separated by a considerable distance (the helix diameter is about 20 Å). The interactions between different double helices in the crystal phase can also be neglected. The most intense interaction between the main DNA and RNA chains occurs owing to the hydrogen bonds between the bases, but such interaction influences mainly the vibrations of the atoms in the bases, virtually without any perturbation of the atomic vibrations in the main chain.

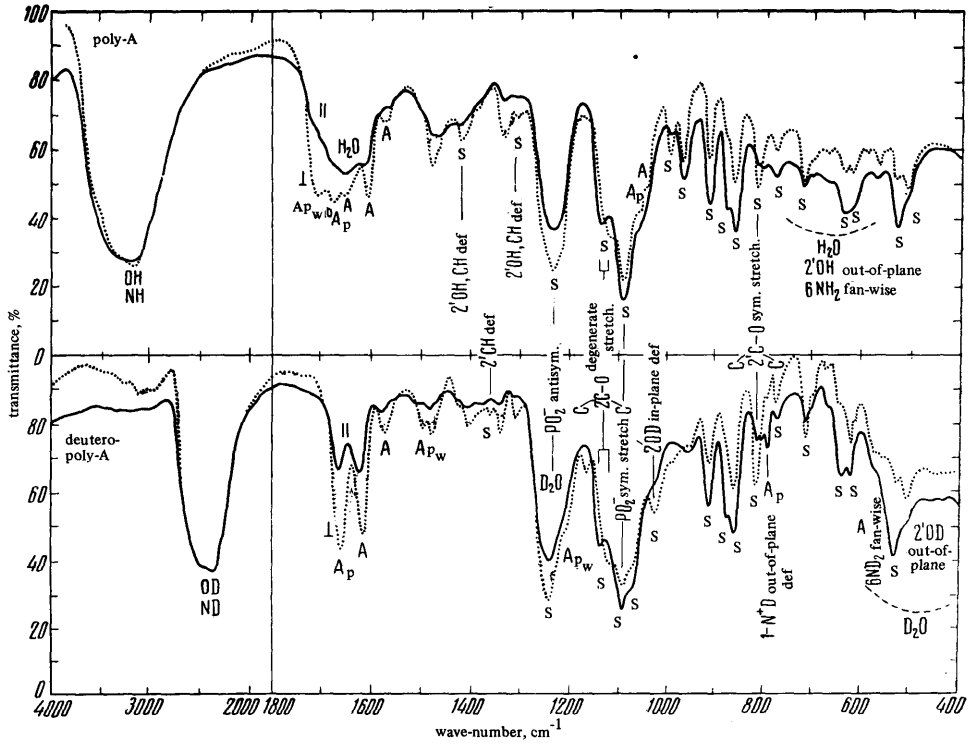


Figure 4. Polarised infrared spectrum of polyriboadenylic acid in the H- and D-forms with a 92% relative moisture content[28]; A—adenine absorption bands; A_p—bands of protonated adenine; A_{pw}—bands disappearing on drying; S—absorption bands due to the vibrations of the molecular skeleton.

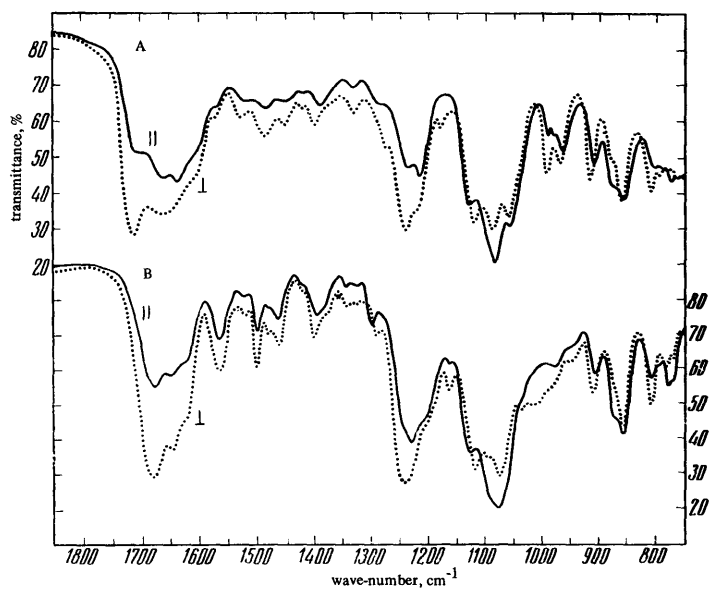


Figure 5. Polarised infrared spectrum of a film of the sodium salt of dwarf rice virus RNA in the H- and D-forms (A and B respectively) for a 75% relative moisture content[27].

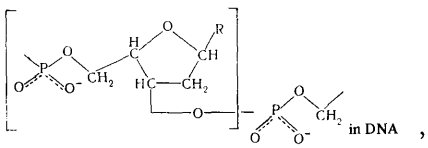
This suggests that both chains in the double helix have identical vibrational spectra and for the analysis of the latter it is sufficient to consider the vibrations of a single chain, formally assuming them to be doubly degenerate.

Summarising the foregoing, one may conclude that the expected vibration spectrum of nucleic acids as polymeric formations is obtained when a single chain of the double helix with a regular structure of the skeleton and a random sequence of the bases is considered.

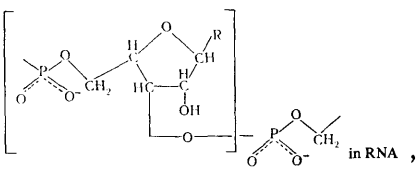
Let us consider the expected vibrational spectrum of the model

$$\underset{M}{\overset{\top}{R}\left[\overset{\top}{R}\right]\overset{\top}{R}},$$

where R is a point mass replacing any of the four bases and M is the monomeric unit which has the following structure:



M = 18 atoms (without R);



M = 19 atoms (without R).

Table 3. The characters, number of normal vibrations, and selection rules for the vibrations of the main DNA chain [rotational axis $C(\pi/5)$].

$C(\pi/5)$	E	C^1	C^2	$C^3 \ldots C^9$	N	Activity	
						i.r.	Raman
A	1	1	1	1...1	3·18—2=52 (T_π, R_π)	act. (∥)	forb.
B	1	—1	1	—1...—1	3·18=54	forb.	act.
E_1	2	$\varepsilon^{\pm1}$	$\varepsilon^{\pm2}$	$\varepsilon^{\pm3} \ldots \varepsilon^{\pm9}$	3·18—1=53 (T_σ)	act. (⊥)	»
E_2	2	$\varepsilon^{\pm2}$	$\varepsilon^{\pm4}$	$\varepsilon^{\pm6} \ldots \varepsilon^{\pm18}$	3·18=54	forb.	»
E_3	2	$\varepsilon^{\pm3}$	$\varepsilon^{\pm6}$	$\varepsilon^{\pm9} \ldots \varepsilon^{\pm27}$	54	»	forb.
E_4	2	$\varepsilon^{\pm4}$	$\varepsilon^{\pm8}$	$\varepsilon^{\pm12} \ldots \varepsilon^{\pm36}$	54	»	»

Note. i.r.—infrared spectrum; N—number of normal vibrations; act.—active vibrations; forb.—forbidden vibrations; T—pure translations of the entire chain parallel (π) and at right angles (σ) to its axis; R_π—rotation of the chain as a whole about the corresponding axis; C^K—rotation by an angle $K\omega$; $\omega = 2\pi/10$; $\epsilon = \exp[i(2\pi/10)]$.

This model probably constitutes a satisfactory first approximation, since here only those skeletal vibrations which are appreciably "mixed" with the vibrations of the bases are distorted.

Assuming the chains of the model to be infinitely long and regular macromolecules, the structure of which may be formulated as $-[M_i]_\infty-$, where M_i is a repeating chain unit, we can derive the general form of the vibrational spectra of such systems. The structure of model chains of this kind belongs to a one-dimensional space group with a $C(\pi/5)$ rotational axis, which is isomorphous with the C_5 point group [61,62]. Table 3 lists the characters, the number of normal vibrations, and the selection rules for the main DNA chain. For viral RNA containing 10 nucleotides per turn of the helix, the factor group remains the same and the characteristics of the spectra are as follows

M=20 (including R)
A=3·20—2=58 (∥) (i.r.)
B=60 (Raman)
E_1 = 59 (⊥) (i.r., Raman)
E_2=60 (Raman)
E_3 = 60, E_4 = 60 } forbidden (i.r., Raman)

The double helix of poly-A has a $C(\pi/2)$ rotational axis, M = 32 (chain unit together with adenine), and the following normal vibrations:

A=3·32—2=94
B=96
E_1=95
E_2=96

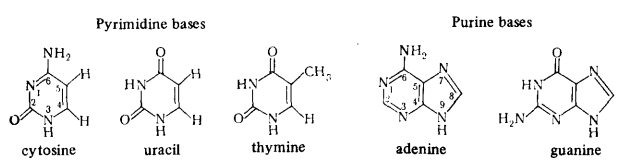
Examination of this model shows immediately that the total number of bands in the real infrared spectra of nucleic acids is less than expected. This finding may be due to several causes: (1) the low intensity of a number of bands; (2) the existence of numerous bands below 400 cm^{-1}; (3) the irregular structure of the molecules, which leads to a broadening of the bands and a pseudo-simplification of the overall pattern.

IV. VIBRATIONS OF NUCLEIC ACID BASES

As stated above, the theoretical calculation of the normal vibrations of nucleic acid bases is difficult owing to the complexity of their structures and very few studies have been made on this problem. Kanaskova [63] calculated the frequencies and the forms of the normal vibrations of free pyrimidine, imidazole, and purine on the basis of the valence force fields of these molecules.

1. Spectra of the Free Bases

The nucleic acid bases are aminoketo-derivatives of purine and pyrimidine—heterocycles containing ring nitrogen atoms.



The problem of the keto–enol and amine–imine tautomerism of the bases in the solid phase was already solved by infrared spectroscopy in the early investigations [29,64-73]. The molecules of the bases lack elements of symmetry and according to crystallographic data [74-81] have a planar configuration. The principal characteristics of the crystals are presented in Table 4.

According to the selection rules [62], all 3N-6 normal vibrations should be active in the infrared spectra of the free nucleic acid bases (30 for uracil, 33 for cytosine, 36 for adenine, 39 for thymine, and 42 for guanine).

The infrared spectra of nucleic acid bases, nucleosides, (base + sugar), and nucleotides (nucleoside phosphates), which are components of the DNA and RNA polynucleotide chains, have been quoted in a number of studies [24,30,32,33,70-72,82-96], where the absorption bands were also assigned.

In the majority of the investigations measurements were made of the spectra of these compounds in the solid phase (potassium bromide discs or mulls) in the light or deuterated forms in the range 4000–700 cm^{-1}, the spectra of the D_2O solutions having been obtained in only a few cases [14,31,35,97-101] over a narrow range (1700–1500 cm^{-1}). The spectra of the bases and nucleosides in the solid phase consist as a rule of narrow well resolved bands (Figs. 6 and 7).

Table 4. Crystallographic characteristics of the nucleic acid bases [74-77,81].

Base	C	U	T	A	G
Cell	orthorhombic	monoclinic	—	monoclinic	monoclinic
Space group	$P_{2_12_12_1}$	P_{2_1}	$P_{2_1/C}$	P_{2_1}	P_{2_1}
No. of molecules in cell	4	4	4	2	2

For the isolated nucleic acid bases, one may expect the occurrence of intense bands due to the stretching vibrations of NH, NH_2, and CH groups in the range 3500 to 2000 cm^{-1} and intense vibration bands of the carbonyl groups, moderately intense bands of the double bond vibrations in the rings of the bases, and deformation vibration bands of the NH and NH_2 group in the range 1800 to 1500 cm^{-1}; in the range 1500–1000 cm^{-1}, one should expect bands of moderate and low intensity associated with the vibrations of the single C–C and C–N bonds, the deformation vibrations of the C–H and N–H bonds, and the skeletal vibrations of the bases; in the range 1000 to 700 cm^{-1}, there should be weak bands due to the out-of-plane deformation vibrations of the NH and CH groups. The infrared spectra of nucleic acid bases are presented in Figs. 6–8; the positions and assignments of the fundamental absorption bands are given in Tables 2, 5, and 6.

The spectrum of cytosine (Fig. 7) shows intense stretching vibration bands of the amino-group at 3367 and 3155 cm^{-1}, an N–H stretching vibration band at 2778 cm^{-1}, which is displaced on deuteration towards lower frequencies, and moderately intense bands associated with the stretching vibrations of the C–H bonds at 3081 and 2948 cm^{-1}, the positions of which are unaffected by deuteration [29,30,32,104]. Indeed, the rate of isotope exchange involving the hydrogen atoms of the DNA bases has been investigated by infrared spectroscopy and other methods and it has been shown that the H atoms in the NH and NH_2 groups are exchanged almost instantaneously, while the exchange in the CH groups is very slow [17,18,103,105-116]. The stretching vibration frequencies of the amino- and imino-groups of the bases show that in both solutions and crystals there are hydrogen bonds between the bases [76,79-81].

The stretching vibration bands of the double bonds of the bases can be conveniently observed in the spectra of the deuterated forms (Fig. 8), since the NH_2 and NH deformation vibration bands, which overlap them, are then displaced towards longer wavelengths. At the same time there is also a bathochromic shift of the band due to the double bonds, which reflects the occurrence of an interaction between the deformation vibrations of the NH and NH_2 groups on the one hand and the vibrations of the rings double bonds and the C=O groups on the other [10,31]. This type of shift is observed, for example, in the spectrum of a D_2O solution of cytosine for the bands due to the $C_{(6)}$=N and C=C bonds adjoining the amino-group (Table 5). The C=C vibrations and the NH_2 deformation vibrations in the light form of cytosine in the solid phase give rise to two bands in the form of a doublet (1655 and 1667 cm^{-1} respectively). The wave-numbers corresponding to the double bond vibration frequencies of cytosine (1655 cm^{-1} for C=O, 1630 cm^{-1} for C=N, and 1621 cm^{-1} for C=C) are reduced, probably owing to conjugation.

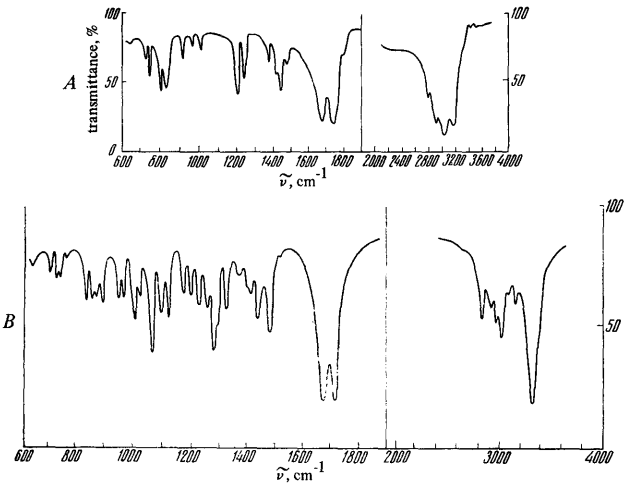


Figure 6. Infrared spectra of solid thymine (A) and thymidine (B) in a neutral medium.

The cytosine ring vibrations are reflected in the spectrum by bands at 1527 and 1365 cm^{-1}, close to the characteristic absorption bands of the pyrimidine ring (1565, 1462, and 1397 cm^{-1}) [89], which have not been assigned to the vibrations of the individual bonds in the ring. These bands and also those at 1236 and 794 cm^{-1} are active in the Raman spectra of cytosine [103].

Substituents in the N_3 position of pyrimidines and the $N_{(9)}$ position of purines in general influence the vibration frequencies of the bases. However, the addition of deoxyribose or ribose leads to only insignificant shifts in the bases. The spectra of nucleosides are complicated owing to the appearance of the sugar absorption bands: bands due to the stretching vibrations of the OH and CH bonds in the range 3700–2000 cm^{-1}, the deformation vibrations of the hydroxy-groups in the range 1350–1110 cm^{-1}, and the vibrations of the ribose ring in the range 1110–900 cm^{-1} (Fig. 6) [10,29,32]. The characteristic bands of the phosphate group (1220 and 1089 cm^{-1}) appear in the spectra of nucleotides and in the case of cytidylic and adenylic acids there are very significant displacements of the bands of the bases owing to the formation of zwitter-ions (a negative charge at the phosphate group and a positive charge at the base) [29,32].

Uracil and thymine, which have similar molecular structures, also have similar infrared spectra (Figs. 6 and 7). Each base has two carbonyl groups, the C_6=O group conjugated with the C=C bond and the $C_{(2)}$=O group

Table 5. Absorption bands of the free bases in infrared and Raman spectra.

Cytosine							Uracil							Thymine	
i.r.[10, 29–31, 33, 35, 82, 97, 98]			Raman [103]				i.r.[10, 29, 31, 33, 34]			Raman [103]				i.r.[14, 33]	
solid phase	solid phase after deuteration	D_2O* pD 7	solid phase	H_2O pH 7	D_2O pD 7	assignment	solid phase	solid phase after deuteration	D_2O* pD 7	solid phase	H_2O pH 7	D_2O pD 7	assignment	solid phase	assignment
3367 s.	3078 m.	—	—	—	—	$\nu_{as}\,NH_2$	3110 s.	3065 m.	—	—	—	—	$\nu\,N_3H$	3175 s.	ν NH
	2940 m.														
3115 s.	2525 s.	—	—	—	—	$\nu_s\,NH_2$	3100 s.	2400 w.					ν CH	3035 s.	$\nu_{as}\,CH_3$
3081 m.															
2948 m.	2320 s.					ν CH	2924 s.	2268 s.					$\nu\,N_1H$	2900 s.	$\nu_s\,CH_3$
2778 m.	2155 s.					ν NH		2135 s.		1710 w.				2780 m.	ν CH
							1724 s.	1712 s.	1690 s.	1662 m.	1680 m.	1690 m.	ν C=O	1735 s.	ν C=O
1667 s.	1680 w.					$\delta\,NH_2$	1680 m.	1690 s.	1655 s.	1655 m.	1640 m.	1650 m.	ν C=O	1670 s.	ν C=O
1655 m.		1652 s.	1660 m.	1660 w.	1650 w.	ν C=O	1666 s.	1640 s.					δ NH		
1630 m.	1637 s.	1615 m.	1635 w.		1610 m.	ν C=N	1623 w.	1630 w.	1620 w.	1620 w.		1633 w.	ν C=C	1640 m.	ν C=C
1621 w.	1605 s.	1582 w.				ν C=C									
								1565 w.	1582 w.	1580 w.				1570 m.	δ NH
1595 m.			1575 w.						1527 s.					1540 m.	
1538 m.	1548 w.					$\delta\,N_3H$	1508 m.	1475 s.	1504 s.			1500 w.		1500 m.	$\delta\,CH_3$
1527 w.		1527 w.	1540 w.	1530 w.	1510 w.	ring	1451 s.	1475 s.		1460 w.			ring	1380 w.	$\beta\,CH_3$
1506 w.	1502 s.	1504 s.	1500 w.			»									
1458 s.				1440 w.		$\beta\,NH_2$	1418 s.	1422 w.		1423 w.	1425 w.		β NH	1245 m.	ν C—CH_3
1362 s.	1374 s.	1365	1366 w.	1365 w.	1388 w.	ring	1389 s.	1390 w.			1390 w.		β NH	1200 m.	
1275 s.	1279 m.		1278 s.	1290 s.	1290 s.	ν C—NH_2	1238 s.	1230 w.		1237 s.	1235 s.	1270 s.	ring	1040 w.	ν C—C
1236 w.	1235 w.		1255 w.	1228 w.		ring	1099 w.	1140 s.		1105 w.		1158 w.	ν C—N	1000 w.	
1156 w.	1178 w.		1125 w.		1140 w.	ν C—N	1004 s.			1011 w.				945 w.	
							994 s.	980 s.		987 w.				900 w.	
1011 w.	1046 w.		1010 w.				851 m.	882 m.						835 m.	} β NH
995 w.	978 w.		965 w.	975 w.	980 w.	ν C—C	822 s.	820 s.				815 w.	β NH	800 m.	
823 m.			820 w.			$\beta\,N_3H$	780 w.			781 s.		783 m.	ring	740 m.	
794 s.	787 s.		794 s.	787 s.	777 s.	ring	760 m.						β NH	705 w.	
784 w.	771 w.					β CH		733 w.		580 w.					

*Nucleoside.

Table 6. Absorption bands of the free bases in infrared and Raman spectra.

Adenine							Guanine					
i.r.[10, 29, 31, 32, 104]			Raman [103]				i.r.[10, 29, 31, 32]			Raman [103]		
solid phase	solid phase after deuteration	D_2O* pD 7	solid phase	H_2O pH 7	D_2O pD 7	assignment	solid phase	solid phase after deuteration	D_2O* pD 0,5	H_2O pH 0,5	D_2O pD 0,5	assignment
3280 m.						$\nu_{as}\,NH_2$	3310 s.	3300 s.				$\nu_{as}\,NH_2$
3105 s.	3096 w.					$\nu_s\,NH_2$	3077 s.	3086 s.				$\nu_s\,NH_2$
3000 w.	2463 m.					ν NH	2840 s.	2872 m.				ν NH
								2688 m.				
2780 w.	2288 s.					ν CH	2680 s.	2494 m.				ν CH
								2278 s.				
2680 w.	2155 m.						1701 s.	1695 s.	1690 s.	1685 w.	1680 w.	ν C=O
1672 s.	1675 w.					$\delta\,NH_2$	1681 s.	1678 w.				$\delta\,NH_2$
1605 m.	1610 s.	1627 s.	1600 w.		1613 w.	ν C=N	1639 w.	1616 s.	1609 m.			ν C=N
1578 m.	1575 s.	1578 m.		1585 m.	1563 m.	ν C=C	1569 m.	1565 m.	1578 s.			ν C=C
1510 w.	1510 s.		1530 w.	1512 m.	1512 m.	ring	1555 m.		1568 w.	1541 w.	1525 w.	δ NH
	1475 m.	1484 w.	1485 m.	1490 w.	1480		1477 m.	1475 m.		1489 s.	1480 s.	ring
1451 m.	1460 m.		1462 w.			$\beta\,NH_2$	1464 m.	1460 m.				δ CH
1418 m.	1420 m.			1425 w.	1435 s.	ring	1418 m.	1418 w.		1412 w.	1400 w.	$\beta\,NH_2$
1368 w.	1375 m.		1370 w.	1380 m.	1350 m.	»	1375 s.	1364 s.		1372 m.	1360 w.	ring
1333 m.	1335 s.		1332 s.	1340 s.	1328 s.	»	1263 s.	1261 w.		1240 w.		νC—NH_2
1305 m.	1310 s.		1312 w.	1310 m.			1215 w.	1215 w.				
1250 m.	1250 w.		1252 m.	1253 w.	1238 w.	β NH	1172 s.	1167 m.		1175 w.	1176 w.	β NH
1215 m.	1226 w.		1225 w.	1216 w.		ν C—NH_2	949 s.	948 w.		1080 w.		$\beta\,NH_2$
1124 m.	1130 w.		1130 w.			ν C—N	877 m.	876 w.		1025 w.		β NH
1022 m.	1010 w.		1025 w.	1010 w.		$\beta\,NH_2$	851 s.	850 m.				
							789 w.					β CH
938 m.	935 m.		945 m.			ν C—C	776 s.	776 m.				β NH
870 w.	855 w.		870 w.	870 w.		$\beta\,N_9H$	703 m.					β CH
847 m.							689 m.	687 w.				β NH
797 m.	796 w.			800 w.		$\beta\,NH_2$						
722 m.	720 w.		725 m.	733 m.	724 w.	ring						

*Nucleoside.

located between the imino-groups. The effect of the environment on the carbonyl groups in both positions should lead to a lowering of their vibration frequencies compared with the characteristic vibration frequencies of the carbonyl group in ketones (in the range 1725–1705 cm^{-1}). Indeed the vibration frequency of the C=O group in imidazolone corresponds to 1684 cm^{-1}.[108] However, two bands at 1735 and 1670 cm^{-1} appear in the spectrum of crystalline uracil[29,32,33], the wave-number of the first being higher than expected. The 1670 cm^{-1} band has been assigned to the vibrations of the $C_{(6)}$=O group conjugated with the C=C bond and the 1730 cm^{-1} band has been assigned to the vibrations of the $C_{(2)}$=O group[29,31–33]. Probably there is an interaction between the carbonyl groups in the uracil and thymine molecules of the type known for dicarbonyl compounds, which leads to a displacement of the vibration frequency of one of the groups towards higher values[109]. It is also possible that the increase of the vibration frequency of the $C_{(2)}$=O group is due to its combination with the N_1H bond vibrations.

Sukhorukov et al.[33] determined the multiplicity n and the length r of the bonds of the carbonyl groups of uracil from

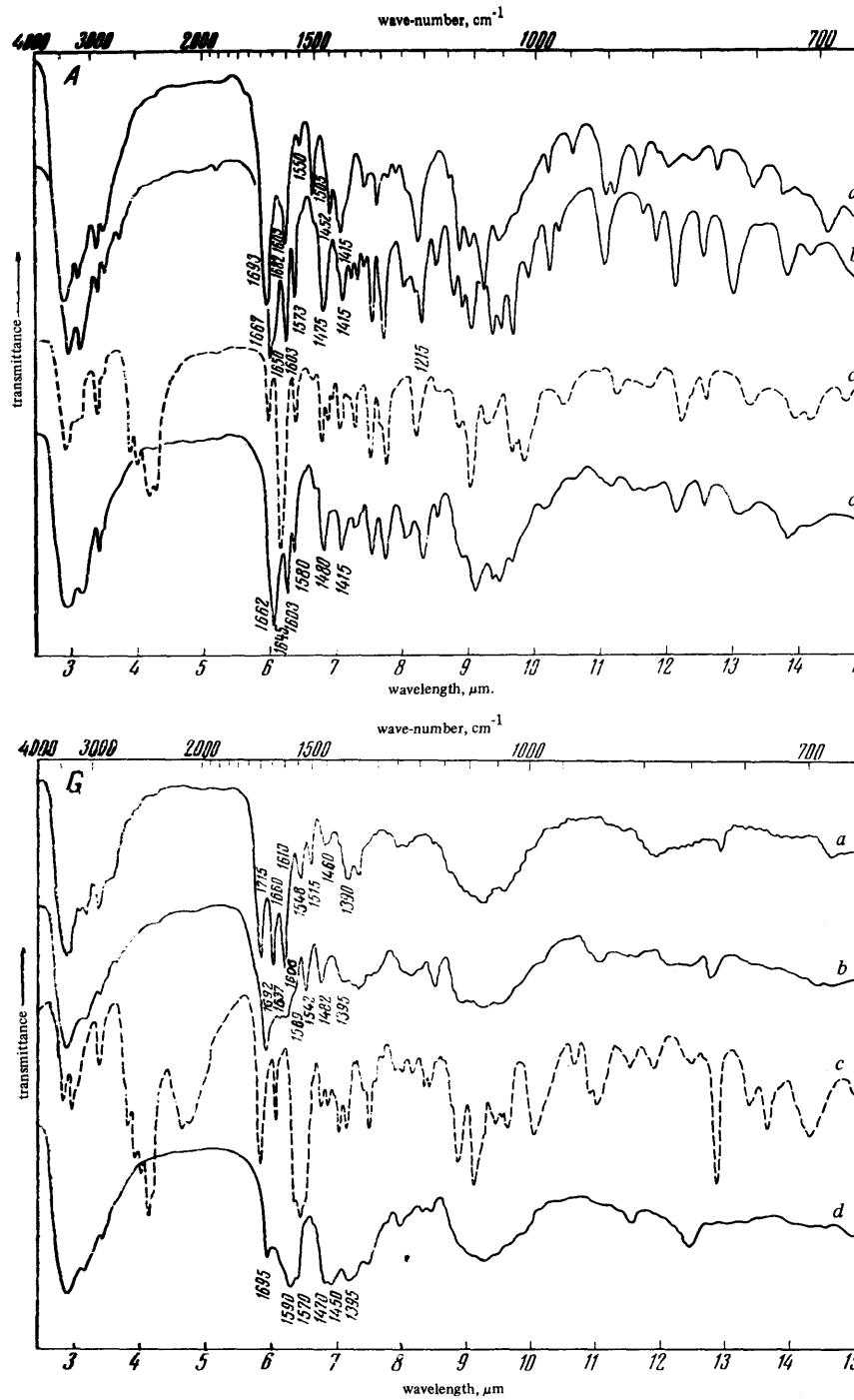


Figure 7. Infrared spectra of solid nucleosides[31]:
a) from an acid solution in H_2O; *b*) from a neutral solution in H_2O;

their vibration frequencies in the infrared spectrum and the empirical relations between these quantities quoted in the literature[110,111]. The vibration frequency of the $C_{(2)}$=O group (wave-number 1735 cm^{-1}) corresponds to $n = 2.07$ and $r = 1.225$ Å, while that of the $C_{(6)}$=O group (1670 cm^{-1}) corresponds to $n = 1.85$ and $r = 1.241$ Å; the values of r obtained agree satisfactorily with the X-ray diffraction data[80]. The intense 1451 and 1238 cm^{-1} bands and the weak 780 cm^{-1} bands have been assigned to the vibrations of the uracil rings coupled with deformation vibrations of the NH bonds.

The spectrum of thymine differs from that of uracil mainly by the presence of bands corresponding to the stretching and deformation vibrations of the methyl group.

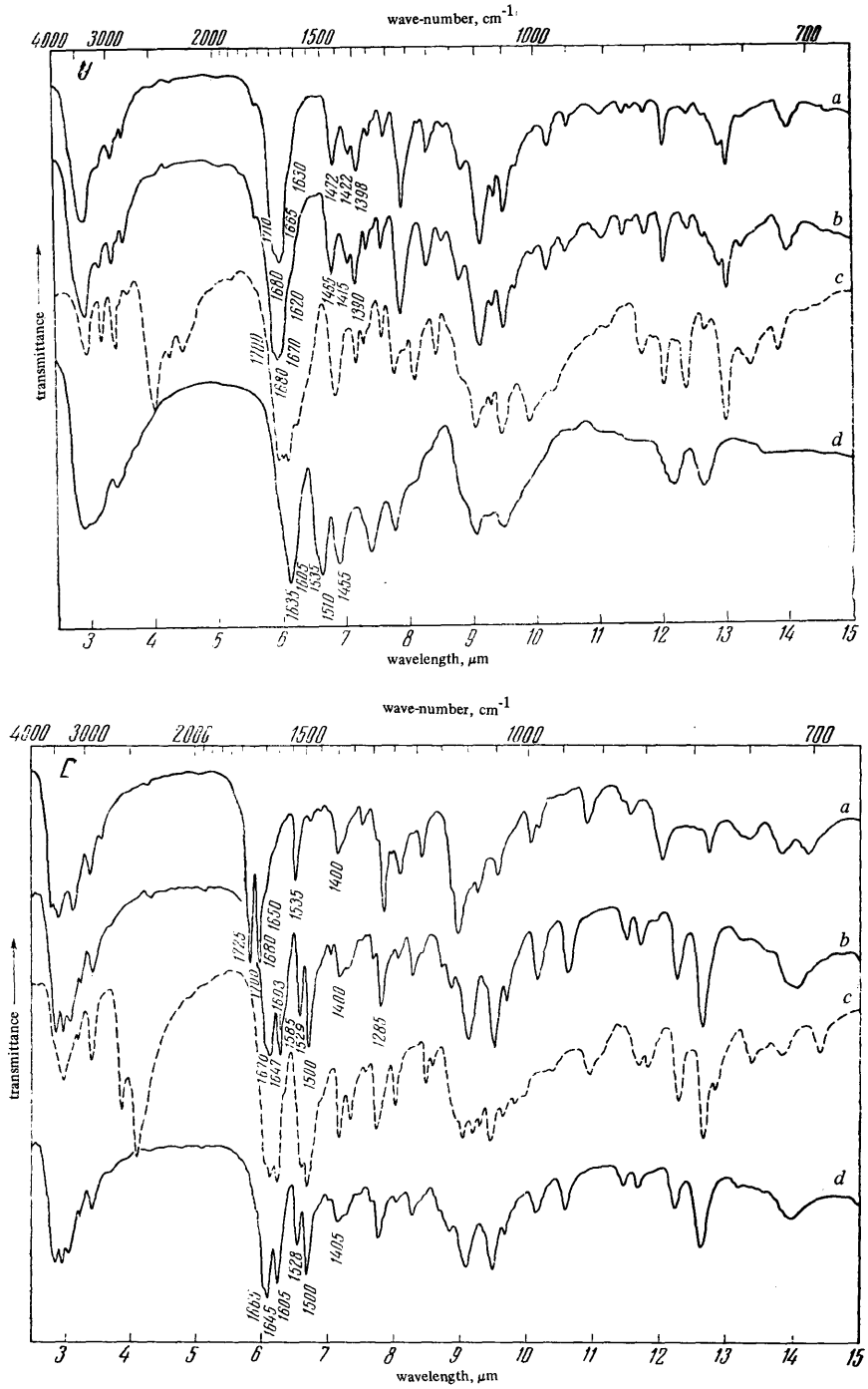


Figure 7 continued. Infrared spectra of solid nucleosides[31]:
c) from a neutral solution in D_2O; *d*) from an alkaline solution in H_2O.

In the low-frequency part of the spectrum (700 to 200 cm^{-1}), polysubstituted pyrimidines have two intense absorption bands at 525 ± 25 and 450 ± 25 cm^{-1}, corresponding to the out-of-plane and in-plane deformation vibrations of the pyrimidine ring[111-115]. The 450 cm^{-1} band can serve for identification, since its position is not affected by substituents. The position of the band due to the out-of-plane vibrations of the pyrimidine ring (525 ± 25 cm^{-1}) depends on the mass of the substituent (NH_2, OH, SH, and S–CH_3 groups), the relation being linear[116].

Guanine has a structure similar to that of cytosine. However, the vibration frequency of its carbonyl group

(wave-number 1700 cm^{-1}) is much higher than in cytosine (1655 cm^{-1}) and higher than the expected vibration frequency of the C=O group conjugated with the C=C double bond. Intense bands characteristic of the purine ring have been observed in the guanine spectrum[88]: 1639 (C=N), 1567 (C=C), 1518, 1477, and 1375 cm^{-1}.[29,31,32] The deuteration of guanine is accompanied by a slight decrease of the C=O and C=N bond frequencies, which may be evidence of a slight interaction of their vibrations with those of the NH and NH_2 groups.

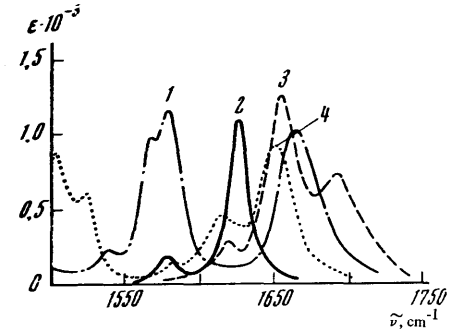


Figure 8. Infrared spectra of D_2O solutions of nucleotides: 1) guanine; 2) adenine; 3) uracil; 4) cytosine; pD 7 (Thomas[102]); ϵ—molar extinction coefficient.

Adenine is the only base without a carbonyl group. Its amino-group gives rise to intense bands at 3280 and 3105 cm^{-1} (ν) and at 1672, 1451, 1022, and 797 cm^{-1} ($\beta + \delta$).[29,32,65] The vibrations of the amino-group interact strongly with those of the adjacent C=N bonds, as a result of which the wave-number of the latter increases on deuteration of adenine from 1605 to 1627 cm^{-1}.[59] Since the structure of adenine differs from that of purine only by the presence of a single substituent, its spectrum shows six characteristic bands due to the skeletal vibrations of purine: at 1605 (C=N), 1575 (C=C), 1510, 1458, 1418, and 1368 cm^{-1}.[10,29,32,97] The C=C bond stretching vibration band usually has a low intensity in infrared spectra, but in the adenine spectrum it is fairly intense, possibly owing to the aromatic nature of the ring of this base.

In a study of the effect of temperature on the infrared spectra of the crystalline bases, Sukhorukov, Kanaskova, Pentin, and coworkers[33,63,117,118] observed that, in the spectra of imidazole, purine, and a number of their derivatives in the range between −160° and 100°C, the bands due to the out-of-plane NH and NH_2 deformation vibrations are the most sensitive to changes in temperature. It was shown that the temperature variation of the frequencies of the out-of-plane vibrations is in all cases linear and may be described by the equation $\nu_{o.o.p.} = A - BT$, where A and B are constants characteristic of each compound. In this connection a method was proposed for the identification of the out-of-plane deformation vibration frequencies of the NH and NH_2 groups of nitrogeneous bases, called the temperature shift method[63,117]. The author used this method to identify a number of bands in the infrared spectra of adenine and its N-methyl derivatives. On the other hand, it was shown that the frequency shift of the proton-donating NH and NH_2 groups depends on the length of the hydrogen bonds (r) formed by them: $B = 0.93 - 0.29r$ (for NH) and $B = 0.83 - 0.27r$ (for NH_2). The temperature shift method proposed makes it possible to distinguish the NH and NH_2 deformation vibration bands and, when combined with other methods, allows a more reliable determination of the structure of the bases in the crystalline state.

The Raman spectra of the free bases and nucleosides in the solid phase and in solution were measured by Lord and Thomas[103]. The frequencies in this spectra are listed in Tables 5 and 6.

The NH and NH_2 deformation vibration bands in the Raman spectra are very weak or altogether absent, but the double bond and ring vibrations give rise to more intense bands than in the infrared spectra. Ribose and phosphate give rise to very weak Raman bands. In the Raman spectra the bands due to the carbonyl groups and those in the range 800 to 750 cm^{-1} in the spectra of pyrimidines and in the range 730–680 cm^{-1} in the spectra of purines are highly polarised. Bands polarised to a moderate extent are observed in the range 1500–1200 cm^{-1}. The depolarisation ratio is determined by the local planar symmetry of the ring of the base, the in-plane deformation vibrations being polarised and the out-of-plane vibrations depolarised.

2. The Spectra of the Nitrogeneous Bases in Polynucleotides

The main problem in the study of the infrared spectra of polynucleotides is the determination of the specific spectroscopic characteristic of the interaction of the bases forming part of nucleic acids, in contrast to those of the free bases.

When the vibration bands of the bases in polynucleotides and nucleic acids are considered, one should expect shifts of certain frequencies owing to the enhancement of the interaction between the complementary bases, since it is known that the specific interaction of such bases via hydrogen bonds is much stronger than their self-association (see Section VI). Moreover, the dispersion interaction between the π electrons of the bases in the vertical "piles" within the polynucleotide chain may make a definite contribution to the change in the vibration frequencies of the bases.

Assignments of the absorption bands due to the vibrations of specific groups in the bases within nucleic acids are available in the literature only for the stretching vibrations of the NH_2 (3350 and 3210 cm^{-1}), NH (2950 cm^{-1}), and CH (3090 cm^{-1}) groups[10,17-19]. The 1705 cm^{-1} band, characteristic of the spectra of native DNA and RNA preparations, which on deuteration shift towards lower frequencies down to 1685 cm^{-1}, has also been discussed. According to Shimanouchi and coworkers[10,38,39] and also Fritzsche[20], the 1705 cm^{-1} band is associated with the stretching vibrations of the carbonyl groups of the bases interacting with the deformation vibrations of the amino-groups. The frequency of this band is higher than those associated with the vibrations of the carbonyl groups of the free bases (with the exception of one of the groups in thymine and uracil). The increase of the C=O vibration frequency of the bases forming part of nucleic acids is probably due to their involvement in hydrogen bonds between the complementary bases.

There are virtually no assignments in the literature for the remaining absorption bands of the bases in nucleic acids. On the basis of published experimental data, we attempted to make a more detailed assignment of the bands

of the bases in DNA. However, for the majority of the bands in the range 1400–600 cm^{-1} such refinement can be regarded as merely conventional. The results are presented in Table 2. Analysis of the data in Table 2 reveals certain regularities. Thus the position of the C=N band in the DNA spectrum is very close to the positions in the corresponding spectra of the individual bases.

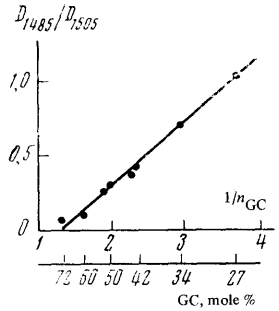


Figure 9. Variation of the ratio of the optical densities of the 1485 and 1505 cm^{-1} bands of deuterated DNA films with $1/n_{GC}$ (n_{GC} = mole fraction of G-C pairs in DNA) [22].

The vibration frequencies of the C=C bonds in the DNA spectrum and in the spectra of free guanine and adenine are almost identical, while in the spectra of free pyrimidines they are somewhat higher. However, the reasons for this effect are so far obscure. The bands due to the in-plane deformation vibrations of the NH_2 and NH groups of the bases are displaced in the DNA spectrum towards lower frequencies compared with the corresponding bands in the spectra of the free bases, which may be caused, as for the C=O groups, by the involvement of the hydrogen atoms of the NH_2 and NH groups in hydrogen bonds. In the remaining parts of the spectrum the vibration frequencies of the bases in nucleic acids are the same as those of the free bases, within the expected limits.

Fritzsche [21,22], who studied the infrared spectra of DNA specimens with different base compositions in the range 1800–1200 cm^{-1}, observed a quantitative linear relation between the intensity ratio of the 1505 and 1485 cm^{-1} bands, which are characteristic of the absorption by guanine and adenine respectively, and the percentage content of the G-C pairs (Fig. 9). He suggested that the infrared spectra of DNA be used for analytical purposes—in order to determine the content of the G-C pairs. In a further development of studies on these lines, Tsuboi et al. [23] measured the infrared spectra of DNA from different species of bacteria with different ratios of the G-C (guanine–cytosine) and A-T (adenine-thymine) base pairs and observed a correlation between the contents of the G-C and A-T pairs and the intensity of the majority of bands in the spectrum. On the basis of these data, they found spectroscopic features characteristic of the presence of the AT and GC base pairs in DNA (listed in Table 7).

Thus the infrared spectrum of DNA does indeed show shifts of certain absorption bands compared with the corresponding bands in the spectra of the free bases and also there are a number of bands characteristic of the AT and GC base pairs.

3. Effect of pH on the Spectra of the Bases

The nucleic acid bases can exist in different ionic forms, since in their molecules there are several sites for proton addition. However, in practice relatively few tautomeric forms are observed, because in many cases protonation is unfavourable on energy grounds. One of the methods for the determination of the site of addition or abstraction of a proton is infrared spectroscopy of the ionic forms of the bases.

Table 7. Absorption bands of DNA bases (92% relative moisture content).

G-C pairs	Deuterated G-C pairs	A-T pairs	Deuterated A-T pairs
1705 s.	1676 s.	1705 s.	1687 s.
			1659 s.
	1648 s.	1636 m.	1638 m.
1609 m.	1623 m.	1605 m.	1618 s.
1576 w.	1576 s.	1574 w.	1571 m.
	1557 m.		
	1537 w.		
1528 m.	1523 w.		
1493 m.	1501 m.	1503 w.	1516 w.
		1477 m.	1476 m.
1456 w.	1453 w.	1450 m.	1458 w.
1444 w.	1439 w.		
1414 w.			
1380 m.	1377 w.	1375 w.	1375 w.
1331 w.	1350 w.	1331 w.	1334 w.
1296 m.	1302 w.	1292 w.	1300 w.
		1276 w.	1153 w.
		932 m.	
893 m.		888 m.	
860 w.	851 w.		
	831 m.	831 m.	834 m.
	797 w.	795 w.	794 w.
778 m.	778 s.	763 w.	773 m.
	727 w.	722 w.	715 w.
	698 w.		

The studies by Sukhorukov [30,32,33,119] and Angell [29] (solid phase), Tsuboi and Shimanouchi [10,31] (solid phase and D_2O solutions), and Sinsheimer et al. [35] (D_2O solutions) were devoted to the elucidation of this problem. The studies showed that, for all the nucleic acid bases, the formation of cations involves a shift of the double bond vibration frequencies towards higher values and the formation of an anion causes a shift towards lower frequencies in both infrared and Raman spectra [31–33,53].

The infrared spectrum of cytidine ($pK = 4.5$) [120] in an acid medium differs considerably from its spectra in neutral and alkaline media (Fig. 10).

The spectrum of protonated cytosine is characterised by an increase of the wave-numbers associated with the C=O bond vibrations (from 1655 to 1680 cm^{-1}) and the vibrations of the NH_2 group (from 1667 to 1725 cm^{-1}). Cytosine may be protonated in two ways: at the amino-group and at the N_1 ring atom. The shift of the absorption bands due to the multiple bonds towards higher frequencies indicates an increase of the multiplicity of the C=O and C=N bonds which is more likely to occur as a result of loss of conjugation following the addition of a proton to the N_1 atom rather than to the amino-group. An analogous shift of the absorption frequencies of the ring double bonds is observed on protonation of pyridine ($C_5H_5NH^+$) but not aniline ($C_6H_5-NH_3^+$). [10,31,32]

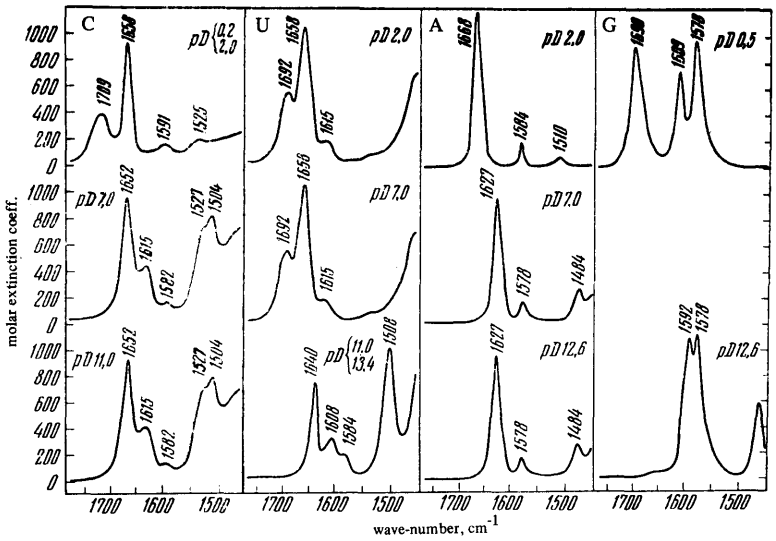


Figure 10. Infrared spectra of D_2O solutions of nucleosides at different pD. [31,100]

A decrease of the intensity of the NH stretching vibration band and a decrease of the frequency of the C=O stretching vibrations (the wave-number decreases from 1680 to 1638 cm^{-1}) are observed in the infrared spectrum of uracil in an alkaline medium ($pK = 9.5$), which is due to the abstraction of a proton from the $N_{(1)}$ or $N_{(3)}$ ring atom and the delocalisation of the electron over the system of conjugated double bonds [31,32]. An empirical relation between the frequency of the stretching vibrations of the carbonyl group and the ionisation potential I, [116]

$$\nu_{C=O} = 29.2\,I + 1409,$$

yields ionisation potentials of 9 and 11 eV for frequencies corresponding to wave-numbers of 1670 and 1735 cm^{-1} respectively; the ionisation potential of the ionic form of uracil is reduced to 7.8 eV; [33] however, all these data require confirmation by independent methods.

In highly alkaline solutions of thymine ($pK = 9.8$), one of the carbonyl groups is enolised and the corresponding band disappears from its infrared spectrum. The remaining bands are displaced towards lower wave-numbers (1735, 1670, 1640, and 1570 cm^{-1} bands in a neutral medium and 1650, 1600, and 1580 cm^{-1} bands in an alkaline medium) [14,98].

The formation of the adenine anion in an alkaline medium ($pK_2 = 9.8$) is accompanied by the disappearance from its infrared spectrum of the band associated with the $N_{(9)}H$ bond, which indicates unambiguously the site of abstraction of the proton from the imidazole ring of adenine [29,32]. In infrared spectra of protonated adenine ($pK_1 = 4.1$), in both the solid phase and in solution, the frequencies of the ring vibrations are enhanced, including the vibrations of the $N_{(1)}=C_{(6)}$ bond (the wave-number increases from 1626 to 1668 cm^{-1} in D_2O solution) [32]. This is most likely to occur as a result of the addition of a proton to the ring $N_{(1)}$ atom rather than to the amino-group. Indeed, it has been shown by X-ray diffraction that in crystalline adenine hydrochloride the proton is located at the ring $N_{(1)}$ atom [121].

As for the remaining bases, the protonation of guanine ($pK_1 = 1.6$) is accompanied by a general shift of the absorption bands in the range 1700–1400 cm^{-1} towards higher frequencies, including the NH_2 deformation vibration band (from 1681 to 1718 cm^{-1}) and resembles the frequency shift on protonation of pyridine (Fig. 10). [12,13,31,32,101] According to crystallographic [75] and NMR [122] data and Pullman's calculations [123], guanine is protonated at the ring $N_{(7)}$ atom and infrared spectroscopic data [31,101,124] are consistent with this postulate. A shift of pH to the alkaline region lowers the intensity of the absorption band due to the guanine carbonyl group ($pK_2 = 9.4$) and gives rise to new absorption bands at 1638 and 1413 cm^{-1}. [31,101] The spectrum of an alkaline solution of guanine resembles that of 2-amino-6-methoxypurine. Probably these changes in the infrared spectrum of guanine are caused by the abstraction of a proton and a redistribution of the charge, resulting in a decrease of the multiplicity of the C=O bond and in increase of electron density at the guanine oxygen atom [31].

The purine and pyrimidine bases may exist in solution in various tautomeric forms, and a different form may predominate than in the solid phase. Miles and coworkers [31,97–101,109] solved this problem by comparing the infrared spectra of D_2O solutions of the bases with those of solutions of model compounds, in which a particular structural element is fixed. For example, *N*-dimethylcytosine and 1-methyl-2-oxopyrimidine served as model compounds for cytosine and 1,9-dimethylguanine and 2-amino-6-methoxypurine were used for guanine. Thus it was shown that the aminoketo-form predominates in cytosine and guanine solutions, the amino-form in adenine solutions, and the diketo-form in uracil and thymine solutions, this being confirmed by NMR data [125].

Thus the study of the spectroscopic changes in the infrared region as the pH of the medium is varied makes it possible to determine the site of abstraction or addition of the proton in purine and pyrimidine bases. Measurements of the infrared spectra of solutions of the bases also make it possible to establish the dominant tautomeric form of the base.

V. INFRARED SPECTROSCOPIC STUDY OF THE SECONDARY STRUCTURE OF NUCLEIC ACIDS

According to the model of Watson and Crick[4,5], the secondary DNA structure consists of a double helix with antiparallel chains (stable in both the crystalline state and in solution[126,127]) stabilised by hydrogen bonds as well as van der Waals forces. The disruption of the DNA helix and the separation of its chains (denaturation) occur both on heating and under the action of acids and alkalies.

The study of the secondary structures of DNA and RNA is one of the most important tasks in molecular biology. In the study of the secondary structure of nucleic acids, X-ray diffraction has hitherto played the main role and infrared spectroscopy an auxiliary role. However, in the study of DNA and RNA structures in solutions, the infrared spectroscopic method has many advantages and yields important information when combined with other methods—hydrodynamic and optical methods and low-angle X-ray scattering[126-129].

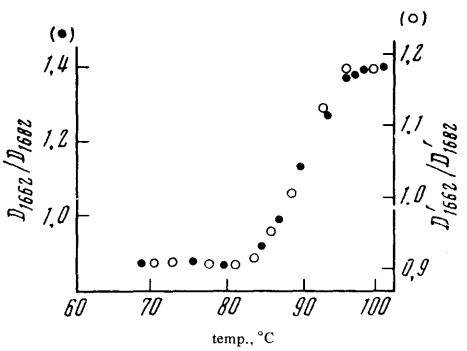


Figure 11. Temperature variation of the ratio of the optical densities of the 1662 and 1682 cm^{-1} bands of D_2O solutions of DNA; 10 mg ml^{-1} of DNA in 0.15 M KCl.[20]

In the infrared spectra of native DNA (in solution or in a film), an intense absorption band, characteristic of its secondary structure, was found; it is located at 1705 cm^{-1} for the light form and at 1685 cm^{-1} for the deuterated form of DNA.[15,20,23,38,37,130] On denaturation, the band is displaced towards lower wave-numbers, for example, from 1685 to 1660 cm^{-1}. The fact that the 1705 (1685) cm^{-1} band is characteristic of the secondary DNA structure has been confirmed by a study of the effect of temperature on its intensity. The curve relating the relative intensities of the 1662 and 1682 cm^{-1} bands to temperature (Fig. 11) resembles the melting curve for DNA in measurements of ultraviolet absorption in the region of 260 nm.[20,39] It has been shown[131] that the temperature variation of the intensity of the maxima in the difference spectra of D_2O solutions of DNA, corresponding to the DNA absorption bands in the region 1700–1550 cm^{-1}, also agrees with the melting curve. This makes it possible to follow the "helix–coil" thermal transition on the basis of the vibrational spectra of DNA solutions and to determine fairly accurately the melting point of the DNA solution (T_{fu}) and the width of the transition (ΔT_{fu}). In contrast to measurements of T_{fu}, ultraviolet spectroscopy, or measurements of the viscosity of DNA solutions, the infrared spectroscopic method makes it possible to determine the melting points of concentrated and gel-like DNA solutions.

At present it is not yet clear why the above bands are sensitive to changes in the secondary DNA structure: whether they are due to changes in the interaction between the two chains or arise in consequence of a breakdown of regularity. However, there is no doubt about the practical usefulness of such data.

Classical X-ray diffraction studies on crystalline DNA specimens[58,59,132] showed that the DNA helix can have different conformations depending on the degree of hydration. The sodium salt of DNA can exist in the B-form, which is stable when the relative moisture content is in excess of 92%, and the A-form is stable for a 75% relative moisture content[58,59,132]. The lithium salt of DNA also has two conformations: the B-form for a relative moisture content in excess of 66% and the C-form for a relative moisture content in the range 44–66%.[139] Further decrease of the water content leads to a gradual disordering of the DNA structure. These conformations of the DNA helix differ from one another in their parameters and the orientation of the bases.

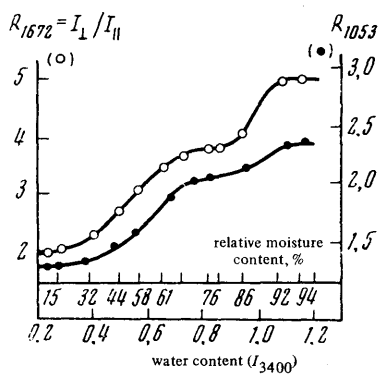


Figure 12. Variation of the dichroic ratios R_{1672} and R_{1053} with the water content in oriented DNA films[18].

The study of the infrared spectra of DNA confirmed and extended the X-ray diffraction data for the conformational transitions in DNA. Bradbury et al.[18] investigated the dependence of the polarisation of a number of absorption bands on the relative moisture content of oriented specimens of the sodium and lithium salts of DNA. A constant dichroic ratio R for the 1672 and 1053 cm^{-1} bands in the relative moisture content ranges corresponding to the stability of the B- and A-forms of DNA, namely above 90% and between 80 and 70%, has been observed for the sodium salt of DNA (Fig. 12). For the lithium salt of DNA, a plateau of this kind was observed on the curve relating R_{1670} to the relative moisture content in other ranges: above 66% and between 56 and 44% for the B- and C-forms respectively. It has been shown[18,134] that, for each conformation of the sodium salt of DNA, there is a characteristic frequency of the antisymmetric vibrations of the phosphate group (two plateaus on the frequency–moisture content curves for this band).

Sutherland and Tsuboi[17] and Falk et al.[19], who carried out similar investigations, also observed an increase of the dichroic ratio of the absorption bands due to the rings of the bases in the sodium salt of DNA with increasing water content in the specimen, but the curves are of a different type, which does not yield a clear proof of the interconversion of the A- and B-forms of DNA. Nevertheless these results show that an ordered helical DNA structure depends on the amount of adsorbed water and its partial dehydration leads to a considerable disorganisation of the molecular structure.

Quite recently Webb and Buther[135] succeeded in finding by the infrared spectroscopic method the structural characteristics of tumour DNA isolated from the tissue of Rous sarcoma and the mammary gland carcinoma in mice[135]. They observed that the tumour DNA is less hydrated for higher values of the relative moisture content and more hydrated for low values than DNA from normal tissues and that the dichroic ratio for tumour DNA is smaller than for normal DNA, remaining constant for relative moisture contents in the range 92–45%. They interpret the infrared spectroscopic data as evidence that the structure of the tumour DNA is intermediate between the B- and A-forms and does not alter for moderate degrees of dehydration.

The study of the vibrational structure of DNA spectra using polarised radiation yielded information about the orientation of its structural elements. Thus the knowledge of the dichroic ratio for the 1670 cm^{-1} band for relative moisture contents of 92 and 75% makes it possible to determine the orientation of the planes of the bases in the A-form of DNA on the hypothesis that in the B-form they are perpendicular to the axis of the helix. The results of such calculations show that in the A-form the planes of the bases deviate from the vertical orientation by 13–15°.[17,18] Measurement of the dichroism of the 1220 and 1089 cm^{-1} bands also permits the calculation of the orientation of the phosphate groups, since it is known that the transition moment of their antisymmetrical vibrations is directed along the O...O line and that of the symmetrical vibrations lies along the bisector of the OPO angle. It has been shown[17,18] that in both the A- and B-forms of DNA the O...O line is inclined to the axis of the spiral by 50–60° and the bisector of the OPO angle is perpendicular to the molecular axis. However, this conclusion is valid only when the form of the vibrations is highly characteristic. But, since the vibrations of the phosphate group are undoubtedly appreciably mixed with other skeletal vibrations, the angles of the orientation of this group in DNA must be assumed to be only approximate. The experimentally observed orientation of the PO_2^- groups in DNA contradicts the Watson–Crick model[4,5], according to which in the B-form of DNA the O...O line is perpendicular to the axis of the helix and the bisector of the OPO angle is parallel to it.

In order to investigate the hydration processes of DNA macromolecules, it is important to elucidate the sequence of the hydration of the structural elements of DNA (bases, deoxyribose, and phosphate groups), which can be achieved with the aid of infrared spectra, since changes in the relative moisture content involve shifts of the absorption bands associated with these structural elements. Thus an increase of the relative moisture content from 0 to 92% causes a shift of the carbonyl band of the DNA bases from 1705 to 1715 cm^{-1}, of the PO_2^- band from 1240 to 1220 cm^{-1}, and of the C–O band of deoxyribose from 1066 to 1053 cm^{-1}.[17,19] By studying the variation of the spectroscopic shifts with the moisture content of the DNA specimens, Falk et al.[19] established that the phosphate groups of DNA are hydrated when the relative moisture content is in the range 0–66% (six water molecules per phosphate group for a 66% relative moisture content), while above 66% the hydration of the bases begins. It has not yet been possible to elucidate the relation between the transition from the A-form to the B-form and the characteristics of the adsorption of water. The positions of the water bands in the infrared absorption spectrum of DNA show that all the adsorbed water layers except the first have a structure similar to that of liquid water[16,137]. Therefore the amount of water adsorbed by the DNA film may be calculated from the intensity of the 3400 cm^{-1} band, assuming that its molar extinction coefficient ϵ is the same as that for liquid water.

The secondary structure of RNA has been investigated much less than that of DNA, because RNA crystallises with difficulty and yields low-quality X-ray diffraction patterns. Nevertheless, it has been established by X-ray diffraction and other methods that RNA molecules consist of single coiled chains with an alternation of helical and non-helical sections[129,137]. The fraction of the A–U and G–C base pairs is different for different types of RNA. The infrared spectra of RNA films, like those of DNA, show a band at 1705 cm^{-1} (1680 cm^{-1} in D_2O), indicating the presence of paired bases in the RNA chain[38]. The variation of the intensity of this band as the temperature is raised from 22° to 90°C is represented by a gently sloping curve, resembling the melting curve of RNA in the ultraviolet region[39]. Infrared spectroscopy has been successfully employed to determine the secondary structure of RNA. The relative amounts of the A–U and G–C pairs have now been found by comparing the observed and calculated spectra for five varieties of transfer RNA: alanine, valine, methionine, formylmethionine, and tyrosine RNA[41,138,139] by the Thomas method[102] and for the ribosomal RNA of *E. coli* by the method of Cotter and Gratzer[42,43]. Both methods of calculation are based on the fact that the absorption by hydrogen-bonded bases differs from that of the free bases and on the hypothesis that the dispersion interaction between the bases in the vertical stacks does not affect the spectrum, although the latter postulate contradicts the data of Maleev and coworkers[140]. The Thomas method consists in the calculation of the RNA spectrum on the hypothesis that the RNA absorption is made up additively from the absorption by the A–U and G–C pairs and the four unpaired bases. The overall RNA spectrum is calculated from the absorption spectra of the four nucleotides and two helical polynucleotides: poly-(A/U) and poly-(G/C). The method of Cotter and Gratzer consists in the calculation of the difference spectrum of RNA (relative to the dialysate) with the aid of the analogous spectra of poly-(A/U) and poly-(G/C), which take into account only the contribution by the paired bases. An advantage of this method over the Thomas method is that of a greater simplicity of the calculation of the spectra, since it involves the analysis of a two-component and not a six-component system.

The data for the secondary structure of RNA obtained from infrared spectra are consistent with the results of other methods, for example the method of optical rotatory dispersion, ultraviolet, spectroscopy, acid–base titration, and the "clover leaf" model of transfer RNA proposed by Dube[141].

It has been shown by X-ray diffraction that the RNA of dwarf rice virus has a secondary structure resembling that of DNA. However, the infrared spectrum of the RNA exhibits a weaker polarisation than the DNA spectrum

(Fig. 5).[27] The bands of the in-plane vibrations of the bases are polarised at right angles to the direction of orientation, as in the DNA spectrum, but the polarisation of the absorption bands of the RNA and DNA phosphate groups is different and hence there is a difference between the orientations of the PO_2^- groups. In the dwarf rice virus RNA helix, the O...O line makes an angle of 70° with the axis and the bisector of the OPO angle makes an angle of 40° (Table 8).

Table 8.

Double helix	1220 cm^{-1} band		1085 cm^{-1} band	
	dichroism	θ, deg*	dichroism	θ, deg*
DNA	nil	55	$\perp$	65
RNA	$\perp$	70	$\parallel$	40
DNA-RNA hybrid	$\perp$	68—78	nil	53—57

*θ—angle between the axis of the helix and the O...O line for the 1220 cm^{-1} band or the angle between the axis of the spiral and the bisector of the OPO angle for the 1085 cm^{-1} band.

The infrared spectrum of the DNA–RNA hybrid contains intense absorption bands characteristic of both DNA and RNA.[142] The dichroism of the absorption bands of the phosphate groups is evidence that the orientation of these groups relative to the axis of the helix of the hybrid is not completely identical with their orientation either in the double-helical DNA or in the double-helical RNA. It has been suggested[142] that the observed dichroism can be explained by the fact that in the hybrid molecule the PO_2^- groups of the DNA chain retain the same orientation as in the double-helical DNA, while in the RNA chain the orientation is the same as in the double-helical RNA.

DNA molecules are known to form nucleoprotein complexes with histones and with polypeptides, for example with poly-L-lysine. Information about the structures of DNA and the histone in deoxynucleoprotein (DNP) is important for the determination of the structure of chromosomes. The spectrum of the DNP film in the range 3500–1400 cm^{-1} resembles more closely the spectrum of the histone than that of DNA, since the bands of the protein are more intense than those of DNA. Below 1400 cm^{-1}, the spectrum of the nucleoprotein is identical with that of DNA[143]. The histone in DNP has an intense band at 3300 cm^{-1} (stretching vibrations of the NH bonds), an Amide I band at 1660 cm^{-1} (stretching vibrations of C=O groups), an Amide II band at 1545 cm^{-1} (deformation vibrations of the NH group), and also weak bands at 1450, 1400, and 1320 cm^{-1}.[144] The absorption bands of the histone are weakly polarised even for high moisture contents. The study of the infrared spectrum and also of the optical rotatory dispersion of DNP in the normal and deuterated states showed that in DNP part of the protein (50%) is in the α-helical form, the conformation of the protein varying with time. Measurement of the dichroism of the absorption bands of DNA for high relative moisture contents of DNP films showed that DNA in the nucleohistone is in the crystalline B-form, in agreement with X-ray diffraction data[134].

The stoichiometric complex of DNA with poly-L-lysine consists of fibres giving rise to distinct X-ray diffraction patterns, resembling that of the B-form of DNA when the relative moisture content is 75 and 66%.[145] The infrared spectrum of this complex is similar in broad outline to that of DNP, the band due to the phosphate groups undergoing only an insignificant shift (from 1224 to 1233 cm^{-1}) as the relative moisture content is reduced to zero, compared with the shift for DNA (from 1225 to 1240 cm^{-1}). In the spectrum of the DNA–histone complex, this shift is somewhat greater than for the DNA–poly-L-lysine complex.

The authors regard the shift of this band on dehydration of DNA as evidence of the liberation of phosphate groups from hydrogen bonding. From this point of view, the slight shift of the band due to the asymmetric PO_2^- vibrations on dehydration of the DNA–poly-L-lysine complex may imply that in this complex there are hydrogen bonds between the PO_2^- groups of DNA and the NH_3^+ groups of the poly-L-lysine side chains. However, the details of the molecular environment of the phosphate groups have not been elucidated and therefore such interpretation cannot be regarded as unambiguous.

VI. INFRARED SPECTROSCOPIC STUDY OF THE INTERACTION OF NUCLEIC ACID COMPONENTS WITH ONE ANOTHER AND WITH OTHER SUBSTANCES

The interaction of the complementary bases of nucleic acids via hydrogen bonding, postulated by Watson and Crick in 1953,[5] is fundamental in biological systems. The linear sequence of nucleotides in DNA molecules stores the genetic information about the cell (the triplet code). An important process in the reading and transmission of this information is the formation of complementary structures with the aid of specific hydrogen bonds. It has been suggested that this type of interaction occurs when DNA controls the synthesis of RNA and also in the interaction between nucleic acids during the synthesis of protein. Therefore it is necessary to find physical and chemical criteria of the specificity and stability of the pairing of the bases in helix nucleic acids, a problem which can be successfully solved by infrared spectroscopy.

The formation of association complexes in aqueous purine and pyrimidine solutions has been observed by measuring the concentration variation of their water vapour pressure[146,147] and also by a chromatographic method in which one of the bases is attached covalently to a column and then eluted by a solution of the other base[148,149]. However, it has been shown by NMR that in aqueous solutions association complexes of the bases can be formed both via hydrogen bonding and via π-electron interaction between the heterocyclic rings and the formation of vertical stacks of the bases[150,151]. On the other hand, Raman spectroscopy failed to show the formation of hydrogen bonds between the complementary bases in aqueous solutions[152]. In water the hydration effect is too large, i.e. the degree of hydrogen bonding between water and the solutes is too high for the interaction between the solutes to be investigated.

It proved possible to demonstrate by infrared spectroscopy the formation of hydrogen bonds between the complementary bases of nucleic acids, initially in the solid phase for mixed crystals of the bases. A new absorption band at 1710 cm^{-1} was observed in the infrared spectrum of a mixed crystal of adenosine and uridine and a 1720 cm^{-1} band was found in the spectrum of a mixed crystal of guanosine and cytidine, both being absent from the spectra of the components[41,153]. Miles[154] failed to reproduce these results, but X-ray diffraction confirmed the presence of hydrogen bonds in mixed A–U and G–C crystals[155–170]. These observations are of fundamental

importance, because they revealed the possibility of determining the ratio of the specific base pairs from infrared spectra.

The study of the hydrogen bonding of nucleic acid bases in a pure form in solution can be conveniently carried out in non-polar solvents such as carbon tetrachloride or weakly polar solvents of the type of chloroform, which form weak hydrogen bonds with the solutes and rule out hydrophobic and electrostatic interactions between the bases and hence the formation of vertical stacks[161-165]. The study of the interaction of the bases in non-aqueous solvents is also important because it may resemble the interaction between the bases within the DNA double helix, where the bases are hardly in contact with water molecules. Such investigations became possible owing to the synthesis of *N*-derivatives of the bases and derivatives substituted in the nucleoside sugar residues, which are soluble in organic media.

Table 9. Vibration frequencies (wave-numbers) of the free NH groups of the bases and the bound NH groups of the homo- and hetero-association complexes.

Compound	$\tilde{\nu}_{NH}$(free), cm^{-1}	$\tilde{\nu}_{NH}$(self-assoc.), cm^{-1}	$\tilde{\nu}_{NH}$(hetero-assoc.), cm^{-1}
Adenine	3530—3527 3415	3485, 3250 3312, 3200	3490 3330
Uracil	3400—3395	3210—3185 3110, 3050	3260 2800
Guanine	3515—3510 3408	3480, 3305 3355	 3490
Cytosine	3531, 3418	3485 3320	3472

The interaction of the bases in solution may be studied on the basis of the infrared spectra in the range 3500 to 2800 cm^{-1}, since the stretching vibration frequencies of the NH_2 and NH groups decrease on formation of hydrogen bonds. The formation of association complexes of the bases is accompanied by a decrease of the intensity of the bands due to the free bases and the appearance of a series of new bands due to the bound NH and NH_2 groups (Table 9). Self-association of the bases in fairly concentrated (10^{-2} M) solutions and association in mixed dilute (10^{-4} M) solutions of the complementary bases of nucleic acids was observed from these spectroscopic changes[161-166] (Fig. 13). Infrared spectroscopy has provided direct proof of homo- and hetero-association of purines and pyrimidines via hydrogen bonding. It also revealed the specificity of this interaction, since, among all possible combinations of the four bases, the G–C and A–U (A–T) combinations proved to be the most stable, the G–C complex being more stable than the A–U complex, probably owing to the formation of three hydrogen bonds. These results have been fully confirmed by studies on the association of the bases and nucleosides by the NMR method[168,169].

In a number of studies[163,170-173] on the concentration variation of the intensity of the NH bands of the monomers, self-association and heteroassociation complexes were obtained for different bases (Table 10). The results of different workers for association in chloroform are satisfactorily consistent with one another and the high values of the constants obtained by Küchler and Derkosch[163] are probably due to the use of carbon tetrachloride as a solvent (see also Drozdova et al.[172]). The values of the constants confirm that the association of adenine with uracil and particularly of guanine with cytosine is much stronger than the self-association of these bases.

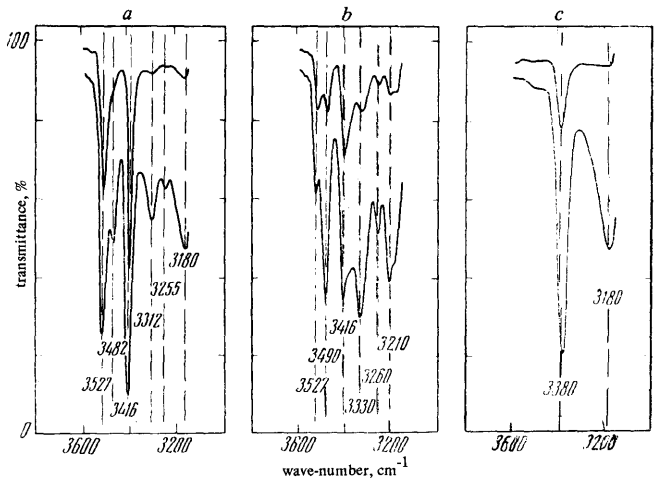


Figure 13. Infrared spectra of solutions in $CDCl_3$: *a*) 9-ethyladenine; *b*) 5-bromouracil; *c*) 1 : 1 mixture of 9-ethyladenine and 5-bromouracil; upper curves—0.019 M; lower curves—0.077 M.

The thermodynamic parameters of the association of the bases in different solvents were calculated from the temperature variation of the association constants. These data are presented in Table 10, which shows that the heats of formation of the association complexes are higher by a factor of 2–3 than the heat of formation of a hydrogen bond of the type H...O–C,[174] which may be due to the cyclic structure of these complexes.

The calculations of the association constants in all the studies mentioned above were made on the hypothesis of dimeric association of the bases, which is supported by the finding that the intensity of the absorption at all the bands of the heteroassociation complexes for a constant overall concentration of the components but with a variable ratio of the latter has a maximum corresponding to a 1 : 1 concentration ratio[161-164,167,170] (Fig. 14). This has been demonstrated[163] by determining the degree of association *n* from the variation of the concentration of the association complex with that of the monomer. For the association of adenine with uracil in their equimolar mixture and for the self-association of adenine and uracil, $n = 2$ was obtained in all cases. The infrared spectroscopic data have also been confirmed by the cryoscopic method.

Another argument in favour of the dimerisation of the bases is the constancy of the ratio of the intensities corresponding to the free NH and NH_2 groups of uracil and adenine in their mixture with variable concentrations of the components[164]. The heats of formation of the dimers, which are 4–6 kcal mole^{-1}, indicate the possibility of a cyclic structure maintained by two hydrogen bonds. X-Ray diffraction showed that in the crystalline state the A–U and G–C dimers have a cyclic structure[155,158].

The majority of the investigators of the specific pairing of nucleic acid bases suggest that one of the several

possible cyclic structures of the dimers predominates in solution, although so far no-one has succeeded in establishing the structure which actually exists. However, the formation of dimers of only one kind has been questioned[172] on the basis of the following facts. Firstly, on dimerisation of the bases a larger number of new bands appear in the infrared spectra than might have been expected for dimers of only one type. Secondly, calculation of the association constants based on the bands of the monomers and dimers leads to different results. The authors of this study believe that the results can be accounted for either by the formation of dimers of different kinds or of trimers of the type A + 2U. The possibility of the formation of trimers in solutions of adenine and a mixture of adenine and uracil is also indicated by NMR data[168,176].

Table 10. Association constants of different bases.

Association complex	Solvent	Association constant, litre mole^{-1}	Temp., °C	ΔH, kcal mole^{-1}	Ref.
A-A	$CHCl_3$	13±2	25	1.3±0.1	173
	$CDCl_3$	3.1±0.3	25	4.0±0.8	170
	CCl_4	83±7	20		163
U-U	$CHCl_3$	11.6±2.0	25		172
	$CHCl_3$	19±4	25	2.0	173
	$CDCl_3$	6.1±0.6	25	4.3±0.4	170
	$CDCl_3$	10±1	25	4.9±0.2	171
	CCl_4	115±8	20		163
A-U	$CHCl_3$	$1\cdot10^2$	25	6.2±0.3	173
	$CDCl_3$	$1\cdot10^2$	25		175
	$CDCl_3$	$1.4\cdot10^2$	25	6.2±0.1	170
	$CDCl_3$	$1.5\cdot10^2$	25		167
	CCl_4	$6\cdot10^2$	20		163
G-C	$CHCl_3$	10^5	30		164

The study of the effect of various substituents (CH_3, SH, Br, and I) on the degree of association of uracil with adenine reveals that substituents which decrease pK_{ass} for uracil or increase electron density in the N(7)-position of adenine also increase their capacity for association. Thus, complexes of 2,6-diaminopurine with uracil and of 5-bromouracil with adenine are the most stable. On the other hand, substitution of the hydrogen atoms in the amino-group of adenine lowers its association constant, for example 6-dimethylaminopurine is completely incapable of association[175].

Miles and coworkers[47-49,52,99] used the infrared spectroscopic method to investigate various structural complexes of synthetic polynucleotides in solution. Polycytidylic (or poly-5-bromocytidylic) and polyinosinic acids are known to interact in solution, forming helices with a structure resembling that of DNA, and polyuridylic and polyadenylic acids form a double or triple helix depending on the experimental conditions[6,45,177-181]. The formation of helical complexes in the interaction between polynucleotides is accompanied by considerable and characteristic changes in the infrared spectra owing to the formation of hydrogen bonds and the change in the chemical environment of the bases (Fig. 15). The bands due to the vibrations of the carbonyl groups are then displaced towards higher frequencies, as for DNA. When the double helix of poly-(A/U) is formed, the infrared spectrum of the complex reveals a shift of the carbonyl band from 1660 to 1672 cm^{-1}.[49,99,154] The spectrum of a solution of the helical poly-(I/2C) complex reveals a shift of the absorption band of polyinosinic acid from 1678 to 1697 cm^{-1} and a decrease of the intensity of the 1651 cm^{-1} band of polycytidylic acid[47,49,50,154]. The 1710 cm^{-1} band, which is characteristic of the secondary structure of double helices, appears in the spectrum of the double-helical polyadenylic acid. A similar increase of the vibration frequency of the carbonyl group of guanine (the wave-number increases from 1666 to 1680 cm^{-1}) occurs on formation of the spirals of 3'- and 5'-guanylic acids.

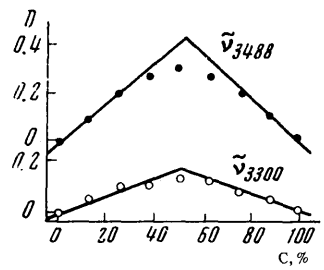


Figure 14. Variation of the optical densities of the 3300 and 3488 cm^{-1} bands of the association complexes with the percentage content of cytosine in a mixture of guanine and cytosine derivatives (overall concentration 1.6×10^{-3} M)[162].

Guanylic acid is known to be capable of forming helical macromolecular complexes which are stable at reduced temperatures[182]. The melting of these helical aggregates has been investigated[48] in the temperature range 10 to 60°C by measuring the intensities of the carbonyl bands.

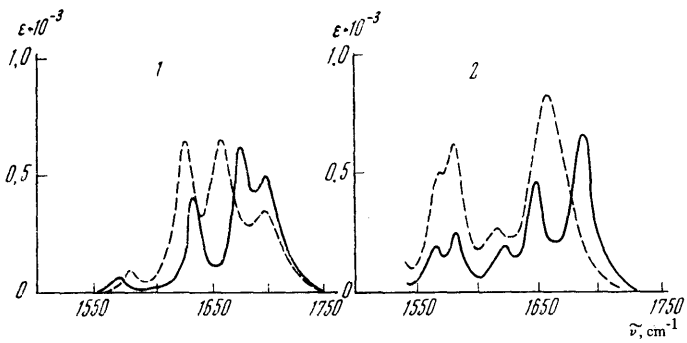


Figure 15. Infrared spectra of D_2O solutions (pD 7.0) of complexes: 1) poly-(A/U); 2) poly(G/C); dashed curves—sum of the spectra of the corresponding mononucleotides[102].

The formation of a helical secondary structure has been observed on the basis of infrared spectra not only in the interaction between complementary polynucleotides but also in the interaction of mono- and oligo-nucleotides with a polynucleotide, for example of guanosine 5'-phosphate or the trimer of guanosine phosphate with polycytidylic acid, of adenosine with poly-(U/C), etc.[51,52] The infrared spectra of these complexes also differ from the sum of the

spectra of the components. However, the complexes are stable only below 40°C (m.p. about 40°C). The parallel variation of the melting curves for the characteristic frequencies of the purine and pyrimidine bases is evidence of the specificity of the pairing of the bases in the complexes of polynucleotides with mono- and oligo-nucleotides.

A number of studies on complexes of nucleic acids and their components with metal ions by different procedures, including the infrared spectroscopic method, have been published recently[183-192]. The physicochemical properties of nucleic acids (intrinsic viscosity, sedimentation rate, and melting point) are known to alter under the influence of metal ions or the acids undergo degradation.

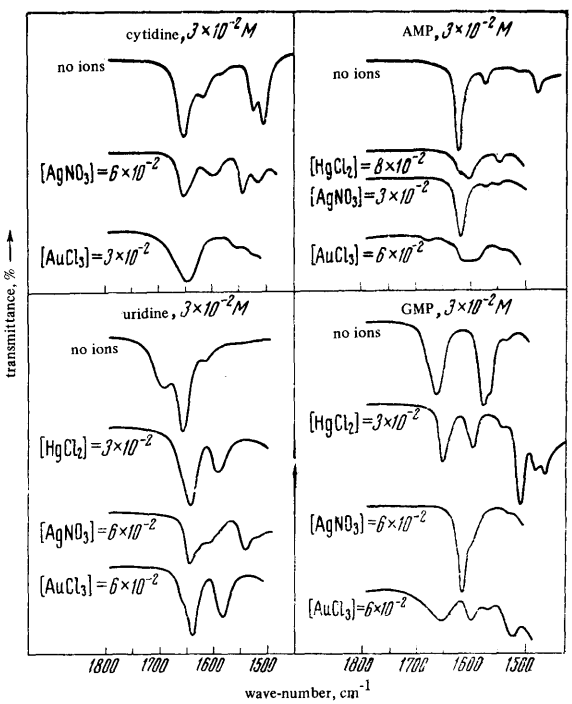


Figure 16. Infrared spectra of D_2O solutions of cytidine, uridine, GMP, and AMP (3×10^{-2} M) in the absence and presence of metal ions[198].

For example, alkali metal, alkaline earth metal, and transition metal ions increase the melting point of DNA and Cu^{2+}, Cd^{2+}, Ag^{2+}, and Fe^{3+} ions reduce it[193,194].

Metal ions can interact with the phosphate groups of nucleic acids (an ion–ion type of interaction) or with the dipoles of the rings of the bases (ion–dipole type of interaction). Therefore in a study of metal complexes of nucleic acids it is important to discover the site of binding of the ion. It has been shown by conductimetric titration that metal ions stabilising the DNA structure combine with its phosphate groups[195,196], while ESR,[197] NMR,[198] and spectrophotometric measurements[193,199-201] showed that destabilising ions (Cu^{2+}, Cd^{2+}, Pb^{2+}, Ag^{+}, and Hg^{2+}) are bound to the DNA bases at the sites of formation of hydrogen bonds. In agreement with these data, it has been observed that Na^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+} do not cause a displacement of the bands in the infrared absorption spectra of nucleic acid bases, while Ag^{+}, Hg^{2+}, and Au^{3+} ions do give rise to a displacement of this kind[202-204]. Shifts of the phosphate bands but not of the bands due to the bases have been observed in the infrared spectrum of a DNA–Fe^{2+} complex[203,204]. Hg^{2+} and Ag^{+} ions lead to considerable shifts of the bands in the region 1800–1500 cm^{-1} in the infrared spectra of all the nucleic acid bases for a metal : base ratio of 2 : 1 (Fig. 16).[202] In the region of the double bond vibrations of the bases, the spectrum of the DNA–Hg^{2+} complex is also appreciably different from that of DNA, which is evidence of the addition of mercury ions to the bases in the macromolecules[202,205,206].

The infrared spectroscopic data concerning the interaction of copper ions with nucleic acid bases are extremely contradictory: some investigators[207] have observed band shifts in the spectra of certain bases in the presence of Cu^{2+} ions, while others[202] failed to observe such shifts. Indeed, Drozdova-Tikhomirova and Kikoina showed[208] that, in the absorption spectrum of solid guanosine and its solution in dimethyl sulphoxide (DMSO) with added copper, the positions of the maxima in the region 1700–1600 cm^{-1} remain unchanged. Changes in the intensities of the carbonyl, amino-group, and double bond bands have been observed in the spectrum of solid guanosine in the presence of Cu^{2+} ions, the changes reaching a maximum for an equimolar ratio of the Cu^{2+} and guanosine concentrations, which is evidence of the formation of a 1 : 1 complex. The existence of a complex in a guanosine solution in DMSO was demonstrated with the aid of a differential spectrum in which analogous changes were observed. The authors postulate that the formation of the G.Cu^{2+} complex is associated with the shift of the tautomeric equilibrium of guanine towards its enolic form, and that this shift may be the reason for the lowering of the melting point of DNA. Uranyl ions form an insoluble complex with DNA, the stoichiometry of which depends on the pH of the medium and the concentration of the ions. Uranyl ions combine with the phosphate groups of DNA, since the spectrum of its complex shows a displacement of the phosphate absorption bands[107]. Thus infrared spectroscopy may be useful for the determination of the location of metal ions in their complexes with nucleic acids.

The interaction of DNA with various compounds, which are of interest from the standpoint of biochemistry or the protection of DNA molecules from various effects, can also be investigated by infrared spectroscopy. One of such compounds is myoinositol, which prevents the destruction of bacterial cells on dehydration and protects them when they are irradiated with ultraviolet light. A considerable increase of the sensitivity of micro-organisms to irradiation and dehydration occurs in the range of relative moisture content between 75 and 50%, i.e. in the region where the packing of the bases in the DNA helix becomes disorganised[209-213]. Preliminary physicochemical studies led to the hypothesis that compounds similar to inositol play the role of combined water on partial dehydration or irradiation of the cells and their components[214,215]. A study of the variation of the polarisation of the infrared spectrum of DNA yielded a direct proof of this hypothesis[206]. As stated above, a decrease of the relative moisture content of DNA films from 75 to 30% is accompanied by a considerable decrease of the dichroic ratio for the 1670 cm^{-1} band. Inositol keeps R_{1670} constant when the DNA film is dehydrated in the relative moisture content range 84–10%. These results may mean that myoinositol replaces the water molecules in the DNA

structure when it is dehydrated and thus stabilises its helical configuration[216]. Unfortunately so far this is the only study of this kind. Nevertheless infrared spectroscopy may prove very promising in the study of the interaction of nucleic acids with agents protecting against radiation, mutagenic and carcinogenic agents, and other biologically active substances.

Measurement of the intensity of the infrared bands of DNA and its components is extremely important in the study of the damage of DNA and various interactions, which has been convincingly demonstrated in the investigation of the specific interactions of DNA bases in solution, discussed in this Section.

In addition, using the area of the absorption bands as a measure of intensity (after Ramsay)[218], Miles[217] showed that the integral intensity of the bands in the range 1700 to 1600 cm^{-1} in the spectra of D_2O solutions of certain nucleotides and polynucleotides is proportional to their concentration. Thus Miles succeeded in demonstrating quantitatively that, when solutions of polyadenylic and polyuridylic acids are mixed, an appreciable deviation from the additivity of the band intensities is observed in the above range as a result of the formation of hydrogen bonds, while for a mixture of mononucleotides additivity is found to hold.

Measurements of the intensity of the absorption bands for DNA solutions and nucleotides apparently make it possible to follow the changes in the structure of the bases in shifts of the tautomeric equilibrium or under the influence of various factors.

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