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IR AND UV SPECTROSCOPY STUDIES OF DNA STABILITY

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ABSTRACT
It is important to analyze and to understand structural and functional relationships of biomolecules such as DNA, RNA, and proteins, in various clinical states. Infrared (IR) absorption spectrum provides an alternative method for the analysis of biological functions of biomedical samples and biomolecules. Purified DNA from whole blood of children with acute lymphoblastic leukemia and normal children were analyzed by UV and IR spectroscopy at different pH solutions and after heating at 100˚C for 30min. IR and UV spectroscopy studies yielded excellent results for analysis of DNA stability in this clinical state.

KEY WORDS: IR, UV, DNA, thermal stability and pH.

INTRODUCTION

The areas of the electromagnetic spectrum that are used commonly in the clinical laboratory include the UV radiation and visible light regions; the infrared radiation (IR, > 780 nm) is also used[1].The absorption of radiant energy by a solution can be described by means of a plot of the absorbance as a function of wave length. This graph is called an absorption spectrum. The absorption spectrum reflects the sum of the energy transitions characteristic of a molecule at each wavelength of light. Absorption spectra are often helpful for qualitative identification purposes. This is particularly true for low energy absorptions such as those found in the IR region[2]. The IR spectral region ranges from the red end to the visible spectrum at 780nm (12820 cm^{-1}) to the onset of the microwave region at a wavelength of 1mm (10cm^{-1}). Traditionally, this range is further subdivided into the near- infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). The MIR region covers the range 400-4000 cm^{-1}, and is the region most familiar to the organic chemist as providing a "finger print" characteristic of molecular species. It is this region that includes the rich spectrum of absorptions corresponding to fundamental vibrations of the species being probed[3]. Spectral analyses of solutions have long been applied to various body fluids for the purpose of clinical study as well as research. Ultraviolet (UV) light absorbance measurements were made for many purposes; to determine the concentration of substance, to assay certain chemical reactions, to identify materials, and to determine the structural parameters of macromolecules[4]. Nuclear DNA may be used as an aid in diagnosis, to predict prognosis and to determine management of certain neoplasia [5-7] . Cancer has been described as a multistage genetic process. The stages of this process include the following; initial of DNA damage, and or chromosome breakdown and rearrangement; gene replication. And selection of successfully growing mutant cells.[8] Acute lymphoblastic leukemia (ALL), is a form of leukemia, or cancer of the white blood cells characterized by excess lymphoblasts. Malignant, immature white blood cells continuously multiply and are overproduced in the bone marrow. All causes damage and death by crowding out normal cells in the bone marrow, and by spreading (metastasizing) to other organs. All is most common in childhood with a peak incidence at 4-5 years of age, and another peak in old age. The overall cure rate in children is 85%, and about 50% of adults have long –term disease – free survival [9,10], such in vivo experiments, in vitro research will continue to explore the effects of buffer, temperature, and other environmental factors on DNA and RNA structure and transitions[11,12,13]. The flexibility and elasticity of single – stranded RNA and single-and double –stranded DNA have been evaluated[14,15]. Another experiments have extensively probed the overstretching transition of double stranded DNA where a 70% increase in length is suddenly observed at high force; the exact force at which the transition takes place varies, *e.g.*, with pH[16,17]. This work was undertaken to identify the IR wave numbers and intensities characteristic of the purified DNA from the blood of ALL patients after heating the sample for 30 minute at 100 ^{0}C in compeer with normal samples. And use UV spectroscopy to characterize the purified samples at different pH values.

MATERIALS AND METHODS

Patients with acute lymphoblastic leukemia were patients of the clinic of pediatric oncoheamatology, Medical city. All patients were within the active stage of disease and 4-10 years old. Twenty children were included in this study, in addition to twenty age matched healthy subject. All laboratory chemicals and reagents were of analar grade. High molecular – weight calf-thymus DNA, triton X-100, phenol, isoamyl alcohol, EDTA, Diphenylamine, glacial Acetic acid, chloroform, SDS, perchloric acid, NaCl and H_2SO_4 were obtained from BDH. HCL and urea were obtained from Fluka. Sucrose from Merk and Tris (hydroxy methyl amino methane) was from Hopkin & williaws. Sephadex G-25 was obtained from Pharmcia. DNA was extracted and purified by the method of Adell and ogbonna[18]. The whole blood was mixed with a lysis buffer (10mmole/ L) Tris – HCL, pH 7.5 containing 300 m mole of sucrose and 10 mL of Triton X-100 surfactant per

liter) to lyse the cells. A fraction containing nuclear material of the leukocytes ,obtained from this mixture by centrifugation was suspended in a buffer containing strong protein-denaturing agents (100m mole/L Tris-HCl, pH 7.0 containing 10 m mole of EDTA ,8mol of urea ,and 10 gm of SDS per liter).The dissociated DNA was extracted twice with phenol/chloroform to remove most of the proteins. The sample was rich in DNA at this stage but still contained some protein. The crud DNA was purified by Sephadex G-25 spin column. The sample was applied to the column and purified DNA was recovered in the first three fractions. DNA concentration was measured by using Burton method[19]. Standard curve was prepared by dilution the stock solution of Calf-thymus DNA to (10, 20, 40, 80, 100, 200) µg/ml .Purified DNA from children with ALL and normal children were used, with concentration of 23 µg/ml, against the blank of TED (10m mole/L Tris-HCL, pH (7.0, 3.0) containing 1m mole of EDTA per liter) at the wavelength from 200 to 300 nm. The UV spectra were recorded with a Shimadzu 160 UV – visible recorded spectrophotometer. Bach of purified DNA samples with concentration (27µg/ml) for children with ALL and normal children at pH (7) after heating at 100˚C for 30minute in water bath were lyophilized. Infrared absorption spectra of DNA were obtained by using DNA films. The water content was rigorously controlled, as this parameter is essential to stabilize the structure. The IR spectra were recorded with PYE Unicam SP3-300 infrared spectrophotometer. Statistical analysis was performed by student's t-test.

RESULTS & DISCUSSION

DNA was isolated by a simple, rapid method, which can be routinely used in clinical chemistry laboratories, then purified by a Sephadex G-25 spin column chromatography. The yield and purity of DNA samples, which were isolated from all patients whole blood, were higher than these of normal children whole blood (table 1), and this may be due to the difference in leukocyte counts.[18] Purified DNA samples of children with ALL and normal children were studied by using UV light. Table (2) show that DNA molecules have the same λ_{max} at (269.5 nm) at pH 7 and 3 for both normal and ALL patients. The absorbance at λ_{max} = 232.5 nm and 235.5 nm for purified samples of normal children and children with ALL respectively at pH 7 were disappeared at pH 3 .

TABLE1: Purity and yield of DNA from whole blood of normal children and children with ALL.

Sample	Purity (A_{260}/A_{280}) mean ± SD	Yield (µg/ml) mean± SD
Normal children	1.5±0.2	21.0±2.5
Children with ALL	1.7±0.5	31.1±3.0

TABLE 2: Wavelengths and absorbance of UV spectrum at different pH (7&3) of DNA purified from whole blood of normal children and children with ALL.

Sample	pH	Wavelength λ (nm.)	Absorbance
DNA of normal children	7	269.5	0.318
		214.0	0.439
		232.5	0.092
	3	269.5	0.283
		216.5	0.328
DNA purified from children with ALL	7	269.5	0.344
		216.0	0.550
		235.5	0.122
	3	270.0	0.116
		213.0	0.018

The values of absorbance at λ_{max} (269.5& 214 nm) for normal children purified samples at pH 7 were decreased at pH 3[λ_{max} (269.5 & 216 nm)] and this is the same for samples DNA purified from whole blood of children with ALL λ_{max} (269.5 & 216 nm) at pH 7 and λ_{max} (270& 213.5 nm) at pH 3. And this is may be due to DNA conformational changes at pH 3 and Hydrolysis of some ionic groups, which were bound to nitrogen bases at pH 7. Most of biological macromolecules absorb Ultraviolet (UV) light in a range of wavelengths that is easily measurable, as a result of their containing aromatic rings. The absorption spectra of some of the amino acids and of the nucleotide bases in nucleic acids have been well studied and are of great use both in identifying substances and in determining the structure of proteins and nucleic acids[20]. All DNA molecules, have the same λ_{max} (259nm) and have nearly indistinguishable spectra, unless, a particular base is present in great excess. The most important aspect of nucleic acid absorption spectroscopy is the decrease in the absorbance of the nucleotide bases that occurs when an oligonucleotide forms [20,21]. After heating of the DNA samples for 30min. at 100˚C as described before, IR absorption spectra were obtained figure (1) and (2), between 4000 cm^{-1} and 200 cm^{-1}. The wave numbers of the main absorptions as well as their intensities are summarized in table(3). From these results some differences between the two spectra were detected; the band at 1140 cm^{-1} appeared as a medium band in normal DNA infrared spectrum, and disappeared in the spectrum of denatured DNA isolated from children with ALL. This band reflects symmetric stretching vibration of the phosphate group of thymine residue [22,23].

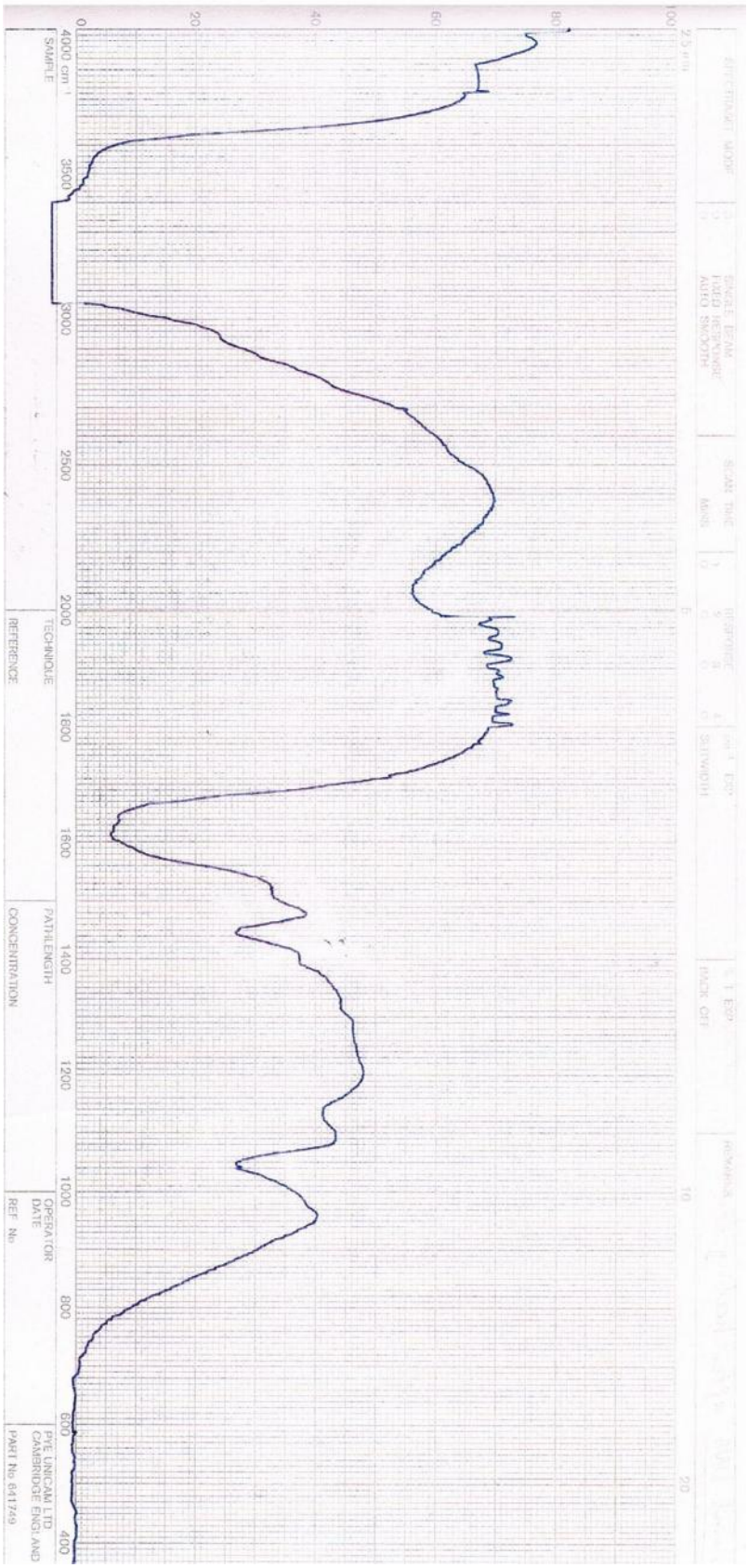

FIGURE 1: Infrared absorption spectrum of purified DNA from whole blood of normal children at pH (7) after heating for 30 min. at 100 ºC.

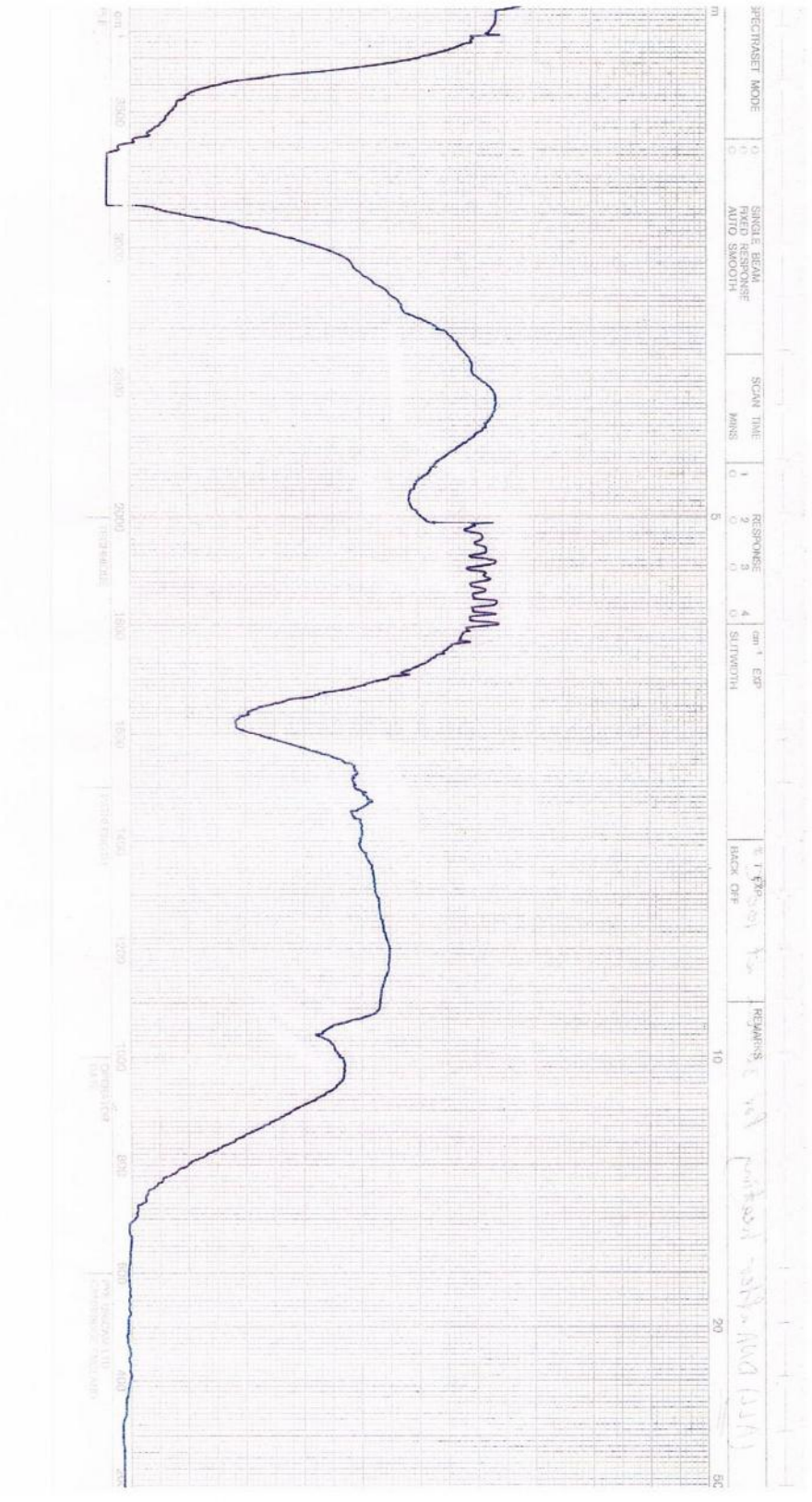

FIGURE 2: Infrared absorption spectrum of purified DNA from whole blood of children with ALL after heating for 30 min. at 100ºC.

Bands at 1930 cm^{-1} and 1905 cm^{-1} appeared as weak bands in normal DNA infrared spectrum, and as a medium bands in spectrum of DNA from the patients. Bands at 1875 cm^{-1} and 1850 cm^{-1} appeared as medium bands in spectrum of denatured DNA isolated from children with ALL, and disappeared in the spectrum of DNA isolated from normal children. These bands were tentatively assigned to the vibrations of groups contained in the nitrogen base residues which were less affected by heating from other molecules in the DNA structure. The spectral region between 1140 $cm^{-1}$and 1875 cm^{-1} is useful to characterize the conformational changes of DNA structure after heating. In summary, the decrease in absorbance at λ_{max}=269.5 with different pH (7&3) for both DNA purified from whole blood of normal persons (from 0.318 to 0.283, table(2)) and DNA purified from whole blood of children with ALL (from 0.344 to 0.116) were proved that the normal samples were more stable than of the pathologic samples against environmental pH changes. The conformational transition of DNA structure can be successfully studied by IR spectroscopy. The facts that both UV-and IR allow the specific analysis of DNA molecules suggest that these methods may also be used to study non covalent complexes between DNA and proteins.

TABLE 3: Infrared wave numbers and intensities of DNA purified from whole blood of children with ALL and normal children after heating for 30min at 100 ºC.

Normal Samples		Patients Samples	
Wave number (cm-1)	Intensity	Wave number(cm-1)	Intensity
3050 - 3450	Very strong broad peak	3160 - 3400	Very strong broad peak
2060	m	2060 -2080	m
1975	w	1975	m
1950	w	1950	w
1930	w	1930	m
1905	w	1905	m
1830	w	1875	m
1815	w	1850	m
1610	s	1830	w
1510 – 1530	sh	1810	w
1445	s	1610 – 1625	s
1140	m	1500 – 1520	sh
1050	s	1450	s
		1040	s

Abbreviations: s: Strong, m: Medium, w: weak, sh: shoulder

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