JABS Journal of Applied Biological Sciences 2 (3): 113-119, 2008
ISSN: 1307-1130, www.nobel0 gp0t

DNA Infrared Absorbency Detection using Photopyroelectric Technique and FTIR Spectroscopy

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Received: March 12, 2008
Accepted: July 28, 2008

Abstract

Absorbencies of different DNA samples were studied using the well known photopyroelectric (PPE) technique and Fourier Transform Infrared (FTIR) spectrometer. In the first method, wideband radiation absorbance from an infrared (IR) pulsed wideband source (PWBS) by DNA samples were detected and compared with FTIR spectrum. It was shown that the PPE technique results are useful and comparable to FTIR in distinguishing different DNA samples of biological interests.

Key words: FTIR, Photopyroelectric, DNA, IR Absorbance.

INTRODUCTION

DNA studies are vital in many fields especially life sciences. This was a driving force behind the many studies involving the different aspects and properties of DNA involving useful experimental techniques. For example, FTIR study of DNA interactions with cationic polyelectrolyte could play an important role as a synthetic transfection agent [1]. Hence, the understanding of such reaction leads to their effective use in genetic engineering and gene therapy [2].

In the past few years much attention is made to the molecular aspects of lipid-DNA complexes and their effects on the aggregation behavior of the gene-delivery complex [3]. The gene therapy is dependant on the replacement of the defective gene by a modified protein–based function into the cell to elicit a therapeutic response, the success of this can be confirmed using FTIR biophysical characterization of DNA complexes [4]. FTIR study in the range 700- 4000 cm^{-1} [5] of DNA isolated from gamma-irradiated epididymis cells of rats from the Chernobyl zone revealed changes related to damage in the primary, secondary and tertiary structure of the nucleic acid due to modification of bases, sugars and redistribution of H-bond network. This allowed the use of DNA absorption as a cancer grading, i.e. a prognostic indicator to assist the treatment decision [6]. Successfully FTIR technique can be used to carryout in situ study of absorption and oxidation of denatured DNA [7-8], structure and formation of supramolecular DNA-protein complexes [9] and more recently is the biological assessment and characterization of multilayered DNA coatings for biomaterials used in the fabrication and their subsequent effect on cell behavior [10]. The technique also employed for study of DNA interaction with cationic and helper lipids on solid supports allowing the formation of ordered structures [11].

The previous applications have made the effective but sophesticated and costly FTIR spectrometer an important tool in the characterization study of DNA complexes. This paper aims to establish a technique that eliminates the laborious work involved in FTIR method. The suggested method is dependant on the measurement of a collective IR radiation absorption signal of DNA complexes in the range 2 – 9.5 µm in conjunction with photopyroelectric film (PVDF). IR radiation from a wideband pulsed IR source is allowed to fall on an aqueous DNA sample placed on top of a 1×1 cm^2 photopyroelectric film and the resulting signal from absorption was measured. The attained signal is then employed as a characterization of the type of DNA whose absorption of IR radiation follows the nucleotides structure. The pyroelectric effect [12] is well known and has good potential for use to measure the temperature change [13-18] resulting from radiation absorption. To our best knowledge, this technique is used for the first time to measure temperature change resulting from collective wideband IR absorption by DNA samples on top of PVDF**.** The suggested technique is rather important in establishing a simple characterization method for DNA structures. Radiation absorbed by sample on top of PVDF initiates a Pyroelectric (PE) signal related to the Pyroelectric effect (PPE). The signal is measured as a potential difference across the PVDF and is expected to follow any variations in the DNA structure, hence allowing differentiation between different samples under study. This makes the technique practical to study DNA samples for typing purposes by an in-expensive easy to handle method.

EXPERIMENTAL DETAILS

Photopyroelectric cell design.

The photopyroelectric cell used in the present study consists of a square piece of biaxially PVDF foil metallised on both

sides with aluminum foil to provide for reflecting surfaces and electrode connections. The foil dimension is 1cm × 1cm, 25 μm thickness. The aluminum foil is important to make sure that only heat resulting from radiation absorption and propagating through the sample is detected and not that generated by direct interaction between radiation and detector. The foil was glued to a block of Perspex glass, which acts as a holder and support for the PVDF. The sample is placed on top of the foil using a micropipette. Perspex block and foil on top constitute the detection cell, it was enclosed in an aluminum box to minimize the ambient electromagnetic interference with the foil, prevent evaporation of the liquid sample during measurements and reduce air turbulence. The infrared radiation was allowed in through a small opening 1cm diameter bored in the aluminum box side. Two electrodes were connected to the top and bottom of the foil with silver paint constituting terminals for the PPE output signal. This cell design was first used [19] to study essential oils and olive oil adulteration. Detection cell shown in Fig.1 is connected to the electronic monitoring system for signal processing as shown in Fig.2.

Wideband infrared (IR) source

The infrared source used in this study is high-intensity IR emitter normally used in gas sensors and provided by Ion Optics Ltd. It has long term stability and can make a shot-to-shot repeatedly, with high Signal-to-noise ratio. The source is a broadband infrared light source emitting in the range 2 – 9.5 μm, it has pulsed operation at large temperature modulations and an active area of 32.7 m^2, rated temperature is 850 ^0C, minimum and maximum resistances are 2.8 ohms and 4.5 ohms respectively. The source looks dull red at 300 mA, and red at 320 mA, its rated drive power ~ 0.460 watts. It is an electrically-pulsed and radiates with low thermal-mass filament tailored for high emissivity in the specified range. This high-efficiency device minimizes drive power, greatly reduces parasitic heating of detectors and optics; it also eliminates the mechanical choppers, permitting a sealed optical path. The high emissivity enables the source to efficiently and rapidly cool via thermal radiation. The hot filament nearly cools to background temperature before the next pulse, thus providing several hundred degrees of temperature modulation. In this study results were obtained at source driving current of 300 mA and modulation frequency of 12.0 Hz.

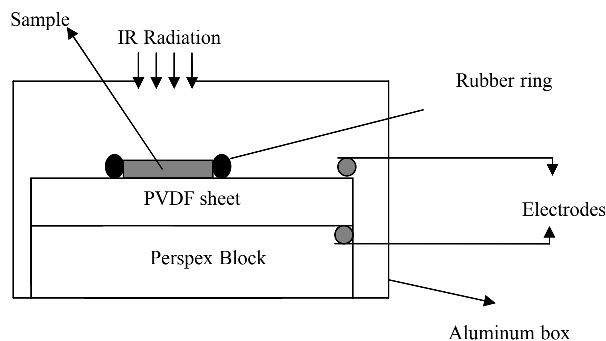

Figure. 1. Schematic showing the Photopyroelectric cell used to study DNA samples .

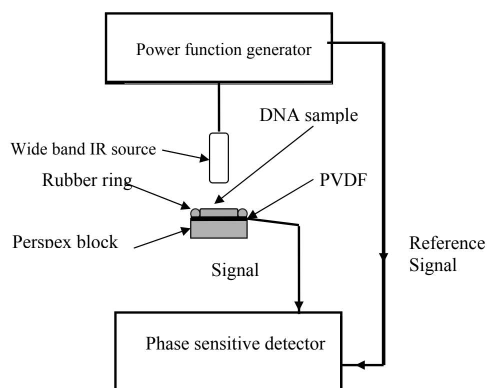

Figure 2. Schematic illustration of the complete photopyroelectric detection scheme used to study DNA samples.

The FTIR spectrometer

FTIR system used in this study is of the type IFS 66/S, manufactured by the German Broker Instruments company and has a resolution of ~ 4.5 cm^{-1}. It is capable of sample analysis up to ~11 millimeters in diameter measured either in bulk or just the top ~1 micrometer layer. System is equipped with an infrared microscope capable of analyzing samples as small as ~10 x 10 micrometers, making it ideal for the analysis of fibers, fragments, captured filter debris, surface imperfections, microelectronic systems, biological tissues, paint fragments, multilayer polymers, and others. Using this system DNA sample absorbencies in the frequency range ~ 600-7500 cm^{-1} were studied.

DNA sample preparation and study

Three complementing sets of DNA primers (P) were used. The lyophilized primers were reconstituted with ultra pure water and the final concentration of each primer was 100 pmols./μ*l*, i.e. (100 μM). The first set of primers labeled P1 and P2 mixed with each other by the same volume ratio of each sample (271μ*l*) to form DNA1, the second set P3 and P4 at 210 μ*l*, to form DNA2 and the third set P5 and P6 at 248 μ*l* form DNA3. The complement primers were mixed together in equal concentration for annealing to take place leading to the formation of double stranded DNA. These primers were of known molecular weight, AT/CG ratio and concentration. Other tested DNA sample was of PCR products of known size and sequences. Only 4 μl is needed from each sample to be used on top of the PVDF as shown in Fig. 2. DNA samples were kept in the refrigerator at -20 ^0C till the time of experiment. The experiment is aimed to test the PPE technique ability to distinguish between DNA samples of different chemical composition.

Radiation is allowed to fall on single drop sample placed on top of a polyvinylidene film PVDF. The absorbed radiation as a consequence generates a PPE signal which can be picked up by a phase sensitive detector as seen in Fig. 2. One single drop ~ 4 μ*l* of sample is placed directly on top of a PVD film in a region bounded by a rubber O-ring. The resulting absorption of the IR radiation by the drop of DNA generates heat wave that is sensed by the PVD film and transformed into a voltage i.e. PPE

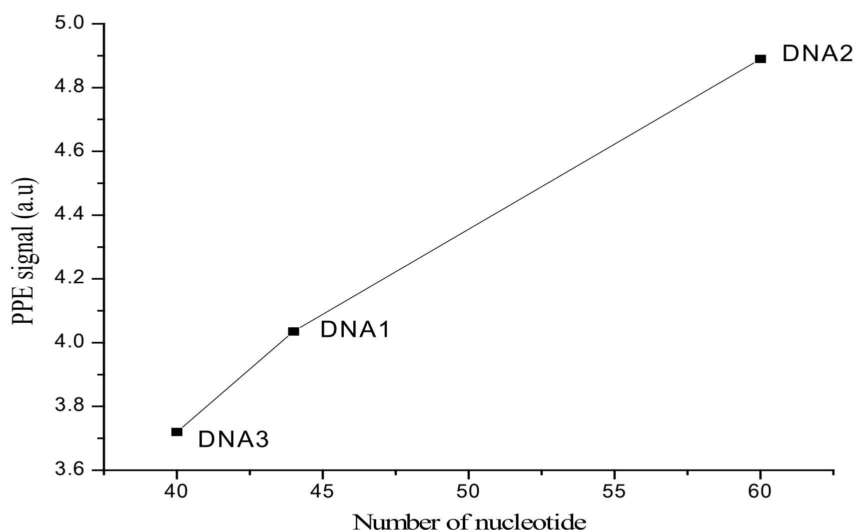

Figure 3. PPE signal versus number of nucleotide for three manufactured DNA samples.

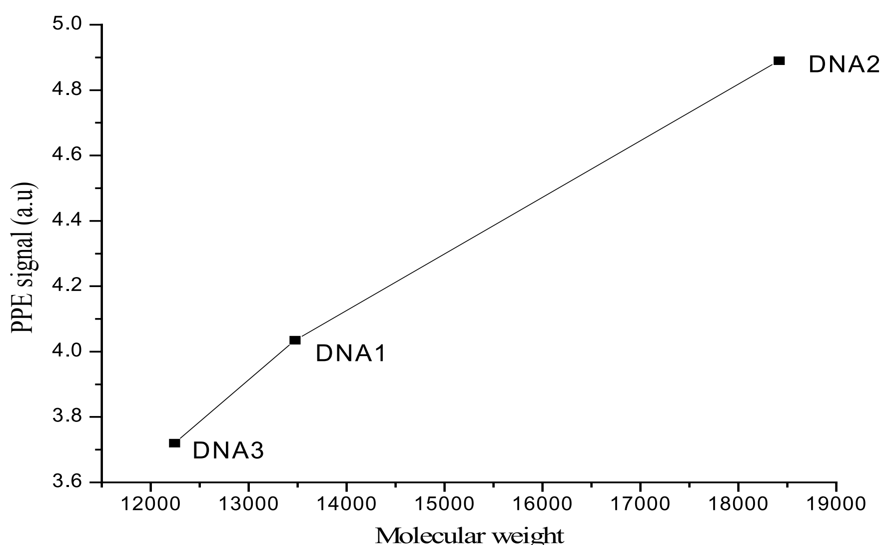

Figure 4. PPE signal versus molecular weight for three manufactured DNA samples.

signal that can be detected by feeding signal from the detection unit into the input of the electronic signal processing system. As far as the FTIR is concerned it is used in the normal way to study DNA samples as radiation is scanned over the range 600-7500 cm^{-1}.

RESULTS AND DISCUSSION

Used DNA liquid samples are allowed to absorb a wide band IR radiation from pulsed IR source in the region ~ 5000-1000 cm^{-1} while placed on top of the PVDF. For comparison purposes, samples absorbencies were studied using FTIR spectrometer. The resonant or vibrational frequencies are determined by the shape of the molecular potential energy surfaces, the masses of atoms and eventually the associated vibrancies coupling. Infrared radiation cause covalent chemical bonds to vibrate by stretching and contracting, hence part of the infrared spectrum absorbed depends on the strength of the chemical bond between the atoms. Therefore, molecules with several different types of bonds (e.g. a C-H and or a C=O), expect to show at least two different absorption bands. The appearance of several absorption peaks is common for high number double and triple bonds systems. These facts play a discriminating role that shows itself as decrease or increase in the IR absorption signal level of the different samples. Vibrational fine structure is most pronounced in vapor phase spectra, and is increasingly broadened and obscured in solution; in both methods liquid samples were investigated and nothing to worry about in this respect.

DNA nucleotides have an IR spectrum in the range 670 to 1443 cm^{-1}, with strong absorptions around 3333 cm^{-1}, the bands at 1667 cm^{-1} associated with C=C, C==N, and C-O stretching, and the strong compounds show a band in the region of 1042 to 1087 cm^{-1} [20]. This range of IR wavelength is covered by the emission range of the pulsed IR source used in the present study. As the number of nucleotides is increased the level of IR absorption signal is expected to increase and this is confirmed by the results of Fig.3. In this figure DNA3 sample with lowest nucleotides number has the lowest absorption, followed by DNA1 and DNA2 according to their respective nucleotides numbers. Molecular weight of a DNA sample is proportional to

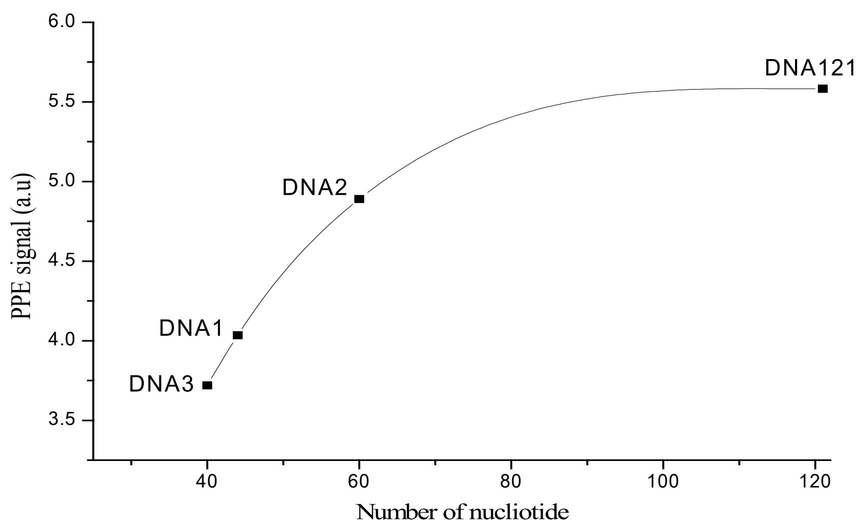

Figure 5. PPE signal versus number of nucleotide for the three manufactured DNA. samples and the three DNA samples of PCR products.

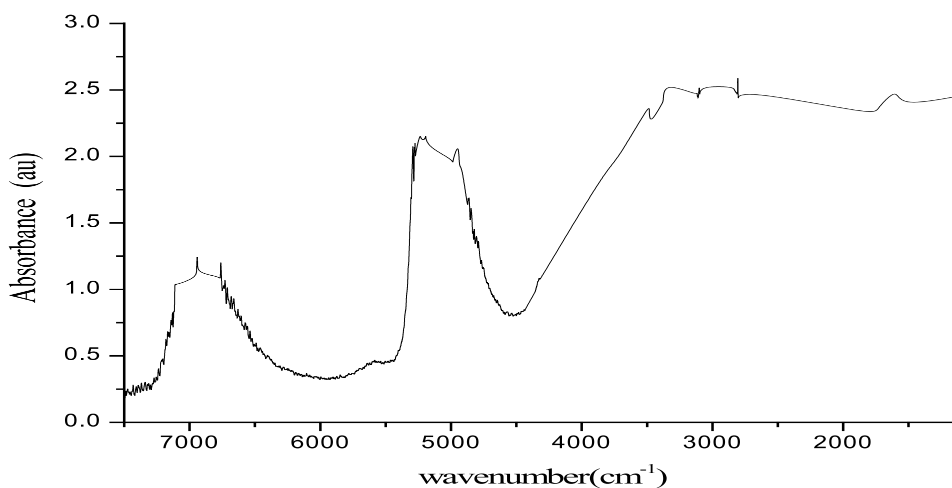

Figure 6. FTIR absorbance spectrum of DNA1 sample.

its number of nucleotides, when absorptions is measured against the number of nucleotides, a result similar to that of Fig.3 is evident in Fig.4. This fact is also confirmed by results drawn in Fig.5. In this figure, it is obvious that the sample DNA121 has the highest absorptions of all samples and this well expected since it has the highest nucleotide numbers of all samples. Hence, results from both techniques correlated very well with each other. Comparing the results of Fig.6 and Fig.7, it is clear that the discrimination ability of FTIR between DNA1 and DNA2 is extremely low compared to band absorption of PPE technique given in Fig.3, Fig.4 and Fig.5. When considering the strong absorption at 3333 cm^{-1} as noticed by Blout [20], and upon normalizing the absorption signals amplitudes at this particular wavenumber with respect to the lowest absorption signal value, namely that of DNA3, it is found that the ratios from FTIR results are: 1: 1.72: 1.78: 2, corresponding to: DNA3: DNA2: DNA1: DNA121 respectively. Comparing this with the PPE results of the band absorptions, it is found that the ratios are: 1: 1.1: 1.3: 1.5, corresponding to: DNA3: DNA1: DNA2: DNA121 respectively. Comparison showed a difference in the order between the two techniques as DNA2 sample preceded DNA1 in the FTIR results, although the number of nucleotides for DNA2 is larger than that of DNA1. This leads to the following conclusion: the distinguishing ability is almost similar for both techniques, but the simplicity and low cost of the PPE technique makes it valuable in this respect. Signals levels indicate a nonlinear behavior due to the existence of hydroxyl and amino stretching vibrations which have a strong absorption around 3333 cm^{-1}. Nonlinear behavior is also enhanced by the existence of C=C, C=N, C-O stretching that absorbed at 1667 cm^{-1}, and the double and triple bonding between the nucleotides itself. This result is confirmed in Fig.5, when results of DNA sample of PCR product and that for the three manufactured DNA samples are drawn in the same figure against the number of nucleotides; it is clear that nonlinearities are more obvious in this figure than in Fig.3 and Fig.4. Figures 6-9 of the FTIR, show that the three manufactured DNA samples shown in Figs. 6, 7, and 8 retain the same spectrum shape, while the results of DNA sample of PCR product shown in Fig.9 has a different spectrum shape, i.e. DNA121 sample structure that introduced none linearity as shown in Fig.5 for PPE technique, showed a different spectrum shape in the FTIR results. This indicates that the PPE is very sensitive to sample's structure

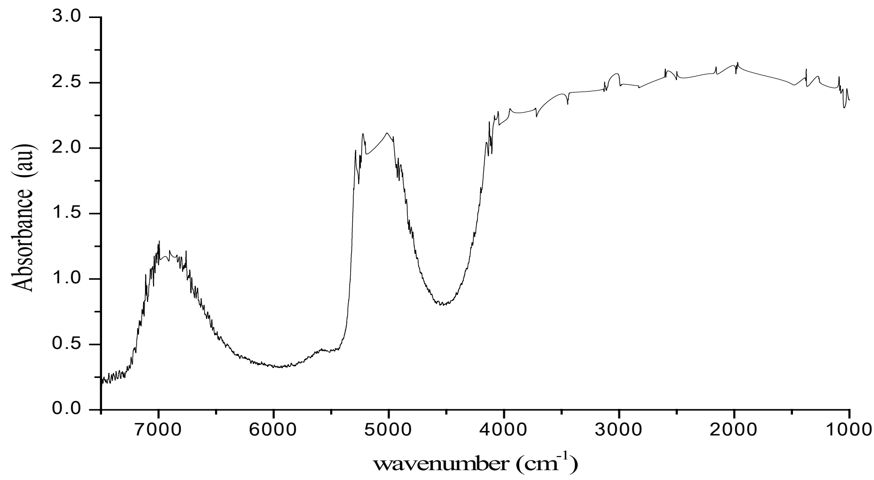

Figure 7. Absorbance versus wavenumber for DNA2 sample.

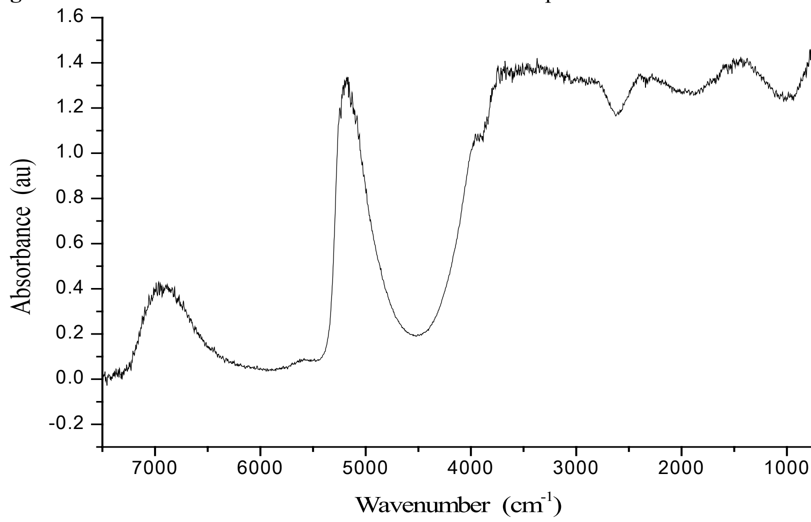

Figure 8. FTIR absorbance spectrum of DNA3 sample.

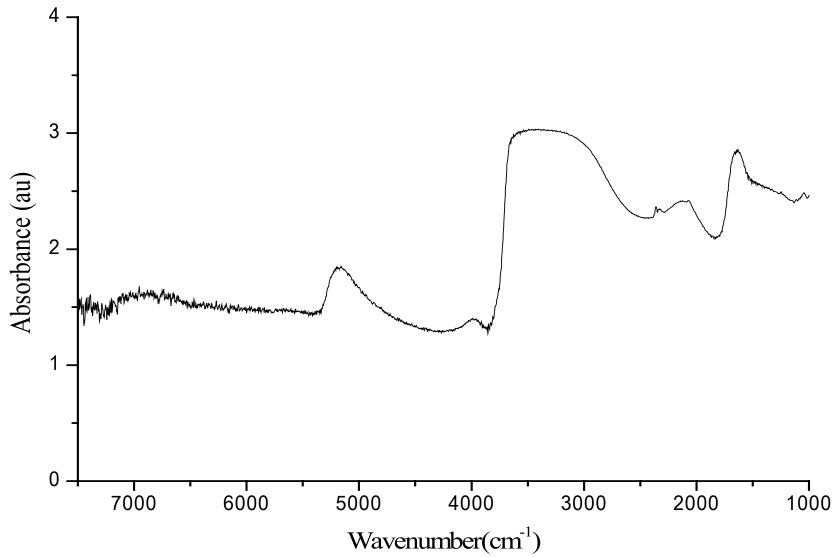

Figure 9. FTIR absorbance spectrum of DNA121 sample.

differences, in spite of the fact it is represented by a wideband absorption signal.

The existence of double and triple bonds shift maximum to a longer wave lengths and hence towards less radiation frequency, this explains the nonlinear behavior in Fig. 5. The DNA121 sample absorption (see Fig.5) is larger than other samples; this is also confirmed by its corresponding FTIR spectrum results shown in Fig.9. At this point it is concluded that the well known FTIR method confirmed PPE absorption results in the concerned radiation region of the pulsed wideband IR source. Both methods show that as the number of nucleotides and sample molecular weight is increased absorption is at low wave number radiation. Nonlinearities due to the existence of hydroxyl and amino stretching vibrations, existence of C=C, C=N, and C-O stretching and the existence of the double and triple bonding between the nucleotides itself showed itself as nonlinear curve in the PPE results and as a change in the spectrum shape for the FTIR results.

CONCLUSIONS

An important conclusion is drawn; the use of PVD film in conjunction with a pulsed wideband IR miniature source emitting in the region ~ 2-10 μm to distinguish different DNA samples is successful. This conclusion is confirmed by the results obtained using FTIR spectrums of the same samples. Detection sensitivity of the PPE scheme is confirmed by the experimentally calculated high signal to noise ratios found to be better than 106. This is rather important result for sensitive detectability at low radiation power in the microwatts range. The above conclusions are encouraging for possible extension of the scheme for further DNA identification and sequencing studies using PVDF. AS far as the level of absorption in the specified radiation region and sample structure is concerned, both methods show almost similar distinguishability of the different samples structures under study, although PPE method is dependant on a wideband absorption signal. Hence, it is concluded that results stress the importance of the PPE scheme as an inexpensive easy to handle tool as compared to the well established FTIR method and it can further be developed for an enhanced design for DNA characterization system.

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