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Spectroscopic Characterization of PEG-DNA Biocomplexes by FTIR

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ABSTRACT

Understanding the mode involved in the binding of certain molecules to DNA is of prime importance, and PEG offers wide-ranging applications in biological, medical and pharmaceutical contexts. FTIR spectroscopy has been used to characterize how the formed biocomplexes bind or dissociate to/from each other between PEG_{400}-$_{ct}$DNA under different conditions. Characterization and investigation of the effect of incubation time on PEG_{400}-$_{ct}$DNA biocomplexes formation were studied through spectroscopic technique FTIR. The influence of time duration and incubation on intermolecular interaction was analysed at three different selected times (Zero, 1hr, and 48 hrs.) at 1:1 PEG_{400}-$_{ct}$DNA monomer to nucleotide ratio. The experiment was carried out at room temperature 22 ºC, with prior vortex stirrer of biocomplex for 10 min to improve homogeneity of sample. The results showed that the binding reaction of PEG_{400}-$_{ct}$DNA proceeds rapidly through DNA base pairs and phosphate DNA backbone, and complexation was reached after a maximum 1hr after mixing PEG_{400} and $_{ct}$DNA at 1:1 ratio. FTIR spectroscopy results suggest that PEG_{400} binds with $_{ct}$DNA by weak to moderate biocomplexes formation, with both hydrophilic and hydrophobic contact through DNA base pairs, with minor binding preference towards phosphate backbone of DNA helix. The mode of interaction most likely referred to an interaction through outside groove binding or electrostatic binding modes. FTIR highlighted the significant effect of incubation time on the stable biocomplexes of non-ionic PEG_{400} and $_{ct}$DNA. Moreover, FTIR spectroscopy technique was rapid, showed good stability, and is a valuable tool for studying the biological properties of biocomplexes of PEG_{400} and $_{ct}$DNA.

INTRODUCTION

Interactions of DNA with various molecules are of interest due to its importance as a biochemical tool for many biomedical applications, such as visualization of DNA (Knee *et al.*, 2008), DNA hybridization (Gooding, 2002), DNA biosensors (Drummond *et al., 2003*, Wang, 2002), action mechanisms and determination of some DNA targeted drugs, and the development of gene and drug delivery systems (Li *et al.*, 2007). Therefore, understanding DNA interaction patterns based on the study of molecules that bind to DNA is one of the significant parameters which can speed up drug discovery and development processes (Elmarzugi and Roberts, 2011).

Several synthetic polymers play a major role as biomaterial and as vehicles for many drug delivery systems, and selecting molecules that bind genomic DNA to form a complex is a central requirement for drug delivery system development, necessitating new *in vitro* methods for rapid and low-cost assessment of the binding affinity and location of molecules bound with DNA molecules (Huang *et al., 2003*). Many applications of DNA-polymer complex have already been demonstrated and characterized. Among synthetic polymers, polyethylene glycols (PEG) show potential in different biotechnical, industrial, and clinical applications (Charles *et al.*, 2009), gene and drug delivery system development due to their solubility, and non-toxicity and biocompatibility (Bello-Roufai *et al.*, 2007, Elmarzugi, 2012). PEG are a class of molecules that show multidisciplinary applications. It is a water-soluble, nontoxic polymer with low conversion value when attached to biological substrate, and well-controlled anionic polymerization, which can produce monofunctional PEG (French *et al.*, 2009).

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Therefore, PEG is a highly investigated polymer for modification of biological macromolecules and surfaces for many pharmaceutical formulations as well as biotechnical applications (Ahmed and Narain, 2013, Fant *et al.,* 2010, Wang *et al.,* 2010).

Fourier transform infrared (FTIR) is a powerful tool that allows information collection on complex biological systems in a non-invasive way (Ami *et al.,* 2011). This technique has been widely employed in recent years to study and monitor in situ biological processes (Ami *et al.,* 2005). In particular, FTIR spectroscopy enabled the characterization and identification of spectral markers of polyplexes of macromolecules (Pijanka *et al.,* 2010).

A well-established technology applied to study the interaction of anticancer drugs and other cytotoxic agents in solutions with proteins and nucleic acids is known as FTIR spectroscopy. FTIR is unique in that it can generate the structural information of an entire molecule and all conformations within the sample in a single snapshot. A systematic study of nucleic acids requires a technology which is fast and non-destructive in addition to operating with small sample amounts (Jangir *et al.,* 2010).

FTIR results are very valuable, and may perhaps lead to design of better and more effective PEG-DNA biocomplexes structure interactions and developing chemical probes of nucleic acid structure (Charak and Mehrotra, 2013).

Detailed structural analysis of PEG_{400} complexes with calf thymus DNA ($_{ct}$DNA) are not well known (Froehlich *et al.,* 2011). Therefore, it was of immense interest to investigate the interaction of PEG with DNA in different incubation time, using the FTIR spectroscopy method.

The main aim of the current study is to assess the influence of incubation time on PEG_{400}-$_{ct}$DNA biocomplex formation at ratio 1:1, and analyzing the resulting effects on binding affinity.

EXPERIMENT

Biocomplexes samples PEG_{400}-$_{ct}$DNA for FTIR were prepared by rapid addition of equal volumes of $_{ct}$DNA to PEG_{400} solution and kept for three different incubation times (Zero, 1hr, 48 hrs.) to allow biocomplexes reaction. The composition of complexes is defined by the complex charge ratio, which is the ratio of PEG to DNA negative charge, monomer to nucleotide ratio based. To improve the stability of the complexes, samples were prepared in buffered purified water, the plots of wave number and absorption of $_{ct}$DNA in plane vibration related to A-T, G-C, base pairs and PO2 stretching vibration versus PEG were obtained after peak normalization (Adali *et al.,* 2013).

FTIR Instrument

FTIR spectra were obtained with Varian 660-IR spectrophotometer, which is equipped with DTGS (deuterated triglycine sulphate) detector and KBr beam splitter assembly. Spectra were collected after three certain incubation times of PEG with ctDNA solution. Background spectra were collected before each measurement. Interferogram were accumulated over the spectral range 4000-400 cm^{-1} with a nominal resolution of 2 cm^{-1} and a minimum of 50 scans. The water subtraction was carried out with 0.1 mM NaCl solution as a reference at pH 7.4. All measurements were carried out at room temperature 22 ºC and controlled ambient humidity of 43% RH. The difference spectra of [($_{ct}$DNA solution + PEG_{400})-($_{ct}$DNA solution)] were then obtained, the spectra are smoothed using Savitzky-Golay procedure (Alex and Dupuis, 1989).

RESULTS AND DISCUSSION

In this paper, we measured the FTIR absorption spectra of PEG_{400} and $_{ct}$DNA individually, then and the PEG_{400}-$_{ct}$DNA biocomplexes at three different times. Incubation times were time zero, after 1hr and after 48 hr. The resulting spectra are reported in figures 1, 2 and 3 respectively.

A single measurement can obtain the infrared response of nucleic acids and PEG polymer. However, three measurements are applied to characterize the band peak positions and to assign them to different biomolecule vibrational modes. Thus, intrinsic heterogeneity of biological samples are present even in the homogenous samples (Ami *et al.,* 2011)

The FTIR spectral features and major peaks for PEG_{400} were presented in figure 1, $_{ct}$DNA fingerprint spectra were presented in figure 2, and PEG-ctDNA biocomplex samples at the different incubation times were presented in figure 3 below.

FTIR Characterization of PEG_{400}

FTIR total absorption spectra analysis of PEG_{400} figure 1 showed many characteristic peaks at (885, 944, 1096, 1146, 1287, 1296, 1349, 1556, 1772, 2710, 2886, and 3711 cm^{-1}). The strong absorptions of PEG_{400} are assigned to -CH2CH2- stretching around 2886 and 3749 cm^{1} demonstrating the presence of saturated carbons (CH2CH2) n-

FTIR Characterization of $_{ct}$DNA

FTIR total absorption spectra of ctDNA finger print spectrum presented in figure 2 show $_{ct}$DNA without the addition of PEG_{400}. Indeed, $_{ct}$DNA possess characteristic peaks at (958, 1017, 1086, 1219, 1313, 1398, 1541, and 1651 cm^{-1}). Ring vibrations of nitrogenous bases (C=O, C=N stretching), PO2 stretching vibrations (symmetric and asymmetric) and deoxyribose stretching of DNA backbone are found predominantly in the spectral region 2000 - 750 cm^{-1}.

Therefore, this particular region is of interest. The vibration bands of $_{ct}$DNA at 1716, 1651, 1611, and 1491 cm^{-1} are assigned to guanine (G), thymine (T), adenine (A) and cytosine (C) nitrogenous bases, respectively [13]. Bands at 1219 and 1086 cm^{-1} are most likely related to phosphate asymmetric and symmetric vibrations, respectively (Sun *et al.,* 2011). These are major bands of pure DNA, which are monitored during the current study.

Changes in these bands (shifting and intensity) based on incubation time require further study.

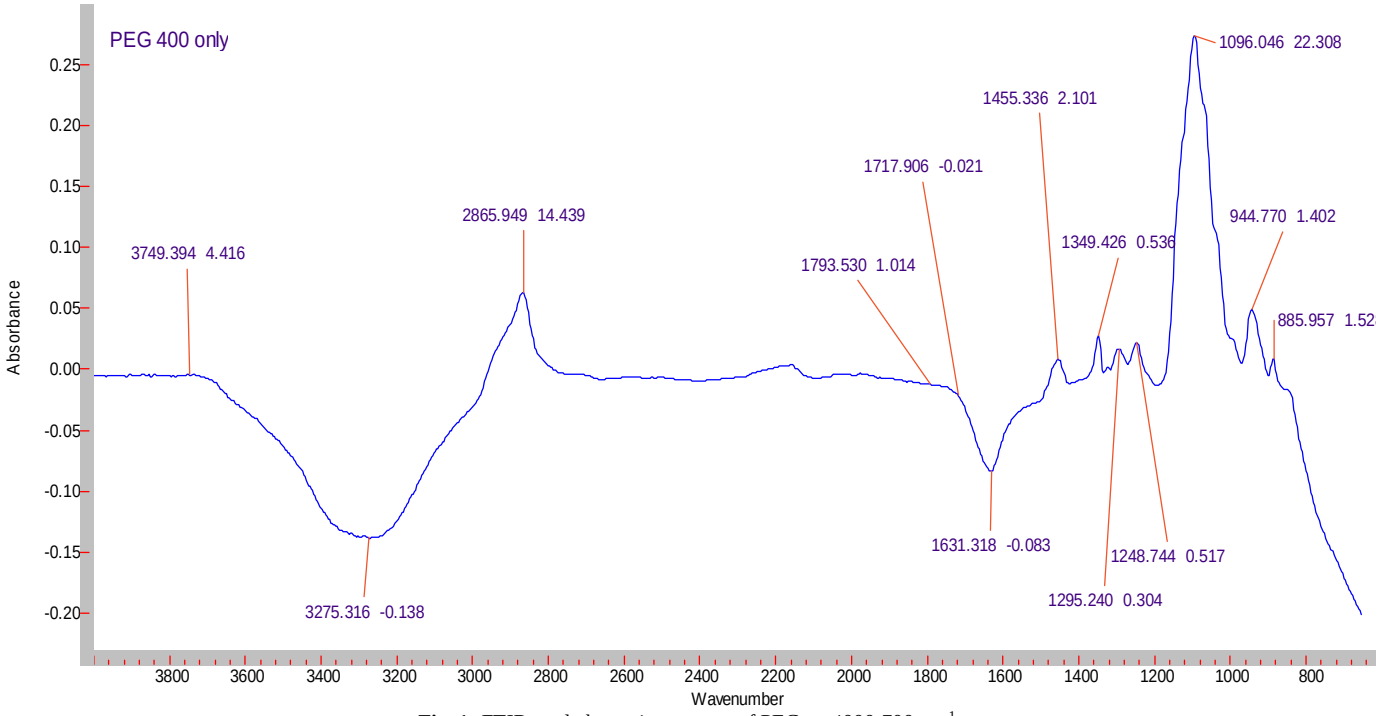

Fig. 1: FTIR total absorption spectra of PEG_{400}. 4000-700 cm^{-1}

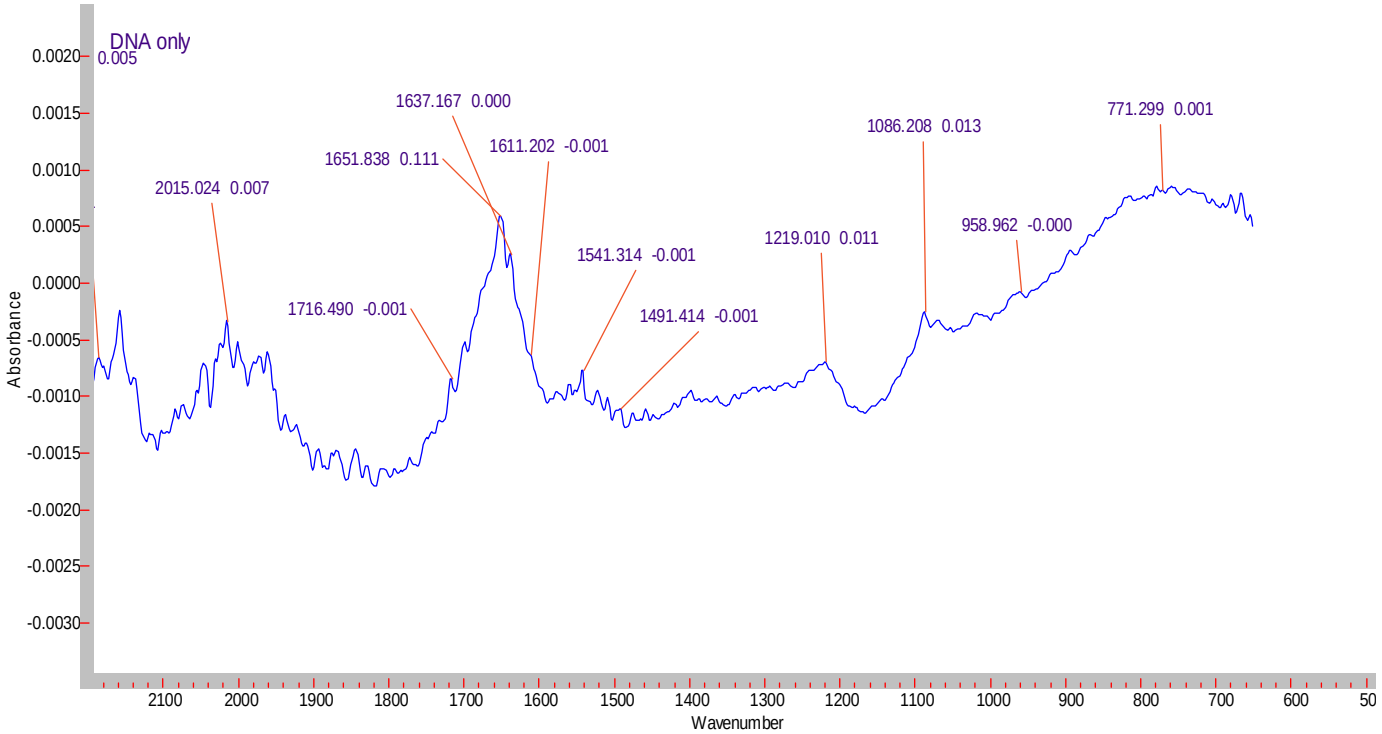

Fig. 2: FTIR finger print total absorption spectra of $_{ct}$DNA, 4000 - 700 cm^{-1}

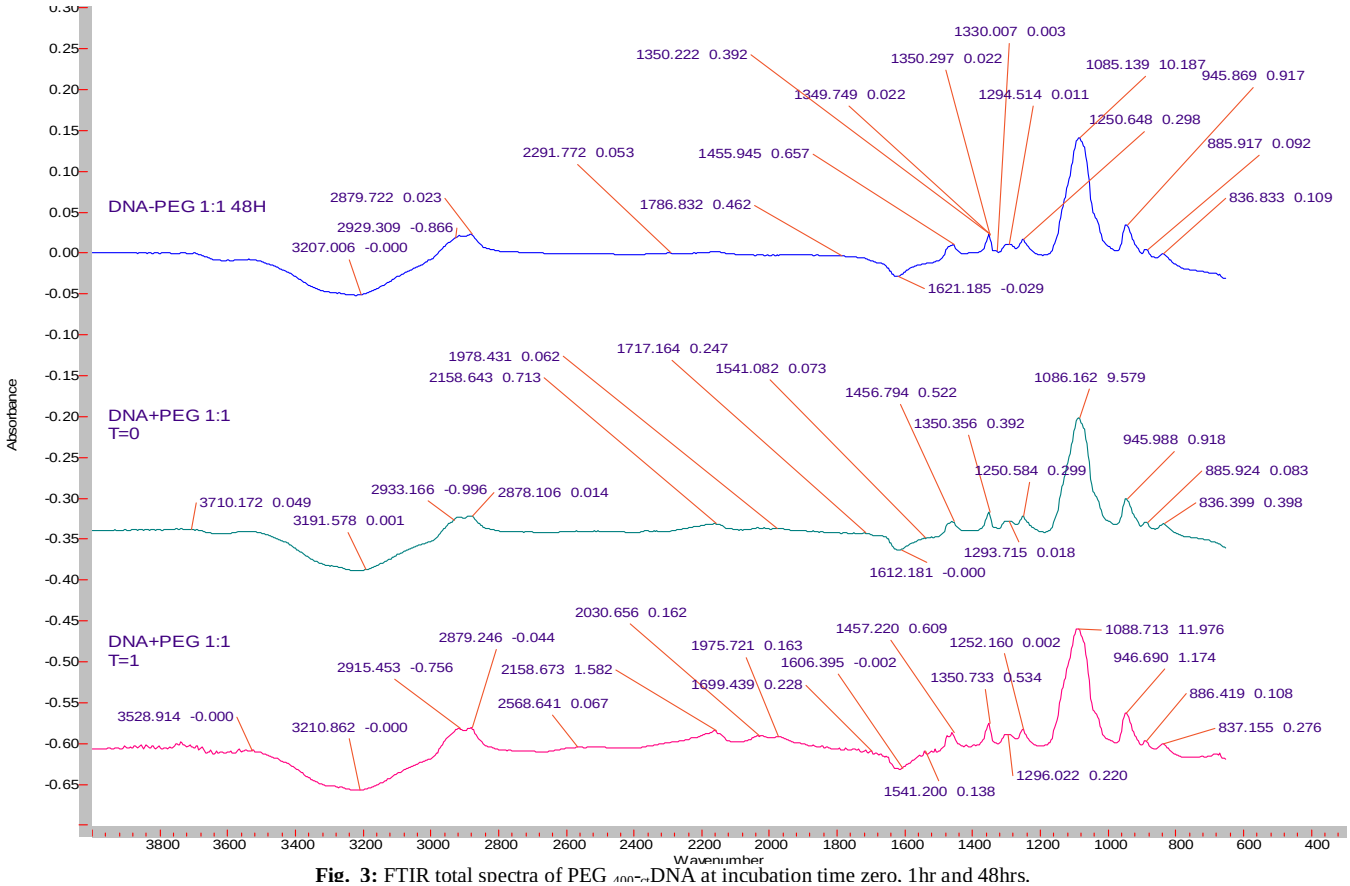

Fig. 3: FTIR total spectra of PEG $_{400}$-$_{ct}$DNA at incubation time zero, 1hr and 48hrs.

Effect of incubation time on FTIR spectra of PEG$_{400}$-$_{ct}$DNA

Spectra features of PEG$_{400}$-$_{ct}$DNA biocomplexes are presented in order to characterize and determine the effect of incubation time on the chemical binding between PEG$_{400}$ and $_{ct}$DNA, FTIR background analysis for pure PEG$_{400}$, and pure $_{ct}$DNA are previously collected. Spectral analysis comparing PEG$_{400}$-$_{ct}$DNA biocomplex samples were also obtained at zero time, after 1hour, and after 48 hours as shown in Figure 3. New absorption peaks can be seen in spectral analysis of PEG$_{400}$-$_{ct}$DNA after zero time, one hour, and 48 hours at 1:1 ratio in buffer pH 7.4 at 2878 and 2933 cm^{-1}, 2875 and 2933 cm^{-1} after 1 hour, and 2879-2929 cm^{-1} after 48 hours. These peaks correspond to some of the characteristic peaks of PEG$_{400}$. Obviously, these peaks were not present in the pure PEG$_{400}$ sample spectra analysis, and the only difference is the addition of PEG$_{400}$ to $_{ct}$DNA, the predominant effect of which is attributed to the incubation time on the chemical binding of PEG$_{400}$ to the $_{ct}$DNA.

PEG$_{400}$ and PO2 Binding

PEG$_{400}$-PO2 interaction was evident from an increase in the absorption and shifting of the PO2 anti symmetric band at 1219 cm^{-1} in the spectra of the PEG$_{400}$-$_{ct}$DNA biocomplexes. The PO2 band at 1219 cm^{-1} shifted toward higher frequencies after zero time to 1250 cm^{-1}, and to 1252 cm^{-1} after 1 hour, returning to 1950 cm^{-1} after 48 hours. The band at 1086 cm^{-1} related to the phosphate symmetric stretching vibration exhibited minor absorption changes and shifted to a lower frequency at 1085 after 48 hours, higher frequency at 1088 after 1 hour, and with no shifting changes after zero time upon $_{ct}$DNA complexation (Pal *et al.*, 2013).

PEG$_{400}$ and base pairs binding

Evidence of PEG-base binding were observed from the spectral changes for free $_{ct}$DNA upon PEG$_{400}$ biocomplexation. After zero time, a minor shifting of the bands at 1716 cm^{-1}(guanine) initially increased the wave number to 1717 cm^{-1} before dropping to 1699 cm^{-1} after 1 hour, and increasing to 1786 cm^{-1} after 48 hours. For cytosine at 1491 cm^{-1}, it has been seen that there is high band shift toward lower wave number after zero time (1456 cm^{-1}), after 1 hour (1457 cm^{-1}), and after 48 hours 1455 cm^{-1}. Over all these changes could be attributed to direct PEG$_{400}$ binding to the base pairs of the ctDNA (Zhang *et al.*, 2005).

CONCLUSION

The spectroscopic results presented here for PEG$_{400}$-$_{ct}$DNA biocomplexes indicate that PEG$_{400}$ binds with $_{ct}$DNA, by weak to moderate complex formation with both hydrophilic and hydrophobic contacts through $_{ct}$DNA base pair, with little binding preference towards phosphate backbone of DNA helix. Binding reaction of PEG$_{400}$ and $_{ct}$DNA proceeds rapidly at room temperature and biocomplex formation varies by time. However,

the presence of the hydrophilic non-ionic moieties PEG on the surface macromolecules decrease their interactions (Ahmed and Narain, 2013), hence increasing the stability of biocomplexes in solution and decreasing their non-specific interactions *in-vivo* and *in-vitro*, which is presumably one strategy for effective gene delivery (Ping *et al.*, 2013, Wang *et al.*, 2001). The mode of interaction between PEG_{400} and $_{ct}$DNA was via outside groove binding or electrostatic binding modes. The study has shown the significant effect of incubation time in PEG_{400}-$_{ct}$DNA biocomplex formation. FTIR technique in this experiment was rapid, offering good stability, and is a valuable tool for studying the biological properties of PEG_{400} with $_{ct}$DNA biocomplexes.

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