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Full Length Research Paper

Interaction of DNA and polyethylenimine: Fourier-transform infrared (FTIR) and differential scanning calorimetry (DSC) studies

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Polyplexes, which are formed spontaneously between cationic polymer and negatively charged nucleic acids, are commonly used for gene and oligonucleotide delivery *in vitro* and *in vivo*. Many kinds of cationic polymers have been used to condense DNA by electrostatic interaction into small particles (Polyplexes), for protecting the DNA from degradation and enhancing its uptake via endocytosis. Polyethylenimine (PEI) appears to be one of the most advanced delivery materials that can condense DNA efficiently forming PEI/DNA complexes. The PEI/DNA interaction was evaluated by measuring zeta potential and DNA secondary structures using Fourier-transform infrared (FTIR) technique. IR results show that the antisymmetric PO^{2-} vibration of DNA (at 1236 cm^{-1}) shifts toward lower frequencies when complexed with PEI. The secondary conformation of DNA in aqueous solution clearly remains in B-form. Thermal behaviors of plasmid DNA, polymer and their formed complexes were recorded to give insights into their conformational changes when temperature was raised. DSC results showed a disappearance of the main DNA transition band at 94°C at all examined N/P (0.3 to 5) ratios of PEI/DNA complexes. These findings could be useful for the developing polymer-based gene delivery systems with better *in vitro* and *in vivo* performance.

Key words: Polyethylenimine, DNA, Fourier-transform infrared (FTIR), differential scanning calorimetry (DSC), zeta potential.

INTRODUCTION

Gene therapy has become the research focus for many laboratories in pharmacy, medicine, biochemistry and chemical engineering worldwide. However, the growing potential of gene therapy for both genetically based and infectious diseases will not achieve its goals until the issue of gene delivery will be resolved.

Recently, a lot of attention is directed to the new development on gene delivery (Hosseinkhani, 2006; Hosseinkhani et al., 2008). The use of cationic polymers confers several advantages, due to the fact that they are durable, inexpensive, easy to prepare, purify and chemically modify, as well as their enormous stability (Lungwitz et al., 2005). Numbers of different cationic polymers are under investigation for delivering DNA (Barteau et al., 2008; Midoux et al., 2009). When DNA is complexed with cationic polymers, the resulting polymer/DNA complexes (polyplexes) are able to transfect eukaryotic cells both *in vitro* and *in vivo* (Hosseinkhani et al., 2004; Hsiue et al., 2006; Roques et al., 2009).

Among the polycations presently used for gene delivery, polyethylenimine (PEI) has a prominent position due to its potential for endosomal escape. It is a cationic polymer that has long been used in non-pharmaceutical processes, including water purification and paper and shampoo manufacturing. PEIs are also reported to be

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relatively safe for internal use in animals and humans (Braun et al., 2005). These polymers are highly positively charged because of the presence of protonable amino nitrogen at every third atom of the polymeric backbone. As the polymer contains repeating units of ethylamine, PEIs are also highly water soluble. Over the past decade, PEIs have been extensively studied as a vehicle for nonviral gene delivery. In fact, PEI is one of the most promising nonviral vectors and has produced excellent transfection efficiencies in both *in vitro* and *in vivo* models (Prevette et al., 2008). Polycation-mediated gene delivery is based on electrostatic interactions between the polycations and the negatively charged phosphate groups of DNA. PEI condenses the DNA in solution and the resulting PEI-DNA complexes, carrying positive surface charges that interact with the negatively charged cell membrane, are readily endocytosed by many cell types (Yanga et al., 2006, Mady et al. 2011).

Fourier transformed FTIR spectroscopy has been used to characterize the secondary structure of DNA and to assess DNA interactions with drugs, organic chemicals and cationic metal ions in aqueous solution (Tajmir et al., 1995; Neault et al., 1996; Neault and Tajmir, 1999; Chooakoonkriang et al., 2003a). Thermodynamic profiles from differential scanning calorimetry offer insights into the molecular forces that cannot be obtained by spectroscopic or other analytical studies (Chaires, 1997; Mrevlishvili et al., 1998). However, only a few attempts of characteri-zing the nature of cationic polymer/DNA complexes in aqueous solutions by FTIR spectroscopy and differential scanning calorimetry (DSC) to correlate their transfection efficiency have been made. In the present study, the mechanism of interaction between the PEI and DNA is further studied by FTIR spectroscopy by measuring the alteration of DNA secondary structures in solution before and after the addition of polymers. Also, DSC is used to understand the thermodynamics of the cationic polymer binding to plasmid DNA.

MATERIALS AND METHODS

25 kDa branched PEI is purchased from Sigma–Aldrich (Madrid, Spain), salmon sperm DNA from MP Biomedicals (llc, France). All other reagents are of analytical reagent grade and the doubly distilled water (H_2O) is used all along.

Preparation of PEI/DNA complexes

7-Polyplexes formation was performed according to Boussif procedure (Boussif et al., 1995) as follows: PEI/DNA com-plexes were prepared at different (N/P) ratios. The ratio of PEI nitrogen to DNA phosphates (N/P) is important in terms of transfection efficiency and cell toxicity. The N/P ratios were calculated based on PEI nitrogen per nucleic acid phosphate (1 µg of DNA is 3 nmol of phosphate, and 1 µl of PEI stock solution (36 nM) contains 10 nmol of amine nitrogen). Appropriate amounts of DNA and polymer solution were mixed and vortexed. The resulting polyplexes were incubated for 30 min at room temperature before use.

Fourier-transform infrared (FTIR) spectroscopy

Microscopic FTIR-spectra of lyophilized samples (DNA, PEI 25k, and PEI/DNA complexes) were investigated using Jasco, FT/IR 460 plus (Japan) spectrometer. Sixteen scans in the range 400 to 2000 cm^{-1} with scanning speed 2 mm/s were signal averaged at a resolution of 2 cm^{-1} at room temperature. The complexes (prepared as the standard procedure) containing 300 µg of DNA were lyophilized, and then the powder was dispersed in KBr and the mixtures compressed to form disks.

Zeta potential measurements

Zeta-potential measurements for different polyplexes were carried out in the standard capillary electrophoresis cell of the Zetasizer 2000 HS (Malvern, UK) at 25°C. Average values were calculated with the data from four runs.

Differential scanning calorimetry (DSC)

Calorimetric measurements of samples (DNA, PEI 25k, and PEI/DNA complexes) were carried out on DSC-60 Shimadzu (Japan). Measurements consisted of a single upward scan from 20 to 150°C/min at a heating rate of 5°C/min. Data analysis was performed using the Tasys software supplied with the instrument.

RESULTS AND DISCUSSION

Microscopic FTIR-spectra of PEI/DNA polyplexes

FTIR can be potentially used to obtain useful in-formation about the fine structure of both polymers and nucleic acids upon their association. The FTIR absorption spectra of the DNA, 25 k PEI and PEI/ DNA complexes (of different N/P) were measured.

The infrared spectrum of DNA in the region of 2000 to 400 cm^{-1} contains a variety of information on the conformational arrangement. Polymers with amido or amine groups can interact with DNA via electrostatic attractions after their protonation. However, the nitrogen base region (1700 to 1500 cm^{-1}) in the DNA spectrum overlaps with the amine signals of the polymers, requiring spectra between 1300 and 800 cm^{-1} to be designated for monitoring this possible interaction. Typical IR spectra of DNA; PEI and PEI/DNA complexes at N/P 2 are shown in Figure 1. The absorption band at 1236 is known to be the antisymmetric stretching vibration of the phosphate groups (PO^{2-}), IR absorption at 1064 cm^{-1} is typically assigned to the vibration of ribose (C-C sugar) and absorption at 965 cm^{-1} is as an indication of the existence of DNA. The band at 1236 cm^{-1} is a marker of B-form DNA (Hackl et al., 2005). However, the antisymmetric vibration of PO^{2-} (1236 cm^{-1}) exhibits a greater change either in band position or intensity before and after DNA condensation. The secondary conformation of DNA in aqueous solution clearly remains in B-form because the assignments of DNA at 1236, 1086, 1064 and 956 cm^{-1} were observed.

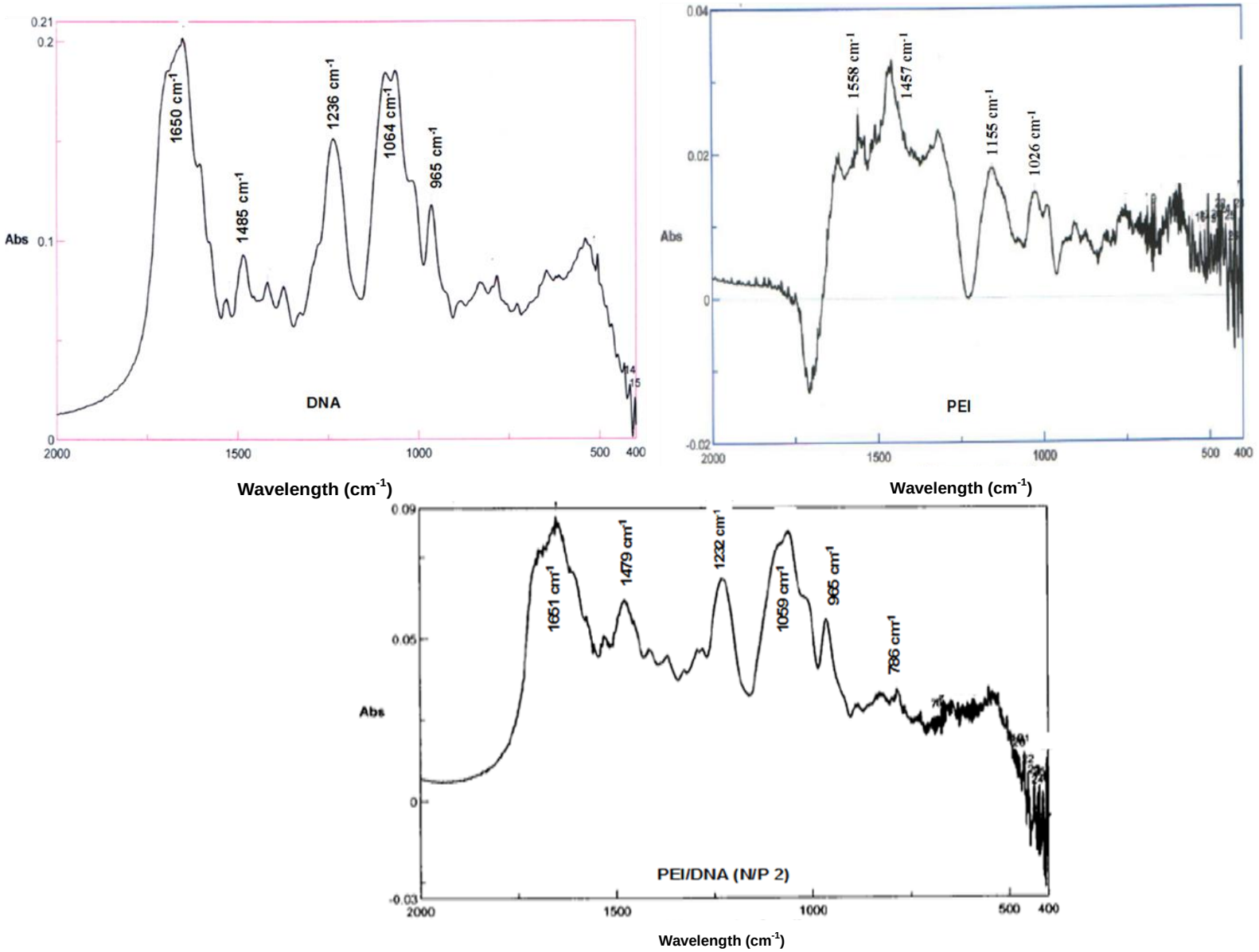

Figure 1. FTIR absorption spectra of salmon sperm DNA; PEI and PEI/DNA complex at N/P 2.

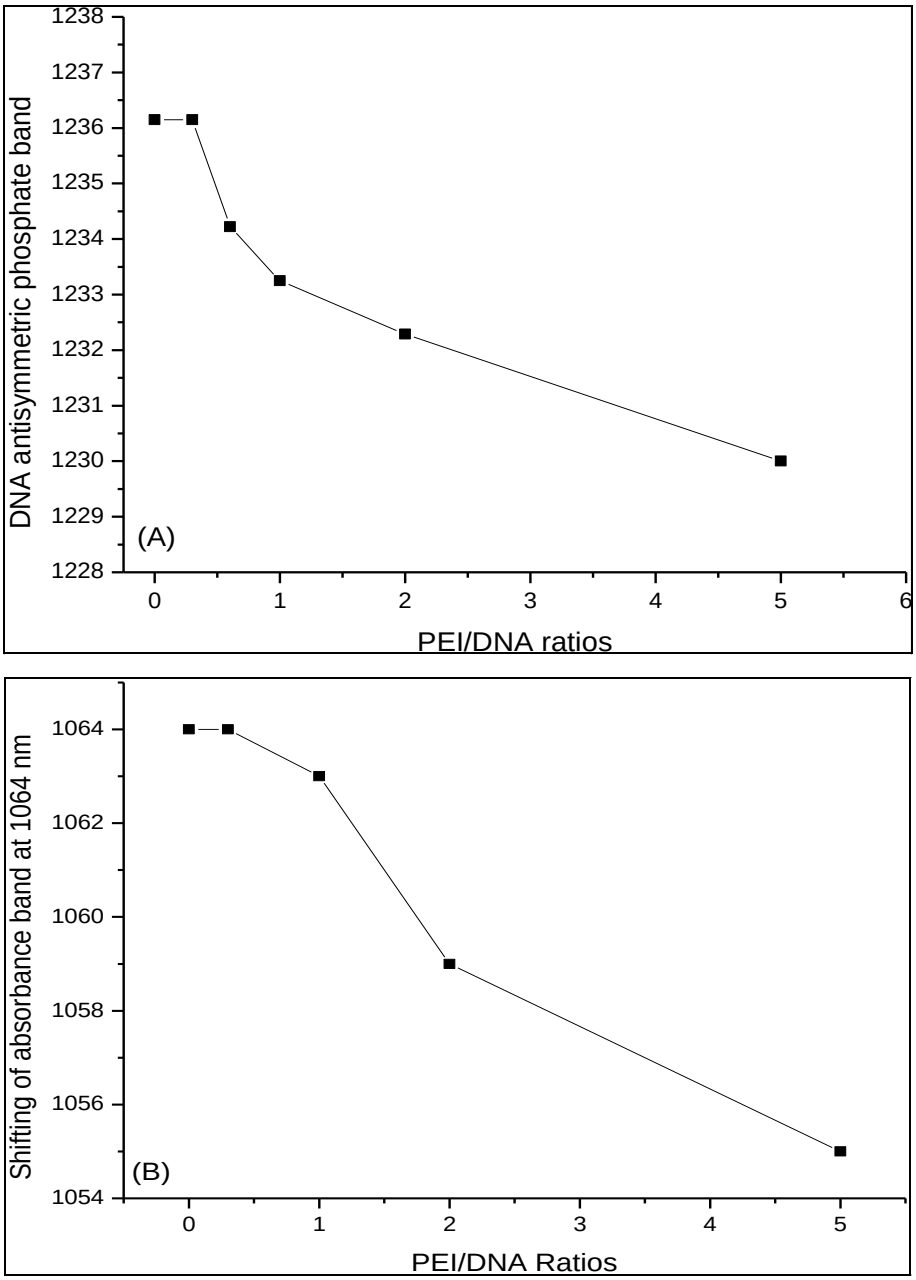

Figure 2. Plots of selected peak position of FTIR spectra of PEI/DNA complexes as a function of various (N/P) ratios of PEI/DNA complexes (A) 1236 cm^{-1}, (B) 1064 cm^{-1}.

At low PEI/DNA ratio (r = 0.3) (data not shown), the spectra of DNA in complexes were nearly identical to naked DNA and the antisymmetric phosphate band stands is close to 1236 cm^{-1} (Figure 1). This indicates that there is fairly less interaction between DNA and PEI. At PEI/DNA ratio of 2, a shift of the antisymmetric phosphate band from 1236 to 1232 cm^{-1} was observed (Figure 2A). The shift to a lower frequency means an occurrence of a stronger interaction between PEI and DNA and smaller particles were formed. Also, this shift to a lower frequency suggests an increase of DNA hydration state (Choosakoonkriang et al., 2003b). These alterations are all factors which may result in the higher transfection efficiency of polyplexes. Binding of DNA to PEI is thought to be mainly driven by entropic forces arising from the release of counter ions. However, other interactions, such as hydro-gen bonds and Van der Waals forces may also contribute to complex formation. Polycations with a high charge density, such as PEI can release more counter ions upon binding with DNA, thus forming more stable

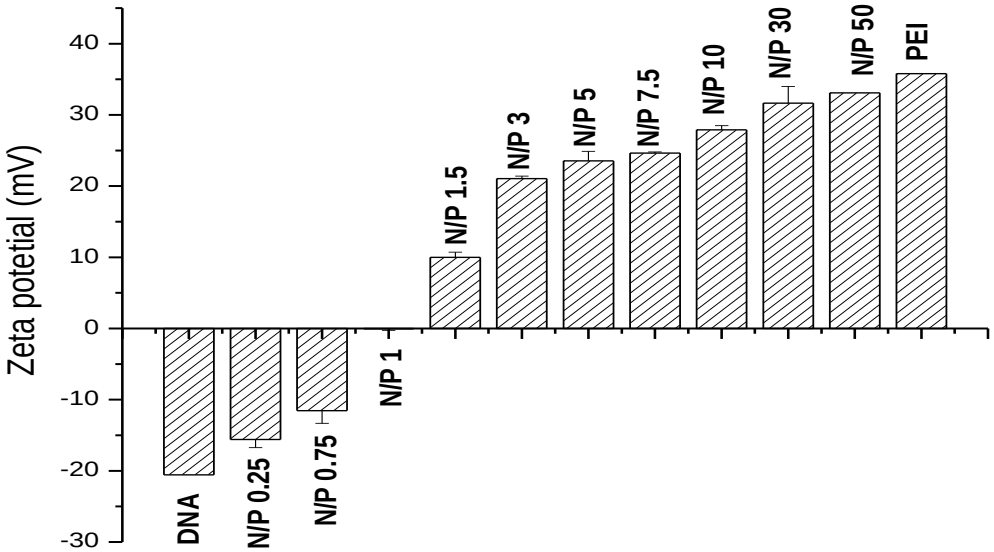

Figure 3. Zeta potential of various N/P ratios of PEI/DNA complexes (n=4).

complexes (van de Wetering et al., 1997). The overall helical form of the DNA does not seem to be affected after complexation with PEI, since DNA remained in its B form, independent of N/P ratio (ratio of nitrogen-containing groups of the polymer to phosphate groups of the nucleic acid).

The observed spectral changes for the phosphate bands were due to a direct interaction between the cationic polymer and the phosphate group of the DNA double helix to form the polyplexes. This interaction was due to the electrostatic interaction between the negative charges of the phosphate group and the positive amine group of the PEI.

The carbonyl stretching vibration of furanose ring (of deoxyribose sugar of the DNA) appeared as a strong band at 1064 cm^{-1} (Banyay et al., 2003). It was unchanged till N/P 0.6, and then it was shifted to lower wavenumber 1054 cm^{-1} after the addition of PEI (Figure 2B). This shift to lower wavenumber may be due to direct interaction between the PEI and deoxyribose sugar of the backbone which may alter the hydrogen bonding profile of this group.

Zeta potential of PEI/DNA polyplexes

Zeta potential is a measure of the surface electrical charge of the particles. Gene transfer was optimal when particles were positively charged and able to bind to anionic cell surface proteoglycans to be taken up by the cells (Honore et al., 2005; Guzin and Julide, 2006). Values of the zeta-potential of PEI/DNA indirectly reflect complex surface net charge and can therefore be used to evaluate the extent of interaction of the cationic polymer with DNA (Mady et al., 2004). On this basis, zeta potentials of DNA, 25 k PEI and their complexes at different N/P ratios were investigated (Figure 3). The zeta potential of DNA was found to be around -21±0.6 mV and that of PEI polymer has a positively charged of 35.8±0.05 mV. PEI/DNA complexes had a negative surface charge for low N/P ratios up to N/P 1 at which the complexes had a nearly neutral surface charge (-0.05±0.21 mV). At N/P ≥ 1.5, the zeta potential became positive and reached to 28±0.7 mV at N/P ratio ≥ 10.

Differential scanning calorimetry of PEI/DNA polyplexes

Differential scanning calorimetry (DSC) was considered as a convenient measure for the stability of the poly-nucleotide involving thermal unfolding of the double helix. It was used to characterize the protection of DNA by the cationic polymer 25 k PEI at different N/P ratios. The scan of heat flow (mW) versus temperature for salmon sperm DNA (Figure 4) exhibited two major peaks. A small peak with splits was observed at range of 50 to 70°C and the largest melting temperature peak at 94°C. This peak identifies DNA denaturation (dissociation of DNA double strands). The temperature transition around 50 to 70°C could be attributed to the process of supercoiled plasmids to deform a liquid crystalline phase. Also, it is known that heating will cause the conversion of supercoiled DNA to nicked-circular DNA (Cherng et al., 1999). This transition may be due to limited melting of a close circular plasmid DNA resulting in a change in the twist and writhe status of the conformation. The formation of melted regions within supercoiled plasmid DNA reflects an unwinding process indicating topological changes. The 25 k PEI polymer had a characteristic peak at 65.5°C (Figure 4).

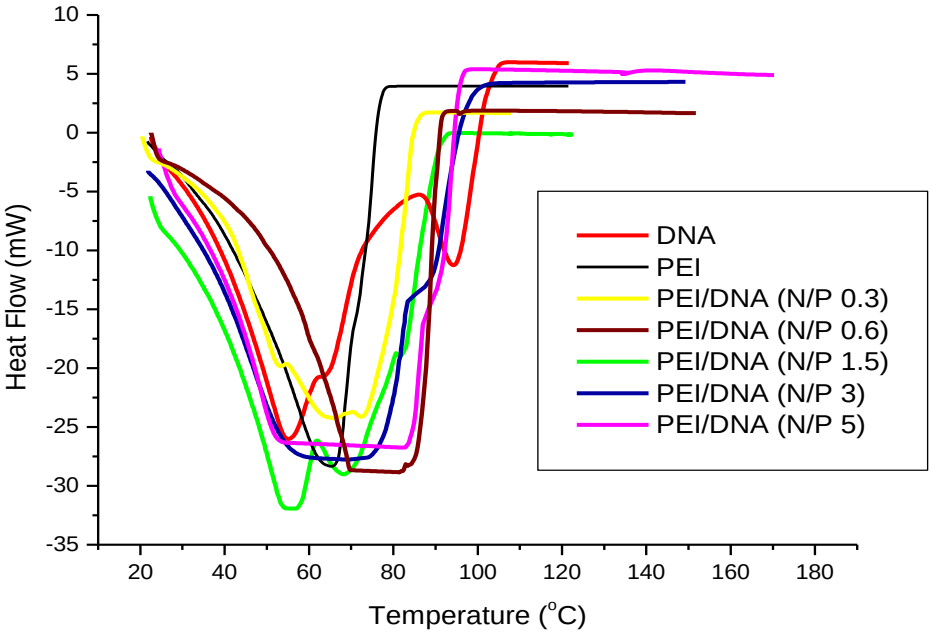

Figure 4. DSC thermograms as heat flow recorded against the temperature of salmon sperm DNA, 25k PEI and PEI/DNA complexes at various N/P ratios and a heating rate of 5°C/min.

There is a disappearance of the largest melting temperature peak of DNA at 94°C, which identifies DNA denaturation or dissociation of DNA double strands, (at all examined N/P ratios from 0.3 to 5). This may be attributed to the protection of DNA by the cationic polymer 25 k PEI. The binding of the cationic polymer to plasmid DNA protects the melting process of plasmid DNA and more energy is required for the complete DNA denaturation. However, there is broadening and shift in the transition temperature of the cationic polymer PEI from 65.5 to 67.5°C at N/P ratios of 3 and 5, respectively. This shift may be due to the fact that the thermal transition of the cationic polymer is ‘‘restricted’’ by the presence of DNA. On the basis of these results, we conclude that the positively-negatively charged interaction retards the endothermic transitions of both cationic polymer and plasmid DNA molecules. The thermal effects shown by cationic polymer–plasmid DNA complex formation will be correlated with known structural features for better designing cationic polymer-based gene delivery systems.

Conclusion

We have demonstrated the structure-function relationship of PEI/DNA complexes and the DNA antisymmetric PO^{2-} vibration is sensitive to the binding of cationic polymers. FTIR data has shown a reduction in the frequency of the asymmetric phosphate stretching vibration of plasmid DNA after its complexation with PEI, which may be attributed to electrostatic interactions between DNA and the polymer. Although the shift of the PO^{2-} antisymmetric band to lower frequencies was found after complexation, the presence of cationic polymers does not alter the secondary structure of DNA from the B conformation in buffer solution. DSC results showed a disappearance of the main DNA transition band at 94°C at all examined N/P (0.3 to 5) ratios of PEI/DNA complexes. These findings could be very useful for the development of polymer-based gene delivery systems with better performance *in vitro* and *in vivo.*

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