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Predicting the UV Spectrum of Oligodeoxynucleotide by 2D-Matlab

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Abstract. A 2D-Matlab model was developed to predict the UV absorbance spectra and thus concentration of oligodeoxynucleotide samples. This model will be valuable for researchers designing experiments involving oligodeoxynucleotides. Initial data was acquired from UV absorbance spectra of oligodeoxynucleotides 23, 24 and 34 bases long. The model, which would predict the concentration of DNA from the R_{260} value, is shown to predict absorbance spectra and the extinction coefficient of DNA as shown when compared across a range of concentrations.

Introduction

Application of small molecules for DNA studies have been of great interest to some researchers [1-3]. Investigating the binding of small molecules to DNA has been of great interest due to the importance in understanding the drug DNA interactions and the consequent design of new efficient drugs targeted to DNA [4-6]. It is important to accurately determine the concentration of the components, which is usually achieved via spectrophotometric analysis. Absorbance measurements are straight forward as long as any contribution from contaminants and the buffer components are taken into account. In addition to fluorescence assays, they are less prone to interference than single wavelength (A_{260}) measurements and are also simple to perform [7,8]. However, absorption of the nucleic acids is measured at several different wavelengths to assess purity and concentration of DNA. A_{260} measurements are quantitative for relatively pure nucleic acid preparations in microgram quantities. Absorbance readings cannot discriminate between DNA and RNA; however, the ratio of absorbance at 260 and 280 nm can be used as an indicator of nucleic acid purity [9,10]. In this paper, the use of various ssDNA and duplex simulated the UV spectrum of absorbance, extinction coefficient of the oligonucleotide at wavelength of 260 nm and concentration of sample. They are calculated using a Microsoft Excel application [11,12] and with Matlab software.

Experimental details

The ssDNA oligonucleotide probes used were 23, 24 and 34 bases in length. The sequence of this ssDNA consist of 5'-ATG AGA ATG AAC TCC AAC TTT AA-3'(23 bases), 5'-GAT GGA AAC GGT AAC GAA TCT GAA-3'(24 bases) and 5'-CAG AGC CTA GTC TAT CAA TCA TTT CCT TGG TGT T-3'(34 bases), respectively. The sequence of this complementary (duplex) was CTT CGT CGG T (11 bases). Firstly, the absorbance and concentration can be predicted by excel software [11,12] after that they are simulated using matlab software. However, the values of various parameters are obtained using lambert beer's law, equation (1).

$$R_\lambda = \varepsilon_\lambda l c \tag{1}$$

Where, R_λ is the absorbance value at 260 nm; ε_λ is the extinction coefficient L/(mol.cm) of the oligonucleotide at 260 nm; l is an optical path length in cm; c is the concentration of oligonucleotide in mol/L.

Results and Discussion

Figure 1 (a,b) demonstrates the values of extinction coefficient (ε_λ) of the oligonucleotide at wavelength of 260 nm of ssDNA at 23, 24 bases and 34 bases. It was found that $\varepsilon_\lambda$of ssDNA increased significantly with increasing sequence length and the peak shifted to longer wavelength due to a higher content of C and G bases which are more absorbing. However, the absorption and full width at half maximum were 33.710 nm (0.764) for ssDNA at 23 bases, increasing to 34.191 nm (0.774) at 24 bases. These different values are due to increasing amount of nucleotide bases. Figure 1 (c,d) show the relationship between ssDNA and duplex of sequence lengths 24 and 34 bases. It was observed that the values of ε_λ increased dramatically with high sequence 34 bases at 312x10^3 mol/(L.cm.strand), while the sequence 24 bases decreased at 250x10^3 mol/(L.cm.strand). Furthermore, the predicted uv spectrum of oligodeoxynucleotides had an accurate value with increasing sequence base for each condition.

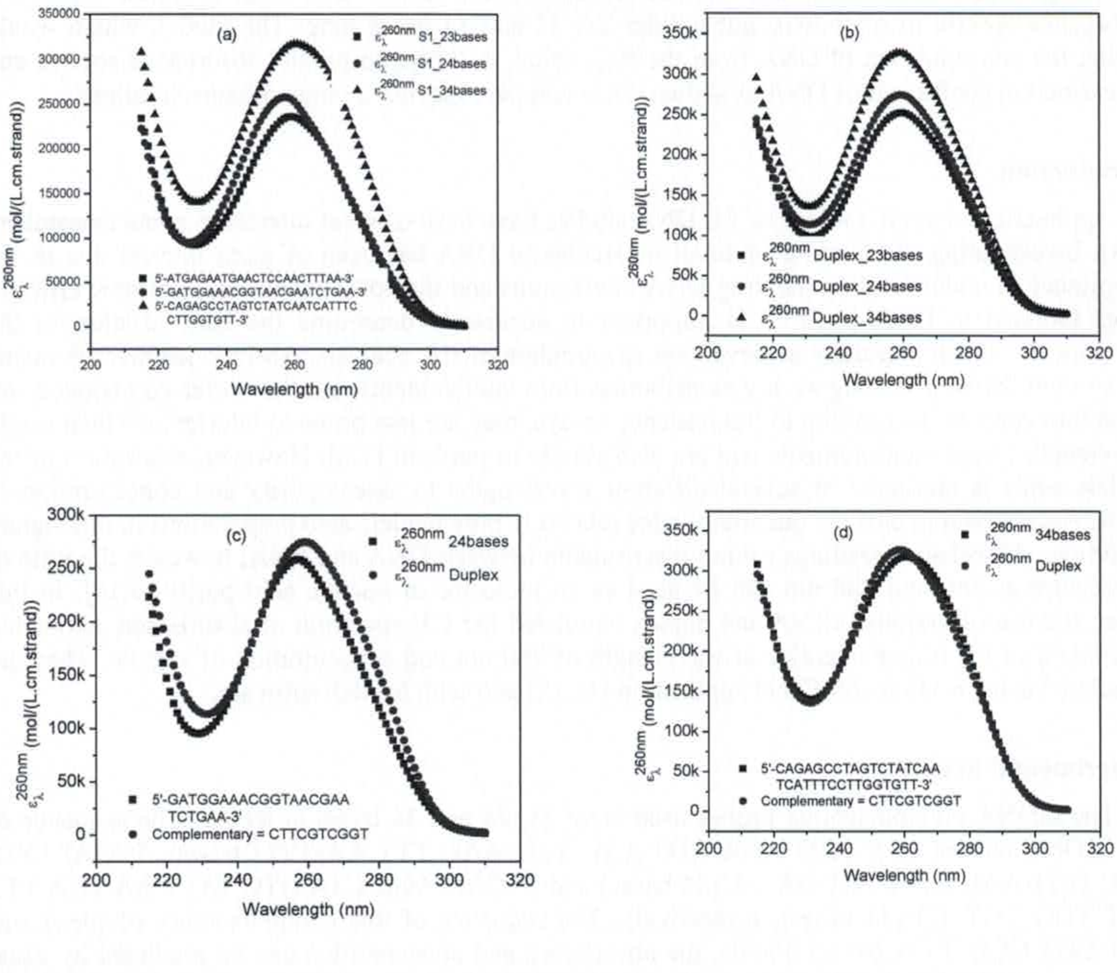

Figure 1. Extinction coefficient (ε_λ) of the ssDNA at wavelength of 260 nm of various sequence (a,b), and hybridization of 24 base and 34 bases (c,d).

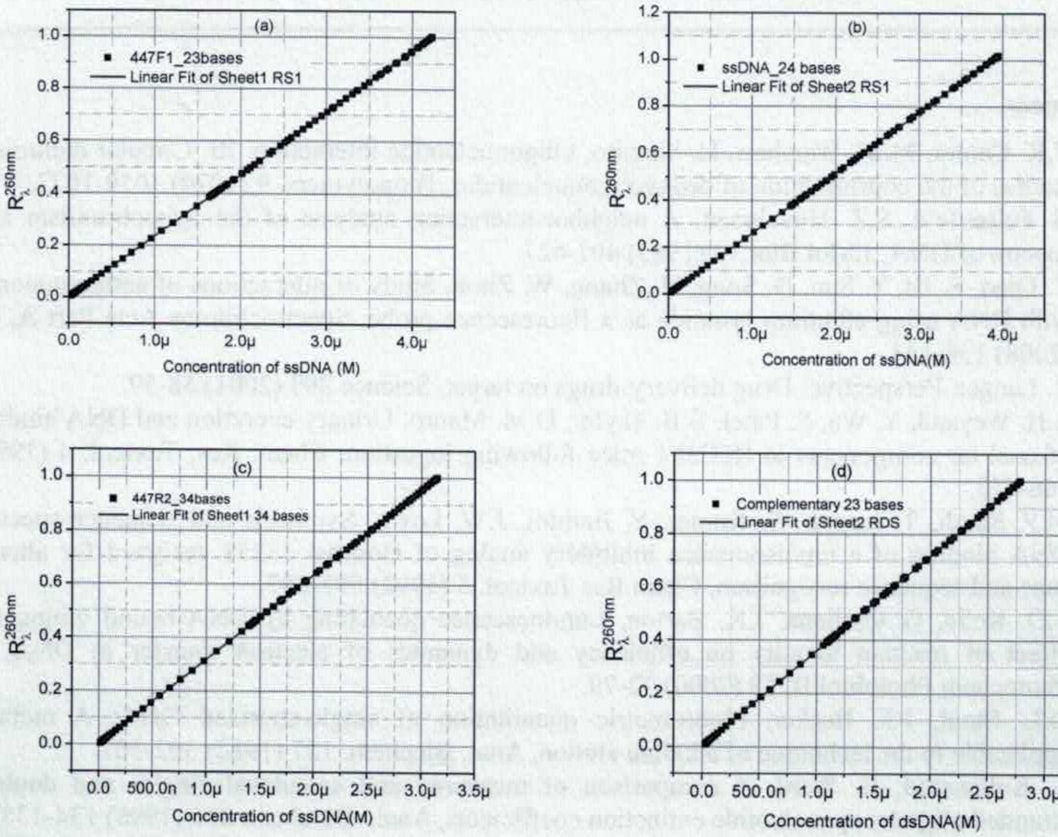

Figure 2. Linear fitting of absorbance spectra and concentration for ssDNA (a-c), dsDNA(d).

Figure 2 presents the predicted concentration for various ssDNA and complementary DNA which was simulated by 2d matlab software but it can be compared with Microsoft Excel. According to figure 2 the gradients were 2.36×10^5, 2.58×10^5, 3.17×10^5 and 3.59×10^5 at 23, 24, 34 and complementary, respectively. It can be observed that the gradient increased with increasing sequence length of DNA. Furthermore, the predicted absorbance values of 3.0μM ssDNA were 0.7, 0.8 and 0.98 for 23, 24 and 34 bases. The benefit of simulation is that it can predict the UV spectra and concentration of the oligonucleotide solution from the nucleotide sequence.

Summary

The absorbance spectra of ssDNA oligodeoxynucleotides was determined with simulation in excel and 2d matlab. Then, the spectra were used to generate a model which would predict the concentration of ssDNA and complementary from the R_{260} value. Finally, the model is shown to predict absorbance spectra, extinction coefficient of oligonucleotide as shown when compared to the increasing sequence base length.

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