Interactions of Novel Sets of Naphthalene Diimides with G – Quadruplex Structures in Human DNA

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We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

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ABSTRACT

The discovery of naphthalene diimide derivatives ability to intercalate DNA and stabilize G – quadruplex structures have drawn interest for investigations. Binding affinities of different NDI derivatives to these DNA assemblies depend on various parameters including the planar structure of ligand and attractive molecule.

Here we present the interaction of *N,N'*-bis(4-sulfophenyl)-1,4,5,8-naphthalene diimide with G – quadruplex DNAs such as telomeric DNA (a-coreTT) & non – telomeric DNA (c-myc). The interactions of the ligand with different types of oligonucleotides were measured by UV – Vis spectroscopy & emission spectroscopy. Additionally, a polymerase chain reaction amplified region of the human beta globin gene was used to confirm the formation of G – quadruplex structures within human DNA via gel electrophoresis method.

Absorption and emission spectroscopy measurements have indicated notable variations between pure compound and the complexes of compound & oligonucleotide. Moreover, gel electrophoresis results have displayed significant difference between the PCR amplified product alone and PCR amplified product with the ligand where the complex migrated slower. Therefore, the examined S-NDI is a promising DNA – binding ligand within the human genome which requires further investigations to understand the reliability and stability of this particular NDI derivative as DNA – binding ligand.

Keywords: Naphthalene diimide derivative, G – quadruplex, human beta globin gene, telomeric region, non – telomeric region

ÖZ

Naftalin diimit türevlerinin DNA'ya bağlanma ve G – dörtlüsü yapılarını stabilize etme kabiliyetinin keşfi, bu tarz araştırmalara olan ilgiyi arttırmıştır. Farklı NDI türevlerinin bu DNA yapılarına bağlanma eğilimleri, hem NDI türevlerinin hemde bu DNA yapılarının düzlemsel yapıları dahil olmak üzere, çeşitli parametrelere bağlıdır.

Burada, *N,N'*-bis(4-sulfophenyl)-1,4,5,8-naftalin diimidinin, telomerik bölgede bulunan DNA (a-coreTT) ve telomerik bölgede bulunmayan DNA (c-myc) gibi G – dörtlüsü yapılarına sahip DNA'lar ile olan etkileşimini gösteriyoruz. Bu türevin bahsedilen farklı oligonükleotit tipleri ile olan etkileşimlerini, UV – Vis spektroskopisi & emisyon spektroskopisi ile ölçülmüştür. Ek olarak, insan beta globin geninin polimeraz zincir reaksiyonu ile çoğaltılmış bir bölgesi, insan DNA'sındaki G – dörtlüsü yapılarının oluşumunu kanıtlamak için jel elektroforez metodu kullanılmıştır.

Absorpsiyon ve emisyon spektroskopisi ölçümleri, tek başına ölçülmüş olan bileşik ve bileşik & oligonükleotit kompleksleri arasında kayda değer farklılıklar olduğunu göstermiştir. Ayrıca, jel elektroforez sonuçları, sadece polimeraz zincir reaksiyonu ürününün ve bu ürün ile bileşikten oluşan kompleksten daha hızlı gittiği görülmüştür. Bu nedenle, incelenen S-NDI insan genomunda DNA'ya bağlanabilme özelliği umut verici olan bir bileşiktir ama bu bileşiğin DNA'ya bağlanma özelliğinin güvenilirliğini ve istikrarını anlamak daha fazla araştırma gerektirmektedir.

Anahtar Kelimeler: Naftalin diimit türevi, G – dörtlüsü yapıları, insan beta globin geni, telomerik bölge, telomerik olmayan bölge

TO

MY FAMILY

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Celsius
3’	Three prime carbon
5’	Five prime carbon
A	Adenine base
Ar	Aromatic
bend	Bending
bp	Base pairs
C	Cytosine base
CD	Circular dichroism
DNA	Deoxyribonucleic acid
dNTP	2’–deoxynucleoside 5’–triphosphates
FNDI	Ferrocenyl naphthalene diimide
FT – IR	Fourier Transform Infrared Spectroscopy
G	Guanine base
g	gram
G4	G – quadruplex
h	hour
H	Hydrogen atom
mL	Milliliter
mM	Millimolar
M_w	Molecular weight
N	Nitrogen atom
NDI	Naphthalene diimide

nm	Nanometers
NMR	Nuclear magnetic resonance
O	Oxygen atom
P	Phosphorus atom
PCR	Polymerase chain reaction
pmol	Picomole
RNA	Ribonucleic acid
S-NDI	Sulfonyl naphthalene diimide derivative
str	Stretching
T	Thymine base
TAE	Tris – acetate - EDTA
TRAP	Telomerase repeated amplification protocol
Tris – HCl	Trisaminomethane hydrochloride
u	Unit
UV	Ultraviolet
UV/Vis	Ultraviolet – visible
V	Volt
V	Volume
µL	Microliter
µM	Micromolar
λ_{exc}	Excitation wavelength
λ_{max}	Wavelength of maximum absorbance

Chapter 1

INTRODUCTION

Naphthalene diimide derivatives have the ability to intercalate DNA. Donor and acceptor chromophores on these molecules revealed electron mobility via intramolecular and intermolecular charge transfer interactions. Results of similar studies on fluorophores conjugated DNA segments could be significant for environmental and biological applications.

Interactions of two types of NDI derivatives – cyclic and noncyclic – with different nucleotides have been studied with a strategy based on the stabilization ability of by NDI molecules. Both types of NDI derivatives have been used as ligands to intercalate g – quadruplex structures of DNA. Comparison of the results showed that cyclic NDI derivatives have higher affinity than noncyclic NDI derivatives which indicates that these bindings depends not only on the attractive molecule but also the structures of ligands as well [1].

Molecular recognition of NDI derivatives by g – quadruplex structures of DNA in telomeres have been studied for the indication of importance of protonation states. Two types of analyses – electronic structure calculations and conformational scans - have been used to determine recognition of these NDI derivatives by G4 structures of DNA. Results of the analyses revealed that interactions of NDI derivatives and G4

structures represented better outcomes in the case of protonation of nitrogen atoms [2].

Also, intercalations of multiple ferrocenyl NDI derivatives to G4 structures of DNA have been studied. Additionally, TRAP assay has been used to determine the telomerase inhibition abilities of all FNDI derivatives. Results revealed that all NDI derivatives showed higher affinity for G4 structures of DNA than both single or double stranded DNA. Moreover, some FNDI derivatives revealed promisingly effective inhibition of telomerase [3].

Moreover, intercalation ability and cytotoxicity of water soluble NDI derivatives bearing specific side chains have been synthesized and studied. Results revealed that cytotoxicity of these novel NDI derivatives had extremely good anti – cancer activity against cancer cells. These NDI derivatives displayed higher affinity with calf thymus DNA than G4 DNA which indicated that these molecules are more effective intercalators to double stranded DNA [4].

Furthermore, interactions between DNA and small ligand molecules have been analyzed and studied. Several instrumental techniques (UV –Vis spectroscopy, fluorescence spectroscopy and cylic voltammetry) have been used to study mechanisms of these interactions. The interactions represented variations in electrochemical properties of nucloebases which can be corresponded to chemical and conformational modifications [5].

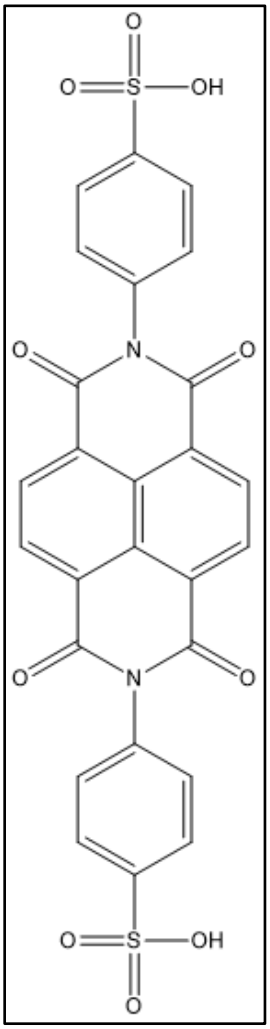

Figure 1.1: The Chemical Structure of Investigated Naphthalene Diimide Derivative

Overall, NDI derivatives are potential DNA intercalators. Their intercalating ability depends not only on their planar structure but also on various parameters such as structure, region and groove dimensions of DNA itself.

The main aim of this project is to investigate and study the stability of the interactions of the NDI derivative (S-NDI) in Figure 1.1, *N,N'*-bis(4-sulfophenyl)-1,4,5,8-naphthalene diimide, as DNA – binding ligand with two different oligonucleotides via various spectroscopy methods; and also to prove the formation of G – quadruplex structures within human DNA via gel electrophoresis method.

Chapter 2

THEORETICAL

2.1 Deoxyribonucleic Acid

Deoxyribonucleic acid (DNA), a hereditary macromolecule, carries the instructions necessary for various crucial mechanisms in the survival of all living organisms and they are located in the nucleus (nuclear DNA) & in mitochondria (mitochondrial DNA) of the cells.

It is composed of four different nucleotides (building blocks of DNA) and two strands of these nucleotides pair up with each other in a specific pattern to form the double helix shape of the DNA [6]. Each nucleotide contains a phosphate molecule, a sugar molecule (deoxyribose) and a nitrogenous base.

2.1.1 Building Blocks of DNA

There are two different types of nitrogenous bases: purines and pyrimidines. **Purines** are adenine and guanine; **pyrimidines** are thymine and cytosine [7]. Each base has different physical and chemical properties and their structures are distinct from one another which can be seen in Figure 2.1 [8]. These four different nitrogenous bases attach to a sugar and a phosphate molecule to form the building blocks of DNA (nucleotides).

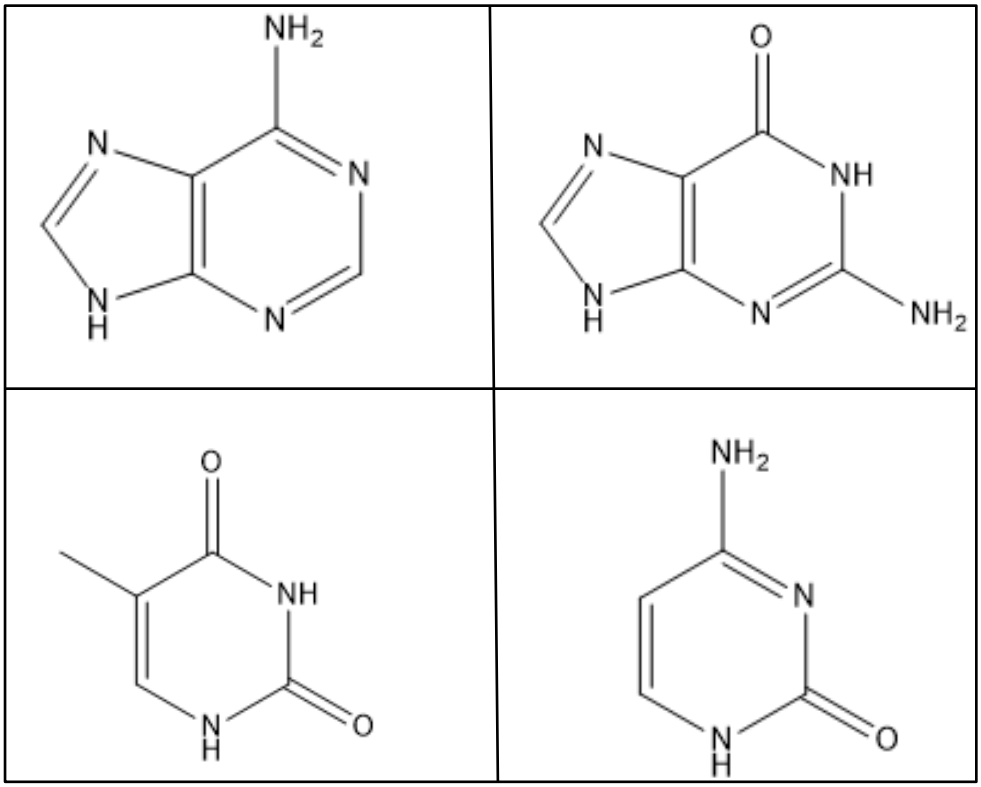

Figure 2.1: Chemical Structures of All Nitrogenous Bases in DNA
Adenine, Guanine, Thymine and Cytosine (from left to right & top to bottom)

2.1.2 Arrangement of DNA Strands

Strands of DNA are arranged in a specific pattern: There are different types of bonds within and between strands. Covalent bonds form within each strand that forms the sugar – phosphate backbone of the DNA and hydrogen bonds form between two strands that forms the double – stranded shape of the DNA [8].

Hydrogen bonds occur between a nitrogenous base of a nucleotide with the base of another nucleotide. These bonds are non – random; adenine pairs up with thymine and guanine pairs up with cytosine [9]. There are two hydrogen bondings between A – T and three hydrogen bondings between G – C.

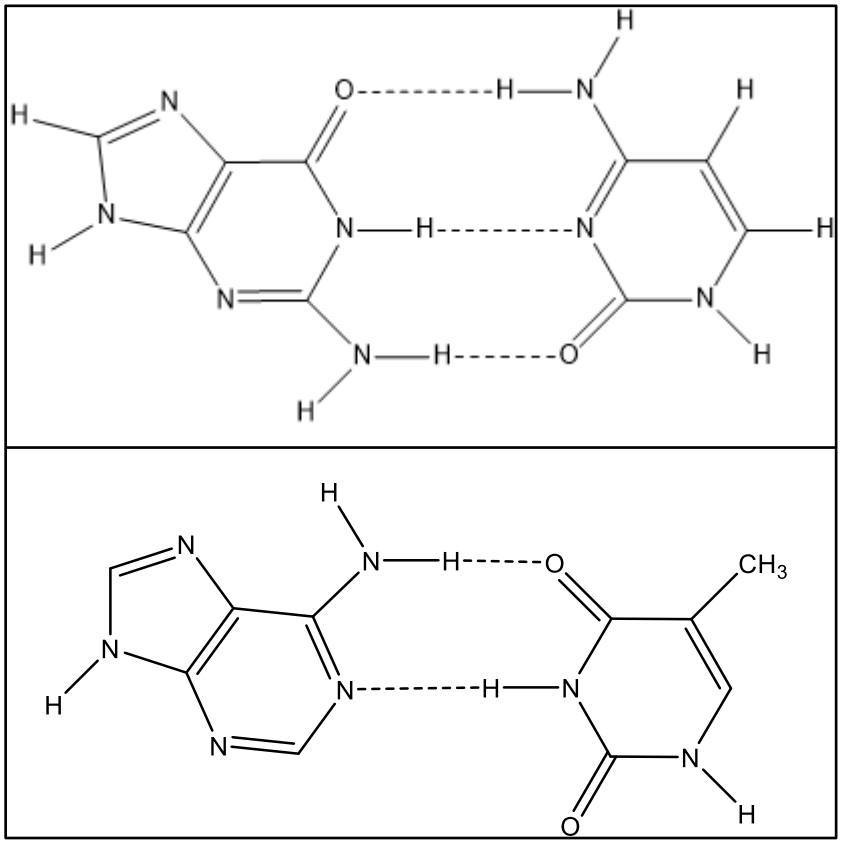

Figure 2.2: Hydrogen Bonds Occuring between G – C (top) and A – T (bottom)

Oxygen and Nitrogen atoms are electronegative. Hydrogen atoms tend to bind to these O and N atoms [10]. Although these type of bonds are very weak, the number of occurence of these bonds between the strands of DNA strengthens the connection of two strands and it can be observed in Figure 2.2.

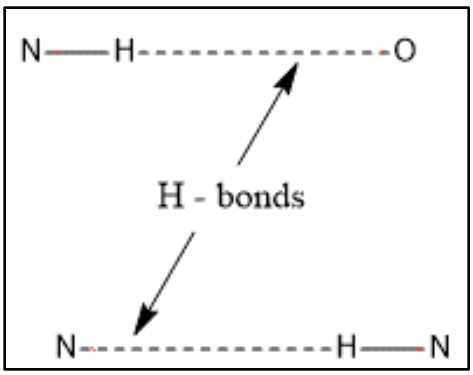

Figure 2.3: Simple Representation of H Bondings with O and N Atoms

Furthermore, covalent bonds occur between phosphate of a nucleotide and sugar of another nucleotide which forms the backbone of the DNA. A 5’ end phosphate group of a nucleotide covalently binds to 3’ end hydroxyl group of another nucleotide within each DNA strand which is illustrated in Figure 2.4 [11].

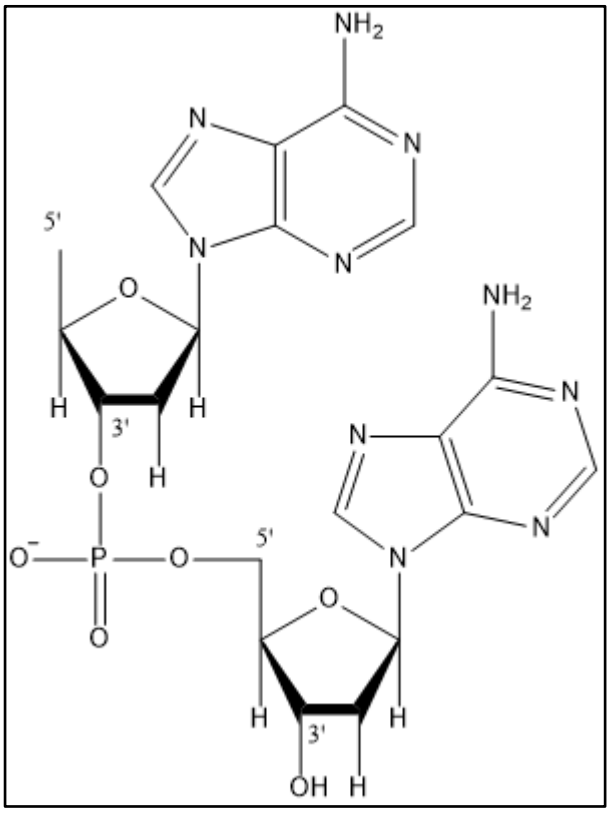

Figure 2.4: Covalent Bond Occurring between 5’ Phosphate and 3’ Hydroxyl Group

2.1.3 G – Quadruplexes

G – quadruplexes are sequences of nucleotides rich in **G** bases (Figure 2.5). These sequences can arise from a single strand or two strands of DNA (& RNA). They form in the ends of chromosomes (telomeres) and in specific regions of various genes [12].

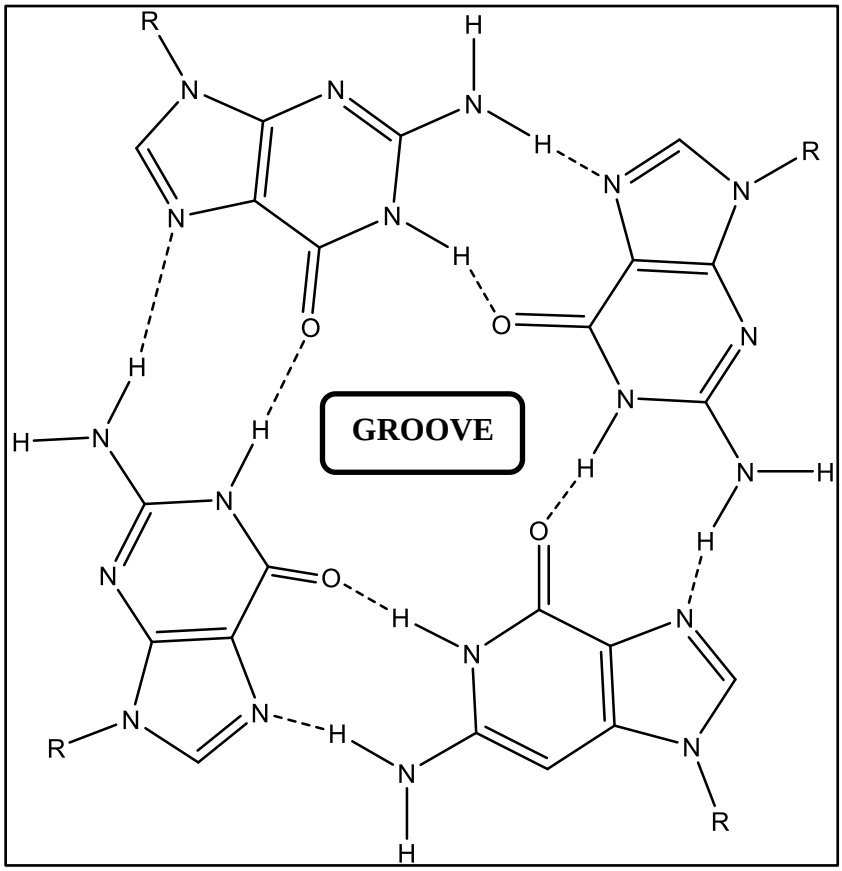

Figure 2.5: An Example of G – quadruplex Structure

Classification of g – quadruplex structures can be done by their locations in a sequence, strand polarities and loop locations that connect a single guanine strand or multiple guanine strands [13]. There are different types of studies for the detection of G4 structures. Most common studies are CD, NMR and UV/Vis spectroscopies. It allows the detection of distinctions between G4 structures. Also, analyses in the human genome showed that there are more than 3000 sequences that have the possibility of forming these structures [13].

Stabilization of G4 in telomeres by specific ligands reduces the telomerase enzyme activity, an active target for anti – cancer drug discovery, which leads to shortening of telomeres [14]. This enzyme is active in most cancer cells. Impairment of its activity prevents elongation of telomeres that ends up in apoptosis of these cells [12].

Moreover, stabilization of G4 in specific (transcriptional & translational) regions of various genes by specific ligands influences the activity of the gene [15]. Approximately half of the genes in human comprise G4 structures near their promoter (transcription initiator) regions. Influencing these regions of genes regulate their transcription and translation [12].

2.1.4 Chemical Modifications of DNA

Comprehension of the chemistry of DNA is the most significant discovery in history of DNA research. The molecule of DNA is negatively charged due to its phosphate backbone which is an important property that can be utilized in different applications related with electrophoresis. Storage of DNA, soluble in water, is possible and it can be stored within a buffered solution containing a chemical buffer that controls pH and chelating agent that suppresses damage of enzymes [16].

Additionally, DNA can absorb UV light. Its nitrogenous bases have maximum absorption wavelength of 260 nm [17]. Moreover, DNA molecule is naturally invisible in solutions but it can be stained which allows visualization when exposed to UV light.

2.2 Intercalating Agents

Intercalation, in biochemistry, is the addition of specific molecules between the nitrogenous bases of DNA. Intercalating agents, also known as ligands, have the ability to bind to nucleotides via conjugation [18].

Initiation of intercalation process occurs when convenient sized ligand binds to the grooves between nucleotides. DNA has to carry out a change bearing a separation between nucleotides to create appropriate groove for the ligand [19].

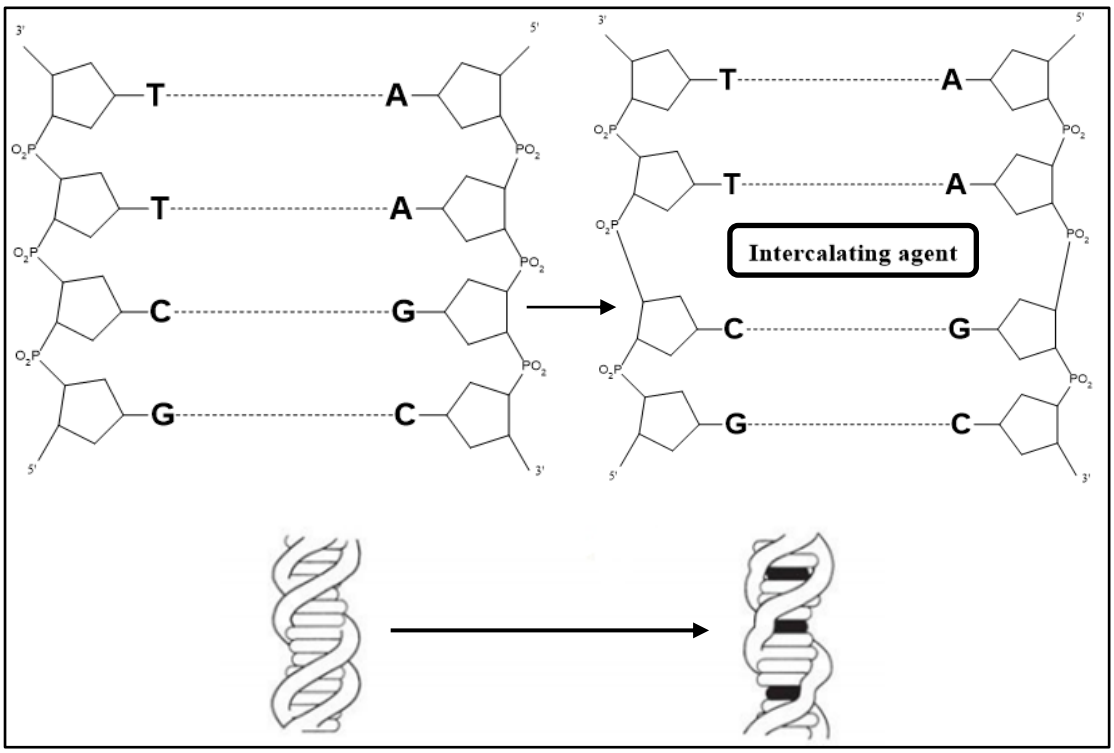

Figure 2.6: An Illustration of Intercalation Process. Intercalating Agent Binds between the Nitrogenous Bases of DNA
(Black Fillings Represent Intercalating Agents)

There are various ligands with distinct sizes, structures and properties. Ligands are being used in many types of applications; but mostly in anti – cancer drug researches (Table 2.1) [19].

Table 2.1: Examples of Different DNA – Binding Agents

NAME	APPLICATION(S)
Ellipticine	Anti – tumor activity
Proflavine	Anti – cancer drug research
Naphthalene diimide derivatives*	Anti – cancer drug research Transcription & Translation regulation of multiple genes

** Molecule of interest*

2.2.1 Naphthalene Diimide Derivatives

Naphthalene diimides are potential DNA – binding ligands. Ligands are ions or molecules that can bind to an atom of another molecule to form a complex. Interactions of different types of NDI derivatives bearing alternative side chains with nucleotides differ immensely [1].

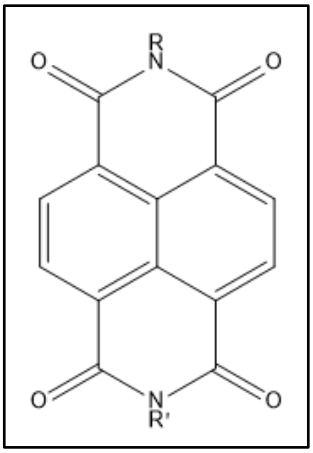

Figure 2.7: Main Structure of NDI Derivatives

They have the ability of forming charge – transfer complexes which provides a stabilizing effect for the formed complex. Also, they can selectively bind to G4 structures in both telomeric sequences and non – telomeric sequences.

NDI derivatives which are unique DNA – binding ligands interact with DNA in a mode called threading intercalation in which intercalation happens between the grooves of DNA (Figure 2.5) [20].

Binding affinities of different ligands depend on not only on their planar structure and the structure of the attractive molecule but also on many other factors related with DNA its region (telomeric or non – telomeric) [21]. Moreover, alternative side chains of naphthalene diimides and their structural formation (cyclic or non-cyclic) play major role in tendency of intercalation to different types of DNA [1].

Chapter 3

EXPERIMENTAL

3.1 Materials and Instruments

The two G – rich oligonucleotides: a-coreTT (5’ – AGGGTTAGGGTTAGGGTTAGGGTT – 3') and c-myc (5’ – TGAGGGTGGGGAGGGTGGGGAA – 3’) were purchased from OLIGOMER [22] and used without any further purification. Also, DNA has been extracted from human cheek sample using PURELINK Genomic DNA purification Kit [23] with permission from Eastern Mediterranean University Research and Publication Ethics Board. The ligand (Figure 1.1) was synthesized by Nur Paşaoğluları Aydınlık. 1.0 M Trisaminomethane hydrochloride (pH 7.4) buffer was prepared with a protocol from Horizon [24]. Compound solutions were prepared within appropriately diluted (50 mM) Tris – HCl buffer and all measurements were performed accordingly.

The Blirt dNTPs, Blirt Extractme DNA clean – up and gel – out kit, Blirt 50X TAE buffer and Blirt 2xPCR TaqNova – RED – Mastermix with Taq polymerase were purchased from Energon [25]; additionally, Biorad Lysis buffer, Biorad 5x Orange – G loading dye, Axygen DNA ladder, Thermo Scientific Proteinase K, Invitrogen UltraPure agarose were used in polymerase chain reaction and gel electrophoresis methods.

Moreover, FT – IR Spectra for the ligand was performed by PerkinElmer Spectrum Two; absorption spectra was performed by PG T90+ spectrophotometer; and emission spectra was performed by Varian Eclipse fluorescence spectrophotometer.

3.2 Methods

3.2.1 Polymerase Chain Reaction

PCR was carried out for a specific region of human beta globin gene which contained repetitive guanine bases from the extracted human DNA from cheek sample.

The components for the polymerase chain reaction, 1X PCR buffer, 0.2 mM dNTP mix, 1u Taq polymerase, H_2O, 10 pmol forward (PC03) and reverse primers (CD6 were added to DNA template.

The PCR were started after the addition of the tubes into the thermal cycler. Primary denaturation was performed at 94 ℃ for 120 seconds and then another denaturation was performed at the same temperature for 30 seconds to separate the DNA into two single strands. Annealing was performed at 57 ℃ for 30 seconds which enabled the primers to attach to the DNA. Elongation was performed at 68 ℃ for 60 seconds to make a new strand of DNA with the help of Taq polymerase. The whole procedure took total of 30 cycles to complete.

1% agarose gel was prepared by the addition of 1g of agarose into 100 mL 1X TAE Buffer. The gel was loaded with 5 µL of DNA ladder (100 bp) & 3 µL of Orange – G loading dye and 10 µL of PCR product & 10 µL of Orange – G loading dye which were then run at 115V for 60 minutes to confirm the presence of the PCR product.

3.2.2 Gel Electrophoresis

4% agarose gel was prepared by the addition of 4g of agarose into 100 mL 1X TAE Buffer. The PCR product alone (control) and different concentrations of naphthalene diimide derivative which was mixed with the product of PCR were loaded into the wells of agarose gel with 3 µL of Orange – G loading dye. The layout of all the wells is shown in Table 3.1. The gel was run at 115V for 80 minutes and then immediately it was taken under UV – light to obtain the image of the gel.

Table 3.1: The Layout of Wells in 4% Agarose Gel

Wells	**Samples**
Well 1	100 bp DNA Ladder (5 µL)
Well 2	PCR product alone (10 µL)
Well 3	PCR product (10 µL) + 5 µM S-NDI (10 µl)
Well 4	PCR product (10 µL) + 10 µM S-NDI (10 µl)
Well 5	PCR product (10 µL) + 20 µM S-NDI (10 µl)
Well 6	PCR product (10 µL) + 40 µM S-NDI (10 µl)
Well 7	PCR product (10 µL) + 80 µM S-NDI (10 µl)
Well 8	100 bp DNA Ladder (5 µL)

3.2.3 Emission Spectroscopy Measurements

Emission spectra were measured and recorded within 365 – 800 nm range (λ_{exc}= 360 nm) for compound alone & complexes; and 225 – 800 nm range (λ_{exc}= 220 nm) for oligonucleotides on a Varian Cary Eclipse fluorescence spectrophotometer with quartz cell.

Measurements were carried out at different time gaps for compound alone after the removal of the compound from sonicator (15 minutes at room temperature) to observe and examine the aggregation of the ligand in the buffer: 0 hour (immediately after removal), 2 hours, 6 hours, 12 hours and 24 hours without disruption. Moreover, measurements for complexes were carried out at same time gaps after the addition of 10 µM/strand of oligonucleotides to the quartz cell containing 10 µM ligand solution with a 1:10 volume ratio, respectively. The measurements were performed within 50 mM Tris – HCl buffer (pH 7.4) and the changes were monitored.

3.2.4 UV- Vis Spectroscopy Measurements

Absorption spectra were measured and recorded within 200 – 800 nm range on a PG T90+ spectrophotometer with quartz cell.

Measurements were carried out at different time gaps for compound alone after the removal of the compound from sonicator (15 minutes at room temperature) to observe and examine the aggregation of the ligand in the buffer: 0 hour (immediately after removal), 2 hours, 6 hours, 12 hours and 24 hours without disruption. Moreover, measurements for complexes were carried out at same time gaps after the addition of 10 µM/strand of oligonucleotides to the quartz cell containing 10 µM ligand solution with a 1:10 volume ratio, respectively. The measurements were performed within 50 mM Tris – HCl buffer (pH 7.4) and the absorption changes were monitored.

Chapter 4

DATA

4.1 FT – IR Spectroscopy Measurement

The result of FT – IR spectroscopy measurement of S-NDI is shown below:

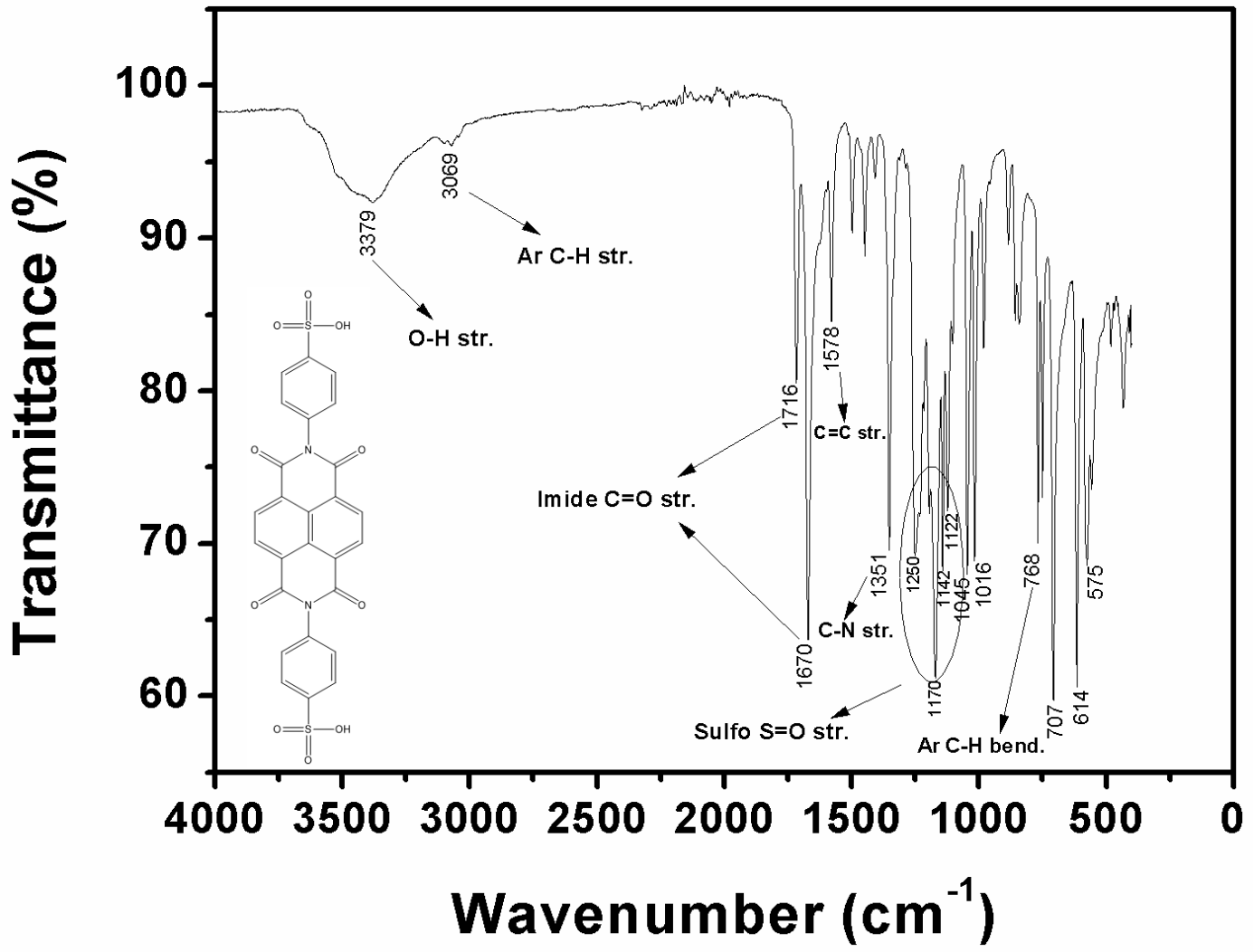

Figure 4.1: S-NDI, Infrared Spectrum

4.2 Gel Electrophoresis

The result of agarose gel electrophoresis is shown below:

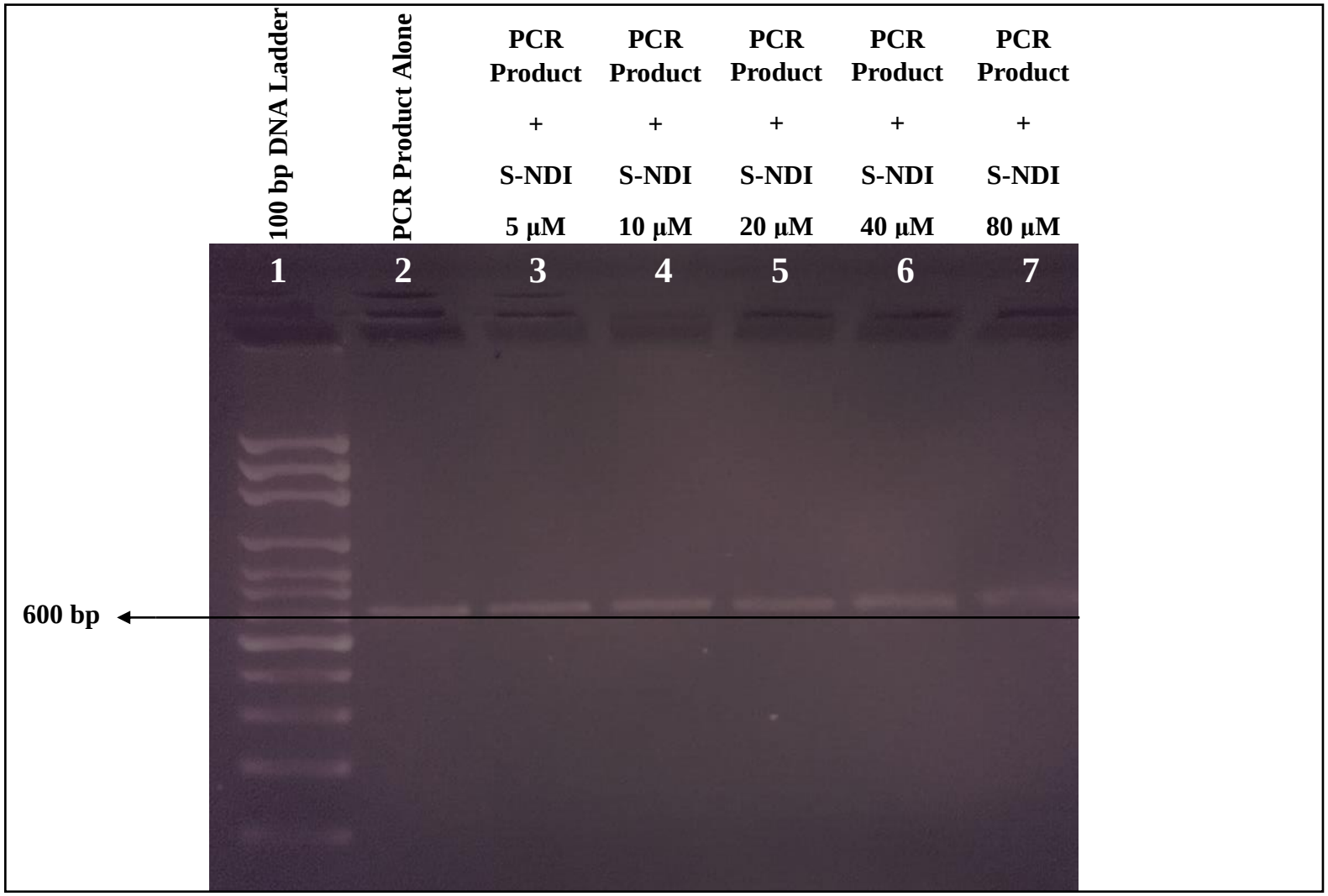

Figure 4.2: Results of G – quadruplex Formation from Low to High Concentration of S-NDI

4.3 Emission & UV – Vis Spectroscopy Measurements

The results of emission and UV – Vis spectroscopy measurements of primers, compound, and complexes are shown below:

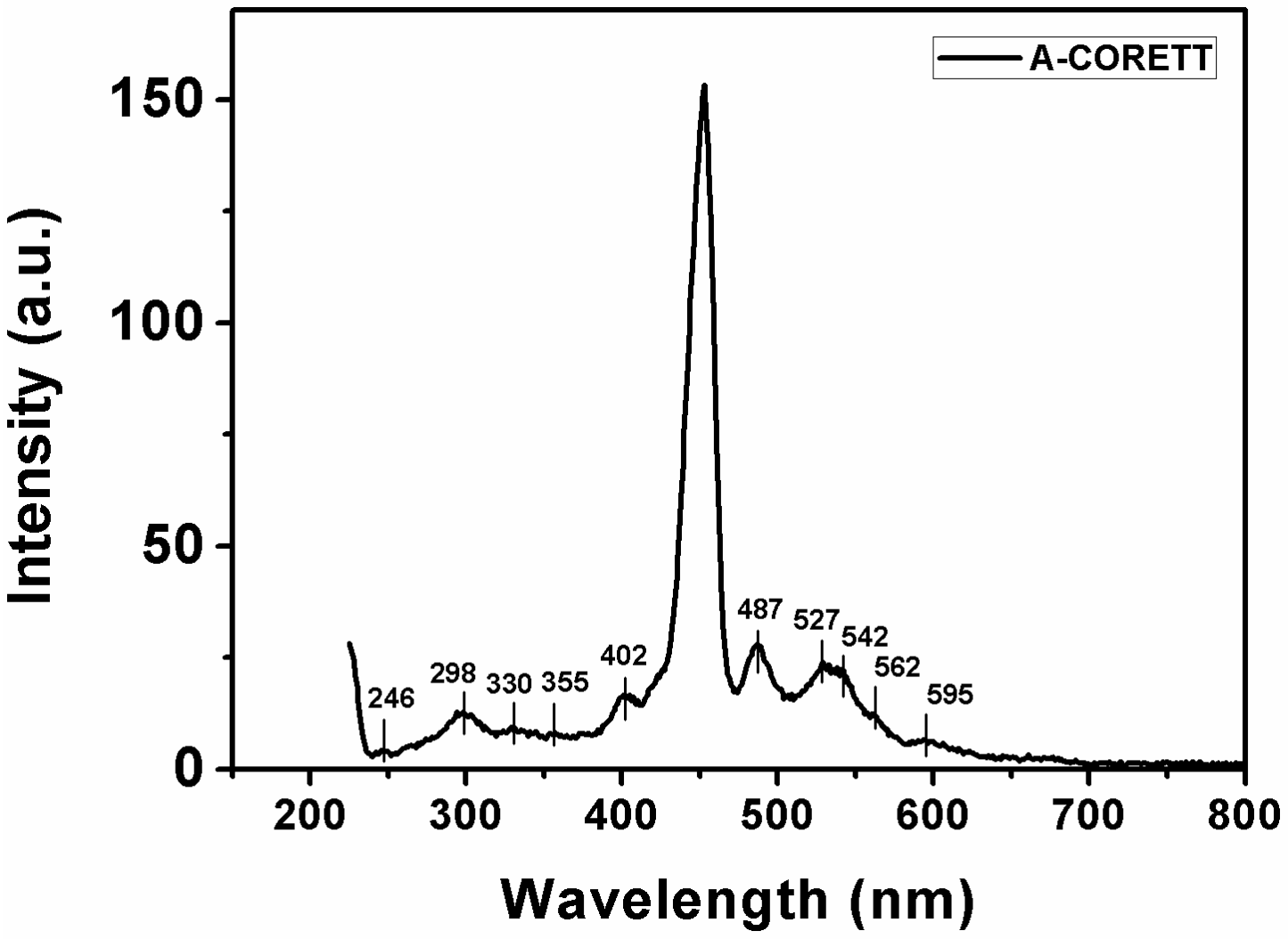

Figure 4.3: a – coreTT, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

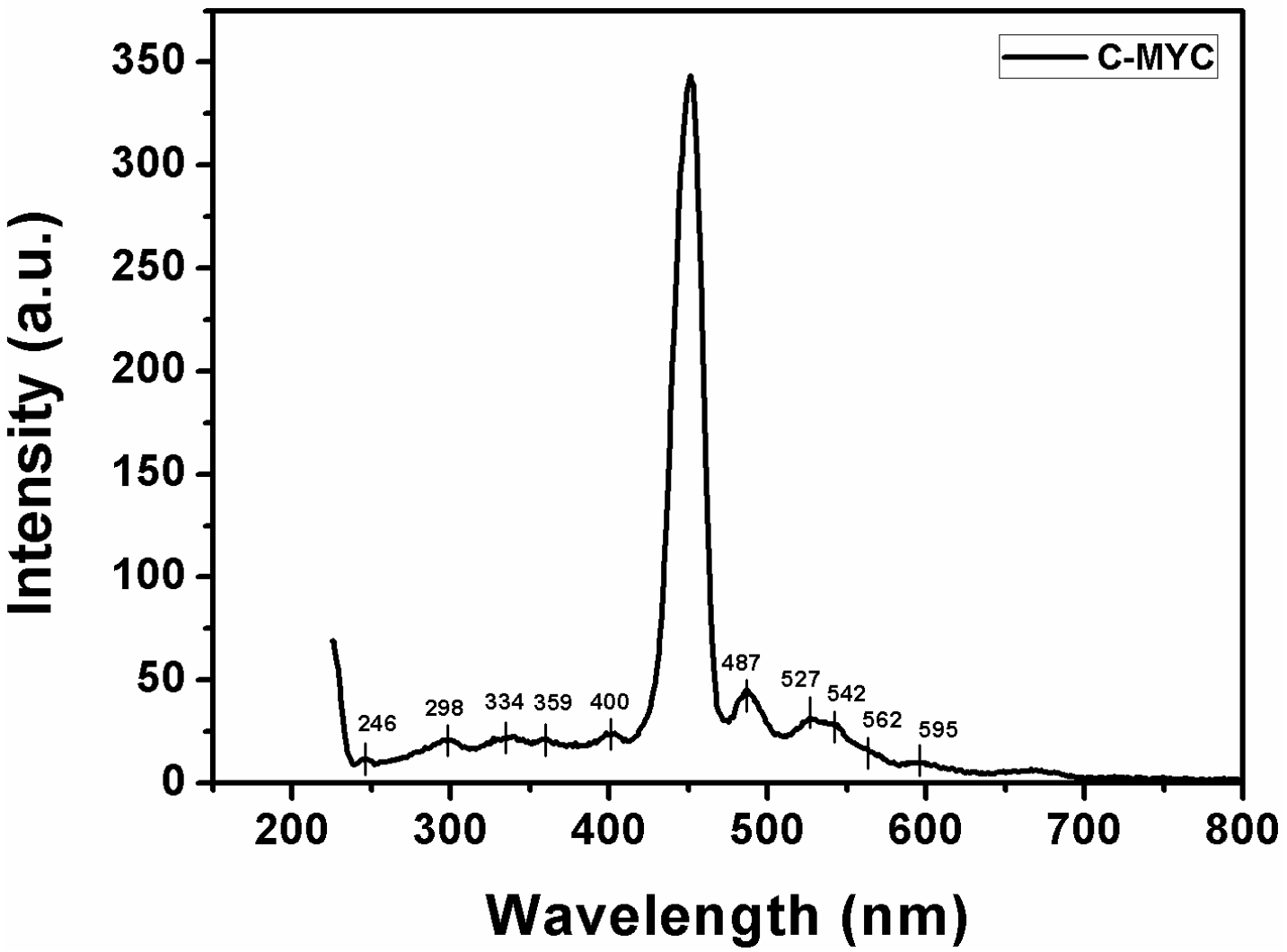

Figure 4.4: c – myc, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

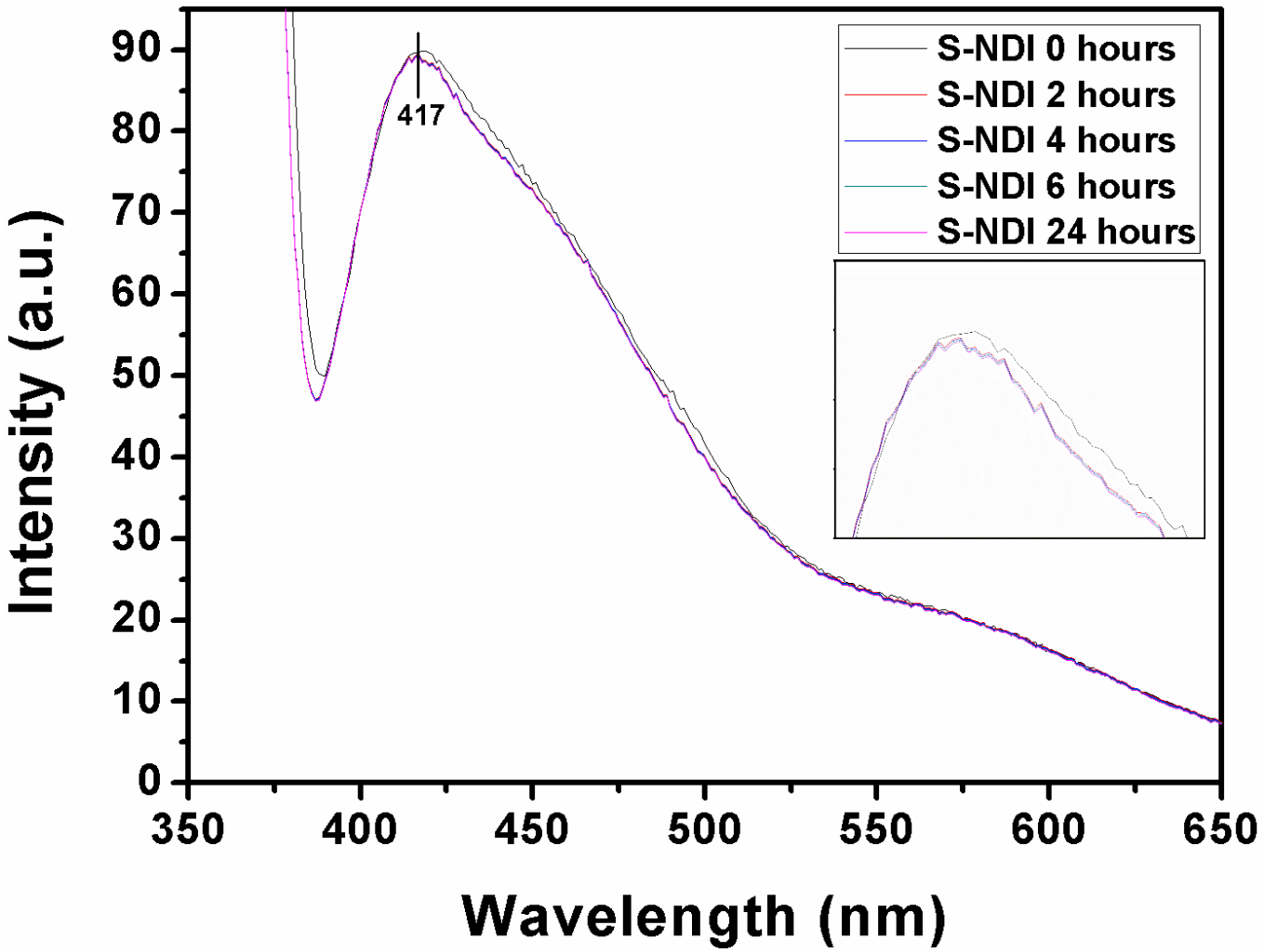

Figure 4.5: S-NDI, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

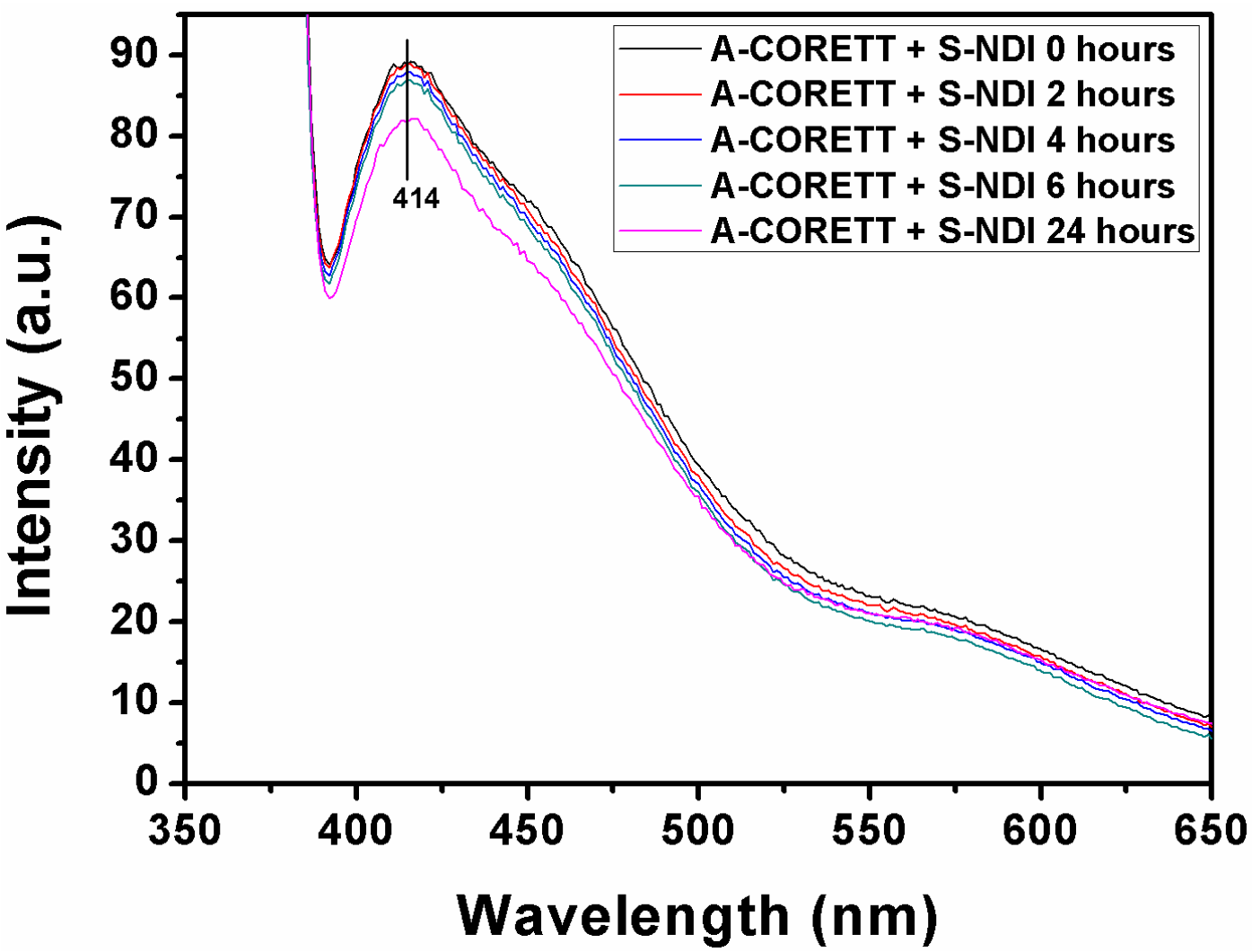

Figure 4.6: a – coreTT/S-NDI Complex, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

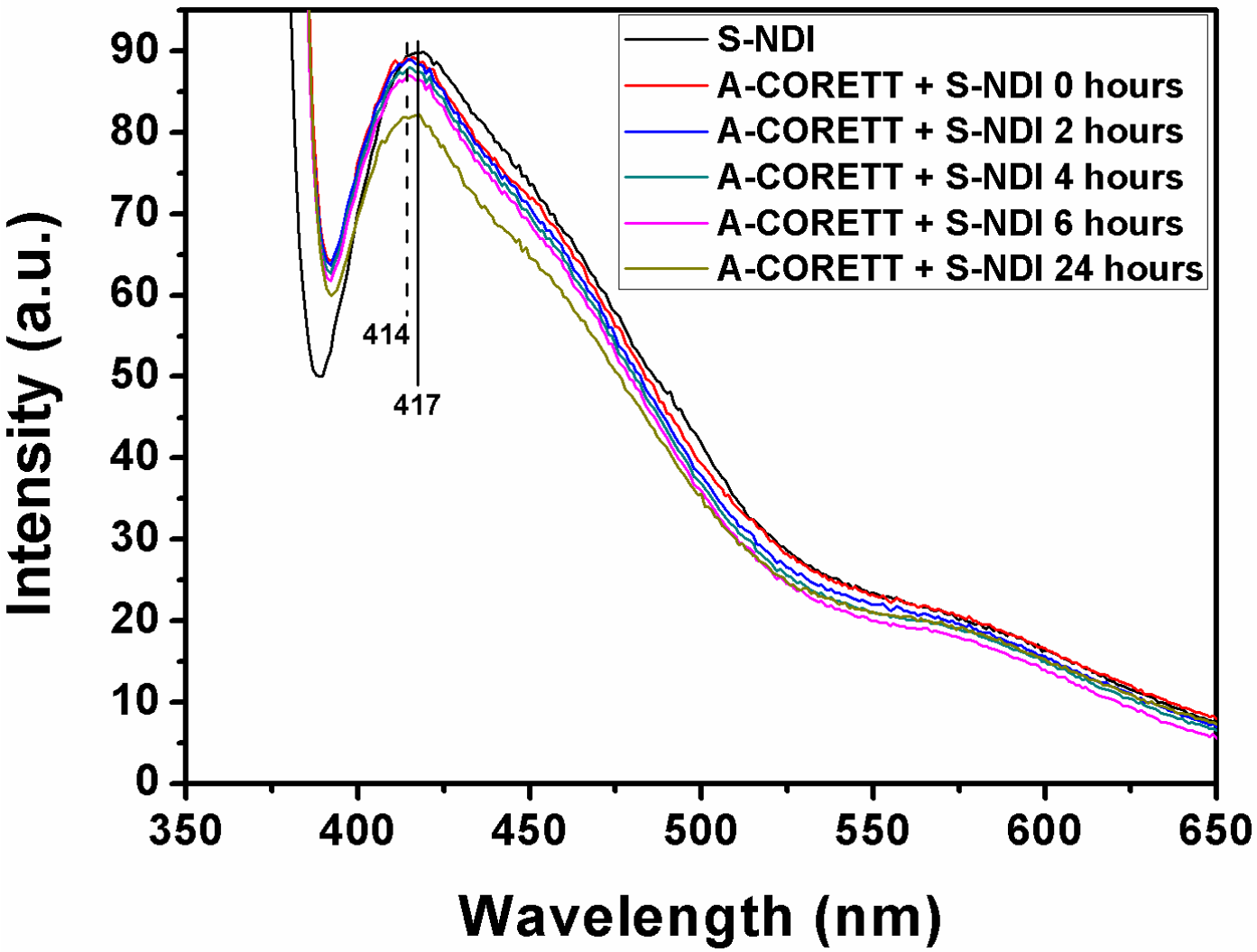

Figure 4.7: a – coreTT/S-NDI Complex and S-NDI, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

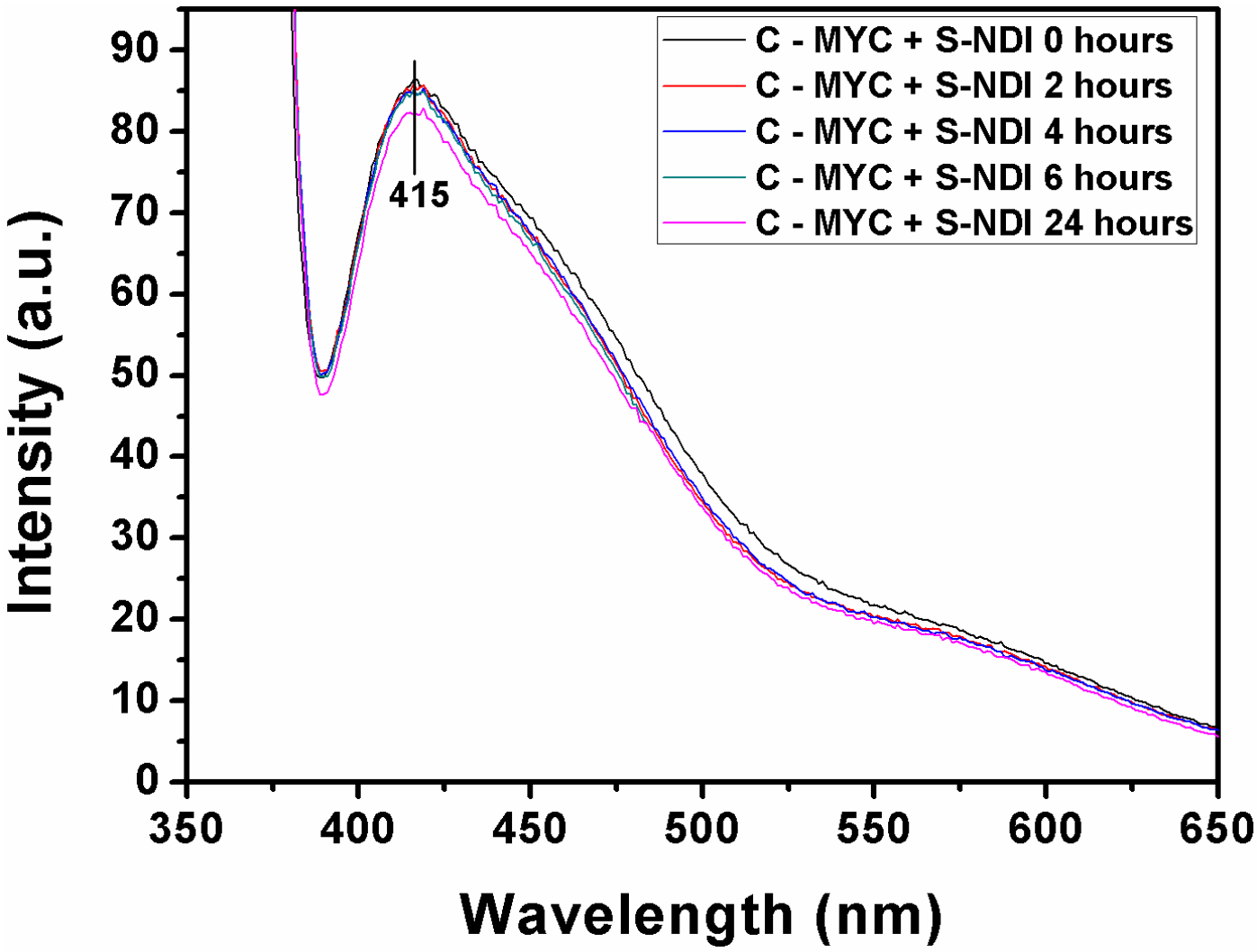

Figure 4.8: c – myc/S-NDI Complex, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

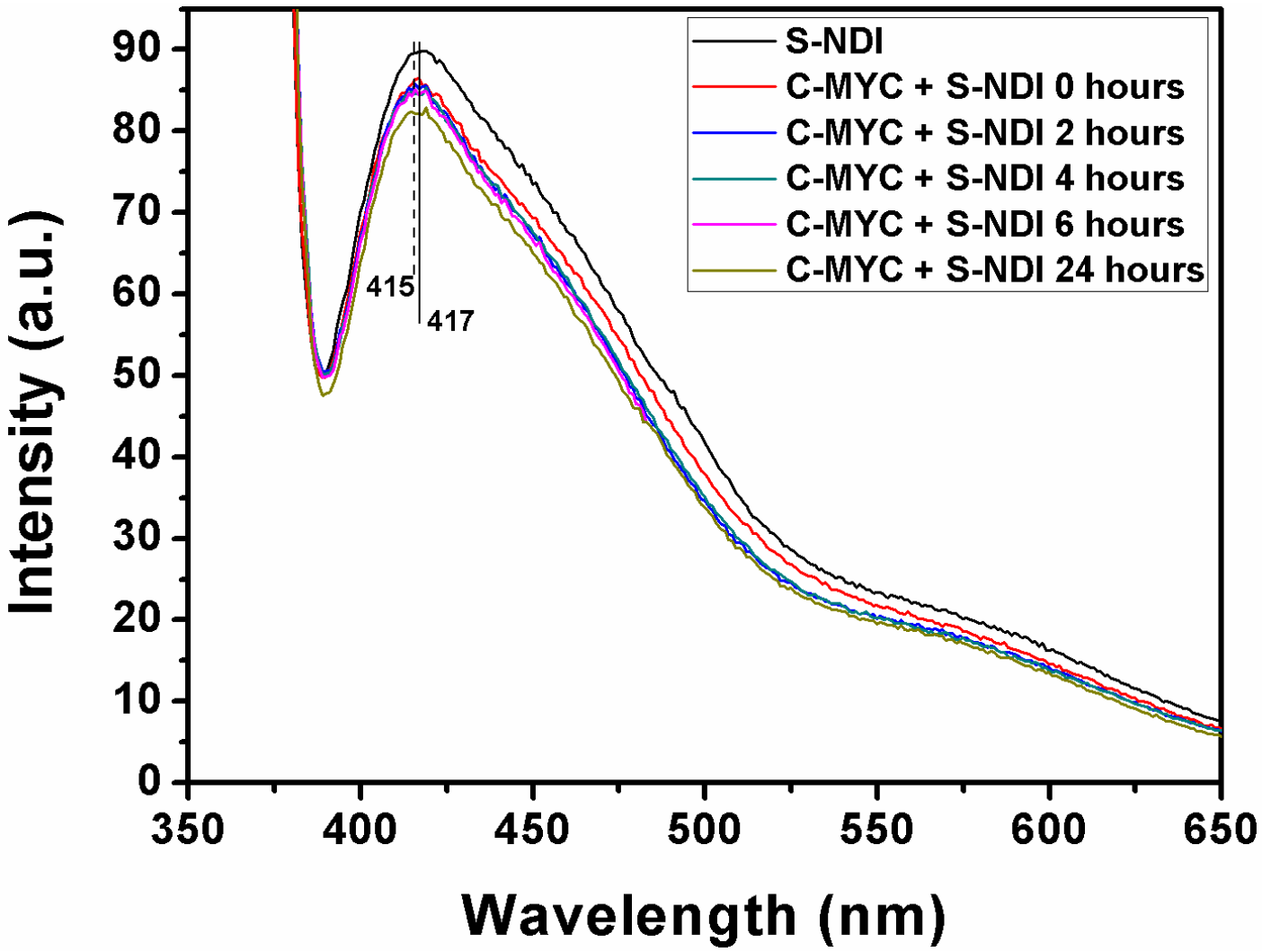

Figure 4.9: c – myc/S-NDI Complex and S-NDI, Emission Spectrum (50 mM Tris – HCl Buffer pH 7.4)

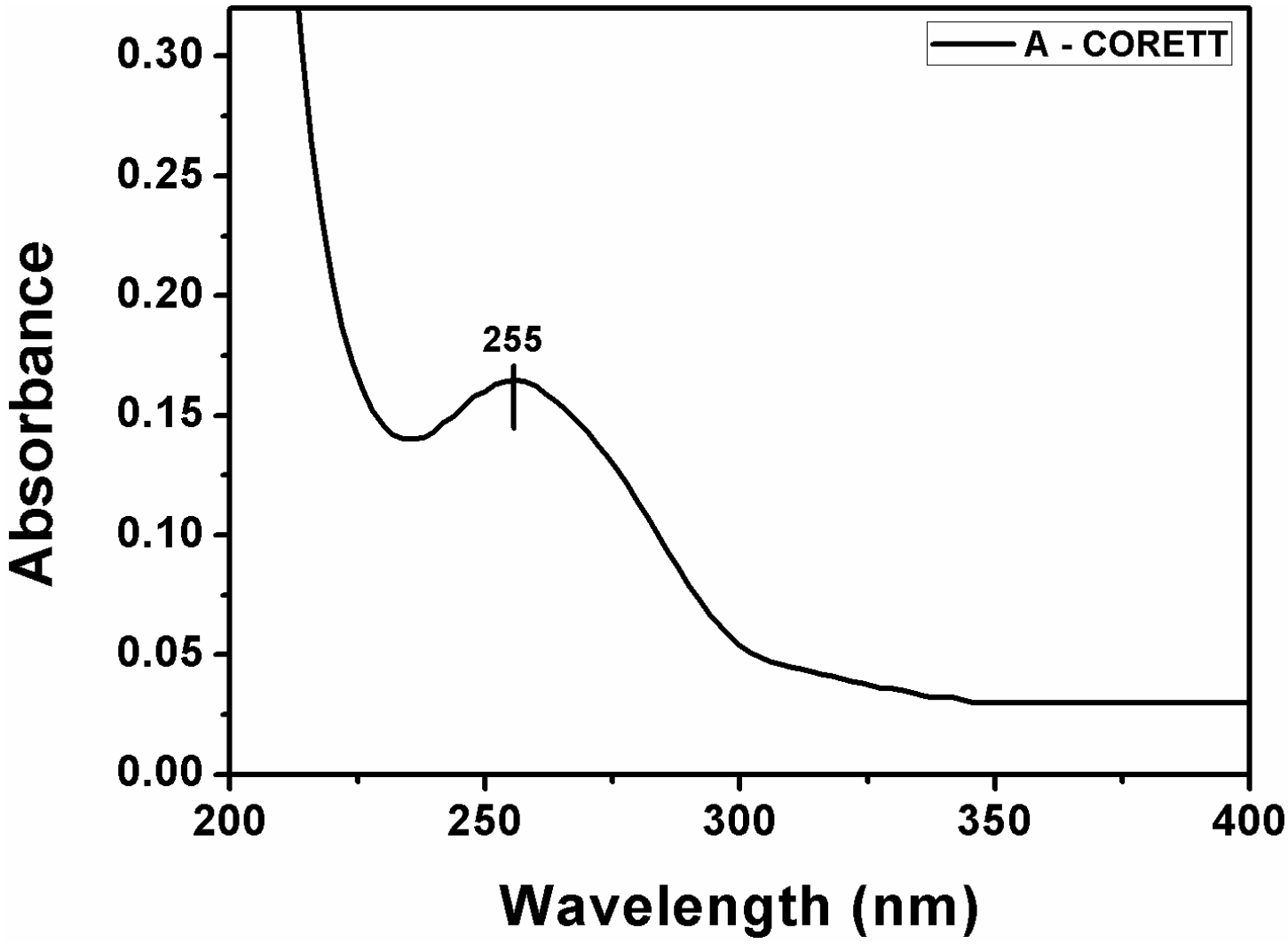

Figure 4.10: a – coreTT, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

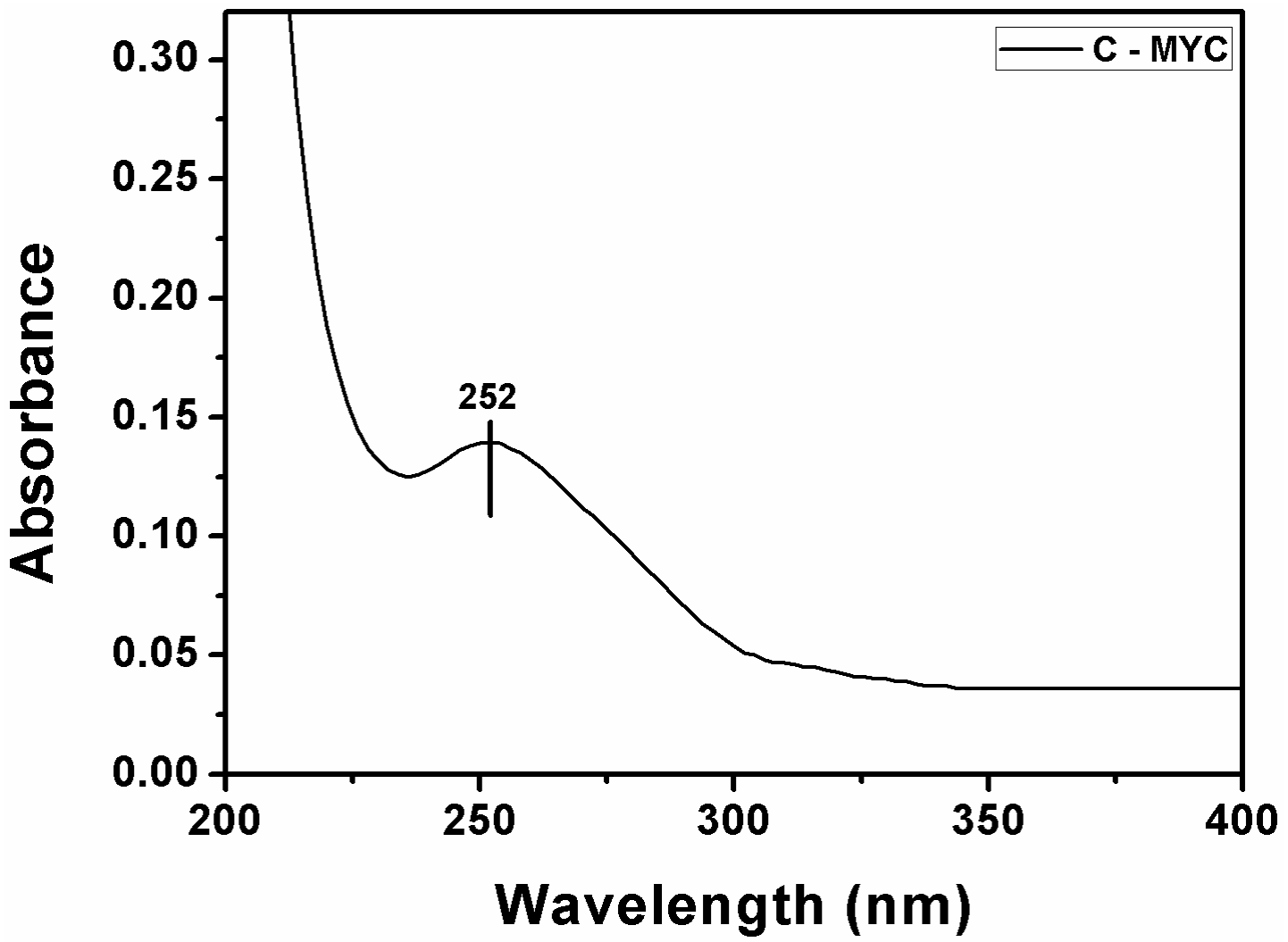

Figure 4.11: c – myc, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

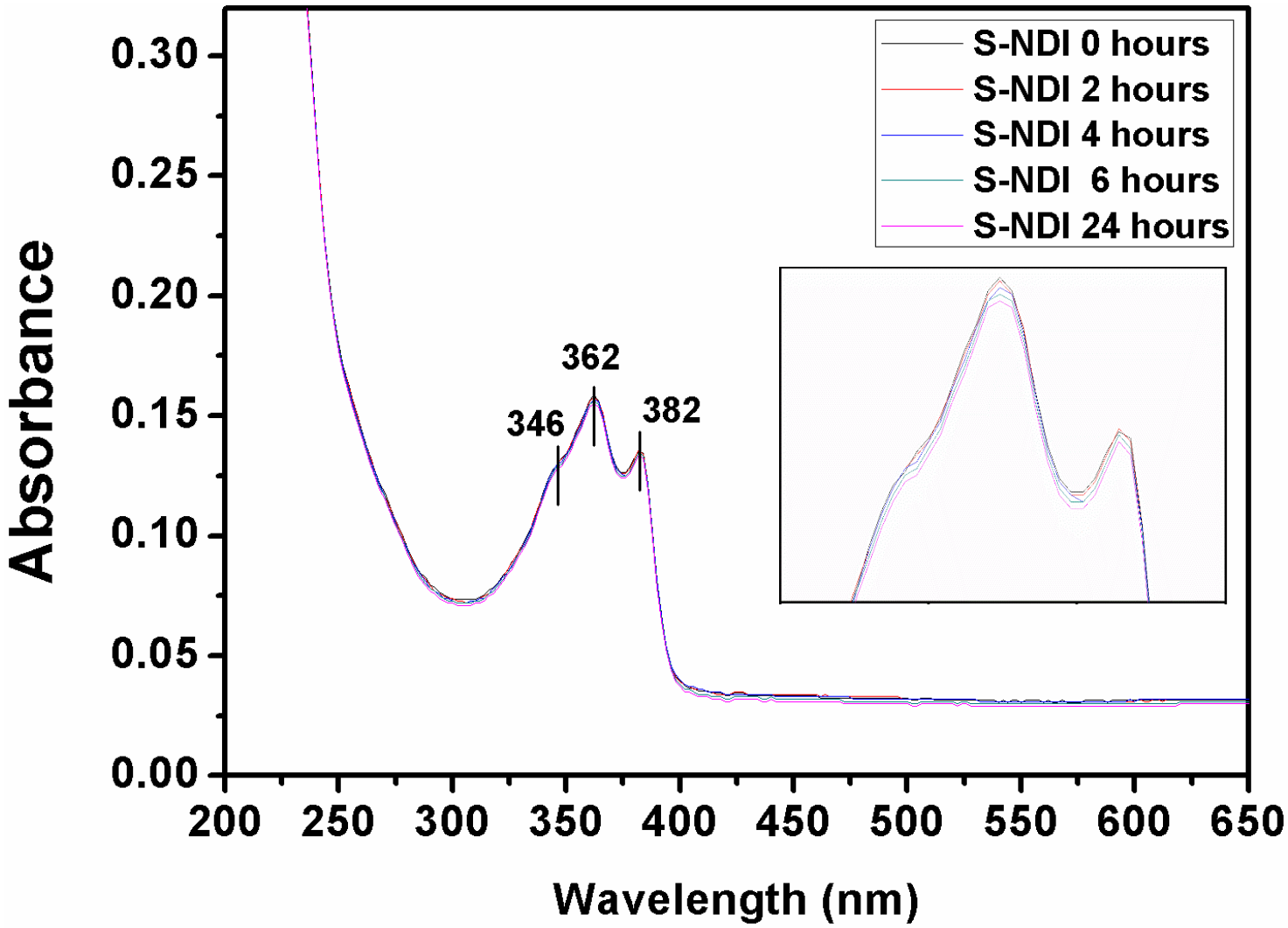

Figure 4.12: S-NDI, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

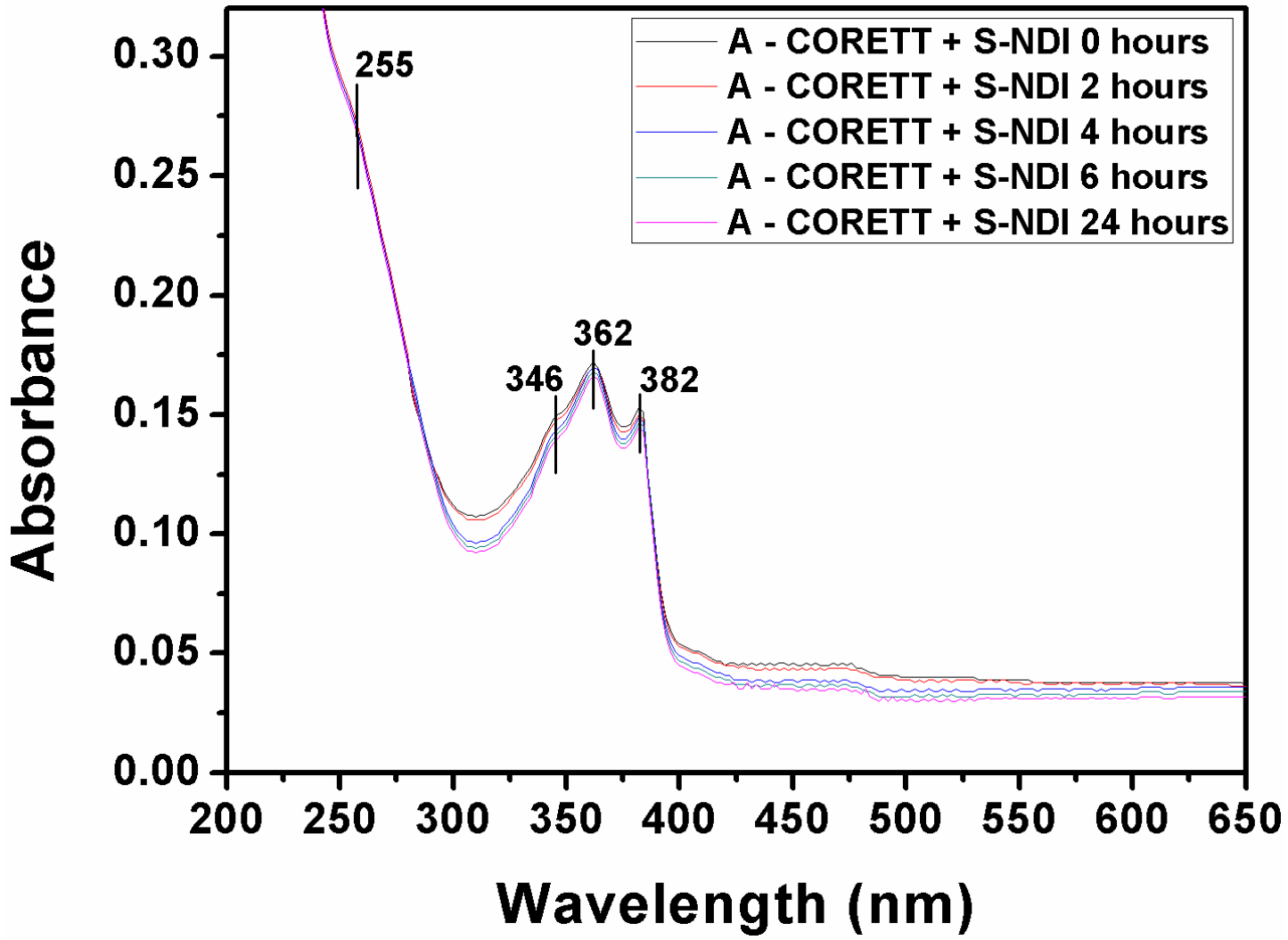

Figure 4.13: a – coreTT/S-NDI Complex, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

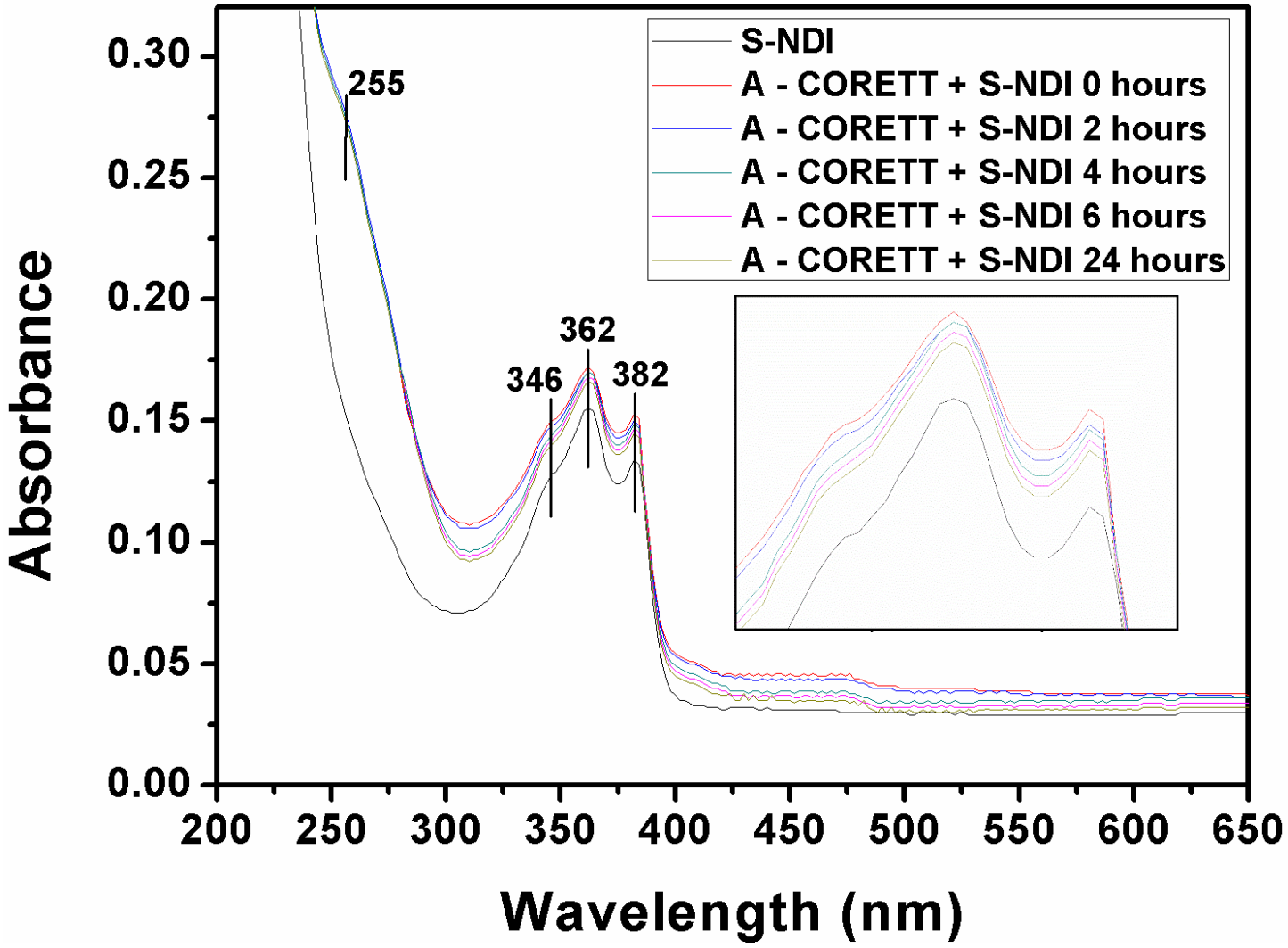

Figure 4.14: a – coreTT/S-NDI Complex and S-NDI, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

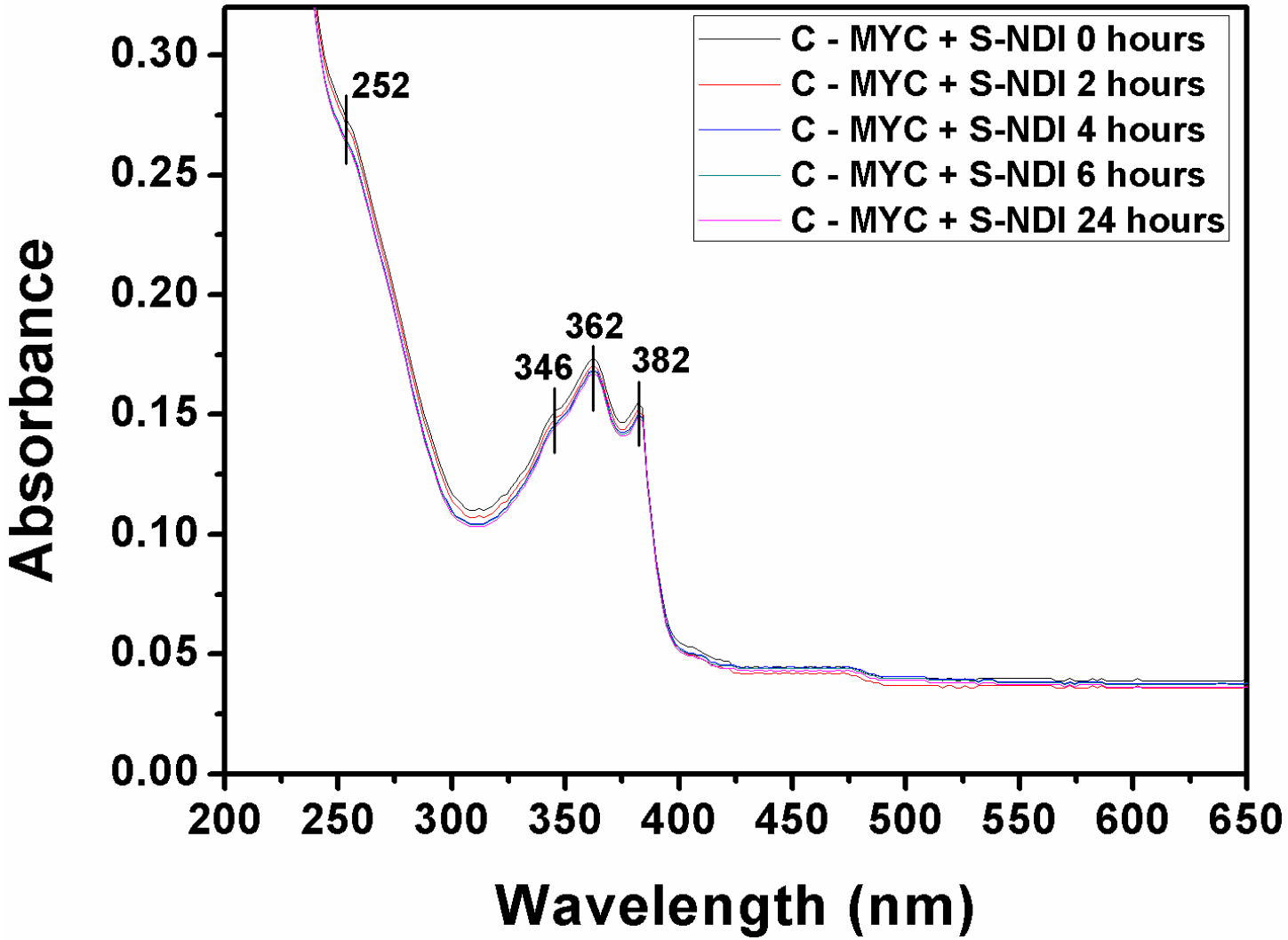

Figure 4.15: c – myc/S-NDI Complex, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

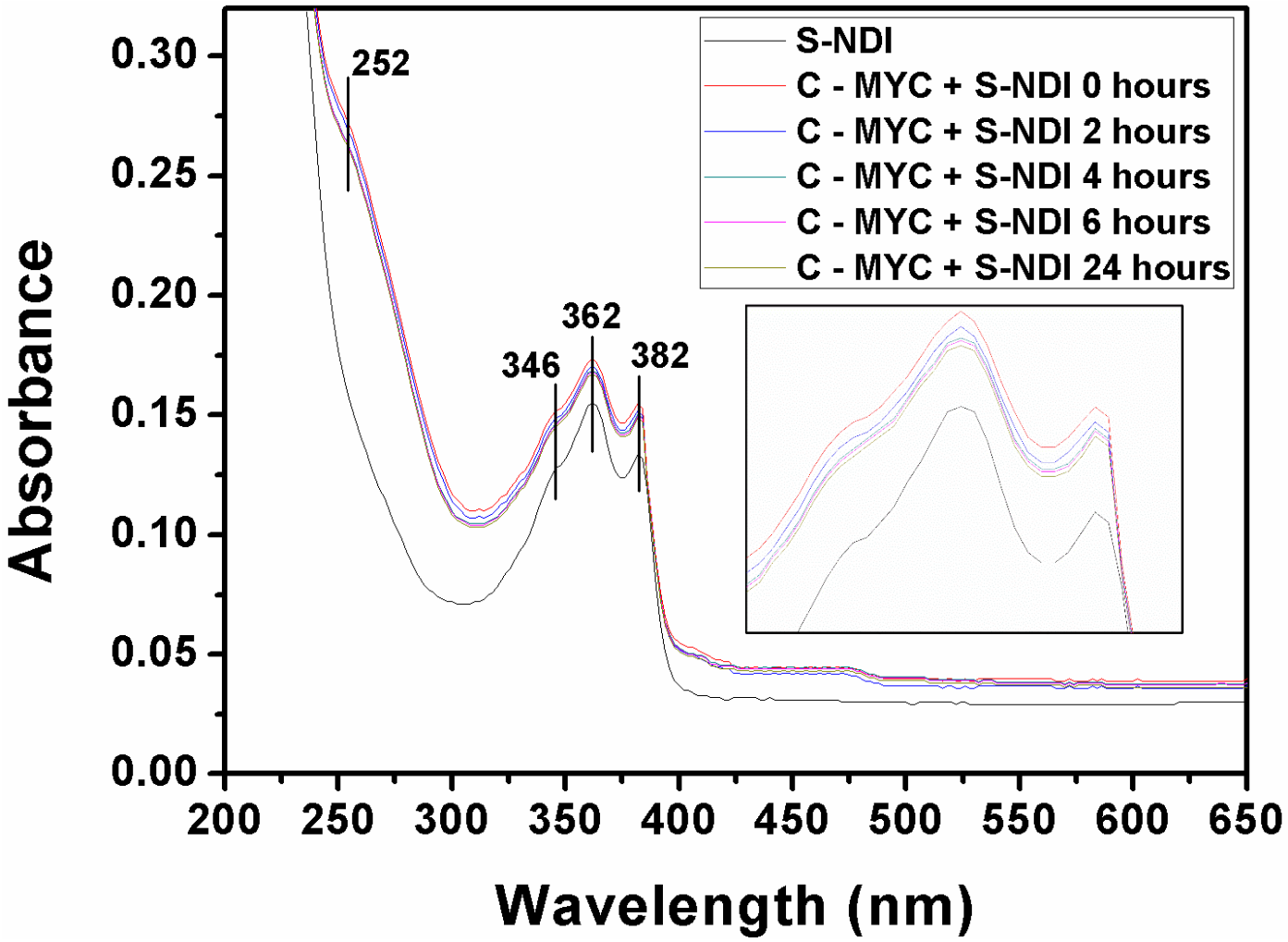

Figure 4.16: c – myc/S-NDI Complex and S-NDI, Absorption Spectrum (50 mM Tris – HCl Buffer pH 7.4)

Chapter 5

RESULTS AND DISCUSSION

5.1 Gel Electrophoresis

The interaction of S-NDI with G4 structures within the specific region of human beta globin gene which contained repetitive guanine bases was examined to prove the formation of G4 structures within human DNA via gel electrophoresis and documented via UV – light.

Different concentrations (5 µM, 10 µM, 20 µM, 40 µM, 80 µM) of the S-NDI were loaded into the wells of agarose gel with PCR product to examine and understand the concentration dependent interaction of S-NDI.

The results showed that the bands of S-NDI with PCR product migrated slower than the band of PCR product alone which is a sign that there are formation of G4 structures within human DNA. Also, the difference in the distance run by different concentrations of S-NDI can be clearly observed in Figure 4.2. The bands with higher concentrations run slower than the lower concentrated bands which leads to the result that S-NDI can interact with G4 structures even at low concentrations.

5.2 Emission Spectroscopy

The emission spectra of pure primers (a – coreTT and c – myc) were recorded with variations in the intensity of peaks which are provided in Figure 4.3 and 4.4, respectively. Moreover, emission spectra of S-NDI alone was measured at 417 nm

and recorded over time to examine and understand its aggregation (0h, 2h, 4h, 6h, and 24h). There was a slight change in the intensity of the peaks of S-NDI alone over time where the intensity mildly decreased and it is illustrated in Figure 4.5.

The spectra of complexes of S-NDI with primers a – coreTT and c – myc are provided in Figure 4.6 and Figure 4.8, respectively. The emission spectra of complexes were measured and recorded to observe the interactions of S-NDI with two different primers over time (0h, 2h, 4h, 6h, and 24h).

The spectra of a – coreTT & S-NDI complex was recorded at 414 nm. There is ≈ 3 nm blueshift upon addition of a – coreTT into S-NDI solution in comparison to S-NDI alone which was changed from 417 nm to 414 nm and it can be observed in Figure 4.7. There is increased change in the intensity of the peaks of the complex where the intensity decreased rapidly in comparison to S-NDI alone which can be observed in Figure 4.6. These suggest that there is an energy transfer from the primer to S-NDI which corresponds to a notable interaction between these molecules but the increased aggregation indicates that the complex is not very stable.

The spectra of c – myc & S-NDI complex was recorded at 415 nm. There is ≈ 2 nm blueshift upon addition of c – myc into S-NDI solution in comparison to S-NDI alone which was changed from 417 nm to 415 nm and it can be observed in Figure 4.9. The aggregation of this complex is similar to the aggregation of a – coreTT & S-NDI complex where the intensity decreased rapidly in comparison to S-NDI alone and it can be observed in Figure 4.8. These suggest that there is an energy transfer from this particular primer to S-NDI as well and the increased aggregation denotes that this complex is not very stable neither.

5.3 UV – Vis Spectroscopy

The spectra of pure primers a – coreTT and c – myc were recorded at 252 nm and 255 nm, respectively which can be observed in Figure 4.10 and 4.11. Additionally, absorption spectra of S-NDI alone was measured as a shoulder at 346 nm and two different peaks at 362 nm & 382 nm. Also, it was recorded over time to examine and understand its aggregation (0h, 2h, 4h, 6h, and 24h). There was slight change in the peaks of S-NDI alone over time where the absorbance lightly decreased and it is illustrated in Figure 4.12.

The spectra of complexes of S-NDI with primers a – coreTT and c – myc are provided in Figure 4.13 and Figure 4.15, respectively. The absorption spectra of complexes were measured and recorded to observe the interactions of S-NDI with two different primers over time (0h, 2h, 4h, 6h, and 24h).

The spectra of a – coreTT & S-NDI complex was recorded as a shoulder at 346 nm and two different peaks at 362 nm & 382 nm as well with a remarkable absorption increase upon addition of particular primer into S-NDI solution when compared to S-NDI alone which can be observed in Figure 4.14. Moreover, the aggregation of the complex is faster than the aggregation on of S-NDI alone which is illustrated in Figure 4.13. These suggest that the S-NDI is completely attracted by G4 structures of a – coreTT and formed a complex at increased absorbance which indicates a considerable interaction between these molecules but the increased aggregation illustrates that the complex is not very stable which overlaps with the aggregation results of the emission spectra.

The spectra of c – myc & S-NDI complex was identically alike as a – coreTT & S-NDI complex with a similar increment in the absorption in comparison with S-NDI alone and it is shown in Figure 4.16. Also, the rapidness of the aggregation of the complex over time is faster than the aggregation of S-NDI as well and it can be observed in Figure 4.15. These suggest that there is similar interaction between the molecules of c – myc & S-NDI complex as a – coreTT & S-NDI complex; and also the increased aggregation corresponds to instability of this particular complex as well.

Chapter 6

CONCLUSION

Briefly, PCR was carried out for a specific region of human beta globin gene which contained repetitive guanine bases from the isolated human DNA from cheek cells. This particular PCR product was mixed with different concentrations of naphthalene diimide derivative to observe the interaction of this compound with G4 structures in this PCR product via gel electrophoresis method. Additionally, two different primers (a – coreTT & c – myc) were used as oligonucleotides (telomeric and non – telomeric respectively) to examine their interaction within the S-NDI via emission and UV – Vis spectroscopy measurements.

Gel electrophoresis method results illustrated a difference in the migration of bands between the complexes and PCR product alone which indicates that there are formations of G4 structures within human DNA. All of the wells of complexes with different concentrations migrated slower than the well of PCR product alone. Also, complexes with higher concentrated S-NDI migrated slower than the complexes with lower concentrated S-NDI which suggest that this particular S-NDI can induce G4 structures even at low concentrations.

Emission spectroscopy observations indicated similar blueshift in both complexes in comparison to S-NDI alone but the complexes aggregation over time showed that the complexes may not be very stable. Moreover, absorption spectroscopy observations

illustrated an absorption increase in both complexes in comparison to S-NDI alone which corresponds to precise attraction of S-NDI by G4 structures of both primers with similar aggregation results as emission spectra. These spectroscopic measurement results propose a notable interaction between the S-NDI and G4 structures of both primers.

Overall, the interactions of S-NDI with G4 structures of telomeric, non – telomeric oligonucleotides, and also with the guanine rich specific region of human beta globin gene indicates that this particular type of NDI may be a promising DNA – binding ligand but further investigations are required to understand the stability and reliability of the interactions of this S-NDI within the G4 structures throughout human DNA.

REFERENCES

[1] Interactions of cyclic and non-cyclic naphthalene diimide derivatives with different nucleic acids. Czerwinska I., Sato S., Juskowiak B., Takenaka S. Issue 9, 2014, *ELSEVIER*, Vol. 22, pp. 2593-2601.

[2] Molecular recognition of naphthalene diimide ligands by telomeric quadruplex-DNA: the importance of the protonation state and mediated hydrogen bonds. A. Spinello, G. Barone, J. Grunenberg. Issue 4, 2016, *Physical Chemistry Chemical Physics*, Vol. 18, pp. 2871-2877.

[3] Ferrocenyl naphthalene diimides as tetraplex DNA binders. Sato S., Takenaka S. 2017, *ELSEVIER*, Vol. 167, pp. 21-26.

[4] Cytotoxicity and DNA-binding property of water-soluble naphthalene diimide derivatives bearing 2-oligoethoxy ethanamine side chain end-labeled with tertiary amino groups. Haiying Wei, Mengjiao Lv, Xiaoxu Duan, Shuai Li, Yuchao Yao, Kerang Wang, Pingzhu Zhang, Xiaoliu Li, Hua Chen. Issue 5, 2014, *Medicinal Chemistry Research*, Vol. 23, pp. 2277–2286.

[5] Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltammetry. Sirajuddin, Muhammad. 2013, *ELSEVIER*, Vol. 124, pp. 1 - 19.

[6] National Institutes of Health. What is DNA? June 12, 2018.

[7] PubChem. Compound Summary. 2004.

[8] Nature. DNA Is a Structure That Encodes Biological Information. 2014.

[9] LSBU. Nucleic Acid Hydration. 2001.

[10] MSOECBM. Hydrogen Bonding in DNA Base Pairs. 2000.

[11] CyberBridge - Harvard University. Nucleotides and The Double Helix. 2007.

[12] Quadruplex DNA: sequence, topology and structure. Sarah Burge, Gary N. Parkinson, Pascale Hazel, Alan K. Todd, Stephen Neidle. Issue 19, 2006, *Nucleic Acids Research*, Vol. 34, pp. 5402–5415.

[13] G-quadruplexes and their regulatory roles in biology. Daniela Rhodes, Hans J. Lipps. Issue 18, 2015, *Nucleic Acids Research*, Vol. 43, pp. 8627–8637.

[14] Human Telomerase and Its Regulation. Yu-Sheng Cong, Woodring E. Wright, Jerry W. Shay. Issue 3, 2002, *Microbiology and Molecular Biology Reviews*, Vol. 66, pp. 407-425.

[15] 5′-UTR RNA G-quadruplexes: translation regulation and targeting. Anthony Bugaut, Shankar Balasubramanian. Issue 11, 2012, *Nucleic Acids Research*, Vol. 40, pp. 4727-4741.

[16] Mandal, Ananya. DNA Chemical Modifications. May 17, 2017.

[17] Ganske, Franka. UV absorbance DNA quantitation. 2018.

[18] Biotech Khan. DNA Damage. October 14, 2014.

[19] DNA intercalators and using them as anticancer drugs. Masoud Karimi Goftar, Nasrollah Moradi Kor, Zahra Moradi Kor. Issue 3, 2014, *International Journal of Advanced Biological and Biomedical Research*, Vol. 2, pp. 811-822.

[20] Recent advances in small organic molecules as DNA intercalating agents: Synthesis, activity, and modeling. Antonio Rescifina, Chiara Zagni, Maria Giulia Varrica, Venerando Pistarà, Antonino Corsaro. 2014, *ELSEVIER*, Vol. 74, pp. Pages 95-115.

[21] Chemistry of naphthalene diimides. Sheshanath V. Bhosale, Chintan H. Jani, Steven J. Langford. Issue 2, 2008, *Chemical Society Reviews*, Vol. 37, pp. 331-342.

[22] Oligomer. Oligomer Biyoteknoloji. 2015. http://www.oligomer.com.tr/.

[23] Invitrogen. Purelink Genomic DNA Kits for purification of genomic DNA. 2012. https://www.thermofisher.com/order/catalog/product/K182001.

[24] Horizon. Tris-HCl Buffer 7.4 pH. 2016. https://dharmacon.horizondiscovery.com/uploadedFiles/Resources/crrna-tracrrna-resuspension-buffer-protocol.pdf.

[25] Energon. 2015. https://energon.com.cy/.

APPENDIX

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Sayı: ETK00-2019-0010 04.02.2019

Konu: Etik Kurulu'na Başvurunuz Hk.

Sayın Ertuğrul Özbil
Kimya Bölümü Yüksek Lisans Öğrencisi

Doğu Akdeniz Üniversitesi Bilimsel Araştırma ve Yayın Etiği Kurulu'nun **04.02.2019** tarih ve ***2019/04-04*** sayılı kararı doğrultusunda **"Yeni Naftalin Diimide Setlerinin İnsan DNA'sındaki G-Kuadruplex Yapıları ile Etkileşimi"** adlı çalışmanızı, Doç. Dr. Şükrü Tüzmen ve Prof. Dr. Huriye İcil'in danışmanlığında araştırmanız, Bilimsel ve Araştırma Etiği açısından uygun bulunmuştur.

Bilginize rica ederim.

Prof. Dr. Fatma Güven Lisaniler
Etik Kurulu Başkanı

FGL/ba.