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Design and Characterization of Photoresponsive Supramolecular Aggregates

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Abstract

Supramolecular chemistry concerns the manner in which molecular building blocks associate via non-covalent interactions and form aggregates. The particular building block in this research is a photoresponsive molecule 4,4'-azobenzene dicarboxylic acid (ADA), a molecule that isomerizes reversibly around an N-N double bond upon irradiation with different wavelengths of light. The large structural changes in the molecule that result from isomerization have the potential to modulate the properties of a supramolecular aggregate.

ADA was studied under a variety of environmental conditions for the purpose of understanding aggregation behavior and geometries. A UV-Vis and Circular Dichroism (CD) spectroscopic study of ADA showed that it is soluble only at very basic pH (above 10.8) and that a decrease in pH gives rise to the formation of an aggregate. Further studies of the self-aggregation process at low pH showed that this process is under hierarchical control. Four procedures for the formation of homo- or hetero-aggregates of ADA were characterized. Each gave rise to the formation of a different structure showing the importance of the pathway undertaken during the aggregation process. CD spectra of the ADA aggregates showed that, typically, they have a preferential asymmetric geometry despite the symmetry of the constituent molecules. Moreover, the use of a chiral template macromolecule (poly-glutamate) during the formation of the aggregate can affect the structure of the supramolecular species and direct its chirality.

The study of the photoisomerization of ADA in the aggregated form showed that the highly packed aggregates of the *trans* isomer were unable to photoisomerize to the *cis* isomer upon UV (365 nm) irradiation. On the contrary the aggregates containing the *cis* isomer of ADA were able to retain their ability to photoisomerize to the aggregates of the *trans* form

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LIST OF ABBREVIATIONS AND NOTATIONS

ADA	Azobenzene-4,4'-dicarboxylic Acid
HCl	Hydrochloric Acid
H_2SO_4	Sulfuric Acid
NaOH	Sodium Hydroxide
poly-D-Glu	Poly-D-Glutamic Acid
poly-L-Glu	Poly-L-Glutamic Acid

1. INTRODUCTION

1.1 Supramolecular Chemistry

Supramolecular chemistry refers to a branch of chemistry in which the self-assembly of multiple molecular subunits is studied. Supramolecular chemistry is distinguished from other types of chemistry due to the fact that the primary type of atomic interaction one deals with is not a covalent bond, but rather all varieties of non-covalent interaction such as electrostatic forces, hydrogen bonds, van der Waals forces, and donor-acceptor interactions to name a few.

In solution, certain molecules containing polar regions, π-bonds, or hydrophobic regions may find it more energetically favorable to interact with themselves or other molecules in solution than to associate with their solvent. The molecules come together with an organization dictated by the non-covalent interactions they are taking advantage of, which results in the formation of an aggregate. The geometry and properties of the resulting supramolecular structure depend on both the molecular building blocks used and the environmental conditions in which the self-assembly process occurred.

The formation of aggregates containing chromophores can be observed and characterized using UV-Vis spectroscopy. Typically, the formation of a supramolecular aggregate is associated with a decrease in intensity, known as a hypochromic effect, and broadening of the peak corresponding to the main absorption band of the monomer. In some cases the position of the maximum of the band shifts to longer or shorter wavelengths.

Chirality is a common feature of many biological molecules and the possibility of creating a supramolecular structure with a defined configuration increases the potential applications and significance of these materials. Chiral molecules lack symmetry elements and have non-superimposable mirror images. Often, this is caused by a carbon with four different substituents or by an asymmetric arrangement of the geometry of a macromolecule. Circular Dichroism spectroscopy (or CD) has the ability to reveal if the aggregate is chiral or achiral and facilitates the study of the inherent or induced chirality of the assembly.

It has been shown that, starting from an achiral monomer, chirality can be induced in the aggregate by the presence of a chiral "template molecule" in solution as the molecular building blocks self-assemble[1]. Usually in the absence of any chiral template an achiral molecular building block gives rise to the formation of an achiral aggregate. Nevertheless some exceptions where the achiral monomer yields a chiral aggregate have been observed.

In the design of a supramolecular species, it is therefore important to understand the relationships between the characteristics of the molecular components (structures and sites of interaction), the types of processes that lead to their association, and the properties of the resulting aggregate.

Factors that have an impact on non-covalent interactions include but are not limited to solvent, charges, ionic strength, pH, concentration, presence of a chiral template and light[2]. Since the process of self-assembly is determined by non-covalent interactions, any changes to these environmental conditions have the potential to alter the

assembly process and the resulting aggregate structure and properties. Supramolecular chemistry has the potential to yield new and interesting functional materials while employing less complex procedures than traditional synthesis methods.

A desirable option for directing the self-assembly process and modulate the properties of the resulting aggregate is represented by the use of light to perform *in situ* morphological changes of the aggregate. Prime candidates for exploiting this manner of control are photoresponsive building blocks[3]. Molecules that change conformation in response to a light stimulus are ideal because the reaction is specific to the chromophore, and the stimulus does not change any other component of the solution. Photochemical reactions occur quickly, produce no chemical waste, and can often be repeated reversibly[3].

1.2   Azobenzenes

Azobenzenes, which contain two phenyl rings separated by an azo bond, are a family of chromophores often used in materials research. In addition to being chemically stable, azobenzenes are widely used due to the ability of ring substituents to affect the electronic absorption band of the nitrogen double bond, allowing it to absorb anywhere from the ultraviolet to visible regions of the electromagnetic spectrum[4]. Azobenzene undergoes a reversible photoisomerization from a more stable *trans* isomer to a *cis* isomer upon irradiation with UV light and reverts back to the *trans* upon exposure to visible light.

The mechanism through which azobenzene photoisomerization occurs is as of yet uncertain and either takes place via a rupture of the π bond and rotation around the N-N

single bond or through an inversion in which the π bond remains intact[5]. In the dark, azobenzenes will exist in the thermally stable *trans* form due to the energetically favorable separation of the phenyl groups across the double bond. When exposed to light that includes a wavelength in the *trans* absorption band, the absorption of a photon induces the azobenzene to isomerize to the *cis* form. A second photon corresponding to the wavelength of the *cis* absorption band can cause the reverse of the process, regenerating the *trans* isomer. Upon irradiation, the azobenzene will reach a photostationary state in which the two opposing photoisomerization reactions occur at an equal rate. The amount of each isomer at the photostationary state is dependent on the ratio of their extinction coefficients and the rate of thermal relaxation back to the *trans* isomer[6]. The steady-state composition varies by system, but neither the *trans* nor the *cis* isomer can ever exist alone in solution.

The azobenzene isomerization is completely reversible and does not yield any side reactions. As shown in **Figure 1.2.1**, the change in the molecule's length from 9.0 A in the *trans* isomer to 5.5 A in the *cis* isomer is one of the largest known for a reversible reaction[7]. Additionally, azobenzene undergoes an increase in dipole moment upon isomerization from the *trans* to *cis*. When an azobenzene is incorporated into a supramolecular structure, this isomerization has the potential to be translated into large structural changes and also provides a potential mechanism for the conversion of photochemical energy into mechanical motion.
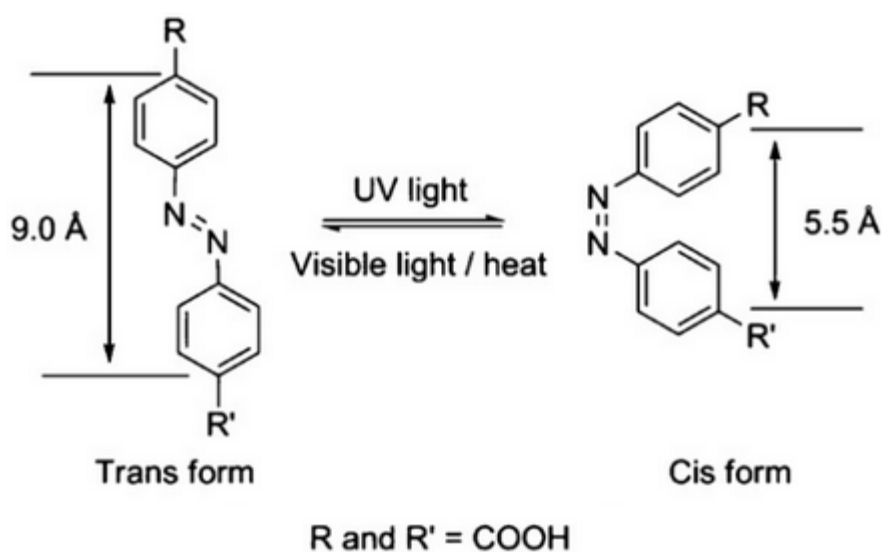
Figure 1.2.1. Conformational changes of azobenzene upon exposure to UV and visible light

Another interesting property of azobenzene is that the bent conformation of the *cis* isomer is less conducive to dense molecular packing than is the flat *trans* isomer[3]. This means that the degree of aggregation depends on the isomer in solution, and, since isomerization can be triggered by a light stimulus, azobenzene provides a molecular building block that has great potential in modulating self-assembly process.

The specific azobenzene used in this research was azobenzene 4,4'-dicarboxylic acid (ADA). ADA is ideal for working in aqueous solution because its carboxyl groups are able to ionize and engender solubility at high pH. At low pH, the carboxyl group hydrogen and the electronegative carbonyl oxygen can form hydrogen bonds, so the molecule provides opportunities for self-assembly. In addition, the two benzene rings in the molecule make it an ideal candidate for an aggregation-oriented system due to the ease with which they can participate in π-π stacking.

UV-Vis spectroscopy represents a useful tool to characterize the formation of an aggregate and in some cases can help predict the geometry of supramolecular species.

The spectral shifts that might accompany the hypochromic effect associated with the aggregation process have been explained in terms of coupling and geometry of the transition moments reflecting, therefore, the geometry of the aggregate.

In the schemes below the two limiting cases related to the interaction between two monomer units whose dipole moments lie in the molecular plane are shown.

If the monomers are oriented in a face-to-face or parallel manner (H aggregate), the splitting of the interacting excited states will give rise to a blue shift of the absorption band of the aggregate compared to the band associated with the monomer. This can be explained by the fact that only the higher energy transition will give rise to a non-zero dipole moment and will therefore be responsible for the absorption (**Figure 1.2.2**)[8].

If the monomers are oriented in a head-to-tail or linear fiber-like manner (J aggregate), the splitting of the interacting excited states will give rise to a red shift of the absorption band of the aggregate compared to the band associated with the monomer. This can be explained by the fact that only the lower energy transition will give rise to a non-zero dipole moment and will therefore be responsible for the absorption (**Figure 1.2.3**)[8].
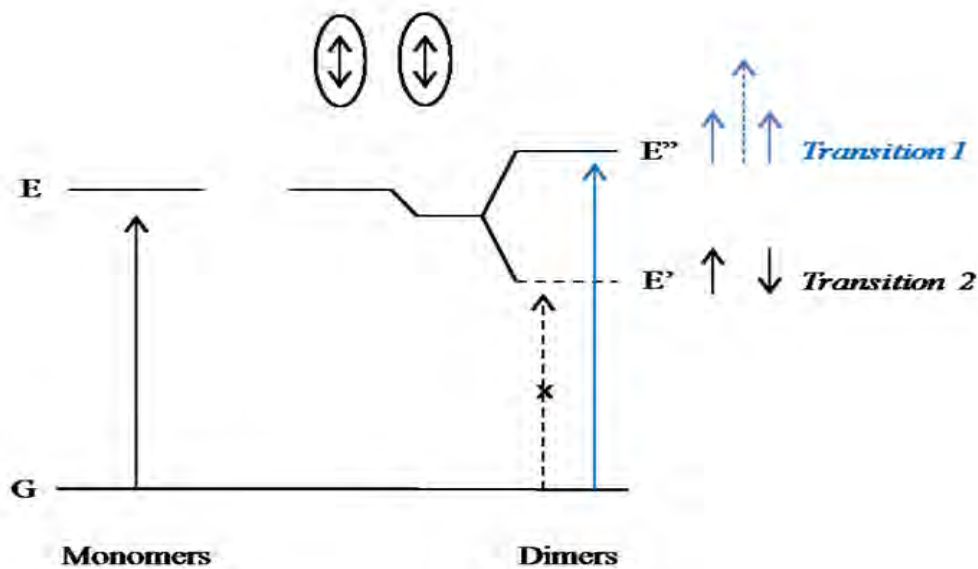
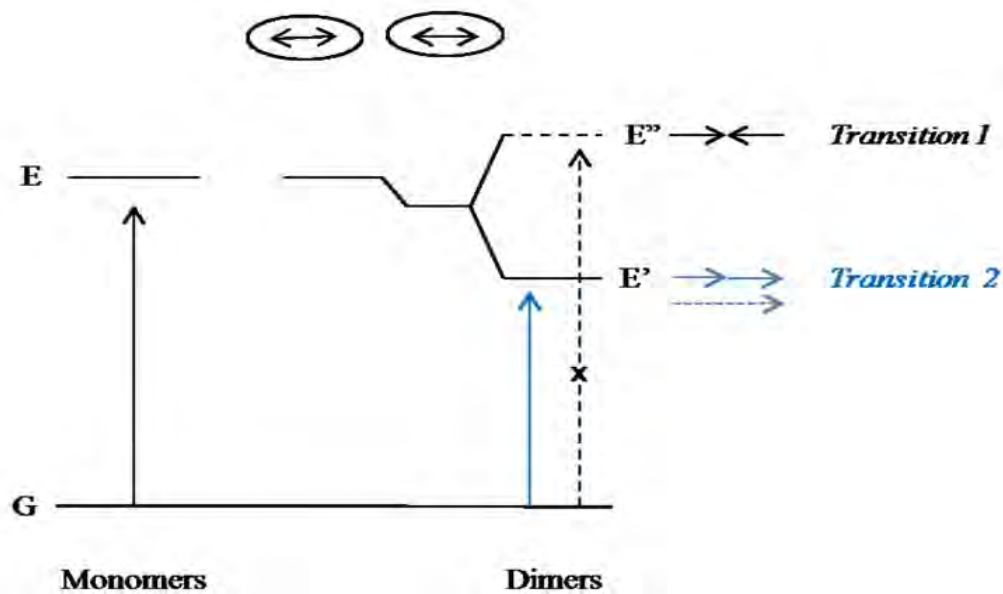**Figure 1.2.2**. Blue shift of the absorption band.

Figure 1.2.3. A red shift of the absorption band.

2.   MATERIALS AND METHODS

In the following procedures, UV-Vis spectra were recorded using a Jasco V-630 Scanning UV-Vis spectrophotometer. Circular dichroism spectroscopy was run on a Jasco J-815 CD spectrophotometer. Temperature was held constant at 20.00° C using a peltier thermoelectric temperature control system. All solutions were prepared and analyzed in a 1.0 cm quartz cuvette. All experiments were performed in water purified with a Milli-Q system. A Mettler Toledo Seven Easy pH meter was used for all pH measurements. UV irradiation was performed using a UVP 100 Watt Mercury Lamp (365 nm). Visible irradiation was performed using a 120V 300W/MR16 incandescent light mounted on a Kodak Ektagraphic projector.

Starting materials were purchased from commercial sources and were used without further purification.

Sodium hydroxide (NaOH) Pellets Certified ACS ≥97.0 %, sulfuric acid (H_2SO_4) Suprapur™ ACS 96% and hydrochloric acid (HCl) 12.1 N Certified ACS Plus were purchased from Fisher Scientific.

Azobenzene-4,4'-dicarboxylic acid (ADA) >95.0% was purchased from TCI America.

Poly-L-glutamic acid sodium salt (poly-L-Glu) and poly-D-glutamic acid sodium salt (poly-D-Glu) were purchased from Sigma Aldrich. The poly-L-Glu had a molecular weight of 750-5,000 g/mol which corresponds to 6-40 amino acids per polymer. The poly-D-Glu had a molecular weight of 2,000-15,000 g/mol which corresponds to 17-177 amino acids per polymer. Stock solutions of the two enantiomers were made by massing

a small quantity of polymer in a 25.00 mL volumetric flask and dissolving the solid in water. The concentration of the solutions were calculated per amino acid using the molar mass of the glutamic acid residue (MM=129.13 g/mol).

The secondary structure of poly-glutamate ($pK_a \sim 4.5$) is determined by the ionization of its carboxylic acid side chains. At acidic pH (less than 5), electrostatic repulsion is reduced by protonation to the extent that the polymer can assume an α-helical conformation[1]. When the side chain is ionized at more basic pH, the electrostatic repulsion between carboxylate anions causes the polymer to adopt an extended random coil conformation. These characteristics are confirmed by the change in characteristic CD signal between 200 and 300 nm. The CD spectra of α-helices have two negative peaks of similar magnitude at 222 nm and 208 nm while the CD spectra of a random coil usually have a strong negative peak around 200 nm and, in many systems, a small positive peak at about 218 nm (**Figure 2.1**).
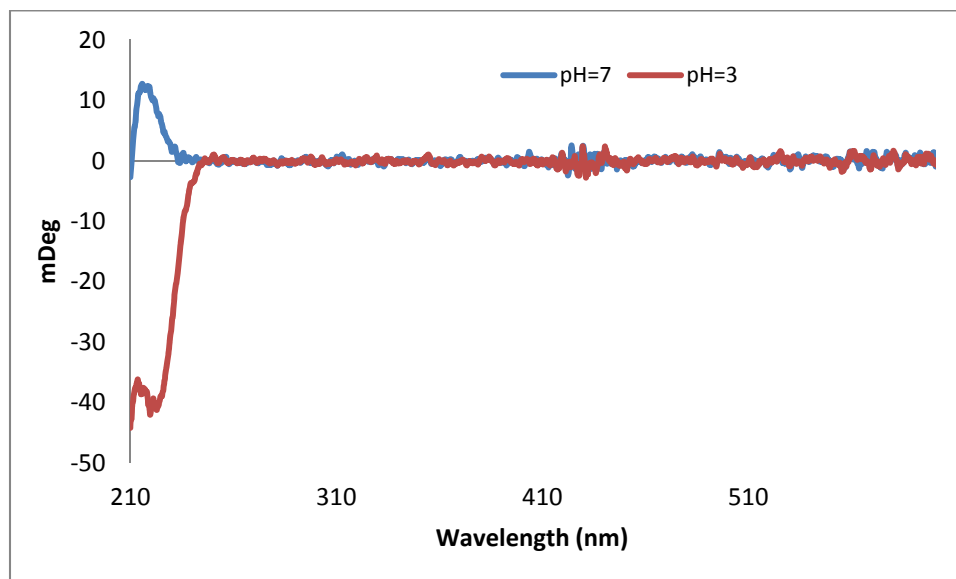

Figure 2.1. CD spectra of poly-L-Glu at pH=7 (blue) and pH=3 (red).

2.1   Solubilization of ADA

The solubility in water of the azobenzene 4,4'-dicarboxylic acid is limited and depends on the pH of the solution reaching the highest value at very basic pH (above pH 10).

Some preliminary data had shown that a decrease in pH of a solution of ADA was promoting its self-aggregation. This process is usually dependent on several factors such as concentration, pH, and temperature. In order to study the self-aggregation of ADA, its dependence on the mode of solubilization of ADA itself was studied.

A stock solution of ADA was made by massing a small quantity of the solid molecule into a 25.00 mL volumetric flask, adding water, and employing either of the following solubilization techniques:

Method A: pH of ADA stock solution was slowly increased to 11 in approximately 0.2 pH unit steps using a 0.1 M aqueous solution of NaOH.

Method B: pH of ADA stock solution was rapidly increased to 11 by a few additions of a 1.0 M aqueous solution of NaOH

In **method A**, absorbance was monitored with each addition of NaOH in order to observe when ADA was fully solubilized (**Figure 2.1.1**). The increase in absorbance leveled off at approximately pH=10.83 and remained constant upon further addition.
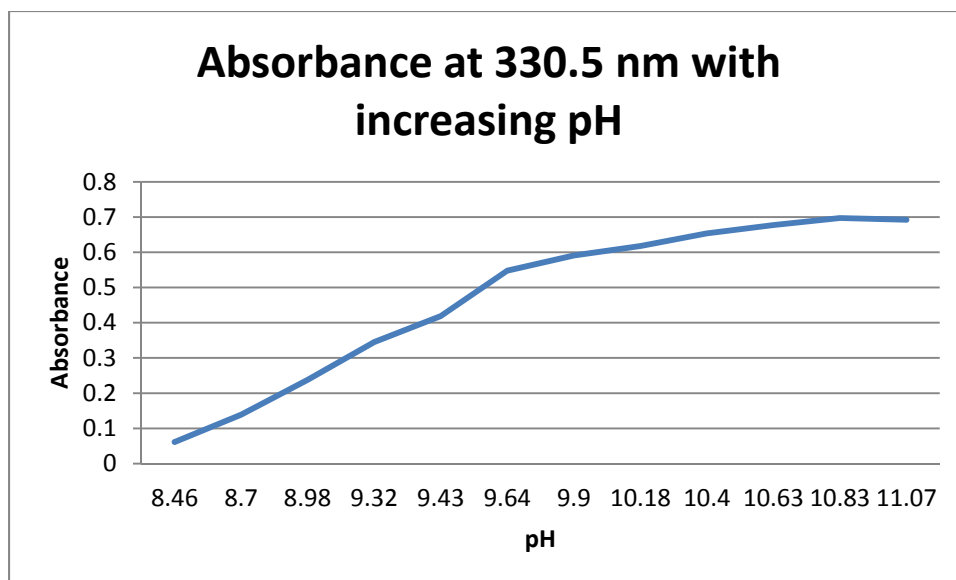
Absorbance at 330.5 nm with increasing pH

Figure showing absorbance (y-axis, 0 to 0.8) vs. pH (x-axis, 8.46 to 11.07). The curve rises from about 0.06 at pH 8.46 to about 0.7 at pH 10.83–11.07.

Figure 2.1.1. Graph of absorbance vs. pH for solubilization **method A.**

The pH was then decreased using a 0.1 M aqueous solution of HCl and it was observed that ADA did not precipitate out of the solution as it became acidic. Although no precipitation was observed, UV-Vis measurements showed a marked decrease in absorbance and a blue shift of the peak from 330.5 to 302 nm (**Figure 2.1.2)** indicating the formation of an aggregate. CD measurements showed that despite the symmetry of the monomer, the aggregate in solution was chiral (**Figure 2.1.3**).
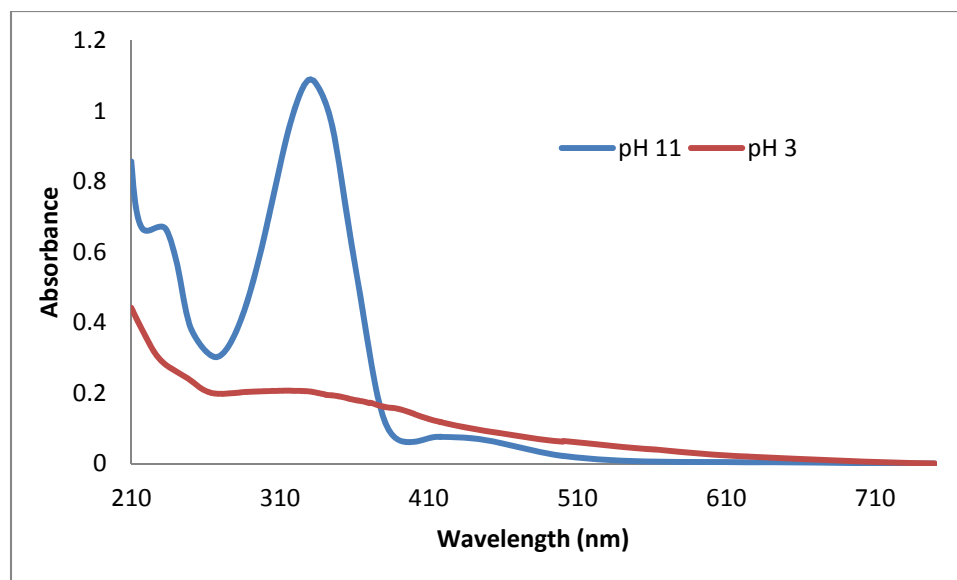
Figure 2.1.2 UV-Vis spectra of the solution of ADA solubilized by **method A** at pH 11.07 (blue) and after decreasing the pH to 3 (red).
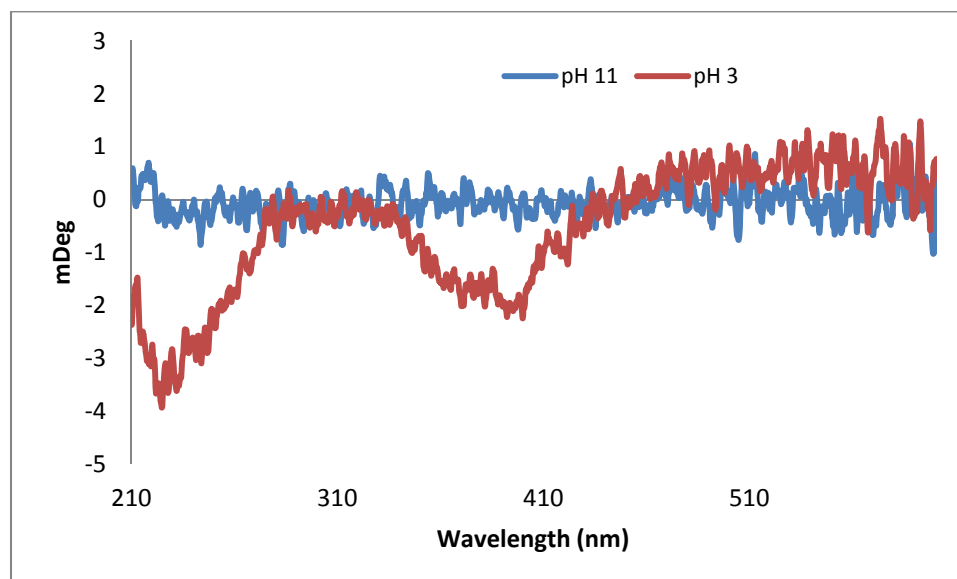
Figure 2.1.3. CD spectra of the solution of ADA solubilized by **method A** at pH 11.07 (blue) and after decreasing the pH to 3 (red).

In **method B**, pH was increased abruptly to 11 using a 1 M aqueous solution of NaOH. Following the addition of base, the solution sat and stirred until the UV-Vis signal stopped rising. In the same manner as **method A**, pH was decreased from 11 to approximately 3 without the formation of a precipitate. A decrease in intensity of the 330 nm peak in the UV-Vis spectra along with the presence of a CD signal again confirmed the existence of a chiral aggregate, but neither the UV-Vis nor the CD signals obtained in **method A** and **method B** were superimposable (**Figure 2.1.4** and **2.1.5**), suggesting that the method of solubilization impacts the subsequent aggregate geometry at low pH.

Since solubilization **methods A** and **B** were evidently not interchangeable, **method B** was chosen as a standard method of making ADA stock solutions.
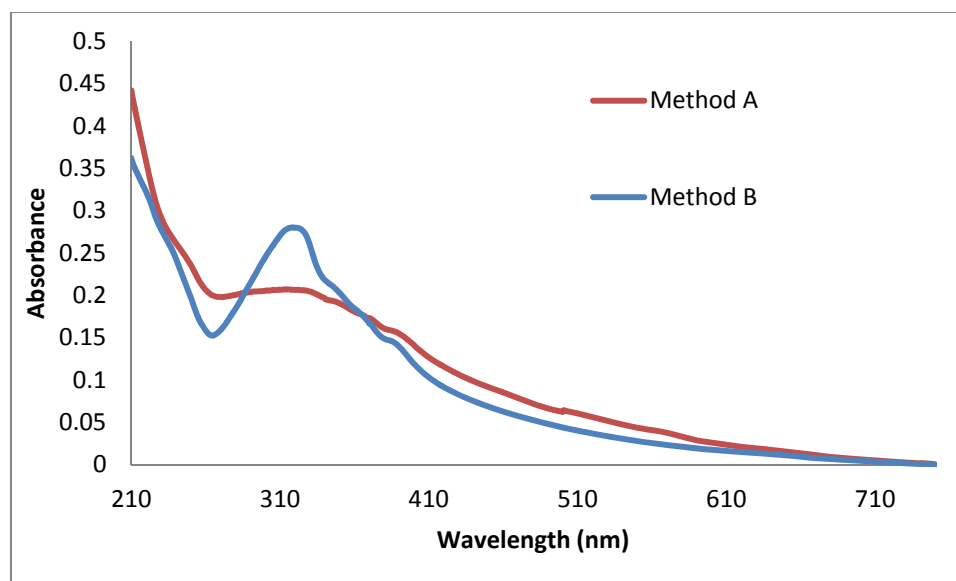
Figure 2.1.4. UV-Vis spectra of two ADA solutions solubilized using **method A** (red) and **method B** (blue) after decreasing their pH to 3.
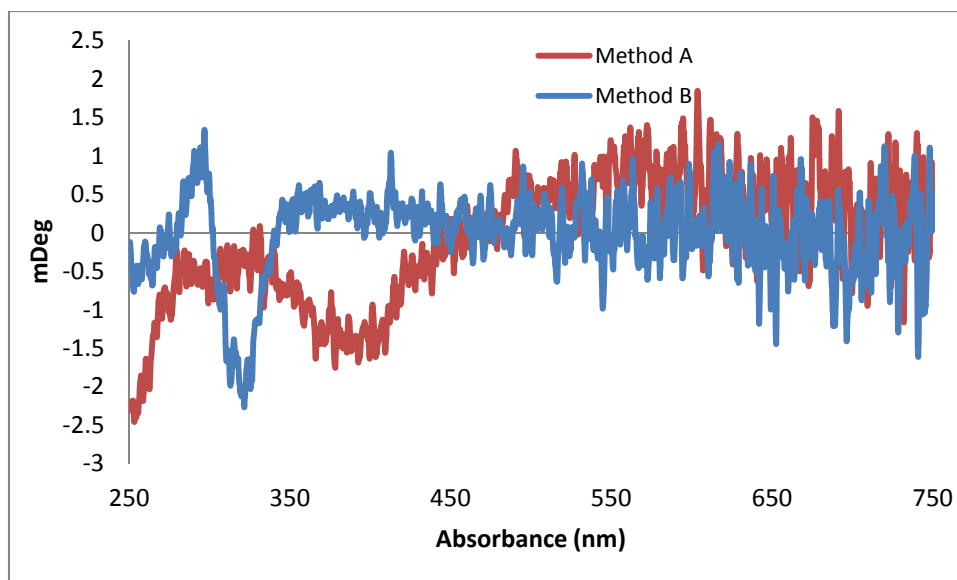
Figure 2.1.5. CD spectra of two ADA solutions solubilized using **method A** (red) and

method B (blue) after decreasing their pH to 3.

2.2   Extinction Coefficient

An extinction coefficient for azobenzene-4,4'-dicarboxylic acid was calculated by plotting the UV-Vis absorbance value versus concentration for a series of ADA solutions. The solutions were prepared by sequential dilutions starting from a stock solution of known concentration prepared by massing a small amount of ADA into a 10.00 mL volumetric flask and adding water and NaOH by **method B**.

All the spectra were measured at pH 11 in a 1.0 cm cuvette and the range of concentrations were chosen so that all the absorbances measured were between 0.1 and 1.

Using the Lambert-Beer Law

$$A = \varepsilon c l \tag{1}$$

where A is the absorbance, ε is the molar absorptivity or extinction coefficient in $M^{-1}cm^{-1}$, c is the concentration in M and l is the path length in cm, a plot of absorbance versus concentration has a slope equal to ε when the path length is 1.0 cm. The plots used to determine the extinction coefficient are given in **Figure 2.2.1**. From the graph, it is determined that the extinction coefficient of ADA at pH 11 is 8286.5 $M^{-1}\cdot cm^{-1}$.
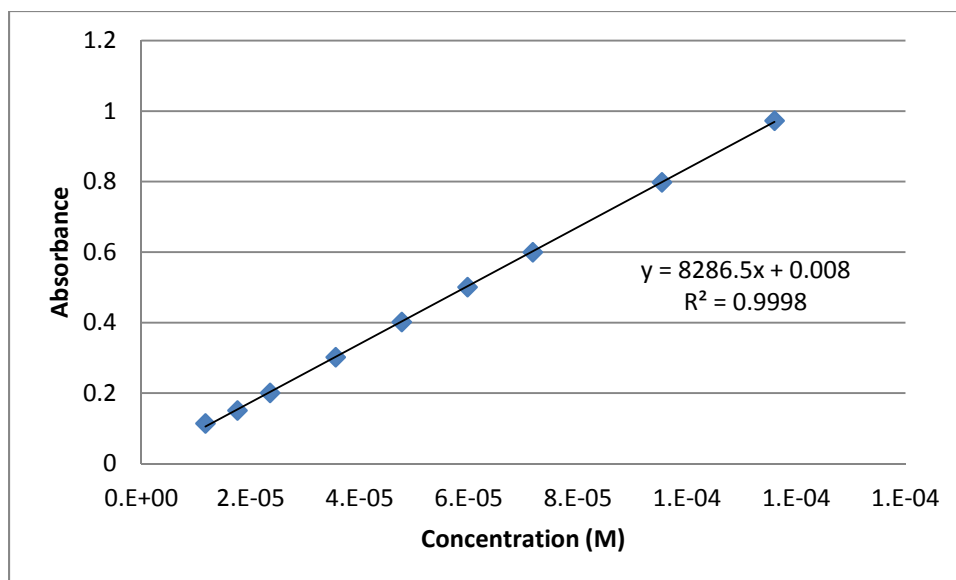
Figure 2.2.1 The Determination of the Extinction Coefficient for Azobenzene 4,4'-

dicarboxylic acid at pH 11.

2.3   Photoisomerization

As shown in **Figure 2.3.1**, the UV-Vis spectrum of ADA in water at pH 7 has two main bands at 228 nm and 330 nm corresponding to the *trans* isomer with the intense peak at 330nm attributed to the π-π* transition of the *trans* isomer. Irradiation of the solution with UV light results in a marked decrease in absorbance at both the 228 and the 330 nm bands and an increase of two bands at 250 nm and 430 nm corresponding to the *cis* isomer, with the weak peak at 430 nm attributed to the n-π* transition of the *cis* isomer[14].

It was determined that irradiating the sample for 15 minutes with a 100 Watt mercury lamp (365 nm) causes the solution to reach a photostationary state after which further irradiation does not result in changes to the UV-Vis absorbance. The *trans* form can be restored by irradiation with visible light or via thermal relaxation. Starting from the *trans* isomer, it is possible to perform a full cycle of photoswitching by irradiation with UV light followed by irradiation with visible light. **Figure 2.3.1** shows how the spectra changes after irradiation with UV light and **Figure 2.3.2** shows how it is restored after irradiation with visible light, demonstrating that the photoisomerization is reversible.
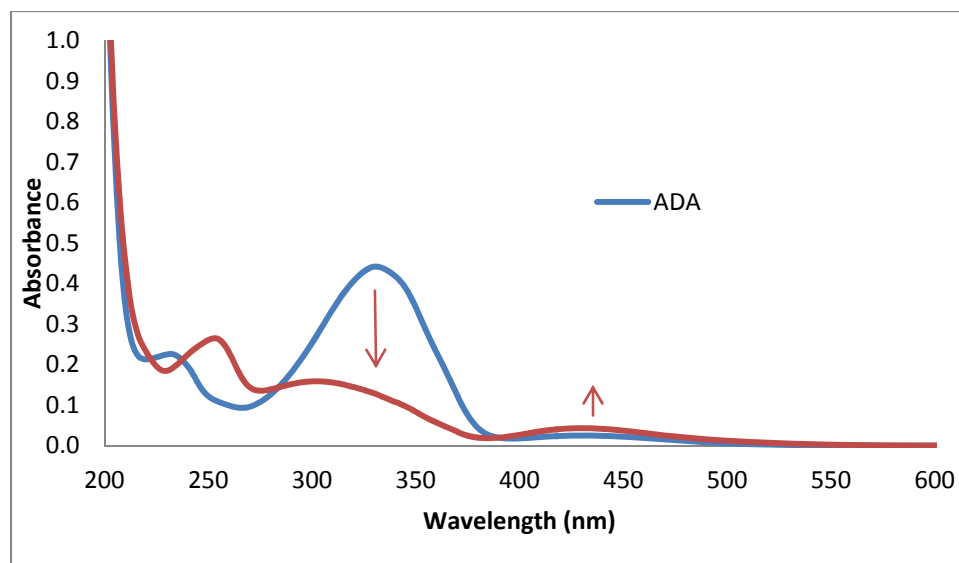
Figure 2.3.1. UV-Vis spectra of *trans*-ADA (blue) and *cis*-ADA (red); *cis*-ADA is photogenerated after irradiation with UV light (365 nm) for 15 minutes.
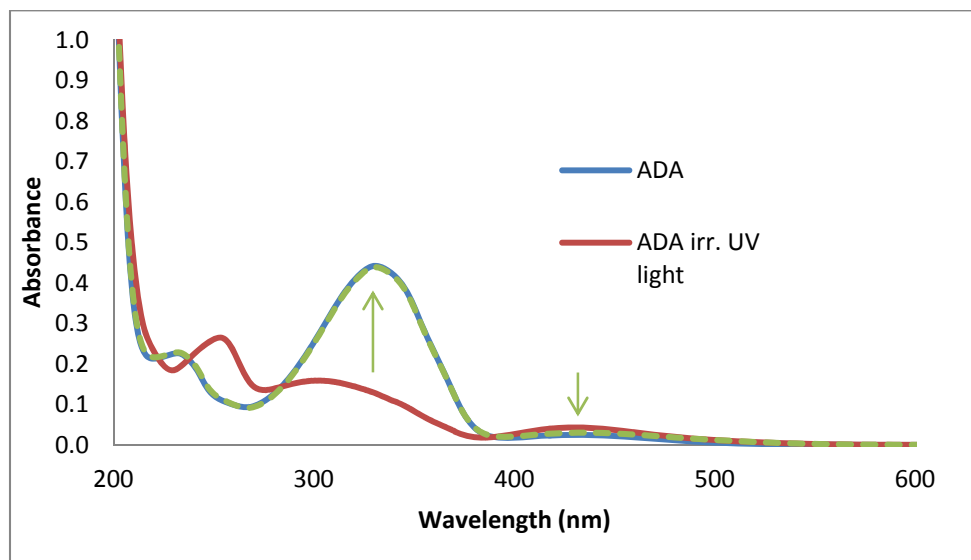

Figure 2.3.2. UV-Vis spectra of a full irradiation cycle: cycle: (⸺) *trans*-ADA before irradiation, (⸺) *cis*-ADA after UV irradiation, and (⸻) *trans*-ADA after irradiation with visible light.

2.3 **Aggregation Procedures**

The *trans*-ADA solutions were prepared in cuvettes by adding an aliquot of a stock solution of azobenzene 4,4'-dicarboxylic acid prepared by **method B** to either Milli-Q water of a 0.6 mM aqueous solution of poly-Glu in order to obtain a 15uM solution of *trans*-ADA. For all the experiments the initial pH of the solution was brought to 7 using either H_2SO_4 of NaOH

In order to study self-aggregation phenomena at acidic pH, the following four procedures were employed for the formation of the aggregates:

- **Procedure 1 (Fast-*trans*):** The pH of the **_trans_-ADA solution** was decreased from 7 to 3 by a 6 μL addition of 1M H_2SO_4. The solution was monitored until the UV-Vis and CD spectra no longer changed.

- **Procedure 2 (Slow-*trans*):** The pH of the **_trans_-ADA solution** was decreased by increments of 0.5 pH units by adding H_2SO_4.

- **Procedure 3 (Fast-*cis*):** The **_trans_-ADA solution** was irradiated with UV light for 15 minutes. The cuvette remained under exposure to UV light for the duration of the pH measurement. The pH was decreased from 7 to 3 by a large addition of H_2SO_4. The solution was monitored until the UV-Vis and CD spectra no longer changed. The solution was then irradiated with visible light for 15 minutes in order to switch back to the *trans* isomer.

- **Procedure 4 (Slow-*cis*):** The **_trans_-ADA solution** was irradiated with UV light for 15 minutes. The cuvette remained under exposure to UV light for the duration of the pH decrease. The pH was decreased by increments of 0.5 pH units by

adding H_2SO_4. The solution was then irradiated with visible light for 15 minutes

in order to switch back to the *trans* isomer.

3. ADA SELF-AGGREGATION

3.1   Fast-*trans* pH Decrease

An aqueous solution of *trans*-ADA was prepared according to the **fast**-*trans* procedure (**Figure 3.1.1 and 3.1.2**). The kinetics of the aggregation was monitored using UV-Vis and CD spectroscopy. The band at 330 nm decreased in intensity with pH reduction and continued to decrease with time. After 60 minutes the signal no longer changed. The decrease was accompanied by a blue shift from 330 nm to 300 nm, which suggests the formation of an H aggregate.

At pH 3, the circular dichroism spectrum confirms the presence of an asymmetrical aggregate, and exhibits a broad bisignate signal spanning from approximately 400 nm to 200 nm. The intensity of this signal did not change with time. The chirality observed is an emergent property of the aggregate structure, since the ADA itself is achiral. Although the hypochromic affect observed in the UV-Vis spectra is quite pronounced, the CD signal is very small. Since the CD only testifies to the presence of chiral aggregates, it seems that a majority of the aggregation in solution is achiral in nature.
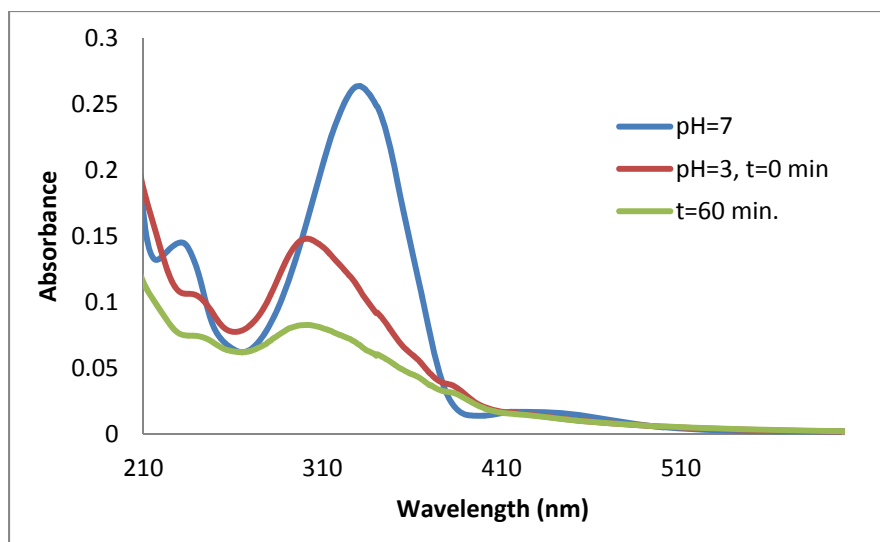
Figure 3.1.1. Effect of decreasing pH on UV-Vis spectra of *trans*-ADA according to procedure **fast-*trans***: *trans*-ADA at pH=7 (blue); *trans*-ADA at pH=3 at t= 0 minutes (red); *trans*-ADA at pH=3 at t= 60 minutes (green).
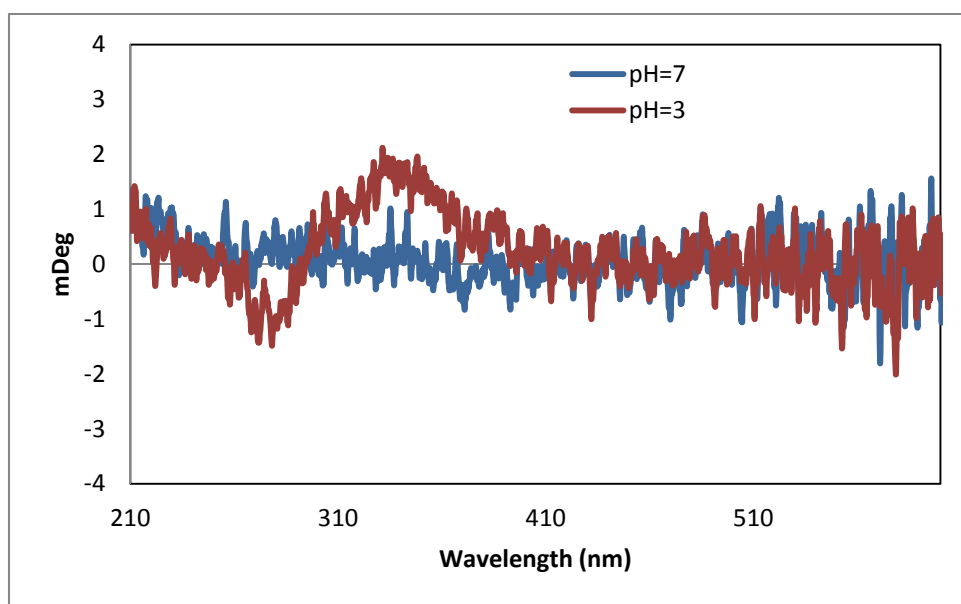

Figure 3.1.2 Effect of decreasing pH on CD spectra of *trans*-ADA according to procedure **fast-*trans***: *trans*-ADA at pH=7 (blue); *trans*-ADA at pH=3 (red). The CD signal does not change with time.

Upon irradiation, the sample did not exhibit the characteristic spectroscopic changes associated with *trans* to *cis* photoisomerization. There are several possibilities for explaining this loss of photodynamic control, but the most likely reason is that the tight packing of *trans*-ADA in an H aggregate geometry provides a hindrance to motion that the energy of excitation cannot overcome.

Although *trans*-ADA loses its photoswitching capabilities when aggregated at low pH, an increase in pH through addition of NaOH breaks the aggregate, as shown by the disappearance of the CD signal and the increase of the absorption band at 330 nm. Upon disassembly of the aggregate, the azobenzene regains its photochromic behavior in response to UV and visible light. Aggregation is accordingly a reversible process dependent on pH, and the ability to isomerize from the *trans* to the *cis* forms of ADA is determined by the degree of aggregation. The cycle of *trans*-ADA aggregation, loss of photoswitching capabilities, disaggregation, and reinstatement of photoswitching abilities is shown in **Figure 3.1.3.**
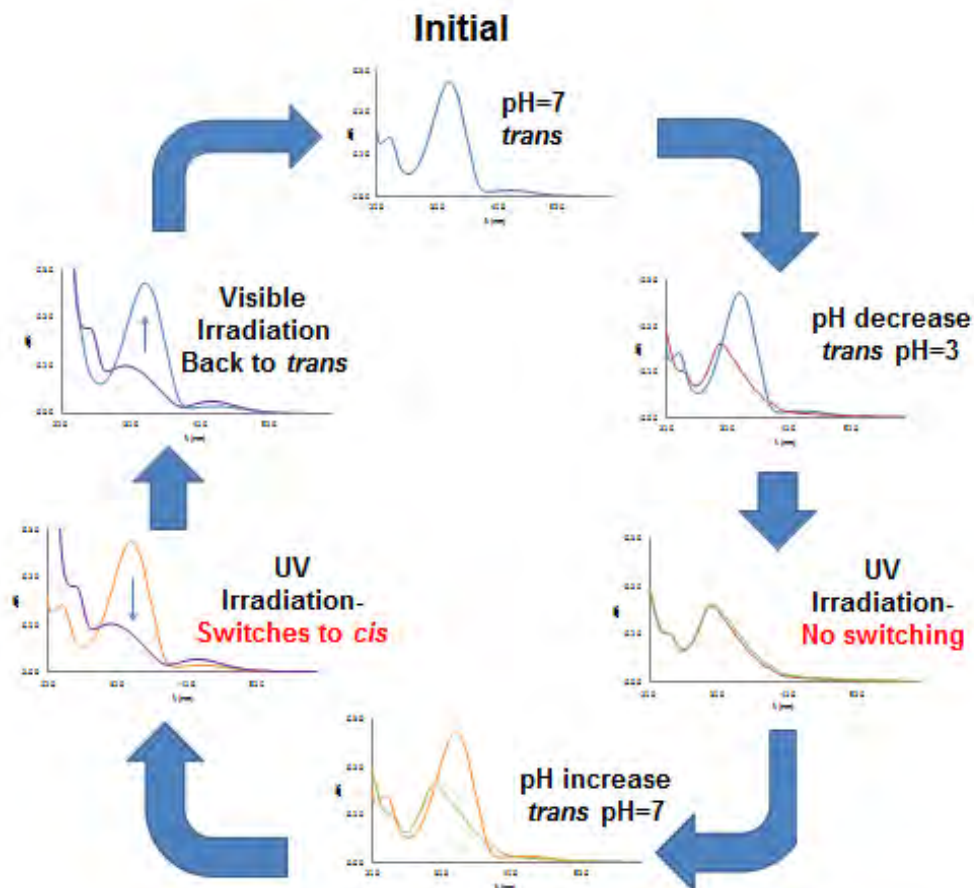
Figure 3.1.3 Cycle showing the inability of *trans*-ADA to switch while aggregated and regaining ability to photoisomerize at pH=7 due to disaggregation (See Appendix for individual spectra, **Figures 7.1-7.6**).

3.2   Slow-*trans* pH decrease

An aqueous solution of *trans*-ADA was prepared according to the **slow-*trans***

procedure. UV-Vis and CD spectra (**Figures 3.2.1** and **3.2.2**) were taken for each pH

value. As pH decreases, the 330 nm band undergoes a hypochromic effect that attenuates

the peak as the pH decreases until the signal is almost nonexistent. The CD spectrum

shows two negative bands at 355 nm and 230 nm with intensities of -2.81 mdeg and -2.30

mdeg, respectively, that appeared at pH=5 and did not increase in intensity as further

additions of acid were made. Although the CD signal did not increase, the 330 nm band

in the UV-Vis spectra continued to decrease with further additions. The absence of a blue

shift and the different CD signal show that the aggregate formed is not an H aggregate.

Additionally, the absence of a red shift suggests that it is not a J aggregate. A plot of

absorbance at 330 nm vs. pH for the **slow-*trans*** pH decrease yields a sigmoidal curve

with an inflection point around pH=5.3 that corresponds to the pK_a of the *trans*-ADA.

The higher pK_a value of the carboxyl groups on the ADA compared to those on a benzoic

acid ($pK_a = 4.2$) suggests that the protonation process is favored by the subsequent

formation of the aggregate (**Figure 3.2.3**).
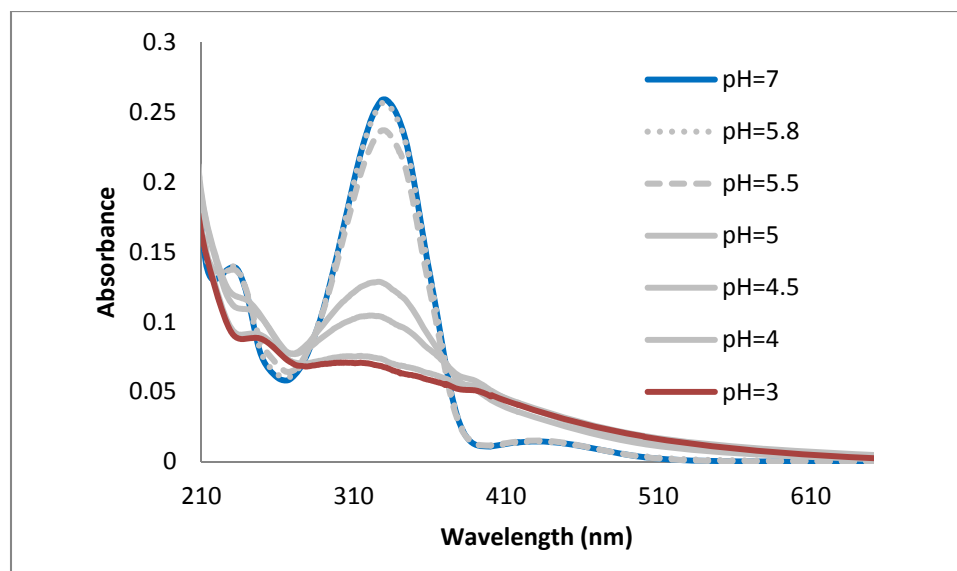
Figure 3.2.1 Effect of decreasing pH on UV-Vis spectra of *trans*-ADA according to procedure **slow-*trans***: *trans*-ADA at pH=7 (blue); *trans*-ADA at pH=3 (red); intermediate pH steps (gray).
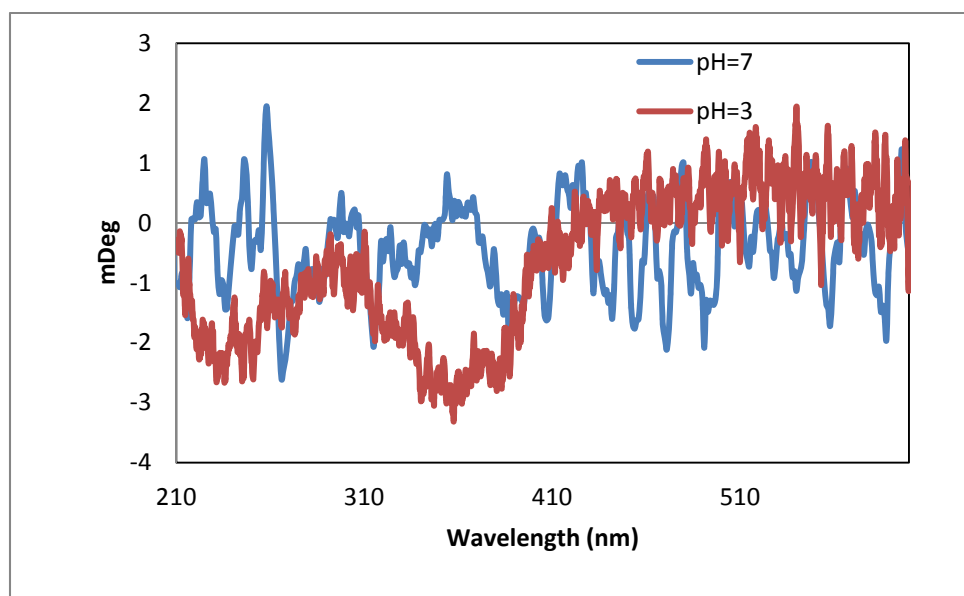

Figure 3.2.2. Effect of decreasing pH on CD spectra of *trans*-ADA according to procedure **slow-*trans***: *trans*-ADA at pH=7 (blue); *trans*-ADA at pH=3 (red). The intermediate steps omitted for clarity.
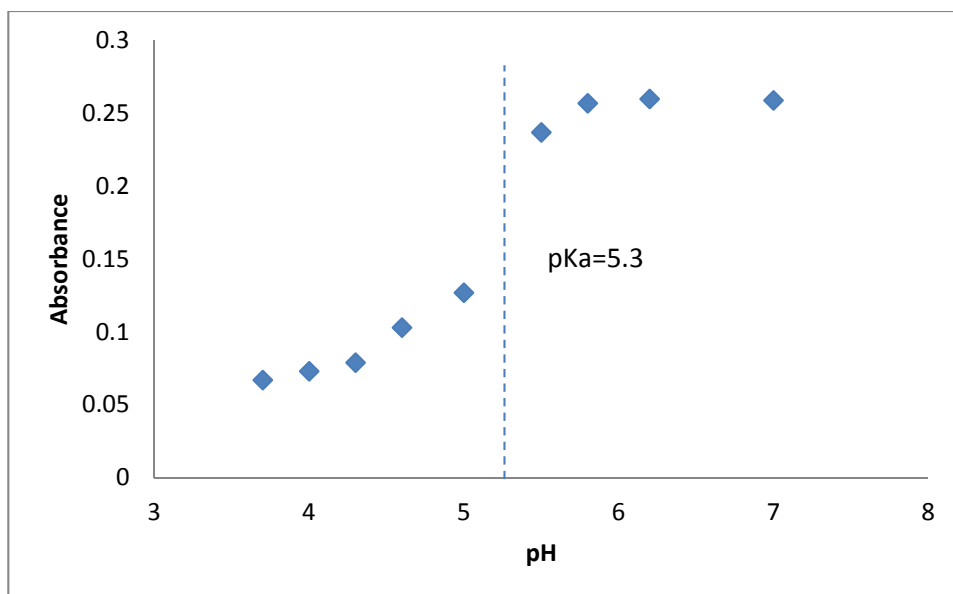
Figure 3.2.3. Plot of absorbance at 330 nm vs. pH for a pH decrease of a solution of

trans-ADA according to procedure **slow-*trans***.

3.3   Fast-*cis* pH Decrease

A solution of *trans*-ADA was irradiated with UV light to obtain the *cis* form of the molecule. pH decreases were performed in the dark with continued UV irradiation to maintain the highest *cis-trans* ratio possible. After approximately 15 minutes of irradiation, the ADA reaches a photostationary state in which the molecule attains equilibrium between the *trans* and *cis* isomers with the ratio of *cis* to *trans* depending on the ratio of their respective extinction coefficients at the wavelength of irradiation. Due to overlap of the absorption bands, we can never assume that our solution is entirely in the *cis* form. The aggregate structures obtained could, therefore, contain both the *cis* and *trans* isomers in the overall geometry.

A fast decrease in pH was performed on aqueous *cis*-ADA according to the **fast-cis** procedure. The kinetics of the aggregation was monitored using UV-Vis and CD spectroscopy. Immediately following the addition of H_2SO_4, the 230 nm band exhibited a slight red shift and hypochromic effect while the 330 nm and 430 nm bands lost their maxima and were replaced by a heavily scattered baseline (**Figure 3.3.1**). After 60 minutes, the band at 230 nm disappears and a new weak broad band appears at approximately 360 nm (intensity of 0.05). The CD spectra (**Figure 3.3.2**) suggest that the initial aggregate is continually changing until it reaches its final structure after 60 minutes. The initial signal has a small positive peak, at 390 nm with an intensity of +2.23 mdeg and three small negative peaks, at 330 nm, 280 nm and 220 nm with intensities of -1.43 mdeg, -2.77 mdeg and -2.33 mdeg, respectively. After 60 minutes, the CD spectrum shows only three small negative peaks, at 350 nm, 220 nm and 280 nm with intensities of 2.45 mdeg, -3.0 mdeg and -3.0 mdeg, respectively.

The sample was irradiated with visible light in order to isomerize *cis*-ADA to the *trans* form. The UV-Vis spectrum shows a slight increase in intensity and a blue shift of the peak at 340 nm to 300 nm with an absorbance of 0.078. The shape, position and intensity of the absorption spectrum are analogous to those of the spectrum of the *trans*-ADA aggregate obtained following the **fast-*trans*** procedure (see paragraph 3.6), indicating that the photoisomerization took place in the aggregated form. The CD spectrum shows the disappearance of the three negative bands and the appearance of a bisignate signal superimposable with the one obtained with the **fast-*trans*** procedure (see paragraph 3.6).

Therefore, the isomerization of the aggregated *cis* form to *trans* either breaks the existing aggregate and provides environmental conditions similar to those obtained by a quick protonation, promoting the formation of the H aggregate, or takes place in a pre-oriented geometry that favors the H aggregate.

In order to confirm that the *cis*-ADA had indeed isomerized to the *trans* isomer, the pH of the solution was increased to 7 with NaOH. The UV-Vis spectrum obtained was superimposable with the initial spectrum of the *trans*-ADA sample before irradiation with UV light, demonstrating the high efficiency of the photoisomerization process for this supramolecular system. The cyclical nature of this process is shown in **Figure 3.3.3**.
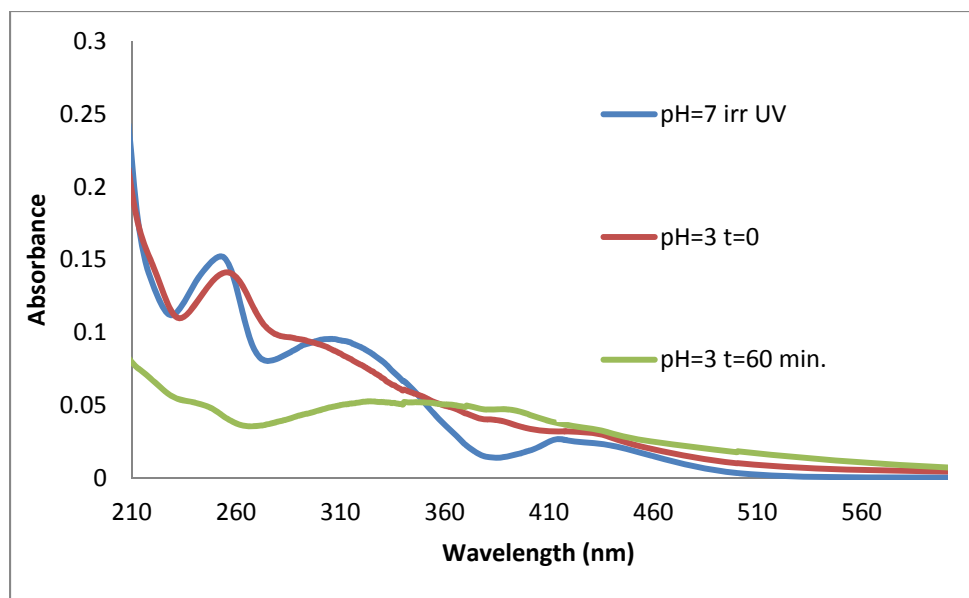
Figure 3.3.1. Effect of decreasing pH on UV-Vis spectra of *cis*-ADA according to procedure **fast-*cis***: *cis*-ADA at pH=7 (blue); *cis*-ADA at pH=3 at t-0 minutes (red), *cis*-ADA at pH=3 at t=60 minutes (green).
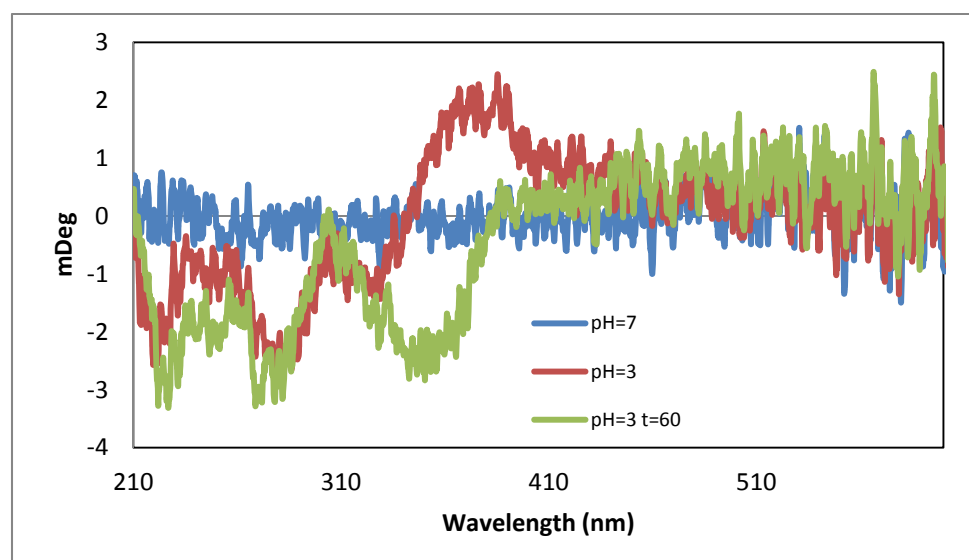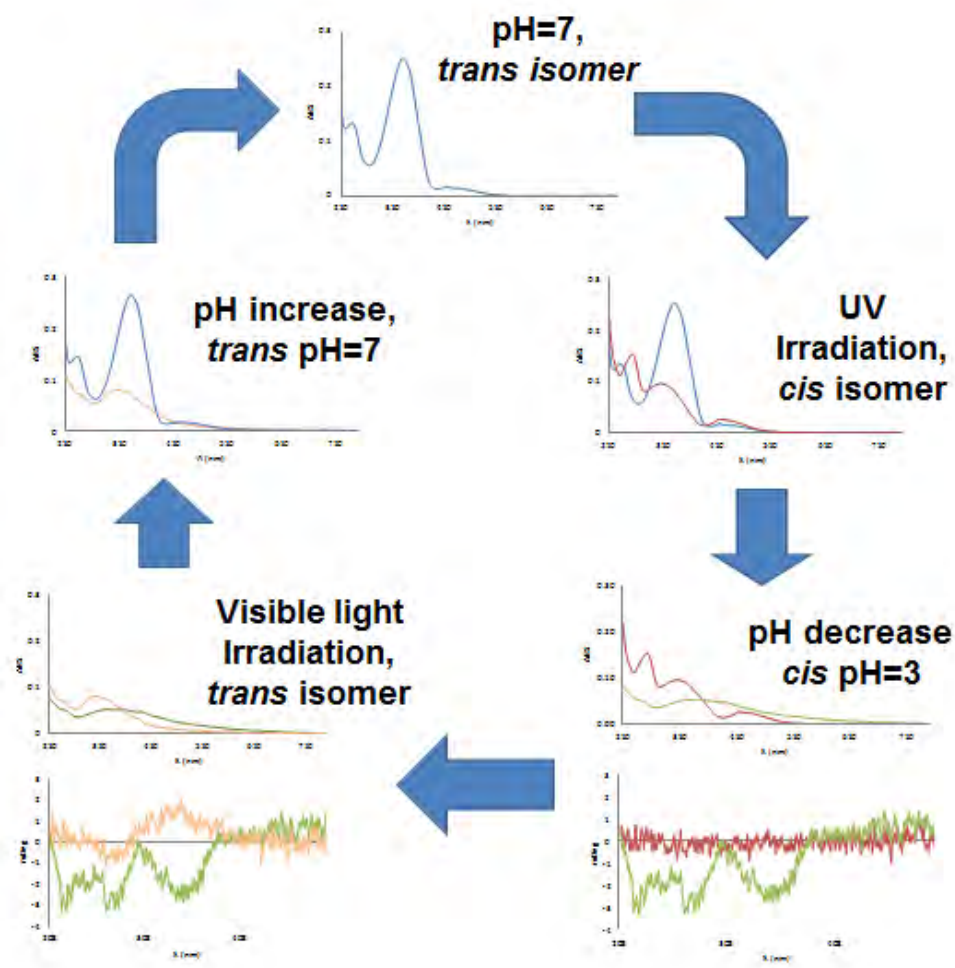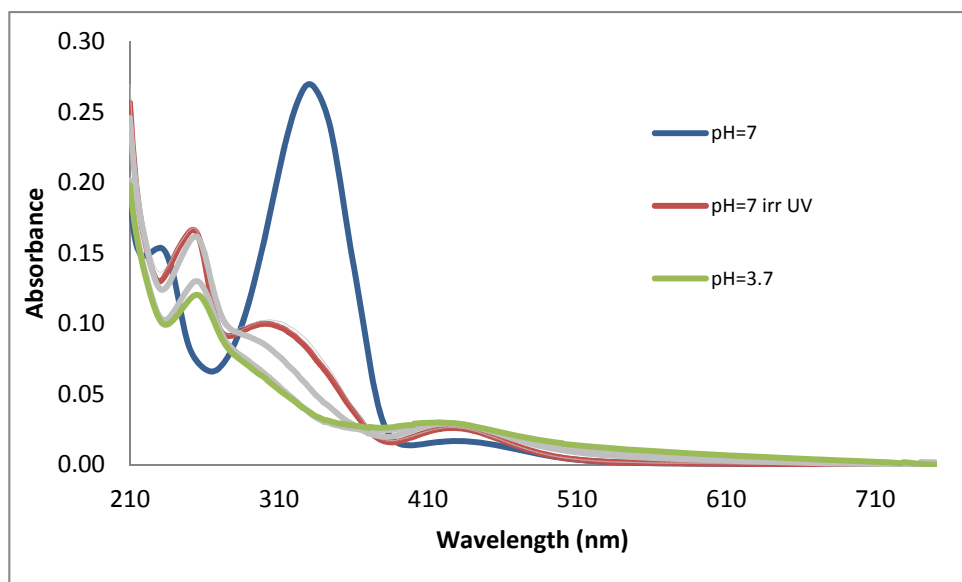

Figure 3.3.2. Effect of decreasing pH on CD spectra of *cis*-ADA according to procedure **fast-*cis***: *cis*-ADA at pH=7 (blue); *cis*-ADA at pH=3 at t-0 minutes (red), *cis*-ADA at pH=3 at t=60 minutes (green).

Figure 3.3.3. Cycle that involves the photoisomerization of the *cis* aggregate to the *trans* aggregate. The steps of the cycle are: *trans*-ADA at pH=7 (top), photoisomerization at pH=7 to the *cis* form (top right), pH decrease and formation of the *cis* aggregate (bottom right), visible irradiation at pH=3 with photoisomerization of *cis* aggregate to *trans* aggregate (bottom left), increase of pH to 7 and disaggregation (top left), and closing of the cycle (See Appendix for individual spectra, **Figures 7.7-7.12**).

3.4   Slow-*cis* pH decrease

An aqueous solution of *cis*-ADA was prepared according to the **slow-*cis***
procedure. UV-Vis and CD spectra (**Figures 3.4.1** and **3.4.2**) were taken for each pH
value. The UV-Vis shows a hypochromic effect and slight red shift of the 230 nm band
and blue shift of the 430 nm band. The baseline of the UV-Vis spectrum for the aggregate
undergoes a marked hyperbolic increase and is attributed to scattering of photons, giving
an apparent increase of the 430 nm band. A CD signal first appears at pH= 4.0, and the
spectra exhibits a weak, broad negative signal with a maximum intensity of -1.99 mdeg
around 370 nm, and slightly more intense and narrow signal of -2.9 mdeg at 250 nm.

The solution at pH= 3 was irradiated with visible light. There was a marked
decrease in the 230 nm and 430 nm peaks in the UV-Vis spectrum (**Figure 3.4.3**). The
CD signal does not change so much in position but increases slightly in magnitude
(**Figure 3.4.4**). The broad negative signal, spanning from 680 to 340nm, shows a peak at
408 nm with an intensity of -3.21 mdeg. The narrow band red shifts slightly and increases
in intensity to -4.0 mdeg. The only slight change of the CD signal upon irradiation
suggests that perhaps the initial signal was primarily due to the self-aggregation of the
portion of *trans*-ADA present in solution at the photostationary state rather than an
aggregation of the *cis* isomer.

Figure 3.4.1. Effect of decreasing pH on UV-Vis spectra of *cis*-ADA according to procedure **slow-*cis***: *trans*-ADA at pH=7 (blue), *cis*-ADA at pH=7 (red), *cis*-ADA at pH=3.7 (green), intermediate steps (gray).
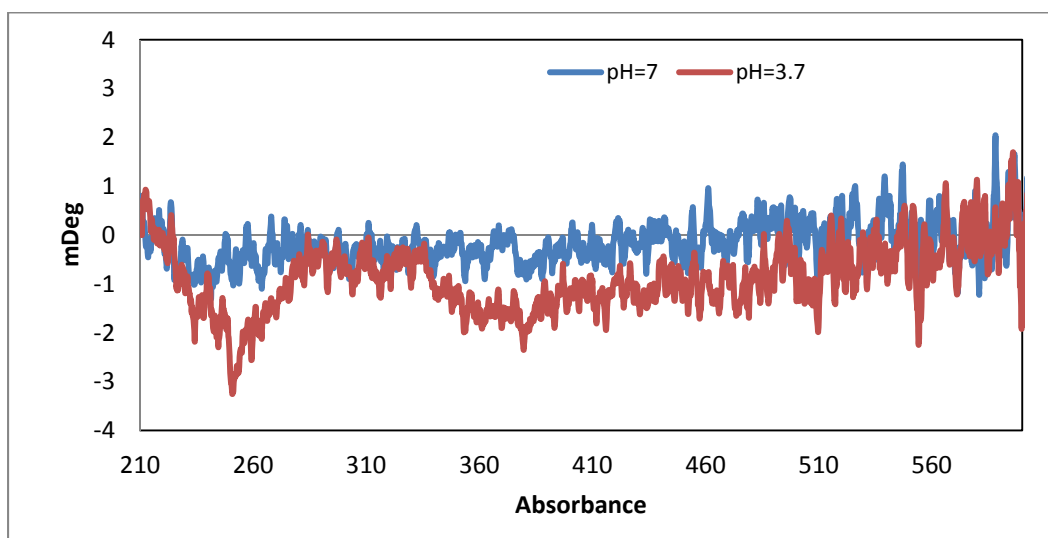

Figure 3.4.2. Effect of decreasing pH on CD spectra of *cis*-ADA according to procedure **slow-*cis***: *cis*-ADA at pH=7 (blue); *cis*-ADA at pH=3.7 (red). The intermediate steps omitted for clarity.
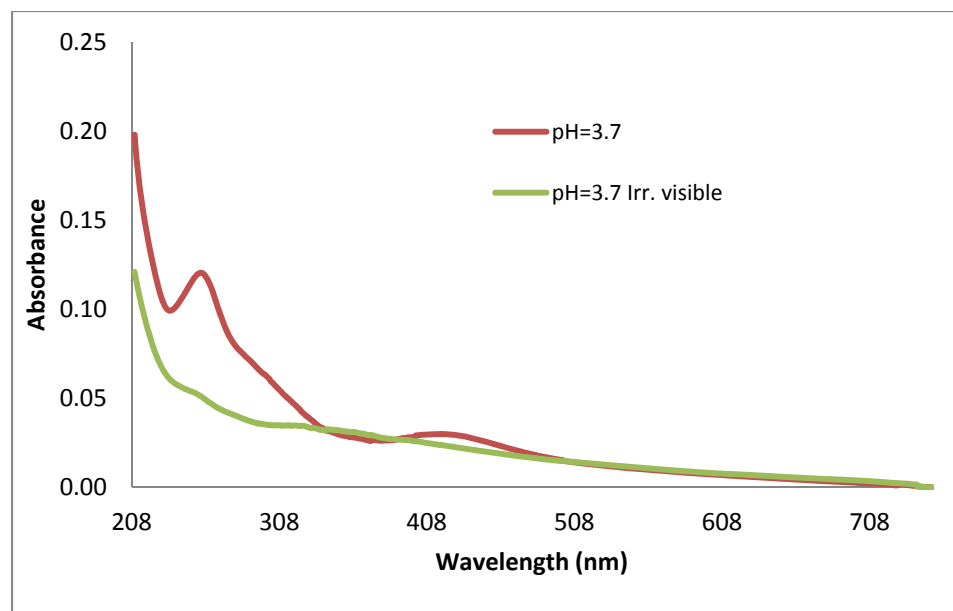
Figure 3.4.3. Effect of visible irradiation on UV-Vis spectrum of *cis*-ADA brought to pH=3.7 by procedure **slow-*cis***: *cis*-ADA at pH=3.7 (red), *trans*-ADA (green).
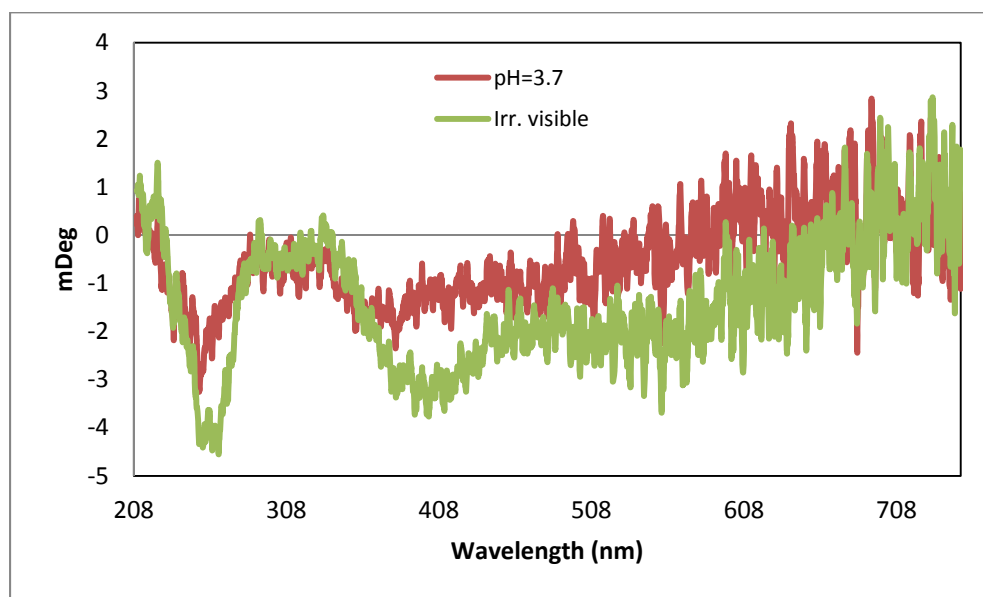
Figure 3.4.4. Effect of visible irradiation on CD spectrum of *cis*-ADA brought to pH=3.7 by procedure **slow-*cis***: *cis*-ADA at pH=3.7 (red), *trans*-ADA (green).

3.5   Hierarchical Control

Although the final conditions for both the fast and slow pH decreases were the same, comparison of spectra for the two methods reveals that the steps through which these conditions were reached affected the geometry of the resulting aggregate. This is not surprising since several supramolecular systems are formed under hierarchical control, with the aggregation process depending on the order and timing of addition of the molecular components.

Both pH decreases of the *trans* isomer yielded a chiral aggregate, as shown by the presence of a CD signal in both cases. The structure of the aggregate resulting from the fast pH decrease is far more defined than the structure resulting from the slow pH decrease. In the fast decrease, the 330 nm band exhibits a marked blue shift as it decreases in intensity which is the characteristic optical response of a stacked H aggregate (**Figure 3.5.1**). The slow decrease, however, showed a more pronounced hypochromic effect with no blue shift indicating that the geometry of the aggregate is neither parallel (H aggregate) nor head-to-tail (J aggregate), but a sort of intermediate arrangement. The CD signals of the aggregates showed a slightly asymmetric bisignate curve suggesting the presence of an exciton coupling between the chromophores combined with other intrinsic chiral signals for the aggregate obtained with the **fast-*trans*** procedure. The geometry resulting from the **slow-*trans*** procedure seems unfavorable for exciton coupling, giving rise to two negative bands (**Figure 3.5.2**).

The difference in aggregate structure between the two methods of pH decrease can possibly be attributed to the different extents to which *trans*-ADA molecules are

protonated during the aggregation process. In the fast decrease, all of the trans-ADA is protonated at the same time, causing it to snap into the most favorable geometry in order to take advantage of hydrogen bonds and π-π stacking. This results in the orderly parallel H-aggregate. In contrast, during the slow pH decrease, the molecules of trans-ADA are in various stages of protonation as the aggregation is occurring. For this reason it is assumed that the solution contains species with various degrees of protonation. The protonated groups are available to form hydrogen bonds with oxygen and/or nitrogen, but the erratically dispersed negative charges might introduce some constraints due to their reciprocal electrostatic repulsion. The solution eventually reaches a point when all carboxyl groups are protonated, but the presence of negative ions during the aggregation process prevents *trans*-ADA from stacking neatly into an H aggregate and causes a different structure to result. One possibility is that the structure consists of a staggered, stacked trans-ADA aggregate with protonated carboxyl groups aligned with azo groups, allowing for the formation of hydrogen bonds between the –OH and the -N=N groups and π-π stacking between the benzene rings. Further measurements such as x-ray crystallography and computational analysis are being considered in order to elucidate the structure.

Similar to the *trans* aggregate, the *cis* aggregate also assembles in a hierarchical manner. In the two different methods of pH decrease for the *cis* isomer, both the different UV-Vis (**Figure 3.5.3**) and CD (**Figure 3.5.4**) spectra at pH 3 indicate that the aggregates formed in the two cases have different structures, confirming a hierarchical control of the aggregation process for the *cis* isomer.
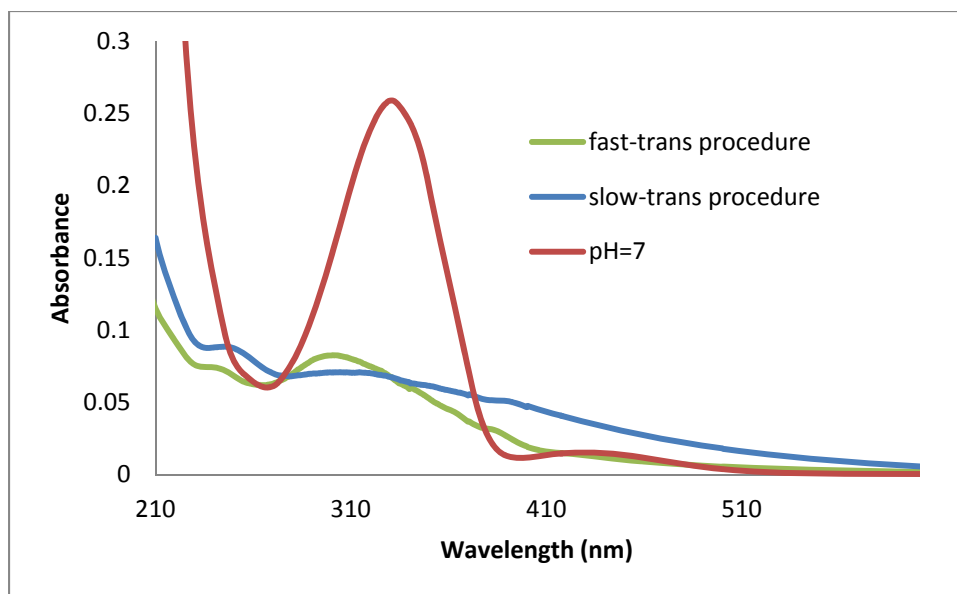
Figure 3.5.1. Comparison of UV-Vis spectra of solutions of *trans*-ADA at pH=7 (red), at pH=3 obtained by **fast-*trans*** procedure (green) and at pH=3 obtained by **slow-*trans*** procedure (blue).
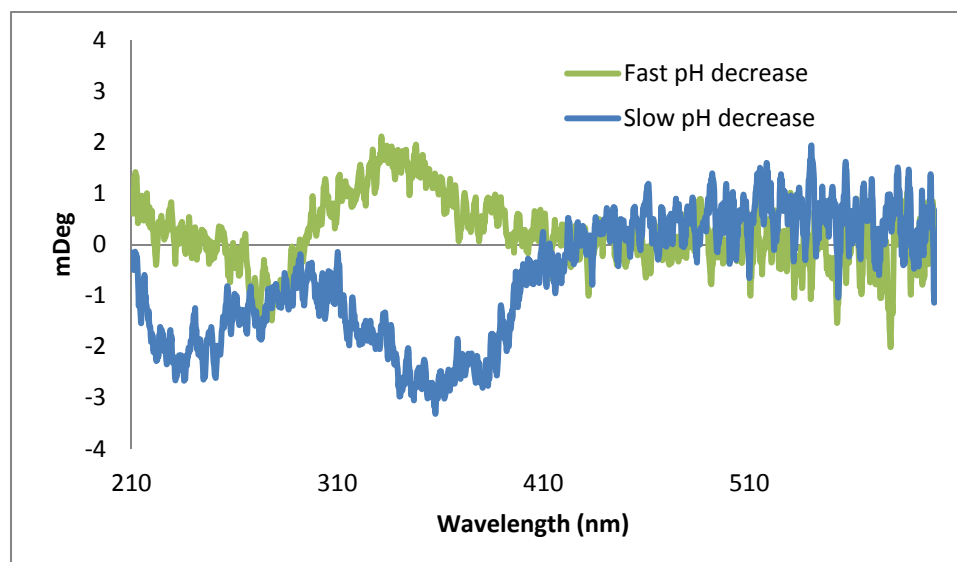

Figure 3.5.2. Comparison of CD spectra of solutions of *trans*-ADA at pH=3 obtained by **fast-*trans*** procedure (green) and at pH=3 obtained by **slow-*trans*** procedure (blue).
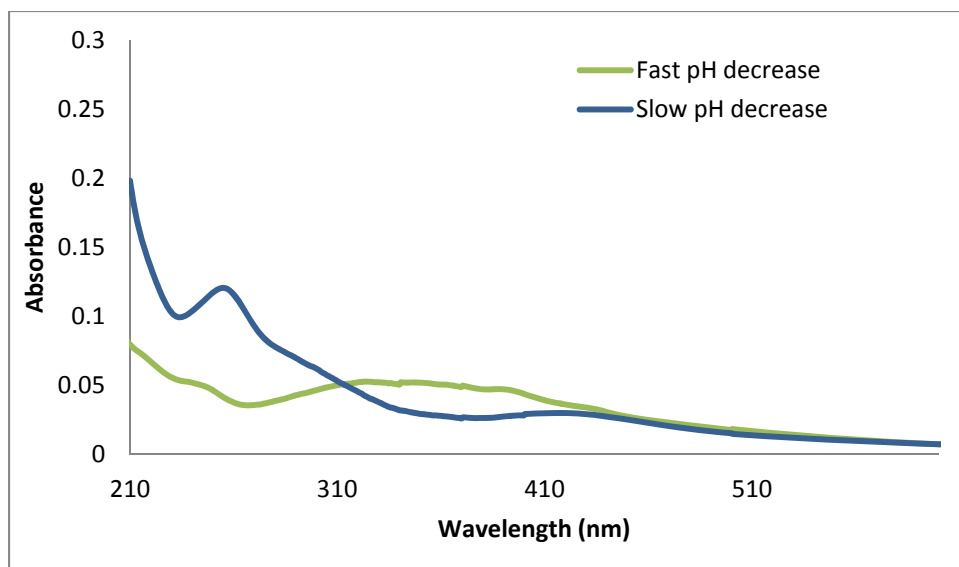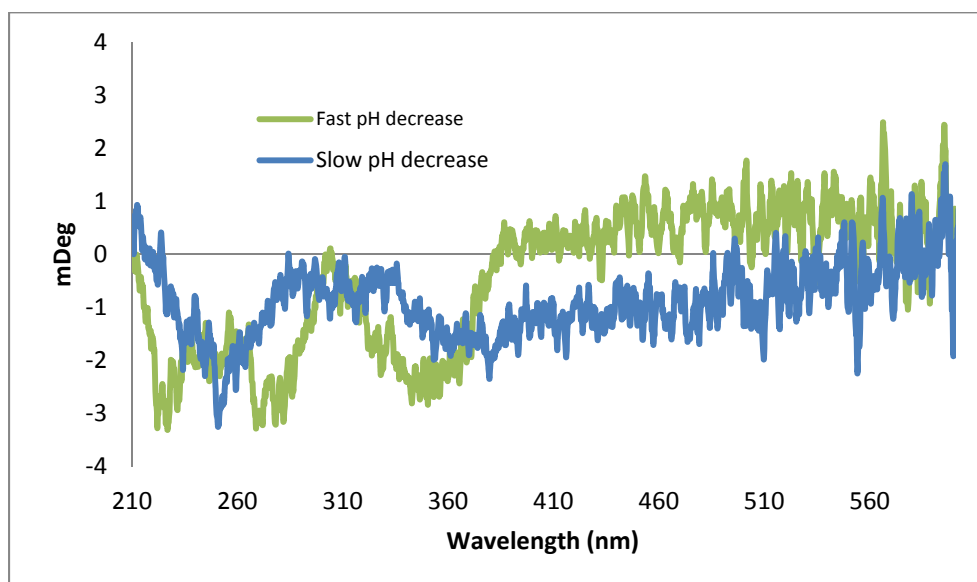
Figure 3.5.3. Comparison of UV-Vis spectra of solutions of *cis*-ADA at pH=3 obtained by **fast-*cis*** procedure (green) and at pH=3 obtained by **slow-*cis*** procedure (blue).

Figure 3.5.4. Comparison of CD spectra of solutions of *cis*-ADA at pH=3 obtained by **fast-*cis*** procedure (green) and at pH=3 obtained by **slow-*cis*** procedure (blue).

3.6   *cis* and *trans* Aggregate Photoisomerization

At basic pH ADA switches from the *trans* to the *cis* isomer upon exposure to UV light, and from the *cis* to the *trans* isomer upon exposure to visible light. After a fast pH decrease, however, it appears from the unchanging UV-Vis band at 330 nm that the photoswitching of *trans*-ADA is impaired to the point that isomerization to the *cis* could not occur (*appendix* **Figure 7.3**). This phenomenon could be attributed to the tightly packed stacking of the *trans*-ADA in the H aggregate. Since the *trans* is already the more energetically stable of the two isomers, the non-covalent interactions might provide too high of an energy barrier for the photomechanical effect to surpass. Isomerization also might occur, but the *cis* might revert back to the *trans* too quickly for spectroscopic analysis to detect.

In the case of the fast pH decrease for the *cis*-ADA isomer, the molecule retains its photoswitching ability while aggregated and upon isomerization assumes the same aggregate geometry attained in the fast pH decrease of the *trans* (*appendix* **Figure 7.9** and **Figure 7.10**). Visible irradiation causes the UV-Vis and CD signals to change markedly and become superimposable with the spectra obtained during the quick *trans*-ADA pH decrease. *cis*-ADA's retention of its ability to photoisomerize while aggregated could be explained in one of two ways: 1) the relative instability of the *cis*-isomer makes isomerization more energetically favorable than the non-covalent interactions of the aggregate; 2) the presence of the *trans* isomer in the aggregate disrupts interactions, leading to a less compact structure in which the *cis* isomer is less hindered, allowing for isomerization to proceed. Regardless, the fact that the *cis* isomer forms one type of aggregate and then is able to switch upon irradiation and take on the same aggregate

geometry obtained in the **fast-*trans*** procedure demonstrates the photoresponsive nature of the system. The photoisomerization is not reversible in the aggregated form, and once the aggregated *trans* isomer is obtained the structure is locked unless the aggregate is broken by increasing the pH.
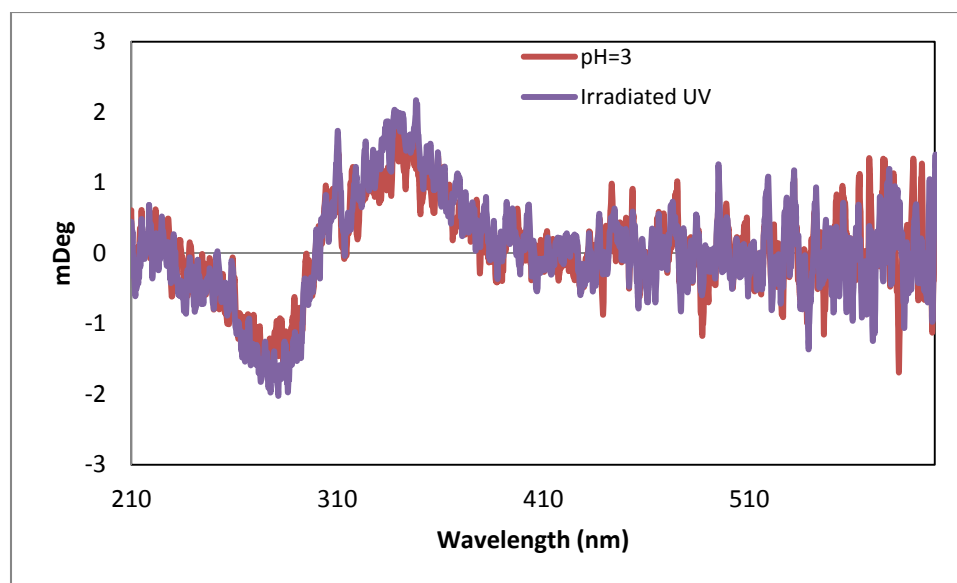

Figure 3.6.1. CD spectra showing the effects of UV irradiation on the aggregate of *trans*-ADA (**fast-*trans*** pH decrease).

4. AGGREGATION OF ADA AND POLY-GLUTAMIC ACID

4.1   ADA and Poly-Glutamic Acid Interaction

Aqueous solutions of ADA showed a tendency for both *cis-* and *trans-*ADA to form inherently chiral aggregates. Poly-glutamic acid (poly-Glu) was introduced into the system to determine if the chirality could be directed or induced by the presence of a chiral template molecule. Both the D and the L enantiomers were studied. In order to determine if an interaction occurred, a series of experiments were performed in which a solution containing ADA (either *cis* or *trans* isomer) was decreased from pH 7 to pH 3 with H_2SO_4, followed by the addition of poly-Glu. Several varieties of this experiment were performed, but only the results relevant in demonstrating the interaction of ADA and poly-glutamic acid will be included in this thesis. The results discussed are from experiments using poly-D-Glu, but equivalent results were observed in experiments with poly-L-Glu.

A solution containing *trans-*ADA was brought down in pH via the **fast-*trans*** procedure. Upon the addition of poly-D-Glu, there was no marked change in either the UV-Vis or CD spectra, except for the introduction of the band corresponding to poly-D-Glu in solution, from 245 nm to 210 nm (**Figure 4.1.2**). The decrease in absorbance at the 330 nm band in the UV-Vis spectrum can be attributed to the slight dilution of the ADA in solution upon the addition of poly-D-Glu (**Figure 4.1.1**). This demonstrates that once the self-aggregate of the *trans-*ADA has formed it is inert and does not rearrange in the presence of poly-Glu.

Using the same procedure, a solution of *cis*-ADA was decreased in pH via the **fast-*cis*** procedure and then poly-D-Glu was added. Like the *trans*-ADA solution, there was no change in the UV-Vis or CD spectra (except for the CD signal corresponding to poly-D-Glu) (**Figure 4.1.3**). Upon irradiation with visible light, however, isomerization of the *cis* to the *trans* is accompanied by the appearance of a bisignate CD signal with peaks at 370 nm (-7.18 mdeg) and 300 nm (+2.18 mdeg) (**Figure 4.1.4**). As shown in the previous section, when *cis*-ADA isomerizes at low pH, the resulting *trans* isomer quickly assembles or rearranges to give rise to the formation of a new aggregate. The presence of poly-Glu during this rapid aggregation directs and orders the organization of the *trans*-ADA and results in a larger CD signal than that attained by ADA alone in solution. This shows that if the chiral template is present during the aggregation process, it is able to interact with the monomer and affect the chirality of the aggregate.

In the following experiments, poly-Glu was introduced before any pH decrease and was present during the entire aggregation process. Differences in spectra resulting from the solution of ADA alone and solution with ADA and poly-Glu can thus be attributed to the ordering influence of the polymer.
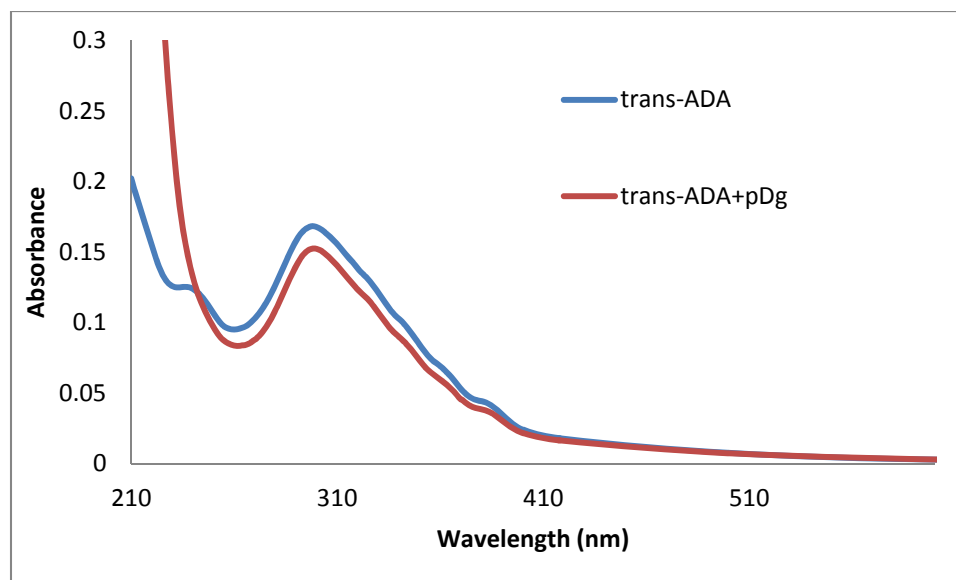
Figure 4.1.1. Comparison of UV-Vis spectra before (blue) and after (red) addition of poly-D-Glu to a solution of *trans*-ADA at pH 3 prepared by the **fast-*trans*** procedure.
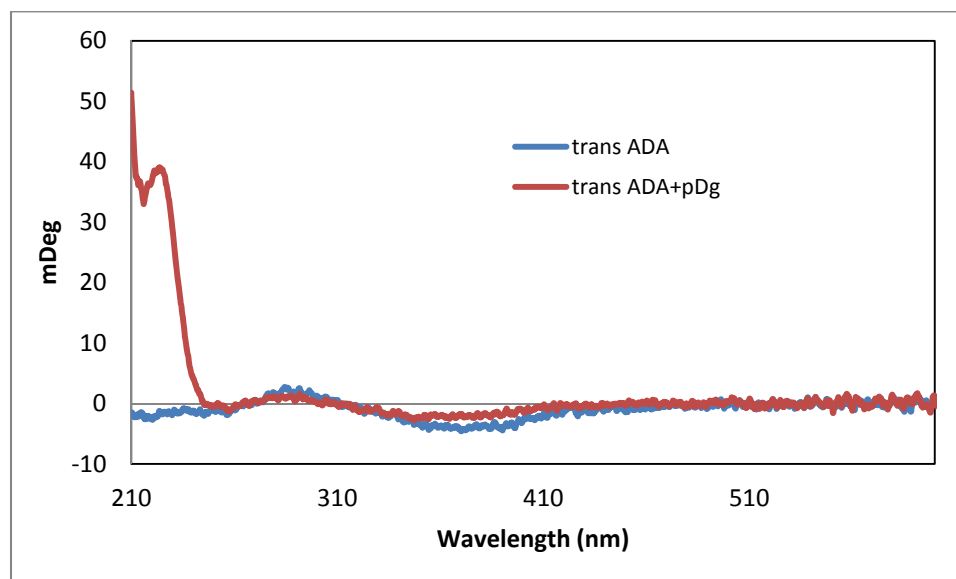
Figure 4.1.2. Comparison of CD spectra before (blue) and after (red) addition of poly-D-Glu to a solution of *trans*-ADA at pH 3 prepared by the **fast-*trans*** procedure.
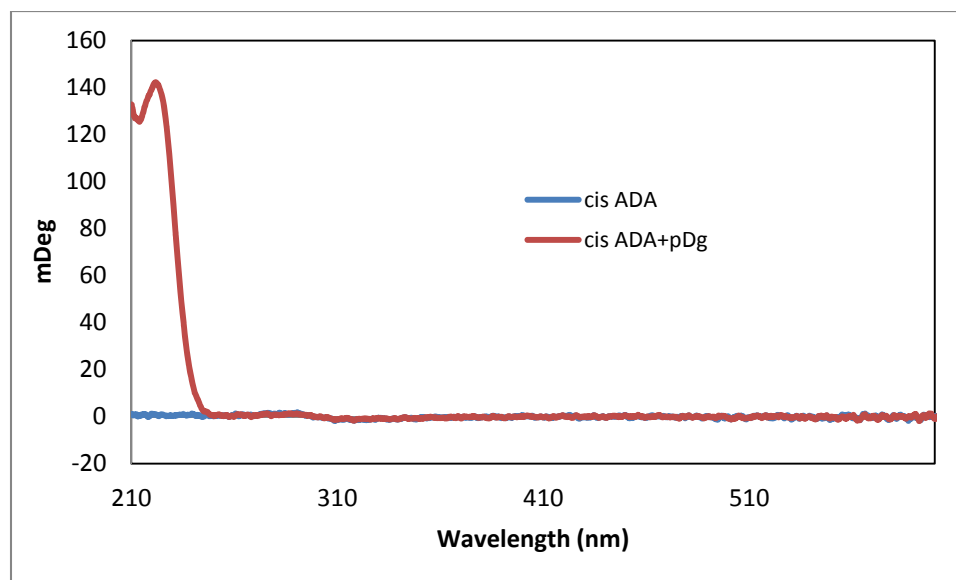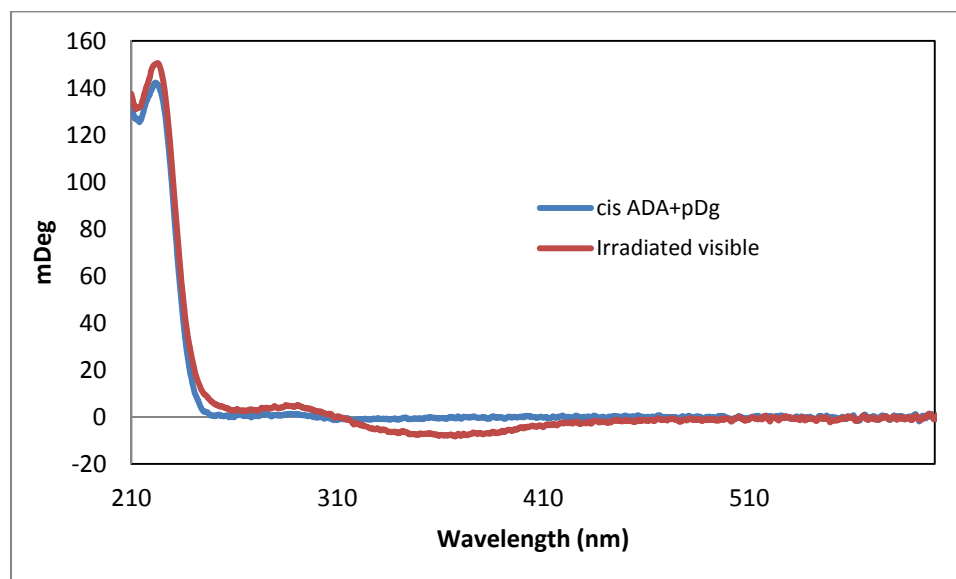
Figure 4.1.3. Comparison of CD spectra before (blue) and after (red) addition of poly-D-

Glu to a solution of *cis*-ADA at pH 3 prepared by the **fast-*cis*** procedure.

Figure 4.1.4. Comparison of CD spectra before (blue) and after (red) irradiation with

visible light of the solution obtained by addition of poly-D-Glu to the *cis*-ADA aggregate

prepared by the **fast-*cis*** procedure (see **Figure 4.1.3**).

4.2   Fast-*trans* poly-Glu pH Decrease

An aqueous solution containing *trans*-ADA and poly-L-glutamic acid was prepared according to the **fast-*trans*** procedure and the aggregation was monitored using UV-Vis and CD spectroscopy. The UV-Vis spectrum exhibits a blue shift similar to the solution containing *trans*-ADA alone, but of a smaller magnitude (**Figure 4.2.1**). The CD spectrum shows a completely different signal compared to the analogous solution containing *trans*-ADA alone. The signal is about ten times more intense and presents three peaks at 340 nm (-17.7 mdeg), 307 nm (-9.3 mdeg), and 280 nm (+13.3) (**Figure 4.2.2**). These spectra support our hypothesis that the poly-L-Glu plays a role in directing and amplifying chirality in a *trans*-ADA aggregate, and that it can be used to yield a more organized aggregate geometry. The solution was monitored for a total of 60 minutes, and it was observed that the intensity of the CD signal decreased with time (down to -10.1/+9.3 mdeg) possibly due to breakage or partial loss of organization of the aggregate.

The same **fast-*trans*** procedure was performed on a solution containing *trans*-ADA and poly-D-Glu. The resulting spectra show greater chiral organization than the spectra of ADA alone in solution. The CD spectrum gives a broad monosignate signal, unlike the one observed in the case of poly-L-glutamate (**Figure 4.2.4**). Comparing the spectra of the *trans*-ADA aggregated in the presence of poly-D-Glu with the one aggregated in the presence of poly-L-Glu, it is evident from the intensity of both the UV-Vis and the CD spectra that there is a lesser degree of aggregation in the case of poly-D-glutamate than poly-L-glutamate (**Figure 4.2.3** and **4.2.4**). The difference between the

two signals could be attributed to preferential interactions of the *trans*-ADA with poly-L-Glu rather than poly-D-Glu.
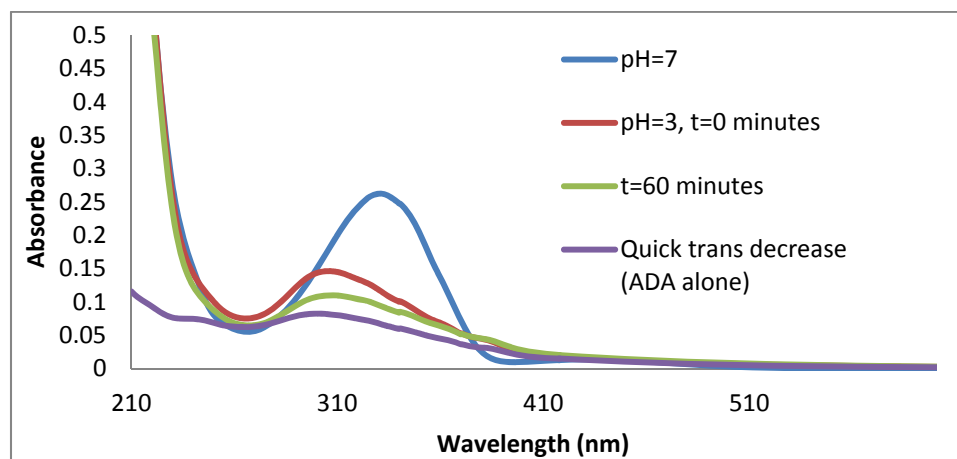

Figure 4.2.1: UV-Vis spectra for **fast-*trans*** pH decrease of a solution of *trans*-ADA/poly-L-Glu from pH 7 (blue) to pH 3 (red) monitored for 60 minutes (green) and compared to ADA-only system under same conditions (purple).
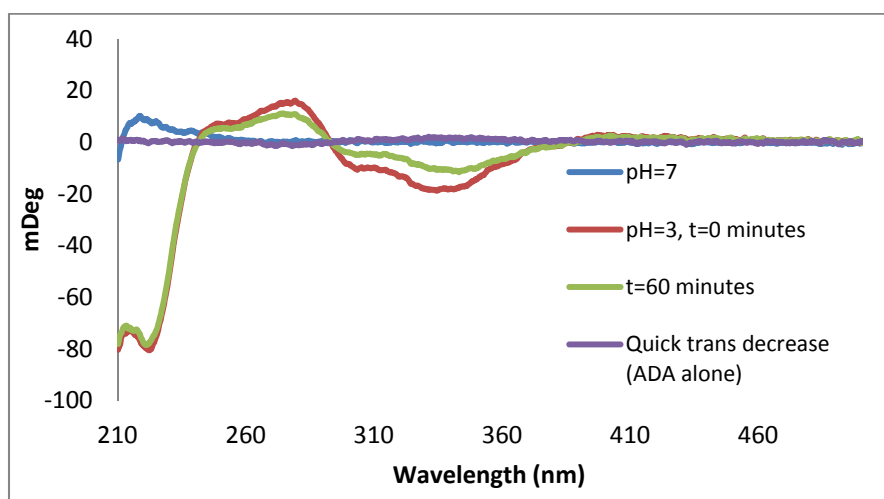

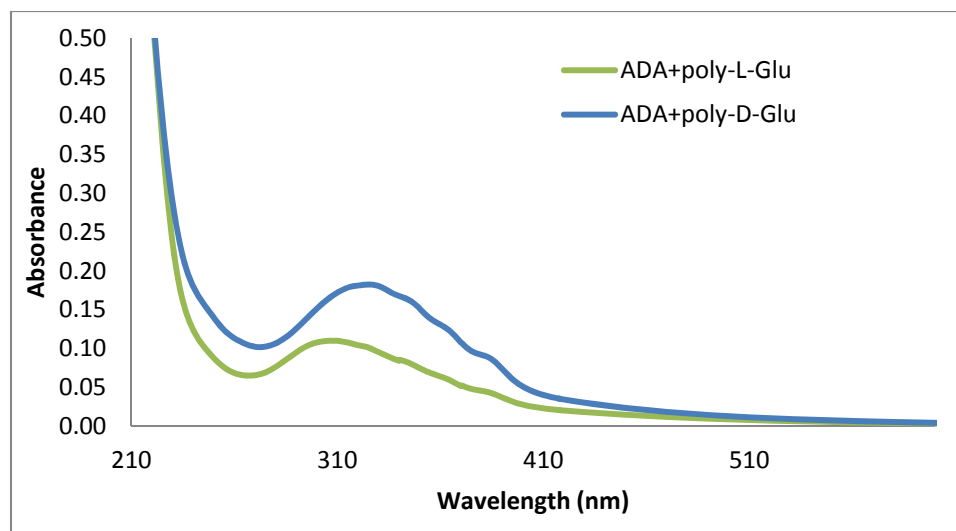**Figure 4.2.2.** CD spectra for **fast-*trans*** pH decrease of a solution of *trans*-ADA/poly-L-Glu from pH 7 (blue) to pH 3 (red) system monitored for 60 minutes (green) and compared to ADA-only system under same conditions (purple).

Figure 4.2.3. Comparison of UV-Vis spectra for **fast-*trans*** pH decrease of solutions containing *trans*-ADA/poly-D-Glu (blue) and *trans*-ADA/poly-L-Glu (green).
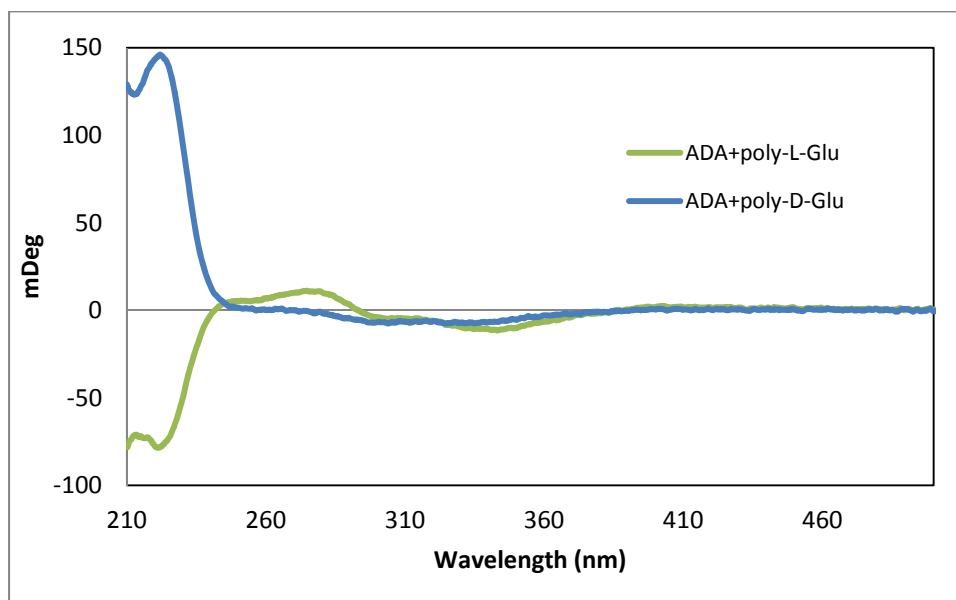
Figure 4.2.4. Comparison of UV-Vis spectra for **fast-*trans*** pH decrease of solutions containing *trans*-ADA/poly-D-Glu (blue) and *trans*-ADA/poly-L-Glu (green).

4.3 Slow-*trans* poly-Glu pH Decrease

Aqueous solutions containing *trans*-ADA and poly-glutamic acid were prepared according to the **slow-*trans*** procedure. Aggregation was monitored using UV-Vis and CD spectroscopy. In the case of *trans*-ADA and poly-L-Glu, a CD signal first appeared at pH=5, and kept increasing and slightly shifting with further pH decrease. The final signal shows a complex profile with an intensity that goes from -18.5 mdeg to +12 mdeg (**Figure 4.3.2**). A comparison of the spectra of the two aggregates formed in the presence of poly-L-Glu, the one obtained following the **fast-*trans*** procedure and the one formed following the **slow-*trans*** procedure, shows that the CD signal corresponding to the slow decrease has a substantially different profile and is more intense than that obtained with the fast decrease (**Figure 4.3.3**). Once again, the aggregation process, suggests a hierarchical control that generates different structures even in the presence of a chiral template. The lack in the spectra of the solution prepared according to the **slow-*trans*** procedure (**Figure 4.3.1**) of the blue shift of the absorption band observed when using the **fast-*trans*** procedure confirms the hierarchical control of the aggregation process.

In the case of *trans*-ADA and poly-D-Glu prepared according to the **slow-*trans*** procedure, the UV-Vis spectrum shows a flattening and broadening of the 330 nm band without a blue shift, similarly to the L enantiomer (**Figure 4.3.4**). The CD spectrum (**Figure 4.3.5**) shows a reduced ability of the D enantiomer to transfer chirality in the *trans*-ADA aggregate as similarly found for the **fast-*trans*** procedure. Nevertheless, a comparison of both the UV-Vis and CD spectra of the aggregates obtained in the presence of the D enantiomer following either the **fast-*trans*** or the **slow-*trans*** procedure shows a hierarchical control of the aggregation process (**Figure 4.3.7**).
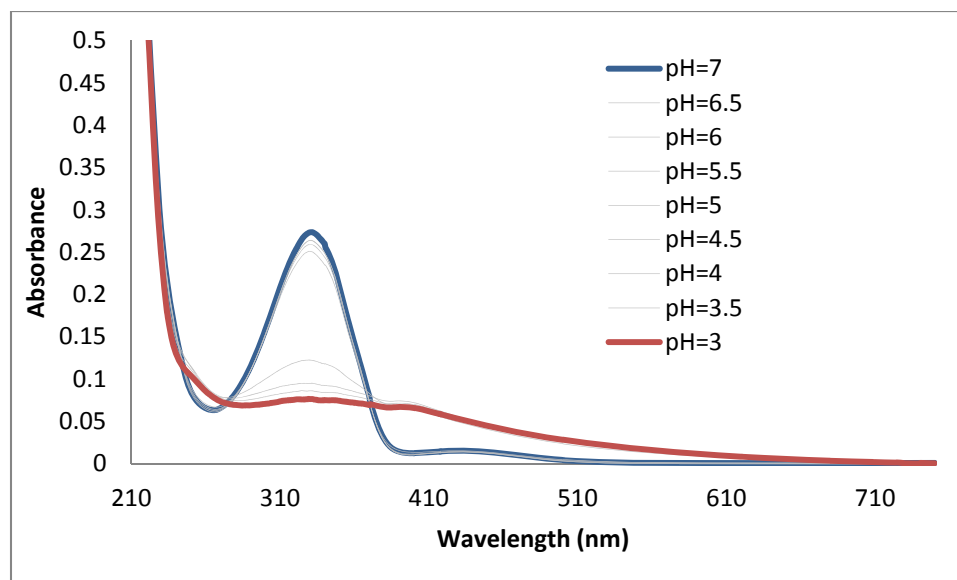
Figure 4.3.1. UV-Vis spectra of **slow-*trans*** procedure for a solution of *trans*-ADA and poly-L-Glu: *trans*-ADA and poly-L-Glu at pH=7 (blue), *trans*-ADA and poly-L-Glu at pH=3 (red), intermediate steps (gray).
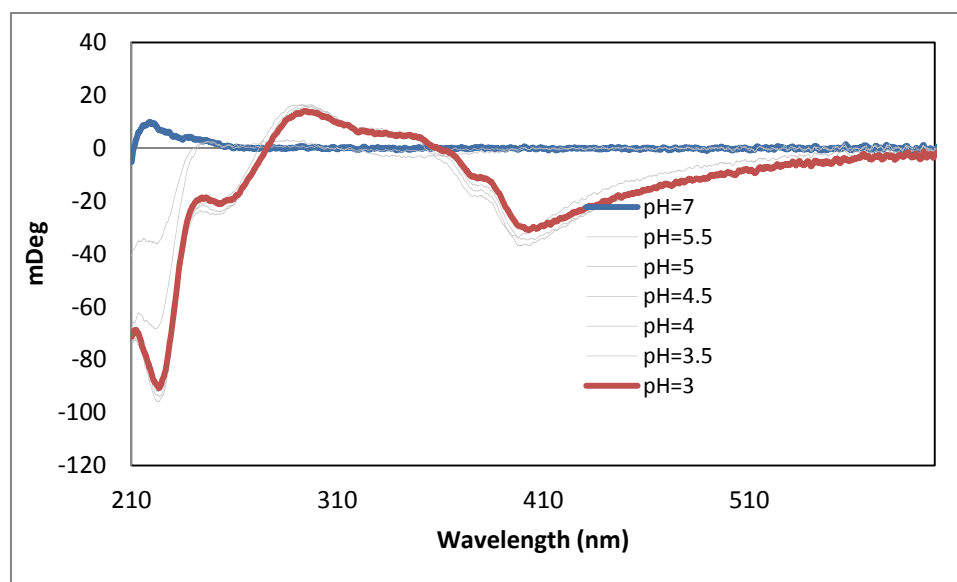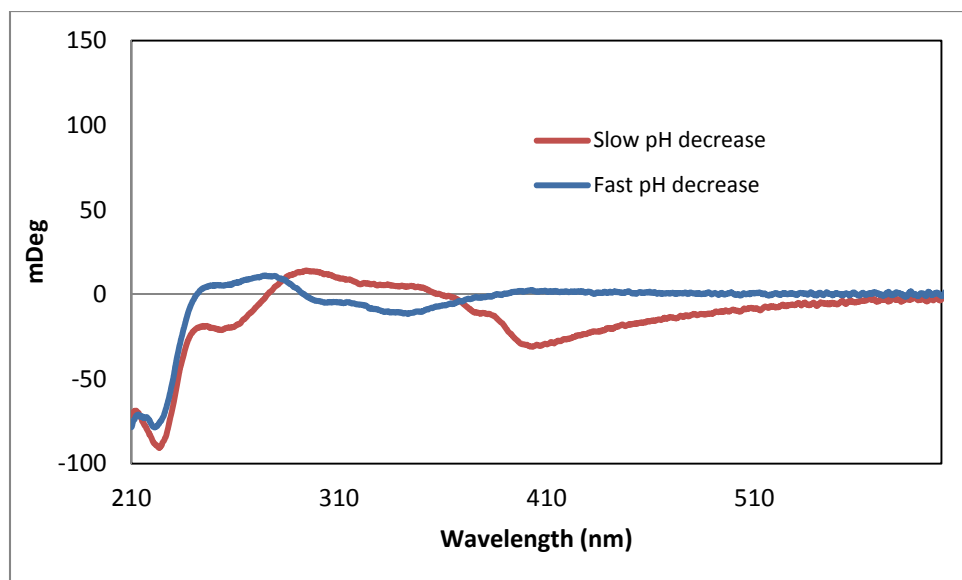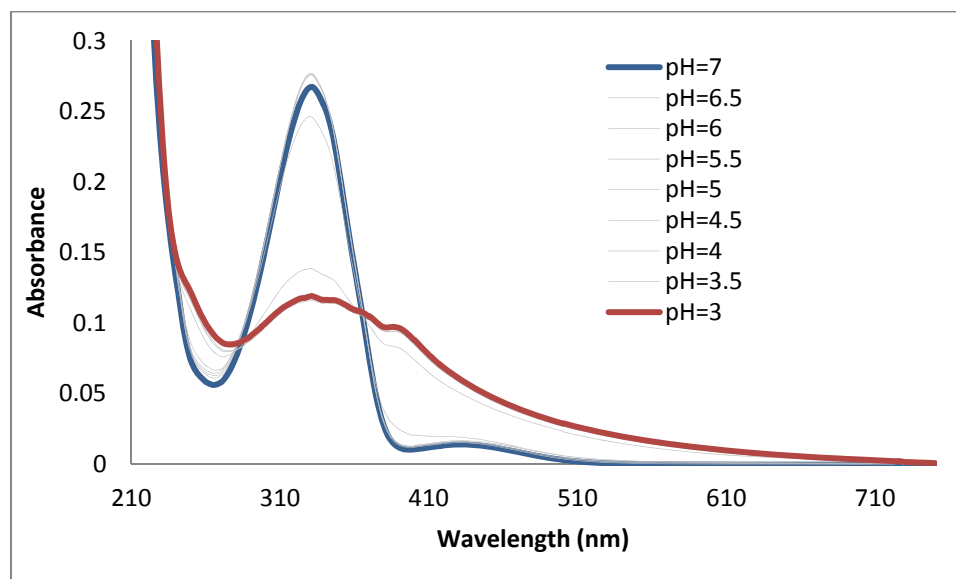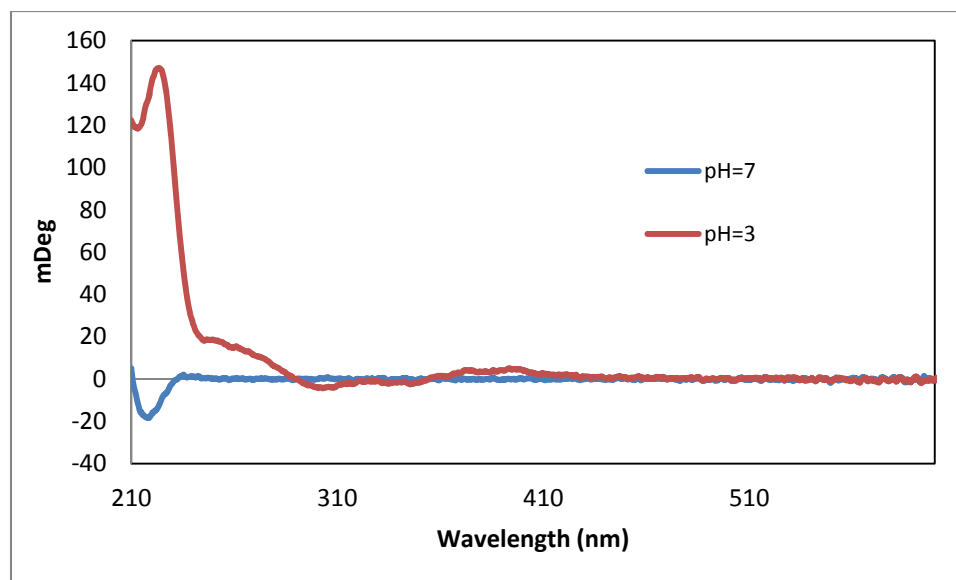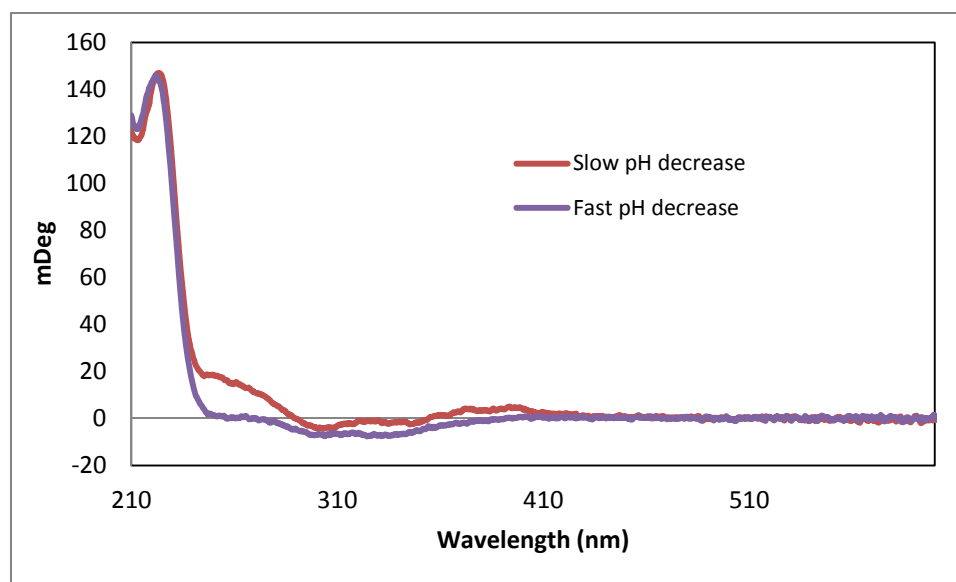

Figure 4.3.2. CD spectra of **slow-*trans*** procedure for a solution of *trans*-ADA and poly-L-Glu: *trans*-ADA and poly-L-Glu at pH=7 (blue), *trans*-ADA and poly-L-Glu at pH=3 (red), intermediate steps (gray).

Figure 4.3.3. Comparison of the CD spectra of solutions containing *trans*-ADA and poly-L-Glu decreased in pH via the **slow-*trans*** (red) and **fast-*trans*** (blue) procedures.

Figure 4.3.4. UV-Vis spectra of **slow-*trans*** procedure for a solution of *trans*-ADA and poly-D-Glu: *trans*-ADA and poly-D-Glu at pH=7 (blue), *trans*-ADA and poly-D-Glu at pH=3 (red), intermediate steps (gray).

Figure 4.3.5. UV-Vis spectra of **slow-*trans*** procedure for a solution of *trans*-ADA and poly-D-Glu: *trans*-ADA and poly-D-Glu at pH=7 (blue), *trans*-ADA and poly-D-Glu at pH=3 (red), intermediate steps omitted for clarity.

Figure 4.3.7. Comparison of CD spectra of solutions containing *trans*-ADA and poly-D-Glu brought down to pH=3 via the **fast-*trans*** (purple) or **slow-*trans*** (red) procedures.
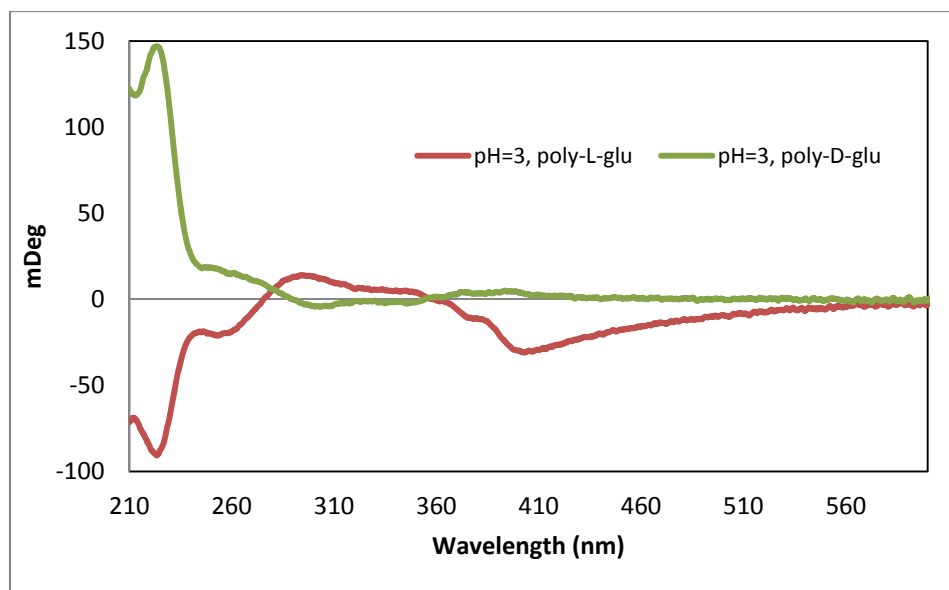
Figure 4.3.8. Comparison of CD spectra for solutions containing *trans*-ADA and either poly-L-Glu (red) or poly-D-Glu (green) brought down in pH to pH=3 via the **slow-*trans*** procedure.

4.4   Fast-*cis* poly-Glu pH decrease

Aqueous solutions containing *trans*-ADA and poly-glutamic acid were prepared according to the **fast-*cis*** procedure and the aggregation was monitored using UV-Vis and CD spectroscopy. In the system containing poly-L-glutamate, pH decrease resulted initially in only slight aggregation that continued to increase in magnitude with time as shown by the increase of the CD signal (**Figure 4.4.2**). The change in the UV-Vis spectra was unusual for an aggregation process since it was not a hypochromic effect of the initial bands but rather a change toward a signal that resembles the signal of an aggregated form of the *trans* since the maximum was around 330 nm (**Figure 4.4.1**). Considering the extensive number of carboxyl groups on the polymer, it is possible that the exothermic protonation process could provide enough energy to promote the thermal isomerization of the *cis* isomer to the *trans* isomer. This could account for the reemergence of the peak close to 330 nm and the change with time of the CD signal. The hypothesis of a thermal isomerization finds support in the absence of a change in both the CD and UV-Vis spectra when the solution was irradiated with visible light (**Figure 4.4.3** and **Figure 4.4.4**).

In the system containing *cis*-ADA and poly-D-Glu, similar phenomena are observed. Again, the degree of aggregation increases with time (**Figure 4.4.5** and **4.4.6**) and, as in the case of poly-L-Glu, there is no change in the UV-Vis or CD spectra upon irradiation with visible light (**Figure 4.4.7** and **4.4.8**). This supports the hypothesis that an exothermic, rapid protonation provides the thermal energy necessary to isomerize to the *trans*-ADA. This also could explain why the CD signal increases over time. The initial signal is weak because there are more *cis*-ADA than *trans*-ADA molecules in solution,

but the energy released in the protonation of poly-glutamate causes more *cis*-ADA to isomerize into *trans*-ADA. The increasing concentration of *trans*-ADA facilitates the formation of a more ordered and chiral aggregate, leading to the observed increase in CD signal.
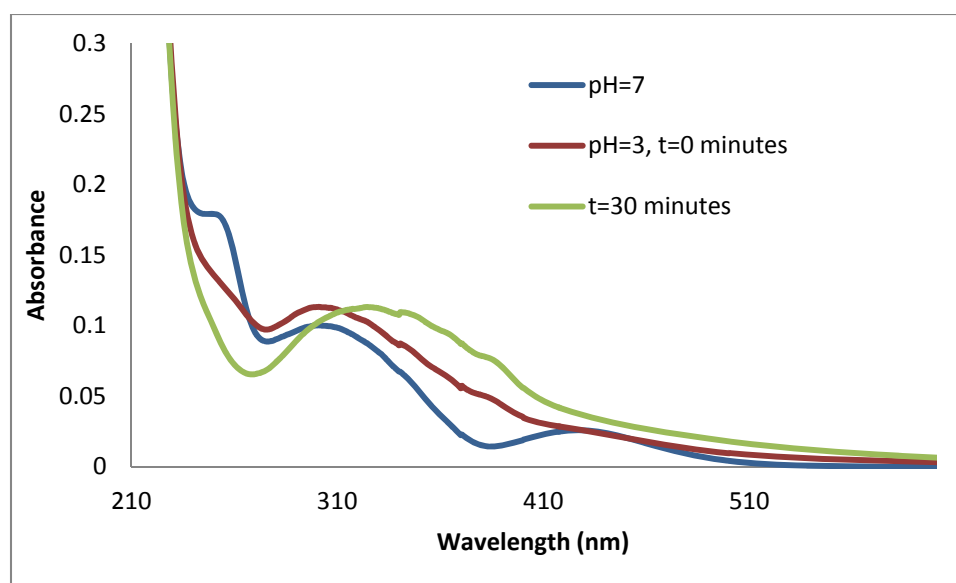

Figure 4.4.1. Comparison of UV-Vis spectra for a solution of *cis*-ADA and poly-L-Glu, decreased in pH via **fast-*cis*** decrease from pH=7 (blue) to pH=3 (red) and monitored for 30 minutes (green).
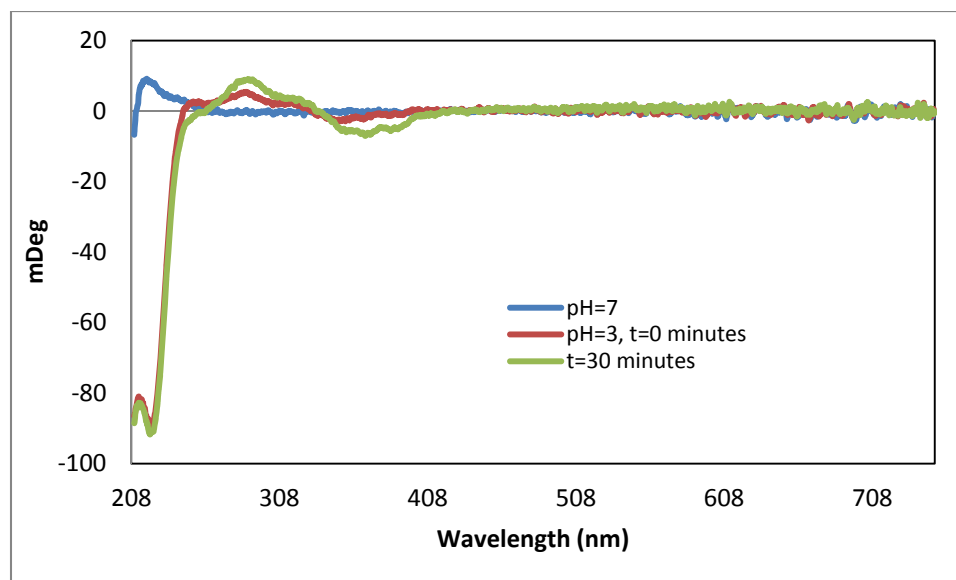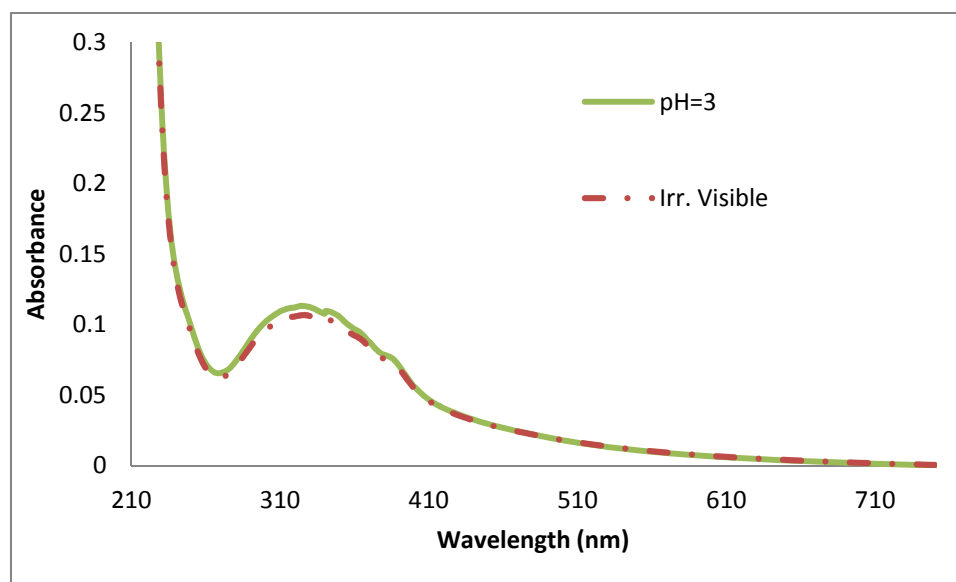
Figure 4.4.2. Comparison of CD spectra for a solution of *cis*-ADA and poly-L-Glu, decreased in pH via **fast-*cis*** decrease from pH=7 (blue) to pH=3 (red) and monitored for 30 minutes (green).

Figure 4.4.3. Effect of visible irradiation on the UV-Vis spectrum of a solution of *cis*-ADA and poly-L-Glu decreased via the **fast-*cis*** procedure: solution before irradiation (green), solution following visible irradiation (red).
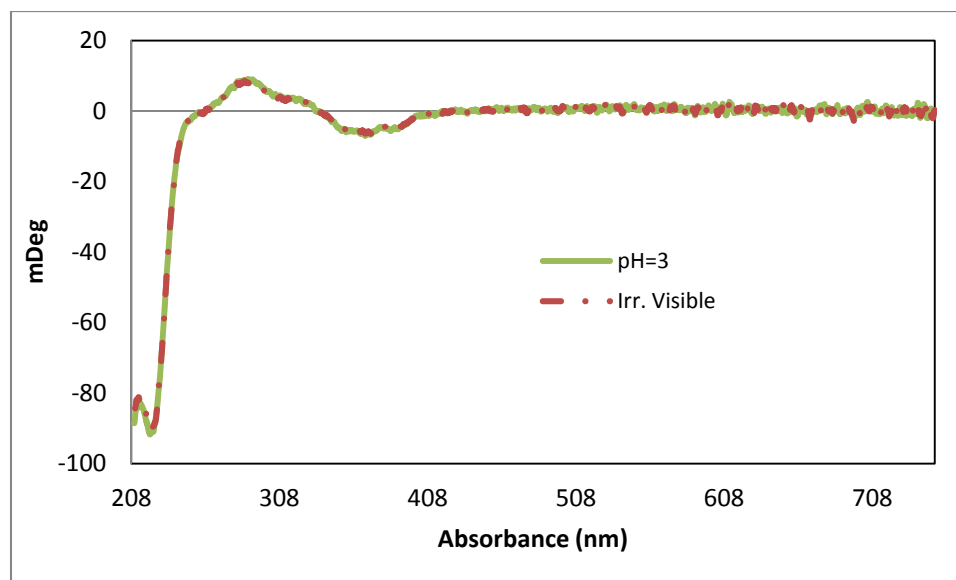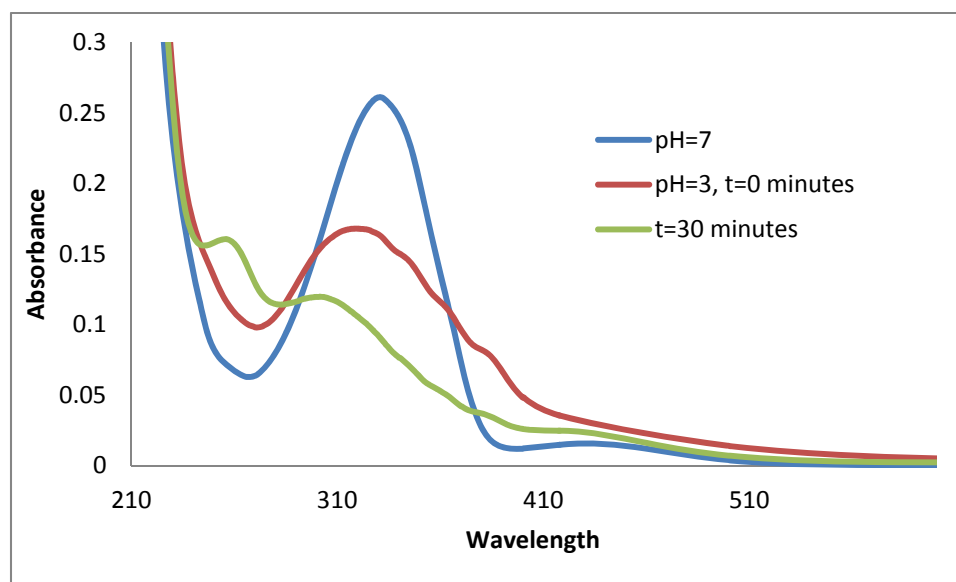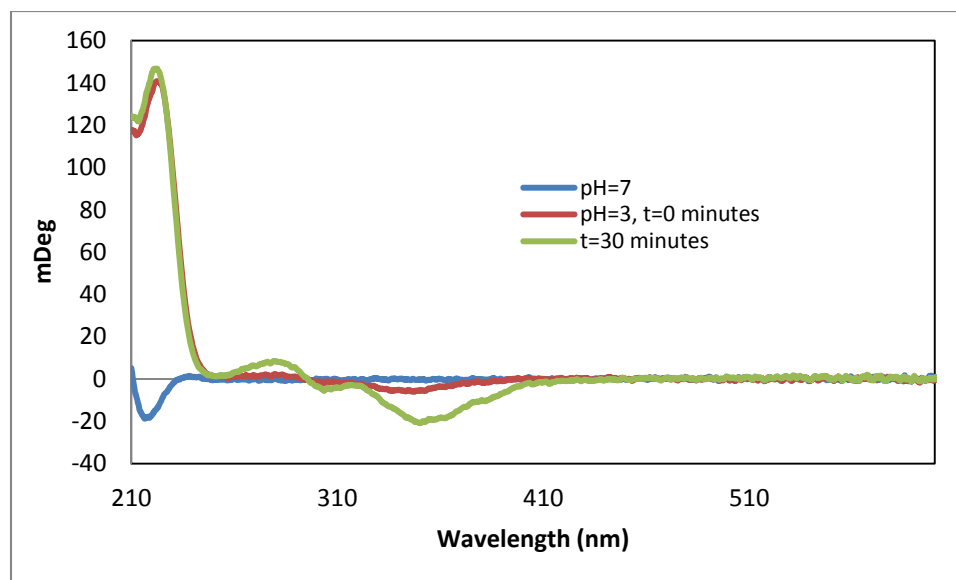
Figure 4.4.4. Effect of visible irradiation on the CD spectrum of a solution of *cis*-ADA and poly-L-Glu decreased via the **fast-*cis*** procedure: solution before irradiation (green), solution following visible irradiation (red).

Figure 4.4.5. Comparison of UV-Vis spectra for a solution of *cis*-ADA and poly-D-Glu, decreased in pH via **fast-*cis*** decrease from pH=7 (blue) to pH=3 (red) and monitored for 30 minutes (green).
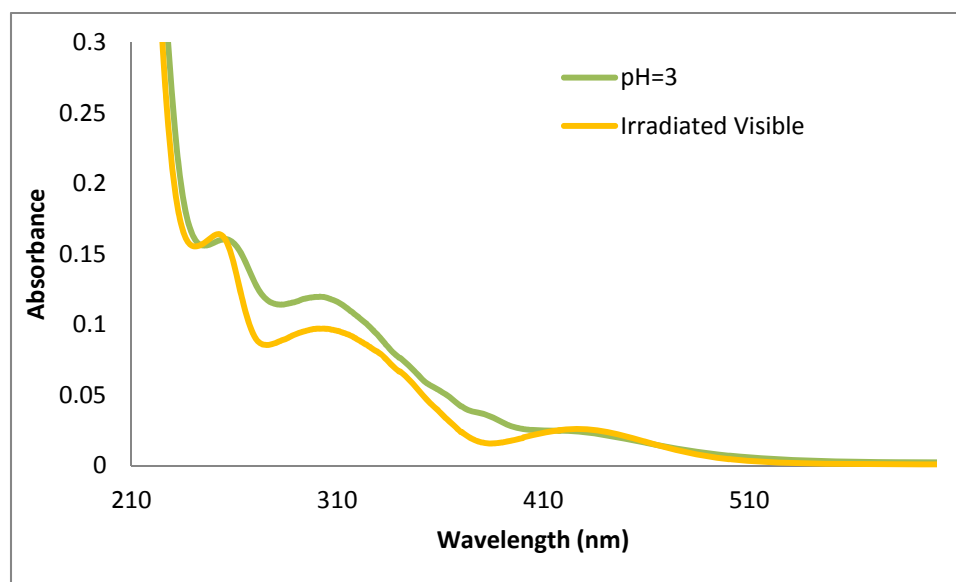
Figure 4.4.6. Comparison of Cd spectra for a solution of *cis*-ADA and poly-D-Glu, decreased in pH via **fast-*cis*** decrease from pH=7 (blue) to pH=3 (red) and monitored for 30 minutes (green).

Figure 4.4.7. Effect of visible irradiation on the UV spectrum of a solution containing *cis*-ADA and poly-D-Glu at pH=3 (via **fast-*cis*** procedure): solution before visible irradiation (green), solution following visible irradiation (orange).
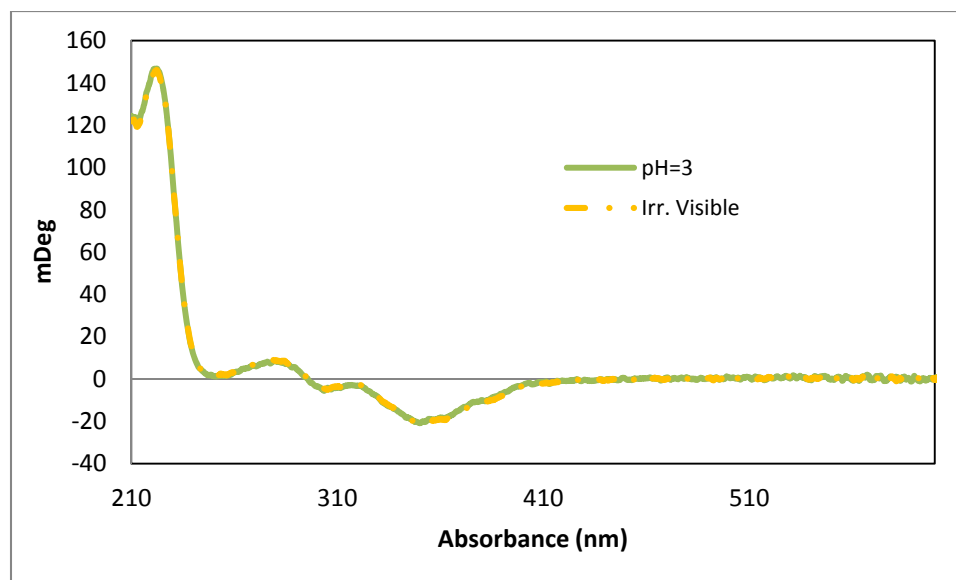
Figure 4.4.8. Comparison of CD spectra for a solution of *cis*-ADA and poly-D-Glu, decreased in pH via **fast-*cis*** decrease from pH=7 (blue) to pH=3 (red) and monitored for 30 minutes (green).

4.5   Slow-*cis* poly-Glu pH decrease

Aqueous solutions containing *cis*-ADA and poly-glutamic acid were prepared according to the **slow-*cis*** procedure. Aggregation was monitored using UV-Vis and CD spectroscopy. In the case of *cis*-ADA and poly-L-glutamic acid, unlike the **fast-*cis*** pH decrease, no CD signal is observed (**Figure 4.5.2**), and the UV-Vis 330 nm peak does not reappear at low pH (**Figure 4.5.3**). This further supports the hypothesis that energy released upon protonation can cause isomerization. When the pH decrease is performed in small steps, the quantity of heat released is not large enough to induce thermal isomerization. When protonation occurs rapidly by a large addition of acid, enough heat is generated to overcome the energy barrier of thermal isomerization. Upon irradiation with visible light, there is a slight increase in the 330 nm band (**Figure 4.5.3**) and a mild increase in CD signal (**Figure 4.5.4**), which would suggest a certain degree of photoisomerization.

When the **slow-*cis*** procedure was performed on a solution containing *cis*-ADA and poly-D-Glu, no CD signal was observed (**Figure 4.5.4**). Upon irradiation with visible light, both the UV-Vis and CD spectra change in such a way as to suggest the isomerization from *cis* to *trans*. The CD exhibits a chiral signal with the most intense peak with a signal of -17.9 mdeg at 370 nm and the UV-Vis spectrum shows a broad band near 330 nm (**Figure 4.5.5** and **4.5.6**).

It is evident that upon slow pH decrease in the presence of *cis*-ADA and poly-Glu, no or very little aggregate is formed, signifying that the *cis* isomer is possibly unable to interact with or be directed by the poly-glutamate. Upon irradiation to the *trans* isomer,

a chiral aggregate is formed, and its CD spectrum has an intensity and profile that

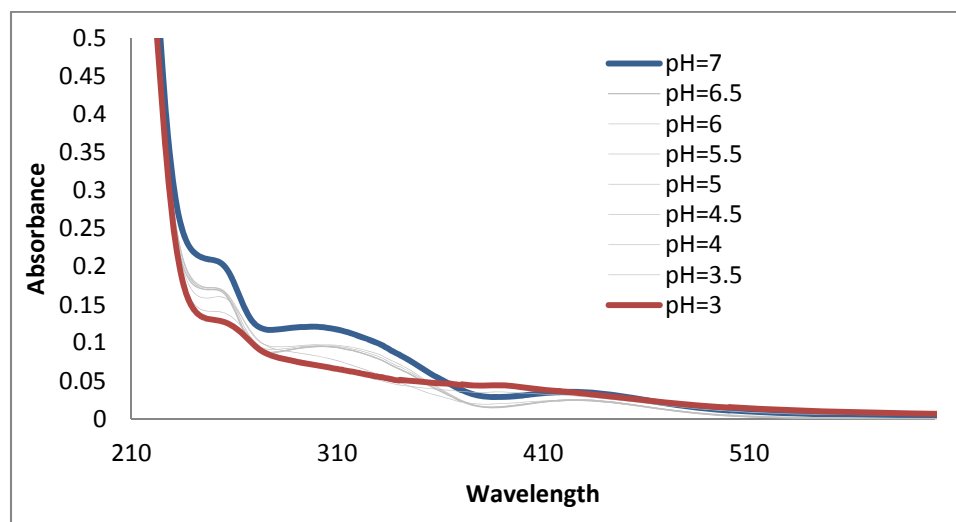suggests interaction with the poly-glutamic acid template.

Figure 4.5.1. UV-Vis spectra of a solution containing *cis*-ADA and poly-L-Glu and

decrease in pH via the **slow-*cis*** procedure: *cis*-ADA and poly-L-Glu at pH=7 (blue), *cis*-

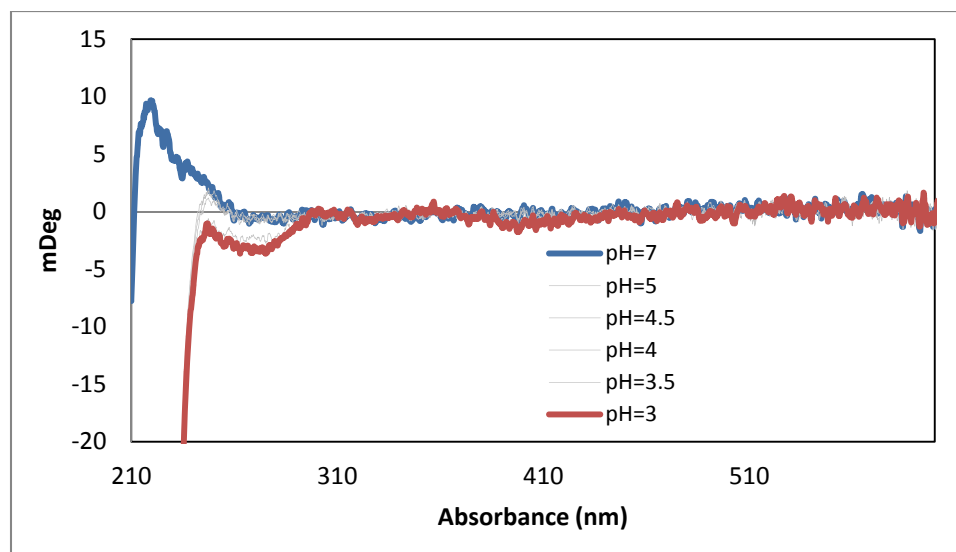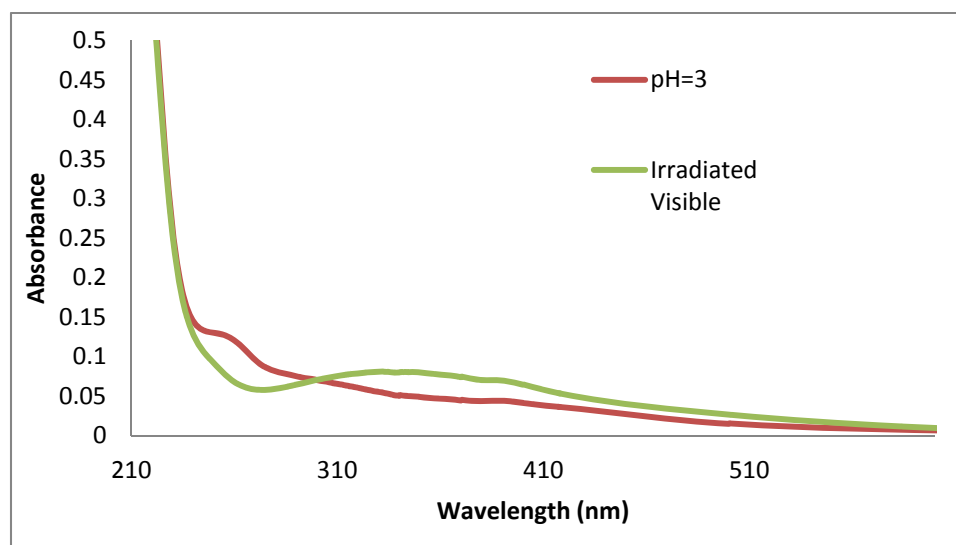ADA and poly-L-Glu at pH=3 (red), intermediate steps (gray).

Figure 4.5.2. CD spectra of a solution containing *cis*-ADA and poly-L-Glu and decrease in pH via the **slow-*cis*** procedure: *cis*-ADA and poly-L-Glu at pH=7 (blue), *cis*-ADA and poly-L-Glu at pH=3 (red), intermediate steps (gray).

Figure 4.5.3. Effect of irradiation with visible light on UV-Vis spectra of a solution containing *cis*-ADA and poly-L-Glu brought down to pH=3 via the **slow-*cis*** procedure: *cis*-ADA and poly-L-Glu at pH=3 (red), *trans*-ADA and poly-L-Glu following visible irradiation (green).
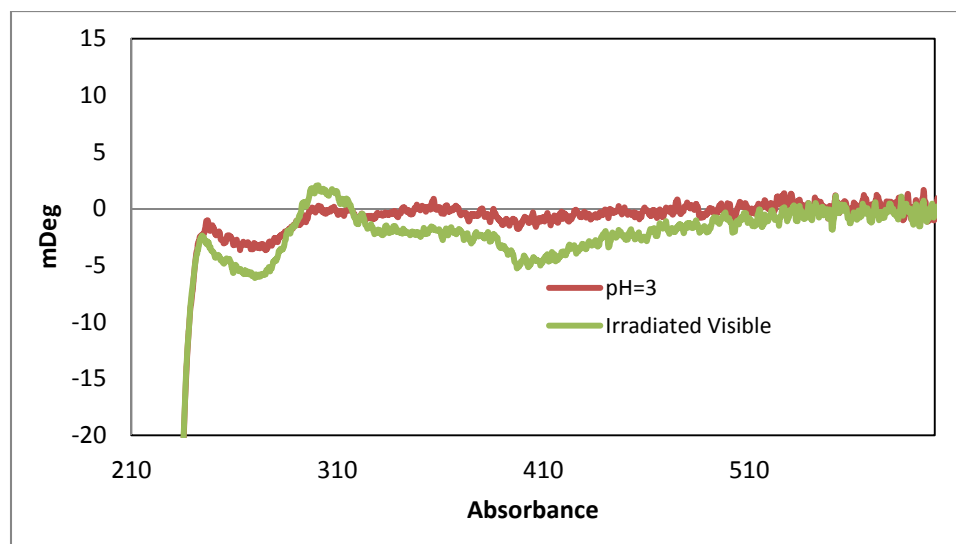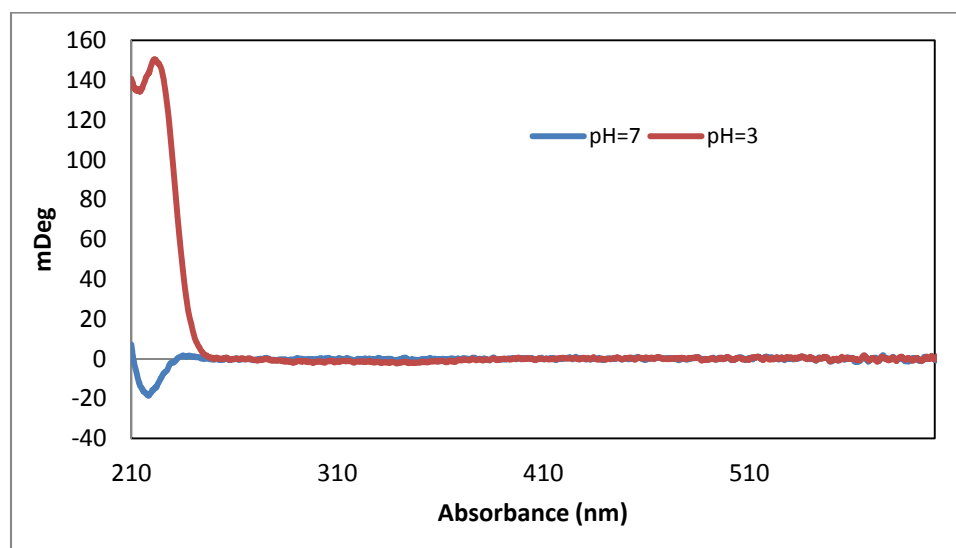
Figure 4.5.4. Effect of irradiation with visible light on CD spectra of a solution containing *cis*-ADA and poly-L-Glu brought down to pH=3 via the **slow-*cis*** procedure: *cis*-ADA and poly-L-Glu at pH=3 (red), *trans*-ADA and poly-L-Glu following visible irradiation (green).

Figure 4.5.4. CD spectra of a solution containing *cis*-ADA and poly-D-Glu and decrease in pH via the **slow-*cis*** procedure: *cis*-ADA and poly-D-Glu at pH=7 (blue), *cis*-ADA and poly-D-Glu at pH=3 (red), intermediate steps (gray).
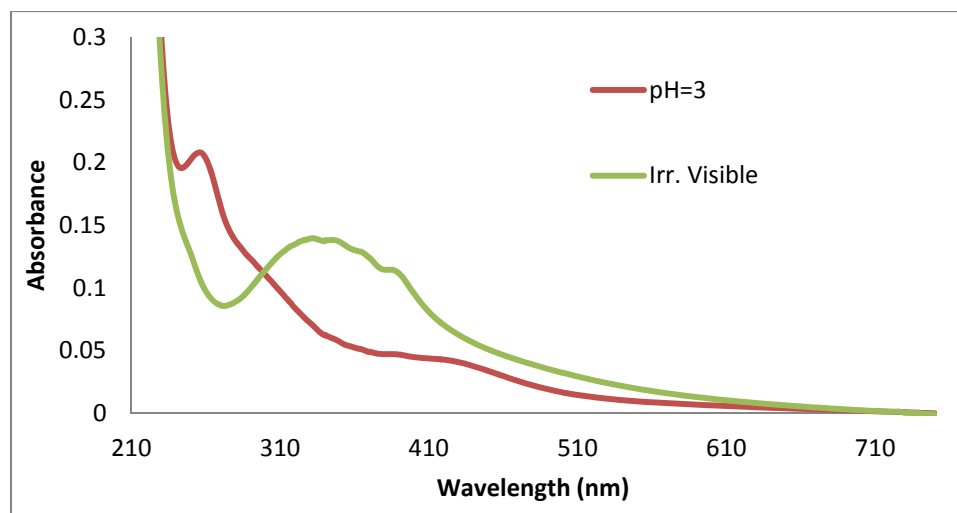
Figure 4.5.5. Effect of irradiation with visible light on UV-Vis spectra of a solution containing *cis*-ADA and poly-D-Glu brought down to pH=3 via the **slow-*cis*** procedure: *cis*-ADA and poly-D-Glu at pH=3 (red), *trans*-ADA and poly-D-Glu following visible irradiation (green).
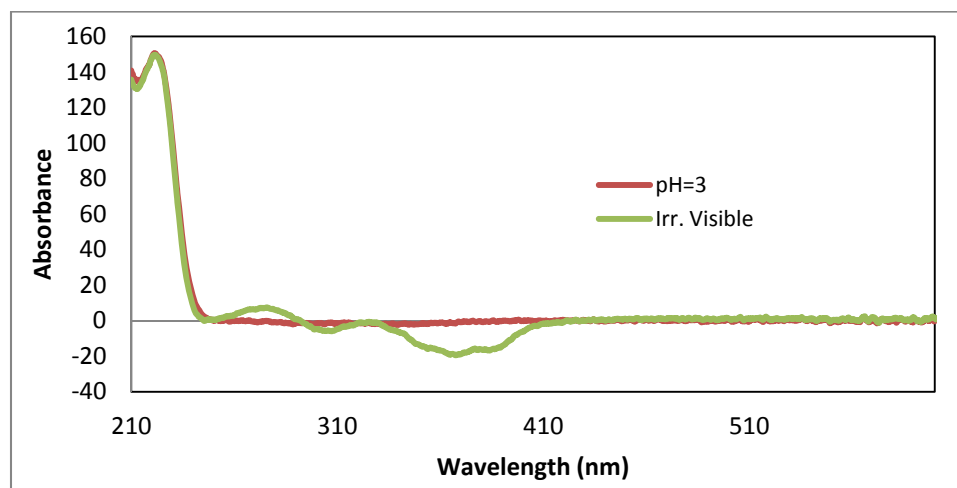

Figure 4.5.6. Effect of irradiation with visible light on CD spectra of a solution containing *cis*-ADA and poly-D-Glu brought down to pH=3 via the **slow-*cis*** procedure: *cis*-ADA and poly-D-Glu at pH=3 (red), *trans*-ADA and poly-D-Glu following visible irradiation (green).

5.   CONCLUSIONS

Through the manipulation of environmental conditions including pH, light exposure, and the presence of a chiral template molecule, the response of ADA to various stimuli in both its *cis* and *trans* isomers in aqueous solution was investigated.

The study of the pH dependence of the solubility of ADA showed that it needs a very basic environment, over pH 10.8, in order to maximize its already poor solubility. Moreover, some preliminary data indicated that a decrease in pH of a solution of ADA results in its self-aggregation. This process is dependent on several parameters such as mode of solubilization, isomeric form, concentration and mode of pH decrease, showing that aggregation is subject to a highly hierarchical mechanism.

In order to reduce the number of variables that can affect the aggregation of ADA its concentration and the mode of solubilization of its stock solution were kept constant. The dependence of aggregation on the isomer and mode of pH decrease was studied.

When the pH of a solution of *trans*-ADA was reduced quickly (**fast-*trans*** procedure), a blue shift and hypochromicity of the absorption band at 330 nm (*trans* isomer) indicate that a parallel assembly, known as an H-aggregate, is formed. In this geometry, both π−π stacking and hydrogen bonds are possible, making the aggregate very compact and strong. As expected in such a tightly packed aggregate, *trans*-ADA temporarily loses its photoswitching ability. If the pH is brought back to 7, the *trans*-ADA returns to its monomeric form and is able to photo-isomerize again from *trans*-ADA to *cis*-ADA. When an aqueous solution of *trans*-ADA is made by the **slow-*trans*** procedure the absorption shows only a hypochromic effect indicating the formation of an

aggregate with a different geometry. As in the previous case, the photo-isomerization is prevented until the aggregate is disrupted at pH 7 or higher. CD spectra show that in both cases the achiral ADA has an intrinsic tendency to interact in an asymmetric fashion, giving rise to the formation of chiral aggregates. Consistent with the absorption data, the signal for the two species is different, indicating a different geometry and a hierarchical control of the aggregation process that depends on the mode of pH decrease.

When the pH of a solution of mainly *cis*-ADA is quickly reduced according to the **fast-*cis*** procedure, both the UV-Vis and CD spectra are consistent with the formation of an aggregate as observed with the *trans* isomer, but with different properties. The *cis* aggregate changes with time before reaching a stable state 60 minutes after the initial pH decrease. The maxima at 330 nm and 430 nm are lost instantaneously, while after 60 minutes a new weak broad band appears at approximately 360 nm. The simultaneous presence of the two isomers, *cis* and *trans,* might be responsible for this complex spectroscopic response. Presumed, the aggregate has a less tightly packed geometry, which would explain the observed retention of the photo-isomerization ability of the *cis* isomer in this aggregated form. Irradiation with visible light switches the *cis*-ADA back to the *trans*-ADA and gives rise to the formation of an aggregate that is analogous to the H aggregate obtained following the **fast-*trans*** procedure, as the superimposition of the UV-Vis and CD spectra shows. When a solution of ADA is prepared according to the **slow-*cis*** procedure, the aggregate obtained has a different absorption and CD spectrum. The different aggregate structures obtained by the **fast** and **slow** procedures for both *cis* and *trans*-ADA provide, therefore, evidence for hierarchical control of the system. The degree and speed of protonation affect the manner in which the ADA molecules come

together to take advantage of non-covalent interactions, and this changes the geometries that result from the aggregation process.

The introduction of a chiral template, poly-glutamic acid (L and D enantiomers), during the aggregation process was shown to direct the formation of a new asymmetric aggregate that has a chirality induced by the polymer as shown by the intensity and different profile of the CD spectra of these systems compared to the one containing only ADA.

This ability to order the aggregate was employed in systems where pH was decreased both slowly and quickly. As in systems without poly-glutamic acid, the *trans* isomer was unable to switch to the *cis* isomer upon irradiation with UV light while aggregated, but when in solution with poly-Glu, *cis*-ADA reacts differently to pH additions and to irradiation with visible light compared to the behavior of the switch without a chiral template. In systems decreased in pH via the **fast-*cis*** procedure, both the CD and UV-Vis spectra changed with time and ultimately appeared more like the *trans* isomer than the *cis*. Not only did the CD spectra show chiral aggregation, but the UV-Vis spectra showed a return of the 330 nm band after sitting for 30 minutes in the dark. Upon irradiation with visible light, these systems did not show evidence of switching, which is likely because there was already such a high ratio of *trans* to *cis* that the magnitude of change due to photoisomerization was much less than previously observed. It is hypothesized that the release of energy during the fast and exothermic protonation of the poly-Glu carboxyl groups might surmount the energy barrier for the *cis* to thermally isomerize to the *trans* isomer and allow the more thermally stable *trans* isomer to become more prevalent in solution.

This hypothesis is supported by a lack of chiral signal shown in spectra from a **slow-*cis*** procedure of the same system, and the absence of a 330 nm peak at low pH. During a slow protonation process, the energy release is not quite so immediate and has time to be dissipated so that it does not reach a point where it catalyzes thermal isomerization. The systems decreased in slow pH steps also retained their ability to photo-switch and upon irradiation yielded similar signals to the systems in which pH was decreased quickly.

Several other systems were studied containing ADA and other molecules, including various cationic porphyrins and spermine, in order to explore the ability of ADA to create photo-responsive hetero-aggregates but the results obtained so far are still unclear and further investigation is required. Future studies will focus on the hetero-aggregation with porphyrins and the concentration-dependence of the ADA process, which several experiments have suggested play an important role during aggregation. Furthermore, in order to confirm the proposed H aggregate structure and elucidate the undefined aggregate geometries observed, theoretical calculations and, if crystallization is achieved, X-ray analysis will be explored.

6.   WORKING BIBLIOGRAPHY

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7. APPENDIX
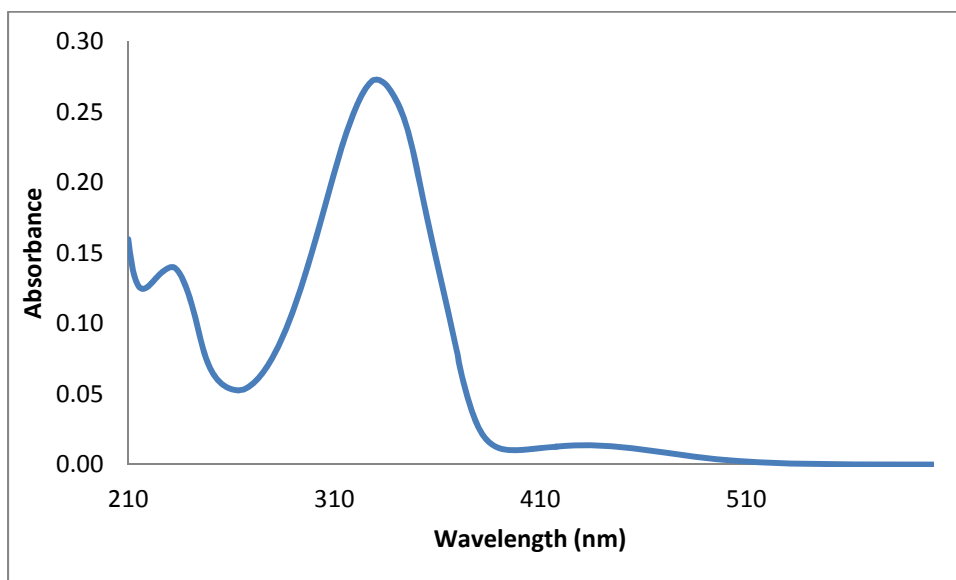
Figure 7.1. Initial step in cycle of **Figure 3.1.3**: UV-Vis spectrum of aqueous *trans*-ADA at pH=7.
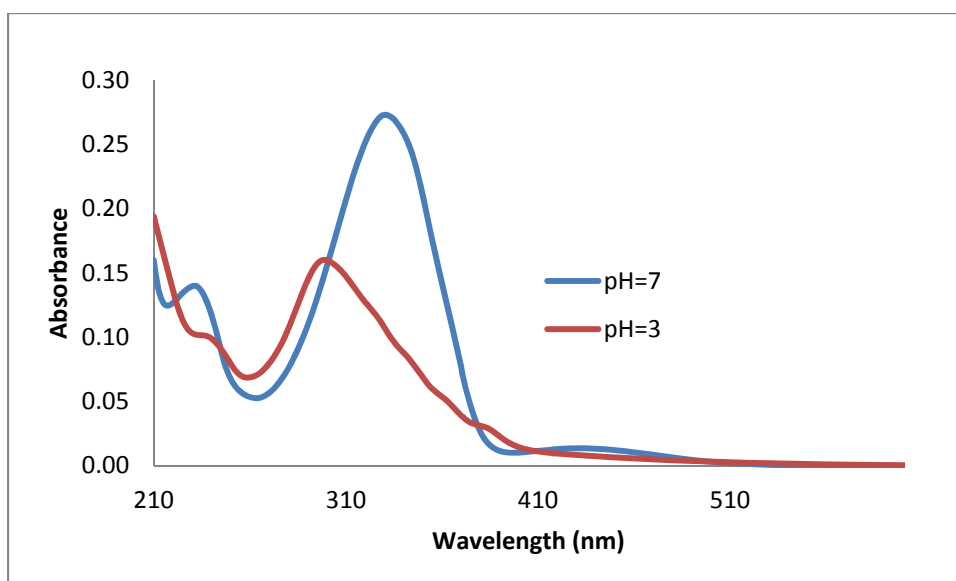
Figure 7.2. Second step in cycle of **Figure 3.1.3**: Effect of **fast-*trans*** procedure on the UV-Vis spectrum of *trans*-ADA: *trans*-ADA at pH=7 (blue), *trans*-ADA at pH=3 (red).
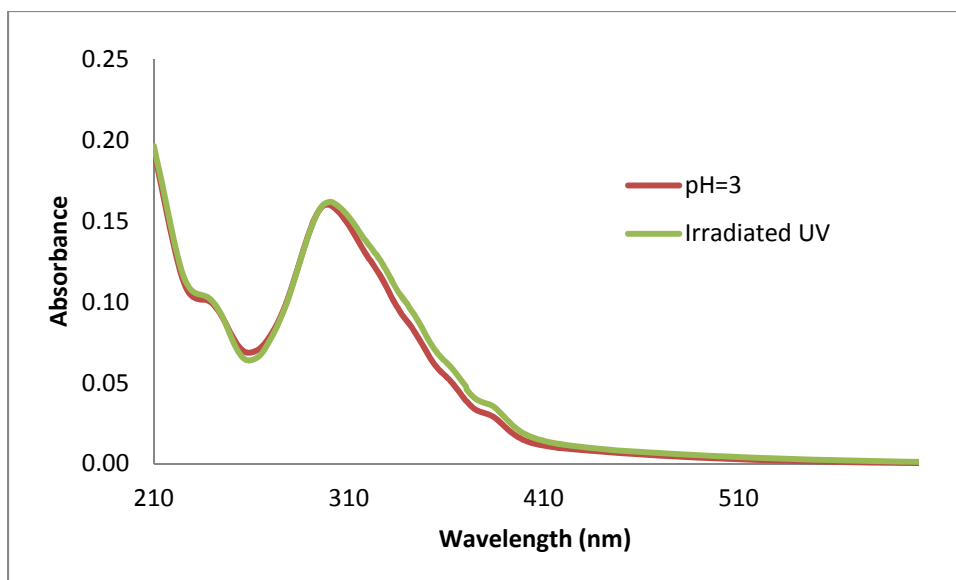
Figure 7.3. Third step in cycle of **Figure 3.1.3**: Effect of UV irradiation on UV-Vis spectrum of *trans*-ADA decreased to pH=3 via **fast-*trans*** procedure: *trans*-ADA at pH=3 (red), *trans*-ADA after irradiation with UV light (green).
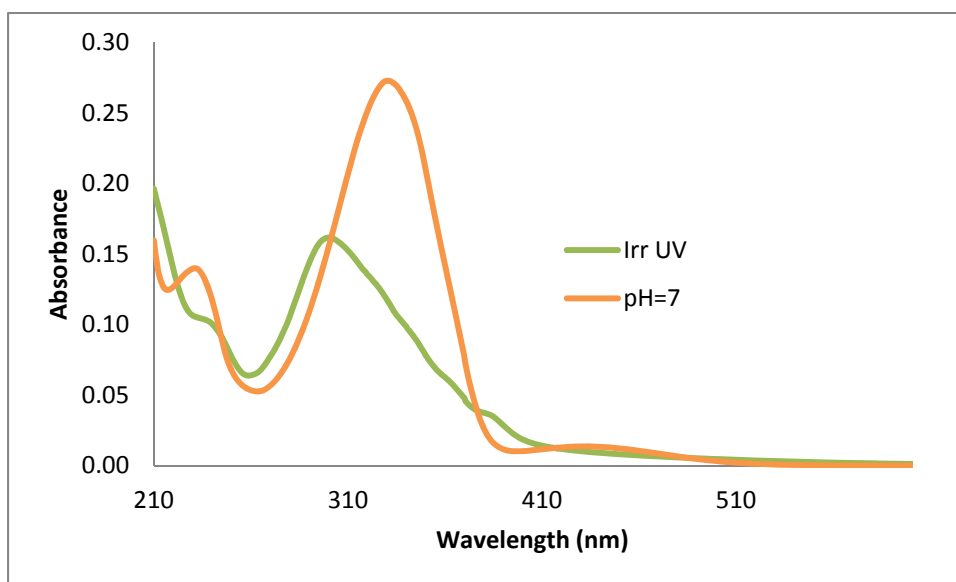
Figure 7.4. Fourth step in cycle of **Figure 3.1.3**: Effect of pH increase from 3 to 7 on the UV-Vis spectrum of *trans*-ADA following UV irradiation: *trans*-ADA at pH=3 (green), *trans*-ADA at pH=7 (orange).
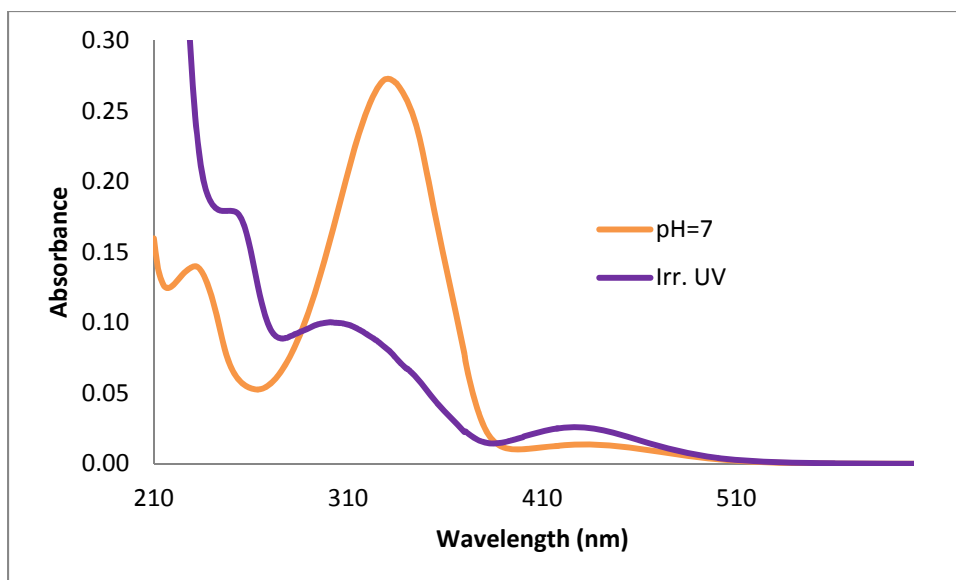
Figure 7.5. Fifth step in cycle of **Figure 3.1.3**: Effect of UV irradiation on the UV-Vis spectrum of *trans*-ADA at pH=7: *trans*-ADA at pH=7 (orange), *cis*-ADA following UV irradiation (purple).
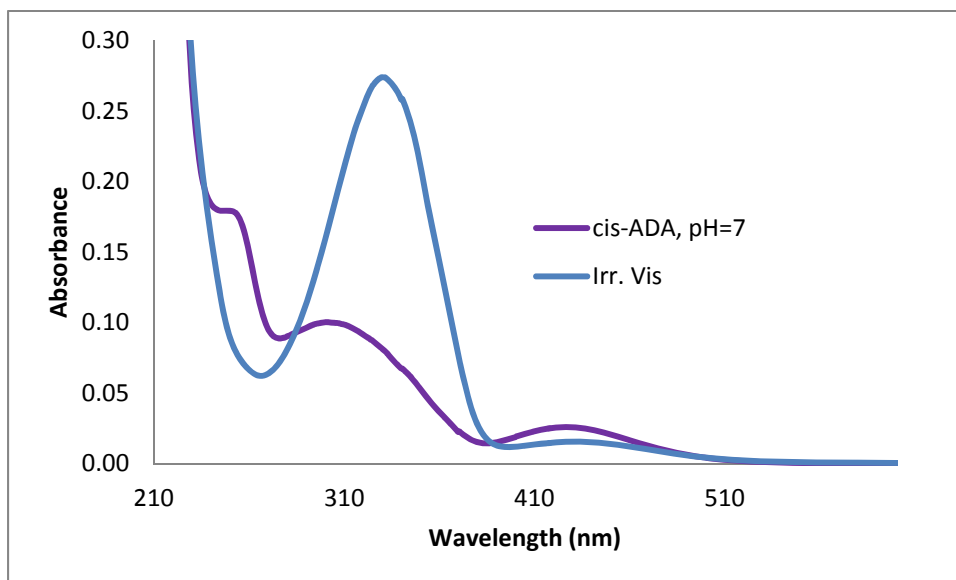
Figure 7.6. Sixth step in cycle of **Figure 3.1.3**: Effect of visible irradiation on the UV-Vis spectrum of *cis*-ADA at pH=7: *cis*-ADA at pH=7 (purple), *trans*-ADA following irradiation with visible light (blue).
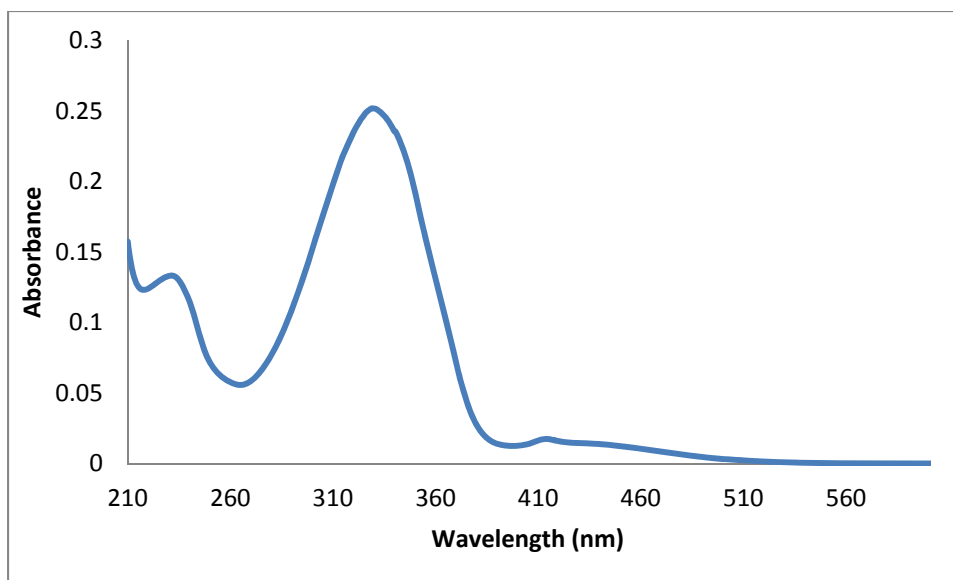
Figure 7.7. Initial step in cycle of **Figure 3.3.3**: UV-Vis spectrum of *trans*-ADA at pH=7.
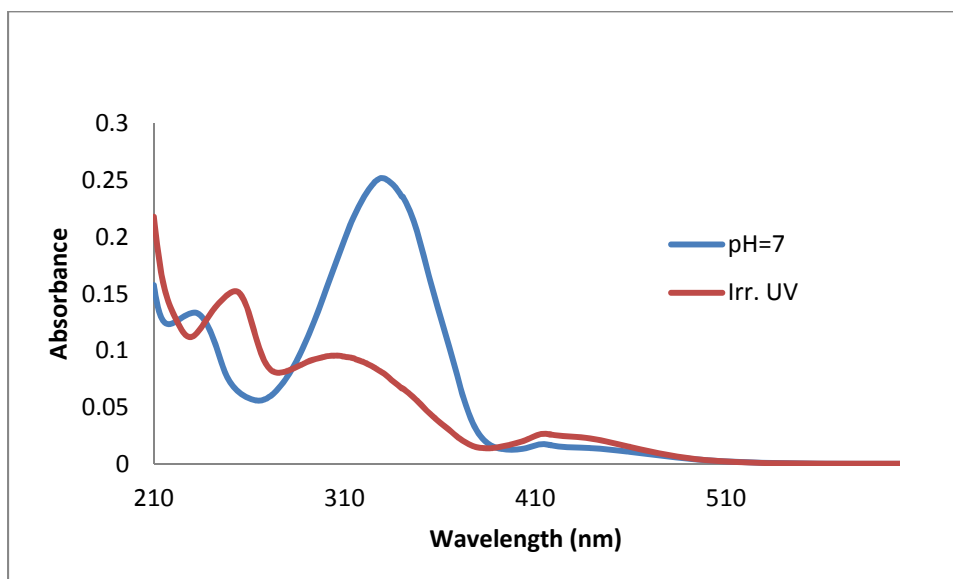
Figure 7.8. Second step in cycle of **Figure 3.3.3** Effect of UV irradiation on a solution of *trans*-ADA at pH=7: *trans*-ADA (blue), *cis*-ADA (red).
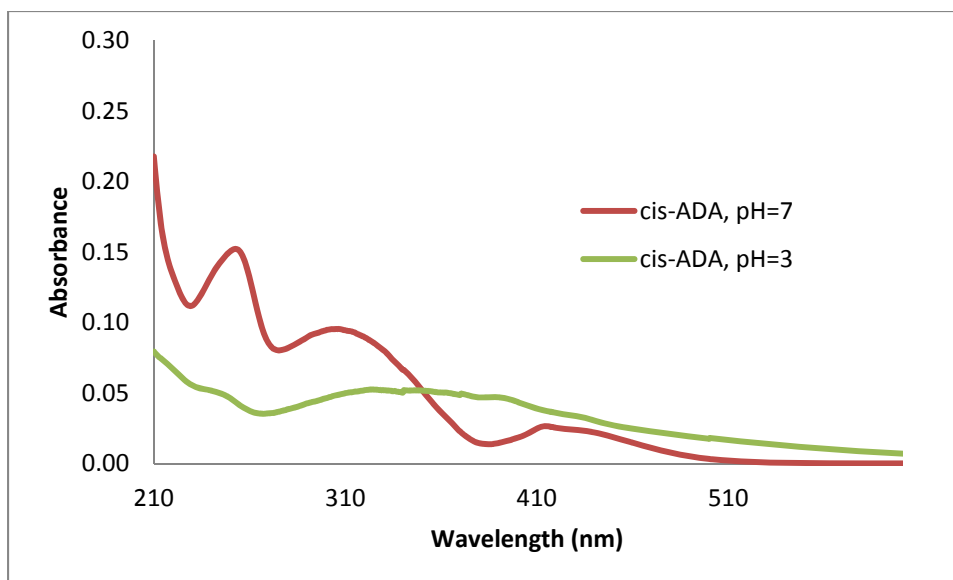
Figure 7.9. Thirds step in cycle of **Figure 3.3.3**: Effect of **fast-*cis*** procedure on UV-Vis spectra of *cis*-ADA: *cis*-ADA at pH=7 (red), *cis*-ADA at pH=3 (green).
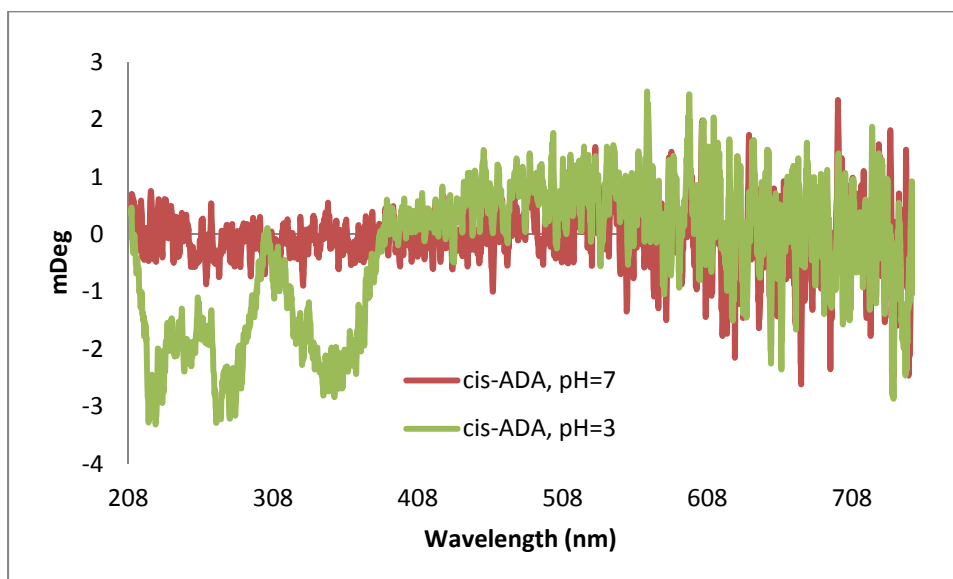

Figure 7.10. Third step in cycle of **Figure 3.3.3**: Effect of **fast-*cis*** procedure on CD spectra of *cis*-ADA: *cis*-ADA at pH=7 (red), *cis*-ADA at pH=3 (green).
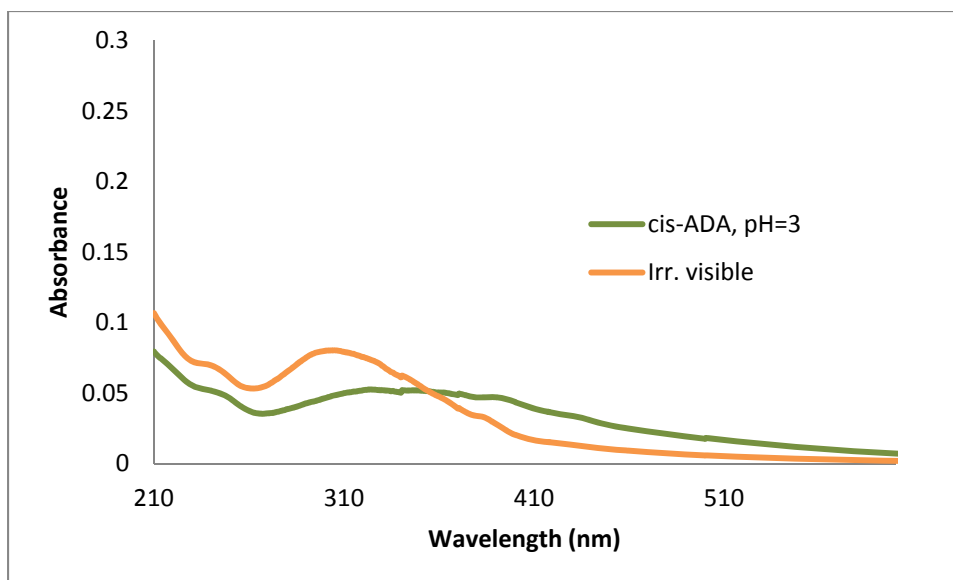
Figure 7.10. Fourth step in cycle of **Figure 3.3.3**: Effect of visible light irradiation on UV-Vis spectrum of *cis*-ADA at pH=3 (decreased in pH by **fast-*cis*** procedure): *cis*-ADA (green), *cis*-ADA irradiated with visible light to *trans*-ADA (orange).
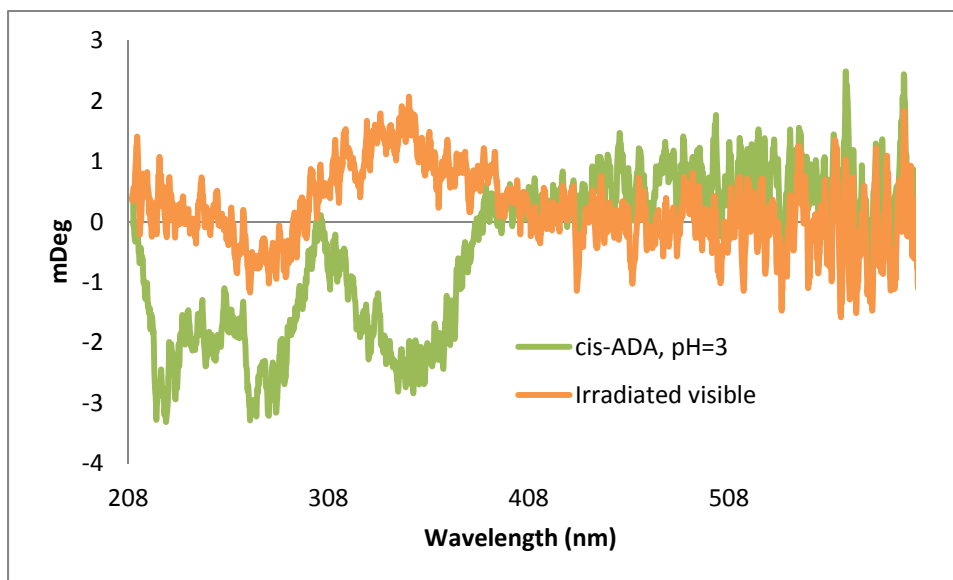

Figure 7.11. Fourth step in cycle of **Figure 3.3.3**: Effect of visible light irradiation on CD spectrum of *cis*-ADA at pH=3 (decreased in pH by **fast-*cis*** procedure): *cis*-ADA (green), *cis*-ADA irradiated with visible light to *trans*-ADA (orange).
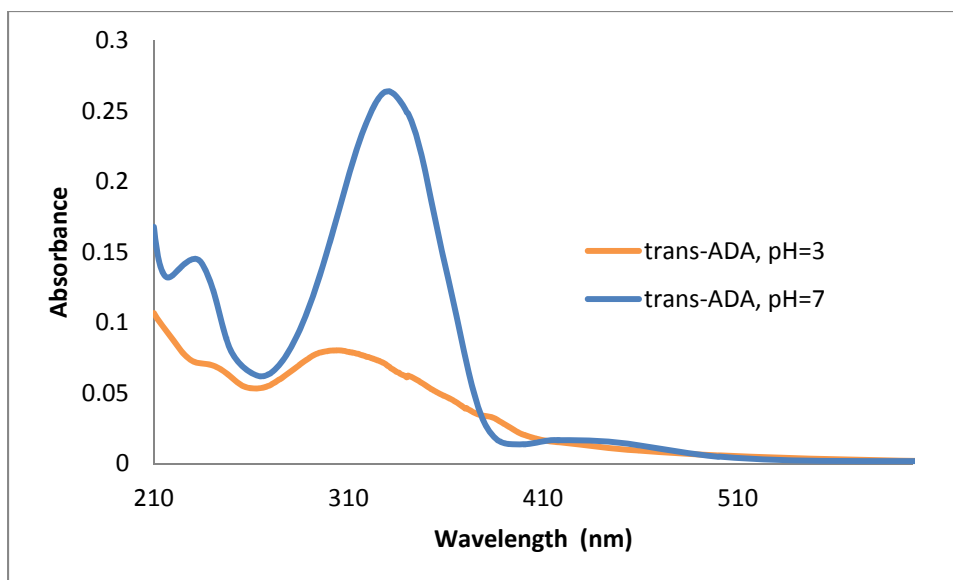
Figure 7.12. Fifth step in cycle of **Figure 3.3.3**: Effect of pH increase from pH=3 to pH=7 on the UV-Vis spectrum of *trans*-ADA: *trans*-ADA at pH=3 (orange), *trans*-ADA at pH=7 (blue).