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# Conformational transitions of a free amino-acid-functionalized polythiophene induced by different buffer systems

K P R Nilsson<sup>1</sup>, M R Andersson<sup>2</sup> and O Inganäs<sup>1</sup>

<sup>1</sup> Biomolecular and Organic Electronics, IFM, Linköping University, SE-581 83 Linköping, Sweden

<sup>2</sup> Department of Polymer Technology, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

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## Abstract

A chiral, 3-substituted polythiophene with an amino-acid function shows pH-dependent visible, emission and circular dichroism spectra in buffered aqueous solution. At pH equal to the *pI* of the amino-acid, the backbone adopts a nonplanar right-handed helical conformation and the polymer chains are separated from each other. Increasing pH leads to a more planar conformation of the backbone and an aggregation of the polymer chains occurs. A lower pH will also lead to a more planar conformation of the backbone, but aggregation of the polymer chains appears to be absent. The aggregates are disrupted by increasing ionic strength in alkaline buffer systems, indicating hydrogen bonding is important for aggregation. On the other hand, ions containing an amino group and one or more hydroxyl groups induce a more planar conformation of the polymer backbone.

## 1. Introduction

The development of materials showing recognition properties toward the chemical environment, such as ions, molecules and biological species, has attracted increasing attention, owing to their great potential for molecular electronics and sensors. In this regard, conjugated polymers (CPs) such as polythiophene and polypyrrole can be used to couple analyte/receptor interactions, as well as nonspecific interactions, into observable (transducible) responses [1–4]. CP-based sensors are very sensitive to very minor perturbations, due to amplification by a collective system response, and offer a key advantage compared with small molecule elements. The possibility of using CPs as detecting elements for biological molecules requires that polymers are compatible with an aqueous environment. This has been achieved by making conjugated (and sometimes luminescent) polyelectrolytes, as recently used to detect biomolecules through their impact on the conditions for photoinduced charge or excitation

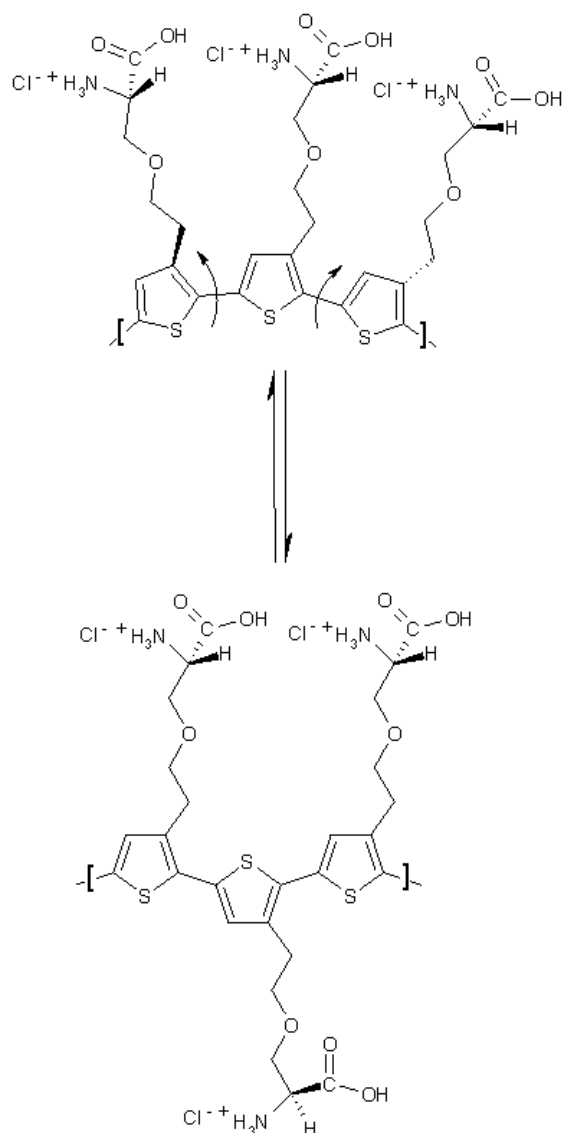
transfer [5,6]. Conjugated polyelectrolytes offer possibilities for very sensitive luminescence measurements, and these materials may become ubiquitous for gene and protein chips in the future.

The physical and chemical properties of polythiophenes can be modified by the introduction of suitable side chains in the 3-position. These polymers exhibit a variety of optical transitions upon external stimuli such as solvents [7], heat [7–11], light [12], metals [13, 14], chemicals [15] and proteins [16, 17], opening the way for design of a variety of sensory devices. Such effects have been termed solvatochromism, thermochromism, photochromism, ionochromism and biochromism respectively. The chromic transitions, from deep violet (maximum of absorption around 550 nm) to bright yellow (maximum of absorption near 400 nm), are believed to be related to a planar-to-nonplanar (from highly conjugated to less conjugated) conformational transition of the backbone. The highly conjugated form is believed to be associated with intermolecular and intramolecular (through chain folding) aggregates.

The side chains in a polymer of 3-substituted thiophene may adopt a *syn* or an *anti* orientation (figure 1). The *anti* conformations are somewhat more stable than the *syn* conformations due to steric factors. The  $\pi$ -electron overlap along the polymer backbone is maximized in a planar *anti* conformation and decreases with increasing twist between neighbouring thiophene rings. There is a strong correlation between the electronic structure and the backbone conformation in CPs, and any change in the main chain conformation will lead to an alteration of effective conjugation length, coupled with a shift of the absorption in the UV–visible range. Studies on different polythiophene derivatives have suggested that this conformational change of the main chain could be affected by order–disorder transitions of the side chains [18]. A chiral side chain, in which the chiral centre is linked to the thiophene ring by a spacer, should perturb the polymer backbone, inducing a small twist along the polymer backbone in an *anti* conformation and a large twist along the backbone, resulting in a helix, for the *syn* conformation. Chiral polythiophenes exhibit optical activity in the  $\pi$ – $\pi^*$  transition. This derives from the main-chain chirality when chains are aggregated to form a supramolecular,  $\pi$ -stacked self-assembly due to intermolecular interactions in poor solvent or at low temperature, whereas they show no activity in the UV–VIS region in a good solvent or at high temperatures [19–22].

Polythiophene derivatives that display biotin [1, 17] and different carbohydrates [3] were synthesized and shown to undergo colorimetric transitions in response to binding of streptavidin and different types of bacterium and virus, respectively. The degree of conjugation in the polythiophene backbone is influenced by external stimuli [8–17, 23–27] and generally involves planar-to-nonplanar transitions. A driving force influencing the conformational change of the polymer backbone is electrostatic interactions, leading to aggregation, gelation or crystallization [15, 28, 29]. Regioregular polythiophenes having carboxylic acid side chains on an ethylene spacer show significant H bonding, that contributes to extended  $\pi$ -orbital overlap along the main chain [15].

The synthesis of poly(3-[(S)-5-amino-5-carboxyl-3-oxapentyl]-2, 5-thiophenylene hydrochloride), POWT (figure 1), with a free amino acid side group, has been reported previously [30]. The polymer adopts different conformations in different solvents (water and methanol) and a red-shift of the absorption maximum is observed in water. We hypothesized, based on previous studies [10, 13–17, 31], that the degree of planarity in the polymer backbone depends on electrostatic, H-bonding, steric or van der Waals interactions within or between polymer chains. As the polymer side chain contains a zwitterionic group, the charge of this group will most certainly induce conformational changes of the polymer backbone. The carboxylic group of serine has a  $pK_a$  of 2.19 and the amino group has a  $pK_a$  of 9.21. Dissolving the polymer in different buffer systems will easily alter the charge of these groups and thereby



**Figure 1.** *Syn* and *anti* conformations of POWT.

force the polymer backbone to adopt different conformations. In deionized water, the polymer side chains will mostly be in the zwitterionic form ( $pI = 5.7$  for serine) and an increase or a decrease in pH will give the polymer a more negative or a more positive net charge, respectively.

In this paper we report the solvatochromism and ionochromism for POWT in different buffer solutions.

## 2. Material and methods

The synthesis of the polymer was reported elsewhere [30]. A stock solution containing  $5.0 \text{ mg polymer ml}^{-1}$  in deionized water was made and the following series of buffer solutions was prepared: 20 mM Na phosphate (pH 2.0 and 3.0), 20 mM Na acetate (pH 4.0

**Table 1.** Absorption maxima for POWT in different buffer systems.

Buffer system	$\lambda_{max}$ absorption (nm)
20 mM phosphate pH 2.0	433
100 mM phosphate pH 2.0	434
20 mM phosphate pH 3.0	424
20 mM acetate pH 4.0	422
100 mM acetate pH 4.0	422
20 mM acetate pH 5.0	400
20 mM phosphate pH 6.0	404
20 mM phosphate pH 7.0	414
100 mM phosphate pH 7.0	411
20 mM Tris-HCl pH 8.0	437
100 mM Tris-HCl pH 8.0	450
20 mM Tris-HCl pH 9.0	459
20 mM carbonate pH 10.0	458
100 mM carbonate pH 10.0	442
20 mM carbonate pH 11.0	475

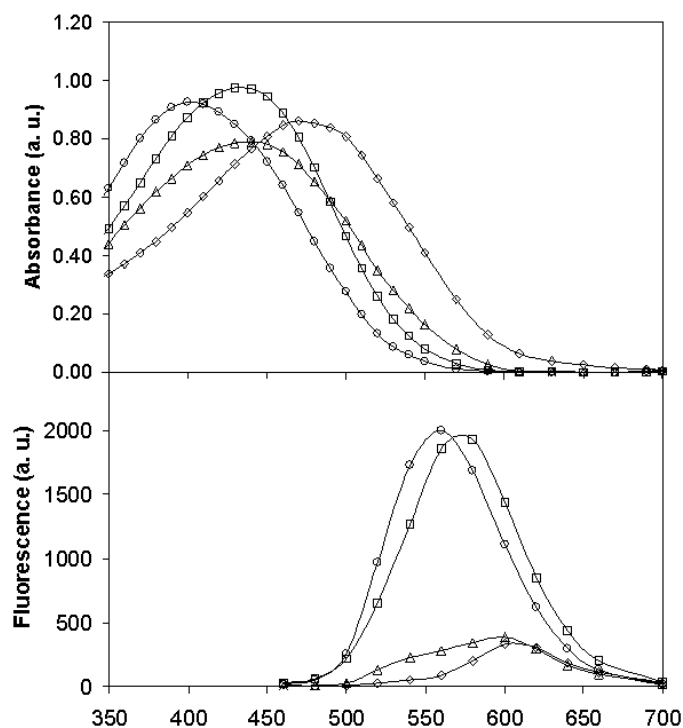
and 5.0), 20 mM Na phosphate (pH 6.0, 7.0), 20 mM Tris-HCl (pH 8.0 and 9.0) and 20 mM Na carbonate (pH 10.0 and 11.0). All the chemicals used were of analytical grade. For the absorption, emission and circular dichroism (CD) measurements the stock solution was diluted with the different buffer solutions to a final concentration of  $37.5 \mu\text{g polymer ml}^{-1}$  solvent and the samples were incubated for 1 h. Optical spectra were recorded on a Perkin-Elmer Lambda 9 UV/VIS/NIR spectrophotometer for UV/VIS, a Hitachi F4500 Fluorescence Spectrophotometer for fluorescence, and an I.S.A. Jobin-Yvon CD6 (5 mm quartz cell) for CD.

### 3. Results and discussions

#### 3.1. Absorption measurements

The absorption spectra and absorption maxima in different buffer solutions for POWT are shown in figure 2 and table 1. The polymer undergoes a pH-induced yellow to orange colour alteration (a shift of the absorption maximum from 400 to 470), indicating that deprotonation and protonation of the amino- and the carboxyl groups have an influence on the coil-to-rod (nonplanar to planar) conformation transition of the polymer backbone. The nonplanar conformation seems to be most abundant at pH 5 and 6. As the side chains become charged, either positive or negative, the polymer chains adopt a more planar conformation, observed as a red-shift. The polymer is probably adopting the rod-shaped conformation due to electrostatic repulsion forces between the polymer side chains [2, 31], or hydrogen bonding [15] and hydrophobic assembly between nearby polymer chains, as these molecular forces are highly influenced by the charge of the polymer side chains. A closer look at the spectra for the polymer in pH 2 and 8 solutions (figure 2) reveals an interesting observation. The absorption maximum is almost the same but the spectrum recorded for the pH 8 solution has a shoulder in the region at 570 nm, suggesting an aggregation of polymer chains takes place at increasing pH but not at acidic pH.

Other properties influencing the polymer conformation are the ionic strength and the ionic composition of the buffer system (table 1). An increase of the ionic strength in the Tris-HCl buffer system will induce a shift of the absorption maximum to longer wavelengths. The shift is stronger for buffer systems when the Tris molecule ( $^+\text{H}_3\text{N}-\text{C}-(\text{CH}_2-\text{CH}_2-\text{OH})_3$ ) has a positive



**Figure 2.** Absorption (top) and emission (bottom) spectra of POWT in different buffer solutions: pH 2 ( $\square$ ), pH 5 ( $\circ$ ), pH 8 ( $\triangle$ ) and pH 11 ( $\diamond$ ). The emission spectra were recorded with excitation at 400 nm.

charge ( $\text{pH} < \text{p}K_a$  for Tris), indicating that an interaction with the negatively charged carboxyl group of the polymer side chain occurs. The polymer backbone is most probably adopting a more rod-shaped conformation due to disruption of the intrachain interactions between amino and carboxyl groups of nearby side chains. The hydroxyl groups of the Tris-molecule may act as hydrogen bond acceptors or as hydrogen bond donors and thereby stabilize the rod conformation by interchain interactions. Alternatively the bulky cation interacting with the negative carboxyl group could function as a steric hindrance between nearby side chains and force the main chains to adopt the *anti* conformation. Adding ethanolamine to the polymer solution (data not shown) will also induce the same shift.

In contrast an increase of the ionic strength in a buffer solution with high pH (7.0 and 10.0) will shift the absorption maximum to shorter wavelengths. An excess of ions will probably remove hydrogen bonds that direct aggregation and consequently breaks up the aggregate, leading to a more nonplanar conformation. The shift is more significant as the pH of the solution increases. This phenomenon is not seen in buffer solutions with acidic pH (2.0 and 4.0), signifying that the molecular forces responsible for the extended conjugation length of the polymer backbone are different from those in alkaline pH.

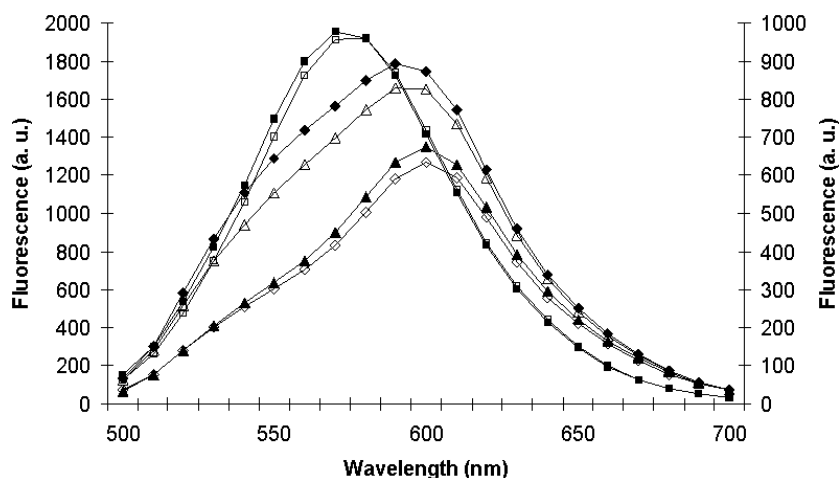
### 3.2. Fluorescence measurements

The pH-induced conformational changes of the polymer chains will also alter the emission spectra for the polymer solutions (figure 2). The polymer emits light with a longer wavelength as the net charge of the polymer side chains become more negative. At pH 11, when almost all

the amino groups are neutral and all the carboxyl groups are negatively charged, the polymer emits light with a wavelength around 605 nm. When the pH decreases and the net charge of the polymer becomes more neutral, light with a shorter wavelength is emitted and as the  $\text{pH} = \text{pI}$  (for serine) the peak is shifted to  $\approx 560$  nm. Earlier studies of POWT have shown an analogous trend in the photoluminescence (PL) of intra- and interchain phenomena in the polymer [32,33]. As the chains were separated the PL maximum was blue-shifted by  $\approx 105$  nm, compared with the dense packing of the polymer chains. This observation indicates that the two distinct separated peaks in the emission spectra of POWT originate from an intrachain event (560 nm) and an interchain event (605 nm). In the pH range from 6.0 to 11.0 a mixture of these two states is present in the solution. Aggregation of the polymer chains seems to be encouraged at higher pH when the polymer backbone adopts a more planar conformation, and this could also be seen in the sample used, as the polymer precipitated faster with an increasing pH. A solution of POWT in the 20 mM phosphate buffer (pH 6.0) remains stable for a couple of months, but in the 20 mM carbonate buffer (pH 11) the polymer precipitates after a couple of days. Apparently the deprotonation of the amino groups is important for the polymer chains to form aggregates. A deprotonated amino group will be able to function as a hydrogen bond acceptor, suggesting hydrogen bonding is important for directing aggregation. The intensity of the fluorescence for the aggregated phase of polythiophene derivatives compared with the fluorescence for the single-chain state has previously been showed to be weaker by approximately one order of magnitude [21, 34], in agreement with the results in this study. The earlier studies of thin films of POWT also showed an analogous trend, as the PL quantum efficiency increased from 4% for the interchain event to 16% for the intrachain event [32, 33]. Interestingly the PL quantum efficiency was also increased in a methanol solution (31%) compared with a water solution (9%), indicating that the polymer backbone adopts different conformations in these two solvents [32].

Surprisingly, for the nonplanar-to-planar conformational transition of the backbone at lower pH, where the net charge of the polymer chains becomes more positive, light with a slightly longer wavelength is emitted, but the intensity of the fluorescence is not decreasing in the same way as for a solution with high pH. These results indicate that the emitted light is due to an intrachain event. Consequently, the positive net charge of the polymer seems to favour a more rod-shaped conformation of the polymer chain, but aggregation of the polymer chains is most probably absent or occurs in another way. The rod-shaped form of the polymer chains at acidic pH is probably induced by electrostatic repulsion forces or hydrogen bonding between nearby polymer chains. At acidic pH the protonated carboxyl group will be able to function as a hydrogen bond donor and this might encourage another type of hydrogen bonding pattern, that will prevent aggregation but induce a more rod-shaped conformation of the polymer backbone. The polymer does not precipitate in solutions with lower pH, in agreement with this assumption.

Increasing the ionic strength of the solutions will also modify the wavelength of the emitted light (figure 3). An increase of ions in the solution with  $\text{pH} = 10.0$  will induce an increase of the emitted light of shorter wavelength by disrupting the planar conformation and breaking up the aggregates, agreeing with the assumption that hydrogen bonding is responsible for directing aggregational ordering. As previously seen in the absorption spectrum the cation in the Tris buffer system ( $\text{pH} = 8.0$ ) will yield a more planar conformation of the polymer backbone. This phenomenon can also be seen in the emission spectrum since light with longer wavelength is emitted while the ionic strength of this buffer system increases. As mentioned earlier, the hydroxyl groups on the Tris molecule can act as hydrogen bond acceptors or hydrogen bond donors, hence it appears that hydrogen bonding will induce aggregation of the polymer chains. An increase of the ionic strength in the buffer solution with  $\text{pH} = 2.0$  will not alter the



**Figure 3.** Emission spectra of POWT in different buffer solutions with varying ionic strength recorded with excitation at 400 nm: pH 2 ( $\square$ , left axis), pH 8 ( $\diamond$ , right axis) and pH 10 ( $\triangle$ , right axis). Filled symbols, 20 mM; open, 100 mM.

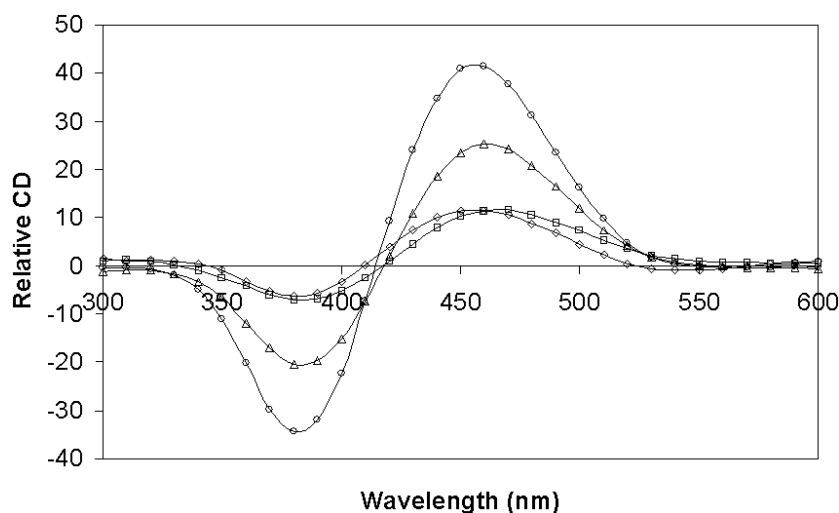
wavelength of the emitted light, clearly demonstrating that the molecular forces responsible for the nonplanar-to-planar conformational transition of the polymer backbone are different in an acidic environment.

### 3.3. CD measurements

As previously reported [30], the polymer exhibits a split-type induced circular dichroism (ICD) in the  $\pi-\pi^*$  transition region. The two major CD peaks in water, interpreted as due to *syn* conformers, disappeared in methanol, where *anti* conformations dominate [30]. Normally  $\pi$ -stacked chiral aggregations of conjugated thiophene polymers, as seen in poor solvents, are accompanied by a colour change from yellow-orange to purple [19–21, 34]. This is due to a transition from a disordered coil-like form to a rodlike,  $\pi$ -stacked one in order to give a chiral supramolecular aggregate with interchain interactions of transoidal polythiophenes. The CD spectra of POWT in different buffer solutions are shown in figure 4. In our case the red-shifts in absorption, induced by different buffer systems, are accompanied by a decrease in the ICD, indicating that chirality introduction may not be derived from  $\pi$ -stacked chiral aggregation of the polymer. Instead the ICDs may be a result of main-chain chirality, such as a predominantly one-handed helical structure induced by the zwitterionic group of the polymer side chain [35, 36]. The CD spectra show that near the zwitterionic point the polymer chain will adopt the nonplanar *syn* conformation with a high helicity. When the side-chains become more charged, negatively or positively, the helicity is reduced. Hence the polymer chain adopts a more rodlike conformation, in agreement with the results previously seen in the absorption measurement.

The absence of CD signals followed by the increase in the PL quantum efficiency, as seen in the earlier studies of POWT [30, 32], suggest that the polymer backbone adopts a random coil conformation in methanol. In water the thiophene rings will be shielded from the solvent and the polymer chains will probably form a micelle-like structure, with the polar side chains pointing outward to the solvent. The helical structures are most probably stabilized by hydrophobic interactions between the thiophene rings, and electrostatic interactions or





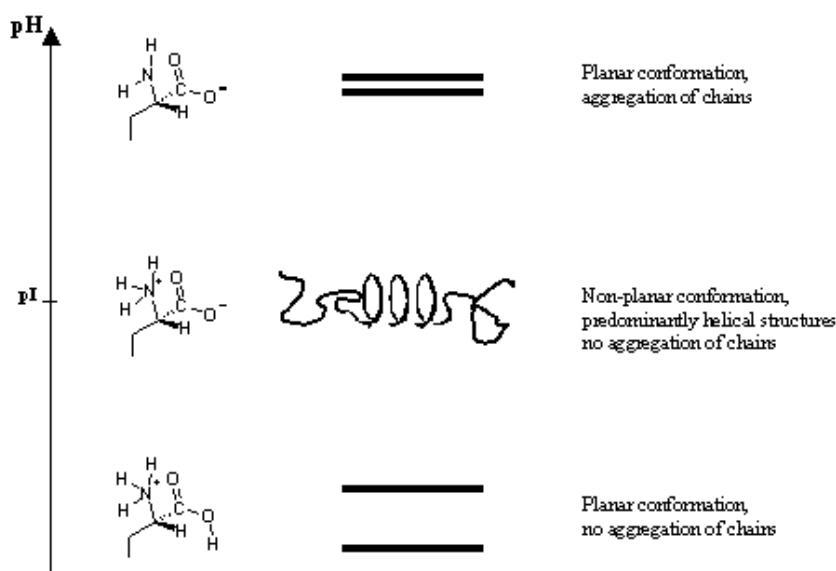
**Figure 4.** CD spectra of POWT in different buffer solutions: pH 3 ( $\square$ ), pH 5 ( $\Delta$ ), pH 6 ( $\circ$ ) and pH 10 ( $\diamond$ ).

hydrogen bonding between the polymer side chains. As the magnitude of the Cotton effect is highest near the isoelectric point for serine and decreases with an alteration of the pH, the hydrogen-bonded ion pair complex seems to favour the helicity of the polymer chains.

Previous studies of a stereoregular poly((4-carboxyphenyl)acetylene) forming acid–base complexes with amines and amino alcohols in DMSO [35] indicate that R- and S-enantiomers induce split-type ICD of mirror images. Interestingly the S-form of serine used in the synthesis of POWT [30] gives a similar ICD pattern (negative/positive) to the primary S-amines in this earlier study [35]. The shape and sign of the ICD pattern are characteristic of a right-handed helical form of polythiophene first observed by Meier [21,37]. Normally a polypeptide made from L- $\alpha$ -amino-acid residues forms a right-handed helix, so apparently the nature of the amino-acid is reflected in the helical conformation of the polymer backbone. Consequently it would be of great interest to synthesize the polymer using the R-form of serine to see whether the sign of the Cotton effect is reversed.

#### 4. Conclusions (summary)

We have shown that the backbone conformation and the separation of different polymer chains of a conjugated polythiophene with a free amino-acid side chain clearly depends on the protonation and the deprotonation of the zwitterionic group. At a pH equal to the  $pI$  of the amino-acid, the backbone adopts a nonplanar right-handed helical conformation and the polymer chains are separated from each other. Increasing pH leads to a more planar conformation of the backbone and an aggregation of the polymer chains occurs. A decrease in pH will also lead to a more planar conformation of the backbone, but aggregation of the polymer chains seems to be absent. A schematic drawing of the different polymer chain conformations is given in figure 5. Different types of ion will also induce the planar-to-nonplanar conformational transition of the polymer backbone. At alkaline pH, ions containing an amino group and one or more hydroxyl groups induce a more planar conformation of the polymer backbone. An increase in ionic strength will disrupt the aggregates, hence it seems that hydrogen bonding is responsible for aggregation. Our future efforts in this system will be



**Figure 5.** The charge of the zwitterionic side chain and a schematic drawing of proposed backbone conformations of POWT at different pHs.

to determine the different types of interaction forcing the polymer to show this extraordinary behaviour and how this can be used in different types of biosensor and bioelectronic system.

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