I.A.U
Karaj branch

Journal of
A p p l ied
C hemical
R esearch
jacr.kiau.ac.ir

Journal of Applied Chemical Research, 15, 2, 55-64(2021)

Synthesis of Alaninemethylester and *N*-(4-aminobenzyl)-9H-purin-6-amine Doped to Polyaniline Nanoparticles and Study on Their Interactions with Ds-DNA by Fluorescence Spectroscopy

Banafsheh Gorji[1*],Reza Zadmard[2], Kian Tavakol[1], Fatemeh Shahkarami[1]

[1]*Department of Chemistry, South Tehran Branch, Islamic Azad University, Tehran, Iran*

[2]*Chemistry & Chemical Engineering Research Center of Iran, Tehran, Iran*

(Received 14Aug. 2020; Final revised received 19Nov.2020)

Abstract

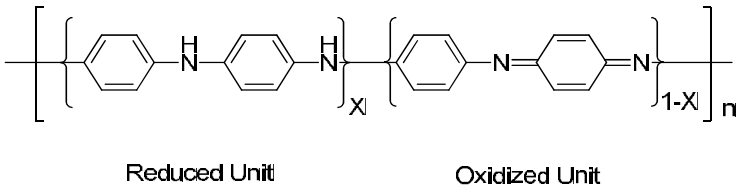
In this paper, two major projects have been successfully accomplished: Firstly, we introduced a novel and highly efficient route which was developed for the synthesis of Polyaniline (PANI) nanoparticles by using Potassium persulfate. Structure of nano PANI was characterized by Fourier-Transform Infrared (FT-IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and elemental analysis and the surface morphology of them was studied by scanning electron microscopy (SEM).Secondly, new nano-biosensors have been synthesized by using a simple and efficient method, from the reaction of nano PANI with the biocompatible branches, such as alaninemethyl ester and *N*-(4-aminobenzyl)-9H-purin-6-amine as a nucleobase and succinic anhydride as a spacer. The structures of 6 and 7 were confirmed by FT-IR and ^{1}H-NMR. The interaction between alaninemethylester and nucleobase doped to nano PANI with short oligo phosphate chains of ds-DNA (45 base pairs), was studied by fluorescence spectroscopy. The results showed that existence of dopants in the structure of nano PANI increases the interactions with DNA and this caused the decrease in the fluorescence emission intensity.

Keywords: Nano Polyaniline, Amino acid, Nucleobase, *N*-(4-aminobenzyl)-9*H*-purin-6-amine, Fluorescence, Biosensor.

[*]***Corresponding author:*** *Banafsheh Gorji,Department of Chemistry, South Tehran Branch, Islamic Azad University, Tehran, Iran. Email: banafsheh.gorji@gmail.com, b_gorji@azad.ac.ir.*

Introduction

Chiral conductive polymers captured intense attention in research fields such as enantioselective analysis [1-3], chiral separations [4] and chemical and biochemical sensors [5,6]. PANI is one of the most important kinds of these materials which can be provided by consecutive connection of aniline monomers in a polymer chain. Existence of nitrogen atom in the phenyl rings produces different oxidation states which influence physical properties of PANI (Scheme 1). Based on the oxidation states of nitrogen, PANI can be categorized in three forms of fully reduced state (x=1) Leucoemenraldine Base (LB), semi-oxidized state (x=0.5) Emeraldine Base (EB) and fully oxidized state (x=0)Pernigraniline Base (PB) [7,8].



Scheme 1.Structure of aniline monomer.

Due to the features such as easy synthesis, low cost and high polymerization efficiency, PANI is one of the most common and most widely used conductive polymers with high environmental stability and unique electrical, electrochemical and optical properties [9,10]. This material has wide applications as a coating, corrosion inhibitors and catalyzer in industries such as pharmaceuticals and electronics.

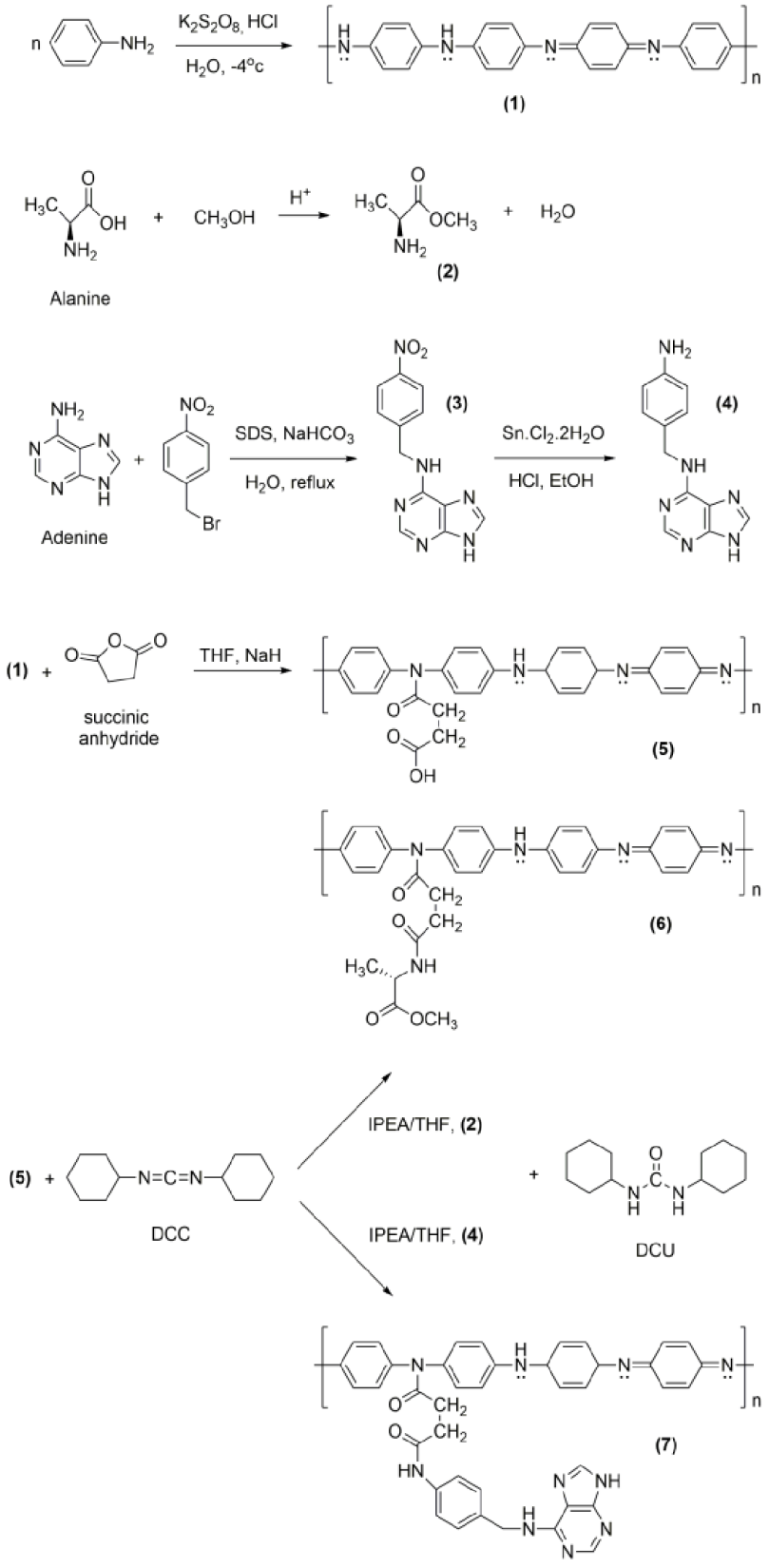
Strong conjugated π-electron system and strong hydrogen bonds between adjacent polymeric chains in PANI, have made it rigid and inflexible material with no specific melting point. This property and low solubility in most organic solvents has limited PANI applications in the industries [11]. In order to improve the structure and process ability of PANI, electrophilic substitution reaction in aromatic ring with a nucleophile can be used [12]. Doping PANI using organic acids such as *p*-toluene sulfonic acid (PTSA), dodecyl benzene sulfonic acid (SSA) and camphor sulfonic acid (CSA) to synthesize sulfonated PANI salt (SPAN) is one example of such reactions [13,14].

DNA is a molecule which contains genetic information. DNA entrance to cells is difficult, because of the existence of negative charges. Phosphate groups on the surface of DNA molecules and anionic phospholipids like serine are the main cause of these negative charges. Researchers are trying to find structures which interact with DNA and they have the capability to enter to the cell membrane. Therefore, positive charge on most of the gene transfer systems is a common practice. For this purpose, artificial carriers such as liposomes, cationic lipids and other surfactants have been designed. But due to the interaction of these compounds with negative charges of blood or anions

which exist in interstitial fluid, they cause side effects. As a result, to optimize transfer methods, researchers work on non-positive carriers or carriers with less positive charges. In this case, synthetic polymers are recommended as a suitable gene transfer method.

Study on the interactions of different compounds with DNA is significant. Polyamines are water-soluble linear amines with low molecular weight. These compounds have $pK_a \simeq 10$. They are available in the form of polycations in physiologic pH and this causes strong interactions with anionic macromolecules such as DNA and RNA [15]. Diamine Putrescine, triamine spermidine and tetraaminespermine are the most prevailing polyamines in living cells. Polyamines have the capability to manipulate DNA structure. The mechanism of polyamine function in DNA condensation involves neutralization of the negatively charged DNA backbone by the positively charged amino groups of Spermidine and Spermine. The literature shows electrostatic interaction between phosphates in DNA and cationic polyamines [16]. X-ray studies illustrate polyamine molecules can be positioned along small and big pores in DNA and have effective interaction with the bases in DNA structure [17]. Proteins also contain specific sites that can have interaction with DNA [18]. Usually, proteins connect to DNA by positioning in the big pores and making hydrogen bonds with bases on the strand pair [19,20].

This research focuses on synthesizing chiral biocompatible polyamides based on PANI. Chiral amino acids or nucleobases were used as side chains to optimize the polymer. For better bonding and reducing space hindrance, the smallest chiral amino acid in the nature, alaninemethylester, was linked to PANI [21]. To bond chains indirectly and to reduce spatial inhibition and better interaction of the final complex with DNA, having a spacer in the nano PANI is a requirement. To have better bonding and reducing spatial inhibition, succinic anhydride was used as spacer and DCC is a coupling agent. For further study, alaninemethylester was replaced with *N*-(4-aminobenzyl)-9*H*-purin-6-amine **4** as a nucleobase. The amine group on **4** should be protected in order to bond to nano PANI, preventing polymerization of adenine and other side groups, increasing flexibility and preventing space hindrance. For this purpose, 4-nitro benzyl bromide was used in the presence of sodium dodecyl sulfate and sodium bicarbonate in aqueous solution at 80 °C [22]. Then, nitro group reduced to amine by using tin chloride and ethanol [23]. Synthesizing steps are shown in Scheme 2.



Scheme 2. Steps for synthesizing alanine and adenine doped to nano PANI.

Experimental

Materials

All materials and solvents were purchased from Merck company. ^{1}H-NMR structure confirmation was obtained using a Bruker 500 MHz. FT-IR spectra was recorded by Bruker, IFS48. The model of Elemental Analysis (CHN) equipment was Quest-Eash EA111. Characterization and morphology is approved by using of SEM model Lecia Cambridge-S360. Fluorescence spectroscopy model was Jasco FP-6500.

Synthesis of nano PANI salt **(1)**

2.5 gr (9.25 mmol) potassium persulfate dissolved in 100 ml 1M HCl at -4 to 0 $^{\circ}$C (oxidant solution). During an hour, 2 ml of double-distilled aniline was added uniformly to the oxidant solution. The color of solution changed from pink to green which was sign of polymerization reactions. The viscose mixture was stirred for 5 hours at the same temperature. The remained green solid filtered and was washed with distillated water and methanol till the solution under the percipitate turned colorless. In order to have neutral product, the percipitate was dissolved in a stirring NH_3 3% for 3 hours at room temperature. Then, it was washed with water and aceton and percipitations dried at 70 $^{\circ}$C in an oven.

Protection of alanine and 9H-purin-6-amine

Synthesis of alanine methyl ester **(2)**

0.5 g of alanine (5.6 mmol) in 10 ml of methanol and under the stream of HCl gas as a catalyst was mixed under reflux for 6 hours to complete the esterification reaction [21]. 0.3 g solid product (2) was obtained.

Synthesis of N-(4-nitrobenzyl)-9H-purin-6-amine **(3)**

1.35 gr (10 mmol) adenine, 0.924 gr (11 mmol) $NaHCO_3$ and 20 mg sodium dodecyl sulfate (SDS) were added to 20 ml distilled water at 80 $^{\circ}$C and stirred for 5 min. 2.16 gr (10 mmol) of 4-nitro benzyl bromide was added to the mixture and the reaction continued for another hour under reflux at the same temperature. Then the solution was cooled at room temperature and the resulted percipitate was dried at oven. To purification, the percipitate was recrystallized with chloroform and ethyl acetate. 0.8 gr of (3) was achieved [22].

Synthesis of N-(4-aminobenzyl)-9H-purin-6-amine **(4)**

1.65 gr (0.007 gmol) $SnCl_2.2H_2O$ and 0.96 mmol HCl were added to 4.8 ml ethanol and stirred at 70 $^{\circ}$C until a clear soultion was achieved. 0.5 gr (0.0018 gmol) of (3) was added drop wise to the mixture in 30 min. The development of the reaction followed by TLC (thin layer chromatography).

At the end of reaction, 20 ml water was added to the solution in order to make the temperature like the room temperature. Then, the solution was put in an ice bath for 30 min to achieve percipitate. The percipitate was washed with distilled water, then 3 ml dichloromethane was added and stirred. The formed suspension was cooled in an ice bath and about 2 ml NaOH was added to pH 12. The organic phase was separated and dried with magnesium sulfate in oven. 0.9 gr of (4) was obtained [23].

Synthesis of Poly-N-[4-Oxobutanoic acid] Aniline **(5)**

0.2 gr (0.6 mmol) of (1) and 10 ml tetrahydrofuran were stirred for 30 min at the room temperature. 0.05 gr (2 mmol) sodium hydrid was added and refluxed for 30 min. After cooling, the flask was placed in an ice bath and 0.16 gr (1.65 mmol) of succinic anhydride was added dropwise and refluxed for 8 hours. 0.27 gr of (5) was obtained.

Synthesis of Poly-N-[(4-Oxobutanamido)Alanine Methhyl ester]Aniline (6) and Poly-N-[(Oxobutanamido)-N-(Aminobenzyl)-Adenine] Aniline **(7)**

In two seperated flasks, 0.1 gr of (5), 27 µl (0.16 mmol) di-isopropyl ethyl amine (IPEA) and 24 µl (0.16 mmol) DCC was dissolved in 5 ml of dry tetrahydrofuran and stirred for 2 hours. Then, 0.14 mmol of (2) and (4) was added to each flask, separately and the mixture was stirred for 20 hours. Black precipitate was obtained and washed with acetone and methanol 10% for 6 hours with Soxhlet extractor to remove the unreacted DCC. The precipitates were dried in an oven at 70 $^{\circ}$C. Finally, 0.06 gr of (6) and 0.05 gr of (7) were obtained.

Study on Fluorescence properties

In the absence of DNA

In three separated vials, 0.01 g of (1), (6) and (7) were poured and 0.6 ml of Hepes buffer 0.1 M was added and mixed intensively. To avoid interference with the nanoparticles, the samples were centrifuged 3500 rpm for 10 min. Then, 200 µl of each samples were brought to 1.5 ml by Hepes buffer (pH=6.5). The fluorescence emission of each it was recorded at an excitation of 284, 298 and 258 nm for compounds (1), (6) and (7), respectively.

In the presence of DNA

The method is as same as previous step, except 0.6 ml DNA was added to each vial and fluorescence emission was measured

Results and discussion

Characteristics of **(1), (6)** *and* **(7)**

Figure 1 illustrates SEM images of nano PANI (1), alanine doped to nano PANI (6) and adenine doped to nano PANI (7). The size of the particles (1), (6) and (7) are about 30-50 nm, 50-70 nm and 70-110 nm, respectivly.

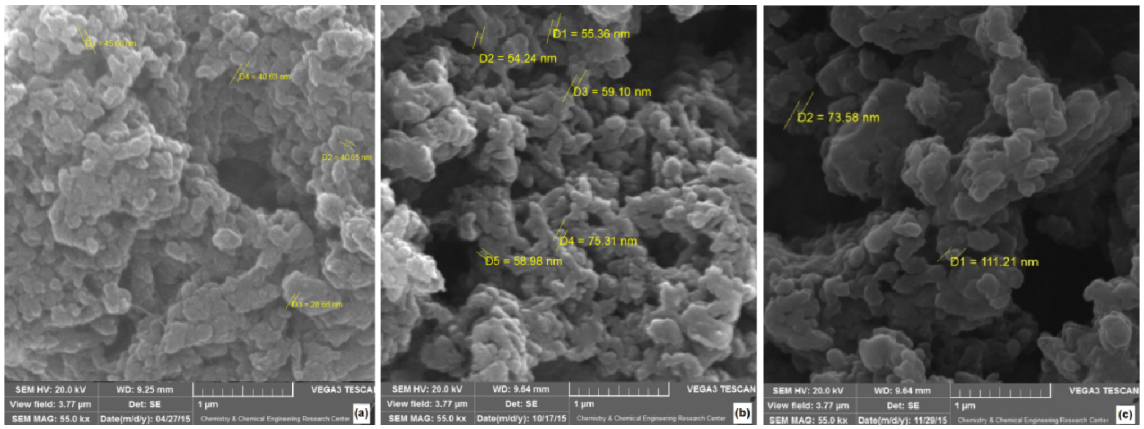


Figure 1. SEM images of a:(**1**), b:(**6**), c:(**7**).

FT-IR spectrums of (1), (6) and (7) are as bellow:

(1): IR (λ_{max}, cm^{-1}): 3430 (NH streching of secondary amine), 2922 (CH streching of aromatic ring), 1585 (C=C streching of the quinoid ring), 1492 (C=C streching of the benzeneoid ring), 1242 (C-N stretching of secondary aromatic amine), 821 (CH out of plan of aromatic ring).

(6): IR (λ_{max}, cm^{-1}): 3390 (NH streching of secondary amine), 1717 (C=O streching of carbonyl), 1589 (NH bending of amine),1254 (C-N stretching of aromatic amine), 1142 (C-O stretching of ester).

(7): IR (λ_{max}, cm^{-1}): 3430 (NH streching of amine), 2923 (CH streching of aromatic ring), 1682 (C=O streching of amid), 1574 (C=C streching of quinoid ring), 1492 (C=C streching of benzenoid ring), 1299 (C-N streching of amine), 766 (C-H out of plan of aromatic ring).

^{1}H-NMR spectrums of (1), (6) and (7) are as bellow:

(1): ^{1}H-NMR δ (500 MHz, DMSO-d_6): 5.79-5.81 (NH polymer), 6.74-7.25 (benzeneoid rings), 7.37-7.46 (quinoid rings), 9.33 (the water protons bonded by NH^+ group).

(6): ^{1}H-NMR δ (500 MHz, DMSO-d_6): 6.92 (benzeneoid and quinoid rings), 3.14-3.18 (CH, q), 2.20-2.23 (CH_2,t), 1.2 (CH_3, d).

(7): ^{1}H-NMR δ (500 MHz, DMSO-d_6): 8.09-8.19 (quinoid rings), 6.87 (aromatic rings), 5.5-5.6 (NH polymer), 3.52-3.57 (CH_2 of aminobenzyl), 2.3-2.4 (CH_2 of oxobutanamide).

Comparison between FT-IR and ^{1}H-NMR spectrums of **(1), (6)** and **(7)**, demostrates bonding of alanine and adenine to PANI nanoparticles as dopant.

The elemental analysis of PANI **(1)** is below: ANAL. Calcd for repeat unit $C_{24}H_{18}N_4$: C, 79.56%; H, 4.97%; N, 15.47.Found: C, 82.70%; H, 4.159%; N, 10.94%.

Study on interactions to DNA by Florescence emission

Nitrogen on PANI can have electrostatic interaction with the phosphate backbone of DNA [24]. Further, alanine and adenine branches which exist in (6) and (7) can establish hydrogen bonding with base pairs in DNA pores. As DNA and PANI have no fluorescence emission, changes in the emission can explain the interactions between (6) and (7) with DNA. Fluorescence emission of (1), (6) and (7) were measured in the absence of DNA and in the presence of DNA (Figure 2).

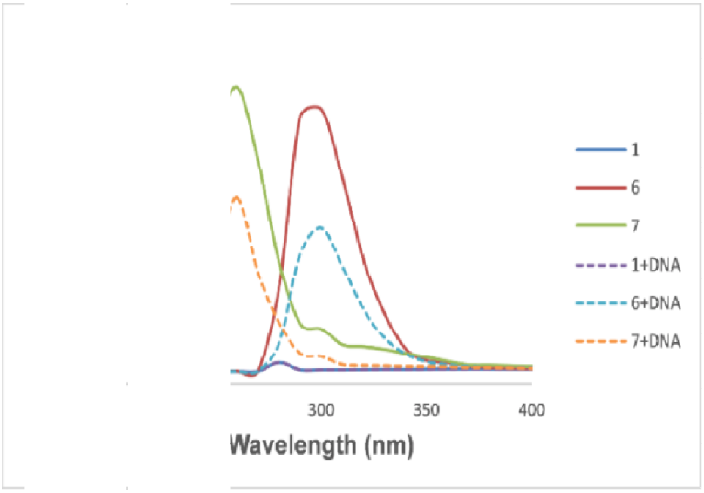


Figure 2. Fluorescence emission of (1), (6) and (7) in the absence and presence of DNA.

As it can be seen, the intensity of fluorescence emission of (**1**) in the presence of DNA is not significantly different, but the interaction of DNA reduces the fluorescence intensity of (**6**) and (**7**), and the maximum emission happens at 298 and 258 nm wavelength, respectively. Due to similarity of (**7**) to DNA and the presence of aromatic rings, the presence of adenine as a nucleobase increased the fluorescence intensity of PANI than amino acid derivative. At compound (**7**), in addition to N chains which bond to the phosphate backbone of DNA with electrostatic interactions, adenine can also have interaction with pair bases in DNA pores through hydrogen bonding. All of these interactions cause formation of polymer-DNA complex that can traverse from lipophilic cell wall.

Conclusions

In this work, a novel and highly efficient route is developed for the synthesis of PANI nanoparticles with small changes in common synthesis methods. The size of particles are 30-50 nm. Then, alanine as an amino acid and adenine as a nucleobase doped to PANI nanoparticles. We have investigated the interaction of these new nanoparticles with oligo phosphates DNA consist 45 Base pairs by fluorescence method. The results showed that nanoparticles of PANI have no fluorescence emission, but the effect of doping with dopants greatly increases the fluorescence emission.

Also, the interaction with DNA and the formation of a polymer-DNA complex reduces this emission intensity. N chains of PANI bond to the phosphate backbone of DNA with electrostatic interactions (such as histones or natural polymers of spermine, spermidine and posterine with DNA). In other hand, amino acids and nucelobases can also have interaction with pair bases in DNA pores through hydrogen bonding (such as the interaction of the leucine zipper proteins with DNA), which causes the polymer to wrap around the DNA. The comparison between (6) and (7) in the presence of DNA shows the fluorescence emission of (7) is lower. It proves that interaction of (7) is more with DNA because of the aromatic and other functional groups on adenine.

Therefore, the structure of alanine and adenine doped to nano PANI has the ability to traverse from lipophilic cell wall, which can be proposed as a polymer for gene transfer.

Acknowledgements

The authors acknowledge the supports of Islamic Azad University South Tehran Branch, Chemistry & Chemical Engineering Research Center of Iran and Sharif University of Technology for the ^{1}H-NMR and SEM measurements.

References

[1] J.C. Moutet, E. Saintl-Aman, F. Tran-Van, P. Angibeaud,J.P. Utille, *Advanced Materials*, 4, 511 (1992).

[2]J. Huang, V.M. Egan,H. Guo,J.Y. Yoon, A.L. Briseno,I.E. Rauda, R.B.Kaner, *Advanced Materials*, 15, 1158 (2003).

[3] M. Schwientek,S. Pleus, C.H.Hamann,*Journal of Electroanalytical Chemistry*, 461,94 (1999).

[4] Y. Okamoto, E.Yashima, *Angewandte Chemie International Edition*, 37, 1020 (1998).

[5] X. Yin,J. Ding, S. Zhang, J. Kong,*Biosensors and Bioelectronics*, 21, 2184 (2006).

[6] J. Huang, S. Virji, B.H. Weiller,R.B. Kaner, *Journal of the American Chemical Society*, 125, 314 (2003).

[7] D. Song, R. Wang, Y. Chen, Sh. Zhang, Ch. Liu, G. Luo,*Reaction Kinetics and Catalysis Letters*, 95, 385 (2008).

[8] A.G. MacDiarmid, A.J. Epstein, *Synthetic Metals*, 65, 103 (1994).

[9] H.D. Tran, I. Norris, J.M. D'Arcy, H. Tsang, Y. Wang, B.R. Mattes, R.B.Kaner,*Macromolecules*, 41, 7405 (2008).

[10] R. Mathew, D. Yang, B.R. Mattes, M.P. Espe, *Macromolecules*, 35, 7575 (2002).

[11] B. Massoumi, O. Badalkhani, H.Gheybi, A.A.Entezami, *Iranian Polymer Journal*, 20,779 (2011).

[12] M.C. Miras, D.F. Acevedo, N. Monge, E. Frontera, C.R. Rivarola, C.A.Barbero, *Open Macromolecules Journal*, 2, 58 (2008).

[13] J. Yue, Z.H. Wang, K.R. Cromack, A.J. Epstein, A.G. MacDiarmid, *Journal of the American Chemical Society*, 113, 2665 (1991).

[14] J. Yue, G. Gordon,A.J. Epstein, *Polymer*, 33, 4410 (1992).

[15] G. Iacomino, G. Picariello, L.D'Agostino, *Biochimicaet Biophysica Acta (BBA)-Molecular Cell Research*,1823, 1745 (2012).

[16] G. Iacomino, G. Picariello, F. Sbrana, A. Di Luccia, R.Raiteri, L.D'Agostino,*Biomacromolecules*, 12, 1178 (2011).

[17] D. Bancroft, L.D. Williams, A. Rich, M.Egli, *Biochemistry*, 33, 1073 (1994).

[18] W.H. Landschulz, P.F. Johnson,S.L. McKnight, *Science*, 240,1759 (1988).

[19] T.E. Ellenberger, C.J. Brandl, K. Struhl, S.C. Harrison,*Cell*, 71, 1223 (1992).

[20] K.T. O'Neil, R.H. Hoess, W.F. DeGrado, *Science*, 249, 774 (1990).

[21] J. Li, Y.Sha,*Molecules*, 13, 1111 (2008).

[22] B.S. Chingakham, K.Veerababurao, K.S. Akshaya,K.P. Bhisma, *European Journal of Organic Chemistry*,2007, 1369 (2007).

[23] C. Hongfeng, N. Christopher, C. Anusuya,L.S. Kirk, *ARKIVOC (xiv)*,1 (2008).

[24] P.P. Sengupta, J.N. Gloria, D.N. Amato, D.L. Patton, B. Murali, A.S. Flynt, *Biomacromolecules*, 12, 3217 (2015).