THESIS ON NATURAL AND EXACT SCIENCES B82

Electrosynthesized Conducting Polymers, Polypyrrole and Poly(3,4-ethylenedioxythiophene), for Molecular Imprinting

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Dissertation was accepted for the defense of the degree of Doctor of Philosophy in Natural and Exact Sciences on March 30, 2009

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Defence of the thesis: May 12, 2009, at 14.00
Lecture hall:
Tallinn University of Technology, Ehitajate tee 5, Tallinn

Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any academic degree.

Anna Menaker

ISSN 1406-4723
ISBN 978-9985-59-900-6

LOODUS- JA TÄPPISTEADUSED B82

Molekulaarselt jäljendatud süsteemid elektrokeemiliselt sünteesitud elektrit juhtivate polümeeride – polüpürrooli ja polü(3,4-etüleendioksütiofeeni) baasil

ANNA MENAKER

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LIST OF PUBLICATIONS

The thesis is based on the following papers referred to in the text by the Roman numerals:

I. Vitali Syritski, Jekaterina Reut, **Anna Menaker**, Róbert E. Gyurcsányi and Andres Öpik, Electrosynthesized molecularly imprinted polypyrrole films for enantioselective recognition of L-aspartic acid, *Electrochimica Acta,* 53(6) (2008) 2729-2736.

II. **Anna Menaker**, Vitali Syritski, Jekaterina Reut, Andres Öpik, Viola Horvath and Róbert E. Gyurcsányi, Electrosynthesized surface-imprinted conducting polymer microrods for selective protein recognition, *Advanced Materials*, 2009, 21, 1–5. DOI: 10.1002/adma.200803597. The work has been judged to be very important and very urgent. It was featured on "**Advances in Advance**" page at www.advmat.de before online publication and on news service "**MaterialsViews.com**" after publication to increase its visibility.

III. Andres Öpik, **Anna Menaker**, Jekaterina Reut and Vitali Syritski, Molecularly imprinted polymers: a new approach for preparation of functional materials, *Proceedings of the Estonian Academy of Sciences*, 58 (1) (2009) 3-11.

In the appendix of the thesis, copies of these papers have been included.

THE AUTHOR'S CONTRIBUTION TO PUBLICATIONS

The contribution by the author to the papers included in the thesis is as follows:

I Carrying out a major part of the experimental work, participation in data processing and in the discussion of the results, minor role in writing.

II Carrying out a major part of the experimental work, participation in data processing and in the discussion of the results, minor role in writing.

III Summation and analysis of the obtained results, major role in writing.

LIST OF ABBREVIATIONS AND SYMBOLS

Av	Avidin
Av-FITC	Fluorescein isothiocyanate labeled avidin
Av-FITC SIP	PEDOT/PSS microrods surface-imprinted with Av-FITC
BSA	Bovine serum albumin
D-Asp	D-aspartic acid
ECPs	Electrically Conducting Polymers
EDOT	3,4-ethylenedioxythiophene
EQCM	Electrochemical Quartz Crystal Microbalance
IC_{50}	The half maximal inhibitory concentration
K_d	The equilibrium dissociation constant
L-Asp	L-aspartic acid
MIP	Molecularly imprinted polymer
NIP	Non- imprinted polymer
oPPy	Overoxidized polypyrrole
oPPy/L-Asp$_{(A)}$	Overoxidized PPy/L-Asp$_{(A)}$
oPPy/L-Asp$_{(B)}$	Overoxidized PPy/L-Asp$_{(B)}$
oPPy/PSS/L-Asp	Overoxidized PPy/PSS/L-Asp
PB	Phosphate buffer solution
PBS	Phosphate buffer saline
PCM	Polycarbonate membrane
PEDOT	Poly(3,4- ethylenedioxythiophene)
PEDOT/PSS	Poly(3,4-ethylenedioxythiophene) doped with PSS
pI	Isoelectric point
PPy	Polypyrrole
PPy/L-Asp	Polypyrrole doped with L-aspartic acid
PPy/L-Asp$_{(A)}$	Polypyrrole doped with L-aspartic acid electrosynthesized from the weakly acidic solution (pH 6)
PPy/L-Asp$_{(B)}$	Polypyrrole doped with L-aspartic acid electrosynthesized from the basic solution (pH 11)
PPy/PSS/L-Asp	Polypyrrole doped with polystyrene sulfonate and L-aspartic acid
PSS	Polystyrene sulfonate
Py	Pyrrole
QCM	Quartz Crystal Microbalance
SEM	Scanning Electron Microscopy
SIP	Surface-Imprinted Polymer

INTRODUCTION

Molecular imprinting is a promising technique for the preparation of synthetic polymers of predetermined specificity. Today, the concept of molecular imprinting has been widely recognized as the most promising methodology for the preparation of different tailor-made materials - Molecularly Imprinted Polymers (MIPs). MIPs have a substantial potential for applications in the areas of analytical separation, catalysis, chemical and biomimetic sensors [1, 2], biotechnology [3], biomedical materials [4], etc.

Since the production of single enantiopure compounds has become increasingly important in the pharmaceutical and agrochemical industries, it is very important to develop tools for efficient chiral separation, as well as analytical methods to be able to control the enantiomeric excess and the optical purity of final products. The molecular imprinting technology offers a unique possibility of obtaining MIPs with enantioselective binding properties for given chiral targets. Such chirally imprinted MIPs have several advantages over conventional chiral selector systems, for example, ease of preparation, low material costs, and flexibility to design various self-supporting formats. The preparation of molecularly imprinted films capable of discriminating between chiral isomers of amino acid is useful and highly efficient with respect to enantioselective separations.

Interest and demand in the preparation imprints to macro-biomolecules, e.g. proteins, nucleic acids, saccharides, or even microorganisms, grow continually in medicine diagnostic, clinical and environmental analysis, and especially in drug delivery systems. At the same time, the practical fabrication of these selective MIP systems remains a challenge and requires specially adapted protocols. The major problem associated with the imprinting of large macromolecules lies in the restricted mobility of them within highly cross-linked polymer networks and the poor efficiency in rebinding. The fabrication of structures with high surface-to-volume ratio having exclusively surface-imprinted binding sites seems to be the most promising way to overcome such difficulties since such sites are more accessible, the mass transfer and the binding kinetics are faster.

The great potentiality of electrically conducting polymers (ECPs) toward a number of applications, such as charge storage devices [5], integrated circuits [6], optoelectronic devices [7], actuators [8], corrosion-protecting coatings [9], and ion-selective electrodes [10], has attracted high attention. Moreover, most ECPs present a number of important advantages for biomedical applications due to their biocompatibility, compatibility with aqueous solutions, ability to entrap and controllably release of biological molecules. These unique characteristics are useful in many fields, such as biosensors [11], tissue engineering [12], neural probes [13], and drug delivery devices [14]. The possibility of highly controllable electrochemical deposition, simplicity of modification and biocompatibility make ECPs a promising material for MIP systems. A number of research papers on the application of molecularly imprinted polypyrrole (PPy) and polyaniline (PANI) as

a recognition matrix for different biological molecules have been published. To our best knowledge, there are no publications on molecular imprinting of poly(3,4-ethylenedioxythiophene) (PEDOT) despite the fact that this polymer is very stable and biocompatible. In general, the area of electrosynthesized ECPs for use in molecular imprinting has had little coverage.

In the present thesis two electrosynthesized ECPs - PPy and PEDOT- were used as a matrix for molecular imprinting. Considering the advantages of enantioselective MIP materials and their usefulness with respect to enantioselective separations, the thin films of overoxidized polypyrrole (oPPy) imprinted with amino acid - L-aspartic acid (L-Asp) - were prepared and their capability to discriminate between L- and D-Asp acid isomers was investigated. Electrochemical Quartz Crystal Microbalance (EQCM) technique was used for both monitoring the selective recognition and the electrochemical modulation of the binding process in the prepared molecularly imprinted PPy films. Taking into account the interest and demand in the preparation imprints to macro-biomolecules, e.g. proteins, the methodology for fabrication of PEDOT microstructures with selective protein-binding sites located on their surface was elaborated. The specific adsorption of avidin (Av-FITC) on the prepared surface-imprinted polymer (SIP) microrods was investigated by fluorescence microscopy.

The work is financially supported by the Estonian Ministry of Education and Research (target financing grant SF0142714s06, base-line financing B612), Estonian Science Foundation (grant G6203), Estonian Doctoral School of Materials Science and Materials Technology (MMTDK).

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the supervisor of my thesis Dr. Vitali Syritski for his excellent guidance, continuous help both in technical and theory issues, fruitful discussions, advice and encouragement during this work. I am also indebted to Vitali Syritski, as my colleague, for giving me the basic skills and necessary knowledge about electrochemistry and electrically conducting polymers.

In addition, I would like to express my deepest gratitude to my co-supervisor Prof. Andres Öpik for proving an excellent opportunity to carry out my doctoral studies at the Chair of Physical Chemistry of Tallinn University of Technology, for financial support, and for his supervision during preparation of my thesis.

My special appreciation belongs to my colleague Dr. Jekaterina Reut who helped me throughout my doctoral study. Due to her unbounded support, assistance, friendship, advice and discussions, I can move ahead to the procedure of defense.

I would like to thank Prof. Enn Mellikov, Head of the Department for providing me the opportunity to work in the Department of Materials Science at Tallinn University of Technology.

I am very grateful to Prof. Róbert E. Gyurcsányi for inviting me to Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, giving an opportunity to carry out the electrochemical experiments and fluorescence microscopy measurements, and also for invaluable contribution to the articles writing process as well as for the pleasant and warm stay in Budapest.

My special thanks belong also to the following associates:

Dr. Viola Horváth and Gyla Jágerszki from the Department of Inorganic and Analytical Chemistry of Budapest University of Technology and Economics for their assistance and help during my stay in their laboratory.

Dr. Olga Volobujeva for the SEM investigations.

I am very grateful to the opponents - Prof. Arkady Karyakin and Dr. Jörg Rappich – for their valuable time and comments.

And finally, I would like to thanks all my colleagues from the Chair of Physical Chemistry, family, friends and, especially, my husband for his understanding and moral support.

Financial support from the Estonian Ministry of Education and Research (target financing grant SF0142714s06, base-line financing B612), Estonian Science Foundation (grant G6203), and Estonian Doctoral School of Materials Science and Materials Technology (MMTDK) are gratefully acknowledged.

Tallinn, May 2009

Anna Menaker

1. LITERATURE REVIEW

1.1. Molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are tailor-made materials capable of selectively rebinding a target molecule, or a group of structurally related molecules based on a combination of recognition mechanisms, such as size, shape, and functionality [15]. The idea of molecular imprinting has gained rapid popularity and has been used successfully in numerous applications, such as analytical separation, catalysis, chemical and biomimetic sensors [1, 2], biotechnology [3], biomedical materials [4], etc. Today, MIPs have been identified as one of the most promising synthetic alternatives of biological origin receptors [16]. In contrast to their biological analogues, major advantages of the MIPs include physical robustness to high pressure and temperature, extreme pH and also inertness to acids, bases and organic solvents [17]. Additionally, low production cost and easy preparation of such materials make them attractive for mass manufacture of MIP-based synthetic receptors. Progress in the development and application of MIPs over recent years is evident from the increasing number of publications and groups working in this area.

The technique of molecular imprinting allows the formation of selective binding sites by co-polymerization of functional monomers and cross-linkers in the presence of a specific target molecule (Fig. 1.1).

Polymerizable functional groups are usually bound by covalent or non-covalent interactions to the template molecule. This procedure makes tailor-made macroporous polymers with a permanent pore structure and a high inner surface area. Under special conditions the template molecules can be removed from the structure in high percentage, leaving matrix cavities in the polymer, ready for selective binding of the target molecules. The selectivity depends both on the orientation of the functional groups inside the cavities and the shape of the cavities.

1.1.1. Historical background

The first report on molecular imprinting goes back to the early 1930s when a Russian scientist M.V. Polyakov prepared a number of imprinted silica gels with an excellent binding capacity [18]. A very similar methodology was used in the experiments of Dickey in the 1950s [19] who was inspired to create affinity for dye molecules in the silica gel according to Linus Pauling's theory of antibodies

Figure 1.1. Schematic representation of the molecular imprinting process

formation [20]. Among other scientists who reported about silica imprinting were Curti [21], Klabunovskii [22], Patrikeev [23], and other research groups, but the number of publications in the area remained low. However, in 1972 Wulff and Klotz, independent of each other, introduced a new synthetic methodology to prepare specific binding sites in cross-linked polymers. Thus, the covalent imprinting in vinyl polymers [24] and in cross-linked polyethyleneimine [25] was reported. The second break through in organic polymer imprinting occurred in 1981 when Mosbach and co-workers presented an organic MIP using non-covalent interactions only [26]. The principles of covalent and non-covalent imprinting are discussed below.

Covalent imprinting

Covalent imprinting is performed by use of templates which are covalently bound to functional monomers. In this case there is a stoichiometric relation between the template and the imprinted binding site. The advantage of this approach is that the functional groups are only associated with the template site, however, only a limited number of compounds (alcohols, ketones, amines, and carboxyl acids) can be imprinted by this method. Also, covalent systems often show kinetically controlled binding, because the binding process involves the formation of stable covalent bonds, and sometimes it may not be suitable for separation methods [27].

Non-covalent imprinting

The non-covalent imprinting is based on the copolymerization of the non-covalently bonded template – functional monomer complexes established in the prepolymerization solution with an excess of a cross-linker. Due to the large number of functional monomers commercially available, ease and simplicity of preparation, and close affinity to the molecular recognition mechanisms of natural receptors have attracted widespread use of this approach. Typical interaction types that have been exploited are hydrogen bonding, ionic interactions, hydrophobic interactions, π-π interactions, and Van der Waals forces. The greater the variety of interactions formed between the template and the functional monomer, the higher the degree of selectivity of the imprinting site. Since the non-covalent interactions are strongly dependent on the polarity of the solvent, the imprinting is usually made in organic solvents, such as chloroform or toluene [3]. A major disadvantage of non-covalent systems is the unavoidable heterogeneity of the binding sites obtained arising from the multitude of bonds/interactions formed between the template and the functional monomers. As a result, these systems are generally not strong, since an excess of the functional monomer is necessary in order to complete the template-functional monomer complex and to maintain its stability during the polymerization [27].

1.1.2. MIPs preparation methods

The classical technique for the preparation of MIPs is bulk polymerization. The bulk or mass polymerization is a process for producing of a polymer in the form of a monolithic block by simply mixing a functional monomer and cross-linking monomers arranged around the imprint molecule, followed by grinding and extraction of the imprint species [28]. Following the grinding procedure, an irregular shape and polydisperse particles have been obtained. This process is time consuming and wasteful (40% lost) [29]. Moreover, most MIPs are hydrophobic in nature that results in their poor recognition in aqueous systems. The low aqueous compatibility of MIPs is the greatest problem in their application for biological recognition like bioassays. Therefore, an increasing number of polymer techniques have been developed, such as suspension polymerization [30], emulsion polymerization [31], precipitation polymerization [32] and two-step polymerization [33]. Besides the general polymerization techniques, other alternative approaches, including the preparation of thin films [34], sol-gel process [35] and surface imprinting [36], are also employed to produce MIPs. These techniques were successfully applied for creating the MIPs against small molecules, such as pesticides, amino acids, steroids, peptides, and drugs [3]. Currently, molecular imprinting of those molecules as imprint templates is a straightforward procedure and extensively described.

However, interest and demand in the preparation imprints to macro-biomolecules, e.g. proteins, nucleic acids, saccharides, or even microorganisms, grow continually in medicine diagnostic, clinical and environmental analysis, and especially in drug delivery systems. At the same time, the practical fabrication of these selective MIP systems remains a challenge and requires specially adapted protocols. The main problem associated with limited mobility of these molecules in the cross-linked polymer network and the poor efficiency in binding kinetics. In addition, the structural complexity and large size of macromolecules lead to non-specific and heterogeneous binding sites that in turn might lead to poor selectivity. To overcome such difficulties, one of the successful decisions is producing polymers with imprinted binding sites located at or close to the surface of the MIPs, enabling easy access to the target protein molecules. Early attempts of proteins imprinting on the surface of porous silica particles [37], on thin film of acrylic polymer [28] or in acrylamide gels [38] were sufficiently complicated and required additional research. The surface-imprinted polymers (SIPs) have many advantages: the sites are more accessible, mass transfer is faster, and hence the binding capacity is stronger. The surface imprinting can be accomplished by various protocols based on the oriented immobilization of the template molecules on sacrificial materials [39-41]. Recently, Li and co-workers reported a protocol for creating SIP nanowires by immobilizing of target molecules within the pores of nanoporous alumina membrane [42]. These nanowires were prepared by chemical polymerization of the functional monomer (acrylamide) and cross-linking monomer in the nanopores of alumina membranes preliminarily modified by proteins. However, the main disadvantage of this elegant method is the weak

adsorption of the protein on alumina membrane that requires a multistep treatment of the alumina before attaching the proteins and also it is difficult to control the polymerization.

One of the very promising approaches for MIP fabrication is the use of electrosynthesized polymers as a matrix for molecular imprinting [43]. The electropolymerization process provides a superior control of the thickness, morphology and spatial localization of polymeric films. These features enable creating a direct communication between the coating and the surface of the transducer in a very simple way. Thus, the electrosynthetic approach could be very helpful both to improve the molecular imprinting polymerization procedure itself and to extend applications of MIPs as sensing elements for chemical sensors with various transduction mechanisms.

1.2. Electrically conducting polymers

A new class of polymers known as electrically conducting polymers (ECPs) was first introduced in the mid-1970s. In 2000 Nobel Prize in Chemistry was awarded for the discovery and development of ECPs. Fig. 1.2 shows the structures of four most widely studied ECPs in their neutral forms.

ECPs are organic materials that have both electrical and optical properties similar to those of metals and inorganic semiconductors, but which also exhibit the attractive properties associated with conventional polymers, such as ease of synthesis and flexibility in processing [44]. A common feature of the ECPs structure is extended π-conjugated backbone, but this alone is not sufficient to produce appreciable conductivity. The increase in the electrical conductivity of organic polymers (from ~10^{-5} to ~10^{3} S/cm) can be obtained by the doping process, i.e. incorporation of some concentration of dopants (counter-ions) from the electrolyte solution into the matrix of an initial conjugated polymer. The doping process can be achieved either by chemical or electrochemical oxidation (*p*-doping) or reduction (*n*-doping) of the polymer:

p-doping process: $$[M]_x + (xy)(A)^- \rightarrow \left[M^{y+}(A)_y^-\right]_x + (xy)e^-$$

n-doping process: $$[M]_x + (xy)(A)^+ + (xy)e^- \rightarrow \left[M^{y-}(A)_y^+\right]_x$$

where M – monomer, x – degree of polymerization, y – doping level, which is defined as the number of unit charges per the monomer ring, and A^-/A^+ - dopant anion/cation. In the course of this process the neutral polymer chain becomes either positively charged (oxidative) or negatively charged (reductive). The conductive form of the polymer contains dopants, which serve to maintain charge neutrality. Doping is a reversible process; therefore in the case of dedoping (ejection of dopant ions) a polymer with an initial backbone can be easily produced. By controllably adjusting the doping level, conductivity anywhere from insulating or semiconducting (non-doped) to highly conducting (fully doped) form of the polymer can also be changed.

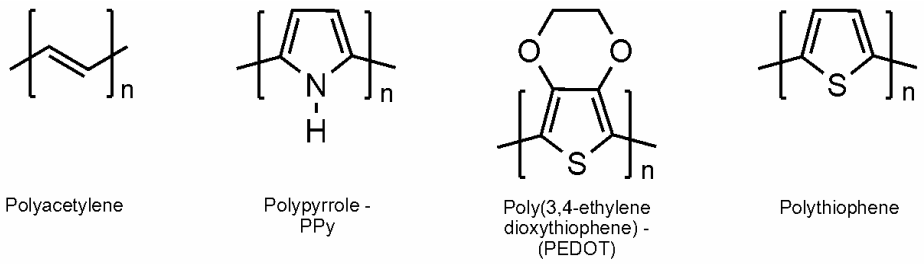

Figure 1.2. Structures of electrically conducting polymers

In this thesis electrically conducting polymers, polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) in particular, were used as matrixes for molecular imprinting.

1.2.1. Polypyrrole

Among various types of ECPs, PPy has been studied in more detail since it can easily be synthesized by a chemical or electrochemical polymerization, has many attractive features, such as excellent conductivity and stability on various substrate materials, even in a neutral pH region. In 1979 Diaz et al. reported on the electrochemically synthesized PPy as a conductive polymer in the form of films [45]. Thanks to the solubility of pyrrole monomers, PPy may be synthesized in both aqueous and organic solvents. The specific properties of PPy are influenced by the dopant and polymerization technique used, as well as by a host of other variables (solvent, pH, substrate and temperature during synthesis). Therefore, careful control over the synthesis process is required to consistently produce films or coatings with desired characteristics. The large variety of ways to synthesize and modify the characteristics of PPy makes this material attractive for a wide range of applications (electrodes for rechargeable batteries and supercapacitor, sensors (gas, humidity), biosensors, corrosion protecting materials, electrochemical actuators, electrochromoc devices, and membranes).

Thanks to its non-toxicity and biocompatibility, PPy is an extremely versatile and promising material for biomedical applications (tissue engineering, neural probe, biosensors, and drug delivery). Thus, glucose biosensor [46] and cholesterol biosensor [47] were fabricated by entrapment of glucose oxidase or cholesterol oxidase, respectively within electropolymerized PPy films. PPy has also been used as immunosensors incorporating anti-human serum albumin [48] and antibodies [49]. According to literature, PPy is a good candidate as the material of micropump drug delivery systems [50]. The controlled release of adenosine 5-triphosphate (ATP) [51], glutamate and dopamine [52, 53] using PPy membranes has been reported.

PPy undergoes irreversible overoxidation at potential values higher than those for its reversible doping and undoping. This process has often been regarded as an undesirable degradation process, which leads to the loss of conductivity and dedoping. Despite these disadvantages, overoxidized polypyrrole (oPPy) has been

used in some electroanalytical applications. The group of Brajter-Toth has studied oPPy films as a model for the design of permeselective electrodes [54]. It was found that the thin oPPy films exhibit permselectivity against anions and excellent selectivity toward cations. Later, this group reported on the application of ultramicroelectrodes coated with oPPy thin films for the detection of dopamine in the presence of ascorbic acid suggesting that such permselective electrodes may be useful for in vivo measurements to minimize the interference of biological anions [55].

1.2.2. Poly(3,4-ethylenedioxythiophene)

During the second half of the 1980s, scientists at the Bayer AG research laboratory developed a new electrically conducting polymer – poly(3,4-ethylenedioxythiophene) (PEDOT). PEDOT has been explored as an alternative to PPy because it is much more thermally stable and resistant to oxidation. In addition to high conductivity (ca. 400-600 S cm^{-1}), PEDOT has a high degree of optical transparency to visible light at its doping state. Moreover, PEDOT-coated electrodes have higher charge capacity than that of electrodes coated with PPy [56]. Both chemical and electrochemical methods have been applied to the preparation of PEDOT films. Unfortunately, the low solubility (ca. 2.1 g/L) of EDOT monomers in water and the high oxidation potential make the electropolymerization process in an aqueous solution difficult. This drawback can be improved by addition of water-dispersible polyelectrolyte, poly(styrenesulfonate) (PSS), used as a dopant during the polymerization. As colloidal dispersion, PEDOT/PSS is stable in water and convenient to form a thin film with many methods [57]. This PEDOT/PSS complex is commercially available as an aqueous dispersion under the trade name CLEVIOS™ (older name BaytronP) from H.C. Starck GmbH (Germany) [58]. This material has been utilized as an antistatic coating for photographic films, electronics packaging, displays and video panels, LCD polarizer films, and textiles [59]. Other very practically useful application areas for PEDOT are transparent conductor in electroluminescent devices, cathodes in capacitors, photovoltaics [60, 61], hole transport layers for light-emitting diodes [62], organic thin film transistors [63], and sensors [64].

Due to its aqueous compatibility and biocompatibility, PEDOT has been suggested as a promising candidate for biosensing purposes and biomedical devices. Thus, considering the growing interest in the development of implantable electrodes, the group of Richardson-Burns made a significant contribution to the study of interactions between cultured neural cells and PEDOT [65]. Thanks to the long-term stability, PEDOT can be used as an alternative for PPy as the electroactive matrix for glucose biosensors [66].

1.2.3. Electrically conducting polymer as a matrix for molecular imprinting

The possibility of highly controllable electrochemical deposition, simplicity of modification and biocompatibility make ECPs a promising material for molecular recognition systems. The first attempts to imprint electrosynthesized PPy by charged and neutral species were reported by Spurlock et al. [67].They prepared and characterized the ultrathin overoxidized polypyrrole (oPPy) films templated with adenosine, inosine and adenosine 5'-triphosphate (ATP) to improve the selectivity and sensitivity of a glassy carbon electrode in the determination of adenosine. However, only poor recognition ability of the prepared oPPy films was found in this report. A more straightforward path to synthesize a molecularly imprinted PPy film through overoxidation has been proposed by the group of Deore [68]. They reported on oPPy films templated with L-glutamate, which exhibited high enantioselectivity towards L-glutamic acid over D-glutamic acid [69]. The results showed that the complementary cavities within electropolymerized PPy films can be created with simultaneous expulsion of anionic template molecules through an overoxidation/dedoping process. This is considered as an advantage of molecularly imprinted oPPy over conventionally prepared MIPs because in this case the template extraction procedure and undesired leakage effects are avoided. Moreover, the authors pointed out the importance of the overoxidation procedure, which hardens the PPy texture to allow the templated cavity to memorize the molecular details after dedoping. There are a number of publications on the use of molecularly imprinted oPPy as a sensing material for bile acids, structural isomers of naphtalenesulfonate [70], and as a synthetic receptor for the direct detection of bovine leukemia virus glycoproteins [71]. The preparation of a biomimetic sensor through the combination of electrosynthesized PPy film imprinted with caffeine molecules and the piezoelectric quartz transducer has been reported [72].

The other molecularly imprinted conducting polymer, polyaniline (PANI), was investigated as a sensing material for atropine [73], saccharide [74]. At the same time, to our knowledge, there are no reports presently available on the molecular imprinting of PEDOT.

Thus, the use of ECPs in molecular imprinting seems to have been little studied so far. In this thesis PPy and PEDOT were investigated as matrices for molecular imprinting.

1.3. Target molecules

This thesis aims to imprint biomolecules, amino acid and protein in particular, on the electrosynthesized ECPs. These molecules are briefly described in this section.

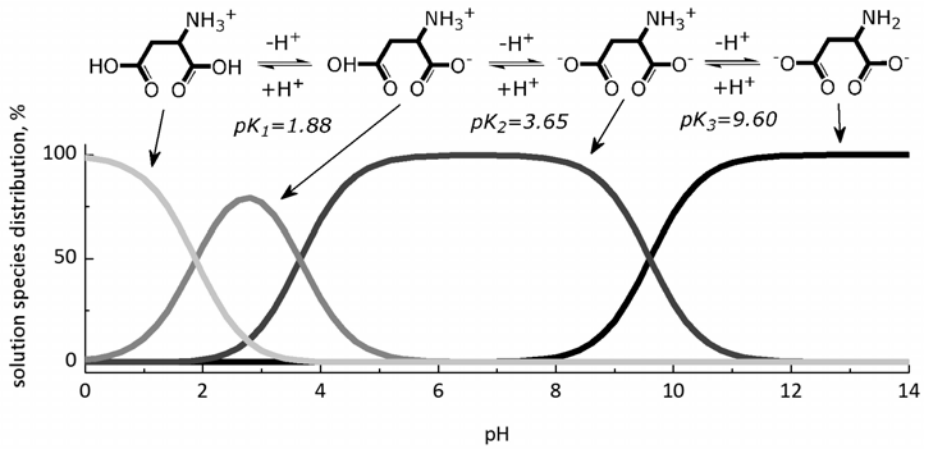

Figure 1.3. Ionization of aspartic acid as a function of pH

1.3.1. Amino acids

Amino acid is a small biomolecule that consists of a basic amino group (-NH2), an acidic carboxyl group (-COOH), and an organic R group (or side chain) that is unique to each amino acid. The term amino acid is short for "α-amino carboxylic acid". Each molecule contains a central carbon atom called the α-carbon, to which four different groups are attached. As a result, there are two asymmetrical isomer forms, known as L- and D-enantiomers. With the exception of glycine, all other amino acids exist in either L or D optically active forms. Nevertheless, most of the amino acids occurring in nature are of the L-type. Hence, proteins are always composed of L-amino acids, whereas D-amino acids are present in some microorganisms, particularly in the cell walls of bacteria and in several antibiotics. However, the optically pure enantiomers for a long period of time can be spontaneously transformed into a racemic mixture which leads to the loss of optical activity. It is known that enantiomers differ in their therapeutical effects, and thus the production of single enantiopure compounds has become increasingly important in the pharmaceutical and agrochemical industries [75]. Therefore, it is very important to develop tools for efficient chiral separation, as well as analytical methods to be able to control the enantiomeric excess and the optical purity of final products [76].

Since amino acids have both an amine and a carboxylic groups and are therefore both acid and base at the same time. Depending upon the solution pH, amino acids can be cations, anions, and zwitterions (a zwitterion carries an equal number of positively and negatively charged groups). Each amino acid has an isoelectric point (pI) numerically equal to the pH at which the molecule has a net zero charge. Isoelectric point for the aspartic acid is 2.77, and hence on average the molecules carry a net positive charge at lower pH and vice versa a net negative charge at a higher pH. The ionization states of aspartic acid are presented in Fig. 1.3.

In this work L-aspartic acid was used as a template/target molecule for molecular imprinting of electrosynthesized conducting polymer – PPy. This choice was motivated by the importance of L-aspartic acid in human metabolism, such as removing toxins from the bloodstream and the use of L-aspartic acid racemization rations in human serum for age estimation [77]. One of the significant aspects is that only glutamic and aspartic acids can be used as dopants, because the other amino acids do not carry a negative charge in neutral and acidic pH levels, which is suitable for pyrrole polymerization [78].

1.3.2. Proteins

Proteins are macromolecules consisting of one or more polypeptides. Each polypeptide consists of a chain of amino acids linked together by peptide bonds. The amino acid sequence is determined by the gene coding for that specific polypeptide. The structural organization of protein can be divided into four different levels, such as primary, secondary, tertiary, and quaternary (Fig. 1.4). The primary structure is defined as the linear sequence of amino acids in polypeptide chain bound to each other by covalent linkages. The secondary structure refers to certain regular geometric figures, such as α-helix and β-sheet, of the chain maintained by hydrogen bonds. Tertiary structure describes the three-dimensional structure of the polypeptide units, which are supported by several forces: hydrogen bonding, hydrophobic interactions, electrostatic interactions, and van der Waals forces. The quaternary structure is the association of multiple polypeptide chains that are held by the same non-covalent forces that stabilize the tertiary structures of proteins. The physicochemical properties of a protein are identical to the amino acids, since amino acids are monomer units of proteins. Proteins usually are amphoteric molecules, i.e. act as an acid or a base depending on the pH environment and the ratio of acidic and basic amino acids.

Proteins are an important class of biological molecules, which have many functions: they are used as structural (connective tissue and hair - collagen, keratin), transport (oxygen supply - hemoglobin), storage (ferritin), contractile (proteins are the major component of muscles), and protective (antibodies) molecules; they are used as enzymatic catalysts, they serve as a mechanical support for skin and bones, they code and transcribe genetic information, they control growth (hormones) and perform many others functions.

Protein selective adsorption on solid surface is a very important and active area of research due to its potential applications from fundamental studies in cell biology to the development of various “biochip” platforms. Exploitation of biological-origin receptors for this purpose despite their excellent selectivity/specificity does not always fulfill the expectations, due to the fragile nature of these molecules. Therefore, implementing synthetic analogs with improved stability and means for cost-effective, rapid fabrication is expected to have great impact on technologies based on molecular recognition.

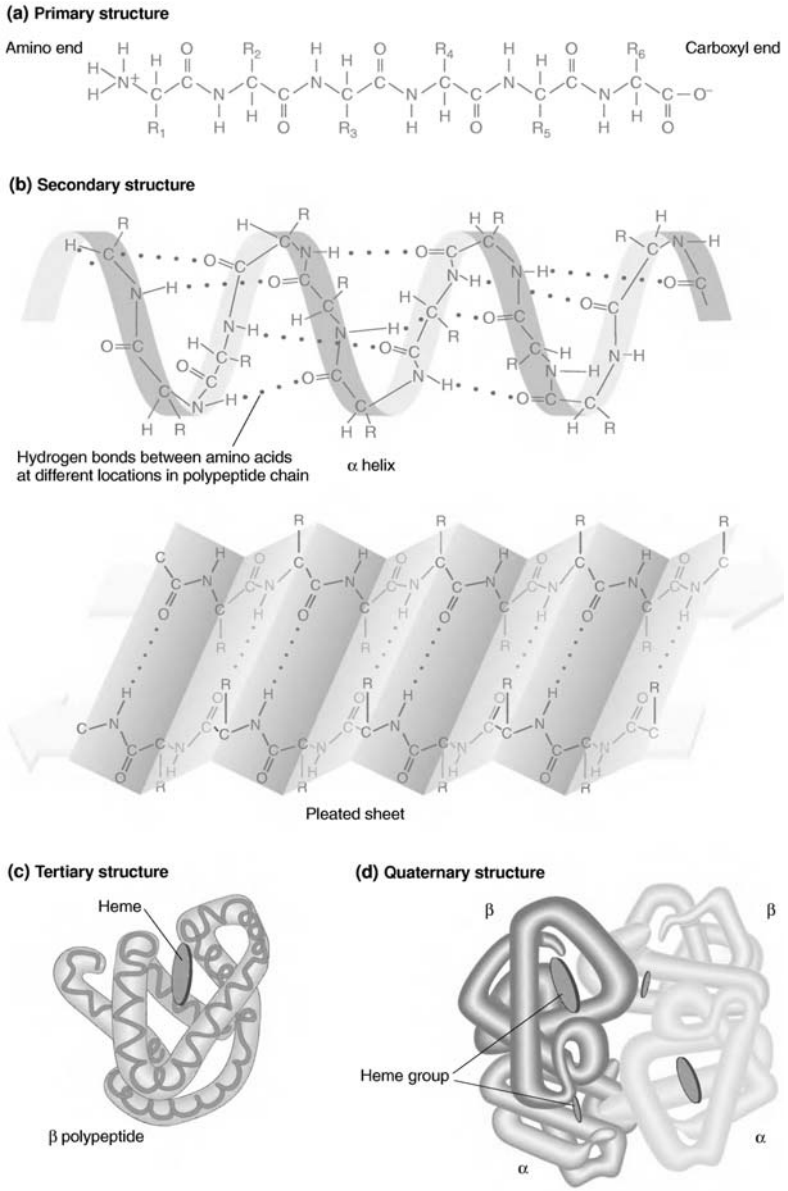

Figure 1.4. Levels of structure in protein (Image from [79])

In this work avidin was utilized as a template protein molecule for the creation of surface imprinting PEDOT microrods. Avidin is a tetrameric glycoprotein present in hen egg white and tissues of birds, reptiles and amphibians. It consists from four identical subunits with a mass of 67 kDa, each subunit consisting of 128 amino acids and binding one molecule of biotin (vitamin B7 and H). The protein is

very soluble in water, up to 20 mg/ml, and is extremely stable over a wide range of pH and temperature [80]. Because of the basic pI of ~ 10, avidin has relatively high non-specific adsorption. Due to its high affinity (Kd = 10^{-15} M at neutral pH 4), specificity for biotin, as well as the stability of the avidin-biotin complex, avidin has been developed in a variety of applications, such as immunoassays and drug delivery [81], and diagnostic tests [82].

1.4. Investigation methods

1.4.1. Quartz Crystal Microbalance

The Quartz Crystal Microbalance (QCM) is a piezoelectric transducer capable of extremely sensitive mass measurements (nanogram level) both in air and liquid. QCM consists of a thin disk-shaped quartz crystal with electrode metallic layers on both sides (Fig. 1.5a). The electrodes are connected to an oscillation circuit board and an alternating high frequency electrical field is applied across the plane of the quartz crystal inducing its vibration in a mechanically resonant shear mode (Fig. 1.5b). In this oscillation circuit board the quartz crystal is the frequency determining element and the resonance frequency (f) and/or the frequency change (Δf) are measured. The mass sensitivity arises from a dependence of the resonance frequency change on the total mass of the crystal, its electrodes, and any materials present on the electrode surface. In 1959 G. Sauerbrey described this phenomenon by an equation (the Sauerbrey equation) where the resonant frequency change is linearly proportional to the mass load at the quartz crystal electrode sensing surface [83]:

$$\Delta f = -\frac{f_0^2 \Delta m}{N\rho} = C_f \cdot \Delta m \tag{1.4}$$

where f_0 is the fundamental frequency of the crystal (Hz), Δm is the change in mass per unit area ($g \cdot cm^{-2}$), N is the frequency constant for quartz (167 kHz·cm), and ρ is the density of quartz (2.65 g cm^{-3}). Thus, for a 5 MHz quartz resonator operating in its fundamental mode, the sensitivity factor C_f is –56.6 $Hz \cdot \mu g^{-1} \cdot cm^2$.
However, it must be kept in mind that the Sauerbrey equation is only strictly applicable for thin, uniform, and rigid films, otherwise viscoelastic changes will also contribute to the frequency shift, leading to an erroneous interpretation of the mass. Nevertheless, even if the material is not entirely rigid, the crystals modified with very thin layers usually follow the Sauerbrey equation.

The QCM can be combined with other surface-analytical instruments. The electrochemical QCM (EQCM) is particularly advanced. EQCM has been used in many types of electrochemical studies, such as underpotential deposition of metals [84], dissolution studies [85] and monitoring of interfacial processes at electrode surfaces [86]. Moreover, QCM offers a large range of applications in the field of biochemical analysis. These applications include the study of DNA immobilization and subsequent hybridization [87]; adsorption of proteins [88]; online detection of antigen-antibody reactions [89], etc. Also, QCM technique has become very

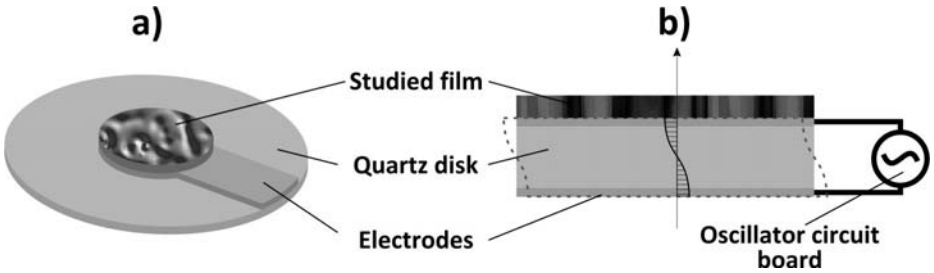

Figure 1.5. a) disk shaped QCM sensor with electrodes, b) schematic representation of vibration for a QCM plate vibrating in a mechanically resonant thickness shear mode

popular in combination with MIPs [43, 69]. Analyte accumulation in the MIP leads to a mass change, which can be easily quantified by QCM.

The main advantage of the QCM in MIP applications is that it is label free, i.e. the sample flowing over the immobilized surface layer does not need to be modified or labeled in any way. EQCM provides additionally excellent in-situ mass monitoring during the electrochemical modulation of a MIP film. In this doctoral thesis EQCM technique was used for the electrosynthesis, overoxidation of L-Asp imprinted PPy films as well as for monitoring the selective recognition process.

1.4.2. Fluorescence microscopy

The technique of fluorescence microscopy has become an essential tool in biology and biomedical sciences, as well as in materials science. This extremely sensitive method is based on the phenomenon that certain materials when irradiated with the light of a specific wavelength emit energy detectable as visible light. Therefore, the biological molecules that are not fluorescing by their nature should be labeled with fluorescing probes (fluorophores) in order to be identified by fluorescence microscopy with a high degree of specificity. The basic function of a fluorescence microscope is to irradiate the specimen with a desired and specific band of wavelengths, and then to separate the much weaker emitted fluorescence from the excitation light. A schematic diagram of the epifluorescence microscope is illustrated in Fig. 1.6. The light source sends full-spectrum light to the excitation filter where selection of the desired band and blockage of unwanted wavelength occurs. Then, the selected wavelengths reach the dichroic beam-splitting mirror, which is a specialized interference filter that efficiently reflects shorter wavelength light and efficiently passes longer wavelength light. The dichroic mirror is tilted at a 45-degree angle with respect to the incoming excitation light and reflects this illumination at a 90-degree angle directly through the objective optical system and onto the specimen. Before the emitted fluorescence can reach the eyepiece or detector, it must first pass through the emission filter. This filter blocks any residual excitation light and passes the desired longer emission wavelengths. All these filters are incorporated into a fluorescence "cube" that selectively illuminates the specimen with wavelengths that excite a particular fluorophore. Thus, for

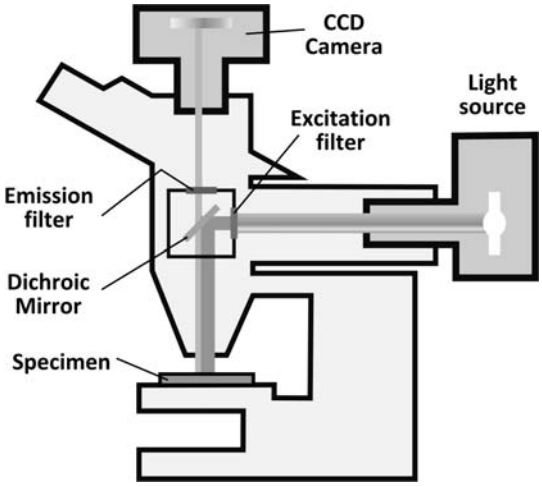

Figure 1.6. Schematic diagram of fluorescence microscopy

example, the excitation of fluorescein isothiocyanate (FITC) with a light wavelength of 492 nm induces a light emission maximum of 517 nm (green light).

Successful applications of fluorescence microscopy to the quantitative and qualitative study of the fluorescently labeled protein adsorption on different substrates were reported in [90, 91]. In the present work, the fluorescence microscopy was applied to investigate quantitatively the specific adsorption of Av-FITC on the prepared Av-FITC SIP microrods.

1.5. Summary of the literature review and objectives of the study

Molecular imprinting is a versatile technique providing functional materials able to recognize biological and chemical agents. MIPs have been successfully applied in separation science, catalysis, and chemical and biochemical sensing where they closely compete with natural receptors in term of sensitivity and selectivity. The better understanding of the imprinting processes and conditions driving the recognition of molecules in these networks have thus made MIPs an attractive, cheap and robust alternative for the detection of small as well as large molecules. However, the challenges remain in the imprinting of macromolecules like proteins, and also in the spatially controlled integration of MIPs onto transducer surfaces for sensing application.

The major problem associated with the imprinting of large macromolecules lies in the restricted mobility of them within highly cross-linked polymer networks and the poor efficiency in rebinding. The fabrication of structures with high surface-to-volume ratio having exclusively surface-imprinted binding sites seems to be the most promising way to overcome such difficulties since such sites are more accessible, mass transfer and binding kinetics are faster.

The electrosynthesis approach in MIPs fabrication allows for creating a direct communication between the polymer and the surface of the transducer in a very simple way, provided the latter is conductive. In the same direction, miniaturization, one of the major goals of chemical sensor technology, could also be easily realized. Nevertheless, there are very few reports on the electrosynthesized MIP materials.

Due to its biocompatibility and ease of electrochemical synthesis, ECPs seem to be very promising as matrices for molecular imprinting. A number of research papers on the application of molecularly imprinted PPy, oPPy, PANI as recognition matrices for different biological molecules have been published. However, to our knowledge, there are no reports on the molecular imprinting of PEDOT.

The preparation of molecularly imprinted oPPy films capable of discriminating between chiral isomers of the amino acid is useful and highly efficient with respect to enantioselective separations.

The main advantage of QCM applications in the field of biochemical analysis is that it is label free, i.e. the sample flowing over the immobilized surface layer does not need to be modified or labeled in any way. The attractive features of the QCM method, such as the possibility *in-situ* monitoring of the electrochemical process and also a superior control of the mass changes (nanogram level) make it very popular in the studies of molecular recognition events.

The fluorescence microscopy technique is an essential tool in materials science and biology, since it helps to reveal the presence of a given type of molecules on the substrate surface or in living cells. This method can be successfully applied to study quantitatively and qualitatively the fluorescently labeled protein adsorption on different substrates.

Based on the abovementioned conclusions the objectives of the present doctoral thesis were as follows:

- Electrochemical preparation of PPy thin films molecularly imprinted with L-aspartic acid. A study of their individual selectivity for L- and D-aspartic acid isomers. The application of the EQCM technique for both monitoring the selective recognition and the electrochemical modulation of the binding process in the prepared molecularly imprinted PPy films.
- Electrochemical fabrication of the PEDOT microstructures surface-imprinted with Av-FITC. Investigation of the specific adsorption of Av-FITC on the prepared microrods. Evaluation of the feasibility and selectivity of detecting avidin by surface-imprinted PEDOT microstructures.

2. EXPERIMENTAL PART

2.1. The enantioselective molecularly imprinted oPPy thin films

2.1.1. Preparation of oPPy/L-Asp thin films

The thin films of PPy were electropolymerized in the presence of L-Asp as template molecules and subsequently overoxidized to create shape complementary cavities.

The electropolymerization was done at constant current (i=0.01 mA/cm^2) in different aqueous electrolyte compositions: (A) 0.2 M Py and 0.5 M L-Asp (pH 6); (B) 0.2 M Py, 0.5 M L-Asp, and 0.01 M NaOH (pH 11); (C) 0.2 M Py, 0.5 M L-Asp, and 0.01 M NaPSS (pH 6). For the formation of complementary cavities the prepared PPy/L-Asp films were overoxidized in the PB (pH 7) by applying constant current (i=0.025 mA/cm^2) until the potential reached the value of 1.2 V. The detailed description procedure was given in the article [I].

2.1.2. EQCM studies

The electrosynthesis, overoxidation and characterization of L-Asp imprinted PPy films were performed by using the EQCM technique (Fig. 2.1). The system consisted of QCM (Stanford Research Systems, Inc) connected to the potentiostat/galvanostat (Reference 600^{TM} Gamry Instruments, Inc.) and a high performance frequency counter (PM 6680B Fluke). All components in the EQCM system were controlled by software written in LabView^{TM} programming language.

The measurements were carried out in a custom-made Teflon electrochemical cell with the QCM sensor holder attached from a side. The disk-shaped Ti/Au electrode of the 5 MHz 1 inch in diameter QCM sensor (Stanford Research Systems, Inc.) was used as a working electrode (Fig. 2.2) while a rectangular shaped platinum plate (4×1.5 cm) was used as the auxiliary electrode and Ag/AgCl/3M KCl) as the reference electrode. Before the deposition of the films, the electrode surface of the quartz crystal was cleaned with hot piranha solution consisting of 30% H_2O_2 and concentrated H_2SO_4 in 1:3 ratio. The deposition of the films was carried out until the resonant frequency of the QCM sensor dropped with 680 Hz, resulting in the film thicknesses of approximately 80 nm.

The enantioselectivity of overoxidized PPy/L-Asp films (oPPy/L-Asp) was determined in KCl–HCl aqueous solutions (pH 1.6) containing 10mM L-Asp (or D-Asp) under stationary conditions with externally applied potential control. The experiments were carried out in a 25 mL volume EQCM cell by applying negative potential (−0.4 V) to the working electrode. The frequency response was monitored in a buffer solution until a constant base line signal was reached, followed by the injection of the sample containing one of the aspartic acid enantiomers. The frequency change was monitored until the steady-state value was reached.

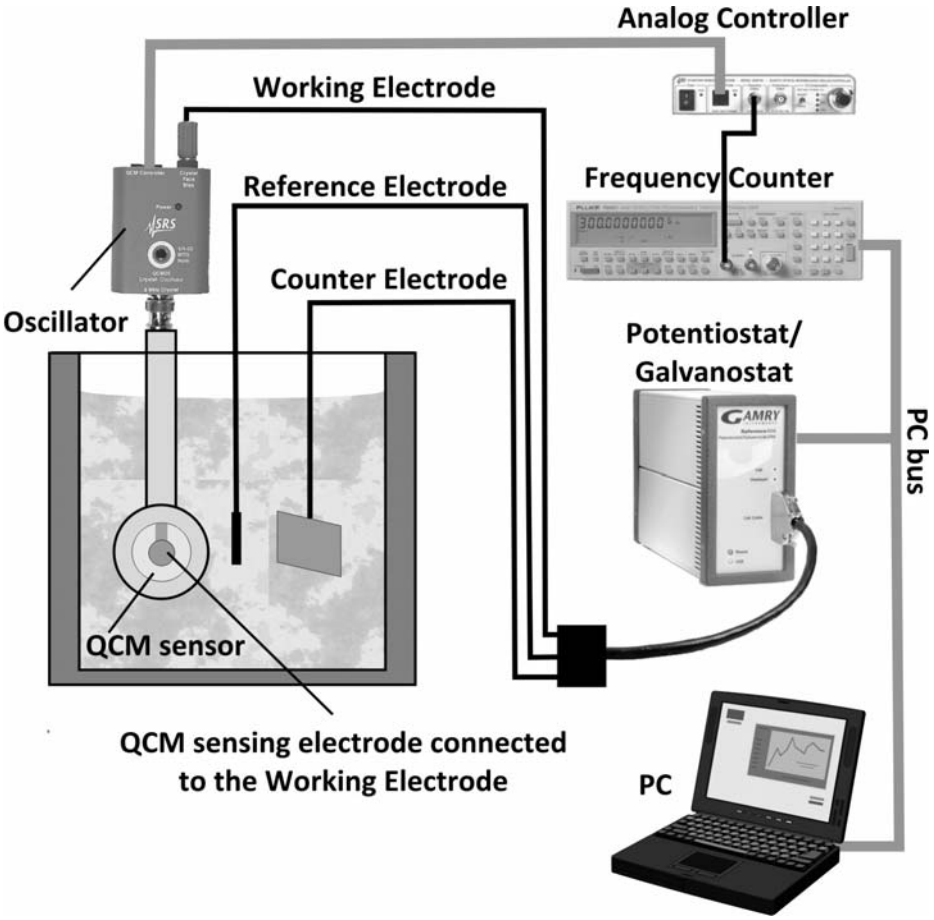

Figure 2.1. Simplified diagram of the electrochemical setup used for EQCM experiments

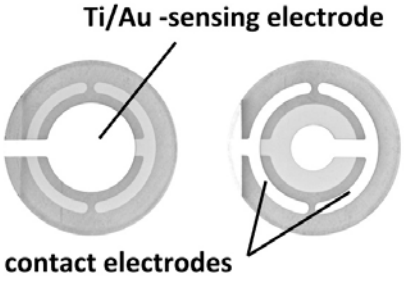

Figure 2.2 Standard 1 inch QCM sensor crystal with Ti/Au as electrode material (Stanford Research System Inc.)

2.2. Surface-imprinted PEDOT/PSS microstructures for selective protein recognition

2.2.1. Fabrication of the surface-imprinted PEDOT/PSS microrods

A template synthesis method was used to generate the surface-imprinted polymeric (SIP) microrods (Fig. 2.3). Track-etched polycarbonate membranes (PCMs) (Whatman Inc.) with 8 μm diameter cylindrically shaped pores were used as sacrificial microreactors for confined electrochemical growth of the PEDOT/PSS. The PCM was subjected to ultrasonic agitation for 3 min and it was incubated for 30 min in the target protein solution (1 mg mL^{-1} avidin-FITC). After rinsing with PBST-20X the protein modified PCM was tightened onto the working electrode (a 2.0 mm diameter gold disc, CH Instruments, Inc. USA) by using the special Teflon made ring-shaped cap. The electrode was placed into a deoxygenated aqueous solution of EDOT (0.01 M) and NaPSS (0.025 M) in a 10 mL volume conical glass vial accommodating additionally a Pt plate counter electrode and a Ag/AgCl/3M KCl reference electrode. The electrochemical

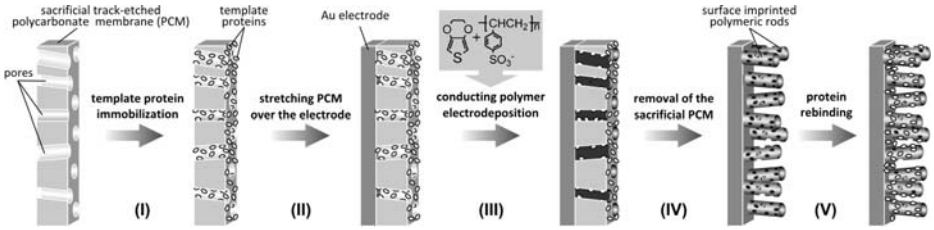

Figure 2.3. Schematics of the surface-imprinting strategy for fabrication of molecularly imprinted polymers for protein assays

deposition of the PEDOT/PSS within the pores of the protein modified PCM was performed by a potentiostat/galvanostat (Reference 600^{TM}, Gamry Instruments, Inc., USA) with a potentiostatic pulse program consisting of switching the potential at a rate of 5 Hz between -0.2 V and 1.1 V for 5000 cycles. After the PEDOT/PSS confined into the micropores were formed, the PCM was removed from the electrode surface by immersing it for 15 min into chloroform. The removal of the sacrificial PCM led to PEDOT/PSS microrods possessing the complementary imprint of the target protein on their surface (Av-FITC SIP microrods). The SIP microrods on the gold electrode surface were observed with an optical microscope (Eclipse LV100D, Nikon) and a field emission scanning electron microscope (SEM) (Ultra 55, Zeiss). All procedures were performed at room temperature. The fabrication process of the Av-FITC SIP microrods was described in detail in [II].

2.2.2. Fluorescence microscopy studies

The existence of Av-FITC binding sites on the surface of the Av-FITC imprinted PEDOT rods was confirmed by binding assays. The microrods were incubated in Av-FITC solutions of various concentrations, thoroughly washed and

then imaged by epifluorescent microscopy. The epifluorescent microscope imaging system was based on a conventional microscope (Eclipse LV100D, Nikon), equipped with a Lumen 200 Fluorescence Illumination System (Prior Scientific), and a longpass emission filter set (B-2A, Nikon). Plan Fluorite objectives were used with 20X and 50X magnification and numerical apertures of 0.45 and 0.8, respectively. NIS-Elements imaging software was used to acquire the fluorescent images from a Peltier-cooled CCD camera (DS-5Mc, Nikon) with subsequent processing and analyzing with respect to the following protocol. First, the gold electrodes with SIP microrods confined to their surface were incubated in various concentration avidin-FITC solutions for 15 min. To remove nonspecifically bound proteins the electrode was immersed in 25 ml stirred PBST-20X for 10 min. After the washing procedure the SIP microrods were imaged by the epifluorescent microscope. The CCD camera's gain control was set to a value of 16 and once the exposure time was adjusted kept constant throughout the subsequent fluorescence measurements. To avoid overexposure of the fluorescence signal after rebinding from more concentrated Av-FITC solutions the gain was regulated as needed. The electrode was positioned in a way that an initially selected region of interest was always present in the center part of the captured fluorescent image. On the image relevant areas (fluorescent rings) were selected and the mean pixel intensity and its standard deviation for the selected area were calculated automatically by NIS-Elements imaging software. The obtained mean values of the fluorescence intensity measurement were corrected with respect to the camera's gain control and plotted as arbitrary units in all the graphs depicting fluorescence.

3. RESULTS AND DISCUSSION

3.1. The enantioselective molecularly imprinted oPPy thin films

3.1.1. Electrochemical deposition of the PPy/L-Asp films. Effect of the electrolyte composition

Considering the acid/base properties of aspartic acid (Fig. 1.3), the pH value of electrolyte solution should be higher than 2.77 (pI value for Asp). In this case Asp exists in the form of anions and consequently can be incorporated into PPy films as dopants during the electropolymerization. Three different solution media were used for the deposition of imprinted PPy films: weakly acid media (A), alkaline media (B), and in the presence of NaPSS (C).

Weakly acid media (A)

The dominant L-Asp species in the synthesis solution with composition (A) is the single negatively charged Asp^{1-} (Fig. 99.5%). Therefore, during oxidative polymerization of PPy, L-Asp^{1-} anions were expected to compensate the positive charge of the PPy backbone resulting in the formation of PPy film doped with L-Asp ($PPy/L\text{-}Asp_{(A)}$). As it can be seen from Fig. 3.1a, the mass increased linearly

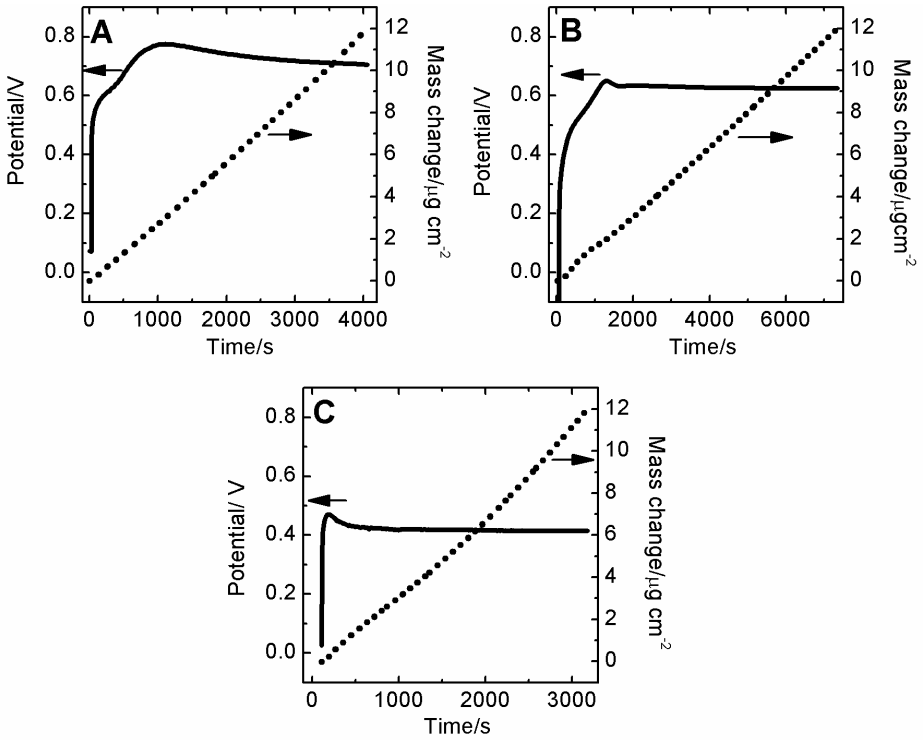

Figure 3.1. Simultaneous potential and mass responses recorded by EQCM during galvanostatic (0.01 mA/cm^2) polymerization of pyrrole from different electrolytes: (A) 0.2M Py and 0.5M L-Asp (pH 6); (B) 0.2M Py, 0.5M L-Asp and 1M NaOH (pH 11); (C) 0.2M Py, 0.5M L-Asp and 0.01M NaPSS (pH 6)

during the synthesis, which indicates the formation of a homogeneous polymer layer on the EQCM electrode. However, the film growth rate was very slow, probably due to protonated amino group bearing a positive charge (the amino acid has two $–COO^-$ groups and one $–NH_3^+$ group in the pH range 4-10), so that higher energy is presumably required to include the aspartate in the polymer network [92]. Additionally, the slow rate might be induced by the local acidification of the solution in the microenvironment of the growing film by protons generated during the electropolymerization. These can shift locally the protonation equilibrium of the aspartic acid reducing the concentration of negatively charged Asp^{-1} in favor of neutral species.

Alkaline media (B)

There is some controversy about the effect of alkaline pH on Py electropolymerization. Several authors have reported that alkaline electrolytes interfere with Py electropolymerization due to the deprotonation of cation radicals to form neutral radicals [93] and nucleophilic attack to the cation radical sites [94].

It was found that only the cation radicals, but not the neutral radicals, produce higher molecular weight oligomers, which explains the inhibition of the polymerization by strong bases.

However, the inhibiting effect is obviously very much dependent on the experimental conditions, such as pH, applied potential or current density. Since overoxidation of the film might also readily occur in these conditions, the applied potential and pH are especially important. Therefore, there are publications demonstrating that PPy films can be electrodeposited from alkaline media as well [95]. In this work the electropolymerization of pyrrole at pH 11 in the presence of L-Asp anions was found to proceed readily and reproducible. Thin films of oPPy/L-$Asp_{(B)}$ with smooth surface were formed on the working electrode by galvanostatic deposition. A linear increase of the mass during the electrochemical growth of the oPPy/L-$Asp_{(B)}$ film was observed (Fig. 3.1b). According to the acid/base properties of aspartic acid (Fig. 1.3) at pH 11 the amino group of Asp is deprotonated and the dominant species is Asp^{2-} (96.2%). This should further favor the insertion of L-Asp anions by charge compensation mechanism into the PPy film. However, the film growth rate decreased as compared to the electrodeposition from electrolyte A at pH 6, which is most likely due to the previously mentioned inhibiting effect of strong bases on the polymerization.

In the presence of NaPSS (C)

It is known that the incorporation of polyelectrolytes into the polymer matrix of conducting polymer can simultaneously improve its stability and mechanical properties [96]. Moreover, infrared, Raman and energy-dispersive spectroscopic results demonstrated that the polyanion of PSS^- is co-inserted into the PPy matrix and can not be extensively expelled from the film by dedoping [97]. We expected that these PPy/PSS/L-Asp films would promote the retention of cavities formed in the polymer matrix during the overoxidation/dedoping process and also to compensate for the hindered diffusion of the L-Asp anion. Moreover, since large polyanion such as PSS is readily adsorbed on the electrode surface, accelerating the first stage of the electropolymerization process [98]. Indeed, Fig. 3.1c demonstrates that a much faster growth of the PPy/PSS/L-Asp films can be achieved than either of solution A or B, while preserving also the linear form of the mass increase during electrosynthesis.

When comparing the potential transients of the three different synthesis methods employed, the highest potential to maintain the polymerization rate is required only in the case of solution A. While all solutions have the same initial concentration of aspartic acid, the concentration of the dominant form, and consequently the ionic strength, depends on the pH. Solution A has the lowest ionic strength, i.e. no added electrolyte and the dominant aspartic acid species being the single negatively charged Asp^{-1}. In addition, solution C has PSS while in solution B the dominant aspartic acid species is Asp^{2-}. Interestingly, when comparing curves A and B it seems that the potential needed to maintain the electropolymerization

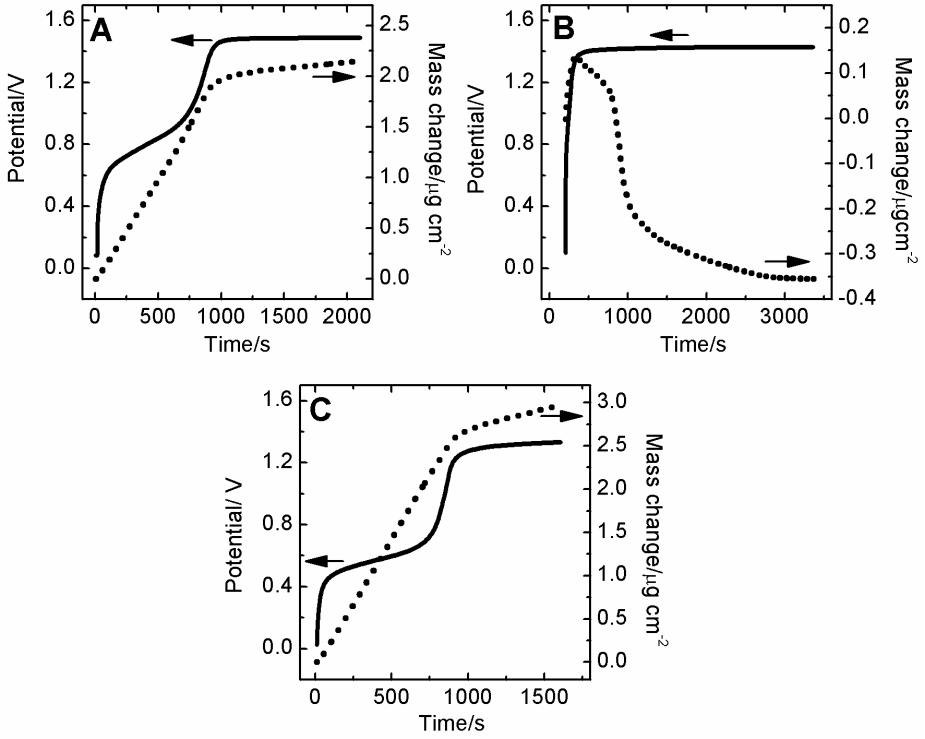

Figure 3.2. Simultaneously recorded potential and mass response during galvanostatic overoxidation (0.025 mA/cm^2) of the PPy films in PBS (pH 7): (A) PPy/L-Asp$_{(A)}$, (B) PPy/L-Asp$_{(B)}$, (C) PPyPSS/L-Asp

rate is mainly governed by the ionic strength of the solution (the charge number and concentration of the anion to be incorporated).

The obtained smooth and homogeneous films of the PPy/L-Asp$_{(A)}$, PPy/L-Asp$_{(B)}$, PPy/PSS/L-Asp (Fig. 4, I) were then overoxidized in order to form complementary cavities for the recognition of L-Asp in the PPy matrix

3.1.2. Overoxidation of PPy films: formation of complementary cavities

It is known that during the overoxidation/dedoping process PPy loses its electroactivity in parallel with the ejection of the dopants and generation of oxygen-containing groups such as carbonyl and carboxyl on the polypyrrole backbone [99]. This increases and diversifies the functionality of the native films. According to Spurlock et al. [67], the molecular sites after imprinting were expected to remain in oPPy since the overoxidation of PPy does not significantly alter polymer morphology, disrupting conjugation but maintaining the integrity of the polymer network. Moreover, Shiigi et al. [100] suggest that concomitantly with dedoping, curing of the polymer texture occurs to retain the complementarity of the cavity. The carbonyl oxygen facing towards the cavity surface can be a driving

force for the uptake of cationic template molecules. The formation of shape-complementary cavity by extracting the anionic template molecule through overoxidation/dedoping of PPy was also demonstrated by Deore et al. [68].

Fig. 3.2 shows the mass and potential responses recorded by EQCM during the galvanostatic overoxidation of PPy/L-Asp$_{(A)}$, PPy/L-Asp$_{(B)}$, PPy/PSS/L-Asp films synthesized at the conditions specified in Fig. 3.1. Observing the potential and mass change responses, at least two different stages during the galvanostatic overoxidation can be identified. At the first stage, the potential slowly changes from 0.5V to 1V. After certain time, depending on the film, the potential is rapidly raised, indicating the beginning of the second stage, up to values higher than 1.3V a with simultaneous change of the mass response slope.

It may be supposed that at the first stage the current was consumed for the irreversible oxidation of the polymer accompanying with an uptake of anions from the solution, which led to a net mass increase. It is likely that at the end of the first stage the polymers had been completely oxidized and the overoxidation process began. Since, during the overoxidation process the polymer loses its electroactivity and dopant anions, the potential in the chronopotentiometric experiment sharply increases while the slope of the mass transients decreases. This behavior was characteristic for the overoxidation of PPy films prepared from solutions A and C (Fig. 3.2a, c).

However, the films prepared from solution B (PPy/L-Asp$_{(B)}$) acted very differently. While the PPy/L-Asp$_{(A)}$ and PPy/PSS/L-Asp films had switched to the second stage after 60% and 73% of the charge required for the synthesis had passed, respectively, for the PPy/L-Asp$_{(B)}$ this stage was entirely absent, suggesting that the film achieved a much higher level of oxidation during its synthesis, i.e. it had already been overoxidized to a great extent. This has been unambiguously demonstrated by Cyclic Voltammograms (CVs) recorded before and after overoxidation of the films (Fig. 3.3), which confirmed the poor electroactivity of the synthesized PPy/L-Asp$_{(B)}$ layer. At the same time the other two types of films showed the redox activity after the synthesis, which however disappeared when overoxidized (Fig. 3.3a, c). It should be noted that the CVs of PPy/L-Asp$_{(B)}$, remained practically unchanged after the overoxidation step (Fig. 3.3b). Fig. 3.2 shows a negative net mass change only for PPy/L-Asp$_{(B)}$. The other films have exhibited only a reduction in the net mass increase right after the overoxidation process started. The mass decrease due to the dedoping in the case of PPy/l-Asp$_{(B)}$ was 0.5 μg/cm^2, which corresponds to ca. 4% of the synthesized mass. Since there are several competitive and opposite mass processes contributing to a mass change, this value is an apparent one providing no information on the individual processes going on and on the type and charge of the species involved in the dedoping process. This loss of mass may not only involve ejection of doping anions but also of soluble shorter chain oligomers incorporated into the film during its synthesis [101]. A leveling of the film surface can be observed after the overoxidation for all samples with the smoothest surface resulting for oPPy/L- Asp$_{(B)}$ film (Fig. 4, I).

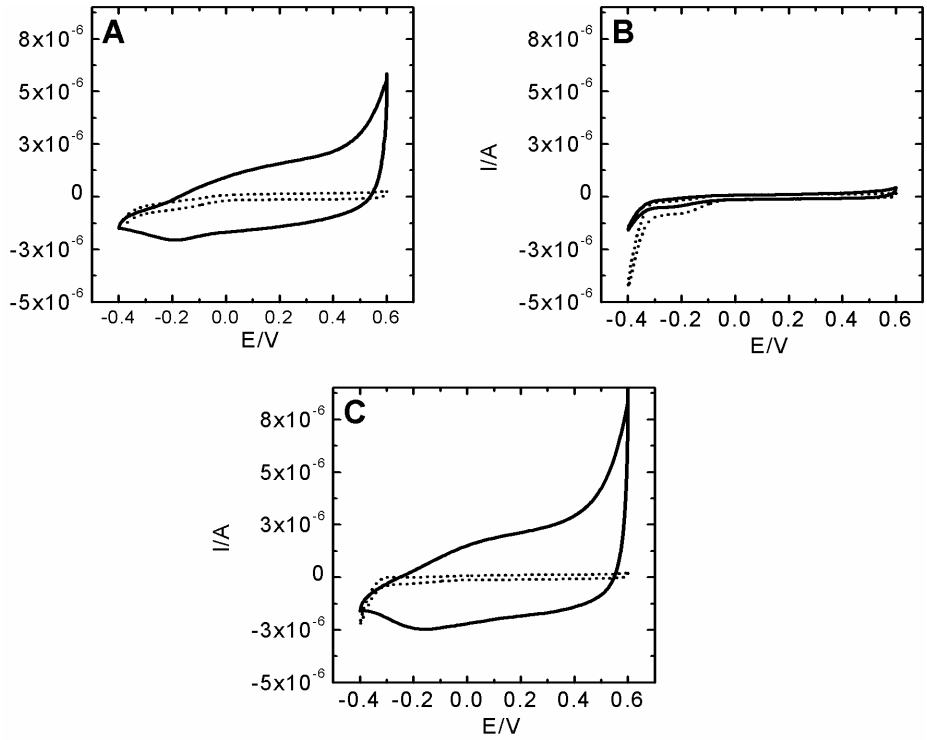

Figure 3.3. Cyclic voltammograms of the PPy films before (—) and after (···) the galvanostatic overoxidation in PB solution (pH 7): (A) PPy/L-Asp$_{(A)}$, (B) PPy/L-Asp$_{(B)}$, (C) PPy/PSS/L-Asp

3.1.3. Recognition properties of the molecularly imprinted oPPy films

To examine the enantioselectivity of the prepared oPPy/L-Asp films they were tested in the KCl-HCl solution (pH 1.6) containing L- or D-Asp acid isomers. These experiments were carried out under potentiostatic conditions (-0.4 V). According to the acid/base properties of aspartic acid (Fig. 1.3) L-Asp dominantly exists as a cation in the solution of pH 1.6. Consequently, applied negative potential assists the uptake of L-Asp cations into the oPPy/L-Asp film. However, the oPPy/L-Asp$_{(A)}$ films prepared at pH 6 do not exhibit any mass increase that would indicate the uptake of the respective template molecule (data not shown). This may be attributed either to the difficulty of doping PPy with L-Asp in the weakly acidic media or to the destruction of the recognition sites during overoxidation. In the case of the oPPy/L-Asp$_{(B)}$ films a mass increase of 1.0 μg/cm^2 was observed as a result of L-Asp addition to the carrier solution, while the response of the same film for D-Asp was only 0.05 μg/cm^2 (Fig. 3.4a). This means that the oPPy/L-Asp$_{(B)}$ films have a ca. 20-fold selectivity for the enantiomer used as a template, which compares very favorably with the enantioselectivity of

previously reported PPy based MIPs: maximal uptake ration for tyrosine L/D=9.4±4.7 [102] and for glutamic acid L/D being approximately 10 [69].

In the case of the oPPy/PSS/L-Asp, the mass increase of 0.45 μg/cm^2 was observed after injection of L-Asp solution during the testing (Fig. 3.4b). The mass change is markedly smaller in comparison with the oPPy/L-Asp$_{(B)}$. Obviously, this indicates that during the synthesis the PSS anions are predominantly incorporated into the PPy film, resulting in a lower concentration of L-Asp ions in the film and consequently, a lower density of the recognition sites is formed during overoxidation. The oPPy/PSS/L-Asp films illustrate also smaller selectivity than oPPy/L-Asp$_{(B)}$ films; the uptake ration of L/D enantiomers being only: 6.5. Since in case of both oPPy/L-Asp$_{(A)}$ and oPPy/PSS/L-Asp films the pH of the polymerization solution was the same, the overoxidation process seems to affect to some extent the binding sites formed during the polymerization and this effect in less severe when additional doping polyanions are present in the film. The experiments demonstrate also that higher a doping level of the template molecule in the film enhances the selectivity of the reuptake.

When oPPy/L-Asp$_{(B)}$ and oPPy/PSS/L-Asp films were tested in the same way but without externally applied potential in the flow-injection analysis system the resonance frequency changes observed could be only attributed to the resonant resistance change caused most likely by the solution viscosity change. No significant mass change and chiral selectivity were obtained for any of these films. Therefore, it seems that the potential stimulus is needed for an effective and selective uptake of the template molecules.

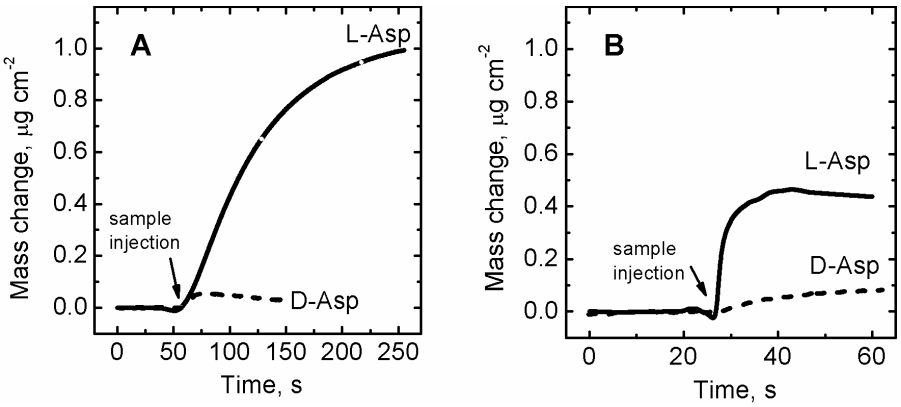

Figure 3.4. Mass change of the oPPy/L-Asp$_{(B)}$ (A) and oPPy/PSS/L-Asp (B) coated EQCM electrode held at a constant potential of -0.4 V due to injection of L-Asp and D-Asp(10 mM) into KCl-HCl solution (pH 1.6)

3.2. Surface-imprinted PEDOT/PSS microstructures

3.2.1. The surface imprinting strategy for fabrication SIPs for protein assays

A novel approach and materials for producing surface-imprinted micro- or nanorods with selective protein-binding sites located on their surface was introduced (Fig. 2.3). The proposed method is based on the electrochemical synthesis of PEDOT/PSS within the pores of the protein (Av or Av-FITC) modified PCM. Precisely sized cylindrical pores of PCM membranes served as sacrificial microreactor for the synthesis. Native membranes (without impregnation with a wetting agent) are hydrophobic in nature and adsorb readily protein molecules. This offers a straightforward method for fixing the target protein onto the pore walls by simple physical adsorption. In these microreactors, positioned on the surface of a gold electrode, conducting polymer rods of PEDOT/PSS were electrochemically grown. After polymerizing the microrods into the pores, the PCM could be easily removed by dissolution in chloroform. The removal of the sacrificial material resulted in the formation of microrods confined to the surface of the gold electrode possessing the complementary imprint of the target protein on their surface. The PEDOT/PSS material has functionalities that are expected to generate hydrogen bonds, and electrostatic and π–π interactions with the protein template. Since avidin is rich in tryptophan, the formation of the latter type of interactions is especially probable.

The physical adsorption of the protein onto the PCM surface in the first step of the synthesis was demonstrated using Av-FITC. Epifluorescent optical microscopy images after incubation of the PCM in Av-FITC solutions of various concentrations clearly indicated the adsorption of the protein (Fig. 2a, II). The adsorption isotherm was recorded to determine the saturation concentration (1 mg mL^{-1}, data not shown). This value was used from then on for membrane modification in order to maximize the binding capacity of the resulting SIPs.

3.2.2. Electrochemical growth of the SIP microrods

The surface-imprinted PEDOT/PSS microrods for selective recognition of proteins (Av or Av-FITC) were obtained by using a potentiostatic squarewave pulse sequence that allows polymer preparation with enhanced adhesion to the electrode surface [103] and being also suitable for localized deposition of ECPs [104]. Fig. 3.5 shows oxidative and reduction current profiles versus time obtained during the PEDOT/PSS electrodeposition on the gold electrode surface with the fastened PCM. According to the electrochemical characteristics there are two stages of microrods formation. During the fist stage, polymerization starts with the oxidation of the monomer in the thin solution layer penetrating between the electrode surface and the PCM. The resulting thin polymeric film is beneficial later for the adhesion of the synthesized microrods. After depositing the thin film of PEDOT/PSS on the electrode surface, the polymerization process continues within the pores of the membrane, accompanying with the decrease oxidation current

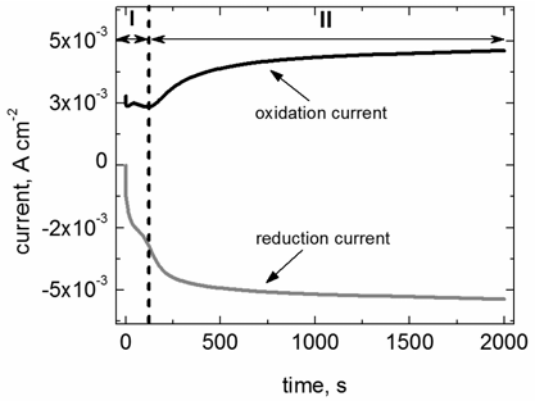

Figure 3.5. Electrochemical current response versus time during the formation of the PEDOT/PSS microrods in the pores (d_N=8 μm) of PCM by applying of squarewave potential pulse sequence (E_1= - 0.2V for 0.2 s; E_2=1.1V for 0.2 s; 5000 cycles)

since the effective electrode area reduces quickly during this stage. It was shown earlier that nucleation of the polymer preferentially proceeds on the pore walls and is regulated by the diffusion of monomers into the pores [105]. The PEDOT/PSS microrods were grown until the pores were filled up to the top surface of the membrane, determined *ex-situ* by using optical microscopy. This stage was characterized by an oxidation current value of 4.45 mA/cm^2. Since the synthesis of the polymeric microrods proved to be remarkably reproducible, a simple adjustment of either the upper current limit or polymerization time provided consistent results.

SEM images of the electrode surface demonstrate both the confinement of the polymerization into the micropores (Fig. 3.6a) and the localization of the PEDOT/PSS microrods on the gold electrode surface after dissolution of the PCM (Fig. 3.6b, c). The diameter of these microrods (ca. 8 μm) corresponds to the pore diameter and their height (ca. 7 μm) to the thickness of the membrane. The nanosized PEDOT/PSS rods can be easily fabricated by using a PCM with the pores of an appropriate size. Fig. 3.6d demonstrates nanometer-scaled PEDOT/PSS rods, which are about 80 nm in diameter.

3.2.3. Investigation of specific binding of Av-FITC by SIP microrods

The existence of Av-FITC binding sites on the SIP microrods was confirmed by binding assays. The microrods were incubated in Av-FITC solutions of various concentrations, thoroughly washed and then imaged by epifluorescent microscopy. As negative controls PEDOT/PSS microrods synthesized in the absence of the target protein (non-imprinted PEDOT microrods – NIPs) were tested also for Av-FITC assay.

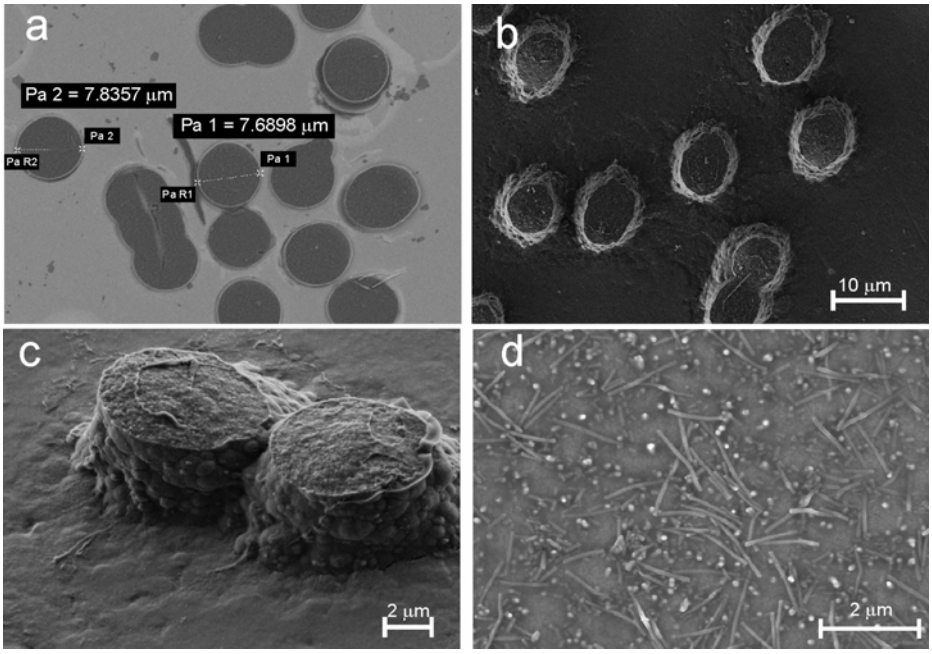

Figure 3.6. Scanning electron micrographs of the PEDOT/PSS rods confined to the micropores (a) and stand-alone microrods on the electrode surface after removal of the PCM (b-d)

Fluorescent images of Av-FITC SIP microrods, presented in Fig. 3.7b show green fluorescent rings around the microrods indicating the adsorption of Av-FITC. There is an excellent contrast between the fluorescence of the top surface of the microrods (non-imprinted) and their mantle (surface-imprinted). The fluorescence is observed exclusively on the lateral surface, proving the formation of complementary cavities on the surface of PEDOT microrods capable of rebinding the target protein molecules. However, it should be noted that due to the thin PEDOT/PSS layer covering the electrode surface under protein modified PCM, the formation of imprinted sites on its surface is also possible. The lack of fluorescence upon Av-FITC binding therefore is remarkable and can only be partly attributed to the lower sensitivity in detecting fluorescent monolayers as opposed to imaging fluorescence in the finite thickness of 3D microstructures. It is likely that the polymer film remains non imprinted due to the limited amount of monomer entrapped between the membrane and electrode, which is not enough to supply the growth of the polymer until it properly contacts the PCM (Fig. 3.7, Area 1). The resulting polymer film can fill the gap only in the close vicinity of the rods where the monomer supply through the pores is still effective. This assumption is supported by the fact that the surface is showing clear fluorescence merely in the close vicinity of the microrods where optical micrographs indicate a thickening of the PEDOT film (Fig. 3.7, Area 2).

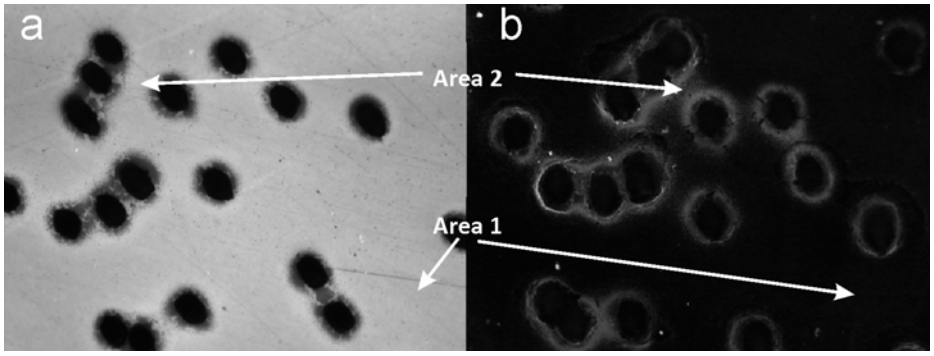

Figure 3.7. Optical (a) and fluorescent (b) images of the SIP microrods on the electrode surface as a result of Av-FITC rebinding on the surface-imprinted mantle of the microrods

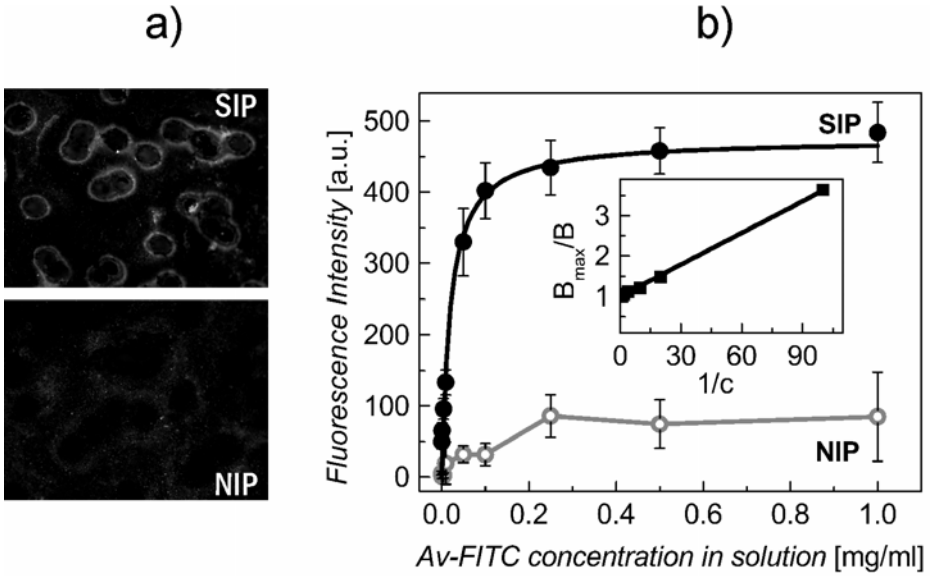

Figure 3.8. a) fluorescent micrographs of Av-FITC binding on Av-FITC SIP and NIP microrods, b) Fluorescence intensity of the imprinted and non-imprinted PEDOT/PSS microrods as a function of Av-FITC concentration in the range of 10^{-4} to 1 mg/ml. The data obtained for SIP was fitted with the hyperbolic equation for specific adsorption. The insert shows the Scatchard Rosenthal linearized plot for the evaluation of K_d

The intensity of the fluorescent rings resulting upon binding of Av-FITC was measured and related to its concentration (Fig. 3.8). The data were fitted with the hyperbolic equation for a specific binding: $B=B_{max}\times c/(K_d+c)$, where c is the concentration of the target protein in the solution, B and B_{max} are the fractions of the bound protein and its saturation value, respectively and K_d is the dissociation constant. The Scatchard-Rosenthal transformation applied to the experimental data

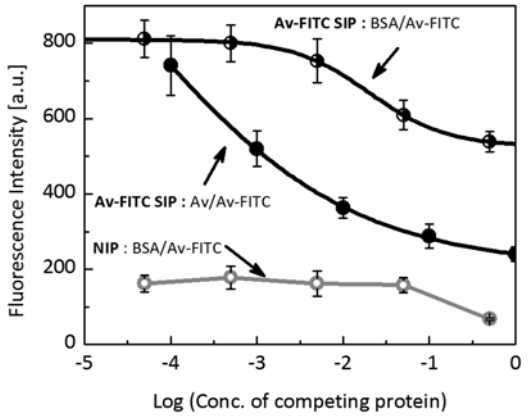

Figure 3.9. Competitive rebinding of Av and BSA on the Av-FITC and BSA surface-imprinted polymer rods as function of the competing protein concentration (mg mL^{-1}). The rebinding was carried out at pH 7 in 0.05 M PBS, with a fixed concentration of Av-FITC (0.05 mg/ml) and increasing concentration of the competitive protein, either Av or BSA

set resulted in a K_d of 394 nM. This indicates a rather strong interaction between the target protein and imprinted polymer. In contrast, the binding of Av-FITC to the NIP is insignificant even at 1 mg mL^{-1} concentrations. This clearly indicates that the PEDOT/PSS is a suitable material for molecular imprinting exhibiting extremely low non-specific binding, even for a protein such as avidin which due to its high pI (10) is notorious for providing high non-specific backgrounds on most materials.

3.2.4. Evaluation of the selectivity of SIP microrods

Competitive binding assays were performed to evaluate the feasibility and selectivity of detecting Av by SIPs. Avidin-FITC imprinted PEDOT microrods were used for the assays while different concentrations of either avidin or BSA were allowed to compete with constant amount of fluorescently labeled avidin for the binding sites. Still, little is known about the nature of protein recognition sites in MIPs or SIPs, since fully specific responses have not yet been reported, it was reasonable to expect that Av-FITC imprinted polymers are going to be responsive to avidin. The Av-FITC differs from Av only in the four FITC molecules, on average that are covalently attached to the protein. As demonstrated by Fig. 3.9, a decrease in the fluorescence signal is indeed observed for Av-FITC SIP microrods with increasing concentrations of avidin. The data are well fitted by 4 parameter dose response curves with a variable Hill slope. The log IC_{50} value was -4.4, however associated with a rather high standard error (1.26). Fixing the Hill slope at 1 provided a value of -2.9 (0.2). Performing the very same experiment with BSA

resulted in an IC_{50} value significantly shifted towards higher concentrations (log IC_{50} = -1.7 (0.02)). These values indicate clearly a preferential binding of avidin as opposed to BSA since the lower the IC_{50} value the higher the competition faced by Av-FITC. The selective response is also supported by a smaller decrease in fluorescence in the case of BSA, i.e., the displacement of Av-FITC from the binding sites is less effective. The same competitive assays performed on NIPs show only a remarkably low fluorescent background that is featureless throughout the relevant concentration range (Fig. 3.9).

CONCLUSIONS

The essential objective of this thesis was to apply electrosynthesized conducting polymers as matrices for the MIP systems. PPy and PEDOT were successfully used for the specific purposes, namely for: (a) enantioselective recognition of amino acid isomers (L-Asp/D-Asp), (b) selective binding of proteins (Av-FITC). Consequently, the results comprise two parts and can be presented as follows:

(a) Enantioselective recognition of amino acids

- Thin films of PPy, electropolymerized in the presence of L-Asp as template molecules with subsequent overoxidation to create shape complementary cavities, were evaluated as MIPs.
- The synthesis parameters such as electrolyte composition and pH value strongly influence the enantioselectivity of the resulting oPPy/L-Asp films. Only the films prepared in alkaline NaOH solution (pH 11) exhibit afterwards rather good enantioselectivity.
- The potential stimulus is needed for an effective and selective uptake of the template molecules. It was demonstrated that under potentiostatic conditions (-0.4 V vs. Ag/AgCl/3M KCl) and strongly acid media (pH 1.6) the oPPy/L-Asp films have a ca. 20-fold selectivity for the enantiomer used as a template.
- EQCM technique is suitable for both monitoring the selective recognition and the electrochemical modulation of the binding process in the prepared molecularly imprinted PPy films.

(b) Selective binding of proteins

- A novel approach and materials for producing surface-imprinted microstructures with selective protein binding sites located on their surface were proposed.
- The protocol for electrochemical preparation of the PEDOT microstructures surface-imprinted with Av-FITC (Av-FITC SIP) was elaborated. The proposed method has the following advantages: (a) simple preparation without involving chemical reactions during template immobilization, (b) excellent control of the polymerization and localization of the microstructures by electrosynthesis, (c) the resulting 3D microstructures as compared to planar systems are beneficial in terms of binding capacity and sensitivity, (d) it is reasonable to expect that the system is scalable down to the nanosizes.
- It was found that the PEDOT/PSS polymeric material exhibits superior resistance against non-specific protein (Av) adsorption

- The Av-FITC SIP microrods show relatively high specific adsorption for Av-FITC, with K_d value of 394 nM.
- Competitive binding assays demonstrated a preferential adsorption of Av-FITC as opposed to BSA by the Av-FITC SIP microrods.

ABSTRACT

In the present thesis two electrosynthesized ECPs - PPy and PEDOT- were used as matrices for molecular imprinting. The thesis comprises two parts. Part one describes the preparation of the thin films of oPPy imprinted with amino acid L-Asp and their capability to discriminate between L- and D- Asp acid isomers, and part two deals with the fabrication and investigation of the surface-imprinted PEDOT microrods for selective recognition of avidin-FITC (Av-FITC).

In part one, thin films of PPy, electropolymerized in the presence of L-Asp as template molecules with subsequent overoxidation to create shape complementary cavities, were evaluated as MIPs. The electrosynthesis, overoxidation and characterization of PPy/L-Asp films were performed by the EQCM technique. It was found that synthesis parameters such as electrolyte composition and pH value strongly influence the enantioselectivity of the resulting oPPy/L-Asp films. Thus, the electrodeposition from a weakly acidic solution containing pyrrole and L-Asp salt (pH 6) leads to films that do not exhibit enantioselectivity for, L-Asp. The electropolymerization of pyrrole in the presence of L-Asp in alkaline media (pH 11) results in adherent smooth and homogeneous PPy/L-$Asp_{(B)}$ films that following the overoxidation/dedoping exhibits rather good enantioselectivity at pH 1.6 (ca. 20-fold selectivity for the enantiomer used as a template). The sensitivity of the measurements is clearly affected by the doping level of the films, as suggested by the smaller frequency change obtained in the case of oPPy/PSS/L-Asp. It was demonstrated that the uptake of L-Asp on oPPy/L-Asp films occurs only in the case of potential-induced uptake/release of targeted molecules. The results suggest the feasibility of preparing molecularly imprinted films by electropolymerization for the enantioselective recognition of amino acids and the suitability of EQCM for both to monitor the selective recognition as well as to modulate electrochemically the binding process.

In part two, a novel approach and materials for creating surface-imprinted micro- or nanorods with selective protein-binding sites located on their surface was introduced. The proof of principle was done by synthesizing surface-imprinted PEDOT/PSS microrods for avidin recognition. The protocol for electrochemical preparation of the PEDOT microstructures surface-imprinted with Av-FITC (Av-FITC SIP) was elaborated It was found that the PEDOT/PSS polymeric material exhibits superior resistance against nonspecific protein (Av-FITC) adsorption and is effectively turned into a selective protein sorbent upon imprinting. The fluorescence microscopy study of target protein specific adsorption on the obtained Av-FITC SIP microrods demonstrated relatively high specific adsorption for Av-FITC, with Kd value of 394 nM. Competitive binding assays demonstrated a preferential adsorption of Av-FITC as opposed to BSA by the Av-FITC SIP microrods. The proposed method for the synthesis of SIPs for protein recognition benefits from the following advantages: (a) simple preparation, without involving chemical reactions during template immobilization or harsh conditions, (b)

excellent control of the polymerization and localization of the microstructures by electrosynthesis, (c) the resulted 3D microstructures as compared to planar systems are beneficial in terms of binding capacity and sensitivity; (d) it is reasonable to expect that the system is scalable down to the nanosizes.

KOKKUVÕTE

Antud doktoritöös on uuritud elektrit juhtivate polümeeride - polüpürrooli (PPy) ja polü(3, 4-etüleendioksütiofeeni (PEDOT) - kasutamise võimalusi molekulaarselt jäljendatud süsteemides maatriksina. Doktoritöö esimeses osas valmistati L-aspartaamhappega (L-Asp) jäljendatud PPy õhukesed kiled ning uuriti nende võimet eristada L-Asp ja D- Asp optilisi isomeere. Doktoritöö teises osas töötati välja metoodika valgumolekulidega (Av-FITC) jäljendatud pindmiste mälupesadega mikrovarraste (SIP/Av-FITC) valmistamiseks ning uuriti nende pinnal Av-FITC spetsiifilist adsorptsiooni. Saadud tulemused võib tinglikult jagada kaheks ja kokkuvõtlikult kirjeldada alljärgnevalt.

L-aspartaamhappega jäljendatud PPy õhukesed kiled (oPPy/L-Asp) sünteesiti elektrokeemiliselt L-Asp juuresolekul ning seejärel tekitati polümeeri struktuuris nn. ”üleoksüdeerimise” teel, suurendades mälupesade tekitamiseks sünteesi-potentsiaali kuni 1,2V-ni. PPy/L-Asp kilede sünteesiks, üleoksüdeerimiseks ja iseloomustamiseks kasutati EQCM meetodit. Leiti, et molekulaarselt jäljendatud oPPy/L-Asp kilede enantioselektiivsus sõltub peamiselt sünteesi tingimustest nagu elektrolüüdi koostis ja pH väärtus. Katsetulemused tõestasid, et leeliselises keskkonnas (pH 11) sünteesitud PPy/L-Asp(B) kile on ligi 20-korda suurema selektiivsusega sihtmolekuli L-Asp suhtes võrreldes D-Asp-ga. NaPSS legeeritud kile oPPy/PSS/L-Asp testimisel L-Asp lahuses saadud väiksem sageduse muutus võrreldes PPy/L-Asp(B) kilega tõestab valmistatud PPy kilede legeerimisaste mõju kilede selektiivsusele. Lisaks leiti, et L-Asp molekulide sidumine oPPy/L-Asp kiledel toimub ainult negatiivse potentsiaali rakendamisel. Kokkuvõtlikult tõendavad saadud tulemused elektrokeemilise sünteesimeetodi sobivust EJP baasil MIP kilede valmistamiseks aminohapete enantioselektiivseks äratundmiseks ning EQCM tehnika eeliseid nii sihtmolekulide selektiivse äratundmise jälgimiseks kui ka nende sidumise protsessi elektrokeemiliseks juhtimiseks.

Doktoritöö teises osas on välja pakutud uudne metoodika valgumolekulidega jäljendatud pindmiste mälupesadega PEDOT mikro- ja nanovarraste (Av-FITC SIP) valmistamiseks. Katsed näitasid, et jäljendamata PEDOT/PSS on äärmiselt madala adsorptsioonivõimega valgumolekulide (avidin) suhtes ning seega sobilik maatriks peale jäljendamist valgu sihtmolekule spetsiifiliselt siduma. Metoodika põhineb šabloon-sünteesi meetodil. Elektrit juhtiva polümeeri PEDOT/PSS elektrokeemiline süntees viidi läbi mikropoorse polükarbonaat membraani (PCM) poorides, kuhu sisestati eelnevalt valgu (Av-FITC) molekulid. Pärast membraani lahustamist ning samaaegset valgu väljapesemist jäävad alles pindmiste mälupesadega jäljendatud PEDOT/PSS mikrovardad. Fluorestsentsmikroskoopia abil saadud tulemused näitasid, et Av-FITC SIP mikrovarrastel on adsorbeerinud valgu fluorestsentsi intensiivsus oluliselt suurem võrreldes mittejäljendatud mikrovarrastega. Samadele järeldustele jõuti ka uurides Av-FITC SIP mikrovarraste selektiivsust sihtmolekulide (Av-FITC) suhtes konkureeriva adsorptsiooni meetodil (competitive binding assays).

Kokkuvõtlikult võib valgumolekulidega jäljendatud pindmiste mälupesadega mikrovarraste valmistamise protsessi eeldused välja tuua järgnevalt i) lihtne elektrokeemilise valmistamise protsess, milles on välistatud struktuuri rikkuda võivad keemilised reaktsioonid valgu sihtmolekulide kinnitamisel; ii) elektrokeemilise polümerisatsiooni ja mikrostruktuuride tekkemehhanismi väga hea kontrollitavus elektrokeemilise sünteesi käigus; iii) valmistatud 3D mikrostruktuurid on märkimisväärselt suurema tundlikkuse ja sidumise võimega võrreldes planaarsete struktuuridega; iv) võimalus ka SIP nanostruktuuride valmistamiseks.

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100. Shiigi, H., Kijima, D., Ikenaga, Y., Hori, K., Fukazawa, S., Nagaoka, T. Molecular recognition for bile acids using a molecularly imprinted overoxidized polypyrrole film. *Journal of the Electrochemical Society*, 2005, **152**(8), H129-H134.
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APPENDIX A

Publication I

Vitali Syritski, Jekaterina Reut, **Anna Menaker**, Róbert E. Gyurcsányi and Andres Öpik, Electrosynthesized molecularly imprinted polypyrrole films for enantioselective recognition of L-aspartic acid, Electrochimica Acta, 2008, 53(6), 2729-2736.

APPENDIX A

Publication II

Anna Menaker, Vitali Syritski, Jekaterina Reut, Andres Öpik, Viola Horvath and Róbert E. Gyurcsányi, Electrosynthesized surface-imprinted conducting polymer microrods for selective protein recognition, Advanced Materials, 2009, 21, 1–5. DOI: 10.1002/adma.200803597. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

APPENDIX A

Publication III

Andres Öpik, **Anna Menaker**, Jekaterina Reut and Vitali Syritski, Molecularly imprinted polymers: a new approach for preparation of functional materials, Proceedings of the Estonian Academy of Sciences, 2009, 58 (1), 3-11

APPENDIX B

Curriculum Vitae

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi	Anna Menaker
Sünniaeg ja -koht	03.04.1979, Tallinn
Kodakondsus	Eesti

2. Kontaktandmed

Aadress	Vilisuu 4-15, 13624 Tallinn, Estonia
Telefon	+372 6202820
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3. Hariduskäik

alates 2005	Tallinna Tehnikaülikool, doktoriõpe, keemia- ja materjalitehnoloogia eriala
2004	Tallinna Tehnikaülikool, Loodusteaduste magistri kraad
2002	Tallinna Tehnikaülikool, Tehnikateaduste bakalaureuse kraad
1997	Tallinna Mustamäe Reaalgümnaasium

4. Keelteoskus (alg-, kesk-, või kõrgtase)

vene	kõrgtase (emakeel)
eesti	kesktase
inglise	kesktase

5. Täiendusõpe

juuni 27-29, 2006	MMTDK suvekool „New trends in material science”, Eesti
aug. 21-25, 2006	MMTDK suvekool „Young scientists summer school on photovoltaics”, Eesti
nov. 06-25, 2006	Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Ungari, praktikant
juuni 26-28, 2007	MMTDK suvekool „Functional materials”, Eesti
nov. 04-24, 2007	Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Ungari, praktikant
mai 06-11, 2008	Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Ungari, praktikant

6. Teenistuskäik

2002-2003	Insener, TTÜ, Keemiainstituut
2005-	Erakorraline teadur, TTÜ, Materjaliteaduse instituut
2005 - 2008	Erakorraline teadur, TTÜ, MMTDK

7. Kaitstud lõputööd

"Põlevkiviõli ekstraktsioon ülekriitilise CO2-ga", bakalaureusetöö, juhendaja Prof. Jüri Soone

"Ekstraktsioon ülekriitilise süsinikdioksiidiga", magistritöö, juhendaja vanemteadur Mihkel Koel

8. Teadustöö põhisuunad

Elektrit juhtivate polümeermaterjalide füüsikaliste ja keemiliste omaduste uurimine. Molekulaarse äratundmise ja eristamise süsteemide valmistamine molekulaarselt jäljendatud elektrit juhtivatest polümeeridest (molecularly imprinted polymers – MIP). MIP baasil valmistatud sünteetiliste retseptorite eelisteks võrreldes bioloogiliste retseptoritega, on nende omaduste stabiilsus ka kõrgematel temperatuuridel ja rõhkudel, samuti ekstreemsetel pH väärtustel, orgaanilistes lahustites ning lõpuks nende oluliselt odavam süntees.

9. Teised uurimisprojektid

Projekt: SF0142714s06 "Elektrit juhtivate polümeermaterjalide omaduste uurimine ja modifitseerimine kasutamiseks funktsionaalsete materjalidena ning elektronseadiste komponentidena".

Projekt: baasfinantseerimine B612 "Sünteetilised retseptorid molekulaarselt jäljendatud elektrit juhtivatest polümeeridest".

CURRICULUM VITAE

1. Personal data

Name	Anna Menaker
Date and place of birth	03.04.1979, Tallinn

2. Contact information

Address	Vilisuu 4-15, 13624 Tallinn, Estonia
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3. Education

since 2005	Tallinn University of Technology, Faculty of Chemical and Materials Technology, doctoral study
2004	Tallinn University of Technology, Faculty of Chemical and Materials Technology, MSc.
2002	Tallinn University of Technology, Faculty of Chemical and Environmental Technology, BSc.
1997	Tallinna Mustamäe Reaalgümnaasium, Secondary education

4. Language competence/skills (fluent, average, basic)

Russian	fluent (mother tongue)
Estonian	average
English	average

5. Special Courses

June 27-29, 2006	MMTDK summer school “New trends in material science”, Estonia
Aug. 21-25, 2006	MMTDK summer school “Young scientists summer school on photovoltaics”, Estonia
Nov. 06-25, 2006	Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Hungary, trainee
June 26-28, 2007	MMTDK summer school “Functional materials”, Estonia
Nov. 04-24, 2007	Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Hungary, trainee
May 06-11, 2008	Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Hungary, trainee

6. Professional Employment

2002-2003y	Engineer, Tallinn University of Technology, Department of Chemistry
2005 y-	Researcher, Tallinn University of Technology, Department of Materials Science
2005 – 2008y	Researcher, Tallinn University of Technology, Doctoral School in Materials Science and Technology

7. Defended thesis

"Supercritical carbon dioxide extraction of shale oil", bachelor thesis, supervisor Prof. Jüri Soone

"Supercritical carbon dioxide extraction", master thesis, supervisor senior research Mihkel Koel

8. Main areas of scientific work/Current research topics

Synthesis of electrically conductive polymers and investigation of their electrochemical properties. Preparation of molecularly imprinted polymer (MIP) systems base on electrically conducting polymers (polypyrrole and poly(3,4-ethylenedioxythiophene). In comparison to their biological analogues, along with possessing antibody-like molecular selectivity, the major advantages MIPs include physical robustness, resistance to elevated temperatures and pressures, and inertness to acids, bases, and organic solvents, as well as low production cost and ease of preparation.

9. Other research projects

Project: SF0142714s06 "Electrically conductive polymers for functional materials and electron devices. Investigation of the physical and chemical properties and possibilities for practical applications."

Project: base-line financing B612 "Synthetic receptors from molecularly-imprinted electrically conducting polymers".

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *NATURAL AND EXACT SCIENCES*

1. **Olav Kongas**. Nonlinear dynamics in modeling cardiac arrhytmias. 1998.

2. **Kalju Vanatalu**. Optimization of processes of microbial biosynthesis of isotopically labeled biomolecules and their complexes. 1999.

3. **Ahto Buldas**. An algebraic approach to the structure of graphs. 1999.

4. **Monika Drews**. A metabolic study of insect cells in batch and continuous culture: application of chemostat and turbidostat to the production of recombinant proteins. 1999.

5. **Eola Valdre**. Endothelial-specific regulation of vessel formation: role of receptor tyrosine kinases. 2000.

6. **Kalju Lott**. Doping and defect thermodynamic equilibrium in ZnS. 2000.

7. **Reet Koljak**. Novel fatty acid dioxygenases from the corals *Plexaura homomalla* and *Gersemia fruticosa.* 2001.

8. **Anne Paju**. Asymmetric oxidation of prochiral and racemic ketones by using sharpless catalyst. 2001.

9. **Marko Vendelin**. Cardiac mechanoenergetics *in silico*. 2001.

10. **Pearu Peterson**. Multi-soliton interactions and the inverse problem of wave crest. 2001.

11. **Anne Menert**. Microcalorimetry of anaerobic digestion. 2001.

12. **Toomas Tiivel**. The role of the mitochondrial outer membrane in *in vivo* regulation of respiration in normal heart and skeletal muscle cell. 2002.

13. **Olle Hints**. Ordovician scolecodonts of Estonia and neighbouring areas: taxonomy, distribution, palaeoecology, and application. 2002.

14. **Jaak Nõlvak**. Chitinozoan biostratigrapy in the Ordovician of Baltoscandia. 2002.

15. **Liivi Kluge**. On algebraic structure of pre-operad. 2002.

16. **Jaanus Lass**. Biosignal interpretation: Study of cardiac arrhytmias and electromagnetic field effects on human nervous system. 2002.

17. **Janek Peterson**. Synthesis, structural characterization and modification of PAMAM dendrimers. 2002.

18. **Merike Vaher**. Room temperature ionic liquids as background electrolyte additives in capillary electrophoresis. 2002.

19. **Valdek Mikli**. Electron microscopy and image analysis study of powdered hardmetal materials and optoelectronic thin films. 2003.

20. **Mart Viljus**. The microstructure and properties of fine-grained cermets. 2003.

21. **Signe Kask**. Identification and characterization of dairy-related *Lactobacillus.* 2003.

22. **Tiiu-Mai Laht**. Influence of microstructure of the curd on enzymatic and microbiological processes in Swiss-type cheese. 2003.

23. **Anne Kuusksalu**. 2–5A synthetase in the marine sponge *Geodia cydonium*. 2003.

24. **Sergei Bereznev**. Solar cells based on polycristalline copper-indium chalcogenides and conductive polymers. 2003.

25. **Kadri Kriis**. Asymmetric synthesis of C_2-symmetric bimorpholines and their application as chiral ligands in the transfer hydrogenation of aromatic ketones. 2004.

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