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Applied Chemistry and Materials Science Faculty
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Nr. Decizie Senat _________din ____________________

PhD Thesis

Abstract

Materiale polimerice compatibile/mimetice cu substraturile biologice cu potențiale aplicații medicale

Compatible/mimetic polymeric materials with biologic substrates with potential medical applications

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Bucharest 2021

SUMMARY

ABBREVIATIONS

2PA – 2 photons absorption process
ABVN – 2,2'-Azobis (2,4 dimethylvaleronitryle)
ACV – 4,4′-Azobis (4-cyanovaleric acid)
DNA – Deoxyribonucleic acid
Ag/AgCl – silver / silver chloride reference electrode
AIBN – 2,2'-azobis-isobutyronitrile
RNA – Ribonucleic acid
Au – gold
BPO – benzoyl peroxide
BSA – bovine seric albumin
C_d – condenser
CHNS – carbon, hydrogen, nitrogen and sulfur analysis
Ch-Ph – chiral-photochromic compounds
CHPMA – 3-chloro -2-hydroxypropyl methacrylate
CNF – carbon nanofibers
CNT – carbon nanotubes
C_p – thermal capacity
CP – conjugated polymers
CPE – constant phase element
CV – cyclic voltammetry
DMAP – 4-dimethylaminopyridine
DMF – N,N-dimethylformamide
DMSO – dimethyl sulfoxide
DOP – dioctyl phthalate
DPE – diphenyl ether
DPEDA-Li – (N, N-diphenylethylenediamine) monolithic
DPPC – 1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DPV – differential pulse voltammetry
DSC – differential scanning calorimetry
*ds*DNA – double stranded DNA
EDC – N- (3-dimethylaminopropyl) -N'-ethylcarbodiimide hydrochloride
EDX – energy dispersive X-ray spectrometry
E_f – final potential
E_i – initial potential
EIS – electrochemical impedance spectroscopy
E_{pa} – the maximum potential of the anode
E_{pc} – the maximum potential of the cathode
FET – field effect transistor
FID – free induction decay
FILi – 9 fluorenilithium
FIR – far infrared
FRET – fluorescence resonance energy transfer
FT-IR – Fourier transform infrared spectroscopy
GA – glutaraldehyde
GC – gas chromatography
GOx – glucose oxidase
HCSA – (+) - / (-) - camphor sulfonic acid
HDPE – high density polyethylene
HPDB-R – 4 - [(4-hydroxyphenyl) diazenyl] benzo-R
HTAB – hexadecyltrimethyl ammonium bromide
I_C – capacitive current
I_f – faradic current
I_{pa} – anodic maximum current
I_{pc} – cathodic maximum current
LC – liquid crystal
LCD – liquid crystal display
LCST – lower critical solution temperature
LDPE – low density polyethylene
LEC – light-emitting electrochemical cells
LED – light emitting diodes
LiCl – lithium chloride
LPO – lauryl peroxide
MB – Methylene Blue
Metal/PMMA/PET – electrode obtained from metallized electrospun PMMA fibers PET foil
Metal/Ti/SiO_2/Si – electrode obtained from metallized silicon/silicon dioxide wafers
mm – isotactic fraction
MPA – multi-photon processes
mr – atactic fraction
MUA – 11-mercaptoundodecanoic acid
NAM – N-acryloyl morpholine
NHS – N-hydroxysuccinimide
NIPAM – N-isopropyl-acrylamide
NIR – near infrared
NLC – nematic liquid crystal
NMMAM – N-methyl methacrylamide
NMNVA – N-methyl-N-vinyl acetamide
NPV – normal pulse voltammetry
OCP – open circuit potential
ODN – oligonucleotide
NLO – nonlinear optics
PC – photonic crystal
PET – polyethylene terephthalate
Ph3P – triphenylphosphine
PMMA – poly (methyl methacrylate)
PNIPAM – poly (N-isopropyl acrylamide)
ppm – parts per million
PPV – poly (p-phenylene vinyl)
PS-ODN – phosphorothiolated oligonucleotide
PVC – Polyvinyl chloride
PVK – Poly (N-vinyl carbazole)
RAFT – Reversible addition−fragmentation chain-transfer polymerization
RF – radio frequency
NMR – Nuclear magnetic resonance
R_p – polymerization rate
rr – syndiotactic fraction
RU – resonance units
R_Ω – ohmic resistance
SEM – scanning electron microscopy

SPR – surface plasmons resonance
*ss*DNA – single stranded DNA
SSL – solid state lighting
tBDB – tert-butyl dithiobenzoate
Tc – crystallization temperature
TEA – triethylamine
TEMED – tetramethyl ethylene diamine
Tg – glass transition temperature
THF – tetrahydrofuran
Tm – melting temperature
TrMA – trityl methacrylate
UV-Vis – ultraviolet-visible spectroscopy
Z_f – faradic process impedance
Z_w – impedance Warburg

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INTRODUCTION

The most common materials found both in nature and as synthetic products are organic compounds. Although their applicability is wide, specialized materials for optics and photonics have recently gained the interest of researchers.

In the last two decades, the study of materials with unconventional properties has stimulated the development of research in the field of manufacturing new materials with structure / composition that allow the control of properties. Thus, material structures that allow direct control of optical properties as well as materials for the field of sensors are already established.

Although until the middle of the last century, for electro-optical applications, the classic was the use of inorganic materials, respectively their composites, after 1960 with the new industrial revolution, organic materials are imposed in the field of specialized applications. This orientation was the consequence of structural / compositional features that give them flexibility and/or application versatility. In addition, this period was also marked by the acceleration of the industrial launch of a new class of organic-polymeric materials. What differentiates the class of polymers in the large organic family to which it belongs, is the level of thermo-mechanical properties that are an immediate consequence of the molecular size. Moreover, the beginning of the ’80s and’ 90s completes the studies of the composition-size-property relationship by substantiating some behavioral peculiarities under the action of electromagnetic stimuli, pH, polarity, etc.

Historically, for the science of polymers is the peak of evolution, especially since it outlines similarities with natural giant molecules such as nucleic acids which led to their use in the manufacture of intrusive / non-intrusive devices for diagnosing various diseases.

The field of photonics as well as that of sensors is practically "conquered" by the class of polymers which offers several advantages compared to classical inorganic materials, respectively to inorganic / organic assemblies and they refer to:

- mechanical flexibility that guarantees long life-time
- structural / compositional versatility
- relatively high thermal resistances, aspect that allows their use in devices with cyclic operation accompanied by thermal effects
- “own memory”- the storage of one's own history, as well as the action of external stimuli
- response to the action of stimuli, modulated both at the temporary level and at the level of property characteristic: amplitude, intensity, frequency, direction, response time, etc.
- the possibility of modulating the level of properties through minimal architectural changes, etc.

Thus, the elucidation of the structure and action of nucleic acids, created the premise of the development of synthesized materials that in material assemblies with DNA (RNA) stimulate / generate specific properties, useful in the field of biophotonics and biosensors.

Interest in the development of wearable sensors and biosensors has grown in recent decades due to the need to obtain real-time information on changing the physiological processes of living organisms through a non-invasive approach. One of the challenges of making these wearable devices is the quantification of biomarkers (including electrolytes) in sweat. Such a device involves the integration / immobilization of a recognition element or ligands of ionic complexation at the sensor surface. On the other hand, electrolyte sensors in clinical diagnosis require high selectivity and sensitivity.

Starting from all these general evolutionary aspects in materials science, the doctoral thesis addresses the synthesis of (co) polymers of N-substituted amides, monomers and polymers based on carbazole chromophores, polymer-DNA assemblies and verifying the applicability of these materials in the field of sensors. Polymeric platforms and wearable (bio) sensors for electrolyte quantification have also been developed.

Thesis structure

The doctoral thesis entitled "***Polymeric materials compatible / mimetic with biological substrates with potential medical applications***", is structured in two parts and it is composed of 12 chapters.

The first part, which includes the first three chapters, is dedicated to the documentary study, which highlights essential aspects of construction, testing and implementation of polymeric materials compatible / mimetic with biological substrates with potential medical applications.

Part second part - is structured in nine chapters (Chapters 4-12) and details the original contributions on the synthesis and characterization of new DNA-based materials with biomimetic abilities, as well as the principles of their use in the development of biosensor devices.

The doctoral thesis includes 147 figures, 36 tables and 320 bibliographical references, and the results obtained were capitalized by publishing 6 articles, out of which 2 in the UPB Bulletin and 4 in ISI-listed journals. The results were presented at 11 scientific events, out of which 3 national and 8 international (10 oral presentations and 3 posters).

Below are presented the main results obtained during the doctoral stage. In this abstract the numbers of chapters, subchapters, figures, tables and bibliographical references are kept as in the thesis.

CHAPTER 1. ORGANIC MATERIALS WITH APPLICATIONS IN OPTICS AND PHOTONICS

In this chapter the literature information of the moment that underlines the constitutive and functional features of organic materials with applications in optics and photonics, emphasizing the aspects related to multiphoton systems; biomolecules and derivatives; liquid and photonic crystals; conjugated polymers; types and principles of conjugation in the polymer structure; synthesis techniques are summarized.

The application field based on the exploitation of optical / photonic phenomena has been for a long time dominated exclusively by inorganic materials / assemblies, mainly due to their inertia to the action of external factors in the operating environment.

With the fundamentation / generalization of synthesis / supramolecular characterization techniques at the level of organic materials, researchers attention is directed towards the obtaining of structures with highly specialized properties. Among the advantages that differentiate them from the inorganic class are: transparency in the ultraviolet-visible domain (UV-Vis), obtaining structures with specific properties by controlled synthesis, solubility in a wide range of solvents, compatibility with biological substrates (DNA, RNA, collagen), structural flexibility, low loss of properties in aging, nonlinear optical properties (ONL), long response time, electrospinning, filming capacity, etc. [1]. Specialized applications require the existence of a local anisotropy to regulate / enhance the sensitivity of the material to the action of stimuli of interest. From a constructive point of view, it becomes necessary the structural insertion of sequences sensitive to external stimulus, which do not affect the fundamental properties of the material, but which ensure an adequate response. **Figure 1.1** shows the generalized operating principle of these materials.

Fundamental to the functioning of living structures are the induced assembly/self-assembly properties. They become evident in the detection, isolation and healing of wounds. In this context, a priority in the development of material science is becoming the development of compatible/mimetic assemblies with biological substrates. A particular aspect is the synthesis of the stereoregulated polymer materials. In addition, the special features of the electrical nature of the new materials make it necessary to adjust its sensitivity. Thus, polymeric zwiterions - mostly cationic / anionic - become ideal candidates for the controlled-directed release of active principles [3]. At the same time, the ability of these materials to organize into complex structures recommends them for gene therapy [4].

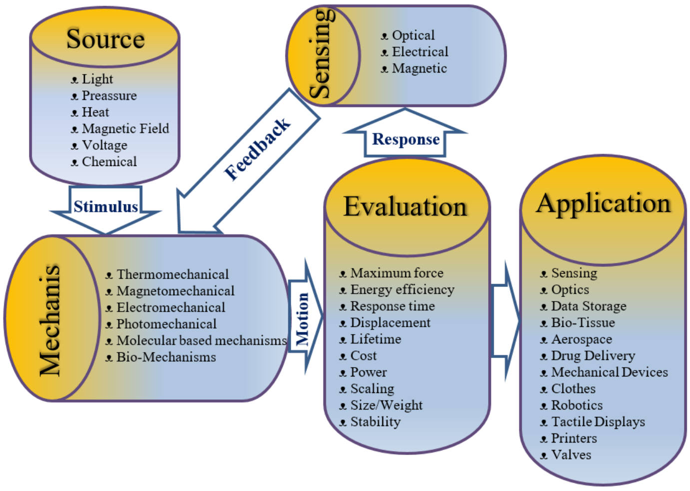

Figura 1.1: Principles of stimulus-response materials [5]

CHAPTER 2. POLYMERIC MATERIALS AND THEIR APPLICATIONS

In this chapter is particularized the information presented in the previous chapter by analyzing the experimental studies reported in the last two decades, with direct reference to the classes of materials and applications to be developed in the experimental study. Here are references to structural features of N-substituted amide polymers; polymeric biomaterials; carbazole-derived chromophores extending to application domains as light emitting diodes; polymeric actuators, field effect transistors.

It can be concluded that the design of the structure of polymers and their synthesis can lead to materials with specific properties depending on the applications of the devices considered.

CHAPTER 3. BIOSENSORS

This chapter, although very short, makes the connection between materials science and the way these devices are manufactured / operated.

Thus, many types of sensors and biosensors can be obtained using polymeric materials. The polymeric materials used in this field can have multiple roles such as substrate / platform, immobilizer / surface modification agent or, through the proper processing of certain types of polymers, the latter turning into a sensor. Both the polymers used in the construction of (bio) sensors and the methods of their functionalization / modification are determined by the proposed application.

RESEARCH OBJECTIVES

Polymers are the materials with the widest structural-compositional properties, and presents multitude of possibilities for synthesis and processing. The architectural structure of these materials continues to evolve, with the change of focus from compounds with exclusively synthetic composition to biological compositions such as polysaccharides or proteins [96]. The synthesis of stereoregulated polymers with assembly and / or self-assembly ability is the key point for obtaining materials with multiple applications able to generate novel and complex material structures based on DNA [189]. The structure of DNA allows various types of functionalization from hybridization to complexation reactions or even the binding of chromophore groups. Such materials can be used in the development of photonic or sensor-type devices for the detection of enzymes, antibodies, DNA sequences, etc. [159,190].

In this context, the main objective of this Ph.D. thesis was to obtain and to characterize polymeric materials based on N-substituted amides containing chromophores and / or DNA that can be used to develop devices with applications in sensors or photonics.

Thus, in the begining, new polymeric materials suitable for sensory devices were synthesized following the specific objectives:

► Synthesis of N-substituted amide homopolymers and their kinetic and structural characterization to determine the optimal synthesis conditions in order to obtain isotacticity;

► Synthesis of copolymers of N-acryloyl morpholine with N-methyl N-vinyl acetamide or 3-chloro 2-hydroxypropyl methacrylate and their kinetic and structural-compositional characterization to determine the copolymerization parameters and the tacticity of the obtained materials;

► Synthesis and characterization of new chromophore monomers based on carbazole rings and azo-benzene derivatives;

► Polymerization of chromophore monomers synthesized with N-substituted amides and determination of the influence of the reaction parameters on the obtained materials;

► Obtaining polymer-DNA systems and their kinetic, structural-compositional and dimensional characterization;

The second part had as general objective the development of sensors. For this purpose, two directions were followed: the development of materials for new types of electrodes and the modification of the surfaces of these electrodes in order to obtain sensors with specific applications.

In order to achieve this objective, the following specific objectives were taken into consideration:

► Development and electrochemical characterization of electrospun polymeric fibers for (bio)sensors fabrication and their comparison with silicon substrates;

► Functionalization of these new platforms obtained from electrospun polymeric fibers for sensors and biosensors manufacture;

► Development of sensors for detection and quantification of small molecules;

► Development of biosensors for enzymatic activity evaluation

► Development of biosensors for genetic mutations detection.

CHAPTER 4. MATERIALS AND DEVICES

This chapter presents the materials, reagents, equipment and characterization techniques used to carry out the experimental part of this thesis.

Because polymerization is a volume contraction reaction that can be quantified using dilatometry, this technique has been used to kinetically characterize new N-substituted amide-based materials and carbazole-azobenzene chromophores.

CHAPTER 5. N-SUBSTITUTED AMIDE HOMOPOLYMERS

The study developed in this chapter contributes to the completion of the kinetic structural-configurational acquaintances of the NAM and NMNVA polymers, respectively.

Thus the kinetic, compositional and structural aspects of the synthesis of N-substituted amide polymers – potential assembly materials with DNA structures or oligoprotein sequences, with complementary structure and configuration were investigated.

The values of $k_p/(k_t)^{0,5}$, determined in this study (**Table 5.1**), classify the NAM polimerization processes from the reaction point of view in the specific range of acrylamide / methacrylamide derivatives [225].

Tabel 5.1: Influence of the solvent on the kinetics of the polymerization reaction

Sample	**Solvent polarity**	**10^2 K_D**	**C_f (%)**	**R_p (mol/L s)**	$k_p/\sqrt{k_t}$
Poli(NAM) în 1,4-dioxan	4.8	10.00	1.1	7000 10^{-6}	0.130
Poli(NAM) în THF	4.0	5.22	13.0	3.440 10^{-4}	0.611
Poli(NAM) în toluen	2.4	2.00	3.4	3.500 10^{-5}	0.064
Poli(NMNVA) în 1,4-dioxan	4.8	2.60	7.7	1.033 10^{-4}	0.233
Poli(NMNVA) în toluen	2.4	2.20	5.9	7.100 10^{-5}	0.150

For all the experiments $[M]_0 = 2$ *mol/L;* $[I]_0 = 5 \times 10^{-3}$ *moli/L; T = 60 °C where:* K_D *contraction factor;* C_f *-final conversion; Rp – polymerization rate;* $k_p/\sqrt{k_t}$ *- the ratio between the propagation and termination constants*

The compositional-conformational analysis highlights, by proton NMR investigations, in the case of NMNVA, distinct regions for the two methyl zones of the substituent (α region - for methyl bound to the carbonyl group and β region for the one bound to the nitrogen atom) which prove different conformations relative to the base chain (**Figure 5.10**).

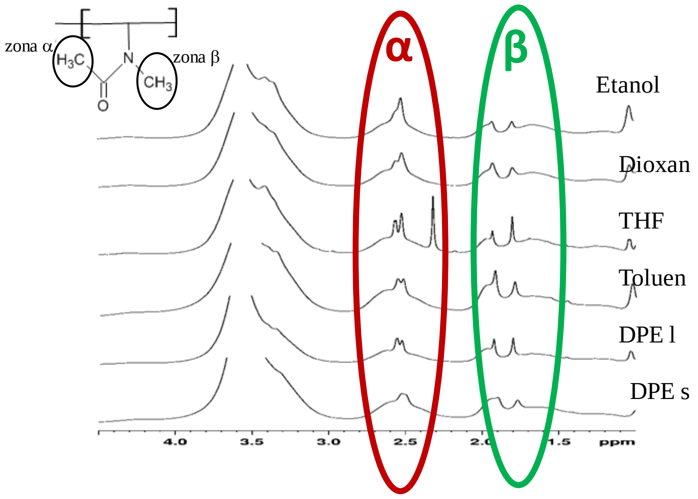

Figure 5.10: ^{1}H-NMR spectra (solvent DMSO) recorded for poly (NMNVA) synthesized in different solvents

CHAPTER 6. N-ACRYLOYL MORPHOLINE COPOLYMERS

Logically succeeding the polymerization study in this chapter are developed the synthesis and the characterization of N-acryloyl morpholine copolymers, in the attempt to optimize compositional-conformational synthetic material structure with the highest probability of complementarity with natural self-organized sequences.

The kinetic study underlines, under the same polymerization conditions, both the high reactivity of NAM and a more rigorous control of the stereostructure compared to the case of monosubstituted counterparts. This behavior can be justified by changing the energy charge of the intermediate radicals. Thus, the stability of the intermediate radicals is a consequence of the stronger donor effect, the reactivity of the macroradical being changed during the chaining process of the monomer units.

The PROCOP technique was used to evaluate the reactivity reports [232]. This technique, based on the use of exhaustive experimental data, uses the discrimination criterion F (**equation 6.8**) to optimize the value of reactivity reports.

$$F = \sqrt{\frac{\sum_{1}^{n}\left(m_{1}^{exp} - m_{1}^{calc}\right)}{n-p}} \tag{6.8}$$

where n represents the number of data points and p represents the number of reactivity ratios in the given kinetic model, m_1^{exp} *represents the experimentally determined copolymer composition (FT-IR and NMR) and* m_1^{calc} *represents the copolymer composition determined based on the reactivity ratios.*

The value of *F* is a discrimination criterion between calculation methods and an indicator of the quality of the experimental data. This optimization by *F* prioritizes the hierarchy of the values distribution obtained by the complementary methods Fineman-Ross (FR); Yezrielev- Brokhina- Roskin (YBR); Kelen-Tudos (KT); Tidwell-Mortimer (TM)) in full form (**Tabelul 6.3**) [232–237].

The compositional data, experimentally determined, allowed a discriminatory analysis of the most probable evolutionary kinetic model. The comparison of the terminal model with the antepenultimate model validates the terminal model kinetic. Consequently, the real values of the reactivity ratios are: r_{NAM} = 1,986; r_{NMNVA} = 0.572 and r_{NAM} = 1.119, respectively; r_{CHPMA} = 0.055. The macroradical with NAM terminal unit will promote homoadition.

Tabel 6.3: Reactivity ratio values obtained by applying differential methods to copolymers obtained from molar fractions of equal comonomers

			FR	***YBR***	***KT***	***TM***	***PROCOP***
NAM (1) - NMNVA (2)	75°C	r_1	1.264	0.926	0.930	1.631	1.986
		r_2	0.523	0.330	0.248	0.591	0.572
		$10^3 F_0^*$	63.768	78.821	86.746	58.494	-
		$10^3 F_c$	67.79	77.24	79.26	55.74	4.222
	85°C	r_1	2.159	0.858	1.857	1.998	3.680
		r_2	0.047	0.018	-0.056	0.058	0.102
		$10^3 F_0^*$	73.60	123.91	-	71.42	-
		$10^3 F_c$	149.65	174.42	-	143.30	9.26
NAM (1)- CHPMA (2)	75°C	r_1	1.298	1.184	1.125	1.309	1.119
		r_2	0.077	0.042	-0.003	0.072	0.055
		$10^3 F_0^*$	43.543	44.938	-	43.435	-
		$10^3 F_c$	53.87	50.40	-	55.03	25.16
	85°C	r_1	1.214	0.853	0.974	1.307	3.112
		r_2	0.019	-0.079	-0.170	0.039	0.039
		$10^3 F_0^*$	44.401	-	-	44.404	-
		$10^3 F_c$	44.93	-	-	43.45	140.47

r_1- reactivity ratio of NAM, r_2 -comonomer reactivity ratio, F_0^*- estimated discrimination factor, F_c - calculated discrimination factor

6.4. Stereo-structural characterization

The arrangement of the substituents relative to main chain axis is strongly affected by different factors such as: polarity of the reaction medium, monomer concentration; the ratio of the comonomers, as well as the working temperature. Generally, the decrease of the process temperature diminishes the freedom of rotational-translational movement of the constitutive molecular sequences, favoring the syndiotactic orientation (rr), relatively independent of the electrical nature of the solvent.

NMR spectra allow a quantitative assessment of the stereotactic disposition at the triad level, under the influence of temperature. Taking into account the composition of the substrate, there are two areas of independent arrangement of methyl groups in the amide structure of NMNVA: α for the methyl substituent in the carbonyl group, and β in the amide nitrogen atom (**Figure 5.10**).

Correlating the influence of the nature of the substrate with that of the temperature (**Figure 6.11**) there is a distinct evolution of the tactics at the level of the two regions α, respectively β. At low temperature values, the syndiotactic insertion (rr) is favored by the increase of the NMNVA fraction in the substrate. At 85°C for x_{0NMNVA}> 0.5 in the substrate the syndiotactic insertion is drastically decreased. The only explanation is the major change in the polarity of the environment.

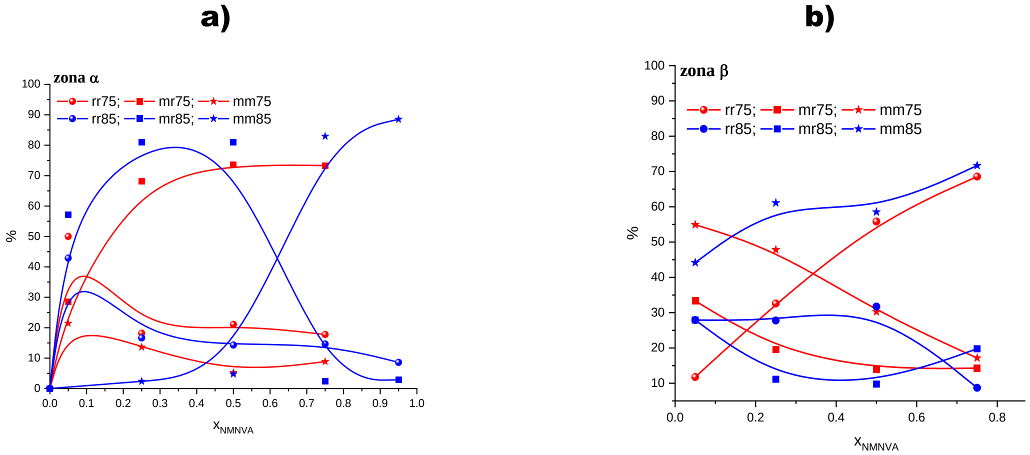

Figura 6.11: Variation of the polymer stereostructure: a) for the alpha region; b) for the beta region

6.5. Partial conclusions

NAM copolymerization products with NMNVA and CHPMA in 1,4-dioxane were characterized using FT-IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy. Synthesized from substrates throughout the compositional field, the products were constitutively characterized by elemental analysis, FT-IR spectroscopy and ^{1}H-NMR. The results obtained are similar, indicating a good agreement between the analytical methods and validating the values of the reactivity reports.

The copolymerization processes of NAM with NMNVA and CHPMA were investigated both compositionally and kinetically. The PROCOP optimization technique allowed the exact evaluation of the reactivity reports, validating the terminal kinetic model in the case of both systems. At the same time, the increased reactivity of the radical terminated in NAM regardless the partner (NMNVA/CHPMA) was highlighted, allowing the compositional assimilation of copolymer block products with short comonomer sequences. In fact, this is a feature of N-substituted acrylamides.

The structural-conformational analysis of copolymers emphasizes the influence of working parameters, thus the relatively simple synthesis methods can be prefigured with control of the orientation of the NMNVA configuration to propagation. This is due to the fact that the syndiotactic propagation is favored enthalpically when the working temperature decreases, while the isotactic configuration is governed entropically if the working temperature increases.

CHAPTER 7. CARBAZOLE-AZOBENZENIC MONOMERS AND POLYMERS

Based on the literature information, to diversify the material structures with NLO properties (by capitalizing the specific properties of natural biomolecules DNA and RNA), and on the compositional-conformational peculiarities of carbazole azo-derivatives (as a result of composing the specific properties of constituent sequences), in this chapter were performed studies concerning the particularities influence on the process of radical polymerization of NAM and NMNVA.

Starting from the methacrylic polymerizable sequence, two monomers with azo group hanging on the carbazole ring and methacrylic polymerizable group were synthesized, characterized and tested in polymerization (**Figure 7.2**).

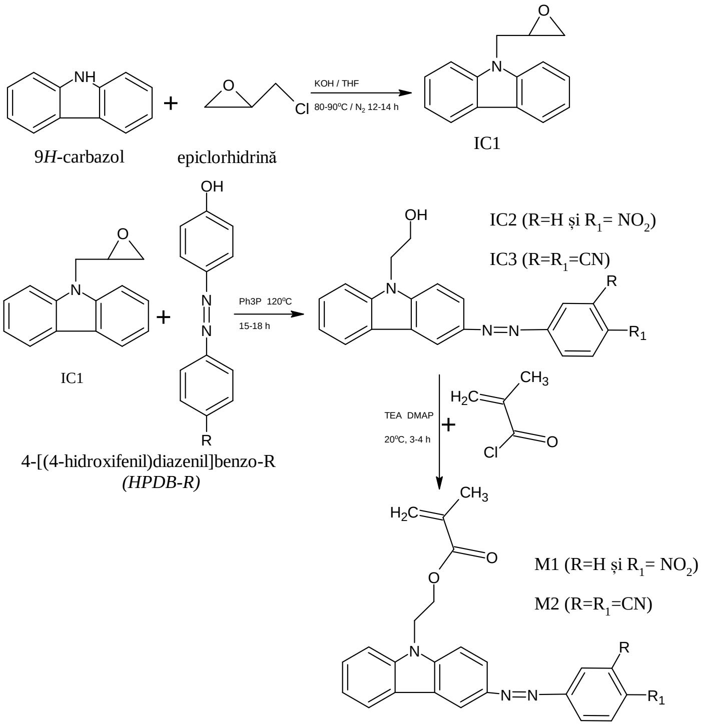

Figura 7.2: Monomer synthesis reactions

7.7. Partial conclusions

Based on the synthesis of carbazole chromophore monomers, the investigation of the kinetic behavior in (co)polymerization, as well as the particularities observed at the action of selected radiation (UV-Vis), it can be concluded:

The used synthesis method led to monomers with the desired structure. The advantage of this method is that the resulting monomer has a precise structure, even if its synthesis involves several steps and strict conditions which leads to a low reaction yield, and a slow polymerization due to bulky substituents.

The positions of the UV-Vis bands are not strongly affected by the length of the conjugate bridge or by the electronic nature of the substituent groups. The polarity of the solvent affects the width and intensity of the absorption bands and at the same time, they are shifted to longer wavelengths.

Comparing the absorption spectra of the synthesized materials was observed in the case of monomer M2 an increase in absorption with increasing polarity of the solvent, which suggests a higher mobility and thus a better reorganization of the alkyl chain around the carbazole ring.

Both NAM homopolymers and its copolymers with carbazole monomers as well as their mixtures define two types of behavior in polymerization reactions depending on the nature of the substituent.

NAM polymerization with carbazole comonomer is faster, with polymerization rates only one order of magnitude lower than in the case of homopolymerization of N-acryloyl morpholine. The reaction rate is not influenced by the concentration of carbazole comonomer. In contrast, when copolymerizing the M2 monomer with N-methyl N-vinyl acetamide, very low copolymerization rates are obtained, below the homopolymerization rates of the monomers.

Regarding the spectral characterization, if the substituent is NO_2 all transitions are stimulated with increasing solvent polarity as a consequence of the solvent-material interaction. The position and the intensity of specific signals are determined by the charge transfer interactions. These types of interactions can alter trans / cis photoisomerization and, as a consequence, the NLO response is affected. For CN substituents, the polarity only affects the intensity of the spectrum which decreases as the polarity of the medium increases.

This study may be useful as a tool to explain the property-structure correlation for these new polymers, being a first step in the optical characterization of the synthesized compounds.

These new chromophore polymers / copolymers are expected to interact with DNA to obtain biomaterials for photonics applications.

CHAPTER 8. CHARACTERIZATION OF MATERIALS WITH INCORPORATED DNA

A preliminary study of the synthesis of DNA-based material assemblies for applications in medical imaging and various branches of biophotonics is presented in this chapter.

Based on the kinetic and conformational-constitutive investigations, the present material argues at semiquantitative level a way to achieve the polymer-DNA assemblies starting from the NAM-NMNVA copolymerization.

Being a semi-quantitative investigation, for the compositional calculation as well as for the DNA binding percentage, the specific signals provided by the FT-IR analysis were used: with reference to the signal from 1111 cm^{-1}. Considering the variation of the vibrational intensities specific to the signal amide I from monomer units, the compositional calculation based on the relationships presented in **Table 8.2** was proposed. The percentage of DNA binding was calculated by the same method, this time using DNA spectrum signals as a reference.

Tabel 8.2: Equations used to determine molar fractions and binding percentage

Molar fractions	**Binding percentage**
$\frac{X_1}{X_2} = \frac{\Delta A_{m1}}{\Delta A_{m2}} \cdot \frac{M_2}{M_1}$	$\%binding = \frac{\Delta A_i^{3423} - \Delta A_i^{3493}}{\Delta A_i^{3423}}$
$\Delta A_{mi} = \frac{A_{mi}^{\ i}}{A_r}$	$\Delta A_i^{3423} = \frac{A_i^{3423}}{A_r}$
Where M_1, M_2 are the molar masses of the monomers	$\Delta A_i^{3493} = \frac{A_i^{3493}}{A_r}$
$A_{mi}^{\ i}$ – intensity of absorption specific to the monomer *i*	A_i^{3423} is the intensity of absorption from the spectrum of the compound *i* and the frequency 3423 cm^{-1}
Ar- intensity of reference absorption (1111 cm^{-1})	A_i^{3493} is the intensity of absorption from the spectrum of compound *i* from which the spectrum of DNA has been subtracted at the frequency 3493 cm^{-1}

The insertion of NAM into the copolymer is stimulated by the increase of polymer production. It is noted the advance of the insertion of NAM when ACV was used as initiator, both in the presence and in the absence of the stabilizer. The presence of DNA supports the formation of active reaction nuclei where the consumption of monomers is determined only by the concentration of free radicals (**Figure 8.5a**). This is also supported by the representation revealed in **Figure 8.5b** in which the compositions obtained in the absence of DNA are represented as unique points and characterized by low yield values. It can be said that the presence of DNA supports the creation of reaction centers in which monomer consumption is stimulated only by the concentration of the initial active centers. At the same time, this semiquantitative compositional evaluation supports the difference in reactivity of the two monomers, demonstrated in our previous studies, both in homo and copolymerization processes that support the formation of reaction zone in which monomer consumption is determined only by the concentration of active radicals [85].

Tabel 8.3. and **Figure 8.5** summarizes the values and the compositional evolution / degree of complexation with the degree of transformation / composition of the copolymer.

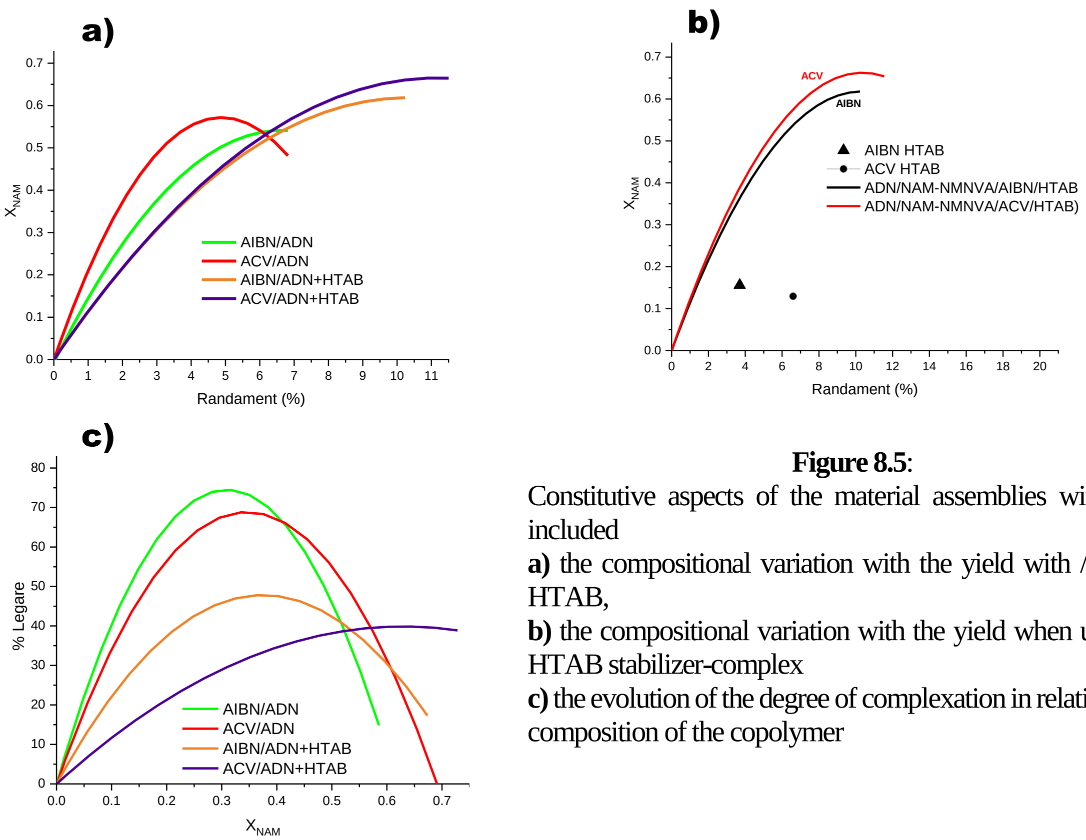

Figure 8.5:
Constitutive aspects of the material assemblies with DNA included
a) the compositional variation with the yield with / without HTAB,
b) the compositional variation with the yield when using the HTAB stabilizer-complex
c) the evolution of the degree of complexation in relation to the composition of the copolymer

One last argument, but not least, in favor of a micro heterogeneous process is the evolution of the percentage of complex by binding in relation to the NAM fraction (**Figure 8.2c**): there is a wider maximum (although weaker in intensity) in the case when HTAB was used. According to this evolution, for small NAM fractions, the number of ascending chains is higher than in the case of NAM fractions higher than 0.5. The decrease in "growth sites" is certainly the consequence of the bimolecular termination processes generated by the rapid consumption of the monomer in the "initial" cuacervates.

8.5. Viscozimetric study

The viscometric study performed for the synthesized materials aims to accentuate the molecular size importance in the assembly process.

Being unconventional materials, for which there are no data in the literature related to size or size-freedom of movement dependence, a rheological behavior study through monitoring the reduced viscosity with respect to the solution concentration (η_{sp}/c) was preformed.

The extrapolation of reduced viscosity to zero concentration to determine intrinsic viscosity [η] was not possible. Therefore the empirical processing using the Rao equation, useful / applicable for describing the viscosity of the dilute solution or of moderate concentration of neutral polymer solutions was used [257,258]. In all cases analyzed, the dependencies (η_{sp}/c- c) were linearized by applying the Rao equation to determine the relative viscosity values.

$$\frac{1}{2\left(\eta_r^{1/2}-1\right)}=\frac{1}{[\eta]c}-\frac{a-1}{2{,}5} \qquad (8.2)$$

where η_r is the relative viscosity, $[\eta]$ is the intrinsic viscosity and a is a specific constant for each solvent-polymer system.

$$[\eta]=k_H\,(M_V)^a \qquad (8.3)$$

The c^* ($1/[\eta]$) value obtained from both the Rao equation (**Figure 8.9**) and from the extrapolation, supports the idea that for large size materials (long polymer chains) (NAM-NMNVA // DNA // ACV) the ionic content is high while for small size materials (short polymer chains) (NAM-NMNVA // DNA // ACV // RAFT) ionic content is low. The high value in case of NAM-NMNVA // DNA // ACV // RAFT // HTAB is due to the probability of the existence of cuacervates such as emulsion-specific mycelium.

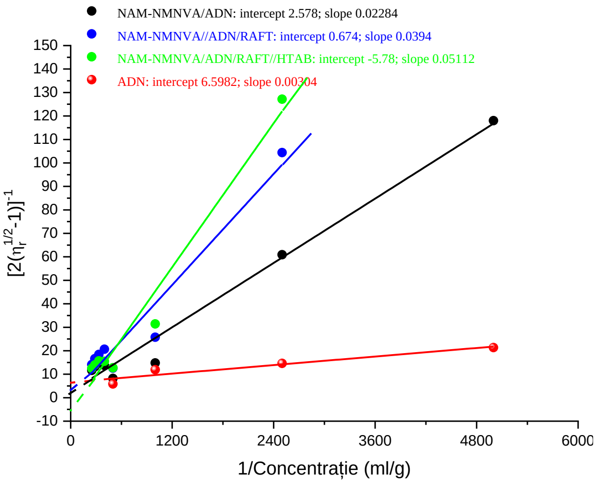

Figura 8.9: Graphical representation of the linearization resulting from the application of the Rao equation

Tabel 8.3: Viscosity values calculated from extrapolation (^{a}) and Rao equation (^{b})

Sistem	$[\eta]^a$ (mL/g)	Slope	k_H	$[\eta]^b$ (mL/g)	$c^*=1/[\eta]$	
NAM-NMNVA/ADN/ACV	38.3/ 33.1	1.679/ -0.292	11.45/ -2.663	43.8	$2.61\ 10^{-2}$/ $3.02\ 10^{-2}$	$2.28\ 10^{-2}$
NAM-NMNVA/ ADN/ACV/RAFT	23.91/ 21.89	0.944/ -0.108	16.52/ -2.254	25.38	$4.18\ 10^{-2}$/ $4.57\ 10^{-2}$	$3.94\ 10^{-2}$
NAM-NMNVA/ ADN/ACV/RAFT/HTAB	16.74/ 34.16	1.223/ -0.365	43.62/ -3.127	19.6	$5.97\ 10^{-2}$/ $2.93\ 10^{-2}$	$5.10\ 10^{-2}$
ADN				329		$3.04\ 10^{-3}$

8.6. Partial conclusions

The polymerization reaction is both, kinetically and efficiently influenced by the initiator. When a stabilizer is used, both initiators have the same behavior, and the use of HTAB favors the introduction of NAM units and favors the formation of stabilizer-DNA complexes.

Intermolecular interactions, understood as the possibility of supramolecular structuring, are favored by small macromolecules, which is generated at the beginning of the process when the number of molecular species is high. When these particles grow, the mobility is decreased and form micro-structures by segregation and the DNA-polymer interaction remains only at the micro-structural level.

The presence of DNA supports the formation of reaction nuclei, and the consumption of monomers is determined only by the concentration of active radicals, obtaining a maximum binding percentage for NAM fractions less than 0.3.

The rheological study of the diluted solutions of the synthesized materials shows that the materials have polyelectrolyte behavior and that the decrease in size and the presence of surfactants leads to an increase in the density and intensity of electrostatic interactions.

Therefore, it can be stated that by developing polymerizations in DNA media, complex, stable materials can be obtained, whose behavior is determined by the size of the resulting polymeric species.

CHAPTER 9. CHARACTERIZATION OF PLANAR AND FLEXIBLE ELECTRODES

This chapter opens the area of investigations to prefigure the manufacture of biosensors with DNA sequences, either on the basis of hybridization of complementary or partially complementary chains.

For a better understanding of the entire electrode manufacturing process, **Figure 9.1** shows the steps required to obtain them.

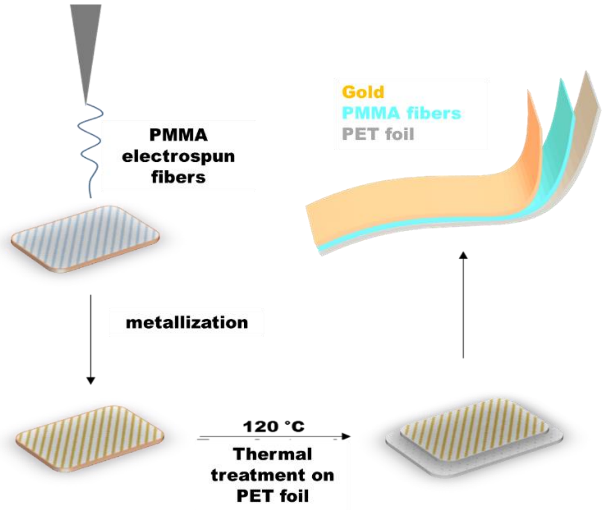

Figure 9.1: Steps of flexible electrodes fabrication

Also, in this chapter are presented: manufacturing techniques, morphological, electrochemical and electrochemical impedance spectroscopy characterization. Through the systematic approach of the general principles specific to the presented methods, the preliminary results in the realization of flexible biosensors are presented.

9.5 Morphological characterization

The micromorphology of flexible electrodes was investigated by scanning electron microscopy (SEM) and compared to that of planar electrodes Metal/Ti/SiO_2/Si (**Figure 9.9**).

Figure 9.9 shows a similar behavior for electrodes coated with gold and silver. It can be seen that Metal/Ti/SiO_2/Si electrodes **(Figure 9.9 A3, A4, B3, B4**) have a granular structure with a grain size of about 50 nm, uniform distributed over the entire surface. In the case of Metal/PMMA/PET electrodes (**Figure 9.9 A1, A2, B1, B2**) several layers of fiber network were observed, with fibers evenly distributed over the entire surface. The fibrillar structure of the polymers was constant with an average diameter of about 0.5 µm. The fibers are evenly coated with metal, which in turn maintained the granular structure with a grain size of about 20 nm. In the case of both substrates a difference appears in the case of palladium coating (**Figure 9.9 C1-4**) where the morphology of the metal is in the form of acicular structures with larger dimensions than in the case of the use of gold and silver.

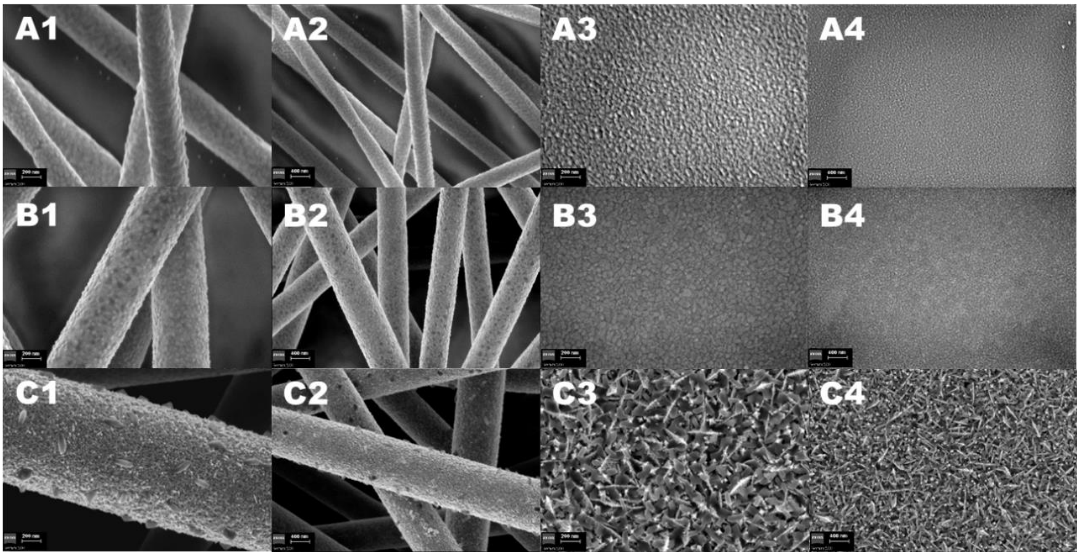

Figure 9.9: SEM images at different magnifications (50k and 100k) of Metal/PMMA/PET (1 and 2) and Metal/SiO_2/Si (3 and 4) electrodes metallized with silver (A), gold (B) and palladium (C)

9.3. Electrochemical characterization by cyclic voltammetry

9.3.1. Gold metallized electrodes

Au/PMMA/PET electrodes were characterized by cyclic voltammetry in 0.1 M H_2SO_4 and compared with Au/Ti/SiO_2/Si electrodes characterized under the same conditions, **Figure 9.10**.

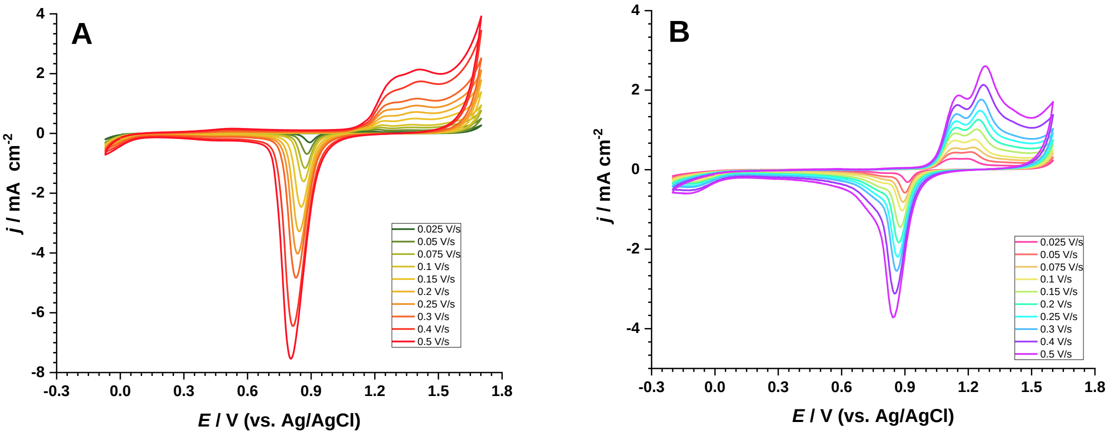

Figure 9.10: Cyclic Voltametry at different scan rates in 0.1M H_2SO_4 for A) Au/PMMA/PET and B) Au/Ti/SiO_2/Si.

The recorded voltamograms for both types of electrodes showed the typical behavior of gold electrodes. Two oxidation processes were observed on the anodic part of the voltammograms. The first observed process at potential values higher than +1.00 V, is due to the formation of the oxide / hydroxide layer. The peak observed at potential values higher than + 1.50 V, at the upper limit of the voltammogram characterize the oxygen oxidation. On the cathodic part, the maximum current at approximately + 0.80 V, is due to the reduction of the oxide / hydroxide layer, while at a more negative potential value the process of reducing the dissolved oxygen in the solution takes place.

The electrochemical behavior of the Au/PMMA/PET electrodes was further characterized in potassium hexacyanoferrate and compared to that of Au/Ti/SiO_2/Si, the electrode characteristics being shown in **Table 9.1** for each electrode.

The difference between the values of the anodic and cathodic potential maxima $\Delta E = E\text{pa} - E\text{pc}$ is higher than the ideal value of $57/n$ mV for the electrode fast kinetics and the difference increased with the scanning rate for both electrodes. The variations of the potential maximum with respect to the decimal logarithm of the scanning rate $dEp/d(\log(v)) = 29.6/an$ are shown in **Table 9.1**, resulting in the charge transfer coefficient a of 0.5 for Au/PMMA/PET and of 0.9 for Au/Ti/SiO_2/Si. The standard constants of electron transfer rate for both electrodes were also calculated and summarized in **Table 9.1**.

Table 9.1. Electroactive area, roughness factor (defined as the ratio between the electroactive area and the geometric area), the slope value in the graphs j vs. $v^{1/2}$ (for the anodic-b_a and cathodic-b_c process), the variation of the anodic maximum potential with the decimal logarithm of the scanning rate and the electron transfer rate constants.

Electrod	***electroactive area/ cm^2***	***Roughness factor***	**b_a / mA cm^{-2} (V s^{-1})$^{-1/2}$**	**b_c / mA cm^{-2} (V s^{-1})$^{-1/2}$**	***dE_{pa} / d(log(v)) /*** **mV decade^{-1}**	**$k_0 \times 10^3$/ cm s^{-1}**
Au/PMMA/PET	0,59	2,5	7,93	-6,1	60	72,6
Au/Ti/SiO_2/Si	0,13	0,5	1,75	-1,60	34	37,6

9.5 Partial conclusions

In this chapter the electrochemical properties of planar and flexible electrodes were investigated. The flexible electrodes were obtained by coating the electrospun polymeric fibers with metals and the planar ones by metallizing the Si/SiO_2 wafers.

Morphological characterization showed uniform metal coating of both types of surfaces regardless the method of metal deposition.

Baesd on the electrochemical characterization, it was concluded that the flexible electrodes obtained by metallization of polymeric fibers have an electroactive area about 5 times larger than that of planar electrodes.

Electrochemical impedance spectroscopy was used to investigate the physical and interface properties of the electrodes in phosphate buffer solution and the results were consistent with those obtained from cyclic voltammetry suggesting a diffusion-controlled process.

CHAPTER 10. IONOPHORE-BASED SENSORS FOR DETECTING IONS FROM BIOLOGICAL FLUIDS

The objective of this chapter was to investigate the electrochemical properties of immobilized ionophores on the surface of planar / flexible electrodes to develop ionophore-based sensors for electrolytes quantification in sweat.

The steps required to fabricate these sensors are shown schematically in **Figure 10.1**.

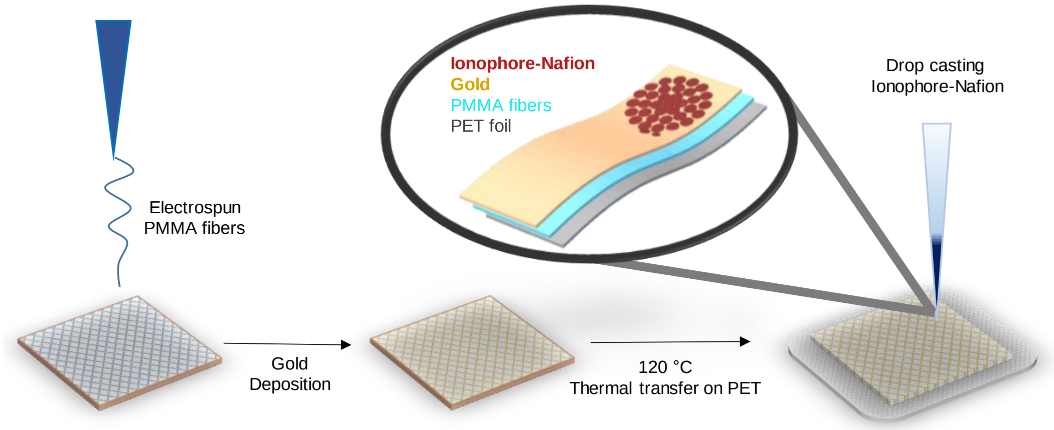

Figure 10.1: Schematic representation of the steps required for the manufacture of ionophore-based sensors on Au/PMMA/PET electrodes

Thus, this study is dedicated to obtaining modified sensors with ionophores whose characterization required the use of specialized material techniques: SEM-EDX, infrared spectroscopy and techniques specific to demonstrate their operation: cyclic voltammetry, potentiometry and electrochemical impedance spectroscopy.

10.3. Applications of flexible sensors in artificial sweat

10.3.2. Potentiometric sensors for the determination of Ca^{2+} in artificial sweat

The evaluation of Ca^{2+} ions using the Ca^{2+} ionophore immobilized in a nafion matrix on the surface of Au/PMMA/PET electrodes involved the potentiometric measurement of the open circuit potential (OCP) value in an electrochemical cell configuration.

The typical response of the Ca^{2+} ionophore measured in artificial sweat (AS) is shown in **Figure 10.11**. **Table 10.5** summarizes the sensitivity and detection limit for each ionophore-modified sensor.

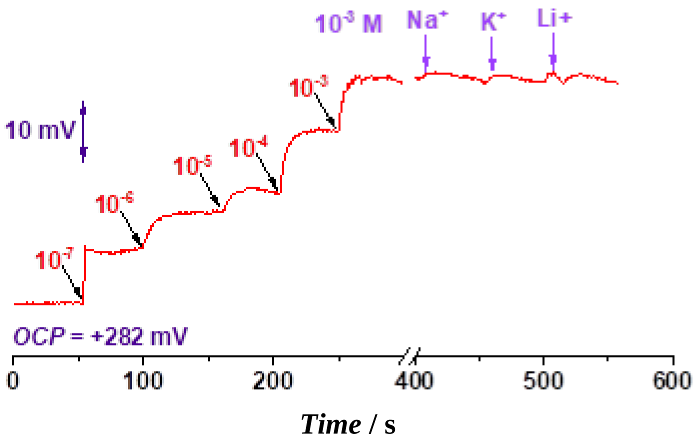

Figure 10.11. The potentiometric response of the Ca^{2+} ionophore immobilized in a nafion matrix on the surface of the Au/PMMA/PET electrode in A.S. at successive injections of different concentrations of Ca^{2+} ions and interferences

Table 10.5. Sensitivity and detection limits of sensors with Ca^{2+}, NH_4^{+}, Cl^{-} and H^{+} ionophores in artificial sweat and other electrolytes

ionophore	medium	Sensitivity / mV per decade	LOD / mol L^{-1}
Ca^{2+}	0,1 M KCl	24.6	4.7×10^{-7}
	A.S.	6.5	1.4×10^{-8}
NH_4^{+}	A.S.	12.9	1.1×10^{-7}
Cl^{-}	0,1 M K_2SO_4	-9.54	1.5×10^{-6}
	A.S.	-8.12	5.4×10^{-6}
H^{+}	A.S.	55.4	7.6×10^{-7}

10.4. Partial conclusions

In this chapter, the electrochemical properties of ionophores immobilized on the surface of planar/flexible electrodes were investigated to develop an ionophore-based sensor for the analysis of electrolytes in sweat. Flexible electrodes were obtained by coating electrospun polymeric fibers with gold. The ionophores were immobilized on the surface of these electrodes in different matrices.

Infrared spectroscopy showed that the Ca^{2+} ionophore can be immobilized in the nafion matrix, maintaining its conformation. SEM-EDX investigations have shown that the immobilization matrix and ionophore are oriented along the fiber direction, which opens up the prospect for target ion detection using an ionophore-based configuration immobilized in the nafion matrix. At the same time, cyclic voltammetry and electrochemical impedance spectroscopy have shown that the ionophore allows the diffusion of target ions through this membrane by changing the electrical properties of the device.

To demonstrate the concept of flexible ionophore-based sensors for the analysis of sweat electrolytes, ionophores for Ca^{2+}, NH_4^+, Cl^- and H^+ were immobilized in a nafion matrix on the surface of Au/PMMA/PET flexible electrodes. The evaluation of the target ions was performed by potentiometry in different media, including artificial sweat, with adequate values of sensitivity, detection limits, selectivity and selectivity coefficients.

CHAPTER 11. BIOSENSORS FOR ENZYMATIC ACTIVITY DETERMINATION

This chapter describes the development and characterization of flexible electrodes obtained from conductive polymeric fibers coated with metals and their applications in biosensing. This type of flexible electrodes can be further integrated into wearable devices for continuous monitoring of analyzes in biological fluids. In this context, the chapter is divided according to the methods of electrochemical characterization of these electrodes, following the proposed applications for these platforms i) detection and quantification of hydrogen peroxide and ii) immobilization of glucosoxidase and detection of glucose.

11.2. Glucose detection by glucosoxidase. Glucose biosensor

11.2.2. GOx/Au/PMMA/PET biosensor applications

The GOx/Au/PMMA/PET biosensor was used to detect glucose in 0.1 M phosphate buffer solutions pH = 7.0 by consecutive injections of different glucose concentrations, at an applied potential of -0.20 V, the optimal value of the potential for the detection of hydrogen peroxide, as previously demonstrated. Less interference occurs during the cathodic interval and for this reason, operationally, negative values of the potential for blood glucose are preferred.

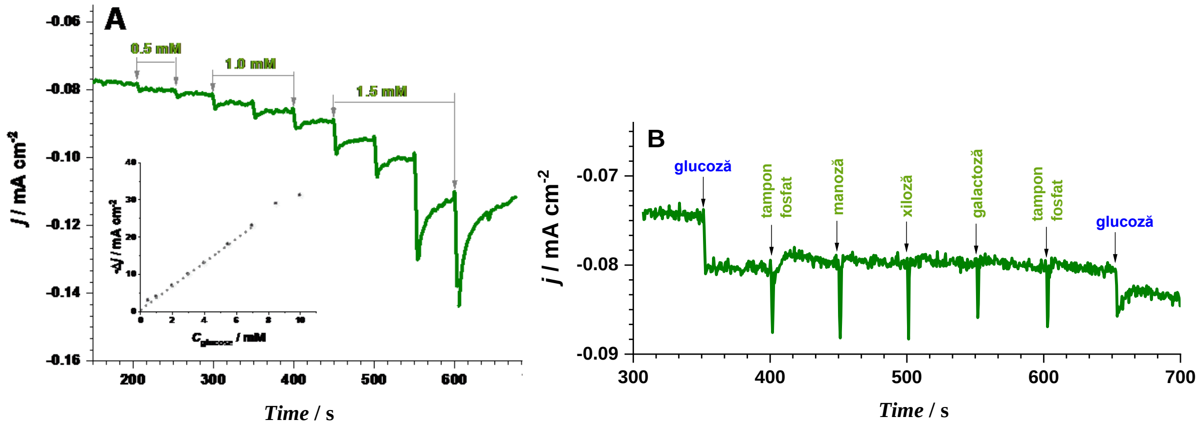

Figure 11.6. The chronoamperometric response of the GOx/Au/PMMA/PET biosensor measured at -0.20 V in phosphate buffer solution pH = 7 at successive injections of A) different glucose concentrations and B) 1.0 mM glucose and interferences.

The recorded amperogram, **Figure 11.6A**, showed a cathodic change in current after glucose injection, suggesting that the working principle of the biosensor is based on the detection of peroxide formed on the electrode surface during the enzymatic reaction. The current increased proportionally to the glucose concentration and linearity was observed up to about 8 mM. The sensitivity of the calibration curve (**Figure 11.6A**-insertion) was 3.10 ± 0.06 mA cm^{-2} mM^{-1} (RSD = 8.3%, n = 3) with a detection limit of 0.33 ± 0.05 mM (RSD = 6.8%, n = 3). The interference study was performed for the GOx/Au/PMMA/PET biosensor, **Figure**

11.6B, and showed insignificant interferences for compounds such as mannose, galactose and xylose.

11.3. Partial conclusions

This study describes the electrochemical properties of electrodes obtained from conductive electrospun polymeric fibers and their applications for biosensing. These electrodes were obtained by electrospinning a solution of poly (methyl methacrylate), leading to submicrometric fibers that were coated with a layer of gold and attached to a flexible polyethylene terephthalate substrate. The performance of gold-coated electrophilated submicron polymeric fibers, as a new electrode platform for biosensitivity applications, has been demonstrated by fixed-potential amperometry at -0.20 V (vs. Ag/AgCl) and quantification of: i) hydrogen peroxide with a sensitivity of 6.14×10^{-6} mA cm^{-2} nM^{-1} and a detection limit of 127 nM; and ii) glucose, after immobilization of glucose oxidase, with a sensitivity of 3.10 µA cm^{-2} mM^{-1}, a detection limit of 0.33 mM and reduced interference.

The technology described for obtaining conductive polymeric fibers and flexible electrodes is important for obtaining wearable devices for continuous monitoring of various analytes to provide a real-time profile of the physiological state of an organism.

CHAPTER 12. BIOSENSORS FOR GENETIC MUTATIONS DETECTION

This chapter is dedicated to biosensors for the detection of genetic mutations and highlights particular aspects in the preparation of surfaces and manufacturing techniques. In addition to the morphological characterization, exploratory analytical composition techniques were used: RAMAN spectroscopy, surface plasmons resonance, XPS respectively.

The strategy used to investigate hybridization reactions in the case of ODN-based biosensors includes three steps, which are: i) immobilization of the oligonucleotide probe on the surface, ii) hybridization with the complementary chain and finally iii) transduction (**Figure 12.1**).

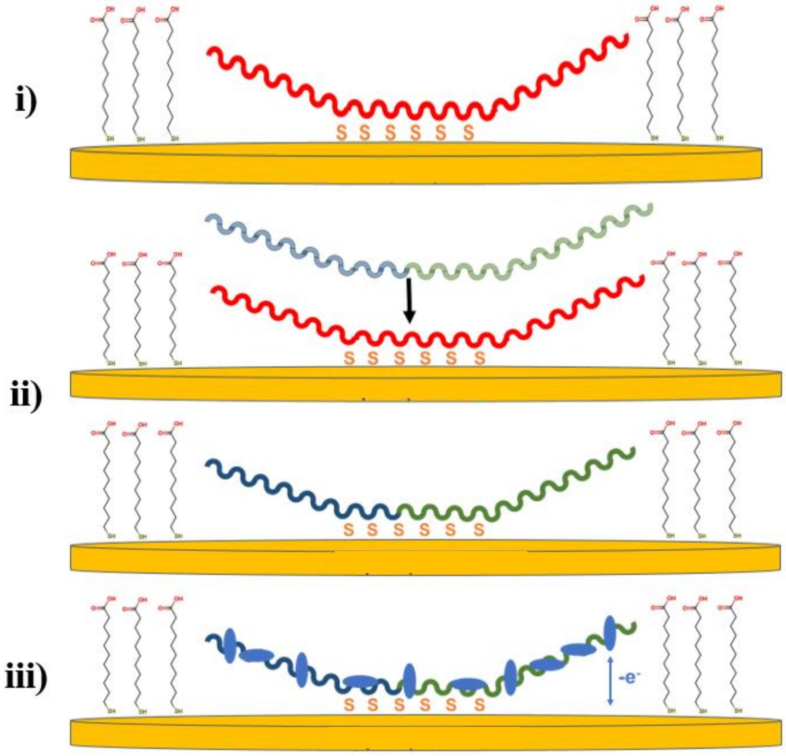

Figure 12.1. The manufacturing steps of the surface-adsorbed ODN biosensor: i) probe immobilization, ii) complementary chain hybridization and iii) transduction (interaction with MB)

The objectives of this study were to obtain two biosensors: a biosensor for detecting the fused sequence in BCR / ABL genes specific to form b2a2 and a second biosensor for detecting the fused sequence in BCR / ABL genes specific for form b3a2, both forms being BCR / ABL biomarkers of chronic myeloid leukemia.

12.2.1.3. Surface Plasmon Resonance

The sensograms obtained by the SPR measurements are presented in **Figure 12.5**. It can be observed the dependence of the adsorption on the concentration of PS-ODN used as well as the fact that in the case of the control there is no adsorption on the gold surface. Thus, it was demonstrated the importance of sulfur groups in the oligo #2 structure in its immobilization on the gold surface.

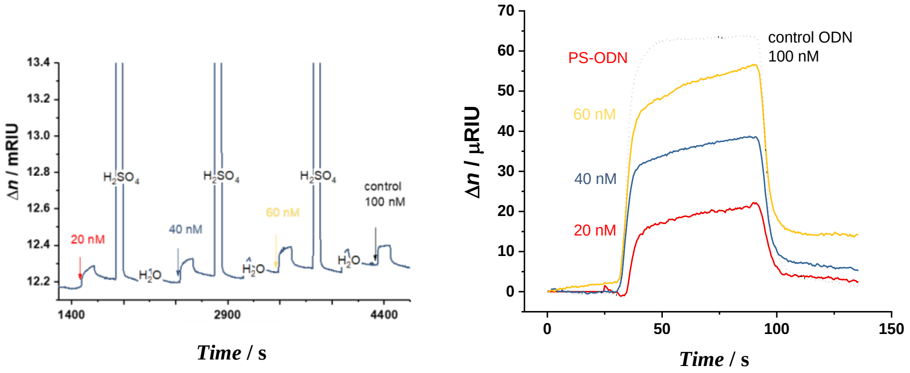

Figure 12.5. SPR sensograms obtained for PS-ODN oligo # 2 adsorption at different concentrations and control performed with unmodified oligonucleotide (oligo # 7)

In order to highlight the effect of the complementarity of the sequences used in the hybridization stage, the results obtained for each case were compared. (**Figure 12.9**). Thus, an increase in current can be observed as the degree of complementarity of the used oligonucleotide increases.

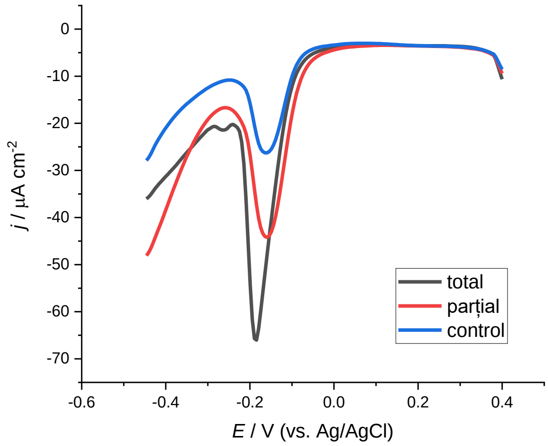

Figure 12.9: Comparison of voltamograms obtained on the passivated sensor after hybridization with **fully complementary**, **partially complementary** and **control** ODN

12.3. Genosensor for detection of BCR / ABL fused gene sequence specific to form b3a2 on Au / PMMA / PET substrate

12.3.1 Surface immobilization of the probe

Table 12.2 shows the DNA sequences required for the construction and testing of the biosensor for the a3b2 probe system-b3a2-specific sequence.

Table 12.2: DNA sequences required for the construction and testing of the biosensor for the detection of the BCR / ABL biomarker of chronic myeloid leukemia

Name	Sequence
Oligo #1	5'-GCT GAA GGG C*T*T*T*T*G* AAC TCT GCT TA-3' Probe to be immobilized to obtain the biosensor (probe a3b2)
Oligo #3	5'-TAA GCA GAG TTC AAA AGC CCT TCA GC-3' The sequence of the BCR / ABL fused gene is specific to the b3a2 form
Oligo #5	5'-TAA GCA GAG TTC AAA TCT GTA CTG CA-3' ABL gene sequence
Oligo #7	5'-CTG TTA TCT GGA AGA TCT GTA CTG CA-3' Non-complementary control sequence

12.3.1.1. Morphological characterization

The investigation by scanning electron microscopy (**Figure 12.10**) of the flexible sensors led to completely different results compared to the results obtained for Au/Ti/SiO_2/Si electrodes.

Thus, the PS-ODN islands observed in the case of Au/Ti/SiO_2/Si are no longer visible the compositional maps obtained by X-ray spectrometry with energy dispersion showing that the adsorption of PS-ODN is realized only on the gold surface, along the fibers (maps for PS-ODN components, phosphorus, nitrogen, sulfur (**Figure 12.10.D-F**).

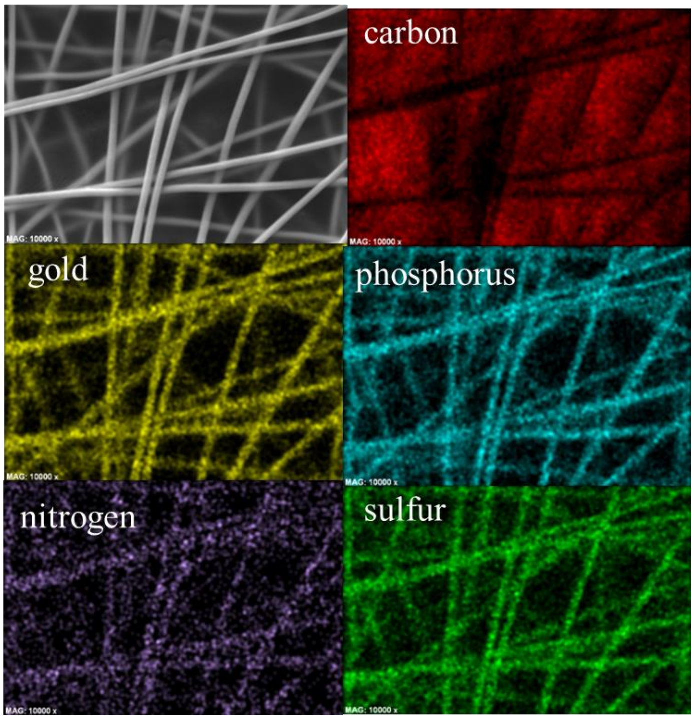

Figure 12.10. SEM images and compositional maps of the Au/PMMA/PET surface functionalized with PS-ODN **A**) analyzed area and map **B**) carbon, **C**) gold, **D**) phosphorus, **E**) nitrogen, **F**) sulfur

12.3.2. Complementary sequence hybridization and transduction

In the case of electrospun polymeric fibers used as a substrate, it was observed that it is not necessary to passivate the surface, PS-ODN being adsorbed on the length of the fibers, the coating being uniform. Differential pulse voltammetry (DPV) measurements were also performed on sensors modified with oligo #1 PS-ODN subjected to oligo #3 ODN hybridization and methylene blue interactions. The obtained results are presented in **Figure 12.13**. The linearization of the current was performed depending on the ODN concentration used in the hybridization step. The detection limit obtained for biosensors manufactured on flexible PMMA/PET support was 0.09 pM, below the detection limit obtained when using the passivated Au/Ti/Si/SiO_2 substrate.

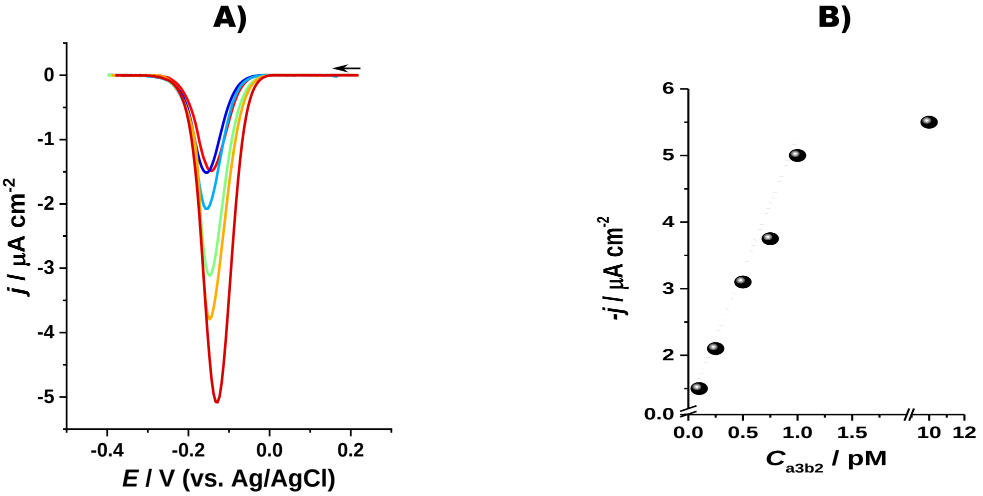

Figure 12.13: A) Voltamograms obtained on the Au/PMMA/PET biosensor and B) Peak current variation with oligo #3 concentration

12.4. Partial conclusions

This chapter describes the production of biosensors for the detection of genetic mutations and is based on the functionalization of electrodes with various sulfur-modified oligonucleotides.

The study was performed comparatively for two types of Si/SiO_2 substrates and electrospun polymeric fibers, both metallized with gold. The most important aspect demonstrated was that in the case of the use of electrospun PMMA fibers is not necessary to passivate the surface.

By functionalizing the surfaces with oligonucleotides, regardless the substrate used, the hydrophilicity of the surface increases as a result of the functionalization. Concerning the performance of the manufactured biosensor, it was found that the biosensor obtained on a flexible substrate is easier to manufacture in terms of the required steps and has lower detection limits.

C.1. GENERAL CONCLUSIONS

The field of materials is constantly evolving in order to obtain new materials with improved and specific properties for different applications, from the medical field to food, electronics, photonics and much more. By designing new controlled syntheses in order to insert functional groups, structural properties can be induced in compounds that can lead to stimulus-response materials driven by certain external factors. Two important aspects are biocompatibility and biodegradability. A suitable material in this regard is DNA, which comes from renewable resources that have a multitude of interesting properties for the field of photonics and sensors.

The aim of this PhD thesis consisted in the synthesis and the characterization of new materials obtained by controlled syntheses in order to induce specific properties. In this sense the main conclusions are:

Polymers of N-substituted amides (N-acryloyl morpholine and N-methyl N-vinyl acetamide) were synthesized and the optimal reaction conditions were identified based on the understanding of the influence of the reaction parameters (solvent polarity, initiator, reaction temperature) on the structure of the polymers obtained. It was concluded that the polarity of the solvent affects the polymerization of the monomers from a kinetic, dimensional and structural point of view.

The two monomers have different kinetic behavior and their polymerization rates are influenced by the nature of the solvent, although in both cases the highest conversions were obtained when 1,4-dioxane was used as the solvent.

Another factor that influences the stereostructure is the nitrogen atom in the amide group of both monomers. This leads to the formation of hydrogen bonds with the carbonyl group resulting in decreased syndiotactic specificity. Also, the thermal properties are affected by the stereostructure and it has been shown that for syndiotactic fractions below 50% there are no changes in the transition from amorphous to crystalline state.

Based on the information obtained from the homopolymerization of N-substituted amides, copolymerization reactions were performed and the reactivities of the monomer pairs were calculated. NAM copolymerization products with NMNVA and 3-chloro-2-hiroxypropyl methacrylate were characterized both structurally and compositionally. The composition of copolymers synthesized in a variety of monomer ratios in the substrate was determined by elemental organic analysis, FT-IR and ^{1}H-NMR spectroscopy leading to similar results, which indicates a good agreement between analytical methods and validates the values of reactivity reports.

Kinetically, the estimation of the reactivity ratios indicates that the macroradical terminated in NAM monomer unit ($r_{NAM} = 1.986$ and $r_{NAM} = 1.119$, respectively) is more reactive than that terminated in NMNVA units ($r_{NMNVA} = 0.572$) or CHPMA ($r_{CHPMA} = 0.055$). The reactivity ratios were evaluated by different differential methods that take into account several reaction parameters, and the best correlation was obtained by the Tidwell-Mortimer method. Concerning the kinetic model, the terminal mechanism is the one that fits the results. For both systems analyzed, the composition of the copolymers is of copolymer block type with short comonomer sequences.

The characterization of the copolymers aimed to highlight the structural features induced by the temperature and comonomers used together for NAM. For NAM-NMNVA systems it can be concluded that a relative stereochemistry of NMNVA units is obtained. This study showed that syndiotactic propagation is favored by increased enthalpy.

Another objective of this doctoral thesis was the synthesis of new chromophore monomers with carbazole groups and azobenzene derivatives. An advantage of the method used is that the resulting monomers have the desired structure, although their synthesis involves multiple reactions and strict conditions. The polymerization of these monomers is slow due to the voluminous substituents.

Spectral investigations have shown that the length of the conjugate bridge or the electronic nature of the substituent groups does not affect the positions of the UV-Vis bands, which are instead influenced by the polarity of the solvent used. Increasing the polarity of the solvent leads to an increase in absorption, which suggests greater mobility and better reorganization of the alkyl chain around the carbazole ring.

Chromophore monomers were copolymerized with NAM and NMNVA, observing two types of behavior in polymerization reactions depending on the nature of the carbazole monomer substituent. Thus, by copolymerization with N-acryloyl morpholine, polymerization rates by only one order of magnitude lower than in the case of homopolymerization of N-acryloyl morpholine are obtained, the reaction rate being not influenced by the concentration of carbazole comonomer. In contrast, when copolymerizing the M2 monomer with N-methyl N-vinyl acetamide, the copolymerization rates obtained were very low being below the homopolymerization rates of the monomers.

Following the spectral characterization, for the monomer with NO_2 substituent all transitions are stimulated by the increase of the solvent polarity as a consequence of the solvent-material interaction. The position and intensity of specific signals are determined by the load transfer interactions. These types of interactions can alter trans / cis photoisomerization and, as a consequence, the ONL response is affected. For CN substituents, the polarity only affects the intensity of the maxima which decreases as the polarity of the medium increases.

To obtain biomaterials for photonics applications, new chromophore polymers / copolymers were tested to highlight the types of interactions that can occur in the presence of DNA. The first aspect investigated was the influence of the initiator on kinetics and productivity. Because the behavior was different for the two initiators (ACV and AIBN), hexadecyltrimethyl ammonium bromide was used as a stabilizer (surfactant). The use of the stabilizer leads to similar behaviors for the two initiators, favoring the introduction of NAM units and leads to the formation of stabilizer-DNA-chromophore complexes.

Although the presence of DNA supports the formation of reaction centers and the consumption of monomers is determined only by the concentration of active radicals, a maximum percentage of binding was obtained only for NAM fractions smaller than 0.5 in the substrate.

Based on the rheological study of dilute solutions of synthesized materials, it was shown that the materials have polyelectrolyte behavior and the decrease in size and the presence of surfactants leads to an increase in the density and intensity of electrostatic interactions. The synthesis of DNA-NAM-NMNVA copolymers results in the formation of stable complex combinations whose behavior is determined by the size of the resulting polymeric species.

This synthesis study of DNA-based material assemblies is a starting point for obtaining materials for applications in medical imaging and various branches of biophotonics.

In order to obtain sensors with DNA sequences that are based on the hybridization of complementary or partially complementary chains between DNA sequences, several studies have been performed to optimize the surfaces and the methodology.

The first step consisted in the morphological characterization of the two types of metallized substrates by different deposition methods, demonstrating that regardless the metallization method used, the coating is uniform. The second step was the investigation of the electrochemical properties of the planar electrodes obtained by metallization of Si/SiO_2 wafers and of the flexible electrodes obtained by coating the electrospun polymeric fibers with metals. Thus, it was concluded from cyclic voltammetry studies and tracing of calibration curves that the flexible electrodes obtained by metallization of electrospun polymeric fibers have an electroactive area about 5 times larger than that of planar electrodes. To investigate the physical and interface properties of the electrodes, electrochemical impedance spectroscopy was performed and the results were consistent with those obtained from cyclic voltammetry experiments for a diffusion-controlled process.

Another important step was the functionalization of the electrodes and the starting point was the modification of the surface in order to detect small molecules such as ions. Thus, the electrochemical properties of immobilized ionophores on the surface of planar/flexible electrodes were investigated to develop an ionophore-based sensor for the analysis of electrolytes in sweat. Gold surface electrodes were used for this study, and ionophores were immobilized by several methods. Of all the immobilization methods (until the completion of this doctoral thesis) only immobilization with nafion matrix and DPPC had satisfactory results. Following the morphological characterization, a high uniformity was observed in the case of ionophore immobilization in the nafion matrix, and infrared spectroscopy showed that the Ca^{2+} ionophore immobilized in the nafion matrix maintains its conformation. For this reason, only this device has been tested in artificial perspiration.

SEM-EDX investigations have shown that the ionophore is oriented along the fiber direction, and cyclic voltammetry and electrochemical impedance spectroscopy have shown that the ionophore allows target ions to diffuse through this membrane by changing the electrical properties of the device. The configuration based on ionophores immobilized in the nafion matrix opens a new perspective for ion detection.

To demonstrate the concept of flexible ionophore-based sensors for the analysis of sweat electrolytes, ionophores for Ca^{2+}, NH_4^+, Cl^- and H^+ were immobilized on the surface of flexible Au/PMMA/PET electrodes in a nafion matrix. The determination of the target ions was performed by potentiometry in different media, including artificial sweat, with adequate values of sensitivity, detection limits, selectivity and selectivity coefficients.

Another study was to investigate the performance of gold-plated electrospun submicron polymeric fibers as a new electrode platform for biosensitivity applications. Thus, their applicability was demonstrated for the quantification by amperometry with fixed potential at -0.20 V (vs. Ag/AgCl) of: i) hydrogen peroxide with a sensitivity of 6.14 x 10^{-6} mA cm^{-2} nM^{-1} and a detection limit of 127 nM; and (ii) glucose, after glucose oxidase immobilisation, with a sensitivity of 3.10 µA cm^{-2} mM^{-1}, a detection limit of 0.33 mM and reduced interference. This proposed technology for obtaining conductive polymer fibers and flexible electrodes is important for obtaining wearable devices for continuous monitoring of various analyzes and to provide a real-time profile of the physiological state of an organism.

The last chapter describes the obtaining of biosensors for the detection of genetic mutations and is based on the functionalization of electrodes with different sulphur-modified oligonucleotides. The study was performed for two types of Si/SiO_2 substrates and electrospun polymeric fibers, both metallized with gold. The most important aspect demonstrated was that in the case of the use of electrophilated PMMA fibers it was not necessary to passivate the surface. The oligonucleotides covalently bind through the sulphur atom to the gold surface of the electrodes and this is done exclusively in the direction of the fibers. By functionalizing the surfaces with oligonucleotides, regardless the substrate used, the hydrophilicity of the surface increases. Concerning the performance of the manufactured biosensor, it was found that the biosensor obtained on a flexible substrate is easier to manufacture and has lower detection limits.

This latest study demonstrates the applicability of polymers and DNA sequences to the manufacture of biosensors for the detection of genetic mutations.

In this context, the doctoral thesis consisted in obtaining and characterizing polymeric materials based on N-substituted amides containing chromophores and / or DNA that can be used for the development of devices with applications in sensors or photonics. Derived from the main objective of the thesis, (bio) sensors were obtained from two complementary perspectives: the development of materials for new types of electrodes and the modification of the surfaces of these electrodes in order to obtain sensors with specific applications.

C.2. ORIGINAL CONTRIBUTIONS

The Ph.D. thesis brings major original contributions in obtaining and characterizing materials based on N-substituted amides, chromophores and DNA that can be used to develop devices with applications in photonics, as well as new sensors and biosensors for quantification of electrolytes, biomarkers or genetic mutations.

Within this Ph.D. thesis the following elements of originality are distinguished:

► The kinetic study of the homopolymerization of N-substituted amides which led to the selection of the optimal reaction conditions for stereo-control.

► In the case of N-methyl N-vinyl acetamide homopolymerization, it has been shown that a structural change can be induced by radical polymerization in suitable solvents. The information obtained in this study is useful in developing methods for controlling stereoregularity for the radical polymerization of N-substituted amides.

► Through the copolymerization study, useful information related to kinetics, methods for estimating reactivities, kinetic models for each investigated system and the mutual influence of monomers were obtained. This information is useful in the development of stereocontrol methods for the radical copolymerization of N-alkyl substituted acrylamides.

► New chromophore monomers based on carbazole groups and azobenzene derivatives were synthesized. The synthesis method leads to precise structures and thus the study of their polymerization explains the property-structure correlations for these new materials which depend on the reaction parameters.

► New polymer-DNA systems were obtained by copolymerization in organic-aqueous medium and investigations related to the influence of the initiator, the presence of stabilizers and the type of polymerization on conversions and binding percentages were performed.

► New polymer-DNA systems have been shown to exhibit polyelectrolyte behavior
► New platforms based on electrospun polymeric fibers for the manufacture of biosensors were obtained and their applicability was demonstrated depending on the nature of their functionalization in the detection of ions, hydrogen peroxide, glucose and mutations of DNA sequences.

C.3. PERSPECTIVES

● The results obtained regarding the synthesis and applications of polymeric materials that are the subject of this Ph.D. thesis confirm that there will always be new aspects to investigate, the research in the field of materials tending to infinity. In addition, the areas in which these new types of materials find their applicability constantly require the development and improvement of the resources used.
● Thus, the synthesized polymeric materials can be embedded in different types of sensors to obtain devices sensitive to external stimuli.
● It is also possible to synthesize polymer-DNA systems with ssDNA chains in order to obtain sensors that operate on hybridization interactions, thus obtaining devices for detecting any type of mutation.
● Amperometric sensors will be manufactured based on metallized electrospun polymeric fibers functionalized with nanostructured materials.
● Other biosensors will be obtained based on the model of the one already manufactured with glucosoxidase to determine the activity of other enzymes.
● In the case of sensors for ion detection, research will continue in order to optimize the manufacturing method but also for the use of other types of immobilization on the surface.
● All flexible (bio)sensors obtained will be incorporated in wearable devices for real-time and continuous monitoring of electrolytes and beyond.
● Starting from the results presented in this study, the research activity will be continued in order to develop new materials for biosensing applications.

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- **Anca Aldea**, Elena Matei, Ricardo J. B. Leote, Ileana Rău, Ionuț Enculescu, Victor C. Diculescu, *Ionophore-nafion Modified Gold-coated Electrospun Polymeric Fibers as Flexible Sensors for Determination of Electrolytes in Sweat*, Electrochimica Acta, Volume 363, 10 December 2020, 137239 (**IF=6.215**)
- **Anca Aldea**, Ana Maria Albu, Ileana Rău *New carbazole based materials: synthesis and spectral characterization* UPB Scientific Bulletin, Series B: Chemistry and Materials Science, **80**(4), 39-48, 2018 (CODEN: SBUPD, ISSN 1454-2331)
- **Anca Aldea**, Ana Maria Albu, Ileana Rău *Solvatochromic study of new carbazole based materials*, UPB Scientific Bulletin, Series B: Chemistry and Materials Science, **80**(2), 149-158, 2018 (CODEN: SBUPD, ISSN 1454-2331)
- Natalia V. Kamanina, Svetlana V. Likhomanova, Vladislav I. Studeonov, Ileana Rău, **Anca Diblă**, Agnieszka Pawlicka, *Rotation of the Polarization Plane of Light Via Use of the DNA-Based Structures for Innovative Medical Devices*, Journal of Nanotechnology in Diagnosis and Treatment, **4**, 1-4, 2016 (ISSN: 2311-8792/16)
- **Anca Aldea**, Ana Maria Albu, Ileana Rău *New polymeric materials for photonic applications: Preliminary investigations*, Optical Materials, **56**, 90-93, 2016 (https://doi.org/10.1016/j.optmat.2016.01.032) (**IF=2.779**)
- **Anca Aldea**, Ana-Maria Albu, Alina Nicolescu, Victorita Tecuceanu *Kinetics of Free Radical Polymerization of N-substituted Amides and Their Structural Implications*, Advances in Materials Science and Engineering, **2016**, 1-9, 2016, Article ID 6430416, (https://doi.org/10.1155/2016/6430416) (**IF=1.271**)

Total IF = 1.271+2.779+6.215=10.265

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2. Anca Aldea, Elena Matei, Victor C. Diculescu, *Ionophore-Based Sensors for Continuous Monitoring of Electrolytes* -XXV International Symposium on Bioelectrochemistry and Bioenergetics, Limerick, Irland, 2019 (ORAL)

3. Anca Aldea, Victor C. Diculescu, Ionut Enculescu, Nicoleta Preda, Mihaela Beregoi, Alexandru Evanghelidis, Ileana Rău *High Surface Flexible Electrodes for Biomedical Applications* -69th Annual International Society of Electrochemistry Meeting, Bologna, Italy, 2018 (ORAL)

4. Anca Aldea, Mihalea Beregoi, Alexandru Evanghelidis, Ileana Rău, Victor C. Diculescu, *New Flexible Materials for Wearable Biosensors* – 17th International Conference on Electroanalysis, Rhodes, Greece, 2018 (ORAL)

5. Anca Diblă, Ana-Maria Albu, Ileana Rău, *Dimensional study of N-acryloyl morpholine copolymers with potential application in biophotonics* - 19th Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), Sibiu, Romania, 2015 (ORAL)

6. Anca Diblă, Ana-Maria Albu, Ileana Rău, *Synthesis and characterization of N-substituted amides copolymers with DNA mimicking properties*- International Multidisciplinary Scientific GeoConferences & Expo SGEM 2015, Albena, Bulgaria, 2015 (POSTER)

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