"ALEXANDRU IOAN CUZA" UNIVERSITY
OF IAŞI
PHYSICS FACULTY

SCIENCES ET TECHNIQUES DU
LANGUEDOC
MONTPELLIER, FRANCE

- COTUTEL THESIS -

RESUME

Obtaining the polymeric structures in the plasma for medical applications

Drd. Bogdan-George RUSU

Scientific committee

Prof. Dr. Diana Mardare (Romania)	President
Dir. Rech. Dr. Mihai BARBOIU (France)	Scientific coordinator
Prof. Dr. Nicoleta DUMITRAŞCU (Romania)	Scientific coordinator
Prof. Dr. Gheorghe POPA (Romania)	Reviewer
Prof. Dr. Bogdan Simionescu (Romania)	Reviewer
Prof. Dr. Frederique Cunin (Romania)	Reviewer

September 2013

Table of Contents

Introduction

This thesis was performed in collaboration between the European Institute of Membranes, the team of Adaptive Supramolecular Nanosystems (NSA) (Montpellier, France), and Plasma Physics Laboratory, Faculty of Physics (Alexandru Ioan Cuza, University Iasi, Romania).

Between 2010 and 2012, for 12 months, I received a Research Training scholarship from the Francophone University Association (AUF) and a scholarship for 6 months in 2013 from IEM / ICG.

The purpose of this thesis is to provide a better understanding of the absorption, recognition and self-assembly mechanisms of biological molecules onto complex systems such as different surfaces obtained by atmospheric pressure plasma, mesoporous materials, and membranes.

This thesis is structured in five chapters, followed by general conclusions, list of publications, list of tables, list of figures and bibliography.

The first chapter presents the main notions about biomedical implants, a short classification of materials used in medical applications and physical and chemical methods concerning the biomaterials functionalization.

The second chapter describes the experimental setup used for plasma polymerization reactions in atmospheric pressure conditions in order to obtain the polymer thin layers of polyethylene glycol (PEG) and copolymer thin layers of polyethylene glycol (PEG) with polystyrene.

The characterization of this polymer and copolymer layers was made using some analysis techniques: atomic force microscopy (AFM), scanning electron microscopy (SEM), the contact angle measurements, Fourier transform infrared spectroscopy (FTIR), UV-VIS spectroscopy and X-ray photoelectron spectroscopy (XPS).

The third chapter describes the protein absorption mechanisms with the aid of atomic force spectroscopy and quartz crystal microbalance techniques on the surfaces obtained by plasma polymerization in the atmospheric pressure conditions.

The studies concerning the ions and amino acids diffusion by functionalized mesoporous structures are presented in the fourth chapter. Has been demonstrated the selectivity character of the membrane for a particular class of amino acids, for those amino acids that has hydrophilic character.

The fifth chapter illustrated the self-assembly and the stabilization mode of the G type molecules inside of porous silicon surfaces obtained by electrochemical etching in hydrofluoric acid solution (HF). By analyzing the optical properties of these types of surfaces were observed a different behavior with regard to the stability of the molecules according to the ionic strength of the bonds.

The thesis ends with the main conclusions drawn from these studies and with references used.

Chapter 1: Materials with biomedical applications

The most common types of materials used in medicine are ceramic, composite, polymeric and metallic materials. Among all these, the polymer materials have a wider applicability. They consist of high molecular weight molecules, composed of a large number of repeating units [1] and are classified into natural and synthetic polymers. Natural polymers used as biomaterials can be divided into two categories: vegetable polymers (cellulose, sodium alginate and natural rubber) or animal polymers (collagen, hyaluronic acid and other natural materials such as DNA encoding the genetic material of living cells). Although these polymers have a wide range of applications and are widely used, they are sometimes overshadowed by a seemingly endless variety of synthetic polymers available today. Synthetic polymers used as biomaterials are also divided into two broad categories: hydrophobic polymers that do not absorb water (PP polypropylene, polymethylmethacrylate PMMA, polyethylene terephthalate PET, polyethylene PE) and hydrophilic materials (which absorb water), such as polyethylene glycol.

Ceramics are inorganic solid materials with various combinations of ionic and covalent bonds. These types of materials have a strongly connected structure and are packaged such that each elementary cell of the network is electrically neutral. Aluminum oxide or alumina (Al2O3) is an example of a ceramic material that has these types of properties used in biomedical applications to obtain orthopedic implants [2].

In order to introduce them in medical applications, all materials must be functionalized. Surface functionalization is the method trough which the functional groups are created or anchored on the surface of solid materials. Using this method can be created surfaces with controllable properties and chemical reactivity. This type of procedure can be performed either by chemical or by physical methods, generally depending on the material properties: the surface roughness, the hydrophobic or hydrophilic nature of the material, the surface energy, bio-compatibility, electrical surface charge.

Chapter 2: Plasma polymerization reactions in atmospheric pressure condition

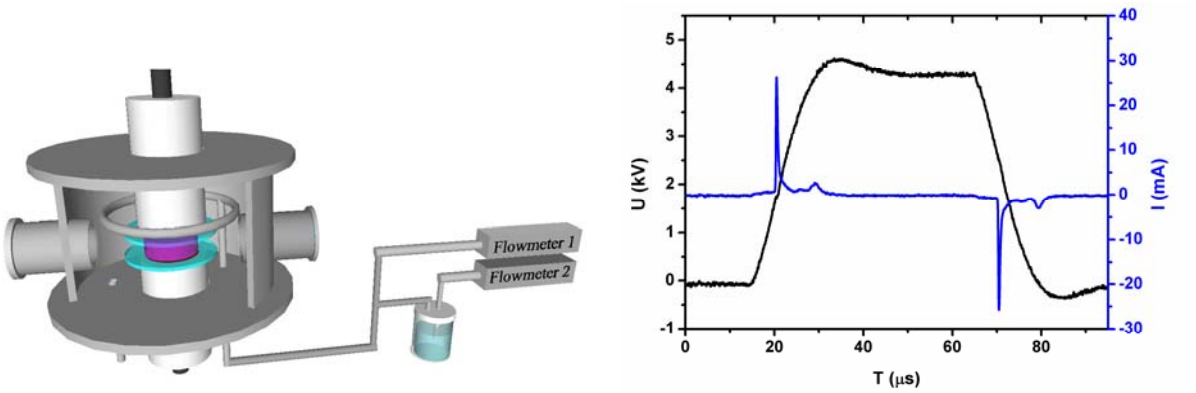
Experimental set-up. Plasma diagnosis methods

Polyethylene glycol (PEG) is a biodegradable polymer having a large number of repeating units of type -CH2CH2O-, by adding, the terminal ends of the polymer can be formed by hydroxyl groups. This polymer is soluble in water, methanol, dichloromethane, diethyl ether, and it is not toxic, providing a wide range of applications in the fields of production of medical and cosmetic materials.

In medicine, because of its low capacity to form biomolecules layers to the surface, the PEG is used in cardiovascular implants, biosensors or tests "in vitro". The role of these layers is to reduce non-specific coupling between the implant and the biological molecules in its immediate vicinity. These types of surfaces were synthesized in low pressure plasma systems, demonstrating the importance of the surface chemistry of the implant and the effectiveness of the PEG films obtained by plasma polymerisation in the rejection of biological molecules [3].

Plasma polymerization at atmospheric pressure is a very good technical solution to for covering various substrates with functional polymer layers. The plasma polymerization has unique practical advantages including (i) the ultra thin film deposition, (ii) good adhesion to the substrate and (iii) the formation of chemically and physically durable surfaces [4, 5

The characterization of thin polymer layers was made with the aid of following techniques: atomic force microscopy (AFM), scanning electron microscopy (SEM), the contact angle measurements, Fourier transform infrared spectroscopy (FTIR),, UV-VIS spectroscopy and X-ray photoelectron spectroscopy (XPS).



(a) (b)

Figure 2.1. (a) Experimental set-up, (b) changes over time of the applied voltage and the current intensity trough plasma

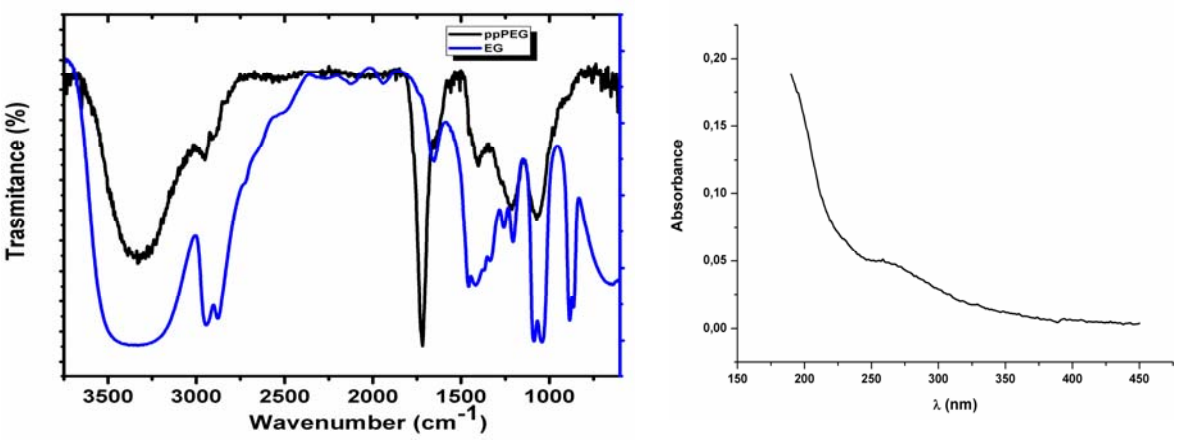
The experimental setup used for obtaining the plasma polymer films is shown schematically in Figure 2.1 a. It consists of two circular copper electrodes obtained by magnetron sputtering deposition on one of the faces of the two glass plates having a thickness of 1.2 mm and serving as a dielectric material. Those two electrodes are disposed in a parallel way, separated by a variable distance, in a closed chamber which is equipped with windows in order to achieve the optical diagnosis of discharge. With the aid of a circular tube, which surrounds those two electrodes, the working gas is introduced together with the monomer which wants to be polymerized, in this case the ethylene glycol (EG). The chamber is at atmospheric pressure and is provided with an orifice for removing the residual gas.

The discharge used in order to obtain polymeric surfaces by plasma polymerization in atmospheric pressure conditions is characterized by well-defined current pulses, with pulse duration of a few microseconds, contrary to the filamentary regime which is characterized by the presence of current peaks corresponding to micro-discharge or strimers regim with a lifetime in the order of nanoseconds. The typical discharge current signals (Figure 2.1 b) shows the homogeneous (diffuse) mode in which the discharge are operating.

Polymer characterization methods

The chemical structure of the films was investigated using the Fourier Transform IR Spectroscopy technique (FTIR), and spectra were recorded using a Bomem MB-104 spectrometer in the range 4000-400 cm-1 (4 cm-1 resolution).

The FTIR spectra of pPEG films (polyethylene glycol plasma polymerization) showed the presence of the ether group-COC-to 1068 cm -1, the elongation vibration of the bond -CC-1203 cm-1 and 1404 cm-1, -C = O bonds at 1722 cm-1 and C-H at 2961 cm-1. The wide band between 3200 and 3600 cm-1 is corresponding to the various modes of OH-type bonds vibration in the volume of the polymer film.



(a) (b)

Figure 2.2. (a) Typical FTIR spectra of ethylene glycol monomer and ppPEG obtained by APP, (b) UV spectra

The FTIR spectra of pPEG films (polyethylene glycol by plasma polymerization) showed the presence of the ether group-COC-to 1068 cm -1, stretching vibration of the bond -CC-1203 cm-1 and 1404 cm-1 and -C = O bonds at 1722 cm-1 and C-H at 2961 cm-1. The wide band between 3200 and 3600 cm-1 is corresponding to the various modes of OH- type bonds vibration from the volume of the polymer film.

The ppPEG films present a low UV absorption, which is between 50 nm and 300 nm. More information about the purity of the chemical composition were given by XPS spectra.

The chemical composition of the films was obtained by analyzing the XPS spectra of the polymers. XPS spectra were acquired using a Versa Probe 5000 spectrometer (Physical Electronics), equipped with a monochromatic Al-Kα X-rays source (hν = 1486.7 eV). During of measurements, the pressure from the chamber was maintained at a value of 5.9x10-8 Pa.

The low resolution XPS spectra of ppEG show the presence of two maxims corresponding to the C1s peak at 284 eV and O1s at 534 eV. The deconvolution of C1s and O1s revealed the contribution of C-C bonds at 284 eV, the C-O groups at 286 eV and 288 eV C-O-C. (Figure 2.3).

All peaks identified by FTIR and XPS technique are in a good correlation with the conventional chemical formula of PEG: HO-CH2-(CH2-CH2-O) n-CH2-OH, which means that the polymer obtained by DBD (dielectric barrier discharge) plasma maintains the chemical composition of the monomer.

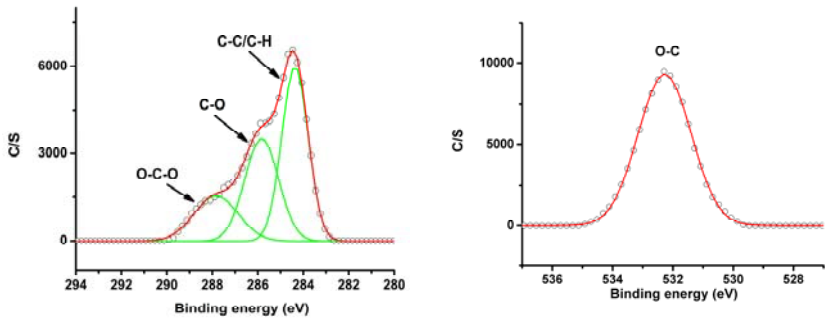


Figure 2.3. Deconvolution of the XPS spectra of ppEG films corresponding to the peak (a) C1s and (b) O1s

The information concerning the surface topography of polymer films were obtained by atomic force microscopy technique (AFM), using an device NT-MDT Solver type M-Pro in tapping mode with a resolution of 0.1 nm in Z direction using a standard silicon nitride tip (NSC21) with a typical tip radius of 10 nm. The study of the mean square roughness (RRMS) and the topography of PPEG films were also made immediately after polymerization and after a week of aging.

The hydrophilic character was demonstrated with the aid of contact angle technique. It can be observed that the mean contact angle to the substrate (glass) is 55 degrees and for pPEG films is only 6 degrees. It also been noted that the morphology of the pPEG films was changed after a week.

Furthermore, the Rrms increased from an amount of 8 nm at an amount of 16.2 nm for a scan area of 10 mm x 10 mm and from 6.9 to 15.5 nm for a scanned surface of 3 mm x 3 mm,.

This phenomenon of water molecules adsorption was demonstrated with the aid of X-ray photoelectron spectroscopy technique (XPS) where was observed an increase in the concentration of C-O groups from a value of 34.71% to 42.87%.

Hydrophilic nature of the polymer was demonstrated with the aid of the contact angle technique, where its average is approximately 6 degrees, in comparison with the contact angle average of the substrate which is 55 ° (glass).

Using the atmospheric pressure plasma polymerization method in a mixture of helium as working gas and ethylene glycol vapors, have been found the optimal parameters needed to control the polymerization reaction. Chemical analysis using X-ray photoelectron spectroscopy technique shows that the polymer formed is PEG due to the presence of CC bonds at 284 eV, CO at 286 eV and 288 eV COC.

From the morphological analysis of the layers, it was observed the appearance of the grain structures having a diameter in micrometer range and has also been observed that they changed very fast their morphology over time.

Plasma copolymerization

In order to obtain the co-polymer films of pST-EG whit the aid of plasma, was used a mixture of ethylene glycol (Merck CHIMIQUE, purity> 99.5%) and styrene (Sigma Aldrich, 99.9% purity) in a volume ratio of 3 / 1.

The IR spectra of the obtained films contain specific adsorption bands of the chemical groups of both types of monomers used. (Figure 2.7.) The presence of the methyl group-CH is an indicator of plasma polymerization process consisting in formation of the polymer chain. All the bands are specific to the polystyrene obtained under the plasma conditions pST, which indicates that for the pST-EG films newly formed, the polymer matrix is that of the styrene. The hydroxyl groups OH, which has a wide band between 3600 and 3200 cm-1 are specific to pPEG films.

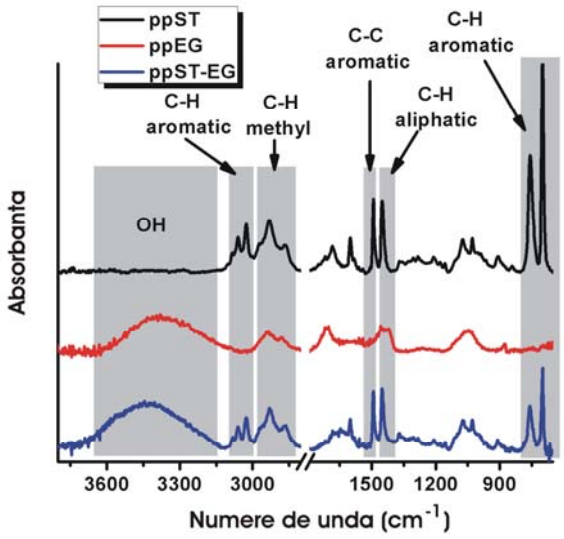


Figure 2.7. FTIR spectra of pST-EG, pST and pEG films obtained by atmospheric pressure plasma polymerization

Using the atmospheric pressure plasma polymerization method in helium and using styrene, ethylene glycol and a mixture of them (25% styrene and 75% ethylene glycol) as the monomer, was obtained a stable polymer layer. The chemical groups of the new polymer obtained from a mixture of ethylene glycol and styrene contain the polystyrene signature, but is incorporating onto the polymeric matrix the hydroxyl structures which are corresponding to ethylene glycol.

Chapter 3: Proteins adsorption on the surfaces of the polymers obtained in the plasma conditions

The study of protein adsorption using atomic force spectroscopy

Another application of plasma polymerization in atmospheric pressure conditions is the AFM tips functionalization in order to measure the adhesion force of bovine serum albumin (which were immobilized on the tip) and a polyethylene terephthalate (PET) surface using atomic force spectroscopy technique of AFM's.

The identification of bovine serum albumin (BSA) immobilized on AFM tips was performed using of X-ray photoelectron spectroscopy (XPS), electron microscopy (SEM) and fluorescence microscopy techniques. XPS spectra show the presence of silicon, carbon and oxygen peaks. After plasma polymerization, the tip was covered with a polystyrene

layer, and the peaks observed were those corresponding for carbon and oxygen. Albumin immobilization has been demonstrated by the presence of nitrogen corresponding to amino groups at 399 eV.

After AFM tips functionalization, the deflection curves show rupture events which indicate the breakage of BSA molecules. The adhesion force between the molecule and the PET surface was calculated using the Hooke equation.

Where k is the spring constant of the cantilever and Δx is the piezoceramic tube displacement in nm. The adhesion forces values calculated in this mode are hundreds of pico-Newtons. Thus, it could be concluded that the adhesion force value for one molecule is around 760 pN [8].

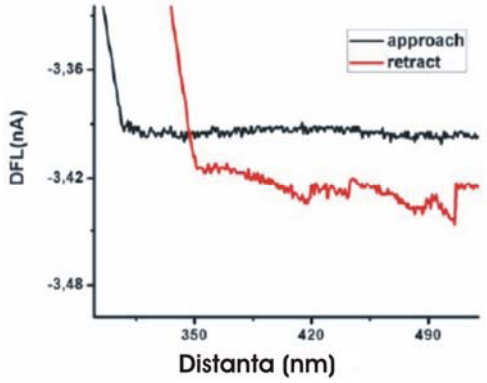


Figure 3.3. Deflection curves according to distance, in liquid medium for a tip which was 5 minutes polymerized by plasma and immersed in BSA

The study of protein adsorption using the quartz microbalance

For measuring the amount of BSA and Concanavalin A (Con A) adsorbed on the films surfaces obtained under atmospheric pressure plasma conditions, have used a quartz crystal microbalance (QCM). For this purpose, the standard gold electrodes was cleaned with piranha solution and ethanol, then dried in nitrogen flow, following to be functionalized by covering them with polymer layers ppEG, ppST and ppST-EG. For QCM measurement, the buffer solution flow was kept constant at 25 μl / min and the injection time of the protein solutions was 60 seconds. After the adsorption signal ΔF saturation, the electrode surface was cleaned with a solution of sodium dodecyl sulfate (SDS) 3% and a PBS solution at a 7.4 pH. Thus, it was observed that after washing the surface with SDS, the basic signal ΔF value returns to the same value it was before the injection of the protein solution, which confirms

that the surface of polymers is stable and the nature of interactions between proteins and the surface is physical one (H bridges, Van der Walls bonds).

Comparing the changes in frequency for all the surfaces obtained at the same protein concentration (10 μM), it could be seen that the polystyrene surfaces obtained in plasma adsorb the highest amount of biomolecules, in comparison with the commercial polystyrene surfaces obtained in a conventional manner.

The reduction of the proteins adsorption on the polystyrene surfaces that was obtained under the plasma conditions was done by introducing simultaneous whith the working gas into discharge a percent of 75% ethylene glycol in relation to the monomer used (styrene). In the case of polyethylene glycol surfaces obtained under the plasma conditions, as can be seen in Figure 3.5, the amount of adsorbed protein is minimal in comparison with other types of surfaces, demonstrating that these films maintains its character to reject biological molecules.

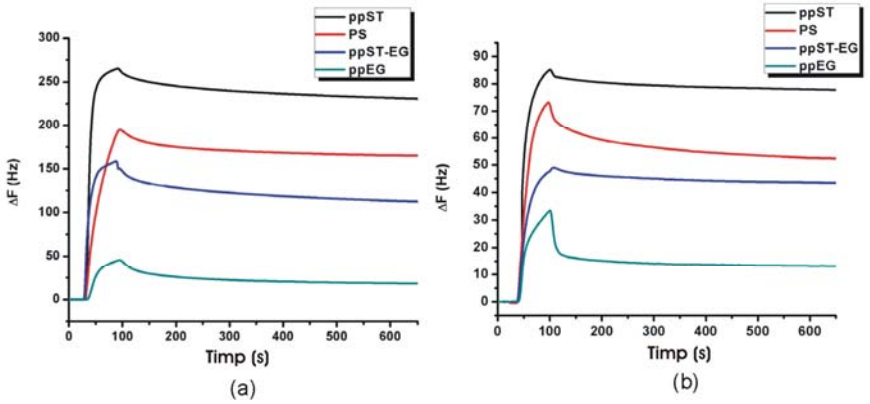


Figure 3.5. The absorption of Con A (a) and BSA (b) diluted to a concentration of 10 μM on different surfaces

Table 1. The affinity constants K calculated for polymer surfaces obtained in atmospheric pressure plasma

…….	K_a (Con A)	K_a (BSA)
ppST	$1,4x10^8$ M^{-1}	$1,1x10^8$ M^{-1}
ST	$4x10^7$ M^{-1}	$2,7x10^7$ M^{-1}
ppST-EG	$1,1x10^7$ M^{-1}	$7,4x10^6$ M^{-1}
ppEG	$6,7x10^6$ M^{-1}	$3,7x10^6$ M^{-1}

From the Table 1, it can be observed that the minimum value of the affinity constants K, for all type of proteins used is found for ethylene glycol (ppEG) and the maximum value for polystyrene (ppST), both surfaces were obtained under atmospheric pressure plasma conditions. The amount of adsorbed protein can be controlled by introducing into discharge simultaneous whit working gas a quantity of ethylene glycol.

Chapter 4: Mesoporous structures used for controlling the diffusion of biological constituents

Mesoporous materials (MCM-41)

Due to their very high active surfaces, which can be easily modified both by chemical and physical processes, the porous materials have found in recent years wide applications in various fields. They are mainly used in controlled absorption and release of drugs, chromatography, separation of biological molecules, photonic and electronic devices [9]. The synthesis of these porous materials is often more difficult than dense material because the first has a repetitive structure from both topographical and chemical point of view [10]. Thus, have developed new methods for obtaining and modifying these materials, from which the most common being the mesoporous M41S family, that can be found now under the MCM acronym (Mobil's Composition of Matter).

The first research in the mesoporous materials field have been reported by Kresage and coworkers in 1992, [11] and from then until now, these methods have been developed and improved [9-12]. These materials are prepared using cationic surfactants, which lead to obtaining pores with a lamellar (MCM-50), hexagonal (MCM-41) or cubic (MCM-48) structure with dimensions of them in nanometer range. MCM-41 is by far the most used mesoporous material due to ordered distribution of the pores, which have the diameter between 2 and 10 nm.

The synthesis of hexagonal mesoporous materials MCM-type can be done by various methods. One of these is to use the tetraetoxisilan (TEOS) 98% purity as a precursor and as surfactant alchiltrimetilamoniu bromide (CnH2n +1 (CH3) 3N+, Br– where n = 12). The method was reported by M. Koltzos and coworkers in 2001 [13].

The chemical composition used was: TEOS, acidic water (pH = 2), ethanol and hexadeciltrimethilamoniu bromide (n = 16) in the following molar ratios: 1:8,2:3,5:0,14. The synthesis was done in two stages. In the first stage was made a mixture of alkoxide, ethanol and half of the total quantity of water, so that in the second stage, after that the solution was kept under stirring for one hour at a room temperature, was added the surfactant and the other half of water. After two hours of stirring, the solution was deposited on a silicon substrate in order to be analyzed both from chemically and physically point of view.

The surfaces thus obtained was dried for 12 hours at a temperature of 20 ° C and then maintained for two hours at 100, 150, and 175 ° C. For the release of the surfactant, the samples were heated for one hour at a temperature of 450 ° C under a nitrogen atmosphere.

Artificial bilayers or nanotubulare membrane systems have been developed in recent years to simulate the natural ionic membranes for the chemical separation, the development of biosensors or storage and controlled release of drugs. Thus, molecular self-assembly of is an elegant method for the construction of these types of ion channels.

The achieving of these types of membranes is based on molecules self-assembling in order to form ion channels into membrane. For this purpose we used a mesoporous silica matrix MCM 41, whose pores were functionalized with octadeciltriclorosilane. As a matrix support was used a commercial alumina anodisc membrane (Anodisc, Whatman International Ltd) (AAO) having a pore dimension of 200 nm.

For a more accurate control of the MCM 41 thickness, the alumina membrane pores were filled with a solution of polyvinyl alcohol (PVA), and with a low-pressure discharge radio frequency (RF) using air from the atmosphere as the working gas, the PVA was etching, were obtained a gap of about 600 nm in the pores of alumina membrane. This space was filled with a matrix of mesoporous silica MCM 41 and the excess was removed from the surface of the membrane with the aid of a "spin-coating" technique at a speed of 4000 rev / min for a period of 30 s. The remaining of PVA within the pores was removed by holding the membrane for a period of 24 hours in a bath of distilled water and under stirring at the room temperature. Thus, the membrane was functionalized with octadeciltriclorosilane (ODS) for 12 hours at a 60 ° C temperature. A diagram of the functionalization protocol is shown in Figure 4.1.

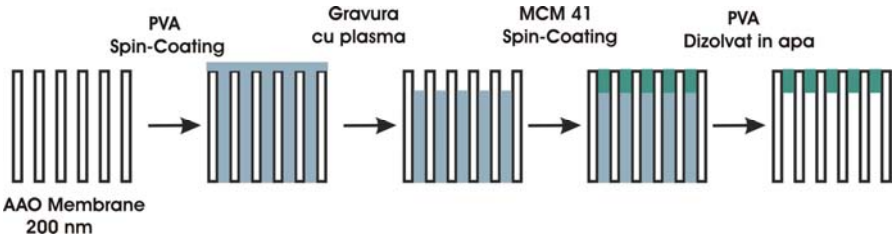


Figure 4.1: Experimental protocol used for functionalization and to obtaining the anodisc alumina membranes in order to measure the diffusion of ions (Na + and K +)
and amino acids (serotonin, tryptophan, tyrosine and phenyl alanine)

Diffusion of Na + and K + ions in mesoporous structures (MCM-41)

In order to determine the Na + and K + ions diffusion trough mesoporous membranes type MCM 41, after their functionalization whit octadeciltriclorosilane (ODS) over a period of 12 hours at a temperature of 60 ° C, they were kept for 48 hours in a solution 15C5 crown ether dissolved in toluene at a concentration of 1mg/ml. A. Cazacu and his colleagues reported in the literature the arrangement and structuring mode of crown ether 15C5 inside the MCM 41 pores, making the first measurements of diffusion through the entire alumina membrane [14].

For the diffusion measurements were using a Teflon cell, with two chambers separated by a functionalized membrane. One of the chambers ("the source chamber") was filled with 50 ml solution of NaCl or KCl at a concentration of 0.1 M, and the other chamber ("the acceptor chamber") was filled with the same volume of Milli-Q water. In the first part of the experiment, the samples were collected every hour and in the second part of the experiment, were collected one sample at an interval of three hours, following that in the third part of the experiment, one sample will be collected at 24 hours. To avoid the pressure gradient at each sample collected, was extracted the same amount of solution from the source chamber. The amount of solution collected was 1 ml, and was diluted with 3 mL of milli-Q water and analyzed by flame atomic absorption spectrometer.

From the diffusion data measured by flame atomic absorption spectrometer (Figure 4.2) can be observed for the first 100 hours a slow diffusion for both types of ions. This is caused by the rearrangement of molecules inside the pore, facilitating their passage through the "crown" of ether. After this period of time, the ions diffusion is facilitated and is accomplished more quickly, reaching the absorption signal saturation corresponding to half of the absorption signal for a blank sample which is at a concentration of 0.1 M. Thus, we

conclude that diffusion occurs until an equilibrium of concentration gradient is reached, in other words until the concentration in the "source" chamber is equal to the concentration in the "acceptor" chamber.

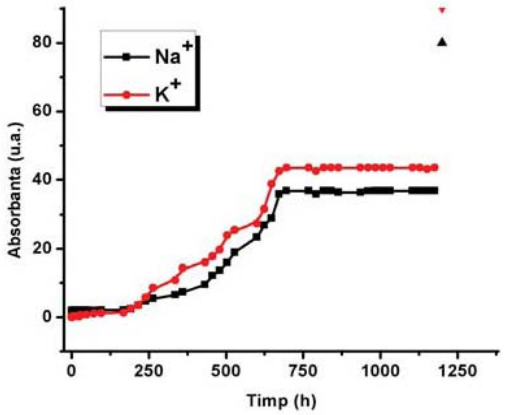


Figure 4.2: Diffusion of ions (Na + and K +) measured using a flame atomic absorption spectrometer through the pores of MCM 41 functionalized with crown ether 15C5

Figure 4.2: Diffusion of ions (Na + and K +) measured using a flame atomic absorption spectrometer through the pores of MCM 41 functionalized with crown ether 15C5

Diffusion of amino acids trough mesoporous structures (MCM-41)

In order to determinate the diffusion of amino acids, after the membranes functionalization whit octadeciltriclorosilane (ODS) for 12 hours at a temperature of 60 ° C, they were kept for 24 hours in a solution of myristoyl dissolved in absolute ethanol at a concentration of 1mg/ml. As in the case of ions diffusion, after the functionalization, the membrane was placed between the chambers of two teflon cells and one of them (the "source" chamber), was filled with 50 ml amino acid solution at a concentration of 0.01 M, and the other (the "acceptor" chamber) was filled with the same volume of milli-Q water. In the first part of the experiment, the samples were collected every hour and in the second part of the experiment, one samples were collected at three-hour intervals, so that in the third part of the experiment the sampling is made by one to 24 hours.

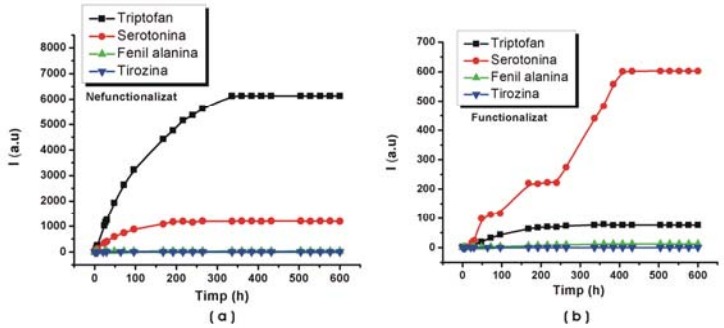


Figure 4.3: Comparison of the diffusion of amino acids (phenyl alanine, serotonin, tyrosine and tryptophan) measured by mass spectrometer coupled with liquid chromatography through the pores of MCM 41 non-functionalized (a) and functionalized with myristoyl (b)

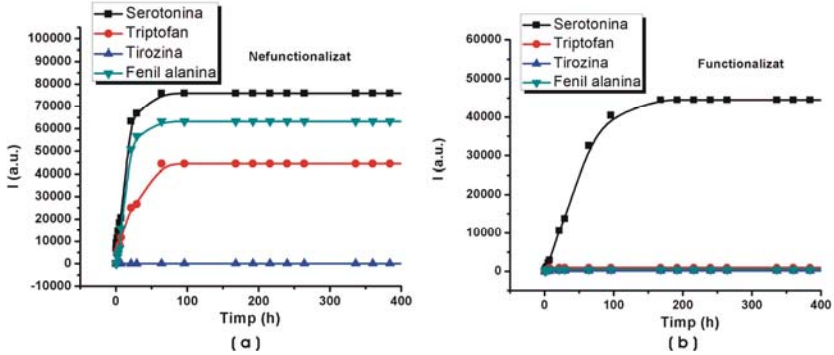


Figure 4.4 Comparison of the mixtures diffusion of amino acids (phenyl alanine, serotonin, tyrosine and tryptophan) measured by mass spectrometer coupled with liquid chromatography through the pores of MCM 41 non-functionalized (a) and functionalized with myristoyl (b)

The analysis of the amount of diffused molecules was performed using the technique of liquid chromatography coupled with mass spectrometry (HPLC-MS). In order to know the quantity of molecules which diffuses through the membrane was draw a calibration curve of all amino acids used at different concentrations. By analyzing the peak`s integral corresponding to the each amino mass and representing it graphically according to time durations at which the samples were taken, we can observe that for all the molecules, the signal value is higher for non-functionalized membranes. This is due to the fact that the space inside the pore in which the amino acids are diffused decreases after the functionalization occurs.

By comparing the diffusion for each membrane, is observed that after it has been functionalized, it becomes selective, and the amount of serotonin which diffuses is much higher in comparison with the diffusion of tryptophan for the same type of membrane. (Figure 4.3.) This may be due to the more hydrophilic nature of serotonin than of the

tryptophan, the selectivity is manifested in the number of polar groups present in the molecule.

The selectivity character of the functionalized membrane with myristoyl was demonstrated by amino acid diffusion analysis where in the acceptor chamber was a mixture of serotonin, tryptophan and phenyl alanine at the same concentration of 0.005 M. Thus, in Figure 4.4 b it can be observed that the only molecules who was transported from the "source" chamber into the "acceptor" chamber is the serotonin, in comparison with the non-functionalized membrane where it was observed a transport of phenyl-alanine and tryptophan, but in smaller amounts (Figure 4.4).

Chapter 5: Absorption of macromolecules (3AcG) onto porous structures

Obtaining and characterization of porous silicon structures

The porous silicon was obtained by electrochemical etching of a silicon monocrystalline substrate used as an anode in an electrolytic cell that contained a solution of hydrofluoric acid (HF). This technique is named also anodizing [15, 16]. The silicon substrate was disposed at the bottom of the electrolysis cell so that the hydrofluoric acid solution will fully cover the substrate (Figure 5.1.). The substrate acts as the anode and the cathode was made of a platinum wire and was imersed in the acid bath. By applying an electrical current between those two electrodes, the porous silicon will form on the surface of the silicon substrate. In fact, the passage of electric current causes an inhomogeneous dissolution located in several small areas of silicon. The porous silicon is actually composed of network canals, and the morphology and thickness are depending of the nature of substrate (the type of doping, the crystal orientation) and the working conditions (current intensity and duration of anodization).

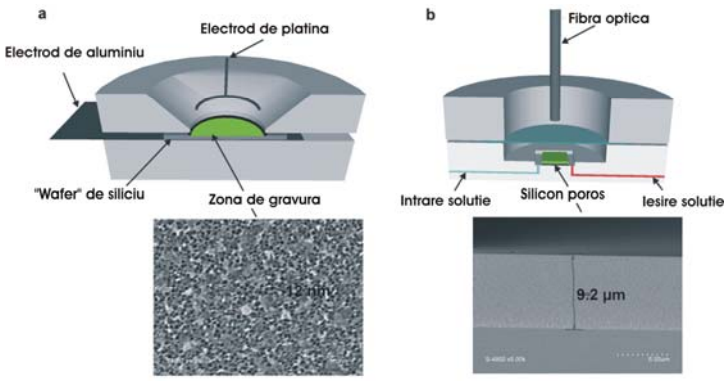


Figure 5.1. Experimental device used for obtaining the silicone pores (a) and for the quantification of the amount of molecules which pass by the interior of the porous silicon (psi) (b)

The porosity of a porous silicon film is proportional to the current density that was applied, and is normally between 40 and 80 percent of the film. The pores are formed only at the interface Si porous - Si, and once formed, the porosity of a layer that is under development can be altered by changing the amount of the applied current. Thus, the film thickness will increase with the new porosity, which is given by the new etching current value. This allows the development of nanostructures layers by modulating the applied current during the etching process.

The ability to control the volume and pore size during the etching process is a unique property belongs to the porous silicon, and is very useful in some applications, such as in the controlled release of drugs. Other types of porous materials have in general a more elaborate protocol for the pore size control, and even so, those dimensions are limited to short intervals.

The photoluminescence and photo-reflectance properties of porous silicon PSi, make from this a highly desirable material in various applications such as molecular absorption sensors or controlled release of drugs.

Study of the absorption of molecules on the porous structures

The modes of arranging of the different types of molecules, provides an evolutionary approach to the new macromolecular structures generation with various applications. These self-assembly methods of the different types of molecules are due to presence of the H-bonds between them. A representative example is the packaging of 3-acetyl-guanosine molecules, which in the presence of ions, assembles itself into a tubular network in which the ion is surrounded by "G"- type molecules, thereby forming the ionic channels.

Due to the fact that silicon pores surface is produced by electrochemical etching, has special optical properties and the amount of molecules which was "trapped" in these pores can be quantified, and in order to stabilize the G-cuadruplex structures, they were placed inside of the pores.

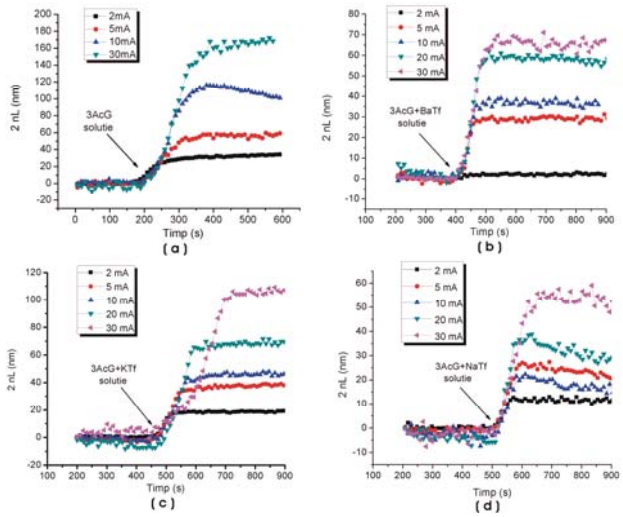


Figure 5.3. Changing in the real-time ot the effective refractive index (2NL) of the silicon pores from different etching currents when introducing the 3AcG (a) 3AcG + Na + (b) 3AcG + K + (c) et 3AcG + Ba2 + (d)

For kinetic measurements of the molecules absorption in the pores was used a liquid cell shown in Figure 5.4 (b). After placing the the porous silicon sample inside the liquid cell, the acetonitrile was introduced at a constant rate of 10 ml / min in order to acquire the baseline of the measured signal 2NL. After its stabilization, was introduced the 3AcG solution and 3AcG + ions at the same flow. Thus, was observed an increase in the measured signal 2NL, the increase depends on the amount of absorbed molecules in the porous silicon psi and is proportional to the pore size and the diameter of the molecules. The highest value of the absorption was found for the sample obtained at a current density of 30 mA/cm2, where the pore size is about 26 nm, and the lowest absorption is found for the sample obtained at a current density of 2 mA/cm2 cases which has an average pore diameter of 5 nm. (Figure 5.9 (a) (b) (c) (d)).

In order to have a better idea of the value of the amount of molecules trapped inside the pores of PSI were performed colored samples, composed of successive layers of different pore diameter. Immediately after functionalization, as shown in Figure 5.11, the samples of

PSI have a green color. When "G" type molecules are placed inside the pores may observe a change in color of PSI film from green to red. This change in color is also stable after keeping the samples for 12 hours at a low pressure.

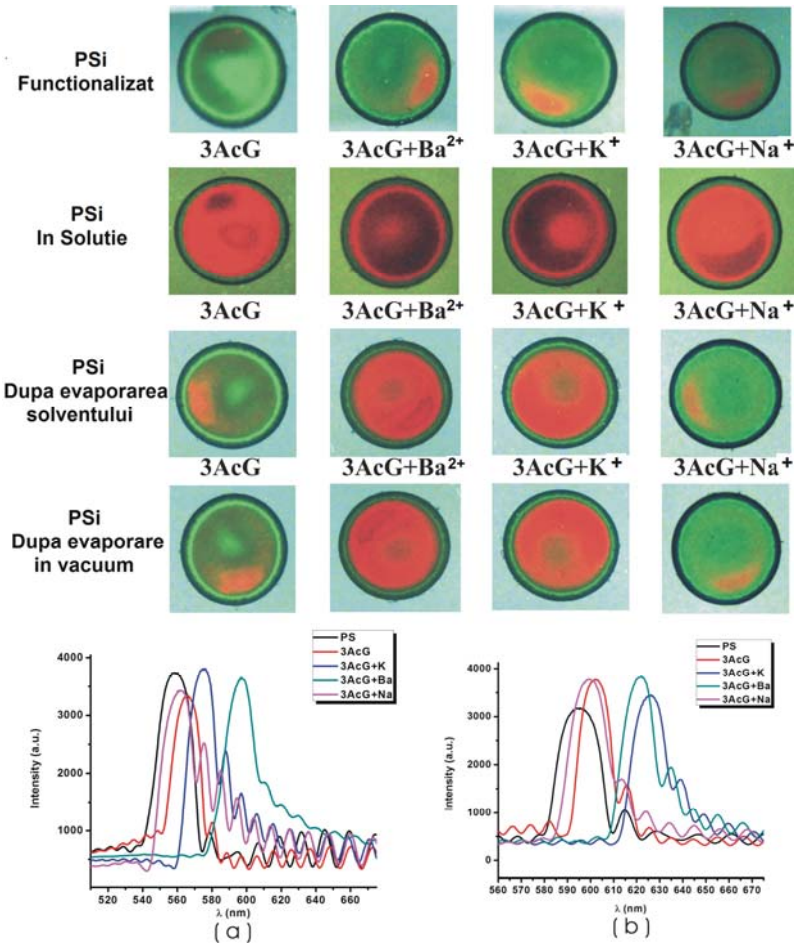


Figure 5.4. Changing in the color of the porous silicon film and the Bragg's "peack" from the reflection spectrum (a) and (b) at the time of their introduction into the liquid solution, and after evaporation of the solvent at room temperature and then in a vacuum conditions

Conclusions

This thesis was focused on obtaining and testing from the bio-functionality point of view of new materials obtained using the plasma techniques (atmospheric pressure plasma polymerization, plasma etching) as well as by chemical methods (electrochemical etching, attachment of functional groups by chemical methods). The functions that have been tested was the biomolecules adsorption on the surface of polymeric materials obtained in the atmospheric pressure plasma conditions, selective diffusion of ions and amino acids in the porous material MCM 41 type and the stabilization of G-cuadruplex systems inside of the silicon pores. The general conclusions of the thesis and experiments which was performed are described briefly as follows:

1. Knowing the importance of polyethylene glycol films in the biomedical applications, with the aid of atmospheric pressure DBD discharge, we succeeded to find the optimum parameters for obtaining these films ("PEG like films"). Once the monomer is inserted in the discharge, this remains homogeneous. The ICCD images have demonstrated a delay in the ignition of the gas discharge when with the working gas (He) is introduced also the ethylene glycol. This is due to the energy loss of charged particles by the fragmentation of the monomer.

The chemical analysis of these films performed by X-ray spectroscopy (XPS) and by IR spectroscopy (ATR-FTIR) tehniques showed the presence of the C-C, C-O and C-O-C that are in a good correlation with the polymers obtained using the conventional chemistry. The morphology of the pEG films present grain structures with a diameter less than 1 μm. These structures are no longer observed after 7 days after obtaining, due to absorption of water molecules from the atmosphere.

2. The polymerization in a mixture of ethylene glycol and styrene led to obtaining of new polymeric film which has the characteristics of both types of monomers. FTIR spectra of the ppST-EG film shows that the polymer matrix is polystyrene, which has incorporated the hydroxyl groups. The wettability studies show that the contact angle of ppST-EG is lower than the polystyrene film and higher than the of polyethylene glycol film, both obtained in the plasma under atmospheric pressure conditions.

3. All three types of polymers (pST, pEG and pST-EG) produced by plasma polymerization at atmospheric pressure were tested in terms of the proteins absorption to the surface with the aid of the quartz microbalance (QCM). The pST film have the maximum of protein absorption to the surface, which is higher than the polystyrene films (PS) obtained by "spin coating" for both types of molecules (ConA and BSA). This is due to the presence of C-O and C=O bonds, that are evidenced by X ray spectroscopy. The pEG films have the minimum of absorption. These films are known by their property to minimize the adsorption of proteins and cells to the surface.

Thus it can be concluded that the surfaces of polystyrene obtained by atmospheric pressure plasma absorb a greater number of molecules on their surface than those obtained by conventional chemistry. This amount of molecules adsorbed on the surface can be reduced by simultaneous introducing in the discharge a percentage of ethylene glycol.

4. From the data analysis of Na + and K + ions diffusion through the functionalized membranes type MCM 41, measured using flame atomic absorption spectrometry it was observed that for the first 100 hours occur a slow diffusion for both types of ions. This is caused by the rearrangement of molecules inside pore, facilitating their passage through the interior of the "crown" of ether. After this period of time, the diffusion of ions is facilitated, is accomplished much more rapidly reaching the absorption signal saturation corresponding to half of the absorption signal of the blank sample at a concentration of 0.1 M. , we conclude that diffusion occurs until an equilibrium of concentration gradient is reached, in other words until the concentration in the "source" chamber is equal to the concentration in the "acceptor" chamber.

In the case of amino acids diffusion, by comparing the diffusion for each membrane was observed that after it has been functionalized, it becomes selective, and the amount of serotonin which diffuses is much higher in comparison with the diffusion of tryptophan for the same type of membrane. (Figure 4.3.) This may be due to the more hydrophilic nature of serotonin than of the tryptophan, the selectivity is manifested in the number of polar groups present in the molecule.

5. The spatial confinement of the G-cuadruplex systems inside of silicon pores is a new method for stabilization and formation of these types of systems. Using optical reflectance technique could determine and quantify the amount of absorbed molecules inside

pores, and the transition from linear to cylindrical systems (ion channels) when introducing different types of ions. By obtaining the porous silicon films it could be correlate the color changing effect of films with the stabilization of molecules, demonstrating that these types of materials can easily be used as biosensors.

List of publications

Published articles

1. G. B. Rusu, M. Asandulesa, I. Topala, V. Pohoata, N. Dumitrascu, M. Barboiu, " Atmospheric pressure plasma polymers for tuned QCM detection of protein adhesion" Biosensors and Bioelectronics (trimis spre publicare)
2. Bogdan-George Rusu, Frederique Cunin, Mihail Barboiu, " The color of self-assembly-Real-time optical detection of stabilized artificial G-quadruplexes under confined conditions" Angewandte Chemie (trimis spre publicare)
3. Alina Silvia Chiper, Rusu Bogdan George, Gheorghe Popa, "Influence of the dielectric Surface nonhomogeneites on the dynamic of the pulsed DBD plasma" IEEE Transactions on Plasma Science, Vol. 39, No. 11, Novembre 2011
4. Chiper Alina Silvia, Rusu Bogdan George, Nastuta Andrei Vasile, Popa Gheorghe, On the discharge parameters of a glow-mode DBD at medium and atmospheric pressure. IEEE Transactions on Plasma Science, 37(10):2098-2102; OCT 2009,
5. A. S. Chiper, G. B. Rusu, C. Vitelaru, I. Mihaila, G. Popa, A comparative study of helium and argon DBD plasmas suitable for thermosensitive materials processing, ROMANIAN JOURNAL OF PHYSICS Volume: 56 Supplement: S Pages: 126-131
6. Schrittwieser R., Ionita C., Murawski A., Maszl C., Asandulesa M., Nastuta A., Rusu G., Douat C., Olenici S. B., Vojvodic I., Dobromir M., Luca D., Jaksch S., Scheier P., Cavity-hollow cathode-sputtering source for titanium films. Journal of Plasma Physics, 76(3-4):655--664; IAN 2010,

Oral presentations

1. I. Topala, G. Rusu, M. Asandulesa, M. Totolin, V. Pohoata, N. Dumitrascu, Functionalization of AFM tips by atmospheric pressure plasma polymerization, 20th International Symposium on Plasma Chemistry – Philadelphia, USA. July 24 - 29, 2011
2. M. Asandulesa, G. Rusu, I. Topala, V. Pohoata, M. Dobromir, and N. Dumitrascu, Synthesis of functional polystyrene-type films in atmospheric pressure dielectric barrier

discharge, 4th International Workshop and Summer School on Plasma Physics (IWSSPP), Kiten, Bulgaria, 2010

Poster Presentations

1. G.B. Rusu, C. Luca, E. Falos, R. Schrittwieser, Polystirene-TiO2 thin films produced in a hollow cathode, 39th European Physical Society Conference on Plasma Physics, Stockholm, Sweden, 2-6 July 2012
2. Roxana Jijie, Rusu Bogdan George, Ionut Topala, Valentin Pohoata, Nicoleta Dumitrascu, Study of protein aggregation and enzymatic activity after exposure to dielectric barrier plasma jet in helium, 4th International Conference on Plasma Medicine, Orléans, France, June 17 to June 21, 2012
3. Roxana Jijie, Ionut Topala, Bogdan George Rusu, Marius Dobromir, Valentin Pohoata, Nicoleta Dumitrascu, Atmospheric pressure plasma treatments of protein films and powders, 10th International Conference on Global Research and Education (interAcademia), Sucevita, Romania, 2011
4. A.S. Chiper, C. Vitelaru, I. Mihaila, G.B. Rusu, G. Popa, A comparative study of a He and an Ar DBD plasmas suitable for use in thermosensitive materials processing, XVth International Conference on Plasma Physics and Applications (CPPA), Iasi, Romania, 2010
5. G.B. Rusu, I. Topala, M. Dobromir, V. Pohoata, N. Dumitrascu, Synthesis of PEG in plasma at atmospheric pressure, XVth International Conference on Plasma Physics and Applications (CPPA), Iasi, Romania, 2010
6. I. Topala, R. Jijie, G.B. Rusu, V. Pohoata, N. Dumitrascu, Structure-function relationships in the case of plasma modified proteins, 21th Europhysics Conference on Atomic and Molecular Physics of Ionized Gases (ESCAMPIG 21), P2.3.7, (2012)
7. G.B. Rusu, D. Spridon. I. Topala, N. Dumitrascu, Characterization of PEG-like films obtained in plasma condition, 23rd European Conference on Biomaterials (ESB 23), Tampere, Finland, Conference CD, 3894 (2010)
8. D. Spridon, G.B. Rusu, I. Topala, Adsorbtion studies of L-asparaginase on polymer surfaces, PhD Students Workshop on Fundamental and Applied Research in Physics (FARPhyis), Facultatea de Fizica, Iasi 2009

9. J. Kluson, E. Falos, C. Luca, G.B. Rusu, R. Niedrist, C. Ionita, N. Y. Sato, R. Perekrestov, P. Kudrna and M. Tichy, Diagnostic study of the discharge in the low pressure plasma jet sputtering systems, 26th Symposium on Plasma Physics and Technology, Prague, Czech Republic, 18 June, 2012 to 21 June, 2012
10. R. Schrittwieser, C. Ionita, A. Murawski, C. Maszl, M. Asandulesa, A. Nastuta, G.B. Rusu, C. Luca, E. Falos, R. Niedrist, C. Douat, S.B. Olenici-Craciunescu, I. Vojvodic, M. Dobromir, D. Luca, S. Jaksch, P. Scheier, "Hollow cathode sputtering experiments for titanium thin films and related phenomena", Technical Meeting on "Plasma Science and Technology " IEE Japan PST-13-001~009 (Hitachi, Ibaraki, Japan, 15-16 March 2013; 15 March 2013)

Selective Bibliography

[1] Chu, P.K. & Liu, X. (1998). Biomaterials Fabrication and Processing HANDBOOK. CRC Press.

[2] Kingery, W.D., Bowen, H. & Uhlmann, D. (1976). Introduction to ceramics, Jhon Willey & Sons, New York.

[3] Kingshott P, Wei J, Bagge-Ravn D, Gadegaard N, Gram L. Covalent attachement of poly (ethylene glycol) to surfaces, critical for reducing bacterial adhesion. Langmuir 2003; 19: 6912-21.

[4] Miguel Manso Silvan, A. Valsesia, D. Gilliland, G. Ceccone, F. Rossi, An evaluation of poly(ethylene-glycol) films stabilized by plasma and ion beam methods, Applied Surface Science 235 (2004) 119–125

[5] N. Inagaki, Plasma Surface Modification and Plasma Polymerization, Technomic, Lancaster, (1996).

[6] A. S. Chiper, G. B. Rusu, A. V. Nastuta, G. Popa, 'On the Discharge Parameters of a Glow-Mode DBD at Medium and Atmospheric Pressure', IEEE Transaction on Plasma Science, Vol. 37, Is. 10,Part 2, p. 2098-2102, (2009);

[7] Butt, H.J., Cappella, B. & Kappl, M. (2005). Force measurements with the atomic force microscope: Technique, interpretation and applications. Surface science reports, 59, 1–152.

[8] Lilia A. Chtcheglova, George T. Shubeitaand al. "Force spectroscopy with a small dithering of AFM tip: a methode of direct and continuous measurements of the spring constant of single molecules and molecular complex" Biophysical Journal 86 (2004) 1177 – 1184.

[9] Vadia, N. & Rajput, S. (2011). Mesoporous material, mcm-41: A new drug carrier. Asian Journal of Pharmaceutical and Clinical Research, 4, 44–53.

[10] Al-Othman, Z.A. (2012). A review: Fundamental aspects of silicate mesoporous materials. Materials, 5, 2874–2902.

[11] Kresge, C., Leonowicz, M., Roth, W., Vartuli, J. & Beck, J. (1992). Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. nature, 359, 710–712.

[12] Goethals, F., Levrau, E., De Canck, E., Baklanov, M.R., Detavernier, C., Van Driessche, I. & Van Der Voort, P. (2013). Pore narrowing of mesoporous silica materials. Materials, 6, 570–579.

[13] Klotz, M., Ayral, A., Guizard, C. & Cot, L. (2001). Synthesis and characterization of silica membranes exhibiting an ordered mesoporosity. control of the porous texture and effect on the membrane permeability. Separation and purification technology, 25, 71–78.

[14] Cazacu, A., Legrand, Y.M., Pasc, A., Nasr, G., Van der Lee, A., Mahon, E. & Barboiu, M. (2009). Dynamic hybrid materials for constitutional self-instructed membranes. Proceedings of the National Academy of Sciences, 106, 8117–8122.

[15] Y.-Q. Chen et al. / J. Chromatogr. B 875 (2008) 502–508

[16] A.F. Beloto et al. / Nucl. Instr. and Meth. in Phys. Res. B 206 (2003) 677–6813) 677–681