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RESEARCH PROJECTS FOR ENCOURAGING ESTABLISHMENT

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HIGH PERFORMANCE POLYMERIC BIOMATERIALS BASED ON FUNCTIONALIZED POLYSULFONES WITH MEDICAL APPLICATIONS

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BRIEF REPORT

concerning the results obtained by the project team between December 16th, 2015 – September 30th, 2016

Objectives and activities proposed for Stage 4 - 2016:

Objective 7. *Optimization of the properties in solution and solid state to obtain semipermeable composite membranes*

Activity: 7.3. Achievement of some semipermeable membranes with controllable pore size. Study of surfaces by specific tests: permeability studies.

Objective 8: *Specific properties of composite membranes for biomedical applications*

Activities: 8.1. Establishing the water adsorption degree, compatibility of polymeric composites/blends with blood components.

8.2. Establishing the correlation between hydrophilicity and biocompatibility for specific applications.

8.3. Testing of antimicrobial activity of polymeric composites/blends using bacterial species (*Staphylococus Aureus and Escherichia Coli*).

Performed objective and activities. All activities proposed for these stages were achieved and are summarized in this report.

7.3. Achievement of some semipermeable membranes with controllable pore size. Study of surfaces by specific tests: permeability studies

The project analyzes the possibilities to obtain new semipermeable biomembranes with controlled degee of hydrophilicity and porosity, obtained by mixing the polysulfones functionalized with quaternary ammonium groups (PSFQ) with various hydrophilic polymers (CAP - cellulose acetate phthalate and polyvinyl alcohol - PVA), achieving thus a balance between the properties of the individual components. The analyzed property is “permeability effect" due to the interaction of the surface chemistry and surface topography at macro- and nanoscale. In polymers, the water sorption can provide vital informations about materials; the moisture sorption properties are recognized as critical factors in determination of their storage performance, stability, processing and application. In this sense, the capacity of the water vapor sorption for the composite membranes based on quaternized polysulfone (PSFQ/CAP and PSFQ/PVA) was determined in dynamic conditions from the sorption isotherms using DVS technique - Dynamic vapor sorption. Figure 1 shows the sorption/desorption isotherms for studied composite systems.

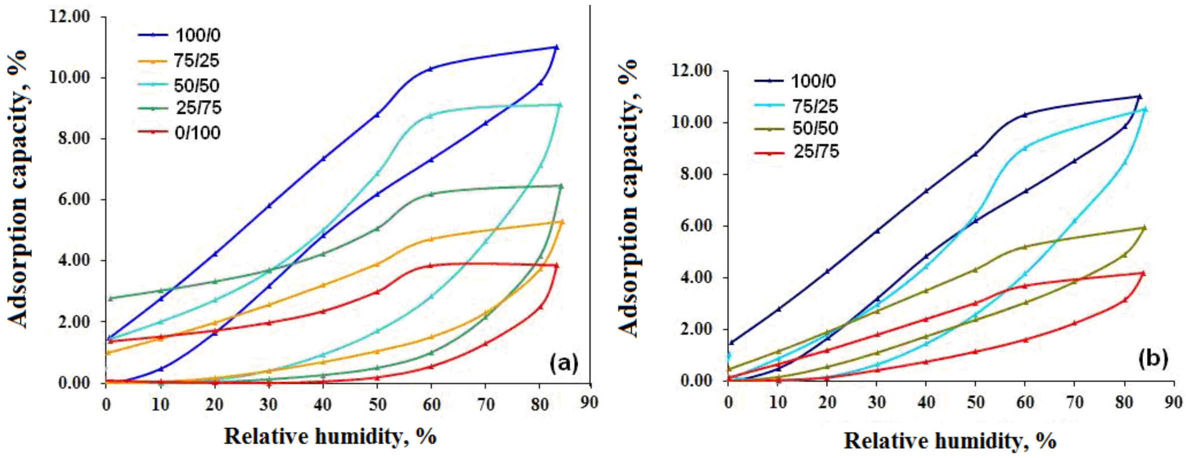

Figure 1. Sorption/desorption isotherms for composite systems of PSFQ/PVA (a) and PSFQ/CAP (b) for different compositions of polymers mixtures at 25 °C

According to IUPAC classification, taking into account the isotherms shape, these may be associated with Type V isotherms. This type represents the adsorption isotherms with hysteresis characteristic to the porous materials [1]. In addition, they describe the sorption of the hydrophilic or weak hydrophobic materials with sorbent-water weak interactions, low sorption at small humidity, sometimes moderate sorption at medium humidity, and a sharp increase in water sorption at humidity close to 100% [2-4]. Table 1 list the values of the surface parameters evaluated from sorption/desorption isotherms, for all systems studied.

Table 1. Surface parameters evaluated from sorption/desorption isotherms, for PSFQ/PVA and PSFQ/CAP composite systems

Polymer/System	Sorbtion capacity, %	Average size of pores, nm	BET Method	
			Specific area, m^2/g	Monolayer, g/g
PSFQ	11.0123	1.046	210.532	0.0599
PVA	3.8602	1.331	58.030	0.0165
CAP	3.6914	0.813	62.230	0.0132
PSFQ/PVA				
75/25	9.1212	1.855	81.990	0.0233
50/50	6.4677	1.291	199.674	0.0568
25/75	5.2915	0.913	69.723	0.0198
PSFQ/CAP				
75/25	10.5182	1.107	201.104	0.0573
50/50	5.9307	1.053	107.094	0.0305
25/75	4.1771	1.046	79.294	0.0226

For studied composite systems the differences between the sorption capacities can be explained by differences in their structure and morphology. In such investigations the structure-solubility in water relationship is a key parameter, especially for the systems with polar groups. By analyzing the experimental results obtained from sorption/desorption graphs, the variation of the dynamic sorption capacity of water vapor shows the same trend for both composite systems. Therefore, the sorption capacity is influenced by the chain's charge density (quaternary ammonium groups) and also by polar groups that are strong protono-acceptors in the formation of the hydrogen bonds. In addition, presence of the flexible groups allows the mobility or relaxing of the macromolecular chains in the polysulfone matrix, providing enough space for the water molecules to integrate into composite system.

Brunauer-Emmett-Teller method (BET Equation 1) [5] was used to evaluate the specific surface area (Table 1) based on water vapor sorption data registered under dynamic conditions. The difference between the order of water sorption capacity and values obtained for specific surface may be caused by the nature of the functional groups of the polymers from the system.

$$W = \frac{W_m \cdot C \cdot RH}{(1 - RH) \cdot (1 - RH + C \cdot RH)} \qquad (1)$$

where W – weight of sorbed water, W_m – weight of water forming the monolayer, C – sorption constant, RH – relative humidity.

Average pore size was estimated by applying the Barrett, Joyner and Halenda model (BJF, Equation 2) [6] assuming a cylindrical geometry of the pores; Table 1 lists these values.

$$r_{pm} = \frac{2 \cdot n}{100 \cdot \rho_a \cdot A} \quad (2)$$

where: A surface area evaluated by BET method; n absorption percentage; ρ_a the density of the adsorbed phase.

Investigations have demonstrated that on their surface can be observed the existence of nanosized pores whose number varies from sample to sample (evaluations from AFM technique) depending on the polymers structure from blend. It is distinguished the porogen effect of the cellulose acetate phthalate and polyvinyl alcohol that generate structures with a high specific surface area and small pore size into the polysulfone matrix. It can be asserted that through the realised researches were also highlighted the transport properties of the composite polysulfone membranes.

In conclusion, the obtained results confirm the possibility of obtaining new semipermeable membranes, by technique proposed in this project, the control of the porosity and topographic features being adjusted through the polymer choice. All these results certify the PSFQ/CAP and PSFQ/PVA composites as potential candidates for practical applications in membrane technology in the biomedical field, controlled release of drugs, wastewater treatment, etc.

Objective 8: *Specific properties of composite membranes for biomedical applications*

8.1. Establishing the water adsorption degree, compatibility of polymeric composites/blends with blood components

A key factor in the design of the biocompatible polymers with an important role in the materials science, biochemistry, cell biology, and biomedicine consists in the establishment of a relationship between the surface characteristics of the polymer and blood components. It is well known that the response of a cell and the way in which adhere to the polymer surface when they are in contact, greatly influence the materials biocompatibility. The biocompatibility of polymers can be improved by changing the polarity and therefore, the watering capacity, addition of functional groups to prevent thrombogenicity, and also the coverage of the surface with biologically compatible species, *e.g.*, proteins and antibiotic [7].

In addition, the compatibility is dictated by the way in which the polymer surfaces interact with blood components, such as red cells and platelets.

Polysulfone and chemically modified polysulfones are the most diverse class of biomaterials more used in biomedical applications, thus significantly contributing to the quality and efficiency of health [8]. In the context of those presented, another objective of the cationic polysulfones surface analysis in mixtures with hydrophilic polymers, aims a better understanding of the interfacial chemistry of adhesion not only with water, but also with some blood components (red blood cells - RBC, platelets) and plasma proteins (albumin, immunoglobulin G (IgG), fibrinogen), representing the necessary condition for the evaluation of the possibilities of using the composite systems based on functionalized polysulfones in biomedical applications and to determine their compatibility with some compounds from blood. Therefore, the values obtained for the interfacial tension γ_{sl} (Equation 3) [(s) - polymeric surface PSFQ/CAP and PSFQ/PVA and (l) - blood or plasma protein components] and work of spreading W_s, (Equation 4) [9,10], have revealed the existence of the attraction forces between two polymeric particles, (s) immersed in blood medium (l) (Figures 2-4).

$$\gamma_{sl} = \left(\sqrt{\gamma_{lv}^{p}} - \sqrt{\gamma_{sv}^{AB}}\right)^2 + \left(\sqrt{\gamma_{lv}^{d}} - \sqrt{\gamma_{sv}^{LW}}\right)^2 \quad (3)$$

$$W_s = W_a - W_c = 2[(\gamma_{sv}^{LW}\gamma_{lv}^{d})^{0.5} + (\gamma_{sv}^{+}\gamma_{lv}^{-})^{0.5} + (\gamma_{sv}^{-}\gamma_{lv}^{+})^{0.5}] - 2\gamma_{lv} \quad (4)$$

γ_{lv}^{d} and γ_{lv}^{p} - represent the disperse and polar components (with the electron donor, γ_{lv}^{-}, and electron acceptor, γ_{lv}^{+}, contributions) at total surface tension, γ_{lv}, of the liquids test used; γ_{sv}^{LW} and γ_{sv}^{AB} - represent the disperse and polar components of the total surface tension of the composite polysulfone films, $\gamma_{sv}^{LW/AB}$, with the electron donor, γ_{sv}^{-}, and electron acceptor, γ_{sv}^{+}, contributions, evaluated by LW/AB method (***data obtained in stage 2 – Objective 4***).

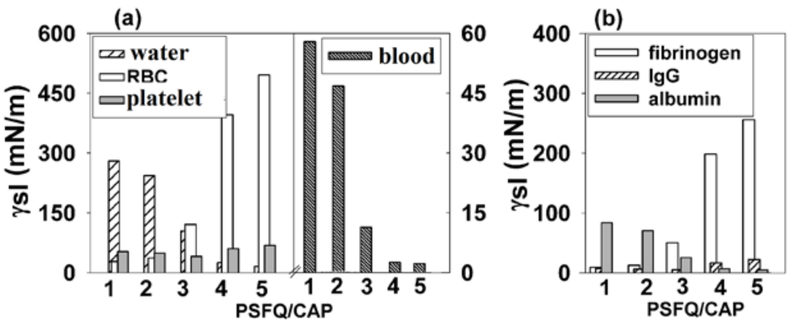

Figure 2. Interfacial tension of water and blood components (a), and of some plasma proteins (b), at the PSFQ/CAP composite films surface (Notations for mixing ratios: „1” - 100/0; „2” - 75/25; „3” - 50/50; „4” -25/75; „5” - 0/100)

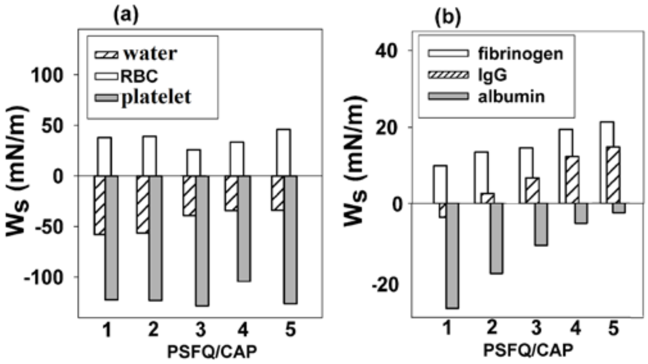

Figure 3. Spreading work of water and blood components (a), and of some plasma proteins (b), at the PSFQ/CAP composite films surface (Notations for mixing ratios: „1” - 100/0; „2” - 75/25; „3” - 50/50; „4” -25/75; „5” - 0/100)

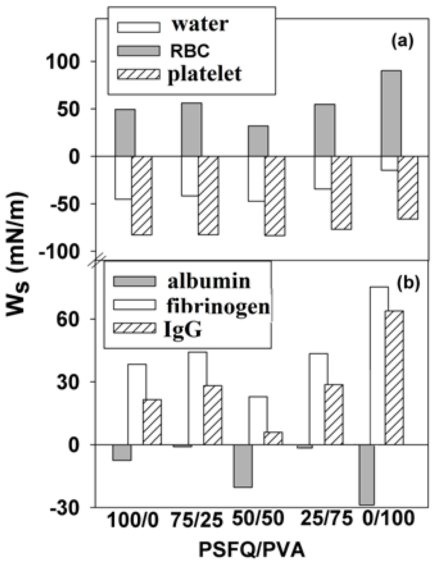

Figure 4. Spreading work of water and blood components (a), and of some plasma proteins (b), at the PSFQ/PVA composite films surface (Notations for mixing ratios 100/0; 75/25; 50/50; 25/75; 0/100)

The positive values obtained for the spreading work of red blood cells, and negative values of the spreading work of platelets (Figure 3 (a), 4 (a)) on the studied composite films surface *were used for the biomaterials characterization, in terms of cell adhesion*. In this context, it is known that materials exhibiting low values of the adhesion work have low capability of cell adhesion compared with those that have a high work of adhesion [11].

8.2. Establishing the correlation between hydrophilicity and biocompatibility for specific applications

The interaction between the polymer surface and red blood cells is mediated mostly by the hydrophobic interaction with the lipid bilayer, electrostatic interaction due to the charged surface and/or direct interaction with plasma proteins membrane, depending on the polymer characteristics. For the analyzed composite systems, the electrostatic interaction may appear between the cationic polysulfone surface and red blood cells as a result of the positively charged of the quaternized groups and of negative charge of red blood cell surface. *This type of interaction becomes decisive, along with the increasing of these polysulfone contributions, especially as genetic carriers in biomedical applications*.

The platelets are essential in hemostasis maintaining, these being very sensitive to the microenvironment blood changes. Considering the exposure to the blood platelets, the negative values of the spreading work (Figures 3 (a) and 4 (a)) *shows that all samples evidence a pronounced cohesion, suggesting that the composites do not interact with platelets, preventing thus the activation of the coagulation at the biomaterial-blood interface*.

An important aspect in the biocompatibility evaluation refers to the competitive or selective adsorption analysis of the blood proteins on the biomaterial surface. Thus, Figures 3 (b) and 4 (b) shows the negative values of the spreading work for albumin that together with platelet rejection highlights the important role that has in the "*host-material*" interaction. On the other hand, the *quaternized polysulfone can be considered as being compatible with certain elements from physiological environment* (*e.q., tissue, cells*), because their interaction with studied biological materials do not generate damage of the blood cells or do not cause changes in plasma proteins structure. In addition, the positive values of the spreading work obtained for fibrinogen and IgG (Figure 3 (b) and 4 (b)) designate the studied polysulfone composite *as being materials with promising properties used in the creation of devices that come in contact with blood (including vascular grafts, stents, pacemakers, extracorporeal circuits, etc)*.

All these properties, correlated with films microarchitecture (Activity 7.2), recommend the blends/composites based on quaternized polysulfones as good candidates for applications in cell and tissue engineering.

Therefore, the compatibility with blood means the preventing of platelet adhesion and the deactivation of the clotting system, phenomena generated by the competitive adsorbtion of blood protein on the polymer surface [12].

Finally, these results appear to be applicable for evaluation of the bacterial adhesion on polymeric surface and then, it could be used to study the possible infections induced by various implants or for obtaining biomembranes.

8.3. Testing of antimicrobial activity of polymeric composites/blends using bacterial species (*Staphylococus aureus and Escherichia coli*)

In addition to cell-material interaction, the antibacterial properties play an important role in medical implants. Thus, for the correct operation of an implant is very important that the attachment of bacteria to be prevented. Some polymers with antibacterial properties that kill the bacteria or prevent their attachment can be used to cover or deposit on the implant surface an antimicrobial coating that offers resistance against the bacterial colonization [13]. In this sense, the polymers with quaternary ammonium groups are polymeric biocide most explored. Testing the antimicrobial activity of the polysulfones containing quaternary ammonium groups is one of the most important properties directly related to possible new applications of these compounds. In the context of those mentioned the antimicrobial efficacy of composites based on quaternized polysulfone (PSFQ/CAP and PSFQ/PVA) was examined, from clinical point of view, on two representative microorganisms *Escherichia coli* ATCC-10536 (Gram-negative) and *Staphylococcus aureus* ATCC-6538 (Gram-positive). The experimental results obtained after establishment of inhibition zones diameters (mm) of blends/composites on the two microorganisms test are shown in Table 2 and Figure 5.

Table 2. Evidencing of the antibacterial action of the compounds tested against to the reference strains, for solutions of 0.5 g/dL in NMP (NMP - reference)

Sample	**Inhibition zones diameters (mm)**			
	Conventional sample notation	Microorganism test *Staphylococcus aureus*	Conventional sample notation	Microorganism test *Escherichia coli*
PSFQ	1 A	18	1 E	23
PVA	2 A	16	2 E	22
CAP	3 A	20	3 E	20
70/30 PSFQ/ PVA	4 A	16	4 E	21
70/30 PSFQ/CAP	5 A	19	5 E	20

*A and E notations refers to the *Staphylococcus aureus* and *Escherichia coli* bacterial species

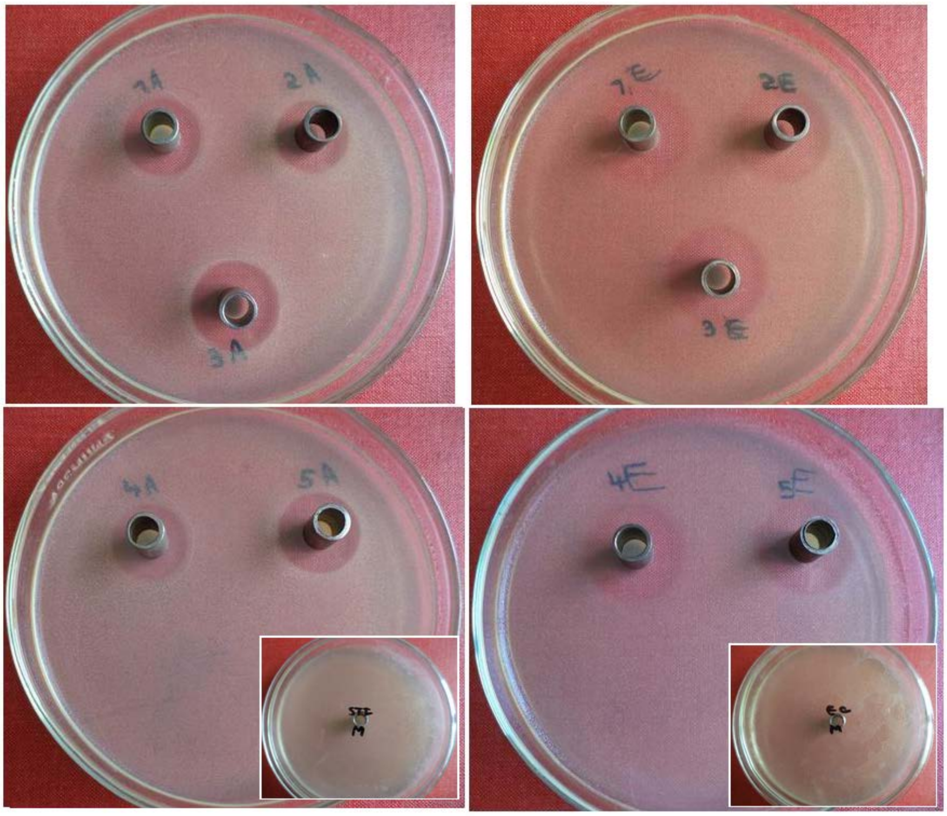

Figure 5. Testing the antimicrobial activity of the studied composite systems (PSFQ/PVA and PSFQ/CAP at various compositions according to notations from Table 2) in NMP against *Staphylococcus aureus and Escherichia coli*, expressed through inhibition zone diameter. Small graph inserted to the right of each figure corresponds to the control sample - NMP

Analysis of the presented data highlights the following:

- for all tested polymer solutions/mixtures is observed the existence of bactericidal action against Gram - negative (*E. coli*) and Gram - positive (*S. aureus*) bacteria; inhibition is more intense compared to the control sample - NMP;

- the analyzed mixtures inhibit the microorganisms growth; being observed small differences concerning the inhibitory effects of these polymers with hydrophilic characteristics. Thus, *S. aureus* is less sensitive than *E. coli* on more hydrophilic polymeric surfaces, inhibition becoming stronger with increasing of CAP content;

- as the inhibition zone diameter is larger, the seed is more sensitive, meaning that the amount of polymer/composite required for inhibition of tested bacteria is less and inverse.

The cationic polysulfones modified with quaternary ammonium groups interfere with bacterial metabolism by the electrostatic interactions that take place at bacterial cell surface

[14-16]. This conclusion is supported by the values of the inhibition zone diameter listed in Table 2; the results show that the bacterial activity of the tested components depends on the microorganism nature. Thus, has been found that the *E. coli* is more sensitive at polymers/composites investigated against to *S. aureus*. On the other hand, differences in the cell wall composition of Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria determine different resistance to destruction by antimicrobial agents (PSFQ). Therefore, all these aspects indicate that the antimicrobial activity depends on the functional groups of the quaternized polysulfone, polyvinyl alcohol and cellulose acetate phthalate and hydrophilic/hydrophobic character of the microorganism, generating interactions between the different functional groups and membranes that form the cell wall of the microorganism.

The antimicrobial activity of the composites based on polysulfone with quaternary ammonium groups is considered to be one of the most important properties directly related to possible new applications. This property is useful in investigations concerning the specific biomedical applications and in use of these composite as semipermeable membranes.

We conclude that the optical properties, degree of hydrophilicity and surface composite membrane morphology obtained from a previous conformational characterization in solution, recommends these polymeric materials for membranes obtaining. Furthermore, the information obtained from the experimental results concerning the antimicrobial activity contributes to the enlarging of the possibilities for application of these composites in the biomedical field.

All the objectives were fully achieved and the most results represent part from the content of the published/submitted for publication scientific papers, books/book chapters, and presentations at international and national scientific conferences.

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Results dissemination: 2016

o 7 papers were elaborated and submitted to journals indexed by Web of Knowledge (4 published, 3 in press);

o 3 chapters book (Ed. InTech and Formatex Research Center)

o 9 participations at scientific events (2 oral presentation and 7 posters).

Chapters book:

1. Anca Filimon, *Perspectives of Conductive Polymers toward Smart Biomaterials for Tissue Engineering, In* Conducting Polymers, Faris Yilmaz (Ed.), InTech Publisher, Chapter 6, pp. 119-143, (2016) ISBN: 978-953-51-2691-1, DOI: 10.5772/63555

2. Adina-Maria Dobos, Anca Filimon, *Polymeric Membranes: From Basic Concepts and Separation Mechanisms to Their Impact on Daily Life*, In: Polymer Science: Research Advances, Practical Applications and Educational Aspects, Formatex, Polymer Science Book Serie Nº 1, A. Méndez-Vilas, A. Solano (Eds.), pp. 429-440, (2016) ISBN-13: 978-84-942134-8-9

3. Mihaela-Dorina Onofrei, Anca Filimon, *Cellulose-Based Hydrogels: Designing Concepts, Properties, and Perspectives for Biomedical and Environmental Applications*, In: Polymer Science: Research Advances, Practical Applications and Educational Aspects, Formatex, Polymer Science Book Serie Nº 1, A. Méndez-Vilas, A. Solano (Eds.), pp. 108-120, (2016) ISBN-13: 978-84-942134-8-9

Papers:

1. Anca Filimon, Ecaterina Avram, Niculae Olaru, Florica Doroftei, Simona Dunca, Electrospun Fibers Containing Cationic Quaternary Ammonium Derivatives with Antibacterial Activity, *IEEE International Conference on E-Health and Bioengineering - EHB 2015, Xplore® ISI Proceedings (Conference Proceedings Citation Index),* ISBN: 978-146737545-0, 2016, DOI: 10.1109/EHB.2015.7391562

2. Adina Maria Dobos, Anca Filimon, Ecaterina Avram, Ghiocel Emil Ioanid, Impact of Surface Properties of Blends Based on Quaternized Polysulfones on Modeling and Interpretation the Interactions with Blood Plasma, *IEEE International Conference on E-Health and Bioengineering - EHB 2015, Xplore® ISI Proceedings (Conference Proceedings Citation Index*), ISBN: 978-146737545-0, 2016, DOI: 10.1109/EHB.2015.7391562

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4. Anca Filimon, Raluca Marinica Albu, Iuliana Stoica, Ecaterina Avram, Blends Based on Ionic Polysulfones with Improved Conformational and Microstructural Characteristics: Perspectives for Biomedical Applications, *Composites Part B-Engineering*, 93(1), 1-11, 2016, DOI: 10.1016/j.compositesb.2016.02.062.

5. Anca Filimon, Adina Maria Dobos, Ecaterina Avram, Factors Controlling the Ionic Transport Processes in Polymer Mixture Solutions Based on Quaternized Polysulfones, Polymer Engineering and Science, 2015 (in press).

6. Adina-Maria Dobos, Anca Filimon, Dielectric Constant and Conductivity as Predictors for Physico-Chemical Properties of Blends Based on Quaternized Polysulfones, Romanian Reports in Physics, 2016 (in press).

7. Mihaela-Dorina Onofrei, Anca Filimon, Iuliana Stoica, Control of Surface Properties of Charged Polysulfone/Cellulose Acetate Phthalate Films with Implications in Water Treatment, Romanian Reports in Physics, 2016 (in press).

Oral presentations:

1. Anca Filimon, Ecaterina Avram, Conformational restructuration induced by plasticizer effect of polyvinyl alcohol in functionalized polysulfone systems, A XXXIV-a Conferinta Nationala de Chimie, Calimanesti-Caciulata, 4-7 Octombrie 2016.
2. Mihaela-Dorina Onofrei, Anca Filimon, Iuliana Stoica, Impact of Microstructures on Surface Properties in Quaternized Polysulfone/Cellulose Acetate Phthalate Blends, A XXXIV-a Conferinta Nationala de Chimie, Calimanesti-Caciulata, 4-7 Octombrie, 2016.

Posters:

1. Anca Filimon, Adriana Popa, Ecaterina Avram, Niculae Olaru, Florica Doroftei, Simona Dunca, Design of Biologically Active Polymer Surfaces: cationic Polyelectrolyte Fibers as multifunctional Platform to Prevent Bacterial Attachment, 9th Edition of Symposium with International Participation - New trends and strategies in the chemistry of advanced materials with relevance in biological systems, technique and environmental protection, Timisoara, Romania, 9-10 Iunie 2016.
2. Anca Filimon, Valentina Elena Musteata, Ecaterina Avram, Conductive Polymer Blends: Dielectric Behavior-Chemical Structure Relationship in Quaternized Polysulfone/Cellulose Acetate Phthalate System, 16th International Balkan Workshop on Applied Physics and Materials Science - IBWAP, Constanta, Romania, 7-9 Iulie 2016.
3. Adina-Maria Dobos, Anca Filimon, Ecaterina Avram, Dielectric Constant and Conductivity as Predictors for Physico-Chemical Properties of Blends Based on Quaternized Polysulfones, 16th International Balkan Workshop on Applied Physics and Materials Science - IBWAP, Constanta, Romania, 7-9 Iulie 2016.
4. Mihaela-Dorina Onofrei, Anca Filimon, Ecaterina Avram, Control of Surface Properties of Charged Polysulfone/Cellulose Acetate Phthalate Films with Implications in Water Treatment, 16th International Balkan Workshop on Applied Physics and Materials Science - IBWAP, Constanta, Romania, 7-9 Iulie 2016.

5. Anca Filimon, Ecaterina Avram, Quaternized Polysulfone/Polyvinyl Alcohol Multicomponent System: Computational Modeling of Hydrogen Bonding and Electrostatic Interactions, International Conference of Physical Chemistry– ROMPHYSCHEM, Galati, Romania, 21-24 Septembrie 2016.
6. Adina Maria Dobos, Anca Filimon, Ecaterina Avram, Modeling the Conformational Characteristics of Ionic Polymers in Solution Induced by the Presence of Polyvinyl Alcohol, International Conference of Physical Chemistry – ROMPHYSCHEM, Galati, Romania, 21-24 Septembrie 2016.
7. Mihaela-Dorina Onofrei, Anca Filimon, Iuliana Stoica, Influence of Hydrophilic Polymer on Polysulfonic Membrane Properties: Rheological and Microstructural Aspects, International Conference of Physical Chemistry – ROMPHYSCHEM, Galati, Romania, 21-24 Septembrie 2016.

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