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# ***BIOACTIVE/BIOCOMPATIBLE POLYMERIC MATERIALS***

## **Spring Training Course Program**

**Centre of Polymer and Carbon Materials, Polish Academy of  
Sciences, Zabrze**

**March 7 – 11, 2011**

supported by

**„Cristofor I. Simionescu” Postdoctoral  
Fellowship Programme**

Project co-financed by European Social Fund, Sectoral Operational  
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2007 – 2013

**Priority Axis 1:** Educational and professional training to support  
economical growth and development of the knowledge-based society  
Major domain 1.5: Doctoral and Postdoctoral Fellowship Programmes  
intended to support research

Contract: POSDRU/89/1.5/S/55216

Beneficiary: “Petru Poni” Institute of Macromolecular Chemistry, Iasi

<http://www.postdoc-icmpp.ro/>



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Prof. Andrzej Dworak

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Dr. Valeria Harabagiu  
Prof. Marek Kowalczyk  
Dr. Magdalena Aflori

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## „Cristofor I. Simionescu” Postdoctoral Fellowship Programme

### Consortium

- ✓ "Gh. Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection; contact: Prof. Nicolae Hurduc
- ✓ “Nicolae Simionescu” Institute of Cell Biology and Pathology, Bucharest; contact: Acad. Maya Simionescu
- ✓ Fundamental and Advanced Technical Research Centre of the Romanian Academy, Timisoara; contact: Dr. Ladislau Vekas
- ✓ Institut Européen des Membranes (CNRS / École Nationale Supérieure de Chimie Montpellier Université Montpellier-II), Montpellier, France; contact: Dr. Mihail Barboiu
- ✓ Centre of Polymer and Carbon Materials, Zabrze, Poland; contact: Dr. Andrzej Dvorak

### Objectives

Research in biomaterials represents a national and European priority (hot area), whose approach requires an interdisciplinary cooperation between chemistry, biology, physics, (bio)materials science, medicine, informatics and an exceptional and expensive research infrastructure, as well as a critical mass of highly trained researchers. The main objective of the project is to train, for three years, 40 young researchers having obtained the PhD degree in the preparation, investigation (structure – properties – application), characterization and use of biomaterials. A second objective consists in increasing the attractiveness of research/innovation career for young PhD researchers through the facilities offered by the project (post-doctoral fellowships, free access to an outstanding research infrastructure, training courses, short trainings in specialized European laboratories, financial support for attending international symposia/congresses). Last but not least, the project will increase – through publications in highly specialized journals – the visibility of Romanian research on the international scientific scene.

### Activities

The proposed objectives will be attended through a clear **set of activities**:

- ✓ Project management
- ✓ Preparation of postdoctoral program and of the scientific curricula
- ✓ Selection of postdoctoral fellows
- ✓ Scientific coordination
- ✓ Research, development and innovation activities stated in the postdoctoral projects
- ✓ Interdisciplinary specialization through national and international stages
- ✓ Participation to national and international scientific events
- ✓ Organization of the scientific/technical training sessions of the postdoctoral fellows
- ✓ Organization of (research) management and entrepreneurial culture courses for the researchers

### Contact:

Acad. Bogdan C. Simionescu, project coordinator ([bsimion@icmpp.ro](mailto:bsimion@icmpp.ro))

Dr. Mihaela Olaru, project manager ([olaruma@icmpp.ro](mailto:olaruma@icmpp.ro))

Dr. Valeria Harabagiu, scientific responsible ([hvaleria@icmpp.ro](mailto:hvaleria@icmpp.ro))

Dr. Magdalena Aflori, dissemination responsible ([maflori@icmpp.ro](mailto:maflori@icmpp.ro))

Ec. Roxana Matache, financial responsible ([matache.roxana@icmpp.ro](mailto:matache.roxana@icmpp.ro))

Jr. Raluca-Oana Andone, juridic responsible ([ralucaandone@yahoo.com](mailto:ralucaandone@yahoo.com))



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## Program of Spring Training Course

**Sunday, March 6**      **Arrival to Krakow**, departure to Zabrze by bus accommodation at hotel ALPEX

**Monday, March 7**      **Centre of Polymer and Carbon Materials, Polish Academy of Sciences in Zabrze**

10:00 Opening and welcome remarks - **Prof. Andrzej Dworak**

10:15 **Prof. Andrzej Dworak** – Smart polymers - new route to medical devices  
(*training presentation*)

11:00 Coffee break

11:30 Seminar presentations

**B. Robak** - Encapsulation of peptides in polymeric nanocontainers (*training presentation*)

**R. Trzcińska** - Polymer-peptide conjugates on surface (*training presentation*)

**A. Arvinte** - Nanocomposite materials based on graphene or carbon nanotubes used for electrochemical sensors development

**M. Silion** -  $\beta$ -Cyclodextrin-C6-Sulfate/3-Azido-3'-deoxythymidine Inclusion Complex: Synthesis, anti-HIV Activity and Toxicity.

**D. Ciolacu** - On the synthesis of novel cellulose-BASED hydrogels

**S. Nica** -  $\pi$ -Extended dyes substituted with azulenyl- heterocycles

**A. Manea** - NADPH oxidase-induced redox signaling in vascular physiology and pathophysiology; novel pharmacological strategies to counteract oxidative stress in atherogenesis

**A.D. Bendrea** - Cotton based multifunctional composite by IN SITU surface coating with conjugated polythiophene derivative

**L. Marin** - New symmetric azomethinic dimer. The influence of structural heterogeneity on the liquid crystalline behaviour

**V. Melinte** - Synthesis of amphiphilic block copolymers with fluorescent probe

14:00 Lunch

15:00 **Dr. Valeria Harabagiu** - Bio-oriented surface modification (*training presentation*)

15:45 Seminar presentations

**Dawid Szweda** - Thermosensitive poly[oligo(ethylene glycol)methacrylates] in the solution and on the surface (*training presentation*)

**M. Cristea** - Mechanical relaxation investigations in soybean oil-based polyurethane networks

**A.I. Cosutchi** - Patterning surface morphology of polyimide precursor with a lyotropic liquid crystal matrix

**M.D. Damaceanu** - Polyamides containing benzophenone chromophoric groups

**F. Bucatariu** - Single polyelectrolyte multilayers deposited onto silica microparticles and silicon wafers

**M. Calin** - Design, preparation and characterization of site-specific target-sensitive liposomes for drug delivery at sites of activated endothelium

**M. Mihai** - Nanostructured materials based on weak polyions

17:30 Coffee and discussion between fellows and Polish participants

17:30 Project management team meeting

19:00 Dinner at hotel ALPEX



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### Tuesday, March 8 **Centre of Polymer and Carbon Materials, Polish Academy of Sciences in Zabrze**

10:00 **Prof. Gerhart Braunegg** - Modern approaches to PHA production  
(*training presentation*)

10:45 Seminar presentations:

**M.Kwiecien** - Controlled depolymerization of aliphatic polyesters from natural resources(*training presentation*)

**R.N. Darie** - Physico-chemical characterization of some systems containing biomass derivatives

**M.V. Dinu** - Macroporous composite hydrogels based on synthetic and natural polymers

**T. Vlad-Bubulac** - Hydrogels Based on Poly(vinylalcohol)/Phosphoester/Chondroitin Sulfate Blends for Biomedical Applications

**M. Brebu** - TG-MSD/FTIR, a useful technique for characterization of thermal behavior of biomass waste

**L. Ursu** - Synthesis and characterization of a silsesquioxane nanocomposite with photoluminescence properties

**A. Burlacu** - In vitro studies regarding the cardiac regenerative potential of mesenchymal stem cells

12:30 Coffee break

13:00 **Prof. Marek Kowalczyk** – Synthetic analogues of aliphatic biopolyesters(*training presentation*)

14:00 Lunch

15:00 Seminar presentations

**I. Romanowska** - New forms of pesticides covalently bonded to biodegradable oligomers (*training presentation*)

**I. Titorencu** - Biological approaches to bone regeneration by tissue engineering and implantology

**A. Georgescu** - Identification of new biomarkers for prediction of endothelial vascular dysfunction; strategies to reverse vascular wall function

**N. Alexandru** - The mechanisms of platelet activation in atherosclerosis and the effects of irbesartan administration

16:00 Coffee break

16:30 Seminar presentations

**M. Maksymiak** - Molecular level structure investigation of bioactive conjugates with perspective application in cosmetic industry

**C. Ursu** - Excimer laser-induced surface modification of poly(ethylene terephthalate)

**C.L. Nistor** - Preliminary Studies on the Complex System: Surface-active Substances - Hybrid Silica Particles - Vegetable Oils

**L. Ignat** - Lignin-polyurethane blends. Mechanical, morphological and degradation aspects

17:30 Closing of the seminar + diploma

19:00 Dinner at hotel ALPEX

### Wednesday, March 9 **10:00 Visit at the Foundation of Cardio surgery Development in Zabrze**

13:00 Lunch at Quinn Luisa coal mine

14:30 Sightseeing at Quinn Luisa coal mine

18:00 – 19:00 Dinner at hotel ALPEX



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- Thursday, March 10** 10:00 **Visit and discussions at the Centre of Polymer and Carbon Materials, Polish Academy of Sciences in Gliwice**  
13:00 Lunch at Argentum hotel restaurant in Gliwice  
15:00 Free time  
18:00 – 19:00 Dinner at hotel ALPEX
- Friday, March 11** 9:00 **Visit at the Chemistry Department of the Jagiellonian University in Krakow**  
20:00 Dinner at hotel ALPEX
- Saturday, March 12** **Departure** to Balice airport



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## **Training course presentations**



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## **Smart polymers – a route to new solutions for medicine**

Andrzej Dworak

*Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze*

Smart polymers or stimuli responding polymers are polymers which react with a distinct change of their properties to relatively small changes of the external conditions. Most frequently, temperature responding polymers are studied, but also macromolecules sensitive to pH, presence of certain ions or biochemical factors are studied.

The lecture gives a brief review of the class of temperature and pH responding polymers. The mechanism of the phase transition (lower critical solution temperature in solutions or volume transition in gels) is discussed. Polymer materials forming the base of stimuli sensitive materials are shown and the synthetic routes to them described.

Basing upon this general discussion examples of specific applications: devices and materials are explained, some of them originating from the labs of the lecturer. Temperature sensitive nanoparticles, especially of core-shell structure, functional star-like polymers as drug carriers and temperature sensitive substrates for cell growth are described and analyzed in some details.



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## **Encapsulation of peptides in polymeric nanocontainers**

Barbara Robak, Paweł Weda, Barbara Trzebicka, Jerzy Silberring, Andrzej Dworak

*Centre of Polymer and Carbon Materials, Polish Academy of Sciences 41-819 Zabrze, Marie Curie Skłodowskiej 34, Poland*

The interest in polymeric materials as drug carriers and use of peptides and proteins as efficient drugs brought many of researchers to combine medicinal function of biomolecules together with protective function of polymeric materials to achieve more effective therapy [1-3].

Hydrophilic nature and short-time activity of some peptides make them difficult to be delivered to the desired site of a human body through the digestive system. Very often they can be easily eliminated from the systemic circulation due to enzymatic degradation and excretion. To avoid this problem biomolecules might be encapsulated inside the protective “capsule” made from polymeric material [4-5].

Thermosensitive polymers seem to be good candidates for drug delivery carriers [6]. Materials of this type are supposed to improve enzymatic resistance of active species and provide control over its delivery.

Considering previous results [7] we proposed to use core-shell nanoparticles based upon thermosensitive poly (N-isopropylacrylamide) (PNIPAM) as efficient peptide delivery carriers. PNIPAM properties were investigated in detail [8]. At room temperature it stays water soluble but upon heating above critical temperature (LCST), PNIPAM forms stable nanoparticles called mesoglobules. Formation of mesoglobules is reversible so to avoid mesoglobules dissolution they need to be stabilized [9-11].

We have proposed to encapsulate the selected peptide NOCICEPTIN (PEPTIDE-NC) into PNIPAM mesoglobules during the process of their formation. The studies on peptides/PNIPAM mixed mesoglobules were preceded by the experiments with model substance poly (2-ethyl-2-oxazoline) (PEtOx), mimicking the peptide structure. Mixed mesoglobules were stabilized by the protective shell formed via radical polymerization of 2-hydroxyethyl methacrylate (HEMA) or NIPAM in the presence of cross-linking agent. The size and morphology of mesoglobules and subsequent core-shell nanoparticles were investigated by dynamic light scattering and atomic force microscopy.

### **Acknowledgements**

This work was supported by Polish Ministry of Science and Higher Education grant NN 209 144136 and by the European Community from the European Social Fund within the RFSD 2 project.

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## **Polymer-peptide conjugates on surface**

Róża Trzcińska, Alicja Utrata-Wesołek, Barbara Trzebicka, Andrzej Dworak, Jerzy Silberring

*Centre of Polymer and Carbon Materials, Polish Academy of Science*

The conjugation of peptides with synthetic polymers became in the last few years an important trend in polymer chemistry. Covalent attachment of biologically active compounds, such as peptides linked to polymers is one of the methods for alteration and control of biodistribution, solubility, pharmacokinetics, and often toxicity of bio-compounds [1].

One of the most popular polymeric materials used as polymer chain in bioconjugation is poly(ethylene oxide). It possesses ideal properties: very low toxicity, good solubility in aqueous solution, extremely low immunogenicity, and antigenicity [2].

In recent years, poly(ethylene oxide)-peptide conjugates became a very important and useful application in microarray technology [3]. Microarrays of peptides make possible the simultaneous screening of thousands binding events in biological and pharmaceutical research [4]. Therefore, they emerge as a powerful tool for proteomics and clinical assays. It was shown that the presence of polymer as a linker between surface and peptide gives many advantages. It minimizes non-specific binding on the surface, provides low background in fluorescence measurement, allows steric freedom, and thus makes linked peptide fully accessible [5-7].

In this work, peptide-poly(ethylene oxide) conjugates were obtained by the solid phase synthesis using Fmoc chemistry. The obtained conjugates were characterized by mass spectrometry using electrospray (ESI) ionization. Immobilization of conjugates by grafting-to method on silica surface has been used. In our future work we intend to test such peptide arrays as diagnostic assay for Wegener's granulomatosis disease.

### Acknowledgement

This work was supported by the European Community from the European Social Fund within the RFSD 2 project.

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## **Bio-oriented surface modification**

Valeria Harabagiu

*“Petru Poni” Institute of Macromolecular Chemistry, 700487 Iasi, Romania*

Material properties are interdependently controlled by both their bulk and surface properties. Most of the materials encountered in our day-to-day life, those used in a variety of high-tech applications as well as those that compose living organisms are not pure compounds but formed of mixtures of phases kept together by specific interfaces. That is why the surface modification targeted to provide specific properties/interactions became a prolific topic of research also promoted by the availability of advanced methods for surface investigation at atomic/molecular and nano levels. Polymer chains can be physically or chemically attached to inorganic (metal, oxides, salts, etc) or organic (polymers) flat, curved or porous surfaces to tailor properties such as wettability, resistance to environmental or technological physico-chemical agents, adhesion, biocompatibility, lubricity, etc.

In this respect, the presentation deals with recent advances in surface attached polymer layers. Thermodynamic and kinetic aspects of polymer physisorption and chemisorption are first discussed. A second part summarizes the preparation methods for polymer-grafted surfaces with emphasis on controlled processes able to yield well-defined polymer-surface objects. Finally, the unique properties of polymer brushes compared to bulk characteristics or to physisorbed layers are highlighted in connection with specific bio-oriented applications.



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## **Synthesis and characterization of thermosensitive polymethacrylates in the solution and on the surface**

Dawid Szweda, Alicja Utrata-Wesołek, Agnieszka Kowalczyk, Andrzej Dworak

*Centre of Polymer and Carbon Materials, Polish Academy of Science 41-819 Zabrze, Marie Curie Skłodowskiej 34, Poland*

Poly[oligo(ethylene glycol) methacrylates] (POEGMA) are a new promising class of thermosensitive polymers. Most of oligo(ethylene glycol) methacrylates (OEGMA) are commercially available and easily polymerizable using atom transfer radical polymerization (ATRP) what provide a controlled way of synthesis. The copolymerization of methacrylates with short and long oligo(ethylene glycol) pendant groups permits precise control of phase transitions temperature of obtained polymers. The main advantages of the POEGMA are the lack of hysteresis of the phase transition, small effect of external physical factors (e.g. salts) on the polymer thermosensitivity and a very narrow phase transition.

Homopolymer of tri(ethylene glycol) monoethyl ether methacrylate (TEGMA-EE) and its copolymers with oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA-ME) of average molar mass 475 g/mol were synthesized by atom transfer radical polymerization. The chemical structure of P[TEGMA-EE] and its copolymers P[(TEGMA-EE)-co-(OEGMA-ME)] was characterized using NMR spectroscopy. The absolute molar masses and molar mass dispersities of polymers were studied by gel permeation chromatography. Thermosensitivity and relationship between the phase transition temperature and the composition of received polymers was examined by UV-VIS technique.

Thermosensitive polymer layers based on tri(ethylene glycol) monoethyl ether methacrylate were obtained using "grafting from" technique. Coverslip glasses were used as a substrate. To enable the ATRP polymerization, coverslips were subjected to chemical modification by the immobilization of the bromide initiator. Ellipsometry delivered information about the thickness of obtained polymer layers. The philicity of the polymer surfaces was analyzed by contact angle measurements and their morphology by AFM investigations.

### Acknowledgement

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## **Controlled depolymerization of aliphatic polyesters from natural resources**

Kwiecień, Grażyna Adamus, Marek Kowalczyk

*Center of Polymer and Carbon Materials, Polish Academy of Sciences, 34 M. Curie-Skłodowska Street, 41-819 Zabrze, Poland*

Aliphatic biopolyesters (PHAs) constitute an important group of thermoplastic, biodegradable and biocompatible polymeric materials, which permit their various commercial applications. In order to improve the physical properties of biopolyesters (PHAs) considerable efforts have been directed to the studies of their modification. One of the modification methods is the synthesis of block and graft copolymers containing PHAs structure. The properties of these kinds of copolymers can be controlled by suitable selection of the other structural units. To utilize usually high-molecular weight PHAs biopolyesters in the synthesis of block and graft copolymers, their earlier partial depolymerization is required.

In this communication the method of controlled chemical depolymerization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) biopolyester (PHBH) to obtain lower molar mass oligomers possessing the same composition and sequence distribution as the original one will be presented. The structural studies of the PHBH oligomers obtained with the aid of NMR spectroscopy and mass spectrometry methods will be reported. Moreover, the possible application of the well-defined PHBH oligomers in further synthesis will be discussed.

This work was supported by the European Community from the European Social Fund within the RFSD 2 project.



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## **Synthetic Analogues of Aliphatic Biopolyesters**

Marek M. Kowalczyk and Coworkers

*Polish Academy of Sciences, Centre of Polymer and Carbon Materials 34 M. Curie-Skłodowskiej St., 41-819 Zabrze, POLAND*

Aliphatic biopolyesters represent an interesting group of biodegradable materials for various technical and medical applications. However, until now, there is no unique biodegradable polymer which can fulfill all of the expected requirements needed for specific applications. Therefore, there is a continuous need for new polymeric materials of that kind and their detailed characterization. Successful development of catalytic synthesis of  $\beta$ -lactones offer new opportunities for the synthesis of new analogues of poly(3-hydroxyalkanoate)s, PHA, with desired architectures and controlled properties.

Recent progress and future trends regarding synthetic analogues of aliphatic biopolyesters will be presented. Using anionic ring-opening polymerization (ROP) approach, polyhydroxyalkanoate (PHA) analogues with defined chemical structure of the end groups as well as block, graft and random copolymers have been obtained and characterized by various techniques with a special emphasis to mass spectrometry. The relationship between the structure, properties and function of the novel polymeric materials prepared will be also discussed.



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## **New forms of pesticides covalently bonded to biodegradable oligomers**

I. Romanowska\*, M. M. Kowalczyk\* and G. Adamus\* in cooperation with W. J. Kowalski\*\*, P. Rychter\*\* and A. Siłowiecki\*\*\*

*\*Center of Polymer and Carbon Materials, Polish Academy of Sciences, 34 M. Curie-Skłodowska St., 41-819 Zabrze, Poland, \*\*J. Długosz University, Institute of Chemistry, Environmental Protection and Biotechnology, 13/15 Armii Krajowej Av., 42-218 Częstochowa, Poland, \*\*\*Plant Protection Institute, Sośnicowice Branch, 29 Gliwicka Str., 44-153 Sośnicowice, Poland*

The conventional forms of pesticides are used with a substantial excess therefore they have a negative impact on the environment. The excess is a consequence of a leakage of bioactive compounds due to environmental conditions such as photochemical disintegration, leaching and evaporation. Accordingly, there is a need for pesticides with prolonged activity and new forms of pesticides covalently bonded to biodegradable oligomers should overcome these environmental problems. Oligomers used as a carriers have to be non-toxic and biodegradable. These requirements are achieved by natural aliphatic biopolyesters and their synthetic analogues, for example oligo(3-hydroxybutyrate). Pesticide-oligomer conjugates ought to release the active compounds to the plants at the controlled rate in quantities required over a specified period of time. Consequently, excess of pesticides can be reduced as well as an environmental pollution.

In this project herbicide-oligomer conjugates will be investigated. The selected herbicides will be used as initiators in the anionic ring opening polymerization of  $\beta$ -butyrolactone. Synthesized conjugates will be characterized by  $^1\text{H}$  NMR, GPC and ESI-MS analysis. Usability of conjugates for agricultural application will be determined during research at the greenhouses and experimental fields at the Plant Protection Institute, Sośnicowice Branch.

This work was supported by the European Community from the European Social Fund within the RFSD 2 project



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## **Molecular level structure investigation of bioactive conjugates with perspective application in cosmetic industry**

M. Maksymiak<sup>1</sup>, G. Adamus<sup>1</sup>, M. Kowalczyk<sup>1</sup>, R. Dębowska<sup>2</sup>

<sup>1</sup>*Polish Academy of Sciences, Centre of Polymer and Carbon Materials, Ul. M. Curie-Skłodowskiej 34, 41-819 Zabrze, Poland*

<sup>2</sup>*Dr Irena Eris Centre for Science and Research, Ul. Puławska 107A, 02-595 Warszawa, Poland*

In recent years an extensive research aimed at the design of novel cosmetic delivery systems has been observed. The attachment of bioactive compounds to polymeric carrier molecules via hydrolysable bonds is one of the promising approaches. It is expected that the conjugated form of a bioactive compound shows modified pharmacokinetics and biodistribution allowing control cosmetic release. Polymeric carriers used for cosmetic modifications should be non-toxic, non-immunogenic, biocompatible and biodegradable [1].

Elaborated in Centre of Polymer and Carbon Materials facile synthesis of oligo([R,S]-3-hydroxybutyrate) (OHB) enable production of oligomers with identical structure as poly(3-hydroxybutyrate) (PHB) present in a wide variety of human tissues and in atherosclerotic plaques. Moreover, the synthetically prepared oligomers were found to be non toxic [2]. Therefore, these oligomers are considered to be useful for cosmetic modifications as novel cosmetic carriers.

The knowledge of structure-property relationships is essential for the successful application of polymeric materials, especially in biomedical applications such as tissue engineering, controlled drug delivery and cosmetic modifications. In recent years, the electrospray ionization (ESI) mass spectrometry has become a routine analytical tool for characterization of polymers, complementing NMR and other traditional techniques by providing detailed structural information about the individual molecules in polymer samples.

In this communication the synthesis of [R,S]-3-hydroxybutyric acid oligomers with required molecular weight, dispersity and chemical structure of the end groups as well as their bioconjugates with active lipoic acid and selected phenolic acids will be presented. The structure of individual macromolecules of oligo([R,S]-3-hydroxybutyrate) and its conjugates with lipoic acid and selected phenolic acids (including chemical structure of their end groups) was determined with aid of multistage mass spectrometry technique (ESI-MS<sub>n</sub>).



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Moreover, in the present communication the comparative cytotoxicity studies on [R,S]-3-hydroxybutyric acid oligomers, pure bioactive species as well as macromolecules containing lipoic acid and selected phenolic acids covalently bonded to oligo([R,S]-3-hydroxybutyrate) will be reported.

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## **Presentations of PhD fellows**



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## **Nanocomposite materials based on graphene or carbon nanotubes used for electrochemical sensors development**

A. Arvinte

*„Petru Poni” Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487 Iasi, Romania*

The integration of nanomaterials into sensing systems is presented in this work, regarding the electrochemical detection of some important analytes. A novel nanocomposite was developed based on mixed metal oxide nanoparticles (Ni-Co-oxide) and carbon nanotubes materials (CNT) homogeneously dispersed in a Nafion polymer. A screen-printed electrode body is used as platform for immobilization of the produced nanocomposite. Ni and Co mixed oxides exhibit interesting and improved electrocatalytic activities and the possibility of utilizing these materials as anode electrodes for the oxidation of insulin is evaluated [1, 2].

Insulin is a very important polypeptide hormone synthesized by pancreatic  $\beta$ -cells and used to control glucose level in blood within a narrow concentration range. Direct electrochemical measurement of insulin is of considerable interest for development of in vivo sensors for monitoring insulin secretion and therapeutic insulin formulations. The CNT-Ni-Co-oxide based electrodes enable the oxidative detection of insulin in aqueous solution at physiological pH, with a very good sensitivity and low limit of detection [3].

A further objective of this work was to explore the applications of graphene as a novel electrode material in the electrochemical oxidation of insulin and its quantitative analysis in aqueous solutions. The electrochemical activity of graphene electrode has been studied comparatively with carbon nanotube electrode by using cyclic voltammetry and differential pulse voltammetry in different media. Various types of polymers have been evaluated as matrices for incorporation of graphene and studied towards oxidation of insulin.

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## **Lignin-polyurethane blends. Mechanical, morphological and degradation aspects**

L. Ignat<sup>1</sup>, M. Ignat<sup>1</sup>, C. Ciobanu<sup>1</sup>, F. Doroftei<sup>1</sup>, V.I. Popa<sup>2</sup>

<sup>1</sup> “Petru Poni” Institute of Macromolecular Chemistry, Gr.Ghica Voda Alley 41A, Iasi 700487, Romania

<sup>2</sup> Faculty of Chemical Engineering and Environmental Protection, “Gh. Asachi” Technical University, Mangeron Avenue 71, Iasi 700050, Romania

Lignin use in polymer industry has recently become very attractive from both economical and environmental reasons. In the specific case of blending, the addition of low lignin amounts was found to improve the properties and extends the application field of other natural or synthetic polymers. The current study was carried out to determine the potential effects of small flax lignin concentrations (4.2 and 9.3 wt %) on the mechanical, morphological and degradative properties of polyester-based polyurethanes (PU). Lignin-polyurethane blends were obtained as thin films by mixing and casting the corresponding polyurethane elastomers and lignin (flax/soda pulping) dimethylformamide solutions. The surface structure and morphologies were investigated by attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR) and scanning electron microscopy (SEM), while the impact on bulk was assessed from tensile tests. The thermo-oxidative behavior was determined from thermogravimetric analysis (TGA). The susceptibility to environmental enzymatic degradation was also examined by using two different microbial oxidases. Addition of low lignin amounts (~ 4-5%) was found to have a major impact on the bulk and surface properties of resulting polyurethane blends, highly enhancing the mechanical behavior and overall macromolecular organization, but lowering the resistance at thermal oxidation. Also, the flax lignin has improved the polyurethane enzymatic oxidation. At low concentrations, lignin may successfully modulate the polyurethane elasticity, tensile strength and decomposition in oxidative environments, properties that are relevant in terms of industrial processing, environmental stability, waste management, and outdoor applications.



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## **$\beta$ -Cyclodextrin-C6-Sulfate/3-Azido-3'-deoxythymidine Inclusion Complex: Synthesis, anti-HIV Activity and Toxicity**

M. Silion, A. Dascalu, M. Pinteala, C. Ungurenasu

*Petru Poni Institute of Macromolecular Chemistry - Center for Advanced Research in Bionanoconjugate and Biomacromolecular Chemistry, Aleea Grigore Ghica Voda 41A, 700487 Iasi, Romania*

Antiviral synthetic and natural sulfated polysaccharides as well as sulfated cyclodextrins (CD) have more over two decades history and are presently under investigation, especially, as anti-HIV agents [1-3]. To decrease undesirable effects of 3-azido-3'-deoxythymidine (AZT), the most used RT blocking agent, many approaches have been examined. In addition to polysaccharide formulated with AZT, one of the most promising method involves the covalent attachment of AZT with a sulfated polysaccharide [4-6].

In the present study we report the preparation procedure and characterization of the first  $\beta$ -CD-6-Sulfate/AZT inclusion complex, as well as in vitro anti-HIV activity of the inclusion complex. Compared with a sulfated  $\beta$ -CD-AZT covalent conjugate whose anti-HIV1 activity is 350 less than the AZT molecule<sup>12</sup> ( 0.29 $\mu$ g/mL versus 0.0004  $\mu$ g/mL) and its highest selective index SI (1166) was only 1.6 more potent than free AZT (725), sulfated  $\beta$ -CD-AZT inclusion complex indicated an anti-HIV1 activity of the same order of magnitude (0.02  $\mu$ M for complex versus 0.01  $\mu$ M for AZT molecule) and in terms of cytotoxicity it is 7.5 times (97.000 : 13.000) more potent than AZT molecule.

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## **On the synthesis of novel cellulose-BASED hydrogels**

D. Ciolacu, M. Cazacu

*“Petru Poni” Institute of Macromolecular Chemistry, 700487, Romania*

The hydrogels from natural polymers are promising for application in the biomaterial field, because of their unique advantages, such as abundance, non-toxicity, biocompatibility, biodegradability, and biological functions. Cellulose, the most abundant natural polymer, can be derivatized to yield various useful products.

The paper reports the preparation and characterization of transparent cellulose-xanthan hydrogels. A two-steps procedure consisting in dissolution of cellulose in NaOH aqueous solution and mixing it with xanthan followed by the cross-linking with epichlorhydrine was approached. The hydrogels purified by washing with water and acetone and dried were investigated by optical microscopy, differential scanning calorimetry and swelling measurements. The influence of supramolecular structure of cellulose allomorphs on the swelling behaviour of these polymeric networks was discussed.



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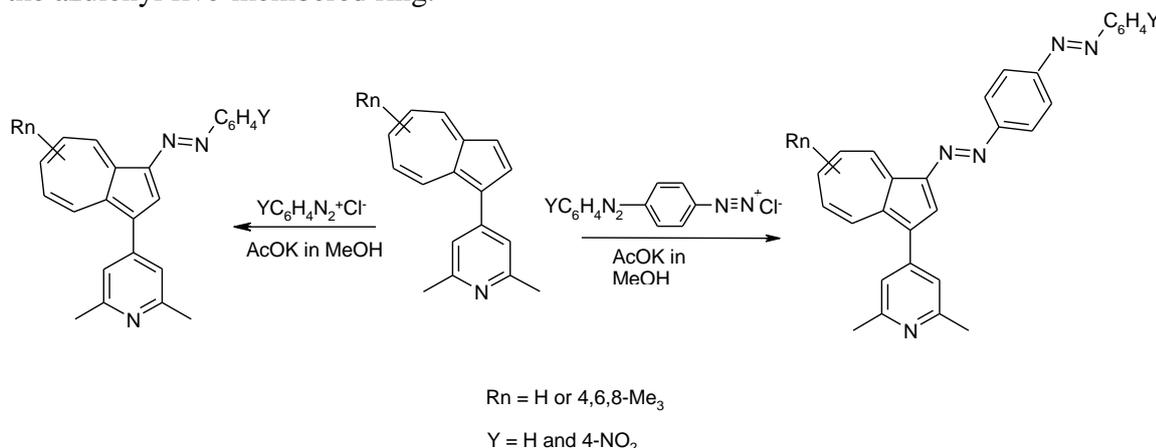
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## $\pi$ -Extended dyes substituted with azulenyl- heterocycles

S. Nica<sup>a,b</sup>, L. Cristian<sup>b</sup>, A.C. Razus<sup>b</sup>*a. "Petru Poni" Institute of Macromolecular Chemistry, 41A, Grigore-Ghica Voda Alley, Iasi, 700847, Romania**b. "C. D. Nenitzescu" Center of Organic Chemistry, 202 B Splaiul Independentei, Bucharest, 060023, Romania.*

The azo-chromophores possesses numerous spectroscopic and photophysical properties, in particular strong electronic absorption in the UV and Vis regions of the spectrum<sup>1</sup> and, because of the planarity of the azo bridge versus stilbenes or other similar systems, they contribute to larger  $\pi$  transmission effects leading to higher optical activity.<sup>2,3</sup> Azulene is a versatile fragment for designing NLO materials possessing a dipole moment of 1.08 D and, for example the azo-azulene derivatives proved to possess remarkable hyperpolarizability.<sup>4</sup> Hence, we synthesized a new family of azo dyes starting from 2,6-dimethyl-4-azulenyl pyridines taking advantage of the reactivity of the azulenyl five-membered ring.



Optical spectroscopy showed that systematic increase of the conjugation along the azobenzene skeleton has affected the spectral properties of the azophenyl substituted 4-(azulene-1-yl)-pyridine, namely a strong bathochromic shift of the visible absorption maxima by increasing the conjugating skeleton and introduction of an electron-withdrawing group. The electrochemical behavior revealed a high stability toward oxidation owing to the higher polarization induced by the azulenylpyridine moiety.

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## **NADPH oxidase-induced redox signaling in vascular physiology and pathophysiology; novel pharmacological strategies to counteract oxidative stress in atherogenesis**

A. Manea<sup>1,2</sup>

<sup>1</sup> "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania

<sup>2</sup> "Nicolae Simionescu" Institute of Cellular Biology, Bucharest, Romania

Oxidative stress is a major contributor to the etiology of all severe vascular pathologies, such as atherosclerosis, stroke, and diabetes. NADPH oxidases (Nox) are a class of multicomponent enzymes whose sole function is the generation of reactive oxygen species (ROS). Different subtypes of Nox are expressed concurrently in the vascular cells and in circulating immune cells interacting with blood vessels. Physiological production of Nox-derived ROS contributes to the maintenance of vascular homeostasis. In pathological state, hyperactivity of Nox, due to modifiable and non-modifiable (i.e., genetic background) risk factors, induces oxidative stress. Nox-derived ROS interact and stimulate other enzymatic sources of oxygen/nitrogen reactive intermediates, and amplify the initial response to insults. In atherosclerosis, Nox-induced lipid peroxidation is highly deleterious since the formation of lipid peroxides expand the free radical reactions initially produced by the activated enzyme complex. Yet, at low, non-cytotoxic concentration, various lipid peroxidation products function as signaling molecules regulating physiological and pathophysiological cell functions. Therefore, understanding the molecular mechanisms of Nox regulation, vascular and subcellular compartmentalization of ROS production and its subsequent biological significance, may lead to a focused and effective anti-oxidative stress therapy. Several drugs that interfere with Nox activation or expression, such as HMG-CoA reductase inhibitors (statins), angiotensin converting enzyme (ACE) inhibitors, AT1-receptor blockers, and calcium channel blockers, reduce vascular oxidative stress, improve endothelial function, and slow down cardiovascular disease progression. In addition to conventional pharmacological approaches small interfering RNA (siRNA) technology represents a promising strategy as therapeutic interventions for targeted specific genes with important role in vascular pathology. The major limitations against the use of siRNA as therapeutic tool are its degradation by serum nucleases, poor cellular uptake and rapid renal clearance following systemic administration. To overcome these hurdles, we developed special formulation of multifunctional nanocarriers (dendrimers, polycationic polymers, antibodyprotamine-complexes) for systemic administration of siRNA.



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## **Physico-chemical characterization of some systems containing biomass derivatives**

R.N. Darie, M. Bercea

*“Petru Poni” Institute of Macromolecular Chemistry, 41A Gr. Ghica Voda Alley, 700487, Iasi, Romania*

Much effort has been focused in recent years to develop environmentally compatible plastic products by incorporating renewable materials, as an alternative to petroleum-based synthetic polymers. These bioplastics cannot replace synthetic plastics in every application, but they can result in appropriate specific products, especially for those applications in which recovery of plastics is not economically feasible, viable, and controllable such as plastic items for one-time use. However, renewables are comparatively less expensive, environmentally friendly, and naturally biodegradable.

Blends of PVA with natural polymers such as starch, chitin, chitosan, cyclodextrin, sugar cane bagasse and other lignocellulosic fillers have been extensively studied in order to confer greater hydrophilicity and accelerated degradation while simultaneously reducing production costs. Through the incorporation of relatively low-molecular-mass lignin fractions, a plasticizing effect occurs, improving the PVA thermal stability. In the present study, some PVA/lignin systems have been realized by melt blending and their physico-chemical characterization were investigated by evaluation of processing behaviour, mechanical tests and FT-IR spectroscopy. A commercial maleated elastomer has been used as compatibility modifier. Particle size distribution of the studied systems has been determined in aqueous solutions.

Also, several composites were prepared by melt mixing using a polyethylene matrix and oak wood flour. The viscoelastic behaviour of the studied samples has been followed by dynamic rheology in melt state.



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## **Cotton based multifunctional composite by *in situ* surface coating with conjugated polythiophene derivative**

A.D. Bendrea

*“Petru Poni” Institute of Macromolecular Chemistry, Iasi, Romania*

A multifunctional textile composite was obtained by using a 3D knitted cotton fabric in conjunction with a thiophene monomer containing a thermotropic moiety in the side chain. The structure of the monomer was designed that by oxidative polymerization a stimuli-sensitive polymer (temperature, electrical field, solvent, interfaces) to be obtained. The structure of the synthesized composite material and polymer formed in the bulk reaction were characterized by infrared spectroscopy and X-ray diffraction. The polymer film morphology and the composite surface topography were examined by microscopic methods SEM and AFM and the surface wettability was evaluated by static contact angle (Cas) measurement. The thermal behavior was assessed comparatively for all the synthesized materials and their anti-adhesive and anti-microbial properties were also investigated.



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## **New symmetric azomethinic dimer. The influence of structural heterogeneity on the liquid crystalline behaviour**

L. Marin, A. Zabulica, M. Sava

*“Petru Poni” Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487 Iasi, Romania*

The study deals with the exploring of the thermotropic liquid crystalline properties of a new azomethinic symmetric dimer and some of its structural heterogeneous mixtures with one of its reagents. Their thermotropic behaviour was monitored by polarized light microscopy, differential scanning microscopy and variable temperature X-ray diffraction measurements. The influence of the structural heterogeneity on the mesomorphic behaviour was established and some similarities with the mesomorphic behaviour of the polydisperse polymers were underlined.



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## **Synthesis of amphiphilic block copolymers with fluorescent probe**

V. Melinte, E.C. Buruiana, T. Buruiana

*Petru Poni Institute of Macromolecular Chemistry, 41 A Gr. Ghica Voda Alley, 700487 Iasi, Romania*

Amphiphilic block copolymers are recognized for the ability to exhibit a complex self-assembling behavior when they are dissolved in a solvent selective for one block. Such polymers have attracted significant attention due to their different conformational transitions occurred into a changing environment. For that reason they represent very promising responsive materials for the fabrication of smart polymer films, micelles and gels. Also, synthesis of block copolymers allows the generation of various morphologies, which can improve their properties and can guide, in proper conditions, the formation of nanoparticles, nanoshells or nanotubes used in pharmacological applications like drug-encapsulation and targeted delivery. Additionally, the inclusion of fluorescent molecules may be exploited in the development of fluorescent nanostructures used in molecular imaging.

In this context, our work describes the synthesis of amphiphilic block copolymers based on poly(ethylene glycol) or poly(acrylic acid) as hydrophilic blocks and poly(n-butyl methacrylate) as hydrophobic block *via* modern polymerization methods (ATRP, RAFT). The characterization of the block copolymers was achieved through specific methods ( $^1\text{H}$  NMR, FTIR, TGA, fluorescence), and the fluorescence response of the chromophores in solutions have been investigated.



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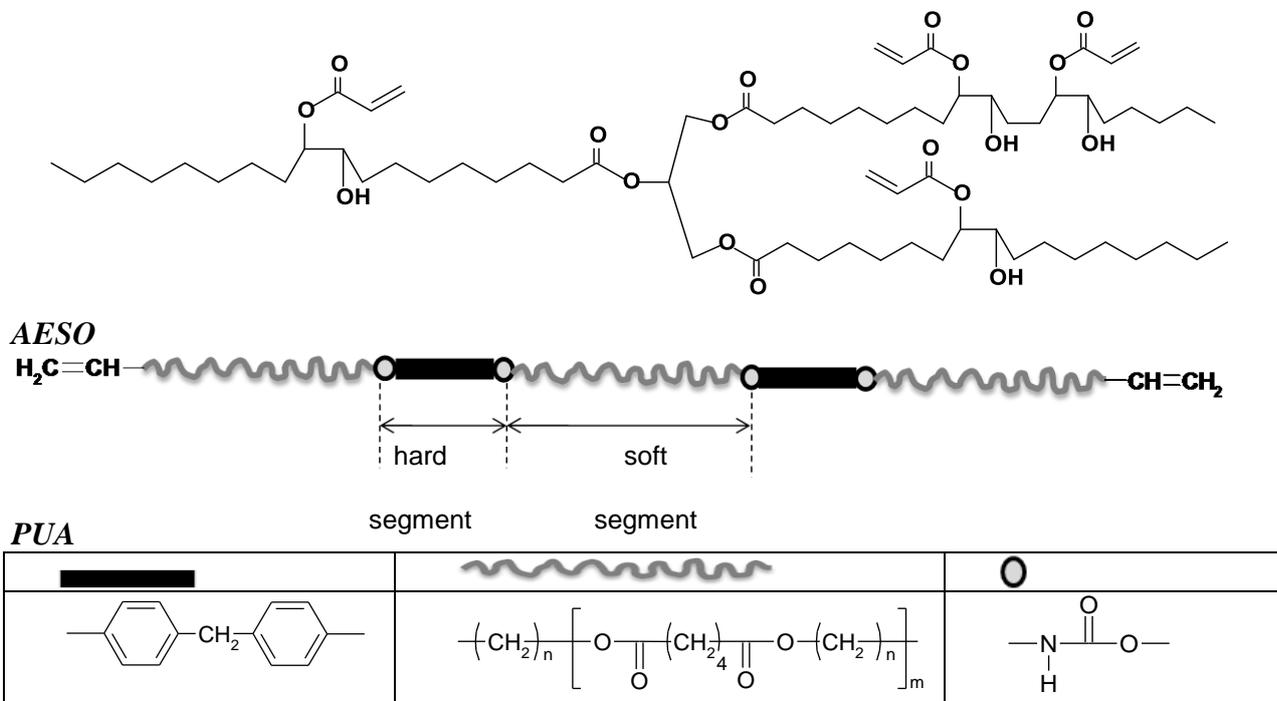
## Mechanical relaxation investigations in soybean oil-based polyurethane networks

M. Cristea<sup>1</sup>, D. Ionita<sup>1</sup>, B.C. Simionescu<sup>1,2</sup>

<sup>1</sup>"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania

<sup>1,2</sup>"Gh. Asachi" Technical University, Department of Natural and Synthetic Polymers, Iasi, Romania

Soybean oil - based polyurethane derivatives were prepared using acrylated epoxidized soybean oil (AESO) and polyurethane acrylate (PUA) by simultaneous thermal polymerization process.



Dynamical analysis performed on films derived from PUA and AESO, in different mass ratios, allows to identify the relaxations in PUA elastomer and to follow the evolution of the relaxations when it becomes part of a network/mixture that includes also AESO. Multifrequency experiments are used for two reasons: to calculate the apparent activation energy and to make the difference between the kinetic phenomena and the relaxation phenomena. Broadly speaking, two effects are produced when including AESO and PUA in the same derivative. Low quantities of AESO break up the hydrogen bonds responsible for the hard domains in polyurethane. High AESO quantities strengthen the AESO/PUA compound and, most important, improves the toughening properties of the PUA elastomer.



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## **Patterning surface morphology of polyimide precursor with a lyotropic liquid crystal matrix**

A.I. Cosutchi, C. Hulubei, I. Stoica, S. Ioan

*“Petru Poni” Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487, Iasi, Romania*

This work aims to obtain high performance alignment layers used in display devices or cell culture substrates with tuned morphology. In this context, blends of hydroxypropyl cellulose (HPC) in lyotropic phase have been prepared with a new epiclone-based poly(amic acid) (PAA) in different ratios. The typical pattern of sheared lyotropic HPC was used as for shaping the morphology of the investigated polyimide precursor. For consolidating the molecular arrangement induced by the well-ordered HPC matrix, cross-linking of the special structure of epiclone moieties was attempted by UV irradiation of the PAA/HPC films. The detailed surface morphology of the PAA/HPC films was examined by polarized light microscopy (PLM) and atomic force microscopy (AFM). The band texture, typical for lyotropic HPC solutions, evidencing different intensities and dimensions can be distinguished even at high PAA content and is also maintained after HPC removal with a selective solvent, namely acetone. The effect of the surface molecular oriented polyimide precursor film on the alignment behavior of N-(4-methoxybenzylidene)-4-butylaniline (MBBA) nematic liquid crystal, was investigated. PLM images revealed successively disposed dark and bright states, which change their luminosity by rotating the sample between crossed polarizers, indicating a uniform homogeneous alignment.



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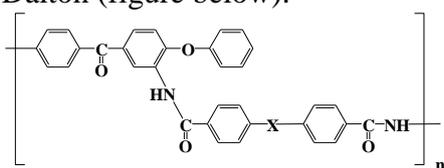
## Polyamides containing benzophenone chromophoric groups

M.D. Damaceanu, R.D. Rusu, M. Bruma

*"Petru Poni" Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda 41A, Iasi-700487, Romania*

Benzophenone chromophoric group, known to undergo light-excitation to  $n, \pi^*$  triplet states, are commonly used as photosensitizers in photochemistry. Triplet benzophenone chromophore can abstract hydrogen atom from weak C-H bond or other hydrogen donor to form a ketyl radical and can be readily quenched by oxygen [1]. Because of high reactivity of the formed radicals, the photo-excited benzophenone polymers could decompose certain toxic chemicals and demonstrate antibacterial functions. Therefore, polymers containing benzophenone chromophoric groups could be employed as self-decontaminating materials for protective clothing, when exposed to UV or visible light irradiation [2].

Design and synthesis of thermally stable polyamides with improved solubility based on an asymmetrical aromatic diamine containing phenoxy substituted benzophenone segment was the main objective of this work. Low-temperature solution polycondensation reactions of this diamine with different aromatic diacid chlorides containing ether, hexafluoroisopropylidene or diphenyl silane groups resulted in polyamides with molecular weights in the range of 102900-106500 Dalton (figure below).



The structures of these monomers and the corresponding polymers were fully confirmed by elemental analysis, infrared and nuclear magnetic resonance spectroscopy. All polyamides were easily soluble at room temperature in polar aprotic solvents and even in less polar solvents such as tetrahydrofuran. The polymers showed excellent thermal stability, up to 385 °C, and exhibited glass transition in the range of 225-256 °C. All the polymers emitted an intense blue light upon irradiation with UV light, due to the emission from the benzophenone chromophore. Further research is necessary to study their great potential to be applied in polymer light-emitting diodes as blue-light-emitting and phosphorescent materials, and as self-decontaminating materials.

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## **Single polyelectrolyte multilayers deposited onto silica microparticles and silicon wafers**

F. Bucatariu<sup>a</sup>, F. Simon<sup>b</sup>, Gh. Fundueanu<sup>a</sup>, G. Hitruc<sup>a</sup>, E.S. Dragan<sup>a</sup>

<sup>a</sup>) "Petru Poni" Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda, 41A, 700487 Iasi, Romania

<sup>b</sup>) Leibniz Institute of Polymer Research, Hohe Strasse 6, 01069 Dresden, Germany

Single polyelectrolyte component multilayers, exemplified by poly(vinyl amine) (PVAm), have been prepared using a method of 3,3',4,4'-benzophenonetetracarboxylic-dianhydride (BTCDA)-mediated electrostatics and hydrogen bondings LbL assembly. The PVAm with low (15000 g/mol) and high (340000 g/mol) molar mass was adsorbed at pH = 9.5 onto silica microparticles with particle diameter of 0.015 – 0.040  $\mu\text{m}$  and onto silicon wafers. The BTCDA cross-linking of the PVAm adsorbed onto silica microparticles results in a surface covered with carboxylic groups. The silica/PVAm-BTCDA, negatively charged over a wide range of pH, can adsorb a new positively charged PVAm layer. A regular increase in the single (PVAm/BTCDA)<sub>n</sub> polyelectrolyte multilayer onto silica microparticles was observed by streaming potential measurements using a Particle Charge Detector. The X-ray photoelectron spectroscopy was employed to monitor the (PVAm-BTCDA)<sub>n</sub> multilayer construction. The topography and the thickness of the films deposited on silicon wafers, as a function of the number of PVAm layers, were investigated by atomic force microscopy. Both molar masses (15 kDa and 340 kDa) of PVAm were suitable for building (BTCDA)-mediated LbL assembly onto silica microparticles.



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## Design, preparation and characterization of site-specific target-sensitive liposomes for drug delivery at sites of activated endothelium

M. Calin<sup>1,2</sup>, D. Stan<sup>1</sup>, I. Manduteanu<sup>1</sup>, V. Simion<sup>1</sup>, M. Pirvulescu<sup>1</sup>, A.M. Gan<sup>1</sup>, M. Simionescu<sup>1</sup>

<sup>1</sup> *Institute of Macromolecular Chemistry „Petru Poni”, Iasi, Romania*

<sup>2</sup> *Institute of Cellular Biology and Pathology “N. Simionescu”, Bucharest, Romania*

### Purpose.

Targeted liposomes can be potentially used as carriers for drug delivery to specific cells. The aim of this study was to develop target-sensitive liposomes (TSL) able to disintegrate after binding to a molecule expressed on the surface of the target cell and release their content in the cell's vicinity.

### Methods.

We selected as the basic component for TSL the phospholipid DOPE (dioleoylphosphatidylethanolamine) that readily forms inverted micelle at physiological conditions and that will be stabilised in a bilayer with a charged phospholipid DOPA (dioleoyl phosphatidic acid) and with a functionalized phospholipidic anchor used to covalently couple targeting moieties (such as antibodies, peptides) to the liposome surface. Vascular cell adhesion molecule-1 (VCAM-1) is a convenient target receptor because its expression is selectively restricted to inflammatory (activated) endothelium under different pathological conditions, such as developing of atheromatous plaque, rheumatoid arthritis or human malignant tumors. Therefore, VCAM-1 binding antibodies were coupled to the surface of liposomes with different compositions (obtained using extrusion method) via an amide bond using as membrane anchors 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(glutaryl) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (a polyethyleneglycol (PEG) modified phospholipid, described to be essential for long circulation of liposomes after i.v. injection). Also, a linear peptide VHPKQHRGGSKGC (shown to specifically bind to VCAM-1) was coupled to micelles obtained from 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000] via a thioether bond and then incubated with pre-formed liposomes resulting in insertion of the complex peptide-derivates of PEG into the outer lipid layer of liposome. The liposomes of different compositions, either classical (C) or sterically stabilized with PEG (SS) were characterized for size (by dynamic light scattering), antibody or peptide coupling to the TSL surface (by bicinchoninic acid protein assay or cysteine assay using Ellman's reagent) and stability (by fluorimetry). The expression of VCAM-1 on the surface of endothelial cell (EC) line EA.hy926 after activation for different periods of time with tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) was determined using ELISA (enzyme-linked immunosorbent assay). To confirm the targeting potential of TSL, non-activated or activated EC were incubated with DiI-labelled TSL. To assess the content release at the surface of activated endothelium by destabilization of liposomes, non-activated or activated EC were incubated with different concentration of C- or SS-TSL containing 100 mM calcein (a fluorescent marker) and further investigated by fluorimetry.



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### Results.

(1) size of liposomes varies with liposome composition being with ~20 % higher in the case of SS-TSL in comparison with C-TSL. (2) coupling efficiency expressed as  $\mu\text{g}$  antibodies (peptide)/ $\mu\text{mol}$  phospholipid are similar using either amide or thioether bond and  $\sim 40 \mu\text{g}$  antibodies (peptide)/ $\mu\text{mol}$  TSL. (3) SS-TSL have higher stability in comparison with C-TSL; in vitro release studies revealed that after 24 hours at  $4^\circ\text{C}$  SS-TSL release  $\sim 20\%$  of content whereas C-TSL release  $\sim 50\%$  (similar to release of content from SS-TSL after 1 week storage). (4) VCAM-1 expression on EA.hy926 cells is maximal after 24 hours of activation with  $1 \text{ ng/ml}$   $\text{TNF-}\alpha$ . (5) The binding of TSL is higher in the case of incubation with activated EC in comparison with non-activated EC. (6) the release of calcein at the endothelium surface depends on liposome concentration and for  $10 \mu\text{M}$  C-TSL reaches 98% release at the surface of activated EC and 18 % release at the surface of non-activated cells after 1 hour of incubation.

### Conclusion and perspectives.

The data indicate a specific release at sites of activated endothelium from TSL composed from DOPE:DOPA:anchor (75:20:5 mols %). Next, more studies will be performed in order to optimize liposome by varying the ratio of DOPE, DOPA and phospholipidic anchor and to obtain more stable TSL that can be stored longer after preparation.



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## **Nanostructured materials based on weak polyions**

M. Mihai<sup>1</sup>, I. Stoica, S. Schwarz<sup>2</sup>

<sup>1</sup>„Petru Poni“ Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda 41 A, 700487 Iași, Romania

<sup>2</sup>Leibniz Institute of Polymer Research Dresden e.V., Hohe Strasse 6, 01069 Dresden, Germany

Among the methods developed to prepare thin films, the layer-by-layer (LbL) deposition of polyelectrolytes has aroused considerable attention due to its versatility. In addition this technique has the advantage of enabling nanoscale control of the thickness, the structure and the composition of the films. The microcontainers made by layer-by-layer deposition of oppositely charged polyelectrolytes onto colloid templates and subsequent removal of the core, are particularly attractive due to their promises for microreactors, microsensors, and drug-delivering systems. This study aims to adsorb polyelectrolyte multilayers on planar or colloidal particles, by assembling weak synthetic and natural polyelectrolytes in which the ionization of the polyelectrolyte is dependent on the pH. The influence of polyelectrolyte nature and number of layers on the properties of new nanostructured materials and their response to the environment pH were monitored by infrared spectroscopy, colloidal titration, zeta potential, and scanning electron microscopy.



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## **Macroporous composite hydrogels based on synthetic and natural polymers**

M.V. Dinu, M.M. Perju, E.S. Drăgan

*“Petru Poni” Institute of Macromolecular Chemistry, Department of Functional Polymers,  
Grigore Ghica Voda Alley 41A, Iasi 700487, Romania,*

Macroporous hydrogels are 3D hydrophilic network polymers that can absorb and conserve considerable amounts of aqueous fluids without dissolving and losing their shape. These materials have been used in a wide range of biological, medical, pharmaceutical, and environmental applications due to their characteristic properties such as biocompatibility, hydrophilicity and lack of toxicity. Although many natural polymers can be used to produce this type of material, the structural versatility available in synthetic hydrogels has given them distinctive properties, which in turn have enhanced their practical interest. Therefore, a combination of natural and synthetic polymers in a composite architecture is expected to induce a synergy in the gel properties. In this study, composite hydrogel networks were synthesized by free-radical polymerization at different temperatures, and cross-linker ratios, using dextran sulfate (DxS) as an ionic physical entrapped polymer, acrylamide monomer and N,N'-methylenebis(acrylamide) as cross-linking agent. Their mechanical, morphological and swelling properties were evaluated as a function of gel preparation conditions. The mechanical analysis revealed that the composite hydrogels formed at  $-18\text{ }^{\circ}\text{C}$  with a cross-linker ratio of 1/40 were very tough and can be compressed up to about 100% strain, without any crack development, while those formed at  $+20\text{ }^{\circ}\text{C}$  were fragile. The porosity measurements showed that the composite hydrogels prepared with a cross-linker ratio of 1/40 exhibited a stable porous structure, which did not collapse during deswelling or drying. The swelling ratio and water retention capacity of the semi-IPN composite hydrogels were also evaluated, as a function of time.



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## **Hydrogels Based on Poly(vinyl alcohol)/Phosphoester/Chondroitin Sulfate Blends for Biomedical Applications**

T. Vlad-Bubulac, A.M. Oprea, D. Serbezeanu, C. Hamciuc

*“Petru Poni” Institute of Macromolecular Chemistry, Aleea Gr. Ghica Voda 41A, Iasi-700487, Romania*

Biodegradable polymers have attracted considerable attention in the very recent years. Their potential applications in biomedical and ecological fields have been generated a great deal of effort in developing new polymeric structures with a biodegradable backbone, having excellent combined properties such as biocompatibility, antifouling properties, tunable mechanical properties, no toxicity, potential functional versatility which allows introduction of bioactive molecules and extensive modification of the physical, chemical and biological properties etc. [1–3]. Actually, the development of novel, stable and economical advanced drug delivery formulations based on hydrogels, which are well known to reduce the problems of conventional dosage forms, is an important topic in the field of biomaterials science.

The present work focuses on the synthesis and characterization of new polymers with phosphoester functions obtained by polycondensation reaction of poly(vinyl alcohol) (PVA) with different phosphonic dichlorides. These compounds were used to prepare hydrogels for biomedical applications. In the first step, the phosphorylated materials, poly(vinyl alcohol)/phosphoesters were synthesized by polycondensation reaction using dimethylformamide as solvent. The products were physically mixed with chondroitin sulfate and then crosslinked with epichlorohydrin. Basic structural characterization was performed and specific investigations for hydrogels were conducted. Swelling studies were performed for all formulations and carried out by direct immersion in phosphate buffer solution (pH=7.4). The drug loading method was performed by soaking or equilibration of superporous hydrogels samples in phosphate buffer solution (pH=7.4) with metoprolol tartrate for their complete swelling. The in vitro release of metoprolol from PVA-P-CS hydrogels was carried out in intestinal pH conditions at 37 °C.

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## **TG-MSD/FTIR, a useful technique for characterization of thermal behavior of biomass waste**

M. Brebu<sup>1</sup>, N. Hurduc<sup>2</sup>

<sup>1</sup> “Petru Poni” Institute of Macromolecular Chemistry, Iasi, Romania, ([bmihai@icmpp.ro](mailto:bmihai@icmpp.ro))

<sup>2</sup> “Gh. Asachi” Technical University of Iasi, Romania

Biomass waste could be a valuable resource to replace fossil fuels for generation of materials and energy. Pyrolysis proved to be a useful technique to convert synthetic polymers into chemicals and/or fuels, therefore it was also largely tested as a method for treatment/valorization of biomass too. It is very useful to determine the thermal behavior of materials prior to pyrolysis in order to establish the temperature range of degradation and the steps in which various products are formed.

Here we present our observations on the thermal behavior of keratin waste and of lignin obtained from thermogravimetry coupled with infrared spectroscopy and mass spectrometry detection of evolved products.



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## **Synthesis and characterization of a silsesquioxane nanocomposite with photoluminescence properties**

L. Ursu, I. Bordianu, M. Olaru, C. Cotofana

*"Petru Poni" Institute of Macromolecular Chemistry", 41A Gr. Ghica Voda Alley, 700487 Iasi, Romania*

*In situ* organically-modified silsesquioxane nanoparticles with vinyl units were obtained combining the sol-gel technique and the radical polymerization of vinyltrimethoxysilane (VTMS) in the presence of a small amount of a cationic surfactant, i.e., cetyl trimethylammonium bromide (CTAB). The surfactant was added in order to improve the optical properties based on controlled nanophase separations. The hydrolysis and polycondensation reactions were followed by Fourier Transform Infrared Spectrometry (FTIR), Scanning Electron Microscopy (SEM) and fluorescence measurements.



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## **In vitro studies regarding the cardiac regenerative potential of mesenchymal stem cells**

A. Burlacu

*„Petru Poni” Institute of Macromolecular Chemistry, Iasi  
Institute of Cellular Biology and Pathology “Nicolae Simionescu” Bucharest*

Understanding the mechanisms by which stem cells interact with host cardiac tissue after transplantation will facilitate their future use in the cellular therapy. Our project has started from the premise that stem cell properties might be modified by the inflammatory conditions existed into the infarcted myocardium at the time of transplantation. Despite the numerous studies on stem cells, a fundamental question is still waiting an answer: how stem cells respond to the host environment and differentiate toward a specific cell phenotype. This question is related to the molecular signals induced by the changes in cytokine levels and secreted inflammatory factors, which complicate the interactions between transplanted and host cells. In order to study the interaction between stem cells and the ischemic cardiac tissue, as well as the succession of stages until stem cells become differentiated cardiomyocytes, several mesenchymal stem cell lines and ischemia models were established and characterized. The paracrine effects of stem cells, as well as their multipotent capacity and cardiac differentiation ability were studied in vitro after exposure to several inflammatory conditions.



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## Biological approaches to bone regeneration by tissue engineering and implantology

I. Titorencu<sup>1,2</sup>; V.V. Jinga<sup>2</sup>; E. Constantinescu<sup>2</sup>; A.V. Gafencu<sup>2</sup>;  
C. Zaharia<sup>3</sup>; M. Albu<sup>4</sup>; A. Vladescu<sup>5</sup>; M. Simionescu<sup>2</sup>

<sup>1</sup> "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania

<sup>2</sup> Institute of Cellular Biology and Pathology, "Nicolae Simionescu", Bucharest, Romania

<sup>3</sup> Clinical Institute "Colentina", Bucharest, Romania

<sup>4</sup> Leather and Footwear Research Institute, Bucharest, Romania.

<sup>5</sup> National Institute for Optoelectronics, Bucharest, Romania

### Background and aim

Bone marrow-derived mesenchymal stromal cells (MSC) represent a potential material for cellular therapy used in a variety of disorders, including bone healing deficiencies. To this aim, osteoprogenitor cells (OPC) from human bone marrow were isolated and their capacity to proliferate and differentiate *in vitro* to osteoblasts was estimated. Also we study the biocompatibility with osteoblast cell lines by *in vitro* colonization of different scaffolds as supports for grafting and alloys used for implants.

### Methods and results

Human MSC were separated on Histopaque and cultured in DMEM supplemented with 15% human serum AB Rh negative. To induce differentiation towards osteoblasts, the cells in culture were exposed to  $10^{-7}$ M dexamethasone (dexa) or/and  $10^{-3}$ M sodium  $\beta$ -glycerophosphate ( $\beta$ -GlyP). In other experiments, after this treatment, the cells were incubated for 48h with 1, 25-dihydroxyvitamin D3 (calcitriol) or 9-cis-retinoic acid (9-RA). At 7, 14, 21 days, alkaline phosphatase (AP) activity, calcium deposits, the expressions of osteocalcin, bone sialoglycoproteins (BSP), osteonectin and cellular ultrastructure were assessed. The biocompatibility of different collagen scaffolds and alloys coated with different (Zr,Ti)CN substrates were assessed with two human osteoblast cell lines: an osteosarcoma cell line - MG 63 and an osteoprogenitor cell line - hFOB 1.19 and with OPC. The colonization capacity of the cells was monitored by fluorescence microscopy, the viability by MTT assay, the differentiation into osteoblast by PCR techniques.

### Results

The AP activity was detected in cells with or without dexa and / or  $\beta$ -GlyP treatment. After 14 days of dexa and  $\beta$ -GlyP treatment, the initiation of extracellular calcium deposition was observed. Gene and protein expression of osteonectin and BSP increased under the combined dexa and  $\beta$ -GlyP treatment. Osteocalcin gene expression was induced only after the additional treatment with calcitriol or 9-RA. BSP gene expression was induced progressively having its maximal after 3 weeks of combined treatment. Ultrastructural analysis revealed the secretory phenotype of OPC, a feature that was maintained during the dexa/ $\beta$ -GlyP treatment, and the presence of large vesicles containing electron-dense structures – representing most likely calcium deposits. *In vitro* biological tests demonstrated that osteoblasts developed on those collagen



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scaffolds and alloys with different substrates. Ultrastructural aspects of the cells integrated within matrix pores were unmodified compared to the control (OPC grown on culture plates).

### Discussions

Under appropriate treatment, MSC can be induced to give rise to OPC that have the capacity to differentiate into osteoblasts characterised by the expression of a variety of osteogenic markers and osteoblastic properties. *In vitro* biocompatibility assessed with OPC and MG 63 and h FOB 1.19 cell lines revealed that all collagen scaffolds and alloys sustained cellular colonization and growth.



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## Identification of new biomarkers for prediction of endothelial vascular dysfunction; strategies to reverse vascular wall function

A. Georgescu<sup>1,2</sup>, N. Alexandru<sup>1,2</sup>, E. Andrei<sup>2</sup>, I. Titorencu<sup>1,2</sup>, E. Dragan<sup>2</sup>, D. Popov<sup>2</sup>

<sup>1</sup>*'Petru Poni' Institute of Macromolecular Chemistry, Iasi, Romania*

<sup>2</sup>*Institute of Cellular Biology and Pathology 'Nicolae Simionescu', Bucharest, Romania*

This study aimed to (a) employ our newly designed model, the hypertensive–hypercholesterolemic hamster (HH) to find whether a correlation exist between circulating microparticles (MPs), endothelial progenitor cells (EPCs) and their contribution to vascular dysfunction and (b) to assess the effect of irbersartan treatment on HH animals (HHI). Aortic arch and mesenteric resistance arteries were explanted from HH, HHI and control (C) hamsters. The results showed that compared to C group, HH displayed: **(i)** a significantly increase in plasma cholesterol and triglyceride concentration, and an enhanced systolic and diastolic arterial blood pressure, and heart rate; **(ii)** a marked elevation of MPs and a significant decrease in EPCs; **(iii)** structural changes of arterial wall correlated with modified protein expression of MMP2, MMP9, MMP12, TIMP1, TIMP2 and collagen type I and III; **(iv)** a considerably altered reactivity of the arterial wall closely correlated with MPs and EPCs adherence; **(v)** an inflammatory process characterised by augmented expression of P-Selectin, E-Selectin, vWF, TF, IL-6, MCP-1, RANTES, eNOS, VEGF and SDF-1. Additionally, the experiments showed the potential of irbersartan to correct all altered parameters in HH. In conclusion, hypertension associated with hypercholesterolemia is accompanied by structural modifications and expression of pro-inflammatory molecules by the vessel wall, alteration of the vascular tone, enhanced release of MPs and reduced EPCs; the ratio between the latter two may be considered a marker of vascular dysfunction. Irbersartan that exhibit a pharmacological control on the levels of MPs and EPCs has the potential to restore the homeostasis of arterial wall.



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## The mechanisms of platelet activation in atherosclerosis and the effects of irbesartan administration

N. Alexandru<sup>1,2</sup>, A. Georgescu<sup>1,2</sup>, E. Dragan<sup>2</sup>, E. Andrei<sup>2</sup>, D. Popov<sup>2</sup>

<sup>1</sup>*'Petru Poni' Institute of Macromolecular Chemistry, Iasi, Romania*

<sup>2</sup>*Institute of Cellular Biology and Pathology- 'Nicolae Simionescu', Bucharest, Romania*

Platelet dysfunction and enhanced oxidative stress have been reported in pathological situations, including hypertension or hypercholesterolemia, that are associated with increased risk of atherothrombotic complications. Angiotensin II is considered a proatherogenic agent playing a considerable role in the development of atherosclerosis. **Aims:** Assessment of the effect of simultaneous hypertension and hypercholesterolemia on platelet activation and nitric oxide (NO) production and evaluation of irbesartan role, an angiotensin II type 1 receptor antagonist. **Material and methods:** Platelets were isolated from the blood of golden Syrian hamsters divided in three experimental groups: controls, C (fed a standard diet), hypercholesterolemic-hypertensive, HH (fed a diet enriched in 3% cholesterol, 15% butter and 8% NaCl, 4 months) and hypercholesterolemic-hypertensive treated with irbesartan, HHI (fed as HH group, plus irbesartan 10 mg/kg/day, 4 months). Electron microscopy, flow cytometry, endogenous NO and immunoblotting assays were used. **Results:** Compared to C group, platelets isolated from HH group showed: (i) morphological modifications; (ii) increased integrin  $\beta_3$  exposed on plasmalemma; (iii) augmented protein expression of molecules implicated in the activation and aggregation process, such as P-selectin, FAK, PI3K, Akt, and Src; and (iv) reduced NO production and eNOS protein expression. Compared to HH group, the irbesartan administration (HHI group) significantly attenuates the level of all the molecules tested and improved de NO generation. **Conclusions:** Experimental hypertension associated with hypercholesterolemia induced major changes in morphology and signaling mechanisms implicated in activation in blood platelets and improved endogenous NO production. The irbesartan treatment has a beneficial effect, diminishing these modifications.



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## **Excimer laser-induced surface modification of poly(ethylene terephthalate)**

C. Ursu<sup>1</sup>, I. Bordianu<sup>1</sup>, M. Dobromir<sup>2</sup>, M. Drobotă<sup>1</sup>, C. Cotofana<sup>1</sup>, M. Olaru<sup>1</sup>, B. C. Simionescu<sup>1,3</sup>

<sup>1</sup>*“Petru Poni” Institute of Macromolecular Chemistry, 41 A Gr. Ghica Voda Alley, 700487 Iasi, Romania*

<sup>2</sup>*“Al. I. Cuza” University, Faculty of Physics, 11 Carol I Blvd., 700506 Iasi, Romania*

<sup>3</sup>*Department of Natural and Synthetic Polymers, “Gh. Asachi” Technical University of Iasi, D. Mangeron Blvd., 700050 Iasi, Romania*

The effects on chemical and physical properties of poly(ethylene terephthalate) (PET) foils as a result of microstructuring induced by KrF (248 nm) and XeCl (308 nm) laser ablation were investigated. Below ablation threshold, roughening of the surface was detected, with the formation of periodic surface structures for 308 nm irradiation, while above the ablation threshold, dendrites or granular protuberances superimpose the densely-packed nap structures. In both cases, the texturing of irradiated surface appears more clearly with increasing pulses number. The changes in surface properties were investigated by scanning electron microscopy (SEM), attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray photoelectron spectroscopy (XPS). The observed texture types originate from different contribution rates of the photothermal and photochemical mechanisms.



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## **Preliminary Studies on the Complex System: Surface-active Substances - Hybrid Silica Particles - Vegetable Oils**

C.L. Nistor<sup>1,2</sup>, D. Donescu<sup>2</sup>, C. Spataru<sup>2</sup>, C. Petcu<sup>2</sup>, M. Ghiurea<sup>2</sup>, C. Deleanu<sup>1</sup>

<sup>1</sup>„Petru Poni” Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487 Iasi, Romania

<sup>2</sup>National Research & Development Institute for Chemistry and Petrochemistry, Splaiul Independentei 202-6, PO Box 174/35, 060021, Bucharest, Romania

Phone: +40.021-316.30.93

Fax: +40.021.312.34.93

The work describes some preliminary studies for encapsulating oils and oil-soluble substances (such as dyes) in mesoporous, mechanically stable silica microparticles. Oil-doped silica particles were prepared by the sol-gel process in aqueous medium, with a base catalyst, using various silica precursors: octyltriethoxy silane (OTES), octadecyltrimethoxy silane (ODTES) and tetraethylorthosilicate (TEOS). Also different types of surfactants were used: Tween 20, Tween 40, Tween 60 and decaethylene glycol oleil ether (Brij 96V). An emulsion was formed, consisting in a continuous phase - comprising an aqueous solution of ethanol, a surface-active substance and NH<sub>4</sub>OH (16%) - and a dispersed phase - of oil, ethanol and silica precursors. The resulted particles sizes were evaluated by DLS and SEM and the thermal behavior was studied by TGA.