

## **DYNAMIC PLATFORMS BASED ON OLIGO/POLYSACCHARIDES NETWORKS OBTAINED THROUGH THIOL-ENE COUPLING FOR BIOMEDICAL APPLICATIONS (THIOLENET)**

**Program: PN-III-P4-PCE**

**Project code: PN-III-P4-PCE-2021-1365**

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**Project director: Assoc. Prof. Dr. Bioeng. Cătălina-Anișoara Peptu**

### **FINAL SCIENTIFIC REPORT**

#### **1. The intended/realized objectives**

The main objective of the project was to develop innovative biomaterials based on chitosan and cyclodextrin using thiol-ene addition reactions. These reactions occur between compounds containing thiol groups and compounds with C=C double bonds in their structure. Due to the complexity of this objective, it was necessary to simultaneously pursue two experimental research directions: modifying cyclodextrin either with thiol groups or with double bonds, and modifying chitosan with thiol groups or with double bonds.

The modified cyclodextrin molecules, either with double bonds or thiol groups, play a dual role in the final polymeric network: on one hand, the cyclodextrin is an integral part of the three-dimensional network formed, while on the other hand, it acts as a small molecular crosslinking agent. This role makes it one of the key design factors for the new formulations, as it allows the adjustment of the release kinetics of the active principle included in these networks.

Therefore, the project aimed to develop systems in the form of hydrogels and/or submicron particles (micro- and nanogels) with potential applications in the field of controlled drug release. Achieving the main objective involved fulfilling several specific objectives carried out over three stages.

During *Phase 1 (2022) of the project, titled Synthesis and characterization of precursors for thiol-ene coupling reactions*, four scientific research activities were planned. All activities were fully completed on time, leading to the achievement of the objectives for the project's first stage as well as the fulfillment of project indicators. A brief description of the main activities conducted during this stage is presented below.

*Activity 1.1 involved determining the optimal conditions for the synthesis and characterization of chitosan modified with double bonds.*

It is currently known that the main limitation of using chitosan in the field of controlled drug release systems is its solubility only in acidic environments. However, this limitation can be overcome through chemical modification of chitosan.

In this stage, experimental studies were conducted to select chemical reagents, optimize the synthesis process, and determine the optimal parameters for obtaining polymeric products with improved properties, such as water solubility, biocompatibility, and biodegradability, which enable their use in the next stage. Three methods were used to obtain chitosan derivatives with lateral double bonds: (1) reaction with maleic anhydride, (2) reaction with itaconic anhydride, and (3) reaction between chitosan grafted with poly(ethylene glycol methyl ether acrylate) and maleic anhydride. All obtained products were characterized in terms of their physical and chemical properties. Additionally, micro/nanoparticles based on chitosan modified with poly(ethylene glycol) methyl ether acrylate were produced using the double crosslinking method in inverse emulsion. The characterization methods employed for analyzing the synthesized polymeric supports and particulate systems included Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), dimensional analysis, scanning electron microscopy (SEM), hemolysis tests, and toxicity analyses.

Within *Activity 1.2. experiments were carried out on the synthesis and characterization of chitosan chemically modified with thiol groups*. Two methods for obtaining thiol-modified chitosan were described: (1) reaction with 2-iminothiolane and (2) reaction with homocysteine thiolactone. The characterization methods used included Fourier-transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR).

In *Activity 1.3, the synthesis and characterization of  $\beta$ -cyclodextrin ( $\beta$ -CD) modified with double bonds using maleic and itaconic anhydrides were performed*. The reactions between  $\beta$ -CD and the anhydrides were carried out in the presence of a catalyst to produce a final product with a degree of substitution suitable for crosslinking reactions at the double bonds, which are essential for obtaining compounds through thiol-ene reactions.

*Activity 1.4. aimed to obtain and characterize thiolated  $\beta$ -cyclodextrin derivatives, achieved through the reaction of  $\beta$ -CD with thiourea*. The characterization method used for the highly accurate determination of the structure and substitution degree of the  $\beta$ -cyclodextrin derivatives synthesized in activities 1.3. and 1.4. was matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). The structural analysis of the synthesized compounds was further confirmed through controlled fragmentation experiments using mass spectrometry (MALDI MS/MS). While mass spectrometry is a highly precise technique for structural analysis, its application is less commonly known. The interpretation of results is specific to the analyzed products, and similar studies are not found in the specialized literature. Consequently, structural analyses using the MS technique, and particularly MS/MS, were presented in detailed form.

In *Stage 2 (2023) of the project, entitled Preparation and characterization of hydrogels and submicron particles through thiol-ene covalent crosslinking reactions*, four scientific research activities were planned. All activities were fully completed on time, leading to the achievement of the stage objectives and the fulfillment of project indicators. A brief description of the main activities conducted during this stage is presented below.

**Activity 2.1: Synthesis and characterization of thiol-modified cyclodextrins** involved determining the optimal conditions for the synthesis and characterization of cyclodextrin modified with thiol groups. The activity described the synthesis and characterization of  $\beta$ -cyclodextrin ( $\beta$ -CD) modified with thiol groups via reaction with thiourea. The characterization method used for highly accurate determination of the structure and substitution degree of the synthesized  $\beta$ -cyclodextrin derivatives was matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). This complex analysis required the development of methods specifically tailored for analyzing the compounds in question. Consequently, the method development took place during Activity 1.4, while the analysis of the obtained compounds and the critical interpretation of the results were conducted during this stage.

**Activity 2.2: Development of methods for preparing hydrogels from the selected precursors** focused on the preparation of hydrogels based on thiol-functionalized chitosan and cyclodextrin modified with double bonds, or only on modified cyclodextrin. During this stage, the optimal experimental conditions for the reaction between the two chemically modified compounds from the previous stage were determined. Additionally, the synthesis of new systems in the form of micro/nanofibers based on modified cyclodextrin was described. The characterization methods employed included Fourier-transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR).

**Activity 2.3: Development of methods for preparing submicron particles from the selected precursors detailed the preparation of submicron particles based on thiol-functionalized chitosan and cyclodextrin modified with double bonds.** This included determining the optimal reaction parameters between the two aforementioned polymers. The activity also provided details on the preparation of micro/nanoparticles based on chitosan modified with poly(ethylene glycol) methyl ether acrylate through the double crosslinking method in a water-oil emulsion. The characterization methods used to analyze the synthesized polymeric supports and particulate systems included FT-IR spectroscopy, NMR, dimensional analysis, scanning electron microscopy (SEM), hemolysis tests, and toxicity analyses.

**Activity 2.4: Influence of crosslinking reaction parameters on the physicochemical properties of the obtained hydrogels/particles studies** provided a detailed description of the steps undertaken to determine the optimal parameters for the crosslinking reaction and to assess their influence on the physicochemical properties of the prepared hydrogels, gels, micro- and nanoparticles, as well as nanofibers.

**Stage 3 (2024) of the project: Demonstration of applicative potential of the new biomaterials** included two planned scientific research activities. Both activities were fully completed on time, achieving the 2024 objectives and fulfilling all project indicators at 100%. A brief description of the main activities carried out in this stage is presented below.

**Activity 3.1: Toxicity studies of the newly obtained materials** involved selecting prepared systems with optimal properties to evaluate their compatibility with different cell types and determine any potential adverse effects on cell viability and normal function. The

tests included cytotoxicity assessments on cell cultures, analysis of membrane integrity, and monitoring of inflammatory responses. These analyses provided a detailed understanding of the toxicological behavior of the synthesized materials, confirming their safety for potential biomedical applications.

**Activity 3.2: Drug encapsulation and release in relation to variable crosslinking reaction parameters studies** presented and described information on the systems' ability to absorb physiological fluids, closely linked to drug loading and controlled release potential. The analysis was performed exclusively on selected systems with optimal parameters. Drug-loaded systems were studied for in vitro release kinetics using spectral techniques (UV-VIS).

*The reported results confirmed the full and timely completion of all activities, leading to the complete achievement of the project's objectives and exceeding the established indicators. In this context, the proposed goal was fully accomplished.*

#### **Details regarding the exploitation and dissemination of results at the project level**

##### **Achieved indicators:**

- Publication of 6 ISI papers (Q1/Q2) - 100% Achieved
- Participation with scientific papers at 12 international conferences - 100% Achieved
- 1 patent application - 100% Achieved
- Writing of annual research reports and final research report - 100% Achieved
- Project website - 100% Achieved

##### **Published ISI articles**

1. Logigan, C.-L.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.; Peptu, C.A. *Chitosan Grafted Poly (Ethylene Glycol) Methyl Ether Acrylate Particulate Hydrogels for Drug Delivery Applications*. Gels 2022, 8, 494, **Impact factor: 5,0**
2. Cristian Peptu, Diana-Andreea Blaj, Mihaela Balan-Porcarasu, Catalina A. Peptu, Valeria Harabagiu. *Custom-modified oligolactide-cyclodextrin derivatives for electrospun drug formulations*. European Polymer Journal, Volume 196, 2023, 112234, **Impact factor: 3.862**
3. Diaconu A-D, Logigan C-L, Peptu CA, Ibanescu C, Harabagiu V, Peptu C. *Polyurethane Degradable Hydrogels Based on Cyclodextrin-Oligocaprolactone Derivatives*. Gels. 2023; 9(9):755, **Impact factor: 4,6**
4. Corina-Lenuța Logigan, Christelle Delaite, Marcel Popa, Elena Simona Bacaita, Crina Elena Tiron, Cristian Peptu and Catalina Anişoara Peptu. *Poly(ethylene glycol) methyl ether acrylate grafted chitosan based micro and nanoparticles as a drug delivery system for antibiotics*. Polymers, **Impact factor: 5,0**
5. T Bibire, R DANILA, Corina-Lenuța Logigan, Catalina Anisoara PEPTU, RS Cozma, Cristina Mihaela Ghiciuc. *Chitosan-gelatin micro/nanoparticles as a controlled delivery of dexketoprofen trometamol for topical applications in wound care*. Med. Surg. J. –Rev. Med. Chir. Soc. Med. Nat., Iași2024, 128(2):411-421, doi: 10.22551/MSJ.2024.02.21, **Impact factor: 1,044**

6. Blaj DA, Peptu CA, Danu M, Harabagiu V, Peptu C, Bujor A, Ochiuz L, Tuchiluş CG. *Enrofloxacin Pharmaceutical Formulations through the Polymer-Free Electrospinning of  $\beta$ -Cyclodextrin-oligolactide Derivatives*. *Pharmaceutics*. 2024 Jul 5;16(7):903. doi: 10.3390/pharmaceutics16070903. PMID: 39065598; PMCID: PMC11279624, **Impact factor: 5,4**

#### ***ISI articles in progress***

1. Corina-Lenuta Logigan, Cristian Peptu, Mihaela Balan-Porcarasu, Maricel Danu, Catalina-Anisoara Peptu. *Development of Novel Gels Based on Cyclodextrin Functionalized with Double Bonds and Thiolated Chitosan via Thiol-Ene Coupling*
2. Corina-Lenuta Logigan, Cristian Peptu, Catalina-Anisoara Peptu. *Innovative Nanoparticulate Polymer Supports via Thiol-Ene Polymer Conjugation for Enhanced Drug Delivery*

#### ***Published book chapter***

1. Corina L. Logigan, Cristian Peptu, Catalina A. Peptu. *Chapter 17 - Liposomes for delivery of substances for other (non-therapeutic) applications*, Editor(s): Sophia G. Antimisiaris, *Liposomes in Drug Delivery*, Academic Press, 2024, Pages 435-460, ISBN 9780443154911, <https://doi.org/10.1016/B978-0-443-15491-1.00014-6>.

#### ***Patent application submitted to OSIM***

1. Patent application no. A00651 /30.10.2024 with title „*Preparation process of micro/nanoparticles from cyclodextrin and chitosan derivatives by photoinduced nanoprecipitation* ”

#### ***Participation at international conferences***

1. **NanoMedicine International Conference - NanoMed 2022**  
*Chitosan grafted poly (ethylene glycol) methyl ether acrylate based micro/nanocarriers for biomedical applications.*  
**Authors:** Peptu, C.A.; Logigan, C.-L.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.
2. **NanoMedicine International Conference - NanoMed 2022**  
*Nanoparticles based on chitosan grafted with PEG derivative as carriers for antibiotics.*  
**Authors:** Logigan, C.-L.; Peptu, C.A.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.
3. **6th International Conference on Chemical Engineering – ICCE 2022**  
*Cyto- and hemocompatible nanoparticles based chitosan grafted poly (ethylene glycol) methyl ether acrylate as a novel drug delivery system.*  
**Authors:** Logigan, C.-L.; Peptu, C.A.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.
4. **Congresul Internațional al Universității „Apollonia” din Iași, Pregătim viitorul promovând excelența, Ediția a XXXIII-a , 2 - 5 Martie, IAȘI, ROMANIA**  
*Chitosan Grafted Poly (Ethylene Glycol) Methyl Ether Acrylate Nanoparticles: Synthesis, Characterization, and Use as Drug Delivery System.*  
**Authors:** Peptu, C.A.; Logigan, C.-L.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.
5. **Progress in Organic and Macromolecular Compounds, 29th Edition, Iasi, Romania, October 4 - 6**

*Design and synthesis of particles based on chitosan grafted poly(ethyleneglycol) methylether acrylate as carriers for antibiotics.*

**Authors:** Peptu, C.A; Logigan, C.-L.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.

**6. 2nd International Conference on Advanced Nanomaterials and Nanotechnology, 20-21 November, Viena, Austria**

*Chitosan grafted poly(ethylene glycol) methyl ether acrylate nanoparticles as drug delivery system.*

**Authors:** Peptu, C.A.; Logigan, C.-L.; Delaite, C.; Tiron, C.-E.; Peptu, C.; Popa, M.

**7. 2nd International Conference on Advanced Nanomaterials and Nanotechnology, 20-21 November, Viena, Austria**

*Design of innovative gels based on thiolated chitosan and double bonds functionalized cyclodextrin formed via thiol-ene coupling.*

**Authors:** Logigan, C.-L.; Peptu, C.; Peptu, C.A

**8. Congresul Internațional al Universității „Apollonia” din Iași, Pregătim viitorul promovând excelența, Ediția a XXXIV-a , 29 februarie - 3 martie 2024, IAȘI, ROMANIA.**

*Synthesis and Characterization of New Films Comprising Cyclodextrin Functionalized with Double Bonds and Thiolated Chitosan Through Thiol-Ene Coupling.*

**Authors:** Logigan, C.-L.; Peptu, C.; Peptu, C.A.

**9. 40th Informal Meeting on Mass Spectrometry 2024.**

*MALDI MS quantification of transesterification reactions in the ring-opening polymerization of lactides.*

**Authors:** Blaj D., Peptu, C.

**10. 26th International Conference, 15-18 August 2024, Burgas, Bulgaria.**

*Gels Derived from Chitosan and Cyclodextrin via Thiol-Ene Coupling.*

**Authors:** Peptu, C.A.

**11. Nanomed International Conference and Exhibition, 23-25 October 2024 Barcelona, Spania.**

*Innovative Nanoparticulate Polymer Supports via Thiol–Ene Polymer Conjugation for Enhanced Drug Delivery.*

**Authors:** Logigan, C.-L.; Peptu, C.; Peptu, C.A.

**12. Nanomed International Conference and Exhibition, 23-25 October 2024 Barcelona, Spania.**

*Advanced Gel Networks from Thiolated Chitosan and Cyclodextrin via Thiol-Ene Coupling.*

**Authors:** Peptu, C.A.; Peptu, C.; Logigan, C.-L.

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