

Proiect: **Cyclodextrins – a “green chemistry” route to aliphatic polyesters / Ciclodextrinele - o cale a chimiei verzi catre poliesteri alifatici -PN-II-RU-PD-2011-3-0127**

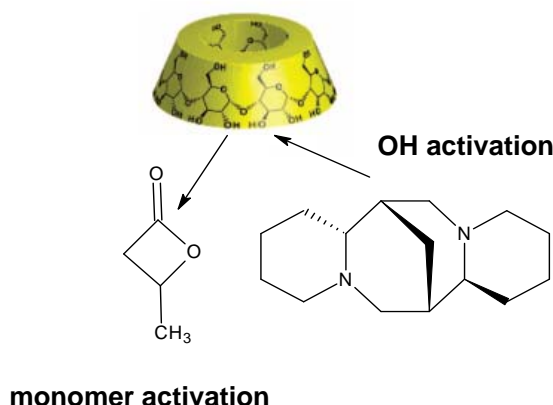
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The project aim: synthesis and characterization at the molecular level, especially through mass spectrometry, of the novel cyclodextrin derivatives obtained through the ring opening polymerization of cyclic esters in the presence cyclodextrins and amines.



Project objectives:

- To prepare novel cyclodextrin-polyester conjugates which combines the biocompatibility and biodegradability of both components with the complexing ability of cyclodextrin. Materials appropriate for bioapplication (drug delivery, bio sensors, tissue engineering) as well as for other applications (chromatographic separation) are envisaged.

The synthetic part is aimed first to develop novel compounds based on different cyclic esters and cyclodextrins. Therefore, different cyclic esters will be subjected to polymerization in the presence of α -, β - and γ -CD and (-)-SP. However, the effect of other amines on the polymerization will be tested. Moreover, different cyclodextrin derivatives and other macrocyclic compounds as calixarenes or cucurbituril will be the subject of preliminary tests.

- To find appropriate methods for preparation of well defined cyclodextrin-polyester conjugates and to optimize them.

So far the β -butyrolactone showed a good propensity for this polymerization system. Further insights over the **bulk and solution polymerization mechanisms** of this particular monomer in presence of the CDs will be provided based on the **polymerization kinetics studies**. A highly accurate structural characterization by LC MS and MS/MS, with close support of NMR, will describe the synthetic processes, step by step. As a result of this, new and rigorous methods for preparing PHB homopolymers, PHB-CD conjugates with low and high molecular weight will be proposed.

- **To provide detailed approaches for separation and structural investigation of the synthesized conjugates.**

The analysis of these compounds is not straight forward since there are no dedicated liquid chromatography and tandem mass spectrometry analytical standards. Thus, new LC separation methods will be created for each synthesized compound. Moreover, the **fragmentation patterns will be established** in order to validate the structural assignments and to identify the possible positional isomers resulted from synthesis. As the fragmentation pathways will have certain specificity to these compounds they will possess a degree of novelty, thus expanding the general knowledge in this field.

- **To propose possible application for the CD-polyester derivatives following a deep characterization of their physico-chemical properties**

Finally, **the possible applications for the newly developed products will be investigated.** As an example, CD-polyester conjugates with low polymerization degree will be tested in view of their capacity to form inclusion complexes with model compounds, while the CD-polyester conjugates with high polymerization degree will be checked in view of their possible properties to form colloidal systems. These would contribute to the expanding the choices for controlled drug delivery systems.

Obiectivele proiectului

1) *Prepararea de noi conjugate pe baza de ciclodextrina si poliesteri alifatici care sa combine biocompatibilitatea si biodegradabilitatea componentelor de plecare pastrand in acelasi timp abilitatea ciclodextrinelor de a forma complexi de incluziune.*

In cadrul proiectului au fost sintetizate si caracterizate cateva tipuri de conjugate covalente pe baza de ciclodextrina, CD, (α -, β - si γ -) avand greutati oligoesteri alifatici pornind de la β -butirolactona (BL), ϵ -caprolactona (CL), δ -valerolactona (VL), DL-lactida (LA). Acesti compusi au fost caracterizati structural la nivel molecular prin tehnici avansate de caracterizare complementare precum spectroscopia RMN si spectrometria de masa. Gradul de polimerizare a esterilor alifatici greutati nu atinge valori ridicate pentru a prezerva capacitatea de incluziune a CD modificate.

2) *Elaborarea unor metode de sinteza adecvate pentru obtinerea de conjugati ciclodextrina-poliester si optimizarea acestora*

Metodele de sinteza propuse sunt polimerizarea in solutie (DMF, DMSO, NMP) a esterilor ciclici utilizand amine drept catalizatori organici.

Rezultatele obtinute prin LC MS in cazul polimerizarii in masa a BL confirma ca gradul mediu de functionalizare a CD este de 12 in timp ce prin polimerizarea in solutie se obtine un grad mediu de 4 unitati monomere per molecula de β -CD. Totusi polimerizarea in masa presupune obtinerea de CD nefunctionalizata, greu de indepartat din amestec.

Polimerizarea DL-lactidei in solutie permite un control adecvat asupra numarului de unitati monomere greutate pe CD. Astfel, reactia in DMSO utilizand SP conduce la grade de polimerizare de 26 unitati lactat per CD iar reactia realizata in DMF sau NMP (la temperaturi ridicate) fara adaos de SP conduce la obtinerea de CD cu ~8 unitati de lactat.

Comparand rezultatele obtinute in cazul celor trei tipuri de ciclodextrina in polimerizare BL, se observa ca numarul de unitati monomere greutate creste odata cu numarul de unitati hidroxilice si implicit odata cu marimea CD. Astfel, in cazul reactiilor catalizate de sparteina (SP) procesele de complexare a monomerilor in CD joaca un rol secundar, activarea grupurilor OH in prezenta compusilor cu caracter bazic precum SP avand un rol predominant.

Polimerizarea in prezenta altor amine mai active, prin comparatie cu cea realizata in prezenta SP, conduce cu preponderenta la formarea a speciilor de CD esterificata cu acid crotonic in paralel cu obtinerea de homopolimer PHB cu masa ridicata si indice de polidispersitate scazut.

Studiile cinetice efectuate prin LC-MS si RMN confirma o crestere initiala rapida a gradului mediu de polimerizare al speciilor CD-BL urmata de o stagnare in timp ce speciile de homopolimer de tip PHB au o crestere lenta a masei moleculare odata cu cresterea gradului de conversie. Au fost identificate procese secundare de degradare a lanturilor polimerice aflate in propagare. Aceasta degradare este redusa in conditiile polimerizarii in masa unde vascozitatea crescuta a mediului de reactie, la conversii relativ ridicate, poate conduce la protejarea speciilor aflate in propagare. Degradarea lanturilor polimerice poate avea loc si prin procese de transesterificare.

3) *Stabilirea unor protocoale de separare si caracterizare structurala a produsilor sintetizati*

Purificarea produsilor de reactie este realizata doar in cazul polimerizarii in solutie. Astfel, produsii pe baza de CD pot fi izolati din amestecul de reactie prin liofilizare sau rotavapare.

Caracterizarea compusilor de tip CD-oligoester a permis elucidarea urmatoarelor probleme: lungimea medie a lanturilor greutate de oligoester (RMN), numarul total de unitati monomere legate de o singura molecula de CD (LC MS) si selectivitatea functionalizarii moleculei de CD (RMN).

Protocolul de caracterizare a produsilor de tip CD-BL obtinuti in solutie consta intr-o abordare complexa utilizand tehnicile LC MS si RMN.

Protocolul general de analiza cromatografica care sa poata fi aplicat produsilor de aceeasi natura cu cei care fac subiectul acestui proiect consta in separarea cromatografica utilizand coloane de tip C18. Separarea are loc prin elutia speciilor CD-BL in functie de gradul de polimerizare in sensul cresterii in timp a masei moleculare a speciilor eluate.

Efectuarea experimentelor de tip MS/MS ale speciilor ionice CD-oligoester, de tip aduct cu Na, permite fragmentarea predominanta a legaturilor esterice in timp ce fragmentarea speciilor protonate conduce cu precadere la ruperea legaturilor semiacetalice dintre inelele glicozidice. Acest fapt a condus la elaborarea unei metode selective de fragmentare care sa permita investigarea structurala specifica la nivel molecular a ciclodextrinelor esterificate.

4) *Propunerea de aplicatii posibile pentru derivatii de CD sintetizati*

Capacitatea de incluziune a derivatilor de CD a fost testata utilizand drept molecula oaspete model nitratul de sulconazol (SC). Includerea SC in derivati de tip CD-lactida conduce la cresterea solubilitatii in apa. Derivatii de tip

CD-LA formeaza prin nanoprecipitare agregate cu dimensiuni medii de 400 nm. Perturbarea mediului de dispersie conduce la compactarea agregatelor fapt ce nu permite studiul acestora in stare uscata.

Activitati de diseminare

Rezultatele obtinute in urma activitatilor de cercetare intreprinse in cadrul proiectului au fost prezentate la trei conferinte internationale:

- **1 poster** - POLYMER MODIFIED CYCLODEXTRINS - MOLECULAR LEVEL CHARACTERIZATION THROUGH LC/MS AND MS/MS **Cristian Peptu**, Valeria Harabagiu, Bogdan C. Simionescu, Marek Kowalczyk Joint Conference of German Mass Spectrometry Society and Polish Mass Spectrometry Society, 4 - 7 martie 2012, Poznań
- **prezentare orala** - LC MS/MS CHARACTERIZATION OF LINEAR AND BRANCHED AZOFUNCTIONAL OLIGOESTERS **Cristian Peptu**, Valeria Harabagiu, Bogdan C. Simionescu, Marek Kowalczyk "30th Informal Meeting on Mass Spectrometry" 29 aprilie-03 mai 2012, Olomouc, Cehia;
- **prezentare orala** - ALIPHATIC OLIGOESTERS FOR BIOLOGICAL APPLICATIONS - STRUCTURAL INSIGHTS AT MOLECULAR LEVEL BY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY **Cristian Peptu**, Valeria Harabagiu, Bogdan C. Simionescu, Marek Kowalczyk "4th Bratislava Young Polymer Scientists workshop" Liptovsky Jan, Slovacia 1-5 octombrie 2012

Studiile bibliografice efectuate au permis elaborarea **unui capitol de carte**:

- **Cristian Peptu***, Leonard Ignat, NANOMEDICAL DEVICES FOR TRANSDERMAL DRUG DELIVERY in Polymeric Nanomedicines, 2013, 671-697 Marcel Popa and Constantin V.Ugela (Eds) All rights reserved - 2013 Bentham Science Publishers Ltd. ISBN: 978-1-60805-628-6

Articole acceptate pentru publicare in reviste cotate ISI:

(1) Modification of β -cyclodextrin through solution ring-opening oligomerization of β -butyrolactone

Cristian Peptu*, Iwona Kwiecień, Valeria Harabagiu, Bogdan C. Simionescu and Marek Kowalczyk

acceptat pentru publicare in Cellulose Chemistry and Technology.

(2) Tandem mass spectrometry characterization of esterified cyclodextrins

Cristian Peptu* and Valeria Harabagiu

acceptat pentru publicare in Digest Journal of Nanomaterials and Biostructures.