Шеста постерна сесия „Младите учени в света на полимерите“

4 юни 2015г.
Институт по полимери, БАН
гр. София

Постерната сесия е организирана от

Института по полимери-БАН

и изцяло финансирана от

проект POLINNOVA

„Укрепване на научноизследователския

капацитет и иновационен потенциал

на ИП – БАН“

от 7-ма Рамкова програма на

Европейската комисия, подпрограма

„Капацитети –Научен потенциал“

СПИСЪК НА УЧАСТНИЦИТЕ

Постер № 1.

Кирилка Младенова, Еми Халаджова, Деница Мелнишка, Магдалена Пенчева, Таня Топузова-Христова, Веселина Москова-Думанова, Станислав Рангелов, Йордан Думанов

"Ефекти на наночастици от поли (винилбензилтриметиламониев хлорид) върху HepG2 клетки"

СУ ,,Свети Климент Охридски'', Биологически факултет, Катедра ,,Биохимия''

ул. Драган Цанков N8, София, България

Постер № 2.

Магдалена Пенчева, Радостина Калинова, Деница Мелнишка, Кирилка Младенова, Таня Топузова-Христова, Йордан Думанов, Ивайло Димитров, Веселина Москова-Думанова

"Ефекти на наночастици от PEGMA-b-PLL върху еукариотни клетки''

СУ ,,Свети Климент Охридски'' Биологически факултет, Катедра ,,Цитология, хистология и ембриология''

ул. Драган Цанков N8, София, България

Постер № 3.

R.Kalinova, J. Doumanov, T. Topouzova-Hristova, I. Dimitrov

"Synthesis and DNA complexation of novel polypeptide-based hybrid block copolymers"

Институт по полимери – БАН

ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 4.

Irena Borisova, Olya Stoilova, Nevena Manolova, Iliya Rashkov

"Reinforced composite materials based on poly(3-hydroxybutyrate) - preparation and properties"

Институт по полимери – БАН

ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 5.

Снежана Тодорова

"Бисфенол А – рискове за човешкото здраве"

Център за оценка на риска, Българска агенция по безопасност на храните бул. ''Цар Борис III'' № 136, ет. 11, 1618, гр. София, България

Постер № 6.

Georgi L. Georgiev and Petar Petrov

"A Novel Approach For Preparation Of Super-Macroporous Dextran Cryogels Via Uv-Irradiaton Technique"

Институт по полимери – БАН

ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 7.

Z. Todorova, N. Koseva, K. Troev

"Synthesis of poly(oxyethylene phosphoramidate)s via Staudinger Reaction"

Институт по полимери – БАН

ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 8.

Pavel Bakardzhiev, Denitsa Momekova, Katarina Edwards, Spiro Konstantinov, Stanislav Rangelov

"Interchain Hydrogen bonds as a stability factor in hybrid polyglycidol-lipid bilayer membranes"

Институт по полимери – БАН

ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 9.

E. Haladjova, G. Mountrichas, S. Pispas, S. Rangelov

"Polyplexes based on poly(vinyl benzyl trimethylammonium chloride) homo- and block copolymers"

Институт по полимери – БАН

ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 10.

Ts. Ivanova, E. Haladjova, M. Mees, D. Momekova, S. Rangelov, R. Hoogenboom

"Ability of poly(n-propyl-2-oxazoline) based copolymers to bind and compact DNA"

Медицински Университет – София, Фармацевтичен факултет

ул. Дунав 2, 1000 София, България

Постер № 11.

Петър Шишков, Милена Недкова

"Characteristics of long time stored single and double base waste propellants"

Минно – Геоложки Университет

ул. "Проф. Боян Каменов", Студентски град, София, България

Постер № 12.

Августина К. Данаилова, Сашка Б. Крумова, Стефка Г. Танева, Румен А. Кръстев, Тоня D. Андреева

"Optimization of Chi/HA polyelectrolyte multilayers for biomedical application"

Институт по биофизика и биомедицинско инженерство – БАН
ул. ,,Акад. Г. Бончев'' Бл. 21, 1113 София, София

Постер № 13.

A. Yakub, S. Vircheva, R. Bryaskova

"Synthesis and characterization of diblock copolymers based on poly(ethylene glycol) and poly(4-vinyl pyridine) via atom transfer radical polymerization"

Химикотехнологичен и металургичен университет
бул. Климент Охридски 8, 1756 София, България

Постер № 14.

D. G. Babikova, I. V. Dimitrov

"Amphiphilic block copolymer bearing various functions intended for targeted drug delivery"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 15.

Ekaterina Stoyanova, Neli Koseva, Petar Petrov and Irina Karadjova

"Stabilized micelles as a delivery system for cisplatin – cytotoxicity and cellular uptake of the loaded micelles"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 16.

P. Mokreva, I. Kostov, V. Uzunova, S. Apostolova, R. Tzoneva, P. Petrov

"Nanocomposite hydroxyethylcellulose/polyaniline cryogels of high electrical conductivity"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 17.

Катерина Колева

"Рециклиране на фармацевтични отпадъци"

Химикотехнологичен и металургичен университет
бул. Климент Охридски 8, 1756 София, България

Постер № 18.

M. Glavcheva, S. Ivanova, D. Christova

"Hydroxyl-functionalized gel polymers with potential biomedical applications: synthesis and characterization"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 19.

Душица Яневска, Денница Мелнишка, Магдалена Пенчева, Кети Илиева, Веселина Москова-Думанова, Йордан Думанов, Таня Топузова-Христова

"Микроскопски техники за визуализиране на наночастици в култивирани клетки"

Софийски Университет"Св.Климент Охридски" – Биологически факултет
ул. Драган Цанков N8, София, България

Постер № 20.

Марин Симеонов, Албена Ледерер, Сузане Бойе, Елена Василева

"Interpenetrating polymer network (IPN) nanogels based on poly(acrylic acid) and polyacrylamide: synthesis and characterization"

Софийски университет "Св. Климент Охридски", Факултет по Химия и фармация, Катедра Фармацевтична и приложна органична химия
Бул. Джеймс Баучер 1, 1164 София, България

Постер № 21.

Boyana Stoyanova , Christo Novakov , Stanislav Rangelov, Christo Tsvetanov

"Studies on self-association behavior of amphiphilic triblock polyglycidol-poly(allyl glycidyl ether)-polyglycidol copolymers in aqueous solution by light scattering"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 22.

Filip Ublekov, Cristina Gutiérrez, Hristo Penchev, Juan F. Rodriguez

"Novel method for exfoliation of sodium montmorillonite in PHB matrix via Supercritical CO_2"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер № 23.

Христина Грънчарова, Марин Симеонов, Павлета Шестакова, Албена Ледерер, Елена Василева

"Синтез и охарактеризиране на комплекси на полисулфобетаини"

Факултет по химия и фармация, СУ "Св. Кл. Охридски"
Бул. Джеймс Баучер 1, 1164 София, България

Постер 24.

Mariya K. Kyulavska, Roza P. Mateva

"Graft Copolymers Of Polyamide 6 Via In Situ Polymerization Of E-Caprolactam"

Институт по полимери – БАН
ул. "Акад. Г. Бончев", бл. 103А, 1113 София, България

Постер №1

Ефекти на наночастици от поли (винилбензилтриметиламониев хлорид) върху HepG2 клетки

Кирилка Младенова[1], Еми Халаджова[3], Деница Мелнишка[2], Магдалена Пенчева[2], Таня Топузова-Христова[2], Веселина Москова-Думанова[2], Станислав Рангелов[3], Йордан Думанов[1]

[1]Софийски университет, Биологически факултет, Катедра "Биохимия"; [2]Софийски университет, Биологически факултет, Катедра "Цитология, хистология и ембриология"; [3]Българска Академия на Науките, Институт по полимери

Увод Полимерите се изучават активно във връзка с тяхната потенциална употреба като сиситеми за пренос на биологично активни молекули. Особено актуален е проблемът с намиране на подходящи преносители на ДНК, които да послужат при разработване на нови продукти за трансфекция и генна терапия. Такива преносители трябва да отговарят на няколко критерия: да не са токсични за клетките, да не повлияват негативно техния метаболизъм и при преноса молекулата на ДНК да остане интактна. При изследване ефекта от нов продукт е необходимо да се установи влиянието му върху различни типове клетки, съставляващи тъканите и органите на даден организъм.

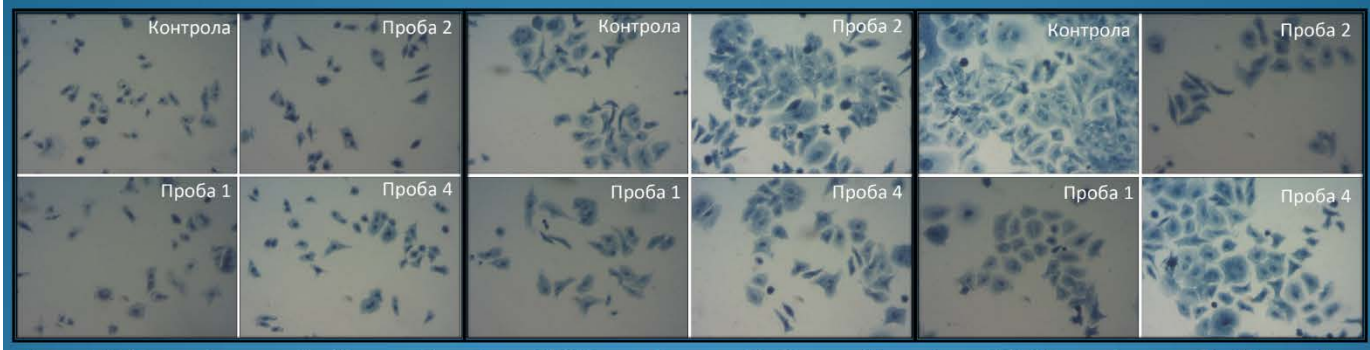
Цел: Целта на проведеното изследване е да се установи влиянието на наночастици от поли (винилбензилтриметиламониев хлорид) върху чернодробни клетки

Методи: Използвана е карциномна клетъчна линия от черен дроб на човек – HepG2. Клетките се култивират в термостат при 37°C и 5% CO_2. Извършени са тестове за метаболитна активност (MTT-тест) на клетките 24- и 72-часа след третиране с наночастици. За установяване на влиянието на наночастиците върху темповете на делене на третирани клетки беше определен митотичен индекс 24, 48 и 72 часа след третиране. Изследват се натоварени наночастици, изготвени при различно съотношение полимер:ДНК (съотношенията са на база заряд): при проба 1 съотношението полимер:ДНК е 1:1, проба 2 – 2:1, проба 4 – 4:1.

Резултати:

Фигура 1 А) MTT-тест на HepG2 клетки, 24h след третиране. Пробите са нанесени с концентрация 0,2μg ДНК за 1x10^{5}клетки.

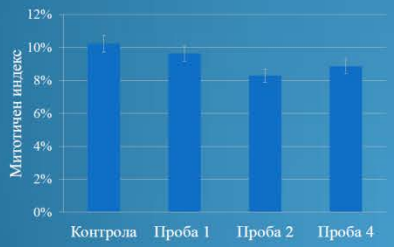
Фигура 1 Б) MTT-тест на HepG2 клетки, 72h след третиране. Пробите са нанесени с концентрация 0,2μg ДНК за 1x10^{5}клетки.



Фигура 2 А) Обща морфология на HepG2 клетки 24 часа след третиране. Оцветяване с метиленово синьо, увеличение 25х.

Фигура 2 Б) Обща морфология на HepG2 клетки 48 часа след третиране. Оцветяване с метиленово синьо, увеличение 25х.

Фигура 2 В) Обща морфология на HepG2 клетки 72 часа след третиране. Оцветяване с метиленово синьо, увеличение 25х.



Фигура 3 А) Митотичен индекс 24 часа след третиране на HepG2 клетки. Пробите са нанесени с концентрация 0,2μg ДНК за 1x10^{5}клетки.

Фигура 3 Б) Митотичен индекс 48 часа след третиране на HepG2 клетки. Пробите са нанесени с концентрация 0,2μg ДНК за 1x10^{5}клетки.

Фигура 3 В) Митотичен индекс 72 часа след третиране на HepG2 клетки. Пробите са нанесени с концентрация 0,2μg ДНК за 1x10^{5}клетки.

Изводи:

1. **Използваните концентрации от натоварени с ДНК наночастици са нетоксични за клетките;**
2. **Няма разлики в общата морфология между третирани и нетретирани клетки;**
3. **Не се забелязва понижение на митотичния индекс на третираните клетки спрямо контролите**

Благодарности: *проучването беше финансирано по проекти ДФНИ – Т02/7 от 12.12.2014 и Докторантски проект към СУ 60/03.04.2015.*

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №2

"ЕФЕКТИ НА НАНОЧАСТИЦИ ОТ PEGMA-B-PLL ВЪРХУ ЕУКАРИОТНИ КЛЕТКИ"

Магдалена Пенчева[1], Радостина Калинова[3], Деница Мелнишка[1], Кирилка Младенова[2], Таня Топузова-Христова[1], Йордан Думанов[2], Ивайло Димитров[3], Веселина Москова-Думанова[1]

[1]Софийски университет, Биологически факултет, Катедра "Цитология, хистология и ембриология"; [2]Софийски университет, Биологически факултет, Катедра "Биохимия"; [3]Българска Академия на Науките, Институт по полимери

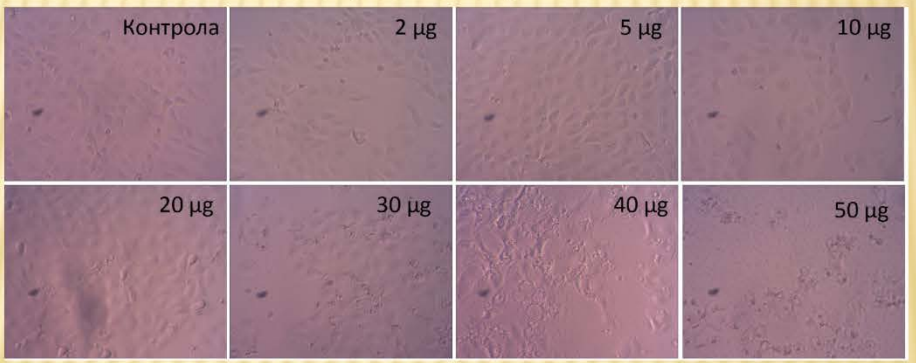
Увод Поли-L-лизинът е подходящ преносител на ДНК тъй като се свързва стабилно с нуклеинови киселини. Това го прави интересен обект за изследванания, насочени към намиране на подходящи препарати за трансфекция и генна терапия. Преносителят трябва да е стабилен, ефективен и нетоксичен. В настоящето изследване вниманието е насочено към влиянието на полиплекси от поли(етилен гликол метакрилат)-b-поли(L-лизин) върху моделна система монослой от еукариотни клетки.

Цел **Целта на настоящето изследване е да се установи влиянието на полиплекси от поли(етилен гликол метакрилат)-b-поли(L-лизин) (PEGMA-b-PLL) върху клетъчни култури от епителни клетки.**

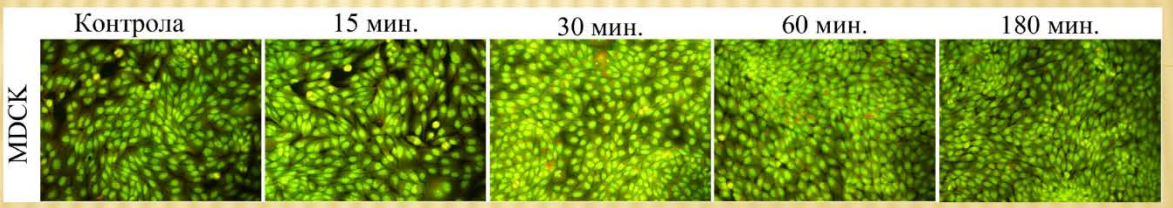
Методи За експериментите се използват бъбречни епителни клетки (MDCK II) от куче. Клетките се третират за 6 часа с различни концентрации на полиплекса. За установяване на метаболитната активност на третирани клетки се използва MTT-тест. Извършва се микроскопско наблюдение на третирани клетки за установяване на морфологични промени. За визуализация на лизозомална фракция се използва флуоресцентрно оцветяване с Акридин оранж. Проследява се оцветяването през определени интервали от време (15-30-60-180мин.) при третиране на клетки с работна концентрация на полиплекса, която съдържа 0.2µg ДНК.

Резултати

Фиг. 1 MTT-тест на MDCK II клетъчна линия, след 6h третиране с полиплекс.



Фиг.2 Обща морфология на MDCK II клетки след 6 часа третиране с полиплекс. Увеличение 250x



Фиг. 3 Оцветяване на третирани MDCK II клетки с Акридин оранж. Увеличение 250x

Изводи:

1. **Полиплексът не проявява цитотоксичност при концентрации под 30µg/ml;**
2. **При ниски концентрации на полиплекса не се забелязват морфологични разлики между третирани и нетретирани клетки;**
3. **Не се забелязва увеличаване на лизозомалната фракция до 180-тата минута от третирането на клетките.**

Благодарности: *проучването беше финансирано по проекти ДФНИ – Т02/7 от 12.12.2014 и Докторантски проект към СУ 60/03.04.2015.*

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №3

Synthesis and DNA complexation of novel polypeptide-based hybrid block copolymers

R. Kalinova[1], J. Doumanov[2], T. Topouzova-Hristova[2], I. Dimitrov[1]

[1]Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl.103A, Sofia 1113, Bulgaria
[2]Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia 1164, Bulgaria

Introduction

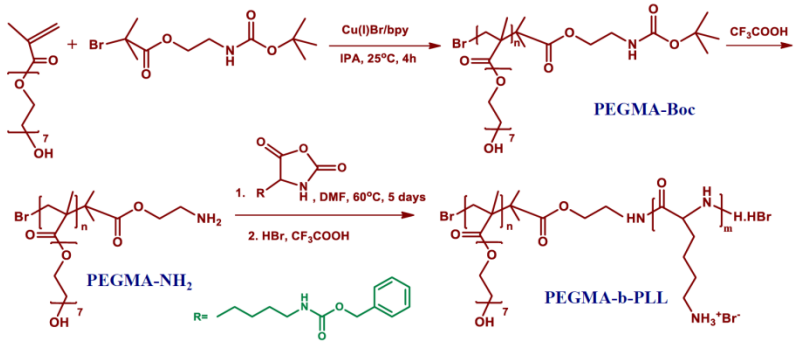
Polymeric gene delivery systems have been studied to transfer nucleic acid therapeutics into specific cells for the treatment of various human diseases. However, the challenge remains in the development of safe, sufficiently stable, specific, and efficient gene delivery systems. Poly(L-lysine) has emerged as one of the suitable non-viral vectors for gene therapy since it can strongly interact with DNA or RNA. In this study poly(ethylene glycol) methacrylate)-b-poly(L-lysine) (PEGMA-b-PLL) block copolymer was synthesized applying a multistep procedure. The resulting copolymer was used to condense DNA (salmon sperm DNA, 2000 b.p.) into a nanosized complexes (polyplexes). The cytotoxicity of hybrid copolymer and its polyplex on human hepatocytes was investigated.

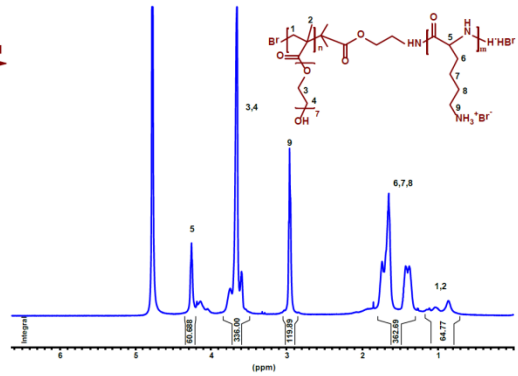
Polymer synthesis and characterization

Poly(ethylene glycol) methacrylate) macroinitiator was prepared by ATRP of oligo(ethylene glycol) methacrylate initiated by t-butyloxycarbonyl (Boc)-protected 2-bromoisobutyryl bromide. After deprotection with trifluoroacetic acid the desired PEGMA-NH_2 macroinitiator was used to initiate the ring-opening polymerization of N^{ε}-(benzyloxycarbonyl)-L-lysine N-carboxyanhydride (ZLLys-NCA) to give the PEGMA-b-PZLL block copolymer. The Z-protecting groups were removed from the peptide block of PEGMA-b-PZLL after treatment of the polymer with solution of HBr in glacial acetic acid.

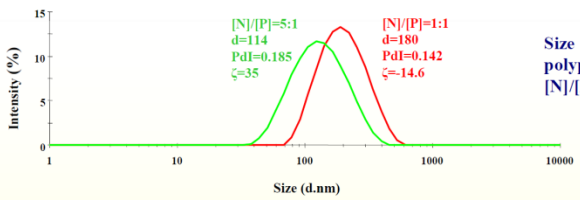
Entry	$M_{n\ th}$ (g mol^{-1})	$M_{n\ exp}$ [a] (g mol^{-1})	$Đ$ [b]
PEGMA-NH_2	5 400	4 300	1.23
PEGMA-b-PLL	15 100	25 900	1.28

[a] determined by ^{1}H NMR; [b] determined by SEC using PEG standards

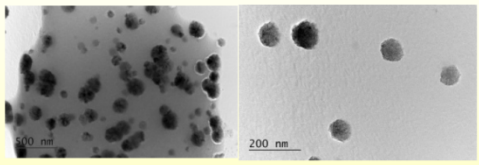




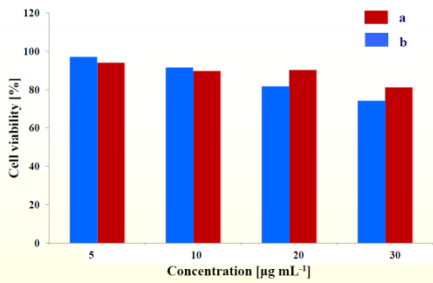
Polyplex formation



Size distribution of polyplex formed at [N]/[P]=5:1 and 1:1



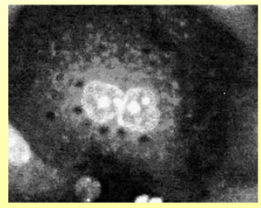
TEM images of polyplex formed at [N]/[P]=5:1



Viability of Hep2G cells by MTT assay after 6 hours of incubation: a polyplex prepared at [N]/[P]=5:1; b hybrid block copolymer PEGMA-b-PLL

Conclusions

- Hybrid poly(ethylene glycol) methacrylate)-b-poly(L-lysine) (PEGMA-b-PLL) copolymers were successfully synthesized by ATRP
- Polyplexes between the hybrid copolymer and DNA were successfully formed and characterized
- Cell viability assay showed absence of cytotoxicity
- Initial evaluation of the polyplex transport pathways in MDCK II epithelial cells was performed.



Fluorescent microscopy image of nanoparticles in MDCK II cell stained with acridine orange (5 μg/ml).

Financial support from Bulgarian National Science Fund through a project No - T02/7 is gratefully acknowledged.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №4

REINFORCED COMPOSITE MATERIALS BASED ON POLY(3-HYDROXYBUTYRATE) – PREPARATION AND PROPERTIES

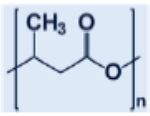
Irena Borisova, Olya Stoilova, Nevena Manolova, Iliya Rashkov

Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences
BG 1113 Sofia, Acad. G. Bonchev St, bl. 103A

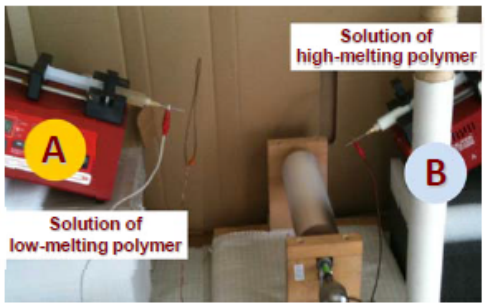
Introduction

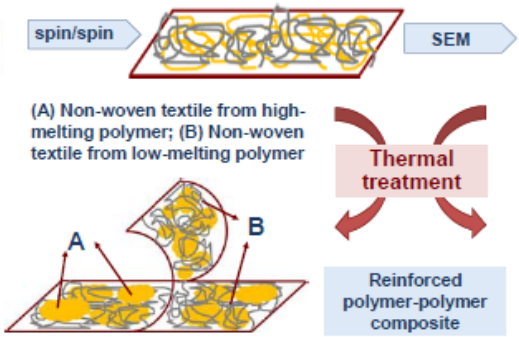
The preparation of electrospun non-woven textile based on biocompatible polymers with different thermal behavior is the strategy used for obtaining polymer-polymer composite materials. The materials are obtained by simultaneous electrospinning of polymers having different thermal behavior. Thermal heating of the electrospun mats results in the formation of a reinforcing matrix. In this work poly(3-hydroxybutyrate)/poly(ε-caprolactone)-based materials were obtained.

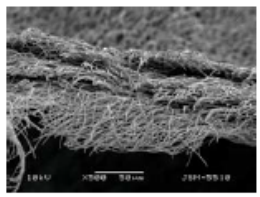
Preparation of reinforced polymer-polymer composite materials

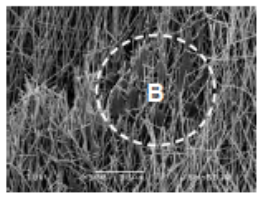


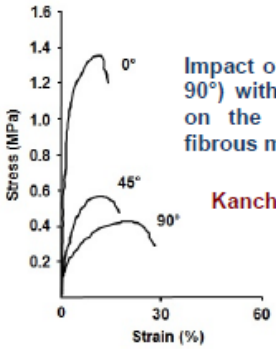
The preparation of multilayered electrospun non-woven textile based on biocompatible polymers with different thermal behavior is the strategy used for preparation of reinforced polymer-polymer composite materials. The materials are built of micro- or nanofibrous non-woven textile (A), incorporated into a polymer matrix (B). The matrix provides mechanical resistance of the composite and were obtained by electrospinning of low-melting polymer. Micro- or nanofibrous non-woven textile provides reinforcement of the composite material and were prepared by electrospinning of high-melting polymer.





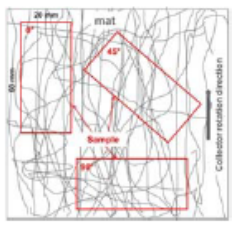


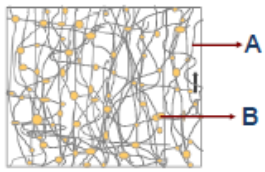


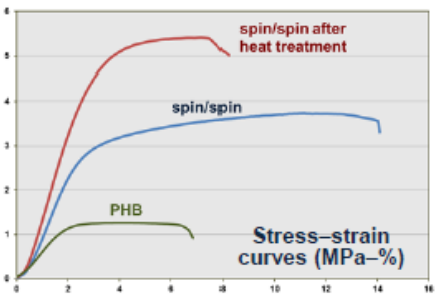


Impact of the direction of cutting of mats (0°, 45° or 90°) with respect to the collector rotation direction on the mechanical behavior of the electrospun fibrous materials.

Kancheva M., Toncheva A., Manolova N., Rashkov I., Express Polym. Lett., 9, 49 - 65 (2015).







Materials	Young's modulus (E, MPa)	Tensile strength at break (σ_B, MPa)	Elongation at break (ε_B, %)
PHB (spin)	33,4 ± 7,8	0,62 ± 0,2	6,8 ± 0,61
PCL (spin)	8,1 ± 3,7	0,96 ± 0,2	106,1 ± 9,1
PHB/PCL (spin/spin)	44,5 ± 18,0	0,74 ± 0,04	47,0 ± 7,2
PHB/PCL (spin/spin) after heat treatment	50,7 ± 11,4	4,0 ± 1,8	7,8 ± 1,5

Mechanical properties were evaluated by stress–strain curves obtained by stretching test at strain rate 20 mm/min. Thermal treatment at the melting temperature of low-melting polymer enabled the reinforcing of the materials thus enhancing the tensile modulus of polymer-polymer composite material.

Conclusion

The design of the polymer-polymer composite materials and their structure were purposely tailored by combining innovative and advanced methods. The relationship between the composition of the reinforced materials and their mechanical properties were evaluated.

Acknowledgment

The authors are thankful to the National Science Fund for financial support (Grant DFNI T02/1).

e-mails:
stoilova@polymer.bas.bg
manolova@polymer.bas.bg

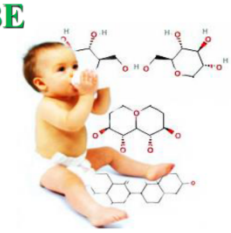
Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №5

БИСФЕНОЛ А - РИСКОВЕ ЗА ЧОВЕШКОТО ЗДРАВЕ

д-р инж. Снежана Тодорова

Център за оценка на риска, Българска агенция по безопасност на храните
бул. "Цар Борис III" № 136, ет. 11, 1618, гр. София, България,
e-mail: snejana.todorova@nvms.government.bg



ВЪВЕДЕНИЕ

Известно е, че поликарбонатите намират широко приложение за направата на шишета за хранене на кърмачета, домакински съдове, кутии за съхранение на храни, шишета и контейнери за вода, а така също и при производството на водопроводни тръби и др.

Въз основа на научни изследвания се доказват потенциалните рискове за здравето на хората от излагането на ниски дози бисфенол А (BPA). Бисфенол А е химично съединение, което намира широко приложение в материалите, влизащи в контакт с храни. Той се използва, като мономер при производството на поликарбонатни и епоксидни смоли. BPA влиза в състава на поликарбонатните пластмаси за направата на CD[та] и DVD[та], в електрическата и електронна апаратура, в строителството, в автомобилната индустрия, както и при производството на редица медицински апаратури.

Епоксидните смоли се използват широко за получаването на защитни покрития на консервни кутии за храни и напитки, съдове, а също и за облицовка на металните капачки на стъклени буркани и бутилки. Те също така успешно се прилагат и като защитни покрития, конструктивни композитни материали, лепила и др.

Целта на настоящия материал е да представи на базата на литературен обзор потенциалните рискове за потребителите по отношение на Бисфенол А.

РИСКОВЕ ЗА ПОТРЕБИТЕЛИТЕ ПО ОТНОШЕНИЕ НА BPA

Считано от 1 юни 2011г. (Регламент на Комисията (ЕС) № 321/2011г.) Европейската комисия е взела решение да забрани производството и разпространението на пазара в Европейския Съюз на пластмасови шишета, предназначени за хранене на кърмачета, съдържащи бисфенол А. BPA е един от химичните вещества, които имат потенциал да взаимодейства с хормоналната система в организма (т. нар. "ендокринно активно вещество"). Известно е още от 1930г., че BPA може да „имитира" женския полов хормон естроген. Ефектите му върху плодовитостта, възпроизводителността, като и влиянието му върху ендокринната система са били обект на много научни дебати, свързани с доклади за така наречения "ефект на малките дози" на BPA, наблюдаван при тестове проведени върху гризачи [1].

Научният комитет на Европейската комисия по храните е направил преглед на безопасността на BPA през 2002 година. През 2006г. Европейският орган по безопасност на храните (EFSA) прави повторна оценка на безопасността на BPA за потребителите, основана на засилени научни проучвания и установява (приема) приемлива дневна доза (TDI) 0,05 милиграм/кг телесно тегло (mg/kg bw). Тази доза представлява размера на дадено вещество (изразено спрямо телесното тегло), което може да се приема всеки ден в продължение на цял живот, без осезаем риск за консуматорите. Човешката хранителна експозиция на BPA, включително и тази на бебета и деца, се оценява да бъде под TDI [1].

EFSA е предоставила допълнителни консултации за BPA през 2008г., 2009г., а така също и през Септември 2010г., когато актуализира своето становище след провеждането на задълбочен и всеобхватен преглед на нови научни изследвания за токсичността на BPA. Експертите от EFSA в панела „Материали в контакт с храни, ензими, ароматизанти и спомагателни средства (CEF)" вземат предвид стотици научни изследвания, както и изследвания от промишлеността и правят заключението, че не могат да идентифицират нови доказателства, които да предизвикат преразглеждане на TDI за BPA от 0,05 мг/кг тегло.

На базата на три годишните си изследвания Френската агенция за безопасност на храните (ANSES) публикува на 9 април 2013г. резултати от направената от тях оценка на риска относно потенциалните рискове за човешкото здраве от бисфенол А.

За *първи път* Агенцията информира, че бисфенол А може да попадне в човешкото тяло посредством *вдишване* (от атмосферния въздух) и чрез *кожата*, при контакт с търговски продукти [2].

ANSES също така в това становище информира, че бисфенол А може да се наблюдава и при *консумация на вода* съхраняваща се в артикули за еднократна употреба, които са произведени от поликарбонати.

В този материал е направена оценка на опасността на други съединения, влизащи в класа на бисфенолите, а имено bisphenols S, F, M, B, AP, AF и BADGE. От *седемте* съединения, анализирани в настоящия доклад, *три* – bisphenols S, bisphenols F и bisphenols AP са потенциални заместители на бисфенол А според ANSES, но се акцентира на факта, че са необходими *допълнителни* токсилогични изследвания за да се подкрепи тази информация.

ЗАКЛЮЧЕНИЕ

Заключенията от оценката на риска, която е направена въз основа на идентифицираните опасности от проучванията, проведени върху животни и охарактеризиране на експозицията, показват потенциален риск при излагане на бисфенол А. Някои от установените вредни ефекти се отнасят до промяна в структурата на млечната жлеза, което може да доведе до образуването на туморни образувания. Отчитането на тези потенциални рискове обаче идва с ниво на доверие, описан в литературата като "умерени" по отношение на текущото състояние на знанията и несигурността за бисфенол А.

Като препоръка е използването на алтернативни артикули на поликарбонатните материали, които не съдържат BPA (например стъклени шишета за хранене на кърмачета или такива от друга пластмаса), отговарящи на изискванията за безопасност, предвидени за материалите, които влизат в контакт с храните.

Съществуват данни, че черният дроб, тънките черва, бъбреците и репродуктивната система са възможните целеви органи/системи за токсичното действие на бисфенол А при лабораторните животни [3]. *Няма данни* за генотоксичност и канцерогенност. Изследванията при животни са показали, че високи дози от бисфенол А могат да доведат до проблеми в развитието, понижаване на теглото и разстежа на поколенията, а също и ефекти по отношение на нервната система и поведението [4].

Токсикокинетичните параметри на BPA се различават при гризачи и хора. При хората BPA бързо се абсорбира от стомашно-чревния тракт и метаболизира до бисфенол А-глюкоронид, който не притежава активност към естрогенните рецептори бързо се елиминира посредством урината с полуживот от максимум шест часа. При плъхове орално приетия BPA също се трансформира до BPA-глюкоронид, но той търпи ентерохепатален кръговрат като се разгражда в стомашно-чревния тракт до изходните вещества и отделения свободен BPA отново се реабсорбира в кръвния поток. Това води до по-бавното елиминиране на BPA, наблюдавано у гризачи, в сравнение с хората.

На 21.01.2015г. на официалния интернет сайт на EFSA бяха публикувани следните материали: *„Научно становище относно рисковете за общественото здраве, свързани с наличието BPA в храни"* и *„Доклад от двуфазно публично обсъждане на проект на научното становище на EFSA за бисфенол А"* [5].

Научното становище относно рисковете за общественото здраве, свързани с наличието на бисфенол А в храни описва оценката на рисковете за общественото здраве, свързани с експозицията на бисфенол А (BPA). Тя (експозицията) е оценена на различни възрастови групи от населението по три различни начина: *(1) външно* - с диета, питейна 2 вода, вдишване, контакт с кожата, използвайки козметика и термочувствителна хартия. Съществуват различни видове принтери, такива като ударни, матрични, безударни, струйни, лазерни, термични-използват термочувствителна хартия. Определена част от термичните принтери (т. нар. главата) е изградена от терморезистори, които се загряват при протичането на ток при употребата на принтера и загряват хартията на определени места, като по този начин се получава образа върху хартията; *(2) вътрешна експозиция отнесена към общия BPA* - абсорбирана доза на BPA, сума от размера на конюгиран и неконюгирана BPA, т.е. размера на свързваните и несвързаните клетки в човешкото тяло с BPA; *(3) агрегирани* - от диета, прах, козметика и термочувствителна хартия, изразена като пероралната еквивалентна човешка доза (human equivalent dose - HED) позовавайки се само на неконюгирания BPA (тя представлява, човешката доза на агент, който се смята, че предизвиква токсичен ефект).

Въз основа на нови данни и методологии, EFSA *понижи приемливата дневна доза до 4 милиграм/кг телесно тегло на ден (mg/kg bw/day).* Това е стойност, която е *12,5 пъти по-ниска от предишното ниво (50 mg/kg bw/day).*

В това научно становище на Панела на EFSA - CEF прави заключение, че най-високата открита хранителната експозиция на BPA, (която включва бебета, деца и юноши), е под приемливата дневна доза от 4 милиграм/кг телесно тегло на ден. *Това показва, че няма опасност за здравето на хората от BPA, в очакваните нива на експозиция.* В допълнение CEF Панелът заключава, че главните оценки за агрегирана експозиция на BPA, чрез хранителен режим и нехранителни източници (прах, играчки, козметика и термочувствителна хартия) за най-застрашените групи (такива, като кърмачета, деца и юноши), също е под приемливата дневна доза от 4 милиграм/кг телесно тегло на ден, което показва, че загрижеността от здравен проблем за хората от BPA е ниска в приблизителните нива на експозиция. Въпреки това, CEF Панелът отбеляза, че съществува значителна несигурност в оценката на експозицията за нехранителни източници.

ИЗТОЧНИК

1. Най-често задаваните въпроси към Европейския орган по безопасност на храните по отношение на Бисфенол А (http://www.efsa.europa.eu/en/faqs/faqbisphenol.htm?utm_source=newsletter&utm_medium=email&utm_content=feature&utm_campaign=20130731#top)

2. Assessment of the health risks of bisphenol A, French Agency for Food, Environmental and Occupational Health & Safety (ANSES) (http://www.anses.fr/en/documents/PRES2013CPA09EN.pdf)

3. Bisphenol A (BPA)-Current state of knowledge and future acsions by WHO and FAO, INFOSAN Inf. Note 5/2009

4. Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A, WHO, 1-5 Nov 2010

5. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs-Executive summary, EFSA Journal 2015;13(1):3978

Постер №6

A NOVEL APPROACH FOR PREPARATION OF SUPER-MACROPOROUS DEXTRAN CRYOGELS VIA UV-IRRADIATON TECHNIQUE

Georgi L. Georgiev and Petar Petrov

Laboratory "Structure and Properties of Polymers", Institute of Polymers, Bulgarian Academy of Sciences "Akad. G. Bonchev" str., bl.103A, 1113 Sofia, Bulgaria.

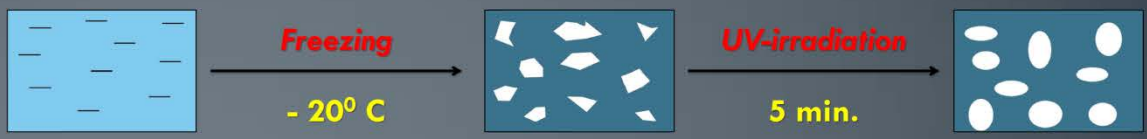
e-mail: ggeorgiev@polymer.bas.bg

Introduction:

Supermacroporous polymer cryogels are attractive materials due to their unique heterogeneous structure composed of large interconnected pores that are filled with solvent and surrounded by thin walls. This structure makes the diffusion of fluids and species within the volume of cryogel easy and, thereby, facilitates mass and heat transfer. Cryogels have been widely used in biomedical and pharmaceutical applications due to their high water content, similar to the tissues.

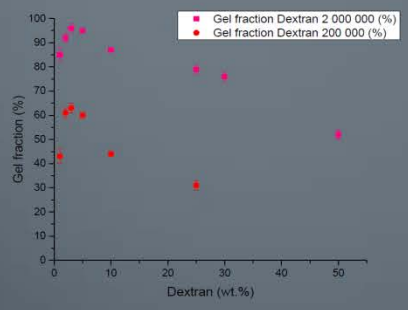
Aim:

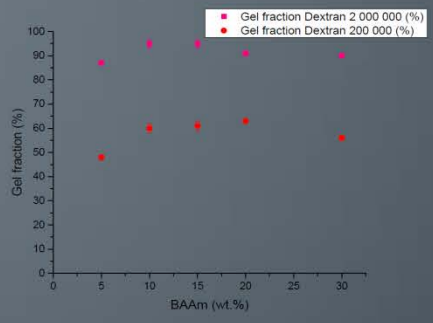
Synthesis of novel super-macroporous cryogels based on Dextran via UV-irradiation technique.

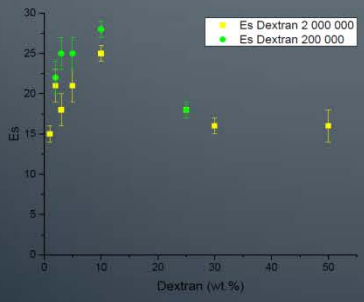


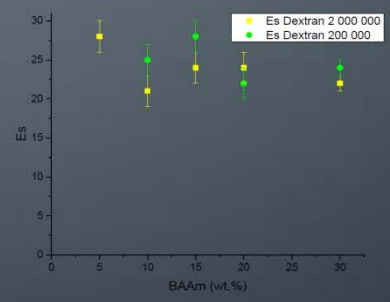
Photocrosslinking of Dextran (Mr ~ 200 000 and Mr ~ 2 000 000), was performed using water-soluble photoinitiator (4-benzoylbenzyl)trimethylammonium chloride in the presence of *N,N*-Methylenebis(acrylamide) as a crosslinker.

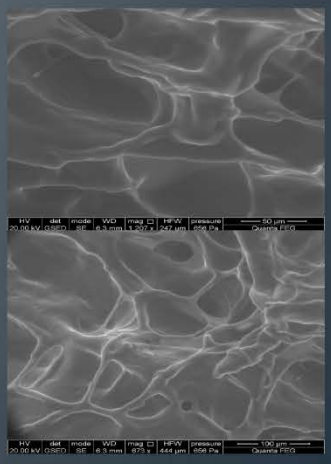
Results:











ESEM images of swollen Dextran (2 000 000) cryogel – pore size 50 ÷ 200 μm.

Conclusions:

Dextran cryogels were synthesized for the first time via UV induced crossslinking in frozen system. It was found that both the initial polymer concentration and amount of crosslinker strongly influence the gel fraction and degree of swelling of the resulting cryogels. High gel fraction (around 90%) was obtained at a relatively low concentration of dextran with Mr ~ 2 000 000 (1 wt.%). Moreover, the optimal content of BAAm required for preparation of cryogels of high gel fraction was in the range of 10 – 15 wt.%.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №7

Synthesis of poly(oxyethylene phosphoramidate)s via Staudinger Reaction

Zornica N. Todorova*, Neli S. Koseva and Kolio D.Troev

Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 103A, 1113 Sofia, Bulgaria

Introduction

In this work we designed a new synthetic route for the preparation of polyphosphoramidates starting from well-defined poly[alkylene trimethylsilylphosphite(III)]s. Since the phosphorus in the obtained polyphosphites is tri-coordinated, we used the new polymers in the chemoselective Staudinger-polyphosphite reaction with different azides. This design can be applied for the synthesis of polyphosphoramide-carbohydrate-conjugates using sugar azides. The products can be used further for peptide and protein modifications.

Methods

•The *polymeric H-phoshonates* were synthesized via a polytransesterification reaction of *dimethyl H-phosphonate* and *poly(ethylene glycol) (PEG 600).*

•The *poly(oxyethylene trimethylsilylphosphite)s* were obtained via a silylation of the *polymeric H-phopshonates* with *BSA, TMCS or HMDS.*

•The structures of the compounds were studied by *MS* and *NMR (¹H, ¹³C and ³¹P) spectroscopy.*

Tools

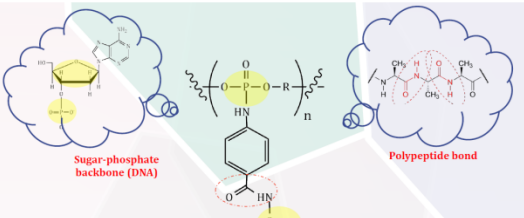
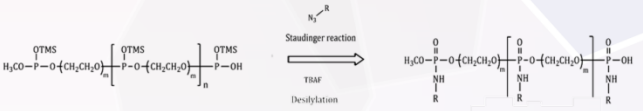


Figure 1: Polyphosphoramidates as mimics of biopolymers.



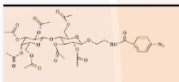
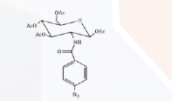
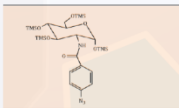
Scheme. 1: Staudinger reaction for the synthesis of polyphosphoramidates.

Results

Table 1: Synthesis of polyphosphoramidates with aryl azides via Staudinger reaction.

Azide	Conversion (according to P{H} NMR)	+TBAF	+H_2O	After Dialysis
Fmoc-p-Azido-L-phenylalanine	Full 5.28 ppm	5.37 ppm (86%)	Not soluble (2 phases)	Can not be performed in water
Fmoc-p-Azido-tetra-fluoro-L-phenylalanine	Full 1.47 ppm	5.39 ppm (98%)	2.15 ppm (~58%) 1.8 ppm (~22%)	2.32 ppm (100%)
Phenyl Azide	full 5.02 ppm	3.39 ppm (~84%)	2.75 ppm (~75%) -0.85 ppm (~25%)	2.56 ppm (~61%) -1.10 ppm (~39%)
p-Azidobenzoic acid	full 4.41 ppm	2.70 ppm (~95%)	Not soluble (Jelly)	Can not be performed in water

Table 2: Synthesis of polyphosphoramidate-carbohydrate-onjugates with via Staudinger reaction.

Sugar Azide	Conversion (according to P{H} NMR) +TBAF
	3.58 ppm (93.33 %) -CH_2O-*P*(O)(NH-Sugar)-OCH_2-
	77.04 % 2.56 ppm (72.00 %) CH_2O-*P*(O)(NH-Sugar)-OCH_2- 1.98 ppm (4.32 %) -CH_2O-*P*(O)(NH-Sugar)-OCH_2- 0.81 ppm (0.72 %) -CH_2O-*P*(O)(NH-Sugar)-OH
	2.19 ppm (28.00 %) -CH_2O-*P*(O)(NH-Sugar)-OCH_2-

Conclusion

✓POETMSPhosphites or their copolymers can be used *in situ* for further reactions incl. the Staudinger reaction;

✓Poly(oxyethylene phosphoramidate)s can be obtained using different azides;

✓Carbohydrate-polyphosphoramidate-conjugate were successfully synthesized and can be used further for multivalent binding studies.

.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №8

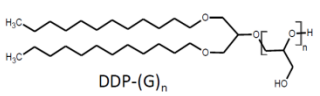
Interchain Hydrogen bonds as a stability factor in hybrid polyglycidol-lipid bilayer membranes

Pavel Bakardzhiev,[1] Denitsa Momekova,[2] Katarina Edwards,[3] Spiro Konstantinov,[4] Stanislav Rangelov[1]

[1] Institute of Polymers, Bulgarian Academy of Sciences, 103-A Acad. G. Bonchev St., 1113 Sofia, Bulgaria
[2] Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University – Sofia, 2 Dunav St., 1000 Sofia, Bulgaria
[3] Department of Chemistry – BMC, University of Uppsala, SE 751 23 Uppsala, Sweden
[4] Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University – Sofia, 2 Dunav St., 1000 Sofia, Bulgaria

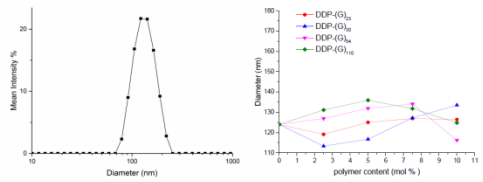
Liposomes are artificial structures, that are formed spontaneously in aqueous media. They are composed of phospholipid molecules, assembled in a self-enclosed bilayer surrounding a water compartment. The liposomal bilayer is mimicking the cell membrane, whereas the liposomes can be considered as artificial cells. Therefore, they have become subject of interest and research in the fields of biophysics, biochemistry and especially in drug delivery. Liposomes can be easily loaded with both hydrophilic and hydrophobic drug molecules, located in the inner water domain and in the phospholipid membrane, respectively. A major drawback, however, is their short half-life in the blood stream due to fast recognition by the immune cells, which is preceded by opsonization with different blood native molecules, mostly lipoproteins. An elegant approach for reducing the opsonization is grafting the liposome membrane with hydrophilic polymer chains, thus creating a repulsive barrier that prevents the liposome-lipoproteins interactions. The aim of the present contribution is to evaluate the potential of linear polyglycidol as a hydrophilic, flexible and biocompatible polymer to provide stabilization properties to liposomes based on dipalmitoylphosphatidylcholine. To achieve the aim, a series of polyglycidol-derivatized lipids with degree of polymerization of the polyglycidol chains in the 23–110 range were synthesized. Liposome formulations with varying copolymer type and content were prepared by film hydration technique followed by extrusion. The hybrid structures were studied by means of dynamic and electrophoretic light scattering, cryogenic transmission electron microscopy, and fluorescence spectroscopy. Cytotoxicity towards OPM-2 and EJ cell lines was assessed as well.

Polymer structure and characteristics



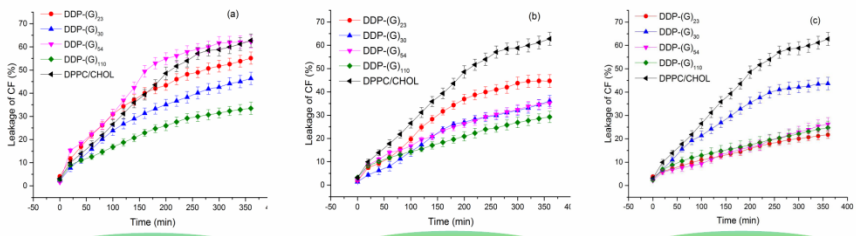
Composition	Mn (^{1}H NMR) g.mol^{-1}	PDI (GPC)
DDP-$(G)_{23}$	2130	1.35
DDP-$(G)_{30}$	2650	1.39
DDP-$(G)_{54}$	4420	1.26
DDP-$(G)_{110}$	8570	1.36

Dynamic light scattering results



Leakage assay

Buffer solution, pH=7,4, T=37 °C

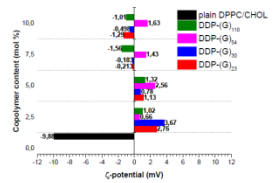


Polymer content 5 mol %

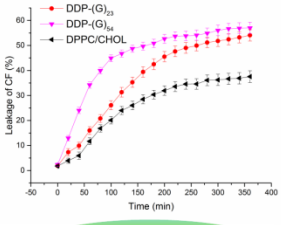
Polymer content 7,5 mol %

Polymer content 10 mol %

ζ-potential data

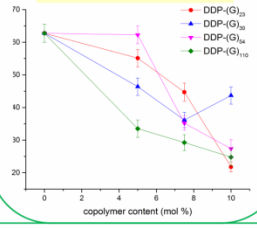


Buffer solution , 0,5 M urea , pH=7,4, T=37 °C

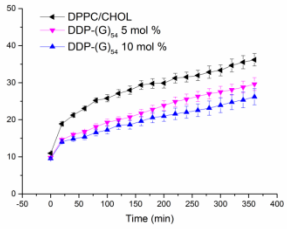


Polymer content 10 mol %

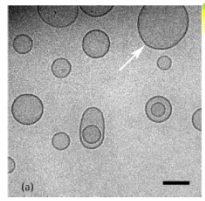
Fraction of the released CF at t=360 min as a function of polymer type and content



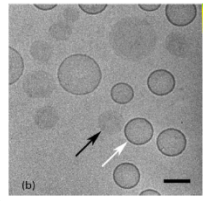
Buffer solution , 50 % human plasma, pH=7,4, T=37 °C



Cryo-TEM micrographs

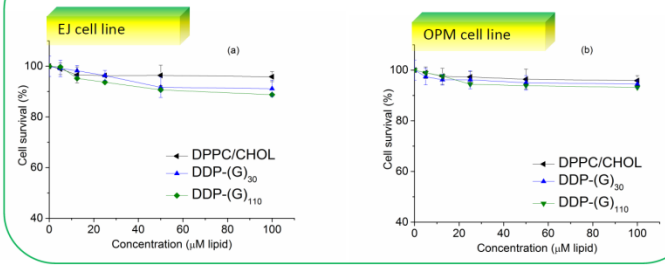


DDP-$(G)_{30}$ 5 mol%



DDP-$(G)_{54}$ 7,5 mol%

Cytotoxicity



DPPC/CHOL liposomes containing different (2.5 – 10 mol %) amounts of DDP-polyglycidol conjugates were successfully prepared. The experimental data, obtained from the *in vitro* leakage assays clearly indicate a stabilization effect of the polymers. Additional experiments in urea containing media revealed the role of intermolecular hydrogen bonding in the polyglycidol layer around the liposomes in reducing the membrane permeability. Furthermore, the enhanced stability in blood plasma strongly indicates that the polyglycidol-derivatized lipids confer resistance to interactions with leakage promoting components present in plasma to the hybrid structures. The liposomes, sizing 113 - 136 nm, were intact and well-separated, judging by cryo-TEM images. Disc-like micelles at high polymer contents were observed. Toxicology tests indicate lack of cytotoxicity against EJ and OPM cells.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №9

Polyplexes based on poly(vinyl benzyl trimethylammonium chloride) homo- and block copolymers

E. Haladjova[1], G. Mountrichas[2], S. Pispas[2], S. Rangelov[1]*

[1] *Institute of Polymers, Bulgarian Academy of Sciences, acad. G. Bonchev st bl.103A, Sofia 1113, Bulgaria*
[2] *Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vass. Constantinou Ave., 116 35 Athens, Greece*
e-mail address: ehaladjova@polymer.bas.bg

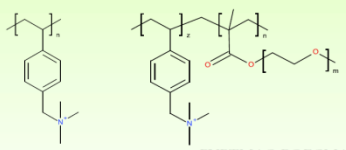
INTRODUCTION

Gene therapy holds promise for treating a wide range of diseases and is of particular interest to researchers. Non-viral DNA delivery systems show important advantages vs. viral systems that are usually associated with an immunological response and safety risks. The documented dangers of the viral systems have motivated the exploration for polymer-based gene delivery systems, which are safer, less pathogenic and less immunogenic alternatives.

In this work we focus on the use of novel homo- and block copolymers based on vinyl benzyl trimethylammonium chloride (VBTMAC) as gene delivery vector systems. DNA/polymer complexes (polyplexes) at a wide range of N/P (amino-to-phosphate groups) ratios were prepared. The ability of novel polymers to form complexes with linear DNA was investigated by light scattering, zeta potential and ethidium bromide fluorescence quenching measurements. The stability of polyplexes was monitored by changes in their hydrodynamic parameters in the presence of salts.

POLYMER STRUCTURES AND CHARACTERISTICS

The polymers were synthesized by RAFT polymerization in aqueous medium, initiated by 4,4'-Azobis(4-cyanovaleric acid), in the presence of (4-cyanopentanoic acid)-4-dithiobenzoate used as chain transfer agent. The molar mass of the polymers were determined by SEC measurements.

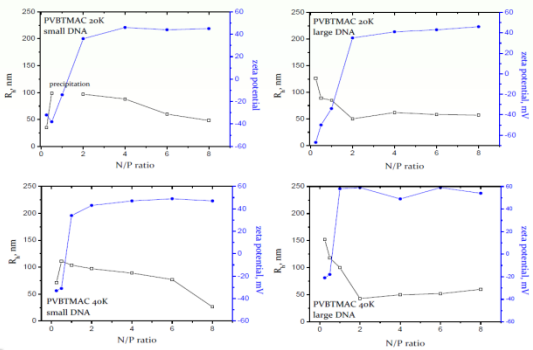


PVBTMAC PVBTMAC-POEGMA

Polymer	Mw g.mol^{-1}	Mw/Mn	Mw PVBTMAC	% POEGMA	ζ, mV
PVBTMAC 20K	20 700	1,16	20 700	-	22,3
PVBTMAC 40K	39 600	1,15	39 600	-	34,6
PVBTMAC-POEGMA 2	22 400	1,19	4 200	81,2	31,5
PVBTMAC-POEGMA 4	33 600	1,18	15 400	45,8	28,9

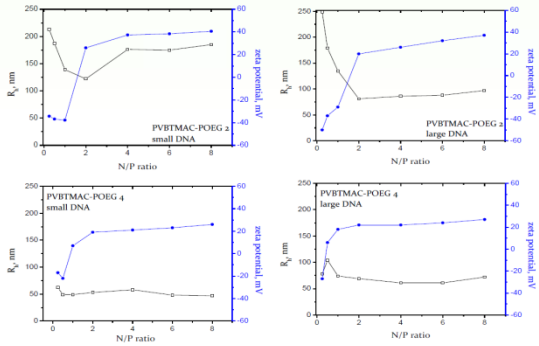
COMPLEXATION WITH DNA

Homopolymers



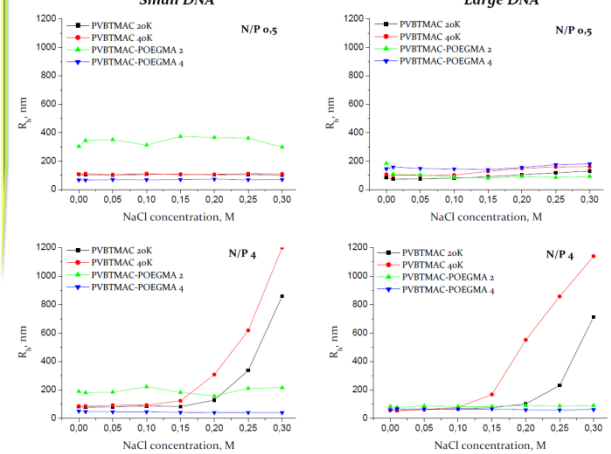
Linear DNA of different molar mass (from salmon testes, Mw ~ 2000 bp and from salmon sperm Mw ~ 113 bp) were used for complexation with PVBTMAC based homo- and block copolymers. Polyplexes at different N/P ratio in the range of 0.5÷8 were prepared. The obtained complexes were colloidaly stable for more than 1 week, preserving their size and polydispersity.

Block copolymers



BEHAVIOR OF POLYPLEXES IN PRESENCE OF SALT

Small DNA ***Large DNA***

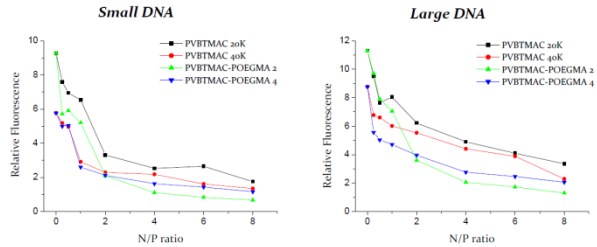


The stability of the polyplexes was investigated in the presence of salt. In the excess of DNA, that is at lower N/P ratios, the polyplexes are not sensitive to presence of salt. At higher N/P ratios, however, a critical salt concentration of about 0.20 M exists, at which the size of the polyplexes, prepared from the homopolymers started to increase presumably due to aggregation. In contrast, no size increase was observed for the polyplexes, prepared from the copolymers, implying a stabilization effect of the POGMA blocks.

ETHIDIUM BROMIDE QUENCHING ASSAY

Quenching of ethidium bromide fluorescence was used to monitor the polyplex formation. The fluorescence intensity decreases due to inhibiting of its binding with DNA. As seen, upon increasing N/P ratio, the relative fluorescence intensity decreased indicating strong complexation. The decrease was somewhat sharper for the polyplexes with the small DNA implying easier polyplex fromation.

Small DNA ***Large DNA***



CONCLUSION

It is shown that homo- and block copolymers of poly(vinyl benzyl trimethylammonium chloride), PVBTMAC, are able to condense DNA molecules. The resulting polyplexes, prepared in a wide N/P range, were of relatively small dimensions (R_h < 100 nm and typically about 50 nm) and displayed narrow size distribution. The fluorescence quenching assay indicated strong interactions between (co)polymers and DNA, that were able to displace the ethidium bromide intercalation in DNA. In the presence of salt, the polyplexes, prepared from the copolymers, exhibited greater stability, which was attributed to the inert POEGMA blocks.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №10

Ability of poly(n-propyl-2-oxazoline) based copolymers to bind and compact DNA

Ts. Ivanova[1], E. Haladjova[2], M. Mees[3], D. Momekova[1], S. Rangelov[2], R. Hoogenboom[3]

[1] Faculty of Pharmacy, Medical University of Sofia, 2Dunav str., Sofia 1000, Bulgaria
[2] Institute of Polymers, Bulgarian Academia of Science, "Acad. Georgie Bonchev St. bl. 103-A 1113 Sofia, Bulgaria
[3] Department of Organic and Macromolecular Chemistry Ghent University, Krijgslaan 281 S4, B-9000 Ghent, Belgium

Introduction

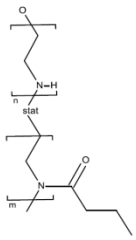
Gene therapy has gained significant attention over the past two decades. It refers to treatment of genetic disorders by modifying gene expression within specific cells. Polyethyleneimine (PEI) has been found to be a very effective transfection agent; therefore, it was called "the gold standard" for gene transfection. However, toxicity issues frequently compromise the high transfection efficiency well documented for systems based on PEI. Poly(2-oxazoline)s (POx) are biocompatible pseudo-polypeptides that have received significant interest for biomedical applications in recent years. They are also known as precursors for the synthesis of PEI. Combining the properties of this two polymers, a robust system can be designed to form polyplexes with DNA.

In this work, partially hydrolised POx were used for complexation with DNA. The formed polyplexes were characterized by dynamic and electrophoretic light scattering. A cytotoxicity investigations of the systhems were carried out. The obtained complexes were stabilized by creating a cross-linked polymer shell on their surface.

Copolymers

Code	DP	PEI %	M_n g/mol	M_w g/mol	Đ
82-2	75	10	12500	15000	1.2
82-3	160	8.5	18900	25377	1.3
82-4	160	7	21200	27800	1.3
82-5	200	9	34000	39500	1.2

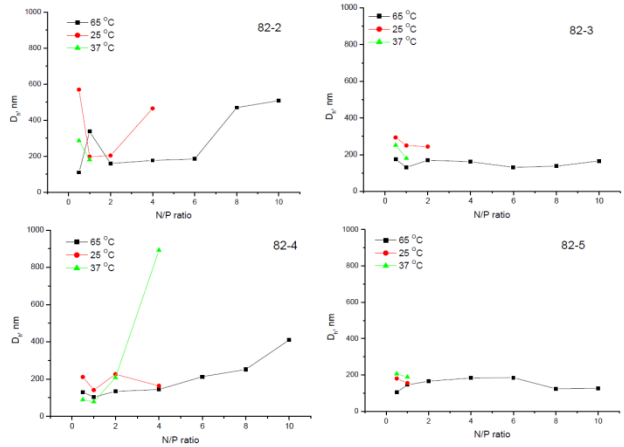
The copolymers were synthesized by partial hydrolysis of the POx. The hydrolysis was performed in 6M HCl at 100° C. The hydrolysis conversion was determined via ^{1}H NMR spectroscopy.



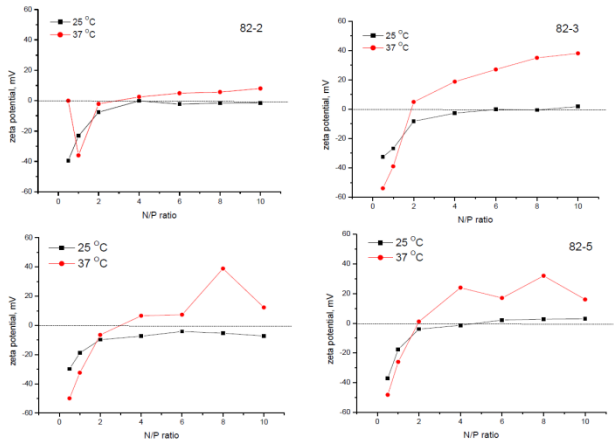
Binding of DNA

DNA/copolymer complexes were prepared by drop-wise addition of appropriate amounts of DNA (salmon testes, 2000 bp) into a heated at 65 °C aqueous solution of the copolymer under stirring. Polyplexes at different N/P ratios were prepared. The size and zeta potential strongly depended on the temperature: above the LCST of copolymers small particles with positive charge were obtained. In contrast, below the LSCT the complexes became larger in size and their surface charge tuned into negative.

Size



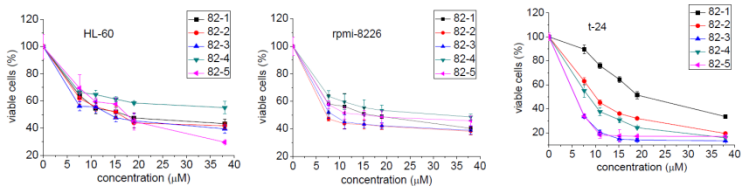
Zeta potential



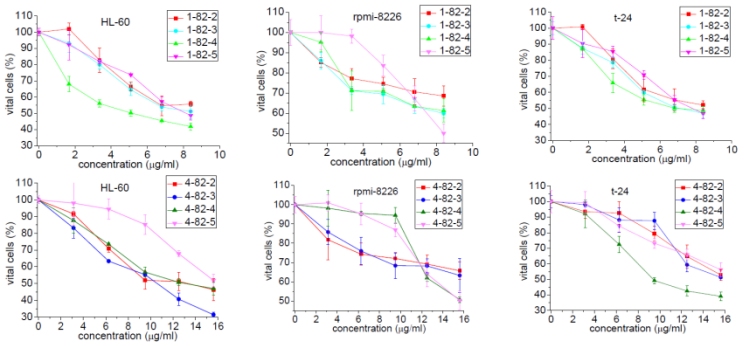
Cytotoxicity

The toxicity of copolymers and their DNA complexes was assessed by MTT-test. As evident from the results the copolymers were characterized with prominent dose dependent cytotoxicity on all tested human cell lines where at highest dose they reduce cell viability with almost 85 %. On the other hand, their counterpart DNA complexes show much lower cytotoxicity. The cell viability at the end of the exposure period was more than 45 %.

Polymers



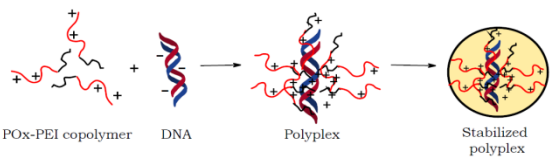
Polymer/DNA complexes



Stabilization of polyplexes by coating

The polyplexes were coated with a surface-cross-linked shell formed by seeded radical polymerization of N-isopropylacrylamide and N,N methylenebis(acrylamide) used as a cross-linker. After coating the complexes retain their small size and remain colloidaly stable.

Sample	D_h before coating at 60 °C (nm)	D_h after coating at 60°C (nm)	D_h after coating at 25°C (nm)	Shell thickness (nm)
82-3 N/P ratio 4	80	142	166	31



Future goals

✓Investigation of complexation behavior of the copolymers with plasmid DNA.
✓Stabilization of the resulting polyplexes with a polymer shell
✓Toxicity screening of the obtained systems
✓Determination of transfection efficiency of the novel DNA delivery vectors

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №11

Characteristics of long time stored single and double base waste propellants

Petar Kr. Shishkov[1*], Milena Nedkova[2],
[1] University of Mining and Geology "Sv. Ivan Rilski, Sofia, Bulgaria; [2]University of Chemical Technology and Metallurgy, Sofia, Bulgaria
[*]P. Shishkov, Corresponding author, e-mail: sfxman86@yahoo.com
Tel.: +359 898 448146, +359 878 448146

Introduction

It is known, that during the ageing single and double base propellants (S and DBP) decreased their nitrogen content [1] and molecular weight of the nitrocellulose, but their polydispersity increased [2]. It was interesting to determined the characteristics of propellants with different techniques, given in [3,4] and in this work, before around 20 year ageing of the same samples of SBP. It were applied the methods for determination of viscosimetric molecular weight, equal for our previous investigations, and different methods for GPC and nitrogen content. In this way was possible to determine the mechanism of ageing of S and DBP. This is the reason for our investigations.

Experimental

The S and DBP were produced in Arsenal OOD, Bulgaria. The propellants were stored in non heated military stores in silk pouch in cartridge – case of gun shells, placed in wooden boxes from date of their production to dismantle of the weapons in 1990 year. From 1990 year to today the propellants were stored at room temperature in UCTM, Sofia in metal cupboard. In this way the samples from all propellants were aged in dark and dry atmosphere. From the S and DBP were made 1% solutions in acetone and the viscosimetric $M_{vis.}$ molecular weights of the polymer were made according method, described in [5] and were given in table 1. The values of number and weight molecular weights M_n and M_w were made by system Waters and were in fig.1 and in table 2. The method for determination of nitrogen content of S and DBP were described in [6].

Results and discussion

The results from determination of $M_{vis.}$ were in table 1

Table 1. Data for $M_{vis.}$ S and DBP

N	Year of production	Kind	Storage, years	Ageing , years	$M_{vis.}x10^{-3}$
1	1944	SBP	(46) 24	70	153
2	1955	SBP	(35) 24	59	642
3	1959	SBP	(31) 24	55	275
4	1983	SBP	(7)…24	31	1335
5	1969	DBP	(21) 24	45	157
6	1977	DBP	(13) 24	37	317

Table 2. Data from GPC for condition of SBP

N	Year of product.	Kind	Storage, years	Ageing, years	M_n	M_w	N
1.	1944	SBP	(46) 24	70	8600	26572	3.09
2.*	1944	SBP	(46) 5	51	37940	256780	7
3.	1955	SBP	(35) 24	59	31498	38160	1.43
4.*	1955	SBP	(35) 5	40	55240	270250	4.86
5.	1959	SBP	(31) 24	55	-	-	-
6.	1977	SBP	(13) 24	37	-	-	-
7.*	1977	SBP	(13) 5	18	59210	280780	4.74
8	1983	SBP	(7)…24	31	44047	52318	1.19
9.*	1983	SBP	(7) 5	12	68560	297260	4.34
10	1969	DBP	(21) 24	45	-	-	-
11	1977	DBP	(13) 24	37	-	-	-

It was evident, that during the ageing the values of $M_{vis.}$ has decreased. The same dependence were with nitrogen content.

The condition of gel permition chromatography (GPC) was given in fig.1

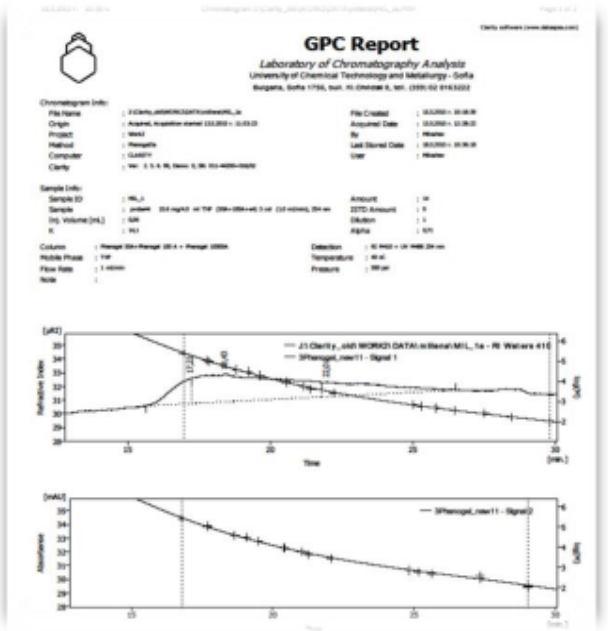


Fig.1 GPC data for sample N 1, SPB, made in 1944 year

Data for other samples were equal. From data for GPC were calculated values for M_n and M_w given in table 2

It was evident, that the dependences M_n – Year of ageing and M_w – Year of ageing for samples of SBP were stretch lines. The dependences n – Year of ageing were equal for samples of SBP, made with different techniques, applied for GPC, made before 20 wears and today. It was confirmed, that were made fractions with different amount and molecular weight after bracing of oxygen bridge between glycoside rings of applied nitrocellulose.

Conclusions

It was confirmed, that during the ageing of S and DBP decreased the values of $M_{vis.}$, M_n and M_w. The values of n increased, connected with bracing of oxygen bridges between glycoside rings of nitrocellulose

Постер №12

IBPBME-BAS

NMI achieving results

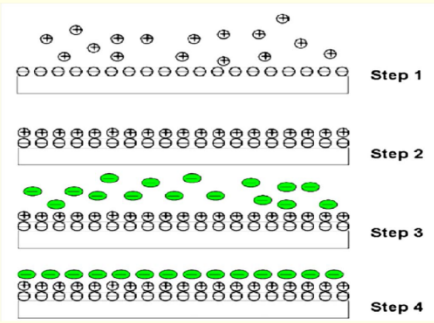
Optimization of HA/Chi polyelectrolyte multilayers for medical application

A. Danailova[1], P. Terziyska[2], S. Krumova[1], S.G. Taneva[1], R. Krustev[3], T.D. Andreeva[1]

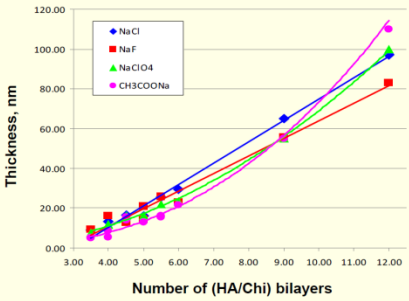
[1]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Georgi Nadiakov Institute of Solid State Physics, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Natural and Medical Sciences Institute, University of Tübingen, department "Biomaterials", Tübingen, Germany.

Coronary artery disease (CAD) is globally one of the major causes of morbidity and mortality, affecting over 17 million people per year (4 million in Europe) (data from the World Health Organization). CAD is responsible for 27% of the death cases in Bulgaria and 24% in Germany (WHO, April 2011). In addition to medical treatment, coronary stent implantation is for many patients the method of choice for the management of coronary atherosclerosis. However bare metal coronary stents can fail to maintain vessel patency due to either restenosis or stent thrombosis. Metal stents coated with an outer polymer layer can be drug-loaded, thus providing controlled and sustained drug delivery, which might allow optimal drug - tissue interactions.

Aim: This study addresses optimization of biocompatibility of (HA/Chi) PEM films by addition of Hofmeister anions and monitoring the thickness, roughness and morphology of the resulting coatings. The two polysaccharides used are biocompatible, nontoxic, and biodegradable. Both have already been widely used in biomedical applications and have interesting intrinsic properties. We focus on the effect of the monovalent chaotropic (ClO_4^-), cosmotropic (F^- and CH_3COO^-), and neutral (Cl^-) anions on the biological response of polyelectrolyte films, by evaluating the blood protein albumin adsorption. The proper conditions for optimal biocompatibility of the studied matrices for blood-exposed cardiac stents were established.



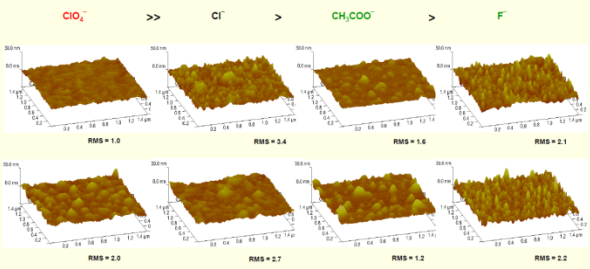
PEM films composed of polyethylenimine **PEI** (as a precursor layer), hyaluronic acid **HA (–)**, and chitosan **Chi (+)**, were prepared by layer-by-layer (LbL) technique on silicon wafers using the hand dipping method. The number of the deposited HA/Chi bilayers varied from 3.5 to 12. Green color represents the adsorption of bovine serum albumin (BSA).



Thickness of the PEM films with different number of Ha/Chi bilayers followed by ellipsometry.

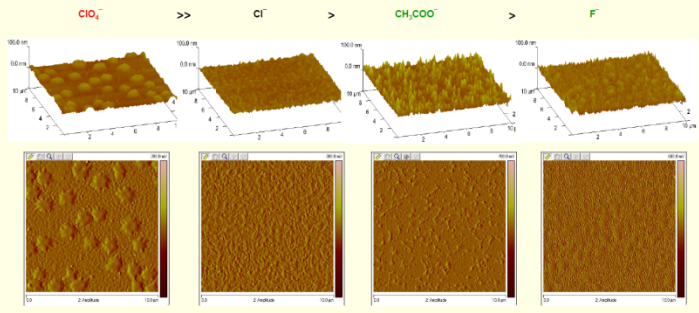
CONCLUSSION 2: In the presence of the neutral Cl^- and the weak cosmotropic F^- anion the HA/Chi films show linear thickness growth, whereas addition of the strongly cosmotropic CH_3COO^- and chaotropic ClO_4^- anions results in exponential thickness growth.

CONCLUSSION 3: The thickness of the HA/Chi coatings built from 12 bilayers increases in the order F^-< Cl^-< ClO_4^-<$CH3COO^-$, following the growth of the ion radius.



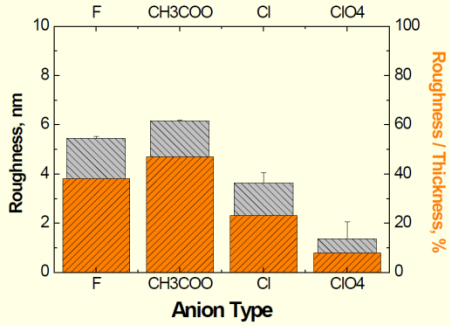
Testing of the biocompatibility of PEM coatings by BSA adsorption.

CONCLUSSION 6: The extent of the protein adsorption depends on the type of the added anions judged by the variation in the surface roughness, the protein adsorption being maximal in the presence of chaotropic, while almost negligible in the presence of cosmotropic anions.



Typical 3D- and deflection-images of 10-bilayers HA/Chi self-assembled films on silicon, taken by AFM (NanoScopeV system, Bruker Inc.) in tapping mode.

CONCLUSSION 1: The surface morphology of the PEMs depends on the type of the Hofmeister anion present in the initial HA and Chi solutions. The size of the surface structures formed during PEMs deposition follows the Hofmeister series $ClO4^-$>> Cl^-> $CH3COO^-$> F^-.



CONCLUSSION 4: The root-mean-square roughness Rrms of the HA/Chi films depends on the Hofmeister anion type. The cosmotropic anions (F^- and CH_3COO^-) favor surface heterogeneity, whereas the chaotropic anion $ClO4^-$ promotes formation of relatively smooth coatings.

CONCLUSSION 5: Rrms of the HA/Chi films grows in the order $ClO4^-$<Cl^-<F^- <CH_3COO^-, thus following the Hofmeister series. The same holds for the relative roughness, expressed by the ratio of Rrms and the thickness of the multilayers.

CONCLUSSION 7: Addition of cosmotropic anions to the initial HA and Chi solutions improves the biocompatibility of the coatings, because it increases the surface roughness and reduces the adsorption of albumin.

Acknowledgements:
This work is supported by the Bilateral academy exchange grant PPP Germany 01/9 2014, Bulgarian Science Fund/DAAD, and partly by INERA, REGPOT-2012-2013-1 NMP.

Постер №13

SYNTHESIS AND CHARACTERIZATION OF DIBLOCK COPOLYMERS BASED ON POLY(ETHYLENE GLYCOL) AND POLY (4-VINYL PYRIDINE) VIA ATOM TRANSFER RADICAL POLYMERIZATIONS

A. Yakub, S. Vircheva, R. Bryaskova

University of Chemical Technology and Metallurgy, Department of Polymer Engineering, Bulgaria

INTRODUCTION

Nowadays, block copolymers have received considerable attention due to their ability to self assemble in solution forming a range of different morphologies and sizes thus finding various applications. Therefore, the design and synthesis of block copolymers with well-defined architecture, controlled molecular weight, polydispersity and chain composition is an intensive developing research area in polymer chemistry.

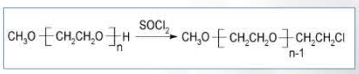
STRATEGY

The general strategy is to prepare diblock copolymers based on poly (ethylene glycol) and poly (4-vinyl pyridine) (PEG-P4VP) via atom transfer radical polymerization (ATRP). For that purpose, a novel ATRP macroinitiator based on PEG was synthesized and used for preparation of diblock copolymers in the presence of 4VP and CuCl/PMDETA as a catalyst system.

RESULTS AND DISCUSSION

Synthesis of chloro terminated ATRP macroinitiator

Poly (ethylene glycol) monomethyl ether (MeOPEG) (Mn=1900g/moL), which has a hydroxyl end group, was converted to an appropriated ATRP macroinitiator for the polymerization of 4-VP. This was achieved by reaction of MeOPEG with thionyl chloride according to scheme 1 thus leading to the formation of suitable chloro-terminated ATRP macroinitiator.

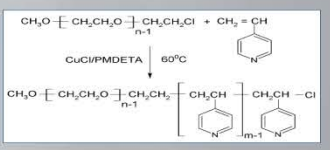


Scheme 1. Chlorination of MeOPEG with thionyl chloride.

IR spectra of MeOPEG and ATRP macroinitiator showed all characteristics signals for the MeOPEG units at: 2878 cm^{-1} (C-H stretching); 1095 cm^{-1} characteristic for -C-O-C- stretching vibration and 1469 cm^{-1} for C-H banding vibration. The chlorination of macroinitiator is clearly evidenced by the appearance a new band at 665 cm^{-1} which is characteristic for C-Cl band (Figure 1). ^{1}H NMR spectrum of macroinitiator showed all characteristic for PEG units signals at 3.38 ppm (3H, -O-CH_3) and 3.45-3.83 ppm (4H, -CH_2-CH_2-O-) (Figure 2). The number average molecular mass and molecular weight distribution of MeOPEG-Cl macrotinitator was determined by SEC. The peak of MeOPEG-Cl macroinitiator was shifted toward higher molar masses (Mn=2050 g/moL, PDI=1.078) in comparison to the pure MeOPEG (Mn=1949 g/moL, PDI= 1.069) indicating the successful chlorination of MeOPEG macroinitiator (Figure 3).

Synthesis of MeOPEG-b-P4VP block copolymer

The synthesis of MeOPEG-b-P4VP block copolymer by ATRP was performed in 2-propanol at 60°C using MeOPEG-Cl macroinitiator in the presence of V4P and PMDETA/CuCl catalyst system according to scheme 2.



Scheme 2. Synthesis of MeOPEG-b-P4VP block copolymer by ATRP.

The ^{1}H NMR spectrum of the MeOPEG-b-P4VP block copolymer shows the peaks for MEOPEG's terminal (a, 3H, -CH_3) protons at 3.38 ppm and at 3.45-3.83 ppm (b, 4H, -CH_2-CH_2-O-) from the main PEG chain. The P4VP characteristic protons at 1.20–2.00 ppm (c, 3H), and its aromatic ring at 6.00–6.90 ppm (d, 2H) and 7.70–8.90 ppm (e, 2H) assigned to the ortho- and meta-protons respectively were observed as well (Figure 4). Figure 5 presents the SEC curve of the diblock copolymers PEG-b-P4VP block copolymer by using MeOPEG-Cl macroinitiator showed elution peak, which is clearly shifted toward higher molar mass (Mn=6470g/moL, PD=1.13) in comparison to the MeOPEG-Cl macroinitiator (Mn=2050g/moL, PDI=1.078) (Figure 5). The maximal degradation temperature for the both were around 400°C - 405°C, which indicated that the second P4VP block did not impaired the thermal stability of the MeOPEG-b-P4VP block copolymer obtained (Figure 6).

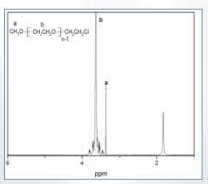


Figure 1. FT-IR spectra of MeOPEG and MeOPEG-Cl .

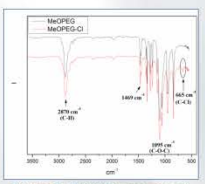


Figure 2. ^{1}H NMR spectrum of MeOPEG-Cl macroinitiator.

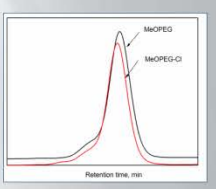


Figure 3. SEC analysis of MeOPEG and MeOPEG-Cl.

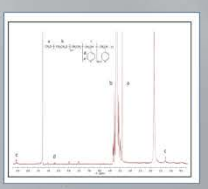


Figure 4. ^{1}H NMR spectrum of MeOPEG-b-P4VP block copolymers

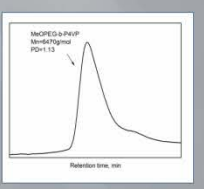


Figure 5. SEC analysis of MeOPEG-b-P4VP block copolymer.

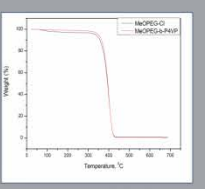


Figure 6. TGA analysis of MeOPEG-b-P4VP block copolymer.

Conclusion

MeOPEG-b-P4VP block copolymers were successfully prepared by atom transfer radical polymerization. For the first time, MeOPEG-Cl macroinitiator obtained by reaction of MeOPEG and thionyl chloride was applied for polymerization of 4VP by ATRP. The synthesis of MeOPEG-b-P4VP block copolymers was proven by ^{1}H NMR spectroscopy with the presence of all typical for the both block signals. SEC analysis also confirms the formation of block copolymers by increasing of MeOPEG-b-P4VP molar mass in comparison to the MeOPEG-Cl macroinitiator keeping low polydispersity index.

Постер №14

AMPHIPHILIC BLOCK COPOLYMER BEARING VARIOUS FUNCTIONS INTENDED FOR TARGETED DRUG DELIVERY

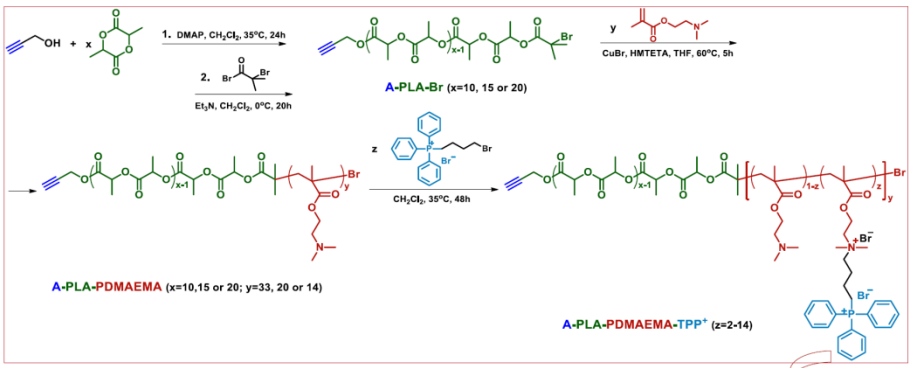
Dimitrina Babikova, Ivaylo Dimitrov

Institute of Polymers,Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

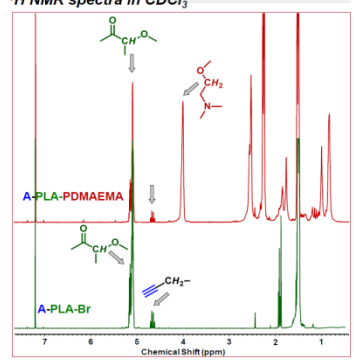
Polymeric nanoparticles play a central role in the systems for controlled drug delivery. The main goal of this work is to create polymer-based multifunctional nanocarriers loaded with active substances which are able to overcome various barriers and are capable of site specific targeting at both cellular and subcellular levels.

Heterobifunctional polyoxyethylene (PEO) with targeting folate (FA), acid-cleavable imine and terminal azide functions was synthesized *via* living anionic polymerization of ethylene oxide followed by polymer chain-end modifications. Separately, poly(D,L-lactide)-*b*-poly(*N*,*N*-dimethylaminoethyl methacrylate) (PLA-PDMAEMA) diblock copolymer bearing "clickable" alkyne end group was synthesized applying controlled ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). Finally, triphenylphosphonium (TPP⁺) subcellular targeting moieties were introduced into the PDMAEMA-block. The two polymers obtained will be coupled applying the highly efficient and selective 1,3-dipolar cycloaddition of azides and alkynes ("click" chemistry) to yield a multifunctional drug delivery nanocarrier for celullar and subcellular targeting.

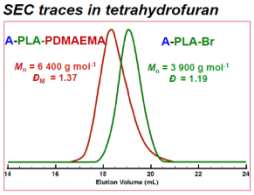
I Controlled synthesis of PLA-PDMAEMA block copolymers with TPP⁺ ligands and terminal alkyne groups



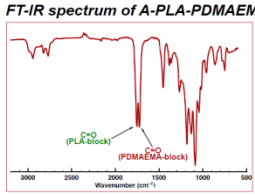
^{1}H NMR spectra in $CDCl_3$



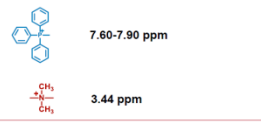
SEC traces in tetrahydrofuran



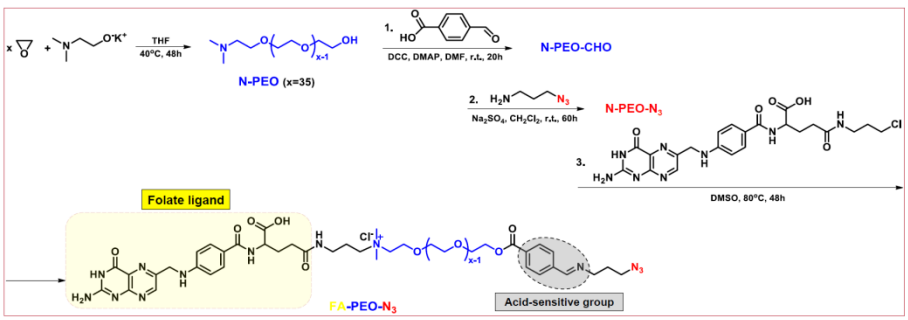
FT-IR spectrum of A-PLA-PDMAEMA



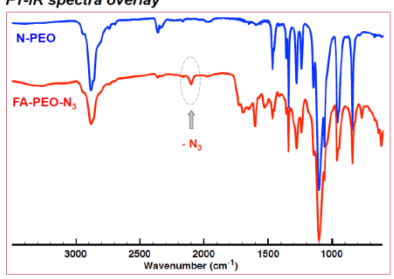
^{1}H NMR of A-PLA-PDMAEMA-TPP⁺ in $CDCl_3$:



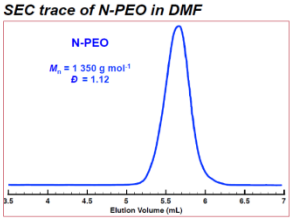
II Synthesis of "clickable" PEO with terminal folate ligand and biodegradable imine group (FA-PEO-N_3)



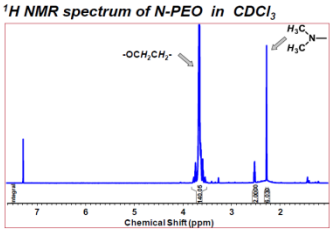
FT-IR spectra overlay



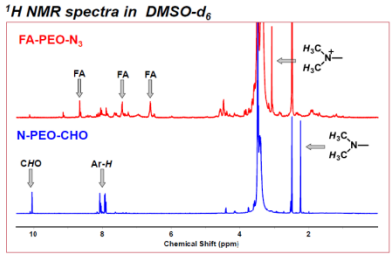
SEC trace of N-PEO in DMF



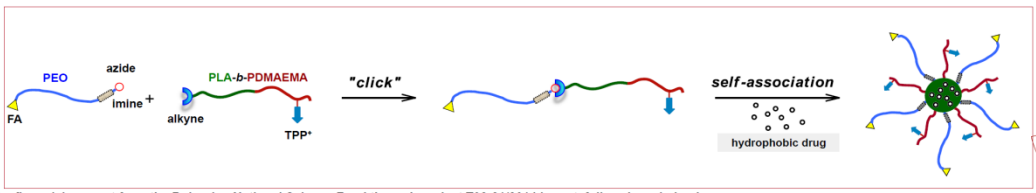
^{1}H NMR spectrum of N-PEO in $CDCl_3$



^{1}H NMR spectra in DMSO-d_6



OUTLOOK



The financial support from the Bulgarian National Science Fund through project T02-21/2014 is gratefully acknowledged.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №15

Stabilized micelles as a delivery system for cisplatin – cytotoxicity and cellular uptake of the loaded micelles

Ekaterina Stoyanova[a], Neli Koseva[a], Petar Petrov[a] and Irina Karadjova[b]

[a]*Institute of Polymers, Bulgarian Academy of Sciences, 103 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria;*
[b]*Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", 1 James Bourchier Blvd, 1164 Sofia, Bulgaria;*
**e-mail: estoyanova@polymer.bas.bg*

Cisplatin is the most widely used platinum-based antineoplastic agent implemented in the treatment of a wide range of cancerous diseases. Yet it is characterized by numerous disadvantages, including severe side toxic effects, short circulation period in the blood, intrinsic or acquired resistance of some tumors to the drug and limited solubility in water.

Drug conjugation to a macromolecular carrier represents a promising strategy for enhancing the efficiency in treatment and reducing the side effects of antineoplastic agents [1,2]. It has been demonstrated that long-circulating carriers can preferentially and effectively accumulate in solid tumors – a phenomenon, assigned as Enhanced Permeability and Retention (EPR) effect [3]. Polymeric micelles are one of the most intensively studied systems for delivery of hydrophobic drugs due to their ability to entrap the drugs in their core.

The aim of the present investigation is an evaluation of the applicability of stabilized polymeric micelles designed with a mixed shell (SPMMS) as carriers for cisplatin, addressing various aspects: drug payload, release profile, cytotoxicity and cellular uptake.

Synthesis and characterization of the SPMMS

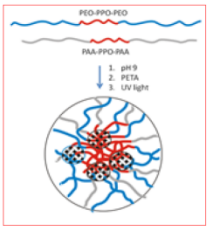


Figure 1. The route to SPMMS.

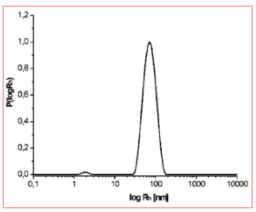


Figure 2. Distribution of the R_h for the SPMMS.

The synthesis of the SPMMS was carried out by mixing of two triblock copolymers: Pluronics 65 and $PAA_{18}PPO_{34}PAA_{18}$. The micelles were then additionally stabilized by loading them with pentaerytroil tetraacrylate which was *in situ* polymerized and cross-linked by irradiation with UV light [4]. A DLS-analysis of the SPMMS revealed a monomodal size distribution of the micelles.

Release of complexes of Pt (II) from the SPMMS

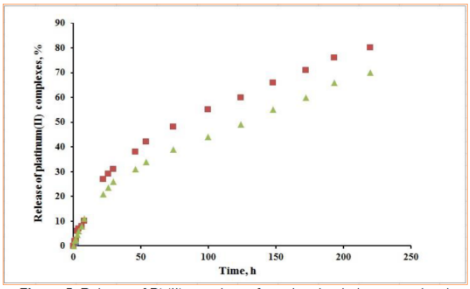


Figure 5. Release of Pt (II) complexes from drug loaded macromolecules at 37°C: (■) PBS; (▲) Citrate buffered solution.

The release of Pt (II) complexes from the micelles was investigated by dialysis in two distinct buffered media – phosphate buffered saline (PBS) and citrate buffered solution. In both of the solutions a high percentage of the loaded cisplatin was released. The release was in a sustain manner and no initial burst effect was observed.

Loading of the SPMMS with cisplatin

Cisplatin (2 mg/mL) was added to the aqueous solution of the particles at the following experimental conditions: pH 8, T = 22°C, molar ratio cisplatin to carboxylate groups 1:4 and duration of reaction 48h. Upon loading, a decrease of the size of the micelles due to the crosslinking of the polyacrylic chains with cisplatin was observed. The loaded particles were assigned as MPAA-Pt. A high drug loading efficiency of 76% was achieved. The amount of the immoblized cisplatin was determined to be 20% of the loaded particles.

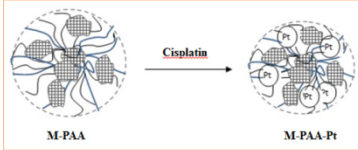


Figure 3. Schematic representation of cisplatin binding to the SPMMS.

180 nm 170 nm

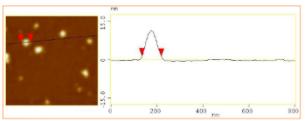


Figure 4. AFM image of the loaded SPMMS revealed spherical in shape particles with a diameter of 85 nm.

Cytotoxicity

In vitro cell viability study was carried out using two human tumor cell lines. Exponentially growing cells were exposed to varying concentrations of free cisplatin and its polymeric formulation MPAA-Pt. Cellular viability was assessed by MTT-dye reduction assay and the corresponding IC_{50} values were calculated.

Table 1. IC_{50} values for free cisplatin and its conjugates with SPMMS.

Проба	IC_{50}, μmol/L			
	MDA[a]		HT29[b]	
	72h	**120h**	**72h**	**120h**
cisPt	20.8	18.6	51.58	52.46
MPAA-Pt	136.6	96.8	69.66	71.81

[a] breast cancer cells
[b] colon cancer cells

Immobilization of cisplatin in the SPMMS results in an increase of the IC_{50} values which is consistent with the sustained manner of drug release. The SPMMS were not toxic at the concentrations used for the conjugates [5]. By virtue of the EPR-effect, the polymeric conjugates are expected to attain higher intra-tumoral levels of platinum *in vivo* as compared to the free cisplatin.

Cellular uptake

Exponentially growing tumor cells were exposed to cisplatin as free drug and as nano-conjugate MPAA-Pt. Free cisplatin is more rapidly and effectively internalized, yet the entrapped drug is effectively accumulated as well. After the 24-hour-long incubation, the levels of the intracellular platinum are even comparable to those attained after the treatment with the free drug. Taking into account the slow rate of drug release, it could be inferred that the accumulation of cisplatin can be ascribed to the uptake of both pre-released complexes and to drug conjugates, entered the cells via endocytosis.

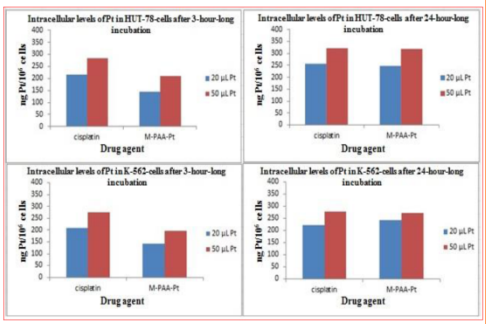


Figure 6. Intracellular levels of platinum after 3-hour-land 24-hour-long exposure of tumor cells to free and immobilized cisplatin (K-562 – chronic myeloid leukemia, HUT-78 – T-cell lymphoma).

Conclusions

A new core-shell type nanocarrier was designed as delivery vehicle of cisplatin. It possess key features which include: (1) hydrophilic shell with high density of carboxylate groups that are able to reversibly exchange ligands with cisplatin and to regenerate the agent at physiological salt concentrations, (2) a high drug payload and (3) sustained manner of release of the Pt (II) complexes. *In vitro* studies reveal: (1) higher IC_{50} values for the immobilized cisplatin which is consistent with the sustained manner of drug release and (2) comparable to the free drug cellular uptake for the immobilized cisplatin. On this ground it can be inferred that from pharmaceutical point of view, the SPMMS represent a promising system for delivery of cisplatin.

Analytical methods for Pt determination

The analytical methods used for determination of Pt in the present study were ICP-OES (inductively coupled plasma optical emission spectrometry) and ETAAS (electrothermal atomic absorption spectrometry). The ICP-OES-measurements were carried out on a ULTIMA 2, Jobin Yvon Spectrometer (Fig. 7). The technique was applied for assessment of the loading efficiency of M-PAA-Pt and for evaluation of cisplatin release profile from the polymer carrier. The ETAAS-measurements were performed on a Perkin-Elmer (Norwalk, CT, USA) Zeeman 3030 spectrometer (Fig. 8). The method was used for determination of the intercellular level of Pt.

Figure 7. ULTIMA Jobin Yvon spectrometer

Figure 8. Perkin-Elmer Zeeman spectrometer

References

[1] Perumal O., Khandare J., Kolhe P. *et al.*, Bioconjugate Chem. 20:5-842, 2009.
[2] Kolhe P., Khandare J., Pillai O. *et al.*, Pharm. Res. 21:12-2185, 2004.
[3] Matsumura Y., Maeda H., Cancer Res., 46:12 I-6387, 1986.
[4] P. Petrov, Ch. Tsvetanov, R. Jerome, *J. Phys. Chem. B 2009, 113, 7527-7533.*
[5] R. Donev, N. Koseva, P. Petrov, A. Kowalczuk, J. Thome, World J. Biol. Psychiatry 2011, 12 (SUPPL. 1), 44-51.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №16

Nanocomposite hydroxyethylcellulose/polyaniline cryogels of high electrical conductivity

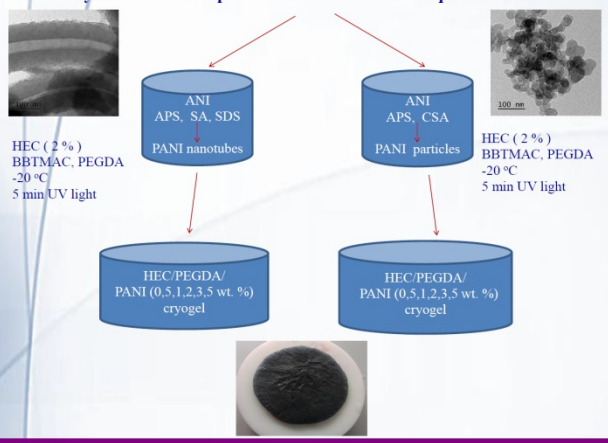
P. Mokreva[1], I. Kostov[1], V. Uzunova[2], S. Apostolova[2], R. Tzoneva[2], P. Petrov[1]

[1]*Institute of Polymers, Bulgarian Academy of Sciences, Akad. G. Bonchev st., bl. 103, Sofia, Bulgaria*

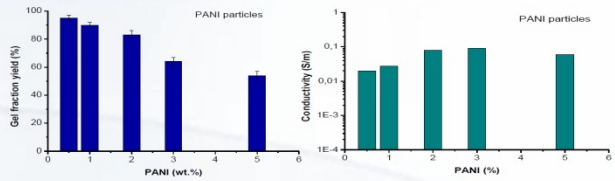
[2]*Institute of Biophysics and Biomedical Engineering, Akad. G. Bonchev st., bl. 21, Sofia, Bulgaria*

Cryogels have recently gained significant interest in the fields of tissue engineering and in vitro cell culture. Cryogels are able to provide the necessary architecture for the three-dimensional (3D) organization of cells and the open pore morphology for supply of nutrients and the removal of waste metabolites. On the other hand, scaffolds based on conducting polymers have been shown, via electrical stimulation, to modulate cellular activities, including cell adhesion, migration, DNA synthesis, and protein secretion.
The present contribution reports on the fabrication of conducting hybrid cryogels via photo-crosslinking of moderately frozen systems.

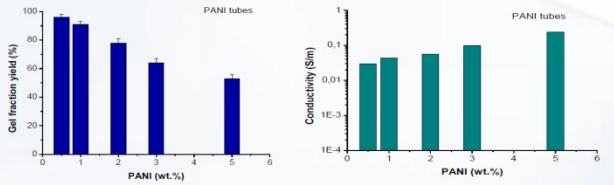
Synthesis of PANI particles and nanotubes in aqueous media



Conducting HEC/PANI (particles) cryogels



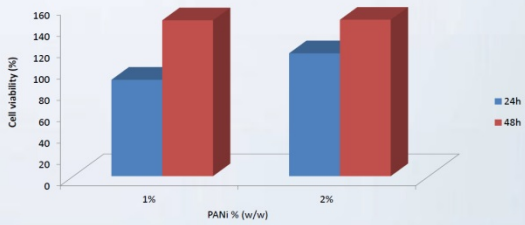
Conducting HEC/PANI (tubes) cryogels



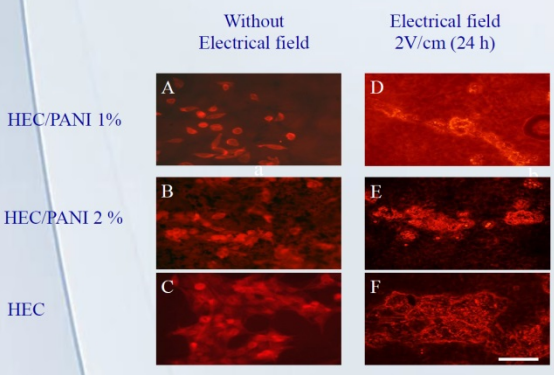
First, polyaniline (PANI) submicron particles and nanotubes were synthesized via oxidative polymerization of aniline in aqueous media. Then, PANI were incorporation into biodegradable 2-hydroxyethylcellulose (HEC) cryogel via cryogenic treatment and photo-crosslinking of HEC in frozen aqueous system

The increase of PANI concentration decreased the gel fraction yield and increased the electrical conductivity of freeze dried cryogels.

MTT test for cell viability



L929 cell proliferation on NEC/PANI cryogels presented in percentage of the control



L929 cells are stained for actin. Bar is 50 mm.

The preliminary experiments with L929 cells confirmed well adhesion and spreading of the cells onto HEC/PANI cryogel matrix. After application of electrical field (2 V/cm for 24 h) the cells were arranged into structures parallel to the electrical field.

CONCLUSIONS

1. ***Novel HEC/PANI cryogels were synthesized via photochemical cross-linking***
2. ***PANI particles/tubes (1-5 wt.%) impart high electrical conductivity of HEC cryogels***
3. ***L929 cells adhere and proliferate very well on HEC/PANI cryogels***
4. ***After the application of EF the cells embedded in HEC/PANI cryogels were arranged in structures parallel to the electrical field***

AKNOWLEDGEMENTS:

FP7 project POLINNOVA – financial support

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №17

Рециклиране на фармацевтични отпадъци

Пластмасовите опаковки – много са удобни за употреба, защото са доста устойчиви – на високи и ниски температури, на удар, на разпадане, разкъсване, разлагане и т. н. Всеизвестно е, че за да се разложат по естествен път полиетиленови отпадъци са необходими млн. години, а затрупването с полиетилен или изгарянето му е вредно за околната среда.

Ето защо се налага да се създадат методи за оползотворяване на полимерните отпадъци.

Методът, който ще представя е получаване на вторични полимери и вторичен алуминий от полимерно-композитни отпадъци. С цел да се опише рециклирането на повече отпадъчни полимерни съединения като обект на изследване ще използвам композитни опаковки – блистерите за хапчета – те са направени от алуминий и полиетилен, полипропилен или поливинилхлорид.

Разработена е нова технологична схема за преработване на химични отпадъци за фармацевтичната индустрия. Композитните материали главно се състоят от два или три слоя: полимер / Al или полимер / Al/полимер, като полимера е Полиетилен - PE, Полипропилен – PP и ПВЦ - PVC. Разработената технология е съчетание на химически процеси и механична обработка на суровината – отпадни композитни опаковки. Получените от разграждането продукти чрез допълнителна обработка – механична и химична, се подготвят за пазара. Предимствата на реализирането на тази идея са:

1.Екологически – оползотворяване на композитните отпадъци и пълна безотпадност на процесите.

2.Спестяване на енергийни ресурси за получаване на същите продукти от чисти суровини, висока енергийна ефективност при обработката на композитните опаковки и като бъдеще - пълно оползотворяване на отделената температура при химичните процеси.

Обработката на различните композитни материали по вид се извършва по отделно – 2 или 3 слойни опаковки: Полиетилен – Al, Полипропилен – Al, Поливинилхлорид – Al. Създават се предпоставки за съвместяване на технологичните потоци, което води до рязко снижаване на енергийните разходи. Осъществява се пълно извличане на основните суровини и се осъществява безотпадност на процесите. Поради това, че работните химични разтвори са на алкална основа и чрез подходяща концентрация, температура и времеви престой в активната зона се постига пълното извличане на алуминиевия слой. Посредством добавяне на вода /от промивните вани/ се получава директно алуминиев хидроксид, като основата, с която се въздейства химически, се регенерира по данни почти до 90 %. Останалите полимери се дообработват до краен продукт – гранулат с въвеждането на съоръжения за механично раздробяване и това ще доведе до превръщането им в търговски продукти с високо качество и оптимална цена за пазара. Алуминиевият хидроксид, може да се получава в 2 разновидности - в кристална и аморфна форма. Кристалната – пряко, а аморфната чрез неутрализация. Чрез комбинирането на 2-та вида или поотделно се произвеждат безотпадно коагуланти за пречистване на води, алуминиев сулфат и алуминиев оксихлорид. Те се явяват също крайни продукти с гарантирана реализация на пазара и висока химична чистота поради чистотата на оползотворения алуминий. Идеята на технологичната схема позволява автоматизиране, което води до оптимизиране, повторяемост и проследимост на процесите, с възможност за активен контрол и висока степен на безопасност. Подходящото предварително механично обработване на опаковките също допринася за оптимизиране на технологията.

Ще опиша приложението на метода за 6 вида блистери, със състав от:

Двуслойни : Поливинилхлорид → $Al_n(-CH_2-CHCl-)_{3n}$; Полиетилен → $Al_n(-CH_2-CH_2-)_{3n}$; Полипропилен → $Al_n(-CH_2-CH(CH_3)_{3n}$;

Трислойни: Поливинилхлорид → $Al_{2n}(-CH_2-CHCl-)_{6n}$; Полиетилен → $Al_{2n}(-CH_2-CH_2-)_{6n}$; Полипропилен → $Al_{2n}(-CH_2-CH(CH_3)_{6n}$

Етапите, през които се минава за да бъдат оползотворени блистерите са следните:

1) Разделяне на слоеве

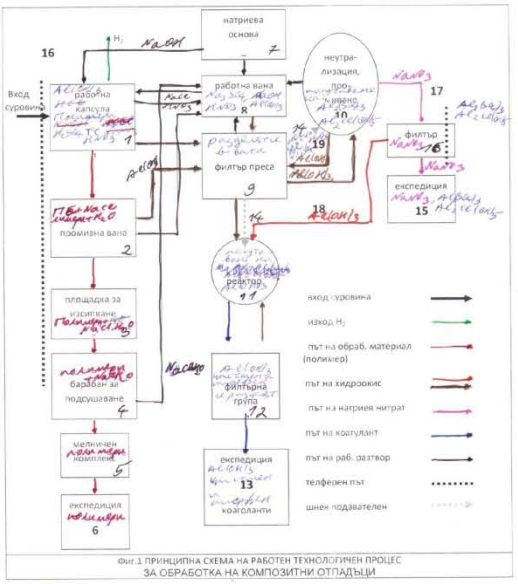
2) Синтез на отработени вторични химични съединения

Разделяне на слоеве

Използва се метода на химическото определяне – чрез осигуряване на условия и набавяне на необходимите съдове и уреди за протичане на определени химични реакции, от които да се получат отделни продукти – съставките на полимерите и други химични съединения, необходими за по-нататъшната обработка.

Необходимо е да протекат следните химични реакции:

Технологична схема



Фиг.1 ПРИНЦИПНА СХЕМА НА РАБОТЕН ТЕХНОЛОГИЧЕН ПРОЦЕС ЗА ОБРАБОТКА НА КОМПОЗИТНИ ОТПАДЪЦИ

Двуслойни полимери

Поливинилхлорид → $Al_n(-CH_2-CHCl-)_{3n}$:

$Al_n(-CH_2-CHCl-)_{3n} + 3nNaCl \rightarrow 3Na_n(-CH_2-CHCl-)_n + AlCl_3$

$2Na_n(-CH_2-CHCl-)_n + 2nHOH \rightarrow 2nNaOH + 2(-CH_2-CHCl-)_n + H_2\uparrow$

$nAlCl_3 + 3nHOH \rightarrow nAl(OH)_3 + 3nHCl$

За полиетилен → $Al_n(-CH_2-CH_2-)_{3n}$ *и полипропилен →* $Al_n(-CH_2-CH(CH_3)_{3n}$ *реакциите са подобни.*

Трислойни полимери

Поливинилхлорид → $Al_{2n}(-CH_2-CHCl-)_{6n}$

$Al_{2n}(-CH_2-CHCl-)_{6n} + 6nNaCl \rightarrow 6Na_n(-CH_2-CHCl-)_{2n} + 2nAlCl_3$

$2Na_n(-CH_2-CHCl-)_{2n} + 2nHOH \rightarrow 2nNaOH + 2(-CH_2-CHCl-)_{2n} + H_2\uparrow$

$nAlCl_3 + 3nHOH \rightarrow nAl(OH)_3 + 3nHCl$

За полиетилен → $Al_{2n}(-CH_2-CH_2-)_{6n}$ *и полипропилен →* $Al_{2n}(-CH_2-CH(CH_3)_{6n}$ *реакциите са подобни.*

Забележителен е методът за получаване на вторичен полиетилен, открит от Даниел Бърд. Ето какво прави той: обогатителна смес от 3г полиетилен (ПЕ) и 3г натриев хлорид ги надробява и изсипва в работната капсула. Тази смес я прелива в промивна вана, където има 1л дестилирана вода. Промива се сместа и след това отделеният пласт полиетиленови частици го изсипва във филтър (филтър преса), промива го 3 пъти, после отива в работната вана, от нея пак в капсулата, пак в промивната вана, където добавя обогатителната смес и растяща среда (пръст от бунище, съдържаща – 0,1%$(NH_4)_2SO_4$; 0,1%$NaNO_3$; 0,1%K_2HPO_4; 0,1%KCl; 0,02%$MgSO_4$ в 1 л. H_2O) 0,01% екстракт от мая в епруветка на 100 мл обогатителна смес.

Съставът на химичната мая е: 25%$NaHCO_3$ – натриев хидрогенкарбонат

60%COOH-CHOH-CHOH-COOK – кисел хидрогенотартарат

Взима проби от почвата във Ватерлоо, Онтарио, за да изолира ПЕ от микроорганизми. 1г почвена проба добавя към обогатителната смес и слага двете в инкубатор (барабан за подсушаване) при 30°C за 4 седмици и после минават през вибрационно сито (мелничен комплекс). Същата процедура се прави и 3ти път. Крайнатаобогатителна култура се филтрува през филтърна хартия, за да се отдели изцяло полиетиленовия прах.

Синтез на отработени вторични химични съединения

Химични реакции:

$Al+3NaOH \rightarrow Na_3[Al(OH)_6]+H_2$

$Al+3NaOH \rightarrow NaAlO_2+H_2O$

$Al_n(PE)3n + 3n(NH_4)_2SO_4 \rightarrow 6(NH_4)_n(PE)_n + Al_2(SO_4)_3$

$3_nNa_2SO_4 + 2_nAl(OH)_3 \leftrightarrow 6_nNaOH + nAl_2(SO_4)_3$

$Al\ PE/(NH_4)SO_4 = 2/3$

$(NH_4)SO_4 = (3.1.10^8)/2 = 1\ 500\ 000$

И за другите се получава:

$NaNO_3 + HCl \rightarrow NaCl + HNO_3$

$NaCl + HNO_3 \rightarrow NaNO_3 + HCl$

$Al_npolimer_{3n} + 3_nNaOH \rightarrow 3_n\ Na_nPE_n + Al(OH)_3$

Работна капсула

$Al_n(-CH_2-CH_2-)_{3n} + 3nNaCl \rightarrow 3Na_n(-CH_2-CH_2-)_n + AlCl_3$

$2Na_n(-CH_2-CH_2-)_n + 2nHOH \rightarrow 2nNaOH + 2(-CH_2-CH_2-)_n + H_2\uparrow$

$nAlCl_3 + 3nHOH \rightarrow nAl(OH)_3 + 3nHCl$

$NaNO_3 + HCl \rightarrow NaCl + HNO_3$

$6_nNaOH + nAl_2(SO_4)_3 \leftrightarrow 3_nNa_2SO_4 + 2_nAl(OH)_3$

NaCl и HNO_3 *се прехвърлят в промивната вана. В нея протичат реакциите:*

$Al+3NaOH \rightarrow Na_3[Al(OH)_6]+H_2$ *и* $Al+3NaOH \rightarrow NaAlO_2+H_2O$

От промивната вана се прехвърлят в площадката за изсушаване, след това в барабана за подсушаване, оттам в работната вана,от нея в филтър пресата и оттам към неутрализация. Реакциите на неутрализация са:

$3nNa_2SO_4 + 2nAl(OH)_3 \leftrightarrow 6_nNaOH + nAl_2(SO_4)_3$

$5HOH + 2Al + HCl \rightarrow Al_2Cl(OH)_5 + 3H_2$ *и* $NaCl + HNO_3 \rightarrow NaNO_3 + HCl$

По телферен път тези съединения стигат до филтъра, филтруват се и се получават отработени вторични съединения.

Алуминиевият хидроксид от неутрализацията се прехвърля във филтър пресата, оттам в реактора, оттам във филтърната група, където той е разделен на кристална и аморфна форма и така се получават вторични коагуланти на $Al(OH)_3$ *– кристална и аморфна форма.*

Като резултат от обогатителна процедура се получава микробен консорциум с възможности за разграждане на ПЕ.

"Найлоновите" торбички и блистерите за лекарства обикновено се заравят в депа или се хвърлят в океаните и околните екосистеми.

Процесът на разграждане полиетилен разработен в този проект може да се използва в промишлен мащаб за биоразграждане на найлонови торбички и блистери за лекарства. Като резултат, това ще спаси живота на милиони видове диви животни и ще спести място в депата.

Да опазим природата чиста! ☺

ИЗГРАЖДАНЕ НА ПОЗИТИВНО ОТНОШЕНИЕ КЪМ ПРИРОДАТА ЧРЕЗ ЕКОЛОГИЧНО ВЪЗПИТАНИЕ

Катерина Колева

Постер №18

Hydroxyl-functionalized gel polymers with potential biomedical applications: synthesis and characterization

M. M. Glavcheva*, D. Ch. Christova, S. D. Ivanova

Laboratory of Amphiphilic and Ionogenic Polymers, Institute of Polymers - Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 103-A, 1113 Sofia, Bulgaria

Introduction

Non-ionic polyacrylamides represent an diverse class of water-soluble and water-swellable polymers comprising some of the most important synthetic materials used to improve the quality of life in our modern society. Hydrophilicity, good water solubility, lack of toxicity and biocompatibility are well-known properties of poly(N-hydroxymethylacrylamide) (PHMAA) and Poly(N,N-dimethylacrylamide) (PDMAA). These properties are mostly valued for applications in biomedical fields such as pharmaceuticals, contact lenses, scaffolds for tissue engineering, but also in cosmetics, adhesive and coating agents, etc. On the basis of above mentioned properties, polymer architectures with tailored features based on PHMA and PDMA can provide advanced materials of promising scientific and commercial value and prospects for application in frontiers of biotechnology and medicine.

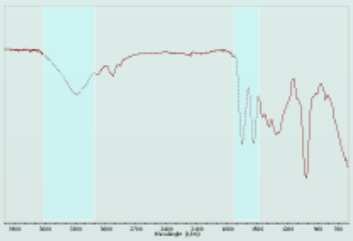
Objectives

This study aimed at one-pot synthesis of hydroxyl-functionalized gel particles based on redox polymerization of N-hydroxymethyl-acrylamide (HMA) and N,N-dimethylacrylamide (DMAA) in aqueous media under moderate reaction conditions. In order to obtain macro- and micro-gel particles of different size and surface properties (respectively OH-content) reaction conditions as well as HMAA-to-DMAA ratio in the initial reaction mixture are varied.

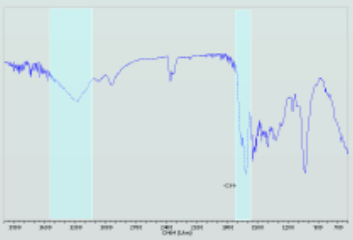
Results and discussion

FTIR spectra

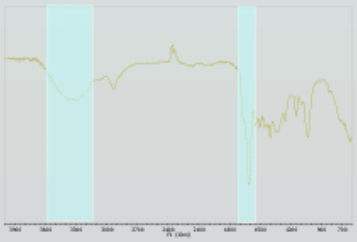
CH-62:



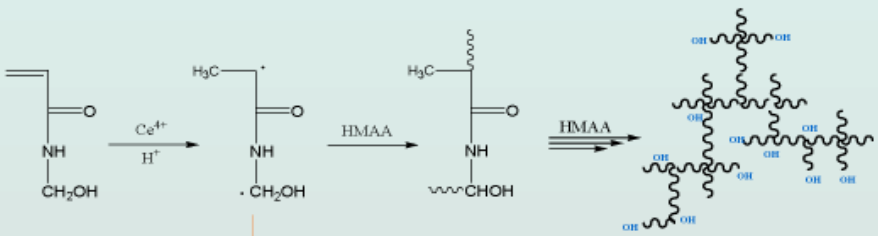
CH-64:

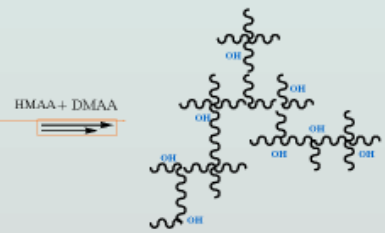


CH-65:

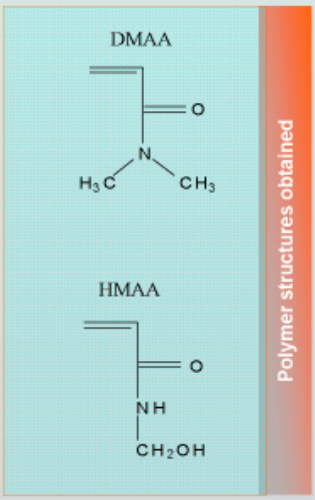


Reaction scheme of Ce-ion mediated polymerisation





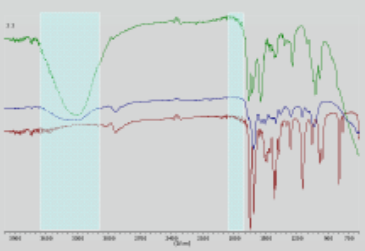
Polymer structures obtained



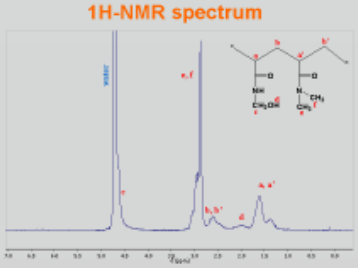
Gel code	Composition by feed (mol.%)		Equilibrium swelling degree (%)
	HMAA	DMAA	
CH-62	100	0	204
CH-64	70	30	73
CH-65	50	50	127
CH-66	50	50	--

CH-66

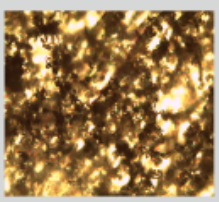
CH-66:

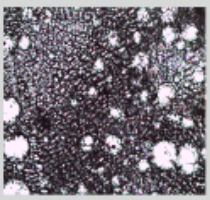


1H-NMR spectrum



Polarizing Optical Microscopy Images





Conclusions

The achieved high content of hydroxyl groups in the obtained gel particles is considered valuable for further particle surface modification, labeling and loading of bioactive substances.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №19

Микроскопски техники за визуализиране на полимерни наночастици в култивирани клетки

Душица Яневска, Деница Мелнишка, Кирилка Младенова, Магдалена Пенчева,
Веселина Москова-Думанова, Йордан Думанов, Таня Топузова- Христова

Биологически факултет, Софийски университет „Св. Климент Охридски“

Фазово-контрастна микроскопия

Основи на метода

Първият микроскоп, който дава добър контраст и подробна картина при нефиксирани и неоцветени обекти. Прави видими разликите на границата на две среди с различен коефициент на пречупване на светлината.

Предимства: Възможност за наблюдение на живи, неоцветени клетки (например във фазите на клетъчния цикъл).

Недостатъци: Намалена разделителна способност

Фиг.1 А Контролни клетки А549

Фиг.1 Б А549 с наночстици

Приложение: Този метод е удобен за проверка на състоянието на клетките преди количествени измервания. Може да се проследи и движението на наночастичките в клетката.

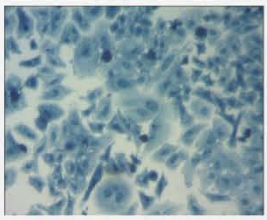
Класическа светлинна микроскопия

Основи на метода

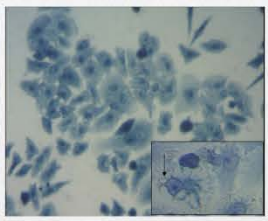
Използват се фиксирани клетки, които могат да се оцветят с различни багрила и се наблюдава общата морфология на клетките.

Предимства: По-добър контраст, възможност за диференциално оцветяване на различни клетъчни компоненти (ядро, митохондрии, цитоплазма).

Недостатъци: По-продължителна обработка на препаратите с различни органични разтворители. Не дава възможност за ясно разграничаване на наночастиците в клетките



Фиг. 2А Контролни клетки А549 оцветени с метиленово синьо



Фиг. 2Б Клетки А549 наночастици, оцветени с метиленово синьо

Приложение: С този метод се оценяват морфологични промени, промени в митотичната активност на клетките

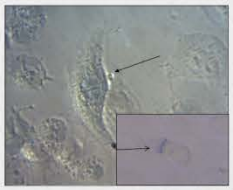
Метод на изключване на трипаново синьо

Използва се за селективно оцветяване на мъртви тъкани и клетки. Наблюдава се жизнеспособността на клетките и пермеабилизацията на мембраната

Предимства: Дава ясна видимост на увреждания на клетъчната мембрана в клетката.

Недостатъци: Не преминава през интактната мембрана. Живите клетки остават неоцветени.

Фиг.3А Контролни клетки А549
Гладки клетки с ясно видими граници



Фиг.3Б А549 клетки оцветени с трипаново синьо. Ядро(синьо) и наночастици

Приложение: С този метод се прави оценка за интегритета на мембраната на клетките

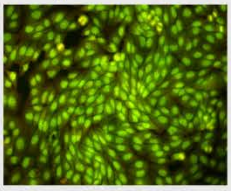
Флуоресцентна микроскопия

Суправитално оцветяване с акридин оранж

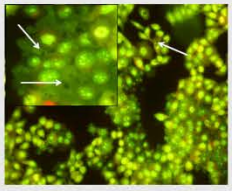
Селективна боя. Преминава и през неувредени клетъчни мембрани. Свързва се с ДНК чрез интеркалация, а с РНК чрез елекростатични взаимодействия. Свързана с ДНК излъчва емисия с максимум 530нм (зелено), а свързаната с РНК – с максимум 650нм (червено). Абсорбционните максимуми са, съответно, 502нм (за ДНК) и 460нм (за РНК). Когато се работи с нефиксирани клетки специфично оцветява лизозомите (червено), ДНК и цитоплазмата (зелено) и РНК (жълто).

Предимства: Голям контраст на изображението. Възможност за диференциално оцветяване с една стъпка.

Недостатъци: Работи се с времени препарати и не могат да се съхраняват и използват повторно.



Фиг.4А Контролни клетки (MDCK).



Фиг.4Б HepG2 клетки оцветени с акридин оранж. Лизозоми (червено), ядро(зелено)

Приложение: Този метод е удобен за визуализиране на пътя на навлизане на наночастиците

Благодарности: Работата е с финансовата подкрепа на договор ДФНИ-Т02/7 от 12.12.2014, МОН.

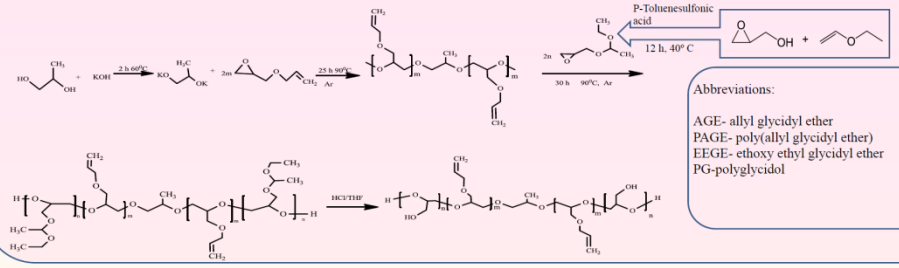
Постер №21

Studies on self-association behavior of amphiphilic triblock polyglycidol-poly(allyl glycidyl ether)-polyglycidol copolymers in aqueous solution by light scattering

Boyana Stoyanova , Christo Novakov , Stanislav Rangelov, Christo Tsvetanov
Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. 103-A, 1113-Sofia, Bulgaria
E-mail address: bstoyanova@polymer.bas.bg

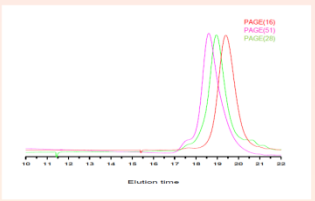
AIM: The aim of this work is to examine novel amphiphilic **polyglycidol-poly(allyl glycidyl ether)-polyglycidol** (PG-PAGE-PG) triblock copolymers with regard to their ability to self-associate in aqueous solution. The critical micellization concentrations (CMCs) were determined by dye-solubilization and spectroscopic methods, whereas the thermodynamic parameters were extracted from the temperature dependence of CMC. The static and dynamic light scattering parameters of the resulting particles such as molar mass, aggregation number, second virial coefficient, hydrodynamic and root mean square radii were determined using combined GPC/MALLS measurements.

Synthetic pathway for preparation of PG-PAGE-PG triblock copolymers



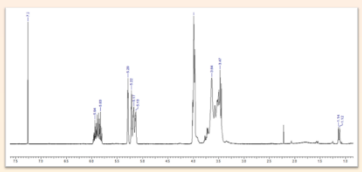
Abbreviations:

AGE- allyl glycidyl ether
PAGE- poly(allyl glycidyl ether)
EEGE- ethoxy ethyl glycidyl ether
PG-polyglycidol



GPC traces of PAGE in THF

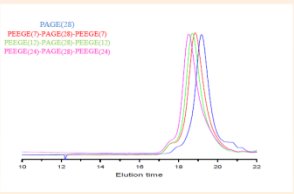
Characterization of PAGE/PG



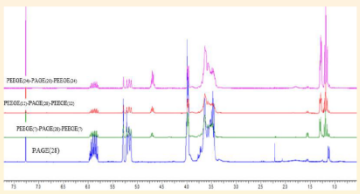
^{1}H NMR spectrum of PAGE (16) in $CDCl_3$

PAGE/PG	M_n (NMR)	M_n (GPC)	PDI
PG(3)-PAGE(16)-PG(3)	2700	2800	1.12
PG(7)-PAGE(16)-PG(7)	4200	4500	1.13
PG(13)-PAGE(16)-PG(13)	5700	5800	1.15
PG(7)-PAGE(28)-PG(7)	5300	5400	1.14
PG(12)-PAGE(28)-PG(12)	6900	7000	1.15
PG(24)-PAGE(28)-PG(24)	10200	10500	1.17
PG(13)-PAGE(51)-PG(13)	9800	10000	1.15
PG(23)-PAGE(51)-PG(23)	12600	13000	1.11
PG(28)-PAGE(51)-PG(28)	14100	14100	1.12
PG(33)-PAGE(51)-PG(33)	15500	15600	1.15

NAME	M_n (NMR)	M_n (GPC)	PDI
$PAGE_{(16)}$	1800	1900	1.12
$PAGE_{(51)}$	5800	6000	1.13
$PAGE_{(28)}$	3200	3400	1.10

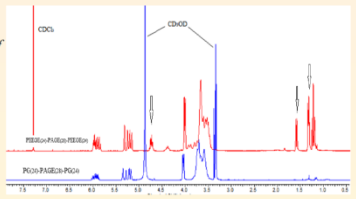


GPC traces of PAGE (28) and copolymers in THF



^{1}H NMR spectra of PAGE and copolymers in $CDCl_3$

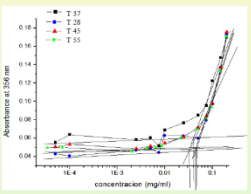
Cleavage of protective ethoxyethyl groups



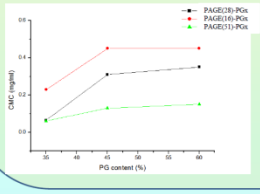
^{1}H NMR spectra of PG-PAGE-PG (cleavage of protective ethoxyethyl groups) in CD_3OD

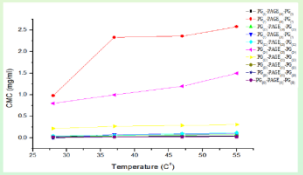
Self-association of the copolymers in aqueous solution

Critical micellization concentrations dye-solubilization (1,6-diphenyl-1,3,5-hexatriene)



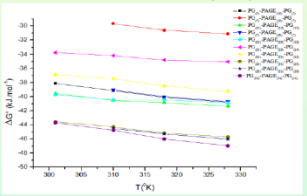
PAGE/PG	CMC (mg/ml)			
	28 C°	37 C°	47 C°	55 C°
PG(3)-PAGE(16)-PG(3)	0.038	0.040	0.045	0.050
PG(7)-PAGE(16)-PG(7)	0.980	2.330	2.360	2.580
PG(13)-PAGE(16)-PG(13)	0.045	0.050	0.070	0.085
PG(7)-PAGE(28)-PG(7)	0.003	0.080	0.090	0.100
PG(12)-PAGE(28)-PG(12)	0.050	0.060	0.098	0.120
PG(24)-PAGE(28)-PG(24)	0.80	1.0	1.200	1.500
PG(13)-PAGE(51)-PG(13)	0.220	0.270	0.290	0.310
PG(23)-PAGE(51)-PG(23)	0.020	0.025	0.031	0.038
PG(28)-PAGE(51)-PG(28)	0.002	0.025	0.032	0.037
PG(33)-PAGE(51)-PG(33)	0.023	0.025	0.027	0.029





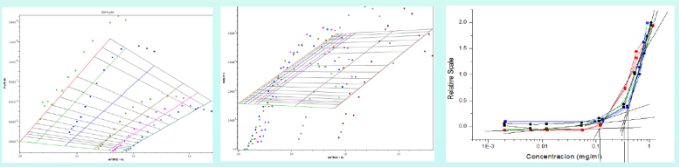
Thermodynamics of self-association

$$\Delta G° = RT\ln(Xcmc) \qquad \Delta G° = \Delta H° - T\Delta S°$$



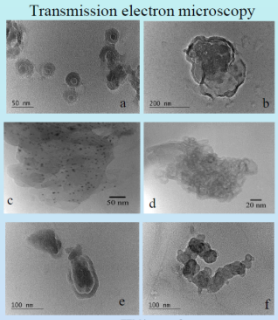
PAGE/PG	ΔH° (KJ mol^{-1})	ΔS° (KJ $mol^{-1}.K^{-1}$)
PG(3)-PAGE(16)-PG(3)	-0.52	0.09
PG(7)-PAGE(16)-PG(7)	-5.26	0.079
PG(13)-PAGE(16)-PG(13)	-20.701	0.06
PG(7)-PAGE(28)-PG(7)	-10.473	0.0921
PG(12)-PAGE(28)-PG(12)	-27.16	0.042
PG(24)-PAGE(28)-PG(24)	-18.64	0.05
PG(13)-PAGE(51)-PG(13)	-9.378	0.091
PG(23)-PAGE(51)-PG(23)	-19.3	0.08
PG(28)-PAGE(51)-PG(28)	-18.475	0.08
PG(33)-PAGE(51)-PG(33)	-5.424	0.127

MALLS measurements



PAGE/PG	CMC mg/ml	R_h nm	R_g nm	A_2 mol ml/g^2	M_w^{app} g/mol	N_{agg}	R_g/R_h
PG(3)-PAGE(16)-PG(3)	0.23	86	55.5	4.110 e-5	1.890 e+7	6750	0.61
PG(7)-PAGE(16)-PG(7)	0.42	45	75.6	1.637 e-7	7.250 e+6	1611	1.66
PG(13)-PAGE(16)-PG(13)	0.45	103	38.3	1.065 e-4	8.566 e+5	147	0.38
PG(7)-PAGE(28)-PG(7)	0.13	140	60.6	2.228 e-5	1.430 e+7	2648	0.42
PG(12)-PAGE(28)-PG(12)	0.31	110	121.7	1.612 e-4	2.480 e+6	354	1.09
PG(24)-PAGE(28)-PG(24)	0.35	172	115.4	1.160 e-3	7.730 e+4	7	0.67
PG(13)-PAGE(51)-PG(13)	0.10	219	53.3		4.573 e+6	824	0.24
PG(23)-PAGE(51)-PG(23)	0.06	47	123.3	3.152 e-5	5.404 e+7	4156	2.61
PG(28)-PAGE(51)-PG(28)	0.13	64	57.8	3.469 e-5	1.247 e+7	2287	0.89
PG(33)-PAGE(51)-PG(33)	0.15	60		1.346 e-5	7.848 e+6	503	

Transmission electron microscopy



TEM images of PG(3)-PAGE(16)-PG(3) (a), PG(7)-PAGE(16)-PG(7) (b), PG(7)-PAGE(28)-PG(7) (c), PG(12)-PAGE(28)-PG(12) (d), PG(13)-PAGE(51)-PG(13) (e), PG(33)-PAGE(51)-PG(33) (f), in aqueous solution.

DLS data for PG-PAGE-PG copolymers in aqueous solution

copolymers	Concentracion (mg/ml)	Particle size, d(nm)
PG(7)-PAGE(28)-PG(7)	0.10	307
	0.05	170
PG(12)-PAGE(28)-PG(12)	1.00	257
	0.10	124
PG(24)-PAGE(28)-PG(24)	5.00	190
	2.50	98
PG(3)-PAGE(16)-PG(3)	0.50	280
	0.10	70
PG(7)-PAGE(16)-PG(7)	2.00	260
	0.10	35
PG(13)-PAGE(51)-PG(13)	0.50	250
	0.10	150
PG(33)-PAGE(51)-PG(33)	0.10	120

Conclusions

1. Three series of amphiphilic PG-PAGE-PG block copolymers were prepared by ring-opening anionic polymerization followed by cleavage of the protective ethoxyethyl groups. The block copolymers differed in molecular weight and PG content. Both the polymerizations and post-polymerization reactions were found to proceed in a controllable and predictable way.
2. In aqueous solution the copolymers were found to self-associate. The self-association was an enthalpically favored process with a small positive entropy contribution. Self-assembled structures were of 50-250 nm in size and aggregation numbers of about hundreds of macromolecules per particle depending on the total copolymer concentration and PG content.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №22

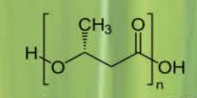
Novel method for exfoliation of sodium montmorillonite in PHB matrix via Supercritical CO_2

Filip Ublekov[1*], Cristina Gutiérrez[2], Hristo Penchev[1], Juan F. Rodriguez[2]

[1] Institute of polymers – Bulgarian Academy of Sciences, Acad. G. Bonchev St. Block 103A, 1113 Sofia, Bulgaria
[2] Instituto de Tecnología Química y Medioambiental, Universidad de Castilla-La Mancha Avda. Camilo José Cela s/n. 13004 Ciudad Real, Spain
Correspondence authors: ublekov.philip@gmail.com

Introduction

Biobased plastics and composites have gained significant interest during the last years. Compared to petroleum-based synthetic polymers, biobased polymers from renewable resources, such as plants and crops, can be naturally recycled by enviromental processes.



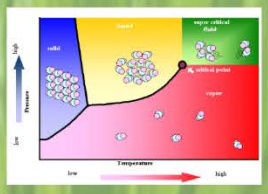
poly (β-hydroxybutyrate) (PHB)

Characteristics
- Highly crystalline
- Biodegradable
- Biocompatible
- Brittle

Limitations
- High cost
- Low impact strength/toughness
- Thermolabile

ALTERNATIVE

Incorporation of dispersed/exfoliated Montmorillonite

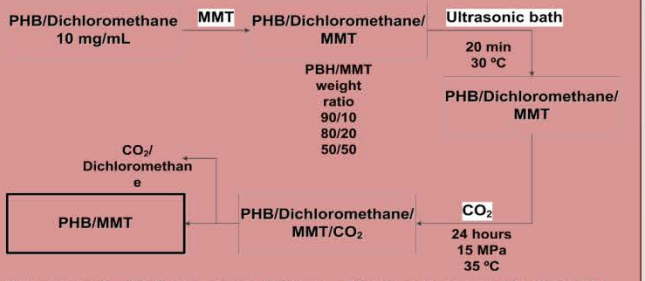


Supercritical fluids (SCFs) have received a considerable attention since they are currently being used as environmentally friendly solvents. $scCO_2$ is proposed for the dispersion of montmorillonite's stacks into individual layers The use of SCFs allows the production of masterbatches with high inorganic content (up to 50 wt. % inorganics) with no viscosity restrictions.

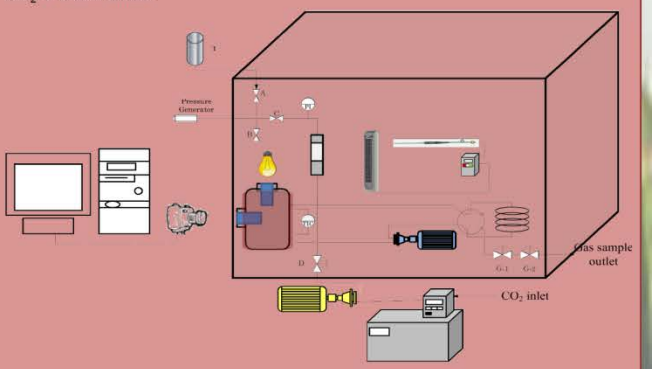
We present a novel method, which exploits the physicochemical properties of supercritical carbon dioxide ($scCO_2$) for dispersing high loadings of natural montmorillonite's stacks in the PHB matrix.

Materials and Methods

PHB Biomer® P350 (Mw=180,000 g mol-1, polydispersity index PDI=1.5) was kindly supplied from Biomer, Krailling, Germany. Molar masses were determined by size exclusion chromatography (polystyrene standards).
The montmorilonite (MMT) used for preparation of nanocomposites was Cloisite® Na+, purchased from Southern Clay Products Inc, USA. Prior to use, Cloisite® Na+ clay and PHB were dried under vacuum at 40°C for 24 hours.
The experimental methodology for the dispersion of montmorillonite in PHB Biomer is schematized below.



Nanocomposite thin films (average thickness - 25 μm) were prepared by drying the suspension on a glass dish for 24 h at 25°C.
The high pressure setup for the homogenization of MMT in PHB using supercritical CO_2 is shown below.



The experimental setup consists on a high-pressure vessel supplied with a front and upper sapphire window and light for visual observation.
The XRD patterns were obtained using powder diffractometer HZG/4A (Freiberger Praezisionsmechanik GmbH, Freiberg). The Cu Kα radiation (λ = 0.154 nm) was used. The scanning range was 2Θ = 1.4°–12° with step 0.1°.

Results and Discussion

Figure 1 shows XRD patterns of pure MMT and PHB/MMT nanocomposites.

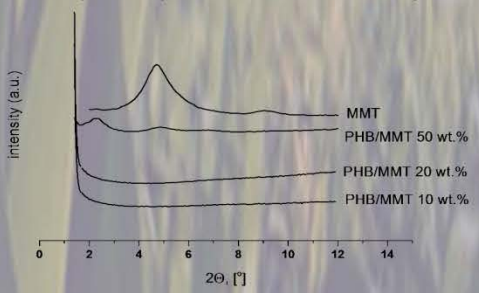


Fig. 1 *XRD patterns of MMT and PHB/MMT nanocomposites with different weight ratio.*

It can be clearly seen, that the main diffraction peak (d_{001}) of MMT, characteristic for silicate layers periodicity, is at 2θ = 4.7°. The diffractograms of the samples with 10 and 20 wt. % MMT shows no diffraction peaks of MMT, which confirms exfoliated nanocomposite structure. The structure of the sample with 50 wt. % is demonstrated by the enlargement degree of d_{001}. The shift of the main diffraction peak of MMT from 4.7° to 2.3° indicates intercalation of PHB chains between silicate layers. However, the structure is partially nanocomposite due to presence of the original peak of MMT at 4.7°.

Conclusions

A promising way for preparation of PHB/MMT nanocomposites in $scCO_2$ has been successfully demonstrated. High degrees of MMT loading have been achieved. Our results indicate that MMT can be delaminated by the $scCO_2$ process and dispersion degree is depended on the MMT loading. XRD data confirms the high degree of MMT exfoliation in the samples with 10 and 20 wt. %. MMT. In this way masterbatches with high loading of MMT can be pre-pared.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086

Постер №23

Polysulfobetaines complexes: synthesis and characterization

H. Guncharova[1,2*], M. Simeonov[2], P. Shestakova[1], A. Lederer[3], E. Vassileva[2]

[1]NMR Laboratory, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, "Acad. G. Bonchev" str., bl. 9, 1113 Sofia, Bulgaria
[2]Laboratory on Structure and Properties of Polymers, Faculty of Chemistry and Pharmacy, University of Sofia, 1, J. Bourchier blvd., 1164 Sofia, Bulgaria
[3]Polymer Separation Group, Institute of Macromolecular Chemistry at Leibniz-Institut für Polymerforschung (IPF) Dresden e.V. , Hohe Str. 6, 01069 Dresden, Germany

I. Introduction

The steadily increasing interest to polymer hydrogels is a result of their specific physico-mechanical behaviour as soft materials and their expanding biomedical and industrial applications. Polyzwitterions (PZIs) are known to provoke very low non-specific protein adsorption thus possessing very good biocompatibility.

Scheme 1. PZIs specific structure[1].

The PZIs biocompatibility is most probably related to their specific structure (Scheme 1) defined by the presence of both positive and negative charges covalently bound. PZIs are also known to expand more in a low molecular salt aqueous solution than in water (antipolyelectrolyte effect). This is related to destroying the clusters formed by dipole-dipole interactions between both opposite charges, i.e. it is also due to the PZIs specific structure. The clusters existence however is still not studied in details.

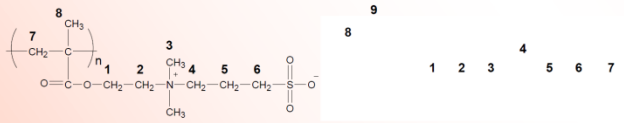
II. Aim

The aim of this study is to shed more light on the interactions in polyelectrolyte complexes formed between PZIs. To this purpose PZIs complexes of two polysulofbetaines were synthesized in solution and their properties were studied as a function of pH, temperature, etc.

III. Experimental part

Synthesis of Polymers: Poly(*N,N'*-dimethyl (methacryloyloxyethyl) ammonium propanesulfonate) (PSB) and poly(*N,N'*-dimethyl (methacryloylamino)propyl ammonium propanesulfonate) (PAmSB) (Scheme 2) were synthesized via free radical polymerization. To this purpose 1M aqueous solution of the respective monomer was prepared containing also initiator $K_2S_2O_8$ (0.1mol.% to the monomer). Polymerization took place at 60°C for 6h. The PSB and PAmSB were dialyzed in distilled water for 1 week to remove the residual chemicals and the obtained solutions were freeze dried.

Formation of Polyelectrolyte complexes (PEC): The formation of the complex between both PZIs was performed using 1:1 molar ratio of PAmSB and PSB. The solution of PAmSB was slowly added dropwise into PSB solution under constant stirring and homogenized.



Scheme 2. Chemical formulas of PSB and PAmSB.

IV. Results

Nuclear Magnetic Resonance (NMR) of PZIs

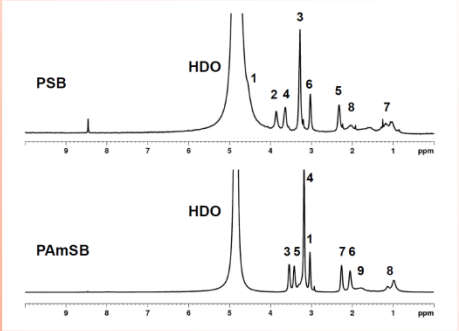
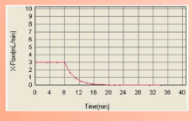


Figure 1. ^{1}H NMR spectra of both PZIs.

The NMR spectra were recorded with Bruker Advance 600 MHz spectrometer in deuterated water (D_2O). ^{1}H NMR spectra (Fig. 1) prove the successful synthesis of PSB and PAmSB. In both PZIs spectra the signals for olefinic protons are not found in the region between 5.5 and 7.0 ppm. This confirms the binding between monomers and formation of polymer chains. The proton signals from the groups numbered in Scheme 2 are assigned on NMR spectra (Fig. 1).

Asymmetrical Flow Field-Flow Fractionation (AF4)



Scheme 3. AF4 separation method profile.

AF4 of PSB, PAmSB and PEC was performed in long asymetric channel with height - 350μm. Water + 0.2% NaN_3 + 50mM $NaNO_3$ was used as eluent. Exponential gradient Vx = 3.0-0 ml/min in 20 Min, Curve factor 2.5 were used. Regenerated cellulose with cut-off 10kDa was used as a membrane. The profile of AF4 separation method is presented in Scheme 3.

PAmSB shows one narrow peak corresponding to linear polymer chains with size between 3 to 11 nm and a very small peak at longer times, most probably due to larger chains as it corresponds to higher molecular weight (Fig. 2).

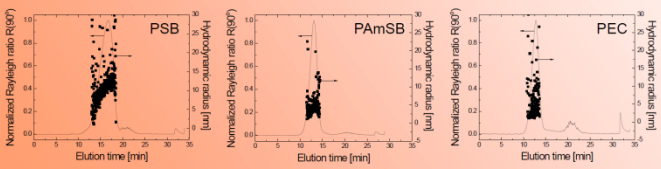


Figure 2. Normalized Rayleigh ratio R(90°) (line) and hydrodynamic radius (points) vs. elution time for nanogels from PSB, PAmSB and their PEC.

PSB is characterized also by one main peak due to the primary particles with size between 5 to 20 nm. These polymer chains however are with wider size distribution compared to PAmSB.

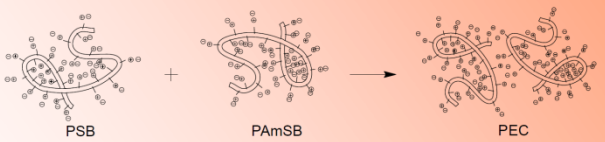
Two smaller peaks appear in PSB fractogram similar to PAmSB most probably due to linear PSB chains with higer molecular weight.

PEC is characterized by one primary peak and two smaller ones at longer times (Fig. 2) similarly to PSB (Fig. 2). The hydrodynamic radius of both PZIs and their PEC revealed by AF4 are presented in Table 1.

Table 1. Hydrodynamic radii (AF4) of PZIs and their PEC.

Sample	PEC	PSB	PAmSB
RH (nm)	25.5 ± 21	17.3±1	11.6±2

PEC of both PZIs has twice higher hydrodynamic radius than its homopolymeric components which is a confirmation for their succesfull complexation (Scheme 4).



Scheme 4. Formation of a polyelectrolyte complex (PEC) between PSB and PAmSB.

Dynamic light scattering (DLS)

Both PZIs show upper critical solution temperature (UCST) which is around 45°C for PSB and 30°C for PAmSB (Fig. 3). The increase in PEC size as a function of the temperature is unusual as it passes through a maximum coinciding with the PSB UCST (~40-45°C). The reason could be that at this temperature both homopolymers are nearly dissolved and thus their chains are in extended conformation. This allows for formation of more dipole-dipole clusters which are the reason for PEC formation (Scheme 5). Thus PZIs complexation is promoted and larger PEC are formed. The further temperature increase results into gradual destroying of the clusters due to polymer dissolution.

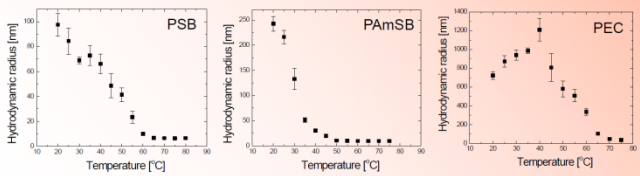
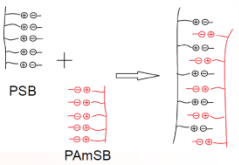


Figure 3. Hydrodynamic radius of PZIs and their PEC as a function of temperature (results from DLS).

DLS was applied to follow the pH influence on PZIs PEC (Table 2). PZIs are not influenced by pH change as they posses equal number of anionic and cationic functional groups in their molecules. However, the media with different H^+ concentration allows for competitive interactions of the charged polymer fragments. For example, at low pH, the high H^+ concentration favors the protonation of the sulfo group thus hampering the formation of dipole-dipole clusters (Scheme 5) resulting in smaller PEC. At high pH, there is an excess of OH- groups which compete with the sulfo group for interaction with quaternary ammonium. Similarly, PEC size is determined mostly by the PZIs components concentration and the size of PEC at high pH is comparable with the size at low pH.



Scheme 5. Dipole-dipole zip cluster formation between PZIs.

Table 2. pH dependence of PEC (PSB-PAmSB)

PEC	pH 4	pH 7	pH 9
Radius (nm)	400.9	657.5	438

At neutral pH, there is a balance between the anionic and cationic groups in water. In this way the dipoles of PSB and PAmSB could more effectively interact to form clusters as they do not have to compete with other charged particles, i.e the dipole-dipole interaction between PSB and PAmSB will be favorable. This results into largest PECs, which is in agreement with the experimental results.

Zeta potential

PSB has negative zeta potential (ZP) while the zeta potential of PAmSB is positive (Table 3).

Table 3. Zeta potential of PZIs and their PEC.

Sample	PEC	PSB	PAmSB
ZP (mV)	-3.33	-33.00	12.40

The zeta potential of their PEC is close to zero and somewhere between the ZP of PEC components which is another indication for successful complex formation.

V. Conclusions

- NMR analysis shows the successful synthesis of both PZIs used for PEC formation.
- For the first time were obtained PEC formed between PZIs (PSB and PAmSB).
- PZIs PEC shows temperature dependence passing through a maximum at temperature which coincides with UCST of PSB and is above UCST of PAmSB. At this temperature PEC has the largest size. The PEC size maximum at this temperature is explained by the more effective dipole-dipole interactions between both PEC components due to polymer expanded conformation.
- AF4 shows narrow size distribution of PEC and its components and twice larger PEC particles compared to its components.
- pH dependence of PEC size is explained by the competitive interactions between charged groups in PZIs and charged groups in water.
- Zeta potential of PEC is close to neutral which confirms the successful dipole-dipole cluster formation between PZIs.

VI. References

[1] E. Mah and R. Ghosh, *Processes* **2013**, *1*(3), 238-262

Acknowledgement: This work was financially supported by Project DFNI-T02/15 of Bulgarian Science Foundation and Project DNTS Germany 01/12 funded by DAAD.

Постер №24

GRAFT COPOLYMERS OF POLYAMIDE 6 VIA IN SITU POLYMERIZATION OF ε-CAPROLACTAM

Mariya K. Kyulavska,[1,2] Roza P. Mateva [2]

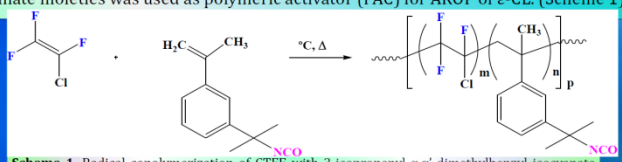
[1]Department of Structure and Properties of Polymers, Institute of Polymers , Bulgarian Academy of Sciences, Acad. G. Bonchev St., bl. 103-A, 1113 Sofia, Bulgaria , email: mkyulavska@polymer.bas.bg

[2]Department of Polymer Engineering, University of Chemical Technology and Metallurgy, Kl. Ohridsky Blvd. 8, 1756 Sofia, Bulgaria

INTRODUCTION

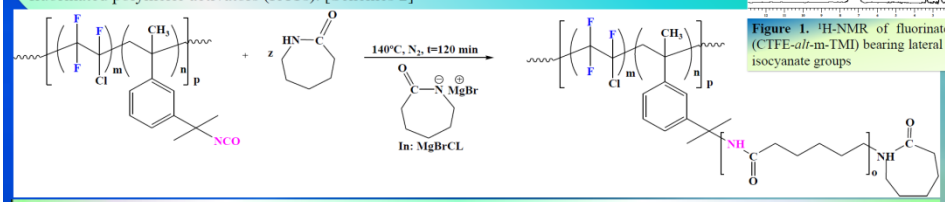
The anionic polymerization of ε-caprolactam (CL) with bifunctional activators has been extensively studied as an effective and beneficial method of improving a chemical and impact resistance, elasticity and other mechanical properties of PA-6. In the presence of activators or polymeric activators also known as macroactivators (MAs) the anionic polymerization of lactams proceeds rapidly at a temperature range of 130-180°C well below the melting point of PA-6 (220°C), permitting direct manufacturing of the product. Thus, the use of polymeric activators (especially elastomeric oligomers) allows desired modifications of some polymer properties (e.g. improved impact resistance). Among the various research investigation those concerning incorporating of the rigid segments (in our case F-containing one) into polyamide 6 polymer chain are scare. [2-6] ***To the best of our knowledge such fluorine-containing polyamides has never been reported.***

Anionic ring opening polymerization (AROP) has been successfully used to obtain novel graft copolymers with polyamide 6 (PA6) grafts onto chlorotrifluoroethylene (CTFE) containing backbone. Initially synthesized poly(CTFE-*alt*-mTMI) oligomer [1], containing pendant isocyanate moieties was used as polymeric activator (PAC) for AROP of ε-CL. (Scheme 1).



Scheme 1. Radical copolymerization of CTFE with 3-isopropenyl-α,α'-dimethylbenzyl isocyanate (m-TMI) in acetonitrile

A series of graft copolymers of polyamide (PA-6) with rigid perfluorinated backbones were synthesized via activated anionic ring opening polymerization (AROP) of ε-caprolactam (CL) in the presence of the Grignard reagent - ε-caprolactam magnesium bromide (MgBrCL) a as initiator and fluorinated polymeric activators (PACs). [Schemes 2]



Scheme 2. Anionic ring opening polymerization of CL in the presence of fluorinated PACs (CTFE-*alt*-m-TMI) and formation of graft copolymers

The obtained graft copolymers were analysed and proved with ^{1}H-NMR and ^{19}F-NMR spectroscopy [Fig.4,5]

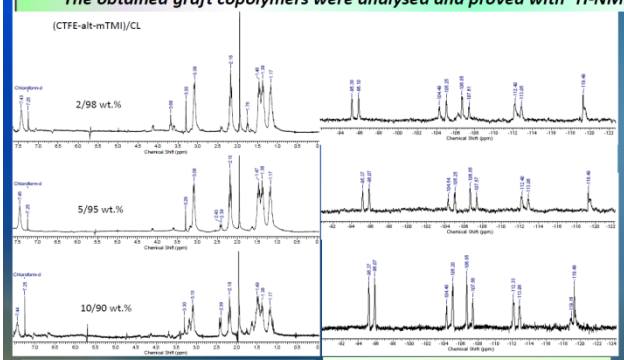


Figure 4. ^{1}H-NMR spectra of obtained fluorine containing graft copolymers of polyamide 6

Figure 5. ^{19}F-NMR spectra of obtained graft copolymers of polyamide 6

^{1}H-NMR and ^{19}F-NMR spectra of used fluorinated polymeric activators (CTFE-alt-mTMI), CTM-70I. [Fig.1,2]

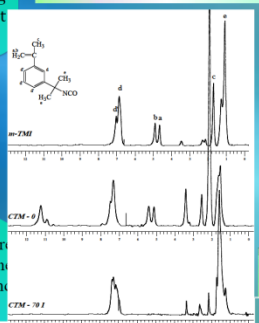


Figure 1. ^{1}H-NMR of fluorinated PAC (CTFE-*alt*-m-TMI) bearing lateral reactive isocyanate groups

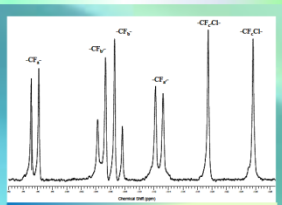


Figure 2. ^{19}F-NMR of fluorinated PAC (CTFE-*alt*-m-TMI)

A part of the structure of the alternating (CTFE-alt-mTMI) copolymer [Fig.3 a and b]

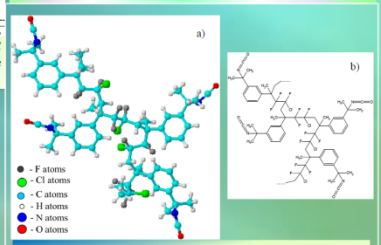


Figure 3. 3D chemical structure (a) and structural formula (b) of fluorinated PAC (CTFE-*alt*-m-TMI) bearing lateral reactive isocyanate groups

The influence of the CL/PACs ratio in feed on the kinetics of anionic ROP, on average molecular weight, and on the structure of the obtained block copolymers were investigated.

Table 1. Main characteristics of used and obtained copolymers

Polymer name	Monomers in feed	Yield [%]	Average molecular weight [g/mol] calc. by (NMR)
CTFE-*alt*-mTMI, CTM-70I	CTFE/mTMI: 70/30	60	1 700
ε-CL homopolymer	0/100	86	24 000
(CTFE-*alt*-Mtmi)-g-CL	(CTFE-*alt*-Mtmi)/CL: 2/98	43	6 200
(CTFE-*alt*-Mtmi)-g-CL	(CTFE-*alt*-Mtmi)/CL: 5/95	59	9 100
(CTFE-*alt*-Mtmi)-g-CL	(CTFE-*alt*-Mtmi)/CL: 10/90	72	19 540

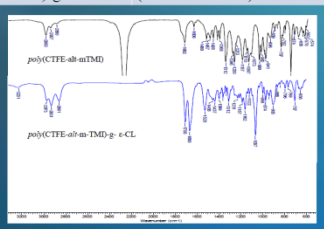


Figure 6. FTIR investigation of fluorinated graft copolymers of polyamide 6

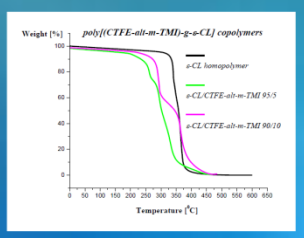


Figure 7. TGA investigation of obtained fluorine containing graft copolymers of polyamide 6

References

[1] M. Kyulavska, G. Kostov, B. Ameduri, R. Mateva, *J. Polym. Sci. A: Polym. Chem.* **2010**, *48*, 2681

[2] W. T. Whang and E. M. Pearce, J. Polym. Sci., Polym. Ch.em. Ed., 25 (1987) 171.

[3] T. Kiyotsukuri, N. Tsutsumi, K. Okada, K. Asai and M. Nagata, J. Polym. Sci., Polym.Chem. Ed., 26 (1988) 2225.

[4] Y. Oishi, S. Harada, M. Kamimoto and Y. Imai, J. Polym. Sci., Polym. Chem. Ed., 27 (1989) 3393.

[5] T. Kiyotsukuri, N. Tsutsumi, T. Sandan and M. Nagata, J. Polym. Sci., Polym. Cha. Ed., 28 (1990) 315. Ann E. Mera, James R. Griffith, Journal of Fltine Chemistry, 59 (1992) 81-89.

[6] B. F. Malichenko, L. N Vilenskaya, Vysokomol. soyed. A14: No. 10, 2079-2085, 1972.

Acknowlegments

The authors are also indebted to Prof. B. Ameduri and Prof. G. Kostov for their invaluable help into the synthesis of the polymeric activator used.

Conclusions

ε-CL was successfully grafted onto perfluorinated backbone trough the lateral isocyanate groups. Such a graft copolymers are reported for the first time. It was proved that using MgBrCL as an initiator of the AROP of ε-CL leads in well defined fluorine containing graft copolymers of polyamede 6.

Acknowledgement:
This work was financially supported by POLINNOVA Project funded under the FP7 Grant Agreement No 316086