

ИНСТИТУТ ПО ПОЛИМЕРИ - БАН

8^{ма} НАУЧНА СЕСИЯ

**„МЛАДИТЕ УЧЕНИ В СВЕТА
НА ПОЛИМЕРИТЕ“**

01.06.2017 г.

СОФИЯ

СПИСЪК НА УЧАСТНИЦИТЕ

ДОКЛАДИ

Доклад №1

Павел Бакърджиев¹, Деница Момекова², Станислав Рангелов¹, Тоня Андреева³, Йордан Думанов⁴

ПОЛИГЛИЦИДОЛ СЪДЪРЖАЩИ ФОСФОЛИПИДНИ НАНОДИСКОВЕ – ПЛАТФОРМА ЗА ИЗСЛЕДВАНЕ И ДОСТАВЯНЕ НА ИНТЕГРАЛНИ МЕМБРАННИ ПРОТЕИНИ

¹ *Институт по полимери, Българска академия на науките*

² *Фармацевтичен факултет, Медицински университет – София*

³ *Институт по биофизика и биомедицинско инженерство, Българска академия на науките*

⁴ *Катедра Биохимия, Биологически факултет, Софийски университет „Св. Климент Охридски“*

Доклад №2

Ивалина Трендафилова¹, Маргарита Попова¹, Деница Момекова², Георги Момеков², Нели Косева³, Агнес Сегеди⁴

НОВИ ЛЕКАРСТВО-ДОСТАВЯЩИ СИСТЕМИ НА ОСНОВАТА НА КОМПОЗИТНИ НОСИТЕЛИ ОТ ТИПА МЕЗОПОРЕСТ СИЛИКАТ-ПОЛИМЕР

¹ *Институт по органична химия с център по фитохимия, Българска академия на науките*

² *Фармацевтичен факултет, Медицински университет – София*

³ *Институт по полимери, Българска академия на науките*

⁴ *Изследователски център за природни науки, Институт за материалите и химия на околната среда, Унгарска академия на науките*

СПИСЪК НА УЧАСТНИЦИТЕ

ПОСТЕРИ

ПОСТЕР №1

E. Veleva-Kostadinova¹, **N. Toncheva-Moncheva**¹, **I. Dimitrov**¹, **D. Momekova**², **Ch. Novakov**¹, **S. Rangelov**¹

THERMALLY RESPONSIVE POLYPEPTIDE-BASED COPOLYMERS AS NON VIRAL VECTORS FOR TRANSPORT OF POLYNUCLEOTIDES

¹Institute of Polymers, Bulgarian Academy of Sciences

²Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University-Sofia

ПОСТЕР №2

Dimitrina Babikova, **Radostina Kalinova**, **Ivaylo Dimitrov**

SYNTHESIS AND MICELLIZATION OF MULTIFUNCTIONAL TRIBLOCK COPOLYMER FOR TARGETED DRUG DELIVERY

Institute of Polymers, Bulgarian Academy of Sciences

ПОСТЕР №3

Emi Haladjova¹, **Grigoris Mountrichas**², **Stergios Pispas**², **Stanislav Rangelov**¹

DYNAMIC AND STATIC LIGHTSCATTERING INVESTIGATIONS OF THERANOSTIC DNA CARRIERS

¹ Institute of Polymers, Bulgarian Academy of Sciences

² Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, Greece

ПОСТЕР №4

M. Ivanov^{1,2}, **E. Haladjova**¹, **M. Smolicek**³, **J. Kronek**³, **S. Rangelov**¹

POLYMER VECTOR SYSTEMS FOR TRANSFECTION OF DNA BASED ON COPOLYMERS OF POLY (2-METHYL-2-OXAZOLINE) AND POLYETHYLENEIMINE

¹ Institute of Polymers, Bulgarian Academy of Sciences

²Biological Faculty, Sofia University "St. Kl. Ohridski"

³Polymer Institute, Slovak Academy of Sciences,

ПОСТЕР №5

Kapka Stoyanova^{1,2}, **Diana Dimitrova**^{1,2}, **Natalia Toncheva-Moncheva**¹, **Stanislav Rangelov**¹

EFFECTS OF PREPARATION PROTOCOL ON SIZE AND CONDENSATION CAPABILITY OF POLYPLEXES BASED ON THERMO-RESPONSIVE PIPOX-RAN-PEI COPOLYMERS

¹Institute of Polymers, Bulgarian Academy of Sciences

²Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University-Sofia

ПОСТЕР №6

L. Ermenkova^{1,2}, **E. Haladjova**¹, **M. Simeonova**³, **S. Rangelov**¹, **Ch. B. Tsvetanov**¹

NOVEL STRATEGY FOR PREPARATION OF MULTI-COMPARTMENT POLYMERIC NANOCAPSULES

¹*Institute of Polymers, Bulgarian Academy of Science*

²*Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski "*

³*Department of Polymer Engineering, University of Chemical Technology and Metallurgy*

ПОСТЕР №7

G. Yakub¹, **A. Toncheva**¹, **N. Manolova**¹, **I. Rashkov**¹, **R. Toshkova**², **A. Georgieva**², **E. Nikolova**²

CURCUMIN-CONTAINING ELECTROSPUN MEMBRANES – UV STABILITY AND *IN VITRO* INTERACTION WITH DIFFERENT CELLS (HELACELLS, LYMPHOCYTES AND MACROPHAGES)

¹*Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences*

²*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*

ПОСТЕР №8

Виктория Пенчева¹, **Елена Маргаритова**¹, **Мария Боринарова**¹, **Деница Момекова**¹, **Петър Петров**²

СИНТЕЗ НА КРИОГЕЛОВЕ ОТ ХИДРОКСИПРОПИЛЦЕЛУЛОЗА С ВКЛЮЧЕНИ СТАБИЛИЗИРАНИ ПОЛИМЕРНИ МИЦЕЛИ

¹*Фармацевтичен факултет, Медицински университет - София*

²*Институт по полимери, Българска академия на науките*

ПОСТЕР №9

Alexander Milkov¹, **Anton Kostadinov**¹, **Georgy Grancharov**², **Valeria Gancheva**², **Boryana Trusheva**³, **Vassya Bankova**³, **Petar Petrov**²

SOLUBILIZATION OF PROPOLIS BY POLY(ETHYLENE OXIDE)-BLOCK-POLY(N-BUTYL ACRYLATE) DIBLOCK COPOLYMER MICELLES: EFFECT OF COPOLYMER COMPOSITION

¹*University of Chemical Technology and Metallurgy*

²*Institute of Polymers, Bulgarian Academy of Sciences*

³*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences*

ПОСТЕР №10

Мирослава Вълчанова^{1,2}, **Станислав Рангелов**¹, **Севдалина Турманова**²

ХИДРОФОБНО МОДИФИЦИРАНИ СЪПОЛИЕТЕРИ С ЛАТЕНТНА ХИМИЧЕСКА ФУНКЦИОНАЛНОСТ

¹*Институт по полимери, Българска академия на науките*

²*Катедра "Технологии, материали и материалознание", Факултет по технически науки, Университет "Проф. д-р Асен Златаров" - Бургас*

ПОСТЕР №11

Ivan Mitkov¹, Emi Haladjova³, Kirilka Mladenova², Ralitsa Veleva¹, Veselina Moskova-Doumanova¹, Svetla Petrova², Jordan Doumanov², Petar Petrov³, Tanya Topouzova-Hristova²

INVESTIGATION OF THE EFFECT OF NOVEL POLYMERIC NANOPARTICLES ON CANCER CELL SURFACE MORFOLOGY BY SCANNING ELECTRON MICROSCOPY

¹Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

²Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski"

³Institute of Polymers, Bulgarian Academy of Sciences

ПОСТЕР №12

Petya Tsekova¹, Mariya Spasova¹, Nevena Manolova¹, Iliya Rashkov¹, Nadya Markova²

ELECTROSPUN CELLULOSE ACETATE FIBERS DECORATED BY ELECTROSPRAYED PVP-CURCUMIN PARTICLES

¹Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences

²Institute of Microbiology, Bulgarian Academy of Sciences

ПОСТЕР №13

Георги Георгиев, Петър Петров

СИНТЕЗ И ОХАРАКТЕРИЗИРАНЕ НА КРИОГЕЛОВЕ ОТ СМЕСЕНИ ПОЛИМЕРНИ МРЕЖИ

Лаборатория „Структура и свойства на полимерите“, Институт по полимери, Българска академия на науките

ПОСТЕР №14

Hristo Penchev¹, Silvia Dimova¹, Filip Ublekov¹, Christo Novakov¹, Katerina Zaharieva²

IRON (III) CHLORIDE CATALYZED CARBONYL OLEFIN METATHESIS POLYMERIZATION OF CHALCONE

¹Institute of Polymers, Bulgarian Academy of Sciences

²Institute of Catalysis, Bulgarian Academy of Sciences

ПОСТЕР №15

Йоанна Йълдъръм¹, Марин Симеонов¹, Христо Цачев², Елена Василева¹

МЕТАКРИЛАТНИ ВЗАИМНОПРОНИКВАЩИ ПОЛИМЕРНИ МРЕЖИ КАТО НОСИТЕЛИ НА ПРОМЕТАЗИН ХИДРОХЛОРИД

¹Лаборатория по структура и свойства на полимерите, Факултет по химия и фармация, Софийски университет „Св. Климент Охридски“

²Лаборатория по Фармацевтична технология, Факултет по химия и фармация, Софийски университет „Св. Климент Охридски“

ПОСТЕР №16

Svetoslava Stoycheva¹, Emi Haladjova³, Kirilka Mladenova¹, Yulia Petseva¹, Zdravko Lalchev¹, Veselina Moskova-Doumanova², Tanya Topouzova-Hristova², Stanislav Rangelov³, Jordan Doumanov¹

INTERACTIONS OF GOLD NANOPARTICLES WITH HUMAN EPITHELIAL CELLS

¹Department Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski"

²Department Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

³Institute of Polymers, Bulgarian Academy of Sciences

ПОСТЕР №17

Ирена Борисова, Оля Стоилова, Невена Манолова, Илия Рашков

ИЗСЛЕДВАНЕ НА ВЛИЯНИЕТО НА ГЕОМЕТРИЯТА НА КОЛЕКТОРА ВЪРХУ ФИЗИКОМЕХАНИЧНИТЕ СВОЙСТВА НА ЕЛЕКТРООВЛАКНЕНИ МАТЕРИАЛИ ОТ ПОЛИ(3-ХИДРОКСИБУТИРАТ)

Лаборатория Биологично активни полимери, Институт по полимери, Българска академия на науките

ПОСТЕР №18

E. Haladjova¹, Ts. Paunova-Krasteva², D. Borisova², M. Kyulavska¹, S. Stoitsova²
NEW APPLICATION OF HYBRID POLYMER SYSTEMS FOR THE DESTRUCTION OF BACTERIAL BIOFILMS

¹Institute of Polymers, Bulgarian Academy of Sciences

²The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

ПОСТЕР №19

Констанс Русева^{1,2}, Кристина Иванова², Елена Василева¹, Цанко Цанов²

АНТИБАКТЕРИАЛНА АКТИВНОСТ И АНТИБИОФИЛМ ОБРАЗУВАЩА СПОСОБНОСТ НА ПОЛИЦВИТЕРЙОННИ ХИДРОГЕЛОВЕ

¹Лаборатория по структура и свойства на полимерите, Факултет по химия и фармация, Софийски университет "Св. Климент Охридски"

²Лаборатория по молекулярни и индустриални биотехнологии, Политехнически университет на Каталуня, Тераса, Испания

ПОСТЕР №20

В. Димитрова, Д. Николова, М. Симеонов, А. Апостолов, Х. Грънчарова, Е. Василева

IN SITU ОТЛАГАНЕ НА КАЛЦИЕВИ ФОСФАТИ В ПОЛИМЕРНИ МРЕЖИ ОТ ПОЛИСУЛФОБЕТАИННОВИ МАТРИЦИ

Лаборатория по структура и свойства на полимерите, Факултет по химия и фармация, Софийски университет „Св. Климент Охридски“

**ПОЛИГЛИЦИДОЛ СЪДЪРЖАЩИ ФОСФОЛИПИДНИ НАНОДИСКОВЕ –
ПЛАТФОРМА ЗА ИЗСЛЕДВАНЕ И ДОСТАВЯНЕ НА ИНТЕГРАЛНИ МЕМБРАННИ
ПРОТЕИНИ**

**Павел Бакърджиев¹, Деница Момекова², Станислав Рангелов¹, Тоня
Андреева³, Йордан Думанов⁴**

¹Българска академия на науките, Институт по полимери, ул. „Акад. Георги Бончев“
103А, София.

²МУ – София, Фармацевтичен факултет, Катедра Технология на лекарствените
средства с биофармация, ул. „Дунав“ 2, София.

³Българска академия на науките, Институт по биофизика и биомедицинско
инженерство, ул. „Акад. Георги Бончев“ 21.

⁴Софийски университет „Св. Климент Охридски“, Биологически факултет, Катедра
Биохимия, бул. „Драган Цанков“ 8, София.

Фосфолипидните нанодискове, подобно на клетъчните мембрани, са агрегати, изградени основно от фосфолипидни молекули, организирани в плосък бислой. Подобна структура предоставя оптимална среда за реконституиране и изследване на трансмембранни протеини. С цел да се постигне структурна и колоидна стабилност нанодисковете биват модифицирани с хидрофилни полимери под формата на амфифилни полимер-липидни конюгати, локализирани предимно по периферния ръб на диска. Настоящото изследване се фокусира върху метода за получаване и физикохимичните параметри на стерично стабилизирани с полиглицидол фосфолипидни нанодискове, изследвани чрез методите на разсейване на светлината, *in vitro* FRET базирани експерименти, целящи определяне на потенциала за сливане с моделни мембрани, както и изследване на процеса на свързване с протеини. Получените наноразмерни агрегати бяха визуализирани чрез атомно-силово микроскопия, а биологичната им поносимост бе изследвана чрез тест за цитотоксичност срещу MDCK II клетъчна линия.

Благодарности: Изследванията са финансирани по проект ДФНП-13/20.04.2016

НОВИ ЛЕКАРСТВО-ДОСТАВЯЩИ СИСТЕМИ НА ОСНОВАТА НА КОМПОЗИТНИ НОСИТЕЛИ ОТ ТИПА МЕЗОПОРЕСТ СИЛИКАТ-ПОЛИМЕР

Ивалина Трендафилова¹, Маргарита Попова¹, Деница Момекова², Георги Момеков², Нели Косева³, Агнес Сегеди⁴

¹Институт по органична химия с център по фитохимия, Българска академия на науките, София, e-mail: ivtrendafilova@gmail.com

² Фармацевтичен факултет, Медицински университет, София

³Институт по полимери, Българска академия на науките, София

⁴ Изследователски център за природни науки, Институт за материалите и химия на околната среда, Унгарска академия на науките, Будапеща

Разработването на нови доставящи системи е едно от най-бързо развиващите се направление в наномедицината. Особено перспективни като носители през последните години се оказаха мезопорестите силикати. Интензивните изследвания на тези материали се дължат на техните уникалните свойства - висока специфична повърхност (700-1500 m²/g), голям поров обем (0.7-1.2 cm³/g), контролируем размер на порите и частиците и повърхност, подлежаща на модифициране. Комбинираното им използване с наноразмерен магнетит предоставя нови възможности в областта на онкологичната терапия за доставяне на лекарствени вещества (ЛВ) до избран орган или тъкан в тялото при прилагане на външно магнитно поле. Допълнителното обвиване на лекарствените системи с подходящи полимери може да подобри тяхната биосъвместимост и чувствителност към различни фактори – светлина, рН на средата, температура. В настоящето изследване се насочихме към разработване на доставящи системи на две лекарства – преднизолон и митоксантрон. Преднизолонът е противовъзпалително ЛВ широко прилагано при състояния свързани с артритни и ракови заболявания. Митоксантронът е цитостатик, широко използван при лечението на различни онкологични заболявания.

В настоящата работа бяха разработени нов тип нанокомпозити от типа магнетит/мезопорест силикат/полимер на базата, на които са разработени доставящи системи за митоксантрон и преднизолон. Нанокомпозитите от типа магнетит/мезопорест силикат със сферична морфология на частиците и размер около 100 нм бяха синтезирани и успешно модифицирани със -SO₃H групи чрез следсинтезна двустъпкова процедура. Изходните и модифицирани магнетит/мезопорест силикат композити бяха ефективно натоварени с ЛВ по метода на омокряне. Получените носители и лекарствени системи бяха характеризирани с прахова рентгенова дифракция, N₂ физисорбция, термичен анализ, ATR-FTIR, ТЕМ и Мьосбауерова спектроскопия. Резултатите от направените анализи показаха добре изградена структура на мезопорестия силикат в композитните материали и ефективно нанасяне на ЛВ в порите им. Системите за доставка на митоксантрон бяха допълнително обвити с полимерен слой от хитозан с цел постигане на забавено освобождаване. Цитотоксичността на свободните ЛВ и на получените доставящи системи е оценена чрез МТТ тестове върху различни клетъчни линии на човешки туморни клетки.

Авторите изказват благодарност на проект ДН 09/18 по Програма финансирана от Фонд „Научни изследвания“.



THERMALLY RESPONSIVE, POLYPEPTIDE-BASED COPOLYMERS AS NON-VIRAL VECTORS FOR TRANSPORT OF DNA

E. Veleva-Kostadinova ¹, N. Toncheva-Moncheva ¹, I. Dimitrov ¹, D. Momekova ², Ch. Novakov ¹, S. Rangelov ¹

¹Institute of Polymers – BAS, Acad. G. Bonchev Street, Block 103-A, 1113 Sofia, Bulgaria

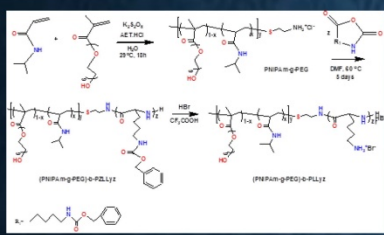
²Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University-Sofia, Bulgaria



INTRODUCTION:

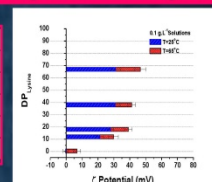
Gene therapy is a powerful approach to treat a wide range of both inherited and acquired diseases, for which the development of a suitable gene delivery system (carrier/vector) is of paramount importance. Cationic polymers are able to condense nucleic acids into nanosized complexes (polyplexes) through electrostatic interactions. Despite the lower transfection efficiency, gene delivery systems based on these polymers show a number of advantages compared to the viral vectors such as easy production, adjustment of size and size distribution, low immunogenicity, biocompatibility, possibilities of composition control. In the present contribution we report on the synthesis of a series of hybrid copolymers comprising of a thermo-responsive poly(N-isopropylacrylamide) (PNIPAm) block with short poly(ethylene glycol) (PEG) side chains and a cationic block of poly(L-lysine) (PLLys) and their complexation behavior with DNA. PNIPAm is probably the most widely used thermosensitive polymer in drug delivery and for other biomedical applications. PEG chains can significantly suppress the adsorption of plasma proteins on the particle surface and impart *Stealth* properties of nanoparticulate carriers, whereas PLLys, being positively charged, is capable to interact electrostatically with DNA. Detailed characterization of the copolymers and further evaluation of their capability to condense DNA into a well-defined and stable polyplexes are performed.

Synthesis of a series of copolymers based on poly(N-isopropylacrylamide)-g-poly(ethylene glycol)-b-poly(L-lysine):



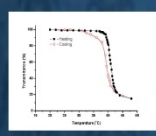
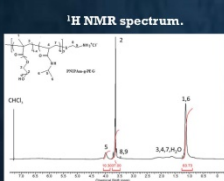
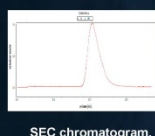
Dynamic and electrophoretic light scattering measurements:

Sample code	D ₀ ,1 (g.L ⁻¹) at 65°C abrupt heating
PNIPAm-g-PEG	103 nm, Intensity, monomodal (PDI=0.916)
NPL-8-0	20 nm, 143 nm, Intensity, bimodal (PDI=0.195)
NPL-15-0	115 nm, Intensity, monomodal (PDI=0.189)
NPL-34-0	105 nm, Intensity, monomodal (PDI=0.172)
NPL-55-0	93 nm, Intensity, monomodal (PDI=0.183)

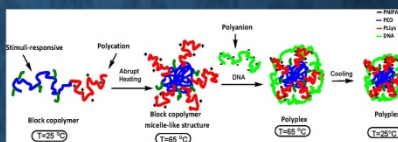


Variations of ζ potential of a series of deprotected (PNIPAm-g-PEG)-b-PLLys copolymers with different polymerization degree of lysine and temperature.

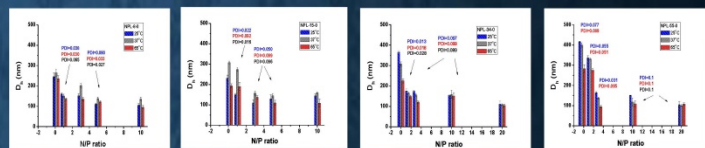
Characterization of thermally sensitive PNIPAm-g-PEG macroinitiator:



Cyclic turbidity measurements of aqueous solutions of the (PNIPAm)-g-(PEG) at 10 g.L⁻¹.



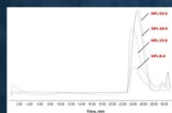
Polyplex investigation:



Variations of hydrodynamic diameters (D_h) of polyplex particles of (PNIPAm-g-PEG)-b-PLLys copolymers: NPL-8-0 (a), NPL-15-0 (b), NPL-34-0 (c) and NPL-65-0 (d) with the amine-to-phosphate groups (N/P) ratio. The polyplexes were prepared at 65 °C (red bars), then cooled the physiologically relevant 37°C (grey bars) and room temperature 25°C (blue bars).

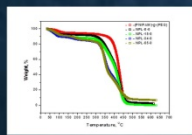
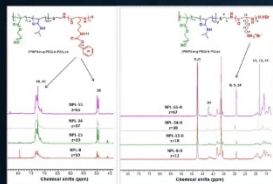
Characterization of poly(N-isopropylacrylamide)-g-poly(ethylene glycol)-b-poly(L-lysine):

Code	Copolymers Composition	M _n (theor.) (g.mol ⁻¹)	TG% (CFP)	Tdmax (°C)
NPL-8-0	[(PNIPAm) ₁₀₀ -g-(PEG) ₁₀]-b-(PLLys) ₁₀	15 600	79	408
NPL-15-0	[(PNIPAm) ₁₀₀ -g-(PEG) ₁₀]-b-(PLLys) ₁₅	16 900	71	404
NPL-34-0	[(PNIPAm) ₁₀₀ -g-(PEG) ₁₀]-b-(PLLys) ₃₄	21 000	70	362
NPL-55-0	[(PNIPAm) ₁₀₀ -g-(PEG) ₁₀]-b-(PLLys) ₅₅	27 100	70	368

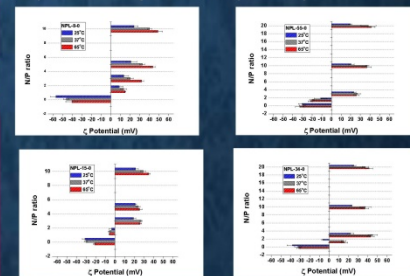


SEC chromatogram of deprotected (PNIPAm-g-PEG)-b-PLLys Copolymers.

The ¹H NMR spectrum of the protected (PNIPAm-g-PEG)-b-PLLys (left) and deprotected (PNIPAm-g-PEG)-b-PLLys (right) copolymers.



TGA curve of the macroinitiator (PNIPAm)-g-(PEG) and deprotected (PNIPAm)-g-(PEG)-b-PLLys copolymers.

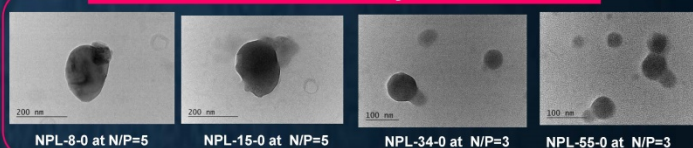


Variations of ζ potentials of polyplex particles of deprotected (PNIPAm-g-PEG)-b-PLLys copolymers:

NPL-8-0 (a), NPL-15-0 (b), NPL-34-0 (c) and NPL-65-0 (d) with the amine-to-phosphate groups (N/P) ratio.

The polyplexes were prepared at 65°C (red bars), then cooled the physiologically relevant 37°C (gray bars) and room temperature 25°C (blue bars).

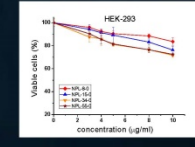
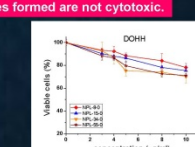
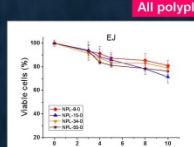
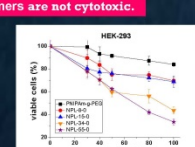
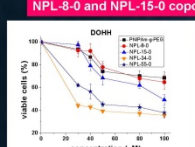
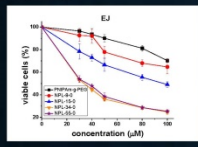
TEM assay:



Cytotoxicity assay:

NPL-8-0 and NPL-15-0 copolymers are not cytotoxic.

All polyplexes formed are not cytotoxic.



Cytotoxicity of tested (PNIPAm-g-PEG)-b-PLLys copolymers against EJ (a); DOHH (b) and HEK-293 (c), as determined by the MTT-dye reduction assay after 72 hours of continuous exposure.

Cytotoxicity of tested polyplex particles at N/P=3 against EJ (a); DOHH (b) and HEK-293 (c), as determined by the MTT-dye reduction assay after 72 hours of continuous exposure.

CONCLUSION:

The synthesis of novel hybrid copolymers comprising of a thermo-responsive poly(N-isopropylacrylamide) (PNIPAm) block with short poly(ethylene glycol) (PEG) side chains and a cationic block of poly(L-lysine) (PLLys) with increasing DP from 12 to 67 is described. The copolymers were synthesised in a two-step procedure. At the first step, a thermally sensitive PNIPAm-g-PEG macroinitiator with a terminal ammonium hydrochloride group was prepared. The macroinitiator was characterised by ¹H NMR and UV spectroscopy. PLLys blocks of different degrees of polymerisation were obtained at the second step involving controlled ring-opening polymerisation of Z-L-lysine N-carboxyanhydride initiated by the macroinitiator. PLLys is positively charged and it is capable to form complexes with various oppositely charged biomacromolecules such as DNA. The physicochemical characterization and cytotoxicity evaluation of the hybrid copolymers and their polyplexes with DNA indicated that they might be promising candidates for gene delivery applications.

FUNDING: Bulgarian NSF project T02/7.

SYNTHESIS AND MICELLIZATION OF MULTIFUNCTIONAL TRIBLOCK COPOLYMER FOR TARGETED DRUG DELIVERY

Dimitrina Babikova, Radostina Kalinova, Ivaylo Dimitrov

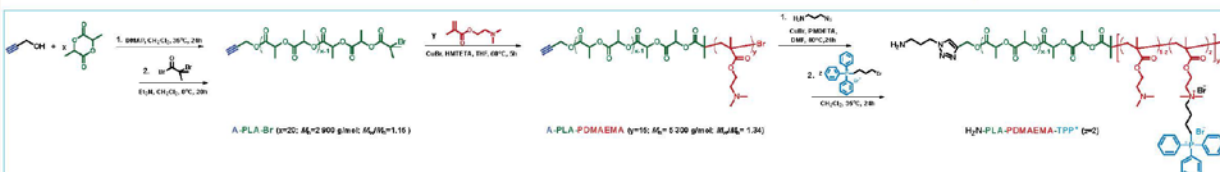
Institute of Polymers, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

Amphiphilic block copolymers have been in use as pharmaceutical excipients for the solubilization of poorly soluble drugs and core/shell association colloids for nanoscale delivery systems. Advances in polymerization chemistries and the application of efficient and orthogonal chemical modification reactions have enabled the engineering of multifunctional polymeric nanoparticles with precise control over the shapes, sizes, internal morphologies, external surface charges and functionalizations. Many drugs have been designed to act on intracellular targets and to affect intracellular processes inside target cells. This subcellular drug targeting approach has been proposed for enhancement of accumulation of these drugs in target organelles and improved efficiency.

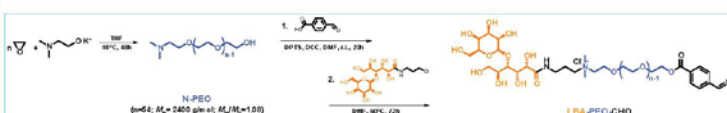
Herein, we present a novel polymeric drug delivery system intended for both cellular and subcellular targeting. The synthetic strategy involves a multistep procedure leading to the formation of amphiphilic triblock copolymer poly(ethylene oxide)-b-poly(D,L-lactide)-b-poly(N,N-dimethylaminoethyl methacrylate), PEO-b-PLA-b-PDMAEMA bearing all the necessary functions ("stealth", targeting and degradable) in the same macromolecule. Initially, amphiphilic diblock copolymer PLA-b-PDMAEMA with terminal amine group and pending triphenylphosphonium (TPP⁺) subcellular targeting ligands was synthesized applying controlled polymerization and modification techniques. In the second step, a heterobifunctional PEO-block, decorated with lactobionic (LBA) cellular targeting ligand and aldehyde end group was attached to the diblock copolymer through the formation of pH-sensitive aromatic imine bond to yield the multifunctional triblock copolymer.

I Synthesis of multifunctional PEO-b-PLA-b-PDMAEMA triblock copolymer (LBA-PEO-PLA-PDMAEMA-TPP⁺)

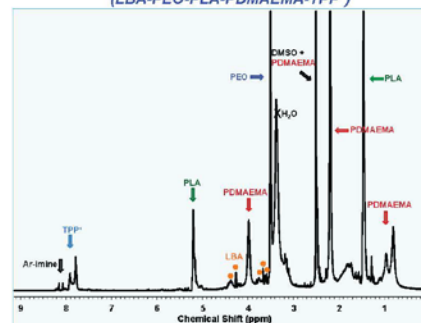
Controlled synthesis of amphiphilic PLA-b-PDMAEMA diblock copolymer with TPP⁺ ligands and terminal primary amine group (H₂N-PLA-PDMAEMA-TPP⁺)



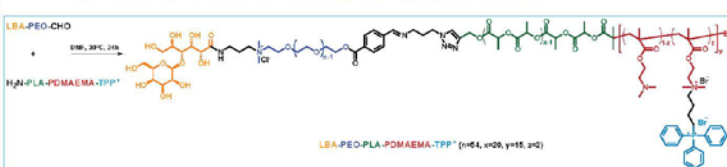
Synthesis of heterobifunctional PEO with lactobionic and aldehyde end groups (LBA-PEO-CHO)



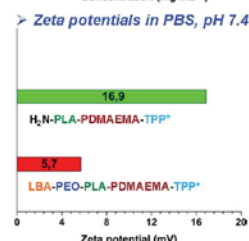
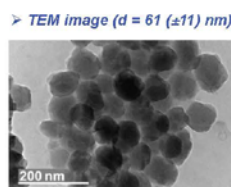
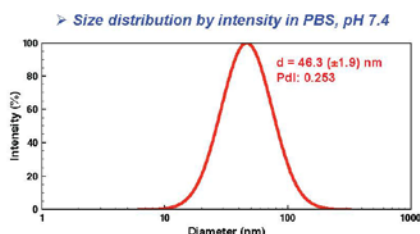
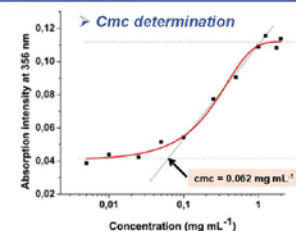
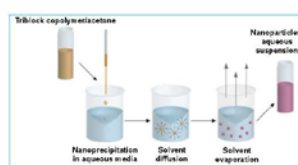
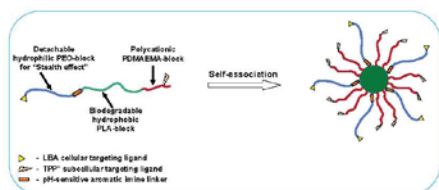
¹H NMR spectrum of multifunctional triblock copolymer (LBA-PEO-PLA-PDMAEMA-TPP⁺)



Multifunctional PEO-b-PLA-b-PDMAEMA triblock copolymer formation



II Self-association of multifunctional PEO-b-PLA-b-PDMAEMA triblock copolymer (LBA-PEO-PLA-PDMAEMA-TPP⁺)



Conclusions

Novel, multifunctional amphiphilic triblock copolymer was designed and successfully obtained applying a multistep synthetic procedure. The system was decorated through modification reactions with lactobionic cellular and triphenylphosphonium subcellular targeting ligands. The amphiphilic triblock copolymer self-associated in aqueous media into multifunctional, nanosized core-shell micelles that consist of biodegradable hydrophobic block of poly(D,L-lactide), polycationic poly(N,N-dimethylaminoethyl methacrylate) block with weakly basic amino groups having sufficient buffering capacity and detachable poly(ethylene oxide) block. The DLS measurements showed formation of nanoparticles with relatively narrow size-distribution. The spherical shape and size of the nanoparticles were visualized by TEM analysis.

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

Dynamic and static light scattering investigations of theranostic DNA carriers

E. Haladjova¹, G. Mountrichas², S. Pispas², S. Rangelov¹

¹ Institute of Polymers, Bulgarian Academy of Sciences, acad. G. Bonchev st bl.103A, Sofia 113, Bulgaria

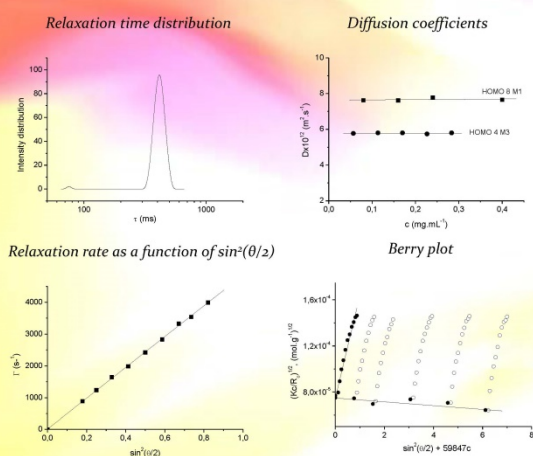
² Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vass. Constantinou Ave., 116 35 Athens, Greece
e-mail address: ehaladjova@polymer.bas.bg

INTRODUCTION

The recent progress in nanotechnology opens new opportunities for creation of specific systems demanded for nanomedicine purposes. An advanced area of nanomedicine is the theranostics that combines therapeutic and diagnostic agents in a single formulation. The field of theranostics responds perfectly to the needs of personalized medicine that has the capacity to detect the onset of disease at its earliest stages, pre-empt the progression of disease, and, at the same time, increase the efficiency of the treatment. The gene therapy is directly related to the personalized medicine and the combination with diagnostics opens new advantages for treatment of genetic disorders. Imaging and therapy, however, require sufficient amount of agents in diseased areas. Therefore, it is very important to control and actually know the precise structure and composition of the multifunctional theranostic nanoparticles.

In the present contribution we combine the properties of a polymer vector with those of gold nanoparticles in new hybrid gene delivery vector systems. The hybrid polyplexes were based on poly(vinyl benzyl trimethylammonium chloride), PVBTMAC, homo- and block copolymers with poly(oligoethylene glycol methacrylate), POEGMA. Using the approach of classic dynamic and static light scattering, we studied these theranostic DNA carriers and determined parameters such as molar mass, intimate composition, particle geometry and density, trying to establish what the polyplexes really carry.

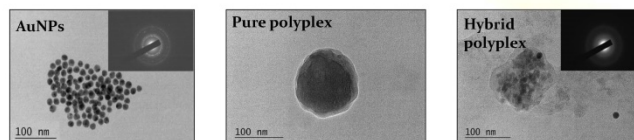
DYNAMIC AND STATIC LIGHT SCATTERING



Sample	$D_0 \times 10^{12}$ (m ² .s ⁻¹)	R_h (nm)	$M_w \times 10^{-6}$ (g.mol ⁻¹)	$A_2 \times 10^5$ (mL.mol.g ⁻²)	R_g (nm)	R_g/R_h	ρ (g.ml ⁻¹)
Homo 0.5	2.69	90.2	169.0	-0.82	97.6	1.08	0.091
Homo 0.5/AuNP	3.02	81.2	96.1	-0.26	80.6	0.99	0.071
BC 0.5	2.53	97.0	116.0	0.18	99.2	1.02	0.050
BC 0.5/AuNP	2.69	91.2	115.0	-1.10	97.0	1.06	0.060
Homo 4	7.04	34.9	4.74	-3.5	30.0	0.86	0.044
Homo 4/AuNP	7.09	34.6	4.39	-6.3	26.7	0.77	0.042
BC 4	6.11	40.2	5.04	-2.89	38.1	0.95	0.031
BC 4/AuNP	6.19	39.6	5.50	-2.49	37.3	0.94	0.035
Homo 8	8.73	28.1	3.10	-2.0	21.7	0.77	0.055
Homo 8/AuNP	7.63	32.1	2.90	-1.20	31.7	0.99	0.035
BC 8	4.18	58.7	9.20	-1.31	55.4	0.94	0.018
BC 8/AuNP	5.12	47.9	4.30	-1.83	40.1	0.84	0.016

VALIDATION OF MORPHOLOGY, STRUCTURE, AND MOLAR MASSES

Transmission Electron Microscopy



INTIMATE COMPOSITION OF THE POLYPLEXES

Sample	DNA molecules per polyplex particle	(co)polymer molecules per polyplex particle	Polyplex particles per AuNP
Homo 0.5	154	1046	n.a.
Homo 0.5/AuNP	87	597	55
BC 0.5	82	1434	n.a.
BC 0.5/AuNP	81	1415	25
Homo 4	1.6	87	n.a.
Homo 4/AuNP	1.4	80	375
BC 4	0.9	128	n.a.
BC 4/AuNP	1	137	257
Homo 8	0.6	66	n.a.
Homo 8/AuNP	0.6	61	492
BC 8	0.9	252	n.a.
BC 8/AuNP	0.4	117	306

Analytical Ultracentrifugation Results

Sample	S^* (s)	D^* (cm ² .s ⁻¹)	$M_w \times 10^6$ (g.mol ⁻¹)
Homo 0.5	6.12×10^{-12}	3.29×10^{-9}	167.7
Homo 0.5/AuNP	7.06×10^{-12}	6.72×10^{-9}	94.9
BC 0.5	4.41×10^{-12}	3.59×10^{-9}	110.9
BC 0.5/AuNP	4.52×10^{-12}	3.50×10^{-9}	116.5
Homo 4	5.86×10^{-13}	1.32×10^{-8}	3.99
Homo 4/AuNP	7.07×10^{-12}	8.84×10^{-8}	7.21
BC 4	3.07×10^{-12}	4.83×10^{-8}	5.75
BC 4/AuNP	6.27×10^{-13}	9.44×10^{-8}	5.99
Homo 8	7.96×10^{-12}	2.02×10^{-7}	3.56
Homo 8/AuNP	3.39×10^{-13}	1.35×10^{-8}	2.26
BC 8	7.60×10^{-13}	1.10×10^{-8}	6.19
BC 8/AuNP	1.07×10^{-12}	1.95×10^{-8}	4.97

CONCLUSIONS

In the present work we investigated binary (DNA:(co)polymer) and ternary (DNA:(co)polymer:AuNPs) polyplexes based on PVBTMAC homopolymer and PVBTMAC-POEGMA diblock copolymer attempting to combine multiple functions within one vehicle: in particular, condensing, protecting, and transport of genetic material, on one hand, and diagnostic and sensing modalities, on the other. The precise structure and intimate composition of the theranostic formulations were determined by classic dynamic and static light scattering. The results obtained were corroborated by transmission electron microscopy and analytical ultracentrifugation. The results revealed strong binding behavior of both PVBTMAC and PVBTMAC-POEGMA so that at higher N/P ratios (N/P = 4 and 8) every single molecule of DNA is complexed and, accordingly, relatively small in size (R_h in the 28 – 59 nm range) and molar mass ($2.90 \times 10^6 - 9.20 \times 10^6$ g.mol⁻¹) polyplex particles were formed. At the lower (0.5) N/P ratio the polyplex particles carried tens of DNA molecules and hundreds of (co)polymer molecules. The N/P ratio did not influence the structure (R_g/R_h slightly below unity, compatible with hairy surface on a compact sphere) of the theranostic carriers. They were characterized as porous and less dense than the familiar copolymer micelles objects with density that decreased with increasing N/P ratio and upon substitution of PVBTMAC with the copolymer. The introduction of AuNPs influenced neither the density, nor the structure of the carriers, which could be correlated with polymer chain flexibility providing conformational and structural adaptiveness to the polyplexes regardless of the complexing components and mainly to the low number fraction of polyplex particles carrying AuNPs. We demonstrated that the classic dynamic and static light scattering measurements constituted a powerful approach for detailed characterization of theranostic carriers and determination of their intimate composition.

Acknowledgement: This work was funded by Project DFNP 12 in the frame of the support program for Young Scientists from Bulgarian Academy of Sciences.

POLYMER VECTOR SYSTEMS FOR TRANSFECTION OF DNA BASED ON COPOLYMERS OF POLY (2-METHYL-2-OXAZOLINE) AND POLYETHYLENEIMINE

M. Ivanov^{1,2}, E. Haladjova¹, M. Smolicek³, J. Kronek³, S. Rangelov¹

¹ Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

² Biological Faculty, Sofia University "St. Kl. Ohridski", 1164 Sofia, Bulgaria

³ Polymer Institute, Slovak Academy of Sciences, 845 41 Bratislava, Slovakia

e-mail: bg_mdi@abv.bg

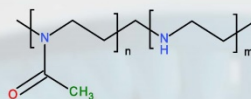
INTRODUCTION

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-oxazolines) (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 linear PEI. Combining the properties of these two polymers, a robust system can be designed to form polyplexes with DNA.

The aim of this work is evaluation of the potential of a series of copolymers based on poly (2-methyl-2-oxazoline) and polyethyleneimine (PMeOx-PEI) with different degree of polymerization and ethyleneimine content. DNA/polymer complexes (polyplexes) at a wide range of amino to phosphate group ratio (N/P=0.5-10) were obtained. The main physicochemical characteristics of the resulting polyplexes, such as hydrodynamic dimensions and ζ -potential, were determined by dynamic and electrophoretic light scattering.

COPOLYMER STRUCTURE AND CHARACTERISTICS

Polymer	DP	% PEI	Mn ¹ H NMR g/mol	Đ	ζ potential mV
PMeOx-PEI	50	41	3410	1,27	11 ± 1
PMeOx-PEI	50	71	2780	1,27	20 ± 1
PMeOx-PEI	50	99	2190	1,27	21 ± 2



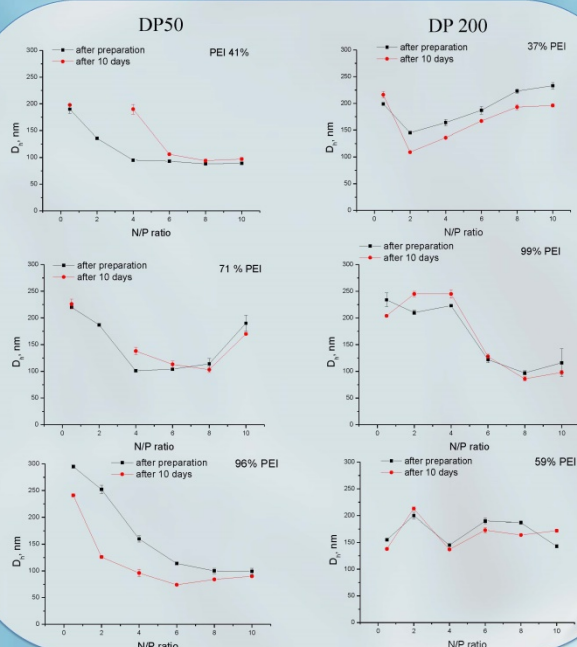
Polymer	DP	% PEI	Mn ¹ H NMR g/mol	Đ	ζ potential mV
PMeOx-PEI	200	37	13890	1,37	8 ± 2
PMeOx-PEI	200	59	12050	1,37	17 ± 4
PMeOx-PEI	200	96	8940	1,37	15 ± 6

Statistical copolymers poly(2-methyl-2-oxazoline-co-ethylene imine) (PMeOx-co-PEI) of two different degree of polymerization and various composition have been prepared in two-step synthesis. In the first step, poly(2-methyl-2-oxazolines) (PMeOx) were prepared through living cationic ring-opening polymerization (LCROP) of 2-methyl-2-oxazoline in 4M acetonitrile at 80 °C using methyl 4-nitrobenzenesulfonate as an initiator. Subsequently, targeted copolymers were prepared by partial hydrolysis of PMeOx in 18.5 vol% aqueous HCl at 100°C using different times of hydrolysis.

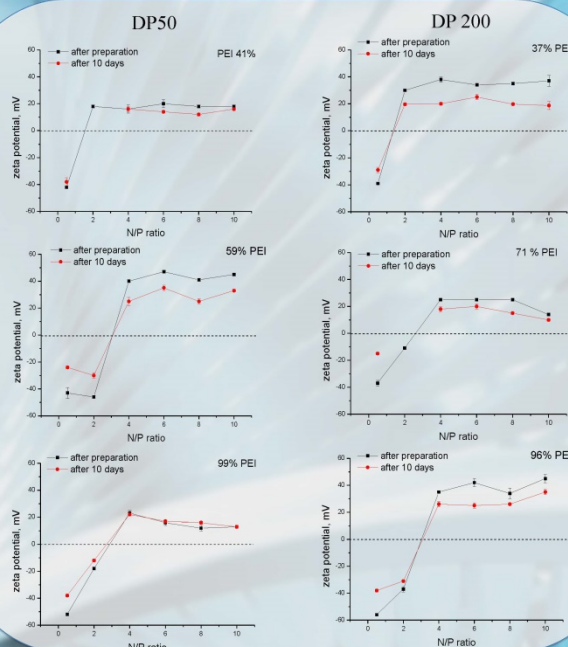
COMPLEXATION WITH DNA

Mixing an appropriate amounts of DNA and polymer solutions at concentrations 0,1 mg.mL⁻¹ and 0,5 mg.mL⁻¹, respectively, polyplexes at different N/P ratios were prepared. The R_h and ζ -potential were investigated as function of N/P ratio. The stability of the resulting vector systems over the time was monitored as well.

Hydrodynamic diameters



ζ -potential



CONCLUSION

- ✓ DNA/polymer complexes based on copolymers of poly(2-methyl-2-oxazoline) and polyethyleneimine of different degree of polymerization and ethyleneimine content were obtained.
- ✓ Formation of well defined particles of small size (ranging from 70 to 240 nm) and narrow size distribution was observed.
- ✓ The surface charge of investigated systems change from negative to positive values with increasing N/P ratio.
- ✓ Influence of degree of polymerization of copolymer chain on the size, zeta potential and colloidal stability of the resulting DNA/polymer complexes was observed.

FUTURE GOALS

- Study of polyplex morphology.
- Monitoring of DNA/copolymer complexation by ethidium bromide quenching assay.
- Cytotoxicity examination.
- Determination of degree of internalization and internalization pathway into the cells.
- Investigations of transfection efficiency.

Acknowledgement:

This work was funded by the National Science Fund (Bulgaria) Project DFNI T02-7.

Effects of preparation protocol on size and condensation capability of polyplexes based on thermo-responsive PIPOX-ran-PEI copolymers

Kapka Stoyanova^{1,2}, Diana Dimitrova^{1,2}, Natalia Toncheva-Moncheva¹, Stanislav Rangelov¹

¹Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University-Sofia, Bulgaria

Abstract:

Gene therapy aims at the treatment of many genetic diseases as it is a technique for correcting defective genes that are responsible for these genetic diseases. Specifically, the delivery of the appropriate, therapeutic gene (DNA) into the cells that will replace, repair or regulate the defective gene that causes the disease is a vital step for gene therapy. DNA, however, is a large negatively charged, hydrophilic molecule. Its delivery to nucleus requires neutralizing, condensing and protecting.

How gene therapy works?

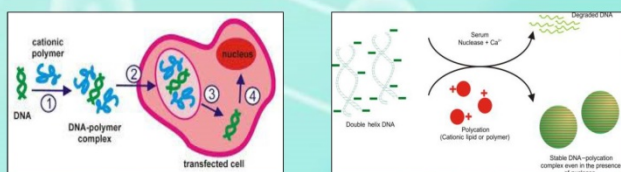
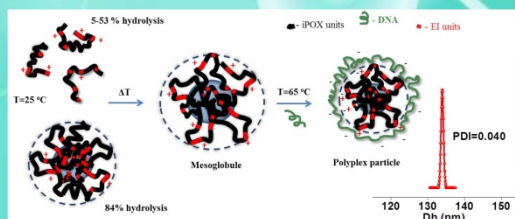


Figure 1: DNA-Polymer complex transportation into the nucleus of the cells

Motivation :

Above the phase transition temperature of the poly(2-isopropyl-2-oxazoline) (PIPOX) moiety in the PIPOX-ran-PEI-5, PIPOX-ran-PEI-15, PIPOX-ran-PEI-53, and PIPOX-ran-PEI-84 copolymers, well-defined nanoparticles (mesoglobules) typically ranging in size 200 – 900 nm and surface potential from +5 to +30 mV, depending on the heating protocol and concentration, were formed. The mesoglobules consisted of a core dominated by PIPOX and a thin positively charged shell from PEI moieties which mediated formation of polyplexes with DNA. Upon complexation with DNA, performed at 65°C and at varying N/P (amine-to-phosphate groups) ratios, well-defined polyplex particles with dimensions ranging between 110 and 150 nm and typically negative ζ potential were formed. The copolymers with PEI contents ≥ 15 % can be considered perspective for delivery of polynucleotides.



Scheme 1. Schematic representation of the formation of mesoglobules from PIPOX-ran-PEI copolymers with degrees of hydrolysis from 5 to 84 % in aqueous solution upon heating and proposed rearrangement of the positive charges in structures formed by PIPOX-ran-PEI-84% at 25 and 65 °C and polyplex particle formation with DNA.

Experimental:

A series of random copolymers of 2-isopropyl-2-oxazoline and ethylenimine (PIPOX-ran-PEI) with PEI contents 5 to 84% were prepared by partial acidic hydrolysis of poly(2-isopropyl-2-oxazoline) (PIPOX).

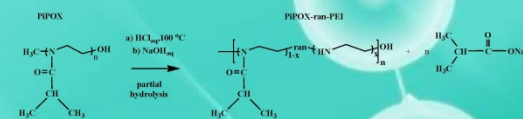


Table 1: Conditions for carrying out partial hydrolysis at T = 100 °C.

Run No	Volume (mL)	HCL [M]	Time (min)	Hydrolysis Degree (%)
1	5.0	11.96	360	5
2	10	11.96	360	15
3	10	11.96	960	53
4	10	11.96	1440	84

Conclusion:

- Copolymers of 2-isopropyl-2-oxazoline and ethylenimine are prepared by acidic hydrolysis.
- They form positively charged mesoglobules at elevated temperatures.
- Several protocols for preparation of mesoglobules have been applied Gradual, abrupt heating and nano-precipitation.
- It was estimated that the protocol of nano-precipitation consisting of drop-wise addition of aqueous solution of proper concentration and temperature below the LCST to a certain volume of water heated at temperatures above the polymer LCST exhibits satisfying reproducibility.
- Well-defined mesoglobules in the lower nanoscale were prepared by nano-precipitation
- Well-defined polyplex particles were prepared upon complexation with DNA ranging 110 - 150 nm, at N/P=3 and N/P=5
- The copolymers with PEI contents ≥ 15 % are perspective for delivery of genes.

Acknowledgements:

Настоящият постер е изработен в рамките на Проект „Студентски практики – Фаза 1“ в Института по Полимери-БАН, лаборатория „Полимеризационни Процеси“ и с подкрепата на Проект „ДФИП – Т02/7 „Нови полимери, полимерни наночастици и нано-капсули за пренос на биологични молекули“.

Results:

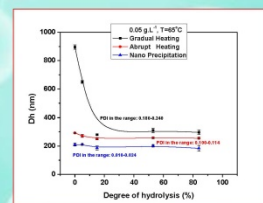


Figure 1. Size (hydrodynamic diameter) and size distribution (PDI) of mesoglobules of PIPOX-ran-PEI copolymers with PEI contents from 0 to 84 % obtained by gradual (black), abrupt (red) and nano-precipitation heating to 65 °C.

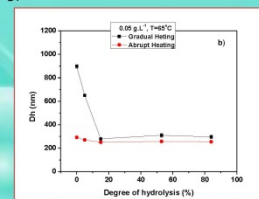
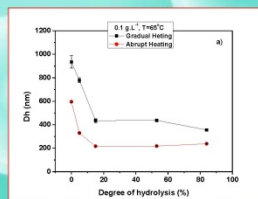


Figure 2. Variations of the hydrodynamic diameters (Dh) with type of heating protocol. Solute concentrations: 0.1 g.L⁻¹ (a) and 0.05 g.L⁻¹ (b).

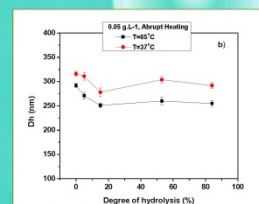
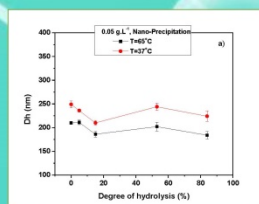


Figure 3. Variations of hydrodynamic diameters (Dh) of mesoglobules of PIPOX-ran-PEI copolymers with temperature and PEI contents from 0 to 84 % obtained by nano-precipitation (a) and abrupt heating (b). The mesoglobules were prepared at 65 °C (black line), then cooled the physiologically relevant 37 °C (red line).

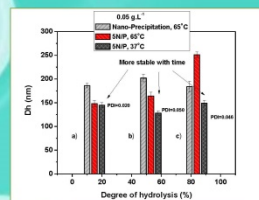
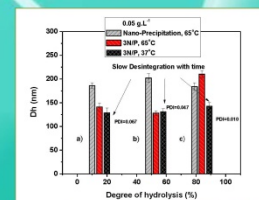


Figure 4 Variations of hydrodynamic diameters (Dh) of polyplex particles of PIPOX-ran-PEI-15% (a), PIPOX-ran-PEI-53% (b), and PIPOX-ran-PEI-84% (c) at amine-to-phosphate groups ratio, N/P=3 and N/P=5. The mesoglobules were prepared by nano-precipitation at 65 °C (grey bars), then polyplexes were formed at the same temperature (red bars), and further cooled the physiologically relevant 37 °C (black bars).

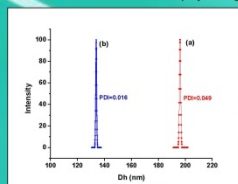


Figure 5. Size distributions from DLS of polyplexes formed from PIPOX-ran-PEI-53% and DNA at N/P=5 and 65 °C (red curve a), cooled polyplexes to the physiologically relevant 37 °C (blue curve b).

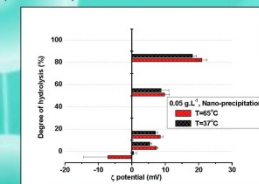


Figure 6. Variations of ζ potential of a series of PIPOX-ran-PEI copolymers in aqueous solution with degree of hydrolysis and temperature.

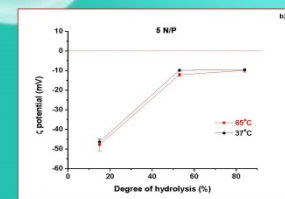
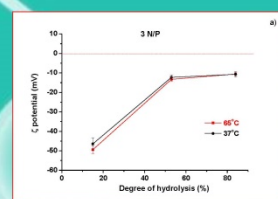


Figure 7. Variations of ζ potential of polyplex particles with degree of hydrolysis of PIPOX-ran-PEI-15%, PIPOX-ran-PEI-53% and PIPOX-ran-PEI-84% at amine-to-phosphate groups (N/P) ratio N/P=3 (a), N/P=5 (b). The polyplexes were prepared by applying nano-precipitation protocol at 65 °C (red squares), then cooled the physiologically relevant 37 °C (black circles).

Literature:

Preparation and properties of positively charged mesoglobules based on poly(2-isopropyl-2-oxazoline) and evaluation of their potential as carriers of polynucleotides, Polymer, 2017

Novel strategy for preparation of multi-compartment polymeric nanocapsules

L. Ermenkova^{1,2}, E. Haladjova¹, M. Simeonova³, S. Rangelov¹, Ch. B. Tsvetanov¹

¹ Institute of Polymers, Bulgarian Academy of Science, "Acad. Georgie Bonchev St. bl. 103-A 1113 Sofia, Bulgaria

² Sofia University, Faculty of Chemistry and Pharmacy, 1 James Bourchier Blvd., 1164 Sofia, Bulgaria

³ University of Chemical Technology and Metallurgy, Department of Polymer Engineering, 8 Kl. Ohridski Blvd., 1756 Sofia, Bulgaria

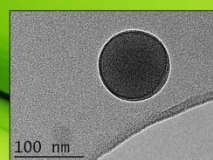
INTRODUCTION

Polymeric hollow nanocapsules are attractive devices with a number of potential applications. They represent colloidal particles composed of a shell or membrane separating the internal cavity from the outside medium thus creating a barrier for various substances that can be encapsulated therein. In the recent years a new generation of carriers have been proposed, composed from numerous compartments. Due to their multi-compartment structure the resulting multi-functional double-walled capsules resemble the cell structure and can be used as artificial cells in medicine and pharmacy as bioreactors or as drug delivery vehicles. Furthermore, in their hollow compartments, substances such as drugs and biomolecules can be encapsulated and released in a controlled manner. These properties make the polymer nanocapsules attractive devices for drug delivery, cancer and gene therapy, protecting enzymes, etc.

In this work we present novel approach for simultaneously preparation and loading of multi-compartment polymeric nanocapsules. First, negatively charged poly(butyl cyanoacrylate), PBCA, nanocapsules were obtained by interfacial polymerization of n-butyl cyanoacrylate, n-BCA. They were used for layer by layer deposition of poly(vinyl benzyl trimethylammonium chloride), PVBTMAC, followed by DNA. Finally, a cross-linked polymeric outer shell based on N-isopropylacrylamide was constructed. The formation of double-walled capsules was investigated during their preparation by dynamic and electrophoretic light scattering.

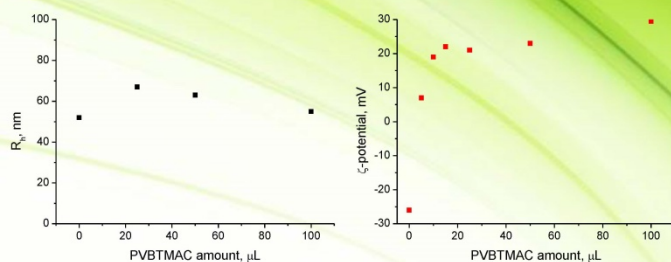
Construction of PBCA nanocapsules

PBCA nanocapsules were obtained by interfacial polymerization of n-BCA. The polymerization was carried out in mixed organic/aqueous phase under magnetic stirring. The resulting milky suspension was used at concentration of 0.1 mg.mL⁻¹. Strongly negative PBCA nanocapsules of small size and narrow size distribution were obtained.



	R _h nm	PDI	ζ mV
PBCA nanocapsules	49.4	0.13	-25.2

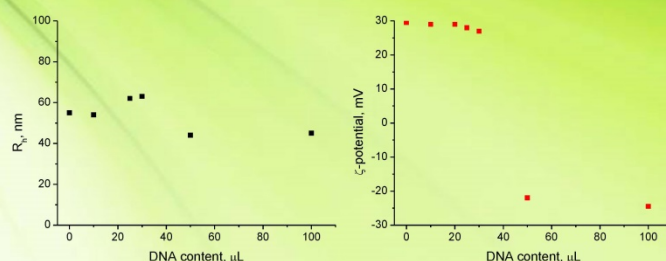
PVBTMAC deposition



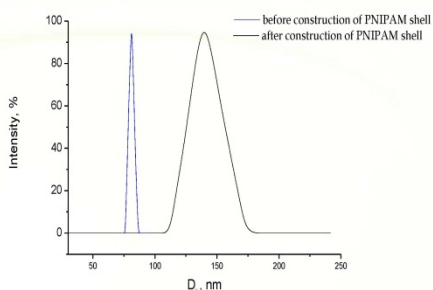
The PVBTMAC deposition was achieved via addition of increasing amounts of aqueous solution of PVBTMAC with concentration of 0.25 mg.mL⁻¹ to the PBCA nanocapsule dispersion. The size of the resulting particles slightly increased, whereas their ζ-potential abruptly changed from negative to positive values with increasing PVBTMAC content.

DNA loading

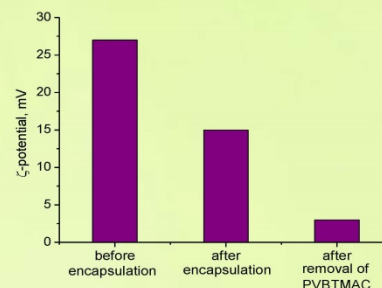
Similarly to the PVBTMAC deposition, various amounts of DNA aqueous solution (0.1 mg.mL⁻¹) were added to the PBCA nanocapsules coated with PVBTMAC (at fixed amount of 100 μL). The sharp transition of the ζ-potential from about +30 to -28 mV evidenced successful DNA deposition.



Preparation of multi-compartment polymeric nanocapsules



On the surface of the PBCA nanocapsules coated with PVBTMAC (100 μL) and DNA (20 μL), a cross-linked shell was constructed by seeded radical copolymerization of N-isopropylacrylamide and N,N-methylenebis(acrylamide). The monomer to initiator molar ratio was selected to give shell thickness of about 30 nm. Removal of PVBTMAC was attempted by extensive dialysis against water after treatment with NaCl. This resulted in a gradual decrease of the ζ-potential with time.



CONCLUSION

A novel approach for simultaneous preparation and loading of multi-compartment polymer nanocapsules has been used. The strategy involved successively layer by layer deposition of oppositely charged polyelectrolytes (PVBTMAC and DNA) onto negatively charged PBCA nanocapsules. The particles were further coated by surface-cross-linked shell based on poly(N-isopropylacrylamide). The polycation PVBTMAC was removed by dialysis against water thus leaving the naked DNA molecules in outer nanocapsules compartment. The multi-compartment double-walled nanocapsules were of size around 70 nm and displayed good colloidal stability.

They can be used in medicine and pharmacy as bioreactors or as multidrug delivery vehicles as well as for theranostic applications in which therapeutic and diagnostic agents are combined in a single formulation. The usage of PBCA nanocapsules give the opportunity to directly load the first capsule compartment with various substances or inorganic particles (e.g. gold, silver, magnetic) that are utilized in diagnostic application.

Acknowledgement:

This work was funded by the National Science Fund (Bulgaria) Project DFNI T02-21.



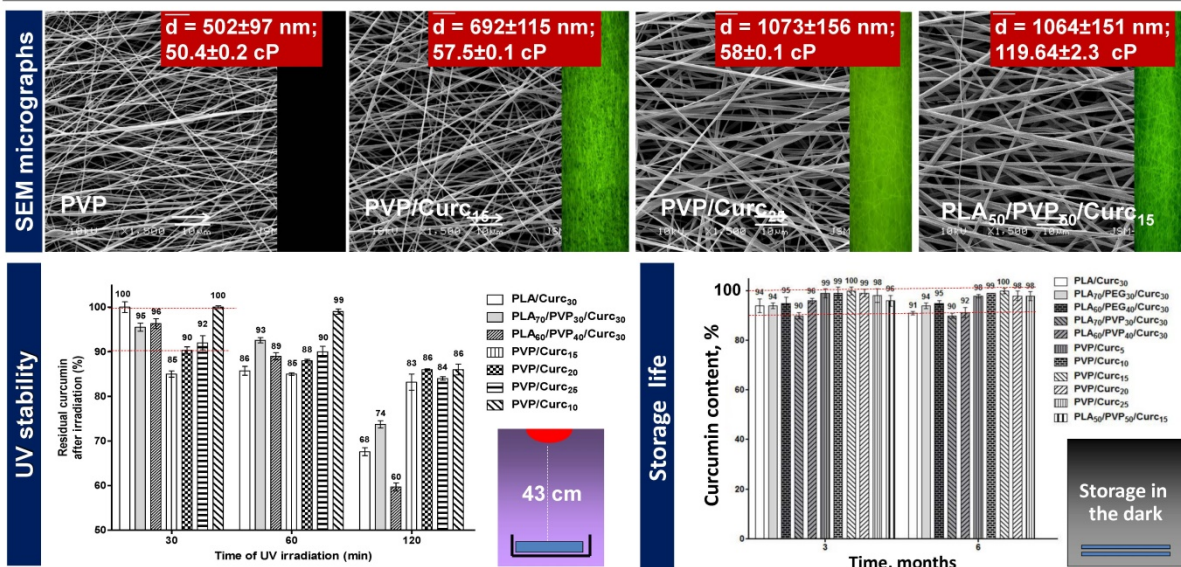
Curcumin-containing electrospun membranes – UV stability and *in vitro* interaction with different cells (HeLa cells, lymphocytes and macrophages)



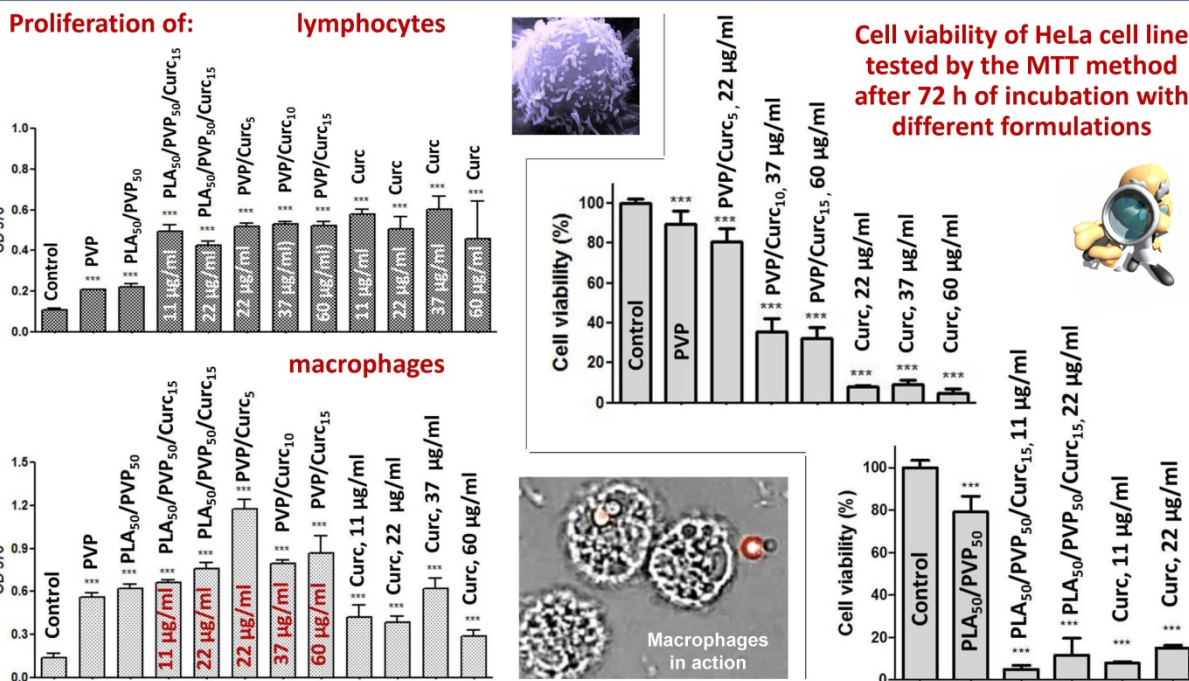
G. Yakub¹, A. Toncheva¹, N. Manolova¹, I. Rashkov¹, R. Toshkova², A. Georgieva², E. Nikolova²
¹Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev St, Bl. 103A, BG-1113 Sofia, Bulgaria; ²Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS, 1113 Sofia, Bulgaria

Aim

To prepare and to characterize curcumin-containing fibrous materials from poly(L-lactide-co-D,L-lactide) (PLA) and polyvinylpyrrolidone (PVP). Exploring the influence of the composition of the polymer matrix on UV stability, storage life, *in vitro* antitumor activity and stimulating effect versus lymphocytes and macrophages.



Biological activity of curcumin-containing fibrous mats



CONCLUSIONS: The fibrous materials containing Curc show good stability, strong *in vitro* antitumor activity and stimulating effect versus lymphocytes and macrophages. They are promising for variety of biomedical applications, including wound dressing materials as well as for application in local drug delivery systems in the treatment of cervical tumors.

ACKNOWLEDGEMENTS Financial support by the NSF of Bulgaria for the research (Grant DFNI T02/1/12.12.2014)

e-mails:
 gyakub@polymer.bas.bg
 manolova@polymer.bas.bg
 rashkov@polymer.bas.bg

СИНТЕЗ НА КРИОГЕЛОВЕ ОТ ХИДРОКСИПРОПИЛЦЕЛУЛОЗА С ВКЛЮЧЕНИ СТАБИЛИЗИРАНИ ПОЛИМЕРНИ МИЦЕЛИ

Виктория Пенчева,¹ Елена Маргаритова,¹ Мария Боринарова,¹ Деница Моменова,¹ Петър Петров²

¹ Фармацевтичен факултет, Медицински университет - София

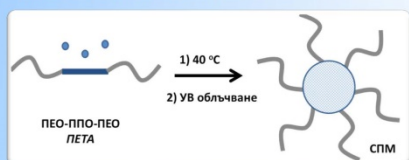
² Институт по полимери, Българска академия на науките

Въведение

Полимерните криогелове намират приложение като носители на клетки, протеини и лекарствени вещества. Криогеловите имат хетерогенна структура, съставена от големи взаимосвързани пори и тънки стени, която улеснява дифузията на активни вещества в обема им. Целта на настоящата работа е да се разработят оригинални полимерни криогелове, съдържащи хидрофобни домени, за имобилизиране на водонерастворими лекарства.

Стратегия

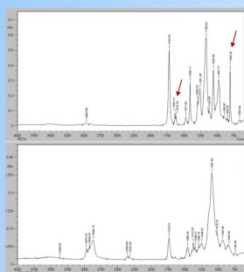
Синтез на стабилизирани полимерни мицели



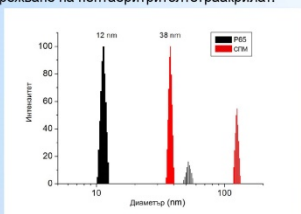
Синтез на криогелове



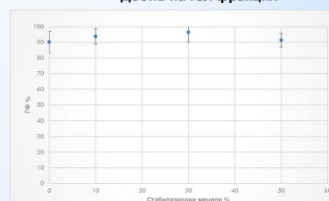
Резултати



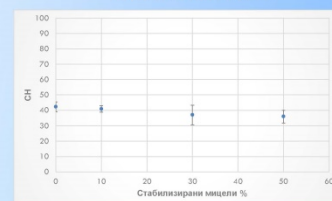
Стабилизирани полимерни мицели от Pluronic P65 са получени чрез имобилизиране и фотохимично омережаване на пентаеритритолтетраакрилат.



Добив на гел фракция

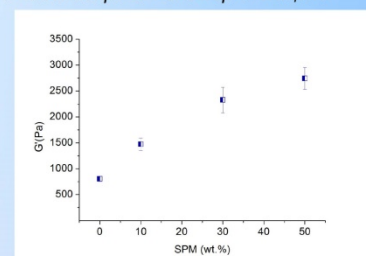
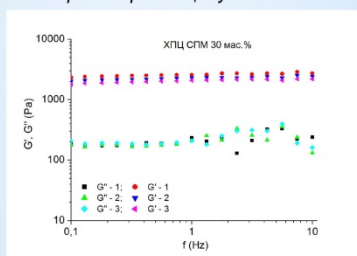
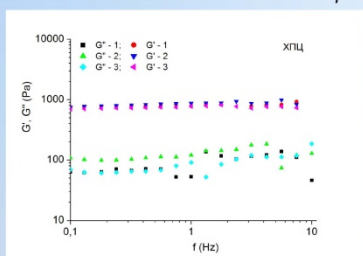


Степен на набъбване

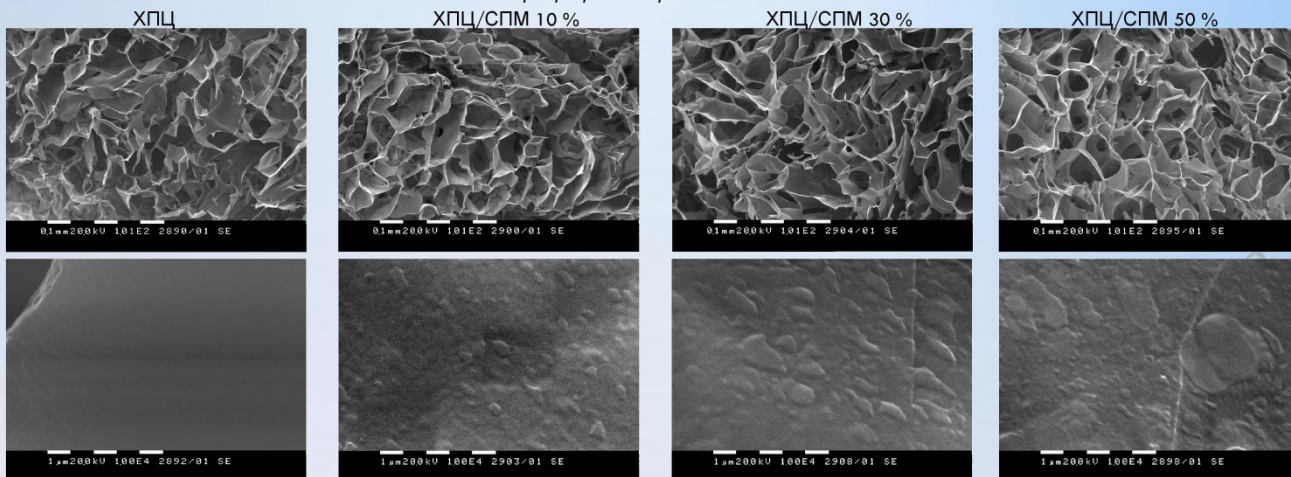


Криогелове от хидроксипропилцелулоза, съдържащи стабилизирани полимерни мицели, с висок добив на гел фракция са синтезирани чрез фотохимично омережаване на замразени водни системи.

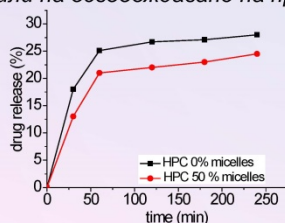
Реологични изследвания на криогелове от хидроксипропилцелулоза без и с включени стабилизирани полимерни мицели



СЕМ микрографии на криогелове от:



Профили на освобождаване на преднизолон



Заключение: Синтезирани са оригинални криогелове от хидроксипропилцелулоза, съдържащи стабилизирани полимерни мицели. Установено е, че с нарастване концентрацията на СПМ се понижава степента на набъбване и се увеличава модула на еластичност на материалите. Доказано е включването и забавеното освобождаване на хидрофобното активно съединение преднизолон от полимерните носители.

Solubilization of propolis by poly(ethylene oxide)-block-poly(n-butyl acrylat) diblock copolymer micelles: Effect of copolymer composition

Alexander Milkov,^a Anton Kostadinov,^a Georgy Grancharov,^b Valeria Gancheva,^b

Boryana Trusheva,^c Vassya Bankova,^c Petar Petrov^b

^a University of Chemical Technology and Metallurgy, 8 St. Kliment Ohridski Blvd., 1756 Sofia, Bulgaria

^b Institute of Polymers, Bulgarian Academy of Sciences, Akad. G. Bonchev St. 103 A, 1113 Sofia, Bulgaria

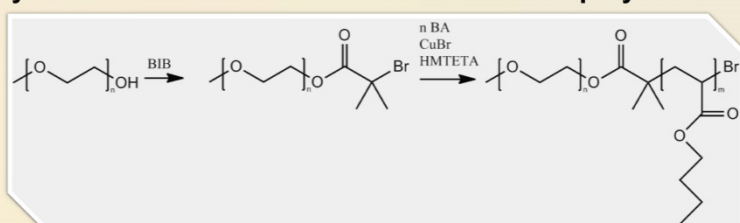
^c Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

E-mail : ppetrov@polymer.bas.bg

Introduction

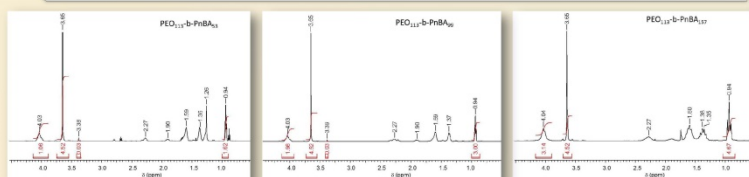
Propolis, a natural product manufactured by bees, possesses immunomodulating, antiinflammatory, antioxidant, antiulcer, antidiabetic, antiobesity and anticancer properties. The poor solubility of the active lipophilic constituents of propolis in aqueous/biological media, however, decreases their bioavailability and limits the practical application of propolis *in vivo*. Block copolymer micelles have been widely exploited for solubilizing a large number of hydrophobic active compounds. The present work reports on the preparation and characterization of colloidal aqueous solution of poplar propolis using poly(ethylene oxide)-block-poly(n-butyl acrylat) diblock copolymers.

Synthesis and Characterization of Block Copolymers

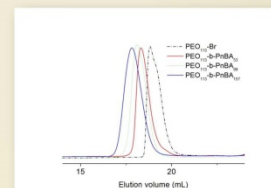


Amphiphilic PEO-b-PnBA diblock copolymers with controlled composition and narrow molar mass distribution were synthesized by ATRP of nBA initiated by PEO-based macroinitiator.

Copolymer composition ¹ H NMR	Mn ¹ H NMR g mol ⁻¹	Mn GPC g mol ⁻¹	PDI ¹ H NMR
PEO ₁₁₃ -Br	5 150	4 253	1.09
PEO ₁₁₃ -b-PnBA ₅₃	11 800	6 977	1.16
PEO ₁₁₃ -b-PnBA ₉₉	17 700	9 143	1.18
PEO ₁₁₃ -b-PnBA ₁₅₇	25 100	12 500	1.31

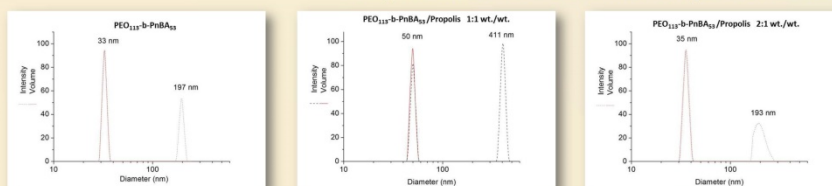


Proton NMR spectra of poly(ethylene oxide)-block-poly(n-butyl acrylat) diblock copolymers

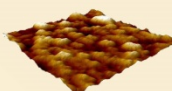
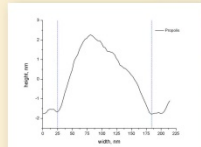
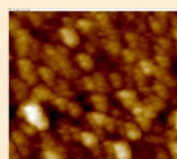
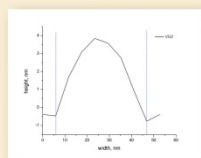
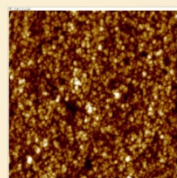


SEC chromatograms of PEO macroinitiator and PEO-b-PnBA block copolymers

Preparation of Colloidal Aqueous Solution



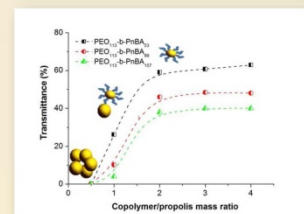
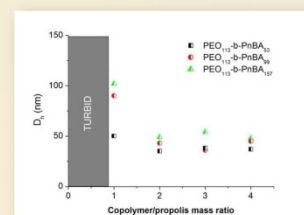
Size distribution of blank PEO₁₁₃-b-PnBA₅₃ micelles and PEO₁₁₃-b-PnBA₅₃ micelles containing different amount of propolis



Atomic Force Microscopy study of micellar and pure propolis

Stable yellowish colloidal aqueous solutions were obtained at copolymer/propolis wt. ratio 2 with all block copolymers studied. The obtained colloidal forms retained their characteristics (transparency, color, stability) upon storage at room temperature for several months. In contrast, pure propolis precipitated in water.

Effect of copolymer composition



Copolymers comprising longer PnBA blocks formed micelles of larger hydrodynamic diameter.

Conclusion: Colloidal aqueous solution of poplar propolis was obtained by encapsulation of the natural product within PEO-b-PnBA micelles. Stable micellar form was prepared at a relatively low copolymer to propolis weight ratio (2). The hydrodynamic diameter of micelles, containing propolis, can be tuned by altering the length of hydrophobic PnBA block. The micellar form of poplar propolis is applicable for *in vivo* experiments.

ХИДРОФОБНО МОДИФИЦИРАНИ СЪПОЛИЕТЕРИ С ЛАТЕНТНА ХИМИЧЕСКА ФУНКЦИОНАЛНОСТ

М. Вълчанова^{1,2}, С. Рангелов¹, С. Турманова²

m.a.valchanova@abv.bg

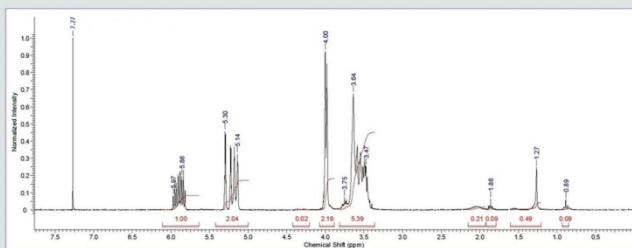
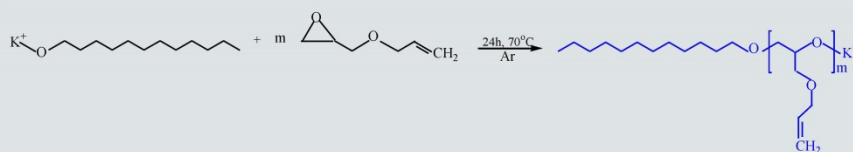
¹Институт по полимери, Българска академия на науките, София

²Катедра "Технологии, материали и материалознание",

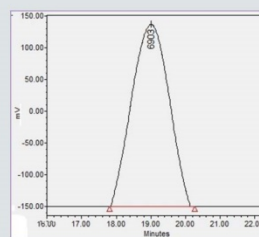
Факултет по технически науки, Университет "Проф. д-р Асен Златаров" гр. Бургас

Синтезирани са нови диблокови съполимери на поли(алилглицидилов етер) и полиглицидол, съдържащи хидрофобен остатък. Първоначално е получен поли(алилглицидилов етер) чрез анионна полимеризация с отваряне на пръстена на алилглицидилов етер, иницирана от калиев додеканолат. При втория етап, поли(алилглицидиловият етер) е използван да иницира анионна полимеризация с отваряне на пръстена на етоксиглицидилов етер, при което са получени серия диблокови съполимери с различни молни съотношения между двата блока. Крайните съполимери, получени след освобождаване на защитните етоксиглицидни групи, съдържат алилови и хидроксилни групи, придаващи латентна химическа функционалност, както и висш алкилов остатък. Съполимерите самоасоциират във водни разтвори и образуват хибридни структури чрез съасоцииране с липидни молекули. Както съполимерите, така и самоасоцираните или съасоцираните структури могат да бъдат химически модифицирани, с което свойствата им могат да се променят радикално.

Синтез на PAGE



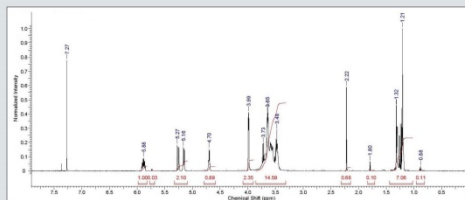
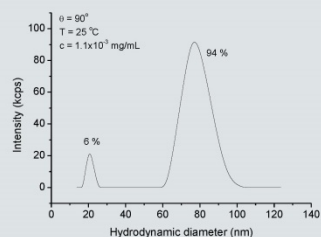
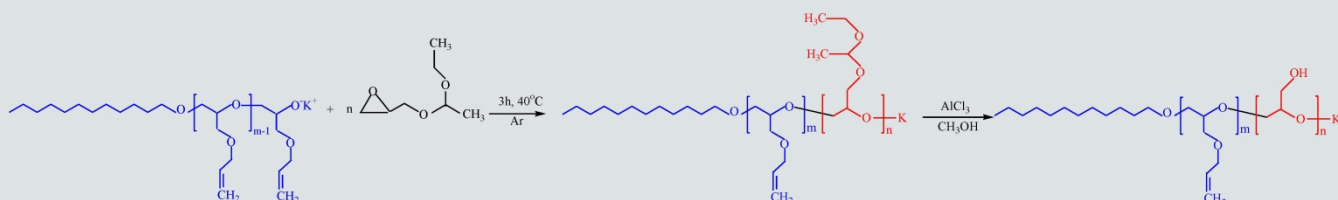
¹H ЯМР спектър на PAGE в CDCl₃



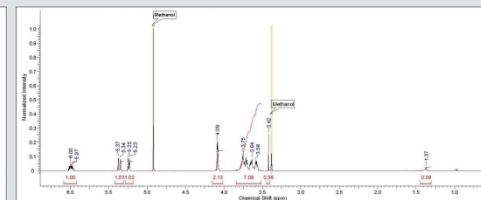
ГПХ на PAGE

M_n = 4900 (¹H ЯМР в CDCl₃)
 DP = 44 (¹H ЯМР в CDCl₃)
 M_n = 6630 (ГПХ)
 M_w = 7530 (ГПХ)
 M_w/M_n = 1.14 (ГПХ)

Синтез на диблокови съполимери PAGE-PG



¹H ЯМР спектър PAGE-PEEGE в CDCl₃



¹H ЯМР спектър PAGE-PG в CDCl₃

Изводи

Чрез анионна полимеризация с отваряне на пръстен са синтезирани серия блокови съполимери поли(алилглицидилов етер)-полиглицидол, съдържащи алилови и хидроксилни функционални групи на всяко мономерно звено от съответния блок. Съполимерите са с фиксирана молна маса на блока PAGE (M_n = 4900), нарастващо съдържание на полиглицидол (25 – 70 mol %) и съдържат хидрофобен остатък. Общата молна маса варира от 7200 до 19200 в зависимост от съдържанието на полиглицидол. Предварителни данни показват, че съполимерите самоасоциират във водни разтвори, като образуват фракция от наноразмерни частици.

PG mol %	DP на PG	M _n (PAGE-PG)
25	16	7200
50	39	10600
70	98	19200

Авторите изказват благодарност за финансовата подкрепа на НИХ при Университет "Проф. д-р Асен Златаров" – Бургас (Проект НИХ-405/2017)

Investigation of The Effect of Novel Polymeric Nanoparticles on Cancer Cell Surface Morphology by Scanning Electron Microscopy

Ivan Mitkov¹, Emi Haladjova³, Kirilka Mladenova², Ralitsa Veleva¹, Veselina Moskova-Doumanova¹, Svetla Petrova², Jordan Doumanov², Petar Petrov³, Tanya Topouzova-Hristova²

¹ Department of Cytology, Histology and Embryology; Faculty of Biology, Sofia University "St. Kliment Ohridski" ² Department of Biochemistry; Faculty of Biology, Sofia University "St. Kliment Ohridski" ³ Institute of Polymers, Bulgarian Academy of Sciences



Introduction

Polymeric nanoparticles have great potential in biology, pharmacy and medicine due to their unique properties, such as the ability to optimize their biodegradability, size and morphology, and to control the mechanisms of their action. They are highly regarded as a competent vector system for gene therapy since they are able to storage, transport and deliver biomolecules (DNA, RNA, proteins) through the cell membrane via the intracellular transport. Therefore, the usage of polymeric nanoparticles is a promising approach in the treatment of cancer and other diseases.

Aims

The aim of this study is to perform comparative analysis of 4 novel types of polymeric nanoparticles in order to determine their effects on A549 human lung cancer cell line regarding alterations of cell morphology.

Materials & Methods

An adenocarcinomic human alveolar basal epithelial cell line – A549, was treated with 4 novel types of nanoparticles in a previously determined non-toxic concentration – 10 µg nanoparticles/mL for 15 and 45 min.

The nanoparticles were based on:

poly(2-(N,N-dimethylamino)ethylmethacrylate) and poly(caprolactone) triblock copolymer

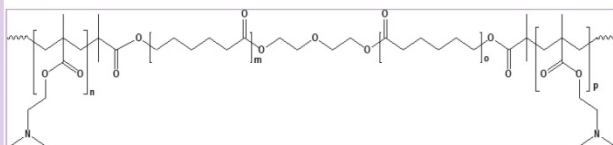


Figure 1. Chemical structure of poly(2-(N,N-dimethylamino)ethylmethacrylate) and poly(caprolactone) triblock copolymer

The 4 types of nanoparticles were as follows:

- m** – Polymer micelles based
- bc** – Micelleplexes (plasmid DNA electrostatically bound to polymer micelles)
- ac** – Micelleplexes coated with polymeric capsule based on N-Isopropylacrylamide
- acd** – Polymer capsules containing plasmid DNA

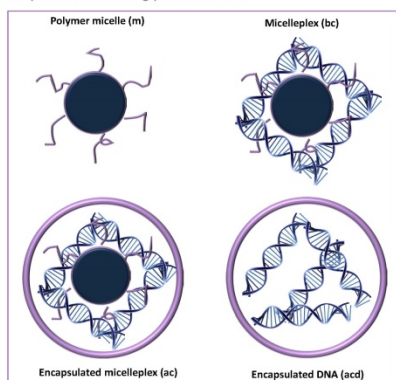


Figure 2. Structures of the used polymeric micelles, micelleplexes and nanocapsules.

For inspection and assessment of possible cell surface alterations caused upon incubation with the nanoparticles, cell probes were fixed, coated with carbon and gold, and Scanning Electron Microscopy (SEM) was performed using a Lyra Tescan microscope.

Conclusions

The analysis of the SEM images showed that incubation of A549 cells with the nanoparticles resulted in slight alterations of cell morphology, manifested as reduction of cell adhesion and membrane perturbation, regardless of the type of nanoparticles used. Moreover, no apoptotic signs or detrimental effects of the nanoparticles on cell surface integrity and morphology were observed, which correlates with our previously collected data of low toxicity of these polymeric nanoparticles on A549 cells. Therefore, these polymeric nanoparticles might be suitable transport systems for gene delivery and transfection.

Results

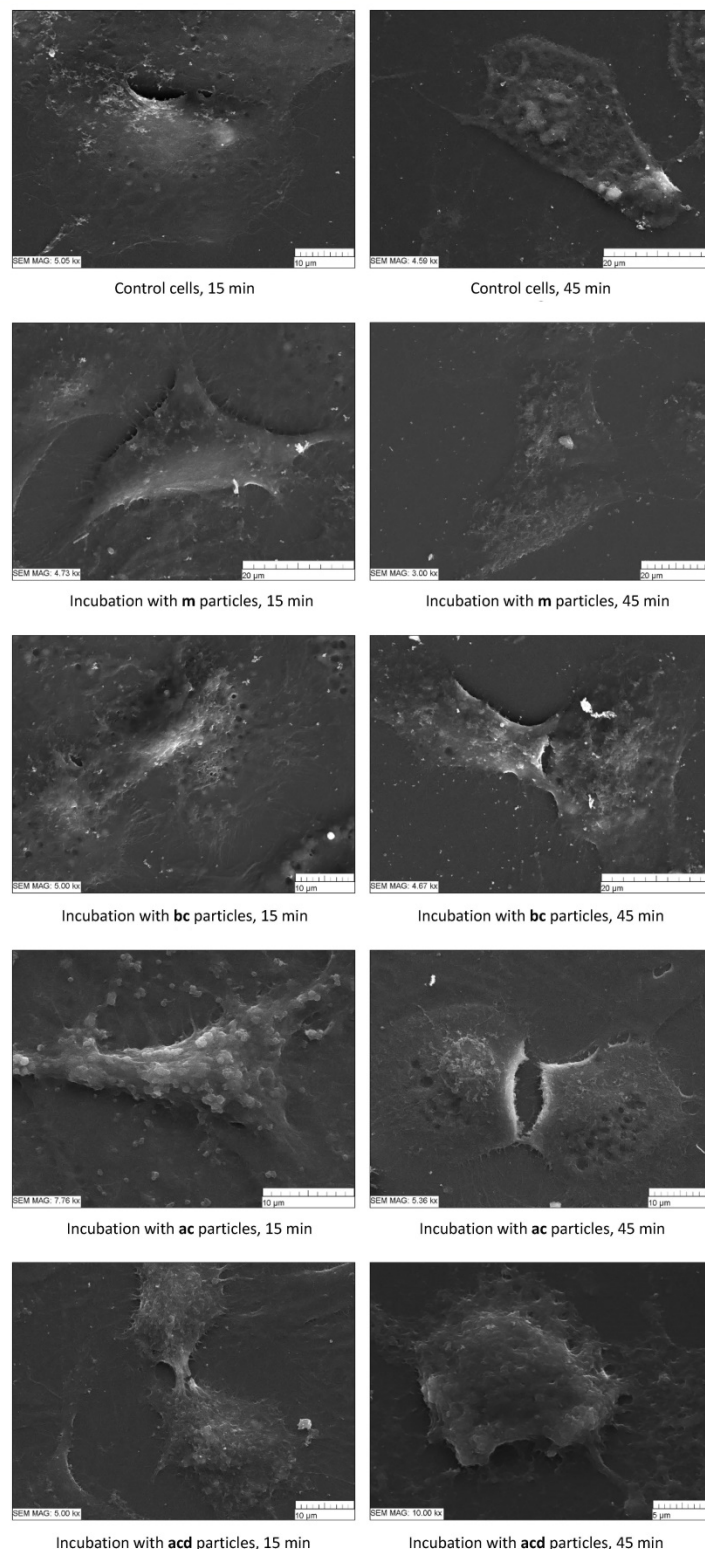


Figure 3. SEM Images of A549 cells incubated with polymeric nanoparticles for 15 and 45 mins.

Acknowledgements: This work was supported by grant DFNI-T02-7 from Bulgarian National Science Fund.



Electrospun cellulose acetate fibers decorated by electrospayed PVP-curcumin particles

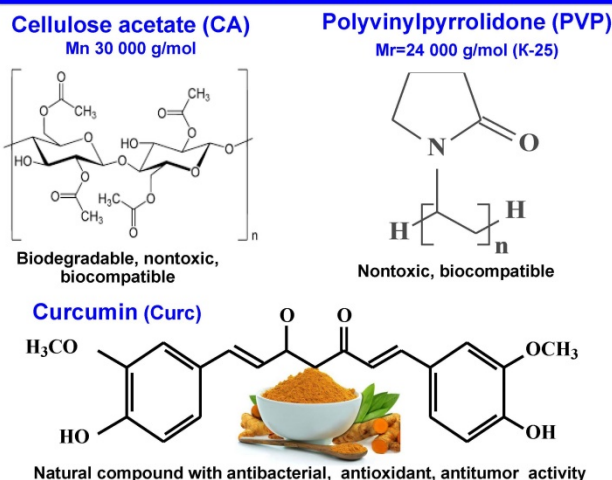


Petya Tsekova^a, Mariya Spasova^a, Nevena Manolova^a, Iliya Rashkov^a, Nadya Markova^b

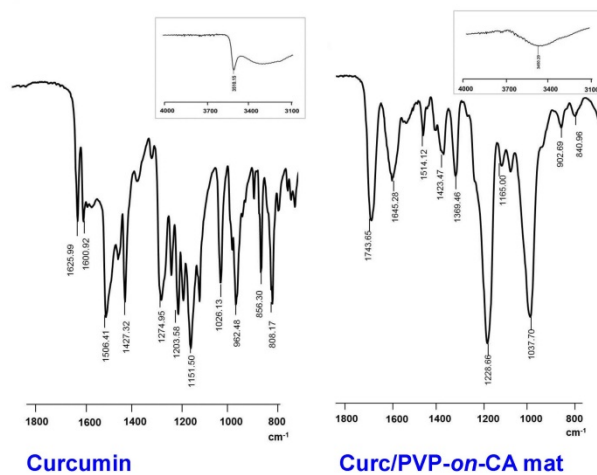
^aLaboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, bl. 103A, BG-1113 Sofia, Bulgaria, ^bInstitute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev St, bl. 26, BG-1113 Sofia, Bulgaria

Curcumin (Curc) exhibits anti-inflammatory, antibacterial and antitumor activity. However, its application is limited by its poor bioavailability related to its extremely low water solubility [1]. The AIM of the present study is to prepare and to characterize novel materials allowing increased release of Curc in aqueous medium. The new materials consisted of electrospun fibers from cellulose acetate (CA) decorated with electrospayed Curc/polyvinylpyrrolidone (Curc/PVP) particles [2].

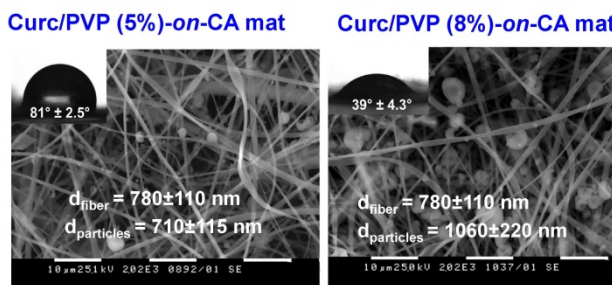
Materials



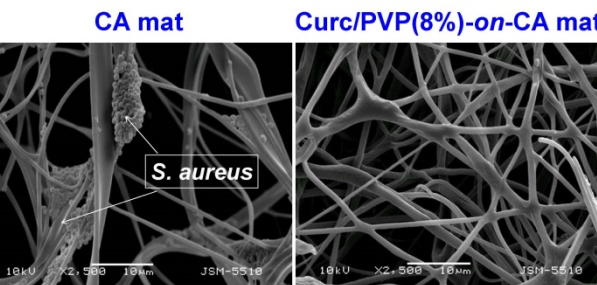
IR-spectra



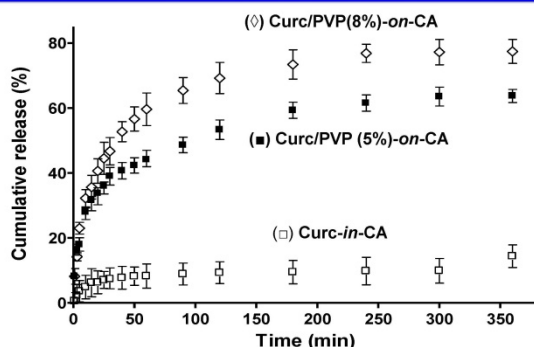
SEM micrographs



Adhesion of S. aureus

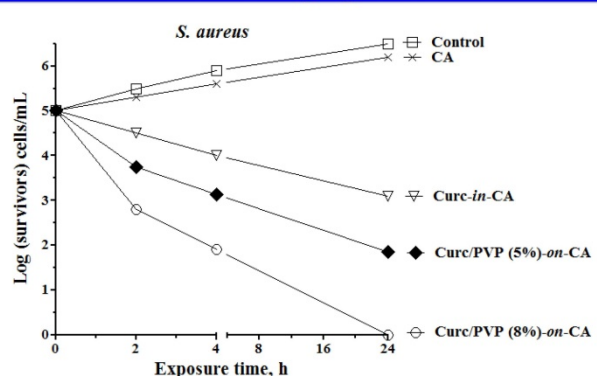


In vitro release of Curc



acetate buffer/PVP (98/2 v/v), pH 5.5, 37°C, ionic strength 0.1.

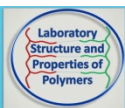
Antibacterial activity against S. aureus



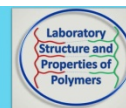
Conclusion. Novel fibrous mats based on CA decorated with Curc/PVP particles have been successfully prepared by conjunction of electrospinning and electrospaying techniques. The incorporation of PVP led to hydrophilization of the mats and facilitated Curc release. Moreover, the Curc-containing mats exhibited antibacterial activity against *S. aureus*.

References [1] G. Yakub, A. Toncheva, N. Manolova, I. Rashkov, V. Kussovski, D. Danchev, *J. Bioact. Compat. Polym.: Biomed. Appl.*, 29 (2014) 607–627.
[2] P. Tsekova, M. Spasova, N. Manolova, N. Markova, I. Rashkov, *Mater. Sci. Eng. C*, 73 (2017) 206–214.

Acknowledgement: The authors thank the National Science Fund of Bulgaria for the financial support (Grant number DFNI–T02/1-2014).



СИНТЕЗ И ОХАРАКТЕРИЗИРАНЕ НА КРИОГЕЛОВЕ ОТ СМЕСЕНИ ПОЛИМЕРНИ МРЕЖИ



Георги Л. Георгиев и Петър Д. Петров

Лаборатория „Структура и свойства на полимерите“
Институт по полимери, Българска академия на науките,
ул. „Акад. Г. Бончев“, блок 103 А, 1113 София, България.

Въведение: Полимерните криогелове са вид гелове, образувани чрез криогенно третиране (замразяване-омрежване-размразяване) на разтвори или колоидни дисперсии. Процесът на замразяване води до образуване на хетерофазна система, съставена от течната и твърда микрофази. В течната микрофаза, съдържаща реагентите и физически свързаната вода, протича омрежването, а твърдата микрофаза, съставена от ледени кристали, играе ролята на пороген. Формираната макропореста структура на криогелите позволява добра дифузия на течности и вещества, поради което тези материали намират широко приложение в медицината и фармацевцията като лекарстводоставящи системи, клетъчни носители, в тъканното инженерство и др.

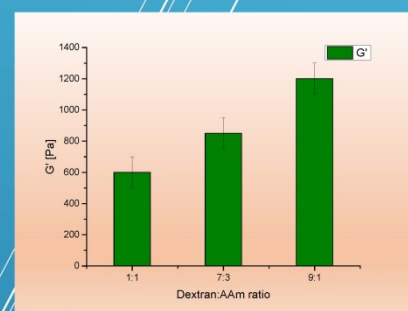
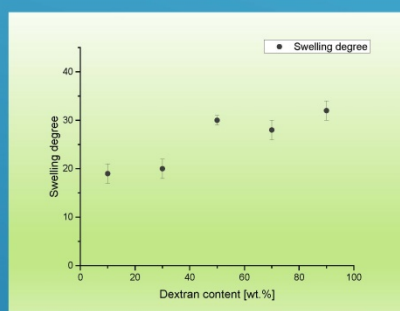
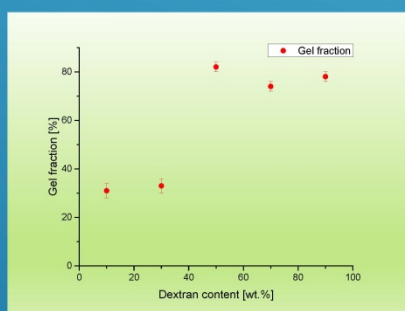
Цел: Получаване на криогелове от смеси декстран/акриламид и декстран/N-изопропилакриламид чрез УВ-облъчване.



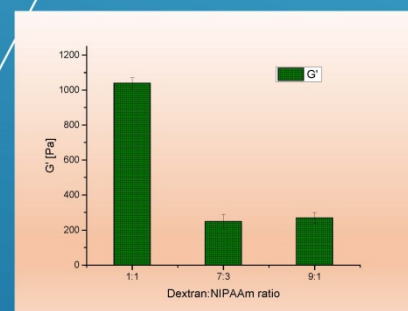
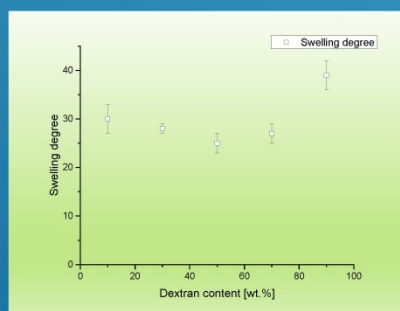
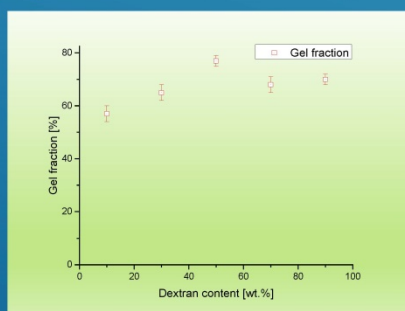
Фотохимичното омрежване на смесите на декстран/акриламид и декстран/N-изопропилакриламид е осъществено чрез използването на смес от два водоразтворими фотоинициатора: (4-бензоилбензил) триметиламониев хлорид и водороден пероксид в съотношение 1:1 и в присъствието на N,N-метиленис-акриламид като омрежващ агент.

Резултати:

1). Криогелове от смеси на декстран и акриламид

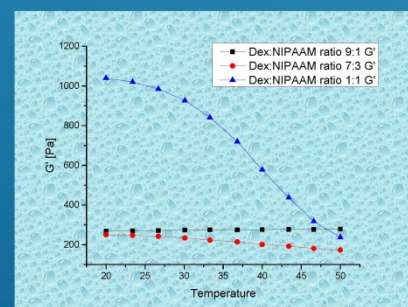


2). Криогелове от смеси на декстран и N-изопропилакриламид



Изводи:

За пръв път са синтезирани криогелове от смеси декстран/акриламид и декстран/N-изопропилакриламид чрез УВ-индуцирано омрежване. Установено е, че криогелове получени от смеси на декстран и N-изопропилакриламид проявяват изразена термочувствителност при минимално съдържанието на N-изопропилакриламид в изходната смес 50 мас. %. Също така, с увеличаване количеството на декстрана се увеличава добива на гел фракцията.



Благодарности: Авторите благодарят за финансовата подкрепа на проект ДФНП-7/20.04.2016.

Iron (III) chloride catalyzed carbonyl olefin metathesis polymerization of chalcone

Hristo Penchev¹, Silvia Dimova¹, Filip Ublekov¹, Christo Novakov¹, Katerina Zaharieva²

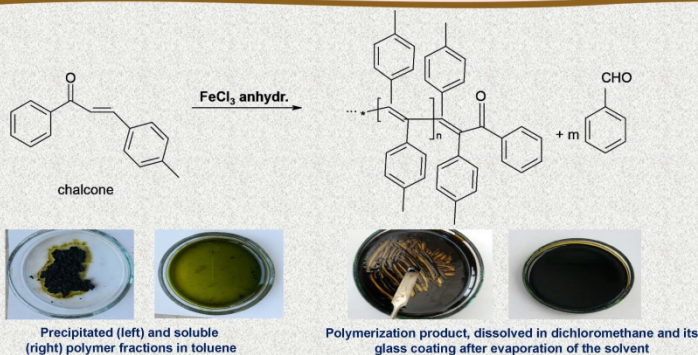
¹ Institute of polymers, Bulgarian Academy of Sciences

² Institute of catalysis, Bulgarian Academy of Sciences

E-mail: dimova@polymer.bas.bg

For the first time, we demonstrate Iron (III) chloride catalyzed carbonyl-olefin metathesis polymerization of chalcone. The element iron, an Earth-abundant and environmentally benign transition metal and its compounds are often used as catalysts. This polymerization is characterized by its operational simplicity and mild reaction conditions. The synthesized conjugated oligomers, in situ doped with FeCl₃, are soluble in common volatile organic solvents and readily form thin films and coatings which broadens the potential application of these oligomers.

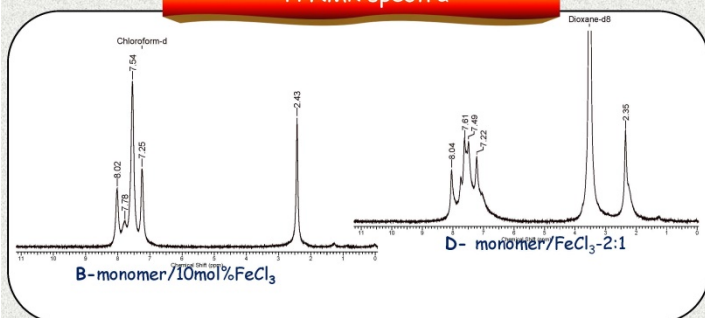
SYNTHESIS AND CHARACTERIZATION



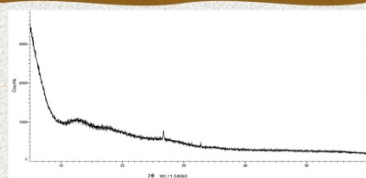
Experimental conditions

Monomer : catalyst = chalcone : FeCl₃, Solvent : 1,2- Dichloroethane
Temperature 60°C, 1h and room temperature, 12h.
The obtained products were isolated as an dark viscous liquid.

¹H NMR spectra

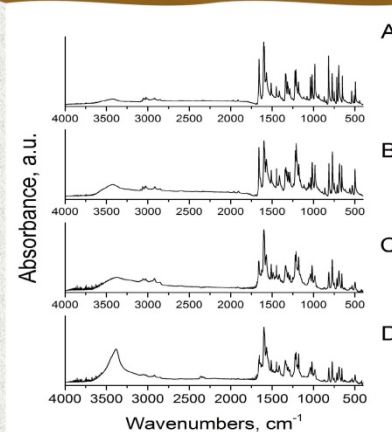


XRD analysis



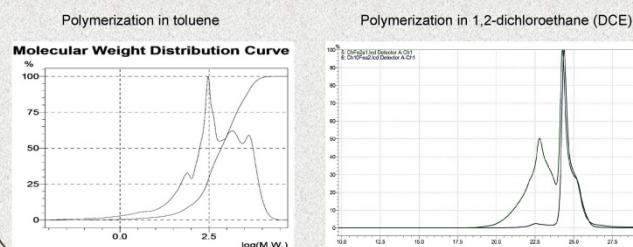
The XRD analysis of the polymerization product in toluene showed amorphous halo and two characteristic crystal reflects which is indication for partial crystallization of the obtained oligomer.

FT- IR spectra



IR spectra of A- monomer, B- monomer/10mol%FeCl₃, C- monomer/FeCl₃-1:1, D- monomer/FeCl₃-2:1

Size exclusion chromatography



The SEC analysis showed significant influence of the used solvent on the polymerization yield molecular weight and MW distribution of the synthesized oligomers. The polymerization in DCE gives only trimeric oligomer, while in toluene M_v 5000 g.mol⁻¹ fraction is achievable.

CONCLUSIONS

- ✓ An effective synthesis of poly(phenyl-substituted vinylidenes) has been demonstrated for the first time by carbonyl-olefin metathesis polymerization of chalcone, catalyzed by anhydrous Iron (III) chloride - cheap and widely available chemical.
- ✓ In situ Fe-doped conjugated oligomers could be obtained by this procedure.
- ✓ Synthetic conditions were varied for preparation of different conjugated oligomers.
- ✓ The FT-IR spectroscopy shows vibration band around 1600cm⁻¹. This are attributed to the presence of conjugated vinylidene backbones. Very strong UV-absorption at 256 nm was observed.

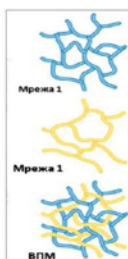


МЕТАКРИЛАТНИ ВЗАИМНОПРОНИКВАЩИ ПОЛИМЕРНИ МРЕЖИ КАТО НОСИТЕЛИ НА ПРОМЕТАЗИН ХИДРОХЛОРИД



Й. Йълдъръм¹, М. Симеонов¹, Х. Цачев², Е. Василева¹

¹Лаборатория по структура и свойства на полимерите, Факултет по химия и фармация, бул. Дж. Баучер 1, София 1164, България;
²Лаборатория по Фармацевтична технология, Факултет по химия и фармация, бул. Дж. Баучер 1, София 1164, България;



Въведение:

Взаимнопроникващите полимерни мрежи (ВПМ) са клас полимерни материали, които комбинират свойствата на две или повече полимерни мрежи, които са частично преплетени, но не са ковалентно свързани помежду си. Подборът на компонентите, изграждащи ВПМ, в съчетание с тяхната фазово разделена структура обуславят перспективното им приложение в сферата на лекарственото доставяне.

Цел:

Настоящото изследване има за цел да разкрие потенциала на ВПМ от поли(метакрилова киселина)(ПМАК) и поли(N,N-диметилакриламид) (ПДМАА) като системи за удължено доставяне на Прометазин хидрохлорид (ПХХ).

Синтез на ВПМ

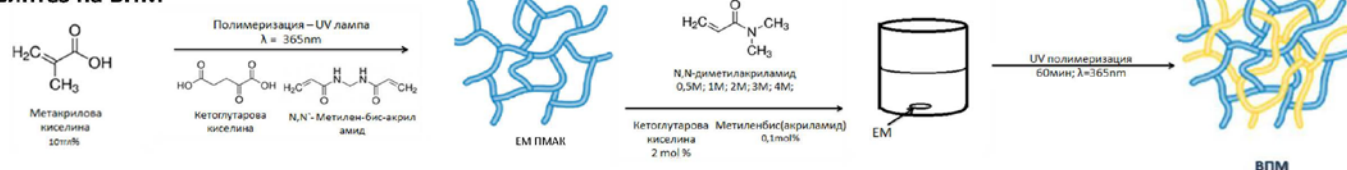
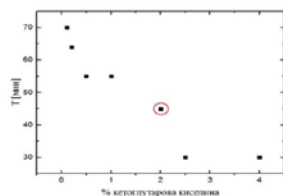


Схема 1. Синтез на ВПМ ПМАК/ПДМАА посредством последователен метод на синтез



Фигура 1. Време на полимеризация на метакрилова киселина като функция на концентрацията на КГК



Фигура 2. Единични мрежи от ПМАК, получени за различни концентрации на КГК

Таблица 1. Състав на получените ВПМ

Проба:	1	2	3	4	5
$C_{\text{ПДМАА}} [M]$	0,5	1	2	3	4
$r_{\text{ПМАК}}$	0,66	0,55	0,22	0,14	0,09



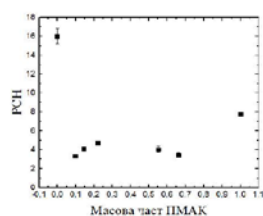
Фигура 3. ВПМ ПМАК/ПДМАА

Оптималните за целта на настоящото изследване условия на полимеризация на МАК са 2% КГК и 45 мин реакционно време.

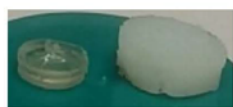
$$r_{\text{ПМАК}} = \frac{m_{\text{ПМАК}}}{m_{\text{ПМАК}} + m_{\text{ПДМАА}}}$$

$r_{\text{ПМАК}}$ - тегловна част на ПМАК във ВПМ

ОХАРАКТЕРИЗИРАНЕ НА ВПМ

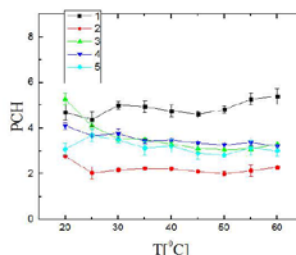


Фигура 4. Равновесна степен на набъване (РСН) като функция от състава на ВПМ ПМАК/ПДМАА

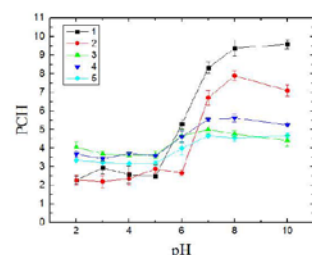


Фигура 5. ВПМ 4 в сухо състояние и в равновесната си степен на набъване

$$РСН = \frac{m_{\text{ПМАК}}^{\text{наб}} - m_{\text{ПМАК}}^{\text{сухо}}}{m_{\text{ПДМАА}}}$$



Фигура 6. Температурна чувствителност на ВПМ ПМАК/ПДМАА



Фигура 7. pH чувствителност на ВПМ ПМАК/ПДМАА

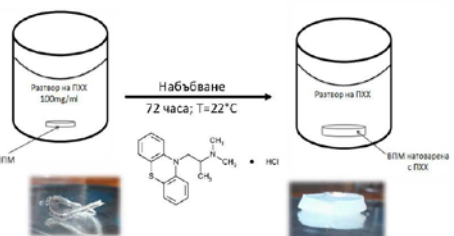
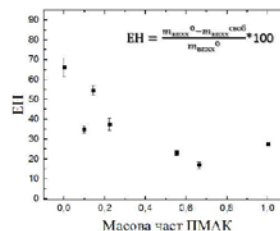
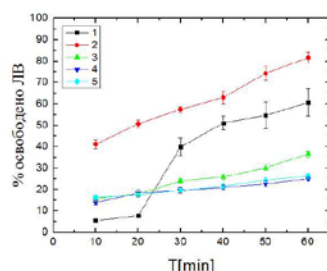


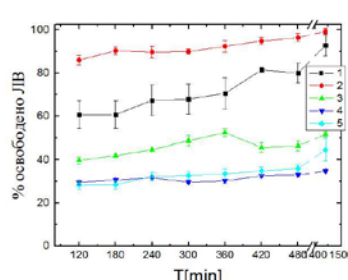
Схема 2. Натоварване на ВПМ с ПХХ



Фигура 8. Ефективност на натоварване (ЕН) на ЛВ във ВПМ



Фигура 9. Освобождаване на ЛВ при pH = 1,2; T = 60мин



Фигура 10. Освобождаване на ЛВ при pH = 6,8; T = 1380 мин

ИЗВОДИ:

- За пръв път ВПМ на ПМАК и ПДМААМ са синтезирани и изследвани като лекарственодоставящи системи.
- Експериментално-получените зависимости на РСН и ЕН от състава на ВПМ дават основание да се предположи, че натоварването на ПМАК се осъществява чрез пасивна дифузия.
- Получените ВПМ имат добре изразена pH чувствителност, но не притежават температурна чувствителност в изследвания температурен интервал.
- Съставът на ВПМ пряко влияе върху профилите на лекарствено освобождаване.

Благодарности: Тази работа е осъществена с финансовата подкрепа на проект 80-10-224, ФНИ при СУ.

INTERACTIONS OF GOLD-CONTAINING NANOPARTICLES WITH HUMAN EPITHELIAL CELLS

Svetoslava Stoycheva*, Emi Haladjova***, Kirilka Mladenova*, Yulia Petseva*, Zdravko Lalchev*, Veselina Moskova-Doumanova**, Tanya Topouzova-Hristova**, Stanislav Rangelov***, Jordan Doumanov*

*Sofia University, Faculty of Biology, Department "Biochemistry" and **Department "Cytology, Histology and Embryology";
***Bulgarian Academy of Sciences, Institute of Polymers

Introduction

Delivery of exogenous gene into cells has great importance in gene function research, genetic modification of cells, and gene therapy. Non-viral gene carriers such as cationic lipids and polymers have been potential alternatives for safe and efficient gene delivery due to their good biocompatibility, versatility, and controllable molecule size. They should be stable, must have no negative effect to the cell metabolism, and DNA should remain intact during transport. The gene delivery efficiency of these materials is usually influenced by many factors, such as the quantity and quality of DNA, ratio of DNA to material, size and toxicity.

Aim

The aim of this study is to investigate the effects of gold-containing nanoparticles on cell viability and morphology of human epithelial cells - A549 and HepG2.

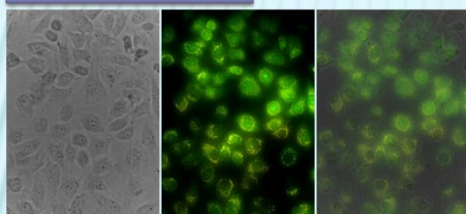
Methods

In this study we used nanoparticles of poly(vinyl butyl trimethyl ammonium chloride) (Homo) and poly(vinyl butyl trimethyl ammonium chloride) - block-poly(oligoethylene glycol metacrylate) containing gold. The working concentration of nanoparticles also contain 0.2 µg/ml DNA.

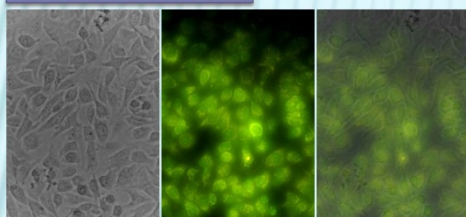
HepG2 (hepatocytes) and A549 (lung) cells were grown in DMEM F-12 Ham and in DMEM respectively with 10% (v/v) FCS, streptomycin (0.1 mg/ml), penicillin (0.06 mg/ml) at 37°C in 5% CO₂ water-saturated atmosphere. For MTT assay both cell types were treated with nanoparticles, containing 0.2 µg/ml DNA for 6h. Then cell viability was determined immediately after treatment (6 h) and at 24, 48, 72, and 96 h by using MTT test, based on metabolic activity of the cells. Fluorescence staining is performed using acridin-orange, which is suitable for visualization of microsomal fraction. Cells were incubated for 30 min with nanoparticles. Fluorescence microscopy images were carried out immediately after treatment with Nikon Eclipse Ti-U (Nikon GmbH, Vienna) using 40x objective lens.

Results

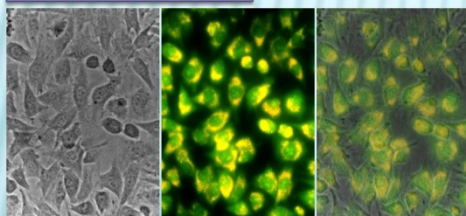
A549 Homo8 M1



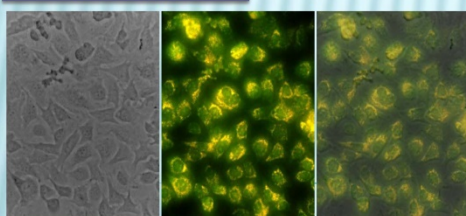
A549 Homo8 M3



A549 BC8 M1



A549 BC8 M3



A549 Controls

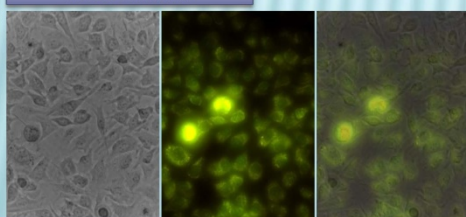


Figure 6: Fluorescent staining with Acridine orange of A549 cells, incubated 30 min with nanoparticles

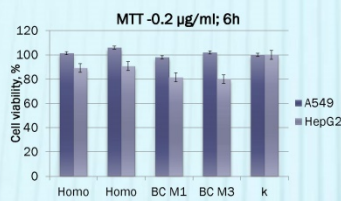


Figure 1: MTT-test for A549 and HepG2 cells, 6h after treatment; working concentration is 0.2 µg/ml

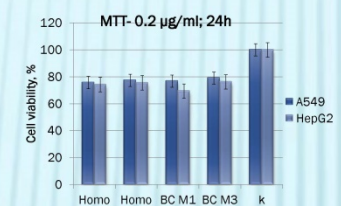


Figure 2: MTT-test for A549 and HepG2 cells, 24h after treatment; working concentration is 0.2 µg/ml DNA

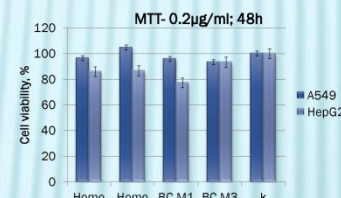


Figure 3: MTT-test for A549 and HepG2 cells, 48h after treatment; working concentration is 0.2 µg/ml DNA

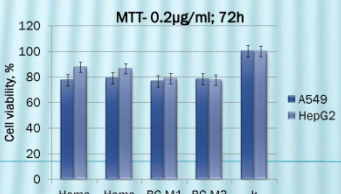


Figure 4: MTT-test for A549 and HepG2 cells, 72h after treatment; working concentration is 0.2 µg/ml

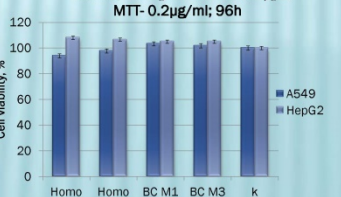
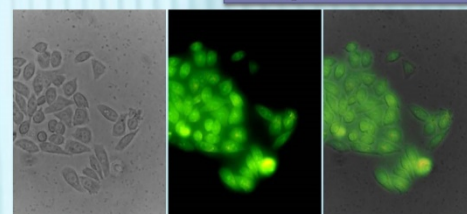
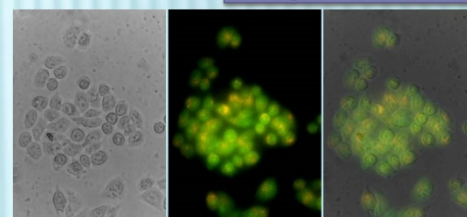


Figure 5: MTT-test for A549 and HepG2 cells, 96h after treatment; working concentration is 0.2 µg/ml

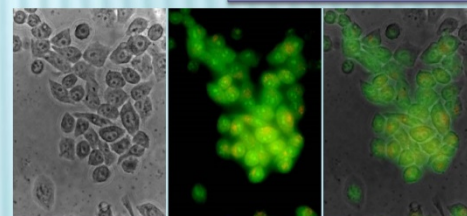
HepG2 Homo8 M1



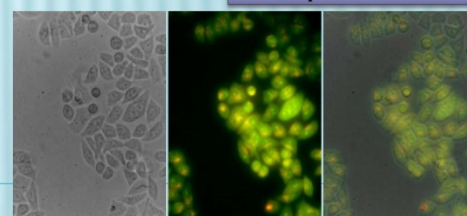
HepG2 Homo8 M3



HepG2 BC8 M1



HepG2 BC8 M3



HepG2 Controls

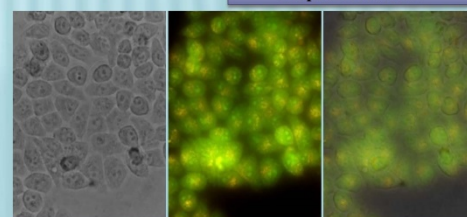


Figure 7: Fluorescent staining with Acridine orange of HepG2 cells, incubated 30 min with nanoparticles

Conclusions:

1. Working concentrations of nanoparticles that were used for experiments are non-toxic to cells;
2. Treated and untreated cells don't show significant difference in their morphology.
3. We didn't observe significant increase in the lysosomal fraction after 30 min treatment of the cells.
4. Acridin-orange staining propose non-endosomal entering of these nanoparticles into the cells. Entering of nanoparticles may cause temporary disruptions of cell membrane, which could explain cell viability rate in the 24 and 48h.

Acknowledgements: The research was funded by project DFNI - T02 / 7 from 12.12.2014

Изследване на влиянието на геометрията на колектора върху физикомеханичните свойства на електроовлакнени материали от поли(3-хидроксибутират)



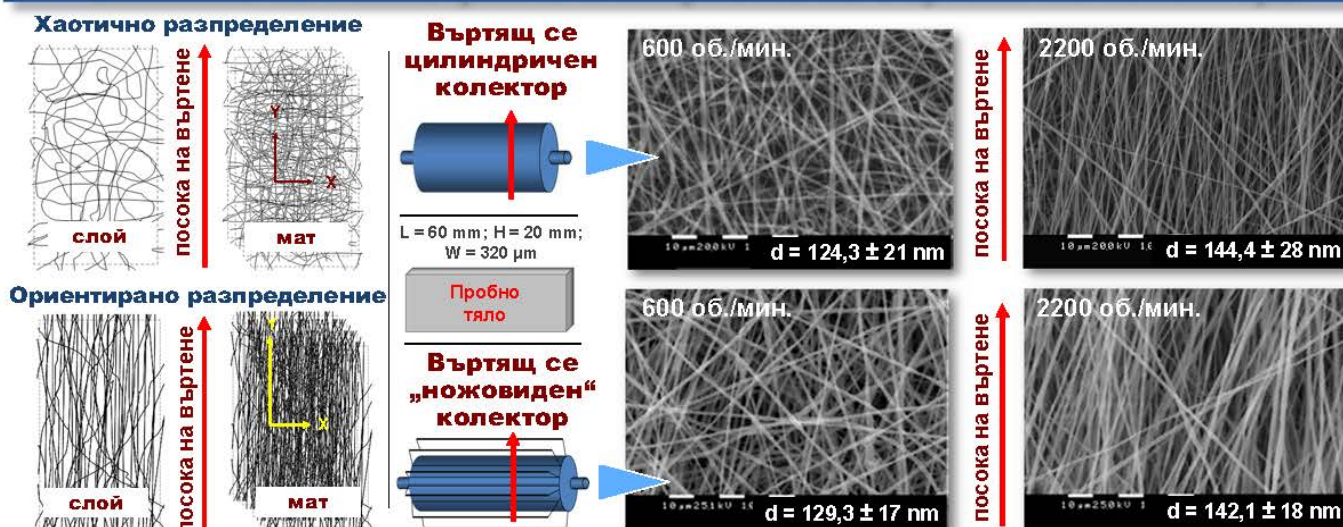
И. Борисова, О. Стоилова*, Н. Манолова*, Ил. Рашков



Лаборатория Биологично активни полимери, Институт по полимери, Българска академия на науките, 1113 София, ул. акад. Г. Бончев бл. 103А

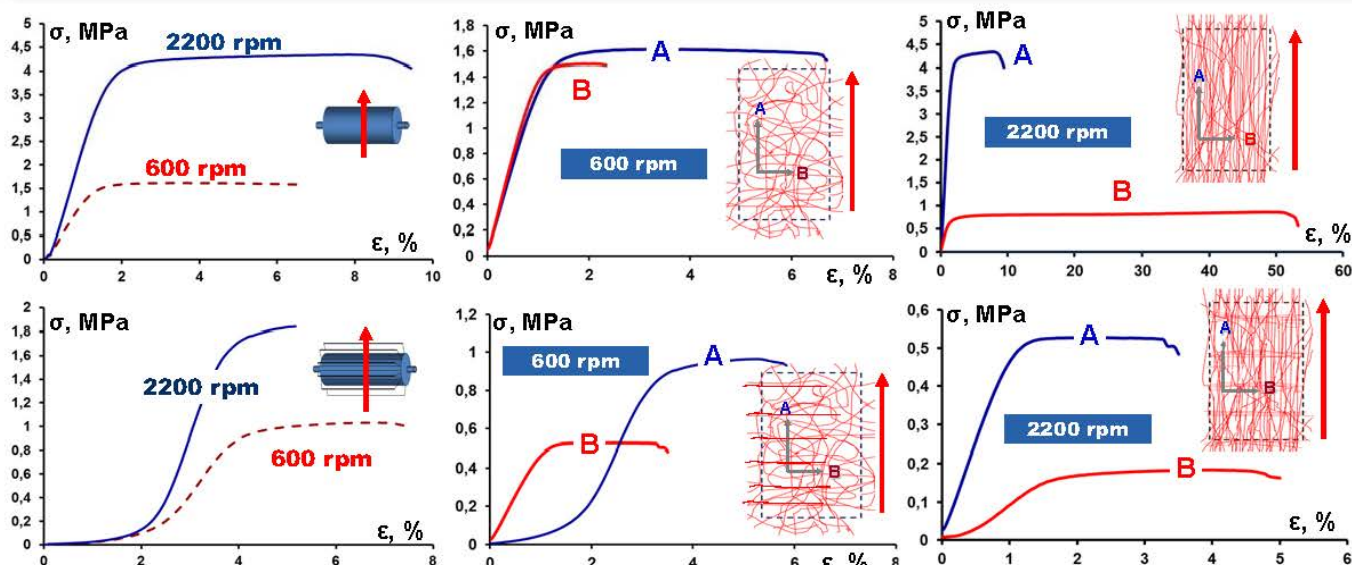
Целта на работата е да се изследват възможностите за получаване на материали от поли(3-хидроксибутират) (ПХБ) чрез електроовлакняване с подобрени физикомеханични свойства и да се изследва влиянието на геометрията на колектора и ориентацията на влакната върху тези свойства.

Влияние на геометрията и скоростта на въртене на колектора



Геометрията на колектора влияе върху ориентацията на влакната. Повишаването на скоростта на въртене на колектора води до допълнителното им ориентирание.

Физикомеханични свойства на матовете



Повишаването на скоростта на въртене на колектора води до повишаване на механичните свойства на получените ПХБ материали. Пробите, изрязани по посока на въртене на колектора (А, при 600 и 2200 rpm) показват по-добри механични показатели спрямо тези, изрязани под 90° (В, при 600 и 2200 rpm).

Благодарност



Авторите изказват своята благодарност на Фонд Научни изследвания за финансовата подкрепа по Договор ДН 09/2.

manolova@polymer.bas.bg
stoilova@polymer.bas.bg



New application of hybrid polymer systems for the destruction of bacterial biofilms



Haladjova E.¹, Paunova-Krasteva Ts.², Borisova D.², Kyulavska M.¹, Stoitsova S.²

¹ Institute of Polymers, Bulgarian Academy of Sciences

² The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

INTRODUCTION

Biofilms are matrix-enclosed communities of bacteria that show increased antibiotic resistance and the capability to evade the immune system. Bacterial biofilm contamination of various surfaces in hospitals and food industry represents a serious health risk. A major concern is their intrinsic resistance to routinely applied antibacterials and disinfectants. This requires the development of novel approaches to biofilm detachment.

The AIM of the study is to evaluate the effectiveness of hybrid polymer systems against a pre-formed bacterial biofilm.

MATERIALS AND METHODS

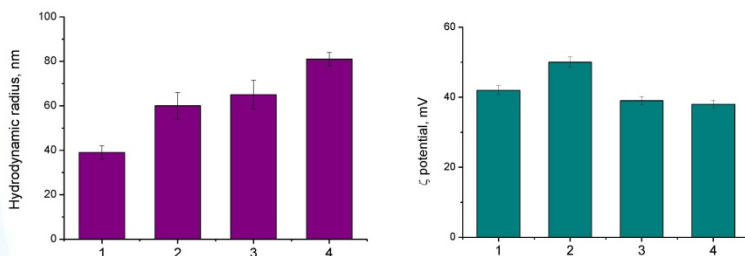
Hybrid polymer systems based on different polycations were used. Some of them were loaded with gold nanoparticles. The loading was performed in two methods. The samples were as follow:

- 1-system based on poly(2-(dimethylamino)ethyl methacrylate)-block-poly(ε-caprolactone)-block-poly(2-(dimethylamino)ethyl methacrylate) triblock copolymer;
- 2-system based on poly(vinyl benzyl trimethylammonium chloride)homopolymer;
- 3-system based on poly(vinyl benzyl trimethylammonium chloride)homopolymer preloaded with gold nanoparticles;
- 4-system based on poly(vinyl benzyl trimethylammonium chloride)homopolymer post loaded with gold nanoparticles.

P. aeruginosa PAO1, previously shown to be a good biofilm former, was used as a model microorganism. The bacterial biofilm was cultivated for 24h on 96-well plates. Then the medium was removed and the biofilm was treated with the nanoparticles for 2 or 4 h respectively.

RESULTS

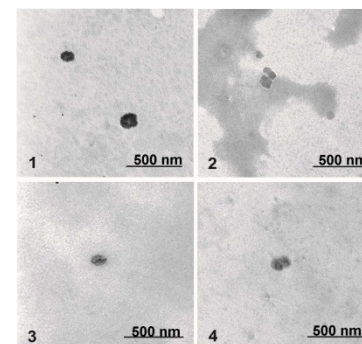
Dynamic and electrophoretic light scattering



The hydrodynamic radius of hybrid polymer systems ranged from 35 to 120 nm depending on polycation used and the presence of gold nanoparticles.

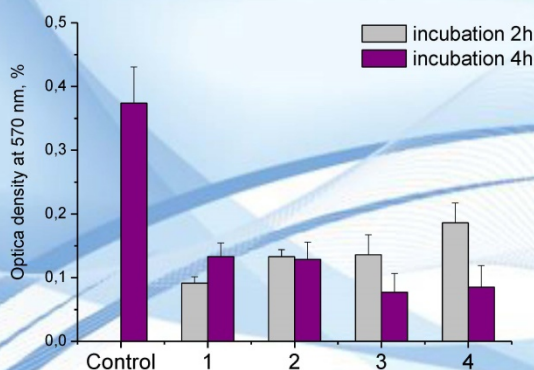
The ζ-potential of hybrid polymer systems was strongly positive independent on polycation used or the presence of gold nanoparticles.

Transmission electron microscopy



The morphology of hybrid polymer systems was visualized by TEM. All systems exhibit spherical shape and their sizes were comparable with those determined from DLS.

Estimation of biofilm detachment



CONCLUSION

The results indicate the applicability of the tested hybrid polymer systems for disinfection of biofilm-contaminated surfaces.



Антибактериална активност и антибиофилм-образуваща способност на полицивтерйони

Констанс Русева^{1,2}, Кристина Иванова², Елена Василева¹, Цанко Цанов²



¹Лаборатория по структура и свойства на полимерите, Факултет по химия и фармация, СУ“Св. Кл. Охридски“, София, България

²Лаборатория по молекулярни и индустриални биотехнологии, Политехнически университет на Каталуня, Тераса, Испания

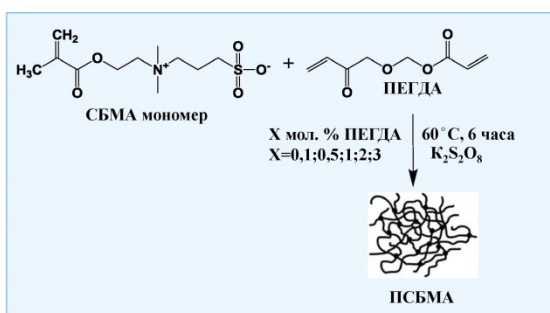
УВОД

Полицивтерйоните (ПЦЙ) са обект на засилен интерес в последните години с цел разработване на нови биосъвместими материали с приложение в медицината. Причината за това е, че те притежават отлична биосъвместимост и едновременно с това проявяват характеристики на „интелигентни материали“ – те могат да откликват на външни стимули като промяна в рН, температурата и йонната сила на средата. Все още, обаче, техните антибактериални свойства и способността им да инхибират образуването на биофилми са неизследвани.

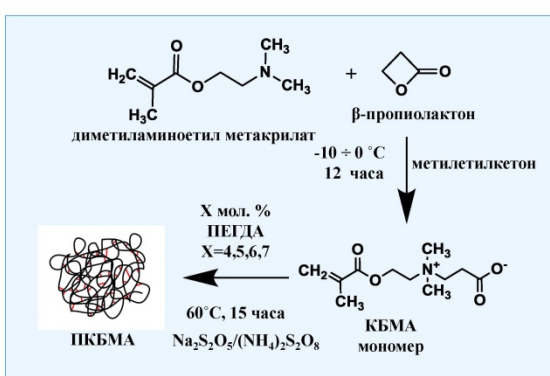
ЦЕЛ

Целта на изследването е да изучи антибактериалните свойства и способността за инхибиране на образуването на биофилми от хидрогелове на два типа ПЦЙ – поликарбоксиветаини и полисулфобетаини. За изпълнение на тази цел бяха синтезирани и охарактеризирани полимерни мрежи от сулфобетаин метакрилат (СБМА) и карбоксиветаин метакрилат (КБМА) с омрежващ агент полиетиленгликолдиакрилат (ПЕГДА).

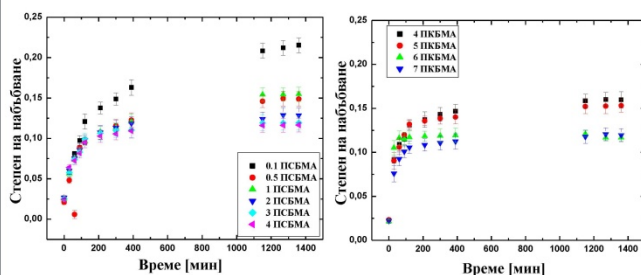
СИНТЕЗ НА ПОЛИМЕРНИ МРЕЖИ ОТ СБМА



СИНТЕЗ НА ПОЛИМЕРНИ МРЕЖИ ОТ КБМА

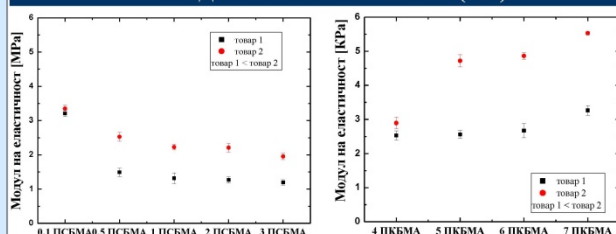


КИНЕТИКА НА НАБЪВАНЕ



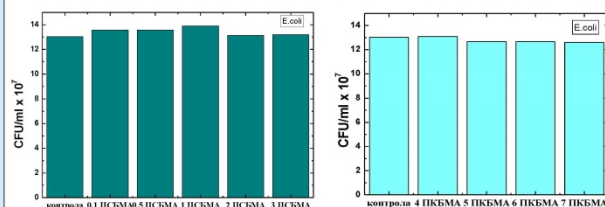
Степента на набъване при двата типа полимерни мрежи намалява с увеличаване на концентрацията на омрежващ агент, тъй като гъстотата на мрежата нараства.

МОДУЛ НА ЕЛАСТИЧНОСТ (МЕ)



МЕ на хидрогелите на ПСБМА показва тенденция на намаляване с увеличаване на концентрацията на ПЕГДА. При хидрогелите на ПКБМА се наблюдава обратна тенденция, като те имат значително по-високи МЕ в сравнение с хидрогелите на ПСБМА. Това се дължи на по-високото количество омрежващ агент, което води до по-гъста мрежа, а оттам и до по-висок МЕ.

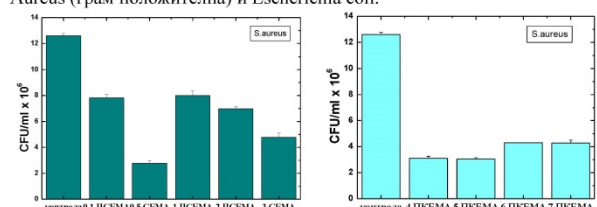
АНТИБАКТЕРИАЛНА АКТИВНОСТ СРЕЩУ E.COLI



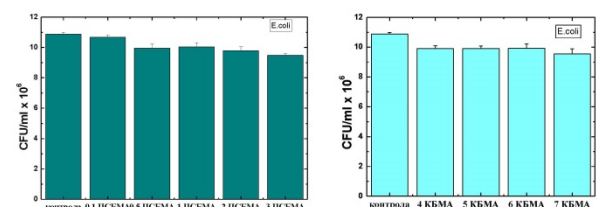
Антибактериалната активност бе изследвана срещу Escherichia coli (грам отрицателна), а като контрола бе използван силикон. Хидрогелите на ПСБМА и ПКБМА не проявяват антибактериална активност срещу Escherichia coli, т.е. те проявяват същата активност като контролата.

АНТИБИОФИЛМ ОБРАЗУВАЩА СПОСОБНОСТ

Способността за инхибиране на образуването на биофилм от страна на хидрогелите на ПСБМА и ПКБМА бе тествана срещу Staphylococcus Aureus (грам положителна) и Escherichia coli.



Двата типа ПЦЙ хидрогелове демонстрират способност да инхибират биофилм образуването от Staphylococcus Aureus, като ПКБМА показват по-силна инхибираща способност в сравнение с ПСБМА.



Двата типа полимерни мрежи демонстрират слабо изразена способност да инхибират биофилм образуването на Escherichia coli.

ИЗВОДИ

- Двата изследвани типа ПЦЙ мрежи:
 - не показват антибактериална активност срещу Escherichia coli
 - имат значителен инхибиращ ефект срещу образуване на биофилм от Staphylococcus Aureus
 - показват слаб инхибиращ ефект срещу образуване на биофилм от Escherichia coli.
- Тези резултати се дължат на ниската неспецифична протеинова адсорбция върху ПЦЙ материали, която предотвратява формирането на биофилм.

In situ отлагане на калциеви фосфати в полисулфобетаинови матрици

В. Димитрова, Д. Николова, М. Симеонов, А. Апостолов, Х. Грънчарова, Е. Василева
 Лаборатория по структура и свойства на полимерите, Факултет по химия и фармация, СУ „Св. Климент Охридски“, бул. „Джеймс Баучер“ No 1, София 1164, България

I. Въведение:

Хибридният материал е съчетание на нано или молекулно ниво между органични и неорганични компоненти. В природата те се получават чрез процеса биоминерализация, при който организми акумулират или формират неорганични материали в биологични тъкани и структури. В човешкото тяло пример за хибридни материали са зъбите и костите, където неорганичният компонент е калциев фосфат, а органичния е най-вече колаген.

Цвитерийоните са неутрални амфотерни молекули, които съдържат ковалентно свързани положителен и отрицателен заряд. Те са често срещани в природата, а техните високомолекулни синтетични аналози са полицвитерийоните. ПЦИ и в частност полисулфобетаините (ПСБ) се отличават с много добра биосъвместимост, дължаща се на тяхната биомиметична структура.

II. Цел:

Целта на настоящето изследване е получаването на нови хибридни материали, които да бъдат използвани за запълване на костни дефекти, и едновременно с това да помагат и тяхната реминерализация. Тази цел бе осъществена чрез *in situ* отлагане на калциеви фосфати в матрици от полисулфобетини. Първоначално бяха получени две полимерни мрежи от ПСБ с различна гъстота на omрежване, т.е. с различна концентрация на omрежващия агент - N,N'-метилден бисакриламид (МБАА). След това, в тях бяха отложени калциеви фосфати и получените хибридни материали бяха охарактеризирани.

III. Експериментална част:

III.1. Синтез на ПСБ матрици

Приготвя се 0,1M разтвор на мономера сулфобетин метакрилат, към който се добавят 0,1 mol% инициатор $K_2S_2O_8$ и omрежващ агент МБАА с две различни концентрации (2 и 4 тегл.%). Полимеризацията е описана на Схема 1. Получените полимерни матрици са с различна гъстота на мрежата, в зависимост от използваното количество omрежващ агент.

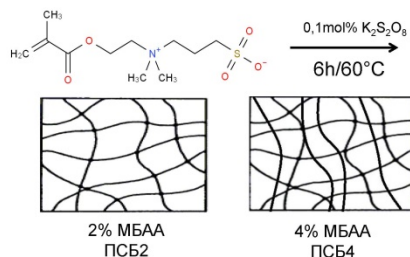


Схема 1: Получаване на полимерните матрици от ПСБ.

III.2. In situ отлагане на калциеви фосфати в матриците от ПСБ:

Получените мрежи от ПСБ бяха потопени в 0.6M разтвор на $CaCl_2$ за 48 часа. След това бяха измити и потопени в разтвори на K_2HPO_4 с две различни концентрации – 0.3M и 0.03 M (Схема 2).

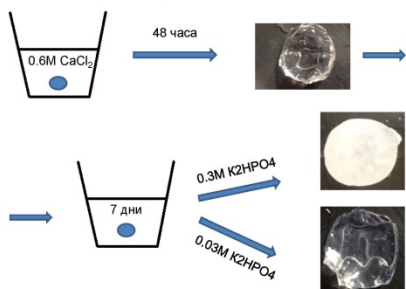


Схема 2: In situ отлагане на калциеви фосфати.

IV. Резултати и обсъждане:

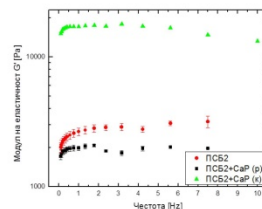
IV.1. Равновесна степен на набъбване (РСН) на полимерните матрици

Таблица 1: РСН на мрежите ПСБ

ПРОБА	Равновесна степен на набъбване
ПСБ 2	1.27 ± 0.07
ПСБ 4	1.07 ± 0.05

Очаквано, мрежата с по-голям процент omрежващ агент има по-малка равновесна степен на набъбване. Това се дължи на по-голямата гъстота на мрежата, която намалява способността на хидрогела да набъбва.

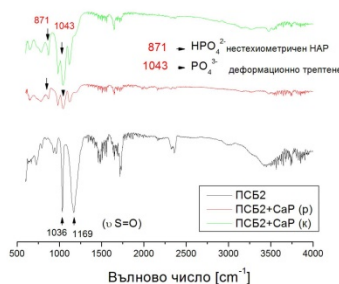
IV.1. Динамичен реологичен анализ



Фигура 1: Реологични свойства на хидрогелите на ПСБ2 и двата хибридни материала.

Модулът на еластичност (МЕ) на хибридният материал, получен с 0.03M K_2HPO_4 , е по-нисък в сравнение с ПСБ2, поради наличието на свободни Ca^{2+} и Cl^- йони, които разрушават физичната мрежа на ПСБ и намаляват МЕ. Хибридният материал, получен с 0.3M K_2HPO_4 , има МЕ, който е значително по-висок в сравнение с другите две изследвани проби. Това се дължи на по-голямото количество отложени калциеви фосфати, за които доказателство е и значителното помътняване.

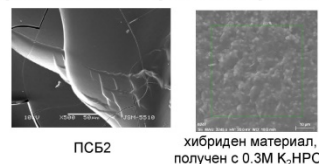
IV.2. Инфрачервена спектроскопия



Отлагането на калциеви фосфати води до появата на нови ивици, характеристични за тях: ивицата при 871 cm^{-1} се дължи на нестехиометричен хидроксиапатит. Ивицата, която се появява при 1043 cm^{-1} се дължи на трептенето на връзката P-O в групата PO_4^{3-} .

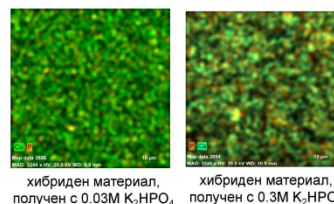
Фигура 2: Отражателно-абсорбционна спектроскопия на ПСБ2 и двата хибридни материала.

IV.3. Сканираща електронна микроскопия (SEM) с енергийно дисперсионна рентгенова спектроскопия (ЕДХ)



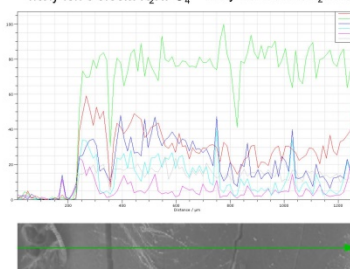
Чистата мрежа от ПСБ2 е хомогенна и не се отличава с фазово разделяне. За разлика от нея, хибридният материал, получен с 0.3M K_2HPO_4 , притежава зърнеста структура.

Фигура 3: SEM на ПСБ2 и хибридният материал, получен с 0.3M K_2HPO_4 .



Наличието на отложени калциеви фосфати (КФ) в хибридният материал бе доказано с ЕДХ, като съдържанието на фосфор е видимо по-малко при по-ниската използвана концентрация на K_2HPO_4 .

Фигура 4: SEM с ЕДХ хибридният материал, получени с 0.03M и с 0.3M K_2HPO_4 .



Равномерното разпределение на отложените КФ в целия обем на ПСБ матрицата бе илюстрирано със SEM-ЕДХ. Анализът е направен по линия, напречна на дебелината на пробата. Количеството на КФ е равномерно в целия обем на хибридният материал.

Фигура 5: SEM с ЕДХ за хибриден материал, получен с 0.3M K_2HPO_4 .

V. Изводи:

1. Синтезирани са нови хибридни материали на базата на ПСБ и калциеви фосфати.
2. In situ отложените калциеви фосфати са равномерно диспергирани в полимерната матрица и водят до увеличаване на модула на еластичност на хибридният материал.
3. Тече изследване на биосъвместимостта на хибридният материал от ИЕМПАМ, БАН.