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EUROPEAN NETWORK OF MULTIDISCIPLINARY RESEARCH TO IMPROVE THE URINARY STENTS

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EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

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"Materials, Technology, and Biomimetics as enabling tools for a new generation of Urinary Stents"

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Polyester micro- and nanosized systems with therapeutic functionality

Magdalena Stevanović

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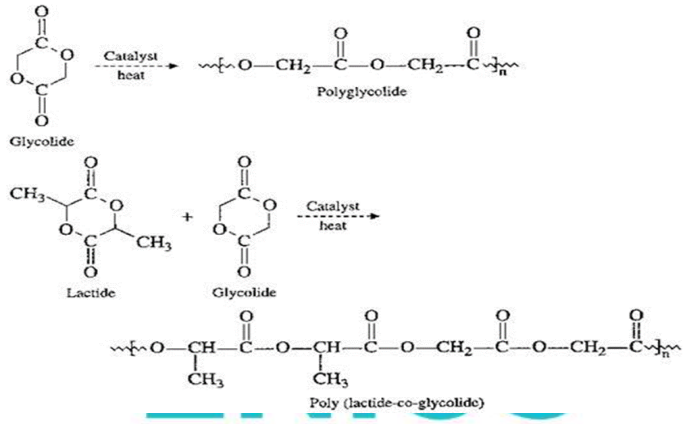
Outline

- Introduction
- Simple method of obtaining polyester micro- and nanospheres
- Encapsulation of medicaments within polymer matrix
- *Structural characteristics, morphology, stability*
- *In vitro* degradation and release tests
- Antimicrobial activity
- The cytotoxicity of nanoparticles with human hepatoma cell lines
- *Induction of intracellular reactive oxygen species*

Introduction

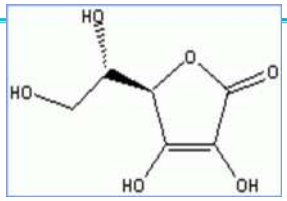
- **Nanomedicine**
- **Controlled drug delivery**
- **Biomacromolecules**
  - **biocompatible, immunocompatible, readily eliminated from the body, preferably through biodegradation.**
  - **natural and synthetic, based on their origin**
- **Aliphatic polyesters can be considered as representatives of synthetic biodegradable polymers.**

Poly (lactide-co-glycolide) (PLGA)



Methods can be divided generally into: dispersion of the polymer solution method, polymerization of the monomer method and coacervation
Key parameters -The size and shape of the particles and degradation

Multifunctional PLGA particles containing poly (L-glutamic acid)-capped silver nanoparticles and ascorbic acid - PLGA/AgNpPGA/AscH particles



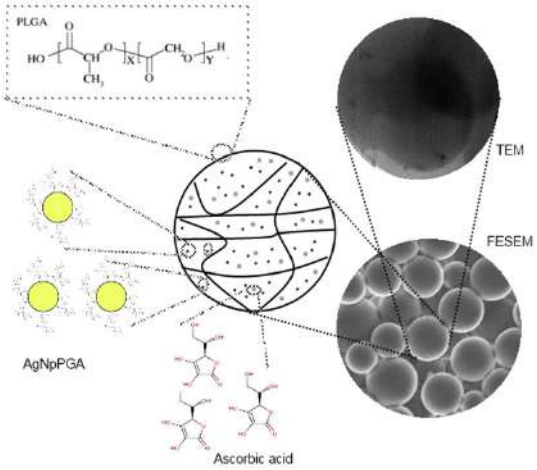
Ascorbic acid is very unstable in air, light, heat, moisture, presence of metal ions, oxygen, and base, and it easily decomposes into biologically inactive compounds.

Among promising nanomaterials with antibacterial and antiviral properties are metallic nanoparticles (Rochelle RA et al, Chem Soc Rev 2012;41:2943–70)

Concerns related to
- **free silver ions in AgNp preparations**
- **nanoparticles' bare metallic surface,** (Beer C, et al. Toxicol Lett 2012;208(3):286–92.)

PLGA/AgNpPGA/AscH particles - system with concurrent antioxidative and prolonged antimicrobial activity

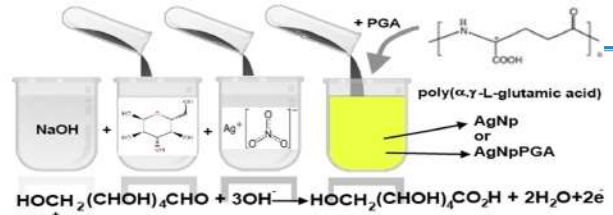
PLGA/AgNpPGA/AscH particles

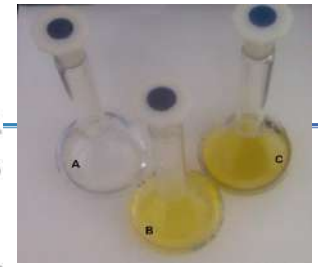


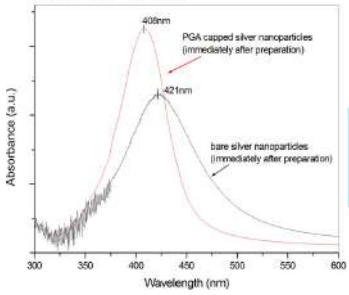
(I) synthesis of silver nanoparticles capped with PGA (AgNpPGA)
(II) homogenization of AgNpPGA with ascorbic acid
(III) encapsulation of AgNpPGA/ascorbic acid within PLGA polymer matrix

Schematic illustration of the multifunctional PLGA/AgNpPGA/AscH particles.

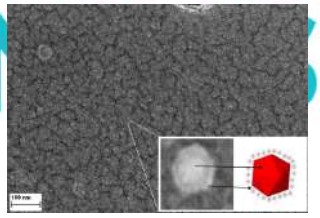
Synthesis of silver nanoparticles





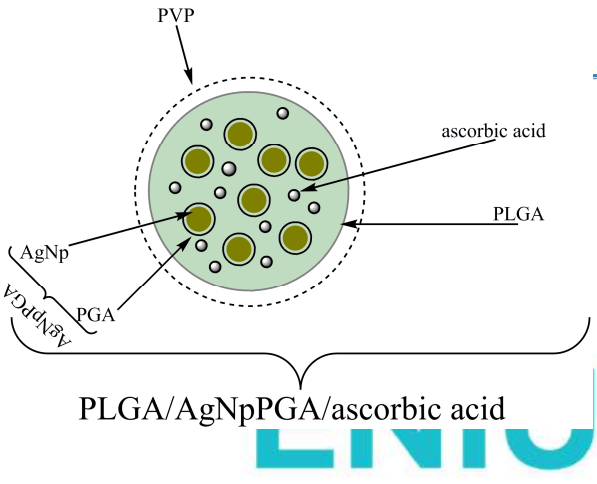


UV–Vis spectra of bare and PGA-capped silver nanoparticles recorded immediately after preparation, and Digital photo showing volumetric flasks containing the negative control (A), bare (B) and capped silver nanoparticles (C).



FESEM image of capped silver nanoparticles with 0.1% PGA (inset shows arbitrarily magnified particle).

PLGA/AgNpPGA/AscH particles



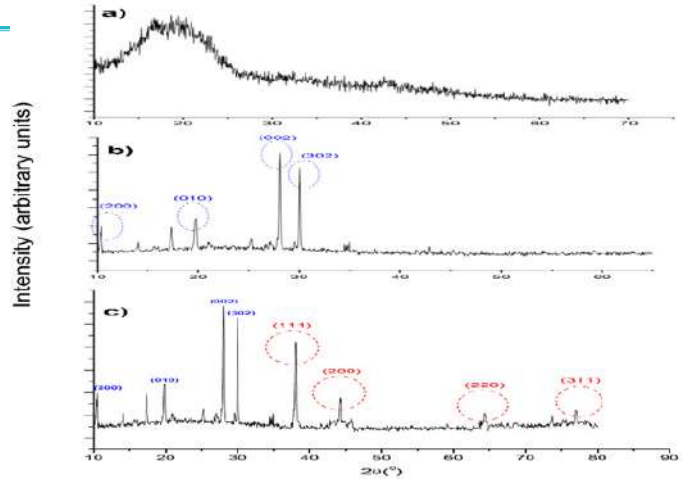
Homogenization - **encapsulation precipitation** with ethanol **stabilization** with PVP

Ascorbic acid - promote **antioxidative** effect, **reduce the free silver ions** in AgNp preparations and **elevate the effectiveness and safety** of silver nanoparticles during the administration

PGA-capped AgNps (AgNpPGAs) together with ascorbic acid were encapsulated within PLGA particles (PLGA/AgNpPGAs) to ensure their **release over an extended period of time**, and therefore their extended antioxidative and antimicrobial effects.

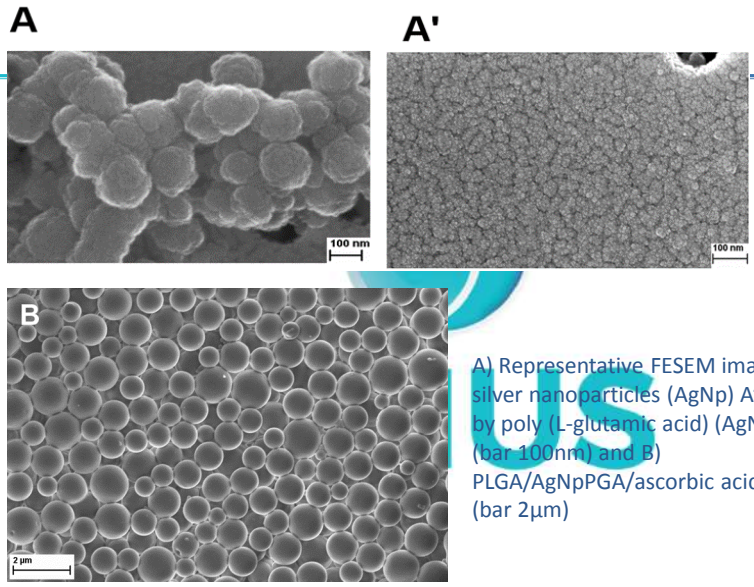
PLGA/AgNpPGA/ascorbic acid 80/5/15 % wt This final solution was centrifuged at 7,000 rpm for 120 min, at 10 ºC (Universal 320R, Hettich, Germany), decanted and dried at room temperature. The encapsulation efficiency was determined to be greater than 90%.

XRD analysis



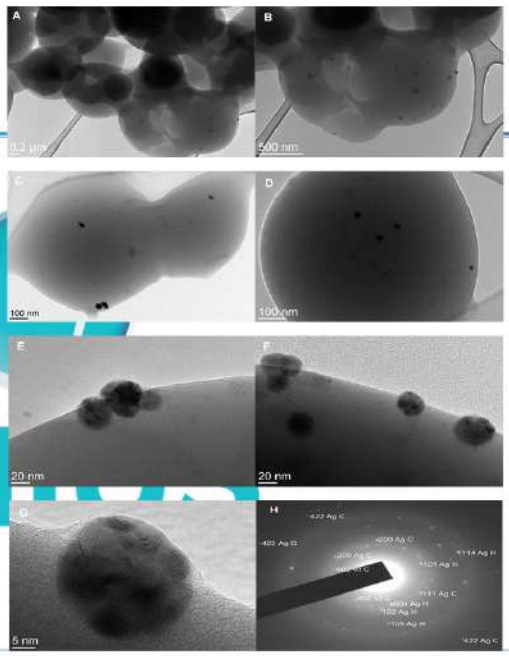
XRD diffraction patterns of a) PLGA, b) PLGA/AscH and c) PLGA/AgNpPGA/AscH

Morphology studies



A) Representative FESEM images of silver nanoparticles (AgNp) A') capped by poly (L-glutamic acid) (AgNpPGA) (bar 100nm) and B) PLGA/AgNpPGA/ascorbic acid particles (bar 2µm)

Morphology studies



Representative TEM images showing AgNpPGAs and ascorbic acid nanoparticles within PLGA polymer matrix (a-g) and corresponding selected-area electron diffraction (SAED) patterns showing the diffractions for silver nanoparticles (H-hexagonal; C-cubic) and ascorbic acid (h).

Zeta potential

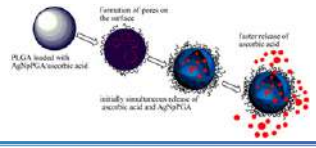
Electrostatic repulsion between particles depends on the value of zeta potential. The higher the zeta potential, the stronger the repulsion, the more stable the system becomes

Characteristics of the AgNpPGAs and PLGA/AgNpPGA/AscH particles in the dispersions.

Sample	Particle size (nm)	PDI	Zeta potential (mV)
AgNpPGA	44.9 ± 5.0	0.206	-43.7 ± 12.0
PLGA/AgNpPGA/AscH	775±5.0	0.158	-30.4±10.5

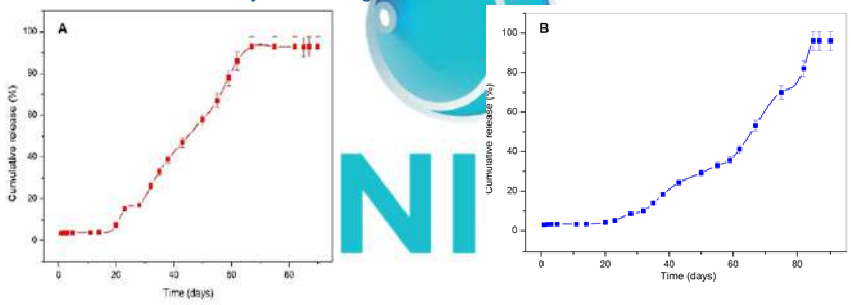
Data are mean ±standard deviation (n=5). The zeta potentials are in the pH range 4.30-4.50.

In vitro degradation



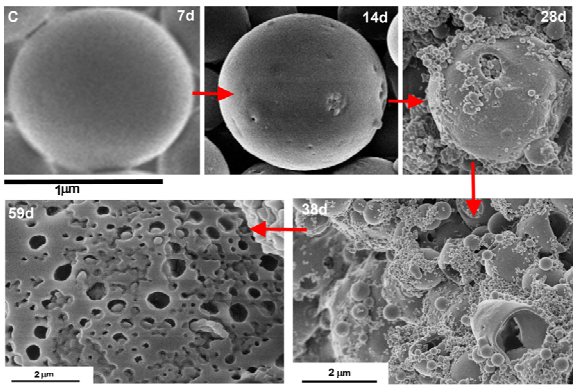
UV-Vis / physiological solution / 90 days

The amount of silver nanoparticles that was released from the PLGA particles during the first two weeks was 3.4% wt and the amount of ascorbic acid was 4.1% wt. The entire amount of ascorbic acid has been released in 68 days of the degradation and the entire amount of silver nanoparticles has been released in 87 days of the degradation.



(A) Cumulative in vitro release of ascorbic acid from PLGA/AgNpPGA/ascorbic acid particles and (B) Cumulative in vitro release of AgNpPGAs from PLGA/AgNpPGA/ascorbic acid particles over time in physiological solution as a degradation medium (pH 7.4; 37 ±1 ºC). Data are means ±SD (n = 3).

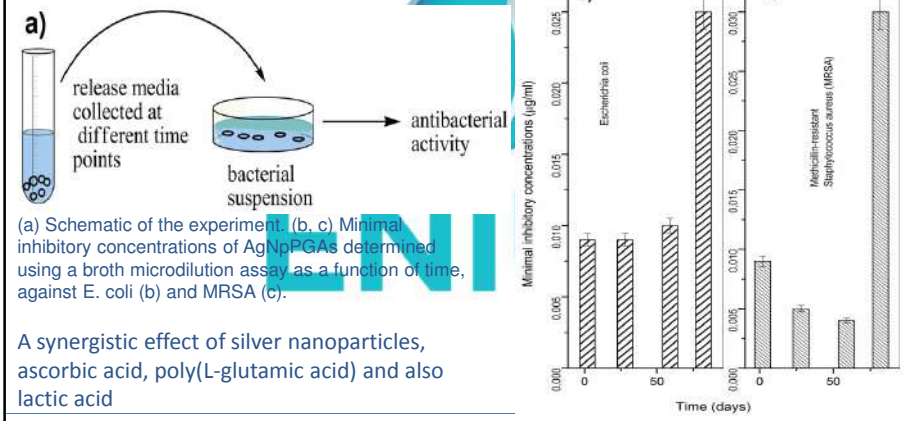
In vitro degradation - morphology studies



Representative FESEM micrographs of dry degraded PLGA/AgNpPGA/ascorbic acid particles after 7, 14, 28, 38, and 59 days of degradation.

Antimicrobial studies

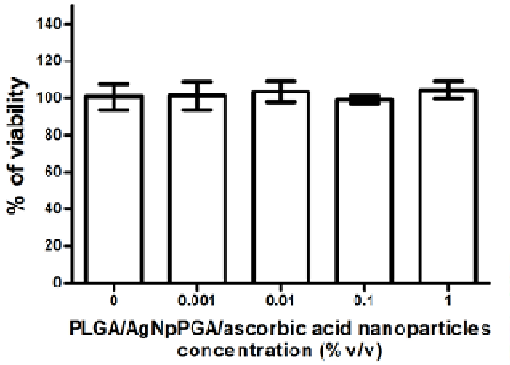
The supernatant, after different periods of time of the degradation of PLGA/AgNpPGA/ascorbic acid particles, was collected for examination of its antimicrobial activity. A broth microdilution method was used to determine MICs of PLGA/AgNpPGA/AscH particles after two, 28, 59 and 82 days of the degradation. All samples exhibited strong antimicrobial activity during the extended period of time.



(a) Schematic of the experiment. (b, c) Minimal inhibitory concentrations of AgNpPGAs determined using a broth microdilution assay as a function of time, against E. coli (b) and MRSA (c).

A synergistic effect of silver nanoparticles, ascorbic acid, poly(L-glutamic acid) and also lactic acid

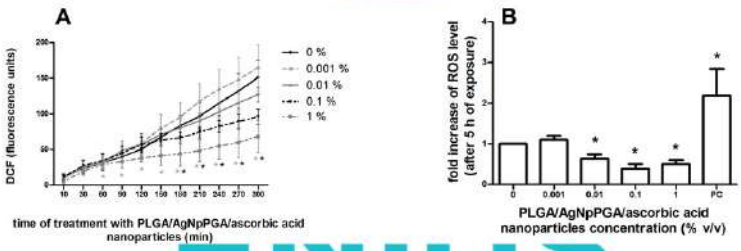
Determining cytotoxicity of PLGA/AgNpPGA/AscH - MTT assay



Viability of HepG2 cells treated with PLGA/AgNpPGA/ascorbic acid particles for 24 h. The data are presented as mean values of three independent experiments (each with five replicates) ± S.D.

Determining intracellular reactive oxygen species formation – DCFH-DA assay

The formation of intracellular reactive oxygen species (ROS) was measured spectrophotometrically using a fluorescent probe, DCFH-DA.



PLGA/AgNpPGA/AscH particles at concentrations 0.01, 0.1 and 1 % (v/v) caused significant decrease in DCF fluorescence intensity, which was after 5 hour exposure two fold lower from that in control cells. This indicates that PLGA/AgNpPGA/AscH either act as scavengers of intracellular ROS and/or reduce their formation.

Summary

PLGA/AgNpPGA/AscH particles are **spherical, uniform and do not agglomerate**.

The degradation of these particles within the physiological solution have been tracked during the 90days and it has been determined that **particles completely degrades** within this period **fully releasing all encapsulated AgNpPGA as well as ascorbic acid**.

PLGA/AgNpPGA/AscH particles **did not affect the viability of HepG2 cells, they diminished the ROS generation and moreover showed superior and extended antimicrobial activity** towards the Gram-positive bacteria methicillin-resistant Staphylococcus aureus (MRSA; ATCC 43300),and the Gram-negative bacteria Escherichia coli (ATCC 25922).

Our data suggest that antioxidative and, at the same time, antimicrobial agent, biodegradable PLGA/AgNpPGA/AscH particles, PLGA/AgNpPGA/AscH **are potential candidate for use in pharmaceutical products and medical devices that may help to prevent the infections** and transmission of drug-resistant pathogens in different clinical environments.

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Miloš Filipović, Department of Chemistry and Pharmacy, University of Erlangen-Nuremberg, Erlangen, Germany

INSTITUTE OF TECHNICAL SCIENCES OF THE SERBIAN ACADEMY OF SCIENCES AND ARTS

Main activities of the department

Fine particles processing and nanotechnologies, drug delivery, colloids, polyesters, poly(lactide-co-glycolide), poly(e-caprolactone), micro and nanospheres, bioactive glass, scaffolds, biomacromolecules, polymers, structure-property relationships of polymers, silver, selenium nanoparticles, characterization of materials

Equipment

FTIR spectroscopy, Uv-Vis spectroscopy, thermal analysis (DSC131 EVO, -170 to 700 °C; SETSYS TMA, up to 2400 °C, SETSYS TG-DTA/DSC, up to 2400 °C) mass spectrometer Omnistar GSD 320, XRD Philips PW1050, optical microscopy, particle analyzer.

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M. Stevanović, I. Savanović, V. Uskoković, S. Škapin, I. Bračko, U. Jovanović, D. Uskoković, A new, simple, green, and one-pot four-component synthesis of bare and poly(alpha,gamma,L-glutamic acid)-capped silver nanoparticles, Colloid & Polymer Science 290, 3 (2012) 221-231, DOI: 10.1007/s00396-011-2540-7

Magdalena Stevanović, Chapter 11, Polymeric micro and nanoparticles for controlled and targeted drug delivery, pages 357-380, In book: Nanostructures for Drug Delivery; 1st Edition, Editors: Ecaterina Andronescu, Alexandru Grumezescu; Publisher: Elsevier, 2017. ISBN:978-0-323-46143-6; PII:B978-0-323-46143-6.00011-7; https://www.elsevier.com/books/nanostructures-for-drug-delivery/andronescu/978-0-323-46143-6

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