RADIOLYTIC SYNTHESIS OF NANOCOMPOSITES BASED ON NOBLE METAL NANOPARTICLES AND NATURAL POLYMER, AND THEIR APPLICATION AS BIOMATERIAL

Aleksandra Krklješ

VINČA Institute of Nuclear Sciences, Laboratory for Radiation Chemistry and Physics – GAMMA, Belgrade, SERBIA

Abstract

Laboratory for Radiation Chemistry and Physics - GAMMA with 60 employees is one of the largest laboratories in the VINČA Institute of Nuclear Sciences. Currently, 26 researchers with Ph.D. degree, 6 with M.Sc. degree and 13 graduated students are working on ongoing projects in experimental research in the field of nanoscience and modifications of materials by irradiation. Laboratory is well equipped for synthesis of nanoscale materials as well as for different types of their characterization. This Laboratory has excellent and long lasting tradition in scientific activities. Based on outstanding results in radiation chemistry, in the Laboratory GAMMA exists radiation unit for industrial sterilization of food and medical equipment.

1. Introduction

Nanotechnology, nanoscience, nanostructures, nanoparticles… These are now some of the most widely used terms in materials science literature. Nanotechnology is one of the fastest growing new areas in science and technology. The preparation and investigation on metal or semiconductor nanoparticles, nanocomposites, nanodevices etc. are of great interest in both research and engineering. Large number of scientists is involved in nanoparticles and nanocomposites research and development. Their current and potential applications in catalysis, photonics, optic, electronics, pharmaceutics and particular in biomedicine have opened a many new challenges.

Recent research efforts have been devoted to the preparation of noble metal nanoparticles because of their unique properties. Large number of synthetic procedures has been employed in order to synthesized noble metal nanoparticles and/or nanocomposites based on them and polymer matrix. It has been shown that their morphology, particles size and size distribution, stability and properties are strongly dependent on the method of preparation and specific experimental conditions. The radiolytic method is very suitable for generating metal particles in solution. The radiolytically generated species, solvated electrons and secondary radicals, exhibit strong reduction potentials and consequently metal ions are reduced at each encounter. The control of particle size is achieved by the use of capping agents such as polymers, which are present during the formation of metal clusters. Polymer molecules interact with the growing metal particles and thus inhibit the aggregation process [1, 2].

Moreover, the radiolytic method has been recognized as highly suitable tool to aid in the formation of hydrogels. The radiation process has various advantages, such as easy process control, the possibility of joining hydrogel formation and sterilization in one technological step, the lack of necessity for initiators and crosslinkers, which are possibly harmful and difficult to remove. The radiation technique is clean, because it does not require any extra substances, does not leave some unwanted residues, and does not need any further purification. These qualities make irradiation the method of choice in the synthesis of hydrogels. On the other hand, although gamma irradiation has proven to be a powerful tool

for synthesis and modification of materials, not so many studies have been reported concerning the radiolytic formation of metal nanoparticles in hydrogel matrix (template synthesis). Hydrogels in the swollen state provide free spaces within the network, which can also serve for nucleation and growth of nanoparticles. In this way, the carrier-hydrogel system acts as a nanoreactor that immobilizes nanoparticles and provides easy handling, giving a new hybrid nanocomposite systems. Therefore, highly stable and uniformly distributed nanoparticles with predetermined dimensions and size-dependent properties have been achieved by a very delicate balance between the reaction conditions, the composition and the structure of hydrogel templates, and the concentration of nanoparticles [3, 4].

In proposed project research activities will be focus on synthesis strategies of formation of silver (Ag) and/or gold (Au) nanoparticles in polymer matrix consisting of chitosan (Ch) or chitosan/poly(vinyl alcohol) (Ch/PVA) blend system. Ag nanoparticles are generally considered as environmentally friendly antibacterial materials. However, their electronic properties recently were reported to be used in biosensors and drug delivery applications as well as drug carriers for biomolecules such as enzymes. Au nanoparticles are the most commonly used nanoparticles for diagnostics and drug delivery. The unique chemical properties of colloidal Au make it a promising targeted delivery approach for drugs or gene specific cells. PVA and Ch are good protectors or dispersants for preparing nanoparticles, and play an important part in the controllable production and stabilization of nanoparticles with definite size. Moreover, they can reduce metal ions to some extent, and promote the nucleation of nanoparticles. These two polymers have been used successfully in many biomedical applications. Preliminary results showed that Ch/PVA scaffolds could integrate with the damaged tissue to promote consistent functional recovery of peripheral nerve tissue, but additional investigation is required for this to be confirmed. Moreover, investigations indicated that Au and Ag nanoparticles had a stimulatory effect on nerve cell proliferation.

Nerve injuries are common in clinical practice. According to statistics, more than 90,000 people are affected by nerve injuries every year. Central nervous system (CNS) is, for the most part, incapable of self-repair and regeneration, while the peripheral nervous system (PNS) has an intrinsic ability for repair and regeneration. Studies on the recovery of PNS functionality after injury have become a rapidly growing field dedicated to the searching of suitable ways for facilitating neuroregeneration. Various approaches have been developed in an attempt to regenerate injured nerves. One such technique involves the actual suturing of the proximal and distal ends of the severed nerve. When a nerve defect of gap is longer, implantation of a graft is often necessary to bridge the stumps for promoting nerve regeneration. Currently, the most widely used material to bridge a peripheral nerve defect is the autologous nerve, e.g., nerve tissue obtained from a second operative site of a patient. However, this treatment raises the possibility of function loss at the donor site, formation of potential painful neuromas, structural differences between donor and recipient grafts, and difficulty in finding a suitable donor site of transplant in patients with diabetic and other chronic diseases, not to mention a potential shortage of graft material where extensive repairs are required. Therefore, it would be desirable to have an alternative nerve graft material that not only fulfills the requirements, but also overcomes many of the shortcomings, of a nerve autograft. A promising alternative for nerve regeneration which avoids the above-mentioned problems is an artificial graft. In fact, many types of biomaterials, natural or synthetic, have been used to make tubes or conduits for guiding peripheral nerve regeneration. Conventionally, nerve conduits are made of silicone rubber due to its chemical stability and elastic properties. However, because silicone tubes are non-biodegradable and non-porous, conduits made of silicone rubber often lead to long term complications including fibrosis and chronic nerve compression in clinical applications, and a second surgery is often necessary for

removal of the tube or conduit. Accordingly, nerve guide conduits fabricated from biodegradable polymers are preferred over non-biodegradable polymers due to the obvious advantage of eliminating the second surgery to remove the conduits. The purpose of a resorbable nerve conduit is to provide unperturbed environment for nerve regeneration in short term and to degrade after nerve reconstruction with little tissue reaction. Although a variety of resorbable nerve conduits have been developed, the results thus far are still not satisfactory, and the search for a better conduit is ever ongoing. Therefore, there is a need for an improved nerve conduit for facilitating the regrowth, repair, or regeneration of nervous tissues.

2. Related work already performed or in progress at institute

As already mentioned, the gamma irradiation method has been proven to be a powerful tool for synthesis and modification of nanomaterials. Our research activities are performed under cover of several national projects (No. 1969, 142066 and III45005) and two international project founded by IAEA (CRP F23028 and TCP RER/8/014), which are related to radiolytic synthesis of polymer based nanocomposites with noble metal nanoparticles. Different architecture of polymer matrix gives two types of nanocomposites - (i) nanocomposites with uncrosslinked polymer matrix and (ii) nanocomposites with crosslinked polymer matrix i.e. hydrogels.

By steady state gamma irradiation Ag and Au nanoparticles were successfully synthesized in PVA solution. Obtained results showed formation of clearly defined spherical nanoparticles with diameter around 10 nm and face centered cubic crystalline structures (Fig. 1). Characterization of Ag-PVA or Au-PVA nanocomposites indicated that it was possible to tune the optical properties of nanoparticles in nanocomposite systems by changing the dielectric properties of surrounding medium and pH value of initial solution, but also with changing the polymer matrix architecture [5, 6].

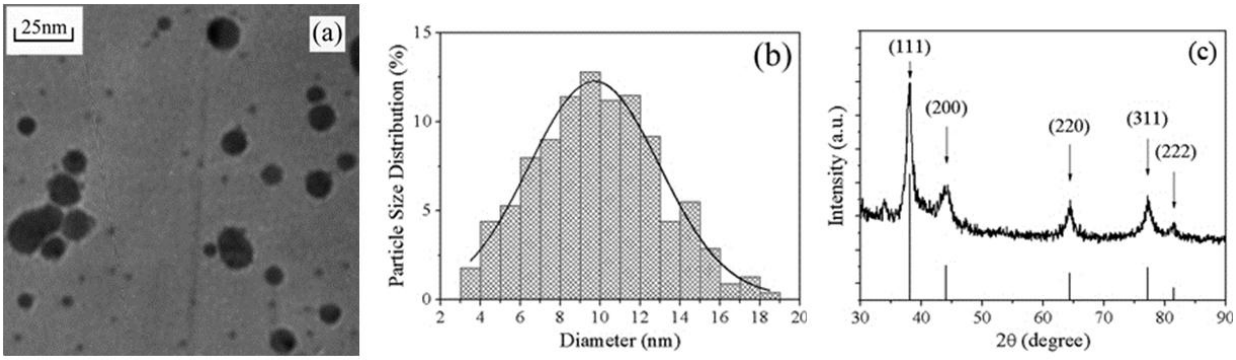

Fig. 1. Typical TEM micrograph (a), size distribution (b) and XRD pattern (c) of Au NPs.

For the largest part, our current work is focused on synthesis and characterization of second type nanocomposites - nanocomposites with crosslinked polymer matrix i.e. hydrogels. We are systematically developing synthetic strategies for *in situ* synthesis of noble metal nanoparticles (Ag and Au) in previous obtained hydrogel matrix (template synthesis). The gels were polymerized or crosslinked by gamma irradiation radical copolymerization or crosslinking, under ambient conditions, to absorbed dose of 25 kGy. Ag/hydrogel nanocomposites were prepared by swelling the crosslinked polymer samples with water solutions of $AgNO_3$ and 2-propanol. In the second step of irradiation, Ag^+ ions were reduced

in hydrogel using electron transfer reactions from radical species formed in water radiolysis. Gamma irradiation was performed until achieving complete reduction of Ag^+ ions. Schematic presentation of the synthesis of Ag/polymer hydrogel nanocomposites is presented on Fig. 2.

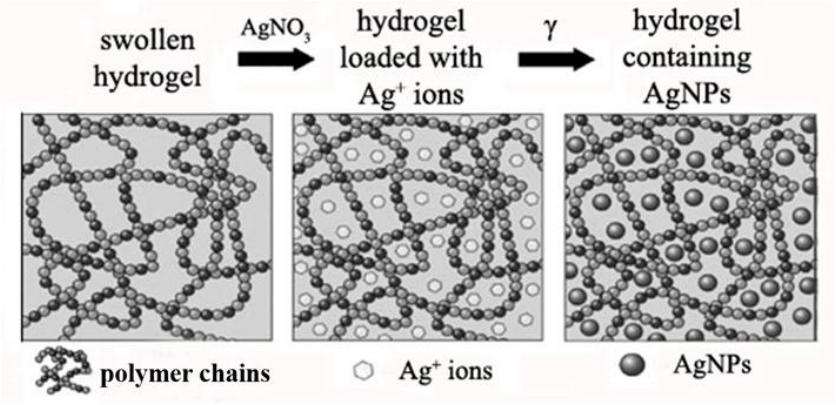

Fig. 2. Schematic presentation of the synthesis of Ag/polymer hydrogel nanocomposite.

By applying described synthetic route, Ag nanoparticles were successfully incorporated in crosslinked polymer matrix such as PVA, PVP, pH-sensitive poly(BIS-co-HEMA-co-IA) copolymer and temperature-sensitive PNIPA hydrogels [7-13]. For example, Fig. 3 shows the thermoresponsive phase transition of PNIPA and Ag/PNIPA hydrogels. During the volume phase transition around 30 ^oC, from a swollen to a collapsed state, the initially clear and soft gel contracts and become hard and opaque, expelling its contents into surroundings [11].

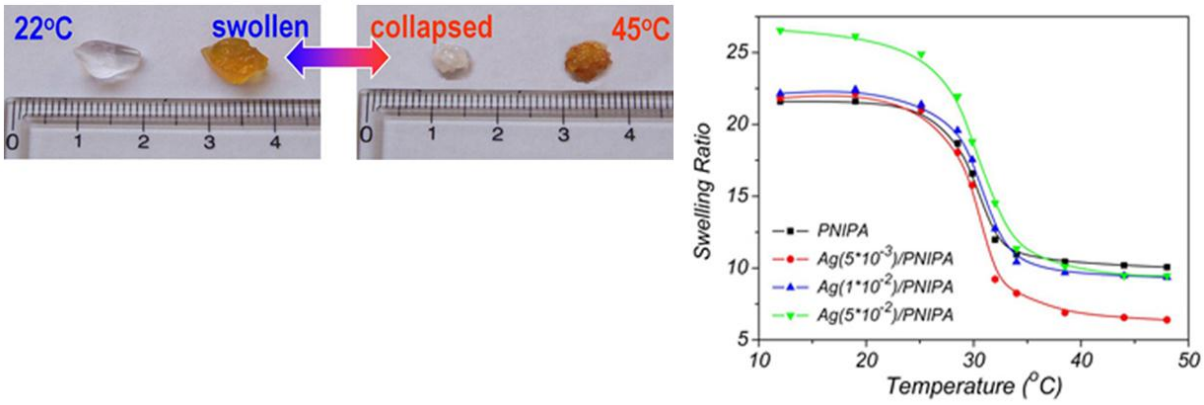

Fig. 3. Photographs of thermoresponsive phase transition from a swollen to a collapsed state: PNIPA (uncolored) and Ag/PNIPA (yellow colored) hydrogels (up) and temperature dependence of swelling ratio of Ag/PNIPA nanocomposite hydrogels (right).

Reduction of Ag^+ ions in different types of investigated hydrogel matrix yielded the typical characteristic surface plasmon band of Ag nanoparticles for all investigated systems, with no broad absorptions at wavelengths longer than the particle plasmon band, as shown in Fig. 4 (left) (example for Ag/PVA hydrogel nanocomposites). Irradiation of $AgNO_3$-loaded hydrogels resulted in a formation of yellow colored Ag/polymer hydrogel nanocomposites, with strong sharp absorption band centered around 390-430 nm. No significant change in the UV-vis characteristics of the Ag nanoparticles formed in these hydrogels, with modification of system's compositions, was observed. The sharp absorption pattern indicates that the

particle size distribution is quite narrow. Moreover, it was shown for the first time that PVA$^{\bullet}$ radicals in PVA hydrogel, obtained by gamma irradiation, have sufficient reducing ability to produce Ag nanoparticles in swollen polymer matrix [10].

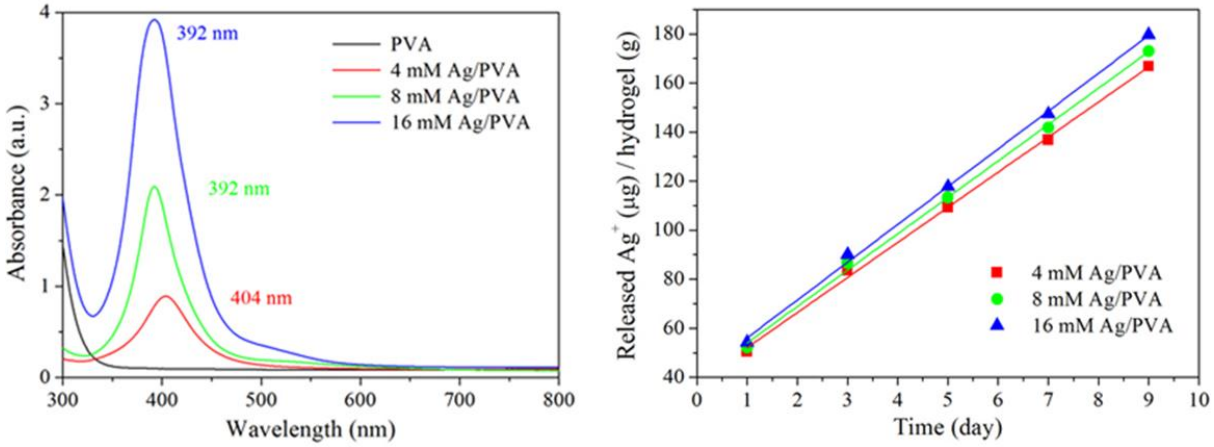

Fig. 4. UV-vis absorption spectra of Ag /PVA hydrogel nanocomposites (left) and release of silver from Ag/PVA hydrogel nanocomposites (right).

Hydrogels are being considered as most promising biomaterials in form of contact lens, burn dressing, artificial corneas, soft tissue substances etc. They are applied from tissue expanders to coating for penile and testicular implants, urology devices and materials as well as for wound dressing. Even in diabetic foot wound therapy, in some cases, a tissue expander can be used for resolving the open wound. On the other hand, nearly synchronous with the advent of the prosthetic medical devices, strategies were developed to attempt to minimize the risk of infection. In recent years nanoscale antibacterial materials as novel antimicrobial species have been seen as promising candidates for application. Nanocrystalline silver has been proved to be most effective antimicrobial agent since silver and silver compounds have powerful antimicrobial capability and broad inhibitory biocidal spectra for microbes, including bacteria, viruses and eukaryotic microorganism. Enhanced antibacterial properties of nanocrystalline silver have been demonstrated both *in vivo* and *in vitro* [7, 8].

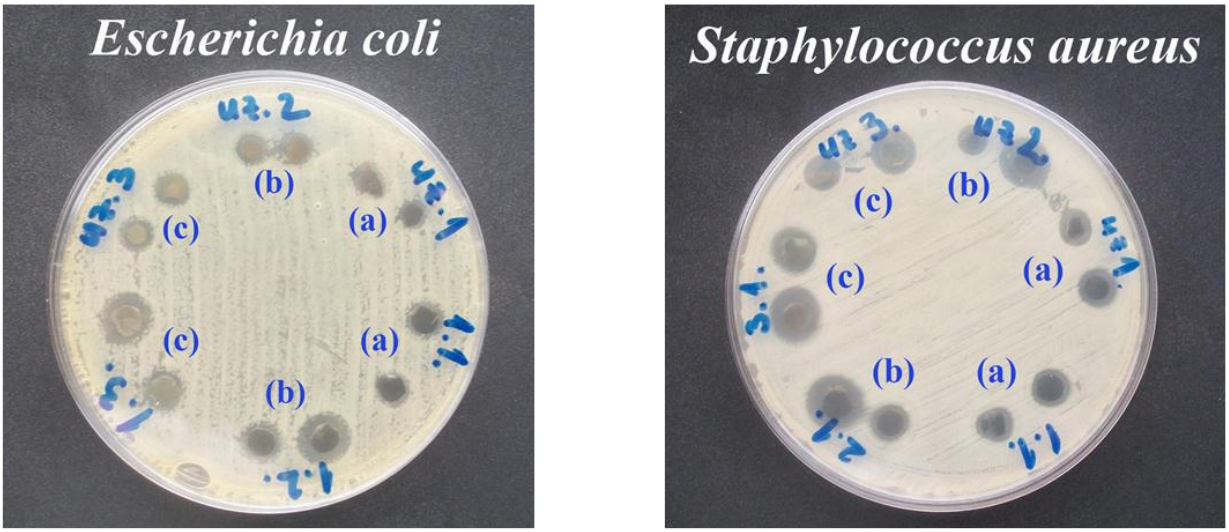

Fig. 5. The antibacterial activity of Ag/PVA hydrogel nanocomposites against Escherichia coli and Staphylococcus aureus by disc diffusion method.Concentration of silver: (a) 35 mM, (b) 70 mM and (c) 100 mM.

The synthesized Ag/polymer hydrogel nanocomposites are suitable for various applications in reconstructive surgery, including wound dressing, tissue expanders etc. Sustained, steady supply of active silver is important property of dressing material. From Fig. 4 (right) (example for Ag/PVA hydrogel nanocomposites) it can be seen that investigated hydrogel nanosystems meet that criteria, showing the continuous release of silver over a long period of time and, as consequence, the test of antimicrobial activity was performed. Antimicrobial efficiency was determined by agar-diffusion test and the obtained results clearly show the formation of inhibition zone towards *Escherichia coli* and *Staphylococcus aureus* in the case of higher nano-Ag concentration (Fig. 5).

3. Experimental

The crosslinking of polymers and reduction of metal ions will be performed by means of radiation chemistry method due to its advantage over chemical methods. The gamma irradiation method will be used because it can offer a mass of reductive radicals, which are totally clean reductive agents from the green chemistry point of view, and thus can avoid by product coming from chemical reducing agents. The synthesis of noble metal/polymer hybrid nanosystems will be performed by two radiation chemistry pathways. First, we will systematically develop synthetic strategies for incorporation of Ag and/or Au nanoparticles in previously obtained Ch or Ch/PVA matrix by gamma irradiation, using liquid filled cavities in polymer matrix as nanoreactors (template synthesis). Second we will investigate the possibility of simultaneously crosslinking of polymers and *in situ* synthesis of noble metal nanoparticles with the goal of joining synthesis and sterilization in one technological step.

Generation of nanoparticles in polymer matrix will be followed by UV-Vis spectroscopy, measuring characteristic plasmon absorption. The strong optical absorption and scattering of noble metal nanoparticles is due to an effect called localized surface plasmon resonance of the electrons in the conduction bands. Characterization of obtained hybrid nanocomposite systems will be performed by FTIR, TEM, SEM, AFM, XRD, MALDI TOF MS, swelling experiments and mechanical testing. The study of biomedical potential of synthesized hybrid

nanocomposite systems will be conducted to investigated their antibacterial activity and evaluate the potential of these systems as a tissue-engineering platform for the treatment of peripheral nerve injury.

4. Preliminary results and discussion

The primary effects of the interaction of high-energy radiation, such as gamma irradiation, with some solution are the excitation and the ionization of the solvent leading to the formation of various species. In the case of aqueous solutions, the absorption of radiation energy occurs in the water and primary products of radiolysis are shown in following equation:

$$H_2O \xrightarrow{\gamma} e_{aq}^-, OH^\bullet, H^\bullet, H^+, H_2, H_2O_2 \quad (1)$$

The main reactive radicals among the primary products are solvated electrons (e_{aq}^-) and hydrogen atoms ($H^\bullet$) as strong reducing agents, while the hydroxyl radicals ($OH^\bullet$) are able to oxidize the ions or the atoms into a higher oxidation state and thus to counterbalance the reduction reactions. For this reason, during the process of metal ions reduction an $OH^\bullet$ radical scavenger must be add in the system. Among various possible molecules, the preferred choice in radiation chemistry is some secondary alcohol, mainly 2-propanol, which converts $OH^\bullet$ and $H^\bullet$ radicals to 2-propanol radicals ($(CH_3)_2C^\bullet OH$), by abstraction of hydrogen from the alcohol:

$$OH^\bullet \left(\text{or } H^\bullet\right) + (CH_3)_2CHOH \longrightarrow H_2O \left(\text{or } H_2\right) + (CH_3)_2C^\bullet OH \quad (2)$$

On the other hand, if the polymer solution without addition of any scavenger was exposed to the gamma irradiation, the polymer network will be formed. It is well known that the radiation crosslinking of polymer molecules (POLYM) is mainly induced by $OH^\bullet$ radical in aqueous medium (G = 0.48 for irradiation induced intermolecular crosslinking):

$$2\,POLYM(H) + 2\,OH^\bullet \longrightarrow POLYM - POLYM\left(\text{crosslinked polymer}\right) + 2\,H_2O \quad (3)$$

Under the certain experimental condition (argon saturated solution of metal ions with addition of 2-propanol), metal nanoparticles in polymer solution or polymer network will be generated mainly by reduction of ions with solvated electrons and with 2-propanol radicals, but in much smaller amounts. For example, reduction processes in the case of silver ions occurs according to the following equation:

$$n\,Ag^+ + n\,e_{aq}^- / (CH_3)_2C^\bullet OH \longrightarrow (Ag)_n \quad (3)$$

4.1. Uncrosslinked Ag-Ch/PVA nanocomposites

In the present work, two different series of Ag-Ch/PVA uncroslinked nanocomposites were synthesized by gamma irradiation, in order to investigate the influence of system composition on the *in situ* formation of Ag nanoparticles. The first series of samples have a constant total weight of polymers in solution, but in different Ch:PVA weight ratio (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100). At the second series of samples the weight of Ch was constant, while the weight of PVA was increased in order to obtained 1:0, 1:0.5, 1:1, 1:1.5 and 1:2 Ch:PVA weight ratio. The concentration of silver ions in all solutions was the same and was 5 mM. After the gamma irradiation, the yellow color of the resulting colloid solutions is characteristic of the reduction of silver from higher oxidation state in its initial salt (Ag^+) to zero valent silver (Ag^0) with formation of Ag nanoparticles.

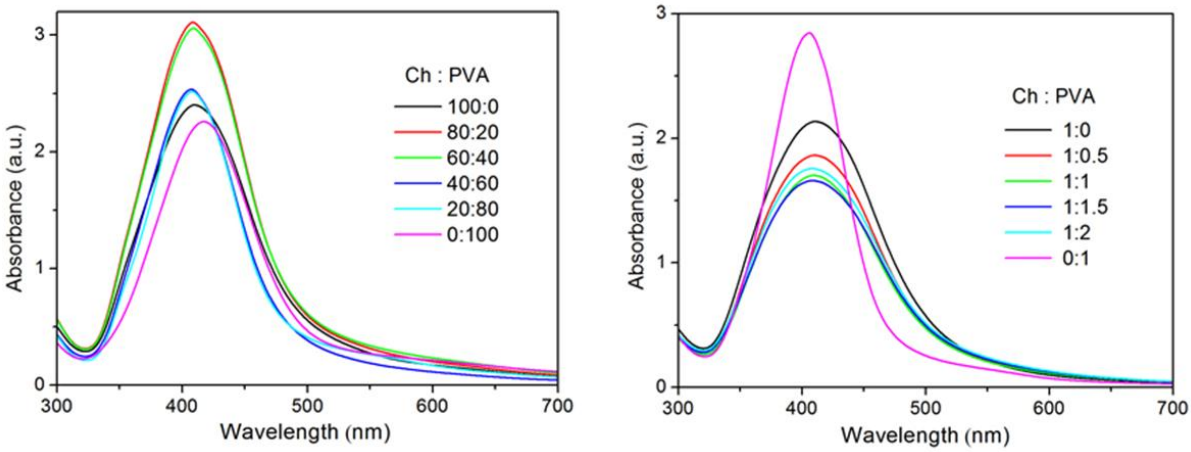

Fig. 6. UV-vis absorption spectra of Ag-Ch/PVA colloid solutions: constant total weight of Ch and PVA but in different weight ratio (left) and constant weight of Ch with increasing weight of PVA (right). The concentration of silver ions was 5 mM.

The formation of Ag nanoparticles in Ch/PVA solutions was confirmed by UV-Vis spectral studies. Fig. 6 depicts the absorption spectra of two different series of Ag-Ch/PVA colloid solution. The absorption spectra shows the characteristic surface plasmon absorption band around 410 nm, due to the plasmon resonance effect originating from the quantum size of the Ag nanoparticles. According to our knowledge this confirms the formation of Ag nanoparticles, with the diameter around 10 nm. Further investigation of Ag nanoparticles and Ag-Ch/PVA uncroslinked nanocomposites, obtained by evaporation of solvent from colloid solutions, will be performed.

4.2.Crosslinked Ch/PVA polymer network (hydrogel)

The Ch/PVA hydrogel systems with Ch:PVA weight ratio of 1:1 and 1:1.5 were crosslinked under the gamma irradiation. The reaction mixtures were irradiated in a ^{60}Co radiation source, under ambient conditions, to absorbed dose of 25 kGy. The swelling properties of obtained Ch/PVA hydrogels were investigated.

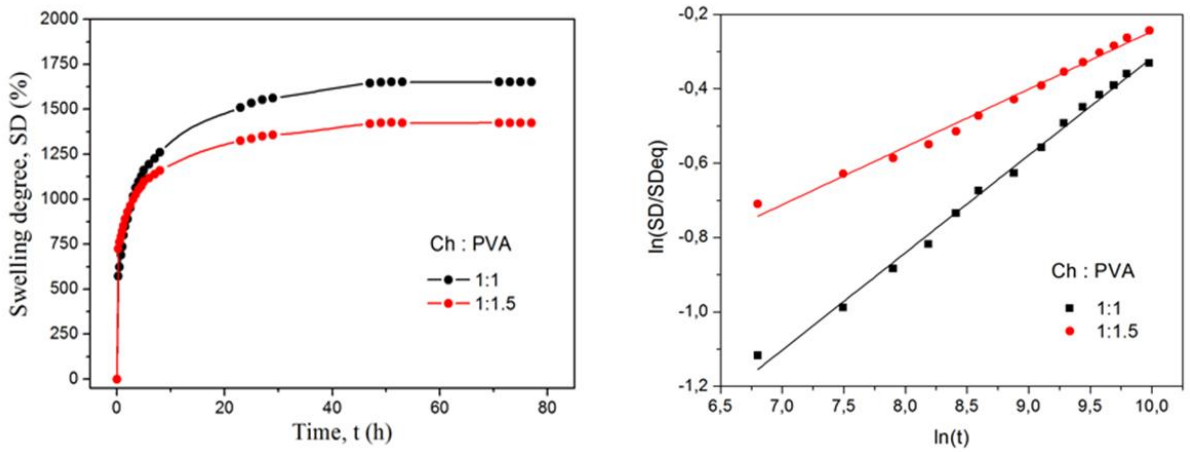

Fig. 7. Swelling curves of Ch/PVA hydrogels, in deionized water at 25 oC (left). Best linear fits of linear parts of swelling curves ln SD/SDeq vs. ln t (right).

The capacity of swelling is one of the most important parameters for evaluation the properties of hydrogels. Fig. 7 (left) depicts the swelling curves of the investigated hydrogels in deionized water at 25 ^{o}C. As can be seen, the swelling isotherms are similar in shape, but introduction of large quantities of PVA has influence on the swelling properties. The equilibrium swelling degree (SD_{eq}) of Ch/PVA (1:1) hydrogel was found to be 1.16 times higher in comparison with Ch/PVA (1:1.5) hydrogel, and swells with the higher initial swelling rate (v_{in}) (Table 1). This can be attributed to the higher crosslinking density with increasing amount of PVA, which was expected from the values of gel content (Table 1).

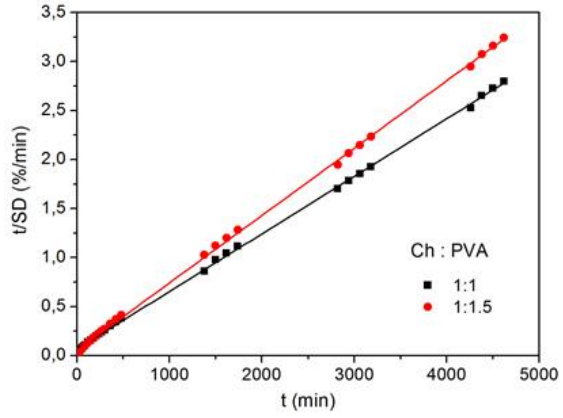

Fig. 8. Relation t/SD vs. t for Ch/PVA hydrogels, in deionized water at 25 oC.

TABLE 4. CHARACTERISTIC PARAMETERS OF SWELLING PROCESS FOR CH/PVA HYDROGELS, IN DEIONIZED WATER AT 25 OC

	Ch/PVA	
	1:1	1:1.5
Gel %	23	29
SD_{eq} (%)	1651	1424
ν_{in} (%/min)	15	5
k (1/s)	0.053	0.165
n	0.262	0.155
D (cm^2/s)	$8.83 \cdot 10^{-7}$	$4.83 \cdot 10^{-8}$

To analyze the model of water diffusion into the polymer networks, the water sorption data was used. The best linear fits of linear parts of logarithmic form of kinetic equation of swelling, $SD/SD_{eq} = k\ t^n$ (*k* is the kinetic constant, related to the structure of the network, *t* is time and *n* is the diffusion exponent), was presented on Fig. 7 (right). The characteristic constant *n* and *k* was calculated from the slope and intercept, respectively, of the linear parts of initial stages of swelling, and they can be related to the specific transport mechanism. In this case, both Ch/PVA hydrogels shows Fickian diffusion ($n \leq 0.5$) when the rates of diffusion is significantly slower than the rate of polymer chain relaxation. The diffusion coefficient (*D*) can be calculated as $D = k^2\ \pi\ \delta^2/16$ (δ is thickness of the xerogel). The values for *n*, *k* and *D* were presented at Table 1. For extensive swelling of hydrogels the equation t/SD = A+Bt was used (Fig. 8), and the linear dependence indicated the Schott second order kinetics ($dSD/dt = k_S(SD_{eq}-SD)^2$).

5. Objective of the project

In this project research will be focus on chitosan biopolymer as component of hybrid nanosystem. Chitosan, a natural polysaccharide with excellent biodegradable, biocompatible, non-toxicity and adsorption characteristics, is a renewable polymer. The main reasons for using chitosan are undoubtedly due to its appealing intrinsic properties in medical applications such as topical ocular application, implantation or injection. Chitosan is metabolized by certain human enzymes such as lysozyme and should be considered as biodegradable and biocompatible. In addition, chitosan biodegradability has been reported that chitosan acts as a penetration enhancer by opening epithelial tight junctions. Due to its positive charges at physiological pH, chitosan is also bioadhesive, which increases retention at the site of application. Importantly, chitosan is abundant in nature, and its production is of low cost and is ecologically interesting. For these reason, chitosan has been used as pharmaceutical and medical materials including hydrogel system. The chitosan hydrogel showed good biocompatibility and biodegradability *in vivo* mouse system.

The overall objective of project being pursued is to develop simple, one step radiolytic methodology to obtain noble metal/natural polymer hybrid nanocomposite system with the goal of exploring favorable characteristics of radiation technology for nanoscale engineering of materials, especially for biomedical application, such as easy process control and the possibility of joining synthesis and sterilization in one technological step. The proposed project is designed to evaluate the potential of radiation technology to process noble metal/natural polymer biomaterial systems as a tissue-engineering platform for the treatment of peripheral nerve injury.

Specific objectives of proposed project are:

- systematically develop synthetic strategies for gamma irradiation induced *in situ* incorporation of Ag and/or Au nanoparticles in previously obtained Ch and Ch/PVA hydrogel networks, using liquid filled cavities in crosslinked polymer matrix as nanoreactors (template synthesis);
- exploring potential of simultaneously crosslinking of Ch or Ch/PVA polymers and *in situ* synthesis of noble metal nanoparticles.

Moreover, these systems should be suitable for controlled release of silver for antibacterial purpose, and therefore they have potential biomedical applications such as wound dressing.

6. Work plan for the research project

First year: Gamma irradiation induced *in situ* synthesis of Ag nanoparticles in mixture of Ch/PVA solutions will be performed. The uncrosslinked nanocomposite systems will be obtained by evaporation of solvent and characterized by UV-vis and FTIR spectroscopy in order to confirm the formation of metal nanoparticles and investigate the mechanism of their stabilization by polymer matrix, respectively. Moreover, by TEM, XRD and MALDI TOF MS analysis will be investigated the shape, structure, size and size distribution of metal nanoparticles stabilized by different ratios of polymers.

Synthesis of polymer hydrogels based on Ch or Ch/PVA and investigation of swelling kinetics, diffusion properties and network parameters. The swelling properties will be investigated in water and Simulated Body Fluid (SBF), at temperatures of at 25 and 37 ^{o}C.

Second year: Synthesis of Ag and/or Au nanoparticles in Ch or Ch/PVA hydrogels previously obtained by gamma irradiation. Investigations of possibility of one-step synthesis of nanocomposite systems in order to achieved simultaneously crosslinking of Ch or Ch/PVA polymers and *in situ* synthesis of noble metal nanoparticles. Hybrid nanocomposite systems will be characterized by swelling measurements in order to investigate swelling kinetics, diffusion properties and network parameters. Generation of metal nanoparticles in hydrogel and interaction between nanofiller and polymer matrix will be followed by UV-vis and FTIR spectroscopy, respectively. Investigation of shape, structure, size and size distribution of metal nanoparticles as well as properties of nanocomposites will be performed by TEM, SEM; AFM, XRD and mechanical measurements.

Third year: The study of biomedical potential of synthesized hybrid nanocomposite systems will be conducted to investigated their antibacterial activity and evaluate the potential of these systems as a tissue-engineering platform for the treatment of peripheral nerve injury.

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