Vladimir Popov, Ivaylo Hinkov*, Svetlomir Diankov, Maria Karsheva and Yordan Handzhiyski

Ultrasound-assisted green synthesis of silver nanoparticles and their incorporation in antibacterial cellulose packaging

Abstract: The antimicrobial activity of nanoparticles (NPs) depends of the surface area in contact with microorganisms. The large surface area of the nanoparticles enhances their interaction with the microbes. In this work, a green, simple, rapid, and efficient ultrasound-assisted reduction method for silver nanoparticles (AgNP) synthesis is presented. For the synthesis, an aqueous solution of silver nitrate, ethanol, and ammonia was used. The adopted method can be easily implemented for any kind of scientific or industrial application due to its cost-effective nature. The effect of sonication time on the nanoparticle formation was investigated. Silver nanoparticles were analyzed through transmission electron microscopy and UV-vis spectroscopy. Antimicrobial additives can be incorporated in mass in different matrixes (polymeric or cellulosic), which is a convenient methodology to achieve antimicrobial activity. In this work, silver nanoparticles were incorporated in cellulose using an ultrasonic bath technique. The most important aspect of cellulose containing silver nanoparticles prepared by this method is its high antimicrobial efficiency. The microbiological study was carried out by a standard agar technique. The analysis showed that cellulose with incorporated silver nanoparticles exhibited strong antimicrobial activity against *Escherichia coli* bacteria. This makes it a promising antibacterial material for food packaging.

Keywords: antibacterial activity; silver nanoparticles; ultrasound.

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***Corresponding author: Ivaylo Hinkov,** Department of Chemical Engineering, University of Chemical Technology and Metallurgy, 8 St. Kl. Ohridski Blvd., 1756 Sofia, Bulgaria, e-mail: hinkov@uctm.edu
Vladimir Popov, Svetlomir Diankov and Maria Karsheva: Department of Chemical Engineering, University of Chemical Technology and Metallurgy, 8 St. Kl. Ohridski Blvd., 1756 Sofia, Bulgaria
Yordan Handzhiyski: Institute of Microbiology, Bulgarian Academy of Sciences, 26 G. Bonchev Str., 1113 Sofia, Bulgaria

1 Introduction

Nanotechnology is an up-to-date and interesting branch of science offering materials with structural features between those of atoms and bulk materials with at least one dimension in the nano-range [1]. Recently there has been an increase in the number of research activities to explore the properties of nano-sized particles. Nanoparticles (NPs) may occur in different shapes such as spheres, platelets, nanorods, dimeric nanorods, and hexagonal discs [2–4]. They can be synthesized by chemical, physical, and biological methods. However, chemical reduction methods are the most commonly used.

Nowadays, silver nanoparticles (AgNPs) prepared by chemical reduction [5] can be assisted by microwave irradiation [6], ultrasonic irradiation [7], or the electrochemical method [8]. However, in most of them, either organic solvents, toxic reducing agents, and stabilizers, which had potential environmental and biological risks, or more than one reactive step was needed [5]. The sonochemical method has been studied to produce different nanomaterials, especially noble metal nanoparticles, such as silver [9, 10], gold [11, 12], and platinum [13, 14].

The physical phenomena associated with ultrasound-assisted synthesis are cavitation (formation, growth, and implosive collapse of bubbles in a liquid) and nebulization (the creation of heated microdroplets like mist in a liquid) [15, 16]. Compared to traditional methods, the main advantages of the sonochemical process are milder conditions, shorter reaction times, and higher yields [17, 18]. Thus, the ultrasonic irradiation method has gradually been introduced in synthesis as a green synthetic approach, safe for environment. Green chemistry provides a number of advantages in process development and manufacturing as well as product design. The three main factors which are very important in the perspective of "Green Nanoscience", are [15]: (i) the choice of the solvent medium, (ii) the choice of an environmentally mild reducing agent, and (iii) the choice of a nontoxic capping agent [19, 20]. The chemical effects of ultrasonic irradiation are due to the very high temperature and pressure that

are transiently formed in collapsing bubbles [10], which increase successfully the conversion, improve the yield, change the reaction pathway, and/or initiate the reaction in biological, (electro)chemical, and even for aerosol systems [21, 22]. Nowadays in the ultrasonic-assisted synthesis of nanoparticles, it has been found that ultrasonic irradiation may affect the size distribution of the particles in a narrower range than conventional heating by thermal convection, because of the penetration property of ultrasonic irradiation through solution, resulting in uniform activation energy for the reaction solution. Moreover, compared to the microwave synthesis of silver nanoparticles [23], the ultrasonic synthesis also can control the morphology and size distribution of the nanoparticles.

Silver nanoparticles can be applied in various areas of practice [5], such as catalysts, antibacterial agents, surface-enhanced Raman spectroscopy, and biochemical sensing, because of their novel physicochemical properties significantly differing from macroscopic metal phases. Silver nanoparticles are amongst the most frequently discussed nanomaterials in medicine [24]. The antibacterial effect of the silver nanoparticles is especially strong and they have been successfully employed in medicine and elsewhere [25]. The antimicrobial activity of synthesized nanomaterials is usually evaluated quantitatively or qualitatively [26] against model organisms. The antibacterial activity of nanoparticles is usually estimated on the representative group of pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Streptococcus mutans*. These bacteria are responsible for various infections in humans of reduced immunity and represent a major threat to public health in food packaging, synthetic textiles, medical devices, dentistry, drinking water, and wastewater treatment [1].

Silver nanoparticles could potentially be used for active antimicrobial surface coatings or packaging films to inhibit microbial growth. International Organization for Standardization (ISO) outlines the official norms regulating antimicrobial action and specifies the methods of evaluating the antibacterial activity of various materials. For example, ISO 20743 specifies quantitative test methods to determine the antibacterial activity of textile products, ISO 22196 specifies a method to evaluate the antibacterial activity of antibacterial-treated plastics and other non-porous surfaces of products, while ISO 27447 describes a method to determine the antibacterial activity of photocatalytic materials [1].

The aim of this work is to develop a rapid green ultrasound-assisted method for silver nanoparticle synthesis, to study the effect of operational parameters, to incorporate the obtained nanoparticles in cellulosic material, and to determine its antibacterial effect.

2 Materials and methods

In a typical reaction procedure, silver nanoparticles were synthesized by reducing silver nitrate (>99.8% $AgNO_3$ purchased from Valerus Co., Sofia, Bulgaria) with distilled water in the presence of ethanol and ammonia. An aqueous solution of silver nitrate (0.01 M) was prepared at vigorous magnetic stirring (Boeco MMS-3000, Boeco, Germany) at room temperature. After 5 min, to this solution were added dropwise 0.8 ml of ethanol (96% P.A. purchased from Valerus Co., Sofia, Bulgaria) acting as reducing agent and 0.8 ml of ammonia aqueous solution (25%). The total volume of each sample was adjusted to 100 ml. The synthesis experiments were performed in a 2.8 l conventional ultrasonic water bath (Siel UST 2,8-100, Siel OOD, Gabrovo, Bulgaria) with an ultrasonic power of 120 W for scheming time. At this stage, the colorless solution of the reaction mixture turned to the evident characteristic yellow color, indicating the formation of silver nanoparticles. The samples were analyzed by UV-vis spectroscopy (VARIAN Cary 100 Scan UV-vis Spectrophotometer, Varian Inc. USA) the same day after the synthesis. Then they were prepared for transmission electron microscopy (TEM) analysis by placing a drop of the solution on a carbon-coated copper grid and drying in air. After 24 h the samples were observed through high-resolution TEM (JEOL JEM 2100, 80–200 kV, Jeol Ltd. Japan).

In order to integrate as-obtained AgNPs in cellulose matrix, samples from raw bleached cellulose fibers were prepared. Chemical additives (aluminium sulfate and mucilage) were added to impart specific properties to paper. A semi-automatic sheet former Rapid Köthen, Gockel Munchen, Germany designed for paper-making was used to prepare cellulose samples with a mass of about 70 g/m^2. It has the advantage of performing filtration and drying in a single piece of equipment (Figure 1).

Cellulose samples were cut into disk shapes with 85 mm diameter. Silver nanoparticles were incorporated by immersing the samples in a flask containing 200 ml of the obtained yellow colored solution with silver nanoparticles. Each sample was sonicated respectively for 3, 5, 7, or 10 min in the ultrasonic bath used for the AgNP synthesis.

The microbiological study was carried out through a standard agar technique. The impregnated cellulose samples were UV sterilized for 2 h. Culture medium Lysogeny broth (LB) was inoculated with 1% suspension of *E. coli* (strain LE392). After sterilization, the cellulose disks were placed on the LB agar into a Petri dishes. The bacterial culture and the plates were incubated for 72 h at 37°C.

3 Results and discussion

The reaction mixture undergoes sonication for different times (8–25 min) at 50°C in the ultrasonic water bath. Figure 2 shows the photographs of the as-synthesized colloidal solutions. As the reaction proceeded, it was observed that the color of the solution changed from colorless to characteristic pale yellow within 8 min of US

Figure 1: Rapid Köthen sheet former used to prepare cellulose samples.

irradiation (Figure 2A), and then to dark yellow within 11 min, indicating the formation of silver nanoparticles (Figure 2B). Finally (after 25 min), the color of the solution turned to dark gray showing the signs of aggregation (Figure 2C). The appearance of the yellow color indicated the formation of silver nanoparticles in the reaction mixture. It is well known that silver nanoparticles exhibit striking colors (light yellow to brown) due to excitation of surface Plasmon vibrations in the particles. The optimal reaction time was fixed at 10 min; when the time exceeds the optimal, the reaction mixture changes to yellow.

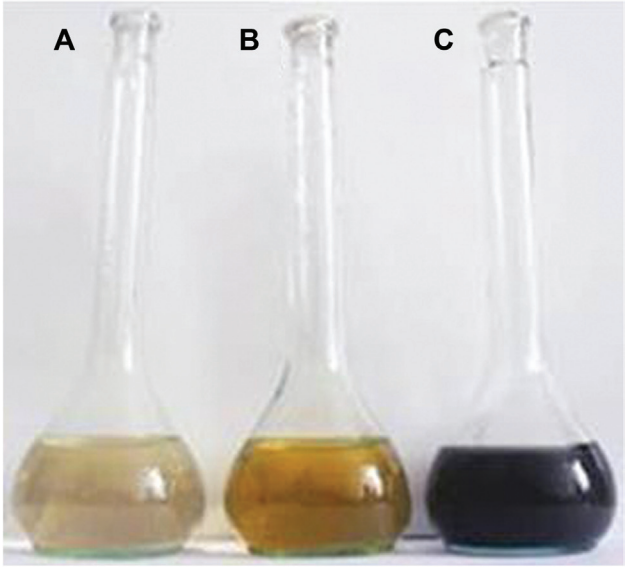

Figure 2: Evolution of the color of the reaction solution of silver nitrate (0.01 M), ethanol (96%), and ammonia (25%) sonicated for (A) 8 min, (B) 11 min, and (C) 25 min.

The results of High Resolution Transmission Electron Microscopy (HRTEM) observation, shown in Figure 3A, proved that the main component of the colloid (yellow color obtained after 11 min of sonication) was composed of uniform particles spherical in shape and only few agglomerates were observed. The size of AgNPs obtained was estimated to be between 5 and 10 nm.

Figure 4 shows a comparison of the UV-vis absorbance spectra of the prepared aqueous solutions after 8, 11, and 25 min sonication time. The intense, broad absorption peak, observed at around 425 nm, corresponds to the surface Plasmon resonance (SPR) band of silver nanoparticles. Usually, the position of the absorption peak depends on the particle size, shape, and the adsorption of nucleophile or electrophile to the particle surface. It is obvious that the absorbance increases with the ultrasonic reaction, which exhibits the occurrence of some change in the solution. The possible explanation of this phenomenon is the formation of greater silver oxide particles or agglomerates, observed with naked eye. This hypothesis needs additional experiments to be confirmed. The TEM and UV-vis analysis revealed that the formation of AgNPs could be achieved in an aqueous solution of $AgNO_3$ and the time required for this reaction was only about 10 min, much shorter than for that performed in aqueous solution (720 min), as reported by He et al. [5].

In order to test best conditions of integrating the as-obtained silver nanoparticles in cellulose matrix, the cellulose samples were immerged in the colloidal solution and

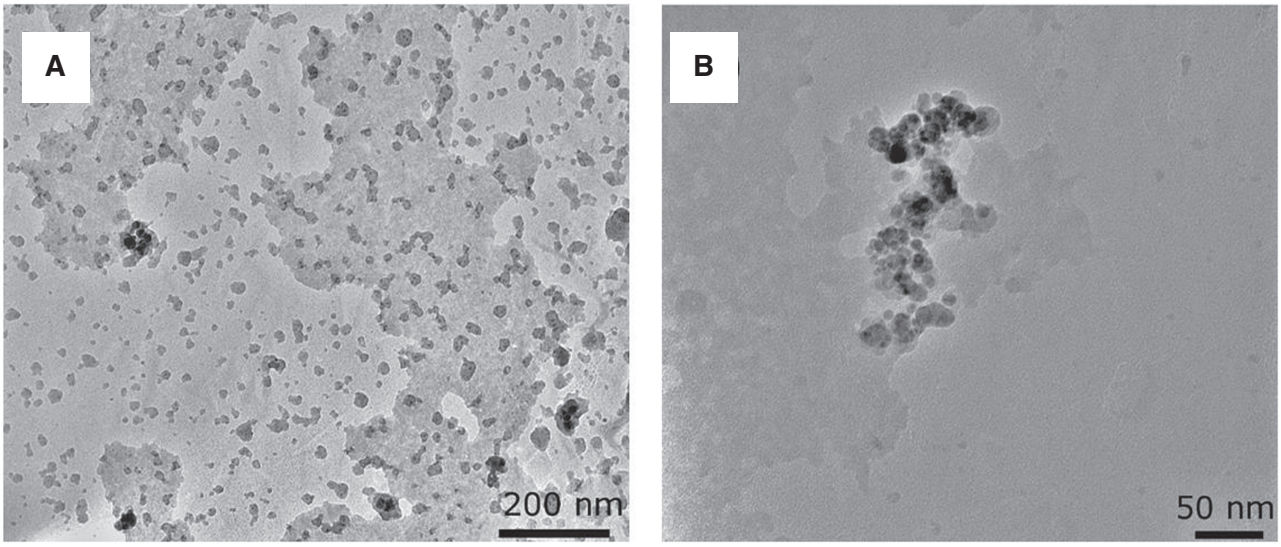

Figure 3: TEM images of silver nanoparticles synthetized in an aqueous solution of silver nitrate (0.01 M), ethanol, and ammonia via sonication for 11 min.

sonicated for 3, 5, 7, or 10 min in the ultrasonic bath. The temperature inside the sonication flask was kept constant (50°C). The ultrasonic waves promote the fast migration of the silver nanoparticles to the cellulose fibers. The deposition of silver was indicated by the change in the color of the cellulose from white to brown for all the samples.

Well-dispersed AgNPs in the cellulose matrix are required; otherwise, the antimicrobial effect decreases. However, important parameters such as particle size distribution, metal content, cationic silver release, and interaction with the surface of cellulose are also relevant parameters that influence the antimicrobial activity of these coated surfaces. In order to compare the effect of the sonication, two control samples were prepared. The first one was pure cellulose and the second one was only immerged for 60 min in the colloidal solution containing AgNPs with no sonication.

The antibacterial activities of Ag-coated cellulose and the control samples were tested against the bacteria *E. coli*. As anticipated, after 72 h of incubation, the pure cellulose control sample and the only immerged not sonicated cellulose sample show no antibacterial activity (Figure 5). In contrast, efficient antimicrobial activity is exhibited by all samples impregnates by sonication (Figure 6).

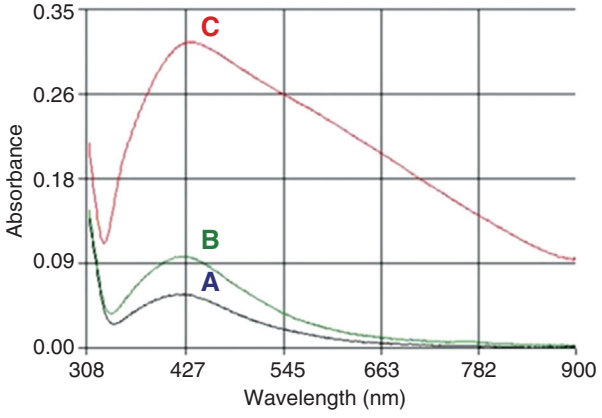

Figure 4: UV-vis absorption spectra of a reaction solution of silver nitrate (0.01 M), ethanol (96%), and ammonia (25%) sonicated for (A) 8 min, (B) 11 min, and (C) 25 min.

4 Conclusions

Silver nanoparticles have been successfully synthesized using the sonochemical route by using an aqueous solution of silver nitrate, ethanol, and ammonia. The proposed method does not involve use of organic solvents or harsh conditions like high temperature. The UV-vis studies show the SPR peak at ~425 nm characteristic of the silver nanoparticles.

In this work, a simple and rapid ultrasound-assisted method to integrate silver nanoparticles in cellulose matrix is presented. This method does not need any seed, or template, and thus is a convenient and fast pathway for large-scale and low-cost production of cellulose-based antibacterial materials.

The impregnated cellulose matrix demonstrates strong bactericidal activity against *E. coli*, and it can be used as an antibacterial packaging material to prevent food stuff from bacterial infection.

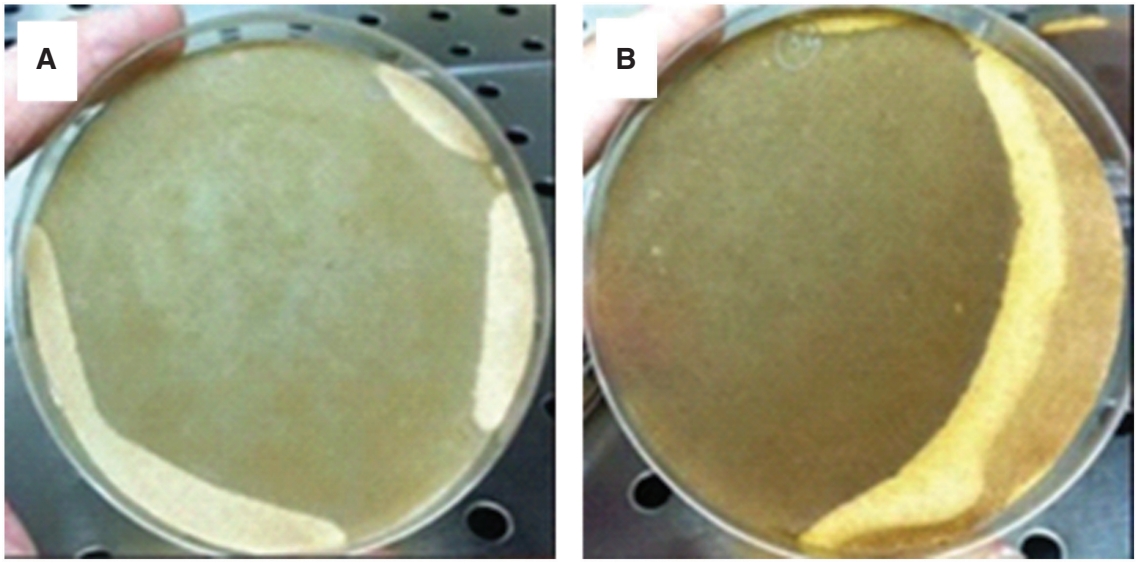

Figure 5: *Escherichia coli* bacteria grown on the control samples: (A) uncoated cellulose, (B) cellulose immerged for 60 min in a yellow colored solution containing AgNPs.

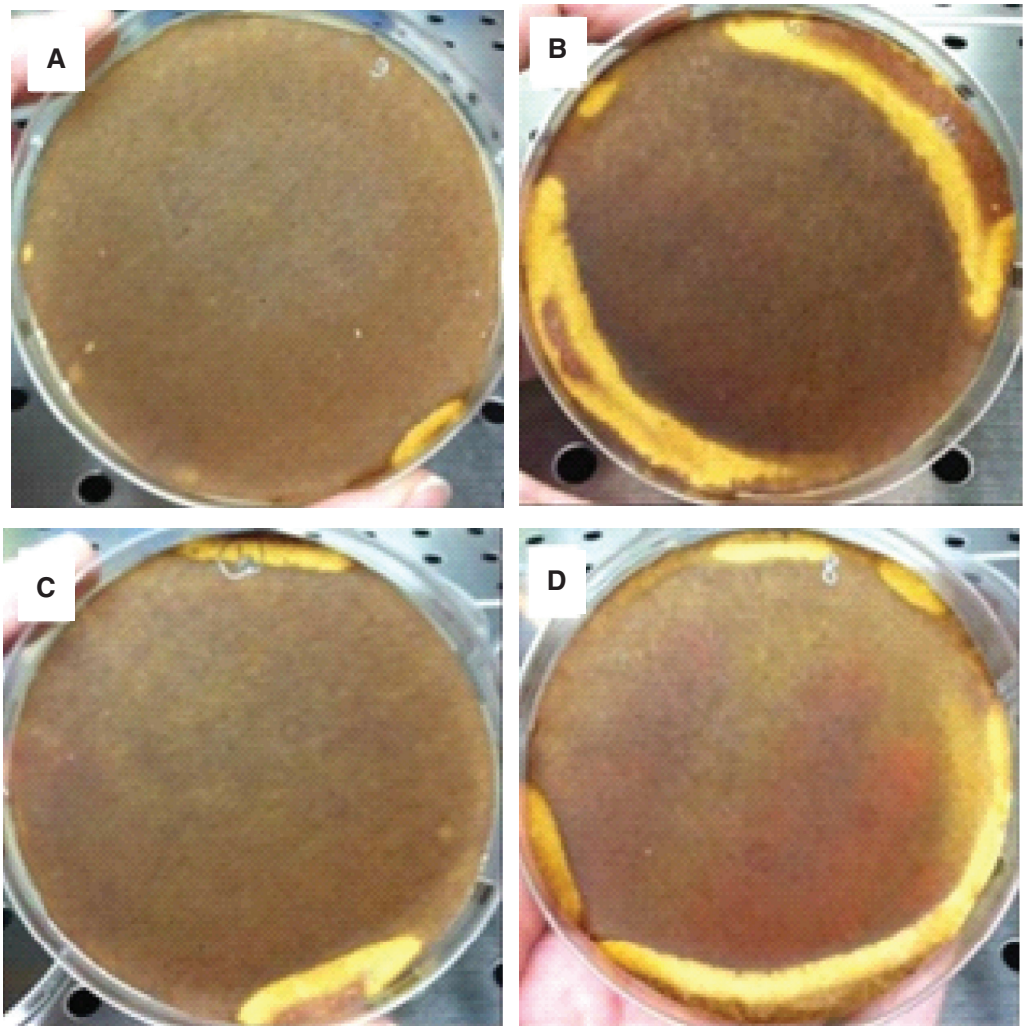

Figure 6: Cellulose samples sonicated in a yellow colored solution containing AgNPs for (A) 3 min, (B) 5 min, (C) 7 min, and (D) 10 min, showing antibacterial activity.

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Bionotes

Vladimir Popov

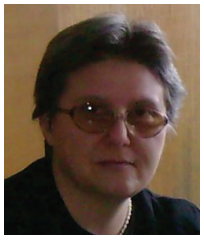
Vladimir Popov graduated from UCTM, Sofia, Bulgaria, with a degree in Chemical Engineering in 2010. He is currently a PhD student in the Department of Chemical Engineering at UCTM. He is focused on the green synthesis of silver nanoparticles by chemical reduction.

Ivaylo Hinkov

Ivaylo Hinkov obtained his PhD in Chemical Engineering from the University Paris 13, France, in 2004 where he worked on carbon nanotube synthesis by electric arc discharge. After postdoctoral research in McGill University, Canada, and the Institute of Materials, University of Nantes, France, on carbon nanostructures he became an Assistant Professor in the University of Chemical Technology and Metallurgy. He is currently working on synthesis and modeling of nanomaterials and nanostructures.

Svetlomir Diankov

Svetlomir Diankov studied Chemical Engineering at the University of Chemical Technology and Metallurgy (UCTM), Sofia, Bulgaria, and graduated in 1999. He obtained his PhD from the University Paris 13, Paris, France. Since 2003 he has been an Assistant Professor in the Department of Chemical Engineering at UCTM. Since 2013 he has been responsible for the Center for Academic Success at UCTM. He is interested in supercritical fluids, adsorption, extraction, and modeling.



Maria Karsheva

Maria Karsheva is an Associated Professor of Chemical Engineering in the University of Chemical Technology and Metallurgy, Sofia,

Bulgaria. Her scientific interests are in the fields of transport phenomena in non-Newtonian systems, in solid-liquid extraction, nanotechnology, and mathematical modeling. Recent works of the team are on the incorporation of plant extracts with antioxidant activity in cosmetic compositions and foods and on the preparation of silver nanoparticles and their use in packaging.

Yordan Handzhiyski

Yordan Handzhiyski obtained his diploma in Chemical Engineering in 1998 and received his PhD from Bulgarian Academy of Sciences in 2008 where he has been studying a process of non-enzymatic glycosylation of *Escherichia coli* chromosomal DNA. His research interests include finding the relationship between the process of glycation and mutagenesis in *E. coli* and optimization of production of recombinants proteins.