Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 2, April - June 2013, p. 787 - 795

PREPARATION OF NANOCRYSTALLINE SILVER USING GELATIN AND GLUCOSE AS STABILIZING AND REDUCING AGENTS, RESPECTIVELY

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The synthesis of nanocrystalline silver (nanosilver, NAg) was a major aim of this work. Nanosilver is a nanomaterial of great interest [1-3] due to its special biocidal properties, which are particularly desirable in various fields such as medicine, cosmetology, materials engineering, construction and others [4,5]. We aimed to develop a new method for the preparation of nanosilver, which would be completely environmentally friendly. This paper discusses the process of obtainingnanosilvervia chemical reduction. Silver nitrate (V) served as the source of silver ions, and glucose was responsible for the reduction. The stabilizing agent was a natural protein substance, gelatin, and was added to inhibit the growth of metallic agglomerates. The use of gelatin as a stabilizing factor and glucose as a reducing agent does not to interfere with the natural environment. The use of these substances renders this method environmentally friendly and is consistent with the principles of green chemistry. We investigated the influence of the independent variables, namely the molar ratio of the reducer to silver ions, the mass content of the stabilizer, the temperature and pH on the resulting suspension properties. Particle size was determined using dynamic light scattering, and a study carried out on the Zetasizer Nano-CI provided information on the electrokinetic potential, which reflects the stability of the colloidal system. Atomic force microscopy was used to image the obtained suspension.

(Received April 10, 2013; Accepted May 10, 2013)

Keywords: Nanosilver, Green chemistry, Glucose, gelatin, Chemical reduction

1. Introduction

Nanotechnology is a rapidly growing field that is used in various branches of science and industry. With the development of ever newer methods of obtaining nanomaterials, nanotechnology is becoming more accessible to a wider range of customers [1]. Nanocrystalline silver is one of the best known nanomaterials [2]. It owes its popularity to its unique biocidal properties, which allow it to be used as a control agent against pathogenic microorganisms. Nanosilver exhibits high antimicrobial activity directed against bacteria, viruses and fungi. It is known, for example, that nanosilver effectively inhibits the growth of bacteria on medical devices, which greatly improves the quality of asepsis. [3] Therefore, its properties are particularly appreciated in medicine, pharmacy, cosmetics, the construction and agricultural industries, textiles and other fields [4]. There are many ways to obtain nanocrystalline silver. In general opinion, they are divided into two groups: physical and chemical methods. Physical methods do not require carrying out chemical reactions and are associated with the mechanical comminutionof larger material, so that the final product has at least one dimension in thenano-scale, from 1 to 100 nm. Chemical methods employ a series of chemical reactions and subsequent organization of less material in ananometric form [6]. The most popular methods for producing nanosilver include chemical reduction, electrochemical reduction, photochemical reduction, laser ablation, microwave irradiation, sonoelectrochemical and sonochemical reduction, reduction with

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polyoxometals, hydrothermal methods, X-ray radiolysis, microemulsification, sputtering and electrospinning and increasingly used biological methods [7-11].

In the last decade, the development of new methods for the synthesis of nanocrystalline silver have become more and more widespread. The emphasis on innovative and modern processes is due to the popularity of the principles of "green chemistry" in the chemical industry and other related industries. Processes which are environmentally friendly and do not endanger living organisms are very popular [12]. Until now, commonly used methods have not always remained neutral to the environment and often a number of dangers are brought with them. Formaldehyde, hydrazine hydrate, sodium borohydride, polyvinylpyrrolidone, sodium dodecylsulfate, chitosan and polyethylene glycol are substrates which are used in the chemical reduction process for the preparation of nanosilver. Some of these may cause allergic reactions and burns. Their bioaccumulation in the environment may have particularly detrimental effects on aquatic organisms. Moreover, when working with the abovementioned substances, it is necessary to take protective measures; the use of polyvinylpyrrolidone as a stabilizing agent for colloidal systemsis an example. Despite the fact that the polymer itself is not classified as a hazardous or toxic substance, there is a likelihood the unreacted mers, which are carcinogenic, are present in the product [13-18].

This article describes how to synthesize nanostructured silver based on a chemical reduction method. Silver nitrate (V) was used as the source of silver ions. In order to reduce Ag^+, glucose was used, and gelatin, which is a natural protein stabilizer and a mixture of proteins and peptides, was responsible for the stabilization of the resulting agglomerates of metallic silver. In comparison with other active proteins, gelatin is a rich source of protein. Many applications of gelatin are based on the colloid effect which it is able to evoke. It can, in fact, act as a colloidal stabilizer, which is achieved by the adsorption of peptide chains on the surface of dispersed particles. Therefore, dispersed particles gain a protective layer prior to their aggregation into larger agglomerates and thus the entire system becomes stable over time [19-21]. Glucose poses no threat to the environment and, according to safety data sheets, it is considered a safe substance and characterized by good biodegradability, so that there is no danger of the accumulation of glucose in the environment [22].

The use of both of these substrates allowed for limiting the use of dangerous substances, making the process environmentally friendly and in compliance with "green chemistry" rules. The synthesis procedure and test results while using different amounts of glucose and gelatin, and at different temperature conditions and at various pH levels are described.

2. Experimental

2.1. Materials

Silver nitrate (V) ($AgNO_3$), gelatin, D(+)glucose and sodium carbonate were purchased from POCh Chemical Company. All reagents were pure per analysis (p.a.). Redistilled water was used to prepare solutions.

2.2. Methods

Spectrophotometric analysis (UV-Vis) was run on Rayleigh UV-1800 spectrophotometer over the wavelength range of 300 to 600 nm with a 2 nm resolution.The size of the obtained nanoparticles was determinedby the dynamic light scattering (DLS) technique using a Zetasizer Nano-ZSparticle size analyzer. This device also served to investigate the value of the electrokinetic potential (ζ). The resulting suspensions of nanosilver were visualized using atomic force microscopy (AFM) on a Veeco Company (USA) NanoScope V.

2.3. Samples preparation

In the order obtain a nanosilver suspension, under continuous stirring, 25 cm^3 of an aqueous solution of gelatin at a given mass concentration was added to 50 cm^3 of an aqueous

solution of silver nitrate (V) at a concentration of 0.001 mol/dm^3 in a round bottom flask placed in a water bath and on a magnetic stirrer. The mixture was then heated to the desired temperature. After establishing the desired temperature conditions, 25 cm^3 of an aqueous solution of glucose, characterized by a given molar ratio of glucose to silver ions, was added to the flask. The pH was adjusted to the desired value by the addition of sodium carbonate at a concentration of 0.1 M. The process parameters are provided in Table 1.

Table 1. Parameters of the nanosilverproduction process; clusters are indicated (blue - cluster 1, red - cluster 2, green - cluster 3)

System No.	Silver ions source	Stabilizer: $C_{gel.}$, [g/dm^3], x_1	Reducer: $n_{gluc.}$: n_{Ag^+}, x_2	pH, x_3	Temperature, [°C], x_4	
1	$AgNO_3$, 0,001 [mol/dm^3]	4	5,0	9	70	
					75	
					80	
2		8	5,0	11	70	
					75	
					80	
3		12	5,0	10	70	
					75	
					80	
4		4	7,5	11	70	
					75	
					80	
5		8	7,5	10	70	
					75	
					80	
6		12	7,5	9	70	
					75	
					80	
7		4	10,0	10	70	
					75	
					80	
8		8	10,0	9	70	
					75	
					80	
9		12	10,0	11	70	
					75	
					80	

Each system (1-10) was obtained in the temperature range from 70 to 80°C, with an interval of 5°C. The observed change in the color of the mixture from colorless to yellow indicated the formation of silver nanoparticles. As the phenomenon of agglomeration occurred, the color of the mixture became more and more intense. The resulting suspensions were stable for more than three months.

3. Results

3.1. Nanoparticle nascency

Spectrophotometric analysis was performed on all samples. The spectrophotometric results, in numerical form, underwent statistical analysis in order to identify major clusters in which the objects belonged (according to their UV-Vis spectra). The purpose of this stage was to find spectra which were identical to the characteristic spectrum ofnanosilver. According to the

literature, the peak characterizing ananosilver suspension occurs at a wavelength range of 400 to 500 nm. It was intended to group the obtained spectra into clusters in which the spectra showed the greatest similarity, with the largest variety of objects belonging to different clusters. Multivariate analysis was performed using the complete linkage method (farthest neighborhood), assuming a Euclidean distance between objects belonging to different clusters. As a result of the analysis, a hierarchical tree was generated which graphically presents the objects (according to their spectra) assigned to each cluster. By adopting the less restrictive Sneath criteria (33%), the collection of objects can be divided into a given number of clusters characterized bynanosilverproduction processes. The results are presented in Figure 1.

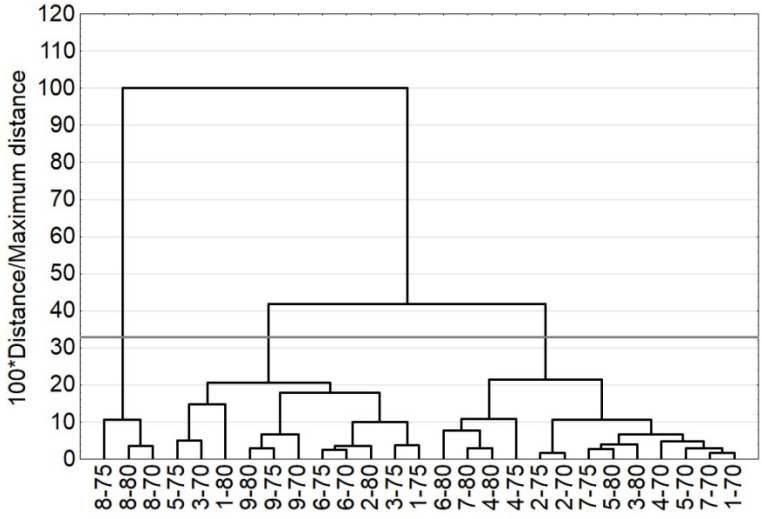


Fig. 1. The tree diagram for the nanosilver spectra (complete linkage, Euclidean distance)

It was found that three clusters were created. This means that the resulting spectra can be divided into three main groups, which contain the maximum amount of related objects within a group and the maximum amount of different objects in the other groups. Subsequently, using the k-means method, the spectra were classified. In this way, objects were grouped into a designated number of clusters and their values were averaged. Figure 2 is a graph of the average spectra obtained in the process for preparing nanosilver.

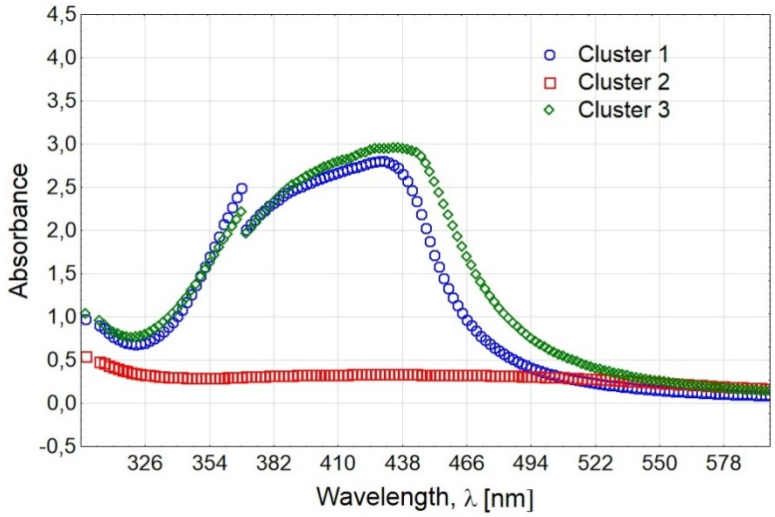


Fig. 2. Graph of the average spectral values

Based on these results, it was found that the spectrum belonging to the second focus did not meet the requirements for convergence with the spectrum characteristic of ananosilver suspension. Systems that belong to the first and third clusters represented a group of selected samples containing silver nanoparticles. Examples of the spectrophotometric data are shown in Figure 3.

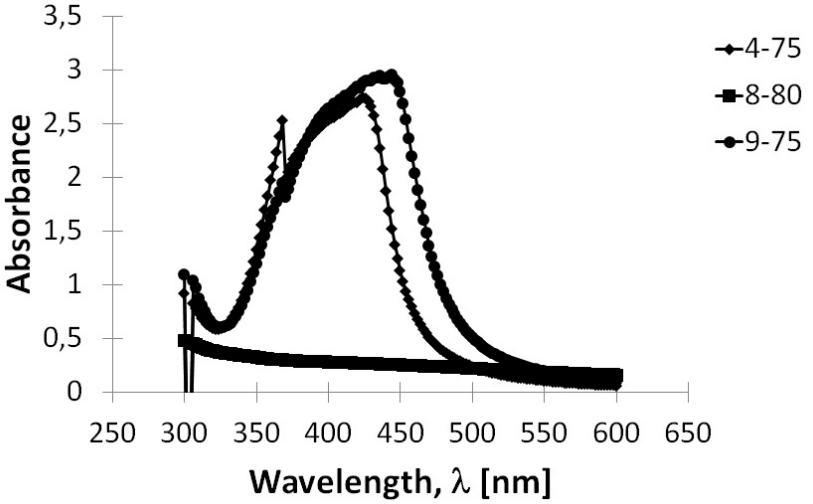


Fig. 3. UV-Vis spectra for systems 4,8 and 9

3.2. The size and stability of nanoparticle suspensions

Analysis of the silver nanoparticle size (y1) and suspension stability (y2) was performed using DLS. As a result, histograms that graphically illustrate the percentage of nanoparticles of a given size were obtained. Electrokinetic potential values, ζ, which indicate suspension stability, were also obtained. Figure 4 shows the DLS results.

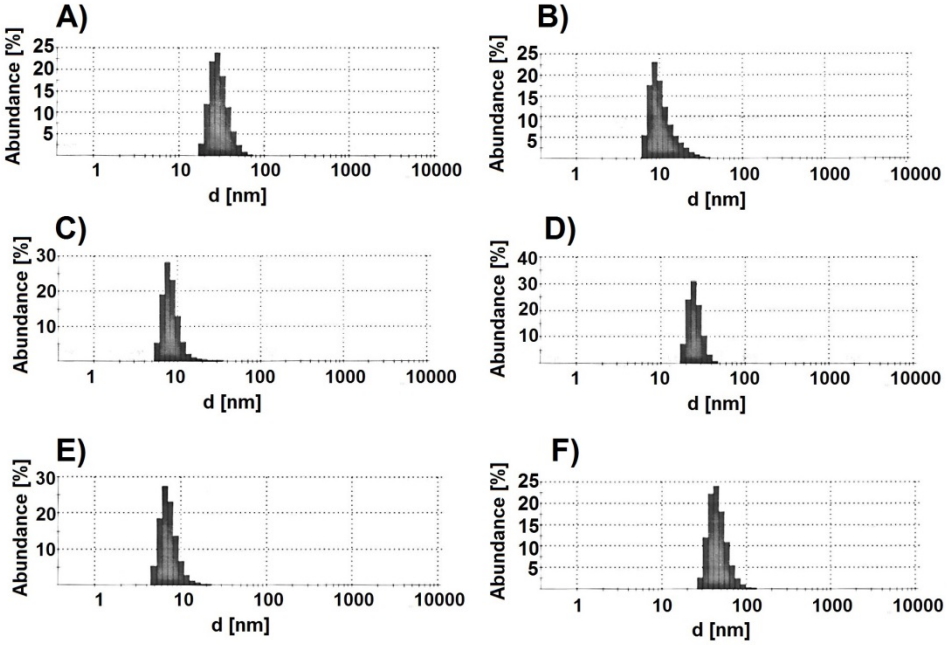


Fig. 4. The results of DLS analysis for selected samples (A – 1-70, B – 3-70, C – 3-80, D – 4-75, E – 5-70, F – 9-70)

Electrokinetic potential measurement results are detailed in Figure 5.

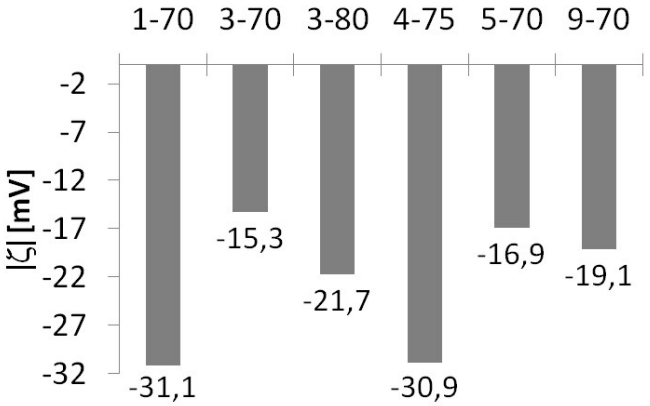


Fig. 5. Electrokinetic potential values for selected samples.

The data were analyzed statistically. The response utility profile was defined.Generating such a profile allows the prediction of the output value when process conditions are proposed. The profile of the approximated response is shown in Figure 6.

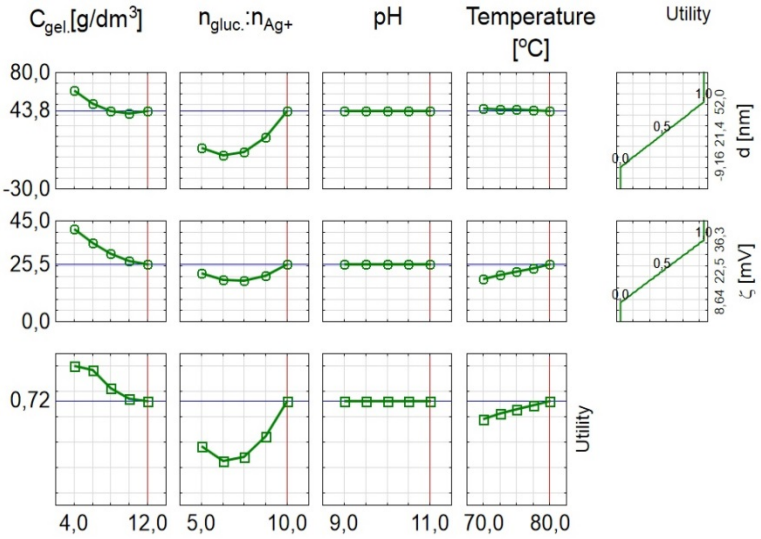


Fig. 6. Profile of the approximated output value for nanosilver suspensions

3.3. Nanoparticle morphology

Images of the suspensions were obtained by AFM (Figure 7).

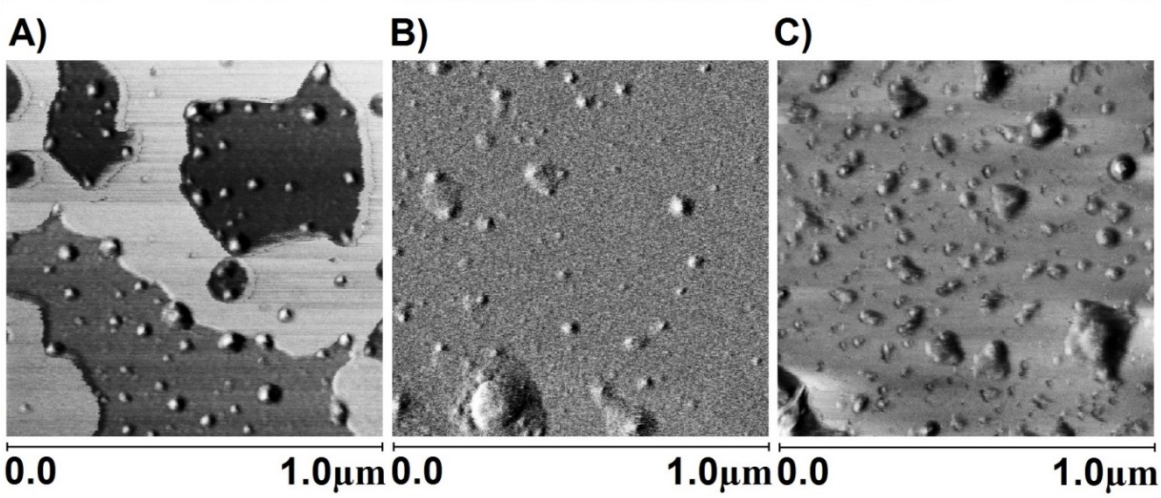
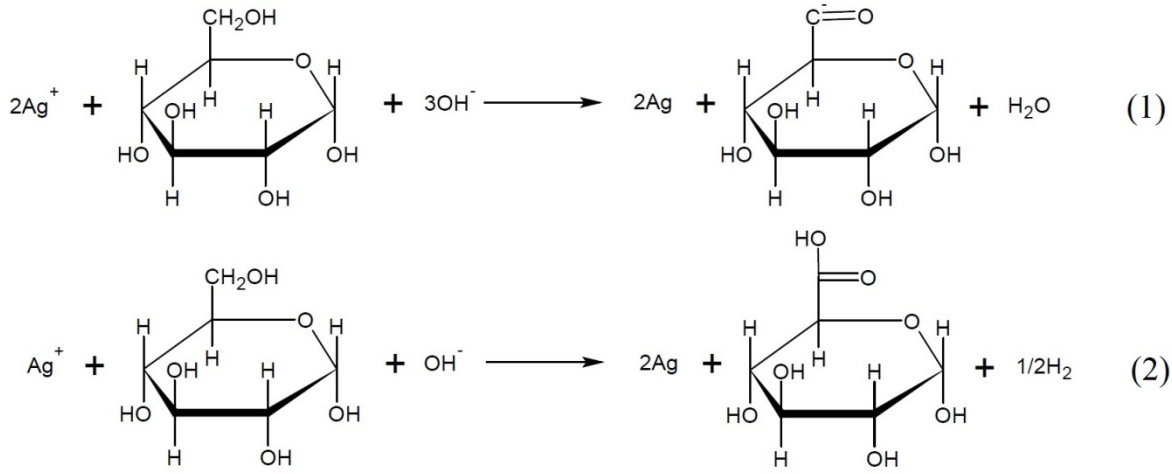


Fig. 7. AFM photographs of the suspensions (A – 1-70, B – 3-80, C – 9-70)
The shape of nanoparticles was determined. The obtained silver nanoparticles had a spherical shape, although there were particles with the shape of a tetrahedron (Fig. 7c).

4. Discussion

Based on the spectrophotometric spectra, it was found that nanosilver was present in all samples, except the sample obtained according to the parameters for system 8, as evidenced by the position of the UV-Vis spectrum peak which is characteristic for a nanosilver suspension, i.e., the maximum occurs in a wavelength range from 400 to 500 nm. In all successfully obtained samples, the peak intensity was similar and the absorbance value was approximately 3.0. This value characterizes the product concentration; it may be concluded that the process efficiency was high. The lack of a characteristic for nanosilver spectrum obtained for system 8 indicates the absence of nanosilver in this sample. This system was prepared using too much reducer in relation to the stabilizer at a low pH. Attention should be paid to the influence of pH on the results. The mechanism of the reaction between glucose and silver ions in an alkaline environment occurs according to the following equations:



Glucose includes a carbonyl group, namely an aldehyde. It is highly polar, since the negative charge is shifted towards the oxygen and a positive charge is focused on the carbon.Thanks to this, the structure is flat (trigonal) and becomes susceptible to nucleophilic attack. During nucleophilic addition, the hydroxyl ion attacks the carbonyl carbon and, in

accordance with the proposed reactions,this can lead to the separation of water from the adduct and the formation of a carbanion, or hydrogen may separate from the resulting product and the adduct may be rearranged into an acidic structure.The set of equations shows that with a greater amount of hydroxyl ions (increased pH value), the reduction reaction is favored since the partial processes take place with a higher yield.Based on the analysis of the approximated output value (Fig. 6), it can be concluded that the pH does not affect the particle diameter or the electrokinetic potential value. However, the results of this statistical analysis should be endorsed with a great deal of criticism because, as shown in the above reasoning, the pH value has a significant impact on the properties of the resulting suspension. This discrepancy can be explained by the fact that too narrow a range of pH values was taken into consideration in these studies (from pH 9.0 to 11.0).

Comparing the curves of the average spectra in the process of obtaining nanosilver (Fig. 2), differences in the position of the peak maximum were seen.Cluster 1 is characterized by a maximum peak at a shorter wavelength, while the maximum peak in cluster 2 is shifted towards the infrared.This reflects the size of the nanoparticles, as in most cases with an increase in the average particle size, the maximum peak is shifted to higher wavelengths.In general, as observed for larger particles, displacement of the peak maximum in the direction of shorter wavelengths with a decrease in size is related to the retardation of the electromagnetic wave.In addition, in the spectra which characterize suspensions of nanosilver, two peak maxima may be seen. This is related to the arrangement of particles relative to the incident electromagnetic wave.The sharp peak maximum located at the shorter wavelength is created due to the radiation on particles which are arranged in a horizontal plane relative to the incident wave.

Analysis of the profile of approximated output value shows that with an increase in the gelatin content in the system, the average diameter of the nanoparticles and the zeta potential decreased. This is due to more effective stabilization when a large amount of stabilizing agent was used and a larger amount of smaller particles was created. Reducing the value of the electrokinetic potential was associated with an intensified process of shielding the surface charge, since more peptide chains are deposited onto the nanoparticles. An increase in temperature favored a slight decrease in the average diameter of the nanoparticles and the stability of the suspension increased, which is reflected in the higher value of the electrokinetic potential. At higher temperatures, the particle size decreased, because the chemical reduction occurred more intensively, associated with the increased activity of glucose and mobility of silver ions. As a result, a larger amount of smaller nanoparticles formed. The profile suggests that the impact of glucose on the results has a minimum impact, and the nature of the effect is non-linear. When the content of the reducing agent in the system increased, while at the same time the content of the stabilizer was constant, it was expected that smaller nanoparticles would be formed. This is due to the intensification of the reduction process, i.e., a larger amount of silver ions would be reduced in a shorter time and a stable suspension would be created, provided that the amount of stabilizer would stabilize the system in an effective way. However, the data are related to the systems that were obtained while changing the amount of the stabilizer. The minima shown in the approximation profile may result from the use of a lower amount of gelatin in relation to the amount of glucose,leading to the formation of an unstable systems, resulting in a lower value of the electrokinetic potential and the simultaneous creation of a larger amount of smaller particles.

The shape of nanoparticles was the only thing which could have been determinedbasing on the AFM photographs. A spherical shape was the main structure of the obtained silver nanoparticles, although there occurred particles with the shape of a tetrahedron as well (Fig. 7c).

5. Conclusion

Anovel method has been developedfor the preparation of nanosilver, using glucose and gelatin as the reducing and stabilizing agents, respectively. Nanosilver suspensions were prepared that were stable for more than three months. The influence of independent parameters (amount of the reducer and stabilizer, temperature and pH) was evaluated in terms of properties of obtained suspensions. The method can be successfully used in ecological processes for the preparation of nanosilver.

Acknowledgements

The scientific work is financed in the years 2010-2013 as research project 4111/B/H03/2010/39.

This work is protected by patent application No. P.399112.

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