Original Research

Microscopic and Spectroscopic Analyses of Selected Agricultural Formulations Containing Various Nanostructures

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Abstract

The aim of the present study was to analyse commercially available compounds containing nanoparticles in the form of Ag, Cu, and Ag sulphate colloids applied in agriculture. The compounds were analysed with TEM and FTIR spectroscopy, i.e., methods that can complement the commonly used research techniques such as EDX or Raman spectroscopy. The results of the microscopic examinations evidenced the presence of single spherical agglomerates (small granules) of the nanoparticle-containing products. Infrared spectroscopy revealed the presence of bands characteristic of vibrations that can be assigned to nanoparticle-X interactions, where X = S, C, or O in aqueous colloid solutions of the analysed samples. Additionally, bands characteristic for C-H stretching vibrations (probably associated with vibrations of the -CH_2 and -CH_3 groups) were observed as bands originating from compounds used as the carriers of the analysed systems. In the case of Ag sulphate, the most distinct band with a maximum at 2,863 cm^{-1}, which is characteristic of SH$\cdots$Ag vibrations, can be observed, and its intensity may be associated with the nanostructure composition of the formulation, which is more diverse than that in the other two compounds.

Keywords: nanoparticles (nanosilver, nanocopper), transmission electron microscopy (TEM), molecular spectroscopy (Fourier transform infrared spectroscopy (FTIR))

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Introduction

Modern agriculture, which is largely based on complex agro-chemical relationships, has achieved an increase in crop yields, thereby posing a serious threat to soil fertility and cleanliness of the natural environment. Adverse effects of the agro-chemical crop cultivation that have been noted in the agro-ecosystem are reflected in the problems of human health. Introduction of the concept of green technology involving the promotion of healthy food, cost-efficient energy, pollution control, etc., has been given priority [1]. The development of nanotechnology motivating individual countries to allocate substantial funds (e.g., the U.S. government spends more than $1 million annually) for implementation thereof can be considered in a similar context [2].

The definition of nanotechnology published and officially accepted by the British Royal Society and the Royal Academy of Engineering [3] specifies that "nanoscience" is the study of phenomena and manipulation of materials at atomic, molecular, and macromolecular scales, where properties differ significantly from those on a larger scale. In 2010 the International Organization for Standardization (ISO) Technical Committee (TC) published a definition of nanotechnology that contains the same elements as those mentioned in recent decades: nanotechnologies involve understanding and controlling nano-scale processes, where the beginning is dependent on the scale of the phenomenon, which usually provides new applications. Nanotechnologies make use of "material properties in the nanoscale, which are distinct from those associated with individual atoms, molecules, or matter for the creation of improved materials, devices, and systems" [4].

It can be claimed that nanotechnology has already revolutionised health care, the textile industry, and information, communications, and energy technologies. A variety of antimicrobial dressings, transparent sunscreens, stain-resistant fabrics, or self-cleaning windows available on the market are examples of products that contain nanoparticles. The achievements of nanotechnology are also applied in the field of renewable energy or, more specifically, the production of biogas, heating mats, and coatings; solar cells with thinner absorbers made of carbon nanomaterials; systems for drying warehouses; and animal husbandry facilities [5]. In agriculture, nanoparticles (NPs) in the form of monocrystalline non-ionic or ionic silver and copper structures have become one of the basic components added for plant protection and the fight against diseases or plant growth stimulants, e.g., in foliar fertilisers [6]. Therefore, one of the major tasks for nanoscience in agriculture is to increase yields and achieve the highest crop quality [7-9].

Characteristics of Nanotechnology

Nanostructures have become a key to the access to a greater range of technology and industry and one of the main priorities for science. Nanotechnology combines such disciplines as physics, chemistry, genetics, information and communication technologies, environmental protection, or military technologies [10].

Nanoscience is also essential for soil science, as many natural compounds in the soil are nanoparticles. In the nanoscale, interactions between particles are dominated either by weak van der Waals forces or by covalent interactions. It should be noted that nanoparticles are not a human invention, as they have always existed naturally (e.g., NPs have been found in a 10,000-year-old glacier ice core) [11-12]. Two mechanisms governing the natural formation of NPs in the environment, i.e., geological and biological processes, have been distinguished [13-14]. The geological mechanisms comprise, e.g., physico-chemical atmospheric conditions and volcanic activity (producing inorganic NPs). The biological mechanisms usually yield organic nanoparticles, although some organisms are able to produce mineral granules in the cells. Various nanomaterials are present in the soil as nanoscale aggregates of organic and bacterial matter, including organic particles such as zeolites, humic acids, allophones, aluminium silicate hydroxides, clay minerals, amorphous materials (Al_0 or Fe_0), $Fe(OH)_2$ (hematite, lepidocrocite, magnetite), $Mn(OH)_2$, uraninite (UO_2), and clays (montmorillonite) [15].

Application of Nanotechnology in Agriculture

The agricultural application of nanotechnology is mainly associated with the food industry for the production of novel functional materials and products and the development of new methods providing food safety. It is assumed that nanotechnology research should follow the principles of sustainable development to ensure the safety of materials and products throughout the transformation cycle. The development of nanotechnology should be based on the design of nanomaterials in their practical aspect accompanied by minimisation of energy use and preventing the production of large amounts of waste.

An advantage of the increasing use of nanotechnology is the greater efficiency of food production methods by the lower consumption of agrochemical products (e.g., pesticides, biocides, antibiotics, or veterinary drugs) [16-17]. Nanotechnology facilitates production of high-quality food and feed supplemented with nano-scale materials and additives. The possibility of the detection of pesticide residues in food is associated with the use of nanosensors as well [16, 18-19]. This application is becoming an alternative to traditional analytical methods, as it not only facilitates increasing the frequency of sampling but also reduces the costs of the use of analytical methods [20].

All products based on Ag or Cu should ideally be chosen to produce a wide range of antimicrobial effects and maintain a suitable level of activity. The emergence of antibiotic-resistant bacteria has led to an increased use of silver and copper as an antimicrobial and detoxifying agent [21-22]. There are more than 100 Ag-containing pesticides. Silver nanoparticles (AgNPs) [23] or their various compound forms are potent antibacterial agents

used currently. Many publications present an AgNP colloidal solution as a safe form and an effective method for treating many diseases in agriculture [24-25]. The application thereof ensures higher efficiency of penetration of herbicides through the cuticle and tissues of weeds [26]. As reported by Lu et al. (2010) [27], citrate used for coating colloidal AgNPs does not induce genotoxic (genetic), cytotoxic (cellular), and phototoxic (photo-degradation toxicity) effects in humans. In contrast, powdered citrate used for coating Ag nanoparticles exhibits considerable toxic properties [28]. Research focused on the discovery of biocompatible coatings able to alleviate the effect of the toxicity of nanomaterials would increase the possibility of the application thereof for accelerating the germination process or plant growth. The use of nanomaterials and biocomposites provides numerous benefits, e.g., permeability, crystallinity, thermal stability, and biodegradability [29].

The size of nanoparticles determines the depth of their penetration into plant tissue. As suggested by Ma (2010) [30], nanoparticles smaller than 5 nm easily pass through the pores in the plant cell wall, which has a diameter of 5-8 mn.

Characteristics of Nanosilver and Nanocopper

Silver is known for its antibacterial properties (mainly against yeast, fungi, viruses, and bacteria) [31]. *In vitro* studies have shown that nanosilver can substantially inhibit the growth of plant pathogens in a dose-dependent manner. Silver nanoparticles are small enough (~5 nm) to act by inactivation of bacterial enzymes, inducing disturbances in DNA replication or impairment of the cell membrane function, and primarily by the generation of reactive oxygen species (ROS) [32]. Copper is one of the oldest plant protection products used in agriculture to ensure normal plant growth and in livestock feeding [33]. Additionally, Cu is actively involved in important cellular processes such as photosynthetic mitochondrial electron transport, respiration, cell wall metabolism, hormone signalling, and protein and iron mobilisation [34]. However, at high concentrations, Cu is highly toxic to plants and causes chlorosis, necrosis, growth inhibition, and root growth inhibition. Nanocopper exhibits antibacterial properties similar to those of nanosilver. In the form of nanoparticles, copper is less toxic than its soluble ionic form [35]. Particle-bound copper has lower bioavailability and therefore lower toxicity than the ionic form. At concentrations of 40-60 mg/ml, it is toxic to various organisms such as algae, bacteria, crustaceans, and fish [36]. The type and application mode of these compounds are important, as the global production of Ag and Cu in the form of NPs is estimated at more than 500 tons per year [37], and their industrial and agricultural use is constantly increasing [38].

Transmission electron microscopy (TEM) is one of the most common and most reliable techniques for measuring the size of metal nanoparticles (NPs); it can provide information about their size and shape [39-41].

The aim of this study was to analyse NP-containing products available on the domestic market and that are applied in agriculture as an antibacterial and antifungal agent and, in a suitable combination, as a simulator of plant growth influencing plant development and inducing plant resistance. The analysis consisted of two tasks: to confirm the content of silver and copper nanoparticles in the formulations and determine their size, which is strictly associated with their plant penetration capability; and to perform a qualitative analysis of the homogeneity of the material and characterise specific bands in the selected components of the formulations with FTIR infrared spectroscopy, which provides information about their interactions. Another aim was to check whether the use of such research techniques as TEM or FTIR infrared spectroscopy could be supplementary to the common research methods (e.g., Raman spectroscopy or EDX elemental analysis).

Materials and Methods

Characteristics of the Material

Our research material consisted of three commercially available formulations. The first two compounds available in 1-liter bottles contained nanosilver and nanocopper colloids at a concentration of ≥0.2% (silver) and ≥0.1% (copper) produced by ITP-SYSTEM Sp. z o.o. in Dąbrowa Górnicza, Poland. The third agent, i.e., fine granules containing sulphates of several elements (Al, Mg, Mn, Ni, and Ag at nanomolar concentrations of 10^{-9} mol) was purchased from AGRARIUS Sp. z o. o. in Dybawka, Poland.

Transmission Electron Microscopy (TEM)

For TEM, specimens of nanoparticles were prepared by pipetting a small drop of solution onto electron microscopy grids coated with support film (formvar-carbon). Due to the metallic/mineral character of the particles, no contrast was added. After short incubation (<5min), the drops were removed from the film by blotting with filter/tissue paper. The grids were finally dried in a vacuum during prolonged transfer into the microscope column. Specimens were observed with the microscope at 25k x and 50k x magnification (Zeiss LEO912AB, at 100kV). For selected images at 50k x, the size of the nanoparticles was measured using standard functions of microscope camera software on automatically calibrated images. The images were enlarged from 2.5 to 3.5x to present a more accurate method for measuring the nanoparticle-containing products.

FTIR Method

The measurements of ATR-FTIR background-corrected spectra were carried out in solvents using an HATR Ge trough (45° cut yielding 10 internal reflections)

crystal plate for liquids and recorded with a VERTEX 70 Fourier transform infrared spectrometer. Typically, 25 scans were collected, Fourier-transformed, and averaged for each measurement. The IR absorption spectra at a resolution of one data point per 1 cm^{-1} were obtained in the region between 4,000 and 400 cm^{-1}. The instrument was purged with argon for 40 min before and during the measurements. The Ge crystal was cleaned with ultra-pure organic solvents, for example methanol, ethanol, acetone,

and propan-2-ol (Sigma-Aldrich Co.). All experiments were carried out at 23ºC.

Results and Discussion

Measurements performed with the TEM method ensure reliable and accurate characterisation of such physical properties as the particle size and shape and
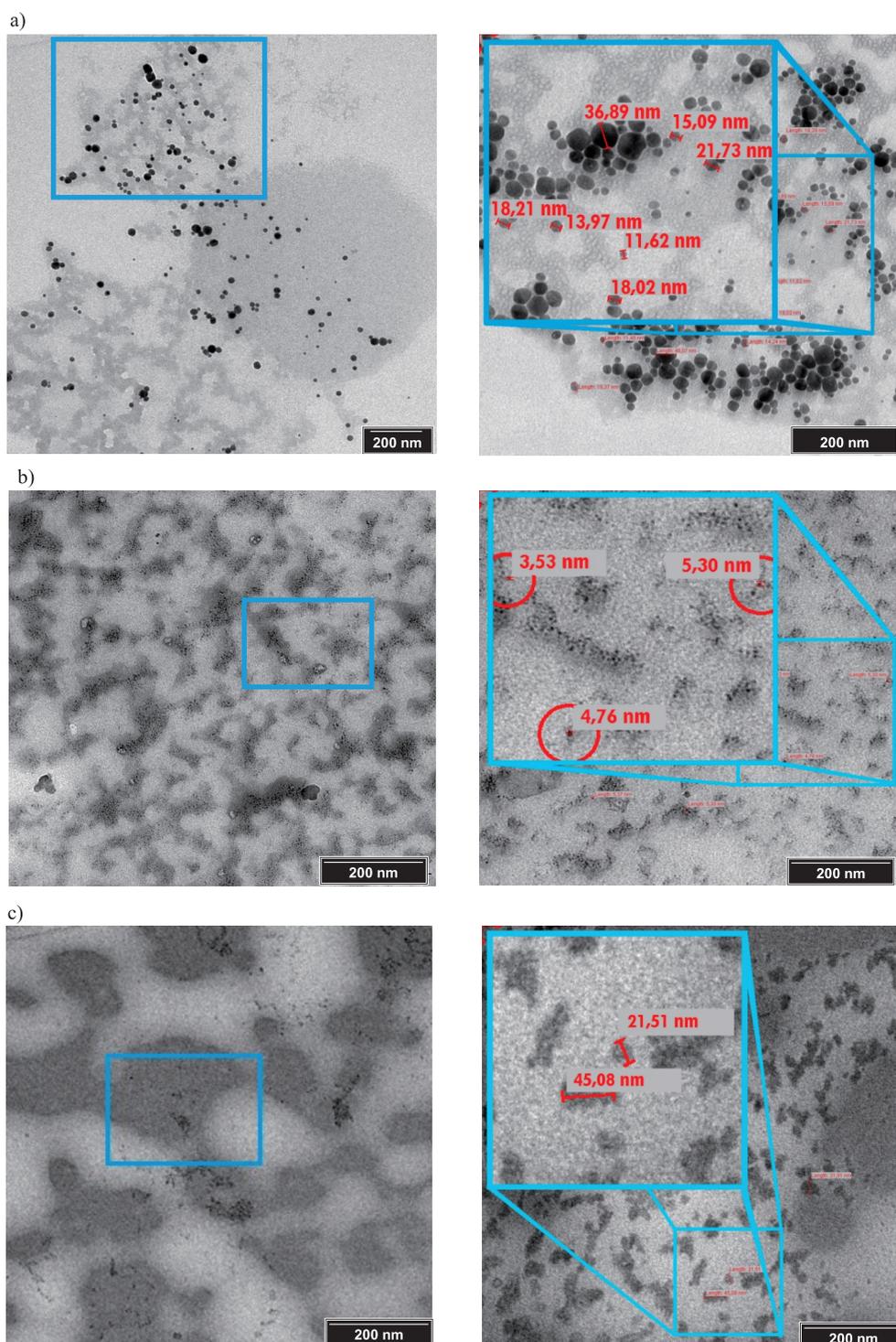
Fig. 1. TEM images of nanoparticles: a) Ag colloid 25 k and 50 k, b) Cu colloid 25 k and 50 k, c) Ag sulphate (10^{-9} mol) 25 k and 50 k.

surface morphology/topology, depending on the type of material and processes involved in the production thereof. The most essential elements are the preparation of the sample, selection of molecules, and the magnification used. Fig. 1 presents the three formulations analysed at two magnifications, i.e., 25k on the left and 50 k on the right. The TEM analysis (Fig. 1) have revealed that the Ag and Cu nanoparticles and the Ag sulphate nanoparticles consist of single agglomerates (small granules), irrespective of the concentration. The particles in the analysed NP-containing formulations are mostly spherical. Clearly, in the case of the Cu and Ag sulphate colloids, the nanoparticles are characterised by smaller sizes and their number is substantially higher, which may enhance the activity of the formulation in plant cultivation.

Fig. 2 shows the mean content of nanoparticles in the Ag colloid in an area of 16 314 nm^2. There were 75,000 nanoparticles with an average height of 8.914 nm and a minimum and maximum height of 2.136 nm and 36.89 nm, respectively. Fig. 3 presents an analysis of the content of nanoparticles in the Cu colloid. The mean content of nanoparticles in the generated image is 101.000 and their average, minimum, and maximum heights are 7.683 nm, 5.085 nm, and 12.041 nm, respectively.

The results of the analysis of the third formulations are shown in Fig. 4. The mean number of Ag sulphate nanoparticles is 322.00 in the analysed area and the average, minimum, and maximum heights are 7.249 nm, 3.560 nm, and 45.08 nm, respectively.
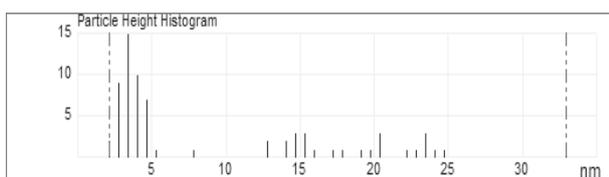
Fig. 2. Analysis of nanoparticles in the Ag colloid.
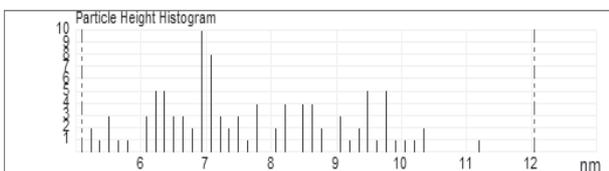
Fig. 3. Analysis of nanoparticles in the Cu colloid.
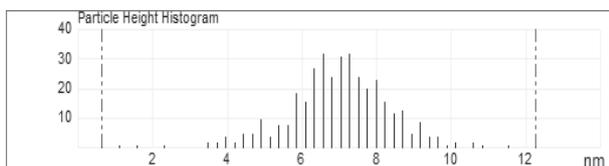
Fig. 4. Analysis of nanoparticles in the Ag sulphate colloid.

Spectroscopic Study: FTIR Infrared Spectroscopy

The next step of the investigations of the formulations involved FTIR infrared spectroscopy analysis. Infrared spectroscopy methods are used for, e.g., qualitative and quantitative analysis and examination of the homogeneity of the analysed material (Table 1 and Fig. 5) [42-43], and primarily for assessing potential molecular interactions in analysed material. Infrared spectroscopy measurements provide information about the purity of analysed formulations, and partial but useful information about the mode of surface adsorption of investigated nanoparticles. It should be noted that the infrared spectroscopy methods are becoming increasingly popular with researchers who identify various NP-containing products [44], mainly due to the immediate acquisition of results, the use of small amounts of the tested compound and chemical reagents, substantially lower costs in comparison with those in other techniques, and the absence of sample destruction. Moreover, the techniques can provide basic information about the interactions between the components of the analysed materials by analysing relevant group frequencies. With FTIR spectroscopy, it is possible to distinguish a regional characteristic of NP-X interactions, e.g., 250-800 cm^{-1}, which indicates the presence of nanostructures in the formulation [44-45]. Fig. 5 shows ATR-FTIR spectra of the analysed samples: Panel A – the solution with Ag nanoparticles, Panel B – the solution with Cu nanoparticles, and Panel C – a spectrum from the solution with Al, Mg, Mn, Ni, and Ag sulphates at nanomolar concentrations. All samples were loaded on a ZnSe crystal and the measurement was performed in the N_2 atmosphere (as described above in the Material and Methods section). The visible very intense and
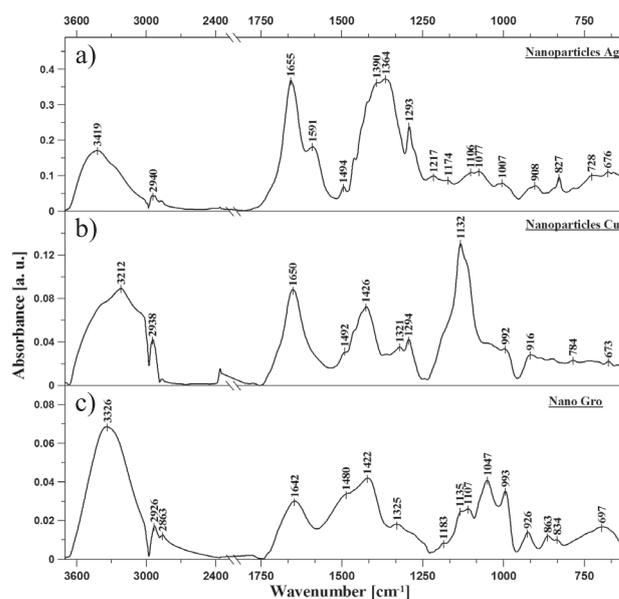
Fig. 5. FTIR infrared spectra for: Ag – Panel A, Cu – Panel B, and Ag sulphate colloids (as well as Al, Mg, Mn, and Ni) – Panel C. The spectra were generated at 23ºC.

Table 1. Position of the maxima of absorption bands assigned to appropriate vibrations for the analysed nanostructures (Ag nanoparticles, Cu nanoparticles, and Ag sulphate, Al, Mg, Mn, and Ni nanoparticles) in the spectral range of 3,700-600 cm^{-1}.

FTIR			Type and origin of vibrations
Position of bands (cm^{-1})			
Ag nanoparticles	Cu nanoparticles	Ag sulphate nanoparticles	
3,419st	3,212st	3,326st	ν (O-H) in H$_2$O
2,940	2,938m	2,926 m	ν(C-H) in CH$_{2, s+as}$, CH$_{3, s+as}$ or SH···Ag
		2,863	
1,655st	1,650st	1,642st	COO$^-$
1,591			C-H bending or ν (NH$_3^-$)
1,494	1,492	1,480 m	C-H bending
	1,426	1,422	ν (C-O-H)
1,390			
1,364st			C-H bending
	1,321	1,325	
1,293	1,294		ν (C-O)
1,217	1,246st	1,242 m	ν (C-H)
1,174		1,183	
1,106	1,132	1,135	M-O, M = Ag, Cu or X
		1,107	ν (C-O-C) or ν (C-O-H)
1,077		1,047	
1,007	992	993	
908	916		ν (C-O-C)
		m863	M-O, M = Ag, Cu or X
827m		834	
728w	784w		
676	673	697m	

ν – stretching vibrations, δ – deformation vibrations, s – symmetric, as – asymmetric, st – strong, w – weak, m – medium

broad bands with maxima at 3,419 (Ag), 3,212 (Cu), and 3,326 cm^{-1} (Ag sulphate) are characteristic of ν (O-H) stretching vibrations in aqueous colloid solutions of the samples, which remained after evaporation of the solvent [44-45], and a colloid mixture in which the analysed nanoparticle structures are kept. The 3,419 cm^{-1} band is also characteristic for N-H (ν (N-H) stretching vibrations (see Panel A). In the case of the Cu colloid, the band is definitely the broadest and exhibits the greatest shift toward lower frequencies, thus underpinning another band with a maximum at 2,938 cm^{-1}. In the spectra with maxima at 2,940 (Ag), 2,938 (Cu), and 2,926 cm^{-1} (Ag) (Al, Mg, Mn, Ni) sulphate, relatively distinct bands characteristic of the stretching vibrations of the aliphatic C-H groups [44] (probably originating from vibrations of the -CH$_2$ and -CH$_3$ groups) can be observed. These bands may be assigned to stabilisers, in which the investigated nanosystems are dissolved [44]. As specified by the

manufacturer of the analysed nanostructure-containing formulations, the mixtures have no clear coatings but are stabilised with organic acid salts, which is not specified in the product description by the producer. Noteworthy is the fact that the bands (in particular those with low intensity from the 2,870-2,600 cm^{-1} region) are characteristic for the stretching vibrations of the S-H group as well. They probably correspond to the vibrations of this group; yet these vibrations are most likely to be generated by the SH···Ag system (or another type of NPs). This may be evidenced by the interactions of the nanoparticles with their stabilisers (see above). In the case of Ag sulphate, the band with a maximum at 2,863 cm^{-1} characteristic also for C-H stretching vibrations is most prominent [44]. Its intensity may also be associated with the more intense metal-stabiliser vibrations in this formulation, compared with the other two products. As mentioned above, besides Ag, there are Al, Mg, Mn, and Ni nanoparticles in the FTIR

spectrum of the formulation presented in Panel C. The intensity is also associated with the apparent interactions between the components contained in the formulation and the aqueous colloid, in which the formulation components stabilised with organic acid salts are suspended.

Next, characteristically intense bands with maxima at 1,642 cm^{-1} in the case of Ag sulphate (Panel A), 1,650 cm^{-1} for Cu nanoparticles (Panel B), and 1,655 cm^{-1} for Ag [and Al, Mg, Mn, and Ni; Panel C] can be observed in the nanoparticles in the three analysed systems. The bands, together with a band with a maximum at ca. 1,591 cm^{-1} (Panel A), may be assigned primarily to the vibration of the COO group combined with the vibrations of the NH$_3^-$ group. This is related to the structure of the products used for stabilizing the solutions. Noteworthy is the band with a maximum at 1,591 cm^{-1}, which is only observed in the case of the formulation containing Ag nanoparticles (Panel A in Fig. 5). According to literature data, the band may represent C-H stretching vibrations and be characteristic for NH$_3^-$ vibrations, which is probably associated with the effect of the stabiliser used. In turn, the band with a maximum at 1,480 (Panel C), 1,492 (Panel B), and 1,494 cm^{-1} (Panel A) is characteristic for C-H stretching vibrations [44] and exhibits the greatest intensity in the case of the Ag sulphate solution, which may result from strengthening the NP-stabiliser vibrations.

The analysis of the other FTIR bands revealed the presence of bands characteristic for C-O-H vibrations with a maximum at 1,422 in the case of Ag sulphate, 1,426 in Cu, and 1,390 cm^{-1} in Ag (Al, Mg, Mn, Ni) nanoparticles. In the case of this material, the characteristic band may be assigned to deformation vibrations typical of the C-H group [45]. The band with the highest intensity with a maximum at 1,364 cm^{-1}, corresponding to C-H stretching vibrations and noted for the Ag nanoparticles, is substantially less intense in the case of Cu nanoparticles (with a maximum at 1,321 cm^{-1}) and the sulphate solution (with a maximum at 1,325 cm^{-1}). The bands present in the "fingerprint" region confirm the manufacturer's information on the stabilisers used in the analysed products. In turn, the bands with a maximum at 1,293 (Panel A) and 1,294 cm^{-1} (Panel B) characteristic for C-O stretching vibrations are poorly visible in the Ag sulphate solution (Panel C). The very important bands with a maximum at 1,106 (Panel A), 1,132 (Panel B), and 1,135 cm^{-1} (Panel C) correspond to the NPs-O vibrations (where NPs are, e.g., Ag, Cu, Ni); in Panel B, the bands are clearly underpinned by the aforementioned vibrations with a maximum at 1,132 cm^{-1}. The greatest intensity of this band is observed in the Cu nanoparticles (Panel B), which evidences the intensity of the NP-stabiliser interactions. The bands with a maximum at 1,047 (Panel C) and 1,077 cm^{-1} (Panel A) probably correspond to the stretching vibrations of the -OH or C-OH groups. In turn, the bands from the 900-1,010 cm^{-1} region in all the analysed samples correspond to C-O-C and C-OH vibrations. The C-O-C or C-OH bands clearly support the presence of stabilising agents in the analysed formulations. Furthermore, the vibrations from the 700-650 cm^{-1} region characteristic for C-S stretching vibrations are visible in all solutions. The last region available for analysis in the solutions is very interesting and characteristic for the NP-stabilising medium interactions. The 650-870 cm^{-1} region (usually even below 650 cm^{-1}) is characteristic for vibrations associated with the interactions of the metal with an O atom (or other atoms) in the medium, in which the formulation was suspended (stabilised) [45]. In the case of the sulphate solution, the region is the most characteristic and exhibits the most intense vibrations, which is probably associated with the presence of metallic particles other than Ag and Cu from Panels A and B, e.g., Al, Mg, Mn, and Ni in the colloids (data provided by the manufacturer). The presence of these vibrations within the specified range clearly indicates the presence of van der Waals interactions between Ag, Cu, or the other NPs mentioned above, and substances stabilising the nanoparticles in the organic acid salt medium.

Conclusions

1. This study focuses on determining the content of nanoparticles (NPs) in formulations with nanomaterials in the form of Ag, Cu, and Ag sulphates (containing small amounts of Al, Mg, Mn, and Ni). The results of the analyses of these compounds can possibly be used in agriculture for crop protection and the fight against pathogens (which is currently being implemented). The transmission electron microscopy TEM analyses have demonstrated the presence of nanoparticles in the formulations available on the market. The maximum height of the nanoparticles was found for the Ag colloid and the lowest for Ag sulphate, which ultimately may have a significant impact on the activity of the formulations in crops.

2. The FTIR infrared spectroscopy analysis clearly evidences that each formulation exhibits distinct bands that can be assigned to vibrations corresponding to respective functional groups bound to the nanoparticle present in the formulation. The bands present in the region below 1,000 cm^{-1} clearly indicate van der Waals interactions in NPs-X (where X is, e.g., an O or H atom). The analysis of the infrared spectra also confirms the strong interaction between the nanoparticles and the organic acid salts used as stabilisers in the colloids.

To summarize, it is worth emphasising that both TEM and FTIR analyses provide clear evidence of the presence of NPs in the analysed products and can indicate efficient applications of the formulations. It is also worth emphasising that the research methods employed in this study are substantially faster and more easily available than the typical analysis performed on this type of system. The proposed approach complements well such research techniques as TEM-EDX, Raman spectroscopy, or chromatographic analysis – which is a common practice among researchers. The investigations also suggest that the biosynthetic NP colloids can be a cost-efficient and

environmentally friendly alternative to the products used at present.

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