DEVELOPMENT OF LONG-LASTING ANTIMICROBIAL AND POTENTIAL HEMOSTATIC NANOCOMPOSITES (PYROPHYLLITE BASED) WITH PVP-COATED COLLOIDAL SILVER NANOPARTICLES

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Abstract: Pyrophyllite clay, modified with PVP coated silver nanoparticles (PYRO-PVP/AgNPs), with recently proved antibacterial activity was prepared. Silver nanoparticles were synthesized by the chemical reduction method of $AgNO_3$ using NaBH4 and poly(vinyl pyrollidone) (PVP) as a stabilizer and excellent dispersant. This research aimed to elucidate the mechanisms and kinetics of AgNPs, along with PVP protective mechanism that are responsible for antibacterial activity towards the microorganisms. Pioneering steps were made toward coagulation studies due to potential of aluminosilicate layered clays to serve as an alternatives to hemostatic agents currently in use. Isoelectric point of pyrophyllite samples with 5, 20, 45 µm diameter particles and PYRO-PVP/AgNPs sample (Ag25mg/L) was evaluated to understand how the anticoagulant or procoagulant properties of the pyrophyllite varied according to the pH of the isoelectric point. Characterization of the PYRO-PVP/AgNPs samples was performed using FTIR spectroscopy, UV/VIS spectroscopy and optical microscope. Release mechanism and kinetics of silver ions were monitored by atomic absorption spectroscopy (AAS). Additionaly, AAS was used for evaluation of heavy metals content in pyrophyllite clay and a simple and cost-effective procedure was proposed for the purification of pyrophyllite.

Keywords: pyrophyllite, nanocomposite, colloidal silver, mechanism of release, kinetics

Introduction: The clays, including pyrophyllite, are widely used in medicine and cosmetics due to their antiseptic, antitoxic and antibacterial properties, chemical inertness and low or zero toxicity. This paper was based on evaluating the possibility of using pyrophyllite as a carrier of silver nanoparticles due to its exceptional antibacterial power. The silver nanoparticles have higher antibacterial efficiency than silver in the form of salts or complexes for two reasons:

1. Silver nanoparticles have a large surface area, so they can make better contact with the surface of bacteria
2. Silver nanoparticles act as efficient reservoirs of Ag + ions, gradual oxidation of silver atoms on surfaces can release biologically active species in a continuous manner.

What is problematic with silver is that even the best designed silver complexes for medical use can lose their activity when transferred to *in vivo* conditions. Also, silver nanoparticles are as toxic to bacterial cells as they are to healthy cells. The side physiological effects attributed to silver include allergies and skin discoloration (argyria). What creates an additional problem is the agglomeration of silver, with the growth of nanoparticles weak antibacterial power. This work allow us to overcome all the above limitations. Namely, PBS (phosphate buffer) was used to simulate the biological environment, so the kinetics of silver release of the submerged silver-pyrophyllite composite was monitored. This also examined the possibility of retention of silver ions on pyrophyllite. PVP (polyvinylpyrrolidone) was used as a stabilizer to prevent agglomeration of silver during the experiment. The key thing is to achieve the highest possible antibacterial power with the lowest possible concentration of silver. Therefore, the antibacterial power depending on the silver concentration was examined. It is anticipated that silver–clay nanohybrid structure would increase the surface activity of the layered clay, while the physical stability of clay will support silver nanoparticles, such that the synergetic effect of silver and clay is to broaden their usage for biomedical applications. In order to achieve the best possible results, the influence of the isoelectric point of pyrophyllite was examined and the purification of pyrophyllite from heavy metals was performed.

Preparation of PYRO/Ag nanocomposite

I step – Synthesis of silver nanoparticles

The $NaBH_4$ solution (c = 0.002M), freshly prepared was mixed with silver nitrate solution (c = 0.001 M). Silver nitrate solution was added drop by drop (1 drop/sec) with constant stirring and afterwards, two drops of 0.3% PVP solution were added.

II step – Synthesis of PYRO/Ag nanocomposite

The obtained silver nanoparticles solution was joined with 100 mg of pyrophyllite clay and stirred for 2h. After stirring, the solution was centrifuged (5000 rpm, 10 min) and obtained nanocomposite was washed by destilled water to move all the residue and impurities. Obtained PYRO/Ag nanocomposite was kept for 24h at 50 °C in an owen and was further subjected to the characterization.

Kinetics – The silver migration

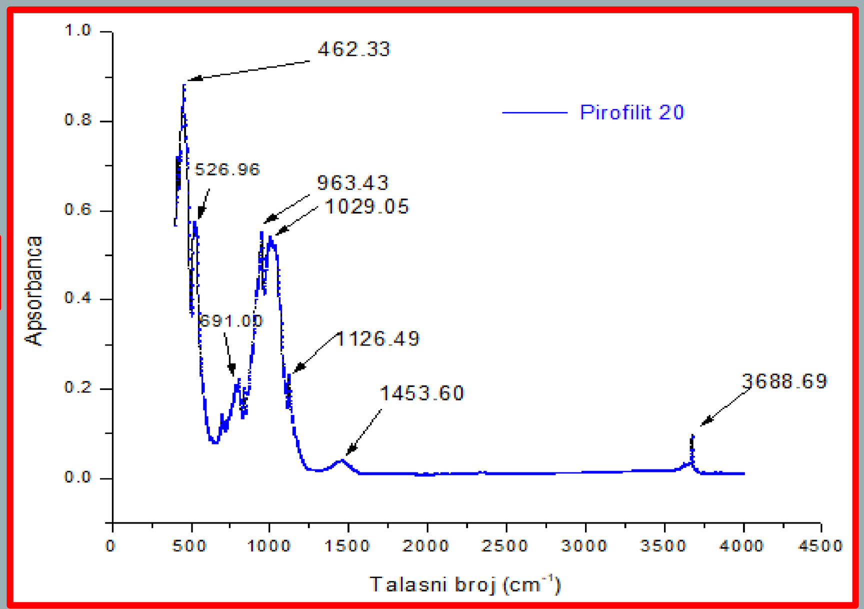
Samples, mg/kg	24h	48h	72h	96h	120h	144h
Ag-5	262	292	304	294	332	382
Ag-15	298	338	346	334	360	436
Ag-25	446	200	298	182	182	182

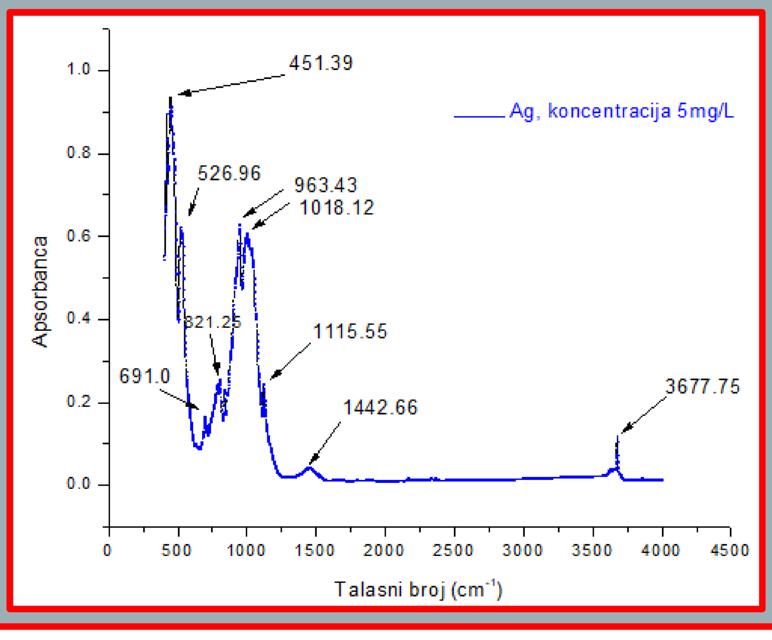
Purification of pyrophyllite

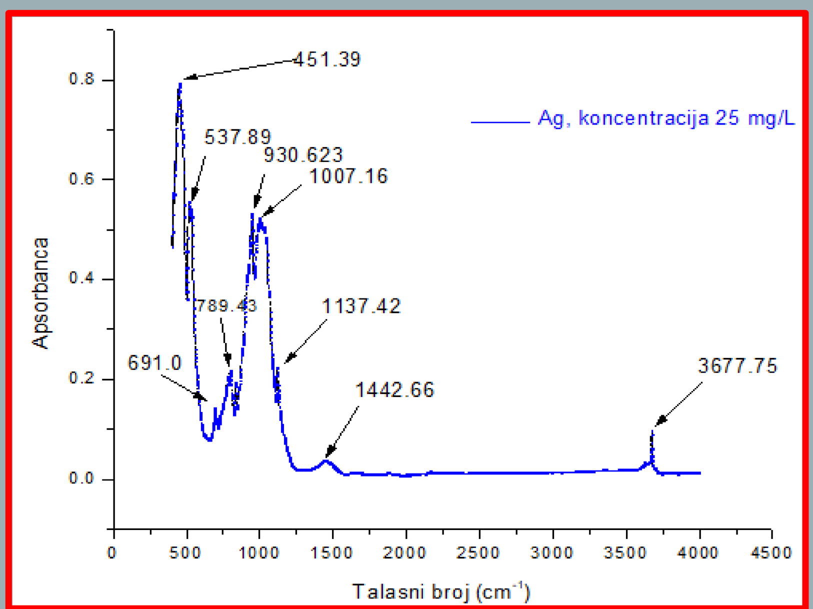
Lab. broj uzorka	Opisna oznaka		Pb, mg/kg	Cu, mg/kg	Zn, mg/kg
1	početni	0-75 µm	34.15	5.37	129.44
2	I talog	0-75 µm	29.32	18.91	162.68
3	I supernatant	0-75 µm	278.03	69.20	132.25
4	II talog	0-75 µm	8.73	<0.01	65.45
5	II supernatant	0-75 µm	9.98	<0.01	16.18
6	početni	tribo	19.20	<0.01	63.92
7	I talog	tribo	45.03	<0.01	110.89
8	I supernatant	tribo	4.82	0.74	33.85
9	II talog	tribo	82.86	<0.01	24.82
10	II supernatant	tribo	34.61	0.83	7.01
11	početni	100 µm	12.33	<0.01	186.46
12	I talog	100 µm	12.49	0.25	38.90
13	I supernatant	100 µm	12.04	2.85	24.11
14	II talog	100 µm	<0.01	<0.01	39.97
15	II supernatant	100 µm	0.25	231.49	16.77

75%

100 %







FTIR spectra of pure pyrophyllite and pyrophyllite doped with silver

In samples of silver-modified pyrophyllite, a zero charge point is determined by the addition of salt to determine surface charge, this information may be of practical interest when using pyrophyllite as a component in formulations used as hemostatic agents (to stop bleeding) or to adsorb toxins that are positively charged particles. Sodium chloride solutions of concentrations: 0.001: 0.01 and 0.1 mol / L were used for the pyrophyllite sample of size 5, 20, 45 µm, and for the Ag-25 sample. The pH of the sodium chloride solution (20 mL) was adjusted by the addition of a 0.1 mol / L hydrochloric acid solution and a 0.1 mol / L sodium hydroxide solution. The solutions were mixed on a magnetic stirrer and 0.05 g of pyrophyllite samples were added to the solutions thus prepared. The prepared samples in parafilm-protected beakers were allowed to stand for 24 h, after which they were filtered and their pH value was measured again.

Pyrophyllite samples	Isoelectric point value
5µm	7.44
20µm	7.23
45µm	7.00
20µm doped with Ag	7.12

Conclusions: Obtained results confirm PYRO/Ag nanocomposite as potential long-lasting antimicrobial material and potential toxins remover.

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