COLLOIDAL NANO-SILVER SOLUTION OBTAINED BY ELECTROCHEMICAL METHOD: IN VITRO AND IN VIVO EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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According to the last years' investigations, silver nanoparticles display unique properties such as excellent electrical conductivity, catalytic activity and nonlinear optical behavior, while still being reasonably priced. Moreover, nano-silver based colloidal solutions are particularly involved in environmental and biological applications due to their specific antimicrobial properties, against a great number of bacteria and fungi.

Colloidal silver solutions (CSSs) involving the electrochemical technique, based on the "sacrificial" Ag electrodes method has been prepared using a mix of different stabilizers and co-stabilizers. A constant current pulse generator with a stirrer and two silver electrodes of 99.999 % purity were used. This method offers the advantage of a high purity of final formed solution, especially for bio-medical applications. Obtained colloidal solutions are very stable, having zeta potential values between 35 ÷ 51 mV. These contain silver nanoparticle with mean diameters of 5…10 nm and concentrations of about 30 ppm [1]. The nanoparticles sizes have been analyzed through dynamic light scattering technique and the silver nanoparticle morphology has been evidenced by transmission electron microscopy (Fig. 1 and Fig. 2).

The tested solutions have the characteristics presented in Table 1

Table 1. Concentration, zeta potential and mean diameter of tested CSSs

No. of sample	Compositions of the electro-formation solutions	Ag nanoparticles concentration, (ppm)	Zeta potential, (mV)	Ag mean diameter, (nm)
I	5 g/l PVP 25 +0.25g/l Na-LS	31.5	- 35.51	4.1
II	5 g/l PVP 10 +0.25g/l Na-LS	28.5	- 51.02	4.4
III	5 g/l PVP 10 +0.75g/l Na-LS	30.7	-37.57	5.5

To evaluate the antimicrobial effect (bacteriostatic and bactericidal) against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, the following parameters have been calculated: minimal concentration with bacteriostatic effect - MCBs, minimal concentration with bactericidal effect – MCBc, the antibacterial effect after short time exposure and antibacterial effect after continuous exposure of culture at nanosilver particles action. The colloidal silver solutions stabilized with a mix of polyvinylpirrolidone, (PVP), and sodium lauril sulfate, (Na-LS), showed some very good results. For in vivo evaluation, dynamics of evolution in time of total number of germs (TNG) has been determined.

- *In vitro* evaluation of CSSs antimicrobial activity.

The obtained CSSs have inhibited bacterial growth and the MCBs and MCBc values are reported in Table2.

Table 2. Minimal concentration with bacteriostatic effect and minimal concentration with bactericidal effect of the tested CSSs

No. of sample	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	MCBs, (ppm)	MCBc, (ppm)	MCBs, (ppm)	MCBc, (ppm)	MCBs, (ppm)	MCBc, (ppm)
I	15.75 – 7.875	31.51	7.875 – 3.937	15.75	7.875 – 3.937	15.75
II	14.25 - 7.125	28.5	3.5625 – 1.781	14.25	3.5625 – 1.781	14.25
III	30.7 – 15.35	-	15.35 – 7.675	30.7	15.35 – 7.675	30.7

The high resistance of S. aureus comparing to the other stems is connected to its morphological characteristics. The results are in agreement with literature data [2]. It has a thick peptidoglicane membrane and as a result, it is very resistant at destroying factors. It is believed that the mechanism of the antibacterial effect of silver ions (Ag^+) involves their absorption and accumulation by bacterial cell and the shrinkage or destroys of the membrane. As a result, the DNA molecules become condensed and lose their ability to replicate [3].

- *In vivo* evaluation of CSSs antimicrobial activity

In vivo tests where carried out on mice, three month old. The total number of germs (TNG) from the wound was determining bacteriological by the classical method of dilution in nourishing agar, evaluating antimicrobial effect against references [4] (Fig.3). The antibacterial effect was more intense in the case of gram negative bacteria compared with gram positive ones.

References

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Figures

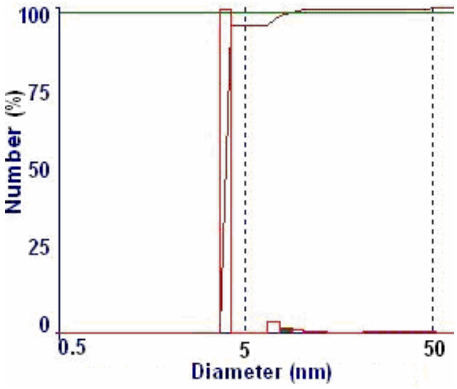

Fig.1. Grain size distribution of Ag nanoparticles (sample II, table 1)

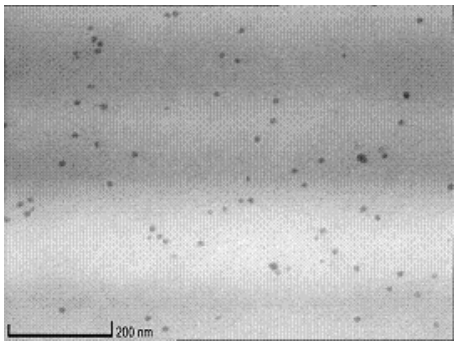

Fig. 2 TEM micrograph of Ag nanoparticles (sample II, table 1)

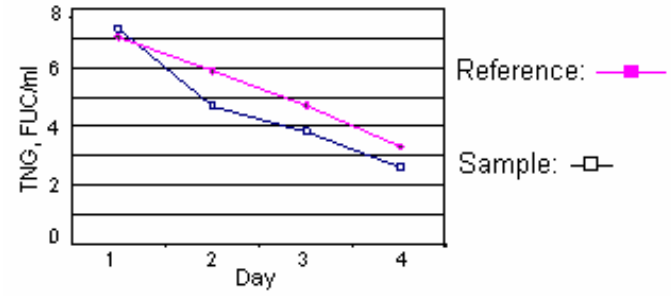

Fig.3. The TNG mean values dynamics of the tested CSSs samples