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FORMULATION AND OPTIMIZATION OF CLAY-BASED DISPERSION FOR TEXTILES FUNCTIONALIZATION

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Abstract: *One of the main factors for diseases transmission is represented by textile materials and an example for textiles that can support bacterial growth is met in hospitals where patients contaminate their cloths and bed covers by bacterial shedding. Thus, there is a need to improve the quality of people's lives by intensive research to develop antimicrobial textiles. Because of the way we live and the environment we live in there is a need for multifunctional textiles with antimicrobial properties. Efficient antimicrobial solutions can be plants derived chemicals and clays. This paper is a part of a Eureka project. The main objective of the project is to develop multi-functional antimicrobial textile materials to prevent bacteria spreading and to create an antimicrobial shield for human body. The work was carried out through 2 steps. The first step consisted of making 4 variants (different in terms of constituent components) and using 3 types of equipment to prepare these 4 variants. The selection of the method for obtaining the most suitable dispersions was made by evaluating the stability over time and by evaluating by molecular absorption spectrophotometry in UV-VIS (on the wavelength range: 200–800 nm). The second step to obtain a stable and physico-chemically and pharmacologically adequate dispersion was to prepare 8 dispersion variants according to the method selected in step I. The evaluation of the obtained dispersions was performed by microbiological methods (inoculation with two strains: Escherichia coli and Staphylococcus aureus), measurements of Zeta potential and by evaluation of the antioxidant character.*

Key words: *antibacterial textiles, nano clays, plant extracts, blue clay.*

1. INTRODUCTION

Textiles can provide a substrate for the development of different populations of microorganisms, especially in conditions of adequate humidity and temperature, in contact with the human body. With increasing attention to hygiene, many investigations have been carried out to make textiles with antimicrobial properties. As many antimicrobial agents are avoided due to possible side effects, a promising alternative is to use inorganic nanoparticles and their nanocomposites.

Chemical finishing of textiles involves the use of chemicals that can perform various functions. They can be applied in the form of aqueous solutions or emulsions, by a variety of methods. Of course, all these treatments also highlight the need to assess and ensure their ecological character [1]. In addition, any antibacterial treatment performed on a textile material, in addition to being effective against micro-organisms, must not be toxic to the user or to the environment [2].

The development of nanotechnologies brings, in the field of textile functionalization, a wide range of new application possibilities [3].

They can be used by:

• introduction of functional nanomaterials in artificial/synthetic fibers, in the synthesis process combining the original characteristics of the fiber with the functionality induced by nanomaterials.

• covering the surface of fibers or textiles with functional nanomaterials, resulting in functional textiles with a higher added value.

• spinning polymers by electrospinning to produce nanometric fibers, which lead to textiles with improved or new characteristics, with multiple applications [4, 5].

The work is part of a project aimed at developing antimicrobial textiles to prevent the spread of bacteria and to create an antimicrobial shield for the human body. The innovation consists in the use of bio-active compounds incorporated in different forms: in blue clay or micro-encapsulated, for the functionalization of textile materials.

2. MATERIALS AND METHODS

The components of the dispersions prepared are blue clay from Râciu (S.C. ROMCOS IMPEX S.R.L.), plant extract (Propolis, *Aloe Vera*, *Calendula officinalis*, and *Plantago major*), Kaolinite CAS 1318-74-7, Halloysite nanoclay CAS 1332-58-7, dimethyl sulfoxide (DMSO) (CAS 67-68-5), and distilled water. To optimize the preparation method, 3 types of stirring equipment have been used: ultrasound bath from Elma, thermostatic water bath equipped with stirring system from mrc, mechanical stirrer from VWR. The stability of the dispersions has been evaluated by measuring their Zeta potential, using the Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The microbiological methods for establishing the most effective antimicrobial dispersions involved the inoculation with two strains: *Escherichia coli* ATCC 10536 and *Staphylococcus aureus* ATCC 6538 from Scharlau. The antioxidant activity assessment has been performed through TEAC method (Trolox Equivalent Antioxidant Capacity), using the Merck reagents and the UV-VIS Spectrometer Lambda 950 from Perkin Elmer.

In the first step, the preparation method has been optimised, by combining the components into 4 aqueous dispersion versions and using the 3 types of stirring equipment: ultrasound bath, thermostatic water bath equipped with stirring system, and mechanical stirrer. The selection of the optime method for mixing the dispersions was accomplished by observing the dispersions visual aspect over time: initial visual assessment, after 24 hours, and after 48 hours.

In the second step, the 4 dispersion versions have been prepared in 2 concentrations (0,1% and 0,3%) and characterized by measuring the Zeta potential and evaluating the antioxidant and antimicrobial activity.

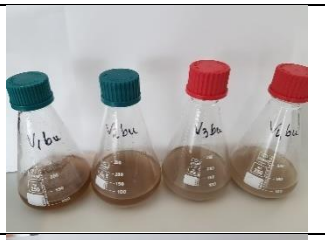
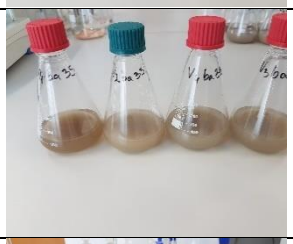
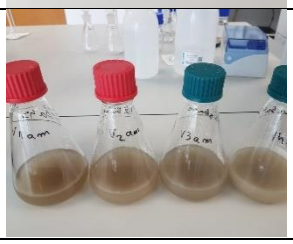
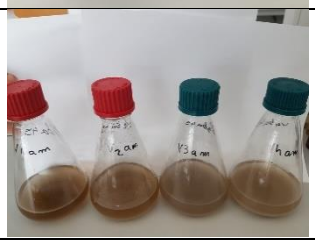
3. RESULTS AND DISCUSSIONS

Table 1 shows the components of the prepared dispersions and table 2 presents their visual aspects, initially, after 24 hours and after 48 hours from stirring the mixed components for 2 hours, at room temperature.

Table 1: *Components of the prepared dispersions*

Version 1 (**V1-1**)	0,1% (w/w) Plant extract	0,1% (w/w) Blue clay from Râciu	-	10% (v/v) DMSO
Version 2 (**V2-1**)			0,1% (w/w) Kaolinite	
Version 3 (**V3-1**)			0,1% (w/w) Halloysite nanoclay	
Version 4 (**V4-1**)			0,1% (w/w) Kaolinite 0,1% (w/w) Halloysite nanoclay	
Version 1 (**V1-3**)	0,3% (w/w) Plant extract	0,3% (w/w) Blue clay from Râciu	-	
Version 2 (**V2-3**)			0,1% (w/w) Kaolinite	
Version 3 (**V3-3**)			0,1% (w/w) Halloysite nanoclay	
Version 4 (**V4-3**)			0,1% (w/w) Kaolinite 0,1% (w/w) Halloysite nanoclay	

Table 2 *Visual aspects, initially, after 24 hours and after 48 hours from stirring the mixed components*

	Initial	After 24 h	After 48 h
Ultrasound bath			
Thermostatic water bath equipped with stirring system			
Mechanical stirrer			

After observing the dispersions for 48 hours, the formation of the sediments deposited on the bottom of the flasks were observed in the case of water bath equipment and mechanical stirrer. The most homogenous dispersions were formed when using the ultrasound bath. This stirring method is the most suitable for cracking the aglomerations of the powders and dissolving the plant extracts, resulting into homogenous and stable in time dispersions.

3.1. Zeta potential measurements

To measure the zeta potential, 300 μL dispersion was added, by pipetting, to 20 mL of distilled water, along with 50 μL of 0.9% NaCl solution and the resulting suspension was subjected to analysis. The mean values of the zeta potential measured are listed in table 3 and illustrated in figure 1.

Table 3 *Zeta potential values obtained for the prepared dispersions*

Conc.	Sample version	Zeta potential (mV)
0,1%	V1	-29,3 ± 1,670
	V2	-35,4 ± 3,610
	V3	-32,9 ±1,640
	V4	-34,8 ± 0,945
0, 3%	V1	-21,6 ± 0,551
	V2	-24,2 ± 0,874
	V3	-25,3 ± 0,115
	V4	-26,8 ± 0,907

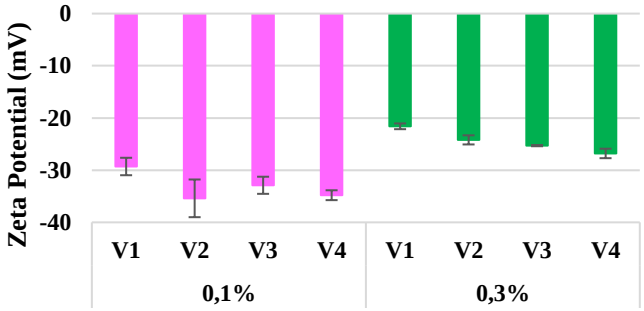


Fig. 1 *Graphic illustration of the zeta potential of the dispersions*

A dispersion is considered stable when the absolute value of the zeta potential is higher than 20 mV. Considering this criterion, all dispersions analysed are stable. However, when comparing the mean values of the zeta potential between the two concentrations, a higher stability of the dispersions with 0,1 % concentration is noticed.

3.2. Antibacterial activity

The antibacterial tests were performed in a 1:1 ratio with the microbial inoculum. Fresh cultures were obtained from each strain, by growing in nutrient broth Nutrient Agar, at 37°C, 48h, this representing the stock culture. For testing, two decimal dilutions of paraffin oil (10^{-2}) were prepared from each culture and the cell concentration in the inoculum used was $1,2 \times 10^5$ CFU/mL (Colony Forming Units) for *Staphylococcus aureus* and $1,5 \times 10^5$ CFU/mL for *Escherichia coli*. The percentage of the microorganism reduction is calculated using the following formula:

$$R = \frac{C_t - T_t}{C_t} X100\% \quad \textbf{(1)}$$

where: R – represents the percentage of antibacterial activity.
C_t – represents the average number of bacteria obtained from the control sample, after an incubation of 18 h to 24 h.
T_t – represents the number of bacteria obtained from the antibacterial effect sample after an incubation of 18 hours to 24 hours.

Table 4 *Antibacterial results for the dispersions prepared with* ***0,1%*** *concentration*

Sample	*Staphylococcus aureus ATCC 6538*			*Escherichia coli ATCC 11229*		
	Result	R%	Log_{10} red.	Result	R%	Log_{10} red.
Inoculum concentration	T_0=1,2x10^5 CFU/mL	-	-	T_0=1,5x10^{5}CFU/mL	-	-
V1	T_0=1,2x10^5 CFU/mL T_{24}= 8,9x10^1 CFU/mL	99,93	3,13	T_0=1,5x10^{5}CFU/mL T_{24}= 1,5x10^2 CFU/mL	99,90	3,00
V2	T0=1,2x10^5 CFU/mL T_{24}=2,1x10^2 CFU/mL	99,83	2,76	T_0=1,5x10^{5}CFU/mL T_{24}=2,4x10^2 CFU/mL	99,84	2,80
V3	T_0=1,2x10^{5}CFU/mL T_{24}= 4,5x10^1 CFU/mL	99,96	3,43	T_0=1,5x10^{5}CFU/mL T_{24}= 1,1x10^2 CFU/mL	99,93	3,13
V4	T_0=1,2x10^5 CFU/mL T_{24}= 1,9x10^2 CFU/mL	99,84	2,80	T_0=1,5x10^5 CFU/mL T_{24}= 2,9x10^2 CFU/mL	99,81	2,71

Table 5 *Antibacterial results for the dispersions prepared with* ***0,3%*** *concentration*

Sample	*Staphylococcus aureus ATCC 6538*			*Escherichia coli ATCC 11229*		
	Result	**R%**	**Log_{10} red.**	**Result**	**R%**	**Log_{10} red.**
Inoculum concentration	T_0=1,2x10^5 CFU/mL	-	-	T_0=1,5x10^5CFU/mL	-	-
V1	T_0=1,2x10^5 CFU/mL T_{24}= 3,5x103 CFU/mL	97,08	1,54	T_0=1,5x10^5 CFU/mL T_{24}= 5,2x10^3 CFU/mL	96,53	1,46
V2	T_0=1,2x10^5 CFU/mL T24=2,2x103 CFU/mL	97,17	1,74	T_0=1,5x10^5CFU/mL T_{24}=7,4x10^3CFU/mL	95,07	1,31
V3	T_0=1,2x10^5CFU/mL T24= 1,7x10^3 CFU/mL	98,58	1,85	T_0=1,5x10^5CFU/mL T_{24}= 4,3x10^3CFU/mL	97,13	1,54
V4	T_0=1,2x10^5 CFU/mL T24= 2,8x10^3CFU/mL	97,67	1,63	T_0=1,5x10^5 CFU/mL T_{24}= 2,1x10^3CFU/mL	98,96	1,85

All samples tested present antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. However, the percentage of the microorganism reduction was higher when the blue clay and plant extract concentration was 0,1%.

3.3. Antioxidant activity

The dispersions analyzed in terms of antioxidant activity are those with 0,1% concentration. The TEAC method is based on spectrophotometric monitoring of the color change and absorbance of the cationic radical 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) - $ABTS^+$.

The discoloration of the solution is related with the percentage of inhibition of the long-life radical $ABTS^+$. Radical inhibition is calculated in relation to the reactivity of Trolox used.

Firstly, a calibration curve was constructed, at 734 nm. Figure 2 shows (a) the calibration curve and (b) the dependence of the degree of inhibition of the $ABTS^+$ radical on the Trolox concentration.

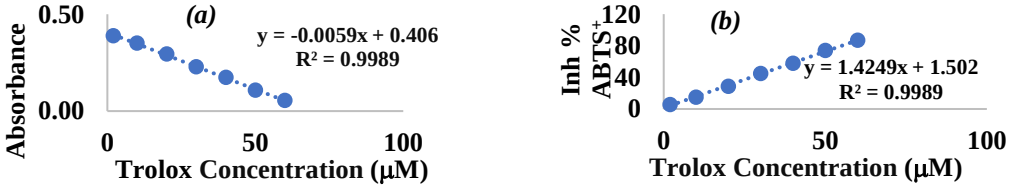


Fig. 2 *(a) Calibration curve of Trolox reagent, (b) Dependence of the degree of inhibition of the $ABTS^+$ radical on the Trolox concentration.*

To determine the antioxidant activity, the absorbance of the samples is measured on the calibration curve performed. The ABTS + radical capture activity is calculated as follows:

$$Inh\ ABTS^+(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad \textbf{(2)}$$

where:

$A_{control}$ – represents the absorbance of the $ABTS^+$ radical distilled water.
A_{sample} – represents the absorbance of the radical $ABTS^+$ sample extract/standard Trolox.

Using the equation y = 1.4249x + 1.502, the antioxidant activity expressed in Trolox equivalent can be evaluated. The results obtained are presented in Table 6.

Table 6. *Results of antioxidant activity of the dispersions with 0,1% concentration.*

Sample	Absorbance	Inh % ABTS	Trolox equivalent (μM)
Control	0,4251	-	-
V1	0,1461	65,6	45.0
V2	0,0245	94,2	65.1
V3	0,0113	97,3	67.3
V4	0,3557	16,3	10.4

The dispersions with the highest values of antioxidant activity (the highest percentage of inhibition of the $ABTS^+$ radical) are the dispersions V2 and V3 (with 0,1% concentration of blue clay and plant extract), with inhibition percentages above 90%. Dispersion V1 presents. Also, a relative high antioxidant activity, with an inhibition percentage of 65,5%. V4 has a low antioxidant activity, with an inhibition percentage of 16.3%.

5. CONCLUSIONS

In the presented paper, 4 dispersion versions were prepared by 3 stirring methods (water bath, ultrasonic bath, and mechanical agitation). Following the visual observations, ultrasonic bath was selected as the stirring method. The concentration of the bioactive compounds was varied (0,1% and 0,3% (w/w) blue clay and plant extract) and the concentration of the other components was kept constant to 0,1% (w/w), resulting 8 dispersion versions. Their stability was further evaluated *via* zeta potential measurement, showing a relative higher stability for dispersions prepared with 0,1% concentration. The antibacterial tests against *Staphylococcus aureus* and *Escherichia coli* revealed the same trend. When assessing the antioxidant character, except for V4, all the other dispersion versions presented antioxidant activity, with inhibition percentages of the $ABTS^+$ radical between 65,5% and 97,3%. Following the tests presented, V1 and V3 (with 0,1% blue clay and plant extracts) were selected to be incorporated in textiles. This work is still in progress.

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