Functionalization of textile materials with bioactive polymeric systems based on propolis and cinnamon essential oil

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LAURA CHIRILĂ
GABRIELA CRISTINA CONSTANTINESCU
ANGELA DANILA

ALINA POPESCU ROXANA RODICA CONSTANTINESCU IRINA-MARIANA SĂNDULACHE

ABSTRACT - REZUMAT

Functionalization of textile materials with bioactive polymeric systems based on propolis and cinnamon essential oil

In order to obtain the textile materials with potential for use in the treatment of inflammatory skin conditions, this study approached the laboratory experiments related to depositing by padding of oil-in-water emulsions type based on chitosan-propolis-cinnamon essential oil. In this regard, seven experimental variants of emulsions were prepared and then characterized by specific methods from physical-chemical and stability point of view. The chitosan-propoliscinnamon essential oil-based emulsions were then immobilized on a knitted textile structure from 100% organic cotton. The functionalized textile materials were characterized from morphological and antibacterial activity point of view. pH values obtained for the synthesized polymeric systems were in the range 4.67-4.88 and electrical conductivity of the emulsions increases with the concentration of the hydrophilic phase in the system, the highest values were registered for the emulsion code R4CSP developed with the smallest volume fraction of water. The stability index over a period of 10 days indicated that emulsions are stable, not showing the presence of one of the flocculation, creaming / sedimentation, coalescence or Ostwald ripening phenomena. The highest values of emulsifying activity index were obtained for the R7CSP, emulsion developed with the smallest volume fraction of water. The highest value of viscosity was obtained for the experimental variant R4CSP in which the highest volume fraction of chitosan and the smallest volume fraction of water were used. The textile materials treated with synthesized emulsions based on chitosanpropolis-cinnamon essential oil have antibacterial effect against the S. aureus test strain, with inhibition zones between 1.5 mm (R2CSP and R7CSP) and 4 mm (R6CSP).

Keywords: functionalized textiles, bioactive compounds, chitosan, cinnamon essential oil, propolis

Funcționalizarea materialelor textile cu sisteme polimerice bioactive pe bază de propolis și ulei esențial de scorțișoară

Pentru a obține materiale textile cu potențial de utilizare în tratamentul afecțiunilor inflamatorii ale pielii, acest studiu a abordat experimentări de laborator cu privire la depunerea prin metoda fulardării a emulsiilor de tipul ulei-în-apă, pe bază de chitosan-propolis-ulei esențial de scorțișoară. În acest sens, au fost preparate șapte variante experimentale de emulsii și apoi caracterizate prin metode specifice din punct de vedere fizico-chimic și al stabilității. Emulsiile pe bază de chitosan-propolis-ulei esențial de scorțișoară au fost apoi imobilizate pe o structură textilă tricotată din 100% bumbac organic. Materialele textile funcționalizate au fost caracterizate din punct de vedere morfologic și al activității anti-bacteriene. pH-ul sistemelor bioactive este cuprins între 4,67 si 4,88; conductivitatea electrică crește odată cu concentrația fazei hidrofile, cele mai mari valori înregistrându-se pentru proba cod R4CSP, care conține cea mai mică fracție volumică de apă. Indicele de stabilitate analizat pe o durată de 10 zile a indicat faptul că emulsiile sunt stabile, neindicând prezența unuia dintre fenomenele de floculare, cremare/sedimentare, coalescență sau maturare Ostwald. Valorile cele mai mari ale indicelui activității emulgatoare au fost obținute pentru R7CSP, emulsia realizată cu cea mai mică fracție volumică de apă. Cea mai mare valoare a viscozității a fost obținute pentru varianta experimentală R4CSP, în care a fost utilizată cea mai mare fracție volumică de chitosan și cea mai mică fracție volumică de apă. Materialele textile tratate cu emulsiile sintetizate pe bază de chitosan-propolis-ulei esențial de scorțișoară prezintă efect antibacterian împotriva tulpinii test S. aureus, cu zone de inhibiție cuprinse între 1,5 mm (R2CSP și R7CSP) și 4 mm (R6CSP).

Cuvinte-cheie: textile funcționalizate, compuși bioactivi, chitosan, ulei esențial de scorțișoară, propolis

INTRODUCTION

The development of multifunctional textile materials based on nano- and micro-technologies represents one of the most competitive and dynamic areas of research worldwide, being a major factor in the sustainable development of the textile industry [1]. The

design of biopolymeric matrices for the delivery of bioactive compounds constitutes a science-based strategy to provide textile materials with the multifunctional properties [2–3]. Natural compounds derivatives from plants can demonstrate various biological effects, including antibacterial and antifungal activity, anti-inflammatory and antioxidant effects [4].

areas of interest. As natural products they have physico-chemical characteristics that are friendly to the environment with added value. It also possesses a relevant biological activity, being used in the medical field thanks to their biocidal activity (bactericides, virucides and fungicides) and medicinal properties. Cinnamon (Cinnamomum zeylanicum) essential oil has been widely applied in food products-and it benefits health in various ways due to its antioxidant and antimicrobial properties [5-6]. Furthermore, cinnamon essential oil has gained popularity for use in skin care products, research on its effects on human skin is largely scarce. The major active components of cinnamon essential oil taken from the bark are cinnamaldehyde, eugenol, carvacrol and linalool. A study [7] conducted on human keratinocytes demonstrated the antioxidant effect of cinnamaldehyde, as well as its potential for treating skin disorders. Using essential oils as bioactive compounds is limited due to their low solubility, instability against environmental conditions and susceptibility to oxidative degradation, resulting in the loss of the functional properties [8-9]. Embedding the biologically active components into polymeric matrices is a procedure studied over the last decades worldwide and can provide an ideal solution for achieving a controlled and targeted delivery of the essential oil [10-11]. The polymer is used as protective carrier for the bioactive compound during the transfer until it is released.

Essential oils have gained new interest in several

Propolis, a natural resinous product, is widely known by its high composition of bioactive compounds and largely used in folk medicine. Propolis has diverse biological activities: it shows antibacterial, antiinflammatory, free radical scavenging, antioxidant, and antitumor effects. The wealth of its bioactive components determines its application in medicine and dentistry as well as in the pharmaceutical, cosmetics, and food industries. Previous studies have shown that propolis is effective in healing wounds and various skin injuries. Propolis is used in the local treatment of burns as an antibacterial, deodorant, anesthetic and stimulant of the regenerative process, forming on the surface of wounds or scars a protective layer, which protects the wound and allows the healing process to begin from its depth.

The aim of the study was to develop textile materials with functional properties by applying bioactive polymeric systems based on chitosan-propolis-cinnamon essential oil. For this purpose, the formulations experiments of bioactive polymeric systems — oil-in-water (O/W) type emulsions have been carried out. The aim was to identify the main factors influencing the formulation process and the stability of the new developed emulsions.

EXPERIMENTAL PART

Materials

Chitosan with low molecular weight, 85% deacetylation degree (Fluka Chemie GmbH, Switzerland) was

used as an embedding agent of bioactive agents. Chitosan flakes were dissolved in solution of CH₃COOH (96%) (Consors, Romania). Tween 80 (Sigma Aldrich, Germany) was used as a surfactant with emulsifying role and glycerol (Honeywell, USA) has been used as a solubilizing with wetting role. Cinnamon leaf essential oil (Mayam, Romania) and propolis tincture (Larix SA, Romania) 70% ethanol solution were used as bioactive agents. For preparation of bioactive systems, the distilled water has been used. Bleached 100% knitted organic cotton was used for the functionalization processes.

Emulsion preparation methodology

For the achievement of the bioactive systems, initially stock solutions of 3% chitosan in 2% CH₃COOH and 30% Tween 80 solution were prepared. Further, over the previously prepared chitosan solution, distilled water was added dropwise, under continuous stirring until a homogeneous mixture was obtained. Glycerol was added to the chitosan/water system, and the system was maintained under magnetic stirring for 10 minutes, after which Tween 80 emulsifier was added. After complete homogenization of the solution, the propolis and cinnamon essential oil were separately added, maintaining the magnetic stirring for 10 minutes for each stage.

The stages of obtaining emulsions based on chitosan-propolis-cinnamon essential oil are shown in figure 1.

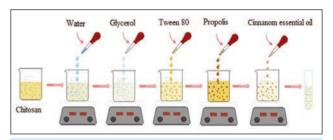


Fig. 1. The stages of obtaining emulsions based on chitosan-propolis-cinnamon essential oil

Thus, 7 variants of emulsions have been developed that differ by: i) the concentration of the embedding agent (chitosan), ii) the concentration of the selected bioactive compounds. For all experimental variants, the ratio between the two selected bioactive compounds was kept constant. The selected experimental variants are presented in table 1.

Immobilization of bioactive polymeric systems on the textile materials

The 7 experimental variants were immobilized on the textile materials by the padding method on the laboratory padder (ROACHES, UK). The treated textile materials were then subjected to the drying operation at 50°C for 3 minutes. Subsequently, the textile materials were analysed from morphological and antibacterial activity point of view.

COMPOSITION OF THE EMULSIONS (REPORTED OF 30 ML EMULSION)								
Code	Chitosan (ml)	Tween 80 (ml)	Glycerol (ml)	Distilled water (ml)	Essential oil (ml)	Propolis (ml)		
R1CSP	9	0.5	2.7	16	0.9	0.9		
R2CSP	12	0.5	2.7	13.0	0.9	0.9		
R3CSP	15	0.5	2.7	10.0	0.9	0.9		
R4CSP	18	0.5	2.7	7.0	0.9	0.9		

2.7

2.7

2.7

11.2

10.6

9.4

0.3

0.6

1.2

Methods

R5CSP

R6CSP

R7CSP

15

15

15

Optical microscopy: The emulsions prepared according to the working recipes were analysed microscopically using an OLYMPUS BX51 optical microscope (Philippines) equipped with the OLYMPUS UC30 photo digital camera.

0.5

0.5

0.5

pH determination: Emulsion pH was directly measured using a HANNA portable pH-meter immersed in the prepared undiluted emulsions. The measurements were performed in triplicate.

Conductometric analysis: The conductivity of the analyzed emulsions was measured directly, using the Consort conductometer, model C1020 WTW (TARA), with SP10T electrode. The conductivity values represent the average of 3 successive readings made on the same sample.

Determination of the emulsion stability index: The stability of an emulsion can be appreciated immediately after preparation and/or after a certain period of time. Immediately after preparation, the emulsions were introduced into test tubes and the evolution was followed for 10 days after their preparation. The stability of the emulsions was evaluated by determining the stability index (ESI) using the equation (1):

$$ESI(\%) = [H - (Hs + Hc)]/H$$
 (1)

where H is the initial height of the emulsion (cm), Hs – the height of the serous layer (cm), Hc – the height of the creamy layer (cm).

Turbidity analysis: In order to determine the turbidity, the transmittance was determined, using a Camspec M501 UV-VIS Spectrophotometer. Turbidity was calculated with the equation (2):

$$\tau = 2.303 \cdot A/L \tag{2}$$

where τ is turbidity (cm⁻¹), A – absorbance, L – path length of cuvette (cm).

Viscosity determination: The viscosity measurements of the obtained bioactive systems were performed with the DV2T Brookfield AMETEK viscometer. Measurements were performed in triplicate.

Electron microscopy: Visualization of the morphology of the cotton fiber surfaces treated with the bioactive systems was performed by scanning electron microscopy using the Quanta 200 electron microscope (FEI, The Netherlands).

Assessment of antibacterial activity: The antibacterial activity of the treated samples in different variants was qualitatively assessed by Agar diffusion method according with the SR EN ISO 20645:2005 standard — Determination of antibacterial activity-agar diffusion plate test, by using of cultures in liquid medium replicated at 24 hours of ATCC 6538 Staphylococcus aureus test strains (Gram-

positive) and ATCC 11229 Escherichia coli (Gramnegative) test strains. Firstly, the Manitol Salt Agar gel for Staphylococcus aureus and Nutrient Agar gel for Escherichia coli were prepared for the lower layer without bacteria. Thus, 10±0.1 ml gel was placed into each sterilized Petri dish and allow gel to solidify. Another amount of gel was prepared for the upper layer and cooled to 45°C in a water bath. 150 ml of gel is inoculated with 1 ml of bacterial working solution 1-5×108/ml. The container is vigorously stirred for the uniform distribution of bacteria. The quantity of 5±0.1 ml are introduced in each Petri plates and allow the gel to solidify. The textile specimens (2 cm diameter) are placed on the surface of the nutrient medium and then incubated at 37°C for 24 h. The antibacterial evaluation was based on the absence or presence of bacterial multiplication in the contact area between the agar and the test specimen and on the occurrence of a possible inhibition area around the specimens. Inhibition zones were calculated using the following equation (3):

Table 1

0.3

0.6

1.2

$$H = (D - d)/2 \tag{3}$$

where H is the inhibition zone (mm), D – the total diameter of specimen and inhibition zone (mm), d – the diameter of specimen (mm).

The evaluation of the results obtained after the calculation of the inhibition zones was performed according to the criteria from SR EN ISO 20645:2005 standard, depicted in the table 2.

Table 2

PCRITERIA FOR EVALUATION OF INHIBITION ZONES ACCORDING TO THE SR EN ISO 20645:2005 STANDARD

Inhibition zone (mm)	Growth	Evaluation
>1 1-0 0	absent absent absent	Satisfactory effect
0	low	Efficiency limit
0 0	moderate important	Unsatisfactory effect

RESULTS AND DISCUSSION

Emulsions appearance

From the visual analysis it is observed that, the emulsions prepared are presented in the form of homogeneous white-yellow-milky solutions without particle agglomerations throughout the sample mass. The images obtained by optical microscopy for the 7 experimental variants are shown in figure 2. According to microscopic images, the dispersed molecule phase is presented as a compact, dense small globule mass. Normally, the destabilization of the emulsions begins with the drops flocculation of essential oil followed by the phenomenon of coalescence and formation of two distinct phases (separation).

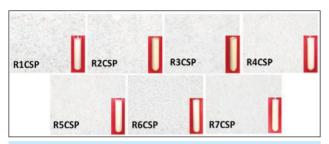


Fig. 2. Optical microscopy of the resulted bioactive systems

Physical-chemical analysis of emulsions

The pH and conductivity values recorded for the synthesized polymeric systems are presented in table 3. Analyzing comparatively the pH values obtained for the synthesized polymeric systems (table 3) it can be seen that they are in the range 4.67–4.88. It can also be observed that the addition of a higher quantity of active principle (essential oil and propolis) causes a lower acid pH. The electrical conductivity of the emulsions increases with the concentration of the hydrophilic phase in the system, highest values registered the for the emulsion developed with the smallest volume fraction of water (e.g. R4CSP). There is no linear dependence between the values of conductivity and the volume fraction of essential oil.

Emulsions stability

The calculated values for the stability index, are shown in table 4. The values recorded for the stability index over a period of 10 days indicate that they were stable, during this time not showing the presence of one of the flocculation, creaming/sedimentation, coalescence or Ostwald ripening phenomena. Generally, the turbidity of the bioactive polymeric systems is correlated to a great extent with their stability. Analysing the values obtained for the 7 experimental variants (table 4) it can be observed that the lowest values of the turbidity were obtained for the R5CSP variant (1.81) in which the smallest amount of active principle is also present (essential oil and propolis). Turbidity decreases with increasing particle size of emulsion.

UV-VIS analysis

The UV-VIS spectra recorded for the obtained polymeric systems are shown in figure 3. UV-vis spectra revealed the presence of absorption bands in the 220–290 nm UV range. The analysed emulsions are characterized by intense absorption in the 270–290 nm area. The major peak in this area is very close to the maximum absorption (286 nm) obtained for cinnamaldehyde, the majority compound present in the cinnamon essential oil [12].

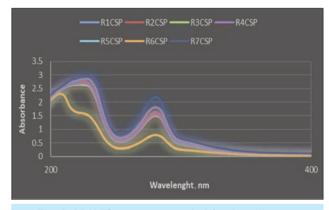


Fig. 3. UV-VIS spectra recorded for the chitosanpropolis-cinnamon essential oil systems

Table 3

CONDUCTIVITY AND pH OF BIOACTIVE SYSTEMS							
Parameters measured		Code					
	R1CSP	R2CSP	R3CSP	R4CSP	R5CSP	R6CSP	R7CSP
рН	4.67	4.68	4.67	4.83	4.84	4.86	4.88
Conductivity (m·S/cm)	1.24	1.54	1.84	2.20	2.01	1.99	1.78

Table 4

STABILITY OF EMULSIONS							
Stability	R1CSP	R2CSP	R3CSP	R4CSP	R5CSP	R6CSP	R7CSP
Turbidity (cm ⁻¹)	4.184	3.762	3.484	3.562	1.816	1.956	5.024
Stability index (%)	100	100	100	100	100	100	100

The main viscosity indices recorded for the synthesized emulsions are shown in table 5.

From the comparative analysis of the viscosities values it is observed that the highest value was obtained for the experimental variant R4CSP (237.5 cP) in which the highest volume fraction of chitosan and the smallest volume fraction of water (R4CSP) were used. It can also be observed that the viscosity of the system is not dependent on the volume fraction of active principle (propolis and essential oil), the highest amount of active principle in the system (R7CSP, ϕ = 0.08 ml active principle/ml

emulsion) leading to obtaining of a viscosity with comparable values to those obtained when using smaller volume fractions (ϕ = 0.06 ml oil/mL emulsion, ϕ = 0.04 ml oil/mL emulsion). The shear stress (SS) has the highest value in the case of the R4CSP variant, the variant obtained when using the largest volume fraction of chitosan, respectively the smallest volume fraction of water.

Electron microscopy of textile materials functionalized with bioactive systems

The images obtained at a magnification of × 8000 for knitted textiles treated with bioactive systems are shown in figure 4. The resulting micrographs reveal that the surface of the cotton fibers is covered with a thin polymeric layer deposited both on the surface of the fibers and inside the space between the fibers. However, the micrographs cannot provide information about the thickness of the deposited layer and its uniformity.

Assessment of antibacterial activity

Images of Petri plates after 24 h incubation are shown in figure 5 and assessment of antibacterial activity is shown in table 6.

VISCOSITY INDICES RECORDED FOR THE EMULSIONS							
Code	Viscosity (cP)	SS (dyne/cm²)	SR (s ⁻¹)	Speed (RPM)	Temperature (°C)		
R1CSP	57.16	106.36	186	200	25.33		
R2CSP	95.16	177.03	186	200	25.33		
R3CSP	151.13	281.03	186	200	25.4		
R4CSP	237.5	441.8	186	200	25.3		
R5CSP	133.1	247.53	186	200	25.43		
R6CSP	147.86	274.96	186	200	25.53		
R7CSP	126.2	243.7	186	200	25.76		

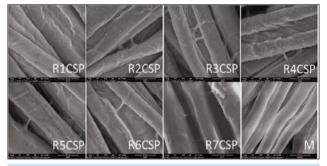


Fig. 4. Electronic images recorded for the knitted fabrics treated with chitosan-propolis-cinnamon essential oil polymeric system

By analyzing the obtained results it can be concluded that the textile materials treated with synthesized emulsions based on chitosan-propolis-cinnamon essential oil have antibacterial effect against the *S. aureus* test strain, with inhibition zones between 1.5 mm (R2CSP and R7CSP) and 4 mm (R6CSP). In the case of *E. coli* strain, the presence of the inhibition zone and the growth of the test strain in the whole culture medium were not observed, being considered a satisfactory effect in terms of antibacterial activity. There is no correlation between the amount of active principle and the obtained antibacterial effect, the sample treated with the emulsion with the

Table 6

EVALUATION OF THE ANTIBACTERIAL ACTIVITY							
Code	E. co	oli	S. aureus				
Code	Inhibition zone (mm)	Evaluation	Inhibition zone (mm)	Evaluation			
R1CSP	- (*)	Satisfactory effect	- (*)	Satisfactory effect			
R2CSP	- (*)	Satisfactory effect	1.5	Satisfactory effect			
R3CSP	- (*)	Satisfactory effect	- (*)	Satisfactory effect			
R4CSP	- (*)	Satisfactory effect	2.5	Satisfactory effect			
R5CSP	- (*)	Satisfactory effect	3	Satisfactory effect			
R6CSP	- (*)	Satisfactory effect	4	Satisfactory effect			
R7CSP	- (*)	Satisfactory effect	1.5	Satisfactory effect			
М	- (*)	Unsatisfactory effect	- (*)	Unsatisfactory effect			

(*) No inhibition zone, no multiplication.

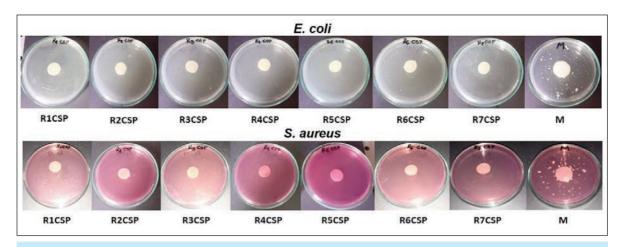


Fig. 5. Images of Petri plates showing antibacterial effect after 24 h of incubation

highest amount of active principle (R7CSP) having the smallest zone of inhibition (1.5 mm).

CONCLUSIONS

Seven exprimental variants of bioactive polymer systems synthesized based on chitosan-propolis-cinamon essential oil were assessed to explore their physico-chemical, biological as well as functional property to understand the possible applications in developing biomedical textiles. From the corroboration of the obtained data it was found that the obtained emulsions have a high degree of stability and a suitable viscosity to ensure a uniform deposition on the textile materials, on the one hand, and on the other hand to be able to be kept in a single phase until

further application on textile materials. Textile materials treated with bioactive polymeric systems have shown antibacterial activity for both gram positive bacteria (*S. aureus*) and gram negative bacteria (*E. coli*) test strains. The research on the potential bioactive polymeric systems based on chitosan-propolis-cinnamon for multifunctional properties development on textile materials is in progress.

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Authors:

LAURA CHIRILĂ¹, GABRIELA CRISTINA CONSTANTINESCU¹, ANGELA DANILA², ALINA POPESCU¹, ROXANA RODICA CONSTANTINESCU³, IRINA-MARIANA SĂNDULACHE¹

¹ National Research and Development Institute for Textiles and Leather, 16 Lucretiu Patrascanu Street, 030508, Bucharest, Romania e-mail: office@incdtp.ro

²"Gheorghe Asachi" Technical University of Iasi, Faculty of Industrial Design and Business Management, 29 Prof. Dr. Doc. Dimitrie Mangeron Blvd., 700050, Iasi, Romania

³National Research and Development Institute for Textiles and Leather, Leather and Footwear Research Institute (ICPI) Division, 93, Ion Minulescu Street, 031215, Bucharest, Romania

Corresponding author:

ANGELA DANILA e-mail: acerempei@tuiasi.ro