Antimicrobial activity of essential oils and their controlled release from the smart PLA fabric

D Štular¹, I Jerman², M Mihelčič³, B Simončič¹ and B Tomšič¹

¹University of Ljubljana, Faculty of Natural Sciences and Engineering, Department of Textiles, Graphic Arts and Design, Aškerčeva 12, 1000 Ljubljana, Slovenia
²National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

Abstract. In this study, “smart”, stimuli responsive PLA fabrics with controlled antimicrobial activity were tailored, by the application of microgel based on temperature responsive poly(N-isopropylacrylamide) (poly-NiPAAm) and pH responsive chitosan, with incorporated β-cyclodextrine (PNCS/CD microgel), and subsequent embedment of essential oils (EO). Antimicrobial activity of lavender, thymol, savory and cinnamon bark EO was tested in 1 – 5 % concentrations. Based on the results, the 4% concentration of savory and cinnamon EO were chosen and prepared as emulsion, allowing further embedment into the PNCS/CD microgel using in-situ procedure on the PLA fabric. Morphological and chemical changes of the studied samples were assessed using scanning electron microscopy (SEM) and Fourier-transform infrared (FT-IR) spectroscopy. Temperature responsiveness was analyzed by determining moisture content (MC) at 20- and 40 °C, antimicrobial activity was determined by evaluating the reduction of gram-positive Staphylococcus aureus and gram-negative Escherichia coli, thus controlled release of the EO was tested by determining the zone of inhibition at 20- and 37 °C. The results show that the embedment of the EO emulsion did not affect the temperature responsiveness of the PNCS/CD microgel. The PNCS/CD microgel proved to be a suitable carrier of antimicrobial agents, assuring the effective controlled release of EO emulsion and excellent antimicrobial activity. Temperature related controlled release of the EO emulsion was proven by the formation of zone of inhibition, which occurred only at conditions dictating shrinkage of the microgel particles.

Keywords—Antibacterial activity, Cinnamon bark essential oil, Controlled release, Smart textiles, Savory essential oil, Stimuli responsive microgel

I. INTRODUCTION

Essential oils (EO) which are distinguished by numerous advantages (i.e. anticancer, antiviral, antioxidant and antimicrobial properties), are indispensable in textile industry, to produce high value-added products which are non-toxic, biocompatible and environmentally friendly [1]. Their antimicrobial activity is based on small phenolic or terpenoid compounds, which hold an ability to invade the cell membrane, thus causing the proton gradient exhaustion, leading to the cell lysis or disruption of adenosine triphosphate synthesis [2]. In order to increase their lifetime on the surface of textile materials, EO can be incorporated into stimuli responsive hydrogels, which enables their release only at predetermined conditions. Namely, the ability of reversible volume change (i.e. swelling/de-swelling), triggered by the presence or absence of different external stimuli, makes stimuli responsive hydrogels ideal carriers of different active ingredients. Among stimuli responsive hydrogels, temperature and pH responsive microgels based on poly-(N-isopropylacrylamide) (poly-NiPAAm) and chitosan (PNCS microgel) has already been proven to be a suitable carrier of different antimicrobials, providing their controlled release only at required conditions [3]. PNCS microgel exists in the swollen phase when the temperature is below 32 °C and pH is below 6.5, thus undergoes transition in to shrunken and collapsed state when the temperature and/or pH rise above these values [4]. Nevertheless, embedding of EO into the structure of the microgel particles represents a challenge, due to the hydrophobic character of the EO. This challenge could be overcome by the addition of cyclodextrines into the microgel structure, as they are widely used as a hydrophobic guest molecules and have an ability to entrap substances in their hydrophobic cavity [5].

In the present research, we incorporated β-cyclodextrine into the structure of the PNCS microgel (PNCS/CD microgel). The suitability of the PNCS/CD microgel as a carrier of different EO was studied. For this purpose, antimicrobial activity of lavender, thyme, savory and cinnamon bark EO in various concentrations was assessed and selected EO were incorporated into the PNCS/CD microgel by the in-situ procedure on PLA fibers. With the use of adequate techniques, temperature responsiveness, antimicrobial activity and controlled release of EO from the functionalized PLA fabric were studied.
II. EXPERIMENTAL

A. Materials
In this study, 100 % poly(lactic acid) (PLA) fabric Revolution® (M+N Textiles, Nederland) was used. Lavender, thymol, cinnamon bark and savory essential oils (Florihana) were purchased from Magnoliija, Nina Medved s.p, dimethyl sulfoxide (DMSO) (sigma Aldrich) was used as a solvent and Tween 20 (Sigma Aldrich) as a surfactant. For the preparation of the PNCS/CD microgel, chitosan (Chitoclear, Primex, Iceland; DD = 95%; η = 159 mPa), glacial acetic acid (Sigma Aldrich), N-isopropylacrylamide (NiPAAm) (Sigma Aldrich), N,N-methylenebisacrylamide (MBA) (Sigma Aldrich), ammonium persulfate (APS) (Sigma Aldrich), β-cyclodextrine (Sigma Aldrich) and sodium dodecyl sulphate (SDS) with high purity (GE Healthcare Life Sciences) were used.

B. Determination of antimicrobial activity of EO
Disk diffusion assay test was used to determine the antimicrobial activity of lavender, thymol, cinnamon bark and savory essential oils. EO were diluted in DMSO in 1, 2, 3, 4 and 5 wt% concentration. The 6 mm discs were impregnated with 20 μL of each diluted EO and air-dried. Blank disc and DMSO impregnated disc were used as controls. Dried discs were placed on agar plate infused with 200 μL of the test bacterium, where gram negative bacteria Escherichia coli (E. coli, ATCC 25922) and gram positive bacteria Staphylococcus aureus (S. aureus, ATCC 6538) were used. Samples were incubated on 37 °C for 24 hours and the zone of inhibition was observed.

C. PNCS/CD microgel synthesis, characterization and application
PNCS microgel, functionalised with β-cyclodextrin was prepared using in-situ synthesis, according to modified methods reported by Lee [6] and Yi [7]. The particle size of dispersed PNCS/CD microgel in correspondence with changes in temperature was determined with dynamic light scattering (DLS) analysis, on Zetasizer Nano S (Malvern, UK), equipped with 4mW He-Ne laser operating at wavelength 633 nm and an avalanche photodiode detector. Scattering light was detected at an angle of 173 °. Microgel was diluted 50 times. Particle size was determined at temperatures ranging from 20- to 40 °C in 5 °C intervals. 60 μL of sample was used for each measurement and results represent an average of 3 measurements. The PNCS/CD microgel was applied using a pad-dry method with a wet pick-up (WP) of 80±5 %.

D. Essential oil emulsion preparation, characterization and incorporation
Emulsions of cinnamon bark and savory essential oils were prepared by diluting 8 g of Tween 20 in 88 g distilled water and mixed for 20 min on room temperature with the speed of 100 rpm. Afterwards, the stirring speed was set on 1500 rpm, while 4 g of essential oil was added dropwise into the mixture. The mixing proceeded for 4 hours and was followed by mixing with lab homogenizer (Hielscher Ultrasonics GmbH) for 5 minutes, using 0.5 ultrasonic impulses. The particle size of EO emulsion was determined using dynamic light scattering (DLS) analysis in the same manner as the PNCS/CD microgel particles. The measurements were conducted at 20 °C and EO emulsion was diluted 10 times.

For the incorporation of EO emulsion into the microgel particles, the PNCS/CD microgel functionalized PLA samples were heated to 50 °C for 15 min, in order to achieve the shrinking of the microgel particles on the fiber surface. Next the EO emulsion was sprayed on the samples until the fabrics were completely wet. The used EO emulsion was previously stored in a refrigerator at 8 °C in order to advance the swelling of the shrunken microgel particles, leading to greater absorption of the EO emulsion. The wet samples were subsequently squeezed with foulard with the pressure of 3 bar in order to achieve homogenous distribution of the EO emulsion. The sample codes are presented in Table 1.

| Sample code | Description of chemical modification |
|-------------|-------------------------------------|
| PLA_UN      | PLA fabric treated with PNCS/CD microgel |
| PLA_M       | PLA fabric treated with PNCS/CD microgel and savour EO emulsion |

G. Analysis and measurements
Add-on was calculated from the average mass of completely dried samples. The samples were placed in the moisture analyzer MLB-C (Kern & SOHN GmbH, Germany) and dried until a constant mass. For each sample, 10 measurements were obtained. The add-on values were calculated according to following equation:

\[
\text{Add-on} = \frac{\text{Mass of completely dried sample} - \text{Mass of sample before drying}}{\text{Mass of sample before drying}} \times 100
\]
\[ Add - on = \left( \frac{m_f - m_{UN}}{m_{UN}} \right) \times 100\% \] (1)

where \( m_f \) [g] is the average of the dry weights of the functionalized sample, and \( m_{UN} \) [g] represents an average of dry weights of the untreated sample.

Morphological changes of the studied PLA samples were determined using JEOL 6060 LV scanning electron microscope, operating at 10 kV. Before SEM images were taken, the samples were coated with a thin layer of mixture of platinum and gold to ensure sufficient electrical conductivity and to avoid charging effects.

Chemical changes were determined using Fourier-transform infrared (FT-IR) spectroscopy, obtained from a Spectrum GX I spectrophotometer (Perkin Elmer, Great Britain) equipped with an attenuated total reflection (ATR) cell and a diamond crystal (n = 2.0). The spectra were recorded over the range 4000–600 cm\(^{-1}\), with a resolution of 4 cm\(^{-1}\) and averaged over 16 spectra.

Temperature responsiveness of the samples was assessed by measuring moisture content using a Moisture analyzer MLB-C (Kern & SOHN GmbH, Germany). Studied samples were preconditioned at 65±2% relative humidity at 20– and 40°C for 24 h, before they were put in a moisture analyzer and dried at 60 °C until the constant mass. Moisture content (MC) was determined by following equation:

\[ MC = \left( \frac{m_0 - m_f}{m_0} \right) \times 100\% \] (2)

where \( m_0 \) denote the initial mass of the pre-conditioned sample and \( m_f \) represents the final mass of the sample after drying. MC was reported as mean values of ten measurements.

From results, the swelling degree of PNCS/CD microgel (SDMC) on the studied PLA samples was calculated by following equation:

\[ SD_{MC} = \left( \frac{MC_f - MC_{UN}}{MC_{UN}} \right) \times 100\% \] (3)

where \( MC_f \) is the moisture content of the functionalized PLA samples, and \( MC_{UN} \) is the moisture content of the untreated PLA sample, determined under the same conditions.

Antibacterial activity of studied cotton samples was estimated by determination of bacterial reduction according to ASTM E 2149–01 standard method. Bacterial reduction of coated samples was evaluated against \( E. coli \) (ATCC 25922) and \( S. aureus \) (ATCC 6538). The reduction of bacteria \( (R) \) was calculated as follows:

\[ R = \left( \frac{U-T}{T} \right) \times 100\% \] (4)

where \( U \) is the number of CFU determined for the untreated sample after 24 h of incubation and \( T \) is the number of CFU determined for the treated samples under the same conditions. For each sample, the value \( R \) represent an average of eight counts.

Additionally, the zone of inhibition was determined referring to the ISO 20645 Agar diffusion plate test, where bacteria was let to grow at 20 °C and 40 °C for the purpose of determining the controlled release. According to the ISO 20645 Agar diffusion plate test, assessment of antibacterial activity was based on the absence or presence of an inhibition zone. All tests were performed in duplicate.

### III. RESULTS AND DISCUSSION

#### A. Determination of antimicrobial activity of EO

Antimicrobial activity of lavender, thymol, cinnamon bark and savory essential oils in 1 – 5 % concentrations was determined against gram negative bacteria \( E. coli \) and gram positive bacteria \( S. aureus \), using disk diffusion assay method. In general, the EO were more efficient against gram positive bacteria \( S. aureus \), in comparison to gram negative bacteria \( E. coli \). Lavender EO did not form any zone of inhibition in the tested concentrations, thus higher concentrations should be used. Furthermore, thymol, savory and cinnamon bark EO formed zone of inhibition in the concentrations ranging from 3 to 5 %, thus it increased with the rising concentrations (data not shown). Based on the results obtained for both bacteria, savory and cinnamon bark essential oils in 4 % concentration (Fig.1) were used for further incorporation into the PNCS/CD microgel.
Fig. 1. Disk diffusion test against *E. coli* and *S. aureus*, of 1-Lavender, 2-Thymol, 3-Savory and 4-Cinnamon bark EO in 4% concentration, where 0-empty disk and S-DMSO impregnated disc were used as control.

**B. PNCS/CD microgel and EO emulsion characterization**

In order to investigate the hydrodynamic particle size of PNCS/CD microgel in dependence of changes in temperature, DLS analysis was used (Fig. 2). At 20 °C microgel particles were swollen and reached the size of 692 nm. Temperature responsiveness is seen as a decrease of the particle size with the rise of temperature, until the temperature reached 30 °C. Values gathered at 35- and 40 °C dramatically increased, which was contributed to fast agglomeration of the microgel particles. Such dramatic respond is in a good agreement with the literature, as Yi [7] reported that the incorporation of β-cyclodextrin into the microgel structure greatly improves its temperature responsiveness. In this case the formation of a complex between the temperature responsive poly-NIPAAm and β-cyclodextrin occurs, providing effective driving force, thus expelling hydrophilic poly-NIPAAm blocks at lower temperatures or pulling hydrophobic blocks of the temperature responsive polymer, upon heating.

Fig. 2. Hydrodynamic diameter of the PNCS/CD microgel as a function of temperature.

DLS was also used to determine the size of EO emulsion particles which was 206.03±1.15 nm with PDI of 0.06 for the savory EO emulsion and 203.03±2.77 nm with 0.10 PDI for the cinnamon bark EO emulsion. Based on the results we can conclude that the type of EO used for preparation of the emulsion did not affect the size of the emulsion particles and that the size of the particles could allow for the emulsion to be embedded into the microgel structure.

**C. Morphological and chemical changes of the functionalized PLA fibers**

The PNCS/CS microgel was applied onto the PLA fabric in the first step and EO emulsion was embedded *in-situ* subsequently. The add-on determination (Table II) suggest that the incorporation of EO emulsion was successful, as the add-on values increased from approximately 3 % for the PLA M sample, to greater than 10 % for the samples PLA M+S and PLA M+C.

| Sample  | Add-on [%] | SD  |
|---------|------------|-----|
| PLA M   | 2.998      | 0.603 |
| PLA M+S | 10.943     | 0.859 |
| PLA M+C | 10.561     | 0.415 |

Next, the morphological changes were examined by SEM and are presented in Fig.3. PNCS/CD microgel formed round bulges on the fiber surface, with 350 - 500 nm in diameter. After the application of EO emulsion the bulges on the fiber surface are much more noticeable, due to the swelling of the microgel and the incorporation of EO emulsion. The diameter of the microgel bulges did not change significantly, yet the contrast is much more noticeable as the microgel particles rise in volume. By comparing the
morphological changes on the PLA_M+S and PLA_M+C samples, we can conclude that the type of the EO used for the emulsion preparation did not influence the morphological changes of the studied samples.

Fig. 3. SEM images of a - PLA_UN, b – PLA_M, c – PLA_M+S and d – PLA_M+C under 6000 times magnification.

Chemical properties of functionalized PLA fabric samples were studied by IR ATR spectroscopy (Fig. 4). The presence of PNCS/CD microgel in the IR ATR spectra was confirmed by the appearance of the absorption band at 1450 cm⁻¹, belonging to the N–H vibration of both poly-NiPAAm and chitosan, as well as by the absorption bands of amide I and amide II at 1645 and 1535 cm⁻¹, manifested by C=O stretching vibration of poly-NiPAAm [8]. The IR ATR spectra of the samples PLA_M+S and PLA_M+C, show the appearance of several additional peaks which can be contributed to the diverse composition of EO [9]. Savory and cinnamon bark essential oils have 20 common components, which was broadcasted in a form of similar additional bands in the area from 3000 to 2850 cm⁻¹ belonging to C–H stretch of alkanes and between 900 and 675 cm⁻¹, belonging to aromatic C=C compounds. Furthermore, the sample PLA_M+C formed absorption band at 1667 cm⁻¹ which can be ascribed to the C=O bond of aldehyde and the bands ranging from 1670 to 1620 cm⁻¹, belonging to C=C stretch of alkenes [10].

Fig. 4. FT-IR spectra a - PLA_UN, b – PLA_M, c – PLA_M+S and d – PLA_M+C.

D. Temperature responsiveness of the functionalized PLA fibers

Temperature responsiveness of the functionalized PLA samples was determined by moisture content (MC) analysis (Fig. 5a). With respect to the untreated PLA fabric, the application of temperature responsive PNCS/CD microgel improved the MC at 20 °C, when the microgel was in the swollen, hydrophilic state. Furthermore, when the EO emulsion was embedded into the microgel structure, the MC values gathered at 20 °C were even higher, as the microgel particles were already hydrated with the EO emulsion. On contrary, at 40 °C the microgel was in its hydrophobic state, therefore the MC of the functionalized samples decreased dramatically. In order to compare and further emphasize the stimuli responsiveness of the PNCS/CD microgel applied on PLA fabric, swelling degree (SD_MC) was calculated (Fig. 5b). It is evident that PNCS/CD microgel applied to the PLA fabric provided approximately 50% higher SD_MC compared to PLA_UN sample. Hence, the presence of the EO emulsion embedded in the microgel
particles did not influence the temperature responsiveness, namely the SDMC values lowered for only 1.6% and 2.6% compared with the PLA_M sample.

![Graph](image)

Fig. 5. a – Moisture content (MC) values and b – swelling degree of the PNCS/CD microgel (SDMC) applied on the studied samples, analyzed at 20- and 40 °C.

**E. Antimicrobial activity of the functionalized PLA fibers**

The antimicrobial activity of the studied samples was tested against Gram-negative *E. coli* and Gram-positive *S. aureus* (Table III). Microgel-coated PLA fabric showed 26% reduction of *E. coli* and 53% reduction of *S. aureus*, which can be contributed to the presence of the chitosan in the microgel structure. Furthermore, excellent reduction was achieved for both PLA samples functionalized with the combination of PNCS/CD microgel and EO emulsion. Surprisingly, combination of PNCS/CD microgel and cinnamon bark EO showed lower bacterial reduction compared to savory EO, which contradicts the disk diffusion test. The reason for such behavior might be in the stableness of the EO emulsion. Namely, after few days some oil bubbles were seen in the cinnamon bark EO emulsion. In a view of that, further optimization of cinnamon bark EO emulsion is needed. Nevertheless, the antimicrobial reduction provided by the PLA_M+C sample is still efficient, namely 97±1.47% for the gram negative bacteria and 87±12.5% for the gram positive bacteria.

| Sample code | *E. coli* | *S. aureus* |
|-------------|-----------|-------------|
| PLA_UN      | 26.4±17.5 | 53.0±10.7   |
| PLA_M       | ≥99.99    | =99.99      |
| PLA_M+S     | 97.8±11.4 | 87.0±12.5   |

Controlled release of the EO emulsion was studied by the determination of zone of inhibition in bacterial growth. The samples were placed on the bacteria infused agar plate and incubated at 20 °C, i.e. conditions where microgel is swollen and 40 °C, when microgel is shrunken. Results gathered for the gram positive bacteria *S. aureus* are shown in Fig. 6. As expected, no zone of inhibition was seen for the samples PLA_UN and PLA_M at any given temperature. In contrast, samples PLA_M+S and PLA_M+C show the controlled release of the EO imposed by the shrinking of the microgel particles. At 20 °C the diameter of bacterial colonies that have grown on the agar plate is greatly reduced. At this temperature the microgel particles exist in the swollen state, which restricted the EO emulsion to be released from the particles on a larger scale. However, the swollen microgel particles are highly porous, thus the wetted agar plate allowed the leakage of the small amounts of EO emulsion. Despite that, bacterial growth next to the sample was still observed. The small amount of the released EO emulsion was not enough to provide the zone of inhibition, yet it was enough to interact with the growth of the bacterial colonies. At 40 °C the microgel particles shrank and released some of the EO emulsion from their structure, which provided a noticeable zone of inhibition (i.e. > 1 mm) next to the studied samples.
IV. CONCLUSION

Based on the antimicrobial tests of the lavender, thymol, savory and cinnamon bark essential oils (EO), tested in 1 – 5 % concentrations, savory and cinnamon bark EO in 4 % concentration were chosen for the incorporation into the PNCS/CD microgel applied on the PLA fabric. The hydrodynamic microgel particles size at 20 °C was 692, thus the size of EO emulsions particles was approximately 200 nm, ensuring that the emulsion could be embedded into the microgel structure. All the samples padded with PNCS/CD microgel exhibited temperature responsiveness, which was preserved even after incorporation of EO emulsion. Accordingly, the type of EO emulsion (i.e. savory and cinnamon bark) did not influence reversible responsive behavior of the studied microgel. Excellent bacterial reduction was obtained for both samples functionalized with EO emulsions. Temperature related release of the EO emulsion was proven by the determination of zone of inhibition, which was formed only at condition dictating shrinkage of the microgel particles, i.e. at 40 °C. This results prove the appropriateness of the functionalization process to create smart textiles with controlled moisture management properties and pro-active antimicrobial activity, triggered only at controlled conditions.

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