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# Application of cellulose acetate for controlled release of thymol

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ABSTRACT

Cellulose acetate (CA) was investigated as a carrier towards development of material with controlled release of thymol as a natural substance with strong antibacterial properties using high pressure techniques. Effect of thymol content on CA was confirmed by SEM, FTIR and DSC methods. Kinetic of thymol release from CA was tested using simulated gastric and intestinal fluids (hydrochloric acid and phosphate buffer saline). Results were correlated with Korsmeyer-Peppas and Weibull model. Depending on the thymol content and chemical nature of the release medium, the time of thymol release varied from one to three days indicating CA as a promising carrier of thymol with potential uses from medicine to agriculture. The impregnated CA showed antibacterial activity against 23 tested bacterial strains including methicillin-resistant *Staphylococcus aureus* (MRSA) which is particularly important bearing in mind that this strain causes fatal infections in humans and animals.



## 1. Introduction

Some of current needs in non-toxic and renewable materials are met by development of cellulose esters based materials with a number of applications such as coatings, controlled release, plastics, composite, optical film, membrane structures, and other types of separation media applications (Edgar et al., 2001). Among cellulose esters, a key role in development of controlled delivery systems has cellulose acetate (CA) due to its established safety, functionality and ease of processing. CA has been applied as pharmaceutical excipient, for control release of agricultural actives and fragrances and as polymer stabilizer (Edgar et al., 2001). One of the ways to increase polymer positive properties and number of its applications is by combining it with bioactive substances. Such an approach can be exploited to prepare antimicrobial coatings with potential use for food packaging, health-care items, wood surfaces, or antimicrobial beads that could be added to liquids or solids for spoilage prevention (Pilati, Degli Esposti, Bondi, Iseppi, & Toselli, 2013). Natural bioactive substance with strong antimicrobial activity against wide range of microorganisms (Gram positive and Gram negative bacteria, fungi, and yeasts) appropriate for incorporation into different polymer carriers is thymol (Markovic, Milovanovic, Radetic, Jokic, & Zizovic, 2015; Milovanovic, Stamenic, Markovic, Ivanovic, & Zizovic, 2015; Pilati et al., 2013; Shahidi, Aslan, Gh oranneviss, & Korachi, 2014). Thymol is a monoterpene phenol abundantly found in plants of Lamiaceae family species like thyme and oregano. Besides antimicrobial activity, it exhibits many positive properties like antioxidant (Aeschbach et al., 1994), anti-inflammatory and cicatrizing activity (Riella et al., 2012). These features make thymol promising bioactive substance for production of polymers with added value.

There are many methods for incorporation of bioactive component into a polymer carrier but the method which proved to be advantageous is supercritical solvent impregnation (SSI). SSI implies dissolution of bioactive compound in a supercritical fluid and contacting of the resulting fluid mixture with a polymer to be impregnated (Kikic & Vecchione, 2003; Milovanovic, Jankovic-Castvan, Ivanovic, & Zizovic, 2015). Main advantages of this technique are avoidance of organic solvents, possibility to work at relatively low temperatures, homogeneous distribution of active principle through the whole volume of polymer, no need for drying step, no waste water, energy savings and easy and total separation of supercritical fluid from the polymer (Dias et al., 2011; Ivanovic et al., 2014; Zizovic, Ivanovic, Milovanovic, & Stamenic, 2014). The most used supercritical fluid is supercritical carbon dioxide ($scCO_2$) because of its established safety and unique physicochemical properties like high diffusivity in organic matter, low surface tension, ease of its recovery *etc.* (Ivanovic et al., 2014; Kikic & Vecchione, 2003; Zizovic et al., 2014).

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In our previous study (Milovanovic, Stamenic et al., 2015), SSI process with carbon dioxide was shown to be an appropriate method for production of CA-based polymer material with antimicrobial properties. The results indicated changes in the CA structure by the influence of thymol content in the impregnated samples. The release kinetic was investigated in water as the release medium. The main objective of this study was to explore CA further as a carrier which would provide controlled release of thymol. In that sense the effects of thymol content on CA, release kinetics in selected release media (simulated gastric and intestinal fluid) as well as antibacterial activity of the obtained CA-thymol system were investigated.

## 2. Experimental part

### 2.1. Materials

Thymol (purity > 99%) was purchased from Sigma-Aldrich (Germany). Cellulose acetate beads (CA-320S NF/EP) with acetyl content 32.0% were generous donation from Eastman (Poland). Commercial $CO_2$ (purity 99%) was supplied by Messer-Tehnogas (Serbia). Hydrochloric acid was purchased from NRK Engineering (Serbia). Disodium-hydrogenphosphate-12-hydrate and sodium-dihydrogenphosphate-2-hydrate were supplied by Centrohem (Serbia).

### 2.2. Supercritical impregnation of cellulose acetate with thymol

Supercritical impregnation of CA with thymol was performed using static method in a high-pressure view cell (Eurotechnica GmbH, Germany) previously described in detail (Milovanovic, Markovic, Stamenic, Radetic, & Zizovic, 2013). The experiments were conducted in a manner described in previous study (Milovanovic, Stamenic et al., 2015). The experiments were carried out at pressure of 10 MPa and at temperature of 35 °C. The impregnation time was 2, 5, 28, and 32 h. Thymol:CA ratio at the beginning of the process was constant 5:1 (2.500 g of thymol and 0.500 g of CA). Each experiment was run in triplicate.

Impregnation yield ($I$) of CA was calculated according to Eq. (1):

$$I(\%) = \frac{m_{thymol}}{m_{CA} + m_{thymol}} \times 100 \quad (1)$$

where $m_{thymol}$ is the mass of impregnated thymol after SSI process, and $m_{CA}$ is the mass of CA at the beginning of the process.

### 2.3. Characterization of the samples

#### 2.3.1. Surface morphology

Field emission scanning electron microscopy (SEM, Mira3Tescan) of the CA beads was used to determine CA morphology before and after supercritical impregnation of thymol. The samples were coated with a thin layer of Au/Pd (85/15) prior to the analysis.

#### 2.3.2. Fourier transform infrared analysis

Fourier transform infrared (FT-IR) spectra of non-treated CA and CA impregnated with thymol were recorded at ambient conditions in the attenuated total reflectance (ATR) mode using Nicolet IS 50 FT-IR Spectrophotometer in the wave number range 4000–400 $cm^{-1}$, with resolution of 4 $cm^{-1}$.

#### 2.3.3. Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) analysis was performed in differential scanning calorimeter (Q10 TA Instruments, USA) previously described in detail (Obradović et al., 2014). In short, samples were examined in nitrogen atmosphere from the room temperature of 25 °C to 300 °C at a heating rate of 10 °C/min. Samples (10 mg) were hermetically sealed in an aluminum pan, put in the DSC cell together with an empty aluminum pan as the reference material, and heated with a continuous nitrogen flow of 50 mL/min.

### 2.4. In vitro thymol release

*In vitro* thymol release tests were conducted in 0.1 N HCl (simulated gastric fluid with pH 1.1), and 0.1 M phosphate buffer (simulated intestinal fluid with pH 7.0) using the shaking water bath WNE 14 (Memmert, Germany) with constant shaking at temperature 37 °C. The experiments were conducted in a manner described in previous study (Milovanovic, Stamenic et al., 2015). Thymol concentration was determined at 274 nm using a spectrophotometer (Cary100 Scan, Varian). Each thymol release test lasted for three days and was run in duplicate.

Release curves were simulated and corresponding parameters were calculated using the equations proposed by Korsmeyer and Peppas (1981) (Eq. (2)) and by Weibull (Papadopoulou, Kosmidis, Vlachou, & Macheras, 2006) (Eq. (3)):

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

$$\frac{M_t}{M_\infty} = 1 - \exp(-a \times t^b) \quad (3)$$

where $M_t$ and $M_\infty$ are the absolute cumulative amounts of bioactive substance released at time $t$ and at infinite release time, respectively; $n$ and $k$ are the release exponent and the kinetic constant, respectively (Dias et al., 2011; Papadopoulou et al., 2006; Ritger & Peppas, 1987); $a$ and $b$ are constants (Papadopoulou et al., 2006). Release exponent $n$ provides information on the involved release mechanisms, and kinetic constant $k$ incorporates structural and geometric characteristics of the release material. It was previously shown that Eq. (2) adequately described the release of drugs or other solutes from slabs, spheres, cylinders and discs regardless of the release mechanism (Ritger & Peppas, 1987) for short time approximation of complex exact relationships and therefore its use is confined for the description of the first 60% of the release curve (Papadopoulou et al., 2006; Pilati et al., 2013).

### 2.5. Antibacterial activity

Qualitative assessments of the antibacterial activity of CA and CA impregnated with thymol against various Gram-negative and Gram-positive bacteria were performed using the agar diffusion method. The following bacterial strains were investigated: *Salmonella Typhimurium, Salmonella Enteritidis*, *Listeria monocytogenes, Listeria ivanovii* ATCC 19119, *Listeria innocua, Corynebacterium spp., Rhodococcus equi, Bacillus anthracis, Bacillus cereus, Bacillus subtilis, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (ATCC 33591), three clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter spp.*, and *Proteus mirabilis*. All investigated bacterial strains were isolated from clinical specimen delivered to routine microbiological examination except the strains from the American Type Culture Collection (ATCC). Conventional bacteriological methods were applied for the purpose of isolation of bacteria with the use of Columbia 5% sheep blood agar (bioMerieux), MacConkey agar (Becton Dickinson), Buffered peptone water (Difco), Rappaport–Vassiliadis semisolid agar (Becton Dickinson), Sabouraud dextrose agar (Becton Dickinson) and XLT4 agar (Becton Dickinson). Identification systems API ID32 (bioMerieux), BBL Crystal Gram Positive ID system and BBL Crystal Enteric/Nonfermenter ID system (Becton Dickinson) were used