Impregnation of Cellulose Acetate Films with Carvacrol Using Supercritical Carbon Ioxide

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Cellulose acetate films were impregnated with carvacrol using supercritical carbon dioxide. The supercritical impregnation process, conducted in a static regime at pressure of 21 MPa and temperature of 50 °C, was optimized by variation in the processing time (30 and 120 min) and decompression rate (from 0.3 MPa/min to 36 MPa/min). Characterization of the obtained cellulose acetate films was performed by Atomic Force Microscopy and Differential Scanning Calorimetry. Effects of glycerol and carvacrol on the properties of the films were discussed. Release kinetics from the cellulose acetate film with 31.4% of carvacrol was investigated in a physiological saline solution. In addition, the Higuchi and Korsmeyer-Peppas release models fitted the carvacrol release curve well. Obtained cellulose acetate films impregnated with carvacrol can be of interest for the application in medicine as wound dressings considering their biocompatibility and biodegradability as well as their potential antimicrobial activity or in the food industry as an active food packaging.

Key words: *carvacrol, cellulose acetate film, supercritical CO_2, supercritical impregnation*

1. INTRODUCTION

Environmental and economic concerns associated with solid waste accumulation and limitation of the resources led to global interest in biodegradable and renewable materials [1]. Considering that cellulose constitutes are the most abundant renewable biopolymers available, their application in different fields has gained much scientific attention [1, 2]. Studies regarding cellulose are focused on cellulose derivates because they have better processability than the pure cellulose [1]. Among cellulosic derivates, cellulose acetate (CA) is of particular interest due to its versatility and broad applications from packaging materials and filters, to textiles [2]. In addition, it can be used in me-

dicine for controlled release of active components due to its biocompatibility [3]. A number of reports have been published on utilization of CA fibers with antimicrobial substances for application as wound dressings [4, 5] as well as on CA films prepared by the solvent casting method intended to be used for food packaging [1, 6, 7] with or without addition of antimicrobial substances. Antimicrobial active packaging can reduce the rate of microbial growth, increase the lag phase and/or inactivate microorganisms present in the target food or on the package itself [6]. Overproduction of antibiotics and their excessive use led to the appearance and spared of resistant bacterial strains [5]. Since a resistance of bacteria to plant extracts has never been recorded, plant extracts' constituents are nowadays again in the focus of scientific attention [5, 8]. One of the natural bioactive components that has shown promising results as bactericide is carvacrol [9, 10]. Carvacrol (5-isopropyl-2-methyl phenol) is a natural monotherpenic phenol found in isolates of Lamiaceae family (i.e. oregano, thyme) [9, 11-13]. It also displays anti-inflammatory and antioxidant activity [9,

11, 12]. Because carvacrol has been approved as a safe food additive by the US Food and Drug Administration (2006) and due to its beneficial biological activities, it can be used both in food packaging and in wound dressings [9, 11-13]. Since carvacrol is prone to degradation and rapid evaporation, it is important to find a suitable technique to prolong its activity [9, 12, 13].

Increase in carvacrol's bioactivity and possibilities for its application can be achieved by its impregnation into appropriate carriers. Lilois et al. [9] reported increase in carvacrol's antimicrobial activity after its incorporation into liposomes. Significant antibacterial activity of carvacrol, even against methicillin resistant strains, was reported for carvacrol impregnated CA beads [10].

Conventional methods of incorporation of bioactive components into polymeric carriers have certain drawbacks such as excessive use of large quantities of organic solvents or high temperatures. These can be avoided by utilization of supercritical fluids that allow production of high purity materials free of residual solvents which can be used in the pharmaceutical and food industry [14, 15]. Impregnation using supercritical fluids implies dissolution of an active component into a supercritical fluid and contact of the resulting mixture with a polymer [14].

Active component can be entrapped by a simple deposition into the polymer matrix or by chemical interaction with the polymer. Supercritical carbon dioxide ($scCO_2$) has been proven as an advantageous medium for polymer processing [2, 15]. Besides having low critical pressure and temperature (Pc=7.3 MPa and Tc=31.1 °C), $scCO_2$ is non-toxic, non-flammable, chemically inert, cheap, and easily available [2, 15]. Physical properties of $scCO_2$, such as low viscosity, high diffusivity and the absence of surface tension effects impart high penetrating capability to $scCO_2$ together with good mass and heat transport properties [2, 14].

This study was focused on the environmentally friendly $scCO_2$ impregnation of biodegradable CA films with a natural antimicrobial agent carvacrol. Obtained films were characterized regarding their topography and thermal properties. Release of carvacrol from the CA film was tested in a physiological saline solution.

2. MATERIALS AND METHODS

2.1. Materials

Cellulose acetate beads (32.0% acetyl content, Eastman, Poland), acetone (Zorka, Serbia) and glycerol (purity 99%, Galafarm, Macedonia) were used for the fabrication of films. The films were impregnated with carvacrol (purity > 99%, Sigma-Aldrich, Germany) using carbon dioxide (purity 99%, Messer-Tehnogas, Serbia).

2.2. Methods

2.2.1. Preparation of cellulose acetate films

CA films were prepared by the solvent casting method. The first type of film was prepared without glycerol as a plasticizer. CA beads (1 g) were dissolved in a mixture of acetone (27 mL) and distilled water (3 mL) by mixing during 2 h at room temperature. Obtained solution was poured onto a ceramic dish (dimensions 15.5 cm x 7 cm) and air dried for 7 days. In fabrication of the second type of film, the same procedure was repeated with addition of glycerol (0.1 g) to the CA/acetone/water solution.

2.2.2. Supercritical impregnation of cellulose acetate films with carvacrol

A high pressure view cell described elsewhere [16] was used for carvacrol impregnation into CA films. A film sample was placed in the cell above a glass container with carvacrol and separated by a fine mesh. Once the temperature of 50 °C was reached, CO_2 was introduced in the cell and pressure was elevated to 21 MPa.

After 30 or 120 min of the impregnation, the CO_2 was removed from the cell. Decompression rates were varied in the range from 0.3 to 36 MPa/min. Impregnation yield was calculated as a ratio between the mass of impregnated carvacrol and the mass of impregnated film multiplied by 100%.

2.2.3. Characterization of cellulose acetate films

CA films were analyzed by Atomic force microscopy (AFM) using a microscope Quesant (Ambios Technology, USA) in oscillating mode. Measurements were performed in air atmosphere using T-shaped silicone consoles with a 40 N/m spring constant. All images were obtained at 1Hz, with 512 × 512 image resolution in different square areas.

Differential scanning calorimetry (DSC) analysis was performed with a calorimeter Seteram 151 equipped with softer SETSOFT 2000. All measurements were performed in a nitrogen atmosphere at temperatures ranging from 25 to 300 °C with a heating rate of 10 °C/min.

2.2.4. Release kinetics

Carvacrol release was investigated by immersion of the impregnated CA films in a physiological saline solution (9 g/L) at 37 ºC in a static conditions. At predetermined time intervals an aliquot (5 mL) of the solution was taken, analyzed and returned to the solution. Carvacrol's concentration was determined by measuring absorption intensity at λ_{max}=273 nm using

an UV-VIS spectrophotometer Cary 100 Scan (Varian). In order to examine the release profile of carvacrol from CA films, following kinetics models were used:

$$Korsmeyer\text{-}Peppas: \frac{M_t}{M_\infty} = k \cdot t^n \qquad (1)$$

$$First\text{-}order: \ln\left(1 - \frac{M_t}{M_\infty}\right) = -k \cdot t \qquad (2)$$

$$Higuchi: \frac{M_t}{M_\infty} = k \cdot t^{1/2} \qquad (3)$$

where: M_t is the amount of carvacrol released in any time t, M_∞ is the amount of carvacrol released at infinite time, k is the release rate constant, and n is the release exponent.

3. RESULTS

3.1. Effect of processing conditions on carvacrol

In order to determine the effect of decompression rate on the impregnation yield of carvacrol in CA films without glycerol, initial impregnation experiments were performed at 21 MPa and 50 °C for exposure times of 30 and 120 min.

Results presented in Figure 1 revealed that the impregnation yield decreases with an increase in the decompression rate from 0.3 to 36 MPa/min for both operating times. Varona et al. [17] reported other trend i.e. that the linalool impregnation yield into starch increases with an increase in depressurization rate.
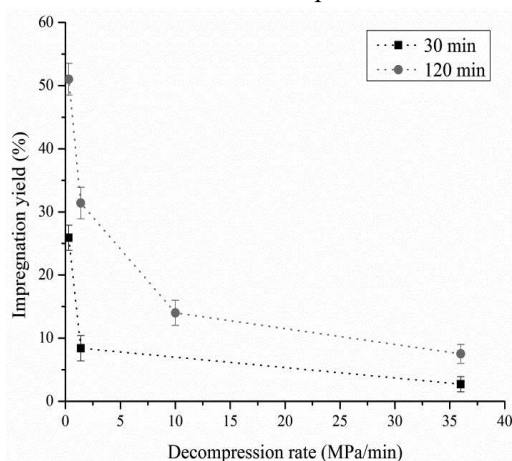

Figure 1 – Effect of decompression rate on impregnation yield

These results confirm that a process of impregnation is strongly dependent on the operating conditions employed as well as on the interaction between an impregnating substance and a polymer. It is postulated that the system with a low affinity between an impregnating substance and a polymer and high decompression rate achieves higher impregnation yields unlike the system with an intensive impregnating

substance-polymer interaction where low decompression rate induces higher impregnation yields [18]. Hence, decompression rate of 1.4 MPa/min was chosen as the optimal considering impregnation yield and duration of decompression.

Figure 2 shows the influence of operating time and addition of glycerol on the impregnation yield of carvacrol. It can be seen that an increase in operating time increases the impregnation yield of carvacrol. The same trend was reported for impregnation of CA beads with thymol and carvacrol [10,19]. Shen et al. [20] showed that compounds with hydroxyl groups, impregnated using scCO_2, had affinity towards hydrogen bonding sites of CA. The highest carvacrol impregnation yield obtained under the employed conditions was 31.4% for the CA film without glycerol. When carvacrol was impregnated into CA beads at 21 MPa and 50 °C for 120 min, the impregnation yield of 19.7% was obtained [10]. Therefore, the film morphology enables faster impregnation in comparison to the bead morphology. This is due to the larger outer surface exposed to supercritical fluid, shorter diffusion paths for sorbed molecules in the polymer and larger void fractions between the polymer chains in the case of films (due to the solvent evaporation in the solvent casting method).

When glycerol was added, the carvacrol impregnation yield decreased compared to films without glycerol. Although glycerol as a plasticizer should decrease interactions between polymeric chains, in this case we can presume that glycerol incorporates itself between polymeric chains and decreases diffusion rates of carvacrol in the polymer matrix.
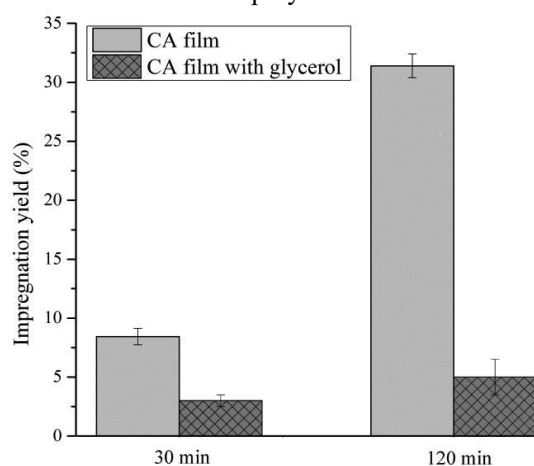

Figure 2 – Effect of operating time and addition of glycerol on impregnation yield

3.2. Characterization of cellulose acetate films

The first type of CA film (without glycerol) had thickness of 0.21±0.05 mm, while the CA film with added glycerol had thickness of 0.26±0.04 mm. Wongsasulak et al. reported [21] an increase in fiber

diameter of zein films containing 10 wt% of glycerol compared to the film without glycerol.

AFM images revealed the presence of small bumps on the surface of the CA film without glycerol (Figure 3a) which was in accordance with the data reported in the literature [3, 6]. The CA film with glycerol was shown to have some holes (Figure 3b). Yuan et al. [3] showed that water and a plasticizer in the solvent system for the CA film casting influenced the morphology and ultimately the properties of the films. Depending on their ratio, they increased or decreased the number and size of the holes onto and inside CA films.
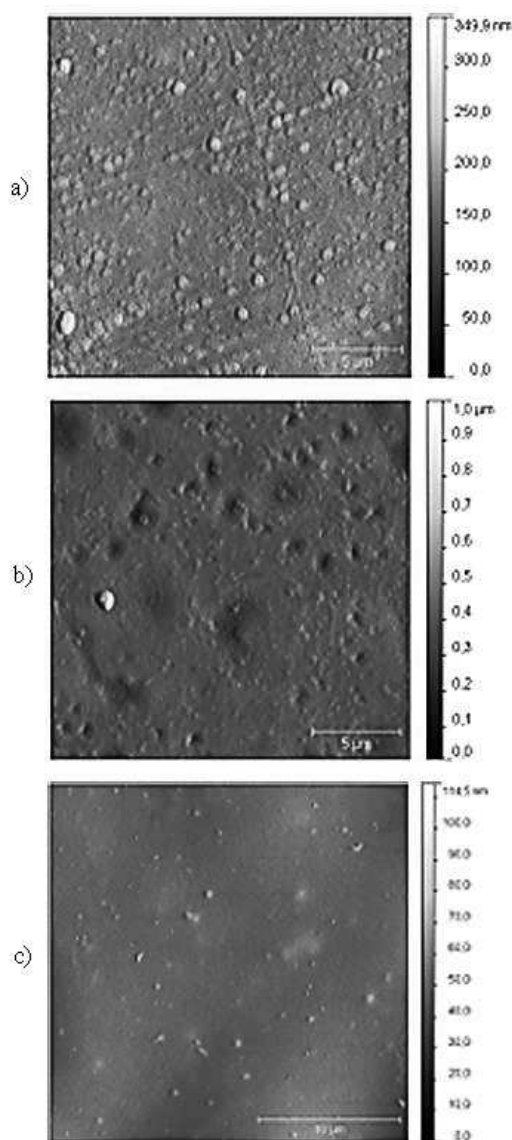

Figure 3 – AFM images of surface of: a) CA film, b) CA flim with glycerol, and c) CA film with carvacrol (31.4%)

The CA film without glycerol impregnated with carvacrol (Figure 3c) is more homogenous with a smaller number of bumps on its surface compared to

the first film. It was previously reported that pure CO_2 doesn't affect CA because chains of CA are interconnected with hydrogen bonds and CO_2 doesn't have enough solvent power to overcome this interactions [19, 20]. So, we can assume that only carvacrol with its OH groups interacted with hydrogen bonds of CA enhancing the polymer chains' mobility and inducing slight changes in the film morphology.

DSC thermograms of the CA films without and with glycerol are presented in Figure 4a. The endotherms detected around 100 °C can be atributed to the removal of water from the sample [1, 19]. In the sample with glycerol this peak is less pronanced and detected around 120°C. The glass transition temperature of the films without and with glycerol was detected at 206.1 °C and 236.7 °C, respectively. Given that results from the literature report decrease in the glass transition temperature of films due to the plasticizer adition [3, 21], the effect of glycerol on the CA films should be further investigated.
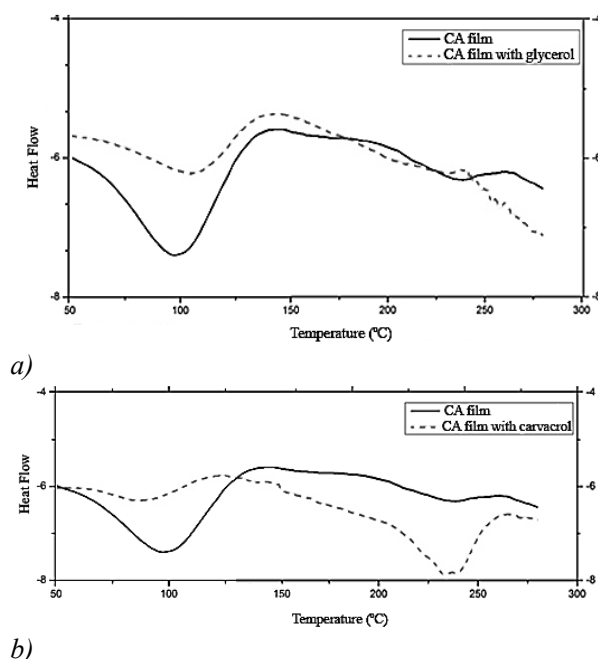

a)

b)

Figure 4 – DSC thermograms of: a) CA films without and with glycerol and b) CA films without and with carvacrol (31.4%)

DSC thermograms of the CA films (the first type, no glycerol added) neat and impregnated with carvacrol are presented in Figure 4b. In the sample with carvacrol, the endotherm around 100 °C attributed to the removal of water is less pronounced compared to the film without carvacrol. Also, impregnation of carvacrol led to the decrease of the glass transition temperature from 206.1°C to 175.4°C confirming plasticizing effect of carvacrol on CA. Decrease in the glass transition temperature of CA beads for 25°C after thymol impregnation with yield of 13.7% was

previously reported [19]. The endothermic peak in the temperature interval 232-240 °C can be atributed to boiling of carvacrol [13].

3.3. Release kinetics

For the investigation of carvacrol release kinetics, physiological saline solution was used as the release medium in order to simulate the body fluids. Cumulative release of carvacrol from the impregnated CA film (31.4% of carvacrol) is presented in Figure 5. As can be seen, the film released almost 85% and 88% of the impregnated carvacrol after 360 min and 24 h, respectively. Bearing in mind that one of the possible applications of CA films impregnated with carvacrol is for wound dressings, a controlled release of carvacrol during short time intervals would be desirable in order to prevent infections.
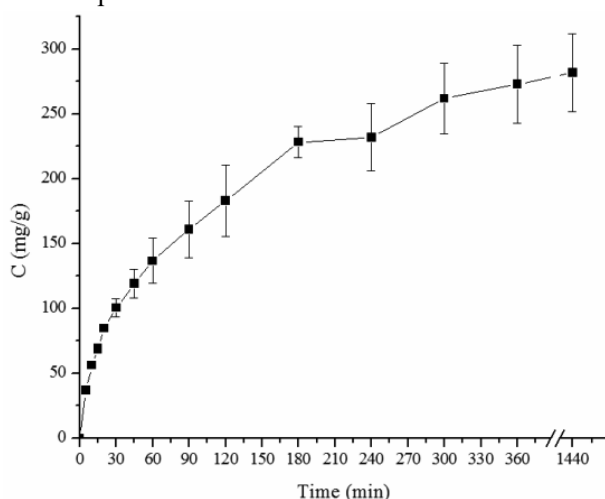

Figure 5 – Cumulative release of carvacrol from impregnated CA films (31.4%)

Release kinetics of carvacrol from the impregnated CA film was examined by fitting with the Korsmeyer-Peppas, first order and Higuchy mathematical models (Table 1). Modeling analysis was performed for the period of time until 60% of carvacrol was released. Table 1 revealed that the Higuchi model fits the best the release profiles of carvacrol. These results implied that the release of carvacrol was governed by the diffusion which controlled the processes. The absence of a significant number of pores on the impregnated CA film's surface (Figure 3c) would explain the phenomenon that the release of the active component was controlled by the diffusion rather than by the capillarity mechanism of this film [1]. The obtained results (Table 1) are in the accordance with the literature since the Higuchi model is widely used in a description of diffusion controlled release from transdermal systems [22, 23]. Table 1 also reveals that the Korsmeyer-Peppas release exponent n is above 0.43 indicating that the carvacrol release occurred by anomalous non-Fickan transport. Bierhalz et al. suggested

that natamicin release from the alginate film was in accordance with the Fickan diffusion [24]. Milovanovic et al. [19] also showed that the mechanisms of release of thymol from CA beads followed Fickan and non-Fickan diffusion depending on the thymol impregnation yields as well as the release medium.

Table 1. Correlation coefficients (R^2), release exponent, and release constants of models used for description of the carvacrol release mechanism

Korsmeyer-Peppas	
$k \times 10^{-2}$, min^{-n}	6.46
n	0.49
R^2	0.991
First order	
$k \times 10^{-3}$, min^{-1}	9.83
R^2	0.951
Higuchi	
$k \times 10^{-2}$, min$^{1/2}$	6.15
R^2	0.998

The antibacterial activity of carvacrol has been already analyzed in many studies [9-13]. Carvacrol's hydrophobic nature as well as its free hydroxyl function are two main factors responsible for its antibcterial nature [12]. Considering the release kinetics of carvacrol from the CA films and its proven antimicrobial activity, we can suppose that the obtained films have a potential for treatment of topical infections and in prevention of food spoilage.

4. CONCLUSION

Cellulose acetate films were successfully produced by the solvent casting method and subsequently impregnated with carvacrol using supercritical carbon dioxide at 21 MPa and 50 C. It was estimated that the increase in impregnation time from 30 min to 120 min led to an increase in the carvacrol impregnation yield, while the increase in decompression spead from 0.3 to 36 MPa/min decreased the carvacrol impregnation yield.

The CA film with 31.4% of impregnated carvcrol released almost 90% of carvacrol within 24 h. In addition, the controlled release of carvacrol from the CA film was obtained. The Higuchi and Korsmeyer-Peppas models fitted the carvacrol release curve well. The obtained carvacrol impregnated CA films have a prospective for application in medicine as wound dressings considering their biocompatibility and biodegradability as well as their potential antimicrobial activity or in the food industry as an active food packaging. Further studies are needed in these directions.

5. ACKNOWLEDGMENT

6. REMARK

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REZIME

IMPREGNACIJA FILMOVA ACETATA CELULOZE KARVAKROLOM POMOĆU NATKRITIČNOG UGLJENIK(IV)-OKSIDA

Filmovi acetata celuloze su impregnirani karvakrolom pomoću natkritičnog ugljenik(IV)-oksida. Proces natkritične impregnacije, izveden u statičkom režimu na pritisku 21 MPa i temperaturi 50 °C, je optimizovan varijacijom procesnog vremena (30 i 120 min) i brzine dekompresije (od 0,3 MPa/min do 36 MPa/min). Karakterizacija dobijenih filmova acetata celuloze je izvedena pomoću mikroskopije atomskih sila i diferencijalne skenirajuće kalorimetrije. Efekat glicerola i karvakrola na karakteristike dobijenih filmova je objašnjen. Otpuštanje karvakrola iz filma koji je ima 31,4% primos impregnacije karvakrola je ispitano u fiziološkom rastvoru. Dodatno, Higuči i Korsmejer-Pepas modeli otpuštanja su dobro fitovali krivu otpuštanja karvakrola. Dobijeni filmovi acetata celuloze impregnirani karvakrolom mogu biti značajni za primenu u medicini za previjanje rana uzimajući u obzir njihovu biokompatibilnost i biodegradabilnost kao i potencijalno antimikrobno dejstvo ili u industriji hrane kao aktivno pakovanje.

Ključne reči: *karvakrol, film acetata celuloze, natkritični ugljenik(IV)-oksid, natkritična impregnacija*