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A Novel Chitosan Gels: Supercritical CO₂ Drying and Impregnation with Thymol

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Supercritical carbon dioxide (scCO₂) technology was used for preparation of functional pH sensitive chitosan-based aerogels characterized with micron size pores and their impregnation with thymol as a natural bioactive substance. Hydrogels based on chitosan, itaconic and methacrylic acids were transformed to alcogels and dried in the air or with scCO₂ to obtain xerobo- and aerogels, respectively. Applying 10 min of static and 120 min of dynamic scCO₂ drying at 11 MPa and 45°C followed with the decompression at a rate of 1 MPa/min yielded an advantageous aerogel with favorable swelling kinetics and elasticity, compared to the xerogel and aerogels obtained at other decompression rates and drying times. This aerogel was successfully loaded with thymol (up to 4.6 wt.%) using supercritical scCO₂ at 10 MPa and 35°C. In vitro studies of swelling in PBS at 37°C indicated a great potential of the obtained stimuli-responsive chitosan gels for topical administration of thymol known for antimicrobial, antioxidant and anti-inflammatory activities. POLYM. ENG. SCI., 58:2192–2199, 2018. © 2018 Society of Plastics Engineers

INTRODUCTION

Chitosan (Ch) represents a vast resource produced at low-cost by deacetylation of chitin (poly-N-acetylglucosamine) - one of the most abundant natural polysaccharide isolated from exoskeletons of arthropods and mollusks, cell walls of fungi and yeasts, and the spines of diatoms [1]. It is composed of randomly distributed β-(1,4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [2, 3]. Preparations of chitosan of various molecular weights, degrees of deacetylation and molecular derivatization have attracted great attention for their potentially beneficial biological properties.

In addition, chitosan is mucocadhesive polycationic polymer which forms intra- and intermolecular hydrogen bonds due to the presence of hydroxyl and amino groups [4]. Therefore, it could be applied as drug and/or gene delivery device, sorbent for water treatment (e.g. removal of heavy metals and textile dyes), functional foods, scaffold for tissue engineering, etc. [5, 6]. The most promising application of chitin and chitosan hydrogels, fibers, membranes, sponges and management of wounds or burn due to its hemostatic, antimicrobial and adhesive properties as well as good permeability to oxygen [7, 8]. Up to date chitosan is approved by the US Food and Drug Administration (FDA) as pharmaceutical excipient for use in wound dressings, dietary additives in Japan, Italy and Finland and hemostatic dressing [9, 10]. It has no or low toxicity [11] and is highly biodegradable and biocompatible [10].

To improve adsorption capacity and sensitivity of the chitosan gels, it is usual to add a hydrophilic monomer and/or polymer [12]. Methacrylic acid (MAA) and itaconic acid (IA) are monomers widely used for the preparation of pH-sensitive gels designed as drug delivery devices because of their biocompatibility and easy reactivity with other functional monomers and polymers [13–17]. In addition, itaconic acid is obtained by fermentation from renewable resources such as carbohydrate materials containing sucrose and glucose (molasses and hydrolyzed starch) [18]. If these acids are incorporated into the chitosan-based gels, even in very small amounts, hydrophilicity of the gel increases, while the swelling behavior of the gels is significantly changed at appropriate pH values due to ionization of COOH groups.

Usually, polymeric gels are prepared by polymerization and cross-linking of proper monomers and/or polymers after which wet gels are obtained. However, a major challenge in the gel preparation is drying and elimination of liquid solvent from the gel without collapsing of the already existing porous structure and thereby avoiding the subsequent shrinkage and cracking of the dried sample. Drying of the gels could be performed by using a suitable drying techniques such as (1) ambient air drying, which often does not preserve the gel structure leading to xerogels [19, 20]; (2) freeze drying technique consists in lowering the temperature of the gel below the crystallization temperature of the solvent, whereby the solvent is removed as a vapor by reducing the pressure and the end product of this process is called a cryogel [20, 21]; (3) drying with supercritical fluids, usually supercritical carbon dioxide (scCO₂). By using scCO₂ drying, the presence of any intermediate vapor–liquid transition and surface tensions in the gel pores is avoided, preventing the gel structure from the pore collapse phenomenon during solvent elimination. Based on the contact time between the gel and the supercritical fluid, supercritical drying can be performed with a continuous flow of scCO₂ throughout the process (dynamic supercritical drying) or in a batch mode (static supercritical drying) [20, 22].

ScCO₂ is a suitable solvent for a wide range of natural bioactive substances. By varying operating pressure and temperature it is possible to tune the solvent power and transport properties of scCO₂ [23]. Besides, it is known for high diffusion rates in organic matter, near zero surface tension and easy recovery from the final product [24–27]. Therefore, scCO₂ is a suitable
solvent for impregnation processes [28], e.g. for the incorporation of active substances into polymeric material especially aimed for pharmaceutical, biomedical, cosmetic and food applications [28–30]. Supercritical solvent impregnation (SSI) implies dissolution of bioactive compounds in a supercritical fluid and contact of the supercritical solution with a polymeric material to be impregnated [28]. The solute can be entrapped by a simple deposition into the polymer matrix after depressurization of the scCO$_2$ or via chemical interactions between the solute and the matrix that would favor the preferential partitioning of the solute in the polymer phase [31].

Emergence and spreading of pan resistant bacterial strains (strains resistant to all existing antibiotics) due to a wide application and misuse of antibiotics, led to an increased interest in plant extracts which may have strong antibacterial activity at the same time with no contribution to further bacterial resistance [32, 33]. Among natural compounds derived from plants, thymol has received a special attention because of its strong activity against many gram-positive and gram-negative bacteria, as well as against fungi [34]. Thymol is a lipophilic monoterpene and a major constituent of oregano and thyme essential oils [35, 36]. It has been recognized as safe to use by FDA, and has the GRAS (Generally Recognized as Safe) status as a food ingredient.

It was already shown that Ch/IA/MAA hydrogels could be applicable as a sorbent for wastewater treatment, e.g. removal of heavy metal ions and textile dyes [37, 38]. Bearing in mind that these hydrogels are stimuli-sensitive, capable to absorb or release active substances and environmentally friendly, it is obvious that they might be very good candidates to be used as gels. Moreover, if they are impregnated with a proper active substance, such as thymol, they could be used as material with antimicrobial properties, as well.

This study was aimed to use scCO$_2$ for preparation of chitosan, methacrylic and itaconic acid aerogels and their further impregnation with thymol. The gels were prepared by free radical copolymerization of methacrylic and itaconic acid in the presence of chitosan [39] and were converted to the alcogels. The effect of drying method and scCO$_2$ drying conditions on the gels’ morphology, crystallinity, mechanical properties, swelling behavior and impregnation with thymol was analysed to testify a potential use of these gels in topical applications.

**MATERIALS AND METHODS**

**Materials**

Chitosan (Fluka, middle viscous, 200–400 mPa-s), itaconic acid (Fluka) and methacrylic acid (Sigma A.G.) were used for hydrogel synthesis. In order to remove inhibitor, methacrylic acid was distilled under vacuum before use. The cross-linking agent N,N’-methylenebisacrylamide (MBA, Acros), redox couple potassium persulphate (KPS, Merck, p.a.) and potassium pyrosulphate (KPyS, Merck p.a.), were applied without further purification. Thymol, 2-isopropyl-5-methylphenol, (purity >99%) was purchased from Sigma–AldrichChemie GmbH (Germany). Commercial CO$_2$ (purity 99%) was supplied by Messer–Tehnogas (Serbia).

**Preparation of Gels**

Chitosan (3 w/v%) was dissolved in an aqueous solution of itaconic acid (5 wt. %). Addition of MAA followed to obtain Ch/IA/MAA gel (G) with a weight ratio Ch/IA/MAA of 1:1.56:10. The redox pair KPS (K$_2$S$_2$O$_8$) (0.2 wt. %) and KPyS (K$_2$S$_2$O$_7$) (0.2 wt. %) was used as initiator. MBA was used as the crosslinking agent (0.2 wt. % with respect to the total weight of the reaction mixture) [39]. Polymerization was carried out at 50°C for 3 h after which Ch/IA/MAA gel, formed in a mold, was cut into discs (d = 10 ± 0.02 mm) and left in distilled water over night in order to remove all unreacted species. By weighing the unreacted materials, it was found that the conversion was practically complete (~99%). Disc shaped hydrogels were immersed in ethanol for 24 h to complete water removal. Ethanol was removed from the alcogels by air-drying or extracted with scCO$_2$ to obtain xero- (XGs) and aerogels (scGs), respectively. XGs were obtained by air-drying of the alcogels to constant weight at ambient temperature and atmospheric pressure. ScGs were obtained by drying of the alcogels with scCO$_2$ in the Autoclave Engineers SCE Screening System described in detail elsewhere [34]. The alcogel sample was placed on the stainless steel support in the extraction vessel and the system was heated. The system was pressurized by pumping CO$_2$ into the vessel until the desired pressure was achieved. The system was kept at 11 MPa and 45°C for 10 min without scCO$_2$ flow. The drying conditions (pressure and temperature) were selected on the basis of the solubility of ethanol in scCO$_2$, while the static time of 10 min was selected on the basis of preliminary visual tests in the high pressure view cell showing that the greatest volume change of the gel samples occurred within this time. Subsequently, dynamic scCO$_2$ drying followed at the same pressure and temperature conditions to replace the scCO$_2$ enriched with ethanol. The flow rate of scCO$_2$ in the dynamic mode was 0.55 L/h. Applied dynamic times (t) and decomposition rates (dP/dt) are given in Table 1.

Monitoring behavior of the Ch/IA/MAA alcogel exposed to scCO$_2$ was performed in the high pressure view cell (Eurotechnica GmbH, Germany) described in detail elsewhere [34] at 11 MPa and 45°C. Drying of the Ch/IA/MAA disc in scCO$_2$ caused volume changes, e.g. contraction. By increasing the exposure time, disc’s diameter and thickness decreases. The volume changes of the sample, as well as the thickness, were monitored by recording images using the IC Capture 2.1. The changes were quantified by image processing program ImageJ. Degree of contraction (DC) was calculated by the following Equation:

$$DC(\%) = \frac{V_0 - V_t}{V_0} \cdot 100$$

Where $V_0$ is the volume of the disc at the ambient conditions at the beginning of the experiment (t = 0), $V_t$ is the volume of the contracted disc at t > 0 and given pressure and temperature.

**TABLE 1. Dynamic times and decomposition rates for supercritical drying (S, M and F denote slow, middle and fast rate of decompression, respectively).**

<table>
<thead>
<tr>
<th>Sample</th>
<th>t  (min)</th>
<th>dP/dt (MPa/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>scG30M</td>
<td>30</td>
<td>1.0</td>
</tr>
<tr>
<td>scG45M</td>
<td>45</td>
<td>1.0</td>
</tr>
<tr>
<td>scG60M</td>
<td>60</td>
<td>1.0</td>
</tr>
<tr>
<td>scG120S</td>
<td>120</td>
<td>6.3</td>
</tr>
<tr>
<td>scG120M</td>
<td>120</td>
<td>6.3</td>
</tr>
<tr>
<td>scG120F</td>
<td>120</td>
<td>6.3</td>
</tr>
<tr>
<td>scG180M</td>
<td>180</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Characterization Methods

Chemical Analysis. ATR FT-IR Spectroscopy. Fourier transform infrared (FT-IR) spectra of the gels were recorded by Nicolet™ iS™10 FT-IR spectrophotometer with ATR cell in the region of 3600 and 700 cm⁻¹ with the resolution of 4 cm⁻¹.

X-ray Diffraction (XRD). XRD patterns of the gels were obtained using a Siemens model D500 X-ray diffractometer. The working voltage and current were 35 kV and 20 mA, respectively. Cu Kα radiation with a wavelength of 0.154 nm was used. The scanning rate was 1.2° min⁻¹ in the 2θ range from 5° to 55°.

Analysis of Gels Morphology. Field Emission Scanning Electron Microscopy (FE-SEM). Sample morphology was characterized by Field-emission scanning electron microscopy (FE-SEM, Tescan Mira 3XMU). The samples for SEM observation were prepared by coating a thin layer of Au/Pd alloy onto previously fractured gels.

Swelling Behavior. Swelling Studies. The gels discs were immersed in an excess of PBS (phosphate buffered saline, pH 7.4) at 37°C [40]. The progress of the swelling process was monitored gravimetrically. The degree of swelling (q) was calculated from the following Equation [39]:

\[ q = \frac{w_t}{w_0} \]

Where \( w_0 \) and \( w_t \) are the initial weight of the dry sample and its weight after swelling for the time \( t \), respectively.

Mechanical Tests. Mechanical Properties. Mechanical properties of gels, swollen to equilibrium at 37°C, were recorded on Hybrid Rheometer HR 2, TA Instruments, with parallel plates geometry (25 mm in diameter). The complex shear moduli were measured as a function of frequency (ω) from 0.1 to 100 rad s⁻¹, while the applied strain was 0.1%.

Supercritical Solvent Impregnation of Gels with Thymol

Thymol was loaded into prepared XGs and ScGs using batch scCO₂ impregnation in the high pressure view cell (Eurotechnica GmbH, Germany) described in detail elsewhere [34]. All the SSI experiments were performed at 10 MPa and 35°C with the impregnation time from 2 h to 6 h. These conditions were selected on the basis of previously analyzed thymol solubility in scCO₂ [33]. Thymol was placed in a glass container on the bottom of the cell, and the gel sample was placed in a porous basket above the thymol. Applied thymol to gel (XG or scG) mass ratio was 1:1. After the SSI, the system was slowly depressurized at a rate of 0.3 MPa/min to avoid collapse of the gel pores. Impregnation yield (I) of the sample was calculated according to the following Equation [34]:

\[ I(\%) = \frac{m_T}{m_T + m_{gel}} \cdot 100 \]

Where the \( m_T \) is the mass of the impregnated thymol, determined gravimetrically as the mass difference between the impregnated gel and the gel at the beginning of the process, \( m_{gel} \) is the mass of gel at the beginning of the process.

RESULTS AND DISCUSSION

Supercritical Drying

Results of monitoring behavior of the Ch/IA/MAA alcogel exposed to scCO₂ are presented in Fig. 1. The fastest rate of the disc contraction (volume contraction from initial 189.96 mm³ to 45.52 mm³) occurred within the first minute of the experiment during the system pressurization, whereby the degree of contraction was 76%. After the first 10 minutes of the disc exposure to CO₂ degree of contraction reached the value of 80.9%. Further exposure to scCO₂ led to slower contraction rate with the degree of contraction of 82.7% after 120 min. On the basis of these results static time of 10 minutes was selected for the drying experiments. After the decompression, increase of the disc volume to 94.53 mm³ was recorded, whereby the final degree of contraction was 50.2%.

ScGs polymer networks were obtained by the combined supercritical drying comprising static drying (10 min) and dynamic drying, whereby the dynamic drying time (t) was varied. All the scGs and XG samples, obtained after drying, were disc shaped. Representative SEM images of the XG and scGs samples are presented in Fig. 2. It was found that xerogel structure was non-porous, while the aerogel structure was found to be porous. Drying in the air leads to the formation of liquid–vapor menisci in the pores of the xerogel. Upon solvent removal, the surface tension on the liquid/solid interface causes collapse of the pores leading to the non-porous structure of the xerogel (Fig. 2a). The porous structure of the aerogels was influenced by the drying time. Comparing Figs. 2b and 2c, it is evident that porous structure is more pronounced for gel obtained after 120 min of the dynamic drying (scG120M) than after 30 min of the dynamic drying (scG30M). In the second case, due to the short time of drying, a certain amount of ethanol was remained in the scG30M gel causing less porous structure comparing to scG120M due to its posterior drying in the air. In Figs. 2e and 2f similar non-porous appearance of the XG and scG30M surfaces due to the collapse of pores can be observed. Therefore, the presence of any intermediate vapor-liquid transition and surface tensions in the gel pores are excluded during complete solvent elimination using supercritical drying with longer dynamic times preventing the collapse of the pores and preserving the gel structure (Figs. 2c and 2d). Furthermore, according to the Figs. 2c and 2d decompression rates applied
did not significantly affect the pore size and density. Based on the presented results, the gel scG120M was selected for further investigation.

Volume and color changes due to transformation of the hydrogel to alcogel and further to XG and scG120M are shown in Fig. 3. The volume of the alcogel is larger than of the hydrogel which is contrary to the previously published results where the solvent led to the gel contraction [41]. It is assumed that during the solvent exchange, additional hydrogen bonds occurred between functional groups of the gel and ethanol resulting in the break of the intramolecular hydrogen bonds inside the gel. Those hydrogen bonds between itaconic acid and chitosan act as additional physical crosslinking in the gel and hence, their breakage leads to lower degree of crosslinking and therefore larger alcogel volume.

Moreover, according to the Fig.3, gel scG120M is white and non-transparent, while the xerogel is fully transparent. Bearing in mind that low porosity allows high transmission and minimizes light scattering at pores through the sample, this could be explained by different degree of light scattering through the samples. Gel scG120M possesses more porous structure than xerogel and therefore the light scattering in pores through the

FIG. 2. SEM micrographs of the cross-section of the dried samples: a) XG; b) scG30M; c) scG120M; d) scG120F; and surface of the e) XG; f) scG30M.
gel is significant giving white sample. In contrast to this, light scattering in xerogel is less pronounced owing to the remarkably lower pore density [42].

Fig. 4A presents the effect of drying time on the visual appearance of the gels. The gels, scG30M and scG45M, are partially transparent in the middle of the discs due to the short drying time which was not enough to completely remove the ethanol. The other gels, dried for a longer time, are non-transparent. According to the Fig. 4B, the decompression rate did not affect the gel appearance.

**FT-IR Analysis**

FT-IR spectra of the xerogel and gel scG120M were presented in Fig. 5. The characteristic peaks in the spectrum of the xerogels at 877 and 1085 cm\(^{-1}\) (asymmetric stretching of the C–O–C bridge) correspond to saccharide structure [43]; peaks at 1487 and 1447 cm\(^{-1}\) are assigned to the CH\(_2\) symmetric deformation mode, while peak at 1045 cm\(^{-1}\) corresponds to the C–O stretching vibration [44].

The band around 3408 cm\(^{-1}\) corresponds to the stretching vibration of O–H and/or N–H [45], while peaks at 2974 and 2928 cm\(^{-1}\) are assigned to the stretching vibration of C–H. The characteristic peak of the carboxylic groups can be observed at 1699 cm\(^{-1}\). A peak at 1550 cm\(^{-1}\) corresponds to the ionic interaction between chitosan and the acids [38].

A comparison of the xerogel and gel spectra implied that significant changes in peaks positions and intensities could not be observed. Drying in scCO\(_2\) did not affect the interactions in the gel and gel structure.

**Effect of the Drying Method on the Gel Properties**

**Swelling Behavior.** The pH environment of chronic wounds has been recorded within the range of 7.15–8.9 [40, 46–48]. Importantly, as the wound progresses towards healing, the pH moves to neutral and then becomes acidic [40, 47, 49].

The swelling behavior of the gels was monitored in PBS (pH of 7.4) at 37°C. Functional groups of chitosan, methacrylic and itaconic acid influenced by pH value of surrounding media are presented in Table 2.

Chitosan is a weak base with a pK\(_a\) of 6.5, below which –NH\(_2\) groups are ionized, i.e. they are in –NH\(_3^+\) form. Further, itaconic and methacrylic acids contain carboxylic groups that are ionized at pH above 3.85 and 5.44 (pK\(_{a1}\) and pK\(_{a2}\), respectively) for IA and above 4.66 (pK\(_{a1}\)) for MAA. Therefore, the investigated hydrogels are amphiphilic, because they have both anionic and cationic groups [38]. At pH values of 7.4 all acid groups become ionized and the electrostatic repulsive forces between anions lead to an increase of swelling of the gels.

The effect of drying method, as well as of drying time, on the swelling kinetics of the gels is presented in Fig. 6a. It can be seen that the xerogel swells faster than aerogels at the beginning of the process. Non-porous structure of the xerogel, confirmed by the SEM analysis, allows swelling due to the electrostatic repulsion between COO\(^{-}\) groups on the xerogel surface. On the other hand, swelling of the porous aerogels includes sorption of the buffer into pores at the beginning, which takes time, and afterwards allows gels to swell. Bearing in mind that swelling of the gels, caused by electrostatic repulsion, is more pronounced because the most of COOH groups are ionized at pH of 7.4 and that it starts immediately, the xerogel swells faster than aerogels.

As can be seen in Fig. 6a, despite faster swelling of the xerogel at the beginning of the process, the equilibrium degree of swelling is higher for the aerogels compared to the xerogel indicating their advantageous properties. It can be also noted that by increasing the drying time in scCO\(_2\), the equilibrium degree of swelling increases due to the higher porosity of the gels and consequently larger specific surface area.

**TABLE 2.** Functional groups of chitosan, methacrylic and itaconic acid influenced by pH

<table>
<thead>
<tr>
<th>pH</th>
<th>Chitosan</th>
<th>Itaconic acid</th>
<th>Methacrylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>–NH(_2)</td>
<td>–COO(^{-})</td>
<td>–COO(^{-})</td>
</tr>
</tbody>
</table>
The effect of decompression rate on the swelling kinetics for gel dried for 2 h is presented in Fig. 6b. The equilibrium degree of swelling is dependent on the decompression rate: it increases by lowering the decompression rate. This difference is more pronounced between slow and medium rate of decompression (samples scG120S and scG120M) compared to medium and fast (scG120M and scG120F).

The observed difference in swelling behavior between the xerogel scG120M could be attributed to lower degree of crystallinity of the aerogel compared to the xerogel (Fig. 7).

The XRD pattern of chitosan reveals a semi-crystalline nature, since sharp peaks are observed at ca. 10.1° and 19.9°, indicating the average intermolecular distance of the amorphous part. Incorporation of the chitosan into gels, the maximum is shifted to lower values due to lower degree of crystallinity [38].

According to the presented XRD patterns, two maxima were observed in both samples: at 2θ = 16.5° corresponding to the chitosan and at 2θ = 34° assigned to methacrylic and itaconic acid incorporated into gels. The XRD pattern of gel scG120M shows the more pronounced maximum compared to xerogel, as a consequence of the drying in scCO₂. Due to the plasticizing effect of scCO₂, the chitosan molecules in the gels may be prone to form more regular crystals thus increasing degree of crystallinity and lowering the rate of swelling.

FIG. 6. Swelling kinetics of the gels: (a) the effect of drying method and drying time; (b) the effect of decompression rate for gel dried 2 h at 45°C and 11 MPa. [Color figure can be viewed at wileyonlinelibrary.com]

FIG. 7. XRD patterns of: (a) xerogel; (b) gel scG120M.

FIG. 8. (a) The effect of drying method and drying time on the mechanical properties of the gels swollen to equilibrium in PBS at 37°C; (b) the effect of the decompression rate on the mechanical properties of the gels swollen in PBS at 37°C. [Color figure can be viewed at wileyonlinelibrary.com]
Mechanical Properties. The shear storage moduli ($G'$) were measured as a function of frequency ($\omega$), by applying an oscillatory shear strain. Figure 8a shows the $G'$ versus $\omega$ (the applied strain was 0.1%) for samples swollen to equilibrium at 37°C in PBS (pH = 7.4). It can be seen that $G'$ slightly increases as the frequency increases. Moreover, the $G'$ is dependent on the drying method and drying time. It is expected that $G'$ of gels decreases with increase of the equilibrium degree of swelling, as it was for gels scG60M, scG120M and scG180M. In contrast, gels scG30M and scG45M showed the opposite behavior, probably due to the heterogeneous gel structure caused by non-completed drying of the gel (Fig. 4). Moreover, drying of the xerogel caused pore collapse and lower $G'$ compared to homogeneous gels (scG60M, scG120M and scG180M). According to the obtained results, it could be concluded that drying of the gels in scCO$_2$ yields more porous gels with improved elasticity which is advantageous for drug delivery applications.

The effect of the decompression rate on the mechanical properties of the gels swollen in PBS at 37°C is presented in Fig. 8b. Faster decompression rate increases $G'$ and mechanical properties of the gel, which is in accordance with the swelling behavior.

**Thymol Impregnation**

Kinetics of the xerogel and scG120M impregnation with thymol is presented in Fig. 9a. As can be seen, during the first period of impregnation, the impregnation yield of xerogel is higher compared to scG120M (Fig. 9a). At the beginning of the impregnation most of thymol is adsorbed at the outer surface of the gels. The xerogel is characterized by the rough outer surface (Fig. 2e) and absence of the pores within the gel. On the other hand, scG120M is porous (Fig. 2g). Due to the larger outer surface, the impregnation was faster in the case of xerogel in the first two hours. In the second part of the SSI process, impregnation within the polymer matrix occurred. This is visible in the rapid increase of the impregnation yield in the case of scG120M during the third hour of impregnation. The porous structure is penetrated by the supercritical fluid leading to the sudden increase in impregnation yield. After 5 h of the SSI, the thymol impregnation yield of scG120M was 4.6%, which was 20% higher than in the case of xerogel. Due to the higher specific surface area of the porous gel the larger amount of thymol was bounded to the matrix and therefore higher impregnation yield was achieved. According to the previously published study [34] obtained impregnation yield of thymol (4.6%) should be expected to provide considerable antibacterial activity of the sample.

Considering possible application of the prepared gels as antimicrobial material, impregnated gel scG120M was investigated by XRD. It is known that drug release characteristics are highly influenced by the state of the drug within the matrix, as well as by the structure of the polymer matrix. Generally, semi-crystalline polymers showed higher extent of burst release due to the drug deposited at the matrix surface and hindered release of the drug from the bulk due to limited water uptake in the semi-crystalline regions [50]. Furthermore, it was shown that drug loading in/on polymer matrix was dependent on its state. If a drug is incorporated in crystalline form, it would be mainly deposited outside the matrix and trigger burst release, while drug in amorphous state will be loaded inside the matrix and be released in a sustained manner [51–53]. Figure 12b presents the XRD patterns of the sample scG120M before and after the thymol impregnation. As can be seen, there are no visible changes in the XRD patterns before and after impregnation indicating that thymol did not crystallize inside the gel and at its surface. Previous investigations showed that drug release was faster if the active substance crystallized during impregnation, while it was prolonged in the case of amorphous active substance [54]. Therefore, a sustained release of thymol from the investigated gels, prepared in this work, is expected.

**CONCLUSIONS**

This paper is the first report on preparation of the novel Ch/IA/MAA aerogels with micron size pores and their impregnation with thymol by using scCO$_2$. By drying the alcogels in scCO$_2$ collapse of the gels’ pores was avoided, while porous structure was affected by the drying time. Hence, swelling is more pronounced for aerogels compared to xerogel and it is dependent on the decompression rate, while the proper mechanical properties were retained making these gels suitable for application in medicine and pharmacy. The obtained impregnation yield of thymol was 4.6% and according to the literature data sufficient to provide antimicrobial activity. The results indicated that scCO$_2$ could be successfully applied for preparation and
impregnation of the Ch/MAA/IA aerogels with thymol, known for strong antimicrobial and antioxidant activities.

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