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Synthesis and characterization of polyvinyl alcohol/cellulose cryogels and their testing as carriers for a bioactive component

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ABSTRACT

Novel physically cross-linked cryogels containing polyvinyl alcohol (PVA) and various amounts of microcrystalline cellulose were obtained by freezing/thawing technique. The main goal of this study was to improve the properties and the performances of the pure PVA cryogels. The morphological aspects of the cryogels were studied by scanning electron microscopy (SEM). The Fourier transform infrared spectroscopy (FT-IR) was used to reveal the presence of the interactions between the two polymers. Changes in crystallinity of the samples were confirmed by X-ray diffraction (XRD) and by FT-IR spectroscopy. The modification of the thermal behavior induced by cellulose was studied by thermogravimetry. Rheological analysis revealed higher values of storage modulus (G′) for the cryogels containing higher amounts of cellulose. The degree and rate of swelling were controlled by the presence of the natural polymer in the network. The potential application as bioactive compound carriers was tested, using vanillin as an active agent.

1. Introduction

Hydrogels are hydrophilic three-dimensional networks of polymer chains which are capable of absorbing large amounts of water or biological fluids [1]. Due to their properties, hydrogels have numerous applications, including contact lenses, membranes for biosensors, carriers for drug/aroma, in tissue engineering as scaffolds for regenerating tissues and organs, as materials for artificial skin, as cell carriers etc. [2–7]. Cast into films and dried, hydrogels are now being used as biodegradable packaging materials for food, cosmetic and pharmaceutical products [8]. Cryogels refer to hydrogels formed at subzero temperature which provide special properties for different bioengineering and biotechnological applications [9].

Polyvinyl alcohol (PVA) is a water-soluble, non-toxic, biodegradable, biocompatible synthetic polymer with excellent film forming properties [10]. Due to its hydroxyl groups present in each repeating unit, PVA exhibits a strong hydrophilic and hydrogen bonding character; thus it is able to form crosslinked hydrogels. Physical hydrogels (cryogels) can be obtained by exposing PVA aqueous solutions to repeated cycles of freezing and thawing, which results in the formation of crystallites. The main advantage of this technique is that no additional chemical crosslinker is being used. Comparing to chemically crosslinked hydrogels, cryogels show higher elasticity and have increased strength due to the crystalline regions which are capable of better distributing a given mechanical load or stress [11,12].

PVA cryogels have been studied as controlled release carriers, as barrier film for food packaging, as material for the preparation of membranes used in chemical separation, as biomaterial in tissue engineering, as wound dressing materials, food packaging etc. [13–16].

Wound dressings are used to promote the wound healing and to create appropriate conditions for this process, while protecting the wound from infections. It is widely accepted that a moist environment is desirable to enhance the wound healing so, some hydrogels are being used with success as wound dressings, because they assure an appropriate level of moisture at the interface between the dressing and the wound, have the capacity to absorb exudates, prevent the wound desiccation, are non-adhesive, have a cooling effect giving a relief feeling to the patient, and in the same time they can deliver an incorporated drug to the wound [17–19].

The use of natural polymers as components of polymeric materials is of great interest, due to the diversity of their properties, but also from economic and ecological reasons. PVA exhibiting high polarity and water solubility is a good candidate for blends with natural polymers [20]. Cryogels based on PVA/polysaccharides are suitable as wound dressing materials because these hydrogels are flexible, mechanically strong, biocompatible, can be easily replaced, can assure a moist wound environment and a barrier against microorganisms [21–23].

Cellulose is the most abundant renewable resource on Earth. Similar to PVA hydrogels, cellulose based hydrogels are also hydrophilic, biodegradable, biocompatible, non-toxic [24]. In the human and

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animal body, cellulose exhibits a relatively low protein adsorption and cell adhesion and a low immune response. In the same time, this polysaccharide is not degradable in the body and is not digestible due to the fact that human and animal cells do not synthesize cellulases, the enzymes capable of degrading cellulose [25]. All these properties made the cellulose hydrogels suitable for various applications, especially in the biomedical field [26–28].

Scientific literature presented different types of celluloses incorporated into PVA films and hydrogels, such as bacterial cellulose [29], cellulose whiskers [30], cellulose nanofibres [31,32], cellulose nanocrystals [33] and cellulose fibrous powder [34]. Various methods of crosslinking, as well as different methods of cellulose dissolution have been used in these kinds of studies. For example, Millon et al. [29] used freezing/thawing method for obtaining PVA/bacterial cellulose cryogels, while Abitbol et al. [33] used the same crosslinking method in order to obtain PVA hydrogels reinforced with cellulose nanocrystals from softwood Kraft pulp. Chang et al. [34] prepared hydrogels using fibrous cellulose dissolved in NaOH/urea aqueous solution and epichlorohydrin as a crosslinking agent.

The novelty of this study refers to the obtaining of PVA cryogels containing microcrystalline cellulose by freezing-thawing method by using a special dissolution method for cellulose in a water/NaOH mixture, at low temperatures [35]. In our study we also tested the obtained cryogels as carriers for the delivery of an antimicrobial and antioxidant agent such as vanillin (4-hydroxy-3-methoxybenzaldehyde). Vanillin, one of the most widely used flavoring agent, is produced naturally in the specialized cells from the pods of the *Vanilla* sp. and synthetically from eugenol, guaiacol or lignin [36]. It is being used as a flavoring and antioxidant agent in food industry, but also in cosmetics and pharmaceutical formulations [37,38]. It has been reported that vanillin exhibits multifunctional effects such as antimutagenic, antiangiogenetic, anti-colitis, anti-sickling, and analgesic effects [39]. The antimicrobial properties of vanillin and its activity have been previously demonstrated against some bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, *Listeria innocua*), but also against some yeast and mold strains (*Candida albicans*, *Penicillum expansum*, *Saccharomyces cerevisiae*) [40,41].

It was expected that the incorporation of microcrystalline cellulose into PVA cryogels lead to an improvement of the properties and an enhancement of the performances. These cryogels were characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), thermal degradation, rheological measurements and "*in vitro*" tested as drug carriers. Also the kinetics of swelling in phosphate buffer solution (PBS) pH 7.4 at 37 °C and of drug release was studied.

2. Experimental

2.1. Materials

PVA with an average molecular weight of 146 000–186 000 and a hydrolysis degree of 99% was purchased from Aldrich. Microcrystalline cellulose Avicell PH-101 was purchased from Fluka.

2.2. PVA/cellulose cryogels preparation

A PVA solution of 8 wt% concentration was prepared by dissolving a certain amount of PVA into double distilled water, at 90 °C under stirring for 8 hours. A clear solution was obtained.

Different amounts of microcrystalline cellulose were dispersed in 11/1 water/NaOH (w/w) mixture, stirred for 5 min and then frozen at low temperature (−30 °C). After defrosting, the obtained solutions were stirred in order to obtain clear, transparent solutions [35].

Cellulose and PVA solutions were mixed in different ratios (Table 1) and stirred for 5 min. The obtained mixtures were poured into Petri dishes and frozen at −20 °C for 12 hours, then thawed at room temperature (+25 °C) for another 12 hours. The freezing/thawing process was repeated three times.

Table 1
Cryogels composition.

Sample	Cryogels composition (wt%)	
	PVA	Cellulose
PVA	100	0
90/10	90	10
70/30	70	30
50/50	50	50

The blank PVA cryogel was prepared pouring a certain amount of PVA solution in a Petri dish and then exposed to three freezing/thawing cycles (12 hours freezing/12 hours thawing).

The PVA and the PVA/cellulose cryogels were washed with distilled water for 3 days and were dried by lyophilization for 24 hours, using a LABCONCO 117 freezing-dryer. Polymeric films were obtained after lyophilization.

Due to the fact that cellulose cryogel could not be obtained by this technique, the cellulosic powder was treated in the same conditions as described above and used as blank sample.

2.3. Investigation methods

2.3.1. Scanning electron microscopy (SEM)

Scanning electron micrographs were taken on liquid nitrogen fractured samples, with a Quanta 200 instrument. The fractured surface was coated with a gold layer. Magnification is given on the images.

2.3.2. X-ray diffraction measurements (XRD)

The measurements were carried out by means of a Bruker AXS D8 Advance X-ray diffractometer with a Cu Kα radiation source. The data were collected in the $2\theta = 2 \div 40°$ region. The crystallinity index (CrI) was calculated by the area method, dividing the area of the crystalline peak by the total area under diffractogram [42].

2.3.3. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR analysis was performed on a Vertex-70 (Bruker) apparatus, using KBr tablets containing a constant mass of 5 mg sample/500 mg KBr. The spectra were recorded in the 4500–600 cm^{-1}, with 16 scans and a resolution of 4 cm^{-1}. Three recordings were done for each system and the average values were used for data interpretation. The energy of the hydrogen bonds (E_H) was calculated using Eq. (1) [42].

$$E_H(\text{kJ}) = (1/k)[(\upsilon_0 - \upsilon)/\upsilon_0] \quad (1)$$

where k is a constant equal to 1.68×10^{-2} $kcal^{-1}$, υ_0 is the standard frequency corresponding to free —OH groups (3650 cm^{-1}), υ is the frequency of the bonded —OH groups (cm^{-1}). The enthalpy of the hydrogen-bond formation (ΔH) was evaluated using Eq. (2) [43]:

$$\Delta H(\text{J/g}) = 0.016\Delta\upsilon_{OH} + 0.63 \quad (2)$$

where $\Delta\upsilon_{OH}$ is the —OH wavenumber shift (cm^{-1}). The hydrogen-bonding distance (R, A°) was obtained using Sederholm Eq. (3) [44].

$$\Delta\upsilon\left(cm^{-1}\right) = 4.43 \times 10^3(2.84 - R) \quad (3)$$

where $\Delta\upsilon = \upsilon_0 - \upsilon$ (υ_0 is the —OH monomeric stretching frequency = 3600 cm^{-1}; υ is the —OH stretching frequency of the sample).

2.3.4. Thermogravimetry

The thermogravimetric analysis was performed on a Jupiter STA 449 F1—Netzsch instrument. Samples of 7 mg were heated up to