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Synthesis of poly(vinyl alcohol) - methyl cellulose hydrogel as possible scaffolds in tissue engineering

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Hydrogels have enormous potential in medical and pharmaceutical applications. Due to their biocompatibility, hydrogels have emerged as a promising option in drug delivery and tissue engineering. In this study, poly(vinyl alcohol) (PVA) and methyl cellulose (MC) interpenetrated network type hydrogels were prepared by simultaneous crosslinking, using epichlorohydrin (EPCl) as crosslinking agent. The structure of the new material was confirmed by IR spectroscopy. Swelling characteristics (maximum swelling degree, swelling rate constant) were determined, in correlation with reaction parameters and network density. A study of drug (Cefoperazone Sodium - CS) release and mechanical tests were also performed.

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1. Introduction

Hydrogels are the most popular materials used as scaffolds for tissue engineering due to their high water content, good biocompatibility and consistency similar to soft tissue. Natural and synthetic hydrogels retain water in a three-dimensional network of polymer chains, which facilitates diffusional exchange of nutrients and waste products with the surrounding environment. A large variety of biomedical applications have been suggested for hydrogels [1, 2], including drug delivery [3-7], contact lenses, corneal implants [8, 9], and substitutes or tissue engineering scaffolds for skin, tendons [10,11], and cartilage [2, 12,13]. The three major parts of tissue engineering are scaffold, cells and the growth factors. The scaffold used for tissue engineering determines the growth and development of the cells in a 3D medium [14-16]. Many types of scaffolds hydrogels were studied up to date: these are based on copolymers, interpenetrating polymer networks (IPN$_s$) or interpolymer complexes. However, considerable improvements of the factors that determine mechanical strength, biocompatibility, cell adhesion and biodegradability are needed before these materials are used for biomedical applications.

Poly(vinyl alcohol) generated by the hydrolysis of poly(vinyl acetate) have been studied as a long-term or permanent scaffold for artificial cartilage, pancreas, bone and aortic heart valve [14, 17]. PVA has a good biocompatibility and suitable mechanical strength. The Young's modulus and ultimate tensile strength of PVA hydrogels are increased with growing of polymer concentration and highly elastic hydrogels can be formed by altering the number of crosslinkable groups per chain [18]. However, highly hydrophilic PVA exhibits poorer cell adhesion (the poor adsorption of adhesion protein is responsible for cell adhesion) [19-21].

Commercial MC is a heterogeneous polymer consisting of highly substituted hydrophobic zones and less substituted hydrophilic zones. Gelation is therefore an intermediate non-equilibrium metastable state in which a three-dimensional network structure is formed due to secondary valence forces [22-25]. MC undergoes thermoreversible sol–gel transformation, when an extremely dilute, semi-dilute or moderately concentrated solution of MC is heated [26]. MC is a biocompatible material with a good adhesion to the cells, hydrosoluble and relatively inexpensive [27-31].

The aim of our study is to synthesize hydrogels based on poly(vinyl alcohol) (PVA) and methyl cellulose (MC) for their possible use in obtaining scaffold for the controlled release of biologically active principles (including growth factors and cells). Despite their adhesive characteristics, methyl cellulose hydrogels doesn't have an adequate mechanical strength to be used as scaffold for tissue engineering. In order to ameliorate this PVA was introduced into MC hydrogels. The presence of hydroxilic groups in both polymers assures the crosslinking with ephychlorohydrin (EPCl) in alkaline medium. A new interpenetrated network type hydrogels based on poly(vinyl alcohol) (PVA) and methyl cellulose (MC) were obtained. The combined advantages of this new material as scaffolding used in tissue engineering (dural substitute), include good biocompatibility and suitable mechanical strength of PVA and the highly hydrophilic properties of MC together with their good adhesion to the cells.

2. Experimental methods

2.1. Materials

Poly(vinyl alcohol) (PVA) (Mw= 16,000 Da) was purchased from Acros. Methylcellulose (MC), acetone and sodium hydroxide were purchased from Sigma Chemical Co. Epichlorohydrin, as crosslinking agent was purchased from SC Victoria SA, Fagaras, Romania. The phosphate buffer solution (PBS) were prepared from the potassium dihydrogen phosphate (Fluka), sodium phosphate dibasic anhydrous (Fluka), according to procedure published in literature. The Cefoperazone Sodium (CS), an antibiotic (purchased from Antibiotice S.A., Iasi).

2.2 Synthesis of PVA-MC hydrogels

PVA-MC IPN$_s$ hydrogels were prepared by chemical crosslinking with epichlorohydrin (EPCl). The experiments were performed according to an experimental program which includes parameters that influences reaction: the MC/PVA ratio (0.29 – 5.51 mole/mole), the polymer mixture/EPCl (r_{PE}) ratio, the time and temperature of the reaction – Table 1.

Table 1. Codification for parameters.

Analyzed parameters	Coding	Domain of variation
Percent of PVA from the polymers mixture (%PVA)	X_1	5-50 %
Rapport polymer mixture/EPCl (r_{PE})	X_2	1÷5 g/g
Time of crosslinking (t) – hours	X_3	4 ÷ 16 hours
Temperature of crosslinking (T) - (ºC)	X_4	40 ÷ 80°C

To a stirred aqueous solution of MC and PVA (2 ml deionised water; 0.5 g polymer mixture) were added 0.28 ml NaOH (10N) and the proper amount of EPCl according to the experimental program. The resulting homogeneous mixture was poured into glass moulds (50 mm × 50 mm × 0.5mm) and kept at different temperatures (40-80 °C) for a period of time (4–16 hours). The formed hydrogel film was washed repeatedly in deionised water until the solution pH was equally with the water pH. An extraction in ethanol (98%) was performed in order to remove the water and unreacted EPCl. The material was dried at 35 °C, for 20 hours in vacuum atmosphere. It was obtained a film in a dry state.

2.3. Analytical instrumentation

A SCINTIMAR SERIES-Digilab IR spectrophotometer, with a wave number between 400-4000 cm^{-1}, 4 cm^{-1} resolution and 48 scans was used to record the Fourier transform Infrared (FTIR) spectra of samples using KBr for sample preparation.

2.4. Swelling assay

The swelling properties were determined gravimetrically. The PVA-MC hydrogels were placed in deionized water and in phosphate buffer solution (PBS) of different pH at room temperature (25°C) until the weight of the hydrated PVA-MC hydrogels did not further increase. The weight of PVA-MC hydrogels was recorded at various times. The weight of dried PVA-MC membranes, m_0, was also recorded. The mass equilibrium degree of swelling, Q_m (%) and the rate of swelling, k (min^{-1}) were calculated using:

$$Q_m = \frac{m - m_0}{m_0} 100\ (\%) \qquad (2)$$

$$kt = -\ln \frac{Q_{max} - Q_m}{Q_{max}} \qquad (3)$$

where: Q_m is the swelling degree (%), m_0 is the initial sample weight (g), m is the hydrogel's weight at swelling equilibrium (g), t = time (min), Q_{max} = maximum swelling degree (%);

The influence of reaction parameters (the MC/PVA ratio, the r_{PE} ratio, the time and temperature) on crosslinking degree was studied indirectly by the hydrogel swelling behavior.

2.5. In vitro drug release

The controlled release experiments were performed using 0,5 g hydrogels that have been placed into 50 ml Cefoperazone Sodium (CS) solution (concentration, c=0.2 g/100 ml). After 24 hours, a hydrogel loaded with 100mg CS/g hydrogel CS was obtained. The *in vitro* release profile of antibiotic was obtained spectrophotometrically using a Cary 50 spectrophotometer (Varian). Calibration was performed using a series of PBS containing known amounts of CS (concentrations range 0.001 – 0.02%, w/w). A drug loaded hydrogel was placed into phosphate buffer solution (100 ml, pH 7.2, 37°C) and the time-profile of CS release was determined by absorbance measurements. At the preset time, 1 ml solution was sampled out and was added back to the release medium after each measurement. Triplicate samples were performed for each formulation. Blank experiments, using polymer hydrogels only, confirmed that the hydrogels did not contribute to the length wave of absorption. The absorption values were converted in concentration values, according to the CS calibration graph.

2.6. Mechanical testing

Uniaxial tensile tests were carried out on swelled samples by an Instron 5565 under tension at a strain rate of 100 mm/s using a 50N loading cell, at 25 °C and 60% relative humidity.

Stress–strain diagrams were obtained and used to calculate Young's modulus and the ultimate tensile strength of each material. Three hydrogel samples of each formulation were tested.

3. Results

3.1. IR spectra

The IR spectra of pure PVA, pure MC and PVA-MC semi-IPN$_s$ show:

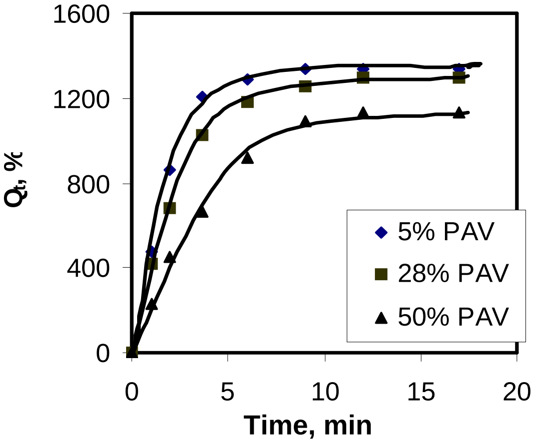

Fig. 1. Swelling degree depending on time for hydrogels at different C_{PAV} (r_{PE} =2, t = 14 h, T = 75 °C).

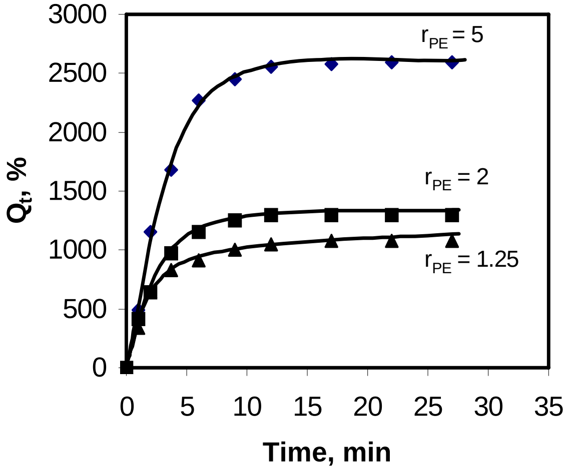

Fig. 2. Swelling degree depending on time for hydrogels at different r_{PE} (C_{PAV} = 28%, t =14 h, T = 75 °C).

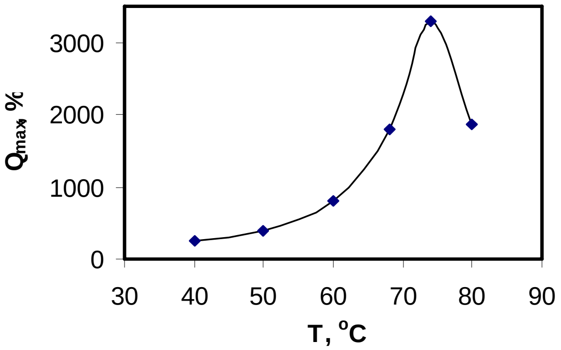

Fig. 3. Swelling degree depending on crosslinking temperature for hydrogels with (C_{PAV} = 12%, r_{PE} =3.5, t =14 h).

MC: -OH: 3386.0 cm^{-1}; -CH$_2$: 2923.3cm^{-1}; -C-H: 1377.9 cm^{-1}; -C=O: 17333.3cm^{-1}; -C-O-C: 1255.9 cm^{-1};
PVA: -OH 3200– 3600 cm^{-1}; -C–O 1050–1150 cm^{-1}
PVA-MC: -OH 3010 – 3171 cm^{-1}; which indicating strong intermolecular hydrogen bonding.

3.2. Swelling behavior of PVA-MC hydrogels

The swelling degree plays a bivalent role in semi-IPN$_s$ materials. The increase of swelling degree has a positive influence on quantity of CS which can be loaded into hydrogels, but negative on their mechanical strength.

The influence of PVA content (C_{PVA}) and the polymer mixture/EPCl ratio (r_{PE}) on hydrogels swelling properties was studied at reaction time t = 14 hours and at temperature T = 75°C, values where the swelling degree was maximum. Hydrogels swelling kinetics follows a first order kinetics (equation 3); K values are represented in Table 2.

Table 2. Maximum swelling degree and kinetic constant depending on hydrogels composition.

Sample	C_{PVA} , %	r_{PE}, g/g	Q_{max} , %	k, min^{-1}
P1	5	2	1340	0.5774
P2	50	1.5	1156	0.4912
P3	12	3.5	3289	0.2483
P4	28	1.25	1080	0.2560
P5	28	2	1296	0.3663
P6	28	5	2594	0.3113
P7	50	3.5	1094	0.7942
P8	34	3.5	2423	0.6176
P9	50	2	1128	0.2888

We observed that swelling degree decreases with increase of PVA content – Fig. 1 and increases with increase of r_{PE} (small content of crosslinking agent) – Fig 2. Maximum swelling degree increases with temperature on the range 40-75 °C, decreasing after that – Fig. 3. The increase of temperature up to 75 °C leads to increases of crosslinking density, which determines the decrease of swelling degree.

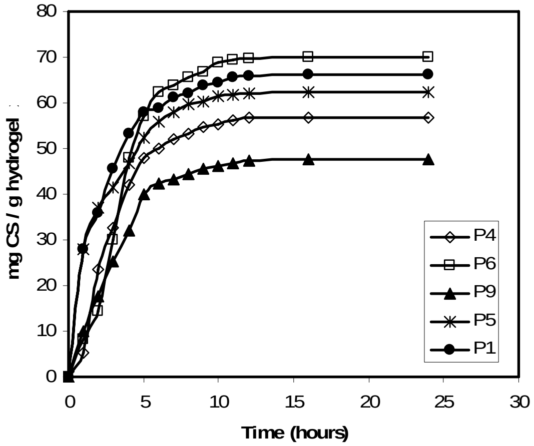

Fig. 4. The quantity of CS released from hydrogels (0.5g hydrogel swelled in 30 ml aqueos drug solution, T=25 ^oC).

3.3. Kinetics of drug release

Referring to the drug release study (Fig. 4), it can be observed that the appropriate systems for such applications contain high percent of MC and a medium ratio of crosslinking agent. It was found that the introduction of the semi-IPN structure lowered the percentage of CS release from the hydrogel.

3.4. Tensile strength of the hydrogels films

Fig. 5 demonstrates that increase of MC content in the interpenetrated network material up to 95% decreased the strength. At a 50% concentration of MC there was however marginal increase in the membrane's strength.

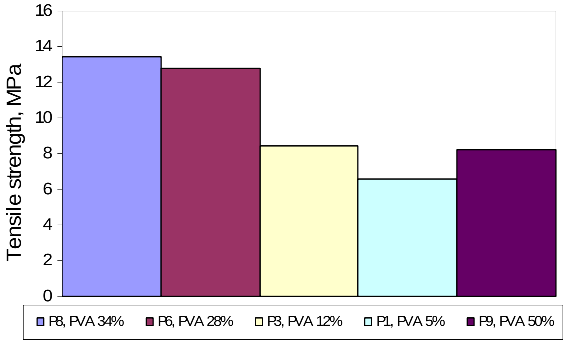

Fig. 5. The tensile strength of PVA- MC hydrogels.

4. Discussion

The IR spectroscopy of Fourier transform shows that interpenetrated network materials were obtained. From the swelling behaviour of the hydrogels, we observed that swelling degree decreases with increase of PVA content (Fig. 1.) and increases with the increase of r_{PE} (Fig. 2.). Hydrogels swelling kinetics follow a first order kinetics (equation 3). Maximum swelling degree increases with temperature on the range 40-75 °C, decreasing after that (Fig. 3.).

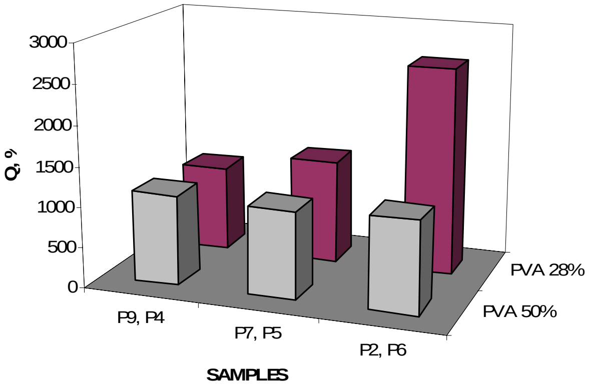

Fig. 6. Swelling degree for hydrogels at different C_{PAV} (r_{EP} =2, t = 14 h, T = 75 °C).

Bound water significantly reduces the glass transition temperature in an almost linear manner and has a significant influence on the uniaxial tensile behavior, while the effect of free water is negligible.

The high water content of the hydrogels provides important characteristics for scaffolds tissue engineering, such as tissue-like elasticity, good permeability toward nutrients and the easy growth of cells. PVA-MC hydrogels are expected to have more capacity for water retention than pure PVA hydrogels. Fig. 6 shows that the maximum swelling degree of the PVA-MC hydrogels increases with the amount of MC. The higher water content and faster swelling can be attributed to the presence of MC.

The way hydrogels swell in aqueous solutions influences both the drug entrapping and its release. The release from these hydrogels is not only dependent on the extent of crosslinking, but also on the amount of the loaded drug. Such hydrogel materials may have more advantages as a potentially interesting platform for the design of scaffolds.

Introduction of the semi-IPN structure improved the mechanical properties of the hydrogel. MC has much poorer mechanical properties than PVA. The addition of MC into PVA hydrogel should weaken the hydrogel and lower its elastic modulus below that of pure PVA hydrogel. A higher MC content in the hydrogel corresponds to a lower modulus. However, the sample with the lowest elastic modulus can still be used to form the scaffold.

5. Conclusions

Chemically crosslinked PVA-MC hydrogels of interpenetrated network type were obtained. The amount of MC from IPNs increases with the growth of initial concentration of these in the polymer mixture and decreases with the increase of r_{PE}. The raise of temperature and the time determines the increase of MC ratio into material as well. It was found that the swelling property of the PVA-MC hydrogels could be controlled by tailoring the reaction parameters: the MC/PVA ratio, the polymer mixture/EPCl (r_{PE}) ratio, the time and temperature of the reaction. The way hydrogels swell in aqueous solutions influence both the drug entrapment and its release.

Mechanical tests show that PVA-MC hydrogels have potential utility in tissue engineering. Further studies will be done for testing the “in vitro” biocompatibility of this material and of the MC’s biological effect on the cells growth in the hydrogels.

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