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Topological characterization of a bacterial cellulose–acrylic acid polymeric matrix

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ABSTRACT

This paper focuses on the micro- and nano-topological organization of a hydrogel, constituted by a mixture of bacterial cellulose and acrylic acid, and intended for biomedical applications. The presence of acrylic acid promotes the formation of two interpenetrated continuous phases: the primary "pores phase" (PP) containing only water and the secondary "polymeric network phase" (PNP) constituted by the polymeric network swollen by the water. Low field Nuclear Magnetic Resonance (LF NMR), rheology, Scanning Electron Microscopy (SEM) and release tests were used to determine the characteristics of the two phases. In particular, we found that this system is a strong hydrogel constituted by 81% (v/v) of PP phase the remaining part being occupied by the PNP phase. Pores diameters span in the range 10–100 μm, the majority of them (85%) falling in the range 30–90 μm. The high PP phase tortuosity indicates that big pores are not directly connected to each other, but their connection is realized by a series of interconnected small pores that rend the drug path tortuous. The PNP is characterized by a polymer volume fraction around 0.73 while mesh size is around 3 nm.

The theoretical interpretation of the experimental data coming from the techniques panel adopted, yielded to the micro- and nano-organization of our hydrogel.

1. Introduction

The use of synthetic fibers for the fabrication of polymer composites is decreasing due to various factors including the expensive cost of materials, the non-biodegradable nature of the synthetic fibers and the environment pollution connected to their synthesis. To minimize the above problems, scientists and engineers are focusing their attention on the use of natural fibers. In this regard, cellulose is very attractive as it is the most abundant and renewable biopolymer in nature. It is the main constituent of plant cell walls, fungi and some algae; additionally, several bacteria have the ability to produce extra-cellular cellulose as their metabolites.

Cellulose is typically obtained from higher plants where it resides as skeletal substance. In particular, cellulose is found in the plant xylem tissues where it is present as cellulosic microfibrils embedded in a matrix of amorphous non-cellulosic polysaccharides (hemicelluloses) and lignin (Dammström et al., 2005). To obtain pure cellulose, solvent extraction is an important method to remove the extractable fraction from cellulosic fibers. However, this procedure may cause fiber damaging due to changes in the chemical composition of the fibers and the rearrangement or transformation of the crystalline structure which determines the switch from cellulose type I to cellulose type II (Ouajai and Shanks, 2005). This cellulose structural change affects the thermal degradation characteristic of the fiber. Due to this problem, cellulose obtained from bacteria is more convenient. Bacterial cellulose is produced by *Acetobacter xylinum* which utilizes a wide range of carbon and nitrogen in liquid medium and transforms them into cellulose in

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the form of floating pellicles (Oikawa et al., 1995). The cellulose produced by this organism is of exceptionally high purity and resembles the same features of plants and algae cellulose in terms of crystalline unit structure and average microfibrillar width (Ross et al., 1991). Therefore, pure cellulose can be easily obtained from this source without having the difficulties associated with the extraction from plants. The cellulose produced by bacteria aggregates to form sub-fibrils which have the width of approximately 1.5 nm thus representing the thinnest natural occurring fibers. Bacterial cellulose sub-fibrils are, then, crystallized into bundles and subsequently into ribbons (Jonas and Farah, 1998). The molecular configuration of cellulose fibrils, which are highly insoluble and inelastic, makes the tensile strength of cellulose comparable to that of steel thus providing mechanical support to the tissues where it resides (Yamanaka et al., 1989). Since cellulose fibrils are natural polymers, the biocompatibility and bio-degradability are key features which are at the base of the vast applications of this amazing material. Cellulose macro- and nano-fibers are used as reinforcing materials which could enhance mechanical, thermal, and biodegradation properties of the composites. Cellulose, commonly fabricated into matrices, became particularly popular biomaterials for controlled-release dosage forms and extended release dosage forms (Alderman, 1984; Longer and Robinson, 1990). Formulations are relatively flexible, and a well-designed system usually gives reproducible release profiles. The large surface area and the negative charge of cellulose nano-fibers are suggested to govern the binding of large amounts of drugs to the surface of this material also ensuring optimal loading and dosing. In addition, the abundance of hydroxyl groups on the outer part of the crystalline nano-cellulose fibers enables surface modification of the material with a range of chemical groups. The surface modification is used to modulate the loading and release of drugs, such as non-ionized and hydrophobic drugs (Lonnberg et al., 2008). This versatility may potentially allow in the future the development of delivery strategies devoted to different administration routes (Grassi et al., 2011) and clinical applications such as drug delivery to the vessel (Zanetti et al., 2008) and to the liver (Baiz et al., 2014; Scaggiante et al., 2014).

Hydrogels, that can expand and have great water absorption capacity and elasticity, demonstrate high capability to cater the release program needed in controlled release therapy (Losi et al., 2006). However, in the hydrated state, hydrogels exhibit a remarkable poor mechanical strength. Previous studies have demonstrated that the incorporation of nano-cellulose has great impact in improving mechanical properties of hydrogels. For example Millon and Wan (2006) reported that nano-cellulose fibers of an average diameter of 50 nm are used in combination with polyvinyl alcohol (PVA) to form biocompatible nano-composites which result in increased mechanical properties similar to that of cardiovascular tissues, such as aorta and heart valve leaflets. In addition, Cai and Kim (2010) set up three different methods to prepare nano-cellulose/poly(ethylene glycol) (PEG) composite that can be used for soft tissue replacement devices. Since the favorable properties of hydrogels lie mainly in their hydrophilicity, the characterization of their water-sorption capabilities is very important (Lin and Metters, 2006). In addition, also the hydrogel nano- and micro-structure is of paramount importance for biomedical applications. Thus, in this paper we report the results of a rheological, low field Nuclear Magnetic Resonance (LF NMR), Scanning Electron Microscopy (SEM) and release study aimed at understanding the macro-, micro- and nano-scopic characteristics of a hydrogel constituted by a mixture of bacterial cellulose and acrylic acid. Indeed, the joint use of these approaches can give important insight about the topology of our hydrogel that is made up by two interpenetrated continuous phases: the primary "pores phase" (PP) containing only water and the secondary "polymeric network phase" (PNP) constituted by the polymeric network swollen by the water. Interestingly, this complex topology can provide an important chance/challenge for the designing of controlled release systems. Indeed, for example, the presence of pores (or channels) can greatly improve the swelling process as solvent uptake can take place by convection (through pores) instead of by diffusion (through polymeric network meshes) as it takes place in homogeneous gels (Chern et al., 2004). Consequently, pores presence reflects into a more rapid gel swelling that, in turn, implies faster drug release kinetics. In addition, it is well known that the average diffusion coefficient of a solute in a mixed structure (PNP plus PP), the so called effective diffusion coefficient (Peppas, 1984), depends on both solute mobility in the PP and in the PNP (Narasimhan, 2000). Thus, depending on the dimension of the solute molecule, a careful tuning of the characteristics of PP (pores size distribution, pores connectivity, strictly related to structure tortuosity) and of PNP can yield to very different release kinetics. Consequently, this great potentiality requires the definition of proper strategies aimed at the characterization of the gel topological properties. This aspect becomes even more important when it is needed to evaluate the effect of different preparation parameters on the gel final structural characteristics.

2. Materials and methods

2.1. Materials

Acrylic acid (ACC) and theophylline (TPH) were purchased from Sigma Aldrich Chemie GmbH Germany, while bacterial cellulose (extracted from Nata de coco – Dayawan Trading (M) Sdn. Bhd.) was purified according to the British Pharmacopoeia. All other reagents were purchased from R&M Chemical (Essex, UK) and were of analytical grade. Distilled water was always used.

2.2. Gel preparation

The purified cellulose was dried and ground to obtain a powder composed of particles with sizes between 20 μm and 200 μm. The powder was dispersed in distilled water at a concentration of 1% (w/v). The acrylic acid was, then, added to the dispersion in a ratio 20:80 (w/w) compared to cellulose. The mixture was poured into a plastic container (size 12 × 12 × 1 cm) and then irradiated in air with an electron beam of 35 kGy intensity (5 kGy per pass, EPS 3000, Japan).

2.3. ESEM

The freshly prepared gel was dried and then left to swell at 37 °C in distilled water for 48 h to reach the equilibrium swelling degree S_d (absorbed water weight/dry matrix weight; =4.3 ± 0.4). A portion of the swollen gel (0.5 × 0.5 × 0.5 cm) was collected and analyzed (ESEM) for no longer than 30 s at 5 °C to prevent drying due to electron bombardment. The images of the surface and the cross sections were obtained by Quanta200F SEM (FEI, USA).

2.4. Image processing and analysis

The ESEM images were processed using the software ImageJ (Abramoff et al., 2004). The procedure used here comprised: (i) a calibration step required to correlate the image dimensions in pixel to physical dimensions; (ii) highlight the pores; and (iii) analysis of the pore areas. From the pores' area and assuming the pores as spherical, was then possible to derive pores diameter and volume.