SYNTHESIS AND CHARACTERIZATION OF ALGINATE-GELATIN HYDROGELS WITH POTENTIAL USE IN BIOMEDICAL FIELD

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This work concerns with obtaining and characterization of new hydrogels based on sodium alginate and gelatin in the form of cross-linked polymer networks, aimed at medical applications, for example controlled release of bioactive agents (pharmaceutical industry) and bioinks (regenerative medicine). Our synthesis strategy was based on the use of mild, ecological reaction conditions in the absence of crosslinking agents and organic oxidants. Only industrially available sodium alginate and gelatin from leather wastes, produced at micro-pilot level at INCDTP-ICPI, were used, without the presence of any additional crosslinking agents, to test their ability to form strong 3D gels. Tunable physical-chemical and mechanical properties of the hydrogels have were obtained by varying the ratio sodium alginate: gelatin. Newly synthesized hydrogels were characterized by both analytical methods, such as ATR-FTIR, TG-DTG and SEM, and standard tests for mechanical resistance.

Keywords: alginate, gelatin, 3D hydrogels

INTRODUCTION

Hydrogels are polymeric systems extensively studied in recent years, due to their wide range of applications in the most diverse fields. Hydrogels are composed of polymeric networks, reticulated, elastic, with hydrophilic properties, capable of retaining impressive amounts of water or other liquids. The structural and compositional similarity with the extracellular matrix is the essential characteristic of hydrogels that ensures their applicability in the bio-medical and pharmaceutical field. In order to be able to cover a wide range of applications, hydrogels must be easy to prepare (e.g. through ecological synthesis methods, with a minimum of operations) and present biocompatibility and biodegradability. In this context, biopolymer materials such as polysaccharides and proteins, and their derivatives, have become a key element in the synthesis of hydrogels, being preferred in applications requiring biodegradability and biocompatibility. Moreover, these hydrophilic biopolymers allow fine-tuning of the physical and chemical properties of hydrogels, enabling thus to obtain materials with optimal properties such as cell adhesion, favorable molecular response, structural integrity, biodegradability, biocompatibility and controlled transport of bioactive molecules.

Due to the current demographic evolution characterized by a continuously growing population in Asia and Africa and an advanced degree of aging in Europe and North America, specialized literature abounds in the design, creation and use of more and more economically efficient and ecological materials aimed at supporting peoples' quality of life. In fact, the impact of demography is reflected by the financial

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parameters: the global regenerative medicine market was estimated at USD 8.5 billion in 2020 and is forecast to grow to USD 17.9 billion in 2025, calculated at a compound annual growth rate (CAGR) of 15.9% (www.biospace.com, 2022).

In recent years, both polysaccharides and proteins have been used for the preparation of hydrogels in order to combine the advantages offered by the two classes of polymers. Polysaccharides, the richest source of natural carbon-based polymers, have the property of being renewable. Natural alginates, the sodium salts of alginic acid, represent one of the most studied polysaccharides in the field of tissue engineering and controlled release of active substances (Zhang *et al*., 2021; Abka-khajouei *et al*., 2022). They are found in large quantities in nature, either as structural components of brown seaweeds or capsular polysaccharides of some soil bacteria. On the other part, collagen is the most abundant protein found in mammalian bodies (about a quarter of all protein in the human body is collagen). Collagen, in the form of colloidal, gelatinous and hydrolyzed solutions, covers a huge range of applications, from high tech (e.g. nanobots), to tissue engineering and regenerative medicine, from biomedical applications to the cosmetic and nutraceutical industries. As a biomedical material, collagen meets essential criteria such as controlled degradation rate, good mechanical strength, excellent biocompatibility and low antigenicity (Sorushanova, 2019; Wang, 2021; Banerjee, 2020).

Starting from these premises, the main objective of this study was to obtain new hydrogels based on sodium alginate and gelatin in the form of cross-linked polymer networks and characterize them. This is a first step in understanding the interaction mechanism and design a tunable synthesis of hydrogels depending on the desired application, for example the controlled release of bioactive agents or bioinks production. The working method used of mild, ecological reaction conditions in the absence of cross-linking agents and organic oxidants. Newly synthesized hydrogels were characterized by both analytical methods, such as ATR-FTIR, TG-DTG and SEM, and standard tests for mechanical resistance.

MATERIALS AND METHODS

Materials and reagents: sodium alginate (Sigma, CAS-No: 9005-38-3), bovine gelatin type B (Sigma, CAS-No: 9000-70-8), gelatin GDCI-1 (obtained at the Leather Research Department, INCDTP-ICPI, Bucharest), glacial acetic acid (SC SILAL TRADING SRL, CAS-No: 64-19-7) and deionized water.

The steps of the adopted synthesis method are presented in Figure 1 (Wang, 2021).

HYDROGELS CHARACTERIZATION

FTIR-ATR Analysis

Spectral behaviour of alginate-gelatin hydrogels is illustrated in Figure 2. A specific absorption band occurred at 1724 cm^{-1} for samples treated with 2% acetic acid solution. This indicates the presence of carbonyl groups suggesting several hypotheses: the formation of carbonyl groups (dialdehyde groups), the formation of ester function groups by reaction with acetic acid or even the formation of carboxyl groups. Given the mild conditions (low temperature, 2% acetic acid, short time), we can retain two of these hypotheses: formation of dialdehyde groups or acetylation of hydroxyl groups.

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The FTIR-ATR spectra of P3-2 and P3-3 hydrogels show the absorption band at 1724 cm^{-1}, too, confirming the oxidation/acetylation mechanism promoted by immersion of alginate-gelatin hydrogel in 2% acetic acid solution.

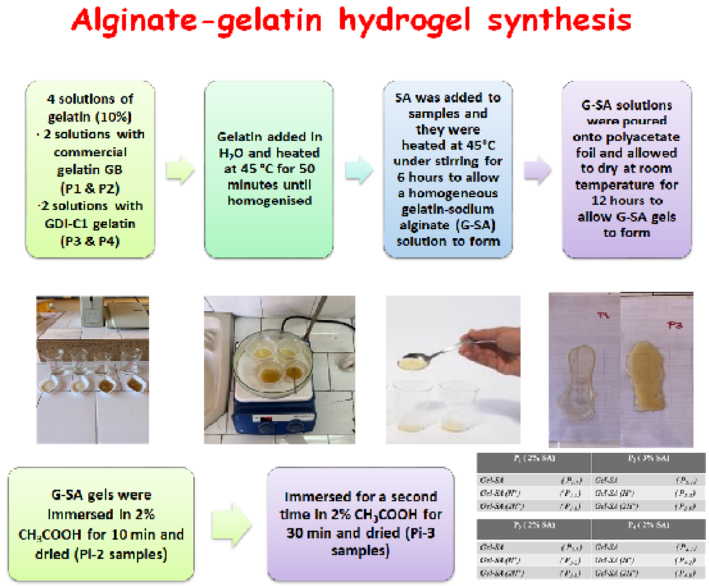

Figure 1. Schematic presentation of the alginate-gelatin hydrogels synthesis together with the list of hydrogels depending on alginate:gelatin ratio and acid immersion treatments

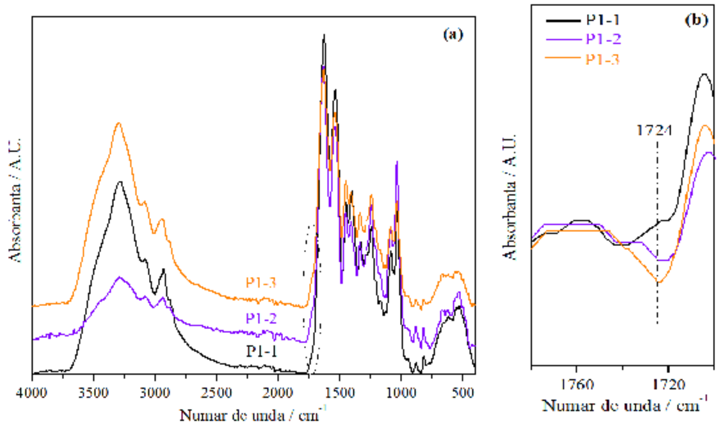

Figure 2. a) FTIR-ATR spectra of samples P1-1, P1-2 and P1-3 (b) the corresponding secondary derivatives show the occurrence of the specific band at 1724 cm^{-1} attributed to the valence vibration of the C = O carbonyl group in the carboxyl/ester function

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TG-DTG Analysis

Several endothermic effects were observed in the hydrogels thermograms in the considered temperature range (Figure 3): while all hydrogels shown thermal effects in the intervals: (200 – 250) °C; (250 – 270) °C and (320 – 350) °C, P3-1 hydrogel (not subjected to immersion in acid solution) showed an exothermic phenomenon at temperatures above 500 °C. According to literature, alginate exhibits three thermal events (mass loss) in the intervals (25 – 200) °C, (200 – 500) °C and (500 – 800) °C (Valido *et al*., 2022). The first event is endothermic and corresponds to alginate dehydration (residual moisture of the polysaccharide and water bound more internally to the alginate structure), while the second and third events are exothermic, and are associated with the degradation of anhydrous sodium alginate and formation of sodium carbonate (Na_2CO_3), followed by its decomposition to sodium oxide (NaO_2) and CO_2 release. The hydrogels' thermograms indicate a different behavior in the interval (200 – 500) °C. This behaviour may be attributed to a different release of water with consequent thermal stabilisation of the structure proved by the fact that sodium carbonate decomposition was not observed in the range up to 600°C.

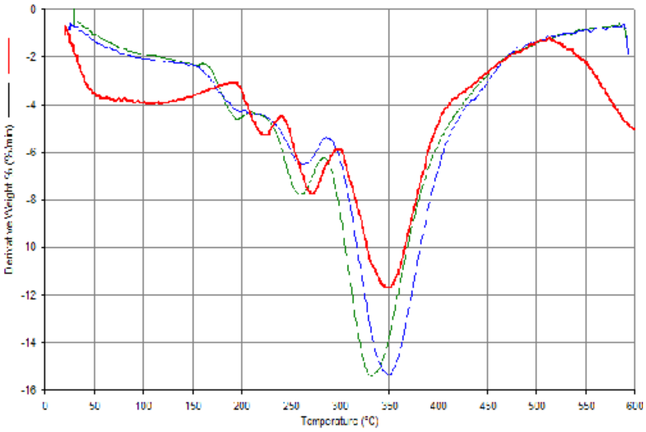

Figure 3. Thermograms obtained for alginate; gelatin hydrogels: P3-1 (red), P3-2 (green) and P3-3 (blue)

SEM Observations

SEM micrographs demonstrate that the microstructure of alginate-gelatin hydrogels, although compact, contains some areas of heterogeneity and air bubbles. Apparently, alginate would act as a filler in the gelatin matrix. However, as the degree of cross-linking increases, through successive immersion in 2% acetic acid solution, the degree of heterogeneity decreases, as well as the density of air bubbles, resulting in smoother and more regular surfaces. The microstructure of the hydrogels suggests that there may be an interaction between the two components of the polymer matrix, with alginate chains interpenetrating more homogeneously with those of gelatin.

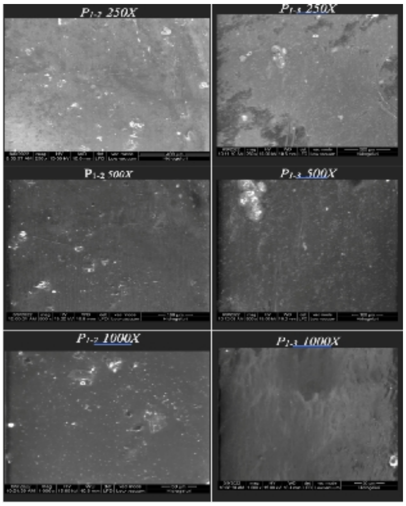

Figure 4. SEM images of samples P1-2 and P1-3 taken at progressive magnifications: 250X, 500X and 1000X

Mechanical Tests

The results of mechanical tests (ISO 7500-1) indicated that by reducing the concentration of gelatin, the mechanical parameters are significantly reduced, i.e., breaking strength and tensile strength are reduced by about 50% and elasticity is drastically reduced by more than 90%.

CONCLUSIONS

Hydrogels based on sodium alginate and gelatin were synthesized using mild, environmentally friendly reaction conditions in the absence of crosslinking agents and organic oxidants. The interpenetration of the polypeptide and polysaccharide networks was obtained by dispersing the biopolymers in deionized water under strong mechanical agitation and moderate heat treatment for 6 hours, followed by air-drying at room temperature. To promote the formation of electrostatic bonds and hydrogen bridges between the specific functional groups of the two biopolymers, a mild acid treatment with dilute acetic acid solution (2% v/v) was applied (i.e, by repeated immersion of the gels in the solution for 10-20 minutes). The effect of acid treatment was assessed by a set of analyzes: molecular analysis of hydrogels by FTIR-ATR spectroscopy, thermal stability analysis by TG-DTG, mechanical analysis and morphological analysis by SEM microscopy. These analyzes led to the conclusion of the formation of a composite system with physical-mechanical properties and thermal stability different from those of the initial biopolymers, properties prone to tailoring by immersion in acidic solution.

The main conclusions regarding the properties of the gelatin-alginate composite suggest a possible interaction between the two components of the polymer matrix, with sodium alginate chains interpenetrating with those of gelatin. At the macroscopic level, the thermal analysis suggests a thermal stabilization highlighted the behavior of the composite in the stages of release of free water and bound water, as well as in the stages

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of thermal degradation of the polysaccharide and protein chains. The temperature range at which water release occurs expands because of the interpenetration of the two polymers, and the decomposition of sodium carbonate no longer occurs in the range up to 600°C for the acidic treated hydrogels. The mechanical analysis demonstrated a reduction in the mechanical resistance to traction and breaking, as well as the reduction in the elasticity of the hydrogel because of the reduction in the concentration of sodium alginate. These first conclusions justify us to optimize the synthesis method by eventually considering the use of ultrasound green technology.

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