CHITOSAN - PVA COMPOSITE HYDROGELS: SYNTHESIS, CHARACTERIZATION AND THEIR ANTIBACTERIAL PROPRIETIES

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INTRODUCTION

Hydrogels are usually polymeric networks, extensively developed as appropriate materials for biomedical and pharmaceutical applications. Natural and synthetic polymers offer many possibilities for the design and development of hydrogels that can be used in biomedical applications. Natural polymers, as polysaccharides, are frequently used in the manufacture of hydrogels, due to the lack of cytotoxicity, biocompatible and biodegradable properties. Chitosan (CS) is a polysaccharide positively charged extensively used for biomedical applications, especially in pharmaceutical formulations due to its attractive proprieties such as biodegradability, biocompatibility, cellular binding capability, antimicrobial, antifungal, antioxidant, and wound healing property. Poly(vinyl alcohol) (PVA) is one of the most used synthetic polymer for the development of hydrogels with biomedical/pharmaceutical applications. Many of its properties such as biocompatibility, the lack of toxicity and non-carcinogenicity are similar with those of natural polymers, except biodegradability.

The original results regarding a novel method for the synthesis of stable CS/PVA-based hydrogels by a double cross-linking procedure: physically by a freeze–thawing method and covalently using an epoxy cross-linking agent are presented in this work. In addition, the natural antimicrobial activity of CS was enhanced by *in situ* generation of silver nanoparticles (AgNPs) under UV irradiation. These new hydrogels could be an candidates for medical applications as wound dressings.

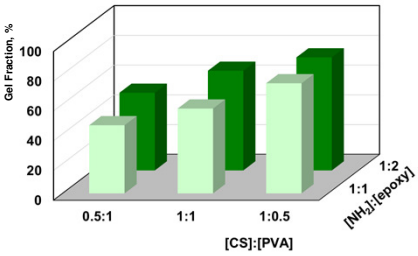
RESULTS AND DISCUSSIONS

Synthesis of CS/PVA hydrogel

Sample	[CS]:[PVA]	$[NH_2]$:[epoxy]	t_T / t_F
$CS_{0.5}/PVA_1B_1$	0.5:1	1:1	24/24
		1:2	
$CS_{0.5}/PVA_1B_1$	1:1	1:1	
		1:2	
$CS_{0.5}/PVA_1B_1$	1:0.5	1:1	
		1:2	

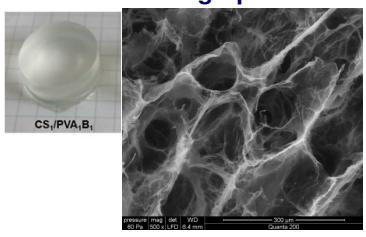
[CS] – the chitosan concentration; [PVA] – the PVA concentration; [CS]:[PVA] – the ratio between partners in hydrogel; $[NH_2]$:[epoxy] – the ratio between the amine groups of chitosan and the epoxy groups of the cross-linking agent; t_T – the time at room temperature, before each freeze step; t_F – the time at freeze step

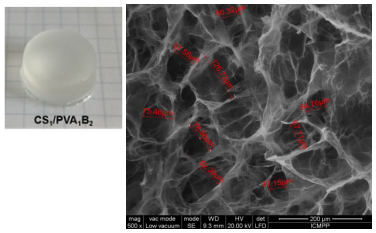
Gel fraction of hydrogels



❑ the gel fraction values of hydrogels were influenced by the synthesis conditions (GF = 38 – 76%)

Optical and Scanning Electron Micrographs

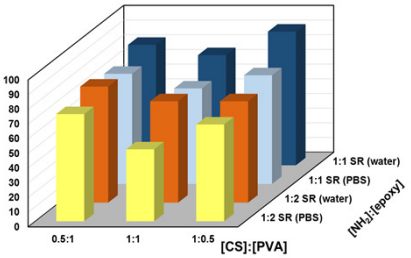


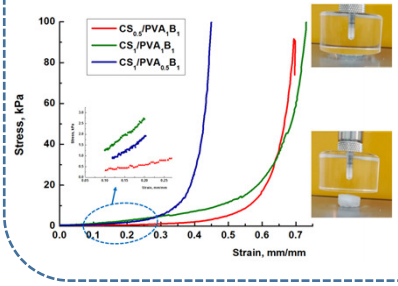


❑ optical photographs of the CS/PVA hydrogels in hydrated state after six F–T cycles. The transparency is probably caused by the longer length and higher hydrophobicity of BDDGE chains which prevents the formation of PVA densely crystalline areas.
❑ scanning electron micrographs of the hydrogels in cross section show a three-dimensional network with well-defined pores with a diameter ranging between 30 and 60 μm.

Swelling behavior

❑ hydrogels showed relatively high water swelling rates of about 60% and reached the swelling equilibrium in the first hour

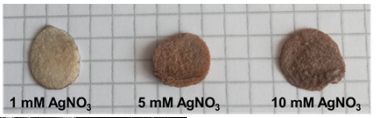




Mechanical properties

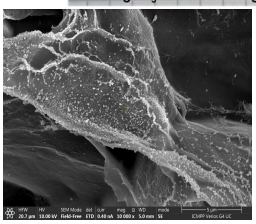
❑ the compression tests of hydrogels showed an elastic behavior with value of elastic modulus of 2.6 – 14.6 kPa

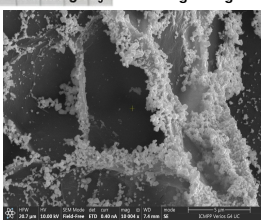
CS/PVA hydrogel loaded with silver nanoparticles



AgNPs:
- *in situ* by immersing in $AgNO_3$ solutions;
- UV lamp (365 nm; 9 W)

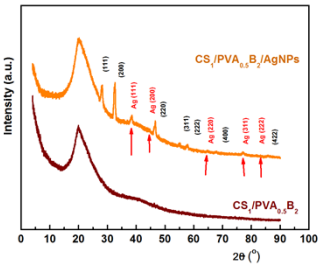
$Ag^+ \rightarrow Ag^0$





❑ the size of the AgNPs increases from 88 nm to 400 nm with the increase of $AgNO_3$ concentration from 1 mM to 10 mM ($p << 0.05$)

X-ray Diffraction



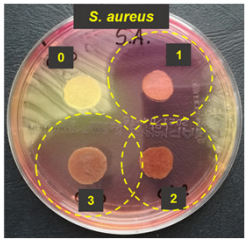
patterns of hydrogel without and with AgNPs after immersion in 10 mM $AgNO_3$

❑ the crystalline planes of face-centered cubic crystals of the metallic Ag structure in hydrogel, which confirms the successful synthesis of crystalline AgNPs (space group 225:Fm-3m, Card No. 9008459)

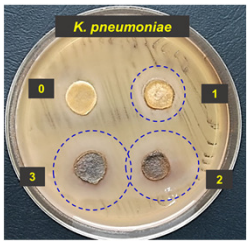
❑ together with Ag^0, of AgCl nanoparticles resulting from the reaction of Ag^+ with the counterion Cl^- from the ionized form of amino groups of chitosan, due to solubilization of CS in HCl.

Antimicrobial Activity

❑ the antibacterial activity of CS/PVA hydrogels with and without AgNPs was investigated using Gram-negative (*K. pneumoniae*) and Gram-positive (*S. aureus*) bacteria by the disk diffusion method.



the inhibitory zone diameter 42 – 45 mm



the inhibitory zone diameter 20 – 24 mm

zone 0 – the hydrogel without AgNPs; zone 1 – hydrogel with AgNPs obtained in 1 mM $AgNO_3$ solution; zone 2 – hydrogel with AgNPs obtained in 5 mM $AgNO_3$ solution; zone 3 – hydrogel with AgNPs obtained in 10 mM $AgNO_3$ solution

CONCLUSIONS

➢ New CS/PVA-based hydrogels were obtained by combining chemical and physical cross-linking methods.
➢ Highest gel fractions were obtained at six F-T cycles and a CS/PVA ratio of 1:0.5 (w/w).
➢ The mechanical tests have shown an elastic behaviour of hydrogels with low elastic modulus values and without cracking up to 70% compression. The values are ideal for future medical applications, as wound or oral dressing that must be strong, soft and flexible materials
➢ The native antimicrobial activity of CS was enhanced by the *in-situ* AgNPs generations under UV irradiation.
➢ SEM micrograph highlighted AgNPs with size of 88 nm and cubic shape
➢ XRD analysis confirmed the face-centered cubic crystal of the Ag metal structure.
➢ The loaded hydrogels with AgNPs present a high inhibitory activity against *S. aureus* (gram-positive bacteria) and *K. Pneumonia.*

ACKNOWLEDGMENT

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PN-III-P2-2.1-PED-2019-1780 within PNCDI III.

D.M. Suflet, I. Popescu, I.M. Pelin, D. L. Ichim, O.M. Daraba, M. Constantin, G. Fundueanu; Pharmaceutics. Pharmaceutical Formulations with Antimicrobial Properties, 2021 (open access), 13(9), 1461 https://doi.org/10.3390/pharmaceutics13091461