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TECHNOLOGY FOR DEVELOPING COLLAGEN COMPOSITES IN THE FORM OF SMART HYDROGELS

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Abstract:
The paper presents an experimental model for obtaining hydrogels with collagenous structure from pelt waste resulting from the liming process. The biotechnology for obtaining protein biocomposites mainly consists in the hydrolysis of pelt waste enriched and/or compounded with biodegradable polymers (PVA, corn starch, maleic copolymers, etc.) for the purpose of using them as soil conditioners and fertilizers. These hydrogels are made using smart processes in order to then be applied in agriculture, for preservation of water in the soil or for controlled release of fertilizers, pesticides but also for the development of additivated agricultural film biodegradable over time. Hydrogels that are based on biopolymers, compared with hydrogels based on synthetic polymers, have the advantage of biodegradability, biocompatibility, and a low level of toxicity. Hydrogels with collagenous structure are tested using a high-performance instrumental analysis system.
Key words: hydrogels, biopolymers, collagen, tannery, soil.

1. INTRODUCTION

A particular segment of agricultural products is the biodegradable sheet. In recent years, foreign specialists have noted that biodegradable sheets of different colours for soil covering have stimulating effects on plants. The biodegradable sheet has a very good transparency, allowing the sunlight to penetrate the seeded soil; it preserves the warmth near the cultivated plant, favouring quicker germination than in the natural environment unprotected by sudden spring temperature drops. Biodegradable sheets are used to prevent weed growth, to protect against insects, and to increase soil temperature. Green soil covering sheet increases soil temperature, reflects the green colour, and thus stimulates the plant to grow faster. Its use is recommended in cold months. The green sheet yields good results in the cultivation of peppers, cucumbers and green courgettes.
The brown soil covering sheet selects the light that falls on it, provides a constant medium temperature in the root area, moderately stimulates the transformation of light into heat. It is recommended for plants that need heat, such as corn. The blue-coloured soil covering sheet has a remarkable greenhouse effect, blocks UV rays and stimulates the transformation of light into heat. It is suitable for cultivation of cantaloupe and pumpkin assortments.

2. EXPERIMENTAL

New hydrogels based on polyvinyl alcohol and collagen were synthesized by radical copolymerization. Polyvinyl alcohol (PVA) is a hydrophilic polymer with suitable characteristics for biomedical applications and for agriculture, such as: high swelling degree, rehydration properties, good oxygen permeability; it is an elastic, biocompatible, biodegradable material with non-toxic, non-carcinogenic and bioadhesive characteristics.
Several processes for obtaining polyvinyl alcohol-based hydrogels are known: a) physical cross-linking through repeated freeze-thaw cycles; b) crosslinking by γ irradiation technique; and c) chemical crosslinking in the presence of glutaraldehyde, ethylaldehyde, boric or maleic acid, etc. (1-3).
A new process was attempted in order to obtain hydrogels in the form of sheets with collagen structure in combination with polyvinyl alcohol. The collagen matrix was obtained by "direct" hydrolysis of pelt waste in dipotassium phosphate acid medium with nutrients (N, P and K) for plant growth (4-6).

Thus, experiments were carried out using a pilot equipment to obtain collagen hydrogels in the form of sheets with additivated and coloured layers (nutrients).
The absorbent hydrogel based on polyvinyl alcohol consists of 50...90% by weight of polyvinyl alcohol (PVA) and 50...10% by weight of collagen.
The process consists in dissolving polyvinyl alcohol (7-10%) by slowly pouring it into industrial water heated to 60-80°C, stirring continuously for 0.5-1.5 hours, then adding collagen hydrolysate (10-20%) and continuing dissolution for another 1.5-2 hours. After total dissolution, 0.2-0.5% polyvinyl alcohol dissolved in alcoholic solution is added, in order to create a separating film between 2 materials of which one is porous. The resulting solution is green, and the liquid hydrogel marked APV-L is obtained.
Table 1 presents the physical-chemical analyses of the APV-L hydrogel performed in the ICPI Testing and Quality Control Department in accordance with Test Report 172/23.06.2017 (Table 1).

Table 1. Physical-chemical characterization of APV-L hydrogel

No.	Characteristics	UM	Determined values * APV-L Hydrogel	Uncertainty	Standard method
1	Dry substance	%	12.23	± 0.42	SR EN ISO 4684 : 2006
2	Ash	%	18.48*	± 0.27	SR EN ISO 4047 : 2002
3	Total nitrogen	%	2.37*	± 0.66	SR ISO 5397 : 1996
4	Protein substance	%	13.33*	± 2.66	SR ISO 5397 : 1996
5.	P and K oxides	%	0.49		
6.	pH		7.37	0.10	STAS 8619/2 1990

*****Values of ash, total nitrogen, protein substance and metal oxides are reported free of volatile matter.

From this hydrogel solution, several additivated and coloured sheets were obtained, as follows:
- by adding 0.2-0.4% cross-linking agent from a 5% glutaraldehyde solution; the mixture was left at room temperature for 24 hours and a green HAPV hydrogel sheet was obtained;
- 1-2% urea and 0.2%-0.3% glutaraldehyde crosslinking agent (or 1-1.5 ml of epichlorohydrin) are added to the APV-L solution to obtain a blue HAPV hydrogel sheet;
- 4-5% cellulose as carboxymethylcellulose (CMC) and 1-5 ml of kymene (or 1-1.5 ml of epichlorohydrin) as crosslinker are added to the APV-L solution, yielding a brown HAPV hydrogel sheet.

3.RESULTS AND DISCUSSIONS

These sheets were analyzed by SEM-EDAX electron microscopy and by Xenotest in order to study light and weather degradation.
Other sheets were also used in the experiments, embedding other polymers such as starch or polyacrylamide in the composition in order to obtain various sheets of different colours using food colouring and other cross-linking agents such as N,N'-methylene bisacrylamide (MBA 99%).
Analysis of nutrients N, P, and K from the 3 PVA/collagen coloured and additivated sheets was performed in the Fertilizer Tests and Quality Control Department (LICCI) of the Research and Development Institute for Pedology, Agrochemistry and Environmental Protection - ICPA Bucharest (Tabelul 2).

Table 2. Characterization of agro sheets

No.	Sample code	Total N (%)	P_2O_5 (%)	K_2O (%)
1	Green sheet	4.35	0.02	0.07
2	Blue sheet	1.09	2.62	3.57
3	Brown sheet	1.59	2.82	4.08

Table 2 shows that the blue and brown sheets are additivated with nutrients in a higher percentage than the green sheet, as expected.
The hydrogels obtained in the form of sheets were studied in terms of structure and composition using modern instrumental methods: electronic SEM-EDAX Microscopy (performed at ICMPP-Petru Poni, Iasi)
The existence of many pores in the collagen and polyvinyl alcohol sheets is visible. If the amount of crosslinking agent is increased, the pore size will be smaller and the porosity and swelling will also be smaller due to increased crosslinking.

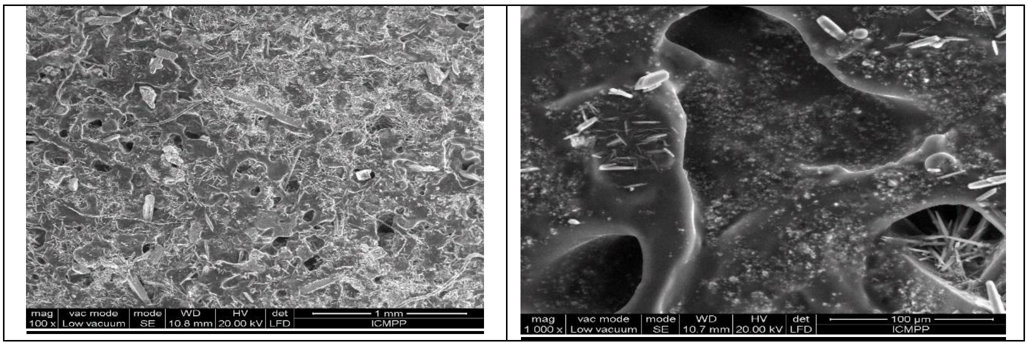
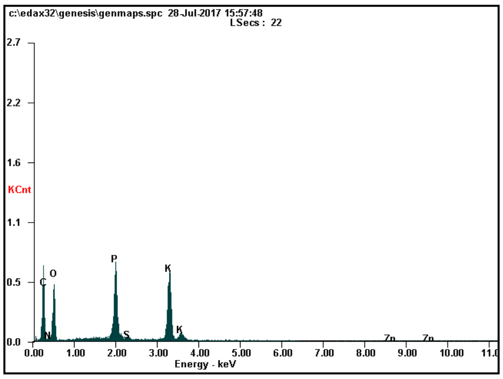


Fig.1. Characterization of green PVA /collagen sheets by electron microscopy

Figure 2 shows the concentrations of micro and macro nutrients contained in the additivated and coloured hydrogel sheets determined by SEM EDAX.



Element	*Wt%*	*At%*
CK	45.12	57.86
NK	04.36	04.80
OK	29.14	28.06
PK	08.49	04.22
SK	00.37	00.18
KK	12.23	04.82
ZnK	00.28	00.07
Matrix	Correction	ZAF

Fig 2. Elemental composition of green PVA/collagen sheet

FT/IR-ATR sheet characterization was performed in the ICPI Testing and Quality Control Department using FT/IR-ATR Attenuated Total Reflectance Spectrophotometer, Perkin Elmer, USA (Fig.3).

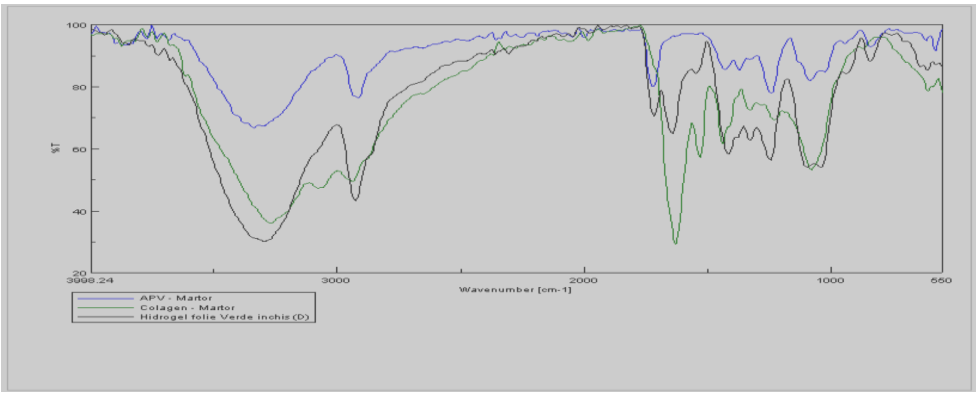


Fig.3 IR spectra of green agro sheet compared to PVA and collagen hydrolysate

The PVA and collagen spectra were studied in comparison with the final IR spectrum of the green hydrogel sheet in this case. It is noticed that by copolymerization the PVA chain is marked by band changes in the 3450-3200 cm^{-1} region which tends to widen and change its transmittance; at the same time, the 1660 cm^{-1} band increases in intensity, because there is also an –OH component in its structure, and the band at 1550 cm^{-1} decreases in intensity. The difficulty in interpreting spectral diagrams results both due to the structural complexity of collagen compositions and the use of trace elements that have chemical affinity for the same functional groups in the respective macrostructure.

The band at 1658 cm^{-1} can be attributed to the valence vibration of the participating CO group or in the vicinity of a ring, the vibration frequency being dependent on the ring size, and the higher frequencies reached in the tensioned rings. This band can provide analytical support in studying an optimal, stable hydrolysate-nutrients composition. Thus, the presence of collagen, polyvinyl alcohol and urea or cellulose are confirmed in the molecular structure of the hydrogel.

Xenotest Characterization. Xenotest is the pilot instrument used to determine the behaviour of materials in sunlight. Sheet analyses were performed at INCDTP using the ICS TEXICON Xenotest Dye Resistance Test Pilot Equipment.

A UV-B 313 (310nm) radiation was applied to the sheets for 10 hours, with a radiation intensity of 0.48 w/m^2, T = 70°C, relative condensation 26%, for 14 days while subjected to climatic conditions (artificial rain) t = 50°C in accordance with ISO 105-B02: 2013 - Method intended for determining the effect on the colour of textiles, with the following assessments :

Table 3 Xenotest colour assessment of PVA/collagen sheets

No.	Fastness to light	Assessment after 10 h at 70°C, 26% humidity	Assessment after 14 days, climatic conditions - artificial rain at 50°C
1	Simple green HAPV sheet	1-2	1-2
2	White HAPV sheet	4-5	4
3	Blue HAPV sheet	4-5	4
4	Green HAPV sheet	4	4
5	Dark brown HAPV sheet	3-4	4
6	Reddish brown HAPV sheet	3	4
7	Turquoise HAPV sheet	5	3

Artificial light testing: with Xenon lamp simulating natural daylight - D65

The colour change was evaluated using the calibration scale, with marks from 1 to 8 (8 being the most resistant colour - no change in colour - and 1 = the weakest, full colour degradation). Because of the weaker crosslinking, green and reddish brown sheets (lower crosslinker concentration) recorded the strongest colour degradation (marks 1, 2 and 3). The white and blue sheets were less degraded (marks 4 and 5).

4. CONCLUSION

This work presents the development of collagen hydrogels in the form of sheets with coloured and additivated layers (nutrients) and the study of sunlight and weather degradation thereof over time.

New polyvinyl alcohol and collagen hydrogels obtained by pelt waste hydrolysis were synthesized by radical copolymerization.

An experimental model for obtaining PVA/collagen sheets additivated with micro/macro elements and coloured was presented in detail.

The resulting sheets were studied in terms of structure and elemental composition by the SEM EDAX method. SEM-EDAX micrographs of PVA/collagen sheets with encapsulated nutrients highlighted the fibrillar structure of polyvinyl alcohol-collagen with phosphorus, potassium, magnesium, etc. nutrient crystals and the pore size.

The elemental composition of the sheets differs because the content of nutrients (N, P and K) varies by adding macro or micro elements to the solution. After examining the PVA/collagen additivated and coloured sheets, cross-linking centres are found as a result of the formation of PVA-collagen-urea or PVA-collagen-cellulose compounds.

FT/IR-ATR spectra of sheets confirmed the presence of functional groups of collagen, polyvinyl alcohol and urea or cellulose in the molecular structure of the hydrogel in the form of sheets.

Sheets tested using Xenotest in artificial light and under environmental conditions (artificial rain) have revealed, by means of colour changes, the degradation of the sheets by sunlight and weather.

This process has established a technology for developing collagen hydrogels in the form of coloured nutrient sheets that can be used as fertilizers in agriculture.

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