

New collagen composites with transdermal application

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INTRODUCTION: Collagen polymers have attracted considerable interest for various biomedical applications, including drug delivery system, control transfer therapeutic agents, substrate culture of living cells [1-4]. The physicochemical properties of such polymers are generally designed to be appropriate for their specific function..

The paper is devoted to a preliminary phase of a large research about a new type of composites based on natural polymers with collagen [2], characterized by a trophic action, which is subject of a patent in preparation. The composite is a collagen matrix with collagen polypeptides bonded with calcium and magnesium ions. This composite is going to be applied as a transdermal dressing and has an effect of fast passing of coetaneous barrier. In this way bioactive components are resorbed by sanguine capillares and reach the general sanguine circulation.

EXPERIMENTAL PART: Collagen membrane is prepared by drying type I collagen fibrillate, in specific condition in order to get micro and nanostructure porous membrane. This structure permits to water vapors to get into membrane by steaming of collagen polypeptide.

- Collagen hydrolysates are obtained by neutral (HO) and acid (HA) hydrolysis of bovine skin at

125⁰C, 2 atm. for various period of time. After hydrolysis, Ca and Mg salts are introduced, last procedure being atomized drying. The amount of calcium was approximately 10 more than magnesium being around 5 mg/g and 0,6 mg/g respectively.

- The collagen composites are realized mixing atomized powder of polypeptide with collagen fibrillate. Drying at about 28⁰C is the last final treatment and the porous membrane is the final product.

The composite materials were characterized using the following techniques:

- Gel chromatography and viscosimetric method for molecular weight determinations
- Liquid porosimetry with Coulter porometer
- Spectroscopy (UV-VIZ, IR)

RESULTS AND DISCUSSIONS: In order to select an optimal polypeptide size, regarding an easy steaming process, under occlusive dressing, a variety of collagen polypeptide were studied, taking into account average molecular weight presented in table 1.

In this table index value 2, 4, 6, 8 for neutral and acid hydrolysates respectively correspond to the hydrolysis time.

Table 1. The average molecular weight for HO and HA collagen hydrolysates

Collagen hydrolysate		Average molecular weight		Variation of molecular weight domain
		Viscosimetric method	Gel chromatography	
Neutral hydrolysates	HO ₂	80.700	72.000	> 80.000- 11.500
	HO ₄	53.350	62.000	> 70.000- 7.000
	HO ₆	20.700	20.500	> 70.000- 3.400
	HO ₈	16.250	16.600	> 69.000- 2.900
Acid hydrolysates	HA ₂	12.500	15.000	> 70.000 - ≤ 3.000
	HA ₄	11.000	11.000	~ 52.000 - ≤ 1.900
	HA ₆	9.600	8.800	~ 43.000 - ≤ 1.600
	HA ₈	7.050	7.300	~ 43.000 - ≤ 1.400

In table 1 varying hydrolysis time (2, 4, 6, and 8 hours) different polypeptides sizes were obtained, having average molecular weight between 7.000-80.000. It is easy to observe that the average molecular weight of collagen hydrolysates are almost similar from both methods.

Therefore, the polydispersion process is very large, as could be seen from table 1, taking into account the lowest value (~7000) and the highest one (~80.000). The lowest value for average molecular weight in the case of acid hydrolysates being less than 1000, in their compositions there are percentage of free aminoacids.

In table 2 physicochemical properties of collagen hydrolysates HO (non atomized) as a function of hydrolysis time. HO₂ with 180000 average molecular weight has a gel aspect and gel process

became over 5% even at temperature around 5°C; HO₄ has a gelatin aspect only at concentration higher than 20%. HO₆ and HO₈ do not present gelatin properties even at very high concentration.

Table 2. Physicochemical values of HO hydrolysate (non atomized)

Physicochemical values of HO hydrolysates	Hydrolysis time (hours)			
	2	4	6	8
Concentration, % g/100ml	22	23	24,6	24,4
pH of solution	8,2	7,8	7,4	7,4
Intrinsic viscosity [η] (dl/g) at 20°C, in 0,5 M KCl solution	0,220	0,136	0,071	0,060
The average viscosimeter molecular weight (M _v)	180.000	67.380	20.450	14.800

The atomized hydrolyses present some changes in physicochemical characteristics as could be seen in table 3. The changes are related to a small influence of thermal denaturation of gelatin macromolecules which is take in place at 45-50°C.

Thermal denaturation in the atomize process is more specific in the case of fibrillate protein than in the case of the globular protein. In fact, at a higher degree of hydrolysis the influence on the average molecular weight decreases.

Table 3. Physicochemical values HA and HO hydrolysates (atomized)

Physicochemical values	Hydrolysis time (hours) for HA hydrolysate (atomized)				Hydrolysis time (hours) for HO hydrolysate (atomized)			
	2	4	6	8	2	4	6	8
Humidity, %	3,90	3,73	3,28	3,78	5,15	5,65	4,7	5,6
Total Nitrogen, %	17,27	17,35	17,24	17,51	17,8	17,50	17,67	17,75
Nitrogen from amino group %	0,97	1,12	1,24	1,64	0,61	0,625	0,65	0,75
Total Nitrogen Ratio (RNH ₂) Nitrogen from amino group	17,80	15,40	13,90	10,67	29,3	28	27,10	23,60
Hide substance, %	97,05	97,50	96,88	98,40	100,05	98,33	99,30	99,75
Ash, %	0,70	0,97	0,72	0,68	0,37	0,53	0,66	0,15
Intrinsic viscosity [η] (dl/g) at 20°C, in 0,5 M KCl solution	0,055	0,0528	0,0480	0,0408	0,145	0,116	0,0715	0,063
The average viscosimeter molecular weight (M _v)	12.500	11.000	9.600	7.050	80.700	53.350	20.700	16.600

Two types of collagen hydrolysates (HO₂ and HA₄) were selected, a neutral and acid one, and Ca and Mg between 0,5-1 % collagen polypeptide.

UV and IR hydrolysates films spectra show that Ca and Mg are banding at carboxyl groups of polypeptide. Electrostatic bond are the indicated one to assure a better ions assimilation and metabolisation into the body.

The composite formation is a three-dimensional fibril structure with pores dimensions between 5-100 nm, for 90% and 0,1-2,5 μ for 10% structure respectively.

CONCLUSION: New type of collagen porous membrane composites with calcium and magnesium were designed and characterized. These composites have a micro and nanostructure, and transdermal applications. Specific area of such a structure is 8 m²/g, and permits water absorption and the transdermal transport of bioactive polypeptide .

REFERENCES:

1. C. A. Scotchford, M. G. Cascone, S. Downes, P. Giusti: Biomaterials 19 (1998)1-11
2. S.D.Gorham: Collagen as a biomaterial In Byron D, editor. Biomaterials, novel materials from biological sources. Basingstoke, Macmillan 1991, 57-121
3. H. Iovu, Ingrid Ionescu Bujor, Gratiela Zgirian, Ioana Demetrescu; Proceeding of CAS Conference 2002 - Vol 1, 295-298.
4. I. Mindru, Mihaela Olteanu, L.I. Dehe; V. Trandafir; Revue Roumaine De Chimie, 28, 7, 1983