

# PHYSICO-CHEMICAL CHARACTERIZATION OF GELATIN-CMC COMPOSITE EDIBLES FILMS FROM POLYION-COMPLEX HYDROGELS

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## ABSTRACT

This study is concerned to elucidate the interaction behavior of films consisted of gelatin and carboxymethylcellulose, which are polyelectrolytes and have applications in tissues engineering. These films were chemically cross-linked using glutaraldehyde.

The decrease of framework in triple helix of gelatin in the presence of polysaccharide and/or the cross-linking agent increases until a total disappearance was showed by XRD, the disappearance of macroporous gelatin structure in the presence of additives has been confirmed by SEM and AFM.

According to DSC analysis, glass transition temperature ( $T_g$ ) increases and decreases as GTA and CMC were added, respectively.

It was shown that swelling of macroporous structure in pseudophysiological mediums was more absorbent and used electrolytes exert an osmotic pressure or ionic strength inducing higher swelling. Also, it was found that diffusion mechanism is directly related to gelatin structure. On the other hand, the incorporation of CMC improves the flexibility of matrice.

**Key words:** Biocompatible polymers, electrostatic interaction, crosslinking, mechanical properties, AFM.

## 1. INTRODUCTION

Natural polymers have large applications notably in packaging materials or coating agents<sup>1</sup>. Gelatin is largely used in the pharmaceutical industry as wound dressing, absorbent film and adhesive, and particularly as excipient in controlled drug release<sup>2-4</sup>. However, it has a very hydrophilic nature and relatively poor in mechanical properties which limited their potential applications<sup>5</sup>. Several studies have been widely used to improve gelatin properties by blending gelatin with other proteins or polysaccharides such as alginate<sup>6</sup>, chitosan<sup>7</sup> and hyaluronic acid<sup>8</sup>.

These, are known for their self adhesiveness and permeability as well as their ability to form physical and chemical hydrogels, self-adhesiveness and permeability properties. Also, they are the support matrices for drug release and tissue regeneration<sup>9</sup>.

Carboxymethylcellulose is one of the endogenous polysaccharides which play an important role in the cellular growth, differentiation and as a buffer in pressure changes. Due to its enormous biological properties, it is used in the medical field<sup>10,11</sup>.

Polysaccharides/proteins mixtures were greatly thermally influenced. Above the isoelectric point of the protein thermodynamic incompatibility between the protein and polysaccharide occurs because of the repulsive electrostatic interactions and different affinities towards the solvent. Therefore, the protein and polysaccharide may co-exist in a single phase but in domains in which they mutually exclude one another or segregate into different phases<sup>12</sup>. Many studies of the interactions between proteins and polysaccharides in solution have been reported<sup>13-16</sup>, that the formation or dissociated of protein-polysaccharide complexes and their solubility depends on parameters such as the type of biopolymers involved and their concentration, the pH, ionic strength, calcium ion concentration, the temperature<sup>17</sup>.

The purpose of this work is to study the influence of sodium carboxymethyl cellulose on the physical and mechanical properties of gelatin.

The formation of integrated materials (permanent films polyionic-complex hydrogels) have been demonstrated in two stages: the first, CMC-gelatin mixture produces a new reversible polyionic biomaterial, whereas, in the latter, the addition of glutaraldehyde as cross-linking agent ensure the formation of a permanent chemical network and its mechanical resistance has been proved to be promising for the development of new renewable biomaterials.

## 2. MATERIALS AND METHODS

Pharmaceutical and food grade pig skin gelatin (Type A, 500 Bloom), powder (isoelectric point, IEP~9.0) and Carboxymethylcellulose sodium (CMC), pKa =3.5, viscosity: 300-600 cps at T=25°C were supplied from Sigma. Glutaraldehyde (GTA) aqueous solution (50wt.%) : Sodium azide (NaN<sub>3</sub>) were purchased from Aldrich and Chemopharma, respectively. These reagents were used as received.

### 2.1. Preparation of films

Biopolymer films were prepared from a mixture of gelatin and CMC at 5 wt. % total solids by casting using the following procedure. 5 grams of gelatin/CMC (80/20) were dissolved in 100 ml distilled water along with 0.02% sodium azide to prevent bacterial contamination for 30 min. Then, the mixture was placed in a water bath at 60°C under a gentle shaking for 30 min to obtain a homogeneous solution. 10 ml of this solution was poured into 9.5 cm diameter polystyrene Petri dish and air dried at ambient temperature for 3 to 4 days. The obtained film was inserted in 20 ml of aqueous solution of GTA (2%) for 24 h. The cross-linked film was washed with distilled water and dried.

The same procedure has been applied for gelatin film.

### 2.2. Fourier transformed infrared spectroscopy analysis

FT-IR spectra of dried films (1%) were obtained using a SHIMADZU 8400S spectrometer. Scanning was carried out in the range 4000-400 cm<sup>-1</sup>.

### 2.3. X-ray diffraction

X-ray diffraction patterns (XRD) were collected on a D8-ADVANCE-BRUKER-AXS diffractometer equipped with a Cu K<sub>α</sub> radiation source ( $\lambda=1.5418 \text{ \AA}$ ) operating at 40KV and 40 mA. Data were recorded in the range of  $2\theta = 5-60^\circ$  at scanning rate (0.02<sup>o</sup>/min).

### 2.4. Differential scanning calorimetry

Calorimetric measurements were performed using a DSC (DSC 200PC). Thermograms were obtained from (2-4 mg) of preheated samples up to 100°C for 30 min using sealed aluminum pans at a heating rate of 5°C/min up to 150°C, under O<sub>2</sub>/N<sub>2</sub> atmosphere (50 ml/min and 20 ml/min), respectively.

### 2.5. Tensile testing

Stress-strain curves of strip-shaped (50 × 20 mm, thickness around 0.26 mm) films equilibrated at 75% relative humidity conditions<sup>18,19</sup> were recorded using a Zwick /Roell Z. 0.1 testing machine, with a crosshead speed of 5 mm min<sup>-1</sup>. The elastic modulus  $E$  (or Young's modulus, MPa), the stress at break  $\sigma_b$  (MPa), and the strain at break  $\epsilon_b$  (%) of the strips were measured in a static mode.

### 2.6. Atomic force microscopy analysis (AFM)

Samples surface was scanned in a liquid medium using a microscope with commercial atomic force (PSIA Xe-100) equipped with a scanner and a liquid quartz cell. The topographic images (512 X 512 pixels) are acquired in semi-contact mode comparable with the tapping mode at ambient temperature using points "silicon nitride V" Si<sub>3</sub>N<sub>4</sub>. In each case, the excitation frequency of the tip is fixed around 300 KHz and the scanning rate on the surface of the sample is 0.5 HZ.

### 2.7. Scanning electron microscopy analysis (SEM)

Film samples were attached by double sided electrically conductive carbon type and their surface morphologies were observed by scanning electron microscopy (SEM) using a JEOL JSM-840 instrument at 5.01V accelerating voltage.

### 2.8. Swelling Mediums

**Solution1:** pseudo extracellular fluid (PECF) which is simulated wound fluid, was prepared by dissolving (0.68g, 11.6 mmol) NaCl, 0.22g KCl, 2.5g of NaHCO<sub>3</sub>, and 0.35g of NaH<sub>2</sub>PO<sub>4</sub> in 100 ml of distilled water to obtain a



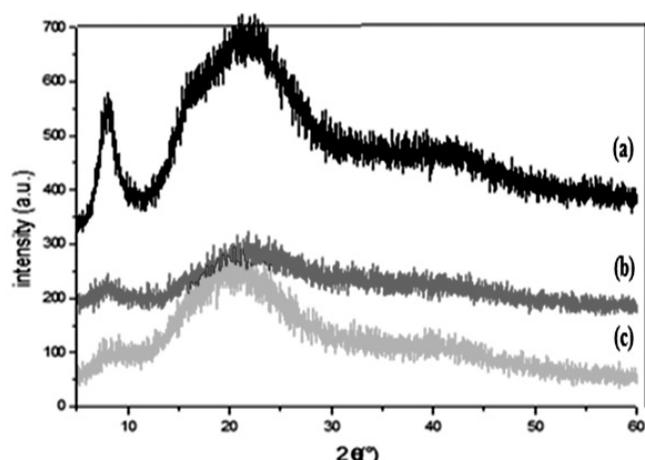


Fig. 3. X-ray diffractograms of G (a), G/CMC (b) G/CMC/GTA (c).

Table 1. X-ray data for gelatin and modified gelatin films.

Formulation	2θ (°)	d <sub>101</sub> (Å)	The triple helix content (%)
Gelatin (G)	8.06	10.97	9.13
	22.12	4.01	-
	43.54	2.07	-
(G/CMC)	7.86	11.24	2.14
	22.7	3.96	-
	41.12	2.18	-
(G/CMC/GTA)	8.73	10.11	0.66
	20.05	4.42	-
	42.51	2.12	-

### 3.3. DSC characterization

The differential scanning calorimetry (DSC) thermograms present in fig. 4 and table 2. All traces display the classical thermal behavior of gelatin samples, with the first drop of the thermogram related to the glass transition, followed by an endothermic peak associated with the helix-to-coil transition.

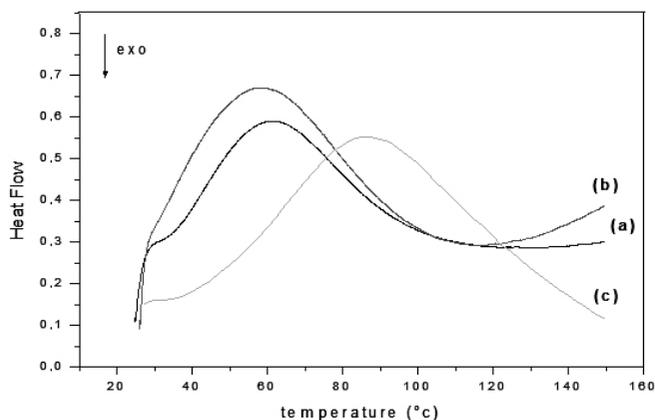


Fig. 4 DSC Thermograms of Gelatin (a), (G/CMC) (b) and (G/CMC/GTA) (c).

Each thermogram exhibits a single endothermic peak ( $T_m$ ) at 61,78 , 58,24 and 84,79 for gelatin, G/CMC, and G/CMC/GTA, respectively. More specifically, the addition of CMC to gelatin decreases  $T_g$  and  $T_m$ , and the increases of enthalpy ( $\Delta H$ ) is due to the evolution of residual water. So, the interaction between two macromolecules increases and CMC tends to plasticize the gelatin. Finally, crosslinked film shows higher stability. Exhibits a higher  $T_g$  and  $T_m$ , as well as  $\Delta H$  than gelatin and gelatin/CMC.

In all cases, the appearance of the endothermic peak in DSC thermograms is normally due to the breakage of hydrogen bonds <sup>29</sup>.

Table 2 The DSC data for films based on gelatin: G, (G/CMC) and (G/CMC/GTA).

	Gelatin	G/CMC	G/CMC/GTA
$T_g$ (°C)	44,19	38,77	69,94
$T_m$ (°C)	61,78	58,24	84,79
$\Delta H_m$ (J/g)	11,49	16,67	21,26

### 3.4. Tensile properties of films

The mean stress-strain curves for the three types of films differ from each other as shown in fig. 5 and confirmed by the results reported in table 3. The elastic modulus values were calculated for all specimens from the slope of the linear climbing tract of the stress-strain plot within the fixed strain region 0.5-1.0%.

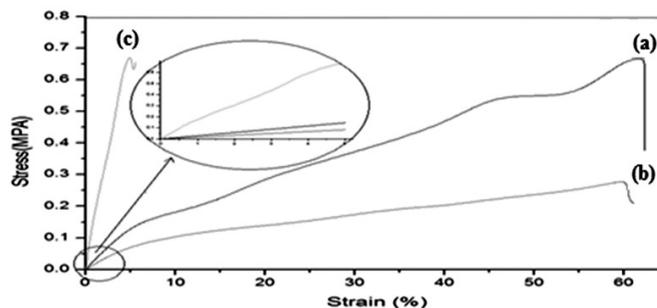


Fig. 5 Tensile stress-strain curves of G, G/CMC, and G/CMC/GTA at RH =75%.

Table 3 Mechanical properties of films based on gelatin.

Formulation	Thickness (mm)	Parameter		
		Elastic modulus (MPa)	Tensile strength (MPa)	Elongation (%)
Gelatin	0,264	2.86	0.66	62.50
G/CMC	0,267	1.48	0.28	60.30
G/CMC/GTA	0,267	13.71	0.67	5.80

According to curves of gelatin, G /CMC, G /CMC/ GTA, it has been observed that the addition of CMC leads to a decrease in tensile strength and  $\epsilon_{max}$  while the Young's modulus decreases after addition of CMC and increases after cross-linking.

### 3.5. AFM

According to Farris *et al.* <sup>35</sup>, gelatin molecules assemble into aggregates containing shorter segments than those expected for collagen triple helices. In our case, this protein presents, uniaxial fibers whose size varies from 0.57 to 1.21  $\mu\text{m}$  length and of approximately 23-40 nm height (fig. 6).

After addition of CMC, the fibrillary homogeneous structure was disappeared and took a relief form (surface in projections and hollows). Whereas, film of (G/CMC/GTA) became thinner and smoother.

### 3.6. SEM

The porosity of biomaterials is a very required field in tissue engineering and drugs release. In our case gelatin film has a macroporous droplet form structure as indicated by Petterson, (2009) <sup>2</sup>.

In notorious way, the presence of CMC and GTA particularly affects the porosity of gelatin as shown in fig. 7.

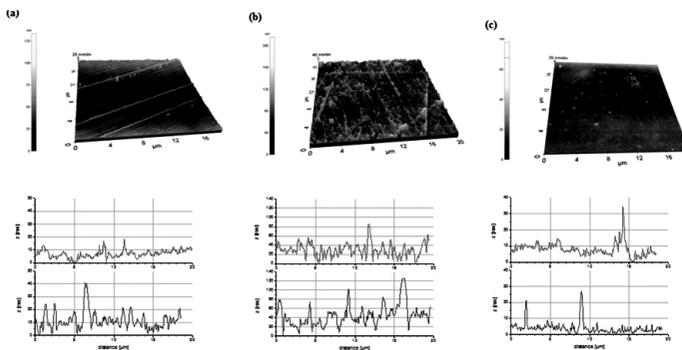


Fig. 6 AFM photographs of G (a), (G/CMC) (b), (G/CMC/GTA) (c).

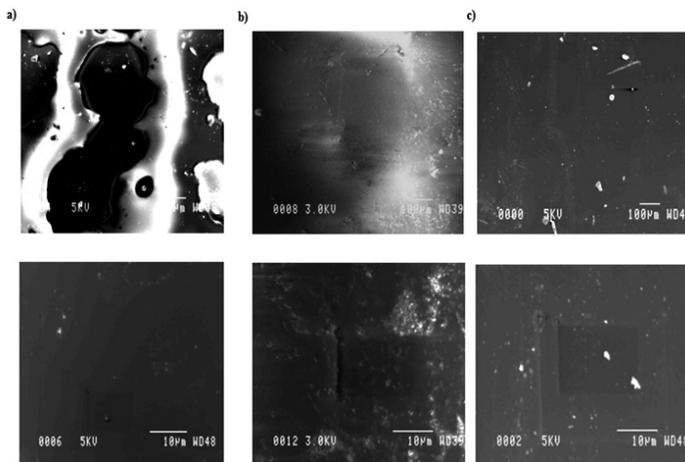


Fig. 7 SEM of G (a), (G/CMC) (b) and (G/CMC/GTA) (c), at two different magnifications (100 X and 2000 X).

Table 5 Swelling kinetics data (n, K, R and D) for G, (G/CMC) and (G/CMC/GTA) at pH=7 and 8.2.

Composition	pH	T	n	K	R	D *10 <sup>7</sup> (cm <sup>2</sup> /s)
G	8,2	25	0,27	0,1892	0,911	0,74
	7		0,19	0,429	0,820	1,83
(G/CMC)	8,2		0,33	0,2005	0,980	1,51
	7		0,33	0,112	0,952	0,48
(G/CMC/GTA)	8,2		0,70	0,0329	0,959	0,93
	7		0,67	0,035	0,978	1,16
(G/CMC/GTA)	8,2	37	0,57	0,0583	0,964	0,95
	7		0,39	0,0647	0,954	1,38

**CONCLUSION**

FT-IR spectra of G/CMC have been confirmed an electrostatic interaction between gelatin and carboxymethyl cellulose. The addition of crosslinking agent (GTA) to gelatin/CMC has been (physically and / or chemically) established leading to three-dimensional networks.

X-ray diffractograms show that the framework in triple helix of gelatin decreases as polysaccharide and/or the crosslinking agent increases until a total disappearance.

According to DSC thermograms, the glass transition temperature is directly related to the denaturation of gelatin (transition helix/coil). Also, it has been observed that T<sub>g</sub> increases and decreases as GTA and CMC were added, respectively.

Atomic force microscopy analysis has been presented the change of morphology of gelatin which was initially in the form of regrouped uniaxial fibers, then, it is transformed into relief, by the addition of CMC. On the other hand, micrograph of G/CMC/GTA film reveals a complete irregular junction.

**3.7. Swelling**

To improve the swelling at pH = 7 and 6.95, it is necessary to use an electrolyte as shown in table 4. On the other hand, it has been observed that the maximum swelling (S<sub>max</sub>) has been increased in the presence of CMC as well with higher pH and lower degree of crosslinking.

Attempts to determine the diffusion mechanisms systems, swelling results have been shown that, the value of n is always lower than 0.5 for non-crosslinked films and agrees with Fickian diffusion “Less Fickian behavior” and the rate of water penetration is lower than that of chains relaxation.

Table 4 Maximum swelling data for G, G/CMC and G/ CMC/GTA at different pH (2.2-8.2).

pH	Swelling					
	2.2	5.95	6.95	7	7.4	8.2
G	905	1187	1515	466	1487	1456
G/CMC	992	1187	1646	762	1355	1518
G/CMC/GTA	127	124	154	51	160	180

The exponent n for crosslinked films is greater than non-crosslinked films, reaching maximum n at 0.7 (G / CMC / GTA) at room temperature and decreases at higher temperatures (table 5).

SEM data are in agreement with AFM. Furthermore, macroporous gelatin structure has been disappeared in the presence of additives.

Swelling in pseudophysiological mediums shows that macroporous structure is more absorbent and used electrolytes exert an osmotic pressure or ionic strength inducing higher swelling.

Also, it was found that diffusion mechanism is directly related to gelatin structure, particularly porosity and T<sub>g</sub>. In general, linear films are Fickian diffusion, which is one of characteristic porous materials. Whereas, crosslinked films (no Fickian) became less porous and T<sub>g</sub> tends to increase, so, the rate of relaxation (toward the glassy state) decreases and becomes much lower than that of water diffusion. At high moisture content (63-75%), tensile tests have been showed that CMC and GTA tend to plasticize and make gelatin more rigid, respectively.

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