

Preparation and Characterization of Biofilms Containing Gelatin in Presence of Two Polysaccharides Starch/Methylcellulose Modified by a Plasticizer

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Abstract

The proteins and polysaccharides are biomaterials very largely used in the medical and pharmaceutical field. It is therefore important to better understand and control the interactions between these elements.

The preparation of the biofilms containing gelatin in the presence of two polysaccharides (starch, methyl cellulose) modified by glycerol is done by a hydrosolubilization of the grains with 2,5% in mass at different temperatures. Drying is done with the free air on hydrophobic polystyrene supports. The crosslinking is done by the glutaraldehyde led to obtaining less friable films thanks to the formation of a tighter crosslinked network. The characterization is made by the following tests: The study of swelling reveals the influence of the temperature on the capacity for absorption of these biofilms, as well as the influence of the presence of plasticizer which is the glycerol on this parameter. The analysis by UV-VIS allows the determination the area of absorption; the mechanical test of the polarity shows that there is improvement of the mechanical properties especially for the biofilms in the presence of glycerol. In end, it should be noted that these tests prove that the biofilms prepared containing methyl cellulose / potato gelatin /starch have properties better than the others biofilms under the same experimental conditions.

Key words: gelatin, glutaraldehyde, methyl cellulose, glycerol, starch

1. Introduction

The past few years have witnessed rapidly expanding interest in renewable agricultural feed stocks and marine food processing wastes as sources of biomolecules with potential to replace synthetic polymers [1] in fabricating biomaterials with bioactivity, biocompatibility, biodegradability, and novel properties for unique applications [2]. As biomolecules have become more available, ever-increasing demand for high-performance “natural” matrices for biomedical and pharmaceutical applications such as tissue engineering [3] and bioadhesives for wound dressing, films, and capsules for or ingestion has stimulated design of ‘smart’ matrices able to ‘sense’ external changes (pH, temperature, humidity).

Gelatin is a complex polypeptide widely used in the food, pharmaceutical, photographic, and cosmetic manufacturing. Gelatin was one of the initial materials used for the formation of biopolymer films and continues to be used in edible film studies given the abundance of raw material, low production cost, global availability, and excellent film forming properties [4].

Starch is the main reserve carbohydrate substances of higher plants which represent a weight fraction important in many agricultural commodities such as cereals (30% to 70%), tubers (60% to 90%) and legumes (25% to 50%).

It is a compound inexpensive, renewable, which is found in medication as a binding agent and as an excipient and food multiple functions as thickening, gelling [5].

Methylcellulose is obtained by chemical modification of cellulose. It is produced by reaction between the alkali cellulose and chloride methylene [6].

In this work, we are interested in the preparation and characterization of films based on gelatin, starch and methylcellulose modified with a plasticizer is glycerol. The physical properties of these dressings are compared. The study focuses on swelling measurements in order to assess the absorbing capacity of the different films. Particular attention is paid to the qualitative characterization of the residue formed before and after crosslinking. This analysis is made by UV spectroscopy.

The mechanical test of the polarity is made in order to reveals the influence of the presence of plasticizer which is the glycerol.

2. Materials and Method

2.1. Materials

The gelatin used is of the animal type and was supplied from Melin Company (France) under the code name A-ph-type B. It is insoluble in cold water but soluble in hot water. Its is electric point is 5.2 and has abloom of 250.

The crosslinking agent used is glutaraldehyde and was purchased from Aldrich Chemical Company in a liquid form and a purity of 50% (v=v).

Sodium azide (NaN_3), used to avoid bacterian contamination, was obtained from Sigma Chemical Company.

Glycerol was obtained from Riedel-de haen Chemical Company and a purity of (86-88%).

Methylcellulose (CMC) obtained from prolabo.

Starch obtained from Roquette Company (Lestrem France).

2.2. Preparation of Hydrocolloid Films Materials

The preparation of the biofilms containing gelatin in the presence of two polysaccharides (starch, methyl cellulose) modified by glycerol is done by a hydrosolubilisation of the grains with 2,5% in mass at different temperatures.

Drying is done with the free air on hydrophobic polystyrene supports.

3.5 grams of gelatin/ starch/ methyl cellulose mixture were dissolved in 150 ml distilled water and a small amount of sodium azide, which was added to each solution to prevent bacterial contamination. After allowing the grains to swell for 35 min, the mixture was placed in a water bath heated at 60°C under slow agitation for 40 min. Once the resulting solution became clear, two different samples (3 ml, 5 ml) were withdrawn from the mixture and poured separately into 8.5 cm diameter polystyrene boxes in order to obtain films with different thicknesses.

These samples were then allowed to dry in free air at room temperature for 3 to 4 days. The resulting films were crosslinked by means of glutaraldehyde (GTA). Crosslinking was carried out by pouring 20 ml of the GTA solution onto the dry films and allowing the reaction to take place

for 24 h. Finally, the crosslinked films were washed with distilled water and dried in free air at room.

2.3. Measurement of Film Thickness

The film thickness was measured by means of a digital micrometer to the nearest 0.01 μm and the measurements were taken at five different positions. The average thickness of the films before crosslinking designated F1, F2 was 31 μm , 42 μm respectively.

After crosslinking and because the glutaraldehyde used was diluted, the resulting crosslinked films absorbed water and got swollen and consequently their thicknesses changed slightly. So the crosslinked films, which are designated as F1C, F2C, have thicknesses of 40 μm , 51 μm corresponding to a thin and thick film, respectively.

2.4. Film characterization

2.4.1. Swelling Measurement

2cm² specimens were immersed in beakers each containing 100 ml distilled water at two different temperatures: room temperature and 37°C, and samples were taken at regular intervals of time ranging from 0 to 120 h. The swollen samples were withdrawn from the medium and weighed again after removal of excess surface water by using a filter paper. The swelling index, I_s (%), was determined using the following equation (1):

$$I_s = \frac{W_s - W_d}{W_s} \times 100 \quad (1)$$

Where W_s is the weight of the swollen film immersed for time t , and W_d is the initial weight of the film.

2.4.2. Analysis of the Residue

After film swelling, the remaining residue, which represents the amount of gelatin/ starch/ methyl cellulose liberated in distilled water, was analyzed by means of a UNICAM–UV Spectrophotometer.

2.4.3. Analysis by Mueller Polarimeter

The films were analyzed by Mueller polarimeter which is one of the major types of polarimeters used in measuring polarization properties.

3. Results

3.1. Swelling Measurement

These tests were carried out at variable film thickness and temperatures. It was noted that certain films have a higher capacity to absorb water than others, liberating protein and polysaccharide until saturation.

The figure 1 shows a significant evolution of the absorption capacity of different biofilms during the early hours to minutes, it was found that the rate of swelling of the biofilm (G / MC / Apt) without glycerol reaches a $G_{\max} = 485\%$ which is superior to other biofilms so it is better absorption capacity.

In figure 2, it was observed a decrease in the swelling ratio of these two biofilms compared to unmodified biofilms by glycerol, this may be due to the saturation of the hydrophilic sites glycerol, while biofilms (G / Mc / Am / g) and (G / Mc / Apt / g) values are close to the rate of inflation over the case in the absence of glycerol, which allows us to say that glycerol improves the properties of absorption of these biofilms.

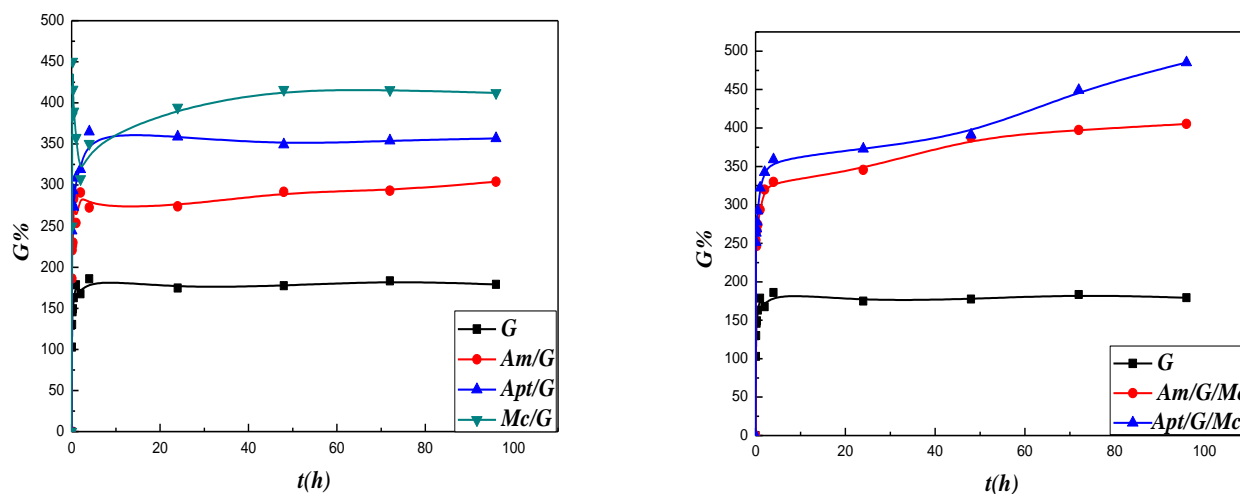


Figure 1. Variation of the swelling index for the different crosslinked films as a function of time at room temperature without glycerol

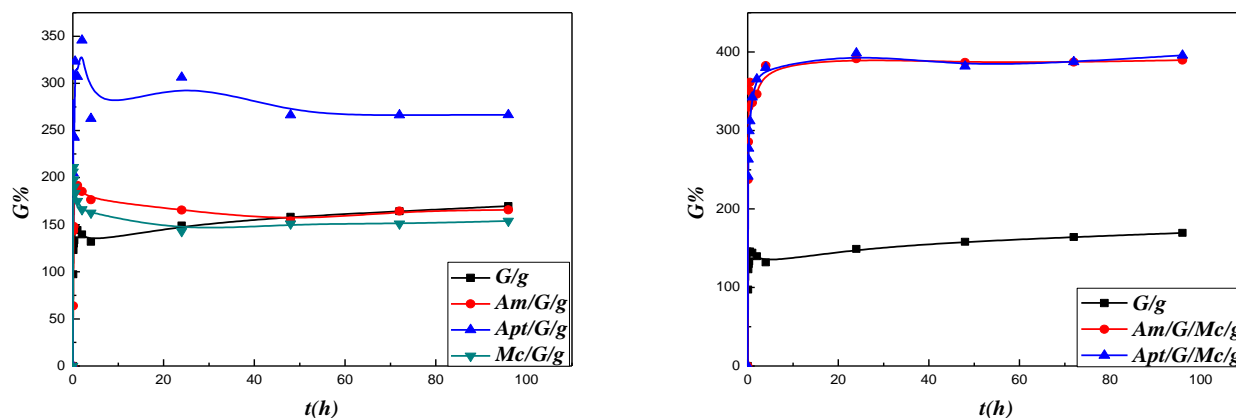


Figure 2. Variation of the swelling index for the different crosslinked films as a function of time at room temperature with glycerol.

As shown in figure 3 After the first four hours, there is a decrease followed by a degree of saturation in the early hours in minutes biofilms (G / Apt) show strong absorption followed by a slight decrease from 406% to 395%. This This decrease a rapid release of the component of biofilms probably due to the increase in temperature, while the other biofilms exhibit a gradual increase in the degree of swelling.

After 24 hours, the biofilm ($G / Mc / Apt$) and ($G / Mc / Am$) exhibit absorption increasing with time, probably due to the ability of biofilms to absorb more, while others biofilms it was found rapid saturation compared to that which was observed at room temperature due to the temperature increase.

According at temperature $37^{\circ}C$ and in the presence of glycerol figure 4 Compared to unmodified case of biofilms by glycerol, these biofilms exhibit better absorption of 256% to 418% for biofilms ($G / Mc / Apt / g$), and of 255% to 328%, while the other biofilms there is a decrease in the rate of swelling.

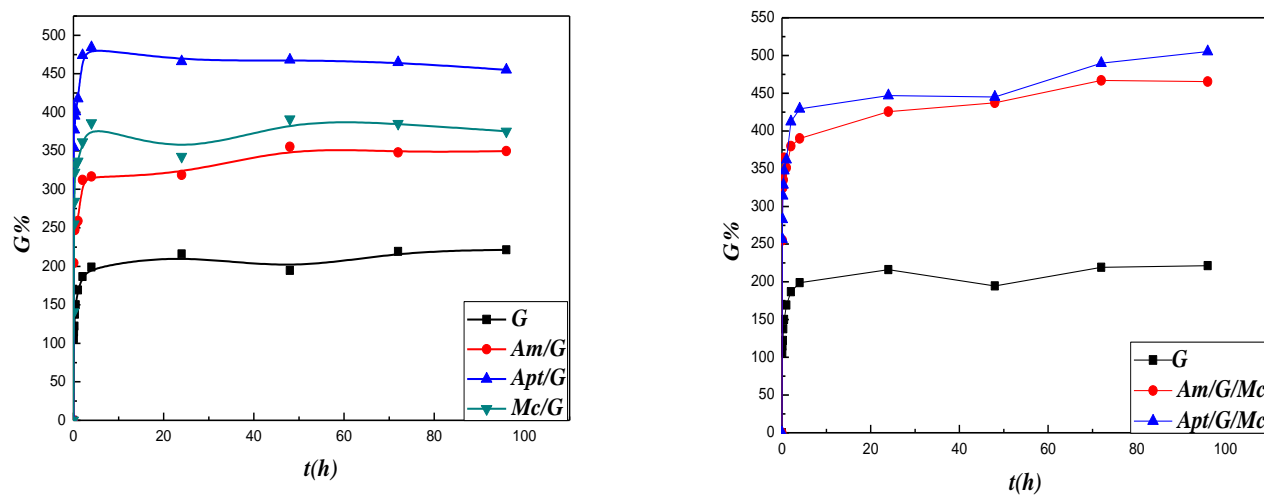


Figure 3. Variation of the swelling index for the different crosslinked films as a function of time at 37°C without glycerol.

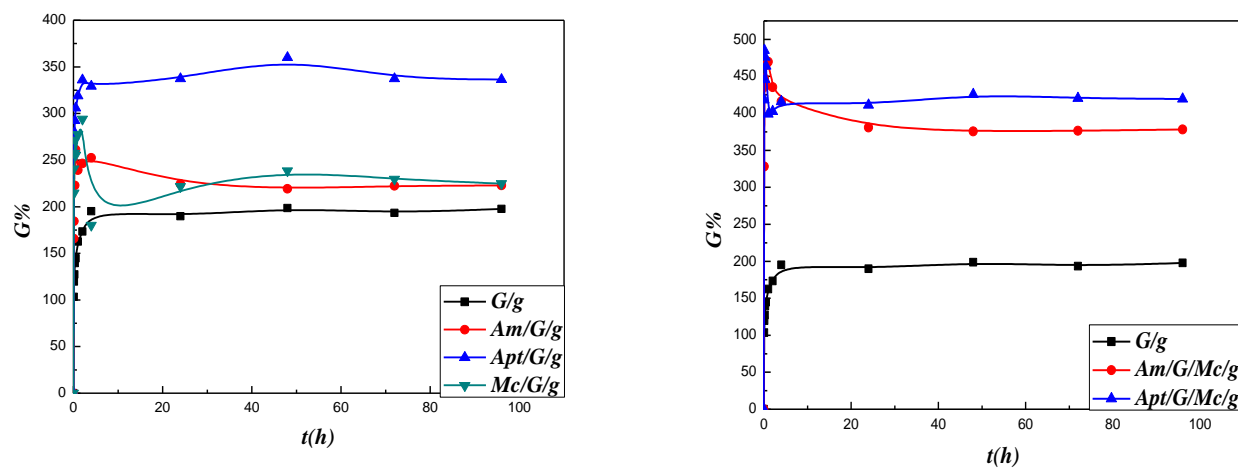


Figure 4. Variation of the swelling index for the different crosslinked films as a function of time at 37°C with glycerol.

3.2. UV Analysis

Amino acids absorb in the ultraviolet in the average of 220 nm, with the exception the three aromatic amino acids (Try, Phe, Tyr) which have a second absorption peak at a higher wavelength in the average of 280 nm.

The UV analysis of the residue of the prepared film is illustrated in Figures 5 and 6. A shoulder can be seen at 228 nm and 230 nm respectively for 22°C and 37°C. In fact, the liberation of the amino acids that was evidenced in the UV spectra is related to the presence of gelatin which is a protein constituted of peptidic bonds that are easy to hydrolyze.

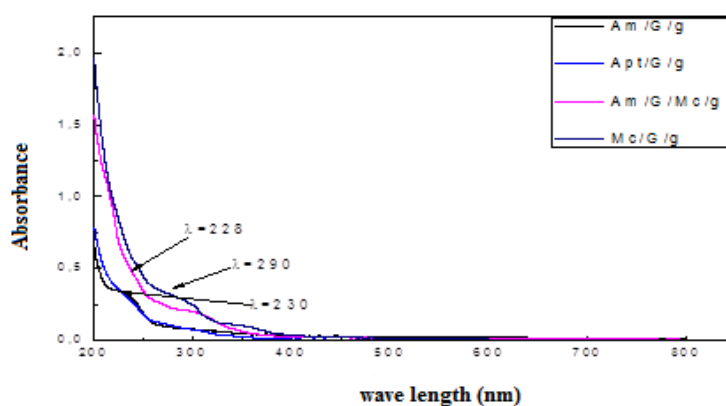


Figure 5. UV spectrum of the crosslinked film residue at room temperature.

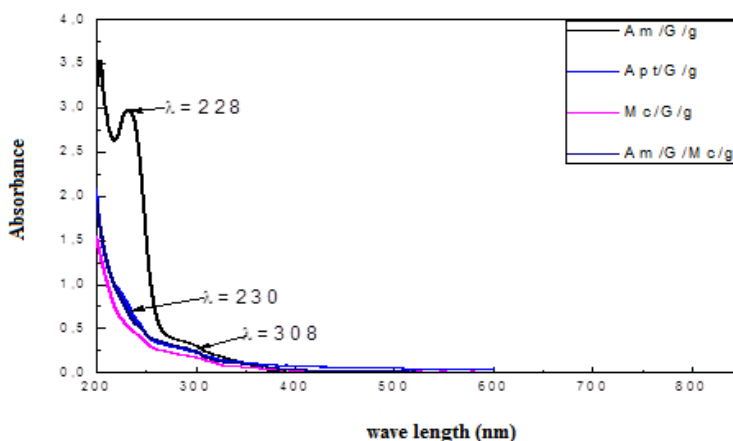


Figure 6. UV spectrum of the crosslinked film residue at 37°C.

3.3. Analysis by Mueller Polarimeter

The objective of this work is to use a method based on the change of polarization during light-matter interaction, to assess the evolution of the distribution, transmission and homogeneity of the films as a function of position and concentration.

We draw the curves representing the variation of the degree of polarization and the position function Diatiniation of different thicknesses.

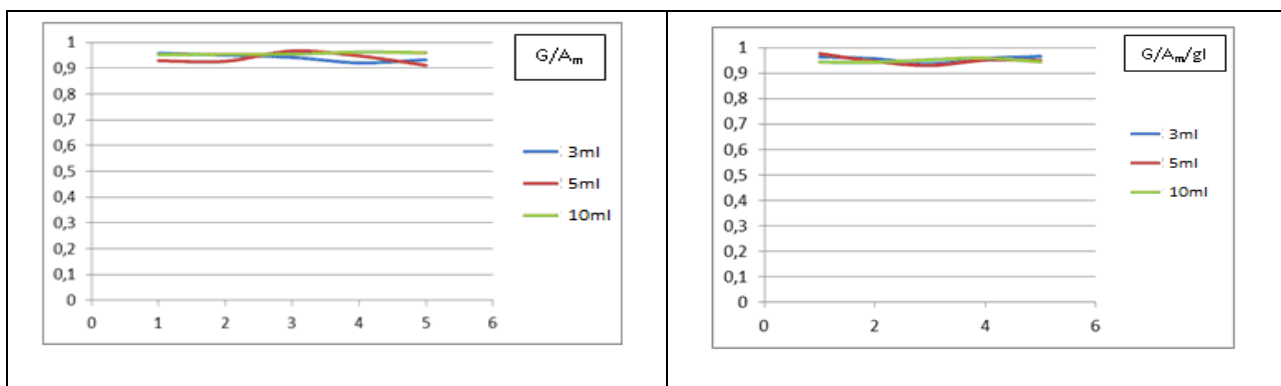


Figure 7. Variation of the degree of polarization as function of position at different thicknesses.

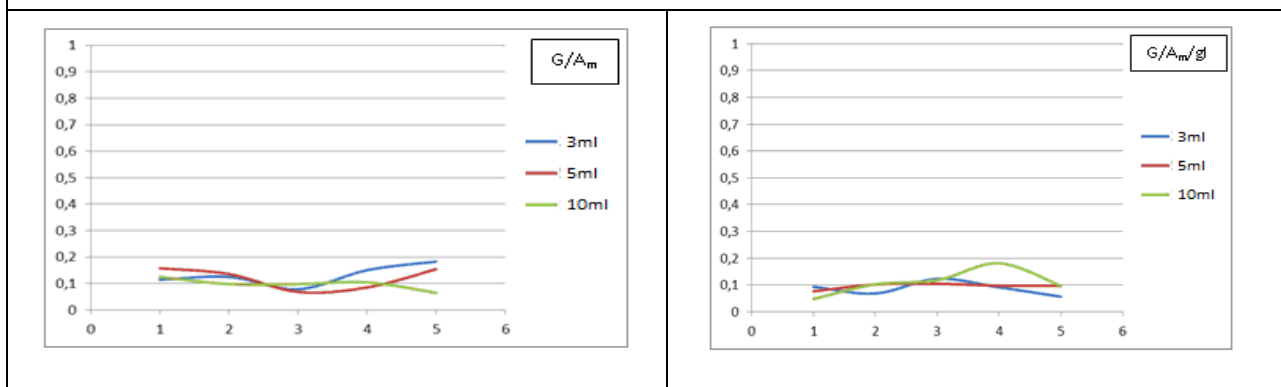


Figure 8. Variation of Diatiniation as function of position at different thicknesses.

From this curves we observe that degree of polarization (DP): Gelatin/Starch and Gelatin /Starch / glycerol is approximately equal1

Table 1. Variation of degree of polarization of different composants of biofilms (42 μ m) as function of position

Position	A(m)	MC	A(m)+G	MC/G	A(m)+G+g	MC/G/g	Mc/A(m)/G	MC/G/g/A(m)
1	0,9637	0,7357	0,952	0,7692	0,9768	0,8615	0,841	0,8423
2	0,9533	0,7526	0,9543	0,873	0,946	0,8679	0,919	0,9189
3	0,9721	0,7777	0,9543	0,7652	0,9301	0,9189	0,9463	0,9463
4	0,9486	0,7969	0,9626	0,7756	0,9516	0,8035	0,9651	0,9652
5	0,9304	0,7627	0,9597	0,7746	0,9501	0,9152	0,9479	0,9481

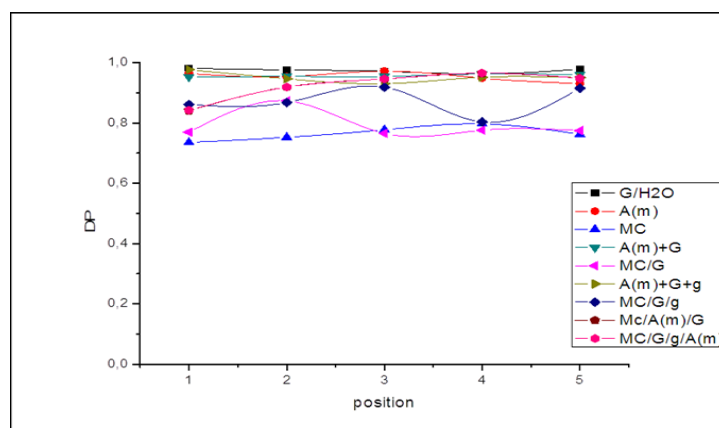


Figure 9. Variation of degree of polarization of different biofilms (42 μ m) as function of position.

Table 2. Variation of Diatiniation of different biofilms as function of position

position	A(m)+G	A(m)+G+g	MC/G/g	Mc/A(m)/G	MC/G/g/A(m)
1	0,1578	0,0763	0,2519	0,434	0,443
2	0,1362	0,1019	0,1541	0,4868	0,4886
3	0,0687	0,1044	0,1142	0,0849	0,0894
4	0,0852	0,0976	0,3518	0,113	0,1128
5	0,1544	0,0965	0,1299	0,117	0,1163

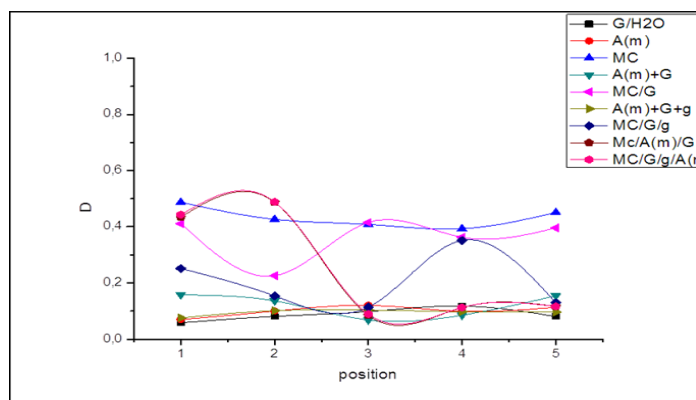


Figure 10. Variation of Diatiniation of different biofilms as function of position

It can be seen from these figures that when:

DP = 1: biofilms G, Am, Am / G, Am / G / g are considered a non-diffusing.

DP < 1: Mc biofilms, Mc/G, Mc/G/g, Mc/G/Am/g are partially diffusing.

D=0: non-absorbing

0 < D < 1: biofilms containing glycerol are partially absorbing.

So the presence of glycerol or methylcellulose makes the film more flexible leading to an increase of the diffusion.

From this test, it was found that the addition of glycerol increases the capacity of the diffusion and absorption. Methylcellulose also has an impact.

Conclusion

The objective of this work was to study the physicochemical properties of different hydrocolloid films prepared from two natural biopolymers. In order to enhance the interactions between the protein and the polysaccharide, the prepared films were crosslinked by glutaraldehyde.

The physicochemical properties of the different films were evaluated through the measurement of the absorption capacity. UV spectroscopy was used to characterize the films. The results showed that the crosslinked films have a higher absorption capacity.

The addition of two polysaccharides (starch and methylcellulose) exhibit a higher absorption so the presence of the plasticizer (glycerol) decrease the swelling index.

The results of the UV spectroscopy of the film residue resulting from the immersion of samples in water showed that the films degrade.

The biofilms analyzed by the technique of mueller polarimeter are made from gelatin/corn starch, gelatin/starch potato in the presence of glycerol. The results found show that the presence of glycerol reduces the transparency of the film. However, the presence of glycerol on gelatin/starch makes the film transparent.

The transparency of biofilm, provides good visual control of cicatrizations wounds.

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Abbreviations

AM: Corn Starch

Apt: Starch potato

CMC: Methylcellulose

G: Gelatin

g: glycerol

GAT: Glutaraldehyde