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Corresponding Author: Prof. Vicente Compañ-Moreno, PhD

Corresponding Author's Institution: Universidad Politécnica de Valencia

First Author: Sergio D. Pasini Cabello, Dr.

Order of Authors: Sergio D. Pasini Cabello, Dr.; Nelio Ariel Ochoa, Dr.; Andres Takara; Sergio Mollá; Vicente Compañ-Moreno, PhD

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Suggested Reviewers: Luis F. del Castillo Dr.
Full Prof., Instituto de Materiales, Universidad Nacional Autonoma de México
lfelipe@unam.mx
Is a good specialist in the field of membranes characterization.

Santiago V. Luis Lafuente Dr.
Full Prof., Organic Chemistry, Universitat Jaume I. Castellón (Spain)
luiss@uji.es
He is the director of one of the best research groups in the preparation of Polymers and membranes in Spain.

Julio Guzman Dr.
Full Prof., Intituto de Polimeros, Consejo Superior de Investigaciones Científicas (CSIC). Madrid. Spain.
jguzman@ictp.csic.es

Is a good specialist in the syntesis and membranes preparation and its characterization.

Vicente Compañ
Departamento de Termodinámica Aplicada
Escuela Superior de Ingenieros Industriales
C/Camino de Vera s/n
46022. Valencia. Spain.
Phone: 34-96-3879328
Fax: 34-96-3877329
E-mail: vicommo@ter.upv.es

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Dear Editor:

I submit to your consideration the publication in Carbohydrate Polymers the manuscript entitled "**Influence of Pectin as a green polymer electrolyte on the transport properties of Chitosan-Pectin membranes",** written in collaboration with Dr. Sergio D. Pasini Cabello, Dr. Noel Ariel Ochoa, Andres Takara and Dr. Sergio Mollá.

Thank you for your consideration.

Sincerely yours,

The authors.

Highlights

1. The effect of weight ratio of Chitosan (CH) to Pectin (PEC) is studied.
2. The methanol permeability of CH/PEC membranes decreases with the amount of Pectin.
3. Proton conductivities of CH/PEC membranes decrease until a ratio composition of 1:1.
4. DSC studies reveal excision of ionic pairs between carboxylic and ammonium groups.
5. Proton diffusion coefficients decrease one order of magnitude in CH/PEC membranes.

Influence of Pectin as a green polymer electrolyte on the transport properties of Chitosan-Pectin membranes.

S.D. Pasini Cabello[a], N.A. Ochoa[a], E.A. Takara[a], S. Mollá[b], V. Compañ[b,*].

[a]: Instituto de Física Aplicada (INFAP). Universidad Nacional de San Luis CONICET. Chacabuco 917 5700 San Luis, Argentina.

[b]: Departamento de Termodinámica Aplicada. Universidad Politécnica de Valencia. 46022-Valencia, Spain.

(*) Corresponding author:

Vicente Compañ

Dpto. Termodinámica Aplicada

Universidad Politécnica de Valencia

46022-Valencia-Spain.

Tel.: +34963879328

Fax: +34963877924

e-mail: vicommo@ter.upv.es

ABSTRACT

Novel composite membranes have been prepared from Chitosan (CH), Pectin (PEC) and their mixtures. The obtained samples were cross-linked and sulfonated before characterization. The results show that CH/PEC membranes display structural changes on the chemical and physical properties as a function of composition. DSC analysis reveals an endothermic peak due to the excision of the ionic pairs between carboxylic group and ammonium groups, which produces a strong change on physical properties such as methanol permeability and proton conductivity. The methanol permeability decreases with the amount of Pectin from $(4.24\pm0.04)x10^{-6}$ cm^2/s for pure Chitosan membrane to $(1.51\pm0.03)x10^{-6}$ cm^2/s for composite CH/PEC membranes when the amount of Pectin is 50% (v/v). The proton conductivities of the composite membranes follow a similar behavior. For a pure CH membrane the conductivity is $2.44x10^{-3}$ S/cm, decreasing with pectin content until the composition 50/50 (v/v), in which the conductivity is about one order of magnitude lower.

Keywords: Biopolymeric membranes; polymer electrolyte membranes (PEM); methanol permeability; proton conductivity; differential scanning calorimetry (DSC); electrochemical techniques.

1. Introduction

The potential applications of biomaterials are numerous and involve different fields such as fibers for the textile industry, medical products, cosmetics, bioimplant, delivery of drugs, herbicides, fungicides, and so on. Biodegradability is nowadays considered to be the most important factor for the introduction of new products. One of the most promising approaches to overcome the safety and environmental problems is the use of renewable resources for obtaining biodegradable polymers useful for various applications in medical, pharmaceutical, agriculture, drug release, and packaging fields. Pectin (PEC) is one of the naturally occurring polysaccharides that has become more and more important over recent years, because this is a material with eco-friendly properties primarily due to the renewability and sustainability of their sources. The pectin has a low cost because of their abundance in nature (Cavallaro, Donato, Lazzara, & Milioto, 2011; Pérez Espitia, Du, Avena-Bustillos, Ferreira Soares, & McHugh, 2014; Meneguin, Cury, & Evangelista, 2014). Pectin is a structural component of the vegetal cell wall, typically isolated from plants of economic importance (citrus, sugar beet, apple, etc.). It is composed of an anionic complex polysaccharide based on chains of linear regions of (1 → 4)-α-D-galacturonosyl units and their methyl esters, interrupted in places by (1→ 2)-α-L-rhamnopyranosyl units. Fractions of these rhamnopyranosyl residues are branch points for neutral sugar side chains of (1 → 5)-α-L-arabinofuranosyl or (1 → 4)-β-D-galactopyranosyl residues (Shols & Voragen, 1994; Shols & Voragen, 1994). On the other hand, Chitosan (CH) is a linear copolysaccharide β-(1→4)-2-amine-2-deoxy-d-glucose (GlcNac) and β-(1→4)-2-acetamine-2-deoxy-d-glucose (GlcN). It is biodegradable, non-toxic, bio-compatible, and renewable (Croisier & Jérôme, 2013; Sevel, Ikinci, & Kas, 2000; Kaminski & Modrzejewska, 1997). Also, it can be easily modified and has a noticeable ability to form many complexes with metal

ions and enzymes (Schmuhl, Krieg, & Keizer, 2001; Khor, 1997). In addition to its many uses in food and agricultural applications, CH exhibits the ability to form films. CH is obtained from Chitin, which is mainly extracted from crustacean exoskeletons (Kurita, 2006; Muzzarelli, 1973). The solubility of polymers in aqueous acidic media solutions has been used to distinguish chitin from CH. The word Chitosan has been used for soluble polymers obtained from the deacetylation of chitin. Incomplete deacetylation reaction allows for the random distribution of GlcNac and GlcN residues (Abdou, Nagy, & Elsabee, 2008; Chang, Tsai, Lee, & Fu, 1997; Knaul, Kasaai, Bui, & Creber, 1998; Tolaimatea et al., 2000). The degree of deacetylation (DDA) and molecular weights (MW) determines some of the characteristics of CH, such as solubility, biodegradability, aggregation properties and intrinsic ionic conductivity (Cho, Jang, Park, & Ko, 2000; Kubota & Eguchi, 1997; Kristiansen, Vårum, & Grasdalen, 1998; Liu, Adhikari, Guo, & Adhikari, 2013; Schatz, Pichot, Delair, Viton, & Domard, 2003). DDA below 40–50% yields soluble polymers (Wang et al., 2008; Wang et al., 2006). However membranes of Chitosan with DDA higher than 80% increases the crystallinity improving their tensile strength and increasing the swelling but they may increase the fragility and ionic permeability (Wan, Creber, Peppley, & Bui, 2003).

In the last years CH membranes has emerged as a new alternative to get membranes with high ionic conductivity at low and moderate temperatures when the membrane is completely or partially hydrated (Wan et al., 2003; Kordesch & Simader, 1996). Chitosan membranes or hybrid Chitosan membranes can be used for an alkaline fuel cell (AFC) where the ion OH^- carrier is required, because they are kinds of polar groups on its backbone (hydroxyl and amino groups) that permit give to this kind of membranes of hydrophilicity, which is the property more important for a fuel cell (Krajewska, 2001; Zawodzinski et al., 1993). The use of this kind of membranes as

polymer electrolyte in a fuel cell has showed, operating at 60ºC, a performance through polarization curves (voltage-current density and power-current density) a current density of 35mA/cm^2 in a PEMFC using hydrogen as feed into the anode and air as the oxidant in the cathode (Wan et al., 2003). In previous studies (Wan et al., 2006), alkaline chitosan-based composite membranes were prepared by incorporating KOH as the functional ionic source The main problem is to hold the KOH permanently inside the composite membrane avoiding the leaching of the bulk KOH into the polymeric matrix. However preliminary studies into a real fuel cell system (FC) with membrane electrode assembly of these composite membranes have shown at 60ºC that a current density of 35mA/cm^2 had been achieved using hydrogen as feed and air as the oxidant (Wan et al., 2006).

Microbial fuel cell (MFC) is a promising technology for wastewater treatment as well as means of energy recovery by microorganism. Various polymer membranes have been developed to facilitate proton transfer between anode and cathode in MFCs (Seol et al., 2012; Chae et al., 2008; Shaari & Kamarudin, 2015). Chitosan is a cheap and environmental-friendly natural biopolymer, which is considered quite promising for its use as a polymer electrolyte in MFCs (Smitha, Sridhar, & Khan, 2004). For these reasons, the physical and chemical structure of CH must be modified to allow its use as a viable polymer electrolyte membrane (Ma & Sajhai, 2013; Tripathi & Shadi, 2011; Holder, Lee, Popuri, & Zhuang, 2016).

Blend, composites and multilayer films of PEC and CH with improved mechanical and barrier properties have been previously described (De Yao et al., 1996; Hiorth, Tho, & Arne, 2003; Hoagland & Parris, 1996; Sriamornsak & Puttipipatkhachorn, 2004; Milkova & Radeva, 2015; Pasini Cabello, Takara, Marchese, & Ochoa, 2015). However, at the best of our knowledge there is not any report on CH/PEC films

functionalized with -SO_3H groups that were characterized in order to reveal their performance as a polymer electrolyte membrane. It is expected that the preparation of composites membranes of CH/PEC in combination of acidic groups may give a mechanical and chemical stability improved by the complexation due to the adsorption of the different charges which originate Coulombic forces between the anions and cations together with the functional groups of the CH and PEC. On the other hand, the formation of ionic complex should produce a diminution of IEC and degree of swelling which compact the polymer matrix. This leads to the formation of an ionic complex with fewer carriers, providing a decreasing of conductivity when the ratio between CH and PEC were 1:1.

In this paper we examine the effect of the weight ratio of CH to PEC following from the chemical functionalization on the key transport properties such as methanol permeability and membrane conductivity. Also, in order to reveal the structure of films an approach based on FTIR spectroscopy and DSC analysis were performed. Water uptake, mechanical properties and ion exchange capacity were also determined.

2. Experimental

2.1 Materials

Pectin from Citrus Fruits (CAS 9000-69- degree of methyl esterification, 6.7%, MW = 30 - 100 kg mol^{-1}) was purchased from Sigma-Aldrich (Denmark). A commercial sample of CH powder of average viscometer molecular weight (890KDa), with approximately 75% deacetylation, was supplied by Sigma-Aldrich. Glutaraldehyde commercialized as 25% Grade II was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glycerol, acetone, H_3PO_4, NaOH, NaCl, HCl, isopropanol and ethanol were

153 provided by BioPack (Campana, Argentina). All solutions were prepared with Milli-Q
154 water (resistivity> 1.8 mΩ cm). 4-formyl- 1.3-benzenedisulfonic acid disodium salt was
155 supplied by Aldrich.
156
157 **2.2 Preparation of the composite membrane**
158 The films made of Chitosan and Pectin and mixtures thereof were obtained by casting.
159 For this a polymer solution of CH and PEC with a mass fraction of 2% (w/v) was
160 prepared in distilled water containing Phosphoric Acid, 1% (v/v) at 40 °C. With
161 appropriate amounts of each solution, mixtures of different ratios were prepared in order
162 to obtain films named CH/PEC 100/0, 80/20, 60/40, 50/50, 40/60, 20/80 and 0/100,
163 respectively. Glycerol was added as a plasticizer at 0.5%. Film forming solutions were
164 cast in Petri dishes (14.5 cm internal diameter) and dried in an oven at 35°C for 48 h.
165 After that, the films were cross-linked, for this the films were immersing for 24 hours in
166 a room temperature bath consisting of 50 ml of acetone solution containing 5% (w/v)
167 glutaraldehyde (Sigma Aldrich) and 1% (w/v) HCl at 25°C. Finally, the films were
168 washed with ethanol and water. Film preparation was carried out in triplicate.
169
170 **2.3. Chemical functionalization**
171 In order to improve the ionic conductivity, sulfonic groups were added following the
172 same procedure reported elsewhere (Mollá & Compañ, 2011). Membranes were
173 immersed in a bath isopropanol/water (70/30 v/v) containing0.04M 4-formyl- 1,3-
174 benzenedisulfonic acid disodium salt and 0,1M HCl for 2 h at 50ºC. Then, several
175 washes of membranes were carried out with 0,3M HCl solution isopropanol/water
176 (70/30 v/v) in order to eliminate possible residual sodium ions. After that, the
177 membranes were dried at 50° C.

178

179 **2.4 Chemical stability of the membranes**

180

181 Vapor phase Fenton test has been developed to determine the membrane degradation as
182 an *ex situ* accelerated test method. Previously doped membranes with 2 ppm of aqueous
183 solution of $FeSO_4$ were vacuum dried and weighted. Then using a chamber similar to
184 reported by Delaney and Liu (Delaney & Liu, 2007), samples were suspended and
185 hydrogen peroxide added (3%) and the temperature was increased up to 80 degrees
186 centigrade using heating tape which was wrapped around the chamber. After test period,
187 the chamber was allowed to reach ambient temperature and the samples were removed,
188 dried and weighted again to determine weight loss.

189

190 **3. Membranes characterization**

191

192 **3.1. Ionic exchange capacity (IEC)**

193 The membranes were equilibrated in a solution of H_3PO_4 acid (2%) and GTA (5%)
194 overnight. The acidic membranes obtained were further washed several times with
195 distilled water and then equilibrated with a 1 M sodium chloride solution. The protons
196 delivered after the ion-exchange reaction $R\text{-}PO_4H_2^+ + Na^+ \rightarrow R\text{-}\ PO4H_2Na + H^+$ were
197 titrated with a 0.01 M sodium hydroxide solution.

198

199 **3.2. Water uptake**

200 Water uptake of the CH/PEC membranes was measured by drying H^+-form samples in a
201 vacuum and then weighed (W_s). For this the composite membranes were immersed in
202 distilled deionized water overnight. After that, the membranes were removed from
203 water, gently blotted with filter paper to remove excess surface water, and then weighed

again (W_W). This operation was repeated five times. Water uptake was calculated from the difference between the weight of the composite wet and dry, by means of the expression (1). The value of water uptake was averaged from three similar membranes crosslinked at the same temperature and the results referred to the wet membrane are given in Table 1.

$$\%\, Water\ uptake = \left(\frac{W_W - W_S}{W_w}\right) \cdot 100 \qquad (1)$$

3.3. Mechanical properties

Tensile tests of the synthesized films were performed at room temperature using a Comten Industries (Series 94 VC) device (Pinellas Park, Tampa FL, USA). Films were cut into strips with a width of 1.1 cm and length of 4.0 cm. To ensure complete relaxation of the polymeric structures and to standardize the experimental procedure, film samples were stored in a humidity- and temperature-controlled chamber for 24 h at 25ºC and 75% R.H. The polymeric strips were then fixed between upper and lower clamps of the tensile tester and the tensile strength was determined at a constant traction speed of 5 mm/min. The mechanical parameter data include the average values from three samples of each film. The film thickness was measured using a Köfer micrometer with a precision of ± 1μm.

3.4. Differential scanning calorimetry (DSC)

The DSC curves were obtained at 10 K/min under a nitrogen atmosphere (Melter Toledo DSC 831). Two scans were performed with each sample, being the first from -50°C to 80°C in order to remove the thermal history of the samples, and the second from -50°C to 250°C.

3.5. Methanol permeability

In order to determine the methanol permeability coefficient through the composite membranes, a typical 2-cell experimental set-up was used such as shown in a previous paper (Mollá & Compañ, 2011), where the donor chamber was filled with 2M aqueous solution of methanol, while the receptor chamber was filled with distilled water, both chambers were kept under stirring and thermostatized at 50ºC.

As it is well described in another paper (Mollá & Compañ, 2015), the variation of methanol concentration with time in the receiver reservoir (c_B) was determined by means of a densimeter (DMA-4500M). A calibration curve of the density versus methanol concentration is previously obtained. During those experiments, a small sample of solution from the receiver compartment was taken at certain time intervals and the density recorded. In order to avoid the volume of liquid in the receiver reservoir (V_B) to change, the samples were recovered from the densimeter after each measurement. Representing the normalized methanol concentration (c_B L V_B)/(AC_A) vs. time, the apparent permeability coefficient (P) of the methanol across the composite membranes can be determined, by mean of the expression (2)

$$C_B = \frac{P \cdot A}{L \cdot V_B} C_A \cdot t \qquad (2)$$

3.6. Membrane conductivity

The conductivity of the composite membranes were measured by electrochemical impedance spectroscopy (EIS) at different temperatures, in the frequency range $10^{-1} < f < 10^6$ Hz. The experiments were performed with 0.1V amplitude, using a Novocontrol broadband dielectric Spectrometer (Hundsangen, Germany) integrated by a SR 830 lock-in amplifier with an Alpha dielectric interface. The membranes were previously

equilibrated with deionized water (Milli-Q) and afterwards placed between two gold electrodes in a parallel plate liquid sample cell (BDS 1308), Novocontrol) coupled to the spectrometer. During the measurements the temperature was maintained below isothermal conditions, controlled by a nitrogen jet QUATRO from Novocontrol with a temperature error of ≈ 0.1 K during every single sweep in frequency. To obtain the conductivity of the membranes four runs were carried out. The value of the conductivity was obtained by taking the average of the four measurements and the uncertainty was the SD from the mean value.

4. Results and discussion

4.1. Water uptake and ionic exchange capacity

In general the conductivity of acidic membranes depends on the water uptake and the Ionic Exchange Capacity (IEC) of the membranes. The IEC represents the density of sulfonic acid groups into the composite membrane. From the water uptake in the channels of the composite hydrated the protons are present in the H_3O^+ form where the hydrogen-bonded to water molecules is very strongly when the composite has higher acid strength. Values of the water uptake and the IEC obtained for all the studied membranes are given in Table 1. The results obtained for the water uptake show that this parameter is very dependent on the amount of CH. For example, when the amount of CH in the composite membrane is 20% and the amount of PEC is 80%, sample (CH20/PEC80), the water uptake decreases about 80% from the value of pure Chitosan membrane. However this decrease is about 60% for composites membranes of CH50/PEC50, and for a pure Pectin membrane the water uptake is about 150% of the previous, which means a 40% decrease from membranes of pure Chitosan. The ionic

exchange capacity (IEC) decreases from 0.37 meq/g for pure Chitosan (CH/PEC 100/00) until 0.09 meq/g when the amount of Pectin is 50%. When the amount of Pectin increases until pure membrane 00/100, the IEC increases up to 0.36 meq/g. From the values given in Table 1 we can see that the IEC of pure membranes CH100/PEC00 and CH00/PEC100 are higher than composite membranes.

Table 1. Water uptake and ionic exchange capacity (IEC) for the CH/PEC membranes.

Membrane	**Water uptake (%)**	**IEC (meq g^{-1})**
CH100/PEC00	249±4	0.37±0.01
CH80/ PEC20	172±9	0.31±0.01
CH60/ PEC 40	141±7	0.26±0.1
CH50/ PEC50	91±5	0.09±0.01
CH40/ PEC 60	56±3	0.20±0.01
CH20/ PEC80	48±3	0.30±0.01
CH00/ PEC100	149±9	0.36±0.01

The change observed in the ionic exchange capacity of the composite membranes can be related with the formation of the ionic complex between the carboxyl groups of PEC and the ammonium groups of CH, since as we increase the amount of one of them, the amount of ionic groups also increase. This therefore increases the formation of interstitial clusters that will result in an increase of ionic carriers into the matrix of the composite membrane. On the contrary, when the ratio is 1:1, then the IEC decreases noticeably as ion carriers have been reduced due to the formation of a chemical structure wherein the polymer has been compacted and where the number of ionic groups has decreased probably due to the existing chain reaction between the two

polymers. As consequence of this, some structural changes related with the starting state are given respect to only PEC or CH. On the other hand, the decreasing of IEC in the composite membrane could be associated with the decreasing of water uptake which is related with the formation of the ionic complex between the carboxyl groups of Pectin and the ammonium groups of Chitosan.

4.2. Mechanical properties

Static tensile strength tests were carried out at room temperature following the experimental procedure described above. The experimental results are gathered in Table 2.

Table 2. Membrane thickness, mechanical parameters, strain at yield point ε_y(%), ultimate tensile strength $\sigma_{ult,}$ and Young's modulus E measured at ambient temperature. In the last columns are given the methanol apparent permeabilities of CH/PEC composite membranes measured at 50ºC.

%Chitosan	L(μm)	E (MPa)	ε_y (%)	σ_{ult} (MPa)	Px10^6 (cm^2/s)
100	395±2	7.2±0.4	4.9±0.6	28.7±1.1	4.24±0.04
80	277±2	7.4±0.5	7.7±0.8	29.3±0.7	3.26±0.06
60	219±2	7.6±0.3	13.8±2.2	30.3±1.5	2.29±0.02
50	222±2	5.6±0.2	6.3±1.3	34.6±1.0	1.51±0.02
40	182±2	5.5±0.1	4.4±0.7	21.7±1.8	2.33±0.06
20	193±2	5.3±0.8	1.8±0.8	19.5±1.9	3.10±0.05
0	257±2	3.9±0.8	10.2±1.9	18.6±1.7	3.90±0.04

From this Table we can see that the increase in Pectin produce changes in the mechanical properties of the membranes. This could be related to the formation of the ion pair. This interaction favors the increase in the yield strength (ε(%)), which can be related to the electrostatic attraction possessing polymers that form the polymeric matrix. This attraction tends to recompose the matrix to its original form and as the amount of ionic bonds has been increased in the matrix then the polymer has greater stretch ability than before fracture. The formation of an ionic pair produces an increase in the ultimate tensile strength until the amount of Chitosan/Pectin is the same (1:1). However the Young modulus increased while the amount of Chitosan decreased until the ratio was 60% of Chitosan and 40% of Pectin. From this percentage the Young modulus decreases by the incorporation of pectin into the chitosan matrix from 7.6MPa for membranes of CH60/PEC40 until 3.9MPa for pure Pectin membranes. The same behavior is observed for the yield strength, where this value increases from pure Chitosan to composite membranes of CH60/PEC40, where the yield strength is about 300% higher than pure Chitosan membranes. From this ratio to CH80/PECc20 the yield strength decreases about 600%. However, in the case of pure Pectin membranes the yield strength is almost double the value of pure Chitosan membranes.

From these results we think that composite membranes of CH60/PEC40 have the best mechanical properties to be used as sustainable membranes in green chemistry applications. A comparison with Chitosan membranes, of high degree of deacetylation and molecular weights (Wan et al., 2003) shows that our composite membranes have a tensile strength and breaking elongation slightly smaller to this one however our composite have a molecular weight of 2 or 3 times lower than the others. It could be due to diminution of crystalline regions in our composite in comparison with the CH membranes with high degree of deacetylation (DDA).

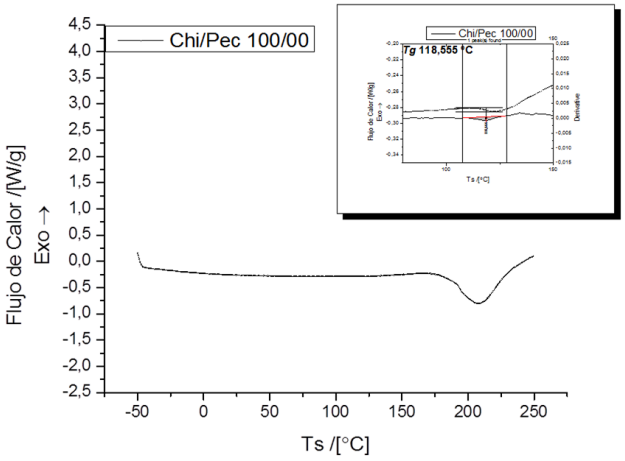
A comparison between CH/PEC and Sorbitol-Chitosan (S-CS) membranes and phosphorylated-Chitosan membranes (CS-P) (Holder et al., 2016), shows that our membranes have a tensile strength 100 times higher when the amount of pectin ranges between 20-40%. On the other hand, CH/PEC membranes have a tensile strength 60% smaller than CS-P with an elongation between 3 and 5 times smaller.

4.3. Differential scanning calorimetry (DSC)

DSC thermograms of sulfonated CH/PEC membranes with compositions 100/00, 60/40, 50/50, 40/60 and 00/100 at 10 K/min rate, under a nitrogen atmosphere, for 100 to 250ºC range, using 0.5% glycerol (GLI) as plasticizer are shown in Figures 1a, 1b and 1c. CH/PEC 100/00 presents the typical Tg(I) and Tg(II) endothermic trials as it has been reported when the second scan is performed (Mucha & Pawlak, 2005). Tg(I) at 118°C refers to water-plasticized chitosan macromolecules. Tg(II) at ~ 190°C attribute to unplasticized chitosan macromolecules. As Mucha & Pawlak have pointed out, referred to Tg (II), this event is a broad jump at 170-220°C, which can be recognized as their Tg reflecting increase of molecular movement due to dissociation of hydrogen bonds and starting of molecular chain scissions. However, those researchers have performed their analysis with uncrosslinked chitosan. Other researchers (Neto et al., 2005; Thacharodi & Panduranga, 1993; Jen & Wen, 2011) have addressed the endothermic peak circa 170°C to the glutaraldehyde use as a crosslinking agent. They informed that there were only endothermic events at 168°C using a single scan. The authors suggested that glutaraldehyde can react with amine groups of chitosan. Thus, water molecules will be bound to hydroxyl groups instead of amino groups, which are less electronegative than hydroxyl ones. Therefore, the endothermic peak was shifted from 133°C to 168°C for uncrosslinked and crosslinked chitosan, respectively. In our

study this explanations are complementary. The thermal behavior of pectin samples has been previously described by Einhorn-Stoll et al. (Einhorn-Stoll, Kunzek, & Dongowski, 2007; Einhorn-Stoll & Kunzek, 2009; Einhorn-Stoll & Kunzek, 2009). These researchers mentioned that pectin thermal behavior depends on the chemical composition and on state transitions, occurring during processing, as well as on the interdependence of both factors. Some pectin samples showed an exothermic degradation peak between 180-270°C. Also, an endothermic pre-peak appeared before the degradation peak, corresponding to a conformational change that might be the transformation from the more stable $^{4}C_{1}$ chair conformation of the galacturonan ring via a boat conformation $^{1,4}B$ to the inverse $^{1}C_{4}$ chair conformation. Other researchers have also observed that this conformational change has a higher free energy G (Marszalek et al., 1999; Jarvis, 2002; Zhang & Marszalek, 2006; Haverkamp, Marshall, & Williams, 2007; Williams, Marshall, Anjukandi, & Haverkamp, 2007). These thermal events are present in the DSC thermograms of CH/PEC 0/100. The pure pectin thermogram shows an exothermic degradation peak at 234°C, and there is an endothermic pre-peak at 205°C. Also, at T=127°C another endothermic event similar to CH is observed related to water-plasticized pectin macromolecules and the sulfonic groups are incorporated by the functionalization of the reaction with 4-formyl-1-3-benzenedisulfonic acid.

From Figure 1 we can see that the thermograms of blend membranes are different from those of pure polymers.



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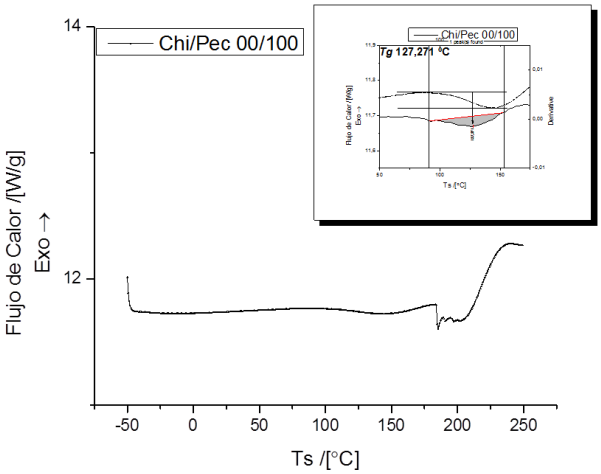

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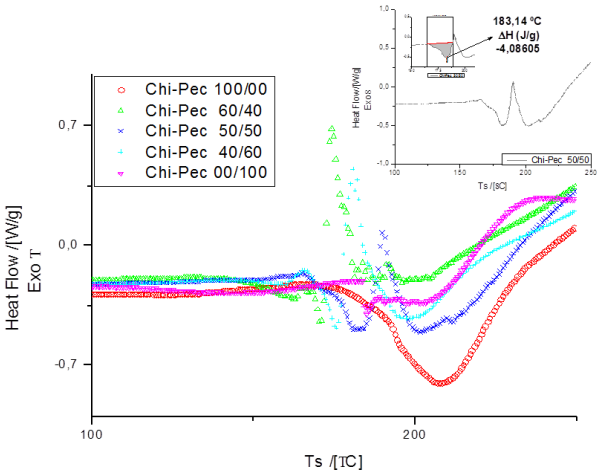

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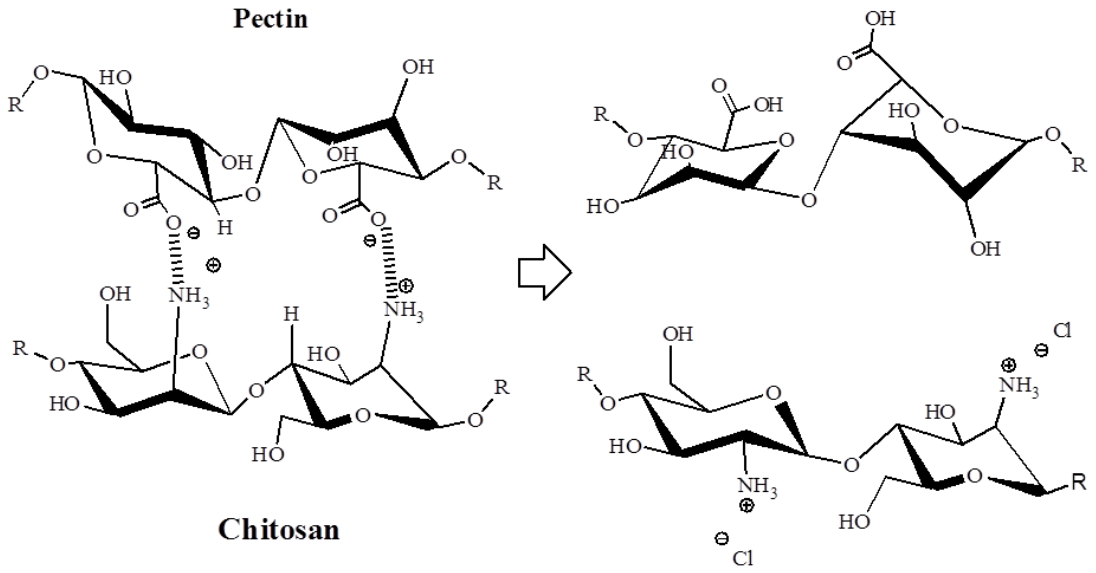
389 **Figure 1.** DSC thermograms of: a) CH100/PEC00, b) CH00/PEC100 and c) DSC of

390 CH/PEC blend membranes

Analysis of the DSC curve for the CH/PEC mixtures showed a pair of endothermic and exothermic peaks below 200°C. Blend membranes have an endothermic peak at 171.2, 175.8 and 183.1ºC for CH60/PEC40, CH40/PEC60 and CH50/PEC50, respectively, like are showed in Table 3. The detected endotherm is attributed to the evaporation of water tightly linked through polar interactions to ionic groups (Ribeiro, Silva, Ferreira, & Veiga, 2005) and can be assigned to the excision of an ion pair between the carboxylic group (-COOH) of the Pectin and the ammonium group ($-NH_3^+$) of the Chitosan. All this is confirmed by the fact that the peak is shifted to the higher temperature as the composition of the membranes tends to be 1:1.

Table 3. Values of temperature and enthalpy corresponding to endothermic and exothermic peaks of blend membranes and pure polymers. In the last column we give the relative average molecular weight of membranes.

Membrane CH-PEC	**Endothermic peak**			**Exothermic peaks**			**M_w**
	T (ºC)	**ΔH (J/g)**	**ΔH (kJ/mol)**	**T (ºC)**	**ΔH (J/g)**	**ΔH (kJ/mol)**	
100-00	-	-		-	-		250000
60-40	171.2	-1.62	-285.01	174.3	5.2	915.2	176000
50-50	183.1	-4.09	-643.55	190.0	3.59	565.5	157500
40-60	175.8	-2.29	-317.74	180.9	7.36	1023.1	139000
00-100	-	-		-	-		650000



408
409 **Figure 2.** Reaction to the excision of an ion pair between the carboxylic group (-
410 COOH) of the Pectin and the amine group ($-NH_3^+$) of the Chitosan.

411

412 The representation of the reaction of the ionic complex ion between the two polymeric
413 components of the CH/PEC membranes is shown in Figure 2, where the thermograms
414 of blend membranes also shows the exothermic peaks corresponding to the subsequent
415 relaxation of the molecular polymeric matrix. The peaks are centered at around 174.3,
416 180.9 and 190.0°C for CH60/PEC40, CH40/PEC60 and CH50/PEC50, respectively,
417 which could be assigned to the formation of an ionic pair between the carboxylate group
418 ($–COO^-$) of pectin and the amine group $–NH3^+$ of chitosan due to dehydration during
419 the DSC scan. Similar results were found in CH/PEC, chitosan/alginate and
420 chitosan/carboxycellulose blends (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani,
421 2007; Mayur, Rajashree, Jolly, & Vijay, 2007; Ostrowska-Czubenko & Gierszewska-
422 Drużyńska, 2009; Rosca, Popa, Lisa, & Chitanu, 2005). From the area of the

endothermic peaks we calculated the enthalpy of the process the excision of an ion pair supposing that the relative average molecular weight of Chitosan and Pectin are 250000 and 65000, respectively. The values obtained are 285.0 kJ/mol (1.62 J/g), 317.7 kJ/mol (2.29 J/g) and 643.5 kJ/mol (4.09 J/g) for CH60/PEC40, CH40/PEC60 and CH50/PEC50, respectively. These results confirm the greater formation of complex ions when membrane composition is tending to 1:1, point where it is necessary to give more temperature and energy to break the complex. Finally in the thermograms we can see that around 235ºC of temperature the degradation of the Pectin begins to occur. Similar results have been found by Gümüsoglu et al. (Gümüsoglu, Albayral, & Deligöz, 2011), where the ionic interaction of the base polyelectrolyte complexes (PEC) membranes formed by Chitosan and poly(acrylic acid (PAA) produce an enhancement of their mechanical properties at least until 230ºC.

4.4. Methanol permeability

The Methanol permeability has been measured analyzing the change of methanol concentration in the receptor chamber. The temperature of the bath was fixed at 50ºC and donor chamber was filled with a 2M methanol solution. Samples of 500μl from receptor chamber were taken within a period between 0 and around 4 hours. The samples were analyzed by gas chromatography and their chromatograms compared with the calibration curve, correlating the chromatograms peak areas with methanol concentrations. The Figure 3 shows the function ($C_B \cdot L \cdot V_B / A \cdot C_A$) versus time measured at 50ºC for CH/PEC membranes. Linear trends are obtained and the slopes estimated. The values obtained from the slope of the plots permit us to obtain the apparent methanol permeability of the membranes by means of eq.(2). In last column of Table 2 we can see the results obtained for the apparent permeabilities of CH/PEC membranes.

A close inspection of this Table shows that the permeability decreases when the concentration of Chitosan and Pectin in the membrane is 1:1, and increases when the membrane is pristine Chitosan and pristine Pectin, respectively. In the case of membranes where the amount of each polymer is different, such as CH60/PEC40 or CH40/PEC60, the apparent permeabilities are practically the same.

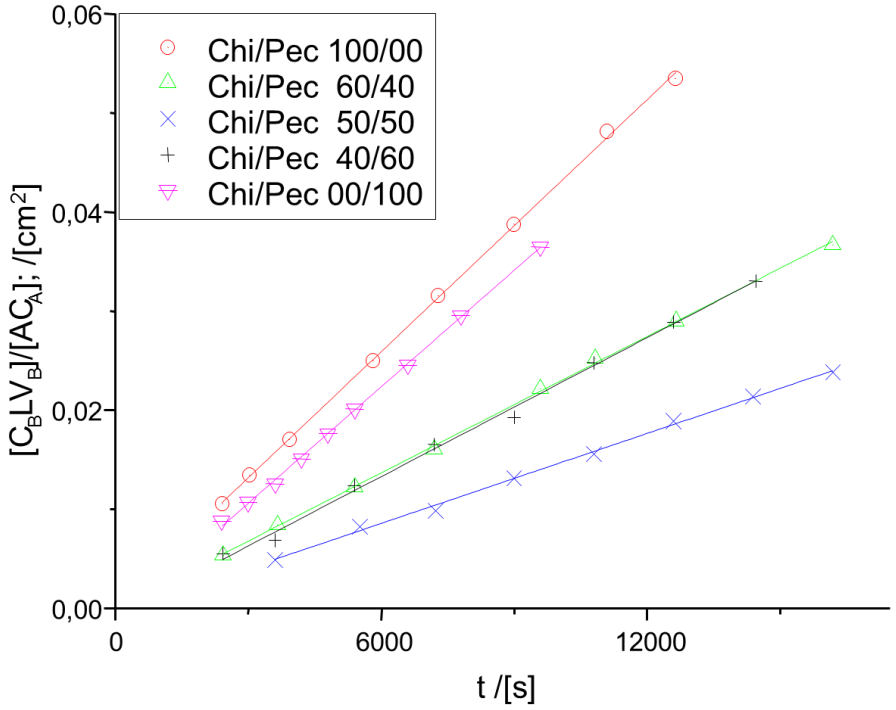


Figure 3. Representation of ($C_B \cdot L \cdot V_B / A \cdot C_A$) versus time of the CH/PEC composite membranes. The slope equates to the apparent methanol permeability.

From the Table 2 we can observe that for CH100/PEC00 membranes the apparent methanol permeability is (4.24±0.04)x10^{-6} cm^2/s, while for CH00/PEC100 this value is (3.90±0.04)x10^{-6} cm^2/s. These values are quite similar to the values found for pristine Nafion® and Alginate90% /Carrageenan10% membranes where the apparent methanol permeabilities are about 2.2x10^{-6} and 4.0x10^{-6} cm^2/s, respectively, but eight times higher than Alginate membranes (Pasini-Cabello et al., 2014). Low concentrations of

Pectin in the membranes have an effect similar to low concentrations of Chitosan in the composed membranes. However, when the amount of Chitosan or Pectin increases in the membrane the permeability decreases. This could be due to the reaction of the formation of complex ions between the carboxyl groups of pectin and chitosan-ammonium groups, producing a chemical structure more compact and stable due to the possible reaction between the chains of both complexes, as shown in Figure 2. These results also can produce a relative change of phase with significant changes in their physical parameters, such methanol permeability, mechanical properties, swelling as consequence of the compacting of its chemical structures in the formation of ionic complex.

4.5. Conductivity results

Impedance spectroscopy measurements were carried out for CH/PEC membranes with compositions 100/00, 80/20, 60/40, 50/50, 40/60, 20/80 and 00/100 at different temperatures in order to obtain the conductivity and the diffusion coefficient of ionic charge carriers. All the impedance measurements were done after hydration of the composite membranes. From the dielectric measurements, the electrical conductivity σ can be obtained from the imaginary part of the complex dielectric permittivity ($\varepsilon^*=\varepsilon'-j\varepsilon''$) where $\varepsilon''= \sigma/(\varepsilon_0 \omega)$, being ε_0 the permittivity of vacuum and ω the angular frequency of the applied electric field. The region where the slope of log ε'' versus log ω is equal to -1 allows extrapolate to low frequencies and the intercept of this straight line is the dc-conductivity (σ_{dc}). Alternatively, the data obtained can be analyzed in terms of the Bode diagram (Compañ, Riande, Fernández-Carretero, Berezina, & Sytcheva, 2008; Paddison, Kruer, & Maier, 2006). Typical plots of Bode diagram are shown in Figure 4. This figure reveals that the real part of the conductivity increases with the frequency and

tends to a constant value when the phase angle, φ, reaches a maximum, for each temperature.

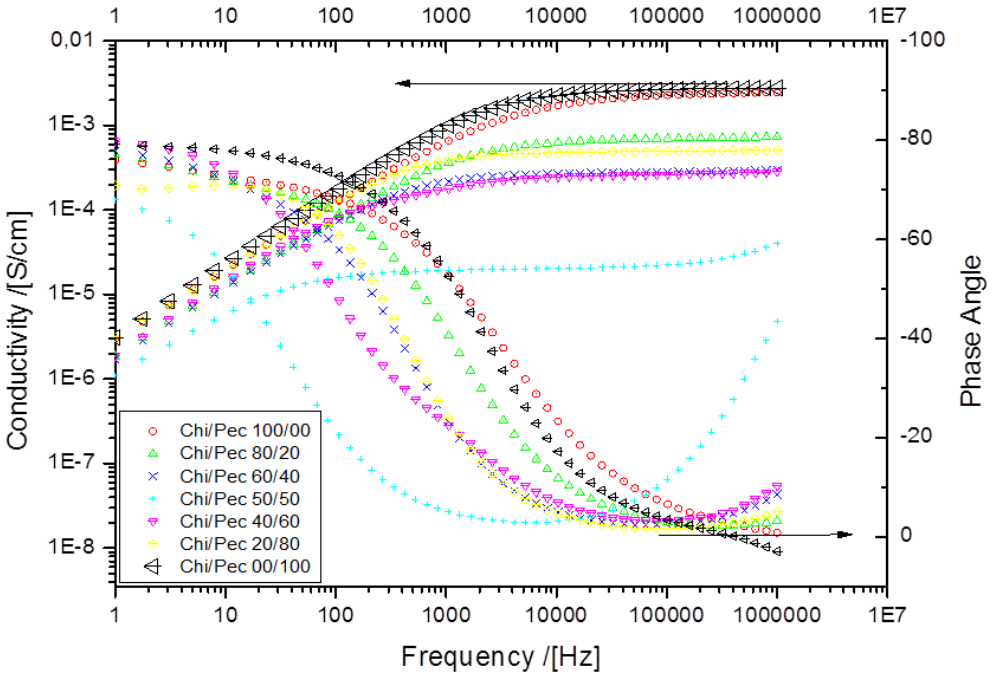


Figure 4. Bode diagram for the CH/PEC membranes obtained at 30ºC of temperature.

As it can be seen in Figure 4, all the samples, at 30ºC of temperature, shown a plateau in the plot of the real part of the conductivity *vs* frequency in the region of moderate and high frequencies (10^3 to10^6 Hz) coexisting with the peak of the phase angle or the tendency to the value zero. The value of the conductivity for which ϕ reaches a value practically equal to zero can be taken as the proton conductivity of the membrane. Values of the conductivity for all the membranes of CH/PEC are given in Figure 5.

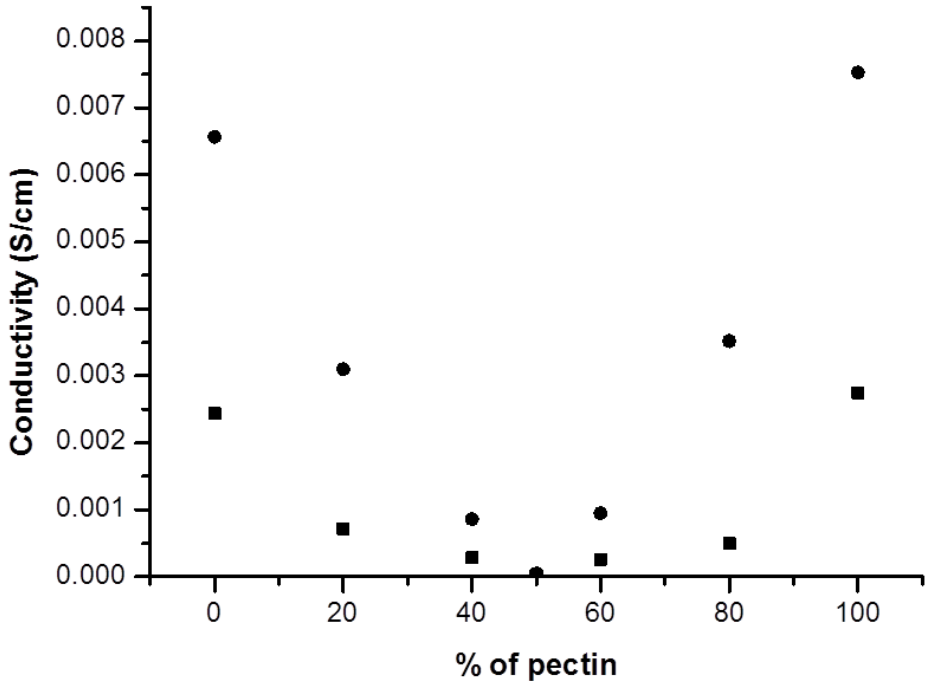


Figure 5. Conductivity of CH/PEC membranes with respect to the amount of pectin measured at 30ºC (■) and 50ºC (●), respectively.

As we can see, the conductivity for pristine Chitosan (CH100/PEC00) was 2.44×10^{-3} S/cm and 6.57×10^{-3} S/cm at 30 and 50ºC respectively, decreasing when the amount of pectin increases, until the proportion of Chitosan and Pectin in the membrane is 1:1, where its conductivity is about two orders of magnitude smaller. These values are higher than estimated for membranes based on Chitosan crosslinked with glutaraldehyde (around 1.3×10^{-4} S/cm) (Majsztrik, Satterfieldf, Bocarsly, & Benziger, 2007), but lower than membranes of Chitosan crosslinked in sulphuric acid (about 1.83×10^{-2} S/cm) (Bass & Freger, 2008). On the other hand, our CH membranes have values of conductivity quite similar to the value found by Shirdast et al. 2.2×10^{-3} S/cm (Shirdast, Sharif, & Abdollahi, 2016), and lower than usually reported by others

516 researchers (Wan et al., 2006; Khiar, Puteh, & Arof, 2006). However our results are
517 more satisfactory due to our membranes have lower IEC (0.37 meq/g) than Shirdast et
518 al. which value was around 0.97 meq/g. The small differences found in conductivity can
519 be attributed to lower degree of deacetylation of Chitosan of this work (around 75%), in
520 comparison with those used by others (between 80 and 90%). On the other hand, our
521 membranes have values higher of IEC than obtained by others (Shirdast et al., 2016),
522 where CH and Sorbitol-Chitosan (S-CH) membranes had values of 0.24 and 0.30
523 meq/g, respectively. (Note that the abbreviation of Chitosan in other references (Holder
524 et al., 2016; Shirdast et al., 2016) is CS in place of CH used in our manuscript).
525 On the other hand, when the amount of Chitosan increases in the composite membrane
526 of CH00/PEC100, the conductivity has the same tendency. The conductivity for pristine
527 chitosan membranes varies from $2.75x10^{-3}$ S/cm at 30ºC and $7.53x10^{-3}$ S/cm at 50ºC to
528 the value of $5x10^{-5}$ S/cm for CH50/PEC50 membranes. The conductivities of our
529 membranes are one order of magnitude higher than the ionic conductivity found in
530 hydrated Chitosan membranes prepared from various degrees of deacetylation (DDA)
531 and different molecular weights from aqueous solutions of Chitosan and acid acetic
532 (Wan et al., 2003). This may be due to increased degree of crystallinity that Chitosan
533 DDA membranes possibly due to restricting the mobility of protons as a result of
534 increased stiffness of the polymer and tortuosity related to increased crystallinity.
535 A comparison between the conductivities of our composite membranes and those CH,
536 S-CH and phosphorylated-Chitosan (CH-P) membranes of other authors (Holder et al.,
537 2016) shown that our composites have an internal resistance comprised between 500
538 and 4500 Ω . These values are in the same order of magnitude than Chitosan (464Ω),
539 Sorbitol-Chitosan (804 Ω) and Sorbitol-Phosphorylated–Chitosan membranes (2984
540 Ω) (Holder et al., 2016).

Electrochemical performance of CH, S-CH and CH-P membranes investigated in single microbial fuel cells (MFCs) for bioelectricity generation have shown an excellent behavior. The results of the performance and durability show that during bath cycle of MFC operating with CH membrane, a maximum voltage of 152.6 mV was reached at day 5 out of 7 days it took to reach cycle completion. The results are better when the membrane used was S-CH, where this membrane was able to sustain a bath cycle for 12 days with maximum voltage of 464 mV with a power density of 94.59 mW/m^2. These results are even better in the case of membranes of CH-P where the maximum voltage was 504 mV and the maximum power density 130.03 mW/cm^2. When the membrane S-CH-P was situated in the MFC system took 15 days to reach the maximum voltage of 294 mV (Holder et al., 2016). That means that this kind of membranes can sustain themselves a lot of days until reach the end of cycle. These results allow us to think that our membranes can also to be used as PEM in fuel cells such as PEMFC, DMFC or MFCs.

The most important parameters to characterize and estimate the ionic transport in polymers and membranes is the mobility associated to the total charge carrier concentration. However, they are in general difficult to quantify and it is still at the present time a matter of discussion in the scientific literature (Compañ et al., 2008). To give an estimation of the diffusivity from the dielectric spectra we have considered the model described previously based on the analysis of the dielectric spectra of the electrode polarization where it is consider neglected the ion-ion interactions and convection flux. Considering in first approximation that all the ionic capacity into the membrane is involved in the distribution of ions that can move freely through the membrane, the proton conductivity and ion diffusivity can be expressed according the

Einstein relation (Paddison et al., 2006), but a misprint in eq.(4) of this reference F was written instead of F^2, now the equation (3) is correctly stated as:

$$\sigma = \frac{c_+ F^2 D_+}{RT} \qquad (3)$$

Where R is the universal gas constant, F the Faraday constant, T the absolute temperature, c_+ the ionic exchange capacity and D_+ the proton diffusion coefficient. The expression (3) permits us to obtain the diffusivity of the protons through the CH/PEC membranes from the values of conductivity obtained from the Bode diagram and the values measured for the ionic exchange capacity given in Table 1. The results found for the diffusion coefficient shows that vary in proportion to the ratio (σ/IEC). The values obtained at 30ºC are 1.70×10^{-6} cm^2/s and 2.0×10^{-6} cm^2/s for pristine Chitosan and Pectin membranes, where this value decreases for the composite membranes until the lowest value of around 0.6×10^{-7} cm^2/s is reached, corresponding to the CH50/PEC50 membranes. These values are quite close to that found in Nafion® 117 (Wan et al., 2006; Khiar et al., 2006), perfluorinated nanocomposite membranes modified by polyaniline (Compañ et al., 2008) and bio-polymeric membranes of Alginate-Carrageenan, but in such cases the membranes had less IEC and water uptake that this one (Pasini-Cabello et al., 2014). At it is known the IEC and swelling of the membranes will affect the ionic permeability through the membrane. In this sense the high degree of swelling into the membrane will allow ions to move through the membrane increasing its diffusivity in the swollen state. Such is shown in Table 1 the values of pristine membranes have higher values of IEC and swelling than composite membranes. This produces a significant increase in conductivity. This behavior could be due to the strong interaction from the intramolecular hydrogen bonds between hydroxyl groups and amine groups which are harder than intermolecular hydrogen interactions bonds between polar groups and water.

590 The interest of CH is the tendency to acquire high hydrophilicity, very easy chemical
591 modification and goods mechanical and thermal resistance. The CH with amino groups
592 present a polycationic character and form ionic cross links in presence of acidic groups
593 (Osugi, Dong, Hexig, & Inoue, 2007) producing polyelectrolytes complexes membranes
594 solving the dopant migration to give effective performance in DMFCs for prolonged
595 periods (Göktepe, Çelik, & Bozkurt, 2008). The main concern of this type of
596 membranes is the possible degradation, during cell operation conditions. In this way
597 some authors (Gümüsoglu et al., 2011), have investigated the oxidative and hydrolytic
598 stability of Chitosan and poly(acrylic Acid) membranes found that can be suitability for
599 use in fuel cells such as DMFCs in which diluted methanol were used. In this way
600 hydrolytic and oxidative stability of the CH/PEC membranes were tested to investigate
601 the convenience for use as PEM in fuel cells. The hydrolytic stability of this type of
602 membranes was characterized controlling the time duration until membrane broken after
603 immersion in hot water. The results found for our membranes were around 125 hours
604 quite similar to PEC membranes which were more than 120 hours. These results show
605 that both kinds of membranes are suitable for fuel cells applications.
606 Several works have reported results from an *ex situ* vapor phase hydrogen peroxide test
607 (Delaney & Liu, 2007; Hommura, Kawahara, Shimohira, & Teraoka, 2008) and found
608 that gas phase hydrogen peroxide is very aggressive toward perfluorosulfonated (PFSA)
609 membrane, causes chain scissions in the backbone and in the side chain (Endoh, 2008).
610 In our membranes the *ex situ* vapor phase hydrogen peroxide test has allowed observe
611 the weight retention of our membranes as function of time, such as is shown in Figure 6.
612 A close inspection of this figure shows that the weight of each membrane decreases
613 gradually. On the other hand, we can observe that pure chitosan membranes (CH/PEC
614 100/00) are completely degraded after 24 h. This behavior has also been reported by

other researchers (Tian, Liu, Hu, & Zhao, 2004) and they have mentioned that this type of change in the fundamental chitosan structure appears to be possible due to the role of the amine group in the degradation of polysacharides by hydrogen peroxide. The glycoside bonds are more susceptible to split since there is an amine group adjacent to the C_2 pyranose ring.

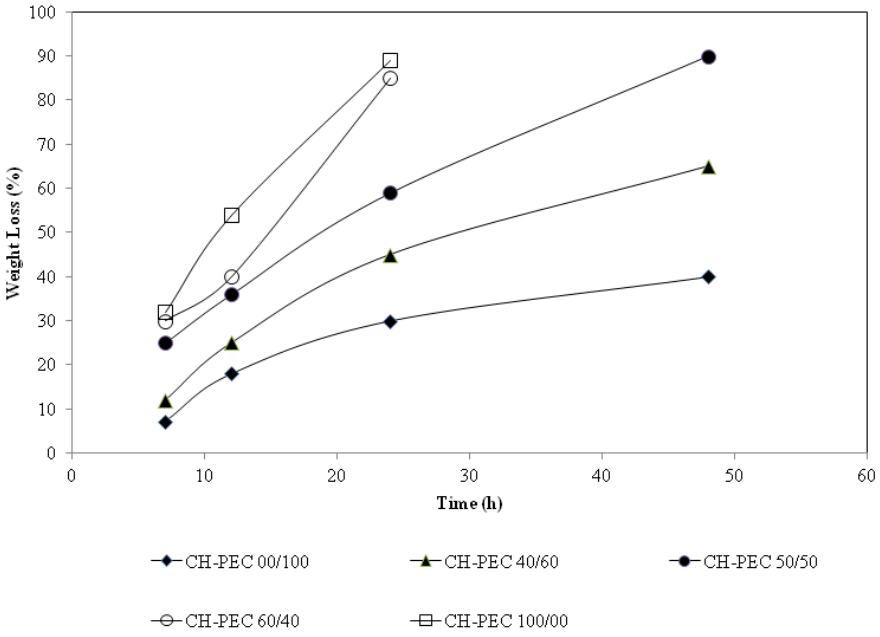


Figure 6. Chemical stability studies of CH/PEC membranes from Fenton test. In this plot we observe the weight loss versus time using as Fenton's reagent in vapor phase 2ppm $FFeSO_4$ in 3wt% H_2O_2 at 80ºC.

However, Pectin based membranes are more resistant to this test such as we can observe from Figure 6 where an increase of amount of PEC produces a significant increase of membranes durability. On the other hand, CH/PEC membranes allow fairly good chemical stability as the sulfonation degree decreases, i.e the IEC decrease.

Therefore, our composite membranes with pectin and lower water uptake are expected to take good chemical stability and durability than pristine CH membranes. As we have seen in our composite membrane, adding Pectin to Chitosan o vice-versa the formation of ionic complex produce a diminution of IEC and degree of swelling which compact the polymer matrix, developing a loss of ionic groups until almost disappear as a consequence of the ionic pair formation where the hydrophilic channels become more compacted increasing the chemical stability and durability of the membranes. Furthermore, we believe that CH/PEC membranes merits further investigation and proportions of CH or PEC in the composite below than 40%, can be use as proton exchange membranes for MFCs and DMFCs, using dilute methanol or ethanol as fuel.

5. Conclusions

To summarize, composite membranes of Chitosan and Pectin were obtained and characterized. The pristine Chitosan and Pectin membranes showed higher proton conductivities, IEC, water uptake and methanol permeability than composite membranes of CH/PEC. This is due to the formation of an ionic complex, which compacts the polymer matrix and therefore reducing the hydrophilicity of the channels. When the ion pair between the carboxylic group (-COOH) of the Pectin and the amine group ($-NH_3^+$) of the Chitosan reacts, it is produced a diminution of content of amine and carboxylic groups decreasing the hydroxyl groups and hence the hydrophilicity and swelling of the membranes. When the content of the composite membrane is 50:50 wt, the ionic strength fraction of the ions is practically the same, reducing around two orders of magnitude its properties with respect to the ionic transport. Our results shown that the methanol permeability is reduced, blocking off methanol molecules passing through the membrane about three times more compared to pristine membranes. On the

654 other hand, the composite membranes with a content 50/50wt have ultimate tensile
655 strength σ_{ult} higher than the others possible due to the channels become more
656 compacted.
657 The *ex situ* vapor phase hydrogen peroxide test has allowed observe that the weight of
658 each membrane decreases gradually. Pristine chitosan membranes (CH/PEC 100/00) are
659 completely degraded after 24 h. This behavior can be possible due to the role of the
660 amine group in the degradation of polysacharides by hydrogen peroxide. The glycoside
661 bonds are more susceptible to split since there is an amine group adjacent to the C_2
662 pyranose ring.
663 However, PEC based membranes are more resistant to Fenton's test where a increasing
664 of PEC produce a significant increase of membranes durability. On the other hand,
665 CH/PEC membranes allow fairly good chemical stability as the sulfonation degree
666 decreases, and by ender the IEC.
667 Then CH/PEC membranes merits further investigation and proportions of chitosan or
668 pectin in the composite below than 40%, can be rise to membranes presumably good
669 candidates for use as proton exchange membranes for MFCs and DMFCs, using dilute
670 methanol or ethanol as fuel. Additionally, the methodology presented in this work may
671 be extendable to other applications considering the influence of the amount of each
672 component into the composite membrane as consequence of the formation of the ionic
673 complex between the carboxyl groups of pectin and amine of chitosan, producing a
674 chemical structure more compact and stable due to the reaction between both groups.
675
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