



## Chitosan/pectin polyelectrolyte complex as a pH indicator

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### ABSTRACT

A polyelectrolyte complex (PEC) matrix formed between chitosan and pectin was developed to entrap a bioactive compound (anthocyanin), obtaining an useful pH indicator device. Polysaccharides of opposite charges such as chitosan and pectin can have a very strong intermolecular interaction. The innovation lies in obtaining a new system based on natural and biodegradable compounds, which is simple to manufacture, to indicate variation in pH by visual changes in colour. This device has potential applications in food packaging. The PEC was studied using chitosan and pectin solutions at different pHs values (3.0, 4.0, 5.0 and 5.5) and pectin/chitosan molar ratios (1.0 to 10/1.0 to 5.0). PEC films were homogeneous and showed the highest yield (60.0%) at pH 5.5. Diffusion tests indicated efficient bioactive compound entrapment in the PEC matrix. Thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy indicate the compatibility between the polymers and bioactive compound.

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### 1. Introduction

A polyelectrolyte complex (PEC) is formed by ionic interactions between polyanions and polycations. It has unique properties, which are significantly different from those of the initial components. Basing a PEC on natural polymers such as chitosan and pectin can improve its mechanical properties. The electrostatic attractions between the ionised amino groups of chitosan ( $\text{NH}_3^+$ ) and the ionised carboxyl acid groups ( $\text{COO}^-$ ) of pectin are the main interactions in the formation of the pectin/chitosan PEC (Rashidova et al., 2004). Different interactions (van der Waals, electrostatic, hydrophobic and hydrogen and coordination bonding) can occur between the different groups in polymer–polymer complexes. In polysaccharide structures such as chitosan and pectin the presence of polar functional groups results in a very strong intermolecular interaction and highly ordered orientation of the rigid-chain polymers (Rashidova et al., 2004; Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007). The stability of these complexes depends on pH, temperature, charge density and ionic strength among other environmental conditions (Recillas et al., 2011).

Chitosan is a linear cationic polysaccharide obtained from chitin, found in the shells of shrimp, lobsters and crabs. It is

characterised by its formation of flexible and resistant films with an efficient oxygen barrier (Yoshida, Bastos, & Franco, 2010; Recillas et al., 2011). It is composed of *N*-glucosamine and a small amount of *N*-acetyl glucosamine and is classified according to degree of deacetylation (Ghaffari et al., 2007). Pectin is a natural, low toxicity and anionic polysaccharide extracted from the cell walls of most plants, such as apples, oranges and pears. It is characterised by its gelling property and branched heteropolysaccharides, which consist predominantly of linear chains of partially methyl-esterified (1,4)  $\alpha$ -D-galacturonic acid residues (Ninan et al., 2013). Depending on the degree of substitution of d-galacturonic carboxyl groups by methoxyl groups ( $-\text{OCH}_3$ ), defined as the degree of esterification (DE), pectins are classified as high-esterified pectins ( $\text{DE} > 50\%$ ) or low-esterified pectins ( $\text{DE} < 50\%$ ) (Jindal, Kumar, Rana, & Tiwary, 2013). This describes the percentage of methoxylated  $\text{C}_6$  atoms in the galacturonic acid backbone and strongly determines the gelling properties. Other important molecular parameters are molecular weight and galacturonan content (GC), which indicates the purity of the pectin (Lopes da Silva & Rao, 2006; Einhorn-Stoll, Kastner, & Drusch, 2014).

Many studies, mainly in medical areas and on drug delivery, have addressed production and characterisation of matrices obtained from chitosan/pectin PEC (Rashidova et al., 2004; Bigucci et al., 2008; Naidu et al., 2009; Cunha & Gandini, 2010; Brinques & Ayub, 2011; Coimbra et al., 2011; Recillas et al., 2011). However, no application of PEC as a pH indicator device was found in the literature.

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The growing concern of consumers over the safety of foods has led to the investigation of alternative natural preservation technologies (Alzoreky & Nakahara, 2003; Latou, Mexis, Badekaa, Kontakos, & Kontominas, 2014). The pH is one of the most important factors influencing the quality, safeness and freshness of food (Smolander, 2003). Zhang, Lub, and Chen (2014) described the advantages of optic or visual pH sensors, such as their small and compact size, safety, long-distance transmission, sensitivity and low cost. One of these new technologies, the colour-based pH indicator, offers potential for the indication of microbial metabolites (Kerry, O'Grady, & Hogan, 2006), since microbiological growth can induce a change in pH (Smolander, 2003). The study of alternative natural packaging devices was developed by Veiga-Santos, Ditchfield, and Tadini (2011), who produced a biodegradable film based on cassava starch plasticised with sucrose and inverted sugar-containing grape and spinach extracts as a pH indicator. Yoshida, Maciel, Mendonça, and Franco (2014) evaluated chitosan films containing anthocyanin as a pH indicator device. Maciel, Yoshida, and Franco (2012) developed a prototype of a colourimetric temperature indicator for monitoring food quality using chitosan suspensions containing anthocyanin applied on card paper sheets. Almeida, Estela, Segundo, and Cerdà (2011) proposed a membrane-less gas-diffusion unit containing a pH indicator to determine ammonium in wastewater and river water. Capel-Cuevas, Cuéllar, Orbe-Payá, Pegalajar, and Capitán-Vallvey (2011) studied different matrices containing different synthetic pH indicators to form an optical pH sensor array based on neural networks. Zhang et al. (2014) prepared a colourimetric pH sensing film based on chitosan and glutaraldehyde, applied it on pork meat and fish and observed visual changes in colour from red to green in the pH range of 2.2–9.0. Lee and Lee (2014) developed a pH-sensitive colourimetric hydrogel using a catechol-conjugated alginate hydrogel and a pyrocatechol violet dye and obtained large colour changes and chemical stability without deformation over a wide range of pH (1.0–13.0). Bigucci et al. (2008) investigated the influence of PEC between chitosan and pectin on the release behaviour of vancomycin, verifying the best results in complexes prepared with 1:9 and 3:7 (chitosan:pectin) at pH 5.0. Ghaffari et al. (2007) studied PEC formation using pectin (high-methoxylated) and chitosan at pH 5.4, obtaining yields in the order of 70.0% with a ratio of 2:1 (pectin:chitosan).

The aim of this study was to develop and characterise PEC formation using chitosan and pectin in different proportions for potential application as an efficient pH indicator device. Zeta potential analysis, FTIR, TGA, SEM and diffusion tests were carried out to study and characterise the degree of interaction between polyions on PEC films.

## 2. Materials and methods

### 2.1. Materials

Chitosan (Primex, molecular weight of  $2.38 \times 10^5 \text{ g mol}^{-1}$  and percentage of acetylation of 9.1%, Iceland), acetic acid (Synth, Brazil), pectin (from citrus fruits) with a high degree of methoxylation (above 50% of groups esterified) (CPKelco, GENU® 105 rapid set, Brazil) and anthocyanin (ATH) powder obtained from grapes (Christian Hansen, AC-12r-WSP, Brazil).

### 2.2. Methods

#### 2.2.1. Determination of the degree of deacetylation of chitosan

An adaptation of the procedure of Raymond, Morin, and Marchessault (1993) and Santos, Soares, and Dockal (2003) was used. A quantity of 0.5 g of chitosan was solubilised in

50.0 mL 0.1 mol L<sup>-1</sup> hydrochloric acid (v/v). The suspension was stirred for 30 min at room temperature ( $25 \pm 2^\circ\text{C}$ ). Samples were titrated with NaOH solution (0.092 mol L<sup>-1</sup>). Changes in conductance were measured using a pH meter with results in mV. The degree of deacetylation (DDA) was calculated by  $\text{DDA} = (16.1 * [\text{base}] * (V_2 - V_1)) / m$ . The degree of acetylation (DA) was calculated by  $\text{DA} = 100 - \text{DDA}$ .

#### 2.2.2. Determination of the degree of esterification of pectin

In accordance with Boček, Zabivalova, and Petropavlovskii (2001), 0.2 g of pectin was placed in a weighing bottle for titration and wetted with ethanol (95.0%). Distilled water was heated to  $40^\circ\text{C}$  (20.0 mL) and added. The polymer was dissolved with magnetic stirring for 2 h. The solution was titrated with 0.1 M NaOH in the presence of phenolphthalein to a pale rose colour and the results were recorded as initial titrated solution ( $T_i$ ). The pH of the solution was measured. Ten mL of 0.1 M NaOH solution was added to neutralise the galacturonic acid in the sample and the mixture was stirred at room temperature for 2 h to saponify the esterified carboxyl groups of the polymer. Then 10.0 mL of 0.1 M HCl was added. Excess HCl was titrated with 0.1 M NaOH. The number of esterified carboxyl groups was calculated from the volume of 0.1 M NaOH titration solution ( $T_f$ ). The degree of esterification (DE) of the pectin was calculated using by the following equation:

$$\text{DE}(\%) = \frac{T_f}{T_i + T_f} * 100 \quad (1)$$

where  $T_i$  is the volume (mL) of 0.1 M NaOH used in the initial titration and  $T_f$  is the volume (mL) of 0.1 M NaOH used in the final titration.

#### 2.2.3. Chitosan/pectin PEC

PECs were prepared according to adaptation of the method of Ghaffari et al. (2007) and Bigucci et al. (2008). Chitosan (0.50 g 100 g<sup>-1</sup>) was dissolved in aqueous acetic acid. The stoichiometric amount of acetic acid was calculated from sample weight, taking into account the value of DA and the weight to achieve protonation of all the NH<sub>2</sub> sites (Notin et al., 2006). Pectin (0.50 g 100 g<sup>-1</sup>) was solubilised in distilled water and stirred magnetically at  $60 \pm 2^\circ\text{C}$  for 30 min. ATH (0.25 g 100 g<sup>-1</sup>) was added to chitosan suspension and homogenised by magnetic stirring at room temperature ( $25 \pm 1^\circ\text{C}$ ) for 45 min. The pH of the chitosan and pectin suspensions was adjusted to 3.0, 4.0, 5.0 or 5.5 using 0.1 M HCl or 0.1 M NaOH solutions. Therefore, chitosan suspension containing ATH was slowly added to the pectin aqueous suspension. The suspension obtained was maintained under magnetic stirring for 10 min (selected by previous testing using different times: 10 min, 1 h, 5 h and 24 h). Different pectin/chitosan ratios were studied (Table 1). The PEC formed between pectin and chitosan was separated by ultracentrifugation (Jouan, MR 1812 model, France) at 12,000 rpm for 30 min at  $5 \pm 1^\circ\text{C}$ . This precipitate was placed in plastic petri dishes and dried at  $40^\circ\text{C}$  during 12 h in an oven with air circulation (Tecnal, TE-394/1 model, Brazil) to form the PEC films.

#### 2.2.4. Determination of zeta potential of PEC suspensions

PEC suspensions were prepared at 0.10 g 100 g<sup>-1</sup>. The suspensions were maintained under continuous magnetic stirring for 2 min and then kept still during four hours to separate the supernatant and complex formed. Zeta potential was measured by dynamic light scattering (DLS) in a Zetasizer Nano-ZS (Malvern, ZEN 3600 model, Germany). All measurements were carried out at  $25^\circ\text{C}$  in triplicate. Different ratios of pectin/chitosan (% v/v) were studied in each pH value (3.0, 4.0, 5.0 and 5.5) to determine the zeta potential of the PEC suspensions.

**Table 1**  
Zeta potential analysis of PEC solutions obtained at different pHs values.

Ratios (P:C) <sup>1</sup>	Zeta potential (mV)			
	pH 3.0	pH 4.0	pH 5.0	pH 5.5
Pectin	-7.99 ± 1.14	-28.23 ± 1.30	-32.63 ± 2.94	-37.10 ± 1.32
Chitosan	55.50 ± 2.16	62.15 ± 0.95	44.45 ± 0.34	37.03 ± 2.21
1.0P:1.0C	55.10 ± 0.07	68.35 ± 3.35	43.00 ± 0.80	33.95 ± 0.15
2.0P:1.0C	52.75 ± 0.95	63.20 ± 2.40	36.90 ± 0.90	27.10 ± 0.90
3.0P:1.0C	45.75 ± 0.85	56.50 ± 3.59	32.48 ± 2.49	21.30 ± 0.58
4.0P:1.0C	34.25 ± 4.05	46.68 ± 3.35	24.20 ± 1.35	12.03 ± 0.43
4.1P:1.0C	-	-	-	11.05 ± 0.05
4.3P:1.0C	-	-	-	5.50 ± 0.06
4.5P:1.0C	-	-	-	-9.04 ± 0.15
4.8P:1.0C	-	-	-	-16.50 ± 0.70
4.5P:1.0C	-	-	17.57 ± 2.57	-
5.0P:1.0C	26.15 ± 1.32	32.83 ± 1.82	-6.06 ± 3.53	-18.24 ± 0.94
5.1P:1.0C	-	32.55 ± 0.05	-	-
5.2P:1.0C	-	31.83 ± 1.72	-	-
5.3P:1.0C	-	24.20 ± 0.10	-	-
5.5P:1.0C	19.80 ± 2.48	9.93 ± 0.27	-15.25 ± 0.45	-
6.0P:1.0C	14.05 ± 0.05	-12.51 ± 2.31	-20.95 ± 0.82	-23.50 ± 0.53
6.5P:1.0C	-	-13.60 ± 0.00	-	-
7.0P:1.0C	8.69 ± 0.29	-18.53 ± 1.86	-23.90 ± 0.55	-25.50 ± 0.10
8.0P:1.0C	-3.66 ± 0.05	-21.20 ± 0.57	-26.00 ± 0.83	-
9.0P:1.0C	-5.64 ± 0.03	-21.42 ± 0.72	-	-
10.0P:1.0C	-7.33 ± 0.30	-23.78 ± 1.65	-	-
1.0P:2.0C	57.85 ± 2.95	66.95 ± 2.15	42.73 ± 2.04	35.35 ± 1.25
1.0P:3.0C	58.75 ± 0.75	65.30 ± 0.20	42.40 ± 0.90	37.00 ± 0.70
1.0P:4.0C	59.70 ± 1.50	66.25 ± 0.35	43.25 ± 0.45	37.70 ± 0.70
1.0P:5.0C	60.65 ± 1.65	-	43.70 ± 1.20	37.20 ± 0.50

<sup>1</sup> "P" and "C" are pectin and chitosan, respectively.

### 2.2.5. Yield of PEC films formed

The yield of PEC films was calculated from the masses of chitosan, pectin and ATH used initially in suspension preparation and the mass of the PEC films formed (Eq. (2)):

$$\text{Yield(\%)} = \frac{M_{\text{complex}}}{(M_c + M_p + M_{\text{Ath}})} * 100 \quad (2)$$

where  $M_{\text{complex}}$  is the complex mass obtained after drying at 40 °C during 12 h and  $M_c$ ,  $M_p$  and  $M_{\text{Ath}}$  are the respective initial masses of chitosan, pectin and ATH used for PEC film formation.

### 2.2.6. Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was performed in the range of 4000–650  $\text{cm}^{-1}$  using a FTIR spectrometer (Thermo Scientific, Nicolet 6700 spectrometer, USA) operating in ATR mode (for the PEC films) and potassium bromide (KBr, FTIR grade, Sigma-Aldrich, Germany) discs for the powder samples (pectin, chitosan and ATH) coupled to a computer with Omnic analysis software. Data collection was performed with a 4  $\text{cm}^{-1}$  spectral resolution and 64 scans.

### 2.2.7. Scanning electron microscopy (SEM) of the PEC films

The SEM analysis of the PEC films was performed on fractured cross sections and the surface of a gold-sputtered CH-Sys using a LEO 440i scanning electron microscope (LEO Electron Microscopy Ltd., England) under the following conditions: accelerating voltage = 15 kV, distance = 25 mm, current = 200 pA, and vacuum =  $10^{-5}$  Torr ( $1.3 \times 10^{-3}$  Pa) (Reis, Yoshida, Reis, & Franco, 2011).

### 2.2.8. Diffusion test of bioactive compound from PEC films at different pHs values

PEC films were preconditioned at  $50.0 \pm 0.2$  °C during 24 h before analysis and cut into 2.0 cm squares and their thickness was measured at five different places using a digital MDC-25 M model micrometer (Mitutoyo, Japan). The PEC films were immersed in 200 mL of phosphate-citric acid buffer solutions with different pHs

values (4.0, 5.5 and 7.0) and kept under constant stirring at 90 rpm (TE-420 model, Tecnal, Brazil). The aliquots (2 mL) were removed at predetermined times during 480 min and the ATH concentration released in the buffer solution was measured using a UV/VIS spectrophotometer (Thermo Fisher Scientific, Genisys 6 model, USA). The specific wavelength ( $\lambda_{\text{max}}$ ) for each pH of the buffer solution was determined by scanning in the range 190–780 nm with a 1 nm resolution, using a UV/VIS spectrophotometer (Varian, Gary 1G model, USA). This was necessary because the ATH structure produces changes in colour at different pH values (Brouillard & Dubois, 1977; Iacobucci & Sweeny, 1983). At pH = 4.0, the  $\lambda_{\text{max}}$  was 530 nm; pH = 5.5, the  $\lambda_{\text{max}}$  was 535 nm and pH = 7.0, the  $\lambda_{\text{max}}$  was 575 nm.

A standard curve was determined for each buffer solution to calculate the concentration of ATH. The amount of the compound released at time  $M_t$  and the fraction of the final value ( $M_t/M_\infty$ ) were calculated and plotted as a function of immersion time ( $t$ ). Tests were carried out in triplicate.

**2.2.8.1. Mathematical diffusion analysis.** The diffusion test was based on a study developed by Yoshida et al. (2010) using the same boundary conditions. To model the bioactive compound (ATH) diffusion mechanism, it was assumed that the process occurred in a thin sheet of film with an initially homogeneous bioactive concentration distribution. Solvent absorption and solute diffusion occurred simultaneously. Film thickness was much smaller than film width, so the diffusivity was considered to be unidirectional and perpendicular to the surface of the sheet. The bioactive compound concentration inside the film was a function of  $x$ , the distance from the surface. The ratio of the total amount of ATH released after time  $t$  ( $M_t$ ) to the amount released at equilibrium ( $M_\infty$ ) (Crank, 1975) was calculated as follows:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{2m+1} \exp \left[ \frac{-2(m+1)^2 \pi^2 D_t}{L^2} \right] \quad (3)$$

where  $M_t$  is the amount of compound release at time  $t$ ;  $M_\infty$  is the amount of compound release at infinite time;  $L$  is the film thickness (mm);  $t$  is the time (s).

A computational program was developed using MatLab® 7.5 software for the mathematical modelling of ATH release results as a function of time. The program provided the diffusion coefficient ( $D'$ ). A reliability test of the method was conducted using the sum of squares of the deviations of the experimental points from the modelling predictions.

### 2.2.9. Colourimetric characterisation of PEC films

The colour parameters of the PEC films were measured before and after exposure to buffer solutions at different pHs (4.0, 5.5 and 7.0). A CR 400 Chroma Meter (Konica Minolta, Japan) was used. The colourimeter was calibrated with a white plate, observation angle of  $2^\circ$  and illuminant D65. Measurements were performed using the CIE  $L^* a^* b^*$  system. The parameter  $L^*$  represents the lightness of colours from 0 (dark) to 100 (light) and  $a^*$ , the greenness/redness (negative  $a^*$  is green and positive  $a^*$  is red) and  $b^*$ , the grade of blueness/yellowness (negative  $b^*$  is blue and positive  $b^*$  is yellow); both  $a^*$  and  $b^*$  move along the two axes that form a plane orthogonal to  $L^*$  and neither has specific numerical limits. Three replicates were conducted per experiment.

### 2.2.10. Thermogravimetric analysis (TGA)

The thermogravimetric analysis of pectin, chitosan, ATH, chitosan/pectin PEC films and chitosan/pectin/ATH PEC films was performed using a thermogravimetric analyser (TGA-50, Shimadzu, Japan). The experiments were run at a heating rate of  $10^\circ\text{C min}^{-1}$  in the range of  $30\text{--}400^\circ\text{C}$  with a nitrogen gas flow of  $50\text{ mL min}^{-1}$ .

### 2.3. Statistical analysis

Statistical analysis was carried out with the Statistic version 7.0 program (Statistic Inc., USA) and differences between the means were detected by the Tukey multiple comparison test.

## 3. Results and discussion

The estimated values for DA of chitosan and DE of pectin were 9.1% and 72.0%, respectively. The pectin is classified as high-esterified. The DA of chitosan and DE of pectin are important factors in obtaining a PEC. These characteristics are related to the available free charges of each polysaccharide, directly influencing its complexation (Ghaffari et al., 2007; Coimbra et al., 2011; Tsai et al., 2014).

### 3.1. Polyelectrolyte complex (PEC)

PEC films were characterised as rugous, homogeneous and without bubbles or defects. The violet colour was due to the ATH entrapped in the PEC matrix (Fig. 1).

For water-soluble PECs, stoichiometrically charged PECs usually precipitate and form turbid PEC solutions. In our study, when mixing chitosan and pectin solutions with the same ionic strength, a non-homogeneous solution with precipitate formation was observed. These results are in accordance with other studies, indicating that soluble PECs often occur due to strong ionic interactions between the polycationic chitosan and polyanionic pectin (Bigucci et al., 2008; Coimbra et al., 2011). According to Tsai et al. (2014), PECs are usually water-soluble and form homogeneous solutions when the densities of positive charges on the polycations and negative charges on the polyanions are not equitable.



Fig. 1. PEC film obtained from chitosan and pectin suspensions containing a colourimetric bioactive compound (ATH).

### 3.2. Zeta potential analysis

It was expected that pectin would interact with chitosan to form PECs through opposite charge interactions. The charge densities of pectin and chitosan solutions at the different pH values are shown in Fig. 2.

For all pHs values studied, the chitosan and pectin solutions had positive and negative charges, respectively. Increasing the pH from 3.0 to 5.5, the zeta potential of chitosan decreased from +55.5 mV to +37.0 mV. This was associated with a pH value quite similar to the pKa of chitosan (6.2–7.0) (Vaarum & Smidsrod, 2005). In this case, at pH below pKa the amino groups of chitosan are deprotonated, which makes the chitosan molecule cationic. The zeta potential was more negative for pectin, increasing the pH from 3.0 to 5.5. Anionic polysaccharides such as pectin have a low range of pKa (3.5–4.5) (Rolin, 2002), which is determined by galacturonic acid, a weak acid found in the pectin structure. At low pH values, the anionic characteristic of pectin is reduced due to dissociation of the carboxylic groups of the pectin structure (Giancone, Torrieri, Masi, & Michon, 2009).

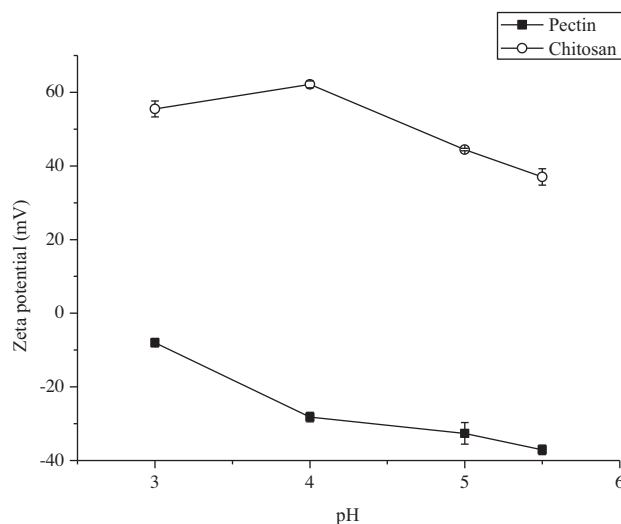


Fig. 2. Zeta potential of pectin 0.01% (w/w) and chitosan 0.01% (w/w) solutions at pH 3.0, 4.0, 5.0 and 5.5.



The interaction between the polymers was evaluated by the PEC charge formed at different pHs values (Table 1). A higher yield of PEC films was obtained when the resultant charge of the solution was near zero, indicated by the charge inversion of the PEC solution. Under this condition, it is possible that there was more interactions between the polymeric charges. At pH 3.0, the best pectin/chitosan ratio was between 7.0P:1.0C and 8.0P:1.0C, and at pH 4.0, 5.0 and 5.5 it was between 5.5P:1.0C and 6.0P:1.0C, 4.5P:1.0C and 5.0P:1.0C and 4.3P:1.0C and 4.5P:1.0C, respectively. When pH was increased from 3.0 to 5.5, smaller amounts of pectin were required to form the higher yields of PEC films. The formation of PECs between a weak polybase (chitosan) and a weak polyacid (pectin) occurs extensively in the pH range between the pKa's of the two polymers, where more than one half of the ionic groups of both polymers are ionised. For the pectin and chitosan complex system, this pH value was between 3.5 and 4.5 (pKa range of pectin, Rolin, 2002) and 6.2 and 7.0 (pKa range of chitosan, Vaarum & Smidsrod, 2005), respectively. A higher homogeneity of PEC films was observed at pH 5.0 and 5.5. Besides pH, the other important factors that affected PEC formation and properties were the proportion and loadings of the two polymers, temperature and ionic strengths. Macleod, Collett, and Fell (1999), Ghaffari et al. (2007), Coimbra et al. (2011) and Recillas et al. (2011) studied the formation of PEC between chitosan and pectin and obtained the best results at a pH range between 3.0 and 6.0 at room temperature.

The resultant charge of the system formed between chitosan and pectin was dependent on pH. Maintaining the proportion of chitosan constant and varying the concentration of pectin, systems with a positive zeta potential were observed in all formulations and at all pHs up to the ratio of 4.3P:1.0C. Varying the proportion of chitosan and maintaining the pectin concentration constant, the charge of the systems was always positive. These results suggest that the amount of pectin added was not sufficient to neutralise all of the chitosan amino groups. The opposite behaviour was observed for the amount of chitosan, which was not high enough to associate with the charges of the pectin, resulting in systems with a negative characteristic due to the excess of pectin concentration.

Ghaffari et al. (2007) studied PEC formation with chitosan and pectin at different pHs and observed that under an extremely acid condition (pH = 1.5), an insufficient ionisation of pectin was promoted to form the PEC at any pectin:chitosan ratio. Increasing pH (3.8 and 5.4), the optimal pectin:chitosan ratios were 3:1 for pH 3.8 and 2:1 and 3:1 for pH 5.4. Similar results for the ionisation limit of pectin at a very acid pH (pH = 2.0) were obtained by Bigucci et al. (2008), where the maximum complex formation was obtained at pH 5.0 using chitosan:pectin ratios from 1:1 to 3:7. For pH 3.0 and 4.0, the best ratios were 1:9 and 3:7, respectively. Rashidova et al. (2004) studied the PEC formed between chitosan (DA of 40%) and pectin (DE of 61–68%) at different molar ratios. They obtained pectin-chitosan interaction in a 2.0% acetic acid solution and observed that structural toughness depended on mixture composition. Naidu et al. (2009) studied the PEC formed between gum kondagogu and chitosan at different ratios, obtaining good results (yield, membrane swelling and drug release) at ratios of 4:1 and 5:1 (gum kondagogu:chitosan). They observed that the gradual increase in the quantity of gum kondagogu used to form the complex reduced the zeta potential of the suspension to negative values, indicating the complete neutralisation of chitosan. Abruzzo et al. (2013) studied complexes formed between chitosan and alginate at pH 5.0 for vaginal delivery of chlorhexidine digluconate. They found the best results of polymeric interaction using a ratio of 1:1.

Based on the zeta potential results, the best formulation of PEC film ratio for each pH was defined (Table 2). The yield of PEC films was calculated based on the complex mass obtained after the drying process and the initial mass of the pectin and chitosan used.

**Table 2**  
Yield of PEC formed between pectin and chitosan at different pHs.

pH	Pectin:chitosan weight ratio (w/w)	Yield (%)
3.0	8.0P:1.0C	48.2 ± 0.80 <sup>a</sup>
4.0	5.5P:1.0C	52.5 ± 1.10 <sup>b</sup>
5.0	5.0P:1.0C	56.6 ± 1.12 <sup>c</sup>
5.5	4.3P:1.0C	60.2 ± 0.89 <sup>d</sup>

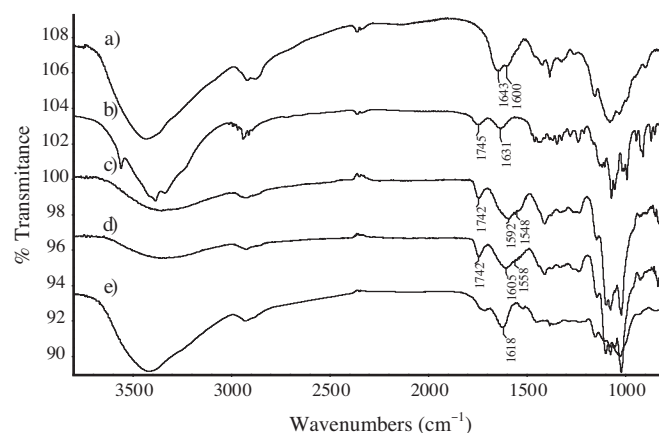
<sup>a,b,c,d</sup> Means on the same line with different superscript letters differ significantly ( $p \leq 0.05$ ) in accordance with Tukey's test.

An increase in pH from 3.0 to 5.5, improved the yield of PEC formed in the order of 25%. The lowest yield (%) was obtained using a high concentration of pectin and pH 3.0. This was associated with the anionic character of pectin that is reduced at low pHs due to the dissociation of the carboxylic groups of galacturonic acid. The maximum yield (60.2%) was observed at a ratio of 4.3P:1.0C at pH 5.5. It is possible that more than one half of the ionic groups of both polymers were ionised at this pH. Based on these results, 4.3P:1.0C at pH 5.5 was defined as the best formulation for continuing the study using ATH entrapped in PEC films.

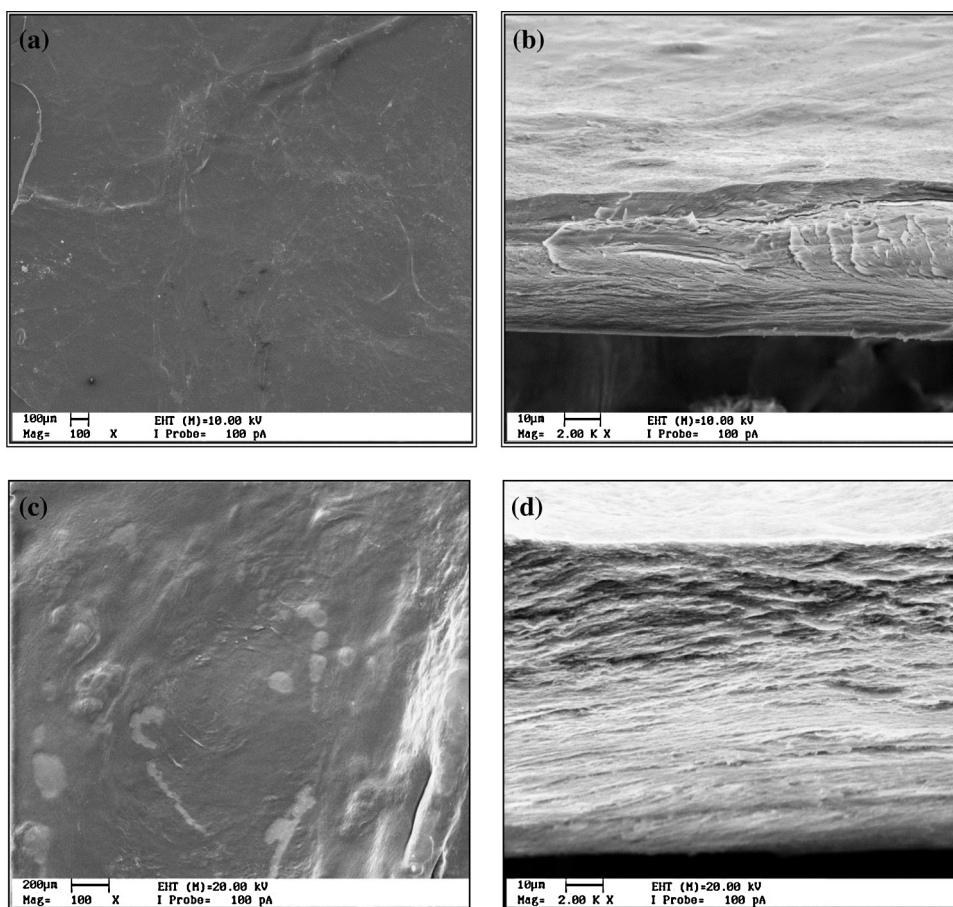
### 3.3. Fourier transform infrared (FTIR) spectroscopy

Samples of chitosan, pectin, ATH and PEC films with and without ATH (4.3P:1.0C) were analysed by FTIR spectroscopy (Fig. 3). It was possible to verify the chitosan-pectin interaction.

The spectral region between 3500  $\text{cm}^{-1}$  and 2800  $\text{cm}^{-1}$  for chitosan (Fig. 3a) and pectin (Fig. 3b) had absorption bands typical of polysaccharides (Stuart, 2004). In the FTIR spectrum of pectin it is possible to observe two bands between 1800 and 1500  $\text{cm}^{-1}$  that are associated with the stretching vibrations of the carbonyl group. The band at 1745  $\text{cm}^{-1}$  corresponds to the methyl ester group ( $\text{COOCH}_3$ ) and the undissociated carboxyl acid ( $\text{COOH}$ ), while the band at 1631  $\text{cm}^{-1}$  is assigned to the asymmetric stretching vibration of the carbonyl group of the carboxylate ion ( $\text{COO}^-$ ) (Coimbra et al., 2011). In the spectrum of chitosan, the band situated at 1643  $\text{cm}^{-1}$  is assigned to the C=O stretching vibration of the amide group (amide I) of the acetylated units of chitosan. The band at 1600  $\text{cm}^{-1}$  is the result of the overlapping of the amide II of the amide groups and the N–H bending vibration (amide II). The spectrums of the PEC films with and without ATH indicate the main changes in the range of 1800–1600  $\text{cm}^{-1}$ , providing evidence of the interaction of the amino and carboxyl groups (Fig. 3c and d). In both spectra a peak can be observed at 1742  $\text{cm}^{-1}$  and can be attributed to the vibration of the carbonyl group of pectin. A broad peak at 1600–1500  $\text{cm}^{-1}$  in the spectrum of the PEC films



**Fig. 3.** FTIR spectra of (a) chitosan; (b) pectin; (c) chitosan/pectin PEC (4.3P:1.0C without ATH); (d) chitosan/pectin/ATH PEC (4.3P:1.0C with ATH) and (e) ATH.



**Fig. 4.** Micrographs of the PEC films: (a) surface area of chitosan/pectin/ATH (4.3P:1.0C with ATH), (b) cross section of 4.3P:1.0C with ATH, (c) surface area of chitosan/pectin (4.3P:1.0C without ATH) and (d) cross section of 4.3P:1.0C without ATH.

indicates a change in environment of the amine group through its interaction with pectin. In fact, the asymmetric stretching vibration of the carbonyl group of the carboxylate ( $\text{COO}^-$ ) groups in pectin ( $\sim 1631\text{ cm}^{-1}$ ) and the amide I ( $\sim 1643\text{ cm}^{-1}$ ) and amide II ( $\sim 1600\text{ cm}^{-1}$ ) vibrations of the amide groups of chitosan indicate the formation of interchain or intermolecular ionic salt bonds, i.e. PEC between amino groups of chitosan and carboxyl groups of pectin (Rashidova et al., 2004; Stuart, 2004; Chen et al., 2010; Brinques & Ayub, 2011).

In Fig. 3e, ATH shows a strong characteristic band at  $1650$  and  $1450\text{ cm}^{-1}$  assigned to the stretching vibration of the C–C aromatic ring. The absorption band around  $1230\text{ cm}^{-1}$  is assigned to stretching of pyran rings, typical of flavonoid compounds (Pereira, Arruda, & Stefani, 2015). The PEC films containing ATH have the same IR spectra, indicating no significant interactions between pectin/chitosan and ATH. In this case, the ATH could be merely entrapped in the PEC matrix.

### 3.4. Scanning electron microscopy (SEM)

The morphology of the PEC films was analysed by SEM to observe the films' surface morphology and cross section as well as the homogeneity of the composite, presence of voids and the films' homogeneous structure. Fig. 4 contains the SEM micrographs of the PEC films using 4.3P:1.0C with (Fig. 4a and b) and without ATH (Fig. 4c and d) at pH 5.5.

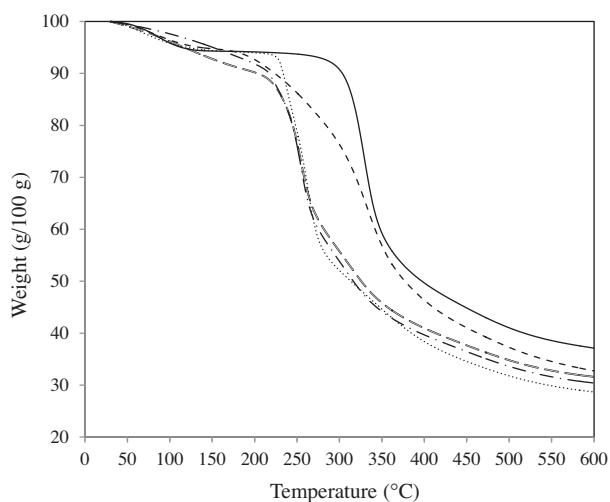
A relatively flat and smooth PEC film surface can be observed (Fig. 4a and c), which indicates that the mixture between chitosan and pectin as well as chitosan, pectin and ATH was homogenous in

these films. This is further supported by the compact cross-section morphologies of both PEC films (Fig. 4b and d), which could indicate a strong interaction between the polysaccharides with and without ATH. Meanwhile, our results agree with those from other research on biopolymers films, where a homogeneous and smooth surface is usually preferred (Khan et al., 2012; Wang et al., 2013; Sun, Wang, Kadouha, & Zhou, 2014).

### 3.5. Thermogravimetric analysis (TGA)

The thermogravimetric analysis (TGA) curves for chitosan, pectin, ATH and PEC films (4.3P:1.0C) with and without ATH were determined. The water distribution within the systems and the temperature limits for PEC film applications were evaluated (Fig. 5).

Typical TGA curves for weight loss as a function of temperature can be observed for chitosan and pectin. Chitosan and pectin were degraded at around  $325^\circ\text{C}$  and  $240^\circ\text{C}$ , respectively. In the thermogram of pure pectin, two stages were observed. The first thermal event was a weight loss in the range of  $50$ – $100^\circ\text{C}$ , which was related to evaporation of the water in the sample and the equilibrium film. Evaporation occurs at the liquid/air interface, and the water is readily available at the surface (Mandanás & Messing, 2000). The second thermal event started from  $200$  to  $240^\circ\text{C}$  and was related to depolymerisation of the pectin chains. For the pure chitosan the first thermal event, related to the evaporation of unbound water, was also seen below  $100^\circ\text{C}$ . The second thermal event for chitosan was observed at about  $230^\circ\text{C}$  with the maximum rate at  $375^\circ\text{C}$  and a 43.7% reduction in weight. The degradation of the PEC films both with and without ATH was similar, which could

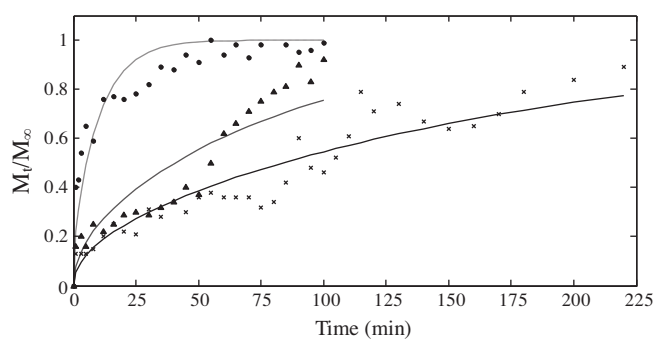


**Fig. 5.** Thermogravimetric analysis: chitosan (···), pectin (-----), ATH (— · — · —), chitosan/pectin PEC (4.3P:1.0C without ATH) (— — —) and chitosan/pectin/ATH PEC (4.3P:1.0C with ATH) (— · — · —).

indicate that the addition of ATH to the PEC did not affect the interaction between the polymers. In these thermograms an event observed at 250 °C could be due to chitosan and pectin complexation (Ghaffari et al., 2007). Structural decomposition of the PEC films started above this temperature. The structural disruption facilitated the movement of water molecules. The thermal degradation of the pure polymers suggests that the formation of ionic bonds between chitosan and pectin can probably be correlated with a loss of organisation (Bigucci et al., 2008).

### 3.6. Diffusion test of bioactive compound from PEC films at different pHs values

A diffusion test of the PEC films containing bioactive compound (ATH) was carried out at different pHs (4.0, 5.5 and 7.0) during 8 h at room temperature ( $25 \pm 0.5$  °C). Adjustment of the curves was based on that proposed by Crank (1975). The proposal of this analysis was to evaluate whether the matrix formed by the pectin-chitosan interaction could entrap the ATH when these films were put into different aqueous buffer solutions. Yoshida et al. (2014) studied chitosan films containing ATH immersed at different pHs (2.0, 4.0, 5.6, 7.0, 8.0 and 13.0) and concluded that despite the excessive change in colour in their films, the ATH migrated from the chitosan films to the aqueous solution almost instantly.



**Fig. 6.** Release of bioactive compound ATH from PEC films (4.3P:1.0C with ATH) immersed in different aqueous buffer solutions: pH 4.0 (x); pH 5.5 (●) and pH 7.0 (▲).

The fractional mass released at pH 4.0, 5.5 and 7.0, defined as  $M_t/M_\infty$  ( $M_t$  is the mass of ATH released into the buffer solution at time  $t$  and  $M_\infty$  is the mass released at infinite time), was plotted against the immersed time (Fig. 6). The average thickness of PEC films was  $0.200 \pm 0.030$  mm.

The release of ATH from the PEC films was gradual and followed Fick's law, tending asymptotically to 1 for all pHs values. A satisfactory correlation was obtained between the experimental data and the proposed model. The maximum quantity of ATH released from PEC films ( $M_\infty$ ) was reached after approximately 4 h, 2 h and 2 h for pH 4.0, 5.5 and 7.0, respectively.

The highest quantity of ATH that migrated from the PEC films to the aqueous buffer solutions was calculated as follows: at pH 4.0, 1.13% of the ATH present in the PEC films migrated to the solution and at pH 5.5 and 7.0 it was 1.51% and 0.77%, respectively. The release of ATH content from the PEC films was lower than 2.0%, which could indicate that the ionic interactions between chitosan and pectin were able to keep the hydrophilic pigment entrapped on the polymeric matrix.

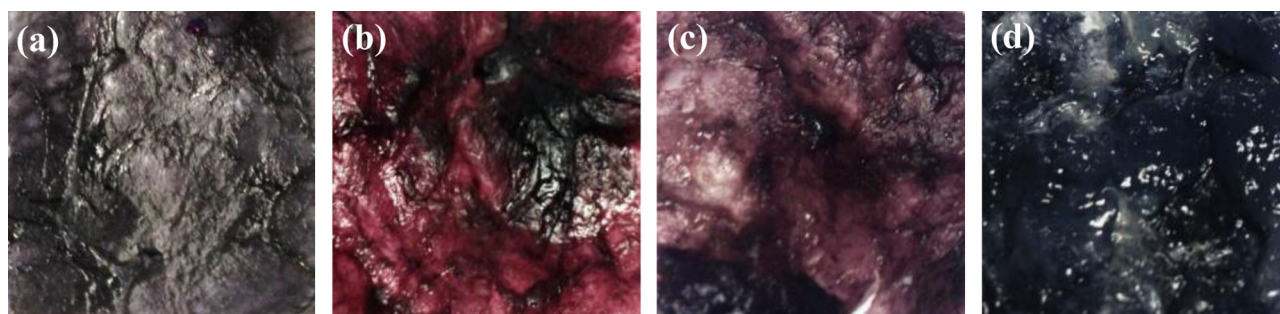
The diffusion coefficients of the bioactive ATH were in the order of  $10^{-11}$   $\text{m}^2 \text{s}^{-1}$  for pH 4.0 ( $D'_{\text{pH}4.0} = 8.40 \pm 0.45 \times 10^{-11} \text{m}^2 \text{s}^{-1}$ ) and 7.0 ( $D'_{\text{pH}7.0} = 0.26 \pm 0.04 \times 10^{-11} \text{m}^2 \text{s}^{-1}$ ) and  $10^{-9}$   $\text{m}^2 \text{s}^{-1}$  for pH 5.5 ( $D'_{\text{pH}5.0} = 1.20 \pm 0.31 \times 10^{-9} \text{m}^2 \text{s}^{-1}$ ).

The lowest diffusion coefficient of ATH in the PEC films was observed at pH 5.5. At this pH the hydrophilicity of the PEC films was low and did not allow absorption. Similar results were observed by Isiklan, Inal, and Yigitoglu (2008) and Pal, Paulson, and Rousseau (2009) for bioactive compound dissolved or dispersed in a polymer network, indicating that the decrease in release rate over time was due to the increased distance of diffusion, which is characteristic of these systems. The solubility of the compounds and their diffusivity in the polymer phase and polymer-compound interactions play an important role in the release process.

According to Korsmeyer and Peppas (1983) and Satish, Satish, and Shivakumar (2006), in certain solvent-penetrating systems, release depends on polymer relaxation, i.e. the stress required to maintain the strain on the polymer decreases as a result of aqueous solution absorption. Immersion of PEC films in an aqueous solution can cause the matrix to relax, thus reducing resistance to compound diffusion. Important parameters associated with the process of release through the polymeric matrix include bioactive compound hydrophilicity and network cross-linking density (Higuchi, 1963).

Yoshida et al. (2010) studied potassium sorbate diffusion in chitosan films and observed that the diffusion coefficient increased at higher concentrations of the bioactive. It was observed that high solubility of the potassium sorbate facilitated absorption of solvent in contact with the aqueous medium. Rivero, Giannuzzi, García, and Pinotti (2013) evaluated the controlled delivery of propionic acid from chitosan films in phosphate buffer at 4 °C and suggested that the process was driven by diffusion at the initial phase release (burst stage) and a slower second release, in which polymer swelling became the main mechanism of agent delivery. Tsai et al. (2014) evaluated chitosan/pectin/gum arabic PEC solutions and various membrane compositions and observed that using low concentration of pectin and gum arabic in PEC films (84/8/8–chitosan/pectin/gum arabic) improved their mechanical properties. A small quantity of polyanionic polymers (pectin and gum arabic) can form network-like PECs that may enhance the tensile strength of the PEC membranes. Larger quantities of pectin and gum arabic (70/15/15–chitosan/pectin/gum arabic) can negatively affect the mechanical properties of the PEC due to the low viscosity of the gum arabic, which interacts weakly with other components and forms globe-like microstructures with chitosan, thereby decreasing the tensile strength of the PEC membranes.





**Fig. 7.** Visual change in colour of the PEC films containing ATH (4.3P:1.0C with ATH) when exposed to different pHs: (a) control (without immersion in buffer solution); (b) pH 4.0; (c) pH 5.5 and (d) pH 7.0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 3**  
Colour parameters of the PEC films containing ATH (4.3P:1.0C with ATH) before and after the diffusion test at different pHs (4.0, 5.5 and 7.0).

pH	Control			After diffusion test		
	$L^*$	$a^*$	$b^*$	$L^*$	$a^*$	$b^*$
4.0	29.11 ± 0.75 <sup>a</sup>	4.43 ± 0.55 <sup>a</sup>	-1.47 ± 0.37 <sup>a</sup>	30.97 ± 1.51 <sup>a</sup>	20.18 ± 1.21 <sup>b</sup>	3.62 ± 0.51 <sup>b</sup>
5.5	30.25 ± 0.85 <sup>a</sup>	4.85 ± 0.98 <sup>a</sup>	-1.66 ± 0.32 <sup>a</sup>	30.86 ± 1.43 <sup>a</sup>	14.34 ± 0.43 <sup>b</sup>	2.17 ± 0.44 <sup>b</sup>
7.0	30.95 ± 1.04 <sup>a</sup>	4.46 ± 0.80 <sup>a</sup>	-1.94 ± 0.37 <sup>a</sup>	27.67 ± 0.91 <sup>b</sup>	0.54 ± 0.11 <sup>b</sup>	-2.43 ± 0.34 <sup>a</sup>

<sup>a,b</sup> Means on the same line and parameters with different superscript letters differ significantly ( $p \leq 0.05$ ) in accordance with Tukey's test.

Furthermore, they observed that the membranes prepared using just chitosan and pectin had a stronger interaction between the polymers.

Associating these results with our results and in accordance with Ghaffari et al. (2007), who claim that at pH 5.4 a strong interaction between chitosan and pectin occurs, we can affirm that the PEC films obtained in our studies at pH 5.5 had the lowest diffusion coefficient due to ionic interactions that occurred to the greatest degree in this region between the pKas of the polymers.

The visual change in colour was observed in all PEC films after immersion at different pHs (4.0, 5.5 and 7.0) (Fig. 7). At pH 4.0 violet colour was observed and at pH 5.5 the PEC films were purple. Bluish-green colour was seen at pH 7.0, indicating significant differences in this parameter according to pH.

Kennedy and Waterhouse (2000) affirmed that these colour variations are associated with the different chemical structures of ATH molecules, which depend on the pH of the solution. Under acid conditions (pH 1.0–3.0), ATH occurs predominantly in the form of the flavylium cation (red colour), contributing to the purple and red colours. In the range of pH 2.0–4.0, the quinoidal blue species are predominant. The increase in pH to 5.0 and 6.0 results in a decrease in colour intensity and the concentration of the flavylium cation, which undergoes hydration to produce a colourless carbinol pseudobase and chalcone, respectively. The equilibrium is shifted towards a purple quinoidal anhydrobase at pH <7.0 and a deep blue ionized anhydrobase at pH <8.0. Re-acidification at any time during this process to a pH below 2.0 will fully restore the original colour of the ATH (Brouillard, 1982; Castañeda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009).

Relating these results to the diffusion test, it can be observed that the proposed system can be used to show changes at different pHs, using a visual parameter to obtain this information. In our work, a device (PEC films) was developed by complexation between chitosan and pectin containing ATH. This new system was able to entrap the ATH in the matrix after immersion at pH 4.0, 5.5 and 7.0, indicating a visual change in colour. The buffer solutions used in these experiments remained limpid and transparent in all cases, confirming that the matrix entrapped the ATH molecules in its structure.

The visual change in colour could be related to the colour parameters  $L^*$ ,  $a^*$  and  $b^*$  (Table 3).

Parameters  $a^*$  and  $b^*$  showed more significant variation after the diffusion test for all pHs studied. At pH 4.0, parameter  $a^*$  increased from 4.43 to 20.18, tending towards violet, while at pH 5.5, it increased from 4.85 to 14.34, showing a tendency towards purple. At both of these pHs, a greater change in colour was observed than for the unexposed PEC films (4.3P:1.0C with ATH) at different pHs. In PEC films in solution at pH 7.0, a significant difference ( $p \leq 0.05$ ) in parameters  $L^*$  and  $a^*$  was observed, showing a tendency towards bluish-green. According to Maciel et al. (2012), the exposure of ATH to different pHs and a high temperature could alter its structure, thereby producing a change in colour. Yoshida et al. (2014) found similar results on colour change in chitosan films containing ATH at different pHs (from 2.0 to 13). They concluded that ATH has a potential application as a natural pH indicator. Kohno et al. (2009) evaluated the stability of ATH at pH 11 and determined that slightly basic conditions changed the ATH structure, accompanied by a marked change in colour.

#### 4. Conclusions

PEC films formed between pectin and chitosan were prepared by the casting/solvent evaporation method. The formation of PEC between pectin and chitosan at pH values in the vicinity of the pKa interval of the two polymers was observed and the PEC was able to entrap ATH on the films. The optimal weight ratio of pectin to chitosan for PEC formation was 4.3P:1.0C at pH 5.5, which produced the highest product yield. The strong interaction between the polysaccharides of opposite charges entrapped the hydrophilic bioactive compound ATH in the PEC matrix, preventing its release into the immersion solution. The proposed system is advantageous due to its simple manufacture and visual change in colour, offering an alternative for indicating pH variations in food products. Indicators associated with intelligent packaging show great potential for assuring the safety and quality of food products.

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