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Research Article

Influence of pH on solution and solid-state properties of citrus pectin

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Abstract

Pectin has been increasingly accepted for pharmaceutical applications including targeted drug delivery due to its biodegradability. For this purpose, the physicochemical characterization of pectin is important for fabrication of dosage forms. The present study focused on the consequence of pH on the properties of pectin. Citrus pectin, including high methoxyl pectin (CU 201, CU 401) and low methoxyl pectin (CU 701) were prepared at different pH and comparatively evaluated for their solution and solid-state properties. The results indicated that rheological behavior was depended on pH and grade of pectin. As increasing pH from 2.9 to 4.0, the viscosity of pectin solutions was significantly decreased. However, the low methoxyl pectin was less sensitive to the increment of pH from 5.0 to 7.0. Regardless of pH, the high methoxyl pectin showed shear thinning behavior while the low methoxyl pectin demonstrated Newtonian flow except for pH 2.9. The results suggested the pH dependent structural change of citrus pectin, especially for low methoxyl pectin, which was later confirmed by the results from powder X-ray diffractometry. The knowledge obtained from this research provides the basic knowledge for the specific selection of pH and grade of pectin in fabrication of drug delivery systems.

Keywords: Pectin; drug delivery; pH; rheology

Introduction

Pectin is a natural biopolymer that has been widely applied in the food and pharmaceutical industry due to its attractive properties including gelling property and ability to modify drug release. It commonly occurs as the major part of cell wall of higher plant; however, the commercial source is still limited to citrus fruits and apple since the desired properties is controlled by the structure and chemistry of pectin from selected sources. Degree of esterification, molecular weight is the major factor that affects physicochemical properties of pectin. With regard to degree of esterification, pectin is categorized as high methoxyl pectin (HMP) and low methoxyl pectin (LMP) with degree of methoxylation of >50% and <50%, respectively.(Sriamornsak, 2003) These two groups of pectin demonstrate different gelling and other related properties which depend on various factors including amount of soluble solid (*e.g.* sugar content), amount of cation species and pH. HMP could form gel at higher solid content (generally more than 55%) as compared with LMP.(Yapo & Koffi, 2013) However, LMP requires divalent cations (usually calcium) for proper gel formation. A higher degree of esterification causes more rapid gel setting at lower soluble solids than pectin with a lower degree of esterification.(Kastner et al., 2014) The gelling behavior is also influenced by pH change. As increasing pH, depolymerization or chain cleavage occurs and results in rapid loss of viscosity and gelling properties while LMP indicate better stability at this conditions. However, there are only few reports exploring the influence of pH on the pectin properties, especially the mechanism and the correlation between solution state and solid-state properties of pectin. Therefore, the purpose of this study was to study the consequence of pH on properties of pectin. High methoxyl citrus pectin (CU201 and CU401) and low methoxyl pectin (CU701) were selected as the representative pectins with different degree of esterification. The pectin solutions were prepared at different pH and comparative evaluated for rheological property, powder X-ray diffractometry, and FTIR spectroscopy. The correlation between those properties was also investigated.(Hua et al., 2015)

Materials and Methods

Materials Citrus pectin (CU701, CU201 and CU401) were received from Herbstreith & Fox KG Pektin-Febriken (Germany). All other chemicals were of reagent grade and used without further purification.

Preparation of pectin solutions and pectin films Each pectin grade was dissolved in deionized water before adjusting the pH to 4.0, 5.0, 6.0 and 7.0 with NH_4OH. The final concentration was adjust to 4% w/w and then characterized for solution properties before casting into thin films. Film was prepared by pouring 150 gm of each pectin solutions in glass plate size 15 x 15 cm^2 and drying by hot air oven (Heraeus, D-6450 Hanau, Germany) at 50 °C for 5 hr. The resulting film was peeled off and subjected to further characterization.

Rheological analysis Rheological measurements were carried out on a rheometer (Kinexus, pro, Malvern, UK) using a cone and plate geometry (CP1/50, SR1233 SS). The temperature was controlled at 25 ± 0.1 °C. Viscosity profiling were investigated to compare rheological behavior at different pH and grade of pectin. The relationship between shear rate (from 0.001-1000 s^{-1}) and shear stress were recorded and the flow property were analyzed by fitting with the Power law model as described by the following equation:

$$\sigma = K\gamma^{\eta} \qquad (1)$$

where σ is shear stress (Pa), K is consistency coefficient (Pa s^n), γ is shear rate (s^{-1}) and n is flow behavior index.(Rao, 2014)

Powder X-ray diffraction Powder X-ray diffraction measurements were performed using a Rigaku (Miniflex II, Japan) diffractometer with Cu-Kα radiation. Each pectin films was placed in sample holder and scanned across a 2θ range of 5-45°.

FTIR spectroscopy Each pectin films was pulverized and mixed with KBr at the ratio of 90:10. The sample was then compressed into the disc by hydraulic press (Specac, 15011). The spectrum was obtained by scanning each sample disc at wavenumber from 4000 to 400 cm^{-1} by using of FTIR spectrophotometer (Nicolet Avatar 360, USA).

Results & Discussion

Rheological analysis The viscosity profiles of pectin solutions prepared at different pH are illustrated in Figure 1-2. Regardless of pectin type, the viscosity had a tendency to decrease as increasing pH, especially as pH change from 2.9 to 4.1. The reduction of viscosity was more clearly observed in solutions of low methoxyl pectin (CU701) as compared to those of high methoxyl pectin (CU201 and CU401). The result suggested the structural dependent viscosity of pectin.

Pectin composes of chain of galacturonic acid units joined by glycosidic linkage in which have free carboxyl groups and some units are methyl esters while the pKa of free carboxyl groups is around 3.5. In this study, the initial pH of pectin solutions was around 2.9 and therefore most carboxyl groups existed in protonated forms. As increasing pH, the protonated carboxyl groups were more ionized and changed to carboxylate groups. The calculated fractions of carboxylate to total carboxyl groups were 20.0, 79.9, 97.5, 99.7 and 99.9 at pH 2.9, 4.1, 5.1, 6.4 and 7.4, respectively. Hence, the dramatically rapid reduction of viscosity as increasing pH from 2.9 to 4.1 should correlate with the rapid acid to salt conversion. The finding was correlated with some previous report.(Gilsenan et al., 2000)

The viscosity is related with the arrangement of polymer chain in solutions. In case of pectin, the polymer chain favored association to form a gel at low pH (less than its pKa) because of hydrogen bonding of unionized carboxyl groups among the polymer chains. However, the increased carboxylate anion as increasing pH led to the formation of inter and intra electrostatic repulsions and forced the polymer chains to dissociate into the more extended form.(Liu & Zhang, 2013) Additionally, the depolymerization, especially at alkali pH, could be also a possible explanation for the reduction of viscosity of pectin solution in this study.

With regard to the effect of DE on viscosity, HMP is more esterified as compared to LMP, resulting in lower number of free carboxyl groups per molecules. Therefore, CU201 might be less sensitive to increasing pH as compared to CU401 and CU701 because of low number of carboxylate groups that formed repulsion force.

In order to study flow behavior of pectin, the viscosity profiles were fitted with the Power law model as illustrated in Table 1. All viscosity profiles showed the coefficients of determination (R^2) values were closed to 1, which indicated the flow behavior of pectin solutions was well predicted by the equations. Regardless of pH, high methoxyl pectin (CU201 and CU401) demonstrated non-Newtonian (shear thinning) as the n values (flow behavior index) were less than 1. However, the low methoxyl pectin (CU701) indicated the Newtonian behavior (n values closed to 1) except at pH 2.9. The result suggested the different polymer arrangement in solution state between HMP and LMP, especially after ionization at increasing pH. It was also noted that n values of HMP were more closed to 1 as increasing pH (or number of carboxylate groups).

FTIR spectroscopy In order to study the consequence of pH on molecular state of pectin after conversion to solid state, all pectin films were comparatively characterize by FTIR spectroscopy. The FTIR spectra of pectin films prepared at various pH from 2.9 to 7.0 (CU201, CU401 and CU701) are shown in Figure 3. All pectin samples demonstrated the high intensity broad band peak around 3450 cm^{-1}, which was assigned to the O-H stretching of hydroxyl of pectin and bound water and the low intensity peak due to C-H stretching at 2940 cm^{-1}, regardless of type pectin and pH change. The peak at 1740 cm^{-1} and 1630 cm^{-1} , attributed to the C=O stretching of methyl ester and carboxylate anions, respectively ($-COOCH_3$, $-COO^-$), were also observed in all pectin samples. However, the higher relative peak intensity of C=O stretching of methyl ester/ C-H stretching (1740/2940) of HMP was observed as compared to LMP. The result was correlated with the degree of methoxylation.(Chatjigakis et al., 1998)

Table 1 Rheological properties of pectin solutions determined by Power law model.(Mierczyńska et al., 2017)

Grade	pH	K	n	R^2
CU201	2.9	16.64	0.52	0.964
	4.1	5.91	0.69	0.988
	5.0	4.88	0.69	0.988
	6.1	4.79	0.68	0.987
	7.2	4.76	0.69	0.988
CU401	2.9	8.41	0.65	0.987
	4.1	4.14	0.74	0.991
	5.1	3.14	0.77	0.992
	6.1	3.08	0.76	0.991
	7.4	1.93	0.80	0.994
CU701	2.9	4.74	0.54	0.985
	4.0	0.08	0.98	0.999
	5.0	0.08	0.98	0.999
	6.0	0.07	0.99	0.999
	7.0	0.07	0.99	0.999

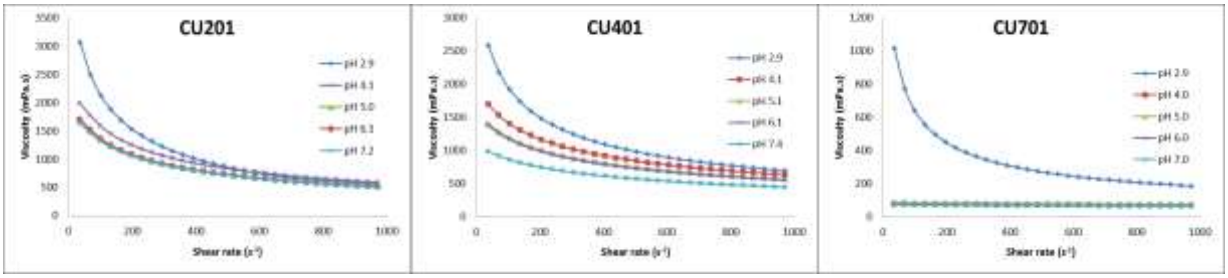

Figure 1. Effect of pH on viscosity profiles of pectin solutions.

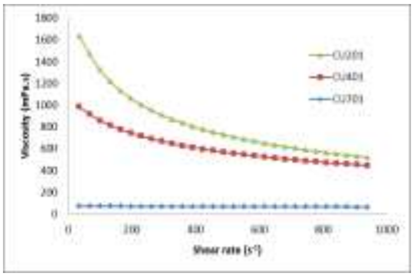

Figure 2. Viscosity profiles of pectin solutions at pH 7.0.

With regard to the consequence of pH on ionization, the relative peak intensity of C=O stretching of CU701 was clearly increased, especially at pH 7.0. The result confirmed the conversion of carboxylic acid to carboxylate. It was also noted that the peak intensity of C=O group due to carboxylate of HMP was not clearly increased. This result may be explained by the lower number of carboxyl group of HMP as compared to LMP.

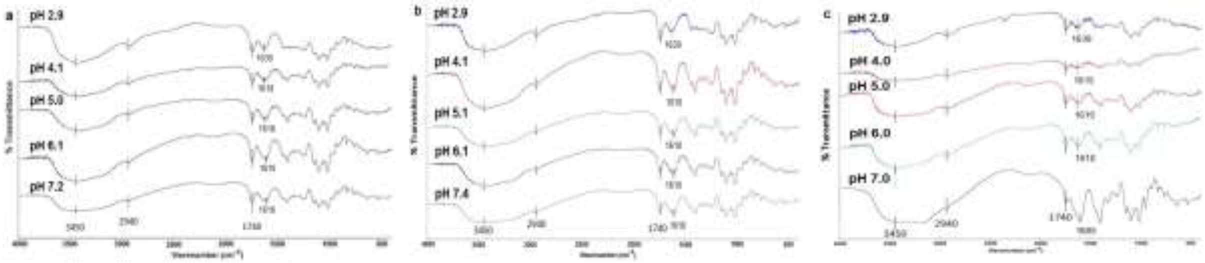

Figure 3. FTIR spectra of pectin films on different pH (a) CU201 (b) CU401 and (c) CU701.

Powder X-ray diffraction (PXRD) Crystallinity directly affects the solid state properties of films and consequently influences on the properties of coated products. As discussed in 3.1, 3.2, the pectin properties was changed as increasing pH and depended on the type of pectin. It was assumed that conversion to carboxylate anion, which depended on pH and degree of esterification, was a major factor affecting the properties. In order to further characterize the effect of these parameters on crystal properties of pectin, the PXRD diffraction patterns of pectin films were comparatively investigated. Figure 4 shows the PXRD pattern of pectin films prepared at various pH. At pH 2.9, all pectin grade showed amorphous characteristic as indicated by halo pattern.(Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007) As increasing to pH 4.1, CU701 demonstrated diffraction peaks at $2\theta = 12.5^\circ$, 14.2° and 20.2°. However, CU201 and CU401 did not show clear diffraction peak even increasing the pH to 7.4. The result indicated that only LMP was crystallized into semi-crystalline structure after changing into dried solid film.

With regard to the property of pectin solutions at increasing pH, the reduced viscosity was related with the free movement of more straight form of pectin containing carboxylate anion. Therefore, it might increase the possibility of polymer chains to come closer and arrange in more order way during drying process. As a result, the more ionized form of CU701 should facilitate the molecular arrangement in a highly ordered structure as compared to CU201 and CU401.

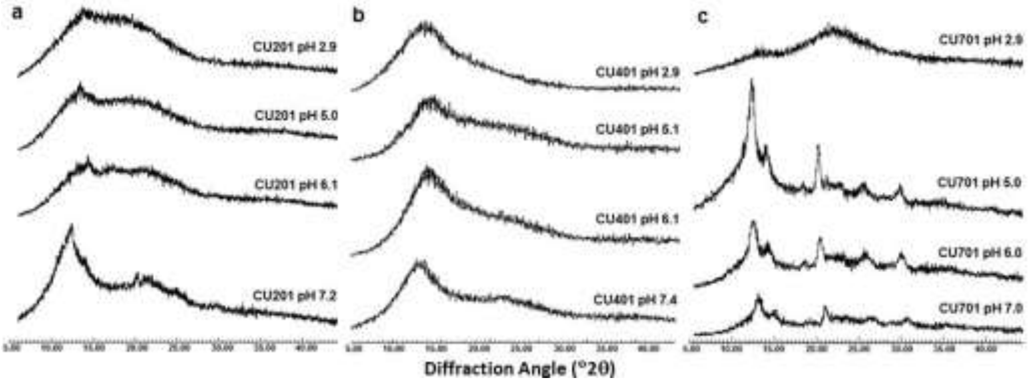

Figure 4. Powder X-ray diffraction of pectin films prepared at different pH (a) CU201 (b) CU401 and (c) CU701.

Conclusion

This study demonstrated the application of rheological analysis, FTIR spectroscopy and powder x-ray diffraction in structural analysis of pectin prepared at various pH. The results revealed that pH and pectin grades directly affected both solution and solid state properties, especially for low methoxyl pectin.

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