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DISSOCIATION OF DISSOLVED PECTINS: FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

F.t.i.r. spectra of neutralized pectic substances having a degree of methyl esterification ranging from 0 to 70 dissolved in pure D_2O solvent and in D_2O containing 0.05M NaCl were measured. Solvent-subtracted F.t.i.r. spectra were obtained before and after activation of pectin dissociation by briefly heating to 110°. Heat activation increased the integrated area under the band at 1607–1604 cm^{-1}. Based on literature assignments, this band was attributed to the antisymmetric stretching vibration of ionized carboxylate groups, and the increase in area was attributed to dissociation of pectin counter-ions simultaneous with the dissociation of pectin aggregates.

INTRODUCTION

In a series of papers, Fishman *et al.*[1–4] have demonstrated self-disaggregation between pH 3.7 and 7.3 for citrus pectin having degrees of methyl esterification (d.m.) up to 73%. Protonated and neutralized pectins form aggregates that can be induced by heat activation to undergo a concentration-dependent disaggregation[5]. This was demonstrated by the variation of reduced osmotic pressure with concentration in the range of 0 to 1.0 g/dL. All neutralized and protonated pectins, except low-methoxy, protonated pectin (d.m. 35 or 37%) exhibited in these plots a minimum at ~0.1 g/dL. Counter-ion binding increased with decreasing d.m., and, at a given d.m., it decreased following heat-activated disaggregation. The decrease in counter-ion binding implies a decrease in charge density upon dissociation. The present work focused on the infrared-spectral characteristics of sodium pectinates (d.m. 35, 58–60, and 70%) and sodium polygalacturonate (d.m. 0) in the 1800–1500-cm^{-1} region. We have measured the infrared spectra of heat-activated poly(galacturonic acid) and pectinates with the intention of confirming the counter-ion dissociation previously observed by membrane osmometry[2]. these studies were conducted on pectic substances dissolved in deuterium oxide in the absence and the presence of 0.05M NaCl.

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EXPERIMENTAL

Materials. — Deuterium oxide (99.8 atom%) was purchased from Cambridge Isotope Laboratories. The source and preparation of the various pectins have been described previously[1].

Infrared spectroscopy. — Sodium salts of pectins (10 ±0.7 mg) for F.t.i.r.-spectral measurements were dissolved in solvent (1 mL). To ensure complete H–D exchange, after dissolution in deuterium oxide, sodium pectinate solutions were kept for a minimum of 24 h prior to F.t.i.r.-spectral measurements. Each solution of polysaccharide in deuterium oxide was divided into two equal portions. The ionic strength of one portion was increased by adding NaCl (3 mg per mL) to give a solution 0.05M in NaCl. The deuterium-ion concentration in these unbuffered solutions was indicated by the pD, which was in the range of 5.0 to 5.5. F.t.i.r. spectra were recorded at room temperature with a Nicolet 7199 infrared spectrometer. The instrument resolution was 2 cm^{-1} for all spectra. The spectrometer was purged with dry nitrogen. Sample solutions and solvents were contained in a 0.15-mm path-length cell with CaF_2 windows. All solutions, solvents, and samples were prepared in a glove box under a dry nitrogen atmosphere. A 0.3-mL aliquot of each pectin solution was sealed in a vial and was heated for 10 min in a laboratory oven at 110 ±10°. Spectra of such heat-activated solutions were obtained within 24 h. All spectra were recorded at 30 ±2°. In order to observe the spectra of the pectins in the region 1900–1300 cm^{-1}, solvent-subtracted spectra of pectinate solutions were necessary. Difference spectra were generated by subtracting the solvent spectrum from the corresponding sample spectrum, using an interactive subtraction routine. Spectral integration and second-derivative spectra were accomplished with appropriate routines in the Nicolet software package.

RESULTS AND DISCUSSION

Four F.t.i.r. spectra between 1800 and 1500 cm^{-1} of sodium pectinate, d.m. 58–60%, 1.0 g/dL in D_2O and in 0.05M NaCl solution, before and after heat activation, are shown in Fig. 1. Deuterium oxide was used to avoid the broad HOH bending near 1640 cm^{-1}, which would overlap with the carboxylate antisymmetric stretch band. For cast films and solutions of self-aggregating poly(methyl methacrylate)[5,6], the ester carbonyl groups appear at 1741 and 1731 cm^{-1}. The carbonyl stretching vibration of pure poly(vinyl acetate) in the solid state (self-associated)[7] occurs at 1739 cm^{-1}. Pectins and alginates[8] produce an ester carbonyl band at 1740 cm^{-1}, and a carboxylate antisymmetric stretching band at 1606 cm^{-1}. Crystalline sodium, potassium, and rubidium salts of D-glucuronic acid exhibit strong, broad carboxylate bands[9] at ν_a 1606–1592 and ν_s 1495–1465 cm^{-1}. Solid-state spectra of cobalt, copper, nickel, and zinc salts of pectic acid (d.m. 0) and pectinic acid (d.m. 7) show carboxylate bands[10] at ν_a 1610 (Cu, ν_a 1620 cm^{-1}) and ν_s 1410 ±5 cm^{-1}. Tipson and co-workers[11] had pointed out a carbon–oxygen stretching band at 1637–

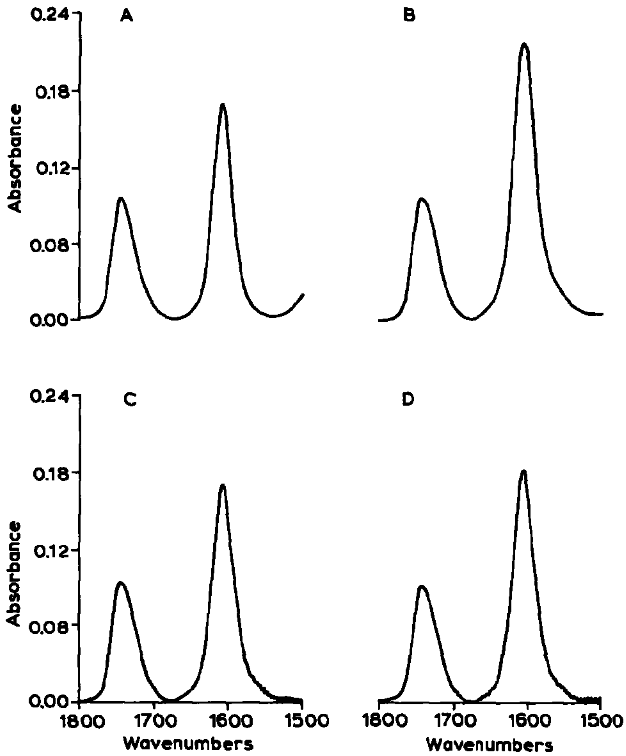

Fig. 1. F.t.i.r. spectra of sodium pectinate, d.m. 58–60%, at 30° in the 1800–1500 cm^{-1} region. [A, 1% in D_2O; B, 1% in D_2O, after heat activation; C, 1% in 0.05M NaCl; and D, 1% in 0.05M NaCl after heat activation.]

1600 cm^{-1} "that distinguishes carboxylate anions from the C=O stretching band of esters, and a band (medium strength)" at 1420–1300 cm^{-1}.

Two outstanding features of the infrared spectra of deuterium oxide solutions of sodium pectinate are bands at 1745–1743 and at 1607–1604 cm^{-1} (see Fig. 1). One other feature which should be mentioned is a band, beyond the aforementioned range, appearing at 1418–1417 cm^{-1}. In the spectra of sodium polygalacturonate, the absence of carbonyl stretching bands above 1680 cm^{-1} demonstrates that the polygalacturonate is de-esterified to a degree of $<2\%$. Likewise, ^{13}C-n.m.r. spectra of the same sample had no peak at 171.3 p.p.m., which also indicated no observable ester groups[4]. In the spectra of sodium pectinate, the broad band near 1745 cm^{-1} is assigned to methyl ester carbonyl groups.

The bands at 1607–1604 and 1417 cm^{-1} correspond to the antisymmetric (ν_a) and symmetric (ν_s) stretching vibrations of the carboxylate groups. The carboxylate stretching frequencies and the separation between them for pectins of different degrees of methyl esterification remain constant. The present results always showed a single, broad band at 1607–1604 cm^{-1} and a less-intense, sharper band, at 1418–

TABLE I

EFFECT OF HEAT ACTIVATION ON SODIUM PECTINATE IN D_2O SOLUTIONS: CARBOXYLATE ANTISYMMETRIC (a) AND ESTER (e) CARBONYL STRETCHING FREQUENCIES (cm^{-1}) AND INTEGRATED INTENSITIES[a,b]

D.m.	*Not heated*		*R1*	*Heat activated*		*R1*	*R2*
	a	*e*		*a*	*e*		
D_2O							
0	1606(14.82)	—	—	1606(20.07)	—	—	1.35
35	1606(9.83)	1743(2.27)	4.33	1605(10.14)	1743(1.96)	5.16	1.19
58–60	1605(5.22)	1743(3.20)	1.63	1605(8.11)	1744(3.32)	2.45	1.50
70	1607(5.17)	1744(5.78)	0.89	1604(7.44)	1744(5.99)	1.24	1.44
NaCl							
0	1606(15.89)	—	—	1606(20.84)	—	—	1.16
35	1607(10.44)	1743(2.39)	4.37	1606(10.07)	1743(2.37)	4.25	0.97
58–60	1606(6.27)	1744(3.56)	1.63	1606(6.90)	1744(3.54)	1.95	1.11
70	1605(5.48)	1745(4.51)	1.21	1604(6.74)	1744(4.59)	1.47	1.23

[a]Values in parentheses are the integrated intensities of the absorbance bands. [b]R_1 = ratio of intensities of ν_a/ν_e; R_2 = ratio of intensities of ν_a after to that before activation for 10 min at 110°.

1417 cm^{-1}, separated by 188 ±1 cm^{-1}. No change in ν_a, ν_s, or band separation was observed, regardless of the degree of esterification. Thus, ester groups do not appear to be involved in the sodium ion coordination.

To observe the dissociation of pectinates we shall consider only the ester carbonyl (ν_e) and carboxylate antisymmetric stretching (ν_a) vibrations. The former provides information for the sodium pectinates (d.m. 0), but not for sodium polygalacturonate (d.m. 0). The spectral effect observed before and after heat activation of sodium pectinates in deuterium oxide solutions is shown in Table I. In the i.r. spectra of pectinates, the ester carbonyl groups produce an asymmetric band which may be a composite of two bands. The effect of self-aggregation on the structure of syndiotactic poly(methyl methacrylate) in solution and in the solid state has been studied by i.r. spectroscopy[5,6]. Solvent-dependent self-aggregation of the polymer introduces a second ester carbonyl band[5], at 1741 cm^{-1}. In solvents where aggregation does not occur, the ester carbonyl band appears at 1731 cm^{-1}. Upon aggregation, a broad, overlapped doublet (1741 and 1731 cm^{-1}) is observed. Similarly, in the spectra of sodium pectinate solutions, a broad, asymmetric band having a maximum at 1745–1743 cm^{-1} is observed. Heat activation of these solutions for 10 min at 110° increases the intensity of the unresolved band only slightly for d.m. 58–60 and 70 (see Table I). Possibly, the breadth and skewness of the ester carbonyl band of sodium pectinate solutions indicate the presence of aggregated (1750–1748 cm^{-1}), as well as somewhat disaggregated (near 1730 cm^{-1}) species. That sodium pectinate is aggregated in solution, and that the tendency to disaggregate is increased by heat activation, had been demonstrated previously[1–4] by other means.

Carboxylate groups are present in sodium polygalacturonate (d.m. 0), as well as in the sodium pectinates. Spectral variations of the antisymmetric stretching of carboxylate groups can be related to counter-ion dissociation. The frequencies and intensities of the carboxylate bands, and their variation with d.m., both before and after heat activation, are also shown in Table I. The variation of i.r. spectra of cast films of sodium and calcium ionomers of an ethylene–methacrylic acid copolymer have been examined by Painter *et al.*[12]. The sodium ionomer gave a single band, which they assigned to a Na^+ ^-O_2C structure. Structural changes induced by elevated temperatures were discussed in terms of increases in the band intensities. The corresponding band frequency remained unchanged. Similarly, for sodium pectinate, the structural changes related to disaggregation and counter-ion dissociation, which are promoted by heat activation, can be observed as changes in the integrated intensity of the carboxylate antisymmetric stretching band.

The intensity ratio of integrated areas, R_1, of the carboxylate antisymmetric stretching band to that of the ester band, before and after heat activation, reflects the spectral changes of the carboxylate band, because pectinate disaggregation induces little if any change in ester band intensity. The ratio, R_2, of the intensities of the carboxylate antisymmetric stretching band after heat activation to that before heat activation demonstrates further that a structural change has occurred, which we correlate with simultaneous disaggregation and counter-ion dissociation of sodium pectinates. The behavior of sodium polygalacturonate and sodium pectinate solution should be compared with caution. Sodium polygalacturonate contains no neutral sugars, whereas neutral sugars are present in sodium pectinates as side chains, or as inserts, as in the case of rhamnose residues.

In conclusion, the increase in the integrated area of the carboxylate antisymmetric stretching band at 1607–1604 cm^{-1} upon brief heat-activation of sodium pectinates and polygalacturonate, appears to be correlated with pectin disaggregation and counter-ion dissociation, as was observed earlier, by membrane osmometry, on heat activation of pectins.

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