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Carbohydrate Research 308 (1998) 123–131

FT-IR study of pectate and pectinate gels formed by divalent cations

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Received 16 December 1997; accepted 5 March 1998

Abstract

The interaction of divalent cations with potassium pectate and three potassium pectinate samples with degrees of esterification E of 23.8, 59.1 and 93.4% were studied by FT-IR spectroscopy. Characteristic shifts occurred in C–O and ring vibrations in the 1200–900 cm^{-1} region as well as in the ν_{as} and ν_s carboxylate bands at around 1617 and 1420 cm^{-1}, indicating a metal coordination by the pectate chains in accordance with the 'egg-box' hypothesis [Grant et al. *FEBS Lett.*, 32 (1973) 195–198]. The FT-IR spectra showed some interaction between pectate and K^+ and Mg^{2+} even when no gels were formed. Ca^{2+} and Sr^{2+} interacted strongly with pectate and low methoxyl pectinates. Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} also showed complex formation with the pectinates at $E = 59.1\%$, and Pb^{2+} and Cu^{2+} to some extent even with very highly esterified pectinate ($E = 93.4\%$). © 1998 Elsevier Science Ltd. All rights reserved

Keywords: FT-IR; Pectate gels; Junction zones; Divalent cations

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1. Introduction

Crosslinking of pectinate molecules by Ca^{2+} ions plays an important role in the organisation of polysaccharides in plant cell walls. The structure of the chain aggregates has been described by the 'egg-box' model [1]. In this model the Ca^{2+} ions are thought to be held in the interstices of adjacent helical polysaccharide chains, complexed by oxygen atoms [2]. As a consequence, the conformation in the crosslinked junction zones in gels is different from that of free pectin chains in solution [3]. It was also reported that the structure of the hydrated gel state was a 2_1 helix rather than the 3_1 helix of dry calcium polygalacturonate [2,3]. In concentrated Ca^{2+}-pectate samples there may be a continuous transition from dimers of 2_1 helices to oligomers of 3_1 helices [4].

Other divalent cations also form pectate gels. The binding affinity of these ions varies widely. Previous studies of interactions of divalent cations with the carboxyl groups of pectates and pectinates have been conducted using various methods, and the results have been evaluated on the basis of binding isotherms, and different selectivity [5–14]. The influence of cation binding on the carboxylate

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PII S0008-6215(98)00065-2

bands at 1617 and 1420 cm^{-1} has already been discussed extensively in previous infrared studies [7,15–17]. These authors found a correlation between the size and charge density of the ions and the position of these carboxylate bands, but did not mention a similar correlation in other pectin bands. Little is known about the conformation of the pectin chains in these systems. Studies of alginate and pectin gels have shown that the divalent cations cause a change in CD spectra, due to the formation of regular 'egg-box' structures [9]. The presence of methyl ester groups affects the ion binding and gelling properties very strongly. The ester groups eliminate negative charges and may sterically hinder the formation of chain aggregates [8,10,18]. The extent of this effect depends mainly on the cation and also on the origin of the pectin sample [14].

CD and small-angle X-ray diffraction of isolated pectate chains have been the main methods of studying the chain conformations so far, but these do not work in more complex systems, e.g. whole cell walls. A ^{13}C CPMAS study has been used to study chain conformations in gel junction zones [4]. IR spectroscopy can also be used to examine carbohydrate polymers in cell walls in situ. In order to interpret the spectra, reference systems of the possible structures are required. The Ca^{2+} crosslinks are one such structure. In this work we have studied gels formed by divalent cations with pectate and pectinates with different degrees of esterification. In order to examine a range of possible geometries of the crosslinks, we have chosen differently sized ions, as well as including copper and magnesium, which have been reported to be special cases [5,9,19].

2. Results

Physical state.—The interaction of divalent cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}) with potassium pectate and three potassium pectinate samples with degrees of esterification E of 23.8, 59.1, and 93.4% were studied. The physical state of the samples is summarised in Table 1. The occurrence of gels reflected the known strength of ion binding [11,14]. Monovalent cations and Mg^{2+} did not form gels, whereas a strong Cu^{2+} gel was observed even at the highest degree of esterification. The other ions were intermediate in their tendency to form gels.

FT-IR spectra.—It has been shown that in pectinate gels all divalent cations given above interact stoichiometrically with the free carboxylate groups, displacing the monovalent cations [6,8,10,18]. We chose the potassium salt to study the pectate and pectinate chains in solution, and used those spectra as a baseline for their interaction with the divalent cations. The observed infrared frequencies and the band assignments are listed in Table 2. Fig. 1 shows the 1500–900 cm^{-1} region of the infrared spectra of potassium pectate and pectinates with different degrees of esterification in solution after subtraction of the water spectrum. With the increasing degree of esterification, band intensities increased at 1442, 1360, and 1265 cm^{-1} as well as at 975 cm^{-1}. At the same time the carboxylate band intensities at ca. 1420 and 955 cm^{-1} decreased. The most characteristic feature of these infrared spectra is a series of strong bands in the 1150–990 cm^{-1} region. Neither hydration nor methylation of the carboxylate group has a strong influence on the positions of these bands [17],

Table 1
Physical states and selected band shifts of the samples

Cation	Pectate, $E=0\%$		Pectinate, $E=23.8\%$		Pectinate, $E=59.1\%$		Pectinate, $E=93.4\%$	
	cm^{-1}	State	cm^{-1}	State	cm^{-1}	State	cm^{-1}	State
Na^{+}	1016	Liquid	1018	Liquid	1018	Liquid	1018	Liquid
K^{+}	1011	Liquid	1019	Liquid	1018	Liquid	1018	Liquid
Mg^{2+}	1004	Liquid	1016	Liquid	1018	Liquid	1018	Liquid
Ca^{2+}	1008	Gel	1009	Gel	1018	Liquid	1018	Liquid
Sr^{2+}	1007	Gel	1007	Gel	1018	Liquid	1018	Liquid
Cd^{2+}	1010	Gel	1011	Gel	1016	Gel	1018	Liquid
Ni^{2+}	1001	Gel	999	Gel	1003[a]	Gel	1018	Liquid
Zn^{2+}	1002	Gel	1001	Gel	1005[a]	Gel	1018[b]	Liquid
Pb^{2+}	1004	Gel	1007	Gel	1005[a]	Gel	1018[b]	Soft gel
Cu^{2+}	1008	Gel	1010	Gel	1012	Gel	1018[b]	Gel

[a] Shoulders.
[b] Small residues of shifted bands remain after subtraction of the 1018 cm^{-1} band.

Table 2
Band positions[a] in the Fourier-deconvoluted FT-IR spectra

Pectate										Pectinate, $E=23.8\%$									Pectinate, $E=59.1\%$							Pectinate, $E=93.4\%$	Band assignment[c]
Na^+	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Pb^{2+}	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Pb^{2+}	K^+, Mg^{2+}, Ca^{2+}	Sr^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Pb^{2+}	K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} [b]	
										1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	1444	OCH_2 deformation of methyl ester
1414	1417	1420	1421	1422	1419	1406	1418	1424	1412	1419	1416	1423	1417	1412	1403	1417	1425	1414	1417	1412	1414	1412	1416	1422	1412		Symmetric stretch of carboxylate
										1363	1362	1377	1372	1367	1366	1367	1362	1367	1372	1369	1368	1362	1371	1366	1369	1367	δ-s CH_2 of ester
1357	1359	1356	1355	1356	1356	1353	1356	1357	1356	1353	1350	1356	1356	1356	1351	1353	1358	1358	1354	1356	1355	1352	1353	1354	1352		Ring vibration (weak)
1331	1334	1332	1337	1338	1333	1331	1332	1332	1337	1332	1331	1336	1336	1333	1331	1331	1333	1335	1330	1331	1333	1331	1333	1331	1331	1331	Ring vibration
										1268	1262	1216	1261	1261	1261	1260	1263	1264	1263	1263	1263	1263	1263	1265	1265	1269	COC of ester
1238	1238	1241	1244	1242	1242	1240	1243	1247	1243	1235	1228	1241	1242	1242	1239	1238	1228	1238	1240	1236	1242	1240	1240	1237	1238	1235	CO, CC of ring
1144	1142	1146	1145	1144	1149	1150	1149	1147	1142	1145	1146	1147	1146	1151	1152	1149	1153	1146	1146	1147	1151	1153	1151	1149	1147	1149	C–O–C of glycosidic link/ring
1102	1101	1103	1101	1097	1103	1104	1105	1105	1100	1105	1105	1107	1109	1105	1105	1103	1105	1105	1107	1107	1105	1109	1109	1107	1111	1109	CO, CC, CCH, OCH, ring
1076	1074	1076	1075	1078	1076	1078	1076	1076	1074	1078	1080	1078	1078	1078	1079	1078	1076	1076	1080	1080	1078	1080	1078	1078	1080	1080	CO, OCH deformation
1045	1046	1048	1045	1043	1047	1046	1047	1047	1043	1048	1049	1045	1045	1047	1047	1047	1047	1049	1049	1049	1049	1049	1049	1049	1049	1051	CO, HCO, OCH, ring
1028	1023	1024	1027	1026	1028	1025	1024	1027	1022	1030	1029	1028	1028	1026	1028	1028	1030	1030	1030	1031	1028	1030	1028	1030	1030	1030	OC, CC, CCH
1016	1011	1004	1008	1007	1001	1008	1002	1010	1004	1019	1016	1009	1007	999	1010	1022	1011	1007	1018	1018	1018	1012	1022	1016	1020	1018	C-2C-3, C-2O-2, C-1O-1
																1001					1003		1005		1005		C-2C-3, C-2O-2, C-1O-1
992	988	982	987	992	976	985	987	988	982	993	991	991	991				991		984	985		980	980	980	984	985	
										978	978	982	972	972	986	978	978	978	972	974	975	972	972	972	972	970	OCH_3 of ester
954	954	957	959	957	957	958	957	959	949	958	960	958	958	960	959	958	958	953	958	957	959	960	958	960	959		Carboxyl group deformation

[a] All band positions are given with an accuracy of ± 2 cm^{-1}.

[b] Sr^{2+} and Ni^{2+} were not measured.

[c] Using refs [16] [20].

which therefore must be regarded as largely uncoupled from the vibrations of the carboxylate side chain. These bands arise from strongly coupled C–C and C–O vibration modes of the saccharide ring, its hydroxyl groups and the glycosidic linkage. A previous normal mode analysis [20] made the following assignments: 1148 cm^{-1}, ν(C–O–C); 1105 cm^{-1}, ring (C–O, C–C); 1080 cm^{-1}, ν(C–O) + δ(OCH); 1052 cm^{-1}, ring (C–O, C–C); 1024 cm^{-1}, ring (C–O, C–C). The strongest peak in sodium pectate (Table 2) appeared at 1016 cm^{-1}. In potassium pectate solution ($E = 0\%$, Fig. 1a) we found this band at 1011 cm^{-1}. In the esterified samples ($E = 23.8$, 59.1, and 93.4%), shown in Fig. 1b–d, it was at 1018 cm^{-1} and the bands did not show any change that could be attributed to potassium ion binding. At $E = 93.4\%$ the spectra of all the studied cations looked very similar to the K$^+$ pectinate. This was due to the fact that the spectra were dominated by the esterified form. The contribution of the few remaining carboxylate groups was only visible after subtraction of the ester spectrum. The absence of band shifts in the K$^+$ pectinate spectra has shown that K$^+$ did not interact with the free carboxylate groups of the esterified pectinate, and this sample was therefore used as reference.

Mg^{2+}.—The spectrum of the magnesium pectate solution (Fig. 2a) showed a band at 1004 cm^{-1}. However, this band was not as strong as with the other divalent cations and it was not found in the magnesium pectinate samples at $E = 23.8$, 59.1, and 93.4% (Fig. 2b–d). At $E = 93.4\%$ the subtraction of the K$^+$ pectinate resulted in a flat line (Fig. 2e).

Ca^{2+}/Sr^{2+}.—The infrared spectrum of calcium pectate gel (Fig. 3a) showed a marked change in some band positions in the 1200–950 cm^{-1} region. The most obvious was a shift of the main maximum of the 1018 cm^{-1} band to 1008 cm^{-1}, with an underlying band at about 1025 cm^{-1} becoming more apparent. This feature was similar to the change indicated in the potassium pectate spectrum, but much more pronounced. In contrast to the potassium pectinate, it was still apparent in the spectra of calcium pectinate with 23.8% esterification (Fig. 3b). The spectra of the samples with $E = 59.1$ and 93.4%, however, no longer showed these band shifts (Fig. 3c,d). As in Mg^{2+} the subtraction of K$^+$-pectinate resulted in a flat line at 93.4% esterification (Fig. 3e). This showed that the state of the pectinate was the same as in the K$^+$ sample. The influence of strontium was found to be identical to calcium (Table 2). The similarity in the IR spectra indicated that the binding of Sr^{2+} is extremely similar to Ca^{2+}. This confirms earlier reports that the structural difference between these samples was minimal [1].

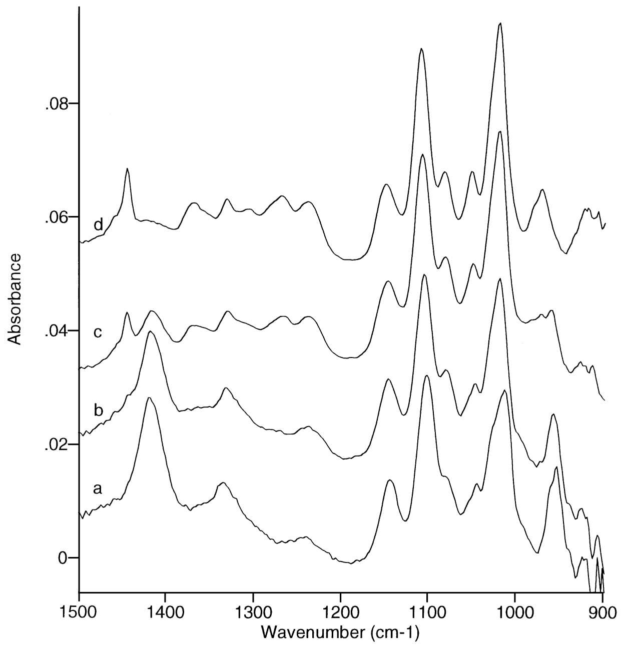

Fig. 1. FT-IR spectra of potassium(I) salt in water: (a) pectate; (b) pectinate $E = 23.8\%$; (c) pectinate $E = 59.1\%$; (d) pectinate $E = 93.4\%$.

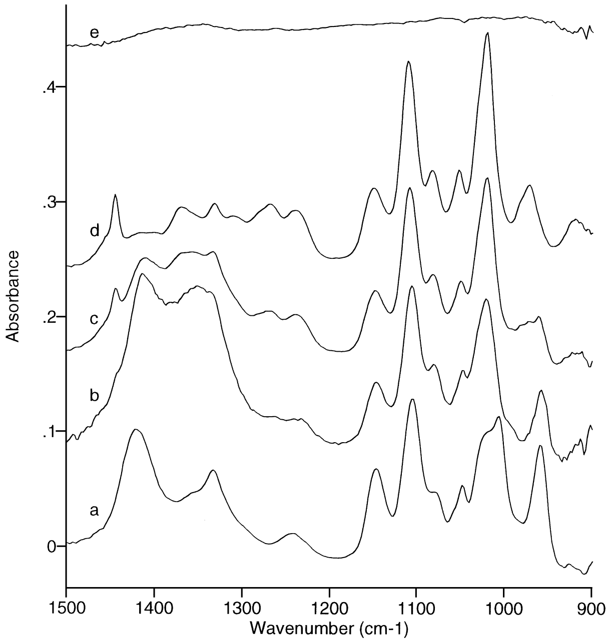

Fig. 2. FT-IR spectra of magnesium(II) salt in water: (a) pectate; (b) pectinate $E = 23.8\%$; (c) pectinate $E = 59.1\%$; (d) pectinate $E = 93.4\%$; (e) Mg^{2+}-pectinate minus K$^+$-pectinate $E = 93.4\%$.

Zn^{2+}/Ni^{2+}.—The spectrum of zinc pectate gel (Fig. 4a) showed a pattern of shifts similar to the calcium pectate gel. Some slight differences in the position of the bands were observed, with the band maximum shifted to 1002 cm^{-1}, and peaks at 1040 and 1240 cm^{-1} more pronounced than in the calcium pectate gel. As with calcium pectinate, the same shifts were observed in the spectra of the pectinate samples at $E = 23.8\%$ (Fig. 4b). In contrast, the spectrum of the zinc pectinate gel at $E = 59.1\%$ (Fig. 4c) still had a strong shoulder at around 1005 cm^{-1}, which was a remainder of the 1002 cm^{-1} band of the pectate. This visible shoulder disappeared in the fully esterified sample ($E = 93.4\%$, Fig. 4d). However, after subtraction of the K^+-pectinate a very small residue of the shifted band at 1006 cm^{-1} remained (Fig. 4e). Comparable behaviour was found with nickel (Table 2).

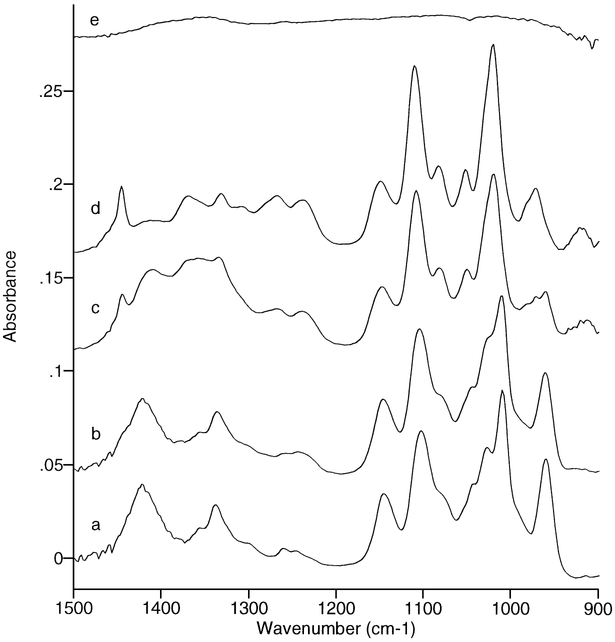

Fig. 3. FT-IR spectra of calcium(II) salt in water: (a) pectate; (b) pectinate $E = 23.8\%$; (c) pectinate $E = 59.1\%$; (d) pectinate $E = 93.4\%$; (e) Ca^{2+}-pectinate minus K^+-pectinate $E = 93.4\%$.

Cu^{2+}/Cd^{2+}.—Fig. 5a shows the infrared spectrum of the copper pectate gel. The values of the band shifts in Cu^{2+} (1008 cm^{-1}) and Cd^{2+} (1010 cm^{-1}) pectate were comparable and these shifts persisted in the spectra of the pectinates at $E = 23.8\%$ (Fig. 5b). The shifted band was still dominant in the Cu^{2+} pectinate at $E = 59.1\%$ (Fig. 5c). However, the spectrum of the Cd^{2+} pectinate at 59.1% contained only a weak shoulder which indicated a remainder of the shifted band of the Cd^{2+} pectate (Table 2). The difference spectrum of the Cu^{2+} pectinate at $E = 93.4\%$ minus K^+ pectinate (Fig. 5e) clearly resembled the spectrum of Cu^{2+} pectate. This showed that even at this high degree of esterification a small population of the Cu^{2+} pectate gel structures persisted.

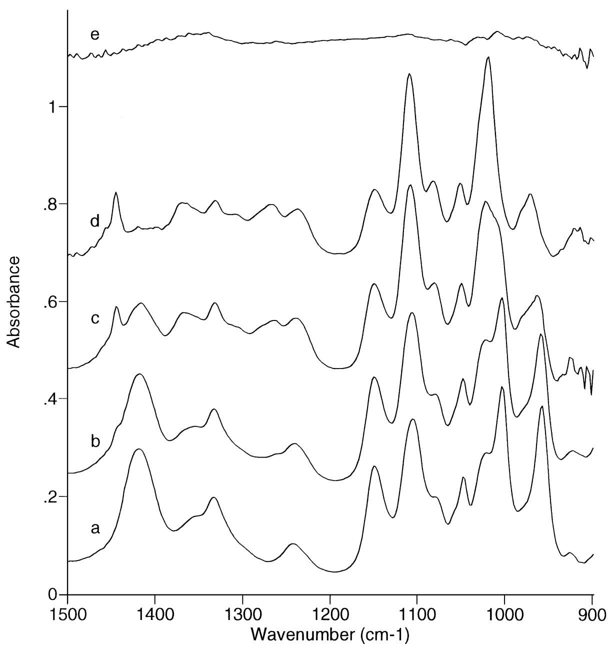

Fig. 4. FT-IR spectra of zinc(II) salt in water: (a) pectate; (b) pectinate $E = 23.8\%$; (c) pectinate $E = 59.1\%$; (d) pectinate $E = 93.4\%$; (e) Zn^{2+}-pectinate minus K^+-pectinate $E = 93.4\%$.

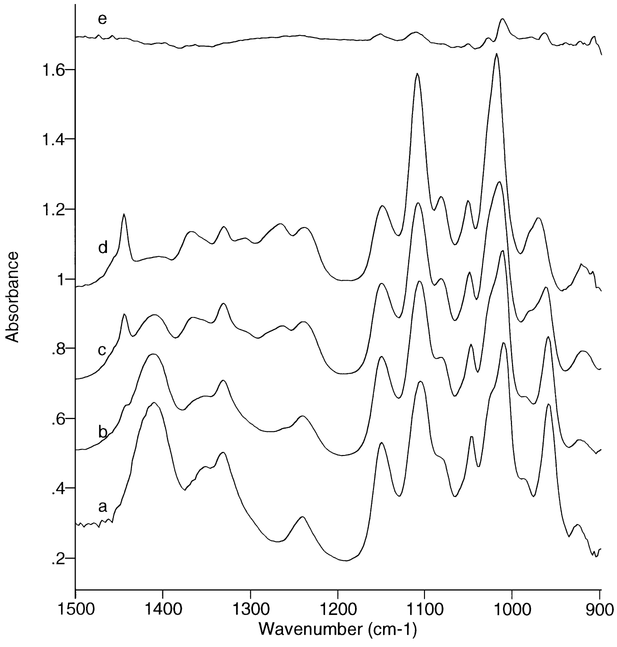

Fig. 5. FT-IR spectra of copper(II) salt in water: (a) pectate; (b) pectinate $E = 23.8\%$; (c) pectinate $E = 59.1\%$; (d) pectinate $E = 93.4\%$; (e) Cu^{2+}-pectinate minus K^+-pectinate $E = 93.4\%$.

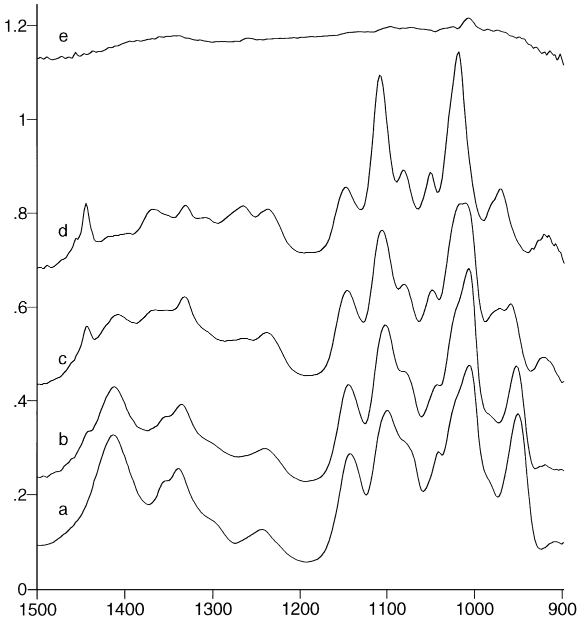

Fig. 6. FT-IR spectra of lead(II) salt in water: (a) pectate; (b) pectinate $E=23.8\%$; (c) pectinate $E=59.1\%$; (d) pectinate $E=93.4\%$; (e) Pb^{2+}-pectinate minus K^+-pectinate $E=93.4\%$.

Pb^{2+}.—Fig. 6 shows that in lead pectate and pectinates the pattern of the infrared bands was much like that observed with zinc. On the whole, the band shapes were broader and less resolved in the 1200–950 cm^{-1} region. In lead pectate (Fig. 6a) the band originally centred at 1018 cm^{-1} was observed at 1004 cm^{-1}. In pectinate with $E=59.1\%$ (Fig. 6c), enough of this shifted band remained to create a shoulder at 1010 cm^{-1} even more pronounced than in the case of zinc. After subtraction of the K^+-pectinate from the spectrum of Pb^{2+}-pectinate at 93.4% a small residue of the shifted band at 1006 cm^{-1} remained (Fig. 6e).

3. Discussion

Earlier infrared investigations of dry pectate gels showed that the positions of the ν_{as} and ν_s carboxylate bands at around 1617 and 1420 cm^{-1} could be correlated with ionic parameters [15–17] such as molecular mass, ionic radius, and electrostatic charge density. But this correlation held only for the group I and II elements, and it was implied that for transition elements the situation could be different [16]. Deiana et al. [7] found that the binding of transition metal cations varied with the structure of the metal complex. Apart from the influence on the carboxylate bands, the most obvious effect of the interaction with the ions was a shift of the strong band centred at 1018 cm^{-1} in pectinate solutions. The shift of this band arising from C–O and C–C vibrations of the pyranoid ring would be difficult to explain if the metal interacted only with the carboxylate group. It is, however, in good agreement with the models of solid and gel structures based on X-ray [21,22] and EXAFS [2] data, which suggested inner sphere complexes where Ca^{2+} interacts with the carboxylate group (O-6), the ring oxygen (O-5), the oxygen (O-4 = O-1′) in the glycosidic bridge, and a hydroxyl group (O-2′) from the next galacturonate residue in the pectate chain [2,22]. Two water molecules occupied the remaining Ca^{2+} coordination sites both in 2_1 and 3_1 helical arrangements [2]. There was a contrasting report of an outer-sphere complex geometry in which some ions would retain their hydration shell [7]. However, recent X-ray data clearly showed that Ca^{2+} can replace bridging water molecules between adjacent helices [23]. Therefore we concluded that the observed shifts in the pectate or pectinate spectra do show the formation of a multidentate metal complex.

The interactions with Ca^{2+}, Sr^{2+}, Ni^{2+}, Zn^{2+} Cd^{2+}, Cu^{2+}, and Pb^{2+} all resulted in a similar pattern of band shifts in the pectate. Therefore we may infer that these ions were bound to the pectate in a similar way. We expected the different radii of the ions to force the polysaccharide chain conformation to adapt somewhat in order to form junction zones. This may have caused some of the small shifts in the infrared bands, but our study did not show a direct correlations of band shifts with ionic parameters such as ion size and charge density. This suggests that the complexes may be governed by different rules, especially the varying coordination arrangements of transition elements. This is particularly noticeable in the case of Cu^{2+}, where the metal–oxygen bonds have at least some covalent character, as was shown by the comparatively low position of the ν_s carboxylate band at 1406 cm^{-1} [7,9]. However, our infrared results indicate a coordination of the Cu^{2+} complex which is comparable to that of the other ions. This differs from a previously reported CD study which indicated a special arrangement for Cu^{2+} [9]. Those CD data may be explained by the different electronic structure of the metal–carboxylate bond in this complex.

Previous experiments [6,8,10,11,14,18] have shown a wide variety of binding constants of ions with pectin in solution. While we could not find a correlation of the size of the band shifts in pectate with the stability of individual pectate ion complexes, the persistence of these shifts in pectinates did seem to coincide with the formation of gels. Table 1 compares the physical state and the position of the above discussed pectate band at $1018\,\mathrm{cm}^{-1}$. The band shifts caused by K^+ and Mg^{2+} in the pectate solution ($E=0\%$) could be interpreted as a weak complex formation, although no gels were formed. The 'egg-box' model is based on cation binding in cavities between two or more adjacent pectate chains, but K^+ and the non-gelling Mg^{2+} system show that a complex may be formed with single chains as well. Large monovalent cations have been reported to effectively interact with low methoxyl pectin and κ-carrageenan [24,25]. In particular the observation of such a complex with K^+ could explain the finding of a synergistic effect of this ion on the Ca^{2+} gelling [24]. In the K^+ and Mg^{2+} pectinate solutions no such interaction was observed, indicating that these ions were dissociated.

Ca^{2+} ions are dissociated from monogalacturonate [11], and the observed cooperativity of its binding with longer oligogalacturonates and pectate suggests that the stability of the gel junction zones is due to the collective effect of many neighbouring complexes [26]. Ca^{2+} and Sr^{2+} formed gels with pectate and with pectinates having 23.8% esterification [27]. In both these samples the changes in the IR spectra indicated complex formation of the ions with the pectin. However, in the pectinate solution with $E=59.1\%$ complex formation was no longer observed. These ion complexes, therefore, are only stable in the gel junction zones. Esterification reduces both the number of negative charges and possible ion binding sites. Also, the structure of the pectate junction zones in gels is supposed to be a two-fold helix, whereas the pectinic acid is said to prefer a three-fold helix geometry [3,28,29], which may indicate a sterical hindrance. These effects should reduce the possible size of the junction zones and their cooperative stabilisation by many neighbouring ion complexes. Ni^{2+}, Zn^{2+}, Pb^{2+}, and Cu^{2+} formed gels, and their spectra showed noticeable complex formation, with the pectinates up to $E=59.1\%$. At this level, the extent of the complex formation increased in line with the increasing binding constants [6,10,11]. Therefore, the individual metal complexes have to be more stable, needing less cooperativity, in order to form gel junction zones in samples with higher degrees of esterification. Our results have shown that Cu^{2+}, and to a smaller extent Pb^{2+} and Zn^{2+}, interacted strongly with the pectinate chain even in highly esterified pectinate ($E=93.4\%$). Thus the Cu^{2+} complex stability is high enough to form a gel even when only 6% free carboxylate groups are present. In this case a statistical distribution of the few remaining free carboxylate groups suggests junction zones formed by single Cu^{2+} complexes.

4. Experimental

Samples.—Sodium pectate (Citrus Colloids, Hereford, UK) was used without purification. The pectin samples with various degrees of esterification of the carboxyl groups ($E=0$, 23.8, and 59.1%) were prepared from purified commercial citrus pectin (Genu Pectin, Medium Rapid Set, Type A, Pectinfabrik Kopenhagen, Denmark) by total and partial alkaline deesterifications, respectively, in 60% ethanol suspensions with a dilute potassium hydroxide solution. The highly esterified pectin with $E=93.4\%$ was obtained from the purified citrus pectin by esterification with methanol in acidic medium (1 M H_2SO_4) at 3 °C for three weeks [6].

The purified commercial pectin contained 85% (dry weight) of D-galacturonan. The esterification degree and the polyuronide content of the prepared pectin samples, as well as the concentrations of free carboxyl groups in solutions of pectate and pectinates for IR measurements, were determined either by the method of precipitation of the insoluble copper pectate and pectinates or potentiometrically [30,31].

The stock solutions of M^{2+} nitrates ($0.1\,\mathrm{mol\,L^{-1}}$; $M^{2+}=Mg^{2+}$, Ca^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}) and $SrCl_2$ were prepared and the concentrations of M^{2+} ions in the solutions determined by chelatometry (Complexon IV, $0.01\,\mathrm{mol\,L^{-1}}$; indicators Murexide or Eriochrome Black T, ammonium buffer solution) employing the spectrophotometric indication of the point of equivalence (with the interference filters Zeiss Jena IF 600 or 650 nm).

Salts of divalent cations of pectins with various degrees of esterification were prepared in the following way. To the solutions of potassium pectate or potassium pectinate with $0.025\,\mathrm{mol\,L^{-1}}$ free carboxylate concentration the solutions of M^{2+} cations were added stoichiometrically (2:1) in $0.0125\,\mathrm{mol\,L^{-1}}$ concentration slowly with thorough stirring. For pectinate with $E = 93.4\%$ these concentrations were halved in order to keep the pectinate soluble. The total concentrations of the polysaccharide were: pectinate $7.26\,\mathrm{g\,L^{-1}}$, pectinate with $E = 23.8\%$ $9.69\,\mathrm{g\,L^{-1}}$, pectinate with $E = 59.1\%$ $15.9\,\mathrm{g\,L^{-1}}$, and pectinate with 93.4% $44\,\mathrm{g\,L^{-1}}$. All samples were left standing to set for 12–16 h. Depending on the degree of esterification of the pectin and the cation added, they either remained viscous solutions or formed clearly visible gels with different consistencies.

IR spectroscopy.—IR spectra were measured on a Nicolet Magna-IR 750 with DTGS detector (OMNIC 3.1 software). The samples were measured in a transmission cell with BaF_2 windows and a 25 mm teflon spacer. 128 scans at $4\,\mathrm{cm^{-1}}$ resolution were coadded and referenced against air. Water spectra recorded under the same conditions were digitally subtracted to obtain the IR spectra of the hydrated polysaccharide samples.

The spectra were Fourier-deconvoluted in the $1500–900\,\mathrm{cm^{-1}}$ region to resolve overlapping bands. Parameters ranged from $19\,\mathrm{cm^{-1}}$ bandwidth/enhancement factor 1.7 to $36\,\mathrm{cm^{-1}}$ bandwidth/enhancement factor 2.1 depending on cations and concentration.

Acknowledgements

The financial support from the BBSRC from its Competitive Strategic Grant is gratefully acknowledged by N.W., R.H.W and P.S.B. M.K. and A.M. are grateful for support from the Slovak Grant Agency (2/4144 and 2/1240). The authors wish to thank the British Council Slovakia for funding the collaboration between IFR and SAS.

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