

Factors Influencing the Properties of Pectic Acid Films

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Synopsis

A pectic acid, extracted in 11% yield from tobacco stems, contains 93% anhydrogalacturonic acid and some galactose. Plasticized films have been cast from water solution and the influences on tensile properties of casting temperature, extent of neutralization, nature of cation, and plasticizer (glycerol) content have been studied. The crystallinity of the pectate films is shown to increase very markedly with neutralization by sodium hydroxide in the range 40–100% neutralization. The ion exchange capacities of sodium hydrogen pectate films for alkaline earth cations are discussed.

INTRODUCTION

We have found that cured and aged tobacco stems (i.e., leaf mid-ribs) are a convenient source of an unesterified pectic acid of moderately high molecular weight. To some extent this product may be regarded as a novel type of industrial raw material, since normal sources of pectin yield an esterified pectic acid which must be subjected to careful enzymic saponification if a free pectic acid is required.¹ Because of this, all of the previous work²⁻⁷ on mechanical properties of pectates has been carried out on pectic acids containing varying proportions of methyl ester groups. The work herein described therefore, comprises the first study of the properties of films of unesterified pectic acid.

Films of pectic and pectinic acids are used in food packaging and coating and also in reconstituted tobacco sheet and their properties are therefore of considerable technological interest. Furthermore, the influence of variables such as extent of neutralization and nature of cation, on properties of pectate films is likely to be relevant to film properties of other polymeric acids, such as alginic acid, carboxymethyl cellulose, and polyacrylic acid.

EXPERIMENTAL

Isolation of Pectic Acid

The stems removed from flue-cured and fermented Connecticut Broad Leaf tobacco (1961 crop) were chopped into ca. 2-cm. lengths and used in all subsequent experiments. Moisture content of the stems was deter-

mined arbitrarily from the loss in weight of a sample kept at 100°C. in a convection oven for 16 hr.

A 60-g. air-dry sample (dry weight 51.5 g.) was covered with 1 liter of 0.1% solution of cetylpyridinium bromide in water and kept at ambient temperature ($15 \pm 3^\circ\text{C}.$) for 16 hr. The stems were removed, further washed with three 500-ml. portions of water, and then stirred and boiled for 3 hr. with 1 liter of a 1% solution of ammonium oxalate in water. During this time the stems disintegrated to a pulp. After pressing the pulp in a filtercloth and washing with three 400-ml. portions of water, the combined filtrate and washings were centrifuged and the resultant clear brown solution stirred vigorously, while 10 ml. concentrated hydrochloric acid was added dropwise to precipitate the pectic acid as a gel.

The gel was separated by centrifuging, washed with four 500-ml. portions of a mixture of acetone and 0.01*N* hydrochloric acid (1:1, v/v) and then with two 500-ml. portions of 50% (v/v) aqueous acetone.

The washed, centrifuged gel had a solids content of 6.0% (w/v), corresponding to a pectic acid yield of 11.6% of the dry stems. It was a pale amber color and a portion was freeze-dried to a spongy beige solid, ash content 0.2%; OCH_3 , 0.2% (Zeisel method); $[\alpha]_D^{20} +244^\circ \pm 8^\circ$ ($c = 0.25$ in 0.155*M* sodium chloride at pH 6). Paper chromatography on Whatman No. 1 paper with ethyl acetate, pyridine, water (9:2:1, v/v), sprayed with silver nitrate/sodium hydroxide,⁸ showed no detectable mono- or oligosaccharides in the gel. A 0.75-g. sample of gel was heated with 2 ml. 1*N* sulfuric acid for 1 hr. at 95°C., neutralized with barium carbonate, and chromatographed in the same way to indicate the presence of galactose (R_A 0.44), a trace of rhamnose (R_A 1.30) and a very faint trace of arabinose (R_A 1.00), produced by the hydrolysis. Paper chromatography in ethyl acetate acetic acid, formic acid, water (18:3:1:4, v/v) indicated the presence of galacturonic acid.

Titration of the diluted gel with 0.1*N* sodium hydroxide using a pH meter with calomel and glass electrodes, gave an inflection at pH 8.3, and equivalent weight 190.

Viscosity of Pectate Solutions

A 6.001-g. sample of a 3.32% (w/w) pectic acid gel was dissolved in 75 ml. 0.16*M* sodium chloride solution, 1.0*N* sodium hydroxide added to pH 6.0, and the resultant solution diluted to 100 ml. and filtered. The viscosity of this solution and of subsequent dilutions with 0.16*M* sodium chloride solution were determined at $25 \pm 0.05^\circ\text{C}.$ in a capillary viscometer; $[\eta] = 185 \text{ ml./g.}$

Preparation of Films

A sample of the pectic acid gel of known free acid content, prepared as described above, was diluted with an equal volume of water, neutralized with alkali as required, and then concentrated at 40°C. (bath temp.)/15 mm. to ca. 3% (w/v). The solution was filtered through a No. 2 sinter

and the accurate concentration determined by drying an aliquot portion at 60°C./15 mm. The required plasticizer was next added (plasticized concentrations are expressed as a percentage of the weight of pectic acid), and after mixing, 15–20 ml. of the solution containing 0.4–0.5 g. pectic

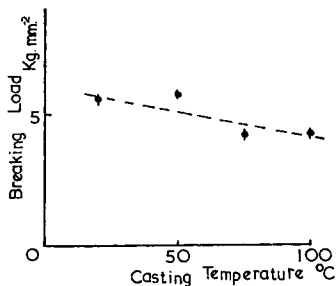


Fig. 1. Breaking load of pectic acid, 50% neutralized with sodium hydroxide, plasticized with 30% glycerol.

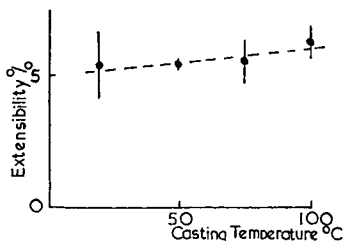


Fig. 2. Extensibility of pectic acid, 50% neutralized with sodium hydroxide, plasticized with 30% glycerol.

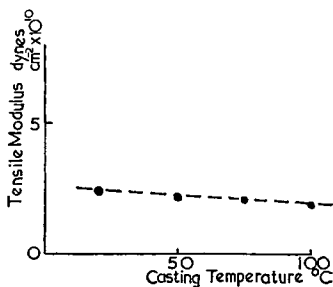


Fig. 3. Tensile modulus of pectic acid, 50% neutralized with sodium hydroxide, plasticized with 30% glycerol.

acid was poured into a rectangular glass dish having a base 14 × 6 cm. The dish was placed on a leveled base in an oven at 75 ± 1°C. through which a slow stream of ambient air was drawn. The film was dry in 2 hr. and the dish was then kept at 20°C., 65% R.H. for 1 hr. before removing the film. Films were similarly cast at 20, 50, and 100°C. from a 50%

neutralized sodium hydrogen pectate solution containing 30% glycerol and their properties are shown in Figures 1-3.

Measurement of Film Properties

All mechanical measurements were carried out on a Cambridge extensometer in a room maintained at $20 \pm 0.5^\circ\text{C}$., $65 \pm 2\%$ R.H., and the films were conditioned in this room for at least 2 days. Film properties are shown in Figures 1-9.

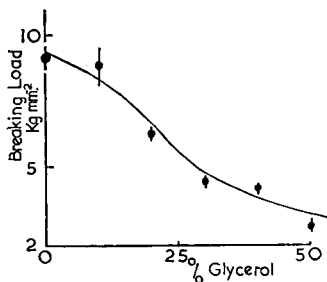


Fig. 4. Breaking load of pectic acid, 50% neutralized with sodium hydroxide, cast at 75°C .

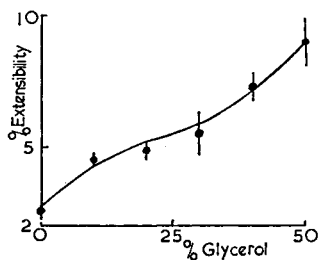


Fig. 5. Extensibility of pectic acid, 50% neutralized with sodium hydroxide, cast at 75°C .

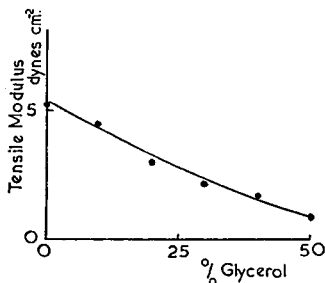


Fig. 6. Tensile modulus of pectic acid, 50% neutralized with sodium hydroxide, cast at 75°C .

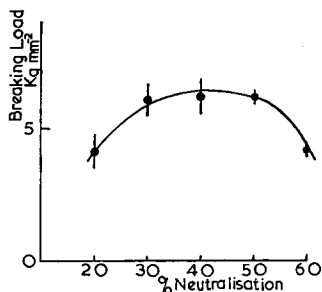


Fig. 7. Breaking load of pectic acid, partially neutralized with sodium hydroxide, plasticized with 20% glycerol, cast at 75°C.

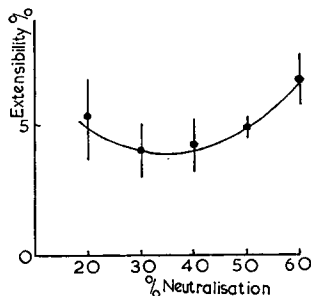


Fig. 8. Extensibility of pectic acid, partially neutralized with sodium hydroxide, plasticized with 20% glycerol, cast at 75°C.

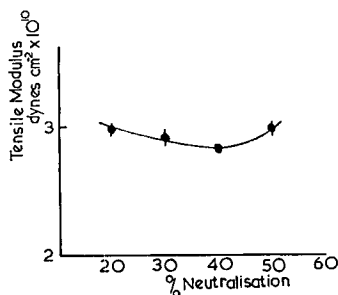


Fig. 9. Tensile modulus of pectic acid, partially neutralized with sodium hydroxide, plasticized with 20% glycerol, cast at 75°C.

For breaking measurements, samples of 40 × 2.1 mm. were cut by stamping with parallel-mounted razor blades, mounted at 30 mm. test length and stressed to break at a constant rate of load of 5.5 g./sec. The sample thickness was measured before test, by means of a micrometer with 10 mm. diameter anvils, and normally showed variation of ± 5% within a given sample. Results are expressed as an average of five breaks. The breaking load measurements were usually more reproducible than extensibility, but spread in both was always considerable and is indicated

as standard deviation in the usual way in the graphs. The stress-strain curves were concave to the strain axis over the whole of their length in all of the experiments, without showing a marked yield point.

For measurement of initial modulus, duplicate samples of 110×2.1 mm. were cut by drawing parallel-mounted razor blades along a straight-edge. The average thickness (ca. 0.06 mm.) was determined after testing from seven equally spaced measurements along the samples with the micrometer, the samples mounted at 100 mm. test length and extended at a constant rate of load 5.5 g./sec. for 1.0 mm. (for a particular sample this corresponds to a constant rate of extension of 0.02–0.06%/sec.). The curve up to this point showed barely measurable deviation from a straight line. The extensometer was immediately reversed to zero and the sample again extended for 1.0 mm. at the same rate. This cycle was repeated for a total of five times, during which the samples acquired a permanent "set" of ca. 0.3 mm., but the 1% secant modulus remained constant within experimental error (estimated $\pm 4\%$ for extensometer and for sample thickness). Modulus determinations on duplicate strips were normally reproducible within the same margin of error.

Determination of the Effect of Cations on Wet Strength

Plasticized films of sodium hydrogen pectate (i.e., pectic acid 50% neutralized with sodium hydroxide), were prepared as described above. Test samples of 40×2.1 mm. were cut, their thickness measured, and then immersed in a 1.0M solution of the required salt (adjusted to pH 2.5 with the acid corresponding to the anion of the salt) at 20°C. for 18 hr. The strips were then washed in several changes of water at 20°C. for 8 hr. In some cases the lengths of representative samples were accurately (± 0.05 mm.) measured at all stages of the above treatment and swelling values calculated on the assumption that the films are isotropic are shown in Table III. Breaking measurements were carried out as above, but with the samples immersed in water at $20 \pm 0.5^\circ\text{C}$. The extensibility values were particularly irreproducible, varying between 6 and 12% without marked change in breaking load.

Determination of Ion Exchange Capacity of Pectate Films for Alkaline Earth Metals

Sodium hydrogen pectate films (50% neutralized) (0.06 g. samples), plasticized with varying amounts of butane-1,4-diol, were treated with 1.0M salt solution at pH 2.5 as described above. The washed films were dried at 40°C./5 mm./CaCl₂, weighed, and then ashed in a platinum crucible. The residue was dissolved in 0.5 ml. concentrated hydrochloric acid and diluted to 50 ml. with water. Aliquot portions were then buffered to the appropriate pH and titrated with ethylenediaminetetraacetic acid.⁹

X-Ray Diffraction by Pectate Films

Samples of film plasticized with 20% glycerol and neutralized to varying extents were folded into glass quills to present ca. 16 thicknesses and irradiated in a 9 cm. Unicam camera with monochromated $\text{CuK}\alpha$ radiation at 40 kv. Photometer plots of the resultant photographs are shown in Figure 10.

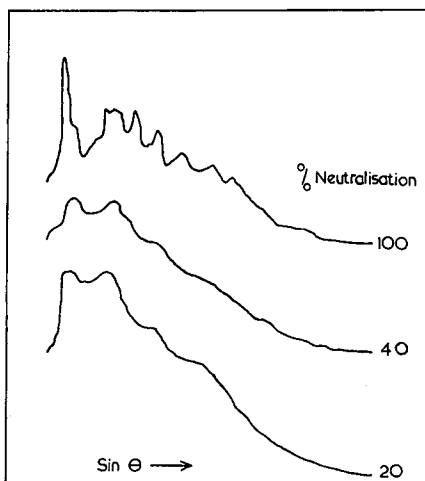


Fig. 10. X-ray diffraction of pectic acid films partially neutralized with sodium hydroxide, plasticized with 20% glycerol, cast at 75°C.

RESULTS AND DISCUSSION

Structure of Pectic Acid

The results of the hydrolysis and paper chromatographic experiments indicate that the pectic acid is a copolymer of galacturonic acid and galactose containing only very small amounts of rhamnose and arabinose. The equivalent weight of 190 corresponds to a copolymer containing 93% anhydrogalacturonic acid units and 7% anhydrogalactose; the low methoxyl content shows that not more than 1% of the uronic acid units are esterified, and the high optical rotation indicates the predominance of α -glycosidic linkages. The pectic acid used in this study therefore probably consists of a main chain of 1,4- α -linked anhydrogalacturonic acid units,¹⁰ with 7% of a galactan, possibly present as side-chains.

The intrinsic viscosity $[\eta] = 185 \text{ ml./g.}$, determined in sodium chloride solution at pH 6, indicates that the polymer is of moderately high molecular weight. Use of the expression

$$[\eta] = 1.4 \times 10^{-4} M^{1.34}$$

derived by Owens et al.¹¹ for pectinic acids gives a value $\bar{M}_w = 3.7 \times 10^4$, which corresponds to a degree of polymerization of ca. 210.

Film-Casting Temperature

Figures 1-3 show that the properties of a 50% neutralized sodium hydrogen pectate film plasticized with 30% glycerol are very little affected by casting temperature over the range 20-100°C. A barely significant decrease in initial tensile modulus and breaking load with increasing temperature was detected, accompanied by a very slight increase in extensibility.

Glycerol Concentration

Increasing the glycerol concentration in a 50% neutralized sodium hydrogen pectate film caused the expected decrease in initial tensile modulus and breaking load and corresponding increase in extensibility. There were indications of a slight discontinuity in the curves of the breaking properties at 20-30% glycerol, but no corresponding discontinuity in the initial tensile modulus.

Extent of Neutralization

Pectic acid containing 20% glycerol was neutralized to varying extents with sodium hydroxide and cast into films at 75°C. Both the free acid film and those neutralized to more than 60% developed multiple cracks on drying, and no tensile measurements were possible. It was, however, possible to obtain x-ray diffraction photographs on samples of 20, 40, and 100% neutralized film and these results are shown in Figure 10. This shows a considerable increase in crystallinity with neutralization, the sodium pectate being highly crystalline, while the sodium hydrogen pectate and presumably the free acid are relatively amorphous. This effect is probably due to the intermolecular polar interaction (crosslinking) of undissociated carboxyl groups in the free and partially neutralized acid, preventing the organization of the polymer molecules into crystallites during the drying out of the gel. Such polar crosslinking would be much less effective between sodium pectate molecules, and the increased molecular mobility during drying-out would be expected to lead to increased crystallinity. The formation of a coherent film appears to require a compromise between an adequate gel concentration (free pectic acid forms a rigid gel at 3% concentration at 20°C.) and sufficiently low crystallinity.

The initial tensile modulus (Fig. 9) shows only very slight change in the range 20-50% neutralization. This is in agreement with the earlier observation¹² that variation of intermolecular cohesive forces in cellulose derivatives has negligible effect on initial tensile modulus, and supports the conclusion that this property is determined by some other effect such as molecular flexibility. The breaking strength and extensibility measured over the same range of neutralization however, showed a marked maximum and minimum, respectively, at ca. 40% neutralization (Figs. 7 and 8). This effect undoubtedly refers to changes in the flow region of the stress-strain curve, but is not susceptible to any simple interpretation.

Effect of Cation on Film Properties

Table I shows the effect on film properties of variation of monovalent cation in the alkali used to prepare 50% neutralized pectate film. The breaking properties show small effects due to the nature of the cation and there is significant variation in the initial tensile modulus. These effects are unlikely to be due to the small differences in moisture content of the films, but the changes in modulus appear to show some dependence on the size of the hydrated cation.

TABLE I
Variation of Monovalent Cation in 50% Neutralized
Pectate Film^a

Properties	Cation			
	Li ⁺	Na ⁺	NH ₄ ⁺	K ⁺
Moisture content, %	10.9	12.0	10.6	13.0
Initial tensile modulus, dynes/cm. ² × 10 ⁻¹⁰	3.0	3.0	2.2	1.8
Breaking strength, kg./mm. ²	5.3 ± 0.14	6.3 ± 0.26	4.7 ± 0.41	3.7 ± 0.24
Extensibility, %	3 ± 0.2	5 ± 0.3	5 ± 0.6	5 ± 0.3
Hydrated cation radius, A. ^b	2.36	1.80	1.225	1.21

^a Plasticized with 20% glycerol.

^b Stokes radii from solution measurements.¹³

The effect of exchanging the cations of a sodium hydrogen pectate film with a polyvalent cation is to render the film insoluble in water.³ When 1.0*M* magnesium and calcium nitrates and barium chloride, respectively, were used as the exchange solutions, however, the resultant magnesium pectate film showed a significantly lower wet strength than the calcium- or barium-treated films. This effect is not due to a lower concentration of magnesium within the film, since Table II shows the same cation content in magnesium and calcium pectate films. The reduced wet strength, however, does indicate a lower degree of crosslinking by magnesium, and this is most probably due to the greater tendency of the magnesium salts of weak acids to form hydrated complexes of the type R—COO⁻ Mg⁺⁺ OH⁻, or R—COO⁻ Mg⁺⁺ X⁻ in comparison with the normal salt R—COO⁻ Mg⁺⁺ -OOC—R. A similar type of explanation has been used to interpret differences in precipitation behavior of polyacrylic acid with magnesium and barium salts.¹⁴

This type of basic salt formation would be expected to lead at the same time to an increased ion exchange capacity of the pectate for magnesium ions and a decreased crosslinking effect. The increased capacity of the pectate film for magnesium in comparison with calcium, thus results in an

TABLE II
Uptake of Alkaline Earth Cations by Plasticized Sodium Hydrogen Pectate Films

Salt (1.0M, pH 2.5)	Plasticizer (butane-1,4-diol) concn., % ^a	Uptake of cations, equiv./equiv. pectic acid
Mg(NO ₃) ₂	25	0.56
	50	0.60
Ca(NO ₃) ₂	25	0.54
	50	0.54
BaCl ₂	25	0.75
	50	0.80

^a With respect to pectic acid.

apparent reversal (Table II) of the normal selectivity scale for exchange of alkaline earth cations on an acidic resin.¹⁵ This effect is presumably due to the fact that the normally accepted selectivity scale (which places the ion "preference" in the order Ba > Ca > Mg) was determined with a resin containing strongly acidic sulfonic acid groups.

The crosslinking of sodium hydrogen pectate films by ion exchange with alkaline earth salts presumably competes with a simultaneous process of swelling and solution in water in the experiments described above. The former process however, appears to occur rapidly, and only very slight swelling is observed in going from an air-dry film to an equilibrium with 1.0M calcium nitrate solution. This absence of extensive swelling makes

TABLE III
Swelling of Plasticized Sodium Hydrogen Pectate Films in 1.0M Calcium Nitrate Solution

Plasticizer (butane-1,4-diol) concn., % ^a	Swelling in salt solution, %	Subsequent swelling in water, %
25	4	0
50	5	0
75	6	0
100	5	0

^a As percentage in original film with respect to pectic acid.

possible the preparation of calcium pectate films of a wide range of porosities. Thus a range of sodium hydrogen pectate films containing varying amounts (25-100%) of butane-1,4-diol have been treated with 1.0M calcium nitrate solution and in each case the film swelled less than 10% (see Table III). Since it is reasonable to assume the complete extraction of the plasticizer by the salt solution and subsequent water washing, the calcium pectate films so produced must differ very markedly in porosity, and the comparison of their properties will form the subject of a later paper.

CONCLUSIONS

An unesterified pectic acid of \bar{M}_w ca. 4×10^4 has been isolated from cured tobacco stem (i.e., leaf mid-rib), in 12% yield. This material is essentially a copolymer of 93% D-galacturonic acid and 7% D-galactose. The properties have been examined of films cast from aqueous solutions of this material under varying conditions.

Variation of casting temperature and of the monovalent cation (Li, Na, K, NH₄) used for neutralization of the pectic acid have only slight effects on film properties. The extent of neutralization however, has a considerable effect on x-ray crystallinity of the films. Completely neutralized sodium pectate films are highly crystalline, while 20% and 40% neutralized films are relatively amorphous. Optimum film properties, however, appear to occur with ca. 40% neutralization.

Ion exchange of mono- for divalent cations in the films renders them insoluble in water, although the wet strength and ion exchange capacity of the resultant films indicates the occurrence of basic salt formation, particularly in the case of magnesium. This type of crosslinking by divalent cations occurs very rapidly and maintains the original air-dry dimensions of the films even in aqueous solution.

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Résumé

Un acide pectique extrait des tiges de tabac, avec un rendement de 11%, contient 93% d'acide galacturonique anhydre et un peu de galactose. Des pellicules plastifiées ont été coulées d'une solution aqueuse. On a étudié l'influence de la température de moulage, du pourcentage de neutralisation de la nature du cation et du contenu de plastifiant sur les propriétés de traction. La cristallinité des films de pectates, croit notablement par neutralisation partielle de la soude de 40 à 100%. Les capacités d'échange ionique de films de pectate acide de sodium à l'égard de cations alcalino-terreux sont discutées.

Zusammenfassung

Eine aus Tabakstengeln in 11% Ausbeute extrahierte Pektinsäure enthält 93% A hydrogalacturonsäure und etwas Galaktose. Weichgemachte Filme wurden aus wässriger Lösung gegossen und der Einfluss der Giesstemperatur, des Neutralisationsgrades, der Natur des Kations und des Weichmachergehaltes (Glycerin) auf das Zugverhalten wurde untersucht. Die Kristallinität der Pectatfilme nimmt im Bereich von 40–100% Neutralisation mit der Neutralisation durch Natriumhydroxyd zu. Die Austauscherkapazität der Natriumhydrogenpektat-Filme für Erdalkalikationen wird diskutiert.

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