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## Research Note

# Investigation of Ion Exchange on Films of Pectic Substances by Infrared Spectroscopy

*SUMMARY*

*A method of infrared (IR) spectroscopy is developed which allows the degree of substitution of pectin carboxyl groups by metal ions to be determined. The strength of $Ca^{2+}$ bonds in films of pectic substances was studied. The dependence of the strength of $Ca^{2+}$ bonds on the degree of methylation of the carboxyls is shown to be different for pectic films compared with solutions. From the results it is concluded that during film formation the macromolecules become orientated to give groups of dimers of non-ionized carboxyls. The $Ca^{2+}$ ions interact with the available carboxyl group dimers.*

## INTRODUCTION

The main advantage of IR spectroscopic studies of ion exchange in pectic substances is that the degree of substitution of hydrogen by a cation can be determined directly within an ion exchanger. The ratio of intensities at the absorption band maxima $\nu_{as}(COO^-)$, $\nu(C{=}O)_E$ (ester groups) and $\nu(C{=}O)_H$ (non-ionized carboxyl) was found to be constant for a given cation and independent of the content of polygalacturonic acid (PGA), of the degree of methylation ($E$) and of the degree of substitution ($\alpha$) of carboxyl hydrogens by metal ions (Filippov, 1978). This allows $\alpha$ to be directly measured by IR spectroscopy in an ion exchanger (pectic film) for a pectin with any value of $E$.

## EXPERIMENTAL

Let us denote the fractions of free (—COOH), methylated ($-COOCH_3$) and metallized ($-COO^-M^+$) carboxyls in a pectin by $H$, $E$ and $M$,



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respectively. Then

$$H + E + M = 1$$

$$\alpha = \frac{M}{M+H} = \frac{M}{1-E}$$

The optical density at the absorption maxima $\nu_{as}(COO^-)$ and $\nu(C{=}O)_H$ can be written as

$$D(COO^-) = D_1 = K_M M + K_W l,$$

$$D(C{=}O) = D_2 = K_E E + K_H(1 - M - E)\, l,$$

where $K$, with the appropriate subscript, denotes the absorption coefficient at the band maximum, $K_W$ is the absorption coefficient of the $\delta(H_2O)$ band at the absorption maximum $\nu_{as}(COO^-)$, and $l$ is the film thickness. The values of absorption coefficients are unknown but their relative values can easily be determined (Filippov, 1978).

By dividing one equation by another and then by $K_H$ we obtain:

$$dD = \frac{J_M M + J_W}{J_E E + (1 - M - E)}$$

where $J$ is the relative intensity of the band in units of the $\nu(C{=}O)_H$ intensity. Solving this equation with respect to $M$ and taking into account that $J_E = 1{\cdot}2$ (Filippov, 1978) we find that

$$M = \frac{dD(1 + 0{\cdot}2E) - J_W}{dD + J_M}$$

and

$$\alpha = \frac{M}{1-E} = \frac{dD(1 + 0{\cdot}2E) - J_W}{(dD + J_M)(1-E)}$$

The value of $J_W$ depends on pectin humidity, and so before the IR spectrum determination the film must be desiccated under standard conditions for 1 h at 70°C and then cooled for 30 min.

We can take the $J_W = f(\nu)$ dependence to be linear in the range of 1590–1630 $cm^{-1}$ where most cations exhibit the $\nu_{as}(COO^-)$ band (Filippov and Rosentul, 1984): $J_W(\nu) = J_W(1590) + K\Delta\nu$, where $\nu$ is the wave number at which $J_W$ is determined, $J_W(1590) = 2 \times 10^{-2}$, $\Delta\nu = \nu - 1590$ $cm^{-1}$, and $K$ is the experimentally determined propor-

tionality factor for the samples with known water content:

$$K = \frac{J_W(\nu) - J_W(1590)}{\Delta\nu} = 0{\cdot}25 \times 10^{-2}$$

or

$$J_W(\nu) = (2 + 0{\cdot}25\Delta\nu) \times 10^{-2}$$

This formula can be used for almost all pectins because previous investigations on 104 samples of various air-dried pectic substances (Filippov and Shkolenko, 1985) showed that all of them contained $15 \pm 5\%$ water. The corresponding values of $J_W$ at the frequency of the maximum 1630 $cm^{-1}$ are in the range $0{\cdot}12 \pm 0{\cdot}03$, the deviation caused thereby being within the accuracy of the $J_M$ measurement. As $J_M$ is determined with an accuracy of 0·03, then a lower limit for reliable measurements of $dD$ exists. This limit is given by the condition:

$$dD(1 + 0{\cdot}2E) \geqslant J_W + 0{\cdot}03.$$

The above method was used to study the dependence of the strength of $Ca^{2+}$-to-pectin film bonds on $E$. The films were prepared from apple pectin with 60% PGA making use of the standard technique described by Filippov (1978), the value of $E$ changing from 0·24 to 0·64. Samples with various degrees of methylation were prepared by alkaline hydrolysis of the initial pectin. In order to achieve an equilibrium state, the pectin films were placed for three days in a $2{\cdot}5 \times 10^{-3}$ M pH 3 solution of $CaCl_2$ in 40% EtOH. The solution volume was chosen to give a ten-fold excess of $Ca^{2+}$ ions over free carboxyl groups. The ion exchange conditions were chosen so that $\alpha$ would not exceed 0·5. The standard deviation ($\bar{S}$) of the mean result $\bar{\alpha}$ lies within the interval 0·02, this value being equal to the method sensitivity at $E = 0{\cdot}2$. The spectra were obtained using a Perkin–Elmer — 577 two-beam spectrophotometer in the range 1500–1900 $cm^{-1}$. The optical densities were measured with respect to the base-line between two absorption minima at 1550 and 1900 $cm^{-1}$ (Fig. 1).

## RESULTS AND DISCUSSION

As has been shown (Kohn, 1975), the activity coefficients ($\gamma_{Ca^{2+}, Sr^{2+}}$) of $Ca^{2+}$ and $Sr^{2+}$ cations in pectin solution decrease with decreasing $E$, indicating that the strength of the cation-to-pectin bonds are increasing. There is a sudden drop in the dependence of $\gamma$ on $E$ in the range

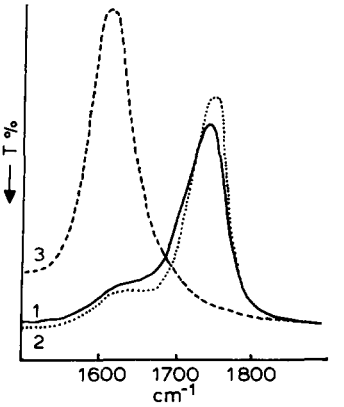


**Fig. 1.** IR absorption spectra of films: pectic acid (1), methyl ester of pectic acid (2) and potassium pectate (3).

$E = 0{\cdot}42-0{\cdot}35$. On the basis of data (Morris *et al.*, 1973) showing differences in $Ca^{2+}$ uronates interaction in solution compared with the more regular gel state, the author (Kohn, 1975) concluded that within the range of $E = 0{\cdot}42-0{\cdot}35$ intramolecular electrostatic binding of $Ca^{2+}$ and

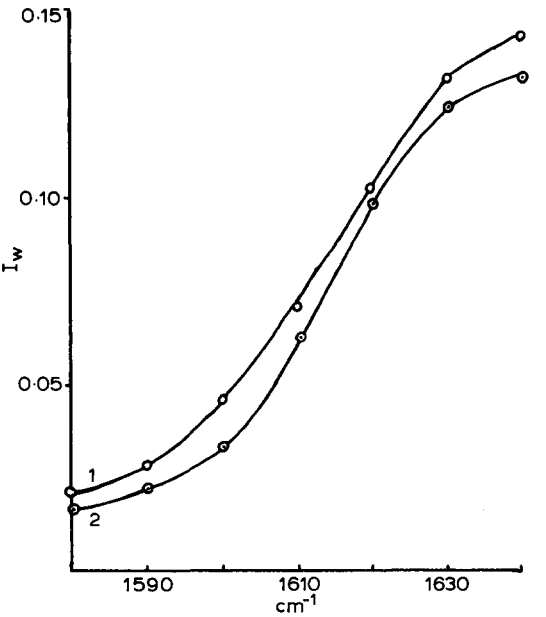


**Fig. 2.** Dependence of the degree of substitution of carboxyl's hydrogen of pectin by $Ca^{2+}$ ions on the methylation degree (40% EtOH, pH 3, $C_{Ca^{2+}} = 2{\cdot}5 \times 10^{-3}$ M).

$Sr^{2+}$ transforms into the intermolecular chelate with the formation of so-called 'egg-box' structures (Grant *et al.*, 1973).

For the solid state pectin samples we have investigated there is no sudden change in the degree of dissociation ($\alpha$) with the degree of methylation ($E$).

Figure 2 shows that $\alpha$ decreases slowly with increasing $E$. This is similar to the solution behaviour at $E < 0{\cdot}35$, and indicates a weak dependence of the strength of the $Ca^{2+}$-to-pectin bonds on $E$. Hence, when the pectin film is formed from solution the polymer molecules predominantly orient with respect to one another, thus forming structures with homogeneous regions of carboxyl groups. From the position of the band assigned to $\nu$(C=O) we can consider that the groups of non-ionized carboxyls dimers are formed. The $Ca^{2+}$ ions interact with the available dimer carboxyl cells forming complexes with a similar coordination sphere independent of $\alpha$ and $E$. This is also confirmed by the independence of position and absorption coefficient of the band $\nu_{as}(COO^-)$ on $\alpha$ and $E$.

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