Determination of the esterification degree of carboxyl groups of pectin with methanol by means of infrared spectroscopy

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A micromethod for determination of the esterification degree of pectin by means of infrared spectroscopy was elaborated. Absorbances in KBr discs were measured in the $\nu_{as}(COO^-)$ and $\nu(C=O)_{ester}$ regions at 1605 and 1745 cm^{-1}, respectively. The error of determination of the esterification degree of pectin did not exceed $\pm 3\%$.

The important properties of pectin as *e.g.* the formation of gel or cation exchange selectivity depend on the esterification degree of carboxyl groups with methanol [1, 2]. A relatively large quantity of the substance is needed both for the alkalimetric determination of the esterification degree of pectin [1, 3] and for the chelatometric analysis employing insoluble copper pectinates and pectates [4, 5]. The esterification degree of pectin could also be well estimated by means of infrared spectroscopy of pectin films [6]. Unfortunately, not all pectin preparations form sufficiently firm films; moreover, not less than 5—10 mg of substance is needed for preparation of the film. On the other hand, the KBr technique required substantially less pectin, roughly 1 mg. This technique of determination of the esterification degree of pectin (E) offered only a semiquantitative relation between E and the ratio of absorptions at $\nu_{as}(COO^-)$ and $\nu(C=O)_{ester}$ [7].

To obtain more precise results, some special properties of the spectra of pectin in this region of wavelength had to be considered. An inevitable condition for determination of E by this method is the complete ionization of all unesterified (free) carboxyl groups. If this condition is not satisfied, the absorption $\nu_{as}(COO^-)$ decreases, whereas that of $\nu(C=O)_{ester}$ increases to the detriment of remaining free carboxyl groups. In pectin preparations the hydrogen of carboxyl groups is sometimes partly replaced by calcium or another metal. In this case, when pressed with KBr, a mixture of K and Ca salts of pectin was formed; this enhanced the determination error of E, since the intensity of the $\nu_{as}(COO^-)$ bands of salts involved was somewhat different (Fig. 1). It was therefore necessary to remove metal cations from pectin over anion exchanger (H^+ form), or by washing pectin with acidified 60% ethanol and to prepare the potassium salt by neutralization with KOH [3].

The band $\delta(H_2O)$, occurring in all samples of pectin, also contributed to the absorption in the $\nu_{as}(COO^-)$ region. At low E values the decreasing part of the $\nu_{as}(COO^-)$ absorption curve overlapped the $\nu(C=O)_{ester}$ band.

Respecting the above-mentioned facts, the absorption of salts of pectic acid in

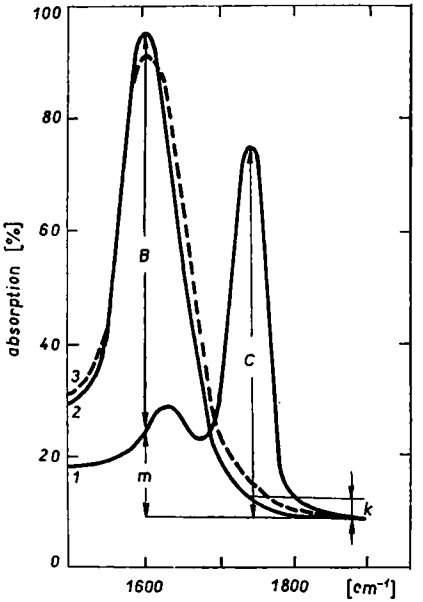

Fig. 1. Absorption spectra of methyl pectate and salts of pectic acid.
1. Methyl pectate; *2.* potassium pectate; *3.* calcium pectate.

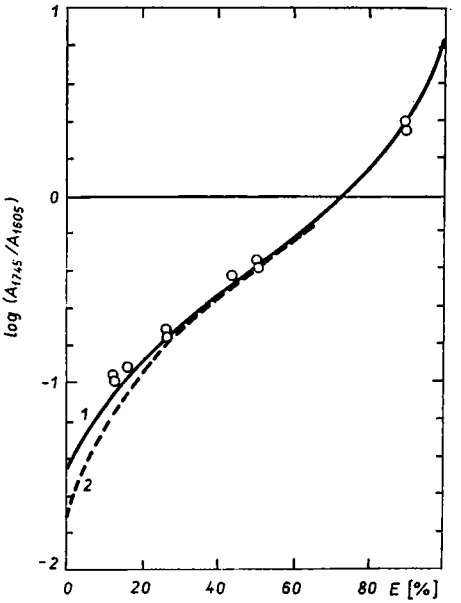

Fig. 2. The relationship of the absorbances ratio A_{1745}/A_{1605} upon the esterification degree of pectin (E).
1. Calculated calibration curve for KBr pellet; *2.* calculated calibration curve for the pectin film. Experimental values of pectin control samples of a known esterification degree E (○).

the $\nu_{as}(COO^-)$ region could be expressed by two factors: the basic absorption $\nu_{as}(COO^-)$, which depended on the number of ionized carboxyl groups, and the constant absorption $\delta(H_2O)$. In the $\nu(C{=}O)_{ester}$ region the absorption of the decreasing part of the $\nu_{as}(COO^-)$ absorption curve was added.

To construct the calibration curve, parameters (constants) C, B, m, and k had to be determined (Fig. 1). These are best expressed by relativ e absorbances I [6]

$$I = \frac{A_\nu}{A_0} 100, \qquad (1)$$

where A_ν stands for the absorbance of the band analyzed and A_0 for that of pyrane ring vibration at 1020 cm^{-1} taken for an internal reference. These parameters were determined with completely deesterified pectin ($E = 0\%$) and methyl pectate ($E = 100\%$). The freeze-dried mixed solution of the sample of pectin with KBr afforded a homogeneous material, the discs of which revealed a minimal deviation in band intensities [8].

The absorption of pectin of partial esterification of carboxyl groups e ($0 \leq e \leq 1$) at 1605 cm^{-1} was expressed by the sum

$$I_{1605} = B(1 - e) + m \tag{2}$$

at 1745 cm^{-1}

$$I_{1745} = C\,e + k(1 - e). \tag{3}$$

Then according to eqn (*1*)

$$\frac{I_{1745}}{I_{1605}} = \frac{A_{1745}}{A_{1605}} = \frac{C\,e + k(1 - e)}{B(1 - e) + m}. \tag{4}$$

The quotient of esterified carboxyl groups e and the esterification degree $E = e \cdot 100$ (%) can be calculated from the known values of parameters C, B, m, and k (Table 1) and from the ratio of absorbances determined A_{1745}/A_{1605}.

Table 1

Parameters C, B, m, and k of methyl and potassium pectates

ν [cm^{-1}]	$e = 0$	$e = 1$
1605	$I_{(COO^-)} = B = 140$	$I_{(H_2O)} = m = 8$
1745	$I_{(COO^-)} = k = 4$	$I_{(C=O)ester} = C = 60$

The esterification degree could be easier determined graphically by means of the calibration curve log $(A_{1745}/A_{1605}) = f(E)$ calculated from eqn (*4*) at variable values e (Fig. 2, curve *1*). This functional dependence was verified on a series of samples of pectin of known E value (Table 2).

All samples were the K salts, *i.e.* they contained carboxyl groups in two forms only: —COOK and —$COOCH_3$. As it follows from Fig. 2, the experimentally estimated E values (circles ○) fit well in the calculated calibration curve *1*.

Table 2

Characteristic data of control samples of pectin

Esterification degree E [%]	Content of polyuronides [%]	$\bar{M}_\eta$
89.5	91	71,000
50.5	91	53,000
43.5	88	63,000
26.5	91	41,000
16.0	91	35,000
13.0	91	44,000

Owing to the fact that the absorption band $\nu_{as}(COO^-)$ of pectin was broader than that of the pectin film and also the parameter $k_{KBr} > k_{film}$, the calibration curve employed for films lay in the region of low E values, little lower (Fig. 2, curve *2*) than that of KBr pellets.

The described method has — besides its advantages, when compared with that reported earlier [6] — also one restriction. It could be applied only to pectins with a low content of acetyl groups, since the latter displayed absorption in the same region as did methyl carboxylates.

Experimental

The starting sample of pectin was an apple pectin, having 88% of polyuronide and 12% of neutral saccharides in the dry substance: D-glucose, L-rhamnose, D-xylose, L-arabinose, and D-galactose in a molar ratio 4 : 7 5 5 : 11 [3]. Samples of pectin of various esterification degree were prepared by partial alkaline deesterification of a highly esterified pectin; the pectin was esterified with sulfuric acid acidified methanol at a low temperature. These processes and analytical methods were reported earlier [3]. Methyl pectate was prepared by reaction of pectin with diazomethane [9]. The average molecular weight of pectin $\overline{M}_\eta$ was estimated viscometrically [10].

The mixture of the sample of pectin and KBr in a 1 : 200 ratio was dissolved in water and freeze-dried [7]. A pellet 12 mm in diameter was made of 150 mg of the lyophilizate.

The spectra were measured with a Perkin—Elmer 457 IR spectrophotometer in the 700—1900 cm^{-1} range. The absorbances for K salts in the maxima of bands $\nu_{as}(COO^-)$ 1605 cm^{-1} and $\nu(C{=}O)_{ester}$ 1745 cm^{-1} were determined by the base-line method. The base line was drawn through the point at 1900 cm^{-1} parallely with the abscissa. The $\delta(H_2O)$ absorption was compensated with pellets of pure freeze-dried KBr.

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