

Studies of the Interaction of Methanol with Pectin in Aqueous Solution

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SUMMARY

The interaction of methanol with pectin (degree of esterification 58%) in aqueous solution has been studied by conductivity measurements, circular dichroism, and equilibrium dialysis. Compared to other aliphatic alcohols methanol has a strong precipitating effect on pectin both in water and in 50% sucrose solutions at pH 7.0 and 3.4. The standard Gibbs free energy of methanol transfer from water into pectin solutions of concentration 0.024-0.032 g-equiv. of ester groups (kg of solvent)⁻¹ is 4 kJ mole⁻¹. The interaction of methanol with pectin gives a complex in which each ester group binds one molecule of alcohol. The reaction is assumed to be caused by the formation of a hydrogen bond involving the hydrogen atom of the hydroxyl group of methanol and the unshared electron pair of the carbonyl group. Methanol interacts strongly with pectin compared to other aliphatic alcohols because of its high acidity.

INTRODUCTION

In studying the adsorption properties of dry pectin samples one of the authors observed increased adsorption of methanol compared to other aliphatic alcohols (Ignatov & Markova, 1983).

The methanol-wetting heat of pectin (degree of esterification (DE) 72%) came to 78.7 J g^{-1} , whereas for the C_2 - C_5 alcohols this value varied between 5.4 and 8.6 J g^{-1} . For these alcohols there was no systematic variation with the length or degree of branching of the hydrocarbon chain. The increase in the heat of wetting of pectin by methanol, compared with other alcohols, rises linearly with the DE of pectin. A dependence of the heat of wetting on pectin DE is not observed for other alcohols nor is it observed for water. It was therefore concluded that water and aliphatic alcohols, starting from C_2 , solvate pectin only at the hydroxyl groups whereas methanol, in addition, interacts with the methoxyl groups. The phenomenon of specific adsorption of methanol by highly esterified pectin was used to remove small amounts of methanol from water-ethanol mixtures (Ignatov *et al.*, 1978; Ignatov & Markova, 1983).

The present study is aimed at determining to what extent the concept of a specific interaction between methanol and pectin, developed from the results of experiments on adsorption of alcohols by dry pectin samples, can be extended to aqueous solutions of pectin.

Information about the level of alcohol required to precipitate pectin from aqueous solutions with different alcohols, the stoichiometry of the methanol-pectin complex, and the transfer constants of methanol from water into the pectin solution were determined respectively by conductivity measurements, circular dichroism spectroscopy (CD), and equilibrium dialysis.

MATERIALS

Citrus pectin obtained by acid-alcohol reprecipitation of a commercial sample supplied by Koch-Light (Great Britain) was studied. Pectin was purified by the method of Plashchina *et al.* (1978). The sample contained 86.5% uronides (determined by the colorimetric method as described by Filippov & Vlasyeva, 1973). Neutral sugars included 3–7% rhamnose, 4–6% arabinose, and less than 1% xylose (determined by

the method of Stromeyer & Linow (1979) at the Central Institute of Nutrition, GDR Academy of Science). The DE was determined as 58% by the colorimetric method described by Filippov & Kuz'minov (1971).

METHODS

Determining the pectin precipitation threshold

The pectin precipitation point was determined from conductivity measurements. A Hungarian conductivity meter type OK-102/1 was used. Alcohol was added at a rate of $(7.8 \pm 0.2) \times 10^{-3}$ ml min⁻¹ into the cuvette containing 15 ml of 1% pectin in water or a 1:1 w/w mixture of water and sucrose. The conductivity of the mixture was continuously recorded during titration. A correction was made for the variation in the conductivity of the solvent during titration. The point where precipitation occurred was obtained from the variation in the slope of the relationship between the conductivity of the pectin solution and the concentration of alcohol as shown in Fig. 1. The precipitation points were reproducible to an accuracy of $\pm 0.5\%$ alcohol (v/v). In the case of the measurements made at pH 3.4 the pectin solutions were first titrated with 0.1 M hydrochloric acid.

Experiments were carried out at $20 \pm 0.1^\circ\text{C}$.

Circular dichroism (CD)

The pectin samples were dissolved in 0.005 M EDTA and dialyzed against 0.1 M NaF for 3 days. The solutions were ultracentrifuged at 1.4×10^5 g for 4 h. The concentration of pectin in the solution was determined by the phenol-sulphuric acid method (Bath, 1958).

20 ml of 0.1% pectin solution in 0.1 M NaF and a calculated quantity of methanol in 0.1 M NaF were mixed in a 50 ml measuring bottle. The ratios of molar concentrations of methanol and ester groups of pectin varied between 0.1 and 10. The final concentration of pectin in our experiment equaled 3.05×10^{-3} g-equiv. of CH_3O -group (kg of solvent)⁻¹.

The CD spectra were obtained on a Jasco-20 (Japan) spectropolarimeter in the wavelength range of 200–250 nm. Measurements were made at $20 \pm 0.1^\circ\text{C}$ in a cuvette of thickness 0.1 dm. Nitrogen was

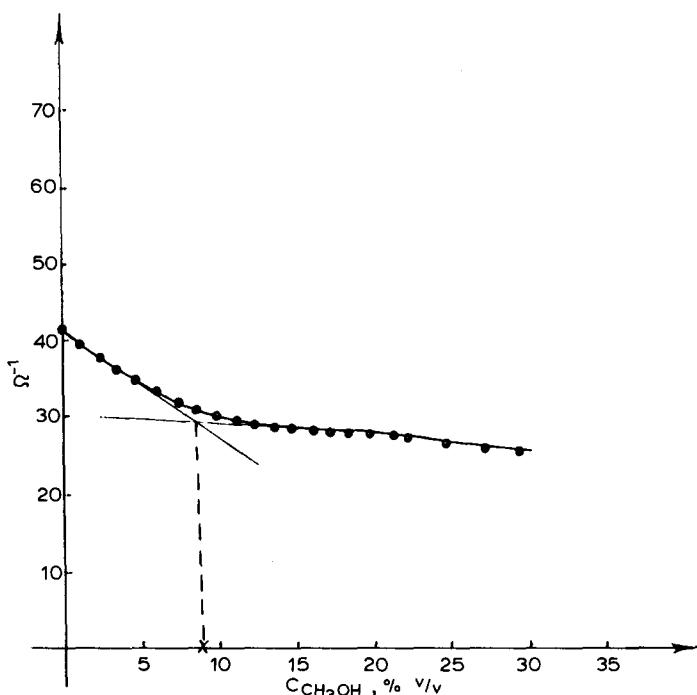


Fig. 1. Plot of conductivity of pectin solution against concentration of alcohol.
Solvent is a 1:1 w/w mixture of water and sucrose, pH 3.4.

blown through the optics of the instrument to avoid distortion of the spectra due to the adsorption of oxygen from the air.

Equilibrium dialysis

Dialysis was carried out in Serva (FRG) cellophane bags 2.5 mm in diameter. The internal diameter of the outer vessel was 7.5 mm. The volume of liquid in the dialysis bag was varied between 24 and 30.5 ml, and in the outer vessel between 319.5 and 326.0 ml. At the polymer concentration used the variation in the liquid level in both vessels during dialysis was negligibly small.

Before starting the experiment the aqueous solution of pectin in the inner vessel was equilibrated at 6°C against 0.1 M NaCl for 1 day. Then,

a calculated amount of methanol was introduced into the outer vessel at a rate of 2×10^{-4} ml min $^{-1}$. The mixture was kept at 6°C for 3 days.

The time for dialysis exceeded that required for equilibration. The latter was determined in preliminary experiments. Methanol was not found to be adsorbed by the membrane.

After dialysis, 5 samples were taken from the outer vessel. The concentration of methanol in these samples was determined by the colorimetric method as described by Filippov & Kuz'minov (1971). The error in the determination of methanol concentration in the outer vessel did not exceed 6×10^{-3} g ml $^{-1}$.

RESULTS

The concentration of alcohol required to precipitate pectin from aqueous solutions is compared for different aliphatic alcohols in Table 1. It is seen from this Table that the precipitation threshold for C₂–C₄ alcohols decreases with increasing hydrocarbon chain length. In spite of this, methanol shows a much lower precipitation threshold than ethanol. This suggests a specific effect of methanol on pectin compared with other alcohols. The ratio of the level of methanol to ethanol required to precipitate pectin depends only weakly on the composition of the original pectin solution.

Decreasing the pH of the aqueous solution of pectin or adding 50% (w/w) sucrose results in a decrease in the amount of alcohol required to precipitate pectin. Perhaps this happens due to the decrease in affinity of pectin to the solvent. Despite the fact that pectin gels under the joint action of acid and sucrose (Rees, 1972), there is some evidence that these substances have an antagonistic effect on the precipitation of pectin with alcohols. This is especially marked in the case of ethanol.

Figure 2 shows the dependence of the molar ellipticity of pectin, at the maximum of the band, associated with $n \rightarrow \pi^*$ transition of carboxyl and ester groups (Plashchina *et al.*, 1978), on the ratios of equivalent concentrations of methanol and the ester groups of pectin. The ratio of the components obtained by intersecting the tangents to the initial and end portions of the curve gives the composition of the complex (Rossotti & Rossotti, 1961). Each ester group is seen to bind a molecule of methanol.

TABLE 1
Concentrations of Alcohols Required to Precipitate Pectin

Alcohol	Precipitation threshold, % v/v of alcohol				Relative precipitation threshold compared with pectin solution in water at pH 7.0			
	Water		1:1 w/w mixture of water and sucrose		Water		1:1 w/w mixture of water and sucrose	
	pH 7.0	pH 3.4	pH 7.0	pH 3.4	pH 7.0	pH 3.4	pH 7.0	pH 3.4
Methanol	14.5	9.0	11.5	11.0	1.00	0.64	0.79	0.75
Ethanol	19.0	13.0	13.0	15.5	1.00	0.68	0.69	0.82
<i>n</i> -Propanol	14.0	10.5	8.0	8.5	1.00	0.76	0.58	0.60
<i>n</i> -Butanol	8.5	—	—	—	1.00	—	—	—
Ratio of thresholds of precipitating pectin with methanol and ethanol	0.76	0.71	0.87	—	—	—	—	—

The stability constant of the complex can, in principle, be estimated by the equilibrium dialysis method. In this case, however, representation of the equilibrium dialysis results in Scatchard coordinates showed considerable scatter and reveals that the intrinsic stability constant of the complex does not exceed 10^2 M^{-1} . Such low values of stability constants cannot be determined with sufficient accuracy by equilibrium dialysis (Coassolo *et al.*, 1980).

Instead of the stability constant of the complex, the constant for the transfer of methanol from aqueous solution into a pectin-containing solution was calculated from the equilibrium dialysis data. The more general nature of thermodynamic description including, as a particular case, an approach from the standpoint of the theory of multiple equilibrium is discussed in the work of Schellman (1978).

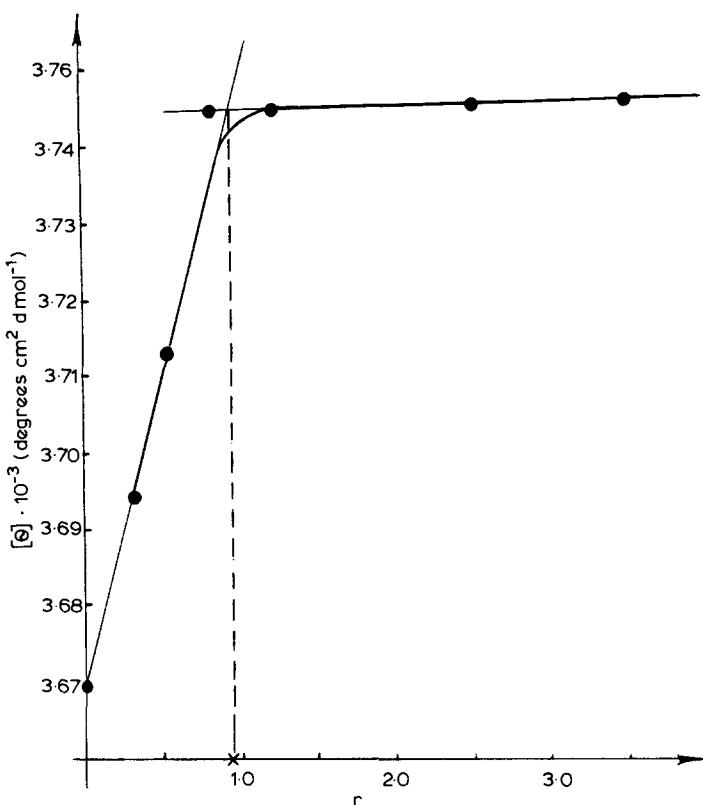


Fig. 2. Molar ellipticity of pectin in 0.1M NaF solution versus ratio of molar concentrations of methanol and ester groups of pectin (r).

According to Carrod & Herrington (1969), the chemical potential of methanol can be written as:

$$\mu_3 = \mu_3^0 + RT \ln \gamma'_3 m'_3 + RT a m'_3 \quad (1')$$

in the inner vessel, and

$$\mu_3'' = \mu_3^0 + RT \ln \gamma''_3 m''_3$$

in the outer vessel.

Here, μ_3^0 stands for the standard chemical potential of methanol; m'_3 and m''_3 are respectively the equilibrium molal concentrations of methanol in the inner and outer vessels; γ'_3 and γ''_3 are the activity

coefficients of methanol in a water-methanol mixture at concentrations conforming to that of methanol in the inner and outer vessels, respectively; m'_2 denotes the concentration of pectin in the inner vessel (g-equiv. of ester groups (kg of solvent) $^{-1}$) and a is the methanol-pectin interaction parameter.

After equilibrium has been established

$$\mu'_3 = \mu''_3$$

$$-a = \ln K/m'_2$$

where $K = \gamma'_3 m'_3 / \gamma''_3 m''_3$ is a constant for the transfer of methanol between water and pectin solution of the given concentration.

According to the data of Butler *et al.* (1933), extrapolated to the working range of concentrations of methanol, $\gamma'_3 \approx \gamma''_3$ (differences do not exceed 2×10^{-3}) and $K = m'_3 / m''_3$.

The results of the equilibrium dialysis studies are presented in Table 2. The differences in the values of transfer constants determined in individual experiments are due to random errors. These errors mask any possible effect due to variations in the polymer concentration.

TABLE 2
Results of Equilibrium Dialysis Studies

Pectin concentration in inner vessel (m'_2), g-equiv. of ester groups (kg of solvent) $^{-1}$	Methanol concentration, mole (kg of solvent) $^{-1}$		$K = m'_3 / m''_3$	Transfer constant, $K = m'_3 / m''_3$	Standard Gibbs transfer free energy ($-\Delta G^\circ$), kJ mole^{-1}	$-a = \ln K / m'_2$ (g-equiv. of ester groups (kg of solvent) $^{-1}$)
	in outer vessel	in inner vessel				
	initial	final (m''_3)		final (m'_3)		
0.0241	0.17	0.14	0.4	2.6	2.3	40
0.0244	0.23	0.18	0.6	3.3	2.8	49
0.0303	0.17	0.09	1.0	10.6	5.5	78
0.0303	0.22	0.13	1.1	8.3	4.9	70
0.321	0.25	0.20	0.6	3.0	2.6	34
				5.6 \pm 3.8	4.0 \pm 1.6	61 \pm 24

Table 2 also contains the values of the parameter a appearing in eqn (1') and of ΔG° , the standard Gibbs free energy of methanol transfer from water into pectin solution.

DISCUSSION

Experimentally it has been established that:

- (1) the specific interaction of pectin with methanol manifests itself in water and a 1:1 w/w mixture of water and sucrose at both pH 7.0 and 3.4;
- (2) in neutral aqueous solution a complex is formed in which each ester group of pectin binds one molecule of methanol;
- (3) the standard Gibbs free energy of methanol transfer from water into pectin solutions (in the range of pectin concentrations investigated) is about 4 kJ mole⁻¹.

Here it should be mentioned that because of the restrictions imposed by the methods used the experiments were conducted at different ionic strengths, that is, conductometric titration was carried out without a background electrolyte, whereas the CD spectroscopy and equilibrium dialysis were performed in solutions with an ionic strength of 0.1. Obviously, the presence of a background electrolyte does not have a critical effect on the interaction of methanol with pectin.

The data reported by Abramzon & Slavin (1974) (see also Abramzon (1981)) reveal that the standard Gibbs free energy ($-\Delta G_0^I$) for the interaction of carbonyl and ester groups of low-molecular compounds with methanol is higher than with other alcohols. Thus, the difference in the values of ΔG_0^I when a carbonyl group interacts with methanol and ethanol amounts to 1.7 kJ mole⁻¹, whereas with ethanol and propanol it equals 0.75 kJ mole⁻¹. In the case of ethanol and octanol this difference amounts to 1.3 kJ mole⁻¹.

Also it should be noted that the values ΔG_0^I obtained by interacting all the studied alcohols (C₁-C₄) with carbonyl and ester groups are close to each other. Apparently, the alcohols interact with the common structural element of these groups, most likely with the oxygen carbonyl atom, the affinity of methanol for this structural element being appreciably higher than that of other alcohols.

Now we shall consider three possible mechanisms for the interaction of the methanol molecule with the carbonyl group:

1. Dipole-dipole interaction of the carbonyl group with a methanol molecule as a whole;
2. Hydrogen bonding involving the hydrogen atom of the hydroxyl group of methanol and the unshared electron pair of the carbonyl group;
3. Interaction of the carbonyl group with the methoxyl part of a methanol molecule.

The dipole-dipole interaction mechanism does not explain the specific nature of the action of methanol, because its dipole moment is not very different from other alcohols that have been studied (Osipov *et al.*, 1971).

To see whether the existence of an intermolecular methoxyl-carbonyl bonding interaction energetically comparable to a hydrogen bond is possible, a non-empirical calculation of a model system containing formaldehyde and methanol molecules was performed using a GAUSSIAN-70 programme in the minimal OST-3G basis set. The molecules were brought closer together in two relative orientations: $\text{H}_2\text{C}=\text{O} \dots \text{H}-\text{OCH}_3$ (conforming to the hydrogen bond) and $\text{H}_2\text{C}=\text{O} \dots \text{CH}_3\text{OH}$ (for which the $\text{C}=\text{O}$ bond of the formaldehyde molecule is oriented along the C_3 symmetry axis of the H_3CO part of the methanol molecule).

Figure 3 shows the results of these calculations, which qualitatively reproduce the existence of a hydrogen bond. Here, due to the limitations of the assumptions made, the $\text{H} \dots \text{O}$ distance is overestimated by 0.15–0.20 Å and the depth of the energy minimum is appreciably underestimated. In the other orientation, no significant bonding interaction was revealed. The observed methoxyl-carbonyl interaction appeared to be restricted by Van der Waals interactions.

Delocalization of electrons upon transition to the carboxylate ion (Ingold, 1953; Pauling, 1960)* apparently results in the loss of the ability of oxygen atoms to serve as proton acceptors in forming a hydrogen bond. This is the reason why the ionized carboxyl groups do not interact with alcohols and the excessive wetting heat of methyl

* A contrary view is suggested as a hypothesis in the work of Crampton & Grunewald (1974).

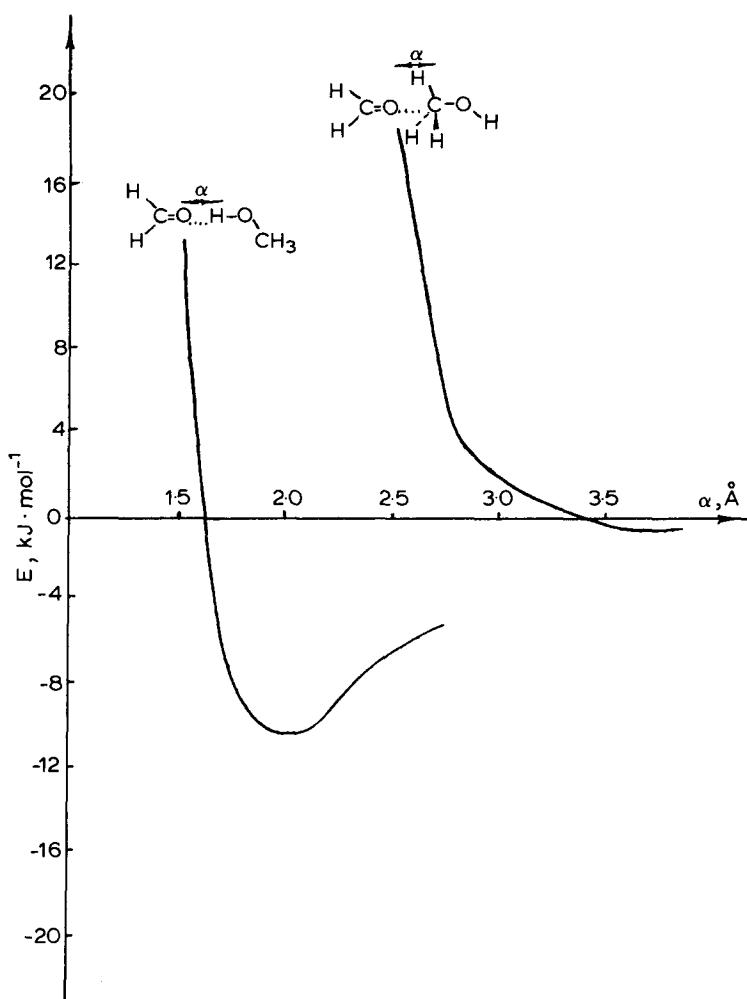


Fig. 3. Potential energy curves of formaldehyde-methanol system.

alcohol for pectin, as shown by Ignatov (1980) and Ignatov & Markova (1983), is proportional to the DE of pectin.

Thus, the results of model calculations show that methanol probably interacts with pectin through the formation of hydrogen bonds with carbonyl groups. This mechanism seems possible for other alcohols. It is, however, realised to a significant extent only in the case of methanol, probably because of its increased acidity compared with other aliphatic

alcohols: for methanol, pK_a equals 16 exceeding by 2 units the value for ethanol (McEwen, 1936)*.

The acidity is known to increase with the introduction of electronegative substitutes into the molecule. Thus the pK_a of glycerol and sucrose, that is of substances in whose presence pectin forms thermo-reversible gels (Rees, 1972), is respectively 14.0 and 12.6. Since glycerol and sucrose are polyfunctional compounds the mechanism of their structure-forming action during gelation may be assumed to involve the formation of networks of hydrogen bonds with ester groups of pectin. This can explain, in particular, the increase in the reduced viscosity of high DE pectin under the action of sucrose and also D-glucose and maltose (Chen & Joslyn, 1967). Also, the formation of hydrogen bonds between hydroxyl and carbonyl groups possibly determines the specific attraction between the macromolecules of high DE pectin in aqueous solutions. This interaction is assumed to contribute both to chain rigidity and gel-forming ability of this material (Morris *et al.*, 1980; Plashchina *et al.*, 1985).

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* The values of pK_a are also reported by Ballinger & Long (1960). For methanol, $pK_a = 15.5$. For ethanol, this value ($pK_a = 15.9$) has been obtained by extrapolating to the value $\sigma = 0$, the linear dependence $pK_a = f(\sigma)$, where σ is a substituent constant.

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