Carbohydrate Research, 60 (1978) 1–8

CIRCULAR-DICHROISM STUDIES OF PECTIN SOLUTIONS*

IRINA G. PLASCHINA, EVGENY E. BRAUDO, AND VLADIMIR B. TOLSTOGUZOV
Institute of Organoelement Compounds, U.S.S.R. Academy of Sciences, 28 Vavilov Street, Moscow (U.S.S.R.)

(Received April 12th, 1976; accepted for publication November 26th, 1976)†

ABSTRACT

The influence of pH, temperature, ionic strength, and some additives on the c.d. spectra of aqueous solutions of pectins of various degrees of esterification has been studied. On changing the pH 7.5 → 2.0, hyper- and hypso-chromic effects are observed. The molecular ellipticity ($[\theta]$) contributions of the CO_2H and CO_2Me groups are equal. Differences in the type of concentration-dependence of pectins of low ester content and fully esterified pectins have been found. The gelling of pectins following the change of solvent composition (addition of propan-2-ol or 5M sodium perchlorate) leads to an increase of $[\theta]$. At a constant level of ionization, $[\theta]$ of the carboxylic chromophore can be used as a probe for intermolecular interactions in pectin solutions.

INTRODUCTION

The capacity of pectins to form iono- and thermo-reversible gels at low and high extents of carboxyl-group esterification, respectively, is well known. However, the nature of the gel-like state of pectins is still not clear[1] and its elucidation requires studies of the process of formation in dilute solutions.

Pectinic substances exhibit[2] a positive Cotton effect with a maximum at ~210 nm corresponding to a n → π^* transition of the carboxyl group. The optical activity of the carboxyl chromophore is determined by the asymmetry of the environment and, hence, it should be affected by intra- and inter-molecular interactions. We now report on chiroptical properties of dilute solutions of pectins having various degrees of esterification.

EXPERIMENTAL

Circular dichroism (c.d.) spectra were recorded with a JASCO-20 spectro-

---

*Physico-chemical Studies of Pectin Solutions: Part I.
†The editors regret that the publication of this paper has been inadvertently delayed by an editorial oversight.

TABLE I

CHARACTERISTICS OF PECTIN SAMPLES[a]

Sample	*Uronide content* (±*0.5%*)	*Degree of esterification* (±*5%*)	pK_{app}[b]	$K_{app} \times 10^4$
F-1	87.0	0	4.10	0.79
F-2	86.5	10	4.05	0.89
F-3	87.0	40	3.67	2.14
F-4	87.0	65	3.55	2.82
F-5	86.5	100		
GalA			3.42	3.80

[a] *c* 0.05%, 0.01M sodium fluoride. [b] See text.

polarimeter in the range 190–300 nm at 27° ±0.1° using a cell of 0.1-dm pathlength. The apparatus was purged with nitrogen.

Measurements of pH (±0.05 unit) were carried out with an OP204/1 Radelkis pH meter.

The absorbance of solutions was determined on a Zeiss Specord UV VIS spectrophotometer.

Centrifugation was performed with a Yanetzki VAC 601 centrifuge.

Sample and solution preparation. — Pectic acid (Fluka) was purified by repeated precipitation from aqueous solution with 0.4M hydrochloric acid and propan-2-ol of increasing concentration (48, 60, and 96%), followed by dialysis against distilled water until free from chloride ions.

Pectins of various degrees of carboxyl-group esterification were prepared by treating[3] a lyophilized sample of pectin with diazomethane at −20°. The uronide content was determined by the carbazole method in the presence of sulphamic and boric acids[4]. The degree of esterification was determined colorimetrically with chromotropic acid[5]. The characteristics of the samples are given in Table I. No traces of ash were detected in the samples.

Solutions were prepared with distilled water (conductivity, $3.6.10^{-6}$ $\text{ohm}^{-1}.\text{cm}^{-1}$). Initial pectin solutions of various degrees of esterification were prepared by stirring with 0.01M sodium fluoride. During solubilization, the pH was maintained at 7.5 by the addition of dilute alkali. Poorly soluble pectic acid was dissolved by heating at 50° for 30 min, and the solutions were centrifuged at 100,000 *g* for 1 h before determining the pectin concentration.

These solutions were diluted as appropriate. Solutions were kept for 3 days at room temperature before measurements were taken. Where appropriate, the pH of the solutions was adjusted with perchloric acid immediately before c.d. spectra were determined, and was checked immediately afterwards. To avoid distortions of pH measurements due to pectin adsorption, the glass electrode was treated with a solution of chlorotrimethylsilane in benzene.

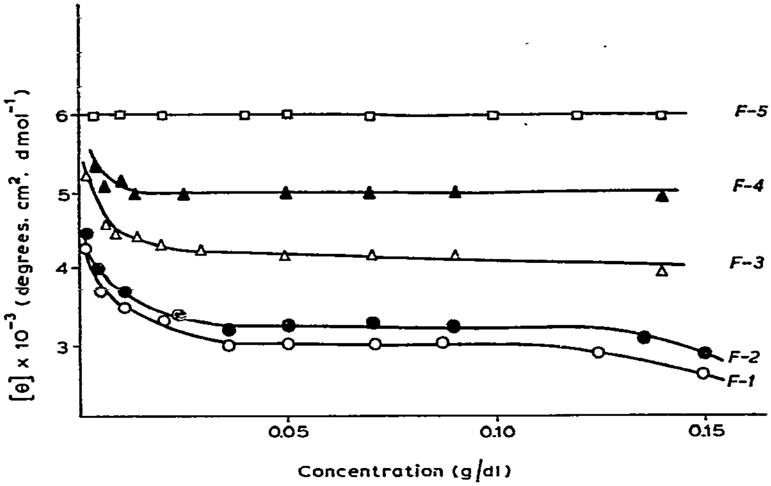

Fig. 1. Concentration dependence of molecular ellipticity of pectins of various degrees of esterification; 0.01M sodium fluoride, pH 7.5.

RESULTS

The pectin samples studied exhibited a positive Cotton effect with a maximum in the range 200–215 nm. The absorption band shifted towards the long-wave region with decrease of charge on the pectin macromolecule (increase of degree of esterification, decrease of pH) and with increase of ionic strength. The values of molecular ellipticity $[\theta]$ presented below correspond to the absorption maximum.

The dependence of $[\theta]$ at pH 7.5 on the concentration of pectins of various degrees of esterification is shown in Fig. 1. Similar data were obtained at pH 4.5. Increase in the degree of esterification causes an increase in $[\theta]$. For fully esterified pectin, $[\theta]$ remains constant throughout the concentration range studied. A concentration-dependence of $[\theta]$ in the range of low concentrations appears at a degree of esterification of 65%. For pectins with a low degree of esterification and for pectic acid, a decrease of molecular ellipticity is also observed at concentrations of 0.1% and higher. Since solutions opalesce in this concentration range, a decrease of $[\theta]$ may be ascribed to light diffusion[6]. All the data presented below were obtained in a concentration range in which molecular ellipticity did not change with concentration.

There is a linear dependence of $[\theta]$ on the degree of esterification of pectin (Fig. 2). Therefore, within the accuracy of the experiment, contributions of esterified and non-esterified carboxyl groups can be assumed to be additive. Data on spectropolarimetric titration are presented in Fig. 3. The curves are plotted according to the equation:

$$[\theta] = [\theta]'' + \frac{[\mathrm{H}^+]}{[\mathrm{H}^+] + K_{\mathrm{app}}}([\theta]' - [\theta]''),$$

where $[\theta]''$ and $[\theta]'$ are the molecular ellipticities of pectins in completely ionized and non-ionized states, respectively. At pH 7.5, $[\theta]''$ was taken as equal to $[\theta]$. Experimental determination of $[\theta]'$ was not possible, as, at low degrees of ionization, the solubility of pectinic substances decreases, the solutions turn turbid, and $[\theta]$ decreases at low pH values. The value of $[\theta]'$ was therefore assumed to be equal to the molecular ellipticity of fully esterified pectin. Values of pK_{app} for pectin ionogenic groups used for plotting the theoretical curves are presented in Table I. It is known, from potentiometric titration data, that pK_{app} decreases with increase in the degree of esterification of pectin[7,8]. As can be seen from Fig. 3, experimental points are in good agreement with the theoretical curves plotted according to selected values of pK_{app}. This result confirms the assumption that the values of $[\theta]$ for pectin in protonated and esterified forms are equal. The curve of the spectropolarimetric titration of galacturonic acid is also presented in Fig. 3. The pK_{app} value of 3.42 agrees with published data[7].

A study of the effect of sodium fluoride concentration (0.01–0.5M) on the c.d. spectra of pectins of various degrees of esterification revealed that $[\theta]$ does not depend on the ionic strength.

Fig. 4 shows a comparison of c.d. spectra of pectic acid and fully esterified pectin at 25 and 75°. Increase in temperature leads to a decrease of $[\theta]$ that is similar in both samples.

In order to determine the effect of solvent on $[\theta]$ of pectins, c.d. spectra were obtained in the presence of propan-2-ol and 5M sodium perchlorate. Because propan-2-ol is a precipitant, the upper concentration level is limited. Sodium perchlorate affects the structure of water and increases the solubility of nonpolar compounds[9], but at high concentrations, it may have a salting-out effect on the pectinic substances.

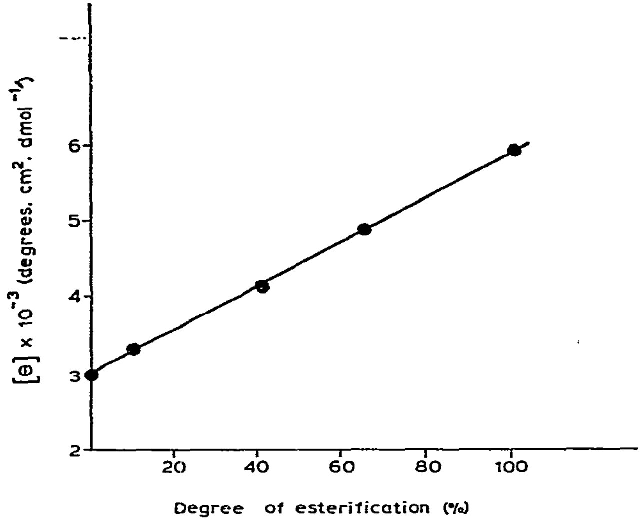

Fig. 2. Dependence of molecular ellipticity on the degree of esterification of pectin; 0.01M sodium fluoride, pH 7.5, c 0.05%.

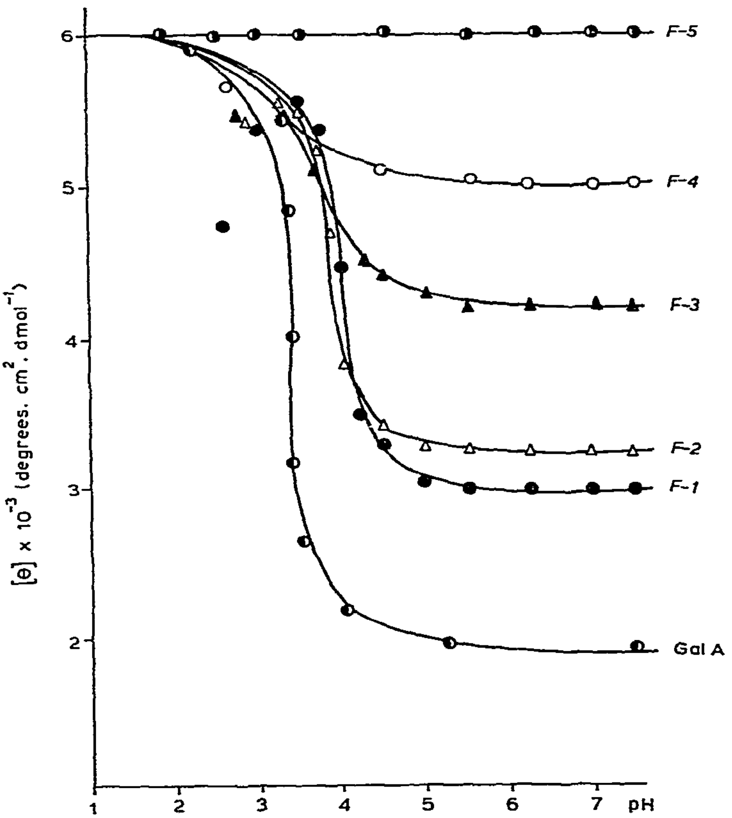

Fig. 3. pH Dependence of the molecular ellipticity of pectins of various degrees of esterification; 0.01M sodium fluoride, *c* 0.05%.

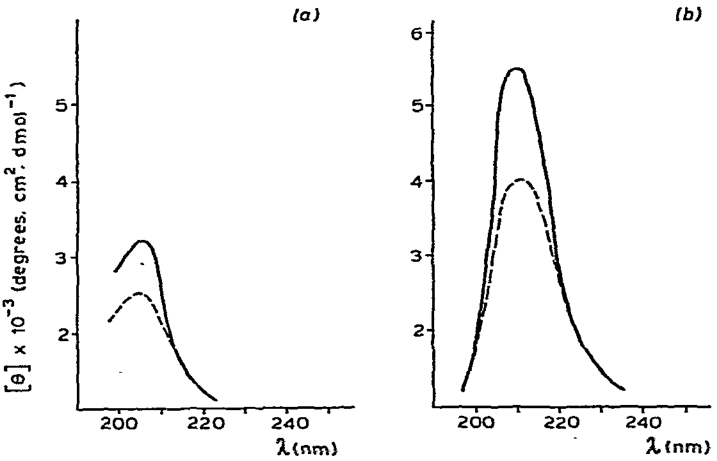

Fig. 4. C.d. spectra of pectic acid *F-1* (*a*), and high-ester pectin *F-5* (*b*), at 25° (—) and at 75° (----).

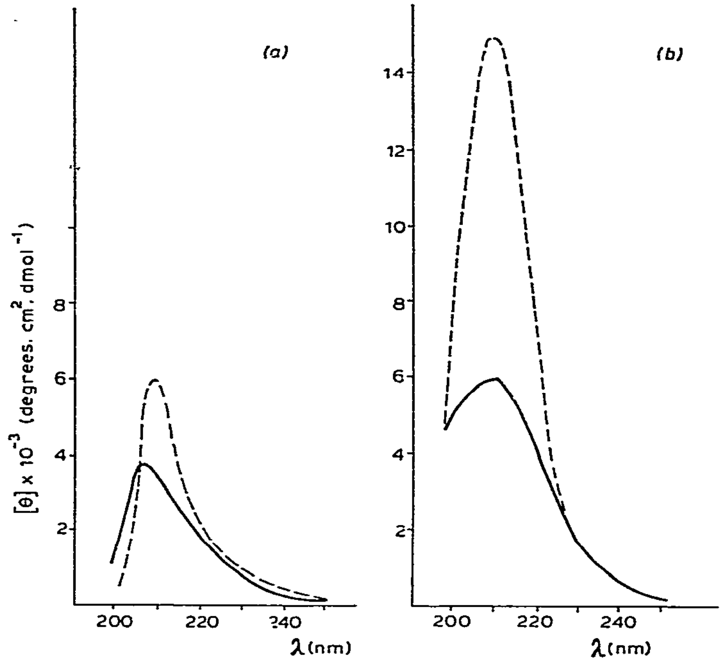

Fig. 5. C.d. spectra of pectic acid *F-1* (*a*), and high-ester pectin *F-5* (*b*), in 0.01M sodium fluoride (—) and in 30% (*c* 0.09%) and 75% (*c* 0.06%) propan-2-ol (----).

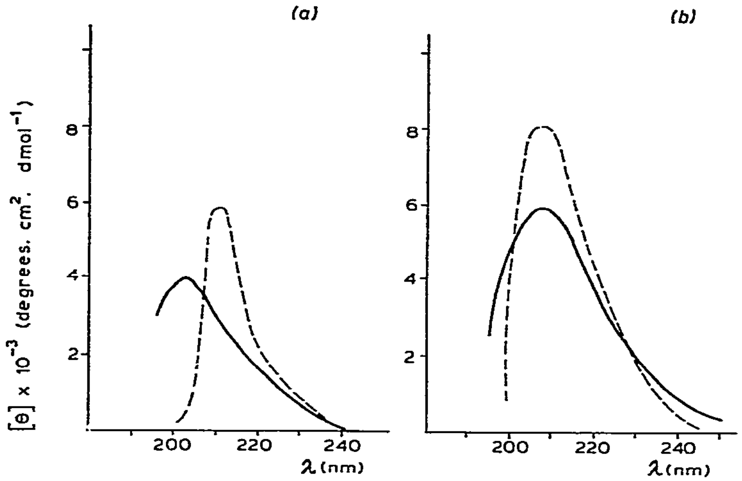

Fig. 6. C.d. spectra of pectic acid *F-1* (*a*), and high-ester pectin *F-5* (*b*), in 0.01M sodium fluoride (—) and 5M sodium perchlorate (----); pH 7.5, *c* 0.07%.

Figs. 5 and 6 show the effect of propan-2-ol and sodium perchlorate on the c.d. spectra of pectic acid and fully esterified pectin. Addition of propan-2-ol or 5M sodium perchlorate results in gel formation and leads to hyper- and hypso-chromic changes in the c.d. spectrum of pectic acid. The c.d. spectrum of fully esterified pectin is not affected by propan-2-ol up to concentrations of 50%. Further increase of concentration, up to 75%, leads to an increase of $[\theta]$. In the presence of sodium perchlorate, $[\theta]$ of high-ester pectin also increases, but, in contrast to pectic acid, it does not form a gel and no hypsochromic effect is observed in the c.d. spectrum.

DISCUSSION

Variation of chiroptical properties under the conditions studied may be connected with variation of the state of the carboxyl group (ionization, esterification) and variation of the asymmetry of the chromophore environment due to intramolecular (conformation change) and intermolecular interactions (association).

The molecule of pectic acid is characterised by a high energy barrier to rotation around the glycosidic bond and, consequently, by low conformational mobility. This conclusion follows from results of conformation calculations[10,11], from experimental data of the dependence of intrinsic viscosity on ionic strength[12], and from the weak dependence of optical rotation on temperature and urea concentration[11,13] (up to 7M). On the basis of X-ray structural data[14], it was assumed that the conformation of the pectinic acid chain is not affected[11] by degrees of esterification up to 70%. The linear dependence of $[\theta]$ on the degree of esterification observed in the present study agrees with this assumption and makes it possible to extend the assumption up to a degree of esterification of 100%. Thus, the contribution of intramolecular interactions to the variation of $[\theta]$ is negligible.

As shown above, the $[\theta]$ contributions of the CO_2H and CO_2Me groups are equal. Carboxyl ionization leads to a sharp decrease of $[\theta]$. At a constant level of ionization of the carboxyl groups, association of pectins may lead to a two-fold increase of $[\theta]$. This conclusion follows from a comparison of c.d. spectra in 0.01M sodium fluoride and in media that induce gel formation (propan-2-ol, 5M sodium perchlorate). Correspondingly, the decrease in molecular ellipticity with increase in temperature may be connected with the decrease in degree of association due to destruction of hydrogen bonds that play an important role[1,15] in the processes of structure formation of pectins, or in the disorientating effect of thermal agitation. Decrease in molecular ellipticity with increase in temperature is observed in both samples. At the same time, different patterns of $[\theta]$–concentration dependence indicate differences in the association of high- and low-ester pectins.

The increase of $[\theta]$ with decrease in concentration in the region of very dilute solutions does not conform with the assumed increase of $[\theta]$ on association of pectins. This problem requires further study.

Thus, it may be noted that molecular ellipticity in the 200–210 nm region increases with a decrease of the extent of ionization of the carboxylic chromophores. At a constant extent of ionization, the molecular ellipticity of the carboxylic chromophore may serve as a probe for intermolecular interactions in solutions of pectinic substances.

REFERENCES

1 D. A. Rees, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 267–322.
2 G. Grant, E. Morris, D. A. Rees, P. Smith, and D. Thom, *FEBS Lett.*, 32 (1973) 195–199.
3 B. Vollmert, *Makromol. Chem.*, 5 (1950) 101–109.
4 M. P. Filippov and T. V. Vlas'eva, *Prikl. Biokhim. Mikrobiol.*, 9 (1973) 134–137.
5 M. P. Filippov and V. I. Kuz'minov, *Zh. Anal. Khim.*, 26 (1971) 143–146.
6 D. W. Urry, T. A. Hinners, and L. Masotti, *Arch. Biochem. Biophys.*, 137 (1970) 214–221.
7 R. Speicer, C. H. Holls, and C. R. Eddy, *J. Phys. Colloid Chem.*, 49 (1945) 328–334.
8 A. Katchalsky, N. Shavit, and H. Eisenberg, *J. Polym. Sci.*, 13 (1954) 69–84.
9 P. H. von Hippel and Th. Schleich, in S. N. Timasheff and J. D. Fasman (Eds.), *Structure and Stability of Biological Macromolecules*, Marcel Dekker, New York, 1969, pp. 320–475.
10 B. K. Sathyanazyana and V. S. R. Rao, *Curr. Sci.*, 42 (1973) 773–775.
11 D. A. Rees and A. W. Wight, *J. Chem. Soc.*, *B*, (1971) 1366–1372.
12 O. Smidsrød and A. Haug, *Biopolymers*, 10 (1971) 1213–1227.
13 S. Hirano, *Life Sci.*, (1971) 151–155.
14 K. J. Palmer and M. B. Hartzog, *J. Am. Chem. Soc.*, 67 (1945) 2122–2127.
15 H. Deuel and E. Stutz, *Adv. Enzymol.*, 20 (1958) 341–382.