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Effect of temperature and concentration on viscosity of orange peel pectin solutions and intrinsic viscosity–molecular weight relationship

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Abstract

The effects of temperature and concentration on the viscosity of orange peel pectin solutions were examined at five different temperatures between 20 and 60°C and five concentration levels between 2.5–20 kg/m^3. The effects of temperature was described by an Arrhenius-type equation. The activation energy for viscous flow was in the range 19.53–27.16 kJ/mol, depending on the concentration. The effect of concentration was described by two types of equation, power-law and exponential. Equations were derived which describes the combined effects of temperature and concentration on the viscosity for two different models in the range of temperatures and concentrations studied. Orange peel pectin was extracted by using HCl (pH 2.5, 90°C, 90 min) ammonium oxalate (0.25%, pH 3.5, 75°C, 90 min) and EDTA (0.5%, 90°C, 90 min) extraction procedures. The best result was obtained with ammonium oxalate extraction in which the pectin content of the final product was 30.12%, although the efficiency among the procedures varied.The average molecular weight was measured by light scattering technique. Magnitudes of intrinsic viscosity and molecular weight of pectins obtained by extraction with HCl, ammonium oxalate and EDTA were 0.262, 0.281, 0.309 m^3/kg and 84 500, 91 400, 102 800 kg/kgmol, respectively. The molecular weight dependence of the intrinsic viscosity of the orange peel pectin solutions was expressed by Mark–Houwink–Sakurada equation. The data were fitted to equation as $\eta_i = 2.34 \times 10^{-5}(M_{w,ave})^{0.8224}$ which helps to evaluate the average molecular weight of pectin solutions from orange peel with a knowledge of their intrinsic viscosity.

Keywords: Orange peel pectin solutions; Jam manufacturing; Viscosity; Temperature and concentration dependence; Molecular weight

1. Introduction

The pectic substances, located primarily in the middle lamella between cells in higher plant tissues, are complex polysaccharides. The predominant structural feature of pectins is a chain of 1 → 4 linked α-D-galacturonic acid residues. They also include the negatively charged rhamnogalacturonans, and the neutral arabinogalactans and L-arabinans (Zitko & Bishop, 1965; Whitaker, 1984).

Citrus pectins find ready application as clouding agents in drinks and due to their gelating and/or stabilizing properties in food and confectionery industries. These functional attributes are dependent on the structure, composition and physical properties of the pectins (Lodge, Nguyen & McIntyre, 1987). Gelling agents are added to commercial products to achieve desired firmness or consistency. Among them, pectic substances find many applications particularly in jam manufacturing. The major sources of commercial pectin are citrus wastes (pulp and peel), apple pomace and sugar-beet pulp (Arslan & Toğrul, 1996; Arslan & Kar, 1998; Donaghy & McKay, 1994; El-Nawawi & Shehate, 1987).

The gelling ability of pectin depend on its solubility and viscosity, which are a measure of its molecular weight (Rao, 1993). The viscosity depends not only on the concentration of the polymer but also on the molecular weight and shape, pH and ionic strength (Thibault & Rombouts, 1986). Higher the molecular weight, the higher is its viscosity and hence, the better is its grade (Brandrup & Immergut, 1975; Rao, 1993), and hence, there is a need to measure the molecular weight of pectin solutions. The pectin molecule can contain from a few hundred units up to approximately one thousand units corresponding to molecular weights of up to 150 000, depending on the raw meterials used (Gregory, 1986). High performance size exclusion chromatography (HPSEC) has been used by Fishman, Gillespie and Sondey (1991) to examine the molecular weights and dimensions of pectins of fruit and vegetable by-products. Chou and Kokini (1987), and Phatak, Chang and Brown (1988) determined the molecular weight distribution of pectin samples by gel permeation

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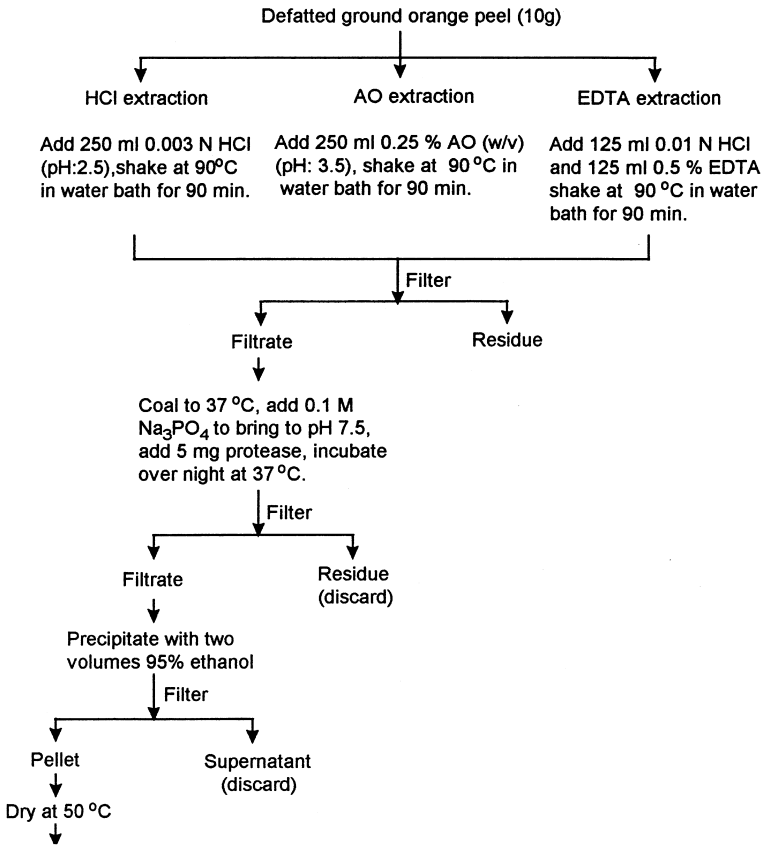

Fig. 1. Scheme for extraction of orange peel pectin.

chromatography. In addition, molecular weight of polymers have been determined using differential refractometer (DRI) and low-angle laser light scattering (LALLS) detection (Pang & Rudin, 1992).

The effect of temperature and concentration on the flow properties must be known for understanding unit operations such as heat transfer and evaporation, respectively. It is unknown how the flow properties of orange peel pectin solutions are influenced by temperature and concentration. The knowledge of the intrinsic viscosity helps the determination of the molecular weight of orange peel pectin. Information on the intrinsic viscosity of orange peel pectin solutions has been reported by Zitko and Bishop (1965). However, at present no attempt has been made to determine the molecular weight of orange peel pectin using light scattering technique and to show the intrinsic viscosity–molecular weight relationship.

The aim of the present study was to extract the pectin from orange peel by different extraction prodecures, to develop models which adequately describe the effect of temperature and concentration on the viscosity of orange peel pectin solutions, to determine intrinsic viscosities of pectin solutions and the average molecular weight of orange peel pectin, and to find the intrinsic viscosity–molecular weight relationship of orange peel pectin solutions.

2. Materials and methods

2.1. Proximate chemical analysis

Dry matter was determined by using the method 40-40 of AACC (1983). Ash content was determined by measuring the residue remaining after incinerating the sample overnight in a muffle furnace at 600°C (AOAC, 1984). Protein content was determined by the kjeldahl method (AACC, 1983: approved procedure 46-12). Crude fat was determined gravimetrically after extraction with petroleum ether. Crude fiber content was determined by using the method of AOAC (1975). Total carbohydrate (nitrogen free extract) was determined by difference. All the results were expressed on a dry weight basis determined by drying samples at 105°C for 12 h. All the experiments in this study were conducted in duplicate.

2.2. Extraction of pectin from orange peel

Pectin was extracted from orange peel by a modificiation of the method of Phatak et al. (1988). Washington oranges (*Citrus sinensis*) were purchased from a local retail market and peeled (albedo plus flavedo). Sun dried orange peel was ground to 50 mesh in a hammer mill to facilitate washing and extraction. Before orange peel was utilized for pectin

production, the peel oil was removed by petroleum ether and dried. Peel was heated to 97°C in a water bath for about 10 min to inactivate the pectic enzymes, and subjected to decolorization process. The orange color in orange peel is mostly due to the carotenoids. To remove these carotenoids, the powder peel was left to stand in water for a while by decanting and changing the water repeteadly until it did not colorize the water.Then, it was filtered through a suction filter, dried at 50°C and sieved. A 50 mesh fraction was used in experiments.

Pectin was extracted from orange peel by HCl, ammonium oxalate (AO) and disodium ethylenediamine tetraacetic acid (EDTA) extraction procedures. The scheme of extraction pectin from orange peel is shown in Fig. 1.

The yields of pectin were expressed in dry weight of extracted material/100 g dry peel. The word "pectin" stands for the pectin obtained in this study.

2.3. Viscometry

In order to determine the intrinsic viscosity of pectin, at first the viscosities of pectin solutions in different concentrations were determined. As pectin is a polyelectrolyte, solutions were prepared by using 0.1 M sodium phosphate buffer (pH = 7.0) instead of water. For viscosity determinations; 2.5, 5, 10, 15, and 20 kg/m^3 pectin solutions were prepared in solvent mentioned above. The mixture was then heated to 20°C and allowed to stand with mixing at ambient temperature for 12 h. The dispersion was filtered. Then, 15 ml of pectin solutions were pipetted into the capillary viscometer for viscosity measurements. The viscosities of pectin solutions at different concentrations were determined at 10°C intervals from 20 to 60°C by means of an Ubbelohde viscosimeter No.13 (capillary no: Ic, I.D.: 0.84 mm). Densities of solutions were measured using Gay–Lussac type pycnometer. The relative viscosity was calculated using the following equation (Chen & Joslyn, 1967):

$$\eta_r = \frac{\eta}{\eta_s} = \frac{t_1 d_1}{t_2 d_2} \tag{1}$$

where η_r the relative viscosity, η the viscosity pectin solution (Pa s), η_s the viscosity of solvent (Pa s), t_1 the time taken by solution to flow in viscometer (s), t_2 the time taken by solution to flow in viscometer (s), d_1 the density of solution (kg/m^3), d_2 the density of solvent (0.1 M sodium phosphate buffer, 1435 kg/m^3).

Relative viscosity values were converted to specific viscosities (η_{sp}) using the following equation (Chen & Joslyn, 1967):

$$\eta_{sp} = \frac{\eta - \eta_s}{\eta_s} = \eta_r - 1 \tag{2}$$

The reduced viscosity (η_{sp}/C) should ideally be independent on the concentration, and it becomes so at the limit of zero concentration (Hwang, Roshdy, Kontominos & Kokini, 1992). The intrinsic viscosity is defined as the limit of the reduced viscosity as the concentration approaches zero. The principal method of determination of the magnitude of intrinsic viscosity is to extrapolate the reduced viscosity to its value at zero solute concentration (Chou & Kokini, 1987).

$$\eta_i = \lim_{C \to 0} \frac{\eta_{sp}}{C} \tag{3}$$

where C the concentration of pectin solution (kg/m^3), η_{sp} the specific viscosity,(η_i the reduced viscosity (m^3/kg).

2.4. Determination of molecular weight

The refractive index of pectin solutions were measured by a refractometer using a mono-chromatic source of sodium vapour lamp. The intensity of light scattered through pectin solutions was measured as the percentage of light transmitted through pectin solutions, as compared to that through 0.1 M sodium phosphate buffer (pH = 7.0) by spectronic 20 spectrofotometer. The experimental measurements were made at five different concentrations (2.5, 5, 10, 15, and 20 kg/m^3).

The average molecular weight of the pectin was calculated by the following equation (Allock & Lampe, 1981; Rao, 1993):

$$\frac{1}{M_{w,ave}} = \lim_{C \to 0} \frac{HC}{\tau} \tag{4}$$

where $M_{w,ave}$ is the average molecular weight (kg/kg mol), τ the turbidity of solution (m^{-1}).

H is given by Allock and Lampe (1981) as

$$H = \frac{32\pi^3 n_0^2}{3\lambda^4 N_0} \left(\frac{n - n_0}{C} \right)^2 \tag{5}$$

where n_0 the refractive index of the solvent (0.1 M sodium phosphate buffer, 1.3368), n the refractive index of the solution, λ the wave length of light (0.5893×10^{-6} m), N_0 the avogadro number (6.023×10^{23}).

The turbidity of the solutions was measured as the decrease in the intensity of a beam of light because of scattering. The decrease or attenuation depends on the length of the light path through the system (Allock & Lampe, 1981) and, by analogy to the Lambert law (Allock & Lampe, 1981), it is possible to write

$$\frac{l}{l_0} = e^{-\tau l} \tag{6}$$

where l/l_0 the fraction of light transmitted through 1 cm length of solution, l the length of the light path in the solution (m).

The fraction of light scattered is generally very small (Allock & Lampe, 1981) and it is a good approximation to express the exponential in the above equation (Allock & Lampe, 1981) as:

$$e^{-\tau l} = 1 - \tau l + (1/2)(\tau l)^2 - (1/6)(\tau l)^3 + \cdots \cong 1 - \tau l \tag{7}$$

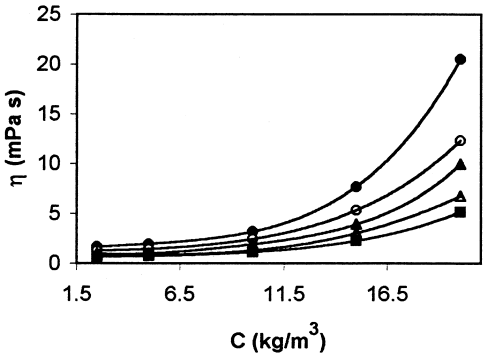

Fig. 2. Change in the viscosity of pectin solutions with concentration at different temperatures (in °C): 20 (●); 30 (○); 40 (▲); 50 (△); 60 (■).

$$\tau l = 1 - e^{-\tau l} = 1 - (l/l_0) \quad (8)$$

$M_{w,ave}$ was measured by plotting HC/τ versus C and interpolating to zero concentration and noting the value of intercept (Allock & Lampe, 1981).

3. Results and discussion

The dried peel contained 2.68% ash, 6.08% crude protein, 17.60% crude fiber, and 71.40% carbohydrate (by difference) on a dry weight basis.

Extraction of orange peel with HCl, AO, and EDTA yielded 29.58, 30.12, and 27.63% pectin, respectively, the highest yield being in case of ammonium oxalate procedure. The extraction process, the variety, and the stage of orange maturity can affect the quantity and quality of extracted pectin and pectin yield depends on the type of raw material and methods of preliminary treatment. It has been established that the extraction of orange peels gives a high yield of highly esterified pectin with good gelling properties (Plöger, 1992).

3.1. The combined effect of temperature and concentration on viscosity

Diluted organic solutions are generally Newtonian in character (Kirk & Othmer, 1970). The hot break tomato paste pectin solutions at concentrations of 2% or lower and citrus pectin solutions at concentrations of 3% or lower were Newtonian (Chou & Kokini, 1987). Therefore, it has been assumed that the pectin solutions studied were Newtonian.

Table 1
Arrhenius model parameters for pectin solutions at different concentrations

C (kg/m^3)	E_a (kJ/mol)	$\eta_0 \times 10^3$ (mPa s)	r^2
2.5	19.53	0.551	0.9887
5	20.61	0.400	0.9700
10	22.04	0.385	0.9919
15	24.32	0.351	0.9964
20	27.16	0.282	0.9892

Fig. 2 shows the experimental results of viscosity versus concentration for different temperatures. When the solution is heated, the viscosity decreases as the thermal energy of the molecules increases and the intermolecular distances increase due to thermal expansion. As seen from Fig. 2, at higher temperature, viscosity decreases and at higher concentration viscosity increases. The effect of temperature was stronger at the higher concentrations. The theory of viscosity also involves intermolecular activity. When the solid concentration increases, the viscosity increases because of the increase in hydrogen bonding with hydroxyl groups and the distortion in the velocity pattern of the liquid by hydrated molecules of the solute. The intermolecular distances which is also a factor that affects the viscosity is inversely proportional to it due to changing temperatures. This theory almost explains the reasons for temperature and concentration dependancy with molecular point of view.

The effect of temperature on the viscosity of fluids can be expressed by an Arrhenius-type equation (Ibarz, Pagan & Miguelsanz, 1992):

$$\eta = \eta_0 \exp(E_a/RT) \quad (9)$$

where η the viscosity of pectin solution (mPa s), η_0 a pre-exponential factor (mPa s), E_a the activation energy of flow (kJ/mol), R the gas constant (kJ/mol K), and T the absolute temperature (K).

Variation of viscosity of fluids with concentration can be described by either an exponential-type or a power-type relationship (Ibarz et al., 1992). For a power-type relationship, the viscosity varies with the concentration raised to a given power:

$$\eta = K_1 C^{A_1} \quad (10)$$

and, for an exponential-type relationship the function is exponential:

$$\eta = K_2 \exp(A_2 C) \quad (11)$$

In both equations, K_1 (mPa s(kg/m^3)$^{-A_1}$), K_2 (mPa s) and A_1 (dimensionless), A_2 (kg/m^3)$^{-1}$ are constants and C the concentration of pectin solutions (kg/m^3).

The values of the constants E_a and η_0 in Eq. (9) were calculated from linear regression analysis of ln η versus $1/T$ by using the equation of Arrhenius. The magnitudes of E_a and η_0 for pectin solutions at different concentrations are shown in Table 1.

As seen from Table 1, the magnitudes of activation energy at high concentrations are higher than those at lower concentrations and the values in this table can be employed to calculate the viscosity of pectin solutions at a specific temperature. At high concentrations, the viscosity is high and the sample requires a larger hole or more space for a molecule to flow into and hence a higher activation energy of flow.

In order to calculate the constants in Eqs. (10) and (11),

Table 2
The combined effect of temperature and concentration on viscosity of pectin solutions

	20°C	30°C	40°C	50°C	60°C
Isothermal data-power model: $\eta = K_1 (C)A_1$					
K_1	0.418	0.360	0.257	0.223	0.201
A_1	1.119	1.027	1.054	0.983	0.933
r^2	0.819	0.835	0.818	0.805	0.824
Isothermal data-exponential model: $\eta = K_2 \exp(A_2 C)$					
K_2	0.975	0.791	0.570	0.467	0.409
A_2	0.144	0.131	0.136	0.127	0.120
r^2	0.970	0.978	0.971	0.965	0.973
Combined Arrhenius and power models: $\eta = K_4 \exp(K_3 \exp(A_3 C)/RT)(C)A_4$					
$A_3 = 0.0184$, $K_3 = 18.62$, $A_4 = -0.2727$, $K_4 = 0.684$					
Combined Arrhenius and exponential models: $\eta = K_5 \exp(K_3 \exp(A_3 C)/RT) \exp(A_5 C)$					
$A_3 = 18.36$, $K_3 = 18.62$, $A_5 = -0.1315$, $K_5 = 0.535$.					

the values of viscosity were fitted to their linear forms obtained by expressing them in a logarithmic form (Table 2). To arrive at a single equation for viscosity, the effects of temperature and concentration can be combined. Such a relationship can be used to model viscosity in operations where both the temperature and concentration change during the process. The relationship of constants E_a and η_0 in Eq. (9) with concentration were fitted to the equations including the concentration. The coefficients are shown in Table 2. The coefficients K_3 (kJ/mol) and A_3 ((kg/m^3)$^{-1}$), K_4 (mPa s(kg/m^3)$^{-A_4}$) and A_4 (dimensionless), K_5 (mPa s) and A_5 ((kg/m^3)$^{-1}$) were obtained by the plot of ln E_a versus C ($r^2 = 0.9927$), ln η_0 versus ln C ($r^2 = 0.9015$) and ln η_0 versus C, respectively ($r^2 = 0.8628$).

The experimental viscosity-concentration data were fitted to the linearized form of Eqs. (10) and (11) by the least-squared method to evaluate constants in Eqs. (10) and (11). The estimates of the parameters of Eqs. (10) and (11) at different temperatures are listed in Table 2.

From the values of the regression coefficient obtained, the exponential model than by the power-type relationship seems to describe the effect of concentration on the viscosity of pectin solutions well (Table 2).

It should be emphasized that the constants in Table 2 are applicable only to the range of temperatures and concentrations studied. The experimental results of this study showed that temperature and concentration have a strong influence on viscosity of orange peel pectin solutions. The model will be useful for the manufacture and applications of pectin from orange peel.

3.2. Intrinsic viscosity–average molecular weight relationship

The quantity intrinsic viscosity of a polymer solution is a measure of the capacity of a polymer molecule to enhance the viscosity (Brandrup & Immergut, 1975). Fig. 3. shows the method of determination of the magnitude of intrinsic viscosity by plotting η_{sp}/C versus the concentration of pectin solutions. It can be concluded that the minumum in Fig. 3 was observed because of the fact that the macromolecules were not free in their movements and could not act as individual units. Magnitudes of intrinsic viscosity and molecular weight of pectins obtained by HCl, AO and EDTA extraction were 0.262,0.281, 0.409 m^3/kg, and 84 500, 91 400, 102 800, respectively.

Intrinsic viscosity is a measure of the hydrodynamic volume occupied by a molecule and the nondimensional parameter $C\eta_i$ can be taken as a measure of the extent of overlapping between polymer molecules. The role of the parameter $C\eta_i$ can best be understood using Huggins equation (Tehchien & Kokini, 1987):

$$\frac{\eta_{sp}}{C} = \eta_i + k'\eta_i^2 C \tag{12}$$

It can be shown (McMillan, 1974) that reduced viscosity can be written in the forms of Martin's equations:

$$\log(\eta_{sp}/C) = \log \eta_i + k''\eta_i C \tag{13}$$

Another method for calculating the intrinsic viscosity by extrapolation to zero concentration is by using Kraemer's equation:

$$\ln \eta_r/C = \eta_i - k'''\eta_i^2 C \tag{14}$$

For very dilute systems, Eq. (14) can be shortened by

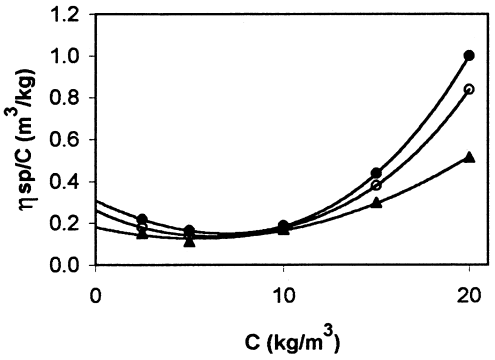

Fig. 3. Plot of η_{sp}/C versus C EDTA (●); HCl (○); AO (▲);

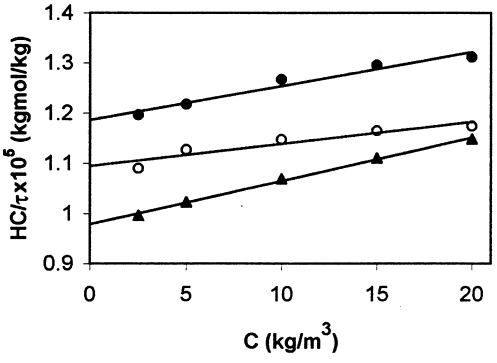

Fig. 4. Light-scattering determination of the molecular weight of pectin from orange peel: HCl (●); AO (○); EDTA (▲).

Table 3
Data for calculating average molecular weight and intrinsic viscosity

Extraction procedure	C (kg/m^3)	Optical rotation	Transmittance (%)	Turbidity $\tau \times 10^2$ (m^{-1})	HC/$\tau \times 10^5$ (kg mol/kg)	η_i (m^3/kg)	$M_{w,ave}$ (kg/kg mol)
HCl	2.5	1.3505	49	0.51	1.196		
	5	1.3590	34	0.66	1.217		
	10	1.3698	30	0.70	1.267	0.262	84500
	15	1.3813	17	0.83	1.296		
	20	1.3912	8	0.92	1.311		
AO	2.5	1.3496	51	0.49	1.090		
	5	1.3570	41	0.59	1.127		
	10	1.3682	30	0.70	1.147	0.281	91400
	15	1.3795	15	0.85	1.165		
	20	1.3886	7	0.93	1.174		
EDTA	2.5	1.3510	44	0.66	0.996		
	5	1.3585	25	0.75	1.023		
	10	1.3698	17	0.83	1.069	0.309	102800
	15	1.3792	12	0.88	1.110		
	20	1.3888	4	0.96	1.148		

retaining only the first order term and η_i can be determined from the slope of a plot of ln η_r versus C (Tanglertpaibul & Rao, 1987).

k, k'' and k''' are Huggins, Martin and Kraemer's constants, respectively. The fitness of viscosity–concentration relationship to Huggins, Martin and Kraemer's equations are also investigated. As η_{sp}/C versus C, $\log(\eta_{sp}/C)$ versus C, $\ln(\eta_r/C)$ versus C graphs were not in a straight line, it could be predicted that the Huggins, Martin and Kraemer's equations could not be fitted.

HC/τ values were calculated and plotted against concentration of pectin solutions (Fig. 4). HC/τ versus C showed similar trends and straight lines with linear regression coefficients in the range 0.99–1.0.

The reciprocal of the intercept is the average molecular weight. The results are presented in Table 3.

Intrinsic viscosity is a characteristic of macromolecules that is related directly to their ability to disturb flow and indirectly to their size and shape. For molecules which can exist with a variety of molecular weights, the relation between intrinsic viscosity and molecular weight is one of the most important properties. Intrinsic viscosity for a long linear molecular species may be written, according to the Staudinger's viscosity rule (Tanglertpaibul & Rao, 1987):

$$\eta_i = KM_{w,ave} \tag{15}$$

where K a constant peculiar to the monomer and the viscosity of the solvent; it also depends upon the configuration of the polymer. Deviations from Staudinger's rule, which are common, are generally attributable to the fact that a polymeric molecule is not "freely drained". Therefore, there is the hydrodynamic shielding effect (Tanglertpaibul & Rao, 1987). Thus, the intrinsic viscosity as functions of average molecular weight are usually represented by well known and widely used Mark–Houwink–Sakurada empirical equations (Isihara, 1992):

$$\eta_i = K(M_{w,ave})^{\alpha} \tag{16}$$

with the constant α taking values between 1.0 and 0.5. Both the exponent α and K are dependent on the nature of the molecule and solvent, and on the temperature.

The equation may be written as follows:

$$\ln(\eta_i) = \ln K + \alpha \ln(M_{w,ave}) \tag{17}$$

Intrinsic viscosity values obtained from plot of η_{sp}/C versus C were used for the plot of $\ln(\eta_i)$ versus $\ln(M_{w,ave})$. The slope of the straight line obtained by Eq. (17) is α and its point of intersection with ordinate gives $\ln(K)$. From this graph, K and α were found to be 2.34×10^{-5} and 0.8224, respectively.

The exponent α and K are dependent on the nature of the molecule and solvent, and on the temperature (Tanglertpaibul & Rao, 1987). The values determined are applicable for pectin solutions in 0.1 M sodium phosphate buffer solvent alone, which limits the applicability of these coefficients. It is possible to determine the average molecular weight of pectin samples by measuring the intrinsic viscosity of pectin with α and K coefficients.

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