Short Communication:

The effect of different storage temperatures on the physical properties of pectin solutions and gels

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Abstract

The stability (in terms of viscosity and gel strength) of pectin solutions and gels potentially plays an important role in their behaviour and functional properties in a wide range of applications and therefore any changes over time must be understood.

The gel strength of pectin gels and intrinsic viscosity of pectin solutions at different temperatures (4°C, 25°C and 40°C) have been investigatied using a "rolling ball" viscometer and a texture analyser respectively. Both the intrinsic viscosity ([η]) and gel strength decrease with increased storage time, although this more pronounced at elevated temperatures. The changes in intrinsic viscosity with storage time and temperature were used to determine the depolymerisation constant (*k*).

Pectin storage conditions and particularly temperature have an influence on depolymerisation, particularly elevated storage temperatures, but whether or not this will be detrimental to its intended application will depend on the functional significance of the changes that occur. In this case based on the previous diffusion studies on a model drug (paracetamol) we conclude that the decreases in viscosity and gel strength within the range observed have no detrimental effect on the drug release properties.

Keywords: pectin; molar mass; intrinsic viscosity; gel strength; stability; drug release

1. Introduction

Pectins are a family of complex polyuronide-based structural polysaccharides, which constitute approximately one third of the dry weight of higher primary plant cell walls [1-3]. These molecules are particularly prevalent in fruit cell walls [4,5], especially citrus fruits and apple pomace. The main pectin chain is composed of α (1→4) linked D-galacturonic acid residues [1,6]. Many of the galacturonic acid residues are esterified at C-6 to form methyl esters. Theoretically the degree of esterification (DE) can range from 0-100% [7]. Pectins with a degree of esterification (DE) > 50% are classified as high methoxyl (HM) pectins and consequently low methoxyl (LM) pectins have a DE < 50% [7]. Low methoxyl pectins interact with calcium ions (or other divalent cations) to form a three dimensional gelled network. This network is usually described using the "egg-box" model [8]. Rhamnose residues are incorporated into the main chain at random intervals, which results in a kink in the otherwise linear chain [9]. Side chains of arabinans and galactans are also present, either randomly dispersed or in localised "hairy" regions [2]. Besides the primary structure [6,8] the conformation and flexibility of pectin molecules are important to the functional properties in the plant cell wall and also significantly affect their commercial use in the food and biomedical industries [2].

Pectins have been used as a gelling agent for a large number of years [2,10,11]; however, there has been recent interest in the use of pectin gels in controlled drug delivery ([10-12]. This is in part due their long standing reputation of being non-toxic [10,11] and their relatively low production costs [12]. It is proposed that pectin could be used to deliver drugs orally, nasally and topically [10,11,13], which are delivery routes generally well accepted by patients

[10,11,14]. As yet pectin has not fully realised its potential as drug delivery system, in part due to the necessity to control the quality of this natural product (variability) and potential instability. The stability (shelf-life) of pectin in terms of viscosity and gel strength is highly relevant to its commercial uses as these properties can play an important role in the function of pectin [2,10,11]. It is therefore fundamentally important to have the means available with which to measure the effects of and understand the relationships between storage conditions and stability.

In this paper we will look at the stability of pectin solutions, in terms of viscosity, across a range of different temperature conditions: 4ºC, 25ºC and 40ºC and the consequent effect on gel strength.

2. Materials and Methods

2.1 Pectins

Pectins with degrees of esterification (DE) of 21 % (P_{21}) and 19 % (P_{19}) were obtained from CP Kelco (Lille Skensved, Denmark) and were used without any further purification. Pectins (5 g) were dissolved in 0.1 M NaCl (500 mL) with stirring for 16 hours. As these two pectins have been standardised with approximately 50% sucrose the true pectin concentrations were 4.7 g/L and 4.9 g/L for P_{21} and P_{19}, respectively (as estimated from the areas under the ls-g(s*) curves from sedimentation velocity in the analytical ultracentrifuge (Morris, *et al*., 2008)[15]). Propyl-4-hydrobenzoate (0.2 g/L) and phenylethyl alcohol (5 mL/L) were added as preservatives to prevent microbial growth.

The stability of pectin solutions and resulting gels was determined by measuring the intrinsic viscosity, [η] and gel strength at different storage durations of up to 6 months at 4ºC, 25ºC or 40ºC.

2.2 Viscometry

The densities and viscosities of sample solutions and reference solvents were determined using an AMVn Automated Micro Viscometer and DMA 5000 Density Meter (both Anton Paar, Graz, Austria) under precise temperature control (20.00 ± 0.01) ºC. The relative, η_{rel} and specific viscosities, η_{sp} were calculated as follows:

$$\eta_{rel} = \left(\frac{\eta}{\eta_0} \right) \quad (1)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (2)$$

where η is the dynamic viscosity (*i.e.* corrected for density) of a pectin solution and η_o is the dynamic viscosity of buffer (1.013 mPas).

Measurements were made at a single concentration (4.7 g/L and 4.9 g/L for P_{21} and P_{19}, respectively) and intrinsic viscosities, [η], were estimated using the Solomon-Ciutâ approximation [16].

$$[\eta] \approx \frac{\left(2\eta_{sp} - 2\ln(\eta_{rel})\right)^{1/2}}{c} \quad (3)$$

2.3 Preparation of Gels

20 mL of pectin solution was added to 5 mL of calcium chloride solution with gentle mixing over 15 seconds. The beaker containing the gel was then covered with laboratory film and cured at laboratory temperature (~21 ± 1) ºC for 1 hour prior to analysis.

2.4 Gel Strength

Gel strengths were determined using a TA-XT2 (Stable Micro Systems Ltd., Godalming, U.K.) in compression mode. Gel strengths were estimated from the area under the curve (g.sec). Measurements were made in triplicate.

3. Results and Discussion

3.1 Intrinsic Viscosities and Gel Strengths of Pectins

The intrinsic viscosities of both pectins (**Tables 1** and **2**) after preparation (t = 0) are in good agreement with previous measurements [15] and are consistent with other studies on citrus pectins [17-22].

The pectins also formed gels of similar strength in terms of area under the force-time curve (AUC) (**Tables 1** and **2**); this is consistent with their solution properties [15].

3.2 Stability of Pectin Solutions and Gels

There was a discernible difference between pectin stability at the three storage conditions (**Tables 1** and **2**). At 4ºC both the intrinsic viscosity and gel strength remained essentially constant throughout the course of the study (6 months). Small decreases in these parameters were evident at 25°C whereas a more notable decrease in both intrinsic viscosity and gel strength was detected at 40ºC. The increase in pectin depolymerisation with increased temperature is consistent with the previous findings [7,21,23], although is generally accepted to be of greater consequence in high methoxyl (HM) pectin as a result of a greater susceptibility to β-elimination [7,21,23,24].

It is also clear that the strength of the pectin gel is directly related to the viscosity of pectin solution used in preparation (**Figure 1**).

3.3 Kinetics of Pectin Depolymerisation

If depolymerisation follows 1st order kinetics the degradation rate constant (k) can be calculated from the following equation [25].

$$\left(\frac{1}{M_{w,t}}\right)-\left(\frac{1}{M_{w,t=0}}\right)=\left(\frac{k}{m}\right)t \quad (4)$$

where $M_{w,t=0}$ and $M_{w,t}$ are the weight-average molar masses, t is time in days and m is the molar mass an average pectin monomer ≈ 180 g/mol [15, 26].

We can convert intrinsic viscosities in to molar mass by rearranging the following Mark-Houwink-Kuhn-Sakurada (MHKS) power law relationship [21].

$$[\eta] = 0.0174\, M_w^{0.84} \quad (5)$$

This enables the estimation of the 1st order rate constant (k) from intrinsic viscosity measurements **(Figures 2** and **3)**.

The data shown in **Table 3** indicates that neither of the two pectins had degraded after prolonged storage at 4°C, whilst for both samples the degradation rate constant (k) at 40°C is an order of magnitude greater than that at 25 °C.

4. Conclusions

The viscosity of pectin solutions decreased marginally, from 4.4 mPas to 4.0 mPas, after 6 months storage at 25ºC and more notably, from 4.4 mPas to 2.5 mPas, after 6 months storage at 40ºC; this is reflected by a decrease in gel strength upon addition of calcium ions. This is explained by a depolymerisation of pectin over time. The rate of depolymerisation is ~ 6 x 10^{-7}/day at 25ºC and an order of magnitude larger at 40 ºC (~7 x 10^{-6}/day).

It has been shown [27] that small decreases in viscosity do not significantly change the drug release rates from pectin gels *in vitro*; this agrees with work in our group [28] which indicates that a change in viscosity from 5 to 2 mPas will have no significant effect on the drug release from pectin gels. This is summarised in **Figure 4** where we can see that the times required to release 10%, 50% and 90% of a model drug (paracetamol) remain essentially constant with decreasing viscosity. These results also suggest that release time (especially 90% release) may be longer at viscosities higher than 7 mPas.

Pectin storage conditions and particularly temperature appear to have an influence on depolymerisation, particularly elevated storage temperatures, but whether or not this will be detrimental to its intended application will depend on the functional significance of the changes that occur. In this case we conclude that the decreases in viscosity and gel strength within the range observed have no detrimental effect on the drug release properties.

Acknowledgements

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Table 1 Solution viscosities, intrinsic viscosities, gel strengths and molecular weights for pectin of degree esterification 21% (P_{21}) stored at different temperatures (4ºC, 25ºC or 40ºC)

Storage Time (days)	**Storage Temperature (ºC)**	**Viscosity (mPas)**	**Intrinsic Viscosity (mL/g)**	**Weight-average Molar Mass (g/mol)**	**Gel Strength Area (g.sec)**
0	-	4.27 ± 0.01	401 ± 1	156000 ± 1000	1485 ± 25
30	4	4.38 ± 0.06	411 ± 6	160000 ± 3000	1445 ± 310
90	4	4.33 ± 0.01	406 ± 1	158000 ± 1000	1345 ± 260
180	4	4.36 ± 0.01	409 ± 1	159000 ± 1000	1395 ± 230
30	25	4.19 ± 0.07	394 ± 5	153000 ± 2000	1360 ± 105
90	25	4.02 ± 0.01	380 ± 1	146000 ± 1000	1025 ± 150
180	25	3.95 ± 0.01	374 ± 1	143000 ± 1000	1060 ± 140
30	40	3.64 ± 0.06	345 ± 5	130000 ± 2000	1210 ± 135
90	40	2.95 ± 0.01	277 ± 1	100000 ± 1000	930 ± 20
180	40	2.45 ± 0.01	220 ± 1	76000 ± 1000	855 ± 85

Table 2 Solution viscosities, intrinsic viscosities, gel strengths and molecular weights for pectin of degree esterification 19 % (P_{19}) stored at different temperatures (4ºC, 25ºC or 40ºC)

Storage Time (days)	**Storage Temperature (ºC)**	**Viscosity (mPas)**	**Intrinsic Viscosity (mL/g)**	**Weight-average Molar Mass (g/mol)**	**Gel Strength Area (g.sec)**
0	-	4.41 ± 0.01	396 ± 1	154000 ± 1000	1645 ± 105
30	4	4.52 ± 0.07	405 ± 6	158000 ± 3000	1305 ± 200
90	4	4.48 ± 0.01	402 ± 1	156000 ± 1000	1400 ± 235
180	4	4.45 ± 0.02	399 ± 2	155000 ± 1000	1320 ± 340
30	25	4.40 ± 0.05	395 ± 4	153000 ± 2000	1340 ± 20
90	25	4.24 ± 0.02	383 ± 1	147000 ± 1000	1340 ± 380
180	25	4.05 ± 0.02	367 ± 2	140000 ± 1000	1000 ± 265
30	40	3.75 ± 0.06	341 ± 5	128000 ± 2000	935 ± 25
90	40	3.12 ± 0.01	282 ± 1	103000 ± 1000	1035 ± 25
180	40	2.45 ± 0.01	212 ± 1	73000 ± 1000	860 ± 80

Table 3 Kinetic rate constants (day^{-1}) for pectins (P_{21} and P_{19}) at 4ºC, 25ºC and 40ºC from intrinsic viscosity determinations

Pectin	Storage Temperature (ºC)		
	4	**25**	**40**
P_{21}	$(-0.5 \pm 1.2) \times 10^{-8}$	$(6.5 \pm 0.6) \times 10^{-7}$	$(7.1 \pm 0.3) \times 10^{-6}$
P_{19}	$(-0.8 \pm 1.1) \times 10^{-7}$	$(5.7 \pm 1.1) \times 10^{-7}$	$(6.7 \pm 0.2) \times 10^{-6}$

Legends to Figures

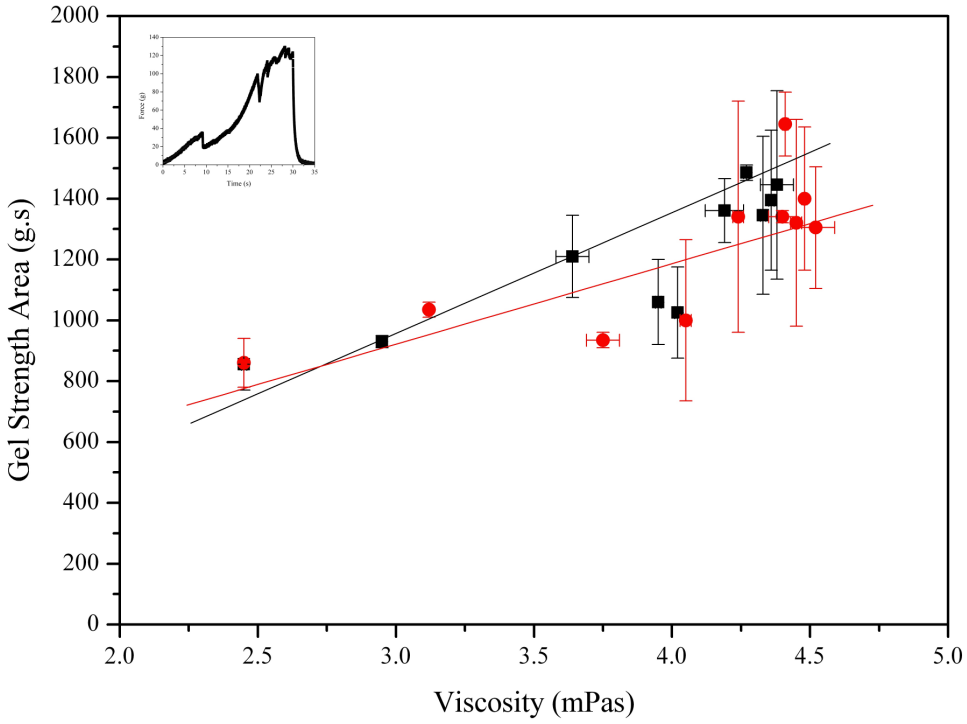

Figure 1 Relationship between gel strength in terms of area under the curve (AUC) for pectins P_{21} (■) and P_{19} (●). Inset: a typical time-force curve (P_{19}, 6 months at 25°C).

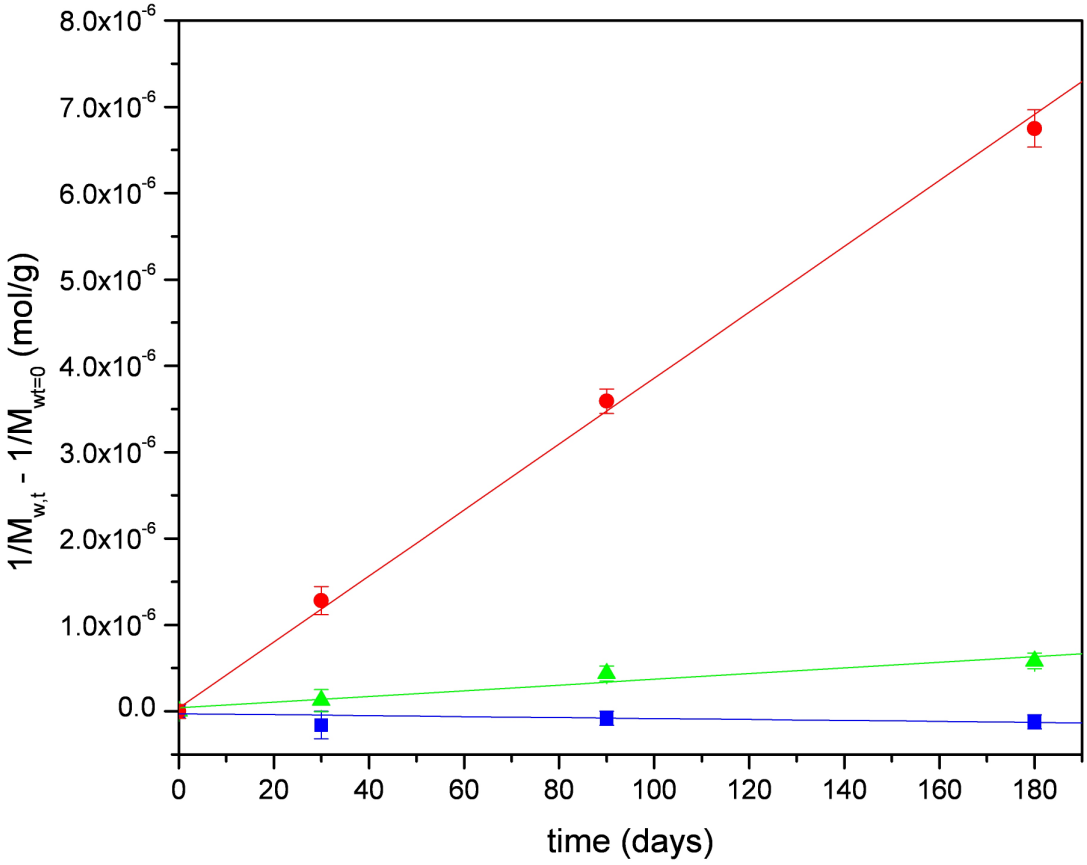

Figure 2 1st order kinetic plots of (mol/g) vs. time (days) for pectin P_{19}, where closed symbols represent molar masses estimated from viscometry at 4 °C (■), 25 °C (▲) and 40 °C (●).

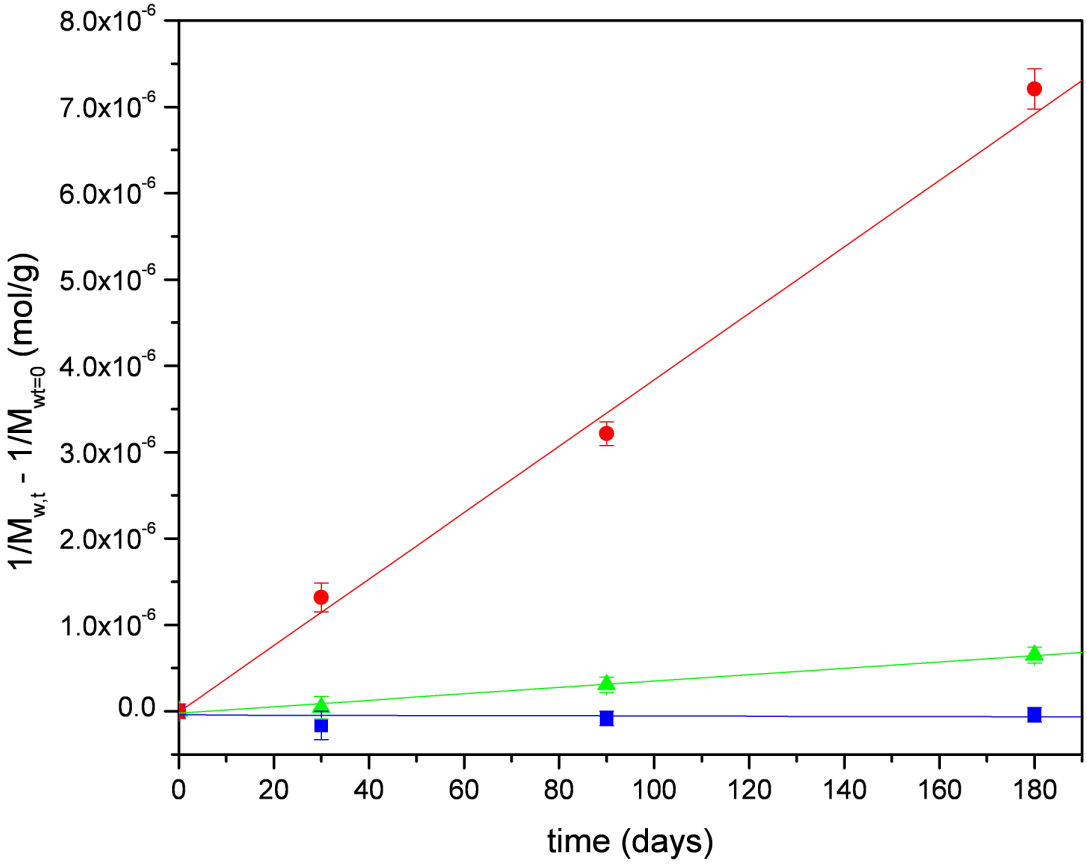

Figure 3 1st order kinetic plots of (mol/g) vs. time (days) for pectin P_{21}, where closed symbols represent molar masses estimated from viscometry at 4°C (■), 25°C (▲ and 40°C (●).

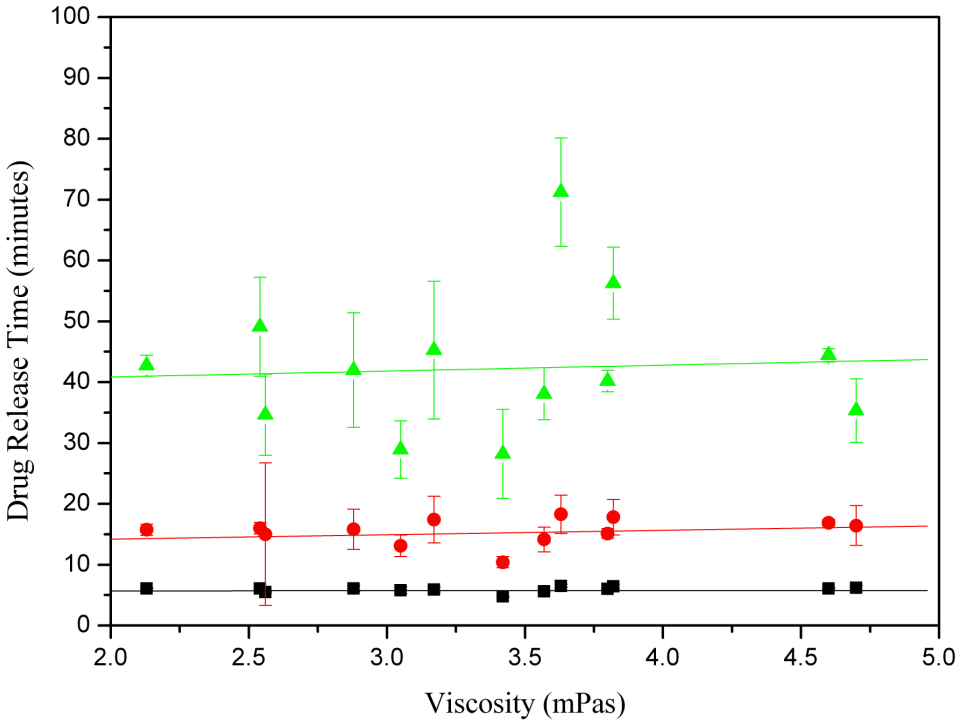

Figure 4 Effect of viscosity on model drug (paracetamol) release from pectin gel systems (adapted from Nessa, 2003). 10% drug release (■), 50% drug release (●) and 90% drug release (▲).