



# University of HUDDERSFIELD

## University of Huddersfield Repository

Morris, Gordon, Castile, Jonathan, Smith, Alan, Adams, Gary and Harding, Stephen E.

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

### Original Citation

Morris, Gordon, Castile, Jonathan, Smith, Alan, Adams, Gary and Harding, Stephen E. (2010) The effect of different storage temperatures on the physical properties of pectin solutions and gels. *Polymer Degradation and Stability*, 95 (12). pp. 2670-2673. ISSN 0141-3910

This version is available at <http://eprints.hud.ac.uk/14603/>

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

For more information, including our policy and submission procedure, please contact the Repository Team at: [E.mailbox@hud.ac.uk](mailto:E.mailbox@hud.ac.uk).

<http://eprints.hud.ac.uk/>

**Short Communication:**

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

Gordon A. Morris<sup>a,✉</sup>, Jonathan Castile<sup>b</sup>, Alan Smith<sup>b</sup>, Gary G. Adams<sup>a,c</sup> and Stephen E. Harding<sup>a</sup>

<sup>a</sup>Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, U.K.

<sup>b</sup>Archimedes Development Limited, Albert Einstein Centre, Nottingham Science and Technology Park, University Boulevard, Nottingham, NG7 2TN, U.K.

<sup>c</sup>Institute of Clinical Research, University of Nottingham, Faculty of Medicine and Health Science, Clifton Boulevard, Nottingham NG7 2RD, U.K.

✉Corresponding author

Tel: +44 (0) 115 9516149

Fax: +44 (0) 115 9516142

Email: [gordon.morris@nottingham.ac.uk](mailto:gordon.morris@nottingham.ac.uk)

## **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 investigated using a “rolling ball” viscometer and a texture analyser respectively. Both the intrinsic viscosity ( $[\eta]$ ) 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  $\alpha$  (1 $\rightarrow$ 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: 4°C, 25°C and 40°C and the consequent effect on gel strength.

## **2. Materials and Methods**

### *2.1 Pectins*

Pectins with degrees of esterification (DE) of 21 % (P<sub>21</sub>) and 19 % (P<sub>19</sub>) 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<sub>21</sub> and P<sub>19</sub>, 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,  $[\eta]$  and gel strength at different storage durations of up to 6 months at 4°C, 25°C or 40°C.

## 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 ( $20.00 \pm 0.01$ ) °C. The relative,  $\eta_{rel}$  and specific viscosities,  $\eta_{sp}$  were calculated as follows:

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

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

where  $\eta$  is the dynamic viscosity (*i.e.* corrected for density) of a pectin solution and  $\eta_0$  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<sub>21</sub> and P<sub>19</sub>, respectively) and intrinsic viscosities,  $[\eta]$ , were estimated using the Solomon-Ciutâ approximation [16].

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

### *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 ( $\sim 21 \pm 1$ ) °C 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 4°C 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 40°C. 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  $\beta$ -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 1<sup>st</sup> 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  $\approx 180$  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 1<sup>st</sup> 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 25°C and more notably, from 4.4 mPas to 2.5 mPas, after 6 months storage at 40°C; 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  $\sim 6 \times 10^{-7}$ /day at 25°C and an order of magnitude larger at 40 °C ( $\sim 7 \times 10^{-6}$ /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**

We thank the United Kingdom Biotechnology and Biological Sciences Research Council (BBSRC) for their financial support.

## **References**

[1] van Buren JP. Function of pectin in plant tissue structure and firmness. In: Walter RH, editor. *The Chemistry and Technology of Pectin* San Diego: Academic Press; 1991. p. 1.

[2] Tombs MP, Harding SE. *Polysaccharide biotechnology*. London: Taylor Francis; 1998.

[3] Mohnen D. Pectin structure and biosynthesis. *Curr Opin Plant Biol* 2008; 11: 266-77.

[4] Ridley BL, O'Neil MA, Mohnen D. Pectins: structure, biosynthesis and oligogalacturonide-related signalling. *Phytochemistry* 2001; 57 : 929-67.

[5] Willats WGT, McCartney L, Mackie W, Knox JP. Pectin: cell biology and prospects for functional analysis. *Plant Mol Biol* 2001; 47: 9-27.

[7] Pilgrim GW, Walter RH, Oakenfull DG. The chemistry of high-methoxyl pectins. In: Walter RH, editor. *The Chemistry and Technology of Pectin* San Diego: Academic Press; 1991. p. 24.

[8] Powel DA, Morris ER, Gidley MJ, and Rees DA. Conformations and Interactions of Pectins II. Influence of Residue Sequence on Chain Association in Calcium Pectate Gels. *J Mol Biol* 1982; 153: 517-31.

[9] Axelos MAV Branger M. The effect of the degree of esterification on the thermal-stability and chain conformation of pectins. *Food Hydrocolloid* 1993; 7: 91-102.

[10] Lui L, Fishman ML, Hicks KB. Pectin in controlled drug delivery – a review. *Cellulose* 2007; 14: 15-24.

[11] Lui L, Fishman M L, Kost J, Hicks KB. Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials* 2003; 24: 3333-43.

[12] Sunghongjeen S, Sriamornsak P, Pitaksuteepong T, Somsiri A, Puttipipatkachorn S. Effect of degree of esterification of pectin and calcium amount on drug release from pectin-based matrix tablets. *AAPS PharmSciTech* 2004; 5: 1-9.

[13] Thiruwong N, Kennedy RA, Sriamornsak P. Viscometric study of pectin–mucin interaction and its mucoadhesive bond strength. *Carbohydr Polym* 2007; 71: 170-9.

[14] Yadav N, Morris GA, Harding SE, Ang S, Adams GG. Various non-injectable delivery systems for the treatment of diabetes mellitus. *Endocrine, Metabolic & Immune Disorders - Drug Targets* 2009; 9: 1-13.

[15] Morris GA, García de la Torre J, Ortega A, Castille J, Smith A, Harding SE. Molecular flexibility of citrus pectins by combined sedimentation and viscosity analysis. *Food Hydrocolloid* 2008; 22: 1435-42.

[16] Solomon O F, Ciutâ IZ. Détermination de la viscosité intrinsèque de solutions de polymères par une simple détermination de la viscosité. *J Appl Polym Sci* 1962; 24, 683-6.

[17] Harding SE, Berth G, Ball A, Mitchell JR, García de la Torre J. The molecular weight distribution and conformation of citrus pectins in solution studied by hydrodynamics. *Carbohydr Polym* 1991; 168: 1-15.

[18] Cros SC, Garnier C, Axelos MAV, Imbery A, Perez, S. Solution conformations of pectin polysaccharides: determination of chain characteristics by small angle neutron scattering, viscometry and molecular modeling. *Biopolymers* 1996; 39: 339-52.

[19] Morris GA, Foster TJ, Harding SE. The effect of degree of esterification on the hydrodynamic properties of citrus pectin. *Food Hydrocolloid* 2000; 14: 227-35.

[20] Ralet M-C, Bonnin E, Thibault, J-F. Chromatographic study of highly methoxylated lime pectins de-esterified by different pectin methyl-esterases. *J Chromatogr B* 2001; 753: 157-66.

[21] Morris GA, Foster TJ, Harding SE. A hydrodynamic study of the depolymerisation of a high methoxy pectin at elevated temperatures. *Carbohydr Polym* 2002; 48: 361-7.

[22] Yoo S-H, Fishman ML, Hotchkiss AT, Lee HG. Viscometric behavior of high-methoxy and low-methoxy pectin solutions. *Food Hydrocolloid* 2006; 20: 62-7.

[23] Axelos MAV, Thibault JF. The chemistry of low-methoxyl pectin gelation. In: Walter RH, editor. *The Chemistry and Technology of Pectin* San Diego: Academic Press; 1991. p. 109.

[24] Morris GA, Butler SNG, Foster TJ, Jumel K, Harding SE. Elevated temperature analytical ultracentrifugation of low-methoxy polyuronide. *Prog Coll Pol Sci* 1999; 113, 205-8.

[25] Zhou G, Yao W, Wang C. Kinetics of microwave degradation of  $\lambda$ -carrageenan from *Chondrus ocellatus*. *Carbohydr Polym* 2006; 64: 73-7.

[26] Norziah MH, Fang EO, Abd Karim A. Extraction and characterisation of pectin from pomelo peels. In: Williams, PA, Philips GO, editors. *Gums and stabilisers for the food industry*, Vol. 10. Cambridge: Royal Society of Chemistry. p. 27.

[27] Chelladurai S, Mishra M, Mishra B. Design and evaluation of bioadhesive *in-situ* nasal gel of ketorolac tromethamine. *Chem Pharm Bull* 2008; 56: 1596-9.

[28] Nessa MU. Physiochemical characterisation of pectin solution as a vehicle for nasal drug delivery. MSc Dissertation, University of Nottingham, U.K; 2003.

**Table 1** Solution viscosities, intrinsic viscosities, gel strengths and molecular weights for pectin of degree esterification 21% (P<sub>21</sub>) stored at different temperatures (4°C, 25°C or 40°C)

| <b>Storage Time (days)</b> | <b>Storage Temperature (°C)</b> | <b>Viscosity (mPas)</b> | <b>Intrinsic Viscosity (mL/g)</b> | <b>Weight-average Molar Mass (g/mol)</b> | <b>Gel Strength Area (g.sec)</b> |
|----------------------------|---------------------------------|-------------------------|-----------------------------------|--|----------------------------------|
| 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<sub>19</sub>) stored at different temperatures (4°C, 25°C or 40°C)

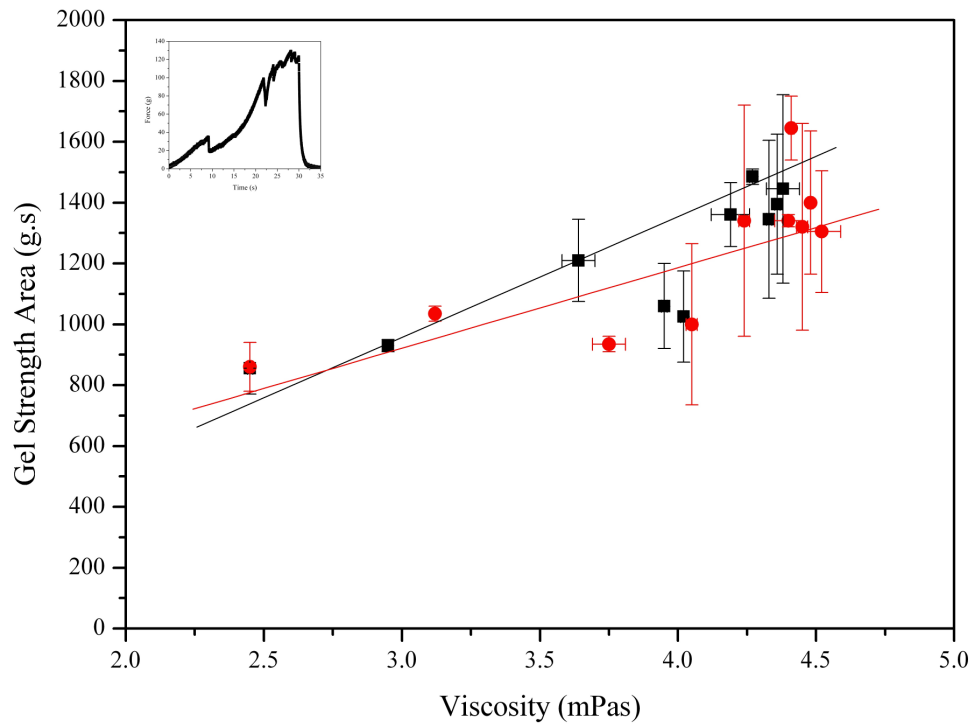
| <b>Storage Time (days)</b> | <b>Storage Temperature (°C)</b> | <b>Viscosity (mPas)</b> | <b>Intrinsic Viscosity (mL/g)</b> | <b>Weight-average Molar Mass (g/mol)</b> | <b>Gel Strength Area (g.sec)</b> |
|----------------------------|---------------------------------|-------------------------|-----------------------------------|--|----------------------------------|
| 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 ( $\text{day}^{-1}$ ) for pectins (P<sub>21</sub> and P<sub>19</sub>) at 4°C, 25°C and 40°C from intrinsic viscosity determinations

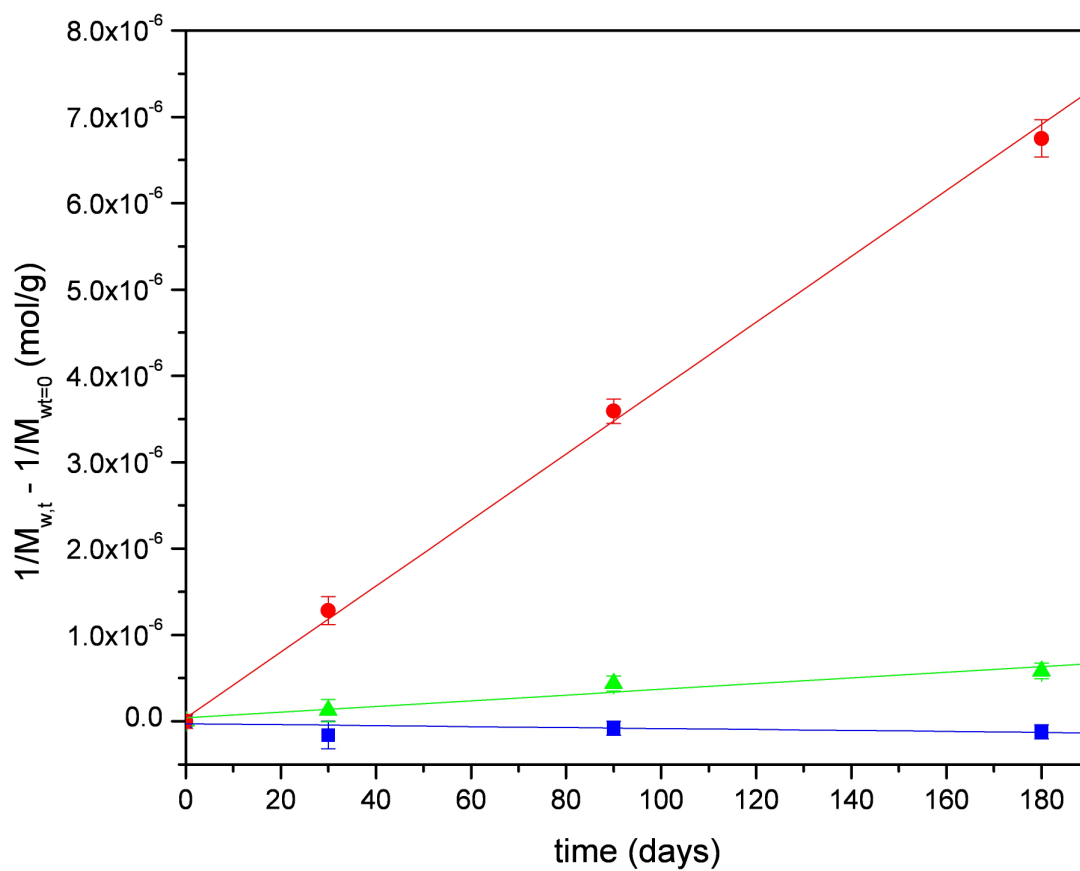
| <b>Pectin</b>         | <b>Storage Temperature (°C)</b> |                                |                                |
|-----------------------|---------------------------------|--------------------------------|--------------------------------|
|                       | <b>4</b>                        | <b>25</b>                      | <b>40</b>                      |
| <b>P<sub>21</sub></b> | $(-0.5 \pm 1.2) \times 10^{-8}$ | $(6.5 \pm 0.6) \times 10^{-7}$ | $(7.1 \pm 0.3) \times 10^{-6}$ |
| <b>P<sub>19</sub></b> | $(-0.8 \pm 1.1) \times 10^{-7}$ | $(5.7 \pm 1.1) \times 10^{-7}$ | $(6.7 \pm 0.2) \times 10^{-6}$ |

## Legends to Figures

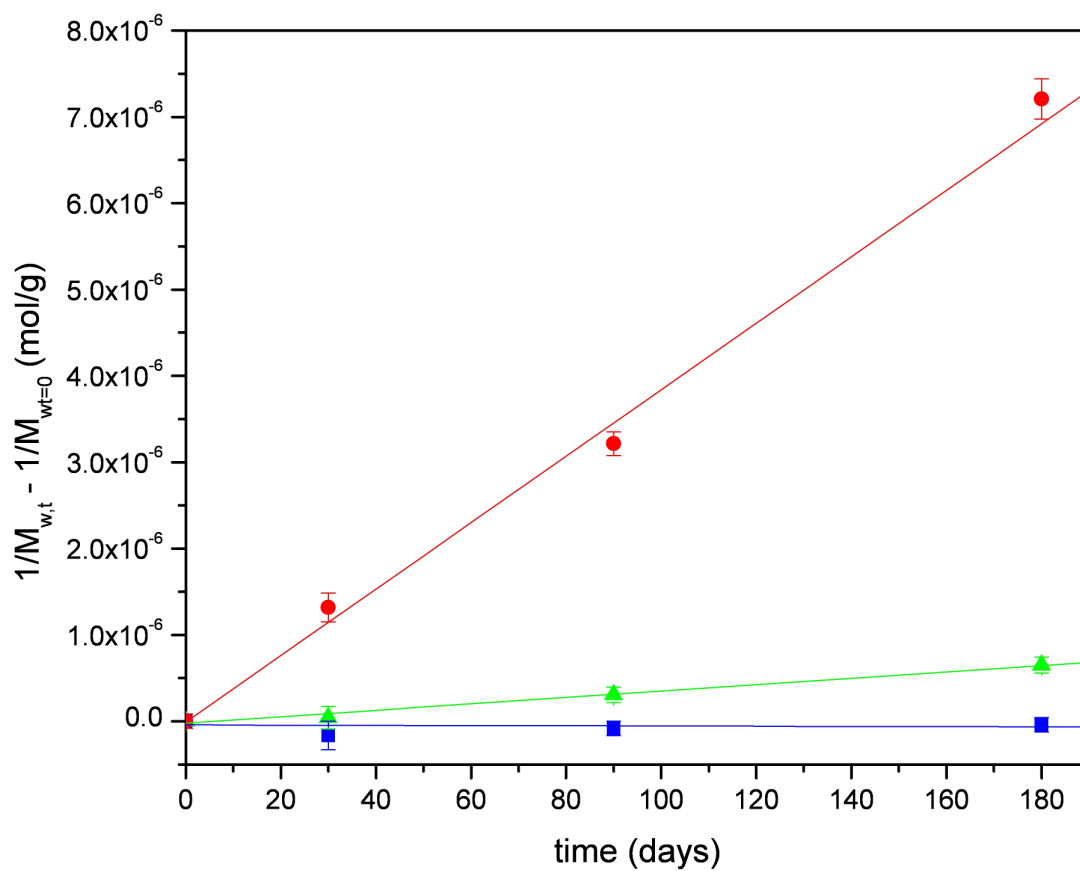


**Figure 1** Relationship between gel strength in terms of area under the curve (AUC) for pectins

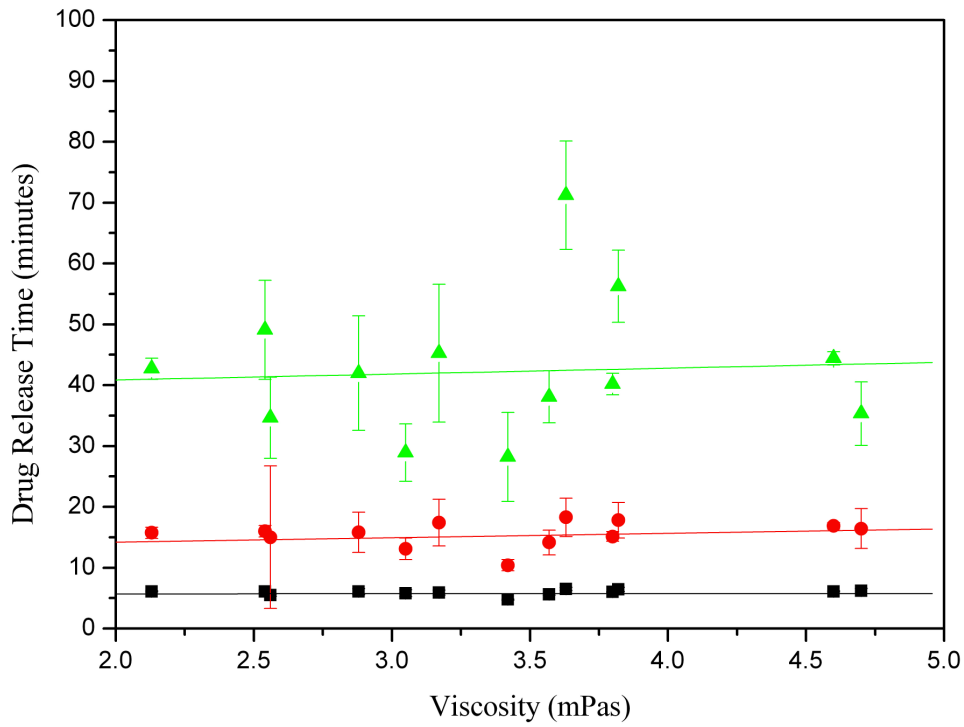
P<sub>21</sub> (■) and P<sub>19</sub> (●). Inset: a typical time-force curve (P<sub>19</sub>, 6 months at 25°C).



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



**Figure 3** 1<sup>st</sup> order kinetic plots of (mol/g) vs. time (days) for pectin P<sub>21</sub>, 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 (▲).