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**Short Communication:**

Global hydrodynamic analysis of the molecular  
flexibility of galactomannans

Gordon A. Morris<sup>a\*</sup>, Trushar R. Patel<sup>a</sup>, David R. Picout<sup>b</sup>, Simon B. Ross-Murphy<sup>b</sup>,  
Alvaro Ortega<sup>c</sup>, Jose Garcia de la Torre<sup>c</sup> and Stephen E. Harding<sup>a</sup>

<sup>a</sup>National Centre for Macromolecular Hydrodynamics, School of Biosciences,  
University of Nottingham, Sutton Bonington, LE12 5RD, U.K.

<sup>b</sup>King's College London, Franklin-Wilkins Building, 150 Stamford Street, Waterloo,  
London, SE1 9NH, U.K.

<sup>c</sup>Departamento de Química Física, Facultad de Química, Universidad de Murcia,  
30071 Murcia, Spain.

\*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**

In the past, intrinsic viscosity and sedimentation velocity analyses have been used separately to assess the conformation and flexibility of guar and locust bean gum galactomannans based on worm-like chain and semi-flexible coil models. Publication of a new global method combining data sets of both intrinsic viscosity and sedimentation coefficient with molecular weight, and minimising a target (error) function now permits a more robust analysis. Using this approach, values for the persistence length of  $(10 \pm 2)$  nm for guar and  $(7 \pm 1)$  nm for locust bean gum are returned if the mass per unit length  $M_L$  is floated as a variable. Using a fixed mass per unit length based on the known compositional data of each galactomannan yields a similar value for  $L_p$  in both cases,  $(8 \pm 1)$  nm for guar and  $(9 \pm 1)$  nm for locust bean gum, with combined set of data yielding  $(9 \pm 1)$  nm: within experimental error the flexibilities of both galactomannans are very similar.

**Keywords:** galactomannan; guar; LBG; worm-like chain; semi-flexible coil; persistence length; mass per unit length

## Introduction

Recent studies have demonstrated the usefulness of the so-called pressure cell method for the solubilisation of galactomannans and subsequent analysis of molecular conformation and flexibility (guar gum and locust bean gum (LBG)) (Picout, Ross-Murphy, Errington & Harding, 2001; Picout, Ross-Murphy, Jumel & Harding, 2002; Patel, Picout, Ross-Murphy & Harding, 2006). It was found that intrinsic viscosity,  $[\eta]$ ; sedimentation coefficient,  $s_{20,w}^{\circ}$ ; z-average radius of gyration,  $r_{g,z}$  and weight average molar mass,  $M_w$  all decrease with increased temperature and heating times, although at temperatures  $> 100^{\circ}\text{C}$  the concomitant application of pressure appears to have a protective effect on polysaccharide chain degradation. Data sets of intrinsic viscosity versus molecular weight, radius of gyration versus molecular weight or sedimentation coefficient molecular weight then permit not only simple estimates of chain conformation type (sphere rod, coil etc) from power law or “Mark-Houwink-Kuhn-Sakurada” types of analysis but also estimates of the flexibility via the chain persistence length  $L_p$  from more sophisticated representations such as the Burchard-Stockmayer-Fixman (BSF) (Stockmayer & Fixman, 1963), Hearst (1963) and Bohdanecky (1983) equations based on data-sets of  $[\eta]$  versus  $M_w$  and the Hearst-Stockmayer (1962) relation of  $s_{20,w}^{\circ}$  versus  $M_w$ , later refined by Yamakawa and Fujii (1973).

*Table 1* gives a comparison of some of the values for  $L_p$  returned. The way these approaches are implemented can lead to significant variability in the results, i.e. contrary to expectation,  $L_p$  is model dependent (Bohdanecky & Petrus, 1991; Ortega & García de la Torre, 2007). This is ably demonstrated by the different persistence lengths calculated by the BSF and Hearst approaches, yielding  $\sim 3$  nm and  $\sim 9$  nm, respectively for these galactomannans (Picout et al., 2002).

<Table 1 near here>

In response to this problem Ortega and García de la Torre (2007) have developed a new procedure which forms the basis of the software package, Multi-HYDFIT. This program gives a combined or “global” estimate of the wormlike chain parameters:  $L_p$  (persistence length) and also the  $M_L$  (mass per unit length) and  $d$  (the chain diameter).

It therefore provides objective criteria for whether, or not, a particular data set of experimental data ( $[\eta]$  versus  $M_w$  for example) is consistent with the other data. We have therefore decided to interpret further our previous data (Picout et al., 2001; Picout et al., 2002; Patel et al., 2006) in the light of this new approach.

### Combined (Global) Analysis Method (HYDFIT)

The linear flexibility of polymer chains are represented in terms of the persistence length,  $L_p$  of equivalent *worm-like chains* (Kratky & Porod, 1949) where the persistence length is defined as the average projection length along the initial direction of the polymer chain and for a theoretical perfect random coil  $L_p = 0$  and for the equivalent extra-rigid rod (see for example Harding, 1997)  $L_p = \infty$ , although in practice limits of  $\sim 1$  nm for random coils (*e.g.* pullulan) and 200 nm for a extra-rigid rod (*e.g.* schizophyllan) are more appropriate (see for example Tombs & Harding, 1998). Chain persistence lengths,  $L_p$  can be estimated using several different approaches using either intrinsic viscosity (Bohdanecky, 1983; Stockmayer & Fixman, 1963; Hearst, 1963) or sedimentation coefficient (Yamakawa & Fujii, 1973; Hearst & Stockmayer, 1962) measurements. For example the Bohdanecky relation (Bohdanecky, 1983):

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2} \quad (1)$$

where  $\Phi$  is the Flory-Fox constant ( $2.86 \times 10^{23} \text{ mol}^{-1}$ ) and  $A_0$  and  $B_0$  are tabulated coefficients (Bohdanecky, 1983)

and the Yamakawa & Fujii (1973) form of the Hearst-Stockmayer (1962) equation:

$$s^0 = \frac{M_L(1-\bar{v}\rho_0)}{3\pi\eta_0 N_A} \times \left[ 1.843 \left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3 \left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \dots \right] \quad (2)$$

Yamakawa and Fujii (1973) showed that  $A_2$  can be considered as  $-\ln(d/2L_p)$  and  $A_3 = 0.1382$  if the  $L_p$  is much higher than the chain diameter,  $d$ . Difficulties arise if the

mass per unit length is not known, although both relations have now been built into an algorithm Multi-HYDFIT (Ortega & García de la Torre, 2007) which estimates the best values (or best range of values) of  $L_p$  and  $M_L$  based on minimisation of a target (error) function,  $\Delta$ . The chain diameter  $d$  can also be floated as a variable but extensive simulations (Ortega & Garcia de la Torre, 2007) have shown that the results returned for  $L_p$  are relatively insensitive to the value chosen for  $d$  (taken here as 1.0 nm for each galactomannan). As before the assumption is made that hydrodynamic interaction between chain elements is so strong as to exclude intramolecular draining effects (Tanford, 1961).

We considered two possible cases:

1. the chain diameter,  $d$  was fixed at 1.0 nm for each galactomannan and the mass per unit length,  $M_L$  was fixed at  $490 \text{ g mol}^{-1} \text{ nm}^{-1}$ ,  $410 \text{ g mol}^{-1} \text{ nm}^{-1}$  and  $450 \text{ g mol}^{-1} \text{ nm}^{-1}$  for guar gum, locust bean gum and combined data sets for both galactomannans (Picout et al., 2002).
2. only the chain diameter,  $d$  was fixed at 1.0 nm.

The Multi-HYDFIT program then “floats” the variable parameters ( $L_p$  in case 1;  $L_p$  and  $M_L$  in case 2) in order to find a minimum of the multi-sample target (error) function,  $\Delta$  (Ortega & García de la Torre, 2007).

In this procedure as defined by Ortega & Garcia de la Torre (2007)  $\Delta$  is calculated using equivalent radii (or the ratio of equivalent radii), where an equivalent radius is defined as the radius of an equivalent sphere having the same value as the determined property. These ‘determined properties’ include the translational frictional coefficient,  $f$  (calculated from either the diffusion or sedimentation coefficients); intrinsic viscosity,  $[\eta]$ ; radius of gyration,  $r_{g,z}$  or the rotation relaxation time,  $\tau$ . In the present study we are interested in the equivalent radii resulting from the sedimentation coefficient *i.e.* translational frictional coefficient and from the intrinsic viscosity.

$$a_T = \frac{f}{6\pi\eta_0} \quad (3)$$

where  $\eta_0$  is the viscosity of water at 20.0 °C, and

$$a_I = \left( \frac{3[\eta]M_w}{10\pi N_A} \right)^{1/3} \quad (4)$$

where  $N_A$  is Avogadro's number.

The target function,  $\Delta$  can be evaluated from this relation:

$$\Delta^2 = \frac{1}{N_s} \sum_{i=1}^{N_s} \left[ \left( \sum_T W_T \right)^{-1} \sum_T W_T \left( \frac{a_{T(cal)} - a_{T(exp)}}{a_{T(exp)}} \right)^2 \right] \quad (5)$$

and this one

$$\Delta^2 = \frac{1}{N_s} \sum_{i=1}^{N_s} \left[ \left( \sum_I W_I \right)^{-1} \sum_I W_I \left( \frac{a_{I(cal)} - a_{I(exp)}}{a_{I(exp)}} \right)^2 \right] \quad (6)$$

where  $N_s$  is the number of samples in multi-sample analysis,  $W_T$  and  $W_I$  are the statistical weights for equivalent radii  $a_T$  and  $a_I$  (from translation frictional coefficient and intrinsic viscosity data respectively) and the subscripts cal and exp represent values from calculated and experimental values respectively.  $\Delta$  is thus a dimensionless estimate of the agreement between the theoretical calculated values for the translational frictional coefficient (consequently the sedimentation coefficient) and the intrinsic viscosity for a particular persistence length,  $L_p$  (and mass per unit length,  $M_L$  in case 2) and the experimentally measured parameters (Ortega & Garcia de la Torre, 2007).

In each case we have treated guar and locust bean gum separately and in combination.

## Results

Fixed diameter,  $d$  and mass per unit length,  $M_L$

The best estimates for the persistence lengths of galactomannans are the minima of the target (error) function,  $\Delta$  – y-axis in *Figure 1*. We can see that the persistence lengths (*Table 2*) of  $\sim 8 - 9$  nm which is in good agreement with the results from the Hearst, Bohdanecky and Yamakawa-Fujii approaches in our previous analysis (Picout et al., 2001; 2002; Patel et al., 2006) and with computer modelled data for hypothetical galactomannan molecules of  $\sim 8 - 12$  nm (Petkowitz, Reicher & Mazeau, 1998).

<Table 2 and Fig 1 near here>

Fixed diameter,  $d$

The best estimates for the persistence lengths and mass per unit lengths for galactomannans are represented on the contour plots shown in *Figure 2a - c*. The values of the target (error) function,  $\Delta$  are represented by the full colour spectrum ranging from blue ( $\Delta = 0.13$ ) to red ( $\Delta = 1.00$ ). We can see that persistence lengths (*Table 3*) are in the range  $7 - 10$  nm and in good agreement with those calculated when the mass per unit length was fixed and with the results from the Hearst, Bohdanecky and Yamakawa-Fujii approaches in our previous analyses (Picout et al., 2001; 2002; Patel et al., 2006) and with the computer modelled data of Petkowitz et al. (1998).

<Table 3 and Figs 2a-c near here>

The calculated values from the mass per unit length differ from the predicted values (Picout et al., 2002) by  $\sim 10 - 20$  %. It should however be noted that all values of target (error) function,  $\Delta$  within each contour in the combined analysis plots vary by  $\sim 5$  % *i.e.* less than the error in the original experimental data; clearly the results for the case of fixed mass per unit length fit well within these minima.



## Discussion

Whilst giving extra confidence in the values for the persistence length obtained the global method has not given results too different from the intrinsic viscosity and sedimentation coefficient data that had previously been used independently (Picout et al., 2001; 2002; Patel et al, 2006). The combined analysis method Multi-HYDFIT (Ortega & García de la Torre, 2007) has been shown to give unbiased estimates for both, the persistence length,  $L_p$  and mass unit length,  $M_L$ .

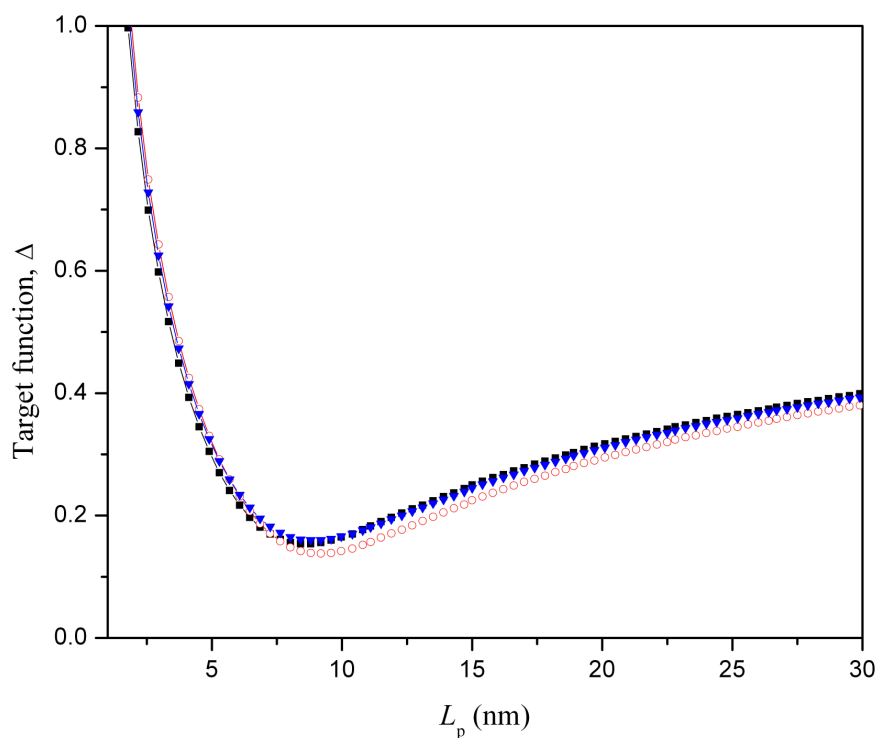
It may have been expected that the stiffness of the wormlike galactomannan chains would increase with decreasing galactose: mannose (G:M) ratio *i.e.* locust bean gum (G:M ~ 1:4) being the more stiff and guar gum (G:M ~ 1:2) being the less stiff, however as previously (Picout et al., 2002) this was not observed. This is most likely due to the reported block-wise (*i.e.* hairy and smooth regions) distribution of galactopyranosyl residues along the mannopyranan polymer chain. This is particularly reported for the case of locust bean gum (Clark, Dea & McCleary, 1985; Dea, 1990; Tombs & Harding, 1998).

## References

- Bohdanecky, M. (1983). New method for estimating the parameters of the wormlike chain model from the intrinsic viscosity of stiff-chain polymers. *Macromolecules*, 16, 1483-1492.
- Bohdanecky, M., & Petrus, V. (1991). Analysis of hydrodynamic data for denatured globular proteins in terms of the worm like cylinder model. *International Journal of Biological Macromolecules*, 13, 231-234.
- Clark, A. H., Dea, I. C. M., & McCleary, B. V. (1985). *The Effect of the Galactomannan Fine Structure on Their Interaction Properties 3<sup>rd</sup> Edition* (pp 429-465). London: Elsevier Applied Science Publishers.
- Dea, I. C. M. (1990). Structure/Function Relationships of Galactomannans and Food Grade Cellulosics. In G. O. Phillips, D. J. Wedlock and P. A. Williams (Eds.). *Gums and Stabilisers for the Food Industry Volume 5* (pp 373-382). Oxford: IRL Press.
- Harding, S. E. (1997). The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution. *Progress in Biophysics and Molecular Biology*, 68, 207-262.
- Hearst, J. E. (1963). Rotatory diffusion constants of stiff-chain macromolecules. *Journal of Chemical Physics*, 38, 1062-1065.
- Hearst, J. E., & Stockmayer, W. H. (1962). Sedimentation constants of broken chains and wormlike coils. *Journal of Chemical Physics*, 37, 1425-1433.
- Kratky, O., & Porod, G. (1949). Röntgenuntersuchung gelöster Fadenmoleküle. *Recueil Des Travaux Chimiques Des Pays-Bas*, 68, 1106-1109.
- Ortega, A., & García de la Torre, J. Equivalent radii and ratios of radii from solution properties as indicators of macromolecular conformation, shape and flexibility (2007) *Biomacromolecules* (accepted) DOI: [10.1021/bm700473f](https://doi.org/10.1021/bm700473f) available on-line 27/07/2007. See also Ortega, A. (2005). *Metodologías computacionales para propiedades en disolución de macromoléculas rígidas y flexibles*. PhD Dissertation, Universidad de Murcia.

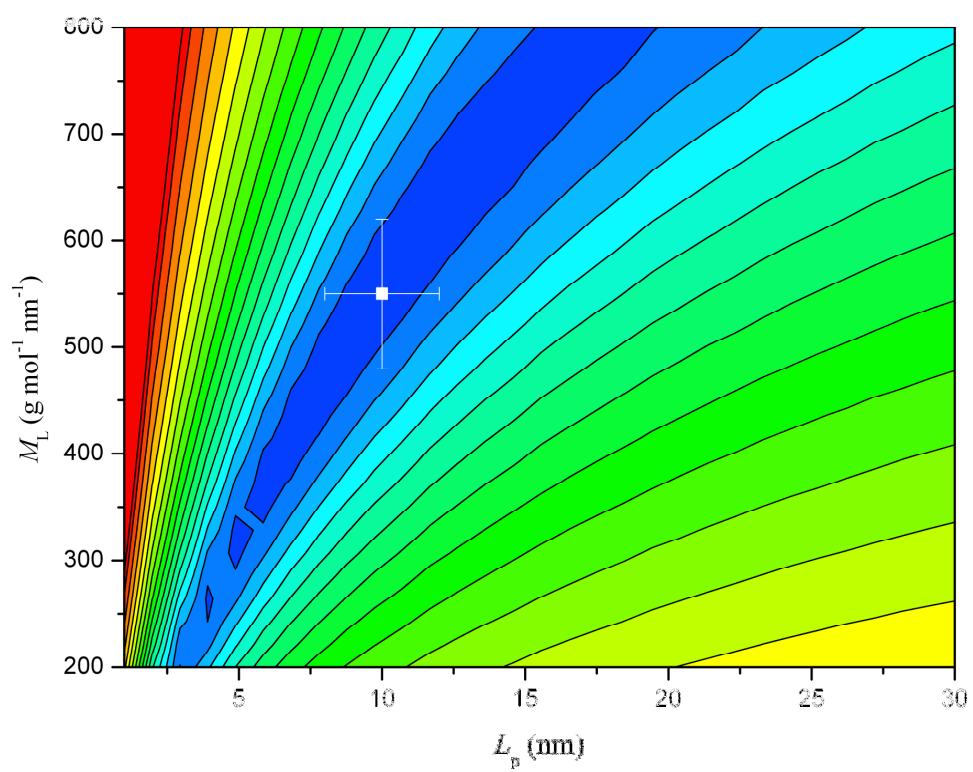
- Patel, T. R., Picout, D. R., Ross-Murphy, S. B., & Harding, S. E. (2006). Pressure cell assisted solution characterisation of polysaccharides. 3. Application of analytical ultracentrifugation techniques. *Biomacromolecules*, 7, 3513-3520.
- Petkowitz, C. L. O., Reicher, F., & Mazeau, K. (1998). Conformation analysis of galactomannans: from oligomeric segments to polymeric chains. *Carbohydrate Polymers*, 37, 25-39.
- Picout, D. R., Ross-Murphy, S. B., Errington, N., & Harding, S. E. (2001). Pressure cell assisted solution characterisation of polysaccharides. 1. Guar gum. *Biomacromolecules*, 2, 1301-1309.
- Picout, D. R., Ross-Murphy, S. B., Jumel, K., & Harding, S. E. (2002). Pressure cell assisted solution characterisation of polysaccharides. 2. Locust bean gum. *Biomacromolecules*, 3, 761-767.
- Stockmayer, W. H., & Fixman, M. J. (1963). On the estimation of unperturbed dimensions from intrinsic viscosities. *Journal of Polymer Science C*, 1, 137-141.
- Tanford, C. (1961). *Physical Chemistry of Macromolecules* (pp. 343-344). New York: J. Wiley & Sons.
- Tombs M. P., & Harding, S. E. (1998). *An Introduction to Polysaccharide Biotechnology* (pp. 14-20). London: Taylor and Francis.
- Yamakawa, H., & Fujii, M. (1973). Translational friction coefficient of wormlike chains. *Macromolecules*, 6, 407-415.

## Legends to Figures

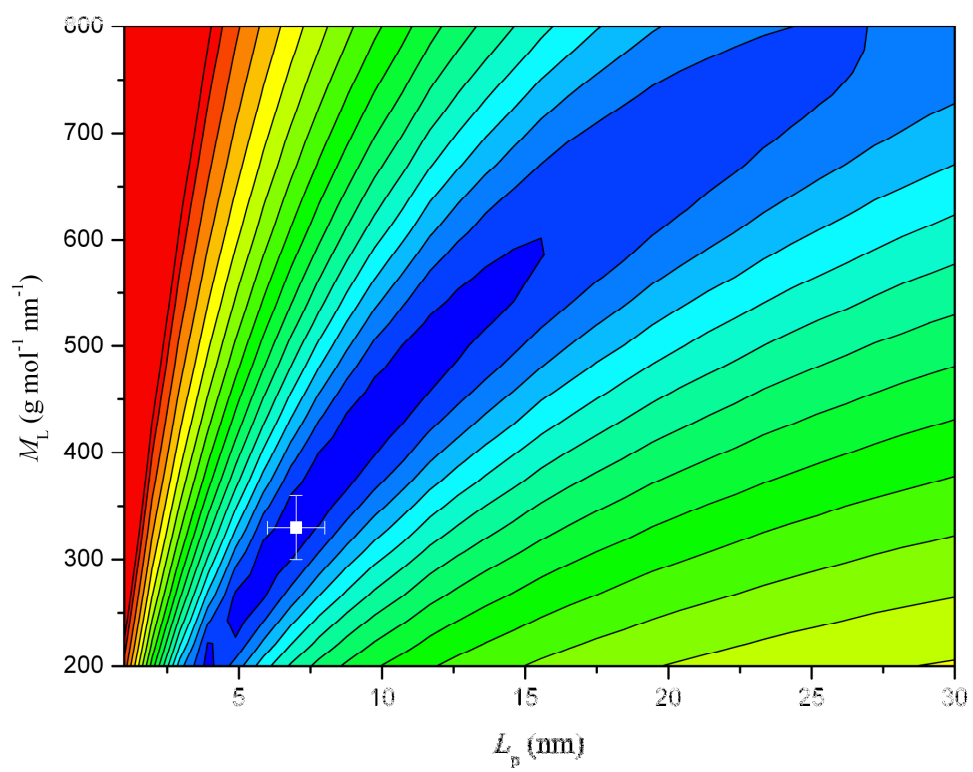


**Figure 1** - Plot of the target function ( $\Delta$ ) vs. persistence length ( $L_p$ ). Guar gum (-■-); locust bean gum (-○-) and guar and locust bean gum (-▼-).

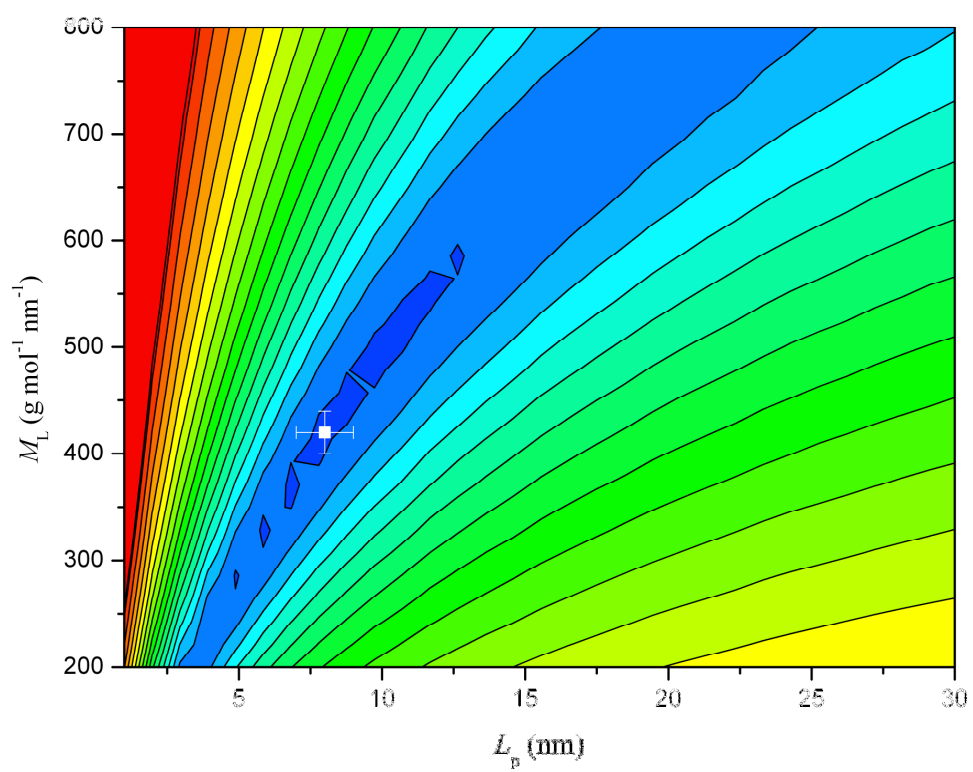
**Figure 2** - The x-axis and y-axis represents  $L_p$  (nm) and  $M_L$  ( $\text{g mol}^{-1} \text{ nm}^{-1}$ ) respectively. The target function  $\Delta$  is calculated over a range of values for  $M_L$  and  $L_p$ . In these representations, the values of  $\Delta$  function are represented by the full colour spectrum, from blue ( $\Delta = 0.13$ ) to red ( $\Delta = 1.00$ ). The minimum value (indicated for clarity) of  $\Delta$  corresponds to the best estimate for  $M_L$  and  $L_p$ . Contour plots from combined sedimentation and viscosity analyses for:



(a) guar galactomannan



(b) locust bean gum



(c) guar and locust bean gum