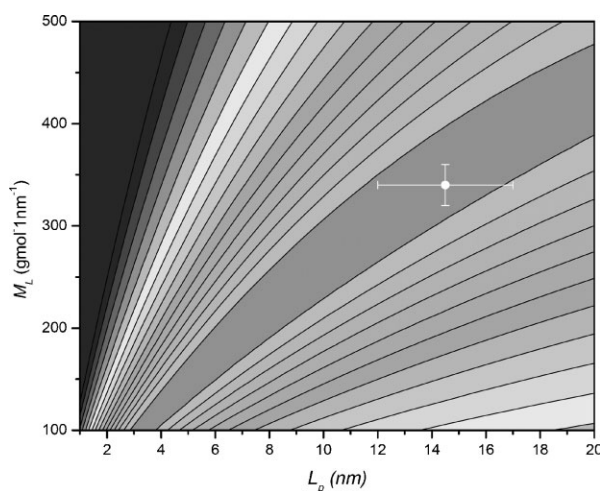# Molecular Flexibility of Methylcelluloses of Differing Degree of Substitution by Combined Sedimentation and Viscosity Analysis

Trushar R. Patel, Gordon A. Morris, Jose Garcia de la Torre, Alvaro Ortega, Petra Mischnick, Stephen E. Harding*

The flexibility/rigidity of methylcelluloses (MCs) plays an important part in their structure–function relationship and therefore on their commercial applications in the food and biomedical industries. In the present study, two MCs of low degree of substitution (DS) 1.09 and 1.32 and four of high DS (1.80, 1.86, 1.88 and 1.93) were characterised in distilled water in terms of intrinsic viscosity $[\eta]$; sedimentation coefficient $(s^0_{20,w})$ and weight average molar mass $(\overline{M}_w)$. Solution conformation and flexibility were estimated qualitatively using conformation zoning and quantitatively (persistence length L_p) using the new combined global method. Sedimentation conformation zoning showed an extended coil (Type C) conformation and the global method applied to each MC sample yielded persistence lengths all within the range $L_p = 12$–17 nm (for a fixed mass per unit length) with no evidence of any significant change in flexibility with DS.

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Introduction

Cellulose is a $(1 \rightarrow 4)$ linked β-D-glucan of primary structure $\ldots \rightarrow 4)$ β-D-Glcp-$(1 \rightarrow \ldots$ The presence of covalent bonds both inside and between glucose units was discovered by Haworth[1] and further early insights into the macromolecular properties of cellulose were given by Staudinger.[2] Early progress in our understanding of its properties was also made from the Svedberg Laboratory by Gralén[3] and Peterlin.[4] A production rate of around 1.5×10^{12} t per year makes cellulose the most abundant natural polymer on earth.[5] It is insoluble in water and other organic solvents because of the presence of the intramolecular hydrogen bond between the −OH group at position 3 and

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the ring oxygen atom of the neighbouring glucose unit as well as the hydrogen bond between −OH group present at sixth carbon and the oxygen of the glycosidic linkages.[6] Approximately 35% of purified commercial cellulose is converted to cellulose esters (25%) or ethers (10%).[7] Methylcellulose (MC) is a methylether derivative of cellulose where a portion of the hydroxyl groups −OH is substituted by −OCH$_3$. There are two types of MCs; (i) 'high' with degree of substitution (DS) from 1.4 to 2.0 which are water soluble and (ii) 'low' with DS from about 0.25 to 1.0 which are soluble in alkali.[8] The DS of a given form of MC is defined as the average number of substituted hydroxyl groups per glucose residue. It is worth noting that DS higher than 2 results in lower solubility as the polar hydroxyl groups are masked. Additionally, MC is insoluble in hot water and will precipitate out.[9]

MC in common with other cellulose ethers have been claimed to be physiologically harmless[10] and are well tolerated by skin and mucous membranes which makes them an attractive biomaterial for many industrial applications, including pharmaceutical excipients,[11] cosmetics[12] and the food industry.[13,14]

Several techniques have been employed for the characterisation of MC or other cellulose ethers including NMR, GLC, HPLC, capillary electrophoresis, mass spectrometry and to some extent, SEC-MALLS.[15] Analytical ultracentrifugation (AUC) has also been used. Pavlov et al.[16] studied a homologous series of 12 MC fractions of the same DS = 1.7 and obtained hydrodynamic scaling or 'Mark–Houwink–Kuhn–Sakurada' power law parameters consistent with a flexible coil polymer. The principal parameters studied were the power law coefficients a and b in the relations $[\eta] \propto M^a$, where $[\eta]$ is the intrinsic viscosity and M the molecular weight, and $s \propto M^b$, where s is the sedimentation coefficient. Values for $a \approx 0.83$ and $b \approx 0.39$ obtained were consistent with a flexible coil model for the series. The flexible coil model was reinforced by measurements of the dependency on the translational diffusion coefficient D with M, D with $[\eta]$, s with $[\eta]$ and s with the concentration dependent sedimentation parameter k_s. Pavlov et al.[16] also estimated the persistence length L_p for this series from a Yamakawa–Fujii plot[17] of s versus $M^{1/2}$ and 'Bushin–Tvestkov–Lysenko–Emelianov'[18] or 'Bohdanecky'[19] plot of $(M^2/[\eta])^{1/3}$ versus $M^{1/2}$ and obtained a value of $L_p \approx (17 \pm 1)$ nm.

In the present study, we focus on six MCs of similar molecular weight but with different DSs to investigate the effect of substitution on the overall conformation and degree of flexibility. We use sedimentation velocity in the AUC and intrinsic viscosity as our principal hydrodynamic techniques, but as our samples are within a narrow molecular weight range and we also do not have a homologous series we adopt a different approach: we use the conformation zoning approach of Pavlov et al.[20] (based on combination of s with k_s) to assess the

conformation type and the newly formulated global approach of Ortega and Garcia de la Torre[21,22] involving combination of the Bohdanecky[19] and Yamakawa–Fujii[17] relations via minimisation of a target function for each sample, to obtain an estimate for L_p for each case. In this way it is possible to show there is no significant change in the conformational flexibility of MCs with the change in DS in the range of 1.3–1.9.

Experimental Part
Preparation of Solutions

In the present study two sets of MC samples were used. MCs of lower DSs MC-1.09 and MC-1.32 were derived from one parental cellulose and MC samples of higher DS (MC-1.80, MC9-1.86, MC-1.88 and MC-1.93) were derived from a different native cellulose as described previously.[23–25] Samples were solubilised in deionised distilled water in screw-capped tubes with constant stirring at low speed at 4.0 °C and stirring was continued at 4.0 °C overnight. Supramolecular aggregates or undissolved particulates which can obscure interpretation of light scattering and viscometry[26] were removed by using an MSE (Crawley, UK) preparative ultracentrifuge at 4 000 rpm for 30 min at 20.0 °C. Sample concentrations were estimated prior to analysis using an ATAGO DD5 (JENCONS Scientific, Leighton Buzzard, UK) differential refractometer based on a refractive index increment (dn/dc) of ≈0.154 mL · g^{-1}.[27]

Sedimentation Velocity Using Analytical Ultracentrifugation

Sedimentation velocity experiments were performed using an Optima XL-I AUC (Beckman Instruments, Palo Alto, USA). Reference solvent (400 µL) and sample solutions (380 µL) were injected into the solvent and sample channels of 12 mm carbon filled centrepieces, and loaded into an eight-hole titanium rotor. Samples were run at 45 000 rpm and 20 °C.

Concentration profiles were registered using the Rayleigh interference optical system. Standard Fourier transform software converted the fringe profiles into plots of fringe displacement, 'j' (relative to the meniscus) versus radial position, 'r'. Data were analysed using the least squares $g^*(s)$ method in SEDFIT[28,29] and curves were fitted using the MULTIG function (A. J. Rowe, NCMH), written pro Fit™ (Quantum Soft, Zurich, Switzerland).

Molecular Weight Determination Using SEC-MALLs

The chromatography assembly consisted of an HPLC pump (Model PU-1580, Jasco Corporation, Tokyo, Japan), a Rheodyne injection valve (Model 7125, Rheodyne, St. Louis, USA) fitted with a 100 µL loop with Phenomenex guard column, TSK (TOSOHAAS Bioseperation Specialists, USA) Gel G 6000PW connected in series with TSK Gel G 4000PW. Light scattering intensity was detected using a Dawn DSP multi-angle laser light scattering photometer and concentration was determined using an Optilab 903 interferometric refractometer (both instruments from Wyatt Technology,

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Santa Barbara, USA). The SEC-MALLs system was equilibrated overnight at a flow rate of 0.8 mL·min^{-1} at room temperature. Samples (100 μL) with accurately known concentration and filtered through 0.45 μm filters (Whatman, Maidstone, UK) were injected at the same flow rate. Signals from the light scattering photometer and the refractometer were captured and analysed (Debye model, second degree of fitting) on a PC using the ASTRA^{TM} (for Windows XP) software supplied by the manufacturer.

Intrinsic Viscosity Measurements

The intrinsic viscosity of each MC sample was measured using a semi-automated viscosity measuring unit (AVS 310, Schott Geräte, Hofheim, Germany) at a temperature of $(20.00 \pm 0.01)\,°C$ in an Ostwald viscometer. Relative dynamic viscosities η_{rel} were calculated using:[30]

$$\eta_{rel} = \left(\frac{t}{t_0}\right)\left(\frac{\rho}{\rho_0}\right) \approx \frac{t}{t_0} \qquad (1)$$

where t and ρ refer to the flow time and density, respectively, and t_0 and ρ_0 are the solvent flow time and density, respectively. Since concentrations were low (<2 mg·mL^{-1}) the density correction term was considered negligible. Reduced specific viscosities η_{red} (mL·g^{-1}) were then determined over a range of concentrations (between 0.1 and 2 mg·mL^{-1}):

$$\eta_{red} = \frac{(\eta_{rel} - 1)}{c} \qquad (2)$$

and intrinsic viscosities $[\eta]$ from extrapolation of η_{red} to zero concentration using the Huggins[27] approach to eliminate nonideality.[31]

$$\eta_{red} = [\eta](1 + K_H[\eta]c) \qquad (3)$$

Results and Discussion

Sedimentation Velocity

From the sedimentation velocity experiments, it was found that all samples are highly pure in a hydrodynamic sense with only one significant component regardless of the DS. The $g^*(s)$ profile for MC-1.80 at various concentrations are shown in Figure 1. Similar profiles were obtained for the other MC samples. The $s_{20,w}$ measured at various concentration were extrapolated to infinite dilution using a reciprocal plot (Figure 2) which gives $s_{20,w}^0$, the sedimentation coefficient (corrected for nonideality), together with the Gralén or concentration dependence coefficient k_s (mL·g^{-1}):[3]

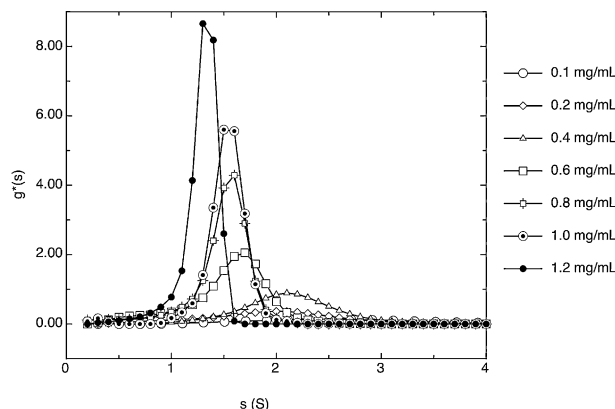$$1/s_{20,w} = (1/s_{20,w}^0)(1 + k_s c) \qquad (4)$$

Figure 1. Sedimentation coefficient distributions [$g^*(s)$] for MC-1.80 at various concentrations.

Sedimentation coefficients are reported in Table 1 along with k_s. In both MCs of low DS (MC-1.09 and MC-1.32) and high DS (MC-1.80, MC-1.86, MC-1.88 and MC-1.93), the $s_{20,w}^0$ and k_s values increases with the increase in DS.

Molecular Weight Distribution

Single elution peaks were observed from the SEC-MALLs profiles for each sample again consistent with homogeneous preparations. The weight average molecular weight, $\overline{M}_w$ for each sample was then calculated using the method described previously by Jumel et al. for the related hydroxypropyl-MC.[32] Estimates for the weight average $\overline{M}_w$ over the principal elution peak are presented in Table 1. The weight average molecular weight increases with increase in DS (in the case of both sets of samples)
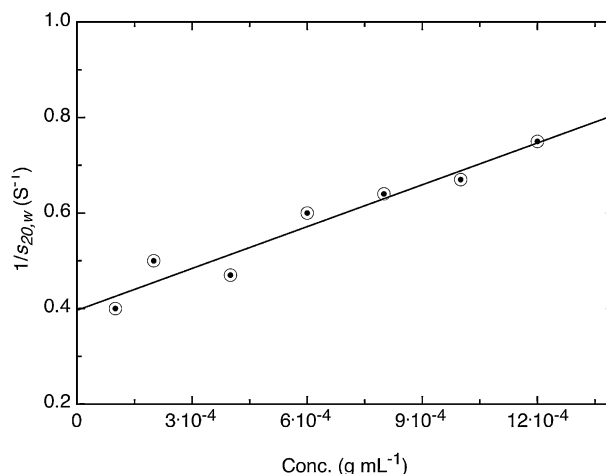

Figure 2. Concentration dependence of the (reciprocal) sedimentation co-efficient, s^{-1} for MC-1.80 ($s_{20,w}^0 = 2.50 \pm 0.11$ S; $k_s = 730$ mL·g^{-1}).

Table 1. Hydrodynamic properties of MCs.

Sample	$s_{20,w}^0$	k_s	$10^{-5} \times \overline{M}_w$	R_{gz}	$[\eta]$
	S	$mL \cdot g^{-1}$	$g \cdot mol^{-1}$	nm	$mL \cdot g^{-1}$
MC-1.09	3.0 ± 0.2	750 ± 50	9.7 ± 1.5	90 ± 10	2500 ± 20
MC-1.32	3.6 ± 0.1	1200 ± 100	11.9 ± 0.9	95 ± 5	2300 ± 20
MC-1.80	2.5 ± 0.1	730 ± 80	6.7 ± 0.1	90 ± 10	1900 ± 10
MC-1.86	2.8 ± 0.2	1160 ± 70	6.7 ± 0.3	85 ± 5	1800 ± 20
MC-1.88	3.1 ± 0.2	1270 ± 60	7.6 ± 0.5	80 ± 5	1700 ± 30
MC-1.93	3.1 ± 0.1	1340 ± 100	9.4 ± 0.5	90 ± 5	2200 ± 10

consistent with observations from sedimentation velocity analysis. Estimate for the radius of gyration, R_{gz} showed, within experimental error, no trend with molecular weight change with values in the range (85 ± 10) nm.

Intrinsic Viscosity

The intrinsic viscosity $[\eta]$ for each MC is given in Table 1 and Figure 3 shows an example extrapolation. Values of the Huggins' constants are in the range of 0.2–0.8 which indicates that water is a good solvent for MC.[33]

It can be seen that the intrinsic viscosity is very high for all the samples, reflecting the high molecular weight and extended conformation of MC. For the radius of gyration, there is no clear trend with molecular weight change.
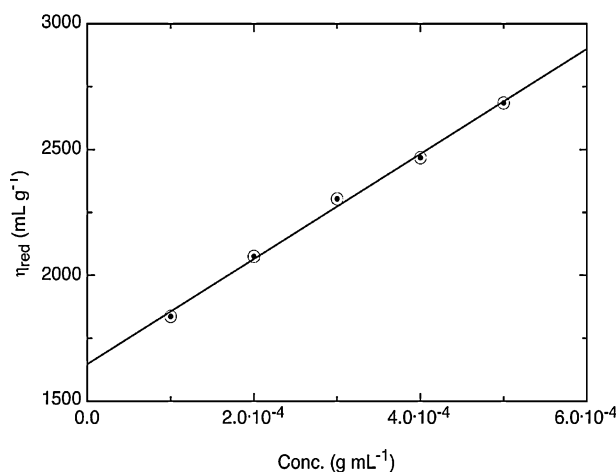
Conformational Analysis

The Translational Frictional Ratio, f/f_o

The translational frictional ratio,[34] f/f_0 is a parameter which depends on molecular weight, conformation and molecular expansion through hydration effects. It can be measured experimentally from the sedimentation coefficient and molecular weight:

$$\frac{f}{f_0} = \frac{\overline{M}_w(1 - \overline{v}\rho_{20,w})}{(N_A 6\pi\eta_{20,w}s_{20,w}^0)}\left(\frac{4\pi N_A}{3\overline{v}\overline{M}_w}\right)^{1/3} \quad (5)$$

where the partial specific volume for MC,[16] $\overline{v} = 0.715$ $mL \cdot g^{-1}$.

Large translational frictional ratios are found for all the MCs studied, and all in the region of 10–12 (Table 2). These values are symptomatic of expanded extended structures for these molecules.

Figure 3. Huggins extrapolation for the intrinsic viscosity of MC-1.88 ($[\eta] = 1700 \pm 30$ $mL \cdot g^{-1}$ and $K_H = 0.69 \pm 0.02$).

Table 2. Conformational parameters for MCs.

Sample	$k_s/[\eta]$	f/f_0	L_p
			nm
MC-1.09	0.30 ± 0.03	12 ± 2	15 ± 2
MC-1.32	0.52 ± 0.04	12 ± 1	12 ± 1
MC-1.80	0.38 ± 0.05	12 ± 1	17 ± 2
MC-1.86	0.64 ± 0.05	11 ± 1	13 ± 2
MC-1.88	0.75 ± 0.05	10 ± 1	12 ± 2
MC-1.93	0.61 ± 0.05	12 ± 1	15 ± 2
Overall	0.53 ± 0.16	12 ± 1	14 ± 1

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Wales–Van Holde Ratio,[35] R

Values in the range of 0.30–0.75 are obtained for $R = k_s/[\eta]$, again consistent with extended structures[5] and are very similar to those found for citrus pectins.[36,37]

Sedimentation Conformation Zoning

The sedimentation conformation zone[20] plot k_sM_L versus $[s]/M_L$ enables an estimate of the 'overall' solution conformation of a macromolecule in solution ranging from Zone A (extra rigid rod) to Zone E (globular or branched). The parameter $[s]$ is related to the sedimentation coefficient by the relation:

$$[s] = \frac{s^0_{20,w}\eta_{20,w}}{(1 - \overline{v}\rho_{20,w})} \quad (6)$$

and M_L the mass per unit length is given by:

$$M_L = \frac{m}{l} \quad (7)$$

The mass of glucose monomer, m is 162 g·mol^{-1} and the average monomer mass will be approximately ≈177 and ≈189 g·mol^{-1} for a low DS 1.09 and a high DS 1.93 respectively. l is the diameter of a monosaccharide[38] ≈0.54 nm. Therefore M_L was fixed at 328 g·mol^{-1}·nm^{-1} (MC-1.09); 334 g·mol^{-1}·nm^{-1} (MC-1.32); 347 g·mol^{-1}·nm^{-1} (MC-1.80); 348 g·mol^{-1}·nm^{-1} (MC-1.86); 349 g·mol^{-1}·nm^{-1} (CM-1.88) and 351 g·mol^{-1}·nm^{-1} (CM-1.93), respectively for each MC. As can be seen from the sedimentation conformation zoning plot (Figure 4) all six MC samples have an extended or 'semi-flexible' conformation (Zone C), which is consistent with previous estimates[20] and the estimates from the translational frictional ratio and the Wales–van Holde ratio. However, to get more quantitative information about the flexibility of the MCs we need to consider the way in which the sedimentation coefficient and intrinsic viscosity changes with molecular weight.

Combined Analysis Method (HYDFIT)

The linear flexibility of polymer chains is represented in terms of the persistence length, L_p of equivalent *worm-like chains* [39] 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[28] $L_p = 0$ and for the equivalent extra-rigid rod[28] $L_p = \infty$, although in practice limits of ≈1 nm for random coils (*e.g.*, pullulan) and 200 nm for an extra-rigid rod (*e.g.*, DNA) are more appropriate.[40] Chain persistence
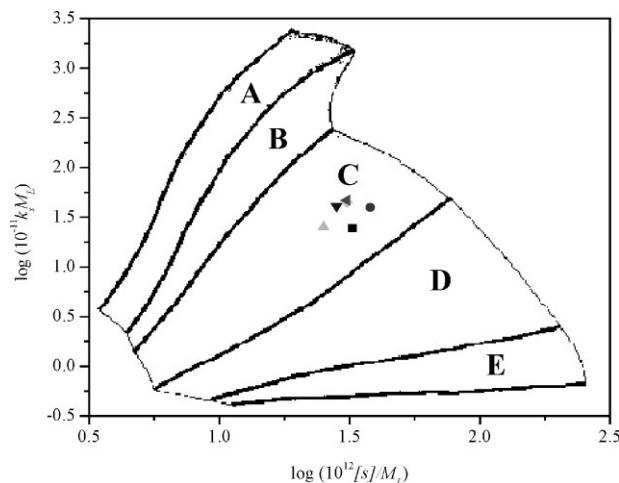

Figure 4. The sedimentation conformation zoning plot (adapted from ref. [20]). Zone A: extra rigid rod; Zone B: rigid rod; Zone C: semi-flexible; Zone D: random coil and Zone E: globular or branched. Individual MCs are marked: MC-1.09 ■; MC-1.32 ●; MC-1.80 ▲; MC-1.86 ▼; MC-1.88 ◆ and MC-1.93 ◀.

lengths, L_p can be estimated using several different approaches using either intrinsic viscosity[19,41,42] or sedimentation coefficient[17,43] measurements. For example, the Bohdanecky relation:[19]

$$\left(\frac{\overline{M}_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3}\left(\frac{2L_p}{M_L}\right)^{-1/2}\overline{M}_w^{1/2} \quad (8)$$

where Φ is the Flory–Fox constant $(2.86 \times 10^{23}$ mol^{-1}) and A_0 and B_0 are tabulated coefficients,[19] and the Yamakawa–Fujii equation:[17]

$$s^0 = \frac{M_L(1 - \overline{v}\rho_0)}{3\pi\eta_0 N_A}$$

$$\times \left[1.843\left(\frac{\overline{M}_w}{2M_LL_p}\right)^{1/2} + A_2 + A_3\left(\frac{\overline{M}_w}{2M_LL_p}\right)^{-1/2} + ...\right] \quad (9)$$

Yamakawa and Fujii[17] 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[21,22] which estimates the best values or best range of values of L_p and M_L based on minimisation of a target function Δ. An estimate for the chain diameter d is also known but extensive simulations 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 MC). The blue contour in Figure 5a–f represents the minimum in the

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target function within experimental error and a continuum of possible values for L_p from 3 to 20 nm and mass per unit lengths M_L from 100–450 $g \cdot mol^{-1} \cdot nm^{-1}$ are allowed within experimental error, reflecting that for samples near the coil-limit, the molecular weight dependence of any property depends essentially on the combined parameter L_p/M_L and cannot be distinguished theoretically. However, if we use the same values of M_L used for the conformation zoning plots a more specific value for L_p can be found for each MC (Table 2 and Figure 6). Therefore, we estimate a persistence length of 12–17 nm for all the MCs, which are again consistent with the conformational estimates from the translational frictional ratio, Wales–van Holde ratio and conformation zoning. Small differences in persistence length between samples are reflected in the radius of gyration and the intrinsic viscosity.

The Multi_HYDFIT[21,22] approach also allows for the estimation of the average persistence length for a homologous series of polymers using sets of $s^0_{20,w}$ and $[\eta]$ versus $\overline{M}_w$ (Figure 5g and 6). Although in this case we are not strictly speaking considering a homologous series it is clear that as all MC molecules are of a similar conformation this can be justified.
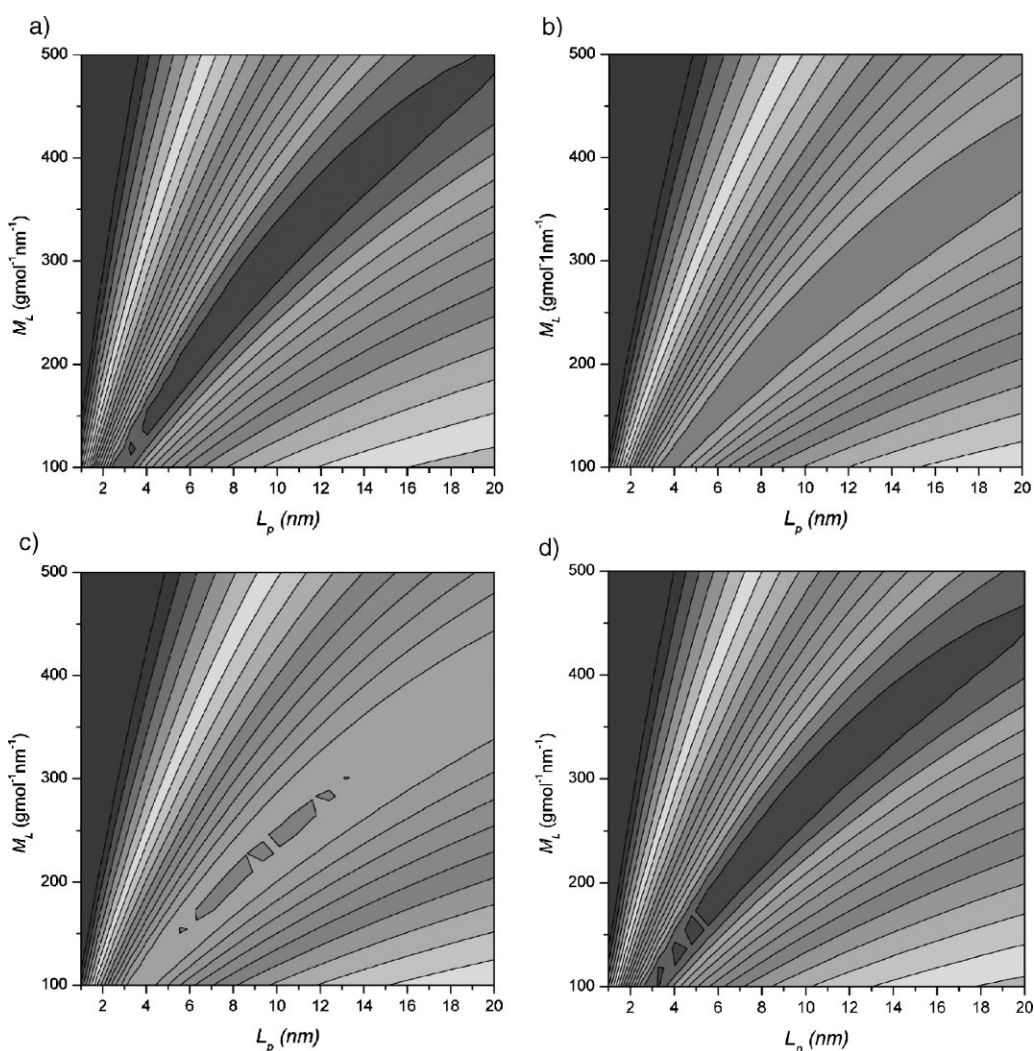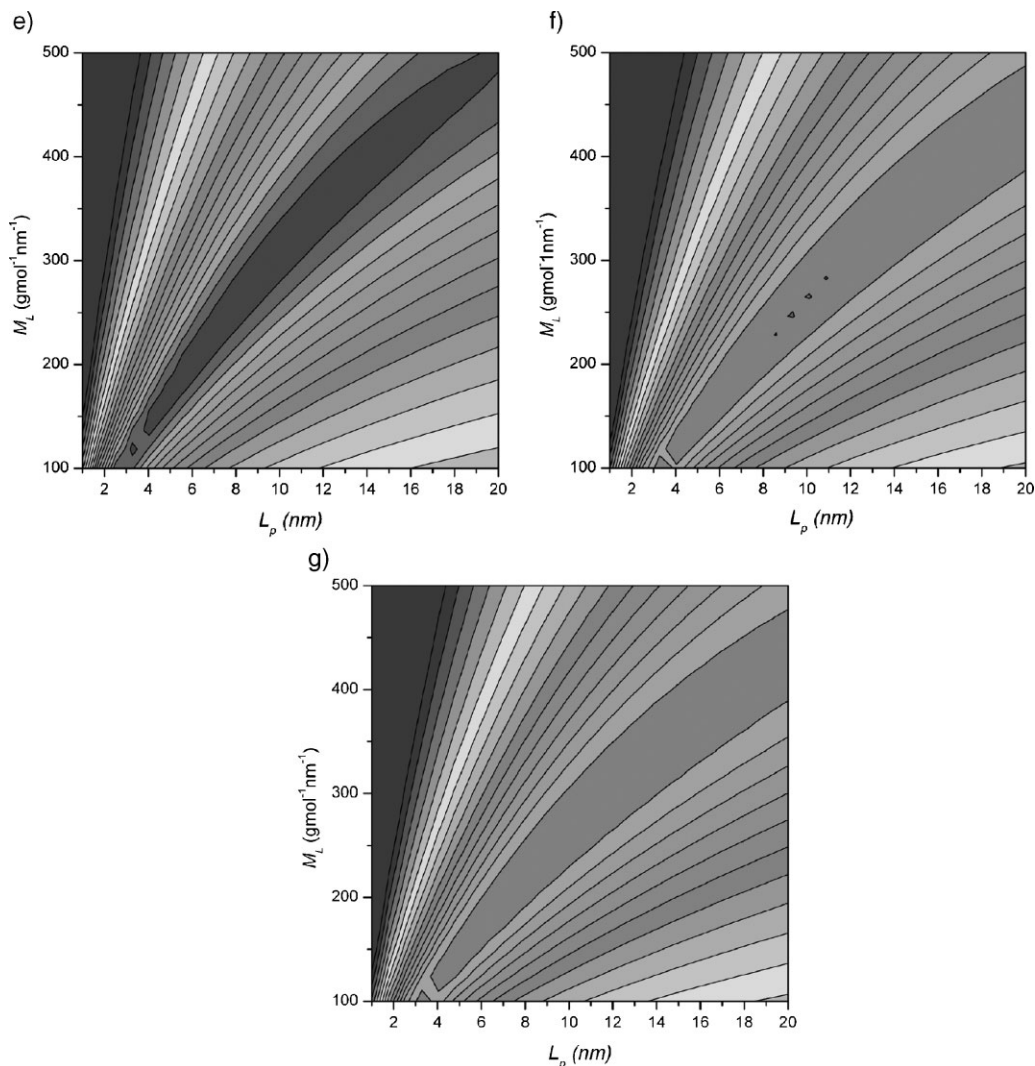

Figure 5. HYDFIT analysis for MC. Solutions of the Bohdanecky [19] and Yamakawa–Fujii [17] relations for L_p with M_L allowed to float. The target function, Δ is then calculated over a range of values for M_L and L_p. In these representations, the values of Δ function are represented different shades of grey ranging from 0.1 to >1.0. (a) Contour plot from *HYDFIT* analysis for MC-1.09. (b) Contour plot from *HYDFIT* analysis for MC-1.32. (c) Contour plot from *HYDFIT* analysis for MC-1.80. (d) Contour plot from *HYDFIT* analysis for MC-1.86. (e) Contour plot from *HYDFIT* analysis for MC-1.88. (f) Contour plot from *HYDFIT* analysis for MC-1.93. (g) Overall contour plot from *HYDFIT* analysis for MCs.

Figure 5 (Continued).

Conclusion

Bueche[44] suggested that cellulose derivatives are within the range of partly expanded coil configuration. In a review article by Clasen and Kulicke,[6] it was reported that cellulose derivatives adopt expanded coiled structure. Our data are also in agreement with the results published by Peterlin[4] for cellulose derivatives and with findings of Jumel et al.[32] for hydroxypropyl-MC. Doty et al.[26] have also reported that cellulose derivatives like carboxy-MC and trinitro cellulose are extended non-Gaussian chains in solution when compared with other polymers. It is worth noting that with increase in DS, there is also increase in s, $[\eta]$, k_s and $\overline{M}_w$. The values of L_p obtained from combined analysis for these samples also suggest that MC adopts an extended coil (Zone C) conformation in solution. The values we obtain for L_p (12–17 nm) are slightly lower than the estimates made by Pavlov et al.[16] based on a homologous series of MC fractions of the same DS.
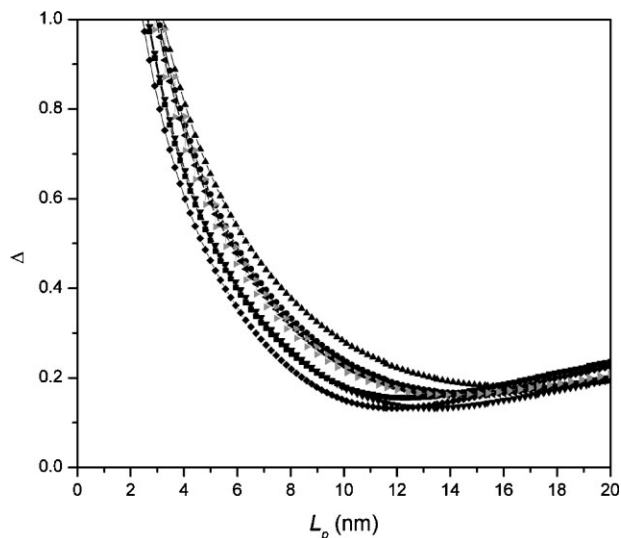
Figure 6. HYDFIT analysis for MC. Solutions of the Bohdanecky [19] and Yamakawa–Fujii [17] relations for the persistence length L_p (for a known mass per unit length). Plot of target function ($\triangle$) versus persistence length for MCs: MC-1.09 ■; MC-1.32 ● MC-1.80 ▲; MC-1.86 ▼; MC-1.88 ◆; MC-1.93◀ and overall ▶.

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