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Synthesis and solution properties of cholesterol end-capped poly(ethylene glycol)

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Abstract

A series of cholesterol end-capped poly(ethylene glycol) (Ch_2PEG) were synthesized by coupling cholesterol at each end of PEG (molecular weight = 4000, 10 000, 20 000 and 35 000 g/mol) with hexamethylene diisocyanate. Unlike hydrophobically modified PEGs, which are end-capped with flexible hydrocarbons or fluorocarbons, Ch_2PEGs are not soluble in water, although they do swell significantly, and the swelling ratio increases with molecular weight. Analysis of the swelling ratios via the Flory–Rehner equation indicates that, as PEG molecular weight increases, the Flory–Huggins interaction parameter decreases slightly from 0.53_4 to 0.49_5 and becomes constant within experimental error when the PEG molecular weight reaches 10 000 g/mol. Addition of small amounts of a co-solvent such as *n*-propanol converts this intractable opaque material to a completely homogeneous, optically transparent, highly elastic fluid whose viscoelastic properties are those of a transient network with relaxation times in the range from 0.1 to 10 s, depending on co-solvent content and temperature.

Keywords: Cholesterol end-capped poly(ethylene glycol); Synthesis; Swelling behavior

1. Introduction

Poly(ethylene glycol) (PEG) or poly(ethylene oxide) is a polymer with many unique properties [1,2]. In the solid state, PEG is a semi-crystalline material with a strong power to solvate alkali ions [3]. The tendency of PEG to crystallize is such that significant crystallinity of PEG has been found even in multi-component systems [4,5]. In solution, PEG exhibits an unusual solubility pattern [2]. It is soluble both in water and in many organic solvents. It is also very effective at excluding other polymers from its presence in an aqueous environment. The combination of these interesting properties makes PEG very attractive in advanced technologies [2,6].

Recently hydrophobically end-capped PEG [7–18] has received attention as a model system for fundamental and technological study of hydrophobically modified water-soluble polymers. Information about the structures formed by hydrophobically end-capped PEG in water has been obtained with the aid of various techniques such as rheometry [7–12], dynamic light scattering [13,14], static and dynamic fluorescence spectroscopy [15,16], NMR self-diffusion [10,14,17], and, at high concentration, small-angle X-ray scattering [18]. Such investigations indicate that molecules of hydrophobically end-capped PEG form aggregates at very low concentrations. As polymer concentration increases, the aggregates increase in size and overlap with each other, at which point the viscosity of the solution increases dramatically. Upon further increase in polymer concentration, the size of the aggregates continues to increase until all the aggregates coalesce, and form a network. This network is transient or reversible in nature reflecting a degree of internal fluidity, which originates in the magnitude of the lifetime of the junction points of the network [7,19–22]. The relaxation time of the network is determined by the exit rates of hydrophobes from aggregates, which is expected to be strongly dependent on the structure of the hydrophobe, e.g. whether hydrocarbon or fluorocarbon, flexible or rigid, etc. While flexible hydrophobes, either hydrocarbons [7–9,12–18] or fluorocarbons [10,11], have been used to end-cap PEG with molecular weights in the range from 2000 to 35 000 g/mol, to our knowledge, no rigid hydrophobes have been used to end-cap PEG in this molecular weight range.

It is well known that cholesterol derivatives have a great tendency to form liquid-crystalline phases because of the rigid anisotropic structure of cholesterol. If we attach cholesterol to each end of PEG, an example of a new class of materials, mesogenically modified water-soluble

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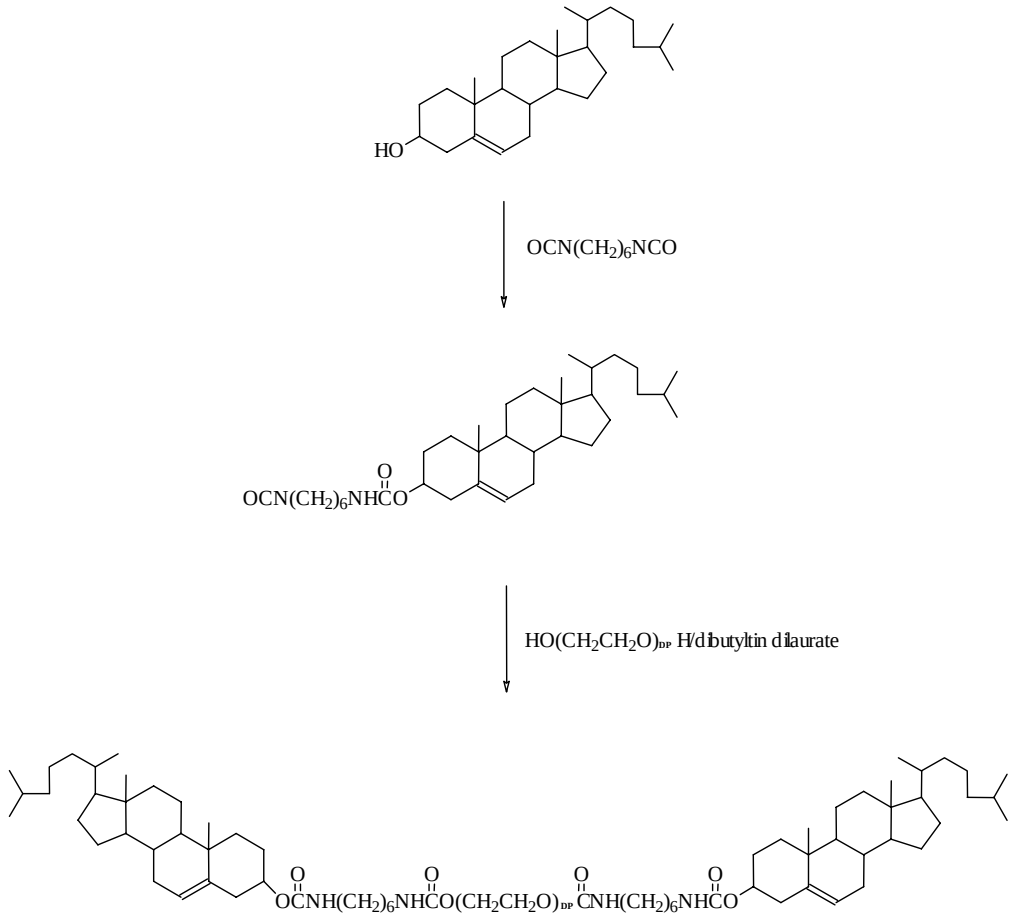

Scheme 1.

polymers, can be synthesized. We expect that this cholesterol end-capped PEG will show strong association in water due to the tendency of cholesterol derivatives to form liquid-crystalline phases. In this paper, we report the synthesis of cholesterol end-capped PEG and some preliminary results on the solution properties.

2. Experimental

2.1. Materials

Cholesterol (Aldrich) was purified by recrystallization from ethanol. Hexamethylene diisocyanate was purified by distillation just before use. Dibutyltin dilaurate and PEG specimens (Fluka) with molecular weight (MW) = 4000, 10 000, 20 000, and 35 000 g/mol, denoted as PEG-4K, PEG-10K, PEG-20K, and PEG-35K, respectively, were used as received.

2.2. Analytical methods

^{1}H NMR spectra were recorded using a Varian XL-200 200 MHz FT NMR spectrometer. Chemical shifts (δ) are given in ppm with the peak (7.24 ppm) of $CHCl_3$ as reference. GPC experiments were carried out on a Waters chromatograph connected to a Waters 410 differential refractometer with poly(ethylene glycol) as standards in THF. Dynamic rheological experiments were performed on a Rheometrics RFS 8500 fluids rheometer with stainless-steel cone-and-plate geometry (cone angle = 0.02 rad and cone radius = 25 mm). The shear strain was 5%.

2.3. Synthesis of cholesterol isocyanate derivative

15.0 g of cholesterol and 100.0 g of hexamethylene diisocyanate were mixed and stirred under nitrogen at 80°C for 24 h. Most of the excess diisocyanate was then removed by distillation under reduced pressure. The residue was dissolved with 300 ml of dry hexane. The resulting solution was kept at −30°C for 24 h. The precipitate obtained was separated and dried in vacuo at 50°C for 24 h. 19.0 g of white solid was obtained (yield: 88%). ^{1}H NMR: δ 0.68 (s, 3H, cholesterol 18-H_3), 0.8–2.1 (38H), 2.32 (m, 2H, cholesterol 4-H_2), 3.15 (m, 2H, CH_2N), 3.29 (t, 2H, CH_2NCO), 4.47 (m, 1H, cholesterol 3-H_1), 4.59 (t, 1H, NHCOO), 5.38 (d, 1H, cholesterol 6-H_1).

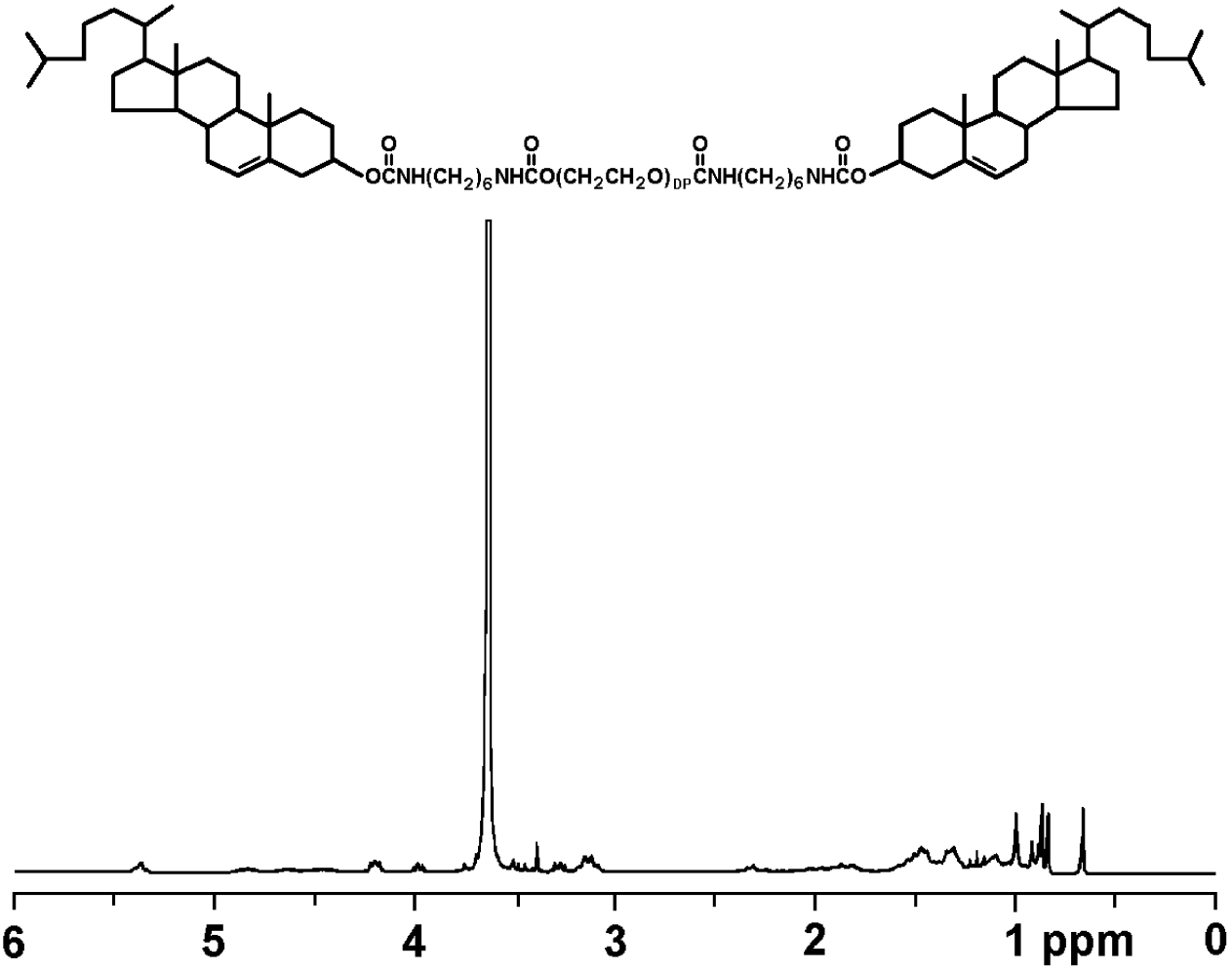

Fig. 1. ^{1}H NMR spectrum of Ch_2PEG-4K in $CDCl_3$.

2.4. Synthesis of cholesterol end-capped PEG (Ch_2PEG)

10.0 g of PEG-20K was dissolved in 150 ml of dry toluene. After 100 ml of toluene was distilled out, 1.1 g of the cholesterol isocyanate derivative and 0.02 ml of dibutyltin dilaurate were added to the PEG/toluene solution. The resulting solution was stirred under nitrogen at 80°C for 8 h. The solution was cooled to room temperature and poured into 500 ml of ethyl ether. The precipitate was separated, washed with ethyl ether and reprecipitated from THF into ethyl ether one more time. After drying in vacuo at 50°C for 24 h, a white solid, which is denoted as Ch_2PEG-20K, was obtained.

Following the same procedure as described above, reactions between the cholesterol isocyanate derivative and PEG-4K, PEG-10K or PEG-35K produced cholesterol end-capped PEGs with different molecular weights, which are denoted as Ch_2PEG-4K, Ch_2PEG-10K, or Ch_2PEG-35K, respectively.

2.5. Swelling study

The swelling behavior of the Ch_2PEGs was investigated at room temperature (20°C) by immersing the weighed dry samples in distilled water. After 10 days, the excess water was removed and the swollen networks were weighed again. The weight swelling ratio, q_w, was calculated as

$$q_w = \frac{W_s}{W_d} \quad (1)$$

where W_s is the weight of swollen sample and W_d the weight of dry sample.

2.6. Preparation of Ch_2PEG solution

0.20 g of Ch_2PEG-20K, 8.82 g of water and 0.98 g of *n*-propanol were mixed and equilibrated at room temperature for 3 days. After removing excess solvent (~40% of the total weight), a transparent solution with highly elastic

Table 1
Characteristics of the cholesterol end-capped PEG

Sample	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$ [a]	DS (%)[b]
Ch_2PEG-4K	5110	1.32	95.8
Ch_2PEG-10K	11110	1.25	95.2
Ch_2PEG-20K	21110	1.22	94.6
Ch_2PEG-35K	36110	1.23	95.9

[a] Measured by GPC in THF.
[b] Measured by ^{1}H NMR in $CDCl_3$.

properties was obtained. The concentration of Ch_2PEG-20K in the solution is about 3.3 wt%.

3. Results and discussion

3.1. Synthesis of cholesterol end-capped PEG

One way to synthesize hydrophobically end-capped PEG is to directly attach a hydrophobe at each end of PEG through linkages such as urethane [9] or ether [18], for example. Another common method is to couple a hydrophobe at each end of PEG using diisocyanates. Isophorone diisocyanate [7,8,10–12] is most frequently used in this approach. The main disadvantage of using isophorone diisocyanate is that the two isocyanates are not equivalent which leads to different reactivity and results in variable structures between hydrophobe and PEG. To avoid this problem, hexamethylene diisocyanate was used in this work to couple cholesterol at each end of the PEG (Scheme 1). The cholesterol was reacted first with excess hexamethylene diisocyanate and the resulting cholesterol isocyanate derivative was then reacted with the PEG in the presence of dibutyltin dilaurate. The molar number of the cholesterol derivative is four times that of the PEG to ensure that most of the hydroxyl end-groups of the PEG are substituted. After reaction, the excess cholesterol derivative can be removed by multiple precipitation.

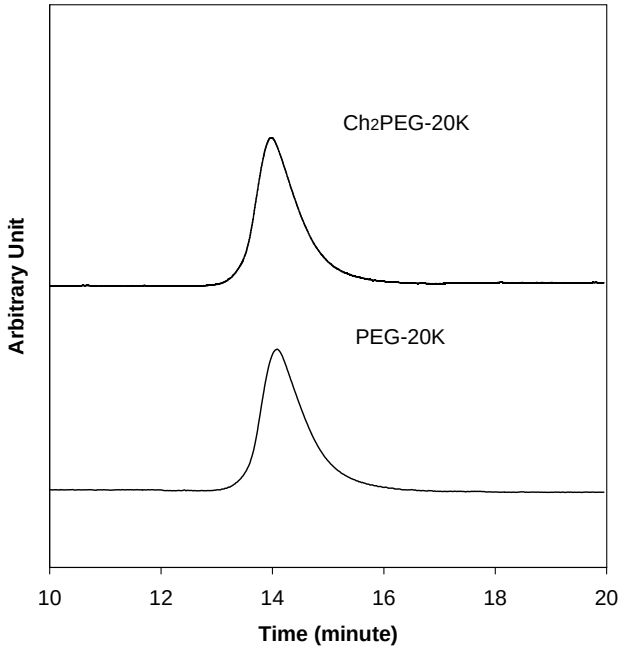

Fig. 2. GPC traces as a function of time for Ch_2PEG-20K and PEG-20K in THF.

The ^{1}H NMR spectrum of the purified cholesterol end-capped PEG (Ch_2PEG-20K) in $CDCl_3$ is shown in Fig. 1. The strong peak at 3.64 ppm is due to the methylene groups in PEG. The peaks in the range from 0.6 to 2.1 ppm are due to 49 hydrogens of the total 58 hydrogens in the cholesterol isocyanate derivative. From Fig. 1, the degree of substitution, DS, of PEG can be calculated via the following equation:

$$\mathrm{DS}\ (\%) = \frac{I_{0.6\text{–}2.1}/(2\times 49)}{I_{3.64}/(4\times \mathrm{DP_{PEG}})}\times 100\% \qquad (2)$$

where $I_{0.6–2.1}$ is the total intensity of the peaks over 0.6–2.1 ppm, $I_{3.64}$ is the intensity of the peak at 3.64 ppm, DP_{PEG} is the degree of polymerization of PEG. The result shows that the degree of substitution of the Ch_2PEGs is at least 95% (Table 1).

To verify that the reaction condition has not degraded the PEG, the uncapped and capped PEG specimens were analyzed by GPC in THF (Fig. 2). From the GPC analysis, we find that the polydispersities of the Ch_2PEGs are approximately 1.25 (Table 1), very close to those of the uncapped PEGs, and the peak positions of molecular weight shift to slightly higher values as expected from the effect of the attached cholesterol derivative. It has been reported [11] that hydrophobically end-capped PEG can also be synthesized by reacting PEG with diisocyanate first and then reacting the resulting PEG isocyanate derivative with hydrophobe. One problem of this approach is that the PEG isocyanate derivative tends to undergo condensation reaction, which results in polymer chains with twice the initial molecular weight [11]. From Fig. 2, it is evident that no condensation occurs during the synthesis of the Ch_2PEG, which suggests that reacting diisocyanate with hydrophobe first, then with PEG is superior to reacting diisocyanate with PEG first, then with hydrophobe.

3.2. Solution properties

Unlike hydrophobically modified PEGs which are end-capped with flexible hydrocarbons or fluorocarbons, the Ch_2PEGs are not soluble in pure water, although they do swell significantly, and the swelling ratio, measured after 10 days equilibrium, increases essentially linearly as molecular weight increases (Fig. 3). The Flory–Huggins interaction parameter χ for the Ch_2PEG and water can be evaluated using the Flory–Rehner equation [23]

$$M_c = -\frac{\rho V_1 \nu_{2m}^{1/3}}{\ln(1-\nu_{2m}) + \nu_{2m} + \chi\nu_{2m}^2} \qquad (3)$$

where M_c is the average molecular weight between

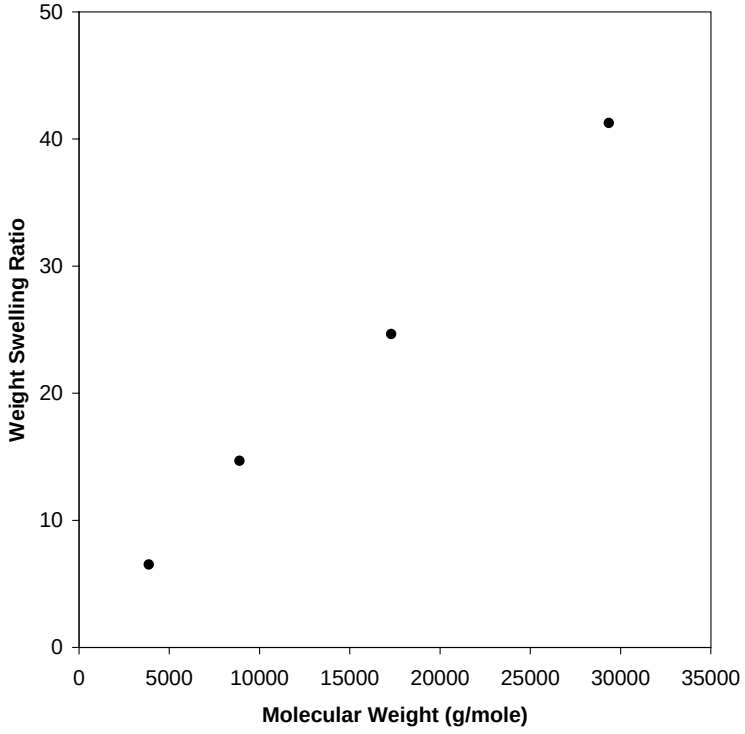

Fig. 3. Weight swelling ratio as a function of molecular weight for Ch_2PEG in water at 20°C.

cross-links, ρ the density of the polymer, V_1 the molar volume of the solvent, and ν_{2m} the volume fraction of the polymer at swelling equilibrium. The results are tabulated in Table 2 which shows that the χ values are in the range from 0.49_5 to 0.53_4, greater than 0.45, the χ value for linear PEG and water [24]. This is due to the fact that χ arises from the enthalpy of mixing of polymer segments and solvent molecules [23] and the cross-links in the Ch_2PEG networks are hydrophobic. When PEG molecular weight increases, the Ch_2PEG becomes more hydrophilic, and consequently, χ decreases (Table 2). When PEG molecular weight reaches 10 000 g/mol, this effect is saturated and χ becomes constant within experimental error. χ values in the range from 0.45 to 0.58, measured from the elastic modulus in uniaxial compression, have been reported for PEG networks formed by end-linking PEG (MW = 1000–10 000 g/mol) with pluriisocyanates in dioxane [25].

We note that Eq. (3) was derived based on a tetra-functionally cross-linked network and the information regarding the functionality of the Ch_2PEG networks is not available at present. The resulting error, however, is negligible since it has been demonstrated, both theoretically [26] and experimentally [27], that ν_{2m} is only slightly dependent on cross-link functionality. For the same reason, the effect of loops in the Ch_2PEG networks on ν_{2m} can also be neglected. The successful application of the Flory–Rehner equation indicates further that the contribution of dangling ends is negligible, consistent with the high degree of end-cap substitution, and the strong tendency for self-association of the cholesterol derivative.

Though the Ch_2PEGs are not soluble in pure water, addition of small amounts of a co-solvent such as *n*-propanol converts this intractable opaque material to a completely homogeneous, optically transparent, highly elastic fluid. The properties of the resulting fluid are strongly dependent on the type and amount of co-solvent. Shown in Fig. 4 is the viscoelastic response to oscillatory deformation of Ch_2PEG-20K (3.3 wt%) in a mixed solvent of water and *n*-propanol (weight ratio = 9 : 1) at 25°C, which indicates that these solutions have viscoelastic behavior typical of a transient network. At very low frequencies, there is a viscous flow ($G'' > G'$) due to the dissociation of cholesterol end groups. The associative effect of the cholesterol end groups is manifested by the highly elastic response of the fluid in dramatic contrast to the low viscosity rheology of the uncapped PEG at comparable concentrations [11]. The data in the low-frequency region of Fig. 4 can be fitted to the following equations derived from the Maxwell model, which consists

Table 2
Flory–Huggins interaction parameter χ for the cholesterol end-capped PEG and water

Sample	$\bar{M}_n$	q_w [a]	ν_{2m}	χ
Ch_2PEG-4K	3870	6.520	0.131	0.53_4
Ch_2PEG-10K	8890	14.688	0.057	0.50_6
Ch_2PEG-20K	17300	24.660	0.034	0.49_9
Ch_2PEG-35K	29360	41.255	0.020	0.49_5

[a] Measured by immersing weighed dry samples in excess distilled water for 10 days.

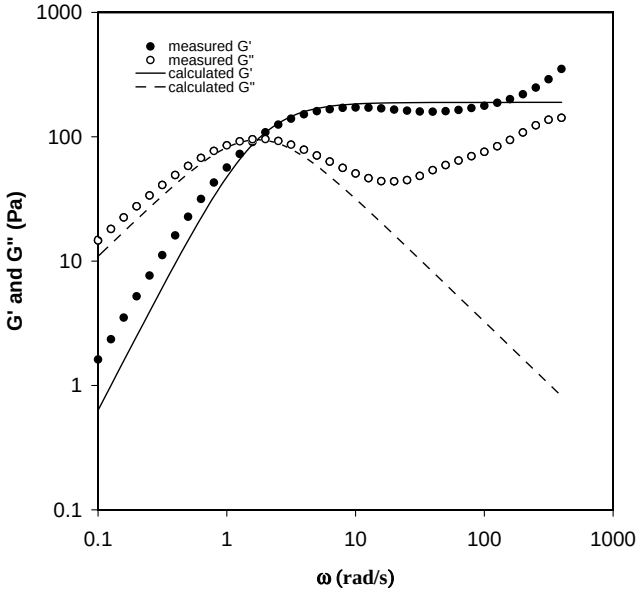

Fig. 4. Storage modulus G' and loss modulus G'' as a function of frequency ω for Ch_2PEG-20K/*n*-propanol/water solution at 25°C.

of an elastic component (spring) in series with a viscous component (dashpot) [28]

$$G'(\omega) = \frac{G_\infty \omega^2 \tau^2}{1 + \omega^2 \tau^2} \tag{4}$$

$$G''(\omega) = \frac{G_\infty \omega \tau}{1 + \omega^2 \tau^2} \tag{5}$$

where G' is the storage modulus, G'' the loss modulus, G_∞ the high-frequency storage modulus, ω the frequency, and τ the relaxation time, such that the viscosity of the dashpot $\eta = G_\infty \tau$. As can be seen the principle features of the low-frequency data, including the existence of the plateau modulus G_∞, and the crossover in G' and G'' at $\omega = 1/\tau$, are closely described by the Maxwell model. These observations are essentially identical to the viscoelastic behavior observed in solutions of PEG end-capped with flexible hydrophobes [8]. The implication of this result is that a very narrow distribution of relaxation times describes the viscoelastic response of the transient network and hence of the dissociation rate of cholesterol aggregates in *n*-propanol/water mixture. At 25°C, we determine $G_\infty = 189$ Pa and $\tau = 0.58$ s. When temperature is raised to 35°C, we find $G_\infty = 131$ Pa and $\tau = 0.33$ s, i.e. increased temperature facilitates end-group dissociation. When the *n*-propanol content of the loaded gel was reduced by extraction with 20 ml of distilled water for 12 h, we find $G_\infty = 60$ Pa and $\tau = 4.33$ s, i.e. increased water content inhibits end-group dissociation. Further investigation is underway to gain better understanding of the structures formed by the Ch_2PEG in water and the influence of co-solvents on the structures.

4. Conclusions

It is clear from our results that the behavior of PEG end-capped with the rigid hydrophobe cholesterol is vastly different from that of PEG with flexible hydrophobic end-groups. The Ch_2PEGs form insoluble networks whereas PEGs with flexible hydrophobes form viscoelastic solutions [7–12]. Addition of the co-solvent *n*-propanol converts the Ch_2PEG into highly elastic solutions whose rheological behavior is similar to that of PEG end-capped with flexible hydrophobes [7–12], presumably because the solvation of the cholesterol by *n*-propanol decreases the aggregation number of end-groups, and increases the dissociation rate of cross-links.

Acknowledgements

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