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Research Article

Theme: Advances in Pharmaceutical Excipients Research and Use: Novel Materials, Functionalities and Testing
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Rheological Evaluation of Inter-grade and Inter-batch Variability of Sodium Alginate

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Abstract. Polymeric excipients are often the least well-characterized components of pharmaceutical formulations. The aim of this study was to facilitate the QbD approach to pharmaceutical manufacturing by evaluating the inter-grade and inter-batch variability of pharmaceutical-grade polymeric excipients. Sodium alginate, a widely used polymeric excipient, was selected for evaluation using appropriate rheological methods and test conditions. The materials used were six different grades of sodium alginate and an additional ten batches of one of the grades. To compare the six grades, steady shear measurements were conducted on solutions at 1%, 2%, and 3% *w*/*w*, consistent with their use as thickening agents. Small-amplitude oscillation (SAO) measurements were conducted on sodium alginate solutions at higher concentrations (4–12% *w*/*w*) corresponding to their use in controlled-release matrices. In order to compare the ten batches of one grade, steady shear and SAO measurements were performed on their solutions at 2% *w*/*w* and 8% *w*/*w*, respectively. Results show that the potential interchangeability of these different grades used as thickening agents could be established by comparing the apparent viscosities of their solutions as a function of both alginate concentration and shear conditions. For sodium alginate used in controlled-release formulations, both steady shear behavior of solutions at low concentrations and viscoelastic properties at higher concentrations should be considered. Furthermore, among batches of the same grade, significant differences in rheological properties were observed, especially at higher solution concentrations. In conclusion, inter-grade and inter-batch variability of sodium alginate can be determined using steady shear and small-amplitude oscillation methods.

KEY WORDS: polymeric excipients; quality-by-design (QbD); rheology; sodium alginate; variability.

INTRODUCTION

Quality-by-design (QbD) principles (1) necessitate the establishment of a design space for each pharmaceutical product encompassing, in part, the active pharmaceutical ingredient(s), the unit operations employed to produce the finished product, and the excipients (2). Polymeric excipients, in particular, comprise mixtures of polymers of different molecular weights and chemical composition and tend to be the least well-characterized components of the design space. This paper focuses on the widely used but poorly characterized polymeric excipient sodium alginate which is commercially extracted from seaweed. Sodium alginates are linear, unbranched, amorphous copolymers of β-D-mannuronic acid (*M*) and α-L-guluronic acid (*G*) linked to each other by 1→4 glycosidic bonds. The *M* and *G* units in the alginates may be randomly or non-randomly arrayed as heterogeneous or homogeneous sequences (Fig. 1). The stiffness of the sequences in aqueous solution increases in the order $MG<M<G$ (3–5).

Sodium alginates have wide application in the pharmaceutical and biomedical areas due to their abundance, low price, and compatibility with biological systems (6). Pharmaceutically, sodium alginates are generally used as binding agents in tablets, as suspending and thickening agents in suspensions, water-miscible gels, lotions and creams, as emulsion stabilizers, or as gel-forming agents in combination with divalent metal ions such as calcium (7). Of particular interest is their potential in the development of alginate-based controlled-release drug delivery systems, such as matrix tablets, microcapsules, *etc.* (6).

More than 200 different alginate grades—varying in molecular weight and chemical composition—are available from manufacturers (6). The heterogeneity of commercial pharmaceutical-grade alginates reflects differences among the botanical sources, seaweed harvesting locations, the season of harvesting, the plant parts employed, and the

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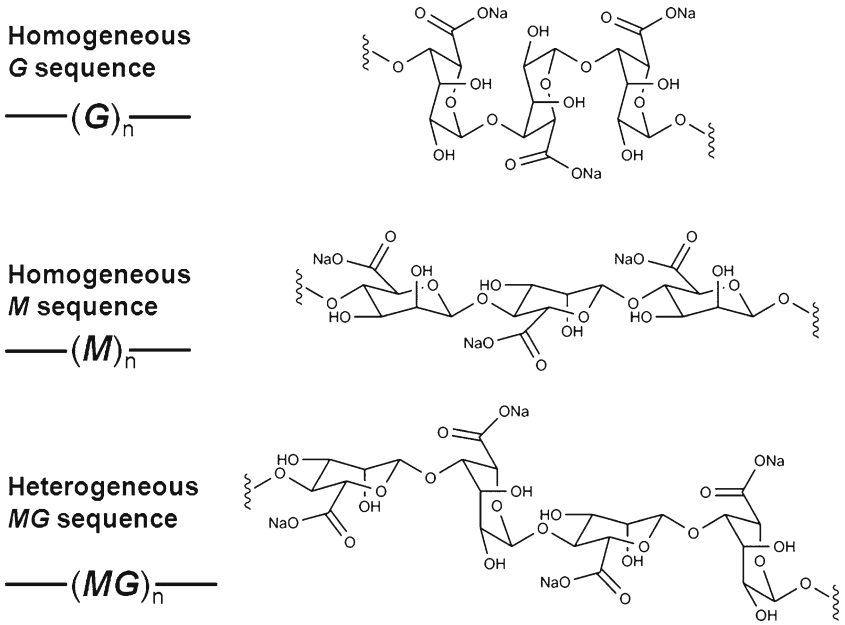

Fig. 1. Sodium alginate sequences (from *top to bottom*): homogeneous *G* sequence, homogeneous *M* sequence, and heterogeneous *MG* sequence [*G*=guluronic acid, *M*= mannuronic acid]

processing methods used. Current pharmacopeial specifications (USP-NF) for sodium alginate do not enable the characterization of variations in the molecular weight distribution and/or chemical composition of sodium alginate. Since these variations can markedly affect the processability or performance of a sodium alginate-containing pharmaceutical product (8,9), adherence to the USP-NF monograph may not ensure the interchangeability of sodium alginates from different sources or even various batches from the same manufacturer. Previous studies have shown that the inter-manufacturer and/or inter-batch (lot) variability of excipients can exert a significant effect on the performance of the final formulations, even though these excipients meet the pharmacopeial specifications (10–14). Since pharmacopeial specifications focus on identity, purity, and safety, they are not guarantors of excipient performance. Thus, it is important for pharmaceutical manufacturers to develop effective methods for the characterization of sodium alginate in order to help establish the design space for sodium alginate-based formulations (1).

An effective method of excipient characterization should reflect the excipient's behavior during processing and its functionality in potential formulations. Since sodium alginates are mostly used as thickeners, binders, and gel-forming agents in both conventional and controlled-release formulations, their processability and functionality can be related to their rheological behavior in solution. Rheological methods have many advantages over other methodologies for the characterization of polymer solutions: simple sample preparation, short times required for tests, and direct measurement of polymer behavior under conditions expected to be encountered during formulation processing or product storage or use. Nonetheless, the rheological properties of sodium alginate solutions are not specified in the United States Pharmacopoeia.

Even when excipient manufacturers do supply rheological data for sodium alginates, they typically only report the apparent viscosities of sodium alginate solutions at one specific concentration and at one temperature—"one-point" measurements—as if the alginate solutions' rheological characteristics were those of Newtonian fluids. In fact, the typical rheological behavior of many polymer solutions is highly shear- and concentration-dependent, encompassing the range from Newtonian to shear-thinning non-Newtonian to viscoelastic behavior (15,16). The shear rates encountered in pharmaceutical manufacturing and in product use can vary considerably, ranging from 10^{-4} to 10^{4} s^{-1} (17). Thus, "one-point" apparent viscosity values provide little or no insight into the selection of suitable polymer grades for a specific formulation or manufacturing process (18). A comprehensive rheological evaluation of sodium alginate solutions is warranted in order to facilitate the identification of criteria that would allow inter-grade or inter-batch comparisons.

Although a number of studies have been published on the rheological behavior of sodium alginate solutions (19–29), most of the sodium alginates employed in these studies were not pharmaceutical-grade. In addition, these studies were limited to the rheological characterization of sodium alginate solutions at concentrations lower than 5% *w/v*. Sodium alginate solutions at these low concentrations exhibit fluid-like behavior, whereas sodium alginate solutions at higher concentrations display a more substantial viscoelastic character. Unfortunately, no studies have been conducted on these more highly concentrated, substantially viscoelastic solutions of sodium alginate.

QbD necessitates an understanding of the rheological behavior of the excipient utilizing experimental conditions and excipient concentrations appropriate to the formulation and processes under consideration. The absence of meaningful published data underscores the need for rheological

Table I. Sodium Alginate Grades and Physicochemical Properties Specified by FMC Biopolymer

Grade	FMC product name	Percent G	Viscosity range[a]
1	Protanal LF10/60LS	35–45	20–70
2	Protanal LF240D	30–35	70–150
3	Protanal LF120M	35–45	70–150
4	Protanal LF200M	35–45	200–400
5	Protanal LF200DL	55–65	200–400
6	Protanal HF120RBS	45–55	600–800

[a] Viscosity data reported in manufacturer's certificate of analysis [viscosity was determined on 1% *w/v* sodium alginate solutions at 20°C using a Brookfield viscometer]

methods that would be appropriate for polymeric excipient evaluation relative to pharmaceutical processing and formulation performance. Since rheological measurements generate numerical test results instead of limit test results, summary statistics of the grade-to-grade and batch-to-batch rheological parameters will benefit both the excipient manufacturer and pharmaceutical manufacturer.

A persistent problem in traditional formulation development stems from the lack of awareness of excipient variability. Following QbD principles, users need to understand the inter-grade and inter-batch variability of excipients and its possible impact on formulation processing and product performance. This study is intended to determine the inter-grade and inter-batch variability of sodium alginate using appropriate rheological methods and conditions, thereby providing insight into the delineation of the design space as part of QbD for sodium alginate-based formulations.

MATERIALS

Six grades of sodium alginate (comprising one batch of each grade)—produced by FMC Biopolymer (Drammen, Norway)—representing a wide range of viscosities, were provided by the manufacturer (Table I) along with ten additional batches of one of the grades (LF120M, Table II). Deionized water was obtained from a *Milli-Q* ultrapure water system (Millipore Corp., Billerica, MA, USA). Sodium chloride (ACS grade) was purchased from Sigma Aldrich (St. Louis, MO, USA) and used as supplied.

METHODS

Calcium Content Determination

Sodium alginate solutions (0.1% *w/v*) were prepared and the calcium content then determined by atomic absorption spectroscopy (Atomic Absorption Spectrophotometer, Model 1100, Perkin-Elmer, MA) (30).

Determination of G/M Ratio by Solid-State ^{13}C NMR (SSNMR)

The G/M ratios in the intact sodium alginate powders were determined by SSNMR:

Solid-state ^{13}C NMR spectra were acquired using a Chemagnetics CMX-300 spectrometer (Varian, Inc., Fort Collins, CO) operating at approximately 75 MHz for ^{13}C. Chemagnetics double-resonance probes equipped with Revolution NMR 7-mm spinning modules (Revolution NMR, LLC, Fort Collins, CO) were used to acquire all spectra. Samples were packed into zirconia rotors and sealed with Teflon end-caps. Spectra were acquired using ramped-amplitude cross-polarization, magic-angle spinning (MAS) with total sideband suppression, and SPINAL64 decoupling. Spectrometer settings were optimized and the reference frequency set using 3-methylglutaric acid. A

Table II. Ten Batches of Sodium Alginate—Protanal LF120M—Produced in 2007 and Physicochemical Properties Specified by FMC Biopolymer

Batch	Manufacturer's batch no.	Manufacturing date	Viscosity[a], mPa·s
A	19,338	01-23-2007	95
B	19,440	02-26-2007	97
C	19,626	04-24-2007	109
D	19,664	05-11-2007	104
E	19,748	06-04-2007	97
F	19,812	06-15-2007	99
G	19,961	08-20-2007	96
H	20,041	09-11-2007	101
I	20,076	10-10-2007	112
J	20,228	11-12-2007	105

[a] Viscosity data reported in manufacturer's certificate of analysis [viscosity was determined on 1% *w/v* sodium alginate solutions at 20°C using a Brookfield viscometer]

contact time of 1 ms, MAS frequency of 4.0 kHz, and a ^{1}H-decoupling field of approximately 80 kHz were used to acquire all spectra. The recycle delays varied based upon $^1HT_1$ values for each sample, which were measured using saturation recovery experiments. Using Chemagnetics Spinsight software, plots of integrated signal intensity *versus* saturation recovery times were fit to Eq. 1:

$$y = amp(1 - e^{\tau/T_1}), \tag{1}$$

where y is the integrated signal intensity, *amp* is the amplitude constant, τ is the saturation recovery time, and T_1 is the spin–lattice relaxation time. Saturation recovery times were arrayed from 0.01 to 10 s, and monoexponential curve-fitting provided an accurate fit for all data sets. A recycle delay equal to five times the $^1HT_1$ value of each sample was used to acquire each spectrum. Some 5,120 transients were acquired in order to achieve a high signal-to-noise ratio. Deconvolution of peaks in the region 60–90 ppm was achieved using Chemagnetics Spinsight software, and peak areas were then used to calculate the amount of guluronic and mannuronic acid present in each sample.

Intrinsic Viscosity

The apparent viscosities of aqueous sodium alginate solutions containing sodium chloride (0.1 M) and of the solvent, $\eta_{solution}$ and $\eta_{solvent}$, respectively, were evaluated at 25°C by using an Ubbelohde viscometer (Cannon Instruments, State College, PA). All alginate concentrations reported in this work were corrected for moisture content as determined by TGA (TA Instruments, New Castle, DE). Intrinsic viscosities $[\eta]$ were determined from the concentration dependence of the reduced specific viscosity $\frac{\eta_{sp}}{C}$ in accordance with the Huggins equation:

$$\frac{\eta_{sp}}{C} = [\eta] + k' \cdot [\eta]^2 \cdot C, \tag{2}$$

where C is concentration in grams per deciliter, k' is a constant, and η_{sp} is specific viscosity:

$$\eta_{sp} = \frac{\eta_{solution}}{\eta_{solvent}} - 1 \tag{3}$$

Weight average molecular weight (M_w) and number average molecular weight (M_n) were calculated according to Mark-Houwink-Sakura equation with the following constants (31):

$$[\eta] = 0.023 \cdot {M_w}^{0.984} \Rightarrow M_w = \left(\frac{[\eta]}{0.023}\right)^{\frac{1}{0.984}} \tag{4}$$

$$[\eta] = 0.095 \cdot {M_n}^{0.963} \Rightarrow M_n = \left(\frac{[\eta]}{0.095}\right)^{\frac{1}{0.963}} \tag{5}$$

where M_w and M_n are expressed in kilodaltons.

Steady Shear

Since sodium alginate is commonly used as a thickening agent in suspensions or emulsions at concentrations ranging from 1% to 3% (7), steady shear measurements were performed on sodium alginate solutions at 1%, 2%, and 3% *w/w* for the six grades and 2% *w/w* for the ten batches of grade 3 using a controlled stress/rate (CS/CR) rheometer (AR-2000, TA Instruments, New Castle, DE) with a cone-and-plate accessory (ϕ=40 mm; θ=1°). Sample temperatures were maintained at 25±0.1°C by a Peltier temperature-control system.

Small-Amplitude Oscillation

Small-amplitude oscillation (SAO) measurements were performed on sodium alginate solutions over a wide range of concentrations using an AR-2000 CS/CR rheometer (TA Instruments, New Castle, DE), equipped with a cone-and-plate accessory (ϕ=40 mm; θ=1°). Frequency sweeps were performed with the angular frequencies (ω) ranging from 1 to 100 rad/s at 25°C and/or 37°C with 10% strain. Strain sweeps from 1% to 100% were carried out to make sure that the 10% strain applied during the frequency sweep was in the linear viscoelastic region for the samples tested. Sample temperatures were maintained at 25±0.1°C or 37±0.1°C by a Peltier temperature-control system.

Data Analysis

Rheological data of the solutions of sodium alginate were analyzed *via* analysis of variance (ANOVA) and Levene's test for homogeneity of variance using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA).

Table III. Calcium Content, % G, $[\eta]$, and Calculated Average Molecular Weights Calculated Based on the Intrinsic Viscosities of the Six Grades of Sodium Alginate

Grade	Calcium (%) (mean±SD)	Percent G per FMC (% range)	Percent G per SSNMR (% range)	[η], dL/g, (mean±SD)	M_w, kDa	M_n, kDa
1	0.42±0.0023	35–45	37–42	5.53±0.15	263	68
2	0.51±0.0023	30–35	33–36	6.04±0.09	288	75
3	0.41±0.0039	35–45	38–42	6.43±0.06	306	80
4	0.26±0.0023	35–45	39–43	8.72±0.24	418	109
5	0.08±0.0039	55–65	48–53	8.54±0.12	409	107
6	0.28±0.0023	45–55	43–47	11.26±1.21	541	142

Mean and standard deviation were calculated from three replicates

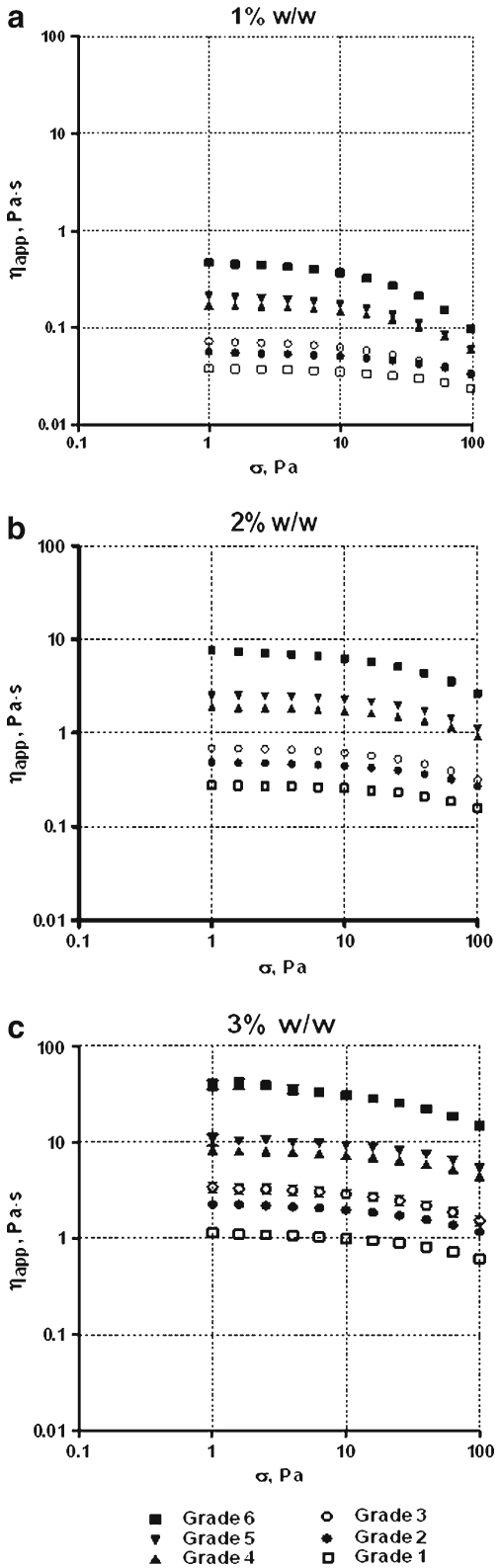

Fig. 2. Steady shear results of sodium alginate solutions at three concentrations at 25°C: apparent viscosity as a function of shear stress for **a** 1%; **b** 2%; and **c** 3% *w/w* solutions. Data are shown as mean and standard deviation of six replicates

Post hoc testing ($p<0.05$) of the multiple comparisons was performed by either the Tukey's HSD (Honestly Significant Difference) test or Games–Howell test depending on whether the outcome of Levene's test was insignificant or significant, respectively.

RESULTS AND DISCUSSION

Inter-grade Variability

Calcium Content, Chemical Composition, and Intrinsic Viscosities

The calcium content, chemical composition, and intrinsic viscosity ([η]) data for the six grades of sodium alginate are listed in Table III. Sodium alginate, extracted from seaweed using the calcium alginate method (32), may have residual calcium that could influence the rheological properties of the resultant sodium alginate solution. In some instances, calcium salts are added to sodium alginate to increase viscosity of the corresponding polymer solutions (32). The calcium content of the sodium alginate employed in this study was found to vary from 0.08% to 0.51% *w/w*. The corresponding molar ratios of calcium to sodium alginate monomer range from 0.004 to 0.025. Since calcium/alginate monomer molar ratios below 0.05 have been reported to exert little or no effect on the apparent viscosities of aqueous alginate solutions measured at different rates of shear (33), the ratios determined for the sodium alginates used in this study do not warrant concerns regarding the possible untoward influence of calcium on sodium alginate solution rheology.

The guluronic acid percentages (% G) of the different grades—as determined in our laboratory—are within the ranges specified by the manufacturer, except for grades 5 and 6 for which the % G values determined by SSNMR are slightly lower than the values listed in the manufacturer's specifications. The range of % G reported by the manufacturer was determined using ^{1}H NMR spectroscopy in solution. However, as sample preparation for solution NMR

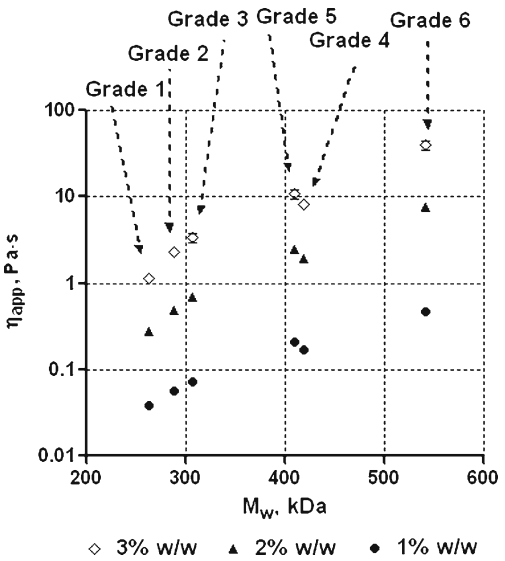

Fig. 3. Apparent viscosities (shear stress=1 Pa; 25°C) of sodium alginate solutions (1%, 2%, and 3% *w/w*) as a function of average M_w (calculated based on intrinsic viscosities). Data are shown as mean and standard deviation of six replicates

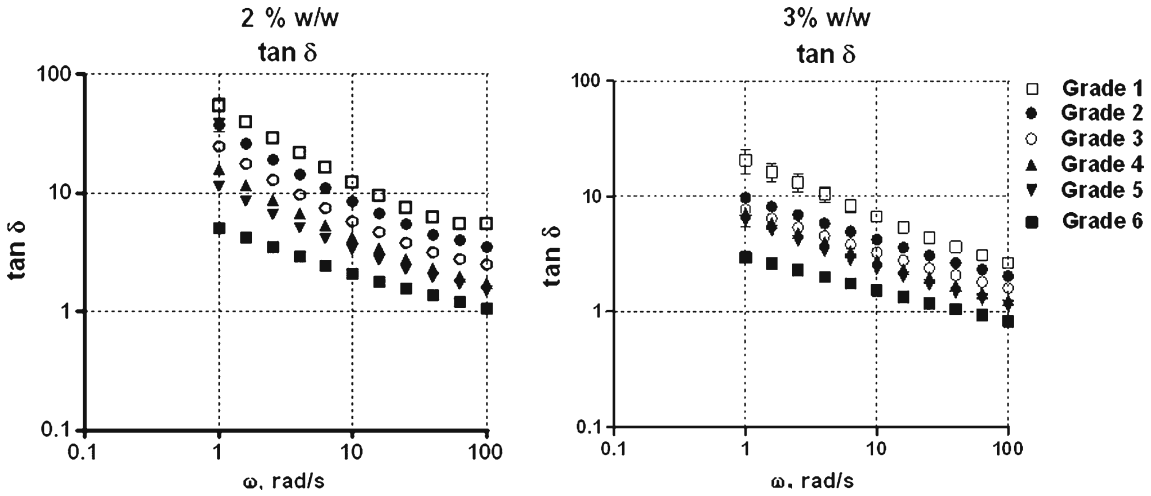

Fig. 4. The tan δ as a function of angular frequency (ω) for sodium alginate solutions at 2% and 3% *w/w* at 25°C. Data are shown as mean and standard deviation of six replicates

requires partial acid hydrolysis of the alginate chain, sample alteration or loss of insoluble material can occur (34,35). Therefore, analysis of the intact solid sample may actually give a better representation of the alginate composition.

The range of intrinsic viscosities (and the corresponding molecular weights) of the sodium alginate differs by approximately twofold among the six grades. The rank-order of intrinsic viscosities of the six grades corresponds, approximately, to the viscosity range specified by the manufacturer: the higher the viscosity grade, the higher the intrinsic viscosity. Interestingly, although there are differences in the intrinsic viscosities of grades 2 and 3, these two grades are characterized by the manufacturer as having the same range of solution viscosities. This is also true for grades 4 and 5.

Steady Shear

The steady shear rheological properties of the solutions of the six grades of sodium alginate at 1%, 2%, and 3% *w/w* concentrations, at 25°C, are depicted in Fig. 2, where the apparent viscosity (η_{app}) is plotted as a function of shear stress. The rank-order of the various sodium alginate grades based on the apparent viscosities of their solutions is grade 1 <grade 2<grade 3<grade 4<grade 5<grade 6 for all three concentrations. Statistical analysis (ANOVA) of the steady shear data (six replicates) shows that apparent viscosities of the six grades are significantly different from each other at all three concentrations ($P<0.001$). *Post hoc* multiple-pair comparisons reveal that all these grades are significantly different from each other at each concentration. Thus, those alginate grades specified by the manufacturer as having the same viscosity range may show significant differences in their rheological behavior under different conditions corresponding to a specific process or product use. As shown in Fig. 2, the apparent viscosities of all the alginate solutions decrease with increasing shear stress, demonstrating the shear-thinning nature of alginate solutions at these concentrations. It is consistent with previous observations of shear-thinning behavior of solutions of sodium alginate at these concentrations (19,21,29,31).

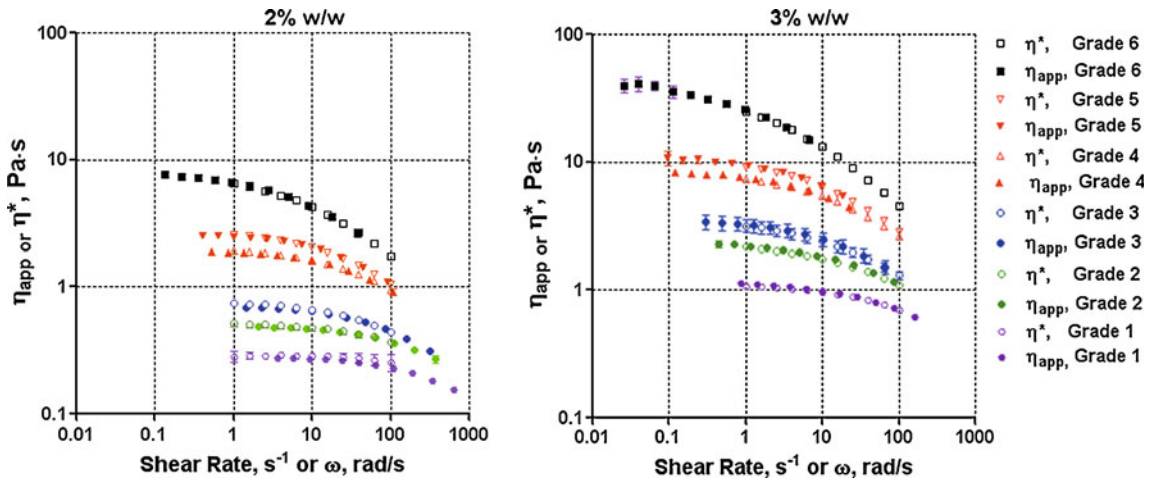

Fig. 5. Complex viscosity (η^*) and apparent viscosity (η_{app}) as a function of angular frequency (radians per second) and shear rate (per second), respectively, for solutions of six grades of sodium alginate at 2% and 3% *w/w* and 25°C (*solid symbols* represent apparent viscosities; *open symbols* represent complex viscosities). Data are shown as mean and standard deviation of six replicates

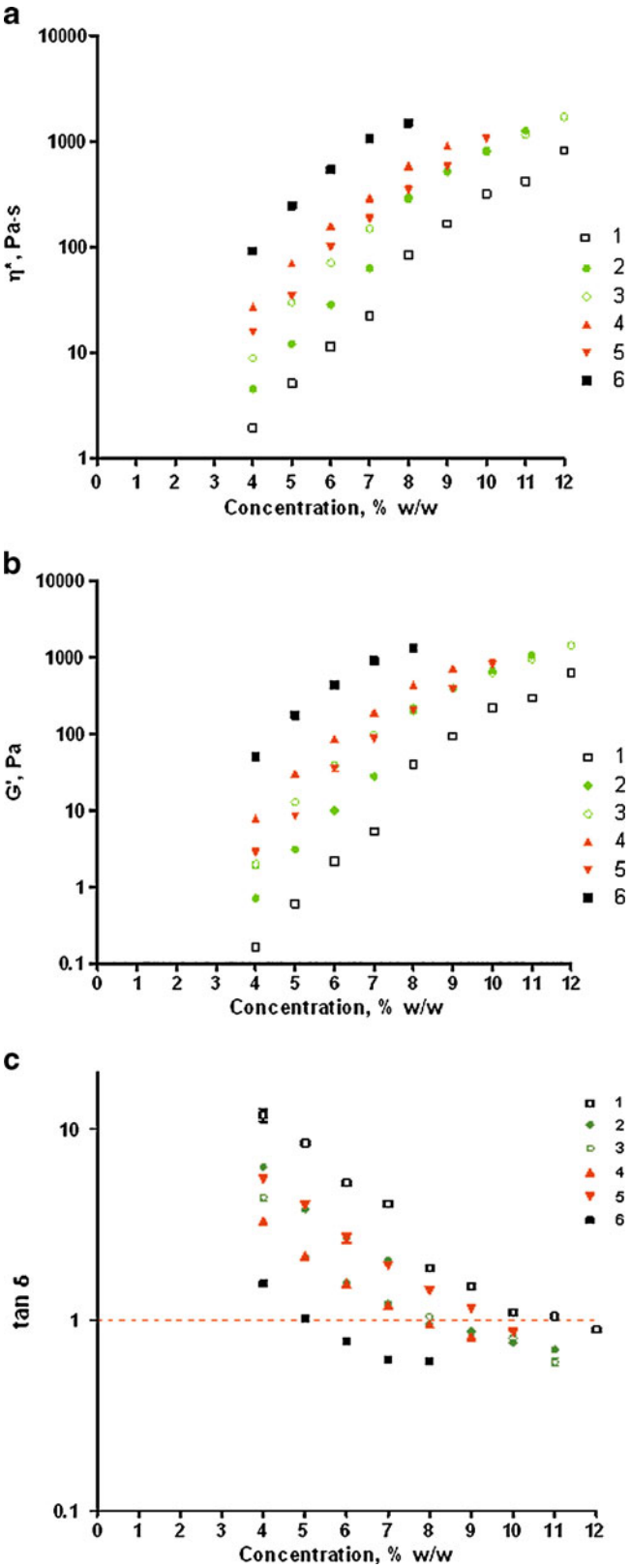

Fig. 6. Concentration dependence of viscoelastic parameters (determined at 1 rad/s) of the solutions of sodium alginate (six grades) at 37°C: **a** complex viscosity (η^*); **b** storage modulus (G'), and **c** tan δ. Data are shown as mean and standard deviation of three replicates

It is evident from Fig. 2 that the differences in apparent viscosity among these grades of sodium alginate become larger at higher alginate concentrations or at lower shear stresses. It emphasizes the importance of determining the apparent viscosities at concentrations and shear conditions relevant to the formulations, *e.g.*, apparent viscosities at low shear (*e.g.*, 1–50 s^{-1}) can be useful in the development of a suspension formulation while apparent viscosities at high shear (> ~5,000 s^{-1}) are more appropriate for solutions used in coating or spray-drying processes.

The apparent viscosities of solutions of these six grades are consistent, for the most part, with the expectation that higher average molecular weights (Table III) would result in higher apparent viscosities. This is depicted in Fig. 3 where apparent viscosities (1 Pa shear stress, 25°C) of sodium alginate solutions are plotted as a function of the M_w values estimated from the corresponding intrinsic viscosities. Since sodium alginate is a linear unbranched polymer, higher molecular weights increase the likelihood of inter-chain interactions in solutions under shear resulting in correspondingly higher viscosities.

One anomaly in the data is that grade 5 results in apparent viscosities that are significantly higher than those for grade 4 at corresponding shear stresses. On one hand, grade 5 has an average molecular weight that is slightly less than that of grade 4. On the other hand, grade 5 is higher in % G than grade 4. Thus, a possible explanation for this anomalous rheological behavior is that the higher percentage of *G* sequences in the alginate molecular chain of grade 5 leads to stiffer, *i.e.*, more extended, alginate chain conformations in solution (3–5) and an increased likelihood of inter-chain interaction under steady shear.

Although size exclusion chromatography has been used to determine the molecular weight distribution of polymeric excipients and NMR has been employed to characterize the chemical composition of such excipients, rheological methods are easier to perform and can measure functionality-related properties of the excipients in a relatively short time period. Furthermore, rheological behavior is indicative of the molecular weight distribution of a polymeric mixture (36–38). Thus, steady shear behavior under specific shear conditions can be employed for assessment of the quality of polymeric excipients used as thickening agents prior to product manufacturing. This work focuses on different grades of excipient from the same manufacturer, but the same methods can be applied to ensure interchangeability or equivalence of an excipient from different manufacturers.

A specific range of apparent viscosities can be achieved by employing different grades of sodium alginate at different concentrations. Therefore, for those formulations whose functionality can be related to apparent viscosity, multiple grades of sodium alginate could be included in the design space as long as alginate concentration and mechanical conditions (*e.g.*, shear rate or stress) were also specified. For example, when developing a suspension with desired apparent viscosities between 150 and 300 mPa·s under low shear conditions (*i.e.*, 1–10 Pa), grades 2 or 3 at 1% *w/w*, or grade 1 at 2% *w/w*, can be included in the design space for this formulation. It is more reasonable and practical to employ the apparent viscosity values as justification for inclusion of excipients in a formulation—by adjusting excipient concen-

Table IV. Calcium Content,% G, [η], and Calculated Average Molecular Weights Calculated Based on Intrinsic Viscosities of the Ten Batches of Sodium Alginate Grade 3

Batch	Calcium content, % (mean±SD)	Percent G per SSNMR (% range)	[η], dL/g (mean±SD)	M_w, kDa (average)	M_n, kDa (average)
A	0.69±0.010	37–41	7.80±0.20	373	97
B	0.73±0.006	38–41	7.14±0.07	341	89
C	0.42±0.008	38–41	7.32±0.18	350	91
D	0.62±0.009	38–40	7.48±0.11	357	93
E	0.54±0.002	38–41	6.67±0.12	318	83
F	0.55±0.012	37–41	6.87±0.27	328	85
G	0.56±0.008	39–41	6.53±0.39	311	81
H	0.45±0.006	38–40	6.91±0.29	330	86
I	0.36±0.008	39–42	7.33±0.19	350	91
J	0.41±0.006	39–42	7.16±0.09	342	89

Mean and standard deviation were calculated from three replicates

trations to achieve the same apparent viscosities—than by selecting excipients based on their apparent viscosity data at one concentration. The inclusion of multiple excipient grades in the design space would be especially important when excipient production or availability is problematic. In fact, the concept of formulation design space was proposed to FDA as a post-approval activity during FDA generic drugs workshop in June 2009 (39).

Small-Amplitude Oscillation

At 1% *w/w* concentrations, sodium alginate solutions show little evidence of elastic behavior or network formation: the values of storage modulus G' are negligible. However, at concentrations≥2% *w/w*, the viscoelastic moduli (storage modulus G', loss modulus G'') are more substantial. SAO results for 2% and 3% *w/w* solutions are shown in Fig. 4 in terms of tan δ, *i.e.*, the ratio of the loss modulus (G'') to the storage modulus (G'). Higher alginate concentration leads to lower tan δ values for all six grades of sodium alginate. ANOVA tests of tan δ of solutions of these grades at both concentrations showed significant differences among these six grades of sodium alginate ($P<0.001$). The results of *post hoc* multiple comparisons test indicate that grades 4 and 5 do not show any significant differences in their viscoelasticity at 2% and 3% *w/w*. Interestingly, there is no significant difference in tan δ between grades 3 and 4 at 3% *w/w* although their apparent viscosities are significantly different at this concentration. Thus, grades that are significantly different in their apparent viscosities may not necessarily be significantly different in their viscoelastic parameters at the same concentration. This phenomenon could be due to differences in intra-polymer, inter-polymer, and polymer–solvent interactions under different shear conditions, *i.e.*, steady flow *versus* SAO.

Cox–Merz Rule

The quasi-empirical Cox–Merz rule (40,41) states that the steady shear apparent viscosity (η_{app}) and the magnitude of the complex viscosity (η^*) of linear polymer solutions are superimposable at numerically equivalent values of shear rate (per second) and angular frequency (radians per second).

The complex viscosity is defined by $\eta^* = \frac{\left(G'^2+G''^2\right)^{1/2}}{\omega}$. As shown in Fig. 5, our rheological data for 2% *w/w* and 3% *w/w* solutions of the six grades of sodium alginate obey this rule. Conformity to the Cox–Merz rule is evidence for the absence of gel structure in these solutions (42). It also confirms that the calcium content of these sodium alginates does not exert a significant effect on the rheological behavior of their solutions at these concentrations.

Concentration Effect on Viscoelasticity

The controlled-release of drug substances from sodium alginate matrices would be expected to correlate with the swelling and erosion of the polymer (8,9). The alginate concentrations in the gel and diffusion layers around the polymer matrix could be expected to range from very high values at the boundary with the unhydrated alginate to relatively dilute concentrations at the boundary with the bulk alginate solution. The inter- and intra-polymer interactions involved in the erosion process are believed to be the same factors resisting strain under small-amplitude oscillation measurements (43). Therefore, the characterization of the rheological properties of sodium alginate solutions under

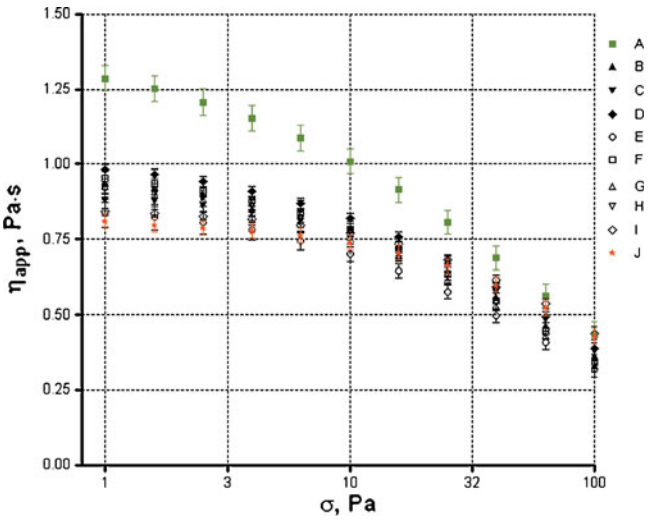

Fig. 7. Steady shear result of sodium alginate (ten batches of grade 3) solutions at 2% *w/w* at 25°C. Data are shown as mean and standard deviation of six replicates

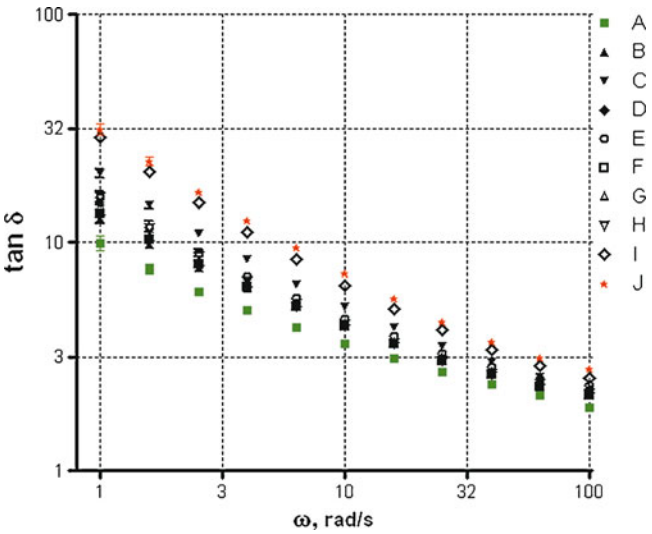

Fig. 8. Angular frequency dependence of tan δ of the 8% *w/w* solutions of sodium alginate (ten batches of grade 3) at 25°C. Data are shown as mean and standard deviation of six replicates

small-amplitude oscillation was extended to a wider range of sodium alginate concentrations in order to mirror the range of conditions that could be encountered in sodium alginate-based controlled-release formulations during use.

The viscoelastic parameters of solutions of the various sodium alginate grades with increasing concentration is illustrated in Fig. 6, which depicts the concentration dependence of the complex viscosity (η^*), storage modulus (G') , and tan δ of these sodium alginate solutions at 37°C, the temperature that peroral alginate formulations would be exposed to in the alimentary tract.

The η^* and G' values increase with increasing concentrations for all six grades, representing an increased degree of chain entanglement. Grades 1 and 6 exhibit the lowest and highest viscoelastic parameters over the concentration range investigated. It is noticed that viscoelastic parameters, *e.g.*, η^* and G' , of grade 4 solutions are larger than those of grade 5 solutions at concentrations higher than 4% *w/w*, in contrast to the rheological behavior seen in their solutions at lower concentrations (2%, 3% *w/w*). Grade 4's η^* and G' values are higher than those of grades 2, 3, and 5 over the whole concentration range while the η^* and G' values for grades 2, 3, and 5 overlap each other at concentrations higher than 8% *w/w*. The tan δ values decrease with increasing concentrations and reach 1 at different concentration for these six grades, indicating that these sodium alginate solutions change from the fluid state to the gel state with increasing concentrations due to increasing polymer chain entanglements. The transition from a polymer solution to a polymer gel occurs at the critical concentration, *i.e.*, when tan δ=1 (44,45). Critical concentrations for the alginate grades 1, 2, 3, 4, 5, and 6 were estimated to be 11.2%, 7.9%, 7.7%, 7.8%, 9.5%, and 5.1% *w/w*, respectively.

Generally, for linear polymers, the higher the molecular weight, the larger the degree of chain entanglement at high concentrations and the lower the critical concentration (46). However, in this study, grades 2, 3, and 4 are substantially different in their molecular weight but similar in their critical concentration. Grade 5, which is relatively high in both molecular weight and % G, showed higher tan δ values (and higher critical concentration) than grades 2 and 3. Although grades 4 and 5 are not significantly different from each other in viscoelasticity at lower concentrations, *i.e.*, 2% and 3% *w/w*, their viscoelasticity profiles at higher concentrations are significantly different from each other. Since the *G* sequence has the most rigid and extended chain conformation in solution (3–5), sodium alginates with higher % G may have a lower degree of chain entanglement at high concentrations than those with a lower % G. Thus, the viscoelasticity of the various grades of sodium alginate at lower concentrations is not indicative of their viscoelasticity profile at higher solution concentrations. There does not appear to be a simple relationship among polymer molecular weight, polymer concentration, chemical composition, and the degree of chain interaction.

The pharmaceutical grades of sodium alginate, as with most polymeric pharmaceutical excipients, are grouped based on the apparent viscosity of their solutions at low concentrations, *e.g.*, 1% *w/v*. In fact, in most studies of sodium alginate matrices in the literature, only the "one-point"

Table V. Results for Multiple Comparisons Test of the Rheological Parameters of the Solutions of the Ten Batches of Sodium Alginate (Grade 3)

Batch										
A	A									
B	a,3	B								
C	a,t,2,3	3	C							
D	a,3	[a]	3	D						
E	a,2,3	3	3	3	E					
F	a,3	[a]	3	3	3	F				
G	a,2,3	[a]	3	3	3	[a]	G			
H	a,1,2,3	3	[a]	2,3	3	3	3	H		
I	a,t,1,2,3	t,2,3	3	t,2,3	3	t,2,3	t,3	t,3	I	
J	a,t,1,2,3	t,1,2,3	1,3	t,1,2,3	t,3	t,2,3	t,2,3	t,3	3	J

The numbers in the cells correspond to significant differences in the paired data for specific rheological outcomes, as follows:
a=in log η_{app} (2% *w/w*, 25°C); *t*=in tan δ (2% *w/w*, 25°C)
1=in log η^* (8% *w/w*, 37°C); 2=in log G′ (8% *w/w*, 37°C)
3=in tan δ (8% *w/w*, 37°C)
[a] No significant differences

apparent viscosities of low concentration sodium alginate solutions were characterized (8,9,47,48). More likely than not, the incomplete rheological characterization of sodium alginate solutions is responsible for the disparity among different studies on the significance of the influence of the viscosity grade of the polymer on drug release from alginate matrices (8,9,47,48). A rational approach to QbD requires a more complete understanding of the rheological behavior of these polymeric excipients as a function of excipient concentration and mechanical condition appropriate to the processing and performance of pharmaceutical formulations. The inclusion of different grades of sodium alginate in the design space for alginate-based formulations necessitates the characterization of both the steady shear and viscoelastic properties of sodium alginate solutions over the range of concentrations that may be encountered.

Inter-batch Variability

The following data reflect the evaluations of the one grade (grade 3) that was available in multiple batches.

Calcium Content, Chemical Composition, and Intrinsic Viscosities

The calcium content, chemical composition, and intrinsic viscosity ([η]) data for the ten batches of grade 3 are listed in Table IV. The calcium content of the sodium alginates employed in this study varies from 0.36% to 0.73% *w/w*. The corresponding molar ratios of calcium to sodium alginate monomer of the multiple batches range from 0.018 to 0.036, which is below the critical ratio (*i.e.*, 0.05) for calcium to exert significant effect on the rheological properties of aqueous alginate solutions (33). The % G values of the multiple batches as determined in our laboratory range from 37% to 41%, within the range specified by the manufacturer. Intrinsic viscosities of the ten batches vary from 6.53 to 7.80 dL/g.

Steady Shear and Small-Amplitude Oscillation

The inter-batch variability of grade 3 is determined by comparing the apparent viscosities of 2% *w/w* solutions of the various batches at 25°C. The inter-batch variability was further investigated by comparing the viscoelastic parameters of the various batch solutions at 8% *w/w* at 37°C, based on the earlier determination of the critical concentration for grade 3. The apparent viscosities of sodium alginate solutions at 2% *w/w* are depicted in Fig. 7. In contrast to the viscosity data provided in the certificates of analysis, batch *A* exhibits substantially higher apparent viscosities than the other batches. ANOVA and the subsequent multiple comparisons tests demonstrate that batch *A* is significantly different from all other batches in the apparent viscosities of its 2% *w/w* solutions while other batches are not significantly different in their apparent viscosities.

The tan δ as a function of angular frequency for 2% *w/w* solutions of multiple batches at 25°C is depicted in Fig. 8. Based on ANOVA, significant differences in tan δ among the ten batches are evident ($P<0.001$). *Post hoc* multiple comparisons test indicated that more batches are significantly different in tan δ than in η_{app} (Table V). In addition, those batches that are significantly different in η_{app} are not necessarily significantly different in tan δ.

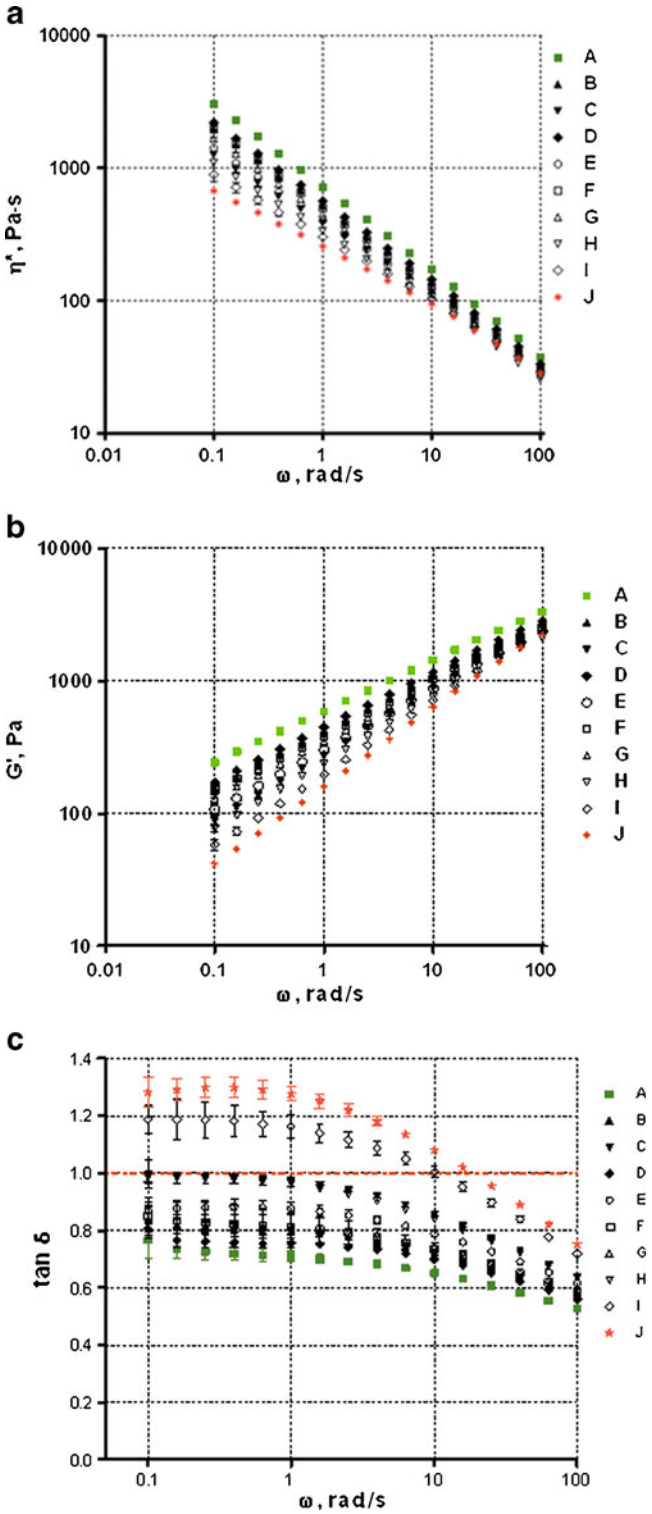

Fig. 9. Angular frequency dependence of viscoelastic parameters of the 8% *w/w* solutions of sodium alginate (ten batches of grade 3) at 37°C: **a** complex viscosity (η^*); **b** G′ , and **c** tan δ. Data are shown as mean and standard deviation of six replicates

Figure 9 depicts η^*, G', and tan δ of the solutions of multiple batches (8% *w/w*) over a wide range of angular frequencies at 37°C. Based upon ANOVA, significant differences ($P<0.001$) among these multiple batches in all three viscoelastic parameters are evident. The result of the *post hoc* multiple comparisons test for rheological parameters is summarized in Table V. These batches show more variability in viscoelastic parameters at this high concentration, especially in their G' and tan δ values.

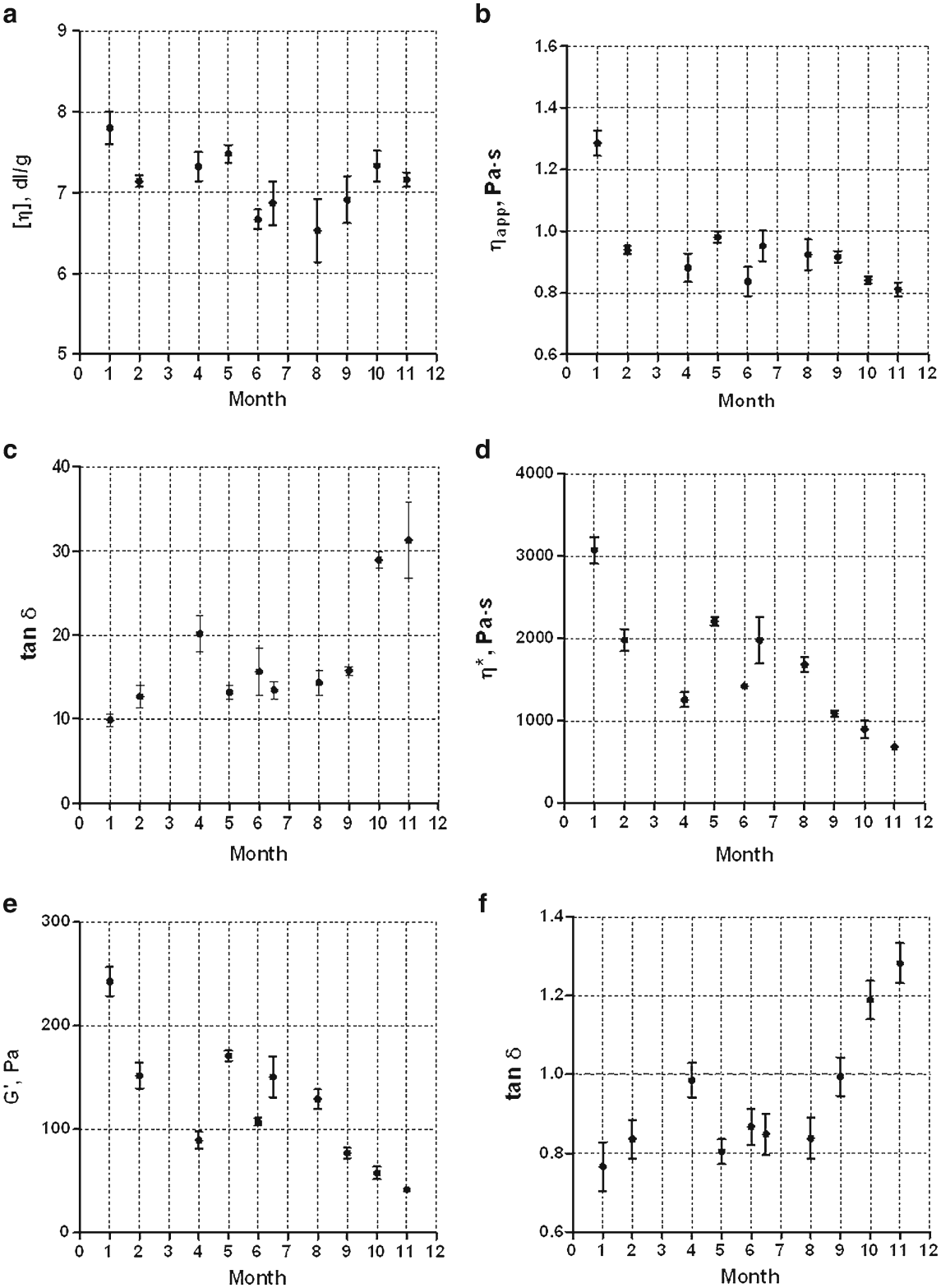

Fig. 10. Temporal variations in rheological parameters of the solutions of multiple batches of grade 3: **a** $[\eta]$; **b** η_{app} (2% *w/w*, σ=1 Pa, 25°C); **c** tan δ (2% *w/w*, ω=1 rad/s, 25°C); **d** η^*; **e** G'; and **f** tan δ (8% *w/w*, ω=0.1 rad/s, 37°C)

Inter-batch Variability as a Function of Date of Sodium Alginate Manufacture

Figure 10 depicts the variations in $[\eta]$, η_{app} (2% *w/w*, σ = 1 Pa, 25°C), tan δ (2% *w/w*, ω=1 rad/s, 25°C), η^*, G', and tan δ (8% *w/w*, ω=0.1 rad/s, 37°C) as a function of time. The statistical analyses presented in Table V show that the batch produced in January is significantly different from the batches produced later in the year. The largest differences in the rheological parameters are evident between the batch produced in January and the last two batches produced in October and November. Batches manufactured between February and September show relatively small variations in their rheological behavior. Thus, the season of harvesting may well be a factor contributing to the batch-to-batch variability of sodium alginate.

In the final analysis, for sodium alginate used as a thickener or binder, the authors recommend the use of steady shear measurements of sodium alginate solutions over a relatively wide range of shear stresses or shear rates. The resultant apparent viscosities or rheograms could be used to ensure batch-to-batch, grade-to-grade, or supplier-to-supplier interchangeability of sodium alginate and to define the design space—in accordance with QbD principles—for specific formulations. For sodium alginate used in controlled-release matrices, both steady shear (at one low concentration, *e.g.*, 2% *w/w*) and small-amplitude oscillation measurements (at one high concentration, *e.g.*, 8% *w/w*) should be performed on sodium alginate solutions to ensure interchangeability and to define the design space.

CONCLUSIONS

When sodium alginate is used as a thickening agent, the rheograms of its solutions at appropriate concentrations and shear conditions should be used as the basis for establishing the interchangeability and equivalence of different grades from the same or various suppliers. At higher concentrations, when sodium alginate is used for alginate-based matrices, the viscoelastic properties of its solutions should be employed among the criteria for including different grades of sodium alginate in the design space for alginate-based matrices. Rheological evaluations of multiple batches of one grade of sodium alginate produced over the course of 1 year showed significant batch-to-batch variability in both rheological behavior at low solution concentration and viscoelastic behavior at high solution concentration. Thus, reliance on "one-point" apparent viscosity measurements is unacceptable.

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