Research Article

Inter- and Intra-Manufacturer Variability in Pharmaceutical Grades and Lots of Xanthan Gum

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Abstract. A pharmaceutical formulation typically contains one or more excipients in addition to the active pharmaceutical ingredient(s). Though excipients have been considered inert components of a formulation, variability in their properties has been shown to affect the performance of drug dosage forms and delivery systems. This study investigates the *inter-* and *intra*-manufacturer variability among different NF grades and lots of xanthan gum made by two manufacturers. As many formulators rely on compendial standards to monitor and control the variability of excipients, this study focuses on the adequacy of the NF specifications, in particular the viscosity specification, to discern the variability in solution properties of different pharmaceutical grades and lots of xanthan gum. All the grades and lots in this study were NF grade materials. Xanthan gum solutions were prepared in accordance with NF test methodology and were rheologically evaluated using a rotational rheometer. Both steady shear measurements and small amplitude oscillatory measurements were carried out on 1% *w/w* xanthan gum solutions. Results showed significant *inter-* and *intra*-manufacturer variability among the NF grades and lots of xanthan gum that was not reflected in the NF viscosity test specifications.

KEY WORDS: excipient variability; rheology; xanthan gum.

INTRODUCTION

Shangraw noted in 1987 that compendial standards have focused on identity, quality, purity, packaging, and labeling and that standards for drugs have taken precedence over standards for excipients (1). This disparity is still evident today, in spite of FDA's revision and support of the *ICH Guidance for Industry—Q8(R2) Pharmaceutical Development* that suggests that "those aspects of drug substances, *excipients.....*that are critical to product quality should be determined and control strategies justified" (2).

Published reports have underscored the effect of inter- and intra-manufacturer variability in excipient properties on processability, quality, and performance of the finished drug product (3,4). Polymeric excipients, in particular, comprise mixtures of polymers of different molecular weights and chemical composition and tend, as a result, to exhibit more variability than other excipients. Moreover, excipients are often produced by a batch process, with the possibility of batch-to-batch variation from the same manufacturer (5). To monitor and control the batch-to-batch variability of excipients, formulators typically rely on compendial specifications provided for excipients in the USP-NF (6).

This study explores the adequacy of USP-NF specifications—in particular, the viscosity specification—to address the issue of variability among different grades and lots of a commonly used excipient, xanthan gum. There are no published data attesting to the effectiveness of the NF viscosity test to discern inter- and intra-manufacturer variability among different lots of xanthan gum.

The NF monograph viscosity specification for xanthan gum requires that the apparent viscosity of a 1.0% *w/w* xanthan gum solution (with the addition of 1.0% *w/w* KCl), measured at 24±1ºC *via* a rotating spindle viscometer at 60 rpm, should not be less than 600 mPa•s. This "one-point" specification for xanthan gums only stipulates the minimum *apparent viscosity* at *one* shear rate/shear stress, as if the xanthan gum solutions' rheological characteristics were those of Newtonian fluids. Furthermore, by specifying only a minimum apparent viscosity and no *upper* boundary, the monograph fails to address the issue of excipient variability. Thus, even if various xanthan gums' solutions differ substantially in their apparent viscosity, as long as their apparent viscosities exceed the minimum of 600 mPa•s, they would meet NF specifications.

Xanthan gum is a high molecular weight exocellular polysaccharide derived from the gram-negative bacterium *Xanthomonas campestris* by an aerobic fermentation process. The structure of xanthan gum consists of a β-1,4-linked D-glucan cellulosic backbone with trisaccharide side-chains composed of β-D-mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-

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D-mannose attached to alternate glucose residues in the backbone by α-1,3-linkages (Fig. 1). The terminal mannose moiety may carry pyruvate residues linked to the 4- and 6-positions. The inner mannose unit is acetylated at C-6. Acetate and pyruvate residues are linked in variable amounts to the side chain, depending upon which *X. campestris* strain the xanthan is isolated from. The pyruvate acid content also varies with fermentation conditions. Usually, the degree of substitution for pyruvate varies between 30% and 40%, whereas for acetate the degree of substitution is as high as 60–70%. The actual distribution pattern of acetal and pyruvate residues is unknown (7). Some of the repeating units may be devoid of the trisaccharide side chain (8). The molecular weight values reported in the literature are very diverse, reflecting the inherent problems associated with xanthan gum (i.e., its very high molecular weight, its tendency to aggregate in solution, and its stiffness.) (9). Intrinsic viscosity measurements—which are used to provide a viscosity-based average molar mass—are also inaccurate due to the yield stress exhibited by xanthan gum solutions even at very dilute concentrations (10).

Xanthan gum is widely used in oral, peroral, and topical formulations as a suspending, thickening, emulsifying, or stabilizing agent (11). It has also been used as a controlled release agent in tablets, a binder in colon-specific drug delivery systems, and as a vehicle in ophthalmic liquid dosage forms (11). The global market for xanthan gum is ~$500 million annually and continues to grow (12).

MATERIALS AND METHODS

Materials

Different lots, manufactured at different times, of three pharmaceutical grades of xanthan gum were provided by two manufacturers—Manufacturers A and B (Table I). According to the manufacturers, the six grades exhibited the same range of (apparent) viscosity but differed in their particle size distributions and the clarity of the resulting aqueous solutions. All lots provided by both manufacturers met NF specifications per their respective certificates of analysis and on that basis alone would be considered interchangeable. All xanthan gum samples were stored at room temperature in tightly closed containers, in accordance with manufacturers' instructions. All samples were within their manufacturers'

recommended shelf-life when tested. Deionized water was produced by a *Milli-Q* ultrapure water system (Millipore Corp., Billerica, MA). Potassium chloride (USP-NF grade), purchased from Fisher Scientific (Waltham, MA), was used as supplied.

Methods

Solution Preparation

In this work, 1% *w/w* xanthan gum solutions were prepared—in accordance with the NF monograph specification—by slowly adding the required amount of polymer powder into a known amount of deionized water in a 400-mL beaker along with 3 g of dissolved KCl. A propeller-type variable-speed mixer (Stir-pak®, Cole-Parmer, Vernon Hills, IL) was used to gently agitate the xanthan gum dispersions for a 2-h period. During this time, the beaker was sealed with Parafilm® (SPI Supplies, West Chester, PA) to prevent water evaporation. In order to facilitate the complete hydration of the polymer, the resultant solutions were kept at rest at room temperature for an additional 12 h. Thereafter, the solutions were centrifuged for 20 min at 2,200 rpm to remove any air bubbles present followed directly by another 12-h rest period prior to conducting the rheological measurements.

The xanthan gum concentrations employed in this study were corrected for moisture content as determined by Computrac MAX 2000XL (Arizona Instrument LLC, Chandler, AZ): 1 to 2 g xanthan gum samples were heated to 105°C until the rate of change of the actual weight loss per minute was less than 0.01% of the original sample weight. Three such measurements were made and the mean value was used for calculations and subsequent solution preparations.

Rheological Evaluations

Rheological measurements of 1% *w/w* aqueous solutions of the various xanthan gums were performed with a Gemini 200 controlled stress rheometer (Malvern Instruments Ltd., Westborough, MA) with a C25 coaxial cylinder geometry, using a software-driven protocol (Bohlin Software Package, Malvern Instruments Ltd., Westborough MA). Sample temperatures were maintained at 24±0.1°C by a Peltier temperature-control system.
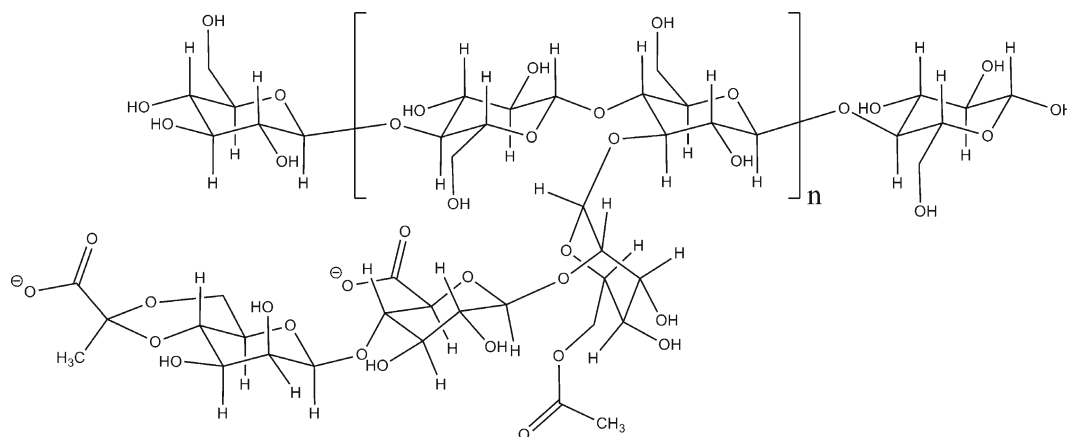

Fig. 1. Structure of xanthan gum

Table I. Xanthan Gum Grades and Lots Provided by Manufacturers A and B

Manufacturer A		Manufacturer B	
Notation	Manufacturing date (Year-Month)	Notation	Manufacturing date (Year-Month)
A1.1 (Grade 1, Lot 1)	2007-06	B1.1 (Grade 1, Lot 1)	2007-03
A1.2 (Grade 1, Lot 2)	2007-02	B2.1 (Grade 2, Lot 1)	2008-02
A2.1 (Grade 2, Lot 1)	2006-06	B2.2 (Grade 2, Lot 2)	2006-12
A2.2 (Grade 2, Lot 2)	2006-02	B3.1 (Grade 3, Lot 1)	2008-02
A2.3 (Grade 2, Lot 3)	2007-12	B3.2 (Grade 3, Lot 2)	2006-07
A2.4 (Grade 2, Lot 4)	2007-08		
A3.1 (Grade 3, Lot 1)	2006-10		

Steady shear behavior of the xanthan gum solutions was evaluated via a stepped-flow protocol over the shear stress range of 10–25 Pa. Small amplitude oscillatory behavior of the xanthan gum solutions was evaluated via frequency sweeps performed at angular frequencies (ω) ranging from 0.05 to ~43 rad/s, with 5% strain. Strain sweeps from 1% to 100% were carried out to make sure that the 5% strain applied during the frequency sweep was in the linear viscoelastic region. Storage moduli (G') and loss moduli (G'') were subsequently determined as a function of angular frequency in this region.

Experimental Design

Lots were randomly selected from among the different lots of the three grades obtained from the two manufacturers: a 1% *w/w* solution of each lot was prepared as described. Each of the solutions was evaluated twice by both steady shear and by small amplitude oscillatory methods. In total, two solutions were prepared and rheologically tested for all lots and grades, making the number of replicates for each 1% *w/w* solution equal to four.

Statistical Analysis

Statistical analyses were performed by using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL). The significance level was set at $\alpha=0.05$. Rheological data of the solutions of xanthan gum were analyzed via ANOVA. *Post hoc* testing of the multiple comparisons was performed either by Tukey's HSD test or by Games–Howell test, depending on the significance of the outcome of Levene's test for homogeneity of variances. The nonlinear regression analyses of rheological data were performed by using GraphPad Prism (version 5, GraphPad Software, Inc., La Jolla, CA).

RESULTS AND DISCUSSION

Although a number of studies on xanthan gum solution rheology have appeared in the literature to date, a comparison of the rheological properties among *pharmaceutical* grades and lots of xanthan gums manufactured by different firms has not been made until now.

Steady Shear Measurements

The steady shear rheological properties of 1% *w/w* solutions of all of the grades and lots are depicted in Fig. 2, where the apparent viscosity (η_{app}) is plotted as a function of shear stress. The apparent viscosities of all the xanthan gum solutions decrease with increasing shear stress, demonstrating the shear-thinning nature of 1% *w/w* xanthan gum solutions. These findings are in agreement with previous results for solutions of xanthan gum at this concentration (9).

Though all lots met the NF viscosity specification ($\eta_{app} \geq$ 600 mPa•s) and were purportedly of the same viscosity range,

Table II. Calculated Power Law Coefficients and Exponents of Apparent Viscosity (K, n), Storage Modulus (K', n') and Loss Modulus (K'', n'')

	$K \pm$ S.E.*	$n \pm$ S.E.*	R^2	$K' \pm$ S.E.*	$n' \pm$ S.E.*	R^2	$K'' \pm$ S.E.*	$n'' \pm$ S.E.*	R^2
A1.1	16.55±0.77	0.155±0.028	0.966	22.98±0.063	0.1911±0.0012	0.989	7.555±0.012	0.1255±0.0007	0.991
A1.2	16.84±1.00	0.157±0.018	0.987	26.86±0.060	0.1708±0.0010	0.991	7.848±0.008	0.1057±0.0005	0.994
A2.1	24.26±1.01	0.167±0.009	0.996	33.36±0.060	0.1472±0.0009	0.991	8.368±0.011	0.0924±0.0006	0.987
A2.2	22.17±0.57	0.156±0.006	0.999	30.78±0.053	0.1549±0.0008	0.993	8.100±0.009	0.0949±0.0005	0.991
A2.3	21.58±1.34	0.133±0.015	0.992	29.33±0.055	0.1600±0.0008	0.992	7.962±0.010	0.1000±0.0006	0.989
A2.4	19.59±0.97	0.131±0.011	0.998	30.81±0.055	0.1526±0.0008	0.992	7.979±0.008	0.0921±0.0005	0.991
A3.1	17.42±0.52	0.147±0.009	0.997	26.27±0.052	0.1686±0.0009	0.993	7.533±0.008	0.1095±0.0005	0.993
B1.1	18.97±5.80	0.148±0.066	0.851	31.75±0.061	0.1351±0.0009	0.988	7.412±0.014	0.0701±0.0009	0.949
B2.1	20.97±0.76	0.162±0.009	0.996	31.71±0.060	0.1384±0.0008	0.989	7.531±0.014	0.0748±0.0009	0.954
B2.2	21.30±0.72	0.138±0.008	0.998	31.52±0.061	0.1483±0.0008	0.990	8.022±0.012	0.0802±0.0008	0.974
B3.1	16.82±0.45	0.100±0.007	0.998	28.26±0.057	0.1493±0.0009	0.989	7.306±0.012	0.0848±0.0008	0.972
B3.2	16.34±1.15	0.063±0.017	0.991	29.74±0.076	0.1470±0.0011	0.982	7.592±0.012	0.0794±0.0007	0.971

*S.E. = Standard Error

Table III. Results of Multi-comparison Tests Among Different *Grades* (P=0.05)

	A1	A2	A3	B1	B2	B3
A1	A1					
A2	1 3,4 5,6	A2				
A3	-	1 3,4 5,6	A3			
B1	3,4 5,6	4 5,6	3,4 6	B1		
B2	1 3,4 6	4 6	1 3,4 6	4 5,6	B2	
B3	2 3,4 6	1,2 3,4 5,6	3,4 6	3,4 6	2	B3

The black and red numerals are indicative of significant differences in steady shear and small amplitude oscillatory rheological parameters, respectively.

$1 = K$ $2 = n$ $3 = K'$ $4 = n'$ $5 = K''$ $6 = n''$

(The shaded region indicates the presence of *inter*-manufacturer variability among grades)

differences in the rheograms for many lots were observed. In particular, lot A1.1 exhibited the lowest viscosities of all the lots.

The shear-rate dependence of apparent viscosity for shear-thinning systems can be characterized by the Ostwald-de Waele power law equation (9):

$$\eta_{\mathrm{app}} = K\dot{\gamma}^{n-1}$$

where K, the consistency index, and n, the power law index, are constants. The higher the consistency index, for a given power–law index, the higher the apparent viscosity. For shear-thinning fluids, n ranges between 0 and 1. The lower the value of the power–law index, the more shear-thinning the solution. K and n were estimated by nonlinear regression analysis of all the replicate data. The results are summarized in Table II.

Inter-and Intra-Manufacturer Variability Among Grades and Lots

Among Grades. Statistical analysis of the steady shear data showed that the consistency indices, K, and the power law indices, n, for the six grades of xanthan gum were significantly different ($P<0.0001$ and $P=0.011$, respectively).

The results of multi-comparison tests among different grades are summarized in Table III. Significant *inter*-manufacturer differences in mean values of K were observed among the various grades of xanthan gum. Significant *intra*-manufacturer differences in mean values of K were found among grades of manufacturer A but not among those of manufacturer B.

The value of the power law index, n, of grade B3 was the lowest of all the grades of xanthan gum and was significantly
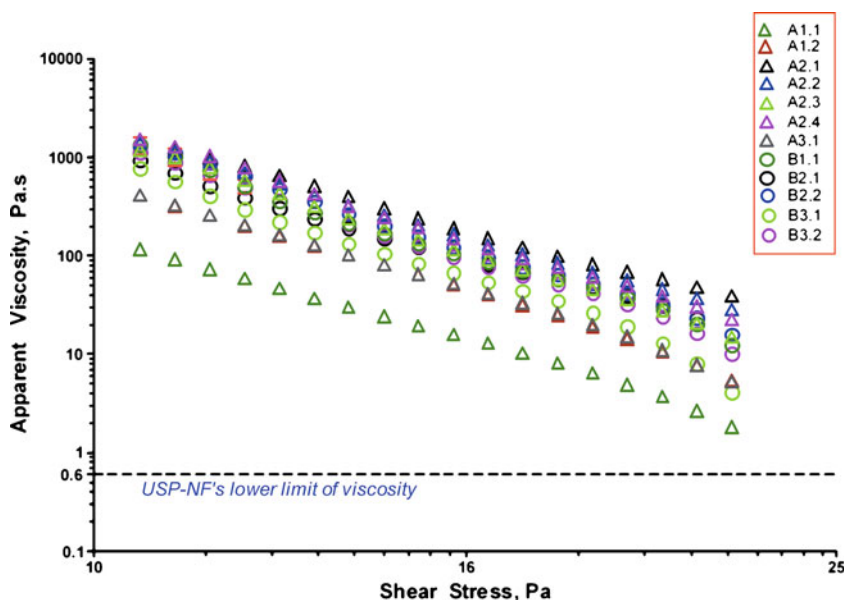
Fig. 2. Steady shear results of 1% *w/w* xanthan gum solutions at 24°C: apparent viscosity as a function of shear rate. Data are shown as mean and standard deviation of four replicates

different from the values for grades A1, A2, and B2. As lower values of *n* are associated with more substantial shear-thinning behavior, solutions prepared from grade B3 would be expected to exhibit more shear-thinning than those prepared from other grades. In contrast to *intra*-manufacturer differences in *K*, significant *intra*-manufacturer differences in the mean values of *n* were observed for the grades of manufacturer B but not for manufacturer A.

Among Lots. Significant differences in both *K* and *n* were observed among lots of grade A2 ($P=0.044$ and $P=0.019$, respectively; Table IV).

Small Amplitude Oscillatory Measurements

Figures 3 and 4 depict the storage modulus (G') and the loss modulus (G'') as a function of angular frequency, respectively. The values of G' were larger than those of G'' for all xanthan gum solutions indicating that the elastic behavior is more substantial than viscous behavior at the solution concentration of 1% *w/w*. These findings are in agreement with previous results reported by Song *et al.* (13) for solutions of xanthan gum at this concentration.

The frequency dependence of G' and G'' can be characterized by the following power–law relationships (13):

$$G' = K' \cdot \omega^{n'}$$

$$G'' = K'' \cdot \omega^{n''}$$

where K', K'', n', and n'' are constants. The coefficients and exponents of these two equations were estimated by non-linear regression analysis of all the replicate data. The results are summarized in Table II. *The higher the value of K', the higher the elasticity of the solution at a given n'. The higher the*

value of K'', the higher the viscous nature of the solution at a given n''. Lower values of n' and n'' indicate a higher degree of chain–chain interactions in the system.

Differences in elastic behavior for many lots were observed as a function of applied angular frequency. In particular, the storage modulus of lot A1.1 was the lowest of all the lots at all values of angular frequency, indicating that solutions prepared from lot A1.1 had the least elastic character compared to that of solutions prepared from all other lots. One possible consequence of the low apparent viscosity and elasticity of lot A1.1 solutions may be an increased sedimentation rate of suspensions made with this lot. The differences in G'' for many lots were observed throughout the range of applied angular frequency, but these differences were not as large as those for G'.

Inter- and Intra-Manufacturer Variability among Grades and Lots

Inter-manufacturer differences in viscoelastic behavior among solutions of the various grades of xanthan gum were more substantial than the differences in their steady shear behavior.

Among Grades. Statistical analysis of the small amplitude oscillation data showed that viscoelastic equation parameters—K', n', K'', and n''—for the six grades of xanthan gum were significantly different from each other ($P<0.0001$). The results of subsequent multi-comparison tests among different grades are summarized in Table III. With the exception of grade pairs A1 and A3 and B2 and B3, all other grades showed significant differences in K', n', K'', and n''.

Among Lots. The results of multi-comparison tests among different lots are summarized in Table IV. The largest lot-to-lot variability was observed for the lots of grade A2. However, significant lot-to-lot differences in viscoelastic

Table IV. Lot-to-lot Differences in Rheological Parameters

A1.1	3,4,5,6
A1.2	
B2.1	4,5,6
B2.2	
B3.1	3,4,5,6
B3.2	

	A2.1		
A2.2	4 5	A2.2	
A2.3	2 3,4 5,6	3,4 5	A2.3
A2.4	1,2 3,4 5	5	3,4 6

The black and red numerals are indicative of significant differences in steady shear and small amplitude oscillatory rheological parameters, respectively.

$$1 = K \quad 2 = n \quad 3 = K' \quad 4 = n' \quad 5 = K'' \quad 6 = n''$$

parameters were observed for all those grades for which multiple lots were evaluated. Lots that were not significantly different in their steady shear behavior nonetheless showed significant differences in their viscoelastic behavior.

CONCLUSION

Steady shear and small amplitude oscillation studies—across a wide range of shear stresses and angular frequencies, respectively—were performed on solutions of six different grades of xanthan gum obtained from two different manufacturers. Results showed significant differences in K, n, K', n', K'',

and n'' not only among different grades but also among different lots of a particular grade manufactured at different times. Even though *all* grades and lots met the NF viscosity specification and have ostensibly similar solution viscosities, the significant differences in K and n of their solutions suggest the potential for variations in solution behavior in unit operations such as pumping (14) or mixing and in the performance or stability of the final product. Overall, more *inter*-manufacturer variability was observed between grades A2 and B3, which were significantly different in *all* the rheological parameters evaluated, than for all other grades. Clearly, formulation and process development scientists need to be aware of the potential variability of xanthan gums' solution rheology and its implications for process
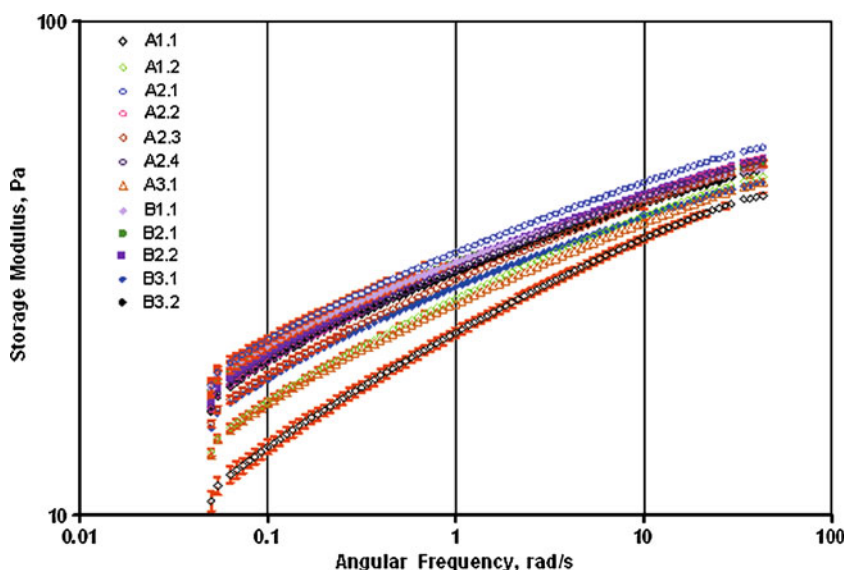
Fig. 3. Storage modulus (G') as a function of angular frequency at constant strain amplitude of 5%. Data are shown as mean and standard deviation of four replicates

control and product performance. As the current "one-point" unbounded NF viscosity specification for xanthan gum is unable to discern such inter- and intra-manufacturer differences, it would be appropriate to consider the revision of the current NF viscosity requirement.

REFERENCES

1. Shangraw RF. Compendial standards for pharmaceutical excipients. Drug Dev Ind Pharm. 1987;13:2421–39.
2. ICH. Guidance for Industry Q8 (R2) Pharmaceutical Development. Washington, DC: US Dept of Health and Human Services FDA, CDER, CBER; 2009.
3. Landín M, Martínez-Pacheco R, Gómez-Amoza JL, Souto C, Concheiro A, Rowe RC. Influence of microcrystalline cellulose source and batch variation on the tabletting behaviour and stability of prednisone formulations. Int J Pharm. 1993;91:143–9.
4. Chatlapalli R, Rohera BD. Study of effect of excipient source variation on rheological behavior of diltiazem HCl-HPMC wet masses using a mixer torque rheometer. Int J Pharm. 2002;238:139–51.
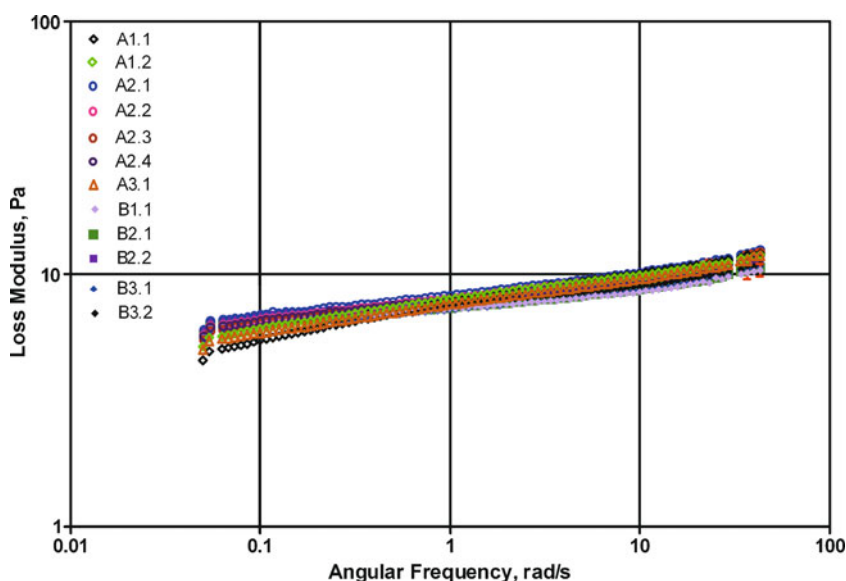5. Armstrong NA. Functionality related tests for excipients. Int J Pharm. 1997;155:1–5.

Fig. 4. Loss modulus (G'') as a function of angular frequency at constant strain amplitude of 5%. Data are shown as mean and standard deviation of four replicates

6. Mohan G. Functionality testing of excipients and their impact on formulation development. Am Pharm Rev. 2005;8:64.

7. Harding NE, Cleary JM, Ielpi L. Food biotechnology. In: Hui YH, Khachatourians GG, editors. Microorganisms. New York: Wiley; 1995. p. 495–514.

8. Born K, Landgendorff V, Boulenguer P, Polysaccharides I. Polysaccharides from Prokaryotes. Germany: Wiley; 2002. p. 262.

9. Song KW, Kim YS, Chang GS. Rheology of concentrated xanthan gum solutions: steady shear flow behavior. Fibers Polym. 2006;7:129–38.

10. Launay B, Cuvelier G, Martinez-Reyes S. Viscosity of locust bean, guar and xanthan gum solutions in the Newtonian domain: a critical examination of the log $(\eta_{sp})_o$-log C$[\eta]_o$ master curves. Carbohydr Polym. 1997;34:385–95.

11. Kibbe AH. Handbook of pharmaceutical excipients. 5th. Washington, DC: American Pharmacists Association; 2006. p. 401–6.

12. Wyatt NB, Liberatore MW. Rheology and viscosity scaling of the polyelectrolyte xanthan gum. J Appl Polym Sci. 2009;114:4076–84.

13. Song KW, Kuk HY, Chang GS. Rheology of concentrated xanthan gum solutions: oscillatory shear flow behavior. Korea Aust Rheol J. 2006;18:67–81.

14. Yaseen EI, Herald TJ, Aramouni FM, Alavi S. Rheological properties of selected gum solutions. Food Res Int. 2005;38:111–9.