Kinetics of Enzymatic Depolymerization of Guar Galactomannan

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A new mathematical model based on Michaelis Menten (MM) kinetics is developed to predict the changes in molecular weight distribution (MWD) during the enzymatic depolymerization of guar galactomannan. The model accounts for the effect of branching by considering the guar molecule as a substrate having three types of bonds with different MM kinetic parameters. The overall kinetics of the enzymatic reactions then can be represented in terms of composite kinetic parameters that are functions of the MM parameters for the individual bonds. The depolymerization is assumed to follow a random scission mechanism, in which an enzyme randomly attacks the substrate molecule at any one of the three types of bonds, and leaves the substrate on cleavage of the bond. Expressions for the variation in molecular weights during depolymerization are developed by applying moment generating techniques to the kinetic model. The model is evaluated against the complete MWD obtained using gel permeation chromatography. During the initial stages of depolymerization, the enzymatic reaction is in the zero-order regime of MM kinetics and the polydispersity index (PDI) increases with time. Subsequently, the PDI decreases as the depolymerization tends to follow first order kinetics. We also show that for a zero-order, random or nonrandom scission, the variation of PDI with time can exhibit a maximum. These analyses confirm that an increase in PDI during the depolymerization is not necessarily due to nonrandom scission, as previously concluded.

1. Introduction

Guar galactomannan is a naturally occurring polysaccharide consisting of a linear backbone of β-1,4-linked mannose units with randomly attached α-1,6-linked galactose branches, with the ratio of mannose to galactose units being approximately 2 (Figure 1)[1]. Guar and its derivatives are extensively used in many applications including food, oil recovery, drug-delivery, and personal and health-care products[2] because of their low cost, natural abundance, and ability to impart thickening and other desirable functionalities. In many of these applications, depolymerization of the guar is required to tailor the rheological and microstructural properties of specific products.[3,4] Consequently, a study of the depolymerization of the native guar to different extents and molecular weights is needed to understand the structure and molecular weight dependence of the physiochemical properties of the polymer.[5−7] Although chemical, thermal, and mechanical methods are traditionally used for degradation,[8,9] enzymes offer a powerful alternative[10] because of their specificity. Guar is susceptible to hydrolysis by three types of enzymes: β-mannanase cleaves the β-1,4 linkages between mannose backbone units; β-mannosidase cleaves the terminal β-1,4 linkages from the nonreducing end of the guar molecule; and α-galactosidase cleaves the α-1,6 linkages between the mannose and galactose units.[5,11] Figure 1 shows a schematic of a guar molecule together with the sites for enzyme action.

Although the effects of enzyme modification on rheology and microstructure of guar solutions and gels have been studied,[12−16] the kinetics of the enzymatic depolymerization of guar is not clearly understood. In previous investigations, the kinetics of depolymerization by β-mannanase enzyme was measured and compared with the kinetics of degradation by acid hydrolysis[6] and ultrasonication.[9] The enzymatic hydrolysis showed similar viscosity and molecular weight reduction patterns compared to those of ultrasonication and acid hydrolysis. Enzymatic hydrolysis resulted in a broadening of the molecular weight distribution (MWD), i.e., an increase of the polydispersity index (PDI). However, ultrasonication and acid hydrolysis resulted in a narrowing of the MWD, i.e., a decrease of the PDI. Although the enzymatic depolymerization was shown to follow a zero-order kinetics,[6,9] the kinetic model could not explain why PDI increased during the depolymerization. In previous studies of β-mannanase catalyzed depolymerization of guar,[6,9] the increase in the PDI was attributed to nonrandom enzyme attack on the β-1,4 linkages in the guar backbone. It was hypothesized that the nonrandomness might result from local confinement

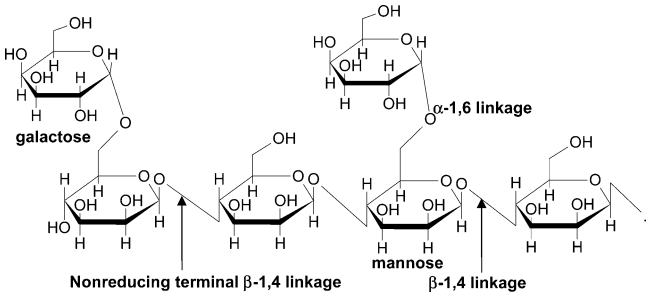

Figure 1. Structure of guar galactomannan showing action of three different enzymes. β-Mannanase cleaves all β-1,4 linkages, β-mannosidase cleaves only terminal β-1, 4 linkages, and α-galactosidase cleaves α-1,6 linkages.

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of the enzymes in the concentrated guar matrix, leading to high levels of hydrolysis in regions around the enzyme and to no degradation in regions inaccessible to the enzyme.[6] This is in contrast to hydrolysis by acids and bases or by mechanical methods, where all degradable bonds in a guar molecule are equally reactive.

The enzymatic hydrolysis of polymers involves the interaction between an enzyme and a polymer molecule, forming an intermediate complex at an active site on the polymer molecule.[17] Some of the factors that affect enzyme−substrate complexation are branching and conformation[18−22] of polymer molecules and the accessibility of bonds to the enzyme attack. Since guar is a branched polymer with galactose side chains randomly attached to the backbone, the presence of the branches can hinder enzyme attack and lead to different reaction rate constants for different bonds in the guar chain. Although nonrandom enzymatic attack was used as one of the possible explanations for the increasing PDI that was observed during the enzymatic hydrolysis of guar,[6] no quantitative kinetic model was developed to show how nonrandom scission increases the PDI.

In this study, we first develop a zero order kinetic model to predict changes in the MWD during the depolymerization of guar by β-mannanase enzyme. The model reveals that both random and nonrandom bond scission lead to a PDI that increases initially and then decreases as the depolymerization reactions progress. Consequently, one cannot conclude that enzymatic depolymerization follows a nonrandom scission mechanism, based solely on changes in PDI with time. In a few studies, enzymatic reactions have been treated as first order with respect to the concentration of degradable bonds.[23−25] In particular, Yoon et al.[25] showed that the PDI increases during microbial depolymerization of polyesters if the reaction follows first-order random scission kinetics. Although some of these models were able to predict changes in MWD during depolymerization, they do not provide a physical interpretation of enzymatic reactions, where the concentration of enzyme−substrate complex determines the rate of reaction.

To gain further insight into the behavior of the enzymatic hydrolysis of guar, we develop a new mathematical model based on Michaelis Menton (MM) kinetics. There are many studies showing that Michaelis Menten kinetics describes the enzymatic depolymerization of polymers.[18,19,26,27] However, their focus has been on estimating the MM kinetic parameters from experimental data. No model has been developed based on MM kinetics to predict the changes in MWD during enzymatic degradation of polymers.

The presence of branches on the backbone of galactomannans and other polysaccharides changes the action pattern and activity of endo-acting glycosidase enzymes on the backbone.[5,11,18,19,28] These effects have been studied via computer simulations.[9,29] In the present model, we treat the effect of galactose branches on the β-mannanase activity by considering the guar molecule as a substrate with three types of active sites (bonds) randomly distributed on the backbone as shown in Figure 2. The three types of bonds are (1) the β-1,4 bond between two unsubstituted mannose units, (2) the β-1,4 bond between an unsubstituted and a substituted mannose unit, and (3) the β-1,4 bond between two substituted mannose units. Each type of bond will have different MM kinetic parameters. However, the β-mannanase enzyme attacks randomly at any of these bonds to form an enzyme−substrate complex, followed by degradation of the bond. We assume that the degradation follows a single scission mechanism,[17] where the enzyme leaves the substrate upon cleavage of a bond. We develop a kinetic rate equation for depolymerization of guar molecules with a distribution of chain lengths assuming that the kinetic parameters are independent of chain length. We then apply moment generating techniques[30] to the kinetic rate equation to develop equations for the rate of change of number-average and weight-average molecular weights as a function of the kinetic parameters.

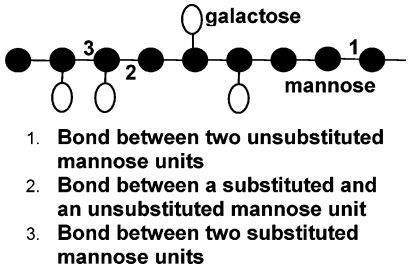

Figure 2. Schematic of a guar molecule showing three types of β-1,4 bonds.

2. Results and Discussion

2.1. Mathematical Modeling. 2.1.1. Nonrandom Zero Order Depolymerization Kinetics. The zero order model assumes the rate of degradation of guar to be independent of substrate concentration but proportional to enzyme concentration. In this model, nonrandom scission is taken into account by considering the rate constant to be a function of both chain length and the position of the hydrolyzable bond on the chain. For zero order scission kinetics, the rate of change of concentration of guar molecules of chain length "i" is given by

$$\frac{dP_i}{dt} = \{\sum_{j=i+1}^{\infty}(k_{j,i} + k_{j,j-i}) - \sum_{j=1}^{i-1}k_{i,j}\}E_T \qquad (1)$$

In the above equation, $k_{i,j}$ is the rate constant for the hydrolysis reaction occurring at the jth bond on a polymer chain of i monomer units. E_T is the total concentration of enzyme in the reaction mixture, and P_i is the concentration of a molecule with chain length "i". The first summation on the right-hand side of the above equation represents the rate of formation of molecules of chain length "i" due to degradation of molecules of chain length $j > i$ at the ith bond or $(j - i)$th bond. The second summation shows the rate of degradation of the molecules of chain length i at any of the $i - 1$ bonds. For random scission kinetics, all k_{ij} are equal.

When a zero-order model is used, it is mathematically possible for the concentrations of polymer molecules with a specific chain length to become negative unless the reaction is "turned off" when all of the molecules of that chain length have been consumed. This can be accomplished by setting all of the rate constants that apply to this chain length equal to zero

$$k_{ij} = 0;\ 1 \le j \le i - 1;\ P_i = 0$$

For molecules with high values of i, $k_{i,j}$ may become zero at relatively short times, especially if the initial concentration of molecules with this chain length is low. On the other hand, the initial concentration of a particular short chain (low i) molecule may be zero such that $k_{i,j} = 0$ for this chain length, initially. However, as molecules of these chain lengths are formed during the depolymerization reaction, the values of $k_{i,j}$ become finite. We do not attempt to account explicitly for these types of

behavior since they depend on the initial chain length and on the final extent of depolymerization.

The following development is based on the assumption that all of the chain lengths that are present in the initial distribution are continuously present until the end of the reaction and that no polymers are formed with a chain length that is not present in the initial distribution. From eq 1, the changes in the moments of chain length distribution can be derived,[30] as follows.

For the *n*th moment

$$\frac{d\mu_n}{dt} = \sum_{i=1}^{\infty} i^n \frac{dP_i}{dt} = -\sum_{i=1}^{\infty}\sum_{j=1}^{i-1}[i^n - j^n - (i-j)^n]k_{i,j}E_T = -k_nE_T \quad (2)$$

Equation 2 can be used to derive the following equations for μ_0, μ_1, and μ_2

$$\frac{d\mu_0}{dt} = \sum_{i=1}^{\infty}\frac{dP_i}{dt} = \sum_{i=1}^{\infty}\sum_{j=1}^{i-1}k_{i,j}E_T = k_0E_T \quad (3)$$

$$\mu_0 = \mu_{00} + k_0E_Tt \quad (4)$$

$$\frac{d\mu_1}{dt} = \sum_{i=1}^{\infty} i\frac{dP_i}{dt} = 0 \quad (5)$$

$$\mu_1 = \mu_{10} \quad (6)$$

$$\frac{d\mu_2}{dt} = \sum_{i=1}^{\infty} i^2\frac{dP_i}{dt} = -\sum_{i=1}^{\infty}\sum_{j=1}^{i-1}[i^2 - j^2 - (i-j)^2]k_{i,j}E_T = -k_2E_T \quad (7)$$

$$\mu_2 = \mu_{20} - k_2E_Tt \quad (8)$$

In eqs 4, 6, and 8, μ_{00}, μ_{10}, and μ_{20} are the initial moments of the chain length distribution before the hydrolysis reactions begin. The equations for changes in number average (X_n) and weight average (X_w) degrees of polymerization (DP) can be derived using eqs 4, 6, and 8

$$\frac{1}{X_n} = \frac{\mu_0}{\mu_1} = \frac{1}{X_{no}} + k_0't \quad (9)$$

$$X_w = \frac{\mu_2}{\mu_1} = X_{wo} - k_2't \quad (10)$$

where

$$k_0' = \frac{k_0E_T}{\mu_{10}};\ k_2' = \frac{k_2E_T}{\mu_{10}} \quad (11)$$

The change in PDI (Q) with time is given by

$$Q = \frac{X_w}{X_n} = \left(\frac{1}{X_{no}} + k_0't\right)(X_{wo} - k_2't) \quad (12)$$

Here, X_{no} and X_{wo} are the initial number average and weight average degrees of polymerization, respectively.

The PDI versus time curve will have an extremum, if

$$\frac{dQ}{dt} = k_0'X_{wo} - \frac{k_2'}{X_{no}} - 2k_0'k_2't = 0 \quad (13)$$

If an extremum exists, it must be a maximum since

$$\frac{d^2Q}{dt^2} = -2k_0'k_2' < 0 \quad (14)$$

The maximum value of Q (Q_{max}) and the time (t_{max}) at which the maximum occurs can be derived using eqs 12 and 13, respectively

$$t_{max} = \frac{1}{2}\left[\frac{X_{wo}}{k_2'} - \frac{1}{k_0'X_{no}}\right] \quad (15)$$

$$Q_{max} = \frac{1}{4k_0'k_2'}\left(\frac{k_2'}{X_{no}} + k_0'X_{wo}\right)^2 \quad (16)$$

Equation 15 shows that if $k_2'/k_0' \geq X_{wo}X_{no}$, a maximum will not occur and the PDI will decline monotonically with time. However, if the initial molecular weight of the polymer is sufficiently high, $X_{no}X_{wo}$ will be greater than k_2'/k_0'. In this case, the PDI will increase to the maximum given by eq 16 and then will decrease. The above results show that the PDI can either increase or decreases for both random and nonrandom scission under zero order kinetics depending on relative values of $X_{wo}X_{no}$ and k_2'/k_0'.

In a previous investigation, it was shown that the PDI can increase for random scission under first-order kinetics.[25] The kinetics of enzymatic depolymerization of guar also were studied for two cases of nonrandom scission: central scission and Gaussian scission.[9] In the central scission case, enzyme preferentially attacks at the center of the polymer chain, whereas in Gaussian scission, the variation in the rate constant along the chain follows a Gaussian distribution with the maximum at the center. However, the experimental data did not fit either of these mechanisms for first-order kinetics. Previous analyses have been based on simplified kinetic models of enzyme−substrate complexation, i.e., either first- or zero-order kinetics. In addition, previous models allowed more than one enzyme to be attached to a guar molecule. However, in view of the very small ratio of enzyme to hydrolyzable bonds, the present model permits no more than one enzyme to be attached to a single polymer molecule. In the following section, we develop a new mathematical model based on the above picture of enzyme−polymer interaction and on a full MM kinetic model to extend the range of previous kinetic analyses.

2.1.2. Michaelis Menten Kinetics. The enzymatic reactions of a single substrate with a single type of active site can be represented as[31]

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \quad (17)$$

The free enzyme (E) attaches to the substrate (S) and forms an enzyme−substrate complex (ES). The enzyme then acts on the substrate to release the products and free enzyme. The kinetic pathway may involve single scission, where an enzyme molecule leaves the substrate on degrading a bond, or multiple scissions, where the enzyme remains attached to a substrate molecule until all the degradable bonds in the molecule have been cut. A pathway intermediate between single scission and multiple scissions also may be followed.[17] We assume that the depolymerization follows a single scission mechanism, as the presence of galactose side chains may preclude the multiple scission mechanism.

In enzymatic depolymerization reactions, every degradable bond is often considered to be a substrate.[26,32] However, it is physically not possible for a guar molecule to hold enzymes at

all degradable bonds, as the MW of a repeat unit between the degradable bonds (~270) is much smaller than the MW of the enzyme (~50 000). Moreover, the enzyme concentration is much lower than that of the guar. In a typical guar hydrolysis reaction, the β-mannanase enzyme concentration is of the order 10^{-10} mmol/mL, whereas the guar concentration is of the order of 10^{-5} mmol/mL. Consequently, in the following development, the guar molecule is considered to be the substrate, rather than a degradable bond. A guar molecule can complex with an enzyme at any of the three types of active sites on the guar backbone, as discussed earlier. The reactions at each of these sites are assumed to follow MM kinetics but with different kinetic parameters at each type of site. We assume these kinetic parameters to be independent of polymer chain length.

Equation 17 can be modified to take into account the multiple types of active sites on a guar backbone. For an enzyme acting on a j type bond on a polymer chain containing i monomer units, eq 17 can be written as

$$E + P_i \underset{k_{2j}}{\overset{k_{1j}}{\rightleftharpoons}} [EP_i]_j \xrightarrow{k_{3j}} E + P_k + P_{i-k} \tag{18}$$

$$\frac{d[EP_i]_j}{dt} = k_{1j}[E]P_i - k_{2j}[EP_i]_j - k_{3j}[EP_i]_j \tag{19}$$

Assuming pseudo steady state for each type of enzyme−substrate complex

$$[EP_i]_j = \frac{k_{1j}}{k_{2j} + k_{3j}}[E]P_i = \frac{[E]P_i}{K_j} \tag{20}$$

where

$$K_j = \frac{k_{2j} + k_{3j}}{k_{1j}} \tag{21}$$

In the above equation, $[EP_i]_j$ represents the concentration of enzyme substrate complex formed at j type bonds on a guar molecule of chain length i, and $[E]$ is the concentration of free enzyme. The mass balance on the total enzyme gives

$$E_T = [E] + \sum_{i=2}^{\infty}\sum_{j=1}^{3}[EP_i]_j = [E] + \sum_{i=2}^{\infty}\sum_{j=1}^{3}\frac{[E]P_i}{K_j} \tag{22}$$

Hence

$$[E] = \frac{E_T}{1 + \sum_{i=2}^{\infty}P_i\sum_{j=1}^{3}\frac{1}{K_j}} \tag{23}$$

Assuming K_j is independent of guar concentration and chain length, eq 23 can be simplified as

$$[E] = \frac{E_T}{1+\sum_{i=2}^{\infty}P_i\sum_{j=1}^{3}\frac{1}{K_j}} \tag{24}$$

where

$$\frac{1}{K_M} = \sum_{j=1}^{3}\frac{1}{K_j} \tag{25}$$

The overall rate of formation of polymer molecules of chain length i is written as

$$\frac{dP_i}{dt} = -\sum_{j=1}^{3}k_{3j}[EP_i]_j + \sum_{l=i+1}^{\infty}\frac{2}{l-1}\sum_{j=1}^{3}k_{3j}[EP_l]_j \tag{26}$$

The two terms in the right-hand side of eq 26 represent the rate of disappearance and rate of formation of polymer molecule containing i monomer units, respectively. The factor $2/(l-1)$ is the probability of formation of polymer molecules of i monomer units from the polymer molecules containing $l(l > i)$ monomer units.

Substituting for $[EP_i]_j$ using eqs 20 and 24, we get

$$\frac{dP_i}{dt} = \frac{k'E_T}{K_M + \sum_{i=2}^{\infty}P_i}\left[-P_i + 2\sum_{l=i+1}^{\infty}\frac{P_l}{l-1}\right] \tag{27}$$

The above equation shows that the depolymerization kinetics can be represented by a modified MM kinetic equation with composite kinetic parameters given by

$$\frac{1}{K_M} = \sum_{j=1}^{3}\frac{1}{K_j};\ k' = K_M\sum_{j=1}^{3}\frac{k_{3j}}{K_j} \tag{28}$$

Eq 27 is applicable for $i > 2$. For $i = 1$

$$\frac{dP_1}{dt} = \frac{k'E_T}{K_M + \sum_{i=2}^{\infty}P_i}\left[2\sum_{l=2}^{\infty}\frac{P_l}{l-1}\right] \tag{29}$$

In the following sections, we use eqs 27 and 29 to derive equations for molecular weight distributions using moment generating techniques.

2.1.3. Determination of Molecular Weight Distribution (MWD) and Polydispersity. The generating function[30] is defined as

$$G(s,t) = \sum_{i=1}^{\infty}s^iP_i \tag{30}$$

From eqs 27 and 29

$$\frac{dG}{dt} = \frac{k'E_T}{K_M + \sum_{i=2}^{\infty}P_i}\left[-\sum_{i=2}^{\infty}s^iP_i + 2\sum_{i=1}^{\infty}s^i\sum_{l=i+1}^{\infty}\frac{P_l}{l-1}\right] \tag{31}$$

The above equation can be simplified, after expanding the summations

$$\frac{dG}{dt} = \frac{k'E_T}{K_M + \sum_{i=2}^{\infty}P_i}\left[-\sum_{i=2}^{\infty}s^iP_i + 2\sum_{i=2}^{\infty}s\left(\frac{s^{i-1}-1}{s-1}\right)\left(\frac{P_i}{i-1}\right)\right] \tag{32}$$

The moments of the MWD, μ_0, μ_1, and μ_2, are given by

$$\mu_0 = \sum_{i=1}^{\infty}P_i = \lim_{s\to 1}(G) \tag{33}$$

$$\mu_1 = \sum_{i=1}^{\infty} iP_i = \lim_{s\to 1}\left(s\frac{\partial G}{\partial s}\right) \tag{34}$$

$$\mu_2 = \sum_{i=1}^{\infty} i^2P_i = \lim_{s\to 1}\left\{s\frac{\partial}{\partial s}\left(s\frac{\partial G}{\partial s}\right)\right\} \tag{35}$$

The equations for the rates of change of the moments can be obtained from eqs 32−35 after applying L'Hopitals rule

$$\frac{d\mu_0}{dt} = \frac{k'E_T(\mu_0 - P_1)}{K_M + (\mu_0 - P_1)} \tag{36}$$

Here, P_1 is the concentration of monomer units and $(\mu_0 - P_1)$ is the concentration of polymers with at least one hydrolyzable bond

$$\frac{d\mu_1}{dt} = 0 \tag{37}$$

$$\frac{d\mu_2}{dt} = \frac{-\frac{k'}{3}(\mu_2 + \mu_1 - 2P_1)}{K_M + (\mu_0 - P_1)} \tag{38}$$

In the following sections, two limiting cases of eqs 36 and 38 are examined to derive equations for average molecular weights and PDI. In a previous investigation,[32] we have shown that the reaction becomes diffusion controlled above 3% (w/w) of guar solution. However, the following analyses will be accurate enough to predict the changes in MWD during enzymatic hydrolysis of guar in the concentration range of <3%(w/w).

Initial Stages Of Depolymerization. In a previous investigation,[32] zero-order kinetics were observed in the guar concentration range of 0.1−3% w/w over a reaction time of 20 h. This can be true only if $K_M \ll (\mu_0 - P_1)$ in eq 36. Initially, the concentration of monomers P_1 is very low. Moreover, the total concentration of substrate molecules containing degradable bonds $(\mu_0 - P_1)$ increases during the initial stages of reaction due to cleavage of interior β-1,4 glycosidic bonds. If the molecular weight of the guar is high, the total probability of cleavage at interior bonds is higher than that of terminal bond cleavage. Hence, the rate of increase in μ_0 is greater than the rate of increase in P_1. If $K_M \ll (\mu_0 - P_1)$, eqs 36 and 38 can be further simplified

$$\frac{d\mu_0}{dt} = k'E_T \tag{39}$$

$$\frac{d\mu_2}{dt} = \frac{-k'E_T(\mu_2 + \mu_1)}{3\mu_0} \tag{40}$$

The rate of change of number (X_n) and weight (X_w) average degree of polymerization (DP) is given by

$$\frac{d}{dt}\left(\frac{1}{X_n}\right) = \left(\frac{k'E_T}{\mu_1}\right) = k'' \tag{41}$$

$$\frac{dX_w}{dt} = \frac{-k''(X_w + 1)}{3\left(\frac{1}{X_n}\right)} \tag{42}$$

Note that the apparent rate constant k'' is proportional to the total enzyme concentration, E_T and inversely proportional to weight percentage of guar in solution $(\sim\mu_1)$.

Integration of eqs 41 and 42 gives the variation of X_n and X_w with time

$$\frac{X_{no}}{X_n} = 1 + X_{no}k''t \tag{43}$$

$$\frac{(X_{wo} + 1)^3}{(X_w + 1)^3} = 1 + X_{no}k''t$$

or

$$\frac{X_{wo}^{\ 3}}{X_w^{\ 3}} = 1 + X_{no}k''t, \quad \text{if } X_w \gg 1 \tag{44}$$

The polydispersity index Q is given by

$$Q = \frac{X_w}{X_n} = Q_0(1 + X_{no}k''t)^{2/3} \tag{45}$$

where Q_0 represents the initial PDI. Equation 45 shows that the polydispersity index increases during the initial stages of depolymerization.

For $X_{no}kt < 1$, eq 44 can be simplified using a Taylor series expansion

$$\frac{X_w}{X_{wo}} = 1 - \frac{1}{3}X_{no}k''t \tag{46}$$

As expected, eqs 43 and 46 have the same form as the corresponding equations for zero-order kinetics given in eqs 9 and 10.

Late Stages of Depolymerization. During the late stages of reaction, $K_M \gg \mu_0 - P_1$, when the number of substrate molecules with degradable bonds, less the number of monomer units decreases below K_M. The reaction then follows pseudo-first-order kinetics, where the rate of depolymerization is proportional to the concentration of substrate molecules with degradable bonds, as shown in eq 47. This stage will occur when X_n is so low that the hydrolysis of guar molecules at the terminal ends results in an increase in concentration of monomer units (P_1) accompanied by a decrease in the concentration of substrate $(\mu_0 - P_1)$ containing hydrolyzable bonds. Under these conditions, eqs 36 and 38 simplify to

$$\frac{d\mu_0}{dt} = \frac{k'E_T(\mu_0 - P_1)}{K_M} \tag{47}$$

$$\frac{d\mu_2}{dt} = \frac{-k'E_T(\mu_2 + \mu_1 - 2P_1)}{3K_M} \tag{48}$$

The variation of polydispersity index is then given by

$$\frac{dQ}{dt} = \frac{k'E_T}{3K_M}\left[2Q - \frac{1}{X_n} - 3\frac{X_wP_1}{\mu_1} + 2\frac{P_1}{X_n\mu_1}\right] \tag{49}$$

As the reaction progresses, $P_1 \to \mu_0$ and the concentration of monomer units will be higher than the total concentration of oligomers and polymers. Hence, eq 49 simplifies to

$$\frac{dQ}{dt} \approx \frac{k'E_T}{3K_M}\left[-Q - \frac{1}{X_n} + 2\frac{1}{X_n^{\ 2}}\right] \tag{50}$$

For

$$Q > 1, \frac{1}{X_n} \gg \frac{1}{X_n^2}$$

$$\therefore \frac{dQ}{dt} \leq 0 \tag{51}$$

Hence, the polydispersity should decrease during the latter stages of hydrolysis.

2.2. Model Evaluation. The enzymatic hydrolysis of guar solution has been studied using β-mannanase enzyme.[6,32] The reaction was carried out with 0.5% (w/w) guar in aqueous solution at room temperature and neutral pH. The enzyme concentration in the reaction mixture was 2×10^{-4} U/mL. The changes in MWD during the reactions were measured using gel permeation chromatography (GPC) with pullulan standards. We use the MWDs obtained from these experiments to evaluate the MM kinetic model.

Figure 3 shows the MWD at various times during the enzymatic depolymerization of guar using β-mannanase enzyme. The data points shown in the figure are reproduced from the curves in ref 6. The MWD was fit to a Zimm Schultz (Z−S) distribution [33] as given by eq 52 using Sigma Plot 8.0. The solid lines in Figure 3 show the resulting Z−S distributions. The parameters "a" and "b" in the Z−S distribution are related to the weight and number average molecular weights by eqs 53 and 54

$$w(P_i) = \frac{a^{b+1}}{b!} M_i^b \exp(-aM_i) \tag{52}$$

$$a = \frac{1}{M_W - M_n} \tag{53}$$

$$b = \frac{M_w}{M_W - M_n} \tag{54}$$

The number average (M_n) and weight average (M_w) molecular weights were calculated using eqs 53 and 54, respectively, at each of the times in Figure 3. The values of X_n and X_w were then calculated from M_n and M_w values as given by eqs 55 and 56

$$X_n = \frac{M_n}{M_r} \tag{55}$$

$$X_w = \frac{M_w}{M_r} \tag{56}$$

In the above equations, M_r is the average molecular weight of a repeat unit. In this analysis, we assumed guar as a graft copolymer of mannose and galactose with a galactose molecule attached as a side group to every alternate mannose molecule. Hence, the average molecular weight of a repeat unit (M_r) was taken as 270, i.e., the average of molecular weights of an unsubstituted mannose unit (~180) and a mannose unit substituted with single molecule of galactose unit (~360).

Figure 4 shows the variation in calculated values of M_n, M_w, and PDI. As expected, both the molecular weights continuously decrease with time. The reductions in M_n and M_w are rapid initially and slower at longer times. However, the PDI increases continuously for the range of reaction times studied.

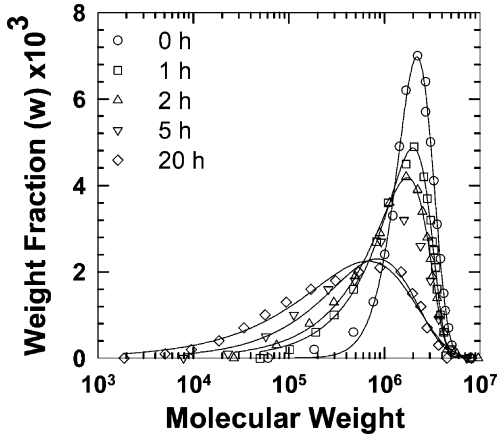

Figure 3. Changes in MWD during hydrolysis of guar by β-mannanase at 25 °C. The data points were obtained from the MWD curves in ref 6. The solid lines are fits of the Zimm-Schultz distribution function to the data points.

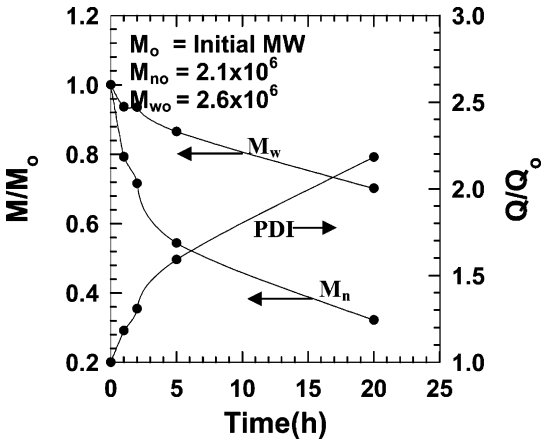

Figure 4. Changes in MW and PDI during the depolymerization of guar by β-mannanase enzyme. The solid lines shown connect the data points, which were obtained from the MWD curves in ref 6.

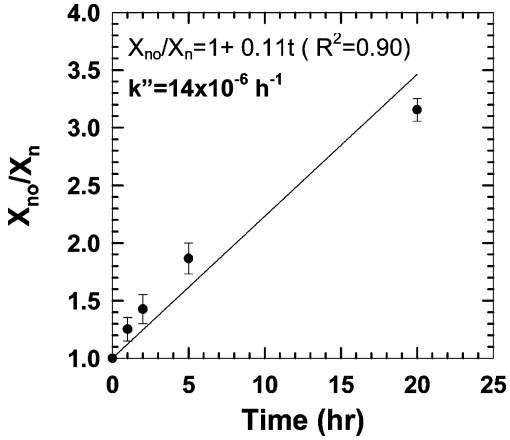

Figure 5. Estimation of kinetic rate constant for the data of ref 6 using eq 43. The error bars represent the residuals estimated from the regression analysis.

The kinetic parameter for guar hydrolysis was obtained using eqs 43 and 44 as shown in Figures 5 and 6. The apparent rate constant (k'') determined from both plots is approximately the same. The values of k'' obtained from Figures 5 and 6 are 14×10^{-6} h^{-1} and 13×10^{-6} h^{-1} respectively. Although the depolymerization was carried out for 20 h, the linearity of Figures 5 and 6, and the agreement of the values of k'' from the plots suggests that the reaction lies within the initial regime

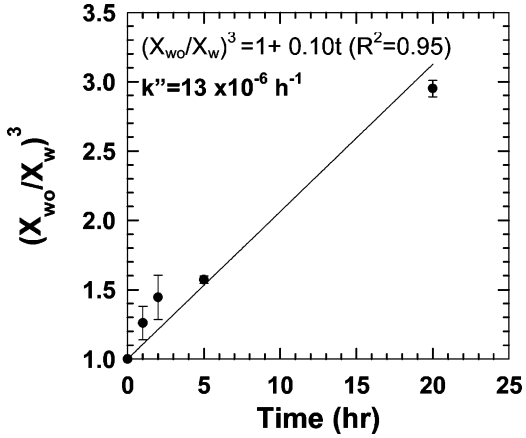

Figure 6. Estimation of kinetic rate constant for the data of ref 6 using eq 44. The error bars represent the residuals estimated from the regression analysis.

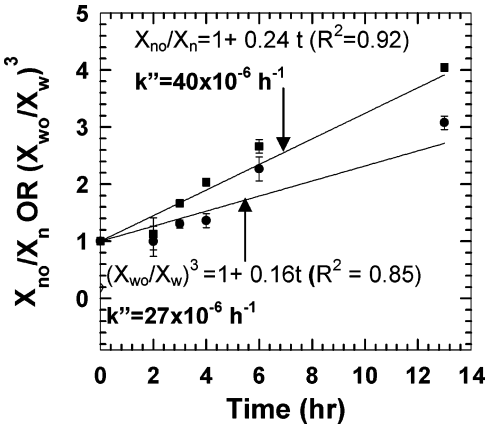

Figure 7. Evaluation of MM kinetic model for the data of ref 9. The error bars represent the residuals estimated from the regression analysis.

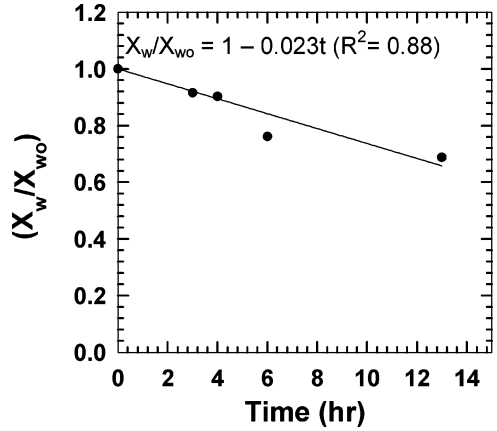

Figure 8. Evaluation of zero-order nonrandom scission kinetic model for the data of ref 9.

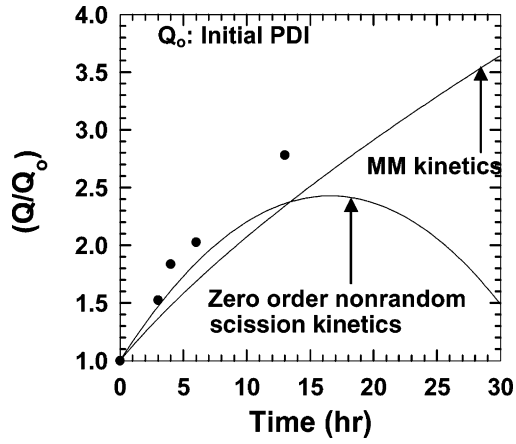

Figure 9. Prediction of PDI using MM kinetics and zero-order nonrandom scission kinetics. The data points are calculated from the MWD data of ref 9. For the MM kinetic model, an average of $X_{no}k''$ (0.20 h^{-1}) estimated from the lines of Figure 7 is used. For zero order nonrandom scission kinetics, the rate constants $k_0' = 0.20$ h^{-1} estimated from Figure 7 and $k_2' = 0.023$ h^{-1} estimated from Figure 8 are used.

of MM kinetics over this whole range of time. Analysis of previous results[6,9] also shows that only a very small fraction (<0.5%) of the original bonds were cleaved during the entire time studied.

In another study[34] on the enzymatic depolymerization of guar, a linear relationship was observed between $1/X_n$ and time, and between the apparent zero-order rate constant (k'') and the reciprocal of guar concentration. These results are in agreement with our derivations (eq 41). In Figure 7, we use the MWDs obtained from ref 9 to estimate the rate constant for the enzymatic degradation of 0.7% guar solution. As discussed previously, the MWDs were fit to a Zimm-Schultz distribution to estimate M_w and M_n. In the Figure 7, we plot (X_{no}/X_n) and $(X_{wo}/X_w)^3$ against time to estimate the apparent rate constant k'' as given in eqs 43 and 44. The good linear fit obtained for both sets of data supports the validity of our approach. The difference in the apparent rate constant obtained from the two lines could be attributed to be experimental error. In Figures 5−7, we estimated the kinetic rate constants assuming linear variations in both $1/X_n$ and $(1/X_w)^3$ with time. This is true only if the entire range of reaction time studied falls in the zero-order regime of Michaelis Menten kinetics as described earlier. Equations 43 and 46 show that the Michaelis Menten kinetics can be reduced to a form of zero order scission kinetics (eqs 9 and 10) for the initial stages of the reaction, when $X_{no}k''t \ll 1$. In Figure 8, X_w is plotted against time for the MWD data of ref 9 and the figure shows that X_w decreases linearly with time as given by eqs 10 and 46. This also corroborates our assumption that the entire reaction time studied falls within the zero order regime of Michaelis Menten kinetics.

The changes in PDI predicted by MM kinetics (eq 45) and zero-order nonrandom kinetics (eq 11) are shown in Figure 9. For predicting the PDI using MM kinetics, the average of the slopes of the two lines in Figure 7 was used as $X_{no}k''$ (=0.2). For predicting the PDI using zero order kinetics, the average of slopes of the lines in Figure 7 was used as k_0' (=0.2), and the slope of the line in Figure 8 was used as k_2' (=0.023). The zero-order kinetic model shows a maximum in the PDI versus time curve, whereas the PDI increases monotonically for MM kinetics, during the reaction time studied. The MM kinetic model predicts that PDI should decrease when the concentration of guar molecule containing at least one degradable bond becomes small compared to K_M. Since guar is used as a rheology modifier in many of its applications, previous kinetic studies have been limited to time durations for which the maximum extent of viscosity reduction occurred. Analysis of previous results[6,9] shows that only a very small fraction (<0.5%) of the original bonds were cleaved during the entire time studied. During this time duration, the concentration of polymer molecules containing at least one degradable bond increases and probably does not go below K_M. Hence PDI should increase during the entire reaction time studied. The zero order models do not take into account the enzyme−substrate complexation and are unable to

accurately predict the kinetics for the later stages of the degradation process where the concentration of enzyme−substrate complex limits the rate of degradation.

3. Conclusions

In this study, we develop two mathematical models to examine the kinetics of enzymatic degradation of guar galactomannans: zero order nonrandom scission kinetics and MM kinetics. These models address many of the unresolved issues in prior investigations such as broadening of molecular weight, random versus nonrandom scission, and effects of branches on degradation kinetics, and provide a detailed analysis of the changes in the entire molecular weight distribution during the enzymatic hydrolysis process.

The enzymatic degradation of guar during the initial stages can be described by zero-order nonrandom or random scission kinetics. The model predicts that for zero order kinetics, the PDI can increase for either random or nonrandom bond scission. Hence the increase in PDI during the enzymatic degradation is not necessarily due to nonrandom scission of bonds, as previously concluded. However, the zero-order model does not take into account the enzyme−substrate complexation behavior and fails to predict accurately the changes in MWD during the entire depolymerization process. As such, a new MM kinetic model has been developed that takes into account enzyme substrate complexation and the effects of galactose branches on the enzyme-polymer complexation. The model is evaluated against two sets of experimental data and fits the data well. The model predicts the PDI to increase and MM rate equation to approximate zero order during the initial stages of reaction, but the PDI to decrease and the rate equation to tend toward first-order kinetics as reaction progresses.

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Nomenclature

E_T = total enzyme concentration (units/mL)
G = moment generating function (mol/mL)
k = rate constant (units/mL)$^{-1}$ (s)$^{-1}$ or (mol/mL) (units/mL)$^{-1}$ (s)$^{-1}$
k'' = apparent rate constant (s^{-1})
K_M = Michaelis constant (mol/mL)
M_n = number average molecular weight
M_r = molecular weight of a repeat unit
M_w = Weight average molecular weight
P = concentration of polymer molecules (mol/mL)
Q = Polydispersity index (PDI)
w = weight fraction
X_n = number average degree of polymerization
X_w = weight average degree of polymerization
μ = moments of molecular weight distribution

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