Manipulation of hydrophobic interactions in associative polymers using cyclodextrin and enzyme

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We examine a new approach to reversibly modulate hydrophobic interactions in associative polymers using cyclodextrins (CD) and enzymes that cause scission of the α-1, 4 linkages in cyclodextrins. The associative polymers have a comb-like structure with pendant hydrophobic groups randomly attached to the polymer backbone. The intermolecular interaction between hydrophobic groups forms a transient network resulting in thickening of solutions containing the polymer. The CDs, doughnut-shaped cyclic polysaccharides, encapsulate the hydrophobes within their hydrophobic cavity and eliminate hydrophobic interactions. This results in several orders of magnitude reduction in solution viscosity and other viscoelastic properties. Subsequent degradation of the CDs by enzymes restores the hydrophobic interactions and the original rheological properties. A rheokinetic model is developed to describe the kinetics of the enzymatic reactions. The model accounts for equilibrium between the CD bound to the hydrophobes and free CD in solution and assumes the enzyme hydrolyzes only the free CD in the solution, which causes the release of the bound CDs in order to maintain equilibrium. The reaction is assumed to follow Michaelis Menten kinetics and the kinetic parameters are determined by tracking the changes in the viscoelastic properties of the polymer solution during the enzymatic scission of CD. The effects of enzyme concentration, polymer concentration and temperature on the rate of recovery of the original rheological properties are experimentally determined, and used to validate the trends of the rheokinetic model.

1. Introduction

Hydrophobically-modified alkali-soluble emulsion (HASE) polymers are water-soluble associative polymers having a comb-like structure with pendant hydrophobic groups randomly attached to the polymer backbone[1,2] (Fig. 1). In aqueous solutions, these hydrophobic groups interact to form a transient network resulting in a concomitant increase in solution viscosity and viscoelasticity.[3–6] As such, HASE polymers are widely used as rheology modifiers in a variety of applications including paints, coatings, and aircraft anti-icing fluids.[7,8]

Although intermolecular hydrophobic interactions between the polymer molecules are attractive from a rheological standpoint, controlling these interactions is often necessary for practical applications. The hydrophobic interactions in the solution are affected by pH,[9] ionic strength,[10] temperature[11] and surfactants[12–15] in the solution. Nevertheless, complete reversibility of the associative phenomena cannot be achieved solely by manipulating these parameters. One approach to eliminate interactions between hydrophobic groups is to use cyclodextrins (CD) for encapsulating these groups within the hydrophobic cavity of cyclodextrins (CD) to form inclusion compounds.[16–20]

Cyclodextrins are doughnut-shaped cyclic oligosaccharides of glucose units linked together by α-1,4 bonds.[21] The most common CDs are composed of 6, 7 or 8 glucose units and denoted as α, β, or γ CDs, respectively. CDs are widely used to encapsulate drug, fragrance, or flavor molecules in pharmaceuticals, consumer products, or food items.[22] Recently, CDs have been found to encapsulate the hydrophobes attached to polymer chains.[19,23,24] The encapsulation of hydrophobic groups by CD reduces the viscosity and other rheological properties of the solution by several orders of magnitude.[16–18,25–28]

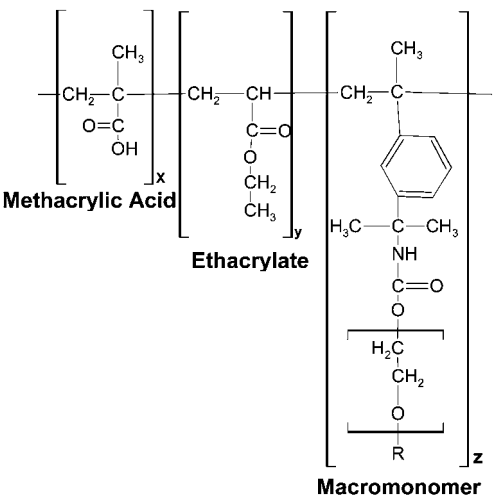

Fig. 1 Structure of HASE polymer in this study: composition of monomers x/y/z = 43.57%/56.21%/0.22% by mole; number of moles of ethoxylation (p = 40), hydrophobic group (R) in the macromonomer is $C_{22}H_{45}$.

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Although the reduction in the viscosity improves the processability of polymer solutions, it is often necessary to restore the original rheological properties for the intended use of the polymer solution. One of the methods to recover the rheological properties is to add a surfactant that has a higher affinity for CD than the hydrophobes.[18,26,29] On addition of surfactants, the CDs attached to hydrophobes are desorbed and they form inclusion compounds with hydrophobic groups of the surfactants. However, polymer-surfactant interaction is inevitable in this process,[30–32] and complete recovery of the original rheological properties cannot be achieved.[18] An intriguing alternative in this regard is to use enzymes that can degrade the CDs.

Cyclodextrins are prone to degradation by microbial and enzymatic attack. Different types of enzymes are found to act on cyclodextrins.[33–36] The amylolytic enzymes that cleave α-1, 4 linkages between glucose units of a starch molecule also degrade CD. Another enzyme, cylodextrinase specifically degrades α-1,4 linkages in CDs.[33–37] These enzymes are found to be capable of releasing the encapsulated molecules from the CD cavity.[38–41] Nevertheless, the mechanism and kinetics of the enzymatic degradation of CD in the presence of inclusion compounds are not clearly understood. The complexation of CD with host molecules is believed to be an equilibrium process, where the CD adsorbed to the host molecule is in equilibrium with free CD in solution.[42] It is not known whether the enzymes degrade adsorbed CDs or free CDs in solution or both. While degradation of the adsorbed CD can break the inclusion compound, the degradation of free CDs can also release the CD from the inclusion compounds to maintain the equilibrium. In fact, if CD equilibrium is established very rapidly relative to the rate of CD degradation, the exact mechanism is not kinetically relevant.

For CDs in aqueous solutions, the activity of α-amylase enzyme increases with increasing CD ring size,[35,43] with almost no activity for α-CD. Studies of the active centers on α-amylase enzyme show that the CDs must bind to hydrophobic groups on the enzyme molecule before ring scission can occur.[44–47] Because of the smaller ring size of α-CDs, it is difficult for them to form a complex with hydrophobic binding sites on α-amylase. Similarly, in the presence of polymers or other molecules containing hydrophobic groups, the rate of CD ring scission will depend on the ability of the CD to complex with enzyme active centers, relative to its ability to complex with the other hydrophobic groups in the reaction mixture.[47,48]

The kinetics of enzymatic degradation of free natural and modified CDs has been extensively studied with different types of enzymes.[43,35,36] The main focus of these studies has been on estimating kinetic parameters for the enzymatic reaction and studying the effects of pH, temperature and ionic strength on the kinetic parameters. However, no kinetic studies have been reported on the degradation of CD in the presence of molecules that form inclusion compounds with the CD.

In previous kinetic studies on degradation of CD, chromatographic techniques were used to analyze the degradation products.[43,49,50] These techniques require dilution of the reaction mixture and the conditions (*i.e.*, temperature, pH, ionic strength) that are quite different from that of the reaction mixture. These methods are not appropriate for reaction mixtures containing HASE polymers, as the dilution and the test conditions may affect the polymer solubility and the complexation of CD with the hydrophobes. Further, reaction mixtures containing hydrophobic polymers pose greater difficulty in chromatographic analysis,[20] since the hydrophobic groups may affect the performance of chromatographic columns. Taking these drawbacks into account, we have developed a new approach, utilizing rheometry, to study the kinetics of the enzymatic degradation of CD in the presence of HASE polymers. The work builds on our previous model on hydrophobe-cyclodextrin complexation,[25] and tracks enzymatic reactions by monitoring changes in the dynamic moduli of the solution as the reaction proceeds.

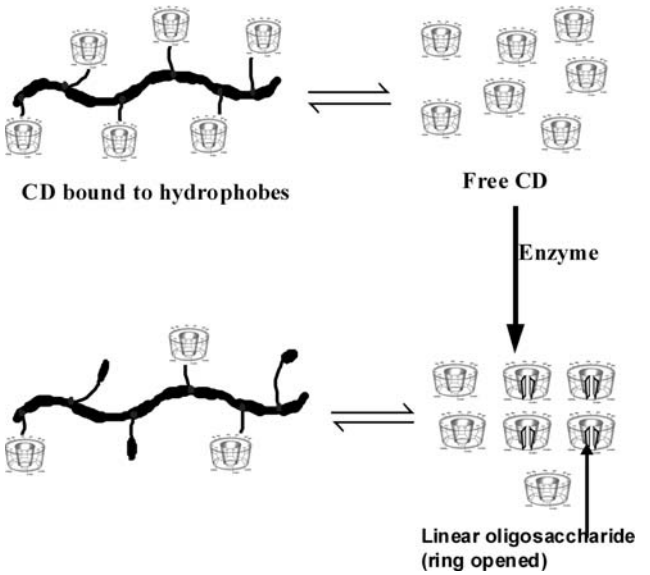

Fig. 2 Schematic of enzymatic degradation of CD in the presence of CD-hydrophobe inclusion compounds. Enzymes degrade free CD in solution and the CD bound to hydrophobes are released in order to maintain the equilibrium between the free CD and the bound CD.

The recovery of rheological properties during enzymatic degradation, schematized in Fig. 2, involves three steps: (1) enzymatic reactions on free CD; (2) dissociation of CD from the hydrophobes to maintain the equilibrium; (3) association of free hydrophobic groups to recover the rheological properties. The degradation of free CD in solution releases the CD adsorbed on the hydrophobes as shown in Fig. 2. On removal of CD from the hydrophobes, hydrophobic interactions are restored that increases the dynamic moduli. In the following section, we develop a detailed kinetic model for the enzymatic degradation of CD and show how the kinetic parameters can be estimated from rheological measurements.

2. Mathematical modeling

2.1. Enzymatic degradation of cyclodextrin

The enzymatic reactions involve two steps, ring opening followed by chain scission, as shown in Fig. 3. In the first step, the CD is cleaved randomly at one of the α-1, 4 linkages between glucose units to form a linear oligosaccharide. In the second step, the enzyme continues to cleave the α-1,4 linkages on the linear oligosaccharide, ultimately leading to the formation of glucose. The ring-opening step and the chain-scission steps will have different kinetic parameters. Moreover, for the chain-scission step, the kinetic rate constant will vary, depending on the

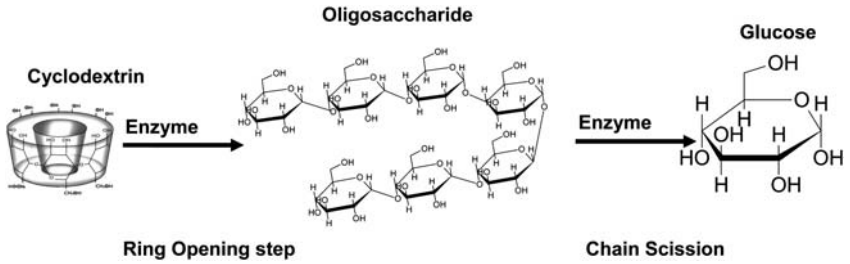

Fig. 3 Schematic of enzymatic reactions showing ring opening and chain scission.

position of α-1,4 bond on the oligosaccharide. The enzymatic reactions in both the steps are assumed to follow the Michaelis Menten (MM) kinetics.

$$E + CD \underset{k_{02}}{\overset{k_{01}}{\rightleftharpoons}} (E - CD) \xrightarrow{k_{03}} E + P_n \tag{1}$$

$$E + P_i \underset{k_{ij2}}{\overset{k_{ij1}}{\rightleftharpoons}} (E - P_i)_j \xrightarrow{k_{ij3}} E + P_j + P_{i-j} \ ,$$

$$for \quad i = 2, n \quad \& \qquad j = 1, i-1 \tag{2}$$

Eqn (1) represents the ring-opening step and the eqn (2) represents the chain- scission step. In the above equations, P_i represents a linear oligosaccharide containing "i" glucose units and "n" is the total number of glucose units in a CD molecule. The enzyme-substrate complexes formed with CD and at the j^{th} bond of an oligomer molecule with "i" glucose units are represented by (E-CD) and $(E\text{-}P_i)_j$ respectively.

Cyclodextrin binds to the hydrophobic sites (H) on the HASE polymer forming an inclusion compound, represented as H-CD.

$$H + CD \underset{k_d}{\overset{k_a}{\rightleftharpoons}} H - CD \tag{3}$$

Since enzymes are proteins that may contain hydrophobic/hydrophilic amino-acids,[51–54] the enzymes may interact with the HASE polymer to form enzyme-polymer complexes, as represented as E-Π.

$$E + \Pi \underset{k_{de}}{\overset{k_{ae}}{\rightleftharpoons}} E - \Pi \tag{4}$$

In eqn (3) and (4), H and Π represent the binding sites on the polymer for CD and enzyme molecules respectively. Applying pseudo steady state approximations for all the complexes in eqn (1), (2), (3) and (4), we get,

$$\frac{d[E - CD]}{dt} = k_{01}[E][CD] - (k_{02} + k_{03})[E - CD] \simeq 0 \tag{5}$$

$$\frac{d[E - P_i]_j}{dt} = k_{ij1}[E][P_i] - \left(k_{ij2} + k_{ij3}\right)[E - P_i]_j \simeq 0 \tag{6}$$

$$\frac{d[H - CD]}{dt} = k_a[H][CD] - k_d[H - CD] \simeq 0 \tag{7}$$

$$\frac{d[E - \Pi]}{dt} = k_{ae}[E][\Pi] - k_{de}[E - \Pi] \simeq 0 \tag{8}$$

The last two equations are equivalent to assuming that Reactions (3) and (4) are in equilibrium.

From eqn (5), (6), (7) and (8),

$$[E - CD] = \frac{k_{01}}{k_{02} + k_{03}}[CD][E] = \frac{[CD][E]}{K_0} \tag{9}$$

$$[E - P_i]_j = \frac{k_{ij1}}{k_{ij2} + k_{ij3}}[P_i][E] = \frac{[P_i][E]}{K_{ij}} \tag{10}$$

$$[H - CD] = \frac{k_a}{k_d}[H][CD] = \frac{[H][CD]}{K_d} \tag{11}$$

$$[E - \Pi] = \frac{k_{ae}}{k_{de}}[E][\Pi] = \frac{[\Pi][E]}{K_\Pi} \tag{12}$$

In the above equations, K_0 is the Michaelis constant for degradation of CD and K_{ij} is the Michaelis constant for the degradation of the j^{th} bond on an oligomer with "i" glucose units. The parameters K_d, K_Π are the binding constants for CD-hydrophobe and enzyme-polymer complexation respectively.

From the enzyme mass balance, the total concentration of enzymes (E_T) is given by,

$$E_T = [E] + [E - CD] + [E - \Pi] + \sum_{i=2}^{n} \sum_{j=1}^{i-1} [E - P_i]_j \tag{13}$$

Using eqn (9)–(12) in (13),

$$[E] = \frac{K_0 E_T}{K_0 + CD + \frac{K_0[\Pi]}{K_\Pi} + \sum_{i=2}^{n} \frac{K_0}{K_i} P_i} \tag{14}$$

where,

$$\frac{1}{K_i} = \sum_{j=1}^{i-1} \frac{1}{K_{ij}} \tag{15}$$

For simplicity, we assume that the enzymatic degradation of CD occurs only in solution, *i.e.*, that CD complexed with the HASE polymer does not react directly (the rationale behind this

assumption is presented later in Section 4.2). Hence, the overall rate of disappearance of free CD from the solution is given by,

$$\frac{d[C]}{dt} = -k_{03}[E - CD] + r_d \tag{16}$$

The first term on right hand side of the above equation is the net rate of degradation CDs by enzyme and the second term (r_d) is the rate at which CD desorbs from the HASE polymer in order to maintain equilibrium between free and complexed CD. The rate "r_d" can be derived as follows.

From the mass balance on hydrophobic binding sites, the total concentration of the binding sites (H_0) can be given by,

$$H_0 = [H] + [H{-}CD] \tag{17}$$

Using eqn (17) in (11),

$$\frac{[H.CD]}{H_0} = \frac{[CD]}{K_d + [CD]} \tag{18}$$

Now, $r_d = -\left(\frac{d[H.CD]}{d[CD]}\right)\frac{d[CD]}{dt} = -\frac{K_d H_0}{(K_d + [CD])^2}\frac{d[CD]}{dt}$

Using eqn (9), (14) and (19) in (16),

$$\frac{d[C]}{dt} = \frac{-k_{03}E_T[CD]}{\left\{K_0 + [CD] + \frac{K_0[\Pi]}{K_\Pi} + \sum_{i=2}^{n}\frac{K_0}{K_i}[P_i]\right\}\left\{1 + \frac{H_0 K_d}{(K_d + [CD])^2}\right\}} \tag{20}$$

From the mass balance on CD, the total concentration of CD (C) is given by,

$$C = [C] + [H - CD] = \left[1 + \frac{H_0}{K_d + [CD]}\right][CD] \tag{21}$$

Using eqn (21) in eqn (20),

$$\frac{dC}{dt} = \frac{-k_{03}E_T[CD]}{\left\{K_0 + [CD] + \frac{K_0[\Pi]}{K_\Pi} + \sum_{i=2}^{n}\frac{K_0}{K_i}[P_i]\right\}} \tag{22}$$

It has been shown that for degradation of CD by amylase or cyclodextrinase enzymes, the substrate binding affinity parameters (or MM constants) K_0, and K_i do not differ significantly from each other.[43,49] Also the ring-opening step is found to be very slow compared to the chain-scission step.[49] Hence, the concentration of oligomers with at least one degradable bond, $\sum_{i=2}^{n} P_i \approx 0$. For CD degrading enzymes, K_0 is of the order of 80mM and the initial concentration of CD in the reaction mixture is 7 mM for 1% HASE solution. Under these conditions, $K_0 + \frac{K_0[\Pi]}{K_\Pi} >>> [CD]$ and eqn (22) can be simplified as,

$$\frac{dC}{dt} = -k_c'E_T[CD] = -k_c[CD] \tag{23}$$

where

$$k_c' = \frac{k_{03}}{K_0 + \frac{K_0[\Pi]}{K_\Pi}} \tag{24}$$

Our experiments as discussed in following paragraphs show that 30 moles of β-CDs are required to completely saturate one mole of the hydrophobes, *i.e.*, only 1 mole of CDs out of 30 moles of CD added to the solution binds to the hydrophobes. Hence we substitute total CD concentration (C) for free CD concentration ([CD]) in eqn (22).

Integrating eqn (23),

$$C \approx [CD] \approx C_0 e^{-k' E_T t} = C_0 e^{-k_c t} \tag{25}$$

In the above equation C_0 represents the initial concentration of total CD in the solution.

2.2. Rheokinetics of enzymatic reactions

According to transient network theory,[55] the high frequency modulus or plateau modulus (G_∞) is directly proportional to number of network junctions in a network,

$$G_\infty = gnkT \tag{26}$$

Where, g is a constant usually taken as unity; n is the junction density, k is the Boltzman constant and T is the absolute temperature. We have observed that the dynamic moduli of HASE polymer solutions decrease on addition of CD to the solution, due to encapsulation of network junctions by CD. We assume that the reduction in the plateau modulus is directly proportional to amount of CD adsorbed to the hydrophobes as given by the following equation.

$$\frac{G_{\infty 0} - G_\infty}{G_{\infty 0}} = a\frac{[H - CD]}{H_0} \tag{27}$$

The parameters G_∞, $G_{\infty o}$ represents the plateau modulus with and without CD, respectively.

The proportionality constant "a" couples the reduction in plateau modulus to the fraction of hydrophobic sites on the HASE polymer occupied by CD ([H-CD]/H_0). It has been shown that the extent of reduction in dynamic moduli with CD is independent of frequency.[25] Hence,

$$\frac{G'_{CD}}{G'_0} = \frac{G_\infty}{G_{\infty 0}} = \theta \tag{28}$$

where, G'_{CD}, G'_0 represent the elastic modulus (G′) values at any frequency with and without CD respectively.

Using eqn (27) and (28) in (18) and assuming $C \approx [CD]$,

$$1 - \theta = \frac{aC}{K_d + C} \tag{29}$$

The eqn (29) indicates that "θ" will become negative at very high CD concentrations, if a>1.0. Since the negative elastic modulus does not have a physical meaning, we assume a = 1 in the following analysis.

The equations for rate of change in θ during enzymatic reactions can be derived using eqn (25) in (29)

$$1 - \theta = \frac{C_0 e^{-k_c' E_T t}}{K_d + C_0 e^{-k_c' E_T t}} \quad (30)$$

Values of θ are obtained by measuring elastic modulus (G′) of the polymer solution during the enzymatic reactions. The variation in θ with time can be fit to eqn (30) to estimate the parameters K_d and k_c'. Thus simple rheological measurements can be used to obtain model parameters.

The kinetic rate constant k_c, varies with temperature according to Arhenius equation.[56]

$$k_c = A \exp\left(\frac{-\Delta E}{RT}\right) \quad (31)$$

Here ΔE is the activation energy, A is the frequency factor, T is the absolute temperature and R is the gas constant.

3. Materials and methods

A model hydrophobically modified alkali-soluble emulsion (HASE) polymer synthesized by UCAR Emulsion Systems (Dow Chemicals, USA) was supplied as aqueous latex of approximately 26% solids. It is a copolymer of methacrylic acid, ethacrylate and a macromonomer containing hydrophobic groups as shown in Fig. 1. The compositions of these components in the polymer are 43.57%, 56.21%, and 0.22% by mole respectively. The hydrophobic macro monomer is composed of C_{22} alkyl hydrophobic groups. These groups are separated from the polymer backbone by a polyethylene oxide spacer containing 40 moles of ethoxylation. The macro monomer is attached to the polymer backbone through a urethane linkage. The polymer was purified using protocols described elsewhere prior to usage.[4,5]

The β-CD was purchased from Cerestar, Inc., USA. The moisture content in CD was measured using a TGA-2950 thermo gravimetric analyzer (TA Instruments, USA) and was found to be approximately 13%. The CD was used as obtained and the moisture content was taken into account while estimating the CD concentrations in the polymer solutions.

HASE solutions were prepared in DI water using sufficient 1M NaOH to neutralize the methacrylic acid in the polymer. The required quantities of polymer, NaOH, β-CD and water were mixed together in a beaker and kept in a water bath at 50 °C to ensure complete dissolution and viscosity development. The samples were removed from the water bath after a clear solution without entrapped air bubbles was obtained. The final solution pH was 7.0 ± 0.2. The samples were used for the experiments within two weeks of their preparation in order to avoid degradation of the polymer in the presence of NaOH.[57]

A commercial α-amylase enzyme (Clarase) used for this study was a gift from Genencor Inc. USA. This enzyme, which is a fungal α-amylase enzyme derived from a selected strain of *Aspergillus Oryzae* was supplied in the form of a liquid solution with a specific gravity of 1.15 g/ml. The activity of the enzyme in the solution was 4000 SKBU/g. One SKBU is the concentration of enzyme required to dextrinize 1.0 g of limit dextrin per hour under assay condition.[58] The optimum pH and temperature ranges for the enzyme activity are 5.5–6.5 and 40–50 °C respectively.

The enzymatic reactions were carried out *in situ* in a TA Instruments AR-2000 stress controlled rheometer using couette geometry. Polymer solution with a known amount of β-CD was equilibrated at the desired temperature in a water bath. The preheated HASE solution with β-CD was then mixed with the desired amount of enzyme and immediately loaded into the rheometer, which had previously been heated to the test temperature. Dynamic time sweep test was carried out to track the enzymatic reactions. The test was carried out at an oscillatory stress of 1 Pa and the dynamic moduli were measured at a frequency of 1 rad/s at five minutes interval. The reaction was stopped when the dynamic moduli increased to a plateau value. High viscosity PDMS oil was used to prevent evaporative loss during the reactions at temperatures above room temperature.

4. Results and discussions

4.1. Effect of CD-hydrophobe complexation on rheology

Fig. 4 shows the effect of β-CD on the dynamic moduli, G′ and G″, of 1% HASE solution measured at 25 °C and pH 7. In the figure, the ratios of elastic moduli with and without CD are plotted against the CD concentration. The symbols show the average values of θ measured over a frequency range of 0.1–10 rad/s; the error bars represent the deviations in θ from the average value. The figure shows that the dynamic moduli decrease on addition of CD and the most reduction is achieved at around 25 moles of CD/mole of macromonomer. The figure also shows that at any CD concentration, the extent of reduction is same at all the frequencies, thus confirming eqn (28),*i.e.*, the ratios G'_{CD}/G'_0 and G''_{CD}/G''_0 are approximately equal at all frequencies. This behavior can be explained as follows.

The hydrophobic interactions between HASE molecules form a transient network with the network junctions that have different moduli and relaxation times depending on the number of polymer chains participating in the junction. The dynamic behavior of such networks can be represented by the generalized Maxwell model with multiple relaxation modes as given by the following equations.[59]

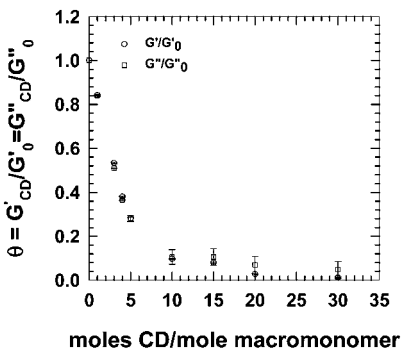

Fig. 4 Effect of CD on the elastic modulus (G′) and viscous modulus (G″) of 1% (w/w) HASE solution measured at 25 °C and pH 7. The symbols show the average of the dynamic moduli over the frequency range of 0.1–10 rad/s and, the error bars represent the deviation in measured values from the average value.

$$G' = \sum_{i=1}^{n} G_i \frac{(\omega\tau_i)^2}{1+(\omega\tau_i)^2}$$

$$G'' = \sum_{i=1}^{n} G_i \frac{\omega\tau_i}{1+(\omega\tau_i)^2} \quad (32)$$

$$where, \quad G_i = gn_i kT$$

In the above equations G_i, is the modulus of a relaxation mode with relaxation time τ_i. The G_i is proportional to the junction density (n_i). On addition of CD, some of the junctions in each relaxation mode are broken, resulting in a decrease in the junction densities. However, the extent of reduction in each junction density is same for all the modes and the relaxation times do not change significantly. Hence the extent of reduction in moduli remains the same at all frequencies. This shows that it is sufficient to monitor changes in dynamic moduli at any one frequency to track the enzymatic degradation of CD.

4.2. Recovery of rheological properties during enzymatic degradation of CD

In Fig. 5, we compare the dynamic spectra of a 1% HASE solution with that of an identical solution that had been treated with CD concentration of 30 moles/mole of macromonomer and then with 37 SKBU/g of α-amylase. The figure shows the dynamic frequency spectra measured five hours after the addition of enzyme. We find that that the degradation of β-CD restores the hydrophobic interactions and recovers the dynamic spectrum so that G′ and G″ match those of the original HASE solution.

We also carried out similar experiments on degradation of α-CD in 1% HASE solution. Although α-CD is able to reduce the viscoelastic properties of HASE solution by several orders of magnitude, no change in solution rheology is observed on addition of α-amylase (data not shown). It has been shown that CDs resist enzymatic reduction by hiding the α-1–4 linkages within their annular cavity.[60] Since α CD has a smaller ring than β-CD, α-amylase may not be able to access α-1–4 bonds inside the CD annulus. Similarly, when CDs are complexed with hydrophobes, the presence of hydrophobes within the annular cavity would obstruct the enzymes in accessing the bonds. This notion validates our model assumption that the α-amylase does not degrade CDs adsorbed to the hydrophobes.

4.3. Estimation of kinetic parameters from rheological experiments

We conducted dynamic time sweep tests, where the dynamic moduli were measured as a function of time. The experiments were conducted at a stress level ensuring that it lied within the linear viscoelastic region during the entire reaction process. The enzymatic reactions were tracked by monitoring the changes in G′ at a frequency of 1.0 rad/s and the results are represented in a plot of θ (ratio of G′ with CD to G′ without CD) vs. time. Fig. 6a shows such a plot for a 1% HASE solution at 25 °C for different concentrations of enzyme [E_T]. In all cases, the initial concentration of the β-CD was maintained at 30moles/mole macromonomer and the pH at 7.0 to obtain maximum enzyme activity while ensuring complete solubility of the polymer. The θ values increased during the degradation of CD, and eventually reached unity indicating complete degradation of CD. The θ *vs.* time curves were fit to eqn (30) using Sigma Plot 8.0 software to estimate the kinetic parameters k_c' and K_d.

Fig. 6b shows a replot of θ as a function of E_T.t, where E_T is the total enzyme concentration in SKBU/g, and t is the reaction time in minutes. We find the data to collapse into a single master curve. In addition, the model eqn (30) fits the experimental data well and also indicates that rate constants ($k_c = k_c'E_T$) increase linearly with enzyme concentration, as expected. The k_c' estimated from the plot is 1.25×10^{-3} (SKBU/g.min)$^{-1}$. The estimated value of the binding constant (K_d) from this experiment is approximately same at all enzyme concentrations with an average of 0.35 mM. This suggests that the presence of enzymes in the solution does not significantly affect the CD-hydrophobe complexation.

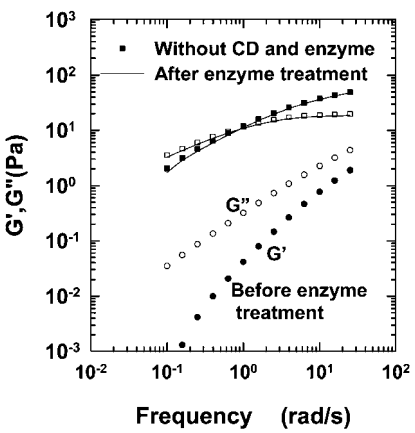

Fig. 5 Recovery of original dynamic properties of 1% HASE solution at 25 °C and pH 7 after enzymatic degradation of β-CD in the solution for 5 h. Initial concentration of CD is 30moles/mole MM, and 37 SKBU/g of enzyme is used for this reaction. Closed symbols show G′ values and open symbols show G″ values.

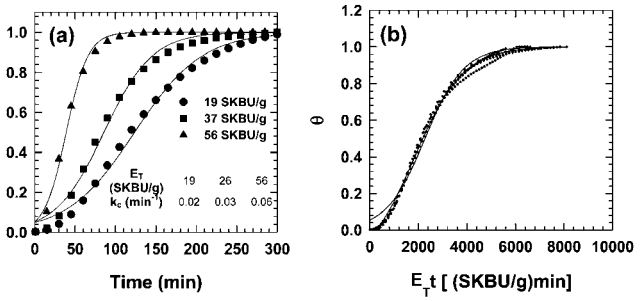

Fig. 6 (a) Evolution of the dynamic elastic modulus as a function of time during enzymatic reactions carried out in 1% HASE solution at 25 °C and pH 7 with enzyme concentrations (E_T) of 19, 37 and 56 SKBU/g. (b) Replot of (6a) showing that evolution dynamic modulus at different enzyme concentrations can be merged on to a single curve when the kinetic rate constant increases linearly with enzyme concentrations. The initial CD concentration in the reaction mixture is 30 moles CD/mole macromonomer. The θ represents the ratio of elastic modulus with and without CD (G'/G'_0) measured at a frequency of 1 rad/s. The solid line shows the model fit.

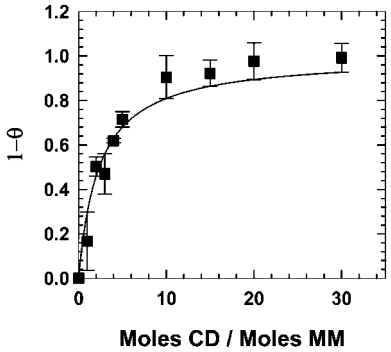

Fig. 7 Effect of β-CD concentrations on the elastic modulus of 1% HASE solution measured at 25 °C. The elastic modulus is measured at an oscillatory stress of 1Pa and frequency of 1rad/s.

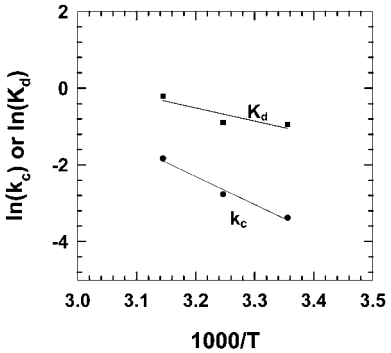

Fig. 9 Effect of temperature on kinetic rate constant and binding constant: The enzymatic reactions are carried out in 1% HASE solution at pH 7 with initial CD concentration of 30 moles CD/mole macromonomer. Enzyme concentration = 26 SKBU/g.

An alternative approach to verify the validity of our model is to estimate K_d using eqn (29) from an independent set of experiments. We measured the dynamic frequency spectrum of 1% HASE solution with different β-CD concentrations at 25 °C. Fig. 7 represents the change in elastic modulus of the HASE solution measured at a frequency of 1 rad/s as a function of β-CD concentration. The experimental data were fit to eqn (29) to estimate the parameter K_d and the value of K_d was found to be 0.5mM respectively. The values of K_d estimated from this experiment and the kinetic experiments are approximately same, and the errors are within the experimental uncertainty limits.

4.4. Effect of temperature on kinetics of enzymatic degradation of β-CD

Fig. 8 shows the changes in θ values of a 1% HASE solution during the enzymatic degradation of β-CD at three different temperatures with an enzyme concentration of 26 SKBU/g. The figure shows that the dynamic moduli recover faster with increasing temperature, indicating the rate of degradation of β-CD increases with temperatures. At a fixed enzyme concentration, the kinetic rate constant is a function of k_{03} and K_0 as given by eqn (24). Both k_{03} and K_0 can change with temperature.[61] As a result the kinetic rate constant can either increase or decrease depending on the effects of temperature on k_{03} and K_0 values. In our case, we find that the rate constant (k_c) to increase with temperature. At higher temperatures, the rate of degradation of the enzyme-substrate complex is more than offset by the decrease in affinity of enzyme to complex with substrate. Hence the rate constant increases with temperature. Fig. 9 also shows that the binding constant (K_d) increases with temperature, suggesting that the affinity of CD to bind to hydrophobes decreases with increasing temperatures.

4.5. Effect of polymer concentration on the kinetics of enzymatic degradation of β-CD

Fig. 10 shows the changes in θ of 1% and 2% HASE solution during the enzymatic degradation of β-CD. The enzyme concentrations used in this study and the kinetic parameters estimated are shown in the Fig. 10. In fact, eqn (24) predicts that k_c should decrease with increasing polymer concentratin as a result of hydrophobic interactions between the polymer and the enzymes. At higher polymer concentrations, more enzymes tend to adsorb on to polymer molecules due to hydrophobic interactions between polymer and enzymes. Hence net enzymes available for degradation of CD decrease resulting in the reduction in rate of degradation of CD. The estimated rate constant for 2%

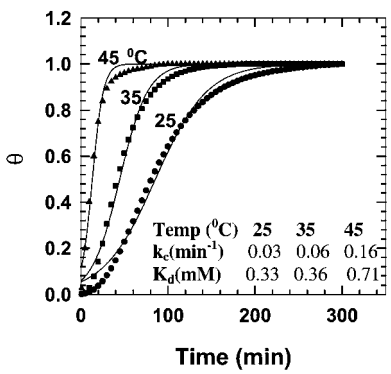

Fig. 8 Effect of temperature on reaction kinetics: The enzymatic reaction is carried in 1% HASE solution at pH 7 with initial CD concentration of 30 moles CD/mole macromonomer. Enzyme concentration = 26 SKBU/g. The solid lines show the model fit.

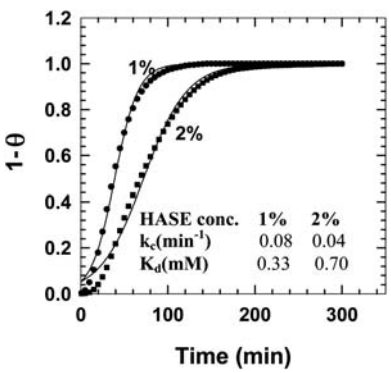

Fig. 10 Effect of polymer concentration on reaction kinetics: The enzymatic reaction is carried out at 25 °C and pH 7 with initial CD concentration of 30 moles CD/mole macromonomer. Enzyme concentration is 56 SKBU/g for 1% solution and 74SKBU/g for 2% solution. The solid lines show the model fit.

polymer solution is approximately half of the rate constant estimated for 1% polymer concentrations. This shows that at the same total enzyme concentrations, amount of enzymes available for reaction is reduced by 50% when the polymer concentration is increased from 1% to 2%. In addition, the affinity of polymer hydrophobes to bind CDs decrease with polymer concentration due to increased hydrophobe-hydrophobe interactions.[25] Hence K_d values increase with increasing polymer concentration as shown in Fig. 10.

5. Conclusions

The encapsulation of hydrophobes present in associative polymers by CD reduces their viscoelastic properties by several orders of magnitudes. Subsequent degradation of CD by enzymes enables complete recovery of the original rheological properties. The rate of recovery is dependent on polymer concentration, enzyme concentration and temperature. A rheo-kinetic model is developed to track the progress of enzymatic reactions *via* monitoring changes in rheological properties of polymer solutions. The model fits the experimental data well and is able to predict the effects of various process parameters on reaction kinetics and on the recovery of the original rheological properties. The kinetic rate constant increases linearly with increasing enzyme concentrations. The kinetic constant is also found to increase with increasing temperature, as represented by Arrhenius equation. At higher polymer concentrations, enzymatic degradation of CD is hindered due to enzyme-polymer interactions. The kinetic model can also be used to estimate the binding constant for CD-hydrophobe complexation. The binding constants estimated using the kinetic model is approximately equal to that obtained using the adsorption equilibrium model.

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