Cationic Polyacrylamides Increase the Rate of Liquefaction and Hydrolysis of Cornstarch

Kendra E. Maxwell, Alison L. Krantz, and Sujit Banerjee
Institute of Paper Science and Technology, School of Chemical and Biomolecular Engineering,
Georgia Institute of Technology, 500 10th St., NW Atlanta, GA 30332

DOI 10.1002/aic.13811

Cationic polyacrylamides (c-PAMs) bind to starch granules and decrease the temperature for the onset of gelatinization by 8°C. c-PAM increases the binding of α-amylase to cornstarch; the rate of cornstarch hydrolysis also increases. By analogy to previous work on the c-PAM promoted hydrolysis of cellulose, it is proposed that the polymer reduces the charge on the surface of starch through a charge-patch mechanism. Because both enzyme and substrate are negatively charged, the bound c-PAM reduces the charge repulsion experienced by the approaching enzyme, which leads to stronger enzyme-substrate binding and faster hydrolysis. Overall, the c-PAM reduces enzyme dose by up to 62% under the conditions used. There is a mirror image relationship between the viscosity of the medium and the hydrolysis rate, which allows optimization of these parameters with enzyme and c-PAM dosage. Low c-PAM levels increase viscosity by agglomerating the substrate, but the viscosity drops at higher c-PAM concentration. © 2012 American Institute of Chemical Engineers *AIChE J,* 59: 79–83, 2013

Keywords: cornstarch, alpha-amylase, polyacrylamide, swelling, viscosity

Introduction

The swelling and subsequent gelatinization of starch granules is the first step in their conversion to sugars and then, potentially, to ethanol. We have recently found that cationic polyacrylamides (c-PAM) and other cationic polymers can catalyze the enzymatic hydrolysis of cellulose.[1] A "patching" mechanism was proposed where the polymer attaches to and neutralizes the negatively charged substrate, thereby decreasing the repulsion experienced by the approaching negatively charged enzyme. Stronger enzyme-substrate binding results.[2,3] Patching is well-established in the literature on fiber agglomeration[4] and on water treatment.[5] The conversion of cornstarch to glucose is more complex than that of the corresponding process with cellulose where swelling and gelatinization are absent. In this article we study the effect of c-PAMs on the several steps through which cornstarch is liquefied and hydrolyzed by α-amylase, and discuss the similarities between the effect of c-PAM on cornstarch and cellulose hydrolysis. c-PAM also affects viscosity, which impacts mixing and pumping. Hence, we specifically examine the interrelationship among viscosity, c-PAM dose and hydrolysis rate.

Methods and Materials

Cornstarch (at 10% moisture content) was obtained from Sigma-Aldrich, St. Louis, Mo. The c-PAM used most extensively was XP10025, a 40% cationicity polymer from Eka Chemicals, Marietta, GA. XP10023 (10% cationicity) and XP10033 (80% cationicity) polymers were also used for some of the Brix measurements. Zeta potential and dissolved organic carbon (DOC) measurements were made with Malvern Zetasizer 3000 and Shimadzu TOC-V_{CSH} instruments, respectively. Viscosity was determined with a Grace Instruments M3500 viscometer. The starch suspension was heated from 20 to 90°C at 2.5°C/min at a shear rate of 400 s^{-1} and held at those conditions for 40 min.

Because c-PAMs gradually lose charge through hydrolysis[6] we measured the zeta potential of c-PAM with time. The results, shown in Figure 1, indicate that while there is some charge loss, the polymer retains most of its charge during the typical hydrolysis period. BAN 480L α-amylase was obtained from Novozymes. The activity was 3.5 ± 0.6 U/mL where 1U is the amount of enzyme that releases 1 μmol/min of glucose from raw cornstarch at pH 6 and 50°C. The uncertainty cited in this example and for other applications in this article is the standard deviation. While there were small differences in enzyme activity over the course of the study, the interpretations are mostly drawn on a relative basis. Swelling measurements were made with 2% starch suspensions containing varying amounts of c-PAM. The samples were heated at different temperatures for 30 min and immediately centrifuged for 15 min at 3,000 RCF. The resulting pellet was weighed, dried at 105°C for 24 h and reweighed. The swelling power was calculated as the ratio of the mass of the hydrated substrate to that of the dry pellet. The DOC of the supernatant was measured; the measurement uncertainty was 11%.

The swelling of cornstarch particles was also monitored with light microscopy. The starch suspension (with and

---

Correspondence concerning this article should be addressed to and S. Banerjee at sb@gatech.edu.

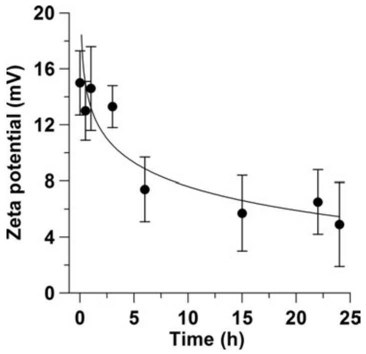

Figure 1. Hydrolysis of 100 ppm of c-PAM (40% cationicity) at 50°C in pH 6 buffer.

The results are averaged over 3 determinations.

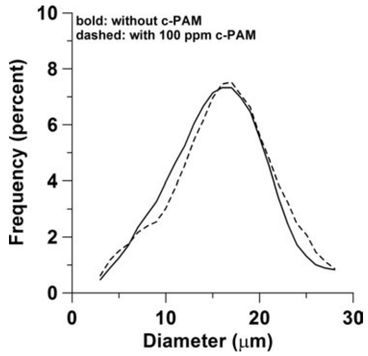

Figure 3. Effect of c-PAM on granule size.

Cornstarch was present at 1%.

without 100 ppm c-PAM) in pH 6 phosphate buffer was placed in a shaker bath at 50°C for 30 min. After cooling to room temperature, photographs were taken through an Olympus BH-2 microscope at 100 X magnification with a Pixera digital camera. Image analysis software (ImageJ) was used to calculate particle size distribution of the images after converting pixels to length (μm) with reference images of known length. Each sample imaged contained more than 300 granules. The results were averaged from four images. The measurement uncertainty was <4%.

The binding of c-PAM to starch was measured by the solution depletion method[7,8] by adding starch at various concentrations to 20 ppm of a c-PAM (40% cationicity). The samples were shaken at 25°C for 30 min and centrifuged at 3,000 RCF for 15 min. The supernatant was charge-titrated against PVSK (potassium polyvinyl sulfate) using a MÜTEK PCD-03 particle charge detector from BTG Americas. The amount of c-PAM adsorbed to the starch was determined from the difference in charge in the supernatant between c-PAM alone and that from the starch/c-PAM samples. The analytical uncertainty was about 6%. The binding of enzyme to c-PAM treated starch was measured by adding c-PAM to a suspension of cornstarch in water at 4°C. Enzyme was added to a concentration of 0.6%, and the suspension shaken cold for 30 min. It was then centrifuged at 13,400 RCF for 10 min and the enzyme in the supernatant determined with a Pierce BCA protein assay kit. The measurement uncertainty was about 1%.

For the hydrolysis runs, enzyme was added to a mixture of c-PAM and starch and the suspension stirred with an impeller. The samples were filtered and the glucose determined with a GOPOD format d-glucose assay kit from Megazyme International, Wicklow, Ireland, adapted to a DA3500 Discrete Analyzer from OI Corp., College Station, TX. The average error from duplicate measurements was 4.5%. Brix was measured with a Sper Scientific 300034 Digital Refractometer.

Results and Discussion

Effect of c-PAM on cornstarch swelling

Because starch granules swell before they rupture we first determined whether c-PAM had any effect on swelling. As Figure 2 shows, swelling power is unaffected by the presence of c-PAM (40% cationicity) across temperatures that straddle the gelatinization temperature of about 70°C. Not all the granules are destroyed at the gelatinization temperature; a significant number survives even at 100°C.[9] The particle size distribution of the starch granules was also obtained by optical microscopy at various times between 40 and 70°C both with and without c-PAM. A typical example with 1% cornstarch taken after 30 min at 50°C is shown in Figure 3. A significant change in particle size was not observed with the c-PAM, which is consistent with the swelling results.

The effect of c-PAM on cornstarch solubilization, as measured by the dissolved organic material (DOC) in a suspension of 2% cornstarch in water, is shown in Figure 4. As will be shown later, the binding of c-PAM to starch is very high so the added c-PAM will be almost completely bound to solids. Hence, the DOC values reported reflect only

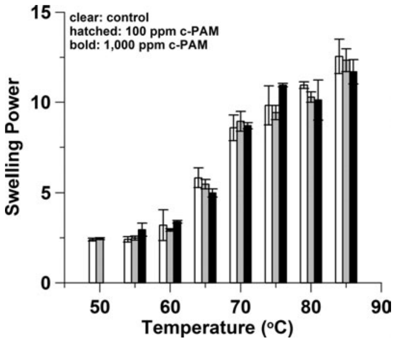

Figure 2. Effect of c-PAM on swelling power.

Cornstarch was present at 2%. The results are averaged over 4 determinations.

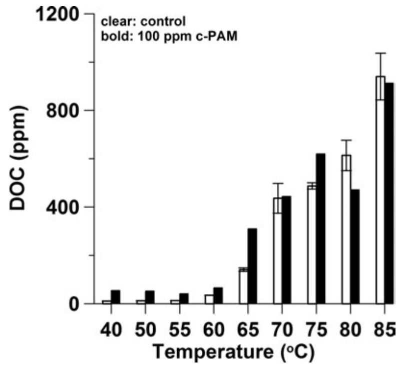

Figure 4. Effect of c-PAM on solubilizing material from cornstarch starch.

The error bars were derived from four replications.

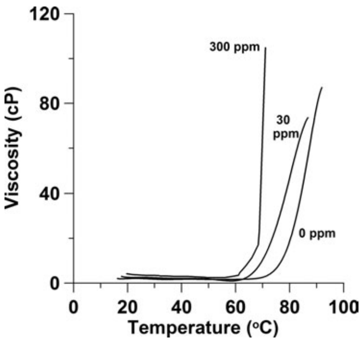

Figure 5. Effect of c-PAM on gelatinization temperature.

Cornstarch was present at 8%.

dissolved material associated with or released from the granules and from the c-PAM. Surprisingly, the c-PAM raises the DOC of the supernatant at temperatures well below the gelatinization temperature of about 70°C; evidently, it induces disintegration of some of the granules. More importantly, it reduces the temperature for the onset of gelatinization (signaled by a sharp rise in viscosity) as seen in the typical viscosity-temperature plot of Figure 5. The average onset temperature for cornstarch was 70 ± 2°C (n = 3), which is in line with literature values.[10] That for c-PAMs ranging between 10, 40 and 80% cationicity c-PAMs used in this study was 62 ± 2°C (n = 8). The p value obtained from a t-test comparing control and c-PAM treated samples was 0.003, demonstrating that the onset temperature for cornstarch gelatinization was significantly lower in the presences of c-PAM. This finding is of considerable practical importance because it allows hydrolysis to begin under milder conditions.

Changes in the onset temperature of gelatinization induced by additives have been observed by many others. For example, Mandala and Bayas[11] found that xanthan increased soluble solids from wheat starch at subgelatinization temperatures from 2 to 3%. Also, the onset temperature can be raised or lowered by the addition of salts.[12,13] Polysaccharides such as guar gum can affect swelling.[14] Although a study of the mechanism through which c-PAM affects the onset temperature is beyond the scope of this study, we recognize that c-PAM is an agglomerant, and the friction among the agglomerated particles could potentially cause early rupture.

Binding of c-PAM and enzyme on cornstarch granules

The distribution of c-PAM between starch and water is illustrated in Figure 6. Surprisingly, the partition coefficient (the ratio of c-PAM associated with starch to that dissolved in an equal weight of water) is very high when the starch content in the suspension is low; it then asymptotically plateaus to a value of 107 at higher starch levels. Partition coefficients are constant if sorption follows a Langmuir isotherm; they typically decrease with increasing solute:solids ratio if a Freundlich equation is obeyed.[15] We have the opposite situation here, where partitioning increases at high solute:solids ratios. The anomaly likely arises from the known ability of c-PAMs to aggregate starchy particles in water.[16] At a high-relative c-PAM dose (low-starch levels in Figure 6) the c-PAM agglomerates starch-derived colloids along with starch particles, which are subsequently pelletized during centrifugation. A high-partition coefficient results because the colloids are largely removed from the water. At low doses the c-PAM is unable to agglomerate all the solids and some of the colloids remain in water. The c-PAM associated with these particles appears in the aqueous phase and gives rise to a lower partition coefficient. The important conclusion here is that c-PAM binds very strongly to starch.

The effect of 30 ppm c-PAM (40% charge) on the binding of α-amylase to cornstarch (0.05–10%) is illustrated in Figure 7. Similar profiles were obtained with up to 300 ppm of c-PAM. Binding constants for the control (without c-PAM) compare well to those reported by Warren et al.[17] It is clear that the c-PAM greatly increases the binding of enzyme to starch. A similar situation applies to the binding of cellulase to cellulose.[2] Increasing sorption through use of c-PAM has been reported in other applications; e.g., Wang et al.[18] showed that c-PAM increased the sorption capacity of modified bentonite. The overall conclusion from these binding measurements is that the c-PAM binds to starch, and, thereby, increases enzyme-substrate binding.

Hydrolysis and viscosity studies

The results in Figure 4 reveal that c-PAM increases the amount of soluble starch in water at pregelatinization temperatures without any added enzyme. It follows that α-amylase should be able to hydrolyze these solubles to glucose. This is, indeed, the case as shown in Figure 8 where 1% cornstarch was hydrolyzed by 0.004% α-amylase. The increase in glucose induced by 30 ppm of c-PAM is about 100 ppm. The carbon content of glucose is 40%. The corresponding increase in DOC in Figure 4 should, therefore, be 40 ppm, which is the result obtained. Hence, the c-PAM solubilizes starch, which hydrolyzes to glucose if enzyme is

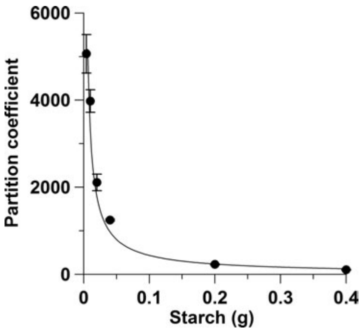

Figure 6. Partitioning of c-PAM between cornstarch starch and water.

The error bars were derived from three replications.

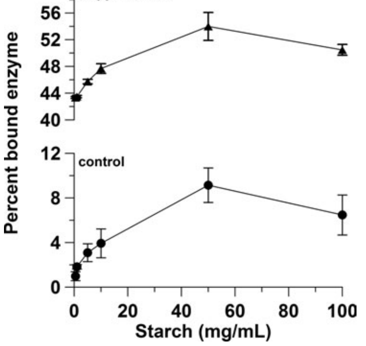

Figure 7. Effect of c-PAM on the binding of α-amylase to cornstarch.

The results were averaged from two determinations.

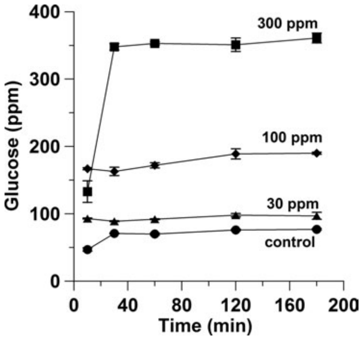

Figure 8. Effect of c-PAM on glucose generation at a pregelatinization temperature of 50°C.

The results were averaged from three determinations.

present. The fraction of the cornstarch solubilized by the c-PAM under pregelatinization conditions is only about 0.2%, so any changes in granular size would be far too small to be detectable in the size measurements of Figure 3.

The effect of c-PAM on enzymatic starch hydrolysis at higher temperature was studied by measuring Brix, a specific gravity-based measurement; 1° Brix is the equivalent of 1 g of sucrose in 100 g of solution. The dependence of peak viscosity and Brix on c-PAM cationicity is illustrated in Figure 9 where 8% cornstarch was hydrolyzed by 1% enzyme by volume. The mirror-image relationship demonstrates that hydrolysis is suppressed at high viscosity. Increasing viscosity retards reaction rates of both chemical[19] and biological[20] processes. The viscosity increase at 10% cationicity comes from agglomeration of the substrate by the polymer. This, by itself, would lead to a lower rate by reducing the exposed surface of the substrate. The particles taken on a net positive charge at higher cationicity and the repulsion among particles reduces the degree of agglomeration and increases the rate. This type of behavior is well established in the literature of sludge dewatering.[21] A mirror image relationship between viscosity and Brix also results when the concentration of a 40% cationicity c-PAM is varied as shown in Figure 10. As previously mentioned, the agglomeration that occurs at low c-PAM concentration is reversed as the c-PAM concentration is raised. Increasing c-PAM concentration is analogous to using a higher charged c-PAM in Figure 9 example in that charge repulsion reduces the tendency of the particles to agglomerate. It follows that for our application a 40% cationicity c-PAM is optimal.

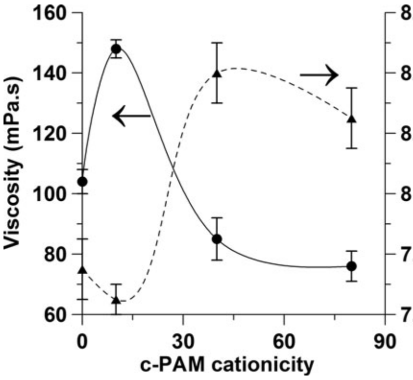

Figure 9. Effect of c-PAM cationicity on viscosity and Brix.

Viscosity and Brix results were averaged from two and three measurements, respectively.

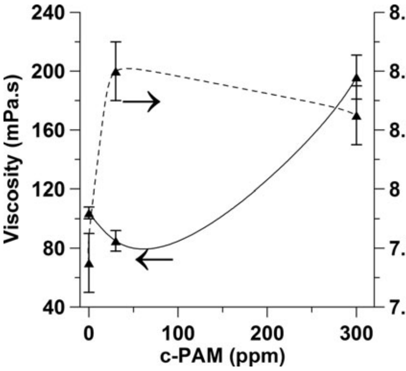

Figure 10. Effect of c-PAM concentration on viscosity and Brix.

Viscosity and Brix results were averaged from two and three measurements, respectively.

The degree to which c-PAM can lower enzyme dosage is demonstrated in Figure 11 where the α-amylase dose was progressively reduced in the presence of 100 ppm 40% cationicity c-PAM. The uncertainty is higher at the intermediate points because viscosity changes sharply in this region. The cornstarch was present at 30% and the enzyme was applied at 0.03% by volume. The hydrolysis was run at 70°C. Interpolation shows that the c-PAM reduces the enzyme loading required to produce the same Brix level obtained in the control by 62%. The c-PAM is cheaper than the enzyme so major savings in enzyme costs are available.

The increase in rate induced by c-PAM for cornstarch parallels that observed for the cellulase induced hydrolysis of cellulose fiber, where the c-PAM provides a similar benefit at a similar dose. A clear difference between the cellulose and cornstarch application lies in the effect of agitation. For cellulose, adequate agitation is critical because the polymer flocculates the fibers and reduces the surface area available to the enzyme; the floc must be disrupted for the c-PAM to show an effect.[22] For the cornstarch work the rate was insensitive to the degree of agitation. Rates obtained with the XP10025 polymer (40% cationicity) at Reynolds numbers of 150–400 differed by only 6%. The smaller particle size of cornstarch reduces its propensity to agglomerate.

Some aspects of cornstarch hydrolysis are strikingly similar to those obtained earlier for cellulose, which suggests a

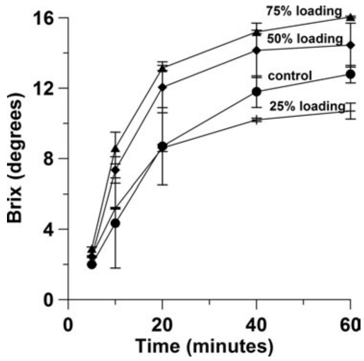

Figure 11. Effect of c-PAM in reducing α-amylase dosage (averaged from three measurements) at 70°C.

common mechanism. It was proposed that the c-PAM accelerated cellulose hydrolysis through a "patching" mechanism through which it neutralized the negative charge of the fiber and reduced the repulsion experienced by the negatively charge cellulase.[2] It seems likely that a similar mechanism applies to cornstarch. The zeta potential of the enzyme and gelatinized corn starch are both negative at −9.1 and −29 mV, respectively, and c-PAM neutralizes the zeta potential of cornstarch. In both cases, the binding of enzyme to substrate increases substantially in the presence of c-PAM.

Conclusions

Cationic polyacrylamides promote the hydrolysis of cornstarch in three ways. First, it increases the solubilization of starch by a small extent before gelatinization. Second, it decreases the temperature of gelatinization. Finally, it increases the amount of glucose produced after gelatinization. The c-PAM promotes cornstarch and cellulose hydrolysis in much the same way. We propose that the c-PAM binds to the substrate and reduces the charge on the surface of the substrate, which increases enzyme-substrate binding by reducing the charge repulsion experienced by the approaching enzyme. The c-PAM is much cheaper than the enzyme and its ability to reduce enzyme dose should be economically attractive. A major finding of this study is the mirror-image relationship between viscosity and the hydrolysis rate, which derives from variable agglomeration caused by the c-PAM.

Acknowledgments

Kendra Maxwell thanks the Institute of Paper Science and Technology for a fellowship. We thank Eka Chemicals for financial support, and Ms Helina Desta for running the pregelatinization liquefaction experiments.

Literature Cited

1. Reye JT, Maxwell K, Rao S, Lu J, Banerjee S. Cationic polyacrylamides enhance rates of starch and cellulose saccharification. *Biotechnol Lett*. 2009;31:1613–1616.
2. Mora S, Lu J, Banerjee S. Mechanism of rate enhancement of wood fiber saccharification by cationic polyelectrolytes. *Biotechnol Lett*. 2011;33:1805–1808.
3. Reye JT, Maxwell K, Banerjee S. Cationic polyacrylamides promote binding of cellulase and amylase. *J Biotechnol*. 2011;154:269–273.
4. Hubbe MA, Nanko H, McNeal MR Retention aid polymer interactions with cellulosic surfaces and suspensions: a review. *BioRes*. 2009;4:850–906.
5. Bratby J. *Coagulation and Flocculation in Water and Wastewater Treatment*. 2nd ed. London: IWA Publishing; 2006.
6. Aksberg R, Wågberg L. Hydrolysis of cationic polyacrylamides. *J Appl Polym Sci*. 1989;38:297–304.
7. Leloup VM, Colonna P, Ring SG. α-Amylase adsorption on starch crystallites. *Biotechnol Bioeng*. 1991;38:127–134.
8. Noh H, Vogler EA. Volumetric interpretation of protein adsorption: Partition coefficients, interphase volumes, and free energies of adsorption to hydrophobic surfaces. *Biomaterials*. 2006;27:5780–93.
9. Chin P, Yu L, Kealy T, Chen L, Li L. Phase transition of starch granules observed by microscope under shearless and shear conditions. *Carbohyd Polym*. 2007;68:495–501.
10. Singh N, Singh J, Kaur L, Sodhi NS, Gill BS. Review: morphological, thermal and rheological properties of starches from different botanical sources. *Food Chem*. 2003;81:219–231.
11. Mandala IG, Bayas E. Xanthan effect on swelling, solubility and viscosity of wheat starch dispersions. *Food Hydrocolloid*. 2004;18:191–201.
12. Jane J. Mechanism of starch gelatinization in neutral salt solutions. *Starch Stärke*. 1993;45(5):161–166.
13. Zhu WX, Gayin J, Chatel F, Dewettinck K, Van der Meeren P. Influence of electrolytes on the heat-induced swelling of aqueous dispersions of native wheat starch granules. *Food Hydrocolloid*. 2009;23:2204–2211.
14. Tester RF, Sommerville MD. The effects of non-starch polysaccharides on the extent of gelatinization, swelling and α-amylase hydrolysis of maize and wheat starches. *Food Hydrocolloid*. 2003;17:41–54.
15. Kinniburgh DG. General purpose adsorption isotherms. *Environ Sci Technol*. 1986;20:895–904.
16. Poraj-Kozminski A, Hill RJ, van de Ven TGM. Asymmetric polymer bridging between starch-coated colloidal particles and pulp fibres by cationic polyacrylamide. *Can J Chem Eng*. 2007;85:580–585.
17. Warren FJ, Royal PG, Gaisford S, Butterworth PJ, Ellis PR. Binding interactions of α-amylase with starch granules: the influence of supramolecular structure and surface area. *Carbohyd Polym*. 2011;86:1038–1047.
18. Wang T, Zhub J, Zhua R, Gea F, Yuan P, He H. Enhancing the sorption capacity of CTMA-bentonite by simultaneous intercalation of cationic polyacrylamide. *J Hazard Mater*. 2010;178:1078–1084.
19. Kramers HA. Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica*. 1940;7:284–304.
20. Uribe S, Sampedro JG. Measuring solution viscosity and its effect on enzyme activity. *Biol Proced Online*. 2003;5:108–115.
21. Vesilind PA. Treatment and disposal of wastewater sludges. *Ann Arbor Sci*. 1974.
22. Reye JT. Cationic polymer enhanced hydrolysis of cornstarch for the production of biofuels, PhD dissertation. Georgia Institute of Technology; 2011.

Manuscript received Feb. 22, 2012, and revision received Mar. 6, 2012.