

Biomimetic polymerization of acrylamide with hydrogen peroxide catalyzed by water-soluble anionic iron(III) 5,10,15,20-tetrakis-(2',6'-dichloro-3'-sulfonatophenyl)porphyrin

Chetna Angrish and Shive M. Singh Chauhan*

Department of Chemistry, University of Delhi, Delhi-110007, India

E mail: smschauhan@chemistry.du.ac.in

Dedicated to Professor P.T. Narsimahan on his 75th birth anniversary

(received 17 Mar 04; accepted 28 Oct 04; published on the web 05 Nov 04)

Abstract

The biomimetic polymerization of acrylamide (**1**) with hydrogen peroxide catalyzed by water soluble iron(III) 5,10,15,20-tetrakis-(2',6'-dichloro-3'-sulfonatophenyl)porphyrin (**3**) gave high yield of polyacrylamide (**2**) in the molecular weight range of 1,30,000-1,50,000 g mol⁻¹ in the absence and presence of 2,4-pentanedione at an ambient temperature in nitrogen atmosphere. The molar ratio of **3**: H₂O₂: **1** was 1: 100: 3300. Yields were high at pH 4.5 but dropped at pH 7.2. Finally no polymer was formed at pH 9.2 in the absence of 2,4-pentanedione, whereas the yield raised to 99% in the presence of 2,4-pentanedione.

Keywords: Iron(III) 5,10,15,20-tetrakis-(2',6'-dichloro-3'-sulfonatophenyl)porphyrin, hydrogen peroxide, biomimetic polymerization, polyacrylamide

Introduction

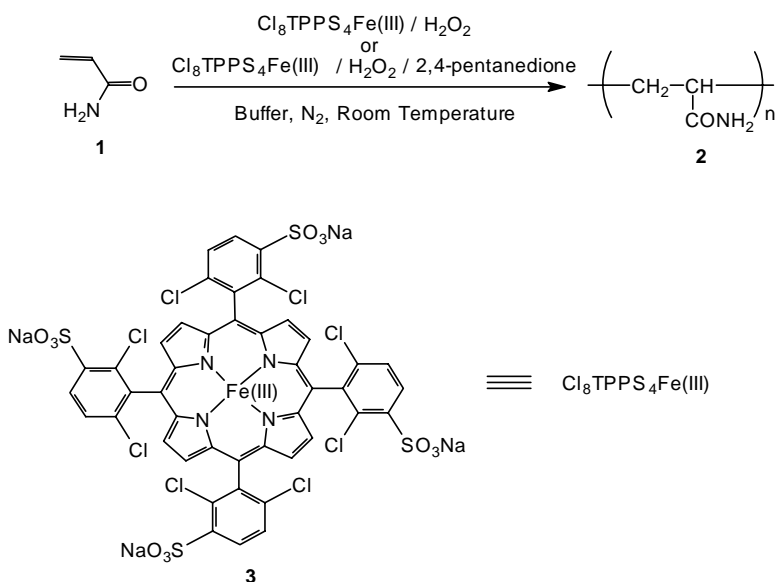
Enzymatic catalysis is involved in both in-vivo and in-vitro polymerizations.¹⁻³ Various biopolymers and non-natural synthetic polymers have been synthesized under ecofriendly and milder conditions by enzyme-catalyzed polymerizations. A selective enzyme catalyzes a specific polymerization reaction with high selectivity. Glycosidase specifically catalyses the synthesis of various natural and unnatural polysaccharides under much milder conditions with high yields.^{4,5} Lipase catalyses the synthesis of optically active polyesters and transesterification of polyesters to produce random copolyesters,^{6,7} whereas papain catalyses the polymerization of amino acids.⁸ Oxidoreductases including peroxidase, laccase and bilirubin oxidase are used as catalysts by the production of novel polyphenolics and related compounds.⁹

The reaction of phenols and anilines with hydrogen peroxide catalyzed by different oxidoreductases is an alternative ecofriendly route to synthesize the industrially important polyaromatics.⁹⁻¹⁶ Similarly the vinylic monomers have also been polymerized with hydrogen peroxide catalyzed by HRP and related enzymes under milder conditions with high yields.^{11,17-20}

Lipid-soluble and water-soluble metalloporphyrins mimic the various reactions of cytochrome P450, HRP and other monooxygenases in different reactions.^{21,22} Herein, we report a novel route for the polymerization of acrylamide with hydrogen peroxide catalyzed by anionic water-soluble iron(III) 5,10,15,20-tetrakis-(2',6'-dichloro-3'-sulfonatophenyl)porphyrin to understand the molecular mechanism of peroxidase and related oxidoreductase enzymes and their utilization in polymerization reactions.

Results and Discussion

Screening of $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (3**) as catalyst in polymerization of acrylamide (**1**).** The reaction of **1** (7mmol) with H_2O_2 (0.106 mmol) in the absence of **3** gave no polymer (**Table 1, entry 1**). The reaction of **1** (7mmol) with H_2O_2 (0.106 mmol) catalyzed by **3** (2.12×10^{-3} mmol) gave polymer **2** (**Scheme 1**) in 85% yield (**Table 1, entry 3**).



Scheme 1. Polymerization of acrylamide (**1**) with hydrogen peroxide catalyzed by $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (**3**).

The formation of polymer **2** was indicated in the absence of a peak at 1612 cm^{-1} for olefinic stretching and a carbonyl absorption from 1672 to 1655 cm^{-1} in its IR spectra. The disappearance of signals for olefinic protons at δ 5.10 and the appearance of characteristic broad signals at δ 1.59 and 2.16 corresponding to the methylene and methine protons in the $^1\text{H-NMR}$ spectra confirmed the formation of **2**. The average molecular weight of **2** was found to be $1,30,000\text{ g mol}^{-1}$ by the viscosity measurements in water at 25°C .²³ The complete thermal degradation of **2** occurred in two stages at 315°C and 423°C when the rate of heating was $5^\circ\text{C}/\text{min}$ in their thermogravimetric analysis²⁴ (**Figure 1**). The ratio of **3**: H_2O_2 : **1** as 1: 100: 3300 was found to be suitable for the polymerization of **1** at room temperature.

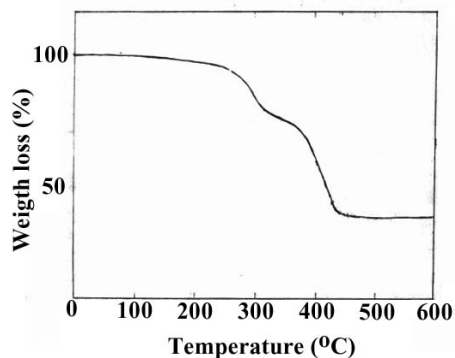


Figure 1. Thermogravimetric analysis of **2** with temperature.

Polymerization of 1 with H₂O₂ catalyzed by Cl₈TPPS₄Fe(III) (3**) in different reaction conditions.** The reaction of **1** with H₂O₂ catalyzed by robust Cl₈TPPS₄Fe(III) (**3**) was carried out in acetate buffer (pH 4.5, 0.2M) at an ambient temperature in a nitrogen atmosphere. The monomer **1** was quantitatively consumed to give the polymer **2** in 85% yield (**Table 1, entry 3**). The yield of **2** dropped to 5% at pH 7.2 while there was no detectable amount of polymer at pH 9.2 under the same conditions (**Table 1, entry 7 and 11**).

Table 1. Effect of pH and the presence of 2,4-PD on the polymerization of **1** with H₂O₂ catalyzed by **3**

S. No.	System ^a	Yield ^b %	Mol. Wt, M _n (g mol ⁻¹) ^c	Polydispersity
1.	H ₂ O ₂ / pH 4.5	-	-	-
2.	H ₂ O ₂ / 2,4-PD ^d / pH 4.5	-	-	-
3.	3 / H ₂ O ₂ / pH 4.5	85	1,30,000	2.45
4.	3 / H ₂ O ₂ / 2,4-PD / pH 4.5	98	1,50,000	2.40
5.	H ₂ O ₂ / pH 7.2	-	-	-
6.	H ₂ O ₂ / 2,4-PD / pH 7.2	-	-	-
7.	3 / H ₂ O ₂ / pH 7.2	5	N.D.	-
8.	3 / H ₂ O ₂ / 2,4-PD / pH 7.2	15	N.D.	-
9.	H ₂ O ₂ / pH 9.2	-	-	-
10.	H ₂ O ₂ / 2,4-PD / pH 9.2	-	-	-
11.	3 / H ₂ O ₂ / pH 9.2	-	-	-
12.	3 / H ₂ O ₂ / 2,4-PD / pH 9.2	99	51,000	2.90

^a Ratio of system **3** : H₂O₂ : **1** = 1 : 100 : 3300.

3: H₂O₂ : 2,4-PD : **1** = 1 : 100 : 110 : 3300.

^b Yield corresponds to weight of **2** obtained to the weight of **1** used.

^c Determined by viscosity measurements at 25°C using $[\eta] = 6.8 \times 10^{-4} M_n^{0.66}$ and $[\eta] = 6.31 \times 10^{-4} M_w^{0.80}$.

^d 2,4-PD = 2,4-Pentanedione, N.D.= Not determined (due to low yield).

Effect of 2,4-pentanedione (2,4-PD) on polymerization of 1 at different pH's. The addition of 2,4-PD to the reaction of **1** with H_2O_2 catalyzed by **3** at pH 4.5 and 7.2 enhanced the yield of polymer **2** to 98 and 15% respectively (**Table 1, entry 4 & 8**). The polyacrylamide (**2**) obtained at pH 4.5 in the presence of 2,4-PD was of comparable molecular weight and polydispersity to that obtained in the absence of 2,4-PD (**Table 1, entry 3 & 4**). At pH 9.2, the addition of 2,4-PD to the above reaction facilitated the polymerization of **1** and produced the polymer **2** of low molecular weight ($51,000 \text{ g mol}^{-1}$) and polydispersity 2.90 in 99% yield (**Table 1, entry 12**).

The polymer was obtained in 40% and 70% yield when the ratio of **3** and 2,4-PD was 1 : 5 and 1 : 50 respectively at pH 9.2. The optimal ratio of **3** and 2,4-PD was found to be 1 : 110 under given reaction conditions to obtain the polymer in 99% yield. Further increase in the concentration of 2,4-PD gave decreased product yield.

The effect of pH on the % yield of the polymer in the absence and the presence of 2,4-PD is also summarized in the **Figure 2**.

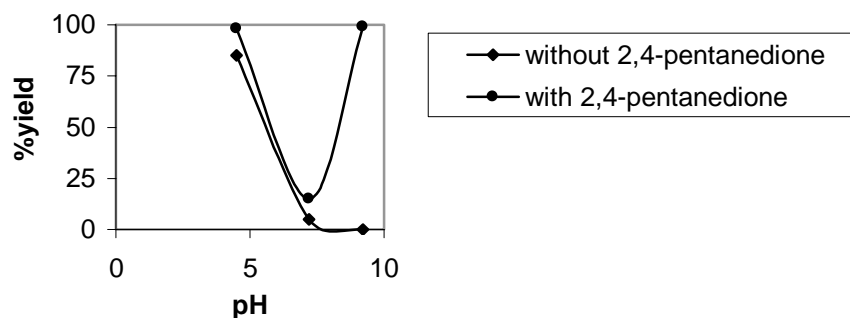


Figure 2. Effect of pH on the %yield of the polymer (**2**) in the absence and the presence of 2,4-PD.

Effect of reaction time on the molecular weight of 2. The effect of the reaction time on the molecular weight of **2** is shown in **Figure 3**. As the reaction time increases the molecular weight of polyacrylamide increases, which indicates the free radical behaviour of the process.

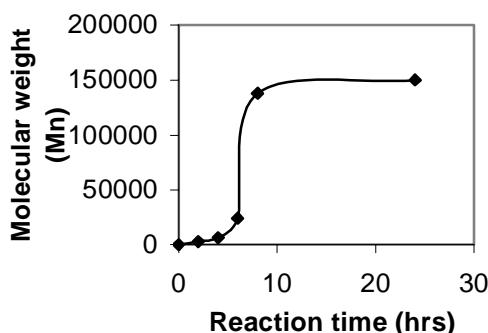
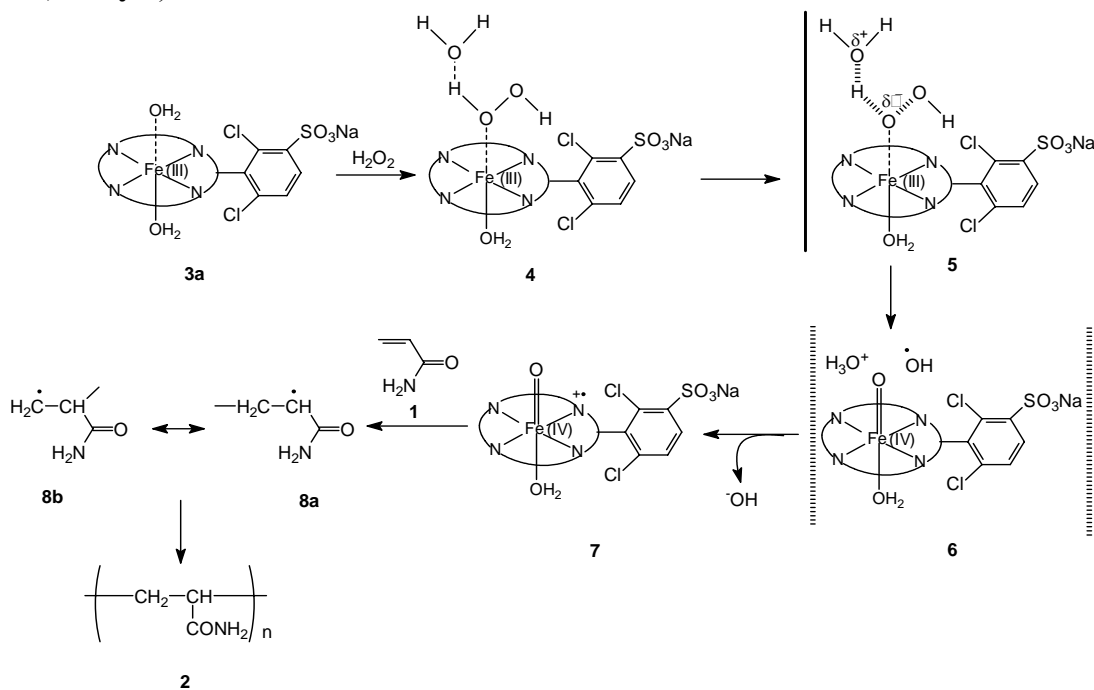


Figure 3. Changes in molecular weight of **2** produced at pH 4.5 (10 mL of 0.2M acetate buffer) using **3** : H_2O_2 : 2,4-PD : **1** in the ratio 1 : 100 : 110 : 3300 at room temperature at different reaction times.

Comparison of polymerization reaction of HRP with water-soluble $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (3**).** The reaction of **1** with H_2O_2 catalyzed by HRP at pH 4.5 in a nitrogen atmosphere gave the polymer in 60% yield at room temperature, the concentrations being $[\text{HRP}] = 2\text{gL}^{-1}$, $[\mathbf{1}] = 0.46\text{M}$ and $[\text{H}_2\text{O}_2] = 11\text{mM}$. No polymer was obtained at pH 9.2 under the same conditions due to deactivation of HRP enzyme at higher pH. This kind of HRP-catalyzed polymerization of vinylic monomers has been reported using large quantities of H_2O_2 (either 1/1 or 9/1 molar ratio) with respect to monomer.^{11,19} The formation of oxoiron(IV) radical cation by the reaction of HRP and H_2O_2 has been proposed to be responsible for the polymerization reaction.

Water-soluble $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (**3**) has been used as a model for HRP to mimic their various reactions. At acidic pH, iron(III) porphyrin exists as $-\text{Fe(III)(H}_2\text{O)}_2-$ (**3a**).^{25,26} On the addition of H_2O_2 to **3a**, the species $-\text{Fe(III)(OOH)(H}_2\text{O)}-$ (**4**) is generated. **4** then spontaneously gives the highvalent oxoiron(IV) radical cation (**7**) through the transient intermediates **5** and **6**. The intermediate **7** is responsible for initiating the formation of acrylamide radical (**8a** and **8b**), which attacks the other acrylamide molecule to form a bigger free radical and hence the reaction further propagates to give the polymer **2**. The reaction is terminated either by the combination of two bigger radicals or by abstraction of a proton from the solvent or another monomer molecule (**Scheme 2**). The stable dimethoxyiron(IV) porphyrin is generated by reaction of iron(III)porphyrin with H_2O_2 in presence of large excess of methanol.²⁷ Thus the polymerization of **1** is quenched by the addition of large excess of methanol. The formation of intermediate **7** could be explained only by the heterolytic cleavage of H_2O_2 , which leads to the polymerization. This was confirmed as no polymer was obtained in the presence of H_2O_2 and absence of **3** (**Table 1, entry 1**).



Scheme 2. Proposed mechanism for the formation of reactive intermediate in polymerization of **1** with hydrogen peroxide catalyzed by $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (**3**).

At neutral and near alkaline pH, the peroxide anion (OOH^-) from H_2O_2 is not able to replace the axial ligand OH on $-\text{Fe}(\text{III})(\text{H}_2\text{O})(\text{OH})-$, which is the species when **3** is present in the buffer of neutral and higher pH. Thus the concentration of highvalent oxoiron(IV) radical cation (**7**) decreases and hence very low or no polymerization is observed at these pH's.

The polymerization of vinylic monomers with H_2O_2 catalyzed by HRP in the presence of 2,4-PD and other β -diketones increases the yield of polymer significantly.^{17-18,20} Polyacrylamide of molecular weight $1,24,000 \text{ g mol}^{-1}$ and polydispersity 2.5 has been obtained by the polymerization of **1** with hydrogen peroxide catalyzed by HRP in the presence of 2,4-PD using H_2O_2 -to-monomer ratio of 1/66 mol/mol at pH 5.1 while no polymer is reported in the absence of 2,4-PD.¹⁷ Thus 2,4-PD was added to the reaction of **1** with H_2O_2 catalyzed by $\text{Cl}_8\text{TPPS}_4\text{Fe}(\text{III})$ (**3**) at pH 4.5 and 7.2 and it enhanced the yield of polymer **2** to 98 and 15% respectively. At pH 4.5, the polymer **2** of comparable molecular weight ($1,30,000 \text{ g mol}^{-1}$) and polydispersity (2.45) was obtained when **3** was used as the catalyst in place of HRP without any need for the addition of 2,4-PD. The addition of 2,4-PD at pH 9.2 offered a new route for polymerization of **1** at this pH, where the use of HRP is limited owing to its deactivation. The reaction of **1** with H_2O_2 in the presence of 2,4-PD in the absence of **3** produced no polymer (**Table 1, entry 10**). At pH 9.2, the reaction of **3** with H_2O_2 in the presence of 2,4-PD may produce a stable intermediate which contributes to the facile polymerization of **1** at this pH.

The large excess of H_2O_2 with respect to the monomer resulted in the degradation of the iron(III) porphyrin. Thus, a 1:66 molar ratio of H_2O_2 to monomer was found to be suitable for the polymerization of **1** both in the absence and the presence of 2,4-PD.

Conclusions

The water soluble anionic $\text{Cl}_8\text{TPPS}_4\text{Fe}(\text{III})$ (**3**) in the presence of hydrogen peroxide is an economical and suitable system to initiate the polymerization of acrylamide at acidic and neutral pH, by using the molar ratio of **3**: H_2O_2 : **1** as 1 : 100 : 3300. The addition of 2,4-PD in this system enhances the yield of the polymer at both acidic and neutral pH and also facilitates the polymerization of **1** at alkaline pH, where HRP is deactivated. Thus the water-soluble anionic iron(III) porphyrin and H_2O_2 mimic the polymerization reaction of oxidoreductase enzyme HRP in the presence or absence of 2,4-PD under milder conditions with excellent yield.

Experimental Section

Material and methods. **1** and 2,4-PD were obtained from Biochemicals unit, India and Merck, Munchen, respectively and were also purified before use. The aqueous hydrogen peroxide (30% v/v) was obtained from CDH India and used without further purification. The 5,10,15,20-tetrakis-(2',6'-dichloro-3'-sulfonatophenyl)porphyrin ($\text{H}_2\text{Cl}_8\text{TPPS-4}$) was prepared by

sulphonation of 5,10,15,20-tetrakis-(2',6'-dichlorophenyl)porphyrin (Cl_8TPPH_2) with oleum at 130°C by following the literature procedure and metallated with ferrous chloride in DMF.^{28,29}

The average molecular weight of polyacrylamide was determined from intrinsic viscosity measurements according to Mark-Houwink equations.²³ Viscosity measurements were obtained at 25°C in water using Ubbelohde viscometer and $[\eta]_{c \rightarrow 0}$ was evaluated by the extrapolation of experimental data. $[\eta]_{c \rightarrow 0}$ values were used to calculate the number and weight average molecular weight according to the relations $[\eta] = 6.8 \times 10^{-4} M_n^{0.66}$ and $[\eta] = 6.31 \times 10^{-4} M_w^{0.80}$. ^1H NMR spectra were recorded on a Bruker Avance Spectrospin 300MHz spectrophotometer. Perkin Elmer FT-IR Spectrum 2000 Spectrometer was used to record IR spectra. Thermogravimetric analysis was obtained on a Rigaku Thermoflex, PTC-10A.

Polymerization of 1 with hydrogen peroxide catalyzed by water-soluble $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (3). The solution of **1** (7 mmol in 10mL acetate buffer of pH 4.5) was charged into a three-necked round-bottomed flask and degassed for 15 minutes. $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (2.12×10^{-3} mmol in 0.5 mL buffer) and 30% H_2O_2 (0.106 mmol) were successively injected into the above flask under stirring. The reaction was stirred for a predetermined time at an ambient temperature under a nitrogen atmosphere. The polymer was obtained by quenching the reaction with a large excess of methanol and filtered off, washing with methanol and drying under vacuum at 50°C . The yield and molecular weight of polymers are given in **Table 1**.

Polymerization of 1 with hydrogen peroxide catalyzed by water-soluble $\text{Cl}_8\text{TPPS}_4\text{Fe(III)}$ (3) in the presence of 2,4-PD. The procedure and molar ratio of reactant (**1**) and catalyst (**3**) used in this case were exactly the same as mentioned above except for the addition of 2,4-PD (0.18 mmol) which was added simultaneously along with hydrogen peroxide. The results obtained in this case are also summarized in **Table 1**.

Acknowledgements

We are thankful to Department of Biotechnology (DBT), India, for financial support. Chetna Angrish is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for Junior Research Fellowship (JRF).

References

1. Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793.
2. Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, *101*, 1097.
3. Uyama, H.; Kobayashi, S. *J. Mol. Cat. B: Enzymatic* **2002**, *19*, 117.
4. Kobayashi, S.; Kashiwa, K.; Kawasaki, T.; Shoda, S. *J. Am. Chem. Soc.* **1991**, *113*, 3079.
5. Kobayashi, S.; Kiyosada, T.; Shoda, S. *J. Am. Chem. Soc.* **1996**, *118*, 13113.
6. Runge, M.; O'Hagan, D.; Haufe, G. *J. Polym. Sci., Polym. Chem. Ed.* **2000**, *38*, 2004.

7. Kumar, A.; Kalra, B.; Dekhterman, A.; Gross, R. A. *Macromolecules* **2000**, *33*, 6303.
8. Anderson, G.; Luisi, P. L. *Helv. Chim. Acta* **1979**, *62*, 488.
9. Ikeda, R.; Sugihara, J.; Uyama, H.; Kobayashi, S. *Macromolecules* **1996**, *29*, 8702.
10. Uyama, H.; Kurioka, H.; Sugihara, J.; Komatsu, I.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3209.
11. Uyama, H.; Loavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554.
12. Dordick, J. S.; Marletta, M. A.; Klivanov, A. V. *Biotech. Bioeng.* **1987**, *30*, 31.
13. Uyama, H.; Kurioka, H.; Kaneko, I.; Kobayashi, S. *Chem. Lett.* **1994**, 423.
14. Akkara, J. A.; Senecal, K. J.; Kaplan, D.L. *J. Pol. Sci. Part A* **1991**, *29*, 1561.
15. Premchandran, R. S.; Banerjee, S.; Wu, X. K.; John, V. T.; McPherson, G. L. *Macromolecules* **1996**, *29*, 6452.
16. Kobayashi, S.; Kaneko, I.; Uyama, H. *Chem. Lett.* **1992**, 393.
17. Teixeira, D.; Lalot, T.; Brigodiot, M.; Marechal, E. *Macromolecules* **1999**, *32*, 70.
18. Kalra, B. Gross, R. A. *Biomacromolecules* **2000**, *1*, 501.
19. Derango, A. R.; Chiang, L.-C.; Dowbenko, R.; Lasch, J. G. *Biotech. Tech.* **1992**, *6*, 523.
20. Singh, A.; Ma, D.; Kaplan, D. L. *Biomacromolecules* **2000**, *1*, 592.
21. Chauhan, S. M. S.; Kalra, B.; Mohapatra, P. P. *J. Mol. Cat. A: Chemical* **1999**, *137*, 85.
22. Chauhan, S. M. S.; Sahoo, B. B. *Bioorg. Med. Chem.* **1999**, *7*, 2629.
23. Shawki, S. M.; Hamielec, A. E. *J. Appl. Polym. Sci.* **1979**, *23*, 3323.
24. Yang, M. H. *Polymer Testing* **1998**, *17*, 191.
25. Panicucci, R.; Bruice, T. C. *J. Am. Chem. Soc.* **1990**, *112*, 6063.
26. Bruice, T. C. *Acc. Chem. Res.* **1991**, *24*, 243.
27. Meunier, B. *Chem. Rev.* **1992**, *92*, 1411.
28. Dolphin, D.; Nakano, T.; Maioni, T. E.; Kirk, T. K.; Farrell, R. In *Lignin Enzymic and Microbial Degradation*; Odier, E. Ed.; INRA: Paris, 1987, 157.
29. Chauhan, S. M. S.; Ray, P. C.; Satapathy, S.; Vijayraghavan, B. *Ind. J. Chem.* **1992**, *31B*, 837.