Vinyl Polymerization. 394.[1] Radical Polymerization of Methyl Methacrylate Initiated with *Saccharomyces Cerevisiae*

Minoru Imoto,* Tatsuro Ouchi, Eijiro Morita, Minoru Yamada,
Tai Tokuyama,[††] and Hitoshi Obata
Laboratry of Synthetic High Polymers, Faculty of Engineering, Kansai University, Suita, Osaka 564
[††] *Laboratory of Applied Biochemistry, Faculty of Engineering, Kansai University, Suita, Osaka 564*

(Received October 4, 1979)

The polymerization of methyl methacrylate with *Saccharomyces cerevisiae* was carried out in water, the reaction being found to proceed through a radical mechanism. The effect of the mass of reagent on the rate of polymerization and selectivity of vinyl monomer were studied. The isolated cell wall showed remarkable activity for polymerization. The change of surface of the polymerization system was observed with a scanning electron microscope.

Recently, it was reported[2] that the cell of yeast, *Saccharomyces cerevisiae*, can initiate the polymerization of methyl methacrylate (MMA) in the presence of Cu(II) ion. The polymerization was found to proceed on the exterior and interior of the cell, particularly on the cell wall. The present paper deals with a precise study of the polymerization. The radical mechanism is confirmed, effects of the mass of reactant on the conversion of MMA, selectivity of the vinyl monomer and polymerization by the cell wall isolated from the cells being given.

Experimental

Materials. Dry yeast *Saccharomyces cerevisiae* (SC-1, Oriental Yeast Co.) was used. MMA and other monomers were purified by the usual methods. Copper(II) chloride (special grade) was used without further purification. The water was ion-exchanged and distilled.

Procedures. MMA, water, and other reagents were placed in a tube which was then sealed in a vacuum after thawing with nitrogen and incubated at 30 or 85 °C with shaking or by being left to stand. After a certain time, the contents of the tube were poured into a large amount of methanol in order to precipitate the polymer formed. After filtration, the product was thoroughly washed with methanol, dried in a vacuum, and then weighed. The conversion of MMA was calculated by the following equation:

$$\text{Conversion}(\%) = \frac{\text{Wt. of products(g)} - \text{Initial wt. of cells(g)}}{\text{Wt. of MMA in the feed(g)}} \times 100$$

It was confirmed by turbidimetry that no multiplication of cell was observed during the course of polymerization at 30 °C for 10 d. The mode of the polymerization system was observed with a scanning electron microscope (SEM).[2] The number-average degree of polymerization ($\bar{P}_n$) of poly(MMA) produced was estimated from the viscosity determined in benzene at 30 °C, using Welch's equation.[3]

Results and Discussion

Confirmation of Radical Mechanism. Copolymerization of MMA with Styrene (St) was carried out (Fig. 1), a radical mechanism being confirmed.

Selectivity of Vinyl Monomer. Polymerization of various monomers was carried out with the system SC-1, Cu(II) ion, and water. The results are given in Table 1.

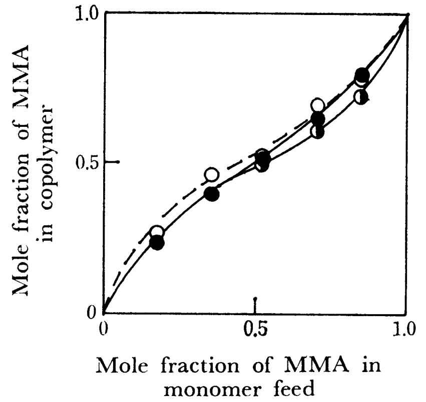


Fig. 1. Copolymerization of MMA and St.
(MMA+St) 3 cm^3, H_2O 10 cm^3, SC-1 0.1 g, $CuCl_2 \cdot 2H_2O$ 1×10^{-3}g. ●(A), Copolymer produced at 85 °C after 3 h with shaking, including both copolymers produced in the water phase and monomer layer. ◗(B), Copolymer produced in the water layer at 30 °C after 10 d on standing. ○(C), Copolymer produced in the monomer layer at 30 °C after 10 d on standing.

Table 1. Polymerization of vinyl monomers[a)]

SC-1/g	$CuCl_2 \cdot 2H_2O$/g	Conversion/%				
		MMA	St	AN	MAA	EMA
0	0	0	1.14	0	0	3.08
0.1	0	0	0.89	0	1.99	0.39
0	1×10^{-3}	0	1.43	0	0	0
0.1	1×10^{-3}	8.63	2.15	0.66	7.25	5.90

a) Monomer 3 cm^3, H_2O 10 cm^3; 30 °C, 10 d, with shaking.
AN: acrylonitrile, MAA: methacrylic acid, EMA: ethyl methacrylate, St: styrene, MMA: methyl methacrylate.

The conversion of St and acrylonitrile (AN) was in the range of thermal polymerization or experimental errors. Thus, no initiating ability of the present initiator system for St and AN was observed. On the other hand, MMA, ethyl methacrylate (EMA), and methacrylic acid (MAA) were found to be polymerized.

Effect of the Mass of Cu(II) Ion on the Polymerization of MMA. The effect of the mass of Cu(II) ion on the conversion of MMA was obtained for polymerization at 85 °C with shaking (Fig. 2) and on standing

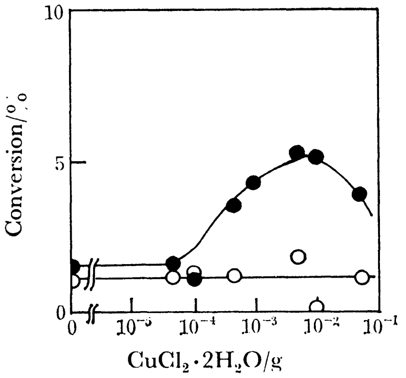


Fig. 2. Conversion of MMA with shaking *vs.* mass of $CuCl_2 \cdot 2H_2O$. MMA 3 cm^3, ● SC-1 0.1 g, ○ SC-1 0 g, H_2O 10 cm^3; 85 °C, 5 h.

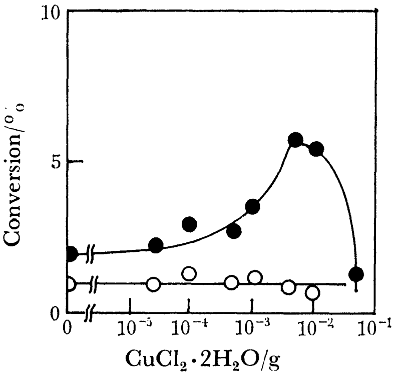


Fig. 3. Conversion of MMA on standing *vs.* mass of $CuCl_2 \cdot 2H_2O$. MMA 3 cm^3, ● SC-1 0.1 g, ○ SC-1 0 g, H_2O 10 cm^3; 85 °C, 5 h.

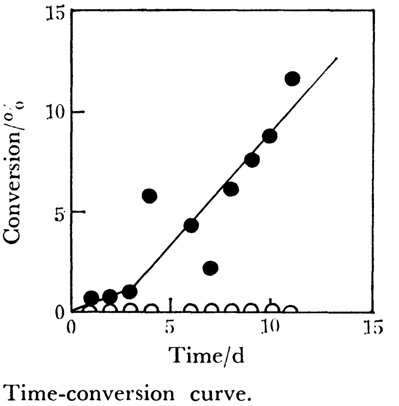
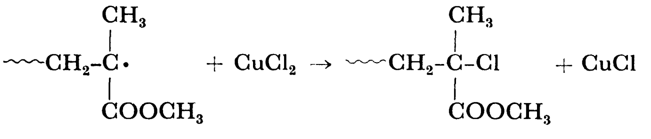


Fig. 4. Time-conversion curve.
MMA 3 cm^3, ● SC-1 0.1 g, ○ SC-1 0 g, H_2O 10 cm^3, $CuCl_2 \cdot 2H_2O$ 1×10^{-3}g; 30 °C, with shaking.

(Fig. 3). In both cases, the maximum conversion was observed at 5×10^{-2} g of $CuCl_2 \cdot 2H_2O$. The decrease in the conversion of MMA by excess Cu(II) ion was due to the following inhibition reaction of the propagating radical with Cu(II) ion:



$$\sim\sim CH_2\text{-}\overset{\displaystyle CH_3}{\underset{\displaystyle COOCH_3}{C}}\cdot \quad + CuCl_2 \rightarrow \sim\sim CH_2\text{-}\overset{\displaystyle CH_3}{\underset{\displaystyle COOCH_3}{C}}\text{-}Cl \quad + CuCl$$

It should be noted that both conversion curves are similar to each other. The time-conversion curve of the polymerization of MMA is shown in Fig. 4. The white and black circles indicate the conversion of MMA in the absence and presence, respectively, of SC-1. There is an induction period of about 2 d.

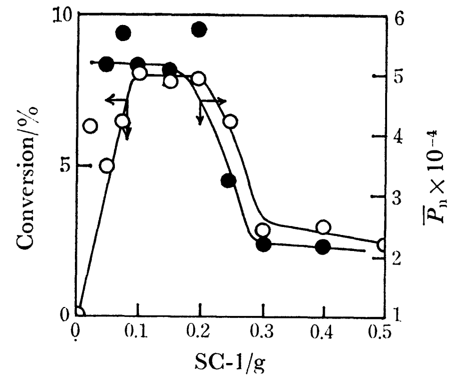


Fig. 5. Conversion of MMA or $\overline{P}_n$ of poly-(MMA) *vs.* mass of SC-1. MMA 3 cm^3, H_2O 10 cm^3, $CuCl_2 \cdot 2H_2O$ 1×10^{-3}g; 30 °C, 10 d, with shaking, ○ conversion, ● $\overline{P}_n$.

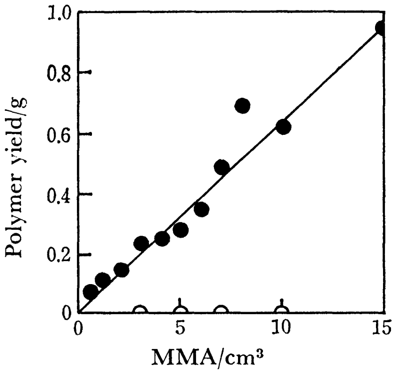


Fig. 6. Polymer yield *vs.* mass of MMA.
● SC-1 0.1 g, ○ SC-1 0 g, H_2O 10 cm^3, $CuCl_2 \cdot 2H_2O$ 1×10^{-3}g; 30 °C, 10 d, with shaking.

Effect of the Mass of SC-1 on the Polymerization of MMA. Varying the mass of SC-1 and keeping that of Cu(II)-ion, MMA, and water constant, we carried out polymerization at 30 °C for 10 d with shaking. The results on the conversion of MMA and $\overline{P}_n$ of poly-(MMA) are shown in Fig. 5.

The conversion increased with the mass of SC-1, reaching maximum immediately and then decreasing. This was confirmed by repeated experiments. $\overline{P}_n$ also decreased with the mass of SC-1.

The only assumption to explain the behavior is as follows: the contents of some unknown free-radical other than the propagating poly-(MMA) radicals increase with the mass of SC-1 in the feed. The free-radicals could be produced by the chain transfer reactions of the propagating poly-(MMA) radicals with the vital macromolecules in the cell, and could scavenge the propagating poly-(MMA) radicals. Thus, above a certain amount of SC-1, the conversion of MMA and $\overline{P}_n$ of poly-(MMA) decreases. This was supported by the results of experimental studies on the chain-transfer reaction of poly-(MMA) radicals with nucleic acid bases and nucleosides.[4]

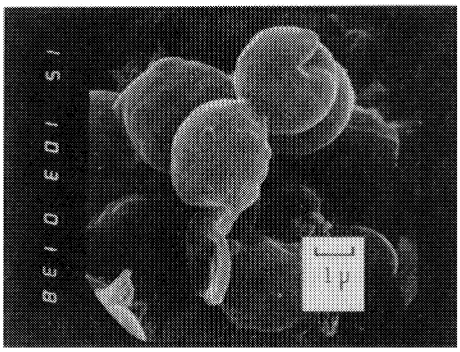


Fig. 7(a). Surface view of the SC-1.

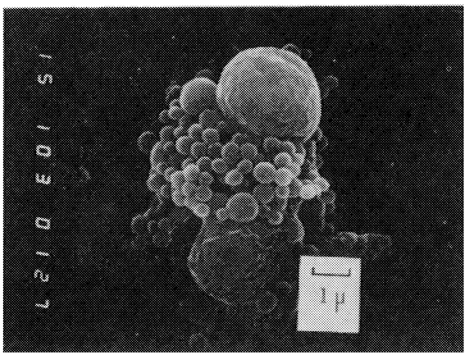


Fig. 7(b). Surface view of the polymerized system (SC-1, 0.1 g; MMA, 3 cm^3; $CuCl_2 \cdot 2H_2O$, 1×10^{-3}g; H_2O, 10 cm^3; 30 °C, 10 d, on standing; Conversion of MMA, 8.8%).

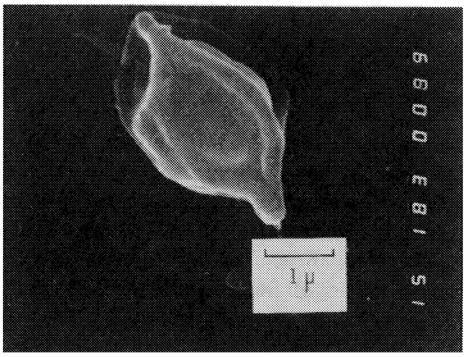


Fig. 7(c). Surface view of the polymerized system. After the polymerization, the product was washed under reflux with benzene for 50 h.

Effects of the Mass of MMA on Polymerization. The masses of SC-1, Cu(II) ion, and H_2O were kept constant. The polymerization was carried out at 30 °C for 10 d with shaking. The results are shown in Fig. 6. The polymer yield in g increased linearly with the amount of MMA.

Scanning Electron Microscopy (SEM). It was observed by transmission electron microscopy that the cell wall is disintegrated at a 3.1% conversion of MMA.$^{1)}$ This is due to the particular easiness of the polymerization of MMA in the cell wall. When the conversion of MMA was only 2.1%, the cell wall became somewhat larger. We have treated the case in which the conversion of MMA reached 8.8%. The results of SEM are shown in Figs. 7(a), (b), and (c). Since the conversion was 8.8%, it seems that the wall was already disintegrated, thus becoming the active center for the polymerization of MMA. The propagating chain radicals were brought into the water phase. A similar polymerization to suspension polymerization takes place. Figure 7(b) is in line with the consideration. The small round particles near the cell are poly-(MMA) produced formed in the water phase. After washing with benzene for 50 h (Fig. 7(c)), the particles of poly-(MMA) produced in the water phase disappeared, only the cell containing poly-(MMA) produced in the interior being observed.

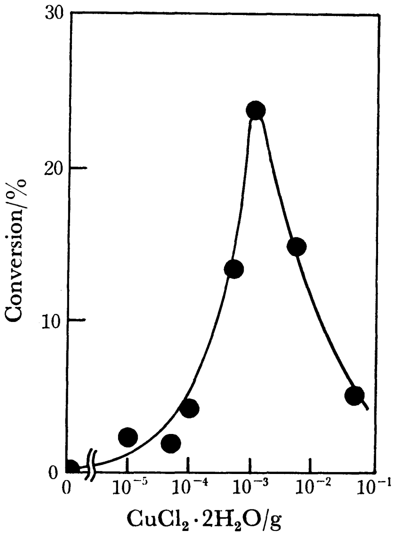


Fig. 8. The polymerization of MMA initiated by the cell wall of SC-1. MMA 3 cm^3, cell wall 0.1 g, H_2O 10 cm^3; 30 °C, 10 d, with shaking.

Polymerization of MMA Initiated with Cell Wall. The cell wall which is particularly active was isolated as follows. SC-1 was disrupted by means of an X-press. The cell wall was separated by centrifugation at 12000 rpm for 60 min, washed twice with cold distilled water and lyophilized, polymerization of MMA then being carried out. The results are shown in Fig. 8. The activity for polymerization of the cell wall became *ca.* 3 times higher than the original SC-1.

References

1) T. Ouchi, N. Murayama, and M. Imoto, *Bull. Chem. Soc. Jpn.* (Part 393), **53**, 748 (1980).

2) M. Imoto, T. Ouchi, Y. Nakamura, E. Morita, M. Yamada, T. Tokuyama, H. Obata, and T. Iwamoto, *J. Polym. Sci., Polym. Chem. Ed.*, **17**, 347 (1979).

3) F. J. Welch, *J. Polym. Sci.*, **61**, 243 (1962).

4) M. Imoto, A. Kuriyama, and T. Ouchi, *Chem. Lett.*, **1979**, 1145.