Asian Journal of Chemistry Vol. 18, No. 2 (2006), 1297–1302

Acrylonitrile Modified Glycidyl Methacrylate/ Methylmethacrylate Terpolymer: A Novel Carrier for Enzyme Immobilization

SANGEETA SRIVASTAVA*, V.C. MALSHE† and M.A. DAVE
Department of Chemistry, K.J. Somaiya College of Science
Vidya Vihar, Mumbai-400 077, India
E-mail: sangeeta@somaiya.com

In this study, a new carrier (*i.e.*, the poly(acrylonitrile/glycidyl methacrylate/methylmethacrylate copolymer beads) for enzyme immobilization is evaluated. Two different types of copolymer beads with different swellabilities with an average diameter of about 200 μm were produced by suspension copolymerization of the respective co-monomers, with or without using cyclohexanone. BPO and PVA were used as the initiator and the stabilizer. The copolymer beads produced without cyclohexanol were non-porous and non-swellable, while porous and swellable (swelling ratio: 14.28%) beads were obtained in which cyclohexanol was used as the porogen. Immobilization of a model enzyme (*i.e.*, glucose oxidase) was studied to show the feasibility of using these beads as an enzyme carrier. More enzymes, but with very low activities, were immobilized on the beads with relatively lower swellabilities, while much higher activities were observed on the beads with relatively higher swellabilities prepared with cyclohexanol. Invertase (from Bio-con India) was immobilized on to a copolymer of glycidyl methacrylate and methyl methacrylate. Polymer particles having glycidyl ether groups were prepared through suspension polymerization in aqueous medium, addition of acrylonitrile provides sufficient space for enzyme immobilization. From the HCl-dioxane back titration method, it was found that about 8–10% of oxirane oxygen remained on the final particles. Invertase 'immobilized' on copolymer exhibited complete inversion of sucrose at neutral pH and 30–37°C. The prepared immobilized invertase could completely hydrolyze sucrose in water at room temperature, at an enzyme concentration of 0.2% based on sucrose weight having invertase activity 1,00,000 U/mL. The enzyme preparation was stable under stirred conditions and could be reused multiple times without loss of enzyme activity. It was found that the nature of the polymer used for enzyme conjugation had a significant effect on the activity of the invertase used.

Key Words: Invertase, Immobilization, Hydrolysis, Sucrose, Polymer support.

INTRODUCTION

Immobilized enzymes may be defined as enzymes whose free movement has been restricted in some manner. One of the major advantages of immobilization is to "fix" the enzyme so as to retain it in a continuous process. Use of soluble enzymes in processing has been limited in part by the cost of the enzymes due

†Department of Chemical Technology, University of Mumbai, Matunga, Mumbai-400 019.

to the difficulty and expense of isolation, instability and the fact that in a freely soluble form they usually can be used only once.

The use of immobilized enzymes has several advantages. An immobilized enzyme, in solid or sometimes semisolid form, is readily separable from the product solution and can be reused, thus increasing utility by a large factor. Additionally, the enzymatic reaction can be terminated easily simply by removing the enzyme, allowing more precise control of the reaction. Finally, it has turned out that enzymes are often much more stable when in fixed form than in solution, allowing an enzymatic conversion to occur over a much longer period without having to replenish the biocatalyst as compared with intact enzymes. Some examples of these effects have already been published[1-11].

For the production of the polymer particles having special functional moieties, suspension homopolymerization and copolymerisation have been studied by several research groups. Suspension copolymerization of methyl methacrylate and glycidyl methacrylate has also been carried out by Lee and Chen[12].

Reactive functional groups assist the formation of a permanent covalent bond between the polymer and the enzyme without affecting its tertiary structure. Oxiranoyl group is perhaps the most preferred functional group since covalent binding of enzyme proceeds under ambient, moderate conditions without significant loss of activity[13].

The pore geometry of oxirane polymers, which offer pendent epoxy groups for covalent bonding of enzyme molecule, can be manipulated by strategic interplay of synthesis variables such as cross link density, volume of porogen and quantities of protective colloid and polymerization initiator[14]. The significance of these synthesis variables on the physical properties of glycidyl copolymers and immobilization of penicillin-G acylase is documented[15].

The sucrose industry is a comparatively minor user of enzymes but provides few historically significant and instructive examples of enzyme technology. The hydrolysis ('inversion') of sucrose, completely or partially, to glucose and fructose provides sweet syrups that are more stable (*i.e.*, less likely to crystallize) than pure sucrose syrups. The most familiar 'Golden Syrup' produced by acid hydrolysis of one of the less pure streams from the cane sugar refinery, but other types of syrups are produced using yeast (*Saccharomyces cerevisiae*) invertase. Enzymatic inversion of sugar requires specific pH and temperature conditions and they were studied[18-20]. Immobilized invertase and whole cells of yeast have been investigated for the continuous inversion of sucrose solutions[21-24].

The enzymatic supports so far described in technical literature or commercially available use glycidylmethacrylate and methylmethacrylate as primary monomers. The mechanical stability of the polymer matrix is limited and the number of cycles of use is about a few hundred. Most of this occurs due to the relatively lower mechanical stability of the polymer beads. These are either brittle or are not able to withstand the osmotic or pH shocks. To enhance the mechanical stability and also to reduce the cost of the matrix, we have modified it with a hard monomer acrylonitrile.

Acrylonitrile produces very stable but flexible polymers. This is indicated by the fact that it is an excellent fibre forming polymer. Its polymer has a high T_g and,

therefore, the polymer resists permanent deformation under conditions of use. Besides, it is a very inexpensive and readily available monomer, that polymerises and co-polymerizes with great ease. There is no example available in literature in which acrylonitrile is used as a component of polymer for immobilizing enzymes. In this work an attempt has also been made to achieve complete inversion of sucrose at neutral pH and at 30–37°C temperature. This immobilized enzyme showed complete inversion of sucrose at room temperature and normal pH. Reusability of this copolymer was tested by performing 100 cycles.

EXPERIMENTAL

Glycidyl methacrylate methyl methacrylate and acrylonitrile were obtained from Lancaster. Polyvinyl alcohol, polyvinylpyrrolidine, cyclohexanol, methanol and divinyl benzene were obtained from Loba Chemie. Invertase was obtained from Biocon India.

Polymer preparation

The porous copolymers were synthesized by suspension polymerization in a jacketed cylindrical polymerization reactor[16, 17]. A volume of water along with polyvinyl pyrrolidone and sodium chloride was heated in the reaction vessel at 70°C, The reaction mixture components were added rapidly at low stirring speed. The mixture contained—initiator, porogen added in the preceding sequence. The addition represented zero time of the copolymerization. At this time the temperature was about 70°C. Then, the stirring speed was slowly increased, temperature remained constant (± 0.5°C) until the end of the reaction. Reaction was allowed to go for completion for 6–7 h. The beads were decanted and washed with water. The porogen was extracted with methanol in a Soxhlet apparatus and finally dried for 24 h at 60°C. The products result as white opaque spherical beads.

Enzyme preparation and loading

The enzyme solution was prepared by taking 0.1 g of enzyme having activity 1,00,000 BU/mL in 100 mL of water for 0.1% enzyme solution and 0.2 g in 100 mL water for 0.2% enzyme solution.100 mL of enzyme solution was used for 10 g of copolymer for immobilization. 100 mL of 10% sugar solution was used in stirred condition for all the experiments.

All the experiments were carried out at an ambient temperature and without adjusting the pH of the sucrose solution.

Analysis

The oxirane oxygen group was found by HCl-dioxane back titration method. The purified particles were redispersed into dry dioxane, after which 0.2 N HCl solution in absolute dioxne was added and the particles were left to react at room temp for 15 min. The unreacted fraction of acid was then determined with 0.1 N methanolic sodium hydroxide solution.

Porosity determination: The porosity of the copolymer at various stages was determined by soaking the sample in water and finding out the difference in the dry and wet weight of the sample. Inversion of sucrose was analyzed by copper ion reducing method of Fehling's solution.

Immobilization of invertase on co-polymer of glycidylmethacrylate

Enzyme invertase was immobilized on copolymer by keeping the polymer in the solution of enzyme for 24 h in temperature controlled shaker.

This immobilized enzyme in polymer was placed on glass column and washed thoroughly. Enzyme activity was analyzed by measuring the inversion of sugar by copper ion reducing method of Fehling's solution.

RESULTS AND DISCUSSIONS

Effect of acrylonitrile polymer on inversion of sucrose

Polymer prepared without using acrylonitrile gave very little inversion, polymer with acrylonitrile gave complete inversion at 30–37°C and at neutral pH. Addition of acrylonitrile provides sufficient space for immobilization of enzyme in such a way which keeps enzyme in active form (Fig. 1).

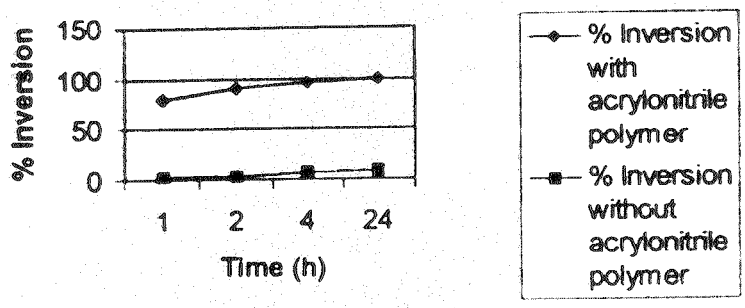

Fig. 1. Effect of acrylonitrile addition in polymer on inversion of sucrose

Stability of immobilised invertase

Effect of pH on inversion of sucrose: Stability of immobilized invertase was determined at pH range 2–7. In general, free invertase was more sensitive to pH shifts. Optimum pH for free invertase was 4.5–5 and for immobilized invertase it was 5–7 pH.

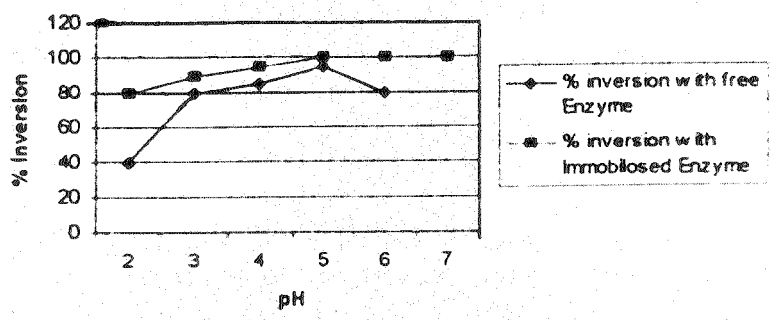

Fig. 2. Effect of pH on inversion of sucrose

Effect of temperature on inversion of sucrose: The stability of immobilized invertase was also determined at various temperatures. In general, free invertase was more sensitive to temperature change. Optimum temperature for free enzyme was 50–55°C and for immobilized enzyme it was ambient temperature.

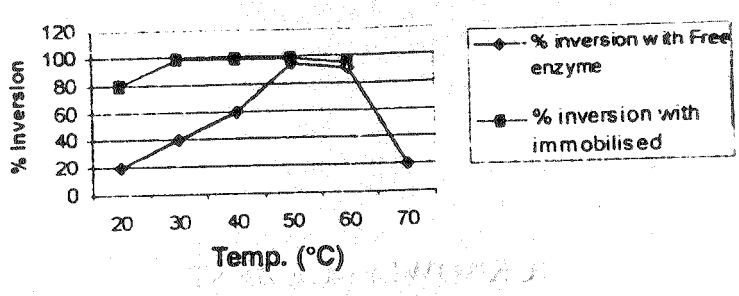

Fig. 3. Effect of temperature on inversion of sucrose

Effect of immobilized enzyme on inversion of sucrose

Inversion of sucrose using immobilized invertase was studied. Immobilized enzyme completely inverted the sucrose solution at neutral pH and at ambient temperature, free enzyme inverted up to 95% sucrose at optimum conditions. Polymer did not show any inversion activity (Fig. 4).

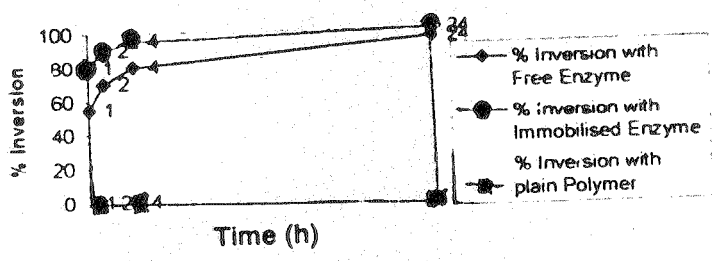

Fig. 4. Effect of immobilization on inversion of sucrose

Reusability of immobilized invertase

Immobilized invertase inverted sucrose completely even after 20 cycles.

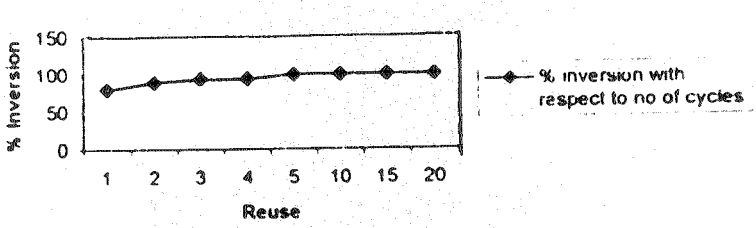

Fig. 5. Reusability of immobilized enzyme

Effect of storage on immobilized invertase stability

The immobilized enzyme was stable for 2 months. After 2 months it showed reduction in catalytic activity. The above results clearly indicate that enzyme has been loaded on the support and it can be used for inversion of sucrose at room temperature and at neutral pH and can be reused 20 times without loss of activity. Addition of acrylonitrile provides sufficient space for immobilization of enzyme in a way that keeps the enzyme in active form.

Conclusion

In conclusion, we report a high performance immobilized invertase obtained by immobilizing invertase on acrylonitrile/glycidyl methacrylate/methyl methacrylat; crosslinked ter polymer which facilitates the inversion of sucrose and allows the inversion to take place without adjusting the pH and temperature and can be repeatedly used. The approach has high potential for translation into a commercial process.

ACKNOWLEDGEMENT

We thank Mr. Samir Somaiya, Director, Somaiya Group, for his constant encouragement during the course of this work. We also thank Dr. K.N. Joyshi, Head of Microbiology Department, K.J. Somaiya College of Science, for his useful and valuable suggestions.

REFERENCES

1. W.-C. Lee and C.-T. Huang, *Enzyme Microbial. Technol.*, **17**, 79 (1995).
2. C.-J. Chiang, W.-C. Lee, D.-C. Sheu and K.-J. Duan, *Biotechnol. Progr.*, **13**, 577 (1997).
3. C.-J. Chiang, L.-T. Hsiau and W.-C. Lee, *Biotechnol. Tech.*, **11**, 121 (1997).
4. W.-H. Liao and W.-C. Lee, *Prep. Biochem. Biotechnol.*, **27**, 39 (1997).
5. H.-P. Chao and W.-C. Lee, *J. Chin. Inst. Chem. Eng.*, **30**, 457 (1999).
6. W.-C. Lee, C.-J. Chiang and P.-Y. Tsai, *Ind. Eng. Chem. Res.*, **38**, 2564 (1999).
7. F.-C. Huang, C.-H. Ke, C.-Y. Kao and W.-C. Lee, *J. Appl. Polym. Sci.*, **80**, 39 (2001).
8. Y.-C. Liu, F.-S. Wang and W.-C. Lee, *Biochem. Eng. J.*, **7**, 17 (2001).
9. L. Koilpillai, R.A. Gadre, S. Bhatnagar, C.R. Rajan, S. Porrathnam, K.K. Kumar, G.R. Ambekar and J.G. Shewale, *J. Chem. Tech. Biotechnol.*, **49**, 173 (1990).
10. J.G. Shewale, S.K. Mujawar, A. Kotha, C.R. Rajan and S. Ponrathnam, An improved process for the preparation of novel crosslonked macroporous glycidyl methacrylate-divinyl benzene copolymers, Indian Patent, Appl. No. 2937/DEL/97 (1997).
11. ———, An improved process for the production of immobilized D-amino acid oxidase, Indian Patent, Appl. No. 2946/DEL/97 (1997).
12. W. Lee and C. Chen, *J. Polym. Sci.*, **37**, 1457 (1999).
13. R.V. Bahulkar, A.A. Prabhune, H. Sivaraman and S. Ponrathnam, *Polymer*, **34**, 163 (1993).
14. A. Kotha, C.R. Raman, S. Ponrathnam, K.K. Kumar and J.G. Shewale, *React. Funct. Polym.*, **28**, 227 (1996).
15. ———, *React. Funct. Polym.*, **28**, 235 (1996).
16. F. Langer, H. Maritz and K. Reichert, *Chem. Eng. Sci.*, **35**, 519 (1980).
17. D. Leng and G. Quardener, *Chem. Eng. Commun.*, **14**, 177 (1982).
18. J. Hradil and F. Svec, *Enzyme Microb.Technol.*, **3**, 331 (1981).
19. ———, *Enzyme Microb. Technol.*, **3**, 336 (1981).
20. S. Ghosh, A method of making a biocatalyst for the preparation of invert sugar syrup from aqueous sucrose solution, Indian Patent 177144 (1996).
21. S.F. D'Souza and G.B. Nadkarni, *Enzyme Microb. Technol.*, **2**, 217 (1980).
22. ———, *Biotechnol. Bioeng.*, **22**, 2179 (1980).
23. S.F. D'Souza, J.S. Melo, A. Deshpande and G.B. Nadkarni, *Biotechnol. Lett.*, **8**, 643 (1986).
24. S.S. Godbole, B.S.Kubal and S.F. D'Souza, *Enzyme Microb. Technol.*, **12**, 214 (1990).

(*Received*: 22 June 2005; *Accepted*: 22 December 2005) AJC-4567