0273-2289/06/265–277/265–277/$30.00

Enzymatic Synthesis of Sorbitan Methacrylate According to Acyl Donors

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Abstract

Recently, sugar polymers have been considered for use as biomaterials in medical applications. These biomaterials are already used extensively in burn dressings, artificial membranes, and contact lenses. In this study, we investigated the optimum conditions under which the enzymatic synthesis of sorbitan methacrylate can be affected using Novozym 435 in *t*-butanol from sorbitan and several acyl donors (ethyl methacrylate, methyl methacrylate, and vinyl methacrylate). The enzymatic synthesis of sorbitan methacrylate, catalyzed by Novozym 435 in *t*-butanol, reached an approx 68% conversion yield at 50 g/L of 1,4-sorbitan, 5% (w/v) of enzyme content, and a 1:5 molar ratio of sorbitan to ethyl methacrylate, with a reaction time of 36 h. Using methyl methacrylate as the acyl donor, we achieved a conversion yield of approx 78% at 50 g/L of 1,4-sorbitan, 7% (w/v) of enzyme content, at a 1:5 molar ratio, with a reaction time of 36 h. Sorbitan methacrylate synthesis using vinyl methacrylate as the acyl donor was expected to result in a superior conversion yield at 3% (w/v) of enzyme content, and at a molar ratio greater than 1:2.5. Higher molar ratios of acyl donor resulted in more rapid conversion rates. Vinyl methacrylate can be applied to obtain higher yields than are realized when using ethyl methacrylate or methyl methacrylate as acyl donors in esterification reactions catalyzed by Novozym 435 in organic solvents. Enzyme recycling resulted in a drastic reduction in conversion yields.

Index Entries: Immobilized enzyme; bioconversion; optimization; biocatalysis; sorbitan; esterification.

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Introduction

"Biomaterials" is a generic term, basically referring to materials that are used for a medical purpose, which maintain direct contact with living tissues. Thus, any biomaterial must be carefully and microscopically fabricated, in order to best adjust to a living organism, regarding both functionality and structure. Biomaterials are also used as essential materials in the manufacture of catheters, contact lenses, and artificial human hearts *(1,2)*. Recently, many researchers have noted the myriad of possible applications for sugar-containing polymeric materials, which can be synthesized from sugar esters *(3–6)*. Sugars constitute an attractive group of multifunctional compounds. They are biologically relevant, and they harbor multiple hydroxyl groups. Sugar esters or esters that contain sugar molecules, have been receiving increasing interest, and are already being utilized in a variety of application fields. They have proven their advantage in a host of industries, and have been employed in flavorings, emulsifiers, lubricants, detergents, and cosmetic additives. Sugar esters are biodegradable, biocompatible, and nontoxic *(3,7)*.

Mild reaction conditions and excellent selectivity associated with lipase-catalyzed reactions permit the generation of pure materials by more efficient and environmentally friendly processes than conventional chemical methods *(5)*. Esterification is the principal process by which sugar esters are synthesized. This process has been studied extensively by both chemical and enzymatic processes. The chemical process is associated with low regioselectivity, resulting in poor selectivity, undesirable side reactions, and low yields. However, the enzymatic process can be applied to the regioselective transformations of mono- and disaccharides, and does not result in any undue complications *(8)*. In this study, we opted to use alcoholysis as the applied esterification process (Fig. 1). Alcoholysis is the esterification of an ester (e.g., vinyl methacrylate [VMA]) with the hydroxyl group in the acyl acceptor (e.g., 1,4-sorbitan), and generating another ester and an alcohol as byproducts.

In the enzymatic process used to synthesize sorbitan methacrylate from 1,4-sorbitan, several factors can affect both the conversion yield and the rate of glycosylation. These factors include the reaction solvent, reaction temperature, the type and concentration of the acyl donor, enzyme content, and initial substrate concentration *(5,9)*. The difficulty inherent to the dissolution of both hydrophobic and hydrophilic substrates in a common reaction solvent of low toxicity has constituted the primary limitation of biological synthesis *(10)*. Reaction solvents used for the synthesis of glycosyl methacrylate must be selected for maximal rates and yields of glycosylation, enzyme activity, and the separation/purification of the product. Thus, *t*-butanol was used as the reaction solvent in all of our experiments, owing primarily to its high degree of substrate solubility, remarkable affinity for glycoside (acyl acceptor), and the ease inherent to the separation/purification of the products, which

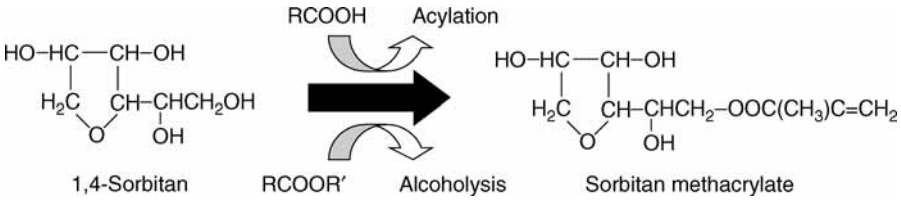

Fig. 1. Enzymatic synthesis of sorbitan methacrylate by acylation and alcoholysis.

is attributable to its low boiling point. In addition, *t*-butanol exhibits a regioselective effect with glycoside (acyl acceptor) *(5,9)*. In our article, *t*-butanol was selected for use in all of our experiments, owing to its ability to achieve a high degree of conversion, as well as the stability of the enzyme as described in previous reports *(5,6)*. Reaction temperature also influences the esterification rate. High reaction temperatures tend to induce enzyme inactivation. The optimum active temperature for Novozym 435 is between 40°C and 60°C. In the sorbitan acrylate *(5)* and β-methlyglucoside acrylate/methacrylate *(6)* esterification experiments, the optimum temperature was determined to be 50°C. In our study, the reaction temperature was set to 50°C in all experiments. We conducted the enzymatic synthesis of sorbitan methacrylate using Novozym 435 (derived from *Candida antarctica*), which is a well-known nonspecific lipase. Novozym 435 facilitates reactions between a wide range of alcohols and vinyl esters, and is a remarkably heat-tolerant enzyme *(11)*.

The objective of this study was to investigate the processes involved with the chemical and enzymatic synthesis of sorbitan methacrylate, which is the basic material used in biocompatible hydrogels.

Materials and Methods

Chemicals

Novozym 435 (lipase B from *C. antarctica*, EC 3.1.1.3), a nonspecific lipase immobilized on a macroporous acrylic resin, with 1–2% water content, and 10,000 propyl laurate units/g, was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO). D-Sorbitol and VMA were obtained from the Sigma-Aldrich Chemical Co., USA. Methyl methacrylate (MMA) and ethyl methacrylate (EMA) were commercially obtained from Junsei Chemicals (Kyoto, Japan) and Acros Organics (NJ), respectively. Acetonitrile and methanol were supplied by Fisher Scientific Korea Ltd. (Korea). All other chemicals were of analytical grade, and the solvent used was dried with molecular sieves for 1 d before use.

1,4-Sorbitan Preparation

All dehydration reactions (sorbitol cyclization) for the synthesis of 1,4-sorbitan using *p*-toluenesulfonic acid (*p*-TSA) in a solvent-free process

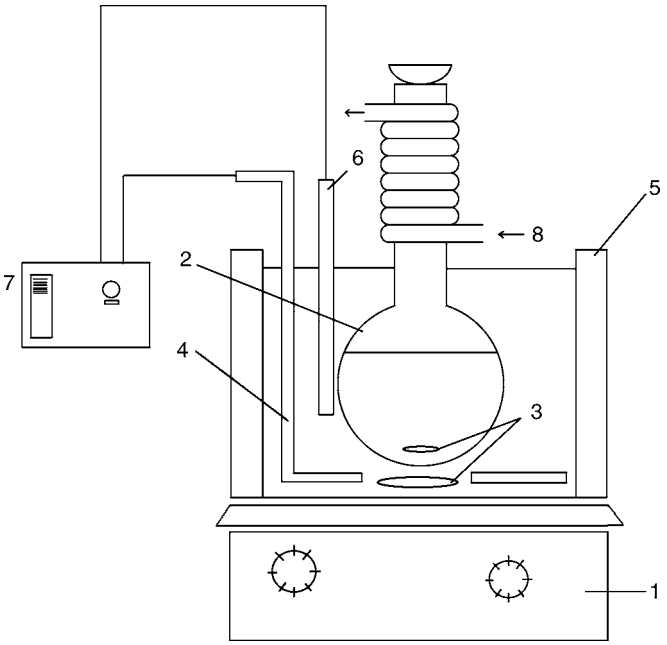

Fig. 2. Schematic diagram of experimental apparatus for the synthesis of 1,4-sorbitan esters. Magnetic stirrer, reactor, magnetic stirrer bar, heating coil, water bath, thermocouple, PID temperature controller, and condenser.

were conducted as previously reported *(5)*. The dehydration reactions were carried out for 2 h at 130 ± 1°C, under 200 mmHg reduced pressure. The reactor volume was 50 mL. The reaction temperature was controlled with an oil bath equipped with a PID (proportional-integral-derivative) temperature controller. Agitation was conducted with a magnetic bar, spinning at approx 200 rpm.

Enzymatic Esterification

In this study, we applied esterification via alcoholysis. Esterification for the synthesis of 1,4-sorbitan esters with immobilized lipase (Novozym 435) was conducted using the apparatus displayed in Fig. 2. Reaction temperature was controlled with a water bath which had been equipped with a PID temperature controller. Mixing was conducted using a magnetic stirrer, spinning at approx 200 rpm. The condenser prevented reactant (*t*-butanol) evaporation. Results are expressed as the mean value of at least two independent measurements.

In order to determine optimal condition of enzymatic esterification, three parameters—molar ratio, enzyme amount, and initial sorbitan concentration—were investigated as follows: Prepared 1,4-sorbitan was added to the bottle, and either EMA, MMA, or VMA was subsequently added, in order to achieve the desired molar ratios (1,4-sorbitan : acyl donor) and enzyme amounts at 50°C, as shown in Table 1. *t*-Butanol was also added to the mixtures, in order to ensure a total volume of 20 mL.

Table 1
Experimental Condition of Enzymatic Esterification of Sorbitan Methacrylate

Reaction parameter	Kind of acyl donor	Reaction temperature (°C)	Molar ratio (acyl acceptor/acyl donor)	Enzyme amount (% [w/v])	Initial acyl acceptor concentration (g/L)	Reaction time (h)
Molar ratio (acyl acceptor/ acyl donor)	EMA, MMA, VMA	50	1:0.5–1:5	3	50	48
Enzyme amount (% [w/v])	EMA, MMA, VMA	50	1:5	1–7	50	48
Initial acyl acceptor concentration (g/L)	EMA, MMA, VMA	50	1:5	5	30–100	48

During the reactions, 0.2 mL of the samples were withdrawn at set intervals, and monitored via high-performance liquid chromatography (HPLC).

In order to determine the effects of repeated enzyme usage on conversion yield, we added 50 g/L of prepared 1,4-sorbitan to the bottle, and VMA was subsequently added up to a 1:5 molar ratio. Reactions were then initiated by the addition of 5% (w/v) Novozym 435 at 50°C for 48 h. Each experiment for repeated enzyme usage was performed with the same enzyme for 48 h. During this experiment, 0.2 mL of samples were withdrawn at set intervals, and monitored via HPLC.

At least a trace of water is essential for bioconversion via enzyme action. Enzymes exhibit different reactions and selectivity in organic solvents than in water. In our experiments, there was no set initial water amount, with the exception of the water content of the enzymes.

Quantitative Analysis

Enzymatic reactions were monitored via analysis of the conversion yield of 1,4-sorbitan with the selected acyl donors (MMA, EMA, and VMA). Acyl donors were measured by HPLC with an ODS2 column (octadecyl [C_{18}], 5 μm, 120 Å, 250 × 4.6 mm, waters), which was constantly maintained at 35°C. A mixture of acetonitrile, methanol, and water (55:40:5 [v/v/v]) was used as a mobile phase, at a flow rate of 1 mL/min, to measure the acyl donors; 0.2 mL of samples were extracted from the reaction mixture at set intervals during the reaction. Enzymes were removed by sample filtering and appropriate dilution. Then we injected 20 μL of the prepared samples. Detection was conducted at 220 nm with MMA, EMA, and VMA as calibration standards.

Results and Discussion

In comparison with conventional chemical processes, the enzymatic synthesis process is accomplished under milder conditions. Therefore, it carries the advantages of high material stability, low energy cost, high selectivity, and low purification cost. The enzymatic process appears to constitute a favorable alternative synthesis process *(4,5)*. In the enzymatic synthesis of sorbitan methacrylate from 1,4-sorbitan, several factors, including the reaction solvent, reaction temperature, the type and concentration of acyl donor, enzyme content, and initial substrate concentration, can impact both the conversion yield and the glycosylation rate *(5)*.

The chemical synthesis of sorbitan, which was used to affect the enzymatic synthesis of sorbitan methacrylate, was accomplished via sorbitol dehydration. This resulted in a fine product, with minimized byproduct formation at 130°C and 200 mmHg reduced pressure, with 1% (w/w) *p*-TSA used as a catalyst, and 120 min of reaction time.

Enzymatic Synthesis of Sorbitan Methacrylate

In this study, we performed alcoholysis for the esterification of several acyl donors (EMA, MMA, or VMA) with the hydroxyl group in 1,4-sorbitan (acyl acceptor). The process was designed to generate sorbitan methacrylate and alcohol as byproducts. Esterifications for the enzymatic synthesis of 1,4-sorbitan ester using immobilized lipase (Novozym 435) were performed using MMA, EMA, and VMA as acyl donors, all in *t*-butanol as the organic solvent.

Effect of Molar Ratio

In the glycosylation of sorbitan, which harbors one primary hydroxyl group, only monoacrylate was synthesized, owing to the regioselective effects of the solvent (*t*-butanol). The theoretical molar ratio of acyl donor to sorbitan is 1:1, but in order to maintain a high reaction velocity and in consideration of the reversibility of glycosylation, we believed that the theoretical molar ratio would not prove to be sufficiently low *(5,9)*.

As shown in Fig. 3A, the enzymatic synthesis of sorbitan methacrylate catalyzed by Novozym 435 in *t*-butanol reached a conversion yield of approx 49% at 50 g/L of initial 1,4-sorbitan concentration, 3% (w/v) of enzyme content, a 1:5 sorbitan to EMA molar ratio, a reaction temperature of 50°C, and duration of 36 h, using EMA as the acyl donor. Using MMA as acyl donor (Fig. 3B) sorbitan methacrylate was synthesized at a conversion rate of approx 55%, at 50 g/L of initial 1,4-sorbitan concentration, 3% (w/v) of enzyme content, a 1:5 sorbitan to MMA molar ratio, a reaction temperature of 50°C, and a duration of 36 h. The degree to which esterification occurred was observed to have increased directly with the molar ratio in both cases.

Effect of Enzyme Amount

For an enzymatic process to be economically comparable with the conventional chemical synthesis process the amount of the added enzyme must be minimized. Higher enzyme loadings result in shorter reaction periods but increase operation costs. The process must be optimized concerning both productivity and enzyme loading. Above optimum enzyme concentrations, the equilibrated conversion yields tend to be reduced as the enzyme begins to form complex compounds not only with the acyl donor, which causes glycosylation, but also with sorbitan methacrylate resulting in the hydrolysis of the desired product *(5,9)*.

Enzyme loading were determined on the conversion of 1,4-sorbitan with EMA (or MMA) to sorbitan methacrylate using Novozym 435. As shown in Fig. 4A, the conversion of 1,4-sorbitan to sorbitan methacrylate was 68% at 36 h, and a 5% (w/v) enzyme amount using EMA as the acyl donor. Enzyme amounts were found to slightly influence conversion yields.

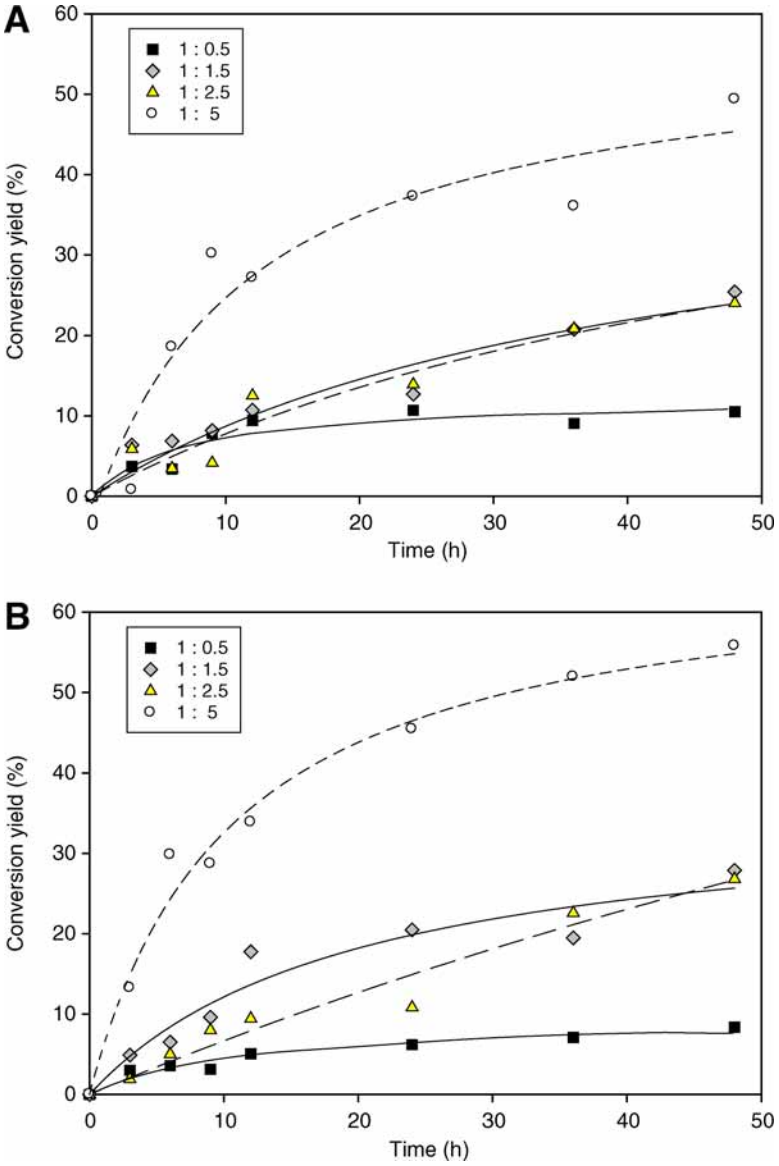

Fig. 3. Effect of molar ratio on conversion of sorbitan methacrylate in lipase-catalyzed glycosylation using **(A)** ethyl methacrylate and **(B)** methyl methacrylate as acyl donor.

However, high enzyme amounts can also result in economic problems. Higher enzyme contents tend to reduce reaction time but the final conversion appears to be independent of the amount of enzyme added. As shown in Fig. 4B, when MMA is used as the acyl donor, sorbitan methacrylate is synthesized at a conversion rate of approx 78%, at 50 g/L of initial 1,4-sorbitan concentration, 7% (w/v) of enzyme content, a sorbitan to MMA molar ratio of 1:5, and a reaction temperature of 50°C, with a reaction duration of 36 h.

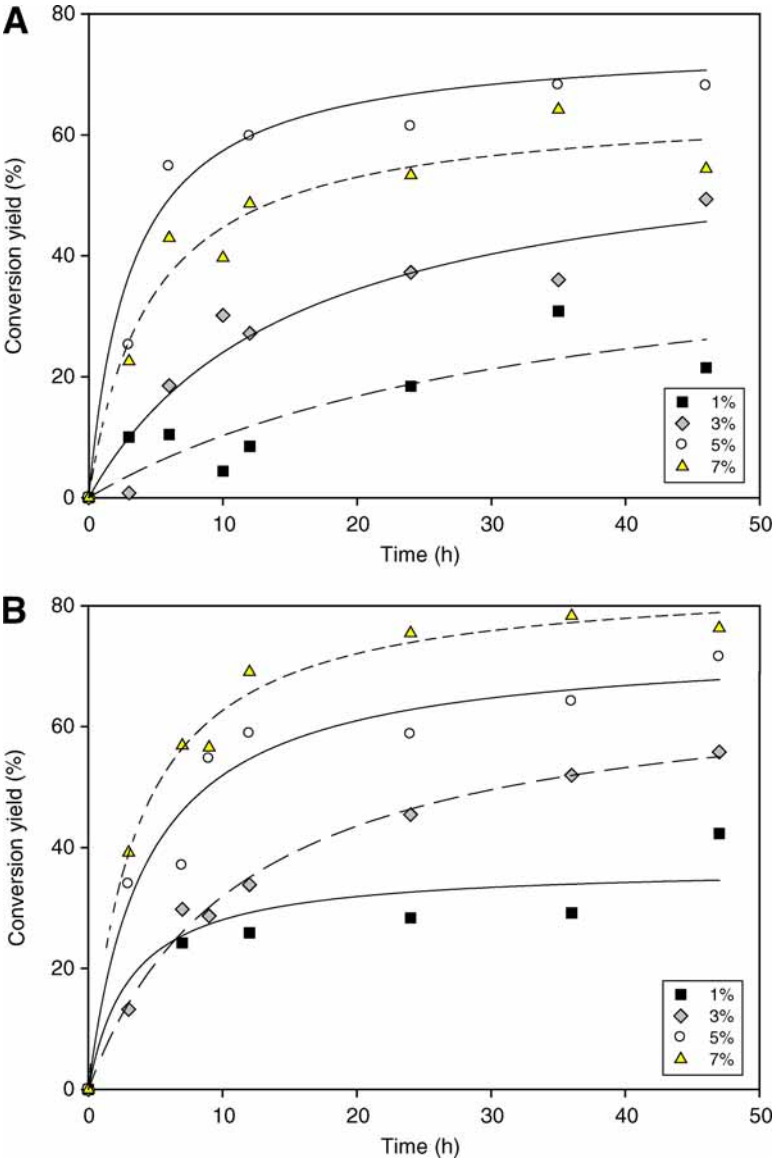

Fig. 4. Effect of enzyme content on conversion of sorbitan methacrylate in lipase-catalyzed glycosylation using **(A)** ethyl methacrylate and **(B)** methyl methacrylate as acyl donor.

Effect of Initial Sorbitan Concentration

As a result of the fact that enzymes exhibit lower activity than do chemical catalysts, reactions such as those conducted in this study often have longer reaction times, and often result in a relatively low conversion yield. This phenomenon can be circumvented by using high initial glycoside concentrations, but may result in an increase in the viscosity. An increase in the viscosity of the reactant at high concentrations of the initial substrate

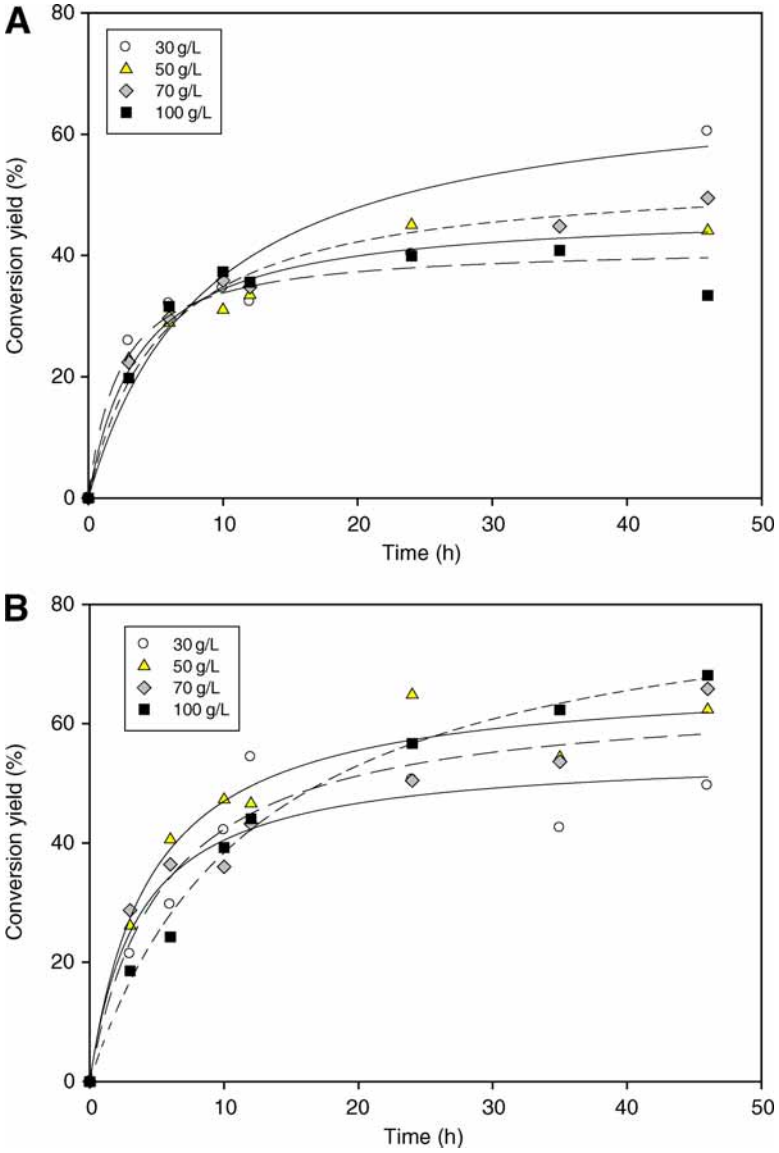

Fig. 5. Effect of initial 1,4-sorbitan concentration on conversion of sorbitan methacrylate in lipase-catalyzed glycosylation using **(A)** ethyl methacrylate and **(B)** methyl methacrylate.

results in lower conversion yields, and increases in the cost of both the raw and purified materials. Therefore, it was necessary to optimize the initial substrate concentration. In a previous experiment involving 1,4-sorbitan acrylate synthesis (5), the initial 1,4-sorbitan concentration was optimized at 50 g/L.

The effects of initial sorbitan concentration on the conversion of 1,4-sorbitan with EMA (or MMA) to sorbitan methacrylate were investigated using Novozym 435. As shown in Fig. 5A, during the reaction periods, the

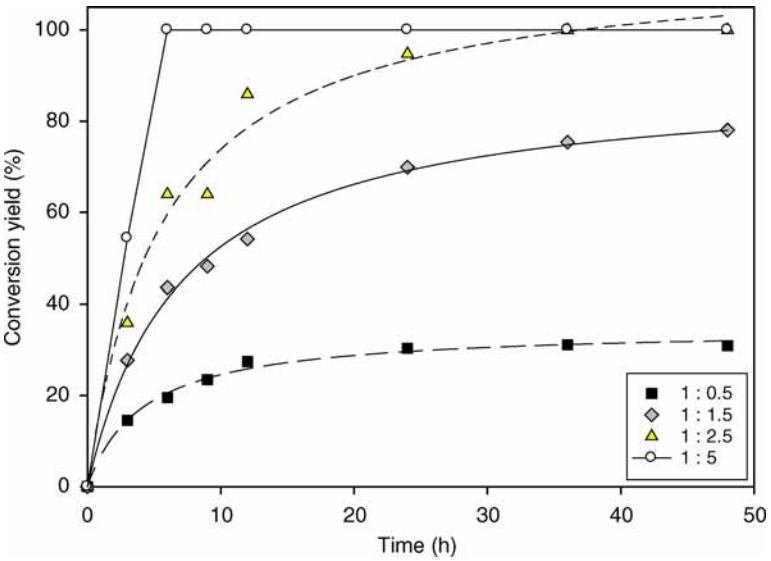

Fig. 6. Effect of molar ratio on conversion of sorbitan methacrylate in lipase-catalyzed glycosylation using vinyl methacrylate.

conversion of 1,4-sorbitan to sorbitan methacrylate was approx 60% at 46 h and a sorbitan concentration of 30 g/L. High initial sorbitan concentrations resulted in low conversion yields. As shown in Fig. 5B, when using MMA as the acyl donor, sorbitan methacrylate was synthesized at a conversion rate of approx 68% at the following conditions: 100 g/L of initial 1,4-sorbitan concentration, 5% (w/v) of enzyme content, 1:5 of molar ratio of sorbitan to MMA, a reaction temperature of 50°C, and a reaction time of 46 h. In contrast to the equilibrated final conversion yield, the initial conversion velocity was found to be higher at 50 g/L sorbitan within the first 12 h than with 100 g/L sorbitan. These results were different from the results of the MMA experiment and the previous sorbitan acrylate synthesis *(1)*.

Enzymatic Esterification With VMA as an Acyl Donor

The synthesis of sorbitan methacrylate using VMA as an acyl donor was expected to result in a superior conversion yield under the following conditions: 3% (w/v) of enzyme content, 1:0.5–1:5 molar ratio, and 50 g/L of initial 1,4-sorbitan concentration. As shown in Fig. 6, during the reaction periods, the conversion of 1,4-sorbitan to sorbitan methacrylate was completed at 46 h and at a molar ratio above 1:2.5. Higher molar ratios of acyl donor resulted in more rapid conversion. The application of VMA to the reaction resulted in a higher conversion yield than is observed with EMA and MMA being used as acyl donors in enzymatic esterification catalyzed by Novozym 435 in organic solvents. In cases in which VMA is employed as an acyl donor, during the glycosylation periods, vinyl alcohol is produced. Simultaneously, tautomeric isomerization to acetaldehyde occurs.

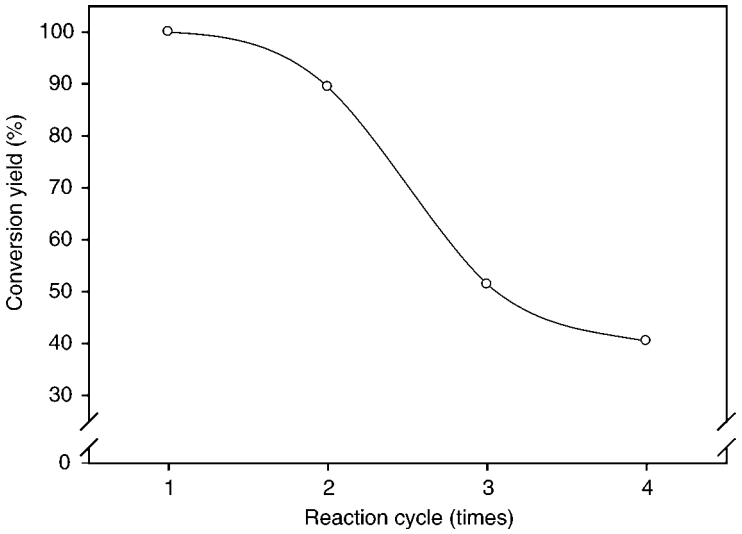

Fig. 7. Effect of enzyme recycling time on conversion of sorbitan methacrylate in lipase-catalyzed glycosylation using vinyl methacrylate.

Therefore, we can conclude that this process facilitates the irreversibility of glycosylation, resulting in higher conversion yields. In experiments involving β-methylglucoside acrylate/methacrylate esterification, higher conversion yields were obtained with the usage of vinyl acrylate and VMA as the acyl donors than when any other acyl donors, including acrylic acid and methacrylic acid, were used *(6)*.

Repeated Usage of Enzyme

In order for the enzymatic process to prove economically feasible as compared with the chemical synthesis process, the amount of added enzyme must be minimized, and must also be recycled in the reaction, as enzymes tend to be fairly expensive. As shown in Fig. 7, sorbitan methacrylate synthesis using VMA as acyl donor was expected to result in a superior conversion yield under the following conditions: 5% (w/v) of enzyme content, a molar ratio of 1:5, and 50 g/L of initial 1,4-sorbitan concentration. During the repeated reaction periods, we observed a reduction in the conversion yield of 1,4-sorbitan to sorbitan methacrylate. On the first reaction, the conversion was completed within 6 h, but during the repeated reactions, the conversion yield decreased dramatically, to approx 40%.

Conclusions

In this study, we attempted to determine the optimum conditions for the enzymatic synthesis of sorbitan methacrylate, using Novozym 435 in *t*-butanol from sorbitan and several acyl donors (EMA, MMA, and VMA). As we achieved an approximate conversion yield of sorbitan methacrylate

catalyzed by Novozym 435 in *t*-butanol of 68% under the following conditions: 50 g/L initial 1,4-sorbitan concentration, 5% (w/v) enzyme content, a molar ratio of sorbitan to EMA of 1:5, and reaction time of 36 h. When MMA was utilized as an acyl donor, sorbitan methacrylate was synthesized at a conversion yield of approx 78% under the following conditions: 50 g/L initial 1,4-sorbitan concentration, 7% (w/v) enzyme content, a molar ratio of 1:5, and a reaction time of 36 h. Sorbitan methacrylate synthesis using VMA as the acyl donor was expected to result in a superior conversion yield at 3% (w/v) of enzyme content, and at a molar ratio of more than 1:2.5. Higher molar ratios of acyl donor resulted in faster conversion rates. VMA can be applied in order to obtain yields higher than those associated with EMA or MMA as acyl donors in our esterification reaction. Enzyme recycling resulted in low conversion yields, depending on recycling time.

Acknowledgment

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (D00250) (R05-2004-000-11185-0).

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