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IMMOBILIZED ENZYMES IN ORGANIC MEDIA: CHIRAL MONOMER PRODUCTION IN ORGANIC MEDIA

DE-FC02-92CH10519
Final Report

March 1996

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1.0 EXECUTIVE SUMMARY

The overall goals of this project were to investigate the critical factors that limit commercial scale applications of enzymes in organic solvents, and to scale-up a process for the production of a precursor to a specialty polymer. In the last phase of the project, we focused on optimizing and scaling up a trans-esterification reaction catalyzed by Subtilisin Carlsberg in very dry organic solvent. The reaction system we have employed has been reported by A. Klibanov et al3. It involves the trans-esterification of vinyl acrylate with (R,S)-sec-(2-naphthyl)ethyl alcohol catalyzed by Subtilisin Carlsberg in tert-amyl-alcohol as a solvent. Only the S ester is produced. The other product, vinyl alcohol, converts spontaneously to acetaldehyde, thus shifting the equilibrium towards production of the desired product. The scaled up reaction was run under various conditions in order to identify the controlling factors.

We have been able to scale up successfully the trans-esterification reaction from 5ml to 75ml. By varying the immobilization and reaction conditions, we increased the initial rate of the reaction by two orders of magnitude and the conversion from 20% to 100%. We have isolated several grams of the S-*sec*-(2-naphthyl)ethyl acrylate product. It contains two minor impurities, none of which is the R enantiomer. This and other chiral acrylic monomers could be polymerized to form polymers with special optical properties.

In our dry enzymatic trans-esterification system, we found that two factors dominate the observed Subtilisin activity: lyoprotection and water control. This is in agreement with other reports[4]. Their influence is exerted mostly through modifying the stability of the enzyme's active form. Small rate effects can also be attributed to the competing hydrolysis. Lyoprotection increases the amount of active enzyme at the start of the reaction. The water level controls the rate of enzyme deactivation during the trans-esterification process. Our results are consistent with the observed initial rate affected mostly by changes in the amount of active protease rather than in the enzyme's intrinsic catalytic rate.

By far, the most influential factor effecting the enzymatic performance in organic media is the pre-treatment of the enzyme[4]. We have examined the effect of polyethylene glycol (PEG) as a lyoprotectant on the performance of the free suspended enzyme. The addition of PEG to the enzyme solution before freeze-drying, brought about a 100 fold increase in initial rate and a 4.3 fold increase in conversion. The positive PEG effect is true for both a free and an immobilized enzyme. The PEG increases greatly the amount of active enzyme which survives the lyophilization step.

Another important factor affecting the trans-esterification of vinyl acrylate with (R,S)-*sec*-(2-naphthyl)ethyl alcohol is the water content. At very low water levels there is a minor contribution from direct competition between hydrolysis and trans-esterification. However, the main water effect is exerted through lowering the stability of the enzyme, since it facilitates its unfolding. We demonstrated that pre drying all components before the start of the process, as well as adding a large amount of molecular sieves to the reactor, greatly improved the enzyme performance. The degree of conversion increased from 67% to 89%.

The overall stability of the enzyme was examined by recycling the biocatalyst after 150 hours in the reactor. The initial rate for the recycled enzyme has dropped by a factor of 25, as compared to the fresh enzyme. The water promoted destabilization is probably the cause for the decreased performance of the recycled enzyme.

The effect of support composition was also studied. We chose to utilize the free fibers themselves as support for the enzyme, since this approach allowed us to isolate the effect of composition, without interference from morphology factors. Two compositions were tested: pure nylon 6 fibers and Hydrofil nylon fibers with 15% Jeffamine. No significant difference between the performance of the two types of fibers was found. As the support's function in this dry system is to provide lyoprotection, any small differences between the two fibers may have been overwhelmed by the presence of PEG.

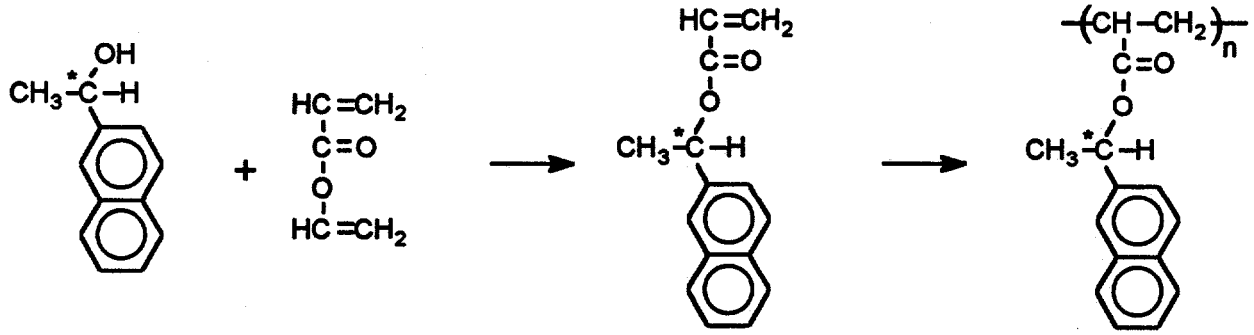
Immobilization of the enzyme in non aqueous systems is not essential for the recovery and reuse of the biocatalyst, since its insolubility in the solvent provides a method for recovery.

However, the handling and reactor design issues are greatly simplified if the enzyme is immobilized on a support. In addition, the support has been shown to confer extra stability to the biocatalyst. We have compared the performance of PEG-treated free suspended subtilisin to fiber-immobilized PEG treated enzyme, and found that for reactions run in very dry organic solvents, immobilization does not provide a significant advantage unless it improves water control.

2.0 INTRODUCTION

The overall goals of this project were to investigate the critical factors that limit commercial scale applications of enzymes in organic solvents, and to scale-up a process for the production of a precursor to a specialty polymer. The overall performance of an immobilized enzyme can be influenced by its intrinsic structure and changes in this structure, as well as by external factors such as water content, support identity and morphology, reactor design, etc.. In the past we have investigated the interrelation between support morphology and water content and its effect on overall enzyme performance. We looked at particulate and fabric supports in wet as well as dry non aqueous environments. In the wet organic solvents, we found mass transfer issues to dominate the enzyme performance[1,2]. Thus particle porosity, as well as the use of a mixed polarity polypropylene/Hydrofil nylon non-woven fabric as an enzyme support, provided an advantage. In dry systems, where mass transfer is generally not an issue, the particulate supports did not provide any benefit, while the non-woven fabric still enhanced the rate of the enzymatic reaction. We believe that this particular fabric with its hydrophilic fibers acts as a lyoprotectant in the process of drying the enzyme.

In the last phase of the project, we focused on optimizing and scaling up a trans-esterification reaction catalyzed by Subtilisin Carlsberg in very dry organic solvent. The reaction system we have employed has been reported by A. Klibanov et al[3]. It involves the trans-esterification of vinyl acrylate with (R,S)-*sec*-(2-naphthyl)ethyl alcohol catalyzed by Subtilisin Carlsberg in *tert*-amyl-alcohol as a solvent. Only the S ester is produced. The other product, vinyl alcohol, converts spontaneously to acetaldehyde, thus shifting the equilibrium towards production of the desired product. The chiral acrylate can then be polymerized to form chiral polymers. Other chiral alcohols might later be used to produce commercially viable polymer products.



The scaled up reaction was run under various conditions in order to identify the controlling factors. Finally, a product isolation methodology has been developed and applied.

3.0 MATERIALS AND EXPERIMENTAL METHODS

Subtilisin Carlsberg was purchased from Sigma. It was utilized either in a free suspension or immobilized. Free enzyme was pre-treated by lyophilization from 0.05 M potassium phosphate buffer pH 7.8. Enzyme immobilization onto nylon 6 and Hydrofil nylon fibers, as well as non woven fabrics, was accomplished by deposition from the same buffered solution. Subtilisin Carlsberg (250 mg or 3070 units) was dissolved in 20 ml of the 0.05 M potassium phosphate buffer containing, in most cases, 150 mg of polyethylene glycol (3400 MW, Polyscience). Dry Nylon fibers (5 grams) were cut into 0.5" lengths, washed with methanol and water in order to remove finishers, and air dried. The enzyme solution was then carefully pipetted onto the fibers. Additional 10 ml of water was added to the enzyme stock in some instances where sufficient wetting did not occur with the 20 ml. The wet fiber ball was frozen and lyophilized. Similar procedure was applied when the support was a piece of fabric.

Vinyl acrylate was purchased from Polyscience, (R,S)-*sec*-(2-naphthyl)ethyl alcohol from Fluka, and *tert*-amyl alcohol from Aldrich. The trans-esterification of vinyl acrylate with (R,S)-*sec*-(2-naphthyl)ethyl alcohol was carried out at 45°C. Reaction conditions followed roughly those reported by Margolin et al [3] Reaction mixtures contained 0.5 M vinyl acrylate, 0.5 M racemic naphthyl ethanol, and 3.33 mg/ml free or immobilized Subtilisin Carlsberg in 75 ml of *tert*-amyl alcohol. Enzyme concentration was selected as a compromise between rate and cost. Molecular sieves (3-7.5 g) were added to some of the runs. In some cases all components were dried extensively before the start of the kinetic run.

The kinetics of vinyl acrylate trans-esterification with (R,S)-*sec*-(2-naphthyl)ethyl alcohol was monitored by HPLC using a HP 1090 LC instrument equipped with DAD. A Phenomenex Chirex Phase 3007 (250x4.6) column and guard with 90% hexane, 9% 1,2-dichloroethane and 1% ethanol in the mobile phase, was utilized to monitor the enantiomers' concentrations throughout the reaction. Since on our time scale only one of the enantiomers reacted, we used the non reactive enantiomer as an internal standard. Reactions were also monitored by non chiral chromatography, using a HP 5890 series 2 GC instrument equipped with a DB-1 30

meter long 0.25mm diameter column and a FID detector. This method, as well as the product isolation gas chromatography, verified that only the S enantiomer of the (R,S)-*sec*-(2-naphthyl)ethyl alcohol reacted with the vinyl alcohol.

Water content was determined by a coulometric Karl-Fisher titration, using a Mitsubishi Moisture Meter model CA-06.

4.0 RESULTS AND DISCUSSION

A series of batch reactions scaled up from 5ml to 75 mls were carried out with free and immobilized Subtilisin Carlsberg under various conditions. A list of all runs with their description is given in Table I. Reactions were typically monitored for 1-2 weeks. Figure 1 provides the reaction progress of two runs with the same conditions. It illustrates the degrees of reproducibility and error. For each run an initial rate was calculated. Table II summarizes the rates, as well as the degree of conversion after 160 hours for all runs. Conversion is expressed as the percent of enantiomer S alcohol converted to the S ester.

4.1 Effect of Lyoprotectant

By far, the most influential factor effecting the enzymatic performance in organic media is the pretreatment of the enzyme4. We have examined the effect of polyethylene glycol (PEG) as a lyoprotectant on the performance of the free suspended enzyme. The trans-esterification reaction was run first with Subtilisin Carlsberg lyophilized from a buffer solution, and then with the enzyme lyophilized from a buffer solution containing PEG. The results are shown in Figure 2. A dramatic difference in both rate of trans-esterification and in overall conversion is evident. The addition of PEG to the enzyme solution before freeze-drying, brought about a 100 fold increase in initial rate and a 4.3 fold increase in conversion (Table II). This suggests that most of the activity is lost before the enzyme is contacted by the organic solvent. The organic solvent, in contrast to water, does not allow the renaturation of the damaged (unfolded) enzyme. The positive PEG effect is true for both a free and an immobilized enzyme. These results are in agreement with other reports. The PEG probably increases greatly the amount of active enzyme which survives the lyophilization step. Without the lyoprotectant the rate is so slow, that deactivation of the enzyme occurs before conversion is complete. In all consequent experiments with either free or immobilized enzyme, we have therefore used PEG in the enzyme pre-treatment.

4.2 Effect of Water Content / Molecular Sieves

Another important factor affecting the trans-esterification of vinyl acrylate with (R,S)-sec-(2-naphthyl)ethyl alcohol is the water content. We have measured the rate of reaction for the free suspended enzyme as a function of water added to the reaction mixture. Small amounts of water, 0.04% to 0.2% (v/v), were added. This water level is below its solubility in the amyl alcohol, so it does not form a separate phase. The result of the experiment are shown in Figure 3. It is clear that there is a direct competition between hydrolysis and trans-esterification. In addition to competition, the presence of water lowers the stability of the enzyme since it facilitates its unfolding. This experiment demonstrated the need to exclude as much water as possible. Even when no water is added to the reaction mixture, some water is present. It originates mostly from the lyophilized enzyme, buffer, and fibers support. Small amounts of water are also contributed by the solvent and reactants. We have measured the water dissolved in the liquid phase for some of the runs. For example, in run 54 (Table I), we observed an increase in the water level from 0.2% at the start of the reaction, to 0.35% at the end. A small change was measured also with run 140: from 0.08% to 0.23%. On the other hand, with runs 56 and 130 we found that the water content was constant throughout the reaction, 0.25% and 0.16%, respectively. The small increases are probably due to slow release of water from the enzyme and support into the solution.

We tested the possibility of using molecular sieves to remove the water from the enzyme's micro-environment. We have used the immobilized subtilisin for that purpose. Reactions were run with the enzyme immobilized on Hydrofil nylon fibers (15% Jeffamine), and 3.0 grams or 7.5 grams molecular sieves added to the reaction vessel. The reaction progress for these conditions, shown in Figure 4, demonstrates the effectiveness of molecular sieves. While the initial rate stayed the same, the conversion increased from 67% to 77%. We have taken yet another measure to remove water by pre-drying all components. The solvent and reactants were

equilibrated with molecular sieves prior to addition of the catalyst. The immobilized enzyme was pre-dried over a desiccant for 48 hours before use. The drying step improved performance as well (Figure 4). The rate improved slightly, and the conversion further increased to 89%.

4.3 Effect of Support Composition and Morphology

In the past we have found that in wet organic systems, the use of a non woven mixed polarity fabric provided an improved enzyme activity over particulate supports and free enzyme2. This was attributed to better distribution of water and better mass transfer. In dry systems, where mass transfer is generally not an issue, this fabric which contains polypropylene and a block copolymer of nylon 6 and Jeffamine, was found again to enhanced the rate of the enzymatic reaction2 (Figure 5). We believe that in the latter system the hydrophilic Jeffamine content acts as a lyoprotectant rather than a mass transfer effector. The Jeffamine block is in fact a modified PEG:

$$\text{Hydrofil nylon} = \text{nylon 6} + \text{terephthalate} + NH_2\text{-(-}CH_2\text{-}CH_2\text{-O-)}_n\text{-}NH_2$$

We have chosen to examine the support in more detail. We have obtained several non-woven polypropylene/cellulose compositions from Kimberley Clark and tested these as enzymatic supports in a dry non-aqueous environment. None of the fabrics was satisfactory. In fact, the enzyme immobilized on all of the fabrics showed very little activity as compared to the AlliedSignal's mixed polarity fabric. Since the reaction system is a dry one, and mass transfer issues are not dominant, the non-woven mixed polarity structure is not as important as the fiber composition and its lyoprotecting effect. Therefore, we have chosen to utilize the free fibers themselves as support for the enzyme. This approach allowed us to isolate the effect of composition, without interference from morphology factors. Two compositions were tested: pure nylon 6 fibers and Hydrofil nylon fibers with 15% Jeffamine.

The enzyme support was prepared by cutting the fibers to 0.5" lengths, washing them to remove dyes and additives and air drying. The length of the fiber pieces was selected to provide an open structure with no clumps. The enzyme was immobilized by wetting the fibers with a minimal volume of a solution containing the enzyme dissolved in a preferred buffer. The buffer contained polyethylene glycol as well. The wet fibers were dried by lyophilization. The fiber supported enzyme was then packed into the reactor together with the molecular sieves. For these comparisons, 7.5 grams of molecular sieves were used. Reaction progress with the nylon 6 and Hydrofil nylon supported enzyme is displayed in Figure 6. The difference between the performance of the two types of fibers is small, and within our experimental error. One can speculate that since Hydrofil nylon retains more water than unmodified nylon 6, the real difference is larger and significant. A repeat of the experiment under less dry conditions might resolve this question. Another factor complicating the interpretation, is the presence of PEG. If all the support's function in this dry system is to provide lyoprotection, any small differences between the two fibers may have been overwhelmed by the presence of PEG. A comparison without PEG should therefore be performed.

4.4 Effect of Enzyme Immobilization

Immobilization of the enzyme in non aqueous systems is not essential for the recovery and reuse of the biocatalyst, since its insolubility in the solvent provides a method for recovery. However, the handling and reactor design issues are greatly simplified if the enzyme is immobilized on a support. Sometimes the support confers extra stability to the biocatalyst. For example, we have observed that in the absence of PEG, subtilisin immobilized on polypropylene/Hydrofil nylon non woven fabric performed better than the free enzyme in the same system2 (Figure 5). We have now compared the performance of PEG treated free-suspended Subtilisin to fiber-immobilized PEG-treated enzyme. This was done using two sets of conditions: in the first comparison the enzyme was immobilized on Hydrofil nylon, and 7.5 grams of molecular sieves were

added to the reaction mixture; in the second comparison no molecular sieves were added. The results are shown in Figures 7 and 8, respectively.

Under both sets of conditions, the free enzyme performs better. However, the difference between the free and immobilized conditions decreases as the water control improves. Under the more dry conditions, the rate with the free enzyme was 60% higher than with the immobilized, and the conversion 10% higher. In the "wet" experiment the differences were 107% and 30% respectively (see Table II). This result further supports our interpretation of the fiber's effect. Since both free and immobilized Subtilisin were PEG pre-treated, the added contribution of stability by the Jeffamine in the fiber is negligible. The Hydrofil fibers are very hydrophilic and retain significant amount of water in addition to the water that adsorbs to the enzyme. The higher the water level, the lower is the trans-esterification rate. Under the more rigorously dried conditions, the difference in water content decreases as does the difference in reaction rate. Thus, for reactions run in very dry organic solvents, immobilization does not provide a significant advantage unless it improves water control.

4.5 Effect of Enzyme Recycling

We have looked at the residual activity of subtilisin immobilized on Hydrofil nylon fibers after it has been reacting for 150 hours under our standard conditions . The enzyme was removed from the reaction mixture, washed with amyl alcohol, and added to fresh reactants. PEG was used in pre-treating this enzyme preparation, but no molecular sieves were added to the reactor. The reaction progress curves for the two runs are given in Figure 9. The initial rate for the recycled enzyme dropped by a factor of 25, as compared to the fresh enzyme (Table II). The water promoted destabilization is probably the cause for the decreased performance of the recycled enzyme. Since no molecular sieves were used, significant amount of water was present, and enhanced the protein's unfolding. However, the recycled enzyme's rate is

still higher than that of a free enzyme lyophilized without PEG (Figure 2). Better water control should improve the enzyme's stability in the solvent.

4.6 Circulating Batch Reactor Experiments

A circulating batch reactor was constructed for the study of reactor design parameters such as, shear sensitivity, packing density, fluid dynamics, residence time and temperature effects. However, even at the slowest circulation rates, no conversion could be detected. The circulating rate was of the order of bed volume per minutes, while with the non circulating batch reactor it took hours to detect reaction. Thus, this reaction is too slow for such a reactor.

4.7 Product Isolation

The naphthyl acrylate enantiomer was isolated in the following fashion. The reaction mixture was first filtered to remove the enzyme. The liquid was then distilled to remove acetaldehyde and amyl alcohol. The residual oil was redissolved in 4:1 hexane/ethyl ether (1 gram in 20 mls), and the product separated out by column chromatography on Davisil Silica (90-130 micron particles with 300 angstrom pore size). The dissolved oil, 65 mls, was applied to a 1.25" diameter column packed with 160 grams of the silica in hexane. The column was eluted with the hexane/ethyl ether. The contents of the various fractions were assayed with chiral HPLC using Phenomenex Chirex Phase. Two typical elution profiles are given in Figures 10 and 11. The fractions containing the desired enantiomeric ester were pooled and the solid ester was recovered by a Rotavap. Two minor products were eluted with the product, S-naphthyl ethyl acrylate, as can be seen from Figures 10 and 11. No attempt was made to identify these products or further clean the naphthyl ester. No trace of the R-naphthyl ethyl acrylate has been detected.

4.8 VEctomer 4010

In 1994 we have examined the economics of a lipase catalyzed synthesis of AlliedSignal's new product, VEctomer 4010. The enzymatic synthesis has the potential to provide a cleaner route to a better performing material. We have demonstrated that the product can indeed be made enzymatically in a non-aqueous environment, but the rate of VEctomer production was very low and significant quantities of by-products were formed. The economic assessment suggested that the enzymatic route will not be cost effective even with major predicted improvements. Therefore we discontinued working on this particular reaction.

5.0 CONCLUSIONS

We have been able to scale up successfully the trans-esterification reaction from 5ml to 75ml. By varying the immobilization and reaction conditions, we increased the initial rate of the reaction by two orders of magnitude and the conversion from 20% to 100%. We have isolated several grams of the S-*sec*-(2-naphthyl)ethyl acrylate product. It contains two minor impurities, none of which is the R enantiomer. This and other chiral acrylic monomers could be polymerized to form polymers with special optical properties.

In our dry enzymatic trans-esterification system, we found that two factors dominate the observed Subtilisin activity: lyoprotection and water control. This is in agreement with other reports[4]. Their influence is exerted mostly through modifying the stability of the enzyme's active form. Small rate effects can also be attributed to the competing hydrolysis. Lyoprotection increases the amount of active enzyme at the start of the reaction. The water level controls the rate of enzyme deactivation during the trans-esterification process.

Our results are consistent with the observed initial rate affected mostly by changes in the amount of active protease rather than in the enzyme's intrinsic catalytic rate. The largest initial rate change, a factor of 100, was observed between enzyme lyophilized with PEG and the one without (Table II). In this case the higher rate resulted from an increase in the active enzyme concentration. The second largest change in activity was observed with the recycled enzyme: a drop in the initial rate by a factor of 25 (Table II). It reflects a continuous denaturation of the enzyme. The recycled enzyme experiment demonstrate loss of over 90% of the activity in approximately 150 hours due to presence of approximately 0.2% water in the liquid phase. The large drop of activity for the recycled enzyme could have only been affected marginally by competition from hydrolysis. A decrease in the rate of catalysis could have occurred over the progress of reaction, since in some cases, we have observed an increase in the dissolved water from 0.02% at the start of the run to approximately 0.25% at the end of the run. However, this water increase could account for up to a factor of two in the rate, but not a factor of 25 (Figure 3). With all other conditions, small rate differences were observed,

and could also be attributed to active enzyme concentration. For example, the initial rates observed with free enzyme in the presence and absence of molecular sieves, are identical within experimental error. However, the conversion in the dried system is improved from 87% to 98% (Table II). This result suggests that when the system is not dried extensively The enzyme unfolds faster during the reaction time.

Lyoprotection can be achieved by additives such as PEG to the pre-treatment buffer, or by immobilizing the enzyme on supports that contain PEG or other hydrophilic polymers. For example, Hydrofil nylon with a high content of Jeffamine might provide effective lyoprotection. However, for enzymatic systems that require extremely low water levels, i.e. trans-esterification, we find that the immobilization does not add any advantage.

Water removal and control is extremely important for such systems, but is more difficult to achieve. Exhaustive pre-drying and addition of molecular sieves improve the enzymes stability and reduce competition by hydrolysis. A choice of hydrophobic support, or no support at all should also help to keep the system dry.

The degree of enzyme stability achieved in our scale up experiments is significant, but is not sufficient. The loss of over 90% of the active Subtilisin in 150 hours, precludes any utilization of the system for commercial production. Better Lyophilization and water control is needed.

TABLE I SUMMARY OF RUN DESCRIPTIONS

RUN	DESIGNATION	CONDITIONS
50	Free	Free Suspended Enzyme
52	Free + PEG	Free Suspended Enzyme pre-treated with PEG
53	Hydrofil + PEG	Enzyme immobilized on Hydrofil nylon, pre-treated with PEG
54	Recycled 53	Enzyme from Run 53 washed in solvent and reacted with fresh reactants
55	Hydrofil + PEG + 3gMS	Enzyme immobilized on Hydrofil nylon, pre-treated with PEG, 3g Molecular Sieves added
130	Nylon + PEG + Pre Dry	Enzyme immobilized on regular nylon, pre-treated with PEG, all components pre-dried, and 7.5 g Molecular Sieves added
140	Hydrofil + PEG + Pre Dry	Enzyme immobilized on Hydrofil nylon, pre-treated with PEG, all components pre-dried, and 7.5g Molecular Sieves added
150	Free + PEG + Pre Dry	Free suspended enzyme, pre-treated with PEG, all components pre-dried, and 7.5g Molecular Sieves added

TABLE II SUMMARY OF KINETIC DATA

RUN	CONDITIONS	INITIAL RATE, %/hr	% CONVERSION
50	Free Enz	0.074	20
52	Free + PEG	7.14	87
53	Hydrofil + PEG	3.44	67
54	Recycled 53	0.137	22
55	Hydrofil + PEG + 3gMS	3.22	77
56	Hydrofil + PEG + 3gMS	2.78	73
130	Nylon + PEG + Pre Dry	4.46	83
140	Hydrofil +PEG + Pre Dry	4.18	89
150	Free + PEG + Pre Dry	6.8	98

REFERENCES

1. Nandi, S., DeFilippi, I. Bedwell, B., and Zemel, H., **Interim Report,** Immobilized Enzymes in Organic Media: Determinants of Water Dependence. June 1993.

2. Nandi, S., DeFilippi, I. Bedwell, B., and Zemel, H., **Progress Statement,** Immobilized Enzymes in Organic Media: Determinants of Water Dependence. August 1994.

3. Margolin, A. L., P. A. Fitzpatrick, P. L. Dubin, and A. M. Klibanov, "Chemoenzymatic Synthesis of Optically Active (Meth)acrylic Polymers", J. Am. Chem. Soc., ***113,*** pp. 4693-4694 (1991)

4. Dabulis, K. and A. M. Klibanov, "Dramatic Enhancement of Enzymatic Activity in Organic Solvents by Lyoprotectants", Biotechnol. Bioeng., ***41***, pp. 566-571 (1993)

FIGURE 1
Reproducibility

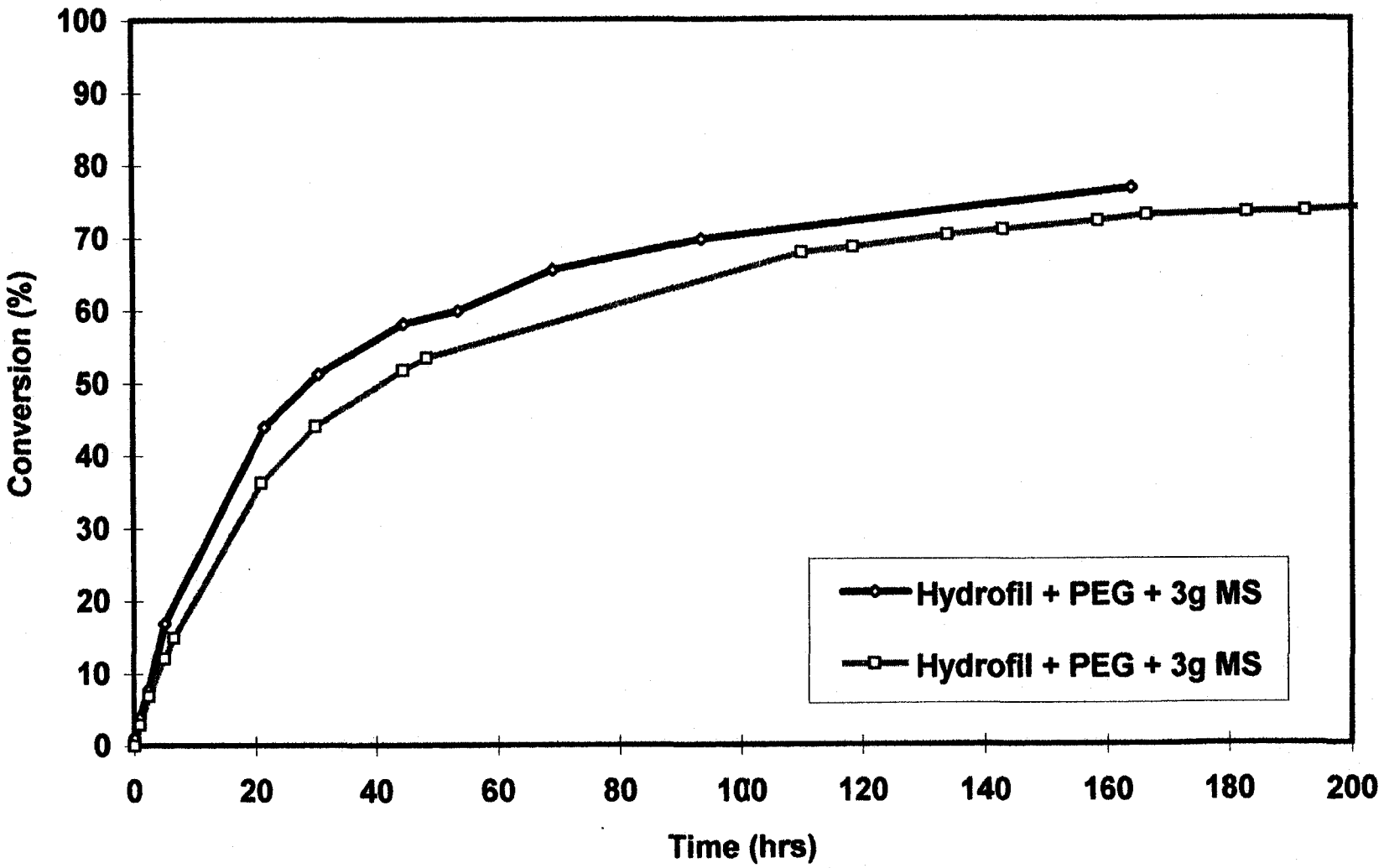


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FIGURE 2
Effect of Lyoprotection

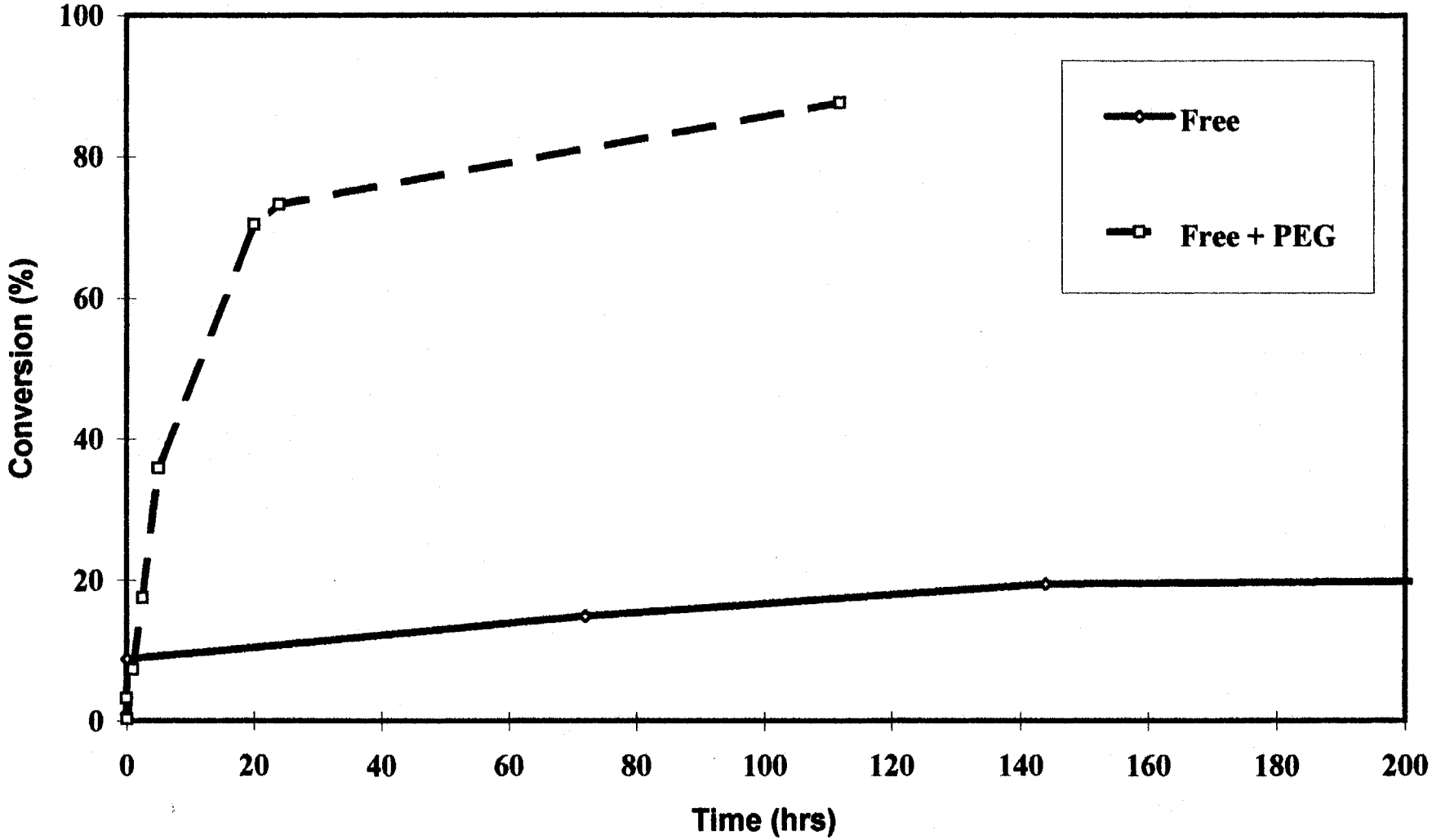

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FIGURE 3
Dependence on Water Concentration

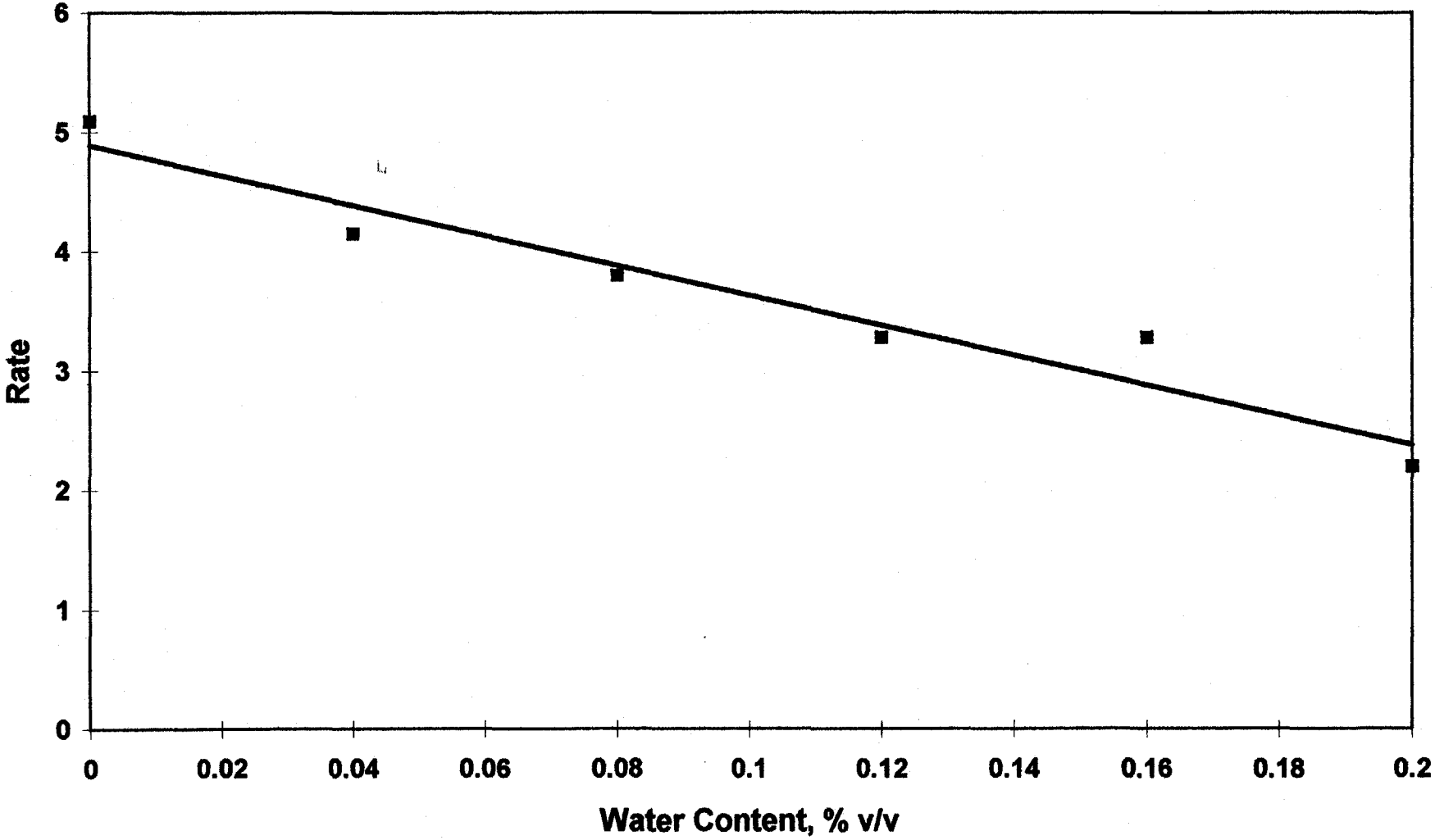

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FIGURE 4
Effect of Drying

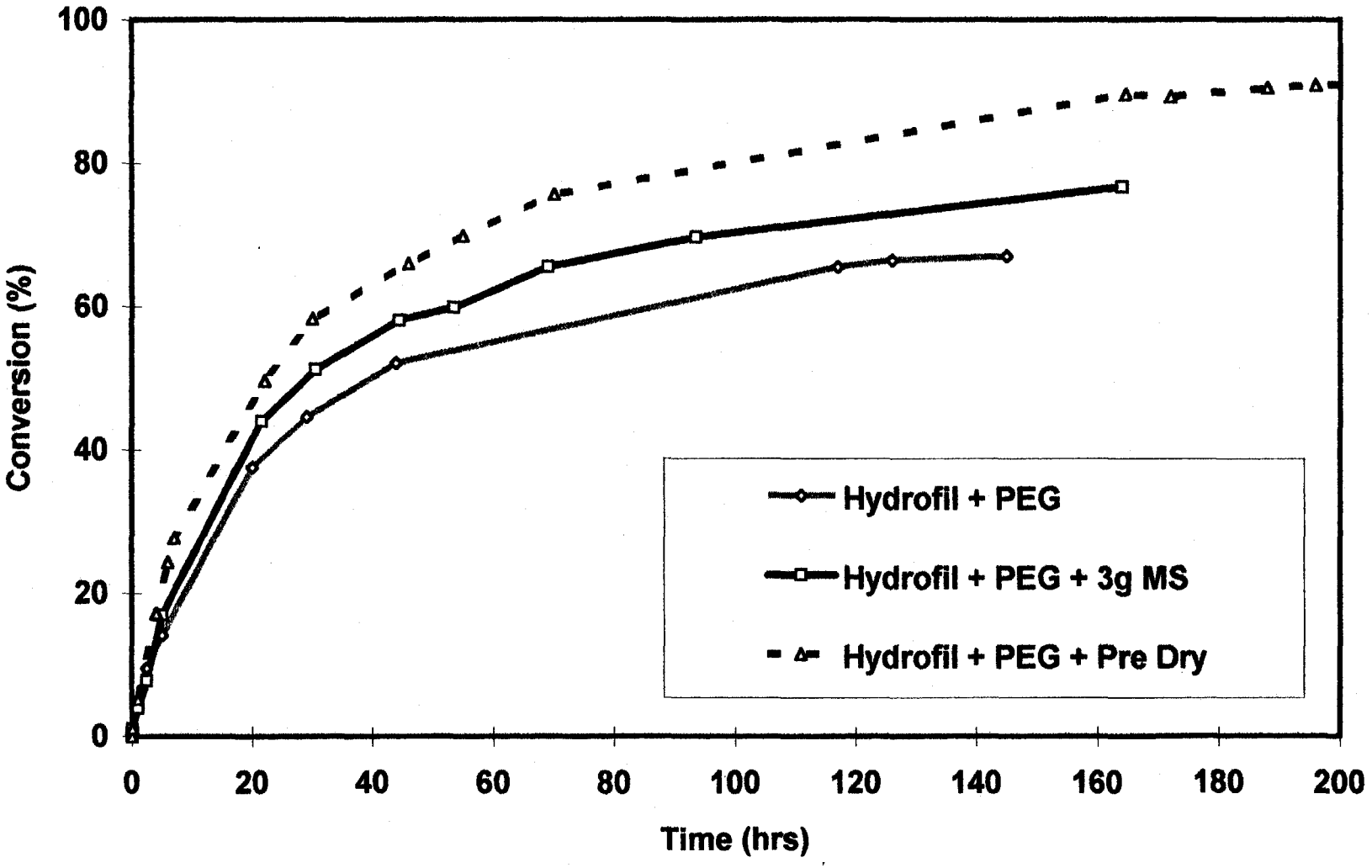

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FIGURE 5
Effect of Mixed Polarity Fabric on Subtilisin Activity

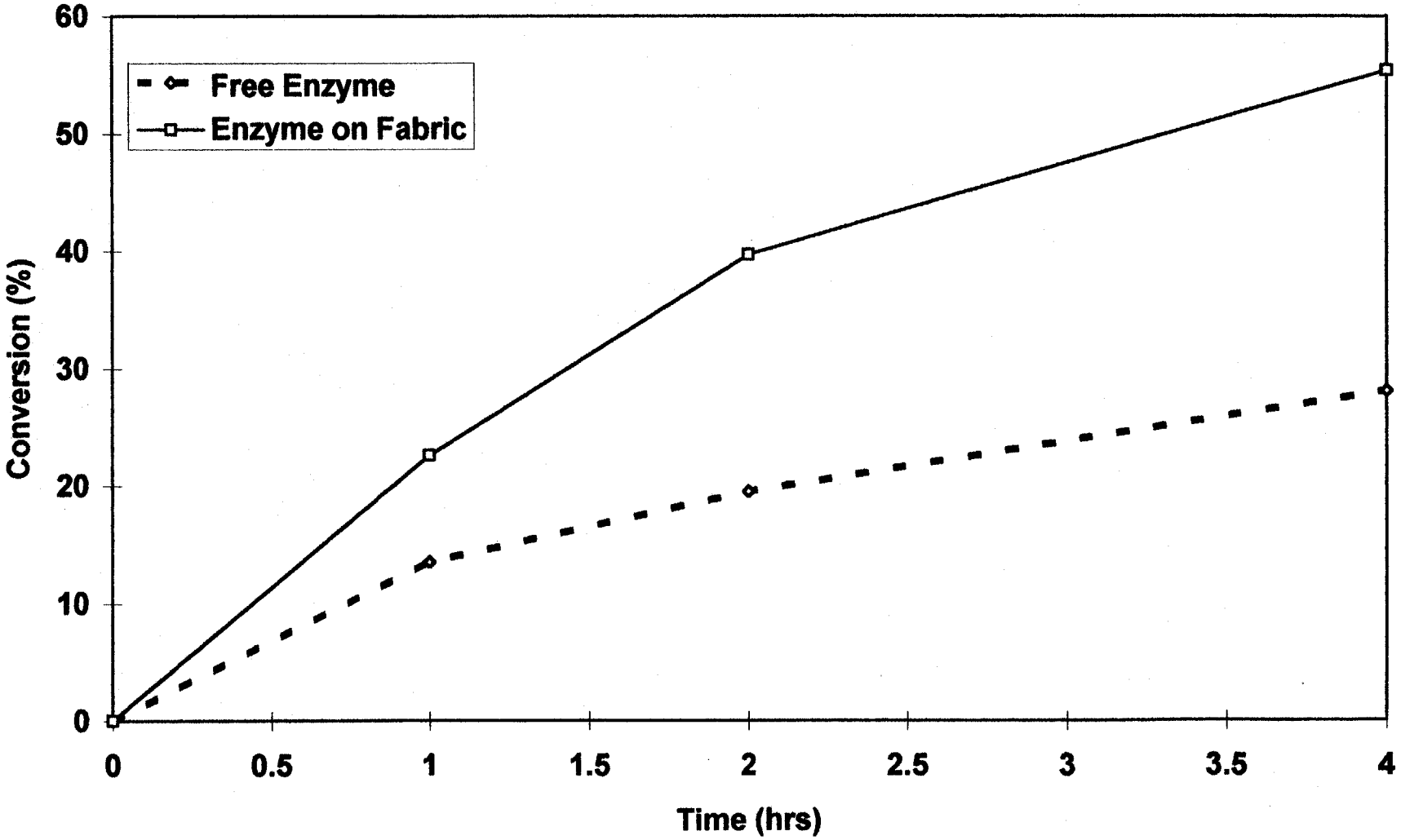

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FIGURE 6
Effect of Fiber Composition

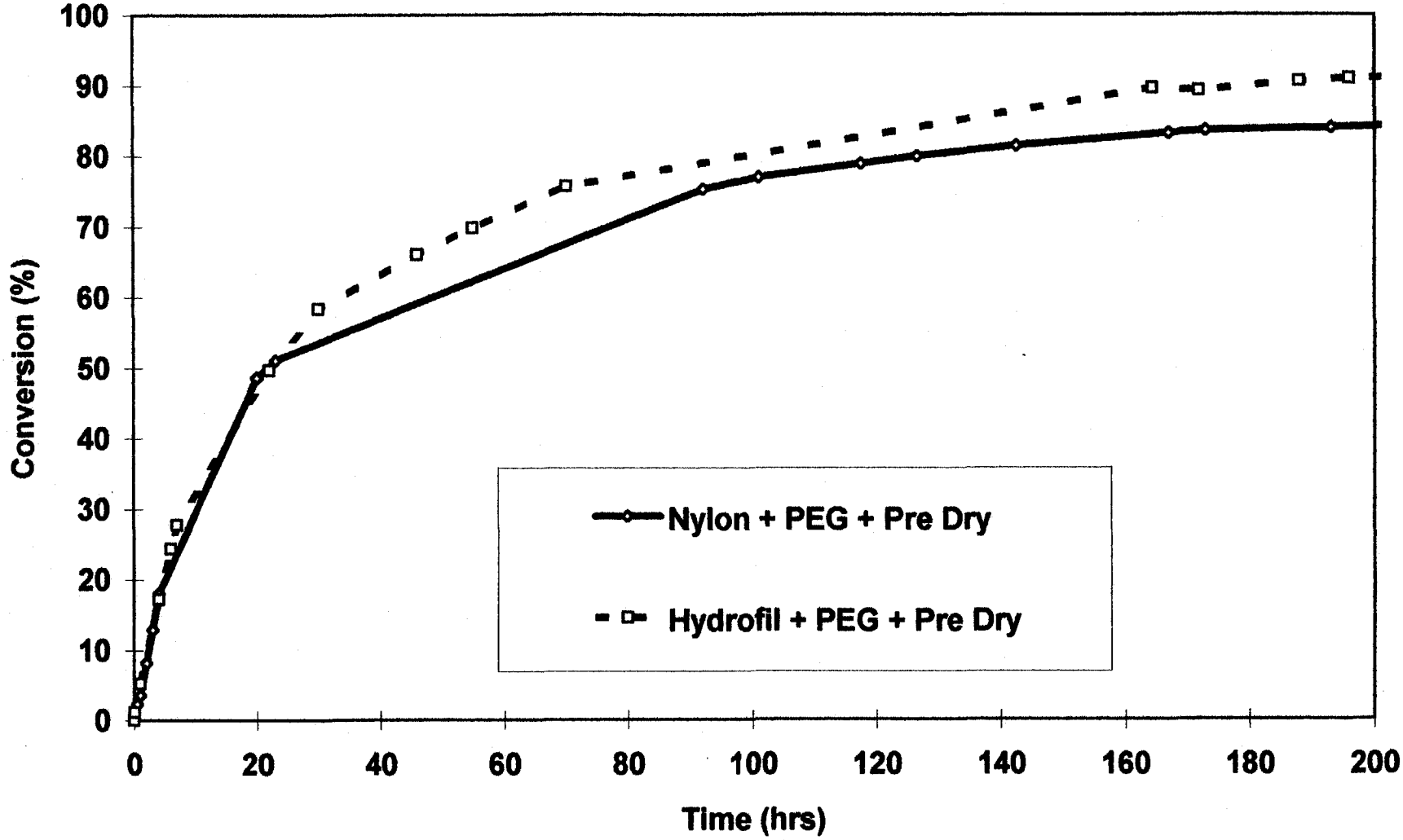

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FIGURE 7
Effect of Immobilization in Presence of MS

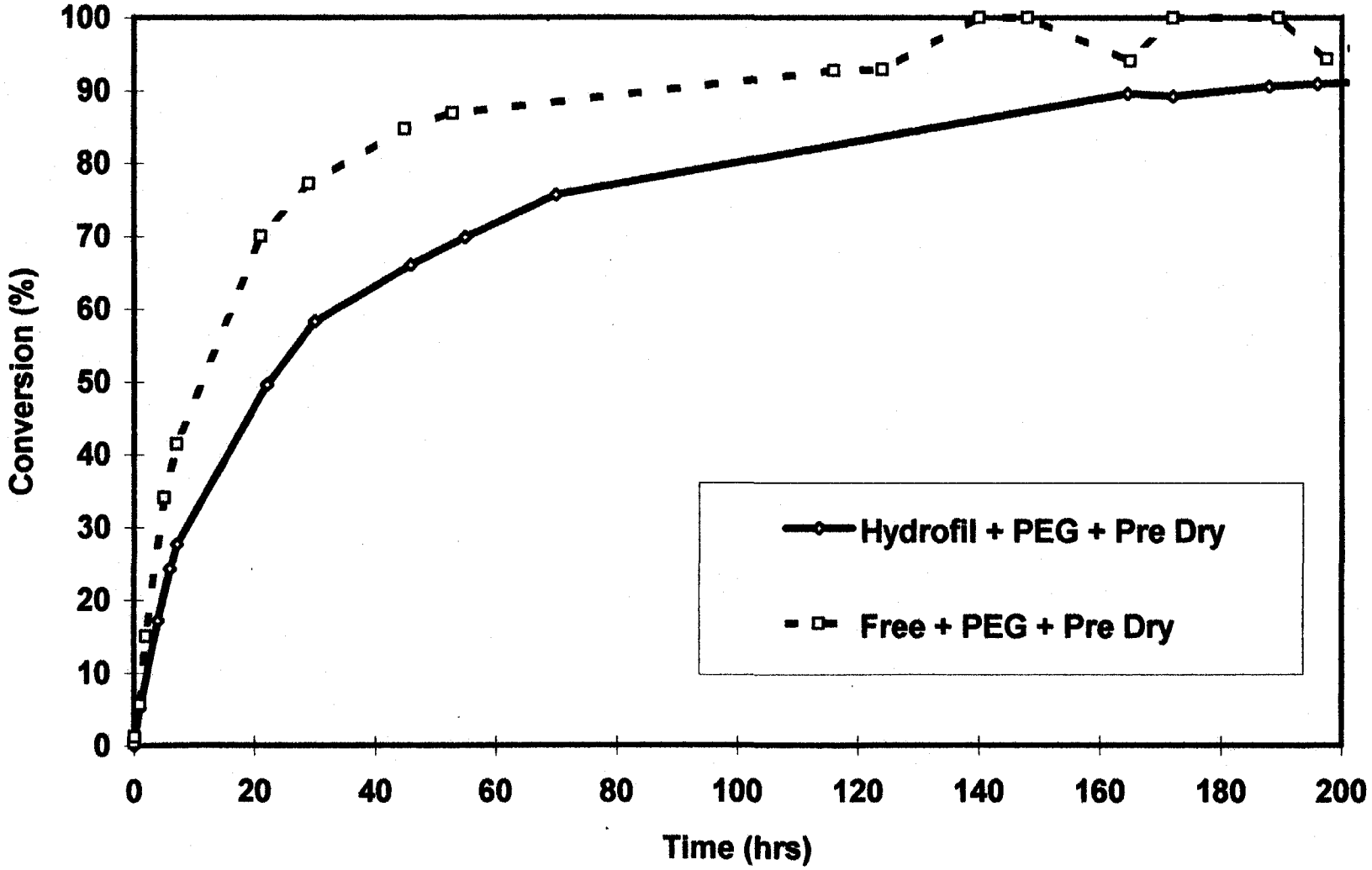

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FIGURE 8
Effect of Immobilization, in Absence of MS

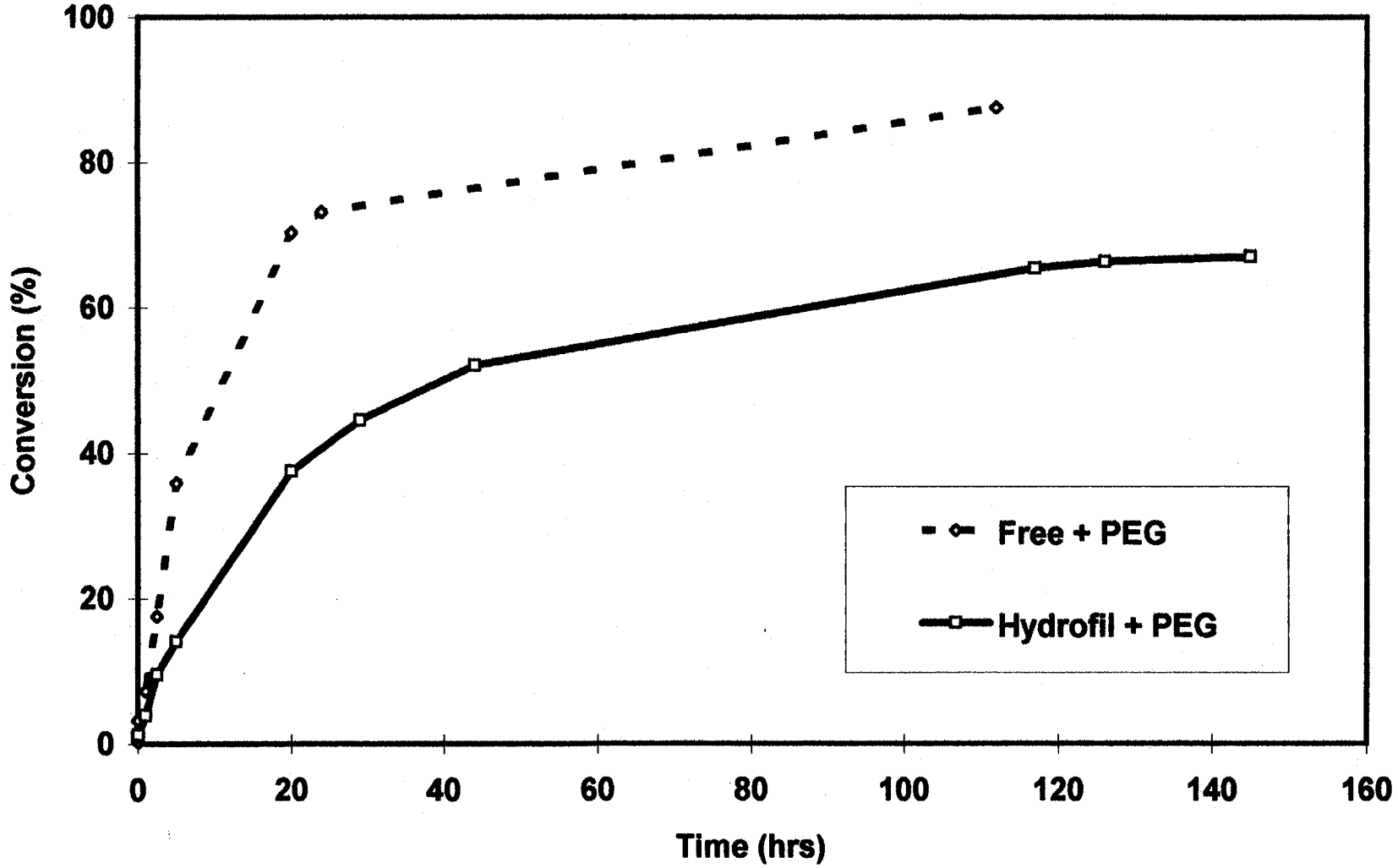

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FIGURE 9
Effect of Enzyme Recycling

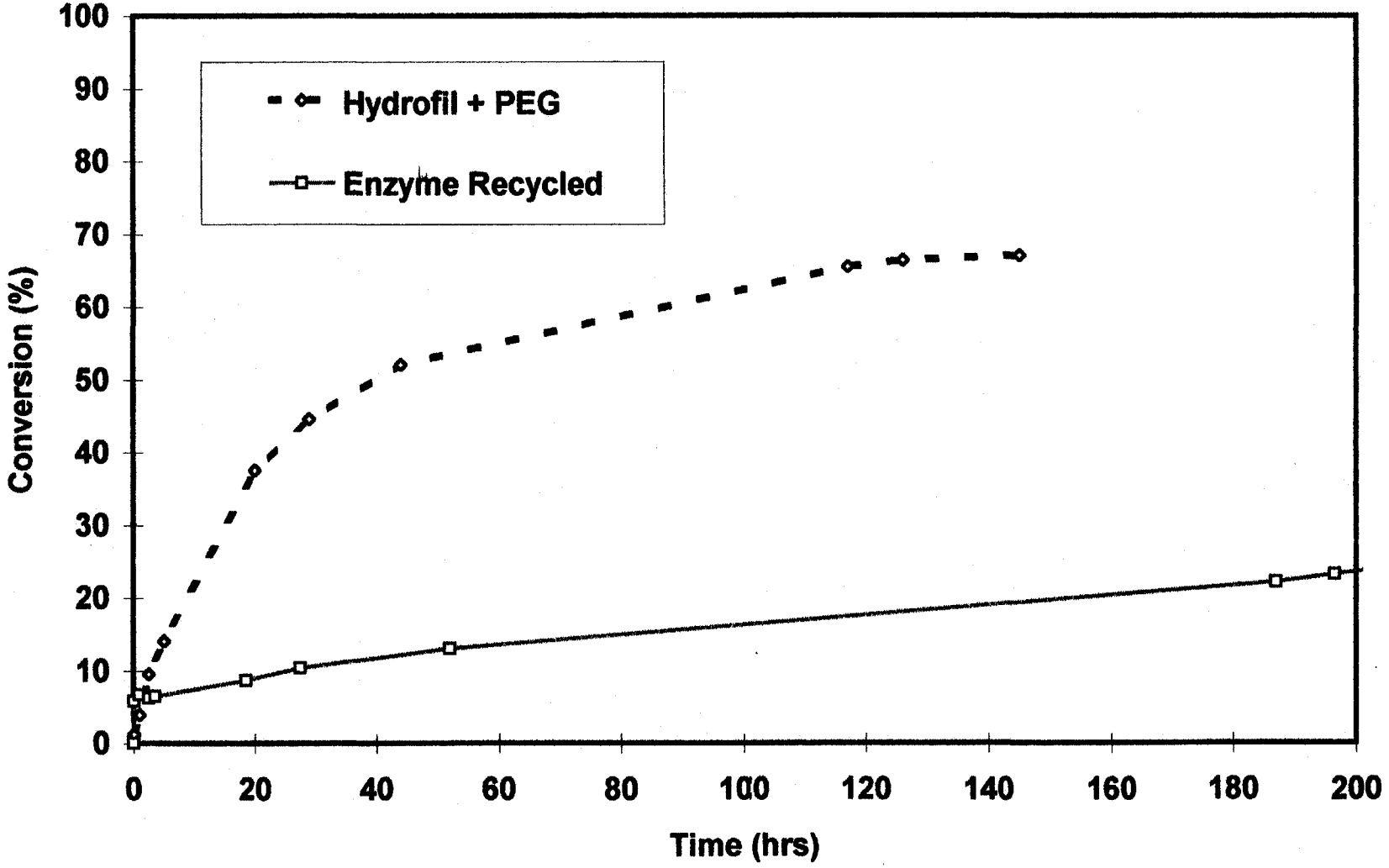

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FIGURE 10
Elution Profile of Reaction Mixture

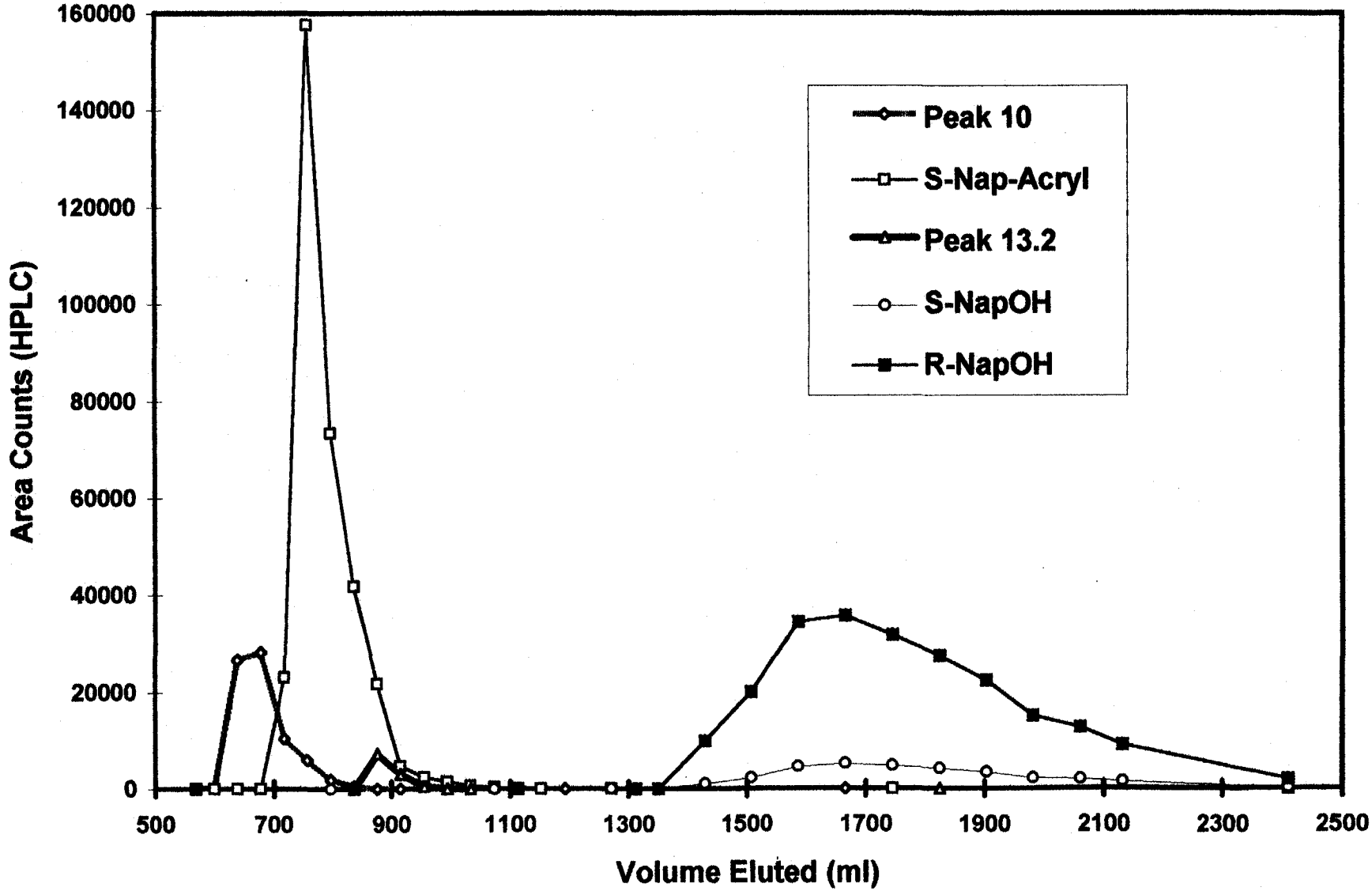

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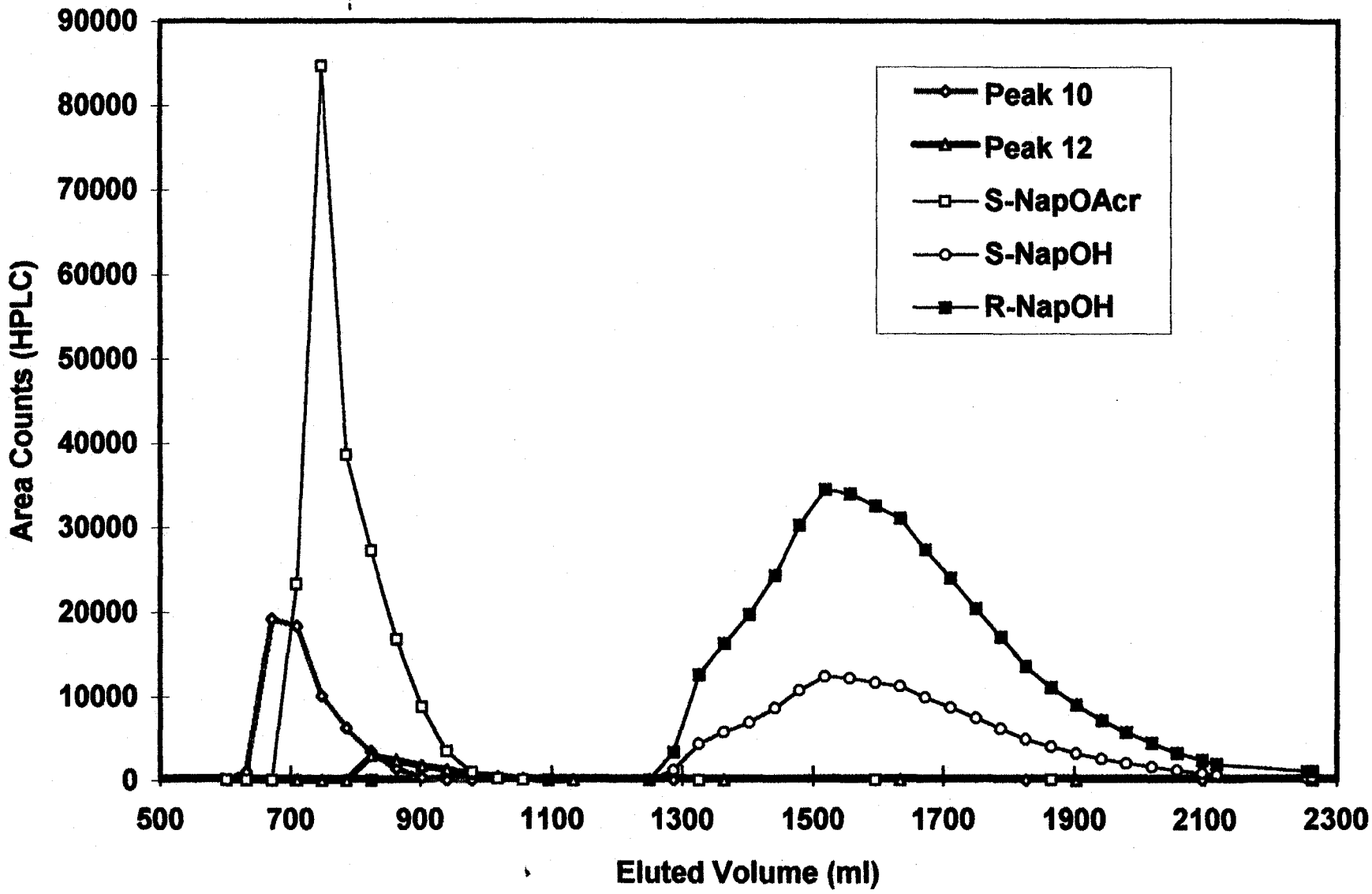
FIGURE 11
Elution Profile of Reaction Mixture



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