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# Selection of Stabilizing Additive for Lipase Immobilization on Controlled Pore Silica by Factorial Design

**Cleide M. F. Soares,[1,2] Heizir F. De Castro,*[,1] M. Helena A. Santana,[2] and Gisella M. Zanin[3]**

*[1]Departamento Engenharia Química, Faculdade de Engenharia Química de Lorena, PO Box 116, 12600-000, Lorena-SP, Brazil, E-mail: decastro@easygold.com.br; [2]Departamento de Biotecnologia, Faculdade de Engenharia Química, UNICAMP, PO Box 6066, 13081-970, Campinas-SP, Brazil; and [3]Departamento de Engenharia Química, Universidade Estadual de Maringá, 87020-900, Maringá-PR, Brazil*

## Abstract

*Candida rugosa* lipase was covalently immobilized on silanized controlled pore silica (CPS) previously activated with glutaraldehyde in the presence of several additives to improve the performance of the immobilized form in long-term operation. Proteins (albumin and lecithin) and organic molecules (β-cyclodextrin and polyethylene glycol [PEG]-1500) were added during the immobilization procedure, and their effects are reported and compared to the behavior of the immobilized biocatalyst in the absence (lacking) of additive. The selection of the most efficient additive at different lipase loadings (150–450 U/g of dry support) was performed by experimental design. Two $2^2$ full factorial designs with two repetitions at the center point were employed to evaluate the immobilization yield. A better stabilizing effect was found when small amounts of albumin or PEG-1500 were added simultaneously to the lipase onto the support. The catalytic activity had a maximum (193 U/mg) for lipase loading of 150 U/g of dry support using PEG-1500 as the stabilizing additive. This immobilized system was used to perform esterification reactions under repeated batch cycles (for the synthesis of butyl butyrate as a model). The half-life of the lipase immobilized on CPS in the presence of PEG-1500 was found to increase fivefold compared with the control (immobilized lipase on CPS without additive).

**Index Entries:** Controlled pore silica; immobilization; lipase; additive; factorial design.

*Author to whom all correspondence and reprint requests should be addressed.

## Introduction

Ester production by enzymatic reactions or biotransformations has stimulated the optimization of processes under nonaqueous media using several enzymes *(1)*. In biotransformations, lipases stand out for carrying out esterification reactions with extreme process simplicity, superior quality of the final product, and high yields *(2)*.

Lipase-catalyzed esterifications can be performed either with solid enzyme powder added directly to the solvent or with immobilized enzyme. The use of lipase immobilized on solid support is usually not necessary, because enzymes generally are insoluble in organic solvents. However, immobilization may protect the enzyme, to some extent, from solvent denaturation. It also helps in maintaining homogeneity of enzymes in the reaction media because it avoids aggregation of enzyme particles. In addition, this technique offers beneficial effect in the stability, as a function of the physicochemical interactions between support and enzyme *(2,3)*.

Several studies have been conducted to establish methodologies for immobilizing lipases on different supports *(3–5)*. Published results suggest that lipases are better immobilized on hydrophobic carriers owing to their peculiar physicochemical character *(4)*. This property and the good characteristics demonstrated by silica-based carriers provided the basis of a choice for controlled pore silica (CPS) as immobilizing support for microbial lipase in previous studies developed by our group *(6)*. In accordance with this work, *Candida rugosa* lipase was immobilized with high activity on silanized CPS activated with glutaraldehyde. Although this procedure has not considerably affected the catalytic properties of this immobilized system, decreased stability was noticed in the synthesis of butyl butyrate under repeated batches (half-life of 36 h) possibly owing to the lipase denaturation or desorption.

To overcome this limitation, several strategies have been reported. Among them, promising results have been achieved by the addition of stabilizing agents that protect the enzyme during the immobilization step *(7–11)*. In the specific case of the lipases, which demand an interface for total catalytic activity, the use of macromolecular additives such as proteins, polyethylene glycol (PEG), and polyvinyl alcohol *(7–9)* have demonstrated stabilizing effects in the activity of the enzyme, avoiding changes in protein structure. On the other hand, the use of low molecular weight compounds such as monomeric carbohydrates (sorbitol, arabitol) and polysaccharides (dextran, starch) had no effect *(8)*. Sometimes the role of these additives is masked by inert impurities included in commercial preparations (9).

The effect of additives on the activity of lipase preparation is not yet well understood. Probably they act through a combination of various effects including enzyme protection from inactivation during the immobilization step, retention of a water layer around the catalyst, and dispersing effects of the enzyme molecules that facilitate mass transport when additives are used together with immobilizing matrices. The kind of additive, its concen-

tration, and the contact time are critical parameters that have to be optimized in each case *(7–11)*.

In the present study, the scope of this technique was explored and the influence of several additives on the activity of CPS-immobilized lipase was studied by employing statistical concepts *(12)*. In particular, emphasis was given to the selection of the most effective additive to improve the performance of the immobilized lipase-CPS in long-term operation. Based on data in the literature *(8–10)*, proteins (albumin and lecithin) and organic molecules (β-cyclodextrin and PEG) were tested as additives. Although polysaccharides are considered to have little effect on lipase activity, β-cyclodextrin was also tested as an additive to CPS derivatives because of its important properties of stabilization against chemicals, heat, light, and oxidation when used to encapsulate guest molecules *(13)*.

The immobilized systems on CPS in the presence of the additive were used in both hydrolysis of olive oil and synthesis of the butyl butyrate. Data were compared with those attained by CPS-immobilized lipase lacking additive under the same operational conditions *(6)*.

## Materials and Methods

### *Materials*

Commercial *C. rugosa* lipase (type VII) was purchased from Sigma (St. Louis, MO). The lipase was a crude preparation with a nominal specific activity of 1440 U/mg of protein based on the Bradford *(14)* method. CPS was supplied by Corning Glass Works, with the following characteristics: average particle porosity (ε) of 0.566, particle matrix density ($\rho s$) of 2.178 g/cm$^3$, particle density (dry) ($\rho p$) of 0.948 g/cm$^3$, particle size of 37.5 nm containing pores of 375 Å *(6)*. The silane γ-aminopropyltriethoxysilane (γ-APTS) and glutaraldehyde (25% solution) were from Sigma. Bovine serum albumin (BSA) (Sigma), soy lecithin (Sinthy), PEG-1500 (Sinthy), and β-cyclodextrin (Sumitomo, S.A.) were used as stabilizing agents. Olive oil (low acidity) was purchased at a local market. Substrates for esterification reactions (*n*-butanol and butyric acid) were from Merck and were dehydrated with 0.32-cm molecular sieves (aluminum sodium silicate, type 13 X, BHD Chemicals, Toronto, Canada). Solvents were standard laboratory grade and other reagents were purchased either from Aldrich (Milwaukee, WI) or Sigma.

### *Immobilization of Lipase on CPS*

Lipase was immobilized by being covalently bound on CPS previously treated with γ-APTS, followed by the reaction of the pretreated beads with glutaraldehyde solution, according to the procedure previously described *(6)*. Suitable amounts of enzyme (0.1–0.3 g) were dissolved in 10 mL of distilled water and mixed with the support (1 g, dry wt) under low stirring for 2 h at room temperature. Proteins (albumin and lecithin) and

organic molecules (β-cyclodextrin or PEG-1500) were added together with the enzyme solution at a fixed amount (5 mg/g of support, 200 µL of aqueous solution containing 50 mg of additive/mL). Next, 10 mL of hexane was added, and the mixture of enzyme, support, and additive was incubated overnight at 4°C. The immobilized lipase was filtered (Whatman filter paper 41) and thoroughly rinsed with hexane. Analyses of hydrolytic activities carried out on the lipase loading solution and immobilized preparations were used to determine the coupling yield (η%) according to Eq. 1:

$$\eta\ (\%) = \frac{Uads}{U_0} \times 100 \tag{1}$$

in which *Uads* is the total activity recovered on the support and $U_0$ is the units offered for immobilization.

### Hydrolytic Activities

Hydrolytic activities of free and immobilized lipase were assayed by the olive oil emulsion method *(6)*. The substrate was prepared by mixing 50 mL of the olive oil with 50 mL of emulsification reagent. The reaction mixture consisting of 5 mL of the emulsion, 2 mL of 100 m*M* sodium phosphate buffer (pH 7.0), and either free (1 mL of lipase, 5 mg/mL) or immobilized (100–250 mg) lipase was incubated for 5 min at 37°C. The reaction was stopped by the addition of 10 mL of acetone-ethanol solution (1:1). The liberated fatty acid was titrated with 25 m*M* potassium hydroxide solution using phenolphthalein as an indicator. One unit of enzyme activity was defined as the amount of enzyme that produces 1 µmol of free fatty acid/min under the assay conditions.

### Protein Assay

Protein was determined according to Bradford's *(14)* method using BSA as a standard. The amount of bound protein was determined indirectly by the difference between the amount of protein introduced into the coupling reaction mixture and the amounts of protein in the filtrate and washing solutions.

### Esterification Reactions

Reaction systems consisted of heptane (10 mL), *n*-butanol (250 m*M*), butyric acid (250 m*M*), and immobilized lipase (0.75 g, dry wt). The mixture was incubated at 37°C for 24 h with continuous shaking at 150 rpm. The remaining butanol and the product formed were determined by gas chromatography using a 6-ft 5% DEGS on a Chromosorb WHP 80/10 mesh column (Hewlett Packard, Palo Alto, CA) and hexanol as the internal standard. Esterification activity was expressed as micromoles of butyl butyrate formed per minute per gram of dry support.

### *Operational Stability*

The operational stability was assayed by the immobilized lipase in successive batches performed under the same conditions as described for esterification reactions. Twenty-four hours after starting each batch, the immobilized lipase was removed from the reaction medium and rinsed with heptane, to extract any substrate or product eventually retained in the matrix. One hour later (length of time required for the solvent to evaporate), the immobilized derivative was introduced into a fresh medium.

### *Experimental Design and Statistical Analyses*

The selected variables were the additives (qualitative variable) and lipase loading (quantitative variable). Two $2^2$ full factorial designs with two replicates at the center point were employed. For qualitative variables, in case of protein additives, (+) represents the presence of albumin and (–) represents the presence of lecithin, while in the case of the organic additives (–) represents the presence of PEG PW-1500 and (+) represents the presence of β-cyclodextrin. For the quantitative variable, (+) is the high level (450 U/g of support) and (–) is the low level (150 U/g of support). Four experiments were carried out at the center point level, coded as 0, for estimation of experimental error. The immobilization yield was used as response. Analysis of the results was based on data generated by the software Statistica (version 5.0).

## Results and Discussion

### *Effect of Additive and Lipase Loading on Immobilization Yield*

In the first factorial design, proteins (albumin and lecithin) were evaluated as qualitative factors. The design of this experiment is given in Table 1, together with the experimental results. Both additive ($X_1$) and lipase loading ($X_2$) affected the coupling yield. When lecithin was used as additive and lipase loading increased from the low level (150 U/g of support) to the high level (450 U/g of support) (runs 1 and 3), the coupling yield decreased from 18.0 to 10.8%. The replacement of lecithin for albumin (runs 2 and 4) promoted an increase in the yield and that effect was more pronounced at the high level than at the low level (32.2 vs 23.6%).

The statistical analysis for the response evaluated is summarized in Table 2. According to the student's *t*-test values, the additive ($X_1$) and the interaction between additives and lipase loading ($X_1X_2$) showed a significant effect at 95% confidence level whereas the lipase loading ($X_2$) did not present a significant influence at the same confidence level. In the experimental range studied, the best stabilizing effect was observed when albumin was used as the additive. Regardless of the lipase loading, runs using lecithin gave much lower coupling yields.

Based on the response evaluated, a mathematical model was developed. Table 3 gives the analysis of variance (ANOVA) for the model used

Table 1
Experimental Design and Coupling Yields
According to First $2^2$ Full Factorial Design

| Run no. | $X_1$ | $X_2$ | Additive | Lipase loading (U/g) | Coupling yield (%) |
|---|---|---|---|---|---|
| 1 | –1 | –1 | Lecithin | 150 | 18.0 |
| 2 | +1 | –1 | Albumin | 150 | 23.6 |
| 3 | –1 | +1 | Lecithin | 450 | 10.8 |
| 4 | +1 | +1 | Albumin | 450 | 32.2 |
| 5 | –1 | 0 | Lecithin | 300 | 12.5 |
| 6 | +1 | 0 | Albumin | 300 | 26.9 |
| 7 | –1 | 0 | Lecithin | 300 | 18.2 |
| 8 | +1 | 0 | Albumin | 300 | 24.8 |

Table 2
Estimated Effects, Standard Errors, and Student's *t*-Test for Coupling Yield
According to $2^2$ Full Factorial Design

| Source | Effect | Standard error | *t* Value |
|---|---|---|---|
| Mean | 20.88 | ±0.85 | 24.56[a] |
| $X_1$ (additive) | 12.01 | ±1.71 | 7.02[a] |
| $X_2$ (lipase loading) | 0.64 | ±2.42 | 0.26 |
| $X_1X_2$ | 7.91 | ±2.42 | 3.27[a] |

[a] $p < 0.05$.

Table 3
ANOVA for Model Regression Representing Coupling Yields
for Lipase on CPS in Presence of Proteins
According to $2^2$ Full Factorial Design[a]

| Source | Sum of square | Degrees of freedom | Mean square | F value | *p* Value |
|---|---|---|---|---|---|
| $X_1$ | 288.48 | 1 | 288.48 | 49.29 | 0.002 |
| $X_2$ | 0.42 | 1 | 0.42 | 0.07 | 0.806 |
| $X_1X_2$ | 62.49 | 1 | 62.49 | 10.68 | 0.031 |
| Total | 23.41 | 4 | 5.85 | — | — |

[a] $R^2 = 0.94$.

to estimate the coupling yield as a function of the experimental factors ($X_1$) and ($X_2$) and their interaction ($X_1X_2$). These data suggest that the coupling yield depends on the type of additive and the lipase loading. The existence of an interaction confirms published data in relationship to the stabilizing effects given by nonenzymatic protein, minimizing in this way the lipase denaturation during its fixation onto solid supports *(9)*.

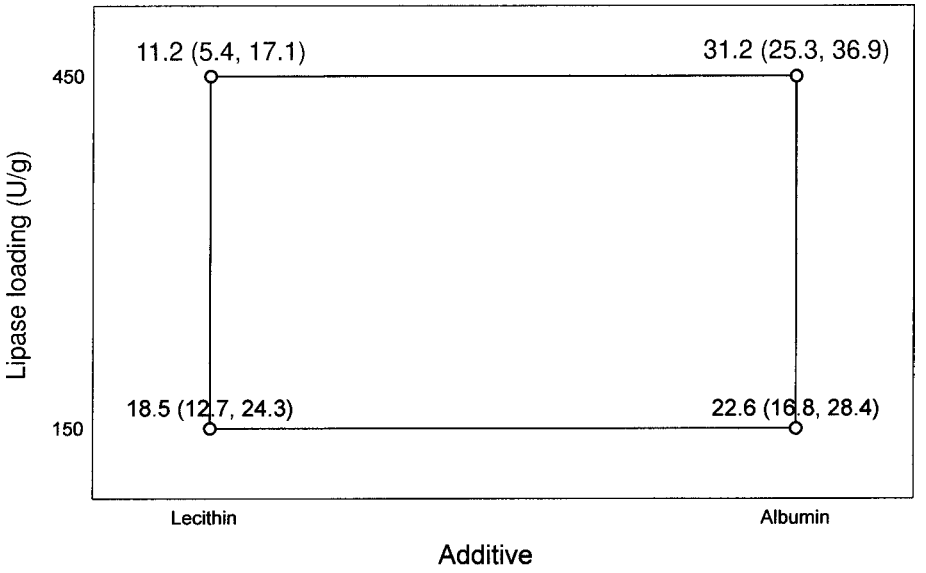


Fig. 1. Response predicted for lipase immobilization yields using proteins (albumin and lecithin) as additives.

The mathematical model representing the process in the experimental range studied (Eq. 2) was found to be appropriate, and the determination coefficient ($R^2$ = 0.94; Table 3) indicated that 94% of the variability in the coupling yield could be explained by this model.

$$\hat{y} = 20.87 + 6.0X_1 + 0.32X_2 + 3.95X_1X_2 \quad (2)$$

in which $\hat{y}$ is the value predicted for the coupling yield, and $X_1$ and $X_2$ are the coded values for additive and lipase loading, respectively.

Data obtained (Table 1) were compared with predicted values by the model (Fig. 1) and demonstrated that the model well represents the immobilization of lipase on CPS in the presence of protein as additives.

The response surface and the contour plot are shown in Fig. 2A and Fig. 2B, respectively. In agreement with the response surface, maximum coupling yield (31%) could be achieved working at high lipase loading (450 U/g of support) using albumin as an additive. The contour plot indicated the behavior for the variable response for future experiments, in which it can be statistically ensured that there is a need to increase the lipase loading if albumin is the additive of choice.

In the second experimental design, the influence of organic molecules (β-cyclodextrin or PEG-1500) on the coupling yield was studied. Table 4 indicates the experimental matrix together with the responses. The coupling yields varied from 7.8 to 59.5%, and the highest value was attained when a minimum level of lipase loading (95 U/g of support) was used in the presence of PEG-1500 (run 2). A decrease of 30% on the immobilization yield was attained at a lipase loading of 450 U/g of support (run 4), showing a negative effect of this additive for high lipase loadings. An inverse

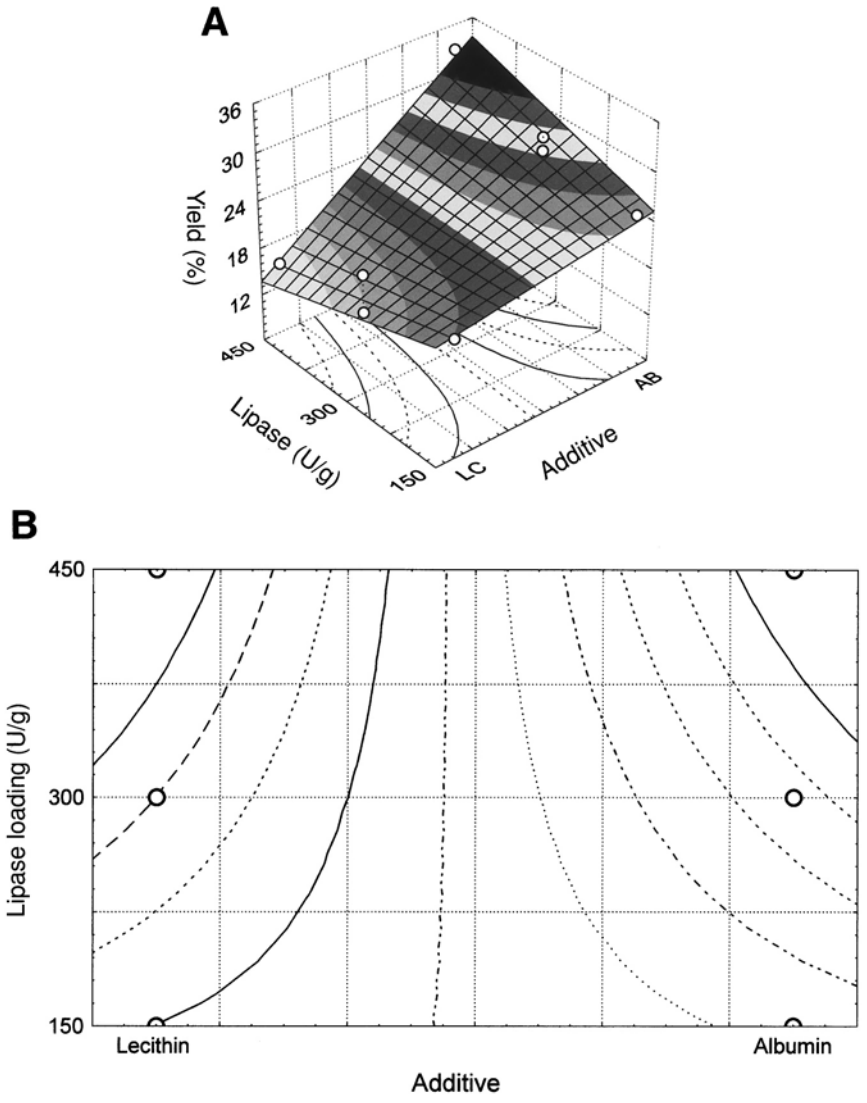


Fig. 2. **(A)** Surface response and **(B)** contour plot described by Eq. 2 using proteins (albumin [AB] and lecithin [LC]) as additives.

behavior was observed for β-cyclodextrin; the immobilization yield increased twice (7.8–14.3%) as the lipase loading increased from 95 to 285 U/g of dry support (runs 1 and 3).

Statistical analysis (Table 5) shows a significant effect for the variable additive ($X_1$) at a 95% confidence level, but not for lipase loading ($X_2$) and its interaction ($X_1X_2$).

The statistical model representing the immobilization coupling ($\hat{y}$) as a function of additive ($X_1$) and lipase loading ($X_2$) and their interaction ($X_1X_2$) can be expressed by Eq. 3:

$$\hat{y} = 26.36 + 14.95X_1 - 2.77X_2 - 6.02X_1X_2 \quad (3)$$

Table 4
Experimental Design and Immobilization Yields
According to Second $2^2$ Full Factorial Design

| Run no. | $X_1$ | $X_2$ | Additive | Lipase loading (U/g) | Coupling yield (%) |
|---|---|---|---|---|---|
| 1 | –1 | –1 | β-Cyclodextrin | 150 | 7.8 |
| 2 | +1 | –1 | PEG-1500 | 150 | 59.5 |
| 3 | –1 | +1 | β-Cyclodextrin | 450 | 14.3 |
| 4 | +1 | +1 | PEG-1500 | 450 | 41.9 |
| 5 | –1 | 0 | β-Cyclodextrin | 300 | 11.0 |
| 6 | +1 | 0 | PEG-1500 | 300 | 29.6 |
| 7 | –1 | 0 | β-Cyclodextrin | 300 | 12.6 |
| 8 | +1 | 0 | PEG-1500 | 300 | 34.2 |

Table 5
Estimated Effects, Standard Errors, and Student's *t*-Test
for Immobilization Yield According to $2^2$ Full Factorial Design

| Source | Effect | Standard error | *t* Value |
|---|---|---|---|
| Mean | 26.35 | ±3.38 | 7.79[a] |
| $X_1$ (additive) | 29.90 | ±6.76 | 4.42[a] |
| $X_2$ (lipase loading) | –5.53 | ±9.56 | –0.58 |
| $X_1X_2$ | –12.03 | ±9.56 | –1.25 |

[a]Significant at 95% confidence level ($t = 4.30$).

Table 6
ANOVA for Model Regression Representing Coupling Yields
for Lipase on CPS in Presence of Organic Molecules
According to $2^2$ Full Factorial Design[a]

| Source | Sum of square | Degrees of freedom | Mean square | F value | *p* Value |
|---|---|---|---|---|---|
| $X_1$ | 1788.32 | 1 | 1788.32 | 19.55 | 0.011 |
| $X_2$ | 30.63 | 1 | 30.63 | 0.33 | 0.59 |
| $X_1X_2$ | 144.84 | 1 | 144.84 | 1.58 | 0.27 |
| Total | 365.89 | 4 | 91.47 | — | — |

[a]$R^2 = 0.84$.

The validity of this model was verified by the ANOVA (Table 6) where it can be observed that the regression was statistically significant ($p < 0.05$) with a determination coefficient of $R^2 = 0.84$. The parameters of the model indicated that the additive was the variable that showed the highest influence on the response variable. This was confirmed by the Fisher's F test, showing a value >2.0, which gave global validity of the resulting equation. According to this equation, the value predicted for immobilization cou-

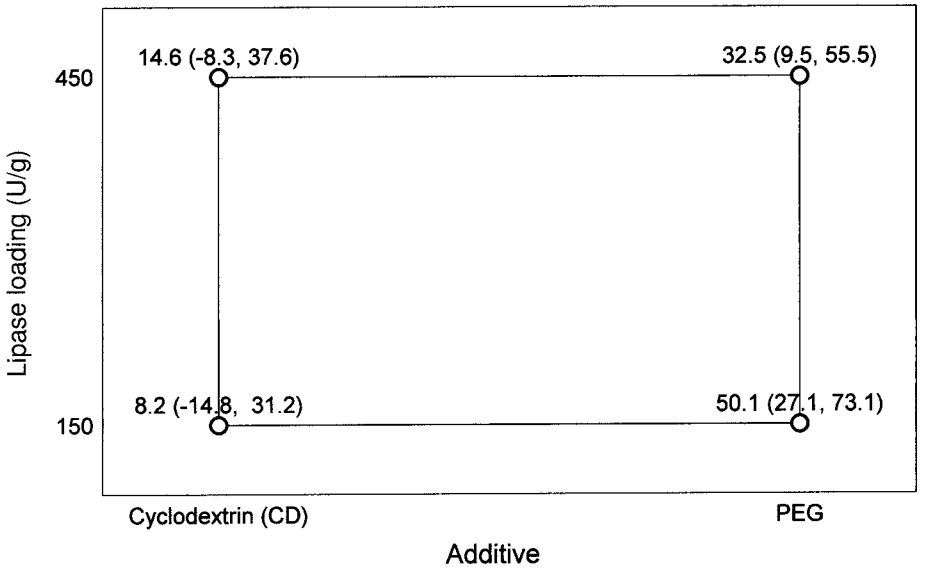


Fig. 3. Response predicted for lipase immobilization yields using organic molecules (β-cyclodextrin and PEG-1500) as additives.

pling was found to be 50% for lipase loading of 150 U/g of dry support in the presence of PEG-1500. The other predicted values for coupling yields according to the model proposed are shown in Fig. 3.

Figure 4 shows that it is interesting to work with β-cyclodextrin at a high level of lipase loading (450 U/g of support) while with PEG-1500 an inverse behavior was observed, a low level of lipase loading is sufficient to attain high immobilization yield.

Based on these results, a defined experimental behavior was observed, demonstrating in general terms that the use of PEG-1500 increases significantly the lipase immobilization yield on CPS. The contour plot justifies the need for performing an additional statistical design in order to optimize the conditions for lipase immobilization on CPS in the presence of PEG-1500. Further investigation of this optimization is under progress.

### *Comparison of Performance of Lipase Immobilized on Silica With and Without Additives in Hydrolysis Reactions*

To allow a better evaluation of all tested additives, immobilization runs (controls) at lipase loading varying from 150 to 450 U/g of dry support without additives were also carried out. Figure 5 shows the hydrolytic activities for resulting derivatives (controls) together with the immobilization preparations with additives. Among the tested additives, lecithin was less effective than albumin, giving an immobilized lipase preparation with hydrolytic activity similar to that obtained without additive (controls).

β-Cyclodextrin showed positive and negative effects, depending on the lipase loading. The positive effect was observed for high lipase loading

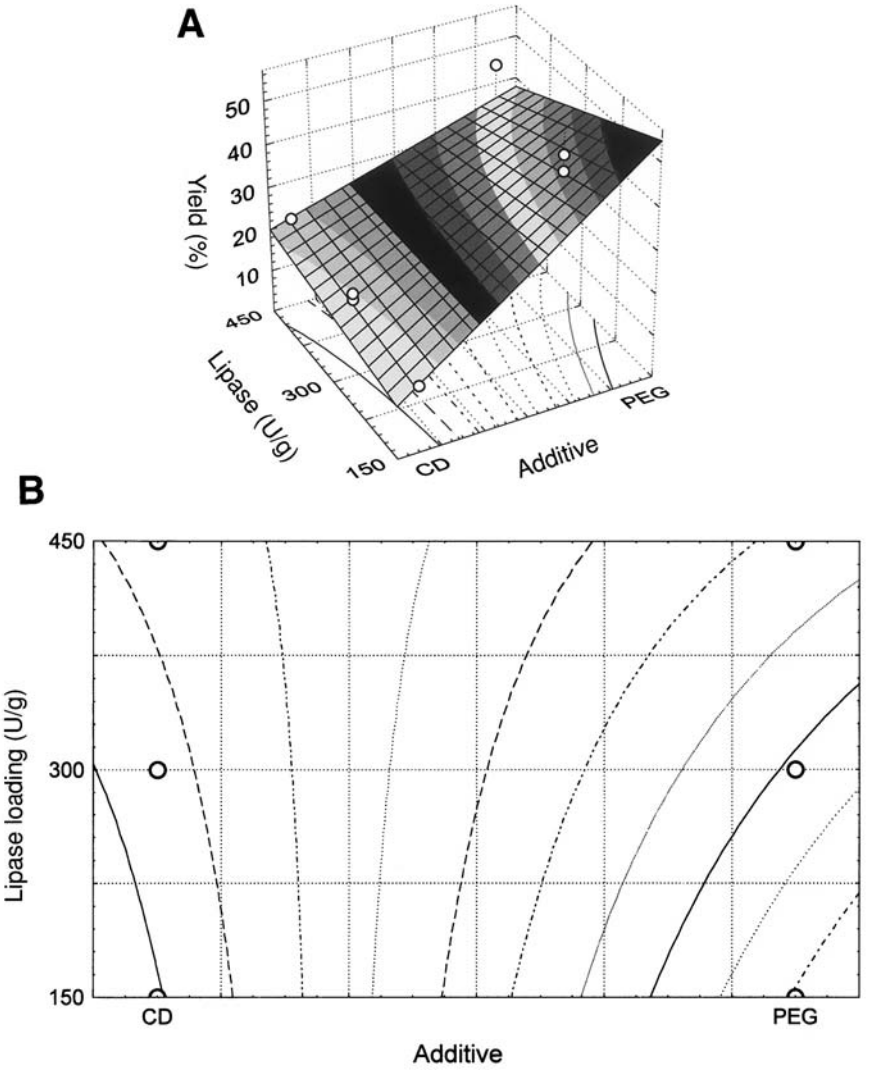


Fig. 4. **(A)** Surface response and **(B)** contour plot described by Eq. 3 using organic molecules (β-cyclodextrin [CD] and PEG-1500) as additives.

(450 U/g of support), and an activity of 70.8 U/mg of supports was obtained. For the other runs (low and medium levels), β-cyclodextrin provided immobilized derivatives with hydrolytic activities lower than those of the controls.

Albumin presented a positive effect for all lipase loadings, and a maximum value of 153.2 U/mg of dry support was obtained at a lipase loading of 450 U/g of support. The preparation of immobilized lipase that showed the highest hydrolytic activity (193 U/mg) was produced in the presence of PEG-1500, confirming the efficiency of this additive, as already reported by several researchers *(5,9)*. This result is quite effective when

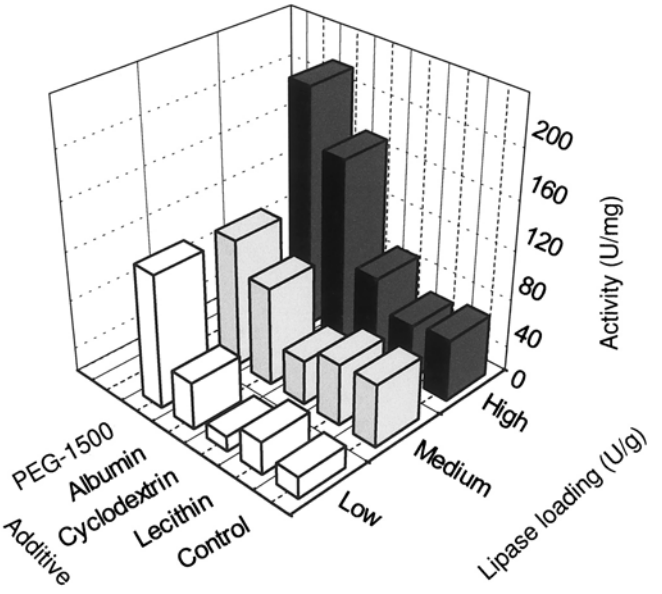


Fig. 5. Comparative hydrolytic activities for CPS immobilized lipase with and without additives for different lipase loadings.

compared to the control (50 U/mg), whose value was approximately four times lower than that obtained in the presence of PEG-1500.

### *Comparison of Performance of Lipase Immobilized on Silica With and Without Additives in Esterification Reactions*

In another set of experiments, immobilized lipases were prepared from CPS such that the lipase loading was kept constant at 300 U/g. The esterification activity was then measured as described. A plot of the data is shown in Fig. 6A–E. Although some additives were not able to increase hydrolytic activities, additive treatment of lipase immobilized on CPS improved the reaction rate and the ester yields in all cases.

With the control (CPS immobilized lipase without additive) the esterification activity was 161 μmol/(g·min) (Fig. 6E). However, when the immobilized lipase was prepared in the presence of lecithin or albumin, the esterification activities increased up to twofold (Fig. 6A,B). CPS lipase derivatives with β-cyclodextrin or PEG-1500 also had superior behavior to that of the control, by promoting an increase of 1.6- and 2.7-fold, respectively, on esterification activity. Therefore, all additives exerted a positive influence on the esterification activities by increasing up to 2.7-fold the activity attained by the control. This beneficial effect is probably owing to the dispersing effects of the enzyme molecules that facilitate mass transfer when additives are used together with the immobilizing matrices. It is also probable that the additives improved the esterification activity by better preserving the native structure of the enzyme in organic media. When the

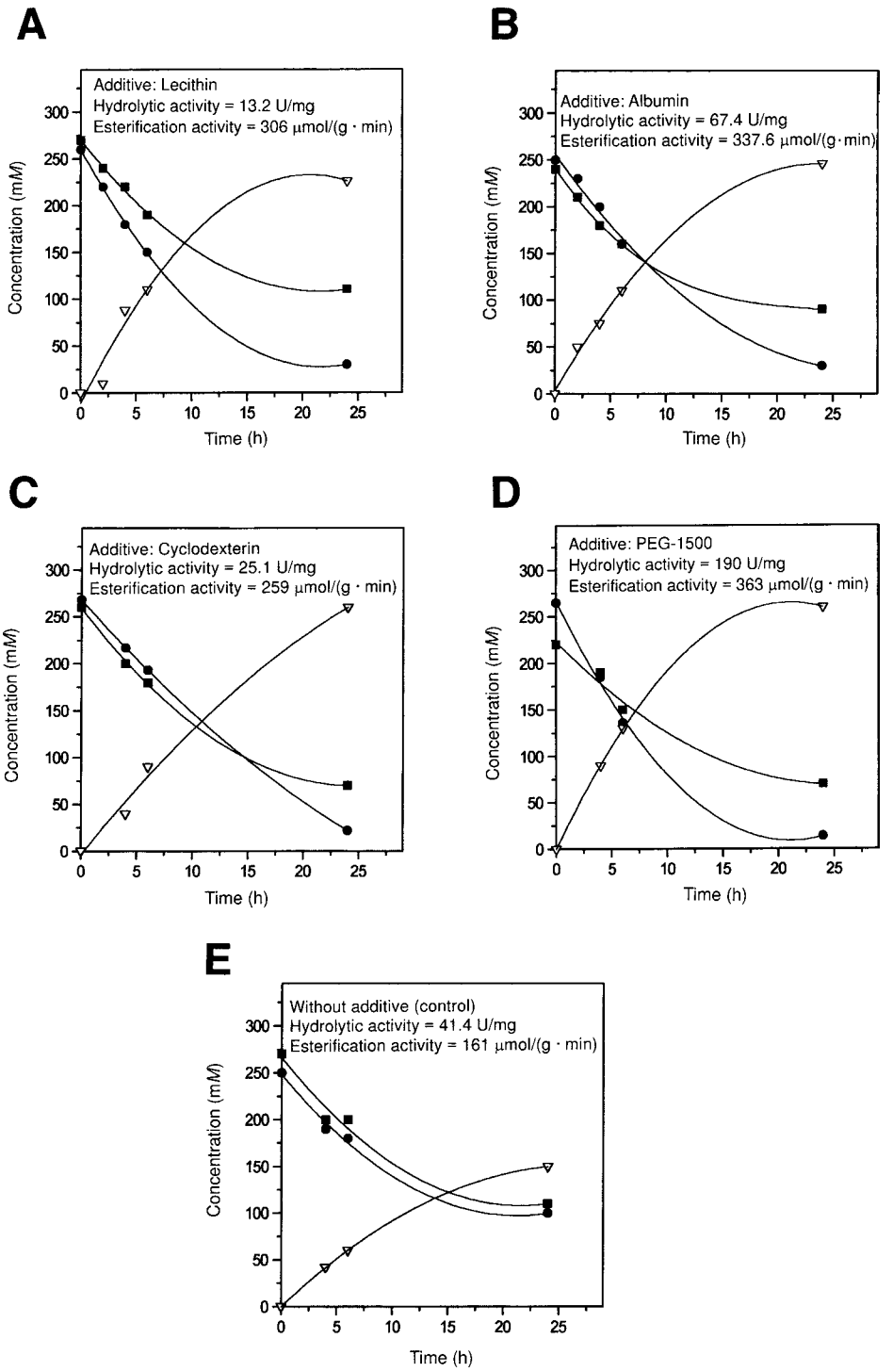


Fig. 6. Synthesis of butyl butyrate (▽) from butanol (●) and butyric acid (■) using lipase immobilized on CPS with additives **(A–D)** and without additive **(E)**. Procedure conditions are as described in Materials and Methods.

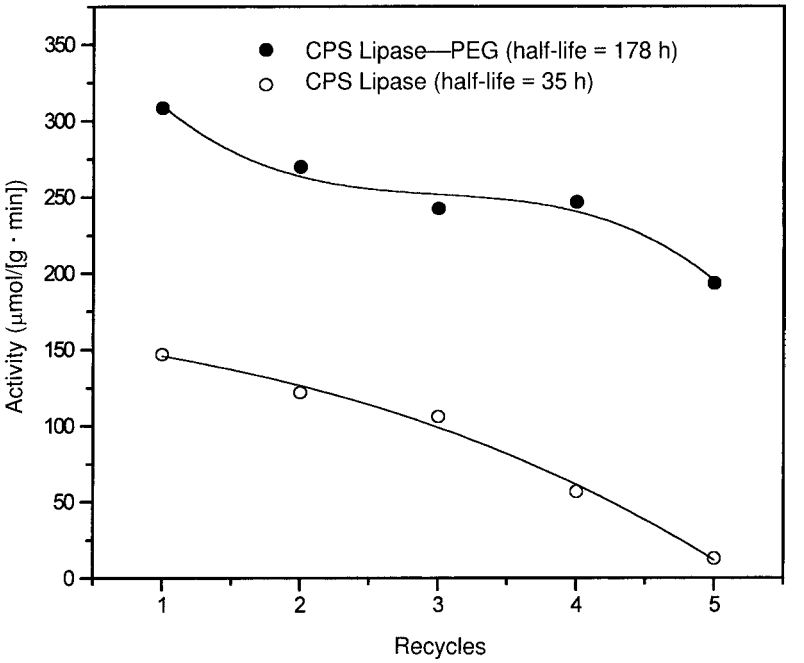


Fig. 7. Batch operational stability test of immobilized lipase derivatives. Esterification assay was carried out with substrate containing 250 m*M* butanol and 250 m*M* butyric acid in heptane. Initial esterification activities were 145 μmol/(g·min) for the lipase CPS (○) without PEG-1500 (control) and 310 μmol/(g·min) for the lipase-CPS with PEG-1500 (●).

water shell around the catalyst was maintained, a significant increase in the accessibility of active sites to the substrate was observed. Additionally, treatment of the immobilized derivatives also promoted a shift in the chemical equilibrium toward synthesis.

The best performance was obtained with PEG-1500 treatment (Fig. 6D). An ester yield of 56% could be obtained in 8 h using lipase immobilized on CPS with PEG-1500, whereas lipase-CPS lacking additive produced an ester yield of 50% in 15 h (Fig. 6E). These results might be explained by the fact that PEG is highly hygroscopic and water in the system might bind to the polyol. In this way, the nucleophilic attack by water was minimized, thus optimizing the butyl butyrate synthesis.

These results confirm data in the literature that indicate a considerable increase in catalytic activity of immobilized lipase by treating the support with additives *(5,9)*.

## *Recycle Potential*

The operational stability of the most active immobilized derivative (CPS-lipase with PEG-1500) was determined during successive batch reactions at 37°C for 24 h using the esterification of *n*-butanol with butyric acid as a model, following methodology previously described *(6)*. Average

values obtained in the operational stability runs are shown in Fig. 7 together with data obtained by the control.

While the lipase immobilized lacking PEG-1500 showed similar behavior to that previously attained *(6)* revealing a half-life of 35 h, the lipase immobilized on CPS with PEG-1500 gave a stable preparation and high esterification activity could be maintained for more than 120 h (five sequential batch reactions), which revealed a half-life of 178 h. As already emphasized, additives to the enzyme preparation are thought to affect stability primarily by affecting the distribution of water around the protein. The water content of the immobilized system has been found to be a decisive factor for the long-term stability of immobilized lipases, even for commercially available preparations, such as lipozyme *(15)*.

## Conclusion

The method for preparing the biocatalyst can influence the catalytic process. In agreement with previous studies *(6)*, *C. rugosa* lipase can be immobilized with high activity on silanized CPS activated with glutaraldehyde. The present work aimed at improving the performance of the immobilized form in long-term operation. Four additives were tested in the immobilization step in order to select the most active derivative for both hydrolysis and esterification reactions. The methodology of experimental design was used to select the most efficient additive considering the coupling yield as a response variable.

The effect of immobilization with the additives on the esterification reaction was exceptionally large, compared with the effects exhibited by the additives on hydrolysis. This enhancement could be attributed to distinct effects of additives. It appears that a certain change in the protein conformation took place when additive bound to the biocatalyst or the retention of the water shell around the catalyst gave additional stability for the enzyme. In addition, by controlling the water system, a shift in the thermodynamic equilibrium toward the esterification reactions also could be obtained.

Among the tested additives, the most promising result was obtained with PEG-1500, which produced preparations with high hydrolysis (198 U/mg) and esterification activities (363 μmol/[g·min]). The performance of this derived in both hydrolysis and esterification reactions was twice superior to that attained with the lipase immobilized on CPS without additive. The half-life (178 h) of the lipase immobilized on CPS with PEG-1500 was found to increase fivefold when compared with previously published results.

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