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Macroporous poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) resins—Versatile immobilization supports for biocatalysts

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ABSTRACT

Crosslinked macroporous hydrophilic poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate)s [abbreviated poly(GMA-*co*-EGDMA)] with identical chemical structure (60% of glycidyl methacrylate) but with varied average pore sizes (from 30 to 560 nm), specific surface areas (from 13.2 to 106.0 m^2/g), specific volumes (from 0.755 to 1.191 cm^3/g) and particle sizes (less than 100–650 μm) were synthesized via suspension polymerization. The influence of the resin properties on the loading of *Candida antarctica* lipase B (Cal-B) during immobilization and on the hydrolytic (hydrolysis of *para*-nitrophenyl acetate) and synthetic (ring-opening polymerization of ε-caprolactone) activity of the immobilized Cal-B were studied. Immobilization of Cal-B was performed at different temperatures and pH values. Cal-B immobilized at 30 °C and pH 6.8 was leading to increased activities. By decreasing the resin diameter: (i) the amount of Cal-B adsorbed onto the resin decreases, (ii) the conversion of *para*-nitrophenyl acetate increases (hydrolytic activity) and (iii) the conversion of ε-caprolactone and the molecular weight of the synthesized poly-ε-caprolactone increases (synthetic activity). Varying the porosity parameters results in different hydrolytic and synthetic activities. Pore sizes of all synthesized resins (from 30 to 560 nm) are big enough to overcome diffusion limitations. Therefore increasing the pore size of the resins resulted in a large increase in the hydrolytic and synthetic activity. Increasing the specific surface area resulted in an increase of activities, as the result of alleviated substrate approach to the immobilized enzyme zones. The obtained results were compared to results from dried Cal-B powder and Novozyme 435. Resin with particle size less than 100 μm and pore size 48 nm had much higher hydrolytic activity than both dried Cal-B powder and Novozyme 435. Nearly similar trends were observed for the synthetic activity.

Via the DMSO leaching technique we could show that about 80% of Cal-B was covalently attached to the macroporous resin.

1. Introduction

Enzymes have excellent features (activity, selectivity, specificity) for designing synthetic processes to obtain a wide range of products under mild and environmental friendly conditions [1,2]. However, enzymes have been optimized, via natural evolution, to fulfill their biological function: to catalyze reactions in complex metabolic pathways exposed to many levels of regulation. Therefore, natural enzymes seldom have the features adequate to be used as industrial catalysts in organic synthesis. Enzymes can denature due to solvent effects and mechanical shear. Recovery of enzymes from reaction solutions and separation of the enzymes from substrates and products are generally difficult. The productivity (space, time, yield) of enzymatic processes is often low due to substrate and/or product inhibition.

An important route to improving enzyme performance in non-natural environments is to immobilize them by either adsorption, covalent attachment or by incorporation in hydrophobic organic–inorganic hybrid materials with the help of a sol–gel process [3,4]. These immobilization procedures have resulted in remarkable improvements in performance: (i) increased enzyme activity (up to a factor of 100) in organic solvents; (ii) increased enantioselectivity; (iii) remarkable long-term stability; (iv) increased temperature stability and (v) convenient recovery by filtration or centrifugation [5–7]. Physical characteristics of matrix supports are crucial for enzyme immobilization and will influence enzyme loading as well as catalytic behavior. Therefore studies on enzyme immobilizations have to focus on (i) choosing the right

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matrix materials with properties that will improve enzyme activity and (ii) choosing the right immobilization conditions such as pH, temperature, initial enzyme loading, time of immobilization, etc.

Macroporous copolymers are used frequently for the preparation of various types of ion exchange resins, as inert component for some types of chromatography, as adsorbents, as support for classical catalysts or enzymes in biosynthesis and as membranes for different purposes [8–12]. A growing interest in copolymers of glycidyl methacrylate, GMA, and ethylene glycol dimethacrylate, EGDMA, can be observed, since the epoxy group of GMA can be easily transformed into a hydroxy, keto, carboxy, amino group, etc. With this, the copolymer characteristics can be easily adjusted to the desired properties. Applications of these copolymers are highly affected by their porosity. Crosslinked macroporous poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate)s [abbreviated poly(GMA-*co*-EGDMA)] can be synthesized in the shape of resin beads by suspension polymerization in presence of low-molecular weight inert component [13]. The inert component is usually a mixture of cyclohexanol and an aliphatic alcohol. The amount of crosslinking agent (EGDMA), the amount and composition of the inert component and the stirring speed have a high influence on the properties of the resins (size, porosity, etc.) and varying those parameters results in a copolymer with the same chemical composition but with different porosities and particle sizes.

It is scientifically and commercially important to move beyond general correlations to a better understanding on a molecular level of how immobilization on surfaces can stabilize and activate protein catalysts. Here we report on the immobilization of *Candida antarctica* lipase B (Cal-B) on poly(GMA-*co*-EGDMA) resins with various particle and pore sizes. Cal-B is a verstile enzyme for enantio- and regioselective transformations on many low molar mass and polymer substrates and has been found to possess a broad range of catalytic activities for chemical synthesis [14–18]. In fact Cal-B, immobilized onto a macroporous acrylic polymer resin (VP OC 1600, Bayer) is the most widely used lipase for specialty chemical manufacture. Commercial utilizations of Cal-B are limited to production of high-priced commercially available Cal-B preparations: Novozyme 435 (Novozymes A/S) and Chirazyme (Roche Molecular Biochemicals).

Optimization of immobilization conditions is crucial which was shown by Gross and co-workers by varying the initially concentration of Cal-B in the immobilization buffer [19]. Increasing the initially added protein per amount of acrylate based macroporous resin resulted in a large increase in the quantity of protein loaded. Further increase of Cal-B concentration in the immobilization buffer resulted in a relatively small increase in Cal-B loading. Further it was shown that prolonging the time of immobilization of Cal-B onto epoxy functionalized macroporous polyacrylic beads resulted in different fractions of physical and chemically bound enzymes [20].

In our laboratory, Cal-B immobilization was performed under different pH value. It was observed that Cal-B immobilized at pH 6.5 had three times higher hydrolytic activity than Cal-B immobilized at pH 11.5. Dumitriu and co-workers observed a similar trend by varying the pH value for immobilization of *Mucor javanicus* Lipase on SBA-15 Silica [21].

Particle size and porosity parameters of the matrix support are extremely important for enzyme activity. Gross and co-workers showed that the polymerization rate of ε-caprolactone catalyzed by immobilized Cal-B strongly depends on the matrix particle size [22,23].

It was shown by Gross and co-workers that Cal-B leaches out very easily into the reaction mixture from the commercial Novozyme 435 [19]. In terms of cost of the biocatalyst and of product safety this is not an ideal situation. Here we were able to show that Cal-B is to 80% covalently attached to the poly(GMA-*co*-EGDMA).

2. Experimental

2.1. Materials

Candida antarctica lipase B (Cal-B) in the form of a dried powder was purchased from BioCatalytics Co. (Grambach, Austria). ε-Caprolactone (ε-CL) was purchased from Sigma–Aldrich Chemical Co. and further dried above calcium hydride overnight and then distilled under vacuum.

Poly(*N*-vinyl pyrrolidone) was purchased from Fluka and all other chemicals were purchased from Sigma–Aldrich and were not purified further.

2.2. Methods

The pore size distributions were determined by mercury porosimetry (Carlo Erba 2000, software Milestone 200). The shape of the beads and the cross-section of the copolymer beads were observed using a scanning electron microscopy (JEOL TSM 6320F). The concentration of the reaction product *p*-nitrophenol (*p*NP) was determined by UV/vis (PYE UNICAM SP8-200 UV/vis spectrophotometer) at the λ_{max} (304 nm) of *p*NP. ^{1}H NMR measurements were performed on a Varian VXR-S 300 spectrometer in chloroform-*d*.

2.3. Preparation of carrier

The suspension polymerizations were performed in a typical reactor with a volume of 0.50 dm^3.

The monomer phase (80.5 g) containing the monomer mixture (20.7 g of GMA and 13.8 g of EGDMA), azobisisobytironitrile (AIBN) as an initiator (0.8 g) and 45.2 g of inert component (40.7 g of cyclohexanol and 4.5 g of tetradecanol or hexadecanol for samples SGE-10/14 and SGE-10/16, respectively, and 36.2 g of cyclohexanol and 9.0 g of tetradecanol for sample SGE-20/14) was suspended in the aqueous phase consisting of 240.0 g of water and 2.4 g of poly(*N*-vinyl pyrrolidone) (PVP). In the labels of copolymer samples, letter S designates suspension copolymerization, G and E stand for the monomers (GMA and EGDMA). The first number in a sample labels stands for the share of aliphatic alcohol in the inert component (w/w) and the second one for the number of C-atoms in the aliphatic alcohol.

The copolymerization was carried out at 70 °C for 2 h and then at 80 °C for 6 h with a stirring rate of 200 rpm. After completion of the reaction, the copolymer particles were washed with water and ethanol, kept in ethanol for 12 h and then dried in a vacuum oven at 45 °C for 24 h.

The particle size distribution was determined by sieve analysis. The particles with diameter in the range of 630–300, 300–150, 150–100 and less than 100 μm were used for further investigation.

The pore size distributions were determined by mercury porosimetry (Carlo Erba 2000, software Milestone 200). The samples were dried at 50 °C for 8 h and degassed at room temperature and a pressure of 0.5 Pa for 2 h. The values of specific pore volume, V_S, and pore diameter that corresponds to half of the pore volume, $d_{V/2}$, were read from cumulative pore distribution curves. The values of specific surface area, S_{Hg}, were calculated on the basis of cylindrical pore model as described in literature [24].

All poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) samples are highly cross-linked (40% of crosslinking) and

therefore swelling in water and organic solvents is insignificant (<1 wt%).

The shape of the beads and the cross-section of the copolymer beads were observed using a scanning electron microscopy (JEOL TSM 6320F).

2.4. Enzyme immobilization

The copolymer beads were added to a Cal-B solution (6.67 mg/ml) in PBS buffer pH 6.8. The ratio of copolymer to Cal-B was 4:1 in all experiments. The samples were incubated in a rotary shaker at 200 rpm at 30 °C. After 24 h the solution was removed by filtering and the resulting immobilized Cal-B was washed with PBS buffer and distilled water, until no protein was detectable any more in the washing solution. Supernatant and washing solutions were collected and using the bicinchoninic acid (BCA) protein assay, the amount of enzyme that is immobilized could be estimated. The resulting resins with immobilized Cal-B were freeze dried for 48 h and then used for hydrolytic and synthetic activity tests.

2.5. Hydrolytic activity

A 1,4-dioxane solution (5 ml) containing *p*-nitrophenyl acetate (*p*NPA) (40 mM) and methanol (80 mM) was added to 20 ml vials containing 0.772 mg of enzyme. The assay reactions were carried out for 50 min at 35 °C (300 rpm) and were terminated by removal of the enzyme by filtration. The concentration of the reaction product *p*-nitrophenol (*p*NP) was determined by UV/vis (PYE UNICAM SP8-200 UV/vis spectrophotometer) at the λ_{max} (304 nm) of *p*NP. Enzymes hydrolytic activities for free Cal-B powder, Cal-B immobilized on poly(GMA-*co*-EGDMA) and Novozyme 435 are defined herein as the nanomoles of *p*NPA hydrolyzed in 1,4-dioxane per unit of weight of enzyme per time (nmol of *p*NP/min mg).

2.6. Synthetic activity

The enzyme synthetic activities were determined for ε-caprolactone (ε-CL) ring-opening polymerizations. In 10 ml flasks containing ε-CL (2.06 g), 5.0 mg of enzyme was added. The reactions were performed at 50 °C for 5 h (300 rpm) under nitrogen. Enzyme synthetic activities are defined as monomer conversion (%) after 5 h. The monomer conversion was determined by ^{1}H NMR experiments (as well as the molecular weight of the product (poly-ε-caprolactone)). ^{1}H NMR measurements were performed on a Varian VXR-S 300 spectrometer in chloroform-*d*.

2.7. Determination of covalent attachment

Immobilized Cal-B (3.0 mg) was added in 1.5 ml of DMSO. The samples were incubated in a rotary shaker at 200 rpm at 30 °C. After 5 h, DMSO and the fraction of the enzyme molecules that are physically adsorbed on carrier were removed and the resulting covalently attached Cal-B was washed with PBS buffer and distilled water, until no protein was detectable any more in the washing solution. Supernatant and washing solutions were collected and using the bicinchoninic acid (BCA) protein assay, the fraction of Cal-B molecules that are covalently attached to the carrier could be estimated.

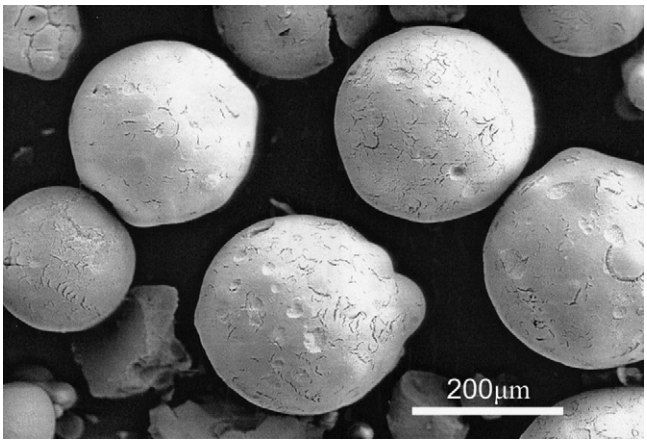

Fig. 1. SEM micrograph of SGE-20/14-d2 beads (resin 4).

3. Results and discussion

A series of poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) resins (see Table 1) with identical chemical structure (60% of glycidyl methacrylate) but with varied particle size (<100–630 μm), average pore diameters (30–560 nm), specific surface area (13.2–106.0 m^2/g) and specific volumes (0.755–1.191 cm^3/g) were studied to assess how these parameters effect Cal-B immobilization, hydrolytic activity (hydrolysis of *p*-nitrophenol acetate) and synthetic activity (polymerization of ε-caprolactone).

3.1. Porous structure

Macroporous poly(GMA-*co*-EGDMA) was synthesized in the shape of beads by suspension polymerization. By varying the content of the inert component and the stirring speed, different porosities and particle sizes were obtained (see Table 1). Figs. 1 and 2 illustrate the scanning electron microscopy (SEM) micrographs. Fig. 1 shows the spherical shape of poly(GMA-*co*-EGDMA) and Fig. 2 indicates the porous structure of some of the copolymer resins.

Table 1
Particle size and porosity parameters of poly(GMA-*co*-EGDMA): average pore diameter, specific surface area and specific volume

Resin	Sample name	Particle size (μm)	Average pore diameter[a] (nm)	Specific surface area[a] (m^2/g)	Specific volume[a] (cm^3/g)
1	SGE-10/16-d1	630–300	30 ± 1.2	82.0 ± 4.1	0.923 ± 0.05
2	SGE-10/16-d2	300–150	87 ± 3.5	36.0 ± 1.8	0.755 ± 0.04
3	SGE-20/14-d1	630–300	92 ± 4.6	36.0 ± 1.8	1.111 ± 0.06
4	SGE-20/14-d2	300–150	270 ± 13.5	27.6 ± 1.4	1.040 ± 0.05
5	SGE-20/14-d3	150–100	59 ± 3.0	46.7 ± 2.3	1.088 ± 0.05
6	SGE-20/14-d4	<100	48 ± 2.4	55.2 ± 2.8	1.100 ± 0.06
7	SGE-20/16-d1	630–300	30 ± 1.2	106.0 ± 5.3	1.191 ± 0.06
8	SGE-20/16-d2	300–150	560 ± 28.0	13.2 ± 0.7	1.125 ± 0.06

[a] Standard deviation values were calculated from three replicate experiments.

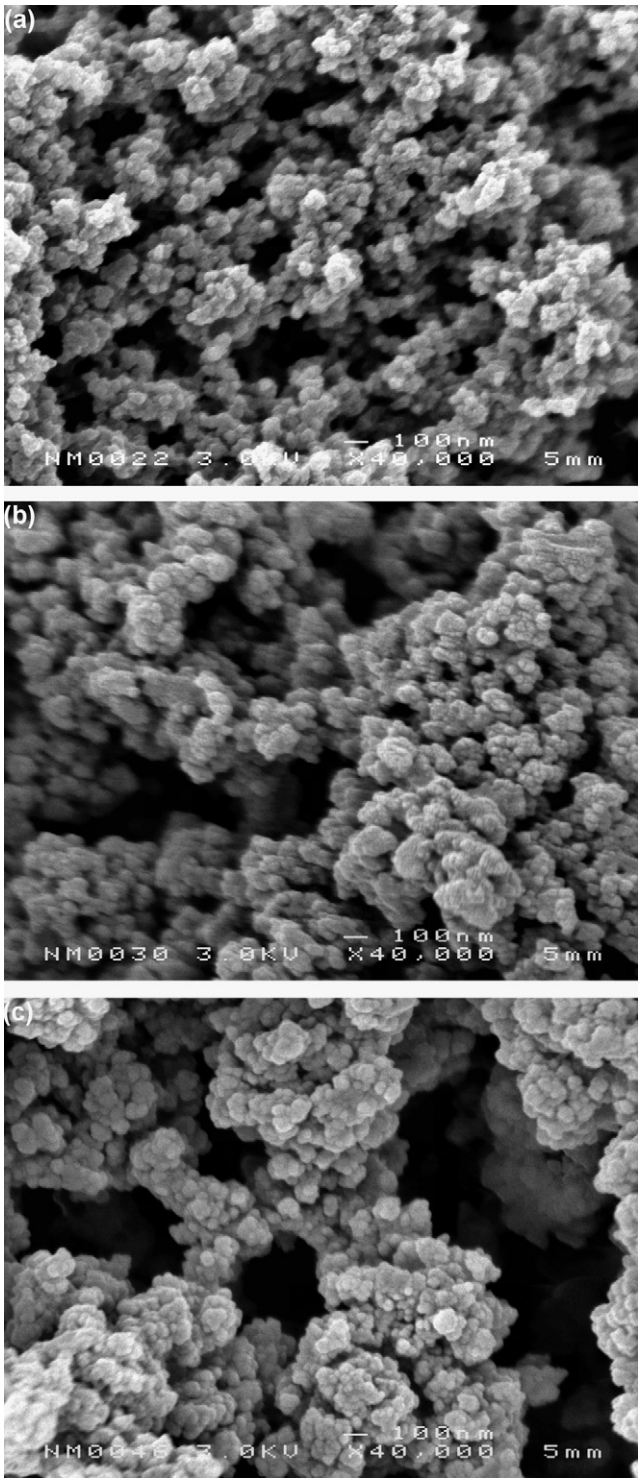

Fig. 2. SEM micrographs of the cross-section of (a) SGE-10/16-d2, (b) SGE-20/14-d2 and (c) SGE-20/16-d2.

3.2. Enzyme loading

Enzyme loading is defined herein as the weight of Cal-B that is immobilized per total weight of carrier. In all samples, amount of Cal-B adsorbed on carrier is given as sum of amount of Cal-B physically adsorbed and amount of Cal-B covalently linked to the carrier.

Candida antarctica lipase B loading also depends on the porosity parameters, particularly on pore size and specific surface area (Table 2). Increasing the pore size of the 300–150 μm beads from 87 to 270 and 560 nm resulted in a decrease in enzyme loading (220.8, 207.8 and 172.1 μg/mg). The same tendency was observed for increasing the pore size of the 630–300 μm beads from 30 to 92 nm (resins 1 and 3, and resins 7 and 3). On the other hand, increasing the specific surface area of the 630–300 μm beads, with identical pore size of 30 nm, from 82.0 (resin 1) to 106.0 m^2/g (resin 7) resulted in a decrease in enzyme loading from 222.7 (resin 1) to 184.8 μg/mg (resin 7). There results are attributed to the fact that the greater amount of enzyme is bound onto polymers with broad pore size distribution [25] and that the pore size distribution can be very different for the copolymers with identical average pore size or specific surface area (Table 3).

Immobilization of *Candida antarctica* lipase B on poly(GMA-*co*-EGDMA) depends on the particle size. Decreasing the particle size from 630–300 μm (resin 3) to 150–100 μm (resin 5) and less than 100 μm (resin 6) resulted in a decrease in enzyme loading (178.2, 166.9 and 157.1 μg/mg). But, changing the particle size for resin 3, resins 5 and 6, also follows changing the pore size (from 92 to 59 and 48 nm). Since decreasing the pore size of the carrier beads resulted in an increase of enzyme loading (discussed above), therefore can be concluded that a decrease of matrix particle size resulted in a decreasing of enzyme loading. Similar behavior was observed with porous polystyrene resins used for Cal-B immobilization [23]. Using infrared microspectroscopy it was shown that Cal-B diffusion was facilitated as the resin size decreased [22,23].

3.3. Immobilized Cal-B activity

The activity of Cal-B immobilized on resins 1–8 (see Table 1), dried Cal-B powder and Novozyme 435 was assessed by hydrolysis of *p*NPA and ε-CL ring-opening polymerization.

The hydrolytic activity of Cal-B immobilized on resins 1–8 was assessed by hydrolysis of *p*PNA (Table 2). The hydrolytic activity strongly depends on the matrix particle size used for Cal-B immobilization. As the particle size for resins 3–6 decreases from 630–300 to 300–150, 150–100 and less than 100 μm, the hydrolytic activity increases from 1928.4, 2775.0, 4534.9 to 5027.2, respectively. The same trend is observed for the resins 1–2 and 7–8. Increasing hydrolytic activity can be observed when resins of smaller particle sizes are used for hydrolysis which is due to the reduced hindrance of substrate and product diffusion in the matrix.

The synthetic activity of immobilized Cal-B was tested by the ring-opening polymerization of ε-CL (Table 2.). The synthetic activity depends on the copolymer particle size as well. As the particle size for resins 3–6 decreased from 630–300 to 300–150, 150–100 and less than 100 μm, conversion of ε-CL after 5 h increased from 18.1 to 21.9, 36.4 to 38.4, respectively. For resins 1–2 and 7–8 the same tendency was observed. However, changing the resin particle size for Cal-B immobilization from 630–300 to 300–150 μm does not affect the molecular weight of poly-ε-caprolactone. For instance, using Cal-B immobilized on resins 1 and 2 in ε-CL ring-opening polymerization, almost the same molecular weights for poly-ε-caprolactone are obtained (467.7 and 453.8, respectively). The same tendency is noticed for particle sizes from 150–100 to less than 100 μm. Most likely, decreasing the particle size from 630–300 to 300–150 μm and increasing the pore sizes from 30 to 87 nm (resins 1 and 2, respectively), which results in a increase in the molecular weight of poly-ε-caprolactone (see below), is voided by decreasing the specific surface areas from 82.0 to 36.0 (resins 1 and 2, respectively), that performs opposite effect (see below). It should be noticed that molecular weights of poly-ε-caprolactone

Table 2
Cal-B Immobilization on poly(GMA-*co*-EGDMA) resins of differing particle size, pore diameter, specific surface area and specific volume, dried Cal-B powder and Novozyme 435: enzyme loading, hydrolytic activity and synthetic activity

Resin	Sample name	Enzyme loading[a] (μg/mg)	Hydrolytic activity	Synthetic activity	
			Activity[a] (nmol pNP/min mg Cal-B)	Conversion of ε-CL[a] (%)	Mn of polycaprolactone[a] (g mol^{-1})
1	SGE-10/16-d1	222.7 ± 3.2	1065.4 ± 22.2	16.9 ± 0.7	467.7 ± 19.5
2	SGE-10/16-d2	220.8 ± 2.2	2125.0 ± 19.0	16.4 ± 0.3	453.8 ± 18.6
3	SGE-20/14-d1	178.2 ± 3.6	1928.4 ± 31.2	18.1 ± 0.3	466.4 ± 13.8
4	SGE-20/14-d2	207.8 ± 4.7	2775.0 ± 69.8	21.9 ± 0.2	485.1 ± 19.6
5	SGE-20/14-d3	166.9 ± 2.7	4534.9 ± 88.9	36,4 ± 0.5	919.5 ± 29.3
6	SGE-20/14-d4	157.1 ± 2.8	5027.2 ± 33.6	38.4 ± 0.3	919.1 ± 30.0
7	SGE-20/16-d1	184.8 ± 1.2	1801.8 ± 19.0	24.9 ± 0.2	614.1 ± 73.9
8	SGE-20/16-d2	172.1 ± 1.2	2875.6 ± 29.3	26.5 ± 0.6	602.7 ± 56.0

[a] Standard deviation values were calculated from three replicate experiments.

are relatively low. It might be that polyester precipitates in the pores of the carrier.

Table 4 shows the hydrolytic activity and synthetic activity results of dried Cal-B powder and commercially available Cal-B preparations, Novozyme 435. Comparison of those results with the ones from Table 2 indicates that Cal-B immobilized on resins 5 and 6 had much higher hydrolytic activity than both dried Cal-B powder and Novozyme 435. Conversion of ε-CL in ring-opening polymerization catalyzed by Cal-B immobilized on resins 5 and 6 is higher than the one catalyzed by dried Cal-B powder and nearly identical than the one catalyzed by Novozyme 435. The highest molecular weight of poly-ε-caprolactone is obtained with Novozyme as biocatalyst.

A strong dependence of the polymerization rate on the matrix particle size that was utilized for Cal-B immobilization is shown in Fig. 3. The ring opening polymerization of ε-caprolactone, catalyzed by Cal-B immobilized on resins 4–7 (resins with different particle and pore size) were followed over time. The kinetic behavior of a polymerization catalyzed by Novozyme 435 is included in Fig. 3a for comparison. It becomes obvious that for instance after 7 h of reaction the conversion of ε-CL increased from 20.3 to 24.9, 38.9 and 41.0%, as the matrix particle size decreased from 630–300 (resin 3) to 300–150 (resin 4), 150–100 (resin 5) and less than 100 μm (resin 6). Cal-B immobilized on resin 5 with particle size 150–100 μm and pore size 59 nm and resin 6 with particle size less than 100 μm and pore size 48 nm showed synthetic activity almost identical of that displayed by Novozyme 435. Fig. 3b shows the plot of molecular weight of poly-ε-caprolactone versus time for Cal-B immobilized on resins 5 and 6 and Novozyme 435. Up to 5 h of reaction, the molecular weight of poly-ε-caprolactone catalyzed by Cal-B immobilized on resin 6 is a little bit higher than catalyzed by Cal-B immobilized on resin 5. If the reaction is prolonged up to 10 h, the situation is reverted. This result can be explained by the very small pore size difference between resin 5 and 6 (59 and 48 nm, respectively), as a consequence of the similar particle sizes (150–100 μm and less than 100 μm, respectively).

Table 3
Pore size distribution of poly(GMA-*co*-EGDMA) samples

Resin	Sample name	Pore size distribution (vol%)			
		<7.5 nm	7.5–150 nm	150–500 nm	>500 nm
1	SGE-10/16-d1	–	66	34	–
2	SGE-10/16-d2	4	88	7	1
3	SGE-20/14-d1	–	70	25	5
4	SGE-20/14-d2	<1	80	16.5	2.5
5	SGE-20/14-d3	2.5	70	14	13.5
6	SGE-20/14-d4	3	75	14	8
7	SGE-20/16-d1	4	88	7	1
8	SGE-20/16-d2	–	21	42	37

The results for synthetic activity are in the great agreement with those for hydrolysis of *p*NPA. Both results can be explained by facilitated substrate access to immobilized enzyme and product outlet from immobilized enzyme when using resins of smaller particle size.

Table 2 illustrates that both *p*NPA hydrolysis and ε-CL polymerization are also strongly dependent on the porosity parameters of the resin. Increasing the pore size of the 300–150 μm beads

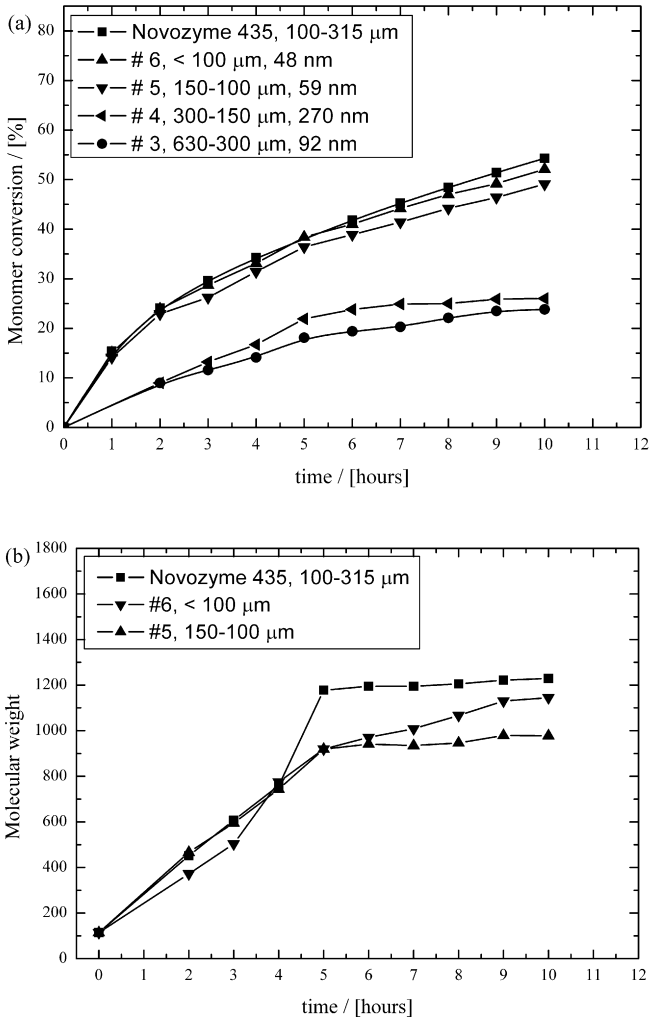

Fig. 3. Influence of matrix particle size used for Cal-B immobilization for ε-CL ring-opening polymerization carried out at 50 °C: (a) monomer conversion followed over time, (b) increase of molecular weight of poly-ε-caprolactone followed over time for Cal-B immobilized on resins 5 and 6 and Novozyme 435.

Table 4
Dried Cal-B powder and Novozyme 435: hydrolytic activity and synthetic activity

Biocatalysts	Sample name	Enzyme loading (μg/mg)	Hydrolytic activity	Synthetic activity	
			Activity[a] (nmol pNP/min mg Cal-B)	Conversion of ε-CL[a] (%)	Mn of polycaprolactone[a] (g mol^{-1})
1	Dried Cal-B powder	–	2396.0 ± 32.9	27.2 ± 0.9	631.0 ± 12.5
2	Novozyme 435	200.0	3795.0 ± 29.9	44.9 ± 0.8	1177.0 ± 22.2

[a] Standard deviation values were calculated from three replicate experiments.

from 87 (resin 2) to 270 (resin 4) and 560 nm (resin 8) resulted in a large increase in the hydrolytic activity (from 2125.0 to 2775.0 and 2875.6), increase in conversion of ε-CL (from 16.4 to 21.9 and 26.5%) and increase in molecular weight of poly-ε-caprolactone (from 453.8 to 485.1 and 602.7). The same trend is noticed when increasing the pore size of 630–300 μm beads from 30 to 92 nm (resins 1 and 3). Resins with the pore diameters of 87, 270 and 560 nm are big enough to overcome diffusion limitation. On the other hand, increase in the pore size of the resin corresponds to an increase in the percent area of beads at which Cal-B is found, so substrate and product diffusion to enzyme regions are facilitated [23].

Increasing the specific surface area of the 630–300 μm beads, with identical pore size of 30 nm, from 82.0 (resin 1) to 106.0 m^2/g (resin 7) resulted in a large increase in the hydrolytic activity (from 1065.4 to 1801.8), increase in conversion of ε-CL (from 16.9 to 24.9) and increase in molecular weight of poly-ε-caprolactone (from 467.7 to 614.1). Increase in the specific surface area alleviates substrate approach to the immobilized enzyme zones.

Poly(GMA-*co*-EGDMA) is suitable for covalent attachment of Cal-B, since epoxy group can easily react with enzyme that contains amino group. Determination of covalent attachment (DMSO extraction) was carried out for Cal-B immobilized on resins 1–8. In all the cases, approximately 80% of Cal-B is covalently attached to the resin. High fraction of Cal-B that is covalently attached to the carrier is due to the strong covalent linkages that are formed between amino-containing Cal-B and the epoxy groups of the poly(GMA-*co*-GDMA).

4. Conclusion

Candida antarctica lipase B (Cal-B) was immobilized on crosslinked macroporous hydrophilic poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) [abbreviated poly(GMA-*co*-EGDMA)] with good results. The activity of the immobilized Cal-B strongly depends on the matrix particle size. By decreasing the resin diameter, the amount of Cal-B adsorbed on the resin decreases, the conversion of *p*NPA increases and the conversion of ε-CL and the molecular weight of poly-ε-caprolactone increases. These results can be explained by enhanced hindrances of substrate and product diffusion to and from the immobilized enzyme with bigger diameter resins.

It was also shown that immobilized Cal-B activity strongly depends on the porosity characteristics of the carrier. Increasing the pore size of the 300–150 μm beads from 87 to 270 and 560 μm resulted in a large increase in the hydrolytic activity, an increase in conversion of ε-CL and an increase in the molecular weight of poly-ε-caprolactone. Also, increasing the specific surface area of the 630–300 μm beads from 82.0 to 106.0 m^2/g added up in a large increase of hydrolytic and synthetic activity. These behaviors were attributed to an increase in the regions where enzyme is immobilized and facilitating substrate diffusion to enzyme in resin with bigger pore size.

It can be concluded from these results that decreasing the resin diameter, increasing the pore size and increasing the specific surface area of poly(GMA-*co*-EGDMA) leads to the highest hydrolytic and synthetic activity.

Immobilized Cal-B activity results were compared with results of commercially available Cal-B preparations, Novozyme 435, and dried Cal-B powder. It was found that Cal-B immobilized on resin with particle size 150–100 μm and pore size 59 nm and resin with particle size less than 100 μm and pore size 48 nm showed a much higher hydrolytic activity than both dried Cal-B powder and Novozyme 435. Nearly similar trends were observed for the synthetic activity.

We were able to show that- in comparison to free Cal-B powder and Novozyme 435–80% of the enzyme is covalently attached to the resin. Unfavorable leaching out of the enzyme during reactions could therefore be prevented. Therefore the poly(GMA-*co*-EGDMA) can be considered good immobilization supports for Cal-B.

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