

Improvement of mechanical and leaching stability of Novo435 by silicone coatings (using a simple dipping and dripping method).

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Introduction

The commercially available Novo435 is a versatile biocatalyst and well recognized for its extraordinary capability to catalyze a broad range of synthetically important reactions (End, 2004). An interesting application of Novo435 on a technical scale is the production of long chain fatty acid esters (so called emollient esters) for cosmetic preparations (Thum, 2004). Novo435 (Novozymes, Bjaeksvard, DK) is a bead-shaped methacrylate carrier (300 – 800 µm in diameter) onto which lipase B from *Candida antarctica* (CalB) is adsorbed (product data sheet, Mei, 2003). The immobilized enzyme exhibits an extremely high catalytic activity (about 10 000 PLU/g_{Novo435} towards esterification of propanol and lauric acid) but the structural integrity is low and severe leaching of enzyme from the carrier occurs, particularly in the presence of media that are of increasing viscosities and surface-activities (Hilterhaus, 2008). Therefore, we decided to attempt further stabilization by coating the particles with a polymer. Based on the findings of Gill (1998), Ragheb (2003) and Buthe (2005) polydimethylsiloxane (PDMS) was chosen as polymeric matrix. Initial experiments demonstrated good activity and stability of Novo435 in this silicone (Thum, 2007 and Wiemann, 2008).

Herein, we now present an efficient process for the application of a thin silicone coating on Novo435 involving simple dipping and dripping and avoiding application of organic solvents. It allows generation of homogenously coated particles with increased mechanical stability and considerably decreased enzyme leaching. Moreover, obtained biocatalysts retain high catalytic activities towards esterification.

Material and methods

Materials

Spherical stainless steel sieve 4.5 cm in diameter was obtained from Real (Mönchengladbach, Germany). Divinylsiloxane CH₂=CH-SiMe₂-(SiMe₂)₉₈-SiMe₂-CH=CH₂ (Mw: 7 438 g/mol) (A100), Si-H-Siloxane Me₃Si-O-(SiMe₂-O-)64.5-(SiMeH-O-)3.5-SiMe₃ (Mw: 5 141.5 g/mol) (B 3.5) and Si-H-Siloxane Me₃Si-O-(SiMe₂-O-)43-(SiMeH-O-)5-SiMe₃ (B5) were prepared according to literature protocols. Syloff4000 was purchased from Ebalta (Rothenburg, DE). Novo435 was obtained from Novozymes (Bagsvaerd, DK). All other chemicals were purchased from Fluka (Neu-Ulm, DE).

Coating

Novo435 particles (400 mg) were placed in a round sieve and dipped into a pure PDMS mixture and slightly stirred for 10-20 min at RT (figure 1 a). The applied PDMS-mixture consisted of a solvent free equimolar mixture of A100 and B3.5 or B5, whereas mixing ratios were related to reactive vinyl functions in A100 and Si-H-functions in B3.5 or B5. An excess of 10 % vinyl functions is expedient. After this incubation, the sieve was retreated and left to drip of dispensable silicone for 2-5 min (figure 1 b). The silicone soaked particles were then placed in a xylene solution containing 10-30 % (v/v) Syloff4000, as shown in figure 1 c and let to polymerise at RT for 10 h. Coated particles were stored at 4 °C.

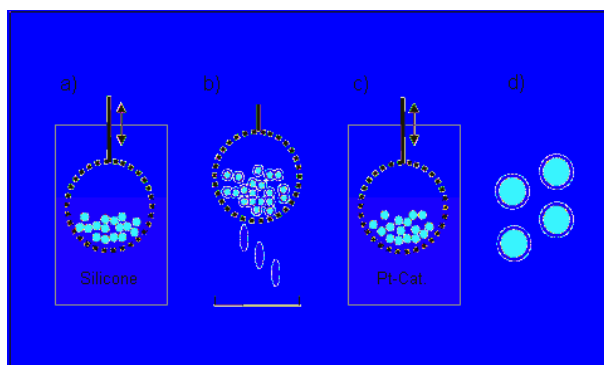


Figure 1: Dipping and dripping method (a) Dipping of Novo435 into a stirred silicone mixture (b) Dripping off dispensable silicone (c) Dipping of silicone soaked Novo435 into Pt-catalyst containing xylene (d) Cross section of silicone coated Novo435

Activity assay

10 mg of catalytically active particles were placed in 20 mL sealable glass vessels containing 5 mL of equimolar substrate solution (lauric acid and 1-propanol) and were gently shaken at 60 °C. Over 25 min samples of 50 µL were taken every 5 min and transferred into 950 µL of decane (containing 4 mM dodecane as internal standard). Initial rates of product formation were detected with gas chromatography (FID) as described by Thum, 2007. One propyllaurate unit (PLU) corresponds to the amount of enzyme that catalyzes the production of 1 µmol of propyllaurate per minute.

Assay for mechanical stability

50 mg of catalytically active particles were placed into 20 mL sealable glass vessels containing 5 mL of lauric acid and were vigorously stirred for 90 min at 60 °C using a magnetic stirrer and a cylindrical stirring bar. The so treated particles were photographed at high resolution using a digital camera (Olympus SP-500UZ, Hamburg, DE) from a height of 15 cm at an angle of 90 ° related to the central point of the glass vessel (figure 2).

Assay for leaching stability

Untreated and coated Novo435 particles were stirred in a mixture of acetonitril and water (1:1 v/v) at 45 °C for 30 min. The so treated particles were separated from the solution, thoroughly rinsed with water, dried at 50 °C and stored at 4 °C until activities were quantified as described before.

Results and discussion

Coating

Coating of Novo435 with a thin film of silicone was accomplished by successive dipping of the particles first into a solution of silicone and secondly into a solution of dissolved polymerisation catalyst. Spare silicone was intermediately dripped off. Polymerisation was completed after five hours curing at RT.

Dipping into a silicone solution of A100 and B3.5 or B5, respectively, for 20 min resulted in an average amount of 50-60 % (w/w) of deposited silicone (table 1). This amount is advantageous to generate fully coated particles of high stability (Wiemann, 2008).

No.	V-Siloxane (A)	H-Siloxane (B)	Novo 435 [g]	Dilution cat.	PDMS content [% w/w]
1	A100	B3.5	1	1:3	53
2	A100	B5	0.4	1:3	58
4	A100	B3.5	0.4	1:10	26

Table 1: Composition of silicones and resulting coating

The amount of deposited silicone decreased to 26 % when the catalyst solution was diluted. A similar effect (data not shown) was observed when the dipping time into the silicone solution was diminished. It is currently under investigation whether the silicone elastomer is solely deposited as exterior layer on Novo435 or permeates into the interior, i.e. soaks the whole particle including the protein layer with silicone.

Mechanical stability of coated Novo435

Silicone coating as described in this study considerably improved the mechanical integrity of Novo435. Strong stirring in viscous lauric acid at 60 °C had no visible effect on coated particles whereas untreated Novo435 disintegrated to a milky turbid suspension (figure 2).

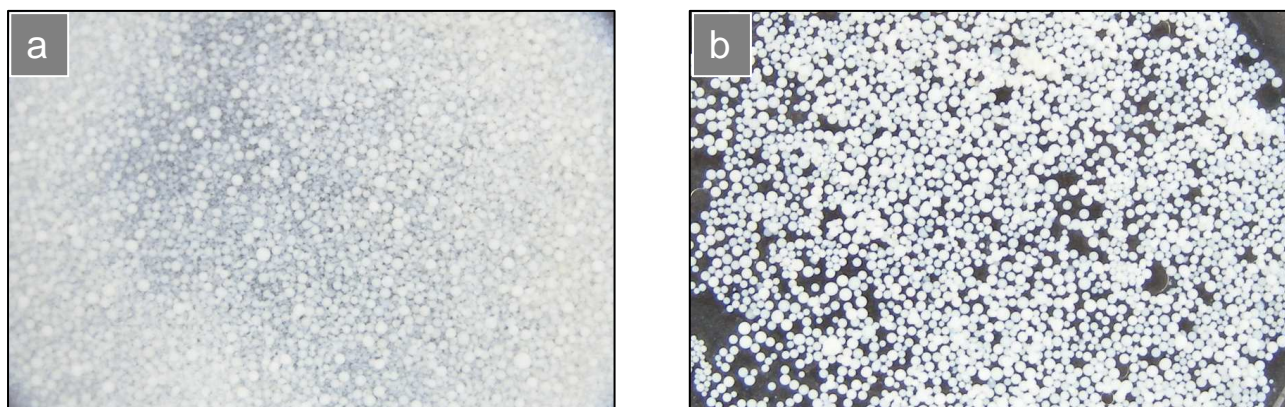


Figure 2: Untreated Novo435 (a) and Novo435 with silicone coating (b), both after 90 minutes of strong stirring in lauric acid (60 °C)

Catalytic activities of coated Novo435

Novo435 coated with silicone according to the novel methodology exhibited a superior quality with regard to esterification activity. The relative activities of coated Novo435 compared to the native Novo435 for the synthesis of propyllaurate were between 72 – 61 %, related to the reactive Novo435 content. With the solvent-based entrapment of pure CalB into PDMS following the protocol by Buthe (2005) activities of 400 U/g_{Imm} were the ultimate limit. Considering the mass transfer limitations expected from these results, the observed activities of coated Novo435 are extraordinary.

	V-Siloxane	H-Siloxane	PDMS content [% w/w]	PLU [U/g _{Imm}]	Relative activity [%]
1	-	-	0	10 200 ± 350	100
2	A100	B 3.5	53	3 457 ± 287	72
3	A100	B 5	58	2 638 ± 262	62
4	A100	B3.5	26	4 581 ± 301	61

Table 2: Esterification in PLU of native and silicone coated Novo435

Leaching stability of coated Novo435

The improved leaching stability of silicone coated Novo435 was shown exemplarily for A100/B5 (as shown in table 2, no. 3). After incubation in a mixture of acetonitril and water (1:1 v/v) almost 45 % of esterification activity was retained, whereas native Novo435 was completely inactivated.

These results are in agreement with the findings for Novo435 coated with a non-optimised method (Thum, 2007 and Wiemann, 2008).

IR-spectrometry was used to proof qualitatively, that the enzyme was in fact desorbed from the carrier and not just inactivated on the particle surface (data not shown). The carrier material, an acrylic resin, showed a strong peak at 1720 cm^{-1} (C=O) and a distinct amide peak at 1650 cm^{-1} . The latter peak, which indicated the factual protein content, maintained in coated Novo435 after treatment with acetonitril/water, while it totally diminished in native Novo435.

Conclusions

The successful application of the dipping and dripping method for the coating of Novo435 with silicone has been demonstrated. This simple method allows an effortless and therefore low-priced production of large quantities of homogenously coated Novo435. The obtained particles maintain high catalytic activities toward the relevant reactions and have a significantly increased mechanical and leaching stability. Therefore, it is feasible that they enable the synthesis of long chain fatty acids for cosmetic and other industrial purposes in stirring tank reactors at repeated use. In addition, using the dipping and dripping method makes application of organic solvents obsolete, which is of special benefit for enzymes with lower stability. A general implementation of this method for other carrier-bound enzymes seems possible, and should soon open up the way to new improved biocatalysts and products.

Acknowledgement

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