

O3-3 Novel method for silicone based particle coating using a fluidized bed reactor

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INTRODUCTION AND OBJECTIVES

Immobilization of enzymes can considerably increase the catalytic stability as a result of molecular interactions between carrier and enzymes. Additionally it is possible to reuse the biocatalysts by separating them from the final reaction media. Especially simple physical adsorption allows immobilization under mild conditions at high retention of activity. One of the most prominent carrier-bound biocatalysts at present time is Novozyme 435 (NZ435). NZ435 is a granular acrylic resin containing adsorbed lipase B from *Candida antarctica*. This biocatalyst is widely used in the industrial production of emollient esters for cosmetic syntheses and other purposes as well. However, the biggest disadvantage of NZ435 is the deficient stability under long-term reaction conditions. Due to the weak noncovalent binding of the enzyme onto the carrier, the use of surfactant-like or some polar organic solvents provokes enzyme leaching. We previously reported that deposition of silicone on NZ435 significantly increases both mechanical stress and enzyme leaching as well, while a respectable specific activity could be retained (Wiemann 2009). Within the published method the deposition of NZ435 with different amounts of silicone was achieved by mixing a comb-like Si-H-siloxane with a divinylterminated polydimethylsiloxane in the presence of a [Pt]-based heterogeneous catalyst (Karstedt catalyst). Cyclohexane was added to achieve homogeneous mixing of the viscous solution. Polymerization by hydrosilylation was completed after 3 h curing at room temperature (25 °C). For using this procedure in a technical scale, large amounts of organic solvent and appropriate equipment for mixing issues would be necessary. This is beside large investment costs especially environmental questionable. Therefore we report herein on an alternative way for producing silicone composite particles where an avoidance of almost all organic solvent is achievable. For this purpose a Wurster-type fluidized bed reactor was used. The principle of operation is appropriate for dispensing a substance homogeneously onto a solid carrier, which matches exactly the intention of silicone disposed NZ435. In a fluidized bed reactor particles are mobilized into a fluid-like state using an airstream from the bottom of the reactor. In the Wurster configuration the coating solution is sprayed into the moving particle stream, which allows an enormous contact area between particles and silicone. Due to using silicone as coating solution the temperature of the used airstream is the critical parameter concerning curing the polymerization reaction. The used [Pt]-catalyst is temperature dependent and therefore the activity should be easily modifiable. A

qualitative characterization of polymerization velocity was made by measuring the viscosity over time and temperature. Likewise an empirically determination of parameters like air stream velocity, air temperature and flow rate of the sprayed silicone was performed as well. Final characterization of the coated NZ435 was carried out by measuring their activity before and after an appropriate enzyme leaching test.

MATERIALS AND METHODS

Materials/ Instruments

Novozyme 435 (NZ435) was obtained from Novozymes A/S (Bagsvaerd, Denmark); Silicones and [Pt]-catalyst was produced and provided from Evonik Goldschmidt GmbH (Essen, Germany). All other chemicals were purchased from Sigma-Aldrich (Germany) and used as obtained. The experimental apparatus was the Uni-Glatt Pilot (Glatt GmbH, Binzen, Germany). The used rheometer was the AR1000 (TA Instruments, New Castle, USA).

Methods

For measuring the viscosity 1,5 g ice cooled silicone was mixed with 1-2 % (v/v) toluene containing different amounts of [Pt]-catalyst. A shear rate of 8 sec⁻¹ was used in all experiments. In coating experiments the used silicone was cooled before spraying with ice and stayed during the process on ice, too. The [Pt]-catalyst was added in the same way as described for the viscosity measurements. The catalytic activity was assessed in propyl laurate units (PLU). 1 PLU corresponds to the amount of NZ435 [g] that catalyzes the production of 1 μmol propyl laurate per minute. The initial product formation was analyzed by gas chromatography as previously described (Thum 2009). The leaching assay for NZ435 particles (100 mg) was performed by stirring them in MeCN/H₂O (1:1, 5 mL) in sealable glass vessels at 45 °C for 15 min.

RESULTS AND DISCUSSION

The original idea of depositing silicone on NZ435 was the formation of a thin membrane of a mesh-like structured polymer on the outer carrier surface in order to prevent the enzyme from leaching. However, the large amounts of silicone required for the so called “coating” indicate that considerable amounts of silicone permeate into the carrier material. The low surface tension of the used silicone, the high porosity of the carrier and reduced silicone viscosity due to the organic solvent used during the deposition are probably responsible for the quick up-

take of the silicone. For the new method the original idea to establish a core-shell structure or at least to fill up only the outer region of the NZ435 was resumed. Major plan for coating the NZ435 is to activate the silicone by adding the catalyst, spray the silicone on the fluidized particles and finish the whole procedure before the silicone polymerizes either in the feed line and the nozzle as well. Since the viscosity of silicone increases significantly during polymerization, the time span before getting viscous was used as evidence to determine the most appropriate time slot between adding the catalyst and end of polymerization. Following this approach different amounts of catalyst were tested whereas 10 ppm was found to be the most suitable concentration. Furthermore the temperature was varied between ice cooled (0 °C) and 60 °C which is also used for activity determination. Figure 1 shows the significantly high temperature dependency of the used [Pt]-catalyst. The viscosity of 1 Pa/sec is reached at 60 °C 150 times faster than at 0 °C. This allows inhibiting the polymerization reaction even after adding the catalyst.

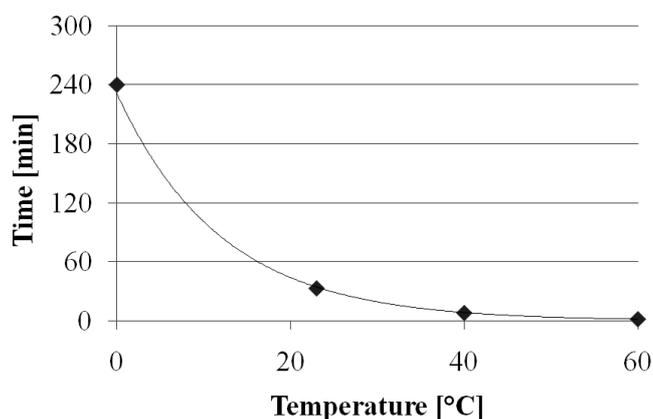


Figure 1 : Time for silicone polymerization at different temperatures (10 ppm [Pt]-catalyst).

Consequently the idea for coating NZ435 with silicone was to cool the silicone to 0 °C, add the catalyst, pump the activated silicone to the nozzle and spray it onto the carrier. In the hot air stream which is used for fluidizing the particles, the droplets should heat up very fast, which finally lead to faster polymerization of silicone on the carrier surface. The optimal fluidization parameters were determined empirically and guaranteed a stable and reproducible fluidized bed. The critical parameter was the determination of the ratio between a polymerization which is fast enough to overcome the cohesive moment of unpolymerized silicone on the carrier surface and a polymerization which is inhibited as long as the silicon is pumped. After repeated empirical testing of different catalyst concentrations, flow rates of silicone and air temperatures, we found optimal coating conditions. It was possible to produce composite particles with 18 % silicone in relation to the total weight of coated particles. Main challenge during test runs were first agglomerations of curing silicone on particles with themselves and second blockages in the spray unit especially in the nozzle.

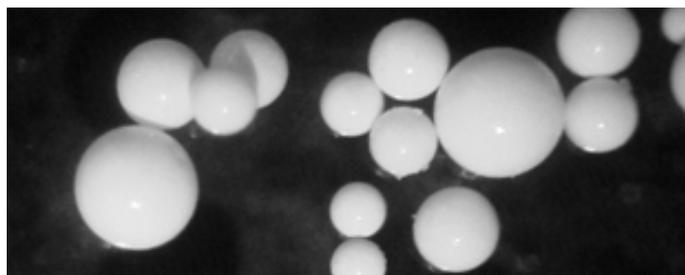


Figure 2 : Coated NZ435 visualized by a reflected-light microscope

Figure 2 shows NZ435 after coating with 18 % of silicone under a reflected-light microscope. Remarkable is the shiny surface which implies besides the silicone bridges between some NZ435 beads that a silicone surface layer was built up during the coating process. Table 1 shows the activities of uncoated and coated particles before and after leaching tests. The previously reported stabilizing effect of silicone against leaching (Wiemann et al 2009) is clearly sustained. The loss of initial activity results from diffusion limitations and was reported for the “default” method as well.

Table 1: Activity of uncoated and coated NZ435 before and after leaching.

	Before Leaching	After leaching	Residual activity
NZ435	9282 PLU/mg	186 PLU/mg	2 %
NZ435 coated	5016 PLU/mg	2962 PLU/mg	59 %

CONCLUSIONS

Since the appropriate stabilization against leaching in Wiemann (2009) needed 50-54 % silicone, the high residual activity after leaching of here coated NZ435 with 18 % silicone clearly shows, that it was possible to build up a core-shell structure wherein the silicone is solely penetrating into the outer carrier parts. Space-resolved EDX-scans will show the thickness of the silicone surface layer and will most likely elucidate how deep the silicone penetrates into the carrier. The adduce evidence of feasibility opens the way to stabilize enzyme carrier with silicone in a more ecological way with fewer silicone and less organic solvent. As fluidized bed systems are easy to be upscale, this method has a great potential to be used in even bigger scales as in this work.

REFERENCES

- Wiemann L.O. et al. (2009) *Enzyme Stabilization by Deposition of Silicone Coatings*. Organic Process Research & Development 13 (3) 617-620
- Thum O. et al. (2009) *Enzyme preparations* EP2011865