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Polyethyleneimine (PEI) functionalized ceramic monoliths as enzyme carriers: Preparation and performance

K.M. de Lathouder [a,*], D.T.J. van Benthem [a], S.A. Wallin [b], C. Mateo [c], R. Fernandez Lafuente [c], J.M. Guisan [c], F. Kapteijn [a], J.A. Moulijn [a]

[a] *Reactor and Catalysis Engineering, DelftChemTech, Faculty of Applied Sciences, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands*
[b] *Corporate R&D, The Dow Chemical Company, 1776 Building, Midland, MI 48674, USA*
[c] *Department of Biocatalysis, Spanish Research Council (CSIC), Universidad Autonoma de Madrid, Calle Marie Curie 2, Cantoblanco, E-28049 Madrid, Spain*

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Abstract

Classical cordierite monoliths and acicular mullite (ACM) monoliths were used as support material for ionic adsorption of β-galactosidase from *Aspergillus oryzae*. Monoliths were silica-coated, then functionalized with polyethyleneimine (PEI) using different methods. In addition to direct adsorption of PEI, monoliths were modified with (3-glycidoxypropyl)trimethoxysilane (GPTMS) and γ-(aminopropyl)triethoxysilane (APTES)–glutaraldehyde for covalent attachment of the polymer. The optimal method, in terms of immobilization capacity and stability, was selected by using a particulate silica support and crushed monoliths. Immobilization of PEI via a GPTMS-functionalized ACM monolith yields the best enzyme carrier. At pH 7, 150 mg β-galactosidase g^{-1} SiO_2 can be adsorbed. In general, a coating with a higher molecular mass polymer results in stronger immobilization, at a higher rate. By using whole monoliths, we find that the molecular weight of the polymer coating influences the adsorption/desorption of the enzyme. The open walls of the ACM monoliths permit significant higher loadings of high molecular weight PEI, which results in a higher enzyme-support strength and increased stability with respect to enzyme desorption. These PEI systems provide an optimal environment for the β-galactosidase, 92% of the free enzyme activity is retained after immobilization. Although adsorption was quite strong and permitted the use of the immobilized enzyme in a wide range of conditions, the enzyme could be completely desorbed after its inactivation. The monolith-carrier combination can be reused several times.

Keywords: Enzyme immobilization; β-Galactosidase; Polyethyleneimine; Ceramic monolith; ACM

1. Introduction

Monolithic catalysts are used for various gas-phase applications and as an alternative for solid catalyzed gas–liquid reactions [1–5]. The honeycomb monolith support offers several advantages over particulate supports, including a high geometric external surface, structural durability, easy catalyst separation [1], a low-pressure drop [6,7], and uniform flow distribution within the matrix. The classical monolith has square parallel channels with very little open and connected porosity within the channel walls. A new type of monolith was synthesized by the Dow Chemical Company and consists of high-porosity acicular mullite (ACM) [8,9].

The ACM monolith has the same macroscopic geometry as classical cordierite monoliths, but the walls consist of an open network of interlocking elongated ceramic grains within the micrometer range (Fig. 1). The ceramic grain size and the pore diameter size are tunable. The open pore structure allows access of reactants to catalysts deposited within the monolith wall [10].

Conventional enzyme carriers are typically inorganic particles, porous beads of synthetic polymers, or gel-like materials. The relatively low mechanical strength and internal diffusion limitations of these carriers can lead to difficulties in industrial operation. If larger beads are used, a fixed-bed reactor can be used, but often at the price of severe intraparticle limitations [11]. In enzymatic systems, this can lead to more than just slow

* Corresponding author. Tel.: +31 104313498; fax: +31 104311499.
E-mail address: karen.delathouder@shell.com (K.M. de Lathouder).

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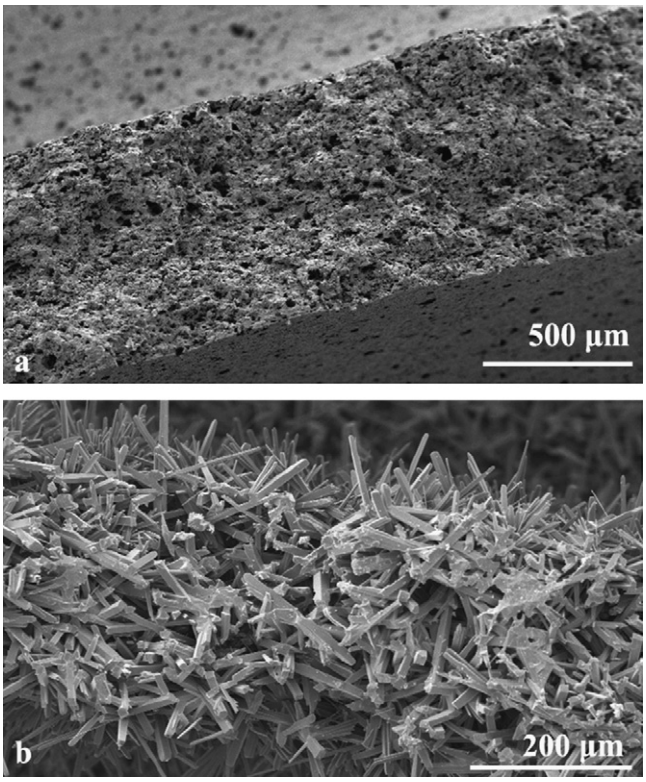

Fig. 1. Structure of the monolith walls: (a) classical cordierite and (b) ACM.

substrate diffusion: intraparticle pH gradients or ionic strength gradients can be equally problematic, affecting enzyme stability, enzyme-support bond, and/or reaction rate. An alternative to large beads in a fixed-bed reactor is a stirred slurry of beads in the size range of 100 μm [12]. However, these small particles tend to be damaged by intense stirring, and often a separate filtering step is required. The use of structured support materials is an attractive alternative to conventional particulate enzyme carriers. The low-pressure drop through monolith-supported enzyme systems allows the use of higher liquid velocities in order to reduce film diffusional effects. Benoit and Kohler [11] used immobilized catalase on a ceramic monolith and compared this with particulate-supported systems. They observed increased mass transfer for the monolithic system. In 1989, Kawakami *et al.*, [13] used ceramic monoliths in a three-phase system operating in both film flow and slug flow with immobilized glucose oxidase.

In order to use ceramic monoliths as enzyme-support material, a suitable carrier material must be applied onto the channel walls. Following this approach, lipase and β-galactosidase were already successfully adsorbed onto monolithic supports with different carbon [14,15] and polymer coatings [16].

When producing industrial biocatalysts using monolithic structures, reversible enzyme immobilization through ionic adsorption can be a convenient protocol. Deactivated enzyme can be desorbed at high ionic strength and the support can become ready to be used again for immobilization of fresh enzyme. A number of protocols for reversible immobilization have been reported over the last decade, enzymes were adsorbed onto supports including polymers and resins [17–20], molecular sieves [21–24], silica and silica–alumina composites [25–29], and carbonaceous materials [30–32]. Probably the most popular method is adsorption on ionic exchange resins (mainly anionic exchangers). This strategy was actually used for the first industrial applications of an immobilized enzyme [33]. However, the electrostatic binding forces are not very strong and most proteins are already fully desorbed at moderate ionic strength (0.2–0.3 M NaCl) or if a pH shift occurs. This leads to an apparent inactivation and causes contamination of the product [34]. Different remedies have been offered such as the addition of cations during immobilization [35]. The use of flexible polymers coated on rigid supports seems to be a very attractive solution: a high mechanical strength combined with a very high density of charges. Adsorption of proteins on this flexible polymer coating should promote minimal conformational distortion, because the polymer may adapt to the protein during multipoint attachment.

The aim of the present work is to explore several methods for the preparation of polyethyleneimine (PEI)-coated monoliths and to compare ACM monoliths with classical cordierite monoliths as the support matrix. PEI is attached to the monoliths by covalent binding and by physical adsorption, and a β-galactosidase from *Aspergillus oryzae* is immobilized on the functionalized monoliths. The effect of the molecular weight of the polymer on enzyme adsorption and desorption is studied and the biocatalysts are compared in terms of activity, stability and ease of reuse. Galactosidases catalyze the hydrolysis of lactose to glucose and galactose. Lactose is the main carbohydrate in milk and whey. The consumption of food with a high-lactose content is problematic for almost 70% of the world population. Together with the low solubility and sweetness of lactose, this has led to an increased interest in developing industrial processes to hydrolyze lactose. Since the use of acid catalysts leads to generation of unwanted odours/flavours, the preferred method is enzymatic hydrolysis. β-Galactosidase consists of four equal subunits and has an approximate diameter of 10–15 nm. The enzyme from *A. oryzae* has a mass of 105 kDa and a stability range between pH 4 and 9 [36]. Optimal activity is observed at pH 4.5 for *o*NPG and at pH 4.8 for lactose hydrolysis. The presence of salts such as NaCl and KCl promotes inactivation [37].

2. Experimental

2.1. Materials

Ludox AS-40 (40% colloidal silica in water) and polyethyleneimine (high molecular weight MW ∼1,000,000, water free; MW = 750,000, 50% in water; low molecular weight MW = 60,000 or 25,000) were from Aldrich. β-Galactosidase from *A. oryzae*, *o*-nitrophenyl-β-galactopyranoside (*o*NPG), and (3-glycidoxypropyl)trimethoxysilane (GPTMS) were purchased from Sigma. (3-Aminopropyl)triethoxysilane (APTES) and CPC-silica carrier (mesh size 50) were purchased from Fluka. Square channel monoliths of ACM, 200 cpsi (cells in.$^{-2}$, 62 cells cm^{-2}) were prepared by a proprietary Dow process to produce honeycombs with "small", medium", and "large" pores. Cordierite monoliths with a cell density of 200 cpsi were used for comparison. The key properties of these monoliths are given in Table 1.

Table 1
Nominal values of key properties of employed square channel monoliths

	ACM-S ("small")	ACM-M ("medium")	ACM-L ("large")	Cordierite
Cell density (cpsi)	200	200	200	200
Wall thickness (mm)	0.24	0.24	0.24	0.3
Wall porosity (%)	60	60	65	35
Pore diameter (μm)	5	18	45	7.5

2.2. Support preparation

Monolith samples with a length of 5 cm and a diameter of 4.3 cm were calcined for 4 h at 1273 K in static air (10 K min^{-1}) to remove possible contaminants. In order to provide sufficient silanol groups on the monolith surface, a colloidal silica layer (Ludox AS-40) [38] was applied before reacting with the silane. Cordierite samples were dipped in 40% Ludox solution, ACM monoliths were washcoated with a diluted solution (4 wt.%). After dipcoating the channels were cleaned with pressurized air and samples were dried (microwave oven (20 min, 150 W) and static air (673 K, 5 K min^{-1}, 4 h) calcination).

Polyethyleneimine (PEI)-functionalized supports were prepared either with a two-step approach via (3-aminopropyl) triethoxysilane (APTES) and glutaraldehyde [17] or through coupling to (3-glycidoxypropyl)trimethoxysilane (GPTMS) [39]. PEI was also directly adsorbed on SiO_2-washcoated monoliths and the particulate CPC-support. Washcoated monoliths and CPC-carrier were treated for 24 h in a 5–10 wt.% solution of APTES or GPTMS in toluene, with 0.1% (v/v) tetraethylene amine. Supports were washed with toluene and acetone and dried at 393 K for 4 h (2 K min^{-1}). GPTMS–aldehyde groups were created by reaction with 1 M H_2SO_4, followed by reaction with $NaIO_3$ (1 M, 1 h). APTES–aldehyde groups were formed by reaction with 2.5 wt.% glutaraldehyde (8 h, 278 K).

PEI was applied to the silica-coated and functionalized supports from a 10 wt.% PEI solution in water at pH 10 by mixing for 24 h on a roller-mixer (particulates) or by continuously recirculating the solution through the monolith in glass reactor [40] at 500 rpm. The GPTMS-coated monoliths were washed with 1 M NaCl, to remove loosely bound PEI. In case of reaction with glutaraldehyde functionalized supports, glutaraldehyde groups were reduced with $NaBH_4$ (10 g l^{-1}, 2 h), followed by washing (50 mM acetate buffer pH 5, 50 mM borate buffer pH 9 and 1 M NaCl and excess distilled water).

Enzyme immobilization was carried out in 5 mM phosphate buffer (pH 7) for 24 h. The enzyme concentration was followed by using UV–vis (Thermo Optek UV-540 with a 1 cm cuvette at 280 nm). The samples were washed with 50 mM phosphate buffer pH 7 and 5 mM acetate buffer pH 4.5, and stored in 5 mM acetate buffer pH 4.5 with 1 g l^{-1} sodium azide at 278 K. The nomenclature of the samples is summarized in Table 2.

2.3. Characterization of the support matrix

Thermogravimetric analysis (TGA) was performed on a Mettler Toledo TGA/SDTA851^e. The samples were heated in air (100 ml min^{-1}) to 1273 K (heating rate 10 K min^{-1}). Diffuse reflectance IR spectra were recorded on a Thermo Nicolet spectrophotometer model Nexus with an MCT detector coupled with a diffuse reflectance accessory model COLLECTOR from SpectraTech.

2.4. Catalyst performance

β-Galactosidase activity was followed spectrophotometrically by the increase in absorbance at 405 nm, promoted by the hydrolysis of *o*NPG in a stirred reactor (V_L = 150 ml, 500 rpm). Experimental conditions were 2 mM *o*NPG in 0.05 M Tris buffer pH 7 and 295 K. Enzyme desorption was studied by slowly increasing the ionic strength of the solution by adding NaCl (concentration range 50–800 mM in 50 mM steps) and monitoring the free enzyme activity of the supernatant at 405 nm (295 K, 4 mM *o*NPG in 0.05 M acetate buffer pH 5). The method for reusing the support was studied by completely desorbing the enzyme in 9 M guanidine solution, followed by thoroughly washing the support. Fresh enzyme was adsorbed as described above.

3. Results and discussion

3.1. Selection of immobilization protocol

To compare the preparation of the PEI-coated support in the absence of mass transfer limitations, crushed cordierite (1–2 mm) was used and compared with CPC-carrier. PEI (MW = 25,000) was attached to the particulate carrier via direct adsorption, reaction with APTES, or reaction with GPTMS. An indirect aldehyde group was created from the epoxy groups of the GPTMS by acid treatment and subsequent oxidation with $NaIO_4$. Agarose–PEI, a generally used enzyme support [17,41] was used as a reference. Crushed silica-coated monolith and CPC-carrier were also included to measure the enzyme adsorption on bare support material. The enzyme was adsorbed from a 1 g l^{-1} β-galactosidase solution. The results for enzyme adsorption on crushed cordierite (open symbols) and CPC (closed symbols) over 21 h are presented in Fig. 2.

Table 2
Nomenclature of the samples

	Indicated with
Support material: particulate silica, cordierite or ACM	CPC, C or ACM
Grain size ACM: small, medium, or large	S, M, L
Silane linker	APTES or GPTMS
GPTMS epoxygroup hydrolyzed to aldehyde	(ald)
Polyethyleneimine (physically adsorbed)	PEI (ads)

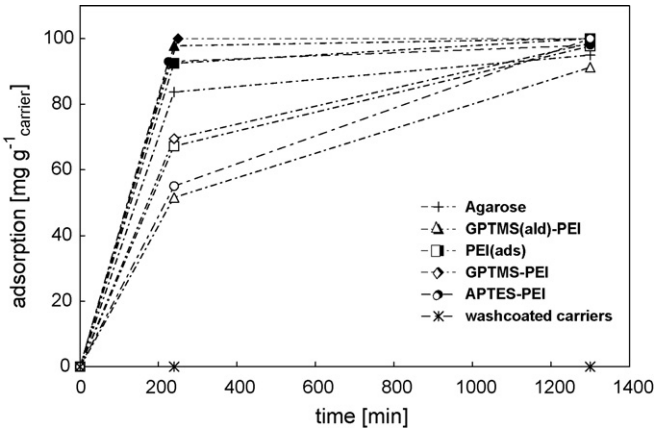

Fig. 2. Adsorption on particulate carriers from a 1 g l^{-1} solution at 295 K. The closed symbols represent the CPC-carriers and the open symbols represent the crushed cordierite.

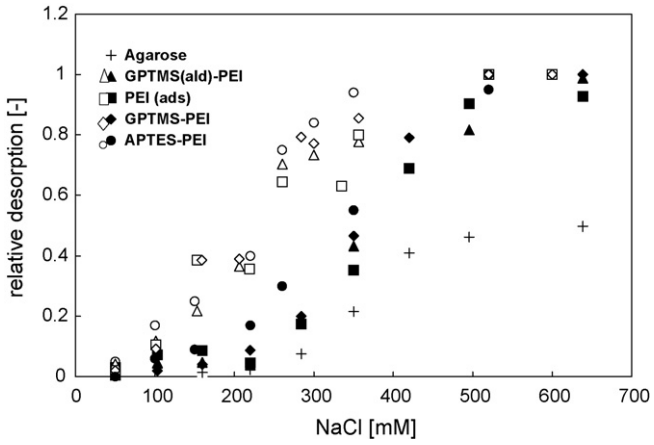

Fig. 3. Desorption at increasing ionic strength in 10 ml NaCl mixtures. The closed symbols represent the CPC-carriers and the open symbols represent the crushed cordierite.

No adsorption is observed for the bare CPC and the crushed silica-coated monolith. The functionalized CPC-carriers reach the maximum adsorption after 5 h, while adsorption on the crushed PEI-functionalized silica-coated cordierite is slower. The final amount of adsorbed enzyme was 100 mg g^{-1} carrier (100%) for all supports. The cordierite supports have a lower PEI-binding yield than the CPC-carriers (from elemental analysis, not shown), because the monoliths have a lower silica content (around 10%) compared to the CPC-carrier (100%). This results in a lower total β-galactosidase adsorption per gram of support. On monoliths, the effective loading is 1 g g^{-1} silica carrier; on CPC this value is 0.1 g g^{-1} silica carrier. The same enzyme loading must be accomplished on a smaller amount of carrier, and is therefore somewhat slower. The PEI loading and rate of adsorption on the agarose-carrier are found at an intermediate level.

The carriers with 100 mg of adsorbed enzyme were subsequently mixed with solutions of increasing concentrations of NaCl. The relative amount of desorbed protein as a function of NaCl concentration is presented in Fig. 3.

The agarose–PEI carrier is very stable with respect to desorption at higher ionic strength. The desorption of adsorbed enzyme from crushed cordierite already takes place at lower ionic strength. This could be expected based on the lower binding strength that is the result of a 10 times higher enzyme loading compared to the CPC-carriers. No significant differences are observed between the different immobilization methods. For both supports, the APTES–PEI method seems less stable against forced desorption. Compared to the GPTMS-based samples, the extra hydrolysis step does not seem to improve the enzyme loading (Fig. 2) or the binding strength of the enzyme at higher ionic strength (Fig. 3). So, there is no advantage in using the GPTMS(ald) method for the preparation of PEI-coated monoliths. Above 0.6 M NaCl all enzyme is desorbed from the CPC- and cordierite-carriers. For Agarose–PEI, a 1–1.5 M concentration was needed for complete desorption [17] (not shown). In the following section, the preparation and use of PEI-coated monoliths will be discussed in more detail.

3.2. Optimization of the procedure using monoliths

PEI,25000 was attached to different monoliths via APTES or GPTMS, or adsorbed directly onto washcoated monoliths. The carrier loading on different monoliths and the resulting β-galactosidase loading (pH 7, 2 g l^{-1}) are presented in Table 3.

The PEI loading is higher for ACM monoliths than for cordierite monoliths (Table 3). This could be expected based on the open structure of these monoliths, and was observed before [16]. On ACM-S monoliths, the total polymer loading is lower than on ACM-M monoliths. This can be ascribed to the higher accessibility of the more open channel wall of the ACM-M samples (larger micrograins) for the viscous PEI solution.

To determine the polymer distribution inside the monolith channels, TGA measurements of different parts of the monoliths were performed (not shown). Based on these measurements, the polymer distribution throughout the channels can be considered to be homogeneous for all methods. The ACM-M-PEI(ads) monoliths were checked for polymer desorption by stirring in water for 5 h. After each cycle TGA was performed (not shown). Initially, 3.2 wt.% PEI was loaded onto the backbone, but after

Table 3
PEI (MW = 25,000) yield and enzyme loading (from 2 g l^{-1}) for 200 cpsi cordierite, ACM-S, and ACM-M

Method	Cordierite		ACM			
	Y_{PEI}C (wt.%)	Protein (mg)	Y_{PEI}ACM-S (wt.%)	Protein (mg)	Y_{PEI}ACM-M (wt.%)	Protein (mg)
GPTMS	7.9	77	9.5	113	12.1	151
APTES	4.7	57	6.1	74	6.3	83
PEI ads	2.5	–	2.5	–	3.2	–

two desorption steps already 30% of the polymer had desorbed from the monolith. Although physical adsorption of PEI is simple and only requires one preparation step, the significant desorption when applied in aqueous environment does not make this the preferred preparation method for polymer–ceramic composites. These PEI(ads)-samples were not used for enzyme adsorption.

Enzyme loading depends on the total PEI loading on the monoliths (Table 3). The highest enzyme adsorption capacity was measured for the carriers with the highest PEI loading (GPTMS-PEI-samples). The use of APTES as a chemical linker leads to a lower PEI loading, and hence a lower enzyme adsorption capacity. As a result of the low loading, the APTES-GA method was not considered a viable route to prepare β-galactosidase–monolith catalysts. This result is in agreement with earlier results for immobilization on monolithic supports by this method [10].

Adsorption on ACM monoliths leads to a higher total enzyme loading compared to cordierite, because of the higher PEI loading inside the fragmented channel wall. With a washcoatloading of 1.5 and 1 g, respectively, on cordierite and ACM-M, the normalized enzyme loading becomes 50 mg g^{-1} SiO_2 for cordierite and 150 mg g^{-1} SiO_2 for ACM-M.

3.2.1. Surface chemistry

To investigate the surface chemistry of the different coatings, the coated monoliths were analyzed with DRIFT-FTIR. The results for washcoated and functionalized ACM-M samples are given in Fig. 4. The bare ACM (not shown) shows typical bands at 801 cm^{-1} (Si–O–Si silica) and 1110 cm^{-1} (Si–O–Si silica) [42,43]. After washcoating with Ludox AS-30 (a), two bands appear at 3750 and 3690 cm^{-1}; a Si–OH-stretching vibration (3750 cm^{-1}) and a hydrogen bonded silanol band (3690 cm^{-1}) [42,44], although the latter is difficult to identify because of the strong contribution of the broad feature of the OH-stretching vibration of physisorbed water. The presence of physisorbed water in various samples is also apparent by the bending mode at around 1625–1650 cm^{-1}.

After silanization with GPTMS (b), the characteristic silica bands disappear and are replaced by the vibrations of the silanes' typical bands (epoxy ring and amino groups) at 3000–3060 cm^{-1}. After addition of the polymer (c), these vibrations disappear. This indicates good polymer coverage; the underlying silane coating is not visible after reaction with PEI. The vibrations of the carbon backbone at 2890–2950 cm^{-1} remain visible because the polymer also has a $–CH_2–$ backbone.

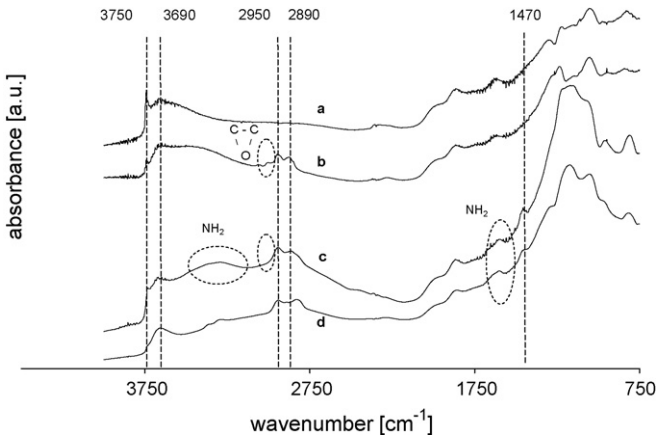

Fig. 4. Drift-FTIR spectra for coated ACM-M monoliths: (a) ACM-SiO_2; (b) ACM-GPTMS; (c) ACM-GPTMS-PEI; (d) ACM-PEI.

The presence of the polymer can also be confirmed by the vibrations for amine groups at 3000–3550 and 1625 cm^{-1}, whereas the intensity of the physisorbed water vibrations has decreased, in agreement with the more apolar nature of the material. The band at 1470 cm^{-1} is most likely also associated with the CH_2 backbone (bending mode).

For direct adsorption onto a washcoated monolith (curve d) the carbon backbone is observed (2890–2950 cm^{-1}), and the Si–OH-stretching vibration has completely disappeared; also with physical adsorption, the monolith surface can be completely covered by the polymer.

3.2.2. Enzyme activity

In Table 4, the protein loading and the specific activity per gram of protein in the hydrolysis of *o*NPG are presented. The activity of the free enzyme under the same conditions is included.

In general the activity per monolith volume is proportional to the amount of immobilized protein. ACM monoliths have a higher activity per monolith volume, because the open wall allows for more PEI to be deposited and more enzyme to be adsorbed. The GPTMS-PEI-samples have the highest enzyme loading, and the highest specific activity. For these samples, 92% of the free β-galactosidase activity is retained after immobilization. If an enzyme content of 5 wt.% is assumed for the β-galactosidase batch, a turnover frequency of 7 s^{-1} is found for this biocatalyst. This is close to the value of 8 s^{-1} that was observed for the free enzyme. The specific activity of

Table 4
Immobilization yield and catalyst performance in the hydrolysis of *o*NPG (295 K 0.2 mM *o*NPG, pH 7) for 200 cpsi cordierite and ACM-M monoliths

Catalyst	Total adsorption (mg)	Enzymatic activity	
		(mmol $m^{-3}_{monolith}$ s^{-1})	(mmol $g^{-1}_{protein}$ s^{-1})[a]
Free β-galactosidase			3.3×10^{-3}
C-GPTMS-PEI	77	3.9	2.9×10^{-3}
C-APTES-PEI	57	1.1	1.1×10^{-3}
ACM-M-GPTMS-PEI	151	9.0	3.0×10^{-3}
ACM-M-APTES-PEI	83	0.9	5.4×10^{-4}

[a] β-Galactosidase content in the crude protein is estimated to be 5%.

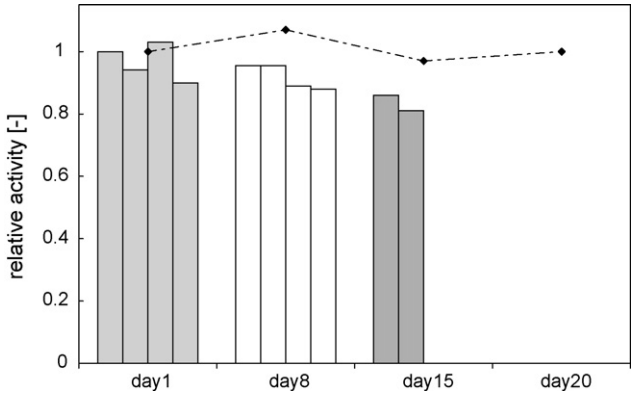

Fig. 5. Stability of a ACM-M GPTMS-PEI,25000 sample over 15 days in the hydrolysis of *o*NPG at 295 K, pH 7 (bars) and lactose 308 K, pH 4.5 (markers).

the APTES-PEI-samples is much lower (15–30% of the original activity). This is probably caused by the presence of free aldehyde groups, which result in chemical modification of the enzyme and a lower specific activity.

3.2.3. Deactivation and reuse

To study the stability of the immobilized β-galactosidase, an ACM-M-GPTMS-PEI-sample was assayed several times in the hydrolysis of *o*NPG during a period of 15 days. Between the subsequent tests, the sample was washed and stored under air at 278 K. A control sample was used in the hydrolysis of lactose at 308 K, pH 4.5 during the same period. This sample was stored in a 1 g l^{-1} sodium azide solution in a 5 mM phosphate buffer pH 7 at 278 K.

During a series of tests on the same day in the hydrolysis of *o*NPG, a slight deactivation is observed (Fig. 5). After storage at 278 K in air, the activity also decreased slightly. In general, slow deactivation of the biocatalysts is observed during both testing and storage. This indicates both product sensitivity and additional effects of storage in air. This is supported by the results of the control sample that was used in lactose hydrolysis and stored in liquid medium. This sample shows no deactivation over the same period of time.

To study the possibilities for repeated use of the monoliths, the enzyme was completely desorbed in a 9 M guanidine solution. Subsequent immobilization resulted in the same enzyme loading. This step was repeated five times, without any loss of capacity or immobilized activity (not shown).

3.3. Effect of polymer size on PEI loading and enzyme adsorption

The influence of the molar weight and bulkiness of the polymer was studied by using 60,000, 750,000 and ∼1,000,000 g mol^{-1} grades of branched PEI. The molecular weight of the polymer strongly influences the viscosity of the PEI solution. It is expected that for mass transport problems inside the monolith channels and/or wall will be more pronounced if a larger PEI size is used. To study the effect of PEI size in the absence of mass transfer limitations in the channels, crushed cordierite was loaded with different PEI sizes via the GPTMS-PEI method. It is expected that larger, branched PEI molecules provide a more stable environment and a stronger bond for the enzyme. So, if mass transfer problems would be present during polymer attachment, the polymer yield will be lower and the enzyme adsorption/desorption behavior will be distinctive. For the crushed carriers the adsorption (a) and desorption (b) curves of β-galactosidase are presented in Fig. 6.

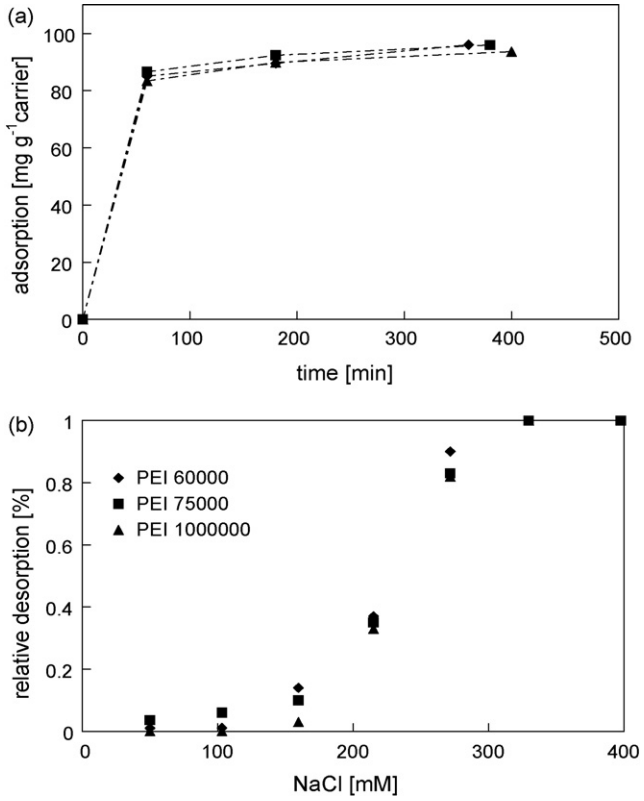

Fig. 6. Adsorption (a) and desorption (b) behavior of β-galactosidase on crushed cordierite with a PEI coating applied via the GPTMS-PEI method. Symbols represent different MW PEI.

No effect of PEI size was observed for adsorption on crushed cordierite. With respect to enzyme–polymer interaction, a stabilizing effect with increasing polymer size can be observed. The larger polymer provides a more stable environment against higher ionic strength, and shows a slower desorption until 160 mM NaCl. The smaller polymer is less effective, and the smallest polymer molecules (MW 60,000) provide the least stable environment, both resulting in a faster desorption. From these results it can be concluded that no large differences in polymer loading are present for the different carriers and that apparently no diffusion limitations are present inside the cordierite matrix during PEI attachment.

By using whole monoliths, possible mass transfer problems inside the monolith channels during PEI-coating can be identified. In Fig. 7 the enzyme adsorption on ACM (open symbols) and cordierite (closed symbols) monoliths, coated with different PEIs is presented.

In general, the ACM monoliths display a faster adsorption, the open symbols are found above the closed symbols after the same time interval, for all MWs of PEI. This suggests that the

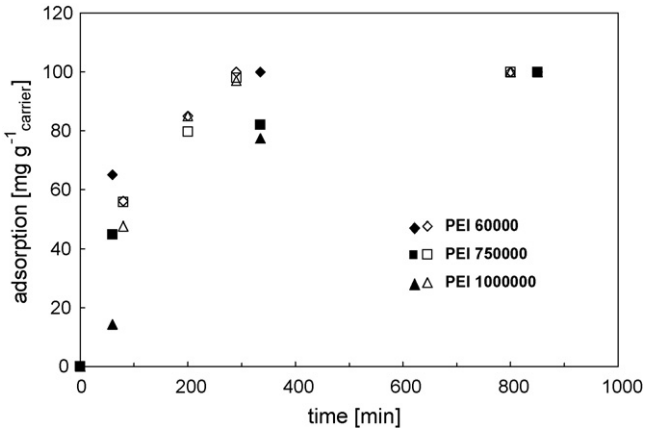

Fig. 7. β-Galactosidase adsorption from a 1 g l^{-1} solution at 295 K, pH 7 on ACM-M (open symbols) and cordierite (closed symbols) on carriers coated with different MW PEI.

open wall of the ACM allows for a higher polymer yield, resulting in a more efficient enzyme immobilization. For cordierite monoliths, the smallest PEI can cover the whole silica layer by relative fast diffusion through the channels, whereas attachment of larger polymer molecules is hindered by transport problems inside the channels. Therefore, the samples that were coated with the larger polymers have a slower enzyme adsorption. In general, all enzymes are completely adsorbed onto the supports after 20 h. But this process is still slower than for CPC-beads (see Fig. 3). This is caused by the higher relative concentration of PEI in the case of CPC (1 g of sample CPC–PEI contains more PEI than 1 g of sample monolith–silica–PEI). It is therefore difficult to compare the results of both experiments in a quantitative way.

From these results it can be expected that the cordierite-PEI samples have a weaker enzyme-carrier bond, causing faster desorption in high ionic strength environment than ACM monoliths. The desorption results are presented in Fig. 8.

As expected, desorption from the cordierite samples is faster than from the ACM samples (compare the open and closed symbols in Fig. 8). Two different effects can be observed in Fig. 8. At comparable loading (no mass transport limitations during polymer attachment), the larger PEI molecules provide a more stable polymer–enzyme bond. This effect can be seen in the

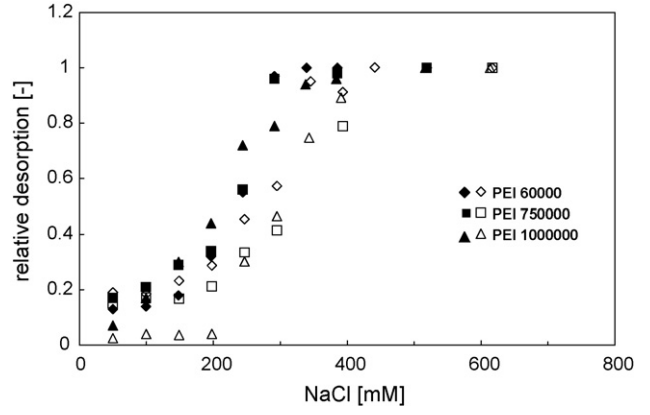

Fig. 8. β-Galactosidase desorption at 295 K from ACM-M (open symbols) and cordierite (closed symbols) carriers with different MW PEI.

ACM-GPTMS series. ACM-GPTMS-PEI,1000000 is very stable with respect to increasing ionic strength compared to the lower MW polymers. Up to 0.2 M NaCl, no desorption takes place. Secondly, a higher polymer loading provides a stronger enzyme–polymer interaction (when transport limitations are present, smaller PEI is easier to load); this effect can be seen in the C-GPTMS samples. The higher PEI loading of the C-GPTMS-PEI-60000 provides a more stable basis against forced desorption. For cordierite monoliths, the positive effect of the polymer size is not observed (see also Fig. 7). This is probably caused by distribution problems of the viscous polymer solutions during PEI-coating. Apparently, the open structure of the ACM monoliths allows for a better polymer distribution of the higher MW PEIs and also for a higher PEI loading than can be obtained for cordierite samples. This results in a stronger enzyme-carrier bond.

Attachment of PEI via a GPTMS linker has been found to be the optimal preparation method in combination with silica carriers. Compared to more common agarose–PEI carriers, the monolith–PEI system has a comparable enzyme adsorption capacity. A higher PEI loading on the monolith results in a stabilizing effect for the enzyme, this was concluded from a slower observed desorption at increasing ionic strength. Increasing the polymer size of the PEI coating also increases the strength of the enzyme-support bond. The use of a higher molecular weight is associated with a more viscous solution and possible mass transport problems inside the monolithic channels. For silica particles, no diffusion problems for addition of larger polymers were observed. The biocatalysts with larger MW PEI showed a delayed desorption at increasing ionic strength. For cordierite monoliths, transport problems during PEI addition lead to a decreased enzyme adsorption efficiency. Also the stabilizing effect of the larger polymer molecules had decreased for these samples. For the open ACM monoliths the diffusion problems during polymer addition are minimized, resulting in a faster enzyme adsorption and increased stability against desorption.

4. Conclusions

Cordierite and ACM monoliths with different microstructure were used as support material for a β-galactosidase from *A. oryzae*. Enzymes were immobilized via ionic adsorption on a polyelectrolyte polymer, polyethyleneimine (PEI). The open structure of the ACM provides a larger carrier deposition capacity, and results in a higher protein loading. Immobilization on a GPTMS-functionalized ACM monolith yields the best enzyme carrier. At pH 7, 150 mg enzyme can be deposited per gram of SiO_2. In general, a higher polymer loading on the monolith surface provides a more stable environment for the enzyme and this stabilizing effect increases with polymer size. With increasing molecular weight of the polymer, the ACM monoliths show an increased enzyme adsorption speed. This indicates a higher polymer loading and implies that the open ACM structure is less affected by mass transfer limitations during PEI-deposition compared to the cordierite monoliths. This results in a higher polymer loading for high molecular weight PEI on ACM and biocatalysts with increased stability at higher ionic strength.

Therefore, the open walls of the ACM monoliths provide an important advantage when used in this ionic adsorption protocol. These PEI systems provide an optimal environment for the enzyme; nearly the full (92%) activity of the free enzyme is retained after immobilization. The enzymes can be completely desorbed in consecutive adsorption–desorption cycles to facilitate reuse of the monolith-carrier combination.

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