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Fine-tuning the second generation sol–gel lipase immobilization with ternary alkoxysilane precursor systems

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ABSTRACT

The sol–gel immobilization of Celite-supported lipase from *Pseudomonas fluorencens* (Lipase AK) was systematically studied using ternary silane precursor systems consisting of alkyltriethoxysilane (alkyl-TEOS), phenyltriethoxysilane (PhTEOS) and tetraethoxysilane (TEOS). The parameters investigated were the surface coverage at various enzyme-Celite ratios (between 1:1 and 1:10) and the effect of molar ratio of alkylTEOS (alkyl = propyl, hexyl, octyl, 1*H*,1*H*,2*H*,2*H*-perfluorooctyl, decyl, dodecyl, octadecyl), PhTEOS and TEOS (seven series of alkylTEOS:PhTEOS:TEOS from 0.1:0.9:1 to 0.9:0.1:1 in 0.1 steps) on the catalytic properties of the sol–gel biocatalysts. For comparison, the corresponding binary systems (1:1 molar ratios of alkylTEOS:TEOS and PhTEOS:TEOS) were also studied. The ternary and binary sol–gel lipase preparations were evaluated by their catalytic behavior in enantiomer selective acetylation of racemic 1-phenylethanol and 2-heptanol. For each alkylTEOS precursor, one or more alkylTEOS/PhTEOS/TEOS ternary systems were superior biocatalysts compared to the corresponding alkylTEOS/TEOS preparations. The best overall results were achieved with the medium-chain octylTEOS and perfluorooctylTEOS-containing ternary systems.

1. Introduction

Lipases (EC 3.1.1.3) are versatile hydrolytic enzymes that can be obtained from animals and plants as well as from natural and recombinant microorganisms with good yields [1,2]. Lipases perform essential roles in the digestion, transport and processing of lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. Microbial lipases serve important everyday life roles as ancient as yogurt and cheese fermentation. However, lipases are also being exploited as cheap and versatile biocatalysts in more modern applications [3–5]. For instance, lipases are used in applications such as baking and laundry detergents and even as biocatalysts in alternative energy strategies to convert vegetable oil into biofuel [6]. Lipases are flexible biocatalysts which can catalyze a wide range of enantio- and regioselective reactions such as hydrolysis, esterifications, transesterifications, aminolysis and ammoniolysis [1,7,8]. The esterification, interesterification reaction or ester hydrolysis usually proceed with high regio- and/or enantioselectivity, making lipases an important group of biocatalysts in organic chemistry.

The development of biotechnology brings along the immobilization of biomolecules or microorganisms. For further industrial applications, immobilization of enzymes has been an active research topic in enzyme technology to enhance their activity, thermal and operational stability, and reusability [9–11].

Among many available immobilization methods, including adsorption, covalent attachment to solid supports and entrapment within polymers [9,10], entrapment of enzymes in inorganic/organic hybrid polymer matrices has received a lot of attention in recent years and has provided new possibilities in the field of material science [12,13]. Sol–gel encapsulation has proven to be a particularly easy and effective way to immobilize purified enzymes, whole cells, antibodies and other proteins [14–16]. Sol–gel immobilization of lipases can enhance their thermostability [19,17], long-term operational stability and storage life [24,25].

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A typical sol–gel immobilization process involves acid- or base-catalyzed hydrolysis, then polycondensation of alkoxysilane precursor [$Si(OR)_4$] in the presence of additives to form a matrix in which the enzyme is encapsulated. Using organically modified silanes of the type $R'–Si(OR)_3$, activity enhancement of the immobilized lipases could be observed with increasing amount and alkyl chain (R′) length of the hydrophobic silanes [18–26]. The optimal molar ratios of the trialkoxy- and tetraalkoxysilane precursors [$R'–Si(OR)_3$ and $Si(OR)_4$] were also investigated [18,24,26]. The presence of additives like polyethylene glycol, polyhydroxy compounds or proteins [25] can significantly enhance the catalytic activity of these enzymes. It was demonstrated that the presence of a small amount of isopropyl alcohol (IPA) during immobilization is beneficial [27,28]. The characterization of the sol–gel biocatalysts is also well documented [29].

The sol–gel immobilized lipases can be supported on inert materials such as Celite to improve the diffusion of the substrates or products to and from the enzyme and thus improve the reaction rate [21,26,30–33]. Mesoporous silica materials can be also effective supports during immobilization [34].

Although it is well known that the nature of the R′ substituent in the trialkoxysilane precursors may significantly influence the properties of the sol–gel lipases [18–26], only the binary sol–gel systems of trialkoxy- and tetraalkoxysilane precursors [$R'–Si(OR)_3$ and $Si(OR)_4$] for lipase immobilization have been studied [18,24,26]. According to our best knowledge, ternary sol–gel systems of a tetraalkoxysilane and two different trialkoxysilane precursors [$R^1–Si(OR)_3$, $R^2–Si(OR)_3$ and $Si(OR)_4$] have been studied only for cutinase (EC 3.1.1.74) [33]. Therefore, our goal was to perform a systematic study of the sol–gel immobilization of a Celite-supported lipase using ternary silane precursor systems of various alkyltriethoxysilanes (alkylTEOS), phenyltriethoxysilane (PhTEOS) and tetraethoxysilane (TEOS).

2. Materials and methods

2.1. Materials

Lipase AK (lipase from *Pseudomonas fluorescens*, Cat. No. 534730), 2-propanol (IPA), vinyl acetate and sodium fluoride (NaF) were products of Aldrich. 1-Phenylethanol, 2-heptanol, polyethylenglycol 1000 (PEG), Celite® 545 (Cat. No. 22140), tetraethoxysilane (TEOS), n-propyltriethoxysilane (PrTEOS) and phenyltriethoxysilane (PhTEOS) were obtained from Fluka. n-Hexyltriethoxysilane (HexTEOS), n-octyltriethoxysilane (OctTEOS), n-decyltriethoxysilane (DecTEOS), n-dodecyltriethoxysilane (DodTEOS), n-octadecyltriethoxysilane (OctdTEOS), and 1*H*,1*H*,2*H*,2*H*-perfluorooctyltriethoxysilane (PFOctTEOS) were obtained from Alfa Aesar.

2.2. Sol–gel immobilization of Lipase AK deposited on Celite® 545 using binary or ternary silane precursor systems

A two-step procedure was applied for sol–gel entrapment of Lipase AK on Celite 545.

Step 1: The Lipase AK powder (50 mg for the standard procedure; 50, 125, 250, 375 or 500 mg for the enzyme loading tests) was added to Tris–HCl buffer (0.1 M, pH 7.5, 780 μl) at 4 °C with stirring for 10 min followed by addition of Celite 545 (500 mg). Acetone (10 ml) was added to the well stirred Celite-enzyme mixture at 10 ml min^{-1} rate at −18 °C. The resulting solid was filtered off and left in air at room temperature for 12 h for drying. The Celite-enzyme preparations were then used for sol–gel immobilization.

Step 2: Tris–HCl buffer (0.1 M, pH 7.5, 390 μl), PEG solution (4%, w/v, 200 μl), NaF solution (1 M, 100 μl) and IPA (200 μl) were mixed in a 20 ml glass vial and the resulting solution was shaken at 1000 cycles per minute at room temperature for 10 min. During the continuous shaking, the corresponding silane precursors alkylTEOS–TEOS (1.5 mmol alkylTEOS and 1.5 mmol TEOS) or the mixture of alkylTEOS:PhTEOS:TEOS (3 mmol; the alkylTEOS:PhTEOS:TEOS molar ratio varied from 0.1:0.9:1 to 0.9:0.1:1 in 0.1 steps) and Celite-enzyme (250 mg) were added to the vial resulting in a sol suspension. To complete the polymerization, the mixture was shaken for 12 h at room temperature. The formed solid was washed with IPA (7 ml), distilled water (5 ml), IPA (5 ml) and n-hexane (5 ml). The resulting white powder was dried in a vacuum exicator for 5 h (until 0.4 mm Hg final level of vacuum). The sol–gel Lipase AK preparations were stored at room temperature.

2.3. Scanning electron microscopy

The surface morphology of the samples was investigated with a JEOL JSM-5500LV scanning electron microscope. The samples of free Lipase AK, Lipase AK on solid support and supported Lipase AK encapsulated in sol–gel matrices were coated with gold prior to analysis. Electron beam energy of 25 kV was used for the investigations.

2.4. Activity and enantiomer selectivity tests of the Lipase AK preparations

The free or sol–gel immobilized Lipase AK (50 mg) was added to the solution of racemic test alcohol (1-phenylethanol, *rac*-**1a**, 49 mg, 0.4 mmol; or 2-heptanol, *rac*-**1b**, 46 mg, 0.4 mmol) and vinyl acetate (100 μl) in hexane – THF 2:1 (1 ml), and the resulting mixture was shaken at 30 °C in a sealed glass vial at 1000 cycles per minute. For GC analyses, samples were taken directly from the reaction mixture (sample size: 10 μl, diluted with CH_2Cl_2 to 100 μl) at 2, 4 and 8 h. The esters were analyzed on an Acme 6100 instrument (Young Lin Instrument Co., South Korea) equipped with flame ionization detector and Hydrodex β-6TBDM [30 m × 0.25 mm × 0.25 μm film of heptakis-(2,3-di-O-methyl-6-O-*t*-butyldimethylsilyl)-β-cyclodextrin (Macherey & Nagel)] column (oven temperature, injector and detector temperatures were 130 °C, 250 °C and 250 °C, respectively; carrier gas:H_2 at a flow of 1.8 ml min^{-1}; split ratio:1:50). Data on conversion, enantiomeric composition of the products [(*R*)-2a,b and (*S*)-**1a**,**b**] and the calculated properties of the enzyme are presented in Tables 1 and 2 and Fig. 3.

The effective specific activity of the biocatalyst [U_B (μmol × min^{-1} × g^{-1})] could be determined in the test reaction from the amount of the racemic alcohol [n_{rac} (μmol)], the conversion [c], the reaction time [t (min)] and the mass of the free biocatalysts (LAK) or the free biocatalyst subjected to the sol–gel immobilized (imm-LAK) [m_B (g)].

$$U_B = \frac{n_{rac}c}{tm_B}$$

As the effective specific activities in the present work were calculated from a single-point experiment, the term "activity" must be considered only for comparative evaluation of the catalytic efficiencies, not for the real kinetic behavior of the biocatalyst.

The activity yield [Y_A (%)] can be calculated from the effective specific activity of the immobilized biocatalyst ($U_{B,imm\text{-}LAK}$) compared to the effective specific activity of the free Lipase AK ($U_{B,LAK}$). $U_{B,imm\text{-}LAK}$ can be determined in the test reaction from the amount of the racemic alcohol [n_{rac} (μmol)], the conversion [c], the reaction time [t (min)] and the mass of the free lipase subjected to the sol–gel immobilization [m_B (g)].

$$Y_A = \frac{100U_{B,imm\text{-}LAK}}{U_{B,LAK}} = 100\frac{(n_{rac}c)/(tm_B)}{U_{B,LAK}}$$

The enantiomer selectivity in a kinetic resolution can be calculated from two of the following three data: conversion [c], enantiomeric excess of the remaining alcohol [$ee_{(S)-1}$], enantiomeric excess of the forming ester [$ee_{(R)-2}$] [35,36].

3. Results and discussion

The beneficial properties of the sol–gel immobilized lipases may be significantly influenced by the nature of the R′ substituent in binary sol–gel systems of trialkoxy- and tetraalkoxysilane precursors [$R'–Si(OR)_3$ and $Si(OR)_4$] [18–26] but ternary sol–gel systems of a tetraalkoxysilane and two different trialkoxysilane precursors [$R^1–Si(OR)_3$, $R^2–Si(OR)_3$ and $Si(OR)_4$] had not been studied for lipase immobilization.

3.1. Selection of the lipase, support and silane precursors

In most cases, alkyltrimethoxysilanes (alkylTMOS's) were preferred for encapsulation of lipases. However, it was indicated that there is no significant difference in properties of encapsulated lipase biocatalysts prepared from alkylTMOS or alkylTEOS silane precursors [31]. Because alkylTEOS's are cheaper and the gelation time is longer and more controllable than with alkylTMOS's, the use of $R'–Si(OEt)_3$ and $Si(OEt)_4$ as silane precursors is preferable. The second generation sol–gel immobilized lipases are supported on inert materials to reduce diffusion limitations [21,26,30–33]. The lipase from *Pseudomonas fluorescens* (Lipase AK) proved to be a good lipase model for studies of factors influencing the sol–gel immobilization of lipases [22,31]. Because the esterification activity of immobilized lipases can be enhanced by

Table 1
Characterization of Celite-supported Lipase AK immobilized in binary sol–gel systems of alkylTEOS/TEOS silane precursors. Effective specific activity (U_B), activity yield (Y_A) and enantiomer selectivity (E) of the free and immobilized forms of Lipase AK are shown in Panel A (with 1-phenylethanol *rac*-**1a**) and Panel B (with 2-heptanol *rac*-**1b**).

No.	Silane precursors[a]	Substrate	Time (h)	c (%)	E[b]	U_B[c] (U g^{-1})	Y_A (%)
Panel A							
1	- (Lipase AK)	*rac*-**1a**	0.25	28	≫200	154.2	100
2	PrTEOS:TEOS	*rac*-**1a**	8	24	≫200	4.1	60
3	HexTEOS:TEOS	*rac*-**1a**	2	28	>200	19.0	181
4	OctTEOS:TEOS	*rac*-**1a**	4	25	≫200	8.4	77
5	PFOctTEOS:TEOS	*rac*-**1a**	2	24	>200	16.8	156
6	DecTEOS:TEOS	*rac*-**1a**	8	33	>200	5.7	54
7	DodTEOS:TEOS	*rac*-**1a**	8	27	≫200	4.6	44
8	OctdTEOS:TEOS	*rac*-**1a**	8	29	>200	5.0	44
9	PhTEOS:TEOS	*rac*-**1a**	2	39	≫200	26.8	242
Panel B							
10	- (Lipase AK)	*rac*-**1b**	1	28	13.8	38.4	100
11	PrTEOS:TEOS	*rac*-**1b**	8	0.3	1.2	0.1	7
12	HexTEOS:TEOS	*rac*-**1b**	8	17	12.6	2.9	109
13	OctTEOS:TEOS	*rac*-**1b**	8	8	10.8	1.3	49
14	PFOctTEOS:TEOS	*rac*-**1b**	8	22	12.2	3.7	139
15	DecTEOS:TEOS	*rac*-**1b**	8	8	11.0	1.3	51
16	DodTEOS:TEOS	*rac*-**1b**	8	4	10.6	0.6	25
17	OctdTEOS:TEOS	*rac*-**1b**	8	5	10.0	0.9	32
18	PhTEOS:TEOS	*rac*-**1b**	8	30	14.7	5.2	187

[a] Lipase AK was preadsorbed on Celite 545 at 1:10 enzyme:support ratio and entrapped with alkylTEOS:TEOS silane precursors at 1:1 molar ratio.
[b] The enantiomer selectivity (E) was calculated from c and $ee_{(S)-1}/ee_{(R)-2}$ [35,36]. Due to sensitivity to experimental errors, E values calculated in the 100–200 range are reported as >100, values in the 200–500 range are reported as >200 and values calculated above 500 are given as ≫200.
[c] In entries 1 and 10 U_B refers to the free Lipase AK ($U_{B,LAK}$) and in entries 2–9 and 11–18 U_B refers to the amount of free Lipase AK subjected to immobilization ($U_{B,imm\text{-}LAK}$).

forming the entrapping-gel on the surface of Celite [19,26], fine-tuning the sol–gel immobilization process of Lipase AK deposited on Celite 545 was selected as the subject for this study. Thus, the properties of Celite-supported Lipase AK immobilized with ternary silane precursor systems of various alkyltriethoxysilanes (alkylTEOS), phenyltriethoxysilane (PhTEOS) and tetraethoxysilane (TEOS) were investigated in this work.

3.2. Adsorption of Lipase AK on Celite 545

A two-step immobilization protocol involving pre-adsorption of the enzyme on solid support followed by sol–gel entrapping was applied. Thus, to select the ideal enzyme distribution on the solid support in the first step, various amounts of Lipase AK were adsorbed on Celite 545 as solid support by precipitation from aqueous solution with the slow addition of cold acetone.

Celite, also known as diatomite or kieselgur, is a naturally occurring, inexpensive fossilic diatomaceous earth. It consists of fossilized remains of diatoms, a type of hard-shelled algae, ranging in size from ca. 2 to 200 μm [37], that are composed of a cell wall comprising silica [38]. This siliceous wall can be highly patterned with a variety of pores, ribs, minute spines, marginal ridges and elevations.

Table 2
Characterization of the best performing Celite-supported ternary sol–gel Lipase AK biocatalysts prepared from alkylTEOS/PhTEOS/TEOS silane precursors. Effective specific activity (U_B), activity yield (Y_A) and enantiomer selectivity (E) of the free and immobilized forms of Lipase AK are shown in Panel A (with 1-phenylethanol *rac*-**1a**) and Panel B (with 2-heptanol *rac*-**1b**).

No.	Triethoxy silane precursors[a]	Substrate	AlkylTEOS amount[b] (%)	Time (h)	c (%)	E[c]	U_B[d] (U g^{-1})	Y_A (%)
Panel A								
1	- (Lipase AK)	*rac*-**1a**	–	0.25	28	≫200	154.2	100
2	PrTEOS:PhTEOS	*rac*-**1a**	60	8	25	≫200	4.3	52
3	HexTEOS:PhTEOS	*rac*-**1a**	60	4	35	>200	12.2	116
4	OctTEOS:PhTEOS	*rac*-**1a**	70	4	34	≫200	11.8	109
5	PFOctTEOS:PhTEOS	*rac*-**1a**	90	2	24	≫200	16.7	181
6	DecTEOS:PhTEOS	*rac*-**1a**	50	8	27	≫200	4.6	18
7	DodTEOS:PhTEOS	*rac*-**1a**	30	2	20	≫200	13.6	53
8	OctdTEOS:PhTEOS	*rac*-**1a**	60	4	30	≫200	10.2	99
9	PhTEOS	*rac*-**1a**	0	2	39	≫200	26.8	242
Panel B								
10	- (Lipase AK)	*rac*-**1b**	–	1	28	13.8	38.4	100
11	PrTEOS:PhTEOS	*rac*-**1b**	60	8	4.7	11.6	0.8	40
12	HexTEOS:PhTEOS	*rac*-**1b**	60	8	6	10.2	1.0	35
13	OctTEOS:PhTEOS	*rac*-**1b**	70	8	19	12.3	3.2	64
14	PFOctTEOS:PhTEOS	*rac*-**1b**	90	8	27	12.3	4.7	205
15	DecTEOS:PhTEOS	*rac*-**1b**	50	8	2.6	8.3	0.1	32
16	DodTEOS:PhTEOS	*rac*-**1b**	30	8	21	13.5	3.6	128
17	OctdTEOS:PhTEOS	*rac*-**1b**	60	8	16	15.6	2.8	108
18	PhTEOS	*rac*-**1b**	0	8	30	14.7	5.2	187

[a] Lipase AK was preadsorbed on Celite 545 (1:10 enzyme:support ratio) and entrapped in a sol–gel from alkylTEOS:PhTEOS:TEOS silane precursors at x:(100 − x):100 molar ratio.
[b] Amount (x) of alkylTEOS in the triethoxysilane mixture.
[c] The enantiomer selectivity (E) was calculated from c and $ee_{(S)-1}/ee_{(R)-2}$ [35,36]. Due to sensitivity to experimental errors, E values calculated in the 100–200 range are reported as >100, values in the 200–500 range are reported as >200 and values calculated above 500 are given as ≫200.
[d] In entries 1 and 10 U_B refers to the free Lipase AK ($U_{B,LAK}$) and in entries 2–9 and 11–18 U_B refers to the amount of free Lipase AK subjected to immobilization ($U_{B,imm\text{-}LAK}$).

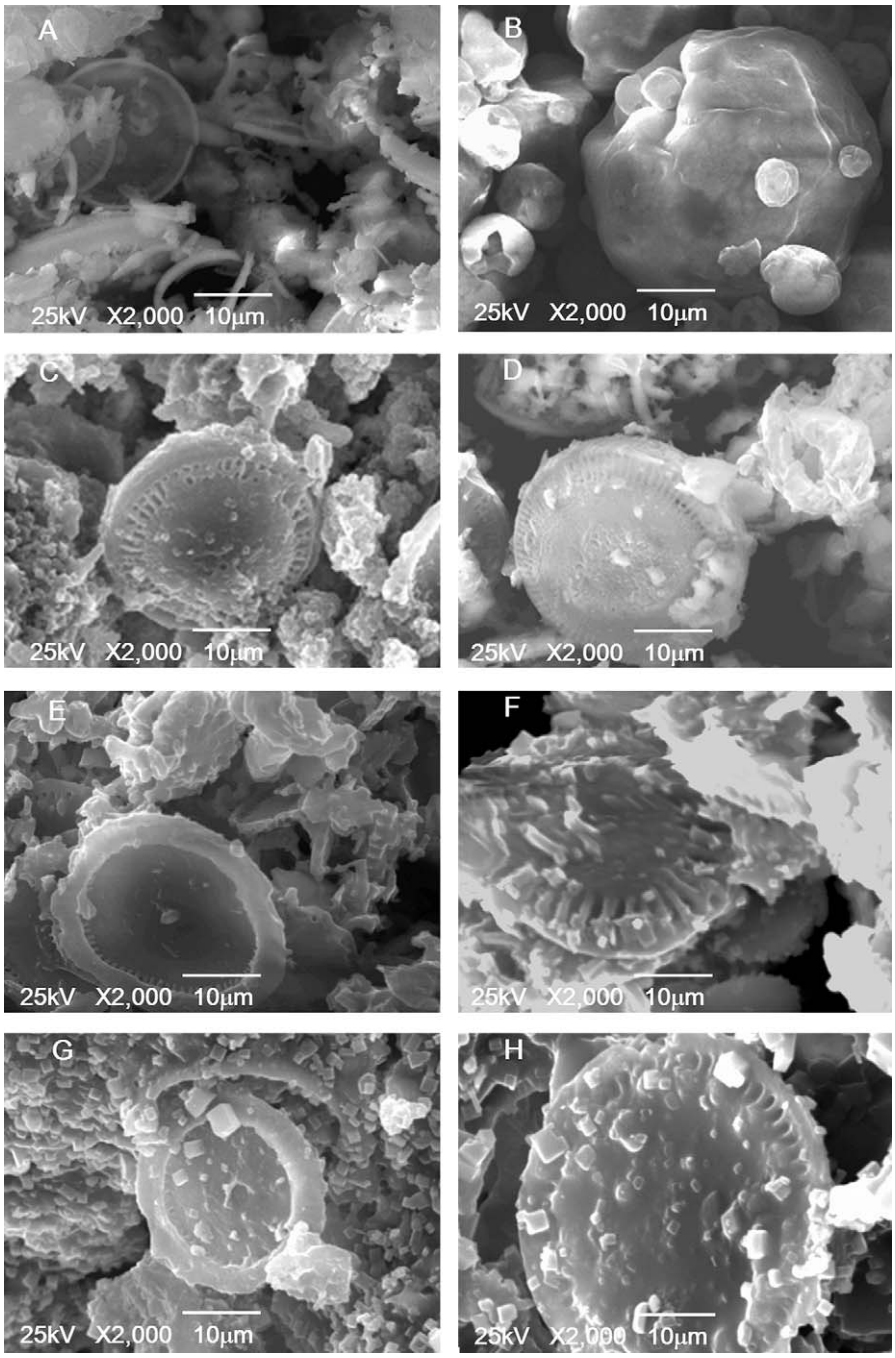

Fig. 1. SEM images of Celite (A), free Lipase AK (B), Celite-supported Lipase AK immobilized in octylTEOS/PhTEOS/TEOS 0.7/0.3/1 sol–gel (C) and various amounts of Lipase AK precipitated onto Celite [Celite/Lipase AK ratio: 10:1 (D), 4:1 (E), 10:5 (F), 4:3 (G) and 1:1 (H)].

To study the surface coverage of Celite, supported lipase AK preparations of different enzyme-Celite ratios (1:10, 1:4, 1:2, 3:4 and 1:1) were made. The free Celite (Fig. 1(A)), free Lipase AK (Fig. 1(B)), a sol–gel entrapped Lipase AK on Celite (Fig. 1(C)) and Celite preparations covered with various amounts of Lipase AK (Fig. 1(D)–(H)) were inspected by scanning electron microscopy (SEM).

The SEM investigations indicated that the commercial Lipase AK consists of relatively large spherical aggregates (4–40 μm diameter, Fig. 1(B)). This size distribution indicates that substantial diffusion limitation may occur when the free form of Lipase AK is used for catalysis in organic media. Visual inspection of the SEM images of the free Celite revealed that the disc-shaped particles in Celite (Fig. 1(A)) are the best forms to investigate the coverage of the solid support (Fig. 1(C)–(H)).

The SEM pictures indicated that at increased amounts of the precipitated enzyme on Celite more and more monoclinic or orthorhombic particles were formed on the surface of the support (Fig. 1(D)–(H)). It is known that lipases from different *Pseudomonas* strains crystallize in similar crystal forms; for example the lipase from *P. cepacia* (PDB code: 1YS1) forms monoclinic crystals [39], while the lipase from *P. aeruginosa* (PDB code: 1EX9) has orthorombic crystal structure [40]. Because the lipase from *P. fluorescens* (Uniprot code A9YY76) exhibits high amino acid sequence homology with lipase of *P. cepacia* (37% identity, 53% homology) and *P. aeruginosa* (45% identity, 60% homology), it can be supposed that

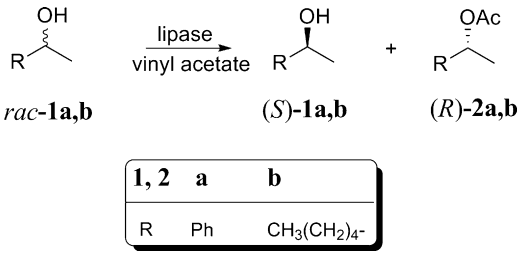

Fig. 2. Kinetic resolution of racemic secondary alcohols *rac*-**1a,b** as test reaction for characterization of the free and immobilized Lipase AK biocatalysts.

Lipase AK of *P. fluorescens* origin forms similar crystals (typically of 1–5 μm size, Fig. 1(F)–(H)) as lipases from *P. aeruginosa* or *P. cepacia*. It is then understandable that the effective specific activity of the enzyme decreases at higher enzyme loading due to the increasing diffusion limitation within these relatively large crystals. According to the morphology studies, the 1:10 enzyme-Celite ratio provided thin and uniform coating on the surface of Celite (Fig. 1(D)) and was selected for the further investigations. The SEM image of one of the best performing ternary sol–gel immobilized preparations (Fig. 1(C); Celite:Lipase AK 10:1, entrapped in octylTEOS/PhTEOS/TEOS 0.7/0.3/1 sol–gel) indicated that surface coating of Celite remained uniform and thin after the sol–gel entrapping step as well.

3.3. Effect of the composition of alkylTEOS–PhTEOS–TEOS silane precursors on the properties of sol–gel Lipase AK supported on Celite 545

The effect of the silane precursor composition on enantiomer selectivity and catalytic ability were investigated in the kinetic resolution of racemic secondary alcohols (Fig. 2). All the binary and ternary sol–gel preparations were tested with the selective acylation of 1-phenylethanol (*rac*-**1a**), in hexane-THF using vinyl acetate as acyl donor (Fig. 3, Panels A in Tables 1 and 2). Acylation of racemic 2-heptanol (*rac*-**1b**), exhibiting moderate enantiomer selectivity with free Lipase AK, was also investigated with the binary (Panel B of Table 1) and selected ternary sol–gel preparations (Panel B of Table 2). To evaluate the efficiency of the immobilization and biocatalysts, the specific activities (U_B), and activity yields (Y_A) and enantiomer selectivities (E) with substrate *rac*-**1a** were compared at ~30% conversion in all the cases. The enantiomer selectivity (E) and activity of the free Lipase AK under identical reaction conditions and conversion ranges were the references in all the cases.

It had already been indicated that mixtures of R′-Si(OEt)$_3$ and Si(OEt)$_4$ at 1:1 molar ratio provided ideal matrices for the sol–gel process [18]. Accordingly, the binary systems [R–Si(OEt)$_3$:TEOS = 1:1] of eight different triethoxysilanes (PrTEOS, HexTEOS, OctTEOS, PFOctTEOS, DecTEOS, DodTEOS, OctdTEOS, PhTEOS) were investigated first in the acylation reaction of 1-phenylethanol (*rac*-**1a**, Panel A of Table 1) and 2-heptanol (*rac*-**1b**, Panel B of Table 1). In the cases of HexTEOS, PFOctTEOS and PhTEOS, the activity yield (Y_A) for the binary sol–gel entrapped Lipase AK exceeded 100% with both test alcohols (Table 1). The best enantiomer selectivities (E) were achieved with the binary systems of PrTEOS, OctTEOS, DodTEOS and PhTEOS for *rac*-**1a** (Panel A of Table 1) and with the binary sol–gel biocatalysts containing HexTEOS, OcTEOS, PFOctTEOS and PhTEOS for *rac*-**1b** (Panel B of Table 1).

From the data with the R–Si(OEt)$_3$:TEOS = 1:1 systems, it was obvious that among the binary systems the PhTEOS:TEOS = 1:1 composition resulted in optimal properties regarding both activity and selectivity.

Next, by keeping the beneficial PhTEOS as one of the three silane precursors, we tried fine-tuning of the sol–gel system by using ternary systems of alkylTEOS:PhTEOS:TEOS silane precursors in various ratios. Thus, the alkylTEOS:PhTEOS molar ratio was varied from 0.1 to 0.9 in 0.1 steps while keeping the trialkoxysilane (alkylTEOS:PhTEOS):tetraalkoxisilane (TEOS) molar ratio at 1:1. The properties of the resulting series of ternary sol–gel biocatalysts were characterized by testing their catalytic behavior in the kinetic resolution of 1-phenylethanol *rac*-**1a** (Fig. 3). The effective specific activity (U_B), activity yield (Y_A) and enantiomer selectivity (E) values of the enantiomer selective acetylation of racemic 1-phenylethanol *rac*-**1a**, calculated at the initial part of the reaction (at 4 h), were compared for the ternary sol–gel Lipase AK biocatalysts, the PhTEOS:TEOS binary sol–gel immobilized Lipase AK and free Lipase AK. Comparison of the ternary systems to the best binary biocatalyst (PhTEOS:TEOS = 1:1) at 4 h indicated that properties of numerous ternary compositions were superior to the PhTEOS:TEOS binary system in all the aspects (Fig. 3). In general, the U_B and Y_A values, related to the productivity of the biocatalysts with racemic 1-phenylethanol *rac*-**1a**, were the best for ternary compositions prepared from medium chain alkylsilane precursors (HexTEOS, OctTEOS, PFOctTEOS), while enantiomer selectivities (E)

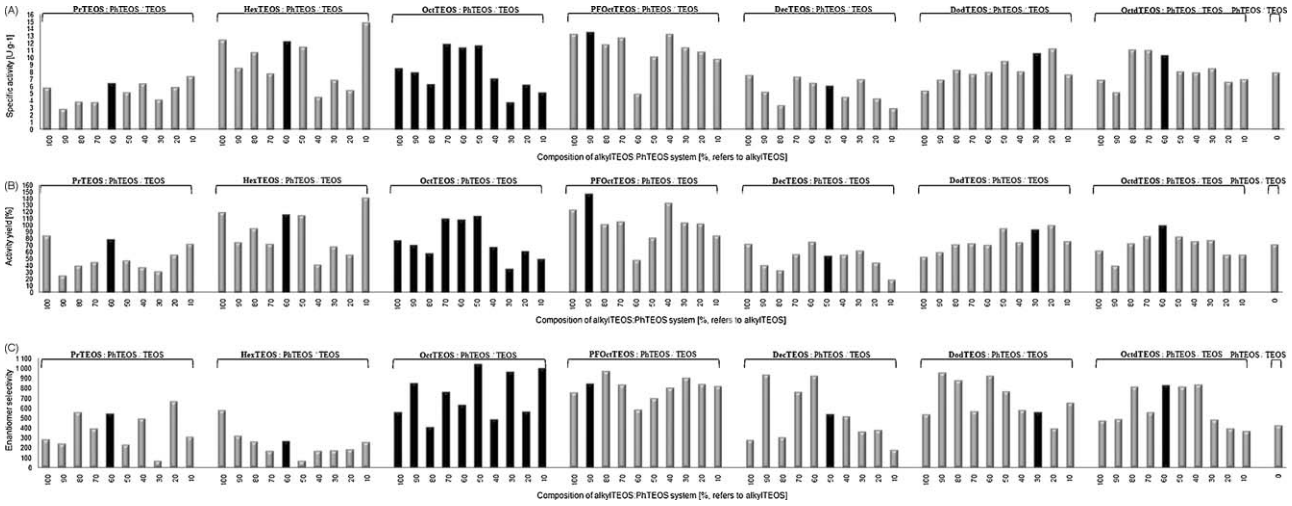

Fig. 3. Characterization of Celite-supported ternary sol–gel Lipase AK biocatalysts prepared from alkylTEOS/PhTEOS/TEOS silane precursors. Effective specific activity, U_B (A); activity yield, Y_A (B) and enantiomer selectivity, E (C) of the free and immobilized forms of Lipase AK are calculated at 4 h reaction time for enantiomer selective acetylation of racemic 1-phenylethanol *rac*-**1a**. The octylTEOS series and representatives of the other alkylTEOS systems included in Table 2 are shown in black.

were sufficient for almost all the longer alkylTEOS precursors (HexTEOS, OctTEOS, PFOctTEOS, DecTEOS, DodTEOS, OctdTEOS). Among all the ternary systems, the perfluorinated chain containing PFOctTEOS series exhibited the best overall performance. Taking the price of PFOctTEOS also into account, however, the OctTEOS series provided the best performance/price result in the kinetic resolution of 1-phenylethanol *rac*-**1a**.

Next, the OctTEOS series and a well performing member of each other alkylTEOS ternary series (marked in black in Fig. 3) were investigated in the kinetic resolution of 2-heptanol *rac*-**1b**, a moderate substrate of Lipase AK. Comparison of the data from the kinetic resolutions of the two different secondary alcohols, 1-phenylethanol *rac*-**1a** and 2-heptanol *rac*-**1b** with the binary (Table 1) and the selected ternary (Table 2) sol–gel systems revealed that different silane precursor compositions resulted in the best activities and selectivities for the two substrates *rac*-**1a**,**b** (Panels A for *rac*-**1a** and Panels B for *rac*-**1b** in Tables 1 and 2, respectively).

In the case of the kinetic resolution of 1-phenylethanol *rac*-**1a** at around 30% conversion (Panels A in Tables 1 and 2), the addition of PhTEOS to longer chain alkylTEOS (OctTEOS, PFOctTEOS, DecTEOS, DodTEOS, OctdTEOS) resulted in ternary sol–gel systems with enhanced activity yield (Y_A) and enantiomer selectivity (E) (entries 4–8 in Table 2) compared to the corresponding binary systems (entries 4–8 in Table 1).

In the case of the kinetic resolution of 2-heptanol *rac*-**1b**, encapsulation in all the alkylTEOS containing binary systems resulted in decreased selectivity (entries 11–17 in Table 1, E = 1.2–12.6) compared to the free Lipase AK (entry 10 in Table 1, E = 13.8). Only the binary PhTEOS system had a slight selectivity enhancement in acylation of *rac*-**1b** (entry 18 in Table 1, E = 14.7). Not surprising, that addition of PhTEOS to alkylTEOS in the ternary systems enhanced the selectivity of the encapsulated lipase in all the cases except DecTEOS (Panel B in Table 2). In the ternary systems, the effective specific activity (U_B) and activity yield (Y_A) were also significantly improved (Panel B in Table 2) compared to the pure alkylTEOS containing binary systems (Panel B in Table 1) with only two exceptions, HexTEOS and DecTEOS (entries 12 and 15 in Table 2). As found earlier with 1-phenylethanol *rac*-**1a**, there were ternary systems that surpassed the selectivity (E = 15.6, entry 17 in Table 2) or productivity (Y_A = 205%, Entry 14 in Table 2) of the pure binary PhTEOS:TEOS system (entry 18 in Table 1) in the kinetic resolution of 2-heptanol *rac*-**1b** as well.

4. Conclusions

Effects of the surface coverage and fine-tuning the properties were investigated for the sol–gel entrapment of Celite-supported Lipase AK.

SEM investigations showed that preparations of lower Lipase AK:Celite ratio (1:10 and 1:4) had uniform and thin enzyme coverage, whereas higher enzyme loading (1:2, 3:4 or 1:1 ratios) resulted in thick enzyme layers containing large crystal-like particles. The enzyme-containing layer remained uniform and thin after the sol–gel entrapment of the Celite-supported Lipase AK of low enzyme loading (1:10) resulting in significant increases in activity yields (Y_A > 200%) by lowering the diffusion limitations which are more pronounced in the larger aggregates of free Lipase AK.

The study of the catalytic behavior of binary and ternary sol–gel biocatalysts in kinetic resolutions of 1-phenylethanol *rac*-**1a** and 2-heptanol *rac*-**1b** using silane precursor systems consisting of alkylTEOS, PhTEOS and TEOS indicated the possibility of fine-tuning with and the importance of ternary sol–gel systems for lipase entrapment. For both substrates *rac*-**1a**,**b**, there were found one or more alkylTEOS:PhTEOS:TEOS ternary systems which surpassed the catalytic properties of any alkylTEOS:TEOS or PhTEOS:TEOS binary systems. Our study also indicated that different sol–gel compositions were optimal for the two different substrates. Although the medium-chain octylTEOS- and perfluorooctylTEOS-containing ternary systems performed the best in general, this study indicated that individual fine-tuning might develop the best biocatalyst for each individual substrate.

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