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Immobilization of ω-transaminases by encapsulation in a sol–gel/celite matrix

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ABSTRACT

Commercially available ω-transaminases ω-TA-117, -113, and *Vibrio fluvialis* (Vf-AT) have been immobilized in a sol–gel matrix. Improved results were obtained by employing Celite 545 as additive. The immobilized ω-transaminases ω-TA-117, -113, and *V. fluvialis* (Vf-AT) were tested in the kinetic resolution of α-chiral primary amines. In contrast to the free enzyme ω-TA-117, the sol–gel/celite immobilized enzyme showed activity even at pH 11. Recycling of the sol–gel/Celite 545 immobilized ω-transaminase ω-TA-117 was performed over five reaction cycles without any substantial loss in enantioselectivity and conversion. Finally, the immobilized ω-TA 117 was employed in a one-pot two-step deracemization of rac-mexiletine and rac-4-phenyl-2-butylamine, two pharmacologically relevant amines. The corresponding optically pure (S)-amines were obtained in up to 95% isolated yield (>99% ee).

1. Introduction

Aminotransferases or transaminases (TAs) (EC 2.6.1.X) are pyridoxal 5′-phosphate (PLP) dependent enzymes which are ubiquitous in nature being responsible for transferring amino groups [1]. Recently, ω-transaminases have gained increased attention due to their potential for the preparation of chiral amines [2–9], which are needed in optically pure form e.g. by the pharmaceutical industry [6,10–15].

For expensive enzyme preparation recycling of the biocatalyst is compulsory to run economic viable processes [16,17]. Recycling/reusing enzymes can be accomplished for instance by immobilization of the enzyme [18–20]. Immobilized enzymes are considered to be more stable with wide potential applications ranging from chemical synthesis to biotechnology and medicines [21–24]. To the best of our knowledge, ω-transaminases have only been immobilized by covalent attachment to different supports [25] but not by sol–gel entrapment. Sol–gel entrapment is a particularly easy and effective method to immobilize enzymes [26,27], since it involves the simple to perform transition of a liquid 'sol' into a solid 'gel' phase. Various mechanisms underlying sol–gel processes have been the subject of several books and reviews [28–30]. Generally, the silane precursor, $R'Si(OR'')_3$ undergoes acid- or base-catalyzed hydrolysis and cross-linking condensation with formation of an SiO_2 matrix in which the enzyme is encapsulated [31]. Regarding to porous structure in nanometer dimensions, sol–gel materials offer unique intrinsic properties, such as high surface to volume ratio, large surface area and porosity. Furthermore, nanoporous materials are usually nontoxic, inert, chemically and thermally stable, so they are applicable where biocompatibility and thermal stability is required. Due to the low temperature processing, the sol–gel technology represents a useful methods to immobilize sensitive biomolecules [32]. The sol–gel encapsulation in silica matrix has been found as an excellent method to prepare high-performance enzymes, since the immobilized catalysts typically showed improved resistance to thermal and chemical denaturation, as well as enhanced storage and operation stability [33].

2. Experimental

2.1. Materials and instruments

The amine rac-1-(2,6-methoxy)-2-aminopropane (rac-mexiletine), rac-4-phenyl-2-butylamine, Tween 80®, Celite 545, tetramethyl orthosilicate (TMOS), trimethoxypropylsilane (TMOPS), isobutyltrimethoxysilane (iBTMOS), poly(vinyl alcohol) (PVA), sodium fluoride, 2-propanol, glucose as well as solvents were commercially available from Sigma–Aldrich (Vienna, Austria) and were used as received unless otherwise stated. The solvents were of analytical grade. ω-Transaminases ω-TA 117, ω-TA-113, ω-TA-114, and from *Vibrio fluvialis* as well as D-glucose dehydrogenase (475 U/mL, order no. 29.10) and NAD-specific formate dehydrogenase (200 U/mL, order no. FDH-101) were obtained from Codexis® Inc. L-Lactate dehydrogenase from bovine heart (Type III, ammonium sulfate suspension, ≥500 U/mg protein, one unit will

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reduce 1.0 μmol of pyruvate to L-lactate per min at pH 7.5 at 37 °C, catalog no. L2625) and L-alanine dehydrogenase from Bacillus subtilis [buffered aqueous glycerol solution, 30 U/mg protein (Lowry), one unit will convert 1.0 μmol of L-alanine to pyruvate and NH_3 per min at pH 10.0 at 25 °C, catalog no. A7653] were purchased from Sigma–Aldrich. Optical rotations were measured on a PerkinElmer Polarimeter 341 in a 1 mL cuvette of 10 cm length. ^1H and ^{13}C NMR were recorded on a Bruker 300 MHz spectrometer at 300 and 75 MHz, respectively, using TMS as internal standard. The conversion of amine was measured by gas chromatography using Agilent GC-MS 5975C that was equipped with a coated DB-1701 DF 0.25 column (30 m × 0.25 mm, Agilent Technologies). All ee values were analyzed by using a Varian GC 3900 that was equipped with a coated CP Chirasil-Dex CB DF 0.25 column (25 m × 0.32 mm, Varian, Inc.).

2.2. Sol–gel entrapment

Sol–gel entrapment of ω-transaminases was performed according to method described by Reetz et al. with slight modifications [34]. A commercial ω-transaminase (lyophilizate) (100 mg) was dissolved in TRIS/HCl buffer (100 mM, pH 7.5, 400 μL, 1 mM pyridoxal 5′-phosphate). Celite 545 (50 mg) or/and Tween 80® (60 mg) were employed as additives if required and the mixture was vigorously shaken with a vortex-mixer. Then aqueous PVA (4% w/v, 100 μL), aqueous sodium fluoride (1 M, 50 μL) and isopropyl alcohol (50 μL) were added, and the mixture was again vigorously shaken with a vortex-mixer. Alkylsilane (2.5 mmol) and TMOS (0.5 mmol, 74 μL) were added and the mixture agitated for 20 s, followed by drying over night at room temperature in opened tubes. The gel was washed with distilled water (20 mL); during this step a spatula was used to crush the gel. In case of immobilized ω-TA-117, 2-propanol (10 mL) was used before water to wash the immobilizate as described by Reetz et al. Thereafter the immobilized ω-transaminase was dried exposed to air at room temperature. The enzyme concentration in the washing liquid was estimated by the Bradford's method [35]. The amount of immobilized protein was estimated to be between 20% and 35% depending on the experiment.

2.3. Transaminase assay

The free (4 mg) and immobilized ω-transaminase (50 mg) was performed at 30 °C for 24 h in sodium phosphate buffer (100 mM, pH 7 with 50 mM sodium pyruvate) containing pyridoxal 5′-phosphate monohydrate (1 mM) in 2 mL eppendorf tube. The reaction mixture contained 50 mM of rac-amine. The conversion to ketone was followed by GC chromatography. The reaction was stopped by adding aqueous NaOH (200 μL, 10N), followed by extraction with ethyl acetate (600 μL, twice). The organic phase was dried (Na_2SO_4) and the conversion was measured by GC using nitrogen as carrier gas.

2.4. Determination of enantiomeric excess

The enantiomeric excess of amines **1a** and **1b** was analyzed by gas chromatography on a chiral phase after derivatization to the acetoamide derivatives, which was performed by adding DMAP and 20-fold excess of acetic acid anhydride. The derivatization reaction was performed in ethyl acetate for 3 h at 40 °C, followed by adding distilled water to hydrolyze the excess of acetic acid anhydride. After drying the organic phase (Na_2SO_4) the ee of the derivatized compound was measured by GC on a chiral phase. Amine **1c** was analyzed after derivatization to trifluoroacetamide, which was performed by adding a 20-fold excess of trifluoroacetic acid anhydride. The derivatization reaction was performed in ethyl acetate for 3 h at 20 °C, followed by evaporating of the excess of anhydride under reduced pressure. After adding a fresh portion of ethyl acetate (600 μL) the ee of the derivatized compound was measured by GC on a chiral phase using hydrogen as carrier gas.

GC program parameters: injector 220 °C; flow 12.5 psi; temperature program 100 °C/hold 2 min; 130 °C/rate 1 °C per min/hold 5 min; 170 °C/rate 20 °C per min/hold 5 min. Retention times: (*S*)-**1a** 38.8 min, (*R*)-**1a** 40.1 min; (*S*)-**1b** 26.5 min, (*R*)-**1b** 28.6 min; (*S*)-**1c** 31.0 min, (*R*)-**1c** 30.1 min.

2.5. Enzyme recycling

The recycling of the sol–gel/Celite 545 immobilized ω-TA 117 (50 mg) was studied for the kinetic resolution of rac-4-phenyl-2-butylamine (**1a**) at 30 °C. The experiments were performed according to Section 2.3. Each reaction was terminated after 24 h by filtration. Collected immobilized enzyme was washed with distilled water (3 × 5 mL) and used in the successive cycle.

2.6. Temperature studies

The temperature study was performed employing sol–gel/Celite 545 immobilized ω-TA 117 545 (50 mg) for the kinetic resolution of rac-4-phenyl-2-butylamine (**1a**) at varied temperature. The conversion was determined according to Section 2.3.

2.7. pH studies

The pH study was performed employing sol–gel/Celite 545 immobilized ω-TA 117 (50 mg) and rac-4-phenyl-2-butylamine (**1a**) as substrate. Depending on the pH three different buffers were used: citrate buffer (pH 4, 100 mM), phosphate buffer (pH 7, 100 mM) and borate buffer (pH 9 and 11, 100 mM). The conversion was determined according to Section 2.3.

2.8. Preparation of optically pure (R)-4-phenyl-2-butylamine (*1a*) using immobilized ω-TA from V. fluvialis via kinetic resolution

In a plastic screw tube (50 mL) rac-4-phenyl-2-butylamine (**1a**) (100 mg, 0.67 mmol, 33 mM) was suspended in phosphate buffer (20 mL, 100 mM, pH 7.0, 1 mM PLP). Sodium pyruvate (74 mg, 0.67 mmol, 33 mM), and the sol–gel/Celite 545 immobilized ω-TA from *V. fluvialis* (300 mg) were added and the reaction was shaken at 30 °C (600 rpm) for 24 h. The pH of the mixture was adjusted to pH 1 employing aqueous HCl (5 M), and the remaining ketone was extracted five times with dichloromethane (5 × 10 mL). After the extraction, no ketone was detectable anymore in the residual aqueous phase. The pH was adjusted to pH 12, and the amine was extracted four times with dichloromethane (4 × 10 mL). The solvent of the combined extracts was evaporated and (R)-4-phenyl-2-butylamine (R)-**1a** was obtained with 48% yield (48 mg) at 51% of conversion; >99% ee; $[\alpha]_D^{20}$ −6.55, c 1.0, $CHCl_3$, [lit. 6.40, c 0.47, $CHCl_3$ for (S)-enantiomer, 98% ee) [36]; ^1H NMR (300 MHz) δ 1.10 (d, 3H, J = 6.70 Hz), 1.32 (s, 2H), 1.50-1.75 (m, 2H), 2.54-2.73 (m, 2H), 2.85-2.95 (m, 1H), 7.10-7.50 (m, 5H).

2.8.1. Synthesis of optically pure (S)-4-phenyl-2-butylamine (*1a*) using immobilized ω-TA 117 via kinetic resolution

In a screw tube (50 mL) rac-4-phenyl-2-butylamine (**1a**) (100 mg, 0.67 mmol, 33 mM) was suspended in phosphate buffer (20 mL, 100 mM, pH 7.0, 1 mM PLP). Sodium pyruvate (74 mg, 0.67 mmol, 33 mM), and the sol–gel/Celite 545/Tween 80® immobilized ω-TA-117 (250 mg) were added and the reaction was shaken at 30 °C (600 rpm) for 24 h. After work-up as described above (S)-

1a was obtained with 43% yield (43 mg); >99% ee; $[\alpha]_D^{20}$ 7.1, c 1.0, $CHCl_3$, [lit. 6.40, c 0.47, $CHCl_3$ for (S)-enantiomer, 98% ee] [36].

*2.8.2. Synthesis of optically pure (S)-phenylethylamine (**1b**) using immobilized ω-TA 117 via kinetic resolution.*

In a screw tube (50 mL) rac-phenylethylamine (**1b**) (100 mg, 0.82 mmol, 41 mM) was suspended in phosphate buffer (20 mL, 100 mM, pH 7.0, 1 mM PLP). Sodium pyruvate (90 mg, 0.82 mmol, 41 mM), and the sol–gel/Celite 545 immobilized ω-TA-117 (250 mg) were added and the reaction was shaken at 30 °C (600 rpm) for 24 h. After work-up as described above (S)-**1b** was obtained with 41% yield (41 mg); >99% ee; $[\alpha]_D^{20}$ −42.1, c 1.0, benzene. The absolute configuration was assigned by comparison of elution order on GC and co-injection with commercially available reference material.

*2.9. Deracemization of rac-4-phenyl-2-butylamine (**1a**) and rac-mexiletine (**1c**) via one-pot two-step procedure on an analytical scale*

*2.9.1. Deracemization of rac-4-phenyl-2-butylamine (**1a**) to the (S)-enantiomer*

The reaction was performed at 30 °C for 24 h in sodium phosphate buffer (100 mM, pH 7) containing pyridoxal 5′-phosphate monohydrate (1 mM) in a 2 mL eppendorf tube. The reaction buffer (1 mL) was mixed with sol–gel/Celite 545 immobilized ω-TA-117 (50 mg) and sodium pyruvate (5.5 mg, 0.05 mmol, 50 mM). The reaction mixture contained 50 mM of the corresponding amine **1a**. After the kinetic resolution (24 h) immobilized enzyme was separated by filtration and lactate dehydrogenase from bovine heart (20 μL, 220 U), NAD^+ (1 mM), glucose (27 mg, 0.15 mmol, 150 mM), glucose dehydrogenase (20 μL, 10 U) were added (to remove pyruvate from the first step) and shaken for 1 h at 30 °C. L-Alanine (5 equiv., 250 mM) and the second ω-transaminase TA-114 (6 mg) were added and shaken at 30 °C. After 24 h aqueous NaOH (200 μL, 10N) and ethyl acetate (600 μL) were added. The organic phase was dried (Na_2SO_4) and analyzed by GC chromatography. The product (S)-**1a** was obtained with 90% of conversion and >99% ee.

*2.9.2. Deracemization of rac-mexiletine (**1c**) to yield (S)-**1c** employing LDH for pyruvate removal in the second step*

The kinetic resolution (24 h) of rac-**1c** (50 mM) was performed as described above but employing sol–gel/Celite 545 immobilized ω-TA-117 (50 mg). The first step of the deracemization sequence was stopped by removal of the immobilized enzyme by filtration followed by the addition of lactate dehydrogenase (20 μL, 220 U), NAD^+ (1 mM), glucose (27 mg, 0.15 mmol, 150 mM), and glucose dehydrogenase (20 μL, 10 U) (to remove pyruvate from the first step) and shaken for 1 h at 30 °C. L-Alanine (5 equiv., 250 mM), and second ω-transaminase TA-113 (6 mg) were added and shaken at 30 °C. After 24 h aqueous NaOH (200 μL, 10N) and ethyl acetate (600 μL) were added. The organic phase was dried (Na_2SO_4) and analyzed by GC chromatography. The product (S)-**1c** was obtained with >99% of conversion and >99% ee.

*2.9.3. Deracemization of rac-mexiletine (**1c**) to yield (S)-**1c** employing L-AlaDH for L-alanine recycling in the second step*

The kinetic resolution (24 h) was performed as described in 2.8.2. After removal of the enzyme by filtration L-alanine dehydrogenase (20 μL, 8 U), NAD^+ (1 mM), ammonium formate (9 mg, 0.15 mmol, 150 mM), and formate dehydrogenase (20 μL, 4 U) were added (to transform remaining pyruvate from the first step to L-alanine) and shaken for 1 h at 30 °C. Additional L-alanine (5 equiv., 250 mM), and the second ω-transaminase TA-113 (6 mg) were added and shaken at 30 °C. The reaction was stopped after 24 h by the addition of aqueous NaOH (200 μL, 10N) and ethyl acetate (600 μL). The organic phase was dried (Na_2SO_4) and analyzed by GC chromatography. The product (S)-**1c** was obtained with 98% of conversion and >99% ee.

*2.10. Preparative deracemization of rac-phenyl-2-butylamine (**1a**)*

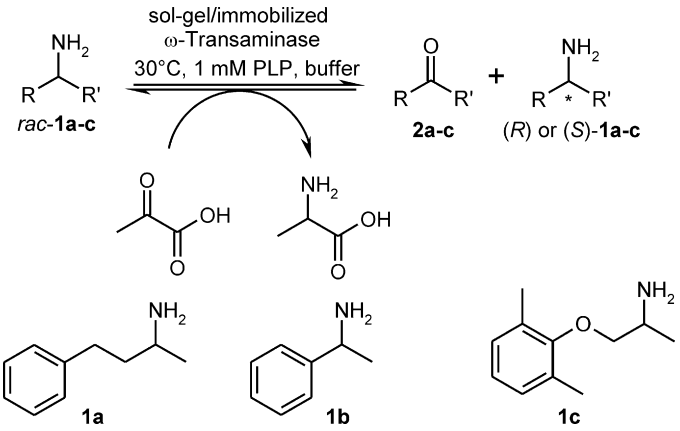
In a screw tube (50 mL) rac-**1a** (50 mg, 0.28 mmol) was suspended in phosphate buffer (20 mL, 100 mM, pH 7.0, 1 mM PLP). Sodium pyruvate (31 mg, 0.28 mmol) and the sol–gel/Celite 545 immobilized ω-transaminase TA-117 (200 mg) were added and the reaction was shaken at 30 °C (300 rpm). After the kinetic resolution (24 h) immobilized enzyme was separated by filtration and lactate dehydrogenase from bovine heart (100 μL), 1 mM NAD^+, glucose (42 mM), glucose dehydrogenase (100 μL) were added and shaken for 1 h at 30 °C. L-Alanine (5 equiv., 100 mM) and the second ω-transaminase TA-114 (30 mg) were added and shaken at 30 °C. After 24 h, the pH of the mixture was adjusted to pH 1 employing aqueous HCl (5 M), and the remaining ketone was extracted five times with dichloromethane (5 × 10 mL). After the extraction, no ketone was detectable anymore in the residual aqueous phase. The pH was adjusted to pH 12, and the amine **1a** was extracted four times with dichloromethane (4 × 10 mL). The solvent of the combined extracts of (S)-**1a** was evaporated and (S)-**1a** was obtained with 88% yield (44 mg); >99% ee; $[\alpha]_D^{20}$ 6.8 c 1.0, chloroform.

*2.11. Preparative deracemization of rac-mexiletine (**1c**)*

In a screw tube (50 mL) rac-mexiletine (**1c**) (50 mg, 0.28 mmol, 14 mM) was suspended in phosphate buffer (20 mL, 100 mM, pH 7.0, 1 mM PLP). Sodium pyruvate (77 mg, 0.70 mmol, 14 mM), and the immobilized ω-ATA-117 (200 mg) were added and the reaction was shaken at 30 °C (600 rpm) for 24 h. After the kinetic resolution (24 h) lactate dehydrogenase (50 μL), NAD^+ (1 mM), glucose (42 mM), and glucose dehydrogenase (50 μL) were added (to remove pyruvate from the first step) and shaken for 1 h at 30 °C. L-Alanine (5 equiv., 70 mM), and the second ω-transaminase TA-113 (30 mg) were added and shaken at 30 °C for 24 h. After work-up as described above (*S*)-mexiletine (**1c**) was obtained with 94% yield (47 mg) at 99% of conversion; >99% ee; $[\alpha]_D^{20}$ +3.50, c 1.0, $CHCl_3$, [lit. +3.0, c 1.05, $CHCl_3$ for (*S*)-enantiomer] [36]; ^{1}H NMR (300 MHz) δ 1.19 (d, J = 6.6 Hz, 3H), 1.71 (bs, 2H), 2,30 (s, 6H), 3.32-3.45 (m, 1H), 3.52-3.65 (m, 1H), 3.67 (dd, J = 4.0, 10.2 Hz, 1H), 6.83-6.95 (m, 1H), 7.05 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz) δ 16.3, 19.8, 47.3, 78.3, 123.8, 128.9, 130.8, 155.5.

3. Results and discussion

For the sol–gel immobilization of the transaminases we expected that the type of silane precursor has an impact on the activity of the encapsulated ω-transaminase; therefore two different alkylsilanes were tested in a first approach [trimethoxypropylsilane (TMOPS) and isobutyltrimethoxysilane (iBTMOS)]. Procedures for sol–gel matrix immobilization have been optimized recently [34] e.g. for optimal reagent ratios (TMOPS/TMOS = 5:1) [37] and have been employed as previously reported. The precursor iBTMOS led to a catalyst preparation with very low residual activity (less then 5%, data not shown). On the other hand TMOPS was found to be a suitable xerogel precursor and used for the further studies. For the immobilization procedure pyridoxal 5′-phosphate (PLP, 1 mM) was added, since it has been shown previously that PLP was needed to stabilize ω-transaminase during immobilization [25]. Immobilized transaminases were tested for the kinetic resolution of racemic pharmacologically relevant amines (Scheme 1). For instance, optically pure 4-phenyl-2-butylamine (**1a**) is a precursor of the antihypertensive dilevalol [38]



Scheme 1. Kinetic resolution of rac-amines catalyzed by immobilized ω-transaminases.

and mexiletine (**1c**) is used to treat arrhythmias within the heart as well as for anesthesia [39–41].

First immobilization experiments employing ω-TAs 113, 117 and *V. fluvialis* (Vf-TA) led only to a reasonable conversion in the case of employing ω-TA 117 for the kinetic resolution of rac-**1a** (Table 1, entries 1–3). In a next step sol–gel immobilization was performed in the presence of Celite 545 (50 mg) as a porous solid support. Celite 545 is described as a highly porous diatomaceous earth composed of silica (SiO_2)/metal oxides [42], and is frequently employed for enzyme immobilization due to its chemical inertness and unique interconnected pore structure [42–44]. Again, only in the case of ω-TA 117 an improvement of conversion was observed (entries 4-6). Reasonable active immobilized enzyme preparations for the two other transaminases Vf-TA and ω-TA 113 were only obtained when omitting the 2-propanol treatment during immobilization by the sol–gel process as well as work-up of the immobilizate (entries 7-8). Obviously the 2-propanol treatment during immobilization did not affect ω-TA 117 but ω-TA 113 and Vf-TA.

Addition of Tween 80® as a further additive during immobilization effected mainly Vf-TA leading to a slightly better conversion than without (entry 9). The ω-ATA 117 immobilized by the sol–gel process in the presence of Celite 545 led to good conversion in the absence as well as presence of Tween 80®. In both cases protein loading yielded up to 35% during immobilization. Employing the best preparations (S)-amine **1a** (entries 6, 11) as well as (R)-amine **1a** (entry 7) were obtained with excellent enantioselectivity for the kinetic resolution (>100). The immobilized ω-TA 117 proved also to be best suited for the two other substrates **1b** and **1c**.

The addition of Tween 80® resulted in an immobilized catalyst which was not as easy to handle as without Tween 80®. Additionally

Table 1
Kinetic resolution employing immobilized enzyme preparations.

Entry	Substrate	ω-TA	Additives[a]	*c* (%)[b]	ee (%)[c]	*E*[d]
1	**1a**	Vf-TA	None	8	n.d.	–
2	**1a**	113	None	2	n.d.	–
3	**1a**	117	None	30	43 (S)	–
4	**1a**	Vf-TA	Celite 545	8	n.d.	–
5	**1a**	113	Celite 545	<1	n.d.	–
6	**1a**	117	Celite 545	48	98 (S)	>100
7	**1a**	Vf-TA[e]	Celite 545	54	>99 (R)	>100
8	**1a**	113[e]	Celite 545	16	18 (R)	–
9	**1a**	Vf-TA	Celite 545, Tween 80®	36	2 (R)	–
10	**1a**	113	Celite 545, Tween 80®	<1	n.d.	–
11	**1a**	117	Celite 545, Tween 80®	58	>99 (S)	>100
19	**1b**	Vf-TA	None	19	11 (R)	–
20	**1b**	Vf-TA	Celite 545	18	42 (R)	–
21	**1b**	Vf-TA	Celite 545, Tween 80®	2	n.d.	–
13	**1b**	117	None	53	>99 (S)	>100
14	**1b**	117	Celite 545	61	>99 (S)	>100
15	**1b**	117	Celite 545, Tween 80®	57	>99 (S)	>100
26	**1c**	Vf-TA[e]	Celite 545	16	15 (R)	–
25	**1c**	113[e]	Celite 545	16	5 (R)	–
22	**1c**	117	None	25	39 (S)	–
23	**1c**	117	Celite 545	58	>99 (S)	>100
24	**1c**	117	Celite 545, Tween 80®	47	97 (S)	>100

[a] Celite 545 (50 mg) or/and Tween 80® (60 mg).
[b] Determined by GC, conversion after 24 h, 50 mM amine concentration.
[c] Determined by GC on a chiral phase.
[d] Enantioselectivity calculated from c and ee [45].
[e] 2-Propanol avoided during immobilization and work-up. n.d. not determined due to too low conversion.

the moderate improvements obtained with Tween 80® for ω-TA 117 did not justify the further usage of Tween 80®; thus, ω-TA 117 was used in the further studies as a sol–gel/Celite 545 immobilized preparation without Tween 80®. Testing the possibility to recycle the sol–gel/Celite 545 immobilized ω-TA 117, the enzyme preparation was employed for a series of kinetic resolutions of rac-**1a**. The gel was separated and washed after each cycle before reusing it. The preparation could successfully be employed in eight cycles: the enantioselectivity remained constant ($E > 100$) over all cycles and the ω-TA 117-immobilizate retained 78% of its initial conversion after eight repetitive uses (Fig. 1).

In a further experiment the conversion was measured at varied temperature (Fig. 2) as well as varied pH values (Fig. 3).

The apparent temperature optimum for the free enzyme was at 30 °C while the one for the immobilized enzyme was between 40 °C and 50 °C. The shift of temperature optimum was postulated previously to result from a multipoint contact through hydrogen bonding as well as ionic and hydrophobic interactions of the enzyme with the matrix [34]. It could be speculated that the ω-transaminase is conformationally arrested in the matrix leading to good activity even at higher temperature (60 °C).

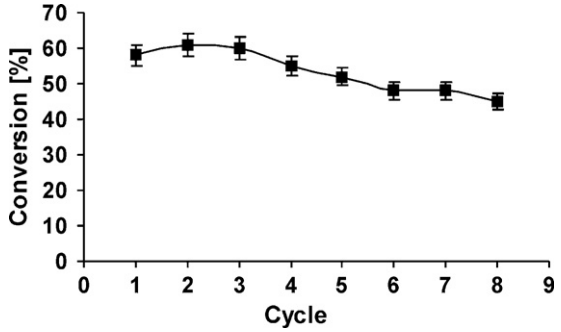


Fig. 1. Recycling of the sol–gel/Celite 545 immobilized ω-TA 117 for the kinetic resolution of rac-**1a**.

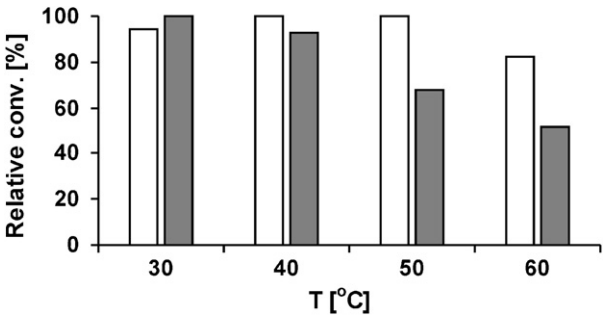
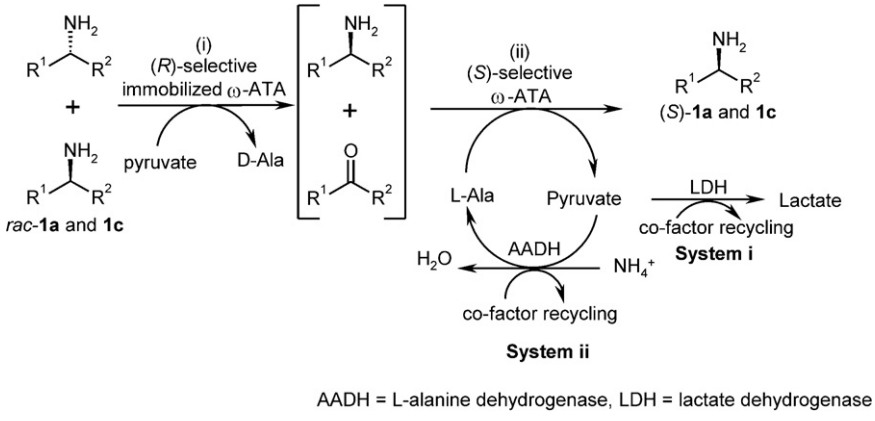


Fig. 2. Conversion of native (gray) and sol–gel/Celite 545 immobilized ω-TA 117 (white) at varied temperature.



Scheme 2. Deracemization of *rac*-amines **1a** and **1c** via a one-pot two-step synthesis procedure.

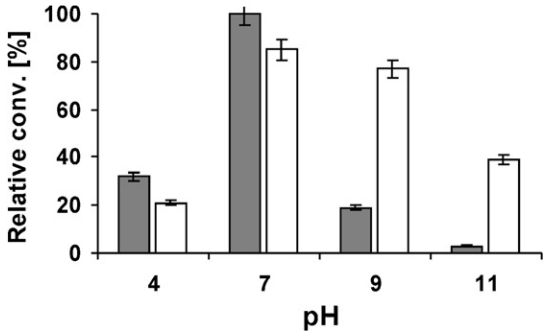


Fig. 3. Relative conversion of native (gray) and the sol–gel/Celite 545 immobilized ω-TA 117 (white) at varied pH values.

Even more pronounced was the apparent stabilization effect of the sol–gel/Celite 545 matrix when testing the conversion at varied pH (Fig. 3).

The pH range of the immobilized ω-TA 117 was broader than the one of the native enzyme; e.g. at pH 9.0 77% conversion was achieved and at pH 11.0 39% conversion while the free enzyme was almost not active anymore at pH 9.0. Thus, the immobilization method preserved the enzyme activity over a broader pH range. Again this result could probably be attributed to the stabilization within the sol–gel matrix. The optimum pH of the immobilized enzyme was found to be similar to the one of native enzyme (pH 7.0).

Finally, the ω-TA 117 and Vf-TA immobilized with Celite 545 were used in a preparative kinetic resolution of 100 mg of rac-amines **1a** and **1b** leading to enantiomerically pure (S)-enantiomer with 43% and 41% yield respectively and (R)-**1a** with 48% yield. In all cases the ee was >99%.Recently, we have published a deracemization concept for prim-amines based on a two-step one-pot process which consists of (i) a kinetic resolution and (ii) a stereoselective amination (Scheme 2) [46]. Substantial limitation of the presented method was a possible interference of the employed (R)- and (S)-ω-transaminases between the two steps. The problem was solved by a heat treatment after the first step to inactivate the enzyme employed in the first step. Thus after the kinetic resolution, the sample was kept at 75 °C for 30 min, before the enzyme required for the second step was added. This method led to complete denaturation and loss of the enzyme which significantly increased the overall cost of this process due to the high price of transaminases.

To improve this process immobilized (R)-transaminase ω-TA-117 was employed in the first step of this sequence. The catalyst preparation could be separated easily by filtration or centrifugation after the first step – the kinetic resolution. The kinetic resolution employing the immobilized enzyme was combined with stereoselective amination using either lactate dehydrogenase (system i) or alanine dehydrogenase (system ii) to shift the equilibrium in the second step to the product side. This sequence enabled an efficient one-pot two-step deracemization leading to optically pure (S)-amines **1a** and **1c** with 90% and up to >99% conversion respectively. Having optimized process conditions, a preparative transformation of 50 mg racemic 4-phenyl-2-butylamine (**1a**) at 14 mM substrate concentration yielded (S)-**1c** at 96% conversion after 48 h with >99% ee and 89% isolated yield. Additionally, 50 mg of racemic 1-(2,6-dimethylphenoxy)-2-propanamine (**1c**) was transformed into optically pure amine (S)-**1c** with complete conversion and >99% ee and 95% isolated yield.

4. Conclusion

Immobilization of ω-transaminases in sol–gel matrix was studied. The ω-TA 117 immobilized with Celite 545 as an additive retained its activity over a broader range of temperature and pH compared to the native enzyme. The enzyme could be recycled eight times with only moderate decrease of conversion for each cycle. Finally, the ω-TA 117 was employed in a two-step deracemization protocol, whereby the enzyme used in the first step could simply be removed by centrifugation.

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