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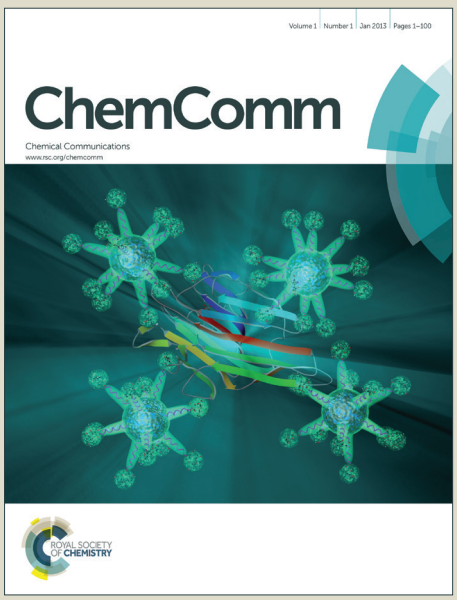

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ARTICLE TYPE

Enhancement of (Stereo)Selectivity in Dynamic Kinetic Resolution Using Core-Shell Nanozeolite@enzyme as Bi-Functional Catalyst

Wanlu Wang, Xiang Li, Zhoujun Wang, Yi Tang, Yahong Zhang*

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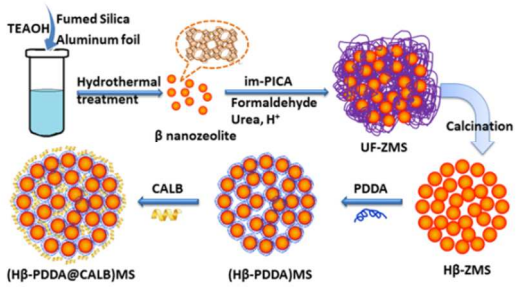
A core-shell nanozeolite@enzyme bi-functional catalyst is constructed, and greatly improves selectivity and stereoselectivity of product in dynamic kinetic resolutions of aromatic secondary alcohols comparing with mixed catalysts, especially those involving small acyl donors.

Methods that generate enantiomerically pure compounds are of great interest in the pharmaceutical and agrochemical industries.[1] Dynamic kinetic resolution (DKR) is one of the most important approaches. In a DKR process, an enzyme-catalyzed trans-esterification process of desired enantiomer and a metal/acid/base-catalyzed racemization process of the undesired enantiomer are combined to offer the prospect of obtaining 100% yield of the desired stereoisomer from a racemic starting mixture.[2] For the DKR of secondary alcohols which is an important class of chiral synthons, lipase is often used as the catalyst of trans-esterification process, while the selection of different racemization catalysts greatly determines the efficiency of the whole DKR process. Although various homogeneous metal complex catalysts provide ideal racemization results and have been studied extensively,[3] the difficulties in separation and regeneration partly limit their application. The acidic heterogeneous catalysts such as acid resins Amberlyst® and Deloxan®, H-zeolite, provide the alternative route of the racemization. Among H-zeolite, H-β zeolite is found to be the most promising acid catalyst because of its moderate acidity and microporous size.[4] However, as indicated by Pellissier[5], the use of H-β zeolite as catalyst could not only cause the formation of some secondary products but also further racemize the resulting product ester when small acyl donors, such as vinyl acetate (VA) and isopropenyl acetate (IPA) were used. Our group has ever employed nanosized H-β zeolite microspheres (Hβ-ZMS) to decrease the secondary products during racemization.[6] Recently, Jaenicke *et. al* used β-Silicalite-1 core-shell composites as racemization catalysts in the one-pot DKR of secondary alcohols, and achieved good selectivity and stereoselectivity of products because of the suppression of the inert Silicalite-1 shell for the dehydration and non-enantioselective trans-esterification of the alcohol.[7] Herein, a core-shell nanozeolite@enzyme bi-functional microsphere catalyst is constructed and applied to DKR of 1-phenylethanol (1-PE). The nanozeolite core is used as both the acid catalyst and the support of immobilized enzyme, while the immobilized enzyme serves as both the enzyme catalyst and the protective layer of substrate and product from acidic core. Benefiting from the architecture advantage of nanozeolite core and enzyme shell, this core-shell structured integrated catalyst can remarkably improve the selectivity and stereoselectivity of product in the DKR of 1-PE compared to that of mechanical-mixed one when using cheaper and smaller sized acyl donors, such as VA and IPA.



Scheme 1 Construction of the core-shell nanozeolite@enzyme bi-functional microsphere.

The construction process of the core-shell bi-functional microsphere catalyst is shown in Scheme 1. Typically, colloidal β nanozeolites were hydrothermally synthesized according to the literature procedures.[8] The colloidal solutions without any treatment were directly used to prepare Hβ-ZMSs with a secondary mesopore by an improved polymerization induced colloid aggregation (im-PICA) method developed by our group.[9] Then, the Hβ-ZMSs with the diameter of 5-7 μm were coated by a layer of cationic polydiallyldimethylammonium chloride (PDDA). The resulting PDDA coated Hβ-ZMSs [(Hβ-PDDA)MSs] were dispersed into the solution of *Candida antarictica* lipase B (CALB) for the purpose of the enzyme immobilization. After removing the free CALB by centrifugation, the catalysts [(Hβ-PDDA@CALB)MSs] were lyophilized at 0.1 mbar for 24 h and equilibrated with saturated $MgCl_2$ solutions for 48 h before they were used. Scanning electron microscopy (SEM) images (Fig. S1A&B†) and X-ray diffraction (XRD) patterns (Fig. S1C†) of Hβ-ZMSs and (Hβ-PDDA@CALB)MSs show that the coating process of PDDA and CALB on the Hβ-ZMSs has little influence on the morphology and framework of Hβ-ZMSs. However, as shown in Fig. S1D† and Table S1†, the coating of PDDA and CALB partly decreases the external surface area and mesopore volume of Hβ-ZMSs, and their micropores are also partly blocked. Furthermore, the results of the

potentiometeric titration (Fig. S2†) indicate the decrease of acid strength and acid site number of (Hβ-PDDA)MSs compared to those of Hβ-ZMSs. The amount and activity of immobilized CALB on both Hβ-ZMS and (Hβ-PDDA)MSs as well as the activity of free CALB are listed in Table S2†. Obviously, the activity of the free CALB is at a low level, which could be assigned to the poor dispersity of CALB in organic solvent. Therefore, an appropriate immobilization of enzyme is necessary. However, according to the previous report,[10] CALB is pH-sensitive and the acidic surface of nanozeolite will inhibit its activity, which can be demonstrated by the complete deactivation of CALB immobilized on Hβ-ZMSs listed in Table S2†. A PDDA layer, thereby, is used to protect CALB from acid sites of Hβ-ZMS external surface.

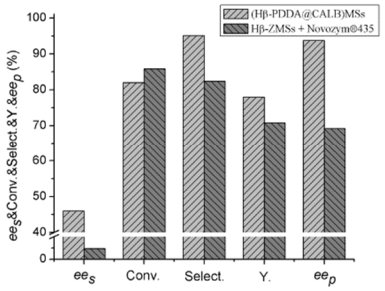


Fig. 1 DKR results of 1-PE catalyzed by core-shell (Hβ-PDDA@CALB)MSs and mixed catalysts using VA as acyl donor at 50 °C. The concentrations of 1-PE and VA are 50 and 100 mmol L^{-1}, respectively. The reaction time is 5 h in (Hβ-PDDA@CALB)MSs system and 0.5 h in mixed catalyst system.

The as-prepared (Hβ-PDDA@CALB)MSs are applied to DKR of 1-PE using VA as acyl donor, and Hβ-ZMSs, associated with commercial enzyme Novozym®435, are used as mixed catalysts. As shown in Fig. 1, a selectivity of 95.1% is observed in the DKR of 1-PE catalyzed by (Hβ-PDDA@CALB)MSs, which is much higher than that (82.4%) catalyzed by mixed catalysts. Moreover, the enantiomeric excess (*ee*) of product (ee_p) in (Hβ-PDDA@CALB)MSs catalytic system reaches 93.8% while that in the mixed one is only 69.8%. It is clear that the core-shell catalyst can bring an improvement of 15% selectivity and 34% ee_p at the similar conversion compared to mixed catalysts. However, the *ee* of substrate (ee_s) catalyzed by core-shell catalyst increases to 46% from 4.7% catalyzed by mixed one. It means the rate of racemization in the core-shell catalyst system is slowed down rapidly, which can be assigned to the decrease of acidity of Hβ-PDDA@CALB)MSs and diffusion limitation of substrate towards acidic sites of nanozeolite cores owing to the encapsulation of CALB and PDDA.

Table 1 DKR results of 1-PE catalyzed by (Hβ-PDDA@CALB)MSs at different temperatures using VA as acyl donor.[a]

T. (°C)	t. (h)	Result of DKR				
		ee_s (%)	Conv. (%)	Select. (%)	Y. (%)	ee_p (%)
50	3.0	36.6	55.4	92.6	51.3	93.6
55	3.0	30.3	59.5	90.3	53.7	91.4
60	2.0	19.6	58.8	86.2	50.7	87.4
	3.0	20.4	71.9	84.9	61.1	85.0

[a] The concentrations of 1-PE and VA are 50 and 100 mmol L^{-1}.

In addition, the influence of reaction temperature and substrate concentration in the DKR of 1-PE using (Hβ-PDDA@CALB)MSs as catalysts is studied, respectively. As shown in Table 1, the rates of both racemization and trans-esterification become faster with the increasing reaction temperature, thus the yield increases notably. However, the selectivity and *ee* of product decrease with the increase of reaction temperature, which suggests that higher reaction temperature will improve not only the yield of product, but also the formation of by products and the further racemization of R-ester, and finally decrease the efficiency of whole DKR process. Furthermore, when the amount of catalyst is fixed and the concentration of 1-PE during the DKR is changed from 25 to100 mmol L^{-1}, it is found in Table 2 that a higher substrate concentration leads to a better result, *i.e.* higher reaction rate, selectivity and ee_p, although their relative conversions decrease with the increasing substrate concentration. For example, when concentration of 1-PE is doubled from 25 to 50 mmol L^{-1}, the amount of product R-ester increases from 0.07 mmol to 0.11 mmol within reaction period of 3 h and their ee_p values also increase from 83.4% to 93.9%. Lower fresh concentration means less substrate and excessive catalyst, which will lead to faster racemization rate and side reaction rate, not only for the substrates, but also for the products. Therefore, changing concentration of substrates and reaction temperature could adjust the reaction rates of racemization and trans-esterification and make them match well each other.

Table 2 DKR results of 1-PE with different concentrations catalyzed by (Hβ-PDDA@CALB)MSs using VA as acyl donor at 50 °C.

$C_{1\text{-PE}}$[a] (mmol L^{-1})	t. (h)	Result of DKR				
		ee_s (%)	Conv. (%)	Select. (%)	Y. (%)	ee_p (%)
25	1.0	24.9	42.0	92.1	38.7	92.5
	2.0	18.7	64.8	90.5	58.7	87.4
	3.0	15.7	80.6	88.5	71.3	83.4
50	1.0	29.8	32.3	93.6	30.3	96.9
	2.0	39.4	49.0	93.3	45.7	95.0
	3.0	43.7	60.5	92.5	56.0	93.9
75	3.0	60.0	52.7	95.2	50.2	96.9
100	3.0	65.5	50.0	96.7	48.4	98.0

[a] The concentration of VA doubles that of 1-PE.

It is worthy to note that all the results in Fig. 1, Table 1 and Table 2 imply that a high ee_s, *i.e.* a slow racemization process during DKR results in a high selectivity and *ee* of product. The slow racemization can decrease not only the transformation between R- and S- alcohol isomers but also the formation of by-products as well as the further racemization of the product R-ester. Obviously, its influence on latter is more significant. Therefore, this core-shell (Hβ-PDDA@CALB)MSs catalyst exerts a clear structural advantage. On one hand, CALB shell can timely remove the R-formed substrate and avoid its meaningless racemization inside the nanozeolite core, which will reduce the production of by-products. On the other hand, CALB shell can work as a protective layer and limit the diffusion of substrate and product R-ester toward nanozeolite core, and decrease their racemization and side reaction rates occurred in the external surface and micropore of nanozeolites. Thus, a much better result of DKR is achieved on this core-shell catalyst. And the slightly

lower DKR rate can be simply compensated by extending the reaction time. In addition, the DKR result of 1-PE catalyzed by only PDDA coated Hβ-ZMSs [(Hβ-PDDA)MSs] and Novozym®435 catalysts further indicates that this core-shell structure is indispensable for a high selectivity and *ee* of product during the DKR (Fig. S3†).

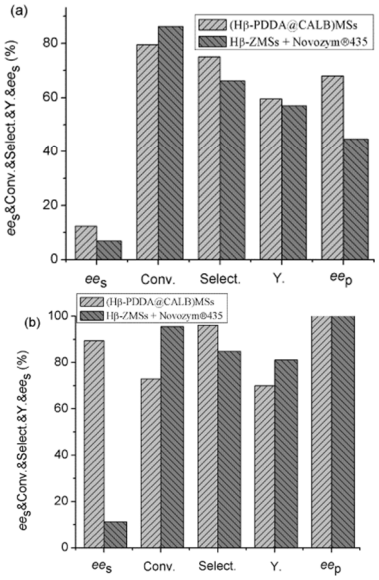


Fig. 2 The DKR results of 1-PE catalyzed by core-shell catalyst using (a) IPA and (b) VO as acyl donors at 50 °C. The concentrations of 1-PE and acyl donor are 50 and 100 mmol L^{-1}, respectively. The reaction time is 3 h in (Hβ-PDDA@CALB)MSs system and 0.25 h (a) and 0.5 h (b) in mixed catalyst system.

To further demonstrate the advantages of this core-shell catalyst, other acyl donors, such as IPA and vinyl octanoate (VO) are applied to the DKR of 1-PE instead of VA. As shown in Fig. 2, when VA is replaced by IPA and VO, similar to that using VA, this core-shell (Hβ-PDDA@CALB)MSs achieve a higher selectivity of product and ee_s compared to those catalyzed by the mixed catalysts, indicating the decrease of racemization rate in core-shell catalyst system. However, the results of ee_p are very different between the two acyl donors. In IPA system, the ee_p catalyzed by the core-shell (Hβ-PDDA@CALB)MSs is increased by 52.1% comparing to the mixed one (Fig. 2a). However, when VO is used as acyl donor (Fig. 2b), there is not a notable difference between ee_p catalyzed by the two kinds of catalysts and both of them stay at a high level (nearly 100%). Obviously, such high ee_p in IPA system should be owned to the protection effect of CALB shell on product. But for the system using VO as acyl donor, the size of its corresponding R-ester is so large that it is difficult to access the acidic sites of nanozeolites even if they are not coated. Therefore, no difference in ee_p is observed in the two catalytic systems. Furthermore, the advantages of this core-shell catalyst can be also verified by the DKR results of other substrates, i.e. 1-(p-tolyl) ethanol and 1-(4-bromophenyl)-ethanol using VA as acyl donor (Table S3†).

In summary, a core-shell nanozeolite@enzyme bi-functional catalyst is constructed, and applied to the DKR of aromatic secondary alcohols. All the results indicate that a slow racemization rate is the key of an ideal DKR process (a high selectivity and stereoselectivity of product). Because the enzyme shell immobilized on the acidic nanozeolite can timely remove R-formed substrate and limit the accessibility and diffusion of substrate/product towards the acidic sites and so decrease the racemization rate of whole DKR, this core-shell bi-functional catalyst displays clear advantages in the selectivity and *ee* of product, especially in those using acyl donors with small molecular weight. Although the advantages of this core-shell bi-functional catalyst are only explored on the DKR of aromatic secondary alcohols, it provides an ideal example for the performance improvement of catalysts just by the reasonable localization of different catalytic active sites in one catalyst.

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Notes and references

Department of Chemistry, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials and Laboratory of Advanced Material, Fudan University, Shanghai, 200433, P. R. China.
E-mail: zhangyh@fudan.edu.cn;
Fax: +86-21-65641740

† Electronic Supplementary Information (ESI) available: Experimental process, SEM images, XRD patterns, N_2 sorption isotherms and pore size distributions, the physical and textural properties of Hβ-ZMSs and (Hβ-PDDA@CALB)MSs. Potentiometric titration curves, the immobilized amount and enzyme activity of CALB on Hβ-ZMSs and (Hβ-PDDA)MSs as well as the activity of free CALB. DKR results of different substrates. See DOI: 10.1039/b000000x/

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