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# The Best Performance of the Combined CAL-B/VOSO$_4$ System Depending on the Good Mutual Coordination of Reactions

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**Abstract.** Enantiomerically pure secondary alcohols were prepared in the combined CAL-B/VOSO$_4$ system in one-pot conditions. Under the optimum conditions, the conversion, enantiomeric excess, selectivity and enantioselectivity reached 92%, >99%, 99% and >200 respectively. The significant improvement of the system performance should be attributed to the mutual coordination of reactions in a chemoenzymatic dynamic kinetic resolution system and reduction of by-reaction. In conclusion, our experimental data and literature data confirm that there must be an optimal amount of V-MPS (VOSO$_4$) to coordinate the in-situ racemic reaction with the kinetic resolution reaction with which the enantiomer required is produced and the adverse kinetic resolution reaction producing adverse enantiomer and minimize the rate of the side reaction in which the substrate was consumed and the selectivity was reduced, resulting in an optimal DKR system. Considering the large activation energy of the in-situ racemic reaction and at least 76% excess catalytic capacity of VOSO$_4$, the strategy adopted is to greatly reduce the amount of VOSO$_4$, and thus an optimal reaction condition should be established, making the DKR system optimal.

**Keywords.** Dynamic kinetic resolution, immobilized lipase, vanadium, heterogeneous catalysis, multi-reaction system

## 1. Introduction

In a recent paper in this journal, Milagre et al. [1] claimed that the compartmentalization of the physical separation of VOSO$_4$ from CAL-B by employing a low-cost, homemade Teflon tube, prevents the incompatibility between VOSO$_4$ and CAL-B, resulting in high recyclability, conversion and selectivity. The Teflon tube, prevents performance loss of the catalysts due to contact, such as the inhibition of VOSO$_4$ on the lipase activity. However, by carefully analyzing the experimental data in this article, our experimental data and literature data, we confirm that VOSO$_4$ itself was not in direct physical contact with the enzyme, therefore there is no need to add the Teflon tube for the compartmentalization of the physical separation of them. The

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significant improvement of the recyclability, conversion and selectivity could be attributed to the mutual coordination of the reactions in the chemoenzymatic DKR (Dynamic kinetic resolution) system and reduction of by-reaction. The reasons will be explained in detail as follows.

## 2. Insoluble $VOSO_4$ cannot Result in Inactivation of the Immobilized Lipase

Firstly, insoluble $VOSO_4$ cannot be in direct physical contact with the lipase on the inner surface of the immobilized lipase particle. The immobilized Candida antarctica lipase B (Novozym 435) was used in this experiment, which is adsorbed on a macroporous resin with microsized pores. Wuyts et al. [2] carried out DKR experiments with lipase and $VOSO_4$ and their result clearly shows the complete absence of any catalytically active vanadium species in solution after the catalyst suspension was filtered with 0.45μm membrane (450 nm). This shows that the particles of the insoluble $VOSO_4$ are greater than 450 nm. The pore size inside immobilized enzyme(12-30 nm) is much smaller than particles size of insoluble vanadium. Therefore, insoluble $VOSO_4$ itself cannot enter the inner surface of the enzyme particles and cannot be in direct physical contact with the enzyme on the inner surface. On the other hand, lipase located on the outer surface of immobilized lipase particles may be in contact with $VOSO_4$ particles, however, their effect can be ignored because they are much less than those on the inner surface. These are the basic knowledge of heterogeneous reactions catalyzed by immobilized enzyme.

## 3. Mutual Coordination of Reactions

Then, in order to obtain the best conversion, yield, enantiomeric excess rate (*ee*) and selectivity for a multi-reaction system, the reactions in the system must be coordinated well with each other. As shown in figure 1, the present DKR system contains multiple reactions as follows, in which reaction 1 is the KR (kinetic resolution)reaction with which the enantiomer required is produced, and reaction 2 is the KR reaction producing adverse enantiomer. Reaction 3 is an in-situ racemic reaction, while reaction 4 is a side reaction in which the substrate was consumed and the selectivity was reduced. For example, reactions 1 and 2 should be coordinated with reaction 3. Because the reaction rate of reaction 1 is higher than that of reaction 2, and the consumption of R-isomer is faster than that of S-isomer, the two substrate isomers in the reaction system are not equal, that is, S-isomer is more than R-isomer. At this time, reaction 3 will play a role in rapidly converting S-isomer into racemate that consists of two optical isomers with equal molar ratio. If excessive amount of $VOSO_4$ is used, the excessive$VOSO_4$ will significantly increase the reaction rate of reaction 4, consuming the substrate and reducing the selectivity. Therefore, there must be an optimal amount of $VOSO_4$ to coordinate reaction 3 with reactions 1 and 2 and minimize the rate of reaction 4, resulting in an optimal DKR system.

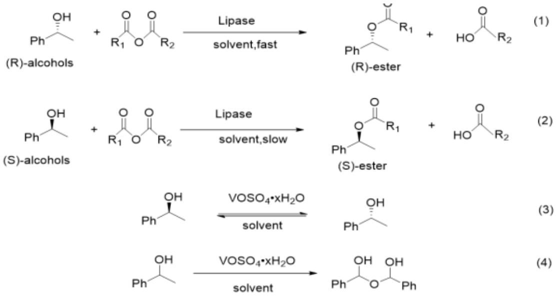


**Figure 1.** Reactions in the chemoenzymatic DKR system.

## 4. Diffusion Resistance

Thirdly, the effect of Teflon tube on the reaction system is to increase a large mass transfer resistance, which reduces reaction rate of reactions 3 and 4 mediated by $VOSO_4$. The resistance consists of the following two aspects.

(1) The diffusion resistance of substrate through the Teflon tube.

(2) The external diffusion resistance. Due to natural convection rather than forced convection inside the tube, the flow boundary layer becomes thicker and the external diffusion resistance increases greatly.

We can roughly estimate the resistance value of the Teflon tube according to the data [1]. As shown in table 1, when the Teflon tube was not added, the conversion rate was 82% (entry 1), while the lipase was placed into the Teflon tube, the conversion rate was reduced to 20% (entry 2). The conversion rate is reduced by about 76% ((82-20) / 82 = 75.61%). The remaining about 24% catalytic capacity is enough to cover the demand of KR. Because the rate of the reaction mediated by $VOSO_4$ is reduced and the by-product is reduced, high conversions, *ee* values and selectivity were achieved (entry 3). Therefore, we believe that the significant improvement of the recyclability, conversion and selectivity could be attributed to the mutual coordination of reactions in the DKR system and reduction of by-reaction, reaction 4.

**Table 1.** Results of the DKR of rac-1-phenylethanol (rac-1) with and without compartmentalization of the catalysts [1].

| Entry | | Time (h) | c (%)[a] | $ee_P$ (%)[b] | Sel (%)[a] | E |
|---|---|---|---|---|---|---|
| 1 | without tube | 2 | 82 | >99 | 92 | >200 |
| 2 | CAL-B into the tube | 2 | 20 | >99 | >99 | >200 |
| 3 | $VOSO_4$ into the tube | 1 | 91 | >99 | 98 | >200 |
| 4[c] | without tube | 1 | 92 | >99 | 99 | >200 |

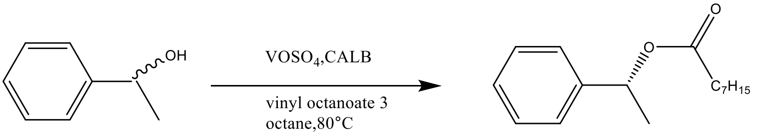
Conditions: *rac*-**1** (0.25 mmol), acyl donor (2 equivalents, 0.50 mmol), heptanes (4 mL), CAL-B (20 mg), $VOSO_4.XH_2O$ (50 mg) into a Teflon tube, 50 °C, magnetic stirring. [a] Determined by GC-FID analysis with *n*-tetradecane (0.050mmol mL-1) as internal standard. [b] Determined by chiral GC-FID analysis. [c] This experiment was carried out by this work, not ref. [1], $VOSO_4.XH_2O$ adsorbed on diatomite (7 mg).

## 5. The Optimal Amount of $VOSO_4$ Affords the Best DKR Performance

As shown in table 1, $VOSO_4$ with the Teflon tube (entry 3), the conversion and selectivity are significantly better than those without the tube (entry 2), and the reaction rate is faster. Such a very good result cannot be attributed to the fact that $VOSO_4$ does not inhibit the enzyme activity because it always does not inhibit the enzyme activity, whether there is with the Teflon tube or without. The direct effect of the Teflon tube may be reduction of the reaction rate of $VOSO_4$ by 76%, due to both the mass transfer resistance through the Teflon tube and external diffusion resistance. From the perspective of mutual coordination of reactions and considering a good experimental result, only 24% rate of $VOSO_4$ reaction (reaction 3) is required to match well with KR, and the extra 76% $VOSO_4$ reaction capacity is used to catalyze side reaction, reaction 4, which should be reduced as much as possible. Similarly, it can also be explained that with the Teflon tube, the number of cycles of the reaction increases from 5 to 8. Of course, we also note that the reaction time is 2 h for 5 cycles, and 1 h for 8 cycles. The reaction time is also one of the factors affecting the enzyme activity, the longer the reaction time, the more the loss of enzyme activity. $VOSO_4$ has very high catalytic efficiency and fast reaction although it is insoluble in organic solvents and its reaction interface is obviously small. Only 24% reaction capacity is enough to meet the demand of in-situ racemization. We speculate that without the tube, if the amount of $VOSO_4$ is greatly reduced, the same effect as with the tube is expected to achieve. So we conducted an experiment, and the experimental results are shown in table 1 (entry 4), c, $ee_p$ and Sel reach 92, >99 and 99, respectively. The experimental results confirm our speculation.

The reported data can also prove this speculation. As shown in table 2 [2], both the yield and *ee* were low, only 67% and 82% (entry 1), respectively, due to the insufficient amount of $VOSO_4$. On the contrary, if excessive amount of V-MPS is used, the dynamic equilibrium between two enantiomers is quickly reached due to the catalyst excess, which is beneficial to KR. Unfortunately; the excessive $VOSO_4$ will significantly increase the reaction rate of reaction 4, consuming the substrate and reducing the selectivity. Moreover, *ee* is also reduced and the reason is not clear. The yield and *ee* decreased to 78% and 90% (entry 3), respectively, owing to excessive $VOSO_4$. Consequently, a more appropriate amount of $VOSO_4$ is 0.2 (entry 2), the yield and *ee* are increased to 94% and 95%, respectively. *ee* significantly increased to 99% (entry 5) because of mitigating of the inhibition of *ee* by acyl donors when acyl donor vinyl octanoate decreased from 4 equivalent to 2 equivalent, reduced by half. In the case, a better mutual coordination of reactions was reached to deliver a better output. Previous data also reveal that *ee*, reactions 3 and 4 may be inhibited by acyl donors [3]. The yield was slightly reduced to 93%, which may be due to the reduction of acyl donor to reduce the rate of reaction 1. There are other data displays that the conversion, yield and *ee* decreased when the amount of $VOSO_4$ deviated from the optimal value [4].

**Table 2.** DKR of rac-1a using vanadyl sulfate and lipase CALB[a] [2].



rac-**1a**

(*R*)-**2a**

| Entry | $VOSO_4$/g | $3^b$( equiv.) | Time/h | $Yield^c$(%) | *ee*$^c$(%) |
|---|---|---|---|---|---|
| 1 | 0.1 | 4 | 4.5 | 67 | 82 |
| 2 | 0.2 | 4 | 3.5 | 94 | 95 |
| 3 | 0.5 | 4 | 4.5 | 78 | 90 |
| $4^d$ | 0.2 | 4 | 3.5 | 93 | 76 |
| 5 | 0.2 | 2 | 3.5 | 93 | 99 |

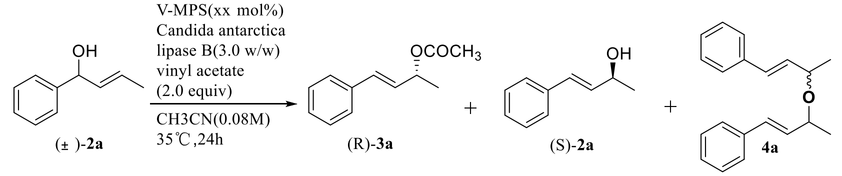
[a] rac-1a (1.27 mmol), vinyl octanoate 3, $VOSO_4 \cdot 5H_2O$ and lipase B(0.11 g) in octane (100 mL) were stirred (300 rpm) at 80 °C. [b] Thetotal amount of 3 was added in two equal portions at 0 and 2.25 h. [c] Ester yield and ester *ee* determined by chiral GC analysis. [d] 100 mL of toluene.

As shown in table 3 [3], with 1.0 mol% of V-MPS 4 (entry 3), reaction 3 can quickly reach equilibrium due to excessive V-MPS 4, the yield of desired enantiomer (R)-3**a** should actually be higher, up to 99%, although it reached 95%. Moreover, by-product 4**a** occurred with the yield of 5%, as a result of reaction 4 activated by the excessive reaction capacity. With 0.5 mol% of V-MPS4 (entry 4), the yield of (R)-3**a** increases to 98% compared with entry 3, while the yield of by-product 4**a** decreases to 1% (entry 4). Obviously, due to the reduction of V-MPS4 from 1.0 to 0.5 mol%, the catalytic capacity is greatly reduced, and the excessive catalytic capacity is not much. Thus the rate of reaction 4 is reduced, and the by-product is reduced to 1%. We speculate that if V-MPS4 is reduced to 0.4 mol%, its reaction capacity may just match reactions 1 and 2 to achieve a good mutual coordination of reactions, resulting in the increases of the yield of (R)-3**a** and no by-product produced. Unfortunately, however, the original authors did reduce the concentration to 0.1 instead of 0.45 as we thought. Because V-MPS4 decreases too much, the V-MPS4 reaction capacity is not large enough to cover reactions 1 and 2, thus, like entry 1, (S)-2**a** is thus yielded with 6%. A similar situation also appeared in other works [3-4].

In conclusion, our experimental data and literature data confirm that there must be an optimal amount of V-MPS ($VOSO_4$) to coordinate reaction 3 with reactions 1 and 2 and minimize the rate of reaction 4, resulting in an optimal DKR system.

If the reaction kinetic model of the DKR system is established, the kinetic details of the four reactions can be clearly displayed [5-7]. Unfortunately, the reaction kinetic model was not established in reference 1, which can deal with the issue that cannot be handled only by experimental data. The reaction kinetic model can be established if some data, such as conversion-time data, can be obtained. Considering the large activation energy of reaction 3 and at least 76% excess catalytic capacity of $VOSO_4$, the strategy adopted is to greatly reduce the amount of $VOSO_4$, and thus an optimal reaction condition should be established, making the DKR system optimal.

**Table 3.** Comparison of three different V-MPS on lipase-catalyzed DKR of (±)-2a [3].



| Entry | V-MPS[mol equiv.] | (R) -3**a** | | (S)-2**a** | | Yield of 4a(%)$^b$ |
|---|---|---|---|---|---|---|
| | | Yield(%)$^a$ | *ee*(%) | Yield(%)$^a$ | *ee*(%) | |
| 1 | V-MPS2[1.0mol%] | 74 | 98 | 24 | >99 | — |
| $2^c$ | V-MPS3[1.0mol%] | 99 | 99 | — | — | — |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | V-MPS4[1.0mol%] | 95 | 98 | — | — | 5 |
| 4 | V-MPS4[0.50mol%] | 98 | 98 | — | — | 1 |
| 5[d] | V-MPS4[0.10mol%] | 94 | 98 | 6 | 99 | — |

[a] Isolated yield. [b] Based on$^{1}$ H NMR analysis of a crude product. [c] Cited from ref. 4c. [d] The reaction was conducted for 120 h.

## 6. Conclusion

Optically active sec-alcohols were prepared in the combined CAL-B/VOSO$_4$ dynamic kinetic resolution system. The conversion, enantiomeric excess, selectivity and enantioselectivity reached 92%, >99%, 99% and >200 respectively, under the optimum conditions. The significant improvement of the system performance should be attributed to the mutual coordination of reactions in a chemoenzymatic dynamic kinetic resolution system and reduction of by-reaction.

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