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Solid-to-solid kinetic resolution. Determination of the enantiomeric ratio

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Abstract

Enzymatic kinetic resolution can be carried out with a suspension of incompletely dissolved solid substrate. In favorable cases, the slowly converted enantiomer may remain as an incompletely dissolved solid and can be easily recovered. Then, such a 'solid-to-solid' resolution process will also show a significantly improved yield and productivity when compared to the reaction with completely dissolved substrate. The determination of the enantiomeric ratio (E) for reactions with suspended racemates requires equations that are different than for reactions with completely dissolved substrate. This determination becomes very complex in case of mass transfer limitation and is greatly facilitated when mass transfer limitations are absent. If the latter condition has been checked, E can be obtained from measurement of the extent of conversion and the enantiomeric excess, either of the product or of the remaining solid substrate, but not of the remaining dissolved substrate.

Keywords: Enzymatic resolution; Enantiomeric ratio; Mass transfer

1. Introduction

Enzymatic kinetic resolution is one of the most important methods to obtain enantiomerically pure compounds [1]. It is usually applied in an aqueous or organic solution of the substrate. However, if high concentrations of the substrate are applied, which may be attractive from an industrial point of view, emulsions or suspensions may occur for liquid and solid substrates, respectively. In this paper, we will consider kinetic resolution of suspended substrates. Kinetic resolution of substrate suspensions by crystallization procedures is a classical technique [1], but enzymatic kinetic resolution of suspended substrates has not received special attention. Nevertheless, the literature contains several successful examples in which a suspension of a racemate is enzymatically converted into a suspension

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of an enantiomerically pure compound (e.g., [2–5]). Recently, we have theoretically worked out such 'solid-to-solid' processes and have shown that under favorable conditions, they show considerable advantages to processes where the same reaction is carried out with fully dissolved substrate: The yield, productivity, substrate concentration, and recovery procedure can be significantly improved [6].

The main parameter determining the course of an enzyme-catalyzed kinetic resolution with dissolved substrate is the enantiomeric ratio (E) [7,8]. The literature contains a considerable amount of examples of the determination of E using a suspension reaction, but it seems now that this determination is complicated by the fact that kinetic resolution for dissolved and for suspended substrate proceeds differently. This is, a.o., due to the solid–liquid mass transfer process as a rate-determining factor. The purpose of this paper is to indicate how E should be determined when enzymatic kinetic resolution is carried out with suspended substrate. Before treating two types of kinetic resolution with suspended substrate, we will first repeat the basic case, i.e., completely dissolved substrate. In all cases, simple (irreversible) reaction kinetics are applied, batch reactors are assumed and the reaction products are assumed to be fully soluble.

2. Kinetic resolution with completely-dissolved substrate

The basic case of kinetic resolution applies to completely dissolved substrate. The bold arrow indicates the faster reaction.

$$\mathrm{A}^R(\text{dissolved}) \rightarrow \mathrm{P}^R(\text{dissolved})$$
$$\mathrm{A}^S(\text{dissolved}) \rightarrow \mathrm{P}^S(\text{dissolved})$$

The ratio of (dis)appearance of (R) and (S)-enantiomers is:

$$\frac{\mathrm{d}c_\mathrm{A}^R}{\mathrm{d}c_\mathrm{A}^S} = \frac{\mathrm{d}c_\mathrm{P}^R}{\mathrm{d}c_\mathrm{P}^S} = \frac{k_\mathrm{cat}^R/K_\mathrm{m}^R}{k_\mathrm{cat}^S/K_\mathrm{m}^S} \cdot \frac{c_\mathrm{A}^R}{c_\mathrm{A}^S} = E \cdot \frac{c_\mathrm{A}^R}{c_\mathrm{A}^S}. \tag{1}$$

The substrate and product concentrations are related to the initial substrate concentration according to:

$$c_\mathrm{AO}^R = c_\mathrm{A}^R + c_\mathrm{P}^R, \tag{2}$$

and a similar equation for the (S)-enantiomer. Using these molar balances, Eq. (1) can be integrated for either substrate or product:

$$E = \frac{\ln\left(c_\mathrm{A}^R/c_\mathrm{AO}^R\right)}{\ln\left(c_\mathrm{A}^S/c_\mathrm{AO}^S\right)} = \frac{\ln\left(1 - c_\mathrm{P}^R/c_\mathrm{AO}^R\right)}{\ln\left(1 - c_\mathrm{P}^S/c_\mathrm{AO}^S\right)}. \tag{3}$$

Eq. (3) contains only a single parameter (E). Thus, in this simple situation, the value of this parameter can be easily determined when the amounts of (R) and (S)-substrate or (R) and (S)-product are measured during the reaction. Usually, these amounts are expressed in terms of extent

of conversion (ξ) and enantiomeric excess (ee) of either substrate or product, and therefore, it is convenient to modify Eq. (3) to [7,8]:

$$E = \frac{\ln\left[(1-\xi)\left(1-\mathrm{ee}_{\mathrm{A}}^{S}\right)\right]}{\ln\left[(1-\xi)\left(1+\mathrm{ee}_{\mathrm{A}}^{S}\right)\right]} = \frac{\ln\left[(1-\xi)\left(1+\mathrm{ee}_{\mathrm{P}}^{R}\right)\right]}{\ln\left[(1-\xi)\left(1-\mathrm{ee}_{\mathrm{P}}^{R}\right)\right]}. \tag{4}$$

3. Kinetic resolution with solid conglomerate suspension

A conglomerate is a 1:1 mechanical mixture of crystals of the two enantiomers, each crystal consisting of either A^R or A^S. In a suspension of solid conglomerate, initially, equal amounts of dissolved A^R and A^S will be present. If an enzyme preferentially converts A^R, this enantiomer will keep dissolving until it is completely consumed, and pure solid A^S remains, resulting in an efficient kinetic resolution process [2,6].

$$\mathrm{A}^R(\text{solid}) \leftrightarrow \mathrm{A}^R(\text{dissolved}) \rightarrow \mathrm{P}^R(\text{dissolved})$$
$$\mathrm{A}^S(\text{solid}) \leftrightarrow \mathrm{A}^S(\text{dissolved}) \rightarrow \mathrm{P}^S(\text{dissolved})$$

If the enantiomeric ratio of the enzyme in such a system is to be determined, one has to realize that the rates of conversion of substrate to product enantiomers are not determined by the *total* substrate concentrations, but only by the *liquid phase* substrate concentrations (with superscript L):

$$\frac{\mathrm{d}c_{\mathrm{P}}^{R}}{\mathrm{d}c_{\mathrm{P}}^{S}} = E\frac{c_{\mathrm{A}}^{\mathrm{L}R}}{c_{\mathrm{A}}^{\mathrm{L}S}}. \tag{5}$$

These liquid phase concentrations are dependent on the rate of dissolution of the *solid crystal* substrate enantiomers (superscript C, in order not to confuse with *S*-enantiomer) according to:

$$\frac{\mathrm{d}c_{\mathrm{A}}^{\mathrm{C}R}}{\mathrm{d}c_{\mathrm{A}}^{\mathrm{C}S}} = \frac{k_{\mathrm{L}}^{R}a^{R}\left(0.5c_{\mathrm{A}}^{*} - c_{\mathrm{A}}^{\mathrm{L}R}\right)}{k_{\mathrm{L}}^{S}a^{S}\left(0.5c_{\mathrm{A}}^{*} - c_{\mathrm{A}}^{\mathrm{L}R}\right)}. \tag{6}$$

These equations show that the concentrations of substrate and product enantiomers are not only determined by the enzyme-kinetic parameter, E, but also by the mass transfer parameters, c_{A}^{*} (the solubility of A) and $k_{\mathrm{L}}a$ (the rate constant for dissolution multiplied by the volume-specific surface area of the solid particles). So, in addition to measuring the substrate and product enantiomer concentrations, the latter parameters have to be known in order to determine E. This will require a number of additional experiments and numerical solution of a complicated set of differential equations [6].

In order to determine E easily, one should be in the situation where the course of the resolution is not dictated by the rate of dissolution, but only by the rate of the enzymatic reaction. If the rate of dissolution is much higher than the rate of enzymatic reaction, the liquid phase concentration of both substrate enantiomers will be constant during the conversion (as long as both *R*- and *S*-crystals are present) and will equal half the substrate solubility ($0.5\ c_{\mathrm{A}}^{*}$). Then, Eq. (5) simplifies to:

$$\frac{\mathrm{d}c_{\mathrm{P}}^{R}}{\mathrm{d}c_{\mathrm{P}}^{S}} = E. \tag{7}$$

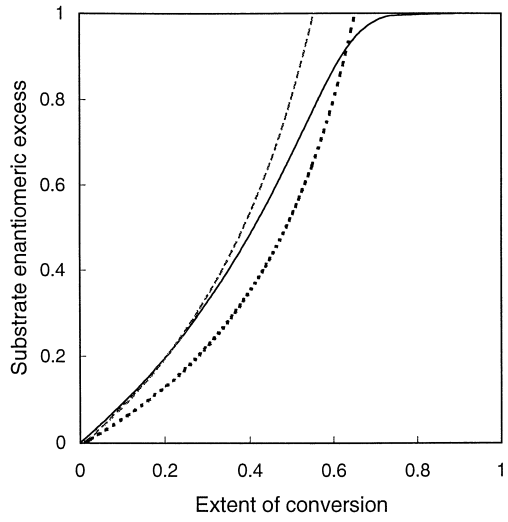

Fig. 1. Enantiomeric excess of the remaining substrate during simulated kinetic resolution for $E = 10$. (—): Completely dissolved racemic substrate (Eq. (4)). (---): Suspended racemic conglomerate, $c_{AO} = 400$ mmol/l and $c_A^* = 4$ mmol/l (Eq. (11)). (· · ·): Suspended racemic compound, $c_{AO} = 400$ mmol/l, $c_A^* = 4$ mmol/l, $U = 3$ (Eq. (13)). For the reactions with suspended substrates, mass transfer is assumed to be absent and the conversion and enantiomeric excesses apply to the undissolved substrate only.

This easily allows determination of the value of E, because integration starting from zero initial concentration leads to:

$$E = c_P^R / c_P^S. \tag{8}$$

Thus, the ratio of product enantiomers remains equal to E as long as there is R-solid, but no mass

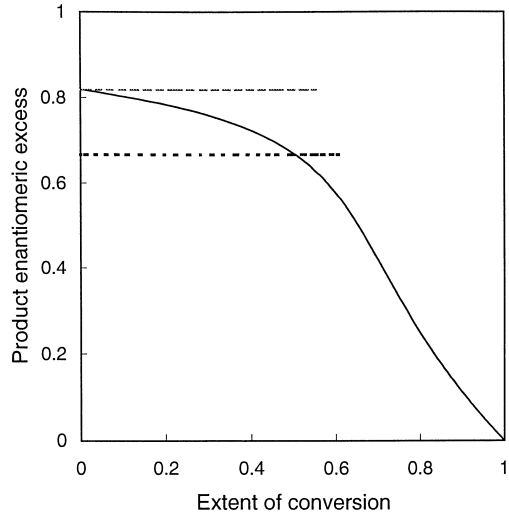

Fig. 2. Enantiomeric excess of the product during simulated kinetic resolution for $E = 10$. Conversion applies to the total conversion of dissolved and undissolved substrate. For other legends see Fig. 1.

Table 1
Apparent E-values that are calculated if a sample is taken at 50% conversion and if the Chen-equations are used instead of the suspension equations

	Conglomerate suspension	Racemic compound suspension
Real E-value (Eqs. (11) and (13), respectively)	10	10
Apparent E-value (Eq. (4), with ee of substrate)	22.1	9.83
Apparent E-value (Eq. (4), with ee of product)	25.1	5.35

For conditions, see legend of Fig. 1.

transfer limitation. Clearly, for this limit situation, Eq. (6) is not valid for solid. However, for either enantiomer, the molar balance can be used, i.e.,

$$c_{\mathrm{AO}}^{R} = c_{\mathrm{A}}^{\mathrm{C}R} + 0.5c_{\mathrm{A}}^{*} + c_{\mathrm{P}}^{R}, \tag{9}$$

and a similar equation for the (S)-enantiomer. Thus, Eq. (8) becomes:

$$E = \frac{c_{\mathrm{AO}}^{R} - c_{\mathrm{A}}^{\mathrm{C}R} - 0.5c_{\mathrm{A}}^{*}}{c_{\mathrm{AO}}^{S} - c_{\mathrm{A}}^{\mathrm{C}S} - 0.5c_{\mathrm{A}}^{*}}. \tag{10}$$

Thus, when the solid substrate concentrations are measured, the solubility is known or negligible, and the mass transfer limitation is absent, E can be easily determined. In this situation, one cannot determine E by measuring liquid phase substrate concentrations. Eqs. (8) and (10) can also be expressed in terms of enantiomeric excess of either product or remaining crystalline solid:

$$E = \frac{1 + \mathrm{ee}_{\mathrm{P}}^{R}}{1 - \mathrm{ee}_{\mathrm{P}}^{R}} = \frac{\left(1 - \xi^{\mathrm{C}}\right)\left(1 - \mathrm{ee}_{\mathrm{A}}^{\mathrm{C}S}\right) - \dfrac{2c_{\mathrm{AO}}^{R} - c_{\mathrm{A}}^{*}}{c_{\mathrm{AO}}^{R} + c_{\mathrm{AO}}^{S}}}{\left(1 - \xi^{\mathrm{C}}\right)\left(1 + \mathrm{ee}_{\mathrm{A}}^{\mathrm{C}S}\right) - \dfrac{2c_{\mathrm{AO}}^{S} - c_{\mathrm{A}}^{*}}{c_{\mathrm{AO}}^{R} + c_{\mathrm{AO}}^{S}}}. \tag{11}$$

Using these equations, one can obtain E from measurement of ee_{P}, or of $\mathrm{ee}_{\mathrm{A}}^{\mathrm{C}}$ as a function of the extent of conversion. Typical curves (for $E = 10$) are shown in Figs. 1 and 2, up to the extent of conversion at which R-solid is completely consumed. (The description of the remaining part of the curve would require an elaborate discussion which is probably of little practical value for E-determination). Clearly, the course of enantiomeric excess vs. conversion is very different from the previous case where the substrate is completely dissolved. So when the Chen-equation (Eq. (4)) is used instead of Eq. (11), wrong values for E are obtained. In the example of Table 1, this is 25 or 22 instead of 10. Fig. 1 also shows that the amount of remaining crystals of ee > 99% can be as high as 45%, whereas this is only 28% for the reaction with completely dissolved substrate. The enantiomeric excess of the product is also better than for the reaction with completely dissolved substrate, but it still is well below 100% if $E = 10$ (see Fig. 2).

4. Kinetic resolution with solid racemic compound suspension

About 90% of the organic chiral solids do not form conglomerates but so-called racemic compounds, in which the two enantiomers are present in a 1:1 ratio down to the unit cell level. In a suspension of A, the rates of dissolution of A^{R} and A^{S} are coupled. Initially, some A^{R} (the dissolved molecules) can be converted rapidly into P^{R} by an enantioselective enzyme, but subsequent

dissolution and conversion of solid A^R will be slow. The reason is that the racemic solid cannot dissolve if the solution is heavily supersaturated in A^S, so A^S must be also be converted, which is a slow process. In the steady state, A^R and A^S dissolve and react at the same slow rate which is not attractive for a kinetic resolution process. However, one may also reach the situation that the concentration of dissolved A^S exceeds the concentration of a eutectic mixture of crystals of the original racemic compound and crystals of pure A^S. Then A^S may start to crystallize, which may lead to a very efficient kinetic resolution process [5,6].

$$\begin{array}{ccccccc} A^R\text{-}A^S\,(\text{solid}) & \leftrightarrow & A^R\,(\text{dissolved}) & + & A^S\,(\text{dissolved}) & \leftrightarrow & A^S\,(\text{solid}) \\ & & \downarrow & & \downarrow & & \\ & & P^R\,(\text{dissolved}) & & P^S\,(\text{dissolved}) & & \end{array}$$

Typical values for the eutectic composition are in the range, $U = c^S_{\mathrm{A,eut}}/c^R_{\mathrm{A,eut}} = 1$ to 10. When the enantiomeric ratio exceeds the eutectic composition ($E > U$), this type of solid-to-solid conversion may be feasible, provided that the rates of substrate dissolution and crystallization are much faster than the rate of the enzymatic reaction [6]. The latter condition also has to be fulfilled when one wants to determine E in such a process. Otherwise, the course of the kinetic resolution process is not only dependent on E, but also on several parameters that are used to describe the dissolution and crystallization processes.

The situation becomes much simpler if no mass transfer limitation occurs. Then the liquid phase concentration of the substrate enantiomers equals the eutectic composition during the whole conversion, as long as *RS*-crystals are present. In this case, E again can be determined from the product concentrations according to Eq. (8), however, with E/U instead of E. For determination of E from solid substrate concentrations, using the molar balances, one can derive:

$$E = \frac{c^R_{\mathrm{AO}} - c^{\mathrm{C}R}_{\mathrm{A}} - c^R_{\mathrm{A,eut}}}{c^S_{\mathrm{AO}} - c^{\mathrm{C}S}_{\mathrm{A}} - c^S_{\mathrm{A,eut}}} \cdot \frac{c^S_{\mathrm{A,eut}}}{c^R_{\mathrm{A,eut}}}. \tag{12}$$

In this equation, the solid phase concentrations are the sum of concentrations in the two types of solids that are present: racemic crystals and pure enantiomer crystals. The enantiomeric ratio can be expressed in enantiomeric excess terms as follows:

$$E = \frac{1 + \mathrm{ee}^R_{\mathrm{P}}}{1 - \mathrm{ee}^R_{\mathrm{P}}} U = \frac{\left(1 - \xi^{\mathrm{C}}\right)\left(1 - \mathrm{ee}^{\mathrm{C}S}_{\mathrm{A}}\right) - 2\dfrac{c^R_{\mathrm{AO}} - c^R_{\mathrm{A,eut}}}{c^R_{\mathrm{AO}} + c^S_{\mathrm{AO}}}}{\left(1 - \xi^{\mathrm{C}}\right)\left(1 + \mathrm{ee}^{\mathrm{C}S}_{\mathrm{A}}\right) - 2\dfrac{c^S_{\mathrm{AO}} - c^S_{\mathrm{A,eut}}}{c^R_{\mathrm{AO}} + c^S_{\mathrm{AO}}}} U \tag{13}$$

Figs. 1 and 2 show that according to this equation, the course of the kinetic resolution is very different from the previous cases, despite the same value of E. For the conditions indicated, the amount of the remaining substrate of ee > 99% is 35%, which again, is better than for completely dissolved substrate, but not as good as for conglomerate suspensions (see Fig. 1). On the other hand, the enantiomeric excess of the product does not reach interesting values (see Fig. 2; for brevity, the course of the product curve after the point where the *RS*-crystals are completely consumed is not dealt with). Nevertheless, to determine the value of E, the enantiomeric excess of either substrate or

product can be measured. Subsequently, the correct equation has to be used, because the Chen-equation leads to erroneous values (see Table 1).

5. Identification of mass transfer limitations

As indicated previously, correct determination of E with either conglomerates or racemic compounds is greatly facilitated if mass transfer limitations are absent. This should be checked by measuring the dissolved substrate concentrations. When these are not constant and not identical to the solubility or to the eutectic composition, mass transfer limitations apparently occurs. Then the formulas given here are not valid for E value determination, and have to be replaced by large sets of coupled non-linear differential equations. Besides, such a situation would indicate that there is still considerable room for improvement of the resolution [6].

To increase the rate of mass transfer with respect to the rate of the enzymatic reaction, one may, e.g., increase the crystal surface area (by using smaller or more substrate particles or seed crystals), improve the stirring or use less enzyme. However, it is impossible to completely avoid mass transfer in the final stage of the reaction, when the surface area of the solid substrate particles is becoming very small. Initial measurements will therefore be more reliable for E-value determination.

6. Conclusion

Enzymatic kinetic resolution of suspended solids proceeds differently than the conventional procedures where a fully dissolved substrate is used. If this is not accounted for, the real enantiomeric ratio may be overestimated or underestimated by orders of magnitude.

In favorable cases, mass transfer is much faster than the enzymatic reaction. Then, kinetic resolution of suspended solids seems to be very attractive for practical synthetic applications. Also, in such cases, one may obtain reliable values of the enantiomeric ratio from experiments with suspended substrates without determining a large amount of additional parameters.

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